Structural and Synthetic Investigations of Diterpenoid Natural Products from Southern African Marine Invertebrates

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Christopher Anthony Gray

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Abstract

This thesis is divided into two parts. The first part (Chapter Two) documents a bioassay guided investigation of the ethyl acetate extracts of four marine invertebrates from Mozambique (an Irciniid sponge, a ***Haliclona*** sp. sponge, an ascidian tentatively identified as ***Diplosoma*** sp., and the soft coral ***Cladiella kashmani***). Eight known compounds [ilimaquinone (**2.1**), renierone (**2.7**), ***N***-formyl-1,2-dihydrorenierone (**2.8**), 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**2.9**), mimosamycin (**2.10**) 7***Z***-allylidene-5-hydroxy-7,7a-dihydro-2***H***-cyclopenta[***b***]pyran-6-one (**2.11**), flaccidoxide (**2.18**) and 11***S***,12***S***-epoxycembra-1***Z***,3***E***,7***E***-trien-14***S***-ol (**2.19**)] and a new diterpene [13***S***,14***R***-diacetoxy-11***S***,12***R***-epoxycembra-1***Z***,3***E***,7***E***-triene (**2.20**)] were isolated and identified using standard spectroscopic techniques. Anomalies in the published spectral data of **2.1** and **2.8** were exposed and corrected, and the absolute stereochemistry of the cembrane diterpenes **2.18** and **2.20** established using the modified Mosher's method. The comparative activities of the nine natural products against four cancer cell lines (A549, LOX, OVCAR3, SNB19) are reported.

The second part of the thesis (Chapter Three – Chapter Six) is concerned with an ecological, structural and synthetic study of diterpenes from the endemic South African pulmonate limpet ***Trimusculus costatus***. Two new labdane diterpenes [6β,7α-diacetoxylabda-8,13***E***-dien-15-ol (**3.10**) and 2α,6β,7α-triacetoxylabda-8,13***E***-dien-15-ol (**3.11**)] were isolated from ***T. costatus*** and evaluated for anti-feeding activity against the common predatory fish ***Pomadasys commersonnii***. A strategy for the semi-synthesis of **3.10** from rhinocerotinoic acid (**4.14**), a diterpene reportedly present in the ubiquitous South African shrub ***Elytropappus rhinocerotis***, was devised in order to allow further bioactivity tests to be performed and unequivocally assign the unknown absolute stereochemistry of the ***T. costatus*** metabolites.

Attempts to isolate rhinocerotinoic acid from local specimens of ***Elytropappus rhinocerotis*** were unsuccessful, and as the repetition of a published synthesis of **4.14** from (–)-sclareol (**4.15**) gave rhinocerotinoic acid in unacceptably low yields with poor stereoselectivity, an improved synthesis of **4.14** is presented.

Comprehensive studies using hispanone (**5.1**) as a model compound showed that 6,7-dioxygenated labda-8-enes could be prepared from compounds possessing a 7-oxo-labda-8-ene skeleton with some degree of stereocontrol. In the process, fourteen new hispanone analogues were prepared and most of these were tested for activity in a suite of ten agro-chemical assays. The novel compound 7β-hydroxy-9α-carbonitrile-15,16-epoxylabda-13(16),14-dien-6-one (**5.34**) exhibited significant activity against the crop fungus ***Phytophthora infestans*** and is currently being subjected to further agro-chemical tests. Unfortunately, the results from the oxygenation study performed on the model compound **5.1** could not be directly extrapolated to rhinocerotinoic acid. Attempts to prepare the naturally occurring **3.10** from **4.14** ***via*** an alternative route were unsuccessful but yielded an analogue of **3.10** in which the substituents at C-6 and C-7 are in a diequatorial rather than a diaxial configuration.

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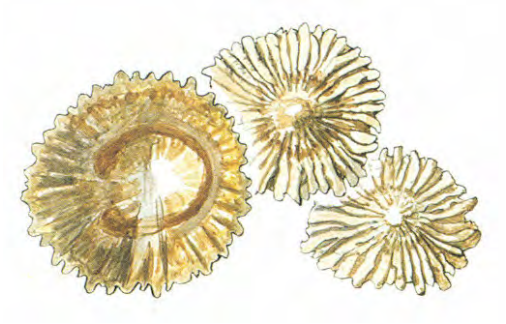
List of Abbreviations

1D	one dimensional
2D	two dimensional
$[\alpha]_D$	specific rotation (determined using the sodium D line at the temperature quoted)
Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
aq	aqueous
br	broad
c	concentration (quoted in g/100 mL)
calcd	calculated
conc.	concentrated
COSY	^{1}H-^{1}H homonuclear correlation spectroscopy
d	doublet
DCC	1,3-dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate
DEPT	distortionless enhancement by polarisation transfer
DIBAH	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMF	***N***,***N***-dimethylformamide
DMSO	dimethylsulfoxide
EIMS	electron impact mass spectrometry
eq	molar equivalent
Et_3N	triethylamine
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
eV	electron-volt
FT	Fourier transform
h	hour/hours
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherrence
HMPA	hexamethylphosphoramide
HPLC	high performance liquid chromatography
HREIMS	high resolution electron impact mass spectrometry
HRFABMS	high resolution fast atom bombardment mass spectrometry
HWE	Horner-Wadsworth-Emmons
IC_{50}	median inhibitory concentration
IR	infrared

LC_{50}	median lethal concentration
LDA	lithium diisopropylamide
lit.	literature
m	multiplet
MCPBA	***meta***-chloroperbenzoic acid
MeLi	methyl lithium
MeOH	methanol
MIC	minimum inhibitory concentration
min	minute/minutes
mmHg	millimetres of mercury
mmol	millimoles
mmu	millimass units
mol	moles
MoOPH	oxodiperoxymolybdenum·pyridine·hexamethylphosphoramide
mp	melting point
Ms	methanesulfonyl
MS	mass spectrometry
MTPA	α-methoxy-α-trifluoromethylphenylacetic acid
N	number of replicates
NBS	***N***-bromosuccinimide
NMR	nuclear magnetic resonance
NOEDS	nuclear Overhauser enhancement difference spectroscopy
NOESY	nuclear Overhauser enhancement spectroscopy
PCC	pyridinium chlorochromate
ppm	parts per million
ppt	parts per thousand
Py	pyridine
q	quartet
R	specified or unspecified alkyl group
rel. int.	relative intensity
RT	room temperature
s	singlet
sat.	saturated
SCUBA	self-contained underwater breathing apparatus
t	triplet
TBAF	tetrabutylammonium fluoride
TBS	***tert***-butyldimethylsilyl
TES	triethylsilyl
tert	tertiary

TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	***para***-toluenesulfonyl
TsOH	***para***-toluenesulfonic acid
UV	ultraviolet



The endemic South African marine pulmonate limpet, ***Trimusculus costatus***.

"The paths of research rarely lead in a straightforward fashion…chance occurrences along the way often enforce a change of course…as we come upon various points of interest which invite us to linger awhile. Ours, like all such rambling tours, possesses that special attraction that comes from knowing that the landscape spread out before us will be open to view, not by intention, but by chance and surprise."

Georg Wittig, Nobel laureate, 1979

Dedicated to Claire with love and gratitude.

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Chapter One

General Introduction

The term 'Organic Chemistry', when first used by Berzelius at the beginning of the nineteenth century, signified the branch of chemistry concerned with the study of compounds found naturally as constituents of living matter.[1] Over the last two centuries, the field of organic chemistry has developed and expanded immensely to yield the vast body of information now available to the organic chemist.[1] It is still evident, however, that the study of biological compounds lies at the very heart of organic chemistry:[1,2] investigations of the substances present in living organisms have not only given a theme to the discipline but have continually enriched its development.[1,2]

Traditionally, one of the most productive areas of enquiry for the organic chemist has been the study of those metabolites having no known role in the primary, protoplasmic metabolism of the organisms that produce them.[2-4] These metabolites are generally structurally complex and exhibit extreme diversity and sporadic existence in their temporal, taxonomic and ontogenetic distribution.[2,3,5] Since the exact metabolic function of many of these compounds was not apparent at the time of their discovery, these natural products were assumed to play a subsidiary role in the biology of the producing organism and have therefore been broadly termed 'secondary metabolites'.[2-4]

Secondary metabolites (natural products) are generally classified by their structural characteristics and biosynthetic origin into one of four groups: phenolics (aromatic and poly-aromatic compounds), isoprenoids (terpenes and steroids), polyketides or alkaloids.[2-4] The division between primary and secondary metabolites, however, is not always clear-cut; there are many rare and unusual amino acids, peptides and fatty acids which could be classed as secondary metabolites, while a large number of steroidal alcohols play an essential structural or hormonal role in most organisms and could conversely be considered primary metabolites.[4] To compound this problem further, the processes of primary and secondary metabolism are intimately related as primary metabolism provides the precursors for the various secondary metabolic pathways.[2,4,5]

1.1 Natural products: a biological perspective

It has been observed that "there is an increasing awareness that the somewhat arbitrary dividing line by which biological compounds are classified as primary or secondary metabolites, and which distinguish metabolites as those that are essential to life (in whatever form) and those that are not, may simply reflect the lacunae in our knowledge of the biological function of secondary metabolites".[2] Indeed, questions concerning the role and function of secondary metabolism in terrestrial systems is one that has excited considerable interest. Research in this area has evolved into the field of chemical ecology[6,7] which has developed during the last century into a mature discipline at the interface of biology and chemistry.[8,9]

Consequently, the role of natural products in the ecology of terrestrial plants and animals has been well documented.[6,10] This research has disclosed the surprising complexity and number of ways in which organisms utilise organic chemicals in ecological interactions,[6,7,11] and chemically mediated interactions are now considered ubiquitous in biological systems.[8] Methods of chemical defence in particular have been observed in all phylogenetic kingdoms[7] and chemical communication has been described as the paramount mode of interaction in most groups of animals.[12]

With the knowledge that many of these terrestrial secondary metabolites possess significant biological activity, it is hardly surprising that plants have formed the basis of traditional medicine systems since ancient times.[13] These plant-based systems continue to play an essential role in medicine; in 1985 the World Health Organisation estimated that approximately 80% of the global population still rely mainly on the traditional medicines for their primary health care.[14] Additionally, natural products also occupy a significant place in the modern pharmaceutical industry. Currently at least one hundred and nineteen natural products, derived from ninety plant species, can be considered as important drugs, and analysis of prescription data from the United States of America in 1993 indicated that over 50% of the most frequently prescribed drugs either contained a natural product or a natural product based compound as the active agent.[13] Considering the medicinal importance of natural products of terrestrial origin, it is not surprising that the study of

terrestrial natural products chemistry continues to be a highly active and productive area of research.[10]

1.2 From land to sea: the development of marine natural products research

In contrast to the extent of knowledge concerning the natural products chemistry and chemical ecology of terrestrial systems, comparable studies of marine systems have only been undertaken relatively recently.[15] The increased availability of SCUBA in the latter half of the twentieth century allowed scientists to observe at first-hand the biologically diverse and ecologically complex marine environment.[16,17] From the outset many hoped that marine organisms would be an exciting source of novel natural products as the oceans represent a very stable physical and chemical environment, rich in nutrients and distinct habitats that encourage a high level of biodiversity. With the link between biodiversity and natural product structural diversity well established from terrestrial phytochemical studies,[1,2] the large number of sessile, soft-bodied, aposematically coloured species present in the marine environment (utilising bioactive chemicals to defend themselves in the absence of physical defence mechanisms) promised rich rewards for the early marine natural products chemists.

Subsequent chemical investigations of marine biota, initially pioneered by Bergmann in the 1950s[18] and further stimulated by the search for 'Drugs from the Sea' in the late 1960s and early 1970s,[19] realised these expectations and have produced a fundamental knowledge-base of marine secondary metabolism.[20] Marine natural products chemistry, which developed rapidly in the late 1970s and 1980s and matured over the last decade[21] has to date yielded more than ten-thousand previously undescribed secondary metabolites.[22,23-25] Many of these natural products differ markedly from terrestrial secondary metabolites in that a large proportion are halogenated and often possess novel chemical structures and functional groups that are unprecedented among terrestrial organisms.[16,20,26] This is hardly surprising when considered from a phylogenetic perspective as many marine plants and invertebrates represent almost exclusively marine phyla and will have evolved aspects of secondary metabolism quite distinct from their terrestrial

counterparts.[20] Regrettably, recent reports tend to indicate that the explosion of novel structures common in the marine natural products isolated in the 1970s and 1980s seems to be fading,[27] and the chances of isolating known natural products from marine organisms is on the increase.

From an early stage, chemical studies of marine organisms have been directed toward defining the biological functions of their secondary metabolites.[15] Initially, advances in the field of marine chemical ecology were largely driven by chemists interested in the biological properties of the structurally novel natural products they had isolated[26] and relied heavily on the concepts, theories and experimental techniques developed through terrestrial chemical ecology research.[15]

More recently, with increased collaboration between organic chemists and marine ecologists, and concurrent improvements in experimental design and chromatographic techniques,[26] many of the secondary metabolites isolated from marine flora and fauna have been shown to serve as efficient deterrents against either predators, fouling organisms, pathogens, or competitors for limited resources.[15,16,26,28] Numerous marine natural products have also been found to play vital roles in reproduction, growth/development processes and succession in tropical, temperate and Antarctic benthic communities.[15,16,26,28]

Despite the extensive progress made in the field of marine chemical ecology, there is much still to be accomplished: Pawlik states that there remains a large body of biological literature that implicates the action of semiochemicals in marine ecological phenomena.[28] Many of the metabolites responsible for these interactions remain unknown as they are frequently present in trace amounts, are not conspicuous in spectroscopic screening of crude organism extracts or may be inactive in routine bioactivity assays.[26] Such limitations have been addressed by placing more emphasis on the design of ecologically relevant bioassay techniques (comprehensively reviewed by Hay ***et al***.[29]). Accordingly, marine chemical ecology has matured into a research area in which chromatographic and spectroscopic investigations are performed in tandem with ecologically relevant assay methods to reveal the underlying molecular basis of many of the inter- and intra-specific interactions observed in the marine environment (for recent reviews see references 15, 26, 28, 30 and 31).

Although a great deal can still be accomplished within the traditional confines of marine chemical ecology, Hay ***et al.*** believe that the most significant future advances in the field will be achieved by better integrating chemical ecology with physiology, neurobiology, molecular biology and population, community and ecosystem ecology.[29] The development of methodologies to allow this integration will be extremely challenging, but if this is successfully accomplished it will afford a much deeper understanding of the mechanisms affecting biological change in marine ecosystems and yield fundamental insights into the ecology and evolution of marine and aquatic communities.[29]

1.3 Marine natural products and the pharmaceutical industry

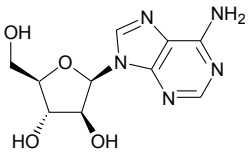
In a recent review,[21] Faulkner observed that "research on bioactive compounds from marine organisms has provided the bread and butter support of marine natural products research throughout the last quarter century". With the continued importance of natural products research on the process of drug discovery,[13,32] and the increasing pressure upon the pharmaceutical industry to find potential therapeutics and launch new chemical entities on the global market,[33] it would seem that this situation will persist into the foreseeable future. Although in recent years numerous patent applications have been filed for the development of marine natural products as therapeutics, food and health-food additives, cosmetics and research tools for the biomedical sciences,[34] the number of marine natural products or their derivatives currently available commercially is very small (Table 1.1).[25,34,35]

Several factors have contributed to this situation. The development of a natural product into a commercially available and viable product requires huge investments of both time and money.[17,23-25,33] The ecological and environmental impact of large-scale collections of marine organisms must be carefully considered.[23-25,35] In addition, recent international proposals concerning the establishment of sovereign rights over biological resources need to be respected, and agreements reached concerning intellectual property rights, licensing fees and payment of royalties to the country of origin.[32,36] The final, and at present probably the most important, factor

controlling the commercial development of a marine natural product is the question of supplying sufficient material for secondary assays once the structure determination and preliminary bioactivity studies are complete.[18,23-25,35,37]

Table 1.1 Some examples of marine natural products currently available commercially (after Pomponi[35]).

Product	Application	Original source	Method of production
Ara-A (**1.1**)	Antiviral drug.	Synthetic analogue[38] based upon the marine sponge metabolites spongouridine (**1.2**) and spongothymidine (**1.3**).[39] Later isolated from the Mediterranean gorgonian ***Eunicella cavolini***.[40]	Microbial fermentation of ***Streptomyces griseus***.[41]
Ara-C (**1.4**)	Anticancer drug.	Synthetic analogue[42] based upon the marine sponge metabolites spongouridine (**1.2**) and spongothymidine (**1.3**).[39]	Chemical synthesis.[42]
Okadaic acid (**1.5**)	Molecular probe: phosphatase inhibitor.	First isolated from the sponge ***Halichondria okadai***[43] and later from the dinoflagelate ***Prorocentrum lima***.[44]	Cell culture.[35]
Manoalide (**1.6**)	Molecular probe: phospholipase A_2 inhibitor.	Marine sponge, ***Luffariella variabilis.***[45]	Wild harvest of sponge.[35,37]
Formulaid®	Docosahexaenoic acid (**1.7**) used as an additive in infant formula nutritional supplement.	Marine microalgae, ***Cryptocodium cohnii.***[46]	Cell culture.[24,34,37]
Resilience®	Marine extract containing pseudopterosins (e.g. pseudopterosin A, **1.8**) added to skin creams.	Carribean gorgonian, ***Pseudopterogorgia elisabethae.***[47]	Wild harvest of gorgonian.[35,37]



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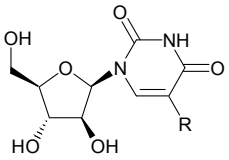

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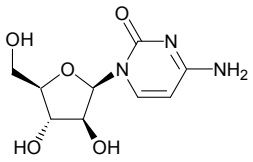

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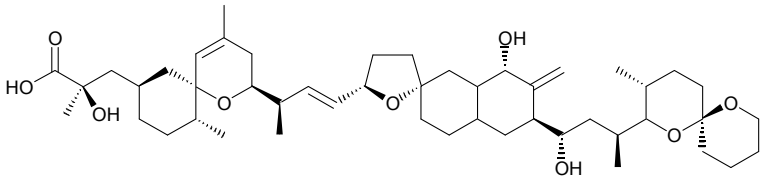

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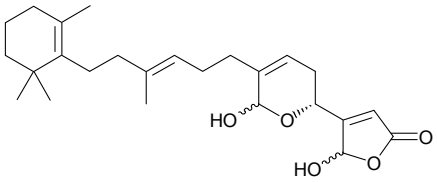

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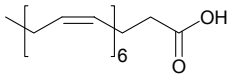

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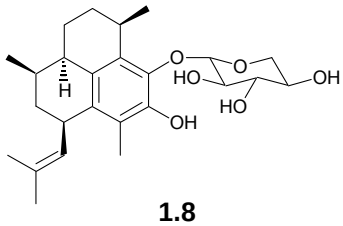
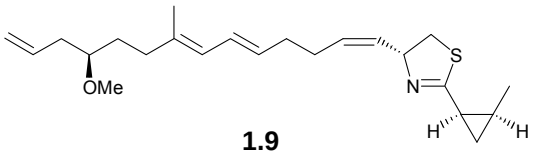


1.8

Many bioactive marine natural products are isolated in extremely small quantities, and whilst technical advances in analytical techniques and high-throughput screening programmes mean that preliminary bioactivity studies and structure elucidation can be carried out on sub-milligram quantities of the natural product, subsequent in-depth demonstration of the desired activity requires quantities of the compound of interest in the range of 10s to 100s of milligrams.[25] If the natural product in question is designated as a potential therapeutic agent, it must then pass into preclinical and clinical trials which generally require gram-quantities of the respective metabolite. Supplying these quantities of potentially important marine natural products represents a considerable challenge.[24,25]

Unfortunately, due to the complex structures of many of the marine natural products recognised as potential commercial products, in many cases obtaining more of the metabolite in question involves further, extensive recollection of the producing organism.[24] Obviously this approach can have deleterious ecological repercussions that have to be carefully evaluated before such recollections are made and alternatives to this approach are now being applied.[24,25,37] Despite these handicaps, the main driving-force behind the isolation of bioactive marine natural products continues to be the desire to obtain new pharmaceuticals.[22] As a result, there are currently many promising marine natural products or synthetic analogues in clinical or preclinical trials and these have been the subject of a number of recent reviews.[17,18,23-25,48-50] The following examples, selected from these reviews, serve to illustrate how the problem of supplying sufficient quantities of potentially important marine natural products for commercial development is presently being addressed.

When the natural product of interest was isolated from a marine cyanobacterium, bacterium or fungus, an alternative method of obtaining sufficient material for in-depth testing and commercial production of the metabolite is through large-scale culture of the producing organism obtained from wild strains.[18,24,35,49] Examples of the successful application of this approach are the culture of ***Lyngbya majuscula***,[24] a Caribbean cyanobacterium that produces the anti-mitotic natural product curacin A (**1.9**);[51] an estuarine actinomycete which yielded the cyclic heptapeptide cyclomarin A (**1.10**)[52] that has exhibited both anti-inflammatory and antiviral activity;[25] and the mangrove derived fungus ***Hypoxylon oceanicum*** which produces 15G256γ (**1.11**),[53] a compound with potential use as an antifungal agent.[24,25] Compounds **1.9**, **1.10** and **1.11** are all currently undergoing preclinical trials,[24] and with mounting evidence that many of the natural products derived from marine organisms are in fact produced by symbiotic marine microbes,[24,54] the future for obtaining biologically active marine natural products through a laboratory culture and isolation protocol seems very bright.



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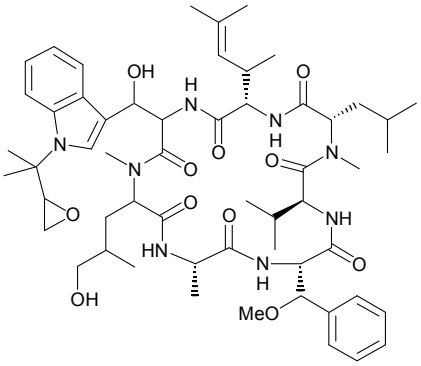

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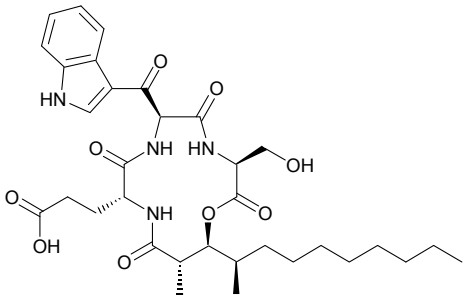

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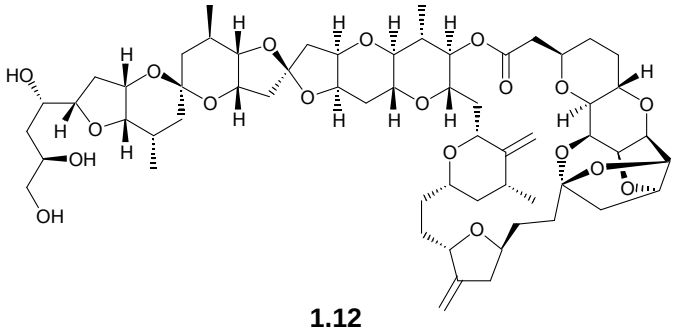
When dealing with macroscopic marine organisms, a second alternative to extensive recollection of natural populations is to propagate and harvest a sustainable stock of the organism through aquaculture. There have been a number of notable successes with this approach that have allowed potentially useful natural products to progress through pre-clinical and clinical trials. The macrolide halichondrin B (**1.12**) was isolated in very limited supply from the Japanese sponge ***Halichondria okadai*** [55] and showed promising ***in vivo*** activity against a number of human cell lines. The anti-cancer potential of **1.12** prompted the National Cancer Institute (USA), the National Institute for Water and Atmospheric Research (New Zealand) and the University of Canterbury (New Zealand) into developing a collaborative programme to obtain further **1.12** through aquaculture of the New Zealand sponge ***Lissodendoryx*** sp.[18,24,25] Two of the biggest successes of the search for 'drugs from the sea' are the potential anticancer drugs bryostatin 1 (**1.13**) from the bryozoan ***Bugula neritina***,[56] and ecteinascidin 743 (**1.14**) from the ascidian ***Ecteinascidia turbinata***.[57] Both of these compounds have advanced to Phase II clinical trials employing sustainable harvesting of wild populations, and more recently the establishment of specialised aquaculture programmes, providing the quantities of **1.13** and **1.14** necessary to support these clinical trials.[24]



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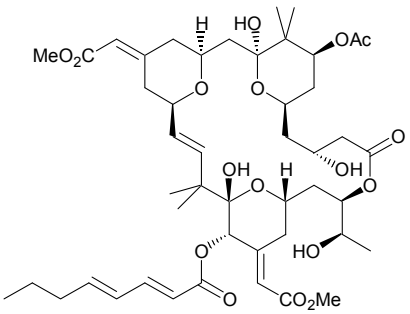

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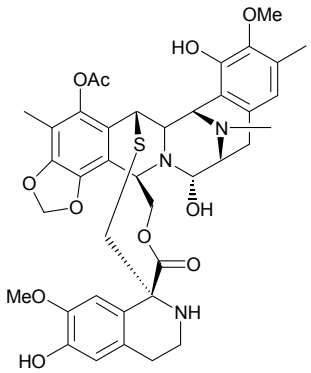
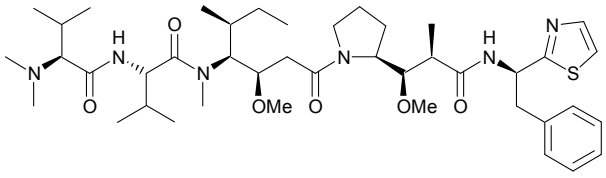


1.14

The final method of procuring stocks of marine natural products for examination of potential commercial applications is through total synthesis. Many natural products with promising bioactivity have complex skeletal structures making them attractive and challenging targets for academic syntheses.[25] This route has been successfully employed to provide a supply of the linear peptide dolastatin 10 (**1.15**),[58] originally isolated in milligram quantities from combined extensive collections (in excess of 1500 kg wet weight) of the mollusc ***Dolabella auricularia***.[59] However, the recent isolation of **1.15** from the marine cyanobacterium ***Symploca*** sp. VP642[60] may present opportunities for production of this peptide through laboratory culture. Dolastatin 10 is now in phase II clinical trials for the treatment of cancer of the pancreas, prostate, lungs, skin, colon, liver, breast and lymphatic system.[24] Discodermalide (**1.16**)[61] from the Carribean sponge ***Discodermia dissoluta***[62] and eleutherobin (**1.17**)[63] from the Australian soft coral ***Eleutherobia*** sp.[64] are also potential anti-cancer therapeutics which are currently undergoing preclinical trials in some part as a consequence of their production ***via*** total synthesis.[24,25] In addition to providing sufficient material during the developmental stages of drug discovery, the synthesis of marine

natural products by research groups working in Academia may also allow for the preparation of related analogues to be used in detailed structure-activity studies and lead to the development of new synthetic procedures required for the construction of these complex metabolites.



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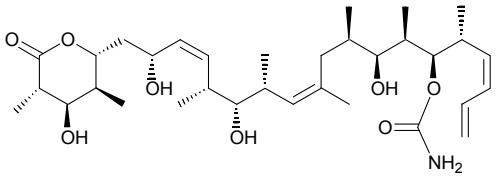

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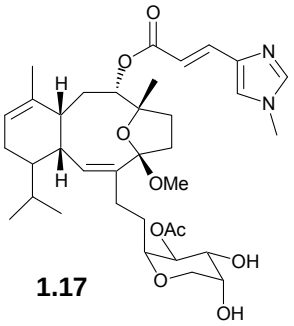


1.17

In conclusion, it is encouraging to see that, despite the present relative paucity of marine pharmaceuticals in the market place, some very promising natural products from marine biota are currently in the advanced stages of development as new chemotherapeutics. Although the goal of finding 'drugs from the sea' has been a major incentive driving research in the marine natural products field, associated lines of research have also vastly increased our knowledge of marine chemical ecology, chemotaxonomy and in some instances uncovered marine species previously unknown to science. It is also clear that marine natural products chemistry has had a major impact on chemistry over the last twenty-five years,[22] stimulating new developments in organic synthetic, chromatographic and analytical methods. With the vast number of invertebrate and algal species present in the marine environment, marine natural products chemists have only begun to uncover the biochemical diversity present in the world's oceans and we can expect the field of marine natural products chemistry to continue to make a significant contribution to both the biological and physical sciences in the future.

This thesis describes research directed towards the isolation, structure elucidation and evaluation of biological activity of natural products from five southern African marine invertebrates. The attempted synthesis of one of the natural products isolated as well as a number of isomeric analogues of this compound is also documented.

Chapter Two

An Investigation of the Natural Products Chemistry of Four Mozambican Marine Invertebrates

2.1 Introduction

As the field of marine natural products chemistry has matured, a general trend has emerged in which tropical marine species have been shown to yield a greater abundance of biologically active natural products when compared to species collected from temperate or cold water habitats.[15,16,26,28,65] This enhanced productivity has been linked to the greater predation pressure experienced by marine algae and invertebrates in tropical habitats. [15,16,26,28,65] As marine natural products chemists working in southern Africa we find ourselves in the fortunate position that relatively few studies have been performed on material collected from the somewhat inaccessible tropical coral reefs of south-eastern Africa along the shores of Northern KwaZulu-Natal and Southern Mozambique. Of the published investigations, most have been carried out by Kashman and co-workers focusing on soft corals,[66] sponges[67] and ascidians[68] from Sodwana Bay, South Africa, although new natural products have also been reported from two sponges[69] and a nudibranch[70] collected off Ponto do Oura, Mozambique. To further augment the incentive of collecting specimens from a relatively unexploited resource, this region of the coast lies upon a transitional zone between two major biogeographic regions; the vast Indo-Pacific faunal province to the north and the Cape endemic faunal province to the south (Figure 2.1).[71] As a consequence, the reefs of southern Mozambique and northern KwaZulu-Natal are remarkably biodiverse (Figure 2.2), incorporating both tropical species and the more temperate species of the Cape endemic province.[71]

As part of the first concerted effort to explore the pharmaceutical potential of southern African marine organisms, a collection of one hundred and eighteen subtidal and intertidal invertebrate and algal species was undertaken at Ponto do Oura, Mozambique in October 1995 in collaboration with SmithKline-Beecham and the Scripps Institution of Oceanography. From a taxonomic perspective, the collection was dominated by sponges (seventy-one specimens) with ascidians and coelenterates being represented in approximately equal proportions (twenty-one and twenty-three specimens respectively). In accordance with the collection protocols of SmithKline-Beecham, sponges, ascidians and soft corals were specifically targeted and therefore few algal and bryozoan species were collected (see Figure 2.3). Although we had high

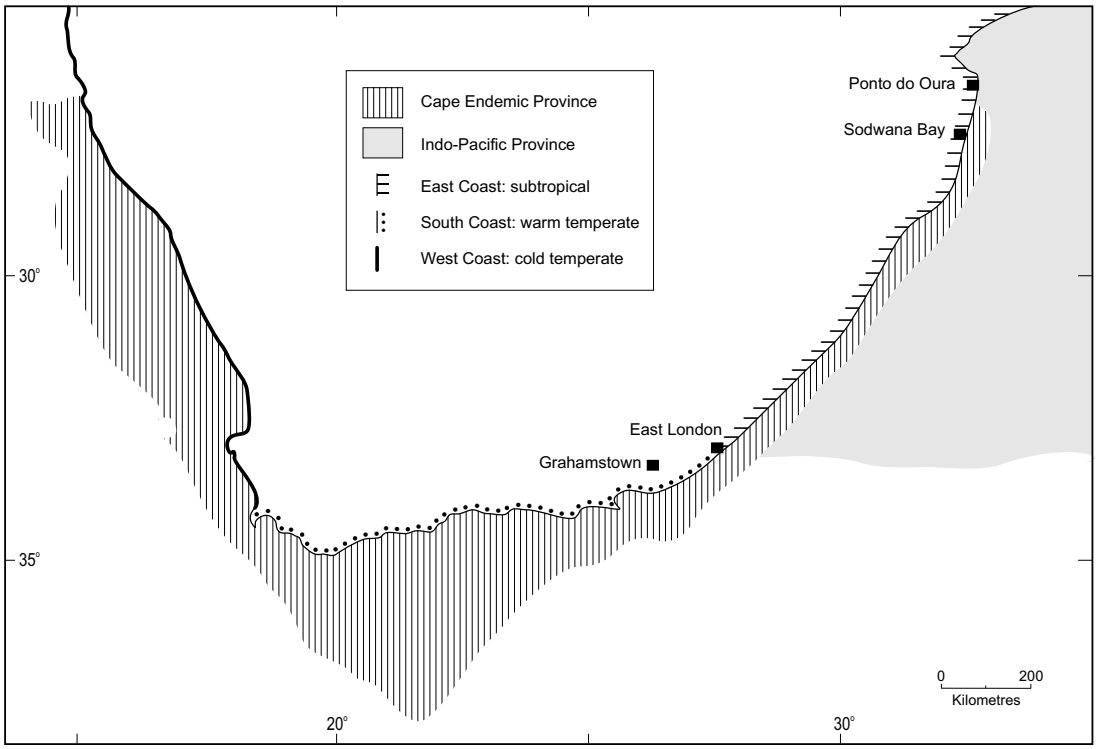


Figure 2.1 Map of southern Africa showing the two major biogeographic zones and sites referred to in this thesis.

Figure 2.2 The diverse coral reef system off Ponto do Oura, Mozambique.

expectations of this collection yielding new and biologically active natural products with significant pharmaceutical potential, unfortunately, due to the termination of the natural products research programme at SmithKline-Beecham in 1996, none of the extracts resulting from the Mozambique collection were assayed for pharmacological activity and were instead returned to Rhodes University. We therefore decided to undertake our own limited in-house screening of this collection in an effort to isolate structurally new and bioactive natural products.

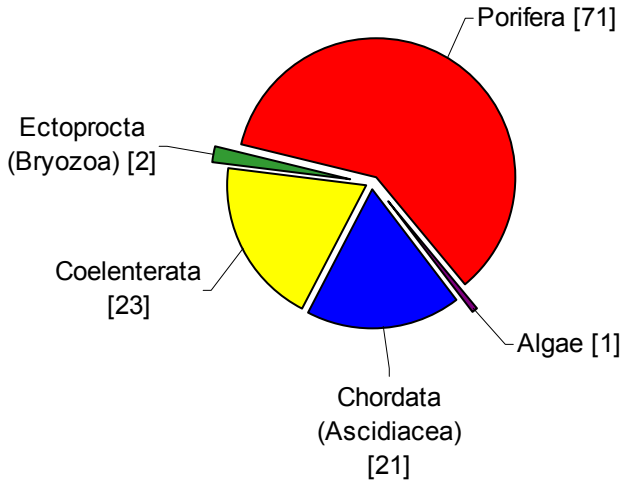


Figure 2.3 The phyletic distribution of the marine invertebrates collected off Ponto do Oura, Mozambique in October/November 1995.

2.2 Results and Discussion

The Mozambique collection was processed by SmithKline-Beecham to yield both ethyl acetate and aqueous extracts for each specimen that were then evaluated by us for broad-scale bioactivity or interesting spectroscopic properties. Out of necessity we were limited to employing the ***Artemia salina*** assay[72,73] and ^{1}H NMR spectroscopic screening to perform this evaluation. Of the two hundred and thirty-six extracts subjected to the ***A. salina*** assay, 36% (eighty four extracts) exhibited larvicidal properties (rated as > 50% mortality of ***A. salina*** larvae at a concentration of 200 μgmL^{-1}). These active extracts were further divided and rated as moderately active (> 50%

mortality of ***A. salina*** larvae at a concentration of 200 μgmL^{-1}), active (> 50% mortality of ***A. salina*** larvae at a concentration of 100 μgmL^{-1}), and highly active (> 50% mortality of ***A. salina*** larvae at a concentration of 50 μgmL^{-1}) as shown in Figure 2.4. From the thirty-nine extracts rated as highly active, four ethyl acetate extracts were chosen for further in-depth study on the basis of their promising ^{1}H NMR spectra and the results of these investigations are described below.

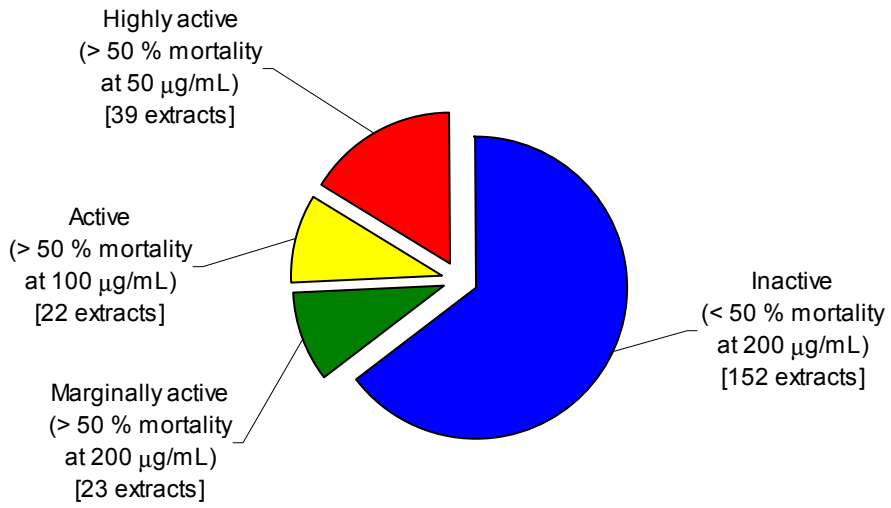


Figure 2.4 Summary of the results obtained from the ***Artemia salina*** bioassay screening of the crude extracts prepared from the Mozambique collection.

2.2.1 Sesquiterpene quinones from a Dictyoceratid sponge of the family Ircinidae

The first extract that was investigated was the ethyl acetate extract of a dark green sponge (MOZ 95-090), which was tentatively identified as belonging to the family Ircinidae. The sponge was collected using SCUBA from a depth of –17 m off Ponto do Oura. Examination of the ^{1}H and ^{13}C NMR spectra of the crude extract suggested that it contained large amounts of a single terpenoid-type natural product. In fact, the extract did not require initial purification by column chromatography and a portion (83 mg) was immediately subjected to normal phase semi-preparative HPLC in 3:2 hexane/ethyl acetate to afford the known compound ilimaquinone[74] (**2.1**,

55 mg, 66.3 % of the crude ethyl acetate extract; unfortunately, records relating to the dry mass of the MOZ 95-090 sponge collected were misplaced by SmithKline-Beecham) as an orange oil.

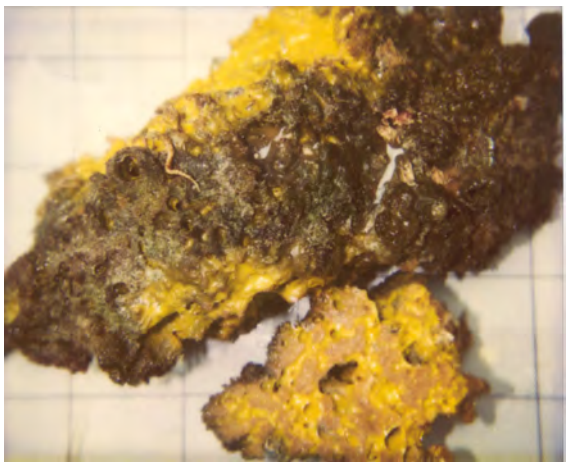


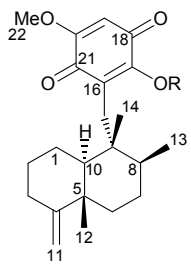
Figure 2.5 Surface photograph of MOZ 95-090 immediately after collection.

The ^{13}C and DEPT135 NMR spectra of the major metabolite **2.1** suggested that this compound possessed a molecular formula of $C_{22}H_{30}O_4$, which was subsequently confirmed by HRFABMS. Examination of the 1D and 2D NMR data indicated the presence of a methoxybenzoquinone moiety [δ_C 56.8 (q), 102.0 (d), 117.3 (s), 153.3 (s) 161.7 (s), 182.0 (s) and 182.3 (s)] which was coupled through a benzylic methylene [δ_H 2.44 (1H, d, ***J*** = 13.7 Hz) and 2.51 (1H, d, ***J*** = 13.7 Hz)] to a rearranged sesquiterpene skeleton possessing two tertiary methyl groups [δ_H 0.82 (3H, s) and 1.02 (3H, s)], a secondary methyl [δ_H 0.95 (3H, d, ***J*** = 6.4 Hz)] and an exocyclic methylene [δ_H 4.40 (1H, s) and 4.42 (1H, s)]. The presence of the quinone moiety was further confirmed by absorptions at λ_{max} 388, 279 and 214 nm in the UV spectrum and ν_{max} 1644 and 1609 cm^{-1} in the IR spectrum of **2.1**. The appearance of an exchangeable proton resonance [δ_H 7.50 (1H, br s)] in the ^1H NMR spectrum and a broad O–H stretch absorption (ν_{max} 3333 cm^{-1}) in the IR spectrum indicated that **2.1** also contained a free hydroxyl group.

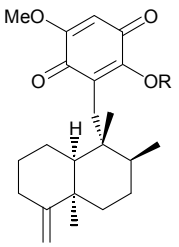
The identification of these structural features in conjunction with the molecular formula of the natural product allowed us to search the literature for accounts of the isolation of similar

compounds from marine sources.[75] Two possible structures were found which were consistent with the data, ***viz.*** ilimaquinone (**2.1**) and 5-***epi***-ilimaquinone (**2.2**).

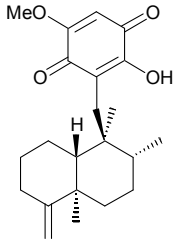
Ilimaquinone (**2.1**) was first isolated from a sponge tentatively identified as ***Hippospongia metachroma*** by Scheuer and co-workers in 1979, and originally assigned the absolute stereochemistry 5***R***, 8***R***, 9***S***, 10***R*** (as shown in **2.3**) on the basis of a weak positive circular dichroism measurement made on a degradation product of **2.1**.[74] Subsequently, Faulkner and co-workers isolated both **2.1** and its C-5 stereoisomer, 5-***epi***-ilimaquinone (**2.2**) as the C-17 acetates **2.4** and **2.5** respectively from a species of ***Fenestraspongia*** from Palau.[76] They established the relative stereochemistry about the A and B rings of **2.2** through chemical degradation and comparison of ^{1}H NMR and MS data with a similar degradation product obtained from arenarol (**2.6**), although the absolute stereochemistry was assumed to be analogous to **2.3**.[76] The absolute configuration of ilimaquinone has since been revised to 5***S***, 8***S***, 9***R***, 10***S*** by Capon and MacLeod.[77] To the best of our knowledge, no similar investigations have been reported for **2.2** and, because its absolute stereochemistry was not related to arenarol through measurement of the optical rotations of their common degradation products,[76] the absolute stereochemistry of 5-***epi***-ilimaquinone will be depicted here, as it is in the literature, as the C-5 epimer of **2.1**.



2.1 R = H
2.4 R = Ac



2.2 R = H
2.5 R = Ac



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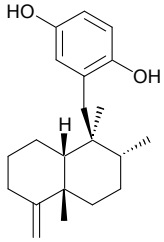


2.6

Since the initial isolation of **2.1** and **2.2**, these and closely related metabolites have been recognised as relatively common and abundant metabolites of Dictyoceratid sponges[78-83] and have been found to exhibit a variety of promising biological activities.[84,85] Ilimaquinone has

become a valuable tool for the investigation of some important biological processes,[21] and as such two total syntheses of **2.1** have recently appeared in the literature.[84,85]

On comparison with literature values,[74] it was found that the spectral data ($[\alpha]_D$, UV, IR, NMR, MS) obtained for the natural product isolated from the Irciniid sponge were consistent with those reported for ilimaquinone (**2.1**), although our assignment of the NMR data for C-4 and C-17 (made from HMBC data; see Figure 2.6) differs from that of the original paper[74] (Table 2.1) but is in agreement with later assignments[80,81] and the observations of Kushlan and Faulkner.[83] Unfortunately, direct comparison of our data with those reported for 5-***epi***-ilimaquinone[81] was not possible as only the literature data pertaining to 5-***epi***-ilimaquinone acetate[76] was available to us at that time. Therefore, to unequivocally confirm the structure of the natural product isolated from MOZ 95-090, **2.1** was acetylated to yield **2.4** which was found to be identical in all respects to ilimaquinone monoacetate[74] and not 5-***epi***-ilimaquinone acetate.[76]

2.2.2 Alkaloids from the intertidal sponge Haliclona sp.

The next extract that was considered worthy of detailed investigation was the ethyl acetate extract of a bright blue encrusting sponge (MOZ 95-080, 184 g dry weight) collected from the intertidal zone at Ponto do Oura. The sponge has been tentatively identified as a species from the genus ***Haliclona*** (synonym ***Reniera***), although it also displayed certain characteristic features of the related Haplosclerida genus ***Xestospongia***.[86] A portion of the crude extract (1.93 g) was subjected to flash chromatography on silica gel in 1:1 hexane/ethyl acetate followed by a final wash of ethyl acetate to elute a brightly coloured polar band that had remained on the column. The fractions collected were combined according to their respective TLC profiles to yield five fractions, and, on the basis of the plethora of deshielded resonances in their ^{1}H NMR spectra, fractions 2 (899 mg) and 5 (101 mg) were chosen for further investigation.

The ^{1}H NMR spectrum of the less polar chromatography fraction (fraction 2) indicated that this contained large amounts of fatty acids and sterols together with a number of compounds of

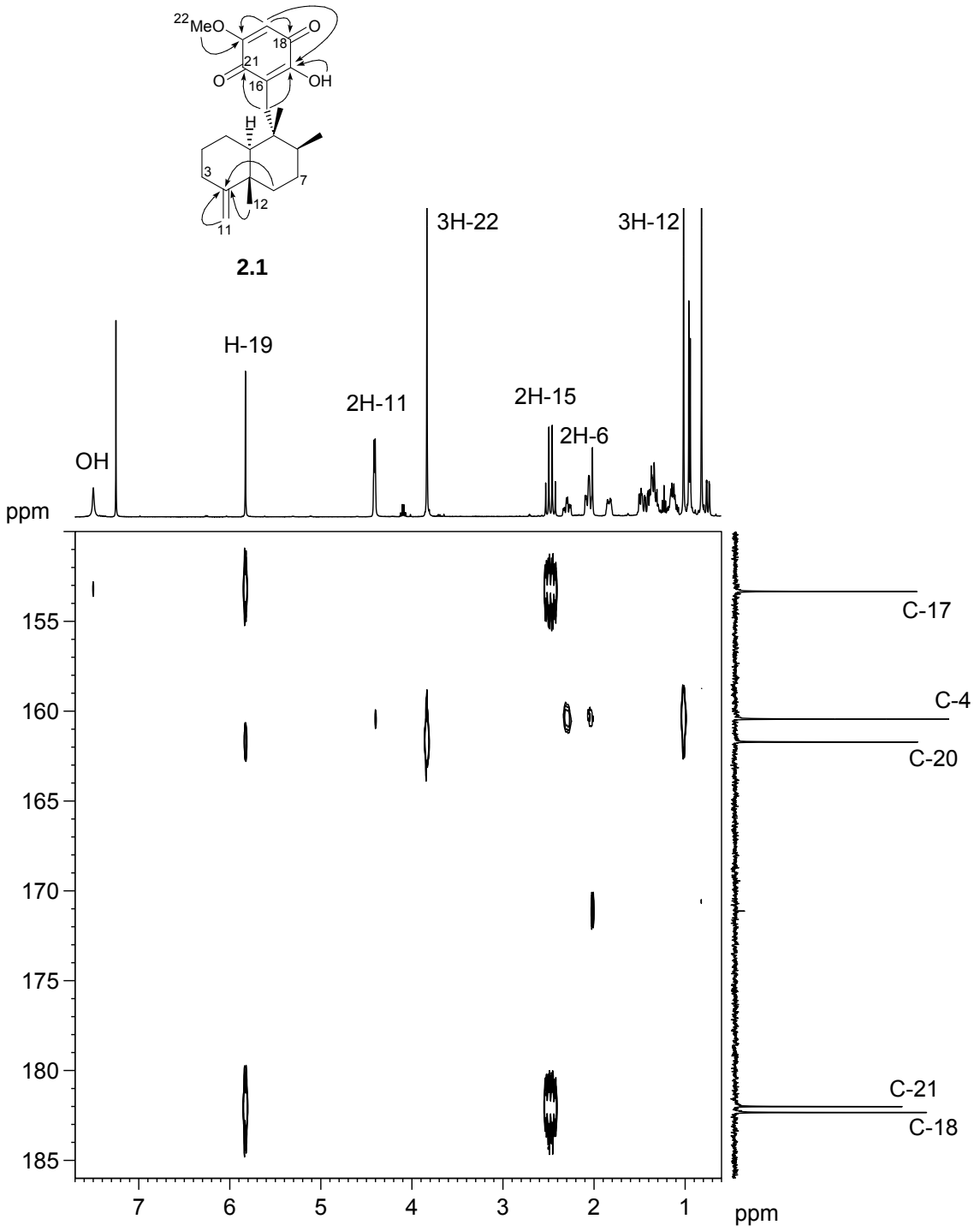


Figure 2.6 A region of the HMBC spectrum ($CDCl_3$, 400 MHz; delay for evolution of long range couplings [d6] = 60 msec; F1 = δ_C 150 – 186; F2 = δ_H 0.6 – 7.7) obtained for ilimaquinone (**2.1**) showing key correlations employed in the assignment of C-4 and C-17 (illustrated in the accompanying figure).

Table 2.1 Comparison of the ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data obtained for ilimaquinone (**2.1**) with the limited data reported by Scheuer and co-workers ($CDCl_3$, ^{1}H, 100 MHz; ^{13}C, field not specified).[74]

	2.1		Literature data[74]	
Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)
1	28.6 (t)	1.16 (1H, m) 1.83 (1H, m)	28.65	
2	23.1 (t)	1.47 (1H, dd, 12.5, 3.0) 2.08 (1H, br d, 16.1)	23.21	
3	36.6 (t)	1.32 (1H, m) 1.50 (1H, dd, 9.6, 2.7)	36.69	
4	160.4 (s)		153.42	
5	40.4 (s)		40.47	
6	32.9 (t)	2.02 (1H, d, 14.0) 2.29 (1H, td, 13.8, 5.3)	33.00	
7	27.9 (t)	1.36 (2H, m)	27.99	
8	38.1 (d)	1.14 (1H, m)	38.16	
9	43.3 (s)		43.34	
10	50.1 (d)	0.76 (1H, dd, 11.7, 2.0)	50.22	
11	102.5 (t)	4.42 (1H, s) 4.40 (1H, s)	102.56	4.44 (2H, br s)
12	20.5 (q)	1.02 (3H, s)	20.56	1.03 (3H, s)
13	17.8 (q)	0.95 (3H, d, 6.4)	17.87	
14	17.3 (q)	0.82 (3H, s)	17.32	0.82 (3H, s)
15	32.3 (t)	2.44 (1H, d, 13.7) 2.51 (1H, d, 13.7)	32.44	2.50 (2H, d)
16	117.3 (s)		117.41	
17	153.3 (s)		160.46	
18	182.3 (s)		182.4	
19	102.0 (d)	5.83 (1H, s)	102.06	5.86 (1H, s)
20	161.7 (s)		161.78	
21	182.0 (s)		182.04	
22	56.8 (q)	3.83 (3H, s)	56.84	3.87 (3H, s)
17-OH		7.50 (1H, br s)		7.50 (1H, s)

interest. As it was desirable to reduce the quantity of these primary metabolites in this fraction before commencing with further fractionation by HPLC, fraction 2 was partitioned between methanol and hexane to yield a methanol soluble fraction as a dark red oil (479 mg). A portion of the oil (118 mg) was then subjected to normal phase HPLC (7:3 hexane/ethyl acetate) to yield the known compounds renierone[87] (**2.7**, 4 mg, 0.017% dry weight) as a yellow powder, ***N***-formyl-1,2-dihydrorenierone[88] (**2.8**, 18 mg, 0.075% dry weight) as a dark red oil, and 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione[88] (**2.9**, 3 mg, 0.013% dry weight) as yellow needles.

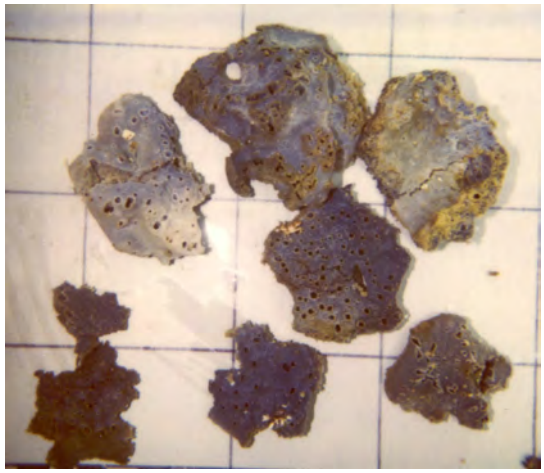
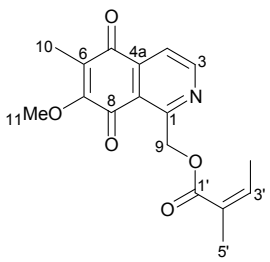


Figure 2.7 Surface photograph of MOZ 95-080 immediately after collection.

The more polar fraction from the initial flash column (fraction 5) was further purified on an open column of silica (eluted with 4:1 ethyl acetate/hexane, ethyl acetate and 1:1 ethyl acetate/methanol). The fraction eluting with ethyl acetate yielded a brown solid, which was subsequently recrystalised from ether-hexane to yield the known metabolite mimosamycin[89] (**2.10**) as an orange powder (27 mg, 0.028% dry weight).



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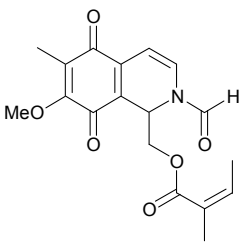

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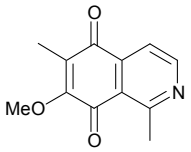

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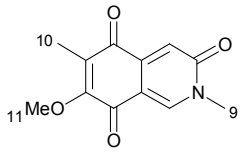


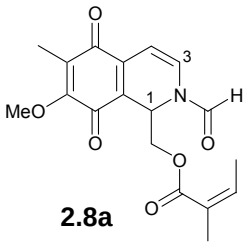
2.10

The molecular formula of **2.7**, $C_{17}H_{17}NO_5$, was assigned from analysis of its ^{13}C NMR and DEPT135 spectra and HRFABMS (***m/z*** 316.1184 [$(M+1)^+$], Δmmu −0.1) data. The ^{13}C NMR spectrum of **2.7** revealed a total of twelve sp^2 hybridised carbon atoms, of which four were assigned to two tetrasubstituted carbon–carbon double bonds [δ_C 158.5 (s), 138.9 (s), 130.5 (s) and 122.7 (s)], two to a trisubstituted double bond [δ_C 137.9 (d) and 127.9 (s)] and two to a disubstituted double bond [δ_C 154.0 (d) and 118.4 (d)]. Three more sp^2 hybridised carbon atoms were assigned to two quinone carbonyls [δ_C 184.6 (s) and 181.8 (s)] and an unsaturated ester [δ_C 167.9 (s)]. The remaining deshielded carbon resonance [δ_C 156.9 (s)] was assigned to an imine moiety. Having thus accounted for only eight of the ten degrees of unsaturation implied by the molecular formula of **2.7**, it was therefore evident that this compound was bicyclic.

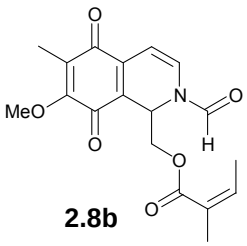
The ^1H NMR spectrum of **2.7** contained resonances corresponding to two mutually coupled aromatic protons [δ_H 8.90 (1H, d, ***J*** = 4.9 Hz) and 7.86 (1H, d, ***J*** = 4.9 Hz)], an esterified benzylic oxymethylene [δ_H 5.77 (2H, s)], a methoxyl group [δ_H 4.13 (3H, s)], a benzylic methyl [δ_H 2.08 (3H, s)] and an angelate ester [δ_H 6.09 (1H, qd, ***J*** = 7.1, 1.4 Hz), 1.99 (3H, dq, ***J*** = 7.2, 1.5 Hz) and 1.97 (3H, m, ***J*** = 1.5 Hz)]. With these fragments of the molecule identified, the full structure was easily and unambiguously identified as that of renierone, an antimicrobial isoquinoline quinone previously isolated from sponges of the genera ***Reniera***,[87,90] ***Cribrochalina***[91] and ***Xestospongia***,[92] through examination of the COSY and HMBC spectra obtained for **2.7** (Table 2.2). All other spectral data (UV, IR and MS) obtained by us were in agreement with those published for renierone.[87]

The ^1H and ^{13}C NMR spectra of **2.8** suggested that, although this compound eluted as a well defined, single fraction during normal phase HPLC, it was actually a 9:5 mixture of two inseparable isomers structurally related to **2.7**. A review of the renierone literature showed that a similar isomeric mixture had been reported for the metabolite ***N***-formyl-1,2-dihydrorenierone (**2.8**) by Frincke and Faulkner during a study of the minor metabolites isolated from the ***Reniera*** sponge which had previously furnished renierone (**2.7**).[88] The authors proposed that, as a consequence of the partial double bond character of the ***N***-formyl bond of **2.8**, two stereoisomers of ***N***-formyl-

1,2-dihydrorenierone (**2.8a** and **2.8b**) were observed on the NMR timescale. The geometries of the major (**2.8a**) and minor (**2.8b**) isomers were determined by comparison of the chemical shifts of H-1 and H-3 proton resonances.[88]



2.8a



2.8b

Table 2.2 The ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR data obtained for renierone (**2.7**).

Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., **J**/Hz)	HMBC correlation to	COSY coupling to
1	156.9 (s)			
3	154.0 (d)	8.90 (1H, d, 4.9)	C-1, C-4, C-4a	H-4
4	118.4 (d)	7.86 (1H, d, 4.9)	C-3 C-5, C-8a	H-3
4a	138.9 (s)			
5	184.6 (s)			
6	130.5 (s)			
7	158.5 (s)			
8	181.8 (s)			
8a	122.7 (s)			
9	65.3 (t)	5.78 (2H, s)	C-1, C-4a, C-8a, C-1´	
10	9.05 (q)	2.08 (3H, s)	C-5, C-6, C-7	
11	61.2 (q)	4.13 (3H, s)	C-7	
1´	167.9 (s)			
2´	127.9 (s)			
3´	137.9 (d)	6.09 (1H, qd, 7.1, 1.4)	C-5´	H_3-4´
4´	15.7 (q)	1.99 (3H, dq, 7.2, 1.5)	C-2´, C-3´	H-3´, H_3-5´
5´	20.6 (q)	1.97 (3H, m, 1.5)	C-1´, C-2´, C-3´	H_3-4´

The ^{1}H NMR data obtained for **2.8** compared favourably with those reported for ***N***-formyl-1,2-dihydrorenierone (Table 2.3) [88] but a number of discrepancies were observed upon comparison of the ^{13}C NMR data. Two of these differences (involving C-4 and C-3´) would seem to be simply due to conflicting assignment of these resonances to the different isomers of **2.8**, although there is a significant inconsistency in the chemical shifts observed for the C-4a resonances of both isomers [δ_C 135.3 (s) and 136.3 (s)] with those reported [δ_C 153.6 (s) and 154.0 (s)].[88]

Table 2.3 Comparison of the ^{1}H ($CDCl_3$, 400 MHz) NMR data obtained for ***N***-formyl-1,2-dihydrorenierone (**2.8**) with that reported by Frincke and Faulkner ($CDCl_3$, 360 MHz).[88]

	2.8a		**2.8b**	
Carbon	δ_H ppm (int., mult., ***J***/Hz)	Literature data[88] δ_H ppm (int., mult., ***J***/Hz)	δ_H ppm (int., mult., ***J***/Hz)	Literature data[88] δ_H ppm (int., mult., ***J***/Hz)
1	5.98 (1H, dd, 4.3, 3.2)	5.99 (dd, 4, 3)	5.34 (1H, dd, 9.2, 3.5)	5.37 (dd, 9, 4)
3	6.89 (1H, d, 7.6)	6.92 (d, 8)	7.42 (1H, d, 7.6)	7.45 (d, 8)
4	6.03 (1H, d, 7.6)	6.03 (d, 8)	6.23 (1H, d, 7.6)	6.25 (d, 8)
9	4.19 (1H, dd, 11.9, 3.2) 4.34 (1H, dd, 12.0, 4.4)	4.21 (dd, 12, 3) 4.37 (dd, 12, 4)	3.88 (1H, dd, 11.4, 3.6) 4.22 (1H, dd, 11.5, 9.3)	3.91 (dd, 12, 4) 4.24 (dd, 12, 9)
10	1.93 (3H, s)	1.95 (s)	1.97 (3H, s)	1.98 (s)
11	4.05 (3H, s)	4.05 (s)	4.04 (3H, s)	4.07 (s)
N-CHO	8.41 (1H, s)	8.43 (s)	8.21 (1H, s)	8.22 (s)
3´	6.05 (1H, qd, 7.2, 1.4)	6.06 (q, 7)	6.13 (1H, qd, 7.2, 1.4)	6.15 (q, 7)
4´	1.90 (3H, dd, 7.2, 1.5)	1.91 (d, 7)	1.98 (3H, dd, 7.3, 1.4)	2.00 (d, 7)
5´	1.76 (3H, t, 1.5)	1.77 (br s)	1.85 (3H, t, 1.5)	1.87 (br s)

It is difficult to comment on the possible reasons for these differences as the quality of the NMR data obtained by Frincke and Faulkner is unknown. The ^{1}H and ^{13}C NMR spectra obtained for **2.8** are reproduced in Figure 2.8 and a comparison of our ^{13}C NMR data and those reported by Frincke and Faulkner is provided in Table 2.4. It should be noted that the ^{13}C NMR data were unassigned in Frincke and Faulkner's paper; the assignments provided here were made by us through analogy with the related metabolite **2.7** and wherever possible confirmed by analysis of the 2D NMR data obtained for **2.8**. In the interest of consistency, the numbering system used here for **2.8** also differs from that used by Frincke and Faulkner and is based on that originally submitted for the parent compound renierone.[87]

Although ***N***-formyl-1,2-dihydrorenierone has also been isolated from the Fijian sponge ***Xestospongia caycedoi*** by Davidson,[93] in this instance the structure of **2.8** was confirmed through comparison of only the ^{1}H NMR data with those reported by Frincke and Faulkner[88] and no other spectroscopic data were reported for ***N***-formyl-1,2-dihydrorenierone. We were thus unable to verify the ^{13}C NMR data obtained for **2.8** by us through comparison with reports of its isolation from natural sources. However, following the synthesis of **2.7**, **2.8** and **2.9** by Kubo and co-workers,[94] during which the authors repeatedly reported that the ^{13}C NMR spectrum obtained for synthetic **2.8** was identical to that recorded for natural **2.8** (supplied to them by Faulkner), this group published a comprehensive study assigning the ^{13}C NMR spectra of thirty isoquinoline quinones and six quinoline quinones.[95] It is somewhat surprising then, that the ^{13}C NMR data reported for **2.8** in this latter paper are identical to those obtained by us, and that Kubo and co-workers make no mention of the inconsistencies observed between their data[95] and that previously reported by Frincke and Faulkner.[88] As all other spectral data obtained for **2.8** (IR, UV, MS) were in agreement with those reported,[88] we remain convinced that this metabolite is ***N***-formyl-1,2-dihydrorenierone despite the ambiguity relating to the published ^{13}C NMR data for **2.8**.

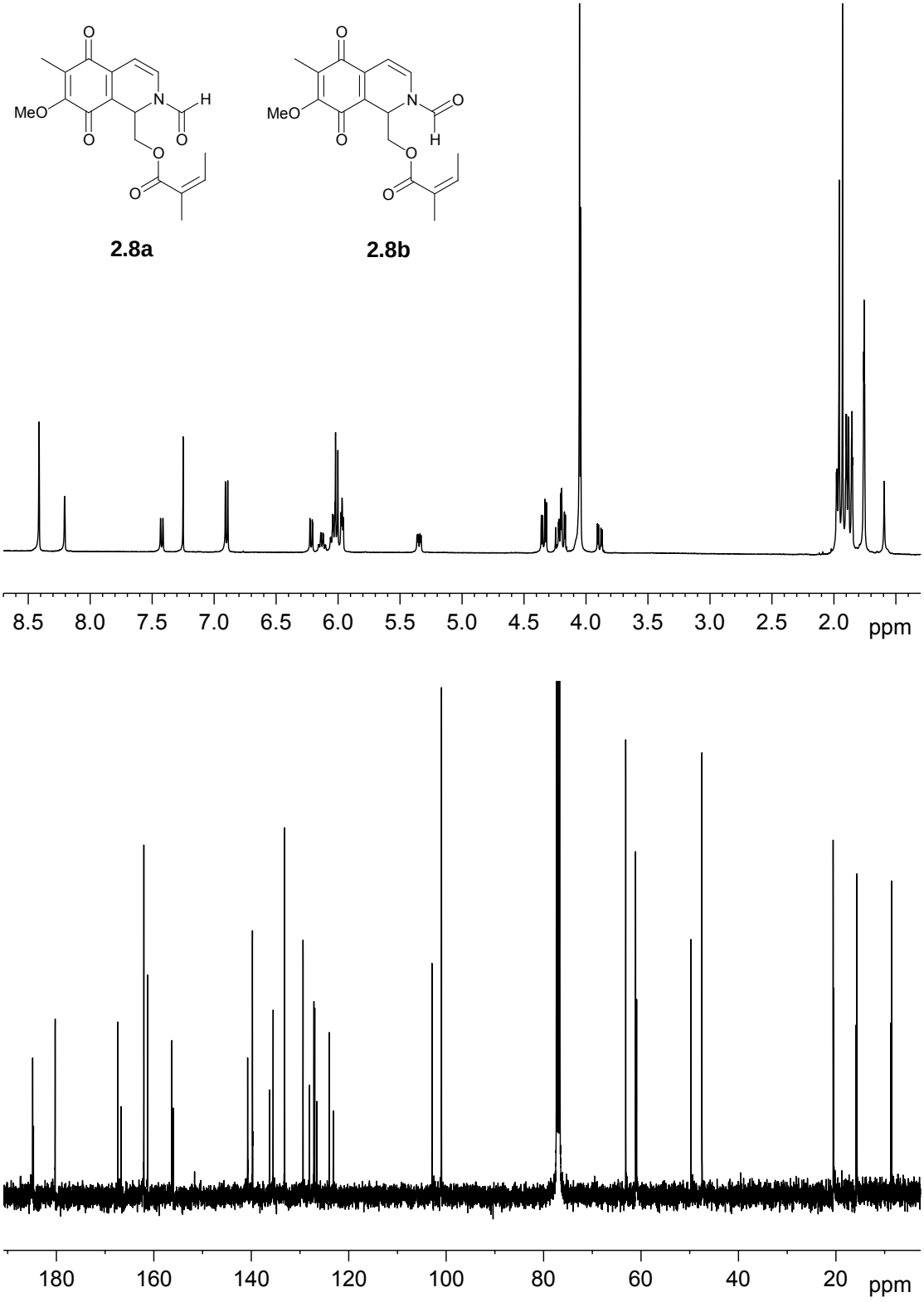


Figure 2.8 ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra obtained for ***N***-formyl-1,2-dihydrorenierone (**2.8**; shown inset as the two rotamers **2.8a** and **2.8b**).

Table 2.4 Comparison of the ^{13}C ($CDCl_3$, 100 MHz) NMR data obtained for ***N***-formyl-1,2-dihydrorenierone (**2.8**) with that reported by Frincke and Faulkner ($CDCl_3$, field not specified).[88]

Carbon	**2.8a**		**2.8b**	
	δ_C ppm (mult.)	Literature data[88] δ_C ppm (mult.)	δ_C ppm (mult.)	Literature data[88] δ_C ppm (mult.)
1	47.5 (d)	47.3 (d)	49.8 (d)	49.6 (d)
3	133.1 (d)	133.1 (d)	129.4 (d)	129.2 (d)
4	100.9 (d)	102.7 (d)	102.9 (d)	100.7 (d)
4a	135.3 (s)	153.6 (s)	136.3 (s)	154.0 (s)
5	184.9 (s)	184.6 (s)	184.7 (s)	184.0 (s)
6	127.1 (s)†	126.9 (s)	128.0 (s)	126.8 (s)
7	156.3 (s)	156.1 (s)	156.0 (s)	155.8 (s)
8	180.2 (s)	180.1 (s)	180.2 (s)	180.0 (s)
8a	124.0 (s)	123.8 (s)	123.1 (s)	123.0 (s)
9	63.1 (t)	62.9 (t)	60.8 (t)	62.9 (t)
10	8.6 (q)	8.4 (q)	8.7 (q)	8.5 (q)
11	61.1 (q)	60.9 (q)	61.1 (q)	60.9 (q)
N-CHO	162.0 (d)	161.9 (d)	161.3 (d)	161.1 (d)
1′	167.4 (s)	167.2 (s)	166.7 (s)	166.5 (s)
2′	127.0 (s)†	126.5 (s)	126.5 (s)	126.4 (s)
3′	139.8 (d)	140.4 (d)	140.7 (d)	139.5 (d)
4′	15.7 (q)	15.5 (q)	15.9 (q)	15.7 (q)
5′	20.6 (q)	20.3 (q)	20.5 (q)	20.3 (q)

† Signals may be interchanged.

With the structures of **2.7** and **2.8** secured, assigning the structure of the remaining metabolites (**2.9** and **2.10**) isolated from the ***Haliclona*** sponge was relatively simple. Comparison of the molecular formula, $C_{12}H_{11}NO_3$ (HRFABMS, ***m/z*** 218.0817 [$(M+1)^+$]), and ^1H NMR spectrum of **2.9**

with that of renierone (**2.7**) indicated that the angelate ester and C-9 methylene group [δ_H 5.78 (2H, s)] had been replaced by a methyl group [δ_H 2.97 (3H, s)] in **2.9**. Examination of the HMBC spectrum of **2.9** and comparison of the remaining ^{1}H and ^{13}C NMR resonances with those of **2.7** indicated that the substitution pattern of the isoquinoline rings was identical in these metabolites. We were thus able to identify **2.9** as 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione, an antimicrobial alkaloid previously isolated from ***Reniera***[88] and ***Xestospongia***[92] sponges. All other spectral data obtained for **2.9** were in agreement with literature values.[88]

The ^{1}H NMR spectrum of **2.10** consisted of only five singlet proton resonances. The presence of two aromatic protons [δ_H 8.24 (1H) and 7.06 (1H)], which displayed a weak mutual correlation in the COSY90 spectrum, indicated that the substitution pattern in **2.10** differed from that evident in the other MOZ 95-080 metabolites. The replacement of the C-3 sp^2 hybridised methine [δ_C ~154 (d)] with a carbonyl [δ_C 162.8 (s)] and the downfield shift of the C-9 methyl group (from δ_C 25.6 in **2.9** to δ_C 38.4 in **2.10**) suggested a 7-methoxy-2,6-dimethyl-3,5,8-trioxo substitution pattern on the isoquinoline skeleton. Comparison of the spectral data obtained for **2.10** with those published for mimosamycin,[89] originally isolated from ***Streptomyces lavendulae*** No. 314[89] and subsequently from marine sponges of the genera ***Reniera***[88,90] (or ***Haliclona***[96]), ***Xestospongia***,[92,97] ***Cribrochalina***[91] and ***Petrosia***,[98] allowed the unequivocal identification of this compound.

2.2.3 7Z-Allylidene-5-hydroxy-7,7a-dihydro-2H-cyclopenta[b]pyran-6-one from the intertidal ascidian Diplosoma sp.

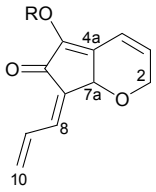
We now turned our attention to the ethyl acetate extract of a green encrusting didemnid ascidian of the genus ***Diplosoma***. The ascidian (578 g dry weight) was once again collected from the intertidal zone at Ponto do Oura. A portion of the crude extract (1.04 g) was first partitioned between hexane and methanol and the methanol soluble portion (782 mg) subjected to flash chromatography on silica gel. The fraction eluted with 1:1 hexane/ethyl acetate was obtained as a brown semi-solid (177 mg) that was washed with both cold hexane and cold methanol and

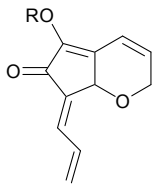
finally precipitated through addition of hexane to an ethyl acetate solution of the compound. In this manner the known compound 7***Z***-allylidene-5-hydroxy-7,7a-dihydro-2***H***-cyclopenta[***b***]pyran-6-one (**2.11**)[99] was obtained as a sticky cream solid (48 mg, 0.017% dry weight).

Figure 2.9 Surface photograph of MOZ 95-111 taken immediately after collection.

The molecular formula of **2.11** was easily assigned as $C_{11}H_{10}O_3$ from ^{13}C NMR, DEPT135 and HRFABMS (***m/z*** 191.0709 [$(M+1)^+$], Δmmu +0.1) data. A search of the literature[75] indicated that only two natural products (**2.11** and **2.12**) with an equivalent molecular formula had previously been isolated from a marine source. In 1978 Wratten and Faulkner reported that the methanol extract of a sponge, ***Ulosa*** sp., contained the isomeric diosphenols **2.11** and **2.12**.[99] These possessed significant antimicrobial activity, but could not be isolated easily: Wratten and Faulkner indicated that only the major metabolite **2.12** could be isolated in low yield after repeated precipitation from methanol/chloroform solution. However, acetylation of the mixture gave the acetates **2.13** and **2.14** that were then separated, once again in low yield, by preparative TLC.[99] The geometry of the Δ^7-double bonds in **2.13** and **2.14** were assigned on the basis of the chemical shifts of the corresponding H-8 olefinic proton resonances as Wratten and Faulkner hypothesised that H-8 in the ***Z***-isomer would be shielded relative to H-8 in the ***E***-isomer due to the shielding effects of the carbonyl group on β-protons.[99] Thus the exocyclic olefinic bond in the

minor acetate **2.13** was assigned a ***Z***-geometry (δ_H 6.65 ppm, H-8) and that of the major acetate **2.14**, the ***E***-geometry (δ_H 7.08, H-8). To the best of our knowledge, this is the only report of the isolation of these compounds in the literature.[75,100]

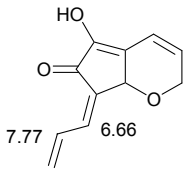


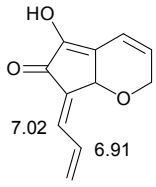


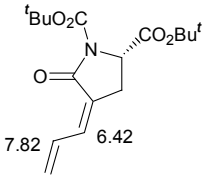
2.11 R = H
2.13 R = Ac

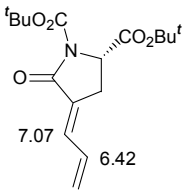
2.12 R = H
2.14 R = Ac

Comparison of the NMR data obtained for **2.11** with the limited data reported for **2.12** by Wratten and Faulkner indicated that the compound isolated by us was not the Δ^7-***E***-isomer **2.12**. Examination of the 2D NMR data obtained for the compound isolated from MOZ 95-111 (Table 2.5) confirmed the structure as 7-allylidene-5-hydroxy-7,7a-dihydro-2***H***-cyclopenta[***b***]pyran-6-one, and comparison of the chemical shift of H-8 (δ_H 6.66) with that reported for the corresponding proton in **2.12** (δ_H 7.05) indicated that it was most probably the Δ^7-***Z***-isomer **2.11**. This stereochemical assignment is supported by comparison of the H-8 and H-9 chemical shifts of **2.11** and **2.12**[99] with those of the corresponding protons in both geometrical isomers of ***tert***-butyl (2***S***)-***N-tert***-butoxycarbonyl-4-(prop-2-en-1-ylidene)-pyroglutamate[101] (**2.15** and **2.16**; see Figure 2.10) and confirmed through the observation of a small nuclear Overhauser enhancement between H-7a and H-8 in the NOESY spectrum of **2.11** (Figure 2.11).









2.11 **2.12** **2.15** **2.16**

Figure 2.10 Comparison of the effect of double bond geometry upon the chemical shifts (in ppm) of the non-terminal allylidene protons in **2.11** / **2.12** and **2.15** / **2.16**.[99,101]

Table 2.5 The ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR data obtained for 7***Z***-allylidene-5-hydroxy-7,7a-dihydro-2***H***-cyclopenta[***b***]pyran-6-one (**2.11**).

Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	HMBC correlation to	COSY coupling to
2	67.1 (t)	4.49 (1H, ddd, 18.4, 4.0, 1.4) 4.57 (1H, dt, 18.5, 2.6)	C-3, C-4,C-4a, C-7, C-7a	H-3, H-4
3	134.1 (d)	6.17 (1H, ddd, 10.0, 4.1, 2.4)	C-2, C-4a	H_2-2, H-4
4	118.4 (d)	6.79 (1H, dt, 10.0, 1.9)	C-2, C-3, C-7a	H_2-2, H-3, H-7a†
4a	128.8 (s)			
5	131.7 (s)			
6	188.0 (s)			
7	147.7 (s)			
7a	71.9 (s)	4.79 (1H, br s)	C-2, C-4a, C-5, C-6, C-7, C-8	H-4†, H-8
8	136.7 (d)	6.66 (1H, dd, 11.5, 0.7)	C-6, C-7a, C-9, C-10	H-7a, H-9, H_2-10†
9	132.1 (d)	7.77 (1H, ddd, 17.1, 11.4,10.0)	C-8	H-8, H_2-10
10	127.2 (t)	5.57 (1H, d, 10.0) 5.61 (1H, d, 17.2)	C-8, C-9	H-8†, H-9

† Weak COSY correlations.

In order to unambiguously identify **2.11**, this metabolite was acetylated in acetic anhydride/pyridine to yield an orange oil. However, on examination of the NMR data recorded for the acetylated product, it became evident that significant isomerisation of the exocyclic double bond had occurred under these conditions. The ^{1}H and ^{13}C NMR spectra indicated that the product obtained from the acetylation was a 1:6 mixture of both isomeric acetates **2.13** and **2.14**. Comparison of the NMR data with those reported[99] indicated that the major product had the ***E***- rather than the ***Z***-geometry about the C-7–C-8 double bond, which would be expected as this is the thermodynamically favoured geometry in C-5 functionalised 2-hydroxycyclopent-2-eneones possessing a C-5 exocyclic olefin.[102]

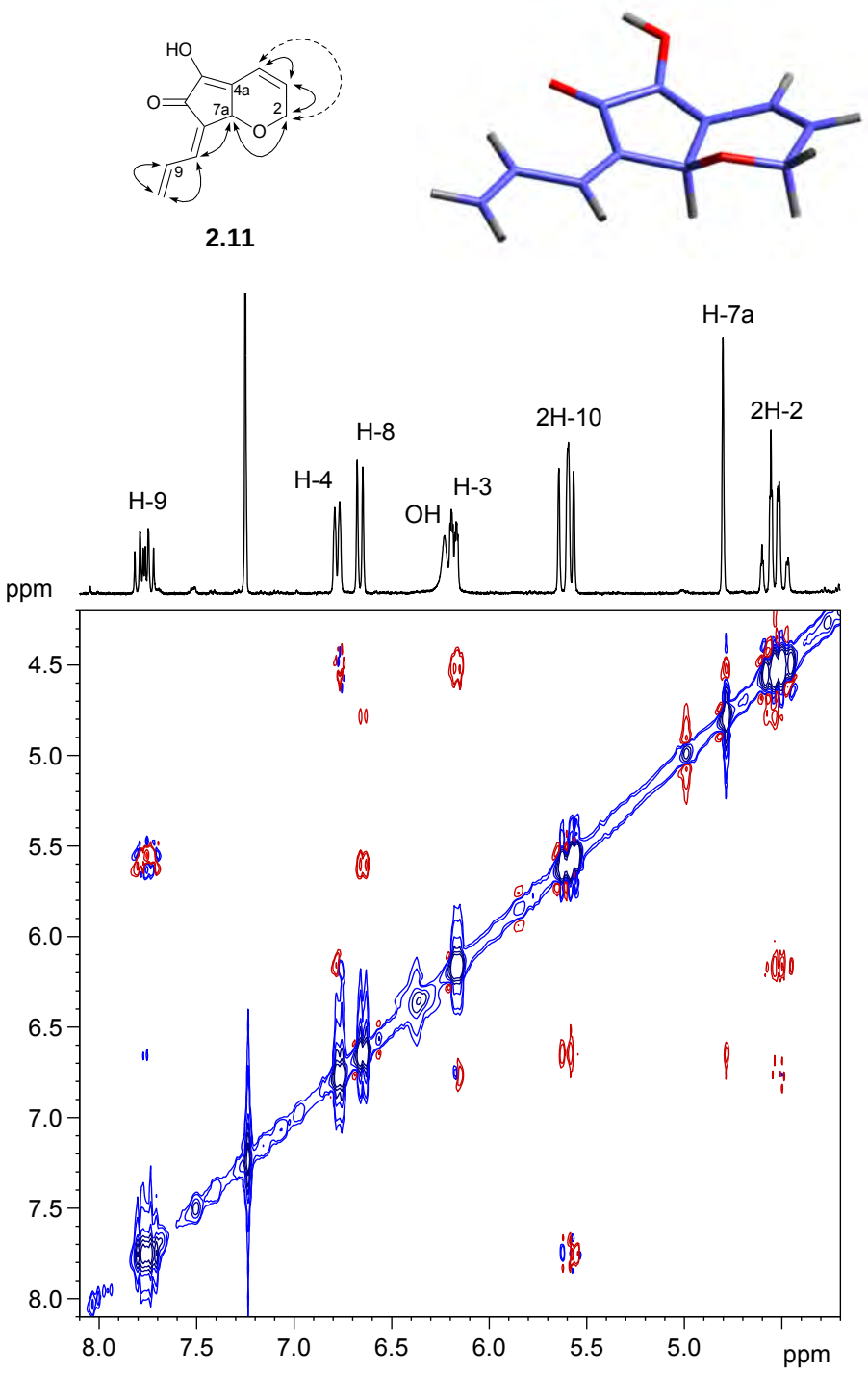


Figure 2.11 NOESY spectrum ($CDCl_3$, 400 MHz; relaxation time [d1] = 1.00 sec; NOESY mixing time [d8] = 800 msec; F1 and F2 = δ_H 4.2 – 8.1) obtained for the diosphenol **2.11**. Red contours indicate positive peaks, blue contours indicate negative peaks. Accompanying figures show the observed NOESY correlations and the computer-modelled global energy minimum conformation of **2.11**.

Following the exclusive isolation of **2.11** from MOZ 95-111 and its ready isomerisation under relatively mild reaction conditions, a careful re-examination of the crude extract of the ascidian by ^{1}H NMR spectroscopy indicated that the ***E***-isomer **2.12** was also present as a minor component of the ethyl acetate extract. Well resolved, characteristic proton resonances for **2.12** were observed [δ_H 5.04 (1H, s, H-7a), 5.73 (1H, d, ***J*** = 16.3 Hz, H-10a), 6.91 (1H, m, H-9) and 7.06 (1H, d, ***J*** = 12.1 Hz, H-8)],[99] and integration of corresponding proton resonances indicated that **2.11** and **2.12** were present in a 8:1 ratio in the crude MOZ 95-111 ethyl acetate extract.

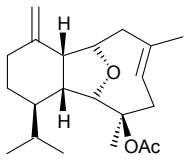
With the knowledge that the C-8 double bond of **2.11** is easily isomerised and that small amounts of **2.12** were present in the crude extract of the ***Diplosoma*** ascidian, we therefore have to question whether the ***E***-isomer isolated by Wratten and Faulkner was an authentic natural product or an artefact produced through isomerisation during the extraction of the ***Ulosa*** sponge.[99] It is also interesting to note that the isolation of **2.11** from both a sponge and an ascidian leaves the true origin of this metabolite in doubt and suggests that it may rather be of microbial origin.

Endeavours to obtain a single crystal of **2.11** suitable for X-ray crystallography from a variety of solvent systems were unfortunately unsuccessful, and the absolute stereochemistry of the diosphenols **2.11** and **2.12** therefore remains unassigned. However, investigations are at present being undertaken in our laboratory in an effort to elucidate the absolute stereochemistry of **2.11** through synthetic manipulation and derivatisation of the natural product.

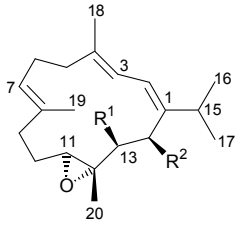
2.2.4 Cembrane diterpenes from the soft coral Cladiella kashmani

The final extract to which we turned our attention was the ethyl acetate extract of the recently described soft coral species ***Cladiella kashmani***[103] that was collected using SCUBA at a depth of –15 m from the Malangaan Reef, Ponto do Oura. This genus has, until now, yielded predominantly tricyclic cladiellane[104-106] (or eunicellane[107]) diterpenes (e.g. cladiellin,[104] **2.17**) and we were expecting to obtain similar metabolites from ***C. kashmani***. However, ***A. salina*** larvicidal bioassay[72,73] guided fractionation of a portion of the ethyl acetate extract (5.0 g) yielded three

bioactive compounds (**2.18** – **2.20**) as colourless oils whose NMR spectral data, although being characteristic of terpenoid metabolites, did not correspond to those expected for cladiellane-type diterpenes.[104-107]



2.17



2.18 R^1 = OH, R^2 = OAc
2.19 R^1 = H, R^2 = OH
2.20 R^1 = R^2 = OAc

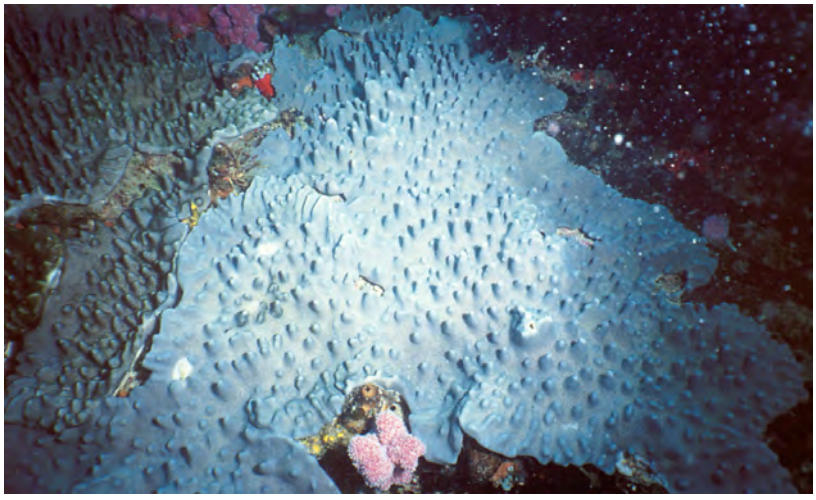
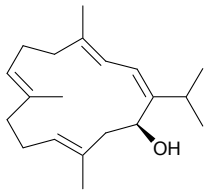


Figure 2.12 A large colony of ***Cladiella kashmani*** with polyps retracted.

The molecular formula of the most polar metabolite **2.18**, $C_{22}H_{34}O_4$, was determined from HREIMS data. The ^{13}C NMR data indicated that this compound possessed an 11,12-epoxy cembranoid skeleton incorporating an acetoxy and a hydroxy moiety. The placement of these functionalities followed from HMQC and HMBC data and the structure of **2.18** was determined to be 14-acetoxy-11,12-epoxycembra-1***Z***,3***E***,7***E***-trien-13-ol, the spectral data of which were

consistent with the spectral data ($[\alpha]_D$, UV, IR, NMR, MS) reported by Kashman ***et al***.[108] for flaccidoxide.

HREIMS data also provided the molecular formula of **2.19** ($C_{20}H_{32}O_2$). Comparison of the spectral data of **2.19** (UV, IR, NMR, MS) with those reported previously[109,110] for 11***S***,12***S***-epoxycembra-1***Z***,3***E***,7***E***-trien-14***S***-ol, isolated from an Australian ***Lobophytum*** sp., confirmed the structure of this compound. It should be noted, however, that although Bowden ***et al***.[109] confidently assigned the absolute configuration of the 14***S***-hydroxyl through chemical manipulation to the known cembrane sarcophytol A (**2.21**),[111] the 11***S***,12***S*** configuration of the epoxide function of **2.19** was assumed from analogy to related compounds and has only recently been determined by Li and co-workers through enantioselective total synthesis of **2.19**.[112] The large, positive optical rotation obtained for **2.19** (+203°) is consistent with those reported by both Bowden ***et al***.[109] ($[\alpha]_D$ +229°) and Li and co-workers[112] ($[\alpha]_D^{20}$ +218°) confirming the 11***S***, 12***S*** and 14***S*** absolute stereochemistry of the compound isolated from ***C. kashmani***.



2.21

Although the ^1H and ^{13}C NMR spectra of **2.20** (Figure 2.13) were very similar to those of **2.18** (see Table 2.6), the spectra of the former compound contained extra resonances in accordance with the presence of an additional acetate moiety signals [δ_H 2.12 (3H, s) and δ_C 170.6 (s) and 20.7 (q)]. The molecular formula of **2.20**, $C_{24}H_{36}O_5$, determined by HREIMS, substantiated the inference made from the NMR data and suggested that **2.20** was the 13-acetate ester of flaccidoxide (**2.18**). This was confirmed by acetylation of **2.18** to yield an oil spectroscopically indistinguishable from **2.20**, unequivocally establishing the structure of **2.20** as 13,14-diacetoxy-11,12-epoxycembra-1***Z***,3***E***,7***E***-triene.

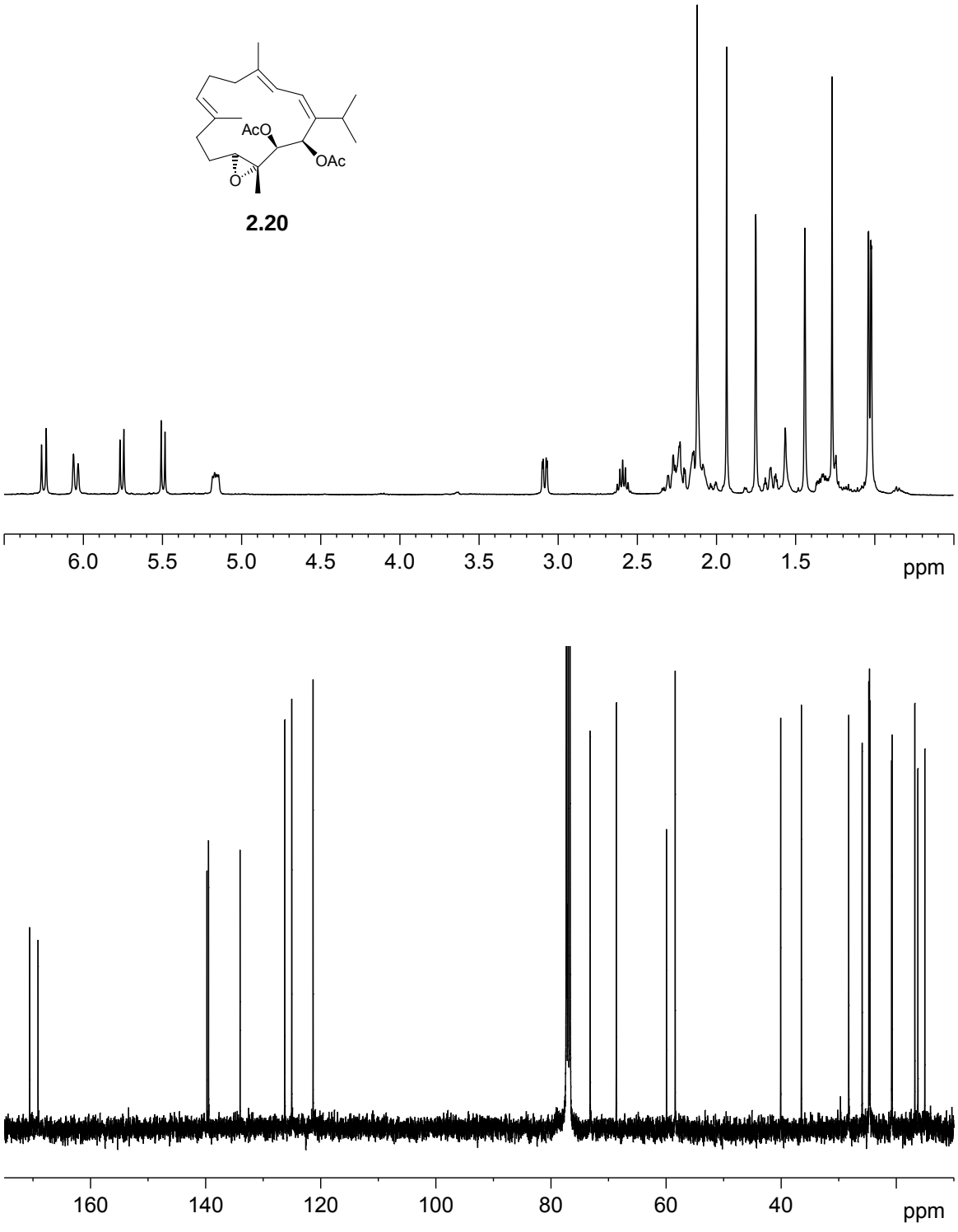
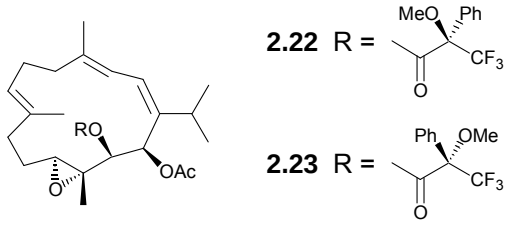


Figure 2.13 ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra obtained for 13***S***,14***R***-diacetoxy-11***S***,12***R***-epoxycembra-1***Z***,3***E***,7***E***-triene (**2.20**; shown inset).

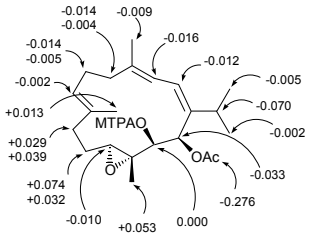
Table 2.6 Comparison of the ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data obtained for flaccidoxide (**2.18**) and 13,14-diacetoxy-11,12-epoxycembra-1***Z***,3***E***,7***E***-triene (**2.20**).

	2.18		**2.20**	
Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)
1	140.4 (s)		139.8 (s)	
2	124.1 (d)	6.22 (1H, d, 11.7)	125.0 (d)	6.26 (1H, d, 11.8)
3	121.2 (d)	6.00 (1H, d, 11.7)	121.3 (d)	6.05 (1H, d, 11.8)
4	139.1 (s)		139.5 (s)	
5	39.9 (t)	2.11 (1H, m) 2.21 (1H, m)	40.1 (t)	2.11 (1H, m) 2.25 (1H, m)
6	25.8 (t)	2.08 (1H, m) 2.27 (1H, m)	25.9 (t)	2.09 (1H, m) 2.29 (1H, m)
7	126.0 (d)	5.13 (1H, m)	126.3 (d)	5.17 (1H, m)
8	134.2 (s)		134.0 (s)	
9	36.4 (t)	2.16 (2H, m)	36.5 (t)	2.19 (2H, m)
10	25.1 (t)	1.38 (1H, m) 1.63 (1H, m)	24.6 (t)	1.33 (1H, m) 1.66 (1H, m)
11	60.8 (d)	3.15 (1H, dd, 8.8, 2.6)	58.4 (d)	3.08 (1H, dd, 8.8, 2.6)
12	62.0 (s)		59.9 (s)	
13	73.4 (d)	3.90 (1H, d, 8.9)	73.2 (d)	5.50 (1H, d, 9.7)
14	71.3 (d)	5.65 (1H, d, 9.0)	68.6 (d)	5.75 (1H, d, 9.7)
15	28.5 (d)	2.49 (1H, septet, 6.8)	28.3 (d)	2.59 (1H, septet, 6.8)
16	24.8 (q)	1.06 (3H, d, 6.8)	24.7 (q)	1.03 (3H, d, 6.8)
17	24.2 (q)	1.01 (3H, d, 6.8)	24.8 (q)	1.03 (3H, d, 6.8)
18	16.3 (q)	1.74 (3H, s)	16.2 (q)	1.75 (3H, s)
19	15.4 (q)	1.46 (3H, s)	15.0 (q)	1.44 (3H, s)
20	17.2 (q)	1.39 (3H, s)	16.8 (q)	1.27 (3H, s)
13-OAc			170.6 (s)	
			20.7 (q)	2.12 (3H, s)
14-OAc	170.2 (s)		169.1 (s)	
	21.1 (q)	2.03 (3H, s)	20.8 (q)	1.94 (3H, s)

The absolute configuration of flaccidoxide (**2.18**) was unknown, and we undertook to assign its stereochemistry using the modified Mosher's method of Kakisawa and co-workers.[113] Accordingly, the ***R***- and ***S***-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters of **2.18** (**2.22** and **2.23** respectively) were prepared employing the esterification procedure of Neises and Steglich,[114] their ^{1}H NMR and COSY spectra recorded and fully assigned, and the Δδ values [δ of protons in the ***S***-MTPA ester (**2.23**) – δ of the corresponding protons in the ***R***-MTPA ester (**2.22**)] calculated (Figure 2.14). Cognisant of possible anomalies in the application of Mosher's method to hindered alcohol functionalities[115] especially on the cembrane skeleton,[116] we approached the interpretation of the Mosher's data with caution. None of the Δδ incongruities observed by Kakisawa and co-workers[116] for hindered cembranoid alcohols were apparent, and the arrangement of positive and negative Δδ values around the cembranoid ring were consistent with only a single anomalous negative Δδ value obtained for H-11 proving to be an exception.



The inconsistent H-11 Δδ value was investigated through molecular modelling studies of the MTPA esters of **2.18**. These revealed that when in the ideal Mosher's conformation (***i.e***. with H-13, the MTPA carbonyl and the MTPA trifluoromethyl all planar and in a ***syn*** relationship),[113,117] H-11 of the ***R***-MTPA ester **2.22** lies both close to, and in the plane of, the aromatic ring and is therefore slightly deshielded and not shielded as expected. The 0.02 ppm downfield chemical shift of H-11 in the ***R***-MTPA ester **2.22** [δ_H 3.10] compared with the acetate **2.20** [δ_H 3.08] possibly lends support to this argument. Similar molecular modelling of the ***S***-MTPA ester **2.23** showed that the H-11 proton is the closest proton to the methoxy moiety of the MTPA ester and is accordingly also weakly deshielded. The net result of these findings is that H-11 is more deshielded in **2.22** than in **2.23** resulting in a small negative Δδ value and explains the observed anomaly.



^{1}H number	***S***-MTPA ester **2.23** δ_H ppm	***R***-MTPA ester **2.22** δ_H ppm	$\Delta\delta$ ppm
2	6.286	6.298	– 0.012
3	6.072	6.088	– 0.016
5a	2.087	2.091	– 0.004
5b	2.188	2.202	– 0.014
6a	2.114	2.128	– 0.014
6b	2.293	2.298	– 0.005
7	5.179	5.181	– 0.002
9a	2.185	2.146	+ 0.039
9b	2.263	2.234	+ 0.029
10a	1.323	1.249	+ 0.074
10b	1.651	1.619	+ 0.032
11	3.086	3.096	– 0.010
13	5.700	5.700	0.000
14	5.786	5.819	– 0.033
15	2.589	2.659	– 0.070
16	1.060	1.065	– 0.005
17	1.047	1.049	– 0.002
18	1.755	1.764	– 0.009
19	1.436	1.423	+ 0.013
20	1.249	1.196	+ 0.053
OAc	1.522	1.798	– 0.276

Figure 2.14 Proton chemical shifts and calculated $\Delta\delta$ values obtained from the application of the modified Mosher's method to **2.18** ($CDCl_3$, 400 MHz).

Substitution of the remaining $\Delta\delta$ values into the model of Kakisawa and co-workers (Figure 2.15),[113] placing the protons with positive $\Delta\delta$ values on the right side and those with negative $\Delta\delta$ values on the left side of the MTPA esterified carbon, allowed the absolute configuration at C-13 of the parent alcohol **2.18** to be defined as 13***S***.

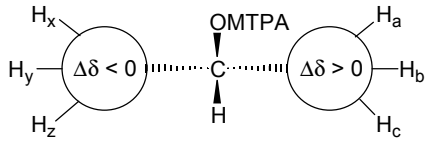


Figure 2.15 Kakisawa and co-workers model for the determination of the absolute configuration of secondary alcohols by the modified Mosher's method.[113]

The 13***S*** stereochemistry of **2.18** was then related to the other stereogenic centres in this compound from a combination of 1D NOEDS experiments (Table 2.7) and molecular modelling studies (Figure 2.16) to assign an 11***S***, 12***S***, 13***S***, 14***R*** stereochemistry for **2.18**. The absolute configuration of flaccidoxide (**2.18**) and flaccidoxide-13-acetate (**2.20**) were shown to be the same from optical rotation measurements, with authentic **2.20** giving a specific rotation of +158° and **2.20** obtained from acetylation of **2.18** a specific rotation of +162°. Taking into account the Cahn-Ingold-Prelog priority reversal at C-12 arising from acetylation at C-13,[118] the absolute configuration of **2.20** is assigned as 11***S***, 12***R***, 13***S***, 14***R***. These stereochemical assignments are consistent with those reported for 13-functionalised[119] and 14-functionalised[109,110] 11,12-epoxy cembranoids.

To the best of our knowledge, this is the first reported isolation of cembrane diterpenes from the octocoral genus ***Cladiella***.[75] Although the occurence of cembranoids in ***Cladiella*** is unusual, it supports the current hypothesis that the cladiellins and related diterpenes (***e.g***. the briarellins, the asbestinins, and the sarcodictyins) are biosynthetically derived from the cembrane skeleton (Scheme 2.1).[106,120] Finally, all three diterpenes were found to be toxic to ***A. salina*** and displayed

an interesting range in activity. The LC_{50} values estimated by probit analysis[121] were flaccidoxide (**2.18**): 50 ppm; compound **2.19**: 110 ppm; and flaccidoxide-13-acetate (**2.20**): 180 ppm.

Table 2.7 Key nuclear Overhauser enhancements observed for flaccidoxide (**2.18**).

Irradiated proton	NOE correlated proton
H-11	H-3, H-7, H-9, H-13, H-14
H-13	H-15, H-20
H-14	H-3, H-11, 14-OAc
H-15	H-13
H-20	H-7, H-10, H-13, H-14
14-OAc	H-11, H-13, H-14

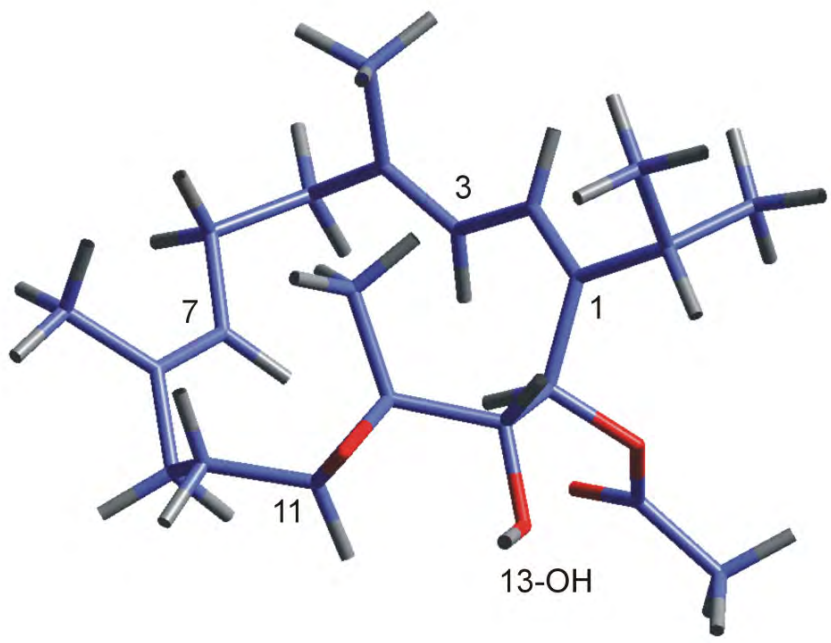
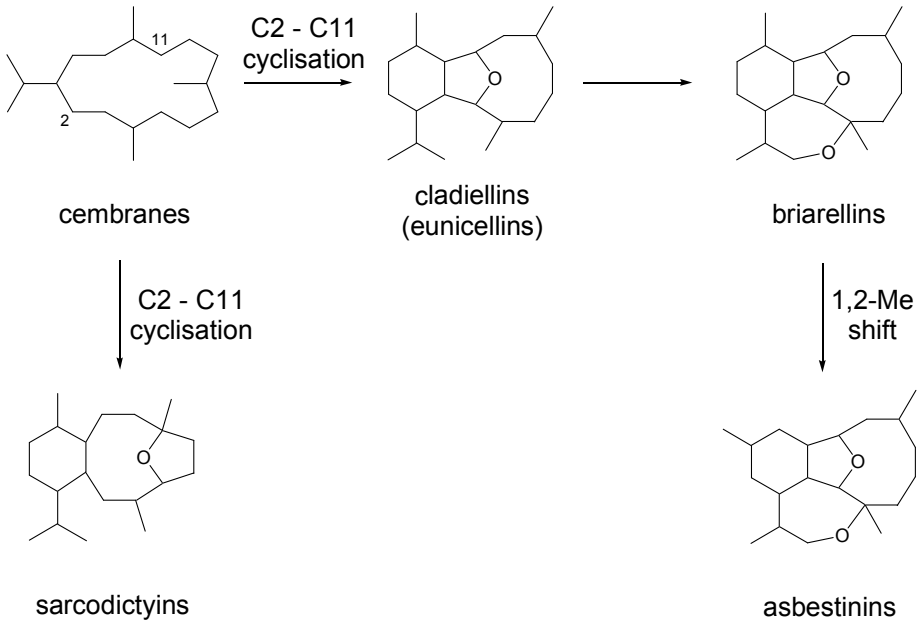


Figure 2.16 Stick representation of the computer-modelled global energy minimum conformation of flaccidoxide (**2.18**).



Scheme 2.1 The proposed biosynthetic relationship between the cembrane diterpenes and the cladiellins, sarcodictyins, briarellins and asbestins.[120]

2.3 An investigation of the potential anti-cancer activity of the metabolites isolated from the Mozambique invertebrates

In 1985 the National Cancer Institute (USA) successfully initiated a project using human cell lines as an anti-cancer primary screen.[122] Incorporating a sixty cancer cell line derived from seven cancer types (brain, colon, leukaemia, lung, melanoma, ovarian and renal), the National Cancer Institute's screening programme identified several potential anti-cancer agents from the many thousands of natural products and terrestrial plant and marine organism extracts submitted for screening over the past twenty-five years. We consider it important that the therapeutic potential of southern African marine invertebrates is comprehensively explored and for this reason the compounds isolated from the Mozambique invertebrates investigated here (described in the preceding sections 2.2.1-2.2.4) were selectively screened against a melanoma cell line (LOX), a leukaemia cell line (A549), a CNS cancer cell line (SNB19) and an ovarian cancer cell line (OVCAR3).[123] The results of these screens are presented in Table 2.8.

Table 2.8 The median inhibitory concentration (IC_{50}) values obtained from screening the nine natural products isolated from the Mozambique 1995 collection against four cancer cell lines.

	IC_{50} (μgmL^{-1})			
Compound	LOX	A549	OVCAR3	SNB19
2.1	11.36	5.22	2.61	7.94
2.7	3.43	39.68	1.99	0.51
2.8	Nil	Nil	Nil	8.48
2.9	Nil	Nil	Nil	Nil
2.10	1.36	1.30	2.84	1.02
2.11	Nil	20.53	Nil	3.80
2.18	Nil	Nil	Nil	Nil
2.19	Nil	26.73	Nil	Nil
2.20	Nil	55.55	Nil	Nil

Ilimaquinone (**2.1**) showed good broad scale activity against the four cancer cell lines which is consistent with data published for related marine sesquiterpenoid quinones.[79,81,82] The best overall activity, however, was exhibited by the microbial metabolite mimosamycin (**2.10**, IC_{50} < 3 μgmL^{-1} against all four cell lines), findings which are once again supported by literature data.[96] Of the remaining ***Haliclona*** metabolites, renierone (**2.7**) exhibited good broad scale activity and ***N***-formyl-1,2-dihydrorenierone (**2.8**) was selectively active against the CNS cancer SNB19. Surprisingly, 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**2.9**) and the cembrane diterpenes **2.18** – **2.20** possessed little or no activity against these cell lines. It is interesting to note that the diosphenol **2.11** showed good selective activity against the SNB19 cell line, although no anti-cancer activity has previously been recorded for this compound. We are currently investigating the activity of **2.11** further by assaying it for activity against oesophageal cancer,[124]

which, although uncommon in other parts of the world, is the fourth most prevalent cancer (out of a total of eighteen common cancers) in the urban populations of the Eastern Cape Province, South Africa.[125]

2.4 Summary and Conclusions

A bioactivity and ^{1}H NMR spectroscopy survey of the ethyl acetate and aqueous extracts of one hundred and eighteen marine invertebrates collected from Ponto do Oura, Mozambique in 1995 prompted us to investigate the ethyl acetate extracts of two sponges, an ascidian and a soft coral. Unfortunately, these investigations proved to be rather disappointing from the perspective of isolating new, biologically active natural products. The four extracts yielded eight known compounds (**2.1**, **2.7** – **2.11**, **2.18** and **2.19**) and only one previously unreported natural product (**2.20**) although we were able to assign the absolute stereochemistry of the soft coral metabolite flaccidoxide (**2.18**) and its acetylated derivative **2.20**. All nine metabolites were tested for anticancer activity against four cell lines. The promising activity observed for the diosphenol **2.11** has prompted us to examine this metabolite further and we are currently subjecting **2.11** to additional anticancer assays whilst attempting to assign the absolute stereochemistry of this intriguing metabolite in our laboratory.

Chapter Three

Labdane Diterpenes from the South African Marine Pulmonate ***Trimusculus costatus***

3.1 Introduction

Following the disappointing results obtained from the survey of invertebrate species collected from Mozambique, we decided to turn our attention to endemic South African invertebrate species in an effort to isolate new natural products. Endemism of marine invertebrate species often signals new chemistry[126] and in South Africa, with its range of subtropical, warm temperate and cold temperate coastal waters, a high level (about 12%) of endemism is evident.[127] One endemic species that we found particularly interesting was the South African button shell, ***Trimusculus costatus***, because of the unusual ecology and established chemistry of the family Trimusculidae (***vide infra***).

The single South African representative of this family of pulmonate mollusc is reported to be a common species on the south and east coasts of South Africa,[128] although no ecological studies have been performed on this species possibly due to its specialised and somewhat inaccessible habitat. The animals are found in tightly clustered colonies on the roofs of caves and rocky overhangs on the seaward side of reef platforms on exposed shores with considerable wave action.[128]

The shell of ***T. costatus*** is that of a typical limpet and adults may obtain a maximum shell length of about 22 mm.[128] The shell is light brown in colour, often irregularly shaped, and is characterised by rough unequal ribs producing a serrated margin (Figure 3.1).[128,129] In common with other members of the family Trimusculidae,[130] the distribution of ***T. costatus*** along the South African coast is patchy and populations proved difficult to locate. At our collection locality only two populations were found along a 2 km section of reef platform. However, within the area where ***T. costatus*** was found the colonies were extensive, comprising of at least six hundred individuals which were tightly packed together to form a cluster of animals often two layers deep.

Figure 3.1 The South African button shell, ***Trimusculus costatus***.

3.1.1 The biology and ecology of the Trimusculidae

The family Trimusculidae is a small group of about eleven species with world-wide distribution. Trimusculid species have been recorded from the Mediterranean, Africa, North and South America, Australia, New Zealand, Japan and many Pacific Islands.[131] Among intertidal limpets, Trimusculid species are uniquely adapted to a specialised habitat in the rocky intertidal zone:[128,130-134] the genus is truly amphibious, possessing a mantle cavity which has evolved to serve as both a lung and a gill.[128] Large groups of animals are found tightly clustered together hanging inverted on the underside of crevices, rocky overhangs and the roofs of caves in the intertidal zone on exposed shores[130,132-134] and have even been found to utilise air-pockets in a subtidal situation.[135]

Constraints imposed by their colonial lifestyle mean that these limpets are essentially sedentary in sites where surface algal growth would be extremely low.[128,133,134] Here they may form such densely packed colonies that their shell margins may interlock like jigsaw-puzzles.[133] The Trimusculids are thus unable to feed by foraging and grazing as do other limpets, and have evolved instead to filter feed.[133,134] During feeding, the shell is lowered slightly to allow water to flow under the mantle, the creature orienting itself tail-end to the current. A curtain of mucus, secreted by mantle glands in front of the head, is then deployed in the current and functions as a net to trap plankton. Once it is laden with food, the net is pulled under the shell by two prominent lobes that flank the head and both the captured food and the mucus net consumed.[128,130,133,134]

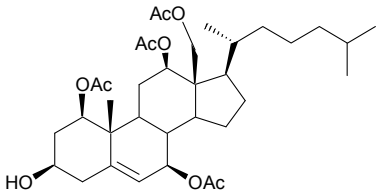
The Trimusculidae lay their eggs in gelatinous collars attached to the rock surface that are brooded beneath the mantle, either as a horse-shoe shaped mass or as two separate crescent shaped collars, one on either side of the foot.[130] Each mass contains numerous eggs which hatch as crawling young with a spiral shell and large foot.[130] Before settling and forming a permanent scar on the rock, each juvenile passes through an active crawling stage to ensure dispersal from brooded eggs or crowded areas of the colony.[130]

Due to the constraints of their sessile lifestyle, Trimusculids do not exhibit the common flight response that most limpets display when in the presence of a predator, ***e.g.*** a seastar.[136] Instead, when disturbed, ***Trimusculus*** species secrete large quantities of a milky white repugnatorial mucus from large sub-epithelial glands on the mantle fold.[132] Indeed, mucus collected from ***T. reticulatus*** from North America has been shown to repel predatory seastars.[136,137] Importantly, this defensive secretion was found to be distinct from the feeding mucus and is produced only in response to attack or trauma of the limpet.[136]

3.1.2 The natural products chemistry of Trimusculid limpets

The reported chemical defence of ***T. reticulatus*** caused Manker and Faulkner to examine the natural products chemistry of ***T. reticulatus***.[138] Their study revealed that the pulmonate contained two new labdane diterpenes: 6β-isovaleroxylabda-8,13***E***-dien-7α,15-diol (**3.1**), present in extracts of the foot, mantle and defensive mucus; and 2α,7α-diacetoxy-6β-isovaleroxylabda-8,13***E***-dien-15-ol (**3.2**), present as a minor constituent of the animals only.[137,138] The structures of **3.1** and **3.2** were elucidated from spectroscopic data and chemical transformation,[138] and the relative stereochemistry at C-6 and C-7 and the geometry of the Δ^{13}-double bond of **3.1** has since been confirmed by the stereoselective synthesis of (±)-**3.1**.[139] The same authors also examined the chemistry of the related species ***T. conica*** (collected from New Zealand) and isolated a further labdane diterpene, 6β-acetoxy-7α-isovaleroxylabda-8,13***E***-dien-15-oic acid (**3.3**) along with the C-18 acetoxylated cholestane sterol 1β,7β,12β,18-tetraacetoxycholest-5-en-3β-ol (**3.4**).[137] Both of

these metabolites were found to be localised in the foot and mantle of the animal and although no mucus was obtained during the collection of this species, it was assumed that these terpenoids would be secreted defensively in a manner analogous to that observed for ***T. reticulatus***.[137]

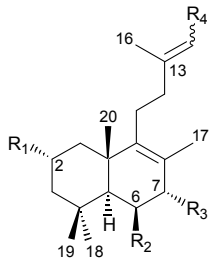


3.4

The situation pertaining to the natural products chemistry of the South American species ***T. peruvianus*** is intriguing. In a preliminary communication relating to the natural products chemistry of this species,[140] San-Martin ***et al.*** report the isolation of a new diterpene, 6β-acetoxylabda-8,13***E***-dien-7α,15-diol (**3.5**), elucidating the structure from spectroscopic methods and through comparison with the NMR data reported for **3.1** and **3.2**.[138] However, a subsequent, poorly edited paper published by this group[141] reports that a second collection of ***T. peruvianus*** yielded four new labda-8,13***Z***-diene analogues (**3.6** – **3.9**) in addition to the previously described diol **3.5** (however, in the second paper **3.5** is erroneously shown to have a ***Z***-geometry for the Δ^{13}-olefin and all of the compounds described are illustrated as possessing a 17-norlabdane skeleton). As we can find no error in the proposed assignment of the Δ^{13}-double bond geometries of compounds **3.5** – **3.9** from the reported NMR data, we have to assume that this species surprisingly produces both Δ^{13}-***E***- and Δ^{13}-***Z***-labdane derivatives.

Although the chemical studies so far performed upon ***T. reticulatus***, ***T. conica*** and ***T. peruvianus*** have yielded chemotaxonomically interesting results, surprisingly none of the investigations mentioned above determined the absolute stereochemistry of the ***Trimusculus*** diterpenes. It therefore remains to be ascertained whether these metabolites belong to the labdane or ***ent***-labdane series.

Table 3.1 A summary of the labdane diterpenes isolated from ***Trimusculus*** species.



Species	Compound	Δ^{13}	Substituents	
T. reticulatus[137,138] (North America)	**3.1**	***E***	R_1 = H	R_2 = $OCOCH_2CH(CH_3)_2$
			R_3 = OH	R_4 = CH_2OH
	3.2	***E***	R_1 = OAc	R_2 = $OCOCH_2CH(CH_3)_2$
			R_3 = OAc	R_4 = CH_2OH
T. conica[137] (New Zealand)	**3.3**	***E***	R_1 = H	R_2 = OAc
			R_3 = $OCOCH_2CH(CH_3)_2$	R_4 = COOH
T. peruvianus[140,141] (South America)	**3.5**	***E***	R_1 = H	R_2 = OAc
			R_3 = OH	R_4 = CH_2OH
	3.6	***Z***	R_1 = H	R_2 = H
			R_3 = OAc	R_4 = CH_2OAc
	3.7	***Z***	R_1 = H	R_2 = OAc
			R_3 = OAc	R_4 = CH_2OAc
	3.8	***Z***	R_1 = H	R_2 = OAc
			R_3 = OAc	R_4 = CH_2OH
	3.9	***Z***	R_1 = H	R_2 = OAc
			R_3 = H	R_4 = CH_2OH
T. costatus (South Africa)	**3.10**	***E***	R_1 = H	R_2 = OAc
			R_3 = OAc	R_4 = CH_2OH
	3.11	***E***	R_1 = OAc	R_2 = OAc
			R_3 = OAc	R_4 = CH_2OH

It should also be noted that in all of the papers relating to the natural products chemistry of the Trimusculids,[137,138,140,141] the numbering system employed deviates from the established labdane nomenclature with respect to the designation of the C-18 – C-20 methyl groups.[142] To avoid unnecessary confusion and maintain consistency in subsequent chapters of this thesis, the numbering system used here will comply with the accepted labdane nomenclature[142] (as shown in Table 3.1) and will therefore vary slightly from that used in the ***Trimusculus*** chemical literature.

3.1.3 A survey of the biological activity of the Trimusculus diterpenes

Manker and Faulkner have investigated the ecological role of the ***T. reticulatus*** labdanes **3.1** and **3.2**.[137] The compounds were screened in a number of bioassays to test their effectiveness as either defensive chemicals or antifouling agents. Once again it was observed that the mucus of ***T. reticulatus*** was repugnant to the sympatric seastar ***Astrometis*** sp., but diol **3.1** alone did not elicit the same response in the echinoderm. The authors suggested that this may be due to the lack of an adequate carrier to mimic a mucus for presentation of diol **3.1** to the seastar, or that more than one component of the mucus is necessary to repel seastars. Surprisingly, the secondary metabolites did not also inhibit feeding of the predatory fish ***Thallasoma lunare***. However, the limpet, its mucus and the diterpene **3.1** were found to be toxic to larvae of the sabellariid tube worm ***Phragmatopoma californica***. The authors concluded that this larvicidal activity might be an important strategy for the members of the genus ***Trimusculus*** because their specialised lifestyle requires that the areas around the limpet colonies be kept free from other intertidal invertebrates.[137]

The diterpenes isolated from ***T. peruvianus*** (**3.5** – **3.9**) were tested for antimicrobial activity against a suite of twelve bacteria employing the standard agar well-diffusion method at concentrations of 100 $\mu g mL^{-1}$.[141] Of the five labdanes, only the triacetate **3.7** exhibited modest antibacterial activity. Further quantitative antibacterial assays performed on **3.7** allowed the determination of this compound's minimum inhibitory concentration against eight bacterial strains

and indicated that **3.7** possessed marginal activity (MIC = 50 μgml^{-1}) against the Gram-positive bacteria ***Bacillus anthracis***, ***B. pumilus*** and ***B. subtilis***, and the Gram-negative bacterium ***Klebsiella bronchiseptica***. Unfortunately, no examination of the ecological role of the ***T. peruvianus*** metabolites was undertaken by the authors.[141]

3.2 Results and Discussion

A sample of small specimens of ***T. costatus*** (TRI 96-001; 422 animals, approximately 50% of a single colony; mean shell length $\pm$ standard deviation = 14.9 $\pm$ 2.9 mm [***N*** = 100]; mean dry weight after extraction $\pm$ standard deviation = 49.0 $\pm$ 21.4 mg [***N*** = 100]) was collected from the seaward edge of a rocky platform at Cintsa West on the south east coast of South Africa during the late summer of 1996.[143] After careful removal from the rock-face, shells of individual limpets were rapidly cleaned with a scalpel (a significant proportion of the shells were encrusted with a thin algal film, spiral fanworms [***Spirorbis*** spp.] and plum anemones [***Actinia equina***][144]) and the whole animals immediately immersed in acetone. The animals were allowed to steep for one week after which the resulting crude acetone extract was filtered, concentrated ***in vacuo*** and partitioned between ethyl acetate and water to give a crude organic extract as a dark green oil (2.03 g). The entire organic extract was subjected to silica gel flash chromatography in 3:2 hexane/ethyl acetate and yielded five fractions, two of which (A; 0.58g and B; 0.49g) displayed significant toxicity in the ***Artemia salina*** larvicidal bioassay.[72,73] Subsequent fractionation was bioassay guided.

The less polar bioactive fraction A had to be subjected to further flash chromatography before semi-preparative HPLC could be performed. Accordingly, a portion (0.32g) of this fraction was passed through a column of silica employing gradient elution, with the bioactive constituent being confined to the major fraction (0.26g) that eluted with 3:2 hexane/ethyl acetate. Semi-preparative normal phase HPLC (3:2 hexane/ethyl acetate) of approximately half (126 mg) of this fraction yielded a single biologically active compound (**3.10**; 57 mg, 0.50 mg/animal or 10 ppt mean dry weight), as a colourless oil ($[\alpha]_D^{27}$ = + 93°).

Figure 3.2 The rocky platform at Cintsa West from which the first collection of ***Trimusculus costatus*** (TRI 96-001) was made.

The molecular formula of **3.10**, $C_{24}H_{38}O_5$, was obtained from HREIMS data (***m/z*** 406.2729, Δmmu +1.2) and all twenty four carbon resonances were clearly resolved in the ^{13}C NMR spectrum of this compound (Figure 3.3). The labda-8,13-dien-15-ol skeleton, common to the known ***Trimusculus*** metabolites, was readily confirmed by the presence of three methyl singlets [δ_H 0.95 (3H, s), 0.96 (3H, s), and 1.27 (3H, s)], a vinylic methyl substituent [δ_H 1.61 (3H, s)] on a tetrasubstituted double bond [δ_C 121.6 (s) and 147.8 (s)], a trisubstituted olefin [δ_C 139.7 (s) and 123.2 (d)] bearing a methyl group [δ_H 1.71 (3H, s)] and a primary alcohol [δ_H 4.16 (2H, d, ***J*** = 6.7 Hz)] in the NMR spectra of **3.10** (Figure 3.3). The ^{13}C NMR spectrum contained additional signals indicative of the presence of two acetylated secondary alcohol functionalities [δ_C 21.1 (q); 21.5 (q); 69.6 (d); 73.5 (d); 169.7 (s); and 169.9 (s)] that were placed at C-6 and C-7 through HMQC and two- and three-bond HMBC correlations. The expected 6β,7α relative stereochemistry was confirmed by a series of 1D nuclear Overhauser enhancement difference experiments (Figure 3.4).

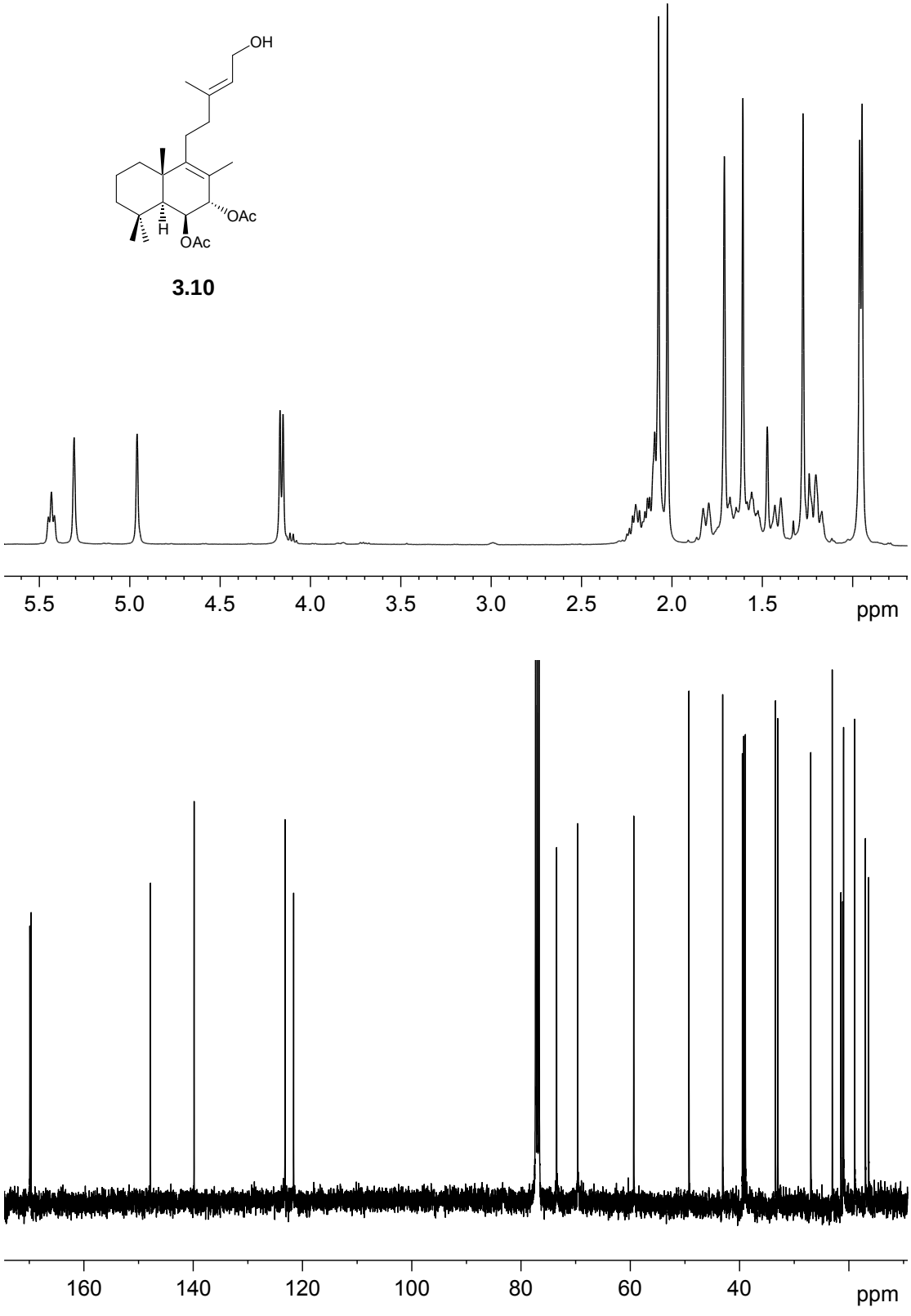


Figure 3.3 ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra obtained for 6β,7α-diacetoxylabda-8,13***E***-dien-15-ol (**3.10**; shown inset).

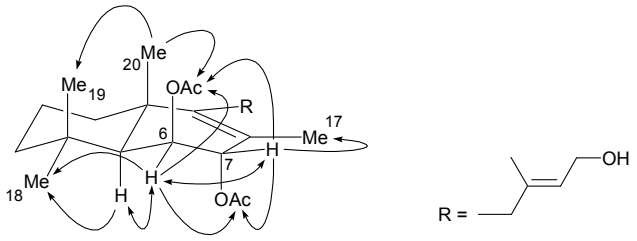


Figure 3.4 Key nuclear Overhauser enhancements observed for 6β,7α-diacetoxylabda-8,13***E***-dien-15-ol (**3.10**).

Comparison of the full NMR data obtained for **3.10** with the NMR assignments reported for 6β,7α-diacetoxylabda-8,13***Z***-dien-15-ol (**3.8**),[141] previously isolated from ***T. peruvianus***, indicated that **3.10** was the Δ^{13}-***E***-geometrical isomer of this compound (see Table 3.2). The stereochemistry of the Δ^{13}-olefin in **3.10** was assigned from the ^{13}C chemical shifts of C-12 (δ_C 39.2) and C-16 (δ_C 16.4) which were in agreement with those expected for labda-13***E***-ene-15-ols (approximately δ_C 39 and 16 respectively)[138,139,145] rather than labda-13***Z***-ene-15-ols (approximately δ_C 33 and 23 respectively).[141,146] The structure of **3.10** was therefore confirmed to be 6β,7α-diacetoxylabda-8,13***E***-dien-15-ol.

The second bioactive fraction (fraction B) from the initial chromatography of the crude organic extract did not require further column chromatography and a portion of this mixture (101 mg) was subsequently purified by normal phase semi-preparative HPLC (2:3 hexane/ethyl acetate). Once again, the ***A. salina*** bioassay indicated that a single compound (**3.11**; 61 mg; 0.69 mg/animal or 14 ppt mean dry weight), isolated as a colourless oil ($[\alpha]_D^{28}$ = + 62°), was responsible for the observed bioactivity of the flash chromatography fraction.

The ^{1}H and ^{13}C NMR data of **3.11** were similar to those of **3.10**, and a molecular formula of $C_{26}H_{40}O_7$ (***m/z*** 464.2760, Δmmu −1.2) from HREIMS data, corroborated by three acetate methyl singlets (δ_H 2.03, 2.04 and 2.08) in the ^{1}H NMR spectrum of **3.11**, indicated that this compound possessed an additional acetoxy functionality. The ^{13}C chemical shifts of C-13, C-14 and C-15 [δ_C 139.3 (s), 123.4 (s), and 59.3 (t) respectively] together with supporting IR data (ν_{max} 3480 cm^{-1})

Table 3.2 Comparison of the ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data obtained for **3.10** with those reported for the Δ^{13}-***Z***-isomer **3.8** (^{1}H, 400 MHz; ^{13}C, 100 MHz; solvent not specified).[141]

	3.10		**3.8**	
Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)
1	38.9 (t)	1.22 (1H, m) 1.81 (1H, d, 12.3)	39.1 (t)	1.18 (1H, br d, 14.0) 1.75 (1H, br d, 14.0)
2	18.9 (t)	1.54 (1H, m) 1.66 (1H. m)	19.2 (t)	1.61 (2H, m)
3	43.1 (t)	1.22 (1H, m) 1.41 (1H, d, 13.2)	43.2 (t)	1.15 (1H, dd, 14.0, 5.3) 1.38 (1H, br d, 14.5)
4	33.4 (s)		33.6 (s)	
5	49.2 (d)	1.47 (1H, br s)	49.3 (d)	1.42 (1H, br s)
6	69.6 (d)	5.31 (1H, br s)	73.7 (d)	5.25 (1H, br s)
7	73.5 (d)	4.96 (1H, br s)	69.7 (d)	4.98 (1H, br s)
8	121.6 (s)		121.9 (s)	
9	147.8 (s)		148.0 (s)	
10	39.5 (s)		39.6 (s)	
11	27.0 (t)	2.15 (2H, m)	27.1 (t)	2.18 (2H, m)
12	39.2 (t)	2.09 (2H, m)	32.3 (t)	2.19 (2H, m)
13	139.7 (s)		139.6 (t)	
14	123.2 (d)	5.43 (1H, t, 6.6)	124.7 (t)	5.34 (1H, t, 7.0)
15	59.3 (t)	4.16 (2H, d, 6.7)	59.1 (t)	4.05 (2H, d, 7.0)
16	16.4 (q)	1.71 (3H, s)	23.6 (q)	1.73 (3H, br s)
17	17.0 (q)	1.61 (3H, s)	17.3 (q)	1.63 (3H, s)
18	33.0 (q)	0.96 (3H, s)	33.2 (q)	0.90 (3H, s)
19	23.0 (q)	0.95 (3H, s)	23.3 (q)	0.89 (3H, s)
20	21.0 (q)	1.27 (3H, s)	21.7 (q)	1.26 (3H, s)
6-OAc	169.7 (s)		169.7 (s)	
	21.5 (q)	2.02 (3H, s)	21.3 (q)	1.97 (3H, s)
7-OAc	169.9 (s)		170.1 (s)	
	21.1 (q)	2.07 (3H, s)	21.2 (q)	2.02 (3H, s)

confirmed that the terminal α,β-unsaturated primary alcohol component of the C-9 side chain was intact and confined the three acetoxy moieties to the decalin ring system. Positioning of the familiar diacetate moiety at C-6 and C-7 followed from analysis two- and three-bond ^{1}H – ^{13}C correlations observed in the HMBC spectrum of **3.11** (Table 3.3).

Unfortunately, HMBC correlations alone could not be used to unequivocally place the third acetoxy group (Table 3.3). However, COSY couplings from ring A methylene protons to the deshielded methine signal at δ_H 5.12 (see Figure 3.5), supported by a series of 1D nuclear Overhauser enhancement difference experiments (Figure 3.6), positioned the third acetoxy group at C-2. In addition, the nuclear Overhauser enhancement data confirmed a 2α,6β,7α-oxygenation pattern in **3.11** in agreement with the other trisubstituted labdane diterpenes previously isolated from ***Trimusculus*** species[138] (Table 3.1) and allowed the structure of **3.11** to be defined as the new labdane 2α,6β,7α-triacetoxylabda-8,13***E***-dien-15-ol.

In order to search for further minor natural products and perform an ecological appraisal of ***T. costatus*** in its natural environment, a second sample of ***T. costatus*** (TRI 96-002; 806 animals) was collected as before from a separate colony at Cintsa West (approximately 2 km from the initial collection site) during the late winter of 1996. This second colony was found on the roof of a natural arch that was sufficiently high to allow a limited ***in situ*** ecological survey to be performed. The colony was estimated to cover 3167 cm^2 of the rock face and comprise approximately 1170 to 1870 individual limpets (mean number of limpets ± standard deviation = 48 ± 11 per 100 cm^2 quadrat [***N*** = 10]; see Table 3.4). A gross examination of the colony revealed that almost all ***T. costatus*** shells were covered with brown filamentous algae, a large proportion were fouled by ***Spirorbis*** spp., and a total of nine ***Actinia equina*** (plum anemones) were observed adhered to ***Trimusculus*** shells within the colony. A series of random quadrats (10 cm × 10 cm, ***N*** = 10) indicated that although this habitat was dominated by ***T. costatus***, intertidal sponges (***Hymeniacedon*** spp.) and to a limited extent barnacles and mussels were sympatric but were not observed overgrowing limpets (Table 3.4). There was very little bare rock around or within the colony with the same filamentous alga covering areas devoid of intertidal invertebrates.

Table 3.3 1D and 2D NMR data ($CDCl_3$, ^1H: 400 MHz; ^{13}C: 100 MHz) obtained for 2α,6β,7α-diacetoxylabda-8,13***E***-dien-15-ol (**3.11**).

Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	HMBC correlation to	COSY coupling to
1	43.9 (t)	1.26 (1H, m) 2.12 (1H, d, 12.3)	C-2	H-2, H_2-3
2	68.4 (d)	5.12 (1H, tt, 11.7, 4.1)		H_2-1, H_2-3
3	47.7 (t)	1.27 (1H, m) 1.80 (1H, m)	C-2	H_2-1, H-2
4	40.5 (s)			
5	48.7 (d)	1.52 (1H, br s)	C-4, C-10, C-19	H-6, H-7
6	69.0 (d)	5.31 (1H, br s)	C-4, C-5, C-7, 6-OAc	H-5, H-7
7	73.3 (d)	5.00 (1H, br s)	C-5, C-8, C-9, C-17, 7-OAc	H-5, H-6, H_3-17
8	122.1 (s)			
9	146.7 (s)			
10	34.3 (s)			
11	26.8 (t)	2.21 (2H, m)	C-12	H_2-12
12	39.3 (t)	2.10 (2H, m)		H_2-11, H-14
13	139.3 (s)			
14	123.4 (d)	5.43 (1H, t, 6.8)		H_2-12, H_2-15, H_3-16
15	59.2 (t)	4.17 (2H, d, 6.8)	C-13, C-14	H-14, H_3-16
16	16.4 (q)	1.71 (3H, s)	C-12, C-13, C-14	H-14, H_2-15
17	16.9 (q)	1.62 (3H, s)	C-7, C-8, C-9	H-7
18	32.9 (q)	1.04 (3H, s)	C-4, C-5, C-19	
19	23.6 (q)	1.04 (3H, s)	C-4, C-5, C-18	
20	21.9 (q)	1.37 (3H, s)	C-1, C-5	
2-OAc	169.5 (s)			
	14.1 (q)	2.03 (3H, s)	2-OAc carbonyl	
6-OAc	169.7 (s)			
	21.0 (q)	2.08 (3H, s)	6-OAc carbonyl	
7-OAc	170.5 (s)			
	21.4 (q)	2.04 (3H, s)	7-OAc carbonyl	

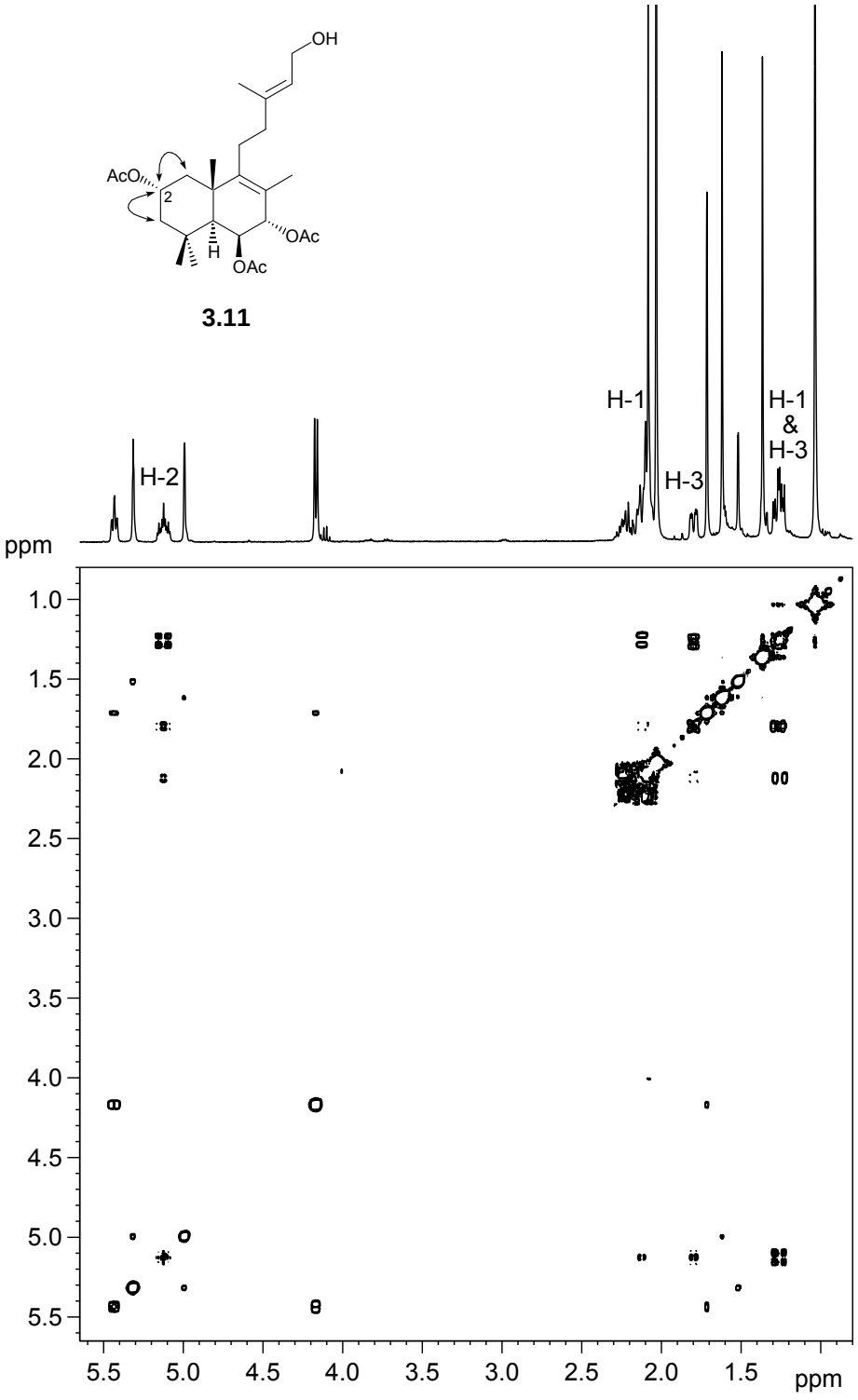


Figure 3.5 COSY90 spectrum ($CDCl_3$, 400 MHz; relaxation time [d1] = 1.00 sec; F1 and F2 = δ_H 0.8 – 5.7) obtained for 2α,6β,7α-triacetoxylabda-8,13***E***-dien-15-ol (**3.11**). Accompanying figure shows COSY correlations used to assign the C-2 acetoxy moiety.

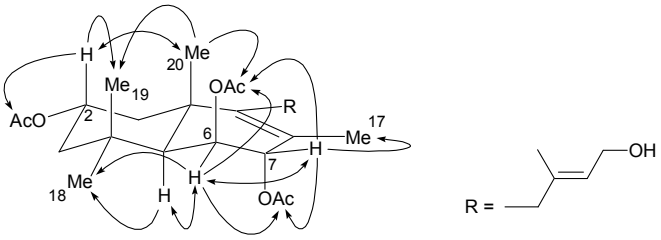


Figure 3.6 Key nuclear Overhauser enhancements observed for 2α,6β,7α-triacetoxylabda-8,13***E***-dien-15-ol (**3.11**).

Table 3.4 Composition of the ***Trimusculus costatus*** colony sampled in the winter of 1996 as determined by random quadrat (10 cm × 10 cm) sampling.

Quadrat	*T. costatus*		Percentage cover of sympatric species	Percentage of bare rock
	Number	Percentage cover		
1	32	56 %	28 % sponge, 14 % algae	2%
2	58	100 %		
3	49	100 %		
4	46	80 %	16 % sponge, 4 % algae	
5	57	100 %		
6	53	100 %		
7	28	42 %	12% sponge, 36% algae, 6% barnacles	4%
8	43	76 %	24 % algae	
9	55	96 %	4 % mussels	
10	57	98 %	2% algae	

On collection, the first 100 limpets were assessed for fouling organisms and a large proportion of the shells (71%) were encrusted with epibionts: 46% were covered with a thin algal film, 3% were found to have epizoic spiral fanworms (***Spirorbis*** spp.), and 22% of shells both algae and fanworms (Figure 3.7). Our observations of both epibiotic species and closely associated

intertidal organisms is in accordance with field observations reported for other Trimusculid species.[130,133]

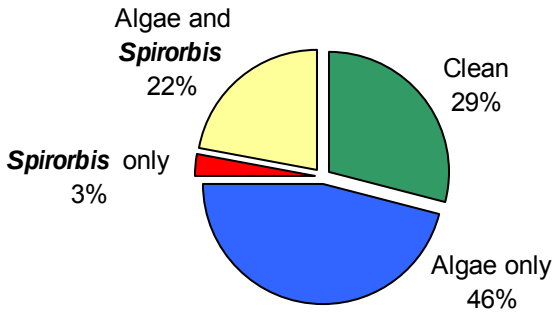


Figure 3.7 The proportion and distribution of epibionts observed on ***T. costatus*** shells (***N*** = 100) from the second collection (TRI 96-002).

The second ***T. costatus*** sample (806 animals) was extracted as before to give a crude organic extract as a dark green oil (2.37 g). The organic extract was subjected to silica gel flash chromatography and yielded three bioactive fractions (0.49g, 0.25g and 0.46g). The two less polar fractions were subjected to semi-preparative normal phase HPLC (3:2 hexane/ethyl acetate) to yield **3.10** (261 mg) and **3.11** (15 mg) respectively as the only bioactive fractions. The third bioactive fraction from the flash chromatography of the crude organic extract was also separated by normal phase semi-preparative HPLC (2:3 hexane/ethyl acetate) to give more **3.11** (260 mg) as the only bioactive compound. None of the other fractions (either from column chromatography or HPLC) contained structurally interesting metabolites (as determined by ^{1}H NMR spectroscopy).

The total yield of **3.10** (261 mg, 0.32 mg/animal or 6 ppt mean dry weight) and **3.11** (275 mg, 0.34 mg/animal or 7 ppt mean dry weight) from the second collection was therefore significantly less than from the initial collection (***c.f.*** 10 and 14 ppt respectively). The difference in diterpene yields could possibly be explained by the different isolation protocols employed for the isolation of the diterpenes from the two collections. However, as the initial extractions for each collection were identical and a more comprehensive isolation procedure was used with the second collection, this rationale is unlikely. A second explanation could be a difference in the mean sizes of the animals collected. Although the second ***T. costatus*** collection comprised individuals of smaller mean shell

length than the initial collection (mean shell length ± standard deviation = 13.7 ± 2.4 mm [***N*** = 100]; t-test: t = 3.531, P < 0.001), the flesh of the animals of both collections were of similar mass (mean dry weight ± standard deviation of the second collection = 49.9 ± 23.2 [***N*** = 100]; t-test: t = –0.289, P = 0.773). We would therefore expect similar yields of **3.10** and **3.11** from both collections.

We obviously cannot draw conclusions from the results of only two extractions, although the marked variability in the quantity of diterpenes isolated from the two collections does pose an interesting question relating to the biosynthesis of these metabolites: does the production of **3.10** and **3.11** vary temporally (***i.e.*** through variations in diet, seasonal spawning ***etc***.) or colonially (***i.e.*** through genetic differences in colonies as a consequence of limited dispersion of offspring)? Although this problem lends itself to examination by the natural products chemist through analytical methods such as HPLC, it would require both periodic and inter-colonial sampling of ***T. costatus*** populations. In our opinion, this would be problematic due to the inaccessible habitat occupied by this species not to mention the difficulty that would be experienced in finding a sufficient number of colonies to sample. For these reasons, pursuit of this inquiry was regretfully deemed to lie outside the scope of this study, although it could be worthy of a further chemical ecology investigation.

3.2.3 Biological activity

The spectrum of biological activity displayed by the diterpenes isolated from ***T. reticulatus***,[137] ***T. conica***[137] and ***T. peruvianus***[141] prompted us to investigate the bioactivity of **3.10** and **3.11**. The compounds were isolated with the aid of the ***A. salina*** assay and the median lethal concentrations of **3.10** and **3.11** were thus determined using both probit analysis[121] and the trimmed Spearman-Karber method[147] (the results from both statistical analyses are reproduced in Table 3.5). Although the acute toxicity data obtained for **3.10** and **3.11** did not fit the probit model well (Pearson's χ^2 goodness-of-fit, P < 0.05 for both diterpenes), in both cases there was excellent

correlation between the median lethal concentrations determined by parametric (probit analysis) and non-parametric (trimmed Spearman-Karber method) analysis procedures. It is interesting to note that the more oxygenated triacetate **3.11** was found to be significantly less active against ***A. salina*** than the diacetate **3.10**. As the ***A. salina*** assay has been shown in certain cases to be a reasonably reliable pre-screen for antitumor assays,[148] the ***T. costatus*** diterpenes were also tested for anticancer activity in four cancer cell lines (LOX melanoma, OVCAR3 ovarian cancer, A549 non-small cell lung cancer and SNB19 CNS cancer).[123] However, both **3.10** and **3.11** failed to show significant activity (IC_{50} > 100 $\mu g ml^{-1}$) against all four cancer cell lines.

The observed activity of **3.10** and **3.11** against ***A. salina*** encouraged us to investigate the possible ecological role of these metabolites. The presence of fouling organisms observed both on and around the ***T. costatus*** colony in the field strongly suggests that neither **3.10** nor **3.11** possesses significant antifouling activity against sympatric species. For this reason we considered it unnecessary to perform laboratory or field antifouling assays on **3.10** or **3.11**. Similarly, the difficulties in performing ecologically relevant assays against seastars described by Faulkner and Manker[137,138] also dissuaded us from performing these types of antipredator assays, even though there is evidence to support the hypothesis that the Trimusculid diterpenes act as a defence against these ecologically important invertebrate predators.[136,137]

Table 3.5 Median lethal concentrations of **3.10** and **3.11** against ***Artemia salina*** larvae.

	Probit analysis ($\mu g mL^{-1}$)			Trimmed Spearman-Karber ($\mu g mL^{-1}$)		
Compound	LC_{50}	95 % Confidence levels		LC_{50}	95 % Confidence levels	
3.10	35	27	43	33	30	37
3.11	155	130	188	154	133	179

The interesting, yet puzzling, observation that neither **3.1** nor **3.2** affected fish feeding,[137] coupled with the fact that fishes are important predators of invertebrates in southern African and other

marine systems (either because they inadvertently remove limpets whilst browsing or because they focus specifically on invertebrate prey)[149] decided us to focus on this aspect of the chemical ecology of ***T. costatus***. The ***T. costatus*** diterpenes were therefore tested for repellent effects against a South African marine generalist predatory fish ***Pomadasys commersonnii*** (spotted grunter). Generalist predatory fish were used because they represent the majority of predatory fish on reefs and antipredatory defences would be directed against them in particular.[150] In addition, generalists would be less likely than specialist predators to have evolved mechanisms to circumvent chemical defences.[150] The laboratory assays were performed using twelve ***P. commersonnii*** based on the method of Pawlik ***et al.***[150,151] and the results of the assays were analysed using the Fisher exact test.[150-152] For any single assay of twelve replicates, a treatment was considered significantly deterrent if four or more fish rejected treated food pellets while accepting control food pellets ($P \leq 0.047$, 1-tailed test).

As shown in Figure 3.8, both the crude ***T. costatus*** extract and the pure compounds **3.10** and **3.11** deterred feeding of ***P. commersonni*** at levels below their assumed natural concentration. Both pure compounds **3.10** (isolated in concentrations of 10 ppt and 6 ppt from the limpets) and **3.11** (isolated in concentrations of 14 ppt and 7 ppt from the limpets) exhibited significant deterency ($P < 0.001$) at a concentration of 5 ppt of the mean mass of the food pellets. Interestingly, the crude extract was found to be an extremely potent feeding deterrent. The first ***T. costatus*** collection gave a yield of crude extract per individual limpet of 4.81 mg (or 98 ppt mean dry limpet mass) while a similar calculation for the second collection gave a yield of 2.94 mg/animal (or 59 ppt mean dry limpet mass). From the feeding assays performed it is evident that the crude extract showed significant activity ($P < 0.001$) at a concentration of 10 ppt mean food pellet mass.

The relative potency of the crude extract may suggest that the active compounds exhibit synergistic activity, a hypothesis which was unfortunately not tested in this study, or that ***T. costatus*** contains other, as yet unidentified, repugnant metabolites which may be inactive against ***A. salina*** and were hence constituents of the crude extract but not isolated as pure natural products.

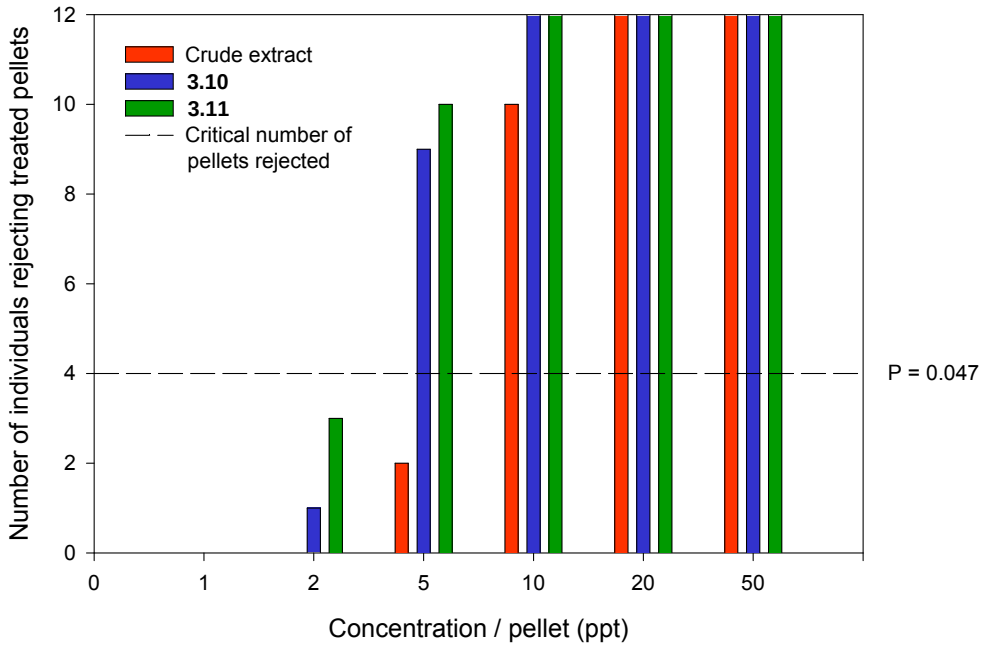


Figure 3.8 Results of the fish feeding deterrence assays shown as rejection of treated pellets by ***Pomadasys commersonnii*** (all control pellets were consumed in each assay). Treatments were considered significantly deterrent if rejected by four or more fish (Fisher exact test, 1-tailed, P = 0.047).

3.3 Summary and Conclusions

An investigation of the natural products chemistry of the endemic South African pulmonate limpet ***Trimusculus costatus*** indicated that this species produces the labdane diterpenes **3.10** and **3.11**, an observation in accordance with previous chemical investigations of Trimusculid limpets.[137,138,140,141] Extractives of collections made from separate limpet colonies suggest that the production of these diterpenes may be variable over time or between ***T. costatus*** populations, although further work would be required to confirm this observation. The structures and relative stereochemistry of **3.10** and **3.11** were elucidated from spectroscopic data, but, as with the other Trimusculid diterpenes,[137,138,140,141] the absolute stereochemistry of these compounds remains to be established.

Although Manker and Faulkner reported that **3.1** possessed significant toxicity against sabellariid tube worm larvae,[137] field observations performed on ***T. costatus*** indicated that this species is frequently fouled with common intertidal invertebrates also found closely associated within and around the colony, an observation which is in accordance with published data for related species.[130,133] Although **3.10** and **3.11** were not tested in any antifouling assays, from our field observations we believe that neither diterpene possesses significant antifouling activity. We therefore have to question whether the activity observed by Manker and Faulkner[137] is simply a manifestation of the inherent biological activity of these compounds rather than a specific defence against intertidal fouling organisms.

It has been reported that diterpenes isolated from ***T. reticulatus*** are not repugnant to fish.[137] However, we found that both diterpenes **3.10** and **3.11** deterred feeding of a common South African generalist predatory fish at, and below, natural concentrations. This, coupled with the fact that the defensive chemicals are most likely secreted in the mucus produced when these animals are disturbed, would seem to add more evidence to the hypothesis that these metabolites play an anti-predator role in the chemical ecology of the Trimusculid limpets.

Chapter Four

The Stereoselective Synthesis of

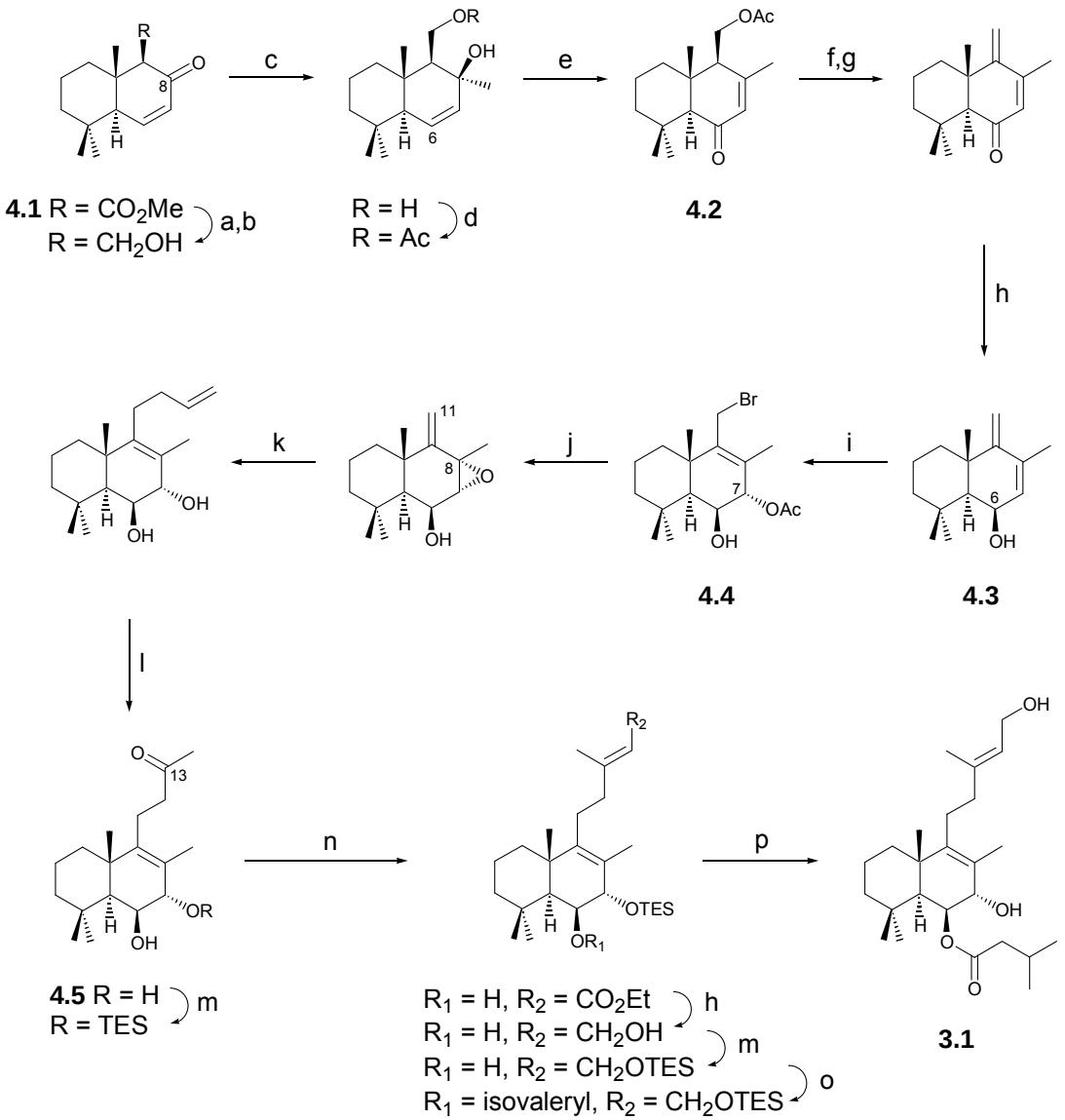
Rhinocerotinoic Acid

4.1 Introduction

The apparent broad spectrum of biological activity observed within the ***Trimusculus*** diterpenes described in the previous chapter identified this group of metabolites as worthy of further study. If an efficient method of obtaining appropriately oxygenated labdanes could be found, then selective esterification would allow a number of the ***Trimusculus*** diterpenes and their 6,7-diastereomers to be prepared. The bioactivity of these synthetic compounds could then be assessed in ecological and pharmaceutical assays and yield valuable structure activity data for this group of metabolites.

Surprisingly, only one synthesis of a ***Trimusculus*** diterpene has been published. Isoe and co-workers synthesised (±)-6β-isovaleroxylabda-8,13-dien-7α,15-diol (**3.1**) stereoselectively from (±)-9-methoxycarbonyl-4,4,10-trimethyl-Δ^6-8-octalone (**4.1**) in 18 steps and 22% overall yield (Scheme 4.1).[139] Isoe's synthesis began with the conversion of **4.1** to the enone **4.2** through hydride reduction, selective C-8 oxidation, stereospecific methylation, esterification and oxidative rearrangement. Hydrolysis of the ester functionality of **4.2** followed by dehydration and reduction with diisobutyl aluminium hydride allowed the preparation of the 6β-allylic alcohol **4.3** in high yield. The required 7α-oxygen functionality was introduced stereospecifically to ring-B through 1,4-addition of acetate to the diene moiety to yield the 6,7-***trans***-diaxially substituted allylic bromide **4.4**. Saponification of the C-7 acetate group of **4.4** followed by two successive S_N2' reactions and Wacker oxidation allowed the preparation of ketone **4.5**. Subsequent selective protection of the C-7 hydroxyl, stereoselective elaboration of the C-9 side chain employing Horner-Wadswoth-Emmons methodology, hydride reduction, selective protection, esterification and finally deprotection yielded the racemic ***Trimusculus*** diterpene **3.1**.

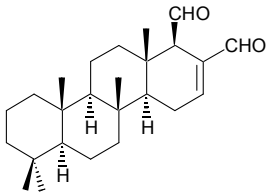
Although Isoe and co-workers synthesis is both elegant and efficient, this route is currently constrained to a racemic synthesis by the starting material, which is obtained as a racemic mixture from β-ionone in 5 steps.[153] Therefore, some other method of preparing the naturally occurring stereoisomers of these compounds is required to allow meaningful analysis of their bioactivity and unequivocally assign their absolute stereochemical configuration.



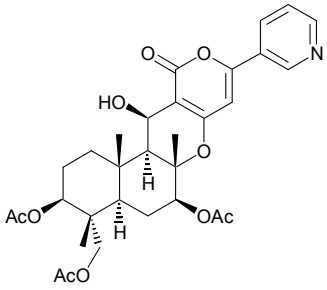
Scheme 4.1 Isoe and co-workers' stereoselective synthesis of (±)-6β-isovaleroxylabda-8,13-dien-7α,15-diol (**3.1**).[139] ***Reagents and conditions:*** a. $LiAlH_4$, Et_2O, 0 °C, 2.5 h; b. $BaMnO_4$, CH_2Cl_2, RT, 24 h; c. MeLi, Et_2O, –78 °C, 3 h; d. Ac_2O, pyridine, RT, 24 h; e. PCC, CH_2Cl_2, RT, 3 h; f. K_2CO_3, MeOH, RT, 5 h; g. TsOH, benzene, Δ, 6 h; h. DIBAH, CH_2Cl_2, –78 °C, 3 h; i. NBS, AcOH, RT, 1 h; j. K_2CO_3, MeOH, RT, 2 h; k. CH_2=CH–CH_2MgBr, CuBr-SMe_2 (cat.), LiBr (cat.), Et_2O, –78 °C, 2 h; l. $PdCl_2$, CuCl, DMF-H_2O, O_2, RT, 1 h; m. TESCl, pyridine, 0 °C, 30 min; n. $(C_2H_5O)_2P(O)CH_2CO_2Et$, NaH, THF, 0 °C to RT, 6 h; o. isovaleric acid, DCC, DMAP, CH_2Cl_2, RT, 24 h; p. TBAF, THF, RT, 6 h.

Various methods have been reported for the enantioselective synthesis of polycyclic terpenoid skeletons, and these fall broadly into three categories:

1) Enantioselective construction of the terpenoid skeleton through biomimetic cyclisation of an appropriate isoprenoid precursor, for example in Corey ***et al.***'s synthesis of scalarendial (**4.6**) from geranylgeranyl acetate[154] and Aggarwal ***et al.***'s formal synthesis of the ***Aspergillus fumigatus*** metabolite (+)-pyripyopene (**4.7**) from geraniol.[155]

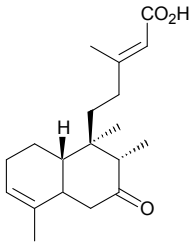


4.6

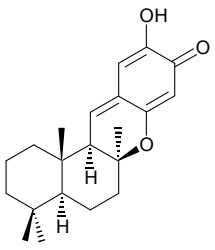


4.7

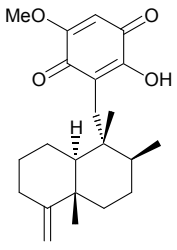
2) Use of an appropriate chiral template to begin the synthesis, for example Kato ***et al.***'s synthesis of (–)-7-oxo-kolavenic acid (**4.8**) from (1***S***,5***S***)-(–)-verbenone,[156] Banerjee and co-workers' synthesis of the marine natural product puupehedione (**4.9**) from (***R***)-(–)-carvone,[157] and Samadi and co-workers synthesis of ilimaquinone (**2.1**) from 5,8a-(***S***)-dimethyl-3,4,8,8a-tetrahydro-2***H***,7***H***-naphthalene-1,6-dione.[85]



4.8



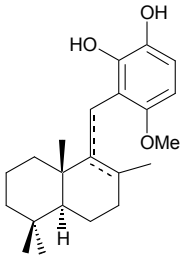
4.9



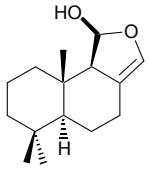
2.1

3) Semi-synthesis from readily available naturally occurring labdanes, which has been effectively applied by Barrero's group in the enantiospecific syntheses of many terpenoid natural

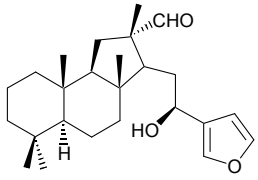
products including puupehedione (**4.9**),[158] wiedendiol-A (**4.10**) and -B (**4.11**),[159] 11,12-epoxydrim-8,12-en-11-ol (**4.12**)[160] and by Urones and co-workers in the preparation of hyrtyosal (**4.13**).[161]



4.10 Δ^{8}
4.11 $\Delta^{9,11}$, 8β

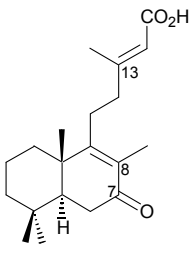


4.12



4.13

Considering economics, ease of synthesis and the ultimate purpose of our synthesis, we decided to choose a naturally occurring diterpene as the starting material for our synthetic studies. We were aware of the existence of rhinocerotinoic acid (**4.14**), a labdane diterpene isolated from the South African medicinal plant ***Elytropappus rhinocerotis*** by Dekker ***et al.*** in 1988,[162] and considered this an ideal chiral template for the semi-synthesis of ***Trimusculus*** metabolites. The presence of an α,β-unsaturated ketone at C-7 of **4.14** was considered an ideal synthetic tool to allow further functionalisation of the labdane B-ring. However, being aware of the difficulties concerning the oxygenation of unfunctionalised A-rings in such systems,[163] we limited our initial objective to the preparation of 6β,7α-diacetoxylabda-8,13***E***-dien-15-ol (**3.10**), isolated from the endemic South African button shell ***Trimusculus costatus***, and its 6,7-diastereomers.

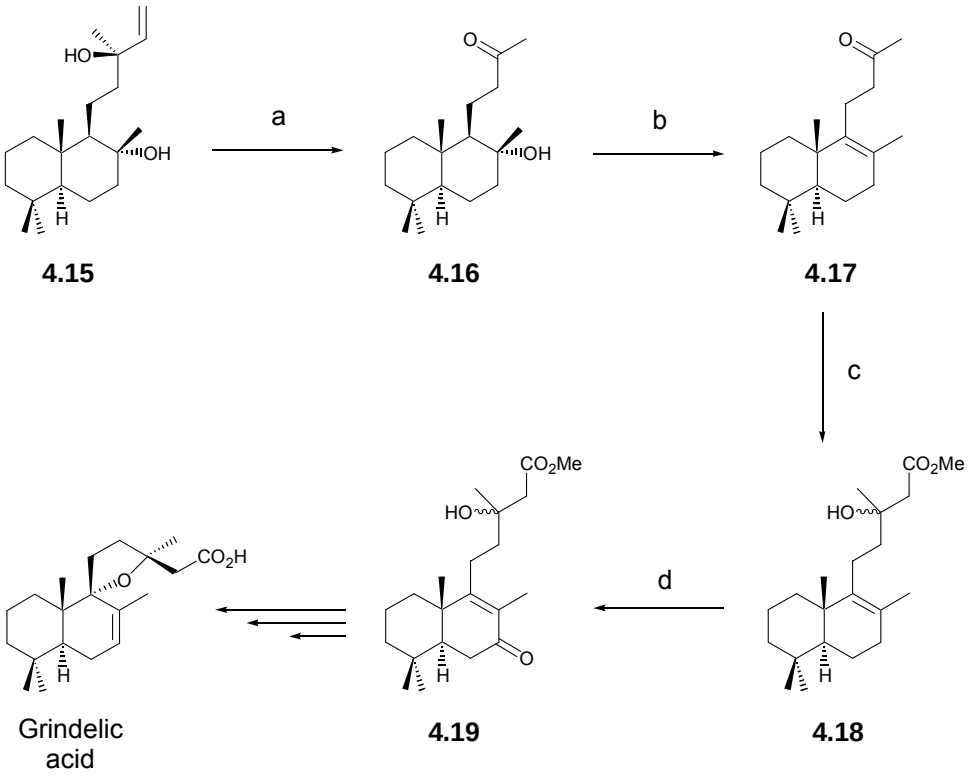


4.14

We initially intended to obtain sufficient **4.14** for semi-synthesis through isolation of this compound from ***E. rhinocerotis***, as this is a common species in the Eastern Cape veld.[164] However, screening of the acidic component of acetone extracts of samples of ***E. rhinocerotis*** collected from Burnt Kraal, near Grahamstown showed that it contained no **4.14** by ^{1}H NMR spectroscopy.

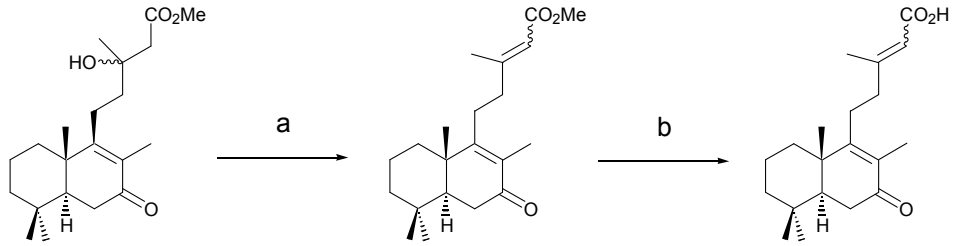
Figure 4.1 The South African medicinal plant ***Elytropappus rhinocerotis***.

In their paper describing the isolation of **4.14**,[162] Dekker ***et al.*** made a brief reference to the synthesis of **4.14** and its Δ^{13}-geometrical isomer, isorhinocerotinoic acid. The authors used this synthesis to unambiguously establish the absolute stereochemistry of **4.14**. In their published synthesis of rhinocerotinoic acid (**4.14**), Dekker ***et al.*** first converted the commercially available diterpene (−)-sclareol (**4.15**) to methyl 13-hydroxy-7-oxo-labda-8-en-15-oate (**4.19**) according to the route described by Mangoni and co-workers in the synthesis of grindelic acid (Scheme 4.2).[165] Dehydration of **4.19** with phosphorous oxychloride in pyridine followed by hydrolysis of the methyl ester in alkaline medium resulted in the formation of rhinocerotinoic acid (**4.14**) and isorhinocerotinoic acid (**4.22**) as shown in Scheme 4.3.[162] Surprisingly, Dekker ***et al.*** gave no experimental details for the synthetic transformations described in their paper, made no reference to the ratio of isomers obtained by dehydration and did not quote an overall yield for their synthesis.



Scheme 4.2 Mangoni and co-workers' synthesis of 13-hydroxy-7-oxo-labda-8-en-15-oic acid methyl ester (**4.19**).[165] ***Reagents and conditions***: a. $KMnO_4$, acetone, < 15 °C; b. I_2, benzene, Δ, 3 h; c. Zn, $BrCH_2CO_2Me$, benzene-Et_2O (5:2), Δ, 2 h; d. CrO_3, H_2SO_4, acetone, RT, over-night.

As we could not isolate **4.14** from its natural source, we decided to follow the seemingly straightforward synthetic routes outlined by Mangoni and co-workers[165] and Dekker ***et al.***[162] and prepare **4.14** from (−)-sclareol. Although the task of following a published synthesis was intended to be a trivial exercise necessary to obtain sufficient **4.14** for our main study, we were unable to reproduce the published results in many of the steps used in the reported synthesis. It was therefore necessary to find alternative methods to carry out a number of the transformations, and this ultimately led to the development of an improved, stereoselective synthesis of **4.14** which is described below.



4.19

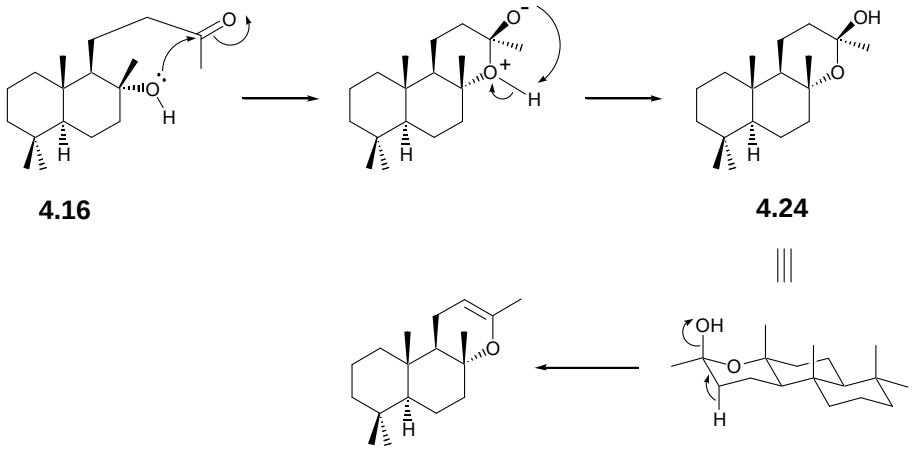
4.20 Δ^{13} ***E***
4.21 Δ^{13} ***Z***

4.14 Δ^{13} ***E***
4.22 Δ^{13} ***Z***

Scheme 4.3 Dekker ***et al.***'s transformation of **4.19** to rhinocerotinoic acid (**4.14**) and isorhinocerotinoic acid (**4.22**).[162] ***Reagents***: a. $POCl_3$, pyridine ; b. Alkaline hydrolysis (reaction conditions and yields were not provided by the authors).

4.2 Synthesis of rhinocerotinoic acid

The synthesis of rhinocerotinoic acid involves the initial preparation of the key intermediate **4.17**. Mangoni and co-workers report that this was accomplished by potassium permanganate oxidation of (–)-sclareol (**4.15**) followed by dehydration of the resulting hydroxy ketone **4.16**.[165] The degradative oxidation of (–)-sclareol with potassium permanganate to yield (+)-8α-hydroxy-14,15-bisnorlabda-13-one (**4.16**) and its dehydration product, (+)-8α,13-epoxy-14,15-bisnorlabda-12-ene (**4.23**), was first reported by Ruzicka ***et al.*** in 1942.[166] Barltrop and co-workers subsequently modified this procedure so that, under controlled conditions, either the hydroxy ketone **4.16** or the enol ether **4.23** may be obtained as the major product of oxidation.[167,168] It is well known that **4.16** may be readily converted to **4.23** by dehydration, with significant dehydration occurring in light petroleum,[167] benzene[169] or pyridine[170] solutions of **4.16** and quantitative conversion occurring in the presence of trace acid[167] or through vacuum distilation.[171] We propose that the dehydration proceeds by the mechanism shown in Scheme 4.4. Due to steric constraints, nucleophilic attack of the ketone occurs from the ***si***-face, giving rise to hemiacetal **4.24** in which the axial hydroxyl readily undergoes ***trans***-elimination to give enol ether **4.23**.

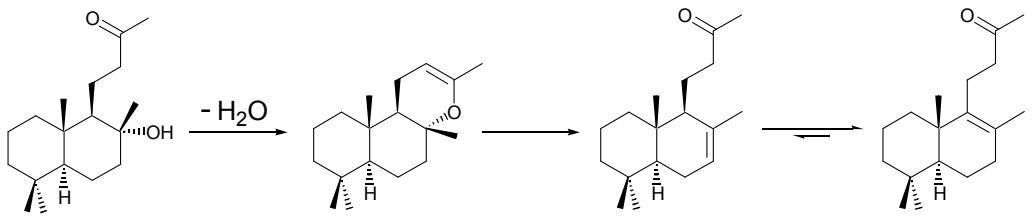


Scheme 4.4 Proposed mechanism for the dehydration of 8α-hydroxy-14,15-bisnorlabda-13-one (**4.16**).

Although Barltrop and co-workers report that both **4.16** and **4.23** may be converted to (+)-14,15-bisnorlabda-8-en-13-one (**4.17**) by treatment with mineral acid in ethanol,[167] it has since been found that this procedure gives rise to a mixture of the Δ^7- and Δ^8-unsaturated ketones.[172] Mangoni and Belardini have also reported that the intermediate prepared by iodine catalysed dehydration of **4.16** differed from that reported by Barltrop and co-workers.[173] As the second step in our synthesis involved the iodine catalysed dehydration of **4.16**, we were careful to optimise the yield of this intermediate and avoid formation of the dehydration product **4.23**.

Accordingly, (–)-sclareol (**4.15**) was oxidised with potassium permanganate following the procedure of Barltrop and co-workers[167] to yield a mixture of the desired ketol **4.16** and its dehydration product, the enol ether **4.23**, as an oily solid. As the ketol **4.16** has been reported to degrade during chromatography on silica gel,[174] presumably through acid catalysed dehydration, the ketol – enol ether mixture was separated by washing the crude product with cold hexane, to give pure **4.16** as a white crystalline solid (mp 81-82 °C, lit.[167,168] 78-80 °C; $[\alpha]_D$ = +5°, lit.[169] +6.7°) in 70% yield and **4.23** as a clear colourless oil (7% yield) which could be crystallised from cold methanol (mp 43-45 °C, lit.[167] 44-46 °C; $[\alpha]_D$ = +4°, lit.[175] +3°). The NMR, IR and MS data obtained for both **4.16** and **4.23** were consistent with published data.[169,176]

The next step in the preparation of the key intermediate **4.17** involved the dehydration of **4.16** using iodine in refluxing benzene.[173] However, initial attempts at this reaction following the literature procedure gave extremely inconsistent results, yielding variable ratios of three products, **4.23**, **4.17** and (+)-14,15-bisnorlabda-7-en-13-one (**4.25**). We therefore undertook a series of experiments to optimise the yield of the desired unsaturated ketone **4.17**. Although literature procedures quoted using a small crystal of iodine,[173] we decided to standardise the iodine concentration used in the dehydration by adding a known volume of a solution of iodine in anhydrous benzene. The reaction was consequently carried out using 0.05 equivalents of iodine, with aliquots of reaction mixture taken after 0, 0.5, 1, 2, 4, 6 and 18 hours reflux. These aliquots were concentrated to dryness and immediately examined by ^{1}H and ^{13}C NMR spectroscopy. The course of the reaction was easily monitored by examination of the vinylic carbon resonances of the three products and a typical series of ^{13}C NMR spectra obtained from selected aliquots (0, 1, 2 and 6 hours reflux) are reproduced in Figure 4.2. The results are summarised in Scheme 4.5. It would seem that **4.16** is rapidly dehydrated to the enol ether **4.23**, which on further reaction undergoes isomerisation, initially to the kinetically favoured Δ^{7}-unsaturated ketone **4.25**, then to the thermodynamically favoured Δ^{8}-unsaturated ketone **4.17**.



4.16 **4.23** **4.25** **4.17**

Scheme 4.5 The observed course of the iodine catalysed dehydration of 8α-hydroxy-14,15-bisnorlabda-13-one (**4.16**).

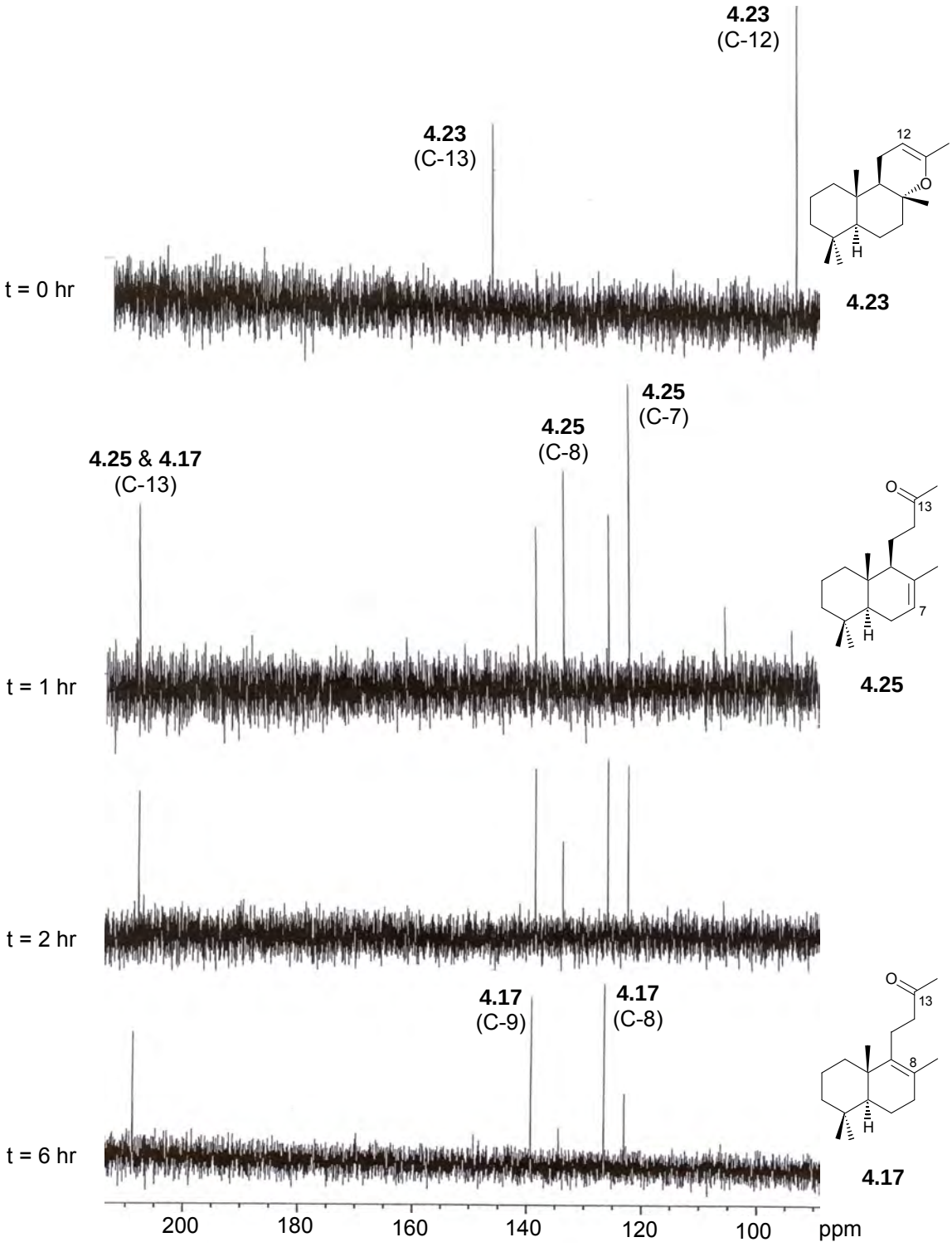
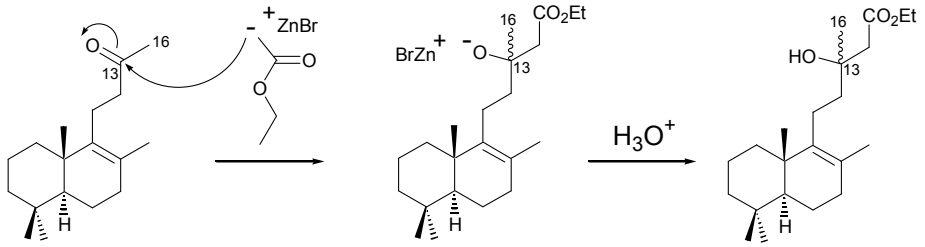


Figure 4.2 The downfield region (δ_C 89 – 214) of the ^{13}C NMR spectra ($CDCl_3$, 100 MHz) obtained for selected aliquots taken from the iodine catalysed dehydration of **4.3**. The structures of the predominant product in each aliquot are shown on the right.

It was evident from the ^{13}C NMR spectra that optimal conditions for this reaction were 6 hours reflux with 0.05 equivalents of iodine, which gave **4.17** ($[\alpha]_D$ = +75°, lit.[173] +78°; ^1H NMR and IR data consistent with literature values[172,177]) and **4.25** ($[\alpha]_D$ = +22°, lit. for enantiomer[178] –23°; ^1H NMR, IR and MS data consistent with literature values reported for the ***ent***-labdane analogue[179]) in 75% and 6% yield respectively as clear colourless oils (which were easily separable by flash chromatography). The proposed reaction steps were supported by subjecting pure **4.23** (prepared by passing **4.16** through a column of silica with chloroform as eluant) to the optimised reaction conditions, which yielded the same products in 76% and 8% yield. This indicated that in subsequent large-scale (5–7 g) preparations of **4.17**, the products obtained from the initial permanganate oxidation step need not be separated before continuing with the dehydration.

When the iodine catalysed dehydration procedure was compared to the acid catalysed dehydration described by Barltrop and co-workers,[167] we found it gave much more favourable results than the latter procedure. In accordance with the observations of Wenkert ***et al.***[172] we also found that when dehydrated under acidic conditions, **4.16** gave a 71% yield of **4.17** and **4.25** in a ratio of 3:2, and **4.23** gave a 67% yield of **4.17** and **4.25** in a ratio of 2:1. It is interesting to note however that in our hands, the products obtained through acid catalysed dehydration were identical to those obtained through iodine catalysed dehydration, contrary to the results reported by Mangoni and Belardini.[173]

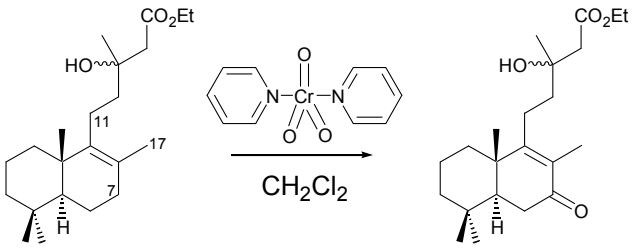
Therefore, with a reliable, gram scale preparation of the key intermediate **4.17** in hand, we now turned our attention to the formation of methyl 13-hydroxylabda-8-en-15-oate (**4.18**) by Reformatsky reaction. At this stage in our synthesis the availability of reagents meant that our route had to differ slightly from that reported by Mangoni and co-workers,[165] and we undertook the preparation of the ethyl ester **4.26** as opposed to the methyl ester **4.18** (Scheme 4.6). Accordingly, the unsaturated ketone **4.17** was converted to ethyl 13-hydroxylabda-8-en-15-oate (**4.26**) by reaction with activated zinc powder and ethyl bromoacetate under classical conditions.[180] This transformation proceeded smoothly in excellent yield (93 %) to give **4.26** as a 1:1 mixture of C-13 epimers (determined from ^1H and ^{13}C NMR data), which were inseparable by normal phase HPLC.



4.17 **4.26**

Scheme 4.6 The mechanism of the Reformatsky reaction of 14,15-bisnorlabda-8-en-13-one (**4.17**) with zinc and ethyl bromoacetate (adapted from Rathke[180]).

Following the synthetic strategy of Mangoni and co-workers,[165] we then attempted the allylic oxidation of **4.26** with Jones' reagent. Although they reported a yield of 86% for this transformation on methyl 13-hydroxy-labda-8-en-15-oate (**4.18**), in our hands this oxidation gave a complex mixture of products in low yield. We attributed this result to competing allylic oxidation at C-11 and C-17 of the labda-8-ene skeleton. We therefore searched for an alternative method of allylic oxidation and eventually decided to use Collins' modification[181] of the Sarrett oxidation.[182] Collins' oxidation has been reported to show both high regioselectivity and favourable yields when applied to allylic oxidation of ***trans***-decalin and conformationally constrained ring systems,[183,184] and the problems concerning the preparation and hygroscopic nature of the required chromium trioxide-pyridine complex have been circumvented by an ***in situ*** preparation.[185] We therefore applied the modified procedure of Chu and Coates[184] to the oxidation of **4.26** using 15 equivalents of dipyridine-chromium (VI) oxide (Scheme 4.7). Although we initially encountered problems with work-up as a black tarry solid formed during the reaction, we found that dissolving the tar in saturated sodium hydrogen carbonate and washing the resulting solution with ethyl acetate gave a good yield (80 %) of the desired α,β-unsaturated ketone [ν_{max} 1661 cm^{-1}; δ_C 200.1 (s, C-7)], ethyl 13-hydroxy-7-oxo-labda-8-en-15-oate (**4.27**), as the only product.



4.26 **4.27**

Scheme 4.7 Regiospecific Collins' oxidation of ethyl 13-hydroxylabda-8-en-15-oate (**4.26**).

The mechanism of allylic oxidation of alkenes by chromium (VI) is not well understood, but is considered to proceed through the initial formation of an allylic radical ***via*** abstraction of a hydrogen atom.[186] Consequently, the regioselectivity of Collins' oxidation in substituted cyclohexenes and steroids has been rationalised to depend on the steric accessibility of the allylic protons, the relative stabilities of the possible allylic intermediates, and the steric and electronic control of the oxygen transfer step at competing sites.[183] Considering these factors, the excellent regioselectivity observed in the oxidation of **4.26** can be explained thus: examination of the energy minimised space filling model of both C-13 epimers of **4.26** (shown in Figure 4.3) indicates that hydrogen abstraction at C-11 will be hindered as these protons occupy a sterically crowded position in both epimers. Of the two remaining allylic sites, in accordance with the observations of Dauben ***et al.***,[183] hydrogen atom abstraction from C-7 will be significantly favoured over C-17 due to the increased ability of secondary C–H bonds to undergo homolytic cleavage over primary C–H bonds.[187]

We now turned our attention to the next transformations reported by Dekker ***et al.***[162] Dehydration of **4.27** with phosphorous oxychloride in pyridine[188] proceeded regiospecifically to give a reasonable yield (76 %) of dehydration products, although the stereoselectivity of this reaction was disappointing. The Δ^{13}-***E***-isomer, ethyl rhinocerotinoate (**4.28**), and the Δ^{13}-***Z***-isomer, ethyl isorhinocerotinoate (**4.29**), were obtained in a 2:1 ratio and could only be separated by normal phase semi-preparative HPLC. Repetition of this procedure using the more active dehydrating agent thionyl chloride[189] gave a complex mixture of products in low yield and was not pursued

further. The unsatisfactory stereoselectivity of this dehydration prompted us to try boron trifluoride etherate, as this reagent has been reported to yield the thermodynamically favoured elimination products in higher yields than classical reagents.[190] However, treatment of **4.27** with boron trifluoride etherate in dichloromethane at room temperature for 24 hours afforded no dehydration products, with only starting material being recovered on work up.

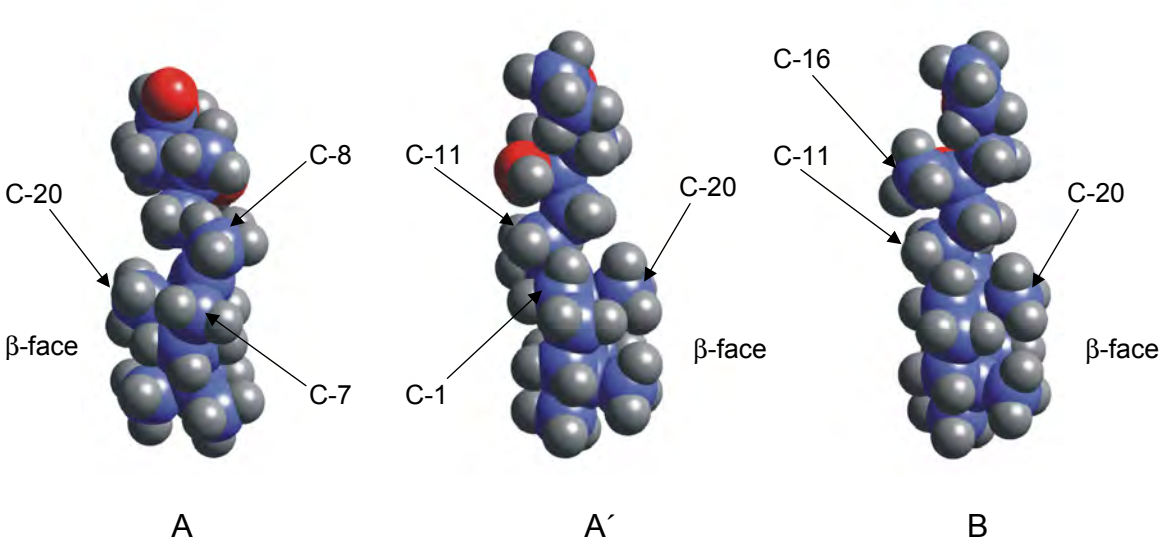
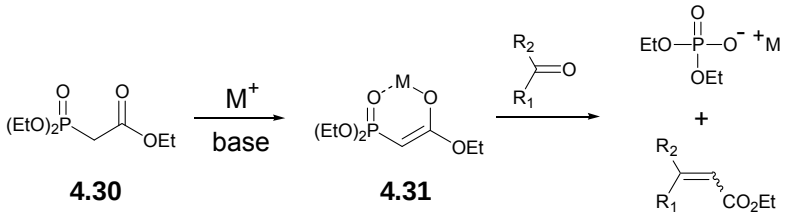


Figure 4.3 Energy minimised space filling models of **4.26** showing the steric environments of the allylic protons H-7 and H-11: A. ethyl 13***R***-hydroxylabda-8-en-15-oate; A´. ethyl 13***R***-hydroxylabda-8-en-15-oate viewed from a different perspective; and B. ethyl 13***S***-hydroxylabda-8-en-15-oate.

As the focus of our synthesis was the preparation the Δ^{13}-***E***-acid **4.14**, we investigated methods of converting **4.29** to **4.28** by isomerisation of the C-13–C-14 double bond. However, refluxing both the 2:1 mixture of **4.28**/**4.29** and pure **4.29** in benzene with catalytic amounts of iodine[191,192] gave no change in the isomeric composition of the mixture and only 6% conversion from the ***Z***- to the ***E***-isomer (determined by ^{1}H NMR spectroscopy) when pure **4.29** was employed. Similarly, prolonged heating of the 2:1 mixture with thiophenol[193] showed no enrichment of the Δ^{13}-***E***-isomer. However, when pure **4.29** was heated with thiophenol for 2 hours under an anhydrous air atmosphere approximately 50% conversion to the Δ^{13}-***E***-isomer, **4.28**, was observed by ^{1}H NMR

spectroscopy. As increased reaction times did not result in further isomerisation of the ***E***/***Z*** mixture, and because of the extensive HPLC required in separating the ***E***- and ***Z***-ethyl ester mixtures, this method was discounted as a means of obtaining large quantities of isomerically pure **4.28**.

We therefore decided to modify the rhinocerotinoic acid synthesis in order to optimise the yield of the Δ^{13}-***E***-ethyl ester **4.28** by employing a stereospecific method of preparing the C-13–C-14 double bond. The obvious method to try was the Horner-Wadsworth-Emmons (HWE) modification of the Wittig reaction which utilises the reaction of metal enolates (**4.31**) derived from triethyl phosphonoacetate (**4.30**) or related α-stabilised phosphonates with carbonyl compounds, yielding olefins and water-soluble phosphate esters (Scheme 4.8).[194] Although the HWE reaction has been widely used in the stereoselective preparation of both ***E***- and ***Z***- disubstituted α,β-unsaturated esters from aldehydes,[195] the stereoselectivity observed when using ketones to prepare trisubstituted alkenes is often significantly diminished.[196] However, we believed that using this approach we could improve on the 2:1 ***E***/***Z*** selectivity achieved by dehydration of **4.27**.

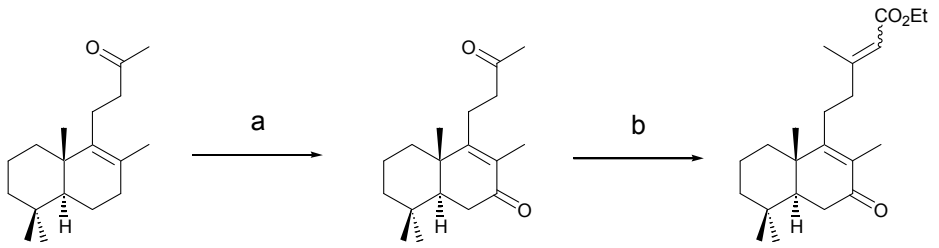


4.30 **4.31**

Scheme 4.8 The Horner-Wadsworth-Emmons modification of the Wittig reaction.[194]

To use this methodology in the synthesis of rhinocerotinoic acid (**4.14**) we would have to perform the allylic oxidation at C-7 of ketone **4.17** prior to elaboration of the side chain and rely on the HWE olefination to proceed selectively at the more electrophilic saturated C-13 carbonyl group in the resulting diketone **4.32** (Scheme 4.9).[197] To this end we oxidised **4.17** ***via*** Collins' oxidation[181] employing the optimised procedure describe before. In this way we were able to prepare the known diketone[198] (+)-14,15-bisborlabda-8-en-7,13-dione **4.32** in 81% yield (mp 78-80 °C, lit.[198] 79.5-80 °C; $[\alpha]_D$ = +58°, lit.[198] +65.8°; NMR, IR and MS data consistent with those reported[198]).

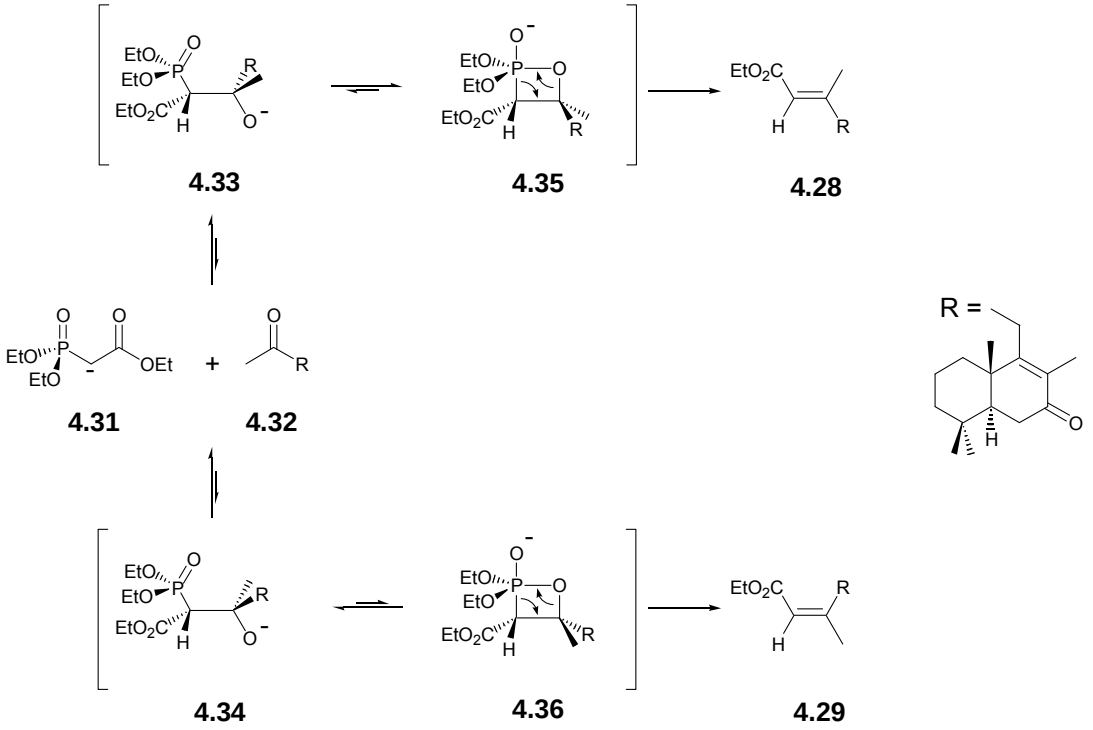
Compound **4.32** was subsequently subjected to HWE reaction with triethyl phosphonoacetate in tetrahydrofuran which proceeded chemospecifically to give the α,β-unsaturated esters **4.28** and **4.29** with vastly improved stereoselectivity (10:1 ***E***/***Z***) and in excellent yield (96%).



4.17 **4.32** **4.28** Δ^{13} ***E*** **4.29** Δ^{13} ***Z***

Scheme 4.9 Stereoselective preparation of ethyl rhinocerotinoate (**4.28**) ***via*** HWE reaction. ***Reagents and conditions***: a. CrO_3-Py_2 (15 equivalents), CH_2Cl_2, RT, 72 h (81%); b. $(C_2H_5O)_2P(O)CH_2CO_2Et$, NaH, THF, RT, 6 h (96%; 10:1 ***E***/***Z***).

As mentioned above, the chemospecificity observed in the HWE reaction between **4.30** and **4.32** was expected prior to performing the transformation primarily due to the comparative reactivities of saturated ***versus*** α,β-unsaturated carbonyls, but steric factors may also have played a role in hindering olefination at C-7. The extent of stereoselectivity observed, however, was unexpected.[199] Examination of the mechanism[196] of attack of the enolate **4.31** on the diketone **4.32** (shown in Scheme 4.10) indicates that initial addition of carbanion **4.31** results in the formation of both ***syn*** (**4.33**) and ***anti*** (**4.34**) isomeric adducts. These adducts may then form oxaphosphetanes **4.35** and **4.36** which decompose by ***syn***-elimination of phosphate to yield the ***trans***-alkene (**4.28**) and the ***cis***-alkene (**4.29**) respectively. As the initial nucleophilic attack by **4.31** and the subsequent oxaphosphetane formation are reversible processes, it is the relative stabilities of **4.35** and **4.36** which ultimately determines the ratio of ***E***- to ***Z***-products obtained. In the case of **4.35** and **4.36**, the difference in steric interaction between the ethoxycarbonyl moiety and the methyl or alkyl groups must therefore be sufficiently large to yield the observed ten-fold selectivity in the alkene products.

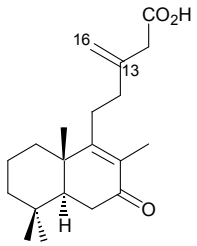


Scheme 4.10 The mechanism of the HWE reaction between bisnorlabda-8-en-7,13-dione (**4.32**) and triethyl phosphonoacetate (modified from Kelly[196]).

Having established a method of preparing the C-13–C-14 double bond stereoselectively, we turned our attention to the hydrolysis of the ester functionality in **4.28** which, following the route reported by Dekker ***et al.***,[162] was hydrolysed under basic conditions. Accordingly, the 10:1 mixture of esters **4.28** and **4.29** was treated with potassium hydroxide in aqueous ethanol under reflux. The reaction was followed by analytical TLC and quenched with acid as soon as there was no evidence of residual ester to give an acidic extract in 95% yield. However, examination of the ^{1}H NMR spectrum of the crude reaction mixture suggested that under these conditions significant isomerisation of the C-13–C-14 double bond had occurred.

The presence of two vinylic methine singlets at δ_H 5.73 and 5.72 indicated that both rhinocerotinoic acid (**4.14**) and the C-13–C-14 ***Z***-isomer, isorhinocerotinoic acid (**4.22**), were present in the reaction mixture, and additional vinylic methylene singlets at δ_H 5.00 and 5.02 suggested that 7-oxo-labda-8,13(16)-dien-15-oic acid (**4.37**) had also been formed. Integration of

these proton resonances indicated that the crude reaction mixture contained the three acids in a 12:3:2 ratio respectively. Attempts to separate this mixture by chromatographic methods (normal phase and reversed phase column chromatography and HPLC) were unsuccessful. Crystallisation from hexane allowed the isolation of approximately half of the ***E***-isomer **4.14** contained within the mixture as a white solid (mp 189-190 °C, lit.[162] 186-189°C; $[\alpha]_D$ = +40°, lit.[162] +42°), and similar crystallisation from methanol-water yielded a small quantity of the pure ***Z***-isomer **4.22** (mp 155-157 °C, lit.[162] 156-158 °C; $[\alpha]_D$ = +50°, lit.[162] +54°). The NMR data obtained for **4.14** and **4.22** are compared with literature data in Tables 4.1 and 4.2.



4.37

The mother liquors from the crystallisation were concentrated to give **4.14**, **4.22** and **4.37** in an approximate ratio of 5:3:2 (by ^{1}H NMR spectroscopy). In order to separate this mixture further and unambiguously identify the third saponification product, it was necessary to methylate the acids with diazomethane (Scheme 4.11). Careful repeated flash chromatography of the resulting oil (the ethyl esters were inseparable under these conditions), followed by semi-preparative HPLC of mixed fractions confirmed our suspicions concerning isomerisation, and gave methyl rhinocerotinoate (**4.20**), methyl isorhinocerotinoate (**4.21**) and methyl 7-oxo-labda-8,13(16)-dien-15-oate (**4.38**). The structure of **4.38** was elucidated by analysis of NMR spectroscopic data (reproduced in Table 4.3) and confirmed by high resolution mass spectrometry.

As Dekker ***et al.***[162] did not report any isomerisation resulting from hydrolysis of the mixed methyl esters **4.20** and **4.21**, nor stipulate the base used for hydrolysis, we also saponified the ethyl esters with a weaker base (potassium carbonate). However, the same products, **4.14**, **4.22** and **4.37**, were obtained in 83% yield and a less favourable ratio of 7:2:7 respectively.

Table 4.1 Comparison of the ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data obtained for rhinocerotinoic acid (**4.14**) with that reported by Dekker ***et al.*** (^{1}H: 500MHz; ^{13}C: 127.5 MHz; recorded in $CDCl_3$ and calibrated relative to TMS).[162]

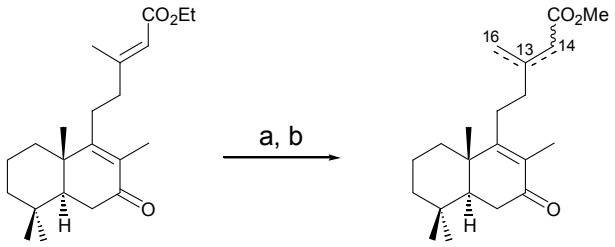
	4.14		Literature data[162]	
Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	δ_C ppm	δ_H ppm
1	35.9 (t)	1.36 (1H, td, 12.4, 3.0) 1.91 (1H, br d, 12.2)	36.0	1.35 1.90
2	18.6 (t)	1.60 (1H, m) 1.73 (1H, m)	18.6	1.58 1.68
3	41.2 (t)	1.21 (1H, td, 13.6, 4.0) 1.48 (1H, br d, 13.5)	41.3	1.20 1.47
4	33.1 (s)		33.1	
5	50.3 (d)	1.69 (1H, dd, 14.3, 3.6)	50.3	1.68
6	35.2 (t)	2.38 (1H, m) 2.50 (1H, dd, 17.6, 3.6)	35.2	2.35 2.49
7	200.1 (s)		200.1	
8	130.6 (s)		130.6	
9	166.3 (s)		166.2	
10	41.0 (s)		41.0	
11	27.6 (t)	2.35 (2H, m)	27.7	2.28
12	39.8 (t)	2.29 (2H, m)	39.8	2.35
13	161.5 (s)		161.5	
14	115.4 (d)	5.73 (1H, br s)	115.4	5.73
15	171.6 (s)		171.6	
16	19.1 (q)	2.21 (3H, d, 0.9)	19.1	2.20
17	11.4 (q)	1.76 (3H, s)	11.4	1.75
18	32.5 (q)	0.87 (3H, s)	32.5	0.86
19	21.3 (q)	0.91 (3H, s)	21.3	0.90
20	18.2 (q)	1.08 (3H, s)	18.2	1.07

Table 4.2 Comparison of the ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data obtained for isorhinocerotinoic acid (**4.22**) with that reported by Dekker ***et al.*** (^{1}H: 500MHz; ^{13}C: 127.5 MHz; recorded in $CDCl_3$ and calibrated relative to TMS).[162]

	4.22		Literature data[162]	
Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	δ_C ppm	δ_H ppm
1	35.9 (t)	1.43 (1H, td, 12.0, 3.7) 2.08 (1H, br d, 11.8)	35.9	1.40 2.04
2	18.6 (t)	1.61 (1H, m) 1.67 (1H, m)	18.6	1.58 1.66
3	41.3 (t)	1.23 (1H, td, 13.3, 4.3) 1.46 (1H, br d, 13.7)	41.3	1.20 1.45
4	33.2 (s)		33.1	
5	50.2 (d)	1.69 (1H, dd, 14.2, 3.7)	50.2	1.68
6	35.3 (t)	2.37 (1H, dd, 17.4, 14.1) 2.50 (1H, dd, 17.6, 3.8)	35.2	2.34 2.49
7	200.4 (s)		200.3	
8	130.7 (s)		130.6	
9	166.9 (s)		167.0	
10	41.1 (s)		41.1	
11	27.7 (t)	2.32 (2H, m)	27.7	2.32
12	32.5 (t)	2.76 (2H, m)	32.5	2.74
13	161.7 (s)		161.6	
14	116.6 (d)	5.72 (1H, br s)	116.2	5.73
15	170.8 (s)		171.6	
16	25.3 (q)	1.99 (3H, d, 1.0)	25.3	1.98
17	11.2 (q)	1.82 (3H, s)	11.2	1.80
18	32.5 (q)	0.88 (3H, s)	32.5	0.86
19	21.3 (q)	0.91 (3H, s)	21.3	0.90
20	18.3 (q)	1.09 (3H, s)	18.2	1.07

Table 4.3 ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR data obtained for methyl 7-oxo-labda-8,13(16)-dien-15-oate (**4.38**).

Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	HMBC correlation to	COSY coupling to
1	35.7 (t)	1.34 (1H, br t, 11.1) 1.90 (1H, br d, 12.4)	C-2, C-10	H_2-2
2	18.6 (t)	1.58 (2H, m)	C-1, C-2	H_2-1, H_2-3
3	41.3 (t)	1.21 (1H, br t, 13.3) 1.46 (1H, br d, 13.1)	C-2, C-19	H_2-2
4	33.1 (s)			
5	50.2 (d)	1.68 (1H, br d, 13.8)	C-4, C-6, C-7, C-10	H_2-6
6	35.2 (t)	2.34 (1H, br t, 13.9) 2.47 (1H, br d, 17.2)	C-5, C-7, C-8, C-9, C-10	H-5
7	200.1 (s)			
8	130.3 (s)			
9	167.2 (s)			
10	40.9 (s)			
11	27.8 (t)	2.31 (2H, m)	C-9, C-10, C-12, C-13,	H_2-12
12	34.6 (t)	2.22 (2H, br d, 7.5)	C-13, C-16	H_2-11, H_2-16
13	141.8 (s)			
14	41.8 (t)	3.08 (2H, br s)	C-12, C-13, C-15, C-16	H_2-16
15	171.6 (s)			
16	114.0 (t)	4.95 (1H, s) 4.98 (1H, s)	C-12, C-13, C-14, C-15	H_2-12, H_2-14
17	11.3 (q)	1.74 (3H, s)	C-7, C-8, C-9	
18	32.5 (q)	0.87 (3H, s)	C-4, C-5, C-10, C-19	
19	21.3 (q)	0.90 (3H, s)	C-3, C-4, C-5, C-18	
20	18.1 (q)	1.07 (3H, s)	C-1, C-5, C-9, C-10	
15-OMe	51.9 (q)	3.68 (3H, s)	C-15	



4.28

4.20 Δ^{13} ***E***
4.21 Δ^{13} Z
4.38 $\Delta^{13,16}$

Scheme 4.11 Base hydrolysis and methylation of ethyl rhinocerotinoate (**4.28**). ***Reagents and conditions***: a. KOH, aq EtOH, Δ, 6 h (95%); b. CH_2N_2, MeOH, 0°C, 1 h (100%).

A search of the literature[100] for the saponification of related β-methyl β-alkyl substituted α,β-unsaturated esters indicated that isomerisation of the double bond through hydroxide catalysed hydrolysis is extremely variable.[200-203] In such systems this transformation has been reported to proceed with no isomerisation,[200] with ***cis***-***trans*** isomerisation,[201,202] as well as with minor α,β- to β,γ-isomerisation of the double bond.[203]

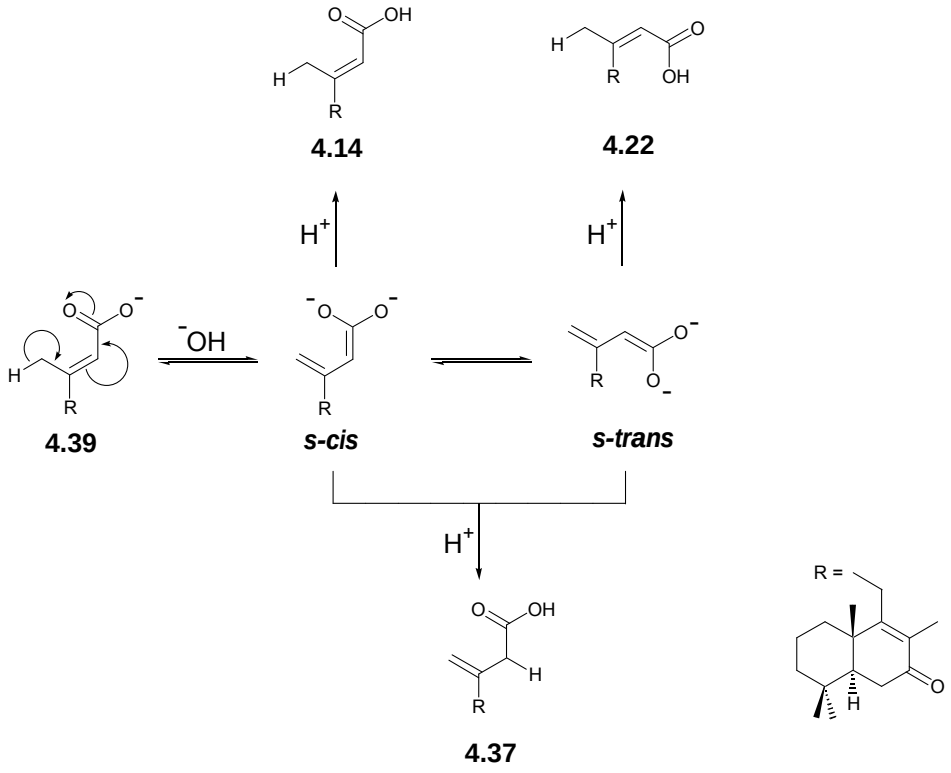
In the saponification of disubstituted α,β-unsaturated esters, ***cis-trans*** isomerisation has been rationalised by the fact that hydroxide is sufficiently nucleophilic to cause isomerisation by the mechanism proposed by Nozaki and Ogg[204] once hydrolysis has occurred.[192] Although this mechanism will adequately explain isomerisation of rhinocerotinoic acid to isorhinocerotinoic acid, it does not account for the formation of the $\Delta^{13,16}$-isomer **4.37** in the hydrolysis of **4.20**. We also consider it highly unlikely that hydroxide attack at the β-carbon of β-disubstituted α,β-unsaturated systems will occur due to the sterically crowded environment occupied by the vinylic carbon atom.

We believe that the observed isomerisation is more likely to occur through a mechanism similar to that proposed in Scheme 4.12. Following cleavage of the ester bond, under basic conditions the rhinocerotinoate anion (**4.39**) formed can lose a proton and move towards equilibrium with the resulting dienolate dianion. Kon and May have proposed that in β-methyl β-alkyl substituted α,β-unsaturated acids, formation of the β,γ-double bond would be expected to occur exclusively in the

alkyl chain due to the comparative instability of the resulting terminal methylene group.[205] However, in their study of γ-alkylation of β-methyl-α,β-unsaturated acids, Katzenellenbogen and Crumrine report that in such species lithium diisopropylamide induced proton abstraction occurs exclusively from the methyl group to yield the kinetically favoured dienolate dianion.[202] In the rhinocerotinoate anion (**4.39**) we consider that the steric crowding around the allylic C-12 inhibits abstraction of a proton from the thermodynamically favoured position resulting in the exclusive formation of the kinetic dienolate dianion, although the participation of an internal base effect acting on the allylic C-16 methyl group in the ***E***-carboxylate anion cannot be discounted.[202]

Once formed, the resulting dienolate can interconvert from the ***s-cis*** to the ***s-trans*** form by rotation about the C-13–C-14 bond.[202] Subsequent quenching of the dienolate dianion by acid would then result in the formation of three possible isomeric products. In reality, the ratio of products obtained will be a consequence of: firstly, the position of the carboxylate-dienolate dianion equilibrium at the instance of quenching; secondly, the relative stabilities of the ***s-cis*** and ***s-trans*** forms of the enolates; and thirdly, the relative affinities of C-13 and C-16 to undergo electrophilic attack by proton.

As the equilibration of α,β- to β,γ-unsaturated β-methyl β-alkyl acids has been found to be a slow process (occurring over a number of days in refluxing 25% potassium hydroxide),[206] it is probable that under our conditions the system will not have attained full equilibrium before the reaction was quenched. Therefore, results obtained for the saponification of ethyl rhinocerotinoate (**4.28**) with the weaker base potassium carbonate, which yielded a mixture of acids containing more of the isomeric acids **4.22** and **4.37** than that obtained from potassium hydroxide hydrolysis, add credence to the proposed mechanism. This observation would be a consequence of the longer reaction times required for complete hydrolysis of the ester by a weaker base, thus allowing the carboxylate anions which have formed longer to convert to, and approach equilibrium with, the dienolate dianions.



Scheme 4.12 Proposed mechanism for the base induced isomerisation of rhinocerotinoic acid (**4.14**).

The proposed mechanism was tested by subjecting the pure methyl esters **4.20**, **4.21** and **4.38** to hydroxide hydrolysis under similar conditions to those used for the **4.28**/**4.29** mixture. In all three cases the product was found to contain the same three isomeric acids (**4.14**, **4.22** and **4.37**) although, as expected, the ratios of the products differed to that obtained from the **4.28**/**4.29** (**4.20** gave 7:2:1, **4.21** gave 2:12:1 and **4.38** gave 10:3:2 respectively).

Although this isomerisation is intriguing and possibly worthy of further mechanistic investigation, the focus of this synthesis was the multi-gram preparation of rhinocerotinoic acid to enable us to undertake our primary goal, ***i.e.*** to examine the possibility of preparing ***Trimusculus*** diterpenes through semi-synthesis from **4.14**. With this in mind, we did not pursue the problem of base induced isomerisation further, but instead examined alternative methods of transforming ethyl ester **4.28** to the desired product **4.14**.

As a consequence of the problems associated with the saponification of the ethyl esters **4.28** and **4.29**, we decided to apply a milder method to the cleavage of the ethyl ester functionality. In this respect we performed trial reactions utilising the chlorotrimethylsilane – sodium iodide system described by Morita ***et al.***[207] and Olah ***et al.***[208] Accordingly, chlorotrimethylsilane was added to a mixture of the esters **4.28** and **4.29** (10:1) and sodium iodide in acetonitrile and the resulting solution heated under reflux for 15 hours. The reaction was quenched with water and the product extracted in the usual manner. Surprisingly, when the crude product was examined by ^{1}H NMR spectroscopy it was evident that it was composed of a complex mixture of products. The mixture was not separated, and the utility of this reagent for the deacylation of **4.28** was not investigated further.

Finally, we decided to try to hydrolyse the 10:1 **4.28**/**4.29** mixture under acidic conditions employing the method outlined by Cativiela ***et al.***[209] This involved heating the esters and hydrochloric acid in tetrahydrofuran at 70 °C. Analysis of the product obtained from this reaction indicated that under these conditions no isomerisation occurred with the desired acid **4.14** and the Δ^{13}-***Z***-isomer **4.22** present in a ratio of 10:1, but these were obtained only in low yield. We considered this a consequence of the prolonged reaction times necessary to facilitate this transformation when using tetrahydrofuran as a solvent. This, coupled with the fact that no isomerism of the C-13 double bond was observed under acidic conditions, prompted us to modify the acid catalysed hydrolysis procedure in an attempt to optimise the yield of **4.14**. Accordingly the reaction was repeated using dimethylsulfoxide as the solvent, and heating at 85 °C for 24 hours gave **4.14** and **4.22** (10:1) as the only acidic products in 62% yield. However, we were still concerned that the prolonged duration of the hydrolysis was diminishing yields of rhinocerotinoic acid as the solution darkened significantly during the reaction.

Our final attempts at optimising this transformation therefore involved the use of acetic acid as the solvent and either hydrochloric or sulfuric acid as the mineral acid. From these experiments it was determined that yields for the hydrochloric acid induced hydrolysis were slightly increased when the reaction was carried out in acetic acid (68%). Remarkably though, the yield of **4.14** was significantly increased when sulfuric acid was used in place of hydrochloric acid. It was found that

heating the 10:1 **4.28**/**4.29** mixture with sulfuric acid in acetic acid at 100°C for 4 hours followed by trituration of the product with hexane and vacuum sublimation afforded isomerically pure (by ^{1}H NMR spectroscopy; see Figure 4.4) rhinocerotinoic acid (**4.14**) in 72% yield.

4.3 Summary and Conclusions

After identifying rhinocerotinoic acid as a promising chiral template for the preparation of 6,7-oxygenated ***Trimusculus*** labdanes, we found that this diterpene acid was not detectable in extracts of samples taken from the local population of ***E. rhinocerotis*** in the Eastern Cape. We therefore decided to synthesise **4.14** from (–)-sclareol as described by Dekker ***et al.***,[162] ***via*** the key intermediate **4.18** prepared by Mangoni and co-workers.[165,173] However, we found a number of discrepancies between our results and those reported involving the dehydration of **4.16**, the allylic oxidation of **4.26**, and the saponification of the ester derivatives **4.28** and **4.29**. The disappointing ***cis***-***trans*** selectivity observed during the phosphorous oxychloride dehydration of **4.27** as employed by Dekker and co-workers and the fact that classical methods of ***cis*** to ***trans*** isomerisation did not show significant enrichment of the desired Δ^{13}-***E***-isomer **4.28**, then prompted us to investigate the possibility of a stereoselective route to **4.14**. This we achieved through HWE reaction of triethyl phosphonoacetate with the key intermediate **4.32** followed by acid catalysed hydrolysis of the resulting ethyl ester. The full stereoselective route employed in the synthesis of **4.14** is summarised in Scheme 4.13 and allowed the preparation of rhinocerotinoic acid in 5 steps and an overall yield of 32%. Direct comparison of this yield with that obtained by Dekker ***et al.*** is not possible as the authors did not quote yields for their synthesis of **4.14**.[162] If, however we assume that they obtained comparable results to those reported by Mangoni and co-workers for the preparation of **4.19**[165,173] and us in the subsequent dehydration and base catalysed hydrolysis steps (we saponified a portion of the 2:1 **4.28**/**4.29** mixture obtained from the dehydration step for comparative purposes), we can calculate that their synthesis would have yielded rhinocerotinoic acid in approximately 12% and isorhinocerotinoic acid in approximately 6% over 6 steps. Our synthetic route therefore constitutes a significant improvement to that described by Dekker ***et al.***

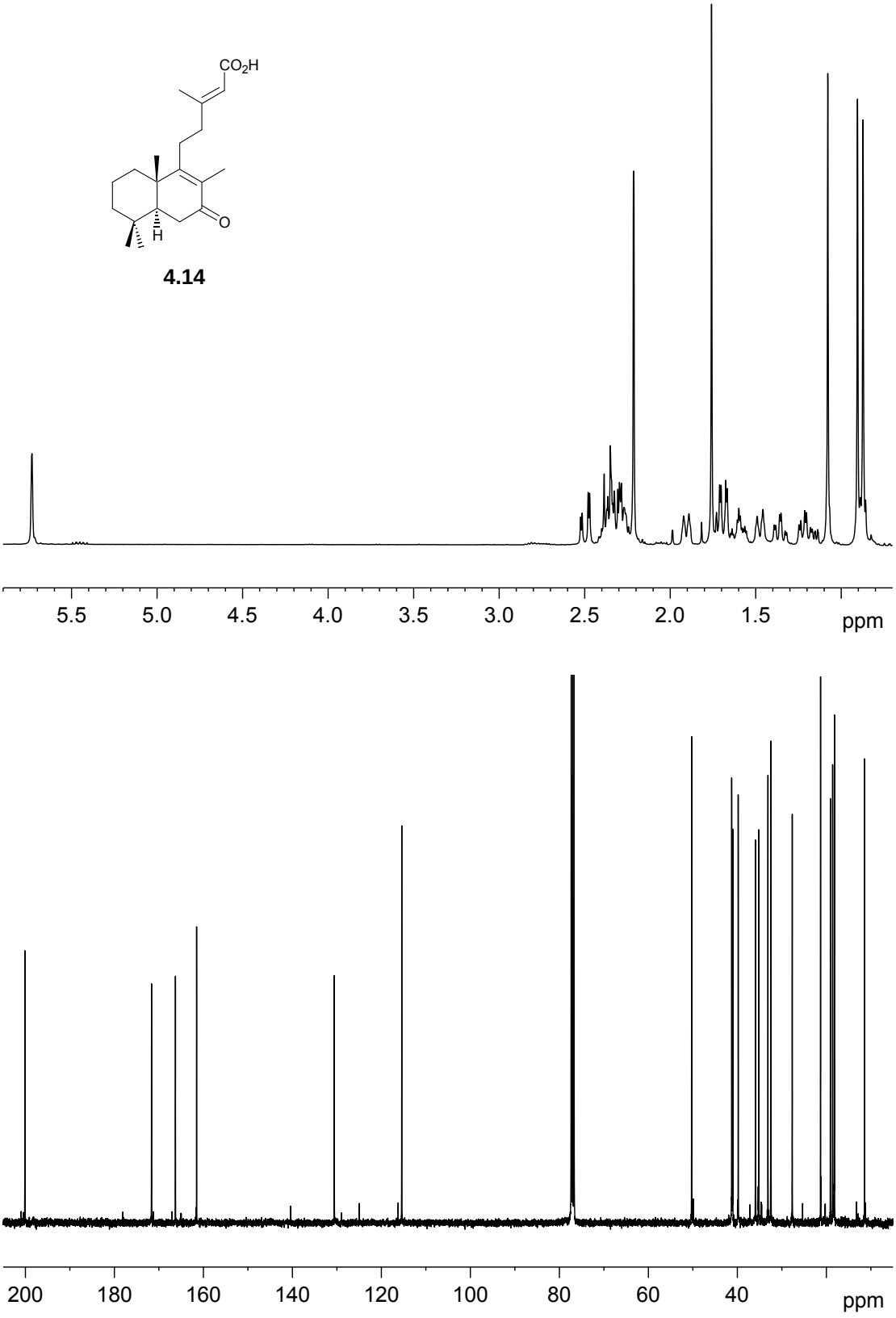
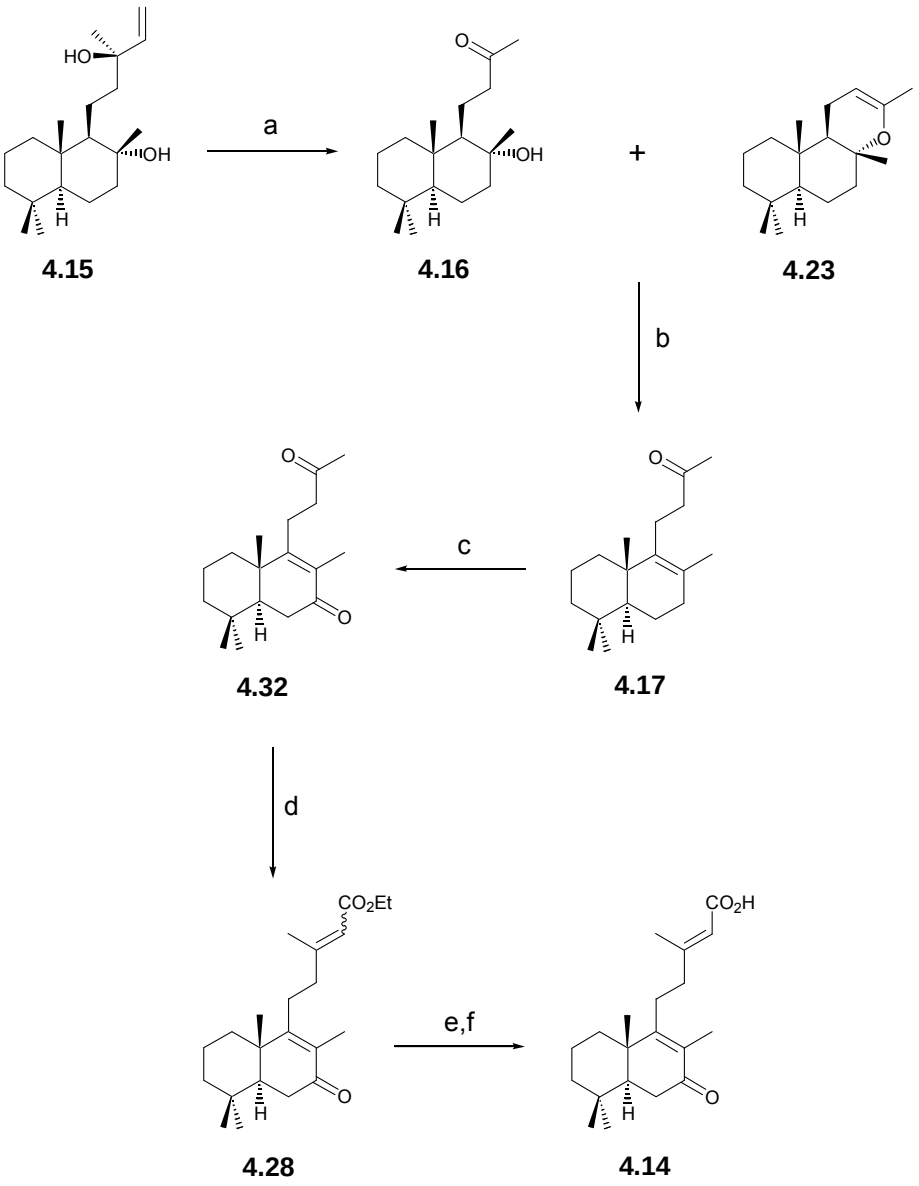


Figure 4.4 ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra obtained for rhinocerotinoic acid (**4.14**; shown inset).



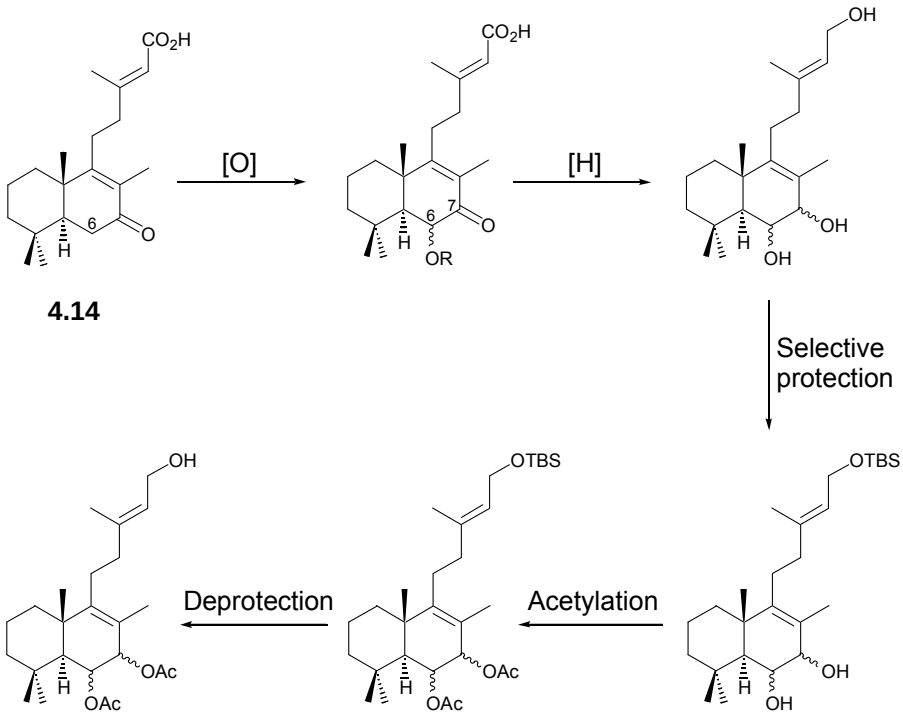
Scheme 4.13 The stereoselective synthesis of (+)-rhinocerotinoic acid (**4.14**) from (–)-sclareol (**4.15**). ***Reagents and conditions***: a. $KMnO_4$, acetone, 15°C, 18 h (70% **4.16**, 7% **4.23**); b. I_2 (0.05 eq), benzene, Δ, 6 h (75%); c. CrO_3-Py_2, CH_2Cl_2, RT, 72 h (81%); d. $(C_2H_5O)_2P(O)CH_2CO_2Et$, NaH, THF, RT, 2 h, (96%, ***E***/***Z*** = 10:1); e. H_2SO_4, AcOH, 100°C, 4 h; f. Trituration from hexane (72% for 2 steps). Overall yield: 32%.

Chapter Five

Investigations Towards the Preparation of 6,7-Dioxygenated Labdanes

5.1 Introduction

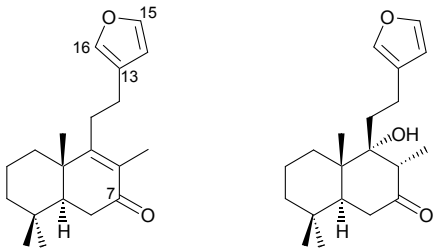
With an efficient, gram scale, method of preparing rhinocerotinoic acid (**4.14**) stereoselectively in hand, we could now turn our attention to transforming this template into a series of 6,7,15-trihydroxylabdanes through synthetic manipulation. Our first objective was the preparation of all of the four possible C-6, C-7 diastereomers of the diacetate **3.10** isolated from ***Trimusculus costatus***, as shown in Scheme 5.1. This would allow us to examine the influence that C-6 and C-7 stereochemistry has on the biological activity of this metabolites in both ecological and pharmaceutical assays. Our second objective was to devise a stereospecific means of synthesising the naturally occurring stereoisomers of the 6- and 6,7-functionalised Δ^{13}-***E Trimusculus*** diterpenes through selective esterification. The work performed towards realising the first goal is described here and the second in Chapter Six.



Scheme 5.1 Proposed strategy for the preparation of diastereomeric 6,7-diacetoxylabda-8,13-dien-15-ols from rhinocerotinoic acid (**4.14**).

At the outset of our synthetic endeavours we envisaged that introducing the C-6, C-7 substituents in the sterically unfavoured 6β,7α ***trans***-diaxial arrangement into **4.14** was going to be

problematic. We therefore initially studied the feasibility of preparing all four diastereomeric 6,7-hydroxylated labdanes from a labda-8,13-dien-7-one skeleton. Although we were now in a position to prepare rhinocerotinoic acid (**4.14**) in gram quantities, we considered it prudent to initially employ an appropriate model compound for this purpose. We considered hispanone (**5.1**) an excellent molecule for this purpose as firstly, it is easily prepared from the natural product hispanolone (**5.2**);[210-212] secondly, the functionality in the A and B rings exactly matches that of **4.14**; thirdly, **5.1** has been relatively well studied from a synthetic perspective (***vide infra***); and fourthly, we were fortunate to have access to an adequate supply of both **5.1** (approximately 1 g) and **5.2** (approximately 2 g),[213] isolated from the Southern African medicinal plant ***Ballota africana***.[214]



5.1 **5.2**

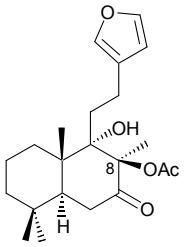
Figure 5.1 The southern African medicinal plant ***Ballota africana***.

5.1.1 Hispanolone and hispanone: isolation and synthetic studies

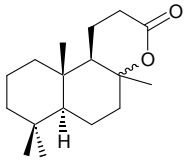
Hispanolone (**5.2**) was first isolated from ***Ballota hispanica*** in 1978[215] and has subsequently been isolated in high yields from other members of the Lamiaceae (formerly Labiateae): ***B.***

andreuzziana,[216] ***B. africana***,[214] ***B. saxatilis*** (subspecies ***saxatilis***),[217] ***Galeopsis angustifolia***[218] and ***Leonuris heterophylus***.[219] Although the isolation of hispanone (**5.1**) as a minor constituent of the aerial parts of ***G. angustifolia***[220] and ***B. saxatilis*** (subspecies ***saxatilis***)[217] has also been reported, Davies-Coleman and Rivett have shown that **5.1** may well be an artefact of the isolation procedure, rather than a genuine natural product.[214]

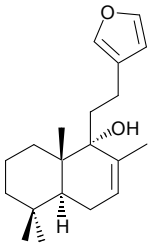
As a consequence of the high yields of hispanolone from natural sources and the ease with which it undergoes dehydration, hispanone has been the employed in a number of semi-synthetic investigations by Rodriguez and co-workers. As such, **5.1** been converted into (+)-galeopsin (**5.3**),[210] (+)-ambrienolide (**5.4**),[211] (–)-8-epiambrienolide (**5.5**)[211] and 15,16-epoxylabda-7,13(16),14-trien-9α-ol (**5.6**)[221] as well as a number of drimane sesquiterpenoids.[212] In addition, the reported fungicidal and antibacterial activity of **5.1**[217] prompted a recent enantiospecific synthesis of this furanolabdane from (***R***)-carvone.[222] Although this was reported as the first total synthesis of **5.1**, Wong and co-workers had previously used hispanone (prepared from the (***S***)-(+)-Wieland-Miescher ketone) as a key intermediate in their enantiospecific synthesis of **5.2**[223,224] ***en route*** to the total synthesis of the natural product prehispanolone (**5.7**) and its hydrogenated analogue 14,15-dihydro-prehispanolone (**5.8**).[224,225]



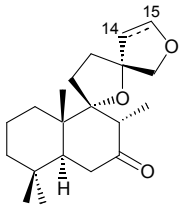
5.3



5.4 8(***R***)
5.5 8(***S***)



5.6



5.7 Δ^{14}
5.8

5.2 Model studies towards the preparation of the 6,7-dihydroxylabda-8,13-dien-15-ols

Although Rodriguez and co-workers reported a 99% yield for the dehydration of **5.2** with thionyl chloride in pyridine,[211] in our hands this transformation only proceeded in moderate yield (62%). For the purposes of preparing sufficient **5.1** for our model studies we therefore employed the

iodine catalysed dehydration procedure described in the previous chapter, which gave **5.1** in 87% yield.

5.2.1 Attempted preparation of the 6,7-dihydroxylated labda-8-enes via *6-acetoxylation and hydride reduction*

The published synthetic transformations performed on **5.1** gave us a good basis upon which to design an appropriate synthetic route to afford 6,7-dihydroxylated derivatives from a 7-oxo-Δ^8-labdane system (Scheme 5.2). It was apparent that C-6 could be oxygenated ***via*** enolate acetoxylation to yield a mixture of 6α- and 6β-isomeric products.[210] Cognisant of the face specificity of hydride reduction in these systems[223,224,226] and from molecular modelling studies, we expected that subsequent lithium aluminium hydride reduction of the acetoxy derivatives **5.9** and **5.10** would yield the 7β-alcohols **5.11** and **5.12** exclusively. The reason for the facial specificity of hydride attack is clearly illustrated by examination of the space filling model of, for example, **5.9** (shown in Figure 5.2): the presence of the α,β-unsaturated ketone significantly distorts the conformation of ring B, causing the C-20 axial methyl group to hinder hydride delivery to the β-face of the C-7 carbonyl group in these systems.

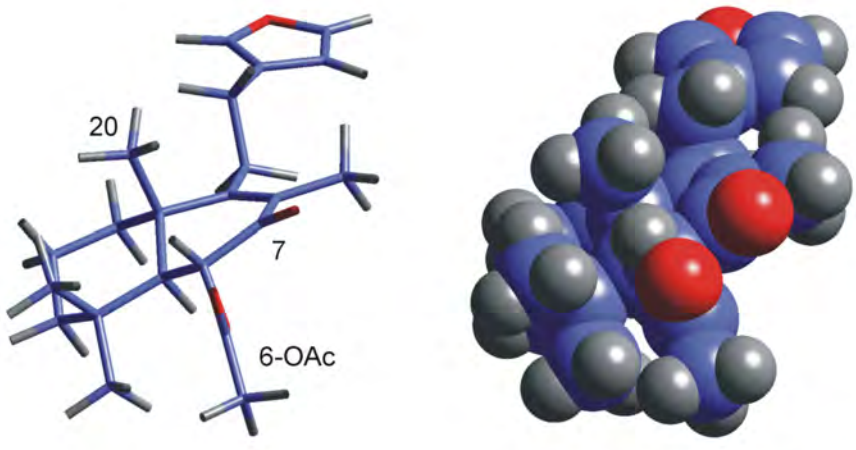
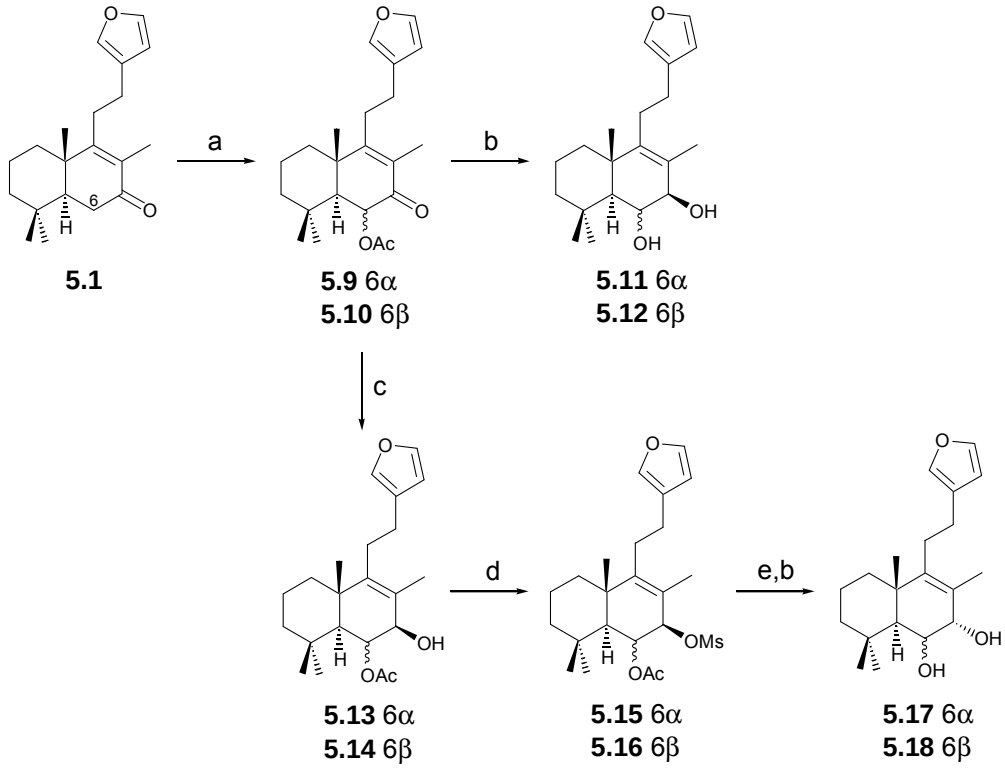


Figure 5.2 Stick and space filling representations of the global energy minimum conformation of 6α-acetoxy-15,16-epoxylabda-8,13(16),14-trien-7-one (**5.9**).



5.1

5.9 6α
5.10 6β

5.11 6α
5.12 6β

5.13 6α
5.14 6β

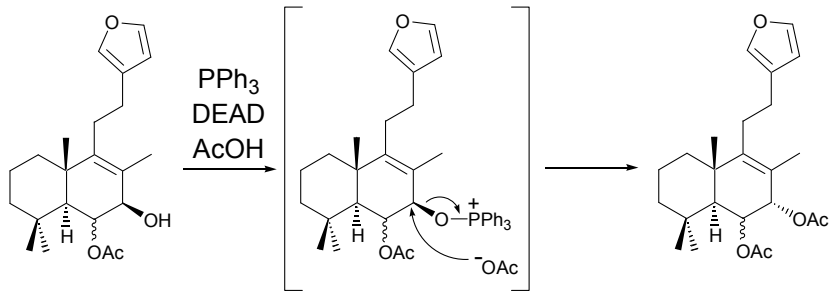
5.15 6α
5.16 6β

5.17 6α
5.18 6β

Scheme 5.2 Proposed route to the four 6,7-diastereomeric 14,15-epoxylabda-8,13(16),14-trien-6,7-diols (**5.11**, **5.12**, **5.17** and **5.18**) from hispanone (**5.1**). ***Reagents and conditions***: a. $Mn(OAc)_3$, benzene, Δ; b. $LiAlH_4$, Et_2O, 0 °C; c. $NaBH_4$, $CeCl_3$, MeOH, 0 °C - RT; d. MsCl, pyridine, DMAP, RT; e. CsOAc, DMAP, toluene, Δ.

The preparation of the 7α-isomers **5.17** and **5.18** would therefore require an alternative synthetic strategy to allow manipulation of the stereochemistry at C-7 subsequent to the C-6 oxygenation step. To this end we envisaged a selective reduction of the C-7 carbonyl using Luche's reagent[227] to give the monoacetylated derivatives **5.13** and **5.14**. With the ester functionality at C-6 acting as a protecting group we would then be in a position to invert the stereochemistry at C-7. Although application of the Mitsunobu reaction would accomplish this transformation in a single step,[228] it was considered that the crowded environment around C-7 in the B-ring of **5.13** and **5.14** would hinder the formation of the required alkoxytriphenylphosphonium intermediates **5.19** and **5.20** (Scheme 5.3). We therefore proposed using a less sterically demanding, two step process and anticipated that formation of the methyl sulfonate esters **5.15** and **5.16** followed by displacement

with cesium acetate[229] would yield the 6α,7α- and 6β,7α-diacetates which could be reduced with $LiAlH_4$ to the desired diols **5.17** and **5.18**.

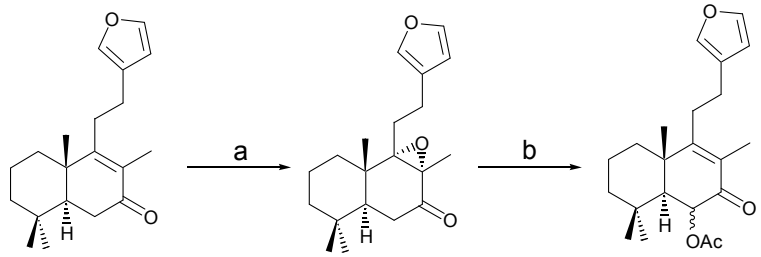


5.13 6α
5.14 6β

5.19 6α
5.20 6β

Scheme 5.3 The mechanism of the Mitsunobu reaction as applied to 6α- and 6β-acetoxy-14,15-epoxylabda-8,13(16),14-trien-7-ol (**5.13** and **5.14**).[228]

Our model study therefore began with the α′-acetoxylation of the α,β-unsaturated ketone **5.1**. Rodriguez and co-workers previously reported achieving this transformation in two steps (19% overall yield) to give a 7:3 mixture of **5.9** and **5.10** (Scheme 5.4).[210] Following this route was, however, undesirable as it entailed epoxidation of the C-8–C-9 double bond under conditions which would cause isomerisation of the C-13–C-14 double bond when applied to rhinocerotinoic acid (see Chapter Four) during our proposed semi-synthesis of the ***Trimusculus*** diterpene analogues.



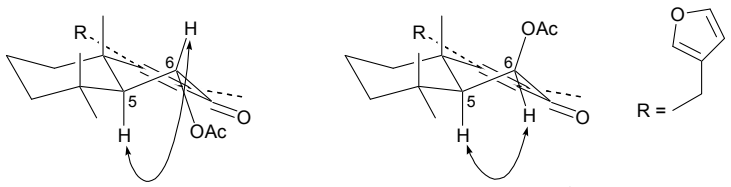
5.1

5.21

5.9 6α
5.10 6β

Scheme 5.4 Rodriguez and co-workers' preparation of 6α- and 6β-acetoxy-14,15-epoxylabda-8,13(16),14-trien-7-one (**5.9** and **5.10**) from hispanone (**5.1**).[210] ***Reagents and conditions***: a. H_2O_2, NaOH, EtOH, 80 °C, 24 h; b. NaOAc, AcOH, 100 °C, 48 h.

It was therefore necessary to find a milder method of preparing **5.9** and **5.10** that would also be suitable to use later with rhinocerotinoic acid. Due to the favourable yields reported for the manganic acetate [manganese (III) acetate] α′-acetoxylation of α,β-enones ***via*** the α-keto radical,[230] we investigated the utility of this reagent for the C-6 acetoxylation of hispanone. Accordingly, a solution of compound **5.1** in anhydrous benzene refluxed for 72 hours to yield a mixture of the desired α′-acetoxy enones **5.9** and **5.10** in 89% isolated yield. The stereochemical preference of the reaction was easily assigned through examination of the product mixture by ^{1}H NMR spectroscopy and comparison of the magnitudes of the H-5–H-6 coupling constants of the two products (Figure 5.3). As expected, the major product was the equatorial acetate **5.9** [δ_H 5.64 (d, ***J*** = 13.4 Hz, H-6)] with the less favoured axial acetate **5.10** accounting for approximately 33% of the product mixture [δ_H 5.79 (d, ***J*** = 3.3 Hz, H-6)]. The diastereomeric acetoxy enones were separated by semi-preparative HPLC before continuing with the reduction step of the proposed synthesis.



Dihedral angle 170^o Dihedral angle 54^o

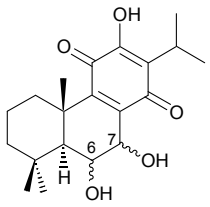
$J_{5,6}$ = 13.4 Hz $J_{5,6}$ = 3.3 Hz

5.9 **5.10**

Figure 5.3 Schematic comparison of the H-5–H-6 dihedral angles (measured from modelled global minimum conformers) and coupling constants in the α′-acetoxy enones **5.9** and **5.10**.

With an efficient means of oxygenating C-6 of hispanone (**5.1**) available, we now turned our attention to the hydride reductions of **5.9** and **5.10**. The individual keto esters were accordingly treated with lithium aluminium hydride and both gave single products in high yield (87% of **5.11** from **5.9** and 99% of **5.12** from **5.10**).

The structure of **5.12** was easily confirmed as 15,16-epoxylabda-8,13(16),14-trien-6β,7β-diol by analysis of the NOESY NMR data obtained for this compound (Figure 5.4) and comparison of the $J_{5,6}$ and $J_{6,7}$ coupling constants with those reported for the four diastereomers of 6,7-dihydroxyroyleanone (**5.24**).[231]



5.24

The C-6, C-7 stereochemistry of **5.11**, however, was more difficult to assign because of overlap of the H-6 and H-7 proton resonances and the complexity of the H-5 multiplet in the ^{1}H NMR spectrum of this compound when recorded in deuterochloroform. The $J_{5,6}$ and $J_{6,7}$ coupling constants were initially estimated from computer simulation of the H-5–H-6–H-7 spin system.[232] The simulated spectrum matched the observed resonances extremely well (Figure 5.5) and the estimated coupling constants ($J_{5,6}$ = 11.5 Hz, $J_{6,7}$ = 8.1 Hz) were in accordance with those reported for the 6α,7β-isomer of **5.24** ($J_{5,6}$ = 12 Hz, $J_{6,7}$ = 8 Hz).[231] However, as the H-5–H-6–H-7 spin system in the 6α,7α-isomer of **5.24** exhibited similar coupling constants ($J_{5,6}$ = 12 Hz, $J_{6,7}$ = 5 Hz)[231] we considered it necessary to confirm our inferences further by repeating the NMR experiments performed on **5.11** in deuterobenzene. In this solvent H-5 appeared as a doublet [δ_H 1.30 (1H, $J_{5,6}$ = 11.3 Hz)] and the H-6 and H-7 resonances, although still overlapping multiplets, were sufficiently separated to allow measurement of $J_{6,7}$ through selective irradiation of H-5 (both H-6 and H-7 collapsed to doublets, $J_{6,7}$ = 7.9 Hz) and unambiguous assignment of the NOESY data obtained for **5.11** (Figure 5.6). The structure of **5.11** was therefore unequivocally established to be 15,16-epoxylabda-8,13(16),14-trien-6α,7β-diol. The full ^{1}H and ^{13}C NMR data (recorded in deuterochloroform) obtained for the two 6,7-dihydroxylated furanolabda-8-ene products **5.11** and **5.12** are presented in Table 5.1.

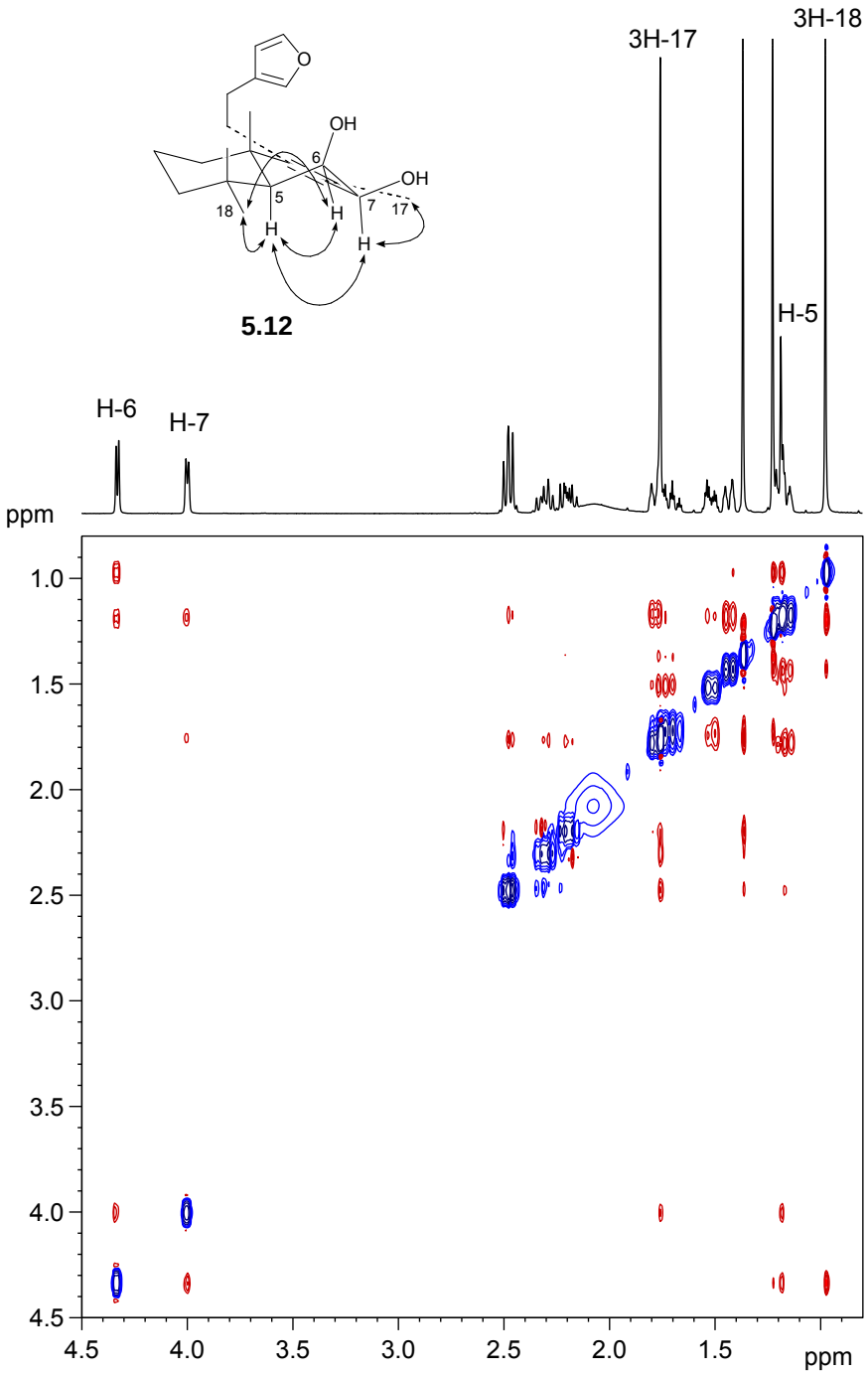


Figure 5.4 The upfield region (F1 and F2 = δ_H 0.8 – 4.5) of the NOESY spectrum ($CDCl_3$, 400 MHz; relaxation time [d1] = 1.00 sec; NOESY mixing time [d8] = 800 msec) obtained for 15,16-epoxylabda-8,13(16),14-trien-6β,7β-diol (**5.12**). Red contours indicate positive peaks, blue contours indicate negative peaks.[233] The accompanying figure shows the key NOESY correlations that confirmed the 6β,7β stereochemistry of **5.12**.

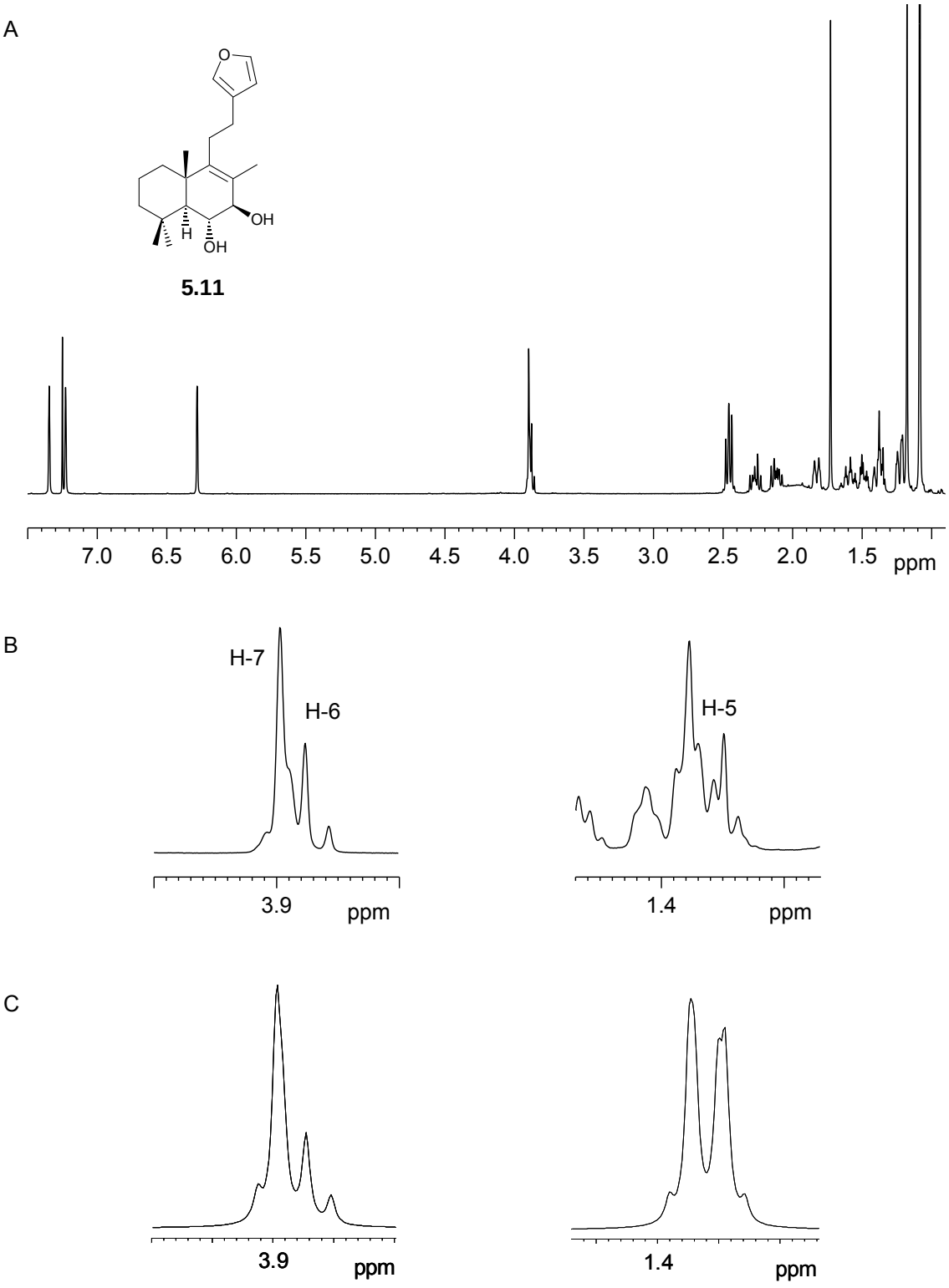


Figure 5.5 A. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of 15,16-epoxylabda-8,13(16),14-trien-6α,7β-diol (**5.11**; shown inset); B. Expansions of the observed H-6–H-7 (δ_H 3.80 – 4.00) and H-5 (δ_H 1.27 – 1.47) multiplets; and C. Similar expansions of the simulated H-6–H-7 and H-5 multiplets ($J_{5,6}$ = 11.5 Hz, $J_{6,7}$ = 8.1 Hz).[232]

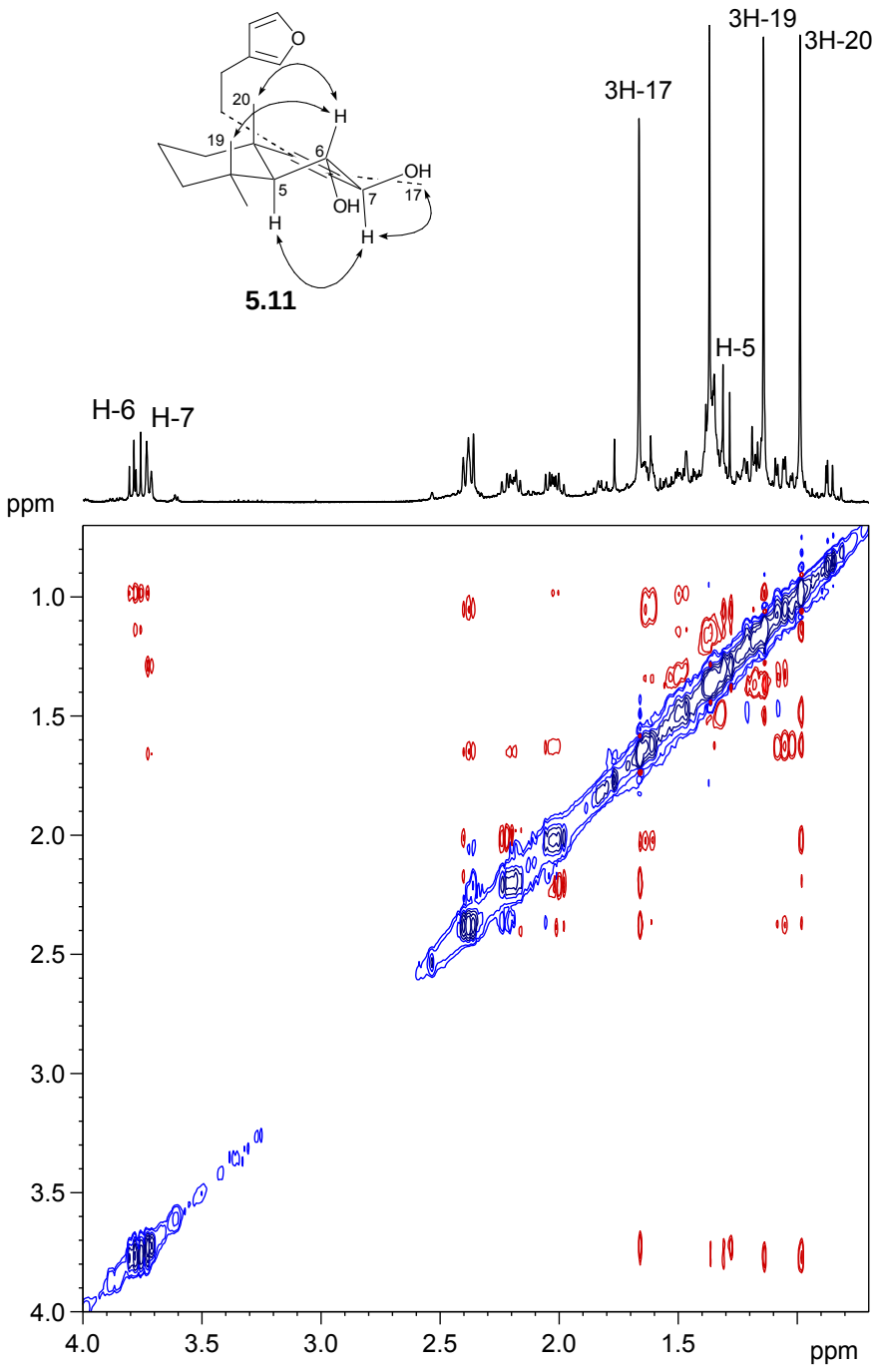


Figure 5.6 The upfield region (F1 and F2 = δ_H 0.7 – 4.0) of the NOESY spectrum (C_6D_6, 400 MHz; relaxation time [d1] = 1.00 sec; NOESY mixing time [d8] = 800 msec) obtained for 15,16-epoxylabda-8,13(16),14-trien-6α,7β-diol (**5.11**). Positive peaks are indicated by red contours and negative peaks by blue contours. The key NOESY correlations used to confirm the 6α,7β stereochemistry of **5.12** are shown in the accompanying figure.

Table 5.1 ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data obtained for the diols **5.11** and **5.12**.

Carbon	6α,7β-diol **5.11**		6β,7β-diol **5.12**	
	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)
1	37.2 (t)	1.24 (1H, br d, 13.1) 1.83 (1H, br d, 12.3)	39.9 (t)	1.18 (1H, m) 1.78 (1H, br d, 12.8)
2	18.7 (t)	1.48 (1H, dt, 13.9, 3.7) 1.58 (1H, tt, 13.3, 3.2)	19.0 (t)	1.52 (1H, dt, 14.2, 3.3) 1.70 (1H, tt, 17.1, 3.4)
3	43.5 (t)	1.23 (1H, br d, 14.4) 1.40 (1H, br d, 14.2)	42.8 (t)	1.17 (1H, m) 1.44 (1H, br d, 12.8)
4	33.4 (s)		33.8 (s)	
5	53.8 (d)	1.36 (1H, m, 11.5)†	53.0 (d)	1.18 (1H, br s)
6	75.1 (d)	3.88 (1H, m, 11.5, 8.1)†	67.9 (d)	4.33 (1H, d, 5.0)
7	80.5 (d)	3.90 (1H, m, 8.1)†	73.3 (d)	4.00 (1H, d, 5.0)
8	126.6 (s)		125.6 (s)	
9	144.0 (s)		143.7 (s)	
10	42.2 (s)		39.9 (s)	
11	28.9 (t)	2.12 (1H, m) 2.27 (1H, m)	28.7 (t)	2.20 (1H, m) 2.31 (1H, m)
12	25.0 (t)	2.46 (2H, m)	25.1 (t)	2.48 (2H, m)
13	125.2 (s)		125.3 (s)	
14	110.7 (d)	6.28 (1H, s)	110.8 (d)	6.29 (1H, s)
15	142.8 (d)	7.35 (1H, s)	142.8 (d)	7.35 (1H, t, 1.4)
16	138.5 (d)	7.23 (1H, s)	138.5 (d)	7.23 (1H, s)
17	14.5 (q)	1.73 (3H, s)	14.9 (q)	1.76 (3H, s)
18	36.2 (q)	1.78 (3H, s)	33.5 (q)	0.98 (3H, s)
19	22.3 (q)	1.09 (3H, s)	24.0 (q)	1.23 (3H, s)
20	21.5 (q)	1.09 (3H, s)	21.9 (q)	1.37 (3H, s)
6-OH		2.25 (1H, br s)		2.08 (1H, br s)
7-OH		1.73 (1H, br s)		2.08 (1H, br s)

† Coupling constants estimated by computer simulation.[232]

Having assigned the stereochemistry of both diols, we had firmly established that hydride attack of both **5.9** and **5.10** occurs exclusively from the α-face at C-7 as expected. With a method of preparing **5.11** and **5.12** identified, we now turned our attention to the synthesis of the 6α,7α- and 6β,7α-diols **5.17** and **5.18** (the latter also being the objective of our stereospecific ***Trimusculus*** metabolite synthesis).

The attempted preparation of the 7α-isomeric diols **5.17** and **5.18** entailed the selective reduction of **5.9** and **5.10** with Luche's reagent (sodium borohydride – cerium chloride heptahydrate) in methanol at room temperature.[227] Using thin layer chromatography to follow the course of the reaction, we observed that the reduction of **5.9** was complete after 8 hours while the reduction of **5.10** was complete in only 10 minutes. These observations implied that the 6α-acetoxy moiety significantly hinders hydride attack of the C-7 carbonyl group of **5.9** (Figure 5.7).

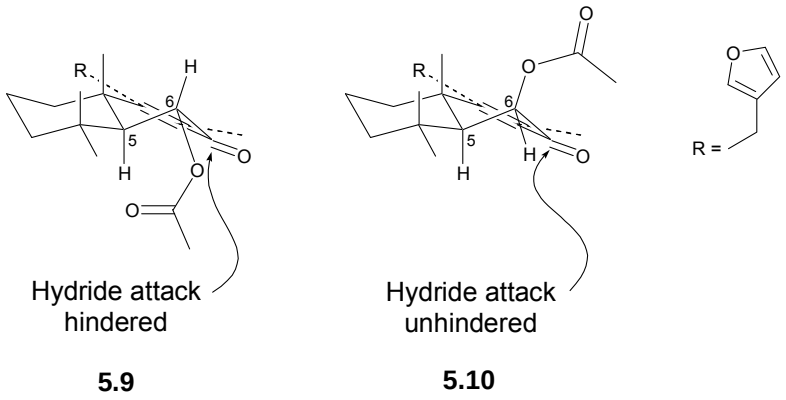
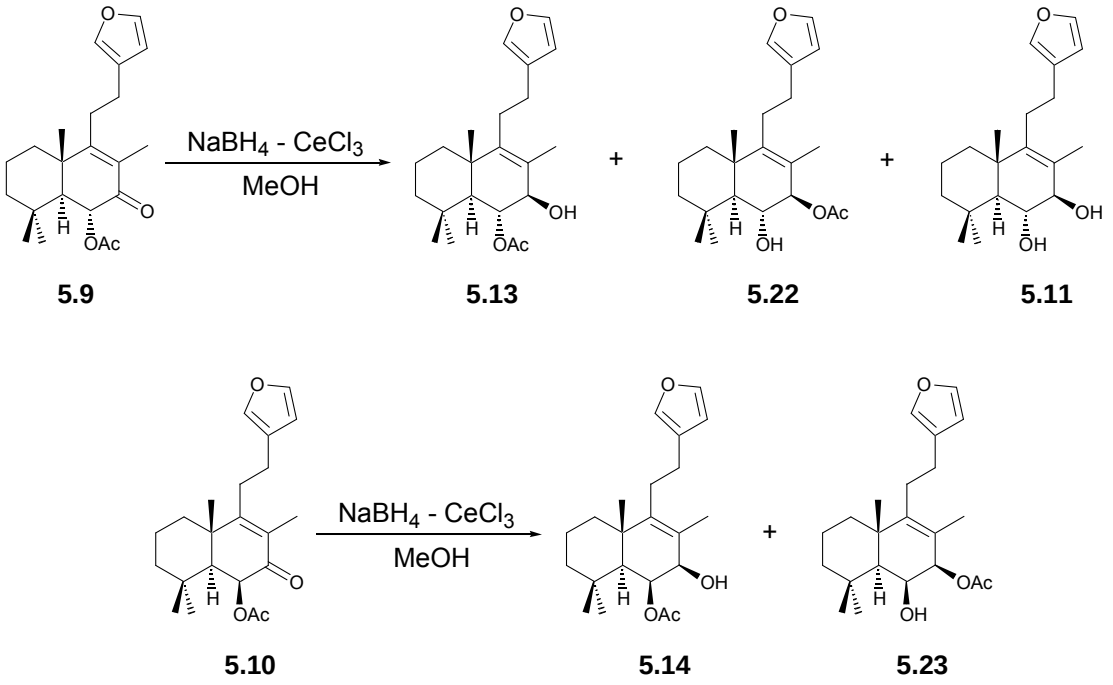


Figure 5.7 Schematic illustration of the possible effect that C-6 functionalisation has on the hydride reduction at C-7 of 6α- and 6β-acetoxy-15,16-epoxylabda-8,13(16),14-trien-7-one (**5.9** and **5.10**).

Surprisingly, examination of the products obtained from the reduction indicated that both acetoxy enones gave a number of products, which had to be separated by normal phase HPLC to allow unambiguous identification. Careful analysis of the 1D and 2D NMR data obtained for the purified products showed that the sodium borohydride reduction of **5.9** gave, in addition to the expected product 6α-acetoxy-15,16-epoxylabda-8,13(16),14-trien-7β-ol (**5.13**) in 32% yield, the isomeric

hydroxy ester **5.22** (24%), the diol **5.11** (10%) and unreacted starting material (21%). Similarly, **5.10** yielded a mixture of the isomeric hydroxy esters **5.14** (56%) and **5.23** (38%). These results are summarised in Scheme 5.5 and the NMR data obtained for **5.13** and **5.22**, and **5.14** and **5.23** are compared in Tables 5.2 and 5.3.



5.9 **5.13** **5.22** **5.11**

5.10 **5.14** **5.23**

Scheme 5.5 Summary of the products obtained from sodium borohydride reduction of 6α- and 6β-acetoxy-15,16-epoxylabda-8,13(16),14-trein-7-one (**5.9** and **5.10**).

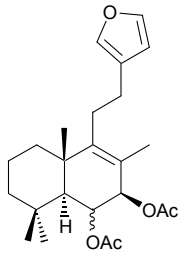
The structures of **5.13**, **5.14**, **5.22** and **5.23** were unequivocally confirmed by acetylation as follows. When treated with acetic anhydride in pyridine, both **5.13** and **5.22** gave 6α,7β-diacetoxy-15,16-epoxy-labda-8,13(16),14-triene (**5.25**). Similarly, **5.14** and **5.23** both gave 6β,7β-diacetoxy-15,16-epoxy-labda-8,13(16),14-triene (**5.26**). The diacetates **5.25** and **5.26** were found to be identical ($[\alpha]_D$, NMR, IR) with the products readily prepared ***via*** acetylation of the diols **5.11** and **5.12**. At this stage it should also be noted that, due to the ease of preparation of the diacetylated derivatives from **5.11** and **5.12**, it would be unlikely that one of the alcohol functionalities in these 6,7-dihydroxylated labdane systems could be selectively manipulated by protection or esterification of the second hydroxyl group.

Table 5.2 Comparison of the ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data obtained for hydroxy esters **5.13** and **5.22**.

Carbon	**5.13** (6α-acetoxy, 7β-hydroxy)		**5.22** (6α-hydroxy, 7β-acetoxy)	
	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)
1	37.2 (t)	1.28 (1H, m) 1.87 (1H, br d, 12.8)	37.1 (t)	1.28 (1H, m) 1.84 (1H, br d, 11.1)
2	18.5 (t)	1.51 (1H, m) 1.57 (1H, m)	18.6 (t)	1.51 (2H, m)
3	43.5 (t)	1.23 (1H, m) 1.40 (1H, br d, 13.5)	43.6 (t)	1.23 (1H, m) 1.39 (1H, br d, 13.6)
4	33.1 (s)		33.6 (s)	
5	52.8 (d)	1.58 (1H, d, 12.1)	54.6 (d)	1.42 (1H, d, 11.9)
6	78.5 (d)	5.24 (1H, dd, 12.1, 6.6)	73.4 (d)	4.04 (1H, m)
7	78.5 (d)	3.98 (1H, br t, 5.1)	83.9 (d)	5.25 (1H, d, 6.7)
8	127.4 (s)		123.6 (s)	
9	144.2 (s)		147.5 (s)	
10	41.7 (s)		41.3 (s)	
11	28.8 (t)	2.18 (1H, m) 2.30 (1H, m)	28.9 (t)	2.17 (1H, m) 2.30 (1H, m)
12	25.1 (t)	2.47 (2H, m)	25.0 (t)	2.46 (2H, m)
13	125.1 (s)		125.0 (s)	
14	110.7 (d)	6.28 (1H, br s)	110.7 (d)	6.28 (1H, d, 0.7)
15	142.8 (d)	7.35 (1H, t, 1.5)	142.8 (d)	7.35 (1H, t, 1.6)
16	138.5 (d)	7.23 (1H, br s)	138.5 (d)	7.28 (1H, d, 0.6)
17	15.2 (q)	1.73 (3H, s)	15.2 (q)	1.35 (3H, s)
18	36.0 (q)	1.06 (3H, s)	36.3 (q)	1.17 (3H, s)
19	22.2 (q)	0.93 (3H, s)	22.0 (s)	1.06 (3H, s)
20	21.9 (q)	1.15 (3H, s)	21.6 (q)	1.08 (3H, s)
OAc	172.6 (s)		173.2 (s)	
	21.8 (q)	2.14 (3H, s)	21.2 (q)	2.15 (3H, s)
OH		2.56 (1H, br d, 5.1)		2.38 (1H, d, 5.3)

Table 5.3 Comparison of the ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data obtained for hydroxy esters **5.14** and **5.23**.

Carbon	**5.14** (6β-acetoxy, 7β-hydroxy)		**5.23** (6β-hydroxy, 7β-acetoxy)	
	δ_C ppm (mult.)	δ_H ppm (mult., int., ***J***/Hz)	δ_C ppm (mult.)	δ_H ppm (mult., int., ***J***/Hz)
1	39.8 (t)	1.19 (1H, m) 1.83 (1H, m)	39.6 (t)	1.18 (1H, br t, 14.2) 1.80 (1H, br d, 14.2)
2	18.9 (t)	1.53 (1H, dt, 14.0, 3.7) 1.68 (1H, tt, 13.7, 3.4)	19.0 (t)	1.53 (1H, m) 1.76 (1H, m)
3	43.1 (t)	1.18 (1H, m) 1.43 (1H, br t, 13.3)	42.7 (t)	1.12 (1H, br t, 13.5) 1.44 (1H, br d, 15.2)
4	33.6 (s)		33.8 (s)	
5	51.5 (d)	1.34 (1H, br s)	52.8 (d)	1.24 (1H, br s)
6	69.4 (d)	5.68 (1H, d, 5.3)	66.0 (d)	4.41 (1H, br d, 4.7)
7	72.3 (d)	4.19 (1H, br t, 4.5)	76.3 (d)	5.40 (1H, br d, 4.7)
8	125.6 (s)		121.7 (s)	
9	143.0 (s)		145.8 (s)	
10	40.1 (s)		40.0 (s)	
11	28.7 (t)	2.21 (1H, m) 2.32 (1H, m)	28.8 (t)	2.24 (1H, m) 2.33 (1H, m)
12	25.1 (t)	2.49 (2H, m)	25.1 (t)	2.49 (2H, m)
13	125.3 (s)		125.2 (s)	
14	110.7 (d)	6.29 (1H, br s)	110.7 (d)	6.29 (1H, br s)
15	142.8 (d)	7.35 (1H, s)	142.8 (d)	7.35 (1H, t, 1.5)
16	138.5 (d)	7.24 (1H, s)	138.5 (d)	7.24 (1H, br s)
17	14.6 (q)	1.75 (3H, s)	14.6 (q)	1.60 (3H, s)
18	33.3 (q)	1.00 (3H, s)	33.6 (q)	0.97 (3H, s)
19	23.1 (q)	0.99 (3H, s)	23.7 (q)	1.22 (3H, s)
20	21.5 (q)	1.34 (3H, s)	21.6 (q)	1.40 (3H, s)
OAc	172.1 (s)		170.5 (s)	
	21.4 (q)	2.09 (3H, s)	21.1 (q)	2.15 (3H, s)
OH		1.85 (1H, br d, 4.6)		Not observed



5.25 6α
5.26 6β

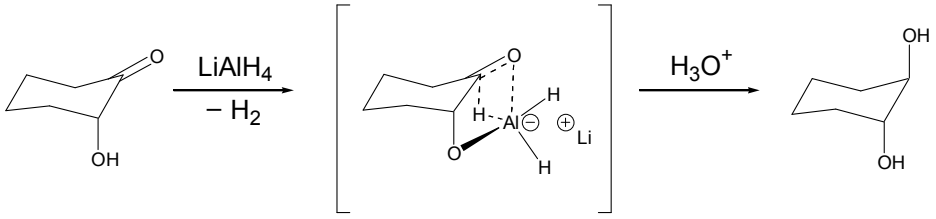
It was therefore obvious that the hydroxy esters obtained from the selective reduction of **5.9** and **5.10** undergo ready transesterification, a process which could have been facilitated by the basic conditions produced on quenching the reaction mixture. However, transesterification was also observed to occur to some extent on standing in deuterochloroform. It was initially considered that the driving force for the transesterification was a reduction in steric strain in the conversion of the C-6 esterified diols to the corresponding C-7 esterified isomers. This hypothesis was discounted by the results of a simple experiment in which each of the four pure isomeric hydroxy esters were left in deuterochloroform for 24 hours at 0 °C. Examination of the solutions by ^{1}H NMR spectroscopy indicated that the isomerisation was in fact an equilibrium process; each of the four hydroxy esters gave rise to mixtures containing the corresponding transesterified products under these conditions.

As a consequence of the unexpected facile transesterification of **5.13** and **5.14**, the proposed route for the preparation of the 6α,7α- and 6β,7α-diols **5.17** and **5.18** ***via*** α′-acetoxylation and selective reduction of hispanone (**5.1**) was not pursued further. Accordingly, an alternative method of synthesising these diastereomers had to be devised.

5.2.2 Attempted preparation of the 6,7-dihydroxylated labda-8-enes via *C-6 hydroxyl directed hydride reduction*

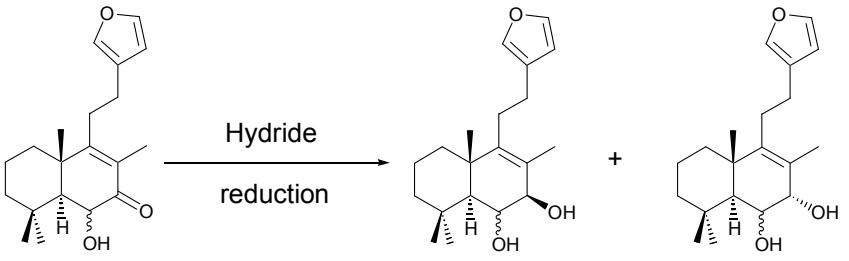
Recently, Senda ***et al.*** reported that a significant directive effect was observed in the lithium aluminium hydride, zinc borohydride or sodium borohydride reduction of 2-hydroxy-

cyclohexanone derivatives in ethereal solvents.[234] The observed stereoselectivity was explained in terms of an initial reaction between the active hydroxyl hydrogen and the hydride reagent to form a complex with evolution of hydrogen gas. Subsequent intramolecular hydride attack results in hydride being delivered from the same side of the ring as the hydroxyl group (illustrated in Scheme 5.6 with lithium aluminium hydride as an example).



Scheme 5.6 The mechanism for the hydroxyl directed complex metal hydride reduction of 2-hydroxycyclohexanone as proposed by Senda ***et al.***[234]

It has already been shown that in 7-oxo-labda-8-ene systems the conformation of the B-ring and the steric bulk of the C-20 methyl group prevent hydride delivery to the β-face of the C-7 carbonyl (***vide supra***). We therefore undertook to investigate the potential of using the directive effect of a 6-hydroxyl group in conjunction with some common co-ordinating hydride reagents to alter the stereochemistry of the reduction and hence prepare the 6α,7α- and 6β,7α-diols **5.17** and **5.18** as well as the previously prepared 6α,7β- and 6β,7β-diols **5.11** and **5.12** as shown in Scheme 5.7.



5.27 6α
5.28 6β

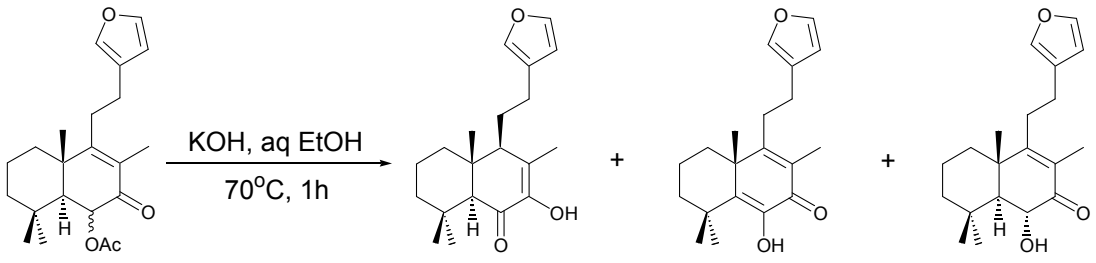
5.11 6α
5.12 6β

5.17 6α
5.18 6β

Scheme 5.7 Proposed preparation of **5.17** and **5.18** through hydroxyl directed hydride reduction.

The first step required in this route is obviously the preparation of 6α- and 6β-hydroxy-15,16-epoxylanbda-8,13(16),14-trien-7-one (**5.27** and **5.28**). As the majority of procedures for the α-hydroxylation of ketones give only moderate yields when applied to α,β-unsaturated systems,[235] we initially considered preparing **5.27** and **5.28** from the acetoxy enones **5.9** and **5.10** for the purpose of trial hydroxyl directed reductions. If the reductions gave promising results, we would then investigate methods of preparing the α′-hydroxylated derivatives direct from the α,β-unsaturated ketone **5.1**.

Attempts to hydrolyse **5.9** and **5.10** under acid conditions similar to those employed in the synthesis of rhinocerotinoic acid (**4.1**) were unsuccessful, giving a complex mixture of products possibly due to cleavage of the furan ring. Saponification of **5.9** and **5.10** was similarly unsuccessful, but gave an interesting mixture of products. Separate treatment of both isomers with potassium hydroxide in aqueous ethanol at 70 °C gave, after normal phase HPLC, dihydro-7-hydroxyhedychenone (**5.29**),[236] 6-hydroxy-15,16-epoxylabda-5,8,13(16),14-tetraen-7-one (**5.30**) and the acyloin (α-hydroxy ketone) **5.27** in 26%, 30% and 4% yield respectively from **5.9** and 38%, 39% and 6% yield respectively from **5.10** (Scheme 5.8).



5.9 6α
5.10 6β

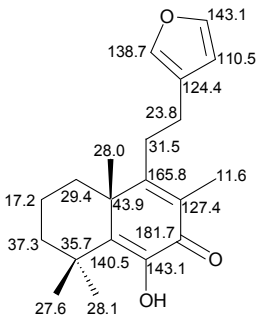
5.29 **5.30** **5.27**

Scheme 5.8 Saponification of 6α- and 6β-acetoxy-15,16-epoxylabda-8,13(16),14-trien-7-one (**5.9** and **5.10**).

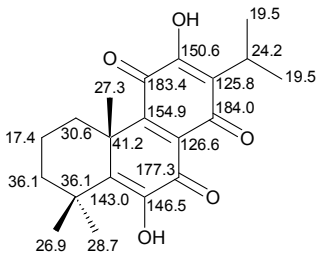
The structure of **5.29** ($[\alpha]_D$ = +2°) was deduced by careful analysis of its 2D NMR spectroscopic data (HMQC, HMBC, COSY and NOESY) and confirmed by HRFABMS (***m*/*z*** 317.2116 [$(M+1)^+$],

Δmmu −0.1). Although **5.29** ($[\alpha]_D$ = +0.7°) has previously been prepared by hydrogenation of the natural product 7-hydroxyhedychenone,[236] only limited 60 MHz ^{1}H NMR data for this compound was reported. The fully assigned NMR data obtained for **5.29** by us is compared to the selected ^{1}H NMR data reported by Sharma ***et al.*** in Table 5.4 and, except for discrepancies in the chemical shifts reported for H_2-11 and H_2-12 (possibly due to the quality of the 60 MHz NMR data obtained by Sharma ***et al.***), there is reasonable agreement between the literature data and those acquired for synthetic **5.29**.

The structure elucidation of **5.30** was rather more challenging, as the B-ring in this compound was composed entirely quaternary or fully substituted sp^2 hybridised carbon atoms. High resolution mass spectrometry indicated that **5.30** possessed a molecular formula of $C_{20}H_{26}O_3$ (HRFABMS; ***m/z*** 315.1960 [(M+1)$^+$]) and the ^{13}C NMR data for the A- and B-rings of the proposed structure compared well with that reported for the natural product coleon-U-quinone (**5.31**; Figure 5.8).[237] Analysis of the heteronuclear 2D NMR data obtained for **5.30** then disclosed a number of key HMBC correlations (from H-1a to C-5, H-1b to C-9, H_2-11 to C-8 and C-9, H_3-17 to C-7, C-8 and C-9, and 6-OH to C-5, C-6 and C-7; see Figure 5.9) that confirmed the configuration of the B-ring and established the structure of **5.30** as 6-hydroxy-15,16-epoxylabda-5,8,13(16),14-tetraen-7-one.



5.30



5.31

Figure 5.8 Comparison of the ^{13}C NMR ($CDCl_3$, 100 MHz) data obtained for compound **5.30** with that reported ($CDCl_3$, field not specified) for coleon-U-quinone (**5.31**) isolated from the leaf-glands of ***Plectranthus argentatus***.[237]

Table 5.4 ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) data obtained for compound **5.29**. The selected ^{1}H (60 MHz, $CDCl_3$) data reported by Sharma ***et al.***[236] are given for comparative purposes [Note: the chemical shift values of the literature data are quoted in both ppm and τ (as originally published)].

Carbon	**5.29**		Literature data[236]	
	δ_C ppm (mult.)	δ_H ppm (mult., int., ***J***/Hz)	δ_H ppm	τ (mult., int., ***J***/Hz)
1	38.4 (t)	1.15 (1H, m) 1.81 (1H, d, 12.9)		
2	18.0 (t)	1.48 (2H, m)		
3	43.0 (t)	1.18 (1H, m) 1.40 (1H, d, 15)		
4	32.5 (s)			
5	62.4 (d)	2.10 (1H, s)	2.11	7.89 (2H, br s)
6	195.2 (s)			
7	143.9 (s)			
8	126.4 (s)			
9	53.9 (d)	2.17 (1H, br d, 6.9)	2.15	7.85 (1H, s)
10	43.6 (s)			
11	28.2 (t)	1.68 (2H, m)	3.37	6.63 (1H, dq)
12	27.1 (t)	2.49 (1H, m) 2.65 (1H, m)	3.80	6.20 (2H, m)
13	124.5 (s)			
14	110.9 (d)	6.29 (1H, s)	6.28	3.72 (1H, m)
15	143.0 (d)	7.37 (1H, s)		
16	138.9 (d)	7.25 (1H, s)	7.33	2.67 (2H, m)
17	13.58 (q)	1.92 (3H, s)	1.90	8.10 (3H, d, 1.5)
18	33.4 (q)	1.17 (3H, s)	1.20	8.80 (3H, s)
19	21.5 (q)	1.13 (3H, s)	1.13	8.87 (3H, s)
20	14.8 (q)	0.84 (3H, s)	0.83	9.17 (3H,s)
7-OH		6.22 (1H, s)		

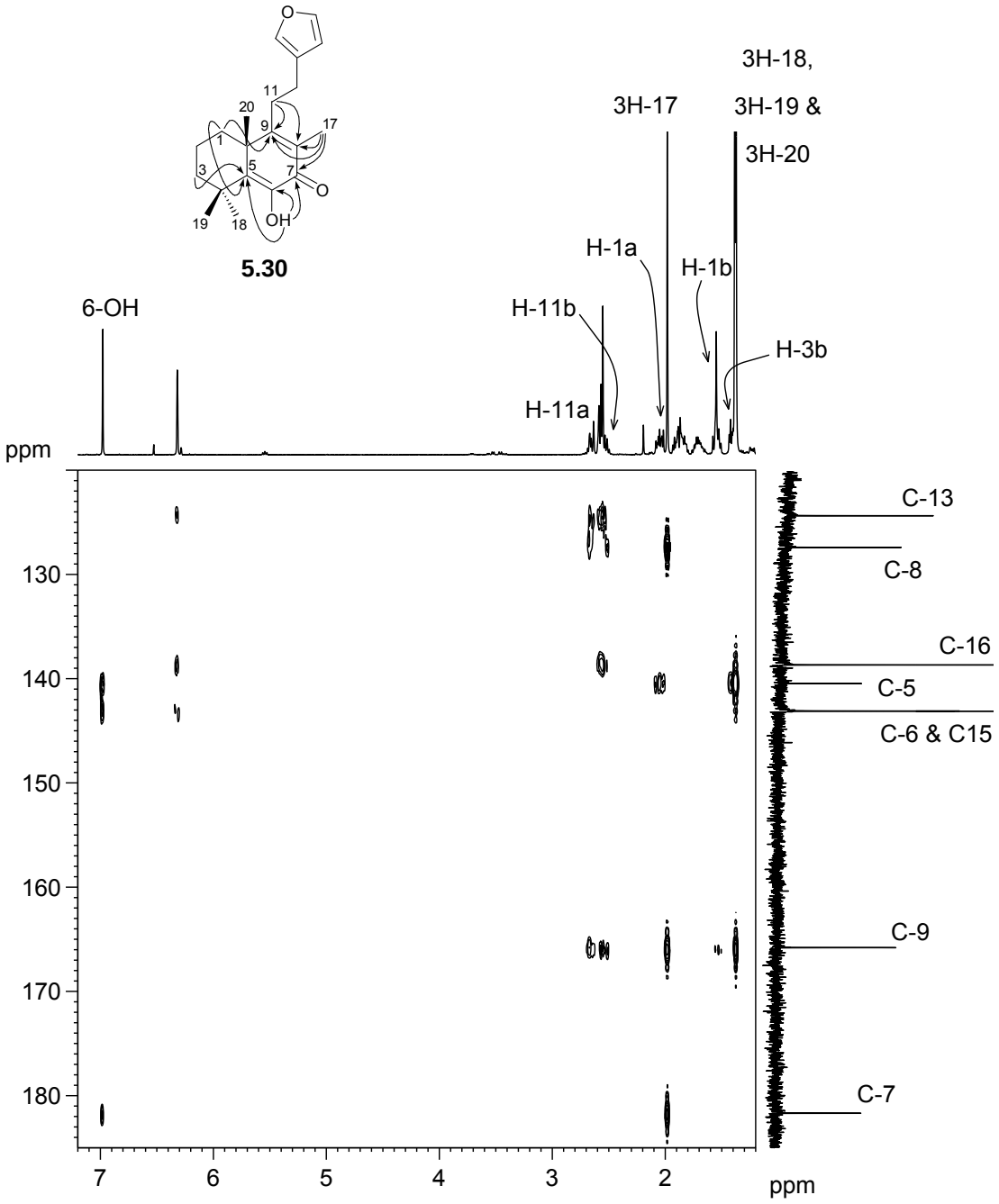
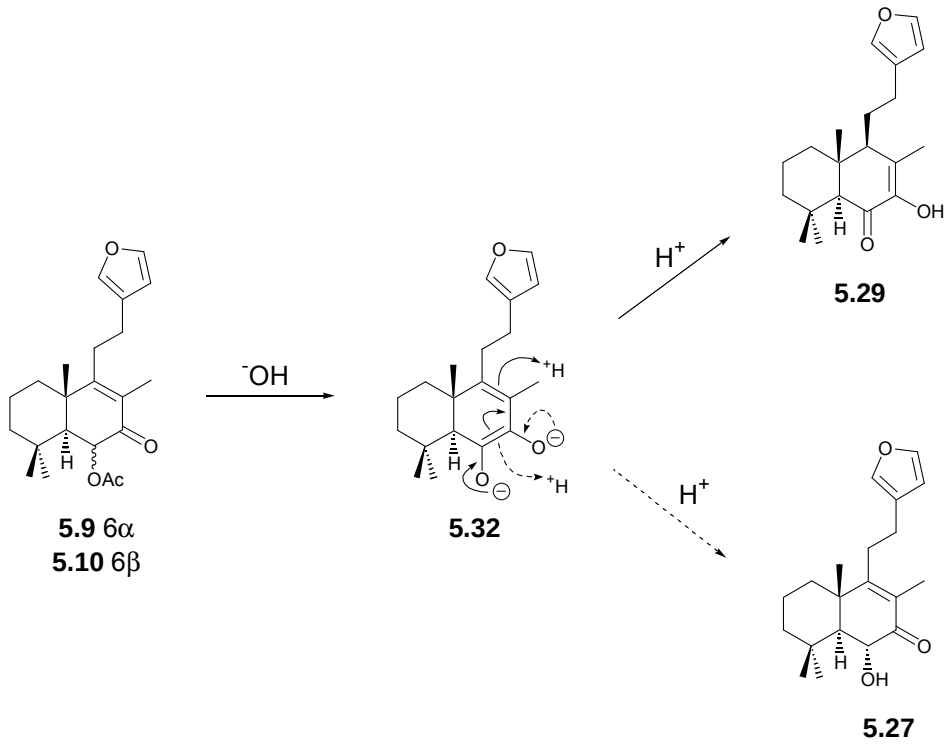


Figure 5.9 A region of the HMBC spectrum ($CDCl_3$, 400MHz; delay for evolution of long range couplings [d6] = 65 msec; F1 = δ_C 120 – 185; F2 = δ_H 1.2 – 7.2) obtained for 6-hydroxy-15,16-epoxylabda-5,8,13(16),14-tetraen-7-one (**5.30**) showing the key correlations that confirmed the configuration of the B-ring (see accompanying structure).

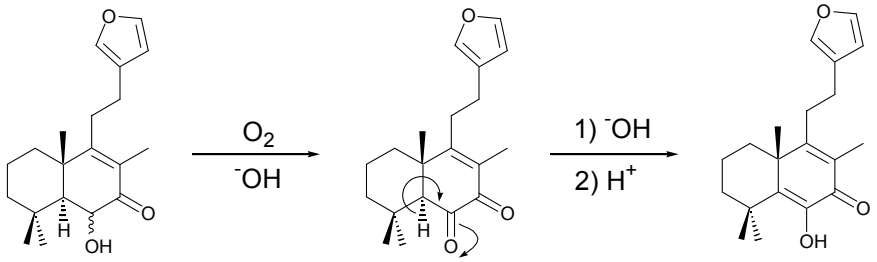
The formation of **5.29** and **5.27** can be easily explained by the formation of the enolate dianion **5.32** under basic conditions. On addition of acid, the enolate could decompose by carbonyl formation at C-6 or C-7 as shown in Scheme 5.9. The absence of the 6β-hydroxy enone **5.28** in the product mixture would be a consequence of the equatorial hydroxyl being favoured through hydrogen bonding between the C-6 hydroxyl and the C-7 carbonyl in the polar solvent system.



Scheme 5.9 Proposed mechanism for the formation of **5.27** (broken arrows) and **5.29** (solid arrows) from the α-hydroxy enones **5.9** and **5.10** through saponification.

The formation of **5.30**, however, is more difficult to rationalise, as this transformation involves oxidation of the substrate during saponification. As the hydrolysis was not conducted under an atmosphere of nitrogen, it is possible that this transformation involves molecular oxygen through some autooxidative process to yield the diketone **5.33**. This supposition may be substantiated to some degree by the observations that ketones may undergo facile α-oxidation in aerated ethanolic potassium hydroxide solution[238] and secondary alcohols can autooxidise ***via*** the hydroperoxy alcohol, which is then easily hydrolysed to the respective ketone.[239] Subsequent

base catalysed tautomerism of **5.33** followed by quenching with acid would yield the diosphenol **5.30** as shown in Scheme 5.10.



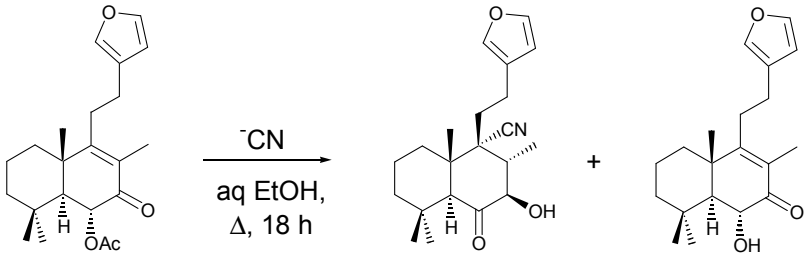
5.27 6α
5.28 6β

5.33

5.30

Scheme 5.10 Tentative mechanism proposed for the formation of diosphenol **5.30** from the hydroxy ketones **5.27** and **5.28**.

It was evident that if the desired ketols **5.27** and **5.28** were to be obtained from **5.9** and **5.10**, a milder method of accomplishing this transformation would be required. We therefore attempted the potassium cyanide catalysed transesterification procedure of Mori ***et al.***[240] Unfortunately, when performed on **5.9** this only yielded the desired product **5.27** in low yield (15%), with the major product (**5.34**) being isolated in 43% yield (Scheme 5.11).



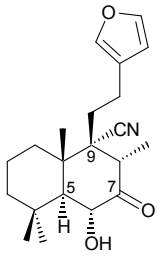
5.9

5.34

5.27

Scheme 5.11 The consequence of applying Mori's potassium cyanide catalysed transesterification procedure[240] to α′-acetoxy enone **5.9**.

The structure of nitrile **5.34** was elucidated by analysis of this compound's 1D and 2D NMR data (reproduced in Table 5.5). Once it was established that in addition to transesterification, a Michael-type conjugate addition involving cyanide had occurred (ν_{max} 2228 cm^{-1}; HRFABMS indicated a molecular formula of $C_{21}H_{29}NO_3$), we initially expected the major product to have the more obvious structure represented by **5.41**.



5.41

However, on closer examination of the NMR data, it was evident that rather than appearing as a doublet through ***trans***-diaxial coupling with H-6, the H-5 resonance was a sharp singlet [δ_H 2.73 (1H)]. Additionally, the H-8 resonance appeared as a double quartet [δ_H 1.87 (1H, ***J*** = 10.8, 6.5 Hz] through coupling to H_3-17 [δ_H 1.42 (3H, d, ***J*** = 6.5 Hz)] and ***trans***-diaxial coupling to the carbinol proton at H-7 [δ_H 3.92 (1H, d, ***J*** = 10.8 Hz)]. These observations, confirmed by examination of the HMBC and COSY data, indicated that the product had structure **5.34** rather than **5.41**.

The stereochemistry of nitrile **5.34** was confirmed from 2D NOESY correlations as illustrated in Figure 5.10. A strong nuclear Overhauser enhancement between H-5 and H-7 indicated that the hydroxyl at C-7 had β-stereochemistry and the absence of any nuclear Overhauser enhancement correlation between H-7 and H-8 confirmed the trans-diaxial arrangement of these protons suggested by the large $\boldsymbol{J}_{7,8}$ coupling constant observed in the ^{1}H NMR spectrum of **5.34**. This was further validated by the observed nuclear Overhauser enhancement between H-8 and the bridgehead methyl H_3-20. The stereochemistry at C-9 was assigned through the observed H-11–H-8 and H-11–H_3-20 correlations, which indicated that C-11 must be equatorially situated and therefore placed the carbonitrile in the axial 9α position.

Table 5.5 ^{1}H (400 MHz, $CDCl_3$), ^{13}C (100 MHz, $CDCl_3$) and 2D NMR data for 7β-hydroxy-9α-carbonitrile-15,16-epoxylabda-13(16),14-dien-6-one (**5.34**).

Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	HMBC correlation to	COSY coupling to
1	35.2 (t)	1.75 (2H, br t, 6.2)	C-2, C-3, C-5 C-9, C-10, C-20	H_2-2, H_2-3, H_3-20
2	18.1 (t)	1.61 (2H, m)	C-3, C-4, C-9,C-10	H_2-1, H_2-3
3	41.6 (t)	1.28 (1H, m) 1.38 (1H, m)	C-2, C-4, C-5, C-18, C-19	H_2-1, H_2-2
4	32.7 (s)			
5	59.7 (d)	2.73 (1H, s)	C-4, C-6, C-9, C-10, C-18, C-19, C-20	H_2-3†, H-7†
6	209.0 (s)			
7	77.3 (s)	3.92 (1H, br d, 10.8)	C-6, C-8, C-17	H-5†, H-8, 7-OH
8	47.1 (d)	1.87 (1H, dq, 10.8, 6.5)	C-7, C-9, C-11, C-17, 9-CN	H-7, H_3-17
9	53.4 (s)			
10	47.0 (s)			
11	31.6 (t)	1.54 (1H, m) 1.98 (1H, m)	C-8, C-9, C-13, C-12, 9-CN	H_2-12
12	24.6 (t)	2.70 (2H, m)	C-11, C-13, C-14, C-16	H_2-11, H-16†
13	123.6 (s)			
14	110.5 (d)	6.27 (1H, d, 0.9)	C-13, C-15, C-16	H-15, H-16
15	143.2 (d)	7.36 (1H, t, 1.6)	C-13, C-14, C-16	H-14, H-16
16	138.8 (d)	7.26 (1H, br s)	C-13, C-14, C-15	H-14, H-15
17	14.7 (q)	1.42 (3H, d, 6.5)	C-6, C-7, C-9, C-10	H-8
18	32.3 (q)	1.02 (3H, s)	C-3, C-4, C-5, C-19	H_3-19†
19	22.1 (q)	1.30 (3H, s)	C-3, C-4, C-5, C-18	H_3-18†
20	15.8 (q)	0.89 (3H, s)	C-1, C-5, C-9, C-10	H_2-1
9-CN	120.3 (s)			
7-OH		3.71 (1H, bd, 3.0)	C-7	H-7

† Weak COSY correlations.

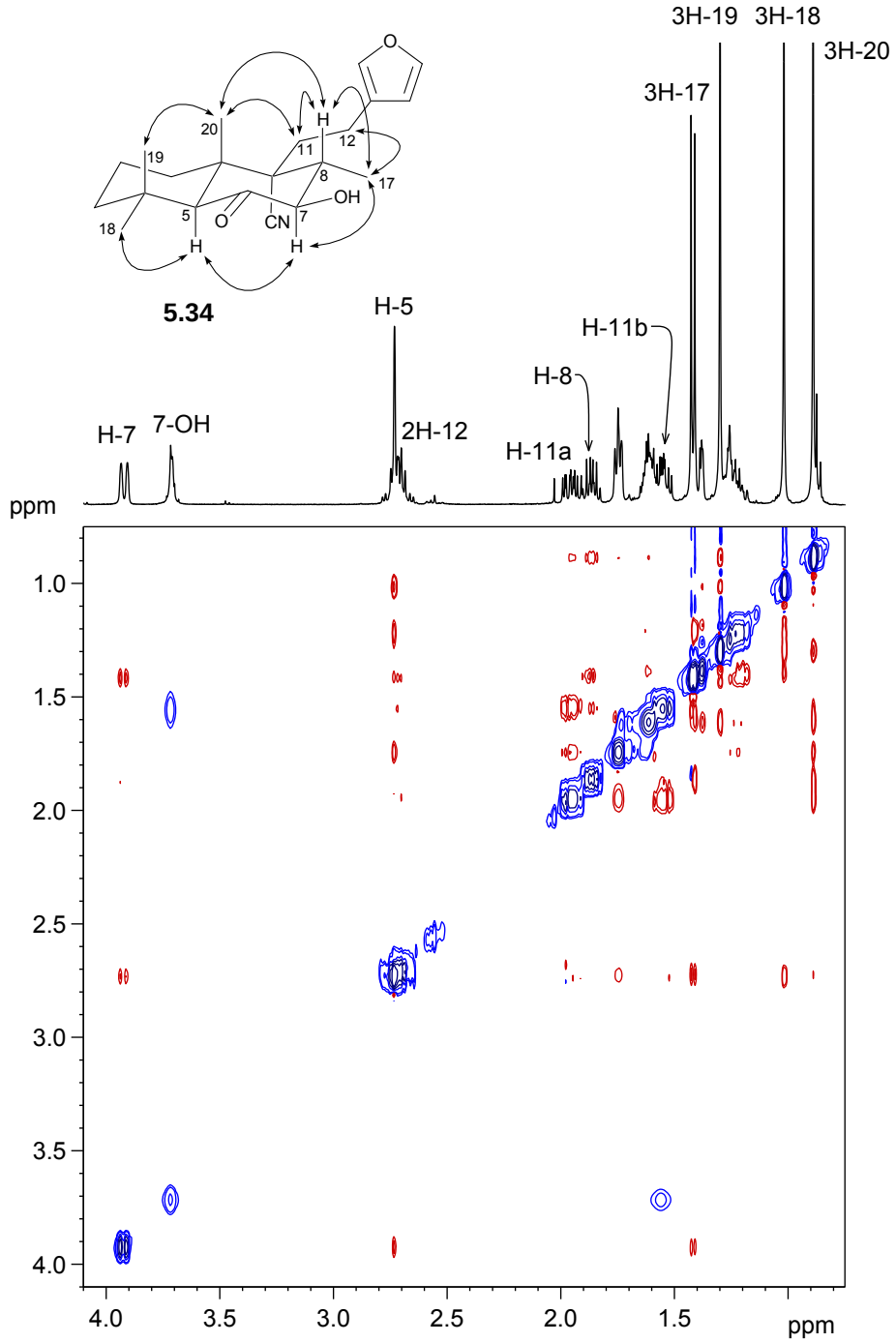
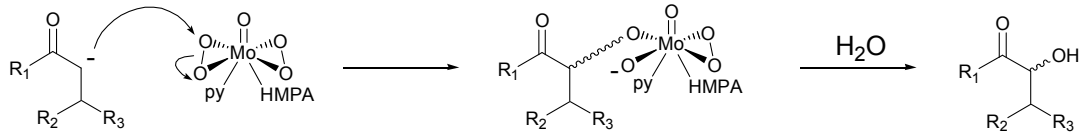


Figure 5.10 The upfield region (F1 and F2 = δ_H 0.75 – 4.1) of the NOESY spectrum ($CDCl_3$, 400 MHz; relaxation time [d1] = 1.00 sec; NOESY mixing time [d8] = 800 msec) obtained for 7β-hydroxy-9α-carbonitrile-15,16-epoxylabda-13(16),14-dien-6-one (**5.34**). Red contours indicate positive peaks, blue contours indicate negative peaks.[233] The accompanying figure shows the key NOESY correlations that confirmed the stereochemistry of **5.34**.

The formation of **5.34** can be envisaged through transesterification of **5.9** with concomitant conjugate addition of cyanide ion to the less hindered α-face of C-9 to yield the expected 7-oxo-6-hydroxy isomer **5.41**. In this transformation the C-17 methyl group will adopt an equatorial position to avoid 1,3-diaxial interaction with the C-20 axial methyl group. Due to the loss of conjugation with the C-8–C-9 double bond, the carbonyl group at C-7 is then easily transposed to C-6 through tautomerism under the basic reaction conditions. As **5.34** was the only addition product isolated from the reaction mixture, the C-6 keto isomer must be significantly favoured over the initially formed adduct **5.41**, although the reason for this preference is unknown.

As it was now obvious that the hydroxy ketones **5.27** and **5.28** could not easily be prepared from **5.9** and **5.10**, we turned our attention to the preparation of these intermediates directly from hispanone. Although there are a number of methods for accomplishing this transformation either in a single step ***via*** the enolate or in two steps ***via*** the silyl enol ether,[235] the problems associated with over-oxidation (***e.g.*** enolate oxidation using molecular oxygen[241]) and the stereochemical course of the reaction in conformationally constrained systems (***e.g.*** stereospecific enolate or silyl enol ether oxidation with ***meta***-chloroperbenzoic acid,[235] dimethyl dioxirane[242] or 2-sulfonyloxaziridines[243]) influenced our choice of reagent for this step of the synthesis. Although Vedejs' oxidation[244,245] generally gives modest yields when applied to α,β-unsaturated systems,[235] it has been shown to give diastereomeric acyloins in reasonable yield when applied to α,β-unsaturated ketones in functionalised trans-decalin systems.[246] We therefore decided to employ this method for the α′-oxidation of **5.1** in an effort to prepare the hydroxy ketones **5.27** and **5.28**.

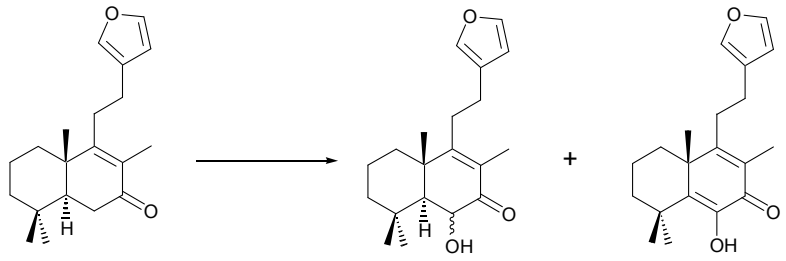
Vedejs' oxidation proceeds by treating a lithium diisopropylamide generated enolate (**5.35**) with oxodiperoxymolybdenum·pyridine·hexamethylphosphoramide (MoOPH, **5.36**) at low temperature to form adduct **5.37**.[245] Subsequent aqueous work up affects the hydrolysis of **5.37** to yield the corresponding acyloin without regioisomerisation (Scheme 5.12).



5.35 **5.36** **5.37**

Scheme 5.12 The mechanism of the Vedejs' oxidation.[245]

Initial attempts at Vedejs' oxidation of hispanone according to literature procedures[245,247] gave a mixture of **5.27** (33%), **5.28** (17%), the α-keto enol **5.30** (19%) and unreacted starting material (29%). This mixture could be separated by normal phase HPLC to yield the pure diastereomeric hydroxy ketones as white crystalline solids (mps: **5.27**, 95-96°C; **5.28**, 99-100°C). The presence of significant amounts of **5.30** in the product mixture suggested that under these conditions hispanone was being over-oxidised to the diketone **5.33** which was isolated as the enol tautomer due to the basic reaction conditions (in accordance with its previous formation through saponification of **5.9** and **5.10**). On the basis of these results, we undertook to optimise the hydroxylation procedure. It was found that using two molar equivalents of both MoOPH and lithium diisopropylamide, significantly increasing the reaction time at – 78°C, and quenching the reaction at 0°C rather than ambient temperature gave an increased yield of **5.27** and **5.28** (43% and 21% respectively), although the reaction mixture still contained **5.30** (5%) and unreacted starting material (13%) as shown in Scheme 5.13. Longer reaction times or increased quantities of lithium diisopropylamide or MoOPH did not improve the yield of **5.27** and **5.28** further.



5.1 **5.27** 6α **5.30**
5.28 6β

Scheme 5.13 α′-Hydroxylation of hispanone (**5.1**). ***Reagents and conditions***: LDA, – 78°C, 1h then MoOPH, – 78°C, 12h. Allowed to warm over 30 min, quenched at 0°C.

Once again, the structures of **5.27** and **5.28** were assigned by careful analysis of their respective spectroscopic data, and the stereochemistry at C-6 confirmed by 1D gradient selected NOESY experiments. It should be noted that **5.28** has previously been isolated from ***Ballota aucheri*** by Rustaiyan ***et al.***[248] and, although there was good agreement between the reported NMR, IR and MS data for the natural product and that obtained for the synthetically derived compound, there was a discrepancy in their optical rotations. Rustaiyan ***et al.*** report a specific rotation of –34° for the natural product,[248] but a specific rotation of +17° was repeatedly obtained for our synthetically derived **5.28**. The validity of the optical rotation obtained for the synthetic compound was determined by acetylation, and treatment of a small portion of **5.28** with acetic anhydride in pyridine yielded an oil that was identical to 6β-acetoxy-15,16-epoxylabda-8,13(16),14-trien-7-one (**5.10**) by ^{1}H and ^{13}C NMR spectroscopy. Although this oil was not purified by chromatography, it gave an optical rotation ($[\alpha]_D$ = –53°) which correlated well with that obtained for an analytical sample of **5.10** ($[\alpha]_D$ = –61°) derived from manganic acetate acetoxylation of hispanone. Although it is possible that the diterpene isolated by Rustaiyan ***et al.*** is the ***ent***-labdane analogue of **5.10**, this is unlikely because, to our knowledge, no ***ent***-furanolabdanes have been isolated to date from the Lamiaceae. It is more likely that the optical rotation reported in the literature is erroneous.

With the hydroxy ketones **5.27** and **5.28** in hand we were now in a position to investigate whether a hydroxyl group at C-6 would have an influence on the facial selectivity of hydride attack at the C-7 carbonyl group in a 7-oxo-labda-8-ene system. Accordingly, each of the isomeric hydroxy ketones was reduced with one molar equivalent of lithium aluminium hydride in tetrahydrofuran, the reaction being initiated at –78 °C and allowed to slowly warm to ambient temperature before being quenched at 0 °C. ^{1}H NMR spectroscopy of the reaction products suggested that under these conditions, **5.27** gave the 6α,7β-diol **5.11** (previously prepared from the acetoxy derivative **5.9**) and a second product, the corresponding 6α,7α-diol **5.17** (indicated by a double double doublet at δ_H 3.97 and a double doublet at δ_H 3.82) in 98% yield and a ratio of 1:1. Similarly, **5.28** gave the 6β,7β-diol **5.12** and the 6β,7α-diol **5.18** (indicated by two broad singlets at δ_H 3.72 and δ_H 4.26) in 99% yield and a ratio of 3:1.

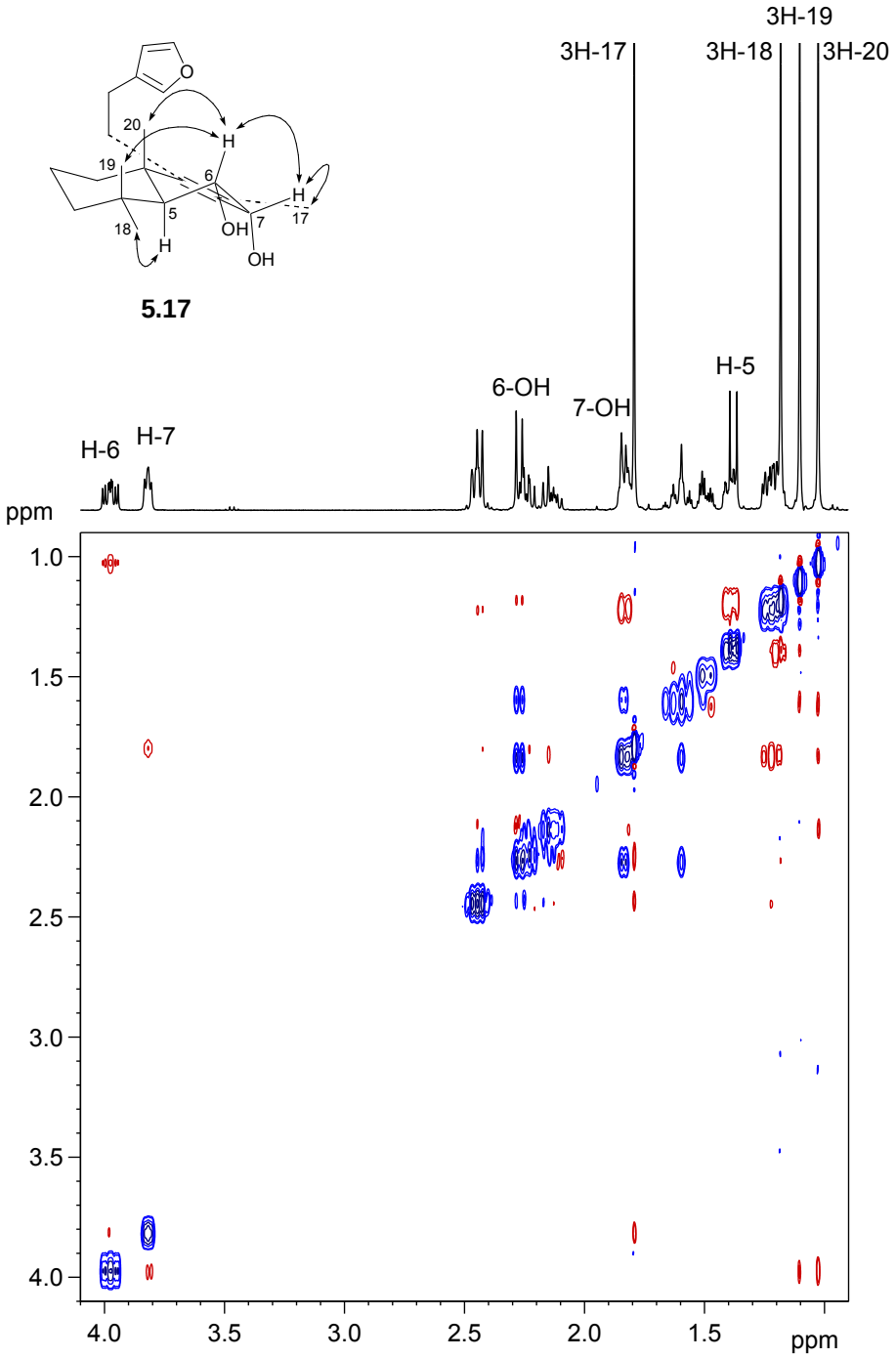
Separation of the diol mixtures by semi-preparative normal phase HPLC followed by scrupulous analysis of the HMQC, HMBC, COSY and NOESY data obtained for **5.17** and **5.18** allowed the unequivocal assignment of their structures as 15,16-epoxylabda-8,13(16),14-trien-6α,7α-diol and 15,16-epoxylabda-8,13(16),14-trien-6β,7α-diol respectively. The ^{1}H and ^{13}C NMR data obtained for **5.17** and **5.18** are reproduced in Table 5.6. The coupling constants of the H-5–H-6–H-7 spin systems in these diols, as with **5.11** and **5.12**, compared well with those reported for the corresponding isomers of 6,7-dihydroxyroyleanone (**5.24**: 6α,7α-isomer, $J_{5,6}$ = 12 Hz and $J_{6,7}$ = 5 Hz; 6β,7α-isomer, $J_{5,6}$ = 0 Hz and $J_{6,7}$ = 2 Hz)[22] and together with key NOESY correlations of H-5, H-6 and H-7 (see Figures 5.11 and 5.12) confirmed the stereochemistry of both isomers.

We now turned our attention to comparing the results obtained with lithium aluminium hydride with those for other common co-ordinating hydride reagents. With the ^{1}H NMR spectra of all four diastereomeric 6,7-diols in hand, we were able to conduct the reductions on a small scale (20 – 40 mg) and analyse the resulting product mixtures for the desired diols by ^{1}H NMR spectroscopy.

Accordingly, the hydroxy ketones **5.27** and **5.28** were reduced with the co-ordinating hydride reagents alane (AlH_3), sodium borohydride and zinc borohydride in tetrahydrofuran under identical conditions to those employed in the lithium aluminium hydride reductions. Alane[249] and zinc borohydride[250] were prepared by literature procedures immediately prior to use. The products obtained from the reductions were analysed by ^{1}H NMR spectroscopy. As the chemical shifts of the H-14 and H-15 singlets vary little in all of the hispanone derivatives, the integrals of these signals in the spectra of the reduction products obtained were considered to represent the total of furanolabdane products in the mixture. Therefore, comparison of the integrals (relative to those obtained for the furanyl protons) obtained for the H-6 and H-7 resonances of the dihydroxylated products and the H-6 resonance of the starting material allowed the conversion to, and ratio of, the desired products to be calculated. As these experiments were of a qualitative rather than a quantitative nature, and because in certain cases the reductions gave unidentified products, the extent of conversion to the desired products rather than absolute yields were used for comparative purposes. The results of these reductions are summarised in Figure 5.13.

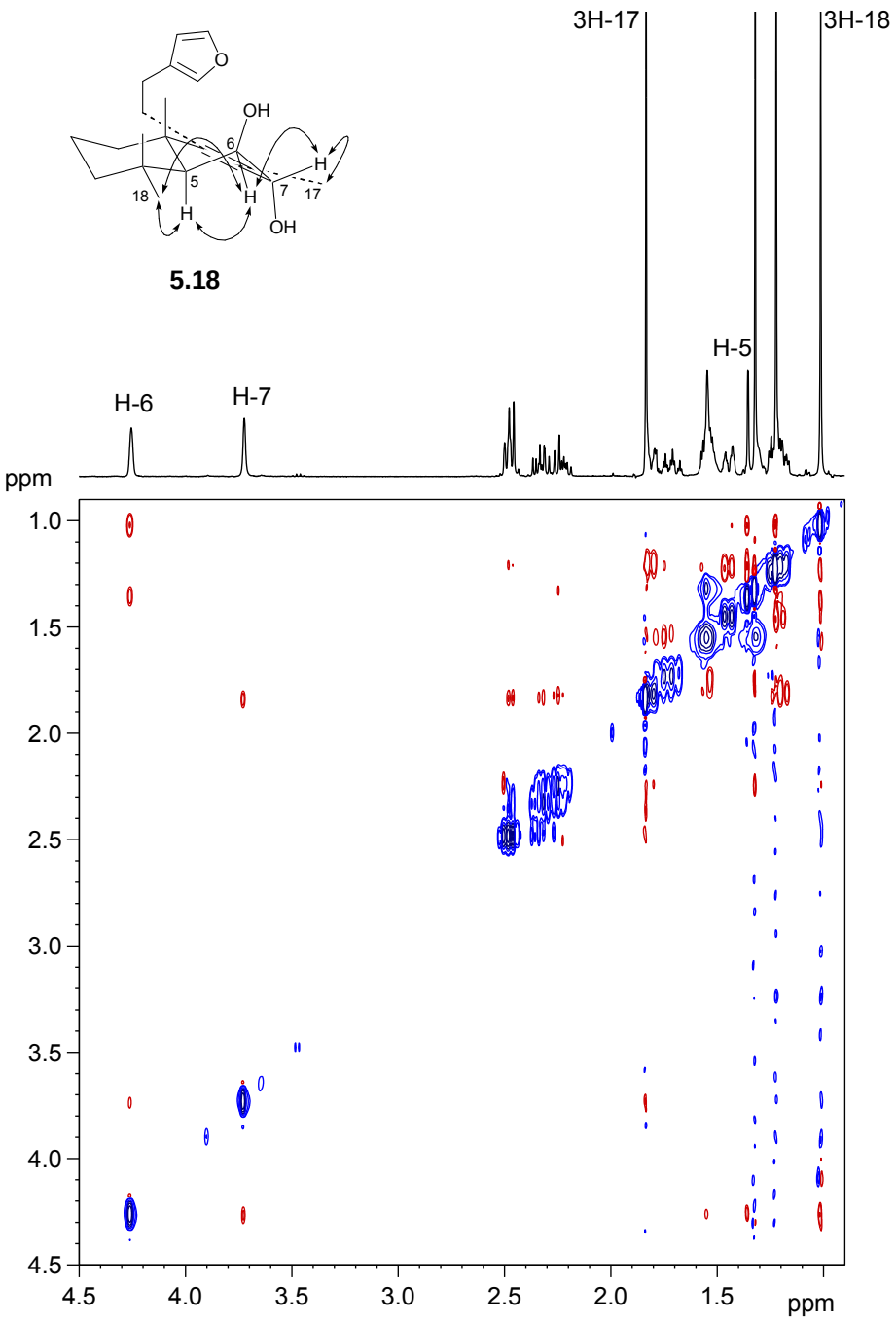
Table 5.6 ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data obtained for diols **5.17** and **5.18**.

Carbon	6α,7α-diol **5.17**		6β,7α-diol **5.18**	
	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)
1	37.1 (t)	1.25 (1H, m) 1.81 (1H, m)	39.4 (t)	1.19 (1H, m) 1.81 (1H, m)
2	18.9 (t)	1.49 (1H, dt, 13.8, 3.8) 1.60 (1H, tt, 13.4, 3.1)	19.1 (t)	1.56 (1H, m) 1.71 (1H, tt, 13.6, 3.6)
3	43.4 (t)	1.21 (1H, m) 1.41 (1H, m)	42.9 (t)	1.25 (1H, m) 1.44 (1H, br d, 13.5)
4	33.3 (s)		33.7 (s)	
5	50.5 (d)	1.38 (1H, d, 11.7)	49.3 (d)	1.36 (1H, d, 0.9)
6	70.1 (d)	3.97 (1H, ddd, 11.8, 9.9, 4.5)	71.1 (d)	4.26 (1H, br s)
7	73.8 (d)	3.82 (1H, dd, 6.2, 4.6)	76.2 (d)	3.72 (1H, br s)
8	126.5 (s)		124.7 (s)	
9	146.4 (s)		146.2 (s)	
10	42.3 (s)		39.4 (s)	
11	29.0 (t)	2.14 (1H, m) 2.26 (1H, m)	29.0 (t)	2.23 (1H, m) 2.34 (1H, m)
12	25.1 (t)	2.46 (2H, m)	25.1 (t)	2.48 (2H, m)
13	125.1 (s)		125.2 (s)	
14	110.7 (d)	6.28 (1H, d, 0.8)	110.7 (d)	6.29 (1H, d, 0.9)
15	142.8 (d)	7.35 (1H, t, 1.6)	142.8 (d)	7.35 (1H, t, 1.6)
16	138.5 (d)	7.23 (1H, s)	138.5 (d)	7.24 (1H, s)
17	17.4 (q)	1.79 (3H, s)	17.8 (q)	1.83 (3H, s)
18	36.3 (q)	1.18 (3H, s)	33.5 (q)	1.01 (3H, s)
19	22.0 (q)	1.10 (3H, s)	24.2 (q)	1.22 (3H, s)
20	20.2 (q)	1.03 (3H, s)	21.5 (q)	1.32 (3H, s)
6-OH		2.27 (1H, d, 10.1)		1.53 (1H, br s)
7-OH		1.84 (1H, br d, 6.8)		1.53 (1H, br s)



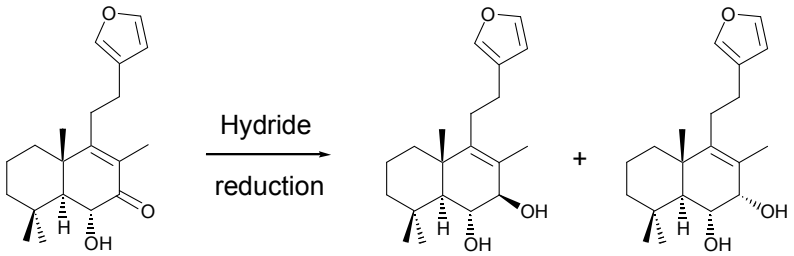
5.17

Figure 5.11 The upfield region (F1 and F2 = δ_H 0.9 – 4.1) of the NOESY spectrum ($CDCl_3$, 400 MHz; relaxation time [d1] = 1.00 sec; NOESY mixing time [d8] = 800 msec) obtained for 15,16-epoxylabda-8,13(16),14-trien-6α,7α-diol (**5.17**). Positive peaks are indicated by red contours and negative peaks by blue contours.[233] The key NOESY correlations used to confirm the 6α,7α stereochemistry of **5.17** are shown in the accompanying figure.



5.18

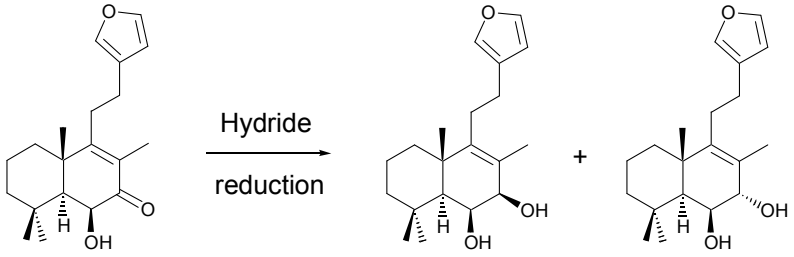
Figure 5.12 The upfield region (F1 and F2 = δ_H 0.9 – 4.5) of the NOESY spectrum ($CDCl_3$, 400 MHz; relaxation time [d1] = 1.00 sec; NOESY mixing time [d8] = 800 msec) obtained for 15,16-epoxylabda-8,13(16),14-trien-6β,7α-diol (**5.18**). Red contours indicate positive peaks, blue contours indicate negative peaks.[233] The accompanying figure shows the key NOESY correlations that confirmed the 6β,7α stereochemistry of **5.18**.



5.27 **5.11** **5.17**

Hydride reagent	Conversion to **5.11** and **5.17**	Ratio of products (**5.11** : **5.17**)	Comments
$LiAlH_4$	98% †	1:1	Exclusive 1,2-reduction
AlH_3	60% ‡	1:3	Exclusive 1,2-reduction, 40% **5.27**
$Zn(BH_4)_2$	24% ‡	8:11	76% other reduction products
$NaBH_4$	41% ‡	1:1	50% **5.27**, 9% other reduction products

† Isolated yield ‡ Calculated from ^{1}H NMR



5.28 **5.12** **5.18**

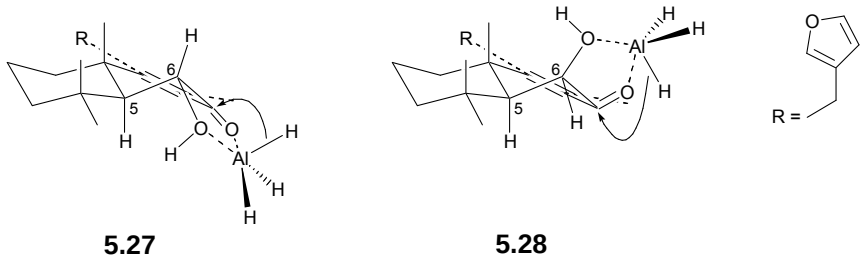
Hydride reagent	Conversion to **5.12** and **5.18**	Ratio of products (**5.12** : **5.18**)	Comments
$LiAlH_4$	99% †	3:1	Exclusive 1,2-reduction
AlH_3	86% ‡	10:1	Exclusive 1,2-reduction, 14% **5.28**
$Zn(BH_4)_2$	0% ‡	–	27% **5.28**, 73% other reduction products
$NaBH_4$	7% ‡	2:1	39% **5.28**, 54% other reduction products

† Isolated yield ‡ Calculated from ^{1}H NMR

Figure 5.13 Summary of the results obtained for the hydride reduction of the 6α- and 6β-hydroxy ketones **5.27** and **5.28**.

Examination of the results presented in Figure 5.13 indicate that the borohydride reagents give disappointing conversion to the desired diols, with both reagents yielding significant quantities of other products. While sodium borohydride exhibited evidence of oxygen directed hydride attack, zinc borohydride did not, possibly due to the increased steric bulk of the latter reagent. The secondary products were identical for both borohydride reagents, yielding a complex multiplet in the ^{1}H NMR spectrum of the 6α-derivatives [δ_H 4.67] and a series of doublets in the spectrum of the 6β-derivatives [δ_H 4.59 (***J*** = 7.8 Hz), 4.64 (***J*** = 7.6 Hz), 4.83 (***J*** = 3.0 Hz) and 4.92 (***J*** = 8.1 Hz)]. Due to the small scale of the reductions, these products were not isolated and identified but it is probable that they result from 1,4-reduction of the α,β-unsaturated carbonyl functionality of **5.27** and **5.28**.[227,251]

The results obtained from the reduction of **5.27** and **5.28** with alane were interesting as this reagent exhibited a distinct preference for β-face attack of the 6α-hydroxy ketone **5.27**, but α-face attack of the 6β-hydroxy-ketone **5.28**. These observations can be rationalised by the ability of alane to co-ordinate to both the C-6 hydroxyl and the C-7 carbonyl oxygens giving rise to a five-membered cyclic transition state (Figure 5.14).[252] In the transition state the carbonyl oxygen will be displaced towards the hydroxyl oxygen and subsequent hydride delivery will occur predominantly at the ***cis***-ring junction to yield the 6,7-cis products **5.17** and **5.12** as the favoured products.[252]



5.27 **5.28**

Figure 5.14 Schematic representation of the transition states and favoured route of hydride delivery in the alane reduction of hydroxy ketones **5.27** and **5.28**.

The results of the comparative reductions of **5.27** and **5.28** indicated that the reagent best suited for this purpose was actually that used initially, ***i.e.*** lithium aluminium hydride. This reagent gave both the 6α,7α-diol **5.11** and the 6α,7β-diol **5.17** in good yield from **5.27** and also effected the highest conversion of **5.28** to the 6β,7α-diol **5.18**. Although the α′-hydroxylation – reduction route investigated yielded all four of the desired 6,7-dihydroxylated isomeric products, the yield of the natural isomer **5.18** after two steps was only 5%. Considering that if applied to rhinocerotinoic acid (**4.14**) in the synthesis of ***Trimusculus*** diterpene analogues this route would require further protection, esterification and deprotection steps, we decided to investigate a final possible route involving oxidation at C-6 of the 7-oxo-labda-8-ene template in an effort to increase the yield of the 6β,7α-dihydroxylated derivative **5.18**.

5.2.3 Attempted preparation of the 6,7-dihydroxylated labda-8-enes via *hydride reduction of the labda-8-en-6,7-dione 5.33*

Our last attempt at procuring the four diastereomeric diols from **5.1** involved the preparation of 15,16-epoxylabda-8,13(16),14-trien-6,7-dione (**5.33**), as molecular modelling studies indicated that the presence of a second carbonyl at C-6 in ring-B of the hispanone template causes the α-face of the C-7 carbonyl to be more available for hydride attack (Figure 5.15). We considered that hydride reduction of the diketone would afford all four diastereomeric diols **5.11**, **5.12**, **5.17** and **5.18** (Scheme 5.14), which could then be separated by semi-preparative HPLC. We hoped that this route would yield the 6β,7α-diol (analogous to the Trimusculid metabolites) in higher yield than was obtained through hydroxyl directed reduction.

The conversion of hispanone to **5.33** could be envisaged in a single step through α′-oxidation to the diketone employing selenium dioxide in acetic acid.[253,254] However, initial attempts at this transformation gave a complex mixture of products, possibly due to competing oxidation at the allylic sites in **5.1**,[254] and this route was not pursued further.

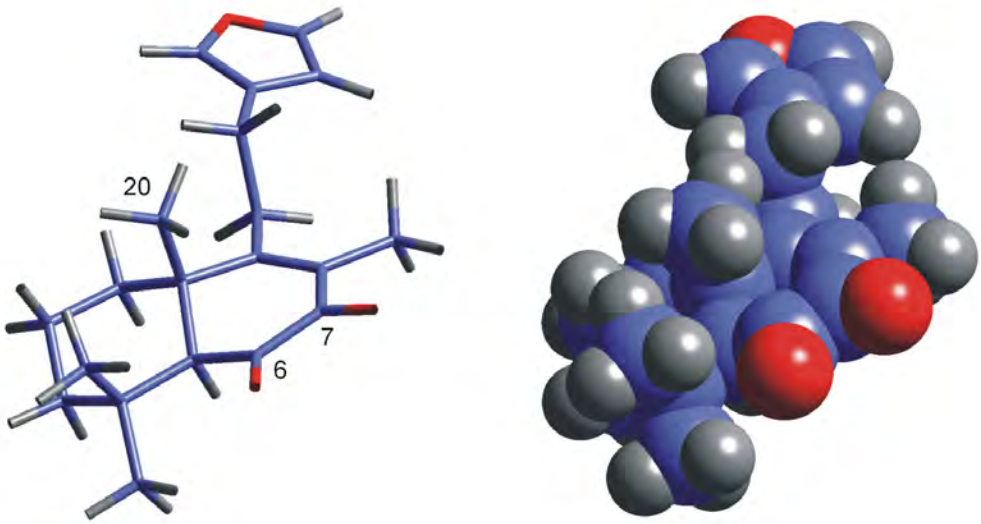
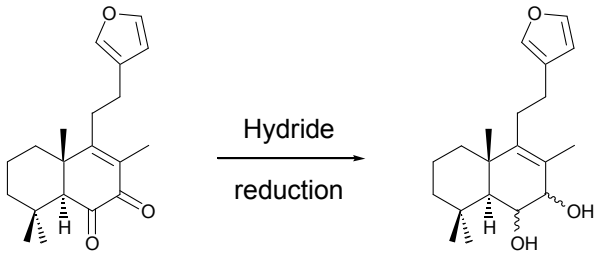


Figure 5.15 Stick and space filling representations of the global energy minimum conformation of 15,16-epoxylabda-8,13(16),14-trien-6,7-dione (**5.33**).



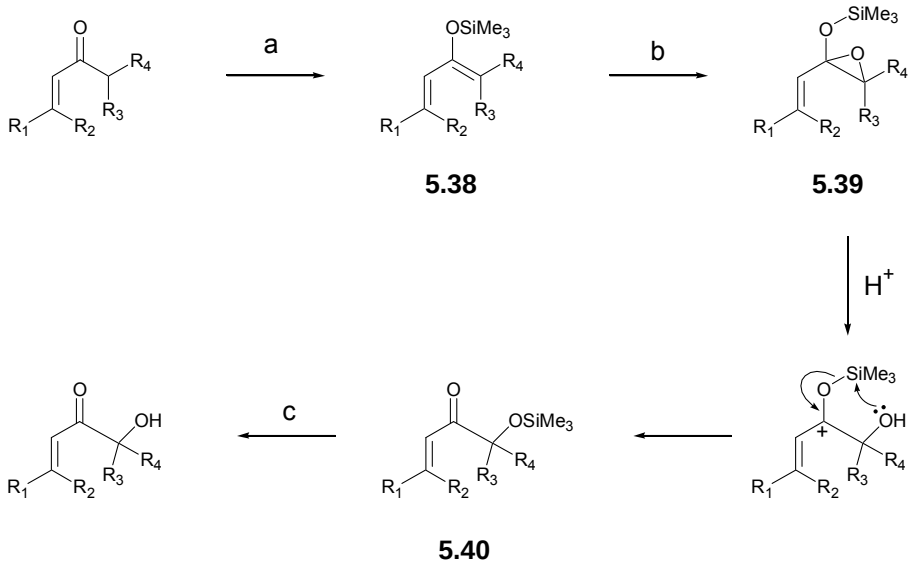
5.33

5.11 6α,7β
5.12 6β,7β
5.17 6α,7α
5.18 6β,7α

Scheme 5.14 Proposed preparation of the 6,7-diastereomeric diols **5.11**, **5.12**, **5.17** and **5.18** from the dione **5.33**.

We therefore undertook to prepare the diketone through oxidation of the α′-hydroxy ketone derivative **5.27**. In an effort to obtain the optimum yield of **5.33** from hispanone, and because the stereochemistry of the α′-hydroxylation was not critical in this instance, we decided to employ the Rubottom procedure[255] in the initial hydroxylation step rather than the MoOPH oxidation procedure of Vedejs.

The Rubottom procedure entails the ***meta***-chloroperbenzoic acid oxidation of the trimethylsilyl dienol ether **5.38** of an α,β-unsaturated ketone to yield the siloxy epoxide **5.39** which undergoes facile 1,4-silyl rearrangement to give **5.40**.[255,256] Subsequent hydrolysis or fluoride ion deprotection of **5.40** furnishes the α′-hydroxy-α,β-unsaturated ketone in good yield (Scheme 5.15).[255,256]



Scheme 5.15 The general mechanism of the Rubottom procedure.[255,256] ***Reagents***: a. LDA, TMSCl, THF; b. MCPBA, CH_2Cl_2; c. TBAF, CH_2Cl_2 or H_3O^+.

When the Rubottom procedure was applied to **5.1**, the intermediate silyl dienol ether, although transiently observed by thin layer chromatography, was found to be extremely susceptible to hydrolysis and could not be isolated. We therefore employed a one-pot Rubottom procedure by careful ***in vacuo*** removal of the tetrahydrofuran after trapping the dienolate with chlorotrimethylsilane, and treating the resulting residue with excess ***meta***-chloroperbenzoic acid in anhydrous dichloromethane. Although replacing the tetrahydrofuran with dichloromethane between the two stages of the reaction may have resulted in the hydrolysis of some of the silyl dienol ether, this action was necessary as it has been reported that the product obtained from the epoxidation step is solvent dependant:[257] the use of polar solvents for this transformation yields the α-***meta***-chlorobenzoyloxy ketone rather than the desired α-trimethylsiloxy ketone. Aqueous work-up of the resulting mixture effected hydrolysis of the trimethylsilyl ether and allowed the

isolation of the 6α-hydroxy ketone **5.27** as the only product in 76% yield; a significant improvement in the yield obtained through MoOPH hydroxylation of hispanone (63% yield of a mixture of 6α- and 6β-isomers).

With an improved method of preparing **5.27** stereospecifically, we now turned our attention to the oxidation of the hydroxy ketone to the diketone **5.33**. As we had already isolated the enol tautomer (**5.30**) of the diketone from a number of reactions, we were careful to select an oxidation reagent that would furnish **5.33** under mild conditions. Due to the relatively crowded environment around C-6 in **5.27**, we were also encouraged to use a reagent which exhibited low sensitivity to steric factors. For these reasons we decided to prepare **5.33** ***via*** Swern oxidation of **5.27**.[258] Accordingly, **5.27** was treated with dimethyl sulfoxide – oxalyl chloride and triethylamine in dichloromethane to afford the diketone **5.33** as a bright yellow crystalline solid (mp 144 – 146 °C) in quantitative yield. The absence of the C-6 carbinol signal [δ_C 73.3 (d)] and the presence of two carbonyl resonances [δ_C 197.7 (s) and 184.7 (s)] in the ^{13}C NMR spectrum of **5.33** along with the key HMBC correlations of H-5 and H_3-17 (see Table 5.7) confirmed the structure of **5.33** as 15,16-epoxylabda-8,13(16),14-trien-6,7-dione.

With **5.33** in hand we were now in a position to attempt the preparation of the isomeric 6,7-diols through hydride reduction. Accordingly the diketone was reduced with lithium aluminium hydride in tetrahydrofuran to yield a mixture of the desired products in 97% yield. Analysis of the product mixture by ^{1}H NMR spectroscopy indicated that the ratio of the diols obtained from the diketone was approximately 10:5:3:1 **5.12** : **5.11** : **5.18** : **5.17** (***i.e.*** overall yields from **5.1** of 39%, 19%, 12% and 4% respectively) which represents a significant improvement in the yield of **5.12** and **5.18** from **5.1** obtained through hydroxyl directed reduction (15% and 5% respectively).

Table 5.7 ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR data obtained for 15,16-epoxylabda-8,13(16),14-trien-6,7-dione (**5.33**).

Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	HMBC correlation to	COSY coupling to
1	36.0 (t)	1.63 (1H, m) 2.04 (1H, br d, 11.4)	C-2, C-3	H_2-2
2	18.3 (t)	1.65 (1H, m) 1.74 (1H, m)	C-1, C-4, C-10	H_2-1, H_2-3
3	41.9 (t)	1.16 (1H, td, 13.1, 3.6) 1.44 (1H, br d, 13.4)	C-2, C-4, C-5, C-18	H_2-2
4	32.6 (s)			
5	66.9 (d)	2.81 (1H, s)	C-1, C-3, C-4, C-6, C-9, C-10, C-18, C-19, C-20	H_3-19†, H_3-20
6	197.7 (s)			
7	184.7 (s)			
8	132.8 (s)			
9	171.2 (s)			
10	45.8 (s)			
11	31.0 (t)	2.59 (2H, m)	C-8, C-9, C-10, C-12	H_2-12
12	24.4 (t)	2.66 (2H, m)	C-9, C-11, C-13, C-14, C-16	H_2-11, H-16
13	123.9 (s)			
14	110.4 (d)	6.31 (1H, dd, 1.6, 0.8)	C-13, C-15, C-16	H-15, H-16†
15	143.3 (d)	7.39 (1H, t, 1.7)	C-13, C-16	H-14, H-16
16	138.7 (d)	7.29 (1H, br d, 0.5)	C-13, C-14, C-15	H_2-12, H-14†, H-15
17	11.5 (q)	1.92 (3H, s)	C-7, C-8, C-9, C-10	
18	32.2 (q)	1.05 (3H, s)	C-3, C-4, C-5, C-19	
19	21.4 (q)	1.34 (3H, s)	C-3, C-4, C-5, C-18	H-5†
20	23.6 (q)	1.20 (3H, s)	C-1, C-5, C-9, C-10	H-5

† Weak COSY correlations.

5.3 Biological activity of the furanolabdanes prepared from hispanone

One of the main underlying objectives of this thesis was the discovery of biologically active compounds from marine invertebrates. However, having prepared a total of eleven furanolabdane intermediates from hispanolone (**5.2**) we decided to take this opportunity to survey these for bioactivity. As hispanolone is a common natural product in several Lamiaceae species, we considered that it would be interesting to subject these compounds to agro-chemical assays accessible through an ongoing collaboration with Dow AgroSciences (Indianapolis, USA).[259] Hispanolone (**5.2**), hispanone (**5.1**) and ten of the derivatives prepared by us (**5.9** – **5.12**, **5.17**, **5.18**, **5.27**, **5.28**, **5.33** and **5.34**) were tested in a series of ten standard agro-chemical assays (four pesticidal, six herbicidal). Of the compounds submitted for screening, only the nitrile **5.34** exhibited significant activity in the agro-chemical screens (80% inhibition of the growth of the crop fungus ***Phytophthora infestans*** at low concentrations) and Dow AgroSciences are currently performing further agro-chemical tests on this compound.

5.4 Summary and Conclusions

The model study investigating the feasibility of preparing all four possible 6,7-diol diastereomers from a 7-oxo-labda-8-ene template revealed that the realisation of this goal was rather more challenging than originally anticipated. Although oxygenation at C-6 could be accomplished in high yield using manganic acetate to yield the α'-acetoxy enones **5.9** and **5.10**, the conformation of the B-ring in these systems was such that it resulted in exclusive α-face delivery of hydride to the C-7 carbonyl functionality during reduction, giving the 6α,7β- and 6β,7β-diols **5.11** and **5.12** only. This facial specificity was expected and an alternative route involving the sodium borohydride – cerium chloride selective reduction of the C-7 carbonyl in the presence of the C-6 acetoxy group was considered as a means of manipulating the stereochemistry at C-7 subsequent to the reduction step through some form of S_N2 inversion process. Unfortunately, the hydroxy esters resulting from the selective reduction of **5.9** and **5.10** undergo facile transesterification giving a mixture of C-6 and C-7 esterified derivatives. This unforeseen

complication inhibited further pursuit of our initial synthetic approach to the 6α,7α- and 6β,7α-diols **5.17** and **5.18**.

A second strategy involving hydride reduction of the hydroxy ketones **5.27** and **5.28** was examined to investigate whether the presence of a hydroxyl group at C-6 would significantly alter the stereochemistry of hydride delivery to C-7 through hydroxyl directed reduction. Attempts at preparing the required hydroxy ketones from their acetylated derivatives **5.9** and **5.10** were unsuccessful but **5.27** and **5.28** were eventually prepared from **5.1** in reasonable yield through enolate oxidation employing MoOPH. Subsequent reduction of **5.27** and **5.28** with various co-ordinating hydride reagents indicated that the stereochemistry of reduction is indeed altered by a C-6 hydroxyl group, with the best spread of reduction products being obtained with lithium aluminium hydride. Despite this, the yield of the hispanone analogue of the naturally occurring ***Trimusculus*** 6β,7α-isomer (***i.e.*** **5.18**) ***via*** this route was poor (5% over two steps from **5.1**) and considered inadequate to warrant the application of this synthetic route to rhinocerotinoic acid (**4.14**).

The last synthetic route investigated in this model study involved hydride reduction of the 6,7-diketone **5.33**. Although this derivative could not be obtained directly from **5.1** by selenium dioxide oxidation, it could be prepared in good yield by Rubottom oxidation followed by Swern oxidation (76% for two steps from **5.1**). Hydride reduction of **5.33** resulted in the preparation of all four diastereomeric diols, and although the yield of the 6β,7α-diol **5.18** was significantly improved (12% over three steps) that of the 6α,7α-diol **5.17** was poor in this case (4% over three steps).

In conclusion, the model oxidation–reduction study performed on hispanone indicated that all four diastereomeric 6,7-dihydroxy-labda-8-enes can be prepared from a 7-oxo-labda-8-ene skeleton although both possible routes which were identified yielded one of the isomers in prohibitively low yield. As the route employing hydroxyl directed reduction gave the three unnatural isomers in reasonable yield (21%, 15% and 21% respectively) we considered that the best method of obtaining all four isomers would be to use this route in conjunction with a stereospecific synthesis

of the natural 6β,7α-isomer isolated from ***Trimusculus costatus***. The work undertaken towards this latter goal is described in Chapter Six.

Chapter Six

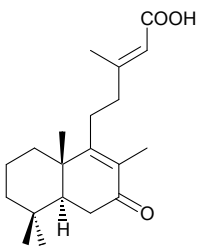
Attempted Stereospecific Synthesis of

6β,7α-Diacetoxylabda-8,13***E***-dien-15-ol and

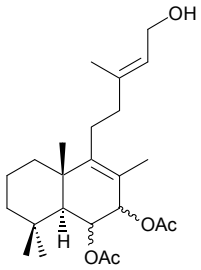
6α,7β-Diacetoxylabda-8,13***E***-dien-15-ol

6.1 Introduction

As a consequence of the unexpected difficulties encountered during the synthesis of rhinocerotinoic acid (**4.14**; see Chapter Four) we were now limited to the extent in which we could explore the use of this template for the stereospecific construction of the 6β,7α,15-funtionalised labda-8,13***E***-diene skeleton (the basic framework of four of the eleven known ***Trimusculus*** diterpenes) by time constraints. However, the experience and insights regarding functionalisation at C-6 of a 7-oxo-labda-8-ene framework afforded by the model studies described in Chapter 5 encouraged us to attempt the stereospecific synthesis of the naturally occurring ***Trimusculus costatus*** metabolite 6β,7α-diacetoxylabda-8,13***E***-dien-15-ol (**3.10**). The work undertaken in this respect and also towards the synthesis of the 6,7-epimeric analogue of **3.10**, 6α,7β-diacetoxylabda-8,13***E***-dien-15-ol (**6.1**), is described here.



4.14



3.10 6β,7α
6.1 6α,7β

6.2 Attempted stereospecific synthesis of 6β,7α-diacetoxylabda-8,13*E*-dien-15-ol

Although it is probable that many synthetic routes could be proposed for the synthesis of **3.10** from rhinocerotinoic acid, we wanted to employ some of the lessons learned during our model work with hispanone (**5.1**). It was evident that obtaining the desired diaxial 6β,7α-functionation pattern of the ***Trimusculus*** labdanes from a 7-oxo-labda-8-ene framework would be no trivial matter. Formation of the 6α,7β-analogue would be somewhat easier, as the conformation of the ***trans***-decalin ring system and the steric bulk provided by the C-20 methyl group should promote diequatorial functionalisation (see Chapter Five). We believed, however, that this could be used

to our advantage if we could find an efficient means of inverting the stereochemistry at C-6 and C-7 subsequent to the initial functionalisation of the B-ring of **4.14**.

The simplest method for this transformation would be to convert the ***trans***-diequatorial functionalised labdane to the 6,7-epoxide. In conformationally constrained ring systems nucleophilic cleavage of oxiranes generally favours the formation of the diaxial adduct,[260,261] obeying what is often referred to as the rule of diaxial opening[260] or the Fürst-Plattner rule.[261] This transformation therefore possesses significant potential as an efficient means of inverting the stereochemistry at C-6 and C-7 of a 6α,7β,15-functionalised labda-8,13***E***-diene substrate.

The preference for diaxial opening of epoxides in cyclohexane derivatives arises from conformational preferences of the two possible products (see Figure 6.1).[260,261] The most favourable transition state for ring opening will have the nucleophile, C-2, C-3 and the epoxide oxygen in as nearly an ***anti***-coplanar arrangement as possible.[260,261] Nucleophilic attack at C-2 (path A) along the axial direction allows the 6-membered ring to be transformed smoothly and directly into a chair conformation with diaxially arranged substituents.[260] This pathway is energetically favoured over path B, in which similar attack at C-3 results in a twist-boat conformation that then converts to the diequatorial chair conformer.[260] However, deviations from the preferred reaction pathway (path A) may arise if ring-cleavage is a two-step rather than a concerted process and also if the epoxide is in a conformation other than the half chair.[260,261]

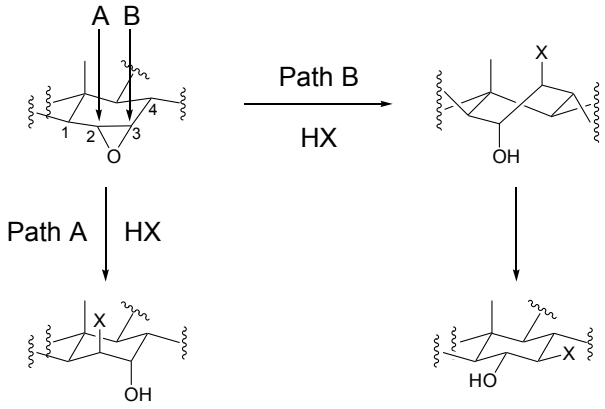


Figure 6.1 Schematic representation of the two possible routes for nucleophilic cleavage of oxiranes in conformationally constrained ring systems (after Haines).[260]

With this transformation in mind, we had to find an appropriate method of preparing a 6,7-epoxylabda-8,13***E***-dien-15-oic acid from rhinocerotinoic acid (**4.14**). Examination of the energy minimised space-filling models of the two possible isomeric epoxides, 6α,7α-epoxylabda-8,13***E***-dien-15-oic acid (**6.2**) and 6β,7β-epoxylabda-8,13***E***-dien-15-oic acid (**6.3**), indicated that axial attack of the epoxide ring of the latter oxirane would be less restricted by steric interactions (see Figure 6.2), increasing the likelihood of obtaining the diaxially substituted product upon nucleophilic cleavage. We therefore wanted to design a synthetic scheme that utilised the epoxide **6.3**, rather than **6.2**, as a key intermediate.

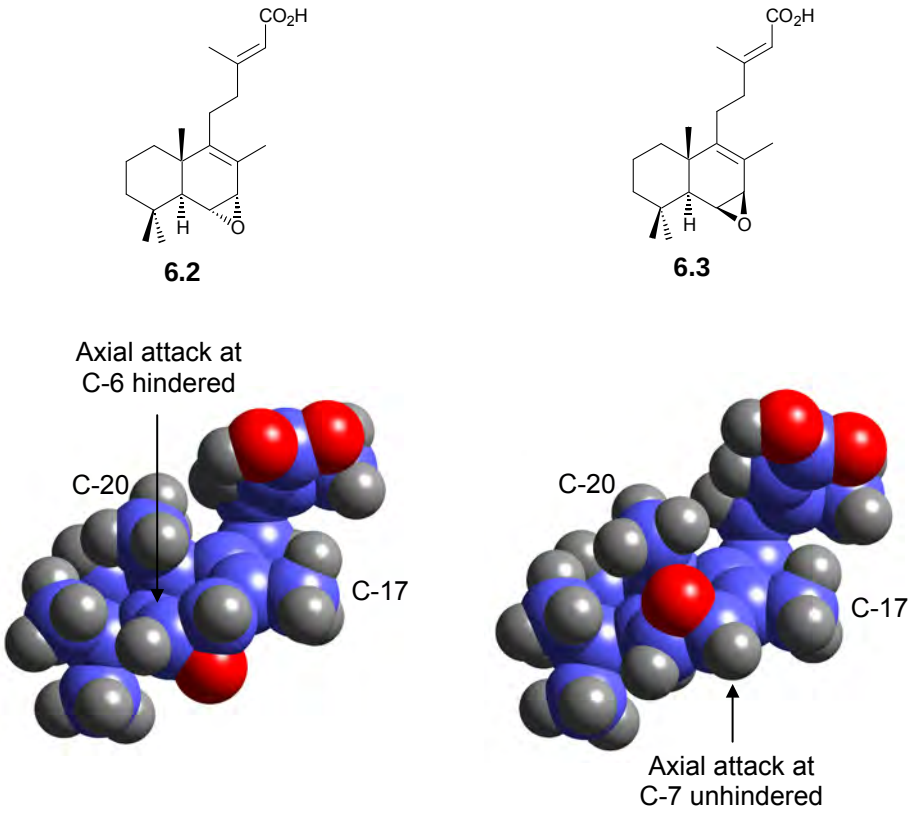
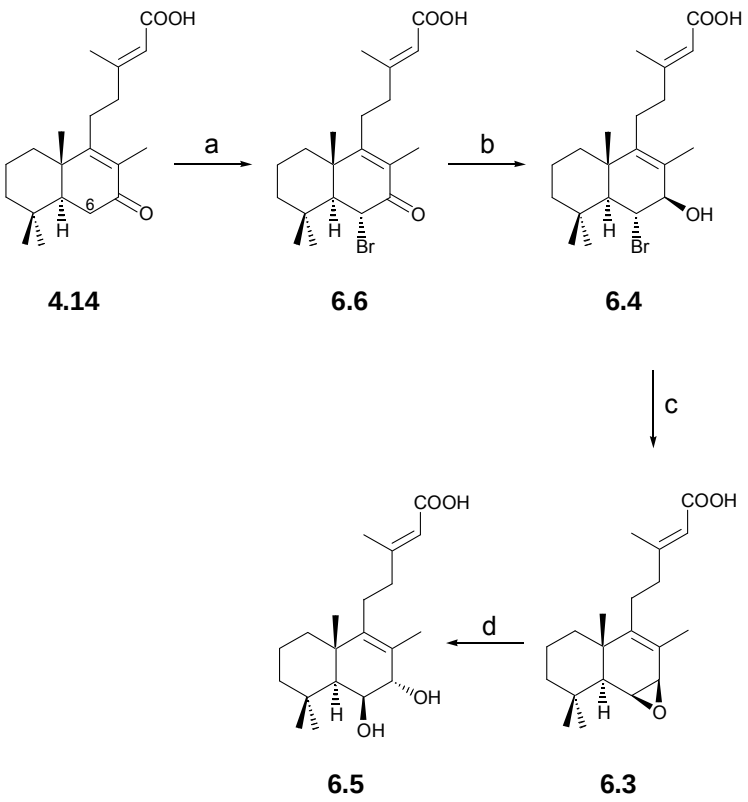


Figure 6.2 Comparison of the steric effects influencing the axial attack of nucleophile on the oxirane rings of 6α,7α-epoxylabda-8,13***E***-dien-15-oic acid (**6.2**) and 6β,7β-epoxylabda-8,13***E***-dien-15-oic acid (**6.3**), shown as energy minimised space-filling models.

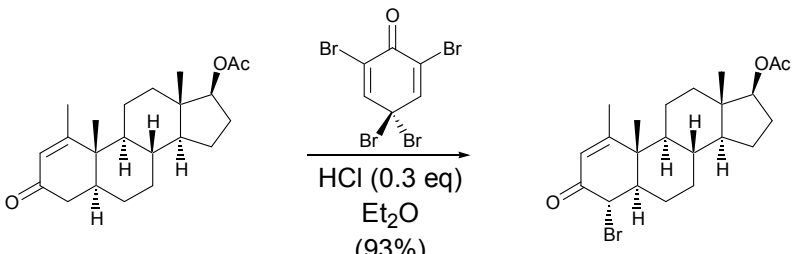
Examination of the literature indicated that diequatorial bromohydrins derived from keto steroids (through treatment with bromine and subsequent hydride reduction) yielded the corresponding

epoxide in reasonable yields on treatment with base.[262] With our acquired knowledge and practical experience of the stereochemical preferences of the 7-oxo-labda-8-ene skeleton to introduction of functionality at C-6 ***via*** the enolate (the 6α-isomer predominates) and subsequent hydride reduction at C-7 (the 7β-isomer is obtained exclusively in the absence of directing groups at C-6), we considered a synthetic route involving the preparation of 6α-bromo-7β-hydroxylabda-8,13***E***-dien-15-oic acid (**6.4**) a promising means of obtaining the key intermediate 6β,7β-epoxylabda-8,13***E***-dien-15-oic acid (**6.3**) from rhinocerotinoic acid (Scheme 6.1). We hoped that nucleophilic cleavage of the 6β,7β-epoxide ring of **6.3** would yield predominantly the diaxial diol **6.5**,[260,261,263] which could then be transformed to **3.10** through hydride reduction, selective protection of the C-15 hydroxyl, acetylation at C-6 and C-7, and deprotection.



Scheme 6.1 Proposed route for the synthesis of 6β,7α-dihrdroxylabda-8,13***E***-dien-15-oic acid (**6.5**) from rhinocerotinoic acid ***via*** the bromohydrin **6.4**. ***Reagents and conditions***: a. 2,4,4,6-tetrabromocyclohexa-2,5-dienone, HCl, Et_2O; b. $NaBH_4$, $CeCl_3$, MeOH, 0°C - RT; c. NaH, THF, 0°C; d. H_2SO_4, 3:1 HMPA/H_2O.

We were now faced with finding an appropriate method of brominating the α′-position of an α,β-unsaturated ketone to yield **6.6**. Consultation with the literature indicated that this transformation may be accomplished in a number of ways: through electrochemical methods employing copper (I) bromide, trifluoroacetic acid and tetraethylammonium tosylate in acetonitrile;[264] ***via*** the silyl dienol ether treated with either bromine (in carbon tetrachloride or pentane) or ***N***-bromosuccinimide (in carbon tetrachloride or tetrahydrofuran);[265] and ***via*** the enolate through treatment of the α,β-unsaturated ketone with 2,4,4,6-tetrabromocyclohexa-2,5-dienone and hydrogen chloride in ether.[266] When reviewing these possibilities, we discovered that the latter procedure of Caló ***et al.***[266] had been employed in the α′-bromination of a number of steroidal systems and yielded the desired equatorial isomer as the major product. In fact, the authors report that when the reaction was carried out on 1-methylandrost-1-en-3-one-17-β-ol acetate (**6.7**), which possesses a substitution pattern about the A-ring very similar to that of the B-ring of **4.14**, the equatorial product 4α-bromo-1-methylandrost-1-en-3-one-17β-ol acetate (**6.8**) was obtained exclusively (Scheme 6.2).[266]

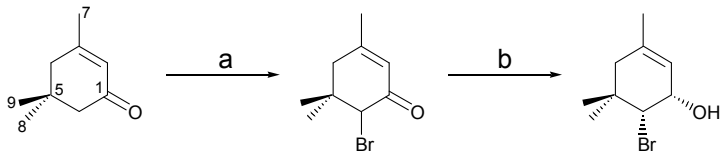


6.7 **6.8**

Scheme 6.2 Caló ***et al***.'s reported stereospecific α′-bromination of 1-methylandrost-1-en-3-one-17-β-ol acetate (**6.7**).[266]

The feasibility of the bromination-reduction strategy for preparing the bromohydrin **6.4** was first tested using isophorone (**6.9**) as a model system (see Scheme 6.3). Treatment of **6.9** with 2,4,4,6-tetrabromocyclohexadienone under the conditions described by Caló ***et al.***[266] gave (±)-6-bromo-3,5,5-trimethylcyclohex-2-enone (**6.10**),[264,265] as a volatile oil in 66% yield. Subsequent reduction of **6.10** with sodium borohydride–cerium chloride[227,262,267] gave the expected

bromohydrin product,[268] (±)-***cis***-6-bromo-3,5,5-trimethylcyclohex-2-enol (**6.11**), in 86% yield. The stereochemistry of the borohydride reduction was confirmed by analysis of the ^{1}H NMR and NOESY data obtained for **6.11**. Strong nuclear Overhauser correlations observed between H-6 and the protons of both the equatorial [δ_C 29.0 (q, C-8)] and axial [δ_C 26.2 (q, C-9)] methyl groups suggested that the bromine substituent at C-6 favoured an axial conformation. A similar correlation between H-1 and the axial methyl H_3-9 in addition to the magnitude of the H-1–H-6 coupling constant ($J_{1,6}$ = 3.9 Hz) indicated that the hydroxyl group at C-1 was equatorial and consequently ***cis*** to the bromine.[268]



6.9 **6.10** **6.11**

Scheme 6.3 Model bromination-reduction study of an α,β-unsaturated ketone employing isophorone (**6.9**). ***Reagents and conditions***: a. 2,4,4,6-tetrabromocyclohexa-2,5-dienone, HCl, Et_2O, RT (68%); b. $NaBH_4$, $CeCl_3$, MeOH, 0°C – RT (86%).

Encouraged by the model reactions performed on isophorone and the reported stereospecificity of Caló's bromination procedure, we undertook to prepare the α´-bromo-α,β-unsaturated ketone **6.6** from rhinocerotinoic acid. Accordingly, **4.14** was treated with 2,4,4,6-tetrabromocyclohexa-dienone and hydrogen chloride in diethyl ether at room temperature. However, on work-up it became evident that although the reaction proceeded in excellent yield (98%), the product obtained consisted of a 4:3 mixture (estimated by ^{1}H NMR spectroscopy) of both the C-6α and C-6β isomers (**6.6** and **6.12**). The stereochemistry of the products was confirmed by separating a portion of the diastereomeric mixture by semi-preparative reversed phase HPLC (4:1 methanol/ water containing 0.05% trifluoroacetic acid) followed by analysis of the respective NMR data obtained for both products (see Table 6.1). As expected, the major product was the pseudo-equatorial 6α-isomer **6.6**, indicated by the large coupling between H-5 and H-6 [δ_H 2.13 (1H, d, ***J*** = 8.3 Hz, H-5) and 4.48 (1H, d, ***J*** = 8.3 Hz, H-6); see Figure 6.3] and strong nuclear Overhauser

Table 6.1 ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data obtained for 6α- and 6β-bromo-7-oxo-labda-8,13***E***-dien-15-oic acid (**6.6** and **6.12**).

Carbon	**6.6**		**6.12**	
	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)
1	36.0 (t)	1.50 (1H, m) 1.84 (1H, br d, 11.3)	39.4 (t)	1.25 (1H, m) 1.90 (1H, m)
2	18.4 (t)	1.60 (1H, m) 1.69 (1H, br d, 13.6)	18.5 (t)	1.62 (1H, br d, 14.3) 1.79 (1H, br t, 14.2)
3	42.0 (t)	1.29 (1H, td, 13.5, 4.3) 1.48 (1H, m)	43.7 (t)	1.19 (1H, m) 1.47 (1H, br d, 13.3)
4	35.2 (s)		34.7 (s)	
5	56.6 (d)	2.13 (1H, d, 8.3)	52.8 (d)	1.84 (1H, d, 2.7)
6	49.6 (d)	4.48 (1H, d, 8.3)	47.2 (d)	4.70 (1H, d, 2.5)
7	194.7 (s)		192.4 (s)	
8	130.8 (s)		128.0 (s)	
9	162.2 (s)		166.4 (s)	
10	40.7 (s)		42.3 (s)	
11	27.1 (t)	2.42 (2H, m)	28.1 (t)	2.41 (2H, m)
12	40.3 (t)	2.30 (2H, m)	39.6 (t)	2.29 (2H, m)
13	161.2 (s)		161.3 (s)	
14	115.6 (d)	5.74 (1H, s)	115.3 (d)	5.74 (1H, s)
15	171.5 (s)		171.0 (s)	
16	19.1 (q)	2.23 (3H, s)	19.1 (q)	2.23 (3H, s)
17	13.5 (q)	1.90 (3H, s)	12.4 (q)	1.87 (3H, s)
18	35.0 (q)	1.21 (3H, s)	32.5 (q)	1.03 (3H, s)
19	22.1 (q)	1.05 (3H, s)	22.2 (s)	1.34 (3H, s)
20	24.0 (q)	0.96 (3H, s)	18.4 (q)	1.55 (3H, s)

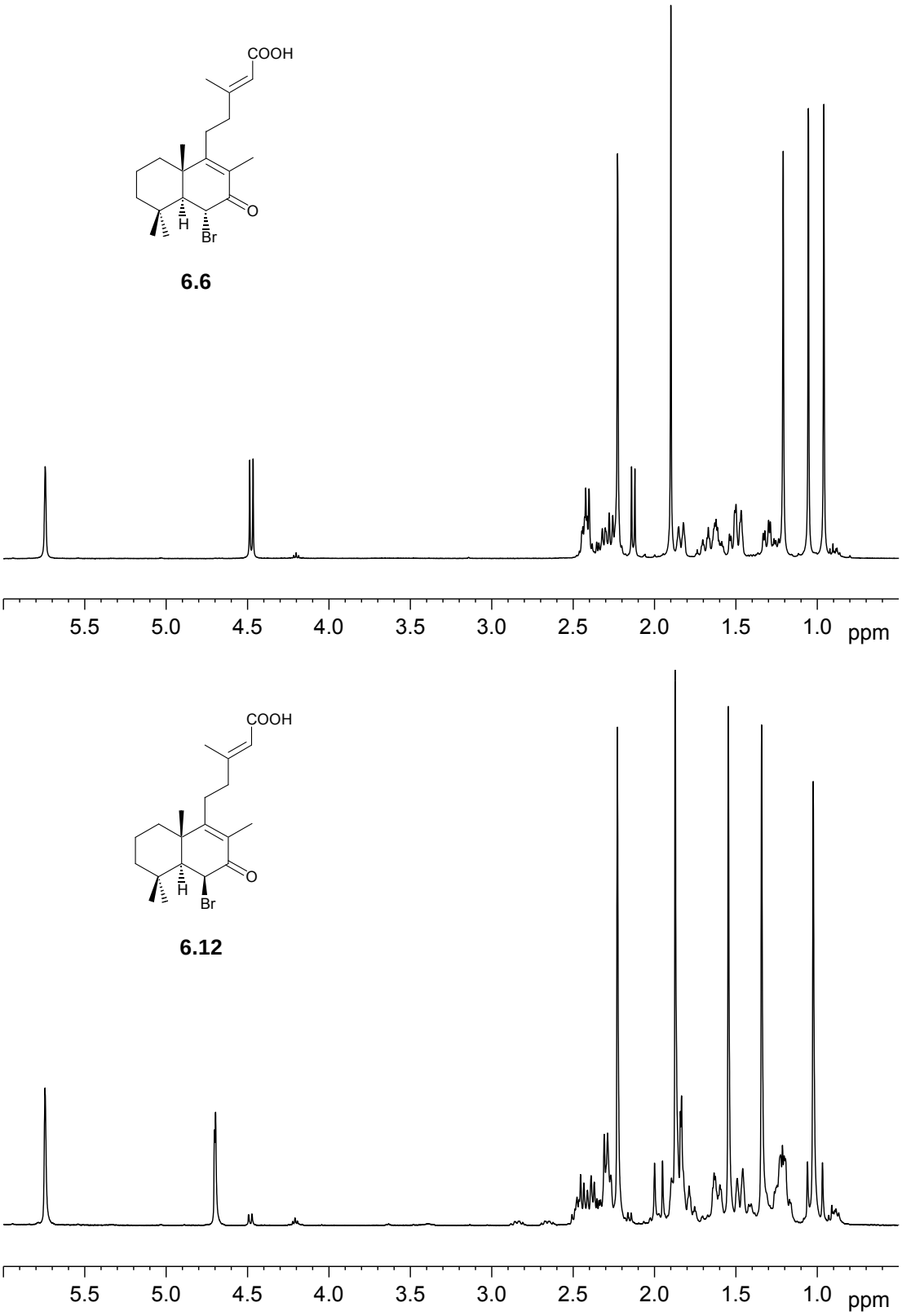
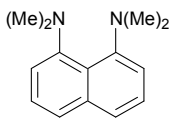


Figure 6.3 The ^{1}H ($CDCl_3$, 400 MHz) NMR spectra obtained for 6α- (top) and 6β-bromo-7-oxo-labda-8,13-dien-15-oic acid (**6.6** and **6.12**; structures shown inset).

enhancements between H-6 and both axial methyl groups H_3-19 and H_3-20. The 6β-isomer **6.12** exhibited a smaller H-5–H-6 coupling constant [δ_H 1.84 (1H, d, ***J*** = 3.7 Hz, H-5) and 4.70 (1H, d, ***J*** = 3.5 Hz, H-6); see Figure 6.3] and a corresponding strong H-5–H-6 correlation in its NOESY spectrum.

As the desired product was the 6α-brominated isomer, we examined the potential of enriching the epimeric mixture through keto-enol tautomerism. This conversion would have to firstly incorporate a non-nucleophilic base to avoid substitution of the halide, and secondly involve a solvent possessing a high dielectric constant to promote the formation of the 6α-isomer through stabilisation of the electrostatic interaction between the equatorial bromine and carbonyl oxygen.[269]

We therefore treated a portion of the 4:3 **6.6**/**6.12** mixture with 2 equivalents of Proton-sponge® (***N,N,N´N´***-tetramethyl-1,8-naphthalenediamine, **6.13**) in anhydrous acetonitrile.[270] The reaction mixture was quenched with 0.5 M hydrochloric acid and the product analysed by ^{1}H NMR spectroscopy. The NMR data indicated that although there had been a substantial reduction in the proportion of the 6β-bromo isomer (from 4:3 **6.6**/**6.12** to 4:1 **6.6**/**6.12**), a secondary product (methyl singlets at δ_H 2.22, 1.99, 1.92, 1.33 and 1.26, and olefinic proton singlets at δ_H 5.76 and 6.39) had been formed in significant amounts under these conditions. Repetition of this procedure using tetrahydrofuran as the solvent also caused significant transformation to the secondary product and gave a less favourable conversion of isomers (^{1}H NMR spectroscopy of the crude product indicated a ratio of 7:4 **6.6**/**6.12**). All attempts to isolate the secondary product produced by action of the base **6.13** on **6.6**/**6.12**, by both normal and reversed phase HPLC, were unsuccessful and therefore the product was not identified.



6.13

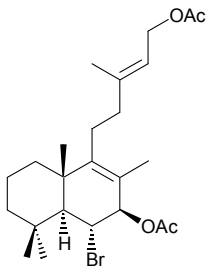
Acknowledging that we could not efficiently convert **6.12** to **6.6** through epimerisation, we decided to establish whether variation of the bromination conditions of Caló ***et al.***[266] would allow us to obtain the 6α-bromo-isomer in higher yields. Accordingly, we performed the bromination in the more polar solvent tetrahydrofuran and obtained **6.6** and **6.12** in a 5:2 ratio. Encouraged by this improvement in selectivity and cognisant that in this type of ring system the 6α-substituted isomer is the thermodynamically favoured product (see Chapter Five), we attempted to further increase the proportion of **6.6** formed in the reaction by conducting the bromination in refluxing tetrahydrofuran. In this way we improved the stereoselectivity of the bromination procedure significantly, and obtained **6.6** and **6.12** in a ratio of 11:2 and an isolated yield of 97%. The undesired axial product was efficiently removed from the mixture of diastereomers by semi-preparative reversed phase HPLC before continuing with the next step of the synthesis.

We now turned our attention to the hydride reduction of the bromoketone **6.6**. Contrary to the results obtained for this transformation when performed on the isophorone derivative **6.10** (which was quantitatively reduced to **6.11**), treatment of **6.6** with sodium borohydride and cerium chloride in methanol did not accomplish the desired transformation to the ***trans***-diequatorial bromohydrin **6.4**, with only starting material being recovered upon work-up of the reaction. Similar results were obtained when a large excess (4 equivalents) of sodium borohydride–cerium chloride was used and the reaction mixture heated at 50°C for 12 hours. When this transformation was attempted at reflux, there was no discernible reduction observed by TLC after 3 hours and continued heating (12 hours) yielded a complex mixture of products (by ^{1}H NMR spectroscopy) that was not investigated further. The apparent unreactivity of the C-7 carbonyl group in **6.6** to hydride reduction was unexpected from the results of both the model reactions performed on 6-bromo-3,5,5-trimethylcyclohex-2-enone (**6.10**) and the sodium borohydride reductions of hispanone derivatives reported in the previous chapter. Presumably the observed unreactivity of **6.6** is due to the increased steric hindrance provided by the equatorial bromine inhibiting axial hydride attack at C-7.

Attempts to prepare a 6,7-bromohydrin using more active hydride reagents (lithium aluminium hydride and diisobutylaluminium hydride) in a variety of solvents were similarly unsuccessful.

When the reductions were performed in ethereal solvents (diethyl ether and tetrahydrofuran) employing a standard aqueous work-up (saturated ammonium chloride), the product obtained was a yellow oil which rapidly darkened on standing at both room temperature and –20 °C. Analysis of this oil by NMR spectroscopy (Figure 6.4) and normal phase HPLC immediately after its isolation indicated that the reduction of **6.6** under these conditions yielded a complex mixture of at least ten different products (by HPLC).

Identical results were obtained when these reductions (using lithium aluminium hydride) were repeated employing milder conditions of work-up (quenching with water or pH 7 buffer) and when the crude reduction product was immediately treated with acetic anhydride–pyridine in an attempt to isolate the C-7,C-15-diacetate **6.14**. The reduction of **6.6** was also attempted with diisobutyl-aluminium hydride in dichloromethane and 2:1 dichloromethane/hexane. The reduction performed in dichloromethane gave similar results to those performed in ethereal solvents, but that performed in the dichloromethane/hexane mixture gave a 93% yield of rhinocerotinoic acid through nucleophilic substitution of the equatorial C-6 bromine substituent.



6.14

It therefore became obvious that due to steric effects and competing reactions, the preparation of the diequatorial bromohydrin **6.4** from rhinocerotinoic acid was not possible. Nevertheless, we still considered the formation of a 6,7-epoxide the best method of furnishing 6β,7α,15-trihydroxylabda-8,13***E***-diene from rhinocerotinoic acid. Our second strategy to this end involved the attempted transformation of the diequatorial vicinal diol **6.15** to a 6,7-epoxide. The conversion of ***trans***-vicinal diols to epoxides is well established in the literature and proceeds by activation of one hydroxyl group that is then displaced through nucleophilic attack by the second hydroxyl in a

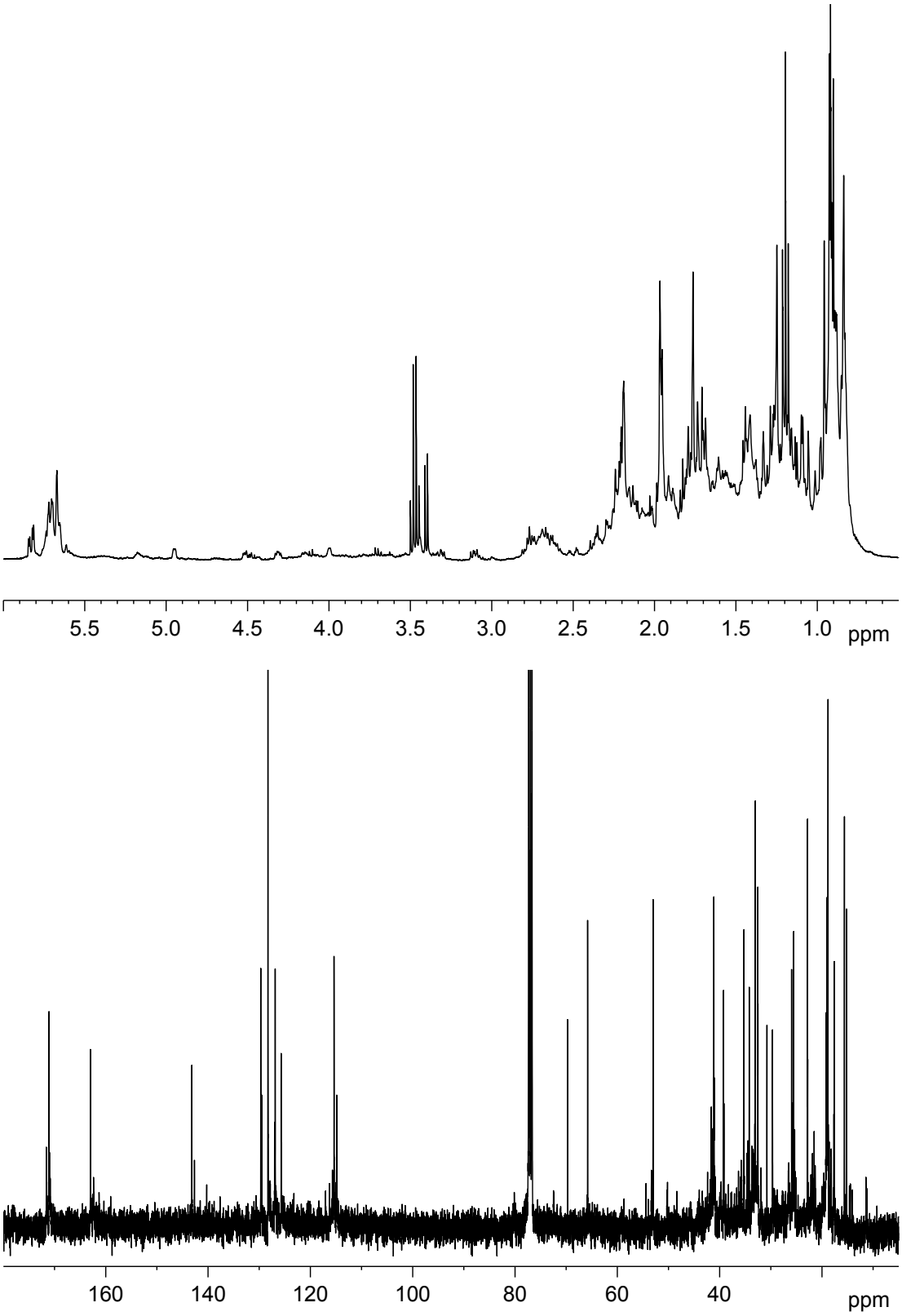
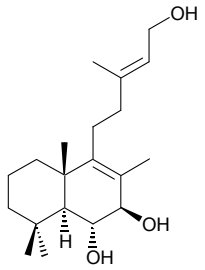


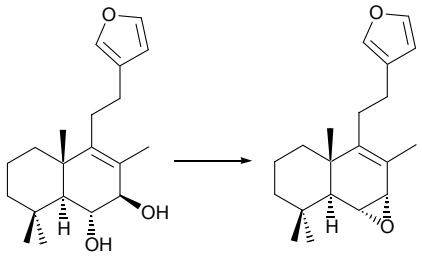
Figure 6.4 Typical ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra of the mixture of products obtained from hydride reduction of 6α-bromo-7-oxo-labda-8,13-dien-15-oic acid (**6.6**).

manner analogous to that observed for ***trans***-halohydrins. The simplest methods of accomplishing this transformation are ***via*** either the Mitsunobu reaction[228] or a one-pot tosylation-substitution approach[271] applied to a suitably oriented diol.



6.15

Although we could find no examples of the Mitsunobu procedure being used for the preparation of epoxides from conformationally constrained ***trans***-diequatorially substituted cyclohexan-1,2-diol derivatives, it has been used to successfully prepare epoxides from both ***quasi***-diaxial and ***quasi***-diequatorial 2´,3´-hydroxynucleosides.[272] The one-pot tosylation-substitution procedure, however, has been used to prepare methyl-2,3-anhydro-4,6-O-benzylidene-α-D-manopyranoside from the diequatorial diol methyl-4,6-O-benzylidene-β-D-glucopyranoside in high yield.[271] Examination of the space-filling model of **6.15** indicated that due to steric interactions, the activating agent would probably react preferentially with the C-7 hydroxyl group and ultimately yield the 6α,7α-epoxide **6.2** as the major product. Although we had reservations regarding the stereochemical outcome of subsequent nucleophilic cleavage of **6.2** (***vide supra***), we believed that careful choice of the correct reagent and solvent system would allow us to obtain at least some diaxial product from this transformation.[263] We therefore considered it a worthy exercise to investigate the potential of using the diequatorial diol **6.15** for the preparation of epoxide **6.2**. Since we had an adequate supply of the hispanone derivative 15,16-epoxylabda-8,13(16),14-trien-6α,7β-diol (**5.11**) prepared during the model study described in Chapter Five, we decided to use this compound to test the applicability of the Mitsunobu reaction[228] and Rao's one-pot epoxidation procedure[271] for the preparation of 6,7-epoxides from a 6α,7β-dihydroxylabda-8-ene skeleton (Scheme 6.4).



5.11

Scheme 6.4 Model reaction examining the potential for transforming a 6α,7β-dihydroxylated labda-8-ene into a 6α,7α-epoxide. ***Reagents and conditions***: PPh_3, DEAD, THF or benzene, RT; or NaH, TsCl, THF, 0 °C.

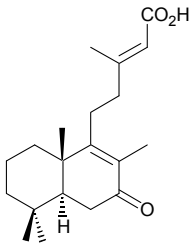
Treatment of the diol **5.11** with triphenylphosphine/diethyl azodicarboxylate in both anhydrous tetrahydrofuran and anhydrous benzene at room temperature and reflux yielded only the diequatorial starting material on work up of the reactions. The total unreactivity of **5.11** under Mitsunobu conditions can be explained by the inability of the required alkoxytriphenyl-phosphonium intermediate to form due to the crowded steric environment surrounding the C-6 and C-7 hydroxyl groups of **5.11**. We were similarly unsuccessful at preparing the desired 6,7-epoxide ***via*** the tosylation-displacement approach: treatment of **5.11** with sodium hydride and ***para***-toluenesulfonyl chloride in anhydrous tetrahydrofuran at 0 °C repeatedly gave a complex mixture of products (determined by ^{1}H NMR spectroscopy). We also considered attempting this transformation ***via*** a two-step approach, but treatment of **5.11** with ***para***-toluenesulfonyl chloride in pyridine also gave a complex mixture of products as a dark brown oil that probably consisted of a mixture of starting material, both monotosylates, the ditosylate and elimination products.

It was therefore evident that the preparation of a 6,7-epoxide from C-6 equatorially functionalised intermediates easily accessible from rhinocerotinoic acid (**4.14**) was not possible and we were grudgingly persuaded to cease our attempts to synthesise the ***Trimusculus*** diterpene **3.10** from **4.14**.

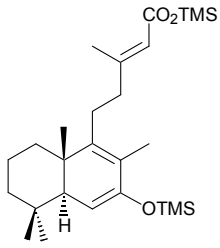
6.3 Attempted stereospecific synthesis of 6α,7β-diacetoxylabda-8,13*E*-dien-15-ol

Due to our apparent inability to easily transform a 6,7-diequatorially functionalised labda-8,13***E***-dien-15-oic acid skeleton into the desired intermediate labda-8,13***E***-dien-6β,7α,15-triol and the now considerable time constraints imposed upon the practical aspect of this research, we attempted the preparation of the 6,7-diepimeric isomer of the natural ***T. costatus*** metabolite **3.10**, 6α,7β-diacetoxylabda-8,13***E***-dien-15-ol (**6.1**), as shown in Scheme 6.5. Although we would gain no information regarding the absolute configuration of the ***Trimusculus*** diterpenes from the preparation of **6.1**, we would be able to compare some aspects of its biological activity with the data obtained for the natural isomer and thus gain some insight into how the stereochemistry of the B-ring affects the efficacy of this diterpene as a fish feeding deterrent and cytotoxic agent (see Chapter Three).

This synthetic route initially required the stereospecific α´-hydroxylation of **4.14**, and for this we employed the Rubottom procedure[255] previously applied in our model studies on hispanone (Chapter Five). It became immediately apparent, however, that this transformation could not be applied directly to **4.14** ***via*** trimethylsilyl 7-trimethylsiloxylabda-6,8,13***E***-trien-15-oate (**6.16**) as this intermediate, although observed by TLC, did not withstand the exchange of solvent necessary for the second step of the Rubottom procedure.



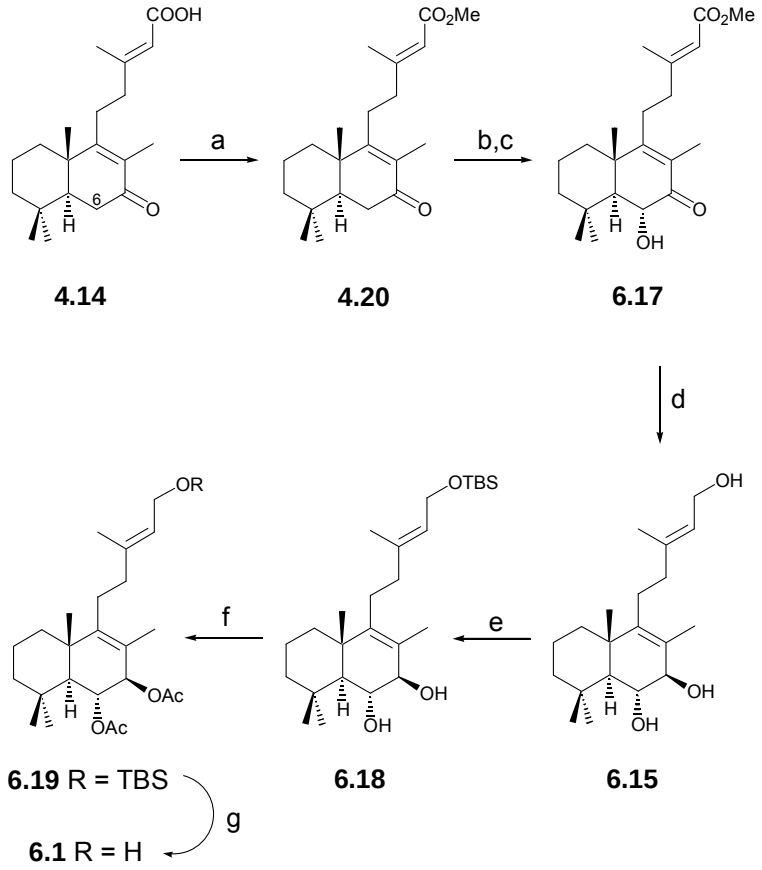
4.14



6.16

To overcome this problem we were forced to methylate **4.14** (see Scheme 6.5), a transformation that was achieved in quantitative yield with diazomethane solution to give methyl rhinocerotinoate (**4.20**) as a white crystalline solid (mp 56–58 °C). Methyl ester **4.20** could then be transformed to the desired product methyl 6α-hydroxy-7-oxo-labda-8,13***E***-dien-15-oate (**6.17**) in 77% yield by

application of the optimised Rubottom conditions formulated in Chapter Five. The hydroxy ketone **6.17** was then smoothly and cleanly reduced with diisobutylaluminium hydride to yield labda-8,13***E***-dien-6α,7β,15-triol (**6.15**) as a colourless oil in quantitative yield, the structure and stereochemistry of which was confirmed by careful analysis of the NMR data (see Table 6.2) and HRFABMS data (***m*/*z*** 323.2587 [$(M+1)^+$], Δmmu +0.1) obtained for this compound. Despite overlap of the H-7 and H_2-15 resonances in the ^{1}H NMR spectrum of the diequatorial reduction product, the stereochemistry at C-6 and C-7 was easily assigned from the magnitudes of the H-5–H-6 (***J*** = 12.1 Hz) and H-6–H-7 (***J*** = 8.3 Hz) coupling constants and the observation of key correlations (H-6 to H_3-19 and H_3-20 and H-7 to H-5) in the NOESY spectrum of **6.15**.



Scheme 6.5 Proposed route for the preparation of the 6,7-diepimer (**6.1**) of the ***Trimusculus costatus*** metabolite 6β,7α-diacetoxylabda-8,13-dien-15-ol (**3.10**). ***Reagents and conditions***: a. CH_2N_2, MeOH, 0 °C; b. LDA, TMSCl, THF, –78 °C - RT; c. MCPBA, CH_2Cl_2, 0 °C - RT; d. DIBAH, CH_2Cl_2, –78 °C; e. TBSCl, imidazole, DMAP, CH_2Cl_2, 0 °C; f. Ac_2O, pyridine, DMAP, RT; g. TBAF, THF, RT.

Table 6.2 1D and 2D NMR data ($CDCl_3$, ^1H: 400 MHz; ^{13}C: 100 MHz) obtained for labda-8,13***E***-dien-6α,7β,15-triol (**6.15**).

Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	HMBC correlation to	COSY coupling to
1	37.4 (t)	1.22 (1H, m) 1.83 (1H, br d, 11.9)	C-2, C-3, C-10, C-20	H_2-2
2	18.3 (t)	1.46 (1H, m) 1.56 (1H, br d, 12.7)	C-10	H_2-1, H_2-3
3	44.1 (t)	1.27 (1H, m) 1.42 (1H, br d, 12.6)	C-1, C-4, C-5, C-18	H_2-2
4	34.3 (s)			
5	54.2 (d)	1.60 (1H, d, 12.1)†	C-4, C-6, C-7, C-9, C-10, C-18, C-19	H-6
6	69.2 (d)	4.29 (1H, dd, 12.1, 8.3)†	C-5, C-7, C-10	H-5, H-7
7	79.8 (d)	4.13 (1H, d, 8.3)†	C-6, C-8, C-9	H-6
8	126.8 (s)			
9	143.9 (s)			
10	43.4 (s)			
11	26.9 (t)	1.98 (1H, m) 2.13 (1H, m)	C-8, C-9, C-10, C-12	H_2-12
12	39.2 (t)	2.04 (2H, m)	C-11, C-13, C-14, C-16	H_2-11
13	139.7 (s)			
14	123.1 (d)	5.40 (1H, tq, 6.8, 1.2)†	C-12, C-15, C-16	H_2-15, H_3-16
15	59.3 (t)	4.13 (2H, d, 6.8)†	C-13, C-14	H-14, H_3-16
16	16.3 (q)	1.68 (3H, d, 1.2)†	C-12, C-13, C-14	H-14, H_2-15
17	15.0 (q)	1.70 (3H, s)	C-7, C-8, C-9	
18	36.2 (q)	1.26 (3H, s)	C-3, C-4, C-5, C-19	
19	21.9 (q)‡	1.08 (3H, s)	C-3, C-4, C-5, C-18	
20	21.8 (q)‡	1.10 (3H, s)	C-1, C-5, C-9, C-10	

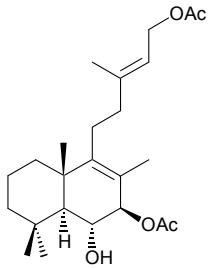
† Coupling constants confirmed by computer simulation.[232]

‡ Signals may be interchanged.

In order to prepare diacetate **6.1** through selective acetylation of the C-6 and C-7 hydroxyl groups in **6.15**, we first had to protect the C-15 primary hydroxyl group. Accordingly, the triol **6.15** was treated with ***tert***-butyldimethylsilyl chloride,[273] imidazole and catalytic 4-dimethylaminopyridine in dichloromethane[274] to afford 15-***tert***-butyldimethylsilanyloxylabda-8,13***E***-dien-6α,7β-diol (**6.18**) in 91% yield.

With the monoprotected triol **6.18** in hand, we considered it a trivial exercise to prepare the diacetate **6.19** (analogous to the hispanone derivative **5.25** prepared in Chapter Five) ***via*** the standard acylation procedure of Steglich and Höfle.[275] However, NMR spectroscopic examination of the product obtained after allowing **6.16** to stir overnight with 4-dimethylaminopyridine in acetic anhydride/pyridine indicated that acetylation had occurred at C-7 only [δ_H 5.64 (1H, br d, ***J*** = 8.6 Hz, H-7) and 2.11 (3H, s, 7-OAc)] to yield 7β-acetoxy-15-***tert***-butyldimethylsilanyloxylabda-8,13***E***-dien-6α-ol (**6.20**; see Figure 6.5). Although the acetylation procedure was repeated on **6.20** with heating (70 °C, 4 hours), ^{1}H NMR spectroscopy indicated that, once again, the C-6 hydroxyl group had not been esterified under these conditions. It should be recalled that a similar transformation performed on the analogous 6α,7β-dihydroxylated hispanone derivative **5.11** proceeded smoothly and rapidly without heating to give the expected diacetylated product in high yield (74%).

Finally, the C-7 monoacetate was treated once more with 4-dimethylaminopyridine in acetic anhydride/pyridine and the mixture heated at 100 °C for 24 hours. Work-up of the reaction in the usual manner followed by normal phase HPLC purification of the product allowed the isolation of 7β,15-diacetoxylabda-8,13***E***-dien-6α-ol (**6.21**; HRFABMS: ***m*/*z*** 406.2719 [M^+]), the NMR data of which were consistent with the protecting group at C-15 being replaced by acetate (Table 6.3).



6.21

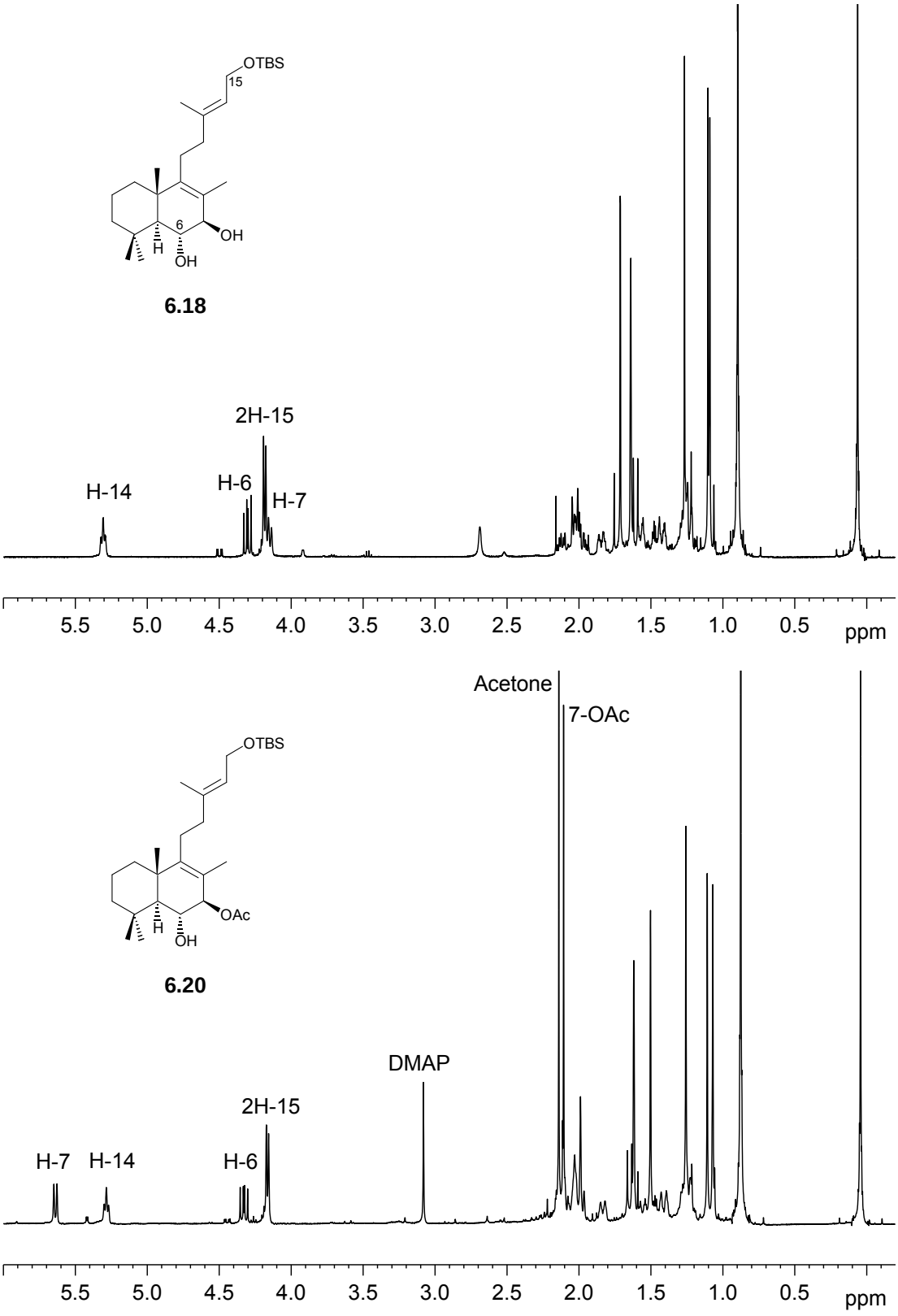


Figure 6.5 Comparison of the ^{1}H NMR spectra ($CDCl_3$, 400 MHz) obtained for 15-***tert***-butyldimethylsilanyloxylabda-8,13***E***-dien-6α,7β-diol (**6.18**; top) and the crude monoacetylated product **6.20** (structures shown inset).

Table 6.3 1D and 2D NMR data ($CDCl_3$, ^1H: 400 MHz; ^{13}C: 100 MHz) obtained for 7β,15-diacetoxylabda-8,13***E***-dien-6α-ol (**6.21**).

Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., ***J***/Hz)	HMBC correlation to	COSY Coupling to
1	37.3 (t)	1.24 (1H, m) 1.84 (1H, br d, 12.5)		H_2-2
2	18.6 (t)	1.49 (1H, m) 1.58 (1H, br d, 13.5)		H_2-1, H_2-3
3	44.1 (t)	1.27 (1H, m) 1.43 (1H, br d, 14.9)		H_2-2
4	34.5 (s)			
5	54.8 (d)	1.67 (1H, d, 12.2)	C-4, C-6, C-7, C-10, C-18, C-19, C-20	H-6
6	63.3 (d)	4.34 (1H, dd, 12.2, 8.6)	C-5, C-10	H-5, H-7
7	80.5 (d)	5.66 (1H, d, 8.5)	C-6, C-8, C-9, 7-OAc	H-6, H_3-17
8	124.7 (s)			
9	146.0 (s)			
10	43.1 (s)			
11	26.9 (t)	2.03 (1H, m) 2.14 (1H, m)	C-12	H_2-12
12	39.2 (t)	2.08 (2H, m)	C-11, C-13, C-14	H_2-11, H-14
13	142.0 (s)			
14	118.3 (d)	5.34 (1H, br t, 6.8)	C-12, C-16	H_2-12, H_2-15, H_3-16
15	61.3 (t)	4.58 (2H, d, 7.0)	C-13, C-14, 15-OAc	H-14, H_3-16
16	16.3 (q)	1.68 (3H, d, 1.2)	C-12, C-13, C-14	H-14, H_2-15
17	14.8 (q)	1.52 (3H, s)	C-7, C-8, C-9	H-7
18	36.3 (q)	1.28 (3H, s)	C-3, C-4, C-5, C-19	
19	21.9 (q)†	1.09 (3H, s)	C-3, C-4, C-5, C-18	
20	21.8 (q)†	1.13 (3H, s)	C-1, C-5, C-9, C-10	
7-OAc	171.0 (s)‡			
	21.1 (q)	2.13 (3H, s)§	7-OAc carbonyl	
15-OAc	171.1 (s)‡			
	21.1 (q)	2.04 (3H, s)§	15-OAc carbonyl	

†, ‡, § Signals may be interchanged.

Surprisingly then, in the rhinocerotinoic acid derivatives acetylation at C-6 would seen to be far more difficult than in the hispanone derivatives prepared in Chapter Five. The next step in the preparation of **6.1** would be to attempt the acetylation of **6.18** using the more active reagent acetyl chloride. Unfortunately this transformation could not be attempted because of lack of time, and we therefore had to bring the synthetic aspect of this research to a close.

6.4 Summary and Conclusions

In this chapter we endeavoured to apply some of the lessons learned from the model studies described in Chapter Five to the stereospecific synthesis of the ***T. costatus*** metabolite **3.10** from our chosen chiral template rhinocerotinoic acid (**4.14**). Aware that a 6,7-diequatorial substitution pattern could be easily obtained from a 7-oxo-labda-8-ene skeleton, we considered a synthetic route involving an efficient method of inverting the stereochemistry at C-6 and C-7 of a 6α,7β-functionalised labda-8,13***E***-diene the best means of attaining a 6β,7α-diaxially oxygenated derivative. The strategy that we chose to employ for this transformation entailed the preparation and subsequent cleavage of a 6,7-epoxylabda-8,13***E***-diene derivative, either ***via*** the ***trans***-diequatorial bromohydrin **6.4** or the ***trans***-diequatorial diol **6.15**. Unfortunately, neither of these routes proved effective in the formation of epoxides due to the rapid degradation of the key intermediate **6.4** and isolation of complex mixtures of products upon treatment of the model compound **5.11** with ***para***-toluenesulfonyl chloride.

We therefore decided to attempt the synthesis of the diequatorial 6α,7β-epimer (**6.1**) of **3.10**, which would allow us to compare the biological activities of the diequatorial versus the diaxial isomer. Although this route began successfully (the key intermediate **6.18** was prepared from **4.14** in 4 steps and 70% overall yield), we surprisingly could not acetylate the 6α-hydroxyl group of **6.18** using standard methodology that accomplished the same transformation on the model compound **5.11**. The reason for the conflicting behaviour observed between the closely related hispanone derivative **5.11** and rhinocerotinoic acid derivative **6.18** is unknown, and brings into

question the utility of hispanone as an appropriate model system that can be extrapolated to the rhinocerotinoic acid system.

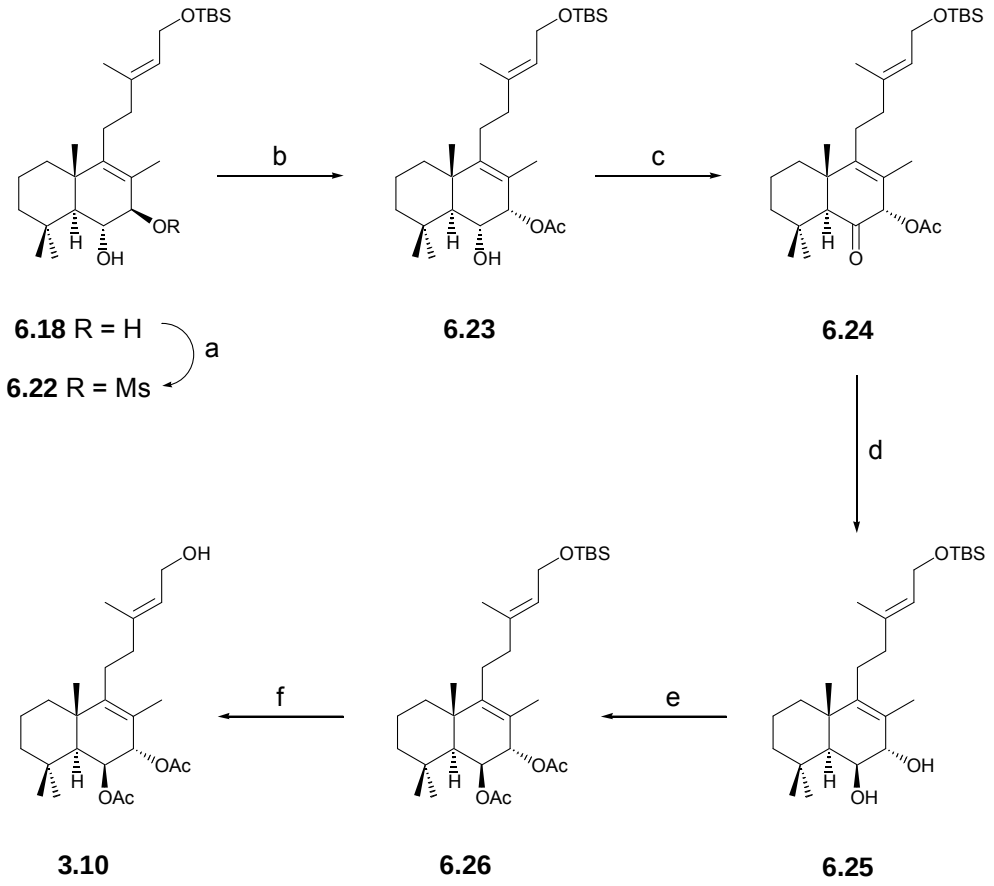
6.5 Avenues for future research

Unfortunately, due to the inevitable time constraints placed upon the laboratory aspect of this research, we were unable to complete the synthesis of both **3.10** and **6.1** from rhinocerotinoic acid (**4.14**). However, in the attempt we have learned valuable lessons regarding the preparation of 6,7-functionalised labda-8-enes from a 7-oxo-labda-8-ene template and can therefore propose a number of avenues for future research. The fact that we were unable to peracetylate **6.18** using acetic anhydride is a minor problem and would probably be easily over-come by employing acetyl chloride for this transformation. However, it is this observation that may be the key to the synthesis of **3.10** and other ***Trimusculus*** diterpenes from **4.14**. With hind-sight, because of the disparity observed between the reactivity of the hispanone derivative **5.11** and the rhinocerotinoic acid derivative **6.18**, it may have been more beneficial to perform the work described in Chapter Five on rhinocerotinoic acid or methyl rhinocerotinoate. However, at the time that this work was being performed hispanone seemed to be the ideal compound for our model studies, especially as we had not completely optimised the rhinocerotinoic acid synthesis when beginning the C-6–C-7 oxidation studies.

The synthetic route which we can now propose centres upon the fact that in **6.18** we would be able to selectively prepare the C-7 mesylate (or tosylate) **6.22**. Although **6.22** could then be used in an attempt to prepare the 6α,7α-epoxide **6.2** (a transformation which could not be completed on the corresponding hispanone derivative), due to the potential for this to revert to the diequatorial diol **6.18** upon nucleophilic cleavage of the oxirane (***vide supra***) we would prefer to follow the route outlined in Scheme 6.6 as follows.

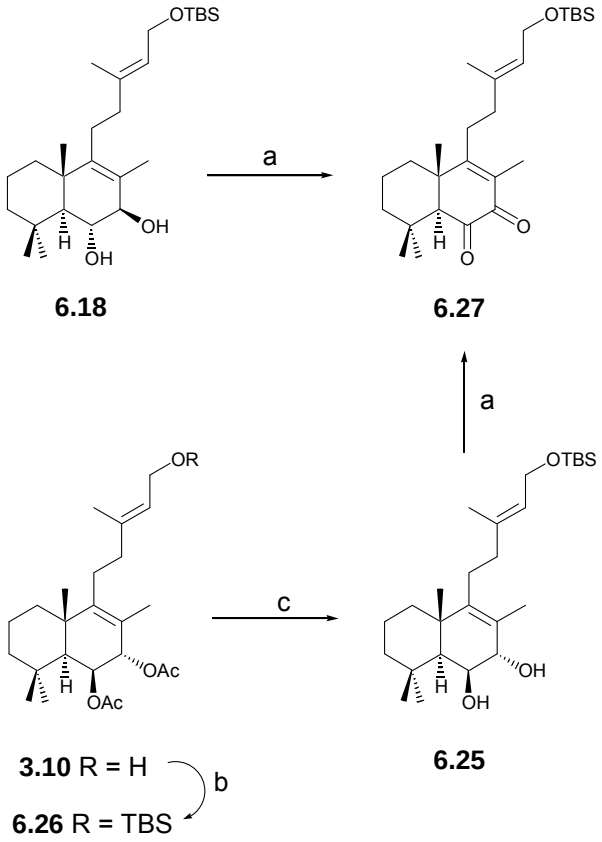
Inversion of the stereochemistry at C-7 of **6.18** could be achieved using a procedure such as the cesium acetate – 4-dimethylaminopyridine inversion procedure of Hawryluk and Snider[229] to yield

the diprotected triol **6.22**. Swern oxidation of **6.23** would then furnish the α-keto ester **6.24** which when reduced with diisobutyl aluminium hydride should furnish the diaxial diol **6.25** as the major, if not exclusive, product. Acetylation of **6.25** with either acetic anhydride/pyridine or acetyl chloride followed by tetrabutylammonium fluoride deprotection[273] of the C-15 primary hydroxyl group would furnish the desired product **3.10**. Although all of these transformations should proceed in reasonable yield, the conversion of rhinocerotinoic acid into 6β,7α-diacetoxylabda-8,13***E***-dien-15-ol ***via*** this strategy would require 10 steps; far more than we originally envisaged when undertaking the synthesis of **3.10**.



Scheme 6.6 Proposed route for the synthesis of 6β,7α-diacetoxylabda-8,13***E***-dien-15-ol (**3.10**) from 15-***tert***-butyldimethylsilanyloxy-6α,7β-diacetoxylabda-8,13***E***-diene (**6.16**). ***Reagents and conditions***: a. MsCl, pyridine, DMAP, RT; b. CsOAc, DMAP, toluene, Δ; c. ClCOCOCl, DMSO, Et_3N, CH_2Cl_2, −78 - 0 °C; d. DIBAH, CH_2Cl_2, −78 °C; e. Ac_2O, pyridine, DMAP; f. TBAF, THF, RT.

The final and probably most attainable suggestion for future work derived from that described here would be to use the experience gained during our synthetic work on 7-oxo-labda-8-ene systems to assign the absolute stereochemistry of the ***Trimusculus*** diterpenes (see Scheme 6.7). It is envisaged that **6.18** could be easily transformed to the 6,7-diketone **6.25**. Conversion of the ***T. costatus*** metabolite **3.10** to the same derivative through ***tert***-butyldimethylsilyl protection of the C-15 hydroxyl group followed by hydride reduction of the C-6 and C-7 acetates and oxidation of the resulting diol **6.25** would allow the optical rotations of the two diketones to be compared and hence unequivocally assign the absolute stereochemistry of **3.10**.



Scheme 6.7 Proposed strategy for the preparation of 15-***tert***-butyldimethylsilanyloxylabda-8,13***E***-dien-6,7-dione (**6.25**) from both 15-***tert***-butyldimethylsilanyloxy-6α,7β-diacetoxylabda-8,13***E***-diene (**6.18**) and the ***T***. ***costatus*** metabolite **3.10**. ***Reagents and conditions***: a. ClCOCOCl, DMSO, Et_3N, CH_2Cl_2, –78 - 0 °C; b. TBSCl, imidazole, DMAP, CH_2Cl_2, 0 °C; c. $LiAlH_4$, Et_2O, 0 °C.

Chapter Seven

Experimental

7.1 General experimental procedures

7.1.1 Analytical

The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded using standard pulse sequences on a Bruker AMX400 spectrometer (Chapters 2 and 3) and a Bruker Avance 400 spectrometer (Chapters 4, 5 and 6). Chemical shifts are reported in ppm and referenced to residual protonated solvent resonances. Coupling constants have been recorded directly from the NMR spectra and corresponding coupling constants have not been matched. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at the sodium-D line (589 nm). Following standard protocol, the concentration of solutions used to determine optical rotations is expressed in g/100 mL. Infra-red spectra were recorded on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer with compounds as films (neat) on NaCl discs. Low resolution mass spectra were recorded on a Hewlett-Packard 5988A (Chapter 3) or a Finnigan GCQ spectrometer at 70 eV. High resolution electron impact mass spectra (Micromass Autospec-TOF spectrometer) were obtained by Dr. P. Boschoff of the Mass Spectrometry Unit, Cape Technikon, Cape Town, South Africa. High resolution fast atom bombardment mass spectra (Micromass 70-70E spectrometer) were obtained by Prof. L. Fourie of the Mass Spectrometry Unit, Potchefstroom University for Christian Higher Education, Potchefstroom, South Africa. Melting points were determined using a Reichert hot-stage microscope and are uncorrected.

7.1.2 Chromatography

General laboratory solvents were distilled from glass before use. Analytical normal phase thin layer chromatography was performed on DC-Plastikfolien Kieselgel 60 F_{254} plates and analytical reverse phase thin layer chromatography was performed on DC-Ferigplatten RP18 F_{254} plates. Plates were viewed under UV light (254 nm) and developed by spraying with 10% H_2SO_4 in MeOH followed by heating. Open column chromatography was performed using Kieselgel 60 (70 – 230 mesh) silica gel and flash chromatography[276] was performed using Kieselgel 60 (230 – 400

mesh) silica gel. Normal phase semi-preparative HPLC separations were performed on a Whatman Magnum 9 Partisil 10 column using a Spectra-Physics Spectra-Series P100 isocratic pump and a Waters 410 Differential Refractometer. Reverse phase semi-preparative HPLC separations were performed on a Phenomenex Luna 10 μm C18 column using a Spectra-Physics IsoChrom LC pump and a Waters R401 Differential Refractometer.

7.1.3 Synthesis

All reactions requiring anhydrous conditions were conducted in flame-dried apparatus under an atmosphere of dry nitrogen or using an anhydrous calcium chloride guard tube. Dry solvents were prepared by standard procedures[277] and stored over the appropriate drying agent under an atmosphere of dry nitrogen. Immediately prior to their use in dry reactions, diethyl ether, tetrahydrofuran and benzene were distilled from sodium metal/benzophenone ketyl, and dichloromethane, acetonitrile and triethylamine were distilled from calcium hydride. Pyridine and dimethyl sulfoxide were distilled at reduced pressure from potassium hydroxide and calcium hydride respectively and stored over 4Å molecular sieves under nitrogen. Organic extracts were dried over anhydrous sodium sulfate or magnesium sulfate. All reactions were magnetically stirred. Lithium diisopropylamide was purchased as a 2.0 M solution in heptane, tetrahydrofuran and ethyl benzene and diisobutylaluminium hydride was purchased as a 1.0 M solution in heptane from Sigma-Aldrich S.A. (Pty) Ltd.

7.1.4 Molecular modelling

Molecular modelling was performed on a Silicon Graphics O2 computer employing the Molecular Simulations Incorporated Cerius2 4.5 platform and the universal force field (1.02). A molecular dynamics routine was used to explore conformational space and establish global energy minima for various possible isomeric structures and conformers of the molecule under examination. For each structure investigated, the local energy minimum conformation was calculated using the

Newton-Raphson algorithm. The local minimum conformer was then subjected to a molecular dynamics routine (constant n, V, T) of 500 annealing cycles (300 to 500 K), each cycle being quenched to a minimum energy conformation using a truncated Raphson algorithm. All of the dynamics runs performed yielded a single lowest energy conformer from more than 10 of the 500 annealing cycles, which was assumed to represent the global energy minimum conformation of the molecule.

7.1.5 Artemia salina cytotoxicity assays

7.1.5.1 Hatching of *Artemia salina* cysts

Brine shrimp cysts (Rainbow Aquarium and Pet Supplies, Port Elizabeth, South Africa) were hatched by incubation in filtered sea-water at 26 °C under constant aeration in the light for 48 h. Hatched instar II and III larvae were separated from cyst shells and unhatched cysts by phototaxis and used immediately.

7.1.5.2 The *Artemia* bioassay

Artemia salina acute toxicity (24 h) bioassays employed in bioassay guided fractionation were performed as described by Solis ***et al.***[73] Crude extracts and chromatographic fractions were tested in duplicate at concentrations of 400, 200, 100 and 50 μgmL^{-1}.

Median lethal concentrations (LC_{50}s) for pure compounds were estimated by analysis of ***A. salina*** mortality data from 12 geometrically diluted solutions across a concentration range of 400 - 12.5 μgmL^{-1}. All assays were replicated three times and included controls for solvent effects and natural mortality (approximately 20 ***A. salina*** larvae were exposed to each replicated treatment for 24 h). Assays were considered void if the natural mortality exceeded 10%. Inter-replicate

variation was examined using a χ^2 test for uniformity.[278] In all cases, variations in mortality between replicate concentrations were found to be not greater than that expected from errors of random sampling (2 degrees of freedom, $\chi^2 < 5.991$, $P > 0.05$). It was therefore considered reasonable to pool replicate data for further analysis.

Before estimation of the LC_{50}, data were adjusted for natural mortality and solvent effects using Abbott's formula.[121,278,279,280] Cognisant of the limitations relating to analysis of quantal response data using parametric models,[147,281] mortality data were analysed by both parametric (probit analysis) and non-parametric (trimmed Spearman-Karber) methods. Probit analysis[121,278] was performed by fitting data to the probit model using the maximum likelihood method.[282,283] When mortality data deviated significantly from the probit model (compounds **3.10** and **3.11**; Pearson's χ^2 goodness-of-fit, $P < 0.05$) a heterogeneity factor was used in the calculation of the 95% confidence intervals for the LC_{50}. Trimmed Spearman-Karber analysis[147] was performed using the Spearman-Karber estimator with 10% trimming.[284] In all cases, median lethal concentrations and 95% confidence intervals obtained by probit analysis were comparable to those obtained using the trimmed Spearman-Karber method.

7.2 Chapter Two Experimental

7.2.1 Irciniid sponge

7.2.1.1 Animal material

The Ircinidae sponge (class Demospongiae, subclass Ceractinomorpha, order Dictyoceratida) was collected at a depth of 17 m from the Malangaan Reef off Ponto do Oura, Mozambique in November 1995. A voucher specimen of the sponge is located in the marine invertebrate collection housed at Rhodes University (MOZ 95-090). The sponge was identified by M. K. Harper, formerly of the Scripps Institution of Oceanography, San Diego, California, USA.

7.2.1.2 Isolation procedures

The sponge was immediately frozen after collection and later freeze-dried (the dry mass of the sponge was not supplied to us by SmithKline-Beecham). All of the freeze-dried material was extracted with EtOAc to give a brown gum (8.40 g), a portion of which (83 mg) was subjected to normal phase HPLC (3:2 hexane/EtOAc) without preliminary fractionation to yield ilimaquinone (**2.1**, 55 mg) as an orange oil.

Ilimaquinone (2.1):[74,77,78,80-82] orange oil; $[\alpha]_D^{22}$ –17° (***c*** 0.52, $CHCl_3$), lit.[74] –23.2°; UV (MeOH) λ_{max} (log ε) 398 (2.59), 279 (4.14), 214 (4.13) nm; IR ν_{max} 3333 (br), 2972, 2927, 2852, 1644, 1609, 1450, 1381, 1349, 1233, 1198, 1034 cm^{-1}; ^1H & ^{13}C NMR data see Table 2.1; EIMS ***m/z*** (rel. int.) 358 [M^+] (12), 191 (18), 189 (18), 170 (31), 168 (100), 135 (22), 95 (41), 79 (21), 67 (29); HRFABMS ***m/z*** 358.2144 (calcd for $C_{22}H_{30}O_4$ [M^+], 358.2144).

7.2.1.3 Acetylation of ilimaquinone

Ilimaquinone (**2.1**, 12 mg) was dissolved in pyridine (0.5 mL) and Ac_2O (0.5 mL) and stirred at room temperature for 24 h. The reaction was quenched with MeOH (2.0 mL) and concentrated ***in vacuo*** to yield ilimaquinone monoacetate (**2.4**, 11 mg, 82% yield) as a yellow oil.

Ilimaquinone monoacetate (2.4):[74] yellow oil; $[\alpha]_D^{22}$ –13° (***c*** 0.52, $CHCl_3$), lit.[74] –8.3°; IR ν_{max} 2929, 2861, 1781, 1730, 1682, 1663, 1609, 1454, 1371, 1232, 1150, 1040 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.88 (1H, s, H-19), 4.46 (1H, br s, H-11a), 4.44 (1H, br s, H-11b), 3.82 (3H, s, H_3-22), 2.54 (1H, d, ***J*** = 13.3 Hz, H-15a), 2.41 (1H, d, ***J*** = 13.3 Hz, H-15b), 2.32 (3H, s, 17-OAc), 2.29 (1H, m, H-6a), 2.07 (1H, m, H-2a), 1.92 (1H, br d, ***J*** = 14.2 Hz, H-6b), 1.87 (1H, m, H-1a), 1.54 (1H, m, H-3a), 1.47 (1H, br d, ***J*** = 12.6 Hz, H-2b), 1.46 (2H, m, H_2-7), 1.35 (1H, m, H-3b), 1.21 (1H, m, H-1b), 1.18 (1H, m, H-8), 1.03 (3H, s, H_3-12), 0.91 (3H, d, ***J*** = 6.4 Hz, H_3-13), 0.83 (3H, s, H_3-14), 0.71 (1H, dd, ***J*** = 11.7, 1.2 Hz, H-10) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.8 (s, C-21),

179.3 (s, C-18), 167.9 (s, 17-OAc), 159.7 (s, C-20), 159.6 (s, C-4), 151.3 (s, C-17), 133.9 (s, C-16), 105.4 (t, C-11), 103.0 (d, C-19), 56.7 (q, C-22), 50.8 (d, C-10), 43.9 (s, C-9), 40.5 (s, C-5), 38.1 (d, C-8), 36.5 (t, C-3), 33.4 (t, C-6), 32.8 (t, C-15), 28.5 (t, C-1), 27.8 (t, C-7), 23.4 (t, C-2), 20.5 (q, 17-OAc & q, C-12), 18.1 (q, 13), 17.1 (q, C-14) ppm; EIMS ***m/z*** (rel. int.) 400 [M^+] (2), 358 (10), 212 (6), 210 (6), 168 (100), 135 (6), 95 (11), 79 (8), 67 (10); HRFABMS ***m/z*** 400.2249 (calcd for $C_{24}H_{32}O_5$ [M^+], 400.2250).

7.2.2 Haliclona sp.

7.2.2.1 Animal material

The ***Haliclona*** sponge (class Demospongiae, subclass Ceractinomorpha, order Haplosclerida, family Chalinidae) was collected intertidally from the underside of rockpool ledges at Ponto do Oura, Mozambique in November 1995. A voucher specimen of the sponge is located in the marine invertebrate collection housed at Rhodes University (MOZ 95-080). The sponge was once again identified by M. K. Harper, formerly of Scripps Institution of Oceanography.

7.2.2.2 Isolation procedures

The sponge was immediately frozen after collection and later freeze-dried (dry weight 184 g). All of the freeze-dried material was extracted with EtOAc to give a reddish-brown oil (3.65 g), a portion of which (1.93 g) was subjected to flash chromatography on silica gel in 1:1 hexane/EtOAc followed by a wash of EtOAc to elute a more polar band that had remained on the column. The fractions collected were combined according to their respective TLC profiles to yield five fractions, and, on the basis of their ^{1}H NMR spectra, fractions 2 (899 mg) and 5 (101 mg) were chosen for further investigation.

Fraction 2 was partitioned between MeOH (15 mL) and hexane (10 mL) to yield a MeOH soluble fraction as a dark red oil (479 mg). A portion of this oil (118 mg) was subsequently subjected to normal phase HPLC (7:3 hexane/EtOAc) to yield the known compounds renierone (**2.7**, 4 mg, 0.017% dry weight) as a yellow powder, ***N***-formyl-1,2-dihydrorenierone (**2.8**, 18 mg, 0.075% dry weight) as a deep red oil, and 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**2.9**, 3 mg, 0.013% dry weight) as yellow needles.

Fraction 5 from the initial flash chromatography column was further purified on an open column of silica (eluted with 4:1 EtOAc/hexane, EtOAc and 1:1 EtOAc/MeOH). The fraction eluting with EtOAc yielded a brown solid (42 mg), which was subsequently recrystalised from Et_2O-hexane to yield the known metabolite mimosamycin (**2.10**) as an orange powder (27 mg, 0.028% dry weight).

Renierone (2.7):[87,90-92] yellow solid; mp 89-91 °C, lit.[87] 91.5-92.5 °C; UV (MeOH) λ_{max} (log ε) 311 (3.62), 285 (3.84), 252 (3.86), 213 (4.33) nm; IR ν_{max} 2954, 1715, 1668, 1651, 1574, 1456, 1309, 1232, 1153, 1053, 869, 748 cm^{-1}; ^1H & ^{13}C NMR data see Table 2.2; EIMS ***m/z*** (rel. int.) 315 [M^+] (41), 301 (23), 286 (12), 273 (17), 258 (10), 232 (30), 216 (100), 204 (19), 82 (19); HRFABMS ***m/z*** 316.1184 (calcd for $C_{17}H_{18}NO_5$ [$(M + H)^+$], 316.1185).

N*-Formyl-1,2-dihydrorenierone (2.8):**[88,93] dark red oil; $[\alpha]_D^{21}$ –196° (c*** 0.09, MeOH), lit.[88] –227° (MeOH); UV (MeOH) λ_{max} (log ε) 509 (3.52), 338 (3.76), 262 (4.21), 209 (4.47) nm; IR ν_{max} 2947, 1704, 1651, 1557, 1436, 1230, 1185, 1146, 1048, 947 cm^{-1}; ^1H NMR data see Table 2.3; ^{13}C NMR data see Table 2.4; EIMS ***m/z*** (rel. int.) 345 [M^+] (4), 315 (18), 245 (7), 232 (72), 216 (13), 204 (100), 189 (12), 176 (12), 161 (10), 117 (10); HRFABMS ***m/z*** 346.1291 (calcd for $C_{18}H_{20}NO_6$ [$(M + H)^+$], 346.1291).

1,6-Dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (2.9):[88,92] mp 186-189 °C dec., lit.[88] 188-190 °C dec.; UV (MeOH) λ_{max} (log ε) 295 (3.71), 280 (3.95), 273 (3.86), 219 (4.23) nm; IR ν_{max} 2916, 1668, 1608, 1568, 1402, 1343, 1210, 1119, 923, 873, 750, 709 cm^{-1}; ^1H NMR

($CDCl_3$, 400 MHz) δ 8.83 (1H, d, ***J*** = 4.9 Hz, H-3), 7.79 (1H, d, ***J*** = 4.9 Hz, H-4), 4.13 (3H, s, H_3-11), 2.97 (3H, s, H_3-9), 2.07 (3H, s, H_3-10); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.0 (s, C-5), 182.1 (s, C-8), 160.2 (s, C-1), 158.8 (s, C-7), 153.7 (d, C-3), 138.9 (s, C-4a), 130.2 (s, C-6), 123.1 (s, C-8a), 117.3 (d, C-4), 61.2 (q, C-11), 25.6 (q, C-9), 9.0 (q, C-10); EIMS ***m/z*** (rel. int.) 217 [M^+] (11), 204 (15), 203 (100), 175 (31), 146 (30), 134 (9), 119 (14), 118 (22), 91 (13), 81 (11); HRFABMS ***m/z*** 218.0817 (calcd for $C_{12}H_{12}NO_3$ [$(M + H)^+$], 318.0817).

Mimosamycin (2.10):[88-92,96-98] mp 217-220 °C, lit.[89a] 219-221 °C; UV (MeOH) λ_{max} (log ε) 312 (4.02), 228 (4.11), 206 (4.36) nm; IR ν_{max} 2922, 1681, 1645, 1634, 1584, 1549, 1319, 1236, 1063, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.24 (1H, s, H-1), 7.06 (1H, s, H-4), 4.14 (3H, s, H_3-11), 3.64 (3H, s, H_3-9), 2.03 (3H, s, H_3-10); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.5 (s, C-5), 177.3 (s, C-8), 162.8 (s, C-3), 159.5 (s, C-7), 142.1 (d, C-1), 138.9 (s, C-6), 133.1 (s, C-4a), 116.7 (d, C-4), 111.3 (s, C-8a), 61.3 (q, C-11), 38.4 (q, C-9), 9.3 (q, C-10); EIMS ***m/z*** (rel. int.) 233 [M^+] (100), 218 (44), 205 (89), 190 (44), 177 (45), 162 (25), 148 (33), 134 (19), 118 (14); HRFABMS ***m/z*** 234.0766 (calcd for $C_{12}H_{12}NO_4$ [$(M + H)^+$], 234.0766).

7.2.3 Diplosoma sp.

7.2.3.1 Animal material

The green ***Diplosoma*** ascidian (class Ascidiacea, order Aplousobranchia, family Didemnidae) was collected intertidally in shallow rockpools from Ponto do Oura, Mozambique in November 1995. A voucher specimen of the sponge is located in the marine invertebrate collection housed at Rhodes University (MOZ 95-111). The ascidian was tentatively identified by Dr P. Coetze, formerly of the University of Port Elizabeth, Port Elizabeth, South Africa.

7.2.3.2 Isolation procedures

The ascidian was immediately frozen after collection and later freeze-dried (dry weight 578 g). All of the freeze-dried material was extracted with EtOAc to give a dark green oil (2.10 g), a portion of which (1.04 g) was initially partitioned between hexane (30 mL) and MeOH (50 mL). The MeOH soluble portion (782 mg) was then subjected to flash chromatography on silica gel using 1:1 EtOAc/hexane, 3:1 EtOAc/hexane and EtOAc. On removal of the solvent, the fraction eluting with 1:1 hexane/EtOAc was obtained as a brown semi-solid (177 mg), which was washed with cold hexane (10 mL) and cold MeOH (5.0 mL) to give a brown solid (98 mg). The solid was then dissolved in warm EtOAc (10 mL, approximately 40 °C) and 7***Z***-allylidene-5-hydroxy-7,7a-dihydro-2***H***-cyclopenta[***b***]pyran-6-one (**2.11**, 48 mg, 0.017% dry weight) was obtained as a sticky cream solid by precipitation on the dropwise addition of hexane.

7*Z*-Allylidene-5-hydroxy-7,7a-dihydro-2*H*-cyclopenta[*b*]pyran-6-one (2.11):[99] mp 264-267 °C (dec.); $[\alpha]_D^{28}$ +27° (***c*** 1.22, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 336 (4.02), 240 (4.30); IR ν_{max} 3269 (br), 2916, 1689, 1627, 1428, 1375, 1351, 1283, 1083, 1008, 929 cm^{-1}; ^1H & ^{13}C NMR data see Table 2.5; EIMS ***m/z*** (rel. int.) 190 [M^+] (11), 167 (12), 165 (9), 155 (28), 154 (100), 137 (61), 136 (79), 120 (19), 107 (36); HRFABMS ***m/z*** 191.0709 (calcd for $C_{11}H_{11}O_3$ [$(M + H)^+$], 191.0708).

7.2.3.3 Acetylation of 7***Z***-allylidene-5-hydroxy-7,7a-dihydro-2***H***-cyclopenta[***b***]pyran-6-one (**2.11**)

Compound **2.11** (6.8 mg) was dissolved in pyridine (0.5 mL) and Ac_2O (0.5 mL) and stirred at room temperature for 18 h. The reaction was quenched with MeOH, concentrated ***in vacuo*** and the resulting oil passed through a small column of silica gel to give a 1:6 mixture (by ^1H NMR spectroscopy) of 7***Z***- and 7***E***-allylidene-5-acetoxy-7,7a-dihydro-2***H***-cyclopenta[***b***]pyran-6-one (**2.13** and **2.14**) as a light brown oil (7.3 mg).

7*Z*-Allylidene-5-acetoxy-7,7a-dihydro-2*H*-cyclopenta[*b*]pyran-6-one (2.13):[99] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (1H, ddd, ***J*** = 17.1, 11.2, 10.0 Hz, H-9), 6.67 (1H, br d, ***J*** = 10.8 Hz, H-4), 6.62 (1H, m, H-8), 6.35 (1H, m, H-3), 5.64 (1H, m, H-10a), 5.58 (1H, d, ***J*** = 10.5 Hz, H-10b), 4.89 (1H, s, H-7a), 4.64 (1H, m, H-2a), 4.51 (1H, m, H-2b), 2.31 (3H, s, 5-OAc); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 186.4 (s, C-6), 167.5 (s, 5-OAc), 143.1 (s, C-7), 137.2 (d, C-8), 134.3 (d, C-3), 132.0 (d, C-9), 130.7 (s, C-5), 128.1 (s, C-4a), 127.8 (t, C-10), 117.9 (d, C-4), 71.9 (d, C-7a), 66.8 (t, C-2), 21.1 (q, 5-OAc).

7*E*-Allylidene-5-acetoxy-7,7a-dihydro-2*H*-cyclopenta[*b*]pyran-6-one (2.14):[99] IR ν_{max} 2921, 1769, 1689, 1634, 1369, 1344, 1262, 1089, 961, 856cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.09 (1H, dd, ***J*** = 11.5, 1.2 Hz, H-8), 6.88 (1H, ddd, ***J*** = 16.9, 11.3, 9.6 Hz, H-9), 6.64 (1H, dt, ***J*** = 9.5, 2.4 Hz, H-4), 6.35 (1H, ddd, ***J*** = 10.0, 4.0, 2.1 Hz, H-3), 5.73 (1H, d, ***J*** = 17.2 Hz, H-10a), 5.65 (1H, d, ***J*** = 9.9 Hz, H-10b), 5.11 (1H, s, H-7a), 4.62 (1H, dt, ***J*** = 18.6, 2.5 Hz, H-2a), 4.53 (1H, ddd, ***J*** = 18.6, 3.8, 1.4 Hz, H-2b), 2.31 (3H, s, 5-OAc); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.7 (s, C-6), 167.0 (s, 5-OAc), 142.8 (s, C-7), 137.6 (d, C-8), 134.3 (d, C-3), 132.5 (d, C-9), 132.2 (s, C-5), 128.1 (s, C-4a), 127.4 (t, C-10), 118.1 (d, C-4), 71.3 (d, C-7a), 66.8 (t, C-2), 20.5 (q, 5-OAc); EIMS ***m/z*** (rel. int.) 232 [M^+] (14), 191 (13), 190 (8), 165 (14), 159 (15), 154 (100), 149 (21), 136 (93), 121 (28), 107 (61); HRFABMS ***m/z*** 233.0614 (calcd for $C_{13}H_{13}O_4$ [$(M + H)^+$], 233.0814).

7.2.4 Cladiella kashmani

7.2.4.1 Animal material

C. kashmani (class Octocorallia, order Alcyonacea, family Alcyoniidae) was collected at a depth of 13 m from the Malangaan Reef off Ponto do Oura, Mozambique in October 1995. A voucher specimen of ***C. kashmani*** is located in the marine invertebrate collection housed at Rhodes University (MOZ 95-021). The soft coral was identified by Dr M Schleyer of the Oceanographic Research Institute, Durban, South Africa.

7.2.4.2 Isolation procedures.

The soft coral was immediately frozen after collection and later freeze-dried (440g). All of the freeze-dried soft coral was extracted with EtOAc to give a brown gum (11.7g), a portion of which (5.0g), was initially flash chromatographed on silica gel (gradient elution; 1:1, 3:2 and 4:1 EtOAc/hexane and 100% EtOAc). The fractions collected were combined on the basis of their TLC characteristics, and excessive amounts of cholesterol were removed from several of the flash chromatography fractions by crystallisation from MeOH. Subsequent fractionation was bioassay-guided, and further silica gel column chromatography (gradient elution; 100% hexane, 9:1, 4:1, and 7:3 hexane/EtOAc and 100% EtOAc) was necessary before selected fractions could be subjected to normal phase HPLC (8:2 and 7:3 hexane/EtOAc) to yield compounds **2.18** (27 mg, 0.014% dry weight), **2.19** (11mg, 0.006% dry weight) and **2.20** (25mg, 0.013% dry weight).

14*R*-Acetoxy-11*S*,12*S*-epoxycembra-1*Z*,3*E*,7*E*-trien-13*S*-ol (flaccidoxide, 2.18):[108] colourless oil; $[\alpha]_D^{21}$ +104° (***c*** 0.17, $CHCl_3$), lit.[108] +118°; UV (MeOH) λ_{max} (log ε) 250 (4.26) nm; IR ν_{max} 3464, 2929, 2868, 1741, 1456, 1371, 1240, 1065, 1034, 945, 868, 755 cm^{-1}; ^1H & ^{13}C NMR data see Table 2.6; EIMS ***m/z*** (rel. int.) 362 [M^+] (2), 302 (2), 193 (6), 163 (10), 151 (16), 139 (21), 137 (40), 123 (58), 109 (45), 95 (32), 81 (29); HREIMS ***m/z*** 362.2459 (calcd for $C_{22}H_{34}O_4$ [M^+], 362.2457); Toxic to ***Artemia salina***, probit analysis LC_{50} = 42 μgmL^{-1} (95% confidence intervals 38, 47), trimmed Spearman-Karber estimator LC_{50} = 45 μgmL^{-1} (95% confidence intervals 41, 50).

11*S*,12*S*-Epoxycembra-1*Z*,3*E*,7*E*-trien-14*S*-ol (2.19):[109,110] colourless oil; $[\alpha]_D^{21}$ +203° (***c*** 0.33, $CHCl_3$), lit.[109] +229°; UV (MeOH) λ_{max} (log ε) 253 (4.30) nm; IR ν_{max} 3421 (br), 2959, 2936, 1457, 1384, 1088, 1002, 878 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.97 (1H, d, ***J*** = 10.8 Hz, H-3), 5.76 (1H, d, ***J*** = 10.8 Hz, H-2), 5.10 (1H, m, H-7), 4.71 (1H, t, ***J*** = 4.9 Hz, H-14), 3.18 (1H, t, ***J*** = 6.8 Hz, H-11), 2.66 (1H, septet, ***J*** = 7.0 Hz, H-15), 2.44 (1H, m, H-9a), 2.21 (2H, m, H_2-6), 2.16 (2H, m, H_2-5), 2.11 (1H, m, H-13a), 2.07 (1H, m, H-9b) 1.97 (1H, m, H-13b), 1.73 (3H, s, H_3-18), 1.58 (3H, s, H_3-19), 1.54 (2H, m, H_2-10), 1.29 (3H, s, H_3-20), 1.10 (3H, d, ***J*** = 7.0 Hz, H_3-16), 1.08 (3H, d, ***J*** = 7.0 Hz, H_3-17) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.5 (s, C-1), 136.7 (s, C-4), 133.6 (s, C-8),

126.9 (d, C-7), 119.6 (d, C-2), 118.3 (d, C-3), 65.8 (d, C-14), 60.0 (s, C-12), 58.6 (d, C-11), 42.2 (t, C-13), 38.4 (t, C-5), 36.5 (t, C-9), 27.7 (d, C-15), 25.0 (t, C-6), 24.2 (q, C-16), 24.1 (t, C-10), 23.8 (q, C-17), 19.4 (q, C-20), 17.2 (q, C-18), 15.0 (q, C-19) ppm; EIMS ***m/z*** (rel. int.) 304 [M^+] (2), 193 (8), 163 (10), 137 (16), 123 (40), 109 (31), 95 (39), 81 (33), 71 (29), 55 (23), 43 (100); HREIMS ***m/z*** 304.2413 (calcd for $C_{20}H_{32}O_2$ [M^+], 304.2402); Toxic to ***Artemia salina***, probit analysis LC_{50} = 107 μgmL^{-1} (95% confidence intervals 91, 127), trimmed Spearman-Karber estimator LC_{50} = 95 μgmL^{-1} (95% confidence intervals 75, 120).

13*S*,14*R*-Diacetoxy-11*S*,12*R*-epoxycembra-1*Z*,3*E*,7*E*-triene (2.20): colourless oil; $[\alpha]_D^{21}$ +158° (***c*** 0.78, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 252 (4.30) nm; IR ν_{max} 2962, 2931, 1745, 1437, 1372, 1243, 1224, 1028, 965 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2.6; EIMS ***m/z*** 404 [M^+] (3), 233 (18), 213 (25), 191 (24), 152 (33), 137 (29), 121 (31), 119 (25), 109 (76), 95 (38), 93 (23), 81 (30), 43 (100); HREIMS ***m/z*** 404.2551 (calcd for $C_{24}H_{36}O_5$ [M^+], 404.2562); Toxic to ***Artemia salina***, probit analysis LC_{50} = 180 μgmL^{-1} (95% confidence intervals 142, 243), trimmed Spearman-Karber estimator LC_{50} = 181 μgmL^{-1} (95% confidence intervals 121, 272).

7.2.4.3 Acetylation of flaccidoxide (**2.18**)

Compound **2.18** (10.8 mg) was dissolved in pyridine (0.5 mL) and Ac_2O (0.5 mL) and stirred at room temperature for 48 h. Excess pyridine and Ac_2O was removed under reduced pressure to give a brown oil (12.0 mg). Normal phase HPLC (7:3 hexane/EtOAc) of the crude product yielded a yellow oil (2.6 mg, $[\alpha]_D^{21}$ +162°) which was identical to the diacetate **2.20** in all respects.

7.2.4.4 Preparation of the ***R***- and ***S***-MTPA esters of flaccidoxide (**2.18**)

R-α-Methoxy-α-trifluoromethylphenylacetic acid (23.6 mg), DCC (35.5 mg) and DMAP (7.1 mg) were added to a solution of **2.18** (6.2 mg) in dry CH_2Cl_2 (1.0 mL). The solution was stirred

overnight at room temperature, diluted with EtOAc (5.0 mL) and H_2O (0.5 mL) and filtered. The resulting solution was washed with 0.2 M HCl (5.0 mL), H_2O (5.0 mL), saturated $NaHCO_3$ (5.0 mL) and H_2O (5.0 mL). The EtOAc solution was dried over anhydrous Na_2SO_4, the solvent removed under reduced pressure and the resultant oil further purified by normal phase HPLC (4:1 Hexane/EtOAc) to yield the ***R***-MTPA ester **2.22** as a colourless oil (1.2 mg).

The ***S***-MTPA ester **2.23** (0.6 mg) was prepared in the same manner as above.

R*-MTPA ester of flaccidoxide (2.22):** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 & 7.42 (2H, m & 3H, m respectively, 13-OMTPA), 6.30 (1H, d, ***J = 11.5 Hz, H-2), 6.09 (1H, d, ***J*** = 11.5 Hz, H-3), 5.82 (1H, d, ***J*** = 9.2 Hz, H-14), 5.70 (1H, d, ***J*** = 9.1 Hz, H-13), 5.18 (1H, m, H-7), 3.58 (3H, s, 13-OMTPA), 3.10 (1H, br d, ***J*** = 8.8 Hz, H-11), 2.66 (1H, m, H-15), 2.30 (1H, m, H-6a), 2.23 (1H, m, H-9a), 2.20 (1H, m, H-5a), 2.15 (1H, m, H-9b), 2.13 (1H, m, H-6b), 2.09 (1H, m, H-5b), 1.80 (3H, s, 14-OAc), 1.76 (3H, s, H_3-18), 1.62 (1H, m, H-10a), 1.42 (3H, s, H_3-19), 1.25 (1H, m, H-10b), 1.20 (3H, s, H_3-20), 1.07 (3H, d, ***J*** = 6.8 Hz, H_3-16), 1.05 (3H, d, ***J*** = 6.8 Hz, H_3-17).

S*-MTPA ester of flaccidoxide (2.23):** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 & 7.41 (2H, m & 3H, m respectively, 13-OMTPA), 6.29 (1H, d, ***J = 11.6 Hz, H-2), 6.07 (1H, d, ***J*** = 11.7 Hz, H-3), 5.79 (1H, d, ***J*** = 9.2 Hz, H-14), 5.70 (1H, d, ***J*** = 9.2 Hz, H-13), 5.18 (1H, m, H-7), 3.57 (3H, s, 13-OMTPA), 3.09 (1H, br d, ***J*** = 8.6 Hz, H-11), 2.59 (1H, m, H-15), 2.29 (1H, m, H-6a), 2.26 (1H, m, H-9a), 2.19 (1H, m, H-5a), 2.19 (1H, m, H-9b), 2.11 (1H, m, H-6b), 2.09 (1H, m, H-5b), 1.76 (3H, s, H_3-18), 1.65 (1H, m, H-10a), 1.52 (3H, s, 14-OAc), 1.44 (3H, s, H_3-19), 1.32 (1H, m, H-10b), 1.25 (3H, s, H_3-20), 1.06 (3H, d, ***J*** = 6.8 Hz, H_3-16), 1.05 (3H, d, ***J*** = 6.8 Hz, H_3-17).

7.3 Chapter Three Experimental

7.3.1 Collection and ecological evaluation of Trimusculus costatus

In January 1996, a collection of ***T. costatus*** (class Gastropoda, order Basommatophora, family Trimusculidae) was made from the seaward edge of a rocky platform at Cintsa West (near East London) on the south-east coast of South Africa.[143] A total of 422 animals were collected (approximately 50% of a single colony) and many of the limpets were found to be encrusted with a thin algal film, spiral fanworms (***Spirorbis*** spp.) and plum anemones (***Actinia equina***).[144] The shells were therefore rapidly cleaned with a scalpel before the whole animals were immersed in acetone. Following extraction, a random sub-sample (***N*** = 100) of this collection was made to assess the mean shell length and mean dry weight (without shell; dried ***in vacuo*** overnight) of the individuals in this collection.

A second ***T. costatus*** collection (806 animals) was made in August 1996 from a separate colony also situated at Cintsa West. This second colony was found on the roof of a natural rock arch near the seaward edge of the reef platform approximately 2 km west of the initial collection site. The dimensions of the arch (approximately 0.8 m wide and 0.3 – 0.4 m high) allowed this colony to be ecologically surveyed whilst lying supine on the reef platform prior to collection of animals. The area inhabited by the ***Trimusculus*** colony was estimated by duplicating its dimensions with a sheet of aluminium foil, the area of which was then measured to the nearest square centimetre using a large sheet of gridded paper. A gross survey of the colony was made in which the number of large invertebrates growing on the limpets was recorded. In addition, the number of limpets, their percentage cover and the percentage cover of other intertidal invertebrates was recorded within, and immediately adjacent to, the colony using randomly placed 10 cm $\times$ 10 cm quadrats (***N*** = 10). The animals were collected in the same manner as in January 1996, although the first 100 individuals removed from the rock face were examined for fouling organisms and this data recorded. Once collected, the animals were again immediately immersed in acetone. The mean shell length and dry mass of a sub-sample (***N*** = 100) of this collection was measured as before.

Voucher specimens from both collections (January 1996: TRI 96-001; August 1996: TRI 96-002) are housed in the marine invertebrate collection at Rhodes University. Both collections were identified by Professor Alan Hodgson, Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa.

7.3.2 Extraction and isolation of Trimusculus costatus metabolites

After collection, the first sample of limpets (TRI 96-001) was steeped in acetone at –20 °C for one week before the acetone extract was decanted and concentrated ***in vacuo*** to yield an aqueous suspension which was partitioned between EtOAc (3 × 50 mL) and H_2O (50 mL). The EtOAc fractions were combined, dried and concentrated to give a green oil (2.03 g). The entire organic extract was subjected to silica gel flash chromatography (3:2 hexane/EtOAc) and the fractions obtained combined according to their TLC profiles to give five main fractions. Of these fractions only the second (583 mg) and third (486 mg) were active against ***Artemia salina*** and subjected to further chromatography.

A portion (317 mg) of the second fraction was applied to a column of silica gel employing gradient elution (4:1, 7:3, 3:2, and 1:1 hexane/EtOAc) with the bioactive constituent being confined to the fraction (257 mg) eluting with 3:2 hexane/EtOAc. A portion (126 mg) of the bioactive fraction was finally subjected to normal phase semi-preparative HPLC (3:2 hexane/EtOAc) to yield 6β,7α-diacetoxylabda-8,13***E***-dien-15-ol (**3.10**, 57 mg, 0.50 mg/animal).

The third fraction from the initial flash column did not require further purification before it could be subjected to normal phase HPLC and as such a portion (101 mg) was thus purified (2:3 hexane/EtOAc) to give 2α,6β,7α-triacetoxylabda-8,13***E***-dien-15-ol (**3.11**; 61 mg; 0.69 mg/animal)

The second ***Trimusculus*** collection (TRI 96-002) was initially treated in the same manner to yield a crude organic extract (2.37 g) as a green oil which was subjected to silica gel flash

chromatography (hexane, 9:1, 4:1, 3:2, 1:1 and 2:3 hexane/EtOAc, and EtOAc). Fractions were collected according to the eluting solvent, and the ***A. salina*** bioassay indicated that the biologically active constituents were confined to the fractions eluting with 3:2, 1:1 and 2:3 hexane/EtOAc (494, 253 and 457 mg respectively). These three fractions were subjected to normal phase semi-preparative HPLC in either 3:2 or 2:3 hexane/EtOAc to give a total of eighteen fractions which were all tested for biological activity in the ***A. salina*** bioassay. Of all eighteen HPLC fractions, the only compounds found to possess significant bioactivity were **3.10** (261 mg in total, 0.32 mg/animal) and **3.11** (275 mg in total, 0.34 mg/animal); all other fractions were inactive, did not exhibit interesting ^{1}H NMR spectra, and were therefore not analysed further.

6β,7α-Diacetoxylabda-8,13*E*-dien-15-ol (3.10): colourless oil; $[\alpha]_D^{27}$ +93º (***c*** 0.69, $CHCl_3$); IR ν_{max} 3460 (br), 2920, 2860, 1740, 1670, 1440, 1365, 1240, 1010, 920 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3.2; EIMS ***m/z*** (rel. int.) 328 (2), 260 (38), 246 (10), 219 (70), 205 (54), 135 (57), 83 (29), 57 (15), 43 (100); HREIMS ***m/z*** 406.2729 (calcd for $C_{24}H_{38}O_5$ [M$^+$], 406.2717).

2α,6β,7α-Triacetoxylabda-8,13*E*-dien-15-ol (3.11): colourless oil; $[\alpha]_D^{28}$ + 62º (***c*** 0.43, $CHCl_3$); IR ν_{max} 3480 (br), 2940, 2870, 1740, 1715, 1470, 1365, 1235, 1025, 940 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3.3; EIMS ***m/z*** (rel. int.) 318 (16), 276 (11), 217 (56), 216 (36), 203 (22), 161 (19), 135 (14), 55 (27), 43 (100); HREIMS ***m/z*** 464.2760 (calcd for $C_{26}H_{40}O_7$ [M$^+$], 464.2772).

7.3.3 Fish feeding deterrence assays

Assays were performed employing a common South African predatory fish, the spotted grunter ***Pomadasys commersonnii***. Twelve small ***P. commersonnii*** (12 – 16 cm in length) were held in separate, opaque-sided compartments of re-circulating aquaria under ambient light and temperature conditions and were trained to accept krill pellets (mean weight ± standard deviation = 50 ± 4 mg [***N*** = 50]) in the laboratory. In a single assay fish were randomly offered either a treated or control food pellet, followed by the other choice. When the second pellet was treated

and was rejected, a further control pellet was offered to determine whether the fish had ceased feeding. In every case when this was necessary, the third pellet was consumed by fish. Pellets were considered rejected if regurgitated three or more times. The significance of differences in the consumption of treated versus control pellets was evaluated at the 5% level with the Fisher exact test.[152] For any single assay performed on twelve fish, an extract was considered significantly deterrent if four or more fish rejected treated pellets but accepted control pellets ($P < 0.047$, 1-tailed test). A single assay was performed with freshly prepared pellets each morning, with the fish being fed untreated pellets each evening.

Food pellets were prepared by applying a measured volume of an Et_2O solution of the pure compounds (**3.10** or **3.11**) or the crude organic extract to food pellets to reach the desired amount per pellet. Control pellets were treated with the corresponding volume of Et_2O alone. Pellets were then dried under nitrogen and placed in a vacuum dessiccator for thirty minutes before being offered to fish. Compounds **3.10** and **3.11** and the crude extract were tested at concentrations (in ascending order) of 0.05, 0.10, 0.25, 0.5, 1.0 and 2.5 mg/pellet (corresponding to 1, 2, 5, 10, 20 and 50 ppt respectively) in 18 separate assays.

7.4 Chapter Four Experimental

7.4.1 Attempted isolation of rhinocerotinoic acid (4.14) through extraction of Elytropappus rhinocerotis

A sample of the aerial parts of ***E. rhinocerotis*** (identified by Mrs Estelle Brink of the Albany Museum Herbarium, Grahamstown, South Africa) was collected at Burnt Kraal near Grahamstown in early November 1999. Some of the collection was dried in a ventilated oven at 45 °C for 48 h and a portion of the dried material (312 g) was extracted into acetone (3 L) employing a Soxhlet apparatus. The acetone extract was concentrated ***in vacuo*** to give a dark green waxy solid (11.8 g). As Dekker ***et al.*** did not report details of the procedure employed for

the isolation of rhinocerotinoic acid (**4.14**) from ***E. rhinocerotis***,[162] we followed the protocol used by Jolad ***et al.*** to isolate a related labdanoic acid, grindellic, acid from ***Isocoma tenuisecta***.[285]

The green waxy solid was stirred with Et_2O (500 mL) for 30 min and the resulting solution allowed to stand overnight at 5 °C after which the deposited waxy precipitate was removed by filtration. A portion of the green filtrate (10 mL) was extracted with 5% $NaHCO_3$ solution (10 mL), the aqueous phase acidified with conc. HCl and the resulting precipitate extracted with Et_2O (20 mL). The organic phase was washed once with water (5 mL), dried (Na_2SO_4) and concentrated ***in vacuo*** to give a light brown oil (0.57 g). A second portion of the filtrate was similarly extracted with 5% Na_2CO_3 (10 mL) to give a brown oil (0.39 g). Both acidic extracts from ***E. rhinocerotis*** were examined by ^{1}H NMR spectroscopy, but no resonances corresponding to those reported for rhinocerotinoic acid[162] were observed in the spectra obtained.

To ensure that an ester of **4.14** was not present in the ***E. rhinocerotis*** extract, the residual Et_2O solutions from the base extractions were combined, concentrated and a portion saponified. Accordingly, the neutral material (0.37 g) was dissolved in EtOH (5.0 mL), 5 M KOH (2.0 mL) added and the resulting solution heated under reflux for 18 h. The EtOH was distilled off ***in vacuo***, H_2O (10 mL) added and the resulting aqueous suspension extracted with Et_2O (10 mL) to remove neutral material. The aqueous phase was then acidified with conc. HCl and extracted with Et_2O (10 ml). The organic phase was dried (Na_2SO_4) and concentrated ***in vacuo*** to give a green oil (0.14 g) that was deemed to not contain **4.14** by ^{1}H NMR spectroscopy.

7.4.2 Synthesis of rhinocerotinoic acid (4.14)

7.4.2.1 Potassium permanganate oxidation of (–)-sclareol (**4.15**)

Sclareol (25.0 g, 81.2 mmol) was dissolved in a mixture of acetone (2.5 L) and H_2O (25 mL) contained in 4 L flask which had previously been rinsed with 5% aqueous Na_2CO_3. The mixture was cooled to a temperature of 15–20 °C and finely powdered $KMnO_4$ (45.0 g, 285 mmol, 3.5 eq)

was added in portions over 2 h. The reaction mixture was stirred at 15–20 °C for 12 h and then at ambient temperature overnight after which 3.5 M NaOH (50 mL) was added and most of the acetone (approximately 1.5 L) removed ***in vacuo***. The resulting heterogeneous mixture was separated by carefully decanting the supernatant solution from the fine MnO_2 precipitate and filtering it through a large plug of cotton wool. The MnO_2 residue was washed with acetone (3 × 500 mL), the washings decanted, filtered as above and combined with the initial filtrate. The acetone from the filtered solution was removed ***in vacuo***, H_2O (50 mL) was added and the resulting aqueous slurry extracted with EtOAc (3 × 100 mL). The organic phases were combined, washed with H_2O (3 × 10 mL), dried and concentrated to give a mixture of 8α-hydroxy-14,15-bisnorlabda-13-one (**4.16**) and 8α,13-epoxy-14,15-bisnorlabda-12-ene (**4.23**) as an oily white solid (17.4 g). The crude product mixture was separated by washing with ice-cold hexane (3 × 10 mL), which left **4.16** (15.9 g, 56.8 mmol, 70 %) as a solid residue. Combination and evaporation of the hexane washings afforded **4.23** (1.43 g, 5.46 mmol, 7 %) as a colourless oil that could be crystallised from cold MeOH (0 °C).

(+)-8α-Hydroxy-14,15-bisnorlabda-13-one (4.16):[167-169,176] white solid; mp 81–82 °C, lit.[167,168] 78-80 °C; $[\alpha]_D^{27}$ +5° (***c*** 1.08, $CHCl_3$), lit.[169] +6.7°; IR ν_{max} 3423 (br), 2925, 2859, 1713, 1448, 1388, 1156, 1117, 1075, 1019 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.60 (2H, m, H_2-12), 2.26 (1H, br s, 8-OH), 2.11 (3H, s, H_3-16), 1.85 (1H, dt, ***J*** = 12.4, 3.2 Hz, H-7a), 1.72 (1H, m, H-11a), 1.66 (1H, m, H-2a), 1.62 (1H, m, H-1a), 1.59 (1H, m, H-6a), 1.52 (1H, m, H-11b), 1.42 (1H, m, H-6b), 1.39 (1H, m, H-7b), 1.34 (1H, m, H-3a), 1.25 (1H, m, H-2b), 1.14 (3H, s, H_3-17), 1.12 (1H, m, H-3b) 1.07 (1H, t, ***J*** = 4.2 Hz, H-9), 0.91 (1H, m, H-1b), 0.88 (1H, m, H-5), 0.86 (3H, s, H_3-20), 0.79 (3H, s, H_3-18) 0.77 (3H, s, H_3-19) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.4 (s, C-13), 73.7 (s, C-8), 60.7 (d, C-9), 56.1 (d, C-5), 46.3 (t, C-12), 44.5 (t, C-7), 41.9 (t, C-3), 40.1 (t, C-1), 39.3 (s, C-10), 33.3 (q, C-20), 33.3 (s, C-4), 30.0 (q, C-16), 24.1 (q, C-17), 21.5 (q, C-19), 20.5 (t, C-2), 18.8 (t, C-11), 18.4 (t, C-6), 15.2 (q, C-18) ppm; EIMS ***m/z*** (rel. int.) 280 [M^+] (2), 262 (77), 244 (41), 191 (56), 229 (95), 191 (56), 177 (46), 121 (63), 109 (100), 95 (91); HRFABMS ***m/z*** 280.2405 (calcd for $C_{18}H_{32}O_2$ [M^+], 280.2402).

(+)-8α,13-Epoxy-14,15-bisnorlabda-12-ene (4.23):[167,169,175,176] fine white solid; mp 43–45 °C, lit.[167] 44–46 °C; $[\alpha]_D^{26}$ +4° (***c*** 3.53, $CHCl_3$), lit.[175] +3°; IR ν_{max} 2925, 2867, 1683, 1449, 1377, 1179, 1127, 1022, 995, 870 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.42 (1H, br d, ***J*** = 3.7 Hz, H-12), 1.92 (2H, dt, ***J*** = 12.3, 3.3 Hz, H_2-7), 1.81 (2H, m, H_2-11), 1.77 (1H, br t, ***J*** = 2.1 Hz, H-6a), 1.67 (3H, s, H_3-16), 1.59 (1H, m, H-2a), 1.56 (2H, m, H_2-1), 1.43 (1H, m, H-2b), 1.39 (1H, m, H-9), 1.37 (2H, m, H_2-3), 1.30 (1H, dd, ***J*** = 12.2, 3.3 Hz, H-6b), 1.15 (3H, s, H_3-17), 0.97 (1H, dd, ***J*** = 12.2, 2.1 Hz, H-5), 0.87 (3H, s, H_3-20), 0.81 (6H, s, H_3-18 & H_3-19) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.9 (s, C-13), 94.6 (d, C-12), 76.2 (s, C-8), 56.2 (d, C-5), 52.5 (d, C-9), 42.0 (t, C-3), 41.2 (t, C-2), 39.3 (t, C-1), 36.7 (s, C-10), 33.5 (q, C-20), 33.2 (s, C-4), 21.6 (q, C-19), 20.4 (q, C-18), 20.1 (q, C-17), 19.8 (t, C-6), 18.6 (t, C-2), 18.3 (t, C-11), 15.0 (q, C-18) ppm; EIMS ***m/z*** (rel. int.) 262 [M^+] (95), 244 (45), 229 (98), 191 (67), 135 (54), 121 (67), 109 (98), 95 (100), 81 (97); HRFABMS ***m/z*** 262.2294 (calcd for $C_{18}H_{30}O$ [M^+], 262.2297).

7.4.2.2 Conversion of (+)-8α-hydroxy-14,15-bisnorlabda-13-one (**4.16**) to (+)-8α,13-epoxy-14,15-bisnorlabda-12-ene (**4.23**)

The hydroxy ketone **4.16** (2.04 g, 7.29 mmol) was slowly passed through an open column of silica gel (100 g) using $CHCl_3$ as eluent. Removal of the solvent ***in vacuo*** gave a quantitative yield of the enol ether **4.23** as a colourless oil (1.91 g, 7.29 mmol).

7.4.2.3 Dehydration of (+)-8α-hydroxy-14,15-bisnorlabda-13-one (**4.16**) and isomerisation of (+)-8α,13-epoxy-14,15-bisnorlabda-12-ene (**4.23**) to (+)-14,15-bisnorlabda-8-ene-13-one (**4.17**)

A. Iodine catalysed dehydration and isomerisation

The hydroxy ketone **4.16** (5.00 g, 17.9 mmol) was dissolved in anhydrous benzene (500 mL) and 0.1 M I_2 in anhydrous benzene (9.0 mL, 0.9 mmol, 0.05 eq) added. The resulting pink solution

was refluxed under anhydrous conditions for 6 h after which the benzene was removed ***in vacuo*** and the residue taken up in Et_2O (100 mL). The Et_2O solution was washed with 5% $Na_2S_2O_3$ (3 × 25 mL) and H_2O (25 mL), dried ($MgSO_4$) and concentrated to give a dark brown oil (4.39 g). The crude product was purified by flash chromatography on silica gel in 9:1 hexane/EtOAc to give (+)-14,15-bisnorlabda-7-ene-13-one (**4.25**, 0.26 g, 0.99 mmol, 6 %) and (+)-14,15-bisnorlabda-8-ene-13-one (**4.17**, 3.51 g, 13.4 mmol, 75 %) as light yellow oils.

The enol ether **4.23** (5.20 g, 19.7 mmol) was treated in the same manner to give a dark brown oil (5.18 g), which after flash chromatography yielded the unsaturated ketones **4.25** (0.41 g, 1.56 mmol, 8 %) and **4.17** (3.91 g, 14.9 mmol, 76%) as light yellow oils.

B. Acid catalysed dehydration and isomerisation

The hydroxy ketone **4.16** (1.43 g, 5.11 mmol) was dissolved in EtOH (50 mL), 5 M HCl (10 mL) added and the solution heated at 70 °C for 2 h. The reaction mixture was concentrated ***in vacuo*** (to a volume of approximately 20 mL), poured into H_2O (100 mL) and extracted with EtOAc (3 × 50 mL). The organic fractions were combined, washed with 5% Na_2CO_3 (50 mL), dried (Na_2SO_4) and concentrated to give a yellow oil (1.21 g). Silica gel flash chromatography of the crude product in 9:1 hexane/EtOAc afforded the unsaturated ketones **4.25** (0.36 g, 1.37 mmol, 27 %) and **4.17** (0.59 g, 2.25 mmol, 44 %) as light yellow oils.

The enol ether **4.23** (1.29 g, 4.92 mmol) was treated in the same manner to give a light yellow oil (1.08 g) that yielded, after flash chromatography, the unsaturated ketones **4.25** (0.30 g 1.15 mmol, 23%) and **4.17** (0.56 g, 2.18 mmol, 44%) as light yellow oils.

(+)-14,15-Bisnorlabda-7-ene-13-one (4.25):[172,173,177] light yellow oil; $[\alpha]_D^{27}$ +22° (***c*** 1.60, $CHCl_3$), lit. for enantiomer[178] –23°; IR ν_{max} 2924, 2847, 1717, 1669, 1457, 1388, 1360, 1214, 1160, 983 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.40 (1H, br s, H-7), 2.62 (1H, m, H-12a), 2.40 (1H, m, H-12b), 2.12 (3H, s, H_3-16), 1.93 (1H, m, H-6a), 1.88 (1H, m, H-6b), 1.85 (1H, m, H-1a), 1.79 (1H, m, H-11a), 1.64 (3H, s, H_3-17), 1.58 (1H, s, H-9), 1.52 (1H, m, H-2a), 1.44 (1H, m, H-2b), 1.41 (1H, m,

H-11b), 1.40 (2H, m, H_2-3), 1.15 (1H, dd, ***J*** = 12.3, 4.9 Hz, H-5), 0.93 (1H, dd, ***J*** = 13.7, 4.3 Hz, H-1b), 0.86 (3H, s, H_3-19), 0.84 (3H, s, H_3-18), 0.76 (3H, s, H_3-20) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.8 (s, C-13), 134.5 (s, C-8), 123.0 (d, C-7), 54.4 (d, C-9), 50.5 (d, C-5), 45.9 (t, C-12), 42.3 (t, C-3), 39.4 (t, C-1), 36.9 (s, C-10), 33.2 (q, C-18), 33.0 (s, C-4), 29.9 (q, C-16), 23.8 (t, C-6), 22.2 (q, C-17), 21.9 (q, C-19), 21.0 (t, C-11), 18.8 (t, C-2), 13.6 (q, C-20) ppm; EIMS ***m/z*** (rel. int.) 262 [M^+] (5), 244 (25), 229 (19), 204 (83), 189 (55), 173 (17), 161 (100), 147 (24), 119 (35), 105 (41); HRFABMS ***m/z*** 262.2296 (calcd for $C_{18}H_{30}O$ [M^+], 262.2297).

(+)-14,15-Bisnorlabda-8-ene-13-one (4.17):[172,173,177] light yellow oil; $[\alpha]_D^{26}$ +75° (***c*** 1.10, $CHCl_3$), lit[173] +78°; IR ν_{max} 2938, 2867, 1716, 1463, 1387, 1360, 1273, 1159, 1070, 999 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.47 (2H, t, ***J*** = 8.3 Hz, H_2-12), 2.28 (1H, m, H-11a), 2.16 (1H, m, H-11b), 2.12 (3H, s, H_3-16), 1.97 (2H, m, H_2-7), 1.77 (1H, br d, ***J*** = 12.2 Hz, H-1a), 1.64 (1H, m, H-6a), 1.57 (1H, m, H-2a), 1.52 (3H, s, H_3-17), 1.47 (1H, m, H-2b), 1.42 (1H, m, H-3a), 1.36 (1H, m, H-6b), 1.12 (1H, m, H-3b), 1.08 (1H, dd, ***J*** = 12.5, 1.9 Hz, H-5), 1.07 (1H, m, H-1b), 0.93 (3H, s, H_3-20), 0.87 (3H, s, H_3-18), 0.82 (3H, s, H_3-19) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.9 (s, C-13), 139.5 (s, C-9), 126.6 (s, C-8), 52.0 (d, C-5), 44.7 (t, C-12), 41.8 (t, C-3), 39.1 (s, C-10), 37.0 (t, C-1), 33.7 (t, C-7), 33.3 (s, C-4), 33.3 (q, C-18), 29.7 (q, C-16), 21.7 (q, C-19), 21.6 (t, C-11), 20.0 (q, C-20), 19.4 (q, C-17), 19.0 (t, C-6 & t, C-2) ppm; EIMS ***m/z*** (rel. int.) 262 [M^+] (9), 244 (58), 229 (100), 204 (32), 189 (73), 173 (71), 159 (63), 133 (67), 105 (67), 91 (60); HRFABMS ***m/z*** 262.2295 (calcd for $C_{18}H_{30}O$ [M^+], 262.2297).

7.4.2.4 Preparation of ethyl 13-hydroxylabda-8-en-15-oate (**4.26**) ***via*** the Reformatsky reaction

Before performing this reaction, all apparatus were thoroughly flame dried under N_2. The reaction was performed under an atmosphere of dry air and meticulously anhydrous conditions were maintained throughout. The zinc powder used was activated immediately prior to use by washing in 10% HCl for 5 min, rinsing twice with water and twice with acetone. The zinc powder was then dried in an oven at 120 °C for 1 h.

Activated zinc powder (2.10 g, 32.2 mmol, 1.2 eq) and ethyl bromoacetate (3.59 mL, 32.2 mmol, 1.2 eq) were stirred in a mixture of anhydrous benzene (30.0 mL) and anhydrous Et_2O (10.0 mL). The mixture was gently warmed at 40 °C until reaction between the zinc and ethyl bromoacetate caused the solvent to reflux. When this reaction was complete, **4.17** (7.04 g, 26.9 mmol) in a mixture of anhydrous benzene (30.0 mL) and anhydrous Et_2O (10.0 mL) was added dropwise over 10 min and the resulting solution gently refluxed for 2.5 h. The solution was cooled, poured into 2 M H_2SO_4 (30 mL) and extracted with Et_2O (3 × 20 mL). The organic fractions were combined, washed with 5% $NaHCO_3$ (2 × 20 mL) and H_2O (10 mL), dried ($MgSO_4$) and concentrated to give a yellow oil (10.3 g). Silica gel flash chromatography in 7:3 hexane/EtOAc afforded ethyl 13-hydroxylabda-8-en-15-oate (**4.26**, 8.74 g, 25.0 mmol, 93%) as a 1:1 mixture of C-13 diastereomers (determined from ^1H and ^{13}C NMR spectroscopy; inseparable by normal phase HPLC) and recovered starting material (**4.17**, 331 mg, 1.26 mmol, 5%).

Ethyl 13-hydroxylabda-8-en-15-oate (4.26): light yellow oil; IR ν_{max} 3523 (br), 2939, 2926, 1716, 1463, 1374, 1332, 1103, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.16 (2H,q, ***J*** = 7.1 Hz, 15-OC$\underline{H}_2$CH$_3$), 3.53/3.52 (1H, s, 13-OH), 2.52 (1H, d, ***J*** = 15.6 Hz, H-14a), 2.48 (1H, d, ***J*** = 15.6 Hz, H-14b), 2.08 (1H, m, H-11a), 1.98 (1H, m, H-11b), 1.88 (1H, m, H-7b), 1.78 (1H, ***J*** = 14.0 Hz, H-1a), 1.67 (1H, m, H-2a), 1.56 (2H, m, H_2-12), 1.54/1.53 (3H, s, H_3-17), 1.43 (1H, m, H-26), 1.38 (2H, m, H_2-6), 1.26 (3H, t, ***J*** = 7.1 Hz, 15-OCH$_2$C$\underline{H}_3$), 1.24 (3H, s, H_3-16), 1.14 (2H, m, H_2-3), 1.11 (1H, m, H-16), 1.07 (1H, m, H-5), 0.93 (3H, s, H_3-18), 0.86 (3H, s, H_3-20), 0.81 (3H, s, H_3-19) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1 (s, C-15), 139.8 (s, C-9), 125.9 (s, C-8), 71.2 (s, C-13), 60,6 (t, 15-O$\underline{C}$H$_2$CH$_3$), 51.9 (d, C-5), 44.6 (t, C-14), 42.1 (t, C-3), 42.0 (t, C-12), 41.8 (t, C-6), 39.1 (s, C-10), 37.0 (t, C-1), 33.6 (t, C-7), 33.3 (q, C-20 & s, C-4), 26.4 (q, C-16), 22.1 (t, C-11), 21.7 (q, C-19), 20.1 (q, C-18), 19.4 (q, C-17), 19.1 (t, C-2), 14.1 (q, 15-OCH$_2$$\underline{C}H_3$) ppm; EIMS ***m/z*** (rel. int.) 350 [M^+] (3), 332 (12), 317 (13), 271 (31), 229 (54), 204 (100), 189 (57), 161 (46), 121 (41), 95 (27); HRFABMS ***m/z*** 350.2821 (calcd for $C_{22}H_{38}O_3$ [M^+], 350.2821).

7.4.2.5 Allylic oxidation of ethyl 13-hydroxylabda-8-en-15-oate (**4.26**)

A. Attempted oxidation employing Jones' reagent

Ethyl 13-hydroxylabda-8-en-15-oate (**4.26**, 140 mg, 0.38 mmol) was dissolved in acetone (2.0 mL) and Jones' reagent (8 N $NaCrO_3/H_2SO_4$, 0.95 mL) added dropwise with cooling (ice-bath). The resulting orange solution was slowly warmed to ambient temperature and allowed to stir overnight, during which the solution turned green and a small amount of tarry solid precipitated from the reaction mixture. The reaction mixture was poured into water (20 mL) and extracted with EtOAc (3×20 mL). The organic fractions were combined, washed with $NaHCO_3$ (2×10 mL) and water (10 mL), dried ($MgSO_4$) and concentrated to give a yellow oil (92 mg). ^{1}H and ^{13}C NMR spectroscopy of the oil indicated that it comprised a complex mixture of products and no resonance indicating the presence of an α,β-unsaturated ketone functionality (δ_C approximately 200 ppm) was observed in the ^{13}C NMR spectrum.

B. Allylic oxidation via the Collins' modification of the Sarrett oxidation

Before performing this reaction, all apparatus were thoroughly flame dried under N_2. The reaction was performed under an atmosphere of dry air and meticulously anhydrous conditions were maintained throughout. The CrO_3 used was dried ***in vacuo*** (0.5 mmHg) over P_2O_5 at 70 °C for 8 h immediately prior to use.

CrO_3 (18.9 g, 189 mmol, 15 eq) was stirred vigorously in CH_2Cl_2 (200 mL) and cooled to –10 °C in an acetone–ice bath. Pyridine (30.5 mL, 378.9 mmol, 30 eq) in CH_2Cl_2 (50 mL) was then added dropwise over 30 min taking care not to allow the temperature to rise above 0 °C. The resulting deep red solution was allowed to warm to ambient temperature over 1 h and stirred for a further 30 min before ethyl 13-hydroxylabda-8-en-15-oate (**4.26**, 4.42g, 12.6 mmol) in CH_2Cl_2 (50 mL) was added dropwise over 30 min. The solution was stirred at room temperature for 48 h, during which time a large amount of waxy, dark brown precipitate formed. The supernatant solution was decanted, the precipitate thoroughly washed with Et_2O (3×100mL) and the washings combined with the supernatant solution. The tarry precipitate was dissolved in sat. $NaHCO_3$ (500 mL) and

this solution extracted with EtOAc (2 × 100 mL) after which the organic fractions were combined with the initial supernatant and the volume of the resulting solution reduced to approximately 200 mL ***in vacuo***. The combined organic fraction was then thoroughly washed with 1.5 M NaOH (3 × 100 mL), 1 M HCl (2 × 100 mL), 5% $NaHCO_3$ (1 × 100 mL) and sat. brine (1 × 50 mL), dried ($MgSO_4$) and concentrated to give a yellow oil (4.17 g). Flash chromatography of the crude product in 3:2 hexane/EtOAc afforded ethyl 13-hydroxy-7-oxo-labda-8-en-15-oate (**4.27**, 1:1 mixture of C-13 diastereomers) as a light yellow oil (3.69 g, 10.1 mmol, 80%).

Ethyl 13-hydroxy-7-oxo-labda-8-en-15-oate (4.27): light yellow oil; IR ν_{max} 3437 (br), 2931, 2863, 1732, 1661, 1600, 1376, 1331, 1154, 1031 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.17 (2H, q, ***J*** = 7.1 Hz, 15-OC$\underline{H}_2CH_3$), 3.64/3.62 (1H, s, 13-OH), 2.53 (1H, d, ***J*** = 15.9 Hz, H-14a), 2.47 (1H, d, ***J*** = 17.5 Hz, H-6a), 2.43 (1H, d, ***J*** = 15.9 Hz, H-14b), 2.33 (1H, m, H-6b), 2.30 (2H, m H_2-11), 1.19 (1H, br t, ***J*** = 11.8 Hz, H-1a), 1.74/1.73 (3H, s, H_3-17), 1.68 (1H, m, H-2a), 1.66 (1H, m, H-5), 1.62 (2H, m, H_2-12), 1.55 (1H, m, H-2b), 1.46 (1H, br d, ***J*** = 13.3 Hz, H-3a), 1.32 (1H, m, H-1b), 1.27 (3H, t, ***J*** = 7.2 Hz, 15-$OCH_2C\underline{H}_3$), 1.27 (3H, s, H_3-16), 1.20 (1H, dd, ***J*** = 13.3, 4.1 Hz, H-3b), 1.07 (3H, s, H_3-18), 0.87 (3H, s, H_3-19), 0.86 (3H, s, H_3-20) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.1 (s, C-7), 172.9 (s, C-15), 167.7/167.6 (s, C-9), 130.2 (s, C-8), 70.9 (s, C-13), 60.8 (t, 15-O$\underline{C}H_2CH_3$), 50.3 (d, C-5), 44.7 (t, C-14), 41.3 (t, C-3), 41.1 (s, C-10), 40.2/40.1 (t, C-12), 35.9 (t, C-1), 35.2 (t, C-6), 33.1 (s, C-4), 32.5 (q, C-20), 26.5/26.3 (q, C-16), 23.7 (t, C-11), 21.3 (q, C-19), 18.6 (t, C-2), 18.2 (q, C-18), 14.1 (q, 15-$OCH_2\underline{C}H_3$), 11.2 (t, C-17) ppm; EIMS ***m/z*** (rel. int.) 364 [M^+] (1), 346 (38), 331 (16), 285 (16), 259 (19), 240 (63), 205 (48), 161 (22), 152 (83), 135 (100); HRFABMS ***m/z*** 365.2691 (calcd for $C_{22}H_{37}O_4$ [$(M + H)^+$] 365.2692).

7.4.2.6 Dehydration of ethyl 13-hydroxy-7-oxo-labda-8-en-15-oate (**4.27**)

A. Dehydration with $POCl_3$–pyridine

Ethyl 13-hydroxy-7-oxo-labda-8-en-15-oate (**4.27**, 6.34 g, 17.4 mmol) was dissolved in anhydrous pyridine (40 mL) and stirred at −10 °C for 30 min. $POCl_3$ (12.7 mL, 139 mmol, 8 eq) in dry

pyridine (20 mL) was added dropwise, the solution slowly warmed to RT and allowed to stir for 16 h. The reaction mixture was quenched by pouring into ice (300 g) and the resulting aqueous suspension extracted with EtOAc (3 × 100 mL). The organic phases were combined, washed with 1 M HCl (3 × 100 mL), 5% Na_2CO_3 (100 mL) and sat. brine (1 × 50 mL), dried (Na_2SO_4) and the solvent evaporated to give a brown oil (5.43 g). Silica gel flash chromatography of the crude product (7:3 hexane/EtOAc) yielded a 2:1 mixture (measured from integration of the H-14 vinylic proton resonances in the ^{1}H NMR spectrum of the purified product) of ethyl rhinocerotinoate (**4.28**) and ethyl isorhinocerotinoate (**4.29**) as a yellow oil (4.58 g, 13.2 mmol, 76%). The two geometrical isomers were inseparable by column chromatography and could only be purified by subjecting the **4.28**/**4.29** mixture to semi-preparative normal phase HLPC in 9:1 hexane/EtOAc.

B. Attempted dehydration with $SOCl_2$–pyridine

Ethyl 13-Hydroxy-7-oxo-labda-8-en-15-oate (**4.27**, 50 mg, 0.14 mmol) in anhydrous pyridine (0.5 mL) was stirred at 0 °C whilst $SOCl_2$ (82 μL, 1.13 mmol, 8 eq) in anhydrous pyridine (0.5 mL) was added dropwise. The solution was allowed to stir at 5 °C overnight before the reaction was quenched with water (2.0 mL) at 0 °C and extracted with EtOAc (3 × 5 mL). The EtOAc phases were combined, washed with water (5 mL) and sat. brine (5 mL), dried ($MgSO_4$) and concentrated ***in vacuo*** (0.5 mmHg) which facilitated the removal of excess pyridine. The product, obtained as a yellow oil (32 mg), was found to be a complex mixture when observed by ^{1}H NMR spectroscopy and no attempt was made to purify and identify any of its components.

C. Attempted dehydration with BF_3–OEt_2

Freshly distilled BF_3-OEt_2 (207 μL, 1.65 mmol, 5 eq) was added dropwise to a solution of ethyl 13-hydroxy-7-oxo-labda-8-en-15-oate (**4.27**, 120 mg, 0.33 mmol) in anhydrous CH_2Cl_2 (3.0 mL) at 0 °C. The reaction mixture was slowly warmed to ambient temperature and stirred for 24 h. The reaction was quenched at 0 °C with sat. $NaHCO_3$ (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The organic fractions were combined, washed with sat. brine (2 × 5 mL), dried ($MgSO_4$) and concentrated ***in vacuo*** to give unreacted **4.27** (108 mg) exclusively.

Ethyl rhinocerotinoate (4.28): colourless oil; $[\alpha]_D^{26}$ +34° (***c*** 1.95, $CHCl_3$); IR ν_{max} 2931, 2863, 1715, 1662, 1608, 1463, 1330, 1222, 1147, 1036 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.69 (1H, br d, ***J*** = 0.9 Hz, H-14), 4.14 (2H, q, ***J*** = 7.1 Hz, 15-OC$\underline{H}_2CH_3$), 2.49 (1H, dd, ***J*** = 17.6, 3.7 Hz, H-6a), 2.34 (1H, m, ***J*** = 17.6 Hz, H-6b), 2.32 (2H, m, H_2-11), 2.24 (2H, m, H_2-12), 2.21 (3H, d, ***J*** = 0.9 Hz, H_3-16), 1.90 (1H, br d, ***J*** = 12.2 Hz, H-1a), 1.75 (3H, s, H_3-17), 1.69 (1H, dd, ***J*** = 8.6, 3.6 Hz, H-2a), 1.66 (1H, m, ***J*** = 6.5 Hz, H-5), 1.60 (1H, m, ***J*** = 3.6 Hz, H-2b), 1.47 (1H, br d, ***J*** = 13.3 Hz, H-3a), 1.37 (1H, td, ***J*** = 12.3, 3.8 Hz, H-1b), 1.27 (3H, t, ***J*** = 7.2 Hz, 15-$OCH_2C\underline{H}_3$), 1.19 (1H, dd, ***J*** = 13.4, 4.2 Hz, H-3b), 1.07 (3H, s, H_3-20), 0.90 (3H, s, H_3-19), 0.87 (3H, s, H_3-18) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.0 (s, C-7), 166.6 (s, C-9), 166.3 (s, C-15), 158.4 (s, C-13), 130.5 (s, C-8), 115.9 (d, C-14), 49.6 (t, 15-O$\underline{C}H_2CH_3$), 50.3 (d, C-5), 41.3 (t, C-3), 41.0 (s, C-10), 39.6 (t, C-12), 35.9 (t, C-1), 35.2 (t, C-6), 33.1 (s, C-4), 32.5 (q, C-18), 27.7 (t, C-11), 21.3 (q, C-19), 18.8 (q, C-16), 18.6 (t, C-2), 18.2 (q, C-20), 14.3 (q, 15-$OCH_2\underline{C}H_3$), 11.4 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 346 [M^+] (42), 331 (12), 318 (32), 300 (54), 285 (31), 258 (67), 245 (30), 205 (37), 161 (21), 135 (100); HRFABMS ***m/z*** 347.2586 (calcd for $C_{22}H_{35}O_3$ [$(M + H)^+$], 347.2586).

Ethyl isorhinocerotinoate (4.29): colourless oil; $[\alpha]_D^{26}$ +47° (***c*** 0.48, $CHCl_3$); IR ν_{max} 2931, 2863, 1714, 1662, 1603, 1444, 1377, 1165, 1144, 1036 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.67 (1H, br d, ***J*** = 1.1 Hz, H-14), 4.14 (2H, q, ***J*** = 7.1 Hz, 15-OC$\underline{H}_2CH_3$), 2.75 (2H, m, H_2-12), 2.49 (1H, dd, ***J*** = 17.5, 3.8 Hz, H-6a), 2.35 (1H, dd, ***J*** = 17.6, 14.1 Hz, H-6b), 2.34 (2H, m, H_2-11), 2.05 (1H, br d, ***J*** = 12.4 Hz, H-1a), 1.94 (3H, d, ***J*** = 1.3 Hz, H_3-16), 1.84 (3H, s, H_3-17), 1.71 (1H, dd, ***J*** = 14.2, 3.9 Hz, H-5), 1.69 (1H, m, H-2a), 1.62 (1H, tt, ***J*** = 12.1, 3.6 Hz, H-2b), 1.47 (1H, br d, ***J*** = 13.4 Hz, H-3a), 1.42 (1H, td, ***J*** = 12.6, 3.9 Hz, H-1b), 1.26 (3H, t, ***J*** = 7.1 Hz, 15-$OCH_2C\underline{H}_3$), 1.23 (1H, td, ***J*** = 13.3, 4.1 Hz, H-3b), 1.10 (3H, s, H_3-20), 0.91 (3H, s, H_3-19), 0.88 (3H, s, H_3-18) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.3 (s, C-7), 167.2 (s, C-9), 166.0 (s, C-15), 157.9 (s, C-13), 130.6 (s, C-8), 117.0 (d, C-14), 59.6 (t, 15-O$\underline{C}H_2CH_3$), 50.2 (d, C-5), 41.3 (t, C-3), 41.1 (s, C-10), 35.9 (t, C-1), 35.3 (t, C-6), 33.1 (s, C-4), 32.5 (q, C-18), 32.2 (t, C-12), 27.6 (t, C-11), 24.9 (q, C-16), 21.3 (q, C-19), 18.7 (t, C-2), 18.3 (q, C-20), 14.3 (q, 15-$OCH_2\underline{C}H_3$), 11.3 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 346 [M^+] (37), 301 (18), 285 (22), 258 (39), 220 (50), 205 (57), 176 (33), 135 (92), 127 (100); HRFABMS ***m/z*** 347.2587 (calcd for $C_{22}H_{35}O_3$ [$(M + H)^+$], 347.2586).

7.4.2.7 Attempted conversion of ethyl isorhinocerotinoate (**4.29**) to ethyl rhinocerotinoate (**4.28**)

A. Attempted I_2 catalysed isomerisation

A portion of the 2:1 **4.28**/**4.29** mixture (47 mg, 0.14 mmol) and a small crystal of I_2 were dissolved in anhydrous benzene (10 mL) and refluxed under anhydrous conditions for 18 h. The solution was then concentrated ***in vacuo***, taken up in Et_2O (10 mL) and washed with 5% $Na_2S_2O_3$ (2×5 mL) and H_2O (5 mL). Drying over $MgSO_4$ and removal of the solvent yielded a yellow oil (44 mg). Comparison of the ratio obtained from the integrals of the H-14 vinyllic proton resonances in the ^{1}H NMR spectrum of the product mixture with that of the starting material indicated that no isomerisation of the Δ^{13}-olefin had occurred under these conditions.

Pure **4.29** (58 mg, 0.17 mmol) was treated in exactly the same manner to yield a yellow oil (56 mg) which was found to contain only 6.4% **4.28** by ^{1}H NMR spectroscopy.

B. Attempted isomerisation employing thiophenol

A portion of the 2:1 **4.28**/**4.29** mixture (57 mg, 0.16 mmol) was heated at 100 °C with thiophenol (0.5 mL) under an anhydrous air atmosphere for 24 h. The resulting viscous yellow liquid was dissolved in Et_2O (5.0 mL), washed with 2M NaOH (2×5 mL) and H_2O (2 mL), dried (Na_2SO_4) and concentrated ***in vacuo*** to yield a dark yellow oil (51 mg) which was examined by ^{1}H NMR spectroscopy. Once again, the ratio of the integrals of the H-14 vinyllic proton resonances in the ^{1}H NMR spectrum of the product indicated that no isomerisation of the Δ^{13}-olefin had occurred under these conditions.

Pure **4.29** (46 mg, 0.13 mmol) was treated in a similar manner (heated for only 2 h) to yield a yellow oil (44 mg). Examination of the ^{1}H NMR spectrum of the product indicated that 48.8% conversion from **4.29** to **4.28** had occurred. This reaction was repeated on three separate portions of **4.29** (each approximately 20 mg) incorporating a longer period of heating in the procedure for each portion (6 h, 12 h and 24 h). Examination of the products obtained indicated

that prolonged heating had little effect upon the extent of isomerisation observed in the reaction (52.1%, 47.9% and 49.3% conversion from **4.29** to **4.28** respectively).

7.4.2.8 Preparation of (+)-14,15-bisnorlabda-8-en-7,13-dione (**4.32**)

Strictly anhydrous reagents and reaction conditions were observed as with the oxidation of **4.26**. CrO_3 (28.5 g, 285 mmol, 15 eq) was stirred vigorously in CH_2Cl_2 (300 mL) and cooled to −10 °C in an acetone–ice bath. Pyridine (46.0 mL, 570.3 mmol, 30 eq) in CH_2Cl_2 (50 mL) was then added dropwise over 1 h taking care not to allow the temperature to rise above 0°C. The resulting deep red heterogeneous mixture was stirred at −10 °C for 30 min before (+)-14,15-bisnorlabda-8-en-13-one (**4.17**, 4.98 g, 19.0 mmol) in CH_2Cl_2 (100 mL) was added dropwise over 30 min. The solution was slowly warmed to room temperature and stirred for 72 h, during which time a large amount of waxy, dark brown precipitate formed. The supernatant solution was decanted, the precipitate thoroughly washed with EtOAc (3 × 100 mL) and the washings combined with the supernatant solution. The tarry precipitate was dissolved in sat. $NaHCO_3$ (600 mL) and this solution extracted with EtOAc (3 × 100 mL) after which all the organic fractions were combined and concentrated to a volume of approximately 300 mL ***in vacuo***. The combined organic fraction was then thoroughly washed with sat. $NaHCO_3$ (3 × 100 mL), 2 M HCl (3 × 100 mL), 5% $NaHCO_3$ (1 × 100 mL) and H_2O (1 × 100 mL), dried ($MgSO_4$) and concentrated to give a brown oil (4.53 g). Flash chromatography of the crude product in 7:3 hexane/EtOAc afforded (+)-14,15-bisnorlabda-8-en-7,13-dione (**4.32**) as a colourless oil (4.26 g, 15.4 mmol, 81%) which could be crystallised from cold hexane to give large off-white crystals. A small amount of starting material (**4.17**, 156 mg, 3%) was also recovered from the flash column.

(+)-14,15-Bisnorlabda-8-en-7,13-dione (4.32):[198] off-white solid; mp 78–80 °C, lit.[198] 79.5–80 °C; $[\alpha]_D^{26}$ +58° (***c*** 1.89, $CHCl_3$), lit.[198] +65.8°; IR ν_{max} 2931, 2870, 1716, 1661, 1607, 1417, 1366, 1330, 1162, 1071 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.56 (2H, m, H_2-12), 2.51 (1H, m, H-6a), 2.46 (2H, m, H_2-11), 2.34 (1H, dd, ***J*** = 17.5, 14.3 Hz, H-16), 2.17 (3H, s, H_3-16), 1.88 (1H, br d, ***J***

= 12.4 Hz, H-1a), 1.70 (1H, m, H-2a), 1.71 (3H, s, H_3-17), 1.67 (1H, dd, ***J*** = 14.2, 3.6 Hz, H-5), 1.57 (1H, m, H-2b), 1.47 (1H, br d, ***J*** = 13.4 Hz, H-3a), 1.25 (1H, td, ***J*** = 12.6, 3.7 Hz, H-1b), 1.23 (1H, td, ***J*** = 13.4, 4.1 Hz, H-3b), 1.08 (3H, s, H_3-20), 0.91 (3H, s, H_3-19), 0.87 (3H, s, H_3-18) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 206.8 (s, C-13), 199.9 (s, C-7), 166.7 (s, C-9), 130.4 (s, C-8), 50.4 (d, C-5), 42.4 (t, C-12), 41.3 (t, C-3), 41.1 (s, C-10), 35.9 (t, C-1), 35.2 (t, C-6), 33.1 (s, C-4), 32.5 (q, C-18), 29.8 (q, C-16), 22.7 (t, C-11), 21.3 (q, C-19), 18.5 (t, C-2), 17.9 (q, C-20), 11.3 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 276 [M^+] (13), 258 (7), 233 (37), 215 (11), 205 (41), 136 (24), 135 (100), 107 (12), 91 (17); HRFABMS ***m/z*** 277.2167 (calcd for $C_{18}H_{29}O_2$ [$(M+H)^+$], 277.2168).

7.4.2.9 Horner-Wadsworth-Emmons reaction of (+)-14,15-bisnorlabda-8-en-17,13-dione (**4.32**)

NaH (336 mg, 14.0 mmol, 1.2 eq) was stirred in anhydrous THF (15.0 mL), whilst triethylphosphonoacetate (2.78 mL, 14.0 mmol, 1.2 eq) in anhydrous THF (5.0 mL) was added dropwise at room temperature. The resulting solution was stirred at room temperature for 30 min then the dione **4.32** (3.22 g, 11.7 mmol) in anhydrous THF (20 mL) was added dropwise and the reaction mixture stirred at ambient temperature overnight. The reaction mixture was concentrated ***in vacuo*** and taken up in Et_2O (50 mL), washed with H_2O (3 × 10 mL), dried ($MgSO_4$) and concentrated to give a light yellow oil (6.91 g). Silica gel flash chromatography (7:3 hexane/EtOAc) of the oil yielded a mixture of ethyl rhinocerotinoate (**4.28**) and ethyl isorhinocerotinoate (**4.29**) as a colourless oil (3.87 g, 11.2 mmol, 96%). ^{1}H NMR spectroscopy of the **4.28**/**4.29** mixture indicated that it was composed of a 10.1:1 mixture of **4.28**/**4.29**. Subsequent HWE reactions of **4.32** indicated that this ratio of products (approximately 10:1) was representative.

7.4.2.10 Saponification of esterified 7-oxo-labda-8,13-dien-15-oic acid derivatives

A. KOH saponification of the 10:1 ethyl rhinocerotinoate (4.28)/ethyl isorhinocerotinoate (4.29) mixture (prepared via the HWE reaction)

The 10:1 **4.28**/**4.29** mixture (3.40 g, 9.83 mmol) and 5 M KOH (10 mL, 50 mmol, 5.1 eq) were refluxed in EtOH (40 mL) for 6 h. The reaction mixture was then concentrated ***in vacuo*** to give a cloudy aqueous solution (15 mL), which was acidified with 1 M HCl and extracted with $CHCl_3$ (3 × 25 mL). The organic fractions were combined, dried and concentrated to give a 12:3:2 mixture (determined by ^{1}H NMR spectroscopy) of rhinocerotinoic acid (**4.14**), isorhinocerotinoic acid (**4.22**) and (+)-7-oxo-labda-8,13(16)-dien-15-oic acid (**4.37**) as a colourless oil (2.98 g, 9.37 mmol, 95%). Treatment of this oil with hexane and vacuum sublimation (150 °C, 0.5 mmHg) of the resulting off-white solid allowed the isolation of isomerically pure (by ^{1}H NMR spectroscopy) **4.14** (1.01 g, 48% of the total **4.14** in the mixture) as fine white crystals. The oil obtained from the mother-liquor was similarly treated with cold MeOH-water (10:1) to yield isomerically pure **4.22** (47 mg, 9% of the total **4.22** in the mixture). The mother-liquors from the second crystallisation were combined to give a 3.11:1.37:1 (≈ 6:3:2, estimated from ^{1}H NMR spectroscopy) mixture of **4.14**/**4.22**/**4.37** as an oil (1.91 g, 6.01 mmol) that was inseparable by both normal phase and reversed phase HPLC. The acid **4.37** could therefore not be obtained pure and was isolated as the methyl ester **4.38** as described below.

The 6:3:2 **4.14**/**4.22**/**4.37** (1.91 g, 6.01 mmol) mixture was dissolved in MeOH (10 mL) and treated with an ethereal diazomethane solution (20 mL, approximately 34 mmol, 5.7 eq)[286] at 0 °C. The bright yellow solution was allowed to stand in ice for 30 min before being slowly warmed to room temperature. After 1 h at room temperature the ether and excess diazomethane was removed by evaporation at 70 °C in a fume-hood. The residual MeOH solution was concentrated ***in vacuo*** to give a mixture of the methyl esters **4.20**, **4.21** and **4.38** in the same ratio of 6:3:2 as a light yellow oil (1.99 g, 5.99 mmol, 100 %). A portion of the methylated mixture (1.69 g) was purified by repeated flash chromatography in 19:1 and 9:1 hexane/EtOAc and normal phase HPLC of mixed fractions in 17:3 hexane/EtOAc to yield methyl rhinocerotinoate (**4.20**, 0.68 g), methyl

isorhinocerotinoate (**4.21**, 0.34 g) and methyl 7-oxo-labda-8,13(16)-dien-15-oate (**4.38**, 0.19 g) as colourless oils. It should be noted that the masses of the methyl esters isolated are not representative of their yields due to the losses incurred through the extensive chromatography required to separate these isomers.

B. KOH saponification of the 2:1 ethyl rhinocerotinoate (4.28)/ethyl isorhinocerotinoate (4.29) mixture (prepared via $POCl_3$ dehydration)

The 2:1 **4.28**/**4.29** mixture (5.82 g, 16.8 mmol) and 5 M KOH (17 mL) was refluxed in EtOH (70 mL) for 6 h. The reaction mixture was then concentrated ***in vacuo*** to give a cloudy aqueous solution (25 mL), which was acidified with 2 M HCl and continuously extracted with Et_2O (100 mL) for 4 h. The resulting Et_2O solution was dried ($MgSO_4$) and concentrated ***in vacuo*** to give a 10:5:2 mixture (determined by ^{1}H NMR spectroscopy) of rhinocerotinoic acid (**4.14**), isorhinocerotinoic acid (**4.22**) and (+)-7-oxo-labda-8,13(16)-dien-15-oic acid (**4.37**) as an orange semi-solid residue (5.01 g, 15.8 mmol, 94%). The mixture was taken up in hexane (60 mL) and stored overnight at 0 °C when an off white solid (2.03 g) precipitated from solution. The solid was vacuum sublimed (150 °C, 0.5 mmHg) to give isomerically pure **4.14** as white needles (1.81 g, 61% of the total **4.14** in the reaction mixture). The mother-liquor from the crystallisation was discarded.

C. ***K_2CO_3 saponification of the 10:1 ethyl rhinocerotinoate (4.28)/ethyl isorhinocerotinoate (4.29) mixture (prepared via the HWE reaction)***

The 10:1 **4.28**/**4.29** mixture (20 mg, 0.06 mmol) and K_2CO_3 (41 mg, 0.30 mmol, 5.0 eq) was refluxed for 14 h in EtOH (4.0 mL) and H_2O (1.0 mL). The reaction mixture was then acidified with 1 M HCl and extracted with $CHCl_3$ (3 $\times$ 5 mL). The organic fractions were combined, dried and concentrated to give a 7:2:7 mixture (determined by ^{1}H NMR spectroscopy) of rhinocerotinoic acid (**4.14**), isorhinocerotinoic acid (**4.22**) and (+)-7-oxo-labda-8,13(16)-dien-15-oic acid (**4.37**) as a colourless oil (16.3 mg, 0.05 mmol, 83%).

D. KOH saponification of methyl rhinocerotinoate (4.20), methyl isorhinocerotinoate (4.21) and methyl 7-oxo-labda-8,13(16)-dien-15-oate (4.38)

The pure methyl esters **4.20** (19 mg, 0.06 mmol), **4.21** (21 mg, 0.06 mmol) and **4.38** (20 mg, 0.06 mmol) were each separately treated with 0.3 M KOH (1.0 mL, 0.30 mmol, 5.0 eq) and refluxed in EtOH (4.0 mL) for 6 h. The reaction mixtures were then acidified with 1 M HCl and extracted with $CHCl_3$ (3 × 5 mL). The organic fractions from each reaction were combined, dried and concentrated to give three yellow oils (16 mg from **4.20**, 15 mg from **4.21** and 17 mg from **4.38**) that were examined by ^{1}H NMR spectroscopy. All three products consisted of mixtures of the isomeric acids **4.14**, **4.22** and **4.37** in the following approximate ratios: 7:2:1 from **4.20**; 2:12:1 from **4.21**; and 10:3:2 from **4.38**.

Rhinocerotinoic acid (4.14):[162] fine white needles; mp 189-190 °C, lit.[162] 189-190 °C; $[\alpha]_D^{27}$ +40° (***c*** 2.52, $CHCl_3$), lit.[162] +42°; IR ν_{max} 3145 (br), 2931, 2853, 1745, 1694, 1657, 1630, 1596, 1436, 1338, 1219, 1146 cm^{-1};^{1}H & ^{13}C NMR data see Table 4.1; EIMS ***m/z*** (rel. int.) 318 [M^+] (24), 300 (26), 290 (32), 258 (31), 231 (19), 205 (54), 177 (23), 135 (100), 121 (29); HRFABMS ***m/z*** 319.2272 (calcd for $C_{20}H_{31}O_3$ [$(M + H)^+$], 319.2273).

Isorhinocerotinoic acid (4.22):[162] white crystalline solid; mp 155-157 °C, lit.[162] 156-158 °C; $[\alpha]_D^{27}$ +50° (***c*** 1.26, $CHCl_3$), lit.[162] +54°; IR ν_{max} 3214 (br), 2931, 2863, 1693, 1651, 1634, 1592, 1442, 1384, 1254, 1164, 1002 cm^{-1}; ^{1}H & ^{13}C NMR data see Table 4.2; EIMS ***m/z*** (rel. int.) 318 [M^+] (24), 300 (10), 285 (13), 258 (22), 241 (9), 220 (45), 205 (44), 176 (22), 135 (100); HRFABMS ***m/z*** 319.2273 (calcd for $C_{20}H_{31}O_3$ [$(M + H)^+$], 319.2273).

Methyl rhinocerotinoate (4.20):[162] colourless oil; $[\alpha]_D^{27}$ +40° (***c*** 2.64, $CHCl_3$); IR ν_{max} 2947, 2930, 2863, 1721, 1663, 1607, 1435, 1223, 1150, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.71 (1H, d, ***J*** = 1.2 Hz, H-14), 3.69 (3H, s, 15-OMe), 2.49 (1H, dd, ***J*** = 17.6, 3.7 Hz, H-6a), 2.37 (1H, m, ***J*** = 17.6 Hz, H-6b), 2.31 (2H, m, H_2-11), 2.24 (2H, m, H_2-12), 2.21 (3H, d, ***J*** = 1.3 Hz, H_3-16), 1.90 (1H, br d, ***J*** = 12.7 Hz, H-1a), 1.76 (3H, s, H_3-17), 1.69 (1H, dd, ***J*** = 14.3, 3.7 Hz, H-5), 1.65 (1H, m, H-2a), 1.59 (1H, m, H-2b), 1.47 (1H, br d, ***J*** = 13.2 Hz, H-3a), 1.35 (1H, td, ***J*** = 12.7, 3.7 Hz, H-

1b), 1.21 (1H, td, ***J*** = 13.5, 4.1 Hz, H-3b), 1.08 (3H, s, H_3-20), 0.91 (3H, s, H_3-19), 0.87 (3H, s, H_3-18) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.0 (s, C-7), 167.0 (s, C-15), 166.3 (s, C-9), 158.9 (s, C-13), 130.5 (s, C-8), 115.5 (d, C-14), 50.9 (q, 15-OMe), 50.3 (d, C-5), 41.3 (t, C-3), 41.0 (s, C-10), 39.6 (t, C-12), 35.9 (t, C-1), 35.2 (t, C-6), 33.1 (s, C-4), 32.5 (q, C-18), 27.7 (t, C-11), 21.3 (q, C-19), 18.8 (q, C-16), 18.6 (t, C-2), 18.2 (q, C-20), 11.4 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 332 [M^+] (69), 317 (26), 304 (43), 300 (35), 285 (36), 258 (28), 245 (28), 205 (25), 135 (100), 121 (24); HRFABMS ***m/z*** 333.2420 (calcd for $C_{21}H_{33}O_3$ [(M + H)$^+$], 333.2430).

Methyl isorhinocerotinoate (4.21):[162] colourless oil; $[\alpha]_D^{27}$ +40° (***c*** 3.00, $CHCl_3$); IR ν_{max} 2948, 2931, 2862, 1718, 1662, 1606, 1440, 1166, 1145, 1022 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.68 (1H, br s, H-14), 3.68 (3H, s, 15-OMe), 2.74 (2H, m, H_2-12), 2.49 (1H, dd, ***J*** = 17.5, 3.8 Hz, H-6a), 2.35 (1H, dd, ***J*** = 17.3, 14.6 Hz, H-6b), 2.32 (2H, m, H_2-11), 2.06 (1H, br d, ***J*** = 12.3 Hz, H-1a), 1.95 (3H, d, ***J*** = 1.0 Hz, H_3-16), 1.84 (3H, s, H_3-17), 1.71 (1H, dd, ***J*** = 14.1, 3.7 Hz, H-5), 1.69 (1H, m, H-2a), 1.63 (1H, tt, ***J*** = 11.1, 3.5 Hz, H-2b), 1.47 (1H, br d, ***J*** = 13.3 Hz, H-3a), 1.42 (1H, td, ***J*** = 12.6, 3.8 Hz, H-1b), 1.23 (1H, td, ***J*** = 13.2, 4.2 Hz, H-3b), 1.10 (3H, s, H_3-20), 0.91 (3H, s, H_3-19), 0.88 (3H, s, H_3-18) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.3 (s, C-7), 167.1 (s, C-9), 166.4 (s, C-15), 158.4 (s, C-13), 130.6 (s, C-8), 116.5 (d, C-14), 51.0 (q, 15-OMe), 50.2 (d, C-5), 41.3 (t, C-3), 41.1 (s, C-10), 35.9 (t, C-1), 35.3 (t, C-6), 33.1 (s, C-4), 32.5 (q, C-18), 32.3 (t, C-12), 27.6 (t, C-11), 25.0 (q, C-16), 21.3 (q, C-19), 18.7 (t, C-2), 18.2 (q, C-20), 11.3 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 332 [M^+] (45), 300 (15), 285 (26), 258 (35), 220 (55), 205 (28), 161 (39), 135 (100), 113 (58), 91 (38); HRFABMS ***m/z*** 333.2430 (calcd for $C_{21}H_{33}O_3$ [(M + H)$^+$], 333.2430).

Methyl 7-oxolabda-8,13(16)-dien-15-oate (4.38): colourless oil; $[\alpha]_D^{27}$ +57° (***c*** 3.03, $CHCl_3$); IR ν_{max} 2951, 2931, 2870, 1742, 1662, 1607, 1435, 1332, 1154, 1017 cm^{-1}; ^{1}H & ^{13}C NMR data see Table 4.3; EIMS ***m/z*** (rel. int.) 332 [M^+] (100), 317 (53), 299 (11), 285 (27), 259 (24), 243 (13), 231 (44), 205 (13), 135 (79), 91 (25); HRFABMS ***m/z*** 333.2420 (calcd for $C_{21}H_{33}O_3$ [(M + H)$^+$], 333.2430).

7.4.2.11 Attempted preparation of rhinocerotinoic acid (**4.14**) employing TMSCl – NaI

A portion (20 mg, 0.06 mmol) of the 10:1 ethyl rhinocerotinoate (**4.28**)/ethyl isorhinocerotinoate (**4.29**) mixture and NaI (45 mg, 0.30 mmol, 5 eq) were stirred in anhydrous CH_3CN (2.0 mL) whilst TMSCl (38 μL, 0.30 mmol, 5 eq) was added ***via*** syringe. The reaction mixture was refluxed for 15 h after which it was diluted with H_2O (2.0 mL) and stirred at room temperature for 30 min. The reaction mixture was concentrated ***in vacuo***, taken up in Et_2O (10 mL) and washed with 2% $Na_2S_2O_3$ (5.0 mL) and H_2O (5.0 mL). The organic phase was then dried ($MgSO_4$) and concentrated to give a light yellow oil (15 mg). Examination of the product by ^{1}H NMR spectroscopy indicated that it was a complex mixture of products that was not investigated further.

7.4.2.12 Acid hydrolysis of the 10:1 ethyl rhinocerotinoate (**4.28**)/ethyl isorhinocerotinoate (**4.29**) mixture (prepared ***via*** the HWE reaction)

A. Hydrolysis with HCl in THF

The 10:1 **4.28**/**4.29** mixture (25 mg, 0.07 mmol) and conc. HCl (0.5 mL) in THF (1.5 mL) was heated at 70 °C in a sealed vessel for 13.5 h. The reaction mixture was basified with 10% Na_2CO_3 (7 mL) and extracted with EtOAc (2 × 5 mL). The aqueous phase was then acidified with HCl and extracted with EtOAc (3 × 5 mL), the organic phases combined, washed with H_2O (1 mL) and dried (Na_2SO_4). Concentration of the EtOAc solution ***in vacuo*** yielded a 10:1 mixture (confirmed by ^{1}H NMR spectroscopy) of rhinocerotinoic acid (**4.14**) and isorhinocerotinoic acid (**4.22**) as a colourless oil (3 mg, 0.01 mmol, 14%).

B. Hydrolysis with HCl in DMSO

The 10:1 **4.28**/**4.29** mixture (230 mg, 0.66 mmol) and conc. HCl (1.0 mL) in DMSO (8.0 mL) was heated at 85 °C for 24 h. The reaction mixture was diluted with H_2O (20 mL) and worked up as before to afford a mixture of **4.14** and **4.22** in a ratio of 10:1 (determined by ^{1}H NMR spectroscopy) as a yellow solid (131 mg, 0.41 mmol, 62%).

C. Hydrolysis with HCl in acetic acid

The 10:1 **4.28**/**4.29** mixture (546 mg, 1.58 mmol), conc. HCl (2.0 mL) and H_2O (1.0 mL) in AcOH (6.0 mL) was heated at 100 °C for 3 h. The reaction mixture was diluted with H_2O (20 mL), extracted with EtOAc (2 × 10 mL) and the combined organic fractions extracted with 20% Na_2CO_3 (5 × 10 mL). The aqueous phases were combined, acidified with HCl and extracted with EtOAc (3 × 15 mL). These organic phases were combined, washed with H_2O (5 mL), dried (Na_2SO_4) and concentrated to yield a mixture **4.14** and **4.22** in a ratio of 10:1 (determined by ^{1}H NMR spectroscopy) as a yellow solid (345 mg, 1.08 mmol, 68%).

D. Hydrolysis with H_2SO_4 in acetic acid

The 10:1 **4.28**/**4.29** mixture (719 mg, 2.08 mmol) and 5 M H_2SO_4 (6.0 mL) in AcOH (7.5 mL) was heated at 100 °C for 4 h. The reaction mixture was worked up as above to give a mixture of **4.14** and **4.22** in a ratio of 10:1 (determined by ^{1}H NMR spectroscopy) as a light yellow solid (542 mg, 1.70 mmol, 82%). Trituration of the product with hexane followed by vacuum sublimation (150 °C, 0.5 mmHg) afforded rhinocerotinoic acid (**4.14**, 478 mg, 1.50 mmol, 72%) as fine white needles (found to be isomerically pure by ^{1}H NMR).

7.5 Chapter Five Experimental

7.5.1 Dehydration of hispanolone (5.2)

A. Dehydration using $SOCl_2$–pyridine

Hispanolone (**5.2**, 245 mg, 0.77 mmol) in anhydrous pyridine (2.0 mL) was stirred at 0 °C whilst $SOCl_2$ (335 μL, 3.86 mmol, 5 eq) in anhydrous pyridine (1.5 mL) was added dropwise. The solution was stirred at 0 °C for 1 h, warmed to ambient temperature and stirred for a further 18 h. The reaction mixture was poured into cold water (0 °C, 10.0 mL) and extracted with Et_2O (3 × 10 mL). The organic phases were combined, washed with 0.5 M HCl (3 × 10 mL), 5% $NaHCO_3$ (10 mL) and H_2O (5 mL), dried ($MgSO_4$) and concentrated to give an orange oil (211 mg). Open

column chromatography of the crude product in 19:1 hexane/EtOAc yielded hispanone (**5.1**) as pale yellow needles (143 mg, 0.48 mmol, 62%).

B. Dehydration using I_2 in benzene

Hispanolone (**5.2**, 1.00 g, 3.14 mmol) was dissolved in anhydrous benzene (100 mL) and 0.1 M I_2 in anhydrous benzene (1.60 mL, 0.16 mmol, 0.05 eq) added. The resulting pink solution was refluxed under anhydrous conditions for 6 h after which the benzene was removed ***in vacuo*** and the residue taken up in Et_2O (40 mL). The Et_2O solution was washed with 5% $Na_2S_2O_3$ (3 × 25 mL) and H_2O (25 mL), dried ($MgSO_4$) and concentrated to give a brown oil (1.07 g). The crude product was purified by column chromatography on silica gel in 19:1 hexane/EtOAc to give hispanone (**5.1**, 0.82 g, 2.73 mmol, 87 %) as pale yellow needles and hispanolone (**5.2**, 91 mg, 0.29 mmol, 9 %) as a colourless oil.

Hispanone (5.1):[210-212,214,217,220-222,224] fine white needles (from MeOH); mp 60-61 °C, lit.[210] 58-60 °C; $[\alpha]_D^{24}$ +41° (***c*** 3.94, $CHCl_3$), lit.[210] +39.7°; IR ν_{max} 2930, 2869, 1661, 1606, 1471, 1326, 1025, 874, 781, 600 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.36 (1H, t, ***J*** = 1.6 Hz, H-15), 7.26 (1H, br s, H-16), 6.30 (1H, d, ***J*** = 0.7 Hz, H-14), 2.52 (2H, m, H_2-12), 2.48 (1H, m, H-6a), 2.45 (2H, m, H_2-11), 2.36 (1H, m, H-6b), 1.96 (1H, br d, ***J*** = 12.0 Hz, H-1a), 1.79 (3H, s, H_3-17), 1.71 (1H, dd, ***J*** = 14.2, 3.8 Hz, H-5), 1.68 (1H, m, H-2a), 1.58 (1H, m, H-2b), 1.48 (1H, br d, ***J*** = 12.8, 3.7 Hz, H-3a), 1.40 (1H, td, ***J*** = 12.8, 3.7 Hz, H-1b), 1.22 (1H, td, ***J*** = 13.4, 4.1 Hz, H-3b), 1.08 (3H, s, H_3-20), 0.91 (3H, s, H_3-19), 0.88 (3H, s, H_3-18) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.3 (s, C-7), 167.0 (s, C-9), 143.0 (d, C-15), 138.6 (d, C-16), 130.3 (s, C-8), 124.5 (s, C-13), 110.6 (d, C-14), 50.3 (d, C-5), 41.3 (t, C-3), 40.9 (s, C-10), 35.9 (t, C-1), 35.2 (t, C-6), 33.1 (s, C-4), 32.5 (q, C-18), 30.2 (t, C-11), 24.2 (t, C-12), 21.3 (q, C-19), 18.6 (t, C-2), 18.1 (q, C-20), 11.4 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 300 [M^+] (39), 285 (60), 282 (80), 267 (87), 177 (68), 176 (100), 161 (54), 135 (81), 133 (83), 81 (48); HRFABMS ***m/z*** 301.2167 (calcd for $C_{20}H_{29}O_2$ [$(M + H)^+$], 301.2168).

7.5.2 Attempted preparation of the four 6,7-diastereomeric 14,15-epoxylabda-8,13(16),14-trien-6,7-diols (5.11, 5.12, 5.17 and 5.18) via *6-acetoxylation and hydride reduction of hispanone (5.1)*

7.5.2.1 Preparation of 6α- and 6β-acetoxy-14,15-epoxylabda-8,13(16),14-trien-7-one (**5.9** and **5.10**)

A. Preparation of manganous acetate [manganese (II) acetate tetrahydrate, $Mn(OAc)_2{\cdot}4H_2O$]

$MnCO_3$ (30 g, 0.26 mol) was refluxed in a mixture of AcOH (60 mL) and H_2O (90 mL) for 5 h, cooled, diluted with H_2O (50 mL) and filtered under vacuum. The precipitate was washed with H_2O (50 mL) and the filtrate evaporated on a steam bath. The residue was re-suspended in H_2O (30 mL) and left in an open dish for 1 week to yield manganous acetate (34.5 g, 0.14 mol, 54%) as large pink crystals.

B. Preparation of hydrated manganic acetate [manganese (III) acetate dihydrate, $Mn(OAc)_3{\cdot}2H_2O$] [230a]

Powdered $Mn(OAc)_2{\cdot}4H_2O$ (12.0 g, 49 mmol) and AcOH (150 mL) were heated under reflux for 30 min. Powdered $KMnO_4$ (2.0 g, 12.7 mmol, 3.9 eq) was then slowly added to the hot solution and the mixture refluxed for a further 30 min. After stirring at ambient temperature for 5h, H_2O (20 mL) was added and the reaction mixture left to stand overnight. The resulting precipitate was filtered off, washed with AcOH (15 mL) and air-dried for 5 days at RT to yield manganic acetate (12.0 g, 45 mmol, 92%) as a reddish-brown powder.

C. α′-Acetoxylation of the α,β-unsaturated ketone 5.1

Before performing this reaction, all apparatus were thoroughly flame dried under N_2. The reaction was performed under an atmosphere of dry air and meticulously anhydrous conditions were maintained throughout. The $Mn(OAc)_3$ used was dried ***in vacuo*** (0.5 mmHg) over P_2O_5 at 70 °C for 6 h immediately prior to use.

Hispanone (**5.1**, 499 mg, 1.66 mmol) was dissolved in anhydrous benzene (50 mL), $Mn(OAc)_3$ (2.50 g; 1.5 g per mmol of substrate[230b]) added and the resulting brown suspension heated under reflux for 72 h. The heterogeneous reaction mixture was then cooled and the fine brown precipitate dissolved by vigorous stirring with 10% aqueous $Na_2S_2O_5$ (30 mL) for 30 min. Conc. HCl (3.0 mL) was added (to give a 0.9 M HCl solution in the aqueous phase) and the mixture stirred for a further 10 min. The organic and aqueous phases were then separated, the aqueous phase thoroughly washed with EtOAc (4 × 25 mL) and the combined organic phases washed with 5% $NaHCO_3$ (10 mL) and H_2O (10 mL). Drying with $MgSO_4$ and evaporation of the solvent gave a yellow oil (636 mg) that was purified by silica gel column chromatography (7:3 hexane/EtOAc) to yield a 2:1 mixture (by ^{1}H NMR spectroscopy) of 6α- and 6β-acetoxy-14,15-epoxylabda-8,13(16),14-trien-7-one (**5.9** and **5.10**) as a pale yellow oil (529 mg, 1.48 mmol, 89%). Normal phase semi-preparative HPLC of a portion (501 mg) of the diastereomeric mixture in 9:1 hexane/EtOAc afforded the pure acetoxy enones **5.9** (323 mg) and **5.10** (169 mg) as colourless oils.

6α-Acetoxy-14,15-epoxylabda-8,13(16),14-trien-7-one (5.9):[210] colourless oil; $[\alpha]_D^{25}$ +50° (***c*** 1.00, $CHCl_3$), lit.[210] +38°; IR ν_{max} 2932, 2872, 1747, 1674, 1614, 1470, 1373, 1235, 1025, 874 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (1H, t, ***J*** = 1.6 Hz, H-15), 7.26 (1H, br s, H-16), 6.29 (1H, d, ***J*** = 0.8 Hz, H-14), 5.64 (1H, d, ***J*** = 13.4 Hz, H-6), 2.55 (2H, m, H_2-12), 2.49 (1H, m, H-11a), 2.44 (1H, m, H-11b), 2.19 (3H, s, 6-OAc), 2.04 (1H, d, ***J*** = 13.2 Hz, H-5), 1.99 (1H, br d, ***J*** = 11.6 Hz, H-1a), 1.76 (3H, s, H_3-17), 1.68 (1H, tt, ***J*** = 13.6, 3.2 Hz, H-2a), 1.60 (1H, m, H-2b), 1.45 (1H, br d, ***J*** = 13.3 Hz, H-3a), 1.42 (1H, td, ***J*** = 12.9, 3.7 Hz, H-1b), 1.29 (1H, td, ***J*** = 13.6, 4.1 Hz, H-3b), 1.28 (3H, s, H_3-20), 1.05 (3H, s, H_3-18), 1.01 (3H, s, H_3-19) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.6 (s, C-7), 170.4 (s, 6-OAc), 166.9 (s, C-9), 143.1 (d, C-15), 138.7 (d, C-16), 128.9 (s, C-8), 124.2 (s, C-13), 110.5 (d, C-14), 74.7 (d, C-6), 53.8 (d, C-5), 42.7 (t, C-3), 42.6 (s, C-10), 36.5 (t, C-1), 35.6 (q, C-18), 33.5 (s, C-4), 30.4 (t, C-11), 24.1 (t, C-12), 21.9 (q, C-19), 21.3 (q, 6-OAc), 19.9 (q, C-20), 18.5 (t, C-2), 11.6 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 358 [M^+] (4), 343 (25), 298 (62), 283 (84), 265 (45), 255 (100), 203 (57), 189 (78), 175 (41), 161 (91); HRFABMS ***m/z*** 359.2222 (calcd for $C_{22}H_{31}O_4$ [$(M + H)^+$], 359.2222).

6β-Acetoxy-14,15-epoxylabda-8,13(16),14-trien-7-one (5.10):[210] colourless oil; $[\alpha]_D^{26}$ –61° (***c*** 1.04, $CHCl_3$); IR ν_{max} 2932, 2863, 2747, 1668, 1606, 1471, 1370, 1230, 1027, 874, 600 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.37 (1H, t, ***J*** = 1.6 Hz, H-15), 7.27 (1H, br s, H-16), 6.31 (1H, d, ***J*** = 0.8 Hz, H-14), 5.79 (1H, d, ***J*** = 3.3 Hz, H-6), 2.58 (2H, m, H_2-12), 2.53 (1H, m, H-11a), 2.50 (1H, m, H-11b), 2.06 (3H, s, 6-OAc), 1.96 (1H, br d, ***J*** = 12.5 Hz, H-1a), 1.85 (3H, s, H_3-17), 1.77 (1H, tt, ***J*** = 13.8, 3.3 Hz, H-2a), 1.76 (1H, d, ***J*** = 3.3 Hz, H-5), 1.62 (1H, dt, ***J*** = 14.1, 3.5 Hz, H-2b), 1.46 (1H, dd, ***J*** = 13.2, 1.2 Hz, H-3a), 1.42 (3H, s, H_3-20), 1.37 (1H, td, ***J*** = 13.1, 3.8 Hz, H-1b), 1.24 (1H, td, ***J*** = 13.4, 3.8 Hz, H-3b), 1.05 (3H, s, H_3-18), 1.04 (3H, s, H_3-19) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 193.5 (s, C-7), 169.6 (s, 6-OAc), 168.5 (s, C-9), 143.1 (d, C-15), 138.7 (d, C-16), 129.3 (s, C-8), 124.4 (s, C-13), 110.5 (d, C-14), 70.2 (d, C-6), 53.0 (d, C-5), 43.6 (t, C-3), 41.1 (s, C-10), 37.6 (t, C-1), 33.8 (s, C-4), 32.5 (q, C-18), 30.7 (t, C-11), 24.3 (t, C-12), 23.0 (q, C-19), 21.8 (q, C-20), 21.4 (q, 6-OAc), 18.6 (t, C-2), 11.7 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 358 [M^+] (2), 298 (58), 283 (63), 265 (36), 255 (100), 234 (46), 203 (68), 192 (88), 161 (95), 81(21); HRFABMS ***m/z*** 359.2222 (calcd for $C_{22}H_{31}O_4$ [$(M + H)^+$], 359.2222).

7.5.2.2 $LiAlH_4$ reduction of the α′-acetoxy enones **5.9** and **5.10**

$LiAlH_4$ (10 mg, 0.26 mmol, 2 eq) was stirred in Et_2O (4.0 mL) at 0 °C whilst **5.9** (45 mg, 0.13 mmol) in Et_2O (4.0 mL) was added dropwise ***via*** cannula. The reaction was stirred under N_2 at RT for 1 h before being cooled to 0 °C and quenched with H_2O (5.0 mL). The resulting suspension was acidified with 1 M HCl (5.0 mL) and the aqueous phase extracted with Et_2O (3 × 5 mL). The organic layers were combined, washed with 5% $NaHCO_3$ (5 mL) and H_2O (5 mL), dried (Na_2SO_4) and concentrated ***in vacuo*** to give pure (analysed by normal phase HPLC in 3:2 hexane/EtOAc) 15,16-epoxylabda-8,13(16),14-trien-6α,7β-diol (**5.11**) as a colourless oil (35 mg, 0.11 mmol, 87%).

Compound **5.10** (19 mg, 0.05 mmol) was reduced in exactly the same manner to yield pure (analysed by normal phase HPLC in 3:2 hexane/EtOAc) 15,16-epoxylabda-8,13(16),14-trien-6β,7β-diol (**5.12**) as a white crystalline solid (16 mg, 0.05 mmol, 99%).

15,16-Epoxylabda-8,13(16),14-trien-6α,7β-diol (5.11): colourless oil; $[\alpha]_D^{26}$ +47° (***c*** 1.84, $CHCl_3$); IR ν_{max} 3401(br), 2930, 2870, 1470, 1384, 1067, 1025, 874, 777, 599 cm^{-1}; ^1H & ^{13}C NMR data ($CDCl_3$) see Table 5.1; ^1H NMR (C_6D_6, 400 MHz) δ 7.16 (1H, s, H-15), 7.09 (1H, s, H-16), 6.12 (1H, s, H-14), 3.78 (1H, dd, ***J*** = 11.3, 7.8 Hz, H-6), 3.72 (1H, d, ***J*** = 7.8 Hz, H-7), 2.38 (2H, m, H_2-12), 2.19 (1H, m, H-11a), 2.02 (1H, m, H-11b), 1.66 (3H, s, H_3-17), 1.63 (1H, m, H-1a), 1.50 (1H, m, H-2a), 1.43 (1H, m, H-2b), 1.37 (3H, s, H_3-18), 1.35 (1H, m, H-3a), 1.30 (1H, d, ***J*** = 11.3 Hz, H-5), 1.19 (1H, m, H-3b), 1.14 (3H, s, H_3-19), 1.07 (1H, dd, ***J*** = 12.6, 3.9 Hz, H-1b), 0.99 (3H, s, H_3-20) ppm, irradiation of H-5 (δ_H 1.30) caused both H-6 (δ_H 3.78) and H-7 (δ_H 3.72) to resonate as doublets, ***J*** = 7.9 Hz; ^{13}C NMR (C_6D_6, 100 MHz) δ 143.5 (s, C-9), 143.1 (d, C-15), 138.9 (d, C-16), 127.6 (s, C-8), 125.6 (s, C-13), 111.0 (d, C-14), 80.5 (d, C-7), 74.8 (d, C-6), 53.9 (d, C-5), 43.9 (t, C-3), 42.4 (s, C-10), 37.4 (t, C-1), 36.6 (q, C-18), 33.6 (s, C-4), 29.2 (t, C-11), 25.4 (t, C-12), 22.5 (q, C-19), 21.7 (q, C-20), 19.1 (t, C-2), 14.7 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 318 [M^+] (1), 300 (6), 236 (89), 218 (16), 203 (24), 175 (100), 149 (41), 135 (28), 105 (17), 81 (19); HRFABMS ***m/z*** 318.2194 (calcd for $C_{20}H_{30}O_3$ [M^+], 318.2195).

15,16-Epoxylabda-8,13(16),14-trien-6β,7β-diol (5.12): white crystalline solid; mp 103-104 °C; $[\alpha]_D^{26}$ +31° (***c*** 1.55, $CHCl_3$); IR ν_{max} 3402 (br), 2928, 2863, 1471, 1385, 1066, 1026, 901, 874, 777 cm^{-1}; ^1H & ^{13}C NMR data see Table 5.1; EIMS ***m/z*** (rel. int.) 318 [M^+] (8), 300 (29), 236 (18), 203 (26), 175 (42), 165 (40), 149 (100), 135 (56), 121 (37), 81 (40); HRFABMS ***m/z*** 318.2194 (calcd for $C_{20}H_{30}O_3$ [M^+], 318.2193).

7.5.2.3 Reduction of the α′-acetoxy enones **5.9** and **5.10** using Luche's reagent

Compound **5.9** (72 mg, 0.20 mmol) and $CeCl_3 \cdot 7H_2O$ (75 mg, 0.20 mmol, 1 eq) were stirred in MeOH (5.0 mL) at 0 °C and $NaBH_4$ (30 mg, 0.79 mmol, 4 eq) added in portions over 15 min. The solution was allowed to warm to RT and the progress of the reaction followed by TLC (7:3 hexane/EtOAc). After 8 h, the reaction was quenched with acetone (2.0 mL), the resulting cloudy solution concentrated ***in vacuo***, the residue taken up in sat. brine (5.0 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic fractions were combined, dried ($MgSO_4$) and concentrated to give a colourless oil (71 mg), which, after semi-preparative normal phase HPLC (3:2 hexane/EtOAc), afforded unreacted starting material (**5.9**) as a colourless oil (15 mg, 0.042 mmol, 21%), 7β-acetoxy-15,16-epoxylabda-8,13(16),14-trien-6α-ol (**5.22**, 17 mg, 0.047 mmol, 24%) and 6α-acetoxy-15,16-epoxylabda-8,13(16),14-trien-7β-ol (**5.13**, 23 mg, 0.064 mmol, 32%) as white crystalline solids, and the diol **5.11** as a colourless oil (6 mg, 0.019 mmol, 10%).

The 6β-acetoxy enone **5.10** (56 mg, 0.16 mmol) was treated in the same manner as above (except that the reaction was quenched after 10 min at RT) to give a colourless oil (56 mg). Normal phase semi-preparative HPLC of the oil in 7:3 hexane/EtOAc afforded 7β-acetoxy-15,16-epoxylabda-8,13(16),14-trien-6β-ol (**5.23**) as a white crystalline solid (22 mg, 0.061 mmol, 38%) and 6β-acetoxy-15,16-epoxylabda-8,13(16),14-trien-7β-ol (**5.14**) as a colourless oil (32 mg, 0.089 mmol, 56%).

7β-Acetoxy-15,16-epoxylabda-8,13(16),14-trien-6α-ol (5.22): white crystalline solid; mp 117-119 °C; $[\alpha]_D^{27}$ +23° (***c*** 0.75, $CHCl_3$); IR ν_{max} 3470 (br), 2930, 2863, 1726, 1716, 1456, 1373, 1246, 1025, 874 cm^{-1}; ^1H & ^{13}C NMR data see Table 5.2; EIMS ***m/z*** (rel. int.) 360 [M^+] (8), 300 (17), 285 (39), 267 (17), 260 (22), 218 (100), 162 (18), 149 (62), 135 (33), 91 (18); HRFABMS ***m/z*** 361.2377 (calcd for $C_{22}H_{33}O_4$ [$(M + H)^+$], 361.2379).

6α-Acetoxy-15,16-epoxylabda-8,13(16),14-trien-7β-ol (5.13): white crystalline solid; mp 88-90 °C; $[\alpha]_D^{24}$ +52° (***c*** 1.03, $CHCl_3$); IR ν_{max} 3437 (br), 2929, 2855, 1732, 1714, 1385, 1248, 1026, 874,

775 cm^{-1}; ^1H & ^{13}C NMR data see Table 5.2; EIMS ***m/z*** (rel. int.) 300 [$(M - AcOH)^+$] (7), 285 (15), 267 (16), 218 (99), 203 (20), 175 (23), 162 (51), 149 (100), 135 (39), 91 (26); HRFABMS ***m/z*** 361.2377 (calcd for $C_{22}H_{33}O_4$ [$(M + H)^+$], 361.2379).

7β-Acetoxy-15,16-epoxylabda-8,13(16),14-trien-6β-ol (5.23): white crystalline solid; mp 107-109 °C; $[\alpha]_D^{24}$ +8° (***c*** 1.06, $CHCl_3$); IR ν_{max} 3437 (br), 2926, 2854, 1738, 1463, 1384, 1245, 1026, 874, 775 cm^{-1}; ^1H & ^{13}C NMR data see Table 5.3; EIMS ***m/z*** (rel. int.) 360 [M^+] (1), 300 (18), 278 (37), 260 (16), 218 (100), 203 (26), 175 (27), 162 (45), 149 (85), 135 (46); HRFABMS ***m/z*** 360.2302 (calcd for $C_{22}H_{32}O_4$ [M^+], 360.2301).

6β-Acetoxy-15,16-epoxylabda-8,13(16),14-trien-7β-ol (5.14): colourless oil; $[\alpha]_D^{24}$ +11° (***c*** 1.64, $CHCl_3$); IR ν_{max} 3402 (br), 2924, 2852, 1738, 1730, 1459, 1385, 1250, 1027, 874 cm^{-1}; ^1H & ^{13}C NMR data see Table 5.3; EIMS ***m/z*** (rel. int.) 360 [M^+] (1), 300 (13), 278 (24), 267 (14), 218 (55), 175 (25), 162 (29), 149 (100), 135 (46), 91 (38); HRFABMS ***m/z*** 360.2301 (calcd for $C_{22}H_{32}O_4$ [M^+], 360.2301).

7.5.2.4 Acetylation of the diols **5.11** and **5.12** and the monoacetates **5.13**, **5.14**, **5.22** and **5.23**

The 6α,7β-diol **5.11** (15 mg) and the two 6α,7β-hydroxy esters **5.13** (5 mg) and **5.22** (5 mg) were separately treated with Ac_2O (0.5 mL) and pyridine (0.5 mL) at ambient temperature for 48 h. The reactions were quenched by adding MeOH (2.0 mL), stirred for 1 h and the excess MeOH, pyridine and MeOAc removed in vacuo (0.5 mmHg) to yield three pale yellow oils (24 mg from **5.11**, 6 mg from **5.13** and 7 mg from **5.22**). Each of the oils were separately purified by normal phase HPLC in 4:1 hexane/EtOAc to yield 6α,7β-diacetoxy-15,16-epoxy-labda-8,13(16),14-triene (**5.25**) exclusively (14 mg from **5.11**, 3 mg from **5.13** and 4 mg from **5.22**). The diacetylated products obtained from **5.13** and **5.22** exhibited identical HPLC retention times and NMR spectra (^1H and ^{13}C) to those of the product obtained from the diol **5.11**.

The 6β,7β-diol **5.12** (18 mg) and the two 6β,7β-hydroxy esters **5.14** (6 mg) and **5.23** (5 mg) were treated in an identical manner to yield 6β,7β-diacetoxy-15,16-epoxy-labda-8,13(16),14-triene (**5.25**) exclusively (15 mg from **5.12**, 4 mg from **5.14** and 4 mg from **5.23**). Once again, the diacetylated products obtained from **5.14** and **5.23** exhibited identical HPLC retention times and NMR spectra (^{1}H and ^{13}C) to those of the product obtained from the diol **5.12**.

6α,7β-Diacetoxy-15,16-epoxy-labda-8,13(16),14-triene (5.25): white crystalline solid; mp 71-72 °C; $[\alpha]_D^{28}$ +5° (***c*** 1.45, $CHCl_3$); IR ν_{max} 2931, 2872, 1739, 1441, 1368, 1246, 1094, 1024, 874, 778 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (1H, t, ***J*** = 1.6 Hz, H-15), 7.23 (1H, br s, H-16), 6.27 (1H, d, ***J*** = 0.8 Hz, H-14), 5.52 (1H, m, H-6), 5.49 (1H, m, H-7), 2.47 (2H, m, H_2-12), 2.29 (1H, m, H-11a), 2.17 (1H, m, H-11b), 2.04 (3H, s, 7-OAc), 2.02 (3H, s, 6-OAc), 1.87 (1H, br d, ***J*** = 11.2 Hz, H-1a), 1.65 (1H, d, ***J*** = 11.9 Hz, H-5), 1.59 (1H, m, H-2a), 1.53 (3H, s, H_3-17), 1.50 (1H, m, H-2b), 1.38 (1H, br d, ***J*** = 13.5 Hz, H-3a), 1.27 (1H, m, H-1b), 1.23 (1H, m, H-3b), 1.17 (3H, s, H_3-20), 1.02 (3H, s, H_3-18), 0.91 (3H, s, H_3-19) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.4 (s, 7-OAc), 170.6 (s, 6-OAc), 146.3 (s, C-9), 142.8 (d, C-15), 138.5 (d, C-16), 124.9 (s, C-13), 123.9 (s, C-8), 110.7 (d, C-14), 79.0 (d, C-7), 73.5 (d, C-6), 52.9 (d, C-5), 43.4 (t, C-3), 41.8 (s, C-10), 37.0 (t, C-1), 35.9 (q, C-18), 33.1 (s, C-4), 28.8 (t, C-11), 25.0 (t, C-12), 22.0 (q, C-19), 21.8 (q, 6-OAc), 21.4 (q, C-20), 21.0 (q, 7-OAc), 18.5 (t, C-2), 14.7 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 342 [(M – AcOH)$^{+}$] (4), 300 (12), 282 (14), 267 (26), 260 (27), 219 (45), 218 (100), 197 (20), 149 (30), 135 (33); HRFABMS ***m/z*** 402.2405 (calcd for $C_{24}H_{34}O_5$ [M^{+}], 402.2406).

6β,7β-Diacetoxy-15,16-epoxy-labda-8,13(16),14-triene (5.26): white crystalline solid; mp 123-124 °C, $[\alpha]_D^{26}$ –5 ° (***c*** 1.48, $CHCl_3$); IR ν_{max} 2931, 2868, 1741, 1441, 1366, 1250, 1229, 1025, 874, 779 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (1H, t, ***J*** = 1.6 Hz, H-15), 7.24 (1H, d, ***J*** = 0.8 Hz, H-16), 6.29 (1H, dd, ***J*** = 1.7, 0.8 Hz, H-14), 5.70 (1H, br d, ***J*** = 4.9 Hz, H-6), 5.46 (1H, br d, ***J*** = 5.1 Hz, H-7), 2.49 (2H, m, H_2-12), 2.35 (1H, m, H-11a), 2.24 (1H, m, H-11b), 2.06 (3H, s, 6-OAc), 2.01 (3H, s, 7-OAc), 1.83 (1H, br d, ***J*** = 11.7 Hz, H-1a), 1.68 (1H, tt, ***J*** = 13.7, 3.3 Hz, H-2a), 1.58 (3H, s, H_3-17), 1.55 (1H, m, H-2b), 1.43 (1H, br d, ***J*** = 14.2 Hz, H-3a), 1.40 (1H, br s, H-5), 1.38 (3H, s, H_3-20), 1.21 (1H, m, H-1b), 1.18 (1H, m, H-3b), 0.99 (3H, s, H_3-18), 0.96 (3H, s, H_3-19)

ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.7 (s, 6-OAc), 170.6 (s, 7-OAc), 144.9 (s, C-9), 142.8 (d, C-15), 138.5 (d, C-16), 125.1 (s, C-13), 122.4 (s, C-8), 110.7 (d, C-14), 72.6 (d, C-7), 66.4 (d, C-6), 51.3 (d, C-5), 42.8 (t, C-3), 40.1 (s, C-10), 39.6 (t, C-1), 33.7 (s, C-4), 33.4 (q, C-18), 28.7 (t, C-11), 25.0 (t, C-12), 23.1 (q, C-19), 21.4 (q, C-20), 21.3 (q, 6-OAc), 20.8 (q, 7-OAc), 18.8 (t, C-2), 14.4 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 341 [$(M - AcOH_2)^+$] (1), 300 (4), 285 (8), 259 (10), 218 (100), 205 (10), 162 (20), 149 (39), 135 (24), 92 (9); HRFABMS ***m/z*** 402.2406 (calcd for $C_{24}H_{34}O_5$ [M^+], 402.2406).

7.5.3 Preparation of the four 6,7-diastereomeric 14,15-epoxylabda-8,13(16),14-trien-6,7-diols (5.11, 5.12, 5.17 and 5.18) via *hydroxyl directed hydride reduction of 6α- and 6β-hydroxy-15,16-epoxylanbda-8,13(16),14-trien-7-one (5.27 and 5.28)*

7.5.3.1 Attempted acid hydrolysis of 6α- and 6β-acetoxy-14,15-epoxylabda-8,13(16),14-trien-7-one (**5.9** and **5.10**)

The α′-acetoxy α,β-unsaturated ketones **5.9** and **5.10** (15 mg each, 0.04 mmol) were separately treated with conc. HCl (100 μL) in THF (1.0 mL), heated at 50 °C and the reactions followed by TLC. After 2h, the reaction mixtures were neutralised with 10% Na_2CO_3 (1 mL) and extracted with Et_2O (3 × 5 mL). The respective organic phases were combined, washed with H_2O (1 mL), dried (Na_2SO_4) and concentrated to give two brown oils (9 mg from **5.9** and 12 mg from **5.10**). Analysis of the oils by ^{1}H NMR spectroscopy indicated that both comprised a complex mixture of products that were not investigated further.

7.5.3.2 Saponification of the acetoxy enones **5.9** and **5.10**

6α-Acetoxy-14,15-epoxylabda-8,13(16),14-trien-7-one (**5.9**, 163 mg, 0.46 mmol) in EtOH (30 mL) and 1.5 M KOH (5.0 mL) was heated at 70 °C for 1 h. The reaction mixture was cooled, 1.0 M

HCl (10.0 mL) added and the EtOH removed by rotary evaporation to give a cloudy suspension that was extracted with CH_2Cl_2 (3 × 15 mL). The organic phases were combined, washed with 5% $NaHCO_3$ (10 mL), dried ($MgSO_4$) and concentrated to give a yellow oil (106 mg). Column chromatography (4:1 hexane/EtOAc) and normal phase semi-preparative HPLC of the oil (19:1 hexane/EtOAc) gave dihydro-7-hydroxyhedychenone (**5.29**, 38 mg, 0.12 mmol, 26%), 6-hydroxy-15,16-epoxylabda-5,8,13(16),14-tetraen-7-one (**5.30**, 45 mg, 0.14 mmol, 30%) and 6α-hydroxy-15,16-epoxylabda-8,13(16),14-trien-7-one (**5.27**, 7 mg, 0.02 mmol, 4%).

6β-Acetoxy-14,15-epoxylabda-8,13(16),14-trien-7-one (**5.10**, 58 mg, 0.16 mmol) in EtOH (10 mL) and 1.5 M KOH (2.0 mL) was treated in the same manner except that the product mixture obtained (yellow oil, 50 mg) was separated by HPLC only. The same products, **5.29** (19 mg, 0.06 mmol), **5.30** (20 mg, 0.06 mmol) and **5.27** (4 mg, 0.01 mmol) were obtained in 38%, 39% and 6% yield respectively.

Dihydro-7-hydroxyhedychenone (5.29):[236] yellow oily solid; $[\alpha]_D^{25}$ +2° (***c*** = 0.89, $CHCl_3$), lit.[236] +0.7°; IR ν_{max} 3382 (br), 2930, 2863, 1692, 1615, 1615, 1471, 1385, 1026, 874, 774 cm^{-1}; ^1H & ^{13}C NMR data see Table 5.4; EIMS ***m/z*** (rel. int.) 316 [M^+] (6), 301 (100), 283 (43), 255 (17), 229 (12), 192 (17), 175 (12), 161 (26), 133 (14), 96 (11); HRFABMS ***m/z*** 317.2116 (calcd for $C_{20}H_{29}O_3$ [$(M + H)^+$], 317.2117).

6-Hydroxy-15,16-epoxylabda-5,8,13(16),14-tetraen-7-one (5.30): pale yellow oil; $[\alpha]_D^{25}$ –41° (***c*** 0.88, $CHCl_3$); IR ν_{max} 3367 (br), 2927, 1624, 1600, 1455, 1384, 1029, 872, 772 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.38 (1H, br s, H-15), 7.28 (1H, br s, H-16), 6.32 (1H, br s, H-14), 2.66 (1H, m, H-11a), 2.57 (2H, m, H_2-12), 2.53 (1H, m, H-11b), 2.05 (1H, m, H-1a), 1.98 (3H, s, H_3-17), 1.89 (1H, m, H-3a), 1.83 (1H, m, H-2a), 1.72 (1H, m, H-2b), 1.41 (1H, m, H-3b), 1.39 (3H, s, H_3-18), 1.38 (6H, s, H_3-19 & H_3-20) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.7 (s, C-7), 165.8 (s, C-9), 143.1 (s, C-6 & d, C-15), 140.5 (s, C-5), 138.7 (d, C-16), 127.4 (s, C-8), 124.4 (s, C-13), 110.5 (d, C-14), 43.9 (s, C-10), 37.3 (t, C-3), 35.7 (s, C-4), 31.5 (t, C-11), 29.4 (t, C-1), 28.1 (q, C-18), 28.0 (q, C-20), 27.6 (q, C-19), 23.8 (t, C-12), 17.2 (t, C-2), 11.6 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 314

$[M^+]$ (76), 299 (69), 281 (72), 267 (14), 245 (19), 233 (23), 215 (100), 205 (58), 177 (39); HRFABMS ***m/z*** 315.1960 (calcd for $C_{20}H_{27}O_3$ $[(M + H)^+]$, 315.1960).

6α-Hydroxy-15,16-epoxylabda-8,13(16),14-trien-7-one (5.27): white crystalline solid; mp 95-96 °C; $[\alpha]_D^{25}$ +51° (***c*** 4.29, $CHCl_3$); IR ν_{max} 2437 (br), 2924, 2855, 1660, 1464, 1345, 1315, 1118, 874, 775 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.37 (1H, t, ***J*** = 1.6 Hz, H-15), 7.26 (1H, s, H-16), 6.29 (1H, d, ***J*** = 0.8 Hz, H-14), 4.38 (1H, dd, ***J*** = 13.1, 2.0 Hz, H-6), 2.54 (2H, m, H_2-12), 2.52 (1H, m, H-11a), 2.44 (1H, m, H-11b), 1.95 (1H, br d, ***J*** = 12.4 Hz, H-1a), 1.85 (3H, s, H_3-17), 1.69 (1H, d, ***J*** = 13.1 Hz, H-5), 1.68 (1H, m, H-2a), 1.57 (1H, m, H-2b), 1.46 (1H, br d, ***J*** = 13.6 Hz, H-3a), 1.39 (1H, m, H-1b), 1.26 (1H, m, H-3b), 1.25 (3H, s, H_3-20), 1.17 (6H, s, H_3-18 & H_3-19) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.7 (s, C-7), 168.9 (s, C-9), 143.1 (d, C-15), 138.7 (d, C-16), 127.4 (s, C-8), 124.3 (s, C-13), 110.5 (d, C-14), 73.3 (d, C-6), 56.7 (d, C-5), 42.8 (t, C-3), 42.5 (s, C-10), 36.7 (t, C-1), 35.8 (q, C-18), 34.0 (s, C-4), 30.6 (t, C-11), 24.0 (t, C-12), 22.0 (q, C-19), 19.4 (q, C-20), 18.3 (t, C-2), 11.6 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 316 $[M^+]$ (5), 301 (100), 283 (45), 255 (16), 229 (11), 192 (10), 175 (12), 161 (29), 135 (12), 91 (17); HRFABMS ***m/z*** 317.2117 (calcd for $C_{20}H_{29}O_3$ $[(M + H)^+]$, 317.2117).

7.5.3.3 Attempted preparation of hydroxy ketone **5.27** by application of Mori's potassium cyanide catalysed transesterification procedure[240] to α′-acetoxy enone **5.9**

The 6α-acetoxy enone **5.9** (54 mg, 0.15 mmol) and KCN (20 mg, 0.31 mmol, 2.1 eq) were dissolved in 95% EtOH (1.0 mL; to give a 2% solution with respect to KCN[240]) and the solution heated under reflux for 8 h (the reaction was halted when no **5.9** was detected by TLC). After removal of the solvent, the residue was taken up in H_2O (5.0 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The organic fractions were combined, dried (Na_2SO_4) and concentrated to give a pale yellow oil (38 mg), which, after normal phase HPLC (9:1 hexane/EtOAc), yielded 7β-hydroxy-9α-carbonitrile-15,16-epoxylabda-13(16),14-dien-6-one (**5.34**, 22 mg, 0.064 mmol, 43%) as an off-white solid and the 6α-hydroxy ketone **5.27** (7 mg, 0.022 mmol, 15%) as a white solid.

7β-Hydroxy-9α-carbonitrile-15,16-epoxylabda-13(16),14-dien-6-one (5.34): off-white solid; mp 69-71 °C; $[\alpha]_D^{26}$ +56° (***c*** 1.59, $CHCl_3$), IR ν_{max} 3469 (br), 2980, 2932, 2874, 2228, 1715, 1464, 1366, 1046, 1025, 874, 780 cm^{-1}; ^1H & ^{13}C NMR data see Table 5.5; EIMS ***m/z*** (rel. int.) 343 [M^+] (6), 326 (17), 314 (5), 262 (8), 182 (21), 139 (29), 121 (25), 95 (100), 67 (46); HRFABMS ***m/z*** 344.2226 (calcd for $C_{21}H_{30}NO_3$ [$(M + H)^+$], 344.2226).

7.5.3.4 Preparation of 6α- and 6β-hydroxy-15,16-epoxylanbda-8,13(16),14-trien-7-one (**5.27** and **5.28**) ***via*** Vedejs' oxidation

A. Preparation of the hydrated oxodiperoxymolybdenum·hexamethylphosphoramide complex MoO_5·H_2O·HMPA and the anhydrous complex MoO_5·HMPA[287]

MoO_3 (2.00 g, 13.9 mmol) was stirred vigorously in 30% H_2O_2 (10.0 mL) at 40 °C for 4 h, cooled to ambient temperature and filtered. The resulting yellow solution was cooled to 10 °C and HMPA (2.50 mL, 14.3 mmol, 1.03 eq) added with stirring. The resulting yellow crystals were collected on a Büchner funnel, washed with H_2O (5 mL) and dried overnight in a vacuum desiccator to give MoO_5·H_2O·HMPA as yellow needles (4.28 g).

The anhydrous complex MoO_5·HMPA (3.95 g, 11.1 mmol, 80%) was prepared by drying the hydrated complex (4.28 g) over P_2O_5 ***in vacuo*** (0.3 mmHg) at 20 °C for 30 h.

B. Preparation of the oxodiperoxymolybdenum·pyridine·hexamethylphosphoramide complex MoO_5·pyridine·HMPA [MoOPH (5.36)][245,287]

MoO_5·HMPA (3.95 g, 11.1 mmol) in anhydrous THF (10.0 mL) was stirred at 20 °C while anhydrous pyridine (930 μL, 11.5 mmol, 1.04 eq) in THF (5.0 mL) was added dropwise over 5 min. The resulting yellow precipitate was collected by rapid filtration, washed with anhydrous Et_2O (10.0 mL) and dried ***in vacuo*** to give MoOPH (**5.36**; 4.31 g, 9.9 mmol, 89%) as fine yellow crystals. MoOPH was stored under N_2 in the dark and refrigerated (< 4 °C) until required (the complex was always used within 4 weeks of its preparation).

C. α′-Hydroxylation of the α,β-unsaturated ketone 5.1

Before performing this reaction, all apparatus were thoroughly flame dried under N_2. The reaction was performed under an atmosphere of N_2 and meticulously anhydrous conditions were maintained throughout.

Hispanone (**5.1**, 300 mg, 1.0 mmol) in THF (5.0 mL) was added dropwise ***via*** a cannula to lithium diisopropylamide (2.0 M, 1.00 mL, 2.0 mmol, 2 eq) in THF (5.0 mL) at −78 °C. The resulting solution was stirred at −78 °C for 1 h before MoOPH (**5.36**, 870 mg, 2.0 mmol, 2 eq) was added in a single portion using a solids addition tube. The deep red reaction mixture was stirred at −78 °C for 12 h, warmed to 0 °C over 30 min and immediately quenched with sat. aqueous Na_2SO_3 (5.0 mL). After stirring for a further 30 min at 0 °C, the mixture was warmed to RT, H_2O (5.0 mL) added and the aqueous and organic phases separated. The aqueous fraction was extracted with Et_2O (3 × 5 mL) and the combined organic phases washed with 0.5 M HCl (10 mL), 5% $NaHCO_3$ (5 mL) and H_2O (5 mL). Drying ($MgSO_4$), removal of solvent ***in vacuo*** and passage of the resulting brown oil (456 mg) through a column of silica (4:1 hexane/EtOAc) yielded a mixture of products as a pale yellow oil (264 mg). Semi-preparative normal phase HPLC of the mixture in 9:1 hexane/EtOAc yielded (in order of elution) 6-hydroxy-15,16-epoxylabda-5,8,13(16),14-tetraen-7-one (**5.30**, 16 mg, 0.05 mmol, 5%) as a colourless oil, 6α-hydroxy-15,16-epoxylabda-8,13(16),14-trien-7-one (**5.27**, 137 mg, 0.43 mmol, 43%) as a white crystalline solid, hispanone (**5.1**, 40 mg, 0.13 mg, 13%) as a yellow crystalline solid and 6β-hydroxy-15,16-epoxylabda-8,13(16),14-trien-7-one (**5.28**, 65 mg, 0.21 mmol, 21%) as a white crystalline solid.

6β-Hydroxy-15,16-epoxylabda-8,13(16),14-trien-7-one (5.28):[248] white crystalline solid; mp 99-100 °C; $[\alpha]_D^{26}$ +17° (***c*** 1.01, $CHCl_3$), lit.[248] −34°; IR ν_{max} 3402 (br), 2930, 2856, 1651, 1604, 1470, 1385, 1026, 874, 782 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.37 (1H, t, ***J*** = 1.6 Hz, H-15), 7.27 (1H, s, H-16), 6.31 (1H, br s, H-14), 4.32 (1H, dd, ***J*** = 3.6, 2.6 Hz, H-6), 2.57 (2H, m, H_2-12), 2.51 (2H, m, H_2-11), 1.90 (1H, br d, ***J*** = 12.2 Hz, H-1a), 1.86 (3H, s, H_3-17), 1.81 (1H, tt, ***J*** = 13.6, 3.3 Hz, H-2a), 1.60 (1H, m, H-2b), 1.57 (1H, d, ***J*** = 3.6 Hz, H-5), 1.45 (1H, br d, ***J*** = 14.3 Hz, H-3a), 1.39 (3H, s, H_3-20), 1.37 (1H, td, ***J*** = 12.6, 3.8 Hz, H-1b), 1.31 (3H, s, H_3-19), 1.23 (1H, td, ***J*** = 13.3, 3.8

Hz, H-3b), 1.06 (3H, s, H_3-18) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.4 (s, C-7), 169.6 (s, C-9), 143.1 (d, C-15), 138.7 (d, C-16), 128.5 (s, C-8), 124.5 (s, C-13), 110.6 (d, C-14), 71.2 (d, C-6), 52.3 (d, C-5), 43.4 (t, C-3), 41.1 (s, C-10), 37.5 (t, C-1), 34.2 (s, C-4), 32.4 (q, C-18), 30.7 (t, C-11), 24.4 (t, C-12), 24.0 (q, C-19), 22.2 (q, C-20), 18.8 (t, C-2), 11.7 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 316 [M^+] (10), 314 (15), 301 (63), 283 (46), 255 (29), 203 (68), 192 (100), 175 (47), 161 (94), 151 (53); HRFABMS ***m/z*** 317.2117 (calcd for C_{20} $H_{29}O_3$ [$(M + H)^+$], 317.2117).

7.5.3.5 Hydride reductions of the α′-hydroxy α,β-unsaturated ketones **5.27** and **5.28**

The reactions described below were performed under an atmosphere of N_2 in flame dried apparatus and meticulously anhydrous conditions were maintained throughout.

A. Preparation of AlH_3 and $Zn(BH4)_2$

Alane was prepared according to the published procedure of Finholt ***et al***.[249] immediately prior to use as follows. A solution of $LiAlH_4$ (57 mg, 1.50 mmol, 3 eq) in THF (40.0 mL) cooled to 0 °C and anhydrous $AlCl_3$ (67 mg, 0.50 mmol, 1 eq) added. The solution was stirred at 0 °C for 30 min during which time a white precipitate of LiCl formed. The precipitate was allowed to settle and the required volume of AlH_3 solution removed using a gas-tight syringe (the AlH_3 concentration of the supernatant was assumed to be 50 mM).

Zinc borohydride was prepared according to the published procedure of Crabbé ***et al***.[250] immediately prior to use as follows. $ZnCl_2$ (5.04 g) was heated to 360 °C under a rapid stream of N_2 for 30 min. After being allowed to cool under N_2, the $ZnCl_2$ was stored over P_2O_5 at 0.4 mmHg for 18h. The freshly fused $ZnCl_2$ (476 mg, 3.5 mmol, 1 eq) was added to $NaBH_4$ (266 mg, 7.0 mmol, 2 eq) in anhydrous THF (20.0 mL) and the mixture stirred for 3 h. The resulting fine precipitate was allowed to settle and the required volume of $Zn(BH_4)_2$ solution removed using a gas-tight syringe (the $Zn(BH_4)_2$ concentration of the supernatant was assumed to be 175 mM).

B. Reduction with $LiAlH_4$

The hydroxy ketones **5.27** (36.2 mg, 0.115 mmol) and **5.28** (35.6 mg, 0.113 mmol) in THF (3.0 mL each) were separately treated with $LiAlH_4$ (0.115 mmol) as a 50 mM solution in THF (2.30 mL) at –78 °C. The reactions were stirred at –78 °C for 1 h, at 0 °C for 1 h and warmed to RT over 6 h. The reactions were quenched with H_2O (2.0 mL) at 0 °C, the organic layers separated and the aqueous phase extracted with Et_2O (3×5 mL). The respective organic fractions were combined, dried ($MgSO_4$) and concentrated to give a white solid (35.8 mg) from **5.27** and a clear colourless oil (35.5 mg) from **5.28**. Analysis by ^{1}H NMR spectroscopy and normal phase HPLC (7:3 hexane/EtOAc) of the product obtained from **5.27** indicated that it contained 15,16-epoxylabda-8,13(16),14-trien-6α,7β-diol (**5.11**) and 15,16-epoxylabda-8,13(16),14-trien-6α,7α-diol (**5.17**) exclusively in a ratio of 1:1.05, corresponding to a 98% (0.113 mmol) isolated yield of reduction products. Similar analysis of the product obtained from **5.28** indicated that it contained 15,16-epoxylabda-8,13(16),14-trien-6β,7β-diol (**5.12**) and 15,16-epoxylabda-8,13(16),14-trien-6β,7α-diol (**5.18**) exclusively in a ratio of 3.08:1, corresponding to a 99% (0.112 mmol) isolated yield of reduction products.

C. Reduction with AlH_3

Compounds **5.27** (38.3 mg, 0.121 mmol) and **5.28** (30.8 mg, 0.097 mmol) were separately reduced with one molar equivalent of 50 mM AlH_3 in THF (2.40 mL and 2.00 mL respectively) by the procedure described above to yield a white solid (36.0 mg) from **5.27** and a colourless oil (28.1 mg) from **5.28**. Analysis by ^{1}H NMR spectroscopy indicated that the product obtained from **5.27** contained 40.1% **5.27**, 15.4% **5.11** and 44.5% **5.17**, and that obtained from **5.28** contained 14.1% **5.28**, 78.0% **5.12** and 7.9% **5.18**.

D. Reduction with $Zn(BH_4)_2$

Treatment of **5.27** (20.7 mg, 0.066 mmol) and **5.28** (18.1 mg, 0.057 mmol) with one molar equivalent of 175 mM $Zn(BH_4)_2$ in THF (377 μL and 326 μL respectively) in the same manner yielded two yellow oils (23.1 mg from **5.27** and 18.3 mg from **5.28**). Analysis by ^{1}H NMR spectroscopy indicated that the product obtained from **5.27** contained 10.1% **5.11**, 13.9% **5.17**

and a large quantity of other reduction products (indicated by a complex multiplet at δ_H 4.67), and that obtained from **5.28** contained 27.2% **5.28** and other reduction products [indicated by a series of doublets at δ_H 4.59 (***J*** = 7.8 Hz), 4.64 (***J*** = 7.6 Hz), 4.83 (***J*** = 3.0 Hz) and 4.92 (***J*** = 8.1 Hz)] only.

E. Reduction with $NaBH_4$

Treatment of **5.27** (22.3 mg, 0.071 mmol) and **5.28** (21.4 mg, 0.068 mmol) with one molar equivalent of 100 mM $NaBH_4$ in THF (710 μL and 680 μL respectively) in the same manner yielded two yellow oils (22.3 mg from **5.27** and 18.9 mg from **5.28**). Analysis by ^{1}H NMR spectroscopy indicated that the product obtained from **5.27** contained 50.0% **5.27**, 21.5% **5.11**, 19.2% **5.17** and a large quantity of the same reduction products observed in the $Zn(BH_4)_2$ reduction of **5.27**. Similarly, the product obtained from **5.28** was found to contain 39.1% **5.28**, 4.6% 5.12, 2.2% 5.18 and significant amounts of the same reduction products observed in the $Zn(BH_4)_2$ reduction of **5.28**.

15,16-Epoxylabda-8,13(16),14-trien-6α,7α-diol (5.17): white crystalline solid; mp 160-161 °C; $[\alpha]_D^{26}$ +100° (***c*** 1.10, $CHCl_3$); IR ν_{max} 3370 (br), 2931, 2868, 1470, 1373, 1085, 1024, 874, 776, 600 cm^{-1}; ^{1}H & ^{13}C NMR data see Table 5.6; EIMS ***m/z*** (rel. int.) 318 [M^+] (1), 300 (26), 285 (29), 267 (36), 217 (26), 175 (44), 161 (47), 149 (100), 91 (55); HRFABMS ***m/z*** 319.2274 (calcd for $C_{20}H_{31}O_3$ [$(M + H)^+$], 319.2273).

15,16-Epoxylabda-8,13(16),14-trien-6β,7α-diol (5.18): white crystalline solid; mp 93-94 °C; $[\alpha]_D^{27}$ +71 ° (***c*** 0.52, $CHCl_3$); IR ν_{max} 3401 (br), 2930, 2863, 1471, 1385, 1111, 1026, 874, 777, 599 cm^{-1}; ^{1}H & ^{13}C NMR data see Table 5.6; EIMS ***m/z*** (rel. int.) 318 [M^+] (7), 300 (27), 285 (39), 267 (36), 217 (30), 204 (47), 189 (99), 149 (100), 135 (99), 91 (92); HRFABMS ***m/z*** 318.2194 (calcd for $C_{20}H_{30}O_3$ [M^+], 318.2195).

7.5.4 Preparation of the four 6,7-diastereomeric 14,15-epoxylabda-8,13(16),14-trien-6,7-diols (5.11, 5.12, 5.17 and 5.18) via *hydride reduction of 15,16-epoxylabda-8,13(16),14-trien-6,7-dione (5.33)*

7.5.4.1 Attempted preparation of 15,16-epoxylabda-8,13(16),14-trien-6,7-dione (**5.33**) *via* selenium dioxide oxidation of hispanone (**5.1**)

SeO_2 (74 mg, 0.67 mmol, 1 eq) was added to a solution of hispanone (200 mg, 0.67 mmol) in 70% AcOH (5.0 mL) and the reaction mixture stirred at RT for 8 h. Analysis of the reaction mixture by TLC indicated that it still contained a large proportion of **5.1**, so the oxidation was continued with heating (100 °C) for a further 1 h. The reaction mixture was cooled, filtered, the selenium residue washed with AcOH (1.0 mL) and the combined acidic solutions poured into water (10 mL). The resulting aqueous suspension was extracted with Et_2O (3 × 15 mL), the combined organic phases washed with sat. $NaHCO_3$ (2 × 15 mL) and H_2O (20 mL), dried ($MgSO_4$) and concentrated to give a red-brown oil (248 mg). Analysis of the oil by ^1H and ^{13}C NMR indicated that it was a complex mixture of products that was not investigated further.

7.5.4.2 Stereospecific preparation of the 6α-hydroxy enone **5.27** by application of a modified Rubottom procedure

Before performing this reaction, all apparatus were thoroughly flame dried under N_2. The reaction was performed under an atmosphere of N_2 and meticulously anhydrous conditions were maintained throughout.

Hispanone (**5.1**, 52 mg, 0.17 mmol) in THF (1.0 mL) was added to LDA (2.0 M, 100 μL, 0.20 mmol, 1.2 eq) in THF (4.0 mL) at –78 °C and the solution stirred for 1 h before TMSCl (26 μL, 0.20 mmol, 1.2 eq) was added ***via*** gas-tight syringe. The reaction was stirred at –78 °C for 1 h, warmed to RT over 1 h and stirred at ambient temperature for a further 2 h. Analysis of the

mixture by TLC indicated the presence of the desired trimethylsilyl dienol ether only, therefore the THF was carefully removed ***in vacuo*** (the rotary evaporator was fitted with a large $CaCl_2$ guard tube so that dry air would be introduced on release of the vacuum) and the residue taken up in anhydrous CH_2Cl_2 (5.0 mL). TLC analysis of the CH_2Cl_2 solution indicated that no significant hydrolysis of the silyl dienol ether had occurred during the exchange of solvent and a fine white precipitate of LiCl was evident in the reaction vessel. The silyl dienol ether solution was cooled to 0 °C and MCPBA was added as a 0.2 M solution in CH_2Cl_2 (1.00 mL, 0.20 mmol, 1.2 eq, solution dried over $MgSO_4$ before use). The reaction was warmed to RT, stirred for 1 h (TLC indicated that no silyl dienol ether was present), quenched with H_2O (0.5 mL) and the CH_2Cl_2 removed ***in vacuo***. The resulting cloudy suspension was taken up in Et_2O (5 mL), washed with 1 M HCl (5 mL), sat. $NaHCO_3$ (5 mL) and H_2O (2 mL), dried ($MgSO_4$) and concentrated to give a yellow oil (57 mg). Normal phase HPLC of the oil in 9:1 hexane/EtOAc yielded 6α-hydroxy-15,16-epoxylabda-8,13(16),14-trien-7-one (**5.27**, 41 mg, 0.13 mmol, 76%) and unreacted **5.1** (10 mg, 0.03 mmol, 18%) as white crystalline solids.

7.5.4.3 Swern oxidation of the 6α-hydroxy enone **5.27**

Oxalyl chloride (144 μL, 1.65 mmol, 5 eq) was added to anhydrous CH_2Cl_2 (2.0 mL) at −78 °C under N_2 and stirred for 30 min. Anhydrous DMSO (234 μL, 3.30 mmol, 10 eq) was added dropwise and the solution stirred for 30 min at −78 °C before **5.27** (103 mg, 0.33 mmol) in anhydrous CH_2Cl_2 (3.0 mL) was added dropwise. The reaction mixture was stirred for 1 h, Et_3N (1.38 mL, 9.90 mmol, 30 eq) added and then slowly warmed to 0 °C. After 1 h at 0 °C, hexane (2.0 mL) was added, the reaction mixture filtered and the residue washed with Et_2O (3 × 2.5 mL). The combined filtrate and washings were washed with 0.5 M HCl (5 mL), 5% $NaHCO_3$ (5 mL) and H_2O (5 mL), dried ($MgSO_4$) and concentrated ***in vacuo*** to give 15,16-epoxylabda-8,13(16),14-trien-6,7-dione (**5.33**, 103 mg, 0.33 mmol, 100%) as brilliant yellow needles. An analytical sample of **5.33** was obtained by recrystallisation from MeOH.

15,16-Epoxylabda-8,13(16),14-trien-6,7-dione (5.33): yellow needles; mp 144-146 °C; $[\alpha]_D^{28}$ +289° (***c*** 2.12, $CHCl_3$); IR ν_{max} 2934, 2864, 1723, 1663, 1603, 1471, 1380, 1325, 1158, 1021, 874, 784, 600 cm^{-1}; ^1H & ^{13}C NMR data see Table 5.7; EIMS ***m/z*** (rel. int.) 314 [M^+] (80), 299 (63), 281 (75), 268 (17) 245 (20), 215 (100), 205 (77), 177 (35), 161 (21), 91 (16); HRFABMS ***m/z*** 315.1961 (calcd for $C_{20}H_{27}O_3$ [$(M + H)^+$], 315.1960).

7.5.4.4 $LiAlH_4$ reduction of diketone **5.33**

Diketone **5.33** (77 mg, 0.25 mmol) in THF (3.0 mL) was stirred at −78 °C while $LiAlH_4$ (9.5 mg, 0.25 mmol, 1 eq) in THF (2.30 mL) was added dropwise. The reaction was allowed to slowly warm to RT over 3 h and quenched with H_2O (2.0 mL) at 0 °C. Work-up in the usual manner gave a white cloudy oil (85 mg) that was found to be a mixture of all four diols in a ratio of 10:5:3:1 **5.12** : **5.11** : **5.18** : **5.17** (by integration of the H-6 and H-7 oxymethine ^1H NMR resonances) and the diols accounted for a total of 97% of the furanolabdane derivatives in the sample (by comparison of the oxymethine integrals with the integrals of the furanyl protons H-14, H-15 and H-16).

7.6 Chapter Six Experimental

7.6.1 Attempted synthesis of 6β,7α-diacetoxylabda-8,13E-dien-15-ol (3.10) from rhinocerotinoic acid (4.14)

7.6.1.1 Preparation of (±)-6-bromo-3,5,5-trimethylcyclohex-2-enone (**6.10**) ***via*** Caló's bromination procedure

A. Preparation of 2,4,4,6-tetrabromo-2,5-cyclohexadienone[288]

Tribromophenol (13.2 g, 39.9 mmol) and $NaOAc{\cdot}3H_2O$ (5.43g, 39.9 mmol, 1 eq) in AcOH (80 mL) was warmed to 60 °C and cooled to RT to afford fine crystals of tribromophenol. The suspension

was rapidly stirred whilst Br_2 (6.4 g, 40.0 mmol, 1 eq) in AcOH (40 mL) was added with cooling (15 °C) over 1 h. After stirring for a further 30 min, the reaction mixture was poured onto ice (400 g), the resulting yellow precipitate collected by vacuum filtration and washed well with water. The precipitate was crystallised from $CHCl_3$ (110 mL) to give 2,4,4,6-tetrabromo-2,5-cyclohexadienone[288,289] (12.9 g, 31.5 mmol, 79 %) as yellow needles [mp 131-132 °C (dec.), lit.[288] 125-130 °C (dec.)].

B. Preparation of anhydrous ethereal HCl

Conc. H_2SO_4 was slowly dropped onto oven-dried NaCl using a long-stemmed capillary dropping-funnel and the HCl evolved was bubbled through anhydrous Et_2O (contained in a three-necked flask fitted with a septum and a $CaCl_2$ drying tube) at 0 °C until a suitable HCl concentration was attained. The concentration of the ethereal HCl solution was periodically determined by removing aliquots of the solution (1.00 mL), immediately mixing these with H_2O (5 mL) and titrating the resulting aqueous HCl solution with 450 mM NaOH.

C. Bromination of isophorone (6.9)

Anhydrous ethereal HCl (1.94 M, 4.1 mL, 8.0 mmol, 1 eq) was added to isophorone (1.10 g, 8.0 mmol) and 2,4,4,6-tetrabromo-2,5-cyclohexadienone (3.30 g, 8.0 mmol, 1 eq) in anhydrous Et_2O (20 mL) and the resulting deep yellow solution stirred at RT for 45 h. The reaction mixture was washed with 10% Na_2CO_3 (2 × 10 mL) and H_2O (3 × 5 mL), dried (Na_2SO_4) and concentrated to give a brown oil (2.59 g). The oil was passed through a column of silica gel in 2:1 hexane/EtOAc to yield an orange oil (2.55 g), a portion of which (206 mg) was subjected to semi-preparative normal phase HPLC (9:1 hexane/EtOAc) to yield tribromophenol (102 mg) as a white crystalline solid and (±)-6-bromo-3,5,5-trimethylcyclohex-2-enone (**6.10**, 94 mg, 0.43 mmol, corresponding to a yield of 66%) as a colourless oil.

(±)-6-Bromo-3,5,5-trimethylcyclohex-2-enone (6.10):[264,265] colourless oil; IR ν_{max} 2972, 2870, 1661, 1673, 1633, 1435, 1380, 1281, 1160, 742 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.88 (1H, br s, H-2), 4.03 (1H, s, H-6), 2.46 (1H, d, ***J*** = 18.5 Hz, H-4a), 2.00 (H, d, ***J*** = 18.6 Hz, H-4b), 1.96 (3H,

s, H_3-7), 1.17 (3H, s, H_3-8), 1.12 (3H, s, H_3-9) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 192.2 (s, C-1), 159.9 (s, C-3), 122.6 (d, C-2), 61.3 (d, C-6), 42.3 (t, C-4), 37.2 (s, C-5), 27.2 (q, C-8), 24.5 (q, C-9), 24.3 (q, C-7) ppm; EIMS ***m/z*** (rel. int.) 218 [$(C_9H_{13}{}^{81}BrO)^+$] (4), 216 [$(C_9H_{13}{}^{79}BrO)^+$] (8), 189 (13), 187 (12), 162 (13), 160 (15), 137 (100), 109 (91), 81 (28), 79 (32); HRFABMS ***m/z*** 217.0226 (calcd for $C_9H_{14}{}^{79}BrO$ [$(M + H)^+$], 217.0228).

7.6.1.2 Reduction of (±)-6-bromo-3,5,5-trimethylcyclohex-2-enone (**6.10**) with Luche's reagent

$NaBH_4$ (11 mg, 0.29 mmol, 2 eq) was added in portions to (±)-6-Bromo-3,5,5-trimethylcyclohex-2-enone (31 mg, 0.14 mmol) and $CeCl_3{\cdot}7H_2O$ (52 mg, 0.14 mmol, 1 eq) in MeOH (5.0 mL) at 0 °C. The reaction mixture was allowed to warm to RT, stirred over-night, diluted with water (5.0 mL) and concentrated ***in vacuo***. The residue was taken up in sat. brine (5 mL), extracted with Et_2O (3 × 10 mL) and the combined organic fractions dried ($MgSO_4$) and concentrated to give (±)-***cis***-6-bromo-3,5,5-trimethylcyclohex-2-enol (**6.11**, 26 mg, 0.12 mmol, 86%) as a colourless oil.

(±)-*cis*-6-Bromo-3,5,5-trimethylcyclohex-2-enol (6.11): colourless oil; IR ν_{max} 3402 (br), 2961, 2870, 1681, 1463, 1385, 1368, 1207, 1150, 1048 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.26 (1H, br s, H-2), 4.29 (1H, d, ***J*** = 3.9 Hz, H-6), 4.22 (1H, br d, ***J*** = 9.5 Hz, H-1), 2.22 (1H, d, ***J*** = 10.2 Hz, 1-OH), 2.14 (1H, d, ***J*** = 17.7 Hz, H-4a), 1.66 (1H, d, ***J*** = 17.6 Hz, H-4b), 1.69 (3H, br s, H_3-7), 1.15 (3H, s, H_3-8), 1.08 (3H, s, H_3-9) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 136.0 (s, C-3), 121.5 (d, C-2), 72.4 (d, C-6), 67.4 (d, C-1), 41.4 (t, C-4), 36.0 (s, C-5), 29.0 (q, C-8), 26.2 (q, C-9), 23.0 (q, C-7) ppm; EIMS ***m/z*** (rel. int.) 220 [$(C_9H_{15}{}^{81}BrO)^+$] (7), 219 (25), 218 [$(C_9H_{15}{}^{79}BrO)^+$] (7), 217 (23), 137 (40), 121 (100), 105 (73), 91 (95), 81 (38), 79 (54); HRFABMS ***m/z*** 219.0381 (calcd for $C_9H_{16}{}^{79}BrO$ [$(M + H)^+$], 219.0385).

7.6.1.3 Preparation of 6α-bromo-7-oxo-labda-8,13***E***-dien-15-oic acid (**6.6**)

A. Bromination of rhinocerotinoic acid (4.14) in Et_2O

Rhinocerotinoic acid (160 mg, 0.50 mmol) and 2,4,4,6-tetrabromo-2,5-cyclohexadienone (210 mg, 0.51 mmol, 1 eq) in anhydrous Et_2O (10.0 mL) were treated with anhydrous ethereal HCl (1.94 M, 0.26 mL, 0.50 mmol, 1 eq) at ambient temperature and the reaction stirred under anhydrous conditions for 40 h. The reaction mixture was extracted with 10% Na_2CO_3 (3 × 5 mL), the aqueous fractions combined, acidified (HCl) and extracted with Et_2O (2 × 10 mL) to give, after drying ($MgSO_4$) and removal of solvent, a colourless oil (288 mg). Analysis of the crude product by ^{1}H NMR spectroscopy indicated that it contained a significant amount of tribromophenol that was subsequently removed by silica gel column chromatography (3:2 and 2:3 hexane/EtOAc) to yield a 3.9:3 mixture (by ^{1}H NMR spectroscopy) of 6α- and 6β-bromo-7-oxo-labda-8,13***E***-dien-15-oic acid (**6.6** and **6.12**, 194 mg, 0.49 mmol, 98%) as a cloudy oil. Semi-preparative reversed phase HPLC of a portion (45 mg) of the isomeric mixture in 4:1 MeOH/H_2O containing 0.05% TFA furnished the pure acids **6.6** and **6.12** as a colourless oil and a white solid respectively.

B. Attempted enrichment of the 6α-isomer 6.6 in the 4:3 6.6/6.12 mixture using Proton-sponge®

The 4:3 mixture of α′-bromo-α,β-unsaturated keto acids **6.6** and **6.12** (104 mg, 0.26 mmol) was stirred in dry CH_3CN (15.0 mL) at −10 °C and ***N,N,N´N´***-tetramethyl-1,8-naphthalenediamine (**6.13**, 111 mg, 0.52 mmol, 2 eq) added. The solution was allowed to warm to ambient temperature and stirred for 1 h before being quenched with 0.5 M HCl (4.0 mL), concentrated ***in vacuo*** and diluted with H_2O (10 mL). The resulting aqueous solution was extracted with CH_2Cl_2 (3 × 10 mL), the organic fractions combined, dried and concentrated to give a brown solid (132 mg). Passage of the crude product through a column of silica gel (1:1 hexane/EtOAc) yielded an orange oil (96 mg). Analysis of the oil by ^{1}H NMR spectroscopy indicated that it contained the two acids **6.6** and **6.12** in a ratio of 4.1:1 along with significant amounts of a secondary product (methyl singlets at δ_H 2.22, 1.99, 1.92, 1.33 and 1.26, and olefinic proton singlets at δ_H 5.76 and 6.39). All attempts to isolate the secondary product by both normal phase and reversed phase

HPLC were unsuccessful due to pronounced tailing of peaks on silica gel and poor resolution on octadecasilane.

Repetition of the above reaction at reduced temperatures and shorter reaction times in both CH_3CN and THF gave less favourable conversion to the desired 6α-isomer **6.6**. In every case, ^1H NMR analysis of the product obtained indicated that the secondary product was present in significant amounts. It should be noted that attempts to accomplish the conversion of **6.12** to **6.6** using the weaker base Et_3N were also unsuccessful: no change in the 4:3 ratio of **6.6**/**6.12** was observed after prolonged treatment (1 week) with Et_3N in CH_3CN.

C. Bromination of rhinocerotinoic acid (4.14) in THF

Caló's bromination procedure was applied to rhinocerotinoic acid (15 mg, 0.05 mmol) in anhydrous THF (3.0 mL) using 2,4,4,6-tetrabromo-2,5-cyclohexadienone (25 mg, 0.06 mmol, 1.2 eq) and anhydrous ethereal HCl (0.42 M, 150 μL, 0.06 mmol, 1.2 eq) at ambient temperature. After stirring for 18 h, the reaction mixture was concentrated ***in vacuo***, analysed by ^1H NMR spectroscopy and found to contain **6.6** and **6.12** in a ratio of 4.7:2.

Application of Caló's bromination procedure to 4.14 (200 mg, 0.63 mmol) in refluxing THF (6.0 mL, reaction time 1 h) using 2,4,4,6-tetrabromo-2,5-cyclohexadienone (312 mg, 0.76 mmol, 1.2 eq) and anhydrous ethereal HCl (2.4 M, 317 μL, 0.76 mmol, 1.2 eq) yielded, after concentration of the reaction mixture, a dark brown oil (568 mg). Silica gel column chromatography of the crude product (3:1 hexane/EtOAc) yielded a mixture of **6.6** and **6.12** (243 mg, 0.61 mmol, 97%) in a ratio of 11.4:3 as a pale yellow oil. The desired product 6α-bromo-7-oxo-labda-8,13***E***-dien-15-oic acid (**6.6**) was easily obtained pure by semi-preparative reversed phase HPLC (4:1 MeOH/H_2O, 0.05% TFA) of the isomeric mixture.

6α-Bromo-7-oxo-labda-8,13*E*-dien-15-oic acid (6.6): Colourless oil; $[\alpha]_D^{27}$ −13° (***c*** 1.95, $CHCl_3$); IR ν_{max} 3313 (br), 2992, 2931, 2870, 1682, 1645, 1441, 1383, 1251, 1173 cm^{-1}; ^1H & ^{13}C NMR data see Table 6.1; EIMS ***m/z*** (rel. int.) 317 [$(M - Br)^+$] (97), 299 (100), 257 (39), 203 (54),

189 (40), 175 (56), 161 (41), 135 (61), 81 (12), 79 (26); HRFABMS ***m/z*** 397.1379 (calcd for $C_{20}H_{30}{}^{79}BrO_3$ [$(M + H)^+$], 397.1378).

6β-Bromo-7-oxo-labda-8,13*E*-dien-15-oic acid (6.12): white solid; mp 161–163 °C; $[\alpha]_D^{27}$ +12° (***c*** 1.26, $CHCl_3$); IR ν_{max} 3183 (br), 3002, 2933, 2856, 1694, 1667, 1645, 1606, 1442, 1381, 1250 cm^{-1}; ^1H & ^{13}C NMR data see Table 6.1; EIMS ***m/z*** (rel. int.) 317 [$(M - Br)^+$] (60), 299 (87), 203 (55), 189 (100), 173 (52), 161 (57), 135 (63), 91 (53), 81 (8), 79 (28); HRFABMS ***m/z*** 397.1378 (calcd for $C_{20}H_{30}{}^{79}BrO_3$ [$(M + H)^+$], 397.1378).

7.6.1.4 Hydride reductions of 6α-bromo-7-oxo-labda-8,13***E***-dien-15-oic acid (**6.6**)

A. $NaBH_4$ reductions

The α′-bromo-α,β-unsaturated keto acid **6.6** (43 mg, 0.11 mmol) in MeOH (3.0 mL) was treated with $NaBH_4$ (4.3 mg, 0.11 mmol, 1 eq) and $CeCl_3{\cdot}7H_2O$ (42 mg, 0.11 mmol, 1 eq) at 0 °C, allowed to warm to RT and stirred for 14 h. The reaction was worked-up in the usual manner to give unchanged starting material (**6.6**, 36 mg) as a pale yellow oil.

Similar results were obtained when **6.6** (50 mg, 0.13 mmol), $NaBH_4$ (19 mg, 0.53 mmol, 4 eq) and $CeCl_3{\cdot}7H_2O$ (194 mg, 0.52 mmol, 4 eq) in MeOH (10.0 mL) was heated at 50 °C for 12 h. When **6.6** (68 mg, 0.17 mmol) in MeOH (10.0 mL) was treated with $NaBH_4$ (26 mg, 0.68 mmol, 4 eq) and $CeCl_3{\cdot}7H_2O$ (254 mg, 0.68 mmol, 4 eq) at reflux, there was no discernable reduction after 3 h (by TLC). However, work-up of the reaction after 12 h reflux yielded a complex mixture of products (by ^1H NMR spectroscopy) that was not investigated further.

B. $LiAlH_4$ reductions

Compound **6.6** (207 mg, 0.50 mmol) was treated with $LiAlH_4$ (76 mg, 2.0 mmol, 4 eq) in Et_2O (5.0 mL) at –78 °C for 6 h. Work up in the usual manner using sat. NH_4Cl (5.0 mL) gave a yellow oil (197 mg) that rapidly darkened on standing at RT and –20 °C. Immediate analysis of the oil by ^1H

NMR spectroscopy and normal phase HPLC (4:1 hexane/EtOAc) showed it to be a complex mixture of at least 10 products.

Identical results were obtained when the $LiAlH_4$ reduction of **6.6** was repeated employing THF as the solvent, when pH 7 buffer or H_2O was used in the reaction work-up (for reactions performed in both Et_2O and THF), and when the reaction product was immediately treated with Ac_2O, pyridine and DMAP an effort to isolate the C-7, C-15 diacetate **6.14**.

C. DIBAH reductions

The reduction of bromo ketone **6.6** was also attempted using DIBAH (4 eq) in Et_2O, THF, CH_2Cl_2 and 2:1 CH_2Cl_2/hexane. Reactions performed in the former three solvents gave results identical to those obtained using $LiAlH_4$ (***vide supra***). However, when **6.6** (100 mg, 0.25 mmol) in CH_2Cl_2 (15 mL) and hexane (7.5 mL) was treated with a 1.0 M solution of DIBAH in heptane (1.00 mL, 100 mmol, 4 eq) at –78 °C and the reaction slowly warmed to RT and allowed to stir at ambient temperature over-night, work-up of the reaction in the usual manner and column chromatography (3:2 hexane/EtOAc) of the resulting yellow oil (95 mg) yielded rhinocerotinoic acid (**4.14**) as a white crystalline solid (74 mg, 0.23 mmol, 92%).

7.6.1.5 Attempted conversion 15,16-epoxylabda-8,13(16),14-trien-6α,7β-diol (**5.11**) into a 6,7-epoxide ***via*** Mitsunobu reaction

The 6α,7β-diol **5.11** (39 mg, 0.12 mmol) and PPh_3 (68 mg, 0.26 mmol, 2.2 eq) were stirred in anhydrous THF (5.0 mL) for 10 min at RT before DEAD (42 μL, 0.26 mmol, 2.2 eq) was added. The reaction was stirred for 24 h at ambient temperature, concentrated ***in vacuo*** and subjected to column chromatography on silica gel (9:1 hexane/EtOAc) to give unreacted starting material (37 mg). Identical results were obtained when the above reaction was performed at reflux (12 h) in anhydrous THF and at RT (24 h) and reflux (24 h) in anhydrous benzene.

7.6.1.6 Attempted conversion 15,16-epoxylabda-8,13(16),14-trien-6α,7β-diol (**5.11**) into a 6,7-epoxide ***via*** a tosylation – substitution strategy

A. Attempted preparation of a 6,7-epoxide by application of Rao's one-pot procedure

The 6α,7β-diol **5.11** (63 mg, 0.20 mmol) in THF (2.0 mL) was added dropwise ***via*** cannula to a stirred suspension of NaH (11 mg, 0.46 mmol, 2.3 eq) in THF (1.0 mL) at 0 °C. After stirring for 30 min, TsCl (46 mg, 0.24 mmol, 1.2 eq) was added in portions over 1 h and the reaction mixture stirred at 0 °C for 30 min. The reaction was quenched with H_2O (5.0 mL) at 0 °C, the resulting suspension extracted with Et_2O (3 × 5 mL), and the combined organic phases washed with 0.5 M HCl (5 mL), 10% $NaHCO_3$ (5 mL) and H_2O (2 mL). Drying (Na_2SO_4) and evaporation of the solvent yielded a complex mixture of products (by ^1H NMR spectroscopy and TLC) as a yellow oil (48 mg).

B. Attempted tosylation of 5.11 employing TsCl in pyridine

TsCl (92 mg, 0.48 mmol, 3 eq) was stirred in anhydrous pyridine (1.0 mL) at 0 °C for 10 min before **5.11** (51 mg, 0.16 mmol) in pyridine (1.0 mL) was added ***via*** syringe. The resulting solution was stirred at 0 °C for 2 h and RT for a further 12 h. Analysis of the reaction mixture by TLC indicated that under these conditions no reaction had occurred, therefore the mixture was heated at 70 °C for 4 h after which there was no **5.11** detectable in the mixture by TLC. The volume of the reaction mixture was reduced ***in vacuo*** (1 mmHg), the residue taken up in EtOAc (5 mL) and washed with 1 M HCl (3 × 5 mL), 10% Na_2CO_3 (2 × 5 mL) and H_2O (5 mL). Drying with $MgSO_4$ and evaporation of the solvent yielded a complex mixture of products (by NMR spectroscopy) as a dark brown oil (80 mg).

7.6.2 Attempted synthesis of 6α,7β-diacetoxylabda-8,13E-dien-15-ol (6.1) from rhinocerotinoic acid (4.14)

7.6.2.1 Attempted Rubottom oxidation of rhinocerotinoic acid (**4.14**)

Repeated attempts to oxidise rhinocerotinoic acid (reactions carried out on a 0.2 mmol – 0.4 mmol scale) following the procedure described in section 7.5.4.2 and using LDA (2.4 eq), TMSCl (2.4 eq) and MCPBA (1.2 eq) yielded unchanged starting material upon work-up of the reactions. TLC analysis (1:1 hexane/EtOAc) of the reaction mixture before and after exchanging the THF for CH_2Cl_2 indicated that although the desired enol ether **6.16** (R_f = 0.91) was formed in the first step of the reaction, this was rapidly converted back to **4.14** (R_f = 0.42) during the substitution of the solvents. Similar results were obtained when anhydrous (sodium dried) hexane was used as the solvent for the second step of the reaction.

7.6.2.2 Methylation of rhinocerotinoic acid (**4.14**)

Rhinocerotinoic acid (1.00 g, 3.14 mmol) was dissolved in MeOH (25 mL) and treated with an ethereal diazomethane solution (10 mL, approximately 6.67 mmol, 2.1 eq)[286] at 0 °C. The bright yellow solution was allowed to stand in ice for 1 h before being slowly warmed to room temperature. After 1 h at room temperature the ether and excess diazomethane were removed by evaporation at 70 °C in a fume-hood. The residual MeOH solution was concentrated ***in vacuo*** to give methyl rhinocerotinoate (**4.20**) as a low-melting (mp 56-58 °C) white crystalline solid (1.04 g, 3.14 mmol, 100%).

7.6.2.3 Preparation of methyl 6α-hydroxy-7-oxo-labda-8,13***E***-dien-15-oate (**6.17**)

Methyl rhinocerotinoate (**4.20**, 100 mg, 0.30 mmol) was treated with a solution of LDA (2.0 M, 225 μL, 0.45 mmol, 1.5 eq), TMSCl (76 μL, 0.60 mmol, 2 eq) and MCPBA (208 mg, 1.20 mmol, 4 eq) following the procedure described in section 7.5.4.2 to give, after column chromatography (9:1 hexane/EtOAc), a 26:3 mixture (by ^{1}H NMR) of methyl 6α-hydroxy-7-oxo-labda-8,13***E***-dien-15-oate (**6.17**) and unreacted **4.20** as a colourless oil (91 mg). Semi-preparative normal phase HPLC of the **6.17**/**4.20** mixture in 9:1 hexane/EtOAc yielded pure **6.17** (80 mg, 0.23 mmol, 77%) as a colourless oil.

Methyl 6α-hydroxy-7-oxo-labda-8,13*E*-dien-15-oate (6.17): colourless oil; $[\alpha]_D^{27}$ +11° (***c*** 2.17, $CHCl_3$); IR ν_{max} 3355 (br), 2948, 2873, 1716, 1681, 1651, 1615, 1435, 1225, 1150 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.71 (1H, q, ***J*** = 1.1 Hz, H-14), 4.45 (1H, d, ***J*** = 9.9 Hz, H-6), 3.69 (3H, s, 15-OMe), 2.40 (2H, m, H_2-11), 2.26 (2H, m, H_2-12), 2.21 (3H, d, ***J*** = 1.2 Hz, H_3-16), 1.97 (1H, d, ***J*** = 9.8 Hz, H-5), 1.86 (1H, m, H-1a & 3H, s, H_3-17), 1.68 (1H, dt, ***J*** = 13.7, 3.1 Hz, H-2a), 1.60 (1H, m, H-2b), 1.52 (1H, m, H-1b), 1.47 (1H, m, H-3a), 1.31 (1H, m, H-3b), 1.19 (3H, s, H_3-18), 1.09 (3H, s, H_3-19), 1.04 (3H, s, H_3-20) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.1 (s, C-7), 166.9 (s, C-15), 164.4 (s, C-9), 158.2 (s, C-13), 130.3 (s, C-8), 115.8 (d, C-14), 59.8 (d, C-6), 56.6 (d, C-5), 50.9 (q, 15-OMe), 42.6 (t, C-3), 41.1 (s, C-10), 39.8 (t, C-12), 36.3 (t, C-1), 35.3 (q, C-18), 34.8 (s, C-4), 27.5 (t, C-11), 23.4 (q, C-20), 22.2 (q, C-19), 18.8 (q, C-16), 18.4 (t, C-2), 12.8 (q, C-17) ppm; EIMS ***m/z*** (rel. int.) 331 [$(M–OH)^+$] (27), 330 (37), 292 (38), 261 (86), 256 (100), 241 (58), 229 (55), 174 (71), 135 (56), 91 (49); HRFABMS ***m/z*** 349.2378 (calcd for $C_{21}H_{33}O_4$ [$(M + H)^+$], 349.2379).

7.6.2.4 DIBAH reduction of methyl 6α-hydroxy-7-oxo-labda-8,13***E***-dien-15-oate (**6.17**)

A 1.0 M solution of DIBAH (840 μL, 0.84 mmol, 4 eq) was added dropwise to hydroxy ketone **6.17** (73 mg, 0.21 mmol) in CH_2Cl_2 at –78 °C, the reaction stirred at that temperature for 1 h and then

slowly warmed to RT over 3 h. The reaction was quenched with sat. aqueous NH_4Cl (2.0 mL) at 0 °C, and the organic phase separated and washed with 1.0 M HCl (5 mL), 5% NaHCO3 (5 mL) and H_2O (2 mL). Drying with $MgSO_4$ and evaporation of the solvent gave labda-8,13***E***-dien-6α,7β,15-triol (**6.15**, 67 mg, 0.21 mmol, 100%) as a colourless oil.

Labda-8,13*E*-dien-6α,7β,15-triol (6.15): colourless oil; $[\alpha]_D^{28}$ +56° (***c*** 3.10, $CHCl_3$); IR ν_{max} 3367 (br), 2932, 2871, 1470, 1444, 1382, 1220, 1101, 1018, 757 cm^{-1}; ^1H & ^{13}C NMR data see Table 6.2; EIMS ***m/z*** (rel. int.) 286 [$(M-2H_2O)^+$] (18), 271 (37), 254 (58), 219 (61), 203 (46), 175 (100), 159 (72), 145 (75), 135 (79), 105 (56); HRFABMS ***m/z*** 323.2587 (calcd for $C_{20}H_{35}O_3$ [$(M + H)^+$], 323.2586).

7.6.2.5 Selective protection of the C-15 hydroxyl group of triol **6.15**

Labda-8,13***E***-dien-6α,7β,15-triol (**6.15**, 54.8 mg, 0.170 mmol), imidazole (23.1 mg, 0.340 mmol, 2 eq), and DMAP (4.1 mg, 0.034 mmol, 0.2 eq) were stirred in anhydrous CH_2Cl_2 (1.0 mL) at 0 °C. TBSCl (30.3 mg, 0.201 mmol, 1.2 eq) in CH_2Cl_2 (1.0 mL) was added dropwise ***via*** cannula, upon which a white precipitate immediately formed. Analysis of the reaction mixture 2 min after all of the TBSCl had been added indicated that the reaction was complete, therefore sat. NH_4Cl (1.0 mL) was added and the resulting mixture vigorously stirred for 10 min. The organic and aqueous fractions were separated, the aqueous fraction was washed with CH_2Cl_2 (3 × 2 mL) and the washings added to the original organic fraction. The combined organic phases were washed with H_2O (2 mL), dried ($MgSO_4$), and concentrated to give, after passage through a short column of silica gel in 7:3 hexane/EtOAc, 15-***tert***-butyldimethylsilanyloxy-labda-8,13***E***-dien-6α,7β-diol (**6.18**, 67.4 mg, 0.154 mmol, 91%) as a colourless oil.

15-*tert*-Butyldimethylsilanyloxy-labda-8,13*E*-dien-6α,7β-diol (6.18): colourless oil; $[\alpha]_D^{25}$ +43° (***c*** 2.02, $CHCl_3$); IR ν_{max} 3401 (br), 2930, 2857, 1471, 1384, 1256, 1111, 1067, 1040, 836 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.31 (1H, br t, ***J*** = 6.4 Hz, H-14), 4.30 (1H, dd, ***J*** = 12.1, 8.4 Hz, H-6),

4.18 (2H, d, ***J*** = 6.3 Hz, H_2-15), 4.15 (1H, br d, ***J*** = 8.3 Hz, H-7), 2.12 (1H, m, H-11a), 2.02 (2H, m, H_2-12), 1.96 (1H, m, H-11b), 1.84 (1H, br d, ***J*** = 12.7 Hz, H-1a), 1.71 (3H, s, H_3-17), 1.64 (3H, s, H_3-16), 1.61 (1H, d, ***J*** = 12.1 Hz, H-5), 1.56 (1H, m, H-2a), 1.48 (1H, m, H-2b), 1.42 (1H, m, H-3a), 1.28 (1H, m, H-3b), 1.27 (3H, s, H_3-18), 1.22 (1H, m, H-1b), 1.10 (3H, s, H_3-20), 1.09 (3H, s, H_3-19), 0.90 (9H, s, 15-OSi$(CH_3)_2$C$(C\underline{H}_3)_3$), 0.08 (6H, s, 15-OSi$(C\underline{H}_3)_2$C$(CH_3)_3$) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.2 (s, C-9), 137.0 (s, C-13), 126.7 (s, C-8), 124.3 (d, C-14), 79.8 (d, C-7), 69.4 (d, C-6), 60.3 (t, C-15), 54.2 (d, C-5), 44.2 (t, C-3), 43.5 (s, C-10), 39.3 (t, C-12), 37.4 (t, C-1), 36.2 (q, C-18), 34.4 (s, C-4), 27.0 (t, C-11), 26.0 (q, 15-OSi$(CH_3)_2$C$(\underline{C}H_3)_3$), 21.9 (q, C-19 & q, C-20), 18.4 (s, 15-OSi$(CH_3)_2\underline{C}(CH_3)_3$), 18.3 (t, C-2), 16.4 (q, C-16), 15.0 (q, C-17), –5.0 (q, 15-OSi$(\underline{C}H_3)_2$C$(CH_3)_3$) ppm; EIMS ***m/z*** (rel. int.) 436 [M^+] (4), 360 (47), 268 (35), 211 (39), 173 (53), 157 (100), 131 (69), 119 (54), 115 (40), 91 (47); HRFABMS ***m/z*** 437.3451 (calcd for $C_{26}H_{49}SiO_3$ [$(M + H)^+$], 437.3451).

7.6.2.6 Attempted preparation of the 6α,7β-diacetate **6.19**

The monoprotected triol **6.18** (31 mg, 0.07 mmol) and DMAP (2 mg, 0.02 mmol) were stirred in dry pyridine (0.5 mL) and Ac_2O (0.5 mL) at RT over-night. Analysis of the reaction mixture by TLC indicated that acetylation had occurred and so the reaction was quenched with MeOH (1.0 mL) and concentrated ***in vacuo*** (1 mmHg) to give a pale yellow oil (35 mg). Analysis of the 1D and 2D NMR data obtained for the product indicated that it contained the monoacetate 7β-acetoxy-15-***tert***-butyldimethylsilanyloxy-labda-8,13***E***-dien-6α-ol (**6.20**) and DMAP only. Treatment of **6.20** with the same reagents at 70 °C for 4 h did not result in further acetylation (by 1H NMR spectroscopy), therefore **6.20** (31 mg, 0.06 mmol) was treated with DMAP (8 mg, 0.07 mmol, 1.2 eq) in pyridine (1.0 mL) and Ac_2O (1.0 mL) and the mixture heated at 100 °C for 24 h. Concentration of the reaction mixture ***in vacuo*** (1 mmHg) yielded a dark brown oil (201 mg) that was taken up in EtOAc (5 mL), washed with 1.0 M HCl (2 $\times$ 5 mL) and sat. $NaHCO_3$ (3.0 mL), dried ($MgSO_4$) and concentrated to give a yellow oil (86 mg). Silica gel column chromatography

and normal phase HPLC of the crude product (both in 9:1 hexane/EtOAc) yielded 7β,15-diacetoxylabda-8,13***E***-dien-6α-ol (**6.21**) as a colourless oil (14 mg, 0.03 mmol, 50%).

7β-Acetoxy-15-*tert*-butyldimethylsilanyloxy-labda-8,13*E*-dien-6α-ol (6.20): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.64 (1H, br d, ***J*** = 8.6 Hz, H-7), 5.28 (1H, br t, ***J*** = 6.3 Hz, H-14), 4.33 (1H, dd, ***J*** = 12.2, 8.6 Hz, H-6), 4.16 (2H, d, ***J*** = 6.3 Hz, H_2-15), 2.13 (1H, m, H-11a), 2.11 (3H, s, 7-OAc), 2.03 (2H, m, H_2-12), 1.97 (1H, m, H-11b), 1.83 (1H, br d, ***J*** = 12.4 Hz, H-1a), 1.65 (1H, d, ***J*** = 12.2 Hz, H-5), 1.62 (3H, s, H_3-16), 1.56 (1H, m, H-2a), 1.50 (3H, s, H_3-17), 1.47 (1H, m, H-2b), 1.41 (1H, br d, ***J*** = 14.3 Hz, H-3a), 1.28 (1H, m, H-3b), 1.26 (3H, s, H_3-18), 1.23 (1H, m, H-1b), 1.11 (3H, s, H_3-20), 1.07 (3H, s, H_3-19), 0.88 (9H, s, 15-OSi$(CH_3)_2$C$(C\underline{H}_3)_3$), 0.04 (6H, s, 15-OSi$(C\underline{H}_3)_2$C$(CH_3)_3$) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (s, 7-OAc), 146.2 (s, C-9), 136.7 (s, C-13), 124.4 (s, C-8), 124.3 (d, C-14), 80.6 (d, C-7), 63.4 (d, C-6), 60.2 (t, C-15), 54.7 (d, C-5), 44.1 (t, C-3), 43.0 (s, C-10), 39.1 (t, C-12), 37.3 (t, C-1), 36.2 (q, C-18), 34.4 (s, C-4), 26.9 (t, C-11), 26.0 (q, 15-OSi$(CH_3)_2$C$(\underline{C}H_3)_3$), 21.8 (q, C-19), 21.7 (s, C-20), 21.0 (q, 7-OAc), 18.4 (s, 15-OSi$(CH_3)_2\underline{C}(CH_3)_3$), 18.2 (t, C-2), 16.4 (q, C-16), 14.7 (q, C-17), –5.1 (q, 15-OSi$(\underline{C}H_3)_2$C$(CH_3)_3$) ppm.

7β,15-Diacetoxylabda-8,13*E*-dien-6α-ol (6.21): colourless oil; $[\alpha]_D^{25}$ +53° (***c*** 0.42, $CHCl_3$); IR ν_{max} 3366 (br), 2932, 2870, 1741, 1440, 1384, 1368, 1230, 1021, 933 cm^{-1}; ^{1}H & ^{13}C NMR data see Table 6.3; EIMS ***m/z*** (rel. int.) 288 [$(M - 2 \times OAc)^+$] (2), 286 (20), 271 (20), 268 (46), 253 (84), 218 (100), 183 (58), 171 (48), 157 (91), 142 (87); HRFABMS ***m/z*** 406.2719 (calcd for $C_{24}H_{38}O_5$ [M^+], 406.2719).

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