Structural and stereochemical investigations of terrestrial and marine pyrone metabolites.

THESIS

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by

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To my parents

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LIST OF ABBREVIATIONS

CD	Circular Dichroism
COSY	^1H-^1H homonuclear COrrelation SpectroscopY
DEPT	Distortionless Enhancement by Polarisation Transfer
EIMS	Electron Impact Mass Spectrometry
FABMS	Fast Atom Bombardment Mass Spectrometry
GCMS	Gas Chromatography - Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
HREIMS	High Resolution Electron Impact Mass Spectrometry
HRFABMS	High Resolution Fast Atom Bombardment Mass Spectrometry
IR	InfraRed
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Överhauser Enhancement
NOEDS	Nuclear Överhauser Enhancement Difference Spectroscopy
ORD	Optical Rotatory Dispersion
ROESY	Rotating frame nuclear Överhauser and Exchange SpectroscopY
TLC	Thin Layer Chromatography
UV	UltraViolet

br	broad (used inconjuction with s, d or t)
d	doublet
eV	electon Volt
fr.	fraction
m	multiplet
q	quartet
s	singlet
semiprep.	semi preparative
sh	shoulder (in IR spectrum)
soln	solution
t	triplet

ABSTRACT

This thesis presents an investigation into the chemistry of 6 substituted 5,6-dihydro-α-pyrone compounds. A comprehensive review of these compounds was published in 1989 and the subsequent literature is covered in an updated review presented below. Eight 6-substituted 5,6-dihydro-α-pyrone metabolites from three different South African plant species *Cryptocarya latifolia*, *Syncolostemon densiflorus*, and *Syncolostemon argenteus* have been the subject of structural and stereochemical investigations. The absolute stereochemistry of the known compound "triacetate" from *C. latifolia* has been established as 6R-[2R,4S,6S-(triacetyloxy)-heptyl]-5,6-dihydro-2H-pyran-2-one (**74**) using CD and acetonide formation with subsequent application of the modified Moshers method. The absolute stereochemistry of the related metabolite "diacetate", also from *C. latifolia*, has been assigned as 6R-[2S,4S-diacetyloxy-pentyl]-5,6-dihydro-2H-pyran-2-one (76). In addition, the outstanding stereochemistry at C-5' in syndenolide, from *S. densiflorus*, followed from conversion to its diacetonide and subsequent NMR analysis. Syndenolide is therefore 6R-[5S-(acetoxy)-1R,2R,3S-(trihydroxy)-heptyl]-5,6-dihydro-2H-pyran-2-one.

The genus *Syncolostemon* has proved to be a rich source of α-pyrone compounds and the chemistry of *S. argenteus*, not investigated previously, was examined as part of an ongoing search for new 5,6-dihydro-α-pyrones. The study yielded five new α-pyrone natural products, synargentolide A - E. The structure of synargentolide A (**82**) has been assigned as 6R-[4R,5R,6S-triacetyloxy-1E-heptenyl]-5,6-dihydro-2H-pyran-2-one using CD and NMR techniques. The structures of synargentolide B (**87**), C (**92**) and E (**94**) also followed from a detailed NMR analysis and the stereochemistry tentatively assigned based on CD and NMR data. Synargentolide D (**93**) was thermally unstable, and a paucity of material prevented stereochemical investigations, however the structure was determined from initial NMR analysis.

The marine molluscs of the genus *Siphonaria* have only become the subject of chemical studies in the last fifteen years. These molluscs characteristically produce polypropionate type natural products. A review of Siphonarian polypropionate metabolites containing a pyrone functionality is presented. Examination of an endemic South African species *Siphonaria serrata* yielded one novel polypropionate metabolite containing a γ-pyrone functionality, siserrone A (**131**). The

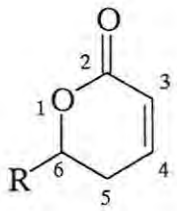
structure of this compound was unambiguously established using standard NMR experiments. The relative stereochemisty of the hemi-ketal moiety was assigned from a careful analysis of the ROESY NMR spectrum and the stereochemisty of the acyclic portion determined from a comparison of the ^{13}C and ^{1}H NMR data of a degradation product with the corresponding data of a synthetic compound. It was also established that the modified Moshers method could not be used to determine the absolute stereochemistry of the secondary hydroxyl substituent at C-11. The absolute stereochemistry of **131** was thus assigned in accordance with the proven stereochemistry of Siphonarian metabolites.

CHAPTER 1

6-Substituted-5,6-dihydro-α-pyrones

1.1 INTRODUCTION

6-Substituted-5,6-dihydro-α-pyrones are a class of compounds that occur widely in nature, particularly in plants and bacteria. These are compounds possessing an α,β-unsaturated-δ-lactone ring (**1**) with an alkyl, alkenyl or aryl substituent at C-6 and occasionally a varied substitution pattern around the ring. Interest in α-pyrones has been stimulated by their frequent occurrence in plants used for medicinal purposes. It is therefore not surprising that many of these compounds are biologically active, exhibiting phytotoxicity, cytotoxicity against tumour cells and antifungal or antimicrobial activity.



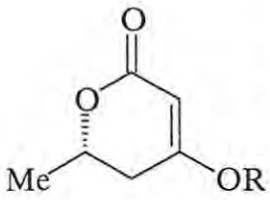
1

The 6-substituted-5,6-dihydro-α-pyrone literature covered by Chemical Abstracts up to December 1987 has been comprehensively reviewed by Davies-Coleman and Rivett.[1] In 1993 the literature pertaining to fungal pyrone compounds, including several 6-substituted 5,6-dihydro-α-pyrones isolated up to December 1991, was reviewed.[2] Some forty new compounds, not covered by either review, have subsequently been isolated. Their sources, bioactivity and structure determination, including stereochemistry, as discussed in the literature until December 1995 are presented in the following review. The nomenclature and numbering system used by Davies-Coleman and Rivett are retained in this updated review. Also in accordance with the earlier review, only naturally occurring 6-substituted-5,6-dihydro-α-pyrones will be discussed and the discussion will exclude compounds, such as the withanolides, with a steroid nucleus attached at C-6. Syntheses will only be mentioned where they were necessary for the structural determination of the α-pyrone natural products.

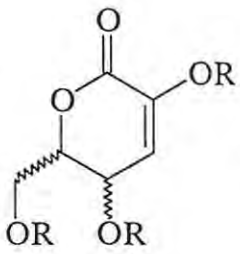
1.1.1 6-Alkyl-5,6-dihydro-α-pyrones

Although the plant families Annonaceae, Lamiaceae, and Lauraceae continue to be excellent sources of 6-substituted-5,6-dihydro-α-pyrones they are not exclusive sources of these compounds. Gerberin (**2**) has been isolated by Nagumo *et al.* from *Gerbera jamesonii hybrida*

of the family Compositae.[3] This is a commercially grown plant and **2** is present in the aerial parts in high yield (3.7%). Although **2** itself is not biologically active it can be readily methylated to the methoxy derivative (**3**) which is a potential synthetic precursor for bioactive compounds. The large scale cultivation of this species could therefore provide an excellent source of chiral precursors for the synthesis of pharmaceuticals. Unfortunately the isolation of **2** from a polar methanol extract of the plant is not trivial and involves a complex series of chromatographic separations on polyamide, ion exchange and gel permeation columns. Standard spectroscopic and chemical techniques were used to determine the structure of **2**. The 6(S) stereochemistry was determined from the positive sign of the $n\rightarrow\pi^*$ Cotton effect in the circular dichroism (CD) spectrum of its acetate derivative (**4**).[4] It must be noted here that there is an error pertaining to the CD of 5,6-dihydro-α-pyrones in the review by Davies-Coleman and Rivett.[1] The structures on page 25 (82) and (83) give rise to negative and positive Cotton effects respectively and not the other way around.



2 R = β-D-glucopyranosyl
3 R = Me
4 R = Ac



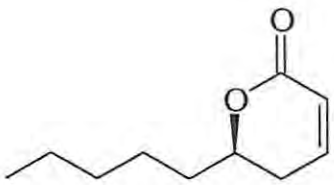
5 R = H
6 R = Ac

A similar polyhydroxylated compound (**5**) has been isolated as its triacetate (**6**) from a liquid culture of the soil fungus *Taleromyces flavus*.[5] The stereochemistry of the two chiral centres in this compound was not established. Although the fungus displays antifungal activity against Verticillium wilt of eggplant (*Solanum melongena* L.), a disease caused by the fungus *Verticillium dahliae*, it appears that **5** is not responsible for the antifungal properties of *T. flavus*.

6-Heptyl-5,6-dihydro-α-pyrones with various levels of oxygenation in the heptyl side chain occur widely. Two such compounds gamahonolide A (**7**) and B (**8**) were isolated by Koshino and co-workers[6] from Timothy chokes, i.e. the grass *Phleum pratense* suffering from the choke disease caused by the phytopathogenic fungus *Epichloe typhinia*. Surprisingly, plants suffering from the disease are resistant to the leaf spot fungus *Cladosporium herbarum*. Compounds **7** and **8**, which are more abundant in plants suffering from the disease, are thought to be

responsible for the systemic and mutualistic behaviour attributed to *E. typhinia*, since they are active against the related leaf spot fungus *C. phlei*. The structures of **7** and **8** were determined by NMR spectroscopic methods including extensive proton decoupling experiments, while the molecular formulae were established by HREIMS. ^{13}C NMR data suggested the presence of the ethyl succinate moiety in **8**, the ethyl ester of which is probably an isolation artifact formed during ethanolic extraction of **8** from the Timothy chokes. A comparison of the ORD spectra of **7** and **8** with the ORD spectrum of massoilactone (**9**) afforded a 6(R) stereochemistry for **7** and **8**. The 6'(R) stereochemistry in **7** was established using Trosts method for the stereochemical determination of secondary alcohol substituents[7,8] in which the (R)- and the (S)-O-methylmandelyl esters are prepared and the chemical shift differences in their ^1H NMR spectra calculated.

7 R = H
8 R = - $COCH_2CH_2CO_2Et$



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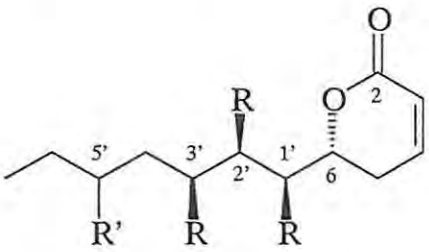
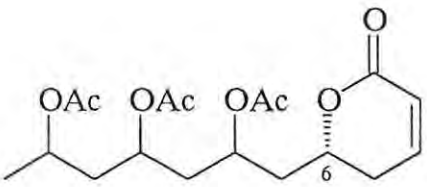


10 R = OH, R' = OAc
11 R = OH, R' = H
12 R = R' = OAc
13 R = OAc, R' = H

Syndenolide (**10**) was the major 5,6-dihydro-α-pyrone isolated from *Syncolostemon densiflorus* (family Lamiaceae) by Davies-Coleman and Rivett.[9] The known α-pyrone compound deacetylboronolide (**11**) was also isolated from this plant. Elemental analysis and spectroscopic methods were used to determine the chemical structure of **10**. The presence of the acyclic triol was demonstrated by a D_2O induced collapse of three hydroxyl proton signals in the ^1H NMR spectrum of **10** and the presence of four acetate methyl signals in the ^1H NMR spectrum of the peracetylated derivative (**12**). A 6(R) stereochemistry for **10** was deduced from the positive sign of the $n\rightarrow\pi^*$ Cotton effect in the CD spectrum and the stereochemistry of the 1', 2' and 3' chiral centres was proposed to be the same as **11** from biosynthetic arguments. This

stereochemical assignment was supported by a comparison of the relevant coupling constants of **12** with those of boronolide (**13**). Initially the stereochemistry at C-5' was not assigned since although **10** was crystalline, no crystals suitable for X-ray crystallography could be obtained. A subsequent investigation and assignment of the stereochemistry at this chiral centre is presented in section 1.2.



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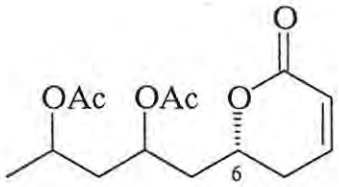
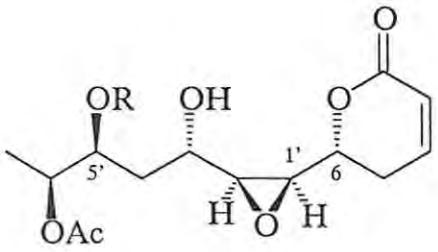
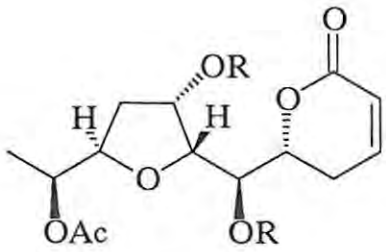


15

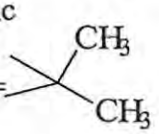
The presence of acetate substituents on the 6-heptyl side chain is very common. Compounds (**14**) and (**15**) were isolated by Drewes *et al.* from the milled bark of trees *Cryptocarya latifolia* in their investigations of the chemistry of plants used for magical and medicinal purposes by the Zulu people of Kwazulu-Natal, South Africa.[10] The structures were determined using two dimensional NMR techniques, but the absolute stereochemistry was not assigned. Although not substantiated experimentally, a 6(R) configuration was predicted for both compounds. An assignment of the outstanding stereochemistry of the chiral centres is presented in section 1.3.

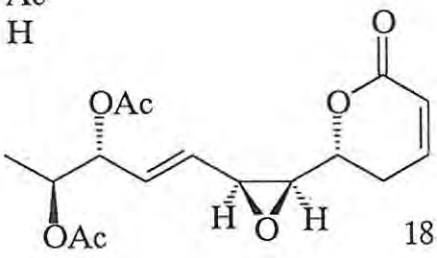


16 R = Ac
21 R = H



17 R = H
19 R = Ac
20 R, R =

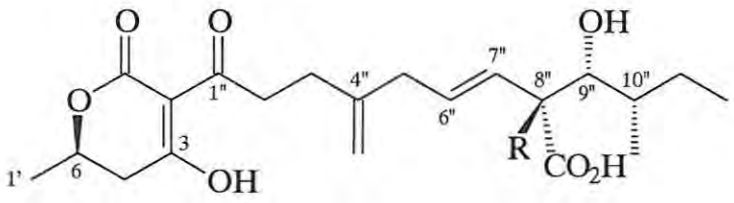




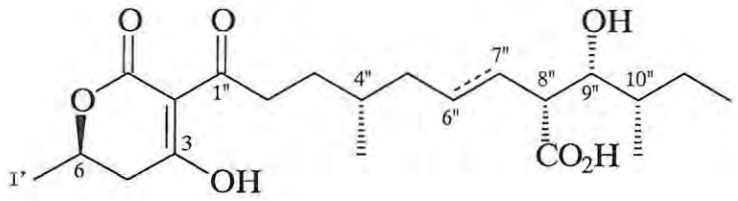
18

Other compounds with acetate groups on the 6-heptyl side chain are synparvolide A (**16**) and C (**17**). These compounds were isolated by Davies-Coleman and Rivett from the leaves of *Syncolostemon parviflorus,*[11] a plant traditionally used, also by the Zulus, as an emetic to treat

appetite loss in adults and children. The chemical structures of **16** and **17** were determined by standard spectroscopic techniques. A comparison of the ^{13}C NMR data of **16** and 5-deacetoxy-5'-epi-olguine (**18**)[12] suggested the presence of an oxirane ring in **16**. CD analysis afforded the 6(R) configuration of **16** and **17**, while the stereochemistry of C-1' and C-2' in **16** was tentatively assigned by comparing the coupling constants $J_{6,1'}$ and $J_{1',2'}$ with those of **18**. The absolute stereochemistry of the secondary alcohol in **16** was established as 3'(S) using the modified Mosher's method and the stereochemistry of C-5' and C-6' were assigned from biosynthetic arguments. Acetylation of **17** gave a triacetate (**19**) with well-resolved signals in the ^{1}H NMR spectrum, thus making this compound suitable for NOE difference experiments. These NMR experiments enabled the relative stereochemistry of the cyclic ether moiety of **17** to be determined. The 1',3'-*syn*diol structure was deduced from the chemical shifts of the methyl groups in the ^{13}C NMR spectrum of the isopropylidene derivative (**20**). Monodesacetyl-synparvolide A (**21**) is a probable precursor for **17** and the remaining C-6' stereochemistry of **17** was assigned accordingly.



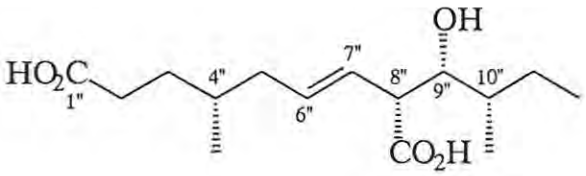
22 R = OH
23 R = H



24
25 $\Delta^{6"}$ (E)

The absolute stereochemistry of alternaric acid (**22**) first isolated in 1949[13] from the fungus *Alternaria solani*, has been established by total synthesis.[14, 15] Another three 6-methyl-5,6-dihydro-α-pyrones (**23**), (**24**) and (**25**), structurally related to alternaric acid, have subsequently been isolated from this fungus.[16] The structures of these latter compounds were determined by spectroscopic methods with the C-3 hydroxyl functionality on the lactone ring affording a characteristic deshielded proton signal at 17 ppm in the ^{1}H NMR spectrum. Spectroscopic methods, chemical degradation and chemical interconversions determined the stereochemistry of compounds **23** - **25**. Oxidative cleavage of the ketone adjacent to the pyrone ring in **25**

yielded the diacid (**26**). Esterification of **26** followed by application of Mosher's method yielded the stereochemistry of the secondary alcohol moiety. To determine the relative stereochemistry at C-8", the two ester groups were first reduced to primary alcohols with $LiAlH_4$. Subsequent preparation of the isopropylidene derivative of the resulting 1,3-diol enabled an NMR investigation of the H-8" and H-9" coupling constant that suggested a *trans*-diaxial orientation for these two protons. The configurations at C-4" and C-10" were determined by comparison of chemical degradation products with synthetic analogues of known stereochemistry. The 6(R) stereochemistry of the lactone ring in **25** was determined from CD data. Since the CD and NMR data of **23** were in agreement with those of **25**, it was proposed that the absolute stereochemistry of the four chiral centres in **23** were the same as that of the corresponding centres in **25**. Catalytic hydrogenation of **25** yielded **24** with the CD and NMR data consistent with the natural product, thus establishing the absolute stereochemistry of natural **24**.[16]



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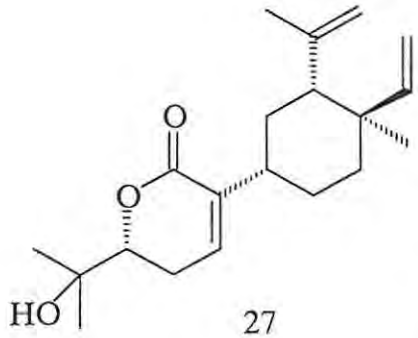
There have been very few reports of 5,6-dihydro-α-pyrones isolated from the marine environment. In 1992, Hamada *et al.* reported the isolation of lobatrienolide (**27**) from an Okinawan soft coral *Sinularia flexibilis*.[17] That same year Tsuda *et al.* reported the isolation of cytotoxic sesterterpenes from the Okinawan marine sponge *Luffariella sp.* among which was a compound with a 5,6-dihydro-α-pyrone moiety, luffariolide E (**28**).[18, 19] Compound **28** showed cytotoxicity against murine leukaemia L1210 cells (IC_{50} 1.1-7.8 μg/ml) in vitro. The structure of **27** was established by spectral techniques and confirmed by reduction under strong UV conditions to the known compound lobatriene (**29**) isolated from the same sponge collection. This interconversion also gave the absolute stereochemistry of **27** since the physical data, including the optical rotation of the derivative were consistent with those of the natural product. NMR techniques, including a series of proton decoupling experiments, were used to determine the structure of **28**. The (*E*)-stereochemistry of the double bond, assigned by an examination of coupling constants, was confirmed by NOE experiments. A series of chemical

degradations of a related compound 4(R)-luffariolide B (**30**) afforded the 6(R), 1'(R) absolute configuration of **28**.



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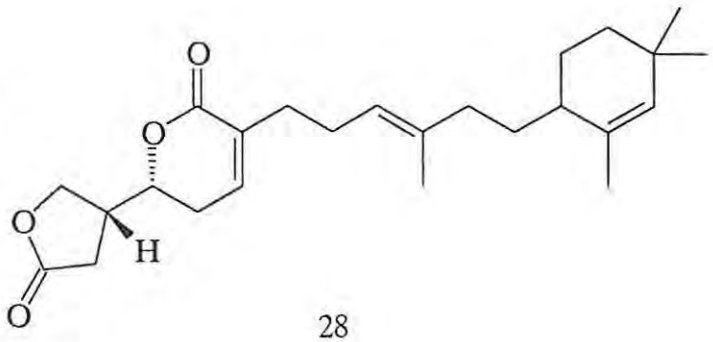

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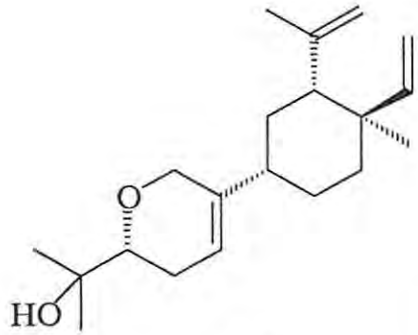

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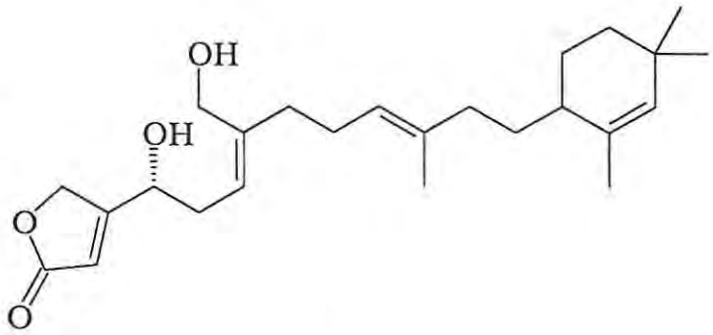


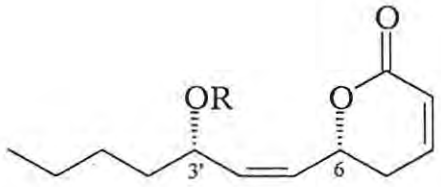
30

1.1.2 6-Alkenyl-5,6-dihydro-α-pyrones

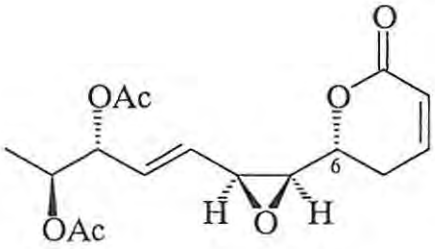
6-Heptenyl-5,6-dihydro-α-pyrones are as common as their saturated counterparts. The position and stereochemistry of the double bond, although usually consistent for compounds from a particular genus, varies widely. The stereochemistry of the olefinic bond can generally be derived from the coupling constants of the vinylic protons J = 12-18 Hz for a *trans* and J = 7-11 Hz for a *cis* configuration.[21] Where coupling constants cannot be obtained for example with low field NMR instruments, recourse is made to infra red spectroscopy. In the IR spectrum, *trans* disubstituted alkenes show only one strong band near 965 cm^{-1} due to a C-H out of plane bend, while *cis* disubstituted alkenes show a strong band near 690 cm^{-1}.[22]

Umuravumbolide (**31**) and deacetylumuravumbolide (**32**) were initially isolated from *Tetradenia riparia* by Van Puyvelde *et al.*. A *trans* configuration was assigned to the double bond based on IR evidence,[23] but a subsequent high field NMR examination[24] of these compounds, isolated

from a South African *Tetradenia sp.* showed that the double bond was *cis*. A 3'(S) configuration for the single acyclic chiral centre in **32** was deduced from the application of the modified Mosher's method to **32**.



31 R = Ac
32 R = H



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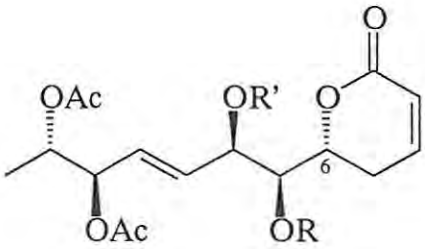
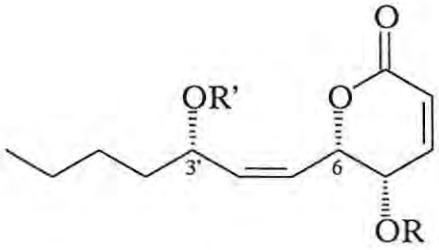


33 R = R' = H
34 R = H, R' = Me
35 R = R' = Ac

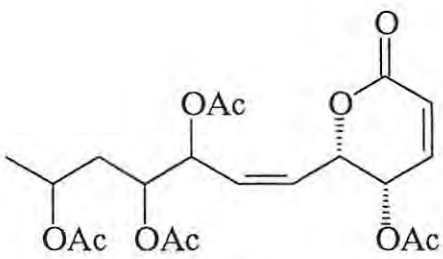
The absolute configuration of another known compound has been established following the 1989 review.[1] Isolation of 5-deacetoxy-5'-epi-olguine, **18** was first reported by Delgado, Pereda-Miranda and Romo de Vivar in 1985.[23] The relative stereochemistry was determined by X-ray analysis and one enantiomer was chosen to represent the natural product. More recently a CD study by the same researchers afforded a 6(R) configuration for **18** confirming that the chosen enantiomer was indeed the natural product.[12] An examination of the minor constituents of the plant *Hyptis oblongifolia* from which **18** had been isolated yielded three new bioactive compounds (**33**), (**34**) and (**35**). The chemical structures of these latter three compounds were determined using NMR spectroscopy. Similarities in their ^1H spectra with that of **18**, particularly with respect to the ^3J coupling constants, supported the proposed stereochemistry of **33**, **34**, and **35**. Acid catalysed methanolysis of the epoxide in **18** took place both regio- and stereoselectively to yield **34** as the only major product. The physical and chemical data for this product were consistent with the natural product, confirming the stereochemical assignment of naturally occurring **34**. Similarly the physical and chemical data of **35** were identical to those of the diacetate of diol **33**. The 6(R) configuration of **35** was determined from the chiroptical analysis of this compound.

Pectinolide A (**36**) and its monodeacetyl analogues pectinolide B (**37**) and pectinolide C (**38**) have been isolated from another *Hyptis* species, *H. pectinata*.[25] These compounds exhibit antimicrobial activity and strong cytotoxicity against a variety of tumour cells (ED_{50} < 4 μg/ml). The chemical structure of **36** was established from spectral and chemical evidence and a $J_{1',2'}$ coupling constant of 10.5 Hz implied an (*E*) stereochemistry for the double bond. A pseudo-equatorial orientation of the C-6 side chain could be inferred from the $J_{5,6}$ coupling constant of 2.9 Hz validating[4] the assignment of a 6(S) configuration from the CD data. Ozonolysis of **36** yielded 2-acetyloxyhexanoic acid and a 3'(S) configuration in **36** was determined from the CD spectrum of the α-hydroxy acid which revealed a weak negative CD maxima at $\Delta\epsilon_{244} = -0.01$ and a positive Cotton effect at $\Delta\epsilon_{209} = +1.58$.[26] Acetylation of **37** and **38** yielded **36** and hence defined their respective stereochemistries.



36 R = R' = Ac
37 R = Ac, R' = H
38 R = H, R' = Ac

Structurally related compounds hyperticin (**39**)[27] and synparvolide B (**40**)[11] have been isolated from yet another *Hyptis* species, *H. urticoides* and *Syncolostemon parviflorus* respectively. The structures of **39** and **40** were determined spectroscopically with the stereochemistry of **40** derived from its biosynthetic relationship to **16**. The genus *Syncolostemon* is a rich source of 6-alkenyl-5,6-dihydro-α-pyrones and the isolation of five new α-pyrones from *S. argentium* is presented in section 1.4.



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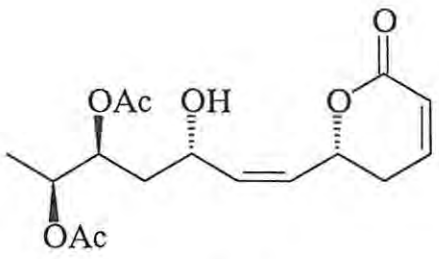
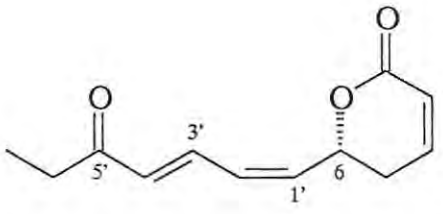


40

In their investigations of Brazilian medicinal plants, Matsuda *et al.* have reported the bioactivity guided isolation of two cytotoxic compounds, (**41**) and (**42**), with conjugated diene side chains,

from *Chorisia crispiflora*.[28] These plants are used as folk medicines for rheumatism and menorrhagia. The $\Delta^{1'},\Delta^{3'}$-Z,E-stereochemistry of the dienone **41** was determined from the coupling constants ($J_{1',2'}$ = 11.0, $J_{3',4'}$ = 15.0 Hz). Split Cotton effects ($\Delta\epsilon_{264}$ -4.7 and $\Delta\epsilon_{209}$ +9.0), due to the interaction of the $\pi\rightarrow\pi^*$ transitions of the enone and the dienone, were observed in the CD spectrum of **41**.[29] Characteristically, Davydov split positive and negative maxima are of equal amplitude[30], thus the CD data suggest that a positive $n\rightarrow\pi^*$ Cotton effect overlaps with the $\pi\rightarrow\pi^*$ transition, implying a 6(R) configuration in **41**. The structure of **42** was determined by spectral techniques and confirmed by oxidation with CrO_3 to give **41**. The 5'(R) configuration in **42** was determined using the modified Mosher's method.



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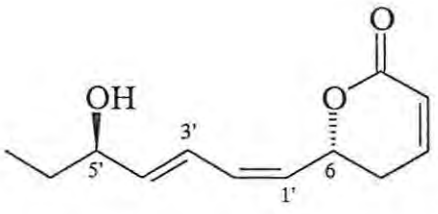
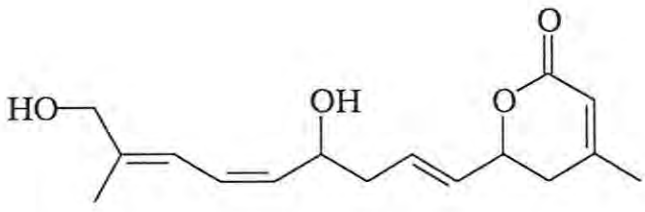


42

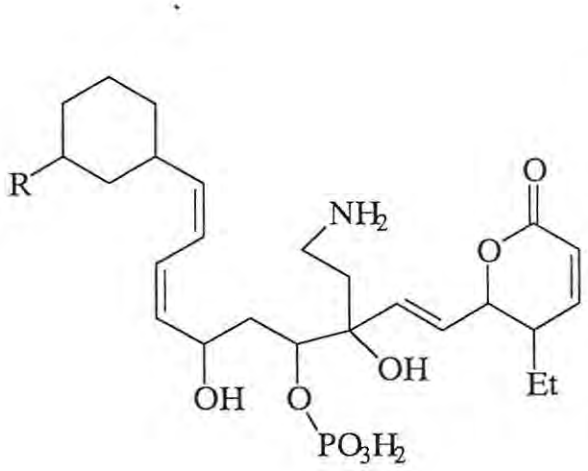
Oncorhyncolide (**43**) is the first α-pyrone to be isolated from marine bacteria. It was obtained from a bacterial isolate #157 taken from surface sea water near a chinook salmon (*Oncorhyncus tshawytscha*) net pen farming operation by Needham and co-workers.[31] Thermally unstable **43** was acetylated to afford its stable diacetate and the structure of the diacetate was determined using two dimensional NMR techniques. NOE experiments were used to determine the stereochemistry of the double bonds, but the configurations of the two chiral centres were surprisingly not assigned. CD studies would have yielded the stereochemistry at C-6 while the modified Mosher's method could have been used to establish the configuration of the acyclic chiral centre.



43

The phoslactomycins A - F (**44** - **49**) also of bacterial origin have potent antibiotic activity, and several patents have been recorded for these compounds. They were isolated from a culture broth of soil isolate actinomycete *Streptomyces nigrescens* by Fushimi *et al.*.[32, 33] These

compounds show weak antibacterial activity, but strong antifungal activity particularly against *Botrytis cinerea*, a phytopathogenic fungus responsible for a grey mould disease affecting vegetable production. Because the bioactivity of the six compounds is very similar, the substituents on the cyclohexane ring are not considered important for biological activity. Current chemical treatments for the grey mould disease differ structurally to the phoslactomycins. Accordingly it is hoped that these α-pyrones will be an effective new approach to combating the resistant strains of this disease.[32] The structure of **48** was determined using HRFABMS and NMR techniques. Decoupling experiments were used to investigate the substitution pattern of the double bonds. The presence of the amino and phosphate groups was confirmed by the ninhydrin and the ammonium molybdate perchloric acid tests respectively. A comparison of the spectral data showed that all the compounds had the same basic skeleton. GCMS of the aliphatic carboxylic acids, produced on saponification of **48** and **49**, were compared with authentic samples and the substituents on the cyclohexane ring of **48** and **49** were thus established.[33]



44 (A) R =—O

45 (B) R = H

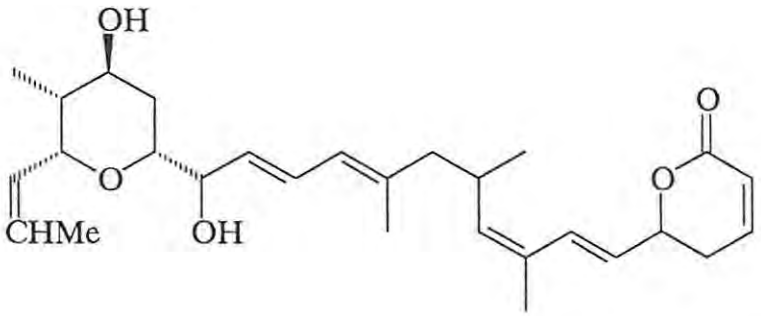
46 (C) R =—O

47 (D) R =—O

48 (E) R =—O

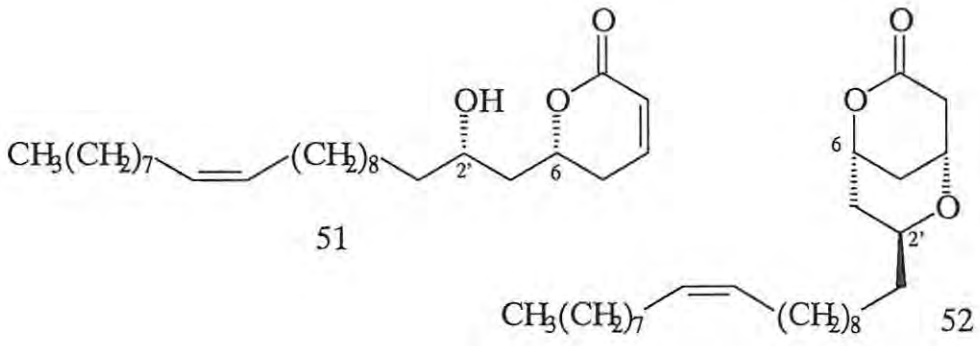
49 (F) R =—O

An antibiotic with a very narrow spectrum of activity, ratjadone (**50**) has been isolated from the culture broth of a myxobacterium *Sorangium cellulosum*.[34, 35] Compound **50** is specific to certain species of Oomycetes, including important phytopathogenic fungi. These fungi are inhibited by very low concentrations of **50**. The structure of ratjadone was determined by standard spectroscopic methods.



50

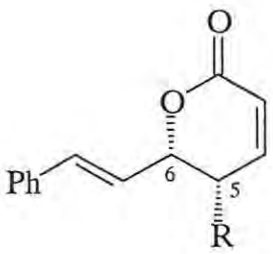
Merlin and co-workers reported the isolation of compound (**51**) from the unusual source of freshly dissected hypertrophied Dufour's gland in worker ants, *Tetramorium acleatum*.[36] Compound **51** is thought to be responsible for the skin irritating (urticating) properties of the ant and it has been proposed that the lipophilicity of the side chain aids penetration through the skin, or the insect exoskeleton. Structural assignments of **51** and its analogue (**52**), which forms slowly as **51** cyclises in solution, were based on spectroscopic evidence. The Z-configuration of the side chain double bond was assigned from an examination of the IR spectrum that contained no peaks near 965 cm^{-1}. Allyl chemical shifts in the ^{13}C NMR spectrum also suggested the presence of a Z-double bond. An abnormally shielded H-2' signal in the ^{1}H NMR spectrum of **52** indicated an axial orientation for this proton. This result together with an examination of the long range coupling in the COSY spectrum of **52** implied a *syn* relative configuration for H-6 and H-2' in **51**.



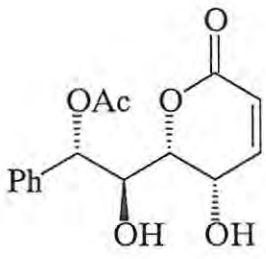
1.1.3 6-Aryl-5,6-dihydro-α-pyrones

In their review,[1] Davies-Coleman and Rivett reported that the genus *Goniothalamus* (Annonaceae) is a rich source of 6-aryl-5,6-dihydro-α-pyrones with goniothalamin (**53**) and its analogues being isolated from five different species within this genus. Subsequent examinations

of four other *Goniothalamus* species have yielded four new goniothalamin analogues. Goniothalamin and the previously unknown compound, 5-acetylgoniothalamin (**54**) have been isolated from *G. uvaroides*.[37] The 6(S) configuration of **54** was proposed to be the same as the 6(R) configuration in **53** from biosynthetic arguments. To avoid confusion, it must be noted here that oxygenation at C-5 can lead to a Cahn Ingold Prelog sequence priority reversal and hence a reversal of the C-6 stereochemical assignment. The 5(S) configuration in **54** was deduced from the $J_{5,6}$ coupling constant which suggested that H-5 and H-6 are *syn*. (+)-5β-Hydroxygoniothalamin (**55**), the deacetyl analogue of **54**, was isolated from *G. dolichocarpus*.[38] The structure and stereochemistry of **55** were established by NMR, X-ray crystallography and partial synthesis. Bioactive analogues 8-acetylgoniotriol (**56**) and goniodiol-8-monoacetate (**57**) have been isolated from *G. giganteus*[39, 40] and *G. amuyon*[41] respectively. Both these compounds exhibit strong cytotoxicity to human tumour cells. The structure of **56** was determined by NMR while X-ray crystallographic analysis afforded the relative stereochemistry. An unambiguous synthesis of both enantiomers of **56** from D-*glycero*-D-*gulo*-heptono-γ-lactone resolved the absolute stereochemistry of the natural product.[39] A comparison of the chemical and physical data of the acetate of **57** with the known peracetylated derivative goniodiol monoacetate (**58**) yielded the stereochemistry of **57**.[41] The relative configuration at C-1' and C-2' was supported by an examination of the relevant coupling constants.



53 R = H
54 R = OAc
55 R = OH



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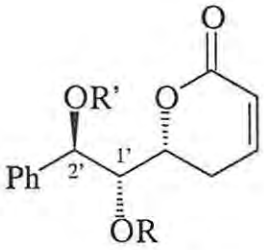
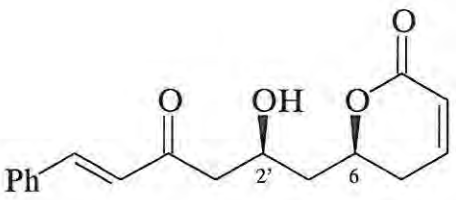


57 R = H, R' = Ac
58 R = Ac, R' = H

Two styryl compounds kurzilactone (**59**) and cryptofolione (**60**) have been isolated from *Cryptocarya kurzii*[42] and a mixture of two *Cryptocarya* species,[43] *C. latifolia* and *C. myrtifolia* respectively. Compound **59** exhibited marked cytotoxicity against KB cancer cells (IC_{50} = 1 μg/mL). NMR was used to establish the chemical structures of the two compounds with coupling constants of 16 Hz indicating *trans* double bonds. NOE experiments afforded the

relative stereochemistry of C-6 and C-2' in **59** and the relative configuration of the 1,3-diol in **60** was explored by preparing the isopropylidene derivative (**61**).[43]



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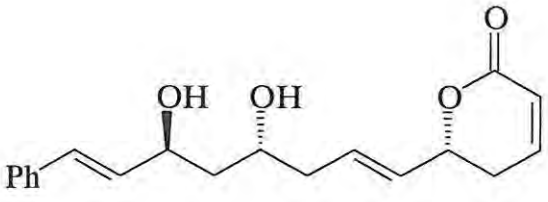

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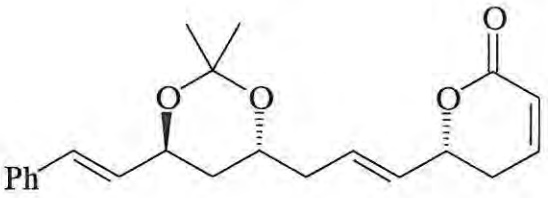

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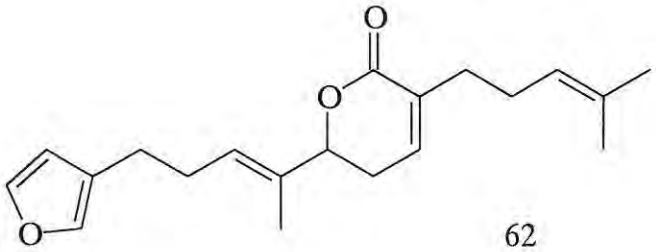

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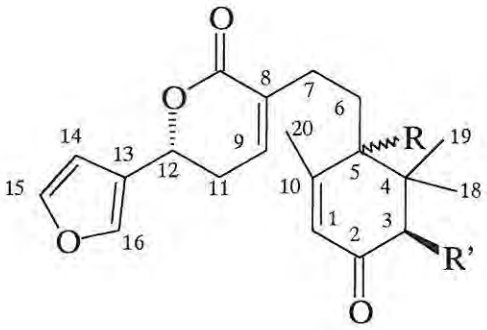


63 R =α H, R' = H
64 R =β H, R' = H
65 R =α H, R' = OH
66 R =β H, R' = OH
67 R =α H, R' = OAc
68 R =β H, R' = OAc
69 R =α OH, R' = H

α = ·····
β = ▬

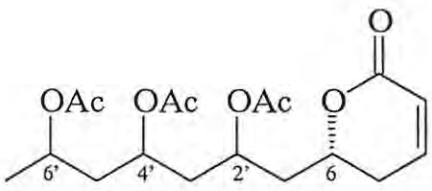
A geranyl geraniol derivative, with a heterocyclic furanyl substituent, conyzaleucolide A (**62**) was isolated by Zdero *et al.* from the aerial parts of *Conyza hypoleuca*.[44] Its structure was elucidated by high field NMR techniques. Another group of α-pyrone compounds also contain a furanyl substituent. Two pairs of epimeric compounds hebecliniolide (**63**) and (**64**) and 3β-hydroxyhebecliniolide (**65**) and (**66**) have been isolated from *Hebeclinium macrophylum* of the family Eupatorieae.[45] A 7:3 epimeric ratio for **63** and **64** was determined from the ^{1}H NMR spectrum. The elucidation of the chemical structures of **63** and **64** was reported in 1977,[46] but

these compounds were omitted from the earlier 5,6-dihydro-α-pyrone review.[1] Epimers **65** and **66** were separated as their acetates (**67**) and (**68**) and the structures were determined from a comparison of the NMR spectra with the spectrum of **63** and **64**.[46] NOE difference experiments indicated the relative stereochemistry of the acetates while the absolute configurations were deduced from the Cotton effects in the CD spectra of the natural products, **63** and **64**, and chemically transformed products.[46] Subsequently, *Tamaulipa azurea* (Eupatorieae) yielded another hebecliniolide derivative 5α-hydroxyhebecliniolide (**69**).[47] A CD study revealed a negative n$\rightarrow\pi^*$ Cotton effect defining the 12(S) [usually 6(S)] configuration of this compound. The chemical structure of **69** was determined through spectroscopic analysis and correlation to the known compound **63**.

1.2 *CRYPTOCARYA LATIFOLIA*

1.2.1 Background

Cryptocarya latifolia, also known as the broad-leafed laurel, is a member of the Lauraceae family.[43] The bark of this large tree (up to 20 m) which can be found along the coast of Kwazulu-Natal is increasingly being used by the Zulu people for mythical purposes[48] and as a medicine to treat chest complaints.[49] The bark of another member of the Lauraceae, *Octea bullata* (the black stinkwood) was originally used to treat these ailments however as a result of over exploitation by traditional healers, *O. bullata* has become increasingly scarce and traditional healers are being forced to make use of alternatives. Since the bark of the two species *C. latifolia* and *O. bullata* have the same aromatic smell and patients are thus unable to detect a change in treatment, *C. latifolia* is a convenient substitute. The substitution of *C. latifolia* for *O. bullata* prompted an investigation into the chemistry of the species *C. latifolia* by Drewes *et al.* and their analysis has shown that the chemistry of this species is considerably different to that of *O. bullata*.[10, 43]



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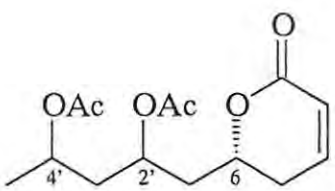

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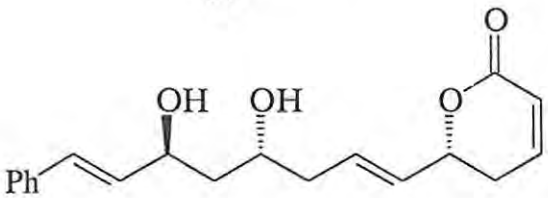

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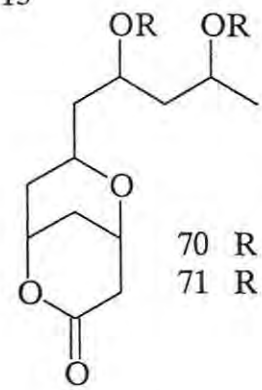


70 R = H
71 R = Ac

Initial chemical investigations into *C. latifolia* yielded cryptofolione **60** (0.003 % dry wt.) from a benzene extract of the bark.[43] Subsequent investigations of an ethanolic extract of the bark of this species by the same researchers[10] yielded two new α-pyrone compounds namely the triacetate **14** (0.008 % dry wt.) and the diacetate **15** (0.015 % dry wt.). Two bicyclic

tetrahydro-α-pyrone compounds structurally related to **14**, (**70**, 0.003 % dry wt.) and (**71**, 0.006 % dry wt.), were also isolated. The structures of these compounds were elucidated by standard NMR techniques, but the absolute stereochemistry was not assigned. A 6(R) configuration was predicted for compounds **14**, **15** and **60** and has been confirmed for **14** and **15** from our investigations into the stereochemistry of these two compounds.

Compound **14** has three chiral centres in addition to the chiral C-6 carbon of the lactone ring. Drewes supplied us with sufficient material for the determination of the absolute configuration at carbons 2', 4' and 6' in **14** by high field NMR techniques. The stereochemistry at C-6 was determined from CD measurements. A sample of the diacetate **15** was also obtained for analysis of the relative configuration of C-2' and C-4' and the absolute configuration of C-6. These stereochemical investigations are described below.

1.2.2 Results and Discussion

In 1968 Snatzke proposed a method for determining the absolute stereochemistry of the C-6 carbon of $\alpha\beta$-unsaturated-δ-lactones using circular dichroism (CD)[4] and this method has subsequently been widely applied to stereochemistry determinations of such systems. The chirality of the 5,6-dihydro-α-pyrone ring at C-6 induces a chirality in the energy levels of the carbonyl chromaphore and the $n\rightarrow\pi^*$ electronic transition is thus chiral. Consequently, the molecule has different molar absorption coefficients for left and right circularly polarised light and a measurement of this difference is used to assign the configuration at the C-6 carbon. The empirical rule for assigning the absolute stereochemistry for these systems is that a positive $n\rightarrow\pi^*$ Cotton effect denotes the stereoisomer depicted in Figure 1a and negative $n\rightarrow\pi^*$ Cotton effect denotes the stereoisomer depicted in Figure 1b. This rule has been based on the CD measurements of a large number of compounds where the absolute stereochemistry has been determined by chemical methods. In order to satisfy this rule, the C-6 substituent of the compound under investigation must adopt a *pseudo*-equatorial orientation. If such an orientation can be inferred from consideration of steric factors, the absolute stereochemistry at the C-6 carbon of $\alpha\beta$-unsaturated-δ-lactones can be unequivocally assigned. As yet, there has been no evidence to contradict the stereochemical assignment based on this rule (Table 1). Hence, the positive Cotton effect observed at λ_{max} 256 nm ($\Delta\epsilon$ = +2.5 and +2.8) indicates a 6(R) configuration in both **14** and **15**.

Table 1 : The $n\rightarrow\pi^*$ CD and ORD data for 6-substituted-5,6-dihydro-α-pyrones[†]

Compound	No.	CD $\Delta\epsilon$	$n\rightarrow\pi^*$ λ nm	ORD Φ	$n\rightarrow\pi^*$ λ nm	Stereochemistry at C-6 implied from Snatske's rules
C_{12} Lactone	**18**	+2.8	256			(R)
C_{12} Lactone	**35**	+2.4	257			(R)
10-Deoxyalternaric acid	**23**	-3.9	266			(S)
10-Deoxy-6,19-dihydro-alternaric acid	**24**	-3.9	260			(S)
10-Deoxy-6,8,9,19-tetra-hydroalternaric acid	**25**	-3.2	259			(S)
Desacetylumuravumbolide	**32**	+0.9	255			(R)
Diacetate	**15**	+2.8	256			(R)
Gamahonolide A	**7**	-1.96	249	-6176	268	(R)
Gamahonolide B	**8**	-0.93	249	-2380	267	(R)
Gerberin acetate	**4**	+7.78	256			(S)
Pectinolide A	**36**	+2.4	265			(S)
Synargentolide A	**82**	+3.5	265			(R)
Synargentolide B	**86**	+3.2	258			(R)
Synargentolide C	**91**	+2.0	266			(R)
Synargentolide E	**93**	+1.1	265			(S)
Syndenolide	**10**	+2.35	257			(R)
Synparvolide A	**16**	+2.37	255			(R)
Synparvolide B	**40**	+2.97	258			(R)
Synparvolide C	**17**	+3.4	256			(R)
Triacetate	**14**	+2.5	256			(R)

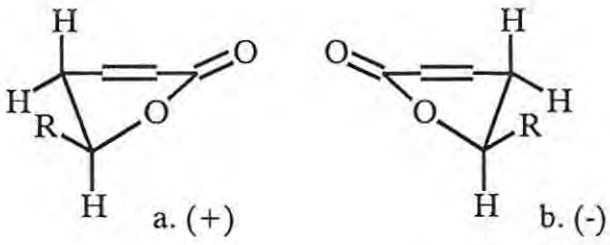


Figure 1 : Configurations assigned by circular dichroism

[†]This table includes CD data published for compounds isolated after 1987 and thus not included in a similar table presented in the review by Davies-Coleman and Rivett.

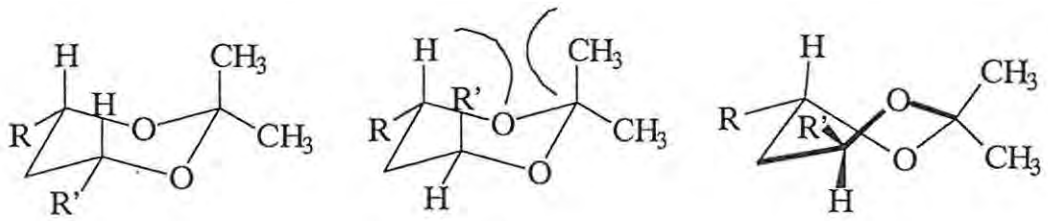
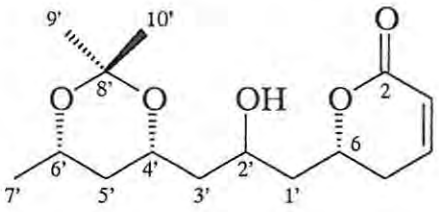
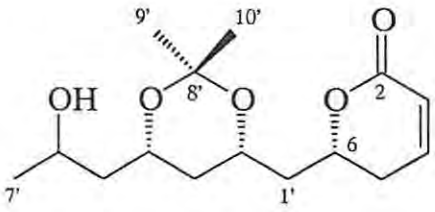


Figure 2 : The conformations of *syn* and *anti* 1,3-diol acetonides

In 1990, Rychnovsky and Skalitzky[50] developed a technique for determining the relative stereochemistry of 1,3-diols using the cyclic isopropylidene (acetonide) derivatives. The *syn*-1,3-diol acetonide exists in a chair conformation with the two alkyl substituents in equatorial positions and the one methyl group axial and the other equatorial (Figure 2a). Since these methyl groups are in different chemical and hence different magnetic environments, they resonate at different frequencies (δ 19 to 20 ppm axial and δ 30 ppm equatorial). The *anti*-1,3-diol acetonide exists in a twist boat conformation (Figure 2c) in order to relieve the 1,3-diaxial interaction (Figure 2b)[51] and both the isopropylidene methyl groups are thus neither axial nor equatorial, exhibiting ^{13}C chemical shifts between δ 24 and 25 ppm.



72



73

Having established the configuration at C-6, the configuration of the asymmetric carbon atoms in the heptyl side chain was investigated as follows. A mixture of acetonides was prepared by filtering an acetone solution of saponified **14** through a column of Amberlyst-15 resin. Amberlyst-15 resin is an acidic, sulphonated polystyrene cation exchange resin which is stable in organic solvents. Although no mechanism has been suggested for the role of the resin in acetonide formation,[52] it is plausible that the acid resin catalyses acetal formation. This is an efficient method to make acetonides and is particularly well suited to small scale work unlike the more traditional methods of acetal formation which require the use of an acid catalyst in an organic solvent and removal of water e.g. by a Dean-Stark apparatus. As expected a

mixture of two acetonides (**72**) and (**73**), which was separated by normal phase high performance liquid chromatography (HPLC), was obtained.

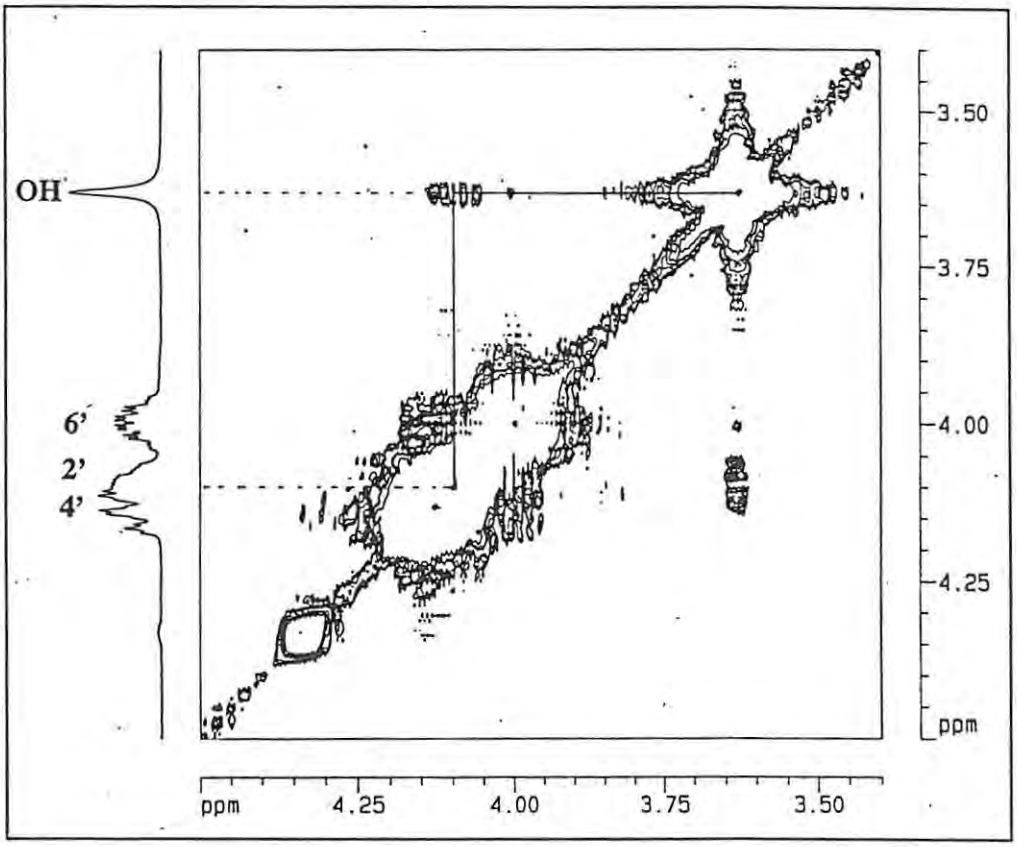


Figure 3: A section of the COSY NMR (400 MHz, $CDCl_3$) spectrum of acetonide **72**

The contiguous coupling sequence from H-3 to H-7' was clearly evident in the COSY NMR spectrum of **72** and made the unambiguous assignment of ^1H chemical shifts (Table 2) possible. Careful examination of the COSY spectrum of **72** (Figure 3) also revealed coupling between the hydroxyl proton (δ 3.65 ppm) and the oxymethine proton H-2' (δ.4.2 ppm) thus tentatively placing the OH moiety at C-2' in this compound. The ^{13}C spectrum showed well resolved resonances accounting for all fifteen carbons of **72** while a DEPT-135 NMR spectrum showed that this compound contained three methyls, four methylene and six methine carbon atoms. As expected, the two quaternary carbons were absent from the DEPT spectrum. All the ^{13}C chemical shifts, presented in table 3, could be assigned from the ^{13}C-^1H correlations observed in the HMQC spectrum. $J_{2,3}$-HMBC correlations (Figure 4) between the hydroxyl proton and the 1', 2' and 3' carbons (δ 41.78 , 68.13 and 42.67 ppm respectively) unequivocally

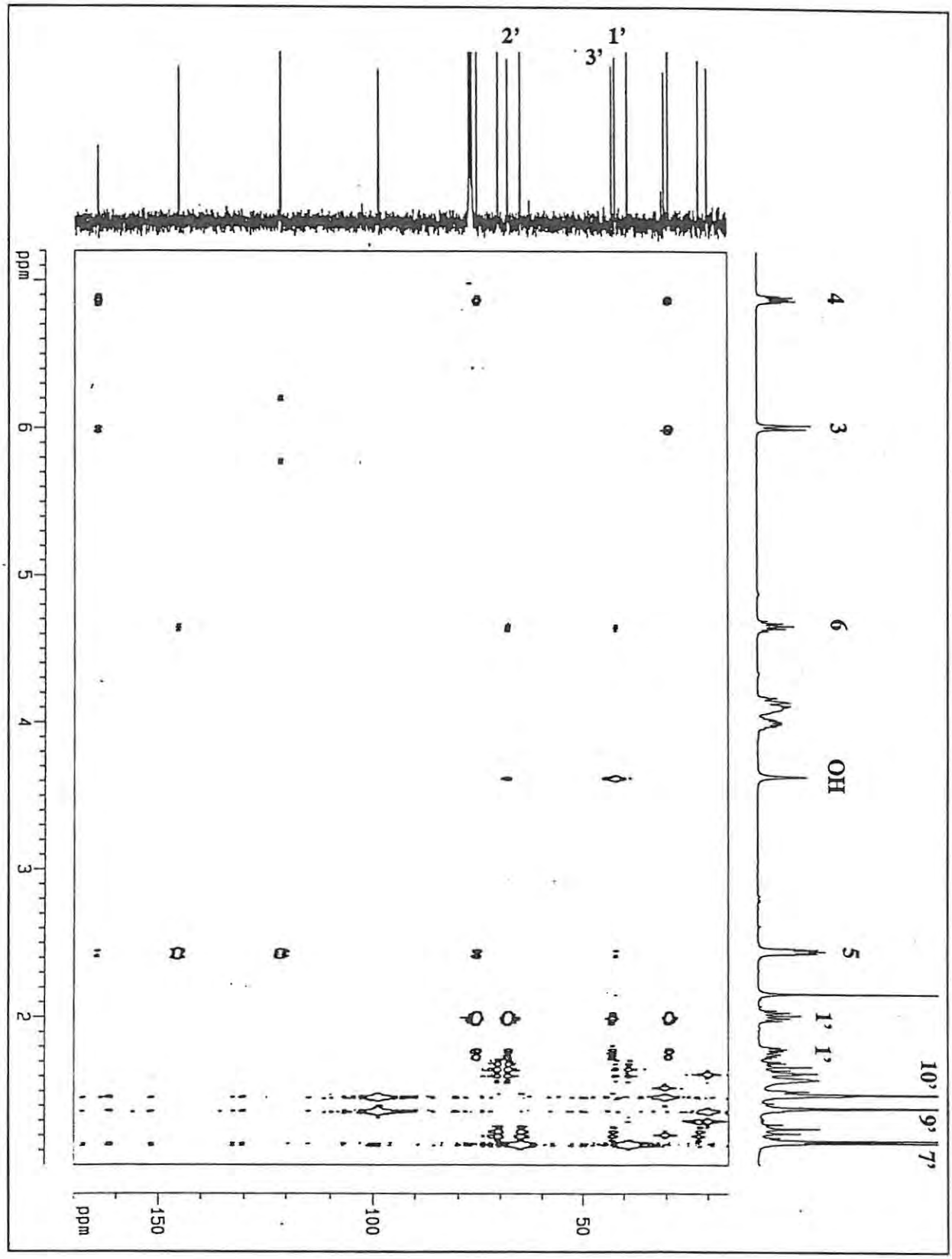


Figure 4 : HMBC NMR (400 MHz, $CDCl_3$) spectrum of acetonide **72**

established the position of the hydroxyl group at C-2' and hence the position of the six membered heterocyclic acetonide ring between C-4' and C-6'. Similarly, COSY and HMQC experiments were used to assign the ^{1}H and ^{13}C chemical shifts of compound **73** and these data are presented in Tables 2 and 3. Examination of the isopropylidene methyl ^{13}C chemical shifts of **72** (δ 19.99 and 30.23 ppm) and **73** (δ 20.0 and 30.1 ppm) suggested that both pairs of 1,3-diols (2',4' and 4',6') in saponified **14** have a *syn* relationship.

Table 2 : ^{1}H chemical shifts for compounds **72**, **73** and **75** †

	72	**73**	**75**
3	6.01 *d*	6.01 *dd*	6.02 *d*
	9.8 Hz	9.7, 1.3 Hz	9.8 Hz
4	6.88 dt	6.88 *ddd*	6.87 *m*
	9.8, 4.4 Hz	9.7, 5.9, 4.5 Hz	
5	2.44 *m*	2.39 *m*	2.39 *m*
6	4.66 *m*	4.59 *m*	4.60 *m*
1'	1.76 *m*, 2.00 *m*	1.75 *m*, 2.04 *m*	1.75 *m*, 2.04 *m*
2'	4.09 *m*	4.17 *m*	4.13 *m*
3'	1.62 *m*, 1.67 *m*	1.54 *m*, 1.28 *m*	1.50 *m*, 1.2 *m*
4'	4.14 *m*	4.12 *m*	3.98 *m*
5'	1.48 *m*, 1.23 *m*	1.54 *m*, 1.60 *m*	1.16 *d*
			6.1 Hz
6'	4.00 *m*	3.99 *m*	----
7'	1.15 *d*	1.15 *d*	----
	6.1 Hz	6.2 Hz	
9'	1.38 *s*	1.36 *s*	1.37 *s*
10'	1.47 *s*	1.46 *s*	1.43 *s*
OH	3.63 *s*	3.22 *s*	

† 400 MHz, $CDCl_3$

Table 3 : ^{13}C chemical shifts for compounds **72**, **73** and **75** ‡

	72	**73**	**75**
2	164.4 *s*	164.3	164.4
3	121.3 *d*	121.3	121.3
4	145.3 *d*	145.2	145.2
5	29.3 *t*	29.3	29.3
6	75.5 *d*	74.5	74.6
1'	41.8 *t*	40.7	42.4
2'	68.1 *d*	65.0	64.9[a]
3'	42.7 *t*	36.7	38.2
4'	70.4 *d*	70.1	65.0[a]
5'	38.9 *t*	44.7	22.1
6'	65.0 *d*	67.7	----
7'	22.0 *q*	23.4	----
8'	98.7 *s*	98.7	98.5
9'	30.2 *q*	30.1	30.2
10'	20.0 *q*	20.0	19.9

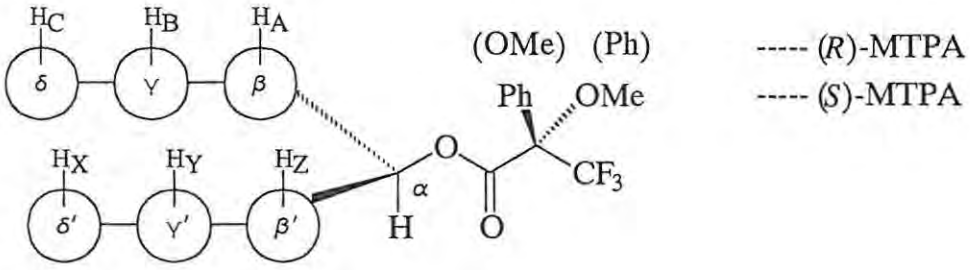
‡ 100 MHz, $CDCl_3$

[a] assignments may be interchanged

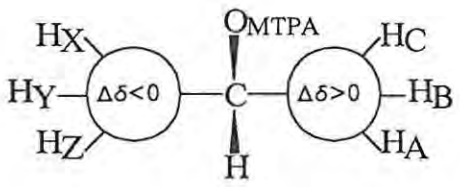
The *syn* stereochemistry of H-4' and H-6' in **72** was confirmed by NOE difference experiments. Irradiation of H-6' (δ 3.95 ppm) gave significant enhancement of the axial acetonide C-10' methyl signal (δ 1.45 ppm). The expected enhancement of H-4' (δ 4.15 ppm) was not observed because the chemical shifts of H-4' and H-6' were too close and both signals collapsed on irradiation of H-6'. Irradiation of the equatorial C-9' methyl signal (δ 1.35 ppm) gave no enhancements but irradiation of the axial C-10' methyl signal resulted in enhancements

of H-4', H-6' and of one of the H-5' (δ 1.25 ppm) signals. NOE experiments were not run for compound **73** because the overlapping proton signals prevented adequate interpretation of NOE data.

Mosher's method for predicting the absolute configuration of secondary alcohols requires the preparation of diastereomers of the compound containing a chiral secondary alcohol.[53] The derivatising agent most commonly used is 2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA). It has been predicted that the most stable conformation of the molecule in solution is one in which the carbinyl proton, the ester carbonyl and the trifluoromethyl groups of the MTPA moiety lie in the same plane and *cis* to one another (Figure 5a). In this conformation, the protons of the alkyl chain on the side of the phenyl substituent will be shielded due to the diamagnetic effect of the benzene ring. Conversely, the protons on the other alkyl substituent



a.



b.

Figure 5 : (a) The conformation of the (R)- and the (S)-MTPA esters of a secondary alcohol.
(b) The model used to determine the absolute configurations of secondary alcohols.

are deshielded by the methoxy group. When this method was first suggested in the early 1970's, only 60-100 MHz NMR instruments were readily available giving poorly resolved proton spectra from which proton shifts were not easily assigned. Use was thus made of ^{19}F-NMR and lanthanide shift reagents. The steric repulsion of the β and β' groups results in a chemical shift difference of the CF_3 (^{19}F) or OMe (^{1}H) groups. The assignment of configuration was thus determined by only two data points. The use of high field FT NMR including two dimensional techniques allows one to assign the chemical shifts of many if not all the protons in the molecule and thus provides many more data points. In the modified Mosher's method the difference in chemical shift ($\Delta\delta = \delta_S - \delta_R$) for each proton in the (R)- and (S)-MTPA esters can be calculated. Assuming the molecule adopts the conformation illustrated in Figure 5a, protons A, B and C will be upfield in the (R)-MTPA ester relative to those in the (S)-MTPA ester. The opposite is true for the protons on the left of the MTPA plane. The $\Delta\delta$ value for the protons on the left is thus negative and for those on the right positive (Figure 5b). Having measured the $\Delta\delta$ values for the protons in a molecule, it is thus possible to determine the stereochemistry of the chiral secondary alcohol. Ohtani *et al* reported that the absolute values for $\Delta\delta$ tend to be proportional to the distance of the proton from the MTPA moiety.[53]

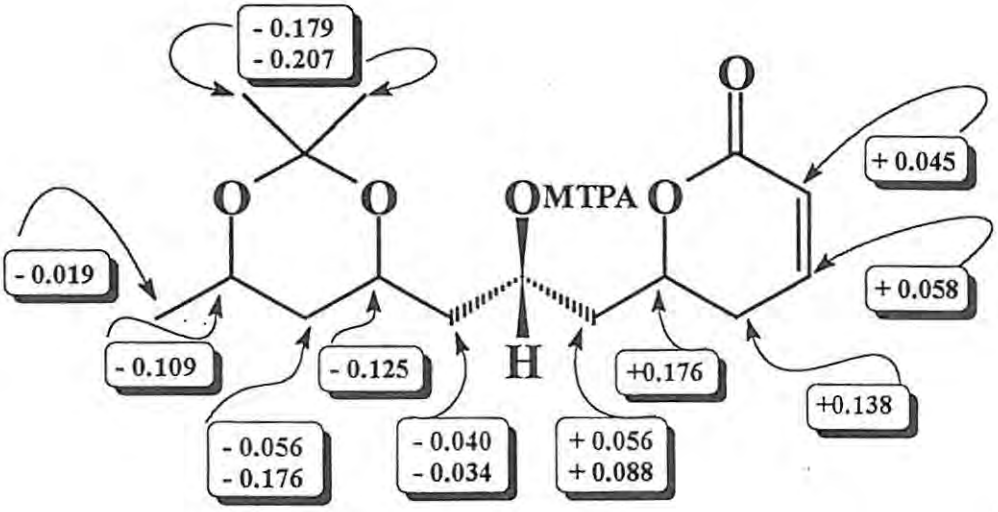


Figure 6 : The $\Delta\delta$ values for the MTPA esters of compound **72**

In order to apply the modified Mosher's method for determining the absolute stereochemistry of chiral secondary alcohols a minimum of approximately 10 mg of the compound is required and it was therefore necessary to prepare more of acetonides **72** and **73**. After further

saponification, acetonide formation and chromatography, the (R)- and the (S)-MTPA esters were prepared from **72** and **73**. The proton signals of each MTPA ester were assigned using COSY experiments and the $\Delta\delta$ values for each pair of diastereomers were calculated as shown in Figures 6 and 7. The positive and negative $\Delta\delta$ values observed for the signals of the protons in the left and right segments clearly indicated a (2'R, 6'S)-stereochemistry in **14**. The absolute $\Delta\delta$ values were not consistently proportional to the distance from the MTPA group. An explanation for this observation lies in the folding of the molecule to bring protons e.g. H-9' and H-10' in the MTPA esters of **72**, into the cone of sheilding of the phenyl group. The (S)-absolute configuration of the remaining chiral centre at C-4' followed from the *syn*-diol relationships of the two acetal oxygen atoms in **72** and **73**. The structure of the triacetate **14** was accordingly established as 6R-[2R,4S,6S-(triacetyloxy)-heptyl]-5,6-dihydro-2H-pyran-2-one (**74**).†

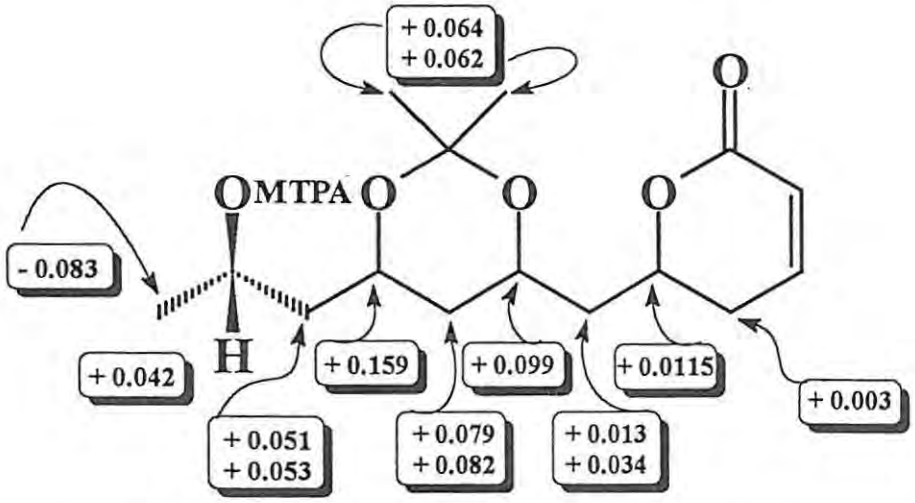
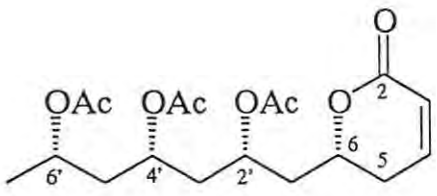


Figure 7 : The $\Delta\delta$ values for the MTPA esters of compound **73**



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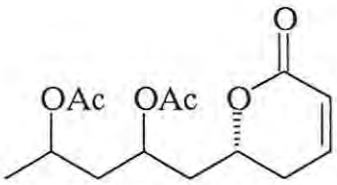
† The assignment of a 2'R configuration is not immediately apparent and careful application of the Cahn Ingold Prelog sequence rules is required.

The acetonide (**75**) prepared from the diacetate **15** also posessed a chair conformation with the one methyl group axial (δ 19.9) and the other equatorial (δ 30.2). Accordingly the acetoxy groups in **15** are *syn*. Since it is not possible to correlate the stereochemistry of the C-2' methine hydrogen with that at C-6 by NMR due to the intervening freely rotating C-1' methylene group, the absolute stereochemistry at C-2' and C-4' remains unassigned. However, because of the proven stereochemistry at the corresponding asymmetric centres in the heptyl side chain of the triacetate **74**, it follows from biosynthetic arguments that the diacetate **15** probably possesses a 2'S, 4'S configuration. A Cahn Ingold Prelog order priority reversal results in a reversal of the C-2 stereochemical assignment. The structure of the diacetate is thus proposed as 6R-[2S,4S-(diacetyloxy)-pentyl]-5,6-dihydro-2H-pyran-2-one (**76**).



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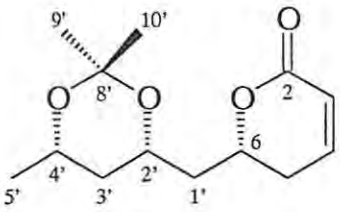

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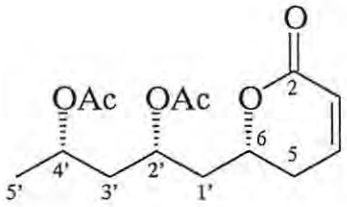

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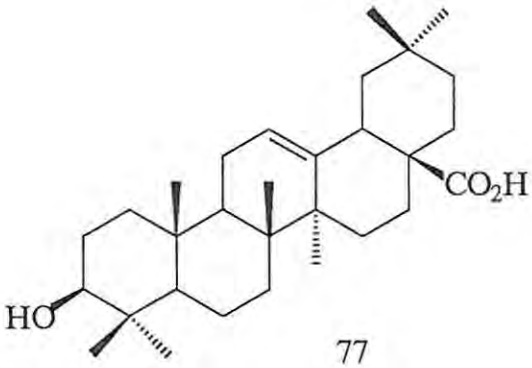
1.3 *SYNCOLOSTEMON DENSIFLORUS*

1.3.1 Background

Syncolostemon, a South African genus comprising nine species which belongs to the family Lamiaceae,[9] has proved to be a rich source of α-pyrone compounds.[54] *S. densiflorus* occurs in semi-coastal grasslands and forest margins from the North Eastern Cape to Northern Kwazulu-Natal.[55] Initial investigations into the acetone extract of the aerial parts of the plant yielded oleanolic acid (**77**, 1.6 % dry wt.) as the major natural product. The tetramethyl ether (**78**, 0.1 % dry wt.) of the polyphenolic compound quercetin (**79**) was also isolated from the acetone extract.



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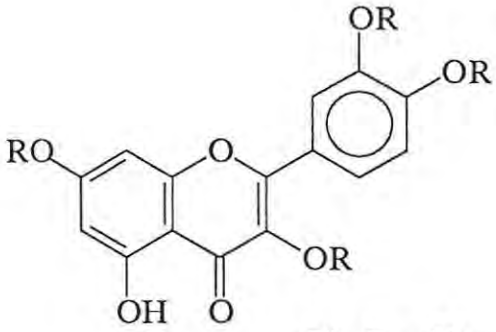
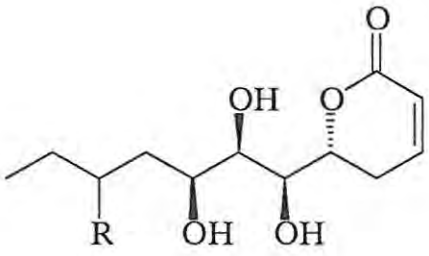


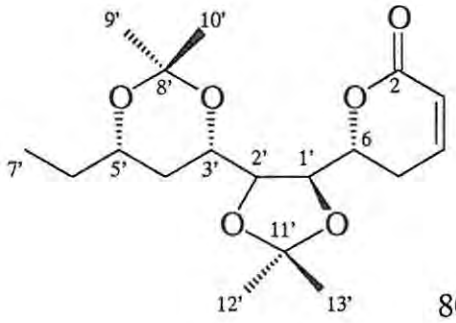
78 R = Me
79 R = H



10 R = OAc
11 R = H

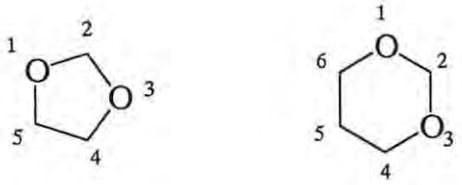
It was only a re-examination of the same extract nearly ten years later that yielded 5,6-dihydro-α-pyrone compounds. Syndenolide (**10**, 0.02 % dry wt.) and the known compound deacetylboronolide (**11**, 0.004 % dry wt.) were isolated by flash chromatography of polar (MeOH-EtOAc) column chromatography fractions of the acetone extract.[9] CD and NMR techniques were used to assign the structure and stereochemistry of **10**, with the stereochemistry at the 1', 2' and 3' carbons following from the biosynthetic relationship with **11** and supported by decoupling experiments. In the following discussion the determination of the configuration at C-5' is described.

1.3.2 Results and Discussion



80

The unknown stereochemistry at C-5' in syndenolide followed from a detailed NMR analysis of its diacetonide (**80**). An acetone solution of saponified syndenolide was filtered through a column of Amberlyst-15 resin[52] to give a mixture of products which were separated by HPLC. The molecular formula of the main product **80** was established as $C_{18}H_{28}O_6$ from HREIMS data (*m/z* 340.1873 Δmmu -13).



a. 110 ppm b. 100 ppm

Figure 8 : The ^{13}C chemical shifts of C-2 in 1,3-dioxolane rings

The ^{1}H and ^{13}C chemical shifts of **80**, presented in Table 4, were assigned using a combination of COSY, HMQC and HMBC NMR experiments. The ^{13}C and DEPT-135 NMR spectra of compound **80** showed that the compound contained three quaternary carbon atoms corresponding to two acetonide rings (δ 98.8, δ 110.6) and a carbonyl carbon (δ 162.9) in the α-pyrone ring. Since the ^{13}C chemical shifts of C-2 in five membered dioxolane rings are typically near 110 ppm (Figure 8a) while the C-2 in six membered 1,3-dioxolane rings resonates near 100 ppm (Figure 8b),† it is probable that compound **80** contains both a five membered acetonide ring bridging C-1' and C-2' and a six membered acetonide ring bridging C-3' and C-5'. This is the only possible arrangement of a five and a six membered diacetonide

† Personal communications with Prof. H. Parolis

in this molecule and the preparation of a five and a seven membered diacetonide is improbable. A DEPT-135 experiment indicated the presence of four methyl groups resonating at δ 30.0, 27.7, 26.7 and 19.8 ppm. The resonances at δ 30.0 and 19.8 are characteristic of a six membered acetonide ring in the chair conformation with one methyl equatorial (δ 30.0) and one axial (δ 19.8).[50, 51] The resonances at δ 27.7 and 26.7 ppm are atypical of six membered acetonide rings. The above observations are best explained by the presence of a six membered acetonide ring prepared from a *syn* 1,3-diol as well as a puckered five membered acetonide ring with magnetically equivalent methyl groups. Evidence for this assignment was provided by the HMBC NMR spectrum of the diacetonide (Figure 9). Correlations between the H_3-9' and H_3-10' methyl protons and the quarternary carbon C-8' belonging to the six membered acetonide ring established that the C-3' and C-5' hydroxy groups in saponified **10** are *syn*. Correlations were also observed between the acetonide methyl H_3-12' and H_3-13' protons and the quaternary acetonide carbon C-11' belonging to the five membered ring which is consistent with the 1',2' *anti* relative stereochemistry predicted for **10** by Davies-Coleman and Rivett.[9]

Table 4 : NMR chemical shifts for compound **80**

	δ ^{13}C †	δ ^{1}H ‡	HMBC to C
2	162.9 *s*	----	
3	121.3 *d*	6.02 *dt* (9.8, 1.7 Hz)	
4	144.9 *d*	6.90 *m*	6[a]
5	26.0 *t*	2.57 *m*	3, 4, 6
6	78.8 *d*	4.42 *dt* (7.3, 7.3 Hz)	4, 1', 2'[a]
1'	75.9 *d*	4.28 *dd* (7.2, 6.6 Hz)	6, 2'[a], 3'
2'	81.7 *d*	4.00 *m*	6, 3'
3'	68.5 *d*	4.00 *m*	
4'	32.2 *t*	1.5 *m*	2'[a], 3', 5'
5'	70.3 *d*	3.72 *m*	
6'	29.2 *t*	1.5 *m*	4'[a], 5'[a], 7'[a]
7'	9.4 *q*	0.90 *t* (7.5 Hz)	5', 6'
8'	98.8 *s*	----	
9'	30.0 *q*	1.38 *s*	8', 10'
10'	19.8 *q*	1.41 *s*	8', 9'
11'	110.6 *s*	----	
12'	26.7 *q*	1.43 *s*	11', 13'
13'	27.7 *q*	1.40 *s*	11', 12'

† 100 MHz, $CDCl_3$
‡ 400 MHz, $CDCl_3$
[a] very weak coupling

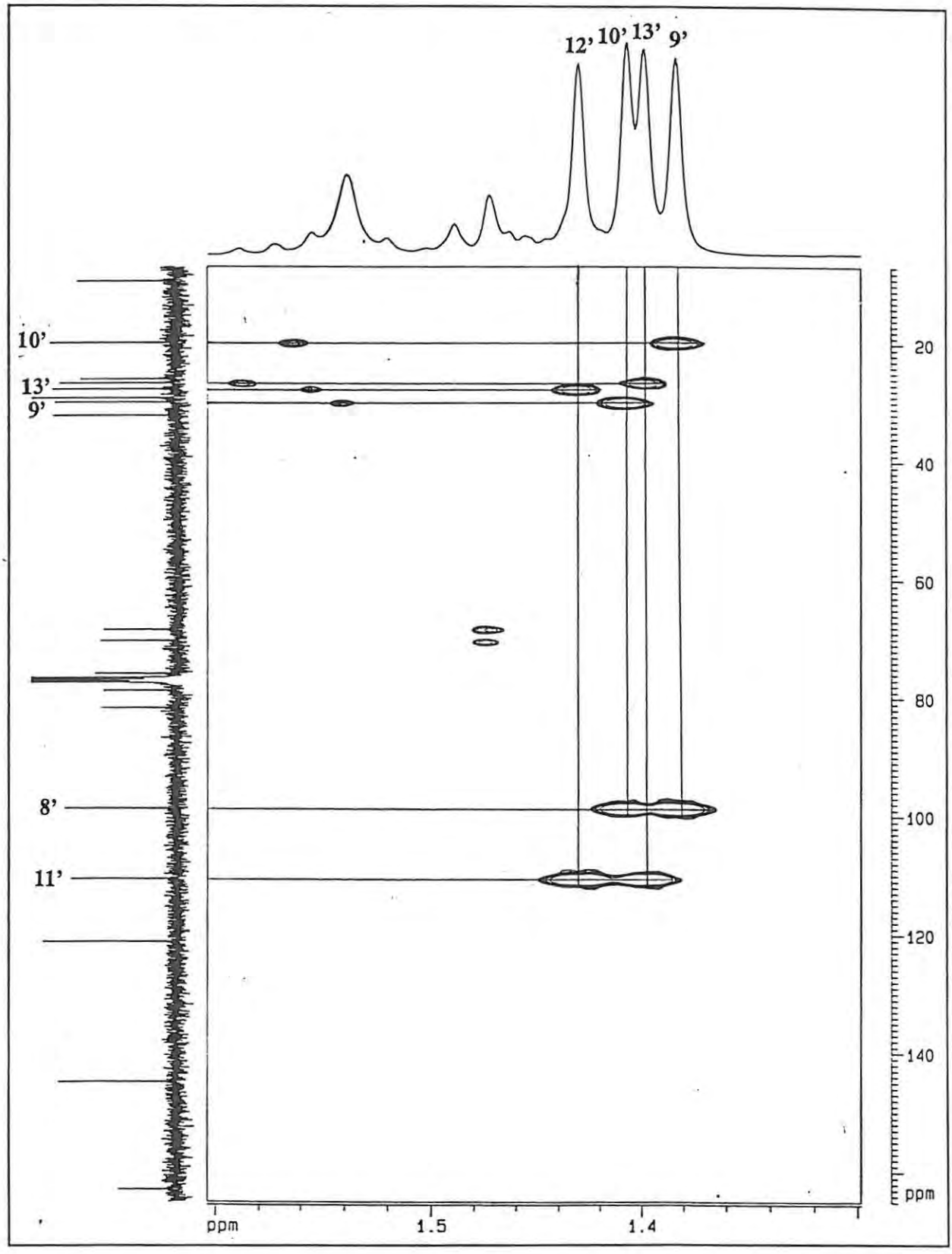
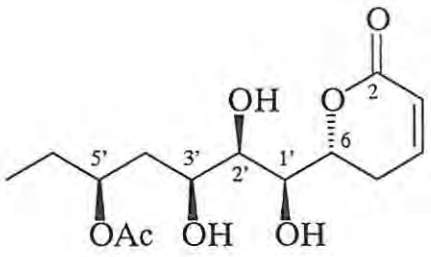


Figure 9 : A section of the HMBC NMR (400 MHz, $CDCl_3$) spectrum of compound **80**

Further support for the predicted relative stereochemistry of **80** was provided by a series of 1D-NOE difference experiments. These experiments also supported the predicted relative stereochemistry of **80**. Irradiation of H-5' resulted in an enhancement of the axial C-10' methyl signal and of the overlapping H-2' and H-3' signal which is attributed to the enhancement of H-3'. This proves that there is a 1,3-dioxolane ring bridging C-3' and C-5' is in a chair conformation with the H-3' and H-5' protons on the same side of the ring and axial. Irradiation of H-1' resulted in enhancements of the C-5 methylene protons, the overlapping H-2' and H-3' signal and a weak enhancement of the H-12' methyl signal belonging to the *anti* acetonide ring. Once again, the enhancement of the overlapping H-2', H-3' signal is ascribed to the enhancement of H-3' and not H-2' in accordance with the predicted stereochemistry. Irradiation of both H-2' and H-3' resulted in strong enhancement of H-6 as well as weak enhancement of the C-5 methylene signal due to the irradiation of H-2' and enhancements of the C-10' axial methyl protons, H-1' and the H-5' methine proton signal owing to the irradiation of H-3'. These results supported the stereochemistry that had previously been assigned to C-6, C-1', C-2' and C-3' and confirmed that the 1,3-dioxolane ring was in a chair conformation. Hence the 3',5' and 1',2'-diols in saponified **10** are respectively in *syn*- and *anti*-arrangements. Syndenolide is therefore 6R-[5S-(acetoxy)-1R,2R,3S-(trihydroxy)-heptyl]-5,6-dihydro-2H-pyran-2-one (**81**).



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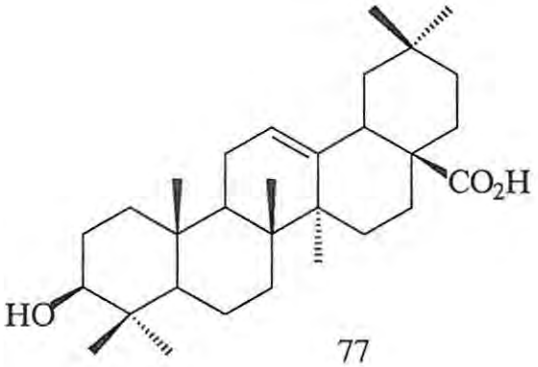
1.4 *SYNCOLOSTEMON ARGENTEUS*

1.4.1 Background

Syncolostemon argenteus is a herb or soft shrub which can grow to a height of 1.3 m and is commonly found in the Kwazulu-Natal midlands and semi-coastal areas occurring in dense grasslands and adjoining forests at altitudes of 300 to 1000 m.[56] The chemistry of this species has not previously been investigated and since the genus *Syncolostemon* has proved to be a rich source of α-pyrone compounds,[9, 11] the chemistry of *S. argenteus* was examined as part of an ongoing search for new 5,6-dihydro-α-pyrone compounds.

1.4.2 Results and Discussion

Isolation of compounds



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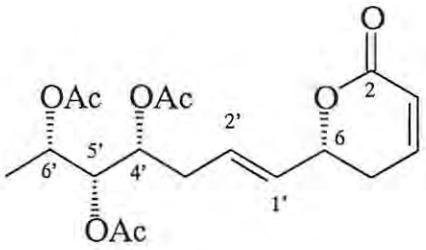
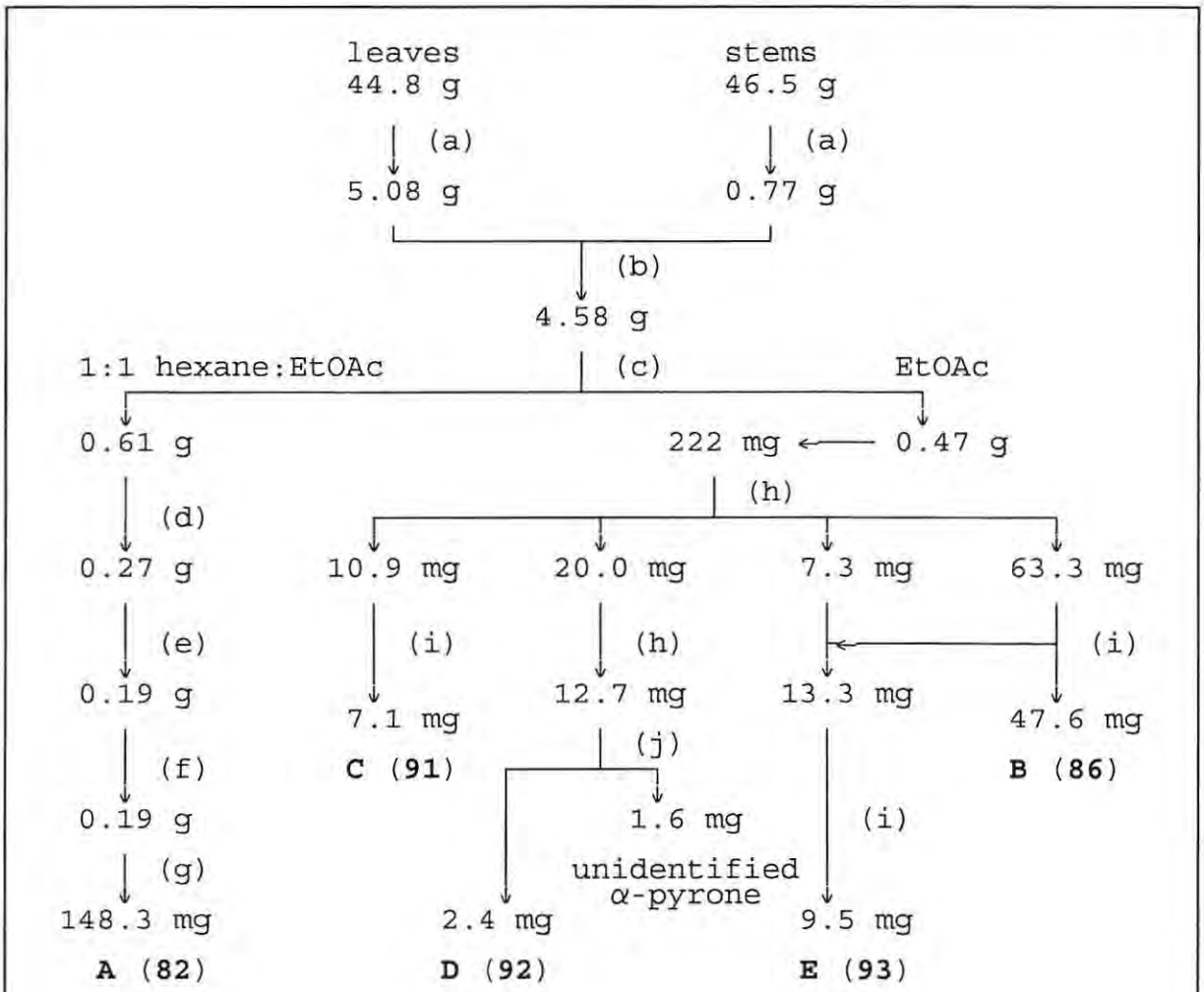


82

The decolourised acetone extracts of the dried stems and leaves of *S. argenteus* (initially separated in the field) were combined as they were shown to contain the same compounds by thin layer chromatography. Unwanted EtOAc insoluble material, largely consisting of oleanolic acid (**77**) was then removed firstly by filtration and secondly by column chromatography. Characteristic α-pyrone proton resonances near δ 6.9 and δ 6.1 ppm were present in the ^{1}H NMR spectra of two of the fractions from this latter chromatography. The less polar of these two fractions was further purified by flash chromatography, filtration to remove $CHCl_3$ insoluble material, and HPLC to yield the α-pyrone compound synargentolide A (**82**, 0.16 % dry wt.). The isolation of **82** has been summarised in Scheme 1.



(a) Soxhlet extraction with acetone (300 ml), leaves decolourised; (b) boiled in acetone (100 ml), cooled and filtered to remove insoluble material; (c) normal phase column chromatography (2:1 hexane/EtOAc, 1:1 hexane/EtOAc and EtOAc); (d) filtered to remove EtOAc insoluble material; (e) normal phase flash chromatography (1:1 hexane/EtOAc); (f) heated in $CHCl_3$ and filtered to remove insoluble material; (g) normal phase HPLC (2:3 hexane/EtOAc); (h) normal phase HPLC (1:4 hexane/EtOAc); (i) normal phase HPLC (EtOAc); (j) reverse phase HPLC (^{18}C, 1:1 MeOH/H_2O).

Scheme 1 : The isolation of 5,6-dihydro-α-pyrone compounds from *Syncolostemon argenteus*

Structural elucidation of synargentolide A

The molecular formula of **82** was established as $C_{18}H_{24}O_8$ by HREIMS data (*m/z* 368.1464 Δmmu +5). From the formula, it was deduced that the compound had seven degrees of unsaturation. The IR data are consistent with the presence of an α,β-unsaturated δ-lactone (ν_{max} 1735 cm^{-1}) and a *trans* disubstituted double bond (ν_{max} 940 cm^{-1}).

Although ^{13}C NMR only accounted for seventeen of the eighteen carbons, it was clear from an HMQC experiment (Figure 10) that an oxymethine carbon signal was concealed beneath the $CDCl_3$ signal in the ^{13}C NMR spectrum. From the chemical shifts of the resonances in the ^{13}C NMR spectrum it was apparent that **82** contained four carbonyl carbons (δ 163.8, 170.0, 170.1 and 170.2) arising from three acetate moieties and the carbonyl carbon of the 5,6-dihydro-α-pyrone ring, and four vinylic carbons (δ 121.6, 128.3, 130.9, 144.5) from an endocyclic and an exocyclic double bond. These data were supported by the ^{1}H NMR spectrum which revealed resonances in accordance with the α and β protons of the α-pyrone ring (δ 6.01, 6.84), two further vinylic protons (δ 5.64, 5.73), four oxymethine protons (δ 4.86, 4.96, 5.08, 5.15) and three acetate methyl singlets (δ 1.99, 2.02, 2.12). These data accounted for all seven degrees of unsaturation implied by the molecular formula. The contiguous coupling sequence from H-3 to H-7' in the COSY spectrum provided evidence for the positions of the exocyclic double bond and the acetate functionalities and made the assignment of the ^{1}H chemical shifts (Table 5) possible. The ^{13}C chemical shifts (Table 6) were assigned using a combination of HMQC and HMBC NMR experiments.

Stereochemical investigations of synargentolide A

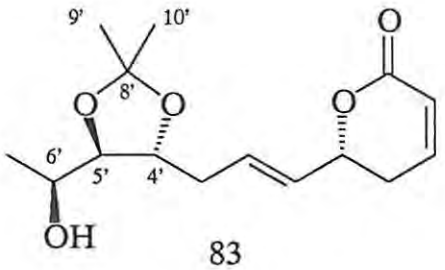
The E configuration of the exocyclic double bond in **82** was established using spin decoupling NMR experiments to determine the vinylic proton coupling constants. Irradiation of the 3' methylene protons reduced the signal for H-2' from a double triplet, which overlapped with the signal for H-1', to a doublet with a $J_{1',2'}$ coupling constant of 15.7 Hz. This coupling is indicative of a *trans* disubstituted double bond[21] (a *cis* double bond requires J = 9-11 Hz) and is consistent with the IR data.

Synargentolide A has four chiral centres. A positive $n\rightarrow\pi^*$ Cotton effect at λ_{max} 265 nm in the CD spectrum confirmed the expected 6(R) stereochemistry for the α-pyrone ring. The configurations of the remaining 4', 5' and 6' asymmetric carbons were established as follows: Compound **82** was saponified and the acetonides prepared by filtering an acetone solution of saponified **82** through a column of Amberlyst-15 resin. The acetonides, obtained in poor yield, were separated by HPLC to yield compound (**83**) as the major product (27 % yield). Paradoxically, the only other dioxolane derivative prepared was compound (**84**, 2 % yield) which possibly formed from the reaction of saponified **82** with an acetaldehyde impurity in the

Table 5 : ^{1}H Chemical shifts for 5,6-dihydro-α-pyrones isolated from *S. argenteus*

	82[†]	**86**[‡]	**91**[‡]	**92**[‡]	**93**[§]
3	6.01 *dt*	5.97 *m*	6.21 *d*	5.98 *d*	6.20 *d*
	9.8, 1.7 Hz	9.7 Hz	9.7 Hz	10.5 Hz	9.6 Hz
4	6.84 *m*	7.06 *m*	7.17 *dd*	6.76 *dd*	7.09 *dd*
			9.7, 6.0 Hz	10.1, 3.5 Hz	9.6, 6.0 Hz
5	2.39 *m*	2.56 *m*	5.39 *dd*	4.68 *dd*	5.31 *m*
			6.0, 2.4 Hz	3.5, 8.0 Hz	
6	4.86 *m*	4.53 *td*	4.64 *dd*	5.14 *dd*	4.31 *dd*
		3.2, 6.5 Hz	2.4, 9.6 Hz	8.1, 4.8 Hz	2.4, 9.2 Hz
1'	5.64 *dd*	3.65 *m*	3.78 *d*	4.32 *dd*	4.22 *m*
	5.6, 15.7 Hz		9.5 Hz	4.8, 3.9 Hz	
2'	5.71 *m*	4.31 *m*	4.45 *d*	4.40 *dd*	5.87 *m*
			5.4 Hz	3.8, 6.5	
3'	2.28 *m*	5.94 *ddd*	5.99 *dd*	5.87 *dd*	5.87 *m*
		5.8, 15.7, 0.9 Hz	5.5, 15.7 Hz	6.5, 15.9 Hz	
4'	5.15 *m*	5.79 *ddd*	5.87 *ddd*	5.76 *dd*	5.30 *m*
		15.7, 6.8, 1.2 Hz	15.7, 6.8, 1.0 Hz	15.9, 6.4 Hz	
5'	5.08 *dd*	5.40 *dd*	5.45 *dd*	5.40 *dd*	3.58 *m*
	7.2, 4.0 Hz	6.8, 3.5 Hz	6.7, 3.4 Hz	6.4, 3.5 Hz	
6'	4.96 *m*	5.04 *m*	5.06 *dq*	5.03 *dq*	4.71 *m*
			3.5, 6.5 Hz	3.5, 6.6 Hz	
7'	1.18 *d*	1.20 *d*	1.22 *d*	1.19 *d*	1.17 *d*
	6.4 Hz	6.6 Hz	6.5 Hz	6.6 Hz	6.3 Hz
Ac-Me	1.99	2.05 *s*	2.01 *s*	2.05 *s*	2.04 *s*
Ac-Me	2.02	2.01 *s*	2.05 *s*	2.00 *s*	2.00 *s*
Ac-Me	2.12		2.06 *s*		1.95 *s*
1'-OH					5.56 *s*
5'-OH					5.30 *s*

[†] 400 MHz, $CDCl_3$; [‡] 400 MHz, CD_3OD; [§] 400 MHz, DMSO-d6



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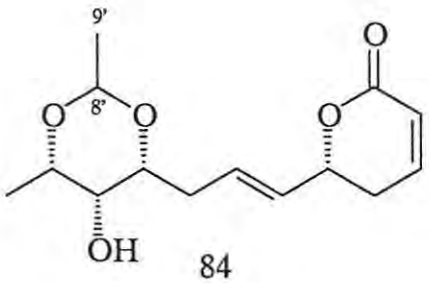

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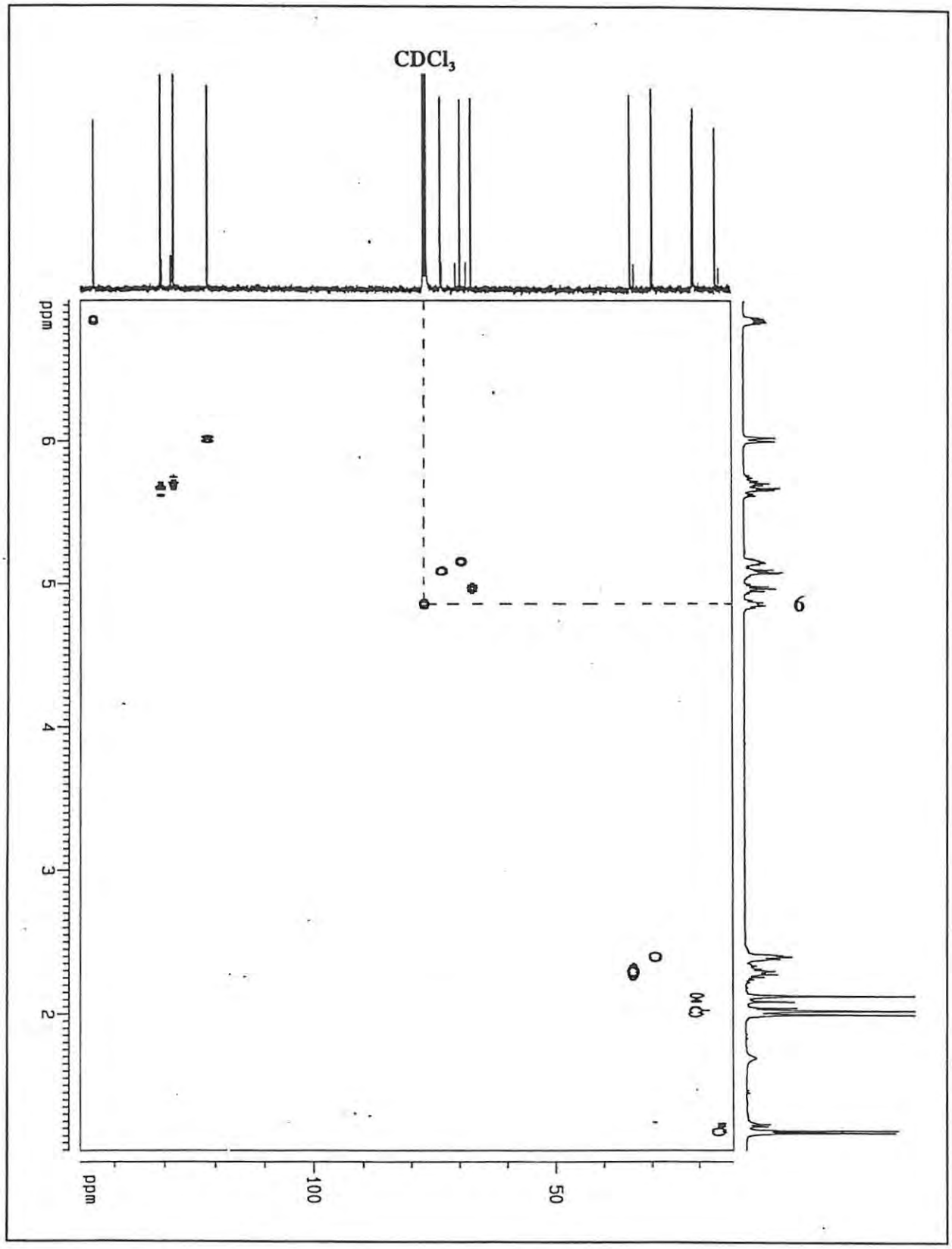


Figure 10 : HMQC NMR (400 MHz, $CDCl_3$) spectrum of **82**

acetone solvent. The ^{1}H NMR spectrum of **84** clearly showed an additional oxymethine proton signal (δ 4.73) when compared with the ^{1}H NMR spectrum of **83**. The COSY coupling between this proton quartet and the methyl doublet at (δ 1.31) in addition to the HMQC correlation with the deshielded bridging carbon C-8' corroborated the structure proposed for this compound.

Table 6 : ^{13}C Chemical shifts for 5,6-dihydro-α-pyrones isolated from *S. argenteus*

	82†	**86**‡	**91**‡	**92**‡	**93**§
2	163.8	166.3	164.5	164.0	162.1
3	121.6	121.2	125.5	121.8	124.3
4	144.5	148.6	142.9	144.1	141.1
5	29.4	26.4	62.9	68.5	60.7
6	$CDCl_3$	79.0	78.5	81.1	79.9
1'	130.9	75.8	71.5	74.6	66.9
2'	128.3	71.5	70.5	80.2	133.0
3'	34.0	136.6	136.8	131.9	127.2
4'	69.7	126.7	126.6	128.9	72.9
5'	73.8	76.3	76.3	76.0	73.1
6'	67.4	72.2	72.3	72.0	69.3
7'	16.0	15.2	15.1	15.1	15.8
Ac-CO	170.2 [a]	172.2	172.2 [c]	172.2 [e]	169.4 [g]
Ac-CO	170.1 [a]	171.8	172.0 [c]	171.8 [e]	169.3 [g]
Ac-CO	170.0 [a]		171.5 [c]		169.3 [g]
Ac-Me	21.0 [b]	21.0	21.1 [d]	21.0 [f]	20.8 [h]
Ac-Me	20.9 [b]	21.1	21.0 [d]	20.9 [f]	20.7 [h]
Ac-Me	20.8 [b]		20.5 [d]		20.3 [h]

† 100 MHz, $CDCl_3$; ‡ 100 MHz, CD_3OD; § 100 MHz, DMSO-d6

Resonances with like superscripts may be interchanged

Although the chemical shift of the C-8' quaternary carbon (δ 108.6) belonging to the acetonide **83** demonstrated the presence of a five membered acetonide ring,† no HMBC two or three bond H-C correlations could be observed to define the position of the ring which could be placed either at C-4'-C-5' or at C-5'-C-6'. Unfortunately the position of the hydroxyl substituent could also not be determined from the chemical shifts of the 4', 5' and 6' oxymethine proton signals. However, on formation of the MTPA esters of **83** the downfield shift of the H-6'

† Personal communications with Prof. H. Parolis

multiplet made it clear that the C-6' hydroxyl had been esterified and the acetonide ring thus bridged C-4' and C-5'. The chemical shifts of the H-4' and H-5' proton signals in **83** were insufficiently separated for successful 1D NOE difference experiments, accordingly a Rotating frame nuclear Överhauser and Exchange SpectroscopY (ROESY) NMR experiment was used to investigate the relative stereochemistry of these protons.[57] A ROESY experiment is a phase sensitive two dimensional NMR experiment in which proton to proton NOE correlations appear as positive cross peaks while diagonal peaks have a negative phase. Although generally suppressed, "through bond" COSY type coupling is sometimes observed in the ROESY spectrum and such coupling appears as a mixed positive and negative cross peak.† Only through bond coupling and no clearly defined NOE correlations were observed between H-4' and H-5' in the ROESY spectrum of acetonide **83** which tentatively suggested an *anti* stereochemistry for the acetonide ring. However, careful examination of the ROESY NMR spectrum of the (S)-MTPA ester of **83** revealed NOE correlations between H-4' and one of the acetonide methyl groups and between H-5' and the other acetonide methyl group thus providing further support for the proposed *anti*-relative stereochemistry of the acetonide ring.

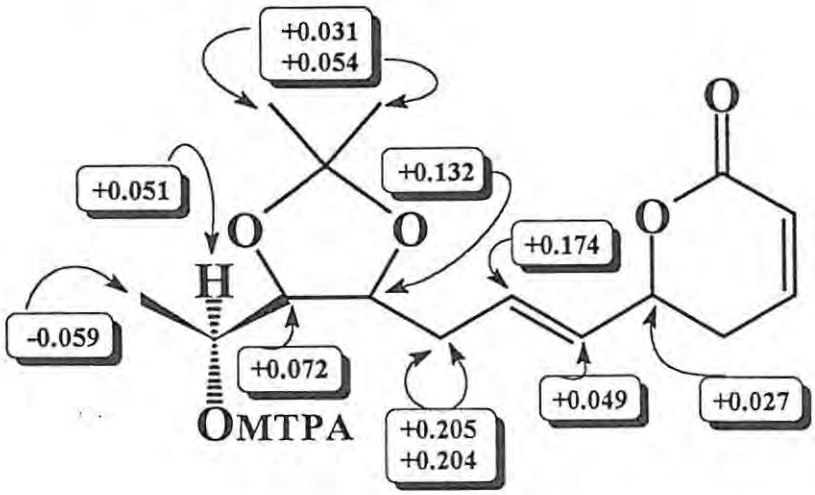


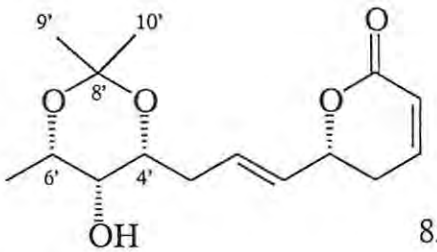
Figure 11 : The $\Delta\delta$ values for the MTPA esters of **83**

Having positioned the acetonide ring, the absolute stereochemistry of the 6' secondary hydroxyl group in **83** was determined using Mosher's method by conversion of **83** to the corresponding (R)- and (S)-MTPA esters[58] and application of the MTPA determination rule.[53] The positive and negative $\Delta\delta_H$-values observed for the signals of the protons in the left and right segments

† Personal communications with Dr David Reid, Smithkline Beecham UK

(Figure 11) indicated a 6'(S)-stereochemistry in **83** and hence in **82**. This absolute configuration is consistent with the observation made by Davies-Coleman and Rivett[11] that all 6-substituted 5,6-dihydro-α-pyrones isolated thus far from the Lamiaceae possess a 6'(S) stereochemistry.

In order to assign the absolute stereochemistry of C-4' and C-5', it was necessary to relate their stereochemistry to that of C-6'. This could be achieved through preparation of the six membered acetonide (**85**). The absence of **85** in the reaction products from the initial preparation of acetonide derivatives of **82** was puzzling and the acetonide preparation was accordingly repeated using fresh Amberlyst-15 resin from a new bottle. Once again two compounds were obtained including acetonide **83** and a very low yield (1 mg, 5.8 %) of the expected compound **85**. None of the artifact, **84**, was evident in the reaction products from the second acetonide preparation. The ^{13}C and ^{1}H chemical shifts of **85** were assigned using COSY and HMQC experiments. Unfortunately the signal of the quaternary carbon belonging to the α-pyrone carbonyl in the ^{13}C NMR spectrum was obscured by baseline noise due to the exceptionally low sample concentration. The *syn* stereochemistry of the 4',6' carbons in synargentolide A could be inferred from the ^{13}C chemical shifts of the two acetonide methyls (19.5 and 29.5 ppm) which revealed that the six membered dioxolane ring was in a chair conformation with one of these methyls axial and the other equatorial.[51] These data confirmed the structure of synargentolide A as 6R-[4R,5R,6S-triacetyloxy-1E-heptenyl]-5,6-dihydro-2H-pyran-2-one.



85

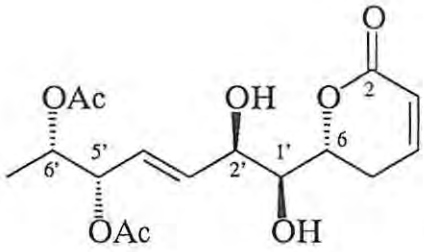
Further isolation of α-pyrones

An examination of the ^{1}H NMR spectrum of the other column chromatography fraction which contained the α,β unsaturated δ-lactone proton signals showed that this fraction contained a number of different α-pyrone compounds. Normal phase HPLC of a portion of this fraction

yielded four new α-pyrones which were insoluble in chloroform. These were separated and purified by repeated HPLC as depicted in Scheme 1.

Structure of synargentolide B

Synargentolide B (**86**), $[\alpha]_D^{25}$ = +45.6° (MeOH; c 1.2), was isolated in 0.11 % yield (dry wt.). The HREIMS data suggested a molecular formula of $C_{16}H_{22}O_8$ for this compound (*m/z* 342.1298 Δmmu -14). IR data clearly showed the presence of hydroxyl groups (ν_{max} 3450 cm^{-1}), a *trans* double bond (ν_{max} 950 cm^{-1}) and the carbonyl of an α-pyrone ring (ν_{max} 1735 cm^{-1}).



86

^{13}C NMR accounted for all sixteen carbons with resonances consistent with three carbonyls, two pairs of vinylic carbons, and five oxymethine carbons. The ^1H NMR data also indicated two pairs of vinylic protons with the H-3 and H-3' olefinic proton signals (δ 5.97, 5.94) overlapping and five oxymethine protons (δ 3.65, 4.31, 4.53, 5.05, 5.40). In addition, two methyl singlets (δ 2.01, 2.05) in the ^1H NMR spectrum clearly revealed that compound **86** contained two acetate moieties which accounted for two of the carbonyl carbons (δ 171.8, 172.2) and two of the oxymethine protons (δ 5.05, 5.40). The α-pyrone ring accounted for the third carbonyl carbon (δ 166.3), one of the pairs of double bond protons (δ 5.97, 7.06) and a third oxymethine proton (δ 4.53). It was thus concluded that the remaining two oxymethine protons were indicative of two hydroxyl substituents at these positions. However, no hydroxyl protons were observed in the ^1H spectrum probably due to deuterium exchange with the methanol-d4 NMR solvent. The ^1H and ^{13}C chemical shifts of **86** (Tables 5 and 6) were assigned using a combination of COSY (Figure 12), HMQC and HMBC (Figure 13) NMR experiments. ^1H chemical shifts were readily assigned from the contiguous coupling sequence in the COSY spectrum with no confusion arising from the overlapping H-3 and H-3' proton signals since H-3 only couples to H-4 and all other cross peaks could thus be attributed to H-3'. With the exception of the two acetate methyl resonances, the ^{13}C signals were sufficiently well resolved

for the assignment of ^{13}C chemical shifts from the ^{1}J H-C correlations in the HMQC spectrum. However, HMBC two and three bond H-C coupling from H-5 to C-3 and H-4' to C-3' were crucial in distinguishing between C-3 and C-3'. HMBC correlations from H-5' and H-6' were used to distinguish between the two acetate carbonyls.

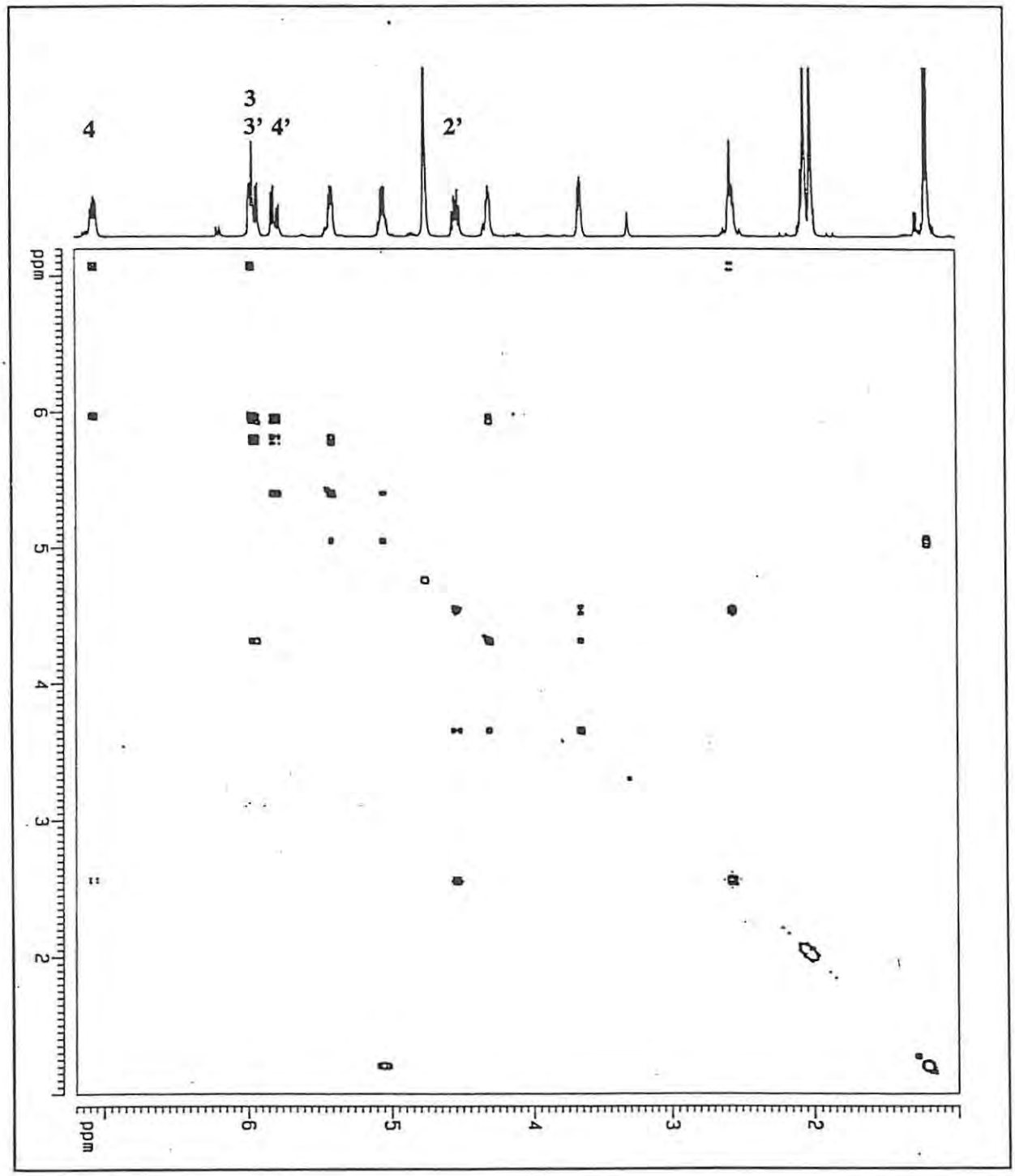


Figure 12 : COSY NMR (400 MHz, CD_3OD) spectrum of **86**

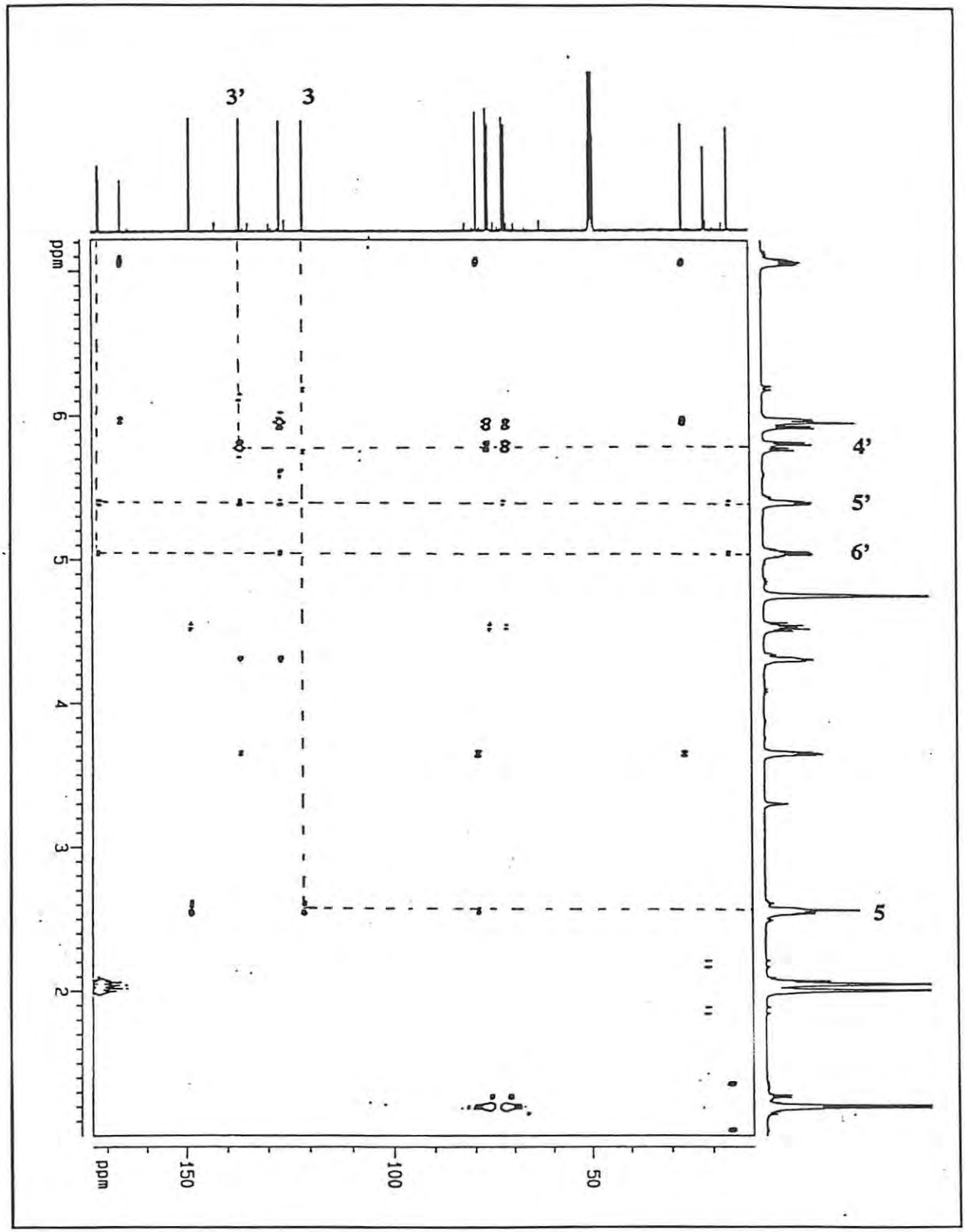
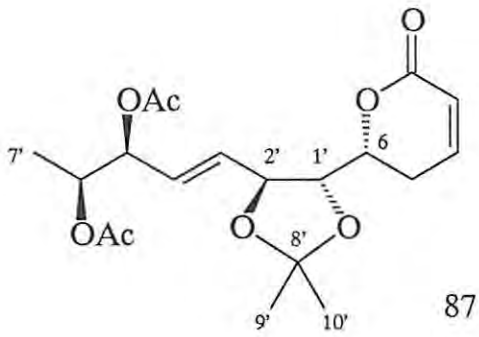


Figure 13 : HMBC NMR (400 MHz, CD_3OD) spectrum of 86

Stereochemistry of synargentolide B

The E stereochemistry of the exocyclic double bond in compound **86** could be deduced from the $J_{3',4'}$ coupling constant of 15.7 Hz. This value was obtained directly from the ^1H NMR spectrum where the H-4' signal was a well resolved double doublet with coupling constants of 15.7 Hz and 6.8 Hz. A measurement of the coupling constants of H-5' ($J_{4',5'}$ = 6.8; $J_{5',6'}$ = 3.5 Hz) confirmed that the large H-4' coupling was due to H-3'. This stereochemical assignment was supported by the IR data (ν_{max} 950 cm^{-1}).



87

Synargentolide B has five asymmetric carbons. Once again a 6(R) configuration was assigned from the positive sign of the $n\rightarrow\pi^*$ Cotton effect at λ_{max} 258 nm in the CD spectrum. The absolute stereochemistry at C-6' was tentatively assigned as (S) from biosynthetic arguments since the stereochemistry of the corresponding chiral centre in synargentolide A and other α-pyrones from the Lamiaceae has consistently been shown to be (S). The relative stereochemistry of the C-1', C-2' diol was investigated through the preparation of a C-1', C-2' five membered acetonide derivative (**87**). The acetonide was prepared in the usual manner by passing an acetone solution of the diol **86** through a column of Amberlyst-15 resin. The $J_{1',2'}$ coupling constant of 7.4 Hz which was measured by ^1H spin decoupling experiments suggested that H-1' and H-2' were at a dihedral angle of approximately 18° or 154° (calculated from the Karplus equations). The former small angle is unlikely since it requires the bulky alkyl substituents to adopt a highly strained nearly eclipsed diaxial conformation. The angle of 154°, however, implies that the 1', 2' protons have adopted a trans-diaxial arrangement which allows the alkyl substituents to adopt pseudo equatorial orientations. This trans-diaxial orientation was confirmed by 1D NOE difference experiments. Although the H-2' and H-6 signals were too close for meaningful irradiation of H-2', it was clear that irradiation of H-1' did not produce any enhancement of the H-2' signal, confirming that H-1' and H-2' are *anti* to each other.

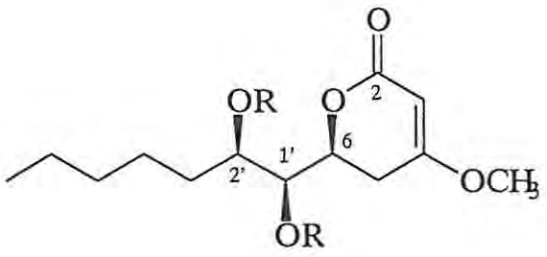
Similarly the ROESY spectrum did not display NOE correlations between H-1' and H-2', only through bond correlations between these protons were observed.

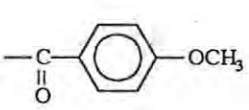
negative chirality
left handed screw

positive chirality
right handed screw

Figure 14 : Chiralities of dibenzoates

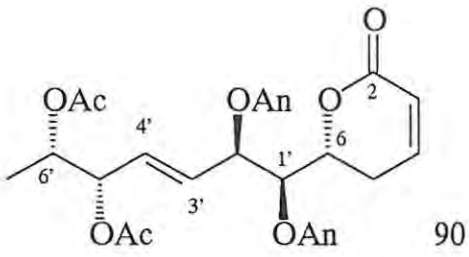
The exciton chirality method[30] is a CD technique which is used to investigate the absolute stereochemistry of dihydroxy systems, particularly vicinal diols, through examination of the CD spectra of their dibenzoate derivatives. Benzoate chromophores undergo a $\pi \rightarrow \pi^*$ charge transfer electronic transition with the electronic transition moment parallel to the alcoholic C-O bond. The dipole-dipole interaction of the transition moments in suitably orientated 1,2-dibenzoates gives rise to two strong Cotton effects equal in magnitude, but with opposite sign separated by Davydov splitting. The chirality of these dibenzoates, defined as positive or negative according to the sense of rotation of a right or left handed screw (Figure 14), reflects the chirality of the transition moment which is the same as the sign of the first Cotton effect.[30] Although this method which requires the benzoate moieties to be gauche to one another, works well for cyclic systems where the conformation of the molecule is fixed, it may sometimes be applied to acyclic systems where rotation is hindered. For example, the 1',2' stereochemistry of



88 R = H
89 R = (An) 

compound (**88**) was clearly indicated through a combination of the Davydov-split Cotton effects of the p-methoxy benzoate chromophore of the dianisate derivative (**89**) ($\Delta\epsilon$ −19.0 λ_{max} 250 nm; $\Delta\epsilon \approx$ +18.6 λ_{max} 243 nm) and an examination of the $J_{1',2'}$ coupling constant to determine

the conformation of the compound.[59] Sometimes the use of substituted benzoates increases the amplitude of the Cotton effect and since the split is observed at a different wavelenth, provides better peak separation when the compound contains other chiral chromophores.[30]



90

The dianisate of synargentolide B was prepared via reaction of **86** with anisoyl chloride in benzene with pyridine as a catalyst. The dianisate derivative (**90**), obtained in poor yield (6.4 %), was separated from the two monoanisate derivatives by HPLC. Having already determined the relative stereochemistry of C-1' and C-2', there were only two possible stereoisomers for this compound. The six rotational conformers with their respective predicted Davydov-split Cotton effects for the two stereoisomers are shown in Figure 15. Irradiation of H-6 in compound **90** reduced H-1' to a doublet with a coupling constant of 3.8 Hz, indicating that the gauche conformation was dominant. An average value for the coupling constant of *cis*-glycol protons in a gauche conformation is 4.0 Hz,[59] but the coupling constant is expected to be less as the protons move further apart due to the bulk of the other substituents. Irradiation of the H-3', H-4' overlapping olefinic proton signal reduced H-2' to a broad doublet with a coupling constant of 2.5 Hz, a value which is probably inaccurate due to the broad nature of the signal. The coupling constants were measured in $CDCl_3$ and CD_3OD and were found to be the same in both solvents indicating that the conformation of **90** initially determined by NMR ($CDCl_3$) is the same as the conformation adopted during CD analysis (methanol). Therefore with the options limited to the gauche conformations (b), (c), (e) and (f) in Figure 15, a positive or negative chirality Davydov-split would define the absolute stereochemistry. The CD curve shows $\Delta\epsilon$ +3.45 (278.0 nm), +1.42 (258.8 nm), +1.76 (236.8 nm), +2.05 (221.4 nm) and -2.79 (212.5 nm). The expected Davydov split Cotton effect at 250 and 243 nm was thus not observed so the preferred conformer must be (c) or (f) which unfortunately does not give any useful information because it does not distinguish between the two possible stereoisomers. Since compounds **89** and **90** are structurally the same near the anisate groups while their CD spectra are completely different, the stereochemistry at the three adjacent chiral centres must

differ and we tentatively conclude that while H-6, H-1' and H-2' are *syn* to one another in **89**, H-1' and H-2', while *syn* to each other, must be *anti* to H-6 in compound **90**.

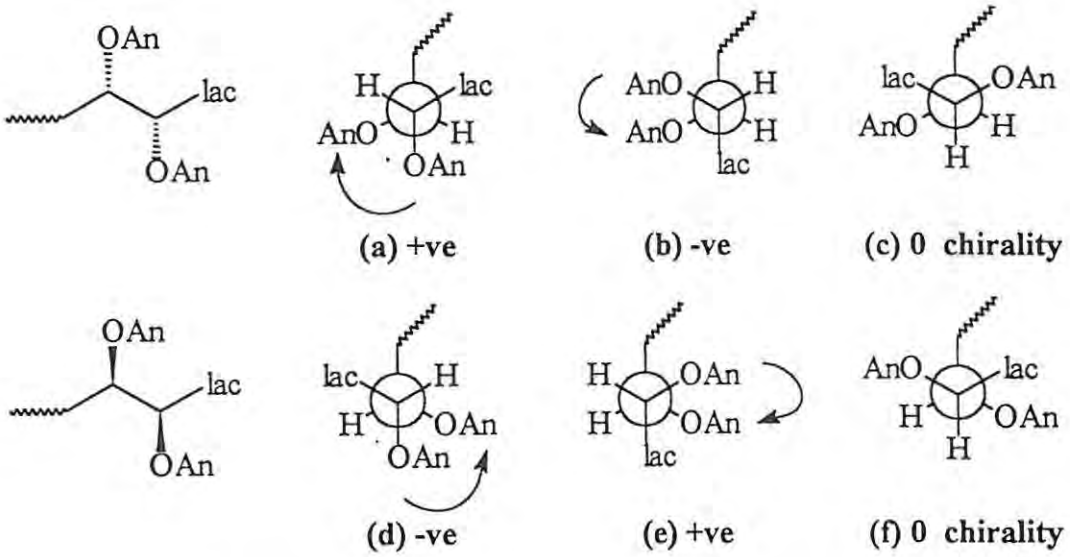
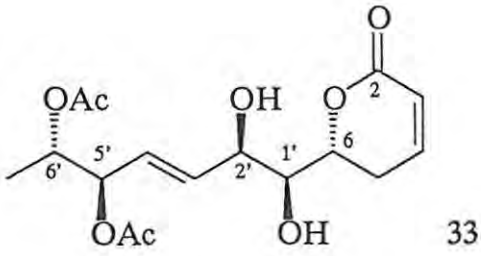


Figure 15 : The six rotational conformers for the two 1',2'-*syn*-stereoisomers of **90**

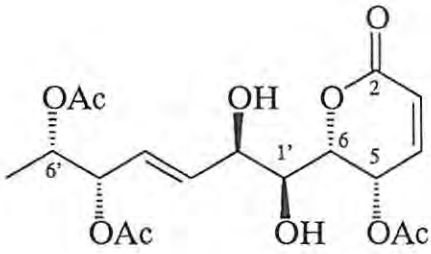
Compound **33** ($[\alpha]_D^{25}$ = +28.8° ($CHCl_3$; c 0.18)) has been isolated from another member of the family Lamiaceae, *Hyptis oblongifolia*.[12] Surprisingly, although not even a trace of compound **86** was observed in the $CDCl_3$ ^1H NMR spectrum, compound **33** is soluble in chloroform. The two compounds must be diastereomeric to account for their different physical properties. Preceeding investigations suggest that four of the five chiral centres in **86** have the same configurations as the corresponding carbons in **33**, the stereochemistry at C-5' in **86** is therefore probably (S) in contrast to the 5'(R) configuration in **33**. The 5'(S) assignment is in accordance with the chirality of the corresponding asymmetric carbon in synargentolide A. The structure of **86** is tentatively proposed as 6R-[5S,6S-diacetyloxy-1S,2R-dihydroxy-3E-heptenyl]-5,6-dihydro-2H-pyran-2-one.



33

Structure elucidation of synargentolide C

Synargentolide C (**91**), $[\alpha]_D^{25} = +140°$ (MeOH; c 0.72), was obtained as a yellow oil (0.016 % dry wt.). A molecular formula of $C_{18}H_{24}O_{10}$ was determined from the weak molecular ion in the HREI mass spectrum (*m/z* 400.1356 Δmmu -13). The IR spectrum including the hydroxyl absorptions (ν 3450 cm^{-1}), α-pyrone carbonyl (ν 1735 cm^{-1}), and a *trans* disubstituted double bond (ν 945 cm^{-1}), was comparable to the spectrum of synargentolide B suggesting that the structure must be very similar. All the proton signals in the ^1H NMR spectrum were well resolved and were assigned from the clear contiguous coupling sequence from H-3 to H-1' and from H-2' to H-7' in the COSY spectrum (Figure 16). A careful examination of the COSY spectrum also revealed the coupling between H-1' and H-2' (Figure 17). The ^1H NMR spectrum of **91** was analogous to the ^1H NMR spectrum of compound **86**, but contained an additional acetate methyl singlet. There were no characteristic endocyclic methylene proton signals and it was apparent from the chemical shift of H-5 that the α-pyrone ring contained a C-5 acetate substituent. This was confirmed by the appearance of a cross peak showing the long range coupling between H-5 and one of the acetate carbonyl carbon's in the HMBC spectrum (Figure 18). The ^1H and ^{13}C chemical shifts were all assigned from a combination of COSY, HMQC and HMBC experiments and are presented in Tables 5 and 6.



91

The stereochemistry of synargentolide C

A $J_{3',4'}$ coupling constant of 15.7 Hz confirmed the predicted E-stereochemistry of the exocyclic double bond. A positive $n \rightarrow \pi^*$ Cotton effect in the CD spectrum established a 6(R) stereochemistry for the 5,6-dihydro-α-pyrone ring. A paucity of material prevented a complete stereochemical investigation of the four acyclic chiral centres in **91** as described previously for synargentolides A and B. No precedents exist for the occurrence of diastereomeric mixtures of 5,6-dihydro-α-pyrones in individual Lamiaceae species. Based on this tenuous biosynthetic

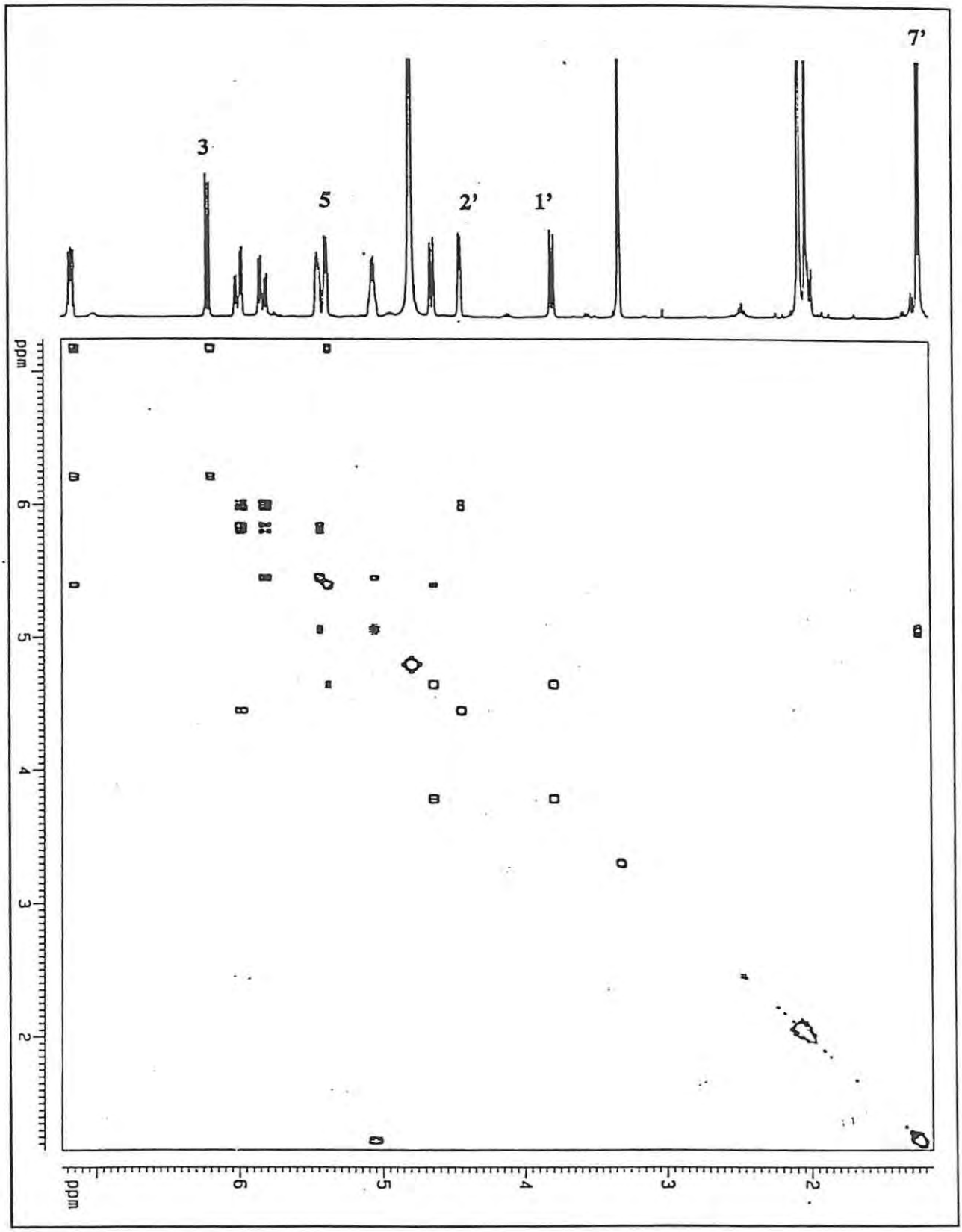


Figure 16 : COSY NMR (400 MHz, CD_3OD) spectrum of **91**

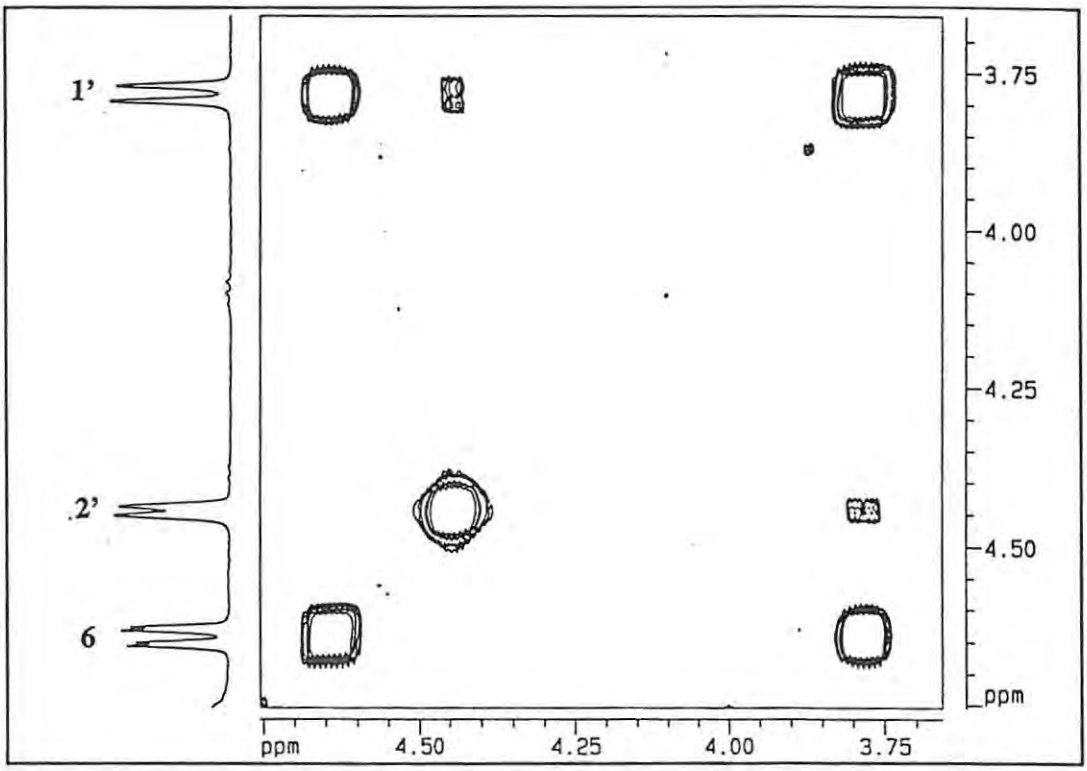


Figure 17 : A section of the COSY NMR (400 MHz, CD_3OD) spectrum of **91**

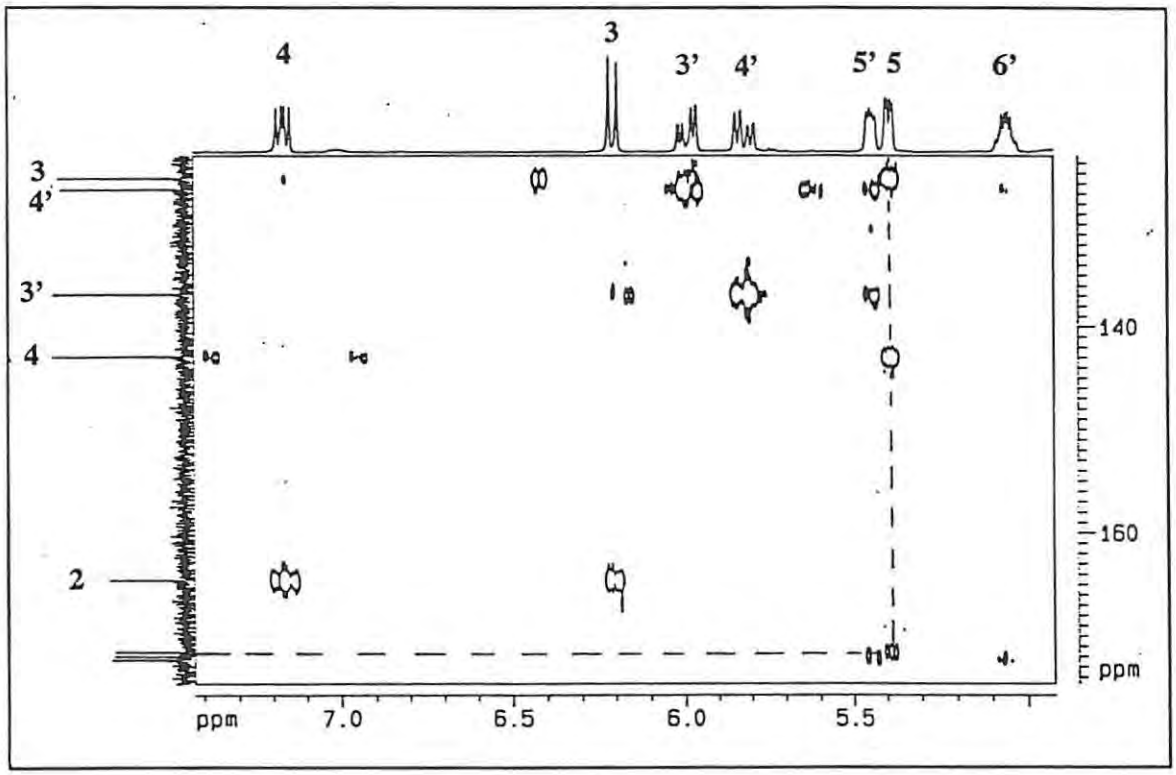
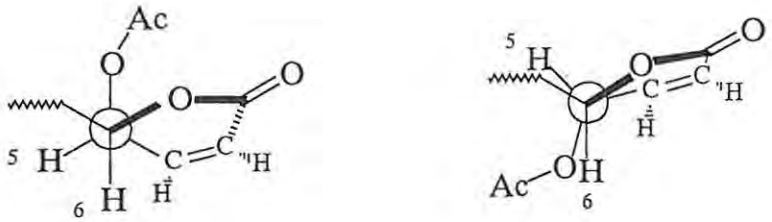


Figure 18 : A section of the HMBC NMR (400 MHz, CD_3OD) spectrum of **91**

argument, the same 1'S, 2'R, 5'S and 6'S stereochemistry established for **86** is also suggested for **91**. The configuration at C-5 was determined from NOE difference experiments. Irradiation of H-6 resulted in a significant enhancement of H-5 and incomplete irradiation of H-5 also resulted in a large enhancement of the H-6 proton signal indicating a *syn* relative stereochemistry. The $J_{5,6}$ coupling constant of 2.4 Hz requires the protons to be at an angle of 56° (gauche) or 122° (eclipsed) as depicted in Figure 19. The gauche conformation is energetically more stable and it supports the NOE data, while the eclipsed conformation would suggest the opposite stereochemistry. It has been predicted that polar substituents on cyclohexane rings have a lesser preference for the equatorial position than alkyl substituents (the free energy difference between equatorial and axial acetoxy substituents on a cyclohexane ring is of the order of 3 kJ.mol^{-1} *cf* C_6H_{11} of 9 kJ.mol^{-1}).[60] Since the 6-heptyl substituent prefers an equatorial arrangement, the small $J_{5,6}$ coupling constant must be associated with an axial equatorial interaction of the protons in a well stabilised pyrone ring. The magnitude of the $J_{5,6}$ coupling constants for other *syn* 5,6-disubstituted 5,6-dihydro-α-pyrones presented in Figure 20 are consistent with the value measured for **91**. The structure of synargentolide C is therefore proposed as 5S-acetoxy-6R-[5S,6S-diacetyloxy-1S,2R-dihydroxy-3E-heptenyl]-5,6-dihydro-2H-pyran-2-one.



gauche (*syn*) eclipsed (*anti*)

Figure 19 : The stereochemistry of **91** implied by the $J_{5,6}$ coupling constant

The structure and stereochemistry of synargentolide D

Synargentolide D (**92**, 0.006 % dry wt.), the 5-deacetyl derivative of **91**, was found to be thermally unstable and decomposed before the accurate mass, IR data and optical rotation could be obtained. However, the structure could clearly be established from one and two dimensional NMR studies. The ^{1}H NMR spectrum revealed that the compound contained two acetates, six oxymethine protons, four olefinic protons and a methyl group all of which could be assigned

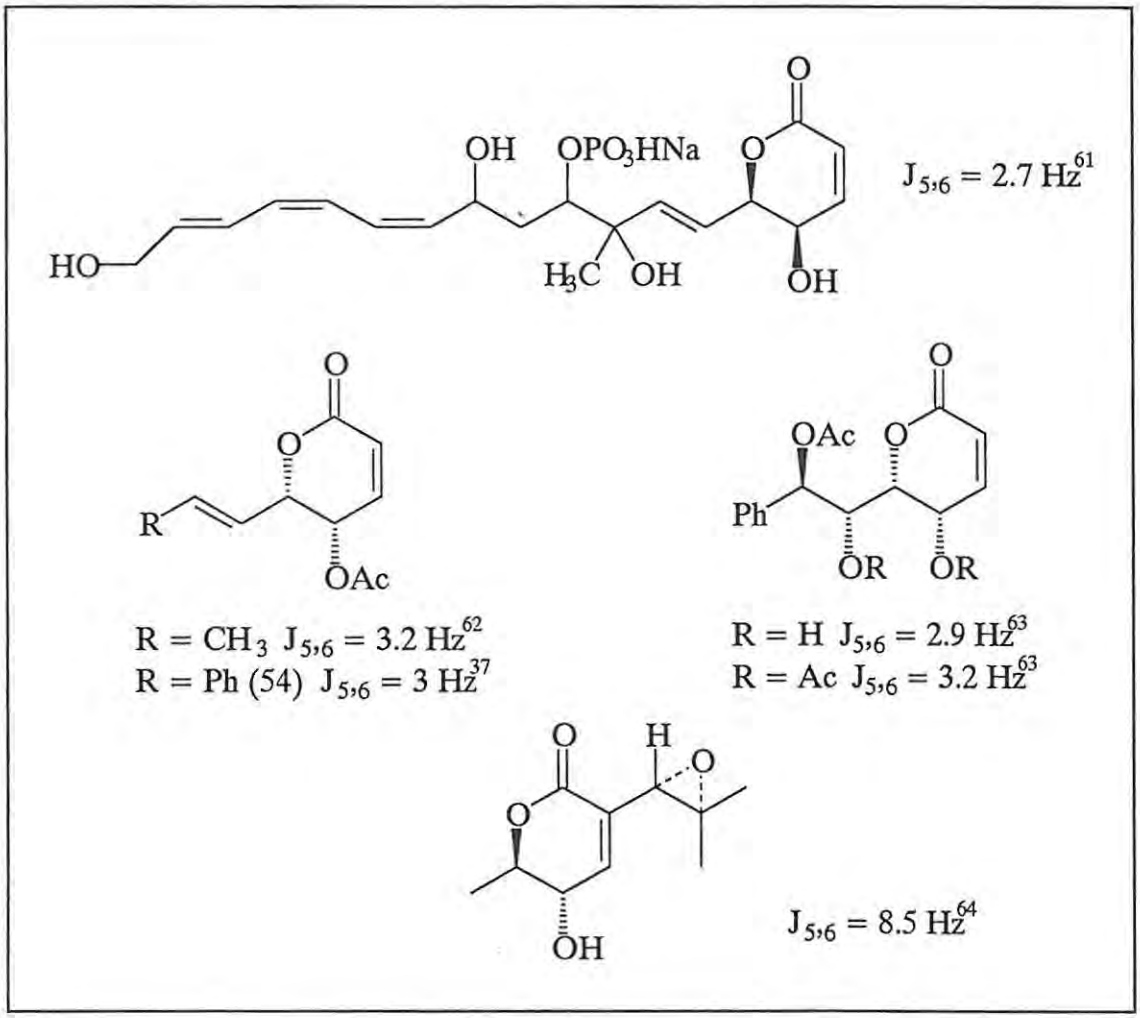
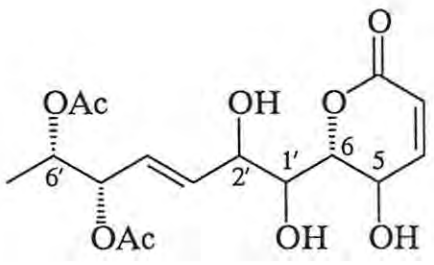


Figure 20 : The $J_{5,6}$ coupling constants of examples of 5,6-disubstituted 5,6-dihydro-α-pyrones

from the contiguous coupling in the COSY spectrum. The upfield shift of H-5 (δ 4.68 ppm) relative to H-5 in compound **91** (δ 5.39 ppm) confined a hydroxyl moiety to C-5 in **92**. Similarly the chemical shifts of the H-1' and H-2' protons (δ 4.32, 4.40) indicated the presence of C-1' and C-2' hydroxyl substituents. The ^{13}C chemical shifts (Table 6) were assigned from the HMQC spectrum.



92

The E stereochemistry of the double bond in synargentolide D was determined from the $J_{3',4'}$ coupling constant (15.9 Hz) measured directly from the ^1H NMR spectrum. Acetylation of **91** and **92** would have established if these two compounds have the same configurations, unfortunately decomposition of compound **92** prevented further stereochemical studies. In order to make some inferences about the stereochemistry of **92**, the ^3J coupling constants which could be calculated directly from the ^1H NMR spectrum were examined. Comparison of the coupling constants of the protons at C-5' and C-6' ($J_{4',5'}$ = 6.4 Hz, $J_{5',6'}$ = 3.5 Hz, $J_{6',7'}$ = 6.6 Hz) in **92** with those of **91** ($J_{4',5'}$ = 6.7 Hz, $J_{5',6'}$ = 3.5 Hz, $J_{6',7'}$ = 6.5 Hz) suggested a 5'(S), 6'(S) absolute configuration for **92**. In contrast, the coupling constants of the protons at C-3, C-4, C-5, C-6 and C-1' ($J_{3,4} \approx$ 10.3 Hz, $J_{4,5}$ = 3.5 Hz, $J_{5,6}$ = 8.0 Hz, $J_{6,1'}$ = 4.8 Hz) in **92** are considerably different to those of **91** ($J_{3,4}$ = 9.7 Hz, $J_{4,5}$ = 6.0 Hz, $J_{5,6}$ = 2.4 Hz, $J_{6,1'}$ = 9.6 Hz). Since all 5,6-dihydro-α-pyrone compounds which have been isolated from plants, including this species, possess the same configuration at C-6, a 6(R) configuration is also predicted for synargentolide D. These data intimate that either there is a change in stereochemistry at C-5 and C-1' or that the conformation of the ring differs. Although the latter is unlikely, a change in orientation may be possible owing to hydrogen bonding between the C-5 hydroxyl moiety and the hydroxyl substituent at C-1' in **92** (Figure 21) which is not possible in **91**. Such a change in conformation could also account for the significantly different $J_{6,1'}$ coupling constant. The $J_{4,5}$ and $J_{5,6}$ coupling constants in **92** are indicative of coupling to an axial proton at C-5 ($J_{4,5a}$ = 2-4 Hz, $J_{5a,6}$ = 9-12 Hz)[1] to give a 5(R) configuration. Such a change in stereochemistry is however insufficient to account for the difference in the $J_{6,1'}$ coupling constant and as stated previously, the occurrence of diastereomeric mixtures of α-pyrone compounds within individual Lamiaceae species is unlikely. The configurations of the the C-5, C-6, C-1' and C-2' chiral centres thus remain unassigned and the structure of **92** is possibly 6R-[5S,6S-diacetyloxy-1,2-dihydroxy-3E-heptenyl]-5-hydroxy-5,6-dihydro-2H-pyran-2-one.

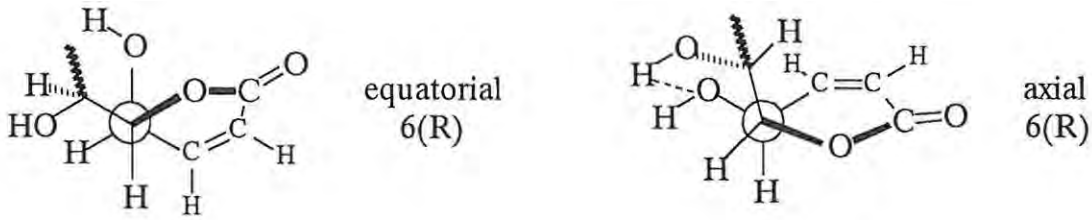
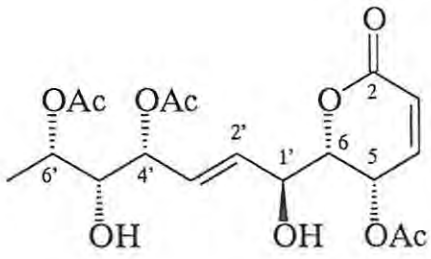


Figure 21 : Two conformations for the α-pyrone ring in **92**

The structure of synargentolide E

The molecular formula of synargentolide E (**93**), $[\alpha]_D^{25}$ = +53.4° (MeOH; c 0.61), the fifth α-pyrone to be isolated from *S. argenteus* was established as $C_{18}H_{24}O_8$ by HREI mass spectrometry. The ^1H NMR spectrum in methanol-d4 had several overlapping signals which complicated the structural elucidation. However the ^{13}C NMR spectrum in methanol-d4 clearly showed that this compound contained three acetate moieties, an α-pyrone ring, an exocyclic double bond and six oxymethine protons. From these data it could be deduced that the compound contained two hydroxyl substituents. The NMR spectra were rerun using DMSO-d6 as a solvent which caused two of the overlapping proton signals to be resolved and allowed the hydroxyl protons to be observed. Examination of the HMQC spectrum (Figure 22) and a comparison of the integrals of the ^1H NMR spectrum identified the hydroxyl proton signals and revealed that two olefinic proton signals were overlapping and that two oxymethine protons were resonating at the same frequency. Using this information, it was possible to interpret the coupling pattern in the COSY spectrum (Figure 23) and assign the ^1H NMR chemical shifts (Table 5). From a careful examination of the COSY and HMQC spectra it was evident that the chemical shifts of H-5 and H-4' differed sufficiently for the ^{13}C chemical shifts for these protons to be assigned. The assignment of the ^{13}C chemical shifts for the vinylic carbons C-2' and C-3' was based on a comparison of the shifts for the exocyclic vinylic carbons in the other α-pyrone compounds isolated from *S. argenteus*. The carbon closest to the pyrone ring consistently resonates downfield relative to the carbon further from the ring. The other ^{13}C chemical shifts were assigned from the HMQC correlations (Table 6).



93

The stereochemistry of the exocyclic double bond proved to be problematic. It has been reported[65] that the vicinal coupling constant for the J_{AB} spin system is approximately equal to the difference in the chemical shifts measured in Hz. Since the chemical shifts for each of the exocyclic olefinic protons in **93** were the same, it is expected that the protons H-2' and H-3'

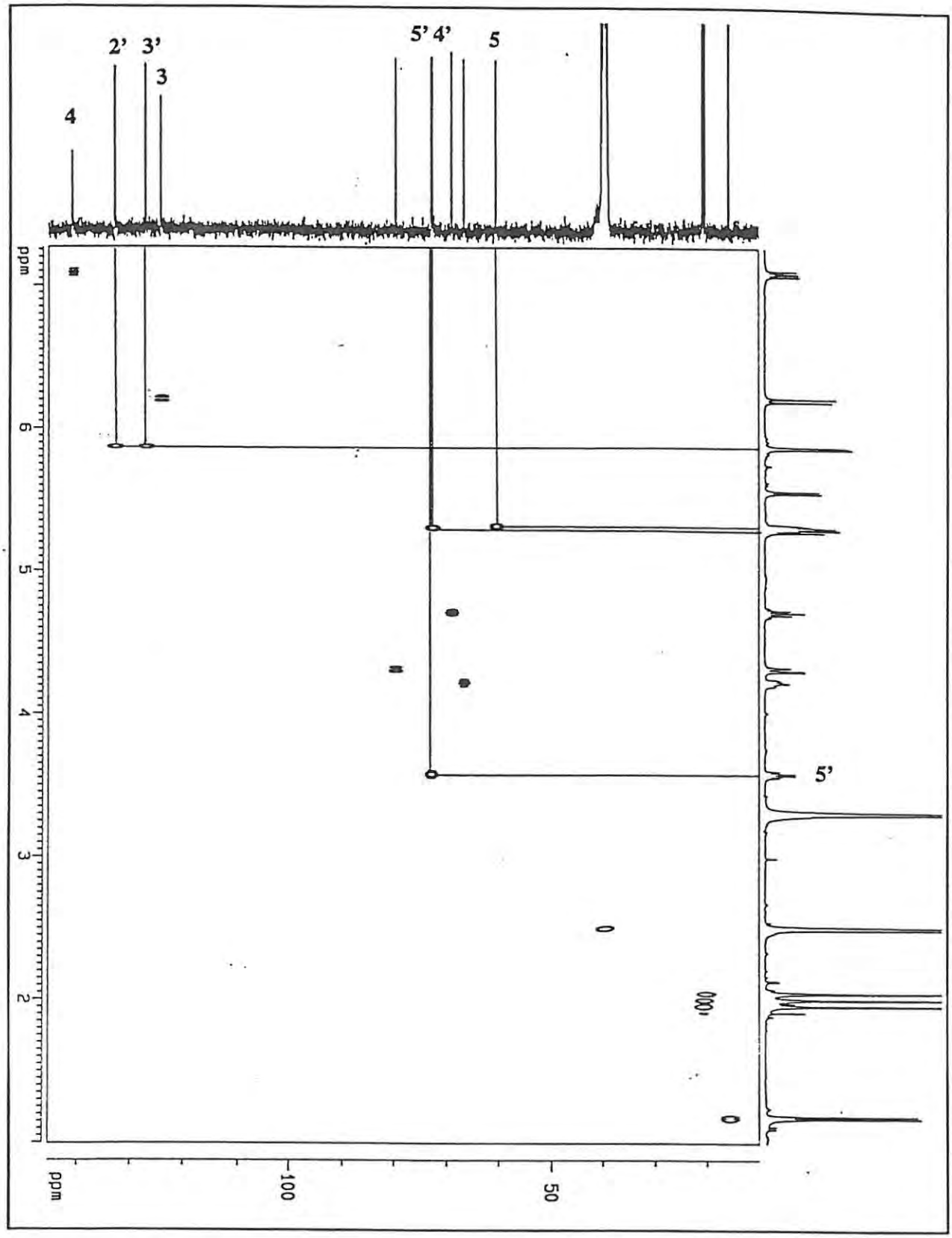


Figure 22 : HMQC NMR (400 MHz, DMSO-d6) spectrum of **93**

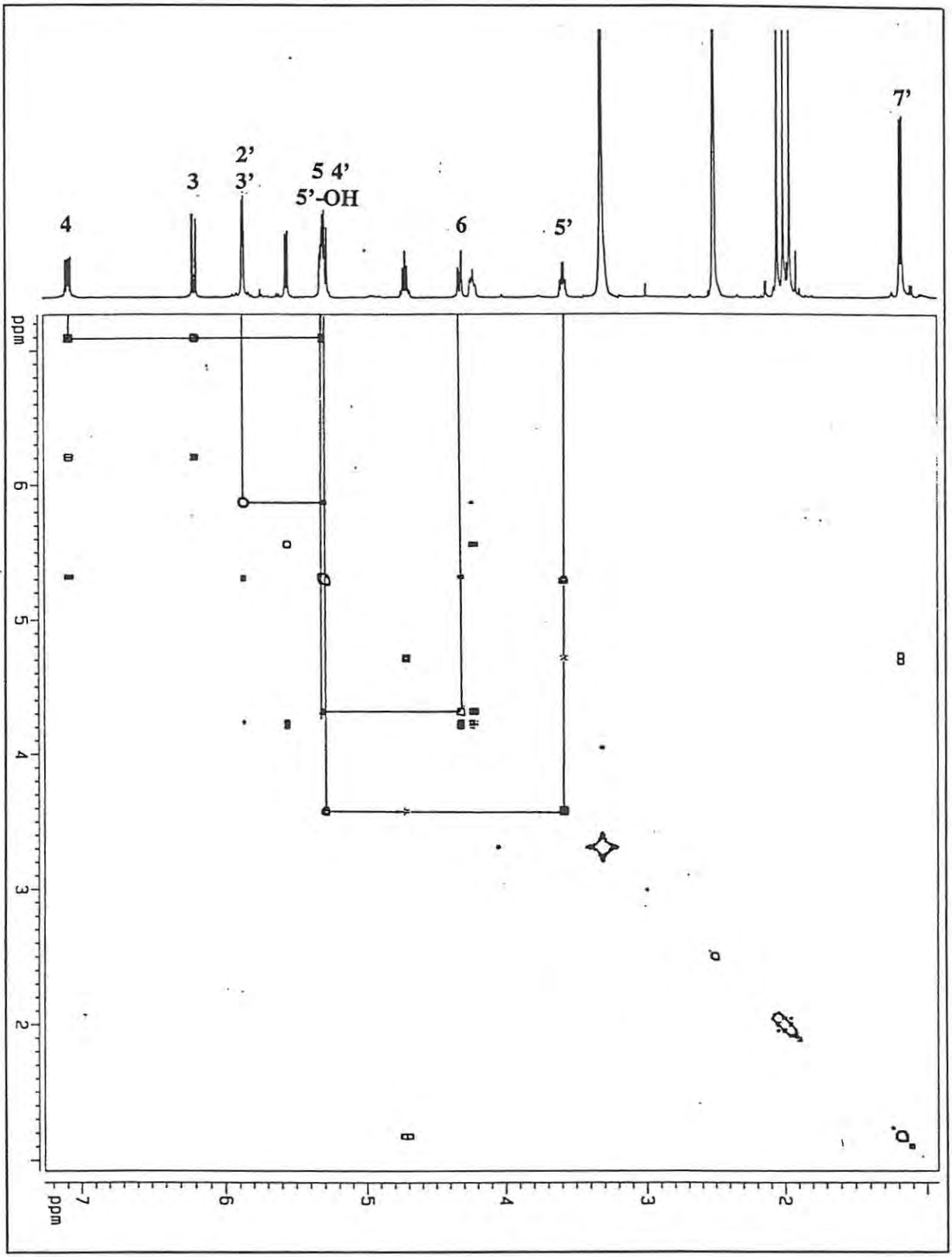


Figure 23 : COSY NMR (400 MHz, DMSO-d6) spectrum of **93**

will have a very small coupling constant irrespective of the stereochemistry of the double bond and it was therefore impossible to determine the E or Z configuration from a measurement of the vicinal coupling constant. The stereochemistry of the exocyclic double bond was thus assigned from an examination of the IR spectrum of **93**. The IR spectrum was very similar to the spectrum of synargentolide C suggesting that the compounds have the same functional groups. The absence of a strong absorption peak between 730 cm^{-1} and 675 cm^{-1} (characteristic of a *cis* disubstituted double bond), and the presence of peaks at 995 cm^{-1} and 927 cm^{-1} suggested the presence of a *trans* exocyclic double bond.[22] This assignment is in accordance with the stereochemistry of the exocyclic double bonds established for synargentolides A-D. It is suspected that the C-H deformation near 800 cm^{-1} is due to the cis double bond of the lactone ring.

The 6(S) stereochemistry followed from the CD data as usual.† Careful examination of the ROESY spectrum showed a strong NOE correlation between H-6 and the H-5, H-4' and 5'-OH multiplet. This correlation, attributed to a nuclear Överhauser effect between H-6 and H-5, suggests a *syn* stereochemistry for these protons. The configurations of the other four chiral centres in synargentolide E have been tentatively assigned in accordance with the stereochemistry established at the corresponding chiral centres in the other *S. argenteus* α-pyrone compounds from biosynthetic arguments as discussed earlier. The structure of **93** is thus tentatively assigned as 5S-acetoxy-6S-[4R,6S-diacetyloxy-1S,5R-dihydroxy-2E-heptenyl]-5,6-dihydro-2H-pyran-2-one.

† A Cahn Ingold Prelog order priority reversal has taken place

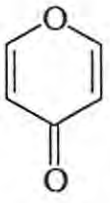
CHAPTER 2

Siphonarian Pyrone Metabolites

2.1 INTRODUCTION

Siphonaria (or 'false' limpets) are air breathing gastropod marine molluscs of the subclass Pulmonata and are common cosmopolitan inhabitants of rocks in the intertidal region. They are often mistaken for limpets† with which they co-occur because they resemble 'true' limpets in both appearance and behaviour. True limpets (Sub Class Prosobranchia) do not have a lung and use a ring of gills around the foot to breathe. Siphonariids are however better suited to their harsh intertidal environment than 'true' limpets because they posses a primitive lung in addition to a secondary gill.[67] During high tide, *Siphonaria* remain clamped to crevasses in rocks, but when the water has receded, they move about on the rocks feeding on algae and microorganisms. Siphonariids are therefore susceptible to both marine and terrestrial predators, and when molested produce a white mucous containing polypropionate metabolites which is thought to act as a chemical defence system to deter predators.[68]

Polypropionate compounds, derived from multiple condensations of three carbon propionate units, are frequently the products of metabolism in bacteria, insects and molluscs.[69] Although these compounds are particularly characteristic of *Siphonaria*, occurring widely throughout the genus, they do not occur in 'true' limpets.[70, 71] The structure and stereochemistry of Siphonarian polypropionates are remarkably similar to the actinomycete antibiotics such as erythromycin and monensin and it is thus not surprising that a number of these compounds posses antibiotic activity. A feature of the Siphonarian polypropionates is their complex cyclisations to yield pyrone, furanone or hemi-ketal functionalities. The Siphonarian pyrone polypropionates include γ-pyrones (**94**), dihydro-γ-pyrones (**95**), tetrahydro-γ-pyrones (**96**) and α-pyrones (**97**). Although γ-pyrones are biologically active, structure activity relationships indicate that the presence of an α-pyrone substituent is responsible for enhanced antibiotic activity.[72]



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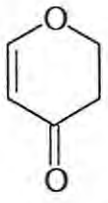

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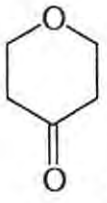

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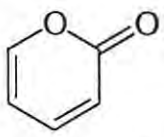

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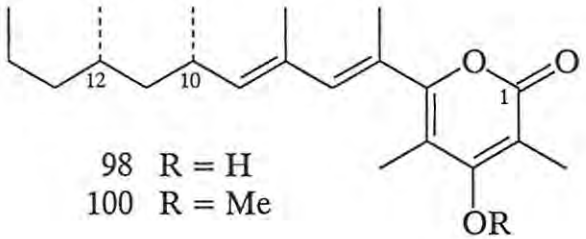
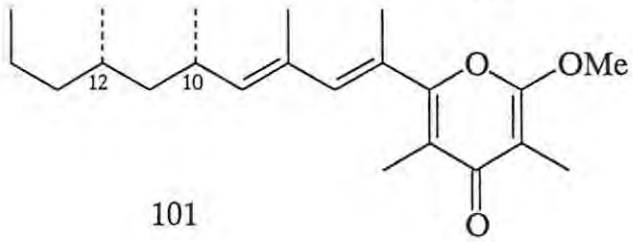
† The term "limpet" is often applied to any mollusc with a cap-shaped shell.[66]

In 1983, the first isolation of Siphonarian pyrone metabolites was reported by Hochlowski and Faulkner. The two compounds, diemenensin A (**98**) and diemenensin B (**99**, which slowly isomerised to A on standing) were isolated from the Australian species *S. diemenensis* in a bioactivity guided investigation.[73] The structure of the two compounds was largely elucidated from their ^{13}C and ^{1}H NMR chemical shifts. The E geometry of the two exocyclic double bonds in **98** was determined from the ^{13}C chemical shifts of the C-6 and C-8 methyl substituents (δ 16.2, 16.7) while analogous methyl carbon chemical shifts (δ 23.0 and 14.9) in **99** indicated that one of the double bonds had a Z geometry in this compound. The position of the Z-double bond was established through NOE experiments. The absolute stereochemistry of the two chiral carbons C-10 and C-12 in **98** was determined from a comparison of the rotation of the methyl ester of the oxidative ozonolysis product (2S,4S)-2,4-dimethylheptanoate with the literature values for the rotations of the enantiomer and a (2S,4R) diastereomer. The same configurations were assigned to the corresponding chiral centres in **99** because isomerisation of the *cis* double bond in **99** yielded compound **98**. The structure of the pyrone rings was determined by comparison of the ^{13}C NMR data of **98** with the corresponding data of the derivatives (**100**) and (**101**), arising from methylation of **98** with diazomethane. Diemenensin A inhibited division of fertilized sea urchin eggs and was active against the bacteria *Staphylococcus aureus* and *Bacillus subtilis*.[73]



98 R = H
100 R = Me



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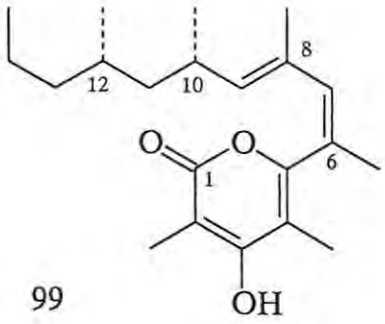
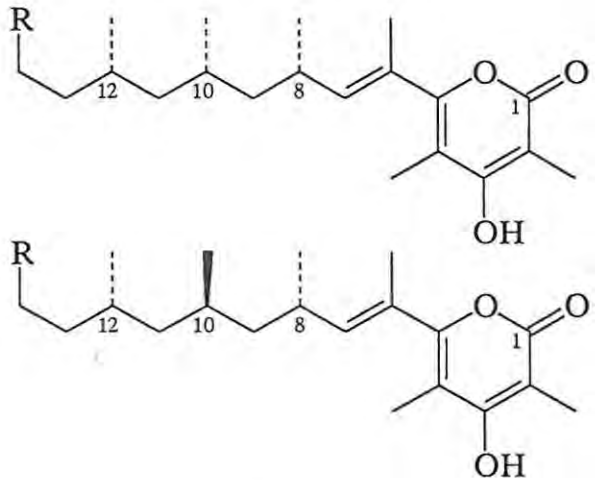


99

Pectinatone (**102**), a crystalline natural product, is another polypropionate metabolite containing an α-pyrone ring. This compound was first isolated from *Siphonaria pectinata* collected off the coast of Florida.[74] Structure (**103**) was proposed for this compound from a comparison of the ^{13}C and ^{1}H chemical shifts of pectinatone with the corresponding data of structurally related

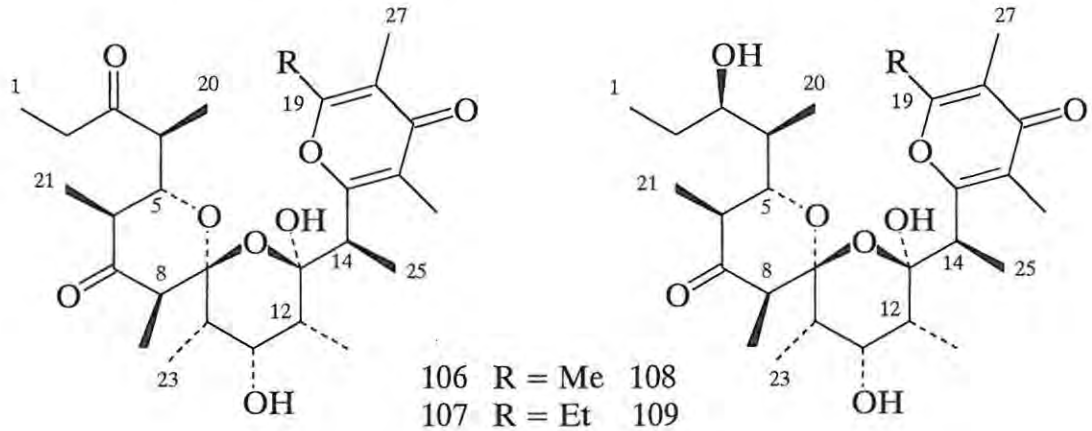


102 R = Me
104 R = H

103 R = Me
105 R = H

compounds and supported by proton decoupling experiments. The stereochemistry of the three chiral centres was initially incorrectly established from comparison of the optical rotations of the methyl ester of the product of oxidative ozonolysis with the literature value for methyl 2S,4R,6S-trimethyloctanoate.[74] The homologue of **102**, norpectinatone (**104**) was subsequently isolated as an oil from *Siphonaria lessoni*.[75] Differences in the NMR data of pectinatone and norpectinatone were consistent with a replacement of the terminal propyl group in **102** with an ethyl group in **104**. Once again the stereochemical assignment of the three chiral centres, structure (**105**), followed from comparison of the rotation of the methyl ester of the acid produced from oxidative ozonolysis compared with published values for isomers of methyl 2,4,6-trimethylnonanoate and methyl 2,4,6-trimethyloctanoate.[75] The stereochemistry of norpectinatone was first questioned in 1986 when a compound matching the proposed stereochemistry **105** was synthesised and found to have different physical properties.[76] Many subsequent studies of Siphonarian polypropionates have revealed a significant stereochemical correlation between the polypropionate natural products suggesting that they share a common genetic origin.[72] By 1990, pectinatone and norpectinatone were the only exceptions to the rule prompting a reinvestigation of their stereochemistry.[72] Both pectinatone and norpectinatone were isolated as crystalline materials from *Siphonaria virgulata*. The structure and relative stereochemistry of pectinatone was determined from an X-ray crystal analysis and one enantiomer **102**, consistent with the common stereochemistry of this class of polypropionate compounds, was chosen to represent this compound. It is biosynthetically unlikely that the stereochemistry of norpectinatone would differ, and the stereochemistry **104** was assigned accordingly.[72] The stereochemistry of pectinatone was simultaneously reassigned by Norte and

co-workers[77] who isolated pectinatone from the species *S. grisea*[†] and concluded firstly from an analysis of the optical rotation of the methyl ester of the degradation products and secondly from an X-ray crystal analysis that the structure was consistent with compound **102**.[77]



In 1984, Hochlowski *et al.* reported the isolation of four related spiroketals, each with a γ-pyrone moiety, from four species of *Siphonaria*. Siphonarin A (**106**) and its homologue siphonarin B (**107**) were isolated from *S. zelandica* and *S. atra* and the related compounds dihydrosiphonarin A (**108**) and B (**109**) were isolated from *S. normalis* and *S. laciniosa*.[79] Siphonarin A was crystalline and its structure was determined by X-ray crystallography supported by ^{13}C and ^{1}H NMR and high resolution MS and IR data. One enantiomer was arbitrarily chosen to represent the structure of the natural product. It was clear from the ^{13}C and ^{1}H NMR spectra that **107** was the ethyl homologue of **106**. The chemical structure of **108** was similarly established through a comparison of the NMR data with the corresponding data for **106**. The major product from a PCC oxidation of **108** was identical in all respects to the natural product, **106** implying that the configurations at the corresponding chiral centres in the four natural products were thus the same. The stereochemistry of the C-3 hydroxyl substituent in **108** and **109** was assigned on the basis of coupling constants and conformational analysis. Once again, NMR data for **109** was consistent with an ethyl homologue of **108**.[79] The absolute stereochemistry of **106** and hence **107**, **108** and **109**, has recently been established through a low temperature X-ray study on the *p*-bromophenylboronate derivative of **106**.[69] Since the absolute configuration of the chiral boronate moiety was known, the absolute configuration of **106** could be determined and was found to be opposite to the enantiomer initially chosen and

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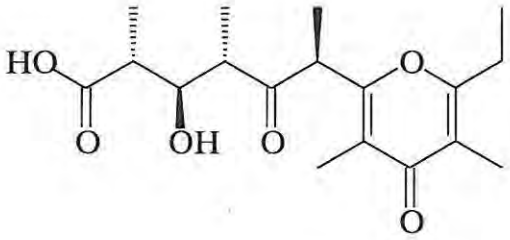
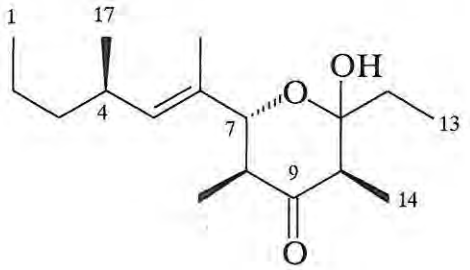
[†] Pectinatone has also been isolated from an endemic South African *Siphonaria* species *S. concinna*.[78]

is correctly depicted in **106**. This assignment was supported by the synthesis of compound (**110**), the enantiomer of the degradation product of **109** by Paterson *et al.*[80]

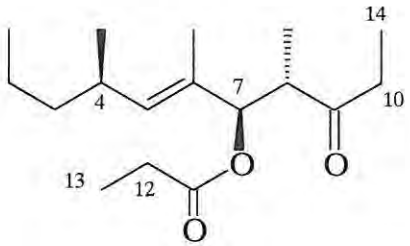


110

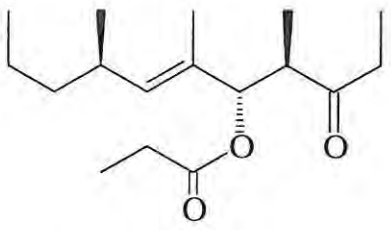
The tetrahydro-γ-pyrone (**111**) was isolated from *S. australis* in 1984.[70] The structure was deduced from the ^{13}C and ^{1}H NMR data, supported by IR analysis. The relative stereochemistry of the ring was elucidated from a careful analysis of the relevant coupling constants in the ^{1}H NMR spectrum. The relative stereochemistry at C-7 and C-8 was determined for the analogous ester (**112**) which was isolated from the same collection. Treatment of **111** with DBU in benzene yielded **112** implying the same relative configurations at these centres. The stereochemistry at C-4 in both compounds remained unassigned. Although attempts to synthesis **111** resulted in the exclusive formation of dihydropyrones, the absolute configuration of **111** has been determined from the synthesis of **112** which had NMR data consistent with the natural product, but different to the diastereomer (**113**) which was also synthesised.[81] The exciton chirality method, applied to the *p*-bromo-benzoate derivatives of **112** and **113**, was used to determine the stereochemistry of the two synthetic diastereomers thus establishing the stereochemistry of the natural product. Compound **111** can be converted to **112** and the stereochemistry of **111** has been assigned accordingly.[81]



111

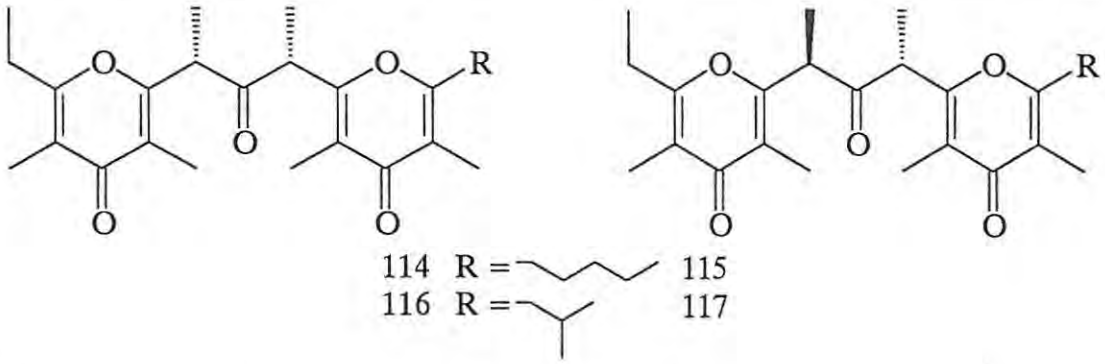


112



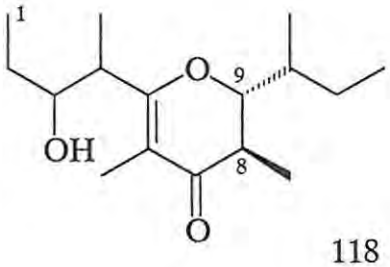
113

The diasteromerically related γ-pyrones with weak antimicrobial activity, maurapyrones A-D (**114-117**) were isolated as racemic mixtures from *S. maura* collected from Costa Rica.[71] Maurapyrone A was crystalline and the stereochemistry of the racemate was determined from X-ray analysis. The partial symmetry of the compound explained the simplicity of the ^{1}H NMR spectrum of **114**. A comparison of the NMR data revealed that racemic **115** had a diastereomeric relationship to maurapyrone A. It was observed that the pure compounds slowly isomerised to give a mixture of diastereomers explaining the occurrence of all four possible stereoisomers in this species. Compounds **116** and **117** were similarly a pair of racemic diastereomers and their structures were determined from an analysis of the NMR spectroscopic data. The dihydro-γ-pyrone maurenone (**118**), also isolated from *S. maura*, was not related to the above compounds and its structure was determined from NMR and IR spectroscopic analyses. The large coupling constant ($J_{8,9}$ = 12.8 Hz) indicated a *trans* relative stereochemistry for H-8 and H-9. No further inferences could be made concerning the stereochemistry.[71]

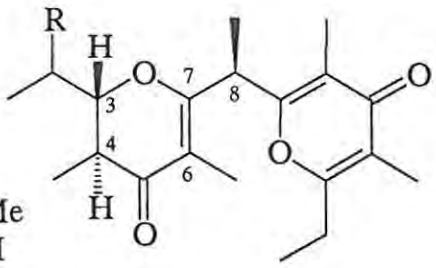


114 R = 115

116 R = 117



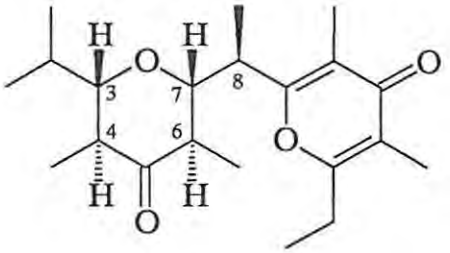
118



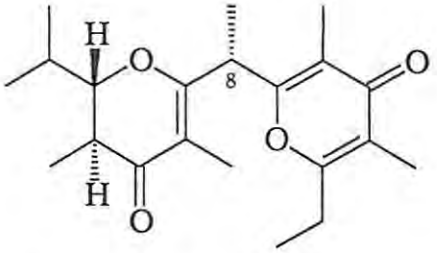
119 R = Me

120 R = H

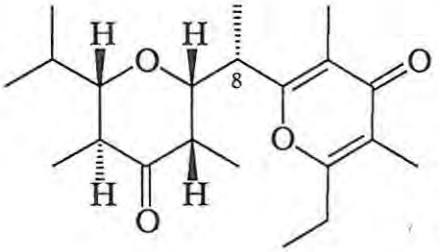
compound (**121**), where the relative stereochemistry of the tetrahydropyran ring protons was inferred from the $J_{3,4}$ and $J_{6,7}$ coupling constants (10.6 and 10.4-10.7 Hz respectively) and the observation of nuclear Överhauser enhancement between H-3 and H-7. By first considering steric effects to predict the most stable conformation of **121** and then examining the $J_{7,8}$ coupling constant (4.0 Hz), the configuration of C-8 relative to C-7 was proposed. In order to substantiate this prediction, the C-8 epimer (**122**) was prepared by stirring **119** in a basic THF solution. The epimer **122** was hydrogenated as before yielding the *cis* H-6, H-7 product (**123**). Again the $J_{7,8}$ coupling constant supported the predicted conformation and hence supported the predicted relative configuration of C-8 in **119**. The absolute configuration of **119** was determined from the sign of the split Cotton effect in the CD spectrum according to the exciton coupling theory.[82] The enone and pyrone $\pi \rightarrow \pi^*$ transitions were identified in the UV spectrum of **119** from a comparison with the UV spectra of model compounds and the exciton coupling theory was applied to the system accordingly. An 8R absolute configuration was thus assigned to **119**. The NMR spectral data of **120** including identical coupling constants corresponded to a lower homologue of **119** and since the optical rotation was of the same sign and order of magnitude, it was considered reasonable to assign the same stereochemistry to **120**.[82]



121



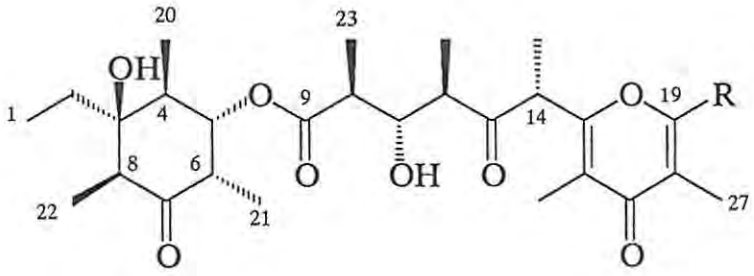
122



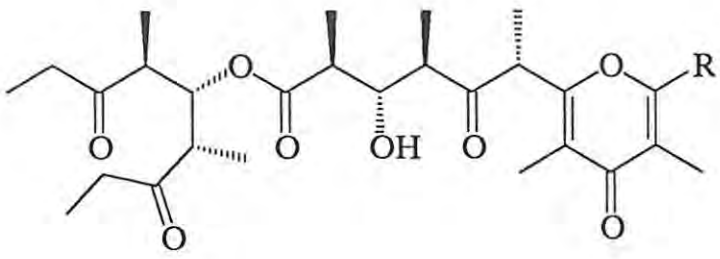
123

Examination of the species *S. baconi* led to the isolation of four new γ-pyrone polypropionate metabolites, the baconipyrones A-D (**124-127**) as well as the known compound siphonarin A, **106**.[67] The molecular formula of baconipyrone B was determined from FABMS. The structure could not be conclusively established using standard spectral techniques because there was no means of linking the isolated spin systems observed in the ^{1}H NMR spectrum. Although **125**

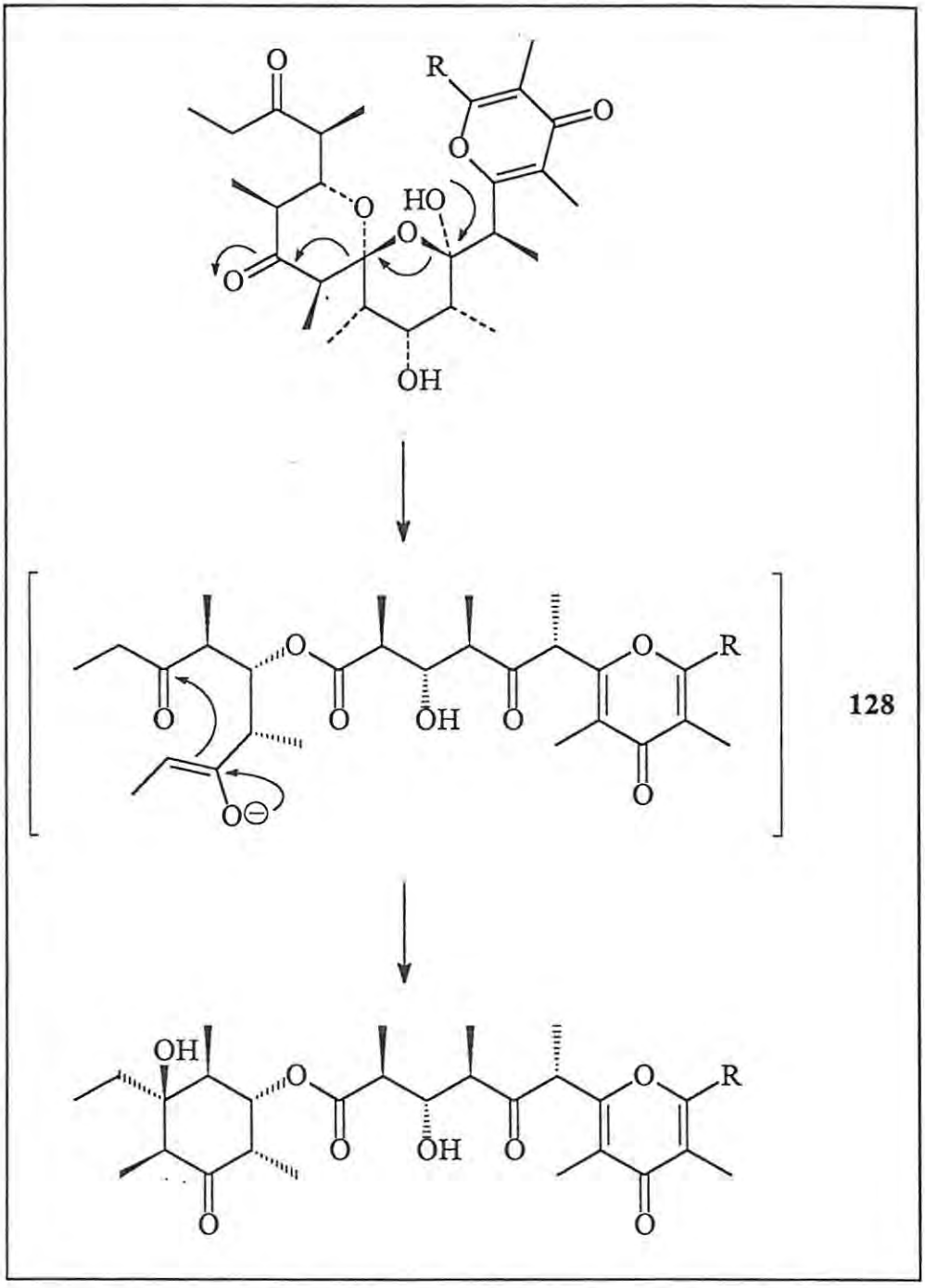
was initially isolated as an oil, it slowly crystallised from ether-hexane yielding colourless cubic crystals which were the subject of an X-ray crystal analysis, from which the structure of **125** was unambiguously resolved. The spectral data were consistent with the X-ray structure. These are the first Siphonarian pyrone compounds with a non-contiguous polypropionate backbone.[67] The co-occurrence of **106** in *Siphonaria baconi* led Manker *et al.* to propose a mechanism for the isomerisation of **106** to **125** as depicted in Scheme 2. Protonation of the intermediate **128** in the proposed mechanism yields baconipyrone D, **127**, that has a prochiral centre at C-5 and could thus cyclise to give two epimers of **125**. Epimers are not observed in the extract of *S. baconi*, leading to the suggestion that the cyclisation is enzyme mediated and the C-3 and C-7 ketone groups distinguished during transformation. It was evident from the spectral data of **124** that this compound was the ethyl homologue of **125**. The structures of compounds **126** and **127** were determined from a comparison of their ^{13}C and ^{1}H NMR chemical shifts with the corresponding NMR data for compounds **124** and **125**. Although the 2-alkyl-3-pentanone chains (C-1 to C-20 and C-22 to C-21) in **126** and **127** are identical in all respects, including the absolute configurations at C-4 and C-6, the corresponding chemical shifts differ as a result of attachment to a prochiral carbon, C-5.[67] The absolute stereochemistry of siphonarin A has now been established,[69, 80] and the enantiomer representative of the structure established by X-ray analysis has been chosen accordingly. In the absence of conflicting data, it is assumed that the stereochemistry of corresponding chiral centres in the four compounds is the same.



124 R = Et
125 R = Me



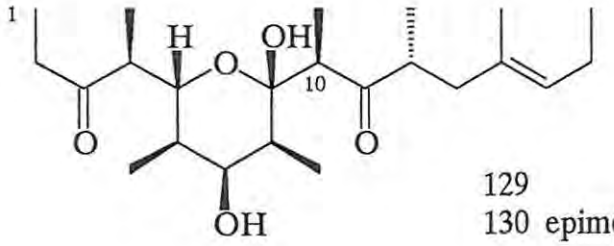
126 R = Et
127 R = Me



Scheme 2: A proposed mechanism for the generation of the baconipyrones from the siphonarins.[67]

As part of an ongoing investigation of endemic South African *Siphonaria* species, the natural product chemistry of the species *S. serrata* was examined. This investigation yielded a new polypropionate compound structurally related to the baconipyrones and the results of the investigation are presented in section 2.2 below.

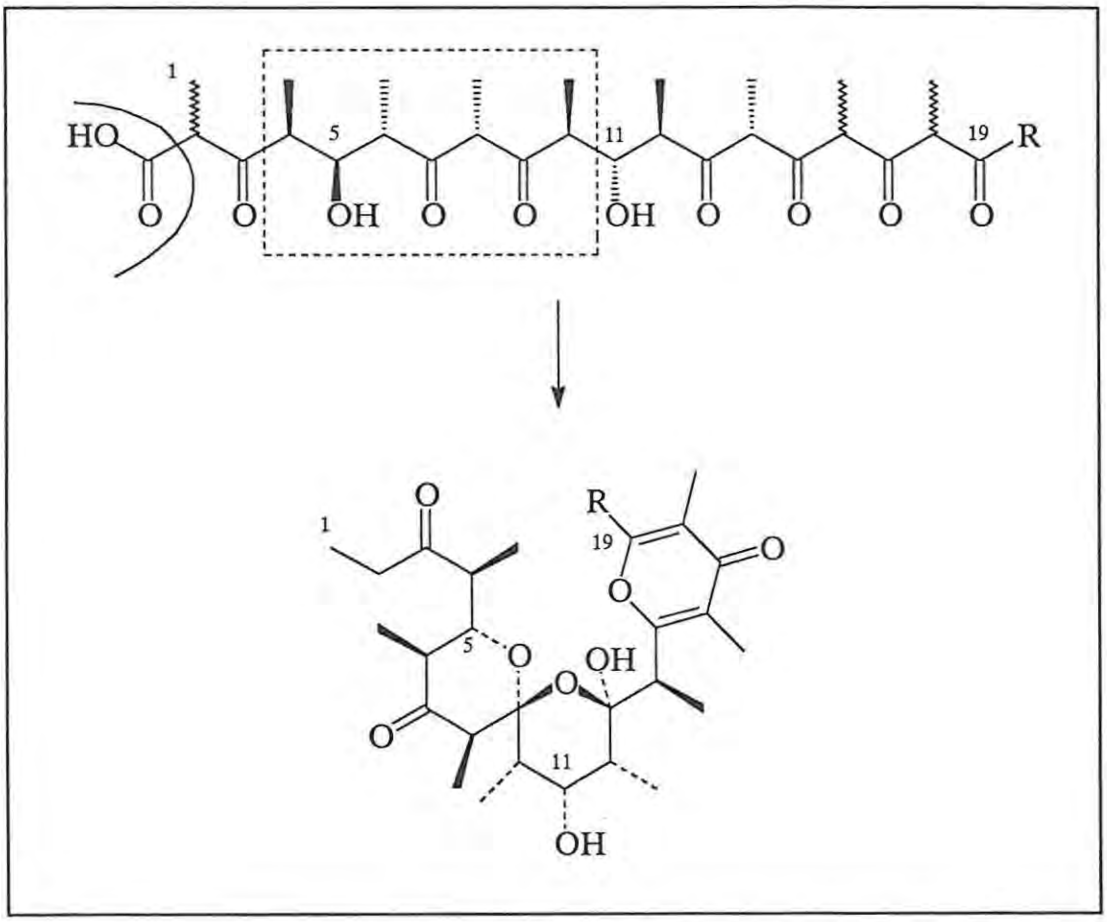
The first study of the biosynthesis of Siphonarian polypropionate metabolites, reported in 1988 clearly demonstrated the polypropionate origin of these compounds.[83] The monitoring of the incorporation of [1-^{14}C]-propionate units into the non pyrone containing compounds denticulatin A (**129**) and B (**130**) was found to be analogous to polypropionate biosynthesis in bacteria. A comparative study of the incorporation of [1-^{14}C]-acetate units revealed that these metabolites were indeed derived from propionate units and were not the methylation products of a polyacetate biosynthesis. An in depth study of the biosynthesis of siphonarin A, **106** and B, **107** also using [1-^{14}C]-propionate units, revealed that although the biosynthesis of these



129
130 epimeric at C-10

compounds started with an acetate (Siphonarin A) or a propionate (Siphonarin B) unit at C-19, the acyclic structure was completed by the sequential addition of propionate building blocks.[69] There is no evidence to suggest at what stage in the biosynthetic sequence reduction of the side chain and subsequent establishment of the stereochemistry takes place, but in the absence of evidence to the contrary it has been proposed that such functionalisation occurs prior to the incorporation of each propionate unit in the growing polyketide chain.[84] Siphonarian polypropionates may be divided into two groups, *viz.*, those with the diemenensin or pectinatone alkenyl backbone often containing an α-pyrone moiety and those with the highly oxygenated siphonarin acyclic backbone often containing a γ-pyrone moiety.[72] The stereochemistry of the carbon atoms bearing methyl groups are consistent for the first group of compounds, while the latter all contain a tetrapropionate unit (Scheme 3) with a stereochemistry common to the Cane-Celmer-Westley PAPA model for polyether antibiotics of bacterial origin.[72, 84] These data point towards a common genetic origin for bacterial and siphonariid polypropionates and Garson *et al.* are currently examining the proteins in *Siphonaria* for enzymes resembling the polyketide or fatty acyl synthases present in bacteria.[84] The presence of cyclic ketals is another structural feature of siphonariid polypropionates. The occurrence of spontaneous cyclisations on silica gel during syntheses of the polypropionate natural products has lead to the proposal that the highly cyclised structures are not the actual

siphonariid metabolites, but are rather artifacts of the isolation process.[85, 86] A number of compounds isolated from *Siphonaria* and considered to be natural products are thus possibly the thermodynamic, non-enzymatic cyclisation products whose mode of cyclisation depends on the oxidation state of the carbon and the configuration of the hydroxyl and methyl groups in the acyclic precursor.[86] This is not necessarily the case for all cyclisations however, and the formation of the γ-pyrone ring in particular, appears to be an integral part of the chemical structure of the secondary metabolite. Similarly the stereospecific formation of the baconipyrones from the siphonarins does not appear to be the result of a thermodynamic process.[67] Presumably the answers to these interesting biosynthetic questions will be forthcoming from further biosynthetic studies, polypropionate synthetic investigations and a re-evaluation of standard isolation procedures.



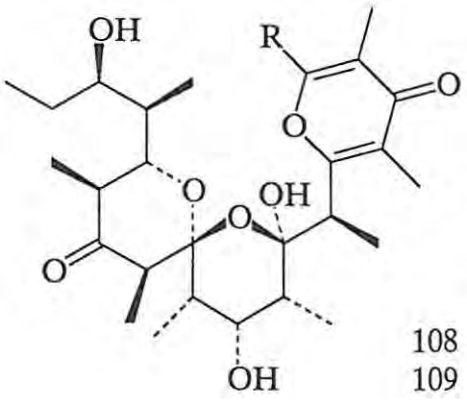
Scheme 3: The tetrapropionate unit common to bacterial and siphonarin type polypropionates.[84]

2.2 *SIPHONARIA SERRATA*

2.2.1 Background

Siphonaria serrata is one of nine South African species of *Siphonaria*.[87] This species is found predominantly along the south and east coasts of South Africa stretching from Cape Town to Durban, with isolated populations occurring on the west coast north of the Orange river and in the Langebaan lagoon.[87] *S. serrata* generally occurs in the upper mid-littoral region and these molluscs are usually 15 to 25 mm in length but have been known to reach lengths of 40 mm. A collection of *S. serrata* was made at low tide in April 1996 from near the Willows Caravan Park, Port Elizabeth.

Preliminary investigations[†] into the chemistry of this species indicated the presence of the known compounds dihydrosiphonarin A (**108**) and B (**109**) as well as a mixture of γ-pyrone containing polypropionate compounds.



108 R = Me
109 R = Et

2.2.2 Results and Discussion

A single γ-pyrone metabolite siserrone A (**131**, 107 mg, 0.40 mg/animal) was purified from the acetone extract of 270 specimens of *Siphonaria serrata* as depicted in Scheme 4. The isolation was specifically designed to target the γ-pyrone compounds suggested from an earlier investigation. Surprisingly, no dihydrosiphonarin A and B were evident in the second collection of *S. serrata*.

[†] M.T. Davies-Coleman, unpublished results.

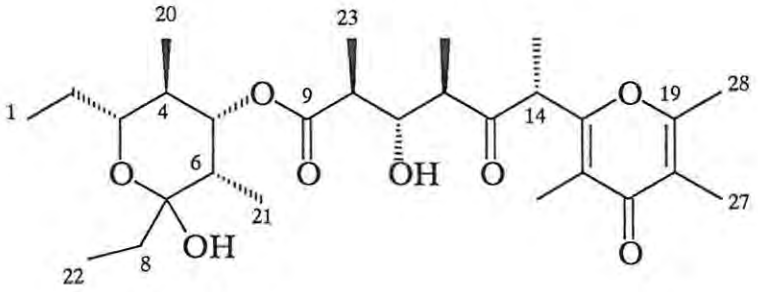
270 molluscs
Acetone extract
↓ (a)
1.385 g
↓ (b)
363.3 mg
↓ (c)
107.9 mg
[131]

(a) EtOAc/H_2O partition. EtOAc fraction dried and concentrated *in vacuo*. Aqueous fraction discarded.

(b) Flash chromatography on silica gel (150 g). Gradient elution with hexane/EtOAc (4:1, 3:1, 2:1, 1:1, EtOAc). The initial fractions from the EtOAc eluent were further purified.

(c) Normal phase HPLC (2:5 hexane/EtOAc mobile phase).

Scheme 4: The isolation of siserrone A (**131**) from *Siphonaria serrata*.



131

Structural Investigations

The molecular formula of **131** was established as $C_{28}H_{44}O_8$ from HREIMS data (*m/z* 508.3040 Δmmu +7). IR data demonstrated the presence of hydroxyl (ν_{max} 3390 cm^{-1}) and ketone (ν_{max} 1720 cm^{-1}) functional groups. Three methyl singlets (δ 1.99, 1.88 and 2.18 ppm), indicative of the presence of a trimethylated γ-pyrone ring, were present in the ^1H NMR spectrum of this compound. The ^{13}C NMR data were also consistent with a γ-pyrone functionality (δ 179.2, 160.7, 160.5, 120.3 and 119.1). The ^{13}C NMR and HMQC spectra provided further evidence for ketone (δ 209.9), ester (δ 175.1) and ketal (δ 99.5) quaternary carbons. The ^1H and ^{13}C NMR chemical shifts are presented in Table 7. Four fragments (A-D, Figure 24) could be identified from the contiguous coupling sequences (H-1 to H-21, H-8 to H-22, H-23 to H-24 and H-25 to H-14) in the COSY NMR spectrum (Figure 25) of **131**. Examination of the long range HMBC H-C correlations (Figure 24 and Table 7) enabled these four isolated spin systems to be linked via the intervening quaternary carbons as follows.

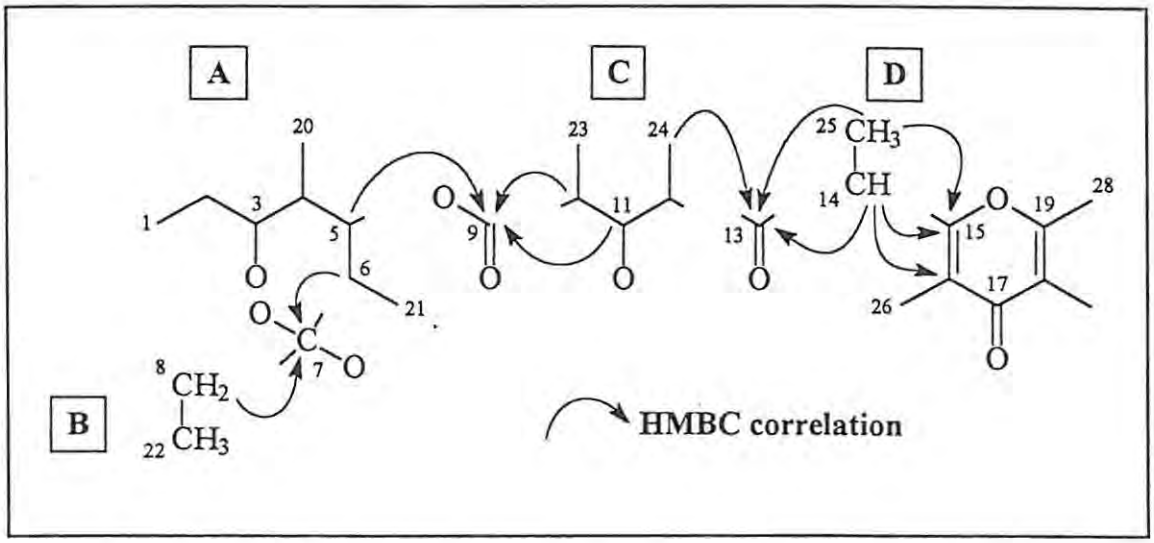


Figure 24: HMBC correlations linking the isolated spin systems (A-D).

HMBC correlations were observed from H-6 and H-8 to the ketal carbon C-7 linking two of the spin systems (A and B). In the absence of evidence to the contrary, we propose that the oxymethine carbon C-3 (δ 74.5) is linked via an oxygen atom to C-7 to form a six membered cyclic hemi-ketal. The chemical shift of H-5 suggested that C-5 contained an ester substituent, an observation supported by the long range H-C coupling from H-5 to the ester carbonyl carbon C-9 in the HMBC NMR spectrum. Correlations from H-10 and H-11 to C-9 linked the third spin system (C) to the first two. HMBC correlations from H-14 to C-15 and C-16 and from H-25 to C-15 identified the CH-CH_3 unit (D) as the fourth substituent of the γ-pyrone ring. Similarly, long range H-C coupling linked the CH-CH_3 spin system to the ketone functionality. Finally, an HMBC correlation from the overlapping H-1, H-22 and H-24 methyl resonances to the ketone carbon was attributed to a ^3J coupling from H-24 to C-13 thereby completing the structural elucidation of **131**. In order to account for the molecular formula the oxymethine carbon C-11 must contain an hydroxyl substituent. The proposed structure therefore accounts for all seven degrees of unsaturation predicted by the molecular formula. Unfortunately, siserrone A was thermally unstable and degraded prior to the determination of the optical rotation.

Compound **131** is clearly a polypropionate metabolite without the usual contiguous carbon backbone and is thus structually related to the baconipyrones. We propose that **131** has a similar biosynthetic origin to the baconipyrones (Scheme 2), however arising from a

dihydrosiphonarin A precursor, shown from a previous collection to be a metabolite of *S. serrata*, and not siphonarin A as in the biosynthesis of the baconipyrones. The proposed biosynthesis of **131** from **108** is depicted in Scheme 5. A stereochemical analysis of **131** would support or refute this proposal and an attempt to investigate the stereochemistry of this compound is described below.

Table 7: NMR chemical shifts for compound **131**.

	^{1}H †	^{13}C ‡	HMBC † to C	ROESY † to H
1	0.89 *m*	9.4	2, 3	4
2	1.38 *m*, 1.63 *m*	25.4	3	
3	3.49 *m*	74.5		5
4	1.63 *m*	33.6	3, 5	
5	5.18 *dd* (11.2, 4.9 Hz)	76.5	9, 20, 21	3, 6, 20
6	1.95 *dq* (4.9, 6.9 Hz)	38.3	4, 5, 7	5
7	----	99.5	----	----
8	1.55 *m*	31.9	7, 22	
9	----	175.1	----	----
10	2.65 *m*	41.2	9, 23	11
11	3.59 *m*	$CDCl_3$	9	10
12	2.73 *m*	48.4	11	14
13	----	209.9	----	----
14	4.00 *q* (6.9 Hz)	51.1	13, 15, 16, 25	12, 26
15	----	160.7 [a]	----	----
16	----	120.3	----	----
17	----	179.2	----	----
18	----	119.1	----	----
19	----	160.5 [a]	----	----
20	0.76 *d* (6.9 Hz)	8.6	3, 4, 5	3, 5
21	0.75 *d* (6.2 Hz)	13.0	5, 6, 7	4
22	0.89 *m*	6.4	5, 7, 8	
23	1.27 *d* (7.2 Hz)	15.1	9, 10, 11	11
24	0.89 *m*	14.0	11, 12, 13	
25	1.32 *d* (6.9 Hz)	12.8	13, 14, 15	
26	1.99 *s*	9.8	15, 16, 17	14
27	1.88 *s*	9.9	17, 18, 19	
28	2.18 *s*	17.4	18, 19	

† 400 MHz, $CDCl_3$ ‡ 100 MHz, $CDCl_3$ [a] assignments may be interchanged

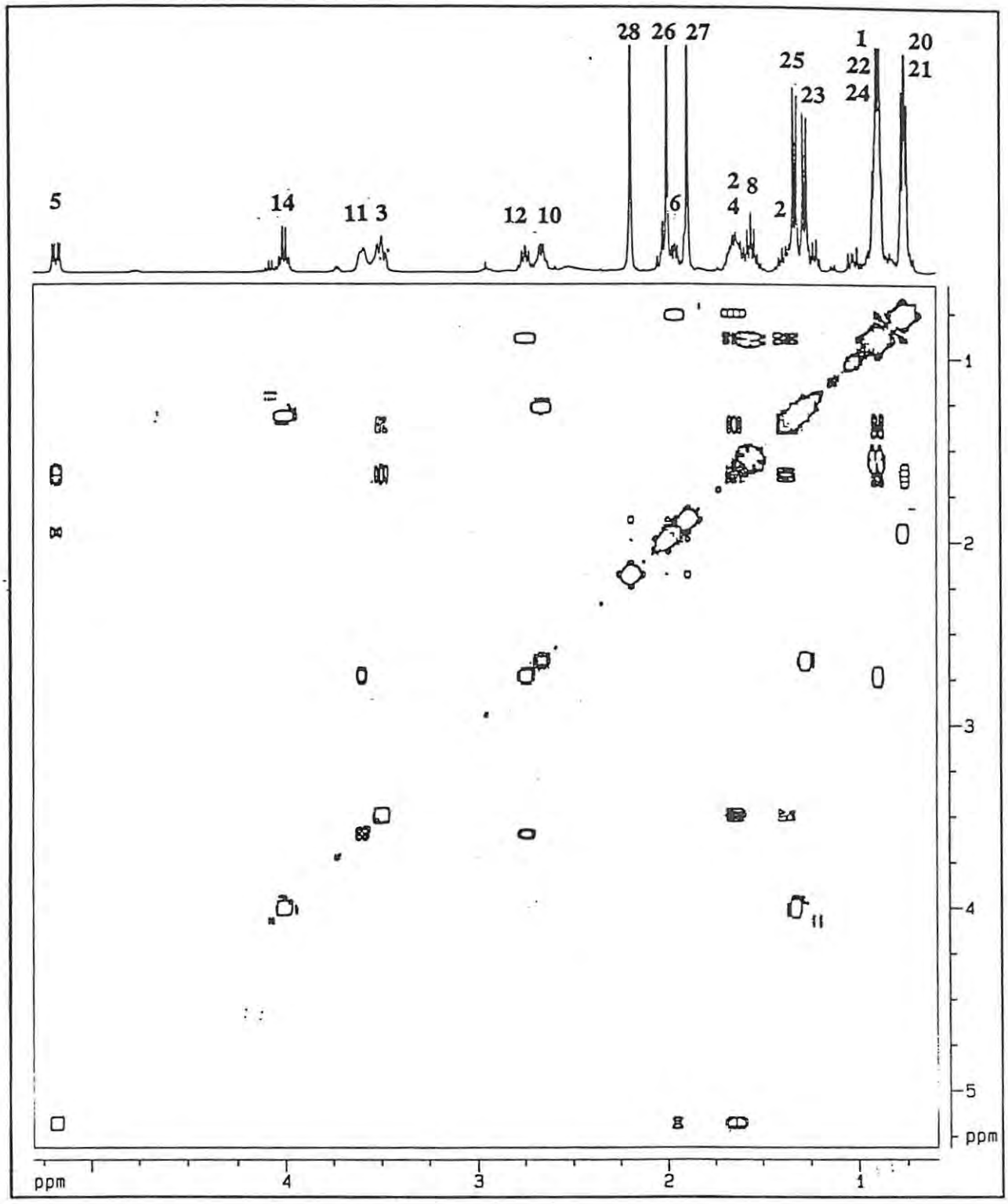
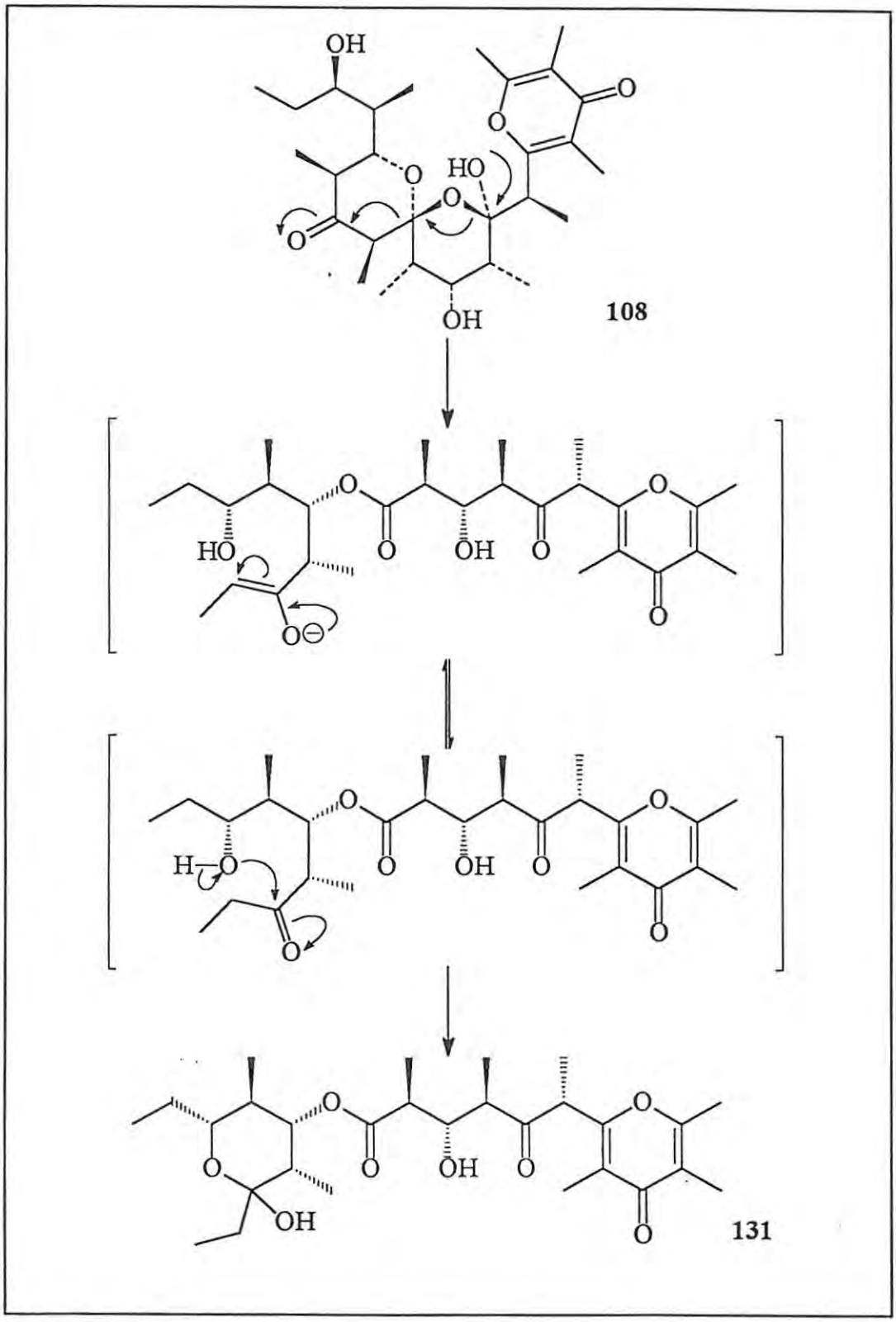


Figure 25 : COSY NMR (400 MHz, $CDCl_3$) spectrum of **131**[†]

[†] The coupling between H-10 and H-11 is very weak, but can be observed in the COSY spectrum by increasing the intensity levels.



Scheme 5: A proposed mechanism for the generation of siserrone A (**131**) from dihydrosiphonarin A (**108**)

The relative stereochemistry of the ring carbons of the cyclic hemi-ketal was investigated using a 2D ROESY NMR experiment (Figure 26) which shows the NOE correlations between protons as positive cross peaks. This spectrum contains a number of negative cross peaks indicative of conformationally or chemically exchanging protons. As was mentioned in the previous chapter, the appearance of through bond, COSY-type correlations, is a problem inherent in the ROESY spectrum. These appear as mixed positive and negative cross peaks. Only true NOE correlations were considered in the interpretation of the spectral data for the assignment of the relative configurations from C-3 to C-6.

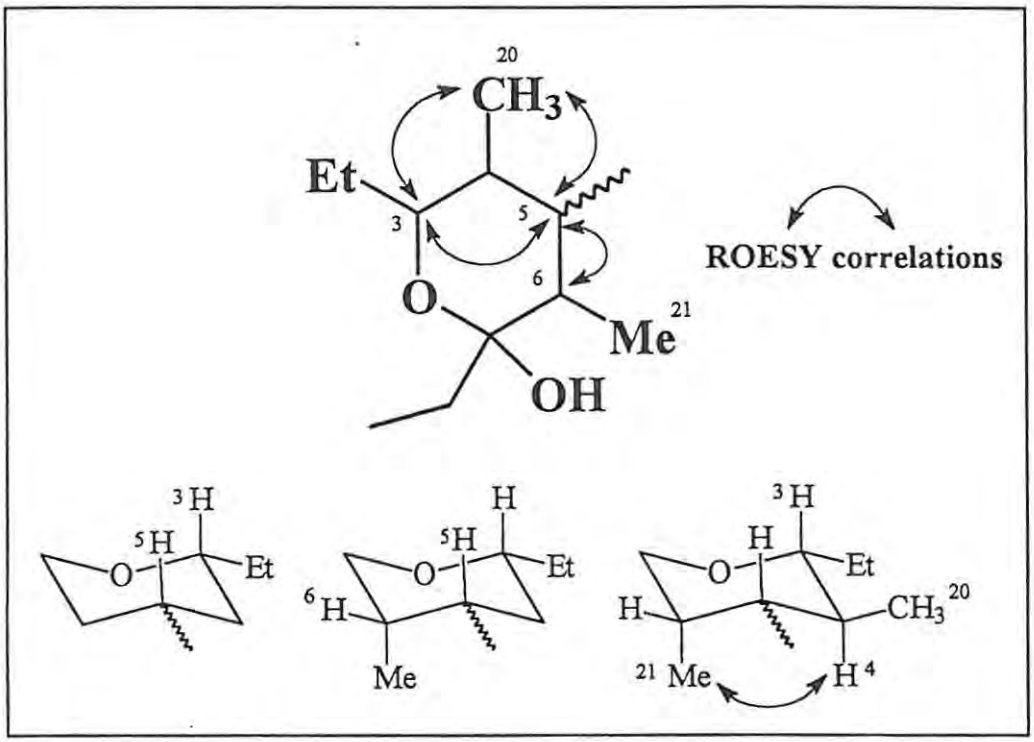


Figure 27: The determination of the relative stereochemistry of the cyclic hemi-ketal in **131** from the NOE correlations in the ROESY spectrum.

A strong NOE correlation between H-3 (δ 3.49) and H-5 (δ 5.18) demonstrated that these protons must be on the same face of the ring, i.e. *syn* to one another. In order for such a correlation to be observed, the protons must be axially orientated suggesting that the ring adopts the thermodynamically preferred chair conformation, with the C-3 and C-5 alkyl substituents equatorial, as the major conformer (Figure 27). Assuming that this chair conformation is dominant, the NOE correlation between H-5 and H-6 (δ 1.95) implies that H-6 is equatorially orientated.

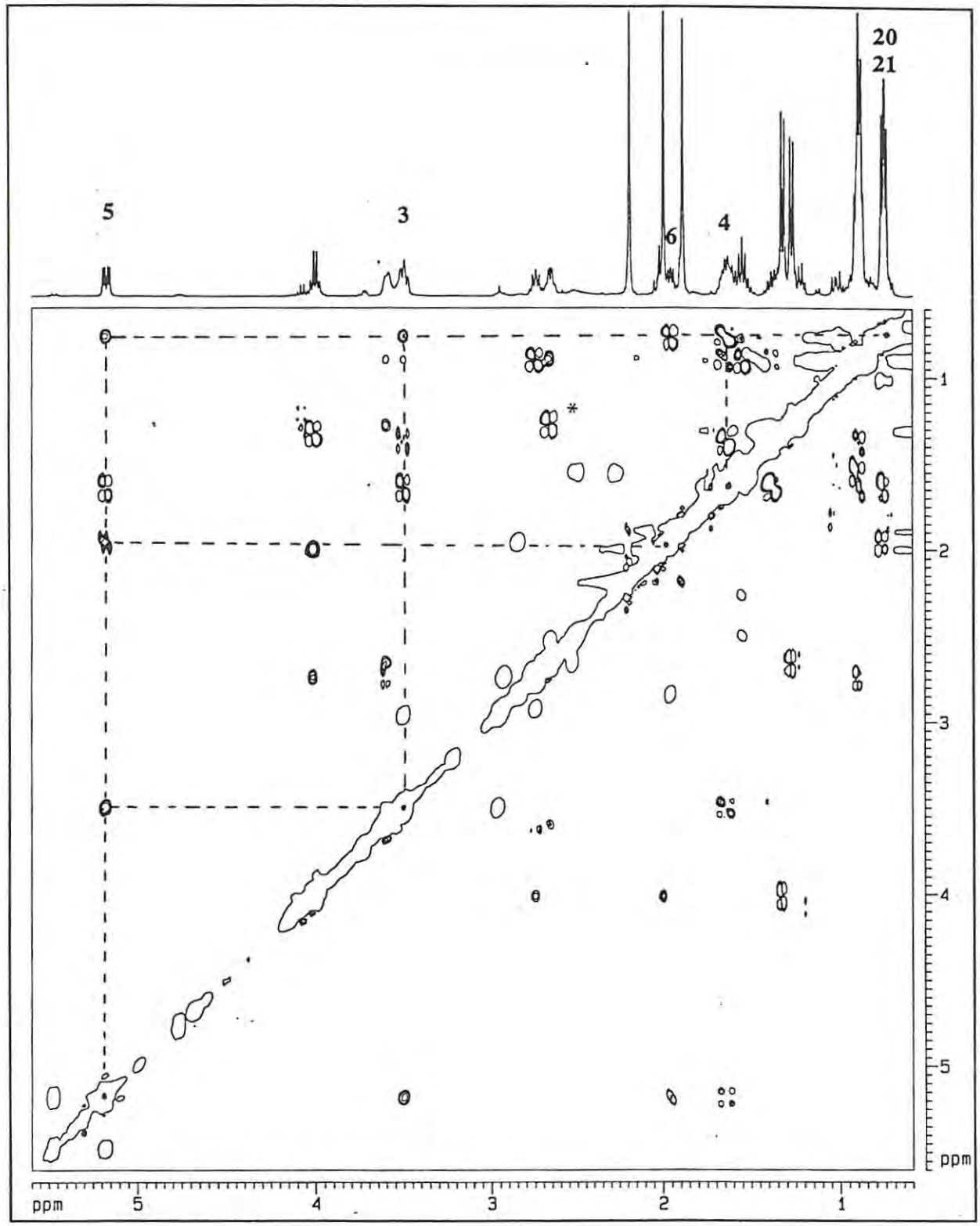


Figure 26: ROESY NMR (400 MHz, $CDCl_3$) spectrum of **131**[†]

[†] Multiple contours are shown for positive cross peaks and single contours for negative cross peaks. A mixed cross peak appears as a combination of two positive and two negative cross peaks (*).

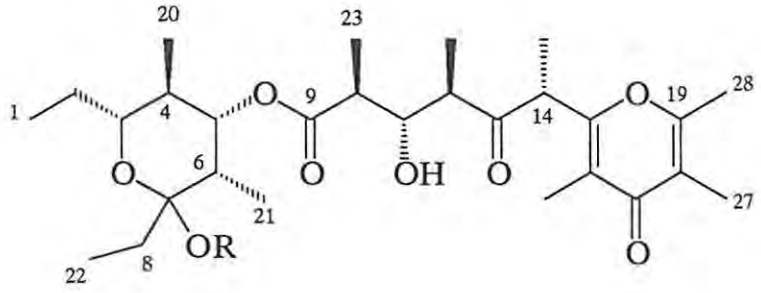
Unfortunately the H-20 (δ 0.76) and H-21 (δ 0.75) methyl resonances were insufficiently separated to enable NOE correlations to be attributed to the one and not the other. However, careful consideration of the system lead to the conclusion that the proton H-3 and the methyl protons H-21 will be spatially too far apart for a nuclear Överhauser effect to be observed irrespective of the configurations of C-3 and C-6. The strong correlation between H-3 and the H-20, H-21 multiplet can thus be attributed to a correlation between H-3 and the methyl protons H-20, thereby placing the C-4 methyl substituent in the equatorial position. The correlation observed between this multiplet and H-5 can thus also be attributed to H-20. There is a very strong correlation between H-4 and the dimethyl multiplet, which from an examination of the stereochemistry proposed is largely attributed to H-21, but may be enhanced by correlation to H-20.

The proposed stereochemistry was supported by an examination of the H-5 and H-6 coupling constants. The large $J_{4,5}$ coupling constant (11.2 Hz) was indicative of a *trans* diaxial relationship between H-4 and H-5, while the $J_{5,6}$ coupling constant (4.9 Hz) supported a gauche relative configuration.[21]

The stereochemistry at C-7 arises from an achiral carbonyl and can readily isomerise in a slightly acidic aqueous medium. It is predicted that the most stable isomer will be one in which the alkyl group is equatorial and the hydroxyl substituent is axial, but the stereochemistry remains unassigned.

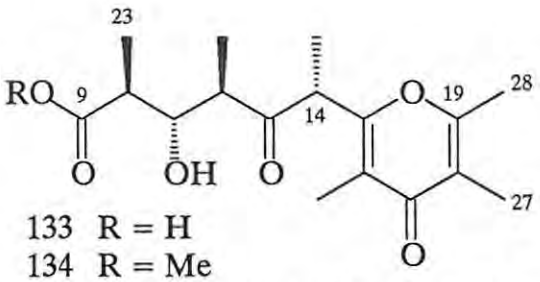
It was proposed that the absolute configuration of the secondary alcohol, C-11, could be established using the modified Mosher's method. Several attempts to prepare the MTPA esters of **131** resulted in low yields (1.5 mg, 16%) of a mixture of the diesters and degradation products. The appearance of the diester in the reaction products necessitated a re-evaluation of the approach to this problem. It was apparent that it was necessary to first protect the hemi-ketal hydroxyl group in order to prepare the mono MTPA esters at C-11. The hemi-ketal hydroxyl functionality was thus protected by stirring a portion of compound **131** in methanol acidified with catalytic amounts of sulphuric acid. TLC analysis revealed that the methyl ketal (**132**) was prepared quantitatively and the Mosher's esters were subsequently prepared from this derivative. Unfortunately TLC analysis revealed the presence of up to seven products in

quantities too small for characterisation and the experiment could not be repeated because the remaining siserrone A had degraded while in solution in ethyl acetate.

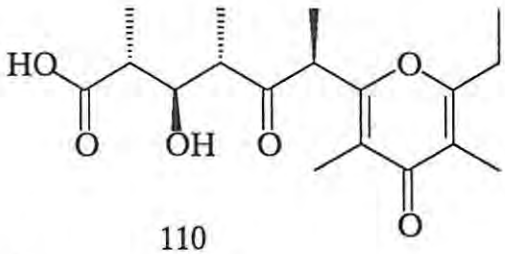


131 R = H
132 R = Me

Further TLC investigation of this ethyl acetate solution of degraded **131** revealed the presence of a number of non-polar compounds and at least one very polar compound (R_f = 0). The ethyl acetate was removed *in vacuo* and the siserrone A degradation products were partitioned between hexane and acetonitrile. The hexane fraction appeared to contain a number of different compounds, while the acetonitrile fraction contained only the acid (**133**) which fortuitously still contained the intact C-11 hydroxyl moiety. The structure of the acid followed from comparison of the ^{1}H and ^{13}C NMR data with the corresponding data for **131**. In addition, comparison of the NMR chemical shifts with those reported for the ethyl homologue, **110** which has been synthesised,[80] suggested that the two acids had the same relative stereochemistry from C-10 to C-14 (Table 8).



133 R = H
134 R = Me
135 R = Me, stereochemistry not assigned



110

In order to prevent trans-esterification in the preparation of the Mosher's esters of **133**, the acid was methylated using an ethereal diazomethane solution. Paradoxically two products (**134** and **135**) in a 4:1 ratio were obtained and the ^{1}H NMR spectra of these two compounds are shown in Figure 28. Standard COSY, HMQC and HMBC NMR experiments confirmed that these two esters and were diastereomeric.

Table 8: A comparison of the NMR chemical shifts for acids **110** and **133**.

	^{1}H †	**110**	**133**	^{13}C ‡	**110**	**133**
9		----	----		177.6	177.6
10		2.71 *qd* (7.2, 2.0 Hz)	2.70 *qd* (7.2, 2.5 Hz)		40.4	40.5
11		3.60 *dd* (9.7, 1.9 Hz)	3.61 *dd* (9.6, 2.3 Hz)		77.6	77.5
12		2.86 *dq* (9.7, 6.7 Hz)	2.86 *dq* (9.6, 6.8 Hz)		48.5	48.4
13		----	----		209.6	209.7
14		4.17 *q* (6.8 Hz)	4.12 *q* (6.9 Hz)		51.4	51.3
15		----	----		162.0	162.0 [b]
16		----	----		120.7	120.7
17		----	----		180.6	180.2
18		----	----		118.6	119.4
19		----	----		166.1	161.8 [b]
23		1.35 *d* (7.2 Hz)	1.32 *d* (7.2 Hz)		15.1	15.0
24		0.89 *d* (6.7 Hz)	0.90 *d* (6.7 Hz)		13.9	14.0
25		1.36 *d* (6.8 Hz)	1.35 *d* (6.9 Hz)		12.7	12.8
26		2.14 *s*	2.09 *s*		10.2 [a]	10.1 [c]
27		1.93 *s*	1.91 *s*		9.7 [a]	10.0 [c]
28		2.58 *q* (7.6 Hz)	2.23 *s*		24.8	17.7
29		1.14 *t* (7.6 Hz)	----		11.3	----

† 400 MHz, $CDCl_3$ ‡ 100.6 MHz (**110**), 100 MHz (**133**), $CDCl_3$

The assignments with like superscripts may be interchanged

Examination of the chemical structure of **134** and **135** reveals that C-14 might be expected to isomerise via a keto-enol tautomerism in which the enol tautomer is resonance stabilised. Comparison of the ^{13}C NMR data for the two compounds revealed that the most significant differences in chemical shift for the two esters occurred at C-14 ($\Delta\delta_{C\text{-}14}$ = 3.3 ppm) supporting the proposal that the esters are epimeric at this carbon. However, the most significant differences in the ^{1}H NMR data occur at the C-23 and C-24 methyl resonances. The methyl ester of **110** was prepared by Paterson *et al.*[80] and a comparison of the ^{13}C and ^{1}H NMR chemical shifts for the synthetic compound and the major diastereomer, **134**, suggests that the relative stereochemistry of these two compounds is the same from C-10 to C-14 (Table 9). It is not known at which stage isomerisation took place, as no replication of signals was evident in the ^{13}C NMR spectrum of the acid, **133**, and no mechanism is proposed for isomerisation in ethereal diazomethane.

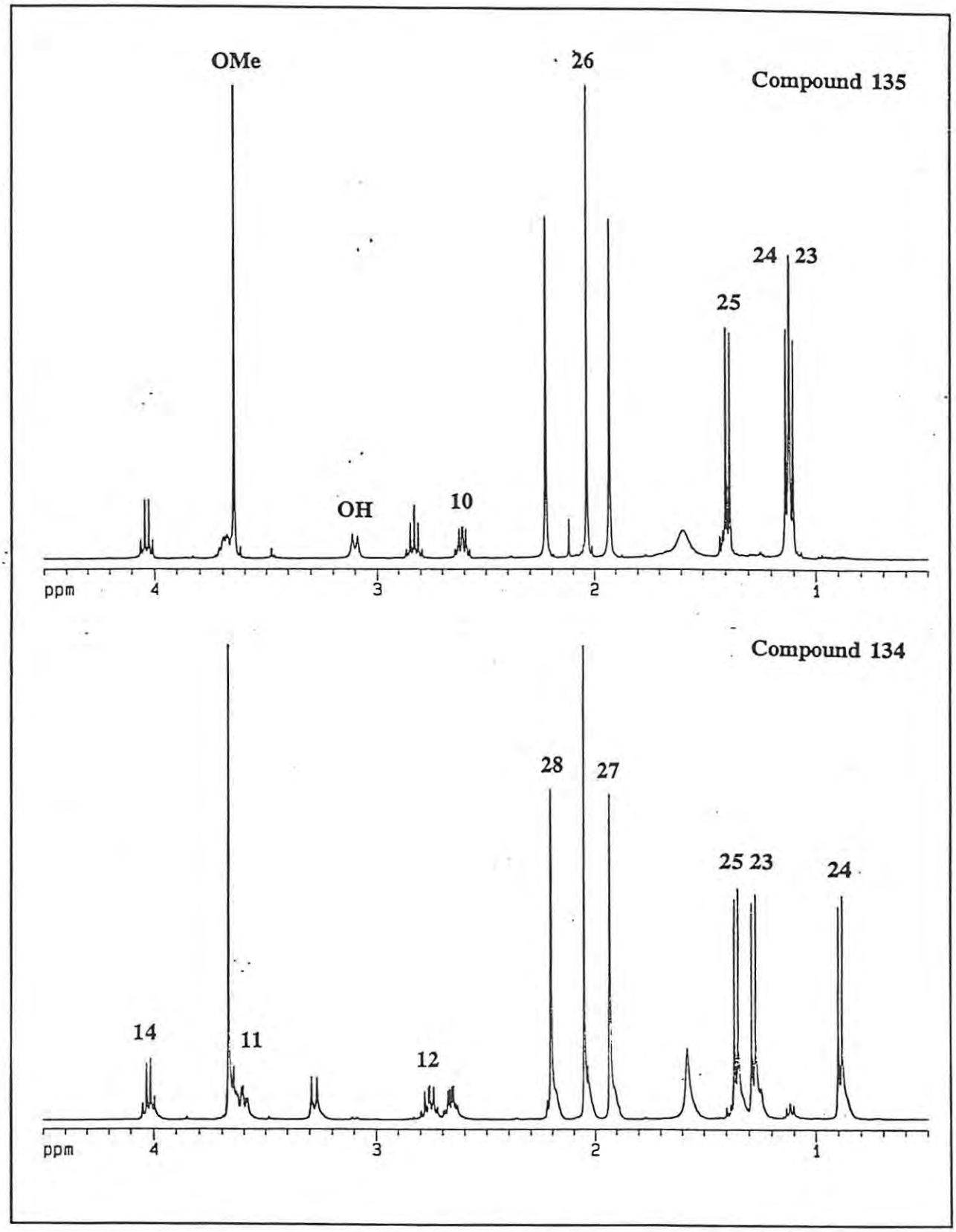


Figure 28: ^{1}H NMR spectra of the esters **134** and **135**

Table 9: A comparison of the NMR chemical shifts for the methyl ester of **110** and the diastereomers **134** and **135**.

^{1}H †	Me-**110**	**134**	**135**	^{13}C ‡	Me-**110**	**134**	**135**
9	----	----	----		175.9	175.8	175.2
10	2.67	2.66	2.61		40.9	41.0	42.1
11	3.61	3.60	3.68		77.4	77.4	76.0
12	2.75	2.76	2.83		48.3	48.2	47.2
13	----	----	----		210.0	210.0	210.4
14	4.05	4.02	4.03		51.2	51.2	47.9
15	----	----	----		160.5	160.6	159.9
16	----	----	----		120.3	120.4	120.3
17	----	----	----		180.6	179.4	179.2
18	----	----	----		118.4	119.3	119.2
19	----	----	----		164.9	160.5	160.7
23	1.28	1.28	1.11		15.1	15.0	14.6
24	0.87	0.89	1.13		14.1	14.1	15.0
25	1.36	1.36	1.34		12.8	12.8	13.2
26	2.07	2.05	2.03		10.0 [a]	9.9 [b]	10.0
27	1.94	1.93	1.93		9.5 [a]	10.0 [b]	10.0
28	2.55	2.21	2.23		24.7	17.5	17.5
29	1.15	----	----		11.3	----	----
OMe	3.68	3.67	3.65		52.0	52.0	51.8
OH	3.33	3.28	3.10		----	----	----

† 400 MHz, $CDCl_3$ ‡ 100.6 MHz (Me-**110**), 100 MHz (**134**, **135**), $CDCl_3$

The assignments with like superscripts may be interchanged

The (R)- and (S)-MTPA esters were prepared from the major diastereomer, **134**, the ^{1}H chemical shifts assigned and the $\Delta\delta$ values calculated (Figure 29). Clearly, the secondary hydroxyl is too sterically hindered to allow the MTPA ester to adopt the ideal conformation necessary for the application of the Mosher's rule. This anomaly in the modified Mosher's method has been reported previously by Kusumi and co workers.[88] They could explain their observations by orientating the MTPA moiety such that the protons above the plane of the macrocyclic compound were deshielded in the (R)- relative to the (S)-ester and vice versa for the protons below the plane of the compound. However, no inferences concerning the configuration of the secondary alcohol could be made.[88] Similarly, the results depicted in Figure 29 can not be used to establish the configuration at C-11. As Kusumi *et al.* point out,

these results highlight the advantage of the modified Mosher's method by revealing that it has a "self-examining function to inspect if the result obtained is valid or not", because of the reliance of the method on many data points.[88]

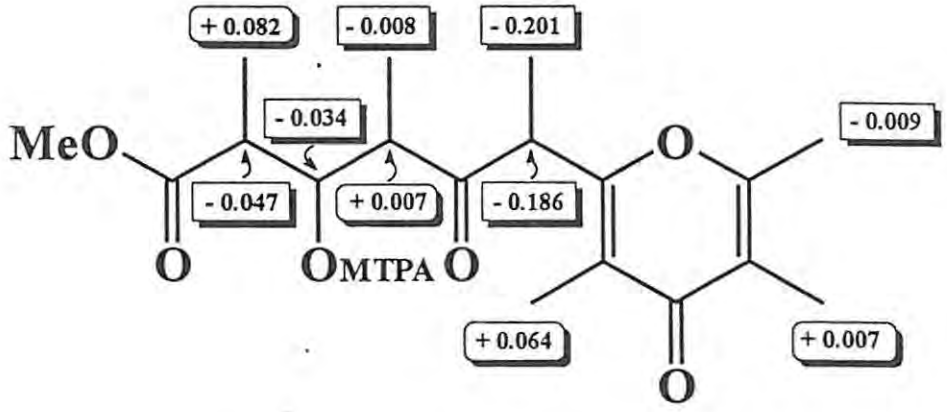


Figure 29: The $\Delta\delta$ values for the MTPA esters of the major diastereomer **134**

In conclusion, it can be said that this study has revealed a relative stereochemistry of the cyclic hemi-ketal in **131** in accordance with the relative stereochemistry predicted from an unfolding of dihydrosiphonarin A (Scheme 5), lending support for the proposal that they arise from a common acyclic precursor. The absolute stereochemistry is thus probably consistent with the enantiomer shown. It has also been established that the modified Mosher's method can not be used to determine the absolute stereochemistry of the secondary alcohol in siserrone A, but the relevant NMR data suggests that the relative stereochemistry of the acyclic chain in **131** is consistent with the stereochemistry of **108.** The absolute stereochemistry of the degradation product **133**, and hence of the natural product **131**, is proposed to be enantiomeric to the synthetic compound **110** in accordance with the proven stereochemistry of related natural products.

CHAPTER 3

Experimental

3.1 GENERAL EXPERIMENTAL

All solvents were redistilled before use. Benzene, hexane and EtOAc were dried over anhydrous Na_2SO_4 prior to distillation. The Me_2CO required for the preparation of acetonides was refluxed over K_2MnO_4, dried over K_2CO_3, distilled, filtered and stored over molecular sieves. Analytical grade solvents were used for HPLC.

Normal phase TLC was performed on DC-Alufolien Kieselgel $60F_{254}$ and reverse phase TLC was performed on DC-Fertigplatten RP-$18F_{254}$ plates. The plates were viewed under 254 nm UV light and were developed by spraying with 10% H_2SO_4 in MeOH followed by heating for a few minutes. Column chromatography was performed using Merck 7734 Kieselgel 60 (230-400 mesh) silica and flash chromatography was performed using Merck 9385 Kieselgel 60 (230-400 mesh) silica.

The HPLC system used in the purification of natural products and their derivatives inlcuded a Spectra-Physics IsoChrom LC or Spectra-Physics SpectraSERIES P100 pump equipped with a Rheodyne injector, a Waters R401 differential refractometer and a Rikadenki chart recorder. A semi-prep. Whatman Magnum 9-Partisil 10 column was used for normal phase separations and in reverse phase separations a Phenomenex Selectosil C18 column was used.

Optical rotations were measured on a Perkin-Elmer 141 polarimeter. All CD spectra were obtained by Professor D. Ferreira at the University of the Orange Free State, Bloemfontein. IR spectra were recorded on a Perkin-Elmer 180 Grating infrared spectrophotometer run neat on NaCl (applied in $CHCl_3$) or AgCl (applied in MeOH) discs. Low-resolution mass spectra (EIMS) were obtained by direct probe analysis on a Hewlett-Packard 5988A spectrometer and all high-resolution spectra (HREIMS) were obtained by Dr P. Boshoff of the Mass Spectrometry Unit at the Cape Technikon (Cape Town) on a Kratos MS 80 RF double-focusing magnetic sector instrument.

The ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra, as well as the NOEDS, ^1H decoupling, DEPT and 2-D experiments, were recorded on a Bruker AMX400 NMR spectrometer. ^1H and ^{13}C chemical shifts are reported in δ units (ppm) and are referenced to residual protonated solvent.

3.2 *CRYPTOCARYA LATIFOLIA*

Acetonides **72** *and* **73**. A solution of **74** (0.20 g) in methanol (2 mL) and 0.22 M NaOH (17 mL) was left to stand overnight, the methanol removed by evaporation under reduced pressure, the soln acidified with 2 M HCl (3.0 mL) and heated (2 min) on a steambath to complete lactonisation of the free acid. The cooled soln was saturated by the addition of NaCl (5 g) to facilitate extraction of the extremely water soluble product with ether in an efficient continuous extraction apparatus (10 h). A soln of the product (132 mg) in dry Me_2CO (2 mL) was placed on a column (1.8 × 14 cm) of Amberlyst-15 resin [previously equilibrated for an hour in dry Me_2CO (120 mL)] for 20 min and then slowly eluted with dry Me_2CO. The product was chromatographed on alumina with benzene to afford an oil (70 mg), shown by TLC in EtOAc on silica gel to consist of two closely moving spots. These spots were separated by semiprep. HPLC on a normal phase column (EtOAc eluent) to give the acetonides **72** and **73** as oils (20 and 25 mg respectively).

Compound **72**. $[\alpha]^{30}_D$ + 83° ($CHCl_3$; c 1.8). EIMS (70 eV) *m/z* (rel. int.): No $[M]^+$, 269 (13), 223 (5), 206 (6), 167 (7), 150 (17), 149 (100), 141 (22), 97 (50), 85 (46), 71 (89), 69 (50), 57 (98), 55 (52), 43 (96), 41 (84). HREIMS: $[M]^+$ 284.1633. $C_{15}H_{24}O_5$ requires 284.1624. ^1H NMR data are presented in Table 2. ^{13}C NMR data are presented in Table 3.

Compound **73**. $[\alpha]^{30}_D$ + 58.5° ($CHCl_3$; c 1.1). EIMS (70 eV) *m/z* (rel. int.): No $[M]^+$, 269 (37), 225 (6), 165 (16), 149 (11), 147 (9), 141 (21), 123 (9), 97 (88), 69 (33), 68 (43), 59 (60), 45 (46), 43 (100) 41 (65). HREIMS: $[M]^+$ 284.1641. $C_{15}H_{24}O_5$ requires 284.1624. ^1H NMR data are presented in Table 2. ^{13}C NMR data are presented in Table 3.

(S)- and (R)-MTPA esters of acetonides **72** *and* **73**. The following preparation is representative of the procedure used. A soln of **72** (8 mg) and DMAP (9 mg) in CH_2Cl_2 (1 mL) was added to a soln of (R)-MTPA (25 mg) and DCC (55 mg) in CH_2Cl_2 (0.2 mL). The mixture was shaken periodically and after 30 min was diluted with water and EtOAc (10 mL). The EtOAc layer was washed with 0.2 M HCl, H_2O, aqueous $NaHCO_3$, H_2O, dried and evaporated. The residue was chromatographed on silica gel in benzene-hexane-EtOAc. The fr. eluted with EtOAc-hexane (1:2) afforded needles (9 mg) of the (R)-MTPA ester.

(S)-MTPA ester of **72**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09 (1H, *m*, H-5'), 1.13 (3H, *d*, $J_{6',7'}$ = 6.1 Hz, H-7'), 1.29 (1H, *m*, H-5'), 1.54 (3H, *s*, H-10'), 1.54 (3H, *s*, H-9'), 1.76 (1H, *m*, H-3'), 1.91 (1H, *m*, H-3'), 2.04 (1H, *m*, H-1'), 2.23 (1H, *m*, H-1'), 2.31 (2H, *m*, H-5), 3.52 (3H, *s*, OMe), 3.81 (1H, *m*, H-4'), 3.82 (1H, *m*, H-6'), 4.48 (1H, *m*, H-6), 5.40 (1H, *m*, H-2'), 6.01 (1H, *dd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 1.4 Hz, H-3), 6.83 (1H, *m*, H-4), 7.41 (2H, *m*, Ph), 7.53 (2H, *m*, Ph).

(R)-MTPA ester of **72**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.15 (1H, *m*, H-5'), 1.15 (3H, *d*, $J_{6',7'}$ = 6.1 Hz, H-7'), 1.34 (3H, *s*, H-10'), 1.36 (3H, *s*, H-9'), *1.47 (1H, m, H-5'),* 1.79 (1H, *m*, H-3'), 1.95 (1H, *m*, H-3'), 1.95 (1H, *m*, H-1'), 2.17 (1H, *m*, H-1'), 2.17 (2H, *m*, H-5), 3.53 (3H, *s*, OMe), 3.93 (1H, *m*, H-4'), 3.93 (1H, *m*, H-6'), 4.30 (1H, *m*, H-6), 5.41 (1H, *m*, H-2'), 5.96 (1H, *dd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 1.3 Hz, H-3), 6.77 (1H, *m*, H-4), 7.39 (2H, *m*, Ph), 7.52 (2H, *m*, Ph).

(S)-MTPA ester of **73**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (1H, *m*, H-3'), 1.27 (3H, *d*, $J_{6',7'}$ = 6.3 Hz, H-7'), 1.33 (3H, *s*, H-10'), 1.36 (3H, *s*, H-9'), 1.52 (1H, *m*, H-3'), 1.63 (1H, *m*, H-5'), 1.74 (1H, *m*, H-1'), 1.94 (1H, *m*, H-5'), 2.02 (1H, *m*, H-1'), 2.37 (2H, *m*, H-5), 3.52 (3H, *s*, OMe), 3.90 (1H, *m*, H-4'), 4.09 (1H, *m*, H-2'), 4.58 (1H, *m*, H-6), 5.30 (1H, *m*, H-6'), 6.02 (1H, *dd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 1.6 Hz, H-3), 6.88 (1H, *m*, H-4), 7.41 (2H, *m*, Ph), 7.52 (2H, *m*, Ph).

(R)-MTPA ester of **73**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (1H, *m*, H-3'), 1.27 (3H, *s*, H-10'), 1.30 (3H, *s*, H-9'), 1.36 (3H, *d*, $J_{6',7'}$ = 6.1 Hz, H-7'), 1.45 (1H, *m*, H-3'), 1.58 (1H, *m*, H-5'), 1.70 (1H, *m*, H-1'), 1.89 (1H, *m*, H-5'), 2.00 (1H, *m*, H-1'), 2.37 (2H, *m*, H-5), 3.53 (3H, *s*, OMe), 3.74 (1H, *m*, H-4'), 3.99 (1H, *m*, H-2'), 4.57 (1H, *m*, H-6), 5.26 (1H, *m*, H-6'), 6.02 (1H, *dd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 1.6 Hz, H-3), 6.88 (1H, *m*, H-4), 7.39 (2H, *m*, Ph), 7.52 (2H, *m*, Ph).

Acetonide **75** *from saponified diacetate* **76**. Diacetate (0.11 g) was saponified and converted to the acetonide as described above and the product purified by HPLC. EIMS (70 eV) *m/z* (rel. int.): No $[M]^+$, 225 (61), 165 (5), 147 (7), 141 (10), 139 (6), 97 (100), 68 (32), 59 (43), 43 (90), 41 (34). HREIMS: $[M]^+$ 240.1354. $C_{13}H_{20}O_4$ requires 240.1361. ^{1}H NMR data are presented in Table 2. ^{13}C NMR data are presented in Table 3.

3.3 *SYNCOLOSTEMON DENSIFLORUS*

Acetonide **80** *from saponified syndenolide* **81**. A soln of syndenolide (28.3 mg) in methanol (0.5 mL) and 0.2 M NaOH (1.5 mL) was left overnight and the methanol evaporated. The soln was acidified with HCl, heated for 2 min, cooled, saturated with NaCl and extracted continuously with ether for 36 h. The product (18.9 mg) was converted to the acetonide as before to afford an oil (6.9 mg) whose ^{1}H NMR spectrum suggested that two α-pyrone products were present in 3:1 ratio. Semiprep. HPLC on a normal phase column (1:1 hexane/EtOAc) afforded the major product, acetonide **80** (2.8 mg). EIMS (70 eV) *m/z* (rel. int.): No $[M]^+$, 325 (21), 265 (3), 207 (8), 151 (7) 143 (39), 97 (42), 85 (41), 69 (23), 59 (53), 55 (16), 43 (100), 41 (32). HREIMS: $[M]^+$ 340.1873. $C_{18}H_{28}O_6$ requires 340.1886. ^{1}H NMR and ^{13}C NMR data are presented in Table 4.

3.4 *SYNCOLOSTEMON ARGENTEUS*

S. argenteus, collected from the Ongoya forest in Natal, was received from Mr Edwards, University of Natal, Pietermaritzburg in August 1995. No voucher specimens could be taken presumably because of lack of flowers, but a specimen had been collected previously from the same population (Hilliard and Burtt 5638, December 1968).

Isolation. 5,6-Dihydro-α-pyrones **82**, **86**, **91**, **92** and **93** were isolated from *S. argenteus* as depicted in Scheme 1.

Synargentolide A, **82**. $[\alpha]_D^{23}$ = +40° ($CHCl_3$; c 1.1). CD (MeOH) λ_{max} 265 nm ($\Delta\epsilon$ = +3.5). IR $\nu_{max}cm^{-1}$ (NaCl): 1735, 1360, 1215, 1055, 1020, 940, 800. EIMS (70 eV) *m/z* (rel. int.): No $[M]^+$, 248 (3), 231 (2), 206 (15), 204 (11), 188 (15), 162 (17), 149 (15), 129 (25), 120 (26), 81 (28), 69 (24), 68 (80), 43 (100). HREIMS: $[M]^+$ 368.1464. $C_{18}H_{24}O_8$ requires 368.1469. ^1H NMR data are presented in Table 5. ^{13}C NMR data are presented in Table 6.

Synargentolide B, **86**. $[\alpha]_D^{25}$ = +45.6° (MeOH; c 1.2). CD (MeOH) λ_{max} 258 nm ($\Delta\epsilon$ = +3.2). IR $\nu_{max}cm^{-1}$ (AgCl): 3450, 1735, 1370, 1240, 1060, 1025, 950, 800. EIMS (70 eV) *m/z* (rel. int.): No $[M]^+$, 206 (3), 167 (5), 149 (100), 141 (13), 128 (17), 113 (18), 97 (37), 85 (47), 81 (34), 71 (59), 69 (39), 57 (67), 43 (33). HREIMS: $[M]^+$ 342.1498. $C_{16}H_{22}O_8$ requires 342.1298. ^1H NMR data are presented in Table 5. ^{13}C NMR data are presented in Table 6.

Synargentolide C, **91**. $[\alpha]_D^{25}$ = +140° (MeOH; c 0.72). CD (MeOH) λ_{max} 266 nm ($\Delta\epsilon$ = +2.0). IR $\nu_{max}cm^{-1}$ (AgCl): 3450, 1735, 1370, 1230, 1090, 1020, 945, 920, 805. EIMS (70 eV) *m/z* (rel. int.): No $[M]^+$, 198 (3), 194 (4), 193 (3), 185 (4), 155 (18), 149 (14), 143 (15), 126 (34), 125 (23), 113 (32), 99 (35), 97 (93), 96 (73), 95 (82), 86 (32), 81 (100), 71 (19), 69 (19), 57 (23), 43 (86). HREIMS: $[M]^+$ 400.1356. $C_{18}H_{24}O_{10}$ requires 400.1369. ^1H NMR data are presented in Table 5. ^{13}C NMR data are presented in Table 6.

Synargentolide D, **92**. ^1H NMR data are presented in Table 5. ^{13}C NMR data are presented in Table 6.

Synargentolide E, **93**. $[\alpha]_D^{25}$ = +53° (MeOH; c 0.61). CD (MeOH) λ_{max} 265 nm ($\Delta\epsilon$ = +1.1). IR ν_{max}cm^{-1} (AgCl): 3450 (OH), 1735, 1725 (sh), 1370, 1235, 1095, 1050, 1020, 995, 927, 805. EIMS (70 eV) *m/z* (rel. int.): No $[M]^+$, 224 (2), 181 (5), 164 (5), 153 (13), 148 (13), 125 (15), 117 (23), 99 (51), 97 (78), 96 (33), 85 (30), 83 (26), 81 (54), 69 (17), 68 (22), 57 (30), 43 (100). HREIMS: $[M]^+$ 400.1353. $C_{18}H_{24}O_{10}$ requires 400.1369. ^{1}H NMR data are presented in Table 5. ^{13}C NMR data are presented in Table 6.

Acetonides **83** *and* **84**. A solution of **82** (118.4 mg) in methanol (2 mL) and 1 M NaOH (2 mL) was left for 2.5 days at 5°C. The soln was acidified with 1 M HCl (2 mL) and heated (2 min) on the steambath. The cooled soln was saturated by the addition of NaCl (1 g) and the product extracted with ether for 24 h. Excess water was removed azeotropically with benzene under vacuum. A soln of product (69 mg) in dry Me_2CO (5 mL) was left on a column (33 × 1 cm) of Amberlyst-15 resin [previously left to equilibrate for 1.5 hours in dry Me_2CO (60 mL)] for 25 min and then eluted with Me_2CO (90 mL) and concentrated *in vacuo*. The product was chromatographed on Merck neutral alumina (20 g) in EtOAc to remove sulphurous acid residues. The acetone condensation product, diacetone alcohol (not encountered previously) was removed under high vacuum at 60° to afford an oil (36.1 mg) which was purified by semiprep. HPLC on a normal phase column (EtOAc eluent) to give the acetonide **83** and compound **84** as oils (24.1 and 1.9 mg respectively).

Acetonide **83**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20 (3H, *d*, $J_{6',7'}$ = 5.9 Hz, H-7'), 1.36 (3H, *s*, H-10'), 1.37 (3H, *s*, H-9'), 2.00 (1H, *s*, OH), 2.34 (1H, *m*, H-3'), 2.43 (2H, *m*, H-5), 2.45 (1H, *m*, H-3'), 3.56 (1H, *m*, H-5'), 3.87 (1H, *m*, H-6'), 4.02 (1H, *m*, H-4'), 4.88 (1H, *m*, H-6), 5.68 (1H, *dd*, $J_{6,1'}$ = 6.7 Hz, $J_{1',2'}$ = 15.5 Hz, H-1'), 5.90 (1H, *m*, H-2'), 6.01 (1H, *dd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 1.8 Hz, H-3), 6.86 (1H, *m*, H-4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3 (C-7'), 27.0 (C-10'), 27.3 (C-9'), 29.6 (C-5), 36.9 (C-3'), 67.5 (C-6'), 76.6 (C-4'), 77.8 (C-6), 83.6 (C-5'), 108.6 (C-8'), 121.5 (C-3), 129.4 (C-1'), 130.8 (C-2'), 144.7 (C-4), 164.0 (C-2). EIMS (70 eV) *m/z* (rel. int.): No $[M]^+$, 267 (20), 167 (3), 149 (9), 145 (17), 138 (34), 133 (8), 101 (16), 68 (63), 59 (85), 55 (23), 45 (32), 43 (100), 41 (47), 39 (37).

Compound **84**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30 (3H, *d*, $J_{6',7'}$ = 6.2 Hz, H-7'), 1.31 (3H, *d*, $J_{8',9'}$ = 5.1 Hz, H-9'), 1.57 (1H, *s*, OH), 2.38 (1H, *m*, H-3'), 2.43 (2H, *m*, H-5), 2.57 (1H, *m*, H-3'), 3.06 (1H, *m*, H-5'), 3.39 (1H, *m*, H-4'), 3.44 (1H, *m*, H-6'), 4.90 (1H, *dt*, $J_{5,6}$ =

7.2 Hz, $J_{6,1'}$ = 7.2 Hz, H-6), 4.73 (1H, *q*, $J_{8',9'}$ = 5.1 Hz, H-8'), 5.71 (1H, *m*, H-1'), 5.95 (1H, *m*, H-2'), 6.04 (1H, *dt*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 1.8 Hz, H-3), 6.87 (1H, *m*, H-4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.9 (C-7'), 20.6 (C-9'), 29.8 (C-5), 34.6 (C-3'), 71.5 (C-5'), 77.2 (C-6'), 78.0 (C-6), 79.5 (C-4'), 98.4 (C-8'), 121.7 (C-3), 129.5 (C-1'), 130.8 (C-2'), 144.6 (C-4).

(R)- and (S)-MTPA esters of acetonide **83**. The following preparation is representative. A soln of **82** (4.4 mg) and DMAP (5.2 mg) in CH_2Cl_2 (1 mL) was added to a soln of (R)-MTPA (16 mg) and DCC (33 mg) in CH_2Cl_2 (0.2 mL). The mixture was shaken periodically and after 90 min was diluted with a few drops of water and EtOAc (10 mL). The EtOAc layer was filtered through cotton wool to remove the dicyclohexylurea precipitate, washed with 0.2 M HCl, H_2O, aqueous $NaHCO_3$, H_2O, dried and evaporated. The residue was chromatographed on silica gel in benzene-hexane-EtOAc. The fr. eluted with 2:1 hexane/EtOAc afforded (7.6 mg) of the (R)-MTPA ester.

R-MTPA ester of **83**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.29 (3H, *s*, H-9' or 10'), 1.34 (3H, *s*, H-9' or 10'), 1.42 (3H, *d*, $J_{6',7'}$ = 6.3 Hz, H-7'), 2.04 (1H, *m*, H-3'), 2.15 (1H, *m*, H-3'), 2.40 (2H, *m*, H-5), 3.57 (3H, *s*, OMe), 3.64 (1H, *dd*, $J_{4',5'}$ = 7.0 Hz, $J_{5',6'}$ = 7.0 Hz, H-5'), 3.74 (1H, *m*, H-4'), 4.83 (1H, *m*, H-6), 5.05 (1H, *m*, H-6'), 5.51 (1H, *dd*, $J_{6,1'}$ = 6.2 Hz, $J_{1',2'}$ = 15.6 Hz, H-1'), 5.64 (1H, *m*, H-2'), 6.04 (1H, *d*, $J_{3,4}$ = 9.8 Hz, H-3), 6.87 (1H, *m*, H-4), 7.38 (2H, *m*, Ph), 7.51 (2H, *m*, Ph).

S-MTPA ester of **83**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.34 (3H, *s*, H-9' or 10'), 1.36 (3H, *d*, $J_{6',7'}$ = 6.8 Hz, H-7'), 1.37 (3H, *s*, H-9' or 10'), 2.24 (1H, *m*, H-3'), 2.35 (1H, *m*, H-3'), 2.39 (2H, *m*, H-5), 3.48 (3H, *s*, OMe), 3.71 (1H, *dd*, H-5'), 3.87 (1H, *m*, H-4'), 4.86 (1H, *m*, H-6), 5.10 (1H, *m*, H-6'), 5.60 (1H, *dd*, $J_{6,1'}$ = 6.3 Hz, $J_{1',2'}$ = 15.6 Hz, H-1'), 5.81 (1H, *m*, H-2'), 6.03 (1H, *dd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 1.6 Hz, H-3), 6.85 (1H, *m*, H-4), 7.41 (2H, *m*, Ph), 7.50 (2H, *m*, Ph).

Acetonide **85**. Surprisingly, the acetonide **85** was not formed previously when acetonides were prepared from **82**. The saponification of synargentolide A (22.4 mg) and acetonide preparation was thus repeated in the usual manner using fresh Amberlyst-15 resin. In order to ensure that all unbonded acid was removed from the column prior to acetonation of saponified **82**, the

column was left to stand for two periods of 1.5 hours in dry Me_2CO (25 mL) each followed by washing with more Me_2CO (25 mL). Once again the diacetone alcohol condesation product was obtained and was removed under vacuum to yield a mixture of acetonides (11.8 mg) which were separated by semiprep. HPLC on a normal phase column (1:9 hexane/EtOAc eluent) to give acetonide **83** as the major product and acetonide **85** (1.0 mg) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (3H, *d*, $J_{6',7'}$ = 5.9 Hz, H-7'), 1.38 (3H, *s*, H-9'), 1.47 (3H, *s*, H-10'), 1.53 (1H, *s*, OH), 2.32 (1H, *m*, H-3'), 2.43 (2H, *m*, H-5), 2.50 (1H, *m*, H-3'), 3.00 (1H, *m*, H-5'), 3.65 (1H, *m*, H-4'), 3.70 (1H, *m*, H-6'), 4.90 (1H, *m*, H-6), 5.69 (1H, *dd*, $J_{6,1'}$ = 6.7 Hz, $J_{1',2'}$ = 15.5 Hz, H-1'), 5.93 (1H, *m*, H-2'), 6.04 (1H, *dd*, $J_{3,4}$ = 9.8 Hz, $J_{3,5}$ = 1.8 Hz, H-3), 6.86 (1H, *m*, H-4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.4 (C-7'), 19.5 (C-9'), 29.5 (C-10'), 29.8 (C-5), 34.9 (C-3'), 69.8 (C-6'), 72.7 (C-4'), 72.8 (C-5'), 78.0 (C-6), 98.5 (C-8'), 121.7 (C-3), 129.3 (C-1'), 131.0 (C-2'), 144.6 (C-4).

Acetonide **87**. Acetonide **87** was prepared from **86** (9.0 mg) as above using a column of Amberlyst-15 resin which had been allowed to equilibrate in dry Me_2CO (45 min). The product was isolated in the usual manner. It was evident from TLC on silica gel in 4:6 hexane/EtOAc that the product consisted of a single compound, acetonide **87** (7.2 mg) and no further purification was required. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20 (3H, *d*, $J_{6,7}$ = 6.5 Hz, H-7'), 1.41 (3H, *s*, H-10'), 1.42 (3H, *s*, H-9'), 2.03 (3H, *s*, Ac-Me), 2.07 (3H, *s*, Ac-Me), 2.52 (2H, *m*, H-5), 3.87 (1H, *dd*, $J_{6,1'}$ = 7.0 Hz, $J_{1',2'}$ = 7.4 Hz, H-1'), 4.43 (1H, *td*, $J_{5,6}$ = 7.6 Hz, $J_{6,1'}$ = 6.9 Hz, H-6), 4.48 (1H, *dd*, $J_{1',2'}$ = 7.4 Hz, $J_{2',3'}$ = 4.3 Hz, H-2'), 5.06 (1H, *dq*, $J_{5',6'}$ = 3.5 Hz, $J_{6',7'}$ = 6.6 Hz, H-6'), 5.39 (1H, *dd*, $J_{4',5'}$ = 5.0 Hz, $J_{5',6'}$ = 3.4 Hz, H-5'), 5.86 (1H, *m*, H-3'), 5.86 (1H, *m*, H-4'), 6.02 (1H, *dt*, $J_{3,4}$ = 9.9 Hz, $J_{3,5}$ = 1.6 Hz, H-3), 6.89 (1H, *dt*, $J_{3,4}$ = 9.9 Hz, $J_{4,5}$ = 4.4 Hz, H-4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.0 (C-7'), 21.0 (Ac-Me), 21.1 (Ac-Me), 26.3 (C-5), 26.9 (C-9'), 26.9 (C-10'), 70.6 (C-6'), 74.5 (C-5'), 78.0 (C-6), 79.1 (C-2'), 80.9 (C-1'), 110.4 (C-8'), 121.5 (C-3), 127.3 (C-4'), 132.4 (C-3'), 144.5 (C-4), 162.6 (C-2), 169.9 (Ac-CO), 170.3 (Ac-CO).

Dianisate **90**. Anisoyl chloride (0.1 mL) and benzene (0.5 mL) was added to a soln of **86** (20.3 mg) in pyridine (0.2 mL) resulting in the formation of an immediate white precipitate. The mixture was shaken, warmed slightly, and left to stand (2.5 days). Water (4 drops) was added to quench the reaction and the mixture was heated on a water bath to dissolve the precipitate. The anisate derivatives were extracted with ether, the extract washed with dilute

HCl, H_2O, aqueous $NaHCO_3$ and H_2O, dried and concentrated *in vacuo*. The mixture of products (131.1 mg) in benzene (0.5 mL) was chromatographed on silica gel (2 g) in hexane-EtOAc. The fractions eluted with 1:1 hexane/EtOAc were shown to contain the dianisate **90** and two monoanisate derivatives by TLC on silica gel in 2:8 hexane/EtOAc. The dianisate (2.3 mg) was purified by normal phase semiprep. HPLC (1:4 hexane/EtOAc eluent). CD (MeOH) λ_{max} 278 ($\Delta\epsilon$ = +3.45),λ_{max} 259 ($\Delta\epsilon$ = +1.42), λ_{max} 237 ($\Delta\epsilon$ = +1.76), λ_{max} 221 ($\Delta\epsilon$ = +2.05), λ_{max} 213 ($\Delta\epsilon$ = -2.79). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, *d*, $J_{6',7'}$ = 6.6 Hz, H-7'), 1.91 (3H, *s*, Ac-Me), 1.94 (3H, *s*, Ac-Me), 2.44 (1H, *m*, $J_{4,5e}$ = 6.0 Hz, $J_{5a,5e}$ = 18.4 Hz, H-5e), 2.60 (1H, *m*, H-5a), 3.86 (6H, *s*, OMe), 4.73 (1H, *m*, H-6), 4.95 (1H, *m*, H-6'), 5.28 (1H, *br-s*, H-5'), 5.63 (1H, *dd*, $J_{6,1'}$ = 6.8 Hz, $J_{1',2'}$ = 2.5 Hz, H-1'), 5.85 (1H, *m*, H-3'), 5.85 (1H, *m*, H-4'), 5.95 (1H, *br-s*, H-2'), 6.02 (1H, *dd*, $J_{3,4}$ = 9.5 Hz, $J_{3,5}$ = 1.4 Hz, H-3), 6.84 (1H, *m*, H-4), 6.92 (2H, *d*, J = 8.6 Hz, Ph), 8.00 (2H, *t*, J = 8.6 Hz, Ph).

3.5 *SIPHONARIA SERRATA*

S. serrata was collected at low tide in April 1996 from near the Willows Caravan Park, Port Elizbeth. The isolation of siserrone A (**131**) is depicted in Scheme 4.

Siserrone A, **131**. IR $\nu_{max}cm^{-1}$ (NaCl): 3390, 2980, 1720, 1450, 1380, 1180. HREIMS: $[M]^+$ 508.3040. $C_{28}H_{44}O_8$ requires 508.3033. ^1H and ^{13}C NMR data are presented in Table 7.

Attempted preparation of the MTPA esters of **131**. The following preparation is representative of the procedure used. A soln of **131** (4.6 mg) and DMAP (9.0 mg) in CH_2Cl_2 (1 mL) was added to a soln of (R)-MTPA (15.7 mg) and DCC (32.8 mg) in CH_2Cl_2 (0.2 mL). The mixture was shaken periodically and after 90 min was diluted with a few drops of water and EtOAc (10 mL). The EtOAc layer was filtered through cotton wool to remove the dicyclohexylurea precipitate, washed with 0.2 M HCl, saturated brine soln, aqueous $NaHCO_3$, saturated brine soln, dried and evaporated. The residue was chromatographed on silica gel in benzene-hexane-EtOAc. The fr. containing the Mosher's esters was purified further by normal phase HPLC (hexane/EtOAc 3:7) to yield the R,R-diester and degradation products (1.5 mg).

Preparation of the methyl ketal **132** *from* **131**. A soln of **131** (10 mg) in methanol (2 mL) was stirred for 7 hours and the reaction monitored by normal phase TLC (hexane/EtOAc 2:8). The addition of a catalytic amount of conc H_2SO_4 to the reaction mixture increased the rate of the reaction and yielded **132** quantitatively after 1 hour. The reaction mixture was partitioned between EtOAc (15 mL) and H_2O (20 mL) and the EtOAc fraction was concentrated *in vacuo* to yield **132** (10.3 mg).

Attempted preparation of the (R)- and (S)-MTPA esters of **132**. The procedure for the preparation of the Moshers esters described above was repeated using the methyl ketal **132**. It was apparent from TLC analysis after initial work-up that a number of different products were present in low yields and the experiment was discontinued.

Isolation of acid **133**. TLC analysis revealed that compound **131** had degraded while in solution in EtOAc. The EtOAc was first removed *in vacuo* and the degradation products (28 mg) partitioned between hexane and CH_3CN. The CH_3CN fraction was washed several times

with hexane and concentrated *in vacuo* to yield **133** (23.4 mg). The ^{13}C and ^{1}H NMR data are presented in Table 8.

Methyl esters **134** *and* **135** *from methylation of* **133**. A solution of **133** (23 mg) in methanol (1 mL) was cooled (-10°C) and treated with an excess of ethereal diazomethane solution (prepared from 1.5 g of N-methyl-N-nitroso-p-toluenesulfonamide),[89] allowed to stand (8 min, -10°C) and concentrated *in vacuo* to yield a mixture of two compounds (13.1 mg). These compounds were separated by normal phase HPLC (hexane/EtOAc 2:8) to yield diastereomers **134** and **135** (8.5 and 2.2 mg respectively).

Major diastereomer **134** *(8.5 mg).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, *d*, $J_{12,24}$ = 6.8 Hz, H-24), 1.28 (3H, *d*, $J_{10,23}$ = 7.2 Hz, H-23), 1.36 (3H, *d*, $J_{14,25}$ = 7.0 Hz, H-25), 1.93 (3H, *s*, H-27), 2.05 (3H, *s*, H-26), 2.21 (3H, *s*, H-28), 2.66 (1H, *dq*, $J_{10,11}$ = 3.0 Hz, $J_{10,23}$ = 7.2 Hz, H-10), 2.76 (1H, *dq*, $J_{11,12}$ = 9.1 Hz, $J_{12,24}$ = 6.8 Hz, H-12), 3.28 (1H, *s*, OH), 3.60 (1H, *dd*, $J_{10,11}$ = 3.0 Hz, $J_{11,12}$ = 9.1 Hz, H-11), 3.67 (3H, *s*, OMe), 4.02 (1H, *q*, $J_{14,25}$ = 7.0 Hz, H-14). The ^{13}C NMR data are presented in Table 9.

Minor diastereomer **135** *(2.2 mg).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.11 (3H, *d*, $J_{10,23}$ = 7.0 Hz, H-23), 1.13 (3H, *d*, $J_{12,24}$ = 7.0 Hz, H-24), 1.34 (3H, *d*, $J_{14,25}$ = 7.0 Hz, H-25), 1.93 (3H, *s*, H-27), 2.03 (3H, *s*, H-26), 2.23 (3H, *s*, H-28), 2.61 (1H, *dq*, $J_{10,11}$ = 5.0 Hz, $J_{10,23}$ = 7.0 Hz, H-10), 2.83 (1H, *dq*, $J_{11,12}$ = 7.1 Hz, $J_{12,24}$ = 7.0 Hz, H-12), 3.10 (1H, *s*, OH), 3.65 (3H, *s*, OMe), 3.68 (1H, *dd*, $J_{10,11}$ = 5.0 Hz, $J_{11,12}$ = 7.1 Hz, H-11), 4.03 (1H, *q*, $J_{14,25}$ = 7.0 Hz, H-14). The ^{13}C NMR data are presented in Table 9.

(R)- and (S)-MTPA esters of **134**. The following preparation is representative. A soln of **134** (3.5 mg) and DMAP (6.5 mg) in CH_2Cl_2 (1 mL) was added to a soln of (S)-MTPA (28.8 mg) and DCC (26.5 mg) in CH_2Cl_2 (0.2 mL). The mixture was shaken periodically and after 30 min was diluted with a few drops of water and EtOAc (10 mL). The EtOAc layer was filtered through cotton wool, washed with 0.2 M HCl, H_2O, aqueous $NaHCO_3$, H_2O, dried and evaporated. The residue (8.4 mg) was chromatographed on silica gel in hexane-EtOAc. The fr. eluted with 1:1 hexane/EtOAc afforded (7.4 mg) of the (S)-MTPA ester.

(R)-MTPA ester of **134**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (3H, *d*, $J_{12,24}$ = 7.0 Hz, H-24), 1.09 (3H, *d*, $J_{10,23}$ = 7.2 Hz, H-23), 1.19 (3H, *d*, $J_{14,25}$ = 6.9 Hz, H-25), 1.91 (3H, *s*, H-27), 1.91 (3H, *s*, H-26), 2.19 (3H, *s*, H-28), 2.99 (1H, *dq*, $J_{10,11}$ = 3.1 Hz, $J_{10,23}$ = 7.2 Hz, H-10), 3.12 (1H, *dq*, $J_{11,12}$ = 9.8 Hz, $J_{12,24}$ = 7.0 Hz, H-12), 3.42 (3H, *s*, OMe), 3.65 (3H, *s*, OMe), 3.67 (1H, *q*, $J_{14,25}$ = 6.9 Hz, H-14), 5.57 (1H, *dd*, $J_{10,11}$ = 3.1 Hz, $J_{11,12}$ = 9.8 Hz, H-11), 7.42 (5H, *s*, Ph).

(S)-MTPA ester of **134**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (3H, *d*, $J_{12,24}$ = 6.9 Hz, H-24), 0.99 (3H, *d*, $J_{14,25}$ = 6.8 Hz, H-25), 1.18 (3H, *d*, $J_{10,23}$ = 7.3 Hz, H-23), 1.92 (3H, *s*, H-27), 1.98 (3H, *s*, H-26), 2.18 (3H, *s*, H-28), 2.95 (1H, *dq*, $J_{10,11}$ = 2.8 Hz, $J_{10,23}$ = 7.3 Hz, H-10), 3.12 (1H, *dq*, $J_{11,12}$ = 9.9 Hz, $J_{12,24}$ = 6.9 Hz, H-12), 3.48 (1H, *q*, $J_{14,25}$ = 6.8 Hz, H-14), 3.56 (3H, *s*, OMe), 3.66 (3H, *s*, OMe), 5.53 (1H, *dd*, $J_{10,11}$ = 2.8 Hz, $J_{11,12}$ = 9.9 Hz, H-11), 7.48 (5H, *m*, Ph), 7.52 (2H, *m*, Ph).

References

(1) Davies-Coleman, M. T.; Rivett, D. E. A. *Fortschritte der Chemie Organischer Naturstoffe*, **1989**, 55, 1.
(2) Dickinson, J. M. *Nat. Prod. Reports*, **1993**, 10, 71.
(3) Nagumo, S.; Toyonaga, T.; Inoue, T.; Nagai, M. *Chem. Pharm. Bull.*, **1989**, 37, 2621.
(4) Snatzke, G. *Angew. Chem. Internat. Edit.*, **1968**, 7, 14.
(5) Ayer, W. A.; Racok, J. S. *Can. J. Chem.*, **1990**, 68, 2095.
(6) Koshino, H.; Yoshihara, T.; Okuno, M.; Sakamura, S.; Tajimi, A.; Shimanuki, T. *Biosci., Biotechnol., Biochem.*, **1992**, 56, 1096.
(7) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.*, **1973**, 95, 512.
(8) Trost, B. M.; Belletire, J. L.; Godleski, S.; Ponticello, G. S.; Varga, S. L.; Springer, J. P. *J. Org. Chem.*, **1986**, 51, 2370.
(9) Davies-Coleman, M. T.; Rivett, D. E. A. *Phytochemistry*, **1994**, 35, 1590.
(10) Drewes, S. E.; Sehlapelo, B. M.; Horn, M. M.; Scott-Shaw, R.; Sandor, P. *Phytochemistry*, **1995**, 38, 1427.
(11) Davies-Coleman, M. T.; Rivett, D. E. A. *Phytochemistry*, **1996**, 41, 1085.
(12) Pereda-Miranda, R.; Garcia, M.; Delgado, G. *Phytochemistry*, **1990**, 29, 2971.
(13) Brian, P. W.; Curtis, P. J.; Hemming, H. G.; Unwin, C. H.; Wright, J. M. *Nature*, **1949**, 164, 534.
(14) Tabuchi, H; Ichihara, A. *Tetrahedron Lett.*, **1992**, 33, 4933.
(15) Tabuchi, H; Hamamoto, T.; Miki, S.; Tejima, T.; Ichihara, A. *Tetrahedron Lett.*, **1993**, 34, 2327.
(16) Tabuchi, H.; Ichihara, A. *J. Chem. Soc., Perkin Trans. 1*, **1994**, 125.
(17) Hamada, T.; Kusumi, T.; Ishitsuka, M. O.; Kakisawa, H. *Chem. Lett.*, **1992**, 33.
(18) Tsuda, M.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.*, **1992**, 57, 3503.
(19) Tsuda, M.; Shigemori, H.; Ishibashi, M.; Kobayashi, J.; Sasaki, T. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, **1992**, 34, 448; *Chem. Abstr.*, **1994**, 120, 116556m.
(20) van Puyvelde, L.; Dubé, S.; Uwimana, E.; Uwera, C.; Domisse, R. A.; Esmans, E. L.; van Schoor, O.; Vlietinch, A. J. *Phytochemistry*, **1979**, 18, 1215.
(21) Williams, D. H.; Fleming, I. *Spectroscopic Methods in Organic Chemistry 3rd edition*, McGraw-Hill, London, **1980**, p 101.
(22) Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry 5th edition*, Longman Scientific & Technical, Essex, **1989**, p 1414.
(23) Delgado, G.; Pereda-Miranda, R.; Romo de Vivar, A. *Heterocycles*, **1985**, 23,

1869.

(24) Davies-Coleman, M. T.; Rivett, D. E. A. *Phytochemistry*, **1995**, 38, 791.

(25) Pereda-Miranda, R.; Hernandez, L.; Villavicencio M. J.; Novelo, M.; Ibarra, P.; Chai, H.; Pezzuto, J. M. *J. Nat. Prod.*, **1993**, 56, 583.

(26) Legrand, M.; Rougier, M. J.; Kagan, H. B. (Ed) *Stereochemistry*, Georg Thieme Publishers, Stuttgart, **1977**, Vol 2, pp 33 - 183.

(27) Romo de Vivar, A.; Vidales, P.; Perez, A. L. *Phytochemistry*, **1991**, 30, 2417.

(28) Matsuda, M.; Endo, Y.; Fushiya, S.; Endo, T.; Nozoe, S. *Heterocycles*, **1994**, 38, 1229.

(29) Koreeda, M., Weiss, G.; Nakanishi, K. *J. Am. Chem. Soc.*, **1973**, 95, 239.

(30) Harada, N.; Nakanishi, K. *Acc. Chem. Res.*, **1972**, 5, 257.

(31) Needham, J.; Andersen, R. J.; Kelly, M. T. *Tetrahedron Lett.*, **1991**, 32, 315.

(32) Fushimi, S.; Nishikawa, S.; Shimazu, A.; Seto, H. *J. Antibiot.*, **1989**, 42, 1019.

(33) Fushimi, S.; Furihata, K.; Seto, H. *J. Antibiot.*, **1989**, 42, 1026.

(34) Schummer, D.; Gerth, K.; Teichenbach, H,; Hoefle, G. *Liebigs Ann.*, **1995**, 4, 685; *Chem. Abstr.*, **1995**, 123, 5217u.

(35) Gerth, K.; Schummer, D.; Hoefle, G.; Irschik, H.; Reichenbach, H. *J. Antibiot.*, **1995**, 48, 973; *Chem. Abstr.*, **1995**, 123, 222498r.

(36) Merlin, P.; Braekman, J. C.; Daloze, D.; Pasteels, A. *Experientia*, **1992**, 48, 111.

(37) Ahmad, F. B.; Tukol, W. A.; Omar, S.; Sharif, A. M. *Phytochemistry*, **1991**, 30, 2430.

(38) Goh, S. H.; Ee, E. C. L.; Chuah, C. H.; Mak, T. C. W. *Nat. Prod. Lett.*, **1995**, 5, 255; *Chem. Abstr.*, **1995**, 122, 128632n.

(39) Shing, T. K. M.; Zhou, Z. H. *Tetrahedron Lett.*, **1992**, 33, 3333.

(40) Fang, X. P.; Anderson, J. E.; Chang, C. J.; Fanwick, P. E.; McLaughlin, J. L. *J. Chem. Soc., Perkin Trans. 1*, **1990**, 1655.

(41) Wu, Y. C.; Chang, F. R.; Duh, C. Y.; Wang, S. K.; Wu, T. S. *Phytochemistry*, **1992**, 31, 2851.

(42) Fu, X.; Sevenet, T.; Hamid, A.; Hadi, A.; Remy, F.; Pais, M. *Phytochemistry*, **1993**, 33, 1272.

(43) Sehlapelo, B. M.; Drewes, S. E.; Scott-Shaw, R. *Phytochemistry*, **1994**, 37, 847.

(44) Zdero, C.; Bohlmann, F.; Mungai, G. M. *Phytochemistry*, **1991**, 30, 575.

(45) Warning, U.; Jakupovic, J.; Friedrich, D.; Castro, V.; Bohlmann, F. *Phytochemistry*, **1987**, 26, 2331.

(46) Bohlmann, F.; Grenz, M. *Chem. Ber.*, **1977**, 110, 1321.

(47) Zdero, C.; Bohlmann, F.; King, R. M. *Phytochemistry*, **1992**, 31, 155.

(48) Cunningham, A. B. *An Investigation of the Herbal Medicine Trade in*

Natal/Kwazulu, Institute of Natural Resources Report No. 29, Pietermaritzburg, **1988**, p 70.

(49) Palgrave, K. C.; Drummond, R. B.; Palgrave, P.; Palgrave, M. C.; Moll, E. J. (Ed) *Trees of Southern Africa*, Struik, Cape Town, **1977**, pp. 178 - 180.

(50) Dann A. E.; Davis, J. B.; Nagler, M. J. *J. Chem. Soc., Perkin Trans. 1*, **1979**, 158.

(51) Rychnovsky, S. D.; Skalitzky, D. J. *Tetrahedron*, **1990**, 31, 945.

(52) Evans, D. A.; Rieger, D. L.; Gage, J. R. *Tetrahedron*, **1990**, 7099.

(53) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.*, **1991**, 113, 4092.

(54) Codd, L. E.; Leistner, O. A. (Ed) *Flora of Southern Africa*, Department of Agriculture and Water Supply, Pretoria, **1985**, Vol 28, Part 4, p 185.

(55) Freeman, R.; Morris, G. P. *J. Chem. Soc., Chem. Commun.*, **1978**, 684.

(56) Codd, L. E.; Leistner, O. A. (Ed) *Flora of Southern Africa*, Department of Agriculture and Water Supply, Pretoria, **1985**, Vol 28, Part 4, p 187.

(57) Nakanishi, K. *One and Two-dimensional NMR Spectra by Modern Pulse Techniques*, Kodansha Ltd, Tokyo, **1990**, pp. 136 - 137.

(58) Kobayashi, M.; Chavakula, R.; Murata, O.; Sarma, N. S. *J. Chem. Res. (S)*, **1992**, 366.

(59) McGahren, W. J.; Ellestad, G. A.; Morton, G. O.; Kunstmann, M. P.; Mullen, P. *J. Org. Chem.*, **1973**, 38, 3542.

(60) March, J. *Advanced Organic Chemistry : Reactions, Mechanisms and Structure 4th edition*, John Wiley & Sons, Inc., New York, **1992**, p 144.

(61) Hokanson, G. C.; French, J. C. *J. Org. Chem.*, **1985**, 50, 462.

(62) Evans, R. H.; Ellestad, G. A.; Kunstmann, M. P. *Tetrahedron Lett.*, **1969**, 22, 1791.

(63) Fang, X.; Anderson, J. E.; Ching, C.; Fanwick, P. E.; McLaughlin, J. L. *J. Chem. Soc. Perkin Trans. I*, **1990**, 1655.

(64) Garson, M. J.; Staunton, J.; Jones, P. G. *J. Chem. Soc. Perkin Trans. I*, **1984**, 1021.

(65) Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry 5th edition*, Longman Scientific & Technical, Essex, **1989**, p 1432.

(66) Branch, G. M.; Griffiths, C. L.; Branch, M. L.; Beckley, L. E. *Two Oceans - A guide to the marine life of Southern Africa*, David Philip, Cape Town, **1994**, p 134.

(67) Manker, C. D.; Faulkner, D. J. *J. Org. Chem.*, **1989**, 54, 5371.

(68) Hochlowski, J. E.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J. *J. Am. Chem.*

Soc., **1983**, 105, 7413.

(69) Garson, M. J.; Jones, D. D. *Tetrahedron Lett.*, **1994**, 35, 6921.

(70) Hochlowski, J. E.; Faulkner, D. J. *J. Org. Chem.*, **1984**, 49, 3838.

(71) Manker, C. D.; Faulkner, D. J. *J. Org. Chem.*, **1986**, 51, 814.

(72) Garson, M. J.; Small, C. J. *J. Chem. Soc., Perkin Trans. 1*, **1990**, 805.

(73) Hochlowski, J. E.; Faulkner, D. J. *Tetrahedron Lett.*, **1983**, 24, 1917.

(74) Biskupiak, J. E.; Ireland, C. M. *Tetrahedron Lett.*, **1983**, 24, 3055.

(75) Capon, R. J.; Faulkner, D. J. *J. Org. Chem.*, **1984**, 49, 2506.

(76) Oppolzer, W.; Moretti, R.; Bernardinelli, G. *Tetrahedron Lett.*, **1986**, 27, 4713.

(77) Norte, M.; Cataldo, F.; González, A. G.; Rodríguez, M. L.; Ruiz-Perez, C. *Tetrahedron*, **1990**, 46, 1669.

(78) Hooper, G. J. *PhD Thesis*, Rhodes University, July **1996**.

(79) Hochlowski, J. E.; Coll, J. C.; Faulkner, D. J.; Biskupiak, J. E.; Ireland, C. M.; Qi-tai, Z.; Cun-heng, H.; Clardy, J. *J. Am. Chem. Soc.*, **1984**, 106, 6748.

(80) Paterson, I.; Franklin, A. S. *Tetrahedron Lett.*, **1994**, 35, 6925.

(81) Sundram, U. N.; Albizati, K. F. *Tetrahedron Lett.*, **1992**, 33, 437.

(82) Manker, C. D.; Faulkner, D. J. *J. Org. Chem.*, **1989**, 54, 5374.

(83) Manker, C. D.; Garson, M. J.; Faulkner, D. J. *J. Chem. Soc., Chem. Commun.*, **1988**, 1061.

(84) Garson, M. J.; Goodman, J. M.; Paterson, I. *Tetrahedron Lett.*, **1994**, 35, 6929.

(85) Paterson, I.; Perkins, M. V. *Tetrahedron Lett.*, **1992**, 33, 801.

(86) Paterson, I.; Perkins, M. V. *J. Am. Chem. Soc.*, **1993**, 115, 1608.

(87) Chambers, R. J.; McQuaid, C. D. *J. Moll. Stud.*, **1994**, 60, 263.

(88) Kusumi, T.; Fujita, Y.; Ohtani, I.; Kakisawa, H. *Tetrahedron Lett.*, **1991**, 32, 2923.

(89) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*, John Wiley and Sons, Inc., New York, **1967**, p 191.