EXTRACTIVES FROM SIX SPECIES OF LAMIACEAE

THESIS

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to my wife, HEATHER

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SUMMARY

In continuation of the phytochemical studies of plants belonging to the Lamiaceae (formerly Labiatae) the acetone extractives of six Southern African species from this family have been examined.

The genus Syncolostemon has not been investigated before and a new 6-substituted-5,6-dihydro-α-pyrone, synrotolide was isolated from S. rotundifolius. The structure of synrotolide was fully elucidated as 6*R*-[3'*R*, 6'*S*-(diacetyloxy)-4'*R*, 5'*S*-(dihydroxy)-1'*Z*-heptenyl] -5,6-dihydro-2H-pyran-2-one. Oleanolic acid is the major component of both S. rotundifolius and S. densifloris. The flavonol quercetin was isolated from S. densifloris as its 3, 3', 4', 7 tetramethyl ether.

A further 6-substituted-5,6-dihydro-α-pyrone, boronolide, was obtained from Tetradenia barberae. The structure of boronolide was known but the absolute stereochemistry was unassigned. Chemical degradation established the total structure of this compound as 6*R*-[1'*R*, 2'*R*, 3'*S*-(trisacetyloxy)-heptyl] -5,6-dihydro-2H-pyran-2-one.

The chemistry of naturally occuring 6-substituted-5,6-dihydro-α-pyrones has not as such been previously reviewed and a comprehensive review covering the literature up to June 1987 is presented here.

Three known labdane diterpenoids nepetaefuran, hispanalone and dubiin were isolated from Leonotis nepetaefolia, Ballota africana and L. ocymifolia var. ocymifolia (formerly L. dubia). Both L. nepetaefolia and L. dubia have been examined before. An X-ray analysis of saponified dubiin and a circular dichroism study of 6-deacetyldehydrodubiin and related compounds corroborated the structure of dubiin as 4*S*, 5*R*, 6*R*, 8*R*, 9*R*, 10*R*-[6-acetoxy-15,16-epoxy-9-hydroxylabda-13 (16), 14-dien-19, 20-olactone]. The chemotaxonomic relationships of the genera Ballota and Leonotis are discussed.

CHAPTER ONE

INTRODUCTION

1. 6-Substituted-5,6-dihydro-α-pyrones:- The necessity for a review

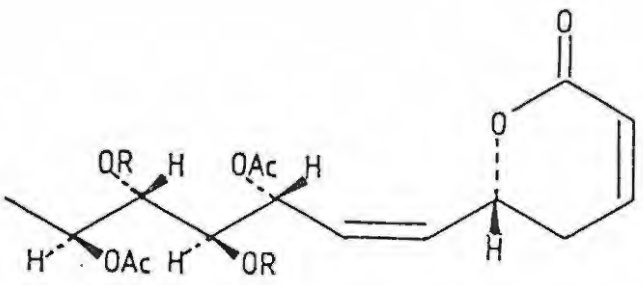
As part of the phytochemical studies of the family Lamiaceae presented in this thesis two 6-substituted-5,6-dihydro-α-pyrones, synrotolide (1)[1] and boronolide (3)[2] , have been isolated from the indigenous South African species Syncolostemon rotundifolius and Tetradenia barberae respectively.

The term 5,6-dihydro-α- or 2-pyrone describes the αβ-unsaturated-δ-lactone ring. 6-Substituted-5,6-dihydro-α-pyrones are an important group of oxygen ring compounds with a diverse range of biological activity. They have been reported as plant growth inhibitors[3] , insect antifeedants[4] , antifungal[5] and antitumour agents[6] , and are widely distributed in both plants and fungi.

5,6-dihydro-α-pyrones have been reviewed together with their unsaturated analogues, α-pyrones, by Mors, Magalhaes and Gottlieb[7] and subsequently by Adityachaudhury and Das[8] . Mors et al have reviewed the pre-1961 literature and comprehensively discussed the earlier work on the structure determination and biosynthesis of nine α-pyrones and four 5,6-dihydro-α-pyrones.

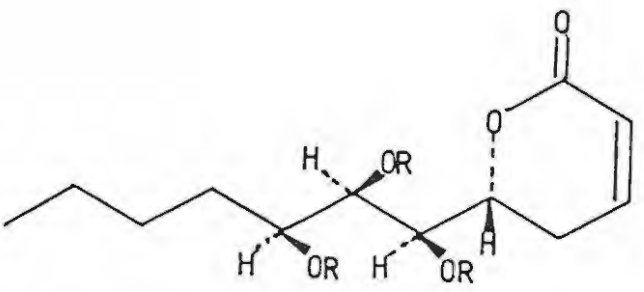
The review of Adityachaudhury and Das, published in 1979, follows the precedent set by Mors et al and is a combined review of both substituted α-pyrones and 5,6-dihydro-α-pyrones. However their review differs from the former by also including a large number of non-aromatic compounds in their list of sixty-eight structures of which a mere twenty-six are 6-substituted-5,6-dihydro-α-pyrones.

The review of Adityachaudhury and Das is deficient in a number of important areas. Firstly, the stereochemistry of more than two-thirds of the compounds listed is known but the review only partially defines the stereochemistry of two related fungal metabolites LL-P880α (5)[9] and



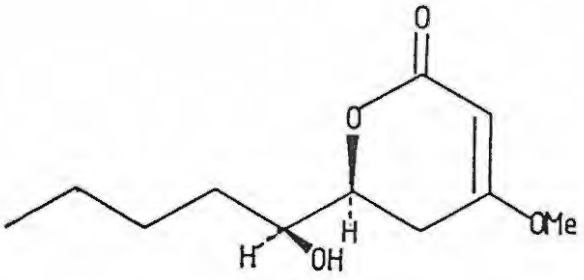
(1) R = H

(2) R = Ac



(3) R = Ac

(4) R = H



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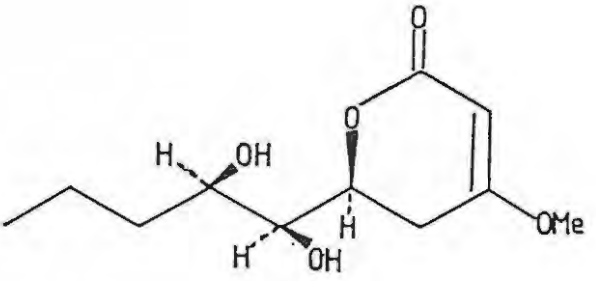

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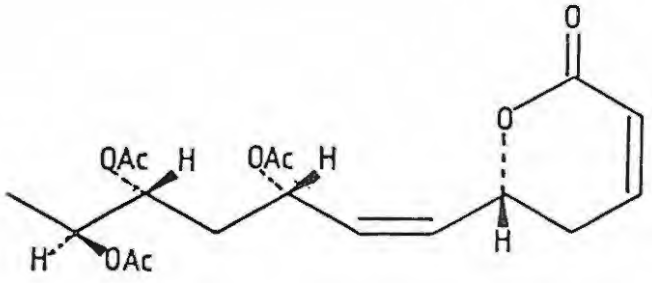

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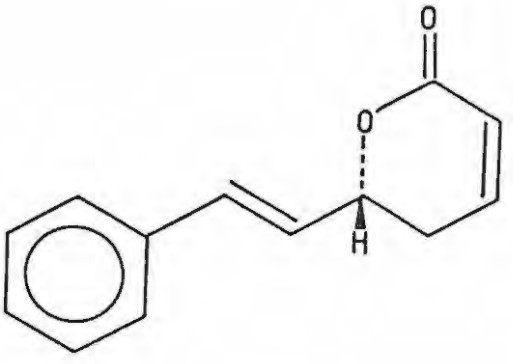

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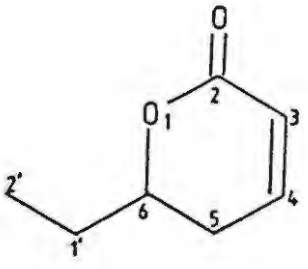

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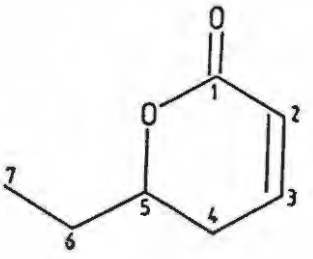

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LL-P880β(6)[10]. There is no discussion of absolute stereochemistry or techniques such as circular dichroism, which has been widely used in the last two decades for stereochemical assignment of the αβ-unsaturated-δ-lactone ring junction. Finally, there are at least six omissions from the list of 5,6-dihydro-α-pyrones isolated prior to 1979. Two of these are important compounds from the Lamiceae, boronolide (3)[11] and hyptolide (7)[12].

Approximately sixty 6-substituted-5,6-dihydro-α-pyrones have been isolated from thirteen families of plants and about twenty fungal species. α-Pyrones and 5,6-dihydro-α-pyrones rarely occur together and α-pyrones have only been isolated from three species which also contain 5,6-dihydro-α-pyrones, an unidentified Pencillium species[10], Alternaria citri[13] and Piper methysticum[14]. Accordingly 5,6-dihydro-α-pyrones warrant a separate review. Also, because of the absence of a comprehensive review, the literature on all known monocyclic and aromatic 6-substituted-5,6-dihydro-α-pyrones, isolated up until June 1987, has been reviewed. The withanolides which have a steroid nucleus attached to the lactone ring in the C-6 position have been excluded.

The main emphasis in this review is on distribution, structure elucidation and absolute stereochemistry. Advances in the physical methods of structure determination and interesting aspects of biosynthesis, biological activity and pharmacology are also discussed.

6-Substituted-5,6-dihydro-α-pyrones provide interesting and challenging synthetic targets as reflected by the many syntheses of these compounds which appear regularly in the literature. A detailed discussion of synthetic methods and strategies is outside the scope of this thesis and will not be embarked upon. However, the synthesis of goniothalamin (8)[15] verifies the classical dictum "synthesis is the final proof of structure".

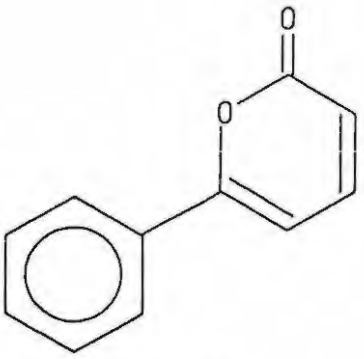
Although this axiom is not infallible, as shown by the most recent synthesis of bostrycin[16], it has certainly stood the test of time. Therefore, in recognitition of the important role of synthesis in ultimate structure determination, syntheses which confirm the structure and absolute stereochemistry of a compound will be discussed.

1:1 Nomenclature and classification

There is little uniformity within the nomenclature of 5,6-dihydro-α-pyrones. The majority of these compounds have been assigned trivial names which are derived from their botanical origin and have the suffixes 'in', 'olide' or 'olactone'. The obvious disadvantage of this system occurs when the same compound is given two or more different names e.g. pestalotin (5)[9,17].

According to IUPAC nomenclature recommendations[18], lactones can be named in either one of two ways. The first or classical method identifies the lactone with a corresponding heterocyclic nucleus. Therefore, a substituted αβ-unsaturated-δ-lactone is named as a substituted 5,6-dihydro-2H-pyran-2-one e.g. 6-ethyl-5,6-dihydro-2H-pyran-2-one (9). This is the most common systematic approach and is the procedure accepted by Chemical Abstracts. Where no ambiguity exists, it is permissible to omit the indicated hydrogen, H, its position and also to reduce pyran-2-one to 2-pyrone. α-Pyrone is synonomous with 2-pyrone and in accordance with common usage will be employed in this thesis.

The lactonisation of a carboxylic acid, hydroxylated at C-5, and replacement of the suffix 'oic' with 'olide' [18], forms the basis of the second recommended nomenclature system, e.g. 2-hepten-5-olide or 5-ethyl-2-penten-5-olide (10).



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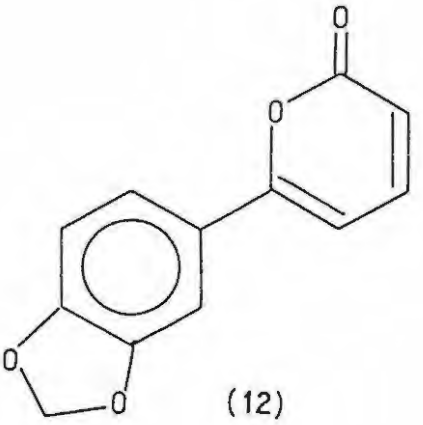

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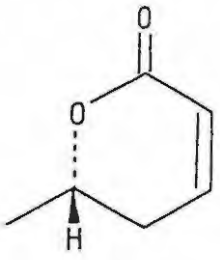

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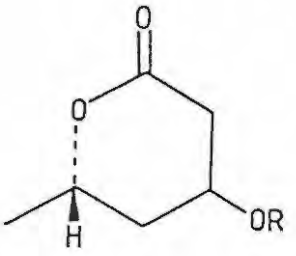
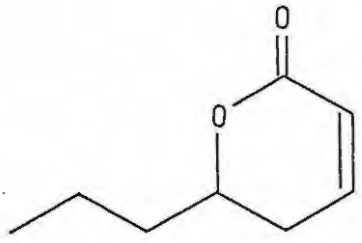


(14) R = β-D-glucosyl



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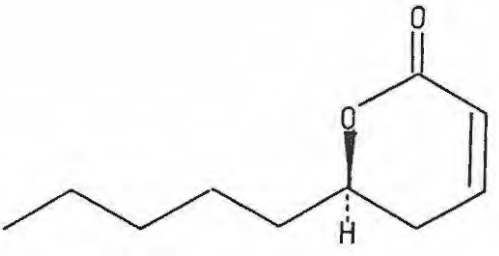

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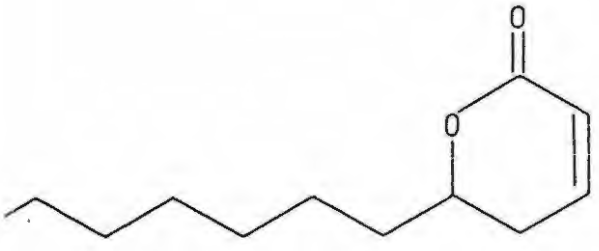

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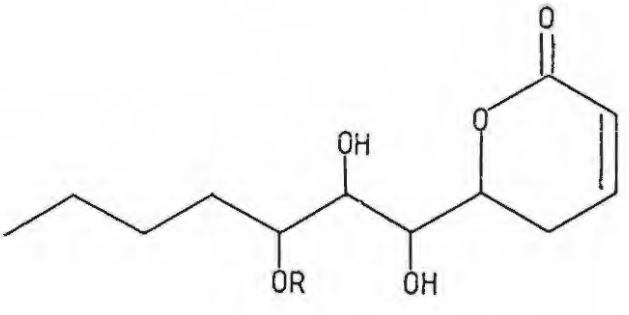


(18) R = H

(19) R = Ac

The numbering of the δ-lactone ring differs in 9 and 10 and this can lead to confusion when specifying ring positions. To minimise confusion the numbering sequence of the former nomenclature system will be used in the tables and text of this thesis. Duplication of numbering between ring and side chain positions is avoided by numbering the C-6 side chain with prime numbers.

The trivial names of the 5,6-dihydro-α-pyrones under review have been retained. Where trivial names have not been given to individual compounds, names in accordance with the former nomenclature system are used.

In the review of Mors et al methoxylation at C-4 was used as the criterion for the classification of α-pyrones. The reason for this originates from the early studies on aromatic α-pyrones where only two compounds, phenyl coumalin (11) and paracotin (12), were shown not to have a C-4 methoxy group. This structural deficit was related to a more recent evolutionary origin of the plant species containing these compounds within their respective genera. Mors et al were able to support this argument with morphological evidence.

In their review Adityachaudhury and Das have divided the α-pyrones and 5,6-dihydro-α-pyrones into groups based on the general type of substituent at C-6. Although this classification does not have any recognisable biosynthetic foundation, it is retained here because of the order it imposes on an otherwise structurally diverse group of compounds. Adityachaudhury and Das restricted their division to 6-aryl- and 6-alkyl-α-pyrones and 5,6-dihydro-α-pyrones. Since nearly a quarter of the 5,6-dihydro-α-pyrones reported have an alkenyl side chain at C-6, these compounds have been separated into a third group. Compounds which have a 6-styryl-substituent are classified with 6-aryl-5,6-dihydro-α-pyrones.

The presence of a methoxy group at C-4 is found in only thirteen of the sixty reported 6-substituted-5,6-dihydro-α-pyrones. It is therefore the exception as opposed to the rule and will not be used for further subdivision within the groups as proposed in the latter review. Instead, compounds are discussed in order of increasing complexity of substitution around the lactone ring.

1:2 Distribution and structure determination

6-Substituted-5,6-dihydro-α-pyrones are widely distributed in the plant kingdom but are particularly associated with the Lamiaceae, Piperaceae, Lauraceae and Annonaceae. They have been found in all parts of plants including the leaves, stems, flowers and fruit. 6-substituted-5,6-dihydro-α-pyrones are also widespread amongst fungal species a number of which remain unidentified.

The structural elucidation of these compounds is a three fold problem: firstly, of identifying the αβ-unsaturated-δ-lactone nucleus, secondly, of identifying the substituent groups and thirdly, of allocating these to their correct position on the lactone ring. The early work on the structures of the 6-substituted-5,6-dihydro-α-pyrones involved the use of a number of chemical degradation methods including periodate oxidation, ozonolysis, oxidation and hydrolysis.

Recent advances in spectroscopic techniques have facilitated the fairly rapid determination of a possible structure. Assignment of absolute stereochemistry with some degree of certainty, is however more difficult. The high level of oxygenation in the acyclic substituents of some compounds often results in a complex sequence of adjacent chiral centres. A wide diversity of methods, both spectroscopic and chemical,

have been used to ascertain the absolute stereochemistry of these compounds and are reviewed here.

1:2:1 6-Alkyl-5,6-dihydro-α-pyrones

The simplest of these compounds is parasorbic acid (**13**). This volatile compound was obtained by Hofmann in 1859 from steam distillation of an acidified extract of mountain ash berries, Sorbus aucuparia (Rosaceae)[19]. The action of alkali on the oily **13** yielded an amorphous salt, later shown to be the salt of an unsaturated hydroxy acid, $C_6H_{10}O_3$[20]. Although **13** was one of the first αβ-unsaturated-δ-lactones to be isolated, it took nearly a century before Kuhn and Jerchel established its 6-methyl-5,6-dihydro-2H-pyran-2-one structure[21].

Almost two decades later two independent groups established the (6S)-configuration of **13** by degradation to (+)-(S)-hexan-1,5-diol[22] and to (+)-(3S)-hydroxybutyric acid[23]. Although a number of syntheses of racemic **13** have been reported the first synthesis of the naturally occuring (+)-(S)-enantiomer was only recently described by Lichtenthaler et al[24]. This synthesis utilizes 3,4-di-O-acetyl-L-rhamnal, accessible from L-rhamnose, as the chiral starting material. The total synthesis of **13** in high yield with complete retention of optical purity further confirms the earlier stereochemical assignment. Tscheche et al showed that parasorbic acid is not present in the plant as **13** but as parasorbiside (**14**)[25].

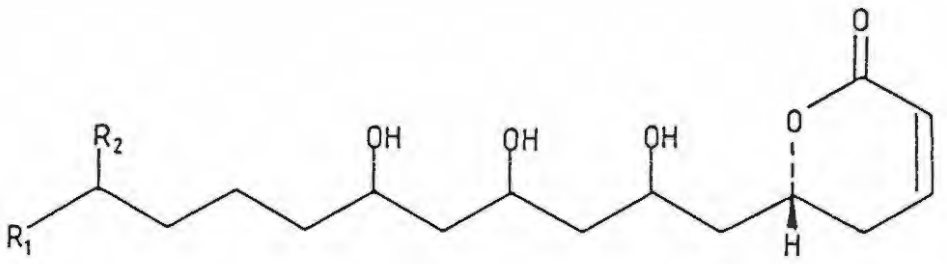
The short chain alkyl homologues of **13** are also volatile, and are important aroma components in both food and beverages. The majority of these compounds have a low odour threshold (approx. 0.1 ppm) and possess significant flavour value. The distribution and odour descriptions of both saturated and unsaturated δ-lactones have been reviewed[26].

6-Propyl-5,6-dihydro-α-pyrone (**15**) was detected by GCMS as a neutral

aroma constituent of burley tobacco, Nicotiana tabacum (Solanaceae)[27]. Massoialactone (16) was first found in the bark oil of Cryptocarya massoia (Lauraceae), a New Guinean medicinal plant[28]. The structure was first proposed by Meyer and later confirmed by Abe and Sato through isolation and identification of the acids obtained from oxidative degradation[29]. The absolute configuration of 16 was assigned as (R) from comparison of its ORD curve with that of 13. This original (R)-configuration for the naturally occuring (-)-enantiomer was confirmed by Mori[30] who synthesised the (+)-(6S)-enantiomer from (+)-(R)-glyceraldehyde. Their synthetic product had an optical purity of 75%. Pirkle and Adams[31] synthesised both the (-)-(R)- and (+)-(S)-enantiomers of 16 by a different route. The key step in their synthesis involved separating diastereomeric derivatives of the racemic lactone precursors by HPLC, thus avoiding the partial racemisation encountered by Mori and yielded the two enantiomers of 16 with total retention of optical purity.

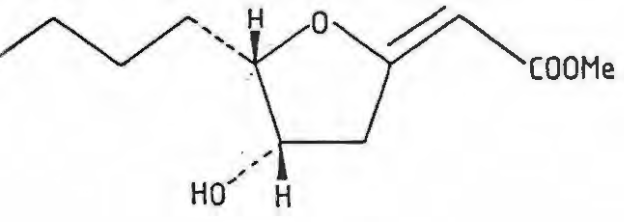
Massoialactone has subsequently been isolated from tuberose flowers[32], wine[33] and molasses[34]. Cavill, Clarke and Whitefield[35] have also extracted 16 from two species of Australian ants where it forms part of the ants' defence mechanism. Cavill et al also reported the isolation of 6-heptyl-5,6-dihydro-α-pyrone (17) as a minor volatile constituent of C. massoia[35].

Oxygenation of the C-6 alkyl side chain or of the lactone ring is accompanied by a decrease in volatility. Boronolide (3) is a crystalline compound first isolated by Franca and Polonsky from a Madagascan species Tetradenia fruticosa (Lamiaceae)[11]. The structure was suggested from NMR studies, and mass spectrometry of 3 and of its dihydro, saponified and methylated derivatives. The stereochemistry at C-6 was proposed as (R) by



(20) $R_1 = C_{11}H_{23}$, $R_2 = H$

(21) $R_1 = C_{11}H_{23}$, $R_2 = OH$



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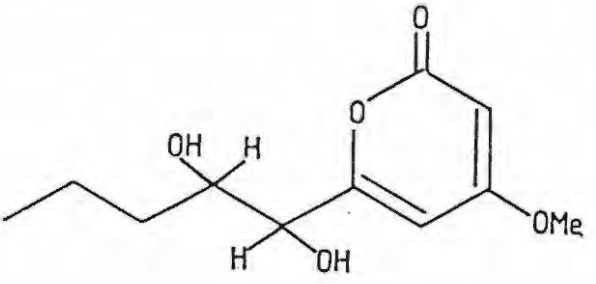

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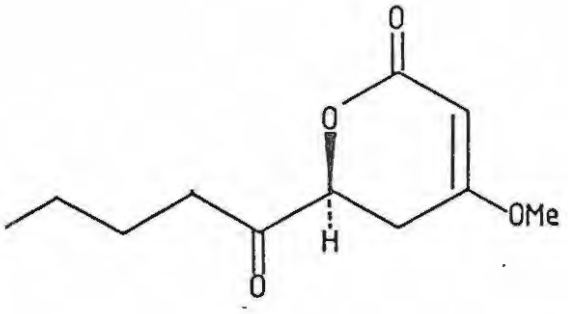

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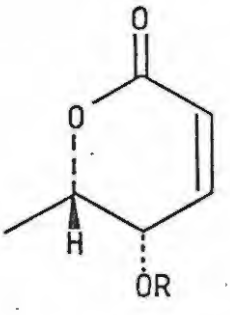
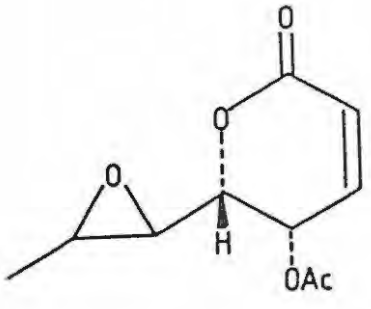


(25) R = β-D-glucosyl

(26) R = H



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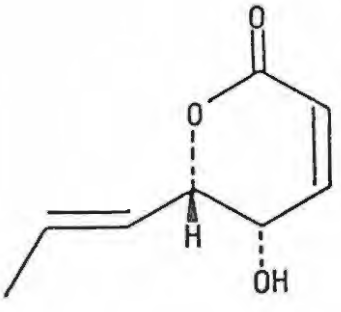

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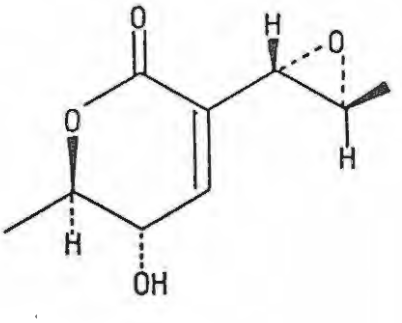


(29)

application of Hudson's lactone rule to the molecular rotation. This result was used in conjunction with a recent crystal structure analysis[36] to assign the relative stereochemistry as shown in 3. Chemical evidence for the (6R)-configuration of 3, isolated from T. barberae, is presented in this thesis.

Two related deacetyl derivatives of 3, deacetylboronolide (18)[37] and 1,2 dideacetylboronolide (19)[38] have been isolated from another Tetradenia species, Tetradenia riparia (formerly Iboza), which is a common medicinal plant from central Africa. The structures of 18 and 19 were suggested from NMR and MS data. Paradoxically, 18 was reported by Van Puyvelde et al to be optically inactive and was thought to be a mixture of three stereoisomers from GCMS of the isolated 18. No optical rotation has been reported for 19 and the stereochemistry remains unassigned.

Two unusual C_{26} polyhydroxy-lactones (20), (21)[39] have been isolated from Eupatorium pilosum Walt., a North American species of the family Compositae. The structures of these compounds were determined using a combination of NMR and MS techniques. Herz et al have incorrectly interpreted the negative CD Cotton effect of 20 and 21 to represent a (6R)-configuration and it should be (6S).

A number of fungal metabolites have been reported which have a 6-alkyl-substituted-5,6-dihydro-α-pyrone structure, with one or more substituents at other positions around the lactone ring.

Three of these compounds are methoxylated at C-4. Pestalotin (5) is a gibberellin synergist, first isolated by Kimura, Katagiri and Tamura[17] from the culture filtrate of a phytopathogenic fungus, Pestalotia cryptomeriaecola. The structure of 5 was established using standard physical techniques. The (6R)-configuration, proposed by Kimura et al, results from an incorrect interpretation of Snatzke's rules[40,41] relating to

the negative sign of the Cotton effect in the CD curve of **5**. Ellestad, McGahren and Kuntsman[9] isolated 5 shortly after Kimura *et al* from an unidentified *Penicillium* species and named it LL-P880α . They did not acknowledge the earlier work of Kimura *et al*, who failed to report the optical rotation, but the melting point and the negative sign of the Cotton effect at 243 nm of LL-P880α would suggest that it is identical with pestalotin. The (S)-configuration at C-6 was proposed by Ellestad *et al* from the CD data and was also inferred from application of the Horeau method[42] to the methylated base hydrolysis product of LL-P880α (22); that is, treatment of 22 with (±)-α-phenylbutyric anhydride liberated (-)-α-phenylbutyric acid. Applying the same method to **5** also liberated (-)-α-phenylbutyric acid and this result was used to assign an (S)-configuration to C-1'.

The first synthesis of naturally occuring (-)-pestalotin was carried out by Meyer and Seebach[43] and confirmed the original stereochemical assignments of Ellestad *et al*. This asymmetric synthesis yielded both (-)-**5** and (-)-epipestalotin which were separated by repeated crystallisation. The two contiguous chiral centres of **5** have been an attractive target for synthesis. Further syntheses of (-)-**5** have been reported by Ichimoto *et al*[44] from D-glucose and by Masaki *et al*[45] from (+)-(R,R) diethyl tartrate. Recently Mori, Otsuka and Oda[46] have reported the synthesis of all four possible stereoisomers of **5**.

Ellestad *et al*[10] later obtained two further lactone metabolites from the same unidentified *Penicillium* species, LL-P880β (**6**) and its unsaturated analogue LL-P880γ (23). The (1'S)- and (2'R)-configuration of the vicinal diol in **6** followed from the application of the excitation chirality technique of Harada and Nakanishi[47], which correlates the

chirality of the diol with the signs of the Cotton effects in the CD curve of its dibenzoate derivative.

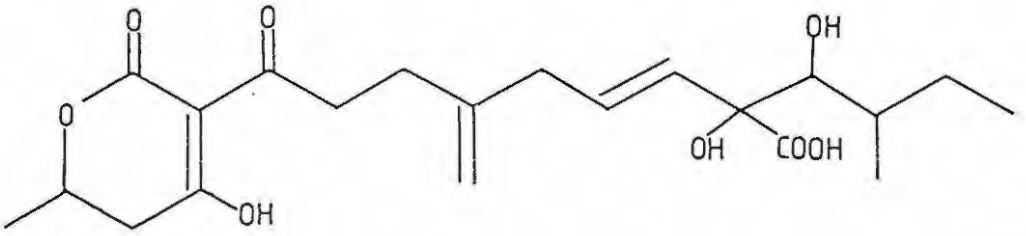
Strunz et al[48] isolated 5 and 6-[1'-oxo-pentyl]-5,6-dihydro-α-pyrone (24) from an unidentified fungus thought to be neither a Pestalotia nor a Penicillium species. The latter was shown to be identical with the Jones oxidation product of 5.

Alkylation at C-6 and oxygenation at C-5 are structural features found in a number of 5,6-dihydro-α-pyrones isolated from both plants and fungi.

A high incidence of possible dietary related gastric tumours in Japan prompted an investigation of the edible ferns Osmunda japonica Thunberg and Osmunda regalis var. spectabilis Willd[49]. The major constituent of the ferns was shown to be osmundalin (25). Acid hydrolysis of 25 yielded osmundalactone 26. Recently 26 was isolated from O. japonica[4] and was shown to possess strong insect anti-feedant activity.

The structure of 26 was proposed using a variety of spectroscopic and chemical methods. Reduction of dihydro-osmundalactone with LAH gave 1,4,5-hexanetriol. Periodate oxidation of the triol yielded acetaldehyde and 4-hydroxybutanal, thus establishing the position of the C-5 hydroxyl group. The (S)-configuration of the chiral centres at C-6 and C-5 was inferred from CD measurements and confirmed by the synthesis of 26 from L-rhamnose[49].

Argoudelis and Zieserl[50] extracted a compound from the fungus Aspergillus nidulans which they named antibiotic U-13,933 (27). This compound later became known as asperline and has also been isolated from A. caespitosus[51], A. carneus[52] and Aspergillus species NRRL 5769[53]. The structure and relative stereochemistry was established from extensive NMR decoupling experiments. Evans, Ellestad and Kunstmann[54] directly related 27 to phomolactone (28) from an unidentified Nigrospora species by oxidation



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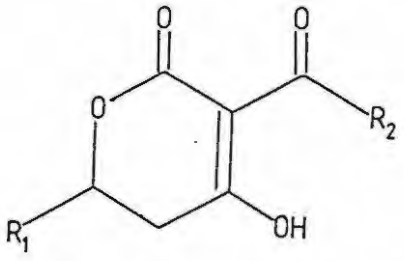
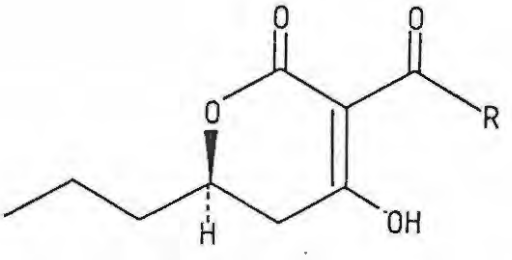


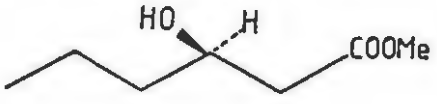
(31) $R_1 = C_5H_{11}$, $R_2 = C_7H_{15}$



(32) R = $CH_3(CH_2)_{10}$—

(33) R = $H_2C=CH-(CH_2)_9$—

(34) R = $CH_3(CH_2)_{12}$—



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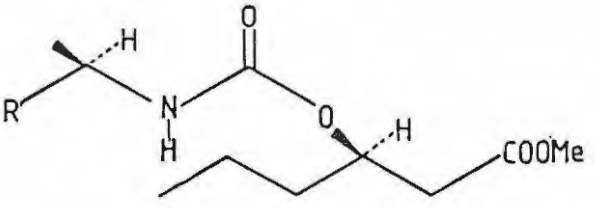
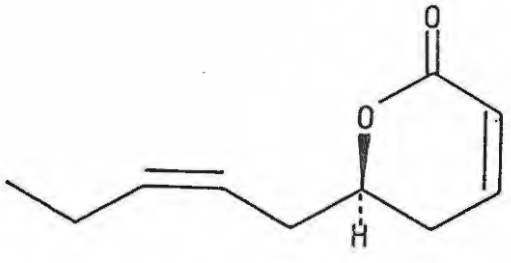


(36) R = α-naphthyl



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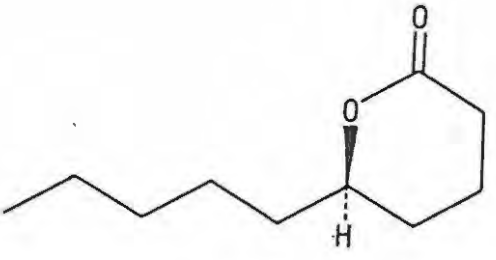


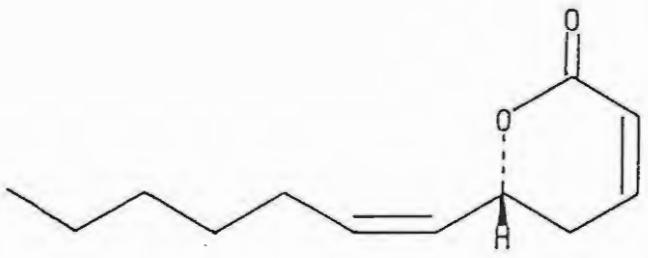
(38)

of the acetylated 28 with m-chloroperbenzoic acid. Phomolactone has also been isolated from Phoma minispora[55] and Nigrospora species Z 1276[56]. The (S)-configuration at the two chiral centres of 28 followed from CD data[54].

Aspergillus elegans[52], A. ochraceus[57], A. melleus[58,59,60,61] and an unidentified penicillic acid producing Aspergillus species[62] have yielded an unusual 5,6-dihydro-α-pyrone, aspyrone (29), related to 27 and 28. The chemical structure of this compound was also proposed from NMR studies. The absolute stereochemistry[61] of 29 followed from ozonolytic degradation to two chiral fragments (-)-1-deoxyerythritol and (-)-2,3-epoxybutyric acid. The absolute stereochemistry of the former fragment was established by comparing the optical rotation of the tris (p-nitrobenzoate) derivative with the corresponding ester of (-)-(2R, 3S)-1-deoxyerythritol, thus confirming the (5S, 6R)-stereochemistry of 29 originally proposed from ORD data. The epoxy acid fragment formed a brucine salt[63] identical with that of (-)-(2R, 3S)-epoxybutyric acid, therefore suggesting an (8S, 9S)-stereochemistry for the epoxypropyl side chain. This stereochemistry was subsequently confirmed by an X-ray structure analysis.

Alkylation at C-6, hydroxylation at C-4 and acylation at C-3 of the lactone ring is found in a number of compounds possessing strong antifungal activity. The first of this type of compound to be isolated was alternaric acid (30) from the fungus Alternaria solani[5]. The structure of the acyl side chain at C-3 was established by Grove[64] and Bartels-Kieth[65,66] from a series of chemical degradations which included alkaline hydrolysis, ozonolysis and periodate oxidation. The absolute stereochemistry at C-6 and the three chiral centres of the acyl substituent are unassigned.

Another compound of this general type was recently obtained from the



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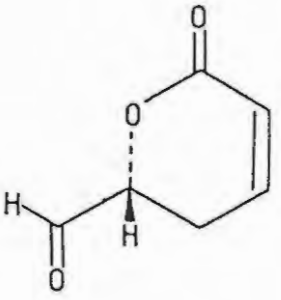

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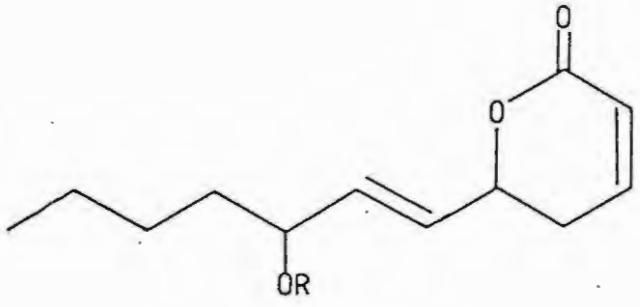
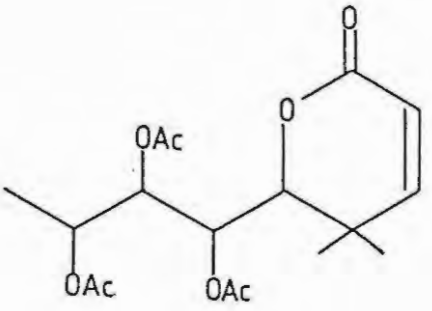


(41) R = Ac

(42) R = H



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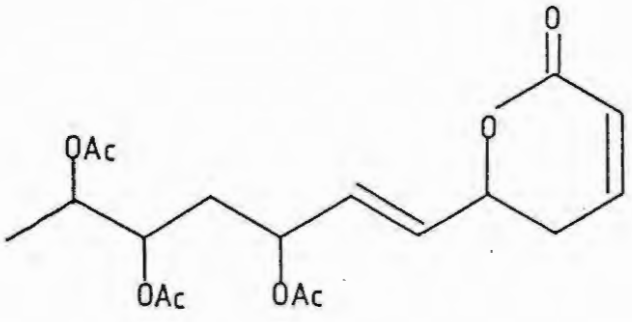

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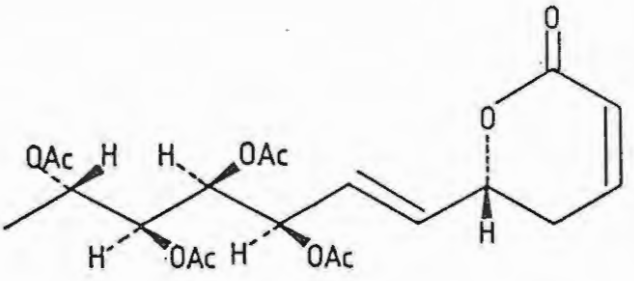

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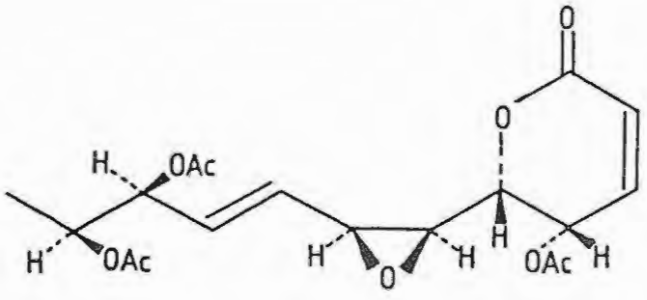

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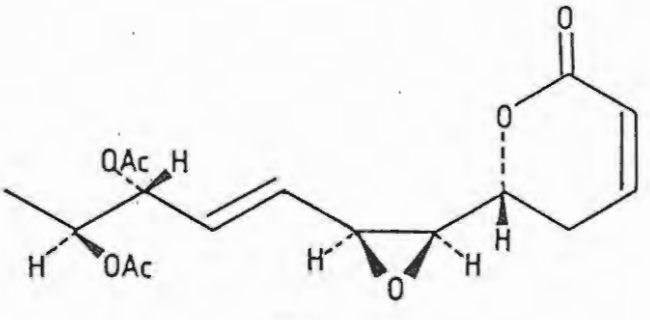


(47)

fungus Lachnellula fuscosanguinea[67]. The structure of lachnelluloic acid (31) was proposed from NMR and mass spectrometry. The synthesis of the racemate of 31 from the symmetrical diketone 6,8-tridecanedione completed the structural assignment.

Podoblastins A, B, C, (32) (33) (34)[68] are antifungal compounds related to 30 and 31 and are found in the higher plant Podophyllum peltatum L.(Berberidaceae). Their chemical structures were proposed from GCMS of the methylated, base catalysed peroxide degradation products. The (6R)-configuration in all three compounds was established using Parkles method[69] in which the carbamate diastereomer of the methylated degradation product (35) was shown, by HPLC, to be identical with N-(R)-1-naphthylethyl-O-(R)-3-(1-methoxycarbonyl)-pentyl carbamate (36).

1:2:2 6-Alkenyl-5,6-dihydro-α-pyrones

The short chain 6-alkenyl-5,6-dihydro-α-pyrones are also volatile and hence resemble their partially saturated analogues. The first decadienolide found in Nature was tuberolactone (37)[70] from the absolute of tuberose flowers Polianthes tuberosa L. (Amaryllidaceae). The structure of 37 was established from NMR and MS studies. The (R)-configuration at C-6 followed from hydrogenation to (+)-5-decanolide (38), previously prepared from microbiological reduction of the corresponding keto acid[71].

Another volatile oil, argentilactone (39)[72], has been extracted from the rhizomes of Aristolochia argentina Gris. (Aristolochiaceae). Both chemical and physical methods were used to resolve the structure. Hydrogenation of 39 followed by saponification of the lactone ring gave 5-hydroxy-dodecanoic acid which yielded a crystalline anilide. Priestap et al used lanthanide shift reagents to assign all the proton signals in the NMR spectrum of 39. The cis configuration of the exocyclic double

bond and the pseudo-equatorial orientation of the side chain at C-6 were also determined. The latter was used, together with the positive Cotton effect in the CD curve, to assign an (R)-configuration to the single chiral centre at C-6.

A synthesis of naturally occuring (-)-39 has recently been published by O'Connor and Just[73]. They synthesised (-)-39 in high yield from the chiral aldehyde 40 via a Wittig reaction, thus confirming the original assignment.

As encountered earlier, oxygenation of the side chain or of the lactone ring reduces the volatility of the 6-alkenyl-5,6-dihydro-α-pyrones. The Lamiaceae have yielded seven compounds with varying degrees of oxygenation of the alkenyl side chain. The simplest of these are umuravumbolide (41) and deacetylumuravumbolide (42) from Tetradenia riparia[37]. Standard spectroscopic techniques were used to determine the structures of 41 and 42. The trans configuration of the exocyclic double bond was proposed from the strong IR absorbance at 965 cm^{-1}. Strangely, these compounds are reported as having no optical rotation.

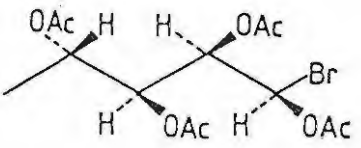
In accordance with the reputed presence of (41) and (42) as racemates in T. riparia, Achenbach and Witzke[74] have reported a synthesis of racemic (41). A tenuous separation of the (6R, 3'S)-diastereomer with its enantiomer (6S, 3'R) from the racemic mixture, by silica gel chromatography, is described.

The first 6-substituted-5,6-dihydro-α-pyrone to be isolated from the Lamiaceae was hyptolide (7) from Hyptis pectinata Poit.. Gorter[75] assigned the structure (43) to hyptolide from identification of the acids obtained from hydrogenation and silver oxide oxidation. A preliminary NMR investigation by Birch and Butler[76] showed that the structure of hyptolide

was at variance with 43 and structure (44) was proposed from exhaustive chemical degradation. Hydrogenation of 44 required 3.2 mole equivalent of hydrogen. Hydrolysis of the hydrogenation products yielded dextrarotatory 10,11-dihydroxydodecanoic acid and 8,10,11-trihydroxydodecanoic acid, which were interpreted as arising from hydrogenolysis of the lactone and the 3'-acetoxy-group. The position of the exocyclic double bond at C1' was thus established and its trans configuration followed from the IR absorbance at 965 cm^{-1}.

The absolute stereochemistry of hyptolide (7) was very recently reported[12]. The X-ray structure proved that the configuration of the exocyclic double bond is cis and not trans. The positive Cotton effect of 7 suggested an (R)-configuration at C-6. The absolute stereochemistry at C5' and C6', and hence of the whole molecule, followed from the synthesis of the dextrarotatory (10S,11R)-dihydroxydodecanoic acid isolated by Birch and Butler.

Hyptis species have yielded a further three 6-alkenyl-5,6-dihydro-α-pyrones which have the same general structure but different stereochemistry at the acyclic chiral centres. Two compounds anamarine (**45**)[77] and olguine (**46**)[78] were extracted from an unidentified Hyptis species, whilst the third compound 4-deacetoxy-10-epi-olguine (47)[79] was obtained from an ubiquitous Mexican plant Hyptis oblongifolia Bentham. Extensive ^{1}H NMR decoupling experiments were used to propose the structures of **45**, **46** and **47**. The trans configuration of the exocyclic double bonds, the cis configuration of the epoxides in **46** and **47** and the relative stereochemistries of all three compounds followed from X-ray analyses. The positive Cotton effect in the CD curve of **45** established an (R)-configuration at C-6 and hence the absolute stereochemistry of the rest of the molecule was assigned. The anomalous X-ray dispersion effect of the oxygen atoms of **46** was used to



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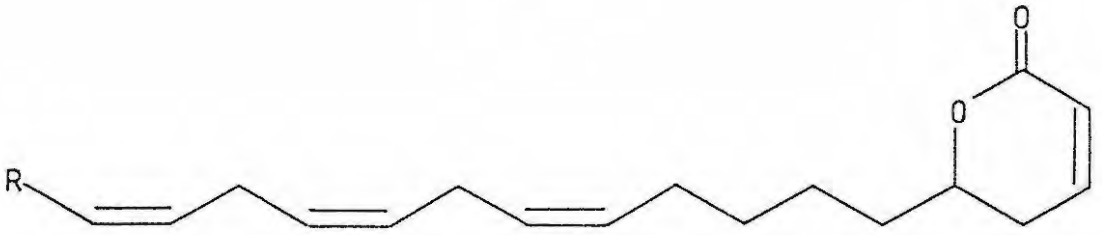
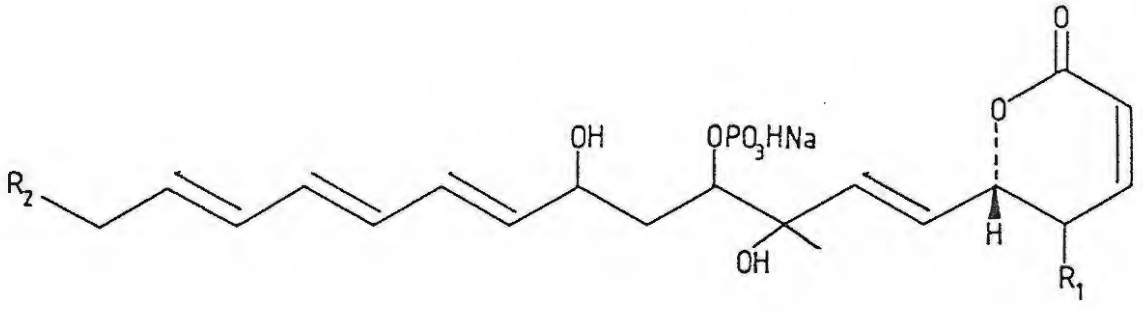


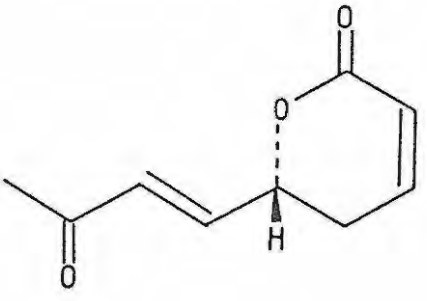
(49) R = C_5H_{11}



(50) R_1 = H, R_2 = OH

(51) R_1 = R_2 = H

(52) R_1 = R_2 = OH



(53)

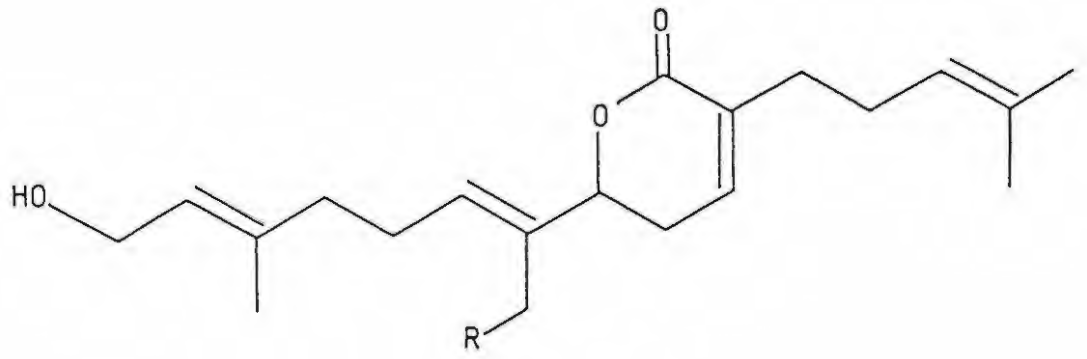
suggest the absolute stereochemistry of this compound. Compound **47** is very similar to **46** and differs only in the absence of the acetoxy group at C-5 and the (R)-configuration at C-5'. The pref relationship of C-5' and C-6' in **47** followed from X-ray and NMR studies, thus establishing a (5'R)- and (6'S)-configuration at these chiral centres.

Retrosynthetic analysis of **45** suggested that synthesis of this compound could be achieved via a Wittig type reaction between aldehyde **40** and the bromide (**48**). The synthesis of **48** as the first stage in the synthesis of **45** has been reported from D-gulonolactone[80].

Synrotolide (**1**)[1] is closely related to compounds **7**,**41**,**42**,**45**,**46**, and **47**. The structure and absolute stereochemistry of **1** is discussed in this thesis.

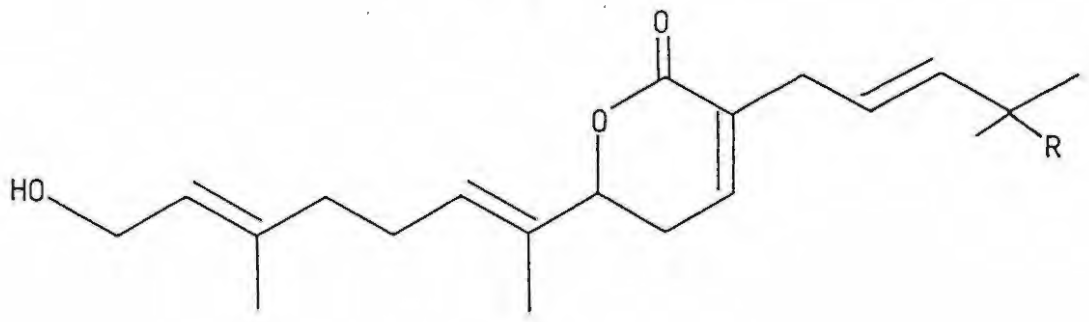
An unusual tridecene, (5'Z, 8'Z, 11'Z)-6-(heptadeca-5', 8', 11'-trienyl-1'-yl)-5,6-dihydro-2H-pyran-2-one (**49**)[81] has been extracted from the red alga Phacelocarpus labillardieri (Sphaerococaeceae). Compound **49** was obtained as a colourless oil and the structure determined by ^{1}H and ^{13}C NMR. The position of the homoallylic triene system in the C_{17} side chain was determined by the isolation of hexanal from ozonolysis of **49**. The stereochemistry at C-6 is unassigned.

Three novel phosphate containing antitumour agents, CI-920 (**50**), PD 113270 (**51**) and PD 11321 (**52**) have been isolated from a subspecies of Streptomyces pulveraceous[6,82]. The chemical structures of these compounds were propsed from extensive NMR analyses and confirmed by chemical degradation[82]. Hydrogenation of **50** and treatment with phosphorus and hydroiodic acid yielded a mixture of iodo compounds, which when treated with LAH followed by catalytic hydrogenation gave 8-methyl-1-octadecanol as the major product. Further evidence for the chemical structure was obtained



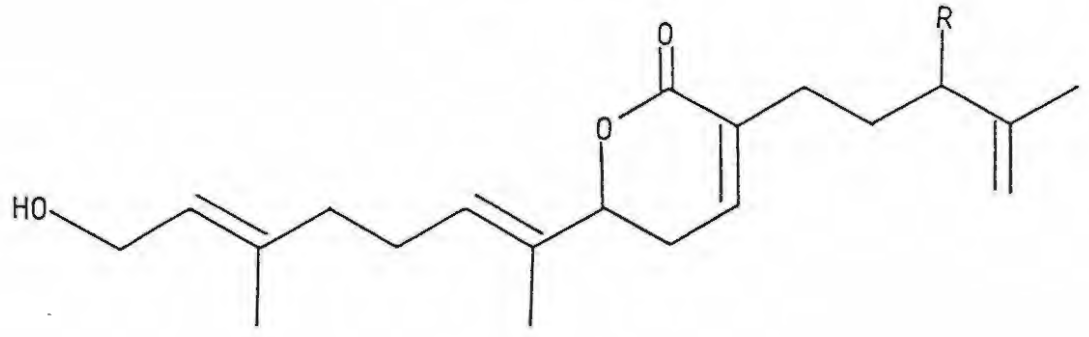
(54) R = H

(55) R = OAc



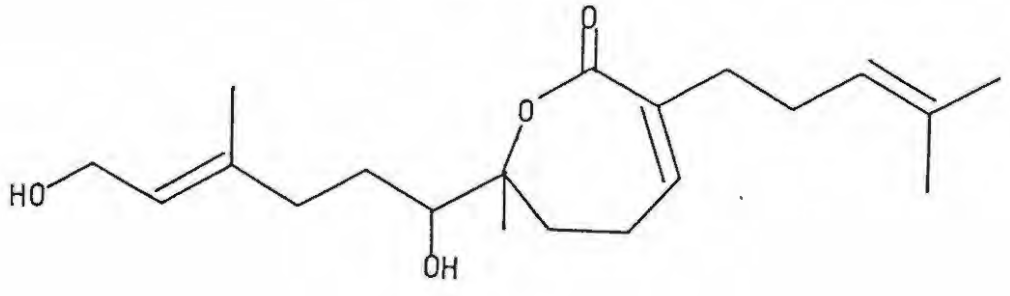
(56) R = OOH

(57) R = OH



(58) R = OOH

(59) R = OH

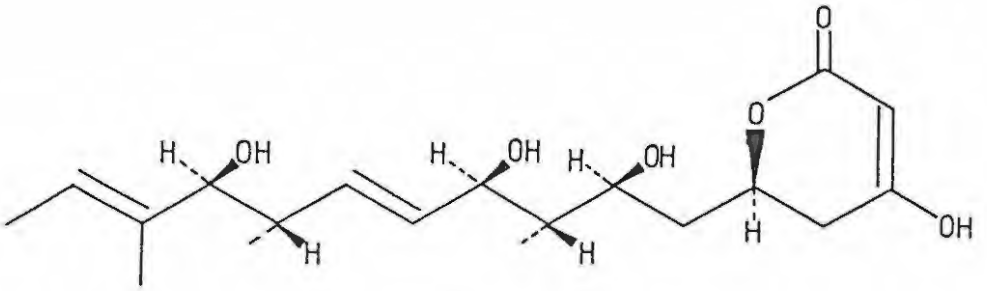


(60)

from periodate oxidation of dephosphorylated 50 to yield the keto-lactone (53) and an unstable triene. The (6R)-configuration of the degradation product followed from NMR comparison with 53 prepared unambiguously by synthesis. The stereochemistry at C-3', C-4' and C-6' is not assigned.

Six compounds, all derived from geranylnerol, have been reported by Bohlmann et al[83] from Ichthyothere ulei Thumb.(Compositae). The structure of (54) and ichthyouleolide (55) followed from ^{1}H NMR data. The chemical shifts of the protons in the NMR spectra of compounds 56 and 58 showed the presence of the hydroperoxide group in the side chain at C-2. Addition of triphenylphosphine to solutions of 56 and 58 afforded the corresponding diols 57 and 59 which were also present in the plant. The configuration at C-6 was unassigned to these compounds which are clearly related to acanthoaustralide (60)[84] from Acanthospermum australe (Compositae).

An unstable compound, Toxin 1 (61)[13,85] has been isolated from the fungus Alternaria citri. Several derivatives of 61, including the acetate and phenylboronate, were prepared in an attempt to stabilise this compound[13]. The degradation product of 61 is the decarboxylated compound A (62), also prepared from base or acid hydrolysis of 61. The chemical structures and relationship of these two compounds followed from NMR and CI-MS studies. An X-ray structure of 62 was used in conjunction with ^{1}H NMR, ^{13}C NMR and CD spectral data to assign the absolute stereochemistry in 62 and hence in 61. The stereochemistry at C-8' followed from the positive Cotton effect at 255 nm of the dibenzoyl derivative which was attributed directly to the contribution from the allylic benzoyl group at C-8'. The (S)-configuration at C-7' was suggested from NOE experiments. The stereochemistry at the other chiral centres followed from the X-ray structure. The negative Cotton effect in the CD spectrum of 61 further confirmed the (6R)-assignment. Four minor toxins with



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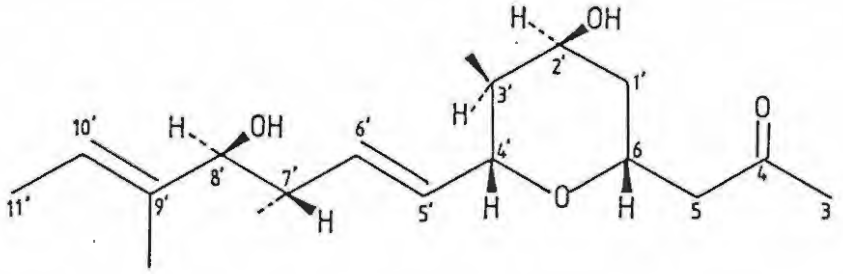
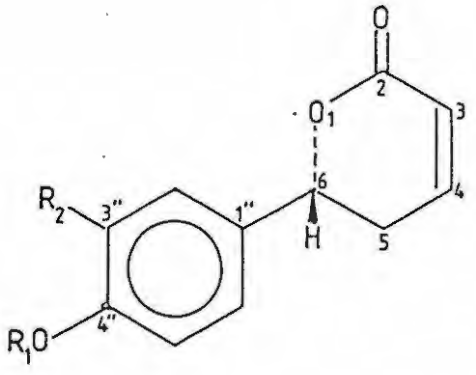


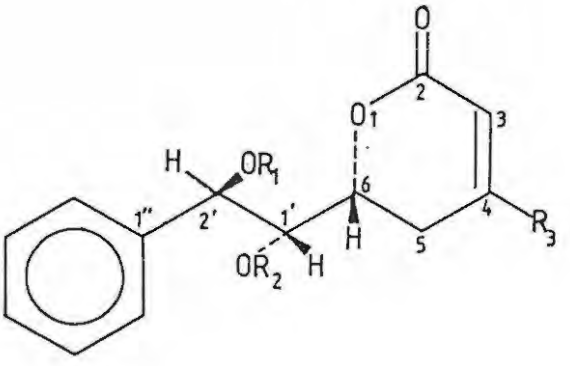
(62)



(63) R_1 = β-D glucosyl, R_2 = H

(64) R_1 = R_2 = H

(65) R_1 = H, R_2 = OH

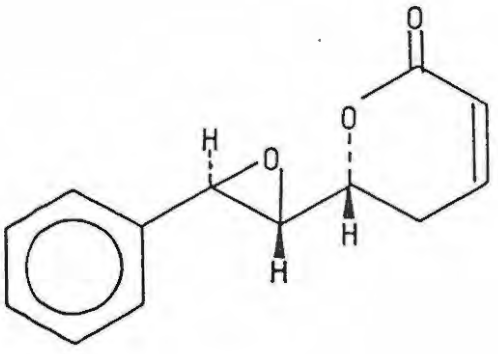


(66) R_1 = R_2 = R_3 = H

(67) R_1 = R_3 = H, R_2 = Ac

(68) R_1 = R_2 = Ac, R_3 = H

(69) R_1 = R_2 = H, R_3 = OH



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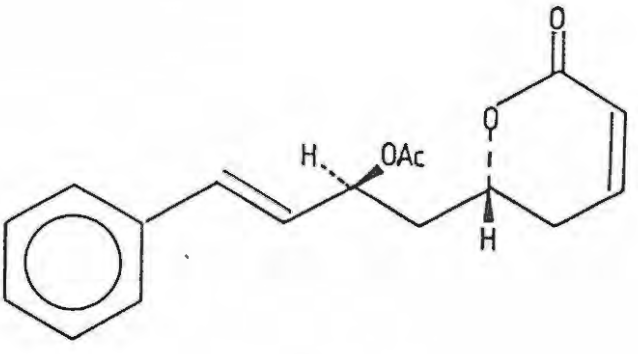


(71)

similar structures to Toxin 1 but possessing an α-pyrone nucleus have also been isolated from A. citri[86].

1:2:3 6-Aryl-5,6-dihydro-α-pyrones

The simplest of the 6-aryl-5,6-dihydro-α-pyrones is psilotin (63) first isolated from Psilotum nudum[87] and subsequently from Tmesipteris tannensis[88]. Both plants are members of the family Psilotaceae.

Hydrolysis of 63 yielded the aglycone psilotinin (64) and β-D-glucose. Chromium trioxide oxidation of 64 yielded p-hydroxybenzoic acid thus establishing the 1,4-benzene disubstitution. The presence of the αβ-unsaturated-δ-lactone ring was shown by IR and NMR studies and confirmed by a synthesis of racemic 64. Although 64 has a single assymetric centre at C-6, psilotinin is suprisingly optically inactive [87]. Racemisation is thought to occur during the enzymic hydrolysis of 63. 3" Hydroxypsilotin (65)[89], a minor compound from P. nudum, was also shown to undergo similar racemisation.

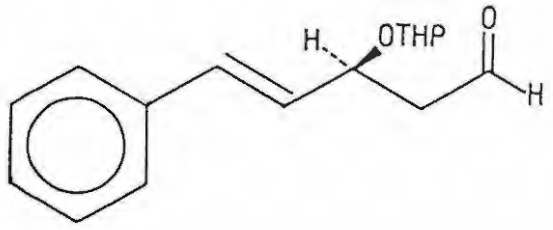
An (S)-configuration at C-6 in 63 was established from CD studies and by a total synthesis of 63 and its epimer[90]. No CD data has been reported for 65, but from the stereochemistry of 63 a (6S)-configuration can be assumed for this compound.

The presence of an exocyclic double bond is a common feature amongst 6-aryl-5,6-dihydro-α-pyrones. Goniothalamin (8), a 5,6 dihydro-α-pyrone with a 6-styryl substituent, is found in four species of plants from two different families. Goniothalamin was first extracted by Hlubucek and Robertson[91] from the bark of Cryptocarya caloneura. NMR and other physical methods revealed the presence of an αβ-unsaturated-δ-lactone ring and a monosubstituted benzene ring. The latter was confirmed by oxidation

of 8 to benzoic acid. The trans configuration of the exocyclic double bond followed from the IR absorbance at 973 cm^{-1}.

The absolute stereochemistry at C-6 in 8 has been uncertain. The original (S)-stereochemistry was proposed by Hlubucek and Robertson from oxidative ozonolysis of 8 and isolation in low yield of L-malic acid as its crystalline xanthate. This assignment survived for twelve years despite its lone contradiction of Snatzke's rules[40,41] relating to the C-6 stereochemistry of αβ-unsaturated-δ-lactones. This anomalous situation prompted Meyer[15] to synthesise the naturally occuring (+)-(R)-8 and its (-)-(S)-enantiomer from (-)-(E,R)- and (+)-(E,S)-3-hydroxy-5-phenyl-4-pentenoic acid respectively. The earlier assignment was thus corrected and further credibility given to the dictum, "synthesis is the final proof of structure".

Goniothalamus species (Annonaceae) are a rich source of 6-aryl-5,6-dihydro-α-pyrones. Goniothalamin has been isolated from three of these species G. andersonii, G. macrophyllus and G. malayanus[92]. G. sesquipedalis and G. grifithii have yielded four analogues of 8 differing in the hydroxylation and' or acetylation patterns at C1' and C2' , namely goniodiol (66), goniodiol monoacetate (67), goniodiol diacetate (68) and goniotriol (69)[93]. The structures of these compounds followed from NMR and MS measurements. Goniothalamin (8) can be assumed to be the logical precursor of these compounds. Talaptra et al[93] used this assumption to assign the stereochemistry at C-6 as (S) in compounds 66-69 from Hlubucek and Robertson's earlier incorrect assignment of the stereochemistry of 8. Although the work of Talaptra et al postdated Meyer's corrected stereochemical assignment of 8 by five years no reference is made to Meyer's paper. Therefore the assumed stereochemistry of C-6 in these four compounds is incorrect and should be (R).



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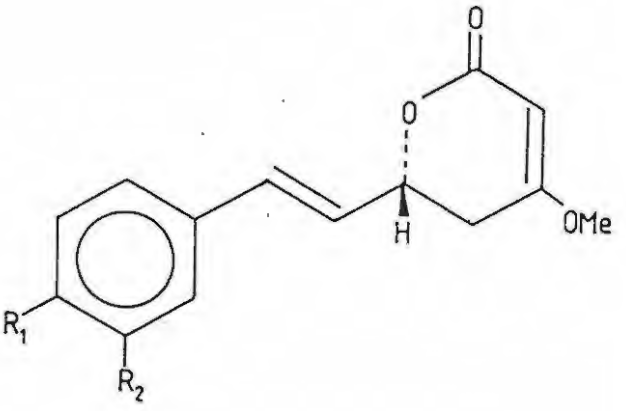
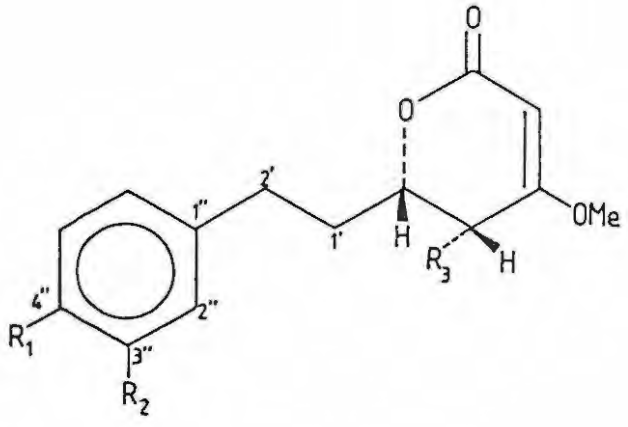


(73) $R_1 = R_2 = H$

(74) $R_1 + R_2 = -O-CH_2-O-$



(75) $R_1 = R_2 = R_3 = H$

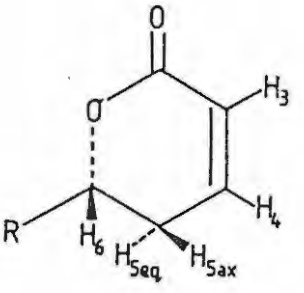
(76) $R_1 = R_2 = -O-CH_2-O-$, $R_3 = H$

(77) $R_1 = R_2 = H$, $R_3 = OH$

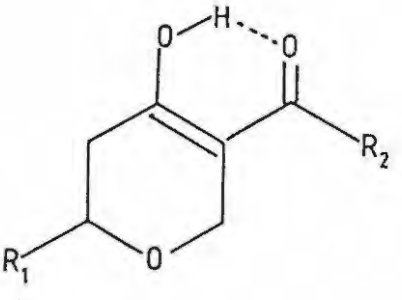
(78) $R_1 = OMe$, $R_2 = R_3 = H$

(79) $R_1 = OH$, $R_2 = OMe$, $R_3 = H$

(80) $R_1 = R_2 = OMe$, $R_3 = H$



(81)



(82)

The stereochemistry at C1' and C2' was shown by interconversion to be the same in compounds **66**-69. The coupling constants of the H-1' and H-2' protons (7.5-8.5 Hz) and consideration of gauche interactions suggested an erythro configuration for the vicinal diol. The (1'S)- and (2'S)-configuration of these four compounds was advanced from a theoretical biosynthetic argument involving epoxidation of **8** and subsequent enzymatic opening of the trans epoxide to give the erythro diol, **66**. The epoxide (**70**) has very recently been isolated from G. macrophyllus[94]. The configuration of the threo epoxide followed from the H_6, H1'coupling constant and the stereochemistry at C-6 was again proposed as (S) from the earlier incorrect assignment of Hlubucek and Robertson.

The genus Cryptocarya has also yielded a novel 6-aryl-5,6-dihydro-α-pyrone, with an exocyclic double bond at the 3'-position. Cryptocaryalactone (**71**) was first isolated by Govindachari and Parthasarathy[95,96] from C. bourdilloni Gamb. and subsequently from C. moschata[97]. The structure of **71** was proposed from application of standard NMR and MS techniques. The stereochemistry at C-6 and C-2' was not defined by Govindachari et al, and the trans configuration of the 3' double bond was once again assumed from an IR absorbance at 965 cm^{-1}.

A total synthesis of naturally occuring (+)-**71** and its epimer from the protected assymetric aldehyde (**72**) was recently published by Meyer[98]. The stereochemistry at C-2' in **71** is directly related to the stereochemistry at C-3 in **72**. The synthesis yielded a mixture of the two diastereomers of **71**, (+)-(2'S, 6R) and (-)-(2'S, 6S) which were separated by chromatography on silica gel. CD measurements on both diastereomers confirmed the (6R)-stereochemistry of (+)-**71**.

A large number of 6-aryl-5,6 dihydro-α-pyrones, methoxylated at C-4,

have been isolated from the tropical shrub Piper methysticum Forst. (Piperaceae), widely known in the South Pacific as kava, kawa or yanqona. It is an important folk medicine and forms the basis of a ceremonial and social drink much favoured by the Polynesians.

The chemistry of the kawa lactones or piperolides **73-80** has been extensively described in the literature. The structure elucidation of kawain (**73**), methysticin (**74**), dihydrokawain (**75**) and dihydromethysticin (**76**) has been thoroughly reviewed by Mors et al[7]. The identical stereochemistry at C-6 in all four of these compounds follows from CD measurements[99]. Hydrogenation of the exocyclic double bond results in a stereochemical order priority reversal about C-6. Accordingly **73** and **74** are assigned a (6R)-configuration and **75** and **76** a (6S)-configuration. An equatorial orientation of the C-6 substituent has been confirmed by NMR studies[100] of compounds **73-80** and this gives further credibility to the CD results.

Further evidence for the stereochemical assignment of methysticin, (+)-(**74**), has been provided by chemical degradation to D-malic acid which was isolated as its bis-phenylhydrazide[101]. Comparison of the optical rotation of this derivative with that from D-malic acid confirmed the (R)-configuration at C-6. Assuming a common biosynthetic origin of the kawa lactones this result confirms the stereochemistry of these compounds proposed from CD measurements.

In addition to the NMR and MS evidence for the structure of dihydrokawain-5-ol (**77**)[102] the positive Cotton effect suggested an (S)-configuration for both chiral centres. This was confirmed by the synthesis of naturally occuring (+)-**77** from **73**[103]. Oxidation of **73** with SeO_2 yielded the two diastereomers of kawain-5-ol which were separated by column chromatography. Surprisingly, catalytic hydrogenation of the

(6R, 5S)-diastereomer over 5% PdC at 0^{o} gave only one product (+)-dihydrokawain-5-ol with almost total retention of optical purity.

The development of a GC-MS technique for the separation and identification of the kawa lactones from P. methysticum Forst.[104] yielded a new lactone, (+)-5,6, 1'2'-tetrahydroyangoin (78), which was also later isolated by column chromatography.

Finally, column chromatography of a methanolic extract of the roots of P. methysticum Forst. yielded two compounds, 4"-hydroxy-3"-methoxy-dihydrokawain (79) and 4",3"-dimethoxy-dihydrokawain (80)[105]. These two compounds have been named in the literature as 11-hydroxy-12-methoxy-dihydrokawain and 11,12-dimethoxy-dihydrokawain respectively. The structures and absolute stereochemistry of 79 and 80 followed from standard NMR, MS and CD methods. Compounds 75, 76, 78 and 80 have also been isolated from a Brazillian species Aniba gigantifolia (Lauraceae)[106].

1:3 Physical methods of structure determination

Nuclear magnetic resonance spectroscopy, mass spectrometry, ultraviolet and infrared spectroscopy and increasingly circular dichroism and X-ray crystallography are used routinely in the structural determination of new 6-substituted-5,6-dihydro-α-pyrones. Detailed spectral data for individual compounds may be found in many of the papers already cited, accordingly this present discussion will focus on the more general applications of these spectroscopic techniques to the structural elucidation of this group of compounds. Obvious trends relating to the spectral behaviour of the αβ-unsaturated-δ-lactone ring and variations induced by substitution into this ring are also considered.

1:3:1 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a powerful and indispensable tool for obtaining structural information from new natural products. NMR has played a major role in the structure determination of more than two-thirds of the known naturally occuring 6-substituted-5,6-dihydro-α-pyrones. Spin decoupling or double resonance experiments have been used extensively to establish the chemical shifts (δppm) and coupling constants (J Hz) in the ^{1}H NMR spectra of these compounds. Lanthanide shift reagents have only been used in the NMR studies of one compound, argentilactone (39)[72], to resolve complex overlapping ^{1}H-resonances. Despite the proliferation in the last decade of high-resolution multiple pulse NMR techniques, especially 2D-NMR[107,108], only limited use has been made of these techniques for the interpretation of the complex spectra of 6-substituted-5,6-dihydro-α-pyrones.

Additive shielding parameters[109] are routinely used to predict the chemical shift of olefinic protons in the NMR spectra of unsaturated compounds. Proton H-3 in structure (81) resonates at δ5.9-6.1 ppm and is coupled to H-4 (J = 9.7-10 Hz) indicative of a cis olefinic function situated adjacent to a carbonyl group. Proton H-3 is also coupled by long-range coupling [12,37,38,72] to the two protons attached to C-5 ($J_{3,5ax}$ = approx. 1 Hz, $J_{3,5eq}$ = 2-3 Hz). Methoxylation at C-4 results in shielding of H-3 which then resonates upfield at δ 5.13-5.18 ppm[17,100]. The deshielding of H-4 (δ 6.78-7.05 ppm) relative to H-3 is typical of a proton attached to the β-carbon of an αβ-unsaturated-carbonyl chromophore. The signal for H-4 appears as a triplet of doublets from its coupling to H-3 and also to $H\text{-}5_{ax}$ ($J_{4,5ax}$ = 2-4 Hz) and H-5eq ($J_{4,5eq}$ = 4-6 Hz). These J-values have been used to determine the relative stereochemistry of

substituents at C-5[49,50,54,62]. The presence of either a hydroxyl or acetoxy group at C-5 has little effect on the chemical shift of H-4.

The two allylic protons at C-5, H-5ax and H-5eq, are not equivalent, exhibiting a typical geminal coupling[17,72,100] to each other ($J_{5ax,5eq}$ = 15-19 Hz) and a coupling to H-4 and to H-6 ($J_{5ax,6}$ = 9-12 Hz, $J_{seq,6}$ = 3-6 Hz). These two protons resonate as a complex multiplet with chemical shifts normally centred between δ 2.3 ppm and δ 2.8 ppm. Methoxylation at C-4[100] has a minimal deshielding effect on the chemical shifts of H-5ax and H-5eq but hydroxylation or acetylation at C-5 results in a downfield chemical shift of the residual axial or equatorial H-5 proton to δ 4.2-5.4 ppm[49,50,54,62].

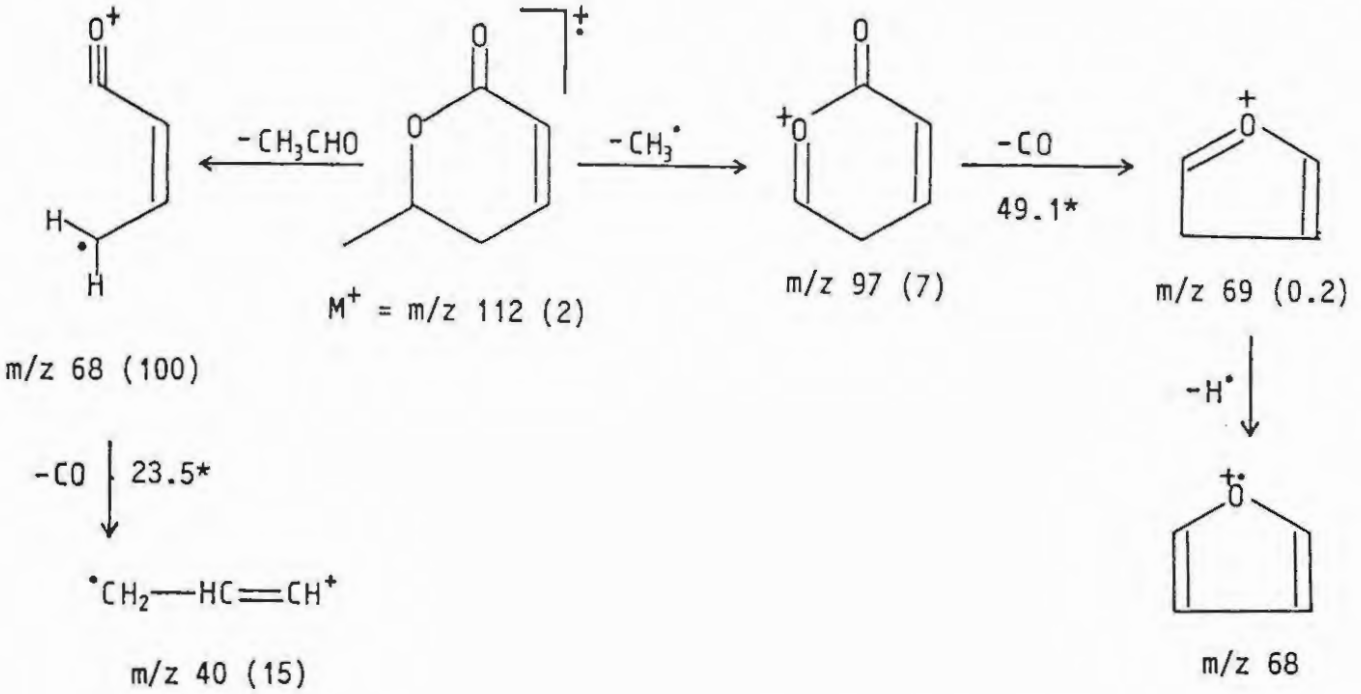
An NMR study[110] of the conformation of parasorbic acid (**13**) suggests from the size of coupling constants ($J_{5ax,6}$ = 10.3 Hz and $J_{5eq,6}$ = 5.4 Hz) that the two H-5 protons make dihedral angles of about 160^{o} and 40^{o} respectively with H-6, and hence the C-6 methyl group is pseudo-equatorially oriented in the most stable conformation of **13**. The H-5, H-6 coupling constants have also been used to assign a pseudo-equatorial orientation to the C-6 side chains of compounds **7**[12], **26**[49], **27**[50], **28**[54], **39**[72] and **73-77**[100]. The chemical shift of the H-6 proton is situated between δ 4.2-5.1 ppm and variations within this range are determined by the type of substituent at C-6. Oxygenation of the side chain results in a number of contiguous chiral centres. The relative stereochemistry of these centres has been determined[13] by application of the Nuclear Overhauser Effect (NOE) which relates the observed proton signal enhancements from the resonant frequency field irradiation of an individual proton, to the internuclear distances between these protons.

Important structural information is also provided by ^{13}C NMR, the use

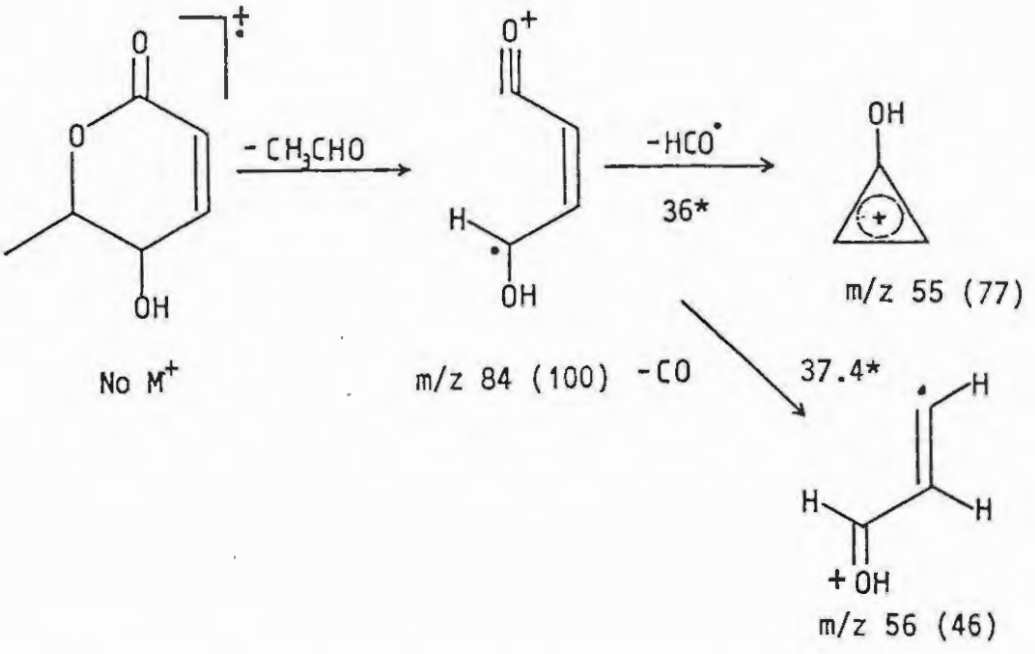
of which has been greatly facilitated by the advent and accessibility of modern high resolution Fourier Transform instruments. Assignments of the individual ^{13}C chemical shifts of 6-substituted-5,6-dihydro-α-pyrones has been achieved mainly from proton noise decoupled and off-resonance decoupled experiments.

The ^{13}C chemical shift of the carbonyl carbon atom at C2, in 6-substituted-5,6-dihydro-α-pyrones (81), is generally between δ 160-169 ppm. Carbon atom C-3 resonates at δ 120-122 ppm but methoxylation at C-4 causes this signal to shift to δ 89-94 ppm[111]. The signal at C-4 (δ 144-148 ppm) is conversely shifted approximately 30 ppm downfield by the presence of this methoxy-group. The C-5 carbon atom resonates at δ 25-30 ppm and this signal is only shifted slightly downfield (2-4 ppm) by methoxylation at C-4. Oxygenation at C-5 generates a downfield shift of its ^{13}C signal by about 40 ppm[111]. It also affects the C-4 chemical shift by 3-6 ppm and the C-6 resonance, normally at δ 72-78 ppm, shifts downfield by about 35 ppm.

The presence of oxygenated side chains obscures the ^{13}C resonance of C-6. The problems inherent in assigning individual ^{13}C shifts in these compounds can be alleviated by modern NMR techniques. A single DEPT (distortionless enhancement by polarisation transfer)[112] experiment can be used to determine the ^{13}C multiplicities while a 2D-COSY (correlated spectroscopy)[113] experiment will give the proton coupling sequence. The ^{13}C shifts are unambiguously assigned when the results from these two determinations are combined with a 2D $^{13}C/^{1}H$ HETCOR (heteronuclear shift correlated spectroscopy)[114] experiment. Applications of this method to the assignment of ^{13}C and ^{1}H shifts in the NMR of synrotolide (1) and boronolide (3) are presented in this thesis.



Scheme 1. Major mass spectral fragments of parasorbic acid[a]



Scheme 2. Major mass spectral fragments of osmundalactone[a]

[a] Relative abundance in parentheses

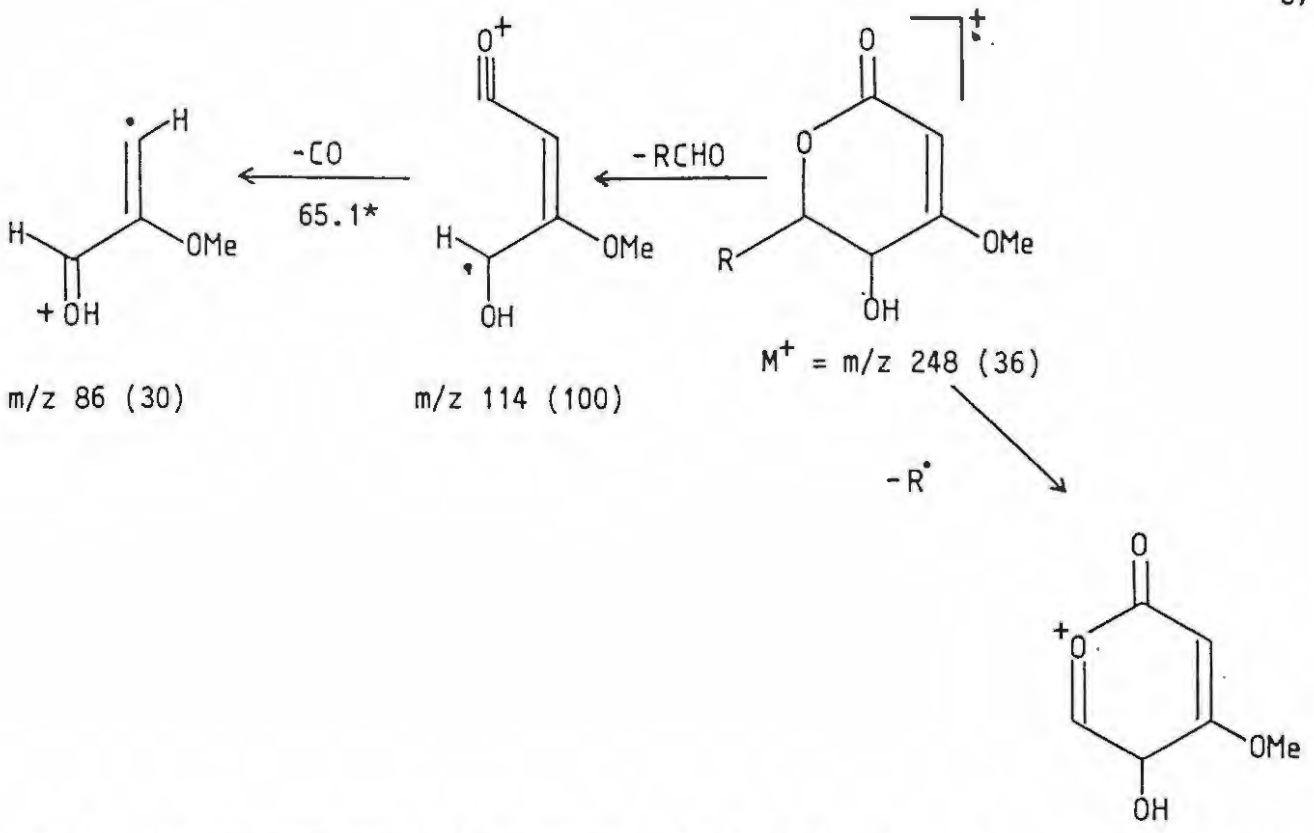
* metastable ions

1:3:2 Mass spectrometry

Mass spectrometry (MS), unlike NMR, has been used predominantly as a supplementary technique for the determination of the chemical structure of 6-substituted-5,6-dihydro-α-pyrones. The great variation in substitution at C-6 and around the lactone ring inhibits a comprehensive presentation of the mass spectra of these compounds. However, the αβ-unsaturated-δ-lactone ring undergoes a series of standard fragmentations in electron impact mass spectrometry (EI-MS) which yield ions of substantial diagnostic value.

The fragmentation modes of eight 6-alkyl-5,6-dihydro-α-pyrones have been studied by Urbach, Stark and Nobuhara[115] using metastable transitions for interpretation of the major fragments. The major mass spectral fragments of the simplest 6-substituted-5,6-dihydro-α-pyrone, parasorbic acid (13), are shown in Scheme 1[115,116]. The two ions at m/z 97 and m/z 68 correspond to the facile α-cleavage of the C-6 side chain which is characteristic of this group of compounds. The ion at m/z 68 is also formed from further fragmentation of the m/z 97 ion[35,72,95]. The structure of the former fragment has been a source of well documented controversy[117] and both proposed structural representations are given in Scheme 1. The cleavage of the C-6 substituent also affords relatively abundant ions in the mass spectrum of multi-substituted-5,6-dihydro-α-pyrones as represented by osmundalactone (26)[49] (Scheme 2) and dihydrokawain-5-ol (77)[102] (Scheme 3). The base peak at m/z 84 in the mass spectrum of 26 can also be interpreted as arising from the loss of CO_2 from the molecular ion. However, high resolution measurements have confirmed the fragmentation as shown in Scheme 2.

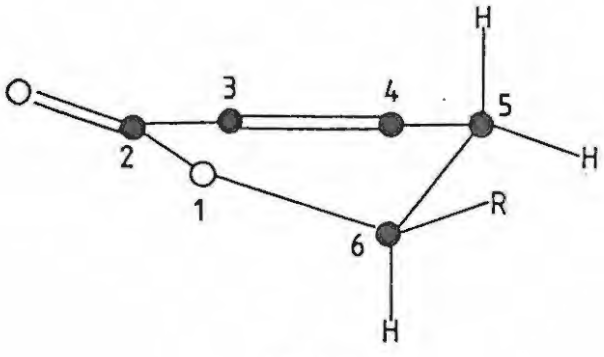
Finally, 6-substituted-5,6-dihydro-α-pyrones, with the exception of



Scheme 3. Major mass spectral fragments of dihydrokawain-5-ol (R = $\not{\text{Ø}}CH_2CH_2$-)[a]

[a] Relative abundance in parentheses

* metastable ions



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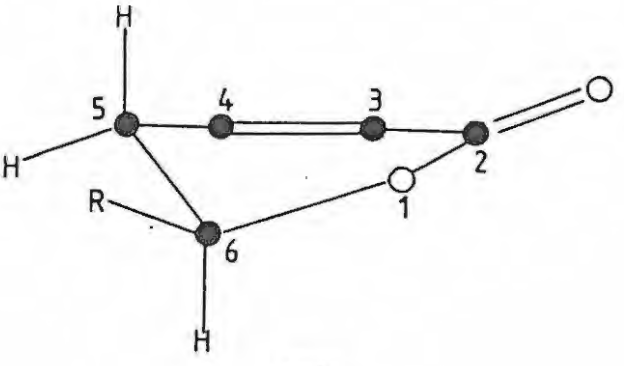

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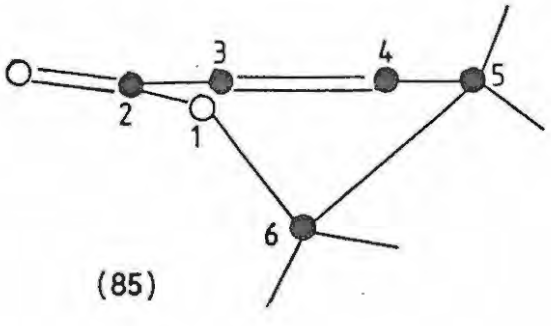

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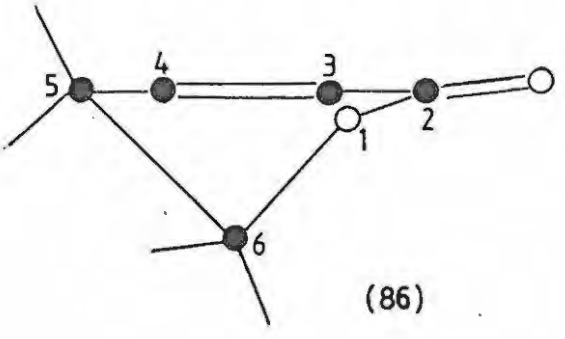


(86)

some polyhydroxylated compounds[13,37,49], exhibit a molecular ion in their mass spectra. The mass spectra of polyhydroxylated 6-substituted-5,6-dihyro-α-pyrones are complicated by thermal decompositions but this problem can be alleviated by trimethyl-silylation.

1:3:3 Infrared and ultraviolet spectroscopy

6-substituted-5,6-dihydro-α-pyrones typically exhibit a strong absorption band in the infrared at 1710-1730 cm^{-1} attributed to the αβ-unsaturated carbonyl group. The lactone double bond absorbs at 1590-1640 cm^{-1} and although this absorption is usually much weaker than that of the carbonyl, its intensity is enhanced by oxygenation at either C-4 or C-5[17,49]. A further absorption at 1555 cm^{-1} displayed by lachnelluloic acid (**31**)[67] and related compounds **32**-**34**[68] has been interpreted as arising from the strongly chelated, enolized β-diketone (82)[67]. A tenuous trans configuration for the exocyclic double bond in compounds **8**[91], **41**[37], **42**[37], **44**[76] and **71**[95] has been assigned from the infrared absorption at approx. 965 cm^{-1}.

The UV absorption maxima (λ) of monosubstituted-6-alkyl- and 6-alkenyl-5,6-dihydro-α-pyrones is normally between 200-215 nm (ε = 800-12000). Methoxylation at C-4 induces a bathochromic shift of approximately 20 nm[17]. Hydroxylation or acetylation at C-5[49] has no effect on the value of λ but acylation at C-3 generates a further UV absorption at 274 nm (ε = 1100)[67]. 6-Aryl-5,6-dihydro-α-pyrones, e.g. psilotin (**63**), also absorb between 275-285 nm. The extended chromophore of the styryl substituent in compounds **8**[91], **71**[95], **73** and **74**[7] is responsible for three characteristic strong UV absorptions at 250 nm (ε = approx. 19000), 283 nm (ε = approx. 16000) and 292 nm (ε = approx. 12000).

1:3:4 Circular dichroism

The chiroptical techniques optical rotary dispersion (ORD) and circular dichroism (CD) have been used as structural tools in the study of natural products for over thirty years[118]. The CD and ORD data of 6-substituted-5,6-dihydro-α-pyrones is presented in Table 1. Prior to 1965 only ORD measurements could be made easily but recent advances in instrumentation, coupled with the simplicity in form of CD spectra (resulting in easier theoretical interpretation), has led to a steady increase in the use of CD and this is now the preferred technique.

ORD is a measure of the difference in refraction and CD a difference in absorption of right and left circularly polarised light by a dissymetric medium. ORD measurements are expressed in units of molecular rotation $[\Phi]$ while CD values are quoted in two different units, the differential absorption $\Delta\epsilon$ and the molecular ellipticity $[\theta]$, related by the expression $[\theta] = 3300\,\Delta\epsilon$.

In the CD or ORD spectra of αβ-unsaturated-δ-lactones the carbonyl group exhibits a distinct $n\rightarrow\pi^*$ Cotton effect (R band) near 260 nm[40,41,119]. The negative or positive sign of this Cotton effect has been interpreted by Snatzke[40] to denote either conformation (83) or (84) respectively for the lactone ring. This interpretation, widely known as Snatzke's rules, is based on the CD spectrum of parasorbic acid (13) and the assumption that the co-planarity through the lactone ester group of saturated-δ-lactones is retained in αβ-unsaturated-δ-lactones.

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

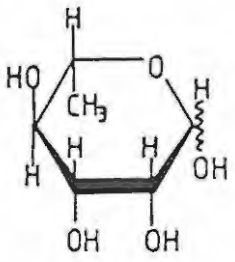
Compound	No	CD $n \rightarrow \pi^*$ $\Delta\varepsilon$	CD $n \rightarrow \pi^*$ λnm	ORD $n \rightarrow \pi^*$	ORD $n \rightarrow \pi^*$ λnm	Stereochemistry at C-6 implied from Snatzke's rules
Anamarine	45	+[a]	260	-	-	(R)
Argentilactone	39	+2	256	-	-	(R)
Asperline	27	+2	265	-	-	(R)
Aspyrone	29	-4.8	263	-1341	263	(R)
C_{26} Lactone	20	-1.32	252	-	-	(S)
C_{26} Lactone	21	-0.59	253	-	-	(S)
Cryptocaryalactone	71	+1.17	265	-	-	(R)
Dihydrokawain	75	+11.2	246	-	-	(S)
Dihydrokawain-5-ol	77	+11.5	247	-	-	(S)
Dihydromethysticin	76	+11.0	246	-	-	(S)
3",4"-Dimethoxy-dihydrokawain	80	+13.13	247	-	-	(S)
Goniothalamin	8	+6.5	253	-	-	(R)
4"-Hydroxy-3"-methoxy-dihydrokawain	79	+8.58	247	-	-	(S)
Hyptolide	7	+2.3	257	-	-	(R)
Kawain	73	+8.3	249	-	-	(R)
Massoialactone	16	-	-	-8900	265	(R)
Methysticin	74	+8.9	242	-	-	(R)
Olguine	46	+[a]	270	-	-	(R)
Osmundalactone	26	+3.86	263	-	-	(S)
Parasorbic acid	13	+2.25	262	+7400	265	(S)
Pestalotin	5	-7.90	243	-	-	(S)
Phomolactone	28	+0.7	265	-	-	(R)
Psilotin	63	-3.3	270	-	-	(S)
Toxin 1	62	-7.75	246	-	-	(R)

[a] Value not cited in literature

The assignment of conformation 84 (R=Me) to parasorbic acid by Snatzke arises from the known stereochemistry[22,23] and the pseudo-equatorial orientation of the substituent methyl group established from NMR studies[110]. Conversely, therefore, Snatzke's rules can be used to assign the absolute stereochemistry at C-6 in 6-substituted-5,6-dihydro-α-pyrones if the sign of the Cotton effect and the orientation of the C-6 substituent are known.

Although the H-5, H-6 NMR coupling constants remain crucial parameters for the unequivocable confirmation of the orientation of the C-6 substituent, a pseudo-equatorial orientation of the C-6 side chain can be inferred from consideration of steric factors. With the exception of the two C_{26}-lactones 20 and 21[39], the absolute stereochemistry at C-6 implied by Snatzke's rules (Table 1) has been confirmed by either chemical degradation, synthesis or biosynthetic relationships. Therefore, in the absence of any evidence to the contrary, a pseudo-equatorial orientation of the C-6 substituent can be assumed for 6-substituted-5,6-dihydro-α-pyrones.

The conformation of the αβ-unsaturated-δ-lactone ring has been the subject of considerable speculation[41,120,121]. Beecham[41] has proposed that co-planarity through the -C-CO-O-C group, as assumed by Snatzke, is not normal in αβ-unsaturated-δ-lactones and suggests that the lactone ring adopts the minimum energy enantiomeric conformations 85 and 86, in which the C_6 atom is displaced by approx. 0.5 Å from a least squares plane containing C-2, C-3, C-4 and C-5. Atom O-1 is slightly displaced towards C-6. The X-ray crystallographic analyses of boronolide (2)[36], hyptolide (7)[12] and goniothalamin (8)[122] further corroborates this suggestion. However, despite this deviation from the original assumption, the empirical interpretation of Snatzke's rules is still valid.



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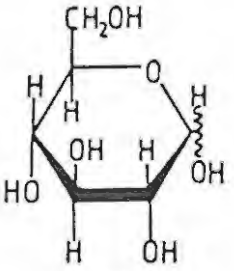

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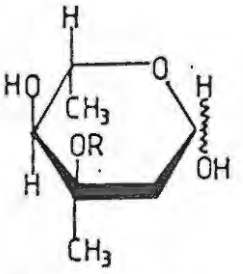
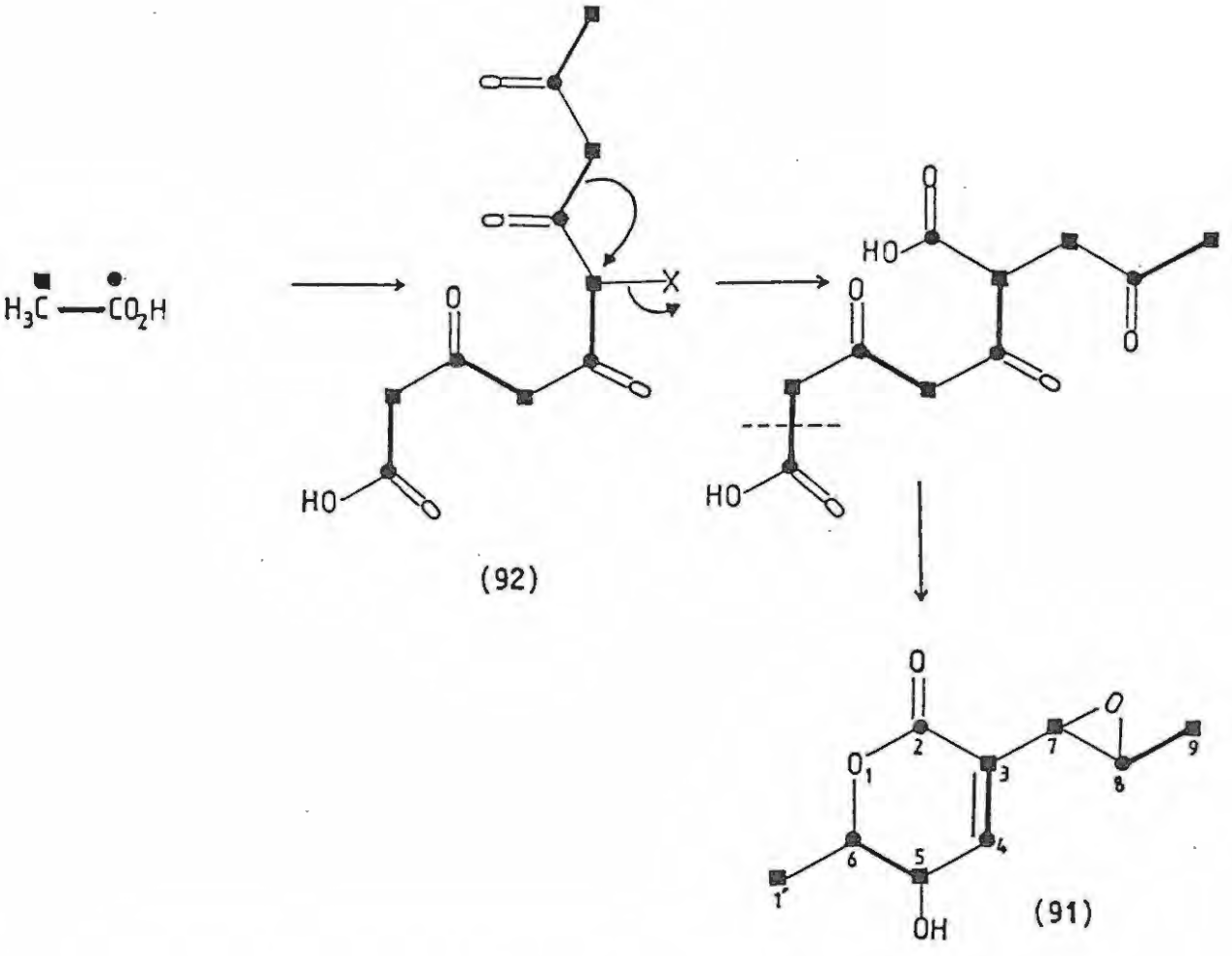


(89) R = H

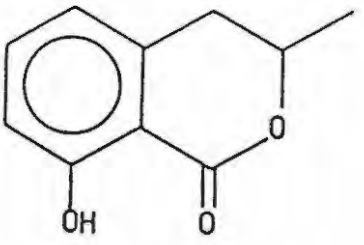
(90) R - Me



(92)

(91)

Scheme 4. The incorporation of ^{13}C labelled acetate in the biosynthesis of aspyrone



(93)

Oxygenation at C-5 results in a strong contribution to the sign of the $\pi \rightarrow \pi^*$ Cotton effect between 205-230 nm in $\alpha\beta$-unsaturated-δ-lactones. From studies on similar compounds Beecham[41] has proposed that the positive or negative Cotton effect generated by the allylic oxygen can be related respectively to a right handed or left handed chirality of the oxygen-olefinic bond. Evidence in support of this proposal is provided by the fungal metabolites asperline (27), phomolactone (28) and aspyrone (29). The chirality of C-5 in these compounds suggested from the positive $\pi \rightarrow \pi^*$ Cotton effect is in accordance with their absolute stereochemistry at C-5, established by NMR studies and the conformation of the lactone ring determined by Snatzke's rules.

1:4 Biosynthesis

The biosynthesis of only three compounds, parasorbic acid (13), aspyrone (29) and psilotin (63), has been reported in the literature.

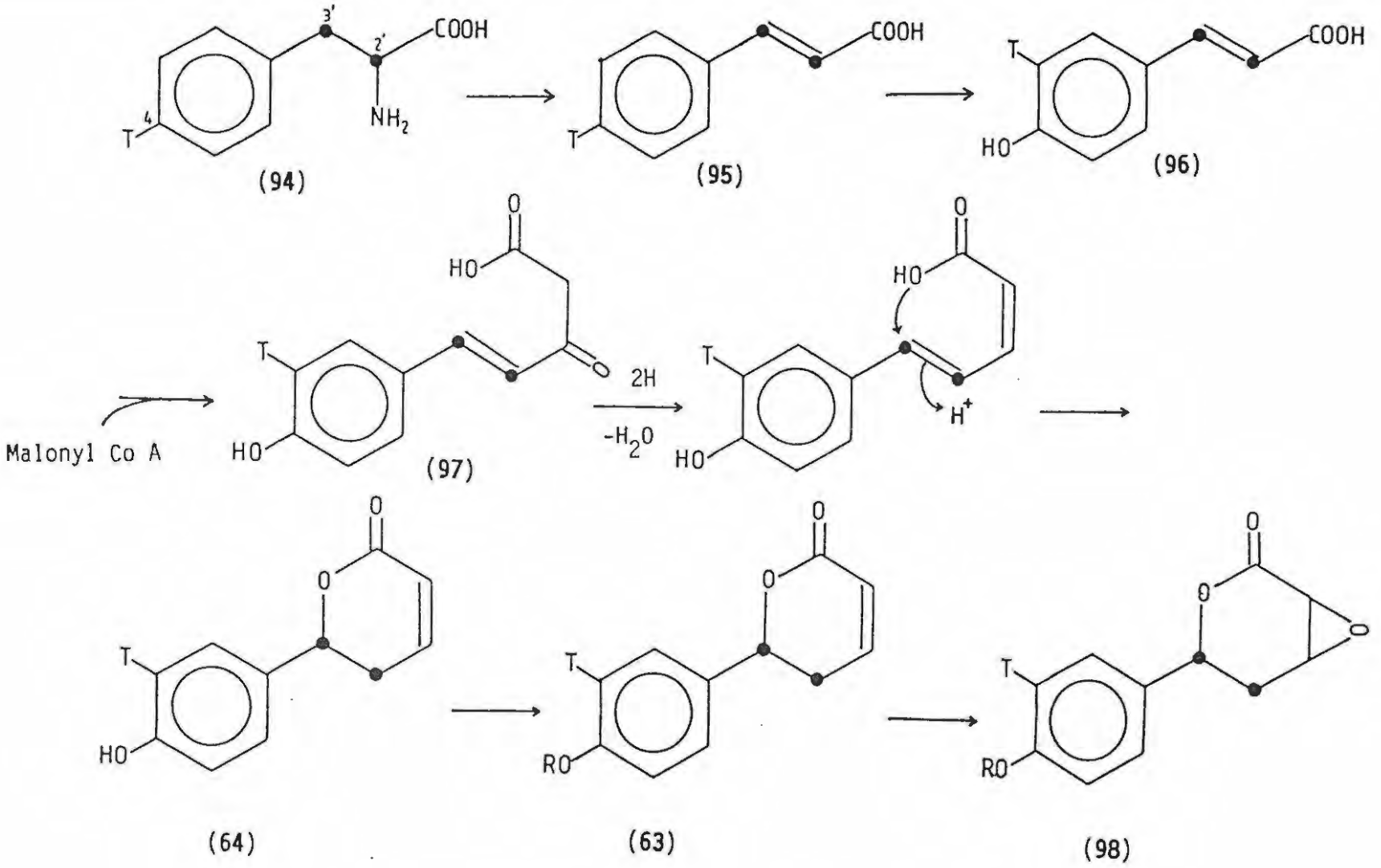
The first biosynthetic investigation of a 6-substituted-5,6-dihydro-α-pyrone was made by Crombie and Firth [123] with parasorbic acid in the Rowan berry (Sorbus aucuparia L.) The chemical structure of 13 suggests that L-rhamnose (87) is a possible 6-deoxy-sugar precursor in which the (6S)-configuration of 13 arises directly from the (5S)-configuration of 87. However, L-rhamnose is absent from Rowan berries and the major monosaccharides are D-glucose (88) and D-sorbitol[21]. Although 88 has a (5R)-configuration, the alteration in the level of oxygenation and the C-5 configurational inversion incorporated in the biosynthesis of L-mycarose (89) and L-cladinose (90) from 88[124,125], does not preclude this compound from being a possible precursor of 13. This hypothesis was tested by injection of $[1\text{-}^{14}C]$-, $[2\text{-}^{14}C]$- and $[6\text{-}^{14}C]$-D-glucose into ripening Rowan berries. The resultant distribution of labelled carbon atoms in 13 was

determined by extraction and chemical degradation of **13** into fragments containing carbon atoms C-1, C-5 and C-6 respectively. The radioactivity of each fragment was measured. The biosynthesis of **13** via an acetate-malonate pathway was also investigated by injecting sodium [1-^{14}C]- and [2-^{14}C]-acetate, and sodium [1-^{14}C]-malonate into Rowan berries.

The substantially higher isotope incorporations and the distribution of the ^{14}C-label in the C-1, C-5 and C-6 fragments of **13** from the latter experiment, suggests that the acetate-malonate pathway is the major biosynthetic route to **13**. Although experiments with the different ^{14}C-D-glucose compounds showed some incorporation of the ^{14}C-isotope at the corresponding carbon atom of **13**, the majority of the label was shifted to other carbon atoms within this molecule and it is therefore unlikely that D-glucose is incorporated as an intact unit into **13**. Crombie and Firth proposed that the ^{14}C isotope from the labelled D-glucose was randomised in the glucose pool via degradation to triose sugars and subsequent resynthesis by the reactions of the pentose cycle.

The biosynthesis of aspyrone (**29**) by the fungus Aspergillus melleus has been well documented. Initial ^{14}C labelling experiments[126] established the polyketide origin of **29** but the unusually branched carbon skeleton differs from that of similar compounds, e.g. asperline (**27**), and does not arise from conventional polyketide transformations.

The incorporation of [1-^{13}C]-, [2-^{13}C]- and [1,2-^{13}C]-acetate into **29** by A. melleus[58,59,60,127,128] has indicated that this compound is derived from three intact acetate units and that the three remaining carbons originate from cleaved acetate units (**91**). The C-3 and C-7 ^{13}C-coupling constant (61 Hz) suggests that the bond between these two atoms is formed from the head to head linkage of two acetate units[58]. Correspondingly



Scheme 5. The proposed biosynthesis of psilotin from phenylalanine

R = β-D-glucosyl

the long range ^{13}C-coupling constant (6.2 Hz) between C-2 and C-7 is indicative of two carbon atoms derived from the same acetate unit[59]. Accordingly the proposed biosynthesis of 29 (Scheme 4) proceeds via a Favorski-type rearrangement of the linear polyketone chain (92)[59,128,129]. Mellein (93) is also a major metabolite from A. melleus[129] and the possibility of an alternative pathway to 29 which includes a mellein type precursor has been disregarded[130] as a result of tritium incorporation studies using [2-^{3}H]-acetate.

Phenylalanine (94) is the major precursor in the biosynthesis of psilotin (63)[131]. The biosynthetic pathway (Scheme 5) was proposed from isotope incorporation studies with (RS)-[2', 3'-$^{13}C_2$]- and (S)-[1'^{14}C]-phenylalanine. The first step in the biosynthesis is the deamination of 94 to yield cinnamic acid (95). Tritium labelled phenylalanine was used to monitor the expected NIH shift which occurs in the conversion of 95 to p-coumaric acid (96)[132]. Chain extension to 96 occurs via the addition of a single acetate unit as malonyl CoA. The structural similarity between 64 and the kawa-lactones suggests a common biosynthetic pathway and kawain is also possibly biosynthesised from 96 with the addition of two acetate units. ^{13}C NMR of the isolated 63 and psilotin epoxide (98) revealed a strong retention of the ^{13}C isotopes from [2', 3'-$^{13}C_2$]-phenylalanine at C-5 and C-6 of these compounds, thus supporting the proposed biosynthetic pathway.

1:5 Biological activity

Allelopathy is the detrimental effect of certain plants on others, instigated by the production of chemical inhibitors or allelochemicals. A number of 6-substituted-5,6-dihydro-α-pyrones have been identified as allelochemicals. Parasorbic acid (13)[133], psilotin (63)[3] and

cryptocaryalactone (71)[134] inhibit seed germination and plant growth. The inhibitory effects of 63 are reversed by glutathione, other thio-compounds and gibberellin A_3, suggesting that 63 forms part of a growth regulatory system[3].

6-Substituted-5,6-dihydro-α-pyrones isolated from fungi exhibit a variety of biological activities. Compounds 27 and 28 possess antibacterial activity[51-53]. Pestalotin (5)[17] enhances the growth-stimulative effect of gibberellic acid in plants, while conversely Toxin 1 (61)[85] and alternaric acid (30)[5] are phytopathogenic. Compound 61 is responsible for brown spot disease on lemons and Rangpur limes while 30 has been shown to cause the collapse of tissues and wilting in plants from the families Solanaceae, Cruciferae and Compositae. Alternaric acid and the other fungal metabolites acylated at C-3 are remarkably specific antifungal toxins. Alternaric acid inhibits germination of the spores of Absidia glauca, Myrothecium verrucia and Stachybotrys atra at very low concentrations, lachnelluloic acid (31)[67] is antagonistic towards Dutch elm disease and wood rotting fungi, while the podoblastins A,B,C, (32), (33), (34)[68] have been reported to possess specific antifungal activity against rice blast. The higher plant metabolite goniothalamin (8) also exhibits antifungal activity[92].

Although many plants utilize allelochemicals as part of a chemical defense against insect attack, only one 6-substituted-5,6-dihydro-α-pyrone has been reported to possess insect antifeedant properties. Osmundalactone (26)[4] inhibits the feeding of the larvae of the yellow butterfly Eurema hecabe mandarina on the fern Osmunda japonica Thunberg. Interestingly, two species of ants from the genus Camponotus employ massoialactone (16)[35] as a defensive substance.

A large number of naturally occuring compounds which exhibit diverse types of physiological action have either an unsaturated δ- or γ-lactone group as a common structural feature, e.g. the cardiac glycosides or the antibiotic penicillic acid. Accordingly, 6-substituted-5,6-dihydro-α-pyrones also display wide ranging physiological activity. The carcinogenic properties of parasorbic acid have been studied[135] and although the αβ-unsaturated-δ-lactone ring is potentially carcinogenic[136], the strong in vivo anti-leukemic activity of compounds (50) (51) and (52) in mice refutes this broad generalisation.

The most diverse pharmacological action of the 6-substituted-5,6-dihydro-α-pyrones is provided by the kawa-lactones (73-78), the active principles from the root, rhizome and lower stem of Piper methysticum Forst[137]. For centuries this plant has been used to prepare an intoxicating and soporific beverage. Kava resin was used in Europe before 1914 for the treatment of gonorrhea and gout. The wide-ranging pharmacological properties of kava which have been reviewed by Meyer[138], include smooth muscle relaxant, local anaesthetic, protection against electro and chemo shock, anti-inflammatory, antipyretic, antimycotic and antiedemic activities.

Finally, compounds closely related to the kawa-lactones also have interesting physiological properties e.g. goniothalamin from Goniothalamus macrophyllus exhibits CNS activity[92]. G. macrophyllus is used as an abortifacient in rural areas of Northern Malaysia and both 8 and the epoxide (70) are reported to induce foetal abnormalities in mice[94].

CHAPTER TWO

DISCUSSION

2. The Lamiaceae

The Lamiaceae (formerly Labiatae) is a large cosmopolitan family of herbs and shrubs which grow in almost all types of habitat and at all altitudes[139]. The family consists of approximately 170 genera and 5 000 species worldwide of which 37 genera and 232 species have been reported by Codd[140] to be indigenous or naturalised to Southern Africa. A number of these Southern African species have afforded new natural products whose structural elucidation has proved both challenging and rewarding[141]. The work presented in this thesis is a continuation of this phytochemical investigation and the extractives of six species from four different genera have been examined.

In recent years the search for novel biologically active natural products from plants has assumed enormous proportions. The National Cancer Institute in the U.S.A. has screened over a 100 000 plant extracts against various forms of cancer. This immense multidisciplinary effort has however only yielded ten compounds[142] with promising anti-leukemic activity. Considering these modest returns the validity of such a costly random exercise seems questionable.

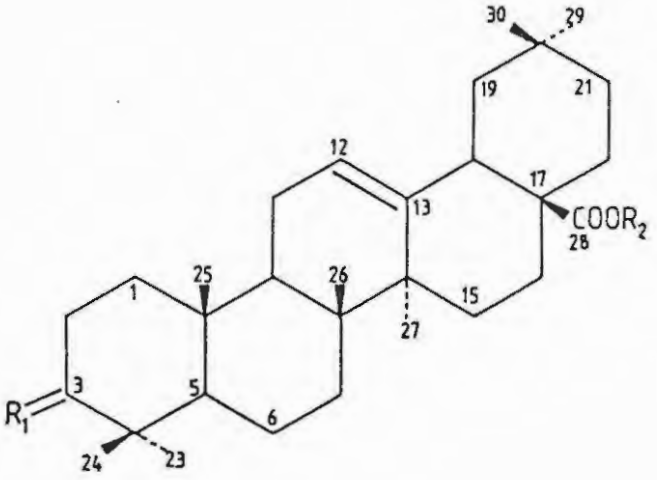
An alternative approach is to direct attention to the investigation of traditional medicines and phytotherapy. In many cultures various plants have been a primary source of medicine for numerous generations. Despite a usage developed over centuries of trial and error, the identification of the active principles in these plants is hindered by a number of factors. These include difficulties in testing scientifically non-specific symptoms of illness, the frequent use of concoctions of different plant extracts and a possible variation in the active ingredient concentration with season, locality and stage of growth of the plant.

For many years the Lamiaceae were thought to be devoid of a species which contained a secondary metabolite with major therapeutic activity[142]. This preconception was dismissed with the isolation of the novel labdane diterpenoid forskolin from the roots of Coleus forskohlii, an Indian medicinal plant. Forskolin is a unique adenylate cyclase activator which lowers normal or elevated blood pressure in various animal species and its pharmacological activity is well documented[142]. The discovery of forskolin has therefore provided impetus to the continued isolation and structure determination of natural products from the Lamiaceae.

Hagenauer[143] has stressed the important relationship between phytochemistry and plant taxonomy. This relationship or chemotaxonomy greatly facilitates the truly natural classification of the many groups of plants occuring in nature. The taxonomy of South African species of the genus Leonotis (Lamiaceae) has recently been reviewed by Iwarsson[140] and the necessity for a parallel chemotaxonomic investigation of this genus has emerged to either refute or support the revised classification. Phytochemical relationships between members of this genus will be discussed here. Finally an understanding of the chemotaxonomy of a particular family or genus not only generates leads in the search for important chemical compounds from plants, but also enables a researcher to recognise unexpected or rearranged products amongst the natural products isolated during an investigation.

2:1 Syncolostemon rotundifolius E. Mey. ex Benth.

The genus Syncolostemon is indigenous to Southern Africa and consists of nine species[140]. The genus is closely related to Hemizygia and has not been subject to a phytochemical examination before. The genus is confined to eastern South Africa and its range extends from the eastern Transvaal



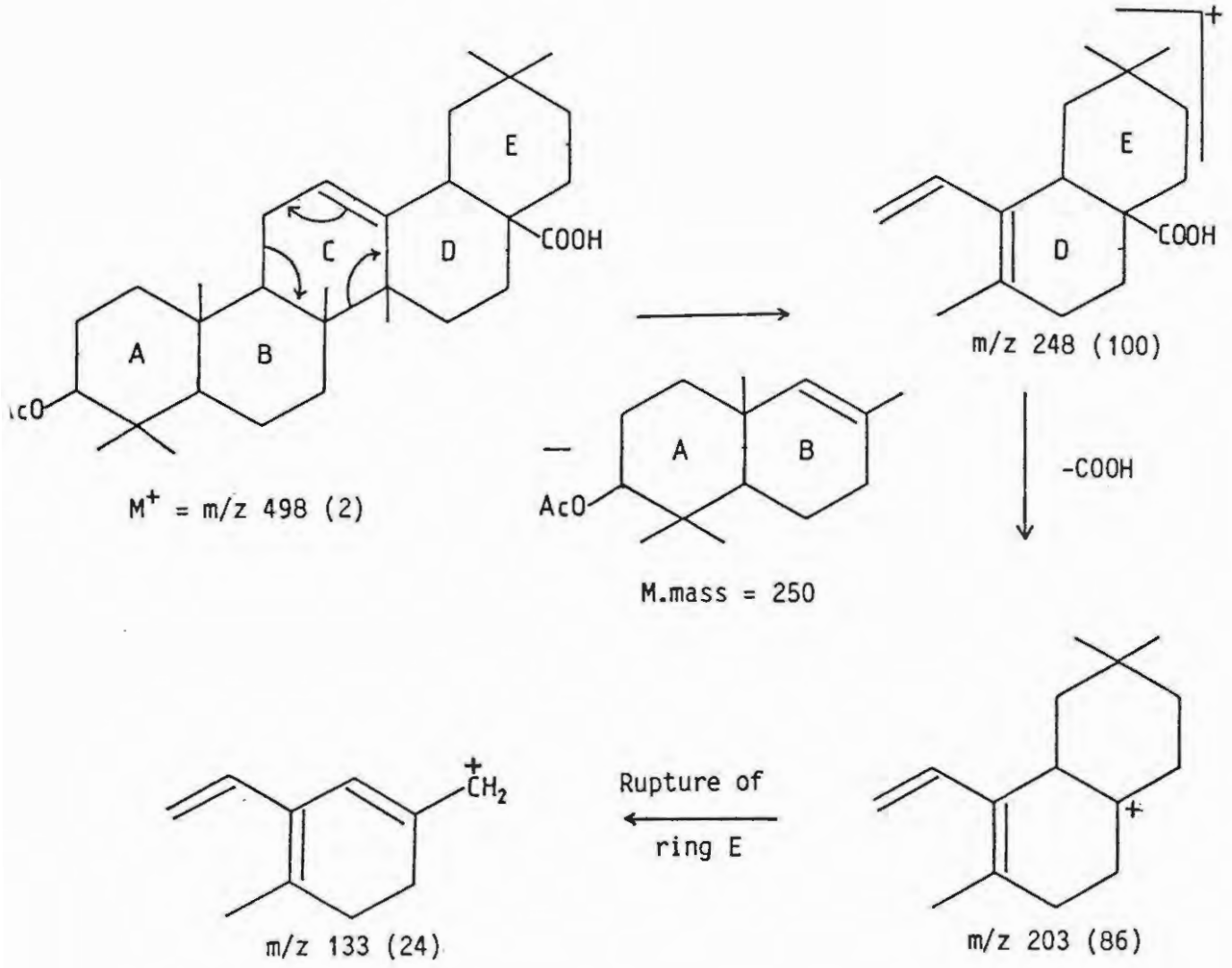
(99) R_1 = H, β-OH, R_2 = H

(100) R_1 = H, β-OAc, R_2 = H

(101) R_1 = H, β-OAc, R_2 = Me

(102) R_1 = H, β-OH, R_2 = Me

(103) R_1 = O, R_2 = Me



Scheme 6. Major mass spectral fragments of oleanolic acid acetate[a]

[a] Relative abundance in parentheses

through Natal and the Transkei to the north eastern Cape Province.

Syncolostemon rotundifolius is a sparingly branched shrub (0.6-2m tall) with small broad leaves and small magenta-pink flowers. The species is found from Port St. Johns in the Transkei to Port Sheptstone in Natal and is confined to grasslands and scrub on rocky coastal slopes[140].

Three batches of S. rotundifolius were collected near Margate in Natal. The first batch was collected at the beginning of winter while the latter two batches (combined) were collected in midsummer. All plant material was air dried in the shade and the leaves extracted with acetone. The acetone extract was concentrated, decolourised and concentrated again to afford a tan-coloured brittle solid. Extensive column chromatography on both silica gel and neutral alumina of this solid extract was used to isolate and purify the major natural products.

Two major compounds were isolated from S. rotundifolius, oleanolic acid (**99**) and a new compound synrotolide (**1**). Oleanolic acid, present in large amounts in the plant (1.4% per mass of dried plant material), could not be crystallised directly from the chromatographed extract and was isolated as its crystalline acetate (**100**). Both spectroscopic and chemical methods were used to determine the structure of **100**.

The broad band noise decoupled ^{13}C NMR spectrum of **100** showed that thirty-two carbon atoms were present which suggested a monoacetylated triterpenoid skeleton. The ^{13}C resonances of a single acetate carbonyl atom (δ 171 ppm) and a carboxylic acid carbonyl atom (δ 184 ppm) were observed. The presence of these two carbonyl groups was confirmed by the infrared spectrum (1720 and 1690 cm^{-1}) which also showed hydroxyl (3230 cm^{-1}), gem dimethyl (1360 cm^{-1}) and trisubstituted double bond (815 cm^{-1}) absorptions[144]. The ^{13}C chemical shifts (δ 122.5 and δ 143.5 ppm)

and the triplet observed in the ^{1}H NMR spectrum (δ 5.24 ppm, J = 4 Hz) corroborated the presence of a trisubstituted double bond. Seven methyl group singlets were observed in the ^{1}H NMR spectrum and the chemical shifts of six quarternary carbon atoms established from a DEPT[112] experiment suggested that **100** was a pentacyclic olean-12-ene[145].

The position of the C-12, C-13 double bond was confirmed by the retro-Diels-Alder fragmentation in the mass spectrum of **100** Scheme 6[146]. The two major fragments (m/z 248 and m/z 203) placed the carboxylic acid group at C-28. The molecular ion (m/z 498), elemental analysis, melting point and optical rotation of **100** was consistent with published data[147,148] for 3β-acetoxy-olean-12-en-28-oic acid ($C_{32}H_{50}O_4$) and the stereochemistry of **100** was thus established.

Saponification of **100** with ethanolic potassium hydroxide gave **99** m.p. 290-296^o while methylation of **100** with diazomethane[149] afforded methyl 3-acetoxy-oleanolate (**101**) m.p. 218-220^o. Mild hydrolysis of **101** gave methyl oleanolate (**102**) m.p. 196-198^o. The methyl ester was not easily saponified to the original acid and this confirmed the hindered nature of the carboxylic acid group[150]. Finally, Jones oxidation[151] of **102** gave methyl 3-oxo-oleanolate (**103**) m.p. 179-181^o. All the melting points and optical rotations of these compounds were consistent with literature values[147,148,152]. The ^{13}C NMR chemical shifts of **100** were assigned by comparison with the shifts published for **99**[153], **101** and **102**[154].

Oleanolic acid is ubiquitous in the plant kingdom and occurs either as the aglycone or as a glycoside coupled with a variety of different sugar moeities. Amongst the Lamiaceae the presence of the aglycone **99** is regularly reported in species of the genus Salvia[155].

The new 6-substituted-5,6-dihydro-α-pyrone, synrotolide (**1**), $C_{16}H_{22}O_8$, m.p. 168-170^o, $[\alpha]_D^{24}$ = -29^o was isolated in 0.8% yield. The presence of an

αβ-unsaturated-δ-lactone ring was shown by the ultraviolet (λ_{max} 210 nm log ε 4.013) and infrared (ν_{max} 1710 and 1695 cm^{-1}) spectra. It also showed hydroxyl (3365 cm^{-1}) and acetoxy absorptions (1740 cm^{-1}), gave a positive test for a 1,2 diol[156] and on acetylation formed a diacetate (2) $C_{20}H_{26}O_{10}$, m.p. 102-103°. Quantitative saponification of 1 with aqueous 0.14 M potassium hydroxide showed that approximately 3 moles of potassium hydroxide were consumed per mole of 1. This suggested that in addition to the lactone ring 1 also contained two acetoxy groups. Saponification of 1 with methanolic potassium carbonate yielded an unstable crystalline product, m.p. 100-104°, in low yield. The instability of this saponified product at room temperature was confirmed by the elemental analysis. One mole of 1 absorbed 2.4 moles of hydrogen indicating the presence of two olefinic double bonds, one of which was incorporated in the αβ-unsaturated-δ-lactone ring.

The chemical, ultraviolet and infrared evidence was confirmed by ^{1}H NMR, ^{13}C NMR and mass spectrometry. Synrotolide was not soluble in chloroform and the ^{1}H and ^{13}C NMR spectra were recorded in deuterated dimethyl sulphoxide. The ^{1}H NMR spectrum indicated the presence of a secondary methyl group (δ1.08 ppm), two acetoxy groups (δ1.97 and 2.00 ppm), one methylene group (δ2.39 ppm), four olefinic protons (3H, complex at δ5-6 ppm), a cis 1H multiplet (δ 7.05 ppm, J = 10 Hz) and two hydroxyl protons (δ3.3 and 3.5 ppm). This spectral data suggested that 1 was a 6-alkenyl-5,6-dihydro-α-pyrone (Sections 1:2:2 and 1:3:1).

The uncertainty in the position and configuration of the olefinic double bond, the 1,2 diol and the two acetoxy groups in the alkenyl side chain of 1 necessitated a detailed analysis of both the ^{13}C and ^{1}H NMR spectra.

The analysis of a ^{13}C NMR spectrum involves firstly determining the multiplicities of the ^{13}C signals i.e. the number of protons attached to each carbon atom, and secondly of assigning unambiguously each ^{13}C signal to an individual carbon atom within a molecule. The classical approach to the first problem has been the use of off-resonance decoupling techniques which scale down the proton-carbon coupling constant and generate the ^{13}C NMR spectrum as a series of multiplets centred at each carbon atom site. The multiplicities can thus be determined from the splitting pattern of these multiplets. The major disadvantages of this technique have been described by Morris[108] and include a reduction in sensitivity and perturbations of the splitting pattern induced by variations in proton coupling strength.

The advent of Fourier transform NMR has seen an enormous growth in the last decade of high resolution multiple pulse NMR techniques. Of the many NMR techniques available two techniques INEPT[157] (insensitive nuclei enhanced by polarisation transfer) and DEPT[112] (distortionless enhancement by polarisation transfer) are widely used to determine ^{13}C multiplicities. Polarisation transfer pulse sequences enhance the sensitivity of the ^{13}C signal and the use of these two techniques to determine multiplicities of ^{13}C signals arises from the fact that different multiplicities result in differential dependencies of signal enhancement on pulse sequence delays (INEPT) or pulse flip angles (DEPT)[108]. The details of the pulse sequences used for these techniques are discussed by Morris[108], and Benn and Gunther[107] and the advantages and disadvantages of the INEPT and DEPT sequences have been described[158]. As both these techniques enhance sensitivity and give spectra which are measured with full proton decoupling they effectively overcome the problems posed by the classical off-resonance decoupled spectra.

For routine use the DEPT sequence is preferred to the INEPT sequence[108] and a single DEPT experiment set with a pulse flip angle of 135^{o} gives a spectrum where methine and methyl groups appear as positive signals while methylene groups appear as negative signals. Carbonyl carbon atoms and quarternary carbon atoms are absent from the spectrum. A broad band noise decoupled ^{13}C NMR spectrum and a DEPT spectrum of 1 are reproduced in Appendix 2. Interpretation of these spectra indicated that one secondary methyl (δ13 ppm), one methylene (δ30 ppm), five oxymethine (δ 69-74 ppm), four olefinic (δ120-147 ppm) and three carbonyl (δ163-169 ppm) carbon atoms were present.

The final stage in the analysis of a ^{13}C NMR spectrum is the assignment of the ^{13}C signals to individual carbon atoms within a molecule. This can be achieved by assigning the proton signals with a COSY experiment[113] and using the assigned ^{1}H chemical shifts in a HETCOR experiment[114] to assign and confirm the otherwise ambiguous ^{13}C shifts.

Overlap of the ^{1}H NMR signals of 1 with the DMSO-d_6 solvent signals (δ 2.5 ppm and absorbed water δ3.3 ppm)[159] prevented a detailed analysis of its ^{1}H NMR spectrum in this solvent. The limited solubility of 1 in other suitable deuterated solvents necessitated the use of synrotolide acetate (2) which was soluble in chloroform, for the COSY and HETCOR experiments. However the disadvantage of using 2 was that although the position of the exocyclic double bond could be established, the position of the 1,2 diol of 1 in relation to the two acetoxy groups obviously could not be determined.

The connectivities and chemical shifts of protons can be determined by two-dimensional homonuclear spin correlated spectroscopy (COSY)[113]. The basis of the COSY technique is the classical Jeener two pulse sequence[107]. A number of variations of this basic pulse sequence exist and the technical

details have been extensively reviewed[107,108]. The COSY experiment provides a two-dimensional spectrum in which both frequency axes are identical and display the ^1H signals as a function of their chemical shift (δ). The data obtained from the COSY spectrum is two fold. Firstly each proton resonance gives a peak along the principal diagonal which is representative of its chemical shift. Secondly each pair of scalar-coupled protons (A and X) give a pair of off-diagonal responses (cross peaks) that are symmetrically disposed with respect to the chemical shifts of the coupled protons and have co-ordinates δ_A, δ_X and δ_X, δ_A. The ^1H connectivities can thus be determined by direct inspection and can be mapped out as illustrated in the COSY spectrum of **2** reproduced in Appendix 2.

The interpretation of the splitting patterns and extraction of the coupling constants for **2** were facilitated by the chemical shift and connectivity data thus obtained and are reproduced in Table 2. The chemical shifts and coupling constants of the H_{5ax} and H_{5eq} protons which form the AB part of a complex ABX type system (Section 1:3:1) could not be resolved. The exocyclic olefinic double bond was unequivocally placed at C-1' from the connectivity of the olefinic proton and the C-6 proton. However, the configuration of this double bond could not be readily determined from the coupling constant of the olefinic protons (11.1 Hz). This value is intermediate between typical literature values[159] for a *cis* configuration (7-10 Hz) and those for a *trans* configuration (14-16 Hz). A *trans* configuration was established for the exocyclic double bond of the closely related compound anamarine (**45**)[77] from X-ray analysis but unfortunately the coupling constant for the olefinic protons was not reported.

TABLE 2.

^{1}H NMR spectral data of synrotolide acetate (2)

(500 MHz, $CDCl_3$)

Position	δ/ppm	$J_{H/H}$ Hz
3	6.01 (1 H, ddd)	$J_{3,4}$ = 9.9, $J_{3,5ax}$ = $J_{3,5eq}$ = 1.7
4	6.85 (1H, ddd)	$J_{4,3}$ = 9.9, $J_{4,5eq}$ = 5.0, $J_{4,5ax}$ = 3.4
5 ax, 5 eq	2.39 (2H, m)	
6	5.27 (1H, ddd)	$J_{6,5ax}$ = 12.0, $J_{6,1'}$ = 8.3, $J_{6,5eq}$ = 3.0
1'	5.81 (1H, dd)	$J_{1'2'}$ = 11.1, $J_{1'6}$ = 8.3
2'	5.61 (1H, dd)	$J_{2'1'}$ = 11.1, $J_{2'3'}$ = 9.6
3'	5.66 (1H dd)	$J_{2'3'}$ = 9.6, $J_{3'4'}$ = 4.0
4'	5.14 (1H, dd)	$J_{4'3'}$ = 4.0, $J_{4'5'}$ = 6.6
5'	5.21 (1H, dd)	$J_{5'4'}$ = 6.6, $J_{5'6'}$ = 4.0
6'	5.07 (1H, dq)	$J_{6'5'}$ = 4.0, $J_{6'7'}$ = 6.7
7'	1.18 (3H, d)	$J_{7'6'}$ = 6.7
Acetyl CH_3	1.97, 2.02, 2.04, 2.05 (4 x 3H, s)	

Conversely the exocyclic double bond in another related compound argentilactone (39)[72] was assigned a cis configuration from the coupling constant (10.5 Hz). An infrared absorption at 965 cm^{-1} (Section 1:3:3) has been used to assign a trans configuration to the exocyclic double bonds of a number of 6-alkenyl-5,6-dihydro-α-pyrones. Synrotolide also has an

absorption at 950 cm^{-1} and therefore the unambiguous assignment of the configuration of the exocyclic olefinic bond in this compound was not possible.

Assignment of the ^{1}H chemical shifts enabled complete assignment of the ^{13}C chemical shifts via a HETCOR experiment. Heteronuclear chemical shift correlation is one of the most powerful of 2D experiments and offers a very good return in chemical information per unit of spectrometer time, as only one experiment is needed to establish connectivities between two different types of nuclei[108]. The pulse sequence generally used contains polarisation transfer and therefore over and above the heteronuclear connectivity information a sensitivity enhancement is also obtained. Details of the HETCOR pulse sequence have been reviewed[107,108]. The HETCOR experiment provides a two-dimensional spectrum in which one frequency axis displays signals as a function of their ^{13}C chemical shifts and the other dimension presents signals as a function of their ^{1}H chemical shifts. In the 2D-spectrum, one signal appears for each directly bonded carbon-proton pair with co-ordinates which represent both the ^{13}C and ^{1}H chemical shifts of the carbon-proton pair. A HETCOR spectrum of **2** is reproduced in Appendix 2.

The ^{13}C chemical shifts of **1** were assigned by direct comparison with those of **2** established from the HETCOR spectrum. The ^{13}C data for these compounds and the reported data for hyptolide (**7**), umuravumbolide (**41**) and anamarine (**45**) are presented in Table 3. The applicability of 2D-NMR techniques for the total assignment of ^{13}C and ^{1}H chemical shifts and ^{1}H connectivities of 6-substituted-5,6-dihydro-α-pyrones was thus established.

Table 3.

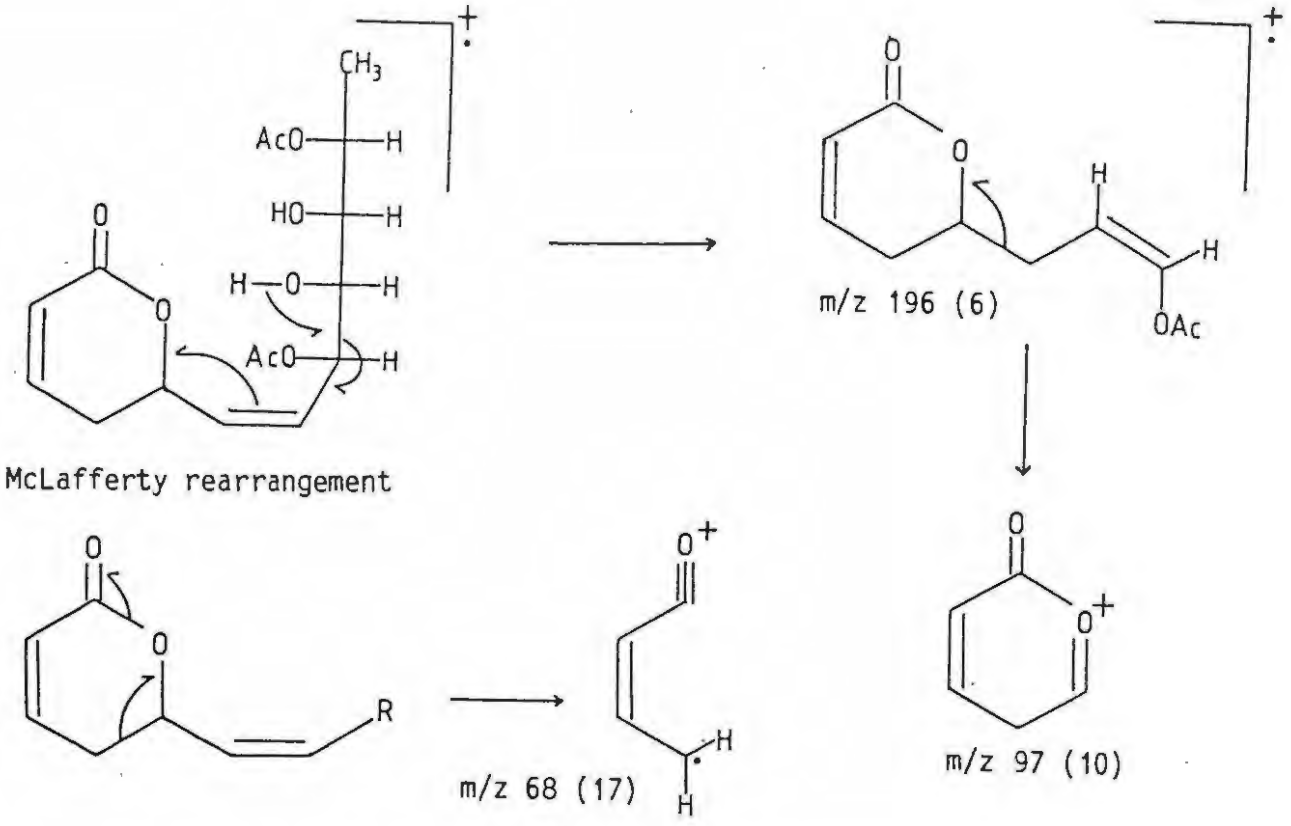
^{13}C NMR spectral data of compounds 1 (75.54 MHz, DMSO-d_6), 2 (75.47 MHz, $CDCl_3$), 7 (125 MHz, $CDCl_3$)[12], 41 (25.15 MHz, $CDCl_3$)[37] and 45 ([a], $CDCl_3$)[77]

C	1	2	7	41	45
2	163.2	163.3	163.2	163.4	163.1
3	120.1	121.5	121.3	121.7	121.4
4	147.0	144.3	144.5	144.1	144.2
5	29.3	29.5	29.3	30.1	29.1
6	74.1	73.7	73.6	74.1	67.3
1'	132.9	132.9	131.0	131.7	125.5
2'	126.7	126.2	130.6	130.1	132.8
3'	69.9	67.6	66.3	69.5	75.7
4'	71.7[b]	71.5	34.6	34.3	71.9
5'	71.6[b]	71.0	70.7	27.6	71.8
6'	71.0	68.5	70.2	22.5	70.4
7'	12.9	14.0	14.5	13.9	15.8
Acetyl CH_3	169.6	170.0	170.8	c	c
	169.3	169.6	170.4		c
		169.5	169.5		c
		169.4			c
Acetyl C=O	21.2	21.11	20.84	c	c
	21.0	20.92	20.82		c
		20.90	20.79		c
		20.89			c

[a] Frequency data not reported in literature

[b] Assignments interchangeable

[c] Values not cited in literature



Scheme 7. The proposed mass spectral fragmentation pattern of synrotolide[a]

[a] Relative abundance in parentheses

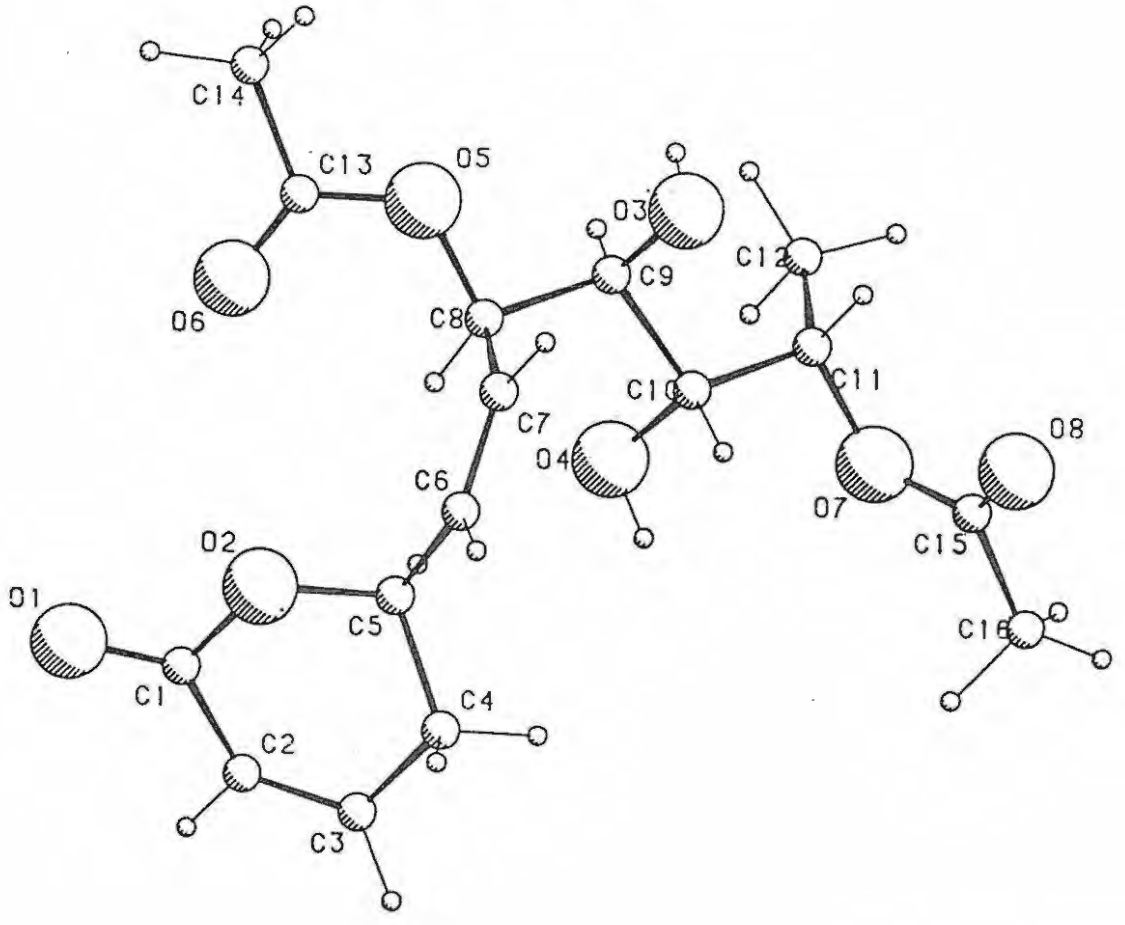


Figure 1. A perspective view of the molecular structure of synrotolide

Mass spectrometry of **1** yielded evidence for the structure of the C-6 side chain. The mass spectrum showed no molecular ion and the only major peak was the base peak at m/z 43 (acetate fragmentation); no other peak exceeded 20% of this peak's intensity. The most useful information came from the peaks at m/z 196, 97 and 68 which were interpreted as arising from a McLafferty-type rearrangement (Scheme 7) and fragmentation of the lactone ring (Section 1:3:2). This placed one acetoxy group at C-3' in the side chain and hence limited the 1,2 diol structure of **1** to two possibilities, with the second acetoxy group at either C-4' or C-6'. In order to settle this uncertainty and also to determine the configuration of the exocyclic double bond and the relative stereochemistry of **1** an X-ray analysis was carried out.

An attempted preparation of the di-p-bromobenzoate derivative of **1** for the solution of the crystal structure by the heavy atom method[160] was unsuccessful and the structure of **1** was accordingly solved by direct methods[161]. Crystals of **1** suitable for X-ray crystallography were crystallised from dichloromethane-chloroform. The intensity data for 1775 reflections were collected of which 1269 reflections with $|F_o| > \sigma|F_o|$ were considered observed. The structure was solved with the direct methods computer program MULTAN[162] and the refinement on F was carried out using the SHELX[163] program. Final agreement values R and R_w of 0.1317 and 0.1083 respectively were obtained for the 1269 observed reflections. The R value could be reduced to 0.0747 for 755 reflections with $|F_o| > 5\sigma|F_o|$ but the variable parameter ratio was considered unacceptably low[164]. The average estimated standard deviations in bond length and angles were approximately 0.02 Å and 1^o respectively (Appendix 1). A PLUTO[165] computer generated stereoscopic drawing of **1** is shown in Figure 1.

The crystal structures of a number of related 6-substituted-5,6-

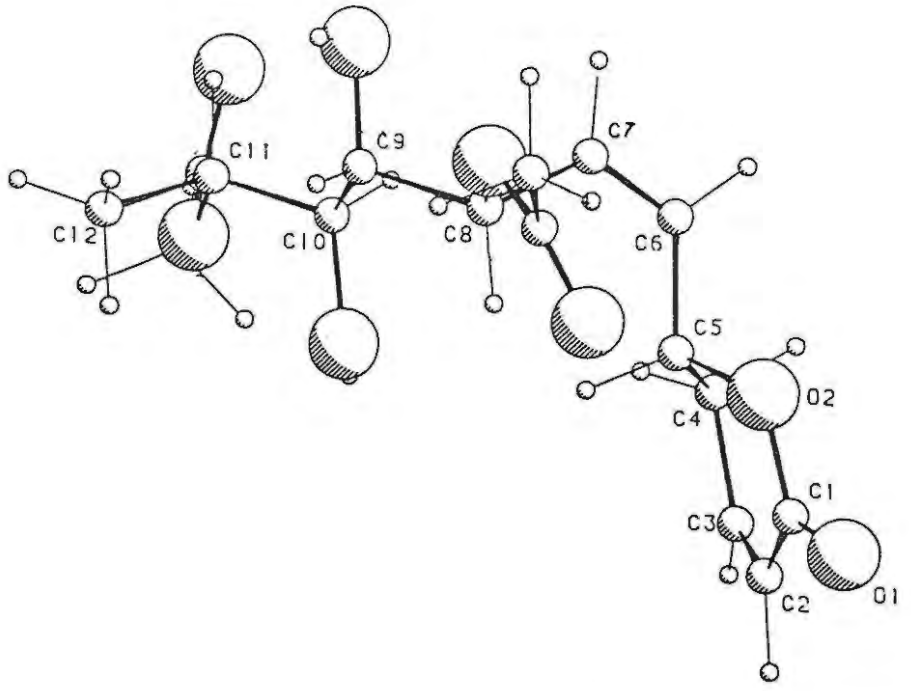


Figure 2. A perspective view of synrotolide illustrating the conformation of the lactone ring

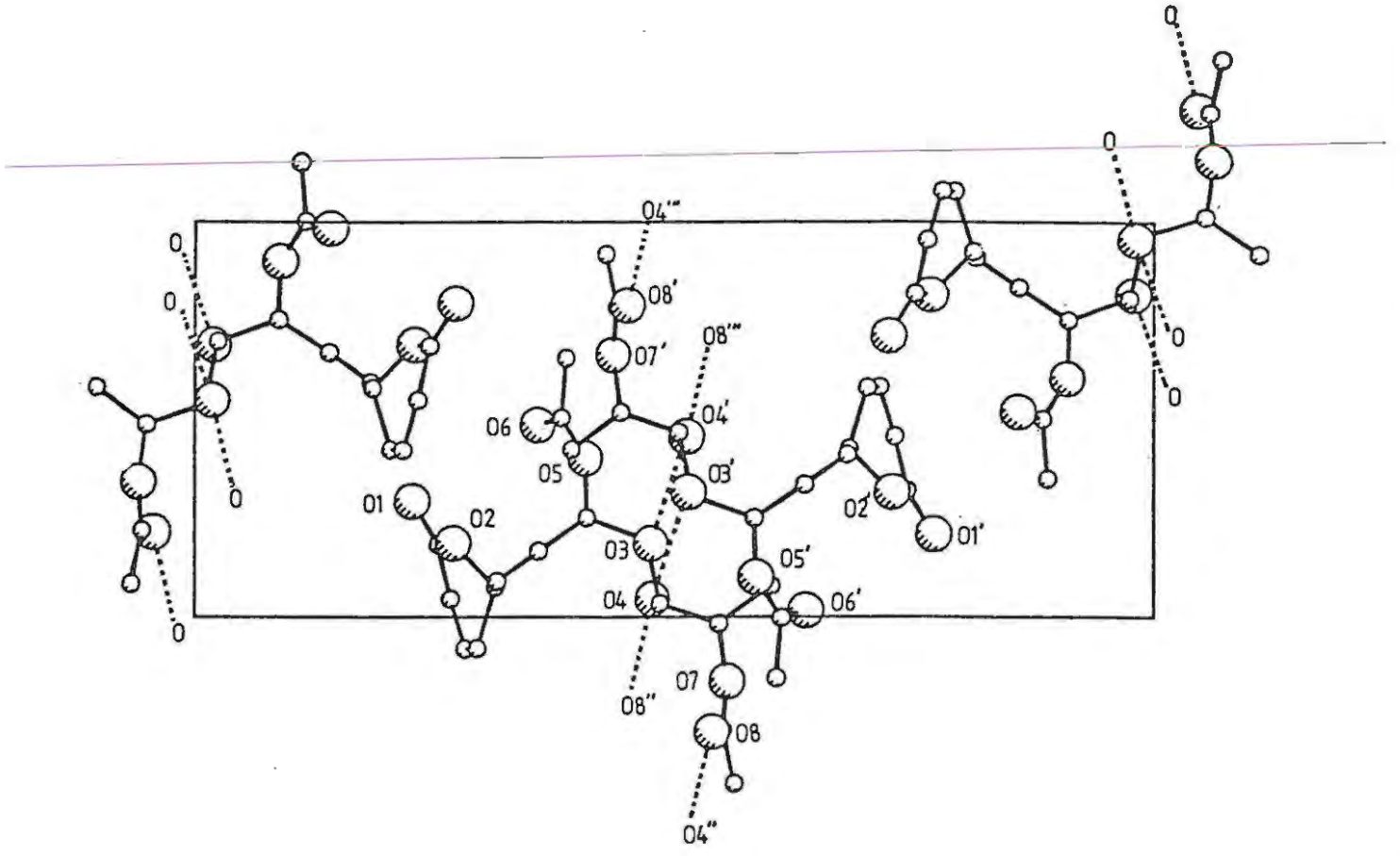


Figure 3. Molecular packing diagram of synrotolide[a]

[a] Hydrogen bonding ------

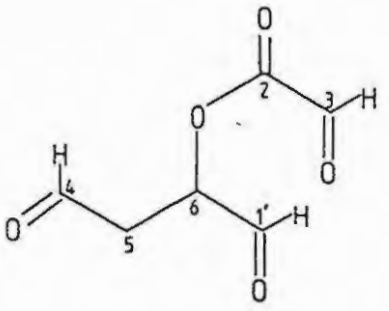
dihydro-α-pyrones, boronolide (3)[36], hyptolide (7)[12], goniothalamin (8)[122], anamarine (45)[77], olguine (46)[78] and 4-deacetoxy-epi-olguine (47)[79] have been reported. A least squares planes analysis[166] of the lactone ring of **1** (Appendix 1) supports Beecham's[41] proposal (Section 1:3:4) that the co-planarity through the ester group of the saturated δ-lactone ring is not normal in αβ-unsaturated-δ-lactone rings. The ester group of the lactone ring of **1** is twisted and its non-planarity can be attributed to the rigidity imparted to the lactone ring by the C-2, C-3 double bond (numbering of carbon atoms as shown in Figure 1). The severe torsional strain in the ring is relieved by C-5 moving far enough out of the plane to relieve this strain[122]. Carbon atom C-5 in **1** is displaced by 0.7 Å from a least squares plane through the other atoms of the ring and the distorted envelope conformation of the lactone ring with C-5 at the apex is shown clearly in Figure 2. The conformation of this lactone ring is consistent with that observed in similar compounds, but the terminology used to describe the lactone ring in these compounds is incongruous and includes half boat[36], distorted envelope[12,77,78], modified skew boat[122] and pseudo-chair[79]. Therefore in accordance with more general usage the term distorted envelope is retained here. The group of atoms C-5, C-6, C-7, and C-8 as well as the two acetoxy groups were each found to be planar (root mean square deviations from the plane less than 0.01 Å). Finally the rectangular unit cell of **1** contains four molecules and the molecular packing diagram with inferred hydrogen bonding, in which the intermolecular oxygen to oxygen bond (O-H...O) is less than 2.8 Å[160], is shown in Figure 3.

The crystal structure established unequivocally a cis or (Z) configuration for the C1'-C2' double bond. The revised structure of 7

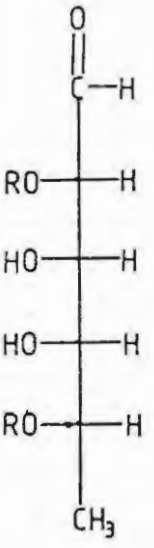
published very recently[12] also established a cis or (Z) configuration for the exocyclic double bond in this compound. The coupling constant of the olefinic protons (10 Hz) of **7** is consistent with that obtained for **2** and **1** (11 Hz). Birch and Butler[76] therefore erroneously proposed the trans or (E) configuration for this bond in **7** on the basis of the infrared absorption of 965 cm^{-1}. Unfortunately this criterion has been used to assign a trans configuration to various 6-alkenyl-5,6-dihydro-α-pyrones (Section 1:3:3) and is clearly not definitive for a trans double bond in these instances. Therefore it is possible that the trans configuration proposed for the exocyclic olefinic bond of **41** and **42** isolated from Tetradenia riparia[37] (Lamiaceae) is incorrect and must be revised.

Paradoxically the X-ray structure determined trans configuration of the C1'-C2' double bond of **45** isolated from a closely related unidentified Hyptis species is at variance with the cis configuration of **1** and **7**. The possibility that isomerisation of the cis double bond of **1** to the more thermodynamically favoured trans configuration might occur with direct exposure to sunlight was investigated. Unchanged **1** was obtained from an acetone solution of this compound exposed to sunlight for five days. The feasibility of isomerisation of the exocyclic double bond of **45** from a cis to a trans configuration occuring during extraction and isolation procedures therefore appears unlikely. Finally Valverde et al have drawn attention to the very short bond length (1.255 Å) of the exocyclic double bond of **45** determined from the X-ray structure. This is at variance with the bond lengths determined for the equivalent bond in **1** (1.33 Å) and **7** (1.312 Å) which are consistent with the bond length (approx. 1.34 Å) normally observed for a C sp^2-sp^2 bond.

The absolute stereochemistry of **1** was determined by chemical degradation. Three approaches to this problem were apparent and all three

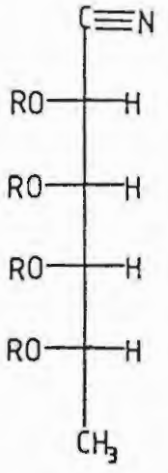


(104)

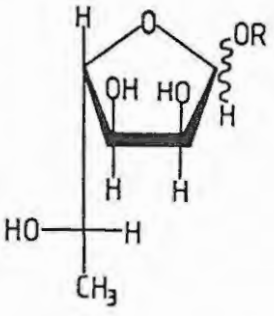


(105) R = Ac

(106) R = H

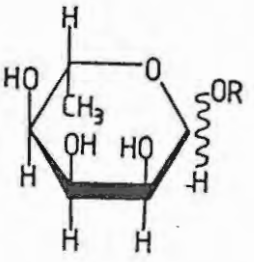


(107) R = Ac



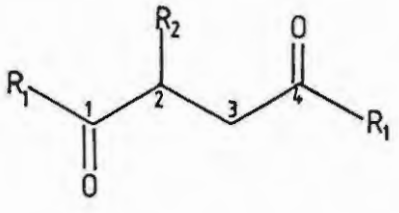
(108) R = α-(-)-2-octyl

(109) R = β-(-)-2-octyl



(110) R = α-(-)-2-octyl

(111) R = β-(-)-2-octyl

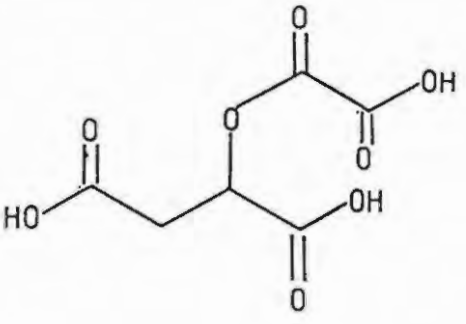


(112) $R_1 = R_2 = OH$

(113) $R_1 = OH$, $R_2 = CH_3CS_2-$

(114) $R_1 = C_6H_5-NH-NH-$, $R_2 = OH$

(115) $R_1 = O-(-)-2-butyl$, $R_2 = OAc$



(116)

were attempted.

The first approach involved periodate cleavage of the C4'-C5' diol of **1** and attempted isolation of the more volatile 2-acetoxypropanal (b.p. 52-55°/15 mm)[147] from the reaction products by steam distillation. The absolute stereochemistry at C-6' in **1** can be related to the configuration at C-2 in 2-acetoxypropanal. The progress of the periodate oxidation of **1** was followed by iodimetry[156] and 1.1 moles of sodium metaperiodate were consumed per mole of **1**. Excess periodate was destroyed, steam passed through the reaction mixture and the distillate collected in ethanolic 2,4-dinitrophenylhydrazine solution[167]. The solution turned a turbid yellow colour but no precipitate of the 2,4-dinitrophenylhydrazone formed. The reaction was repeated and the distillate collected in an aqueous 2,4-dinitrophenylhydrazine solution[156] with similar results. Difficulty in isolating 2-acetoxypropanal from a reaction mixture was also reported by Fleet and Harding[168] in their synthesis of 2-acetoxypropanal from the reduction of the corresponding acid chloride with bis(triphenylphosphine) copper(1) tetrahydroborate. Therefore, because of the difficulties evident in isolating 2-acetoxypropanal, an alternative route to the absolute stereochemistry of **1** was investigated.

Reductive ozonolysis of **1** would yield two six carbon products, a trialdehyde (**104**) derived from the αβ-unsaturated-δ-lactone ring and an aldehyde (**105**) derived from the C-6 side chain. The structure of **105** is consistent with that for a diacetoxy-6-deoxy sugar and the relative stereochemistry which followed from the X-ray structure of **1** limited the absolute stereochemistry of this compound to two possibilities, either D- or L- 2,5-diacetoxy-6-deoxyallose. Accordingly hydrolysis of **105** would yield either 6-deoxy-D-allose or 6-deoxy-L-allose (**106**). The synthesis of

6-deoxy-D-allose has been reported in the literature[169,170] but no reference to the occurence of either one of the enantiomers of this rare monosaccharide in nature could be found. Synthesis of 6-deoxy-D-allose was not attempted and a 20 mg sample synthesised by Reichstein[170] was obtained (Section 3:5:3).

Ozonolysis of **1** was carried out in acetic acid at 10^{o} and dimethyl sulphide was used for the reductive work up of the ozonolysis products. Acetic acid was used in preference to the more commonly used ozonolysis solvent dichloromethane because **1** crystallised from a dichloromethane solution at low temperatures. The use of dimethyl sulphide for the reduction of the ozonide[171] was considered superior to other methods, e.g. the slow addition of the ozonolysis products to a suspension of zinc powder in aqueous acetic acid[172].

The separation and isolation of monosaccharides by ascending or descending preparative paper chromatography is well established[173]. Separation of compounds by these methods is achieved via the differential partition of the compounds between a water miscible solvent or mixture of solvents (mobile phase) and the hydrated paper fibres or stationary phase. Descending paper chromatography was used to separate **104** and **105** using either the butanol-ethanol-water (4:1:1) or ethyl acetate-acetic acid-formic acid-water (18:3:1:4) solvent system. Separation of **104** and **105** using the former solvent system was achieved in approximately 15 hours while an improved separation was accomplished in the latter in only 5 hours and this solvent system was accordingly used for the preparative separation and isolation of these compounds.

Two techniques were used to visualise the paper chromatograms. Firstly the aldehyde specific alkaline silver nitrate/sodium thiosulphate method[174] in which the reducing sugar (**105**) and the aldehyde (**104**) both

gave a dark spot on a pale background, and secondly the more specific ethanolic vanillin/dilute sulphuric acid method (generally used to identify deoxysugars)[175] which only gave a colour reaction with **105**. The position and identity of the two compounds on the paper chromatogram was thus established.

Compound **105** was extracted from the preparative paper chromatogram with water, freeze dried and hydrolysed with trifluoroacetic acid to yield 6-deoxyallose (**106**). Trifluoroacetic acid was used for the hydrolysis because it has the advantage of being volatile and can therefore be removed under reduced pressure. The identity of **106** was confirmed by capillary gas chromatography (g.l.c.).

Since the inception of the packed column into the field of g.l.c. thirty years ago[176] there has been very little fundamental change in the basic fabrication of this type of column[177]. Possibly the greatest drawback of packed columns is the problem of completely coating the solid support with a stationary phase. The presence of residual active sites on a partially coated solid support not only interferes with the analysis of many polar compounds but also leads to a shortened column life and poor reproducibility between different columns coated with the same stationary phase. Many of the problems associated with packed columns are eliminated or minimised by the use of capillary columns. However, for many years there was widespread resistance to the use of capillary columns arising predominantly from the inherent fragility of conventional glass capillary columns. In 1979 the first fused silica glass capillary columns were manufactured[177] which were both flexible and thin-walled. As the advantages of these fused silica columns became apparent much of the resistance to their use was dissipated. Initial problems of coating these

columns were overcome by an improved understanding of surface chemistry which generated a new era of crosslinked stationary phases which could be bonded directly to the surface of the fused silica glass capillary.

The advantages of bonded fused silica capillary columns are numerous and include a high degree of reproducibility between manufactured columns. Also the bonded stationary phase does not readily bleed off the column and the column can be back-flushed to remove impurities, thus prolonging its useful life. The dimensions of capillary columns vary from 10 to 50 metres in length and 0.25 to 0.75 mm in internal diameter while the film thickness of the bonded stationary phase varies from 0.25 to 1.0 μm.

A further attribute of capillary columns is that much less sample is required (< 4 μg) and often a split injection is performed in which only a small proportion of the sample actually passes through the column, the rest being voided. For the capillary g.l.c. carried out as part of this work, a bonded fused silica capillary column (DB-225) was used and found to be suitable for the various separations required. Helium carrier gas and a flame ionisation detector (FID) were used exclusively. The FID is a very good general detector for organic compounds and exhibits a linear detection over a wide range of sample concentrations.

Carbohydrates normally require derivatisation prior to g.l.c. and although trimethylsilyation (as first proposed by Bayer[178] and Sweely et al[179], is commonly used because of the rapid formation of the trimethylsilyl ethers, it has a number of disadvantages. Firstly the ethers are readily hydrolysed by water, necessitating dry preparation conditions, and secondly they yield multiple peaks in the chromatogram attributed to α and β furanose and pyranose forms of the monosaccharide. Ten acyclic derivatives of monosaccharides have been reported which give only single peaks in capillary g.l.c. and the advantages and disadvantages

associated with their use have been discussed recently by Frank et al.[180].

The derivative of **106** chosen to establish its identity by capillary g.l.c. with 6-deoxyallose was the peracetylated aldononitrile (PAAN) derivative (**107**). The PAAN derivatives of both **106** and 6-deoxy-D-allose were similarly prepared using McGinnis' method[181] in which the acyclic monosaccharide is converted to the oxime which is then dehydrated and peracetylated. The initial stage of the reaction is performed in N-methylimidazole which acts as both a catalyst and solvent. Major advantages of using PAAN derivatives is that they are relatively easy to prepare and remain unaffected under aqueous or even mildly acidic conditions. The PAAN derivatives of 6-deoxy-D-allose and **106** gave single peaks in their chromatograms with identical retention times ($^{+}$ 0.3%) thus establishing the identity of **106**.

The absolute stereochemistry of **106** however remained unassigned. 6-Deoxy-D-allose mutarotates in an aqueous solution and values reported for the optical rotation in water vary from -8.5^{o} (after four minutes) to $+ 1.2^{o}$ (after thirty-five minutes)[169]. The unequivocal assignment of absolute stereochemistry based on this mutarotation could be considered tenuous. However, the optical rotation of 6-deoxy-D-allose phenylosazone is pronounced (-79^{o} and -68^{o})[169,170] and a stereochemical assignment proposed from the optical rotation of this derivative would be more tenable. Accordingly, the preparation of the phenylosazone of **106** was attempted. Unfortunately a crysalline product could not be obtained although the same derivatisation of the 6-deoxy-D-allose obtained from Reichstein, yielded fine yellow needles with an optical rotation consistent with the literature value[169]. Therefore an alternative route to the absolute stereochemistry of **106** was necessary.

The enantiomers of aldoses can be separated by capillary g.l.c.. Two approaches are possible; either conversion of the enantiomers into diastereomers with a chiral reagent and separation on a non chiral stationary phase, or direct separation of the enantiomers on a chiral stationary phase. The former method was used here. Various chiral reagents have been reported which can be used to prepare diastereomers separable on a number of different capillary columns. Leontein et al[182] prepared the peracetylated octyl glycosides of a variety of sugars with the commercially available, enantiomerically pure, chiral reagent (+)-2-octanol. The diastereomers were separated on a capillary column of SP-1000. A similar method using (-)-2-butanol was subsequently reported[183] and the trimethylsilylated (-)-2-butyl glycosides of a number of monosaccharides were separated on an SE-30 capillary column. A new method was reported by Little[184] involving the preparation of silylated and acetylated diasteromeric dithioacetals of sugars with (+)-1-phenylethanethiol and subsequent separation on SE-30 and SE-54 capillary columns. Finally, Schweer[185] has separated monosaccharide enantiomers as both their (-)-menthyloxime pertrifluoroacetates and trifluoroacetylated (-)-bornyloximes on an OV-225 column.

The method of Leontein et al was used to establish the absolute stereochemistry of **106**. The method used here differed slightly from the published procedure in that (-)-2-octanol and not (+)-2-octanol was used as the chiral reagent. The (-)-2-octylglycosides of both **106** and 6-deoxy-D-allose were similarly prepared using a sealed ampoule with a catalytic amount of trifluoroacetic acid. The solution was concentrated and the resulting mixture of glycosides acetylated and separated on a DB-225 capillary column. Four peaks were expected in the chromatogram representing the α and β furanose (**108** and **109**) and the α and β pyranose

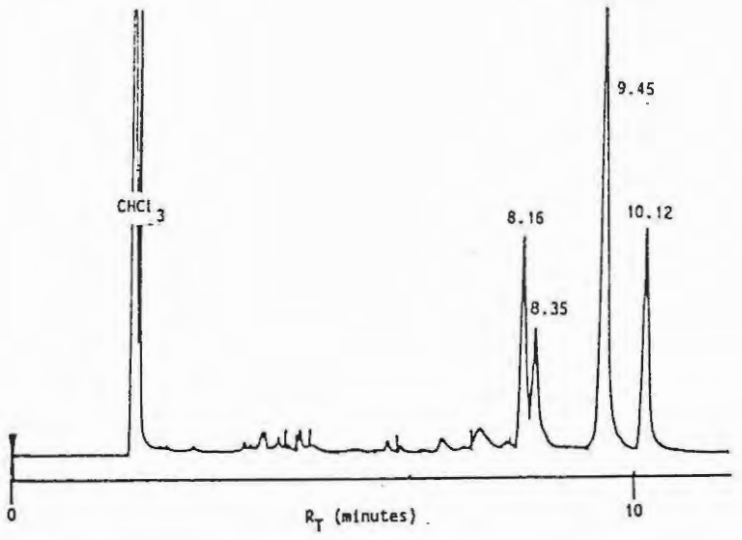


Figure 4. Capillary g.l.c. of the acetylated (-)-2-octyl glycosides of 6-deoxyallose derived from synrotolide

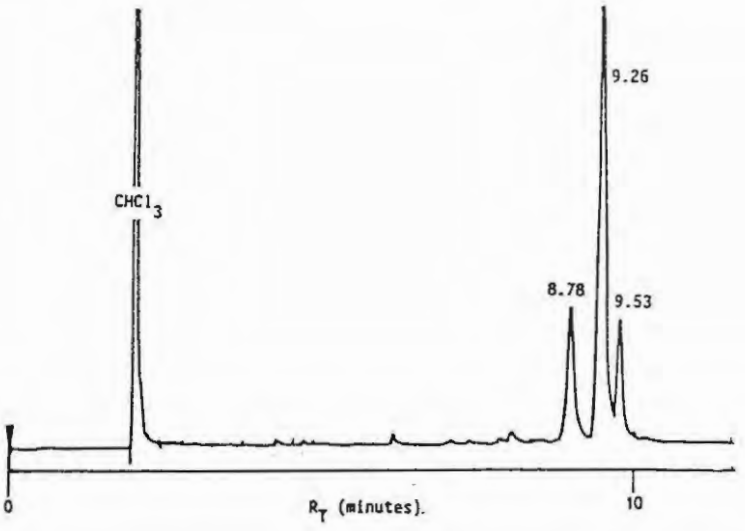


Figure 5. Capillary g.l.c. of the acetylated (-)-2-octyl glycosides of 6-deoxy-D-allose

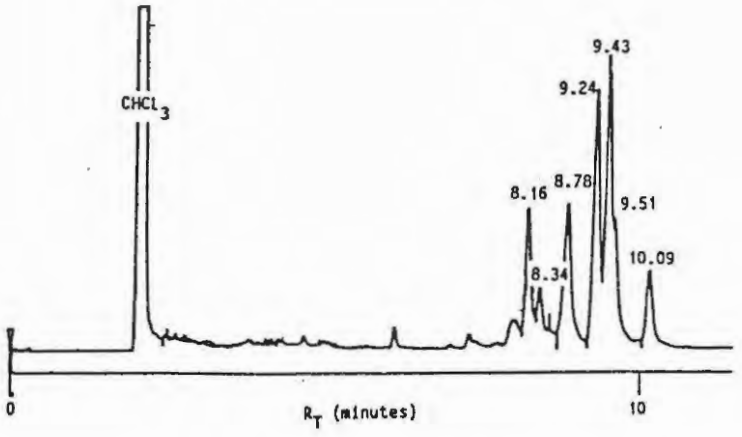


Figure 6. Capillary g.l.c. of the acetylated (±)-2-octyl glycosides of 6-deoxy-D-allose

(110 and 111) diastereomers. The chromatograms of the (-)-2-octylglycosides of 106 and 6-deoxy-D-allose are shown in Figure 4 and Figure 5 respectively. Only three peaks are evident in Figure 5 which were attributed to peak overlap and subsequent attempts to separate the fourth peak by varying the g.l.c. conditions were unsuccessful.

Direct comparison of the retention times and relative intensities of the peaks in Figure 4 and Figure 5 established an L-configuration for 106. This assignment was checked by performing the glycosidation of 6-deoxy-D-allose using ($\pm$)-2-octanol. As anticipated the chromatogram thus obtained (Figure 6) was the sum of the chromatograms shown in Figure 4 and Figure 5. This method is used to establish the expected chromatograms for both enantiomers of a sugar when only one enantiomer is available[182].

A 6-(R) configuration in 1 followed from the absolute stereochemistry established for the side chain and the relative stereochemistry of 1 determined from X-ray analysis. Alternatively the stereochemistry at C-6 could be confirmed by the third and final approach to the absolute stereochemistry of 1, that is, by chemical degradation to malic acid (112). Malic acid can be derived from carbon atoms 4,5,6 and 1' of 1 and thus the absolute stereochemistry at C-6 in 1 can be related to the configuration at the single chiral centre at C-2 in 112.

Chemical degradation of 6-substituted-5,6-dihydro-α-pyrones to 112 has been used to assign the absolute configuration of two 6-aryl-5,6-dihydro-α-pyrones goniothalamin (8) and methysticin (74) (Section 1:2:3). However, the optical rotation of D-malic acid (+ 2.9^{o}) is sufficiently close to zero to make an assignment of absolute configuration based on this value tenuous. Accordingly 112 derived from 8 and 74 was converted to the xanthate or dithiocarbonate (113) and the bis-phenylhydrazide (114)

respectively. Hlubucek and Robertson used Holmberg's[186] original preparation procedure for preparing **113** and reported a very low yield (6%) of this compound. Attempted preparations of **113** using commercial DL-malic acid and Holmberg's method also gave low yields (3-7%). A recently reported procedure[187] for the preparation of the dithiocarbonate of secondary alcohols in high yield was also attempted using DL-malic acid and similar low yields of **113** were recorded. Apart from the very low yields of **113** obtained by these methods, uncertainty in the interpretation of the CD spectrum of this compound has also been clearly demonstrated (Section 1:2:3). Although **114** is a more viable alternative derivative for the stereochemical assignment of **112** than **113** both these derivatives were abandoned in favour of a capillary g.l.c. separation of diastereomeric derivatives of **112**.

The resolution of optical isomers of 2-hydroxycarboxylic acids has been of considerable interest in biochemistry and medicine[188,189]. Two procedures involving capillary g.l.c. can be used to separate and identify the different enantiomers of these compounds. The first method is the conversion of the acids into diastereomeric esters with a chiral alcohol such as (-)-2-butanol[188] or else derivatization of the hydroxyl group with (-)-menthol[189] or a chiral isocyanate[190] and separation of the diastereomers by capillary g.l.c. on a non-chiral stationary phase. The second method involves the direct separation of the enantiomers by capillary g.l.c. on a chiral stationary phase. This latter method is more generally used for the enantiomeric separation of 2-hydroxycarboxylic acids because of the inherent shortcomings of diastereomeric derivatisation as outlined by Frank et al[191].

The first polymeric chiral stationary phase used for the separation of the enantiomers of a 2-hydroxycarboxylic acid as its volatile

pentafluoropropionoxy-N-cyclohexylcarboxamide derivative was Chirasil-Val[191]. Chirasil-Val represented a major advance in the technology of chiral stationary phases because of its relatively high thermal stability and hence greatly reduced bleeding off the column. The Chirasil-Val column could also be directly interfaced with a mass spectrometer thus providing unequivocal identification of the individual g.l.c. peaks. Since the advent of Chirasil-Val a number of closely related chiral stationary phases have been reported [192,193] which have given improved resolution of the enantiomers of 2-hydroxycarboxylic acids as their volatile ester derivatives.

Unfortunately a capillary column coated with a suitable chiral stationary phase was not available in this laboratory and the stereochemical assignment of the malic acid (112) derived from 1 was therefore limited to the preparation of a diastereomeric derivative of 112 followed by capillary g.l.c. on a non-chiral stationary phase. According to Frank et al[191] the disadvantages of this method lie in the quantitative analysis of racemic mixtures arising firstly from differences in the reaction kinetics of derivatisation between enantiomers, and secondly the probability of racemisation of the chiral centres occuring during derivatization. However the 112 derived from 1 would be isolated as a single enantiomer and not as a racemic mixture thus necessitating a qualitative as opposed to a quantitative g.l.c. determination. Therefore, providing that the degree of racemisation during derivatisation remains relatively small, the method of Kamerling et al[188] would be acceptable for the assignment of the stereochemistry of 112 by capillary g.l.c..

Kamerling et al used (-)-2-butanol as the chiral esterifying agent and separated the acetylated-(-)-2-butyl ester derivatives of DL-malic acid

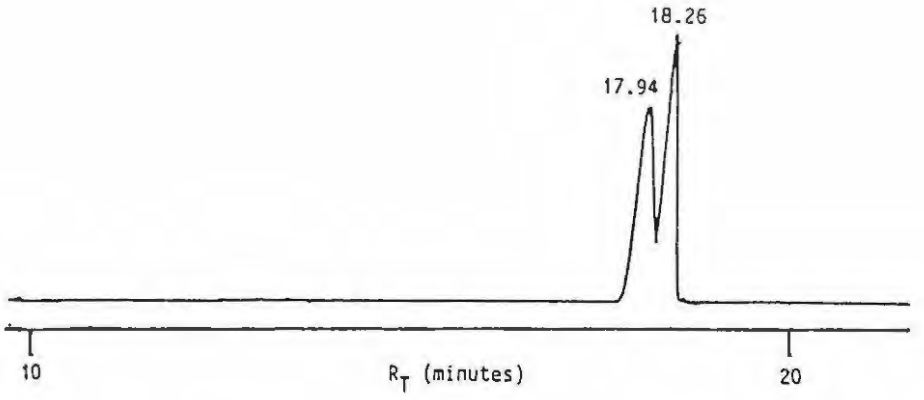


Figure 7. Capillary g.l.c. of the acetylated (±)-2-dibutyl esters of L-malic acid

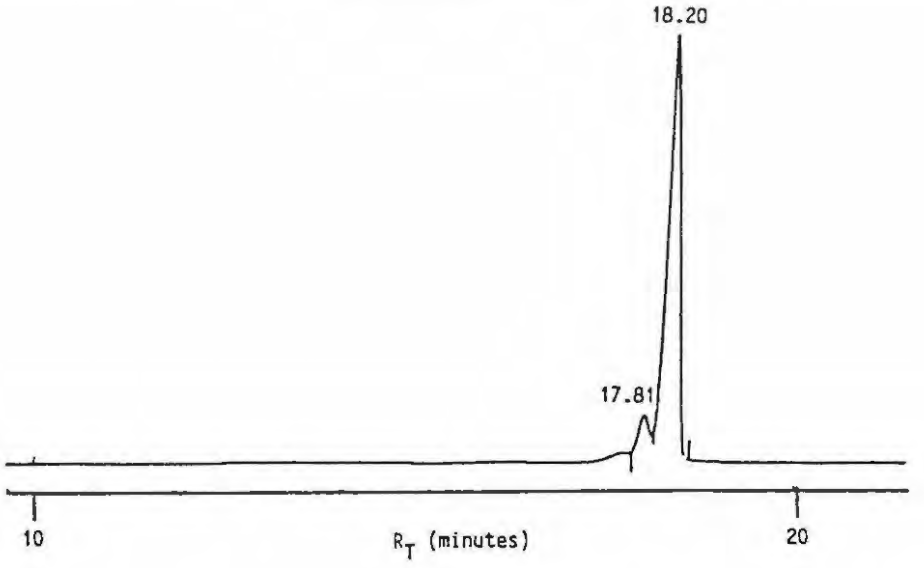


Figure 8. Capillary g.l.c. of the acetylated (+)-2-dibutyl esters of L-malic acid

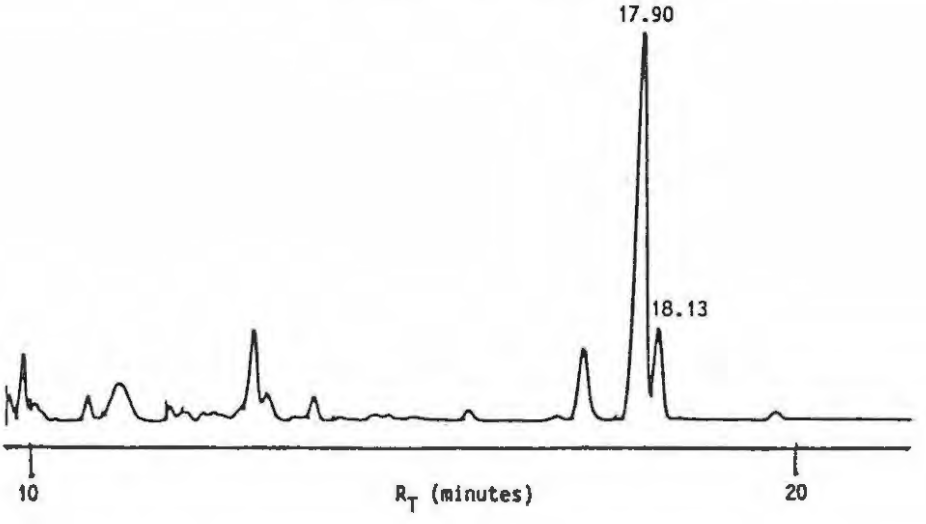


Figure 9. Capillary g.l.c. of the acetylated (+)-2-dibutyl esters of malic acid derived from synrotolide

(115) on an SP-1000 capillary column. Both (-)-2-butanol and (+)-2-butanol are available commercially and the less expensive (+)-2-butanol was used here. Kamerling et al have drawn attention to the fact that the commercially available preparations are slightly contaminated (approx. 6%) with the enantiomer.

The separation of the acetylated (±)-2-dibutyl ester derivatives of L-malic acid was achieved on a DB-225 capillary column which was found to be superior to an OV-1 or OV-17 column. The reaction of L-malic acid with (±)-2-butanol gives rise to four diastereomers (L; +,+), (L; -,-), (L; +,-) and (L; -,+). However only two peaks appear in the chromatogram (Figure 7) and it has been suggested[188] that only the chiral ester group attached directly to the asymmetric centre controls the chromatographic behaviour of the diasteromeric derivative. Hence the (L; -,+) form in which C-1 is attached to the (-)-2-butyl moiety will coincide with the (L; -,-) form and conversely the (L; +,-) form will coincide with the (L; +,+) form. The (D; +,+) and (L; -,-) forms are enantiomeric and will thus exhibit the same chromatographic behaviour. Therefore the chromatogram containing the (D; +,+) and (L; +,+) forms obtained from the acetylated (+)-2-dibutyl esters of DL-malic acid is identical to the chromatogram shown in Figure 7.

The identity of the peaks in (Figure 7) were established by comparison with the chromatogram yielded by the acetylated (+)-2-dibutyl esters of L-malic acid (Figure 8). The major peak with the slower retention time represents the (L; +,+) form while the minor peak can be attributed to the enantiomeric contamination of (+)-2-butanol and represents the (L; -,-) form or (L; -,+) form. Thus the peak with the faster retention time is also equivalent to the (D; +,+) form.

Malic acid (112) can be derived from **1** by oxidative ozonolysis. An

excess of ozone was passed through a solution of **1** in a mixture of acetic and formic acids (9:1). Oxidation of the ozonolysis products arising from ozonolysis of **8** and **74** was achieved with a 30% hydrogen peroxide solution. Recently Bailey *et al*[194] reported a different method of oxidising ozonides to carboxylic acids by passing oxygen through a refluxing solution of the ozonolysis products for several hours until a negative peroxide test is obtained. A comparative study of the oxidative ozonolysis of cyclohexene was made using both these methods. The yields (52-65%) of adipic acid, m.p. 148-150^{o} (lit. 151-152^{o})[147], obtained by the latter method, while slightly lower than those reported by Bailey *et al* (75-80%), were considerably better than those obtained by the former method (27-31%). Hence the method of Bailey *et al* was used for the oxidative work up of the ozonolysis products of **1**.

The products from the oxidative ozonolysis of **1** would be the tricarboxylic acid (**116**) and 2,5-diacetoxy-3,4-dihydroxyhexanoic acid. Base hydrolysis of these carboxylic acids followed by acidification would convert **116** into two dicarboxylic acids, malic acid (**112**) and oxalic acid. Hlubucek and Robertson removed the latter as its insoluble calcium salt in their ozonolysis of **8**. This was not done here because of the small amounts of acid present (less than 0.5 mmol). Attempts to identify **112** amongst the reaction products by t.l.c., with a specific fluoregenic spray reagent[195] were unsuccessful. The spray reagent (a solution of dicyclohexyl-carbodiimide (0.8 M) in n-butanol) was found to be insensitive to the presence of **112** at low concentrations and gave a violet spot only with solutions of **112** greater than 0.5 mmol ml^{-1}. However the presence of **112** was established by capillary g.l.c. of the acetylated dimethyl ester derivative on a DB-225 column.

The absolute stereochemistry of the **112** derived from **1** followed from capillary g.l.c. comparison of the chromatograms of the acetylated (+)-2-dibutyl esters (Figures 7,8 and 9). This method established a D- or (R)-configuration for the derived **112**. The intensity of the minor peak (R_T 18.13 min) is 20% of the major peak (R_T 17.90 min) and this represents racemisation of the derived **112** which can be attributed to both the factors described by Frank et al and the enantiomeric contamination of the (+)-2-butanol. The racemisation is not excessive and accordingly does not confuse the stereochemical assignment.

The major advantage of this g.l.c. method is that only relatively small amounts (approx. 0.5 mmol) of the 5,6-dihydro-α-pyrone are required and extensive isolation and purification of the derived **112** is not necessary. A DB-225 column can be interfaced with a mass spectrometer and thus the identities of all the diastereomeric peaks were unequivocably established. The mass spectrum of **115** showed no molecular ion (m/z 288) and the highest peak in the spectrum (m/z 215) was interpreted as arising from the facile α-cleavage of one of the butyl ester groups with the subsequent loss of an $-OC_4H_9$ fragment. Further loss of $-C_4H_8$ from the m/z 215 ion probably by a McLafferty type rearrangement of the remaining butyl ester group would give the base peak (m/z 159).

Circular dichroism has been used to assign the absolute stereochemistry at C-6 in twenty-four 6-substituted-5,6-dihydro-α-pyrones related to **1** (Section 1:3:4). Synrotolide has a positive Cotton effect ($\Delta\varepsilon$ = +2.45 at 266 nm) and an equatorial orientation of the C-6 side chain was inferred from the NMR coupling constants of the axial and equatorial protons on C-5 with the C-6 proton (Table 2 and Section 1:3:1). Therefore, in accordance with Snatzke's rules the $\alpha\beta$-unsaturated-δ-lactone ring of **1** adopts conformation (84) and the 6-(R)-configuration of

synrotolide is thus confirmed.

The structure of 1 was therefore conclusively established as 6*R*-[3'*R*,6'*S*-(diacetyloxy)-4'*R*,5'*S*-(dihydroxy)-1'*Z*-heptenyl] - 5,6-dihydro-2H-pyran-2-one.

The absolute stereochemistry of 1 is in accordance with that published for other 6-substituted-5,6-dihydro-α-pyrones isolated from the Lamiaceae (Table 4).

Table 4

The absolute stereochemistry of six 5,6-dihydro-α-pyrones isolated from the Lamiaceae

Compound	Chiral centres							
	5	6	1'	2'	3'	4'	5'	6'
Synrotolide (1)	-	(R)	(Z)		(R)	(R)	(S)	(S)
Hyptolide (7)	-	(R)	(Z)		(S)	-	(R)	(S)
Anamarine (45)	-	(R)	(E)		(R)	(S)	(S)	(S)
Olguine (46)	(R)	(R)	(S)	(S)	(E)		(S)	(S)
4-deacetoxy-10-epi-olguine (47)	-	(R)	(R)	(S)	(E)		(R)	(S)
Boronolide (3)	-	(R)	(R)	(R)	(S)	-	-	-

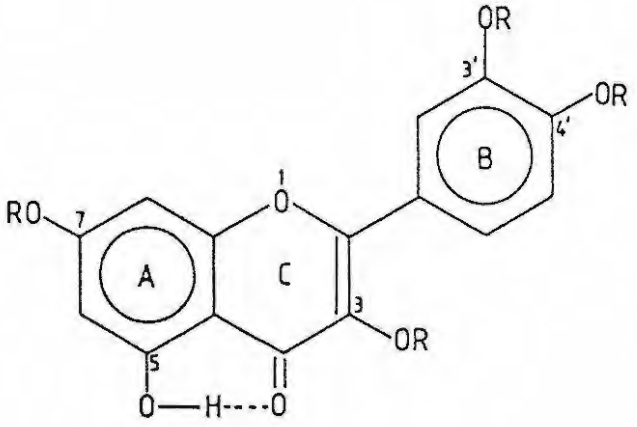
The stereochemistry at C-3' and C-5' in 7 is effectively the same as that in 1 and 45 and differs only because of an order priority reversal brought about by the methylene group at C-4'. Therefore, with the exclusion of the C-1' double bond the absolute stereochemistry of 1, 7 and 45 differs only

at C-4'. These similarities strongly suggest a common biosynthetic origin for these three compounds and hence a probable chemotaxonomic affinity between the genera Syncolostemon and Hyptis.

Previous studies of the biosynthesis of 6-substituted-5,6-dihydro-α-pyrones (Section 1:4) would suggest that despite the structural relationship between 1 and possible carbohydrate precursors the biosynthesis of this compound probably proceeds via an acetate-malonate pathway. In accordance with the varied biological activity of other 6-substituted-5,6-dihydro-α-pyrones (Section 1:5) aspects of the potential biological activity of 1 have been tested. Synrotolide has been screened by the National Cancer Institute (Leukemia KB cell line) with negative results and was also found to be inactive when tested by Maybaker (UK) against the enzyme HMG CoA reductase (cholesterol biosynthesis) and arterial smooth muscle proliferation in hyperlipaemic rabbit serum (in vitro). No reported evidence could be found for the use of this plant in tribal medicine in Southern Africa but a related species S. parviflorus is used by the Zulu people as an emetic for loss of appetite[195]. Hyptis pectinata has a more widespread medicinal usage amongst the people of Africa and has been used from West Africa to Madagascar as a remedy for roundworm, fever and coughing[195].

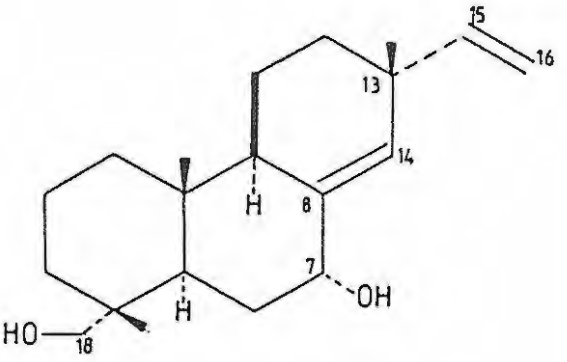
2:2 Syncolostemon densifloris Benth.

Syncolostemon densifloris is ostensibly similar to S. rotundifolius and the two species can be differentiated by the structure of the calyx and the number and position of the flowers on the stems. S. densifloris is found in semi-coastal grasslands and forest margins from the North Eastern Cape through the Transkei to Northern Natal[140].

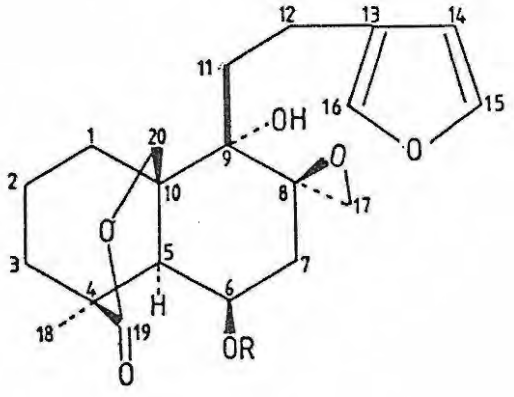


(117) R = Me

(118) R = H

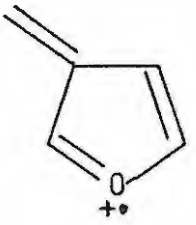


(119)

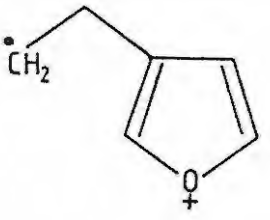


(120) R = Ac

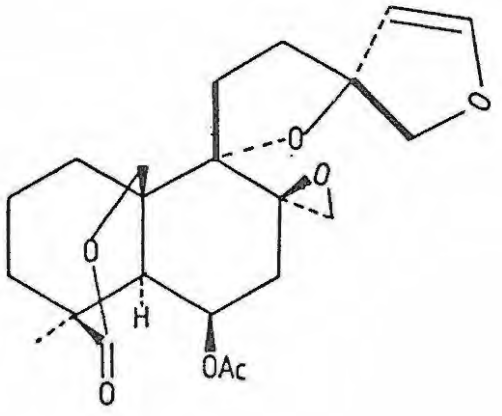
(121) R = H



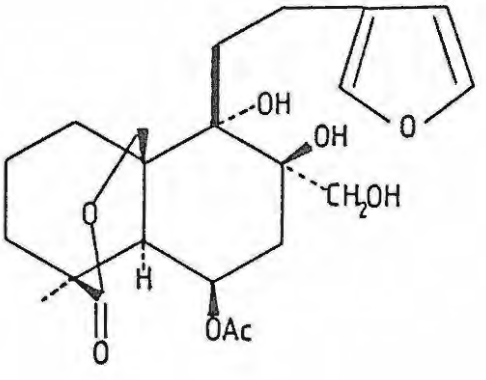
(122)



(123)



(124)



(125)

A single batch of S. densifloris was collected near East London during the autumn of 1984. The leaves and stems were dried and extracted with acetone. Concentration of the acetone extract yielded a tan coloured brittle solid similar in colour and texture to that obtained from S. rotundifolius.

The major natural product present in the tan coloured brittle solid was oleanolic acid (99) which was isolated and identified as the acetate (100). No 5,6-dihydro-α-pyrones similar to 1 were obtained from the S. densifloris extract. However exhaustive column chromatography of the plant extract gave a pale yellow oil which was shown to contain predominantly one compound by t.l.c.. The oil gave a positive test for a phenolic compound with ferric chloride solution and the t.l.c. spot, equivalent to the major compound, fluoresced under ultraviolet light confirming the presence of a conjugated chromophore. The phenolic compound could not be crystallised from the yellow oil and accordingly a portion of the oil was methylated with methyl iodide to yield yellow needles of quercetin 3, 3', 4', 7 tetramethyl ether (117).

The structure of this flavonoid compound followed from IR, NMR and mass spectrometry. The molecular ion peak at m/z 358 (100%) in the EI mass spectrum of 117 and its elemental analysis suggested a molecular formula $C_{19}H_{18}O_7$. The IR spectrum indicated the presence of hydroxyl (3400 cm^{-1}), methoxyl (2900 cm^{-1}) and conjugated carbonyl (1638 cm^{-1}) functions. The ^1H NMR data indicated four methoxyl groups (δ3.83 - 3.95 p.p.m.) and five aromatic protons (δ6.32, 6.42, 6.98, 7.67 and 7.71 p.p.m.) The chemical shifts and coupling constants for the last three aromatic protons above are characteristic[196] for the H-5', H-2' and H-6' protons of ring B respectively. The two remaining aromatic protons (δ6.32 and 6.42 p.p.m.) were assigned as H-6 and H-8 in ring A respectively from

comparison with the NMR data of similar compounds recorded in the literature[197]. Thus the ^{1}H NMR data indicated a flavonol skeleton[198] with a 3, 5, 7, 3', 4' oxygenation pattern. The single hydroxyl group was placed at position 5 from the NMR signal of the hydroxyl proton (δ12.65 p.p.m.) which is indicative of strong hydrogen bonding between this proton and the carbonyl at position 4. The four methoxy groups were accordingly placed at positions 3, 7, 3' and 4'.

Quercetin (118)[147] is ubiquitous in nature and also occurs as its partially or totally methylated ether. Accordingly the major shortcoming of isolating quercetin by the method described is that the degree of methylation of the compound occuring in S. densifloris could not be established.

2:3 Tetradenia barberae (N.E.Br) Codd

Three known species of the genus Tetradenia (formerly Iboza) are found in Southern Africa. One of these species T. riparia has a large range which extends from South Africa to Angola and through tropical East Africa to Ethiopia. A further three species of Tetradenia occur in Madagascar one of which is closely allied to T. riparia[140].

T. barberae is a rare xerophytic shrub which only occurs in the lower Fish River valley near Grahamstown. The T. barberae shrubs are 0.6-1 m tall and have long, irregularly galled, woody, grey-brown stems with small round leaves. The phytochemistry of this species has not been investigated before. A single batch of T. barberae was collected on south west facing slopes of the Fish River escarpment during March, 1986 at the end of the plants' flowering season. The leaves were separated from the stems, air dried in the shade and extracted with acetone. The acetone

extract was decolourized and concentrated to afford a dark brown gum.

Silica gel chromatography of this gum yielded the crystalline 6-substituted-5,6-dihydro-α-pyrone boronolide (3), m.p. 89-90°, $[\alpha]_D^{26}$ = +26°, as the major natural product. The structure of boronolide followed from a combination of spectroscopic techniques. The highest peak in the mass spectrum m/z 371 (M^+ + 1) and the elemental analysis suggested a molecular formula $C_{18}H_{26}O_8$. The presence of an αβ-unsaturated-δ-lactone ring followed from the ions at m/z 97 and m/z 68 in the mass spectrum and also from the UV (λ_{max} 205 nm, log ε 3.92) and IR (ν_{max} 1710 cm^{-1}) spectra. The ^{13}C and ^{1}H NMR spectra showed the presence of three acetoxy groups, the positions of which unequivocally established by a COSY experiment. The COSY experiment (Appendix 2) enabled assignment of the chemical shifts and coupling constants of all the protons in 3 (Table 5). The splitting pattern of the multiplet attributed to the H-5ax and H-5eq protons (δ 2.27-2.49 p.p.m.) is simpler than that observed in the ^{1}H NMR spectrum of synrotolide (1) and the coupling constants of these two protons were accordingly resolved. The ^{13}C chemical shifts of 3 were assigned from a HETCOR experiment (Appendix 2) and are compared (Table 6) with those of saponified boronolide (4) and the reported values for 1,2-dideacetylboronolide (19).

Boronolide was previously isolated by Franca and Polonsky[11] from T. fruticosa, a Madagascan Tetradenia species and the melting point and optical rotation values of boronolide isolated from both these species are in agreement. The relative stereochemistry of 3 was recently determined by an X-ray analysis[36] and although a tentative (6R)-assignment of 3 had been made by Franca and Polonsky (Section 1:2:1) the absolute stereochemistry of 3 was unknown.

The absolute stereochemistry of 3 could be determined from chemical degradation to malic acid (112) in which the absolute configuration at C-6

in **3** could be related to the configuration at C-2 in 112.

Table 5.

^{1}H NMR spectral data of boronolide (3) (300 MHz, $CDCl_3$)

Position	δ/ppm	$J_{H/H}$ Hz
3	5.99 (1H, ddd)	$J_{3,4} = 10$, $J_{3,5eq} = 4$, $J_{3,5ax} = 2$
4	6.85 (1H, ddd)	$J_{4,3} = 10$, $J_{4,5eq} = 6$, $J_{4,5ax} = 3$
5_{ax}	2.49 (1H, ddd)	$J_{5ax,5eq} = 18$, $J_{5ax,6} = 12$, $J_{5ax,4} = 3$, $J_{5ax,3} = 2$
5_{eq}	2.27 (1H, ddd)	$J_{5ax,5eq} = 18$, $J_{5eq,4} = 6$, $J_{5eq,3} = 2$, $J_{5eq,6} = 4$
6	4.50 (1H, ddd)	$J_{6,5ax} = 12$, $J_{6,1'} = 6$, $J_{6,5eq} = 4$
1'	5.32 (1H, dd)	$J_{1'6} = 6$, $J_{1'2'} = 8$
2'	5.31 (1H, dd)	$J_{2'3'} = 12$, $J_{2'1'} = 8$
3'	4.98 (1H, dd)	$J_{3'2'} = 12$, $J_{3'4'} = 6$
4'	1.52 (2H,m)	
5'	1.28 (2H,m)	
6'	1.22 (2H,m)	
7'	0.85 (1H,t)	$J_{7'6'} = 6$
Acetyl CH_3	2.11, 2.07, 2.04 (3 x 3H,s)	

Table 6

^{13}C NMR spectral data of compounds 3 (75.5MHz, $CDCl_3$), 4 (75.47 MH_z, $CDCl_3$) and 19 (50.29 MHz, $CDCl_3$)[38]

C	3	4	19
2	166.2	163.7	163.4
3	121.3	120.8	121.1
4	144.0	145.8	145.4
5	25.1	25.5	25.5
6	75.1	-[b]	77.1
1'	70.6[a]	74.1[a]	70.2[a]
2'	70.6[a]	70.1[a]	70.1[a]
3'	71.5	76.4	75.6
4'	30.3	33.3	30.3
5'	27.0	27.4	27.2
6'	22.4	22.4	22.2
7'	13.9	13.7	13.7
Acetyl CH_3	21.0		21.0
	20.7		
	20.6		
Acetyl C=O	170.2		171.6
	169.7		
	169.5		

[a] Assignments interchangeable

[b] Signal not assigned because of overlap with solvent peaks.

The procedure would involve firstly saponification of **3**, periodate oxidation of the saponified boronolide (**4**), and finally oxidative ozonolysis and capillary g.l.c. identification of the enantiomer of **112** as described previously (Section 2:1).

Saponification of **3** yielded the saponified product (**4**). The ^{13}C NMR chemical shifts of **4** (Table 6) were assigned by direct comparison with the ^{13}C chemical shifts of **3**. The optical rotation of **4** ($+56^{o}$) is in accordance with that reported by Franca and Polonsky but differs from the optical inactivity reported by Van Puyvelde et al for deacetylboronolide (**18**) isolated from T. riparia[37]. Surprisingly the presence of two further compounds, umuravumbolide (**41**) and deacetylumuravumbolide (**42**), as racemic mixtures in T. riparia have also been reported by Van Puyvelde et al[37]. The racemisation of these 6-substituted-5,6-dihydro-α-pyrones is at variance with similar compounds isolated from other species of the Lamiaceae and a re-evaluation of the phytochemistry of T. riparia is necessary.

The progress of the periodate oxidation of **4** was followed by iodimetry [156] and 1.9 moles of sodium metaperiodate were consumed per mole of **4**. Oxidative ozonolysis of the periodate oxidation products was carried out as previously described for synrotolide. No attempt was made to isolate malic acid (**112**) from the carboxylic acid products, and the absolute stereochemistry of the derived **112** followed from capillary g.l.c. comparison of the acetylated (+)-2-dibutyl esters with the chromatograms shown in Figures 7, 8, and 9. The chromatogram (major peak R_T 17.85 minutes and minor peak 18.10 minutes) was similar to Figure 9 and an (R)-configuration for the malic acid derived from **3** was thus established. Accordingly the applicability of this g.l.c. technique for the stereochemical assignment at C-6 in a variety of 6-substituted-5,6-dihydro-α-pyrones was exemplified.

Further evidence for the (6R)-configuration of 3 followed from the CD spectrum. The positive Cotton effect of 3 ($\Delta\varepsilon$ +2.48 at 256 nm) and an equatorial orientation of the C-6 heptyl side chain inferred from NMR coupling constants (Table 5 and Section 1:3:1) suggested conformation 84 for the lactone ring and therefore an (R)-configuration at C-6.

In accordance with the relative stereochemistry determined from X-ray[36] analysis the absolute stereochemistry of 3 was therefore unequivocally established as 6*R*-[1'*R*, 2'*R*, 3'*S*-(trisacetyloxy)-heptyl]-5,6-dihydro-2H-pyran-2-one.

The isolation of boronolide from both T. barberae and the endemic Madagascan species T. fruticosa would suggest a chemotaxonomic relationship between these two species. Although T. barberae has no documented usage in tribal medicine (possibly attributed to its very localised distribution) T. fruticosa is widely recognised and valued in Madagascan folk medicine under the names 'Borona' or 'Boronodahy'[36].

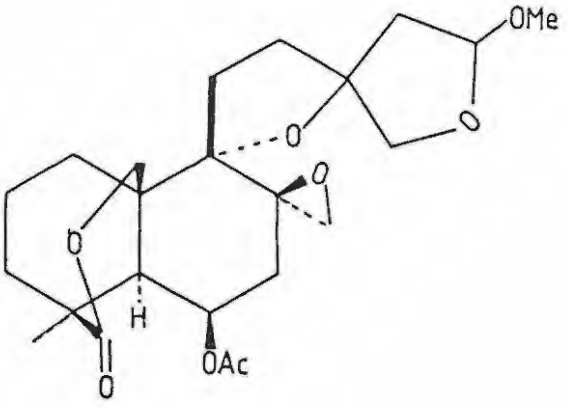
The closely related species T. riparia which is ubiquitous south of the Sahara also plays an important role in indigenous folk medicine. In Rwanda this species is commonly known as 'Umuravumba' and is widely cultivated and used as a remedy for a variety of illnesses including malaria, diarrhoea and several kinds of fevers and aches[37,38]. A similar usage of this plant by the Zulu people of South Africa has been reported[195]. Finally, Van Puyvelde et al[199] have isolated a minor constituent, 8(14), 15-sandaracopimaradiene-7α, 18-diol (119) from T. riparia which exhibits significant antimicrobial and antispasmodic properties.

2:4 Leonotis nepetaefolia (L.) R. Br.

The revised taxonomy of the Southern African species of the genus Leonotis is discussed in Section 2:6. One of the three species reported to occur in Southern Africa is the pantropical weed L. nepetaefolia[140]. This species occurs from Sierra Leone to Kenya and southwards as far as Botswana and the Transvaal. L. nepetaefolia also occurs in Central America and Asia Minor. Phytochemical investigations of specimens from the West Indies and India have been reported by White and Manchand[200] and Connolly et al[201] respectively. However, no investigation of African specimens of this genus has been carried out.

In Africa L. nepetaefolia is an annual or short lived perennial herb which is often found along roadsides and in abandoned cultivated areas. A batch of plant material was collected in North Eastern Botswana during April, 1985 and the leaves and stems were air dried and extracted with acetone in the normal manner. Exhaustive column chromatography of the dark brown acetone extract afforded the major compound nepetaefuran (120) m.p. 228-230^{o}, $[\alpha]_{D}^{25}$ = +31.9^{o}. T.l.c. of the chromatographic fractions and visualisation of the t.l.c. plates with Ehrlich reagent established the presence of a further three minor furanoid compounds. Attempts to isolate these minor components by both open column chromatography and medium pressure chromatography were unsuccessful.

The structure of 120 followed from a combination of spectroscopic techniques and comparison of the melting point and optical rotation of 120 and its saponified product (121) with previously published values. Nepetaefuran gave a positive Ehrlrich test confirming the presence of a β-substituted furan ring[202]. Evidence to support the position of the pendant furan moiety followed from the m/z 81 (122) and m/z 95 (123) ions in the mass spectrum which are attributed to the cleavage of the 11,12- and



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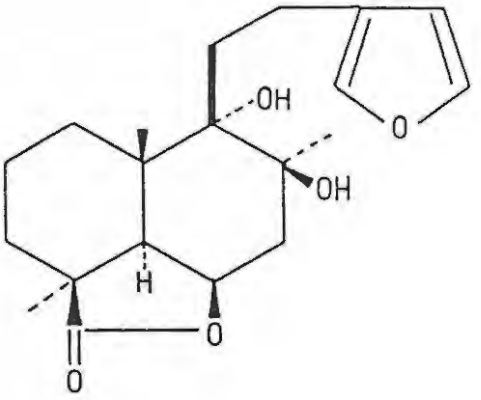

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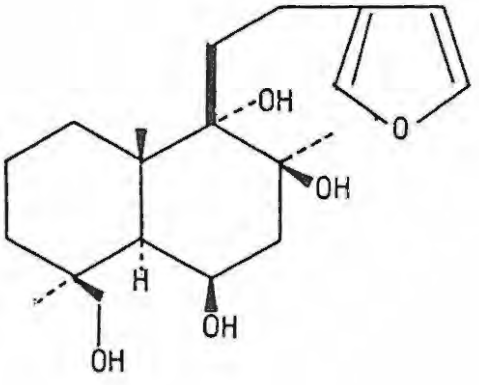

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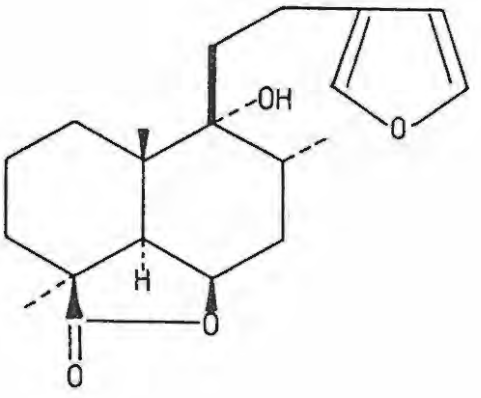

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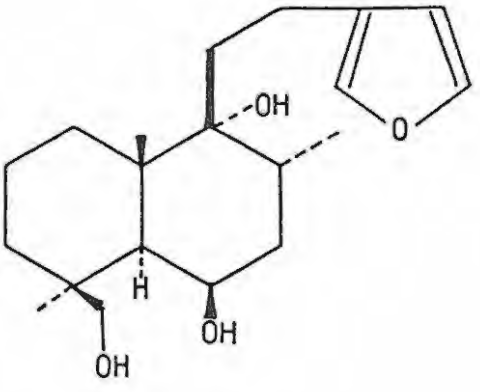

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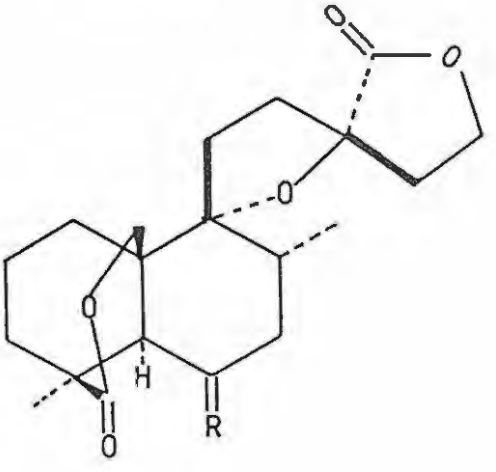


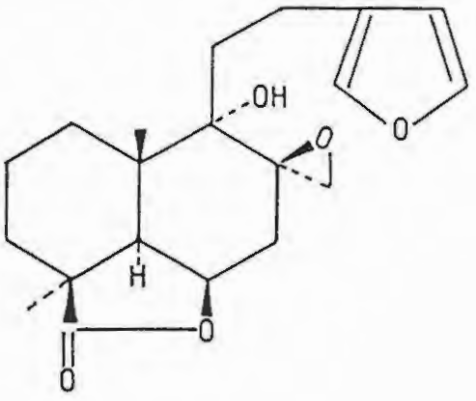
(131) R = α-H, β-OH

(132) R = O

9,11- bonds respectively. The infrared absorption (3500 cm^{-1}) was assigned to the tertiary hydroxyl group at C-9 and the broad absorption (1730 cm^{-1}) was consistent with δ-lactone and ester carbonyl absorptions. The ^1H NMR chemical shifts of **120** were in accordance with NMR data published by White and Manchand[200]. Although the melting point of **120** differed by 10^{o} from the value reported by White and Manchand the optical rotation and the melting point and optical rotation of the saponified product (**121**) were in agreement.

White and Manchand have also reported the presence of nepetaefolin (**124**), isomeric with **120**, in L. nepetaefolia. The instability of **124** was demonstrated by its facile transformation to **120** under very mild conditions including ethanolic chloroform and chromatography on alumina. White and Manchard were only able to separate these two compounds by rigorous chromatography and it is therefore possible that contamination of **120** with a small amount of **124** might explain the depressed melting point and poor elemental analysis obtained here.

Two further diterpenoids, nepetaefuranol (**125**)[200] and methoxynepetaefolin (**126**)[203] have also been isolated from the West Indian species. White and Manchand have determined the absolute stereochemistry of this group of compounds by direct correlation of **120** with 8β-hydroxymarrubiin (**127**)[204,205]. Tosylation of the reduction products of **120** followed by lithium aluminium hydride reduction of the mixture of monotosylates gave the tetraol (**128**) also obtained from lithium aluminium hydride reduction of **127**. The stereochemical relationship between **127** and marrubiin (**129**), of known absolute stereochemistry [206-208], was established by phosphorous trichloride dehydration of **127** and lithium aluminium hydride reduction of the resultant C-8, C-9 epoxide to yield marrubenol (**130**)[205],



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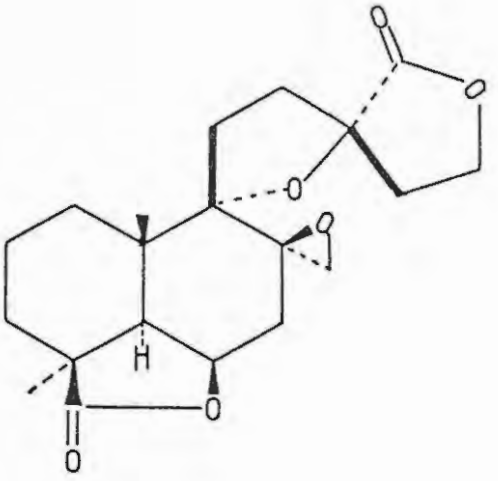

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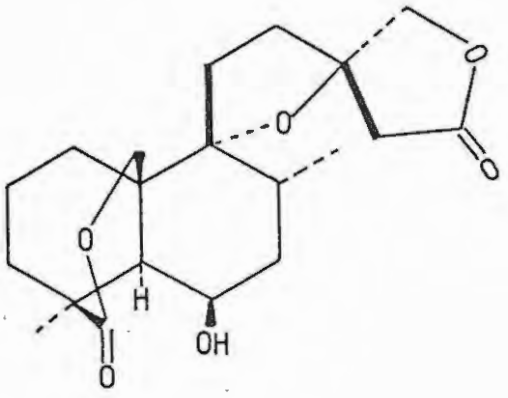

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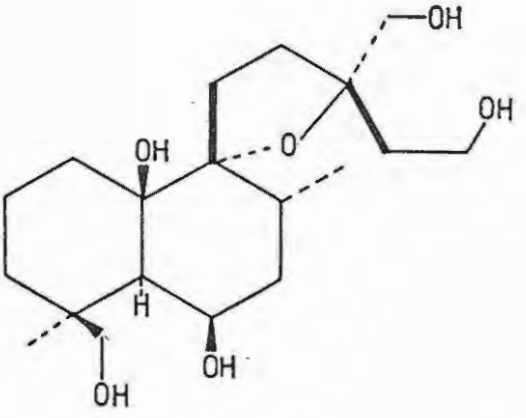

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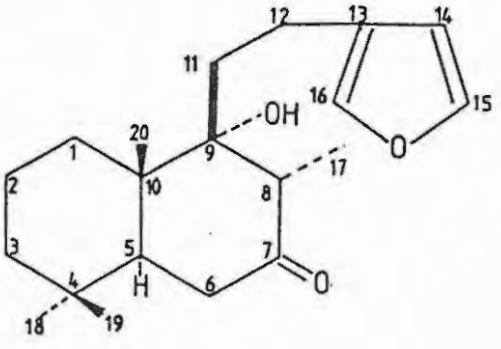

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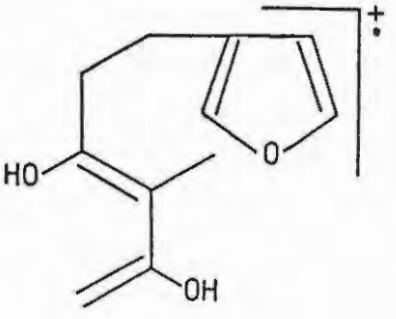


(138)

previously prepared from the reduction of **129**[209]. The (R)-stereochemistry of the C-13 spiro carbon in **124** was ascertained from an X-ray analysis[210].

Leonotis nepetaefolia exemplifies the variation of the natural product composition which may occur in the same species of plant obtained from different localities. Thus a different compliment of diterpenoids were isolated from Indian specimens of L. nepetaefolia by Connolly et al[201] and these include the major compound nepetaefolinol (**131**) and two minor compounds leonotinin (**133**) and the dilactone (**134**). Connolly et al proposed the absolute stereochemistry of **131** from the precedent established by nepetaefuran (**120**)[200] and the positive Cotton effect ($\Delta\varepsilon$ + 1.25) of the ketone (**132**). The relationship between the sign of the Cotton effect of 6-keto diterpenoids and their absolute stereochemistry is discussed in Section 2:6. Rivett et al[211] related the stereochemistry of **131** to that of saponified leonitin(**135**). Reduction of both these compounds with lithium aluminium hydride afforded the same pentaol (**136**). The (S)-stereochemistry of the C-13 spiro carbon of **135** and hence of **131** followed from X-ray analysis of leonitin[212]. The structure of leonotinin(**133**) was confirmed by lithium aluminium hydride reduction of this compound[201] to yield **128** of known stereochemistry.

The usage of L. nepetaefolia in traditional medicine in both Africa and Central and South America is well documented[195]. This plant is used to treat a variety of illnesses including typhoid, rheumatism, headaches, fevers and asthma. In accordance with the reputed anti-cancer properties of L. nepetaefolia ethanolic extracts of West Indian specimens of this plant have shown activity in the National Cancer Institute's Walker carcinosarcoma 256 (intramuscular, WM) screening system[210].

The isolation of nepetaefuran from an African sample of L. nepetaefolia suggests a phytochemical affinity with west Indian specimens of this

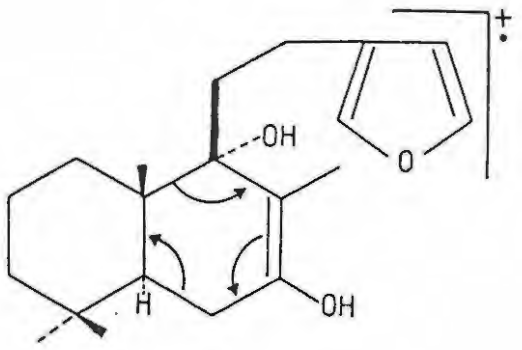
species. The extent of this phytochemical relationship however rests on the isolation and identification of the minor constituents from African L. nepetaefolia which unfortunately was not achieved in this investigation.

2:5 Ballota africana (L.) Benth.

The genus Ballota consists of about thirty-three species occuring mainly in the Mediterranean region and adjoining Asia Minor[140]. The phytochemistry of approximately a third of these species has been examined. Only one Ballota species, B. africana, is indigenous to Southern Africa and has not been investigated before.

The common name for B. africana is kattekrui or catmint which refers to its not unpleasantly aromatic foliage[140] and the distribution of this plant is limited to the arid, winter rainfall regions of the Cape Province and Southern Namibia. Plant material was collected near Grahamstown in November, 1986 at the end of the plants' flowering season and the leaves and stems were air dried and extracted with acetone in the normal manner. Column chromatography of the acetone extract on silica gel yielded large amounts (0.8% yield) of a single, crystalline compound, identified as hispanalone (**137**), m.p. 146-148°, $[\alpha]_D^{23}$ = -18.7°.

The structure of hispanalone was determined from a variety of spectroscopic and chemical techniques. The molecular ion in the mass spectrum (m/z 318) and elemental analysis suggested a molecular formula $C_{20}H_{30}O_3$. Hispanalone gave a positive Ehrlrich test for a β-substituted furan ring[202] and the IR spectrum revealed both hydroxyl (3500 cm^{-1}) and carbonyl (1694 cm^{-1}) absorptions. The ^{13}C and ^{1}H NMR spectra of **137** established the labdane diterpenoid skeleton of this compound. Acetylation of **137** was unsuccessful and this was attributed to



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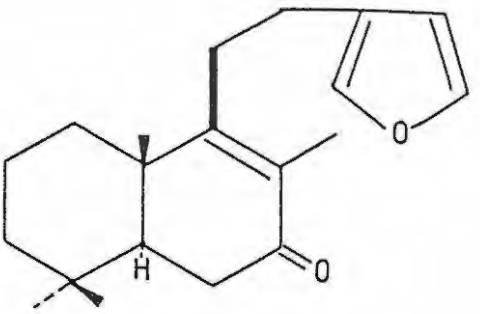

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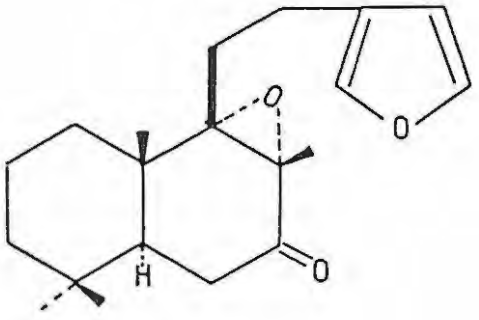

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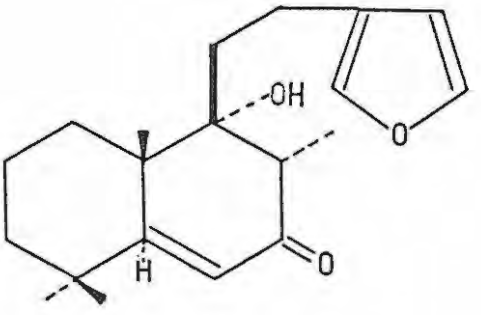

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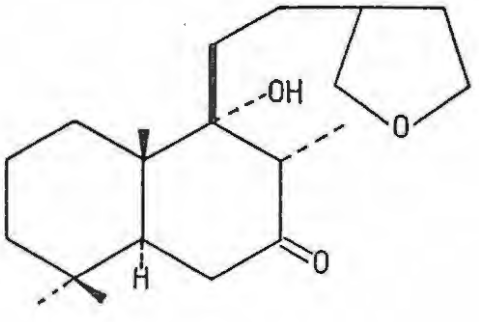

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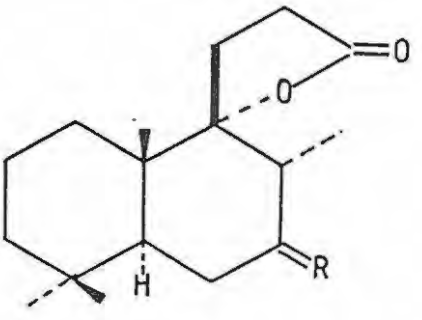


(144) R = H_2

(145) R = O

the difficulty in acetylating a tertiary hydroxyl group, thus placing this group at C-9. The position of the carbonyl group at C-7 followed from the mass spectrum and the peak at m/z 194 (138) which is interpreted as arising from a retro-Diels-Alder cleavage of the $\Delta^{7,8}$ - enolic form of hispanalone (139)[213]. The melting point, optical rotation and CD spectrum was consistent with the values reported by Savona et al for hispanalone previously isolated from B. hispanica[213] and B. andreuzziana[214].

The ^{1}H and ^{13}C chemical shifts of **137** were assigned from 2D-NMR spectroscopy. The DEPT, HETCOR and COSY-45 spectra of **137** are reproduced in Appendix 2. The ^{13}C NMR spectroscopy of labdane diterpenoids is well documented[215-217] and normally the ^{13}C NMR shifts of these compounds are assigned from a combination of proton decoupled and single-frequency off-resonance decoupled spectra, chemical shift theory and from analogy to the chemical shifts of model decalin and labdane compounds[217]. The inherent advantages of using a combination of 2D-NMR techniques to assign ^{1}H and ^{13}C NMR shifts has been described previously (Section 2:1) and these techniques are equally applicable to the NMR spectroscopy of labdane diterpenoids. The ^{13}C chemical shifts of marrubiin (129)[216], hispanalone (**137**) and two closely related compounds (**140**) and (**141**) synthesised from hispanalone[218] are shown in Table 7.

The two protons of a methylene group which either forms part of a ring system or is situated adjacent to a chiral centre are normally non-equivalent and accordingly they give rise to complex splitting patterns in the ^{1}H NMR spectrum[159]. This factor coupled with the close proximity of the cross peaks to the diagonal peaks in the COSY-45 spectrum (Appendix 2) impeded the complete assignment of the ^{1}H chemical shifts of **137** by this method. A COSY-45 spectrum differs from the standard COSY spectrum in that

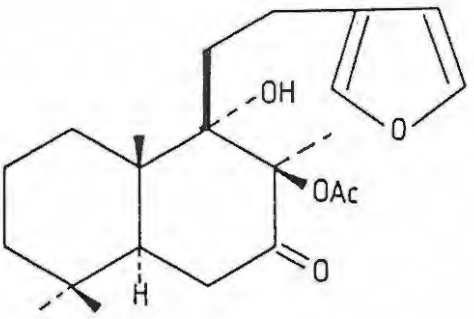
Table 7

^{13}C NMR spectral data of compounds **137** (75.4 MHz, $CDCl_3$), **140** ($CDCl_3$)[a,218], **141** ($CDCl_3$)[a,218] and **129** (25.2 MHz, $CDCl_3$)[216]

C	137	140	141	129
1	31.9	35.2[b]	35.7	35.3[c]
2	18.6	18.6	18.5	18.2
3	41.3	41.3	41.2	28.6[c]
4	33.1	33.1	33.2	43.9
5	46.5	50.3	42.0	44.9
6	39.9	35.9[b]	34.6	76.6
7	211.8	199.6	208.4	31.6[c]
8	50.9	130.2	65.7	32.3
9	81.7	166.6	73.2	75.7
10	43.3	40.9	38.9	39.8
11	34.8	30.2	26.7	28.3
12	21.6	24.2	21.6	21.0
13	124.8	124.3	124.3	125.5
14	110.6	110.4	110.5	111.0
15	142.9	142.8	142.7	143.2
16	138.5	138.4	138.4	138.8
17	8.3	11.5	12.8	16.6
18	33.6	32.5	32.4	23.0
19	21.4	21.3	20.7	184.3
20	16.3	18.2	16.7	22.3[c]

a, Frequency (MHz) not cited in literature

b,c Assignments interchangeable



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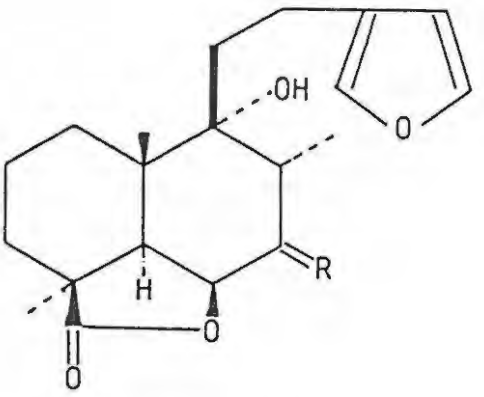
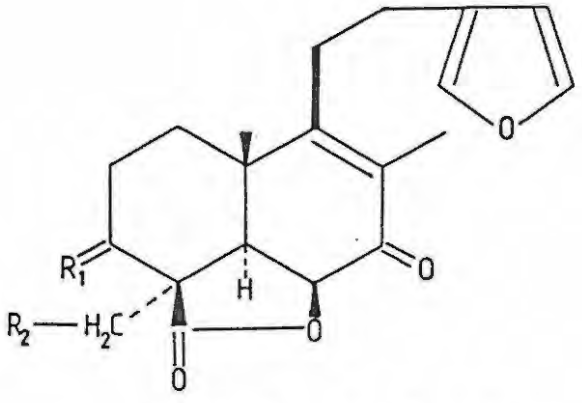


(147) R = O

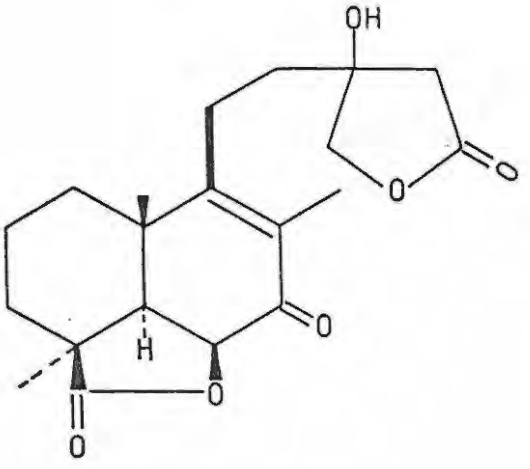
(148) R = α-OAc, β-H



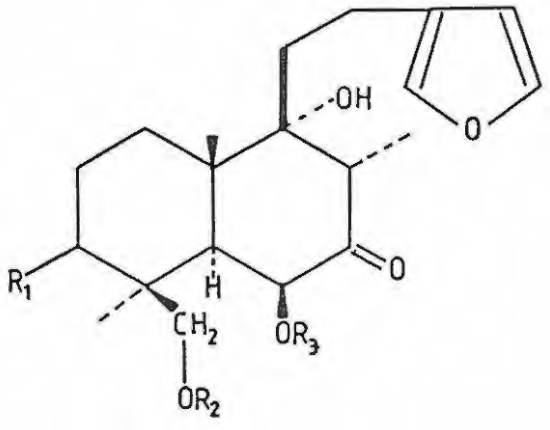
(149) $R_1 = H_2$, R_2 = H

(150) R_1 = O, R_2 = H

(151) $R_1 = H_2$, R_2 = OH

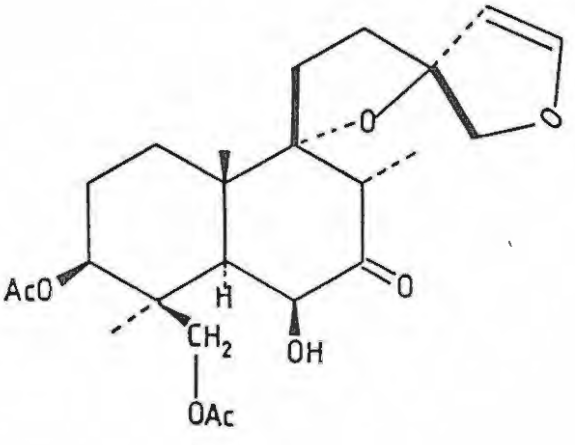


(152)



(153) $R_1 = R_2 = R_3$ = H

(154) R_1 = β-OAc, R_2 = Ac, R_3 = H

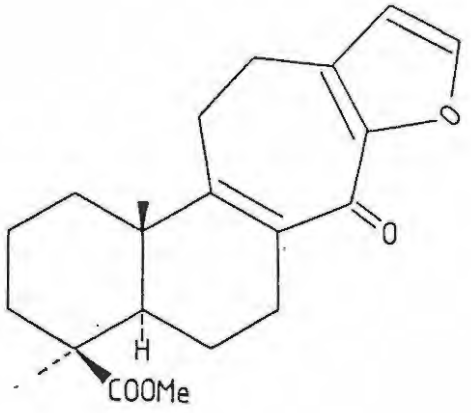


(155)

a 45^{o} instead of a 90^{o} mixing pulse is used and this leads to an asymmetry in intensity of cross-peaks with peaks near the diagonal effectively being suppressed[107]. The net result is a simplified 2D spectrum which enables easier interpretation provided extensive overlapping of cross peaks does not occur[108]. Accurate measurement of the chemical shift and coupling constants of protons in complex spectra can be achieved with a high-resolution double quantum filtered phase-sensitive COSY spectrum[108]. However this experiment was not performed here because the relatively simple structure of **137** has been conclusively established by other means.

The absolute stereochemistry of hispanalone was proposed by Savona *et al*[213] from comparison of the melting point and optical rotation of **137** with values published for dihydro-compound Y previously prepared by Kaplan and Rivett from Li/NH_3 reduction of compound Y (**142**)[219]. Direct comparison of (mixed m.p., t.l.c. and IR spectrum) of **137** with a sample of dihydro-compound Y supplied by Professor Rivett unequivocally established the structural relationship between these two compounds. Hydrogenation of **137** over Paal catalyst[220] yielded tetrahydro-hispanalone (**143**) which has a melting point and optical rotation in agreement with published values[219] for hexahydro-compound Y. Rivett and Kaplan related the structure of compound Y, isolated from *Leonotis leonurus*, to marrubiin (**129**) through a common degradation product isoambreinolide (**144**). Isoambreinolide was prepared from the γ-lactone (**145**), obtained from chromic acid oxidation of dihydro-compound Y, by conversion of the C-7 keto group into a thioketal followed by desulphurisation of this derivative with Raney nickel.

Hispanalone has also been isolated from another genus *Galeopsis* (Lamiaceae) where it occurs in *G. augustifolia*[221] with a similar diterpenoid galeopsin (**146**) or 8β -acetoxyhispanalone. A partial synthesis of **146** from **137** has been reported by Savona *et al*[218].



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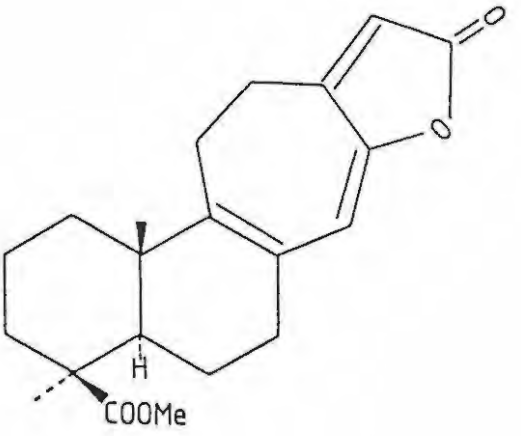
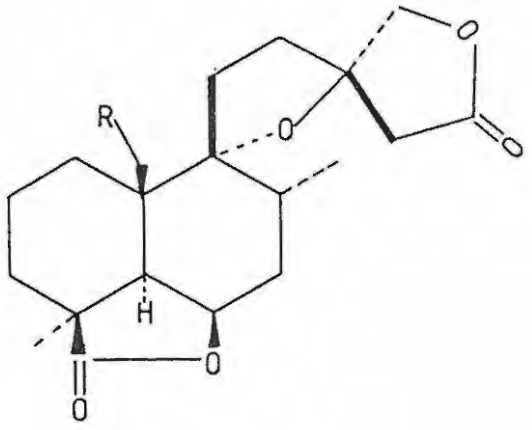
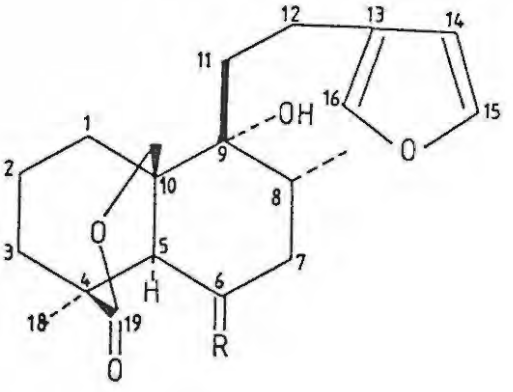


(157)



(158) R = H

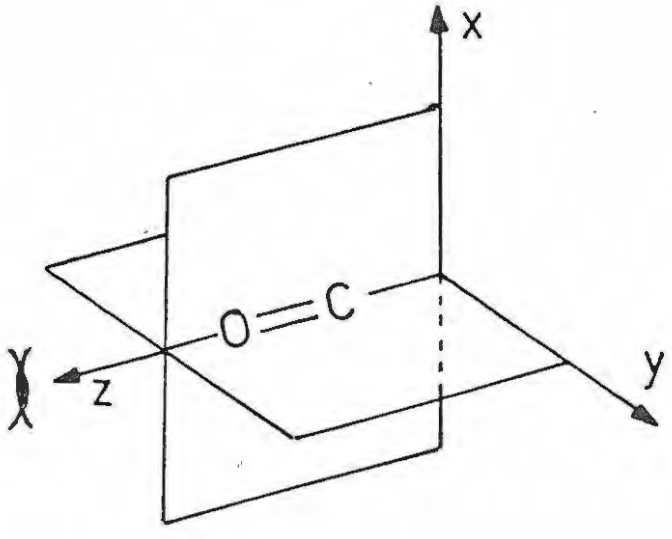
(159) R = OAc



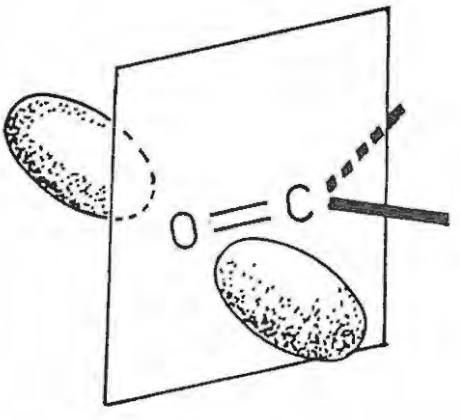
(160) R = β-OAc, α- H

(161) R = β-OH, α- H

(162) R = O



(163)



(164)

Marrubiin (**129**) has been isolated from Ballota nigra and marrubenol (**130**) from B. acetabulosa and B. pseudodictamnus[214]. The chemotaxonomic affinity between B. nigra (black horehound) and Marrubiim vulgarae (white horehound) is further exemplified by the similarities between marrubiin and a number of other diterpenoids isolated from Ballota species. The main difference between compounds from these two genera is the occurence of oxygenation at C-7 in all major diterpenoids from Ballota species. B. nigra afforded the new diterpenoid ballotinone (**147**) shown by ^{13}C NMR comparison with **129** to be 7-oxomarrubiin[222]. A similar compound 7α-acetoxymarrubiin (**148**) was extracted from B. rupestris[223]. Four species,B. nigra, B. rupestris, B. lanata and B. pseudodictamnus all contain an $\alpha\beta$-unsaturated carbonyl compound ballonigrin (**149**) analogous to **147**[214,223,224]. Ballonigrin was prepared from **147** by mild dehydration of the C-9 hydroxyl group with warm alumina in chloroform. The presence of the closely related 13-hydroxyballonigrinolide (**152**) was reported in B. lanata[224] while B. nigra also yielded the diketone ballonigrinone (**150**)[223]. The keto-diol 18-hydroxyballonigrin (**151**)[225] and a keto-triol ballotenol (**153**)[226] were isolated from B. acetabulosa and B. nigra respectively. Recently the 9,13-epoxylabdane preleosibrin (**155**), the prefuranoid derivative of leosibrin (**154**), previously identified in Leonorus sibricus[227], was extracted by Savona et al from B. nigra subsp. feotida[228]. Finally, two diterpene acids hispanonic and hispaninic acids, with an unusual seven membered ring C were obtained as their methyl esters **156** and **157** from methylation of chromatographic fractions of B. hispanica[229].

The value of black horehound and other members of the genus Ballota in herbal remedies is well known[222, 224]. The indigenous peoples of Southern Africa use B. africana to relieve severe colic and for snake bites. In the

Western Province of South Africa this plant is widely used to treat a variety of illnesses including asthma, influenza and insomnia[195].

2:6 Leonotis ocymifolia var. ocymifolia (formerly L. dubia)

The taxonomy of the Southern African genus Leonotis has recently been revised by Iwarsson[140]. Previously approximately twenty-six species of Leonotis were recognised in Southern Africa, but Iwarsson has condensed this classification into three species L. ocymifolia, L. leonurus and L. nepetaefolia of which the former consists of three varieties (Table 8). The phytochemistry of L. leonurus has been investigated by Kaplan and Rivett[219]. Three compounds, marrubiin (129), compound X (158) and compound Y (142) were isolated from this species. The phytochemistry of African specimens of L. nepetaefolia has been discussed (Section 2:4).

The major revision of the taxonomy of this genus has revolved around L. ocymifolia in which the majority of formerly recognised individual species have been reduced to only three varieties. The major natural products from the three species shown in Table 8 have been isolated and identified. L. dysophylla yielded 8β - hydroxymarrubiin (127)[205] and L. leonitis gave leonitin (159)[211]. The structural relationship between these two compounds and other compounds from the genus Leonotis has been described (Section 2:4). Although L. dubia is now classified with L. leonitis as the same species L. ocymifolia var. ocymifolia, the isolation of dubiin (160)[230] as the major natural product from the former refutes this latest classification. The obvious structural differences between dubiin and leonitin provide chemotaxonomic support for the retention of L. leonitis and L. dubia as individual species. Leonitin is 20-acetoxy compound X and this small structural difference would suggest that L. leonitis is more closely related to L. leonurus than it is to L. dubia.

Table 8.

The revised taxonomic classification of

Leonotis ocymifolia

var. *ocymifolia*	var. *schinzii*	var. *raineriana*
L. ocymifolia	*L. schinzii*	*L. raineriana*
*L. leonitis**	*L. randii*	*L. intermedia*
L. parviflora	*L. microphylla*	*L. dysophylla**
*L. dubia**		*L. laxifolia*
L. mollis		*L. malacophylla*
L. hirtiflora		*L. bachmannii*
		L. latifolia
		L. dinteri
		L. urticifolia
		L. hereroensis
		L. brevipes
		L. galpinii
		L. westae
		L. intermedia

* Species subjected to a phytochemical investigation

Conversely the δ-lactone ring structure of dubiin is also found in *L. nepetaefolia* indicating a possible affinity between these two species. Further chemotaxonomic discrepancies in Iwarsson's classification may exist and a phytochemical investigation of the three varieties of *L. ocymifolia* is warranted.

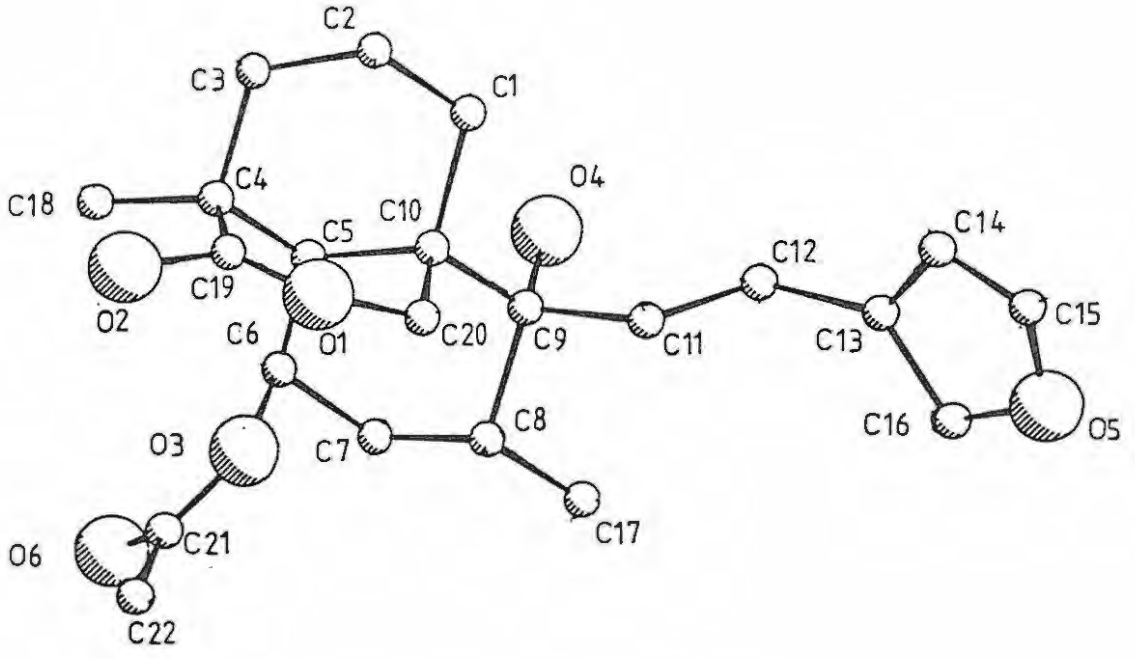


Figure 10. A perspective view of the molecular structure of dubiin

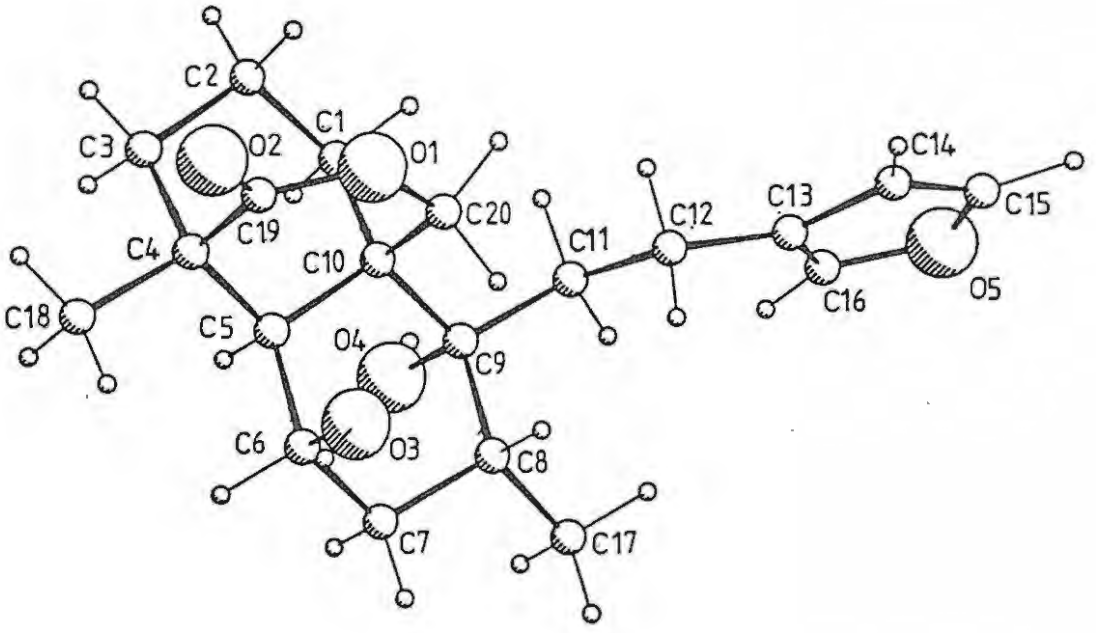


Figure 11. A perspective view of the molecular structure of saponified dubiin

The studies on L. nepetaefolia (Section 2:4) have exemplified the variations in the natural product composition of a species which may occur with changes in locality. During earlier studies on L. dubia Eagle[231] examined plants from various localities within a 30 km radius of Grahamstown. No variation in natural products was reported and the single compound dubiin (**160**) was found in all plant collections in a yield ranging from 0.5-1.0%. This continuity was corroborated by the isolation of **160** from plant material identified as L. ocymifolia var. ocymifolia (synonymous with Herbarium specimens of L. dubia) growing in an area west of Port Alfred about 80 kms from Grahamstown. The **160** obtained from this plant material was identical with a sample isolated by Eagle and Rivett[230] (m.p., mixed m.p., t.l.c. and IR spectrum), but the 1.6% yield per mass of dried plant material was higher than previously reported.

The absolute stereochemistry of **160** remained unassigned and was therefore determined. To confirm the structure of **160** proposed by Eagle and Rivett an X-ray analysis was carried out.

An attempt to prepare the p-bromobenzoate derivative of saponified dubiin (**161**), for the solution of the crystal structure by the heavy atom method[160], was unsuccessful and is due to the well-known difficulty in esterifying the C-6 hydroxyl group of labdanes. The alternative approach is the use of direct methods[161] and a fragment of a thin crystalline plate of **160** was used for the collection of intensity data. Unfortunately the data set was very poor and frequent recalculations of the orientation matrix were observed during the data collection. Despite the inferior data set the structure of **160** was solved by the direct methods program SHELXS[232] with only 983 reflections with $|F_o| > \sigma |F_o|$. However, refinement of the model generated by SHELXS was limited by the quality of the intensity data and final R and R_w values of 0.168 and 0.177 were eventually

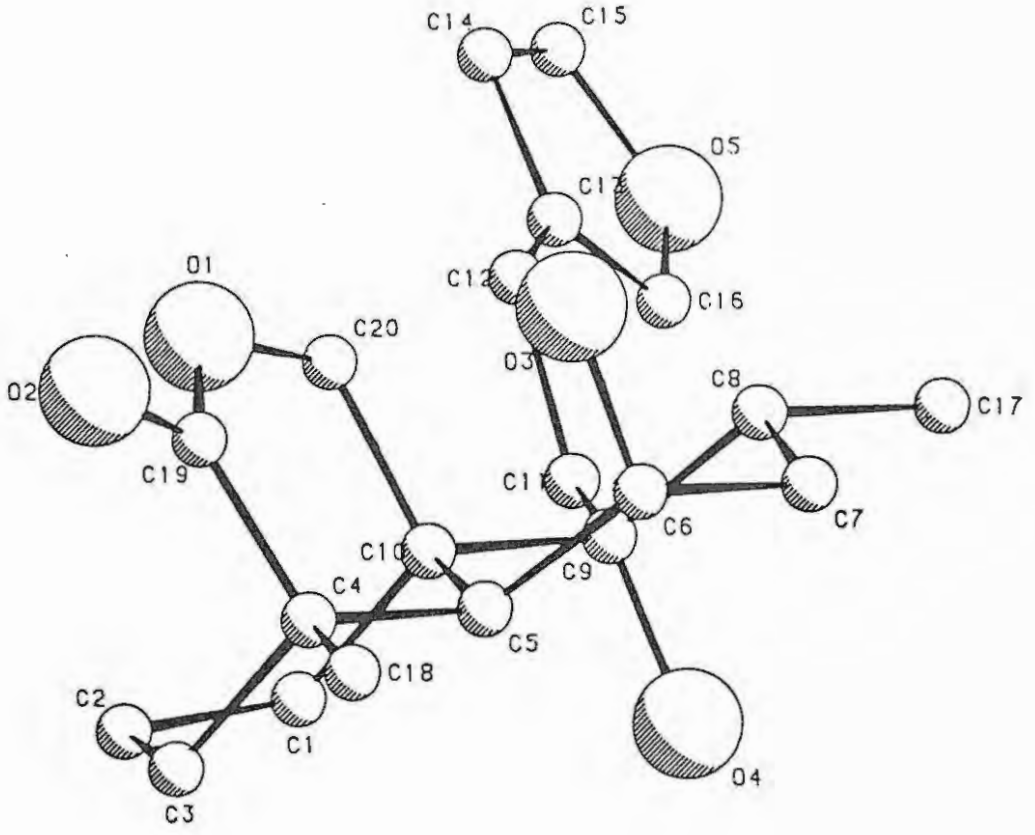


Figure 12. The conformations of the ring systems of saponified dubiin

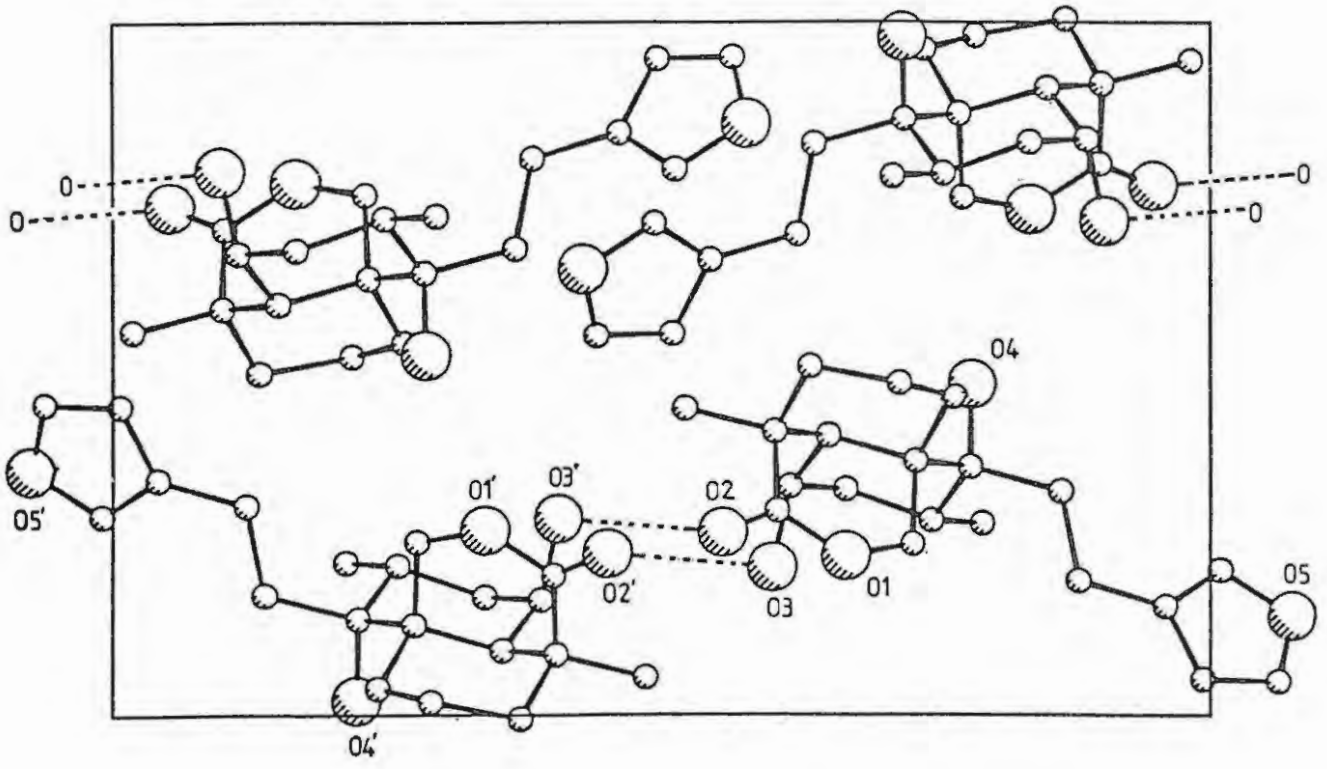


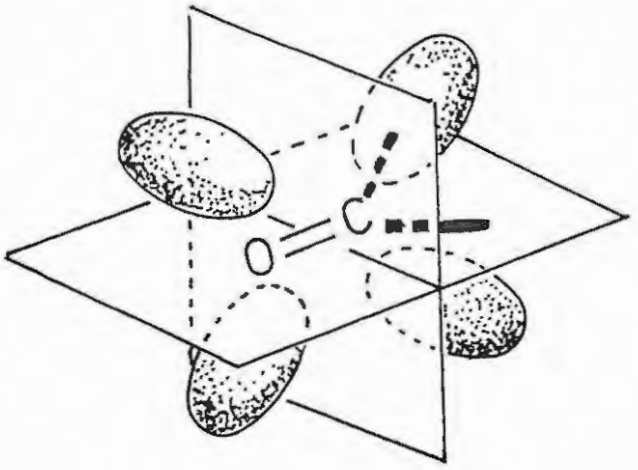
Figure 13. The molecular packing diagram of saponified dubiin[a]

[a] Hydrogen bonding ------

obtained. A perspective view of the molecular structure of dubiin is shown in Figure 10. The large agreement (R) values and estimated standard deviations in both bond lengths ($\pm$ 0.04 Å) and angles ($\pm$ 4°) denoted a poorly refined structure[164] which prevented comparison with the published crystal structures of similar compounds. Accordingly the X-ray analysis was repeated on saponified dubiin (**161**).

Large prismatic crystals of **161** were obtained using Jones' vapour diffusion method[233] which afforded a superior set of intensity data compared to the set collected for **160**. A total of 1663 reflections with $|F_o| > \sigma|F_o|$ were acquired and the structure was solved by direct methods while the refinement on F was carried out by least squares analysis using the SHELX[163] program. The final R value of 0.041 for 1531 reflections with $|F_o| > 2\sigma|F_o|$ and 241 variables (data : parameter ratio = 1:6.4) and estimated standard deviations in bond lengths ($\pm$ 0.004 Å) and angles ($\pm$ 0.3°) represent a significant improvement in the refinement of the structure of **161** compared to **160**. A perspective view of the molecular structure of **161** is shown in Figure 11.

The structure of **160** proposed by Eagle and Rivett was thus confirmed. As expected the two six membered rings of the trans-decalin system of **161** are both in normal chair conformations (Figure 12). Both saponified dubiin and nepetaefolin (**124**)[200,210] possess a similar δ-lactone ring structure. In accordance with the severe crowding between the two tertiary carbon atoms, C-9 and C-10, the C-9, C-10 bond lengths in **161** (1.580 Å) and **124** (1.582 Å)[210] are longer than the value normally reported for an sp^3 - sp^3 bond (1.54 Å). Conversely the C-6, C-7 bond length in both **161** (1.516 Å) and **124** (1.517 Å) is shorter than normally encountered, to offset the longer C-9, C-10 bond lengths. All other C-C, C-O single and double bonds



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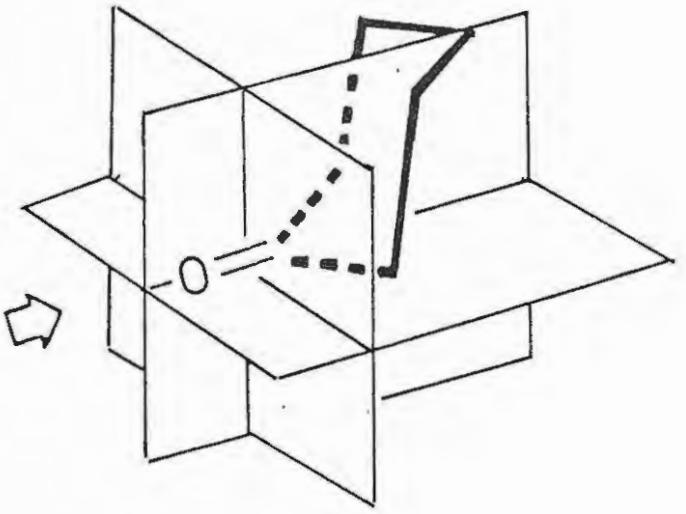
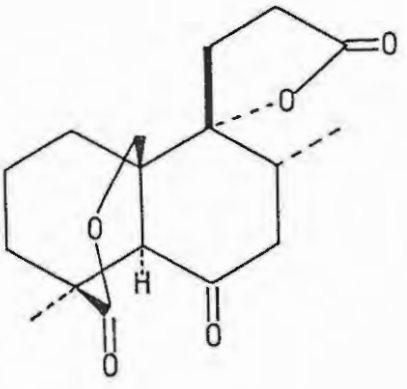


(166)



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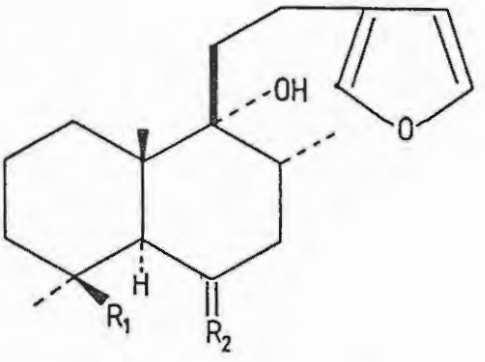
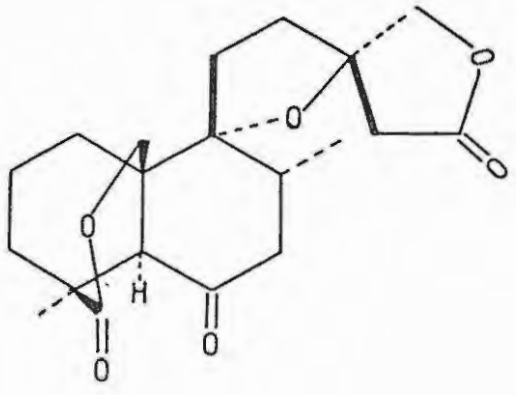


(168) R_1 = COOH, R_2 = O

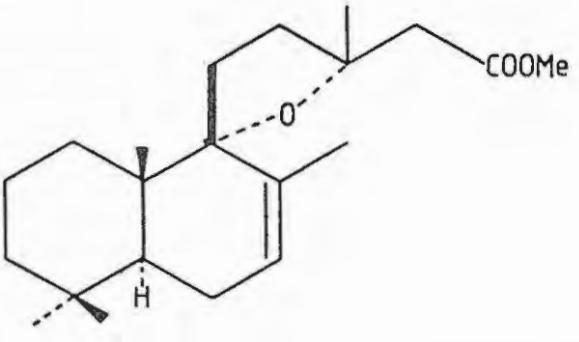
(169) R_1 = COOH, R_2 = β - OH, α -H

(170) R_1 = CHO, R_2 = O

(172) R_1 = $CH(SCH_2)_2$, R_2 = O



(173)

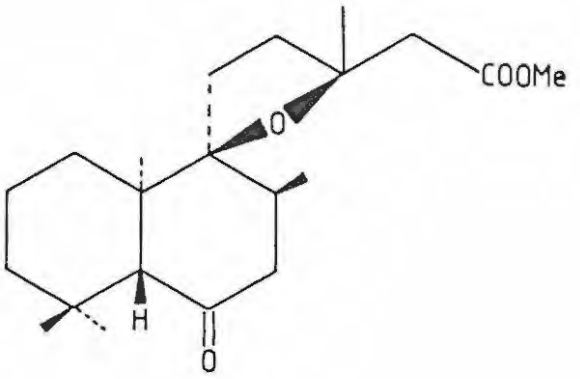


(174)

are of normal length and the bond angles of the sp^3 carbon atoms are all approximately normal tetrahedral angles. Least squares planes analysis[166] of the furan ring and δ-lactone ring of **161** confirmed the co-planarity of the former and showed that a least squares plane exists through C-4, C-10, C-19 and C-20 in the latter (estimated standard deviation from plane ± 0.03 Å). Both the oxygen atoms O-1 and O-2 are displaced to the same side of this least squares plane by 0.17 and 0.11 Å respectively. Finally, the crystal packing of **161** is attributed to the hydrogen bonding between O-2 and O-3 of adjacent molecules (intermolecular oxygen to oxygen bond lengths, O - H --- O less than 2.8 Å)[160] as shown in Figure 13.

The absolute stereochemistry of **160** could be determined from application of the octant rule to the sign of the Cotton effect in the CD curve of 6-deacetyldehydrodubiin (**162**) prepared by Collins oxidation[234] of **161**. The ^{13}C NMR signals of **162** were assigned from 2D-NMR experiments (Appendix 2) and comparison with published values for analogous compounds[217]. All the individual proton signals could not be assigned from the COSY-45 spectrum of **162** and this was attributed to the same problems encountered previously with the ^{1}H assignment of hispanalone (Section 2:5).

The octant rule[235] for the interpretation of the $n \rightarrow \pi^*$ transition of saturated chiral alkyl ketones has been widely used to extract both stereochemical and conformational information from these compounds for over twenty-five years. The $n \rightarrow \pi^*$ transition of alkyl ketones occurs at about 300 nm[40] and the octants are derived from the local C_{2v} symmetry of the carbonyl group (**163**) and a consideration of the relevant orbitals of the $n \rightarrow \pi^*$ transition[119,236]. The n orbital has a nodal plane (**164**) which is identical with the (x,z) local symmetry plane of the carbonyl group while the π^* orbital (**165**) has two nodal surfaces, one identical with the (y,z)



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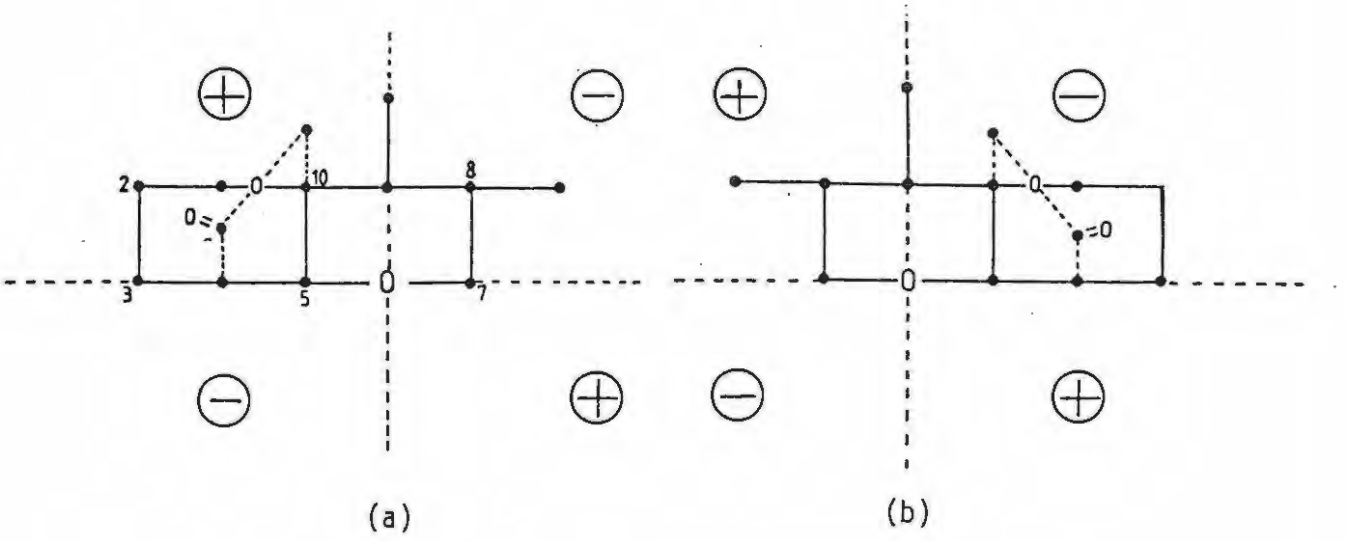

Figure 14. Octant projections of 6-deacetyldehydro-dubiin

(a) positive Cotton effect (b) negative Cotton effect

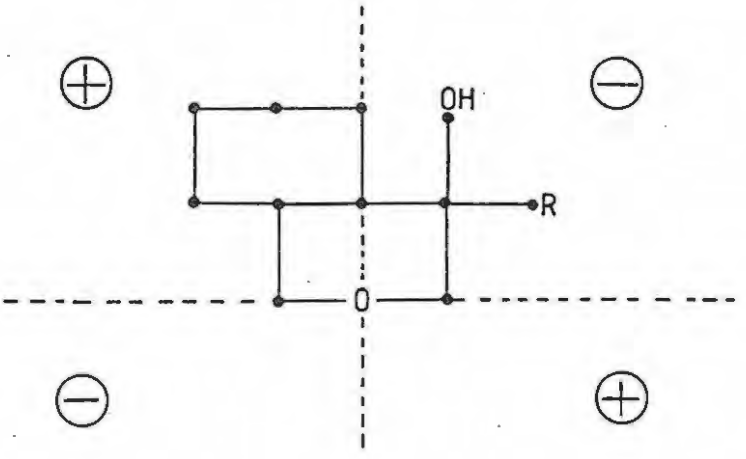

Figure 15. Octant projection of hispanalone

R = $(CH_2)_2C_4H_3O$

symmetry plane and a second nonsymmetry-derived surface perpendicular to this which is normally represented as a plane bisecting the middle of the carbonyl bond. These three planes divide the space around a ketone e.g. cyclohexanone (166) into eight octants. For the application of the octant rule the octants are viewed along the carbonyl bond from the oxygen to the carbon atom. In practice each octant has been assigned a positive or negative sign arising from the contribution to the sign and magnitude of the $n\rightarrow\pi^*$ Cotton effect attributed to the presence of a substituent (s) in that particular octant. The substituents affect the $n\rightarrow\pi^*$ rotatory strength by destroying the molecular symmetry and this effect is further enhanced by the type of substituent and its orientation. Thus α-axial and β-equatorial substituents are reported[119] to exert a greater influence on the Cotton effect than β-axial or α-equatorial substituents. The octant rule also states that substituents lying in the two symmetry planes (x,z) and (y,z) make no contribution to the Cotton effect associated with the carbonyl group. As a rule[40] the rear octants are more important than the front octants and the signs of the former are shown in Figure 14(a) and(b).

As the complexity of the molecule increases beyond that of a monocyclic ketone the interpretation of the contribution to the Cotton effect of the individual substituents becomes more difficult. Accordingly, the sign of the Cotton effect of polycyclic, multifunctional alkyl ketones is normally attributed to the sign of the octant containing the majority of the molecule[119]. Hence the positive Cotton effect of 6-deacetyldehydrodubiin (162) is interpreted as arising from the octant projection shown in Figure 14(a) and the enantiomeric projection which would yield a negative Cotton effect is shown in Figure 14(b).

In accordance with the octant rule the α-axial hydroxyl and the β-

equatorial pendant furan groups substituted at C-9 should make no contribution to the Cotton effect as they lie on the (x,z) symmetry plane. However it is likely that the furan ring deviates slightly from this plane (Figure 12) and thus might contribute to the sign and magnitude of the Cotton effect. Accordingly, the extent of this contribution was investigated by effectively removing the furan ring. This was achieved by the synthesis of the new $\gamma\sigma$-lactone (**167**) from chromium trioxide oxidation of **162** in acetic acid. The sign, magnitude and position of the Cotton effect of **162** and **167** were almost identical confirming the furan rings negligable contribution. Another possible influence on the Cotton effect of **162** is the δ-lactone ring but the absence of a Cotton effect in the CD spectrum of **161** invalidated this possibility .

The standard procedure for determining the relationship between the sign of the Cotton effect and the absolute stereochemistry of a compound is by analogy to the Cotton effects of similar compounds of known stereochemistry[40,119]. Accordingly 6-dehydromarrubic acid (**168**) was prepared from marrubiin (**129**) previously isolated by Professor Rivett from L. leonurus[219]. Alkaline hydrolysis of **129** afforded marrubic acid (**169**)[237] which was oxidised using Jones' method[151] to yield (**168**)[207]. Although **168**, of known absolute stereochemistry, is a suitable standard for CD comparison with other labdane diterpenoids an uncertainty exists as to the contribution of the C-19 carboxylic acid group to the sign and magnitude of the Cotton effect of the C-6 ketone. Thus an attempt was made to prepare the definitive hydroxy-ketone (**170**) from marrubiin.

Compound **170** has been prepared by Anthonsen et al[238] and their method was followed. Reduction of marrubiin with lithium aluminium hydride afforded marrubenol (**130**) which was oxidised by chromium trioxide in pyridine to yield the keto-aldehyde (**171**). The thioketal (**172**) was

prepared from **171** but attempts to separate this product from unchanged starting material were unsuccessful. The reaction mixture was accordingly treated with Raney nickel[239] to afford the hydroxy-ketone (**170**). Initial attempts to separate this component by alumina, silica gel column chromatography and preparative t.l.c. were unsuccessful.

Despite failing to isolate **170** from the reaction mixture the CD spectra of a wide variety of other labdane diterpenoids were recorded and are tabulated in Table 9. The value given for 6-dehydronepetaefolinol (**132**) was obtained from the literature[201] while the value for 6-dehydro-saponified leonitin (**173**) was obtained from a sample prepared by Professor Rivett.

Table 9.

The $n \rightarrow \pi^*$ CD data for 6-keto-labdane diterpenoids

Compound	No.	$n \rightarrow \pi^*$ $\Delta\varepsilon$	λnm
6-Deacetyldehydrodubiin	162	+0.69	289
6-Dehydronepetaelfolinol	132	+1.25	[a]
6-Dehydromarrubic acid	168	+0.64	277
6-Dehydro-saponified leonitin	173	+0.89	287
Dubiin $\gamma\,\delta$-lactone	167	+0.62	288

[a] Value not cited in the literature

The positive Cotton effect observed for all the labdane diterpenoids

in Table 9 establishes the absolute stereochemistry of **161** as shown in Figure 11. Paradoxically Bohlmann et al[240] have recently reported the structure (**174**) for the hydrogenated 6-keto derivative of the methyl ester of grindelic acid (**175**). From the positive Cotton effect of **174** ($\Delta\varepsilon$ = +0.61) Bohlmann et al have proposed a reassignment of all the previously isolated labdane diterpenoids from Grindelia species to the ent-labdane series with the stereochemistry as shown in **174**. The signs and magnitudes of the Cotton effects of the labdane diterpenoids in Table 9, which are in accordance with that reported for **174**, refute Bohlmann et al's proposal and the absolute stereochemistry of the grindelane diterpenoids must therefore remain as shown in **175**.

Finally the negative Cotton effect of the 7-keto labdane diterpenoid hispanalone ($\Delta\varepsilon$ = -0.98), of known absolute stereochemistry, is interpreted as arising from the octant projection as shown in Figure 15. The presence of the hydroxyl group and furan substituent in a negative octant thus determines the sign of the Cotton effect in this instance.

CHAPTER THREE

EXPERIMENTAL

3. General Methods

Melting points (uncorrected) were determined on a Kofler block and elemental analyses were performed at the microanalytical laboratories of the National Chemical Research Laboratory (N.C.R.L.), C.S.I.R.. Optical rotations were measured in a 1 dm tube on a Perkin-Elmer Model 141 polarimeter using the solvent indicated. The ultraviolet spectra were recorded in methanol on a Unicam SP800 spectrophotometer and the infrared spectra (KBr disc) on a Perkin-Elmer Model 180 spectrophotometer. Circular dischroism spectra were run by Professor Ferreira of the University of the Orange Free State. Proton NMR and ^{13}C NMR spectra were determined on Bruker 300 MHz and 500 MHz instruments by Dr. E.V. Brandt of the University of the Orange Free State and by Mr. I. Antonowitz of the N.C.R.L. respectively. Tetramethylsilane was used as the internal standard (δ = 0 ppm) and spectra were recorded in either deuterated chloroform or deuterated dimethyl sulphoxide. The 70 eV electron impact mass spectra were determined on a HP 5988A spectrometer and GCMS experiments were carried out in combination with a J & W Scientific fused silica, bonded-phase (0.25 μm thickness) DB 225 capillary column (0.25 mm x 30 m). This capillary column was also used for the gas chromatography experiments in a HP 5890A gas chromatograph using helium carrier gas and a flame ionisation detector under the conditions indicated.

Both coarse (70-230 mesh ASTM, Merck No. 7734) and fine (230-400 mesh ASTM, Merck No. 9385) silica gel 60 and neutral alumina were used for the chromatography of plant extracts and reaction products. Alumina for chromatography was acid washed, neutralised and activated by heating at 150^{o} for 16 hours. Preparative t.l.c. plates were prepared by coating glass plates (20 x 20 cm) with silica gel 60 PF_{254} (Merck No. 7747) to an approximate thickness of 2 mm. All chromatography and syntheses were

monitored on 0.2 mm thick plastic backed silica gel 60 F_{254} plates (Merck No. 5735). Unless otherwise stated these plates were eluted with ethyl acetate and were visualised by spraying with a 10 : 1 methanol - conc. sulphuric acid spray reagent followed by heating at about 100^{o} for one to two minutes.

The preliminary investigations of the crystal lattices of synrotolide, dubiin and saponified dubiin were carried out on a Stoe reciprocal lattice explorer. The X-ray generator (Siefert Isodebye Flex 1001) was operated at 20 mAmp and 40 KV and nickel filtered Cu Kα radiation (λ = 1,542 Å) was used for the de Jong Boumann and Buerger Precession photographs. The X-ray film was processed in the normal manner. Intensity data for synrotolide were collected by Dr. P. van Rooyen of the N.C.R.L. and those for dubiin and saponified dubiin by Dr. M. Niven of the University of Cape Town.

3:1 Extraction of Syncolostemon rotundifolius

Three batches of plant material were collected by Mr. H. Nicholson near Margate in Natal. The first batch was collected over a two week period at the end of May 1984 and the second and third batches on 8.11.1984 and 22.01.1985 respectively. The latter two batches were combined and a specimen of S. rotundifolius from this plant collection was deposited with the Albany Museum, Grahamstown (Voucher No. A7335). The plant material was air dried in the shade for 8-12 weeks and extracted. The following experiment is typical.

The dried leaves (2.28 kg) were soaked in acetone (80 ℓ) at room temperature for four days, the acetone run off and the plant material washed with a further 15 ℓ of acetone. The combined acetone extracts

were concentrated by flash distillation to approximately 6 ℓ and the dark green solution stirred with decolourising charcoal (BDH, 2 x 50 g) for three hours. The solution was filtered through a Celite 545 pad and concentrated to 2 ℓ by flash distillation. The remaining acetone was evaporated on a rotary evaporator to leave a brown gum which was dried in a vacuum desiccator to give a tan-coloured brittle solid (216 g).

A crude separation of the compounds from the solid plant extract was achieved by chromatography on an open column of silica gel 60 (70-230 mesh ASTM). Ethyl acetate (30 ml) was added to the tan-coloured brittle solid (40 g) and the solution warmed until all the solid had dissolved. Silica gel 60 (40 g, 70-230 mesh ASTM) was added to the warm solution and the ethyl acetate removed in vacuo. The mixture was slurried with hexane (40 ml) and applied to the column of silica gel 60 (350 g) in hexane. The column (665 cm^3) was eluted with a hexane-ethyl acetate gradient (10 ℓ) of increasing ethyl acetate concentration. Fractions (165 ml) were collected and combined from t.l.c. results. T.l.c. of the fractions eluted with 3:1, 5:2, and 2:1 hexane-ethyl acetate gave a single pink spot (R_F 0.70). The remaining 1:1, 2:1 ethyl acetate-hexane and ethyl acetate fractions also contained this compound. The 1:1 fraction yielded two further compounds which gave distinctive green (R_F 0.68) and purple (R_F 0.51) colour reactions on t.l.c.. The major compound in the 2:1 ethyl acetate-hexane and ethyl acetate fractions yielded a dark green spot (R_F 0.37) on t.l.c.. Two minor compounds (R_F 0.46 and 0.27) in these fractions also gave a green colour reaction on t.l.c..

Column chromatography of the crude extract was also attempted with silica gel 60 (70-230 mesh ASTM), deactivated with 15% water, and silica gel 60 (230-400 mesh ASTM). Inferior separation was achieved with the former silica gel while the run time for the chromatography was extended,

without a significant improvement in separation, with the latter. Therefore to ensure optimum separation and to reduce possible isomerisation from prolonged contact with silica gel, the coarser silica gel (70-230 mesh ASTM) was used.

Further chromatography on both silica gel (70-230 mesh ASTM) and neutral alumina of the 1:1 ethyl acetate-hexane fraction (5.5 g) to separate the three compounds in this fraction were unsuccessful. Preliminary trial separations using preparative t.l.c. were also unsuccessful and this was partly attributed to the difficulty in visualizing the compounds on the preparative t.l.c. plates.

3:2 Isolation of crude oleanolic acid (99)

Although the combined 3:1, 5:2 and 2:1 hexane-ethyl acetate fractions (5.8 g) consisted of a single compound, this compound could not be crystallised from the crude fraction. Further chromatography of a portion (115 mg) of the combined fractions through neutral alumina (4 g), did not purify this compound sufficiently for it to crystallise. Accordingly oleanolic acid was isolated from the combined 3:1, 5:2 and 2:1 fractions as its crystalline monoacetate derivative. The mass of crude oleanolic acid (5.8 g) isolated from the solid extract (40 g) represents an overall 1.4% yield from the dried plant material.

3:2:1 Acetylation of crude oleanolic acid

A solution of the combined 3:1, 5:2 and 2:1 fractions (320 mg) in dry pyridine (3 ml) and acetic anhydride (6 ml) was refluxed at 140^{o} for 1 hour. The solvent was removed under reduced pressure and the residue dissolved in warm ethanol (10 ml). The dark brown solution was refluxed

with decolourizing charcoal (BDH, 0.5 g) and filtered though a Celite 545 pad. The pale yellow solution was concentrated under reduced pressure to 3 ml and left in the refrigerator overnight. Fine needles formed (175 mg, 50%), m.p. 242-243°, which were filtered off, washed with cold ethanol (1 ml) and dried in a vacuum desiccator. T.l.c. of this derivative gave a single pink spot (R_F 0.75). Recrystallisation from ethanol gave oleanolic acid acetate (100) m.p. 255-257°, (lit. 258-260°)[147], $[\alpha]_D^{25}$ = +69° (c, 1.20 in chloroform), (lit. $[\alpha]_D^{17}$ = +75°)[148].

IR spectrum ν_{max} cm^{-1}	:	3230, 2860, 1720, 1690(sh), 1455, 1360, 1220, 1020, 815.
H^1 NMR spectrum (300 MHz, $CDCl_3$)	:	δ5.24 (1H, t, J = 4 Hz, H-12), 4.46 (1H, t, J = 8Hz, H-3), 2.80 (1H, m, H-18), 2.05 (3H, s, acetyl) 1-2.05 (23H), 1.11, 0.92, 0.90, 0.88, 0.84, 0.82, 0.71 (3H, s, 7x methyl).
^{13}C NMR spectrum (75.5 MHz, $CDCl_3$)	:	δ184.0 (C28), 171.1 (Acetyl CO), 143.5 (C13), 122.5 (C12), 81.0 (C3), 55.2 (C5), 47,8 (C9), 46.5 (C17), 45.8 (C19), 41.5 (C14), 41.0 (C18), 39.4 (C8), 38.2 (C1), 37.8 (C4), 37.3 (C10), 34.1 (C21), 33.2 (C29), 32.8 (C7), 32.6 (C22), 30.8 (C20), 28.2 (C23), 27.8 (C2), 27.8 (C15), 26.0 (C27), 23.6 (C30), 23.5 (C16), 23.0 (C11), 21.1 (Acetyl CH_3), 18.4 (C6), 17.2 (C26), 16.8 (C24), 15.5 (C25).
Mass spectrum m/z (rel.int.)	:	498 (2), 438 (4), 250 (3), 248 (100), 203 (86), 133 (24), 69 (22), 43 (29).

M^+ calculated for $C_{32}H_{50}O_4$ = 498.

Analysis : A sample was dried (P_2O_5) at 100°/0.2 mm for 3 hours.

Found : C = 77.38 H = 10.37%

$C_{32}H_{50}O_4$ required : C = 77.06 H = 10.10%

3:2:2 Saponification of oleanolic acid acetate (100)

A solution of oleanolic acid acetate (560 mg, 1.1 mmol) in 0.2 M ethanolic potassium hydroxide (20 ml, 4.0 mmol) was refluxed for 1.5 hours at 80°. Water (30 ml) was added and the alcohol removed under reduced pressure. The solution was acidified and a white precipitate formed. The mixture was allowed to stand in the refrigerator for 3 hours and the white precipitate (485 mg) m.p. 275-285° was filtered off, washed with water (2 x 5 ml) and dried in a vacuum desiccator. T.l.c. of this compound gave a single pink spot (R_F 0.70) identical with the t.l.c. of the combined 3:1, 5:2, 2:1 hexane-ethyl acetate fractions. The oleanolic acid (99) was difficult to recrystallise and a sample eventually crystallised from ethanol as very small needles m.p. 290-296° (lit. 306-308°)[147] after standing for 8 weeks at room temperature. $[\alpha]_D^{25}$ = +78° (c, 0.07 in chloroform), (lit $[\alpha]_D^{12}$ = +80°)[147].

Mass spectrum : 458 (2), 249 (100), 203 (92), 133 (36),

m/z (rel. int.) 105 (26).

M^+ calculated for $C_{30}H_{48}O_3$ = 456.

3:2:3 Methylation of oleanolic acid acetate

A solution of oleanolic acid acetate (135 mg) in ether (5 ml) was treated with excess diazomethane (prepared from 2 g of N-methyl-N-nitroso-

p-toluenesulfonamide)[149] and left to stand in ice for 3 hours. The ethereal solution was concentrated to dryness under reduced pressure. The dry residue in benzene (2 ml) was passed through a short column of neutral alumina (2 g) and crystallised from ethanol to afford slender needles, m.p. 218-220° (110 mg, 79%) which also gave a pink spot (R_F 0.76) on t.l.c.. The melting point of the methyl 3-acetoxyoleanolate (**101**) was not raised on recrystallisation (lit. 224-225°)[148]. $[\alpha]_D^{25}$ = +68° (c, 0.06 in chloroform), (lit. $[\alpha]_D^{25}$ = +70°)[148].

Mass spectrum : 512 (1), 453 (1), 262 (52), 202 (40),
m/z (rel. int.) 188 (15), 133 (32), 43 (100).
M^+ calculated for $C_{33}H_{52}O_4$ = 512.

3:2:4 Saponification of methyl 3-acetoxyoleanolate (101)

A solution of methyl 3-acetoxyoleanolate (1.22 g) and dry, finely divided potassium carbonate (2.2 g) in methanol (150 ml) was refluxed for 21 hours. The solution was concentrated to 20 ml under reduced pressure and water (20 ml) added. The remaining methanol was removed under reduced pressure and the white precipitate filtered off and washed with water (2 x 5 ml). The precipitate was dried in a vacuum dessicator and crystallised from methanol to yield colourless needles (0.57 g, 51%) m.p. 196-198° of methyl oleanolate (**102**) (lit. 196-198°)[147] $[\alpha]_D$ = +72° (c, 0.82) in chloroform), (lit. $[\alpha]_D^{26}$ = +75°)[147].

Mass spectrum : 471 (5), 470 (2), 262 (78), 208 (14),
m/z (rel. int.) 202 (100), 133 (19).
M^+ calculated for $C_{31}H_{50}O_3$ = 470.

3:2:5 Oxidation[151] of methyl oleanolate (102)

A solution of methyl oleanolate (51 mg) in acetone (15 ml) (distilled over potassium permanganate) was cooled in ice and 8 N CrO_3/H_2SO_4 (0.075 ml) was added dropwise from a micro-burette until an orange colour was obtained which persisted for about 5 minutes. A few drops of ethanol were added to destroy the excess CrO_3, followed by water (10 ml). The acetone was removed under reduced pressure whereupon the solution became cloudy and was left to stand in the refrigerator overnight. The resulting white precipitate of the ketone (50 mg, 98%) m.p. 165-169° was filtered, washed with water (2 x 10 ml) and dried in a vacuum desiccator. Recrystallisation of methyl 3-oxooleanolate (103) from methanol gave colourless needles m.p. 179-181°. $[\alpha]_D^{25}$ = +87° (c, 0.04 in chloroform), (lit. m.p. 180-182° $[\alpha]_D$ = +76°)[152].

Mass spectrum m/z (rel. int.)	:	468 (1), 410 (3), 262 (34), 202 (100), 188 (32), 133 (29), 55 (57).
		M^+ calculated for $C_{31}H_{48}O_3$ = 468.

3:3 Isolation of synrotolide (1)

The fractions eluted with 2:1 ethyl acetate-hexane and ethyl acetate were combined and the solvent removed under reduced pressure to yield a brown gum (21 g) which was taken up in warm 3:1 ethyl acetate-hexane (300 ml), and left to stand at room temperature for two days. Synrotolide (1.2 g), m.p. 163-166°, crystallised from the solution as large colourless prisms which were filtered off, washed with a small volume of ethyl acetate and dried in a vacuum desiccator. The mother liquors were concentrated and rechromatographed on silica gel (300 g) to yield a further 2.1 g of synrotolide, m.p. 162-165°. The major t.l.c. spot (R_F 0.37) of

the original combined 2:1 and ethyl acetate fractions was identical with synrotolide. Recrystallisation of synrotolide from ethyl acetate afforded colourless prisms, m.p. 168-170°, $[\alpha]_D^{24}$ = -29° (c, 0.06 in methanol). The total mass of synrotolide (3.3 g) isolated from the solid extracet (40 g) represents an overall 0.8% yield from the dried plant material.

UV spectrum	:	λ_{max} = 208 nm (log ε = 4.013)
CD spectrum (in methanol)	:	Δε = +2.45 at 266 nm.
IR spectrum ν_{max} cm^{-1}	:	3365, 1740, 1710 (sh), 1695, 1375, 1230, 1025, 950.
^1H NMR spectrum (300 MHz, DMSO-d_6)	:	δ1.08 (3H, d, $J_{6,7}$ = 6.5 Hz, C-7' methyl), 1.97 (3H, s, acetyl), 2.01 (3H, s, acetyl), 2.39 (2H, m, H-5ae), 3.28 (1H, m) 3.49 (1H, m), 5.01 (1H, td), 5.05 (1H, d, J = 6Hz), 5.31 (1H, m), 5.39 (1H, d, J = 6Hz), 5.60 (1H, dd, J = 9Hz), 5.72 (1H, dd, $J_{1,2}$ = 11Hz, H-2'), 5.82 (1H, dd, $J_{2,1}$ = 11Hz, H-1'), 5.97 (1H, m, $J_{4,3}$ = 10Hz, H-3), 7.05 (1H, m, $J_{3,4}$ = 10Hz, H-4).
^{13}C NMR spectrum	:	Table 3 (Section 2:1)
Mass spectrum m/z (rel. int.)	:	196 (6), 165 (6), 154 (14), 136 (16), 153 (14), 107 (14), 97 (10), 91 (6), 81 (19), 68 (17), 43 (100).
		M^+ calculated for $C_{16}H_{22}O_8$ = 342.
Analysis	:	A sample was dried (P_2O_5) at 25°/0.1 mm for 20 hours.
Found	:	C = 56.06 H = 6.50%
$C_{16}H_{22}O_8$ required	:	C = 56.14 H = 6.48%

3:3:1 Acetylation of synrotolide

A solution of synrotolide (80 mg) in dry pyridine (1.5 ml) and acetic anhydride (3 ml) was left to stand overnight at room temperature. The solvent was removed under reduced pressure and the residue taken up in warm ethanol (2 ml) and left in the refrigerator for 2 days. The diacetate (2) crystallised as colourless plates (85 mg, 85%), m.p. 101-103°, unchanged in melting point on recrystallisation from ethanol. $[\alpha]_D^{24} = -11^\circ$ (c, 0.09 in chloroform). T.l.c. of this compound gave a single green brown spot (R_F 0.62).

UV spectrum	:	λ_{max} = 208 nm (log ε = 4.002)
IR spectrum ν_{max} cm^{-1}	:	1745, 1728 (sh), 1375, 1230, 1210, 1015, 960.
^1H NMR spectrum	:	Table 2 (Section 2:1)
^{13}C NMR spectrum	:	Table 3 (Section2:1)
Mass spectrum m/z (rel. int.)	:	426 (0.1), 280 (2), 231 (7), 204 (10), 178 (19), 136 (31), 135 (26), 107 (17), 97 (6), 68 (32), 43 (100). M^+ calculated for $C_{20}H_{26}O_{10}$ = 426.
Analysis	:	A sample was dried (P_2O_5) at 25°/0.1 mm for 18 hours.
Found	:	C = 56.15 H = 6.16%
$C_{20}H_{26}O_{10}$ required	:	C = 56.33 H = 6.15%.

3:3:2 Attempted saponification of synrotolide

A solution of synrotolide (260 mg) and potassium carbonate (360 mg, dried, finely divided) in methanol (20 ml) was refluxed for 3 hours. Water (20 ml) was added and the methanol removed under reduced pressure.

The aqueous solution was acidified and continuously extracted with ether for 5 hours. The yield of saponified product (54 mg) was considered low and the extraction was continued for a further 15 hours. The ether was removed under reduced pressure to afford an orange residue (total yield 146 mg) which gave a single green spot (R_F 0.22) on t.l.c.. A solution of this residue (146 mg) and decolourizing charcoal (BDH, 0.3 g) in ethanol (10 ml) was refluxed for 1 hour and filtered through a Celite 545 pad. The ethanol was removed under reduced pressure and the pale yellow oil crystallised from butanol as small white needles (43 mg, 22%), m.p. 100-104°. The melting point was raised on recrystallisation from the same solvent to 109-112°, $[\alpha]_D^{24}$ = +36.5° (c, 0.08, in water). The crystals of saponified product yellowed on standing at room temperature for 2 days and after 10 days had decomposed to a brown oil. The reaction was repeated and the rate of decomposition of the crystalline product was found to be reduced by storage at -10°.

Analysis	:	A sample of the crystalline saponified product was dried (P_2O_5) at 20°/0.1 mm for 12 hours.
Found	:	C = 47.46 H = 6.90%
$C_{12}H_{18}O_6$ required	:	C = 55.81 H = 7.02%.

Later a quantitative experiment on the saponification of synrotolide (357 mg, 1.04 mmol) with aqueous 0,145 M potassium hydroxide (40 ml, 5.8 mmol) showed that after 3 hours 3.4 moles of potassium hydroxide had been consumed per mole of synrotolide.

3:3:3 Quantitative microhydrogenation of synrotolide

A solution of synrotolide (12.3 mg, 0.036 mmol) in ethanol (10 ml) was hydrogenated over Adams catalyst (BDH Pt O_2 + H_2O, 10 mg) for 50 minutes during which time 1.99 ml of hydrogen (equivalent to 2.37 moles per mole of synrotolide) were absorbed. T.l.c. of the reaction mixture showed that no starting material remained and that there were at least two hydrogenation products. No attempt was made to isolate these products.

3:3:4 Qualitative 1,2-diol test on synrotolide[156]

A few crystals of synrotolide were added to a 0.5% aqueous paraperiodic acid solution (2 ml) and concentrated nitric acid (0.1 ml) in a small test tube. The mixture was shaken for half a minute and 5% aqueous silver nitrate solution (0.2 ml) was added whereupon a white precipitate formed. The test was repeated with ethylene glycol and a similar precipitate was obtained.

3:3:5 Attempted preparation of synrotolide di-p-bromobenzoate

A solution of synrotolide (76 mg), 4-dimethylaminopyridine (4 mg) and 4-bromobenzoyl chloride (962 mg) in dry pyridine was stirred at room temperature overnight. Water (1 ml) and 1 M sodium carbonate solution (5 ml) was added to the solution and the resultant white precipitate filtered off, washed with water (5 ml) and dried in a vacuum desiccator. Recrystallisation of the precipitate from benzene gave needles (45 mg, 3%) of 4-bromobenzoic anhydride m.p. 212-213° (lit. m. p. 212-213°)[147].

Mass spectrum m/z (rel. int.)	:	384 (8), 185 (97), 183 (100), 157 (28), 155 (30), 76 (35), 75 (34), 74 (11), 50 (29).
		M^+ calculated for $C_{14}H_8O_3Br_2$ = 384.

The aqueous pyridine mother liquor was extracted with ether (2 x 15 ml) and the combined ether layers were washed with 1 M HCl (4 x 20 ml) and water (3 x 20 ml). The ether layer was dried over anhydrous sodium sulphate, filtered and the ether removed under reduced pressure to yield a yellow oil (154 mg). Although this oil showed a single spot (R_F 0.71) on t.l.c. it resisted crystallisation.

3:4 X-ray crystallography of synrotolide

3:4:1 Collection of intensity data

Slow crystallisation of synrotolide from a solution of chloroform and dichloromethane yielded large colourless prisms (approx. 0.2 mm x 0.5 mm) suitable for X-ray diffraction. The symmetry elements evident from de Jong-Boumann and Buerger Precession photographs of a single crystal indicated that the crystal was orthorhombic with space group $P2_12_12_1$. The experimentally determined crystal density, from flotation in hexane-carbon tetrachloride, was consistent with the density calculated from the preliminary unit cell dimensions and the molecular mass of synrotolide.

Data were collected on an Enraf Nonius CAD-4F automated four circle diffractometer using Mo-Kα radiation (λ = 0.7107 Å). Intensity data were collected by the $\omega - 2\theta$ scan technique and were not corrected for absorption or extinction; $\theta_{max} = 25^{o}$; h = 0-10, k = 0-10 and l = 0-25. The intensities of three suitable reference reflections were measured every hour to monitor crystal and instrumental stabilities. No crystal instability was noted and Lorentz and polarization corrections were applied to the 1775 reflections collected of which 1269 with $|F_o| > \sigma |F_o|$ were considered observed.

The crystal data are recorded in Table 10 (Appendix 1).

3:4:2 Structure solution and refinement

The direct methods program MULTAN[162] was used to solve the structure and the refinement on F was carried out by full matrix least-squares analysis using the SHELX[163] program.

The six membered ring with the exception of the carbonyl oxygen and the majority of the heptenyl side chain appeared in a Fourier map calculated using the phase set, with the highest figure of merit, generated by MULTAN. Refinement of this model gave an agreement value (R) of 0.342. The remaining carbon and oxygen atoms emerged from the difference Fourier synthesis and all oxygen and carbon atoms were assigned anisotropic and isotropic temperature factors respectively. The hydrogen atoms could not be identified in the difference Fourier map produced from refinement of this model (R = 0.178). Accordingly the hydrogen atoms were placed in calculated positions with common isotropic temperature factors and further refinement yielded final R and R_w values of 0.1317 and 0.1083 respectively, for 1269 reflections with $|F_o| > \sigma |F_o|$ and 153 variables. A weighting function[163] $w = k(\sigma^2 F + gF^2)^{-1}$ was used and the values of k and g were optimised after each refinement; the final values were 0.1464 and 0.003998 respectively. A final value R based on 755 reflections and 153 variables with $|F_o| > 5\sigma |F_o|$ was 0.0747 but the variable/parameter ratio (1:4.9) was unacceptably low[164].

Additional computer programs used were PLUTO[165] and XANADU[166]. The final atomic co-ordinates, thermal parameters and intramolecular bond lengths and angles of the non hydrogen atoms are set out in Tables 11, 12, 13 and 14 (Appendix 1). Observed and calculated structure factors are available from the Department of Chemistry and Biochemistry, Rhodes

University, Grahamstown.

3:5 The absolute stereochemistry of synrotolide

3:5:1 Attempted periodate oxidation of synrotolide

Synrotolide (250 mg, 0.73 mmol) was dissolved in water (40 ml) on a steam bath. The aqueous solution was cooled and a 0.042 M sodium metaperiodate solution (20 ml, 0.84 mmol) added. The progress of the oxidation was followed by iodimetry[156] and aliquots (1 ml) of the solution were added to a saturated solution of sodium bicarbonate (5 ml), followed by 2.50×10^{-3} M sodium arsenite solution (5 ml, 0.125 mmol) and a 10% aqueous potassium iodide solution (1 ml). The solution was left in the dark for 15 minutes and the excess arsenite was titrated against a standard 1.21×10^{-3} M iodine solution using a starch indicator. After 8 hours 0.78 mmol of sodium metaperiodate had been consumed and the oxidation was considered complete. An excess of 0.01 M sodium arsenite solution (5 ml, 0.05 mmol) was added to the oxidised solution to destroy excess periodate.

Steam was passed through the oxidised solution and the distillate collected in a boiling tube containing freshly prepared ethanolic 2,4-dinitrophenylhydrazine solution (5 ml)[167]. The solution turned a turbid yellow colour but no precipitate formed. The reaction was repeated and the distillate collected in aqueous 2,4-dinitrophenylhydrazine solution (5 ml)[156] with similar results.

Continuous ether extraction (3 hours) of the oxidised solution after steam distillation yielded a brown syrup (35 mg) which also failed to give a precipitate with ethanolic 2,4-dinitrophenylhydrazine solution.

3:5:2 Reductive ozonolysis of synrotolide

An excess of ozone (0.44 mmol O_3 min^{-1}) was passed through a solution of synrotolide (152 mg, 0.44 mmol) in acetic acid (6 ml) at 10^{o} for 15 minutes. Excess ozone was removed by a stream of nitrogen and the reaction mixture was stirred with dimethyl sulphide (0.4 ml) for 2 hours at room temperature. The solvents were removed under reduced pressure to afford a pale yellow syrup.

The ozonolysis products were separated by descending paper chromatography on sheets of paper (Whatmans No. 1, 23 cm x 57 cm) in a butanol - ethanol - water (4:1:1) solution for 15 hours. The paper chromatograms were visualised by sequential immersion in firstly a solution of saturated aqueous silver nitrate (2.5 ml) in acetone (500 ml), secondly in a solution of 40% aqueous sodium hydroxide (25 ml) in ethanol (500 ml), and finally in a solution of sodium acetate (25 g), sodium thiosulphate (25 g) and acetic acid (1 ml) in water (500 ml)[174]. The two major ozonolysis products appeared as intense brown spots (R_F 0.78 and R_F 0.63). Visualisation of the ozonolysis products by spraying the paper chromatogram with a freshly prepared mixture of equal volumes of 1% vanillin in ethanol and 3% aqueous sulphuric acid followed by heating in an oven at 80^{o} for 10 minutes[175], gave a single yellow spot (R_F 0.78). A paper chromatogram of L-rhamnose eluted in the same solvent system and visualised with this technique gave a red spot (R_F 0.34) which faded to yellow on cooling.

An improved separation of the two ozonolysis products was achieved by elution with an ethyl acetate - acetic acid - formic acid - water (18:3:1:4) solution for 5 hours. Visualisation of the chromatogram by the former technique showed two spots (R_F 0.88 and R_F 0.45). Accordingly this solvent system was used to separate the ozonolysis products by descending

preparative paper chromatography on two large sheets of paper (Whatmans No 1, 46 cm x 57 cm). A solution of the ozonolysis products in ethyl acetate (1 ml) was applied to the papers with a fine glass capillary and the papers developed for 4.5 hours. The bands corresponding to the ozonolysis products were identified by removing two strips (2 cm x 57 cm) from each chromatogram and visualising these strips using the former technique.

The section of each chromatogram containing the fast moving compound (R_F 0.88) was separated, cut up into small pieces (approx. 2 cm x 1 cm), combined, and extracted with water (120 ml) for 24 hours. The aqueous solution was filtered through a scintered glass filter (porosity 4) and freeze dried ($-50^{o}/10\ \mu$) to yield a light brown solid (122 mg) (**105**).

3:5:3 GC identification of 6-deoxyallose

A solution of the light brown solid (**105**) (60 mg) in aqueous 1 M trifluoroacetic acid (2 ml) was refluxed for 2 hours. Evaporation of the solution under reduced pressure gave the hydrolysed product (51 mg) (**106**).

A mixture of **106** (3 mg), water (0.2 ml) and 0.4 ml of a solution of hydroxylamine hydrogen chloride (0.25 g) in N-methyl imidazole (10 ml) was heated in a stoppered flask at 80^{o} for 10 minutes[181]. The solution was cooled to 0^{o} and acetic anhydride was added in small portions (5 x 0.2 ml). The solution was stirred at room temperature for 15 minutes and chloroform (5 ml) added. The chloroform solution was washed with water (2 x 3 ml),

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* Professor Tamm of the Institute fur Organische Chemie, der Universitat Basel is thanked for a sample (20 mg) of 6-deoxy-D-allose from the collection of Professor Reichstein[170].

10% aqueous sulphuric acid (2 x 3 ml), saturated aqueous sodium bicarbonate solution (2 x 3 ml) and water (2 x 5 ml). The chloroform layer was dried over anhydrous sodium sulphate, filtered, the solvent removed under reduced pressure and the peracetylated aldononitrile[181] (**107**) residue redissolved in chloroform (50 µl). The same derivative of a standard 6-deoxy-D-allose* (1.7 mg) was similarly prepared.

GC analysis of both peracetylated aldonitrile derivatives was performed on a DB 225 capillary column (oven temperature = 225°, inlet and detector temperature = 250°, helium carrier = 1.5 ml min^{-1}, split ratio = 62.5 : 1, injection volume 1 µl). Both derivatives gave a single peak, at 6.23 minutes for the 6-deoxy-D-allose derivative and at 6.21 minutes for the 6-deoxy-allose derivative prepared from the ozonolysis product of synrotolide.

3:5:4 Preparation of 6-deoxy-D-allose phenylosazone

A mixture of standard 6-deoxy-D-allose (8 mg), saturated aqueous sodium metabisulphite (0.1 ml) and 0.2 ml of a solution of phenylhydrazine (400 mg) in acetic acid (2 ml), and water (1 ml) was heated vigorously on a steam bath for 15 minutes. Clusters of fine yellow needles (6 mg) crystallised from the hot solution and were filtered off, washed with water and dried. $[\alpha]_D^{24}$ = -80° (c, 0.056 in 3:2 pyridine-ethanol) lit. $[\alpha]_D^{19}$ = -68° (c, 0.862 in 3:2 ethanol-pyridine)[170] and $[\alpha]_D^{25}$ = -79° (in 3:2 pyridine-ethanol)[169].

A mixture of the crude 6-deoxyallose (**106**) (24 mg) derived from synrotolide, saturated aqueous sodium metabisulphite (0.1 ml) and phenylhydrazine solution (0.8 ml) in water was heated vigorously for 15 minutes. No crystalline product formed immediately and the solution was

left in the referigerator overnight. A yellow brown precipitate (4 mg) was filtered, washed with water and dried. Attempts to recrystallise this precipitate were unsuccessful.

3:5:5 The absolute stereochemistry of the 6-deoxyallose (**106**) derived from synrotolide

A solution of **106** (3 mg) and trifluoroacetic acid (0.1 ml) in (-)-2-octanol (0.5 ml) was heated in a sealed ampoule at 130° for 17 hours. The solvents were removed in vacuo (130°/0.1 mm) and a solution of the residue in acetic anhydride (0.5 ml) and dry pyridine (0.5 ml) refluxed at 100° for 20 minutes. The solution was cooled and added to cold water (5 ml). The aqueous solution was extracted with chloroform (2 x 4 ml) and the chloroform layer washed with water (5 ml), 10% aqueous sulphuric acid (2 x 5 ml), saturated aqueous sodium bicarbonate solution (2 x 5 ml) and water (2 x 5 ml). The chloroform layer was dried over anhydrous sodium sulphate, filtered and the chloroform removed under reduced pressure (55°/20 mm). The residue was redissolved in chloroform (30 µl).

The peracetylated (-)-2-octyl- and (±)-2-octyl glycosides of standard 6-deoxy-D-allose (2 x 3 mg) were similarly prepared. GC analysis[182] of these compounds was carried out on a DB-225 capillary column (oven temperature = 230°, inlet and detector temperatures = 250°, helium carrier = 1.5 ml min^{-1}, split ratio = 62.5:1, injection volume = 1 µl).

The peracetylated (-)-2-octyl glycosides of 6-deoxy-D-allose gave three peaks with R_T 8.78, 9.26 and 9.53 minutes, while GC of the same derivative of **106** gave four peaks with R_T 8.16, 8.35, 9.45 and 10.12 minutes. GC of the peracetylated (±)-2-octyl glycosides of 6-deoxy-D-allose gave seven peaks with R_T 8.16, 8.34, 8.78, 9.24, 9.43, 9.51, and 10.09 minutes.

3:5:6 Oxidative ozonolysis of synrotolide

An excess of ozone (0.54 mmol O_3 min^{-1}) was passed through a solution of synrotolide (103 mg, 0.30 mmol) in 20 ml of acetic acid - formic acid (9:1) at 10^{o} for 6 minutes. The solution was purged with nitrogen after which it was refluxed at 110^{o} for 12 hours while a stream of oxygen was passed through it[194]. Evaporation of the solvent left a dark yellow residue which was stirred with an aqueous 4% potassium hydroxide solution for 30 minutes. The basic solution was then passed through a column of ion exchange resin (Amberlite IR 120, H^{+} form) and the acidic eluate freeze-dried to afford a brown oil (100 mg).

The presence of malic acid in a portion of this oil (20 mg) was confirmed by esterification with excess ethereal diazomethane followed by acetylation with acetic anhydride (1 ml) and dry pyridine (1 ml) at 100^{o} for 30 minutes. The solution was cooled, cold water (15 ml) added and the aqueous solution extracted with chloroform (3 x 5 ml). The combined chloroform extracts were washed with an aqueous 10% sulphuric acid solution (2 x 10 ml), water (2 x 10 ml), aqueous saturated sodium bicarbonate solution (2 x 10 ml) and water (3 x 10 ml), dried over anhydrous sodium sulphate and the chloroform removed by evaporation under a stream of compressed air. The pale yellow residue was redissolved in chloroform (160 µl). The same derivative of L-malic acid was similarly prepared and both products compared by GC analysis on a DB 225 capillary column (oven temperature = 130^{o}, inlet and detector temperatures = 250^{o}, helium carrier = 1.5 ml min^{-1}, split ratio = 60:1, injection volume = 0.5 µl). A retention time of 11.23 minutes was obtained for both compounds.

3:5:7 Preparation and GC of the acetylated (+)-2-dibutyl esters of DL- and L-malic acid[188]

Dry hydrochloric acid gas, prepared from the dropwise addition of concentrated hydrochloric acid to concentrated sulphuric acid, was passed through (+)-2-butanol (3 ml) for several minutes. Acidified (+)-2-butanol (1 ml) was added to L-malic acid (10 mg) in an ampoule, which was sealed and heated at 100^{o} for 2 hours. The (+)-2-butanol was removed under reduced pressure to yield a brown residue. A solution of the residue in dry pyridine (1 ml) and acetic anhydride (1 ml) was refluxed for 30 minutes, cold water (20 ml) added and the aqueous solution extracted with chloroform (2 x 10 ml). The chloroform extracts were combined and washed with an aqueous 10% sulphuric acid solution (2 x 10 ml), water (2 x 10 ml), aqueous saturated sodium bicarbonate solution (2 x 10 ml) and water (2 x 15 ml). The chloroform layer was dried over anhydrous sodium sulphate, filtered and the chloroform removed under reduced pressure (20 mm/50^{o}) to yield a yellow oil which was redissolved in chloroform (180 μl). The acetylated (+)-2-dibutyl esters of DL-malic acid were similarly prepared.

GC analysis on a DB-225 capillary column (oven temperature = 165^{o}, inlet and detector temperatures = 250^{o}, helium carrier = 1.5 ml min^{-1}, split ratio = 60:1, injection volume = 0.3 μl) of the esters of DL-malic acid gave two peaks of equal area with retention times of 17.90 minutes and 18.26 minutes, while GC of the ester of L-malic acid, under the same conditions, gave a large peak at 18.20 minutes and a very small peak at 17.81 minutes (intensity 8% that of the major peak).

3:5:8 The absolute stereochemistry of the malic acid derived from synrotolide

The acetylated (+)-2-dibutyl ester derivatives of the oxidised ozonolysis products of synrotolide (41 mg) were prepared and GC analysis performed using the procedure and GC conditions outlined previously (Section 3:5:7). The derivatives gave a major peak at 17.90 minutes and the identity of this peak was confirmed using GCMS (oven temperature = 160^{o}, inlet temperature = 250^{o}, helium carrier approx. 2 ml min^{-1}). The increased carrier gas flow rate in the GCMS system necessitated lowering of the column temperature to maintain optimum separation of the diastereomeric peaks as indicated by the total ion current. Thus the (+)-2-dibutyl ester derivatives of DL-malic acid had retention times of 10.61 and 10.81 minutes. The mass spectrum of the acetylated (+)-2-dibutyl ester of D-malic acid derived from synrotolide had m/z (rel. int.) : No M^{+}, 215(7), 188(4), 177(5), 159(100), 132(33), 131(26), 99(21), 89(44), 71(28), 57(30).

3:5:9 Attempted isomerisation of the exocyclic double bond of synrotolide

A solution of synrotolide (12 mg) in acetone (2 ml) was exposed to direct sunlight for five days. The acetone was removed under reduced pressure and the residue recrystallised from ethyl acetate. The colourless prisms (4 mg) were filtered off and dried, m.p. 169-171^{o} and shown (mixed m.p., t.l.c. and IR spectrum) to consist of unchanged synrotolide.

3:6 Extraction of Syncolostemon densifloris

Syncolostemon densifloris was collected on "Grootboom" farm about 40 km north east of East London on 20.5.1984. A specimen was deposited with the Albany Museum, Grahamstown (Voucher No. A7340) and the remaining plant

material was air dried in the shade for 4 weeks. The dried leaves and stems (5.0 kg) were soaked in acetone (130 ℓ) at room temperature for 4 days, the acetone run off and the plant material washed with a further 10 ℓ of acetone. The combined acetone extracts were concentrated by flash distillation to approximately 9 ℓ and the solution divided into two equal volumes. Each volume was stirred with decolourising charcoal (Mcdonald Adams, 120 g and BDH, 50 g) for 12 hours, filtered through a Celite 545 pad, combined and concentrated to 2 ℓ by flash distillation. The remaining acetone was removed on a rotary evaporator and the residue dried in a vacuum desiccator to yield a tan-coloured brittle solid (360 g).

A mixture of the tan-coloured brittle solid (30 g) and coarse sand (40 g) in benzene (100 ml) was refluxed for 1 hour. The mixture was cooled and the benzene removed under reduced pressure. Benzene (50 ml) was added to the mixture which was applied as a slurry to an open column of silica gel 60 (300 g, 70-230 mesh ASTM) in hexane. The column (620 cm^3) was eluted with a hexane-ethyl acetate gradient (6 ℓ) of increasing ethyl acetate concentration. Fractions (165 ml) were collected and combined from t.l.c. results .

The fractions eluted with 3:1, 2:1 and 1:1 hexane-ethyl acetate gave a single pink spot (R_F 0.72) on t.l.c.. This compound was also shown by t.l.c. to be present in the ethyl acetate fractions which also contained at least three other minor compounds. One of the minor compounds (R_F 0.61) gave a bright red fluorescence when the t.l.c. plate was exposed to ultra violet light.

3:6:1 Acetylation of crude oleanolic acid

The 3:1, 2:1 and 1:1 hexane - ethyl acetate fractions were combined to

yield an off-white solid (6.6 g) which represents an overall 1.6% yield of crude oleanolic acid from dried plant material. A solution of this solid (50 mg) in acetic anhydride (1.5 ml) and pyridine (1 ml) was stirred at room temperature overnight. The solvents were removed under reduced pressure and the residue taken up in ethanol (1 ml). Fine needles formed (12 mg, 22%), m.p. 252-255^{o}, which were filtered off, dried and shown to be identical with oleanolic acid acetate obtained from Syncolostemon rotundifolius (mixed m.p., t.l.c. and IR spectrum).

3:6:2 Isolation of quercetin 3, 3', 4', 7 tetramethyl ether (117)

The ethyl acetate fractions were combined and the solvent removed under reduced pressure to yield a brown gum (14.4 g). A portion of this gum (7.0 g) was rechromatographed through silica gel 60 (80 g, 230-400 mesh ASTM) in hexane. The column (262 cm^3) was eluted with 2:1 hexane-ethyl acetate (400 ml), 1:1 hexane-ethyl acetate (400 ml) and ethyl acetate (500 ml). Fractions (110 ml) were collected and t.l.c. of the sixth fraction showed that it contained predominantly one compound (R_F 0.61) with fluoresced under U.V. light. The solvent from this fraction was removed under reduced pressure to yield a yellow oil (2.6 g) which resisted crystallization. An aqueous solution of ferric chloride (1 ml) was added to this oil (0.05 g) in a small test tube and the solution turned a dark green colour.

A mixture of the yellow oil (766 mg), finely divided potassium carbonate (400 mg), methyl iodide (4.5 ml) and dry acetone (20 ml) was refluxed for 5 hours. The solvents were removed under reduced pressure and the residue extracted with 3:1 benzene-ether (100 ml). The organic phase was washed with 0.1 M hydrochloric acid (4 x 50 ml), water (4 x 100 ml), dried over anhydrous calcium chloride and filtered. The solvents were removed

under reduced pressure to give a pale yellow oil (694 mg) which was crystallised from methanol (5 ml) to give fine yellow needles (69 mg), m.p. 152-153.5°, of quercetin 3, 3', 4', 7 tetramethyl ether (117). The melting point was raised on recrystallisation from ethanol (10 ml) to 154-156°. (lit. m.p. 159-160°)[147].

IR spectrum ν_{max} cm^{-1}	:	3400, 2900, 1638, 1580, 1536, 1420, 1305, 1140, 810
^1H NMR spectrum (300 MHz, $CDCl_3$)	:	δ 3.96, 3.95, 3.86, 3.83 (12H, s, 4 x OMe), 6.32 (1H, d, J = 2Hz, H-6), 6.42 (1H, d, J = 2Hz, H-8), 6.98 (1H, d, J = 9Hz, H-5'), 7,67 (1H, d, J = 2Hz, H-2'), 7.71 (1H, dd, J = 2Hz and 9Hz, H-6'), 12.65 (1H, s, OH)
Mass spectrum m/z (rel. int.)	:	358 (100), 343 (86), 315 (84), 285 (29), 231 (32), 165 (56), 149 (34), 122 (28), 79 (30), 68 (33). M^+ calculated for $C_{19}H_{18}O_7$ = 358.
Analysis	:	A sample was dried (P_2O_5) at 80°/0.1 mm for 2 hours
Found	:	C = 62.96 H = 5.08%
$C_{19}H_{18}O_7$ required	:	C = 63.68 H = 5.06%

3:7 Extraction of Tetradenia barberae

A batch of plant material was collected on the border of Selborne farm and the Andries Vosloo Kudu Reserve in the Fish River valley near Grahamstown on 21.3.1986. A specimen was deposited with the Albany Museum, Grahamstown (Voucher No. A7335) and the remaining plant material was air

dried in the shade for 2 weeks. The dried leaves (577 g) were soaked in acetone (25 ℓ) at room temperature for 4 days, the acetone run off and the plant material washed with a further 5 ℓ of acetone. The combined acetone extracts were concentrated to 4 ℓ by flash distillation and the solution stirred with decolourising charcoal (BDH, 120 g) for 4 hours. The solution was filtered through a Celite 545 pad and concentrated to 1 ℓ by flash distillation. The remaining acetone was removed in vacuo and the dark brown residue (49 g) was dried in a vacuum desiccator.

3:7:1 Isolation of boronolide (3)

A mixture of the dark brown residue (16 g), silica gel (16 g, 70-230 mesh ASTM), coarse sand (16 g) was refluxed in ethyl acetate (50 ml) for a few minutes. The ethyl acetate was removed under reduced pressure and the mixture slurried in benzene (20 ml) and applied to an open column of silica gel (220 g, 70-230 mesh ASTM) in hexane. The column (470 cm^3) was eluted with a litre of each of the following hexane-ethyl acetate solutions: 20:1, 10:1, 8:1, 6:1, 4:1, 3:1, 2:1, 1:1 and finally with a litre of ethyl acetate. Fractions (110 ml) were collected and combined from t.l.c. results.

The fractions eluted with 1:1 hexane-ethyl acetate gave a single orange spot (R_F 0.78) and were combined and the solvent removed in vacuo to yield a brown residue (2.2 g), which was refluxed with decolourising charcoal (BDH, 0.5 g) in ethanol (10 ml), filtered through a Celite 545 pad and the ethanol removed in vacuo to yield a yellow crystalline solid (2 g). Recrystallisation of the solid from ethanol (8 ml) gave white needles (1.2 g) of boronolide (3), m.p. 89-90°, (lit. 90°)[11] unchanged in m.p. on further recrystallisation from hexane-benzene (5:1). $[\alpha]_D^{26}$ = +28° (c, 0.08 in ethanol), (lit. $[\alpha]_D$ = +25°). The mass of boronolide (1.2 g) isolated from

the gum (16 g) represents an overall yield of 0.7% from dried plant material.

UV spectrum	:	λ_{max} = 205 nm (log ε = 3.92)
CD spectrum (in methanol)	:	$\Delta\varepsilon$ = 2.48 at 256 nm
IR spectrum ν_{max} cm^{-1}	:	1730, 1710, 1365, 1220, 1020, 952
Mass spectrum m/z (rel. int.)	:	371 (2), 311 (3), 273 (23), 242 (47), 201 (36), 18 (47), 172 (28), 171 (39), 159 (36), 140 (55), 121 (47), 110 (42), 97 (46), 95 (40), 68 (42), 43 (100). M^+ calculated for $C_{18}H_{26}O_8$ = 370
^1H NMR spectrum	:	Table 5 (Section 2:3)
^{13}C NMR spectrum	:	Table 6 (Section 2:3)
Analysis	:	A sample was dried (P_2O_5) 25°/0.1 mm for 20 hours
Found	:	C = 58.01 H = 7.22%
$C_{18}H_{26}O_8$ required	:	C = 58.37 H = 7.08%

3:7:2 Saponification of boronolide

A solution of boronolide (480 mg) and aqueous 0.5 M potassium hydroxide (30 ml) was stirred for 4 hours. The solution was acidified and continuously extracted with ether for 6 hours. The ether was removed under reduced pressure to yield a pale yellow oil which gave a single spot on t.l.c. (R_F 0.39). This oil resisted crystallisation and was passed through a column of neutral alumina (10 g) in benzene. The fractions

eluted with ethyl acetate (50 ml) and methanol (80 ml) were combined and the solvent removed in vacuo to yield a colourless oil which crystallised from benzene-hexane (1:1) to afford small white needles (162 mg, 51%) of saponified boronolide (4), m.p. 93-94°. The m.p. was raised on recrystallisation from hexane-benzene (2:1) to 99-100°, $[\alpha]_D^{22}$ = +56° (c, 0.07 in ethanol) (lit. m.p. 99-100°, $[\alpha]_D$ = +48°)[11].

IR spectrum ν_{max} cm^{-1}	:	3240, 1695, 1440, 1244, 1062, 1015, 970
^{13}C NMR	:	Table 6 (Section 2:3)

3:7:3 Periodate oxidation and ozonolysis of saponified boronolide (4)

A solution of saponified boronolide (150 mg, 0.615 mmol) in water (32 ml) and aqueous 0.041 sodium metaperiodate solution (32 ml, 1.31 mmol) was stirred for 16 hours. The progress of the oxidation was followed by iodometry[156] and after 16 hours 1.16 mmol of sodium metaperiodate had been consumed, equivalent to 1.9 moles of sodium metaperiodate per mole of 4.

The solution was freeze-dried and the pale yellow residue extracted with ethyl acetate (3 x 100 ml). The ethyl acetate was removed in vacuo and the yellow oil (92 mg) was dissolved in 20 ml of acetic acid - formic acid (9:1). An excess of ozone (0.53 mmol O_3 min^{-1}) was passed through this solution at 10° for 3 minutes. The solution was purged with nitrogen after which it was refluxed at 110° for 12 hours while a stream of oxygen was passed through it. Evaporation of the solvent left a dark yellow residue which was stirred with an aqueous 4% potassium hydroxide solution for 30 minutes. The basic solution was then passed through a column of ion exchange resin (Amberlite IR 120, H^+ form) and the acidic eluate freeze-dried to afford a brown oil (70 mg).

3:7:4 The absolute stereochemistry of the malic acid derived from boronolide

The acetylated (+)-2-dibutyl ester derivatives of the oxidised ozonolysis products of boronolide (40 mg) were prepared and GC analysis performed using the procedure and GC conditions outlined previously (Section 3:5:7). The derivatives gave a major peak with a retention time of 17.85 minutes and a minor peak (R_T 18.10 minutes). The intensity of the minor peak was 30% that of the major peak. The identities of these peaks were confirmed using GCMS (Section 3:5:8). The mass spectrum of the acetylated (+)-2- dibutyl ester of D-malic acid derived from boronolide had m/z (rel. int.) : 215 (6), 188 (4), 177 (5), 159 (100), 132 (32), 131 (23), 99 (19), 89 (48), 71 (33), 57 (33).

3:8 Extraction of Leonotis nepetaefolia

Plant material was collected in the Moremi Game Reserve in Botswana on 9.4.1985 and a specimen identified by Dr Jacot-Guillarmod of the Albany Museum, Grahamstown. The leaves and stems were air dried in the shade for 4 weeks. The leaves (880 g) were soaked in acetone (40 ℓ) at room temperature for 4 days, the acetone run off and the plant material washed with a further 3 ℓ of acetone. The combined acetone extracts were concentrated to 4 ℓ by flash distillation and the solution stirred with decolourizing charcoal (BDH, 80 g) for 3 hours. The solution was filtered through a Celite 545 pad and concentrated to 800 ml by flash distillation. The remaining acetone was removed under reduced pressure and the dark brown residue (44 g) was dried in a vacuum desiccator.

3:8:1 Isolation of nepetaefuran (120)

A mixture of the dark brown residue (40 g), silica gel (40 g, 70-230

mesh ASTM) and benzene (200 ml) was warmed on a steam bath. The benzene was removed under reduced pressure using a rotary evaporator and the mixture slurried with benzene (50 ml) and applied to an open column of silica gel (350 g, 70-230 mesh ASTM, deactivated with 15% water) in benzene. The column (770 cm^3) was eluted with benzene (3.5 ℓ), 2:1 benzene-ethyl acetate (3.5 ℓ) and ethyl acetate (3 ℓ). T.l.c. of the 2:1 benzene - ethyl acetate fractions showed that these fractions contained a major compound (R_F 0.67) and a minor compound (R_F 0.74). Both these compounds gave bright pink spots when the t.l.c. plate was visualised by spraying with Ehrlrich reagent (2% ethanolic solution of p-amino-benzaldehyde) followed by development in concentrated hydrochloric acid vapour.

The 2:1 benzene-ethyl acetate fractions were combined and the solvent removed under reduced pressure to yield a brown gum (12 g) which was re-chromatographed on an open column of silica gel (250 g, 230-400 mesh ASTM, deactivated with 15% water) in benzene. The major compound (R_F 0.67) was eluted with 1:1 benzene-ethyl acetate. The fractions containing this compound were combined and the solvent removed to yield a yellow semi-crystalline oil (3 g) which crystallised from benzene to give colourless prisms (0.74 g), m.p. 215-218°, of nepetaefuran (**120**). The melting point was raised on recrystallisation from ethanol to 228-230°, $[\alpha]_D^{25}$ = +31.9° (c, 1.1 in methanol) (lit: m.p. 241-242°, $[\alpha]_D$ = +32.3°)[200].

IR spectrum ν_{max} cm^{-1}	:	3500, 2925, 1730, 1380, 1240, 1145, 1030.
^1H NMR spectrum (60 MHz, DMSO-d_6)	:	δ 0.95 (3H, s), 1.78 (3H, s) 1.1-2.6 (m), 2.76 (1H, d, J = 4), 4.04 (1H, d, 12 Hz),

4.76 (1H, s), 5.01 (2H, m), 6.34 (1H, m), 7.44 (1H, s), 7.51 (1H , t).

Mass spectrum m/z (rel. int.) : 404 (0.1), 231 (5), 185 (4), 172 (7), 159 (6), 105 (10), 95 (23), 81 (76), 67 (14), 53 (19), 43 (100).

M^+ calculated for $C_{22}H_{28}O_7$ = 404

Analysis : A sample was dried (P_2O_5) 80°/0.1 mm for 5 hours

Found : C = 63.85 H = 7.74%

$C_{22}H_{28}O_7$ required : C = 65.33 H = 6.98%

3:8:2 Saponification of nepetaefuran

A solution of nepetaefuran (129 mg) in ethanol (5 ml) was stirred with 10% ethanolic potassium hydroxide solution at room temperature for 40 hours. The solution was diluted with water (50 ml), acidified with cold 5% hydrochloric acid and extracted with ethyl acetate (2 x 75 ml). The extract was washed with water (2 x 100 ml), dried over anhydrous magnesium sulphate and filtered. The ethyl acetate was removed under reduced pressure to yield a crystalline residue (81 mg, 70%) which was recrystallised from ethyl acetate-hexane to give needles of saponified nepetaefuran (121), m.p. 193-194°, $[\alpha]_D^{23}$ = +25.4° (c, 0.04 in methanol) (lit. m.p. 196-198° $[\alpha]_D^{25}$ = +29.6°).

IR spectrum ν_{max} cm^{-1} : 3460, 3350, 1690, 1678, 1450, 1360, 1205, 1160, 1030

3:8:3 Isolation of nepetaefuran by medium pressure chromatography

T.l.c. of the ethyl acetate fractions from the initial crude column

chromatography of the plant extract showed the presence of three compounds (R_F 0.67, 0.54 and 0.44) which gave a positive Ehrlrich test. The major compound nepetaefuran (R_F 0.67) was isolated by medium pressure chromatography from these fractions. The fractions were combined and the solvent removed under reduced pressure to yield a dark brown gum (6 g) which was dissolved in warm ethyl acetate (10 ml). The ethyl acetate solution was applied to a medium pressure column of silica gel (269 g, 230-400 mesh ASTM) in hexane. The column (490 ml) was eluted with 2:1 ethyl acetate-hexane (1.5 ℓ) at a flow rate of approximately 10 ml min^{-1}. Fractions (95 x 20 ml) were collected and every third fraction examined by t.l.c.. Fractions 25-44 were combined and the solvent removed to yield a yellow oil (1.15 g) which crystallised from ethanol to give small prisms (130 mg), m.p. 216-218^{o}. The melting point was raised on recrystallisation from ethanol to 224-226^{o} and this compound was identical with product previously obtained (Section 3:8:3) from its t.l.c., mixed melting point and IR spectrum. The total mass of nepetaefuran isolated (0.87 g) from the extracted residue (40 g) represents an overall yield of 0.1% from dried plant material.

The combined fractions 45-52 (1.2 g) contained a single compound (R_F 0.54) which resisted crystallisation. This compound was also present in fractions 52-73 along with another furanoid compound (R_F 0.44). Attempts to separate these compounds by further chromatography were unsuccessful.

3:9 Extraction of *Ballota africana*

Plant material was collected at Hilton farm near Grahamstown on 7.11.1986 and a specimen deposited with the Albany Museum, Grahamstown (Voucher No. A 7370). The plant material was air dried in the shade for 7

weeks and the dried leaves and stems (6.2 kg) separated into two batches (2 x 3.1 kg). The same extraction procedure was carried out on each batch and they were both soaked in acetone (110 ℓ) at room temperature for 5 days. The acetone was run off, concentrated by flash distillation and the concentrated extract (2 x 5 ℓ) stirred with decolourising charcoal (BDH, 2 x 50 g) for 4 hours. The solutions were filtered through a Celite 545 pad, combined and concentrated again by flash distillation. The remaining acetone was finally removed in a rotary evaporator and the dark brown gum (279 g) dried in a vacuum desiccator.

3:9:1 Isolation of hispanalone (137)

A mixture of the dark brown gum (41 g), silica gel (40 g, 70-230 mesh ASTM), coarse sand (30 g) and ethyl acetate (100 ml) was warmed on a steam bath. The ethyl acetate was removed under reduced pressure in a rotary evaporator and residual water was removed azeotropically with benzene (2 x 100 ml). The mixture was slurried in hexane and applied to an open column of silica gel (350 g, 70-230 mesh ASTM) in hexane. The column (830 cm^3) was eluted with a litre each of the following solvent mixtures: hexane, 10:1, 8:1, 6:1, 4:1, 3:1, 2:1 hexane-ethyl acetate and ethylacetate. Fractions (110 ml) were collected. Evaporation of the solvent from the combined fractions eluted with hexane-ethyl acetate (6:1) afforded a crystalline solid (15.8 g). The solid was recrystallised from ethyl acetate (150 ml) to give colourless plates (3.4 g) of hispanolone (137) m.p. 140-143°. The mother liquors were concentrated and the orange oil (12.3 g) crystallised twice from ethyl acetate-hexane (3:4) to yield a further crop of colourless plates (3.8 g) m.p. 142-144°. Therefore the total yield of 137 (7.2 g) from the gum (41 g) represents an overall 0.8% yield from the dried plant material. The melting point was raised on recrystallisation from benzene-

hexane (3:4) to 146-148°, $[\alpha]_D^{23}$ = -18.7° (c, 1.2 in chloroform) (lit. m.p. 142-144°, $[\alpha]_D$ + -17.6°)[213]. Hispanalone was identical with dihydro-compound Y (m.p., mixed m.p., t.l.c. and IR spectrum)[219].

IR spectrum ν_{max} cm^{-1}	:	3500, 2920, 1694, 1465, 1370, 1155, 1110, 1018, 870
UV spectrum	:	λ_{max} = 208 nm (log ε = 3,88)
CD spectrum (in methanol)	:	Δε = -0.98 at 287 nm
^1H NMR spectrum (300 MHz, $CDCl_3$)	:	δ 0.85 (3H, s, C-18 methyl), 0.88 (3H, s, C-19 methyl), 1.09 (3H, d, $J_{8,17}$ = 6.5 Hz, C-17 methyl), 1.16 (3H, s, C-20 methyl), 1.19 (1H, m, H-3_a), 1.41 (1H, m, H-3_b), 1.47 (1H, m, H-1_a), 1.55 (1H, m, H-1_b), 1.59 (1H, m, H-2_a), 1.64 (1H, m, H-2_b), 1.89 (2H, m, H_2-11), 2.00 (1H, m, H-5), 2.29 (1H, m, H-6_a), 2.39 (1H, m, H-6_b), 2.47 (2H, m, H_2-12), 2.73 (1H, q, $J_{17,8}$ = 6.5 Hz, H-8), 6.35 (1H, m, H-14), 7.21 (1H, m, H-16), 7.33 (1H, t, J = 2Hz, H-15)
^{13}C NMR spectrum	:	Table 7 (Section 2:5)
Mass spectrum m/z (rel. int.)	:	318 (4), 236 (5), 194 (25), 176 (9), 152 (7), 123 (54), 122 (30), 109 (87), 95 (53), 81 (100), 67 (28), 53 (23). M^+ calculated for $C_{20}H_{30}O_3$ = 318
Analysis	:	A sample was dried (P_2O_5) 100°/0.1 mm for 2 hours

Found : C = 75.84 H = 9.85%

$C_{20}H_{30}O_3$ required : C = 75.43 H = 9.50%

3:9:2 Attempted acetylation of hispanalone

A solution of hispanolone (277 mg), 4-dimethylaminopyridine (3 mg) in acetic anhydride (3 ml) and dry pyridine (3 ml) was left at room temperature for 12 hours. The solvents were removed in vacuo and the residue crystallised from ethyl acetate-hexane (3:5) to afford unchanged hispanolone (166 mg, 60%), m.p. 139-141°, mixed m.p. 142-143°.

3:9:3 Hydrogenation of hispanolone

A mixture of hispanalone (516 mg) and Paal catalyst (995 mg)[220] was shaken in ethanol (100 ml) under hydrogen for 5 hours during which time 89.3 ml of hydrogen (equivalent to 1.94 moles per mole of hispanolone) were absorbed. The solution, after filtration through a Celite 545 pad, was shown by t.l.c. to contain a single compound (R_F 0.5) which gave a negative Ehrlrich test. The solvent was removed under reduced pressure to yield a semi crystalline oil which crystallised from benzene-hexane (1:20) to afford colourless needles of tetrahydrohispanalone (143) (352 mg, 56%) m.p. 104-106°. The melting point was raised on recrystallisation from benzene-hexane to 107-109°, $[\alpha]_D^{24}$ = -20.3° (c, 1.1 in chloroform) (lit. hexahydro-compound Y, m.p. 105°, $[\alpha]_D$ = -17°)[219].

IR spectrum : 3420, 2940, 1680, 1410, 1385, 1100,
ν_{max} cm^{-1} 970

Mass spectrum : 322 (0.2), 204 (1.1), 199 (14.8), 180
m/z (rel. int.) (11.0), 127 (30.5), 109 (100), 83 (37).
M^+ calculated for $C_{20}H_{34}O_3$ = 322

3:10 Extraction of Leonotis ocymifolia var. ocymifolia

A batch of plant material was collected 4 km west of Port Alfred on 18.9.1985 and identified by Dr. Jacot-Guillarmod of the Albany Museum Herbarium. Acetone extraction of the plant material as described[230] and neutral alumina chromatography of the extract yielded colourless prisms of dubiin (**160**) (14.0 g), m.p. 183-184°, which represents an overall yield of 1.6% from the dried plant material. The melting point of this product was not raised on recrystallisation from methanol-water and it was found to be identical (mixed m.p., t.l.c. and IR spectrum) with a sample of dubiin isolated previously from Leonotis dubia, $[\alpha]_D^{23}$ = -18.1° (c, 1.16 in chloroform) (lit. m.p. 187-188° $[\alpha]_D^{21}$ = -17°)[230].

IR spectrum ν_{max} cm^{-1}	:	3480, 1737, 1703, 1496, 869
Mass spectrum m/z (rel.int.)	:	390(2), 330(5), 235(9), 188(12), 96(34), 95(55), 81(100), 43(88)
		M^+ calculated for $C_{22}H_{30}O_6$ = 390

3:10:1 Saponification of dubiin (**160**)

Saponification of dubiin as described[230] gave crude **161**, m.p. 171-173° (98%) which on recrystallisation from ethanol had m.p. 184-186°, $[\alpha]_D^{23}$ = +11.6° (c, 1.0 in chloroform) (lit. m.p. 185-186°, $[\alpha]_D^{21}$ = +13°)[230].

IR spectrum ν_{max} cm^{-1}	:	3570, 1702, 1698, 1497, 871
CD spectrum (in methanol)	:	No Cotton effect

Mass spectrum : 348(3), 253(7), 194(6), 177(12), 134(10)
m/z (rel. int.) 121(19), 109(19), 95(46), 81(100).
M^+ calculated for $C_{20}H_{28}O_5$ = 348

3:10:2 Attempted preparation of the p-bromobenzoate derivative of saponified dubiin (161)

A solution of saponified dubiin (161) (9 mg), 4-dimethylaminopyridine (0.3 mg), 4-bromobenzoyl chloride (60 mg) in dry pyridine (2 ml) was refluxed for 24 hours. The reaction was monitored with t.l.c. and after 24 hours only starting material remained. The experiment was discarded.

3.11 Attempted X-ray structure determination of dubiin

3.11.1 Collection of intensity data

Large plates of dubiin were crystallised by the vapour diffusion method[233] from benzene and hexane. The plates were washed with benzene-hexane (2:1), dried in a vacuum desiccator and carefully fragmented to yield smaller plates with well defined edges suitable for X-ray diffraction.

Preliminary investigations of the crystal lattice (de Jong-Boumann and Buerger Precession photographs) indicated that the crystal was orthorhombic with space group $P2_12_12_1$. The experimentally determined crystal density by flotation in hexane-carbon tetrachloride, was consistent with the density calculated from the preliminary unit cell dimensions and molecular mass of dubiin.

The diffraction intensities of a single crystal (0.01 x 0.5 x 0.5 mm) were measured on an Enraf Nonius CAD-4F automated four circle diffractometer with Mo-K_α radiation (λ = 0.7107 Å). A set of 18

reflections in the range $11 < \theta < 12^{o}$ were used for the least squares refinement of the unit cell parameters. The intensity data were collected by the ω-2θ scan technique with an intensity control applied every hour and an orientation control every 200 reflections. A loss in intensity of 2.1% (possibly due to crystal sublimation) and frequent recalculations of the orientation matrix were observed which were partly attributed to the slow scan speed and long exposure time (73 hours). A total of 2132 reflections were collected in the range $1 < \theta < 25^{o}$ of which 1289 reflections with $|F_o| \geq \sigma|F_o|$ were considered observed. The data were corrected for Lorentz and polarization effects, but not for absorption.

The crystal data are recorded in Table 10 (Appendix 1:).

3.11.2 Structure solution and partial refinement

The data set was poor with very few strong reflections (only 698 reflections had $I > \sigma I$) and the direct methods program MULTAN[162] was unable to solve the phase problem. However, a partial solution was obtained with the direct methods program SHELXS[232] which generated a model that contained the three 6-membered rings of dubiin and the substituents at C-4, C-6, C-8 and C-9. The furan ring was not located in the difference Fourier map based on this model and refinement on F was carried out by least squares analysis using the SHELX[163] program. Four cycles of isotropic refinement gave an agreement value R of 0.295 which suggested that the model was correct. The furan ring emerged from the difference Fourier synthesis and after a further 3 cycles of isotropic refinement the R value was reduced to 0.219. At this point hydrogen atoms were included, at calculated positions (excepting the OH hydrogen atom, as yet unlocated) and allowed to 'ride' on their respective C atoms[163] with common isotropic temperature

factors. Oxygen atoms were refined with anisotropic temperature factors. Further refinement yielded final R and R_w values of 0.168 and 0.177 respectively for 983 reflections with $|F_o| > \sigma|F_o|$ and 156 variables. A weighting function[163] $\omega = k\,(\sigma^2 F + gF^2)^{-1}$ was used and the values of k and g were optimised after each refinement to give final values of 0.4736 and 0.05693 respectively.

An additional computer program used was PLUTO[165] and the final atomic co-ordinates, thermal parameters and intramolecular bond lengths and angles of the non-hydrogen atoms are given in Tables 16-19 (Appendix 1). Observed and calculated structure factors are available from the Department of Chemistry and Biochemistry, Rhodes University, Grahamstown.

3.12 X-ray crystallography of saponified dubiin

3:12:1 Collection of intensity data

Large prisms of saponified dubiin were crystallised by the vapour diffusion[233] method from acetone and pentane. The prisms were washed with acetone-pentane (1:4) and dried in a vacuum desiccator.

De Jong-Boumann and Buerger Precession photographs were taken of a single crystal and the crystal class (orthorhombic) and space group ($P2_12_12_1$) was established. The crystal density was determined experimentally by the flotation method (hexane-carbon tetrachloride) and was consistent with the density calculated from the molecular mass of saponified dubiin and the preliminary cell dimensions.

A single prismatic crystal (0.35 x 0.43 x 0.50 mm) was used for the collection of diffraction data on an Enraf Nonius CAD-4F automated four circle diffractometer with Mo-Kα radiation (λ = 0.7107 Å). A set of 24 reflections in the range ($16^o < \theta < 17^o$) were used for the least squares

refinement of the unit cell parameters. Intensity data were collected by the ω-2θ scan technique, θ_{max} = 25°; h= 0-10, k = 0-13 and l= 0-21. An intensity control was applied every hour and an orientation control every 200 reflections to monitor crystal and instrumental stabilities. No crystal instability was noted and a semi-empirical absorption correction[241] and Lorentz and polarization corrections were applied to the 1793 reflections collected of which 1663 with $|F_o| > \sigma |F_o|$ were considered observed.

The crystal data are recorded in Table 10 (Appendix 1).

3:12:2 Structure solution and refinement

The direct methods program SHELXS[232] was used to solve the structure and the 20 carbon and 5 oxygen atoms of the saponified dubiin molecule could be assigned to major atom peaks in the E-map generated by this program. The refinement on F was carried out by least squares analysis using the SHELX[163] program. Three cycles of isotropic refinement gave an agreement value R of 0.129 for this model. The non-hydrogen atom positional parameters and temperature factors were refined anisotropically except for the C-17 and C-18 methyl groups which were entered as rigid structures and refined isotropically. The two OH hydrogen atom positions were located from the difference map while all the other hydrogen atoms were entered in calculated positions with common isotropic temperature factors, and were allowed to 'ride' on their respective carbon atoms[163]. The trial structure was finally divided into two blocks comprising the furan ring plus the C-11, C-12 atoms in one block and the remainder of the molecule in the other block. After 6 cycles of blocked full matrix least squares refinement[163] the R value was reduced to 0.050. The constraints placed on the two

methyl groups were removed and the model refined again through a further 6 cycles of blocked full matrix refinement (with all the non-hydrogen atoms refined anisotropically) to give an R value of 0.041. A PLUTO plot revealed that the incorrect enantiomorph had been refined and the refinement was repeated with inversion of the atomic positional parameters in the point (0, 0, 0) and a final R value of 0.041 (unit weights) for 1531 reflections with $|F_o| > 2\sigma|F_o|$ and 241 variables was obtained. Least-squares planes analyses of fragments of the molecule were performed using the program XANADU[166].

The final atomic co-ordinates, thermal parameters and intramolecular bond lengths and angles of the non-hydrogen atoms are given in Tables 20-22 (Appendix 1). Observed and calculated structure factors are available from the Department of Chemistry and Biochemistry, Rhodes University, Grahamstown.

3:13 Collins oxidation[234] of saponified dubiin

Collins oxidation of dubiin as described[230] gave a crude product (162) m.p. 145-149° (72%) which on recrystallisation from ethyl acetate-hexane had m.p. 152-155°, $[\alpha]_D^{25} = -48°$ (c,1.2 in chloroform) (lit. m.p. 157-159°, $[\alpha]_D = -51°$)[230].

IR spectrum ν_{max} cm^{-1}	:	3490, 1710, 1695, 1497, 810
CD spectrum (in methanol)	:	$\Delta\varepsilon$ = +0.69 at 289 nm
^1H NMR spectrum (300 MHz, $CDCl_3$)	:	δ 1.08 (3H, d, J = 6.5 Hz, C-17 methyl), 1.27 (3H, s, C-18 methyl), 1.46 (1H, m, H-1a), 1.55 - 1.84 (4H, m), 1.87 - 2.11

	3H, m), 2.18 (2H, m), 2.35 (3H, m, H-8, 2xH-12), 2.76 (1H, m, H-7a), 3.04 (1H, s, H-5), 4.21 (2H, m, 2H-20), 6.23 (1H, m, H-14), 7.21 (1H, m, H-16), 7.33 (1H, m, H-15),
^{13}C NMR (75.5 MHz, $CDCl_3$)	δ209.9 (C6), 175.0 (C19), 143.2 (C15), 138.5 (C16), 124.1 (C13), 110.4 (C14), 75.5 (C9), 74.6 (C20), 56.1 (C5), 48.9 (C4), 45.8 (C7), 39.8 (C10), 39.5 (C8), 38.1 (C3), 35.1 (C11), 31.9 (C1), 23.4 (C18), 20.2 (C12), 20.1 (C2), 16.0 (C17).
Mass spectrum m/z (rel. int.)	346 (2.9), 193 (41.1), 165 (17.9), 109 (21.1), 96 (14.4), 95 (24.3), 81(100), 79(41.3), 67 (14), 53 (17.6)
	M^+ calculated for $C_{20}H_{26}O_5$ = 346

3:13:1 Oxidation of 6-deacetyldehydrodubiin with chromium trioxide in acetic acid

A solution of chromium trioxide (1.8 g, 18 mmol) in water (6 ml) and acetic acid (20 ml) was added over a period of 5 minutes to a cold solution of 6-deacetyldehydrodubiin (162) (.68 g, 1.9 mmol) in acetic acid (10 ml). The solution was stirred at room temperature for 4 days, water (70 ml) added and the aqueous solution extracted with chloroform (2 x 40 ml). The chloroform extracts were combined and washed with aqueous sodium carbonate (2 x 40 ml), water (2 x 40 ml), dried over anhydrous sodium sulphate, filtered and the chloroform removed under reduced pressure to yield a colourless gel (0.47 g). T.l.c. of a solution of the gel in ethanol did

not give a visible spot with the methanolic sulphuric acid spray reagent. Crystallisation of the gel from ethanol yielded colourless prisms (0.38 g, 65%) of the γδ lactone (**167**) m.p. 249-251°. The m.p. was raised on recrystallisation from the same solvent to 252-253°, $[\alpha]_D^{23}$ = -53° (c, 1.0 in methanol).

IR spectrum ν_{max} cm^{-1}	:	3420, 2920, 1740, 1720, 1702, 1460, 1405, 1220, 1112, 960
CD spectrum (in methanol)	:	$\Delta\varepsilon$ =+0.62 at 288 nm
Mass spectrum m/z (rel. int.)	:	306(17.5), 249(28), 221(9), 178(15), 125(29), 109(38), 108(100), 95(19), 91(42), 79(47), 55(52)
		M^+ calculated for $C_{17}H_{22}O_5$ = 306

3:14 Preparation of 6-dehydromarrubic acid (**168**)

A solution of marrubiin ex Leonotis leonurus[219] (0.4 g, 1.2 mmol) and potassium hydroxide (0.5 g) in water (10 ml) and ethanol (10 ml) was refluxed for 16 hours. The ethanol was removed under reduced pressure and the aqueous solution continuously extracted with ether for 1 hour. This ether extract was discarded and the aqueous solution acidified with sulphuric acid (5N) and continuously extracted again with ether for 2 hours. The ether was removed under reduced pressure to give a pale yellow residue which was crystallised from methanol-water (1:1) to afford fine needles (0.23 g, 55%) m.p. 191-192° (decomp.) of marrubic acid (**169**) (lit. m.p. 198° decomp.)[237].

A solution of marrubic acid (0.23 g) in acetone (20 ml) (distilled over potassium permanganate) was cooled in ice and 8N CrO_3/H_2SO_4 (0.6 ml)

added dropwise from a microburette until an orange colour was obtained which persisted for about 5 minutes. A few drops of ethanol were added to destroy the excess CrO_3, followed by water (20 ml). The solution was extracted with ethyl acetate and washed with water. The solvents were removed under reduced pressure to yield a pale yellow oil which was crystallised from methanol-water to afford colourless needles of 6-dehydromarrubic acid (**168**) (0.15 g, 66%) m.p. 154-155^{o} (lit. m.p. 156-157^{o})[207].

CD spectrum (in methanol) : $\Delta\varepsilon$ = +0.64 at 277 nm

3:14:1 Reduction of marrubiin (129)

A solution of marrubiin ex Leonotis leonurus (1.9 g, 5.7 mmol) and lithium aluminium hydride (1.5 g) in dry tetrahydrofuran (80 ml) was refluxed for 4 hours. The solution was cooled to 0^{o} and ethyl acetate (10 ml) and cold water (25 ml) added. The tetrahydrofuran was removed under reduced pressure whereupon a white precipitate formed. Dilute sulphuric acid was added and the supernatant decantered and extracted with ether (3 x 50 ml). Ether (50 ml) was added to the white precipitate and the solution was refluxed for 20 minutes. The ether extracts were combined, washed with a saturated aqueous sodium bicarbonate solution (2 x 50 ml) and water (3 x 50 ml), and dried over anhydrous sodium sulphate. The solution was filtered and the ether removed under reduced pressure to yield a colourless oil (1.9 g). A solution of this oil in benzene (5 ml) was passed through a short column of neutral alumina (10 g) in benzene. The filtrate which gave one spot on t.l.c. (R_F 0.47) (marrubiin R_F 0.69)

was evaporated and the residue (1.54 g, 80%) was crystallised from benzene-hexane (2:5) to give colourless needles of marrubenol (130) (1.42 g) m.p. 132-134° (lit. m.p. 138°)[209].

13:14:2 Oxidation of marrubenol (130)

A solution of marrubenol (1.12 g, 3.3 mmol) in pyridine was added to a mixture of finely divided chromium trioxide (1.8 g) in pyridine (20 ml) at 0°. The solution was stirred overnight and added to cold 5M hydrochloric acid (60 ml). The acidic solution was extracted with chloroform (4 x 30 ml) and the combined chloroform extracts filtered through a cotton wool plug, washed with 1M hydrochloric acid (3 x 50 ml), saturated aqueous sodium bicarbonate solution (3 x 50 ml) and water (2 x 50 ml). The chloroform layer was dried over anhydrous sodium sulphate, filtered and the chloroform removed under reduced pressure to yield a reddish brown oil which was passed through a short column of neutral alumina (8 g) in benzene. The filtrate which gave a single spot on t.l.c. (R_F 0.60) was evaporated and afforded a semi-crystalline residue (0.66 g, 60%) which was recrystallised from benzene-hexane (1:3) to give white needles of the crude ketoaldehyde (171) m.p. 100-104° (lit. m.p. 110-111°)[206].

3:14:3 Attempted preparation of the hydroxy-ketone (170)

The crude ketoaldehyde (0.65 g) and ethanedithiol (0.9 ml) were dissolved in sodium dried ether (20 ml) containing freshly distilled BF_3-etherate (7 ml) and the solution stirred for 2 days at room temperature. Ether (40 ml) was added and the etheral solution was washed with a 1M aqueous potassium hydroxide solution (5 x 50 ml) and water (2 x 50). The ether layer was dried over anhydrous sodium sulphate and the ether removed under reduced pressure to yield an oil which was shown by t.l.c. in hexane-

ether (7:3) to be a mixture of unchanged ketoaldehyde (R_F 0.15) and another product (R_F 0.22) presumed to be the thioketal (172). These two products could not be separated by chromatography on neutral alumina and were eluted together from the column with benzene-chloroform (1:1).

The mixture of products (0.61 g) was refluxed with freshly prepared Raney nickel[239] (approx. 3 g) in dry acetone (30 ml) for 28 hours. The solution was filtered and the acetone removed in vacuo. T.l.c. of the residue in hexane-ether (7:3) showed it to consist of three main products: the ketoaldehyde (R_F 0.15), and two other products (R_F 0.26 and R_F 0.34). Very little unchanged thioketal (R_F 0.21) was present. Attempts to separate these products by column chromatography through silica gel (70-230 mesh) and neutral alumina were unsuccessful. Preparative t.l.c. (hexane-ether 7:3) was also attempted without success.

APPENDIX ONE

CRYSTALLOGRAPHIC DATA

Table 10

Crystal data for synrotolide, dubiin and saponified dubiin

	Synrotolide	Dubiin	Saponified dubiin
Formula	$C_{16}H_{22}O_8$	$C_{22}H_{30}O_6$	$C_{20}H_{28}O_5$
Molecular mass	342.35	390.16	348.44
Crystal system	Orthorhombic	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a/Å	8.858(3)	7.232(4)	8.604(2)
b/Å	8.977(2)	8.067(10)	11.351(2)
c/Å	21.741(2)	34.907(11)	17.842(2)
$\alpha = \beta = \gamma/^{o}$	90	90	90
v/Å^3	1728.6	2035.7	1742.5
Z	4	4	4
D_c/gcm^{-3}	1.314	1.262	1.306
D_m/gcm^{-3}	1.292	1.273	1.311
μ(Mo-Kα)/cm^{-1}	0.67	0.54	0.55
F(000)	728.0	840.0	752.0

Table 11

Fractional atomic co-ordinates (x 10^4) and anisotropic temperature factors[a] (x 10^4) for the oxygen atoms [b,c] of synrotolide

Atom	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(1)	1554(11)	2918(14)	7717(5)	801(73)	1499(107)	1339(95)	-253(91)	-46(65)	365(78)
O(2)	3524(9)	1860(9)	7303(4)	548(51)	676(55)	881(59)	11(48)	-51(44)	20(46)
O(3)	7843(9)	1853(8)	5199(4)	217(31)	454(45)	730(65)	-19(39)	61(38)	-38(33)
O(4)	4057(7)	468(7)	5184(4)	236(33)	254(35)	818(49)	39(35)	-27(34)	-56(30)
O(5)	6389(8)	3987(7)	5896(4)	507(42)	320(37)	777(53)	-113(37)	221(43)	-54(34)
O(6)	4371(9)	4836(10)	6413(4)	624(57)	740(61)	1363(81)	49(52)	308(56)	135(52)
O(7)	5376(8)	-1608(7)	4399(4)	438(40)	283(34)	901(57)	-50(37)	-127(42)	77(32)
O(8)	7478(8)	-2867(8)	4563(4)	504(50)	445(43)	1205(74)	144(45)	-54(46)	172(39)

a In the form: $\exp\left[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)\right]$

b For atom labelling see Figure 1, p. 62

c Estimated standard deviations in parentheses

Table 12

Fractional atomic co-ordinates (x 10^4) and isotropic temperature factors ($Å^2$ x 10^4) for the carbon atoms[b,c] of synrotolide

Atom	x/a	y/b	z/c	U_{iso} or U_{11}
C(1)	1996(19)	1817(18)	7452(7)	801(44)
C(2)	1228(17)	454(15)	7332(6)	801(39)
C(3)	1879(17)	-791(18)	7185(6)	866(43)
C(4)	3511(15)	-767(16)	7051(6)	774(39)
C(5)	4016(13)	771(13)	6859(6)	563(29)
C(6)	5742(13)	914(13)	6842(6)	600(31)
C(7)	6473(13)	1669(11)	6406(5)	516(28)
C(8)	5791(11)	2497(10)	5866(4)	371(23)
C(9)	6269(11)	1911(11)	5251(4)	389(24)
C(10)	5661(10)	351(11)	5106(4)	366(24)
C(11)	6119(10)	-154(10)	4484(4)	329(21)
C(12)	5660(13)	773(14)	3949(5)	570(31)
C(13)	5562(14)	5032(15)	6155(6)	588(30)
C(14)	6229(16)	6511(14)	6112(6)	737(38)
C(15)	6166(13)	-2819(12)	4447(5)	488(27)
C(16)	5200(14)	-4169(13)	4329(5)	633(33)

b For atom labelling see Figure 1, p. 62

c Estimated standard deviations in parentheses

Table 13

Synrotolide intramolecular bond lengths (Å)[a,b]

Bond	Length	Bond	Length
C(1) - O(1)	1.21(2)	C(9) - C(10)	1.53(1)
C(1) - C(2)	1.42(2)	C(9) - O(3)	1.40(1)
C(1) - O(2)	1.39(2)	C(10) - C(11)	1.48(1)
C(2) - C(3)	1.30(2)	C(10) - O(4)	1.44(1)
C(3) - C(4)	1.47(2)	C(11) - C(12)	1.49(1)
C(4) - C(5)	1.51(2)	C(11) - O(7)	1.47(1)
C(5) - C(6)	1.54(1)	C(13) - C(14)	1.46(2)
C(5) - O(2)	1.44(1)	C(13) - O(5)	1.32(1)
C(6) - C(7)	1.33(1)	C(13) - O(6)	1.21(1)
C(7) - C(8)	1.48(1)	C(15) - C(16)	1.51(2)
C(8) - C(9)	1.54(1)	C(15) - O(7)	1.30(1)
C(8) - O(5)	1.44(1)	C(15) - O(8)	1.19(1)

[a] Estimated standard deviations in parentheses

[b] For atom labelling see Figure 1, p. 62

Table 14

Synrotolide intramolecular bond angles (°)[a,b]

Bond	Angle	Bond	Angle
O(1) - C(1) - C(2)	123(2)	O(3) - C(9) - C(10)	108(1)
O(2) - C(1) - O(1)	114(1)	C(9) - C(10) - C(11)	112(1)
O(2) - C(1) - C(2)	116(2)	C(9) - C(10) - O(4)	105(1)
C(1) - C(2) - C(3)	125(2)	O(4) - C(10) - C(11)	114(1)
C(1) - O(2) - C(5)	116(1)	C(10) - C(11) - C(12)	118(1)
C(2) - C(3) - C(4)	118(2)	C(10) - C(11) - O(7)	105(1)
C(3) - C(4) - C(5)	112(1)	O(7) - C(11) - C(12)	106(1)
C(4) - C(5) - C(6)	112(1)	C(8) - O(5) - C(13)	118(1)
O(2) - C(5) - C(6)	105(1)	C(14) - C(13) - O(5)	113(1)
C(5) - C(6) - C(7)	123(1)	O(6) - C(13) - O(5)	126(1)
C(6) - C(7) - C(8)	127(1)	C(14) - C(13) - O(6)	121(1)
C(7) - C(8) - C(9)	114(1)	C(11) - O(7) - C(15)	119(1)
C(7) - C(8) - O(5)	108(1)	O(7) - C(15) - C(16)	111(1)
O(5) - C(8) - C(9)	104(1)	O(7) - C(15) - O(8)	125(1)
C(8) - C(9) - C(10)	114(1)	O(8) - C(15) - C(16)	124(1)
C(8) - C(9) - O(3)	111(1)		

[a] Estimated standard deviations in parentheses

[b] For atom labelling see Figure 1, p.62

Table 15

Synrotolide : Least-squares planes[a]

Plane 1 The 5,6-dihydro-α-pyrone ring

Equation (-2.9612)X + (1.4307)Y + (20.1951)Z = 3.7353

Atoms included in calculation	Distance from plane, Å	Atoms not included in calculation	Distance from plane, Å
C(1)	0.073	C(5)	0.729
C(2)	-0.206		
C(3)	-0.138		
C(4)	0.417		
O(1)	0.326		
O(2)	-0.471		

Plane 2 Molecular fragment C(5) - C(8)

Equation (-.4592)X + (7.3808)Y + (12.3237)Z = 3.0343

Atoms included in calculation	Distance from plane, Å
C(5)	0.002
C(6)	-0.005
C(7)	0.005
C(8)	-0.002

[a] The equation of the planes are expressed in orthogonalized space as AX + BY + CZ = D

Table 16

Fractional atomic co-ordinates (x 10^4) and anisotropic temperature factors[a] (X 10^4) for the oxygen atoms[b,c] of dubiin

Atom	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(1)	3511(17)	10998(12)	1216(5)	322(65)	76(52)	911(125)	20(54)	235(71)	125(52)
O(2)	5459(18)	12447(15)	884(5)	423(78)	256(62)	927(126)	175(73)	151(85)	-125(67)
O(3)	3353(20)	9080(14)	496(4)	699(95)	249(59)	327(78)	-4(50)	128(69)	-28(70)
O(4)	4092(19)	4991(14)	1401(5)	592(85)	127(48)	986(141)	82(70)	114(104)	285(65)
O(5)	-3430(39)	4756(37)	2378(9)	1435(194)	1565(247)	1260(257)	869(218)	304(205)	-178(211)
O(6)	3828(25)	8584(18)	-124(6)	902(131)	364(74)	861(140)	198(85)	208(112)	-9(98)

a In the form: $\exp\left[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)\right]$

b For atom labelling see Figure 10, p. 107

c Estimated standard deviations in parentheses

Table 17

Fractional atomic co-ordinates (x 10^4) and isotropic temperature factors ($Å^2$ x 10^4) for the carbon atoms[b,c] of dubiin

Atom	x/a	y/b	z/c	U_{iso} or U_{11}
C(1)	5184(30)	7886(31)	1707(7)	610(66)
C(2)	6396(30)	9556(30)	1735(7)	602(62)
C(3)	7542(26)	9774(27)	1369(6)	492(52)
C(4)	6304(27)	9648(26)	986(7)	522(55)
C(5)	5334(22)	8035(21)	961(6)	338(45)
C(6)	4489(27)	7587(28)	627(7)	435(59)
C(7)	3185(30)	6046(29)	583(8)	581(64)
C(8)	1928(25)	5999(26)	932(7)	487(55)
C(9)	2858(21)	6239(22)	1330(6)	319(43)
C(10)	4000(22)	7901(22)	1339(6)	361(46)
C(11)	1299(32)	6303(32)	1644(8)	677(68)
C(12)	1106(39)	4731(37)	1877(9)	833(84)
C(13)	-583(33)	4805(33)	2145(9)	648(65)
C(14)	-784(45)	5719(57)	2483(13)	1172(124)
C(15)	-2345(57)	5506(58)	2613(15)	1241(147)
C(16)	-2332(43)	4174(41)	2050(11)	872(95)
C(17)	767(32)	4436(29)	903(8)	569(62)
C(18)	7658(25)	9886(32)	690(8)	587(61)

continued on next page

Table 17 continued

Atom	x/a	y/b	z/c	U_{iso} or U_{11}
C(19)	5033(23)	11108(24)	1045(6)	361(47)
C(20)	2639(23)	9443(23)	1349(6)	383(46)
C(21)	3157(30)	9419(32)	132(8)	583(57)
C(22)	2130(42)	10985(39)	76(11)	918(100)

b For atom labelling see Figure 10, p.107

c Estimated standard deviations in parentheses

Table 18

Dubiin intramolecular bond lengths (Å)[a,b]

Bond	Length	Bond	Length
C(1) - C(2)	1.61(4)	C(6) - C(7)	1.57(3)
C(1) - C(10)	1.54(3)	C(7) - C(8)	1.52(3)
O(1) - C(19)	1.26(2)	C(8) - C(9)	1.56(3)
O(1) - C(20)	1.48(2)	C(8) - C(17)	1.52(3)
C(2) - C(3)	1.53(3)	C(9) - C(10)	1.58(2)
C(3) - C(4)	1.61(3)	O(4) - C(9)	1.37(2)
O(2) - C(19)	1.26(2)	C(9) - C(11)	1.58(3)
C(4) - C(5)	1.48(3)	C(10) - C(20)	1.58(3)
C(4) - C(18)	1.44(3)	C(11) - C(12)	1.51(4)
C(4) - C(19)	1.51(3)	C(12) - C(13)	1.54(4)
C(5) - C(6)	1.37(3)	C(13) - C(14)	1.40(5)
C(5) - C(10)	1.64(3)	C(14) - C(15)	1.30(5)
O(3) - C(6)	1.53(3)	C(13) - C(16)	1.40(4)
O(3) - C(21)	1.31(3)	O(5) - C(15)	1.29(5)
C(21) - O(6)	1.22(3)	O(5) - C(16)	1.47(5)
C(21) - C(22)	1.48(4)		

[a] Estimated standard deviations in parentheses

[b] For atom labelling see Figure 10, p. 107

Table 19

Dubiin intramolecular bond angles (°)[a,b]

Bond	Angle	Bond	Angle
C(10) - C(1) - C(2)	110(2)	C(8) - C(7) - C(6)	108(2)
C(20) - O(1) - C(19)	126(1)	C(9) - C(8) - C(7)	117(2)
C(3) - C(2) - C(1)	109(2)	C(17) - C(8) - C(7)	107(2)
C(18) - C(4) - C(3)	102(2)	C(17) - C(8) - C(9)	114(2)
C(18) - C(4) - C(5)	114(2)	C(10) - C(9) - C(8)	111(2)
C(19) - C(4) - C(3)	102(2)	O(4) - C(9) - C(8)	111(2)
C(19) - (C4) - C(5)	114(2)	O(4) - C(9) - C(10)	106(1)
C(19) - C(4) - C(18)	114(2)	C(11) - C(9) - C(8)	108(2)
C(2) - C(3) - C(4)	112(2)	C(11) - C(9) - C(10)	110(2)
C(5) - C(4) - C(3)	112(2)	C(11) - C(9) - O(4)	112(2)
C(6) - C(5) - C(4)	119(2)	C(5) - C(10) - C(1)	110(1)
C(6) - O(3) - C(21)	121(2)	C(9) - C(10) - C(1)	108(2)
C(10) - C(5) - C(4)	107(2)	C(9) - C(10) - C(5)	110(2)
C(10) - C(5) - C(6)	114(2)	C(20) - C(10) - C(1)	110(2)
O(3) - C(6) - C(5)	107(2)	C(20) - C(10) - C(5)	109(2)
C(7) - C(6) - C(5)	124(2)	C(20) - C(10) - C(9)	110(1)
C(7) - C(6) - O(3)	106(1)	O(2) - C(19) - O(1)	119(2)
C(4) - C(19) - O(1)	123(2)	C(16) - C(13) - C(14)	107(3)

continued on next page

Table 19 continued

Bond	Angle	Bond	Angle
C(4) - C(19) - O(2)	117(2)	C(15) - C(14) - C(13)	110(5)
C(10) - C(20) - O(1)	113(1)	O(5) - C(16) - C(13)	100(3)
C(12) - C(11) - C(9)	121(2)	C(16) - O(5) - C(15)	108(3)
C(13) - C(12) - C(11)	112(2)	O(3) - C(21) - C(22)	111(3)
C(14) - C(13) - C(12)	128(3)	O(3) - C(21) - O(6)	124(3)
C(16) - C(13) - C(12)	124(3)	C(22) - C(21) - O(6)	125(3)

[a] Estimated standard deviations in parentheses

[b] For atom labelling see Figure 10, p.107

Table 20

Fractional atomic co-ordinates (x 10^4) and anisotropic temperature factors[a] (x 10^4) for the carbon and oxygen atoms[b,c] of saponified dubiin

Atom	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C(1)	-1729(4)	-5389(3)	-7621(2)	428(21)	449(21)	329(19)	15(17)	24(18)	-109(19)
C(2)	-279(5)	-5192(4)	-7144(2)	442(22)	488(23)	556(24)	20(20)	2(21)	-137(20)
C(3)	-709(5)	-4951(3)	-6328(2)	502(24)	437(22)	485(23)	-52(18)	-203(20)	-49(20)
C(4)	-1821(5)	-5887(3)	-6000(2)	461(22)	417(20)	293(18)	-96(17)	-52(18)	87(19)
C(5)	-3289(4)	-5948(3)	-6491(2)	379(19)	329(17)	281(17)	-33(15)	-22(16)	72(17)
C(6)	-4589(5)	-6678(3)	-6142(2)	479(22)	431(21)	262(17)	-14(16)	111(17)	48(19)
C(7)	-6005(4)	-6723(3)	-6647(2)	383(20)	477(22)	413(20)	5(18)	93(18)	-25(19)
C(8)	-5607(4)	-7176(3)	-7439(2)	370(20)	335(19)	373(18)	-16(16)	0(16)	0(17)
C(9)	-4337(4)	-6398(3)	-7800(2)	394(20)	262(16)	292(17)	-9(14)	-14(16)	40(15)
C(10)	-2832(4)	-6316(3)	-7295(2)	359(18)	300(17)	243(16)	-14(14)	-13(15)	22(15)
C(11)	-4030(4)	-6750(3)	-8626(2)	513(21)	352(17)	257(16)	-13(14)	-39(16)	5(18)
C(12)	-3580(5)	-8047(3)	-8786(2)	550(23)	420(19)	278(17)	-89(15)	-62(17)	60(18)
C(13)	-3903(4)	-8413(3)	-9584(2)	443(20)	424(19)	271(16)	-38(15)	-16(16)	-50(18)

continued on next page

Table 20 continued

Atom	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C(14)	-3335(4)	-9472(3)	-9932(2)	505(21)	459(20)	346(18)	-67(16)	46(18)	-29(20)
C(15)	-3941(5)	-9499(4)	-10621(2)	729(27)	517(23)	382(20)	-141(18)	70(21)	-75(24)
C(16)	-4815(5)	-7889(3)	-10094(2)	760(28)	562(23)	321(18)	-90(17)	-89(20)	34(24)
C(17)	-7094(5)	-7217(4)	-7910(2)	386(21)	555(25)	599(25)	-91(22)	-81(21)	-76(21)
C(18)	-2186(5)	-5554(4)	-5183(2)	639(27)	689(28)	324(20)	-202(20)	-104(20)	137(25)
C(19)	-928(5)	-7033(3)	-6020(2)	537(23)	466(22)	278(18)	-7(17)	-53(18)	85(21)
C(20)	-2017(4)	-7518(3)	-7263(2)	409(20)	397(20)	256(16)	-60(16)	-41(16)	91(17)
O(1)	-930(3)	-7688(2)	-6646(1)	476(15)	449(14)	320(12)	-65(11)	-92(12)	178(14)
O(2)	-52(4)	-7349(3)	-5525(1)	866(22)	680(19)	359(14)	-38(14)	-234(16)	290(20)
O(3)	-4041(4)	-7856(2)	-5976(1)	730(20)	475(16)	326(14)	125(13)	146(15)	70(16)
O(4)	-4976(4)	-5213(2)	-7815(2)	583(17)	273(12)	399(15)	8(12)	-67(15)	86(13)
O(5)	-4862(4)	-8542(3)	-10747(1)	912(22)	689(19)	328(13)	-103(13)	-170(16)	-22(19)

a In the form: $\exp\left[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)\right]$

b For atom labelling see Figure 11, p. 107

c Estimated standard deviations in parentheses

Table 21

Saponified dubiin intramolecular bond lengths (Å)[a,b]

Bond	Length	Bond	Length
C(1) - C(2)	1.526(5)	C(7) - C(8)	1.541(5)
C(1) - (C(10)	1.532(5)	C(8) - C(9)	1.546(5)
O(1) - C(19)	1.342(4)	C(8) - C(17)	1.532(5)
O(1) - C(20)	1.458(4)	C(9) - C(10)	1.580(5)
C(2) - C(3)	1.528(5)	O(4) - C(9)	1.454(4)
C(3) - C(4)	1.546(5)	O(4) - H	0.72(5)
O(2) - C(19)	1.215(5)	C(9) - C(11)	1.549(4)
C(4) - C(5)	1.538(5)	C(10) - C(20)	1.534(5)
C(4) - C(18)	1.539(5)	C(11) - C(12)	1.549(5)
C(4) - C(19)	1.510(6)	C(12) - C(13)	1.510(4)
C(5) - C(6)	1.524(5)	C(13) - C(14)	1.438(5)
C(5) - C(10)	1.545(5)	C(14) - C(15)	1.336(5)
O(3) - C(6)	1.449(5)	C(13) - C(16)	1.340(5)
O(3) - H	0.79(4)	O(5) - C(15)	1.362(5)
C(6) - C(7)	1.516(5)	O(5) - C(16)	1.381(4)

[a] Estimated standard deviations in parentheses

[b] For atom labelling see Figure 11, p.107

Table 22

Saponified dubiin intramolecular bond angles (°)[a,b]

Bond	Angle	Bond	Angle
C(10) - (C1) - C(2)	113.3	C(17) - C(8) - C(9)	112.3
C(20) - O(1) - C(19)	123.8	C(10) - C(9) - C(8)	112.0
C(3) - C(2) - C(1)	111.1	O(4) - C(9) - C(8)	105.6
C(18) - C(4) - C(3)	108.4	O(4) - C(9) - C(10)	105.4
C(18) - C(4) - C(5)	112.5	C(11) - C(9) - C(8)	111.8
C(19) - C(4) - C(3)	105.6	C(11) - C(9) - C(10)	114.7
C(19) - C(4) - C(5)	111.4	C(11) - C(9) - O(4)	106.6
C(19) - C(4) - C(18)	109.7	C(5) - C(10) - C(1)	108.9
C(5) - C(4) - C(3)	108.9	C(9) - C(10) - C(1)	109.4
C(6) - C(5) - C(4)	113.2	C(9) - C(10) - C(5)	109.7
C(10) - C(5) - C(4)	109.4	C(20) - C(10) - C(1)	110.0
C(10) - C(5) - C(6)	114.8	C(20) - C(10) - C(5)	108.8
O(3) - C(6) - C(5)	110.3	C(20) - C(10) - C(9)	110.1
C(7) - C(6) - C(5)	111.4	O(2) - C(19) - O(1)	116.2
C(7) - C(6) - O(3)	110.6	C(4) - C(19) - O(1)	119.8
C(8) - C(7) - C(6)	112.1	C(4) - C(19) - O(2)	123.5
C(9) - C(8) - C(7)	110.4	C(12) - C(11) - C(9)	117.6

continued on next page

Table 22 continued

Bond	Angle	Bond	Angle
C(17) - C(8) - C(7)	109.1	C(13) - C(12) - C(11)	113.0
C(14) - C(13) - C(12)	125.0	C(15) - C(14) - C(13)	106.4
C(16) - C(13) - C(12)	128.7	O(5) - C(16) - C(13)	110.5
C(16) - C(13) - C(14)	106.1	C(16) - O(5) - C(15)	105.8

[a] Estimated standard deviations of all bond angles were 0.3^{o}

[b] For atom labelling see Figure 11, p. 107

Table 23

Saponified dubiin : Least - squares planes[a]

Plane 1 The furan ring

Equation $(-6.7996)X + (5.7146)Y + (6.2318)Z = 3.9656$

Atoms included in calculation	Distance from plane, A
C(13)	0.001
C(14)	-0.001
C(15)	0.001
C(16)	-0.001
O(5)	-0.000

Plane 2 The lactone ring

Equation $(6.8577)X + (4.9517)Y + (-7.4515)Z = -0.3367$

Atoms included in calculation	Distance from plane, A	Atoms not included in calculation	Distance from plane, A
C(4)	0.0301	O(1)	-0.1709
C(10)	-0.0296	O(2)	-0.1058
C(19)	-0.0306		
C(20)	0.0302		

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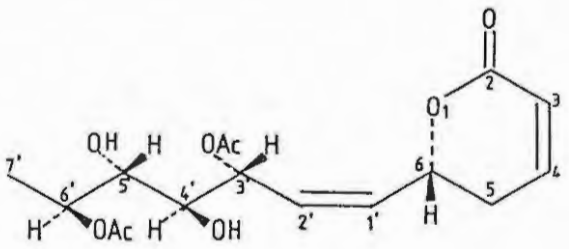
[a] The equations of the planes are expressed in orthogonalized space as $AX + BY + CZ = D$

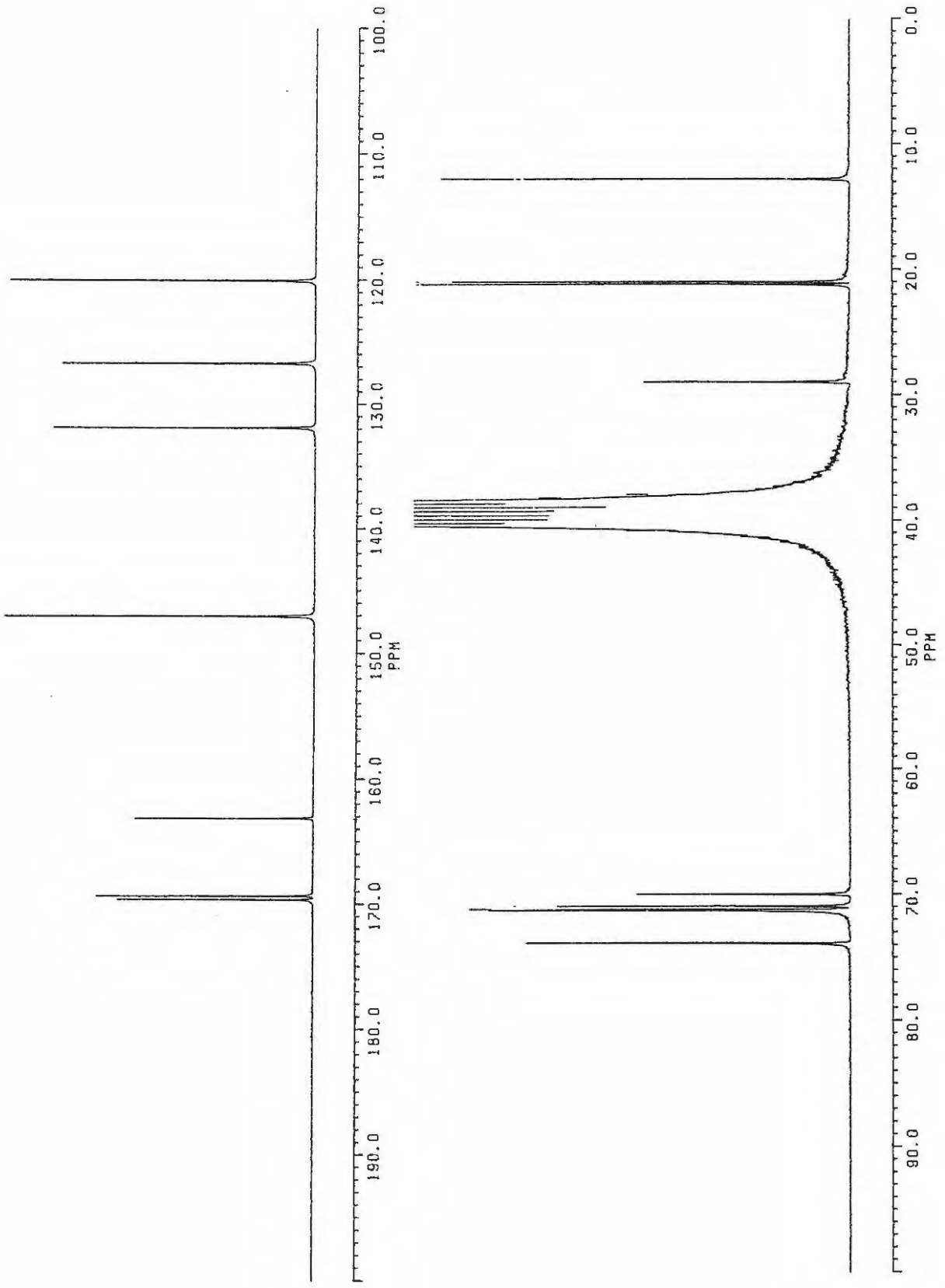
APPENDIX TWO

^{1}H AND ^{13}C NMR SPECTRA

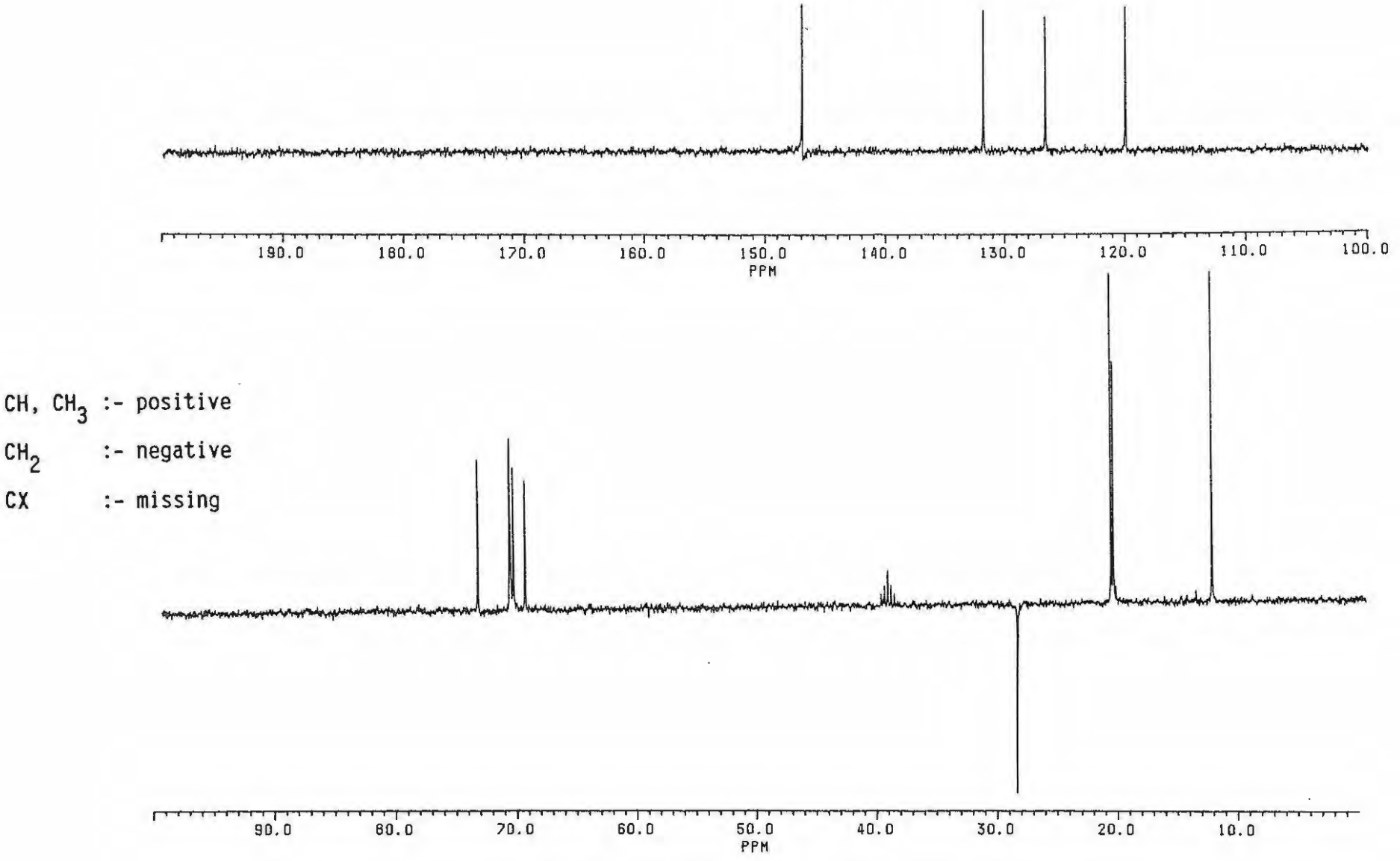


(1)

300 MHz ^{1}H NMR spectrum of synrotolide (1) in DMSO-d_6



75.5 MHz ^{13}C NMR spectrum of synrotolide in DMSO-d_6

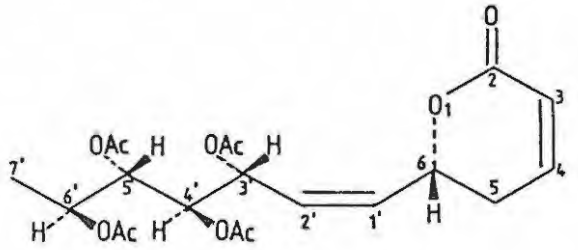


CH, CH_3 :- positive

CH_2 :- negative

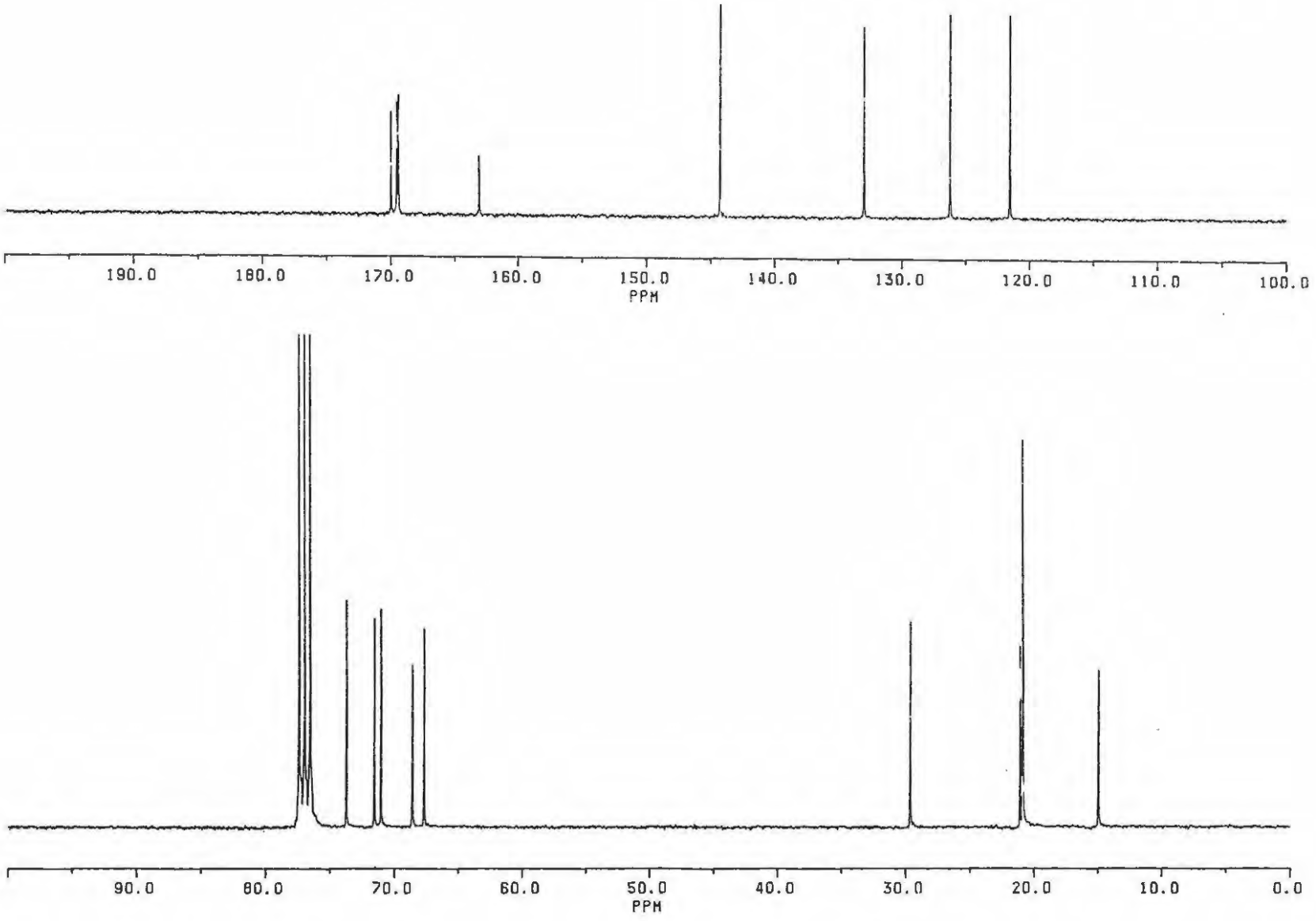
CX :- missing

Synrotolide DEPT experiment in DMSO-d_6

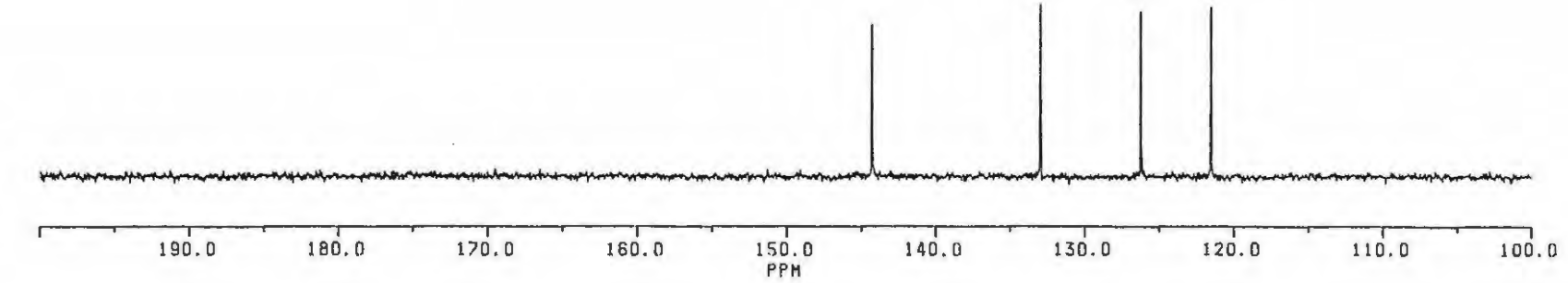


(2)

300 MHz ^{1}H NMR spectrum of synrotolide acetate (2) in $CDCl_3$



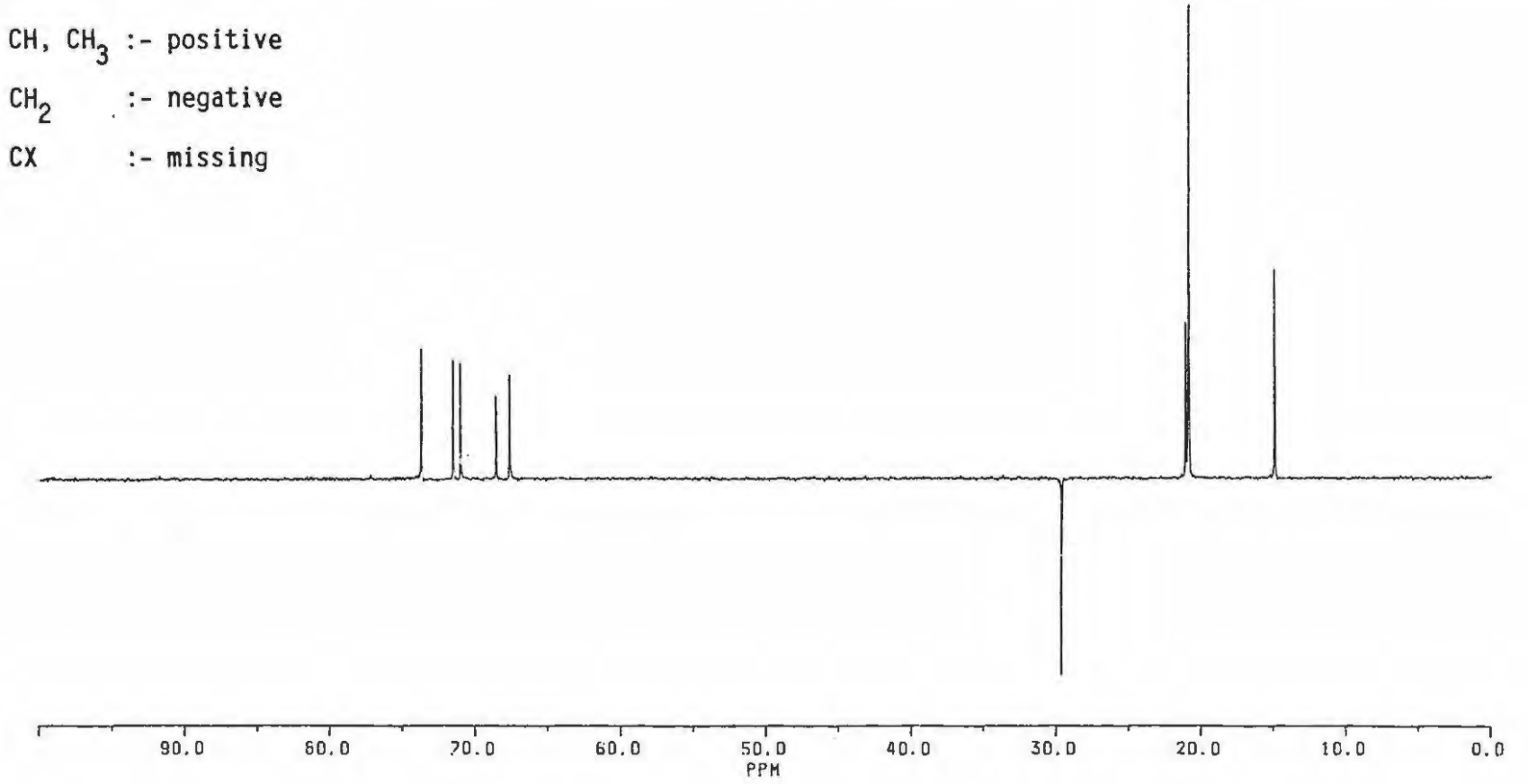
75.5 MHz ^{13}C NMR spectrum of synrotolide acetate in $CDCl_3$



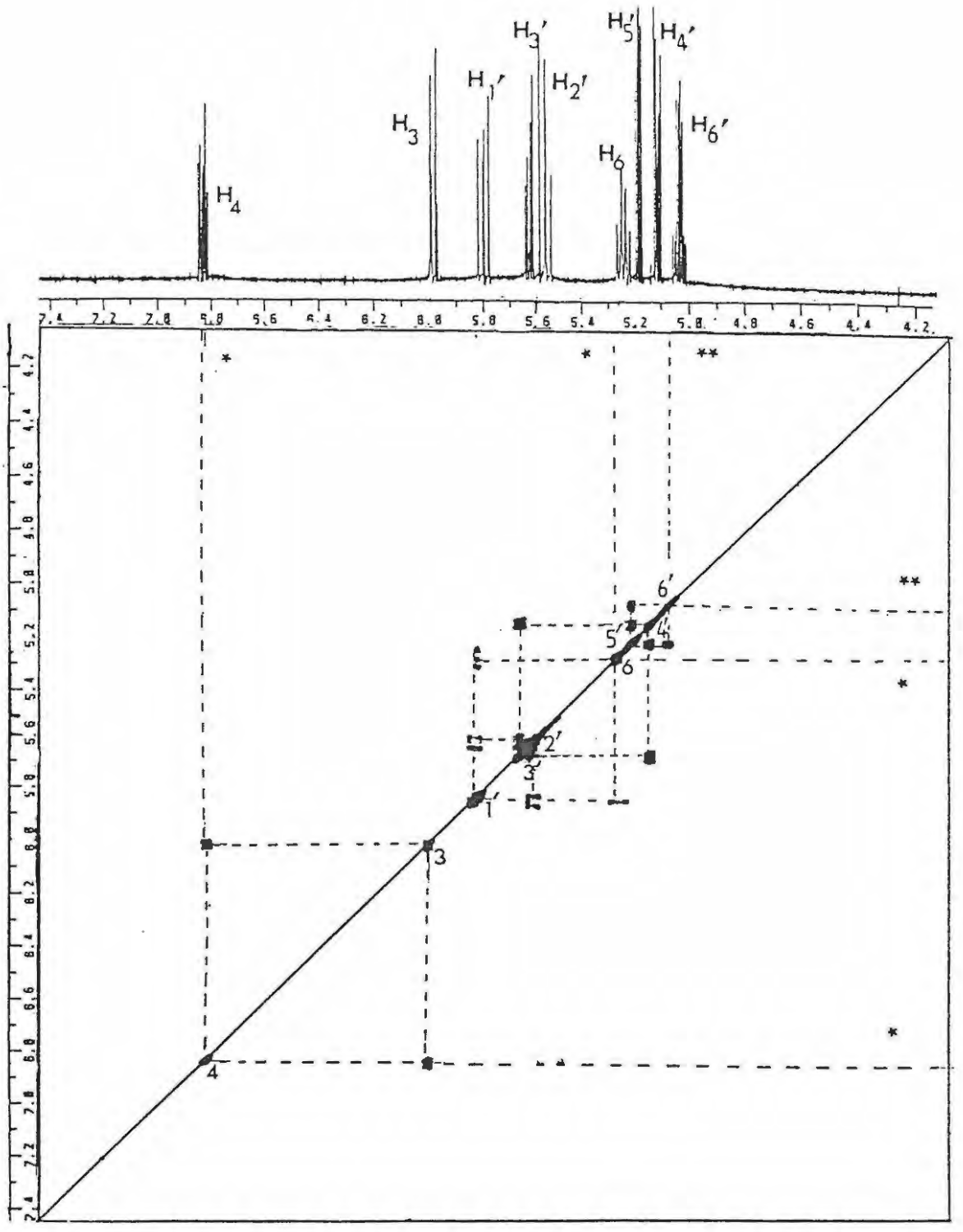
CH, CH_3 :- positive

CH_2 :- negative

CX :- missing

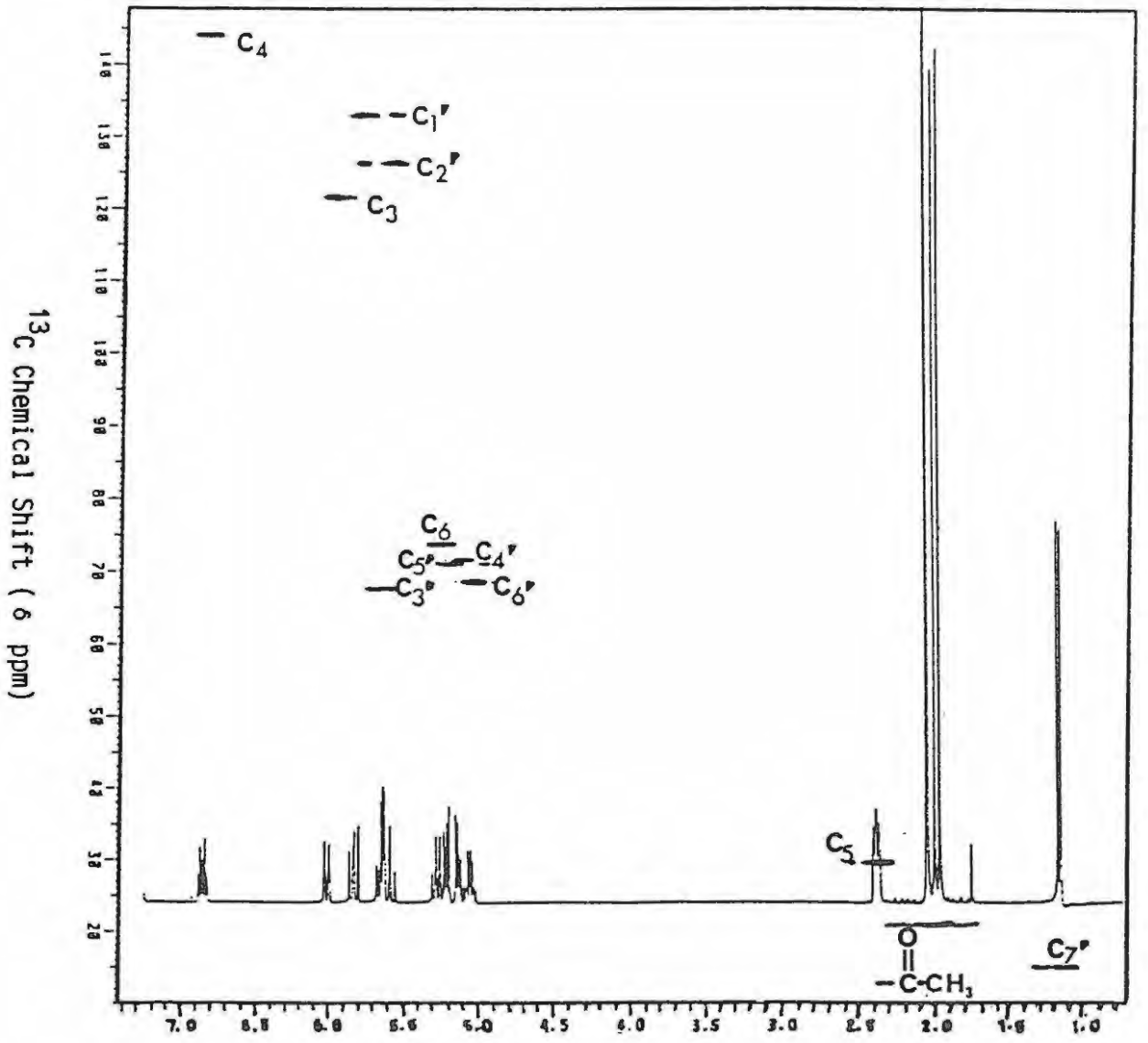


Synrotolide acetate DEPT experiment in $CDCl_3$



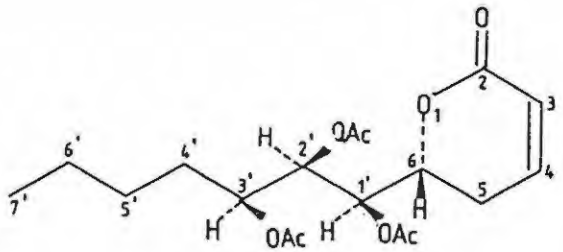
Synrotolide acetate COSY experiment in $CDCl_3$ (δ 4.2 - 7.4 ppm)

* Connectivity to H_{5ax} and H_{5eq} ** Connectivity to H_7.



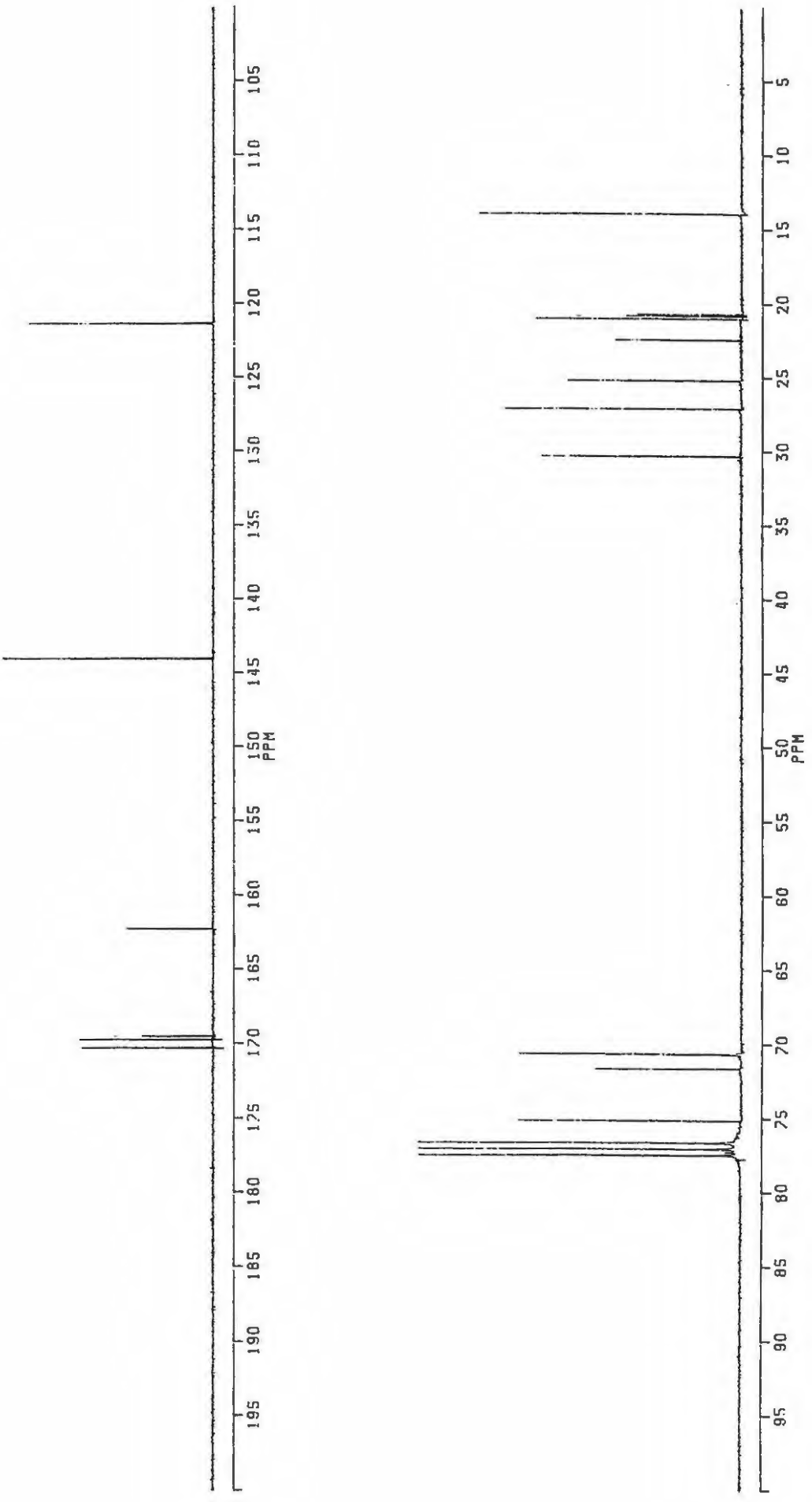
Synrotolide acetate HETCOR experiment in $CDCl_3$ *

* ^1H NMR spectrum superimposed

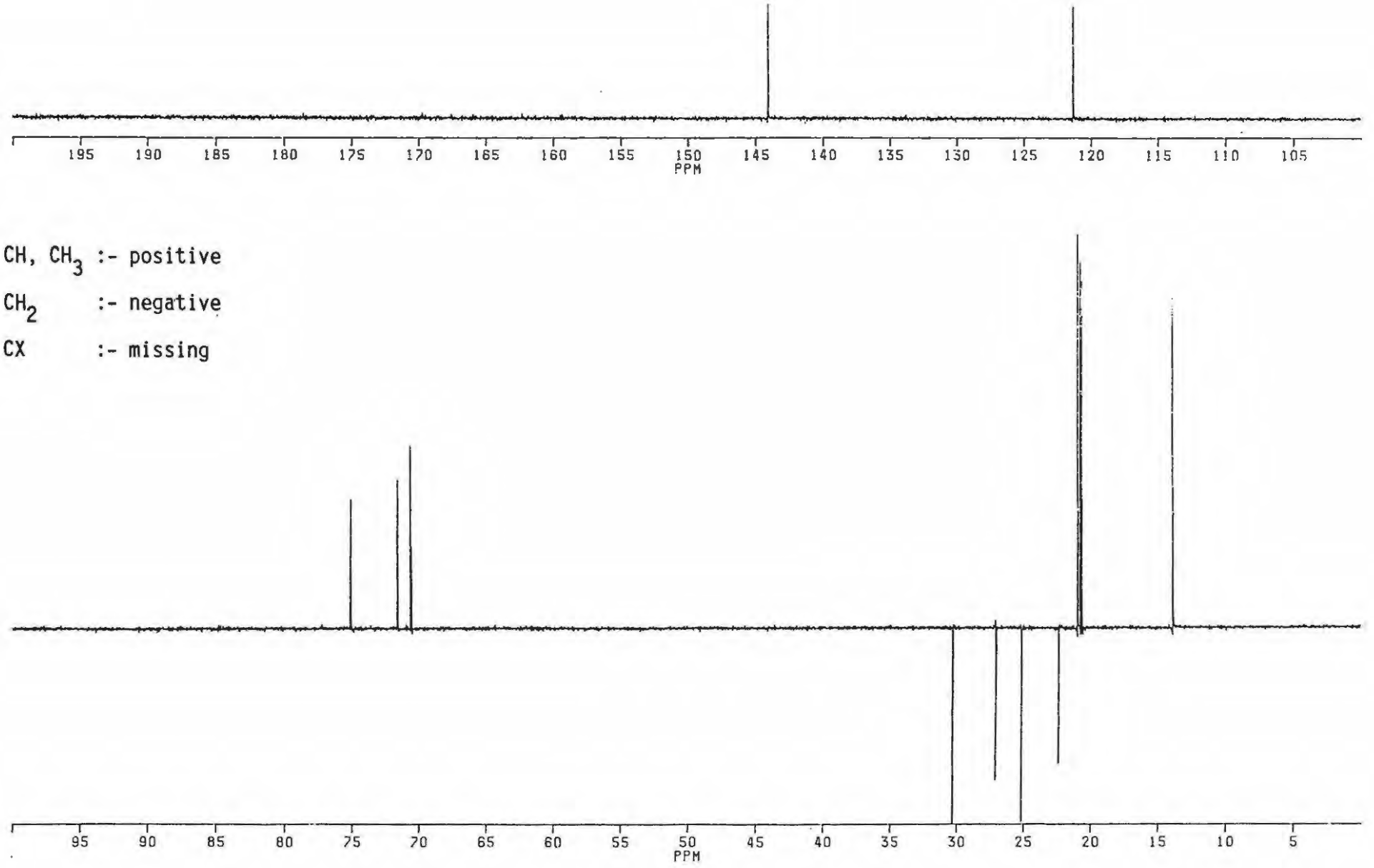


(3)

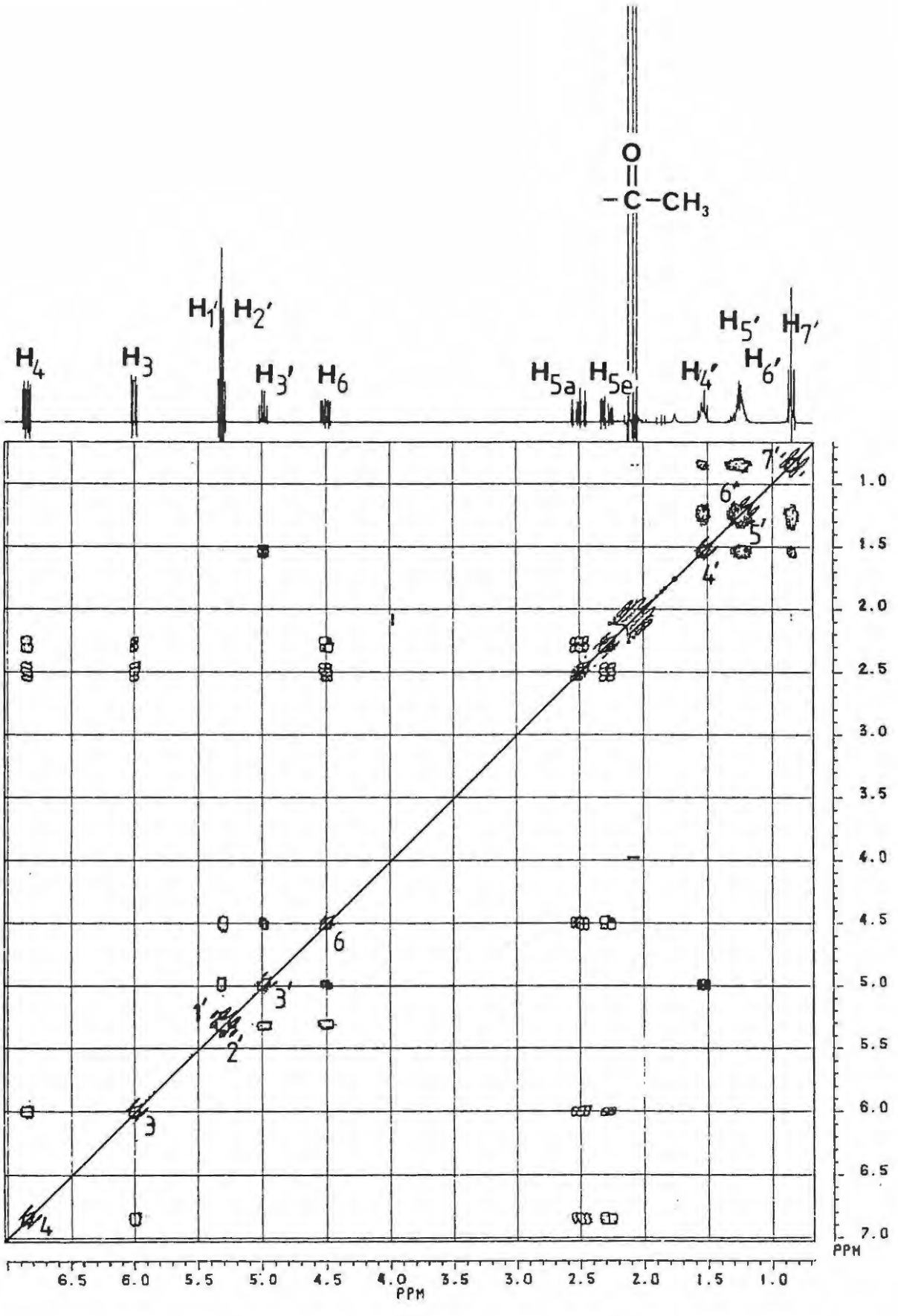
300 MHz ^{1}H NMR spectrum of boronolide (3) in $CDCl_3$



75.5 MHz ^{13}C NMR spectrum of boronolide in $CDCl_3$

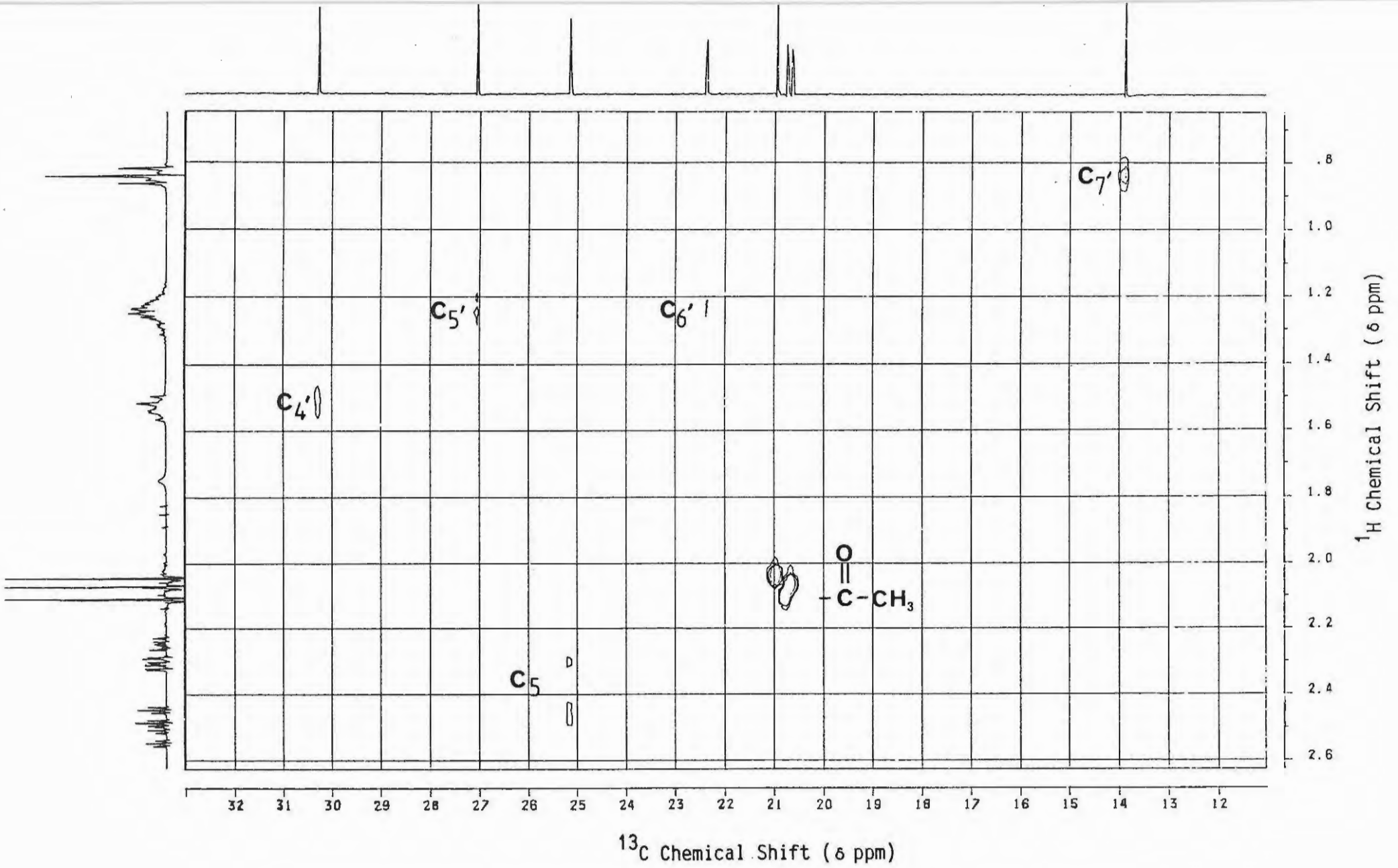


Boronolide DEPT experiment in $CDCl_3$

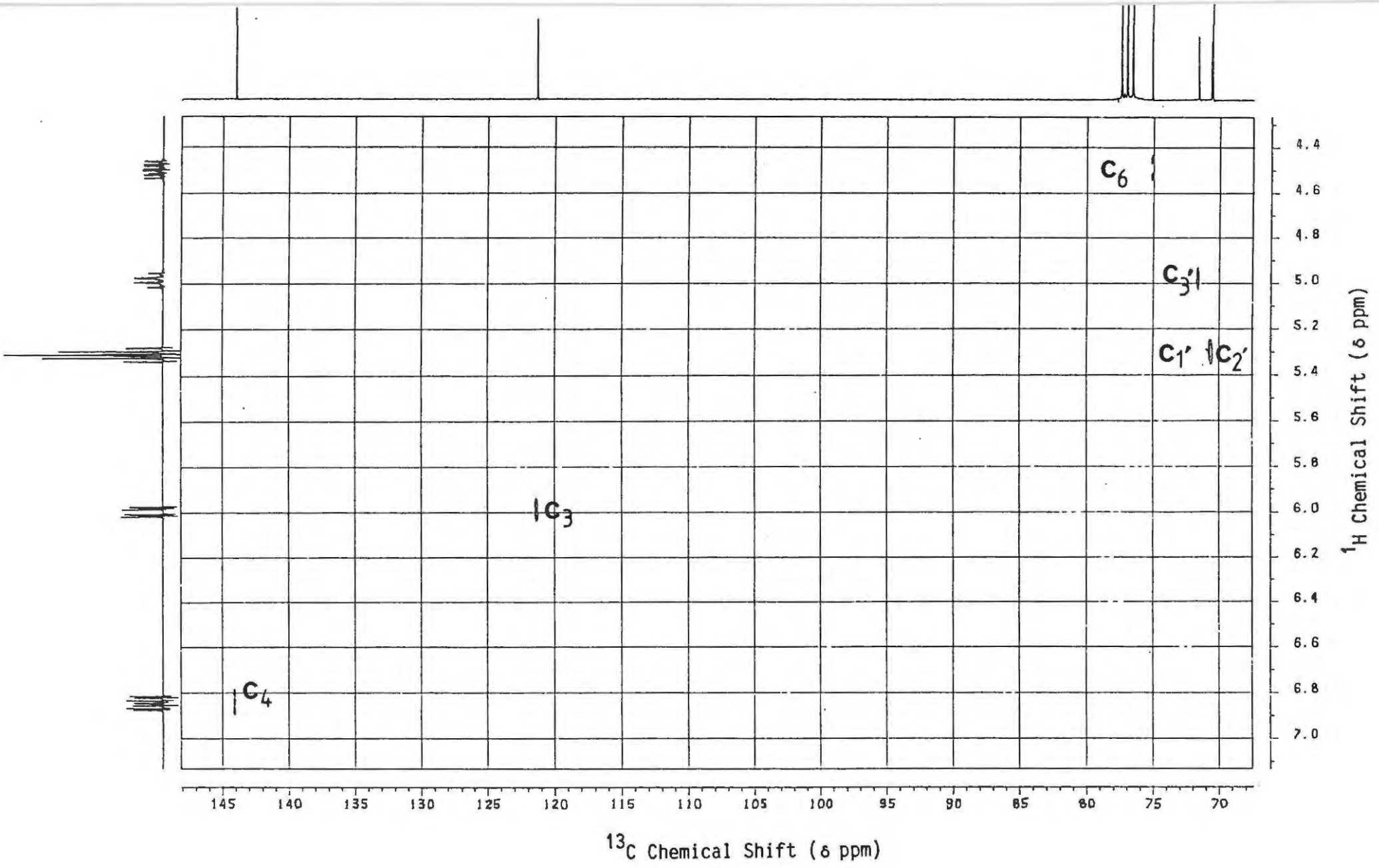


Boronolide COSY experiment in $CDCl_3$ *

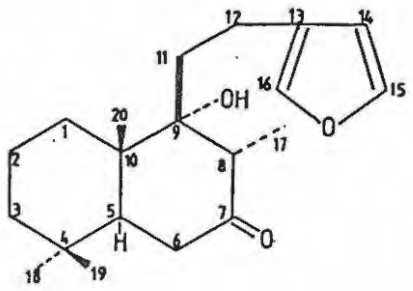
*Connectivities not mapped out



Boronolide HETCOR experiment in $CDCl_3$ (^1H δ 0.8 - 2.6 ppm, ^{13}C δ 12 - 32 ppm)

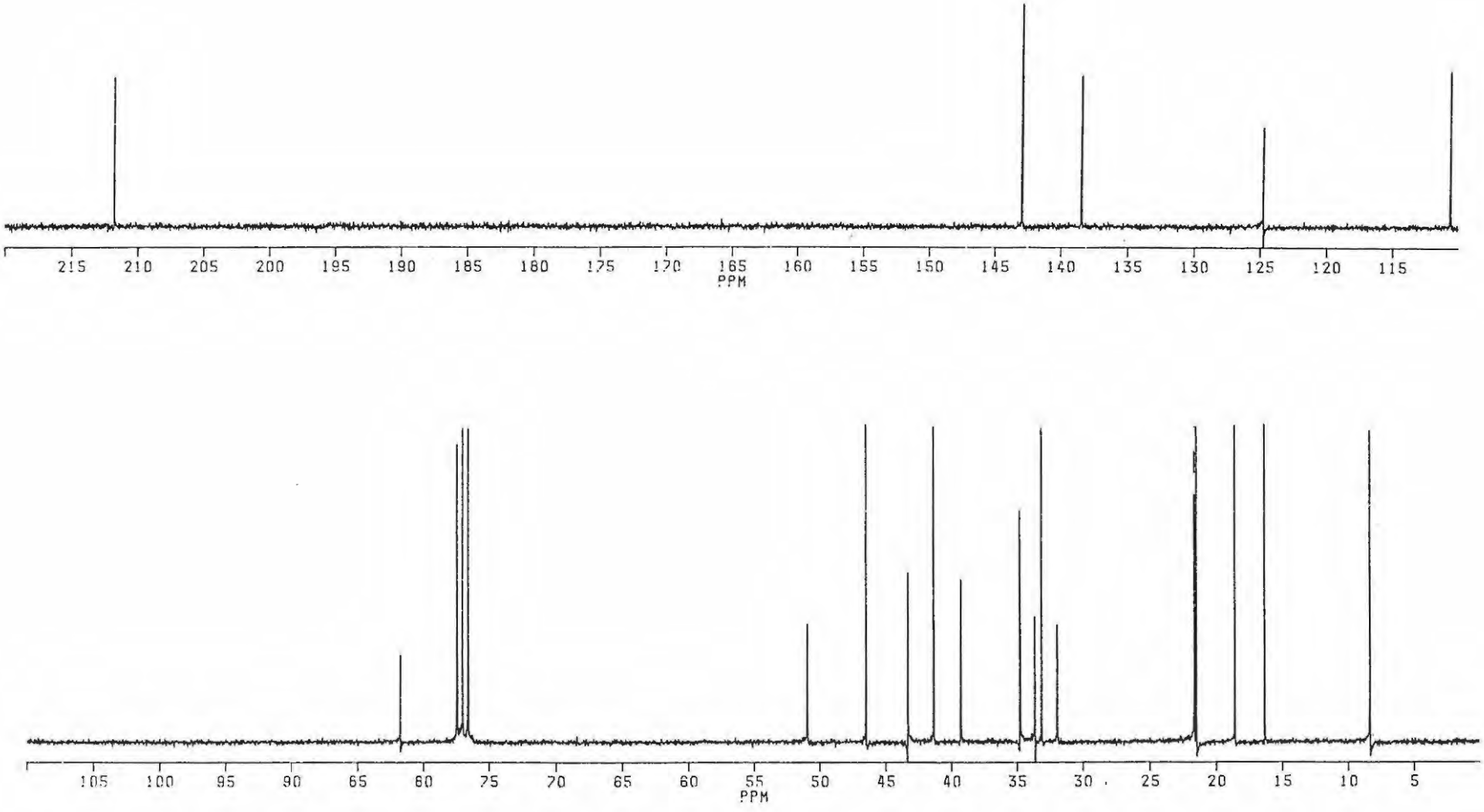


Boronolide HETCOR experiment in $CDCl_3$ (^1H δ 4.4 - 7.0 ppm, ^{13}C δ 70 - 145 ppm)



(137)

300 MHz ^{1}H NMR spectrum of hispanalone (137) in $CDCl_3$

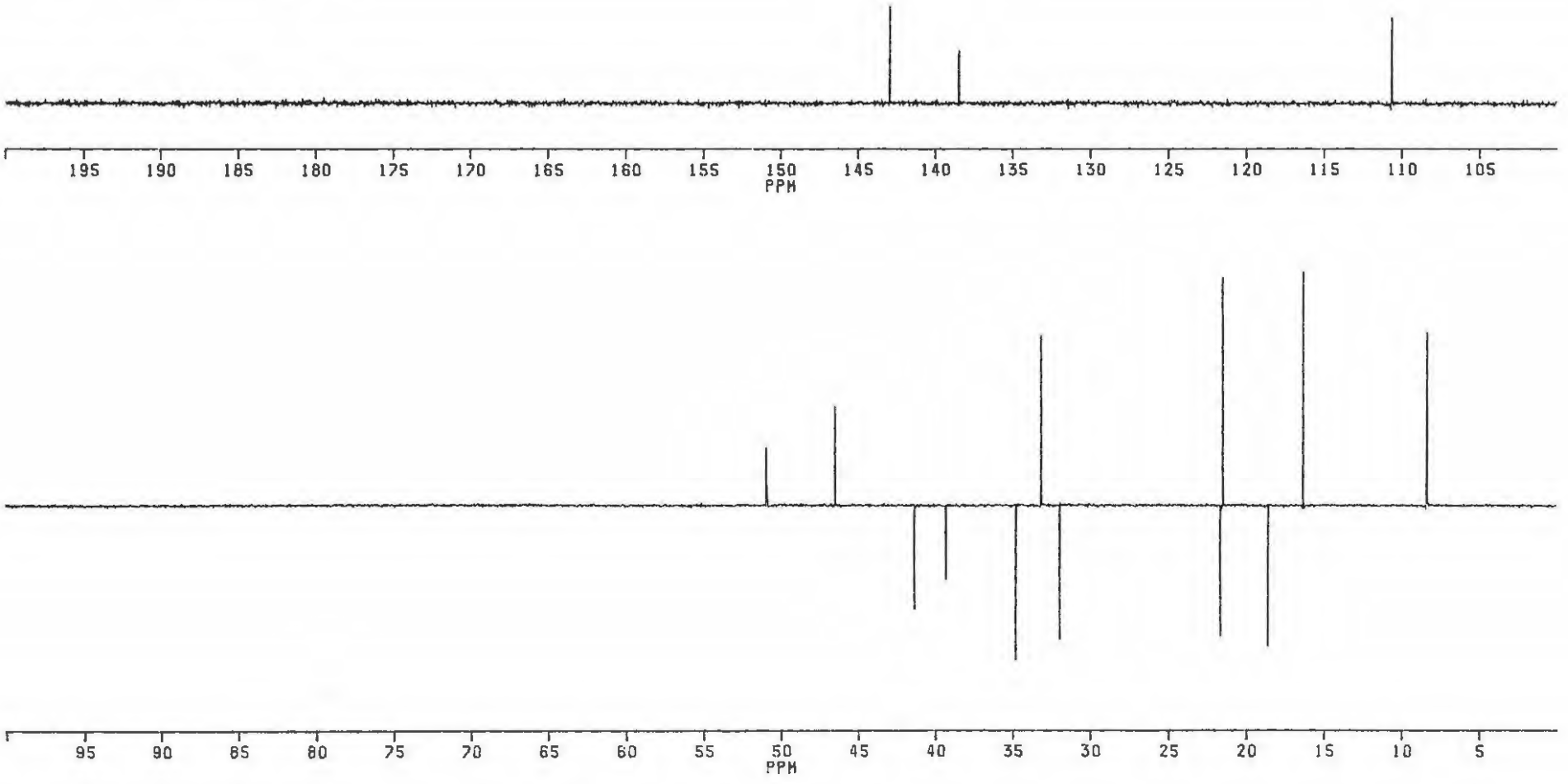


75.5 MHz ^{13}C NMR spectrum of hispanalone in $CDCl_3$

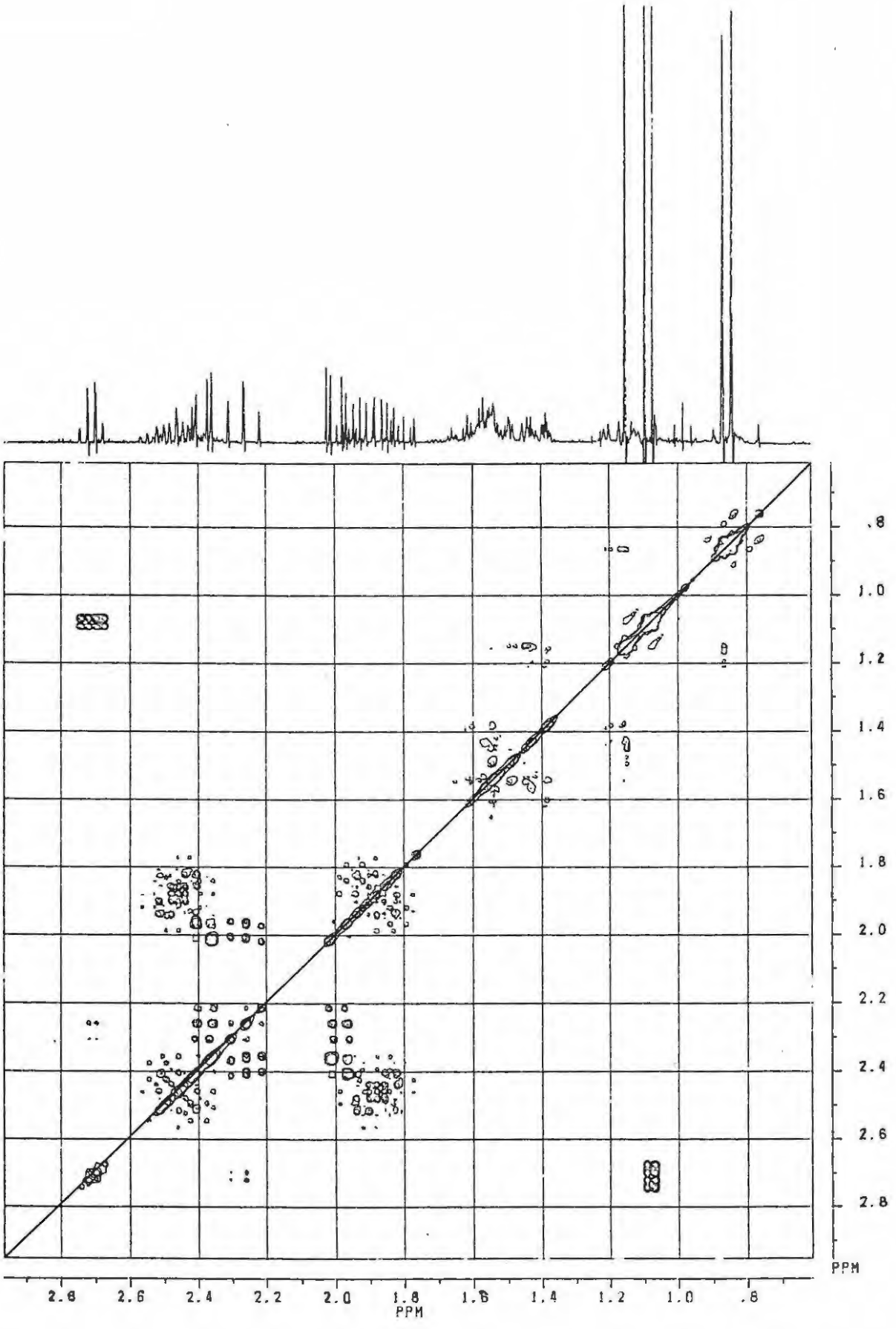
CH, CH_3 :- positive

CH_2 :- negative

CX :- missing

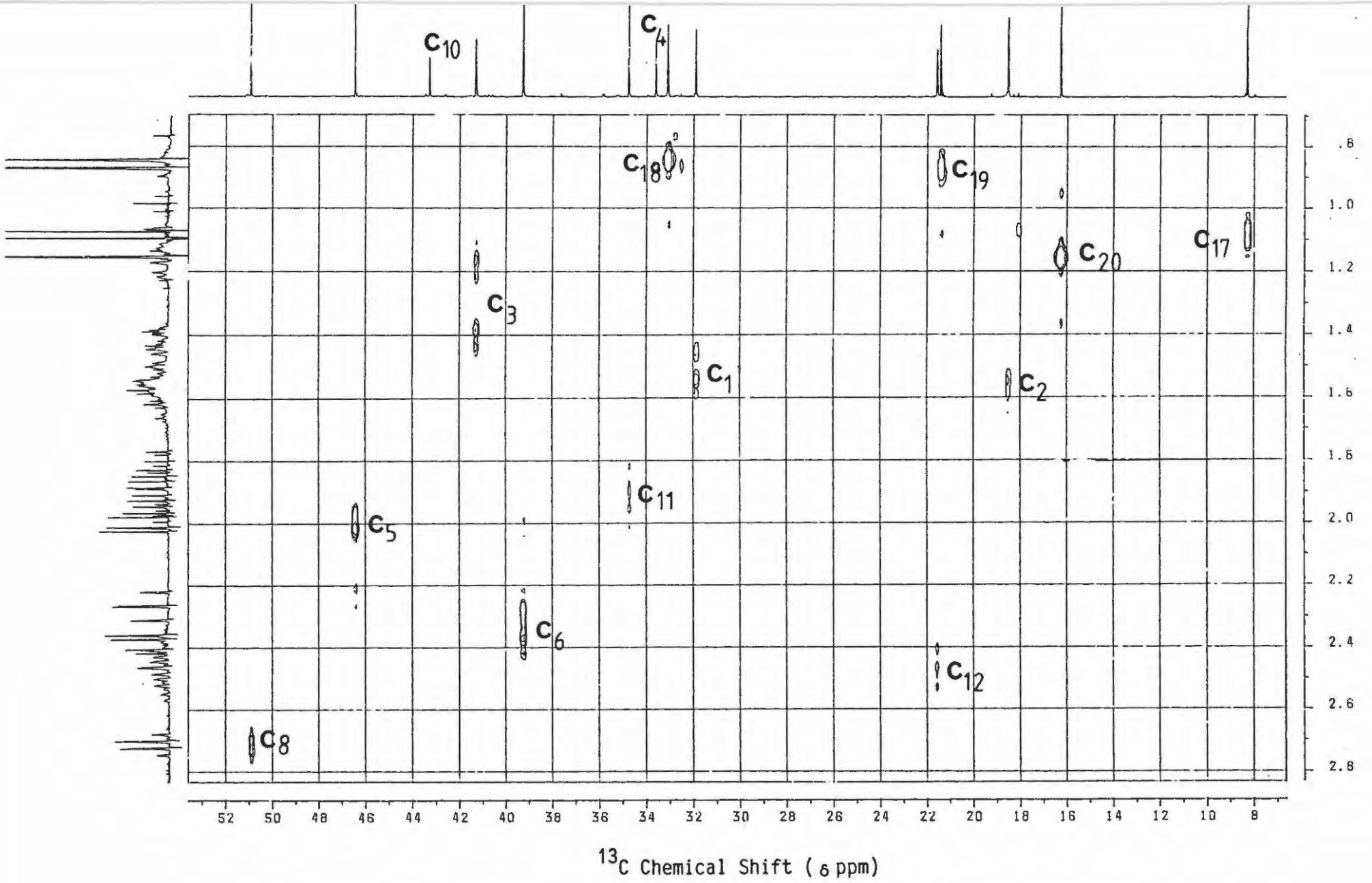


Hispanalone DEPT experiment in $CDCl_3$

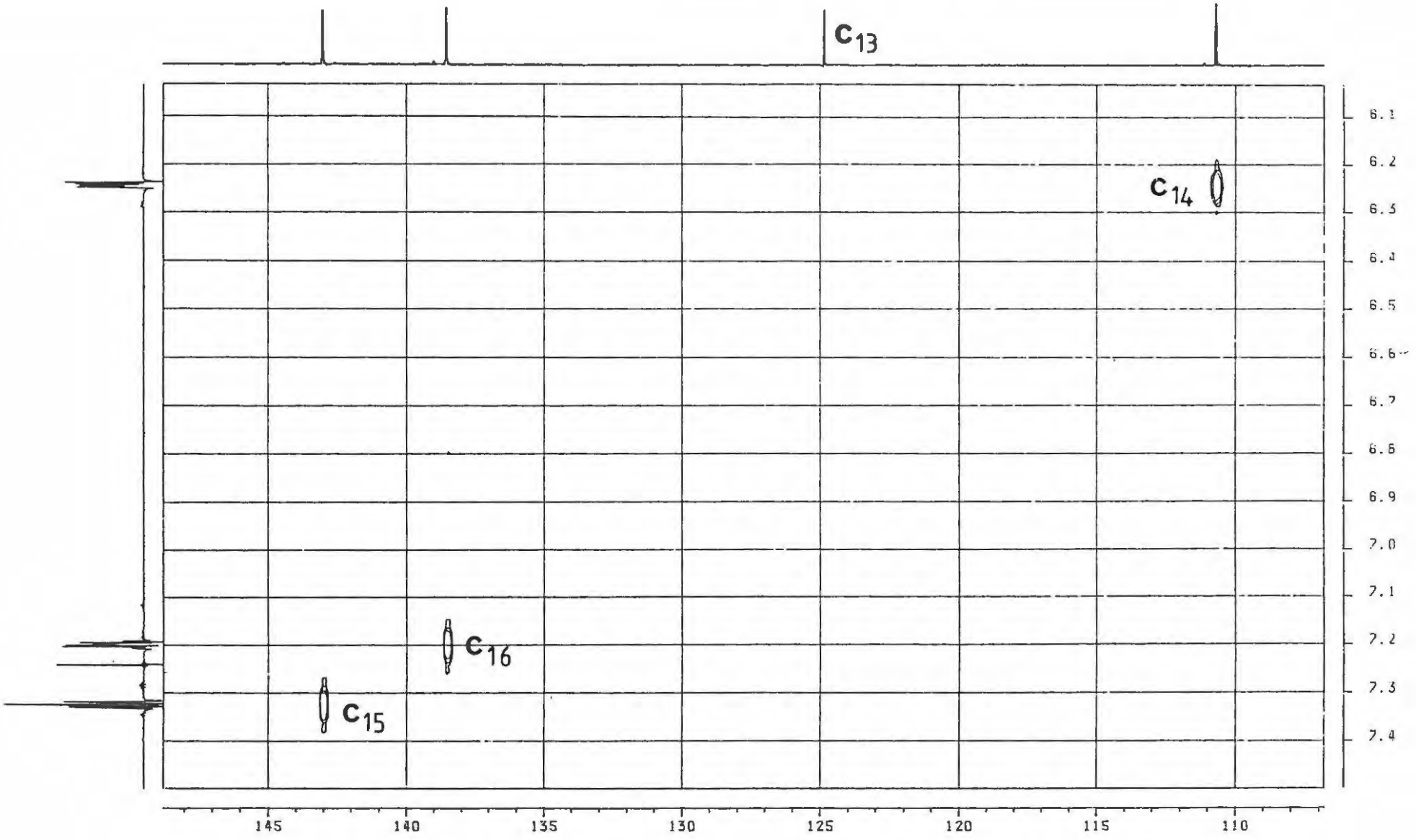


Hispanalone COSY experiment in $CDCl_3$ (δ 0.9 - 2.9 ppm) *

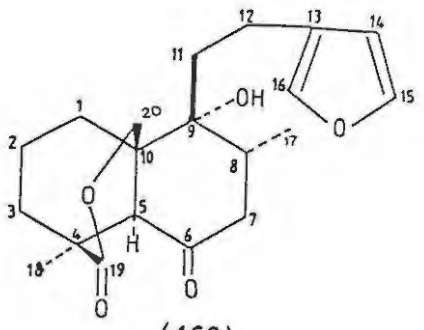
*Connectivities not mapped out



Hispanalone HETCOR experiment in $CDCl_3$ (^1H δ 0.6 - 2.8 ppm, ^{13}C δ 7 - 53 ppm)



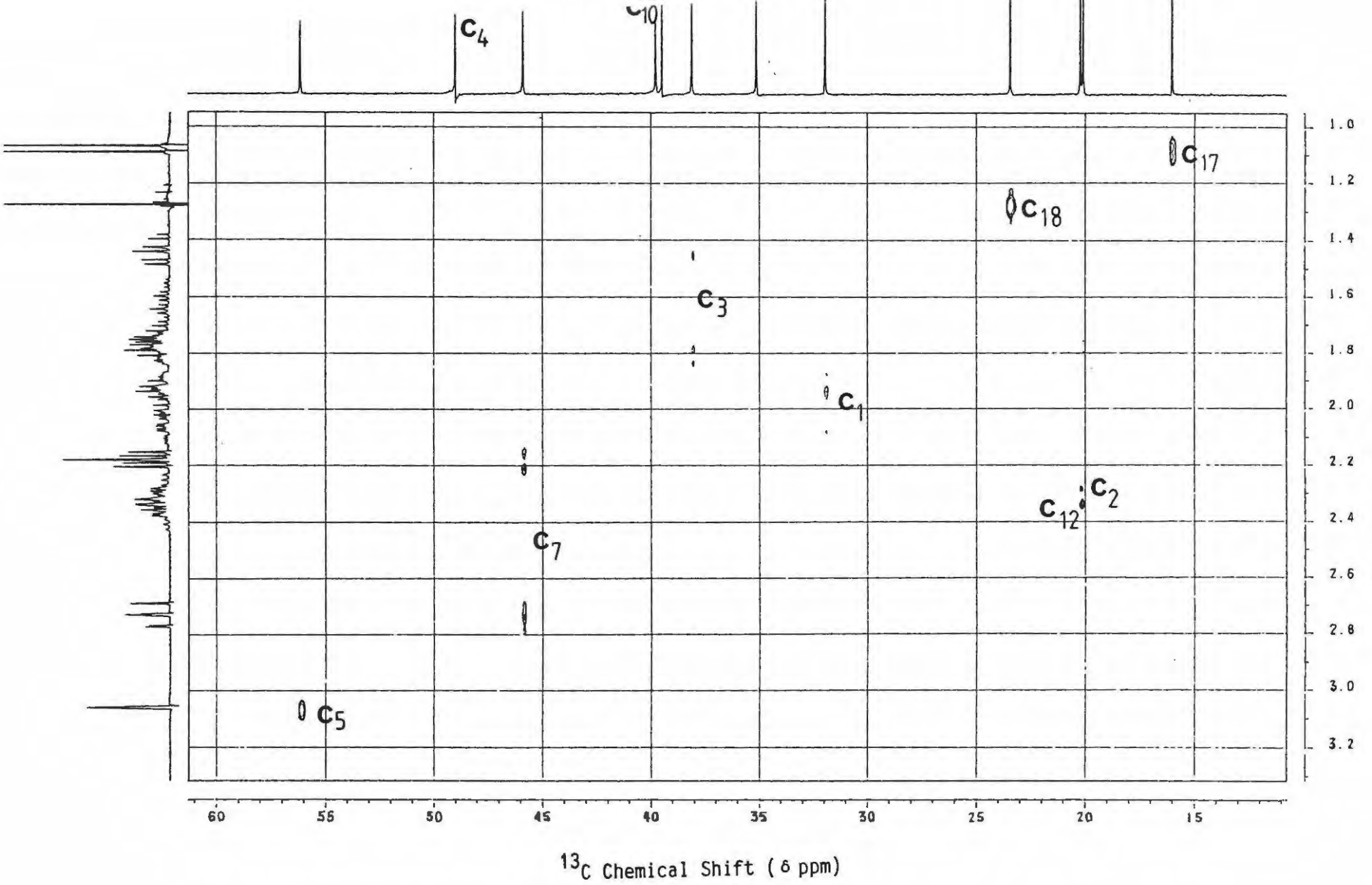
Hispanalone HETCOR experiment in $CDCl_3$ (^1H δ 6.0 - 7.5 ppm, ^{13}C δ 100 - 150 ppm)



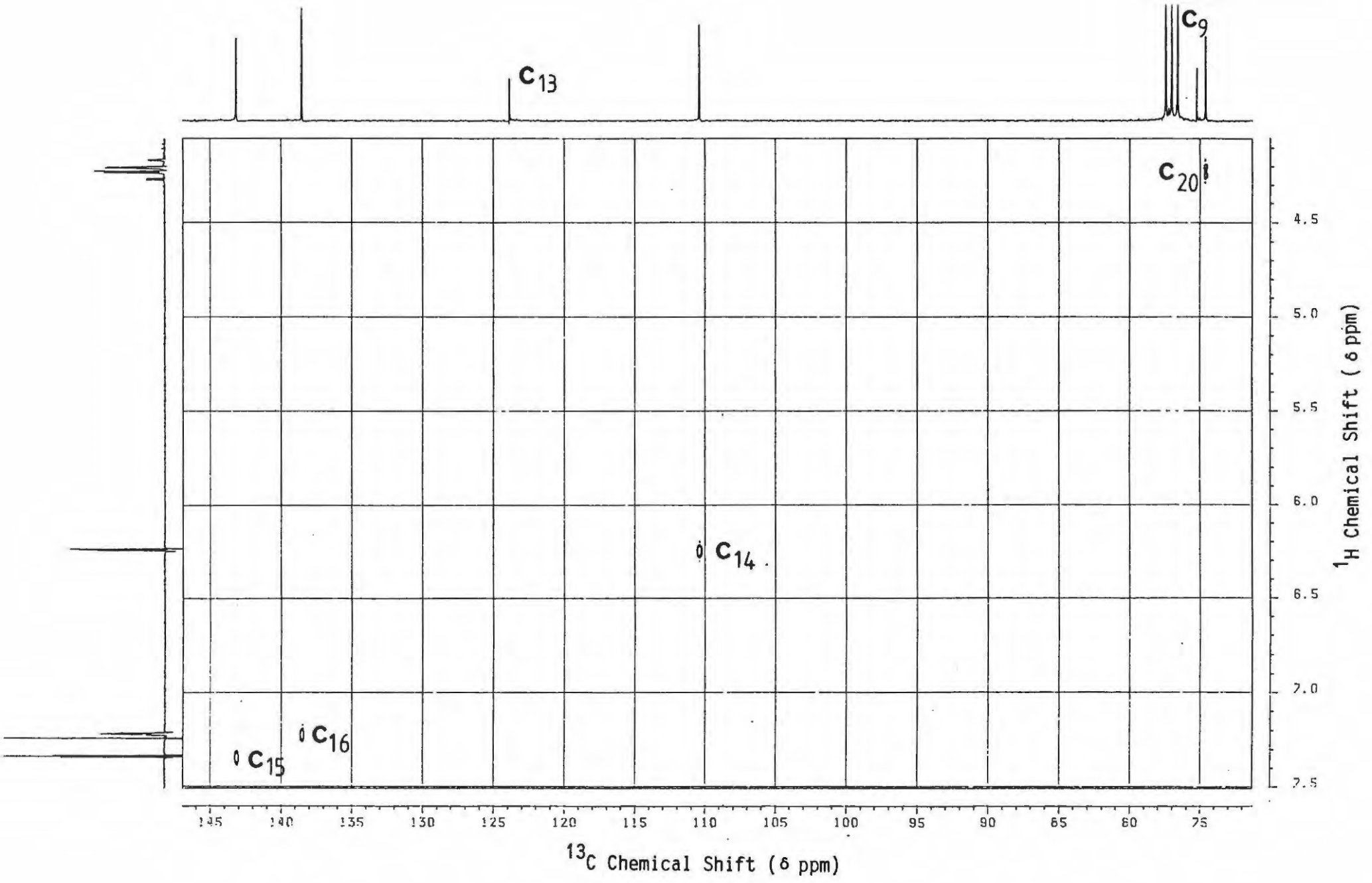
(162)

6- Deacetyldehydrodubiin (162) COSY experiment in $CDCl_3$*

*Connectivities not mapped out



6- Deacetyldehydrodubiin HETCOR experiment in $CDCl_3$ (^1H δ 1.0 - 3.2 ppm, ^{13}C δ 10 - 60 ppm)



6- Deacetyldehydrodubiin HETCOR experiment in $CDCl_3$ (^1H δ 4.0 - 7.5 ppm, ^{13}C δ 70 - 150 ppm)

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