EXTRACTIVES FROM THE MELIACEAE

by

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PREFACE

The experimental work described in this thesis was carried out in the Department of Chemistry, University of Natal, Durban from January 1978 to December 1979, under the supervision of Professor D.A.H. Taylor.

These studies represent original work by the author and have not been submitted in any form to another University.

Signed: D. A. Mulholland
D.A.Mulholland

I hereby certify that the above statement is correct.

Signed: David H. Taylor
Professor D.A.Taylor,
M.A., D.Phil., D.Sc.(Oxon),
Supervisor

SUMMARY

Extractives from the seeds of *Aphanamixis polystacha* and *Trichilia dregeana*, and from the heartwood of *Entandrophragma spicatum* have been examined.

A further two compounds, polystachin (or rohituka 9) (48) and rohituka 10 (49) have been isolated from the *A. polystacha* extract.

The *T. dregeana* extract yielded rohituka 7 (45), dregeana 1 (49), 2 (35), 3 (34), 4 (57) and 5 (58). Dregeana 1 (49) was found to be the same as rohituka 10 (49).

A further two compounds, spicatin (63) and spicata 2 (70) have been obtained from the heartwood of *E. spicatum*.

ACKNOWLEDGEMENTS

I would like to express my appreciation to Professor D.A.H. Taylor for all the interest he has shown, and the advice he has given, throughout the duration of this project.

I would also like to thank all the other members of the Chemistry Department who have assisted me over the past two years.

I am grateful to Dr. P. Bladon of the University of Strathclyde for providing mass spectra, to Mrs. E. Serfontein and Mrs. F. Fletcher for typing this thesis, to my husband, Michael Mulholland for his help and the Council for Scientific and Industrial Research for a post-graduate bursary.

ABBREVIATIONS

AcO	acetate
^{13}C n.m.r.	carbon (13) nuclear magnetic resonance
d	doublet
g.l.c.	gas liquid chromatography
^{1}H n.m.r.	proton nuclear magnetic resonance
Hz	Hertz
Me	methyl
m	multiplet
n.m.r.	nuclear magnetic resonance
p.p.m.	parts per million
q	quartet
s	singlet
t	triplet

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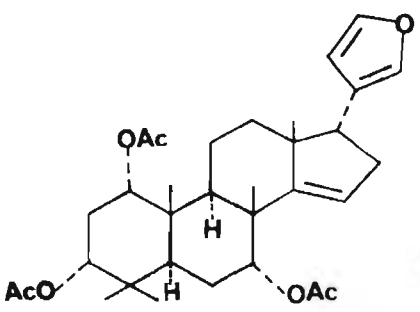
CHAPTER ONE

THE BIOGENESIS OF THE LIMONOIDS

Limonoids are compounds having a C_{22} nucleus attached to a furan ring. The nucleus may occur in many forms. It is thought[1] that the biochemically basic form is the one found in deoxyhavanensin (1) and that the more complex forms are derived from this, or similar types of compounds, by means of oxidative opening of one or more of the carbon rings, usually followed by recyclisation in some other way.

Research in the field of the chemistry of the Meliaceae was begun in the 1950's with the isolation of the compound gedunin (2) by Bevan and co-workers from the timber of *Entandrophragma angolense*.[2] At about the same time, King isolated a related compound, dihydrogedunin (3) from *Guarea thompsonii*.[3]



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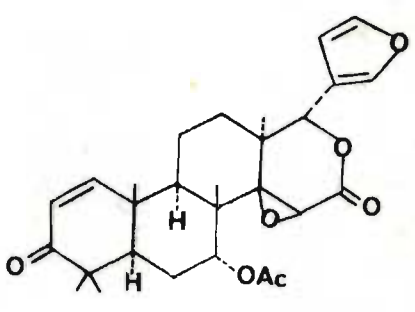
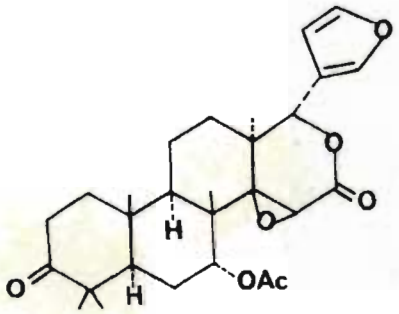


(2)

In 1960, Arigoni, Barton, Corey, Jeger and associates elucidated the structure of limonin (4), an extractive from citrus fruits.[4] The similarity between gedunin (2) and limonin (4) was noticed and the structure of gedunin (2) was worked out.[5]

Further work has shown that these types of compounds are common in the Meliaceae. These substances are referred to as meliacins, tetranortriterpenoids or, more commonly, limonoids.



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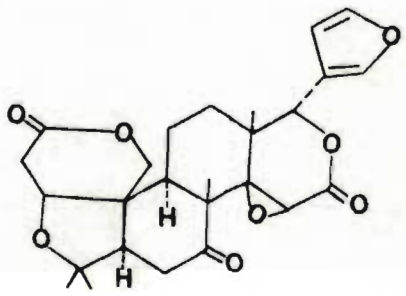
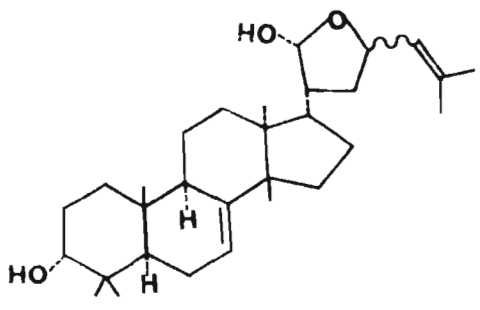


(4)

Many compounds belonging to the C_{30} triterpene group which appear very similar to the limonoids in structure but lack the furan ring, are known. They are called the 'protolimonoids'. The oxygenation pattern of their side chain makes it seem likely that they are degraded to form limonoids. An example of this type of compound is flindissol (5).

It seems likely that the quassinoids, the group of compounds related to quassin (6), whose structure was elucidated in 1962,[6] share a common biosynthetic pathway with the limonoids.



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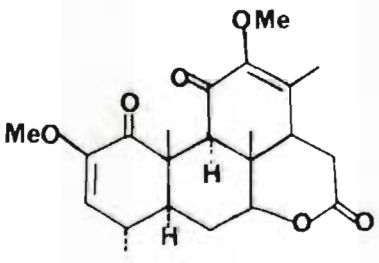


(6)

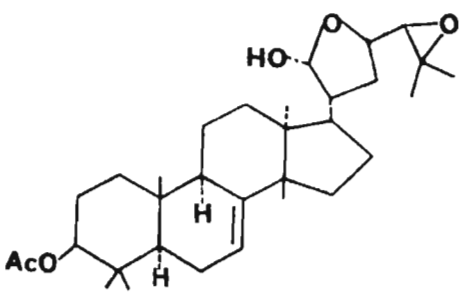
Limonoids have been isolated from the Rutaceae, Meliaceae and Cneoraceae families. The quassinoids are found in the Simaroubaceae.[1]

In the Meliaceae, limonoids occur commonly in the timber, seed and bark and have been found more rarely in the leaves. The timber of a given plant often contains one, or a few related limonoids which are characteristic

and this has been used as a basis of timber identification by means of thin layer chromatography.[1]

Taylor[1] has divided the limonoids isolated from the Meliaceae into seven types.

Type A are the protolimonoids, such as flindissol (5), turreanthin (7) and grandifoliolenone (8). Protolimonoids have been isolated from species belonging to the Meliaceae and Rutaceae families. They may represent incompletely metabolized intermediates in the biosynthesis of the limonoids, or, by-products from the main biosynthetic line.



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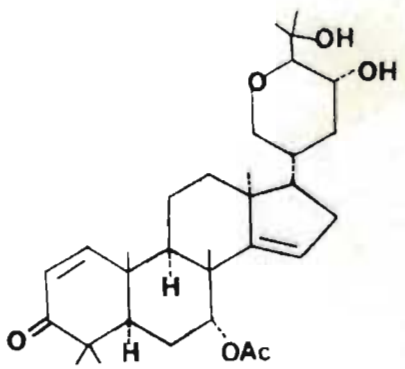
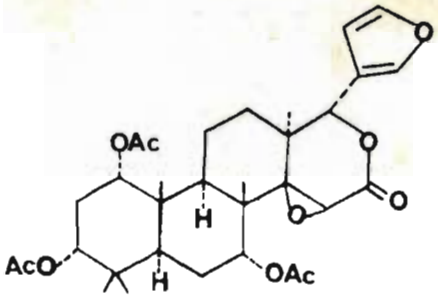


(8)

Limonoids of type B have the ring skeleton of deoxyhavanensin (1). These compounds represent the simplest true limonoids and have been obtained in minor amounts from many species. This type of limonoid is believed to be intermediate in the production of all other types of limonoids.

Limonoids of type C represent the first stage in a major biosynthetic division, being the intermediaries in the biosynthesis of the type D and type E groups, but not the type F or G groups. Type C compounds have the ring D skeleton as in gedunin (2) and khivorin (9).



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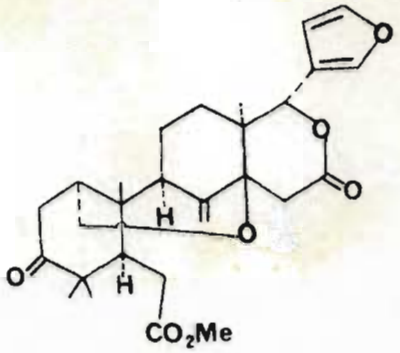

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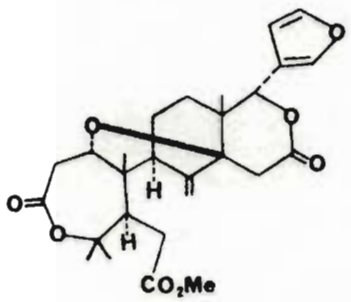
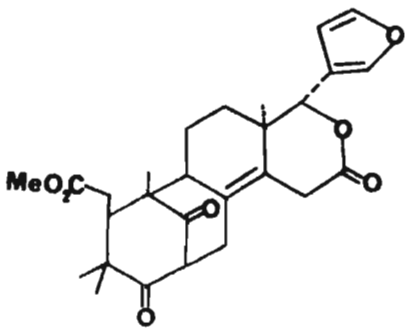


(11)

The type D compounds are derived from type C compounds which have undergone oxidative opening of ring B. Type D compounds may be further divided into two subgroups. The compounds of the first subgroup, of which methyl angolensate (10) is an example, give rise to the type K compounds by the oxidative opening of ring A into a seven-membered lactone ring. An example of a type K compound is methyl ivorensate (11). The second subgroup contains substances in which the opening of ring B has been followed by recyclisation to give a bicyclononanolide skeleton. Mexicanolide (12) is the simplest example of a compound having a bicyclononanolide-type skeleton. Compounds of the mexicanolide-type are probably intermediates in the biosynthesis of the type E compounds.



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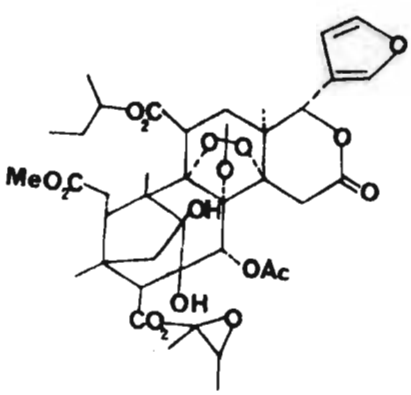
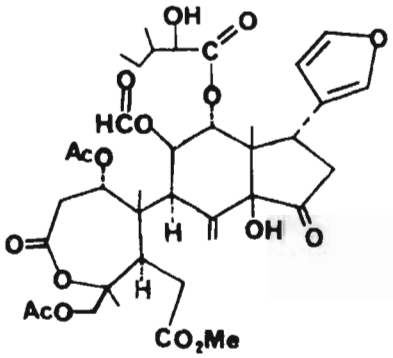


(13)

The type E compounds are the most developed limonoids having a ring D-lactone system. An example of a type E compound is utilin (13). They are thought to arise from a mexicanolide-type compound by the closure of a ring between one of the methyl groups on C-4 and C-1 to give a bridged ring A.

Prieurianin (14) is an example of a limonoid of type F. Rings A and B have been oxidatively opened, but ring D has not. This type of compound probably arises from a type B compound. Further oxidation of class F compounds gives rise to complex molecules.



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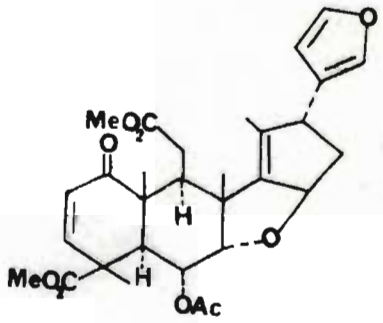


(15)

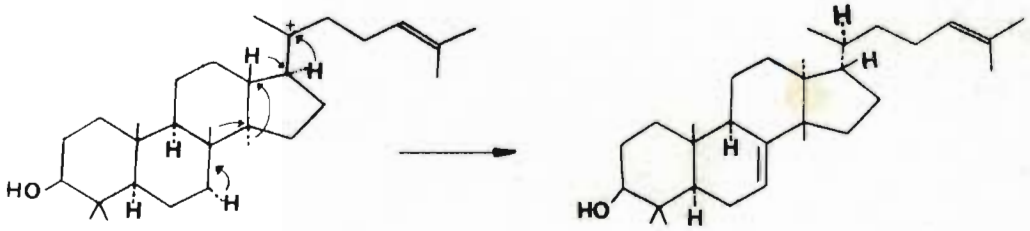
Type G compounds are found in the limonoids from *Melia* and *Azadirachta*. Rings A, B and D remain carbocyclic, but ring C is opened, either to a methyl ester or a lactone. Further oxidative changes may occur giving complex substances such as nimbin (15).

Various schemes have been proposed suggesting how the different limonoids might be produced in the plant.

It is believed[7] that the limonoids are produced from the hypothetical triterpene (16), or its 20-epimer. The triterpene (16) is the Δ7-isomer of tirucallol, the 20-epimer being butyrospermol. It has been suggested[8,9] that this compound (16) may be formed directly from

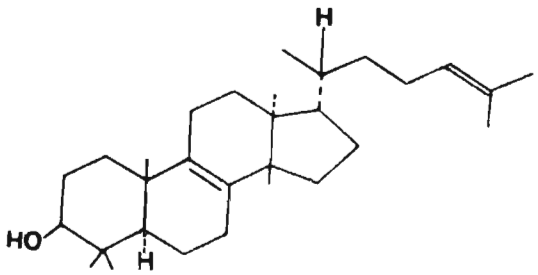
the cationic intermediate (17) resulting from the cyclisation of squalene, without the intervention of euphol (18) (Scheme 1.1).

Scheme 1.1



(17) (16)

It is not certain whether limonoids are derived from a euphol (20βH)- or tirucallol (20αH)-type compound, since C-20 becomes trigonal in the course of rearrangement. Both types of configurations have been found in related C-30 compounds in which the configuration at C-20 has been established. Bourjotinolone B (19) and bourjotone (20) have the tirucallol-, and kulinone (21) has the euphol-, configuration.



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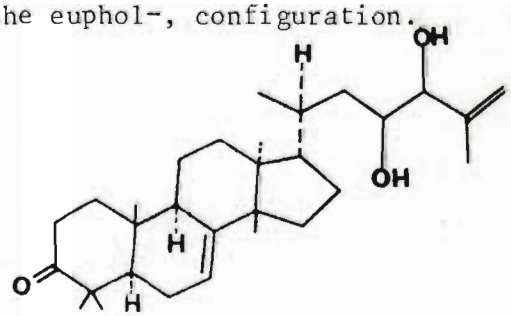

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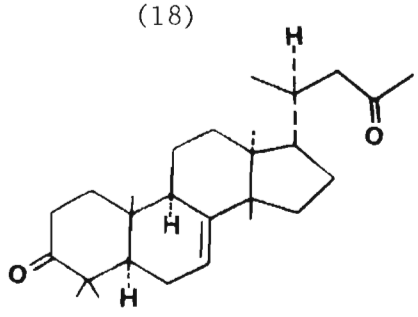

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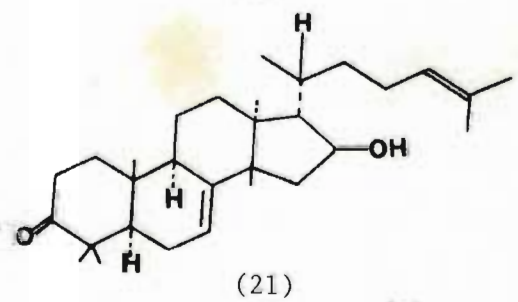
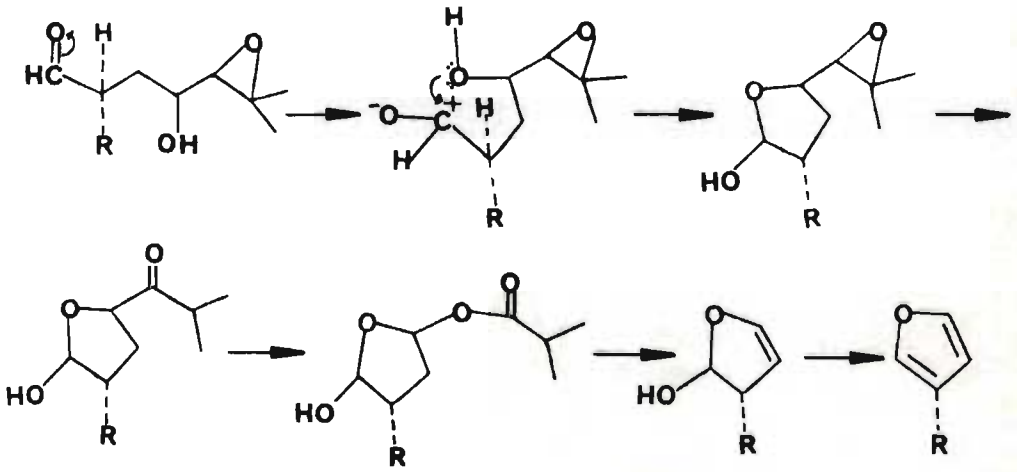


(21)

Arigoni, Barton, Corey, Jeger and their associates[4] have suggested that two processes occur in the formation of limonoids from a tirucallol-, (euphol-), type precursor. These two processes are the oxidative cleavage of the side chain between C-23 and C-24 and an apo-euphol type rearrangement in which a methyl group migrates from C-14 to C-8.

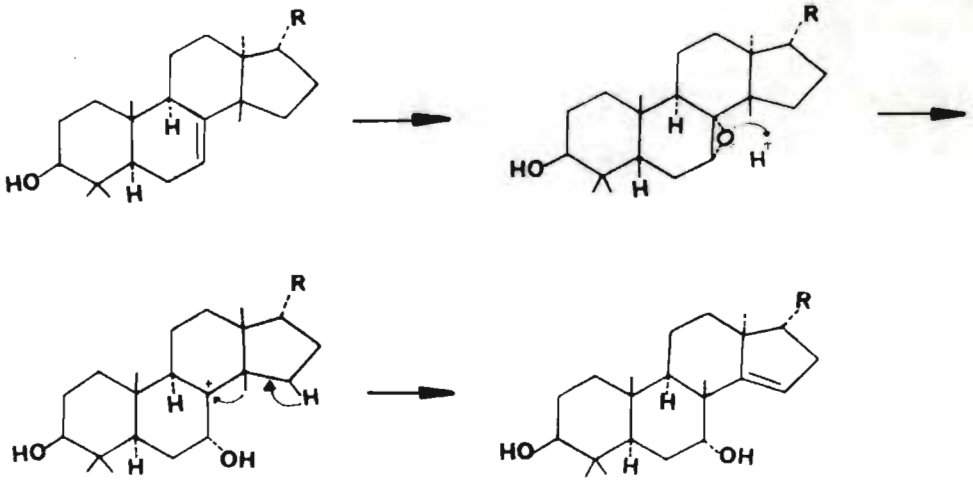
Halsall and co-workers[10] have proposed a scheme for the conversion of the euphol side chain into the β-substituted furan ring (Scheme 1.2). Their proposal is supported by the occurrence of compounds such as flindissol (5) and turreanthin (7). The 24-ketone could arise from the 24,25-epoxide by rearrangement or via formation of the glycol and subsequent oxidation of the 24-hydroxyl group.[11] Fission of the C-23, C-24 bond of the side chain is believed to occur by a Baeyer-Villager oxidation analogous to that which occurs in the biosynthesis of nyctanthic acid (22)[12,13] or methyl angolensate (10)[14], producing the dihydrofuran which, on dehydration, would yield the β-substituted furan.

Scheme 1.2



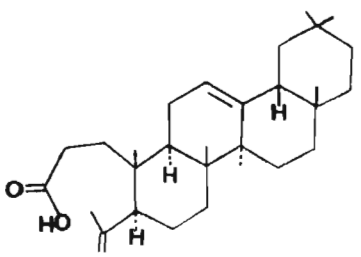
Cotterrell, Halsall and Wriglesworth[9] have proposed Scheme 1.3 for the apo-euphol rearrangement. The nuclear double bond is oxidised to an epoxide which undergoes acid-catalysed isomerisation with a shift of a methyl group from C-14 to C-8 giving a Δ^{14}, 8-methyl, 7α-hydroxy compound.

Scheme 1.3



Although neither Scheme 1.2 nor Scheme 1.3 has been shown to occur in the plant, both have been performed in the laboratory by Halsall and co-workers in the conversion of turreanthin (7) into the diol (25).[15,16]

Turreanthin (7) was transformed, by means of sodium metaperiodate in aqueous dioxan containing a trace of perchloric acid, into the hemiacetal (23), which was dehydrated to the furan (24) by toluene-p-sulphonic acid in benzene. The 7α, 8α-epoxide obtained with monoperphthalic acid rearranged with boron trifluoride etherate to the apo-compound which was hydrolysed to the diol (25).



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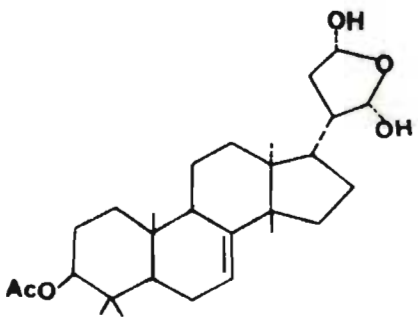

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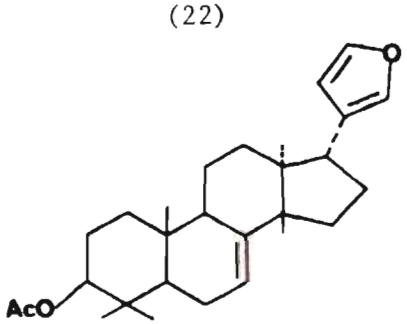

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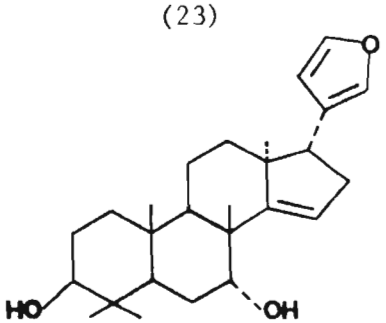
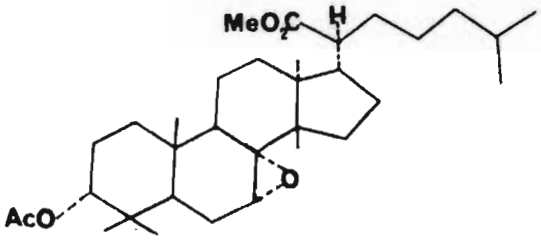


(25)

The methyl group rearrangement has been accomplished prior to side chain degradation in the laboratory.[9] The epoxide from methyl acetyl-dihydro-α-elemolate(26)rearranged with boron trifluoride etherate to the apo-derivative (27).



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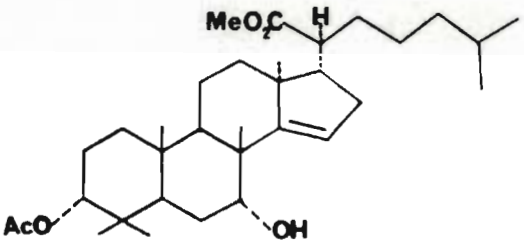
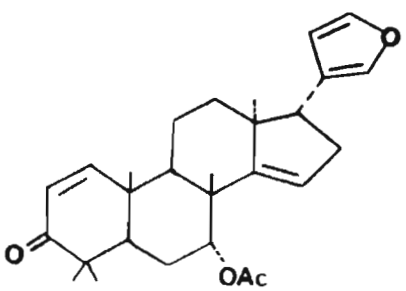


(27)

These experiments indicate that either step could occur first. However, the structure of grandifoliolenone (8), a compound which has undergone the methyl group migration but does not have a furan ring, provides circumstantial evidence that the rearrangement may precede side chain degradation.

By means of Schemes 1.2 and 1.3, the type B limonoids could be formed from the type A protolimonoids.

Arigoni, Barton, Corey, Jeger *et al* have proposed Scheme 1.4 for the oxidation of ring D.[4] Their proposal is supported by the occurrence of compounds representing each of the stages. Azadirone (28) is an example of (i), azadiradione (29) is an example of (ii), grandifolione (30) is an example of (iii) and gedunin (2), an example of (iv). In this manner, type B compounds could be converted to the type C compounds.



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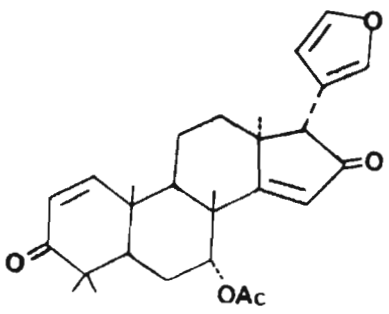

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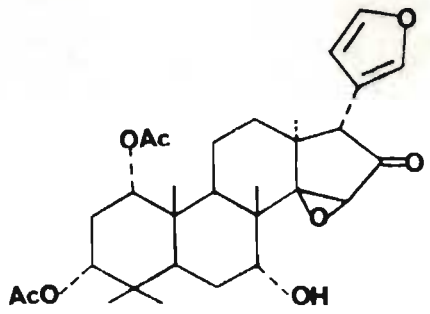
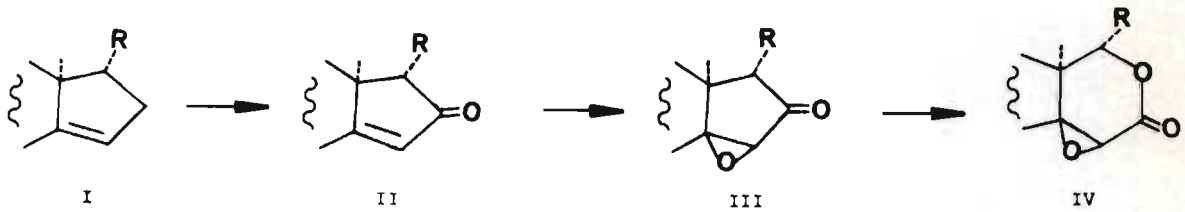


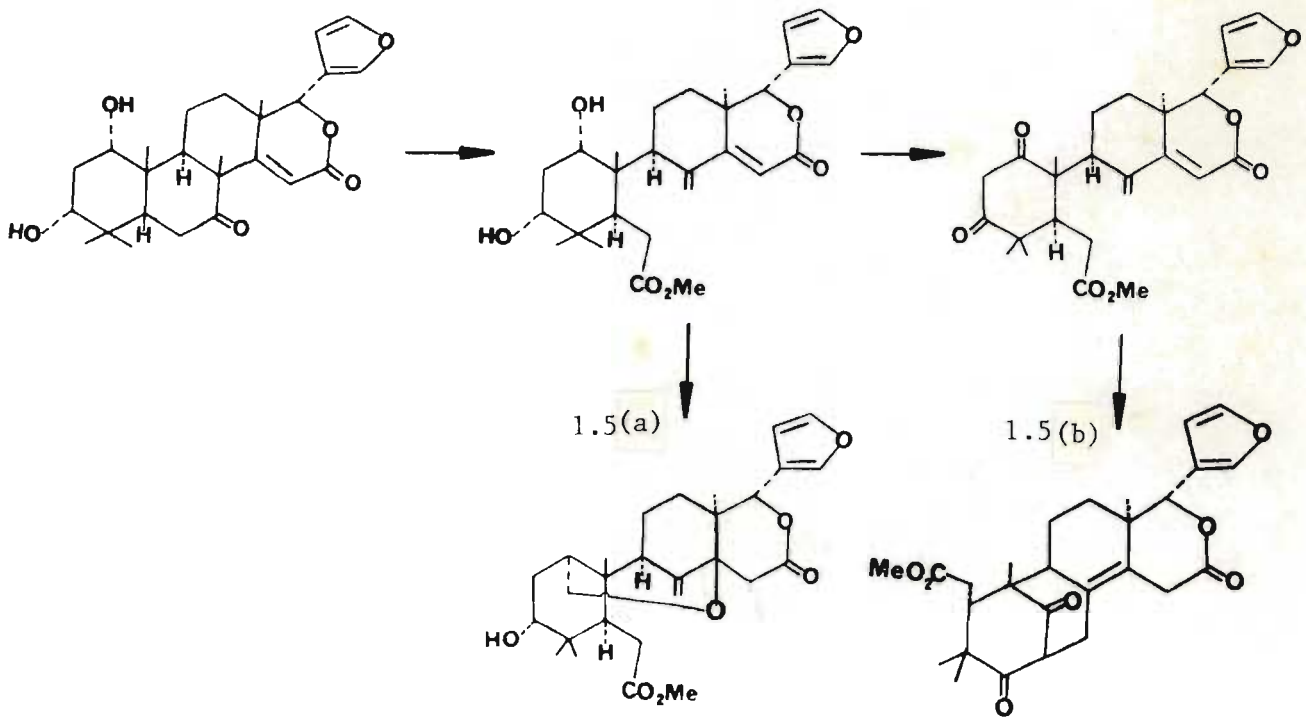
(30)

Scheme 1.4



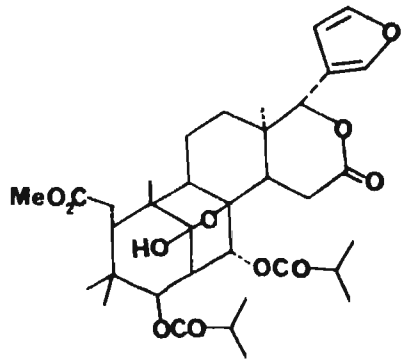
Scheme 1.5 has been proposed[1] to account for the way in which ring B may be opened to form a type D compound from a type C compound. Scheme 1.5(a) shows how a methyl angolensate-type of compound could be formed and Scheme 1.5(b) shows how a further skeletal change might occur to form the cyclononanolide skeleton of the mexicanolide-type of compounds.

Scheme 1.5

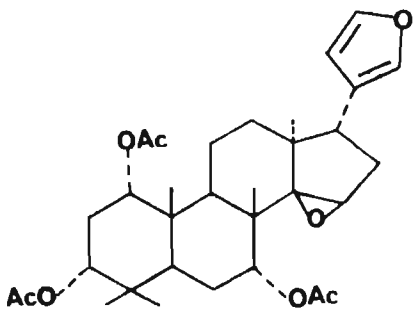


The reactions in Schemes 1.4 and 1.5 have been performed in the laboratory.[17,18,19,20,21] As these reactions consist of simple oxidations and spontaneous cyclisations, it is probable that these are the routes followed in the plant.[1]

The type E compounds are thought to have arisen from a mexicanolide-type compound by closure of one of the methyl groups on C-4 and C-1 to give a bridged ring A. It is not known how this change might occur, but it is possible that the biosynthesis involves remote oxidation of the C-4 methyl group by an oxygen radical at C-1 in a compound such as xyloccensin (31), (the only compound that has been isolated which is an intermediate between stages D and E), followed by a radical cyclisation.[1] Xyloccensin B (31), co-occurs with compounds of types D and E in *Xylocarpus moluccensis*.[22]



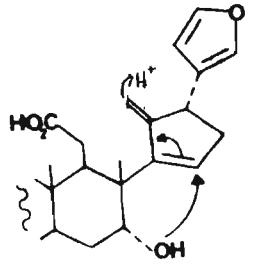
(31)



(32)

Limonoids of the prieurianin (14)-type (Type F) are thought to have arisen from havanensin (32), a simple oxidation product of deoxyhavanensin (1), a type B compound. It is thought that rings A and B are opened by the same type of Baeyer-Villager oxidations described in Scheme 1.5.[1]

The biosynthesis of type G compounds (such as nimbin (15)) seems to involve a cleavage of the C-12, C-13 bond in ring C analogous to the cleavage which occurs in ring B. Addition of a 7α-hydroxy group to C-15 of the diene acid (33), would then produce nimbin (15).[7]

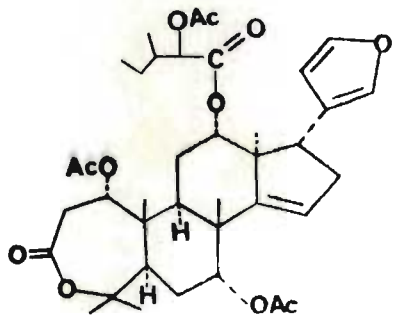


(33)

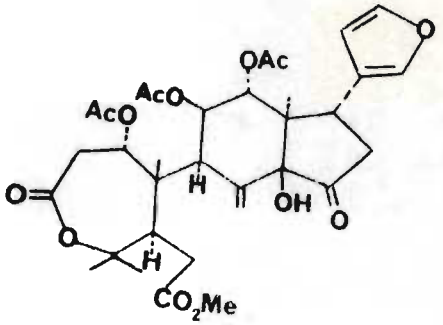
Compounds recently isolated from *Trichilia dregeana*,[23] *Cneorum tricoccon* and *Neochamaelea pulverulenta*,[24] enable this proposed biosynthetic pathway to be extended.

Compounds have been found in these species which have undergone ring A opening to form a 7-membered lactone, but whose ring B structure is intact. These compounds fall into three types, H, I and J.

The type H compounds have been found in the seeds of *Trichila dregeana*. They have unoxidised ring B and D systems, but ring A has been oxidatively opened to form a 7-membered lactone ring. An example is the compound dregeana 3 (34). Co-occurring with these type H compounds are some type F compounds, for example dregeana 2 (35). (See Chapter Three).[23] This suggests that type H compounds could be intermediaries between the type B and type F compounds.

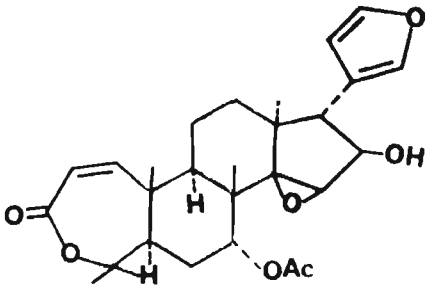


(34)



(35)

The type I compounds are derived from compounds with the azadiradione-type ring D system, which have undergone ring A opening to form a 7-membered lactone ring. An example of a type I compound is S_{22} (36) which has been isolated from *Cneorum tricoccon*.[24]



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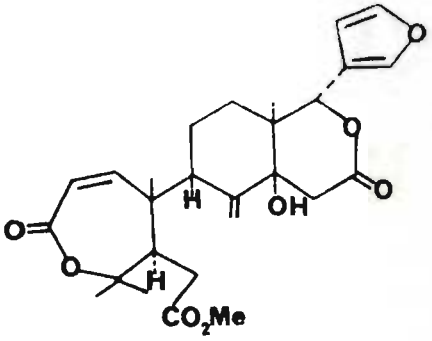
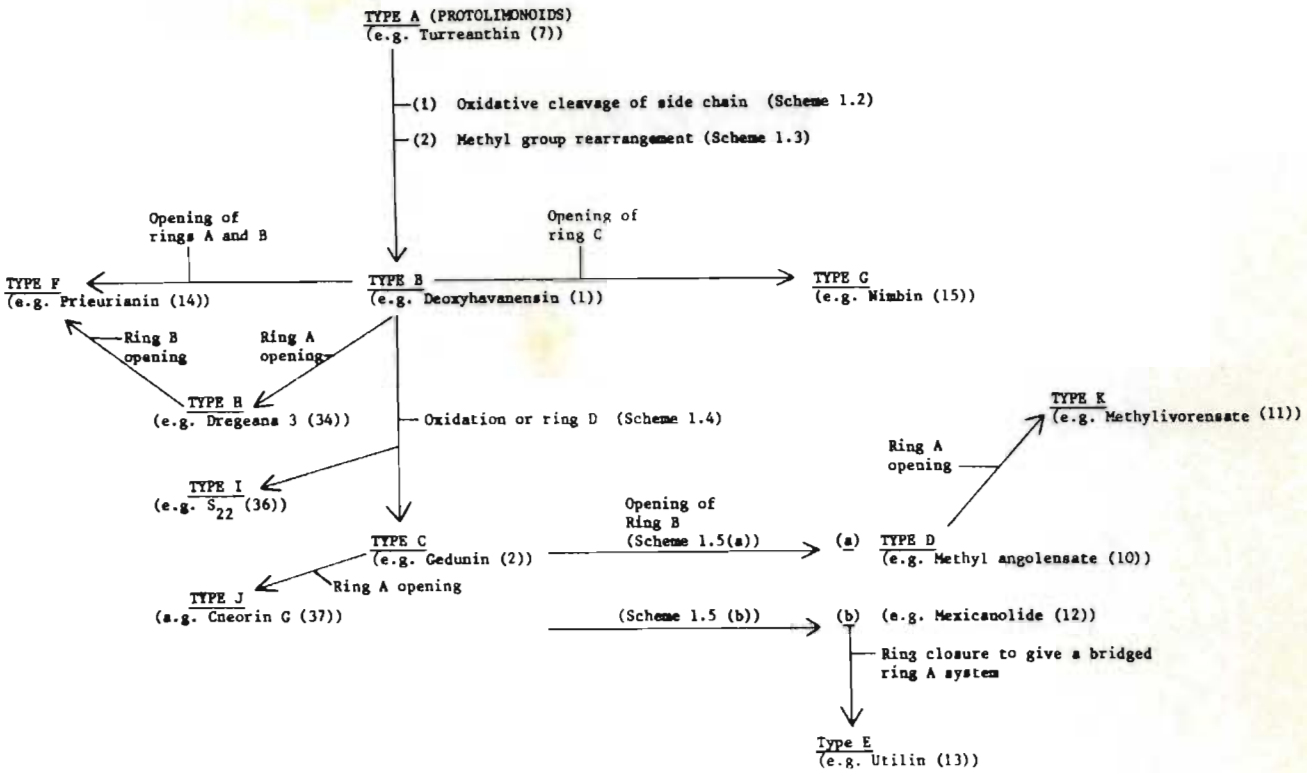


(37)

Type J compounds have the gedunin (2)-type ring D system, but have the ring A lactone system. An example of this type of compound is Cneorin G (37) which has been isolated from *Neochamaelea pulverulenta*.[24]

The biosynthetic scheme proposed here has been summarized in Scheme 1.6. The reactions in this scheme are all peracid oxidations, catalysed by various peroxidases in the plant.

Scheme 1.6



Summary of the scheme proposed for the biosynthesis of the limonoids

CHAPTER TWO

EXTRACTIVES FROM THE SEED OF *Aphanamixis polystacha*

2.1 Introduction

Aphanamixis polystacha (Wall) J.N. Parker (Synonyms *Amoora rohituka* Wight et Arn, *Aphanamixis rohituka* (Roxb. Pierre), is an Indo-Malayan member of the Meliaceae family.

Several limonoids have been isolated from the seeds of this tree.[25,26,27,28] These compounds are of the prieurianin (14)-type and as their structures have been worked out using the structure of prieurianin as a basis, the structural elucidation of prieurianin will be reviewed.

2.1.1 The structure of prieurianin (14)

Prieurianin (14) was isolated in 1965 as a crystalline substance from the wood of *Trichilia prieuriana*.[29] It took about ten years for its structure to be worked out, partly because of the unresolved nature of its ^{1}H n.m.r. and ^{13}C n.m.r. spectra at ambient temperatures. Only when the spectra were recorded at elevated temperatures did the peaks resolve themselves.

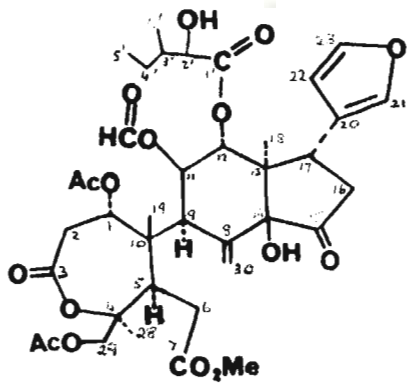
Prieurianin was assigned the molecular formula $C_{38}H_{50}O_{16}$ by

means of the technique of high resolution mass spectroscopy.

Using proton-noise and continuous-wave decoupling techniques, resonances could be assigned to all thirty-eight carbon atoms. The spectra showed resonances which were attributed to a formate group at 161.8(d) p.p.m., five esters or lactones at 168.8(s), 170.3(s), 170.4(s), 174.7(s) and 177.5(s) p.p.m., two ethylenic carbons at 125.7(t) and 139.3(s) p.p.m., two hydroxy groups and a furan ring. This accounted for all the oxygen atoms in the molecule.

The ^{1}H n.m.r. spectrum included resonances attributable to a formate group (δ8.02), a β-substituted furan ring (δ6.44, 7.35 and 7.46), [illegible] (δ1.64, δ1.77, δ1.77), a secondary methyl group (δ1.33), a methoxycarbonyl group (δ3.69) and two acetate groups (δ2.08 and 2.14).

Hydrolysis experiments showed the presence of a 2-hydroxy-3-methylvalerate ester, a formate and two acetate groups in prieurianin.



(14)

From ^{1}H n.m.r. data, the arrangement and stereochemistry of H-9, H-11 and H-12 and also the arrangement of the 16-H and 17-H proton systems could be deduced. The ^{1}H n.m.r. spectrum showed that the furan ring was β-substituted. The J_{gem} of 20 Hz for 16-CH_2 indicated its being adjacent to a ketone. Weak coupling (0.5 Hz) between 1'-H and 11-H indicated that the formate group was positioned at C-11. The position of

the 2'-hydroxy-3'-methylvalerate side chain was established to be at C-12 because H-23 moves from δ7.36 to δ7.44 on acetylation of the 2'-OH group of the ester. The α-configuration of the 13-CH_3 group is common to all limonoids so it was assumed to be in the same configuration here. The stereochemistry at C-1, C-4 and C-14 was not known.

A three-dimensional X-ray analysis of the crystal structure of prieurianin 2'-p-bromobenzenesulphonate confirmed the deductions from the n.m.r. spectroscopy and showed the stereochemistry at C-1, C-4 and C-14.

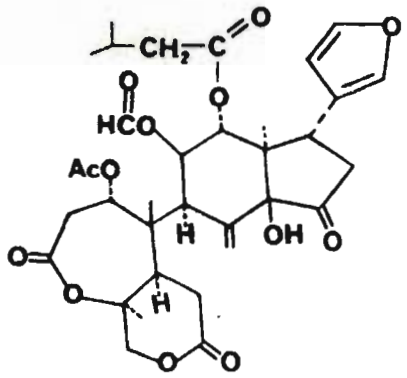
2.1.2 Limonoids from the seed of *Aphanamixis polystacha*

Several specimens of *A. polystacha* are found cultivated in Durban. Seeds gathered from a tree in the Durban Botanical Gardens, from one on the University campus and from several growing in the grounds of D.H.S. Old Boys Club were used.

The hexane extract of the seeds was examined by Bredell.[25,26] The main component of the extract was found to be the crystalline compound, rohitukin (38). The ^1H n.m.r. spectrum of rohitukin showed that it was a prieurianin-type of compound, but that it lacked the methyl ester and one of the acetate groups and had a different ester at C-12. Hydrolysis of rohitukin showed that the acid present at C-12 was 3'-methylbutyric acid, and that an extra lactone ring was present. As there was no methyl ester present, it was deduced that there was a 7,29-lactone, giving the structure (38) for rohitukin.

Examination of the mother liquors resulting from the re-crystallisation of rohitukin yielded a further eight compounds, rohituka

1-8 (39-46) of which only rohituka 7 (45) was crystalline.[27,28]



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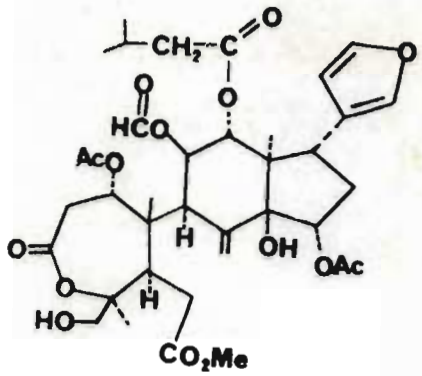

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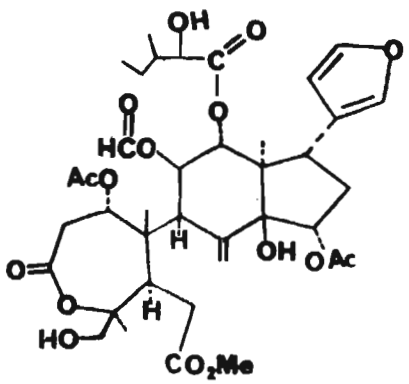

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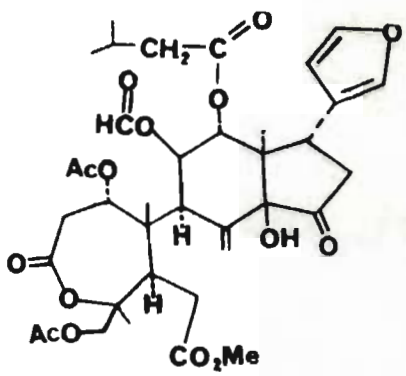

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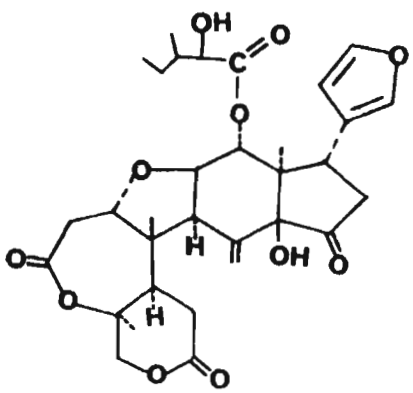

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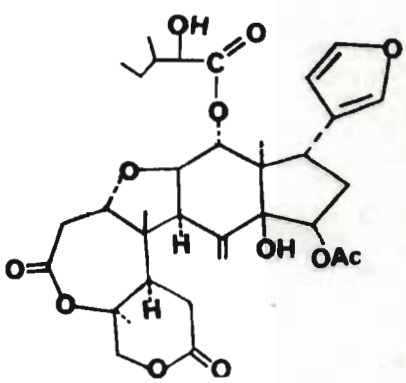

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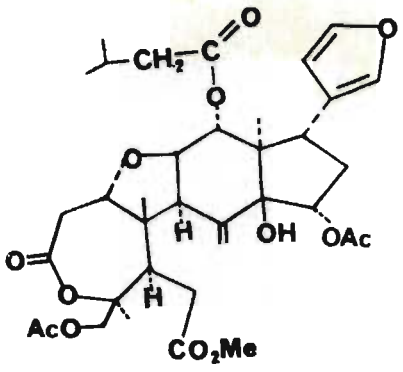

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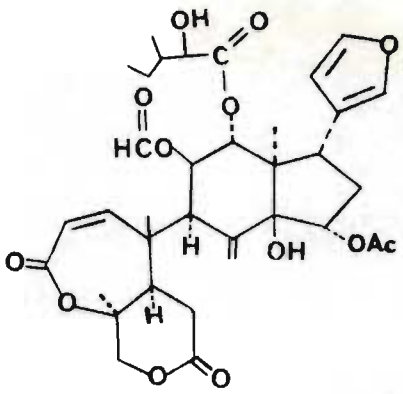

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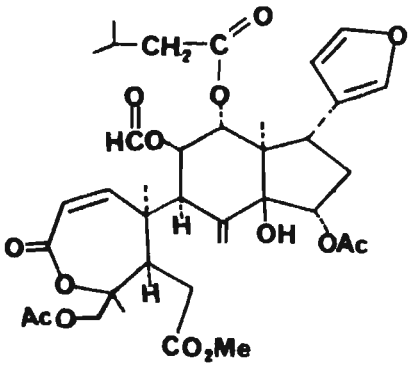

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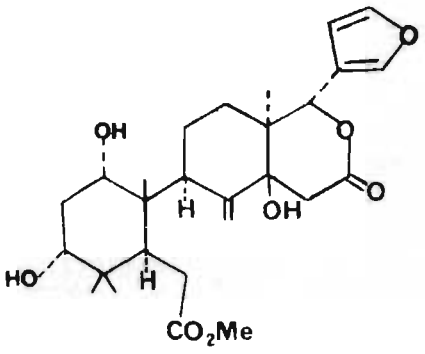


(47)

The ^{13}C n.m.r. spectrum of rohituka 2 differed from that of prieurianin (14) in that the resonance due to the C-15 ketonic carbonyl group of prieuranin was missing but an extra resonance in the C-O region of the spectrum was present. The ^{1}H n.m.r. spectrum of rohituka 2 showed an extra one proton multiplet at δ5.70. This suggested that rohituka 2 differed from prieurianin by the C-15 carbonyl group of prieurianin being replaced by an acyl group. The ^{1}H n.m.r. spectrum had resonances attributable to a methoxycarbonyl group, two acetates, a formate group, and the 2'-hydroxy-3'-methylvalerate ester. Thus, structure (40) was assigned to rohituka 2.

The ^{1}H n.m.r. spectrum of rohituka 1 lacked the resonances attributable to the 2'-hydroxy-3'-methylvalerate ester, but had resonances corresponding to a 3'-methylbutyric acid residue, as in rohitukin (38). In other ways, the spectrum was identical to that of rohituka 2 (40), so structure (39) was assigned to rohituka 1.

The ^{13}C n.m.r. spectrum of rohituka 3 indicated the presence of a ketonic carbonyl group at C-15. The ^{1}H n.m.r. spectrum showed resonances due to the 2'-hydroxy-3'-methylvalerate ester and a 1,11-oxide bridge but no resonances due to formate, acetate nor carbomethoxy groups. On this basis, rohituka 3 was assigned structure (41).

The ^{1}H n.m.r. spectrum of rohituka 4 was identical to that of prieurianin (14) except that it lacked the resonances due to the 2'-hydroxy-3'-methylvalerate group and had resonances ascribable to a 3'-methylbutyrate group. Structure (42) was assigned to rohituka 4.

The ^{1}H n.m.r. spectrum of rohituka 5 closely resembled that of rohituka 3, but it had an extra acetate group, and a one proton multiplet at δ5.57 as was present in rohituka 1 (39) and 2 (40). Thus it was believed that rohituka 5 was the 15-acetate analogue of rohituka 3 (41) and was assigned structure (43).

Rohituka 6 was found to have resonances due to a methoxycarbonyl group, a 1,11-oxide bridge, a 3'-methylbutyrate group, and a 15-acetate group in its ^{1}H n.m.r. spectrum. Structure (44) was proposed for this compound.

Rohituka 7 was crystalline. The ^{1}H n.m.r. spectrum and double resonance experiments showed that the compound had a 7,29-lactone ring, a formate group, a 2'-hydroxy-3'-methylvalerate group, and an α,β-unsaturated ring A lactone. The ^{13}C n.m.r. spectrum showed that rohituka 7 had a 15-acetate group. Structure (45) was assigned to this compound.

The ^{1}H n.m.r. spectrum of rohituka 8 showed the presence of a carbomethoxy group, a second acetate group which was present at C-29 and a 3'-methylbutyrate ester at C-12. In other respects, the spectrum was identical to that of rohituka 7 (45), so structure (46) was assigned to rohituka 8.

The ^{1}H n.m.r. data of rohitukin (38) and rohituka 1-8 (39-46) are included in Table 5.1 and their spectra are given in Chapter Six for purposes of comparison.

2.2 Further compounds from the seed of *Aphanamixis polystacha*

The hexane extract of a second batch of seeds from a tree on the University campus has now been examined and has yielded the crystalline compounds rohitukin (38) and rohituka 7 (45), and after repeated column chromatography of the mother liquors, polystachin, or rohituka 9,[30] and rohituka 10, both of which were amorphous.

Polystachin (rohituka 9)

Polystachin was found to have an ^{1}H n.m.r. spectrum (Spectrum H-13) very similar to that of rohitukin (38) (Spectrum H-1). The mass spectral weight of polystachin was found to be 598.2396 which corresponds to a molecular formula of $C_{32}H_{38}O_{11}$. This is 60 units less than the molecular weight of rohitukin and suggests the loss of an acetic acid

molecule. In agreement with this, the ^{1}H n.m.r. spectrum of polystachin does not show the resonance at δ2.1 (due to the acetate group at C-1) which is present in that of rohitukin. The resonance at δ5.25 in the spectrum of rohitukin, which is ascribed to the H-1β proton, is also missing in the spectrum of polystachin.

The presence of the usual ring C system was established by double resonance experiments. The resonance signal of H-12 appeared as a doublet (δ6.05, J = 10 Hz) coupled to a quartet (δ5.32, H-11). On irradiation of this quartet, the doublet collapsed, the singlet due to the formate proton (δ8.06) sharpened, and a second doublet (δ2.73, J = 5 Hz), attributable to H-9, collapsed. Irradiation of this H-9 doublet caused the H-11 resonance to collapse to a doublet and the two superposed vinyl proton resonances (δ5.48, bs) to become sharpened.

The proton magnetic resonance spectrum also showed three singlets (δ1.97, 1.02, 0,86) due to three tertiary methyl groups, a two proton multiplet at δ4.14, similar to that due to the lactone $-CH_2O-$ at C-29 in rohitukin (38), a single proton multiplet at δ3.88 ($W_{\frac{1}{2}}$= 19 Hz) corresponding to the resonance of H-17 in rohitukin, a single proton multiplet at δ3.78 ($W_{\frac{1}{2}}$= 16 Hz) and several other resonances between δ2 and δ3 which were not clearly resolved. The spectrum was not temperature sensitive in the range 20-60°. This suggests that free rotation about the 9-10 bond does not occur in polystachin.

The ^{13}C n.m.r. spectrum (Spectrum C-1) showed one ketonic carbonyl resonance (δ205.1) corresponding to the C-15 resonance of rohitukin (38), three ester carbonyl singlets (δ172.3, 172.3, 167.5) and a doublet (δ160.2) attributable to the formate carbonyl group.

There were six resonances representing carbon atoms singly linked to oxygens (δ87.6(s), 78.7(s), 74.4(t), 73.9(d), 73.1(d) and 73.0(d)). Of these, one of the singlets, the triplet and two of the doublets may be assigned to C-4, C-29, C-11 and C-12 by comparison with the spectrum of rohitukin. This leaves one singlet and one doublet unassigned. Neither the infrared spectrum (Spectrum I-1) nor the ^{1}H n.m.r. spectrum showed the presence of hydroxy groups. The molecular formula shows that there was only one remaining oxygen atom. This implies that the remaining oxygen atom must occur as an oxide bridge, linking the two carbon atoms which gave the remaining singlet and doublet signals in the ^{13}C n.m.r. spectrum.

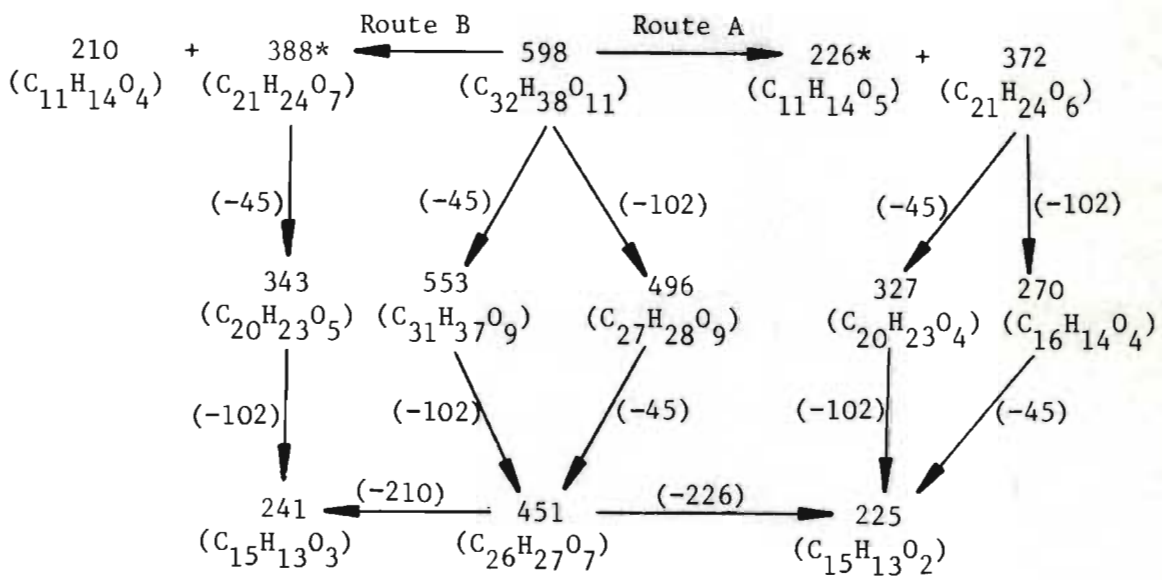
In the partial synthesis of methyl angolensate (10),[21] the dihydroxy compound (47) was found to cyclise spontaneously to methyl angolensate (10). The biosynthesis of polystachin could involve a similar cyclisation in an unsaturated ring A analogue of rohitukin with the formation of the 1,14-ether. Thus structure (48) is proposed for polystachin. The resonance at δ3.78 (W = 16 Hz) may then be ascribed to H-1. The H-1 proton in methyl ivorensate (11) resonates at δ3.41 (W = 10 Hz), suggesting that the stereochemistry might be different in the two cases.

Further evidence for H-1 of polystachin having an α-configuration comes from the examination of a model of polystachin. The model predicts that the 1β-H isomer would have 1-H, 2-H dihedral angles of about 90°, 40°, but the 1α-H isomer would have dihedral angles of about 180°, 60°. Also in the 1β-H isomer the ether oxygen is antiperiplanar to H-2β. For these two reasons, the splitting of H-1 would be expected to be less in the 1β-H isomer than in the 1α-H isomer.[31] The value of 10 Hz for methyl ivorensate (11) compared with 16 Hz for polystachin agrees with this.

Additional evidence for polystachin having a 1,14-ether-bridge comes from the comparison of the mass spectrum of polystachin (Spectrum M-1) with that of dregeana 2 (35) (Spectrum M-2). The mass spectrum of dregeana 2 shows that the molecule breaks across the C-9, C-10 bond to give two fragments (see Scheme 3.1).

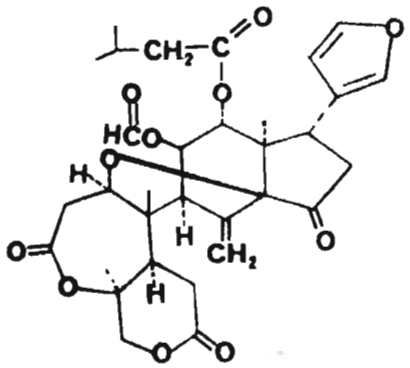
If polystachin has a 1,14-ether, when the compound splits across the C-9, C-10 bond it could also split in such a way as either to leave the oxygen atom attached to C-1 or C-14. Thus the molecule would split into two fragments of m/e = 226 and m/e = 372 or m/e = 210 and m/e = 388. The larger fragments could then lose a formate group (-45 peak) or the C-12 ester (-102 peak) as shown in Scheme 2.1.

Scheme 2.1

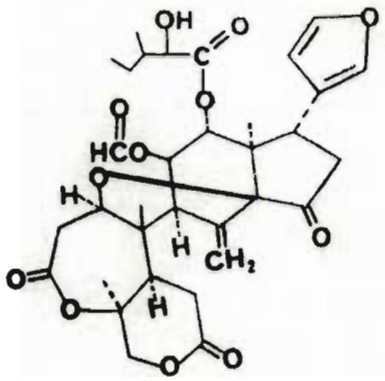


(*indicates that this peak was not detected in the mass spectrum).

Peaks have been found at m/e = 210.0931 (C_{11} H_{14} O_4 requires m/e = 210.0892) and at m/e = 372.1573 (C_{21} H_{24} O_6 requires m/e = 372.1555), so the molecule does split with the oxygen being attached to C-1 or C-14 as predicted. The mass spectrum also verifies that the ester present at C-12 is the 3'-methylbutyrate one.



(48)



(49)

Rohituka 10 (Dregeana 1)

Rohituka 10 was found to be identical to the substance dregeana 1 which has been isolated from the seeds of *Trichilia dregeana*. This compound which has the structure (49) is described in Chapter Three.

An interesting feature of the rohituka compounds is the variation in the position of the resonance due to the formate proton. Rohituka 1 (39) and 2 (40) have resonances due to the formate proton at δ8.02, polystachin (48) and rohituka 10 (49) at δ8.07; rohituka 4 (42) at δ7.91; rohituka 7 (45) and 8 (46) at δ7.97; and rohitukin (38) at δ7.85.

Substitution of the 3'-methylbutyric acid residue at C-12 by the 2'-hydroxy-3'-methylvalerate one does not affect the resonance position. (Rohituka 1 (39) and 2 (40) which have different esters at C-12 have resonances ascribable to the formate proton at δ8.02). Acetylation of the compound does not appear to affect the resonance position of the formate proton either. (Rohituka 2 (41) and its acetate have the same

formate resonance).

The possession of a C-7:C-29 lactone ring does not alter the position of the resonance due to the formate proton. Rohituka 7 (45) has the C-7:C-29 lactone but rohituka 8 (46) does not, but the resonance ascribable to the formate proton is in the same position in the ^{1}H n.m.r. spectra of both.

The possession of a 15-keto group instead of a 15-acetate group alters the position of the resonance due to the formate proton. The only difference between the acetate of rohituka 1 (39) and rohituka 4 (42) is that the former has an acetate group at C-15, and the latter, a 15-keto group. However, the resonance ascribable to the formate proton occurs at δ8.02 in the spectrum of rohituka 1 acetate, but at δ7.91 in that of rohituka 4 (42). This difference in the resonance position could only arise because the substitution at C-15 has been changed. Rohitukin also has a 15-ketone and the resonance due to its formate proton occurs in a similar position to that of rohituka 4 (42).

To test the proposal that the position of the resonance due to the formate proton is affected by the substituent at C-15, the ^{1}H n.m.r. spectrum of prieurianin (14), a compound with a 15-keto group was examined. The only differences between prieurianin and rohituka 2 (40) are the substitution at C-15 and the C-29 acetate in prieurianin. The resonance in the spectrum of prieurianin was found to be at δ7.86 compared to δ8.02 in the spectrum of rohituka 2. It has been shown previously that acetylation of the compound does not affect the position of the resonance due to the formate proton, so the difference must be due to the different substitution at C-15.

The second factor which affects the position of the resonance due to the formate proton, is the substitution at C-1. Three sorts of substitution at C-1 are found in these compounds. There is either an acetate at C-1, a 1,14-ether linkage or the α,β-unsaturated lactone.

The only way in which rohituka 8 (46) differs from the acetate of rohituka 1 (39) is that rohituka 8 has the α,β-unsaturated lactone and rohituka 1 acetate has an acetate group at C-1. The position of the resonance due to the formate proton in rohituka 8 is δ7.97 and that of rohituka 1 acetate has been shifted downfield slightly to δ8.02.

Polystachin differs from rohitukin only in having a 1,14-ether linkage instead of the 1-acetate group. The resonance due to the formate proton occurs at δ8.07, but in rohitukin it occurs at δ7.85.

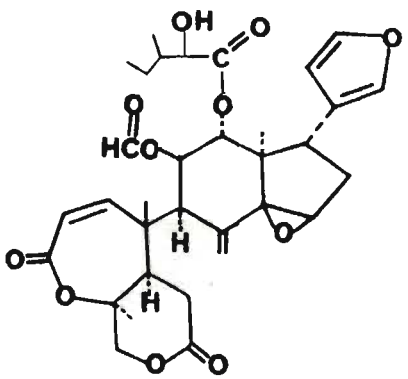
Thus the substitution at C-15 and C-1 does affect the position due to the resonance of the formate proton. Substitution of the 15-acetate group by a 15-ketone shifts the resonance upfield by about 12 Hz. The effect of replacing an acetate at C-1 by the α,β-unsaturated lactone is to shift the resonance upfield by about 5 Hz (cf. rohituka 1 (39) and rohituka 8 (46)) and the effect of replacing the acetate by a 1,14-ether bridge is to shift the resonance downfield by about 22 Hz.

To determine the effect that a 14,15-epoxide would have on the position of the resonance due to the formate proton, the values given for compounds isolated by Connolly *et al* from *Trichilia prieuriana* and *Guarea thompsonii* were examined.[32]

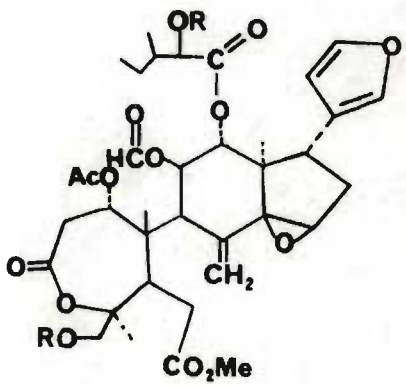
The compound D-4 (50) from *Trichilia prieuriana* differs from rohituka 7 (45) in having a 14,15-epoxide instead of the 14-hydroxyl,

15-acetate group. The formate proton resonance occurs at δ7.93 in D-4, but at δ7.97 in rohituka 7. Thus in compounds having the α,β-unsaturated ring A lactone, the effect of replacing the acetate group at C-15 and the C-14 hydroxyl group by a 14,15-epoxide, is to move the resonance due to the formate proton slightly upfield.

The presence of a 7,29-lactone affects the position of the resonance due to the formate proton in compounds having a 14,15-epoxide. Compounds D-5 (51) and its acetate (52) have the resonance at δ8.05, but in compound B (53) isolated from *Guarea thompsonii*, which only differs from D-5 in having the 7,29-lactone, the resonance was found to occur at δ7.90.

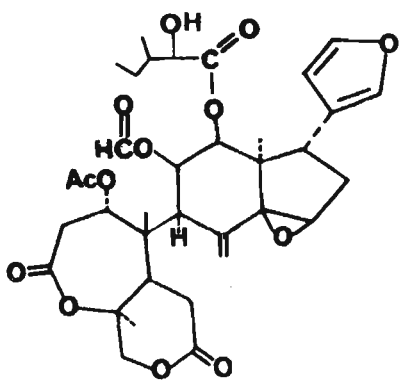


(50)

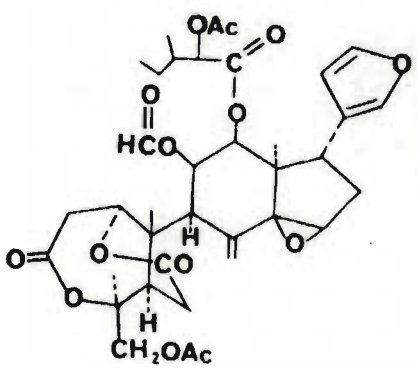


(51) R=H

(52) R=Ac



(53)



(54)

The effect of replacing the 14-hydroxyl, 15-acetate group by a 14,15-epoxide in compounds having an acetate group at C-1, is to shift the resonance due to the formate proton slightly downfield. Rohituka 2 (40) has this resonance at δ8.02 but in the spectrum of compound D-5 (51), it appears at δ8.05.

In dregeanin (54), a compound which is believed to have a 1,7-lactone, the resonance due to the formate proton occurs at δ8.02. No compound is known having a 1,7-lactone and either an acetate or a ketone at C-15, so no comparisons can be made here.

In summary, if a compound has an α,β-unsaturated ring A lactone, the effect of a 14,15-epoxide is to shift the resonance slightly upfield (by about 3 Hz). If the compound has an acetate group at C-1, the effect of the 14,15-epoxide is to shift the resonance downfield by about the same amount. The possession of a 7,29-lactone system seems to cause a much greater shift than the substitution at C-1 does (11 Hz upfield from that of the corresponding 1-acetate compound without the 7,29-lactone.

These observations are summarized in Table 2.1.

Table 2.1 ^{1}H NMR POSITIONS FOR THE RESONANCE DUE TO THE FORMATE PROTON

	15-ketone	15-acetate	14,15-epoxide
1-acetate	rohituka 4 (42) δ7.91 prieurianin (14) δ7.86	rohituka 1 (39) δ8.02 rohituka 2 (40) δ8.02	D-5 (51) δ8.05 D-5 acetate (52) δ8.05
α,β-unsaturated lactone	-	rohituka 7 (45) δ7.97 rohituka 8 (46) δ7.97	D-4 (50) δ7.93
1,14-ether	polystachin (48) δ8.07 rohituka 10 (49) δ8.07	-	-
1,7-lactone and 1-acetate	rohitukin (38) δ7.85	-	compound B (53) δ7.90
7,29-lactone	-	-	dregeanin (54) δ8.02

It is a well known phenomenon that the anisotropy of the carbonyl group causes deshielding of protons lying in a cone extending from the carbonyl oxygen atom, but shielding of protons lying outside this cone (Figure 2.1).[33] This effect is region and angle dependent (Figure 2.2).

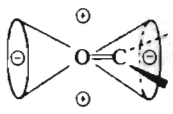


Figure 2.1

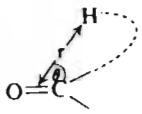


Figure 2.2.

It has been found that if a 15-acetate group is present, the resonance due to the formate proton occurs at δ8.02 and if there is a 15-keto group, it occurs at δ7.86, there being a C-1 acetate group present in both cases.

Examination of a model shows that the least sterically hindered position for the formate group and the 15-acetate group would be for them to occur behind the molecule. With these two groups in this position, the formate proton and the acetate carbonyl group are in close proximity and, furthermore, the formate proton is situated within a region making a small angle θ with the axis of the carbonyl group of the acetate group.

A model of the 15-ketone compound shows that the formate proton is not in close proximity with the keto group and the proton forms a much larger angle θ with the axis of the carbonyl group than it did in the former case.

This accounts for the fact that the formate proton is deshielded in the case of the 15-acetate compounds, but is shielded in the case of the 15-keto compounds.

Compounds with the α,β-unsaturated lactone have resonances due to the formate proton occurring at δ7.97. The reason for this is that the steric hindrance offered by the acetate group at C-1 is present no longer, so the formate group is able to move more freely, thus the deshielding effect would be less.

Compounds with the 1,14-oxide bridge have the resonance due to the formate proton at δ8.07. Examination of a model shows that the formate proton is as close to the carbonyl part of the acetate group as in the case of the 1,15-diacetate, but here the molecule is fixed so that the formate proton would be further deshielded by the 7-carbonyl group. Thus the resonance occurs further downfield than in the case of the 1,15-diacetate compounds.

CHAPTER THREE

EXTRACTIVES FROM THE SEED OF *Trichilia dregeana*

3.1 Introduction

There has been confusion about the taxonomy of the Natal Mahogany, but it is now thought that this name refers to two species of tree: the true Natal Mahogany, *Trichilia emetica* Vahl, a savannah species; and the Forest Natal Mahogany, *Trichilia dregeana* Sonder. Both of these species are found extending northward from South Africa to tropical Africa. At one stage it was believed that *Trichilia roka* was a separate species, but it is now considered to be conspecific with *Trichilia emetica*.

Trichilia dregeana (also referred to as *T. splendida* A. Chev.) is a medium to large tree, often planted for shade.

3.2 Limonoids from the seed of *Trichilia dregeana*

The timber from the tree has been found to contain dregeanin (54),[34] and the bark has been found to yield a similar sort of compound, which has not been identified.[35]

The seeds of *Trichilia dregeana* were collected from the shade trees of the cricket ground of Durban High School. They were shelled

and the seed coats and the seeds were examined separately. Because of the oily nature of the seeds and the fact that the seed coat material was in small pieces, the seeds could not be milled in the usual fashion. The seed coats were ground in a coffee grinder, resulting in a very finely-particulated oily material, which was difficult to extract as the material would not let the solvent pass through it. The material had to be mixed with celite in a ratio of about two parts celite to one part material in order to allow the solvent to pass through it. The seeds were crushed using an ice-crusher and presented no extraction problems.

Extraction of the seed coats with hexane gave a yellow precipitate. Thin layer chromatography showed that this precipitate contained a lot of oil and several limonoids. Preliminary column chromatography over coarse silica gel separated most of the oil, leaving an extract which consisted mainly of limonoids. Repeated column chromatography using fine silica gel yielded pure rohituka 7(45), a crystalline compound, which had been obtained previously from the seed of *Aphanamixis polystacha*, dregeana 1, dregeana 2, dregeana 3 and dregeana 5.

Extraction of the seeds with hexane resulted in a yellow precipitate. Thin layer chromatography of a sample of this extract showed that most of it was a fat, but that there was some limonoid material present. The extract was extracted with aqueous methanol, and the resulting limonoid-containing fraction was chromatographed. The compound dregeana 3(a) was obtained from this extract.

The method of separating the seed coats from the seeds had the advantage of obtaining an extract from the seed coats which did not have so much oil or so many compounds to separate, but the seed-shelling was very time-consuming. However, it showed that most of the limonoid material was present in the seed coat and there were differences between the limonoids present in the seed and the seed coat.

A further sample of the same seeds was extracted with the seed coat and seed intact. Extraction of the minced seeds with hexane gave an oil which was partitioned between light petroleum and methanol to give a limonoid fraction (0.125%), mixed triglycerides and palmitic acid which was identfied by g.l.c. of its methyl ester. The limonoid fraction gave rohituka 7(45) and the compounds dregeana 2 and 4 were obtained from the resulting mother liquor by means of medium pressure column chromatography.

Attempts to separate the limonoid mixture by preparative thin layer chromatography were unsuccessful. It appears that these compounds, like the rohituka ones, undergo reaction on the plates resulting in a more complex mixture than that which was applied to the plate.[28] Other workers do not seem to have this problem. A sample of the extract was given to Mr. Akinniyi of Glasgow University, who obtained rohituka 7(45) and the three of the compounds which had been isolated previously by means of column chromatography. He also obtained the compound dregeana 3(b).

Dregeana 1

Dregeana 1 has also been isolated from *Aphanamixis polystacha* and is also called rohituka 10.

Dregeana 1 gave a ^{1}H n.m.r. spectrum (H-14) which was very similar to that of polystachin (48) (spectrum H-13). Both spectra have a resonance due to the formate proton at δ8.07, neither compound has acetate groups nor a carbomethoxy group. Both spectra are missing the 15-H resonance which usually occurs at about δ5.7. The single proton multiplet at δ3.78 (W = 16 Hz) which has been assigned to the 1-H proton in polystachin, was also present in the ^{1}H n.m.r. spectrum of dregeana 1. The position of this resonance is much further upfield than the resonances due to the H-1 protons in the other rohituka compounds, and as the splitting of this multiplet is the same for both polystachin and dregeana 1, it appears that the 1,14-oxide is present in dregeana 1 as in polystachin. The doublet at δ2.73 (J = 6 Hz) which has been ascribed to H-9 in polystachin as a result of double resonance experiments, is also present in the ^{1}H n.m.r. spectrum of dregeana 1. This resonance is also further upfield than the H-9 resonances of the other rohituka compounds and this is probably the result of free rotation about the C-9, C-10 bond not occurring in these compounds as a result of the 1,14-oxide.

The ^{1}H n.m.r. spectrum of dregeana 1 differs from that of polystachin in that it has an extra doublet at δ3.38 (J = 4 Hz). On acetylation of dregeana 1, this doublet shifts to δ4.72 and there is a resonance ascribable to the protons in an acetate group at δ2.09. On acetylation of the compound rohituka 7(45) the doublet at δ3.30 ascribed to H-2' shifts to δ4.68 and an extra acetate resonance appears at δ2.09. Thus it appears that the 2-hydroxy-3-methylvalerate ester is present at C-12 in dregeana 1 instead of the 3-methylbutyrate one, present in polystachin.

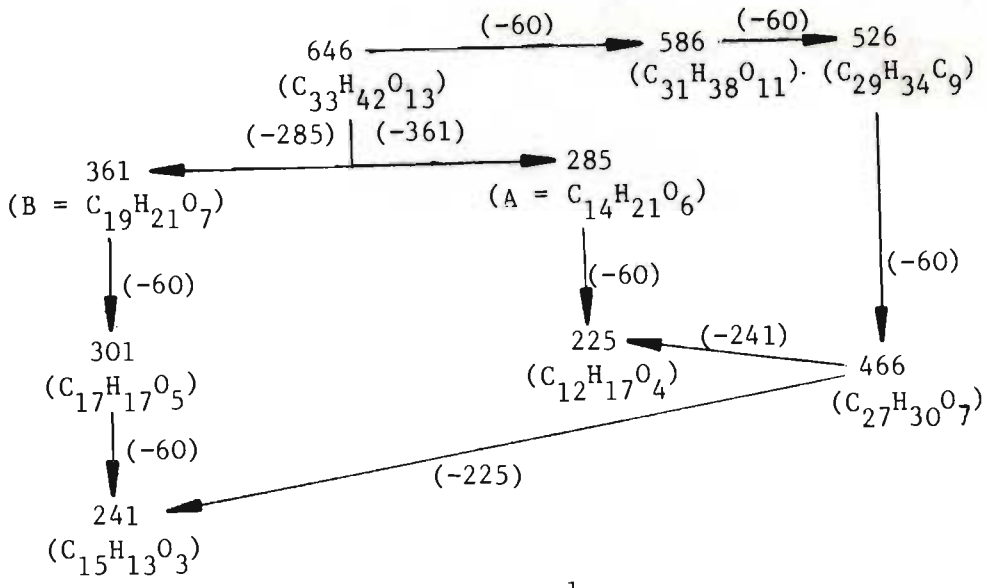
The ^{13}C n.m.r. spectra of dregeana 1 and polystachin (Spectrum C-1) showed singlets at δ205.1 and δ205.0 respectively. These resonances are ascribed to C-15. Thus both compounds have a keto group at C-15, as was suggested by the absence of a H-15 resonance in the ^{1}H n.m.r. spectra. The ^{13}C n.m.r. spectra of the two compounds are the same except for the resonances ascribable to carbon atoms in the C-12 side chain. The C-1' singlet occurs at δ172.3 in the spectrum of polystachin, but at δ174.9 in that of dregeana 1. The spectrum of polystachin has resonances at δ43.2(t), δ37.2(d), δ24.9(q) and δ22.3(q) which are ascribable to C-2', C-3', C-4' and C-5' in a 3'-methylbutyrate ester. The spectrum of dregeana 1 has resonances at δ75.2(d), δ36.8(d), δ38.0(t), δ11.4(q) and δ15.1(q), ascribable to C-2', C-3', C-4', C-5' and C-6' in a 2'-hydroxy-3'-methylvalerate ester.

Thus dregeana 1 was assigned structure (49).

Dregeana 2

Dregeana 2 was found to be very different from previously isolated compounds. The mass spectrum (Spectrum M-2) gave a molecular weight of 646.2674, corresponding to the molecular formula $C_{33}H_{42}O_{13}$. The mass spectrum showed three peaks (M-60, M-120, M-180), corresponding to the loss of three acetic acid molecules. It also showed that the molecule split into two parts, A(m/e = 285.1308) and B(m/e = 361.1300), having the formulae of $C_{14}H_{21}O_6$ and $C_{19}H_{21}O_7$ respectively. Peaks occurred at m/e = 301.1130 and m/e = 241.0904 indicating that fragment B contained two acetate groups, and a peak at m/e = 225.1072 showed that fragment A contained one acetate group. The suggested fragmentation pattern of dregeana 2 is given in Scheme 3.1.

SCHEME 3.1



Single and double resonance ^1H n.m.r. spectra were recorded for dregeana 2. The ^1H n.m.r. spectrum (spectrum H-16) showed the usual furan ring (δ7.21, 7.32, 6.23), a carbomethoxy group (δ3.74) and two acetate groups (δ2.12 and δ2.06). There was a sharp methyl resonance at δ1.72 and no formate resonance was present. Double resonance experiments showed the presence of three coupled systems. In the first, a doublet (δ5.95, J = 11.4 Hz) was coupled to a multiplet (δ5.22), which was further coupled to a doublet (δ3.72, J = 7 Hz) which also had a small coupling to two singlets (δ6.05, δ5.75). Comparison with the spectra of related compounds showed that the singlets represented an exocyclic methylene group at C-30 (δ5.92 and δ5.58 in rohitukin (38)) and that the coupled chain extends through H-9 (δ3.72, J = 7 Hz in rohitukin) and H-11 (multiplet at δ5.49 in rohitukin) to H-12 (doublet at δ6.06, J = 11 Hz in rohitukin), with acyl groups being present at C-11 and C-12, as in the case of the rohituka compounds. In the second chain, a multiplet (δ3.93, W/2 = 20 Hz) was coupled to two multiplets (δ2.41 and δ2.89), which were not further coupled. In rohitukin a multiplet (δ3.98, W/2 = 18 Hz) ascribed to H-17 is coupled to the two H-16 multiplets (δ2.4). Thus the dregeana 2 resonances could be ascribed to H-17, H-16A and H-16B. The lack of further coupling of 2H-16 suggests

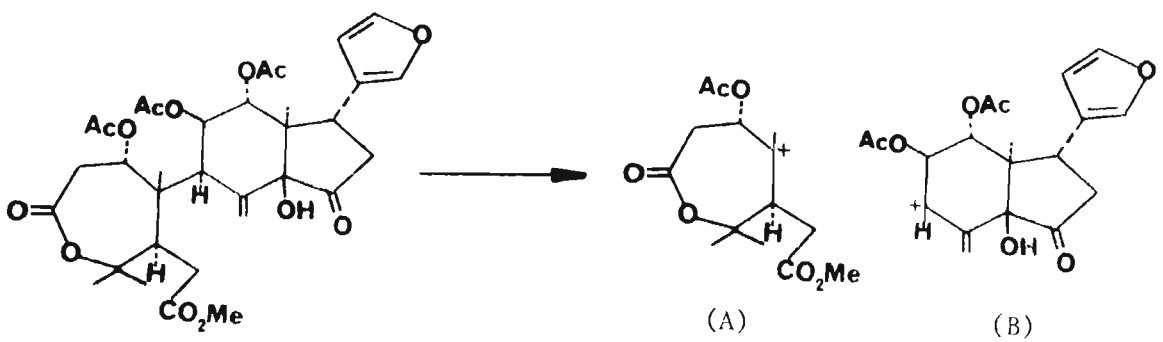
that a carbonyl group is present at C-15. In the third chain, a multiplet (δ5.50, W/2 = 15 Hz) was coupled to a two proton multiplet (δ2.97) which was not further coupled. In the acetate of rohutuka 1 (39) the H-1 multiplet (δ5.78) is coupled to a two proton H-2 multiplet (δ2.98). Thus the resonances in the third chain are ascribable to H-1 and H-2 in a 1-acyloxy ring A lactone.

The ^{13}C n.m.r. spectrum (Spectrum C-2) showed a ketonic carbonyl carbon resonating at δ206.6 and a resonance at δ80.8 (s) corresponding to a tertiary carbon attached to an oxygen. These resonances indicate a 14-hydroxy, 15-keto system, which is common in the rohituka-type compounds (cf δ206.6(s) and δ81.2(s) in rohutukin). There were five resonances due to ester carbonyl groups (δ176.1, δ170.6, δ170.3, δ170.3, δ169.3). These were due to a methyl ester, the ring A lactone, and three other esters located at C-1, C-11 and C-12. Adding up the number of carbon atoms required for the nucleus and the carbomethoxy group leaves a remainder of six carbon atoms for the three ester groups. As there is no formate group present, these must all be acetate groups. This is supported by information obtained from the mass spectrum. The ^{1}H n m.r. spectrum showed only two acetate resonances (δ2.12 and 2.06). It has been found[36] that in 12-α acetylated limonoids, the acetate methyl group is strongly shielded by the furan ring and may resonate as far upfield as δ1.5. Thus the resonance at δ1.72 probably represents the third acetate group, at C-12α.

The ^{13}C n.m.r and ^{1}H n.m.r. spectra showed resonances attributable to four other methyl groups (δ31.95 (q), δ27.8(q), δ18.9 (q) and δ12.8(q)) (δ1.62, δ1.56, δ1.52, δ1.02). There was no resonance due to a CH_2O group in either spectrum, so it appears that there is an

unoxidised *gem* dimethyl group at C-4, as in the simpler limonoids. Structure (35) is proposed for dregeana 2. This structure is in agreement with data obtained from the mass spectrum. Fission of the C-9, C-10 bond would result in fragment A(m/e = 285.1308) and fragment B(m/e = 361.1300). Fragment B has two acetate groups, and fragment A has one acetate group attached to it.

The splitting of the δ5.5 resonance due to the H-1, H-2 coupling was found to be 15 Hz. This is much larger than the normal value for the rohituka compounds where it ranges from 6-12 Hz (cf 8 Hz for the acetate of rohituka 1(39)). It is, however, in agreement with the value obtained for polystachin (48) (16 Hz). The reason proposed for this value for polystachin was that as no rotation could occur about the C-9, C-10 bond, the large coupling constant could be due to the oxygen being in the β configuration. The ^{13}C n.m.r. spectrum of dregeana 2 is seen to sharpen when the sample is heated to 60°, implying that rotation about the C-9, C-10 bond does occur. It seems unlikely that the acetate at C-1 in dregeana 2 would be in the unusual 1-β configuration. The reason for the large coupling constant could be that the conformation of ring A is affected in some way by the replacement of the usual C-11 formate by an acetate, or the absence of the CH_2OH group usually found at C-4.

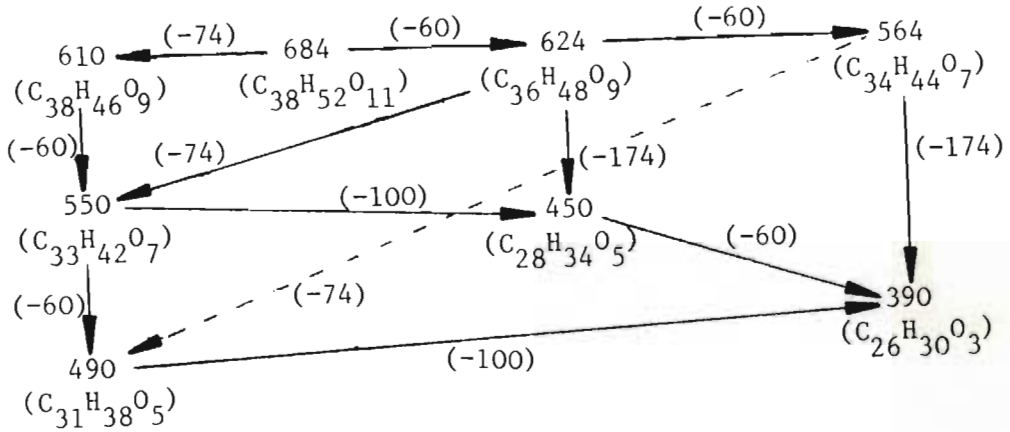


(35) (A) (B)

Dregeana 3

Dregeana 3 has a molecular weight of 684.3538 corresponding to a formula of $C_{38}H_{52}O_{11}$. The mass spectrum of dregeana 3 (Spectrum M-3) shows that the compound has two acetate groups and a 2′-acetoxy-3′-methylvalerate group. The 2′-acetoxy-3′-methylvalerate group was lost either as a -174 peak or a -74 then a -100 peak. (see Scheme 3.2).

SCHEME 3.2



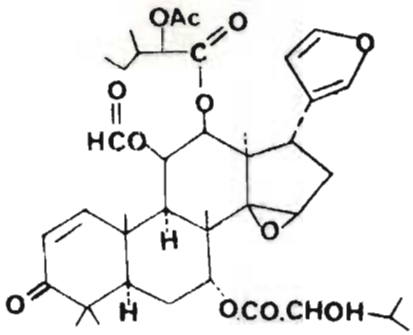
The nuclear magnetic resonance spectra showed that dregeana 3 was more like heudelottin F (55)[37] than the rohituka substances. The ^1H n.m.r. spectrum (Spectrum H-17) showed the usual three furan bands (δ7.15, 7.31 and 6.21), no formate nor carbomethoxy group, three acetate resonances (δ2.2, 2.13, 2.0) five other methyl resonances (δ1.57, 1.55, 1.32, 1.27, 1.05), the X parts of ABX multiplets at δ5.47 (W/2 5 Hz), δ5.26 (W/2 6 Hz), δ5.06 (W/2 17 Hz) and δ4.71 (W/2 8 Hz), a doublet at δ4.92 (J = 4.5 Hz), a two proton doublet at δ3.12 (J = 4 Hz) which was coupled to the multiplet at δ4.71, and a two proton multiplet at δ2.41 coupled to the multiplet at δ5.47 which collapsed into a doublet on irradiation at δ5.47. The doublet at δ4.92 may be assigned to H-2' in the

2'-acetoxy-3'-methylvalerate residue (cf δ4.86 for H-2' in rohituka 5 acetate (43)).

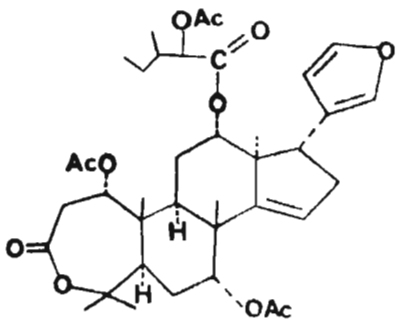
The ^{13}C n.m.r. spectrum (spectrum C-3) showed a double bond (δ156.0(s), 122.1(d)) but no 15-keto group. The two proton multiplet at δ2.41 may be assigned to the two H-16 protons (cf resonance at δ2.4 in rohituka 1 (39)), the multiplet at δ5.47 would be the H-15 proton. Thus the double bond occurs between C-14 and C-15. The multiplet at δ4.71 would be due to H-1 in a ring A lactone of the obacunone-type, the doublet at δ3.12 representing 2H-2. The narrow multiplet at δ5.26 may be assigned to H-7 in a 7-α-acyloxy structure (cf δ5.22 for H-7 in Sapelin E triacetate).[38]

Assuming that dregeana 3 has the usual meliacan structure, the wide multiplet at δ5.06 could only be due to a proton at C-11α or C-12β. A proton at C-11α is coupled to three protons (H-9, 2H-12) giving a widely split resonance (11β - acetoxygedunin has five lines, W/2 21 Hz),[39] but a proton at C-12β is only coupled to two protons, giving a simpler pattern but still a considerable splitting (6,12-α-diacetoxymethylangolensate has four lines, W/2 18 Hz).[36] This suggests that the three line resonance obtained may be due to H-12. It has been found that the resonance position of a C-12α acetate is shifted far upfield by the furan ring.[36] As the acetate resonances are in the usual positions, it may be assumed that an acetate is not present at C-12, but the acyloxy ester is. Therefore the two acetate groups would occur at C-1 and C-7.

Thus structure (34) is proposed for dregeana 3.



(55)



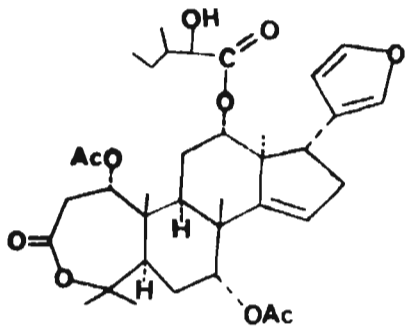
(34)

Two substances closely related to dregeana 3 have been isolated from the seeds of *Trichilia dregeana*.

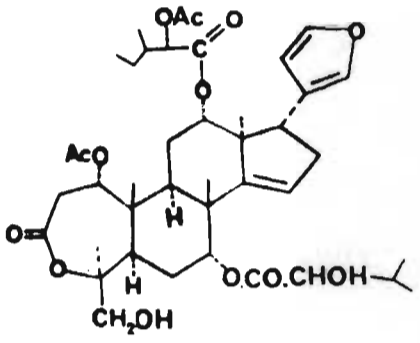
Compound 3(a) was obtained from the extract of the seeds after the seed coats had been removed. The ^{1}H n.m.r. spectrum of this compound shows a very intense band at $\delta 1.32$ and the ^{13}C n.m.r. spectrum has a very intense triplet at $\delta 29.5$, both indicating the presence of many aliphatic methylene groups. For a long time it was believed that this compound was impure, being a mixture of dregeana 3 and a fatty acid. However, the mass spectrum of the compound showed that it had a molecular weight of 936, equivalent to having eighteen more CH_2 groups incorporated into the molecule than dregeana 3. This suggests that a fatty acid ester may be incorporated into the molecule in some way. It is interesting that the seed contains so much fat that fatty acids even become incorporated into molecules like this one.

A ^{1}H n.m.r. spectrum of dregeana 3 (b) provided by Mr. Akinniyi, of Glasgow University, who isolated this compound by means of preparative thin layer chromatography of the extract, was interpreted to be the spectrum of deacetyldregeana 3. The doublet at

δ4.92 present in the spectrum of dregeana 3 was replaced by a doublet at δ4.05 and the acetate band at δ2.2 in the spectrum of dregeana 3 was absent in the spectrum of dregeana 3(b). Structure (56) was assigned to dregeana 3(b).



(56)



(57)

The mass spectrum of dregeana 4 (Spectrum M-4) showed that this compound had a molecular weight of 759.3933. The molecular formula $C_{41}H_{59}O_{13}$ requires a molecular weight of 759.3955. Peaks at M-60 and M-120 indicated that two acetate groups were present in the molecule.

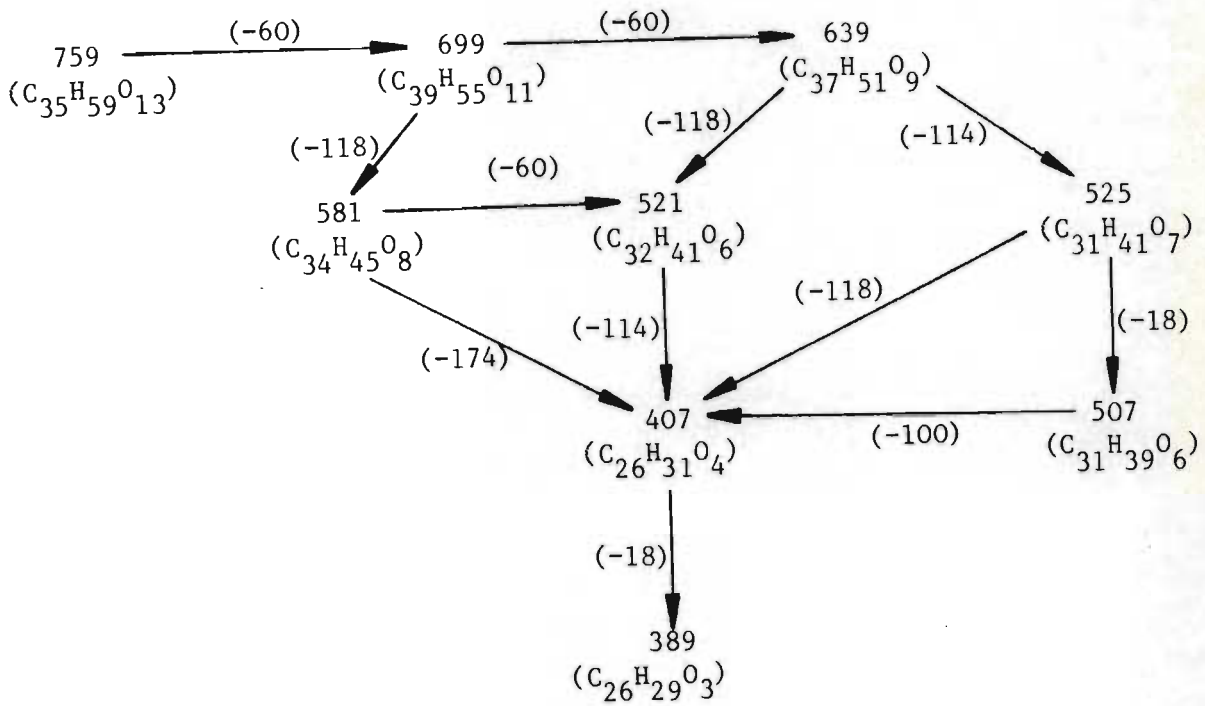
A fragment with the formula $C_8H_{14}O_4$ was lost either to give a -174 peak, a -60 then a -114 peak, or a -74 then a -100 peak. The -60 peak shows that this fragment contained an acetate group. This -174 peak occurs in the mass spectrum of dregeana 3 (Spectrum M-3) and corresponds to a 2′-acetoxy-3′-methylbutyric acid group.

A fragment with a mass of 118 corresponding to the formula $C_5H_{10}O_3$ is lost either as a -118 fragment or a -18 then a -100 fragment. This indicates that a hydroxy group is present in this fragment. The mass of 118 corresponds to a 2″-hydroxy-3″-methylbutyric acid group which has been found to occur at C-7 in heudelottin F(55).

A second -18 peak is found in the mass spectrum of dregeana 4 which does not occur in the spectrum of dregeana 3.

The proposed fragmentation pattern for dregeana 4 is given in Scheme 3.3.

SCHEME 3.3



The ^1H n.m.r. spectrum of dregeana 4 (spectrum H-18) was very much like that of dregeana 3 (34) (spectrum H-17). In agreement with the mass spectrum only two acetate resonances (δ2.13, δ2.0) were present in the spectrum of dregeana 4, one of which corresponded to the acetate group in the 2'-acetoxy-3'-methylvalerate group. The position of the bands near δ5 was shifted slightly, there were additional resonances at δ4.10, 4.00 (each 1 Hd, J apparent = 3 Hz) which could represent a hydroxy methylene group, and there was a new resonance at δ3.90 (1H, bs) which could be ascribed to the H-2" of the 2"-hydroxy-3"-methylbutyric acid residue.

The ^{1}H n.m.r. spectrum of the acetate of dregeana 4 (Spectrum H-19) showed two extra acetate resonances corresponding to the acetylation of the 2"-OH group of the 2"-hydroxy-3"-methylvalerate residue and the hydroxy methylene group. The 2H-29 resonances were shifted to δ4.86, and the δ3.9 resonance also moved, but could not be located.

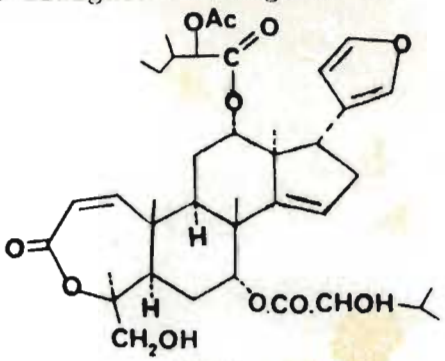
The ^{13}C n.m.r. spectrum of dregeana 4 (spectrum C-4) was very similar to that of dregeana 3 (34) (Spectrum C-3) but lacked resonances due to the third acetate group, had extra resonances which were ascribable to a 2"-hydroxy-3"-methylbutyric acid group and a triplet (δ66.2) ascribable to the hydroxy methylene group.

It appears that dregeana 4 is similar to dregeana 3 (34) but that one of the methyl groups at C-4 has been oxidised to a hydroxy methylene group, and a 2"-hydroxy-3"-methylbutyric acid group occurs at C-7 as in heudelottin F (55) in place of the C-7 acetate group. Thus structure (57) is assigned to dregeana 4.

A small amount of dregeana 5 was obtained, so only its ^{1}H n.m.r. spectrum could be recorded. The differences between this spectrum (H-20) and that of dregeana 4 (57) (spectrum H-18) were that two pairs of coupled doublets at δ6.47, δ5.97 (J = 12 Hz) and δ4.58, δ4.13 (J = 12 Hz) had appeared in place of the 2H-29 resonances near δ4.10, the H-1 multiplet at δ4.81 and the H-2 resonance at δ3.23. The acetate resonance at δ2.13 was also missing.

Therefore dregeana 5 appears to be the same as dregeana 4 (57) but it has an unsaturated ring A system instead of the acetate group at C-1.

Thus structure (58) is assigned to dregeana 5.

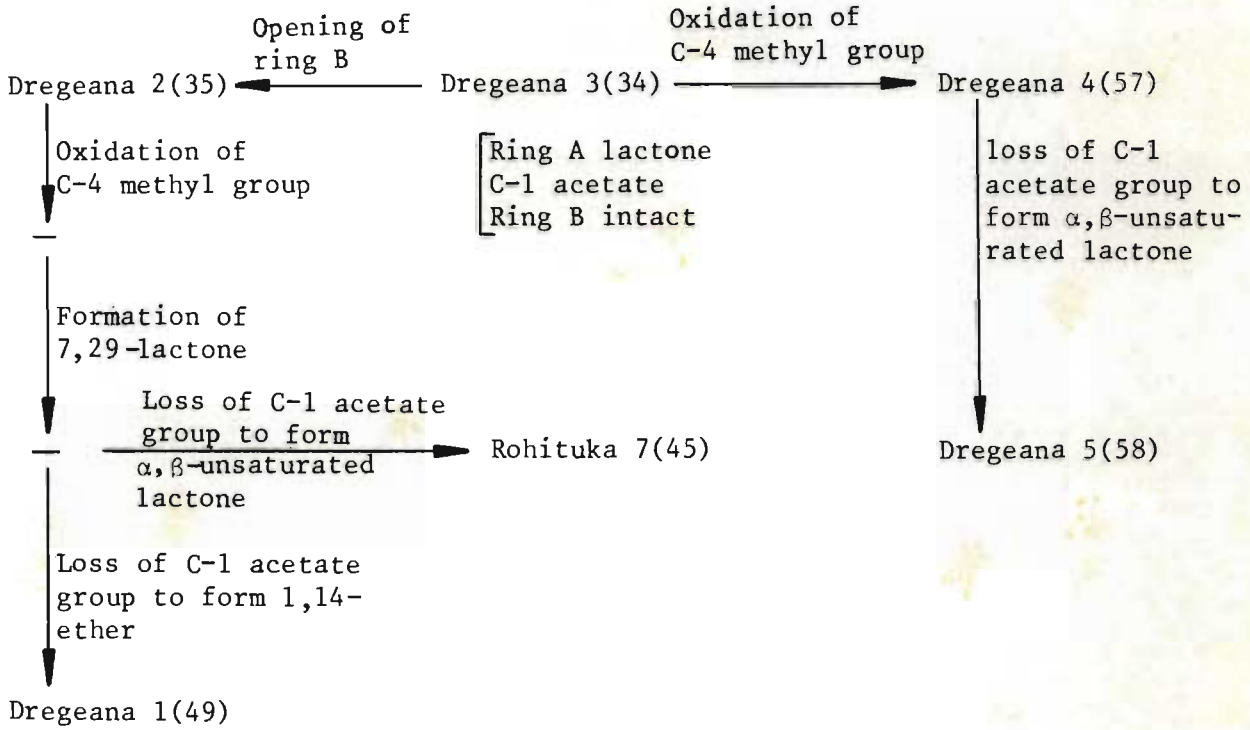


(58)

Dregeana 3 (34) and dregeana 4 (57) are of particular interest as they are the only compounds that have been isolated that are biogenetically intermediate between compounds with the intact meliacan structure, such as deoxyhavanensin (1) and the prieurianin group of compounds. Mondon has isolated similar compounds from *Cneorum-tricoccon*,[24] but the compounds he has isolated have ring D systems that are more highly oxygenated than those found in the prieurianin-type of compounds, so it may be deduced that they are not on the direct biosynthetic route to the prieurianin class of compounds (see Chapter 1).

This extract is of particular interest because of the presence of limonoids at different stages in the biosynthetic pathway found in it. Dregeana 3 (34) has a ring A lactone, an acetate group at C-1, but an intact ring B; dregeana 4 (57) has the ring A lactone, the acetate group at C-1, but one of the methyl groups at C-4 has been oxidised to a hydroxy methylene group; dregeana 5 (58) is the same as dregeana 4 (57) but the C-1 acetate group has been lost to form the α,β - unsaturated lactone; dregeana 2 (35) has the ring A lactone, the acetate at C-1, ring B has been opened, but the C-4 *gem* dimethyl groups have not been oxygenated; dregeana 1 (49) and rohituka 7 (45) have the ring A lactone, an open ring B, a 7,29-lactone, but the C-1 acetate group has been lost to form the α,β-unsaturated lactone or the 1,14-ether. The suggested inter-relationship between these compounds is shown in Scheme 3.4.

SCHEME 3.4



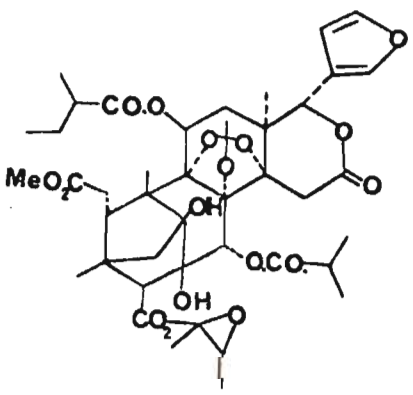
Further investigation of this extract would probably yield more compounds representing intermediate stages in the biosynthetic pathway.

CHAPTER FOUR

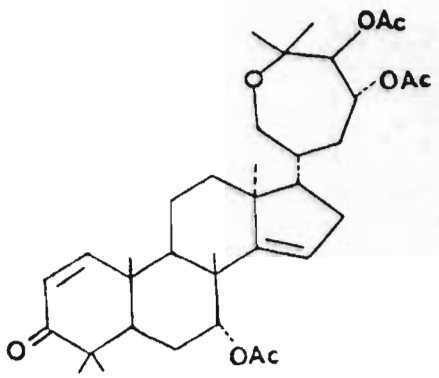
EXTRACTIVES FROM *Entandrophragma spicatum*

The heartwood of *Entandrophragma spicatum* was collected in northern South West Africa. The powdered heartwood was extracted with refluxing hexane and the solvent was decanted leaving a gum. A portion of this gum was investigated by Bredell[25] who obtained entandrophragmin (59) and, after preparative thin layer chromatography, a crystalline compound which was thought to be bussein (69).

More of the same extract has been investigated. Entandrophragmin (59) which comprises about half of the extract was obtained crystalline. Thin layer chromatography, comparing a sample of the extract with a sample of bussein, revealed that there did not appear to be any bussein present in the extract. By means of column chromatography, two compounds were obtained pure. The first, spicatin, was obtained as a gum, the second, spicata 2, was crystalline. The ^{1}H n.m.r. and ^{13}C n.m.r. spectra of spicata 2 were very much like those of bussein, and the fact that spicata 2 is present in large amounts (about 10% of the extract), makes it seem likely that this compound is the same as the one that was previously isolated.



(59)



(60)

Spicatin

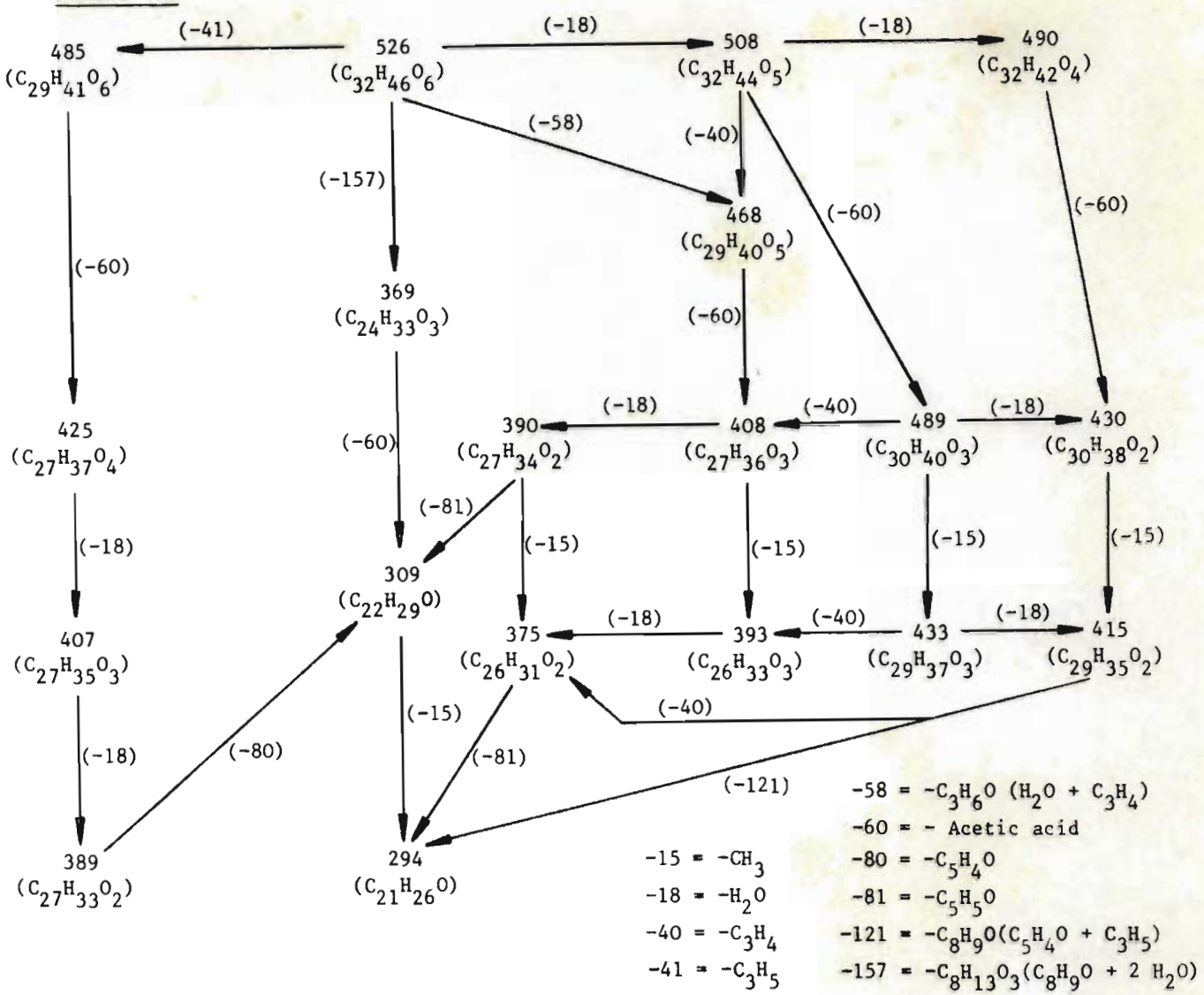
The mass spectrum of spicatin (Spectrum M-5) showed that the compound had a molecular weight of526.3323, corresponding to a molecular formula $C_{32}H_{46}O_6$. The ^1H n.m.r. spectrum (Spectrum H-21) showed that the molecule had no furan, so it was assumed that spicatin had some sort of protolimonoid side chain at C-17. Examination of the mass spectrum led to the proposed fragmentation pattern (Scheme 4.1).

The spectrum has a peak at m/e = 369.2366 (M-157) which corresponds to the loss of a $C_8H_{13}O_3$ fragment, then a peak at m/e = 309.2201 (M-217) showing the loss of an acetic acid molecule. It appears that various fragmentation routes lead to the m/e = 309.2201 peak, so it is assumed that 309.2201 is the mass of the compound without the side chain at C-17 and the acetate group. This mass corresponds to a formula of $C_{22}H_{29}O$.

Several deductions about the side chain may be made from the mass spectrum. The acetate group is not part of the side chain because a fragment, $C_8H_{13}O_3$ is lost before the acetic acid molecule in one of the suggested fragmentation routes. This could not happen if the acetate group was attached to the side chain. Two hydroxy groups must occur in the side chain as M-18 (m/e = 508.3170) and M-36 (m/e = 490.3028) peaks occur in the mass spectrum. In one proposed fragmentation route, a water molecule is lost first, then the acetate from the main part of the molecule, then another water molecule and then the rest of the side chain (C_8H_9O) is lost, resulting in the m/e = 309.2201 peak. In another possible route, a C_3H_5 fragment is lost, then the acetic acid molecule, then the two water molecules, then the rest of the side chain ($C_5H_6O_2$), again resulting in the m/e = 309.2201 peak.

As no metastable peaks were given in the mass spectrum, these proposals could not be confirmed.

SCHEME 4.1



The formula of the side chain, $C_8\ H_{13}\ O_3$, suggests that two rings or double bonds, or epoxide groups must be present. The loss of a $C_3\ H_5$ fragment suggest the presence of a $CH_2{=}\underset{|}{C}{-}CH_3$ group in the molecule. However, no triplets were present in the double bond region of the ^{13}C n.m.r. spectrum of spicatin.

The ^{13}C n.m.r. spectrum of spicatin (Spectrum C-5) was very complicated as there seemed to be extra bands present, possibly as a result of spicatin being a mixture of two stereoisomers. The spectrum showed resonances due to a keto group (δ204.3 (s)), an acetate carbonyl group (δ169.79 (s)) and two double bonds (δ159.02 (s), δ158.07 (d), δ125.28 (d) and δ119.03 (d)). The spectrum had two unusual singlets at δ97.37 and δ95.71.

The ^{1}H n.m.r. spectrum of spicatin (Spectrum H-21) showed an AB pattern with resonances at δ7.15 and δ5.83 (J 10 Hz) which correspond to H-1 and H-2 of a Δ^{1}-3-ketone system. (Sapelin E triacetate (60) has resonances at δ7.13 and δ5.80 (J 10 Hz) for this system in its ^{1}H n.m.r. spectrum).[38]

A resonance ascribable to an acetate group occurs at δ1.95 in the ^{1}H n.m.r. spectrum of spicatin. The mass spectrum of spicatin has shown that this acetate group is attached to the nucleus.

On biosynthetic grounds, the second double bond would occur either at Δ^{7} or Δ^{14}, and, as there is a substituent at C-1 already, the acetate group would occur at C-15 or C-7. In sapelin E triacetate (60), a compound having an acetate group at C-7 and a Δ^{14} system, resonances due to H-7 and H-15 appear superposed as a multiplet at δ5.22. Sapelin F tetra-acetate (61), a compound with a Δ^{7} system has a resonance at δ5.29 due to H-7.[38] The ^{1}H n.m.r. spectrum of spicatin has a multiplet at δ5.22, but no resonance around δ5.29. Therefore it appears that the acetate group is present at C-7 and the 14,15-double bond occurs.

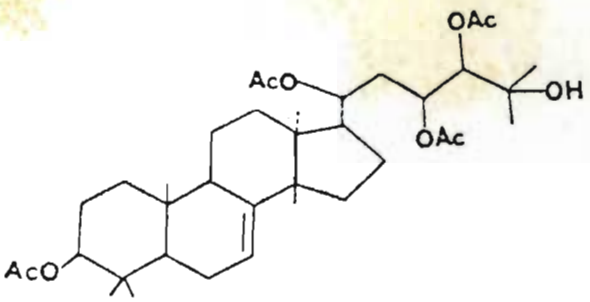
The formula of the nucleus of spicatin is the same as that of the nucleus of sapelin E triacetate (60), so it is assumed that the same nucleus is present in each.

Acetylation of spicatin yielded a triacetate. The ^{1}H n.m.r. spectrum (Spectrum H-22) of this product showed an extra resonance at δ2.07 which, as a result of its intensity, was ascribable to two extra acetate groups.

The ^{13}C n.m.r. spectrum of spicatin triacetate (Spectrum C-6) was

much simpler than that of spicatin, indicating that the two stereoisomers were no longer present. The two singlets at δ97.37 and δ95.71 had disappeared, there was an extra ketone resonance (δ210.81 (s)) and resonances ascribable to the C-7 acetate group and the two new acetate groups (δ170.7 (s), δ170.26 (s), δ169.62 (s)). The Δ^1 and Δ^{14} systems (δ159.23 (s), δ157.84 (d), δ125.60 (d), δ119.24 (d)) were present in spicatin triacetate. There was also a singlet, two doublets and a triplet in the C-O region of the spectrum (δ77.31 (s), δ74.82 (d), δ73.60 (d), δ65.78 (t)). The resonance at δ74.82 (d), was present in the spectrum of spicatin (δ74.69 (d)) and is ascribable to C-7. The other resonances were found to be different in the ^{13}C n.m.r. spectrum of spicatin. The resonances at δ77.31 (s), δ73.60 (d) and δ65.78 (t) in the ^{13}C n.m.r. spectrum of the triacetate occurred at δ76.14 (s), δ67.45 (d) and δ57.13 (t) in the spectrum of spicatin. As acetylation would not be expected to have altered the nucleus in any way, these three resonances and the extra ketone resonance are ascribable to carbon atoms in the side chain. After assignment of resonances to all the carbon atoms in the nucleus, there was a doublet (δ36.5), a triplet (δ31.5), two quartets and resonances for the two extra acetate groups (δ170.70 (s), δ170.26 (s), δ20.65 (q), δ20.63 (q)) as well as the resonances at δ77.31 (s), δ73.60 (d), δ65.78 (t) and δ210.81 (s). By comparison of the spectrum of spicatin with that of the triacetate, the resonances at δ38.58 (d), δ29.60 (t) as well as two quartets and the resonances already mentioned (δ97.37 (s), δ95.71 (s), δ76.14 (s), δ67.45 (d), δ57.13 (t)) were ascribed to carbon atoms in the side chain of spicatin.

As the spicatin side chain has eight carbon atoms, it may be assumed that an oxygenated form of the triterpenoid eight-carbon side chain (62) occurs.



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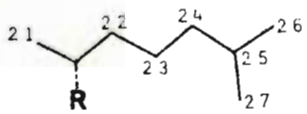


(62)

If no substituent was present at C-21, C-26 or C-27 in the spicatin side chain there would be three quartets present for these atoms in the ^{13}C n.m.r. spectrum. The ^{13}C n.m.r. spectrum of the molecule has shown that no double bonds are present in the side chain, and as there are only two quartet resonances, one of the methyl groups must be substituted in some way. The mass spectrum has shown that a C_3H_5 fragment is lost from the side chain so it appears that the two methyl groups would be geminal and the C-21 methyl group would be modified.

The ^{13}C n.m.r. spectrum of spicatin has shown nine resonances attributable to the eight side chain carbon atoms. The singlets at δ97.37 and δ95.71 which both correspond to a carbon atom attached to two oxygen atoms disappear to give a single ketone resonance (δ210.81 (s)) on acetylation of the compound, so it is assumed that these two resonances are due to a single carbon atom which gives two resonances because two different stereoisomers occur here. On acetylation of the compound it appears that ring opening occurs at this point resulting in a single stereoisomer.

The formula of the side chain indicates that, as no double bonds occur in the side chain, two rings must be present. It is extremely unlikely that a four-membered ring would occur. It is known that two methyl groups are present, so it seems likely that a five-membered and a three-membered oxide ring are present in the side chain. This would

mean that the six carbon atoms which do not occur as part of a methyl group, are all involved in either of the ring structures.

However, no resonances ascribable to the carbon atoms of an epoxide (the three-membered oxide ring) occur in the ^{13}C n.m.r. spectrum of spicatin. Furthermore there is a M-41 peak (m/e = 485.2898), which possibly corresponds to the loss of a $CH_2=\underset{|}{C}-CH_3$ fragment from the side chain on determination of the mass spectrum although no resonances due to a side chain double bond occur in the ^{13}C n.m.r. spectrum of spicatin.

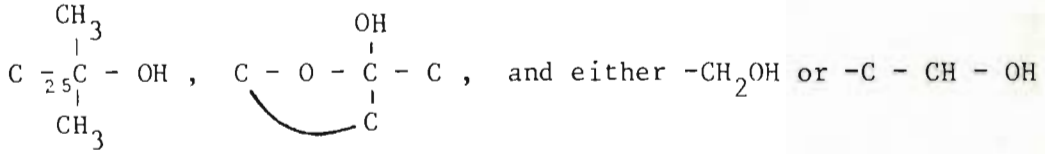
This suggests that a water molecule was lost on determination of the mass spectrum of spicatin so that 526.3323 is not the true molecular weight of spicatin. If a tertiary hydroxy group was present in spicatin at C-25, it might have been so readily lost with a proton from one of the neighbouring methyl groups, that it was not detected in the mass spectrum, which would account for the loss of the isopropenyl group. Thus spicatin would have a molecular weight of 544, not 526, and the formula for the side chain would be $C_8 H_{15} O_4$. Further evidence for the lack of a second oxide group comes from the fact that two further water molecules corresponding to two further hydroxyl groups are seen to be lost in the mass spectrum. If one five-membered oxygen-containing ring is present (see later), the presence of these two hydroxyl groups would exclude the possibility of the epoxide. Thus the side chain formula of $C_8 H_{15} O_4$ includes three hydroxy groups, one of which occurs at C-25.

As the carbon atom giving the two singlet resonances appears to be joined to an oxygen atom that is part of a ring, and three of the four side chain oxygen atoms occur in hydroxy groups, this carbon atom must have one of the hydroxy groups attached to it, and a further hydroxy

group must occur elsewhere in the side chain.

The only two unassigned resonances which could be attributed to a carbon atom attached to an oxygen occur at δ67.45 (d) and δ57.13 (t). One of these resonances would be due to the hydroxy group, and the other to the carbon atom joined to the oxygen atom in the ring.

At this stage the presence of the following parts of the side chain have been established:

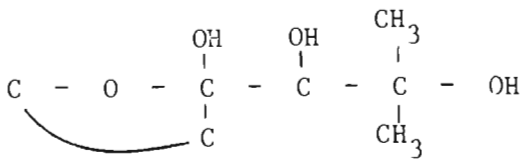


The singlet ascribable to C-25 shifts very slightly on acetylation of the compound (from δ76.14 (s) to δ77.31 (s)). This indicates that, as expected, the tertiary hydroxyl group has not been acetylated. It also indicates that C-25 is not situated next to the carbon atom which gives the two singlet resonances because when the hydroxy group occurring at this atom was changed to a ketone group on acetylation, a much greater shift would occur in the resonance ascribable to C-25. Thus it appears that a -C-OH group, not the O-C-O group occurs adjacent to C-25. The slight shift in the C-25 resonance on acetylation of the compound would be ascribable to acetylation of the neighbouring hydroxy group. The carbon atom adjacent to C-25 (i.e. C-24) would have to give a doublet in the ^{13}C n.m.r. spectrum (δ67.45).

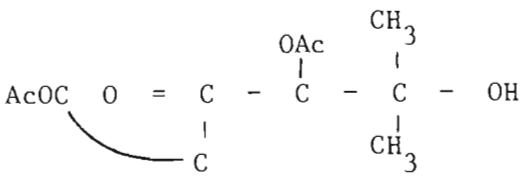
The ketone resonance in the spectrum of the triacetate occurs in a position (δ210.81 (s)) which suggests that it is vicinal to a carbon atom attached to an oxygen atom. Hence it is likely that the ketone occurs at C-23. If C-23 was not part of the ring structure, no

ring could occur as rings containing less than five members are unlikely.

At this stage the existence of the following part of the side chain has been established:

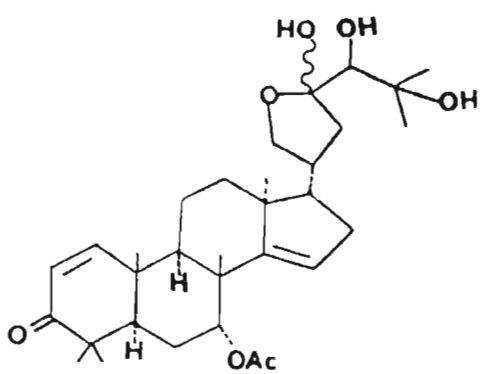


On acetylation of this side chain, the following would be obtained:

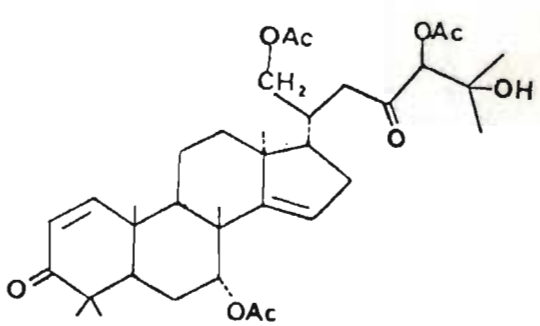


The triplets occurring at δ57.13 and δ29.60 and the doublet at δ38.58 remain unassigned. C-20 could not be a triplet so the resonance at δ38.58 (d) is assigned to C-20. It has been suggested earlier than C-21 must be substituted in some was as it does not occur as a methyl group, so the resonance at δ57.13 (t) is assigned to C-21. The remaining triplet (δ29.60) would have to be assigned to C-22.

Thus spicatin would have the structure (63). On acetylation, the ring opens to give a keto group at C-23 and acetate groups at C-21 and C-24. Thus the triacetate would have the structure (64).



(63)



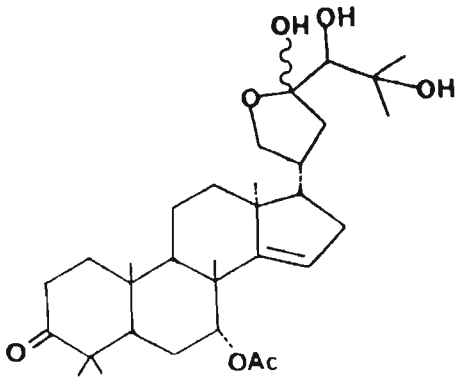
(64)

Various reactions have been carried out with spicatin in order to try to obtain derivatives which were pure isomers and consequently had simpler ^{13}C n.m.r. spectra than spicatin.

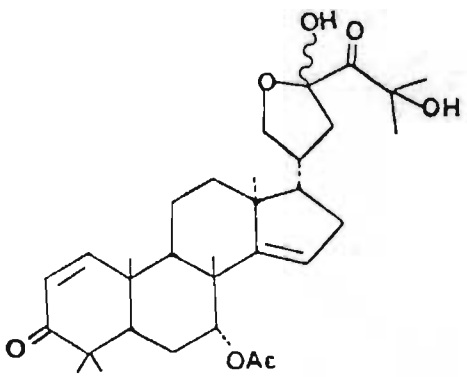
Catalytic hydrogenation of spicatin resulted in the loss of the doublets attributed to C-1 and C-2 at δ157 (d) and δ125 (d) but not the resonances due to C-14 and C-15 (δ158.80 (s) and δ119.19 (d)). The C-3 resonance moved from δ204.36 (s) where it occurs in the ^{13}C n.m.r. spectrum of spicatin to δ216.13 (s) in the spectrum of dihydrospicatin (C-7). This spectrum was complex and contained many extra bands, as did that of spicatin. For example, there were two singlets at δ158 and two doublets at about δ119, showing that the two stereoisomers at C-23 affect the resonance positions of C-14 and C-15. The two singlets present at δ97.37 and δ95.71 in the spectrum of spicatin, ascribable to C-23, were also present in the spectrum of dihydrospicatin. The ^{1}H n.m.r. spectrum of dihydrospicatin showed that the resonances at δ7.15 and δ5.83 (J 10 Hz), which were present, corresponding to 1-H and 2-H in a Δ^{1}-3-ketone system, in the spectrum of spicatin, were missing. Resonances could be ascribed to all the carbon atoms in the nucleus and side chain by means of comparison of the ^{13}C n.m.r. of dihydrospicatin with those of spicatin and spicatin triacetate. From this it was seen that dihydrospicatin was the same as spicatin except that the C-1, C-2 double bond had disappeared. Thus structure (65) was assigned to this compound.

Oxidation of spicatin with CrO_3 gave compound (66). The presence of the two C-23 singlets at δ92.57 and δ92.22 and the fact that two singlets occurred for C-14 (δ157.37, δ156.19) and two doublets for C-15 (δ119.17 and δ118.99), showed that the cyclic hemiacetal was still present. An extra singlet (δ207) occurred in the ^{13}C n.m.r. spectrum of

the oxidation product (Spectrum C-8) but the doublet at δ67.45 present in the spectrum of spicatin which has been assigned to C-24, was missing. The singlet due to C-25 occurring at δ76.14 in the spectrum of spicatin occurred here at δ77.25. In other respects, the spectrum of the oxidation product was very similar to that of spicatin. Thus it appears that the oxidation product is the 24-keto derivative of spicatin and has the structure (66).



(65)



(66)

On reaction of spicatin with $NaIO_4$ in an acid medium, compound (67) was obtained. The ^{13}C n.m.r. spectrum (Spectrum C-9) of this compound showed all the usual resonances corresponding to the carbon atoms in the nucleus, plus two extra singlets (δ177, δ72.0), three doublets (δ103.3, δ55.0, δ46.3), a triplet (δ64.7), two quartets and another triplet (δ66.8) corresponding to dioxan, the solvent used for the reaction which had been retained in the amorphous reaction product. The resonances at δ72.0 (s) and δ55.0 (d) correspond to the carbon atoms at the two ends of an epoxide. (These resonances occur at δ71.5 (s) and δ57.0 (d) in heudelottin F(55) a compound with a 14,15-epoxide.)[37] The resonances at δ177 (s) and δ103.3 (d) correspond to a double bond. Unusual positions like this are known for enolate double bond resonances, for example, in bussein (69) C-15 resonates at δ96 (s) and C-4' at δ186 (d) as a result of the carbonyl group at C-16 and the hydroxy group at C-4.[40] The singlets corresponding to C-23 have disappeared and only one resonance each occurs for C-14 and C-15 and the triplet ascribed to C-22 has disappeared. Thus it appears that the acid medium has caused dehydration of

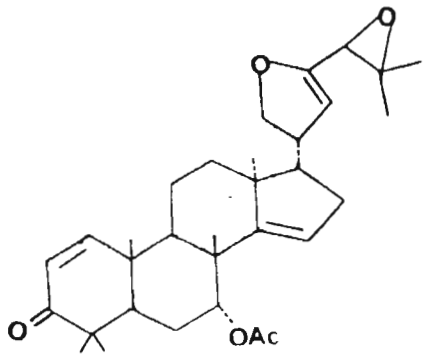
the spicatin side chain to give a 24,25-epoxide and a 22,23-double bond. The singlet at δ177 would be due to C-23 and the doublet (δ103.3) would be due to C-22. These resonances have been shifted downfield and upfield, respectively, because they are part of the cyclic hemiacetal and because of the adjacent epoxide group. The remaining doublet (δ46.3) and triplet (δ64.7) are ascribable to C-20 and C-21, respectively.

The epoxide would be formed by the tertiary hydroxyl at C-25 being lost and the resulting carbocation forming an epoxide with the adjacent C-24 hydroxy group.

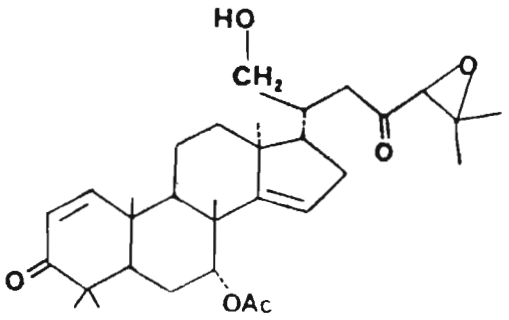
Thus structure (67) is ascribed to this product.

Thin layer chromatography testing a sample of the mixture obtained when spicatin was reacted with $POCl_3$ indicated that compound (67) was present in this mixture.

Refluxing spicatin with methanol and sulphuric acid yielded compound (68). The two singlets attributed to the two stereoisomers at C-23 were missing and single resonances occurred for C-14 (δ159.03 (s)) and C-15 (δ119.29 (d)) in the ^{13}C n.m.r. spectrum of this compound (Spectrum C-10). Resonances ascribable to all the carbon atoms in the usual spicatin nucleus were present. An extra singlet (δ211.12) was present and also resonances at δ72.64 (s), δ39.12 (d), δ58.50 (d), δ59.04 (t), δ29.68 (t) and two quartets, making up the eight side chain carbon atoms. The resonances ascribable to the carbon atoms at both ends of an epoxide were present (δ72,64 (d), δ58.50 (d)). Thus it appears that the cyclic acetal must have opened to give a keto group at C-23 (δ211.12 (s)) and a $-CH_2OH$ group (δ59.04 (t)) at C-21 and that the acidic conditions have again caused dehydration of the 24,25-diol to give a 24,25-epoxide.



(67)



(68)

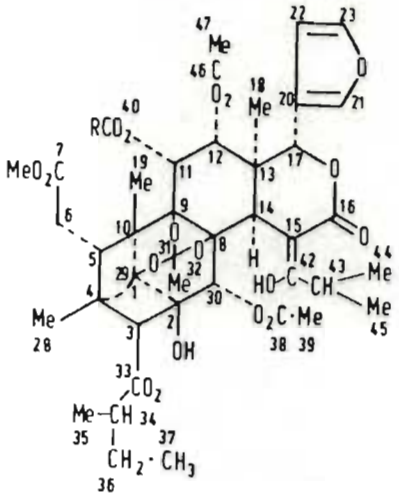
Spicata 2

The ^{1}H n.m.r. and ^{13}C n.m.r. spectra of spicata 2 (Spectra H-25, C-11) were very similar to those of bussein (69). (See Tables 5.6 and 5.7). Spicata 2 was found to have a molecular weight of 914, which is 42 more than that of bussein. It was impossible to obtain an accurate mass for spicata 2 as it was too high for the mass spectrometer to measure accurately.

The ^{1}H n.m.r. spectrum of spicata 2 showed that one of the three acetate groups present in bussein, the one resonating at δ2.17, was absent in spicata 2. Work by Hanni[41] on the partial hydrolysis of bussein has shown that the peak at δ2.17 corresponds to the acetate at C-11. The acetate at C-30 (δ2.0) and C-12 (δ1.6) in the spectrum of bussein were found to be present in the spectrum of spicata 2 but the H-11 resonance (δ5.61 in bussein) was found to have moved slightly upfield. It would appear that the acetate at C-11 has been replaced by another ester. A 2-methyl-butyrate ester would account for the difference of 42 in molecular weight between bussein and spicata 2.

This proposal is substantiated by comparison of the ^{13}C n.m.r. spectra of bussein[40] and spicata 2. The spectrum of spicata 2 lacks the quartet at δ20.8, corresponding to an acetate methyl group, but has gained a doublet (δ41.3), a triplet (δ27.0) and two quartets (δ16.0 and 14.0).

These four extra resonances indicate the presence of a 2-methylbutyrate group. Thus structure (70) was proposed for spicata 2.



(69) R = CH_3-

(70) R = $CH_3 - CH_2 - \underset{\displaystyle CH_3}{\overset{|}{CH}} -$

CHAPTER FIVE

EXPERIMENTAL

5.1 CHROMATOGRAPHIC TECHNIQUES

5.1.1 Thin layer chromatography

Thin layer chromatography was performed using pre-coated 0,2 mm thick silica gel 60 F_{254} sheets (Merck Art 5554). The solvent system used for the *Aphanamixis polystacha* and *Entandrophragma spicatum* extracts was hexane : ethyl acetate (30:60) and the system used for the *Trichilia dregeana* extract was hexane : ethyl acetate (50:50).

The spray reagent used was anisaldehyde : conc. sulphuric acid : ethanol (5:5:90).

5.1.2 Preparative thin layer chromatography

Preparative thin layer chromatography was attempted using the *Aphanamixis polystacha* extract. Plates coated in our laboratory using silica gel 7730 and commercially obtained 2 mm thick silica gel 60 plates (Merck Art. 5745) were used. Samples of 80 mg of extract were dissolved in 1 cm^3 of methylene chloride and applied to the plates which were run three times in a solvent system of hexane : ethyl acetate (30:60). The bands were visualized by spraying a narrow strip on each side of the

plate with the anisaldehyde spray reagent. The silica gel was scraped off the plates in bands and extracted for ten minutes with methylene chloride.

This technique was not found to be successful either because of the similarity of the R_f values of the compounds or because the compounds were isomerising on the plates.

5.1.3 Column chromatography

Four sorts of column chromatography were used. With all three extracts, a rough separation was first obtained by using a column containing coarse silica gel (Merck 7733). The solvent systems used were the same as those described for thin layer chromatography. A ratio of about 30 g of silica gel to 1 g of extract was used.

The *Aphanamixis polystacha* extract was further separated using columns containing silica gel with a particle size of less than 0.08 mm (Merck 7729). Repeated chromatography was necessary in order to obtain pure compounds. This method was found to be very unsatisfactory as the separations obtained were not, in general, very good and the columns took a long time, sometimes two or three days, to run.

At first this method was attempted to separate the extracts from *Trichilia dregeana* and *Entandrophragma spicatum*, but the same problems were experienced here. In order to overcome these problems, the third sort of column chromatography, "medium pressure chromatography",[42] was attempted. The same grade silica gel (Merck 7729) and the same solvent systems were used. Using this method, it took about forty-five

minutes for one run to be performed, the separation obtained was found to be slightly better than previously, and the columns could be re-used five or six times without losing their efficiency. The apparatus required for this technique consisted of a chromatographic column (2,5 cm diameter) and a flow controller valve. The column was a flattened bottomed 50 cm long glass tube fitted with a teflon stopcock and topped with a ground glass joint into which the flow controller fitted. The flow controller had an air inlet which was attached to a pump,and a regulator valve.

A piece of glass wool was placed in the area between the stopcock and the flattened bottom of the column, then a layer of sand (2 mm deep) was added. Silica gel was poured in to a depth of 20 cm, and the column was tapped on the bench until the silica gel level stopped dropping. A further 2 mm deep layer of sand was added on top of the silica gel. The column was filled with solvent, the flow controller attached, the regulator valve closed, the stopcock opened and the pump switched on. When the solvent level reached the top of the sand, the pump was switched off, the regulator valve opened and the stopcock closed. The sample (200 mg dissolved in a minimum of the solvent being used) was applied under pressure, then the walls of the column were washed with a minimum of the solvent and presssure was applied until the solvent level reached the top of the sand. The column was refilled with solvent and the run commenced. Fractions of 15 cm^3 were collected. Usually about thirty fractions were collected.

The silica gel was far more tightly packed using this method than it had been using the previous one, so in order to force the solvent through the silica gel, the regulator valve had to be kept closed, causing

a build-up of pressure in the column. This build-up of pressure was considered rather dangerous.

The fourth sort of column chromatography was used in the separation of some of the spicatin derivatives. It was thought that the products of these reactions might be sensitive to acid, so aluminium oxide was used as an absorbant instead of silica gel. It was found that even with the regulator valve left open, the pressure provided by the pump was sufficient to obtain a flow rate far better than that obtained using silica gel and separation seemed to be as good as with silica gel.

5.2 SPECTROSCOPIC TECHNIQUES

5.2.1 ^{1}H nuclear magnetic resonance spectroscopy

Samples were dissolved in deuteriochloroform and spectra were recorded at 40^{o} using a CFT-20-spectrophotometer at 80 MHz. Absorbances were measured in p.p.m. from internal TMS, either graphically or by print out from the computer.

5.2.2 ^{13}C nuclear magnetic resonance spectroscopy

Samples were dissolved in deuteriochloroform and spectra were recorded at 40^{o} using a CFT-20 spectrophotometer. Shifts were printed out by the computer in p.p.m. relative to the central line of the $CDCl_3$ triplet at δ77.07. Spectra were recorded with proton noise decoupling and with single frequency off resonance decoupling.

5.2.3 Infrared spectroscopy

Samples were dissolved in spectroscopic chloroform and spectra recorded using a Perkin-Elmer 727 infrared spectrophotometer.

5.2.4 Mass spectroscopy

Mass spectra were recorded by Dr. Peter Bladon of the University of Strathclyde on an MS 901 with computer data processing equipment.

5.3 EXTRACTIVES FROM THE SEED OF *Aphanamixis polystacha*

5.3.1 Extraction of the seeds

Seeds (3 kg) of *Aphanamixis polystacha* were minced and then extracted with light petroleum in a soxhlet for 24 hours. Solid material separated out from the petroleum. The petroleum was decanted from the solid material. After recrystallisation from methanol, the crystalline compound, rohitukin (38),was obtained from this solid material. The mother liquor was investigated further.

5.3.2 Chromatography of the *A. polystacha* extract

A thin layer chromatogram of the mother liquor showed that it was a mixture of several compounds. After repeated column chromatography (Section 5.1.3) two compounds, polystachin(48) (or rohituka 9) and rohituka 10 (49) were obtained pure.

5.3.3 Acetylation of rohituka 10

Rohituka 10 (100 mg) was dissolved in pyridine (1 cm^3). Acetic anhydride (1 cm^3) was added, the mixture was briefly warmed on a steam-bath and left to stand overnight. Methanol (2 cm^3) was added and the solvent was removed on a rotor-evaporator. Toluene (2 × 2 cm^3) was added and removed in order to remove remaining traces of pyridine, then methanol (2 × 2 cm^3) was added and removed in order to remove remaining traces of toluene. Thin layer chromatography showed that a pure product had been obtained.

5.3.4 Spectroscopy of compounds isolated from *A. polystacha*

5.3.4.1 ^1H n.m.r. spectroscopy of polystachin (Spectrum H-13) and rohituka 10 (Spectrum H-14)

The data obtained from compounds previously isolated from *A. polystacha*[27] are included for purposes of comparison. (Table 5.1)

5.3.4.2 ^{13}C n.m.r. spectroscopy of *A. polystacha* compounds

^{13}C n.m.r. spectra of rohituka 1[39] acetate, rohituka 2 (40), rohituka 7 (45), polystachin (48), rohituka 10 (49) and rohitukin (38) have been recorded. (Table 5.2)

5.3.4.3 Mass spectroscopy of polystachin (48) (Spectrum M-1)

The molecular weight of polystachin was found to be 598.2396. $C_{32}H_{38}O_{11}$ requires 598.2414.

5.3.4.4 Infrared spectroscopy of polystachin (48) (Spectrum I-1)

The infrared spectrum of polystachin was recorded in spectroscopic chloroform. (η = 1725, 1738, 1761, 1768 cm^{-1}).

5.4 EXTRACTIVES FROM THE SEED OF *Trichilia dregeana*

5.4.1 Extraction of the seeds of *Trichilia dregeana*

The seeds of *Trichilia dregeana* (10 kg) were collected from the grounds of Durban High School. About half of the seeds were shelled.

The seed coats were ground in a coffee grinder, resulting in an oily, finely-particulated material which had to be mixed with about twice its volume of celite before it could be extracted. The material was extracted for 24 hours with refluxing hexane. The solvent was decanted, leaving a yellow precipitate.

The seeds were crushed using an ice-crushing machine and extracted for 24 hours with refluxing hexane. A yellow precipitate was obtained after decantation of the solvent.

The remainder of the unshelled seeds were milled and extracted at a later stage, giving a third extract.

5.4.2 Chromatography of the extracts

Thin layer chromatography of the seed coat extract showed that it contained about half oil and several limonoids. Preliminary column chromatography over coarse silica gel (Section 5.1.3) separated most of

the oil, leaving an extract comprised mainly of limonoids. Repeated column chromatography using fine silica gel yielded rohituka 7 (45), a crystalline compound which had been obtained previously from the seed of *Aphanamixis polystacha*;[27] dregeana 1 (49), an amorphous compound which was found to be present in the same extract from *A. polystacha* and has also been named rohituka 10; dregeana 2 (35); dregeana 3 (34) and dregeana 5 (58).

Thin layer chromatography of a sample of the extract from the shelled seeds showed that most of it was fat, but that a small amount of limonoid material was present. The extract was extracted with aqueous methanol, and the resulting limonoid fraction was chromatographed using fine silica gel. The compound dregeana 3 (a) was obtained from this extract.

The third extract yielded an oil which was partitioned between hexane and methanol to give a limonoid fraction (0.125%); mixed triglycerides and palmitic acid, which was identified by a g.l.c. of its methyl ester. The limonoid fraction gave crystalline rohituka 7 (45), and the compounds dregeana 2 (35) and dregeana 4 (57) were obtained from the mother liquor after medium pressure chromatography. Mr. Akinniyi of Glasgow University obtained rohituka 7 (45), dregeana 1 (49), dregeana 2 (35), dregeana 3 (34) and dregeana 3 (b) (56) from this extract using the technique of preparative thin layer chromatography.

5.4.3 Melting points of crystalline compounds

Rohituka 7 (45) had a melting point of 239-242^{o}. This was the same as that of a specimen that had been obtained from the seed of

Aphanamixis polystacha.[27]

Dregeana 2 (35) and 3 (34) crystallized on evaporation of a methanol solution. Neither compound had a sharp melting point, but dregeana 2 melted at about 150° and dregeana 3 at about 100°.

5.4.4 Spectroscopy of compounds obtained from *Trichilia dregeana*

Details of the ^{13}C n.m.r. and ^{1}H n.m.r. spectra of rohituka 7 (45) and dregeana 1 (49) are given in Section 5.3.4 and 5.3.5.

5.4.4.1 ^{1}H n.m.r. spectra of dregeana 2 (35), 3 (34), 4 (57) and 5 (58)

The data obtained from the ^{1}H n.m.r. spectra of these compounds (spectra H-16, H-17, H-18, H-20) is given in Table 5.3.

5.4.4.2 ^{13}C n.m.r. spectra of dregeana 2 (35), 3 (34) and 4 (57)

The data obtained from the ^{13}C n.m.r. spectra of these compounds (spectra C-2, C-3, C-4) is presented in Table 5.4.

5.4.4.3 Mass spectroscopy of dregeana 2 (35), 3 (34) and 4 (57)

The mass spectrum of dregeana 2 (spectrum M-2) showed that this compound had a molecular weight of 646.2674. (C_{33} H_{42} O_{13} requires 646.2625).

The mass spectrum of dregeana 3 (spectrum M-3) showed that this compound had a molecular weight of 684,3538 (C_{38} H_{52} O_{11} requires 684.2589).

The mass spectrum of dregeana 4 (spectrum M-4) showed that this compound had a molecular weight of 758.3876 (C_{41} H_{58} O_{13} requires 758.3877).

5.4.4.4 Infrared spectroscopy of dregeana 2 (35) and 4 (57)

The infrared spectra of degreana 2 (35) (spectrum I-2) and 4 (57) (spectrum I-3) were recorded in spectroscopic chloroform.

The infrared spectrum of dregeana 2 showed peaks at η = 1720, 1735, 1747, 1750 and 1753 cm^{-1}.

The infrared spectrum of dregeana 4 showed peaks at η = 1731, 1749, 1758 and 1763 cm^{-1}.

5.5 EXTRACTIVES FROM THE HEARTWOOD OF *Entandrophragma spicatum*

5.5.1 Extraction of the heartwood of *E. spicatum*

The powdered heartwood of *E. spicatum* (10 kg) was extracted with light petroleum for 24 hours. The resulting extract was evaporated to dryness and the residue was dissolved in toluene-cyclohexane. A thick gum sank to the bottom and the supernatant was decanted off. Entandrophragmin(59) crystallised out from the supernatant.[25] The gum was examined further.

5.5.2 Chromatography of the *E. spicatum* extract

Thin layer chromatography revealed that the gum contained much entandrophragmin and numerous other compounds. Column chromatography over

silica gel yielded spicatin (63), a gum, and a second compound, spicata 2 (70), which was crystalline. Spicata 2(m.p.272) was recrystallised from a mixture of ethyl acetate and hexane.

5.5.3 Reactions of spicatin

It was found to be very difficult to obtain pure spicatin and reactions generally gave a mixture of products. All the reaction products had to be chromatographed to remove the impurities which had been present in the starting material and the minor reaction products.

5.5.3.1 Acetylation of spicatin

Spicatin (500 mg) was dissolved in pyridine (5 cm^3). Acetic anhydride (5 cm^3) was added, the mixture was briefly warmed on a steambath and left to stand overnight. Methanol (10 cm^3) was added and the solvent was removed on a rotor-evaporator. Toluene (2 × 10 cm^3) was added and removed in order to remove remaining traces of pyridine, then methanol (2 × 10 cm^3) was added and removed in order to remove remaining traces of toluene. Thin layer chromatography showed that all the spicatin had been acetylated, giving one main product but several other components from impurities in the starting material. The mixture was separated by means of column chromatography and spicatin triacetate (64), a gum, was obtained pure.

5.5.3.2 Chromium trioxide oxidation of spicatin

Chromium trioxide (400 mg) was added to a magnetically stirred mixture of pyridine (10 cm^3) and methylene chloride (10 cm^3). The flask was fitted with a drying tube containing calcium chloride and stirring

was continued for 15 minutes. A solution of impure spicatin (200 mg) in methylene chloride (2 cm^3) was added. The mixture was stirred for 12 hours at room temperature, the solvent was decanted, and the residue extracted with ether (2 × 10 cm^3). The organic fractions were combined and taken to dryness under reduced pressure. The product was purified by means of column chromatography, giving compound (66).

5.5.3.3 Sodium periodate oxidation of spicatin

Sodium periodate (2 g) in water (5 cm^3) and 70% perchloric acid (2 drops) were added to a solution of spicatin (1 g) in dioxan (100 cm^3). The mixture was stirred for one hour and then the preciptate of sodium iodate was filtered off and washed with dioxan. Sodium hydrogen carbonate (200 mg) was added to the clear solution, which was concentrated to 25 cm^3 and then poured into water. Extraction with ether yielded a gum which was chromatographed to give a pure product (67) which was amorphous.

5.5.3.4 Catalytic hydrogenation of spicatin

Spicatin (500 mg) was dissolved in methanol (20 cm^3). PtO_2 (100 mg) was added and the magnetically stirred solution was hydrogenated for 10 minutes. The solution was filtered to remove the catalyst, the methanol was removed under reduced pressure, and the product was chromatographed to obtain dihydrospicatin (65).

5.5.3.5 Mild methanolic sulphuric acid treatment of spicatin

Spicatin (0,65 g) was heated under reflux with sulphuric acid

(0.45 cm^3) in methanol (110 cm^3) for 25 minutes. The mixture was poured into water (150 cm^3) and extracted with chloroform (3 × 75 cm^3). Evaporation yielded a gum which was chromatographed to obtain a pure product (68).

5.5.3.6 Reaction of spicatin with $POCl_3$

Spicatin (0.5 g) in pyridine (6 cm^3) was treated with phosphorous-oxychloride (1.5 cm^3) and one drop of water. Next morning the mixture was poured on ice and ether, and the organic layer washed with dilute hydrochloric acid, sodium carbonate and water, dried and evaporated. Thin layer chromatography of the residue against the product obtained from the sodium periodate reaction (67) showed that the residue was a mixture of four components, one of which appeared to be (67).

5.5.4 Spectroscopy of spicatin and its derivatives

5.5.4.1 Mass spectroscopy of spicatin (63)

The mass spectrum (spectrum (M-4)) showed that spicatin had a molecular weight of 526.3323. $C_{32}H_{46}O_6$ requires 526.3294.

5.5.4.2 Nuclear magnetic resonance spectroscopy

^{13}C n.m.r. spectra were recorded for spicatin and the five derivatives prepared from it. (spectra C-5 to C-10). ^{1}H n.m.r. spectra (spectra H-21 to H-24) were also recorded. A comparison of the ^{13}C n.m.r. spectra is given in Table 5.5.

5.5.4.3 Infrared spectroscopy of spicatin (63)

The infrared spectrum of spicatin was recorded in spectroscopic chloroform. Absorption maxima were found at η = 1655, 1667, 1721, 1733 and 1747 cm^{-1}.

5.5.5 Spectroscopy of spicata 2 (70)

5.5.5.1 ^{1}H n.m.r. spectroscopy of spicata 2 (70)

The data obtained from the ^{1}H n.m.r. spectrum of spicata 2 (70) (spectrum H-25) are compared with those of bussein (69)[41] in Table 5.6.

5.5.5.2 ^{13}C n.m.r. spectroscopy of spicata 2 (70)

A comparison between the ^{13}C n.m.r. spectra of spicata 2 (70) (spectrum C-11) and bussein (69)[40] is given in Table 5.7.

5.5.5.3 Mass spectroscopy of spicata 2 (70)

Spicata 2 (70) was found to have a molecular weight of 914, which corresponds to a molecular formula of $C_{47}H_{62}O_{18}$. The internal standard which was used to determine this molecular weight did not have a high enough molecular weight in order for an accurate molecular weight to be determined for spicata 2.

5.5.5.4 Infrared spectroscopy of spicata 2 (70)

The infrared spectrum of spicata 2 (70), recorded in spectroscopic chloroform, had adsorption maxima at η = 1590, 1630, 1740, 1759 cm^{-1}.

5.6 TABLES

Table 5.1: ^{1}H NMR spectra of *Aphanamixis polystacha* compounds

Proton	Rohitukin (38)	1 (39)	1* acetate	2* (40)	2 acetate	3* acetate (41)	4 (42)	5 acetate (43)	6 acetate (44)	7* (45)	7 acetate	8 (46)	9* (48)	10 (49)	10 acetate
1	5.25 m		5.78 m8	5.85 m 6	5.75 m	3.98 m 10	5.26 m	4.16 m		7.58 d 12		7.05 d 12	3.78 m 16	3.78 m 16	
2	3.24 bs		2.98 m			2.50 m				6.11 d 12		6.12 d 12			
9	3.72 d 7		3.25 d 6	3.23 d 5		3.22 d 6.3				3.16 d 10			2.73 d 6	2.73 d 6	
11	5.49 m 19		5.40 m 17	5.47 m 16	5.55 m	3.79 m 15	5.5 m 19	4.06 m		5.70 m		5.67 m	5.32 m 15	5.32 m 15	
12	6.06 d 11		6.12 d 11	6.15 d 11	5.97 d 11	5.84 d 9	6.25 d 12	5.75 d 8		6.25 d 11		6.12 d 12	6.05 d 9	6.13 d 12	
15	NA		5.71 m 16	5.70 m 13	5.70 m	NA	NA	5.57 m 16		5.75 m			NA	NA	
16	2.4 m		2.38 m	2.32 m						2.37 m					
17	3.98 m 18		4.0 m 20	4.05 m 20				3.85 m		4.01 m 10			3.88 m 19	3.97 m 20	
29A	4.18 s	3.86 bs	4.61 d 13	3.92 bs	4.56 d 13	4.35 d 12		4.35 d 12		4.37 d 12		4.72 d 11	4.14 bs	4.14 bs	
29B	4.18 s	3.86 bs	4.15 d 13	3.92 bs	4.07 d 13	4.03 d 12		4.05 d 12		4.10 d 12		4.35 d 11	4.14 bs	4.14 bs	
30A	5.92	5.36	5.23	5.40		5.42	5.98	5.25		5.32		5.35	5.48	5.51	
30B	5.58	5.22	5.18	5.27		5.42	5.74	5.17		5.27		5.35	5.48	5.51	
α furan A	7.36	7.4	7.4	7.37	7.35	7.37	7.38	7.37		7.4	7.37	7.35	7.33	7.36	
α furan B	7.24	7.26	7.26	7.25	7.35	7.37	7.28	7.37		7.25	7.37	7.20	7.22	7.22	
β furan	6.28	6.30	6.30	6.32	6.3	6.3	6.44	6.35		6.32	6.32	6.27	6.22	6.22	
CO_2Me	NA	3.75	3.74	3.77	3.70	NA	3.69	NA	3.80	NA	NA	3.85	NA	NA	
Ac	2.1	2.24 2.07	2.28 2.2 2.16	2.31 2.15	2.27 2.14 2.14 2.11	2.21	2.22 2.15	2.25 2.22	2.22	2.2	2.2 2.09	2.2 2.12	NA	NA	2.09
CMe	1.83 1.75 0.98		1.70 1.47 1.02	1.70 1.56 1.10	1.73 1.51 1.33	1.92 1.27 1.05	1.81 1.66 1.03	1.78 1.3 1.10	1.67 1.36 1.15	1.75 1.27 1.12	1.75 1.27 1.12	1.61 1.36 1.13	1.97 1.02 0.86	1.97 1.00 0.88	
2'					4.72 d 4.5	4.82 d 4.5		4.86 d 3.5		3.30	4.68 d 4			3.38 d 4	4.72 d 4
Formate	7.85	8.02	8.02	8.02	8.02	NA	7.91	NA	NA	7.97	7.97	7.97	8.07	8.07	8.07

Couplings J for doublets $J_{AX} + J_{BX}$ for ABX systems, are given in Hz. NA = Not applicable * = Investigation by double resonance.

Table 5.2 ^{13}C NMR spectra of compounds from *A. polystacha*

Carbon Atom	1 acetate (39)	2 (40)	7 (45)	Polystachin (48)	Dregeana (1) (49)	Rohitukin (38)
1	(d) 73.3	(d) 75.1	153.1 (d)	(d) 73.9	(d) 75.2	(d) 75.8
11	73.0	74.9	(d) 76.2	73.1	75.0	75.7
12	72.7	73.8	75.1	73.0	73.6	71.8
15	70.6	72.6	72.5	205.1 (s)	205.0 (s)	206.6 (s)
2'	(t) 43.0	69.7	71.6	(t) 43.2	72.1	(t) 42.9
6	36.2	(t) 36.1	(t) 36.8	41.1	(t) 41.1	37.6
16	36.0	35.4	30.2	38.6	38.3	32.2
4'	-	35.2	23.3	-	38.0	-
2	33.6	34.2	120.6 (d)	32.8	32.7	22.5
5	44.2	(d) 41.6	51.1 (d)	(d) 41.1	(d) 41.1	(d) 42.8
9	39.7	39.6	(d) 39.3	41.1	40.7	42.0
3'	36.0	38.1	37.9	37.2	36.8	35.4
3 (s)	168.8	168.9	166.6	167.5	167.5	169.3
4	(s) 88.4	(s) 92.1	(s) 84.9	(s) 87.6	(s) 87.3	(s) 81.2
14	84.4	84.3	79.2	78.7	78.6	79.5
7	(s) 173.6	(s) 175.5	(s) 174.9	(s) 172.3	(s) 174.9	(s) 173.3
1'	172.2	175.3	171.9	172.3	172.4	172.3
8 (s)	142.8	142.9	140.9	135.0	134.3	139.2
10	(s) 50.7	(s) 50.9	(s) 51.1	(s) 50.2	(s) 50.0	(s) 49.7
13	48.7	48.8	44.0	49.2	49.1	46.4
17 (d)	52.1	52.0	52.4	55.7	55.2	52.0
20 (s)	123.8	123.7	123.7	122.3	121.9	123.0
21 (d)	142.6	142.9	143.1	143.0	143.3	142.9
22 (d)	110.9	110.7	110.7	110.6	110.3	110.8
23 (d)	140.5	140.7	140.7	140.8	142.7	140.8
29 (t)	67.9	66.2	74.7	74.4	74.4	78.0
30 (t)	119.7	120.0	119.5	118.8	119.3	124.7
Acetate C = O(s)	170.9	171.4	169.4	-	-	169.5
	169.8	170.4				
	169.6					
O-Me (q)	53.0	52.8	-	-	-	-
Formate (d)	160.9	161.3	159.9	160.2	160.3	160.3
C-Me (q)	24.9	22.7	27.2	29.0	29.0	24.9
	22.4	20.7	24.0	24.9	22.2	23.3
	22.3	20.7	20.8	22.3	15.1	22.5
	20.8	19.4	15.3	22.3	12.3	21.4
	20.8	15.1	13.5	12.2	11.4	21.2
	20.7	13.3	11.3			12.9
	20.1	11.4				
	13.2					

Table 5.3 ^{1}H NMR Spectra of Dregeana 2(35), 3(34), 4(57) and 5(58)

Proton	Dregeana 2	Dregeana 3	Dregeana 4	Dregeana 5
1	5.50 m 15	4.71 m 8	4.81 m 8	6.47 d 12
2	2.97 m	3.12 d 4	3.23 d 3	5.95 d 12
7	NA	5.26 m 6	5.27 m 6	5.33
9	3.72 d 7	-	-	-
11	5.22 m 18	-	-	-
12	5.93 d 11	5.06 m 17	5.03 m 18	5.19 m 16
15	NA	5.47 m 5	5.56 m 6	5.52 m
16	2.41 , 2.89 m m	2.41 m	2.50 m	-
17	3.93 m 20	2.93 m 17	-	-
29A	NA	NA	4.1 d 3	4.13 d 12
29B	NA	NA	4.0 d 3	4.58 d 12
30A	5.75 bs	NA	NA	NA
30B	6.05 bs	NA	NA	NA
α furan A	7.21	7.15	7.15	7.23
α furan B	7.32	7.31	7.31	7.35
β furan	6.23	6.21	6.21	6.28
CO_2Me	3.74	NA	NA	NA
Ac	2.12 2.06	2.2 2.13	2.13 2.0	2.03
C Me	1.72 1.62 1.56 1.52 1.02	2.0 1.57 1.55 1.32 1.27 1.05	1.46 1.30 1.25 1.08	-
2'	NA	4.92 d 4.5	4.87 d 4.5 3.90 bs	4.03 bs

Couplings, J for doublets, $J_{AX} \times J_{BX}$ for ABX systems, in Hz. NA = not applicable

Table 5.4 ^{13}C NMR Spectra of Dregeana 2(35), 3(34) and 4(57)

Carbon Atom	Dregeana 2	Dregeana 3	Dregeana 4
C = O 3	(s) 169.3	(s) 170.7	(s) 174.8
Acetates	170.6	170.7	174.0
	170.3	169.9	170.9
	170.3	169.9	-
1'	-	169.7	169.6
1"	-	-	168.8
7	176.1	(d) 76.5	(d) 72.2
1	(d) 71.1	75.1	76.3
2'	-	74.9	75.8
2"	-	-	75.6
12	72.75	71.1	71.1
11	73.0	(t) 36.8	(t) 37.2
2	(t) 41.35	35.2	35.7
6	38.1	34.1	26.8
16	34.35	29.7	26.0
4'	-	26.4	24.4
4	(s) 83.8	(s) 85.4	(s) 85.4
14	80.95	(s) 156.0	(s) 156.0
5	(d) 49.1	(d) 44.1	(d) 44.8
9	35.5	37.5	39.5
3'	-	27.3	37.6
3"	-	-	32.2

Carbon Atom	Dregeana 2	Dregeana 3	Dregeana 4
17(d)	51.6	51.4	50.6
8	(s) 139.0	(s) 51.4	(s) 51.8
10	(s) 49.2	44.4	44.8
13	48.0	41.7	42.0
15	(s) 206.5	(d) 122.1	(d) 122.7
20(s)	125.6	125.2	124.7
21(d)	142.6	142.2	142.6
22(d)	110.9	111.7	111.9
23(d)	140.5	140.4	140.8
29	(q)	(q)	(t) 66.2
30(t)	123.4	-	-
OMe(q)	52.9	-	-
CMe(q)	31.9	29.2	29.3
	27.8	25.0	28.3
	21.1	23.9	21.4
	21.1	23.9	20.8
	20.4	21.2	19.5
	18.9	20.6	16.3
	12.8	15.0	15.6
		15.0	15.6
		14.6	15.1
		11.5	11.8

Table 5.5 ^{13}C NMR Spectra of Spicatin and derivatives.

Carbon Atom	Spicatin 63	64	65	66	67	68
1	158.07(d)	157.59(d)	33.78(t)	159.04(d)	159.0(d)	157.45(d)
2	125.28(d)	125.60(d)	33.05(t)	125.84(d)	125.6(d)	125.76(d)
3(s)	204.36	204.02	216.13	204.06	204.1	203.97
4 (s)	46.39	46.76	46.73	46.85	46.6	46.95
8	43.96	44.17	46.64	44.29	44.1	44.28
10	42.55	42.90	42.03	43.01	42.8	43.00
13	39.67	39.93	37.01	40.09	39.9	40.11
5 (d)	46.03	46.35	43.03	46.48	46.2	46.52
9	38.44	38.83	38.83	38.83	38.6	38.89
6 (t)	34.78	34.67	34.71	34.21	34.4	35.25
11	34.46	34.27	33.78	33.99	34.3	34.04
12	23.67	23.87	23.25	23.92	23.7	23.95
16	16.61	16.85	16.75	16.62	16.3	16.67
7(d)	74.69	74.82	74.98	74.75	74.8	74.84
14(s)	159.02 158.73	159.26	158.96 158.67	157.37 156.19	157.7	159.03
15(d)	119.25 119.03	119.23	119.19 119.01	119.17 118.99	119.2	119.29
17(d)	46.31	56.0	48.22	46.48	56.0	50.48
20(d)	38.58	36.50	34.87	37.51	41.95	39.12
21(t)	57.13	65.78	57.29	58.44	64.7	59.04
22	29.60(t)	31.5 (t)	29.80(t)	34.81(t)	103.4(d)	29.68(t)
23	97.37 95.71 (s)	210.81(s)	96.05 (s) 91.20	92.57 (s) 92.22	177.0(s)	211.12(s)
24	67.45(d)	73.60(d)	67.64(d)	207 (s)	55.0(d)	58.50(d)
25(s)	76.14	77.31	76.22	77.25	72.1	72.64
Acetate C=O(s)	169.79	170.70	169.82	169.71	169.3	169.66
		170.26				
		169.62				
C-Me(q)	27.08	27.71	26.74	28.38	27.2	27.33
	26.88	27.33	26.10	28.27	27.2	27.14
	24.29	27.33	24.37	27.37	27.2	21.27
	23.91	27.07	24.37	27.15	27.2	20.90
	21.03	21.15	20.89	21.29	21.0	20.26
	20.84	20.87	20.89	20.96	20.8	18.92
	19.77	20.65	19.58	20.15	19.5	18.92
	18.78	20.63	15.00	18.92	18.6	18.92
		19.51				
		18.82				

Table 5.6 ^{1}H NMR Spectra of Spicata 2 (70) and Bussein (69).[41]

Proton	Spicata 2(70)	Bussein (69)
Furan	7.63	7.64
	7.35	7.30
	6.43	6.45
CO_2Me	3.72(s)	3.74(s)
H-17	5.80(s)	5.81(s)
H-11	5.56 (d,J = 2.5 Hz)	5.61 (d,J = 2.5 Hz)
H-30	5.46(s)	5.48(s)
H-3	4.94(s)	4.98(s)
H-12	4.62 (d,J = 2.5 Hz)	4.62 (d,J = 2.5 Hz)
H-14	3.26(s)	3.30(s)
H-29	3.15, 2.55 (J = 10 Hz)	3.15, 2.55 (J = 10 Hz)
H-2'	2.95 (m,J = 6.5 Hz)	2.94 (m,J = 6.5 Hz)
H-6	2.75 (m)	2.76 (m)
-OH	2.77*	2.77*
H on acetate groups	-	2.17
	1.98	2.00
	1.60	1.63
Methyl protons	1.58, 1.38,	1.54
	1.30, 1.24,	1.28
	0.97	1.00

(*Peak sharpens on addition of D_2O).

Table 5.7 ^{13}C NMR Spectra of Bussein (69)[40] and Spicata 2 (70).

	Bussein (69)		Spicata 2 (70)	
Carbon types	Number of Carbons	Chemical Shifts	Number of Carbons	Chemical Shifts
Ester carbonyls and C-1'	7(s)	183.2, 176.1, 172.5, 170.3, 169.1, 168.6, 168.4.	7(s)	183.0, 176.2, 172.7, 174.4, 170.4, 168.5, 168.3.
Furan	3(d)	142.6 (C-21)	3(d)	142.6
		141.2 (C-23)		141.4
		109.8 (C-22)		109.9
	1(s)	121.9 (C-20)	1(s)	122.0
Orthoester	1(s)	118.9	1(s)	119.0
C-15	1(s)	90.8	1(s)	91.0
C-O	4(s)	84.9, 83.3, 79.6, 77.2.	4(s)	84.9, 83.4, 79.7, 77.2
	5(d)	82.9, 73.9, 70.0, 70.0, 69.0.	5(d)	83.1, 74.0, 70.1, 70.1, 68.7.
CO_2Me	1(q)	51.8	1(q)	51.8
Saturated (quarternary)	3(s)	45.7, 45.7, 44.9.	3(s)	45.7, 45.0, 45.9.
Methine	4(d)	43.6, 41.4, 36.9, 30.2, - .	5(d)	43.6, 41.5, 36.9, 30.2, 41.3.
Methylene	3(t)	39.8, 33.2, 26.5, - .	4(t)	39.9, 33.2, 26.5, 27.0,
Methyl	11(q)	20.9, 20.7, 20.7, 20.3, 19.8, 18.5, 17.9, 16.7, - , 15.9, 14.2, - , 11.6.	12(q)	- , 20.7, 20.5, 20.3, 19.6, 18.3, 17.7, 16.6, 16.0, 15.8, 14.2, 14.0, 11.5.

CHAPTER SIX

SPECTRA

6.1 ^{1}H NMR SPECTRA

H-1 Rohitukin (38)

H-2 Rohituka 1 (39)

H-3 Rohituka 2 acetate

H-4 Rohituka 2 (40)

H-5 Rohituka 1 acetate

H-6 Rohituka 3 (41)

H-7 Rohituka 4 (42)

H-8 Rohituka 5 (43)

H-9 Rohituka 6 (44)

H-10 Rohituka 7 (45)

H-11 Rohituka 7 acetate

H-12 Rohituka 8 (46)

H-13 Polystachin (48)

H-14 Rohituka 10 (Dregeana 1) (49)

H-15 Rohituka 10 acetate

H-16 Dregeana 2 (35)

H-17 Dregeana 3 (34)

H-18 Dregeana 4 (57)

H-19 Dregeana 4 acetate

H-20 Dregeana 5 (58)

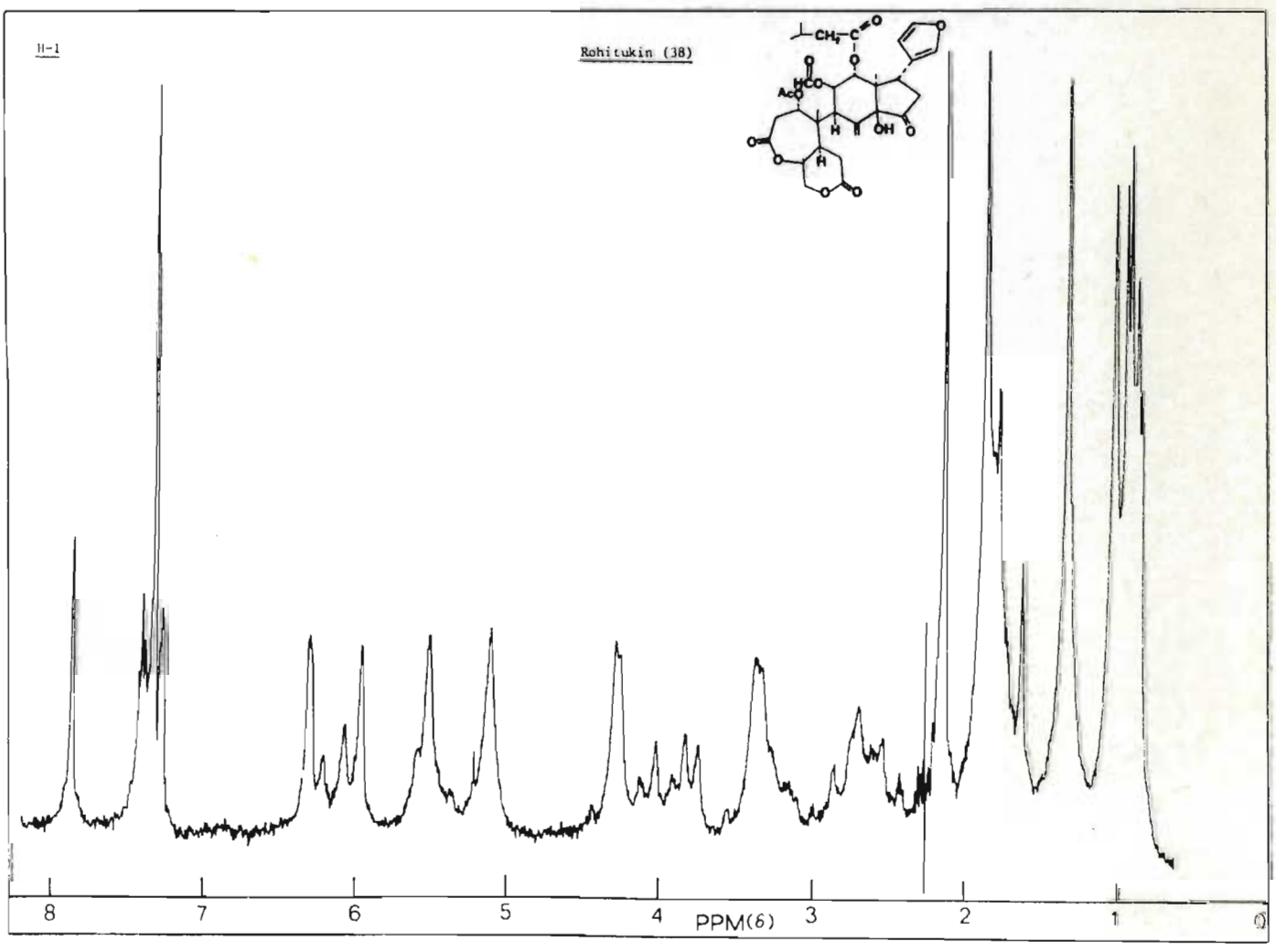
H-21 Spicatin (63)

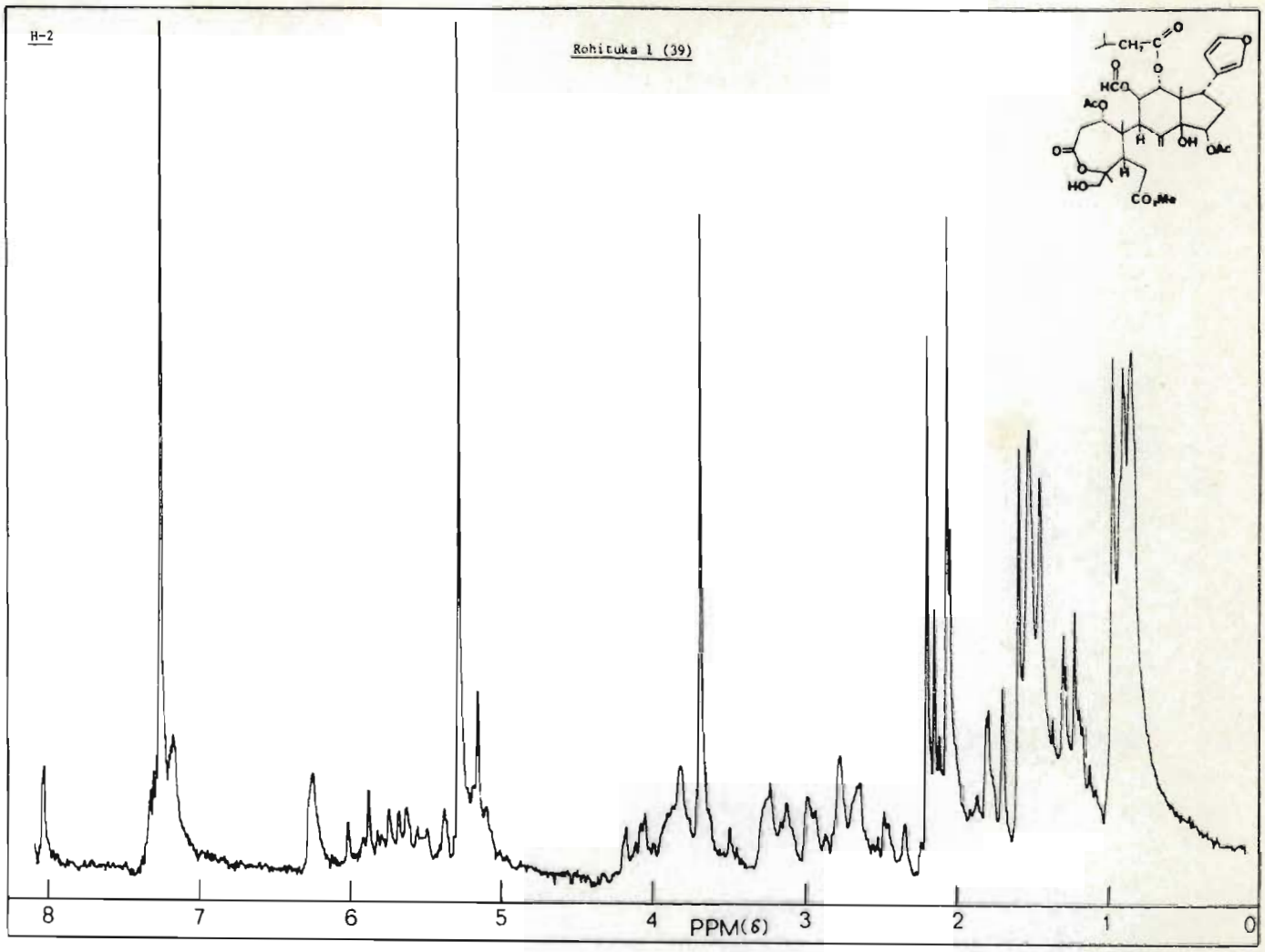
H-22 Spicatin triacetate (64)

H-23 Spicatin -CrO_3 oxidation (66)

H-24 Spicatin -$NaIO_4$ reaction (67)

H-25 Spicata 2 (70)

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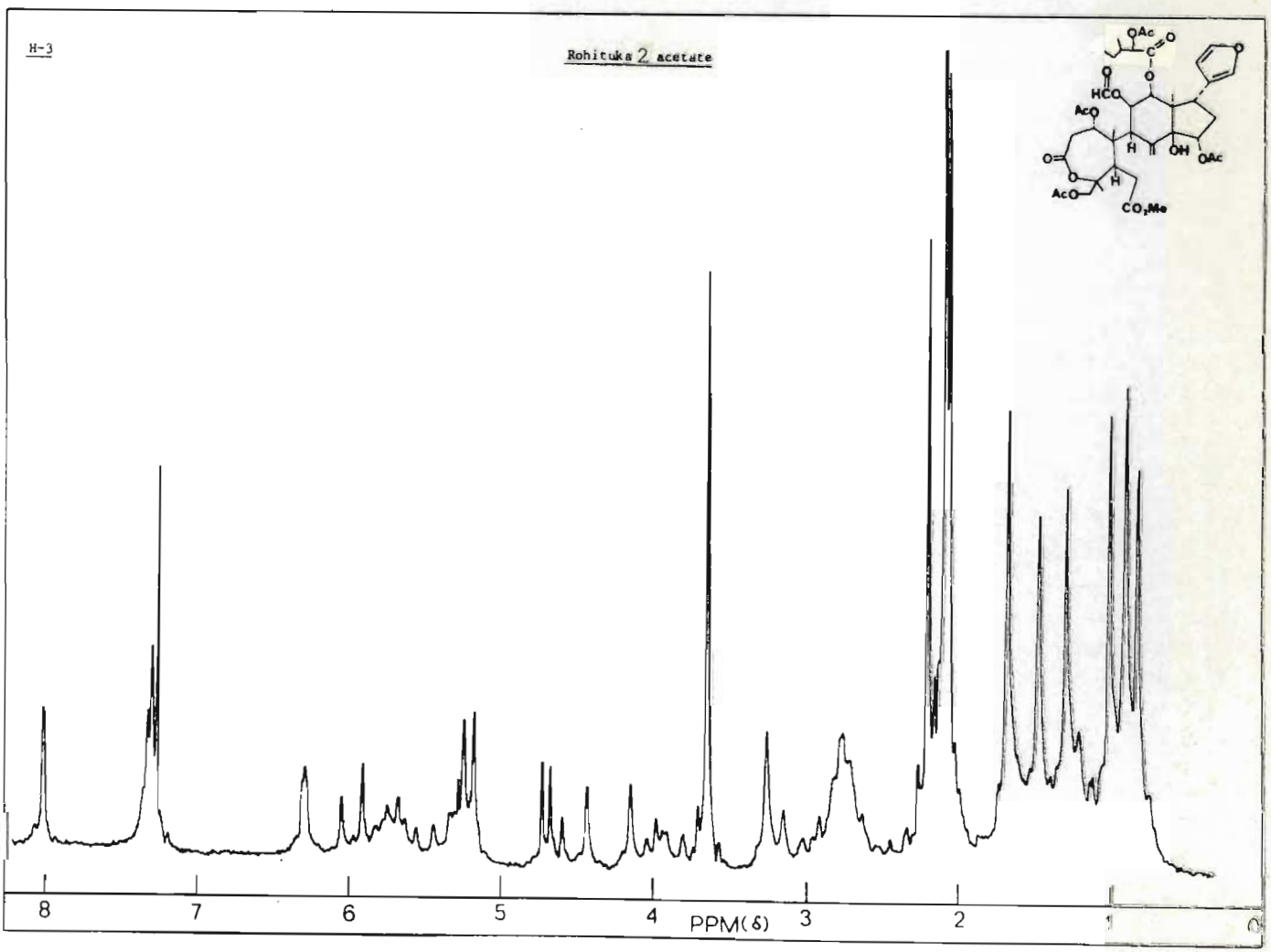
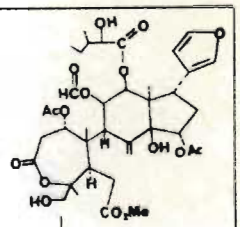
Rohituka 1 (39)
8
7
6
5
4
PPM(δ)
3
2
1
0


H-3
Rohituka 2 acetate
8
7
6
5
4
PPM(δ)
3
2

H-4

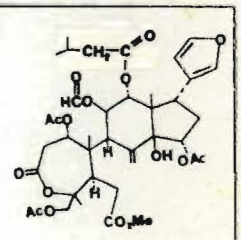
Rohituka 2 (40)



8 7 6 5 4 PPM(δ) 3 2 1 0

H-5

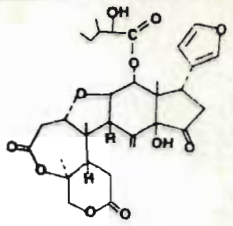
Rohituka 1 acetate



8 7 6 5 4 PPM(δ) 3 2 1 0

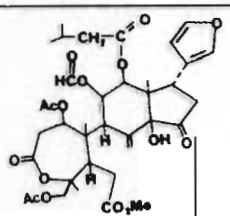
H-6

Rohituka 3 (41)



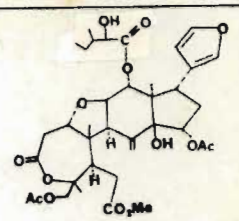
H-7

Rohituka 4 (42)



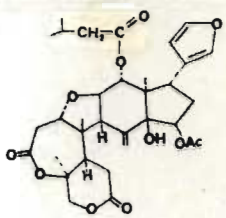
H-8

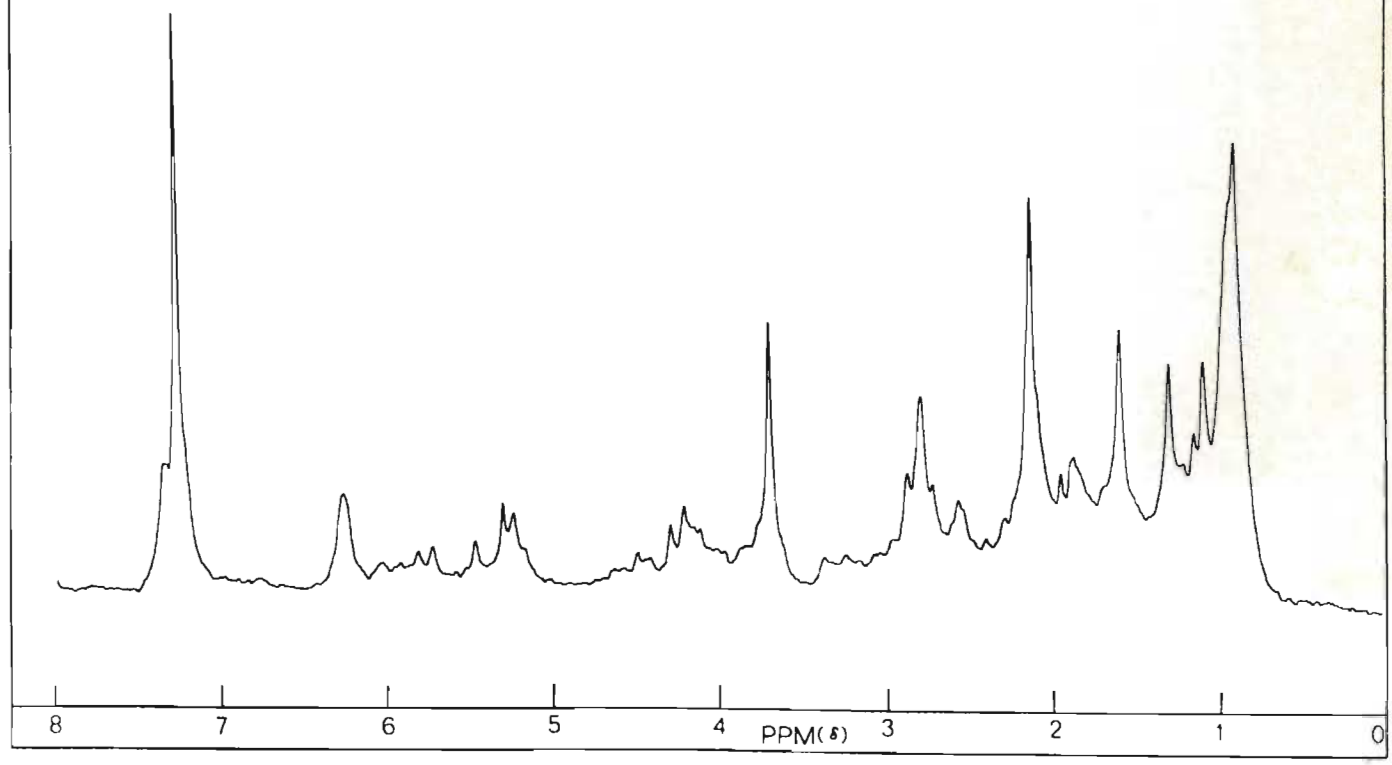
Rohituka 5 (43)



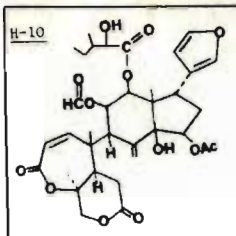
H-9

Rohituka 6 (44)





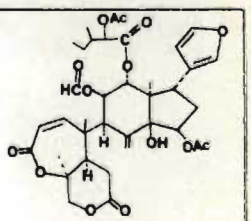
H-10



Rohituka 7 (45)

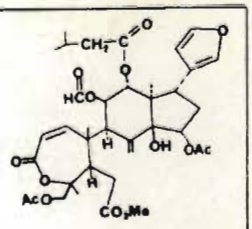
H-11

Rohituka 7 acetate

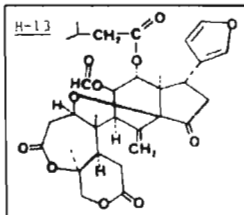


H-12

Rohituka 6 (46)



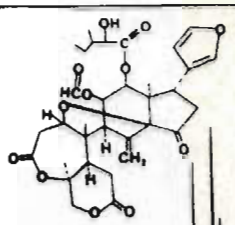
H-13



Polystachin (Rohituka 9) (48)

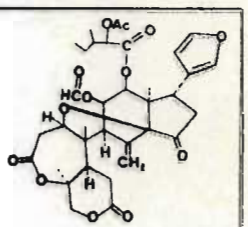
H-14

Rohituka 10 (Dregeana 1) (49)



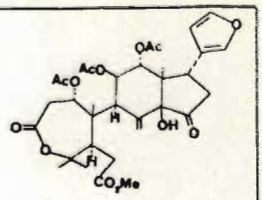
H-15

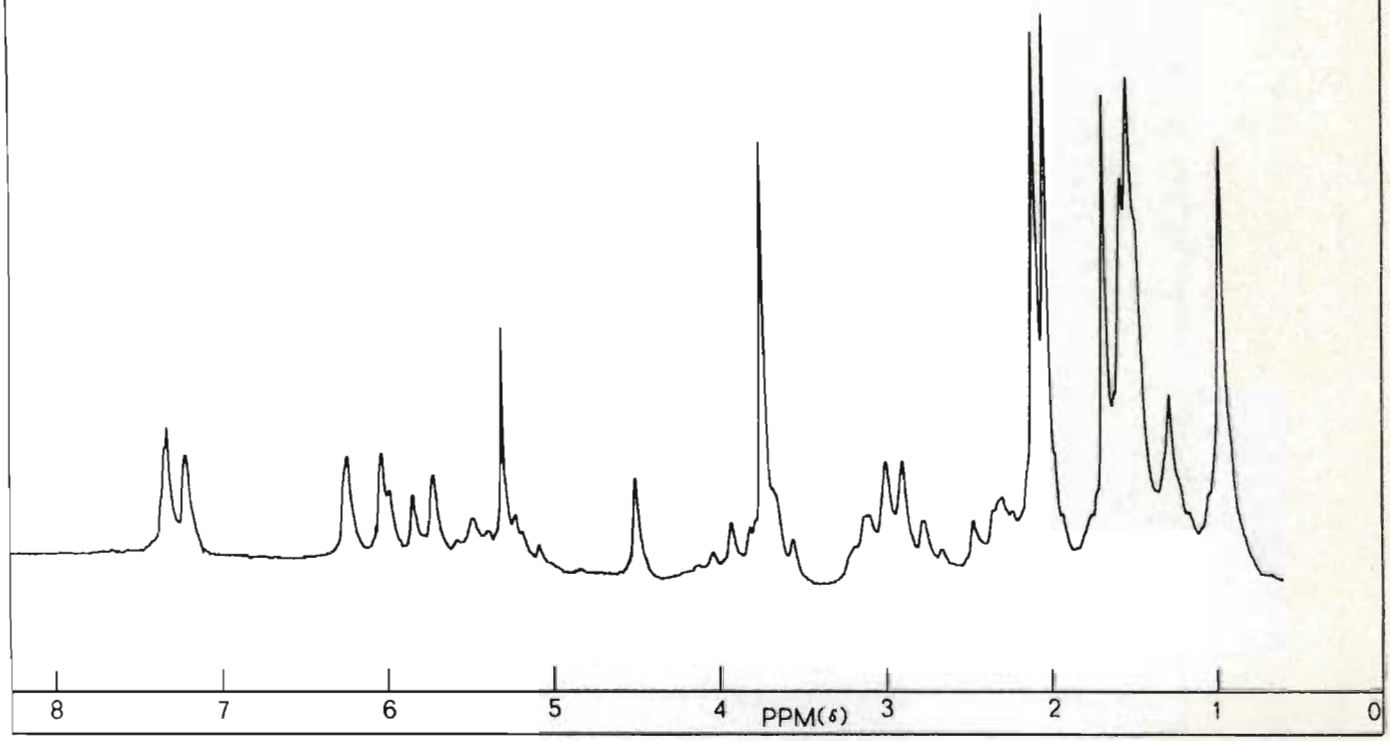
Rohituka 10 acetate



H-16

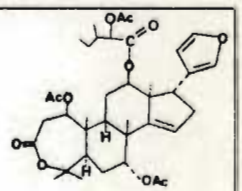
Dregeana 2 (35)

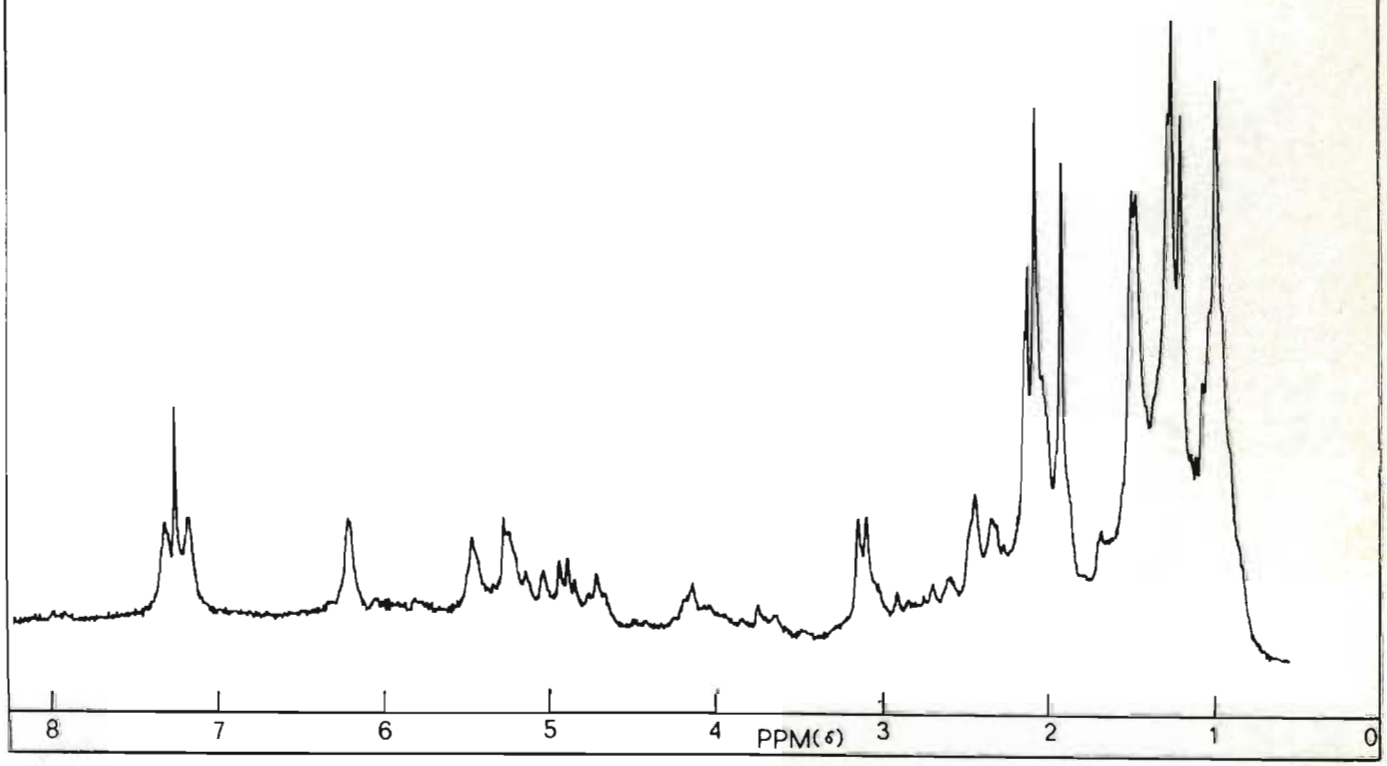




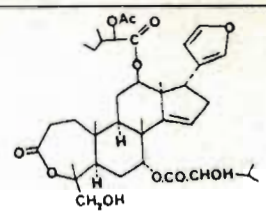
H-17

Dregeana 3 (34)



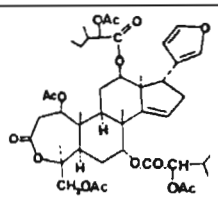


H-18



Dregeana 4 (57)

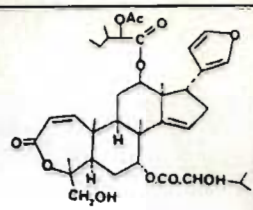
H-19



Dregeana 4 acetate

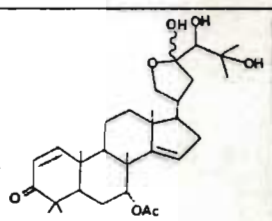
H-20

Dregeana 5 (58)



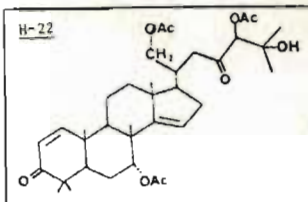
H-21

Spicatin (63)



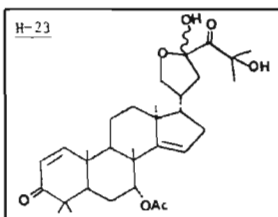
H-22

Spicatin triacetate (64)



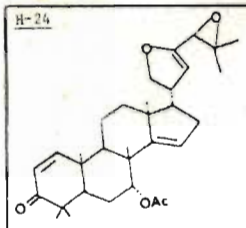
H-23

Spicatin-CrO_3 oxidation (66)



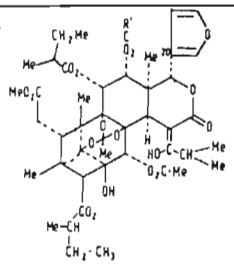
H-24

Spicatin - $NaIO_4$ reaction (67)



H-25

Spicata 2 (70)



6.2 ^{13}C NMR SPECTRA

C-1 Polystachin (48)

C-2 Dregeana 2 (35)

C-3 Dregeana 3 (34)

C-4 Dregeana 4 (57)

C-5 Spicatin (63)

C-6 Spicatin triacetate (64)

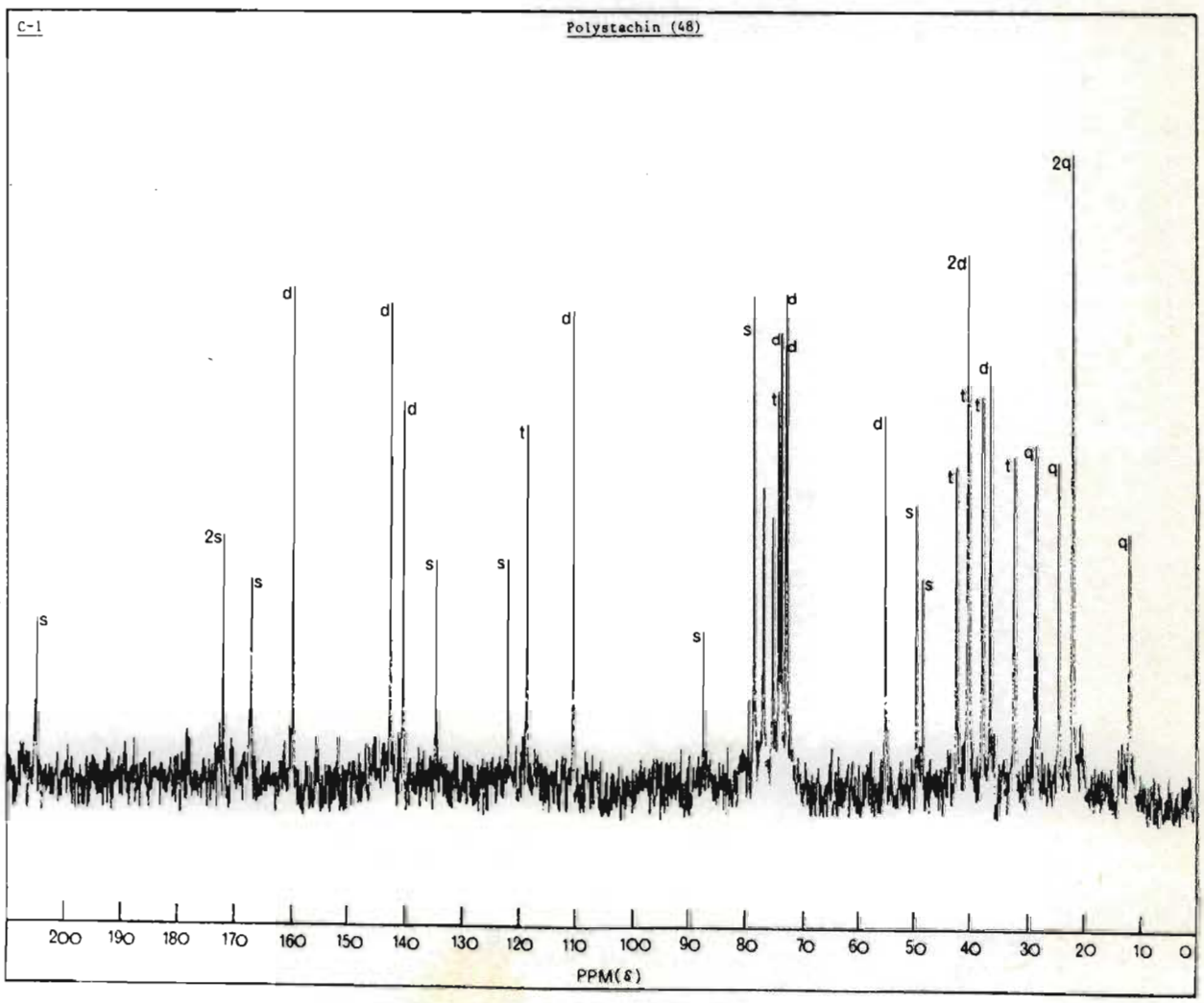
C-7 Dihydrospicatin (65)

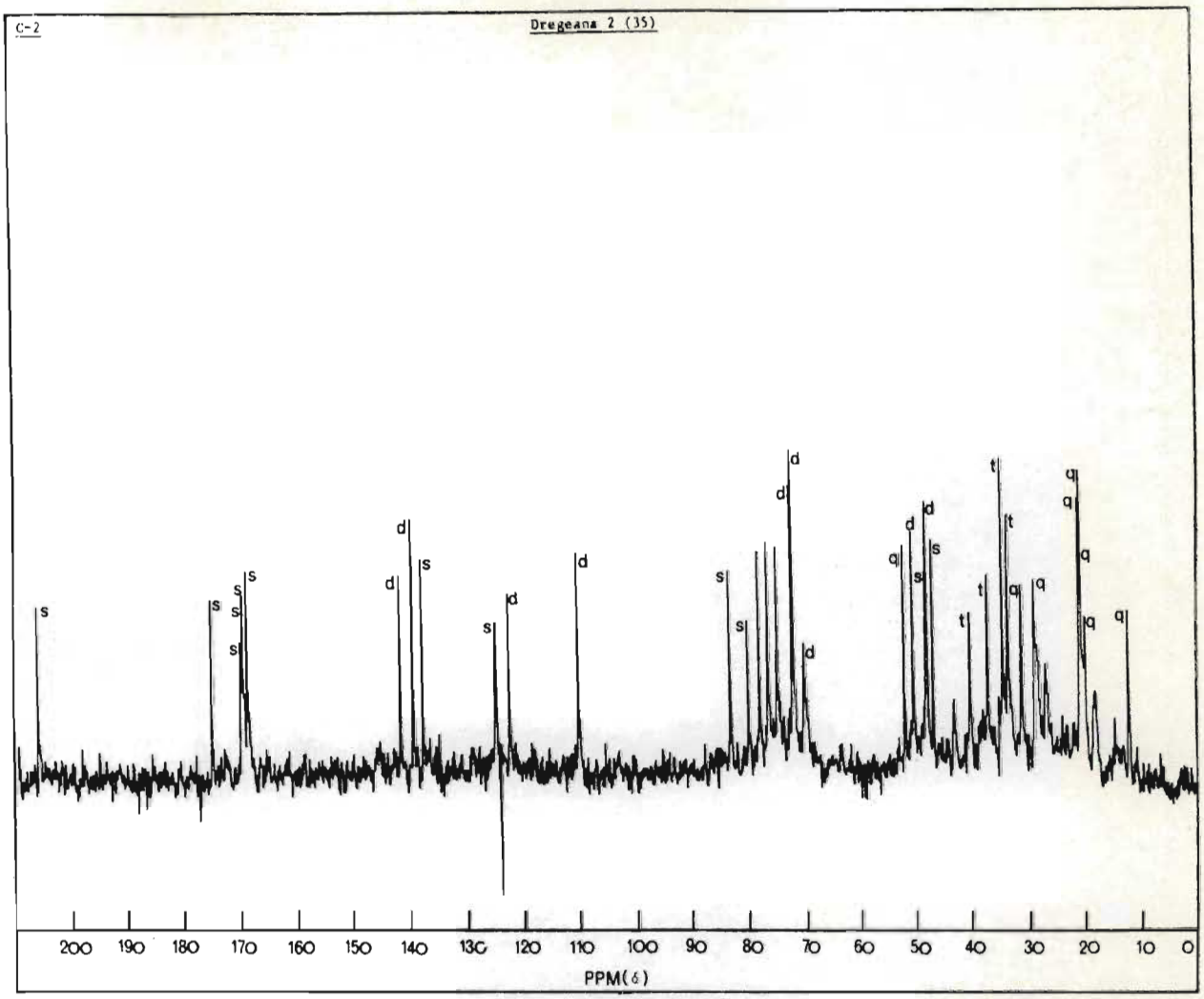
C-8 Spicatin -CrO_3 oxidation (66)

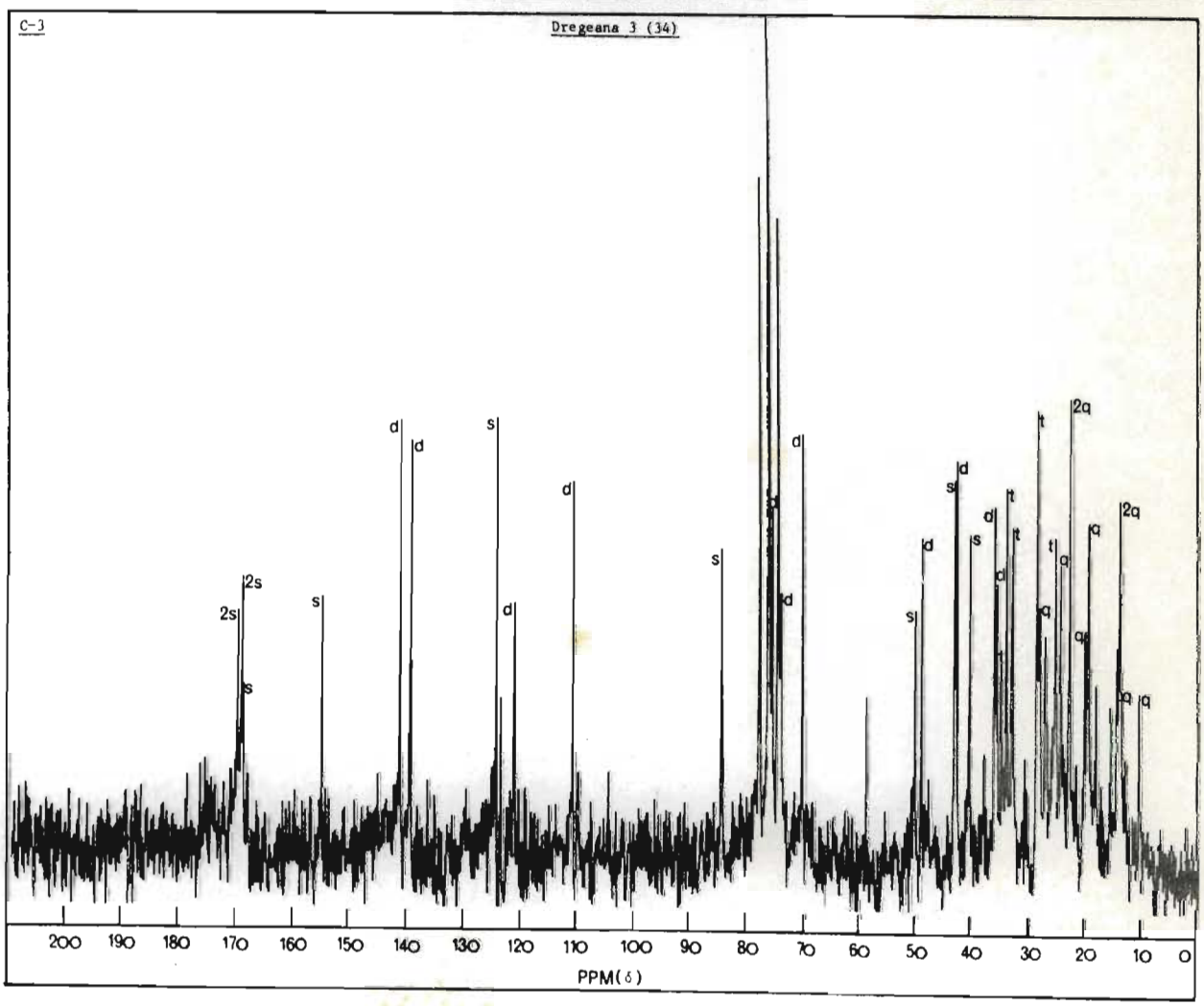
C-9 Spicatin -$NaIO_4$ reaction (67)

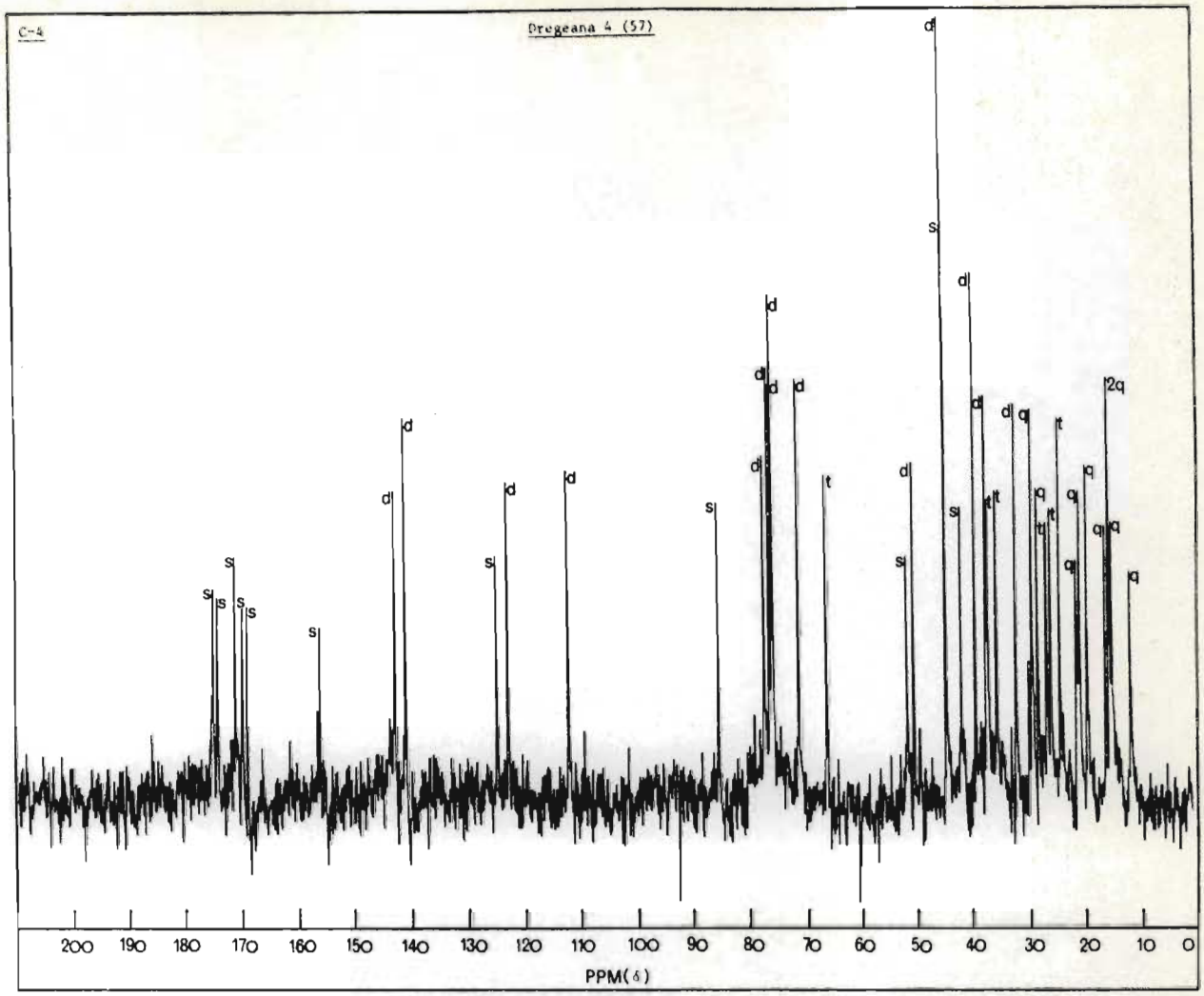
C-10 Spicatin-$MeOH/H_2SO_4$ reaction (68)

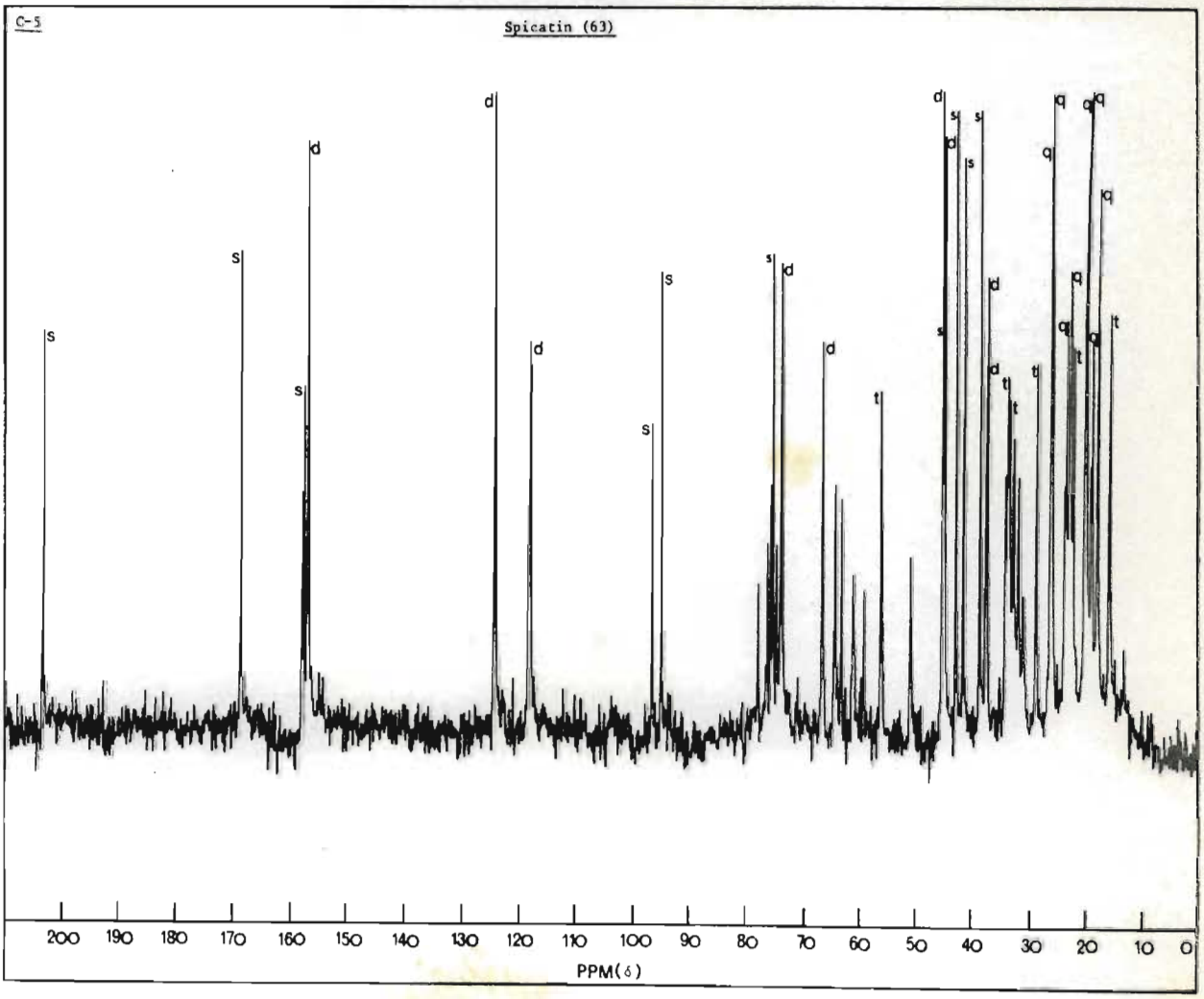
C-11 Spicata 2 (70)




C-2
Dregeana 2 (35)
PPM(δ)

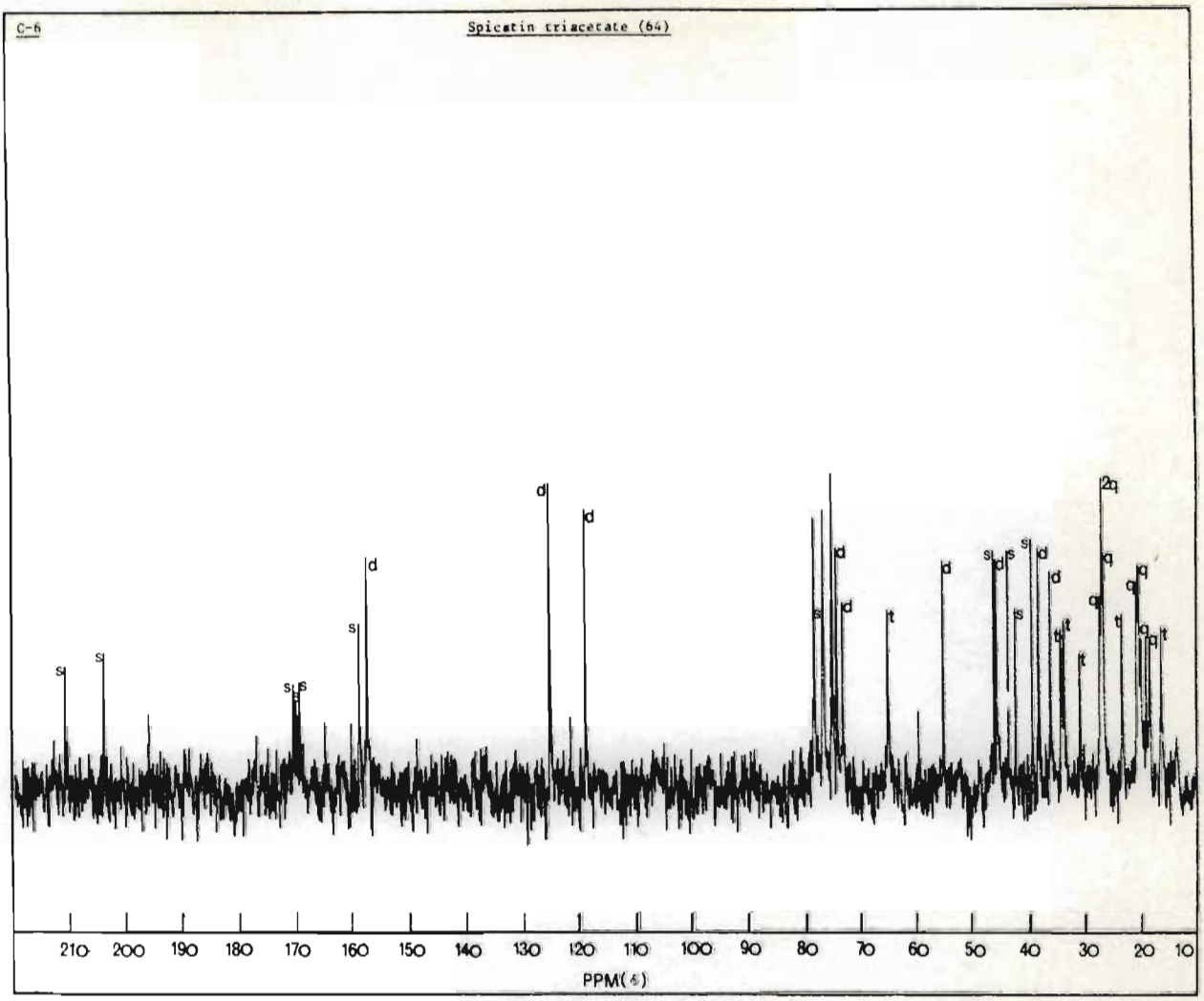

C-3
Dregeana 3 (34)
PPM(δ)


C-4
Pregeana 4 (57)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
PPM(δ)


C-5
Spicatin (63)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
PPM(δ)

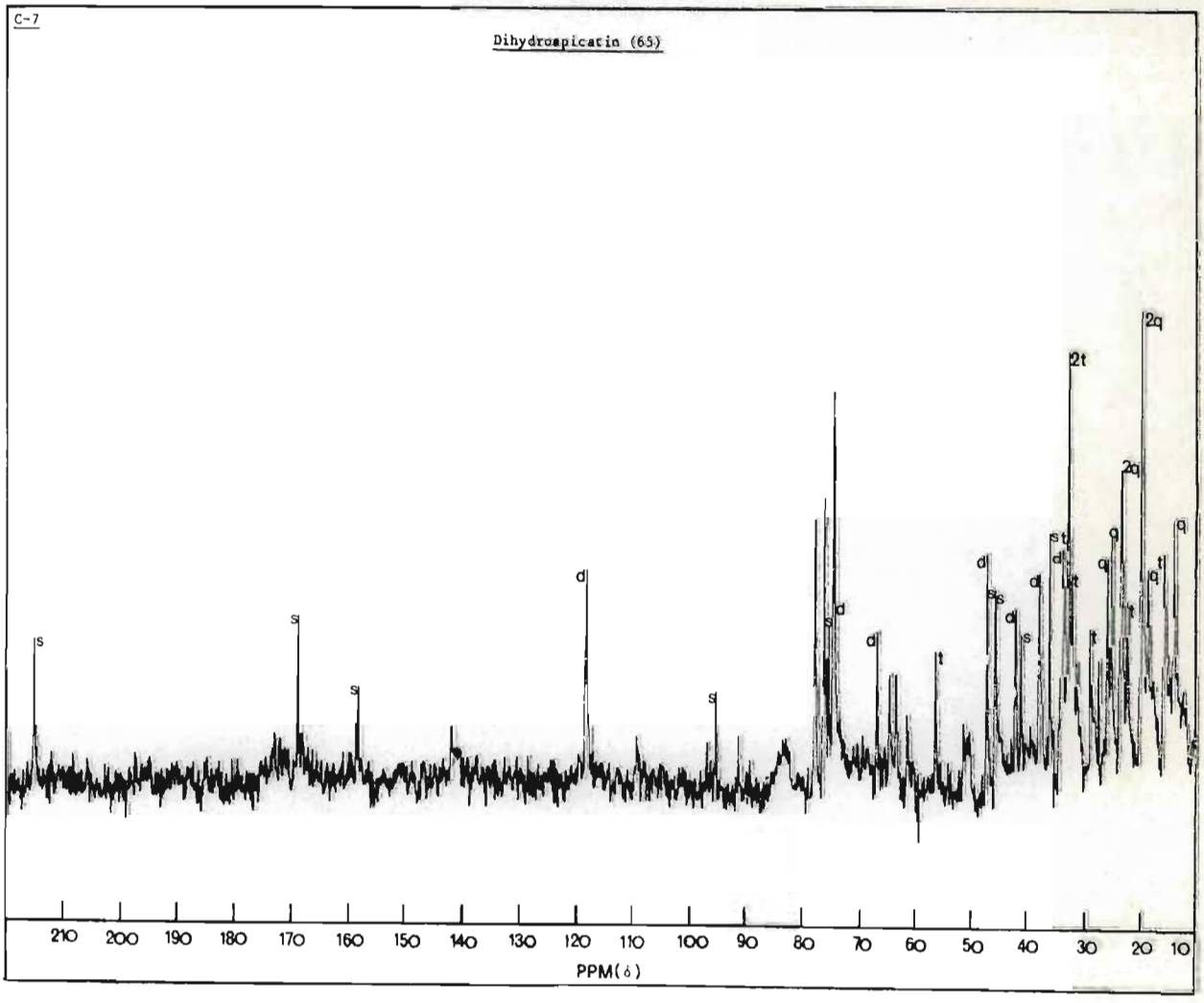
C-6

Spicatin triacetate (64)



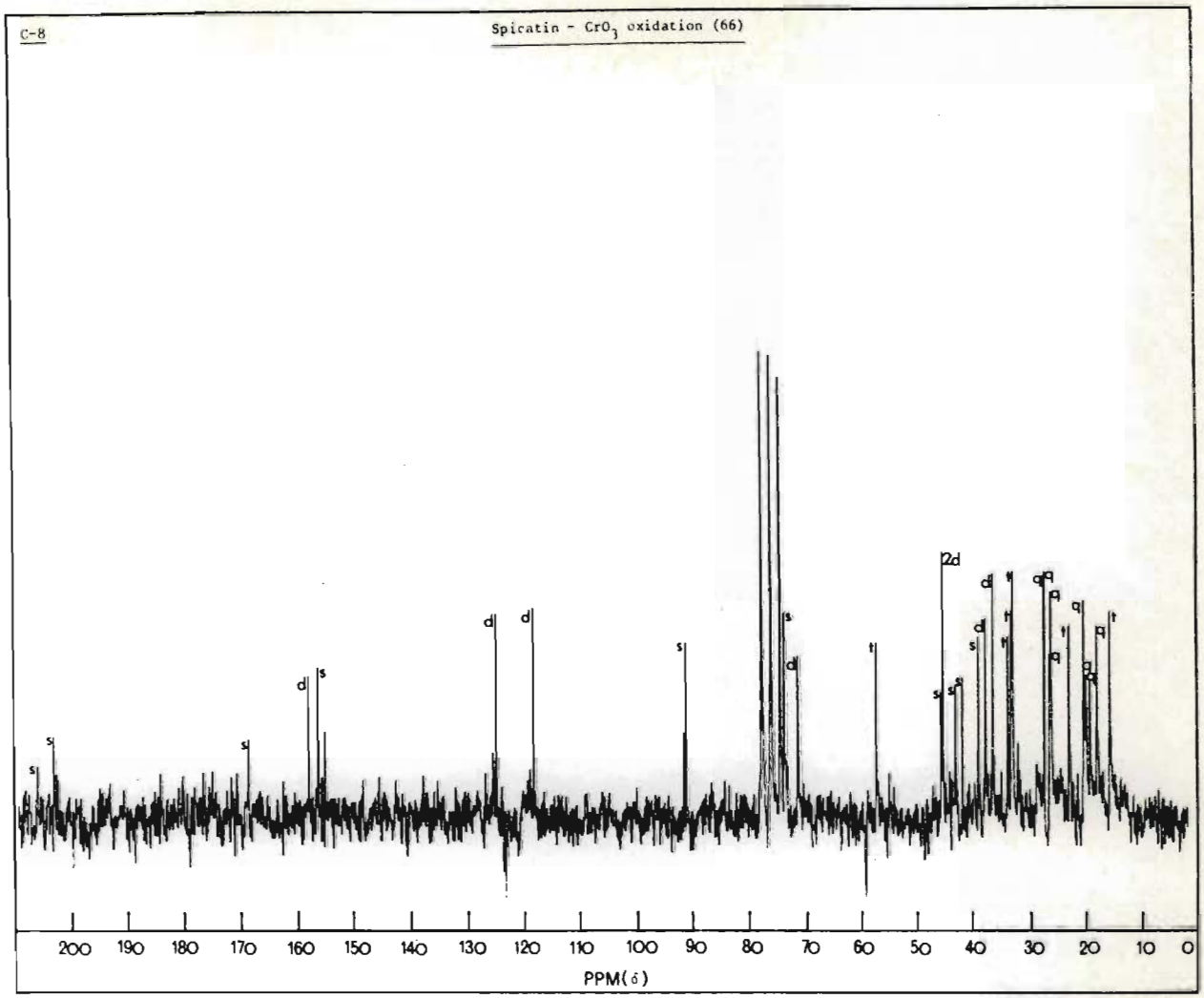
C-7

Dihydrospicatin (65)



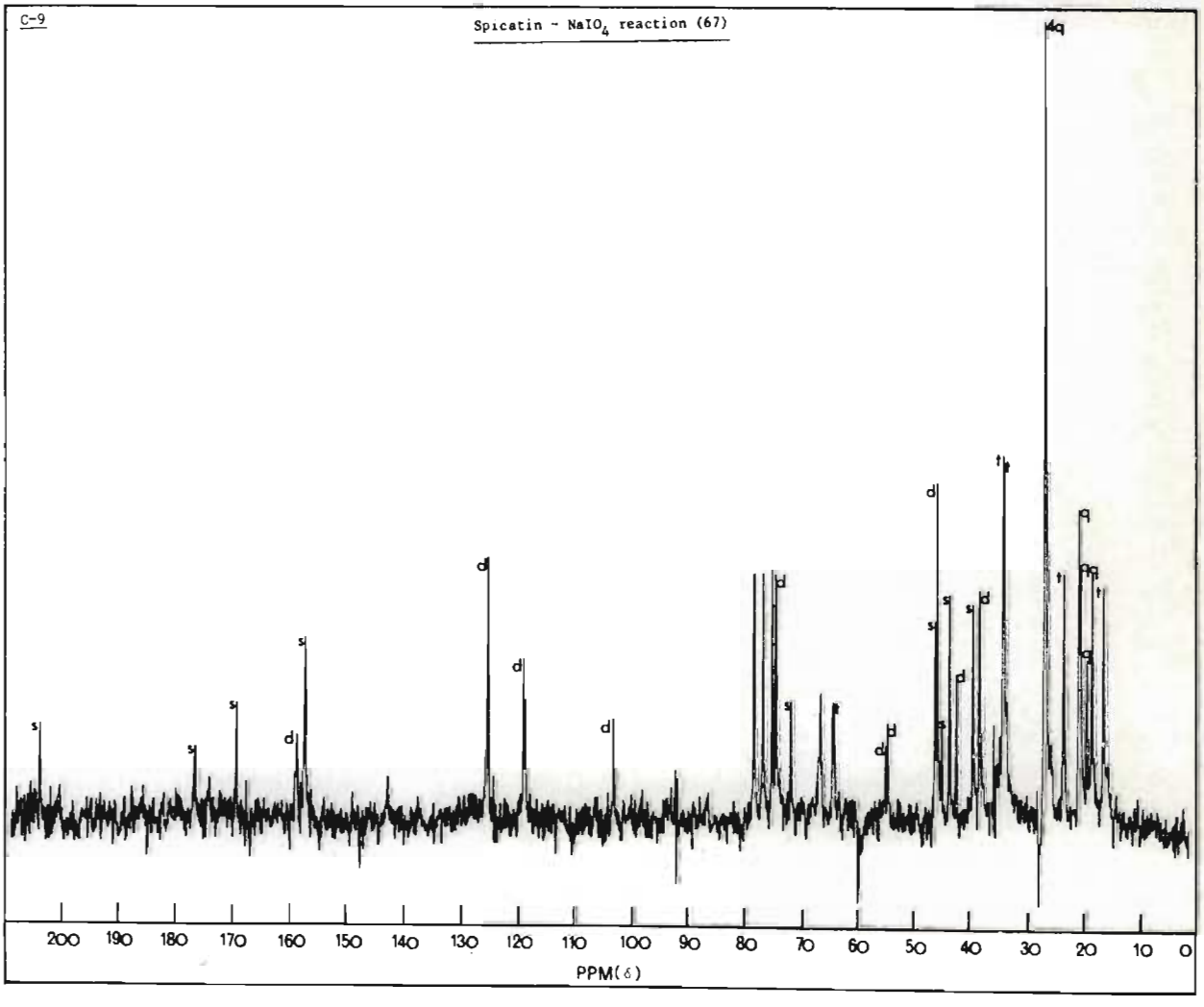
C-8

Spicatin - CrO_3 oxidation (66)



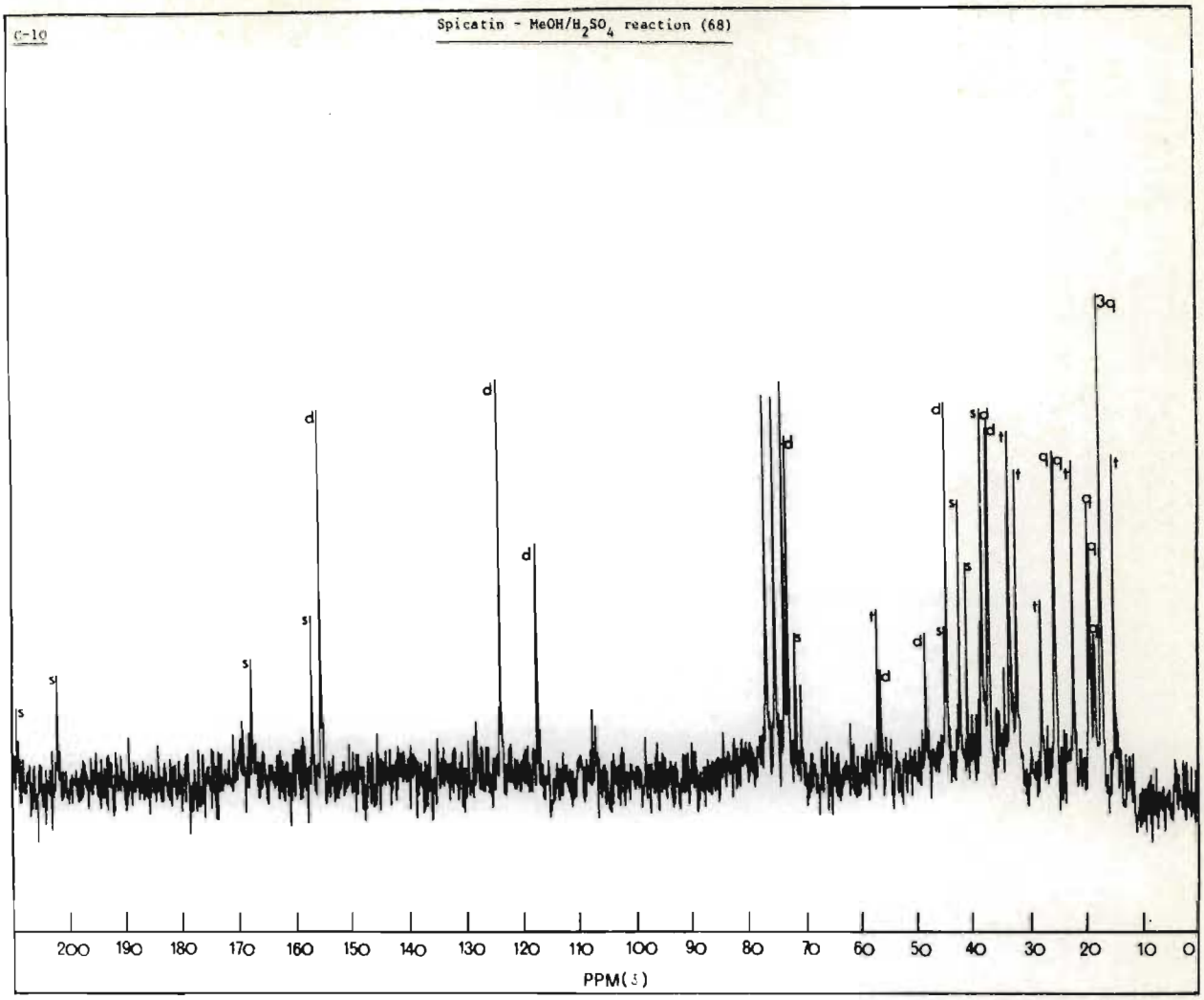
C-9

Spicatin - $NaIO_4$ reaction (67)



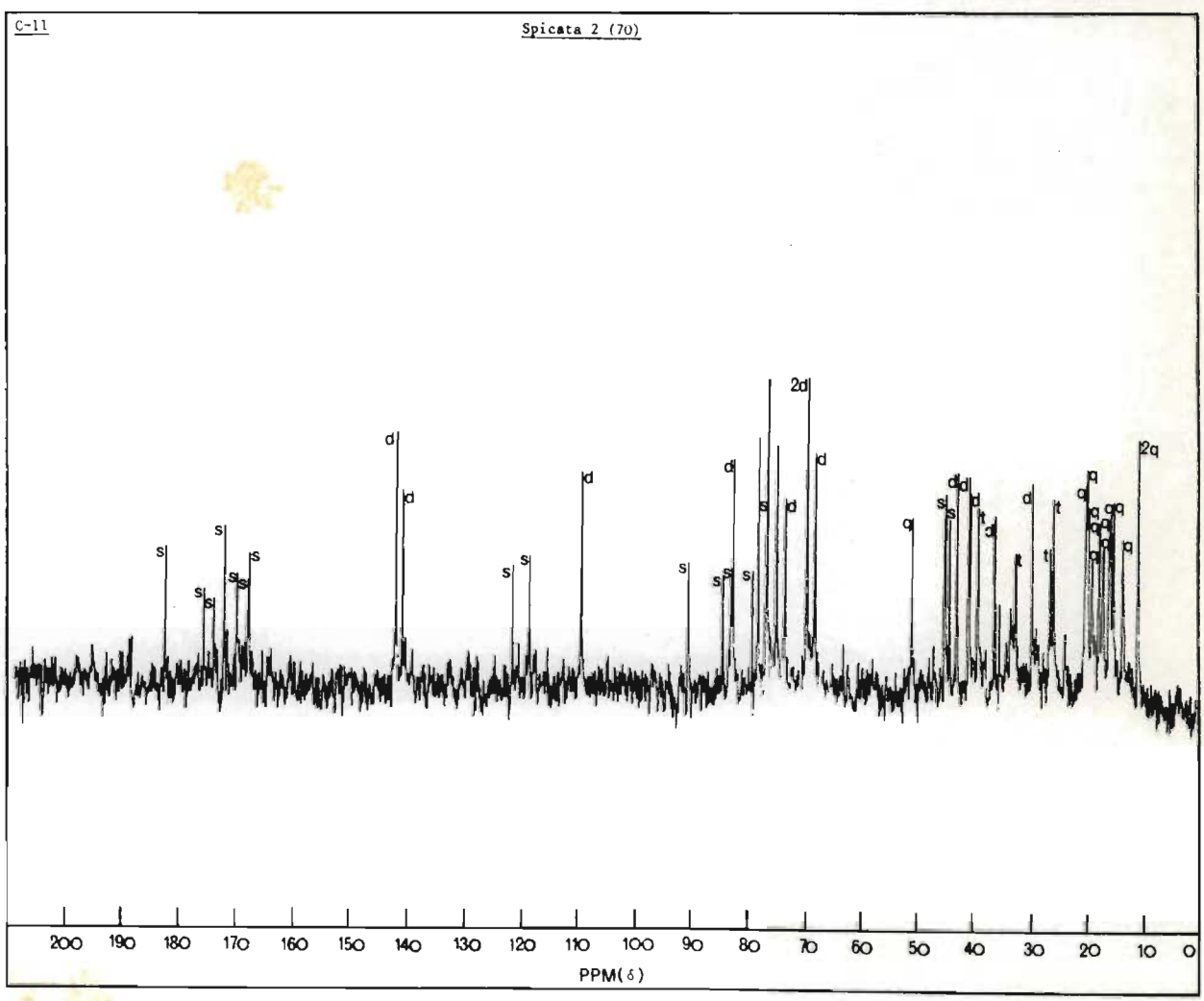
C-10

Spicatin - MeOH/H_2SO_4 reaction (68)



C-11

Spicata 2 (70)



6.3 INFRARED SPECTRA

I - 1 Polystachin (48)

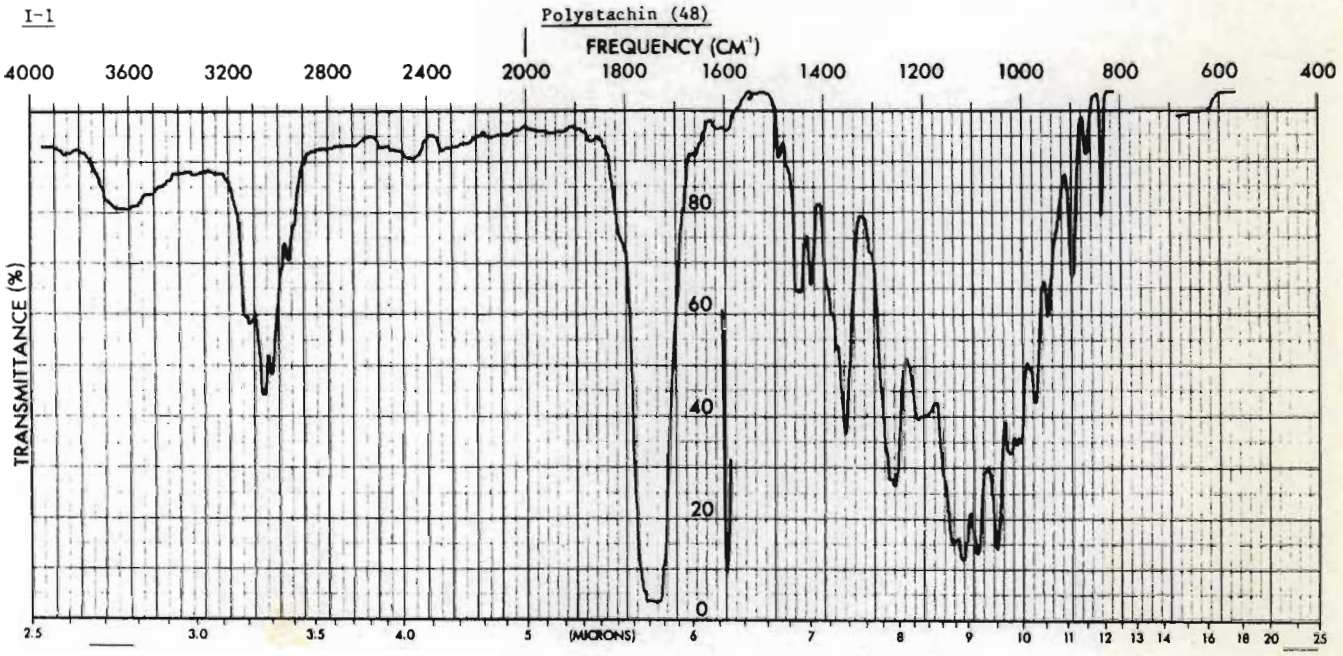
I - 2 Dregeana (35)

I - 3 Dregeana 4 (57)

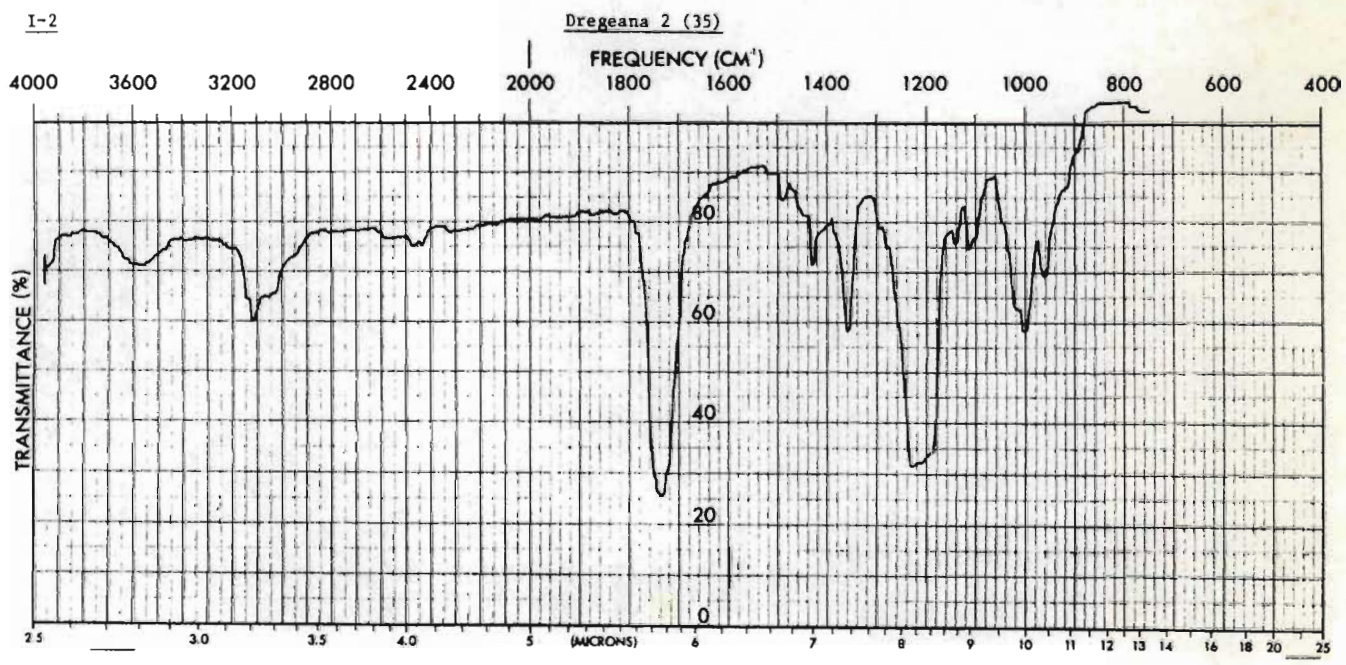
I - 4 Spicatin (63)

I - 5 Spicata 2 (70)

I-1

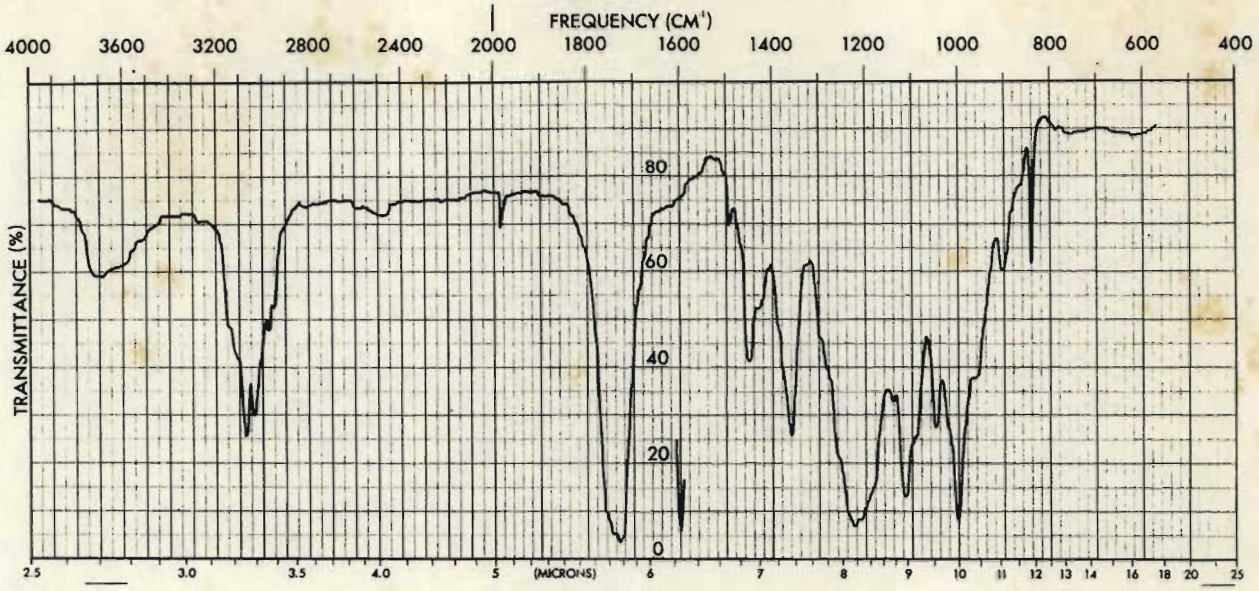


I-2



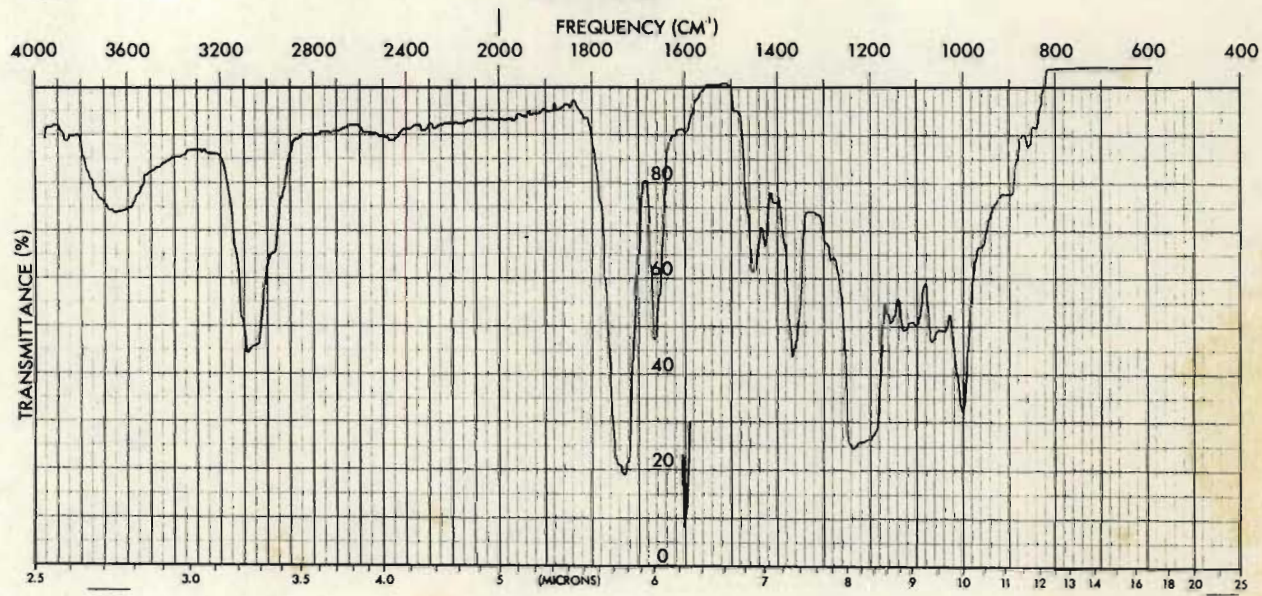
I-3

Dregeana 4 (57)



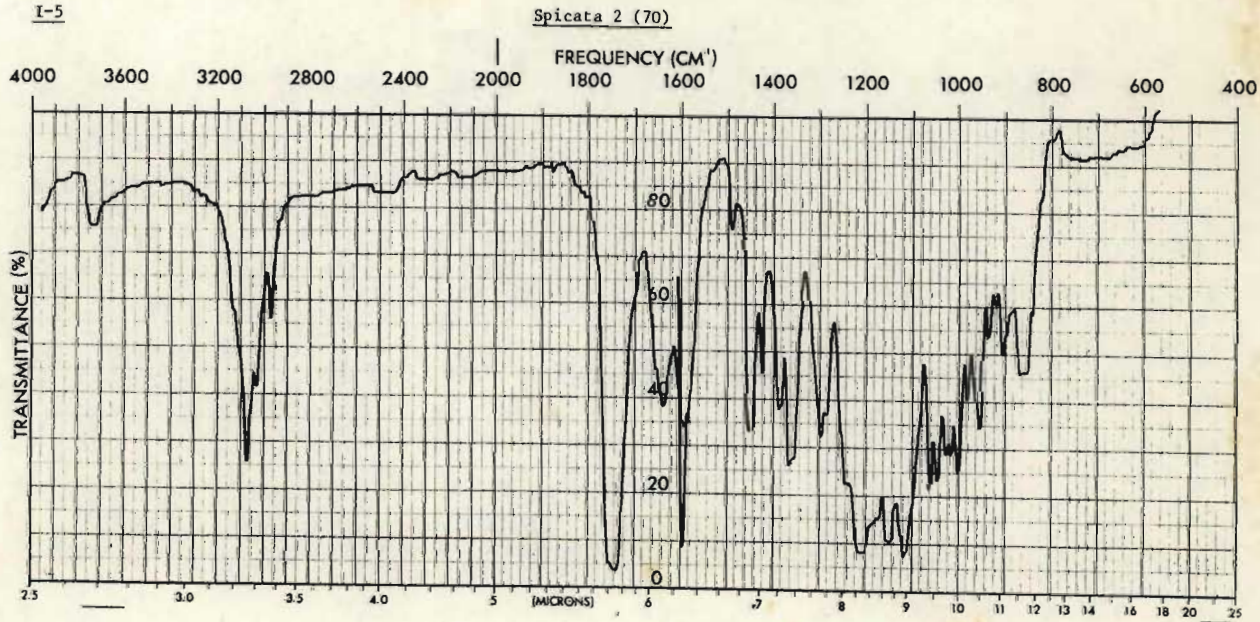
I-4

Spicatin (63)



I-5

Spicata 2 (70)



6.4 MASS SPECTRA

M - 1 Polystachin (48)

M - 2 Dregeana 2 (35)

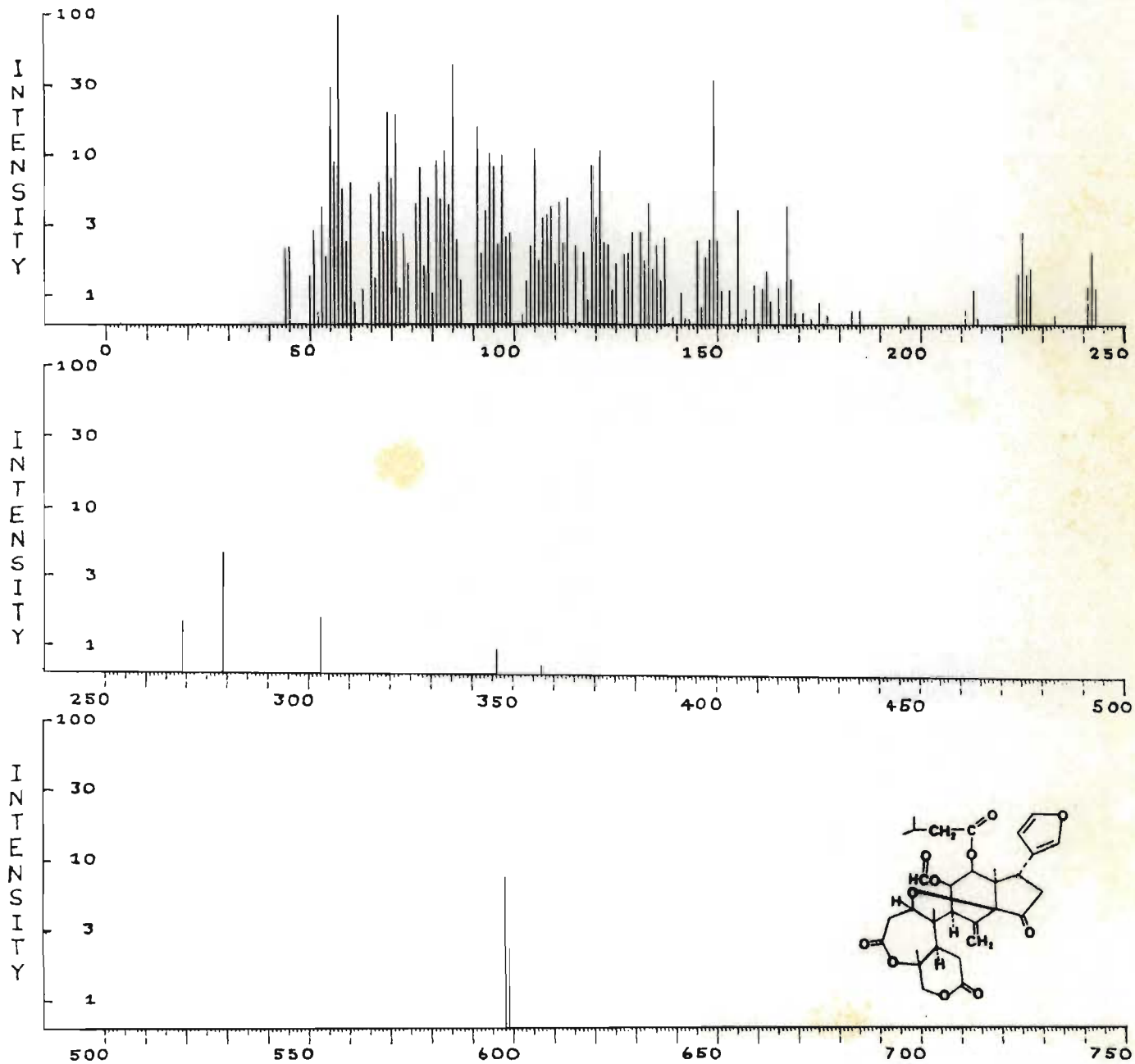
M - 3 Dregeana 3 (34)

M - 4 Dregeana 4 (57)

M - 5 Spicatin (63)

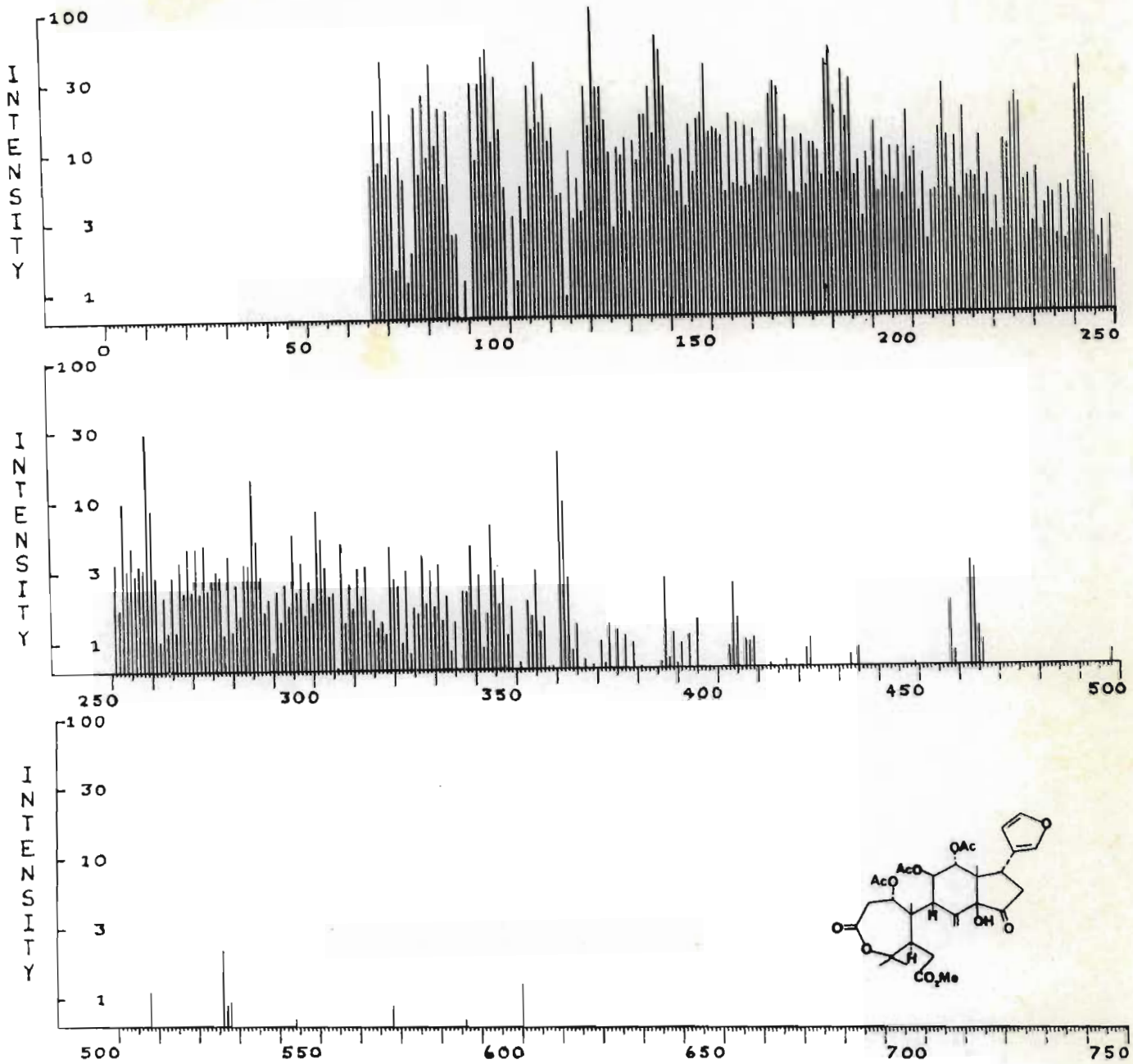
M-1

MASS SPECTRUM OF POLYSTACHIN (48)



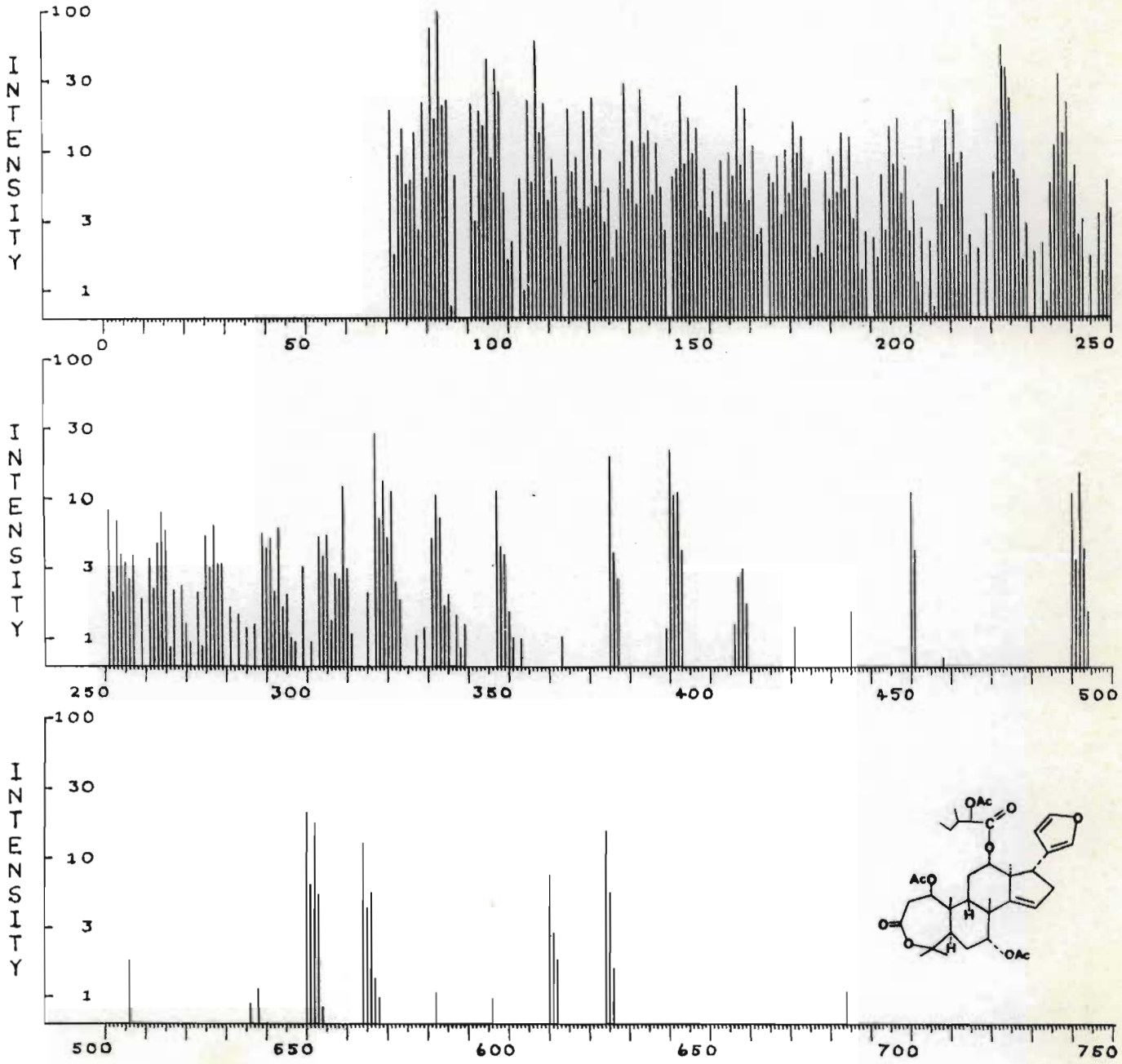
M-2

MASS SPECTRUM OF DREGEANA 2 (35)



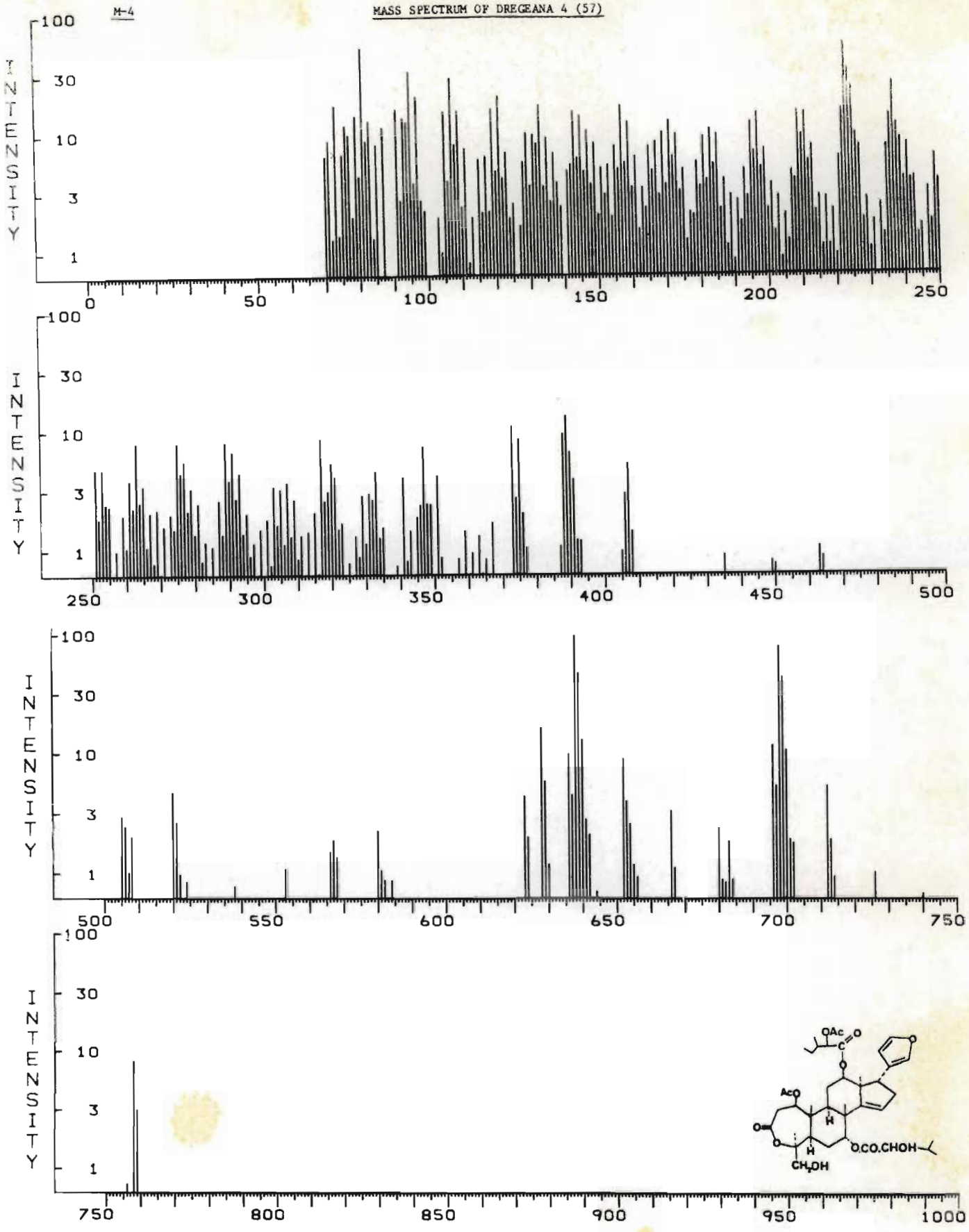
M-3

MASS SPECTRUM OF DREGEANA 3 (34)



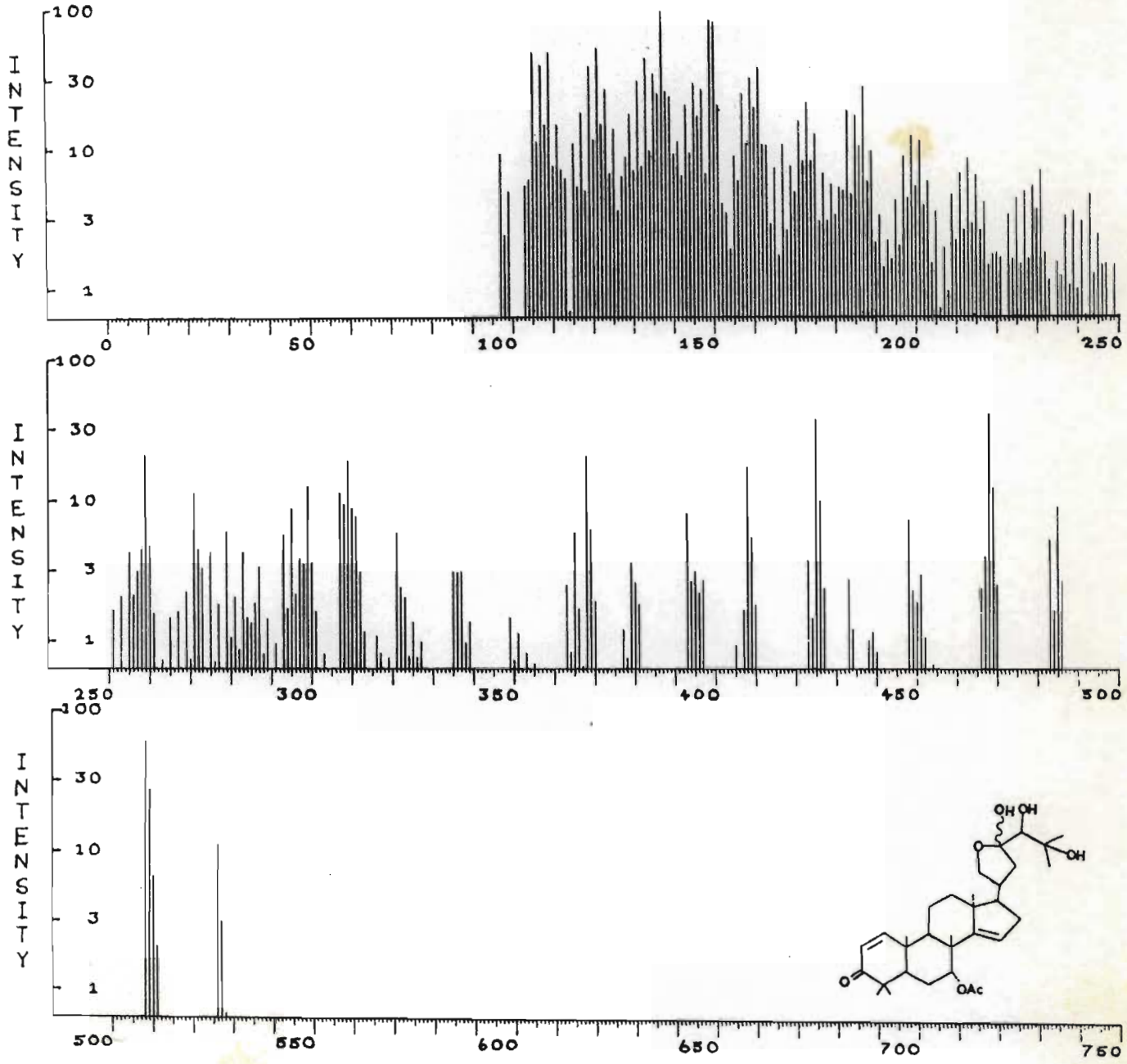
M-4

MASS SPECTRUM OF DREGEANA 4 (57)



M-5

MASS SPECTRUM OF SPICATIN (63)



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