A THESIS ENTITLED

"STUDIES IN THE TERPENOID FIELD"

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BY

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A C K N O W L E D G E M E N T S

I am deeply indebted to Dr. Joseph D. Connolly for his extreme kindness, constant advice, and help and encouragement through the period of his supervision. Without his great understanding and friendship it would have been very difficult for me to achieve the present work. I wish also to express my gratitude to his wife, Effie, and their children, for having been a second family to me during the past three years. Sincere thanks must go to Professor Brian Capon, who has been, above all, a friend to me, and to Dr. David Rycroft for his expert advice in the interpretation of ^{1}H and ^{13}C n.m.r. data, for his friendly criticism, and for his continued patience. To all the technical staff who were involved with the development of this research I extend my thanks. And, finally, I must mention my parents, without whom this work would not have been possible: to them my undying gratitude.

C O N T E N T S

CHAPTER IX · The Structure of a new Coumarin from Leonotis nepetaefolia

"STUDIES IN THE TERPENOID FIELD"

Cecilia Labbe D.

SUMMARY

This thesis consists of a General Introduction, dealing briefly with the biogenesis of terpenoids, and nine chapters. Chapters I to VII are concerned with the chemistry of tetranortriterpenoids, a group of modified triterpenoids from the Meliaceae and Rutaceae families. The present state of knowledge of these compounds is reviewed in Chapter I. This is followed by discussions of the results of investigation into the tetranortriterpenoid constituents of the wood and seeds of Chisocheton paniculatus and the seeds of Chukrasia tabularis (Chapters II and III). Fifteen new compounds were isolated from these sources and their structures elucidated mainly by use of ^{1}H and ^{13}C n.m.r. spectroscopy. Chapter IV describes the several new additions to the small group of tetranortriterpenoids, related to prieurianin, which exist in solution at room temperature as a mixture of sterically hindered conformers. A detailed discussion of the alkaline hydrolysis products of several members of this group is included. Revised structures for atalantin and atalantolide, from Atalantia monophylla, are proposed in Chapter V. Chapter VI is concerned with the interrelation of swietenine and swietenolide, the major tetranortriterpenoids of the seeds of Swietenia macrophylla, and Chapter VII with the structure of ekebergins I and II, complex tetranortriterpenoids from seeds and wood of Ekebergia senegal-ensis. Detailed consideration of the spectroscopic properties of these

compounds leads to biogenetically acceptable structures.

Two casbene-derived diterpenoids, jatropholones A and B, from the roots of Jatropha gossypiifolia, form the subject matter of Chapter VIII. The structures of these compounds have the novel feature of a fully substituted cresol ring and were confirmed by X-ray analysis of jatropholone B acetate.

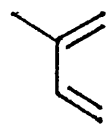
The final chapter deals with a coumarin from Leonotis nepetaefolia. The structure was assigned by ^{1}H n.m.r. and confirmed by synthesis of 2'-hydroxy- 4',5'-dimethoxy-6'-methylacetophenone, the compound obtained on hydrolysis of the natural product.

GENERAL INTRODUCTION

THE TERPENOIDS

Origin, Structure and Function.[1,2,3] The terpenoids are a large group of natural products found mainly in plants. Originally, the simpler members of the group were obtained, by steam-distillation of the parts of the plant, as oily mixtures known as essential oils. Because of their fragrancy they have had an important role since ancient times in the manufacture of perfumes and cosmetics. Chemical interest in the composition of these oils was aroused during the past century. It was then found that they were mixtures of hydrocarbons, whose common characteristic was the presence of one or more isomeric unsaturated hydrocarbons with the molecular formula $C_{10}H_{16}$, in the more volatile fractions of the oils. They were named terpenes from their association with oil of turpentine. Subsequent development in this field led to the more general term terpenoids. In addition to the C_{10} terpenoids present in the more volatile fractions, a group of C_{15} compounds were obtained from the higher boiling fractions, with a structure related to the C_{10} terpenoids. Later, compounds with higher number of carbon atoms (C_{20} and C_{30}) were isolated from non-steam volatile saps, gums, and resins of plants.

Early structural investigation of the C_{10} terpenoids indicated that they were, formally at least, head-to-tail dimers of isoprene (1).



(1)

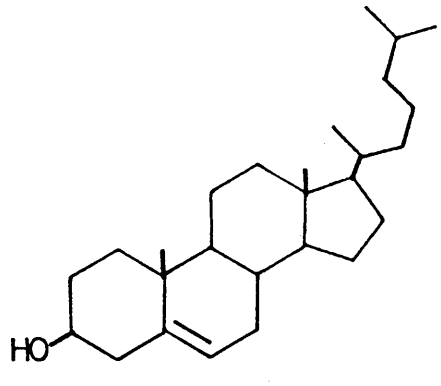
Similarly, the C_{15} terpenoids were found to be formed by three such units

linked in the same fashion. These findings led to the generalization known as the "Isoprene Rule" first proposed by Wallach in 1887 and later developed by Robinson[1], which states that in order to be a terpenoid, a compound must have a carbon skeleton formed by isoprene units linked head-to-tail. It was later modified by Ruzicka[4] who proposed a "Biogenetic Isoprene Rule"to account for a number of substances, related by origin, structure, and chemical behaviour to the terpenoids, but whose skeleton cannot be constructed from isoprene units. The "Biogenetic Isoprene Rule" proposed that they could arise from isoprenoid precursors by removal or addition of one or more fragments or by molecular rearrangements or by a combination of these processes.

Terpenoids are classified according to the number of isopentane units present in the molecule, assuming that the simplest members of the group are formed by two such units. The current classification is as follows:

No. of carbon atoms	Class
10	Monoterpenoids
15	Sesquiterpenoids
20	Diterpenoids
25	Sesterpenoids
30	Triterpenoids
40	Tetraterpenoids
>40	Polyterpenoids

The biological significance of terpenoids is still obscure in most cases, and it has been proposed that they are by-products in the biosynthesis of essential hormones and primary metabolites in the plant.[5]



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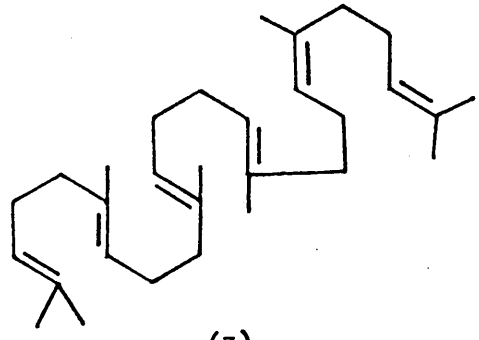

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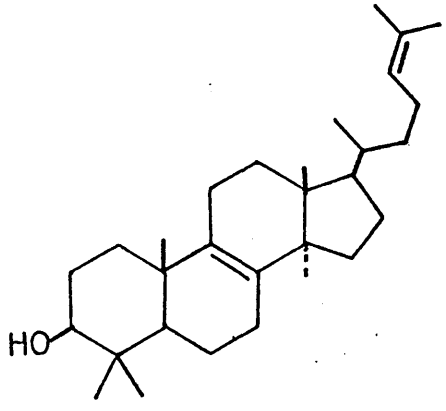

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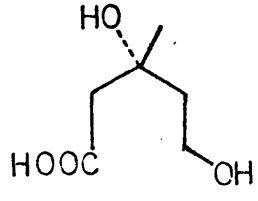


(5)

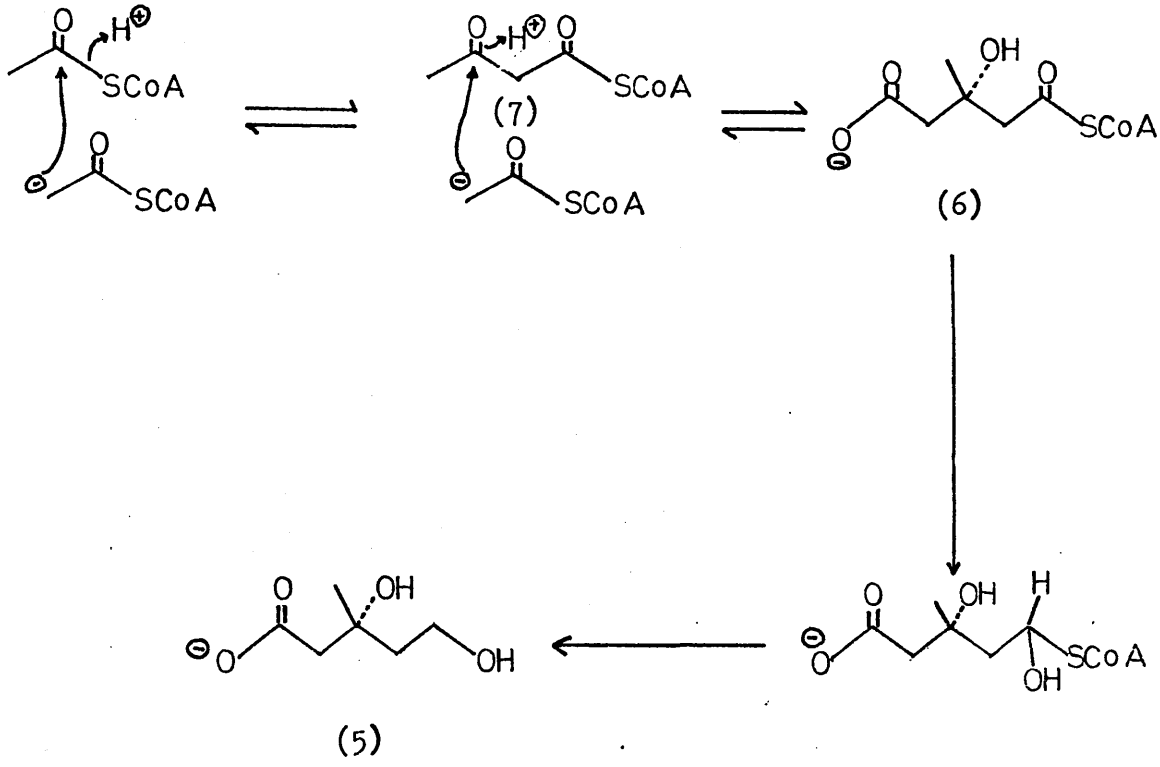
Another theory suggests that they are produced during periods of dormancy in order to keep the enzymatic systems ready for other important functions. Some terpenoids, however, have important roles in plant physiology as growth or dormancy hormones.[7]

Biosynthesis.[8] Considerable effort was spent in the search for the biological equivalent of isoprene (1), in the biosynthesis of terpenoids and steroids, mainly by Cornforth, Bloch, Lynen and Popjack. Early proposals that acetic acid, abundant in the primary metabolism of plants, was the basic building unit, were confirmed experimentally by the use of ^{14}C labelled acetate. Thus, it was demonstrated that cholesterol was ultimately derived from acetic acid, since incubation of rat liver slices with labelled acetate produced labelled cholesterol (2). These findings, however, gave little information about the intermediate stages in the biosynthesis.

In 1952 it was shown that the triterpenoid hydrocarbon squalene (3) found in shark liver oil, was a precursor of cholesterol (2), since labelled squalene was converted into cholesterol in mice. Finally, rat tissue experiments and the observed distribution of the acetate carbon atoms proved that the tetracyclic terpenoid lanosterol (4) is the precursor of cholesterol, and it arises by cyclisation and rearrangement of squalene (3).

A major breakthrough in the search of the proposed C_5 precursor was provided by the discovery of mevalonic acid (5)[9] in 1956. It was found that the labelled R-isomer was incorporated quantitatively into cholesterol on incubation with cell-free rat liver homogenate. Also, under anaerobic conditions, 2-^{14}C-mevalonic acid was converted into squalene (3). In all cases it was observed that C-1 was lost as CO_2. Many other experiments support the intermediacy of mevalonic acid in the biosynthesis of terpenoids. The S-isomer has been found, however, to be inactive.

The formation of mevalonate from acetate was then the subject of many biochemical studies.[10] The current view can be summarized as follows:

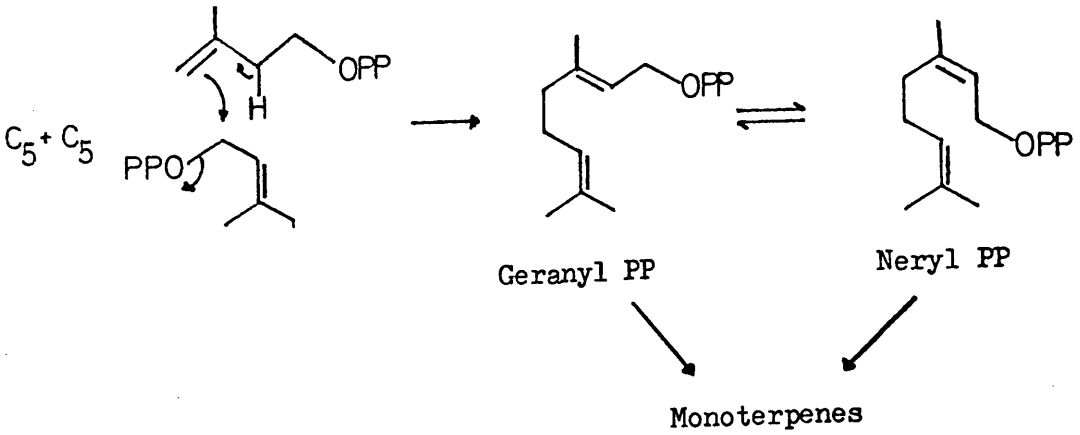


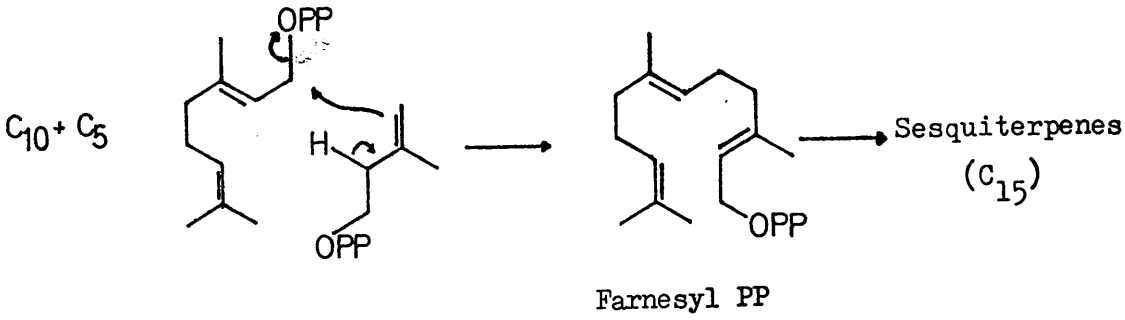
SCHEME 1

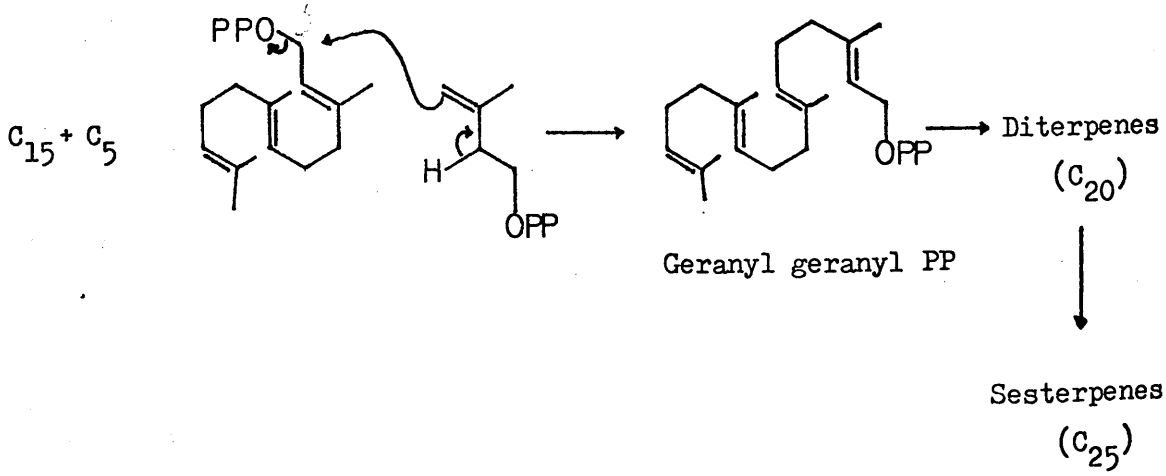
The immediate precursor of mevalonic acid (5) is S-3-hydroxy-3-methyl-glutaryl coenzyme A (6) (see Scheme 1), which is reduced by NADPH to (5) by hydrogen transfer in the presence of an enzyme. (6) arises by an aldol condensation of acetoacetyl coenzyme A (7) with acetyl coenzyme A.

As pointed out earlier, incorporation of mevalonate into a terpenoid containing a multiple of five carbon atoms, requires the loss of C-1. This decarboxylation is known to occur prior to the formation of the terpenoid chain, and a discrete five-carbon unit is formed. The nature of this unit and the mode of formation are illustrated in Scheme 2.

Scheme 3

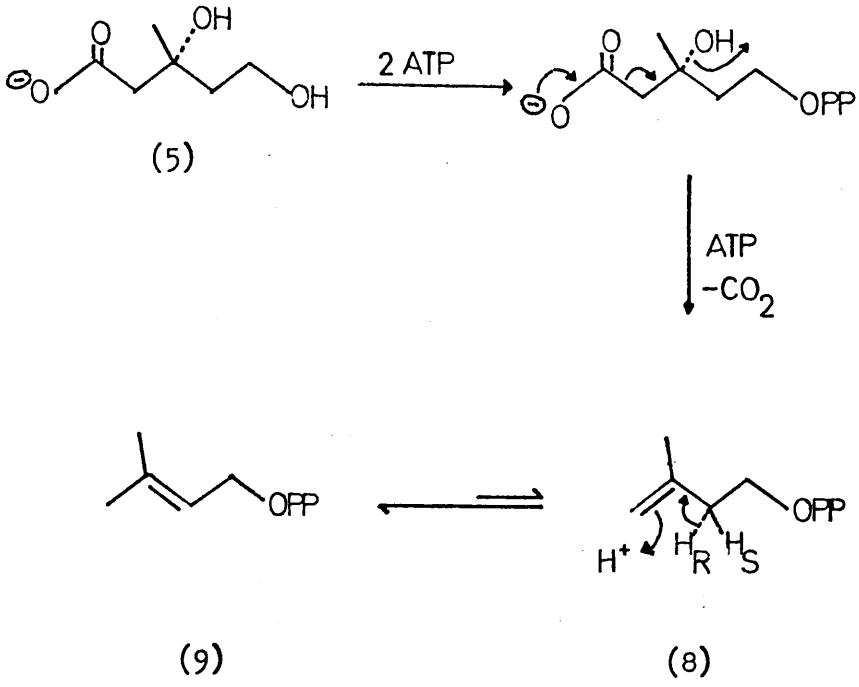






$C_{15} + C_{15} \longrightarrow$ Triterpenes and steroids (C_{30})

$C_{20} + C_{20} \longrightarrow$ Tetraterpenes (C_{40})



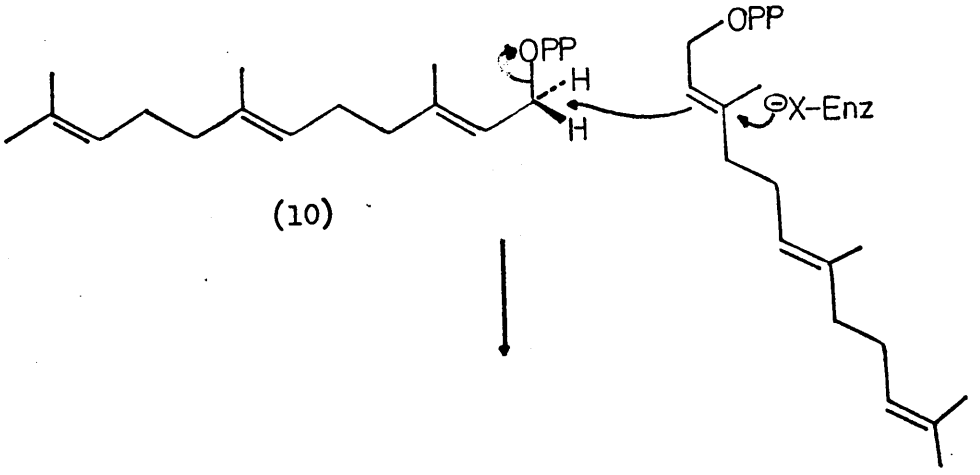
(5) (9) (8)

SCHEME 2

The formation of the acyclic terpenoid precursors from isopentenyl pyrophosphate (IPP) (8) and the isomeric dimethylallyl pyrophosphate (DMAPP) (9) can be visualized as in Scheme 3. It is worth mentioning that most natural acyclic polyisoprenoids are "all-trans".[2]

Formation and Cyclization of Squalene.[11] Squalene (3) is derived from two farnesyl pyrophosphate (10) units joined in the unusual "head-to-head" fashion.[10,11] The stereochemistry of this process is known from tracer studies and is believed to proceed via the intermediate pre-squalene (11).[12] A reasonable mechanism is shown in Scheme 4. The polycyclic structures formed from squalene can be rationalized in terms of the ways in which squalene may be folded on the enzyme surface. The formation of euphol (12) (or tirucallol), the putative precursor of tetranortriterpenoids (see Chapter 1), involves cyclization of squalene in the chair-chair-chair-boat conformation (13) (see Scheme 5). The corresponding chair-boat-chair-boat folding of squalene oxide (14) leads to lanosterol (4), and hence the steroids, on cyclization. Cyclization is usually initiated by acid

Scheme 4



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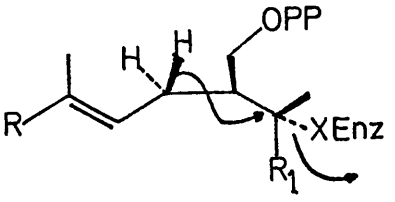

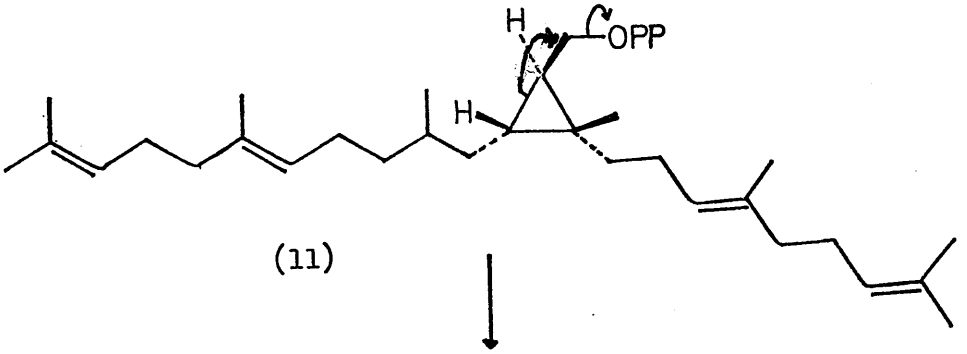

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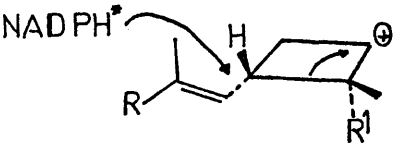

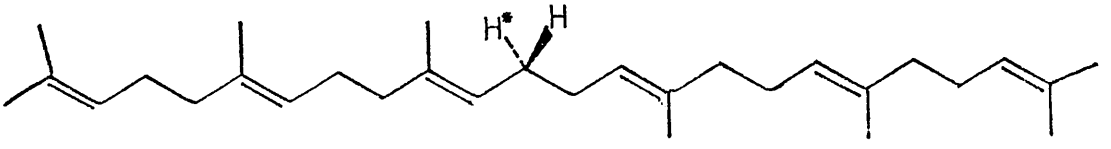

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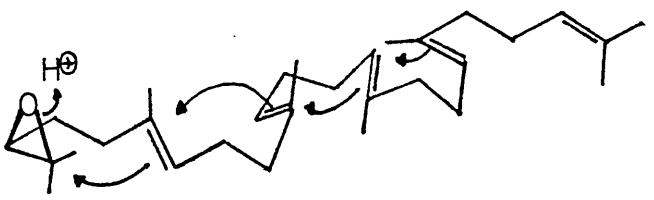
Scheme 5



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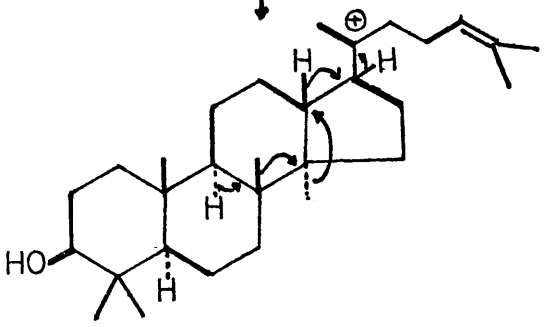

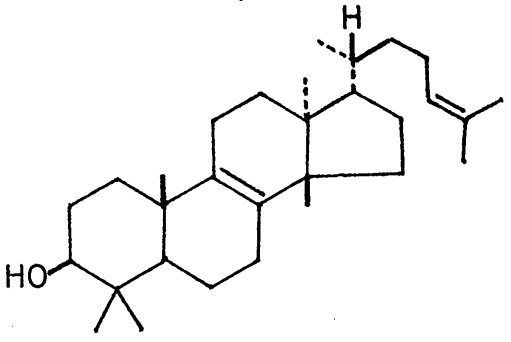

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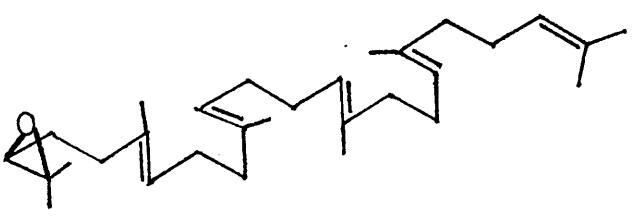

(14)

catalysed opening of squalene monoepoxide (14). Only the (3S)-enantiomer is used by a wide variety of biological systems.[2]

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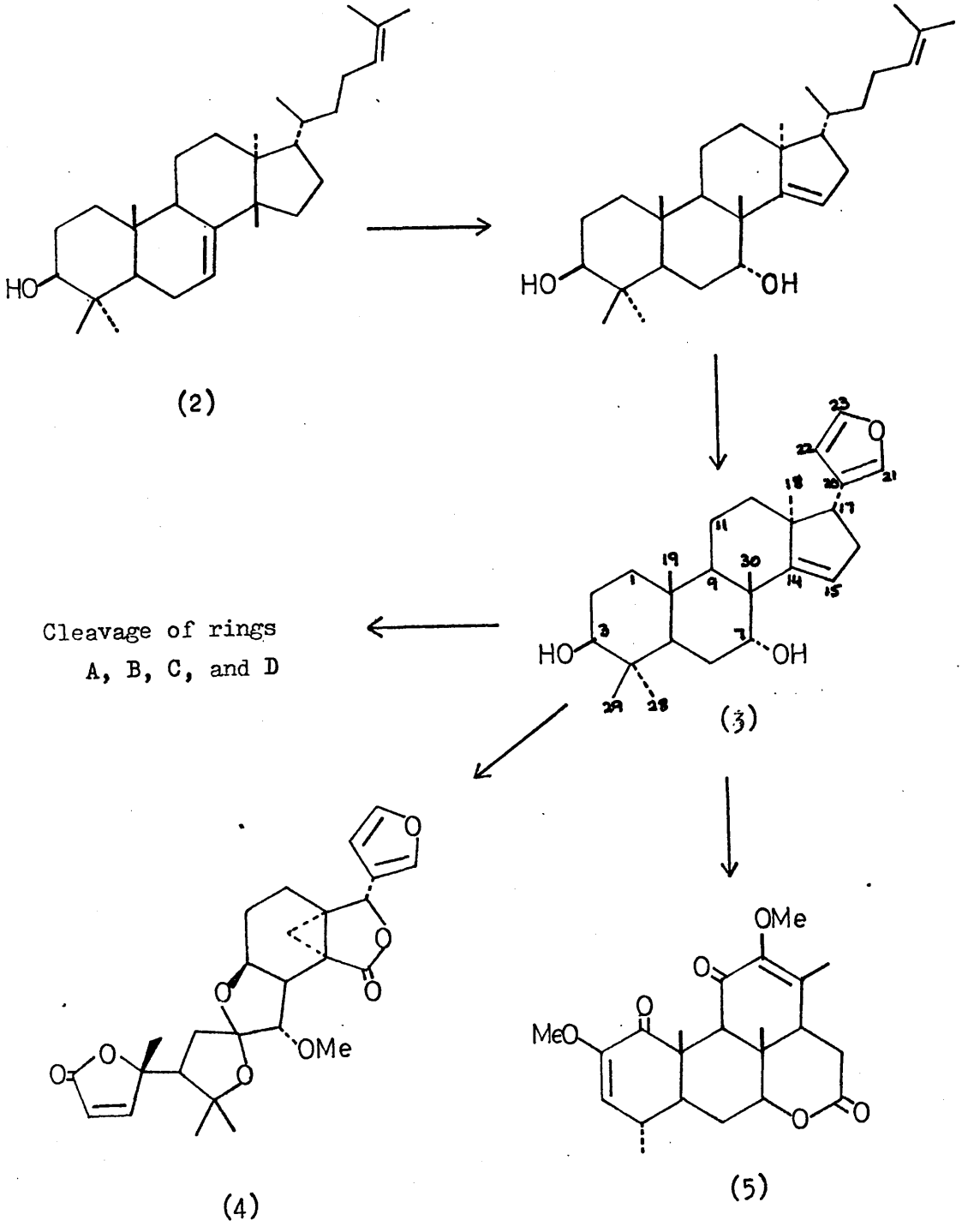
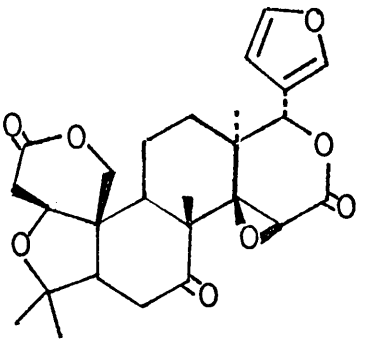
CHAPTER I

REVIEW OF TETRANORTRITERPENOIDS

Scheme 1

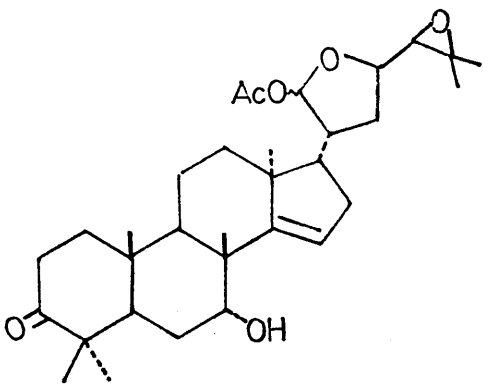

HO
HO
OH
(2)
23
22
21
20
18
17
11
19
30
9
1
14
15
3
7
HO
OH
29
28
(3)
Cleavage of rings
A, B, C, and D
O
O
O
O
O
O
OMe
(4)
OMe
O
O
MeO
O
O
(5)

The tetranortriterpenoids are a large group of furanoid compounds found mainly in the Rutaceae and Meliaceae.[1,2] Their name indicates that they are based on a C_{26} carbon skeleton. They are also called limonoids since it was the structural elucidation of limonin (1), a bitter principle from Citrus fruits, in 1960[3,4] which produced the breakthrough which stimulated subsequent investigations.



(1)

Biogenetically, tetranortriterpenoids may be derived from a euphol or tirucallol precursor (2) (Scheme 1), which first undergoes an apo-rearrangement with simultaneous introduction of oxygen at C-7 and then loss of the four terminal carbon atoms of the side chain with formation of the furan ring to give the simple tetranortriterpenoid (3). Further oxidations, Baeyer-Villiger ring cleavages and rearrangements of (3), can lead to the wide variety of structural types which have been reported. It is interesting to note that (3) can suffer further degradations to give pentanortriterpenoids, eg. (4) found in the Cneoraceae[5], and quassinoids, eg., (5) found in the Simaroubaceae.[1,6] While biosynthetic results for the proposed biogenesis of tetranortriterpenoids are limited, conclusive evidence has been obtained for the formation of quassinoids from mevalonate via a simple tirucallol precursor.



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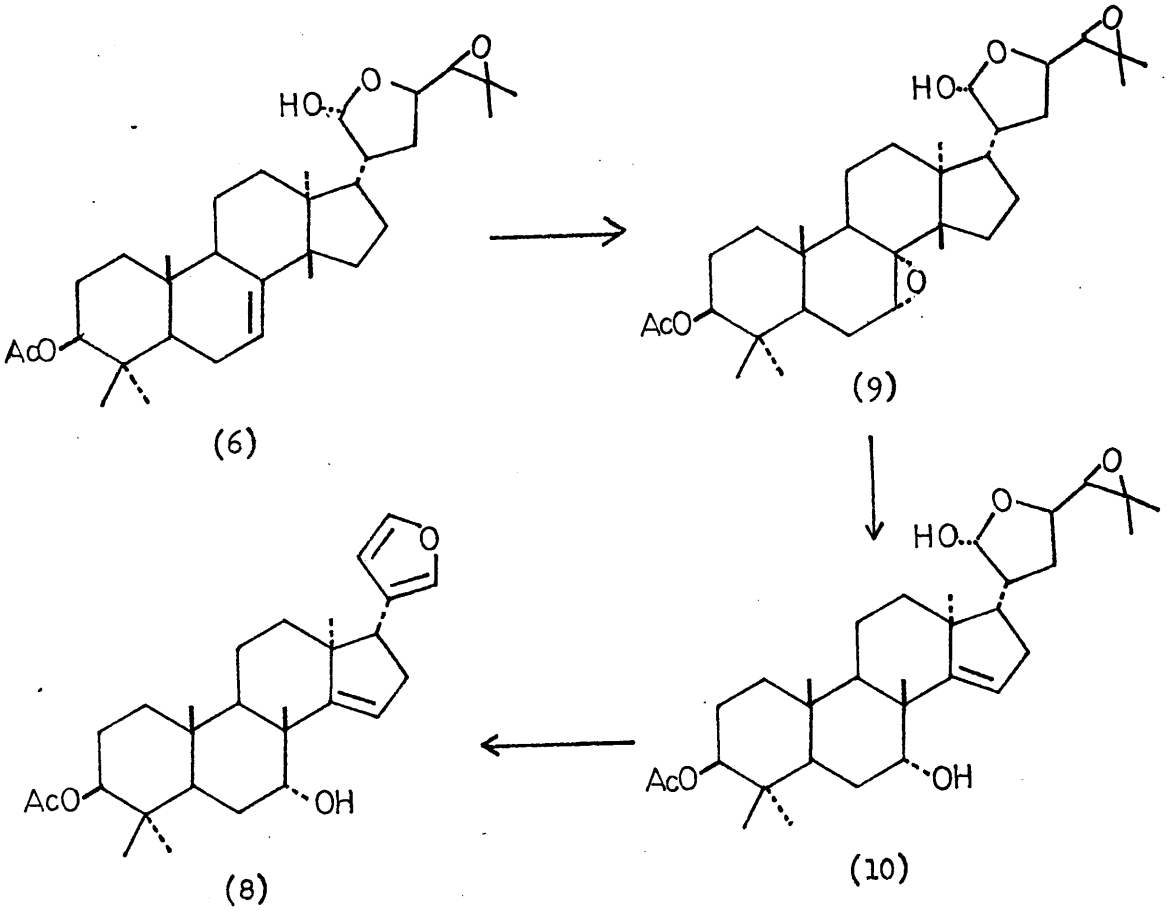
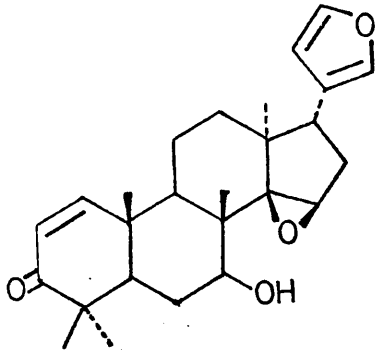


Scheme 2

This brief review is intended to give a broad picture of the present state of knowledge in the tetranortriterpenoid series. It is convenient to consider these compounds in groups, according to the extent of ring cleavage. First, it is appropriate to discuss some of the C_{30} tirucallol and apo-tirucallol derivatives which often co-occur with the tetranortriterpenoids and which seem likely precursors.

(a) C_{30} Precursors.- Turreanthin (6)[7] is one example of the group of compounds with a pattern of side chain oxygenation which very probably represents an intermediate stage between tirucallol and the furan ring of tetranortriterpenoids. The isolation of a growing number of apo-derivatives, including (7)[8], with an intact side chain suggests that skeletal rearrangement precedes furan formation. The possible intermediacy of these C_{30} compounds in the biogenesis of tetranortriterpenoids was demonstrated *in vitro* by Halsall and his colleagues with a conversion of turreanthin into the simple limonoid (8) (see Scheme 2). Lewis acid treatment of the 7α,8α-epoxide (9) smoothly converted it into the apo-derivative (10) with the desired oxygen substituent at C-7 and a 14,15 -double bond.[9]

(b) Intact C_{26}-Skeleton.- At the stage of the simplest limonoid (3), further oxidations can occur in ring D leading to derivatives with oxygen functions at carbons 14, 15, 16 and even 17. A common feature is epoxidation of the double bond as in trichilenone (11)[10], sometimes accompanied by a ketonic carbonyl at C-16 as in nimbinin (12).[11] The carbonyl group is also found in compounds with the double bond intact, eg., azadiradione (13)[12], and it is reasonable to assume that functionalisation of C-16 occurs prior to epoxidation of the double bond. Recently, several compounds (eg.,17β-hydroxyazadiradione (14)[13]) with a hydroxyl group at C-17 have been isolated.



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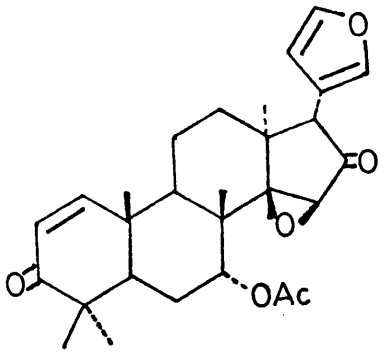

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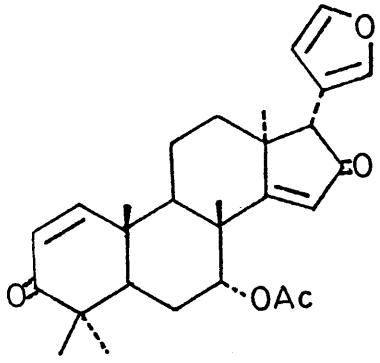

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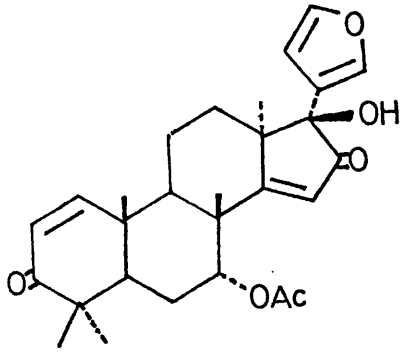

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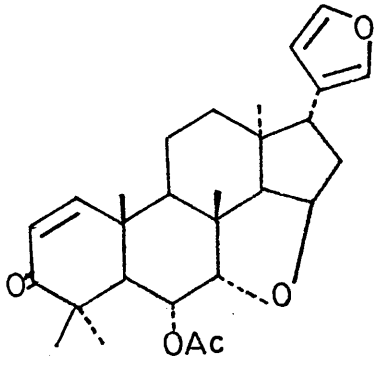

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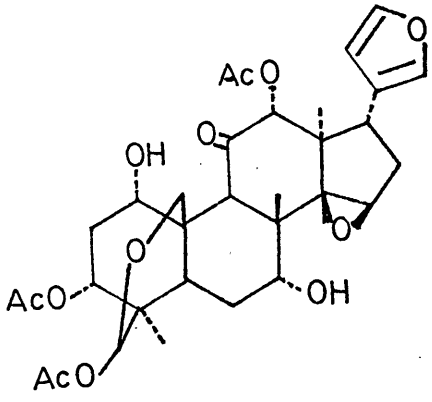

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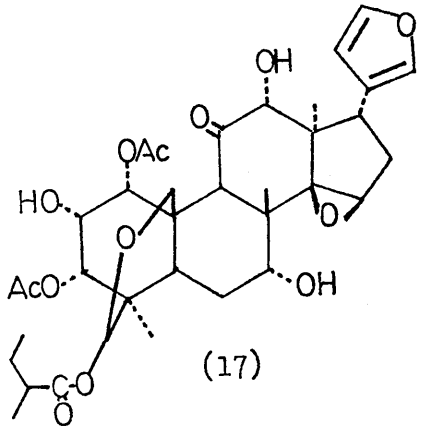

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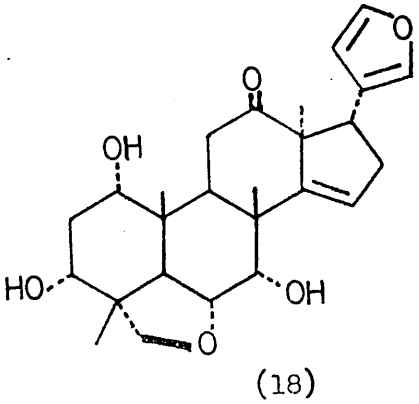

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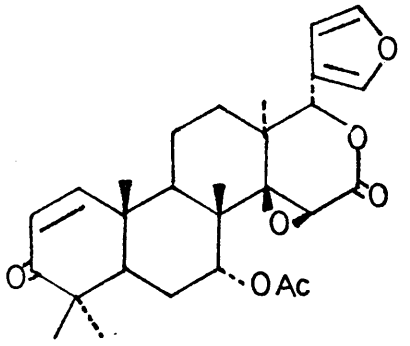

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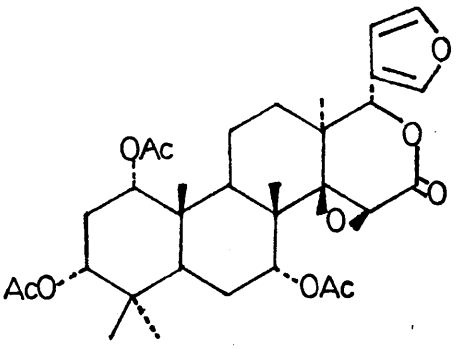

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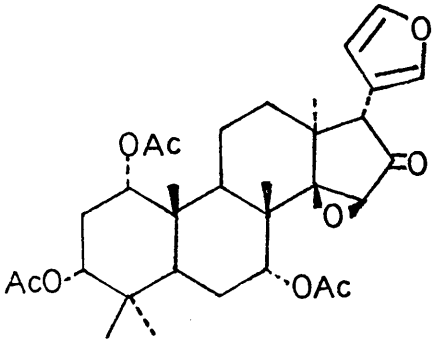

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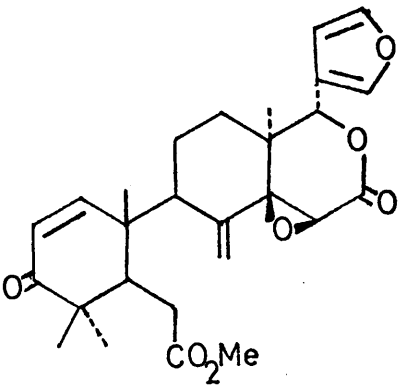

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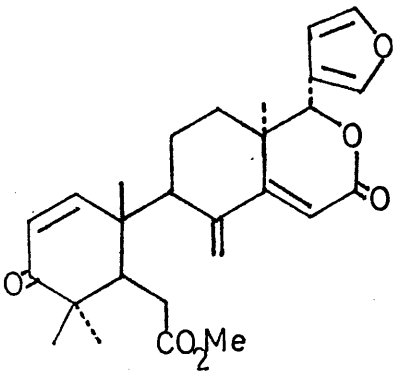

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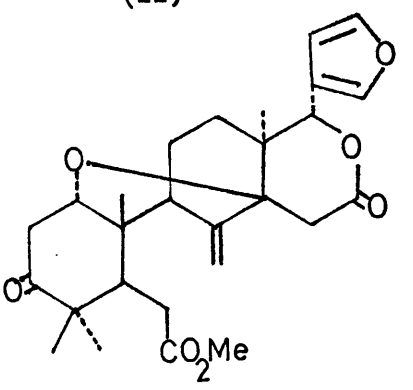

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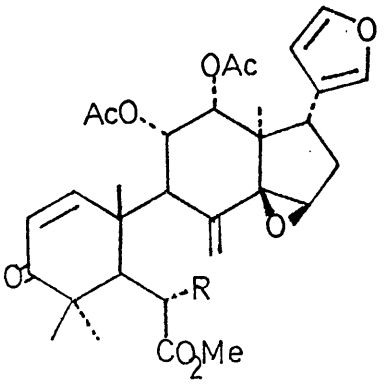
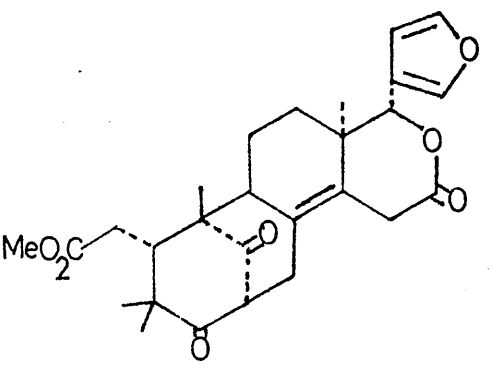


(25) R = H

(26) R = Ac



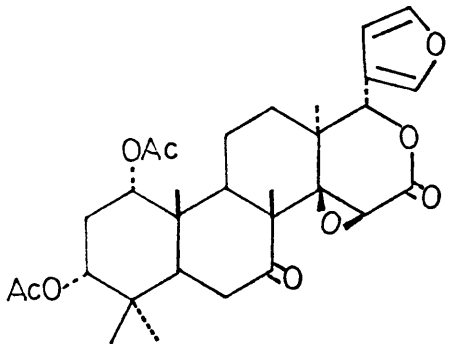
(27)

Other oxidations can take place in rings A, B and C at carbons 1, 2, 6, 11 and 12. Recent examples include vepinin (15)[14] with an ether between C-7 and C-15, sendanin (16)[15], a highly oxygenated compound from Melia azedarach with a hemiacetal between C-19 and C-27, and the related aphanastatin (17)[16] from Aphanamixis grandifolia, which has considerable antitumour activity. The presence of a hydroxyl group at C-6 may lead to the formation of an ether bridge with the 4α methyl group as in nimbidin (18).[17]

(c) Ring D Cleaved.- The next step in the elaboration of the tetranortriterpenoid skeleton leads to the formation of the characteristic ring D epoxylactone by biochemical Baeyer-Villiger oxidation of a 16-oxo-precursor. Two of the most abundant tetranortriterpenoids, gedunin (19)[18] and khivorin (20)[19], belong to this group. They have been prepared in vitro by Baeyer-Villiger oxidation of the putative precursors nimbinin (12) and khayanthone (21) respectively.[20,21] It is reasonable to assume that compounds of this type represent an intermediate stage in the biosynthesis of the complex group of ring B cleaved tetranortriterpenoids with which they often co-occur.

(d) Ring B Cleaved.- Most members of this group have also undergone cleavage of ring D. The typical ring B cleaved system exemplified by andirobin (22)[22] can arise by formal Baeyer-Villiger oxidation of a 7-oxo-compound followed by hydrolytic opening of the lactone and dehydration of the tertiary hydroxyl group to give the 8,30 exomethylene group. The corresponding diene lactone, deoxyandirobin (23)[23] has also been isolated. Methyl angolensate (24)[24] has the interesting 1,14-ether which presumably arises by addition of a 1α-hydroxyl group to the α,β-unsaturated ring D lactone. Both andirobin and methyl angolensate have been prepared in vitro by partial synthesis[25] from khivorin (20).

Scheme 3



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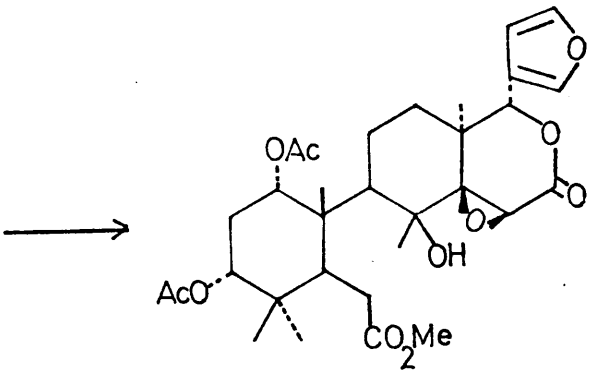

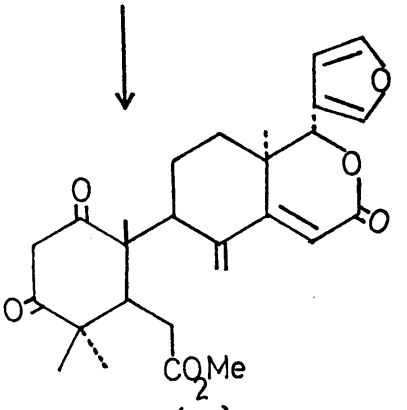

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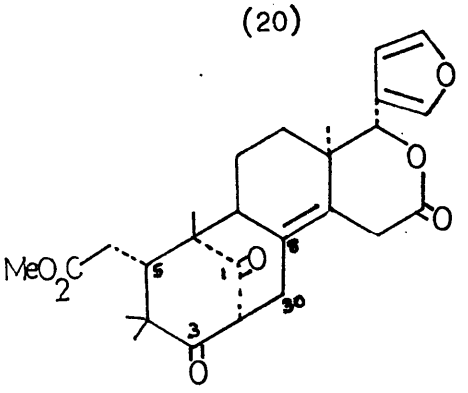

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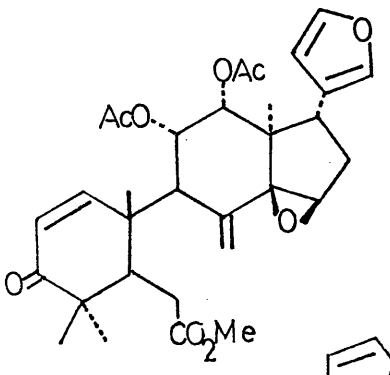

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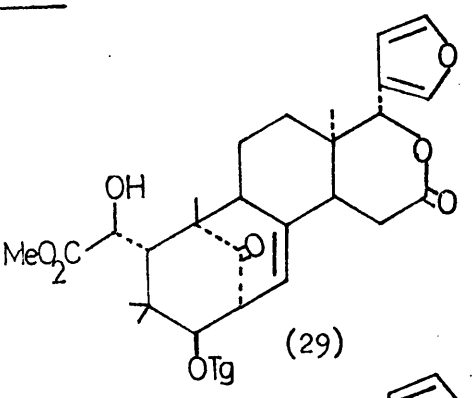

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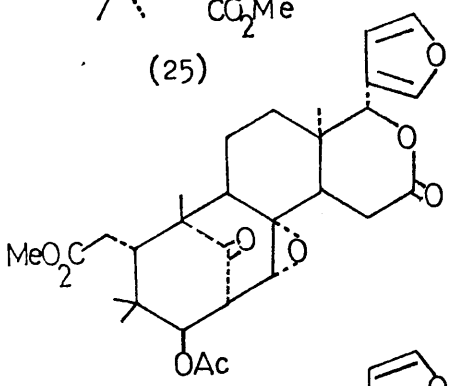

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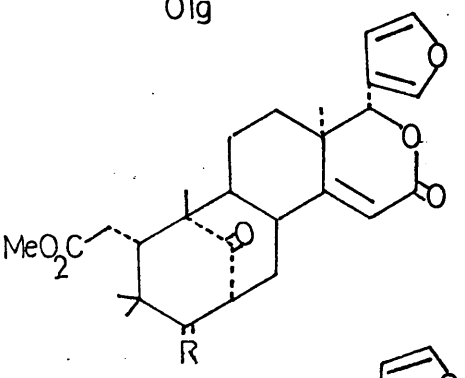
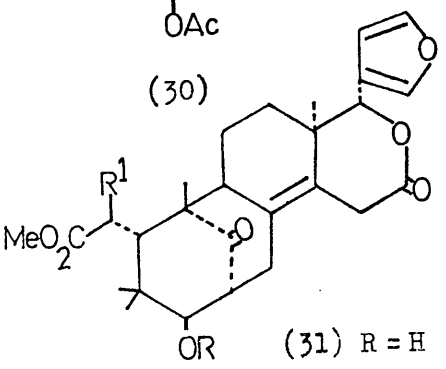


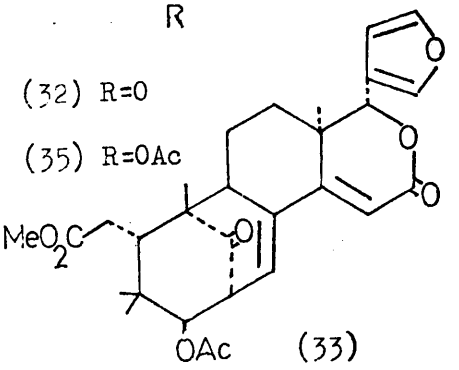
(32) R=O

(35) R=OAc



(31) R = H ; R^1=OH

(34) R = Ac ; R^1=H



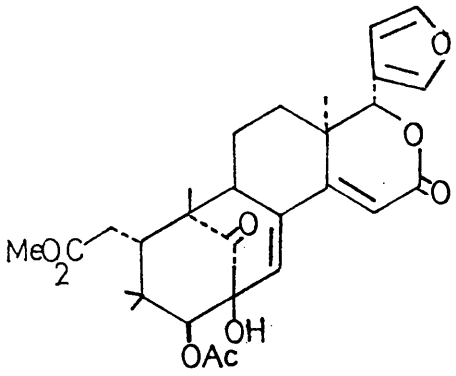
(33)

The first examples of simple ring B cleaved tetranortriterpenoids with an intact ring D have appeared recently. Toonacilin (25) and its 6-acetoxy derivative (26) from Toona ciliata are of special interest in view of their potent antifeedant activity against the Mexican bean beetle.[26]

This group of tetranortriterpenoids is unique in that the initial cleavage of ring B can be obscured by subsequent carbon-carbon bond formation between C-2 and C-30 to give the bicyclononane ring system as in mexicanolide (27).[28] An in vitro partial synthesis of mexicanolide from khivorin (20) has been achieved[25] (see Scheme 3). The diketone precursor (28) undergoes facile cyclisation in mild base to (27).

The first representative of the bicyclononanolide group was swietenine (29) from Swietenia macrophylla[29] whose structure was confirmed by X-ray analysis.[30] The residual 8,30 double bond is very hindered and unreactive. Although the natural epoxide, xylocarpin (30), has been isolated from Xylocarpus granatum[31] attempts to form the epoxide in vitro have been unsuccessful. The nuclear double bond is also found at 8,14 as in mexicanolide (27)[28] and swietenolide (31)[32] and at 14,15 as in carapin (32)[33]. Augustadienolide (33)[34] from Cedrela augustifolia is the corresponding diene. Hydrogenation afforded a mixture of fissinolide (34) and the carapin derivative (35).

2-α-Hydroxyaugustadienolide (36)[35] and xyloccensin A (37)[36] represent an increasing oxidation level in this series which leads to the highly complex compounds like utilin (38)[37] and bussein (39).[38] Two new features are apparent in these structures: a) the formation of a new carbocyclic ring between the 4 α methyl group and C-1; and b) the introduction of the orthoacetate at 1,8 and 9 or 8,9 and 14. The reaction of an unactivated methyl group and a ketonic carbonyl is unusual and finds analogy in photochemistry. The occurrence of compounds of this type is



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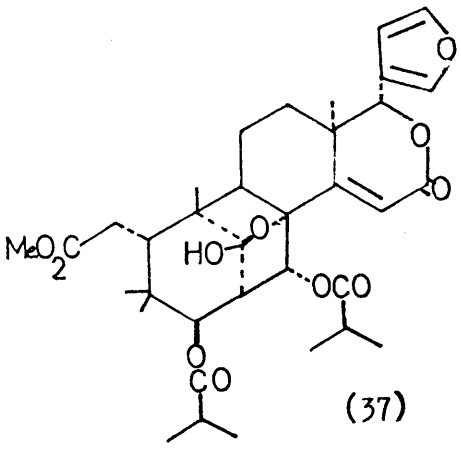

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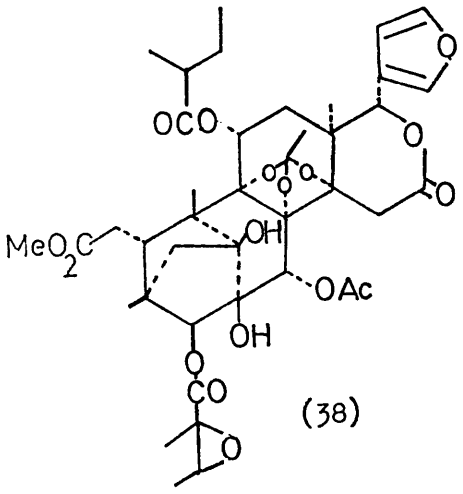

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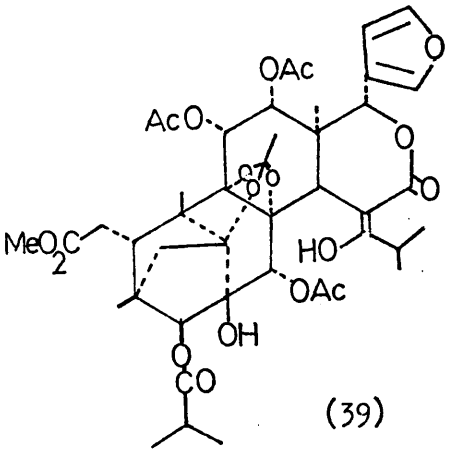

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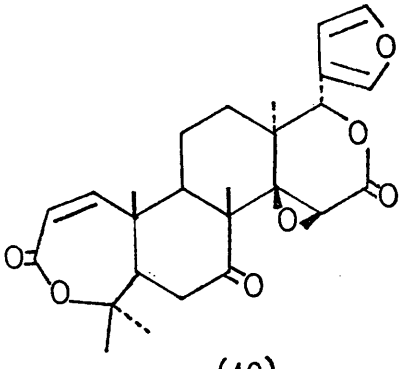

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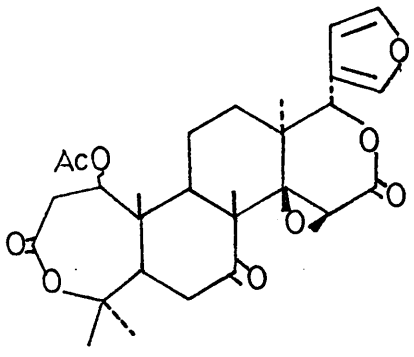

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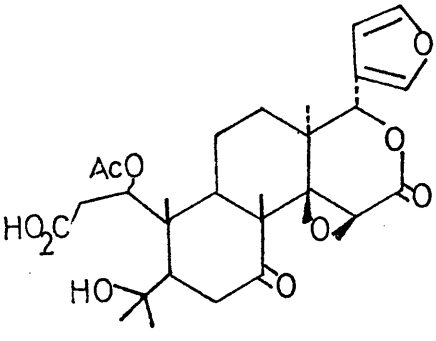

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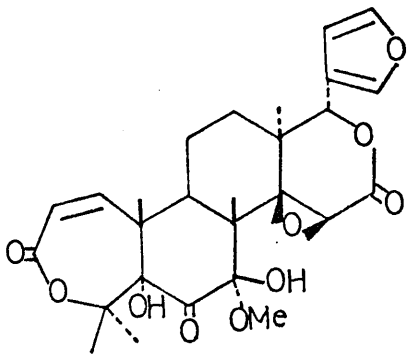

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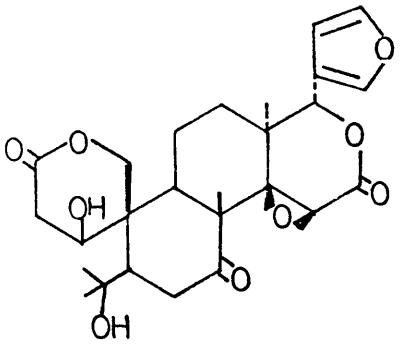

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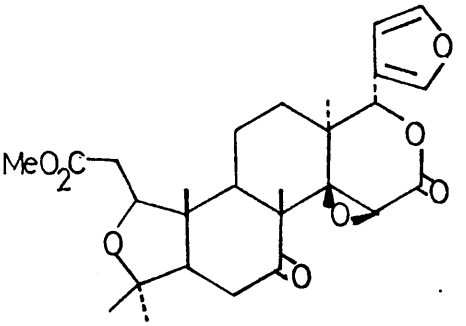

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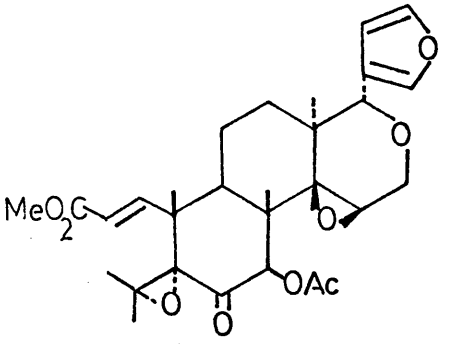

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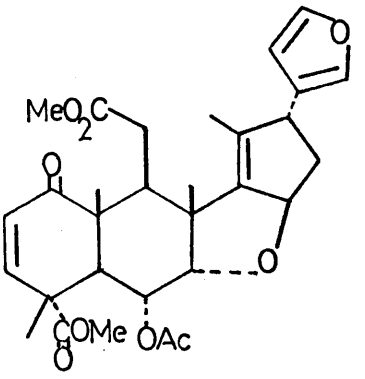

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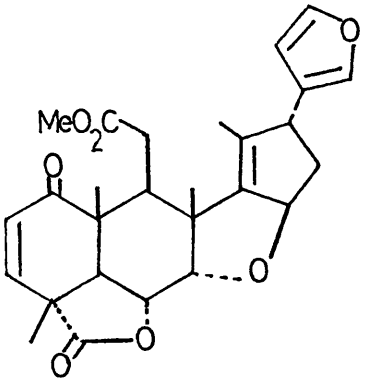

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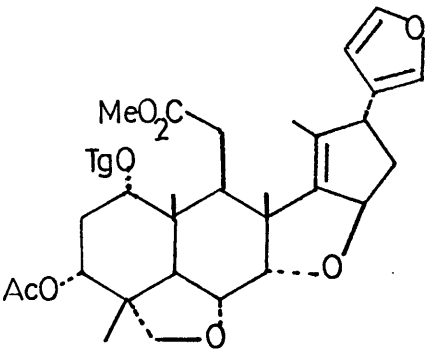

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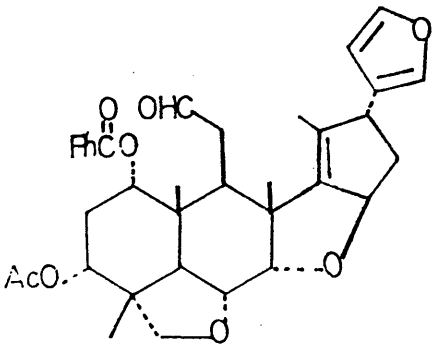

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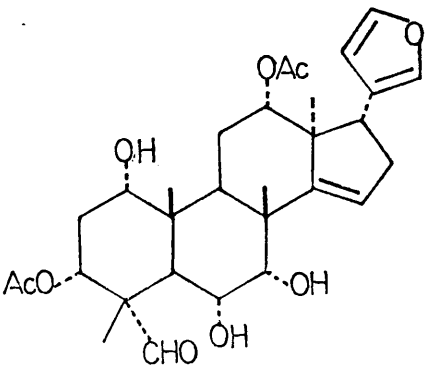


(50)

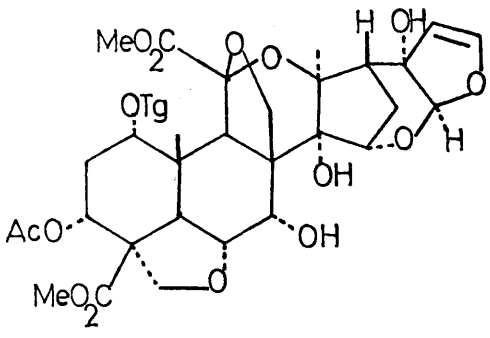
so far restricted to Entandrophragma and Chukrasia species (see Chapter III).

(e) Ring A Cleaved.- All the compounds in this group have the characteristic ring D epoxylactone system and most come from Citrus species. Historically this group is of special interest since the development of the chemistry of tetranortriterpenoids dates from the structure elucidation of limonin (1).[3] In limonin, the initial ring cleavage is obscured by subsequent reactions. The simple Baeyer-Villiger cleavage of ring A is more obvious in obacunone (40)[39] and nomilin (41)[40] two further compounds from Citrus species. Harrisonin (42)[41], from Harrisonii abyssinica has an interesting hemiacetal function at C-7.

The opened form of the ring A ε-lactone is apparent in nomilinic acid (43)[42] which may be regarded as a precursor of the C-19 oxidised derivatives inchangin (44)[43] and limonin (1). Veprisone (45)[44] is a simpler example of the C-1, C-4 ether which is probably formed by addition of the C-4 tertiary hydroxyl group to the unsaturated ester (or lactone as in limonin (1)). Alternatively, dehydration and epoxidation leads to spathelin (46).[45]

(f) Ring C Cleaved.- This is a compact group of compounds restricted to Melia azedarach and Azadirachta indica.[1] The common features are illustrated by nimbin (47)[46], nimbolide (48)[47] and salannin (49).[48] Sendanal (50)[49], recently isolated from M. azedarach, has the appropriate functionality for transformation into the above compounds. Onchinal[50] (51) is biogenetically interesting since it represents simple ring C cleavage of a 12-hydroxy precursor (eg., sendanal (50)). The most interesting and most complex member of this group is azadirachtin (52)[51], a powerful locust antifeedant.

(g) Rings A and B Cleaved.- Prieurianin (53) from Trichilia prie-



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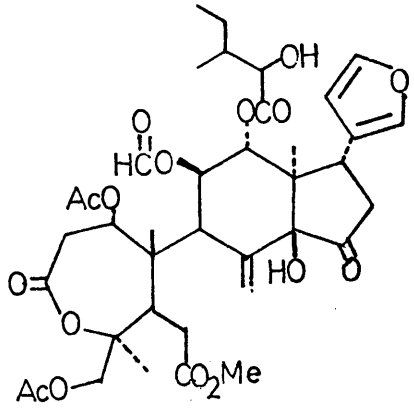

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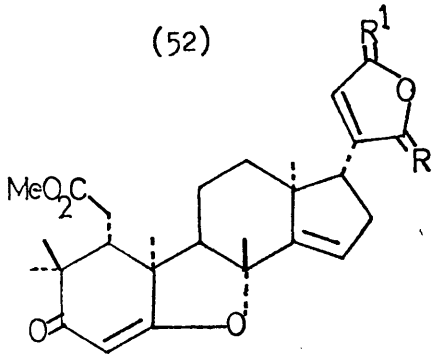
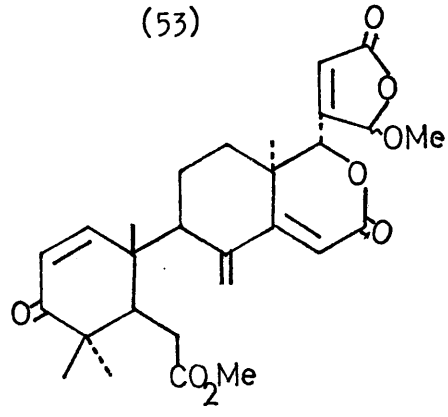


(54) R = O ; R^1 = OH

(55) R = OH ; R^1 = O



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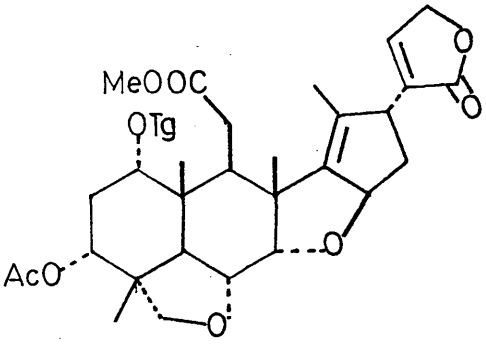

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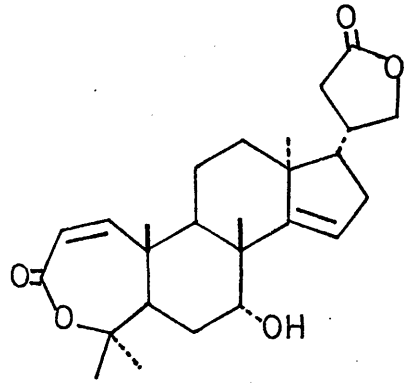

(58)

uriana[52], is the first member of an interesting group of tetranortriterpenoids with both rings A and B cleaved. In solution it exists as a mixture of conformational isomers at room temperature, due to restricted rotation about the C-9, C-10 bond. Other members of this group form part of the subject matter of this thesis (see Chapter IV).

(h) Modified Side Chain.- Recently a growing number of publications have appeared on tetranortriterpenoids with the usual furan ring replaced by other C_4 units, including the isomeric γ-hydroxybutenolides (54) and (55)[53], the methoxybutenolide (56)[54], the butenolide (57)[55] and the γ-lactone (58).[56] It is not clear whether all these compounds are genuine natural products or whether they are artefacts formed by the action of light and oxygen on the furan ring. Photooxidation of several tetranortriterpenoids has been shown to give the corresponding γ-hydroxybutenolides.[57]

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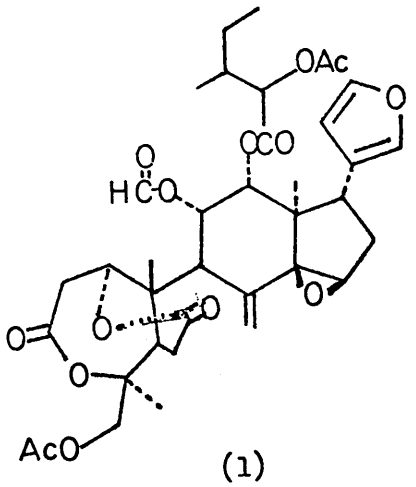
CHAPTER II

NEW APO-TIRUCALLOL DERIVATIVES AND TETRANORTRITERPENOIDS FROM THE WOOD AND SEEDS OF CHISOCHETON PANICULATUS



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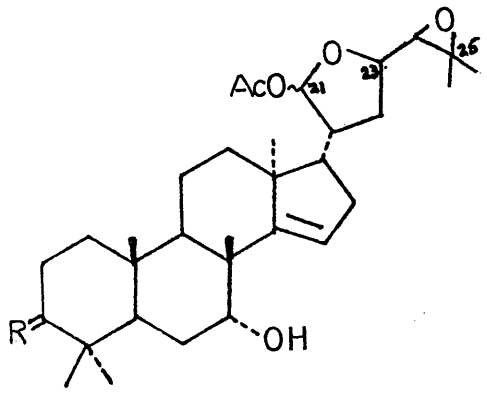
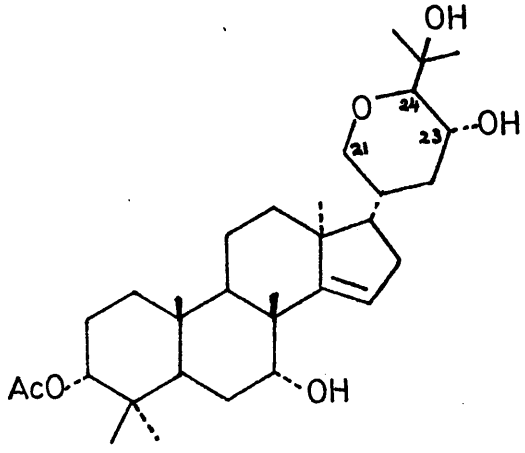


(2) R = O

(6) R = H,OH



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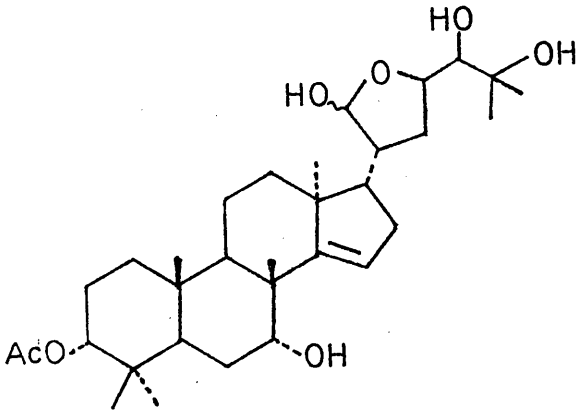

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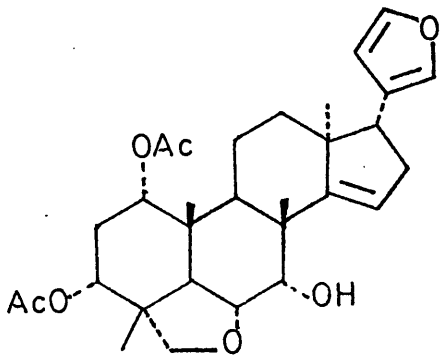


(5)

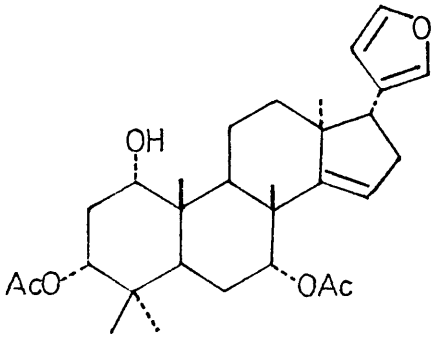
I N T R O D U C T I O N

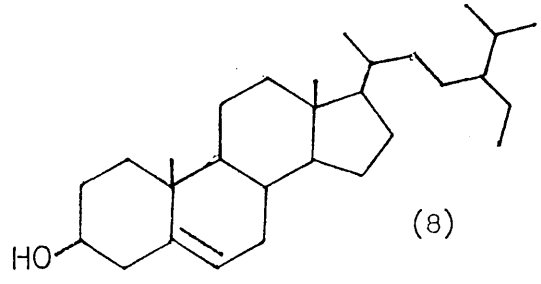
Chisocheton paniculatus (Meliaceae), also known as *Guarea paniculata*, is one of the twenty two species of the genus which originate in India, Burma and Malaysia. The taxonomic characteristics of this tall tree are very similar to those of *Guarea* species, a genus belonging to the same tribe (Guareeae). This accounts for the dual classification of this species.[1]

Chemical studies of the Guareeae tribe, which also includes *Dysoxylum* and *Cabralea* species, have been mainly concerned with *Guarea* species which produce various interesting limonoids and triterpenoids.[2-6] *Cabralea eichleriana*, on the other hand, is the only species of its genus examined, and produces a wide range of limonoids[7,8], while *Dysoxylum* species contain mainly sesquiterpenoids.[9] The Chisocheton genus has not been studied.

The botanical similarities between *Chisocheton paniculatus* and *Guarea* species led us to investigate the wood and seeds of the former species in the hope of finding limonoids related to dregeanin (1), an interesting compound isolated from *G. thompsonii*.[10] In this chapter, we report the isolation of five new compounds (2)-(6), from the wood of *C. paniculatus* and discuss the spectroscopic and chemical evidence that led to their structure elucidation. Compounds (2),(3),(4) and (6) represent new apo-tirucallol derivatives. The extract also contained deoxyhavanensin-3,7,-diacetate (7)[11] and β-sitosterol (8).

(7)





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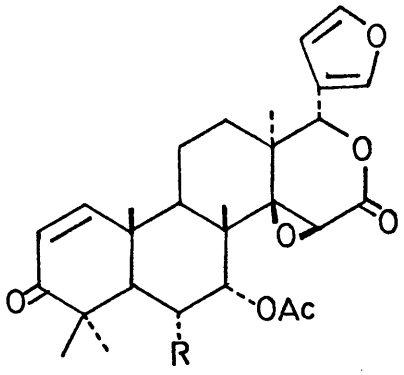
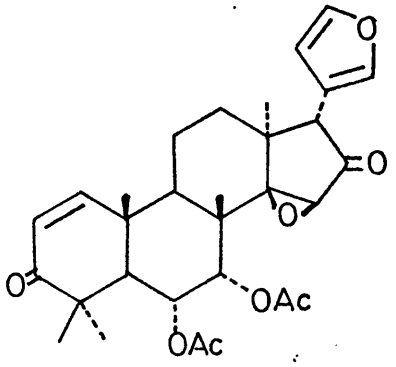


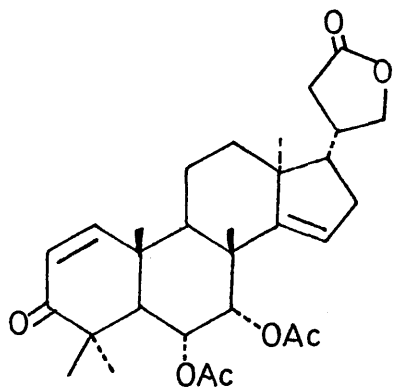
(9) R = H

(10) R = OAc



(11)

From the seeds of the same tree we isolated the known compounds, gedunin (9)[12], 6α-acetoxygedunin (10)[13] and 6α-acetoxynimbinin (11)[14] along with four new compounds, which were assigned structures (12)-(15) on the basis of their chemical and spectroscopic properties. Compounds (12) and (13) are possible precursors of furanoid tetranortriterpenoids.



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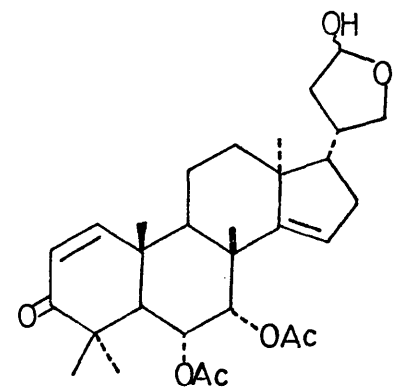

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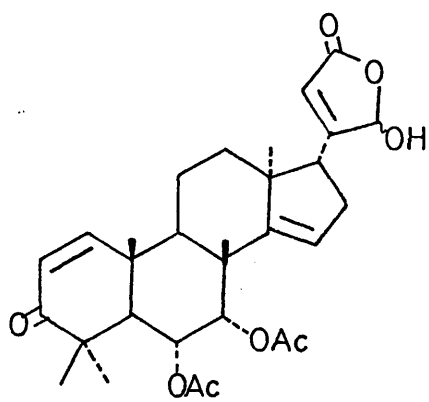

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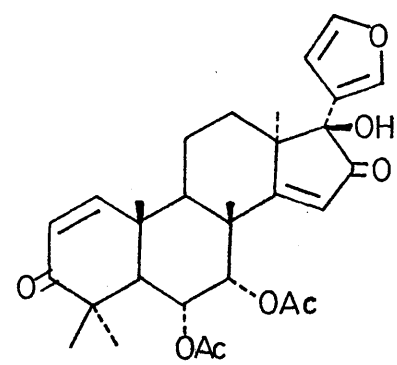


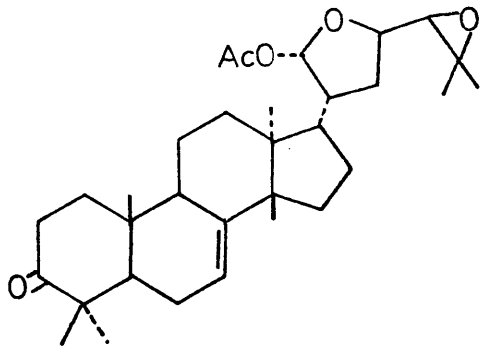
(15)

DISCUSSION

Column chromatography of the light petroleum extract of the wood of Chisocheton paniculatus (Meliaceae) afforded three crystalline substances A-C. Two other compounds, D and E, were obtained by preparative t.l.c. of the mother liquors of A and B respectively.

From the ^{1}H n.m.r. spectra of compounds A, $C_{32}H_{48}O_6$, B, $C_{32}H_{52}O_6$, C, $C_{32}H_{52}O_7$ and E, $C_{32}H_{50}O_6$, it was readily apparent that they were triterpenoids with an intact side chain, as indicated by the presence of signals corresponding to seven tertiary methyl groups. Compound D, $C_{30}H_{40}O_7$, gave a positive test with Ehrlich's reagent, which indicated the presence of a furan ring in the molecule. This was also obvious from its ^{1}H n.m.r. spectrum (see Table 1) and suggested a tetranortriterpenoid skeleton for this compound. The nature of the side chains and the structures of these five compounds were revealed by a study of their spectroscopic properties.

The i.r. spectrum of A (2) had absorptions for a cyclohexanone (1705 cm^{-1}), a hydroxyl (3570 cm^{-1}) and an acetate group (1750 cm^{-1}). The hemiacetal attachment of the latter was deduced by the presence of a doublet at 96.6 p.p.m. ($\underline{CH}\overset{O}{\underset{O}{<}}$, C-21) in the SFORD ^{13}C n.m.r. spectra and by the fact that the proton attached to this carbon gave rise to a sharp doublet [δ_H 6.23 (J 4 Hz, H-21)] in the ^{1}H n.m.r. spectrum. Other signals in the ^{1}H and ^{13}C n.m.r. spectra were assigned to a trisubstituted double bond [δ_H 5.48 (t, H-15), δ_C 119.6 (d, C-15), 161.5 (s, C-14)], a trisubstituted epoxide [δ_H 2.64 (d, J 7 Hz, H-24), δ_C 57.1 (s, C-25) and 66.7 (d, C-24)], a secondary ether oxygen [δ_H 3.9 (m, H-23)] and a



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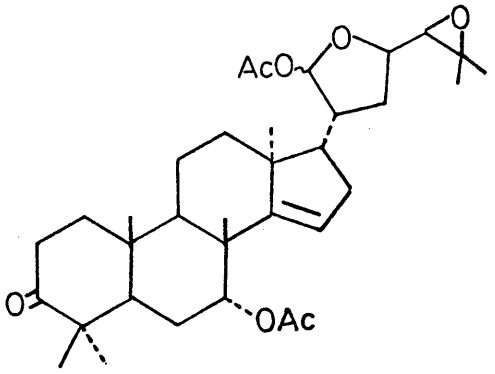

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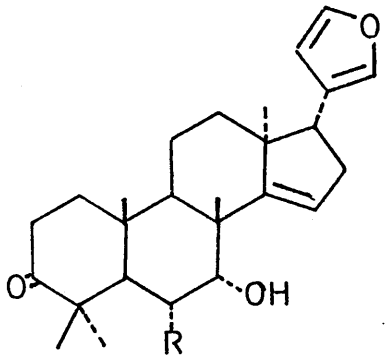
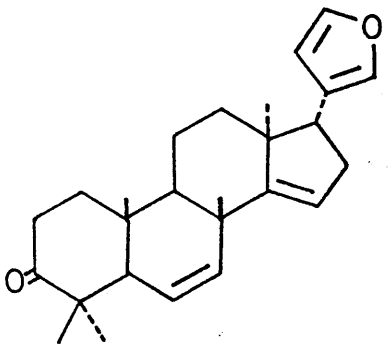


(18) R = H

(19) R = OAc



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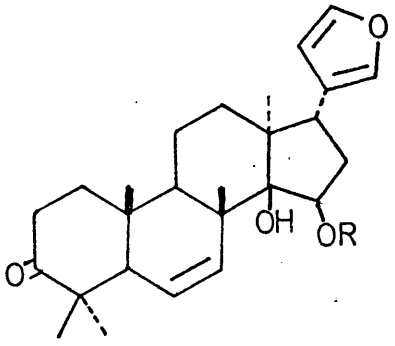
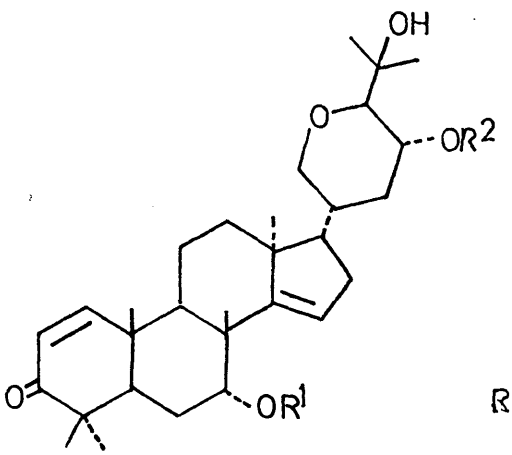


(21) R = H

(22) R = Ac

secondary hydroxyl group [δ_H 3.90 (t, H-7)]. Decoupling experiments indicated that the epoxide proton was coupled to the proton on the carbon bearing the ether oxygen and that this in turn was coupled to a methylene group at δ_H 2.64. Comparison of these data with melianone acetate (16)[15] confirmed the structure of the side chain as in (2). The reported values for (16) for H-21 [δ_H 6.20 (d, J 3 Hz)], H-24 [δ_H 2.73 (d, J 7 Hz)] and H-23 [δ_H 3.90 (m)] are in good agreement with this assignment.[15] The presence of the cyclohexanone, the secondary hydroxyl group and the trisubstituted double bond in the residual tetracarbocyclic nucleus suggested an apo-tirucallol skeleton. This was confirmed by the shift of the vinyl proton, H-15, from δ_H 5.48 in (2) to 5.26 in the ^{1}H n.m.r. spectrum of the corresponding diacetate (17). This indicated that the secondary hydroxyl was at C-7. Similar acetylation shifts have been observed with the tetranortriterpenoid (18).[16] On biogenetic ground the ketonic carbonyl group was placed at C-3.

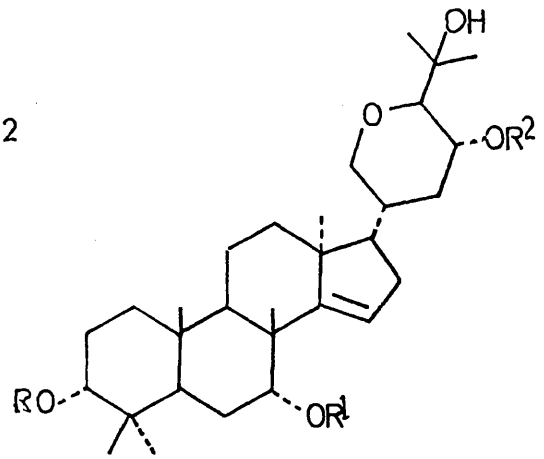
Final proof of structure (2) for compound A was obtained by converting it into the known tetranortriterpenoid (18) using the conditions worked out by Buchanan and Halsall.[16] Thus treatment of (2) with sodium periodate and aqueous perchloric acid followed by p-toluenesulphonic acid in benzene, afforded (18) whose physical and spectroscopic properties accorded with published data. It was our intention to use this compound for a partial synthesis of meldenin (19), a tetranortriterpenoid from Melia azadirachta.[17] Reaction of (18) with thionyl chloride in pyridine gave the diene (20) [δ_H 6.05 (dd, J 9,3 Hz, H-6), 5.4 (d, J 9 Hz, H-7)]. The formation of a 6,7 double bond provided further evidence for the apo-tirucallol nature of (2). Unfortunately, osmium tetroxide reacted preferentially with the trisubstituted 14,15 double bond of (20) to give the diol (21) which under normal acetylating conditions afforded the monoacetate (22) [δ_H 5.3 (t, J 8 Hz, H-15), 5.78 (2H, bs, H-6 and H-7)].



(23) R^1= Ac ; R^2= H

(24) R^1= H ; R^2= H

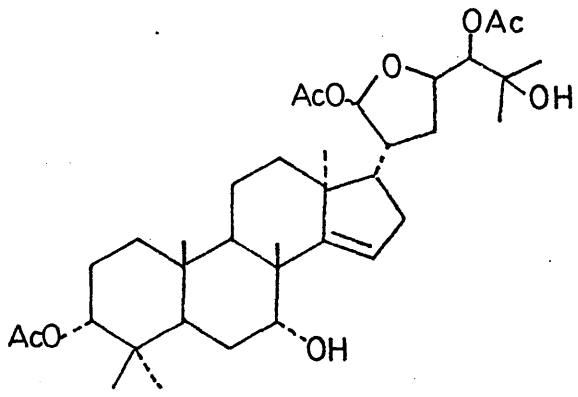
(40) R^1= Ac ; R^2= Ac



(25) R = R^1= R^2 = H

(26) R= R^2= Ac ; R^1= H

(27) R = R^1= R^2= Ac



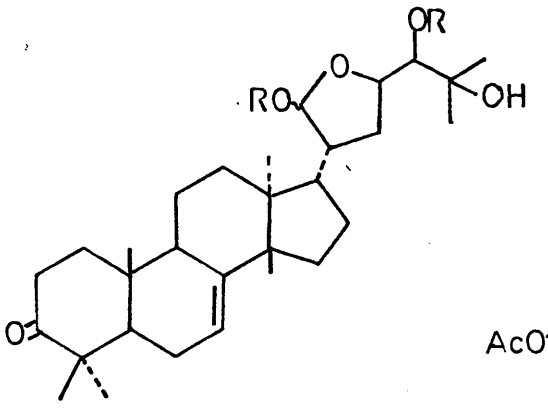
(28)

Lack of material prevented further work on the synthesis of meldenin.

The spectroscopic properties of compound B (3) (see Tables 1 and 2) [ν_{max} (CCl_4, 3610, 3560, 3515, 1727 cm^{-1}] revealed the presence of seven tertiary methyl groups, a trisubstituted double bond, one tertiary and two secondary hydroxyl groups, a secondary acetate and a primary-tertiary cyclic ether. The nature of the side chain as in (3) was readily deduced from the similarity of the appropriate data, especially the H-24 doublet at δ_H 2.85 (J 9 Hz), with those recorded for grandifoliolenone[18] (23) and the closely related sapelins C and D, (24) and (25) respectively.[19] The remaining information suggested an apo-tirucallol skeleton with a 3α-acetate and a 7α-hydroxyl group, and led to structure (3) for compound B. This was confirmed by the ^1H n.m.r. spectra of the corresponding diacetate (26) and triacetate (27) (which is sapelin D triacetate). The side chain resonances of the diacetate (26) paralleled those of grandifoliolenone acetate (40) with H-15 remaining unchanged. In the triacetate (27) H-15 shifted upfield (δ_H 5.49 to δ_H 5.20) as expected on the formation of a 7-α-acetate.[16]

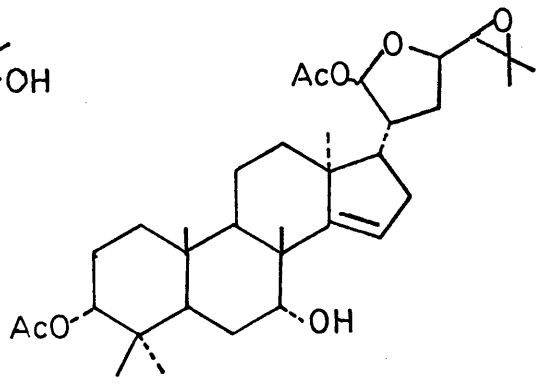
Compound C (4) [ν_{max} (CCl_4) 3610-3300, 1725 cm^{-1}] lacked the epoxide of A (2) and the cyclic ether of B (3). However the presence of a hemiacetal [δ_C 96.5(d)] indicated a side chain related to that of compound A. From the spectroscopic properties we were able to detect in addition to the hemiacetal, seven tertiary methyl groups, a trisubstituted double bond, one tertiary and two secondary hydroxyl groups and a secondary acetate. This information could be satisfactorily assembled to give structure (4) for compound C.

Acetylation of (4) afforded a mixture from which the triacetate (28) was isolated by preparative t.l.c. In its ^1H n.m.r. spectrum it had inter alia signals arising from a hemiacetal acetate [δ_H 6.12 (d, J 3 Hz,

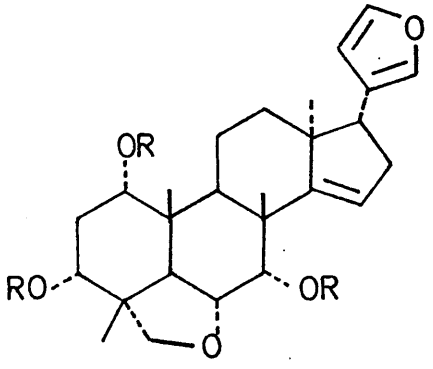


(29a) R = Ac

(29b) R = H



(30)



(31) R = H

(32) R = Ac

H-21) and a secondary acetate proton [δ_H 4.82 (d, J 3 Hz, H-24)]. Decoupling experiments clearly demonstrated that the latter was H-24 and confirmed the side chain sequence. Thus, irradiation at the frequency of H-23 (δ_H 4.51)caused the collapse of the H-24 doublet (δ_H 4.79) to a singlet. Melianodiol acetate (29a)[20] has the same side chain as (28) and the reported value for H-24 (δ_H 3.2, m) in the deacetyl compound (29b), melianodiol, is in good agreement with that found for compound C (4) (δ_H 3.27,m,H-24).

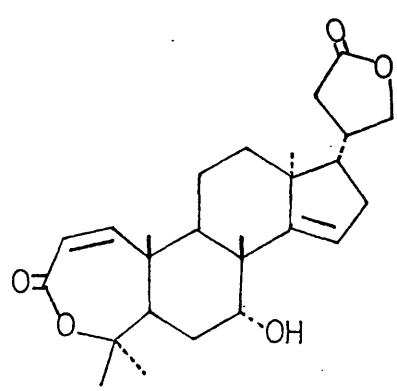
Compound E (6) had similar spectroscopic properties to those of compound A. It differed in the lack of a ketonic carbonyl group and in the appearance of a new secondary hydroxyl group. This suggested that compound E was the dihydro-A derivative (6). The ^{1}H n.m.r. spectrum of the corresponding monoacetate (30) was in accord with this proposal and had a >C<u>H</u>OAc resonance at δ_H 4.65 (H-3). As with the other three compounds, the 7α-hydroxyl group was more resistant to acetylation under normal conditions and the signal for H-15 did not exhibit an upfield shift.

The fifth new compound was the tetranortriterpenoid D (5). It had a tetracarbocyclic skeleton with the characteristic β-substituted furan ring, four tertiary methyls, a trisubstituted double bond, a secondary hydroxyl, two secondary acetates and a primary-secondary cyclic ether. Irradiation at 2.11 p.p.m. caused the collapse of the two >C<u>H</u>OAc triplets to singlets, indicating that they were attached to C-1 and C-3. The H-5, H-6, H-7 spin system and the cyclic ether were readily identified by spin decoupling and by comparison with vilasinin (31).[21] Confirmation of structure (5) was obtained by acetylation, which afforded the known compound vilasinin triacetate (32).[22]

The extract yielded another tetranortriterpenoid whose spectroscopic properties identified it as 14,15-deoxyhavanensin-3,7-diacetate (7).[11]

We also examined the light petroleum extract of the seeds of C. paniculatus. This proved to be a rich source of tetranortriterpenoids but many were present in small amount. Careful preparative t.l.c. resulted in the isolation of four new compounds, F (12), G (13), H (14) and I (15), whose structures were deduced on the evidence presented below. The known compounds 6α-acetoxynimbinin (6α-acetoxy-14,15-epoxyazadiradione) (11), gedunin (9) and 6α-acetoxygedunin (10) were also obtained.[12-14]

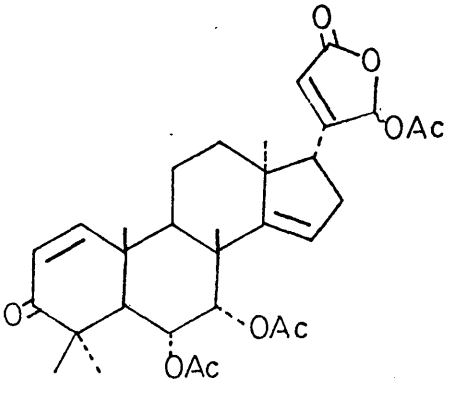
The carbon skeletons of the new compounds were readily identified by their spectroscopic data and by comparison with known compounds. Thus, compound F (12), $C_{30}H_{40}O_7$, [ν_{max} (CCl_4) 1792 (γ-lactone), 1750 (acetate) and 1682 (enone) cm^{-1}] with five tertiary methyl groups, two mutually coupled secondary acetates, a trisubstituted double bond and a Δ^1-3 ketone had a 6α-acetoxyazadiradione skeleton as in (12). The typical furan resonances were absent and were replaced by those of a γ-lactone ring. The position of the lactone carbonyl group (C-23) was established by the ^1H n.m.r. spectrum, which showed the two H-21 protons as a triplet (J 9 Hz) at δ_H 3.91 and a doublet of doublets (J 9,8 Hz) at δ_H 4.46 p.p.m. respectively. These collapsed to an AB quartet (J 9Hz) on irradiation at δ_H 2.76 (H-20). During the course of this work the X-ray structure of a tetranortriterpenoid (33), with the same γ-lactone side chain, from Cneorum tricoccum, appeared.[23] The reported data for the two H-21 protons [δ_H 4.42 (dd, J 9,8 Hz) and 3.90 (t, J 9 Hz)] compare well with those above.



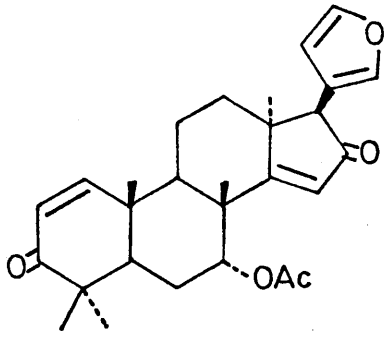
(33)

It was apparent from their spectroscopic properties that compounds G (13), $C_{30}H_{42}O_7$, and H (14), $C_{30}H_{38}O_8$, had the same carbon skeleton as F (12) and differed only in the nature of the degraded side chain. Compound G (13) was unstable and difficult to characterise. The ^{13}C n.m.r. spectrum indicated that it was a mixture. The presence of a carbon resonance at δ_C 97.7(d) suggested a cyclic hemiacetal as in (13). The equilibration at C-23 in such a system could account for the multiple nature of the ^{13}C n.m.r. spectrum. The presence of the hemiacetal function was established by Jones oxidation of (13) to give, in good yield, the γ-lactone (12) described above.

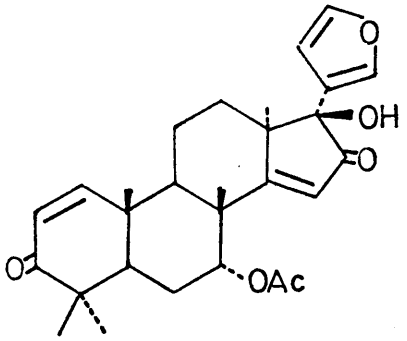
Compound H (14) was insoluble in chloroform and was converted to the corresponding acetate (34) whose spectroscopic properties revealed a β-substituted butenolide [δ_H 6.02 (s, H-22), δ_C 169.0 (s, C-23), 120.3 (d, C-22) and 166.5 (s, C-20)] and a secondary hemiacetal acetate [δ_H 6.84 (s, H-21), δ_C 93.3 (d, C-21)]. These features were accommodated in a γ-acetoxybutenolide side chain as in (34). Several examples of this type have been reported recently.[24] The γ-hydroxybutenolide presumably arises by oxidation of the furan ring and the possibility that it is an artefact has not been excluded. On the other hand, the γ-lactone, at the same oxidation level as the furan, and the hemiacetal, at a lower oxidation level, may represent intermediate stages in the formation of the furan from the intact side chain eg. of (2).



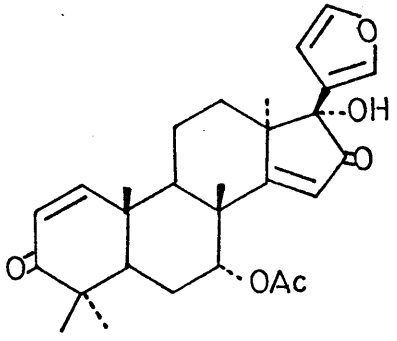
(34)



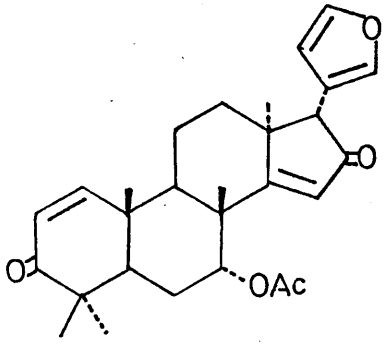
(35)



(36)



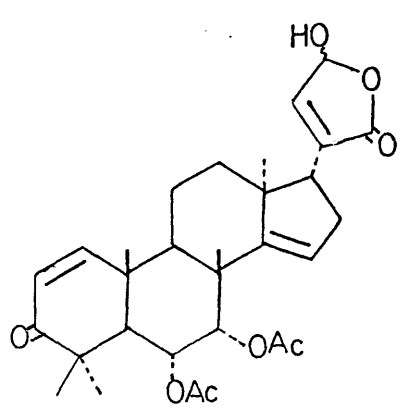
(37)



(38)

Compound I, $C_{30}H_{36}O_8$, was readily assigned the structure (15), 17β-hydroxy-6α-acetoxyazadiradione. It lacked the typical H-17 resonances in its ^1H and ^{13}C n.m.r. spectra and had instead a tertiary hydroxyl group [ν_{max} (CCl_4) 3590 cm^{-1}; δ_H ca. 2.5 (exchangeable with D_2O); δ_C 80.8(s)]. There was no direct evidence for the configuration at C-17. However, during the course of this work Kraus and Cramer reported[25] the isolation of 17-epi-azadiradione (35) and 17β-hydroxyazadiradione (36). Previously Voelter and his colleagues had published[26] a different 17-hydroxy compound without evidence for the configuration at C-17. Therefore this must be 17α-hydroxyazadiradione (37). An examination of the ^{13}C n.m.r. spectra of these compounds revealed that the signal for C-20 shifted from δ_C 118.4 in azadiradione (38) to δ_C 123.6 in 17-epi-azadiradione (35) ($\Delta\delta$5.2) and similarly from δ_C 122.6 in 17β-hydroxy-azadiradione (36) to δ_C 129.0 in Voelter's compound ($\Delta\delta$ 6.4). The corresponding C-20 resonance in our compound appeared at δ_C 122.4, suggesting that it was 17β-hydroxy-6α-acetoxyazadiradione (15).

Several other compounds with the 6α-acetoxyazadiradione skeleton and modified C_4 side chains, including the alternative γ-hydroxybutenolide (39), were present in the seed extract. The most interesting had an aldehyde resonance at δ_H 9.99 (1H,s) and an AB quartet (J 5.5 Hz) at δ_H 6.12 and 6.52. Unfortunately, lack of material prevented full characterisation of these minor constituents.



(39)

Table 1

^{1}H n.m.r. spectra[a] of compounds from C. paniculatus.

	(2)	(3)	(4)	(5)	(6)
H-1				4.92 (t,3)	
H-3		4.64 (t,3)	4.63 (t,3)	4.68 (t,3)	3.40br (t,3)
H-5				2.65 (d,10)[b]	
H-6				4.15 (3,10)[b]	
H-7	3.94br (s)	3.90br (s)	3.90 br (s)	4.20 (3)[b]	3.91br (s)
H-15	5.48 (t,3)	5.49 br (t)	5.47br (s)	5.61br (t,3)	5.47 (t,3)
H-21	6.23 (d,4)	3.42, 3.95 (ABq,12)	5.29br (m)	7.35	6.24 (d,4)
H-22				6.27	
H-23	3.90 (m)	3.85 (m)	4.50 (m)	7.23	3.90 (m)
H-24	2.64 (d,7)	2.87 (d,9)	3.27 (m)		2.66 (d,7)
H-28				3.58 (2H,s)	
OAc	2.04	2.05	2.05	1.98	2.03
				2.01	
C-Me	0.99	0.84	0.85	0.85	0.82
	1.03	0.88	0.90	0.97	0.87
	1.03	0.88	0.90	1.11	0.92
	1.09	1.00	1.06	1.19	1.01
	1.09	1.08	1.06		1.03
	1.26	1.25	1.24		1.26
	1.31	1.28	1.28		1.30

a. Chemical shifts in p.p.m. downfield from internal Me_4Si; solvent $CDCl_3$; multiplicities and coupling constants (Hz) in parentheses.

b. ABX system, J values by first order analysis.

Table 1

^{1}H n.m.r. spectra[a] of compounds from C. paniculatus

	(12)	(13)	(39)	(15)
H-1	7.12(d,10)	7.12(d,10)	7.09(d,10)	7.13(d,10)
H-3	5.93(d,10)	5.92(d,10)	5.92(d,10)	5.96(d,10)
H-5	2.45(d,13)	2.49(d,13)	2.50(d,13)	2.54(d,13)[b]
H-6	5.40(m)	5.40(m)	5.40(m)	5.50(3,13)[b]
H-7	5.40(m)	5.40(m)	5.40(m)	5.58(3)[b]
H-15	5.40(m)	5.40(m)	5.40(m)	5.80(s)
H-21	3.94(t,9) 4.47(dd,8,9)	3.44(t,9) 4.10(m)	6.86br(s)	7.58
H-22			6.01 br(s)	6.40
H-23				7.46
H-24				
H-28				
OAc	1.99 2.03	1.96 2.02	1.99 2.02 2.17	1.98 2.04
C-Me	1.02 1.16 1.16 1.25 1.27	1.00 1.15 1.15 1.24 1.24	0.93 1.16 1.16 1.23 1.30	0.96 1.19 1.23 1.27 1.45

a. Chemical shifts in p.p.m. downfield from internal Me_4Si; solvent $CDCl_3$; multiplicities and coupling constants (Hz) in parentheses.

b. ABX system, J values by first order analysis.

Table 2

^{13}C n.m.r. spectra[a] of compounds from C.paniculatus.

Carbon No.	(2)	(3)	(4)	(6)	(5)	(12)
1	38.5	33.2	33.2	32.5	71.8	156.9
2	33.9	22.8	22.7	25.0	27.7	126.3
3	217.2	78.1	78.1	76.1	72.3[b]	204.4
4	46.9	36.2[b]	36.1[b]	37.0[b]	39.2[b]	40.8
5	46.5	41.9[b]	41.8[b]	40.5[b]	39.6	48.0
6	24.9	23.6	23.6	23.7	74.0	69.8
7	71.9	72.5	72.2	72.3	72.9[b]	74.4
8	44.0	44.5[b]	44.3[b]	44.4[b]	45.8[b]	42.9
9	40.8	41.7[b]	41.5[b]	41.5[b]	33.7[b]	37.0
10	37.1	37.5	37.6	37.7	42.3[b]	44.9
11	16.3[bc]	16.4[c]	16.3[c]	16.3[cd]	15.2[c]	16.3[b]
12	32.3[bc]	34.1[c]	33.2[c]	32.5[cd]	33.0[c]	33.4[b]
13	46.5	46.7	46.6	46.7	47.4	46.5
14	161.5	162.4	162.1	162.2	159.9	158.1
15	119.6[c]	119.8[c]	119.6[c]	119.2[d]	120.7[c]	119.5[b]
16	35.1[c]	34.8[c]	35.0[c]	35.0[d]	34.4[c]	33.9[b]
17	52.6	52.2	52.6	52.5	51.6	58.1
20	44.2	35.9	44.7	44.3	124.5	37.4
21	96.6[b]	70.1	96.5	96.7[c]	139.7	72.4
22	31.3[b]	36.4	30.3	31.4[c]	111.1	34.8
23	79.7	86.5	78.4	79.7	142.6	176.4
24	66.7	64.5	75.2	66.7	-	-
25	57.1	74.1	73.7	57.1	-	-
28					77.9	
C-Me	27.2	28.5	27.8	28.0	-	31.6
	26.2	27.9	27.6	27.9	-	26.8
	24.9	27.6	26.6	24.9	-	20.7
	21.1	24.0	26.6	22.1	26.2	20.4
	19.7	21.8	21.8	19.5	21.2	20.1
	19.3	19.2	19.8	19.3	19.5	-
	14.9	15.2	15.1	15.2	15.4	-
MeCO	21.5	21.4	21.4	21.5	21.2	21.3
					21.2	20.9
MeCO	170.0	171.0	171.0	169.9	170.3	170.2
					170.0	170.0

a. Chemical shifts in p.p.m. downfield, from internal Me_4Si; solvent $CDCl_3$.

b,c,d. These assignments may be interchanged.

Carbon No.	(13)	(39)	(15)
1	157.2	156.6	156.3
2	126.2	126.4	126.6
3	204.6	204.3	204.1
4	40.8	40.7	40.8
5	48.0	48.0	48.0
6	69.9	69.7	69.4
7	74.5	74.3	73.7
8	42.9	43.2	45.0
9	37.0	37.0	37.5
10	44.9	44.9	45.0
11	16.4	16.4[b]	15.8
12	33.2	33.4[b]	22.1
13	46.6	47.5	50.2
14	158.1	157.7	191.9
15	119.7	119.4[b]	120.5
16	35.3	33.0[b]	205.5
17	58.9; 58.2	52.8	80.8
20	37.4	166.5	122.4
21	72.0; 70.4	93.3	142.9
22	39.8	120.3	109.5
23	97.7; 98.3	169.7	141.6
Me	31.6	31.6	31.6
	26.8	26.9	31.1
	20.7	21.3	24.6
	20.4	20.7	20.8
	19.9	20.4	20.4
MeCO	21.3	21.3	21.2
	20.9	20.9	20.8
MeCO	170.2	170.2	170.2
	170.1	170.0	169.7
		169.0	

Assignment of ^{13}C n.m.r. Resonances.- Assignments are based on chemical shift rules, multiplicities in off-resonance-decoupled spectra, correlation with ^{1}H chemical shifts using two off-resonance-decoupled spectra and comparison with published data for similar compounds. Signals at lower field than 60 p.p.m. are easily assigned by these means. Of the quaternary carbons C-13 shows a small residual long range coupling with H-15 in the off-resonance-decoupled spectra (irradiating at 0 p.p.m.). In compounds (2), (3), (4), and (6) C-8 and C-10 are essentially unchanged, C-10 being assigned by comparison.[27] C-4 shows a 10 p.p.m. upfield shift from (2) to (6) and a further small high field acetylation shift in (3) and (4). In (12), (13), (34), and (15) the assignments of C-4 and C-10 are reversed with respect to azadiradione derivatives[25] because of the γ-gauche and anti effects of the 6α-OAc substituent. C-8 varies slightly as the C-17 substituent is changed.

Of the methine carbons C-17 is assigned by its absence in (15). C-5 moves upfield from (2) to (6) on the introduction of a γ-gauche hydroxyl group and C-9 remains unchanged.[28] Because of the 6α-OAc, C-5 is expected to be at lower field in (12), (13), (34), and (15) than in (2) (cf. ref.25). The remaining methine is C-20 in the variable C-17 substituents.

Of the methylene carbons C-11 is at highest field. The resonances at ca. 33 p.p.m. in (34) must be C-12 and C-16; the other assignments for (12), (13) and (15) then follow. For (2) and (6) C-12, C-16, and C-22 are invariant. C-1 and C-2 in (2) are assigned by comparison[27] and both move appreciably to higher field in (6). The assignment of C-6 then follows and does not change in (3) and (4). C-1 and C-2 show acetylation shifts from (6) to (3) and (4).

The methyl carbons are not assigned with the exception of the acetates,

which are recognised by larger residual coupling in the off-resonance-decoupled spectra.

The assignments for (5) are made by comparison as far as possible, but suitable model systems are not available. The spectrum for (13) shows doubling and reduction in intensity of some resonances associated with the substituent at C-17 and it is possible that not all relevant peaks are resolved.

GENERAL EXPERIMENTAL

All melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded on Varian X.L.-100 or Perkin-Elmer R-32 spectrometers using tetramethylsilane as internal reference in deuteriochloroform. Proton noise decoupled pulsed FT ^{13}C n.m.r. spectra with $\leq$ 1.52 Hz per data point were obtained at 25.2 MHz on a Varian XL-100 spectrometer, operated by Dr. D.S. Rycroft, for solutions in $CDCl_3$ at room temperature (ca 25°C), unless otherwise stated. Shifts are given as positive downfield (p.p.m.) from internal tetramethylsilane. Assignments are based on chemical shift rules, multiplicities in off-resonance-decoupled spectra, correlation with ^{1}H chemical shifts using two off-resonance-decoupled spectra , and by comparison with published data for similar compounds.

Ultraviolet absorption spectra were measured in ethanol or methanol solutions using a Unicam S.P. 800 spectrometer. Infra-red solution spectra were recorded by Mrs. F. Lawrie and staff, on a Perkin-Elmer 580 or on a Perkin Elmer 225 instrument using carbon tetrachloride as solvent, unless otherwise stated.

Mass spectra were routinely determined by Mr. A. Ritchie and staff on an A.E.I.-G.E.C. M.S.-12 mass spectrometer, high resolution spectra being obtained on an A.E.I. M.S.-902s instrument. Micro-analyses were carried out by Mrs. W. Harkness and staff. Circular dichroism spectra were recorded by Dr. P.M. Scopes, Westfield College, London.

Chromatographic separations were effected using commercial "Woelm" alumina for column chromatography and Merck's "Kieselgel G" for analytical and preparative t.l.c. Light petroleum refers to the fraction of b.p. 60°-80°C.

EXPERIMENTAL

Isolation.- (a) Wood. Powdered wood (5.4 Kg) of C. paniculatus was continuously extracted with light petroleum in a Soxhlet. The oily extract (41 g) was chromatographed over Grade IV alumina (1 Kg) in light petroleum. The initial fractions eluted with increasing proportions of chloroform in light petroleum yielded β-sitosterol (8) (4 g). The fractions eluted with increasing proportions of ethyl acetate in chloroform crystallised on addition of ether-light petroleum and afforded, in increasing order of polarity, compounds A, B and C. Compound A (2) (700 mg) had m.p. 209°- 211°C (ex. ether-methanol), m/e 510 (M^+-18). (Found: C, 72.6 ; H, 9.25. $C_{32}H_{48}O_6$ requires C, 72.7 ; H, 9.1%). Compound B (3) (300 mg) was crystallised from ether-light petroleum and had m.p. 204°- 206°C, m/e 514 (M^+-18). (Found: C, 72.0 ; H, 10.0. $C_{32}H_{52}O_6$ requires C, 72.15 ; H, 9.85%). Compound C (4) (600 mg) had m.p. 145°- 150°C (ex. chloroform-ether), m/e 530 (M^+-18). (Found: C, 70.0 ; H, 9.2. $C_{32}H_{52}O_7$ requires C, 70.0 ; H, 9.5%). Preparative t.l.c. of the mother liquors of A (2), using ethyl acetate-carbon tetrachloride (6:4) gave compound D (5) (vilasinin 1,3-diacetate) (200 mg) crystallised from methanol, m.p. 128°-131°C [m/e 512 ; ν_{max} (CCl_4) 3560 and 1735 cm^{-1}]. (Found: C, 70.3 ; H, 8.1. $C_{30}H_{40}O_7$ requires C, 70.3 H, 7.9%). Preparative t.l.c. of the mother liquors of B (4), afforded compound E (6) as a gum [m/e 470 (M^+-60)]. Extraction of a minor band on the plate gave the known compound 14,15-deoxyhavanensin-3,7-diacetate (7) (8 mg) whose ^1H n.m.r. spectrum accorded with reported data.[11]

(b) Seeds. Ground seeds (500 g) of C. paniculatus were extracted with light petroleum in a Soxhlet. The oily extract (180 g), obtained on removal of the solvent under reduced pressure, deposited a solid (5.5 g) on

treatment with light petroleum. This solid was chromatographed on Grade IV alumina eluting with increasing amounts of ethyl acetate in chloroform. The early fractions contained mainly fat (2 g). The later fractions showed many spots on analytical t.l.c. Multiple preparative t.l.c. using ethyl acetate-carbon tetrachloride (3:7) and methanol-chloroform (1:9) afforded the following compounds:

(a) Compound F (12) (42 mg) m.p. 236°- 240°C (ex. chloroform - ether), m/e 512. (Found: C, 70.45 ; H, 7.8. $C_{30}H_{40}O_7$ requires C, 70.3 ; H, 7.8%).

(b) Compound G (13) (40 mg) as a gum. This compound could not be characterised as it was present as a mixture in equilibrium. Jones oxidation under usual conditions (10 drops, 0°C) afforded a product identical with compound F (12) (^{1}H n.m.r., m.p., m.m.p., analytical t.lc.) in good yield.

(c) Compound H (14) (120 mg) as an insoluble powder [m/e 508 (M^+-18)]. Acetylation with pyridine-acetic anhydride at r.t. (1 min), afforded a mixture from which the major component, the acetate (34) (60 mg) was obtained by preparative t.l.c. [m/e 568 (M^+)].

(d) Compound I (15) (17β-hydroxy-6α-acetoxyazadiradione) (18 mg) m.p. 288°- 292°C (ex. methanol-ether-light petroleum) [m/e 524 ; ν_{max} (CCl_4) 3590, 1752, 1720, 1682 cm^{-1}]. (Found: 524.24071 (M^+). $C_{30}H_{36}O_8$ requires (M^+) 524.24099).

The known compounds, 6α-acetoxynimbinin (11) (6α-acetoxy-14,15-epoxyazadiradione)[14] (18 mg), m.p. 167°-169°C ; gedunin (9)[12] (20 mg) m.p. 216°- 220°C and 6α-acetoxygedunin (10)[13] (40 mg), m.p. 270°- 274°C were also isolated and readily identified spectroscopically.

The more polar fractions (2.8 g) of the column could not be

separated and were acetylated with acetic anhydride in pyridine on the steam bath for ½ h. Analytical t.l.c. indicated the presence of at least ten compounds. Lack of material prevented full characterisation.

Acetylation Reactions.- Acetates were prepared by treatment of the alcohols with acetic anhydride in pyridine on the steam bath for ½ h.

Compound A Acetate (17) was not obtained crystalline [m/e 510 (M^+-60); δ_H 1.02 (6H), 1.04 (6H), 1.14, 1.29, 1.33 (C-methyls), 1.95, 2.06 (OAc's), 2.68 (1H,d, J 7 Hz, H-24), 3.95 (1H,m, H-23), 5.26 (2H,m, H-7 and H-15), 6.27 (1H,d, J 3 Hz, H-21)].

Compound B (3) afforded a mixture which was separated by preparative t.l.c. using ethyl acetate-carbon tetrachloride (3:1), to give the diacetate (26) m.p. 213°- 215°C (ex. chloroform-ether) [m/e 556 (M^+-18) ; ν_{max} (CCl_4) 3575, 1737 cm^{-1} ; δ_H 0.83, 0.86 (6H), 0.98, 1.06, 1.14 and 1.17 (C-methyls), 2.01 and 2.06 (-OAc's), 3.16 (1H,d, J 9 Hz, H-24), 3.58 and 4.04 (b ABq, J 12 Hz, 2H-21), 3.93 (t, J 3 Hz, H-7), 4.67 (t, J 3 Hz, H-3), 4.98 (m, H-23), 5.49 (bd, J 3 Hz, H-15)] and the triacetate (27) (sapelin D triacetate[19]) m.p. 160°- 162°C (ex. chloroform-ether) [m/e 598 (M -18) ; ν_{max} (CCl_4) 3575, 1732, 1737 cm^{-1} ; δ_H 0.73, 0.86, 0.88, 1.10, 1.14 and 1.17 (C-methyls), 1.94, 2.00 and 2.07 (OAc's), 3.16 (d, J 9 Hz, H-24), 3.56 and 4.04 (b ABq, J 12 Hz, 2H-21), 4.66 (t, J 3 Hz, H-3), 4.97 (m, H-23), 5.16 (t, J 3 Hz, H-7), 5.29 (bd, J 3 Hz, H-15)].

Compound C (4) yielded, after preparative t.l.c., the triacetate (28) as a gum [m/e 572 (M^+-60) ; δ_H 0.81, 0.86 (6H), 1.03 (6H), 1.25, 1.29 (C-methyls), 2.05 (6H), 2.13 (OAc's), 3.91 (t, J 3 Hz, H-7), 4.51 (m, H-23), 4.65 (t, J 3 Hz, H-3), 4.79 (d, J 4 Hz, H-24), 5.48 (bt, J 3 Hz, H-15), 6.08 (d, J 3 Hz, H-21)].

Compound D (5) afforded the known triacetate (32), m.p. 220°C, identified by its spectroscopic data.[22]

Compound E (6) gave the non-crystalline acetate (30) [m/e 512 (M^+ - 60), δ_H 0.81, 0.86 (6H), 1.03 (6H), 1.25, 1.29 (C-methyls), 2.03, 2.05 (OAc's), 2.65 (d, J 7 Hz, H-24), 3.91 (2H, m, H-7 and H-23), 4.65 (t, J 3 Hz, H-3), 5.48 (bt, H-15), 6.25 (d, J 3 Hz, H-21)].

Tetranortriterpenoid (18)[16] Compound A (2) (870 mg) in tetrahydrofuran (150 ml) was treated with sodium periodate (2 g) in water acidified with 70% perchloric acid (3 drops). The solution was stirred at r.t. for 27 h. The precipitated sodium iodate was filtered off and washed with tetrahydrofuran. Sodium bicarbonate (100 mg) was added, the solvent removed *in vacuo* and an excess of water added. Extraction with chloroform yielded a yellow gum which was dissolved in benzene (100 ml) and refluxed for 2 h with toluene p-sulphonic acid (1 mg). The product was chromatographed on Grade IV alumina in ether-light petroleum. The fractions eluted with ether afforded the known furanoid tetranortriterpenoid (18)[16] (400 mg) which was crystallised from methanol-ether and had m.p. 175°- 177°C.

Diene (20) Tetranortriterpenoid (18) (140 mg) in pyridine was treated with thionyl chloride (10 drops) at ice temperature for ½ h. The reaction mixture was poured into ice-water and extracted with chloroform. The product was chromatographed over Grade IV alumina in light petroleum-ether to give the crystalline diene (20) (28 mg) m.p. 180°- 182°C (ex-methanol) m/e 378 (M^+) ; [δ_H 0.80, 0.93, 1.04, 1.08, 1.18 (C-methyls), 5.45 (bd, J 10 Hz), H-7), 5.50 (obsc t, H-15), 6.05 (dd, J 3,10 Hz, H-6), 6.25, 7.22, 7.32 (furan protons)].

Diol - monoacetate (22) Diene (20), in ether-pyridine, was reacted with excess osmium tetroxide and the reaction left in the dark for 24 h.

Preparative t.l.c. of the crude product yielded the non-crystalline diol (21),[δ_H 0.7, 0.97, 1.03, 1.1, 1.21 (C-methyls), 4.27 (dd, J 8,6 Hz, H-15), 5.77 (2H,bs, H-6 and H-7), 6.22, 7.15, 7.32 (furan protons)], which was acetylated on the steam bath for 1 h to give the non-crystalline monoacetate (22) [δ_H 0.72, 0.93, 1.02, 1.08, 1.2 (C-methyls), 2.08 (OAc), 5.32 (t, J 8 Hz, H-15), 5.70 (2H,bs, H-6 and H-7), 6.23, 7.15, 7.32 (furan protons)].

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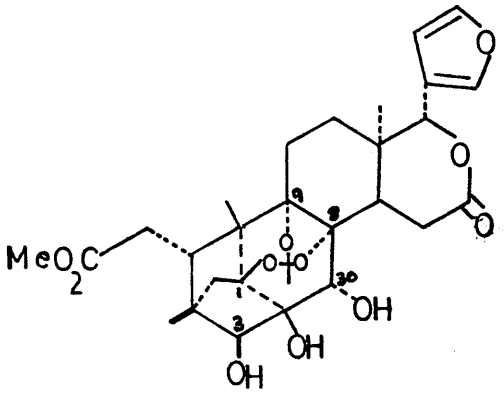
CHAPTER III

TETRANORTRITERPENOIDS FROM THE SEEDS OF CHUKRASIA TABULARIS



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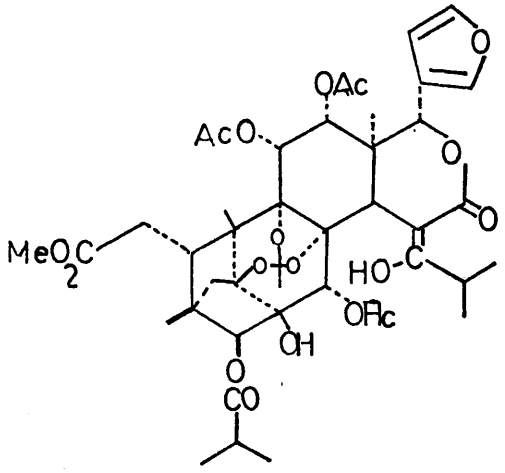

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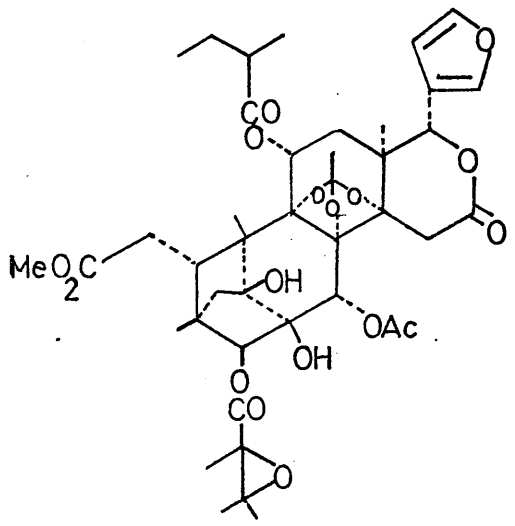

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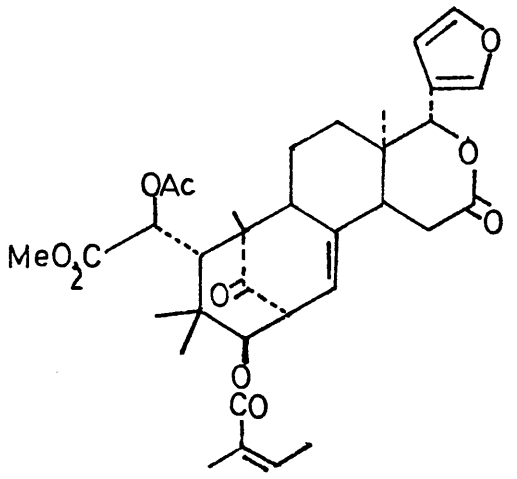
INTRODUCTION

Chukrasia tabularis (Meliaceae) is native to the tropical regions of Asia, mainly India, where it is an important timber tree. It belongs to the Swieteniae tribe which also includes the closely related Entandrophragma, Swietenia, Khaya, Soymida and Lovoa.[1]

Chemical studies of this tribe have been mainly concerned with the tetranortriterpenoids produced by Khaya[2] and Entandrophragma[3] species. The other genera (only one or two species examined) afforded limonoids with a ring B-cleaved skeleton[4-6], similar to those found in Khaya species. Entandrophragma, on the other hand, has aroused particular interest, as all the species examined contained a novel type of limonoid with an unusual bicyclononanolide skeleton, represented by phragmalin[7] (1) and bussein[8] (2). The most striking features of this type of compound are the orthoacetate [at C-1,8,9 in (2), or at C-8,9,14 in utilin (3)[9]], and the methylene bridge between C-1 and C-4. Biogenetically they can arise from a precursor like swietenine (4)[5], by a series of oxidations to produce the orthoacetate and by cyclisation of the 4α-methyl group with the C-1 ketonic carbonyl to form the methylene bridge. The mechanism of this cyclisation is unknown though it may be radical in character.[10] Neither of these features has been found to occur in simpler bicyclononanolides.

It was of interest to examine the related species, Chukrasia tabularis, and isolate, if possible, more compounds belonging to this unique group, and thus shed some light in the processes involved in their formation from simpler limonoids. In this chapter we report the isolation from the seeds of C. tabularis, of four closely related compounds with a phragmalin skeleton. We shall discuss the chemical and spectroscopical evidence



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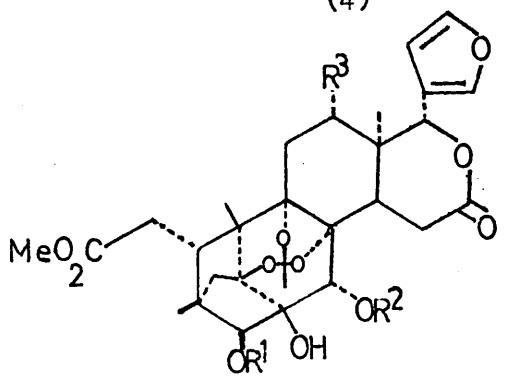
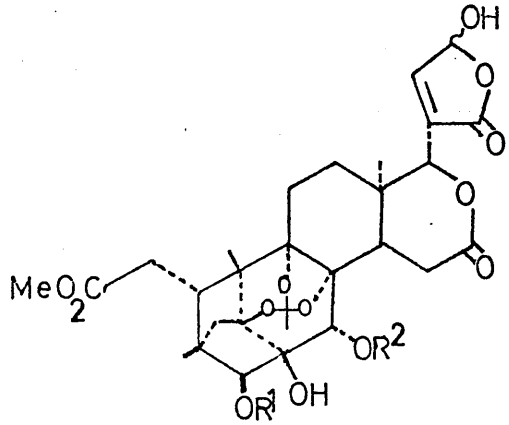


(5) $R^1 = R^2 = Pr^iCO$; $R^3 = H$

(6) $R^1 = Pr^iCO$; $R^2 = EtCO$; $R^3 = H$

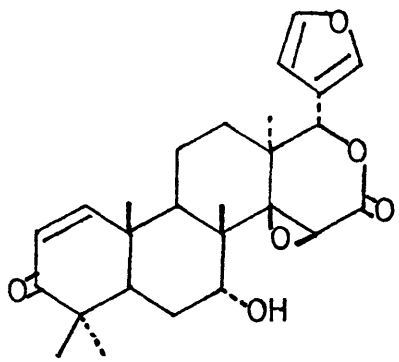
(7) $R^1 = R^2 = Pr^iCO$; $R^3 = Ac$

(8) $R^1 = Pr^iCO$; $R^2 = EtCO$; $R^3 = Ac$



(9) $R^1 = R^2 = Pr^iCO$

(10) $R^1 = Pr^iCO$; $R^2 = EtCO$



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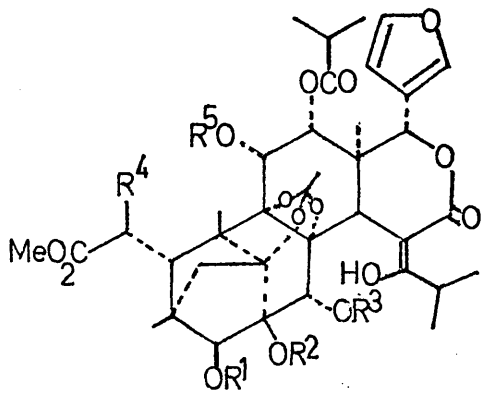


(12) R^1 = ⟩—CO ; R^2 = H ; R^3, R^5 = Ac + ⟩—CO ; R^4 = OH

(13) R^1 = ⟩—CO ; R^2 = H ; R^3, R^5 = Ac + ⟩—CO ; R^4 = H

(14) R^1 = Ac ; R^2 = R^4 = H ; R^3 = R^5 = ⟩—CO

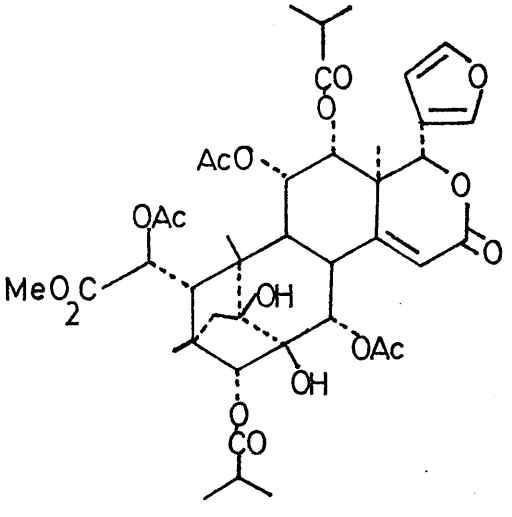
(15) R^1 = Ac ; R^2 = R^4 = H ; R^3, R^5 = ⟩—CO + Ac

(16) R^1 = Ac ; R^2 = Ac ; R^3, R^5 = Ac + ⟩—CO ; R^4 = H

(17) R^1 = Ac ; R^2 = Ac ; R^3, R^5 = ⟩—CO ; R^4 = H

that led us to assign structures (5) - (8) to them. We have also isolated two compounds (9) and (10) with a modified furan ring (γ-hydroxybutenolide). These are obviously formed from the furan derivatives (5) and (6) respectively. Also present in the extract was the known substance 7-deacetoxy-7-hydroxygedunin (11).[11]

Soon after this work was published[12], Taylor[13] and Tamm[14] published independently the isolation of six new bussein ester-derivatives (12)-(17), from the wood of C. tabularis. The bark was reported[13] to contain a small amount of an interesting compound, tabularin (18), which may represent an intermediate in the biosynthesis of the complex bicyclononanolides since it already has the methylene bridge but lacks the orthoester function.



(18)

D I S C U S S I O N

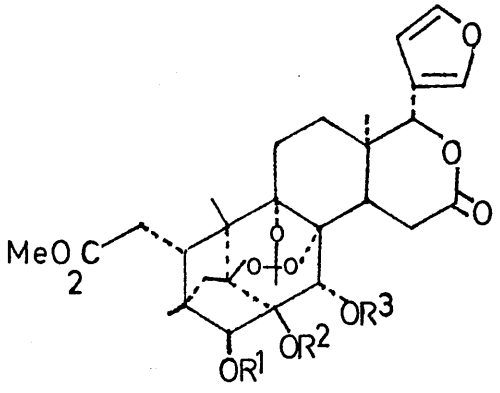
Extraction of the seeds of Chukrasia tabularis (Meliaceae) with light petroleum and concentration of the solution afforded a precipitate containing a complex mixture. The major components were four closely related tetranortriterpenoids, A - D, which were eventually obtained crystalline by extensive preparative t.l.c.

A striking similarity in the spectroscopic properties of compounds A (5), $C_{37}H_{48}O_{13}$, and C (6), $C_{36}H_{46}O_{13}$, (see Tables 1 and 2), suggested that they had the same tetranortriterpenoid skeleton and differed only in the nature of the esters attached to it. Thus, both had in the ^1H n.m.r. spectra signals corresponding to a β-substituted furan ring, a methoxycarbonyl group, a hydroxyl (δ_H 2.65 in A, δ_H 2.71 in C, exchangeable with D_2O), an orthoacetate (δ_H 1.60) and three low field singlets. A difference in the complex methyl region of the spectra, otherwise identical, supported the assumption of the presence of different ester groups in A and C. Additional information about the nature of the skeleton was obtained by a study of the ^{13}C n.m.r. spectra. A characteristic low field singlet at δ_C 119.0 was assigned to an orthoacetate, which together with the lack of signals for an exomethylene group, suggested a bicyclononanolide skeleton for these compounds. The off-resonance spectra on the other hand, revealed the presence of three carbon doublets at δ_C 83.1, 78.5 and 70.7 whose chemical shift indicated that they were attached to oxygen. They were correlated with the low field singlets present in the ^1H n.m.r. spectra, and attributed to H-17 and two other protons attached to esterified positions. The carbon atoms involved in the formation of the orthoacetate group, gave rise to three singlets in the 85-87 p.p.m. region. An extra singlet in this part of the spectrum

was then allocated to the C-OH group, which had therefore to be tertiary.

The positions of attachment of the orthoacetate were decided by a comparison of the structures of phragmalin[7] (1) and utilin[9] (3). We favoured a phragmalin-type of skeleton as it can accommodate two secondary ester groups next to fully substituted positions, to account for the observed multiplicity of the ^{1}H n.m.r. signals. Such positions are not available in utilin, as the hydroxyl group has to be at C-1, for biogenetic requirements.

Definitive proof for these assignments was obtained when alkaline hydrolysis of A (5) and C (6) afforded the same compound, identified as phragmalin (1), by comparison with an authentic specimen and by conversion to the known mono-, di-, and tri-acetates (19)-(21).[7] The nature of the



(19) R^1 = Ac ; R^2 = R^3 = H

(20) R^1 = R^2 = Ac ; R^3 = H

(21) R^1 = R^2 = R^3 = Ac

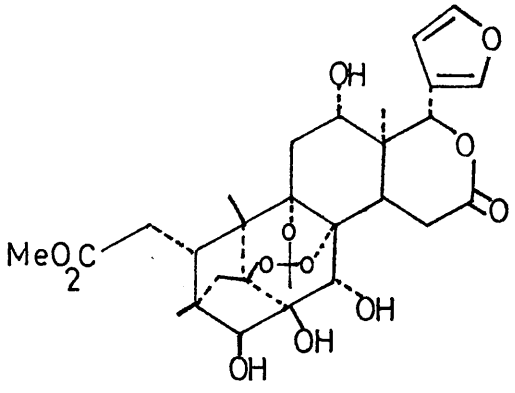
attached ester groups was elucidated in several ways. First, ^{1}H n.m.r. examination of the volatile acids released on hydrolysis[15], showed that compound A (5) gave rise to 2 mol. equiv. of isobutyric acid, whereas C (6) gave 1 mol. equiv. each of isobutyric and propionic acids. Second-

ly, the mass spectrum of A showed a characteristic cleavage for an isobutyrate (m/e 71) whereas C had peaks for cleavage of both, isobutyrate and propionate (m/e 57). These findings were confirmed by the ^{13}C n.m.r. spectra of A and C. The spectrum of C (see Table 2) had an unusually high methyl signal at δ_C 8.62, characteristic of a propionate ester, and a doublet at δ_C 27.7 for the methylene carbon (Me$\underline{CH_2}$CO). These signals were not present in the spectrum of A, which had instead an intense peak at δ_C 34.4, corresponding to the methine carbon of two isobutyrates. The chemical shifts of the remaining ester carbons are listed in Table 2. From this evidence it follows that A is phragmalin-3,30-diisobutyrate (5) and C is the corresponding isobutyrate-propionate derivative.

The question of the position of attachment of the propionate in C is less easily settled. We favoured C-30 on the basis of the ^{13}C frequencies for the carbonyl carbons in the spectra of the series of compounds in Table 2. From the data obtained for the acetate derivatives of phragmalin, (19), (20) and (21), it follows that the carbonyl carbon of an ester group attached to C-30 resonates at higher field than that in the corresponding C-3 ester. This difference is enhanced by the substitution effect on going from isobutyrate to propionate[16] [c.f. (5) and (6)]. These assignments are consistent with structure (6), phragmalin-3-isobutyrate-30-propionate for compound C.

The spectroscopic properties of compound B (7), $C_{39}H_{50}O_{15}$, and D (8), $C_{38}H_{48}O_{15}$, were very similar to those of A (5) and C (6) (see Tables 1 and 2) suggesting the same phragmalin skeleton. Both compounds had an additional >CHOAc group [δ_C 69.1(d); δ_H 4.60 (B) or 4.66 (D) (dd, J 4, 12 Hz)] relative to A and C, and an acetate methyl resonance at abnormally high field (δ_H 1.66). These data require a 12α-acetate group since in this position it becomes shielded by the furan ring[17,9], as reported for

bussein[8] (2), where it appears at δ_H 1.63. The ^{13}C n.m.r. spectra of B and D supported this assignment. Introduction of an oxygen substituent at C-12 caused a downfield shift (relative to A and B) of the signals due to C-12 (40 p.p.m.), C-11 (6.5 p.p.m.) and C-13 (4.5 p.p.m.) and an upfield shift of those due to C-18 (5.5 p.p.m.) and C-17 (2 p.p.m.). Hydrolysis of both D and B afforded 12α-hydroxyphragmalin (22), which has not been described previously. The nature of the ester substituents

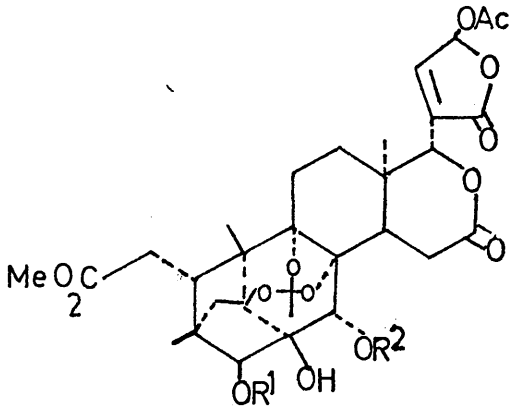


(22)

in B and D was elucidated as before, by a study of the volatile acids released on hydrolysis, the mass spectral fragmentation and the ^{13}C chemical shifts. In this case, hydrolysis afforded 1 mol. equiv. of acetic acid, in addition to isobutyric (B and D) and propionic acids (D). Thus, B is 12α-acetoxyphragmalin-3,30-diisobutyrate (7) and D is 12α-acetoxyphragmalin-3-isobutyrate-30propionate (8).

Column chromatography of a sample of the residual light petroleum extract afforded a polar fraction from which a crystalline mixture of two compounds was obtained. They were separated, with difficulty, by preparative t.l.c. The ^1H n.m.r. spectra of E (9) and F (10) were virtually identical with those of compounds A (5) and C (6) respectively, except for the absence of the characteristic β-substituted furan ring resonances.

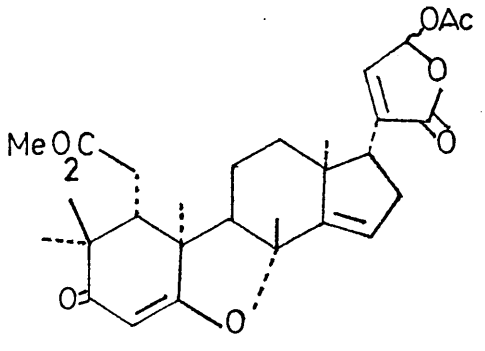
This suggested that the furan ring had undergone oxidative modification.[4] Both E and F were acetylated almost instantaneously at r.t. with pyridine-acetic anhydride to give the monoacetates (23) and (24) respectively.



(23) $R^1 = R^2 = Pr^iCO$

(24) $R^1 = Pr^iCO$; $R^2 = EtCO$

The presence in the acetate (23) of an α-substituted α,β-unsaturated carbonyl system [δ_C 176.6 (C-21), 135.4 (C-20) and 147.2 (C-22); δ_H 7.28 (bs, H-22)] and a secondary carbon attached to two oxygen atoms [δ_C 92.1 (C-23); δ_H 6.91 (H-23, downfield shift from δ_H 6.36 in (9)] indicated a γ-acetoxybutenolide. Thus, compounds E and F have structures (9) and (10) respectively. Tetranortriterpenoids with this type of oxidatively modified furan ring are well known[4] and the spectroscopic values quoted above and in Table 2 correspond well with literature values for compounds with the butenolide carbonyl group at C-21, eg., tricoccin S_8 acetate (25)



(25)

[δ_H 6.88 (H-23), 7.0 (H-22)]. The alternative arrangement with the carbonyl group at C-23 is also known but compounds of this type were not isolated from the extract. The corresponding modified furan derivatives of B (7) and D (8) were not detected. While it is possible that such modified furan derivatives may be natural products, it seems likely that, in this case, they are artefacts formed during prolonged standing of the light petroleum extract in light.

Table 1

^{1}H n.m.r. spectra of phragmalin and related compounds.

	(5)	(7)	(6)	(8)	(1)	(19)	(20)	(21)	(22)	(23)
H-21	7.52	7.48	7.52	7.46	7.50	7.52	7.51	7.50	7.64	-
H-22	6.42	6.43	6.46	6.42	6.46	6.42	6.44	6.42	6.58	7.28
H-23	7.42	7.40	7.43	7.42	7.39	7.42	7.40	7.38	7.46	6.91
H-17	5.54	5.59	5.52	5.56	5.58	5.55	5.48	5.53	5.63	5.48
H-30	5.91	6.02	5.88	5.99	4.74	4.56	5.91	6.29	4.68	5.94
H-3	4.66	4.68	4.61	4.59	3.56	4.70	4.64	5.09	3.54	4.65
H-12	-	4.60 (q,4,12)	-	4.66 (q,4,12)	-	-	-	-	4.00 (t,8 Hz)	-
-CO_2Me	3.69	3.72	3.70	3.72	3.69	3.70	3.69	3.69	3.69	3.75
MeCO(O)	1.65	1.60	1.65	1.60	1.61	1.62	1.67	1.70	1.61	1.65
12α-OAc	-	1.66	-	1.65	-	-	-	-	-	-
C-Me[a]	0.90	0.90	0.91	0.92	0.98	0.94	0.93	0.95	0.98	0.94
	1.04	1.12	1.05	1.13	1.04	1.06	1.11	1.11	1.07	1.06
	1.14	1.18	1.13	1.20	1.13	1.14	1.17	1.19	1.13	1.16

a. Skeletal methyls only.

Table 2

^{13}C n.m.r. spectra of phragmalin and related compounds.

Carbon No.	(5)	(7)	(6)	(8)	(1)	(19)	(20)	(21)	(23)
1[a]	87.2	86.1	87.1	86.0	86.7	86.7	87.2	86.9	87.2
2	79.8	80.0	79.5	79.8	78.6	77.8	79.4	85.3	80.0
3[b]	83.1	83.2	83.4	83.8	83.1	83.0	83.4	81.1	82.9
4	45.4	45.4	45.4	45.4	45.8	45.6	45.4	46.2	45.8
5	36.8	35.9	36.7	35.9	35.9	36.9	36.4	35.5	36.5
6	33.3	33.5	33.5	33.5	34.3	33.8	33.5	33.3	34.2
8[a]	86.4	85.7	86.2	85.5	86.4	86.2	86.2	86.0	86.2
9[b]	85.5	85.3	85.5	85.3	84.4	94.1	85.6	85.3	85.8
10	45.3	45.1	45.2	45.1	45.8	45.4	45.3	45.8	45.5
11	25.2	31.7	25.2	31.7	25.3	25.2	25.3	25.4	25.3
12	29.1	69.1	29.2	69.1	29.3	29.0	29.2	29.2	27.9
13	34.4	38.9	34.4	38.7	34.3	34.6	34.4	34.4	35.0
14	42.7	43.8	42.8	42.8	42.5	42.2	42.9	43.2	42.7
15	26.6	26.8	26.6	26.8	27.4	27.2	26.7	26.6	26.3
17	78.5	76.6	78.7	76.9	79.1	78.3	78.8	78.6	77.1
20	121.3	120.9	121.2	120.9	121.6	121.6	121.2	121.2	135.4
21	140.6	141.0	140.5	141.0	140.6	140.3	140.7	140.8	167.6
22	109.7	109.9	109.7	109.9	109.7	109.7	109.7	109.8	147.2
23	142.9	143.0	142.9	143.0	142.7	143.1	143.1	143.0	92.1
29	39.5	39.8	39.5	39.8	39.5	39.6	39.4	40.2	39.5
30	70.7	70.2	70.9	70.3	68.9	69.2	71.2	69.3	70.4
$-CO_2\underline{Me}$	51.9	51.7	51.9	51.7	52.0	52.1	52.0	52.1	52.4
$MeO\underline{C}(O)O$	119.0	119.1	118.9	119.1	118.9	119.1	119.1	119.1	119.0
$\underline{Me}OC(O)O$	21.1	21.1	21.1	21.1	21.3	21.2	21.1	21.1	21.2
18	19.5	14.0	19.6	14.0	20.2	19.9	19.7	19.6	20.0
19[c]	16.3	16.2	16.3	16.2	15.8	16.0	16.2	16.6	16.1
28[c]	14.4	14.0	14.5	14.0	14.8	14.5	14.5	14.6	14.7
$\underline{Me}_2CHCO$	19.5	19.7	19.3	19.5					19.3
	19.3	19.3	18.3	18.4					19.6
	18.2	18.4							18.1
	18.0	17.9							18.0
$Me_2\underline{C}HCO$	34.4	34.6	34.3	34.7					34.6
	34.4	34.6							34.6
$\underline{Me}CH_2CO$			8.6	8.6					
$MeCH_2CO$			27.7	27.9					
$\underline{Me}CO$		20.1		20.1		20.9	21.2	21.7	20.7
							21.2	21.6	
								21.1	
CO at: 7	172.9	172.1	172.9	172.1	173.4	172.9	172.8	172.7	172.5
16	170.1	169.7	170.1	169.7	172.0	171.3	170.8	170.3	169.3
3	176.5	177.3	176.6	177.5		170.3	170.3	170.3	177.0
	174.9	174.9	172.3	172.5			169.0	168.6	175.2
12/2/23		169.2		169.2				170.2	168.7

a,b,c, These assignments may be interchanged.

EXPERIMENTAL

Isolation.- Powdered seeds (3 Kg) of Chukrasia tabularis were extracted with light petroleum in a Soxhlet apparatus. Concentration of the solution resulted in the precipitation of a gummy material (25 g) which was filtered off and chromatographed over alumina (Grade IV) using gradually increasing proportions of chloroform in light petroleum. The fractions eluted with chloroform were combined to give a mixture (10 g) of four compounds, A-D, of similar polarity. They were subjected to careful preparative t.l.c. (multiple runs) using carbon tetrachloride-ethyl acetate (7:3) as solvent. Subsequent crystallisation afforded the following compounds (in increasing order of polarity):

(a) Compound A (5) (phragmalin diisobutyrate) (0.85 g) m.p. 224°- 228°C (needles ex. ether-light petroleum); [ν_{max} (CCl_4) 3578, 1748 cm^{-1}; m/e 682 (M^+-18)]. (Found: C, 63.6; H, 7.1. $C_{37}H_{48}O_{13}$ requires C, 63.4; H, 6.85%).

(b) Compound B (7) (12-α-acetoxyphragmalin diisobutyrate) (0.3 g) m.p. 226°- 229°C (needles ex. ether-light petroleum); [ν_{max} (CCl_4) 3578, 1748 cm^{-1}; m/e 740 (M^+-18)]. (Found: C, 62.0; H, 6.80. $C_{39}H_{50}O_{15}$ requires C, 61.75; H, 6.6%).

(c) Compound C (6) (phragmalin-3-isobutyrate-30-propionate) (0.6 g) m.p. 195°- 200°C (needles ex. carbon tetrachloride-light petroleum); [ν_{max} (CCl_4) 3578, 1748 cm^{-1}; m/e 668 (M^+-18)]. (Found: C, 53.0; H, 5.45. $C_{36}H_{46}O_{13}\cdot CCl_4$ requires C, 53.0; H, 5.5%).

(d) Compound D (8) (12-α-acetoxyphragmalin-3-isobutyrate-30-propionate) (0.5 g) m.p. 214°- 216°C (needles ex. ether-light petroleum); [ν_{max} (CCl_4) 3578, 1748 cm^{-1}; m/e 726 (M^+-18)]. (Found: C, 61.25; H, 6.55. $C_{38}H_{48}O_{15}$

requires C, 61.3; H, 6.45%).

Preparative t.l.c. of later fractions eluted with methanol-chloroform afforded 7-deacetoxy-7-hydroxygedunin (11) (50 mg) m.p. 249°- 255°C, identified by direct comparison (m.p., n.m.r., m.s.) with an authentic sample.[11]

A sample (32 g) of the residual light petroleum extract was chromatographed over SiO_2 in light petroleum eluting with increasing amounts of chloroform in light petroleum. The early fractions containing fatty material were discarded. The later fractions were combined (10.24 g) and rechromatographed over SiO_2 in chloroform. The intermediate fractions contained a mixture of compounds (5)-(8) (4 g). The final fraction (0.5 g) eluted with methanol was plated and the main band crystallised from methanol-ether-light petroleum to give a mixture m.p. 242°- 248°C of compounds (9) and (10). Separation was difficult but careful preparative t.l.c. afforded pure compound (9) m.p. 243°- 250°C (ex. ether-methanol); [m/e 714 (M^+-18), δ_H 7.28 (H-22), 6.36 (H-23), 5.94 (H-30), 5.46 (H-17), 4.65 (H-3), 3.75 ($-CO_2Me$), 2.67 (-OH), 1.65 ($Me-\overset{O}{\overset{\|}{C}}-O$)]. The corresponding acetate (23), prepared by treatment of (9) with acetic anhydride in pyridine at r.t. for 1 minute, was crystallised from methanol-ether and had m.p. 218°- 220°C, m/e 756 (M^+-18). (Found: C, 60.25; H, 6.60. $C_{39}H_{50}O_{16}$ requires C, 60.45; H, 6.45%). The second component of the mixture, compound (10), was not obtained entirely pure but had m/e 700 (M^+-18); [δ_H 7.28 (H-22), 6.30 (H-23), 5.92 (H-30), 5.46 (H-17), 4.60 (H-3), 7.38 ($-CO_2Me$), 1.66 ($Me-\overset{O}{\overset{\|}{C}}-O$)]. On acetylation it yielded the acetate (24), m.p. 203°- 210°C (ex. methanol-ether); [m/e 742 (M^+-18), δ_H 7.28 (H-22), 6.91 (H-23), 5.92 (H-30), 5.49 (H-17), 4.60 (H-3), 3.78 ($-CO_2Me$), 2.68 (-OH), 2.17 (OAc), 1.66 ($Me-\overset{O}{\overset{\|}{C}}-O$)]. Acetylation of the mixture of (9) and (10) followed by preparative t.l.c. led to a more efficient separation.

Alkaline hydrolysis of compound A (5).- Compound A (5) (100 mg) was dis-

solved in 5% methanolic potassium hydroxide (10 ml) and the solution refluxed for ½ h. Addition of water, acidification with 6M hydrochloric acid and extraction with chloroform gave a gum which was dissolved in methanol and treated with excess ethereal diazomethane. Purification by preparative t.l.c and crystallisation from ether-methanol yielded phragmalin (1) (57 mg) as needles m.p. 148°- 153°C with the same spectroscopic properties as an authentic specimen.[7] A similar result was obtained on hydrolysis of compound C (6).

Alkaline hydrolysis of compound D (8).- Compound D (8) (97 mg) was hydrolysed and methylated as above. Preparative t.l.c. of the product and crystallisation from ether-methanol afforded 12α-hydroxyphragmalin (22) (60 mg) as needles m.p. 160°- 170°C. (Found: C, 58.35; H, 6.5. $C_{29}H_{36}O_{12} \cdot H_2O$ requires C, 58.9; H, 6.45%).

Hydrolysis of compound B (7) gave a similar result.

Acetylation of phragmalin.- Phragmalin (100 mg) was refluxed in acetyl chloride for 3 h. Preparative t.l.c. of the product afforded phragmalin monoacetate (19) (42 mg) m.p. 248°- 255°C (ex. methanol-ether) and phragmalin diacetate (20) (35 mg) m.p. 234°- 238°C (ex. methanol-ether). Longer reaction times led to the formation inter alia of phragmalin triacetate (21) m.p. 165°- 173°C (ex. methanol-ether-light petroleum). These compounds had the expected spectroscopic properties (see Tables 1 and 2).

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CHAPTER IV

COMPLEX TETRANORTRITERPENOIDS

FROM

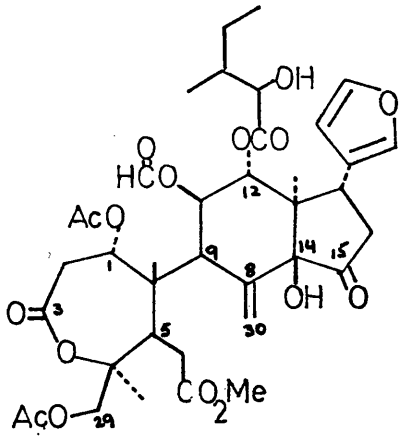
TRICHILIA AND GUAREA SPECIES

INTRODUCTION

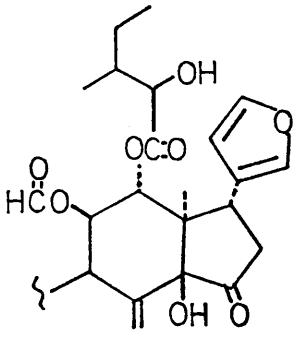
This chapter is concerned with a small group of complex tetranor-triterpenoids, from Trichilia and Guarea species, which unexpectedly exist in solution as a mixture of sterically hindered conformational isomers. This results in broadening of the ^{13}C and ^{1}H n.m.r. spectra (and even in the absence of some ^{13}C resonances) at room temperature and makes interpretation more difficult. No progress was made with this group until it was realised that a conformational problem existed and the spectra were run at elevated temperatures.

The first member of this group, prieurianin, $C_{38}H_{50}O_{16}$, from the wood of T. prieuriana, was isolated in 1965[1], but its structure remained unresolved until 1975 when it was assigned[2] structure (1) on the basis of chemical and spectroscopical evidence, and X-ray analysis.[3] Prieurianin had hydroxyl and carbonyl absorptions in the i.r. (ν_{max} 3560,3370, 1776-1710 cm^{-1}). Its c.d. spectrum [306 nm ($\Delta\varepsilon$ -1.4)] indicated a ketonic carbonyl group. The functional groups revealed spectroscopically (^{1}H and ^{13}C n.m.r.) included a ketone, two acetates, a formate, a carbomethoxyl, a lactone, a 2'-hydroxy-3'-methylpentanoate, and an exomethylene. The remaining oxygens were accounted for by a β-substituted furan ring and a tertiary hydroxyl group. Thus prieurianin was bicarbocyclic and had two rings of the typical tetracyclic apo-tirucallol nucleus cleaved.

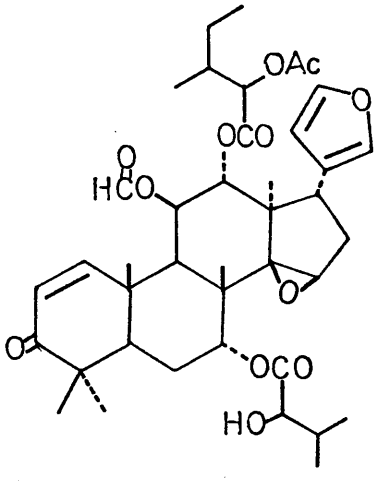
At ambient temperature only one tertiary methyl signal was apparent in the ^{1}H n.m.r. spectrum of prieurianin.[2] However, at 67°C in deuterio-acetone, the spectrum was well defined and three tertiary methyls were observed. Detailed analysis, with spin decoupling, of the high temperature spectrum suggested the partial structure (2) for rings C and D. The


OH
OCO
HCO
AcO
12
1
9
8
14
15
OH
O
3
5
30
CO2Me
AcO
29

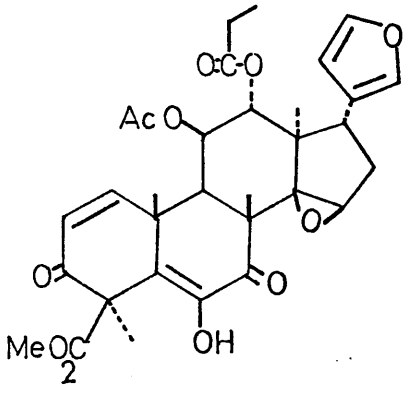
(1)


OH
OCO
HCO
OH
O

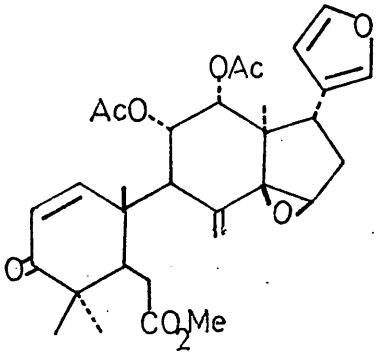
(2)


OAc
OCO
HCO
OCO
HO

(3)


OCO
AcO
MeOC2
OH

(4)

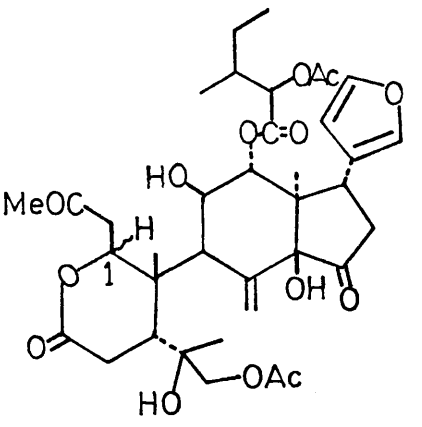

OAc
AcO
CO2Me

(5)

formate was placed at C-11 since there was weak coupling between the formyl proton and H-11. The attachment of the 2'-hydroxy-3'-methyl-pentanoate ester to C-12 followed from the shift of the furan H-23 from δ_H 7.36 to δ_H 7.44 on acetylation of the 2'-hydroxyl group. It is perhaps not without significance that several uncleaved tetranortriterpenoids from other Trichilia species also have oxygenation at C-11 and C-12. These include the heudelottins[4] (c.f., heudelottin F (3)), from T. heudelotii and hirtin (4) from T. hirta.[5] The ring D three spin system was readily identified. The large geminal coupling constant (J 16 Hz) for 2H-16 was consistent with the presence of a ketone at C-15. Finally, the c.d. spectrum and the unsymmetrical environment of the exomethylene protons supported the placing of the t-hydroxyl group at C-14. These results indicated that prieurianin had an A,B-cleaved skeleton.

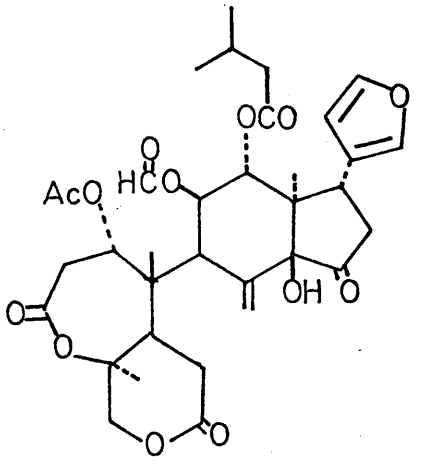
Other features of the ^1H n.m.r. spectrum included two acetates, one primary and one secondary. Two AEX spin systems, one involving the secondary acetate, were identified in the $Eu(dpm)_3$ shifted spectrum of prieurianin. These structural units taken in conjunction with the carbomethoxyl and lactone were readily assembled to give the biogenetically reasonable structure (1) for prieurianin. An X-ray analysis of prieurianin 2'-p-bromobenzenesulphonate confirmed structure (1) and established the full stereochemistry.[3]

The reasons for the conformational problems of prieurianin and related compounds are not yet clear. The atoms whose n.m.r. resonances are affected are all in the vicinity of the C-9,C-10 bond. The simplest explanation is that of restricted rotation about this bond. The ring A ε-lactone seems to be necessary for this effect since ring B cleaved compounds with a carbocyclic ring A, e.g. toonacilin (5)[6], have not been reported to suffer from conformational problems.

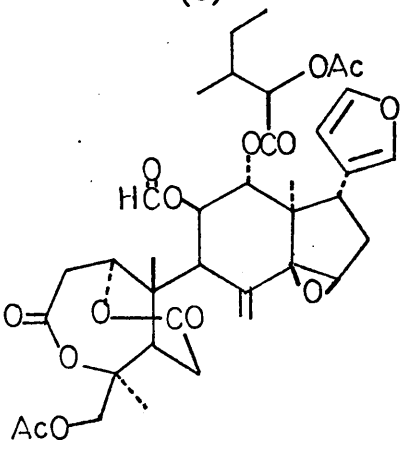


(6)

(7) C-1 epimer of (6)



(8)

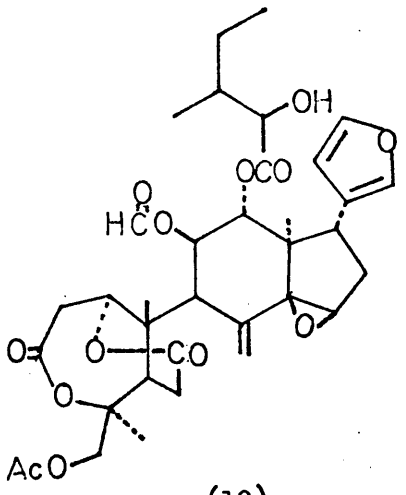


(9)

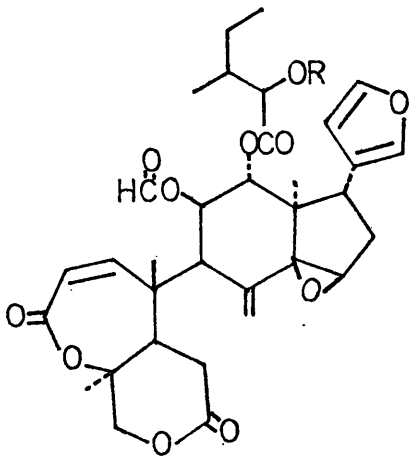
Alkaline hydrolysis of prieurianin (1) was a complex reaction. In the original work[2] two epimeric products were isolated after acetylation and were assigned the structures (6) and (7). The reaction was interpreted in terms of methanolysis of the ε-lactone, β-elimination of the C-1 acetate, and lactonisation of the C-7 carboxyl group to C-1 from both α and β faces. The hydrolysis products, unlike prieurianin, gave sharp ^{1}H and ^{13}C n.m.r. spectra. In the following discussion the structures of these hydrolysis products will be revised.

Two further members of this group of tetranortriterpenoids have been published recently.[7] Rohitukin (8) from T. rokka is very similar to prieurianin with the C-7 carboxyl group lactonised to C-29 to form a δ-lactone. The ester attached to C-12 was identified as 3-methylbutanoate. Dregeanin (9) has been found in T. dregeana and other species.[7] The substituents of rings C and D were readily established by comparison with prieurianin and other model systems. The 14,15-epoxide is a common feature in the tetranortriterpenoid series. Dregeanin had a carbonyl band at 1787 cm^{-1}. Since it was impossible to have both a ring A ε-lactone and a γ-lactone involving the 7-carboxyl group, this carbonyl frequency was interpreted as indicating a strained δ-lactone between C-7 and C-1. This led to structure (9) for dregeanin.

In the following discussion the structures of several new compounds belonging to this group will be considered. These include D-4 and D-5, two minor constituents of the extract of the bark of T. prieuriana, compounds B and C from the root bark of Guarea thompsonii, and a derivative of rohitukin from the seeds of T. rokka. The alkaline hydrolysis of all the compounds in this group was examined in detail and the results will be discussed below.

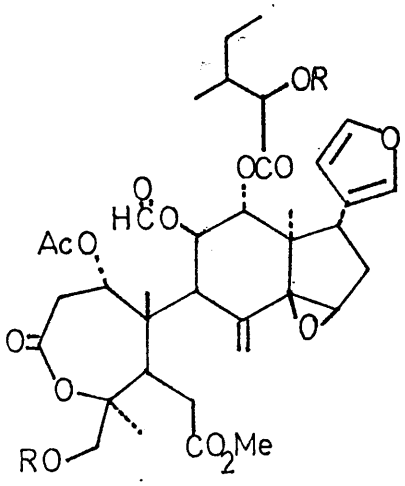


(10)



(11) R = H

(13) R = Ac



(12) R = H

(15) R = Ac

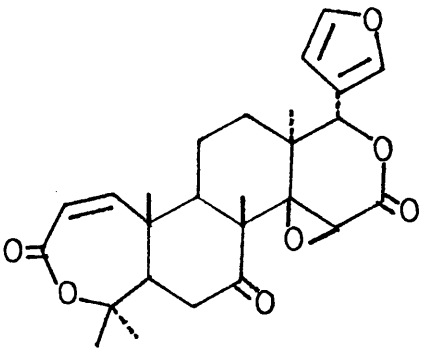
DISCUSSION

Careful chromatography of the light petroleum extract of the bark of T. prieuriana afforded three crystalline substances.[8] The least polar was 2'-deacetyldregeanin (10) which was readily identified by acetylation to give dregeanin (9). The other two compounds D-4, $C_{33}H_{40}O_{11}$, and D-5, $C_{36}H_{48}O_{14}$, were assigned structures (11) and (12) respectively on the evidence presented below.

Similarity of D-4 to dregeanin (9) and prieurianin (1) was immediately suggested by the broadness of the ^1H n.m.r. spectrum at ambient temperature. At 60°C the spectrum sharpened considerably. This facilitated interpretation and at the same time emphasised the relationship with dregeanin. There were signals for three tertiary methyl groups [δ_H 0.94 and 1.57 (6H)], an exomethylene [δ_H 5.68, 5.53 (bs)], a β-substituted furan (see Table 1) and a formate [δ_H 7.93 (s)]. The presence of a 2'-hydroxy-3'-methylpentanoate was indicated by the molecular formula and the appropriate methyl signals (see Table 1) and by the resonance at δ_H 3.44 (d, J 4 Hz, H-2') which shifted to δ_H 4.82 on formation of the corresponding acetate (13). A dregeanin type of ring D followed from the epoxide signals in the ^{13}C n.m.r. spectrum at δ_C 72.3 (s, C-14) and 60.7 (d, C-15) and the H-15 singlet at δ_H 3.86. The lack of coupling of this proton with the C-16 methylene group is well documented.[9] Spin decoupling experiments confirmed the characteristic AMX system involving H-9 [δ_H 3.66 (d, J 8 Hz)], H-11 [δ_H 5.50 (dd, J 11,8 Hz)] and H-12 [δ_H 6.04 (d, J11 Hz)]. Thus D-4 had a ring B cleaved skeleton with rings C and D identical to those in dregeanin (9).

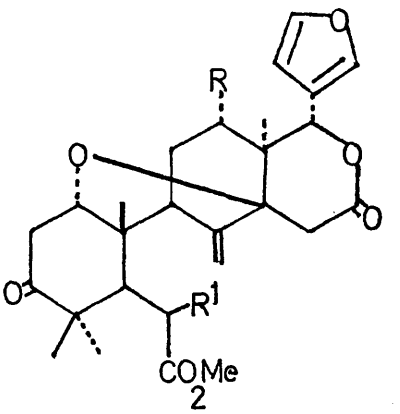
The above functional groups accounted for seven oxygens. Since the

C-7 carboxyl group was not present as a carbomethoxyl it had to be involved in a lactone as in rohitukin (8). The lack of a high carbonyl absorption excluded the dregeanin type of strained lactone. The remaining two oxygens were accomodated in a ring A ε-lactone. Another obvious feature of the ^{1}H n.m.r. spectrum of D-4, was an AB system [δ_H 6.75 and 6.00, (both d, J 12 Hz)] arising from a double bond in conjugation with a carbonyl group. This could only be placed in ring A leading to an α,β-unsaturated ε-lactone as in obacunone (14)[10]. Thus D-4 was assigned the structure (11). The ^{13}C n.m.r. data supported this assignment (see Table 2).



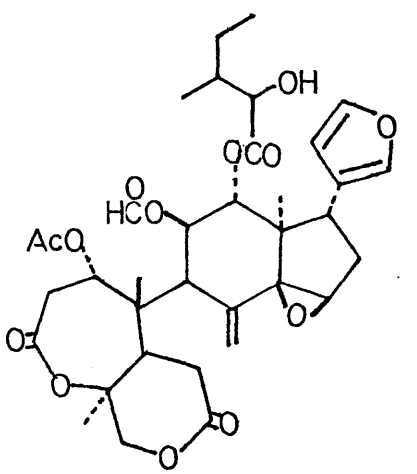
(14)

The ^{1}H n.m.r. spectrum of the second compound, D-5 (12), at 60°C was well resolved but more difficult to interpret than that of D-4 (11) because of overlap of signals, especially in the region between 5 and 6 p.p.m.. The presence of three tertiary methyls, an acetate, a 2'-hydroxy-3'-methylpentanoate, a carbomethoxyl, an exomethylene and a β-substituted furan, was readily established(see Table 1). Spin decoupling experiments identified the familiar H-9, H-11, H-12 three spin system and this, in conjunction with the epoxide proton singlet (H-15) at δ_H 3.90, suggested the same part structure as D-4 (11) for rings C and D. The remaining functionality including a hydroxymethyl group (δ_H 3.76, bs, 2H-29, sharpening on addition of D_2O), a secondary acetate (δ_H 5.55, complex m, H-1) and



(16) R= H ; R^1 = OH

(17) R = OH ; R^1 = H



(18)

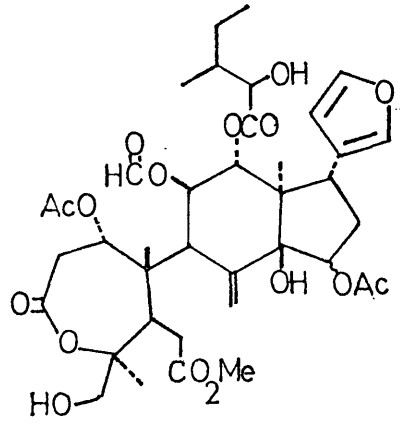
a lactone ring was readily accommodated in the biogenetically reasonable structure (12) for D-5. In support of this structure acetylation of D-5 afforded the triacetate (15) which lacked hydroxyl absorption in the i.r. and which had signals for the new acetates at δ_H 4.32 and 4.14 (ABq, J 12 Hz, $-CH_2OAc$) and δ_H 4.79 (d, J 4 Hz, H-2'). Thus D-5 has the same arrangement in rings A and B as prieurianin (1) though it belongs to the dregeanin series with the 14β,15β-epoxide in ring D.

The root bark of Guarea thompsonii has been reported[11] to contain dregeanin (9), 3-acetyldregeanin (10), methyl 6-hydroxyangolensate (16), and methyl 12α-acetoxyangolensate (17). The last compound is of interest since the 12α acetate methyl group is shielded by the furan ring and appears at δ_H 1.50 in the ^1H n.m.r. spectrum. During the course of this work two further compounds, B and C, were isolated by Professor D.A.H. Taylor, Durban, who sent them to Glasgow for study. The structure of compound C will be discussed later in conjunction with the hydrolysis of dregeanin.

The structure of compound B, $C_{35}H_{44}O_{13}$, (18), was readily deduced from a comparative study of its ^1H and ^{13}C n.m.r. spectra, run at 60°C, with those of the related compounds discussed above (see Tables 1 and 2). The presence of a formate, an exomethylene, a ring D epoxide (δ_H 3.93, s, H-15), a 2'-hydroxy-3-methylpentanoate (δ_H 3.33, d, J 4Hz, H-2') and the familiar H-9, H-11, H-12 spin system indicated the same arrangement in rings C and D as in D-4 (11) and D-5 (12). The lack of both a carbomethoxyl and high carbonyl absorption in the i.r. suggested the same bis-lactone structure for rings A and B as in rohitukin (8). The secondary acetate (δ_H 5.52, m, H-1) was placed at C-1 by analogy with rohitukin and prieurianin (1). Thus compound B was assigned the structure (18). The ^{13}C data (see Table 2) were in full agreement with this assignment.

Rohitukin is only one of several compounds present in the light petro-

leum extract of the seeds of T. rokka.[12] A precipitate obtained by Professor D.A.H. Taylor, Durban, on concentration of the light petroleum extract was sent to Glasgow for examination. Preparative t.l.c. afforded two compounds. The less polar was rohitukin (9). The second substance, R-6, could not be induced to crystallise. Its molecular formula could not be determined by standard mass measurement techniques since it did not give a suitable mass spectrum. Consideration of the ^{1}H and ^{13}C n.m.r. spectra led eventually to the molecular formula $C_{38}H_{52}O_{16}$ and to structure (19) for R-6. The ^{1}H n.m.r. spectrum at room temperature showed the typical broadness associated with the ring B cleaved tetranortriterpenoids with a ring A ε-lactone which have been discussed above. At 55°C it showed resonances for three tertiary methyl groups (δ_H 0.96, 1.43 and 1.57), a secondary methyl (δ_H 0.87, d, J 7 Hz), an ethyl group (δ_H 0.80, t, J 7 Hz), two acetates (δ_H 2.19 and 2.03), a carbomethoxyl (δ_H 3.68), a hydroxymethyl (δ_H 3.83, s , 2H-29), an exomethylene (δ_H 5.19, 5.32, both s), a formate (δ_H 8.03, s) and a β-substituted furan. Decoupling experiments confirmed the presence of the AMX system arising from H-9 (δ_H 3.22, d, J 9 Hz), H-11 (δ_H 5.43, dd, J 9,10 Hz) and H-12 (δ_H 6.08, d, J 10 Hz). The narrow doublet corresponding to H-2' (δ_H 3.24, J 4 Hz) of the 2'-hydroxy-3-methylpentanoate was also readily recognized. These data suggested



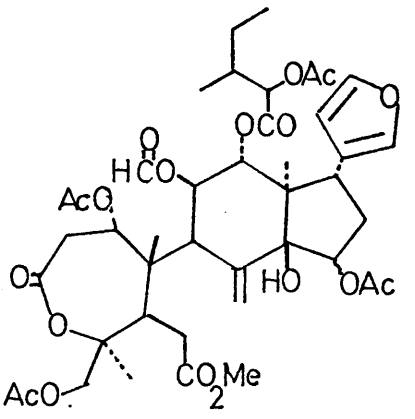
(19)

that R-6 had a structure that was closely related to that of prieurianin (1).

A two-proton resonance at 5.70 p.p.m., consisting of a doublet of doublets superimposed on a broad multiplet, was associated with protons attached to carbons bearing the two acetate groups. It seemed reasonable to assume, on the basis of the structure of prieurianin (1) and rohitukin (8), that one of the acetates was attached to C-1. In rohitukin H-1 resonates as a broad multiplet at 5.17 p.p.m. Allocation of the second acetate presented some difficulty at first glance since it had to be attached to a carbon adjacent to two non-equivalent protons. The ^{13}C n.m.r. spectrum of R-6 provided a ready answer. The typical ring D ketol or epoxide systems were absent. However, a low field singlet at δ_C 84.6 suggested that there was a hydroxyl group attached to C-14. The presence of this group was also deduced from the i.r. spectrum of R-6 (19) and its acetate (20). This suggested that C-15 carried the remaining acetate function and led to structure (19) for R-6 which can therefore be regarded as arising by reduction of prieurianin or by hydrolytic cleavage of the epoxide ring of D-5 (12). Acetylation of R-6 afforded the tetraacetate (20). In the ^{1}H n.m.r. spectrum of (20) the signals for H-2' and 2H-29 moved downfield as expected. The configuration of the 15-acetoxy group was not established (vide infra). The configuration at C-14 is assummed to be the same as in prieurianin (1) and rohitukin (8).

Efforts to prepare R-6 by reduction of the ketonic carbonyl of prieurianin (1) were unsuccessful. Treatment of (1) with sodium borohydride in methanol followed by acetylation afforded a mixture of mainly two compounds. The more polar compound had a complex ^{1}H n.m.r. spectrum with at least six acetate groups. It was not investigated further. The less polar product PR-1, was not obtained crystalline. It had similar polarity to the acetate

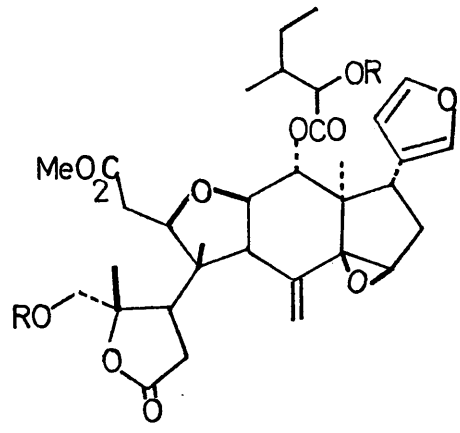
(20) of R-6 on analytical t.l.c. but its ^1H n.m.r. spectrum (at 60°C) indicated that the two compounds, though similar, were not identical (see Tables 1 and 2). It was concluded that R-6 acetate (20) and PR-1 (21) were epimeric at C-15. No definitive evidence was obtained for the configuration at C-15. It is possible that the reduction would occur from the β-face of the molecule, opposite to the furan and the 13α- methyl group, to give a 15α-hydroxyl group.



(20)

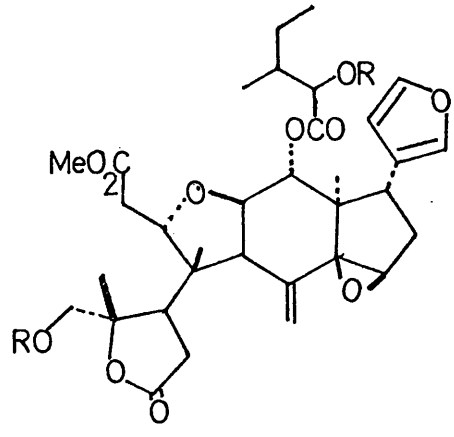
(21) C-15 epimer

Hydrolysis.- An investigation into the alkaline hydrolysis of members of this group of tetranortriterpenoids was undertaken with the object of providing confirmation of the structural proposals discussed above. In addition it was hoped that the use of a variety of available structural types would throw further light on the course of the hydrolysis reaction. It was generally found, as with prieurianin , that a large proportion of the crude hydrolysis product was methyl ester arising from alcoholysis of the ε-lactone ring. Treatment with diazomethane was included in the standard work-up to convert the minor acidic fraction into methyl ester. All the hydrolysis products gave sharp well defined n.m.r. spectra in striking contrast to the parent compounds.



(22) R = H

(24) R = Ac



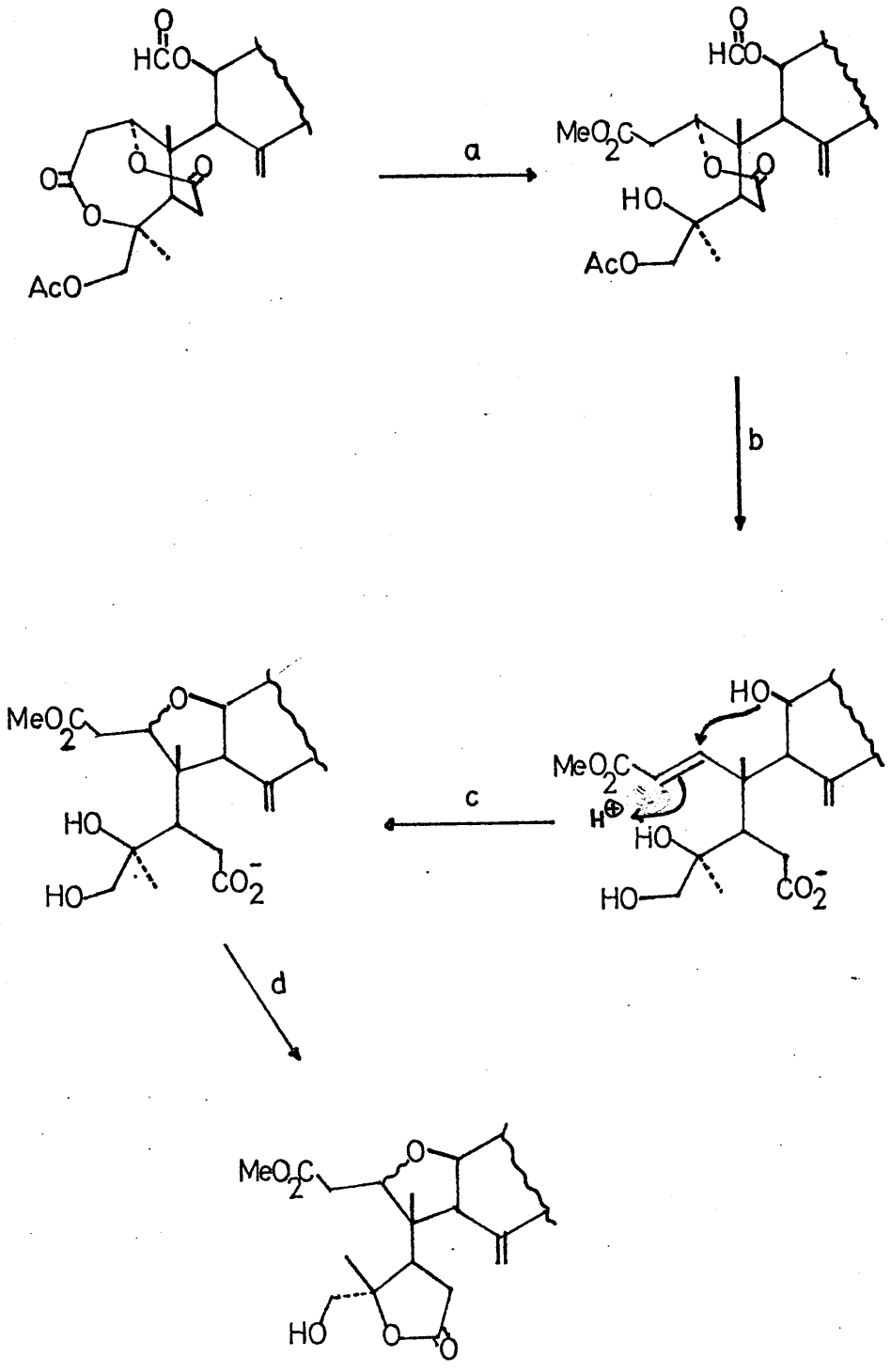
(23) R = H

(23a) R = Ac

The first compound to be examined was dregeanin (9). On hydrolysis it afforded four compounds DM-1, -2, -3, and -4, in variable proportions. The methyl esters DM-2 and DM-3 had similar spectroscopic properties, (see Tables 1 and 2) and were isomeric ($C_{33}H_{44}O_{11}$). It seemed likely that they corresponded to the two epimeric esters, (6) and (7), obtained on alkaline hydrolysis of prieurianin (see Introduction). They were assigned the structures (22) and (23) on the following evidence.

The i.r. spectrum of DM-2 (22) had bands at 3550 (hydroxyl), 1750 (ester) and 1790 (γ-lactone) cm^{-1}. It was clear from the molecular formula and the ^1H n.m.r. spectrum that the 2'-hydroxy-3-methylpentanoate ester attached to C-12 had survived the hydrolysis. The proton spectrum also showed the presence of the furan, epoxide, exomethylene and carbomethoxyl. A doublet of doublets at δ_H 4.19 (J 9,10 Hz) was identified as H-11 since irradiation at this frequency caused collapse of the doublets corresponding to H-9 (δ_H 3.45, J 10 Hz) and H-12 (δ_H 5.53, J 9 Hz). It was apparent that both H-11 and H-1 (δ_H 4.39, t, J 6 Hz) were at significantly higher field than in dregeanin itself. Neither of these resonances was affected by acetylation of DM-2, which gave a diacetate (24) lacking hydroxyl absorption in the i.r. The ^1H n.m.r. spectrum of the diacetate (24) clearly showed that the acetates were attached to C-2' (δ_H 4.85, J 4 Hz) and to C-29 (δ_H 4.28, 4.02, ABq, J 12 Hz). The above functional groups accounted for ten of the eleven oxygens in DM-2. It followed that the remaining oxygen had to be attached to both C-1 and C-11 i.e., an ether ring. The formation of this ether prevents rotation about the C-9, C-10 bond and removes the source of the broadness in the room temperature spectra of the parent compound. The above evidence together with the ^{13}C n.m.r. data (see Table 2) led to structure (22) for DM-2. (For ease of presentation the C-1 terminus of the ether is assumed to be β; this assumption will be discussed later).

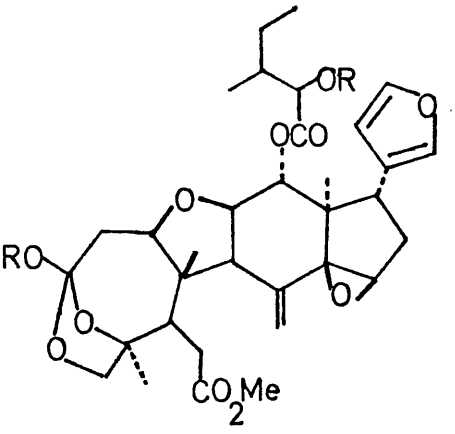
Scheme 1



The second product, DM-3 (23), had similar i.r., ^{1}H and ^{13}C n.m.r. spectroscopic properties (see Tables 1 and 2) to those of DM-2. The minor difference in the ^{1}H n.m.r. spectrum concerned H-1 (δ_H 4.20 from 4.39), 2H-30 (δ_H 5.37 and 5.32 from 5.53 and 5.37) and 2H-29 (δ_H 3.72 and 3.50, from 3.78 and 3.57). Likewise the changes in the ^{13}C n.m.r. spectrum of DM-3 with respect to DM-2 involved C-1 (δ_C 81.1 from 83.8), C-9 (δ_C 53.1 from 50.9), C-2 (δ_C 36.8 from 35.2), C-8 and C-30 (δ_C 136.6, 121.9 from 137.8, 120.9) and C-29 (δ_C 67.5 from 68.4). These spectroscopic differences, taken in conjunction with a rational mechanism for the formation of DM-2 and DM-3 from dregeanin (see below), supported the proposal that DM-3 was the C-1 epimer of DM-2 and had structure (23). The evidence available so far, did not allow a decision concerning the relative configuration at C-1 in the two epimers.

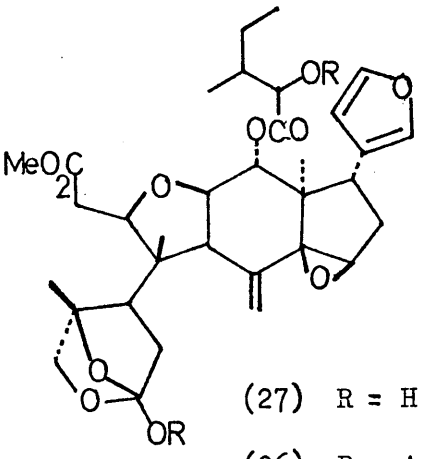
The formation of DM-2 and DM-3 can be rationalised in terms of the following steps: (a) alcoholysis of the ε-lactone ring, (b) β-elimination of the oxygen substituent at C-1, (c) hydrolysis of the formate and addition of the C-11 hydroxyl group to the α,β-unsaturated ester (or lactone) from either the α or the β face, and (d) lactonisation of the C-7 carboxyl group at C-4 to form a γ-lactone. This reaction sequence is summarized in Scheme 1. The order of the steps is arbitrary, e.g., step (b) might well precede step (a). The availability of D-4 (11) allowed the possibility of testing the intermediacy of a Δ^1 compound (see below).

The least polar product of the hydrolysis of dregeanin was DM-1 (25), $C_{33}H_{44}O_{11}$, isomeric with and similar in many respects to DM-2 (22) and DM-3 (23). The carbomethoxyl group, the 1,11-ether and the characteristic arrangement in rings C and D were readily identified in the ^{1}H n.m.r. spectrum (see Table 1). The main differences were the lack of γ-lactone absorption in the i.r. and the size of the geminal coupling constant of the



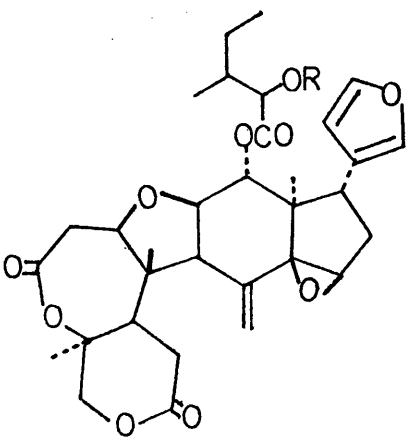
(25) R = H

(26) R = Ac



(27) R = H

(26) R = Ac

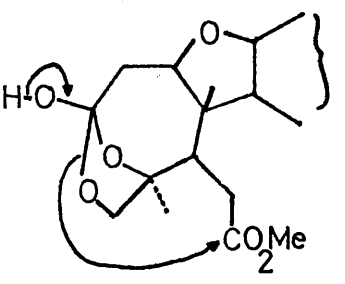


(28) R = H

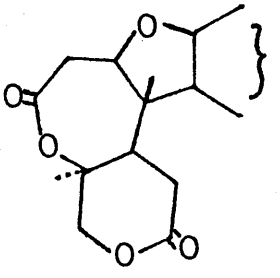
(29) R = Ac

protons attached to C-29 [8Hz as compared to 12 Hz in (22) and (23)]. Acetylation afforded the diacetate (26) which lacked hydroxyl absorption in the i.r. One acetate group was clearly attached to C-2' in view of the shift of H-2' from 3.64 p.p.m. in (25) to 4.92 p.p.m. in (26). It was possible that the second acetate was at C-29 but the slight shift of the 2H-29 protons from δ_H 3.47 and 4.03 (ABq J 8 Hz) in (25) to δ_H 3.85 and 4.09 (ABq, J 8 Hz) in (26) was inconsistent with this suggestion (see Table 1). The ^{13}C n.m.r. spectrum of DM-1 provided the necessary breakthrough. It had signals for only two carbonyl carbons at δ_C 175.2 and δ_C 174.2 instead of the expected three. A singlet at δ_C 119.9 indicated a carbon bearing three oxygens. This suggested that one of the carbonyl groups and the primary alcohol function (C-29) were involved in hemiorthoester formation and led to two possible structures, (25) and (27), for DM-1. The 8 Hz geminal coupling of the C-29 protons supported the presence [13] of a 1,3-dioxolan and the formation of a hemiorthoester acetate accounted for the second acetate group in (26). Lack of material made difficult to obtain definitive evidence to distinguish between these two structures.

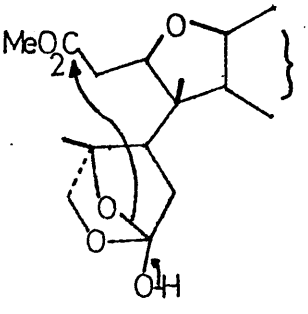
On thin layer chromatography DM-1 (25) was always accompanied by a small amount of DM-4 (28), the most polar product of the hydrolysis of dregeanin. Analytical t.l.c. clearly demonstrated that DM-1 was irreversibly converted into DM-4 on standing. The latter had the molecular formula $C_{32}H_{40}O_{10}$ and was the only one of the hydrolysis products that lacked a carbomethoxyl group. In the i.r. spectrum there were bands for hydroxyl (3535, 3520 cm^{-1}) and ester and lactone (1730 br, 1760 cm^{-1}) absorptions. The ^1H n.m.r. spectrum revealed the same 1,11-ether and arrangement in rings C and D as in the other compounds. Acetylation gave the monoacetate (29) which had no hydroxyl absorption. Again it was clear from the ^1H n.m.r. spectrum that the acetate was at C-2'. The fact that



(25)



(28)



(27)

the 2H-29 protons did not change on formation of the acetate suggested that they were involved in δ-lactone ring formation. This led to the bis-lactone structure (28) for DM-4. The ^{13}C n.m.r. spectrum showed the appropriate carbonyl carbon resonances at δ_C 173.3, 172.5 and 169.6 for two lactones and an ester.

The conversion of DM-1 into DM-4 can be rationalized on the basis of either structure (25) or (27) as opening of the hemiorthoester with reformation of the lactone followed by intramolecular attack by the newly released hydroxyl on the carbomethoxyl to form the second lactone (see (25) and (27), arrows). Initially it was thought unlikely that an ε-lactone would be formed in this way and for this reason the structure (25) was preferred. It was also considered that the formation of the hemiorthoester (25) during the hydrolysis reaction protected the ε-lactone and enabled it to survive alkaline conditions. However, exposure of DM-2 (22) to the hydrolysis conditions afforded a mixture of DM-1 (25), DM-2 (22) and DM-4 (28). This showed that reformation of the ε-lactone was possible. Another important conclusion from this experiment was that these three compounds had the same configuration at C-1. When DM-3 (23) was treated under the hydrolysis conditions it was recovered essentially unchanged. These experiments demonstrated the stability of the 1,11-ether to the hydrolysis conditions. It is not clear from models why one configuration at C-1 should permit the formation of the hemiorthoester while the other configuration does not.

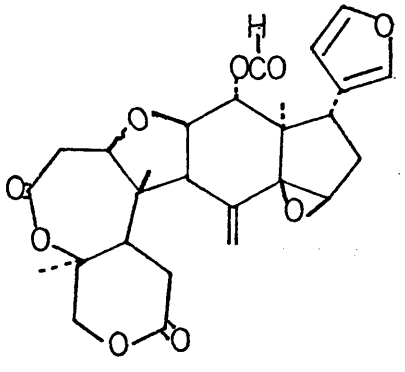
At this stage it is convenient to consider the hydrolysis of the other compounds in this series. Compound B (18) from Guarea thompsonii and D-4 (11) from Trichilia prieuriana were both smoothly converted to DM-3 (23) with little or no trace of any other products. The result with D-4 (11) was particularly gratifying since the intermediacy of an α,β-unsaturated

-lactone or ester had been proposed during the earlier studies on prieurianin. A pilot hydrolysis of D-5 (12) from T. prieuriana paralleled the dregeanin case and yielded all four compounds DM-1 (25), DM-2 (22), DM-3 (23), and DM-4 (28) (analytical t.l.c.).

The reasons for the different results of the hydrolysis reactions are obscure. It seems likely that the relative rates of β-elimination, addition of the 11β-hydroxy group to the α,β-unsaturated system and opening of the ε-lactone determine the observed product ratios. Inspection of models suggests that addition of the 11β-hydroxyl group to the α face of the α,β-unsaturated ε-lactone is less hindered whereas addition from both α and β faces may occur once the lactone has opened to the corresponding methyl ester (by alcoholysis). In the case of D-4 (11) a fast addition step (from the α face) could lead to one product whereas with D-5 (12) the opening of the ε-lactone could compete with the elimination-addition steps allowing addition from both faces to give the C-1 epimeric products. For this reason and for ease of presentation, the stereochemistry of the oxygen substituent at C-1 in DM-3 (23) and related compounds is drawn as α and in DM-2 (22) and related compounds as β. It is clear that some firm evidence for these assignments is desirable.

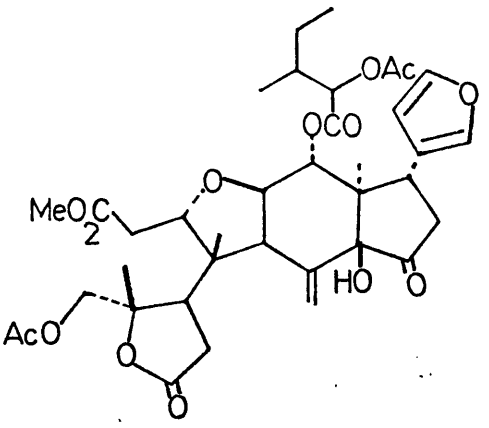
At this point it is convenient to consider the structure of compound C, $C_{29}H_{34}O_{10}$, [ν_{max} (CCl_4) 1733, 1762 cm^{-1}] from Guarea thompsonii. Unlike the other natural compounds, C gave a sharp, well defined proton n.m.r. spectrum at room temperature. Unfortunately, it was available in very small amount (ca 3 mg). The proton spectrum was run in the pulsed F.T. mode on an XL-100 instrument modified to allow homonuclear double resonance. The obvious features of the spectrum included three tertiary methyls, a formate, an exomethylene, a β-substituted furan, a ring D epoxide (δ_H 3.90, s, H-15) and H-12 (δ_H 5.56, d, J 9 Hz). The chemical shift

of H-12 required the attachment of the formate, in the absence of any other ester functions. This was confirmed by the existence of a small coupling between the formyl proton and H-12 (0.5 Hz). The lack of both a carbomethoxyl group and γ-lactone absorption in the i.r., suggested the same bis-lactone arrangement in rings A and B as in DM-4 (28). The resonances for 2H-29 (δ_H 4.24 and 3.98, ABq, J 12 Hz), H-1 (δ_H 4.13, dd, J 6,11 Hz), H-11 (δ_H 4.27, t, J 9 Hz), and H-9 (δ_H 3.26, d, J 9 Hz) were identified with the aid of double resonance. The chemical shifts of H-1 and H-11 and the necessity to accommodate only one more oxygen atom strongly supported a 1,11-ether as in the hydrolysis products of dregeanin and related compounds. This led to structure (30) for compound C. The available information did not allow the assignment of the configuration at C-1.

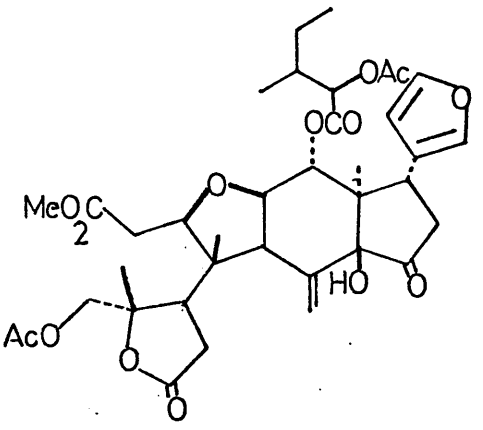


(30)

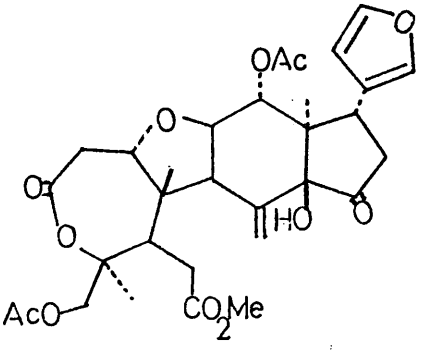
The hydrolysis of prieurianin (1) was re-examined in the hope of isolating some of the minor products. The reaction product was much more complex than with the above compounds containing a ring D epoxide. It was possible to isolate, after acetylation, the diacetates of the two epimeric esters PM-2 and PM-3, originally assigned structures (6) and (7) (see Introduction). Both compounds had γ-lactone absorption in their



(31)



(32)



(33)

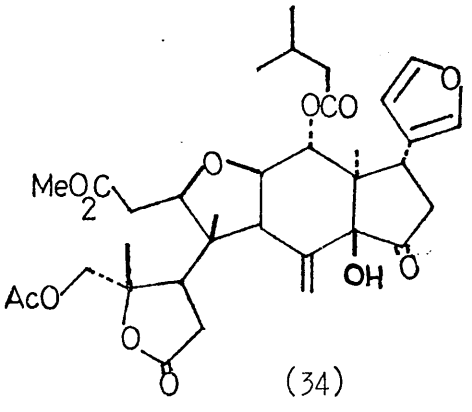
i.r. spectra. A comparison of their spectroscopic data (see Tables 1 and 2) with those of the dregeanin hydrolysis products readily led to the revised structures (31) and (32) respectively. The assignment of the C-1 configuration in (31) and (32) will be dicussed below.

A new compound PM-4, was also isolated from the hydrolysis and characterised as its diacetate. It was assigned structure (33) on the following basis. PM-4 diacetate (ν_{max} 3380 and 1755(s) cm^{-1}) had the molecular formula $C_{31}H_{38}O_{12}$ and it was clear that it had lost the 2'-hydroxy-3-methylpentanoate ester. The ^{1}H and ^{13}C n.m.r. spectra showed a carbomethoxyl group, three tertiary methyl signals and two acetates, one (δ_H 1.88) at a higher field than usual. An acetate attached to C-12 (δ_H 5.86, d, J 10 Hz, H-12) would be expected to come under the shielding influence of the furan ring.[11] The other acetate was attached to C-29 (δ_H 3.90, 4.06, ABq, J 12 Hz). The chemical shifts of H-11 (δ_H 4.06, dd, J 10,6 Hz) and H-1 (δ_H 3.80, dd, J 8,5 Hz) were consistent with the presence of a 1,11-ether. As with the other compounds in this series the H-12, H-11, and H-9 (δ_H 2.99, d, J 6 Hz) three spin system was easily identified by double resonance experiments. The ^{13}C n.m.r. spectrum had resonances for ester or lactone carbonyl groups at δ_C 176.6 and 171.4, in addition to the two acetate carbonyls. The absence of γ-lactone absorption in the i.r. spectrum led to structure (33) for PM-4 diacetate, with the carbomethoxyl group at C-7 and ε-lactone ring A. The configuration at C-1 will be discussed later.

PM-4 is of special interest since it is the only compound to be obtained from the hydrolysis reactions that has lost the ester group at C-12. Its isolation raised the possibility of achieving a chemical correlation between prieurianin (1) and the related rohitukin (8) from Trichilia rokka. The latter has a different ester group at C-12. Hydrolysis of rohitukin

followed by acetylation gave a complex mixture. The limited quantity of rohitukin available for this reaction did not facilitate characterisation of the products. A small amount of material with similar chromatographic and ^{1}H n.m.r. spectroscopic properties to PM-4 diacetate was obtained but attempts at further purification were unsuccessful. The major product and the only one isolated as a crystalline compound in this particular experiment was RM-1 acetate (34), one of the expected C-1 epimers analogous to the diacetates of PM-2 (31) and PM-3 (32). Its similarity to the latter (see Tables 1 and 2) suggested that they had the same configuration at C-1.

Inspection of the ^{13}C n.m.r. chemical shifts of C-1 and C-9 in the various hydrolysis products discussed above reveals a pattern which may be interpreted in terms of their relative configurations at C-1. The respective values for C-1 and C-9 in DM-3 (23) (δ_C 81.1, 53.1), PM-2 diacetate (31) (δ_C 80.4, 53.8) and PM-4 diacetate (33) (δ_C 79.9, 53.8) contrast with those in DM-2 (22) (δ_C 83.8, 50.9), PM-3 diacetate (32) (δ_C 83.2, 49.7), and RM-1 acetate (34) (δ_C 83.0, 49.5). In the discussion on page 99 it was suggested that the C-1 oxygen substituent in DM-3 (23) was α. If this is assumed to be correct it follows that PM-2 diacetate (31) and PM-4 diacetate (34) also have 1-α oxygen substituents while DM-2 (22), PM-3 diacetate (32) and RM-1 acetate (34) have the opposite configuration at C-1.



(34)

Table 1

^{1}H n.m.r. spectra of dregeanin, prieurianin, and related compounds.[c]

	(11)	(13)[d]	(12)	(15)
H-1	6.75(d,12)	6.73(d,12)	5.55[a]	5.46[a]
H-2	6.00(d,12)	5.95(d,12)		
H-9	3.66(d, 8)	3.65(d, 8)	3.28(d, 7)	3.42(d, 7)
H-11	5.50(dd,11,8)	5.38(dd,11,8)	5.55[a]	5.46[a]
H-12	6.04(d,11)	5.97(d,11)	5.79(d,12)	5.83(d,11)
H-15	3.86(s)	3.86(s)	3.90(s)	3.91(s)
H-17	3.02(dd,10,7)	3.08(dd,10,7)	b	b
2H-29	4.45 3.92 (ABq,12)	4.43 3.92 (ABq,12)	3.76(s)	4.32 4.14 (ABq,12)
2H-30	5.68 5.63	5.66 5.49	5.48 5.31	5.49 5.42
furan	7.38 7.15 6.16	7.32 7.24 6.22	7.35 7.14 6.14	7.35 7.27 6.23
C-Me's	1.57 1.57 0.94	1.64 1.64 1.04	1.57 1.45 0.94	1.71 1.62 1.05
HCOO	7.93	7.89	8.05	8.06
OAc	-	2.10	2.04	2.11 2.14 2.17
CO_2Me	-	-	3.62	3.68
H-2'	3.44(d,4)	4.82(d,4)	3.43(d,4)	4.79(d,4)
3H-5'	0.77	0.86	0.80	0.86
3H-6'	0.85	0.89	0.87	0.93

a. Obscured.

b. Not identified.

c. In $CDCl_3$ at 25°C unless otherwise stated.

d. At 60°C.

	(18)	(8)[d]	(30)	(19)[e]
H-1	5.52[a]	5.17(m)	4.13(dd,11,6)	5.74[a]
H-2				
H-9	3.65(d, 7)	3.69(d, 8)	3.26(d, 9)	3.22(d,9)
H-11	5.59(dd,11,7)	5.45(dd,10,8)	4.27(t, 9)	5.43(dd,10,9)
H-12	5.89(d,11)	6.05(d,10)	5.56(d, 9)	6.08(d,10)
H-15	3.93(s)	-	3.90(s)	5.68(dd,9,6)
H-17	3.01(dd,11,7)	3.96(t,10)	b	3.97(t,10)
2H-29	4.24 3.96(ABq,12)	4.16(s)	4.24 3.98(ABq,12)	3.83(s)
2H-30	5.48	5.91 5.48	5.43 5.35	5.32 5.19
furan	7.37 7.16 6.18	7.33 7.22 6.25	7.33 7.13 6.16	7.35 7.21 6.27
C-Me's	1.76 1.54 0.95	1.82 1.74 0.97	1.65 1.25 0.81	1.57 1.43 0.96
HCOO	7.90	7.84	8.04(d,0.5)	8.03
OAc	2.11	2.08	-	2.19 2.03
CO_2Me	-	-	-	3.68
H-2'	3.33(d,4)	-	-	3.24(d,4)
3H-5'	0.75	0.82	-	0.80
3H-6'	0.82	0.79(3H-4')	-	0.87

e. At 55°C.

Table 1 (cont'd)

^{1}H n.m.r. spectra of dregeanin, prieurianin, and related compounds.[c]

	(20)[e]	(21)[d]	(1)[f]	(9)
H-1	5.77[a]	5.34[a]	5.10(dd,7,3)	5.54[a]
H-2				
H-9	3.22(d, 9)	3.73(d, 7)	3.85(d, 8)	3.69(d, 7)
H-11	5.36(dd,11,9)	5.18(dd,11,7)	5.46(dd,11,8)	5.32(dd,11,7)
H-12	6.02(d,11)	6.03(d,11)	6.16(d,11)	5.74(d,11)
H-15	5.69(dd,9,6)	5.66(dd,9,5)	-	3.90(s)
H-17	3.97(t,10)	3.96(t,10)	3.99(t, 9)	3.04(dd,10,6)
2H-29	4.54 4.10 (ABq,12)	4.44 4.15 (ABq,12)	4.13 3.94 (ABq,12)	4.46(s)
2H-30	5.28 5.22	5.63 5.42	6.02 5.74	5.56 5.36
furan	7.31 7.31 6.32	7.31 7.31 6.32	7.36 7.22 6.25	7.33 7.27 6.25
C-Me's	1.68 1.47 0.99	1.57 1.52 0.97	1.85 1.69 1.01	1.59 1.41 0.98
HCOO	8.01	7.92	7.83	8.02
OAc	2.22 2.10 2.08 2.10	2.14 2.08 2.05 2.08	2.10 2.04	2.11 2.09
CO_2Me	3.67	3.79	3.71	
H-2'	4.72(d,4)	4.69(d,4)	3.20(d,4)	4.84(d,4)
3H-5'	0.80	0.78	0.78	0.78
3H-6'	0.84	0.81	0.84	0.85

a. Obscured.

b. Not identified.

c. In $CDCl_3$ solution at 25°C unless otherwise stated. d. At 60°C.

e. At 55°C. f. At 66°C.

	(25)	(26)	(22)	(24)
H-1	4.05(dd,10,6)	4.15[a]	4.39(t,6)	4.32(dd,7,5)
H-2				
H-9	3.34(d,10)	3.36(d,10)	3.45(d,10)	3.37(d,10)
H-11	4.13(dd,10,9)	4.15(dd,10,9)	4.19(dd,10,9)	4.19(dd,10,9)
H-12	5.62(d,9)	5.67(d,9)	5.53(d,10)	5.53(d,9)
H-15	3.82(s)	3.87(s)	3.85(s)	3.85(s)
H-17	b	b	2.97(dd,11,7)	2.99(dd,11,7)
2H-29	4.03 3.47 (ABq,12)	4.09 3.85 (ABq,12)	3.78 3.57 (ABq,12)	4.28 4.02 (ABq,12)
2H-30	5.50 5.26	5.53 5.29	5.53 5.37	5.38 5.29
furan	7.31 7.09 6.12	7.35 7.27 6.23	7.27 7.05 6.08	7.30 7.25 6.18
C-Me's	1.19 1.18 0.74	1.27 1.23 0.75	1.38 1.14 0.80	1.48 1.19 0.79
HCOO	-	-	-	-
OAc	-	2.19 2.05	-	2.15 2.10
CO_2Me	3.65	3.72	3.61	3.65
H-2'	3.64(d,4)	4.92(d,4)	3.63[a]	4.85(d,4)
3H-5'	0.82	0.84	0.79	0.83
3H-6'	0.94	0.96	0.87	0.94

	(31)	(32)	(33)	(34)
H-1	4.5(t,8)	4.11[a]	3.80(dd,8,5)	4.20[a]
H-2				
H-9	3.25(d,10)	3.45(d,10)	2.99(d,6)	3.49(d,10)
H-11	4.09(dd,10,7)	4.03(dd,10,7)	4.06(dd,10,6)	4.17(dd,10,7)
H-12	5.80(d,7)	5.85(d,7)	5.87(d,10)	5.79(d,7)
H-15	-	-	-	-
H-17	3.82(t,10)	3.85(t,10)	3.84(t,10)	3.90(t,10)
2H-29	4.35 4.14 (ABq,12)	4.30 4.05 (ABq,12)	3.90 4.06 (ABq,12)	4.33 4.08 (ABq,12)
2H-30	6.27 5.22	6.07 5.35	5.39 5.33	6.07 5.36
furan	7.37 7.37 6.27	7.37 7.30 6.23	7.36 7.26 6.24	7.33 7.21 6.25
C-Me's	1.55 1.03 0.82	1.54 1.05 0.83	1.79 1.12 0.78	1.55 1.08 0.85
HCOO	-	-	-	-
OAc	2.16 2.10	2.15 2.13	2.10 1.88	2.13
CO_2Me	3.67	3.72	3.63	3.73
H-2'	4.71(d,4)	4.75(d,4)	-	-
3H-5'	0.82	0.82	-	0.89
3H-6'	0.90	0.90	-	0.86(3H-4')

Table 1 (cont'd)

^{1}H n.m.r. spectra of dregeanin, prieurianin, and related compounds.[c]

	(23)	(23a)	(28)	(29)
H-1	4.20[a]	4.20[a]	4.29(dd,9,7)	4.29(t,8)
H-2				
H-9	3.49(d,10)	3.45(d,10)	3.35(d,10)	3.33(d,10)
H-11	4.20(dd,10,8)	4.19[a]	4.22(dd,10,9)	4.21(dd,10,9)
H-12	5.56(d,8)	5.54(d,8)	5.61(d,9)	5.63(d,9)
H-15	3.87(s)	3.86(s)	3.92(s)	3.92(s)
H-17	3.10(dd,10,8)	3.06(dd,11,7)	b	b
2H-29	3.72 3.50(ABq,12)	4.23 3.99(ABq,12)	4.19 4.01(ABq,12)	4.20 4.02(ABq,12)
2H-30	5.37 5.32	5.38 5.30	5.51 5.46	5.50 5.44
furan	7.30 7.08 6.10	7.35 7.27 6.21	7.32 7.10 6.12	7.35 7.29 6.24
C-Me's	1.51 1.22 0.83	1.54 1.14 0.75	1.67 1.28 0.78	1.68 1.30 0.74
HCOO	-	-	-	-
Oac	-	2.15 2.11	-	2.17
CO_2Me	3.70	3.72	-	-
H-2'	3.70(d,4)	4.89(d,4)	3.68(d,4)	4.88(d,4)
3H-5'	0.86	0.83	0.82	0.84
3H-6'	0.96	0.86	0.93	0.96

a. Obscured.

b. Not identified.

c. In $CDCl_3$ solution at 25°C unless otherwise stated.

Table 2.

^{13}C n.m.r. spectra of dregeanin, prieurianin, and related compounds.[e]

Carbon No.	(18)	(11)[f]	(12)[g]	(9)[h]	(22)	(23)	(25)	(28)[g]
1	71.7	155.6	70.9	71.4	83.8	81.1[a]	77.6[a]	77.5[a]
2	37.9	122.8	36.3	37.9	35.2	36.8	39.2	40.1
3	169.5[a]	168.7	172.0[b]	168.8	174.9	174.7[b]	119.9	169.6
4	79.8	81.9	91.5	88.6	89.8	90.1	86.6	81.0
5	43.3	50.5	43.4	45.8	42.8	43.6	50.7	47.7
6	31.0	32.3	34.2[a]	32.9	33.0[c]	32.3[c]	32.6[c]	30.0
7	171.0	174.5[a]	174.7[d]	170.8	171.0	171.8	174.2	172.5
8	136.5	137.2	138.5	136.4	137.8	136.6	138.7	139.3
9	51.5	56.3	53.6	51.4	50.9	53.1	61.9	61.4
10	45.9[c]	46.8[c]	48.6[c]	45.8[c]	49.0	48.8	50.0	50.1
11	73.2	73.5	74.4	72.8	79.7	79.0[a]	81.1[a]	79.5[a]
12	75.6	77.7	76.0	75.6	80.3	80.8	81.4	81.0
13	46.4[c]	46.3[c]	46.3[c]	46.9[c]	44.6	45.1	45.1	45.9
14	71.1	72.3	71.7	71.4	72.0	71.6	71.9	72.3
15	59.5	60.7	59.9	59.5	59.5	59.0	58.9	59.8
16	33.6	34.6	34.9[a]	33.9	33.6[c]	33.5[c]	33.5[c]	34.1
17	37.9	39.8	39.1	38.6	38.2	37.6	37.6	38.2[c]
20	121.7	123.6	123.5	122.0	122.6	122.5	122.6	123.9
21	140.6	141.9	141.5	140.9	140.2	140.2	140.2	141.2
22	111.1	112.4	112.4	111.4	111.1	111.2	111.2	112.4
23	143.0	144.0	143.6	142.9	142.7	142.7	142.6	143.4
29	76.6	74.7	66.5	72.0	68.4	67.5	68.6	75.3
30	123.4	123.9	123.5	123.7	120.9	121.9	121.6	122.9
1'	174.7	175.1[a]	175.2[d]	173.4	175.9	175.0[b]	175.2	175.3
2'	75.0	76.2	75.8	75.9	74.5	74.5	74.6	75.3
3'	37.9	39.5	38.9	36.3	39.3	39.3	39.2	38.5[c]
4'	23.0	24.4	23.8	24.3	23.9	23.9	23.5	24.5
5'	11.5	12.0	11.8	11.3	11.8	11.8	11.8	12.2
6'	15.2	15.7	15.7	15.4	14.8	14.6	14.9	15.5
HC(O)	160.3	162.3	162.5	161.3	-	-	-	-
OAc	21.4	-	20.9	20.8 20.5	-	-	-	-
C-Me	25.4 20.2 13.9	26.9 23.8 14.4	19.4 17.0 13.8	19.1 17.0 13.4	20.1 18.9 14.2	20.6 18.0 13.8	24.9 11.8 14.0	27.1 12.3 14.1
OMe	-	-	52.0	-	52.1	51.8	52.1	-
CH_3COO	169.7[a]		170.8[b]	170.1 170.1				

a,b,c,d. These assignments may be interchanged.

e. $CDCl_3$ solution and at 25°C unless otherwise stated. f. CD_3OD solution

g. $(CD_3)_2CO$ solution. h. At 60°C.

Carbon No.	(1)[h]	(31)	(32)	(33)	(34)	(19)[h]	(21)[h]
1	71.8	80.4	83.2	79.9	83.0	72.7	72.2
2	37.5	36.8	34.7	35.1	34.7	35.8	36.6
3	169.6	173.8	174.7	171.4	174.7	169.8[c]	169.8
4	84.6	87.3	87.1	88.6	87.0	91.9	83.7
5	45.8	49.0	43.8	44.8	43.7	41.9	38.3
6	33.0	32.4	32.3	35.1	32.3	34.2	33.3
7	176.7	172.0	171.3	176.6	171.0	175.1	175.1
8	138.2	139.3	140.2	137.7	140.2	142.9	141.8
9	51.6	53.8	49.7	53.8	49.5	53.1	50.8
10	47.4[c]	50.4	48.0[c]	49.0[a]	48.2	49.0	49.0
11	74.4	80.9	80.7	73.5	80.7	74.1	74.6
12	74.1	78.0	77.9	78.6	75.8	75.2	72.8[a]
13	49.8[c]	48.5	48.3[c]	48.2[a]	48.2	51.1	50.8[c]
14	81.1	80.1	80.1	87.0[c]	80.1	84.6	85.2
15	206.1	209.0	209.7	205.7	209.8	70.0	72.5[a]
16	41.6	41.6	41.8	41.1	42.1	39.8	40.0
17	35.5	35.1	35.0	37.1	34.9	36.5	48.9[c]
20	123.2	122.4	122.4	122.5	122.8	123.9	124.3
21	140.8	141.0	141.0	140.8	140.7	140.7	140.8
22	110.8	110.6	110.6	110.6	110.6	110.8	111.0
23	143.1	143.0	142.9	142.7	142.7	142.9	142.8
29	68.7	68.2	68.2	72.1	68.2	66.5	67.3
30	125.7	120.7	120.3	116.7	120.3	120.0	121.9
1'	174.8	170.4[c]	170.4[c]	-	172.3	174.9	170.0
2'	74.9	75.7	75.6	-	43.1	75.2	75.8
3'	38.2	37.0	37.0	-	25.3	38.2	36.2
4'	23.3	24.4	24.1	-	22.3	23.1	24.5
5'	11.5	11.7	11.9	-	22.3	11.5	11.3
6'	15.2	15.1	15.2	-	-	15.2	15.4
HCO	160.6	-	-	-	-	161.3	161.1
OAc	20.6	20.7	20.7	20.9	20.7	20.7	21.0
	21.0	20.7	20.7	20.9	-	20.7	20.7
							20.5(2)
C-Me	26.3	19.8	20.4	26.2	20.5	19.6	26.5
	23.2	18.1	19.8	20.6	20.0	16.5	20.0
	13.0	13.1	13.6	13.1	13.6	13.4	13.5
OMe	53.2	51.8	52.3	51.9	52.2	52.1	52.8
CH_3COO	170.1	170.2[c]	170.5[c]	170.7	170.5	169.9[c]	170.0(2)
	168.4	169.0	169.0	170.7		171.5	168.9
							168.5

EXPERIMENTAL

Prieurianin (1), D-4 (11) and D-5 (12) were isolated from the wood (prieurianin)[1,2] and bark (D-4 and D-5)[8] of Trichilia prieuriana by Dr. A. Harding. D-4 (11) was obtained as a gum (Found: m/e 612.25735. $C_{33}H_{40}O_{11}$ requires 612.25703). The corresponding acetate (13), prepared in the usual way, was also a gum [ν_{max} (CCl_4) 1750, 1728 cm^{-1}]. Neither D-5 (12) (Found: m/e 686.28942. $C_{36}H_{48}O_{14}-H_2O$ requires 686.29381) nor its acetate (15) [ν_{max} (CCl_4) 1775, 1750, 1730 cm^{-1}] could be induced to crystallise. Dregeanin (9)[7], compound B (18) and compound C (30) were isolated from the root bark of Guarea thompsonii by Professor D.A.H. Taylor, Durban. Compound B (18) was recrystallised from methanol and had m.p. 250°- 255°C, m/e 672, [ν_{max} (CCl_4) 3550, 1768 (sh), 1750 (sh), 1735 cm^{-1}]. Compound C (30) was also crystallised from methanol and had m.p. 290°-300°C (decomp.), m/e 498, [ν_{max} 1762, 1733 cm^{-1}]. (Found: C, 65.0 ; H, 6.0. $C_{27}H_{30}O_9$ requires C, 65.05 ; H, 6.1 %).

A specimen of rohitukin (8)[7] and a light petroleum extract of the seeds of Trichilia rokka were obtained from Professor Taylor.

HYDROLYSIS REACTIONS

The compounds were dissolved in a methanolic solution (5%) of potassium hydroxide and left for 1 hour at room temperature. The usual work-up was addition of water and acidification with acetic acid. Extraction of the aqueous solution with chloroform and removal of the solvent *in vacuo* afforded a gum which was methylated with diazomethane in ether. Purification and separation of the products was achieved in general by preparative t.l.c. using a mixture of ethyl acetate-carbon tetrachloride

(in varying proportions) as solvent system. In the case of prieurianin and rohitukin, the crude hydrolysate was acetylated in pyridine and acetic anhydride.

A. Hydrolysis of Dregeanin (9).- Dregeanin (100 mg) was hydrolysed as above. Preparative t.l.c. afforded the following four products (in order of increasing polarity):

DM-1 (25).- Obtained as a gum (5 mg), m/e 616; [ν_{max} (CCl_4) 3550, 3590, 1775, 1738 cm^{-1}]. It was transformed slowly on standing into DM-4 (28). Acetylation in pyridine and acetic anhydride (1 hour, steam-bath) afforded a non-crystalline diacetate (26), m/e 700; [ν_{max} (CCl_4) 1763, 1748 cm^{-1}]. (Found: m/e 700.30913. $C_{37}H_{48}O_{13}$ requires 700.30946).

DM-2 (22).- As a gum (54 mg), m/e 616; [ν_{max} (CCl_4) 3550, 1790, 1750, 1728]. (Found: m/e 616.28826. $C_{33}H_{44}O_{11}$ requires 616.28833). Acetylation as above afforded the crystalline diacetate (24), m.p. 181°-184°C (ex. methanol-ether), m/e 700; [ν_{max} 1750, 1785 cm^{-1}]. (Found: m/e 700.30934. $C_{37}H_{48}O_{13}$ requires 700.30946).

DM-3 (23).- Crystallised from ether-light petroleum (29 mg), m.p. 206°- 209°C, m/e 616; [ν_{max} (CCl_4) 3550, 1790, 1750, 1738 cm^{-1}]. (Found: m/e 616.28803. $C_{33}H_{44}O_{11}$ requires 616.28833). Acetylation as before afforded a non-crystalline diacetate (23a), m/e 700; [ν_{max} (CCl_4) 1790, 1750 cm^{-1}]. (Found: m/e 700. 30934. $C_{37}H_{48}O_{13}$ requires 700.30946).

DM-4 (28).- Crystallised from methanol-ether (10 mg), m.p. 229°-234°C, m/e 584; [ν_{max} (CCl_4) 3535, 3520, 1720-40 (br), 1760 cm^{-1}]. (Found: m/e 584.2622. $C_{32}H_{40}O_{10}$ requires 584.2621). Acetylation gave the crystalline monoacetate (29), m.p. 243°- 247°C (ex. methanol-ether), m/e 626; [ν_{max} (CCl_4) 1775, 1760, 1750 cm^{-1}]. (Found: m/e 626.27229.

$C_{34}H_{42}O_{11}$ requires 626.27268).

B. Hydrolysis of Prieurianin (1).- Prieurianin (1) (390 mg) was hydrolysed as above. The methylated product was acetylated in the usual way and purified by preparative t.l.c. to give the following three compounds (in order of increasing polarity):

Ac. PM-2 (31).- Obtained as a gum (56.5 mg), m/e 716; [ν_{max} 3570, 1790, 1750 cm^{-1}]. (Found: m/e 716.30410. $C_{37}H_{48}O_{14}$ requires 716.30437).

Ac. PM-3 (32).- Crystallised from methanol-ether (40.9 mg), m.p. 204°- 207°C, m/e 716, [ν_{max} (CCl_4) 3565, 1788, 1750 cm^{-1}]. (Found: m/e 716.30490. $C_{37}H_{48}O_{14}$ requires 716.30437).

Ac. PM-4 (33).- Crystallised from methanol-ether-light petroleum, (33.4 mg), m.p. 244°-248°C, m/e 602, [ν_{max} (CCl_4) 3480(sh), 3380, 1755 cm^{-1}]. (Found: m/e 602.23631. $C_{31}H_{38}O_{12}$ requires 602.23629).

C. Hydrolysis of Rohitukin (8).- Rohitukin (8) (260 mg) was hydrolysed as above. Acetylation of the methylated product afforded mainly one compound, Ac. RM-1 (76.2 mg), which crystallised from methanol-ether, m.p. 148°- 154°C, m/e 644, [ν_{max} (CCl_4) 1745, 1785, 3400, 3580 cm^{-1}]. (Found: C, 63.22; H, 6.98. $C_{34}H_{44}O_{12}$ requires C, 63.67 ; H, 6.83 %). Traces of two other compounds were isolated from the residual complex mixture but they could not be purified and characterised.

A sample of the light petroleum extract of the seeds of T. rokka (500 mg) was separated by preparative t.l.c. using ethyl acetate-carbon tetrachloride (50%) as solvent system. The front band yielded a small quantity of rohitukin (8). The main component, R-6 (19) (116 mg) [ν_{max} (CCl_4) 3550, 3710, 1755, 1730 cm^{-1}] could not be induced to crystallise. Acetylation gave the non-crystalline acetate (20) [ν_{max} (CCl_4) 3600,

1756 cm^{-1}). Neither of these compounds gave suitable mass spectra, but their ^{1}H and ^{13}C n.m.r. spectroscopic properties were consistent with the proposed structures.

D. Hydrolysis of D-4 (11).- D-4 (30 mg) was hydrolysed as above. Analytical t.l.c. indicated that the methylated product was mainly DM-3 (23). Crystallisation from ether-light petroleum afforded DM-3 (23) (18 mg), m.p. 206°- 209°C, identical with authentic material (m.m.p and ^{1}H n.m.r.).

E. Hydrolysis of Compound B (18).- Compound B (40 mg) gave the same result as D above, yielding only DM-3 (23) (30 mg), m.p. 206-209°C.

F. Hydrolysis of D-5 (12).- The hydrolysis of D-5 was carried out on a small scale (1 mg) in view of the scarcity of material. Analytical t.l.c. showed that the product consisted of four spots corresponding to DM-1, DM-2, DM-3 and DM-4.

G. Equilibration Reactions.- Small quantities (1 mg) of the dregeanin hydrolysis compounds were exposed to the hydrolysis conditions and the reaction products, after methylation, examined by analytical t.l.c. DM-1 (25), DM-2 (22), and DM-4 (28) gave the same mixture of three spots corresponding to (25), (22) and (28) whereas DM-3 (23) was recovered largely unchanged as a single spot.

Reduction of Prieurianin.- Prieurianin (250 mg) was dissolved in methanol and an excess of sodium borohydride was added. The reaction was left at r.t. for 20 minutes and then water was added. Acidification to pH-2 with dilute hydrochloric acid, extraction with chloroform and removal of the solvent afforded a gum which was acetylated in the usual way. Preparative t.l.c. using ethyl acetate-carbon tetrachloride (50 %) afforded two main bands. The less polar product, Ac. PR-1 (21) (61 mg), [ν_{max}

(CCl_4) 3510, 1750, 1730(sh) cm^{-1}] had similar chromatographic properties to Ac. R-6 (20). It failed to give a reasonable mass spectrum but its spectroscopic properties were in accord with the proposed structure. The major product (122 mg) was not investigated further since it did not resemble Ac. R-6. The appearance of several acetate resonances in its ^1H and ^{13}C n.m.r. spectra suggested that it was a product of over-reduction.

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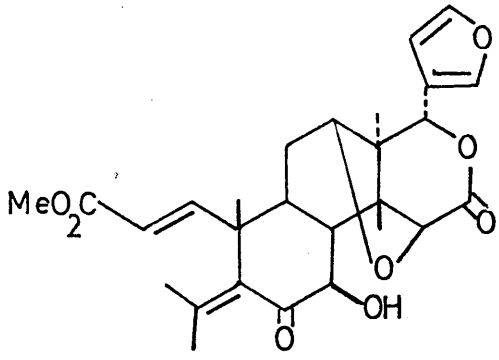
CHAPTER V

ATALANTIN AND ATALANTOLIDE :

LIMONOIDS FROM ATALANTIA MONOPHYLLA



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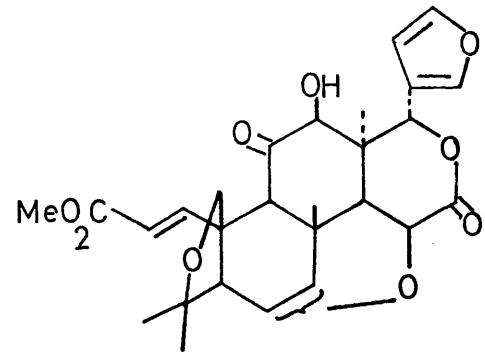

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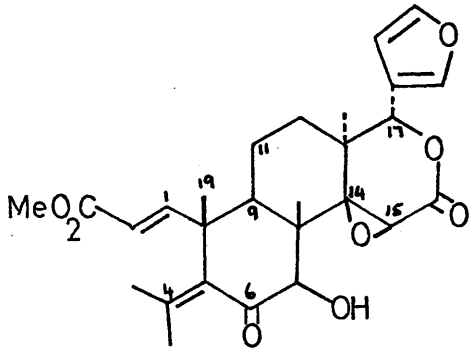

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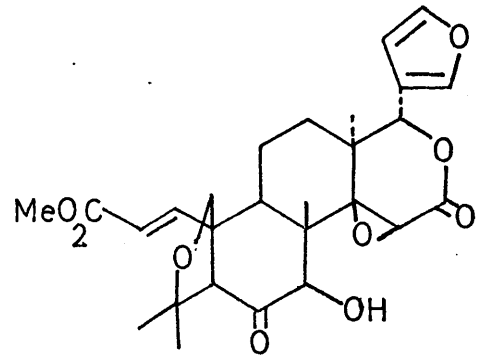

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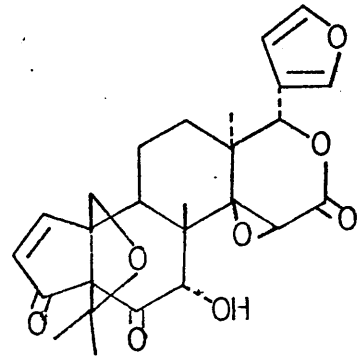

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INTRODUCTION

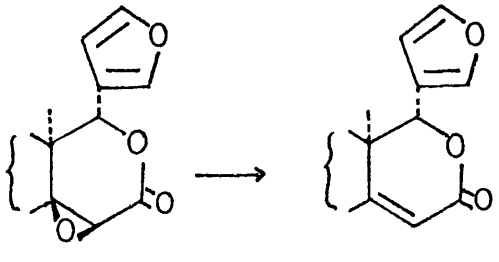
The Rutaceae and Meliaceae families, are the main sources of limonoids in the plant kingdom.[1] Among the genera included in the first family, is Atalantia, closely related to the well studied Citrus.[2] Eleven species of Atalantia are known and they occur throughout the mountains of India, Ceylon and Burma. Their use in local medicine[3], for various purposes, including treatment against snake bite, have made them the object of chemical studies. The main natural products isolated from three species are acridone alkaloids[4,5], which occur along with triterpenes[6], sesquiterpenes[7], coumarins[6,8] and the limonoids, atalantin and atalantolide.[5,9,10,11] The last have been found only in the root bark of A. monophylla.

Our interest in this species was aroused by the structures (1) and (2), proposed for atalantolide and atalantin. These structures appeared to us to be unusual in relation to the generally accepted biogenesis of limonoids.[1] In the following pages, we present and discuss the evidence that led us to the revised structures (3) and (4) for atalantolide and atalantin.

During our work, Dreyer and his colleagues published definitive evidence for the revised structure (4) for atalantin.[11] They also reported the isolation of an interesting compound, cycloepiatalantin (5), closely related to the two compounds mentioned above. Although they did not isolate atalantolide from the extract, they suggested structure (3) for it.

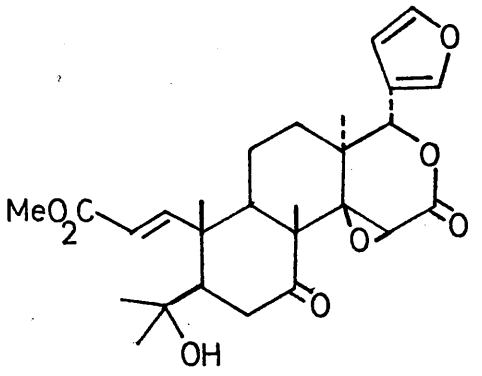
D I S C U S S I O N

The structure (1) proposed by Shringarpure and Sabata[5] for atalantolide, a tetranortriterpenoid from the root bark of Atalantia monophylla had some unusual features. In particular, it had a furan ring, a ring D lactone and oxygenation at C-7 and yet, had not undergone the apo-rearrangement. This made it unique in biogenetic terms. The authors were led to the conclusion that C-8 lacked the expected methyl substituent by the fact that the corresponding diketone, obtained by Jones oxidation of atalantolide, was capable of enolisation. In addition, C-14 had to be fully substituted since H-15 was a sharp singlet. They discarded the possibility of a typical 14,15-epoxide on the basis of the failure of atalantolide to react with chromous chloride. This reagent reduces the characteristic ring D epoxy-lactone system (6) of limonoids, to the corresponding deoxy-lactone (7). It is significant that the successful use of this reagent requires complete exclusion of oxygen from the reaction vessel. The chemical shift of H-15 (δ_H 4.24) indicated that it was



(6) (7)

attached to a carbon atom bearing an ether oxygen. The other terminus of this ether had to be secondary, since it was considered that the multiplet at 3.4 p.p.m. also corresponded to an ether proton. In an unrearranged skeleton the only positions available for this second ether



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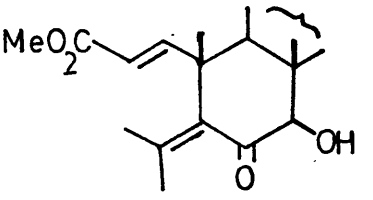

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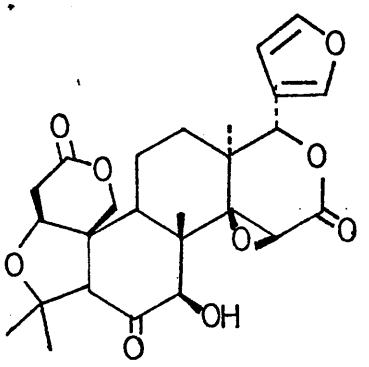

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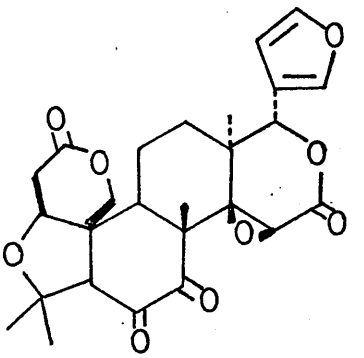
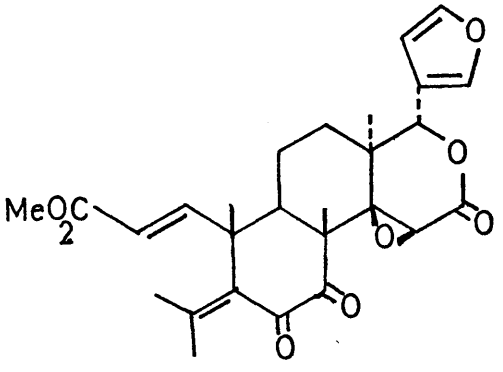


(11)

terminus are C-11 or C-12. Thus they arrived at structure (1).

The lack of convincing support for these assignments, and the ambiguity of the structure, led us to reconsider the original evidence and to supplement it by a study of the ^{13}C n.m.r. spectra of atalantolide for which we propose the biogenetically reasonable structure (3).

The ^{1}H n.m.r. spectrum (Table 1) had resonances for five C-methyl groups, three tertiary and two attached to a tetrasubstituted double bond (δ_H 1.81 and 2.04, both bs). This, together with an α,β unsaturated methyl ester, suggested a ring A cleaved tetranortriterpenoid skeleton, similar to that of methyl obacunoate[12](8). The spectrum also showed the characteristic [1] signals for a β-substituted furan ring, the low field singlet for H-17 indicating the lactonic nature of ring D, H-15, and a secondary hydroxyl group [δ_H 4.83 (H-7), 4.24 (OH); both d, J 2.9 Hz] forming part of an α-ketol, (oxidation gives the α-diketone), as deduced by the i.r. spectrum and the downfield chemical shift of H-7 which normally appears at 4.0 p.p.m. The unsaturated nature of the ketonic function, on the other hand, [ν_{max} (CCl_4) 1667 cm^{-1}; λ_{max} 256 nm (ε 6,300)], required it to be at C-6. These facts led to the partial structure (9) for rings A and B. The β configuration of the C-7 hydroxyl group was assigned on the basis of the low chemical shift of H-15 (Table 1), by analogy with rutaevin[13] (10). Acetylation produced as expected for a hydroxyl group at C-7[14], a shift towards higher field (0.43 p.p.m.) in the H-15 signal. Oxidation to the α-diketone resulted in a slightly bigger shift (0.52 p.p.m.) of H-15. The corresponding shifts in the rutaevin series were 0.33 and 0.54 p.p.m., respectively.[13] Further support for this assignment comes from the observed 15% N.O.E. in the H-7 signal upon irradiation at the frequency of H-9, (δ_H 3.39 - see below).



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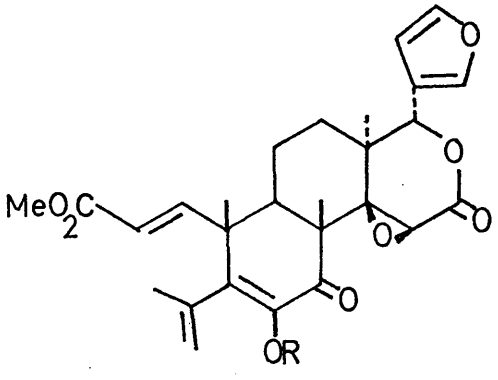

(13) R = H

(14) R = Ac

The similarity between atalantolide and rutaevin strongly suggested the presence of the 14,15 epoxide in the former. Convincing support was obtained by analysis of the ^{13}C n.m.r. spectra (Table 2). The ^{13}C chemical shifts for C-15 and C-14, of the typical ring D epoxy lactone system in limonoids, are well documented[14],[eg. limonin (11), δ_C 67.4 (s, C-14) and 53.9 (d, C-15)]. The presence of this system in atalantolide followed from the resonances at δ_C 67.4 (s, C-14) and 51.3 (d, C-15). The existence of only one secondary carbon atom bearing oxygen, ruled out the possibility of an ether at either C-11 or C-12 as previously proposed. The proton resonating at δ_H 3.39 (m, H-9), which the Indian authors considered to be an oxide proton[5], is associated with a carbon doublet at δ_C 44.3 and hence cannot be attached to a carbon atom bearing oxygen. This was determined by calculation of the value expected for δ_H from the residual splittings in the ^{13}C off-resonance spectra at 0 and 8 p.p.m.[15] In addition, the mass spectrum of atalantolide had a peak at m/e 361 resulting from the characteristic cleavage of the ring D epoxy lactone.[16]

Further evidence to support this structure was obtained by an examination of the products of Jones oxidation. The reported product[5], the yellow crystalline diketone (12) was difficult to purify. Analytical t.l.c. and the ^1H n.m.r. spectrum revealed the presence of a second compound. The u.v. spectrum [λ_{max} 284 (ε 6,000); λ_{max} (OH^-) 352 (ε 6,300); λ_{max} (H^+) 284 nm] suggested that this was the corresponding enol form. Treatment of (12) with base, followed by acidification and preparative t.l.c. afforded the pure, non-crystalline enol, whose spectroscopic properties indicated the structure (13). The significant resonances in the ^1H n.m.r. spectrum (Table 1) included those for three tertiary methyl groups (δ_H 1.10, 1.12 and 1.48), a vinylic methyl (δ_H 1.90), an exocyclic

methylene (δ_H 4.91 and 5.16) and an enolic hydroxyl (δ_H 6.10, exchangeable with D_2O). Irradiation at the vinylic methyl frequency resulted in a sharpening of the exocyclic methylene signals. These results show that enolisation of (12) involves the removal of a proton from one of the methyl groups attached to C-4. Acetylation of (13) afforded the enol acetate (14) [λ_{max} 246 nm (ε 6,200); ^1H n.m.r. parameters in Table 1].

The structure (2) previously proposed for atalantin [9], the second limonoid from the extract, showed two unusual features, namely, an ether bridge between C-19 and C-4, and an unlocalised cyclic ether between C-15 and either C-7 or C-6. The latter was proposed as a consequence of the negative results of chromous chloride reduction, as in the case of atalantolide (3). Dreyer has since prepared deoxy-atalantin by chromous chloride reduction[11] and independently arrived at the proposed structure (4), which we have assigned to atalantin.

The ^1H n.m.r. spectrum of atalantin was very similar to that for atalantolide (see Table 1). It had the characteristic signals for a β-substituted furan ring, H-17 as a low field singlet (δ_H 5.53), indicating the presence of the ring D-lactone, H-15 as a sharp singlet at 4.44 p.p.m. which indicated, as in the case of atalantolide, the presence of a C-15 oxide and a substituent at C-14 (a fact ignored by the Indian authors), an α, β-unsaturated methyl ester, H-9, and an α-ketol system [ν_{max} (CCl_4) 1710 cm^{-1}; δ_H 4.77 (s, after D_2O exchange, H-7)]. The appearence of four tertiary methyl groups and an AB quartet (δ_H 3.79 and 4.17, J 9.5 Hz; 2H-19), suggested that it belonged to the limonin series with C-19 oxidised.[1] To accommodate the tertiary methyls, the cyclic ether in atalantin must be from C-19 to C-4. This is attractive in biogenetic terms since it can arise by hydroxylation of C-19 in atalantolide (3) followed by Michael addition of the hydroxyl group to the enone system.

As with atalantolide, oxidation of atalantin produced an upfield shift of the H-15 resonance (0.66 p.p.m.), indicating that the hydroxyl is at C-7 (β) and not at C-12 as proposed previously.[9] Since the hydroxyl group is part of an α-hydroxy ketone system, the keto group could only be located at C-6. This arrangement was further supported by a one proton singlet at 3.11 p.p.m. (br), (consistent with a proton next to a carbonyl group), attributable to H-5. This resonance is also observed in the ^{1}H n.m.r. spectrum of rutaevin[13] (10). Decoupling experiments demonstrated a long range coupling (1 Hz) between H-5 and H-7 which requires a boat conformation for ring B. The β configuration of the C-7 hydroxyl was corroborated by observation of N.O.E. between H-7 and H-9 (ca. 15% enhancement either way), even though the geometry of a ring B boat conformation is not such as to maximise these effects.

As with atalantolide, the presence of the C-14,15 epoxide followed from the characteristic signals at δ_C 68.5 (s, C-14) and 52.9 (d, C-15), in the ^{13}C n.m.r. spectrum (Table 2). The above information and previous chemical results[9], can be satisfactorily accomodated in the structure (4) for atalantin, which fits well into the general structural pattern of limonoids found in the Rutaceae, although the C-19,4 ether system is a novel feature.

Table 1

^{1}H n.m.r. spectra of atalantin, atalantolide and derivatives

	(3)	acetate	(12)	(13)	(14)	(4)
C-Me	0.68	0.82	1.04	1.10	1.07	0.89
	1.36	1.30	1.08	1.12	1.17	1.24
	1.41	1.41	1.63	1.48	1.48	1.30
	1.81	1.78	1.96	1.90	1.82	1.36
	2.04	2.00	2.16			
2H-19						3.79 (d, J 9.5)
						4.17 (d, J 9.5)
H-9	3.39(m)	3.40(m)	3.46(m)	3.34(m)	3.32(m)	3.34 (m)
H-5						3.11 (bs)
CO_2Me	3.57	3.61	3.52	3.63	3.64	3.69
OH	4.24 (d J 3)					3.84(br)
H-15	4.24	3.81	3.72	4.01	3.87	4.44
H-7	4.83 (d, J 3)	6.00				4.77 (br)
H-17	5.51	5.48	5.44	5.42	5.42	5.53
H-2	5.75 (d, J 12)	5.70 (d, J 12)	5.69 (d, J 12)	5.85	5.86	5.90 (d, J 12)
H-1	6.37 (d, J 12)	6.30 (d, J 12)	6.38 (d, J 12)	5.85	5.86	6.62 (d, J 12)
H-22	6.39 (dd, J 1.0 and 2)	6.36(m)	6.38(m)	6.37(m)	6.37(m)	6.36(m)
H-21 and H-23	7.40(m)	7.39(m)	7.40(m)	7.41(m)	7.39(m)	7.41(m)
OAc		2.26			2.16	
$\rangle$=CH_2				4.91(br)	4.76(br)	
				5.16(br)	5.08(br)	

Table 2

^{13}C n.m.r. spectra[a] of atalantolide and atalantin

Carbon no.	(3)	(4)
1	154.8	163.3
2	118.0	120.1
3	166.3	165.9
4	152.8	84.4
5	135.8	64.5
6	201.1	209.1
7	79.8	80.0
8	45.0[b]	43.9
9	44.3	40.2
10	45.2[b]	52.7
11	20.3	20.4
12	32.4	30.6
13	37.9	38.1
14	67.4	68.5
15	51.3	52.9
16	167.7	167.7
17	78.3	78.1
19		75.0
20	120.5	120.5
21	141.0	141.1
22	110.1	110.0
23	142.9	143.0
C-Me	29.1	31.1
	25.6	25.0
	24.4	
18	20.0	19.7
30	13.0	12.5
CO_2Me	51.4	51.9

a The assignments of C-21 and C-23 agree with ref. 17 but not with refs. 11 and 18; we have confirmed our assignments by means of double resonance correlation for compounds where H-21 and -23 are chemically shifted.

b These assignments may be interchanged.

EXPERIMENTAL

The samples of atalantin and atalantolide were obtained from Dr. B. Sabata, and their isolation and characterisation are described in references 5 and 9.

Oxidation of Atalantolide.- Jones reagent (10 drops) was added slowly to a stirred solution of atalantolide (50 mg) in acetone (5 ml) at 0°C. The solution was left at this temperature for 5 min., diluted with water and extracted with chloroform. The crude product was crystallised from chloroform-light petroleum and then methanol, to give the dione (12) (20 mg) as yellow crystals, m.p. 180°-181°C (lit.[5], 170-172°C). Analytical t.l.c. of the dione and the mother liquors indicated the presence of a less polar compound, which was isolated by preparative t.l.c. to yield the non-crystalline enol (13) (24 mg), [ν_{max} (CCl_4) 3455, 1683, 1731 and 1754 cm^{-1}]. (Found: m/e 482.19411. $C_{27}H_{30}O_8$ requires M^+, 482.19404). Exposure of the dione (12) under mild aqueous alkali followed by acidification and preparative t.l.c., afforded mainly the enol, (13). It is interesting to note that the dione could be obtained pure as crystals, but once in solution or in t.l.c. plates, it equilibrated to produce the enol, which on the other hand, was quite stable in solution.

The Enol Acetate (14).- The enol (13) (30 mg) was acetylated with acetic anhydride-pyridine at r.t. overnight. Preparative t.l.c., of the crude product gave the non-crystalline enol acetate (14) (15 mg), [ν_{max} (CCl_4) 1707, 1730 and 1762 cm^{-1}]. (Found: m/e 524.20457. $C_{29}H_{32}O_9$ requires M^+, 524.2046).

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CHAPTER VI

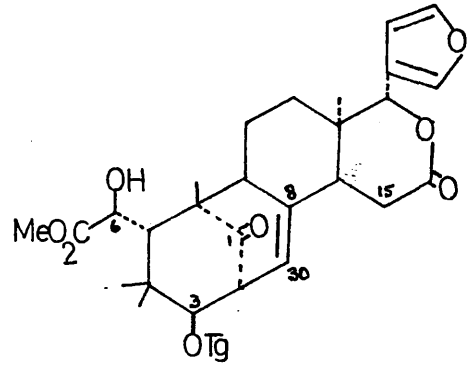
INTERRELATION OF SWIETENINE

AND

SWIETENOLIDE

I N T R O D U C T I O N

The structures of swietenine (1)[1] and swietenolide (2)[2,3] the major constituents of the seeds of Swietenia macrophylla, have been known for more than a decade. They are both bicyclononanolides but differ in the position of the residual double bond. During the course of the structural work on swietenine[1] extensive efforts were made to oxidise or to migrate the $\Delta^{8(30)}$ double bond but without success. The singular lack of reactivity of this double bond thwarted previous efforts to interrelate swietenine(1) and swietenolide (2). This problem has now been overcome by a series of simple reactions which resulted in the conversion of swietenine into swietenolide diacetate (10). The details of this work are described in the following pages.



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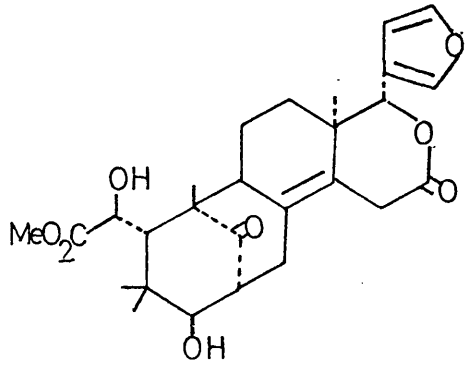

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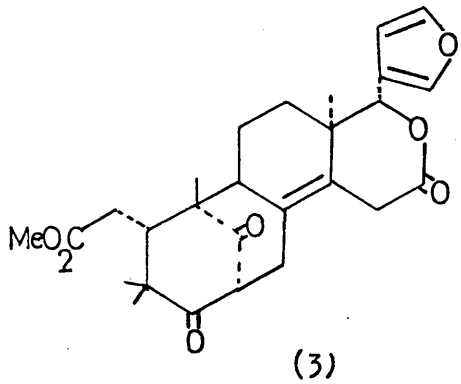

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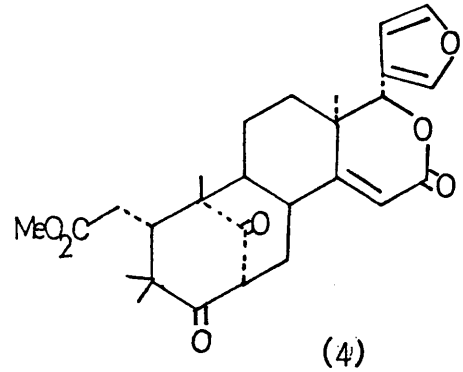

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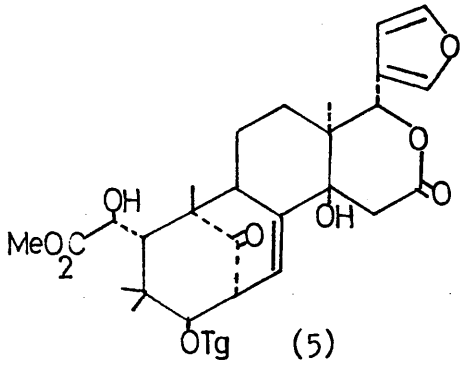

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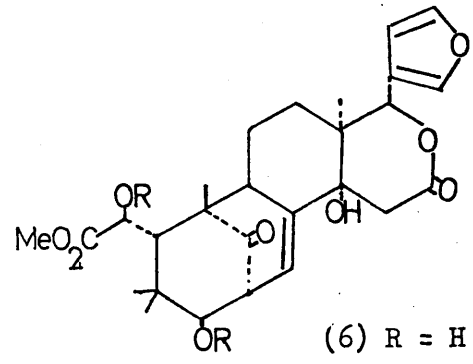
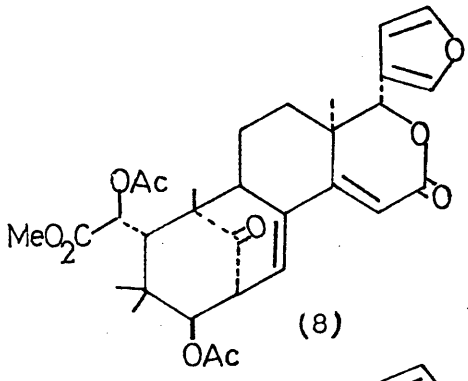


(6) R = H

(7) R = Ac



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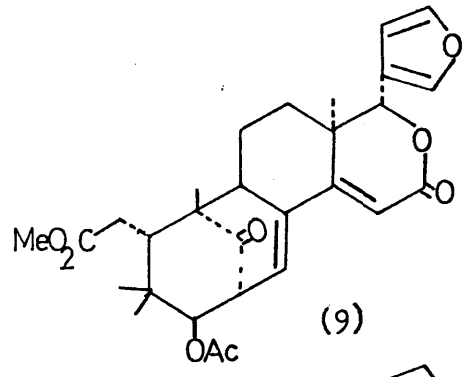

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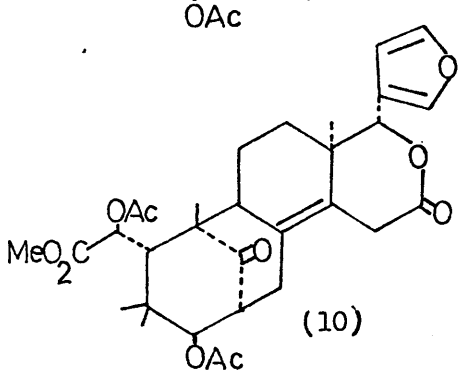

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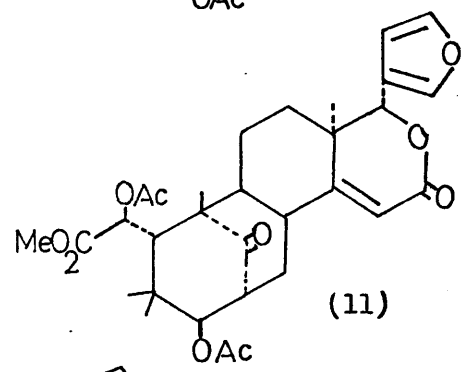

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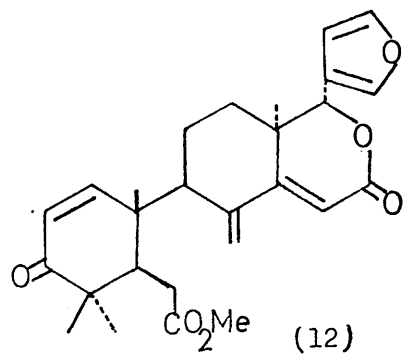

(12)

DISCUSSION

Selenium dioxide oxidation of mexicanolide (3) or carapin (4) has been reported[4] to result in the introduction of a t-hydroxyl group at C-8. On treatment with selenium dioxide swietenine (1) was smoothly converted into hydroxyswietenine (5). The new tertiary hydroxyl group was placed at C-14 rather than C-9 since hydroxyswietenine was transformed into the cisoid diene-lactone (8) on dehydration (see below). The C-14 hydroxyl group was assigned the α configuration by inspection of models since this could better account for the observed downfield shift (0.25 p.p.m.) of H-30 in the ^1H n.m.r. spectrum of (5) relative to swietenine (1). Kupchan cleavage[5] ($OsO_4/NaIO_4/HCO_3^-$) of the tiglate ester afforded the triol (6) which was converted into the diacetate (7) in the usual manner. Reaction of (7) with thionyl chloride in pyridine at 0°C yielded the diene (8) [λ_{max} 281 nm ; δ_H 6.19 (s, H-15), 6.26 (dd, J 6,3 Hz, H-30)] whose spectroscopic properties compared well with those of augustidienolide (9)[6] [λ_{max} 283 nm ; δ_H 6.23 (s, H-15) and 6.31 (dd, J 5.5, 3.5 Hz, H-30)]. Hydrogenation of the diene (8) over 10%/Pd/C in ethyl acetate resulted in 1,4-addition to the cisoid diene-lactone system with formation of swietenolide diacetate (10)[3] identical (^1H n.m.r., t.l.c., m.p., m.s.), with an authentic specimen. Thus the long awaited interrelation of swietenine and swietenolide was achieved. A second product of the hydrogenation was the Δ^{14}-derivative (11) [δ_H 5.85 (s, H-15)]. Similar results have been reported for the hydrogenation of augustidienolide[6] and deoxyandirobin (12).[7]

EXPERIMENTAL

Allylic Oxidation of Swietenine.- Swietenine (650 mg) was refluxed in aqueous acetic acid (10 ml) and selenium dioxide (280 mg) for 1 hour. The solution was allowed to cool at room temperature and then poured into excess water. Extraction with chloroform and removal of the solvent *in vacuo*, afforded a gum which was chromatographed over Grade IV alumina in light petroleum. Elution with ether gave the crystalline 14-hydroxyswietenine (5) (200 mg), m.p. 255°- 258°C (ex. ether-methanol), [m/e 584, δ_H 0.89, 0.94, 1.09 and 1.44 (C-Me), 3.73 (3H, CO_2Me), 3.50 (dd, J 9,6 Hz, H-2), 3.47 (bs, H-5), 4.54 (bs, H-6), 4.65 (d, J 9 Hz, H-3), 5.52 (s, H-17), 5.57 (d, J 6 Hz, H-30), 2.90 and 2.25 (hydroxyls, exchangeable with D_2O), 1.68 and 1.76 (6H, m, tiglate methyls), 6.83 (m, tiglate vinyl proton), 7.59, 7.44 and 6.42 (furan)]. (Found: m/e 584.26230. $C_{32}H_{40}O_{10}$ requires 584.26243).

Diacetate (7).- 14-hydroxyswietenine (147 mg) and osmium tetroxide (65 mg) were kept in ether (1 ml) and pyridine (1 ml) in the dark for 16 hours. A saturated solution of sodium metabisulphite in water (10 ml) was added and the mixture stirred for two hours. The resulting mixture of tetrols was dissolved in methanol and treated with an excess of aqueous sodium metabisulphite solution overnight. The crude pyruvate ester obtained by dilution with water and extraction into chloroform was hydrolysed with aqueous sodium hydrogen carbonate solution at r.t. for 5 minutes. The reaction product was acetylated with acetic anhydride in pyridine for 4 hours over a steam-bath. Analytical t.l.c. of the crude product indicated the presence of a main product which was identified as the 3,6-diacetate (7) by a study of its ^1H n.m.r. spectrum [δ_H 0.80, 1.00, 1.05, 1.15 (C-Me), 2.04 and 2.17 (acetates), 3.72 (CO_2Me), 3.50 (dd, J 9,6 Hz, H-2), 5.55(s, H-6),

4.64 (d, J 9 Hz, H-3), 5.61 (s, H-17), 5.60 (d, J 6 Hz, H-30), 6.47, 7.43 and 7.73 (furan)].

Diene (8).- The diacetate in pyridine was treated with thionyl chloride (10 drops) at 0°C for 5 min. Addition of water and extraction with chloroform afforded a crude product which was purified by column chromatography over Grade IV alumina in light petroleum -ether (9:1). The fractions eluted with ether afforded the non-crystalline diene (8) (60 mg), m/e 568, [δ_H 0.92, 1.04, 1.07, 1.20 (C-Me), 2.16 and 2.18 (OAc's), 3.74 (3H,s, CO_2Me), 3.46 (s, H-5), 3.64 (obsc. m, H-2), 4.75 (d, J 9 Hz, H-3), 5.13 (s, H-17), 5.40 (bs, H-6), 6.26 (dd, J 6,3 Hz, H-30), 7.53, 7.43 and 6.48 (furan)]. (Found: m/e 568.23078. $C_{31}H_{36}O_{10}$ requires 568.23082).

Catalytic Hydrogenation of the Diene.- The diene (47 mg) was dissolved in ethyl acetate (10 ml) and stirred with 10% Pd/C (30 mg) in a hydrogen atmosphere. The reaction stopped after 2 minutes when 3 ml of hydrogen were consummed. Preparative t.l.c. of the product afforded swietenolide diacetate (10) (34.2 mg), m.p. 220°- 225°C (ex. ether-light petroleum), (lit.[3], 225°- 228°C), m/e 570, [δ_H 0.86, 1.04, 1.07, 1.16 (C-Me), 2.16 (6H, OAc's), 3.74 (3H,s, CO_2Me), 3.41 (bs, H-5), 3.15 (m, H-2), 4.87 (d, J 10 Hz, H-3), 5.60 (s, H-17), 5.47 (s, H-6), 6.46, 7.42 and 7.52 (furan)]. Extraction of a minor band in the plate afforded the non-crystalline 3,6-diacetoxydehydrocarapin (11) , (12.9 mg), m/e 570, [δ_H 0.96, 0.98, 1.16, 1.20 (C-Me), 2.09 and 2.14 (OAc's), 3.73 (3H, s, CO_2Me), 2.94 (bs, H-5), 3.17 (m, H-2), 5.10 (s, H-17), 5.58 (s, H-6), 5.45 (d, J 10 Hz, H-3), 5.85 (s, H-15), 7.43 (2H,m) and 6.4 (furan)]. (Found: m/e 570.24632. $C_{31}H_{38}O_{10}$ requires 570.24647).

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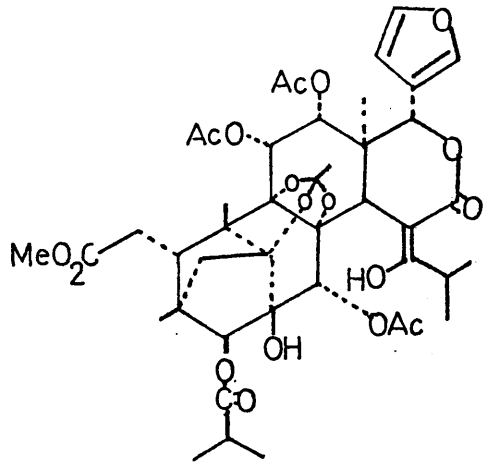
CHAPTER VII

STRUCTURE ELUCIDATION

OF

EKEBERGINS I AND II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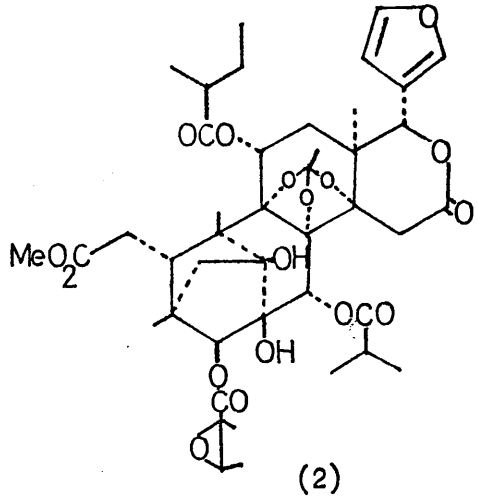
OAc
OH
O
CO

(1)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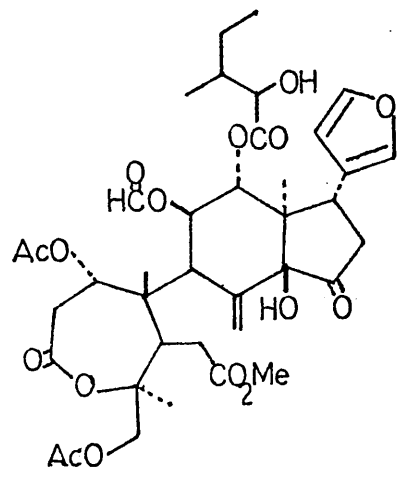
OCO
OH
O
CO

(2)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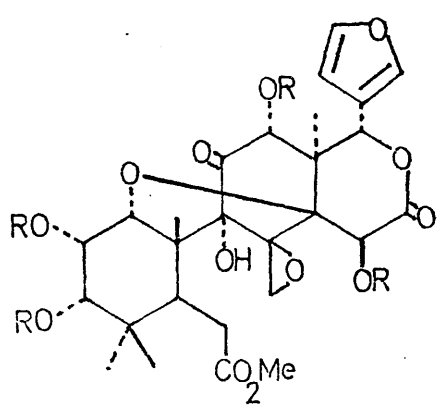
(3)

INTRODUCTION

During the early investigations of the trees belonging to the Meliaceae family, the Ibadan group of Ekong, Bevan and Taylor isolated several highly oxygenated tetranortriterpenoids (eg, bussein (1), entandrophragmin (2), and prieurianin (3), see Chapter 1), with novel structures which were too complex for immediate solution at that time.[1] Since then the structures of most of these compounds have been elucidated, often with the aid of X-ray analysis. One notable exception, however, is ekebergin which was isolated from both seeds and heartwood of Ekebergia senegalensis. Ekebergin occurs in several modifications, described in the original literature[1] as A, B, and C, which differ in the nature of the esterifying groups. Ekebergin I (ekebergin B), kindly supplied by Professor D.A.H. Taylor, Durban, has an isobutyrate, a 2-methylbutyrate and two acetates whereas ekebergin II (ekebergin A), kindly extracted from the seeds by Dr. D.A. Okorie, Ibadan, has an isobutyrate and three acetates.

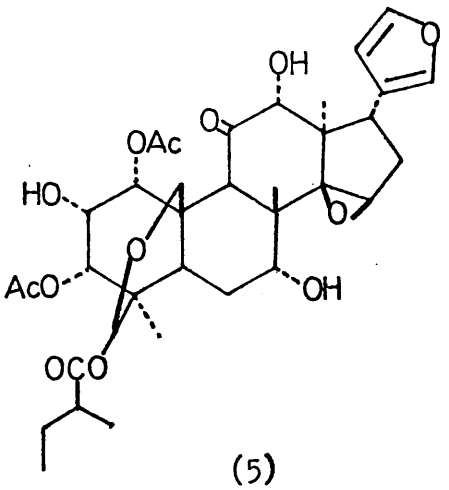
A detailed examination of the spectroscopic properties led to the biogenetically reasonable structure (4) for the ekebergin nucleus. The positions of the different esters were not determined.



(4)

D I S C U S S I O N

Ekebergin I, $C_{40}H_{52}O_{17}$, had hydroxyl and carbonyl absorptions in the i.r. [ν_{max} (CCl_4) 3425, 1778, 1767, 1755, 1738 and 1688 cm^{-1}]. The mass spectrum showed a parent ion at m/e 804 and a small amount of a homologue at m/e 818. The functional groups identified spectroscopically included a ketonic carbonyl (δ_C 207.2), a carbomethoxyl (δ_H 3.68 ; δ_C 173.4 and 52.1), two acetates (δ_H 2.18 and 1.93 ; δ_C 169.7, 168.4, 20.4 and 20.1), an isobutyrate [δ_H 2.69 (septet, J 7 Hz), 1.15 and 1.17 (both d, J 7 Hz) ; δ_C 176.9, 33.5(d), 19.2 and 18.3], a 2"-methylbutyrate [δ_H 2.55(m, H-2"), 1.60(m, 2H-3"), 1.12(d, J 7 Hz) and 0.65 (t, J 7 Hz) ; δ_C 40.2(d, C-2"), 27.1(t, C-3"), 15.5 and 10.9], a ring D lactone [δ_H 6.60 (s, H-17) ; δ_C 79.7(d, C-17) and 166.0(C-16)], a 1,1-disubstituted epoxide [δ_H 2.92 and 2.57(ABq, J 5 Hz, 2H-30) ; δ_C 59.5(s, C-8) and 45.4(t, C-30)] and a tertiary hydroxyl group [δ_H 4.24(s, exchangeable with D_2O) ; δ_C 84.2 or 83.9(s)]. The other ^{13}C signals between 60 and 90 p.p.m. and their correlated ^1H signals were at δ_C 75.5(d) and δ_H 5.34 (s, H-12), δ_C 75.0(d) and δ_H 4.60 (d, J 3 Hz, H-1), δ_C 74.1(d) and δ_H 5.25 (d, J 4 Hz, H-3), δ_C 68.9(d) and δ_H 5.89 (s, H-15), δ_C 65.8(d) and δ_H 5.44 (t, J 4 Hz, H-2) and δ_C 84.2 or 83.9 (s), of which four had to bear ester substituents. The preceding functional groups, and the furan ring accounted for sixteen of the seventeen oxygens. Thus the remaining oxygen was involved in an ether ring. Only one of the lowfield proton signals was unchanged on hydrolysis and this suggested a secondary-tertiary ether. In addition, there remained two double bond equivalents. The molecule was therefore, bicarbocyclic and had one ring cleaved in addition to the ring D lactone. The presence of four tertiary methyl groups (δ_H 0.89, 1.17, 1.29 and 1.34;



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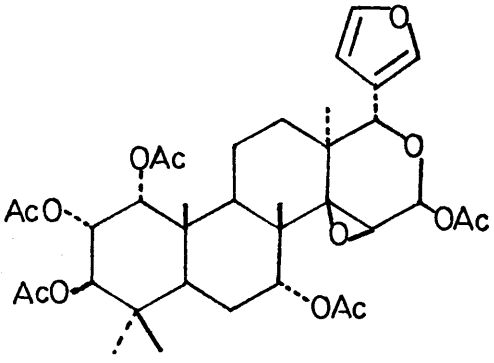

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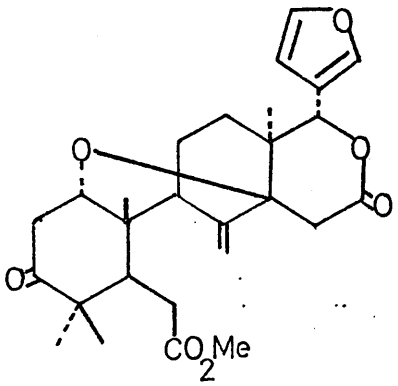
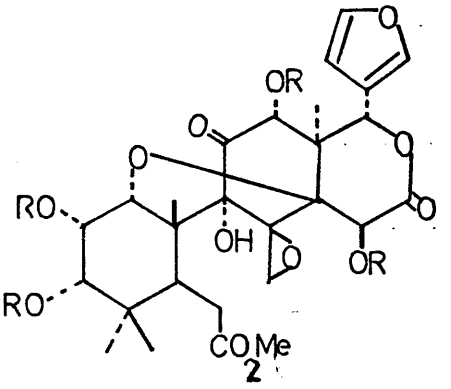


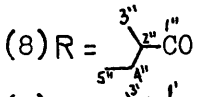
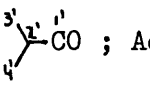
(7)

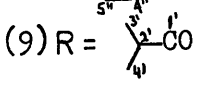
δ_C 11.1, 20.1, 22.9 and 27.3) led to the same conclusion. The fifth tertiary methyl group of the original apo-tirucallol nucleus, which often appears as an exomethylene in a ring B cleaved system, was concealed as the epoxide. It was apparent that ekebergin had a ring B cleaved skeleton since cleavage of ring A or ring C was excluded by ^1H coupling requirements and Nuclear Overhauser Effects (NOE's) respectively (see below).

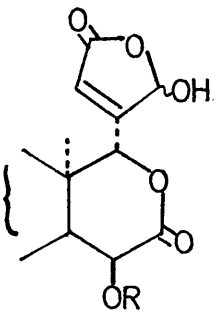
The next stage was the placing of the various functional groups in a ring B cleaved skeleton. A limited amount of information was available from double resonance experiments which demonstrated a three spin system consisting of resonances at δ_H 4.58 (d, J 3 Hz, H-1), 5.44 (t, 3.5 Hz, H-2) and 5.28 (d, J 4 Hz, H-3). This could only be accommodated in ring A. The stereochemistry of the oxygen substituents (1α, 2α, 3α) was assigned by analogy with aphanastatin (5)[2] and by comparison with the gedunin derivative (6). The demonstration by homonuclear decoupling of a large range coupling between H-17 and the furan protons confirmed the ring D lactone. The protons associated with the isobutyrate and 2"-methylbutyrate were readily identified (see Table I). The remaining spin system was an ABX associated with H-5 and 2H-6. The large observed value of J_{AB} (19 Hz) was consistent with the presence of a carbonyl group (carbomethoxyl) at C-7. The ether ring was attached to C-1 and C-14 by analogy with methyl angolensate (7)[3] and since it permitted one of the downfield singlets associated with a secondary ester to be H-15. The remaining oxygen functions were placed in ring C as in (8) by analogy with aphanastatin (5)[2], and confirmed by the NOE experiments below.

The evidence presented so far did not lead uniquely to structure (8) and did not define stereochemistry at C-12 and C-15. However further support was obtained from the observation of several NOE's. These were



(8) R =  ;  ; Ac ; Ac

(9) R =  ; Ac ; Ac ; Ac

as in (9) 

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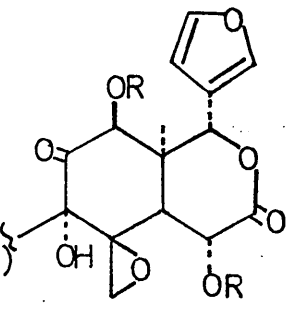
as in (9) 

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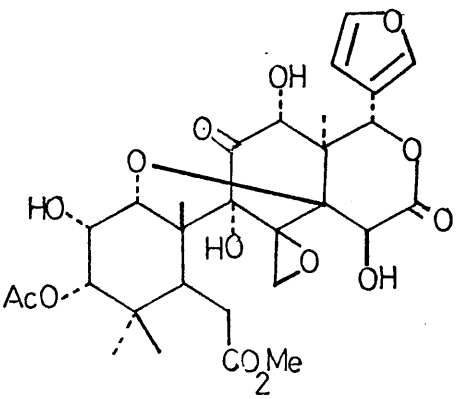

(11)

(H-5) - {H-12} (15%), (H-12) - {H-5} (10%), (H-15) - {C-13 methyl group} (10%) and (H-17) - {H-12} (10%). An examination of models showed that the 12β-H is close in space to both H-5, as a result of the 1,14-ether link, and H-17 in a molecule of structure (8). In addition the 15α-H has a 1,3-diaxial relationship with the methyl group attached to C-13. These observations provided convincing support for the structure (8) for ekebergin I (esters unassigned).

It was clear from the spectroscopic properties of ekebergin II (9), $C_{37}H_{46}O_{17}$ (m/e 762), that it had an acetate in place of the 2"-methylbutyrate of ekebergin I but, otherwise, had the same structure (see Tables 1 and 2). A second compound isolated from the seed extract was the modified furan derivative (10) of ekebergin II. It was identified by its spectroscopic properties (see Tables 1 and 2 and Chapters 1,2 and 3). It was present as a mixture of epimers at C-21.

Mild alkaline hydrolysis of ekebergin II afforded, after methylation, a product (11) which still retained one acetate. The ^{1}H n.m.r. spectrum indicated that it was attached to C-3 (δ_H 5.09). This result showed either that there was an acetate at C-3 in ekebergin I or that it was at C-2 and was transferred during the hydrolysis. C-3 is the only oxygenated carbon that shows a significant chemical shift difference between ekebergins I and II (0.5 p.p.m.), suggesting that C-3 bears the methylbutyrate in ekebergin I, and an acetate in ekebergin II.

In an attempt to demonstrate the presence of α-acyloxy or α-hydroxy carbonyl groups, ekebergin II was refluxed overnight with zinc in acetic acid. Surprisingly this reaction gave an almost quantitative yield of an isomeric compound, isoekebergin II (12). The significant shifts in the ^{1}H n.m.r. spectrum (Table 1) of (12) relative to ekebergin II were:

H-5 ($\Delta\delta$- 0.5), H-12 ($\Delta\delta$ 1.36), H-15 ($\Delta\delta$ - 0.45). These shifts seemed to require epimerisation at both C-12 and C-15 relative to ekebergin II and led to (12) as a possible structure. Further work is required to confirm this.

Table 1

^{1}H n.m.r. spectra of ekebergins I and II and related compounds.[b]

	(8)	(9)	(10)	(11)	(12)
H-1	4.58(d,3)	4.60(d,4)	4.56(d,4)	4.69	4.66(d,5)
H-2	5.44(t,3.5)	5.44(t,4)	5.41(t,4)	4.39	5.33(dd,5,3)
H-3	5.28(d,4)	5.25(d,4)	5.23(d,4)	5.09	5.16(d,3)
H-5	3.25(dd,7,2)	3.27(dd,7,2)	3.05(dd,7,2)	a	ca 2.54
2H-6	2.73(dd,19,2) 2.31(dd,19,7)	2.73(dd,19,2) 2.31(dd,19,7)	a a	a	a
H-12	5.32	5.34(s)	5.34(br)	4.87	6.70
H-15	5.86	5.89(s)	5.81	4.48	5.44
H-17	6.60	6.61(s)	6.36(bs)	6.30	6.02
H-21	7.59	7.63	6.04(m)	7.60	7.35
H-22	6.46	6.46	6.54(bs)	6.62	6.43
H-23	7.44	7.44		7.44	7.43
2H-30	2.91 2.59 (ABq,5)	2.92 2.57 (ABq,5)	2.93 2.57 (ABq,5)	2.77 3.62	3.10 2.59 (ABq,5)
OAc	2.18 1.93	2.17 2.11 1.92	2.15 2.14 2.09	2.13	2.33 2.25 1.82
OH	4.24	4.23	4.29		3.58
C-Me	1.34 1.29 1.17 0.89	1.34 1.29 1.16 0.87	1.39 1.25 1.13 0.80	1.19 1.08 1.08 0.93	1.38 1.11 1.11 0.86
H-2'	2.69[c]	2.67[c]	a	-	a
H-3'	1.15(d,7)	1.15	1.15	-	1.22
H-4'	1.17(d,7)	1.17	1.13	-	1.16
H-2"	2.55(m)	-	-	-	-
2H-3"	1.60(m)	-	-	-	-
3H-4"	0.65(t,7)	-	-	-	-
3H-5"	1.12(d,7)	-	-	-	-

a. Not identified.

b. J values in parentheses (Hz). c. Septet J 7Hz.

Table 2

^{13}C n.m.r. spectra of ekebergins I and II and related compounds.

Carbon No.	(8)	(9)	(10)	(11)
1	75.0	75.0	75.1	77.3
2	65.8	65.7	65.5	64.9
3	74.1	74.6	74.3; 73.7[c]	75.6
4	39.6	39.5	39.7	39.2
5	35.5	35.4	35.7	35.7
6	28.5	28.4	28.8	29.3
7	173.4	173.4	174.9	173.8
8	59.5	59.5	59.1	59.8
9	83.9[a]	84.0[a]	83.9[a]	83.7[a]
10	56.8[b]	56.8[b]	56.7[b]	55.2[b]
11	207.2	207.1	206.5	209.5
12	75.5	75.5	75.1	73.7
13	56.8[b]	56.8[b]	56.7[b]	55.2[b]
14	84.2[a]	84.1[a]	84.2[a]	84.6[a]
15	68.9	69.0	68.6	68.9
16	166.0	166.1	168.1	174.5
17	79.7	79.7	79.1; 79,4[c]	80.3
20	108.8	108.8	169.0	109.0
21	140.5	140.5	121.3; 121.5[c]	140.2
22	120.3	120.2	96.7	120.7
23	143.4	143.6	162.5; 162.9[c]	143.4
30	45.4	45.3	45.2	46.3
OMe	52.1	52.1	52.6	52.4
1'	176.9	176.9	176.9	
2'	33.5	33.6	33.6	
3'	19.2	20.0	19.2	
4'	18.3	19.3	18.2	
1"	176.9			
2"	40.2			
3"	27.1			
4"	10.9			
5"	15.5			
C-Me	27.3	27.2	27.2	27.8
	22.9	22.6	22.5; 22.8[c]	23.0
	11.1	11.2	11.4	8.9
	20.1	20.6	19.9	19.0
$CH_3CO(O)$	20.4	20.5	20.3	20.5
	20.1	20.1	20.3	
		20.0	20.3	
$CH_3CO(O)$	169.7	170.7	170.8	174.5
	168.4	169.7	169.7	
		168.3	165.1	

a,b. These rows may be interchanged. c. Epimeric mixture.

EXPERIMENTAL

Isolation.- An oily light petroleum extract (150 g) from the seeds of Ekebergia senegalensis was obtained from Dr. D.A.Okorie, Ibadan. Addition of cold light petroleum gave a precipitate (1.53 g), which was then chromatographed (preparative t.l.c.) using chloroform-methanol (1%) to give pure ekebergin II (9) (107 mg), m/e 762. Further addition of light petroleum to the extract afforded a more polar compound which was purified by preparative t.l.c. (60% ethyl acetate in carbon tetrachloride) to give the modified furan derivative (10) as a gum.

Hydrolysis.- Ekebergin II (107 mg) was dissolved in 5 % methanolic potassium hydroxide and left for 1 hour at room temperature. The usual work-up yielded a crystalline hydrolysis product (11) (23.7 mg).

Attempted Reduction of Ekebergin II.- Ekebergin II (10 mg) was dissolved in acetic acid and an excess of zinc powder added. The mixture was heated under reflux overnight and worked up as usual. Preparative t.l.c. afforded isoekebergin II (12), m/e 762.

Preparation of the Gedunin Derivative (6).- Gedunin, in solution in ether containing a few drops of pyridine, was treated with osmium tetroxide and the solution left in the dark overnight. The reaction was worked up by stirring with aqueous sodium metabisulphite and extraction with chloroform. The crude diol was dissolved in methanol and reacted with excess sodium borohydride. Acetylation of the product followed by preparative t.l.c. afforded a small amount of the pentaacetate (6) [δ_C 0.82, 1.01 (2), 1.11, 1.25 (C-methyls), 1.94, 2.07, 2.10 (2), 2.17 (OAc), 3.30 (d, J 2 Hz, H-15), 4.65 (m, H-7), 5.00 (d, J 4 Hz, H-1), 5.06 (d, J 4 Hz, H-3), 5.11 (s, H-17), 5.48 (t, J 4 Hz, H-2), 6.18 (d, J 2 Hz, H-16), 6.26,

7.32, 7.37 (furan)].

The above compounds were used for spectroscopic analysis and pilot reactions. Their full characterisation (m.p., i.r., mass analysis) awaits the arrival of more material.

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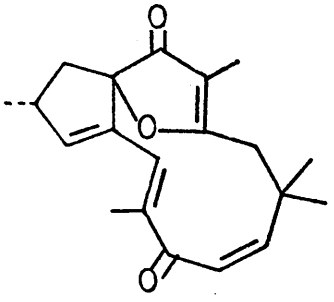
CHAPTER VIII

JATROPHOLONES A AND B:

NEW DITERPENOIDS FROM THE ROOTS

OF

JATROPHA GOSSYPIIFOLIA



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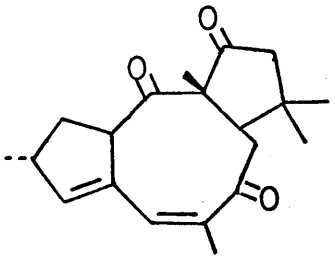

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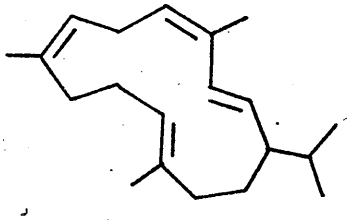

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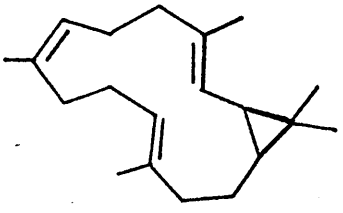

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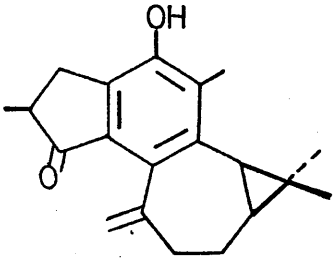

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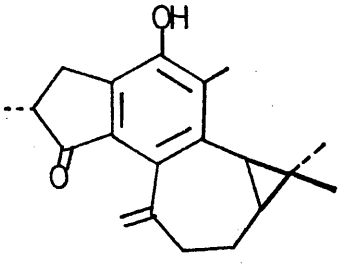

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INTRODUCTION

Jatropha gossypiifolia (Euphorbiaceae) grows widely throughout the tropical regions of Central and South America, from where it originates. It was later introduced into India and Africa, along with other species of the genus.[1] The bark of this small shrub has been used from ancient times for various medicinal purposes mainly related with the relief of stomach disorders.[2] It has been reported, however, that it contains toxic principles which would cause madness or death if consumed in high doses.[3] The plant has also been reported to possess insecticidal properties.[4]

Chemical studies, of different parts of the plant, have afforded an alkaloid[3], β-sitosterol[5], flavonoids[6] and a diterpenoid[7], jatrophone (1). The most interesting feature, however, is the antitumor activity exhibited by jatrophone and the related jatrophatrione (2) from J. macrorhiza.[8] Many diterpenoids from the Euphorbiaceae show this type of biological activity, and their potential importance in medicine has brought as a consequence an increasing amount of research in this field during the past few years.[9] Structurally, most of these diterpenoids show a relationship which favours the proposal of a common biogenesis from a cembrene (3) or a casbene (4) precursor.[9]

We have examined the benzene extract of the roots of Indian J. gossypiifolia and have isolated two new epimeric diterpenoids, jatropholones A and B. In this chapter we discuss the spectroscopic and chemical evidence that led to the assignment of structures (5) and (6) for these compounds. The jatropholones do not have antitumor activity [10], but they fit well into the biogenetic pattern of the Euphorbiaceae diterpenoids.

DISCUSSION

Jatropholones A and B were obtained as a crystalline mixture from the benzene extract of the roots of J. gossypiifolia. Their separation, by the usual chromatographic methods, proved to be rather difficult as their polarities were extremely similar. In fact, they appeared as a single spot on an analytical t.l.c. plate in most solvent systems, even after repeated runs. The existence of a mixture, however, was readily apparent from the ^{1}H n.m.r. spectrum and from the wide range of melting point (220°- 230°C), unchanged by recrystallisation. Careful preparative t.l.c., using a mixture of ethyl acetate-carbon tetrachloride (4:1), afforded the pure compounds after two consecutive runs. This solvent mixture has proved useful on other occasions[11], in the separation of mixtures of close polarities.

Elemental analysis afforded the same molecular formula, $C_{20}H_{24}O_2$, for both jatropholones, thus indicating that they were isomers and highly unsaturated. $C_{20}H_{24}O_2$ requires nine double bond equivalents. Both compounds had similar absorption bands in their i.r. spectra. A strong peak at 1715 cm^{-1} was attributed to either a saturated cyclohexanone or an α,β-unsaturated cyclopentenone.[12] A band at 3603 cm^{-1} accounted for the second oxygen atom, as a free hydroxyl group, probably phenolic in character. The phenolic nature of the hydroxyl group was established by the u.v. spectrum of jatropholone A which changed on addition of alkali, indicating the formation of the phenolate anion[λ_{max} 225, 235, 275 and 325 nm; (OH^-) 257, 290 and 362 nm]. Further confirmation was provided by the formation of the corresponding acetate, (see Experimental).

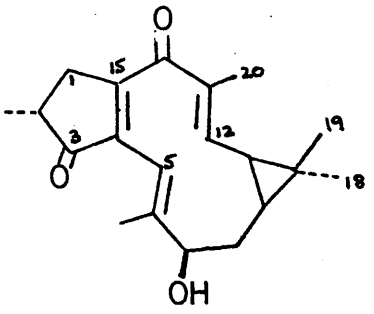
The ^{1}H n.m.r. spectrum of jatropholone A (5) revealed the presence

of four methyl groups [δ_H 0.89, 1.30 (tertiary methyls), 1.33, (3H,d, J 7 Hz, secondary methyl) and 2.32 (aromatic methyl)] , an exomethylene group [δ_H 4.68 and 5.19 (each bs)] and a phenolic hydroxyl proton (δ_H 5.25, 1H, s). The absence of signals for aromatic protons indicated that the benzene ring was fully substituted.

Further information was obtained by a study of the ^{13}C n.m.r. spectrum of jatropholone A. It had signals for a ketonic carbonyl (δ_C 207.9), an exomethylene group [δ_C 146.1(s) and 114.8(t)] , a fully substituted cresol ring [δ_C 150.1, 137.5, 136.7, 134.3, 132.6, 130.8, all singlets, and 28.2(q)] and three methyl groups [δ_C 16.2(q), 15.8(q) and 13.2(q)] . In addition, the off-resonance spectra had a high field singlet (δ_C 19.5), three doublets (δ_C 42.6, 28.4 and 21.5) and three triplets (δ_C 33.6, 30.4 and 26.0). The presence of only <u>one</u> singlet indicated that the tertiary methyl groups were geminal. At the same time, the abnormally high field nature of this signal identified it as part of a cyclopropane ring.[13] Two of the doublets could then be assigned to the other carbon atoms of the cyclopropane ring (δ_C 28.4 and 21.5). The remaining doublet at δ_C 42.6 corresponded to the carbon bearing the secondary methyl group.

As pointed out earlier, the molecular formula requires nine double bond equivalents. The groups found so far account for only seven of them, therefore the molecule has to be bicarbocyclic in addition to the aromatic and cyclopropane rings. As eight carbon atoms were available for the extra two rings required, they must form two six-membered rings or five- and seven-membered rings to accomodate the i.r. carbonyl frequency. This led to several possible structures for jatropholone A, depending on the relative positions of the substituents. Further information was therefore necessary to decide among them.

The presence of a cyclopropane ring in the skeleton suggested that



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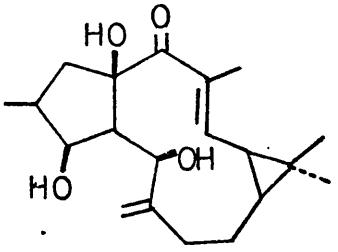

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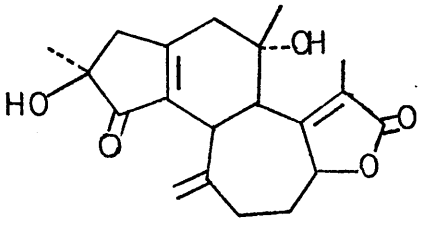

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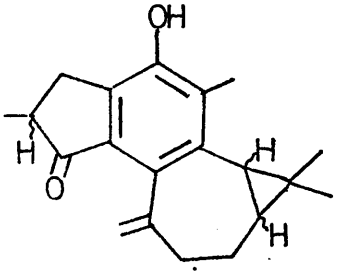

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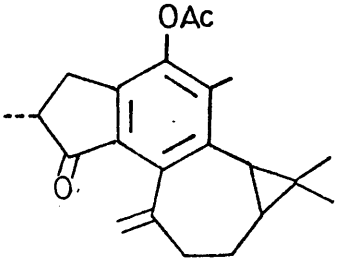

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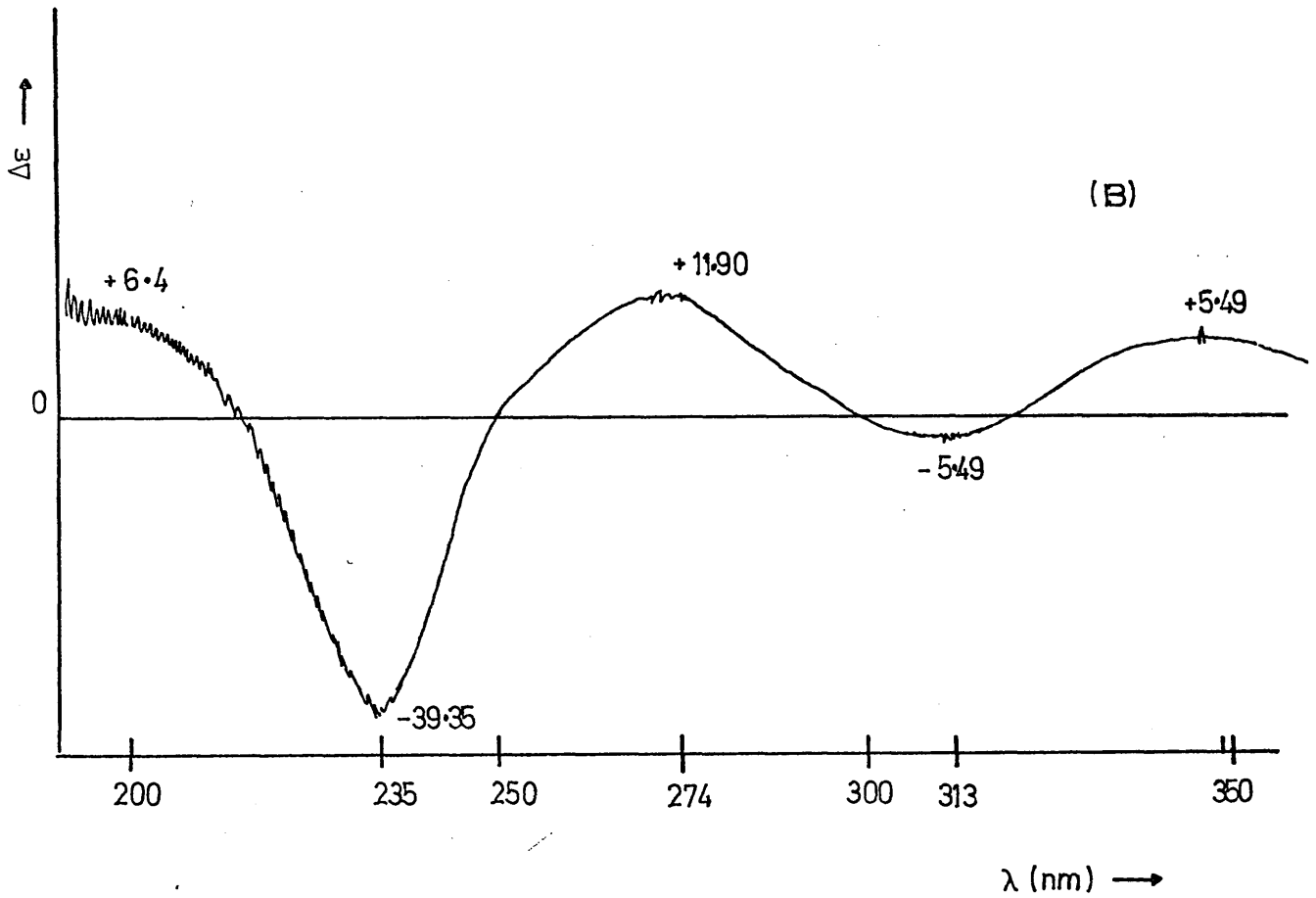
jatropholone A was related to casbene (4). Examination of the structures of lathyrol[14](7), bertyadionol[15] (8) and crotofolin[16] (9), all diterpenoids derived from casbene, provided some useful indications. In all these compounds cyclisation has taken place to form a cyclopentane ring with an oxygenated position 3 and an adjacent secondary methyl group. It seemed reasonable to assume that a similar cyclisation had occurred in jatropholone A, resulting in the formation of a cyclopentenone. Cyclisation of a lathyrol- or a bertyadionol-type of skeleton to form a potential cresol ring can only occur between C-12 and C-5. This type of fusion is found in crotofolin (9). Aromatisation of the resulting cyclohexenone ring leads to structure (10), which is in full agreement with the spectroscopic properties of jatropholone A. There is, however, no basis for deciding the stereochemistry at C-2, or the cyclopropane ring junction. Due to the small amount of material available, it was not possible to provide chemical confirmation of the proposed structure.

As indicated earlier, both jatropholones exhibited almost identical spectroscopic properties, a fact that pointed to a stereoisomeric relationship between them. On the basis of structure (10) for jatropholone A, there are only two possible centres of stereoisomerism ie., the cyclopropane ring junction and the secondary methyl group, adjacent to the carbonyl group. Mild basic treatment of jatropholone A afforded an equimolar mixture which, on careful separation by preparative t.l.c., gave pure samples of both jatropholone A (5) and jatropholone B (6). A similar mixture was obtained on base treatment of jatropholone B. This result clearly established the C-2 epimeric relationship between the jatropholones and in addition, confirmed the attachment of the secondary methyl group α to the carbonyl group. We still lacked any evidence to enable us to decide the relative or absolute configuration of the jatropholones.

Fig. 1

C.D. Spectra of Jatropholones A and B



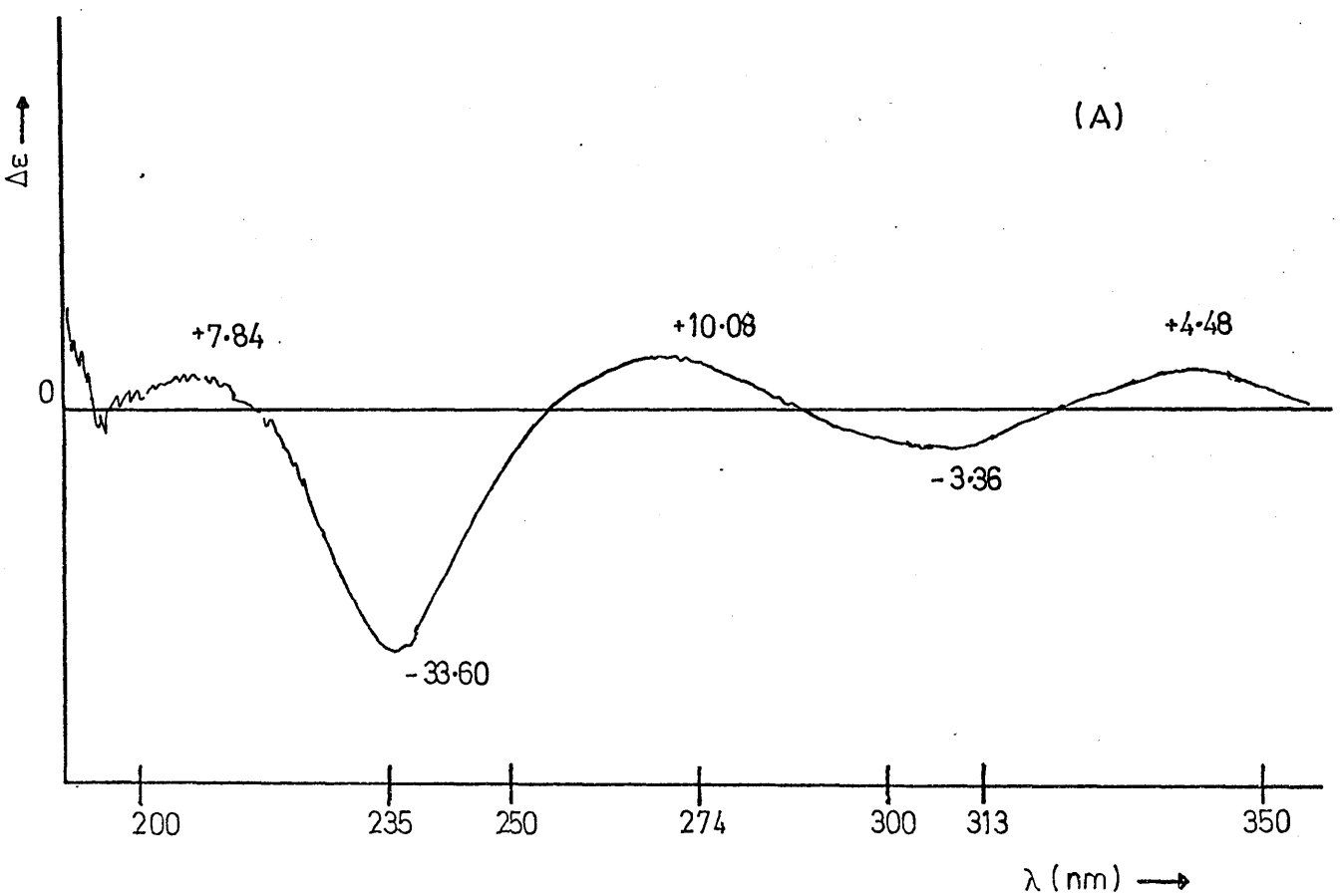
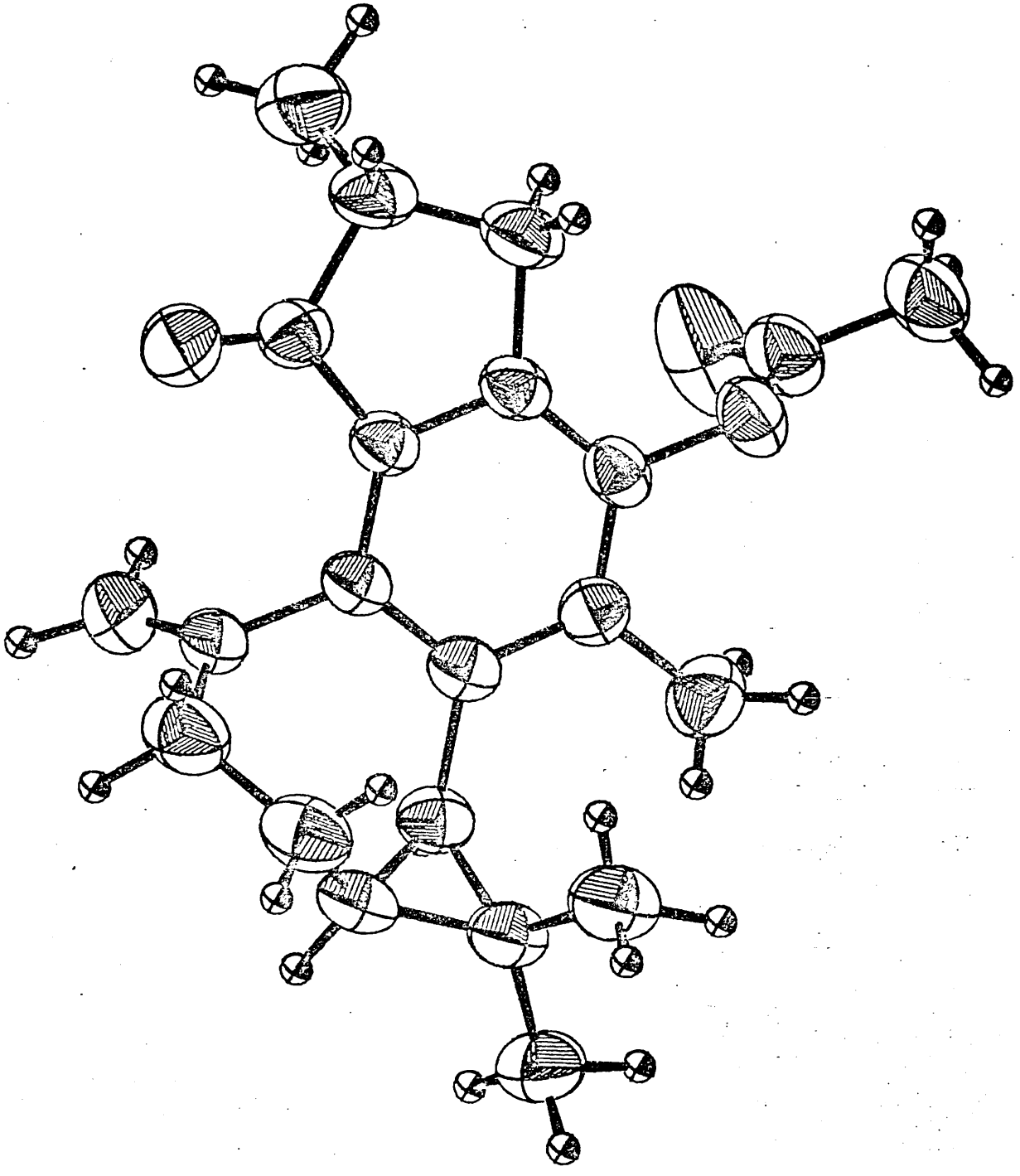


Fig. 2

ORTEP drawing of Jatropolone B acetate



Their c.d. curves were virtually identical but difficult to interpret in the absence of suitable model compounds. (see Fig. 1).

At this point, it was necessary to obtain an X-ray analysis of a derivative of one of the compounds, jatropholone B acetate,† which afforded structure (11). An ORTEP drawing of the molecule, Fig. 2, clearly demonstrates that the exomethylene group is twisted out of conjugation with the aromatic ring. The X-ray result revealed the relative stereochemistry and with the above evidence, led to structures (5) and (6) for jatropholones A and B respectively. It is reasonable to assume that they have the absolute configuration which is common to similar diterpenoids [eg., bertyadionol (8) and lathyrol (7)] from the Euphorbiaceae. Thus, the jatropholones represent a new skeletal type, akin to crotofolin (9).

† K.K. Purushothaman, S. Chandrasekharan, A.F. Cameron, J.D. Connolly, C. Labbe, A. Maltz, and D.S. Rycroft, Tetrahedron Letters, 1979, 979.

EXPERIMENTAL

Isolation.- The jatropholones (5) and (6) were isolated as a mixture by the Indian workers in the following way: Dried and finely crushed roots (25 Kg) of J. gossypiifolia were extracted with cold benzene. The benzene extract was chromatographed over silica gel. The fractions eluted with 1:3 benzene-chloroform, and chloroform, were combined and crystallised from benzene to yield colourless crystals of jatropholones A and B, m.p. 220°- 230°C (200 mg). Separation of the mixture was achieved by careful column chromatography on silica gel or by preparative t.l.c. using ethyl acetate-carbon tetrachloride (4:1, two runs) as solvent. Jatropholone A (5) (80 mg) was crystallised from ether-methanol, m.p. 218°-220°C, [m/e 296; c.d. nm ($\Delta\varepsilon$) 235 (-33.6), 274 (10.08), 3.13 (-3.16) and 348 (4.48)] . (Found: C, 78.0; H, 8.0. $C_{20}H_{24}O_2 \cdot \frac{1}{2} H_2O$ requires C, 78.5; H, 8.3%). Jatropholone B (6) (70 mg) was crystallised from ether-methanol, m.p. 228°-230°C, [m/e 296; λ_{max} 225, 238, 275, 335 nm; (OH^-) 256, 296, 357 nm; ν_{max} (CCl_4) 3603, 1715 cm^{-1}; c.d., nm ($\Delta\varepsilon$) 235 (-39.35), 274 (11.90), 313 (-5.49) and 348 (5.49); δ_H 0.86, 1.29 (3H,s); 1.34 (d,J 7 Hz); 2.3 (3H,s); 4.66, 5.19 (both bs) exomethylene; 5.42 (s, OH); δ_C 208.2 (s) (ketone); 145.6(s), 115.2(t) (exomethylene); 150.2(s), 137.5(s), 137.2(s), 131.8(s), 131.1(s), 134.4(s) and 28.2(q) (cresol); 19.5(s), 29.4(d), 21.5(d) (cyclopropane); 42.2(d), 33.5(t), 30.1(t), 26.0(t), 17.1(q), 16.1(q) and 13.3(q)] . (Found: C, 80.7; H, 8.4. $C_{20}H_{24}O_2$ requires C, 81.1; H, 8.1%).

The corresponding acetate (11) was prepared by heating (6) in pyridine-acetic anhydride on a steam bath for $\frac{1}{2}$ h. It had m.p. 140°- 142°C (ex. chloroform-ether), [m/e 338; ν_{max} (CCl_4), 1765, 1717 cm^{-1}, δ_C 207.1 (ketone), 168.4 (s) and 20.5(q) (acetate); 145.0(s), 115.4(t) (exomethylene); 145.9(s),

143.7(s), 139.8(s), 138.1(s), 137.8(s), 132.4(s) and 28.2(q) (cresol); 28.2(d), 21.4(d), 19.6(s) (cyclopropane); 42.2(d), 33.2(t), 30.7(t), 25.9(t), 16.8(q), 16.1(q) and 13.9(q)]. (Found: C, 78.1; H, 7.6. $C_{22}H_{26}O_3$ requires C, 78.1; H, 7.6%).

Equilibration of (5) and (6) in base.- Jatropholone A (5) (20 mg) was dissolved in 5% ethanolic potassium hydroxide solution (10 ml) and refluxed for 1 hour. Addition of water, acidification with acetic acid and extraction with chloroform afforded an equimolar mixture of (6) and (5) (analytical t.l.c.). Preparative t.l.c. separation gave pure samples of (5) and (6), identical with authentic specimens. A similar result was obtained on exposure of jatropholone B (6) to basic conditions.

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CHAPTER IX

THE STRUCTURE OF A NEW COUMARIN

FROM

LEONOTIS NEPETAEFOLIA

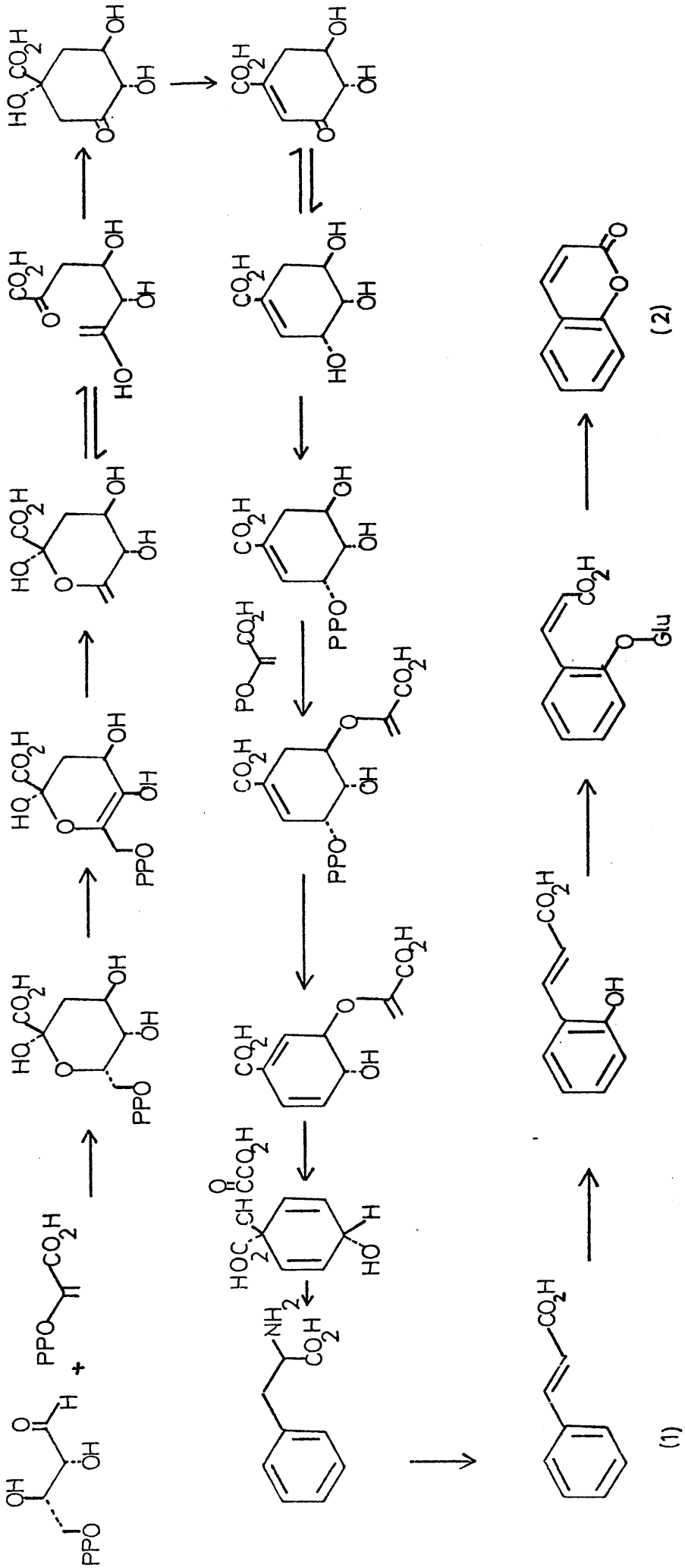


Fig. 1

INTRODUCTION

Occurrence of coumarins in plants.[1] During the process of production of the aromatic aminoacids (eg., phenylalanine and tyrosine) necessary for the metabolism of higher plants, a branching in the "shikimic acid pathway" (Fig. 1) may occur, leading to various by-products.[2] The formation of cinnamic acid (1) is generally considered to be the starting point of a series of oxidations which eventually lead to the synthesis of coumarins. Biosynthetic studies of simple coumarins support this route.[3] It has also been proposed that some coumarins can arise by a different mechanism involving the cyclisation of a polyketide precursor.[4] The biosynthesis of coumarins in fungi, appears to follow the polyketide pathway.[5]

Even though coumarins are secondary metabolites, as are the tetranor-triterpenoids discussed in other chapters, they are found widely throughout the plant kingdom and in the whole plant. This special prominence can be explained[6] by the connection of the "shikimic acid pathway" with pathways in plants of sugar metabolism, through phosphoenol pyruvate and erythrose-4-phosphate (see Fig. 1).

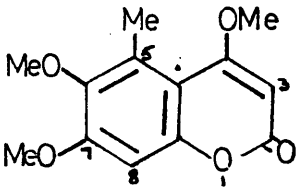
Coumarins are structurally identified by the presence of a bicyclic aromatic nucleus, exemplified by coumarin (2), the simplest member of the group. The unsaturated δ-lactone has a characteristic strong absorption at about 1725 cm^{-1} in the i.r. spectrum[7] which, together with other spectroscopic data, aids identification. The most useful technique for structural assignment is, as with most natural products, nuclear magnetic resonance. Steck and Mazurek[8] have compiled ^{1}H chemical shift-structure correlations that facilitate the rapid identification of the major structural types. Lanthanide shift reagents[9] have also been found very helpful for

the allocation of substituents on the aromatic ring, relative to the carbonyl group. More recently, ^{13}C n.m.r. has been introduced.[10]

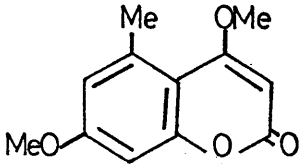
Two main structural types are found[1] among naturally occurring coumarins: (a) coumarins with acyclic substituents, and (b) furano- and pyrano-coumarins. In the first group, oxygenation occurs at one or more of the six available positions (usually C-7), being present as phenolic, ethereal or glycosidic groups. Many coumarins bear isoprenoid chains, either intact or partially degraded, of one, two or three units attached to carbon, oxygen, or to both. The second group arises by oxidative ring closure of the prenyl group to a neighbouring oxygen to form, after loss of acetone, a furan ring. An alternative mode of cyclisation gives rise to pyrano-coumarins.

Extractives from Leonotis species (Labiateae).- . Leonotis species are native of the tropical regions of Central and South America, and widely distributed throughout South Africa. They are frequently associated with Cannabis species through the colloquial name "dagga", and are thought to possess various medicinal properties.[11] Previous work on Leonotis nepetaefolia has resulted in the isolation of seven labdane diterpenoids[12-14] closely related to those isolated from other species of the genus.[15] They have been found distributed in the whole plant.

During the course of the work on the diterpenoids of L. nepetaefolia grown in India[12], a small amount of a crystalline substance was obtained, whose spectroscopic properties indicated that it was a coumarin. In the present chapter we will present the chemical and spectroscopical evidence that led us[16] to assign the structure (3), (4,6,7-trimethoxy-5-methyl-chromen-2-one), to this substance.



(3)



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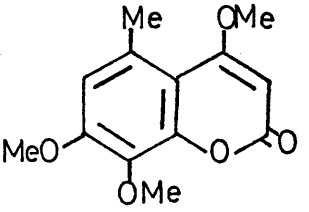
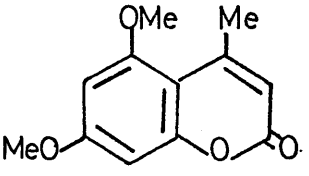


(5)



(6)

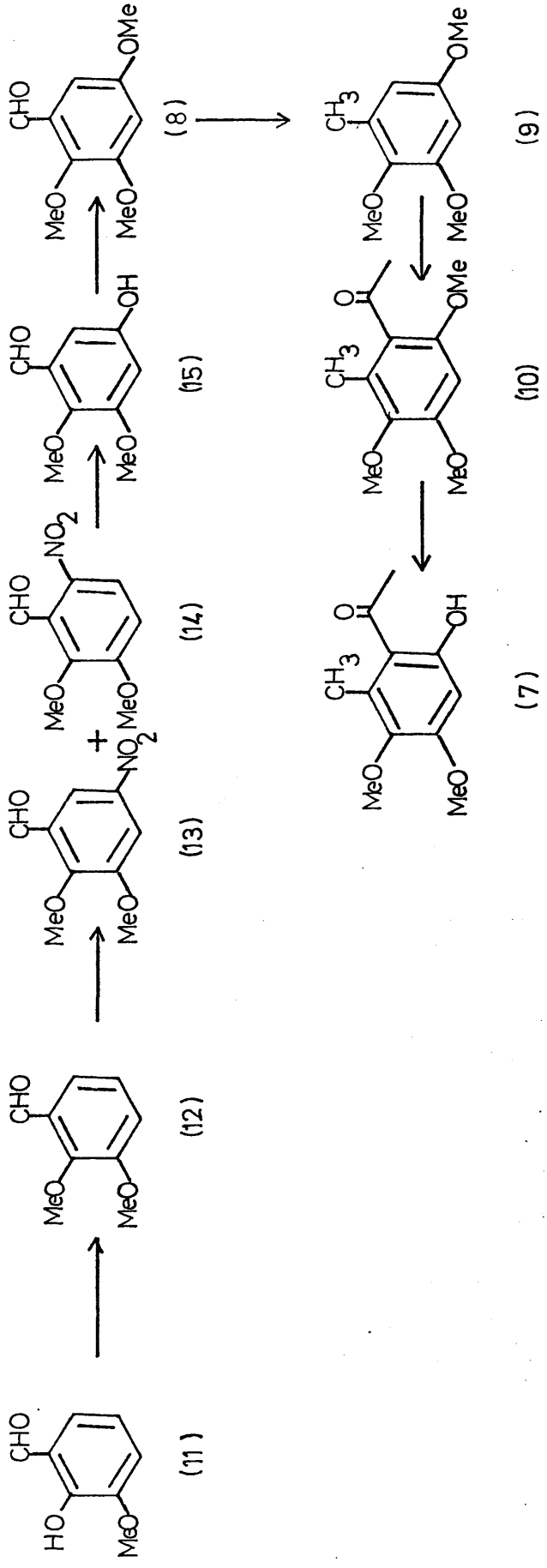
DISCUSSION

Elemental analysis of (3) gave the molecular formula $C_{13}H_{14}O_5$, indicating a high degree of unsaturation. The i.r. spectrum had a strong carbonyl band at 1727 cm^{-1} (CCl_4) and this, in conjunction with the u.v. spectrum (see Experimental), suggested a coumarin nucleus. The nature of the fourteen protons was readily disclosed by the ^1H n.m.r. spectrum which had resonances for three methoxyl groups (δ_H 3.75, 3.91 and 3.94), an aromatic methyl (δ_H 2.59), a vinyl proton (H-3), and an aromatic proton (δ_H 5.53 and 6.68 respectively). Thus the structural problem was rapidly reduced to one of attaching three methoxyl groups and a methyl to a coumarin nucleus.

On biogenetic grounds[1], and by comparison with the structure of siderin[17] (4), we favoured a C-5 methyl group. Attachment at C-4 was discarded on the basis of the resonance of H-3, which appears[18] at 6.05-6.28 p.p.m. when a methyl group is at C-4, whereas it shifts to higher field (5.53 p.p.m.) when a methoxyl group is the substituent.[19] Since we would expect one methoxyl group to be at C-7, we were left with two possible structures, (3) and (5), for the coumarin.

Some authors[9] have found that the lanthanide shift reagent, $Eu(fod)_3$, provides a useful means of determining the substitution pattern in non-phenolic coumarins. The europium complexes preferentially with the carbonyl oxygen. Thus, the signals corresponding to the different protons in the nucleus, shift in proportion to their proximity to the carbonyl group. Experimentally it has been found that the largest shift is experienced by H-3 which is then used as a reference, and the shifts observed for the other substituents are measured relative to it, independent of

Fig. 2



the amount of reagent used. The observed sizes of the shifts follow the decreasing order: 3 >4,8 > 5,7 > 6.

On addition of $Eu(fod)_3$ to a $CDCl_3$ solution of the coumarin, H-3 shifted dramatically, as expected. The shifts of the other signals relative to H-3 were: Me, 0.16; OMe's, 0.17, 0.13 and 0.08; aromatic proton, 0.30. The corresponding shifts for the model compound (6) were: Me, 0.21; OMe's, 0.10 and 0.07; H-6, 0.14 and H-8, 0.29. The size of the relative shift of the aromatic proton of (3) indicated that it was attached to C-8. On this basis we could write structure (3) with some confidence.

Definitive proof was obtained by alkaline hydrolysis, followed by acidic hydrolysis, which resulted in decarboxylation to give the o-hydroxyacetophenone (7), [ν_{max} 3,400 and 1,620 cm^{-1}; δ_H 2.5 (Ar Me), 2.62 (CH_3CO), 3.70 and 3.84 (2 OMe), 6.32 (1H,s, aromatic) and 13.08 (1H,s, phenolic hydroxyl)], whose structure was confirmed by synthesis (see Fig. 2). The formation of the acetophenone (7) provided confirmation for the presence of the C-4 methoxyl in (3).

Wolff-Kishner reduction of the 2,3,5-trimethoxybenzaldehyde (8) (prepared from o-vanillin) afforded the corresponding toluene (9)[δ_H 2.17 (Ar Me), 3.71 (6H) and 3.80 (3 OMe's), 6.27 and 6.33 (both d, J 3 Hz, H-4 and H-6)]. Fortunately, Friedel-Crafts acylation of (9) with acetyl chloride occurred regiospecifically at C-6, to yield the acetophenone (10),[δ_H 2.17 (Ar Me), 2.45 (CH_3CO), 3.71, 3.80 and 3.88 (3 OMe), 6.40 (1H,s, H-3)]. Benzene induced shifts[20] of the methoxy groups of (10), (0.60, 0.43 and 0.11 p.p.m.) supported this assignment. Treatment of (10) with an excess of boron trichloride, a reagent for the specific demethylation of o-methoxy-acylbenzenes[21] readily afforded the o-hydroxyacetophenone (7), identical (m.p., m.m.p, i.r., u.v., n.m.r., t.l.c. and m.s.) with the hydrolysis product of the natural coumarin (3). This confirms the structure of (3) as 4,6,7-trimethoxy-5-methyl-chromen-2-one.

EXPERIMENTAL

Isolation.- The coumarin (3) was isolated by the Indian workers[16] in the following way: Dried powdered Leonotis nepetaefolia (whole plant, 3 Kg) was extracted with cold benzene. Evaporation of the solvent afforded an oily extract which was left overnight. Nepetaefolinol(15) precipitated and was filtered off. Column chromatography of the mother liquors over Grade IV alumina, using light petroleum-chloroform in increasing polarity, allowed the separation of the fatty material. The chloroform eluate was rechromatographed and a gum (100 mg) obtained, which solidified on trituration with ether. Crystallisation from methanol gave 4,6,7-trimethoxy-5-methylchromen-2-one (3), m.p. 209°- 210°C, [λ_{max} 225, 275 infl., 287, 313, 327 infl. nm (ε 20,000; 8,800; 12,800; 15,000 and 11,000)]. (Found: C, 62.2; H, 5.4. $C_{13}H_{14}O_5$ requires C, 62.4; H, 5.65%).

2'-Hydroxy-4', 5'-dimethoxy-6'-methylacetophenone (7).- The coumarin (3) (90 mg) was refluxed with 5% methanolic potassium hydroxide (10 ml) for 2 hours. After removal of the solvent in vacuo, the residue was acidified with 5 M hydrochloric acid, heated for 5 minutes and extracted with ether. Crystallisation from cold hexane yielded the product (7), m.p. 76°- 77°C, [λ_{max} 220, 234, 277 and 318 nm (ε 10,500; 8,500; 5,600 and 3,500)]. (Found: C, 62.6; H, 6.8. $C_{11}H_{14}O_4$ requires C, 62.85; H, 6.7%).

o-Veratraldehyde (12).- o-Vanillin (11) (10 g) was dissolved in dimethyl sulphoxide and slowly added to a stirred solution of sodium hydride (1.6 g) in dimethylsulphoxide, cooled in an ice-bath. Methyl iodide (9.3 g) was then slowly added to the mixture. Once the reaction was over, the unreacted hydride was decomposed by addition of water. Extraction with ether gave o-veratraldehyde (12) (4 g) which recrystallised from chloroform as white

cubes, m.p. 50°- 52°C.

2,3,5-Trimethoxybenzaldehyde (9).- A tenfold excess of concentrated nitric acid[22] was added to o-veratraldehyde (12 g) and the reaction left at r.t. for five minutes. The solution was then cooled and diluted with water, whereupon a yellowish precipitate was obtained. Filtration and washing with water afforded a crystalline equimolar mixture, m.p. 70°- 87°C, of 5- and 6-nitro derivatives (13) and (14) respectively, (11.7 g) which proved to be very unstable on exposure to light. Reduction and diazotisation of the mixture to the corresponding hydroxy derivatives was accomplished by the method of Smith and Laforge.[23] The mixture (11.7 g) was dissolved in 30% sodium bisulphite solution (20 ml) and diluted with water (100 ml). This solution was added to a boiling mixture of calcium carbonate (90 g), ferrous chloride (240 g) and water (600 ml). The mixture was then refluxed for ½ h., and filtered into concentrated hydrochloric acid (50 ml) diluted with two parts of water. The resulting solution was cooled with an ice-bath to 4°- 5°C and an aqueous solution of sodium nitrite (4 g/300 ml) was added. The reaction was allowed to warm to r.t. and then heated on a steam bath for 2 hours. Decolourisation with charcoal and extraction with ether gave the crude product (2.9 g) which consisted mainly of the desired 2,3-dimethoxy-5-hydroxybenzaldehyde (15). It crystallised from methanol as long needles (1.92 g), m.p. 141°- 144°C (lit.[23], 137°C), [δ_H 3.85 and 3.88 (2 OMe), 6.67 and 6.76 (both d, J 3 Hz, H-6 and H-4) and 10.28 (1H,s, CHO)]. Methylation as above afforded 2,3,5-methoxybenzaldehyde (8) which crystallised from aqueous methanol as long needles, m.p. 67°C (lit.[23], 71°C), [δ_H 3.80, 3.85 and 3.88 (three OMe), 6.73 and 6.83 (both d, J 3 Hz, H-4 and H-6) and 10.38 (1H,s,CHO)].

2,3,5-Trimethoxytoluene (9).- The aldehyde (8) (1 g) in diethylene glycol was heated on an oil bath at 150°C for 2 hours with an excess of hydrazine

hydrate. Solid potassium hydroxide, in excess, was added and the mixture heated for three hours at 180°C. Dilution with water and extraction with ether gave a tarry product (0.57 g), which on preparative t.l.c. yielded 2,3,5-trimethoxytoluene (9) as an oil (100 mg), m/e 182; [λ_{max} 228 (ε 5,300) and 283 nm (ε 2,250)].

2', 4', 5'- Trimethoxy-6'-methylacetophenone (10).- 2,3,5-Trimethoxytoluene (100 mg) was dissolved in an ethereal solution of an excess of aluminium chloride. An excess of acetyl chloride was then added dropwise.[24] The mixture was stirred at r.t. overnight, acidified with 5M hydrochloric acid, and heated over steam bath for ½ h. Extraction with ether and recrystallisation from hexane afforded 2', 4', 5'- trimethoxy-6'- methylacetophenone (10) (90 mg), m.p. 84°C, [ν_{max} (CCl_4) 1697 cm^{-1}; λ_{max} 224, 268 and 295 nm (ε 10,000, 4,000, 3,500)]. (Found: C, 64.35; H, 7.25. $C_{12}H_{16}O_4$ requires C, 64.25; H, 7.2%).

2'- Hydroxy-4', 5'- dimethoxy-6'- methylacetophenone (7).- The trimethoxyacetophenone (10), (30 mg) in dichloromethane, was stirred at 0°C for 5 minutes with a large excess of boron trichloride. Water was added and the organic layer separated. The crude product was crystallised from hexane to give 2'-hydroxy-4',5'-dimethoxy-6'-methylacetophenone (7), (24 mg) m.p. 76°-77°C, identical with the hydrolysis product of (3). The isomeric 2'-hydroxy-3',4'-dimethoxy-6'-methylacetophenone has m.p. 94°C.[24]

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