A THESIS

ENTITLED

'STUDIES IN THE TETRANORTRITERPENOID SERIES'

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CONTENTS

CHAPTER I

REVIEW OF TETRANORTRITERPENOIDS

Elucidation of the constitution and configuration of limonin (A28)[1,2,3] in 1960 marked the beginning of a new chapter in triterpenoid chemistry. It initiated the emergence of the tetranortriterpenoids, a group of great structural diversity and chemical interest. It is appropriate at this point to survey the present state of knowledge of this group beginning with the biogenetic sequence originally proposed for limonin.

Tetranortriterpenoids can be derived (see Scheme I) from a precursor possessing the carbon skeleton and stereochemistry of the tetracyclic triterpenoid euphol (A1) (or tirucallol (A2)). Cleavage of the side chain between C-23, C-24 followed by oxidative cyclisation to C-20 leads to the furan ring. The migration of the methyl group from C-14 to C-8 with loss of a proton from C-15 and introduction of oxygen at C-7 gives an intermediate (A3) with the requisite functionality for its conversion to any of the known tetranortriterpenoids. Thus allylic oxidation at C-16 followed by a Baeyer-Villiger cleavage and epoxidation of the olefinic linkage leads to the αβ-epoxy-δ-lactone commonly found in ring D.

Further modifications in the form of oxygenation and ring cleavages can then occur. Strong support for this scheme comes from the co-occurrence in the same plant or in closely related plants of a group of compounds representing various stages along the pathway. It is convenient to discuss the tetranortriterpenoids in terms of these stages.

(I) Recently a number of tetracyclictriterpenoids have been isolated whose side chain oxygenation pattern makes them candidates for subsequent degradation to C_{26} compounds. It is interesting to note that these compounds generally occur together with a variety of tetranortriterpenoids. It is not difficult to see the potential β-substituted furan, a feature of tetranortriterpenoids, in the side chain of flindissol[4] (A4) the first member of the series. Later examples are turraeanthin[5] (A5), melianone[6] (A6), odoratol[7,8] (A7) and bourjotinolone A (A8), and B (A9).[9] Although the original biogenetic scheme is based on euphol (A1), there is a high probability that this should be changed to tirucallol (A2) since the cases in which the C-20 configuration has been established are all tirucallol derivatives.[5,6]

(II) The isolation from Khaya grandifoliola of grandifoliolenone[10] (A10) with its apo-euphol (or apo-tirucallol) skeleton suggests that this rearrangement may precede the formation of the furan ring in the plant. Grandifoliolenone (A10) is the only representative of this skeleton as yet isolated. However analogy for its formation is to be found in the oxidation of dihydro-butyrospermyl acetate[11] (A11) to (A12). Recently two groups of workers have successfully carried out in vitro synthesis of apo-tirucallol derivatives by acid catalysed opening of the 7α, 8α epoxide of methyl 3α-acetoxy tirucallol-7-en-21-oate[12] (A13) and melianone[13] (A6) respectively to give (A14) and (A15).

(III) While a number of furanoids e.g., cedrelone[14] (A16) and anthothecol[15] (A17) representing the cyclo-pentene stage on the biogenetic pathway have been known for sometime, it is only recently the first member of the series based on the intermediate cyclopentenone was isolated. This is grandifolione[16] (A18) whose occurrence in K. grandifoliola with the corresponding αβ-epoxy-δ-

lactone, 7-deacetyl-7-hydroxykhivorin[16] (A19), lends considerable weight to the postulated biogenetic scheme. Further support comes from the series, azadirone (A20), azadiradione (A21), epoxyazadiradione (nimbinin) (A22), and gedunin (A23) which all occur in nim oil[17] and the isolation of khayanthone (A24) along with khivorin (A25) from Khaya anthotheca.[18]

(IV) The αβ-epoxy-δ-lactone system in ring D, a common feature of many tetranortriterpenoids, is found in gedunin[19] (A23) and khivorin[20] (A25). These were among the first structures to be elucidated after limonin and the limonol-merolimonol change (see later) was used in their structural proof. Recently several oxygenated derivatives of gedunin[45,46] and khivorin[47,48,49] have been isolated.

However, in addition to further oxygenation, the basic skeleton can undergo ring cleavages of the type exemplified by nyctanthic[21] (A26) and dammarenolic[22] (A27) acids. This process can occur in rings A, B, and C and leads us to the following groups of tetranortriterpenoids.

(V) Limonin[1,2,3] (A28), the most distinguished tetranortriterpenoid, has ring A cleaved. It can be regarded as arising from the basic gedunin skeleton by cleavage between C-3 and C-4 followed by lactonisation of the carboxyl function at C-3 to the oxidised C-19 methyl group with concomitant cyclisation of the hydroxyl group at C-4 to the olefin linkage at C-1. The chemistry of limonin is too varied to be dealt with in this brief review. However one reaction deserves a mention. Thus treatment of limonol (7α-OH) with base leads to furfuraldehyde and merolimonol[1,3,23] (A29). This reaction is important since it was eventually responsible for the suggestion[41,42] that the compounds related to quassin[24,25] (A30) shared a common biogenetic pathway with tetranortriterpenoids. Several compounds related to limonin are now known e.g., obacunone[3,26] (A31).

(VI) The ring cleavage process can also occur between C-7 and C-8 in the basic skeleton to give ring B cleaved derivatives. The simplest of these is andirobin[27] (A32) which is the cleavage product of gedunin[19] (A23). Other closely related members of the series are methyl angolensate[28] (A33) and the corresponding 6-alcohol[29] and 6-acetate.[29]

The bicyclo[3,3,1]nonane ring system is found in a special group of ring B cleaved tetranortriterpenoids, the bicyclononanolides. This arises by Michael addition of C-2 to C-30 in a precursor of the type (A34). The structure of swietenine[30] (A35), the first member of the group, was solved by chemical and X-ray methods.[44] Swietenine occurs with another bicyclononanolide, swietenolide[31,32] (A36), in Swietenia macrophylla. The bicyclo[3,3,1]nonane system gives rise to a number of interesting rearrangements e.g., treatment of mexicanolide[33] (A37) or its $\Delta^{14(15)}$ isomer, carapin,[43] with mild base results in formation of the dienelactone[33] (A38) by a retro-Michael process.

(VII) Cleavage between C-12 and C-13 gives rise to the ring C cleaved skeleton. This is to be found in nimbin[34,35] (A39), salannin[36] (A40) and nimbolide[37] (A41), all of which are present in Melia azadirachta. These compounds all have ring D intact and are among the few tetranortriterpenoids with oxygenation on one of the C-4 methyl groups.

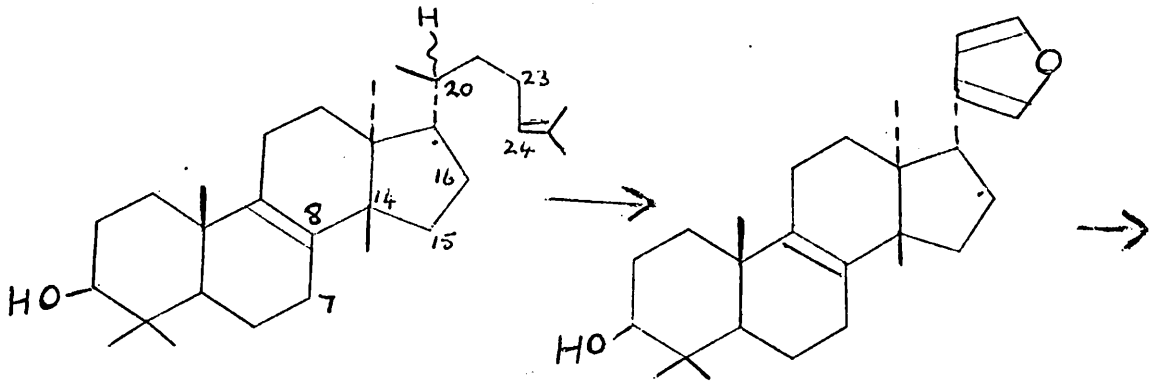
(VIII) Two furanoid compounds which by virtue of their structure and botanical source are thought to have arisen by further modifications of the tetranortriterpenoid basic skeleton are odoratin[38] (A42) (Cedrela odorata) and fraxinellone[39] (A43) (Dictamus albus).

It had been proposed that quassin[24,25] (A30), a C_{20} compound from Quassia amara, is biogenetically derived from a diterpenoid precursor with a pimarane skeleton. Recently it was suggested[41,42] that tetranortriterpenoids can be converted in vivo into the C_{20} bitter principles by loss of a C_5 fragment (furfuraldehyde) in a process analogous to the limonol - meroliminol change followed by loss of a methyl group

from C-4. Support for this proposal comes from the similarity of merolimonol to these C_{20} and related C_{19} compounds and, in particular, from the structure and absolute stereochemistry of simarolide[40] (A44).

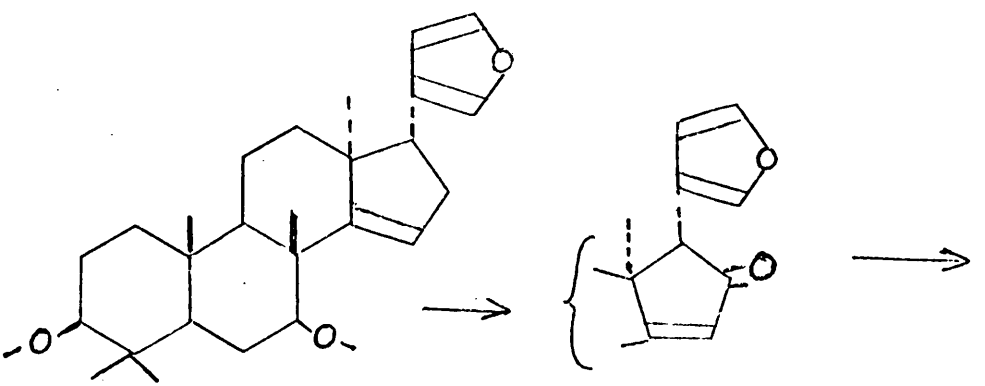
A point of phytochemical interest becomes apparent on examination of the sources of the compounds discussed above. The C_{26} members occur predominantly in the Meliaceae family with the exception of the ring A cleaved derivatives which are found only in the the Rutaceae. The C_{20} and C_{19} classes exist only in the Simarubaceae. These narrow botanical limits may be of use in classification of doubtful genera.

Scheme I



A1 20βH

A2 20αH



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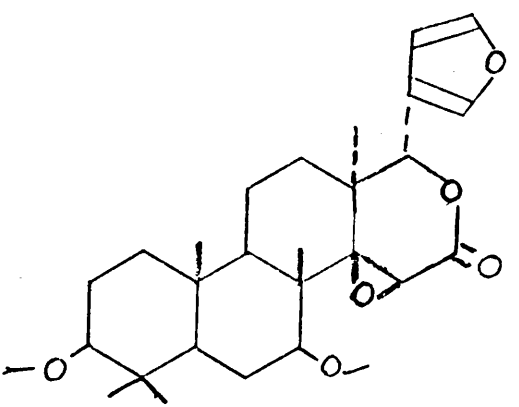

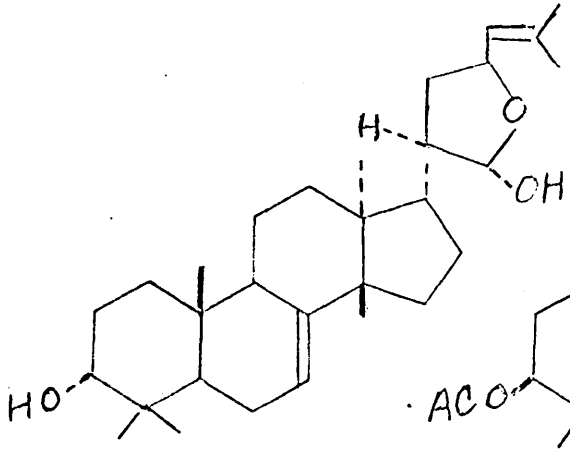

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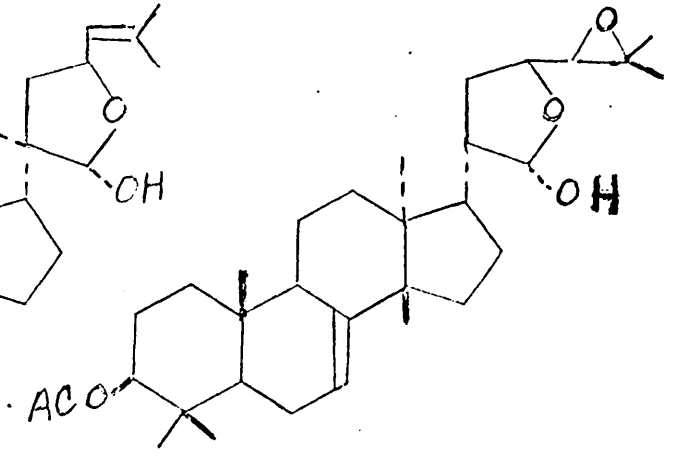

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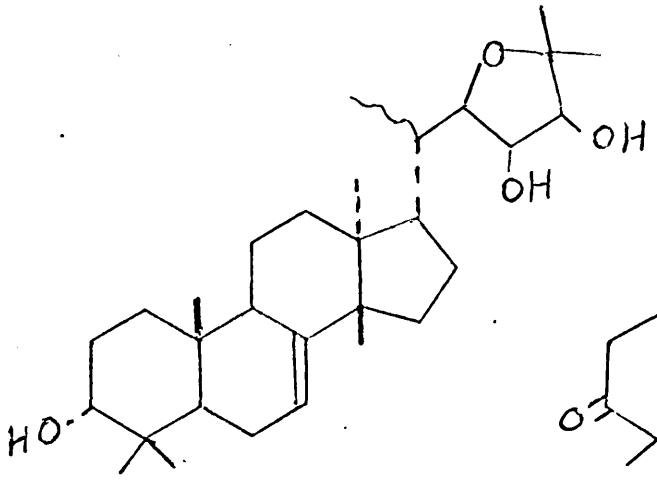

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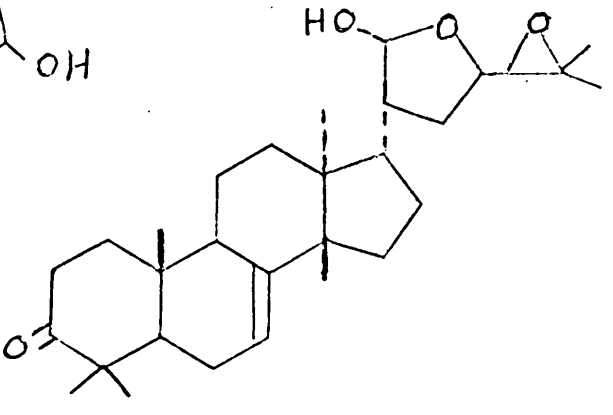

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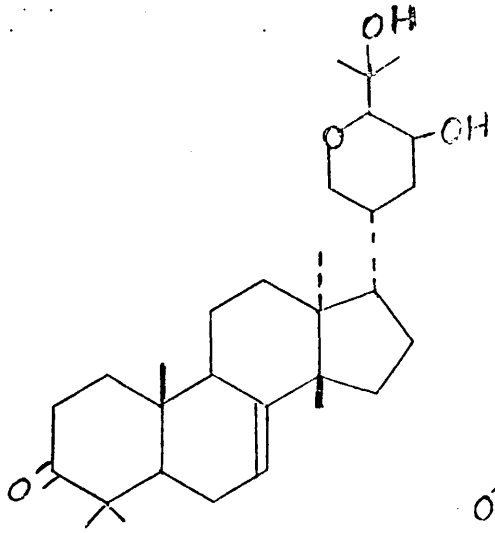

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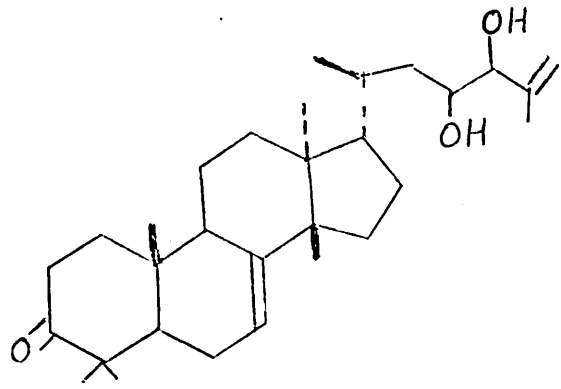

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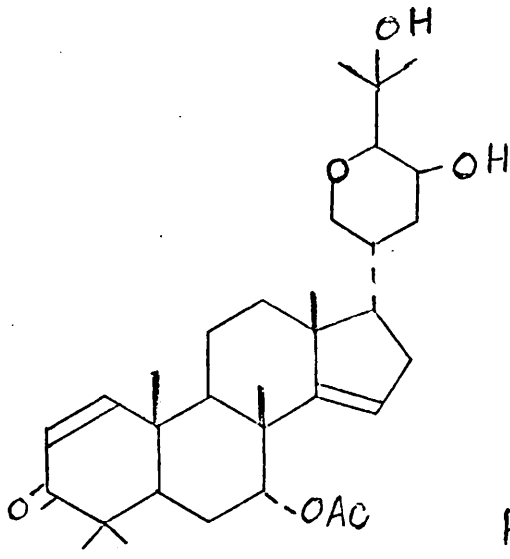

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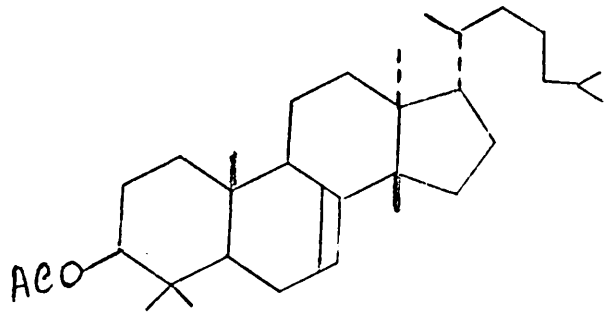

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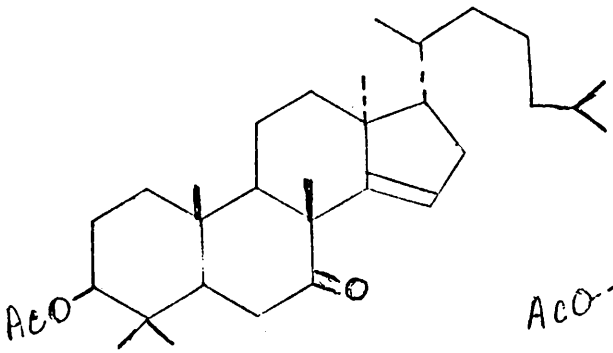

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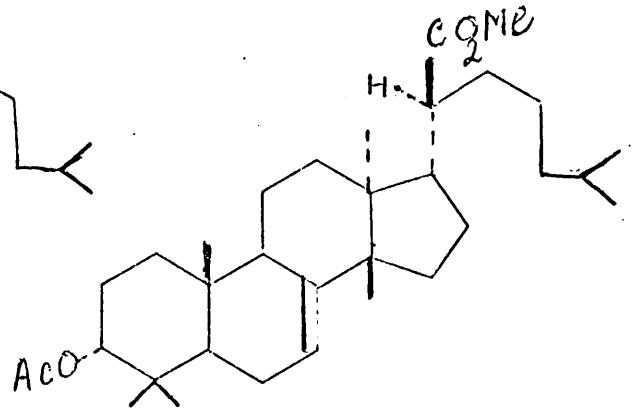

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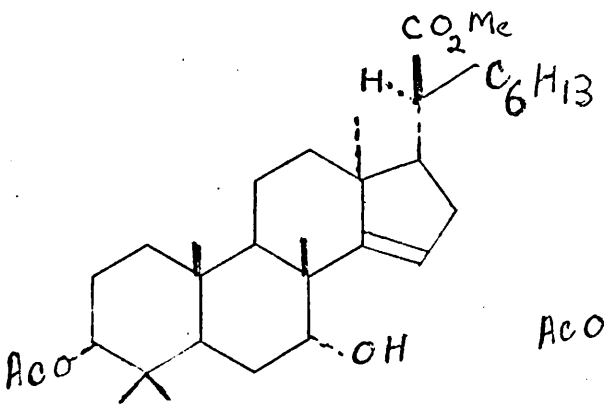

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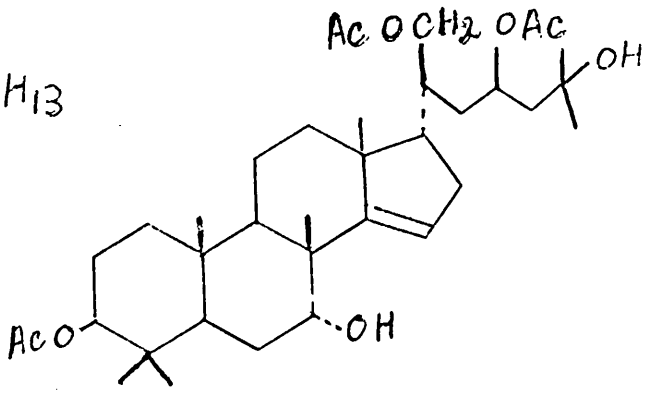

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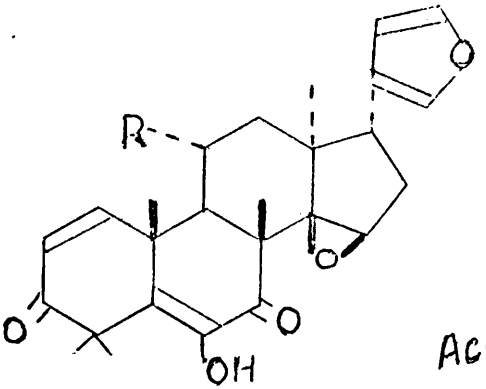
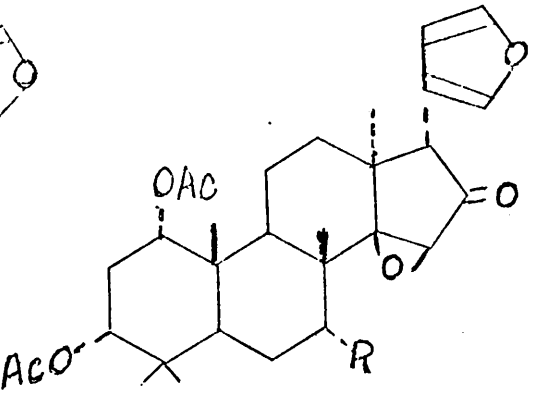


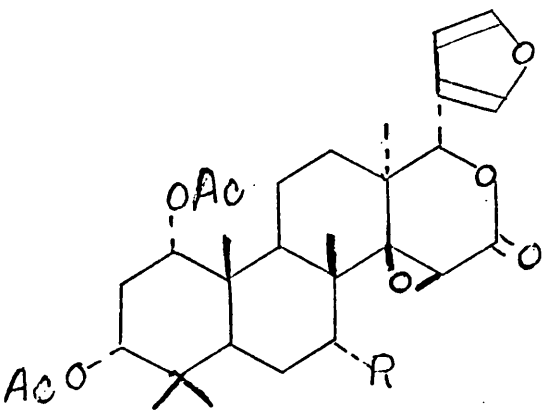
A16 R = H

A17 R = OAc



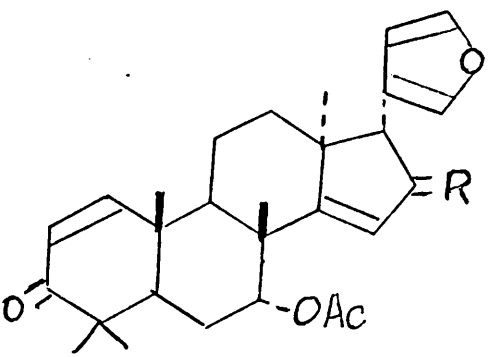
A18 R = OH

A24 R = OAc



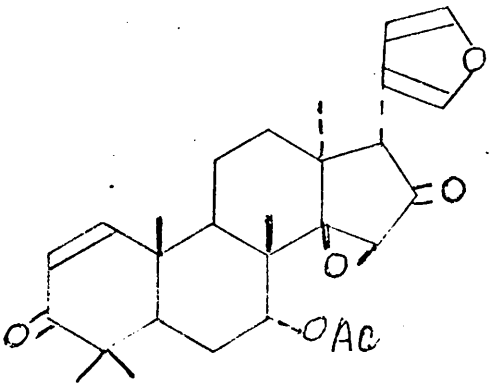
A19 R = OH

A25 R = OAc



A20 R = 2H

A21 R = O



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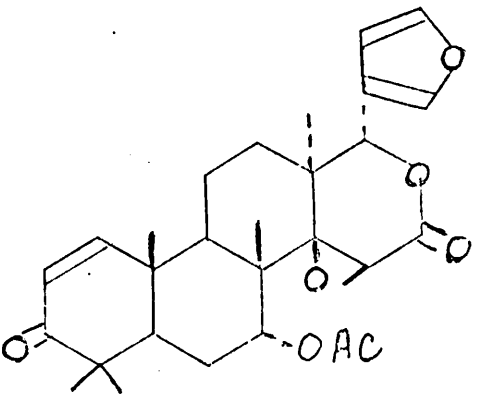

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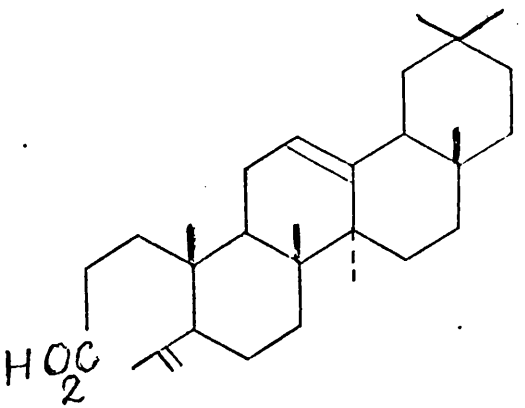

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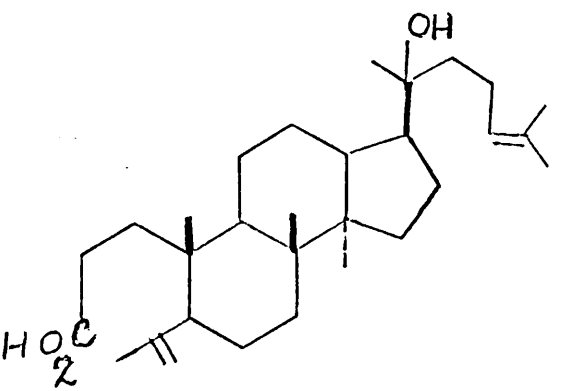

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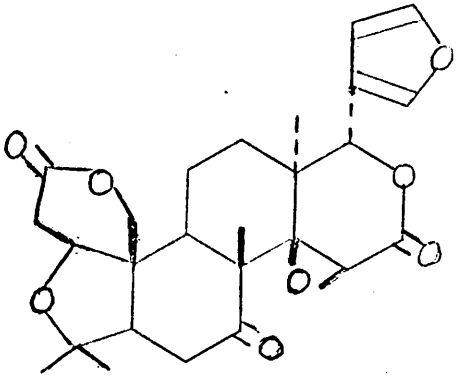

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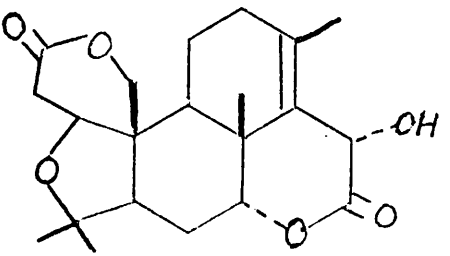

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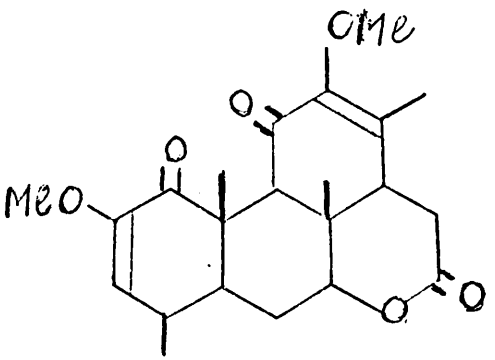

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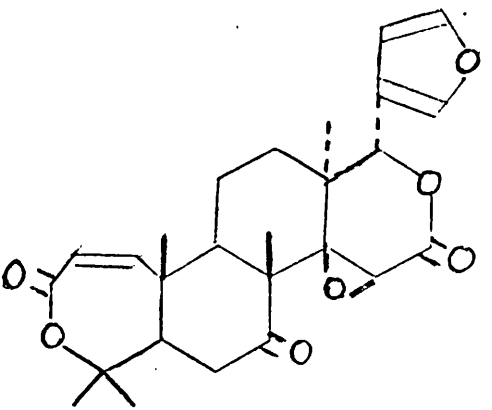

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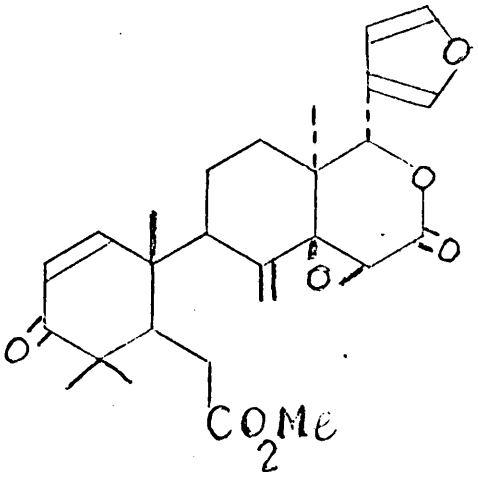

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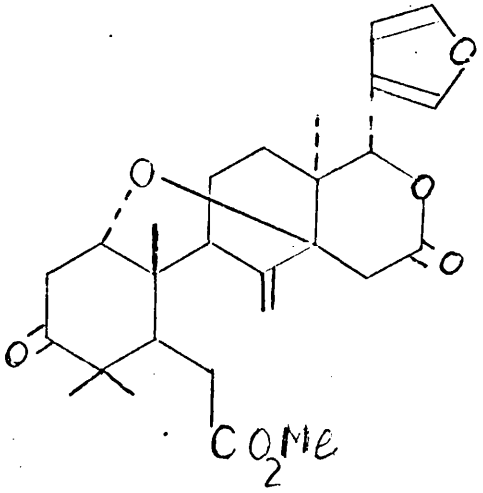

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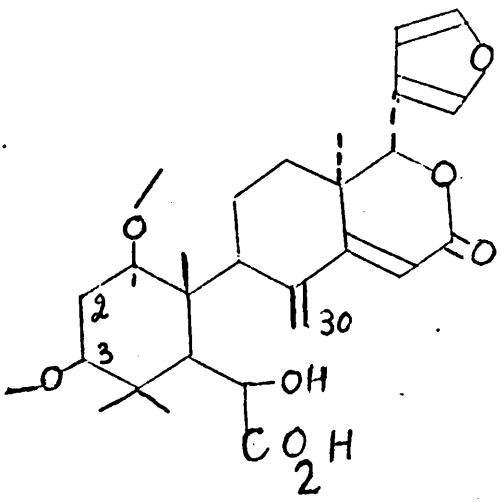

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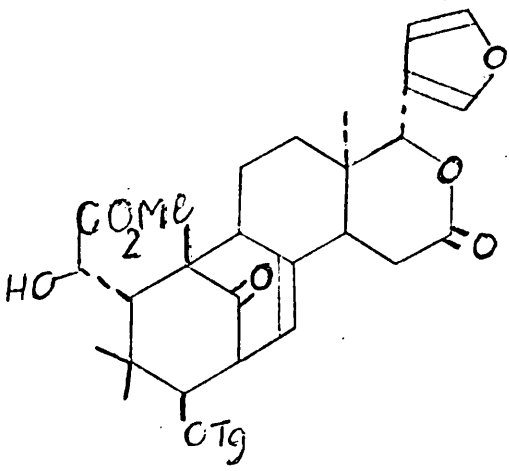

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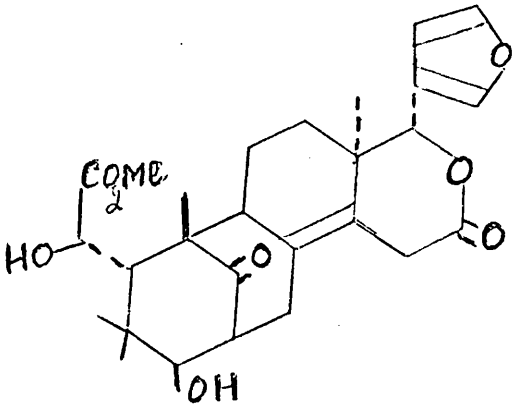

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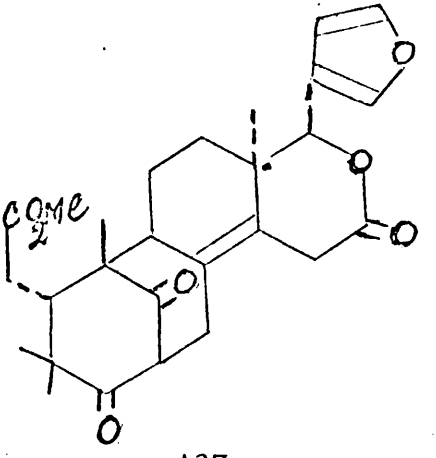

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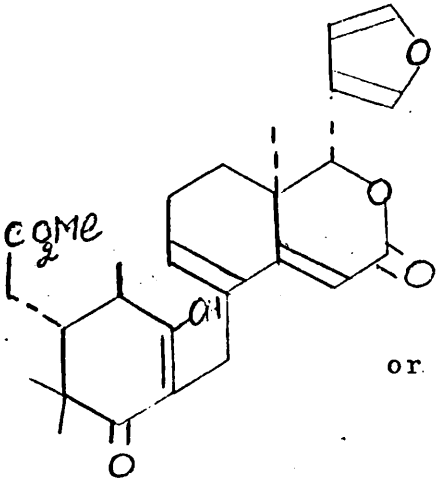

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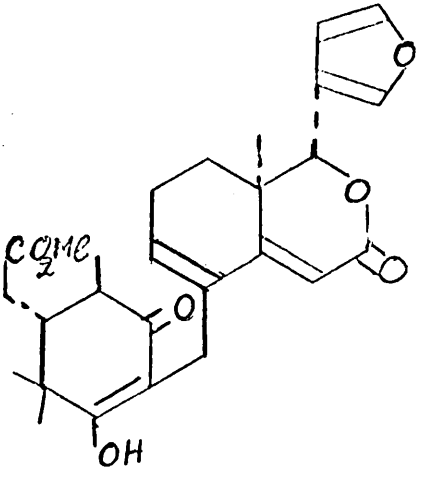

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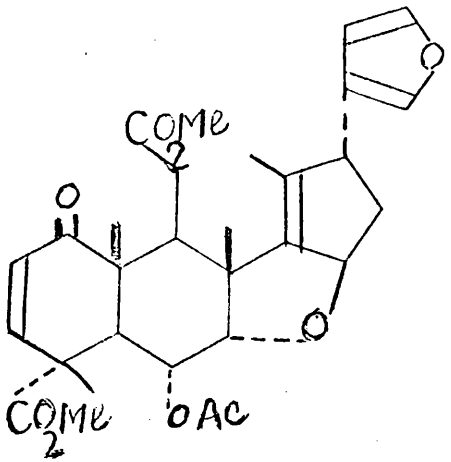

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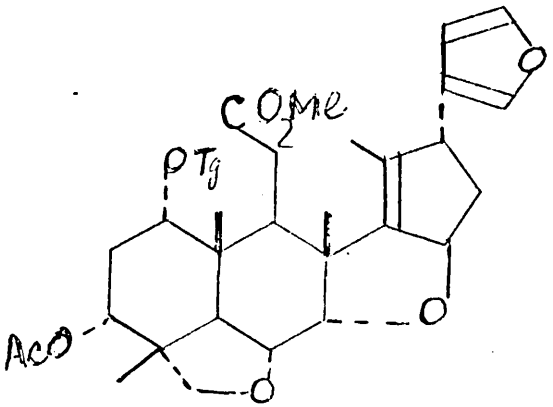

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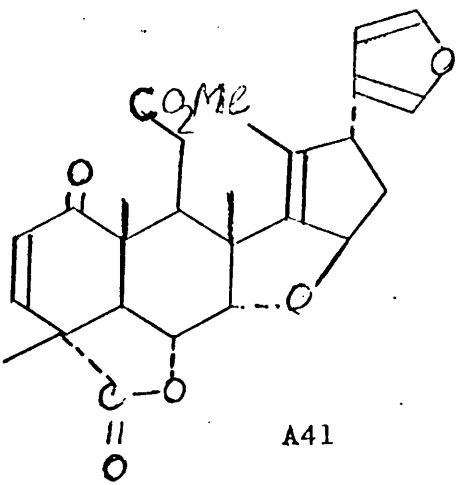

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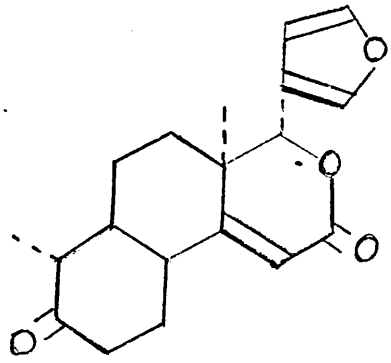

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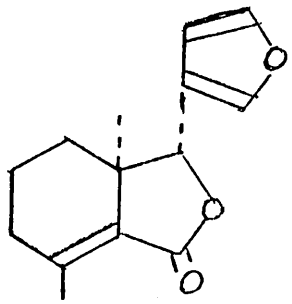

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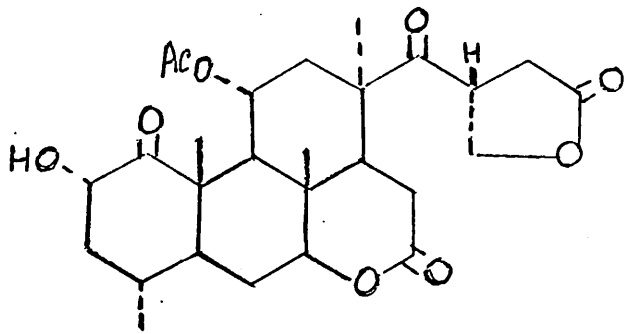

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CHAPTER II

GRANDIFOLIONE AND RELATED COMPOUNDS FROM KHAYA GRANDIFOLIOLA

INTRODUCTION

The genus Khaya (family Meliaceae) is distributed in West Africa and forms the main source of West African mahogany. Many species are found growing there and of these, in addition to Khaya grandifoliola, four have been investigated - K. anthotheca, K. senegalensis, K. ivorensis and K. nyasica. The seeds and heartwood of these species have yielded a number of tetranortriterpenoids whose structures have been elucidated by chemical and spectroscopic methods. The results reported are as follows:

K. anthotheca

(a) Heartwood - anthothecol[1,8] (B1)

(b) Seeds - khivorin (B2), 3-deacetylkhivorin and khayanthone[9] (B4)

K. senegalensis

(a) Heartwood - 7-deacetyl-7-oxokhivorin[1] (B3) and methyl angolensate[6] (B5)

(b) Seeds - khivorin, 3-deacetylkhivorin, methyl angolensate, 7-deacetyl-7-oxokhivorin, 6-deoxy-destigloylswietenine acetate[5,27] (B21)

(c) bark - methyl angolensate, 6-hydroxy methyl angolensate.[5]

K. nyasica

(a) Heartwood - nyasin (B8) and khivorin.[2]

K. ivorensis

(a) Heartwood - khivorin,[3] methyl angolensate and 7-deacetylkhivorin.[2]

(b) Seeds - methyl angolensate.[2]

K. grandifoliola

Earlier work[3] on the heartwood extractive yielded khivorin. Later investigations[4] resulted in the isolation of methyl angolensate, mexicanolide (B9) and grandifoliolin (B10) (fissinolide[11]) which was shown to be the acetate of the 3β-alcohol from sodium borohydride reduction of mexicanolide.[12]

More recent examination[7] of a different sample of heartwood of K. grandifoliola, revealed the presence of methyl angolensate (B5), the previously unreported 6-hydroxy methyl angolensate (B6) and the corresponding

acetate (B7) and a tetracyclic triterpene, grandifoliolenone[10] (B11), whose apoeuphol (or tirucallol) structure is of significance in the biogenetic scheme for tetranortriterpenoids.

No mexicanolide was detected in this sample of wood nor in the specimen to be discussed in the following pages and this, together with other differences, points to considerable regional variations in this particular species of Khaya.

DISCUSSION

We re-examined the extract from a specimen of trunk wood of K. grandifoliola and observed[13] that it contained about 0·002% of a previously undescribed tetranortriterpenoid of novel structure which we named grandifolione and which we formulated as (B12) on the evidence presented below. The extract also yielded the previously unreported 7-hydroxy-7-deacetoxykhivorin (B13) in addition to the known compounds, 7-oxo-7-deacetoxykhivorin (B3), methyl angolensate (B5) and 6-hydroxy methyl angolensate (B6).

Grandifolione, $C_{30}H_{40}O_8$, m.p. 233-235°, $[\alpha]_D$ -35°, had in the i.r. $\nu_{max.}^{CCl_4}$ 1740 (acetates), 1755 (αβ-epoxycyclopentanone) and 3622 $cm.^{-1}$ (free hydroxyl). In view of the limited amount (75 mg.) at our disposal, it was fortunate that the n.m.r. spectrum of grandifolione proved to be most informative. This was particularly so when it was compared with the spectrum of the corresponding oily lactone (B13), $C_{30}H_{40}O_9$, $[\alpha]_D$ -32°, $\nu_{max.}^{CCl_4}$ 3610 (free hydroxyl), 1740 $cm.^{-1}$ (acetate and αβ-epoxy-δ-lactone) which was readily identified by conversion into its acetate khivorin[3] (B2), and also into the previously described 7-ketone[1] (B3) and 1,3,7-triol[1] (see later). Thus grandifolione (B12) and the lactone (B13) both showed resonances of the expected intensity and multiplicity for the protons at C-1, C-3, C-7, C-21, C-22 and C-23, the chemical shift differences for corresponding hydrogens being not greater than 0·2 p.p.m. (Table I). This similarity extended to the C-methyl region and the unresolved region between τ 7 and τ 9 of methylene and methine signals. At the same time the anticipated differences between the ketone (B12) and the lactone (B13) were

clearly discernible. Thus the H-17 signal moved from τ 6·20 in the ketone to τ 4·53 in the lactone while H-15 moved from τ 6·54 to τ 6·16. The H-17 signal could be distinguished from the sharper H-15 signal in the spectrum of (B12) by double irradiation which demonstrated its long-range coupling with the methyl group at τ 8·92 (C-18 methyl).

Oxidation of grandifolione afforded the diketone (B14), $C_{30}H_{38}O_8$, m.p. 229-230°, $\nu_{max.}^{CCl_4}$ 1718 (cyclohexanone), 1745 (acetates), 1758 ($\alpha\beta$-epoxycyclopentanone) $cm.^{-1}$, and acetylation the triacetate (B4), $C_{32}H_{42}O_9$, m.p. 214-216°, $\nu_{max.}^{CCl_4}$ 1742 (acetates), 1756 ($\alpha\beta$-epoxycyclopentanone) $cm.^{-1}$ which was identical in all respects with khayanthone, a compound later isolated[9] from Khaya anthotheca. Comparison of the n.m.r. spectra of these compounds with those of the related lactones, 7-oxo-7-deacetoxykhivorin (B3), and khivorin (B2), again showed the similarities observed between the spectra of (B12) and (B13). Moreover, in each pair of compounds, four of the five tertiary methyl groups were similarly and characteristically grouped, while the fifth was deshielded by $\sim$ 20 c/sec. in the lactone when compared with the corresponding ketone. Comment has previously been

made[14,15] on the shift of the H-15 resonance resulting from change of the substituent at C-7. This was indeed observed for the change 7αOH → 7αOAc → 7CO in both the ketone series, where H-15 changed τ 6·53 → 6·63 → 6·39 and the lactone series, where it changed τ 6·16 → 6·50 → 6·21.

Further support for the proposed αβ-epoxycyclopentanone in grandifolione came from its reduction by chromous acetate which afforded deoxygrandifolione (B15), $C_{30}H_{40}O_7$, m.p. 206-207°, $\nu_{max.}^{CCl_4}$ 1711 (cyclopentenone), 1735 (acetates), 3612 (free hydroxyl) $cm.^{-1}$, $\lambda_{max.}$ 242 mμ (ε 8,300). The epoxidic proton signal (H-15) in grandifolione (τ 6·54) was replaced by a sharp singlet at τ 4·17.

It is reasonable to assign the α configuration (axial) to the oxygen functions at C-1, C-3 and C-7 in grandifolione (B12), since in all the compounds examined the observed width at half height $(W^{\frac{1}{2}})$[16] for all >C<u>H</u>OAc and >C<u>H</u>OH protons was less than 8 c/sec.

The c.d. of grandifolione (B12) and deoxygrandifolione (B15) deserve comment. Cyclopentenones normally show a reversal of sign in the CE for the R (n → π*)

band vis a vis cyclohexenones of the same chirality.[17] The chirality of what from Drieding models appears to be the most favourable conformation of the enone in grandifolione (see B16) leads on the basis of this 'inverse rule' to prediction of a negative CE. This is in fact observed but, interestingly not only in the R-band $\Delta\varepsilon_{max.(330\ m\mu)} = -0{\cdot}4$, but also in the K-band $\Delta\varepsilon_{max.(241\ m\mu)} = -11{\cdot}5\ -12{\cdot}4$, showing that the R and K bands need not necessarily be of opposite sign as they normally are.[18]

The c.d. of grandifolione (B12) is even more interesting. It has been known for some time that αβ-epoxidoketones obey a 'Reversed Octant Rule'.[19,20] However there have previously been lacking examples of this chromophore in a five-membered ring and it was of interest to see whether here, as in αβ-unsaturated ketones, a reversal of the CE compared with the 6-ring compounds, would be observed. The large negative CE of grandifolione $\Delta\varepsilon_{max.(309\ m\mu)} = -4{\cdot}28$ closely parallels that recently described[21] for the equivalent chromophore in the steroidal epoxidoketone (B17) $\Delta\varepsilon_{max.(311m\mu)} = -5{\cdot}47$ and this has the same sign as

the CE of the corresponding αβ-epoxidocyclohexanone (B18)[19]. There is thus no reversal of the effect and αβ-epoxycyclopentanones (like αβ-epoxycyclohexanones) appear to obey the simple 'Reversed Octant Rule'.

The structure of grandifolione has an interesting bearing on the supposed biosynthesis of tetranortriterpenoids since it is the first representative of the intermediate cyclopentenone stage[22] (see Introduction). Isolation of the related epoxido-lactone (B13) from the same extract lends additional support to the postulated biosynthetic sequence. Since the publication[13] of grandifolione, the isolation of four related substances also from Meliaceous sources has been reported.[9,23,24]

GENERAL EXPERIMENTAL

Melting points were taken on a Kofler hot stage apparatus. Specific rotations refer to chloroform solutions except where otherwise specified. Infrared solution spectra were kindly recorded by Mrs. F. Lawrie on the Perkin-Elmer 225 and 257 grating infrared spectrophotometers. Ultraviolet spectra were measured in ethanol on a Unicam SP 800 A spectrophotometer. Microanalysis were performed by Mr. J.M.L. Cameron, B.Sc. and his staff. Woelm grade I alumina deactivated according to the Brockmann[25] scale of activity and Spence grade H alumina deactivated with 10% acetic acid were used for column chromatography. Chromatoplates were prepared by the method of Stahl[26] using Kieselgel G (Merck). Rf values were not recorded but where necessary known compounds were run concurrently for comparison purposes. Circular dichroism curves were recorded by Dr. G. Snatzke and optical rotatory dispersion curves were measured by Professor W. Klyne. Nuclear magnetic resonance spectra were obtained by Mrs. S.P. Hamilton, Miss M. Taylor and Mr. J. Gall on a Perkin-Elmer R 10, and a Varian Associates HA 100 spectrometer operating at 60

Mc/sec. and 100 Mc/sec. respectively, using approximately 0·3M solutions in deuterated chloroform with tetramethylsilane as internal standard. Double irradiation experiments were performed with the latter using a Muirhead D 890A oscillator and calibration was checked with Hewlett-Packard 5212 A electronic counter. The Argon chromatograph of W.G. Pye was used for gas liquid chromatography and the LKB 9000 gas chromatography-mass spectrometry (GC-MS) instrument was used for GC-MS studies. Mass molecular weights were measured on an AEI MS 9 spectrometer by Dr. P. Bladon, Strathclyde University and Dr. J. Martin.

EXPERIMENTAL

Extraction of Grandifolione.

Powdered heartwood (3·5 kg.) of Khaya grandifoliola* was extracted with ethyl acetate in a Soxhlet. The solvent was removed in vacuo and the residual dense oil (150 g.) triturated with hot chloroform. This yielded chloroform-soluble material (20 g.) which was chromatographed over alumina (Grade V; 1 kg.) in light petroleum. The initial fractions [up to benzene-light petroleum (9:1)] yielded β-sitosterol (1·3 g.), methyl angolensate[6] (35 mg.) m.p. 198-200° (ex methanol), and 7-oxo-7-deacetoxykhivorin[1] (3·0 g.) m.p. 227-228° (ex methanol). Benzene eluted a mixture which was separated by t.l.c. into 6-hydroxy methyl angolensate[7] (18 mg.) m.p. 237-239° (ex aqueous methanol), the previously unreported oily 7-hydroxy-7-deacetoxykhivorin (B13) (250 mg.), $[\alpha]_D$ -32° (c 1·0). (Found: C. 65·75; H, 7·35. $C_{30}H_{40}O_9$ requires C, 66·15; H, 7·4%), and grandifolione (B12) (75 mg.) m.p. 233-235° (from ether) $[\alpha]_D$ -35·5° (c 1·0), $\nu_{max.}^{CCl_4}$ 1740 (acetates), 1755 (αβ-epoxycyclopentanone), and 3622 (free hydroxyl) $cm.^{-1}$. (Found: C, 67·9; H, 7·5. $C_{30}H_{40}O_8$ requires C, 68·15; H, 7·65%).

* Obtained from the Forestry Division, Kumasi, Ghana.

Reduction of Grandifolione with Chromous Acetate.

To grandifolione (25 mg.) in acetone (AnalaR; 12 ml.) was added sodium acetate (250 mg.) and glacial acetic acid (2 drops) in water (5 ml.). Chromous acetate (100 mg.) in aqueous acetone (12 ml.; 1:5) was then added and the solution stirred at 20° under nitrogen for 12 hr. The reaction mixture was diluted with water and extracted with chloroform. Preparative t.l.c. of the crude product yielded deoxygrandifolione (B15) (14 mg.) m.p. 206-207° (from ether-light petroleum) $\lambda_{max.}$ 242 mμ (ε 8,300), $\nu_{max.}^{CCl_4}$ 1711 (cyclopentenone), 1735 (acetates), and 3613 (free hydroxyl) cm.$^{-1}$. (Found: C, 70·1; H, 7·85. $C_{30}H_{40}O_7$ requires C, 70·3; H, 7·85%).

Oxidation of Grandifolione.

Grandifolione (25 mg.) was dissolved in acetone (3 ml.) and the solution cooled to 0°. Jones reagent (5 drops) was added and after 1 min. the reaction mixture was diluted with water and extracted with chloroform. The product on crystallisation from ether gave the dione (B14) (19 mg.) m.p. 229-230°, $\nu_{max.}^{CCl_4}$ 1718 (cyclohexanone), 1745 (acetates), and 1758 (αβ-epoxy-cyclopentanone) cm.$^{-1}$. (Found: C, 67·8; H, 7·5. $C_{30}H_{38}O_8$ requires C, 68·4; H, 7·3%).

Acetylation of Grandifolione.

Grandifolione (20 mg.) was heated on the steam-bath with acetic anhydride (1 ml.) in dry pyridine (0·5 ml.) for 1 hr. and then worked up. The acetate (B4) (20 mg.) was purified by t.l.c. and then crystallisation from chloroform-ether-light petroleum. It had m.p. 215-217°, $\nu_{max.}^{CCl_4}$ 1742 (acetates) and 1756 (αβ-epoxycyclopentanone) cm.$^{-1}$. (Found: C, 67·35; H, 7·65. $C_{32}H_{42}O_9$ requires C, 67·35; H, 7·4%).

Oxidation of 7-Hydroxy-7-Deacetoxykhivorin.

The diacetate (B13) (30 mg.) in acetone (3 ml.) was treated with Jones reagent (5 drops) at 0°. The product on crystallisation from methanol had m.p. 227-228°, alone or when mixed with an authentic sample of 7-oxo-7-deacetoxykhivorin (B3) (i.r. and n.m.r. spectra identical).

Acetylation of 7-Hydroxy-7-Deacetoxykhivorin.

The diacetate (30 mg.) was treated in pyridine (0·5 ml.) with acetic anhydride (2 ml.) on the steam-bath for 1 hr. The product was purified by t.l.c. and crystallised from ether to yield khivorin[3] (B2) m.p. 257-260°.

Hydrolysis of 7-Hydroxy-7-Deacetoxykhivorin.

The diacetate (20 mg.) was treated at 20° with methanolic potassium hydroxide(2%, 5 ml.) for 12 hr. Acidification and extraction of this solution yielded the crude product which was purified by t.l.c. and crystallisation from methanol to give the known triol[1] (B19) m.p. 328-330°.

Hydrolysis of 7-Oxo-7-Deacetoxykhivorin.

The diacetoxyketone (50 mg.) was treated for 12 hr. at 20° with methanolic potassium hydroxide (2%, 5 ml.). This solution on acidification with dilute hydrochloric acid and extraction with chloroform gave the diol (B20) m.p. 290-292° (from methanol). (Found: C, 68·2; H, 7·4. $C_{26}H_{34}O_7$ requires C, 68·1; H, 7·45%).

Identification of the Known Compounds.

1. 7-Oxo-7-deacetoxykhivorin (B3) crystallised from methanol, had m.p. 227-228° $[\alpha]_D$ -106° (lit.[1] m.p. 228-232°, $[\alpha]_D$ -106°) $\nu_{max.}^{CCl_4}$ 1719 (cyclohexanol), 1744 $cm.^{-1}$ (acetate and αβ-epoxy-δ-lactone), for n.m.r. data see table I.

2. β-sitosterol, crystallised from methanol, had m.p. 136-138°, m.p. undepressed with authentic sample.

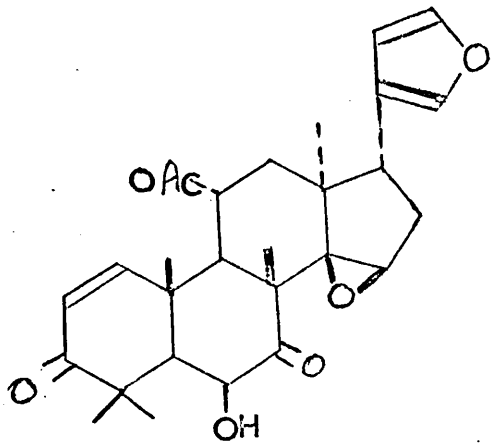
3. Methyl angolensate (B5), crystallised from methanol in rods, showed m.p. 198-200°, m.m.p. undepressed and n.m.r. spectrum identical with authentic sample.

4. 6-Hydroxy methyl angolensate (B6) crystallised from aqueous methanol in needles m.p. 237-239°, $[\alpha]_D$ -85°, m.m.p. undepressed with authentic sample.

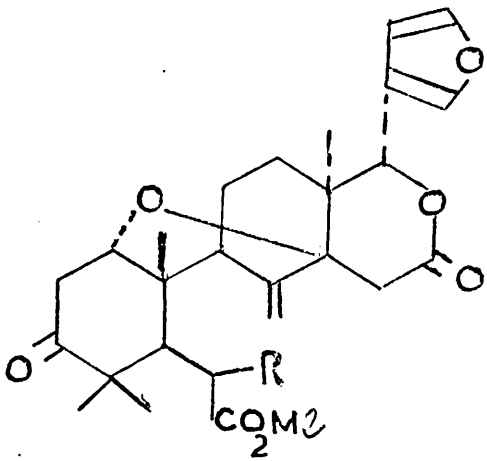
Table I

τ values.

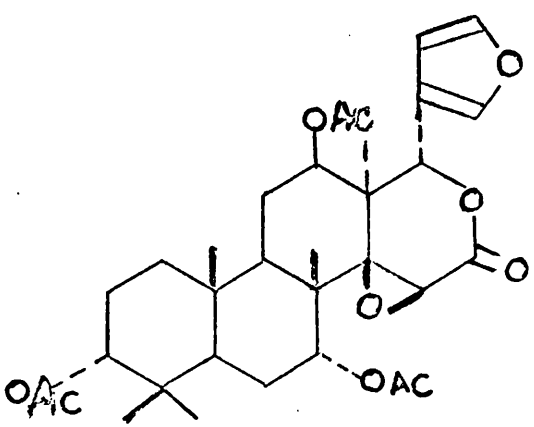
	H-1	H-3	H-7	H-15	H-17	α Furan	β Furan	Acetate	Methyl groups
1 Grandifolione (B12)	5·33m	5·33m	6·45	6·54	6·2	2·57	3·79	7·9,7·8	9·04, 8·99, 8·92, 8·89, 8·83
2 Grandifolione Acetate (B4)	5·32m	5·32m	5·33m	6·63	6·21	2·57	3·79	7·95, 7·87(2)	9·13, 9·02, 8·92, 8·85, 8·81
3 Ketone (B14)	5·33m	5·33m	-	6·39	6·2	2·56	3·77	7·95, 7·97	9·04, 9·00, 8·94, 8·76(2)
4 7-Hydroxy-7-deacetoxy-khivorin(B13)	5·4	5·5	6·55	6·16	4·53	2·65	3·73	7·8(2)	9·07, 9·03, 8·97, 8·95, 8·72
5 Khivorin(B2)	5·34	5·34	5·34	6·5	4·41	2·64	3·71	7·95(2), 7·82	9·12, 9·02, 8·93, 8·88, 8·7
6 7-Oxo-7-deacetoxy-khivorin(B3)	5·3	5·3	-	6·21	4·55	2·63	3·70	7·93, 7·95	9·04, 8·94, 8·78(3)
7 Deoxy-grandifolione (B15)	5·29	5·36	6·05	4·17	6·57	2·57	3·75	7·83, 7·97	9.02, 8·98, 8·92, 8·9, 8·71



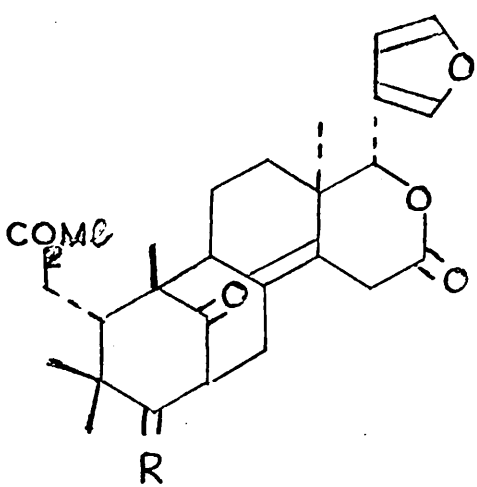
B1



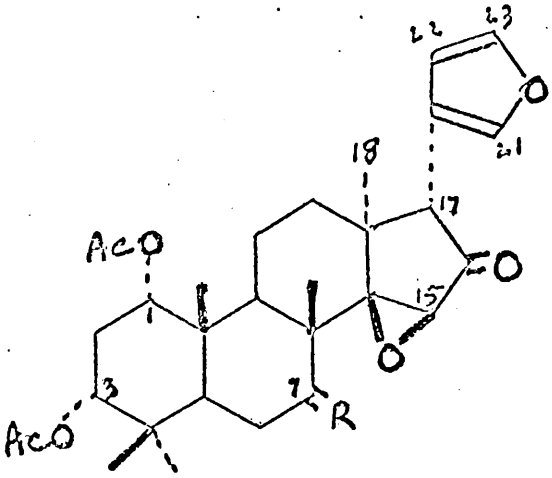
B5 R = H
B6 R = OH
B7 R = OAc



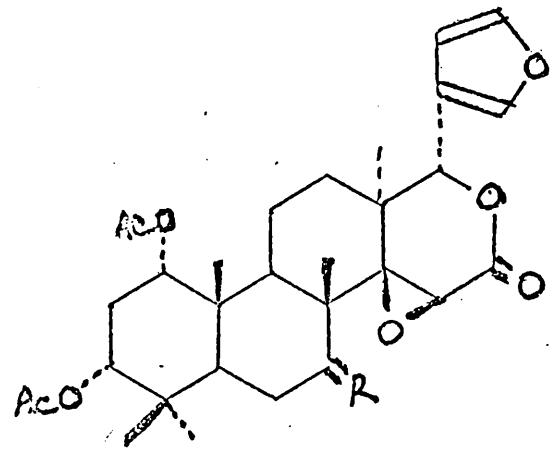
B8



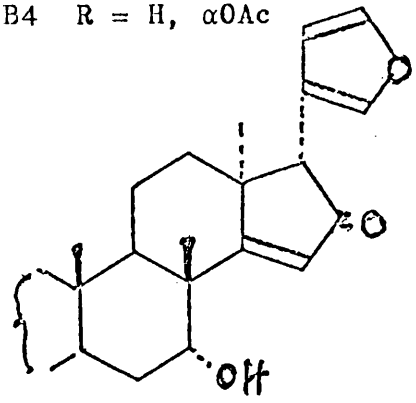
B9 R = O
B10 R = βOAc, H



B12 R=H, αOH
B14 R = O
B4 R = H, αOAc



B13 R = H, αOH
B2 R = H, αOAc
B3 R = O



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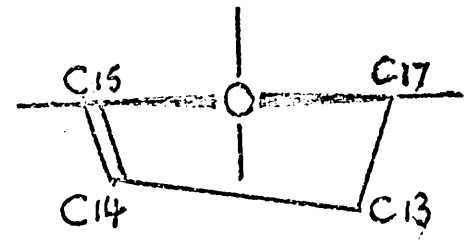

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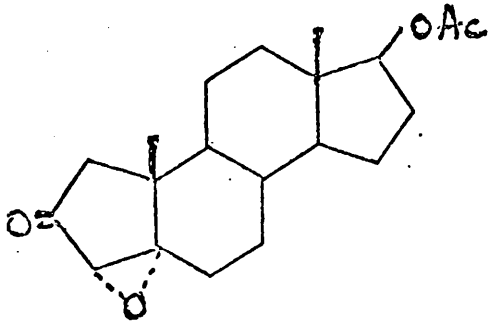

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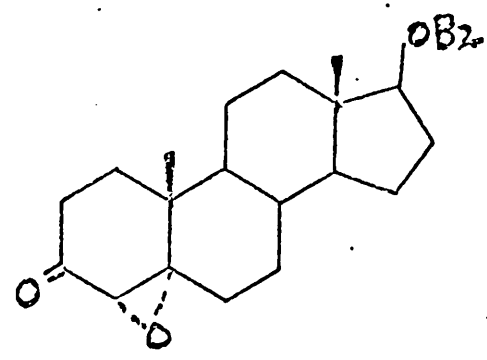

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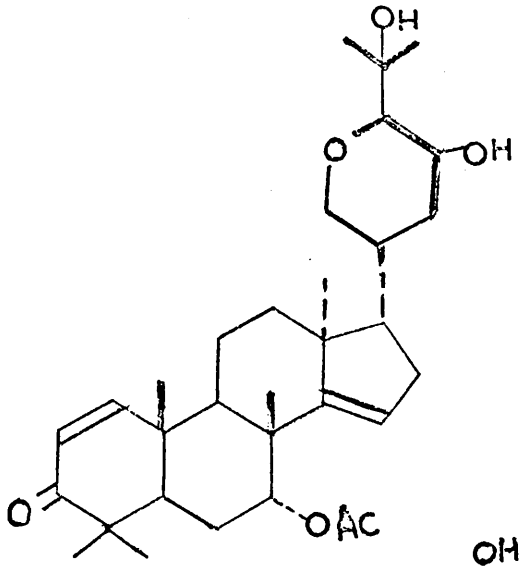

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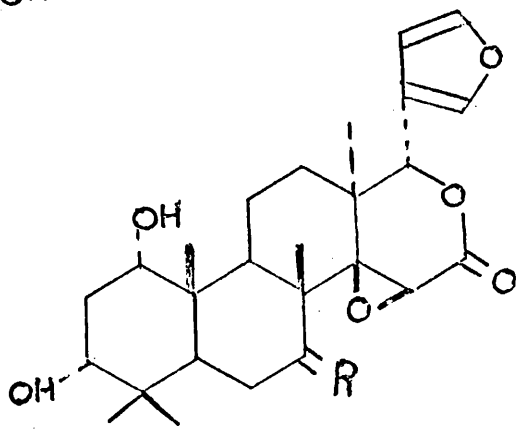
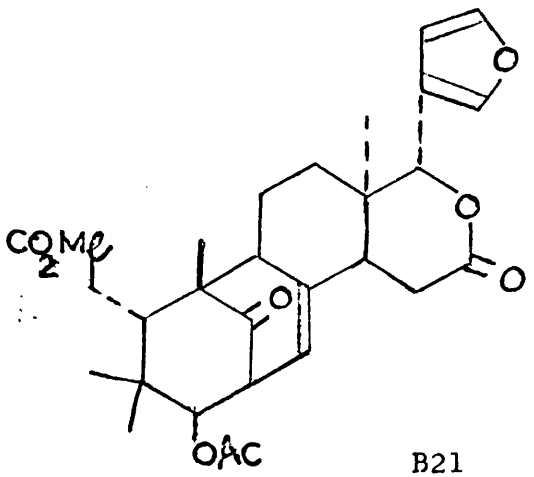


B19 R = αOH, H

B20 R = O



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CHAPTER III

HEARTWOOD CONSTITUENTS OF

CEDRELA GLAZIOVII

INTRODUCTION

The genus Cedrela (family Meliaceae) is distributed in the tropical regions of the world. Chemical studies of the heartwoods of trees belonging to this genus have interested many workers. The benzene extractive of the heartwood of Cedrela toona gave the tetranortriterpenoid cedrelone[1] (C1) and a mobile oil which contained mainly sesquiterpenes.[2,3,4] The structure of cedrelone, arrived at by chemical means,[5] was confirmed by X-ray analysis.[6]

The heartwood of Cedrela mexicana yielded the bicyclononanolide mexicanolide[7,8] (C2). A closely related compound, fissinolide (C3), was isolated from the fruit of C. fissilis.[9] Fissinolide was readily shown to be the acetate of the 3β-alcohol obtained from mexicanolide by sodium borohydride reduction.

It has been observed that the constituents of C. odorata[10] (popularly known as West Indian Cedar) vary, depending upon its growth and environmental conditions. The light petroleum extract of the heartwood yielded mexicanolide and 7-deacetyl-7-oxogedunin (cedrolide)[10] (C5). Examination of the benzene extract of the heartwood resulted in the isolation[11,14] of the known

compounds methyl angolensate (C6), gedunin (C4) and 7-deacetyl-7-oxogedunin together with two new tetracyclictriterpenoids odoratol (C7) and odoratone (C8) which were also obtained independently from C. glaziovii. It is the investigation of the constituents of C. glaziovii that forms the subject matter of this section.

DISCUSSION

We investigated[12,13] the ethyl acetate extract of the heartwood of Cedrela glaziovii and isolated from it the following new compounds:

Odoratol (C7), $C_{30}H_{50}O_4$, m.p. 235-237°, $[\alpha]_D$ -50°.

3-dehydro-odoratol (odoratone) (C8), $C_{30}H_{48}O_4$, m.p. 230-232°, $[\alpha]_D$ -80°.

24-epi-odoratol (iso-odoratol) (C30), $C_{30}H_{50}O_4$, m.p. 251-253°, $[\alpha]_D$ -54°.

Pentaol I acetonide (C35), $C_{33}H_{56}O_5$, m.p. 215-218°, $[\alpha]_D$ -37°.

Pentaol II acetonide (C37), $C_{33}H_{56}O_5$, m.p. 236-238°, $[\alpha]_D$ -34°.

6-hydroxycarapin (C33), $C_{27}H_{32}O_8$, $[\alpha]_D$ +35°.

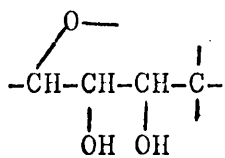
In addition two new non-crystalline tetranortriterpenoids were obtained, together with a number of previously described compounds of this class - carapin[15] (C32), mexcianolide[10] (C2), cedrolide[10,11] (C5), methyl angolensate[11] (C6) and gedunin[16] (C4).

Odoratol, which was also found in C. mexicana,[7] was formulated as (C7) on the following evidence.

The n.m.r. spectra of odoratol and its derivatives clearly indicated one secondary and seven tertiary methyl groups thus suggesting a euphane or lanostane skeleton. The single vinyl proton (τ 4·7 - 4·8; $W^{\frac{1}{2}}$ 10 c/sec.) was characteristic of an isolated trisubstituted olefinic linkage with an adjacent methylene group ($\geq C{=}CH{-}CH_2{-}$).

The i.r. spectrum of odoratol showed only hydroxyl absorption ($\nu_{max.}^{nujol}$ 3372 $cm.^{-1}$) whereas the corresponding acetate (C22) had no hydroxyl groups ($\nu_{max.}^{CCl_4}$ 1750 and 1735 $cm.^{-1}$). The n.m.r. spectrum indicated that it was a triacetate (see Table I). In addition odoratol gave an acetonide (C23), $C_{33}H_{54}O_4$, m.p. 210-212°, when treated with anhydrous copper sulphate in acetone. This was transformed, on oxidation with Jones reagent

into the keto-acetonide (C17), $C_{33}H_{52}O_4$, m.p. 169-170°, $\nu_{max.}^{CCl_4}$ 1720 cm.$^{-1}$, which lacked hydroxyl absorption. Therefore of the four oxygen atoms in odoratol three were present as secondary hydroxyl groups and fourth as an ether. The n.m.r. spectra of the above derivatives (see Table I) showed that two of the hydroxyl groups were vicinal and that the ether linkage was attached α to one of them. This led to the part structure



most probably situated in the side chain of a euphol or tirucallol skeleton.

The hydroxyl groups could be readily distinguished chemically and assigned to their respective environments. Thus oxidation of odoratol with Jones reagent led to six products which were separated by preparative t.l.c.

Three monoketones $C_{30}H_{48}O_4$

(1) (C8), m.p. 230-232°, $\nu_{max.}^{CCl_4}$ 3635, 3563 (hydroxyl) and 1720 cm.$^{-1}$ (cyclohexanone) (identical with odoratone of natural provenance). A detailed analysis of the n.m.r. spectrum of the derived diacetate (C14), m.p. 219-221°, $\nu_{max.}^{CCl_4}$ 1755, 1715 cm.$^{-1}$, made it possible to define the functionality in the side chain of odoratol. The signals from H-22 (τ 6·06; d, J = 6 c/sec.) H-23 (τ 4·96; t, J = 6,7 c/sec.) and H-24 (τ 5·21; d, J = 7 c/sec.), each collapsed upon double irradiation at the interacting proton as expected. Moreover double irradiation at H-20 (τ 8·43) simultaneously collapsed the C-21 methyl doublet to a singlet and sharpened the slightly diffuse doublet (τ 6·06) arising from H-22.

(2) (C9), m.p. 233-234°, $\nu_{max.}^{CCl_4}$ 3615, 3540, 1768 cm.$^{-1}$, which showed in the n.m.r. signals for H-3 at τ 6·57 (bs), H-22 at τ 6·1 (s) and H-24 at τ 6·17 (s). The fact that H-22 and H-24 were singlets showed that the secondary hydroxyl at C-23 in odoratol had suffered oxidation. The singlet arising from H-22 was slightly diffuse due to a small coupling with H-20.

(3) (C10), m.p. 220-221°, $\nu_{max.}^{CCl_4}$ 3615, 3540, 1766 cm.$^{-1}$ whose assignment as 24-dehydro odoratol followed readily from the n.m.r. spectrum in which H-22 (τ 6·19), H-23 (τ 5·84) formed essentially an AB quartet. Again H-22 showed a small coupling with H-20.

Two diketones $C_{30}H_{46}O_4$

(4) (C11), m.p. 194-196°, $\nu_{max.}^{CCl_4}$ 3562, 1768 and 1713 cm.$^{-1}$, whose n.m.r. spectrum showed H-22 (τ 6·11) and H-24 (τ 6·18) as singlets, supporting the 3,23-bisdehydro structure of this compound.

(5) (C12), m.p. 200-202°, $\nu_{max.}^{CCl_4}$ 3540, 1766 and 1713 cm.$^{-1}$, which was assigned the structure (C12) on the basis of the appearance of an AB quartet (J = 11 c/sec.) at τ 6·16 (H-22) and τ 5·81 (H-23). The higher field doublet was sharpened when H-20 (τ 8·04) was irradiated; the signal from the C-21 methyl group (τ 9·03, d), simultaneously collapsed to a singlet.

(6) The triketone (diosphenol), (C13), $C_{30}H_{44}O_4$, m.p. 132-134°, which had $\nu_{max.}^{CCl_4}$ 3540, 1715, 1635 cm.$^{-1}$ and $\lambda_{max.}$ 292 mμ, shifting in base to $\lambda_{max.}$ 342 mμ. The derived acetate had $\nu_{max.}^{CCl_4}$ 1782, 1635 and 1712 cm.$^{-1}$.

The four α-ketols (C9), (C10), (C11), and (C12) showed in the i.r. the expected[17] hypsochromic shifts: $\nu_{max.}^{CCl_4}$ respectively at 1768, 1766, 1768, 1766 cm.$^{-1}$.

Three major points remained undefined: (a) the choice between a lanostane and a euphane/tirucallane skeleton; (b) location of the olefinic double bond; (c) location of the remaining secondary hydroxyl group.

The isolated trisubstituted olefinic double bond in odoratol was placed at position 7(8) in a euphane or lanostane skeleton, since it was readily isomerised into the $\Delta^{8(9)}$-position and oxidised to the 7,9(11)-diene. Thus odoratol was converted almost quantitatively into the $\Delta^{8(9)}$-isomer (C15), $C_{30}H_{50}O_4$, m.p. 211-214°, $[\alpha]_D$ -31° when it was shaken in acetic acid with hydrogen in presence of Adams' catalyst.[18] The diene (C16), $C_{30}H_{48}O_4.H_2O$, m.p. 255-260°, $[\alpha]_D$ -174°, $\lambda_{max.}$ 232, 239, 248 mμ (ε 14,700; 16,150; 10,600), was formed smoothly when odoratol was treated with mercuric acetate in acetic acid solution at 20°.

Both the facile isomerisation of odoratol to its $\Delta^{8(9)}$-isomer[19] and the positions of the triple maximum in the diene[20] (C16) placed odoratol in the euphane/tirucallane rather than the lanostane series, and further support in this direction came from the large negative $\Delta[\Phi]_D$ value (-674°) for the $\Delta^{8(9)}$-isomer (C15) → 7,9(11)-diene (C16) change.[21] These conclusions were reinforced by a detailed comparison of the O.R.D. curves of the 3-oxo-acetonide (C17), $C_{33}H_{52}O_4$, m.p. 208-210°, its $\Delta^{8(9)}$-isomer (C18), m.p. 169-170°, and the diene (C19), $C_{33}H_{50}O_4$, m.p. 206-208° [$\lambda_{max.}$ 232; 239; 248 mμ (ε 14,700; 16,150; 10,600)], prepared by standard methods (see Experimental), with the appropriate reference compounds in the euphane and lanostane series.

Thus the change from the $\Delta^{7(8)}$- to the $\Delta^{8(9)}$-isomer was characterised by a change in the sign of the Cotton curve from -ve to +ve. However, this did not distinguish between the euphane/tirucallane and lanostane series, since the same change of sign was observed in both.[22] (See Figs. 1 and 2).

In contrast, the 7,8(11)-diene did provide a distinction. The O.R.D. curves of lanosta-7,9(11)-dien-3-one (C20), eupha-7,9(11)-dien-3-one (C21) (see Experimental) and the diene (C19) from odoratol are shown in Fig. 3. Interestingly, the Cotton effect of the carbonyl group was negligible in all three cases. The diene Cotton effect, however, was negative for the euphadiene (C21) and the diene from odoratol (C19) but strongly positive for lanosta-7,9(11)-dien-3-one (20).

The comparison thus supported location of the odoratol double bond at C-7(8) in a euphane skeleton and suggested that the hitherto unplaced secondary hydroxyl in odoratol was situated at the biogenetically probably C-3 position. Its α (axial)-configuration could be inferred from the multiplicity of the C-3 hydrogen signal in the triacetates of odoratol and 3-epi-odoratol, the major product from borohydride reduction of odoratone. (See Experimental). Thus in odoratol triacetate (C22) H-3 at τ 5·39 was a broad singlet ($W^{\frac{1}{2}}$ 6 c/sec.) while in the 3-epimer H-3 at τ 5·49 was a triplet ($W^{\frac{1}{2}}$ 18 c/sec.).

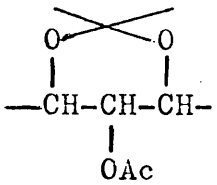
The structure of the naturally occurring iso-odoratol (C30), m.p. 251-253°, $[\alpha]_D$ -54° was defined as follows. It gave a triacetate whose n.m.r. spectrum was similar to that of odoratol triacetate in the chemical shift of the lowfield protons though $J_{H-23,H-24}$ had altered from 7 to 3 c/sec. It did not form an acetonide but on oxidation with the Jones reagent afforded the diosphenol (C13), [$\nu_{max.}^{CCl_4}$ 3550, 1715, 1635 cm^{-1}; $\lambda_{max.}$ (EtOH) 292 mμ (ε 8,680); $\lambda_{max.}$ (0·1N NaOH/EtOH) 342 mμ (ε 8,830). The diosphenol acetate, $C_{32}H_{46}O_5$ had $\nu_{max.}^{CCl_4}$ 1782, 1712, 1635 $cm.^{-1}$; $\lambda_{max.}$ 270 mμ (ε 11,630)], also obtained as one of the oxidation products of odoratol. This clearly limited the possible configurational differences between odoratol and iso-odoratol to positions 22, 23 and 24. Sodium borohydride reduction of 24-dehydro-odoratol (C10) yielded a mixture of odoratol and iso-odoratol, which is therefore 24-epi-odoratol.

In spite of the available coupling constants relating to the system H-20, H-22, H-23 and H-24 (see Table I), we feel unable to assign with any confidence the relative configurations at C-22, C-23 and C-24 in

odoratol or to distinguish between the euphol and tirucallol configurations at C-20, because of the conformational uncertainties inherent in the system under discussion.

The two tetracyclic triterpenoid pentaols present in the polar fraction of the extract could only be isolated as acetonides. Pentaol I acetonide (C35) m.p. 215-218°, analysed for $C_{33}H_{56}O_5$ and gave a diacetate (C36), m.p. 125-128°, which still retained a hydroxyl group ($\nu_{max.}^{CCl_4}$ 3580 cm.$^{-1}$) probably tertiary. The n.m.r. spectrum of the acetate lacked any signal for >C<u>H</u>·OH confirming the tertiary nature of the hydroxyl group. In addition it showed nine tertiary methyl groups (τ 8·61, 8·69, 8·90, 8·92, 9·08, 9·11, 9·24, 9·29, 9·31), one secondary methyl group (τ 9·18, J = 7 c/sec.), a vinyl proton (τ 4·81 (bs)), two secondary acetates (τ 8·02, 8·06), one isolated (τ 5·38 (bs), $W^{\frac{1}{2}}$ 7 c/sec.) and the other (τ 5·19 (t), J = 10 c/sec.) involved in a spin coupled system with two secondary acetonide protons (τ 6·36 (d), J = 10 c/sec. and 6·46 (d), J = 10 c/sec.). Irradiation at τ 5·19 caused the two overlapping doublets arising from the acetonide protons to collapse to two

singlets. In turn irradiation in the centre of the acetonide protons resulted in the secondary acetate appearing as a singlet. These results led to the 1,3-acetonide part structure



When the n.m.r. spectrum of (C36) was run in 50% benzene/$CDCl_3$ solution the two acetonide proton doublets moved apart and it was observed that one of them had a further coupling (2 c/sec.), suggesting the presence of a proton on the neighbouring carbon atom. These facts were satisfactorily accommodated in the structure (C35) which indicated a close relationship with odoratol (C7). Assignment of the stereochemistry in the side chain was not possible for the reasons which applied in the case of odoratol. In view of the small coupling of the proton at C-3 ($W^{\frac{1}{2}}$ 7 c/sec.), the hydroxyl at C-3 must be attached α.

The pentaol II acetonide (C37), m.p. 236-238°, also analysed for $C_{30}H_{56}O_5$ and gave a mono-acetate, m.p. 222-224°, $\nu_{max.}^{CCl_4}$ 3580, 3490, 1730 $cm.^{-1}$. In the n.m.r.

spectrum nine tertiary methyl groups (τ 8·61, 8·64 (2), 8·69, 8·8, 9·02, 9·06, 9·16, and 9·25), one secondary methyl group (τ 9·19 J = 8 c/sec.) and a secondary acetate (τ 7·98, 5·3 (bs), $W^{\frac{1}{2}}$ 7 c/sec.) were present. It seemed likely that pentaol II acetonide was closely related to pentaol I acetonide (C35). The data mentioned above indicated the same skeleton with a 3α-OAc and a double bond at C-7(8). Unfortunately the three protons relating to the acetonide and the hindered secondary alcohol (resistant to acetylation under forcing conditions) formed a complex multiplet centred at τ 6·0 which did not yield to first order analysis. Due to paucity of material the final solution of the problem was not found.

6-Hydroxycarapin (C33), $C_{27}H_{32}O_8$ $[\alpha]_D$ +35° $\nu_{max.}^{CCl_4}$ 3605, 3530 (hydroxyl), 1740 (methyl ester and δ-lactone) and 1712 $cm.^{-1}$ (ketone), was obtained as a gum. The corresponding acetate (C34) showed $\nu_{max.}^{CCl_4}$ 1742 (methyl ester, δ-lactone and acetate), 1712 $cm.^{-1}$ (ketone) and complete absence of hydroxyl absorption. Both 6-hydroxycarapin and its acetate showed characteristic absorption in the u.v. ($\lambda_{max.}$ 287 mμ (ε 30,900) (0·1N

NaOH-EtOH)) for β-diketones of the mexicanolide[7] (C2) type. The n.m.r. spectra of 6-hydroxycarapin and its acetate were very similar to that of carapin (C32) (cf. methyl angolensate and 6-hydroxy methyl angolensate[24] and see Table II) with the additional feature of H-6 (bs) at τ 5·4 and τ 4·35 respectively.

It was reported that carapin (C32) isomerised[15] readily to mexicanolide (C2) over alumina (unspecified). This suggested the possibility of relating 6-hydroxy-carapin to swietenolide (C38) by isomerisation of the double bond followed by reduction of the 3-ketone. However all efforts to effect the isomerisation failed (even with carapin itself) and eventually lack of material prevented any further work.

The polar fraction of the extract also yielded two furanoid triterpenoids which although purified by preparative t.l.c., failed to crystallise. The two had virtually identical spectroscopic properties. Thus in the i.r. they showed bands at 3610, 1740, 1715 $cm.^{-1}$. Their n.m.r. spectra (see Table III) indicated the presence of four tertiary methyl groups and a methyl-ester. This in conjunction with lack of an exomethylene

group suggested a bicyclononanolide skeleton. The sharp singlets at τ 3·45 (vinyl proton) and τ 4·82 (H-17) were consistant with the presence of an $\alpha\beta$-unsaturated δ-lactone system in ring D. (cf. 14,15-deoxy-andirobin[25] (C39) in which H-15 resonates at τ 3·58). Both compounds appeared to contain two tertiary hydroxyl groups since two one-proton singlets disappeared on D_2O exchange. In agreement with the tertiary nature of the hydroxyls the compounds resisted oxidation with Jones reagent and acetylation under forcing conditions. As a result of the small amount of material available no further progress was made.

EXPERIMENTAL

Extraction

Powdered heartwood (4 kg.) of Cedrela glaziovii obtained from the Forest Research Institute, Kepong, Selangor, was extracted with hot ethyl acetate (15 l.). The ethyl acetate was removed in vacuo and chloroform (3 l.) added. The chloroform-insoluble material was filtered off and the soluble material defatted with light petroleum. The residue was dissolved in methanol and allowed to stand overnight when a crystalline mixture (40 gm.) of mexicanolide and cedrolide (7-deacetoxy-7-oxogedunin) separated. The mother liquors were chromatographed on Grade V neutral alumina. Elution with benzene-light petroleum (1:2) yielded β-sitosterol (0·05%), benzene-light petroleum (1:1), gedunin (0·2%), methyl angolensate (0·08%), and carapin (0·012%). Benzene-eluted fractions were complex mixtures. Chloroform-benzene (1:3) gave odoratone (0·025%), 6-hydroxycarapin (0·001%) and two tetranor-triterpenoids (0·005 and 0·0075% respectively), chloroform gave odoratol (0·15%) and methanol-chloroform (1:20) a mixture of more polar compounds from which iso-

odoratol (0·0075%) was isolated by preparative t.l.c. From the residue two tetracyclictriterpene pentaols were isolated (as acetonides - see later)

Odoratol (0·005%) was also obtained by chromatography of the mother liquors from the extraction of Cedrela mexicana, after removal of mexicanolide.[7]

Odoratone (C8) was crystallised from ether-light petroleum and had m.p. 230-232°, $[\alpha]_D$ -80°, $\nu_{max.}^{CCl_4}$ 3635, 3563, 1720 cm.$^{-1}$. (Found: C, 76·25; H, 10·05. $C_{30}H_{48}O_4$ requires C, 76·2; H, 10·25%).

Iso-odoratol (24-epi-odoratol) (C30) was purified by repeated crystallisation from ether-light petroleum. It had m.p. 251-253°, $[\alpha]_D$ -53°. (Found: C, 75·60; H, 10·45. $C_{30}H_{50}O_4$ requires C, 75·90; H, 10·60%). The derived acetate (C31) was non-crystalline, $\nu_{max.}^{CCl_4}$ 1748, 1733 cm.$^{-1}$. (Found: C, 71·9; H, 9·65. $C_{36}H_{56}O_7$ requires C, 71·95; H, 9·4%). Iso-odoratol was recovered unchanged from treatment with acetone-anhydrous copper sulphate under the conditions which converted odoratol into the acetonide.

Odoratol (C7), crystallised several times from methanol, afforded rods m.p. 235-237°, $[\alpha]_D$ -50°, $\nu_{max.}^{nujol}$ 3372 cm.$^{-1}$. (Found: C, 75·5; H, 10·5. $C_{30}H_{50}O_4$ requires C, 75·9; H, 10·6%).

Triacetate (C22)

Odoratol (30 mg.) in pyridine (5 ml.) and acetic anhydride (2 ml.) was left overnight at 20°. The acetate (C22) obtained after the usual work-up was purified by preparative t.l.c. but did not crystallise, $\nu_{max.}^{CCl_4}$ 1750 and 1735 cm.$^{-1}$. (Found: C, 72·10; H, 9·6. $C_{36}H_{56}O_7$ requires C, 71·95; H, 9·4%).

Acetonide (C23)

Odoratol (30 mg.) was dissolved in AnalaR acetone (4 ml.) and anhydrous copper sulphate (60 mg.) added. The reaction was stirred at 20° for 24 hr. The acetonide (C23) (25 mg.), obtained after removal of the copper sulphate and acetone, crystallised from aqueous acetone in needles, m.p. 210-212°. (Found: C, 76·8; H, 10·35. $C_{33}H_{54}O_4$ requires C, 77·0; H, 10·6%).

$\Delta^{8(9)}$ Isomer (C15) of Odoratol

A solution of odoratol (50 mg.) in glacial acetic acid (6 ml.) was shaken in an atmosphere of hydrogen in presence of Adams' catalyst for 2 hr. The residue after removal of catalyst and solvent afforded the olefin (C15) (45 mg.). Crystallised from methanol this had m.p. 211-214°, $[\alpha]_D$ -31°. (Found: C, 75·6; H, 10·5. $C_{30}H_{50}O_4$ requires C, 75·90; H, 10·60%). The derived acetate (C25) did not crystallise, $\nu_{max.}^{CCl_4}$ 1748, 1732 cm.$^{-1}$. (Found: C, 72·1; H, 9·65. $C_{36}H_{56}O_7$ requires C, 71·95; H, 9·4%).

Diene (C16)

Odoratol (50 mg.) in glacial acetic acid (6 ml.) was kept with a solution of mercuric acetate (70 mg.) in acetic acid (3 ml.) for 24 hr. at 20°. Mercurous acetate was filtered off and the solvent removed in vacuo affording diene (C16) (40 mg.) which, crystallised from methanol, had m.p. 255-260°, $[\alpha]_D$ -174°, $\lambda_{max.}$ 232 mμ (ε 14,700), 239 mμ (ε 16,1500), 248 mμ (ε 10,600). (Found: C, 73·45; H, 10·15. $C_{30}H_{48}O_4.H_2O$ requires

C, 73·45; H, 10·25%). The derived acetate (C26) did not crystallise, $\nu_{max.}^{CCl_4}$ 1750, 1732 cm.$^{-1}$. (Found: C, 70·65; H, 9·05. $C_{36}H_{54}O_7.H_2O$ requires C, 70·1; H, 9·15%).

3-Dehydro-odoratol Acetonide (C17)

Odoratol acetonide (C23) (50 mg.) in AnalaR acetone (5 ml.) at 0° was treated with Jones reagent (4 drops) for 1 min. The product, 3-dehydro-odoratol acetonide (C17), crystallised from ether-light petroleum in plates (40 mg.) m.p. 208-210°, $\nu_{max.}^{CCl_4}$ 1720 cm.$^{-1}$. (Found: C, 77·35; H, 10·5. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%).

3-Dehydro-diene Acetonide (C19)

3-Dehydro-acetonide (C17) (50 mg.) was oxidised with mercuric acetate as above. The product was purified by preparative t.l.c. to yield the diene (C19) (35 mg.) m.p. 206-208° (ex ether-light petroleum), $\nu_{max.}^{CCl_4}$ 1712 cm.$^{-1}$, $\lambda_{max.}$ 232 (ε 9,050), 239 (ε 10,450), 247 (ε 7,250) mμ. (Found: C, 77·45; H, 9·8. $C_{33}H_{50}O_4$ requires C, 77·6; H, 9·85%).

Acetonide (C24) of the $\Delta^{8(9)}$ Isomer

The $\Delta^{8(9)}$ isomer (C15) of odoratol was treated with acetone and anhydrous copper sulphate as described above for odoratol acetonide. The acetonide (C24) crystallised from ether-light petroleum, had m.p. 214-216°, $[\alpha]_D$ -20°, $\nu_{max.}^{CCl_4}$ 3625 cm.$^{-1}$. (Found: C, 76·85; H, 10·4. $C_{33}H_{54}O_4$ requires C, 77·0; H, 10·5%).

Oxidation of Acetonide (C24)

The acetonide (50 mg.) was oxidised as above with Jones reagent. The ketone (C18) (35 mg.), when purified by preparative t.l.c. and crystallised from ether-light petroleum, had m.p. 169-170°, $[\alpha]_D$ +7°, $\nu_{max.}^{CCl_4}$ 1720 cm.$^{-1}$. (Found: C, 77·1; H, 10·2. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%).

Oxidation of Odoratol

To odoratol (500 mg.) in AnalaR acetone (20 ml.) at 0° was added Jones reagent (20 drops) diluted with acetone (20 drops). The mixture was left for 30 sec. at 0° and then worked up as usual. The crude product was separated on preparative t.l.c. to give:

(I) 3-Dehydro-odoratol (C8) (100 mg.) identical in all respects (m.p., m.m.p., n.m.r., t.l.c.) with natural odoratone. The derived acetate (C14), crystallised from ether-light petroleum, had m.p. 219-221°, $\nu_{max.}^{CCl4}$ 1755, 1715 cm.$^{-1}$. (Found: C, 73·45; H, 9·4. $C_{34}H_{52}O_6$ requires C, 73·4; H, 9·4%).

(II) 23-Dehydro-odoratol (C9) (5 mg.) crystallised from ether-light petroleum in needles m.p. 233-234°, $\nu_{max.}^{CCl_4}$ 3615, 3540, 1768 cm.$^{-1}$. (Found: C, 75·95; H, 10·25. $C_{30}H_{48}O_4$ requires C, 76·2; H, 10·25%).

(III) 24-Dehydro-odoratol (C10) (5 mg.) crystallised from ether-light petroleum in needles m.p. 220-221°, $\nu_{max.}^{CCl_4}$ 3615, 3540, 1766 cm.$^{-1}$. Mass spectrometric m.wt. 472·35337. $C_{30}H_{48}O_4$ requires 472·35240.

(IV) 3,23-Bisdehydro-odoratol (C11) (60 mg.) crystallised from carbon tetrachloride in needles m.p. 194-196°, $\nu_{max.}^{CCl_4}$ 3562, 1768, 1714 cm.$^{-1}$. (Found: C, 76·5; H, 9·75. $C_{30}H_{46}O_4$ requires C, 76·5; H, 9·85%).

(V) 3,24-Bisdehydro-odoratol (C12) (70 mg.) crystallised from carbon tetrachloride in needles m.p. 200-202°, $\nu_{max.}^{CCl_4}$ 3540, 1766, 1713 cm.$^{-1}$. (Found: C, 76·2; H, 9·75. $C_{30}H_{46}O_4$ requires C, 76·5; H, 9·85%).

(VI) Diosphenol (C13). In a second experiment odoratol (200 mg.) was treated with Jones reagent as above but at 20°. The reaction was left for 5 min. and the product purified by preparative t.l.c. The diosphenol (C13) (45 mg.) crystallised from ether-light petroleum in plates m.p. 232-234°, $\nu_{max.}^{CCl_4}$ 3550, 1715, 1635 cm.$^{-1}$, $\lambda_{max.}$ 292 mμ (ε 8,680) (EtOH), 342 mμ (ε 8,830) (0·1N NaOH/EtOH). (Found: 76·5; H, 9·65. $C_{30}H_{44}O_4$ requires C, 76·9; H, 9·45%). The derived acetate failed to crystallise, $\nu_{max.}^{CCl_4}$ 1782, 1712, 1635 cm.$^{-1}$, $\lambda_{max.}$ 270 mμ (ε 11,630). (Found: C, 75·0; H, 9·35. $C_{32}H_{46}O_5$ requires C, 75·25; H, 9·1%).

Oxidation of Iso-odoratol

Jones oxidation of iso-odoratol as above yielded inter alia the diosphenol (C13) m.p. 232-234° identical in all respects (m.p., m.m.p., u.v., n.m.r., t.l.c.) with the diosphenol (C13) obtained from odoratol.

Sodium Borohydride Reductions

(a) 3-Dehydro-odoratol (30 mg.) was dissolved in methanol and sodium borohydride (10 mg.) added. The reaction was left at 20° for 1 hr., acidified and worked up as usual. The residue was crystallised from methanol to give 3-epi-odoratol, m.p. 244-246°, $[\alpha]_D$ -46°. (Found: C, 75·65; H, 10·65. $C_{30}H_{50}O_4$ requires C, 75·9; H, 10·6%). The corresponding acetate (C27) was obtained as a gum $\nu_{max.}^{CCl_4}$ 1748, 1732 cm.$^{-1}$. (Found: C, 72·1; H, 9·35. $C_{36}H_{56}O_7$ requires C, 71·95; H, 9·4%).

(b) The 3,23-dione (C11) (20 mg.) was treated with sodium borohydride as above. The product was separated into its two components by preparative t.l.c. The less polar compound (6 mg.) had m.p. 244-246° (ex methanol) and was

identical with 3-epi-odoratol. The more polar 3,23-epi-odoratol (12 mg.) was crystallised from methanol and had m.p. 248-249°, $[\alpha]_D$ -48°. (Found: C, 76·0; H, 10·4. $C_{30}H_{50}O_4$ requires C, 75·9; H, 10·6%). The derived acetate (C29) was obtained as a gum, $\nu_{max.}^{CCl_4}$ 1748, 1732 cm.$^{-1}$. (Found: C, 72·0; H, 9·6. $C_{36}H_{56}O_7$ requires C, 71·95; H, 9·4%).

(c) The 3,24-dione (C12) (40 mg.) was reduced with sodium borohydride as above. The product was separated into its two components by preparative t.l.c. The less polar (12 mg.) was identical with 3-epi-odoratol, m.p. 244-246°. The more polar compound, 3,24-epi-odoratol (24 mg.), crystallised from methanol, had m.p. 260-262°, $[\alpha]_D$ -52°. (Found: C, 73·2; H, 10·45. $C_{30}H_{50}O_4H_2O$ requires C, 73·1; H, 10·65%). The corresponding acetate (C28) did not crystallise, $\nu_{max.}^{CCl_4}$ 1748, 1732 cm.$^{-1}$. (Found: C, 71·9; H, 9·6. $C_{36}H_{56}O_7$ requires C, 71·95; H, 9·4%).

(d) 24-Dehydro-odoratol (C10) (4 mg.) was treated with sodium borohydride as above. The product was separated by preparative t.l.c. into odoratol (1 mg.) m.p. 235-237° (ex methanol) and 24-epi-odoratol (3 mg.) m.p. 250-252° (ex ether-light petroleum) identical in all respects (t.l.c., n.m.r.,* mass spectrum, m.p., m.m.p.) with natural iso-odoratol (C30).

Pentaol Acetonides

The polar fractions were treated with anhydrous copper sulphate in acetone. The mixture of acetonides obtained in this way yielded two crystalline acetonides by preparative t.l.c.

(a) Pentaol I acetonide (C35), crystallised from ether-light petroleum, had m.p. 215-218°, $[\alpha]_D$ -37° (ethanol), (yield 0·0035%). (Found: C, 74·15; H, 10·95. $C_{33}H_{56}O_5$ requires C, 74·4; H, 10·6%).

* Obtained with the Varian CAT 1024 spectrum accumulator.

The acetate (C36), prepared in the usual way, crystallised from light petroleum, m.p. 125-128°, $\nu_{max.}^{CCl_4}$ 3580, 1745, 1730 cm.$^{-1}$. (Found: C, 72·3; H, 9·95. $C_{37}H_{60}O_7$ requires C, 72·05; H, 9·8%).

(b) Pentaol II acetonide (C37), crystallised from ether-light petroleum, had m.p. 236-238°, $[\alpha]_D$ -34° (ethanol), (yield 0·0025%). (Found: C, 74·5; H, 10·74. $C_{33}H_{56}O_5$ requires C, 74·4; H, 10·6%). The derived acetate crystallised from ether m.p. 222-224°, $\nu_{max.}^{CCl_4}$ 3580, 3490, 1730 cm.$^{-1}$. (Found: C, 73·1; H, 10·4. $C_{35}H_{58}O_6$ requires C, 73·15; H, 10·18%). The mother liquors of iso-odoratol (C30) on treatment with acetone and anhydrous copper sulphate also yielded inter alia the two crystalline pentaol acetonides (C35) and (C37).

6-Hydroxycarapin (C33)

6-Hydroxycarapin (C33) was repeatedly purified by preparative t.l.c. but failed to crystallise, $[\alpha]_D$ +35°, (yield 0·001%), $\nu_{max.}^{CCl_4}$ 3605, 3530, 1740, 1712 cm.$^{-1}$. The end absorption in the u.v. shown by an ethanolic

solution was immediately supplanted by an intense new maximum at 287 mμ (ε 30,900), when two drops of 4N NaOH were added to the u.v. cell and this, upon acidification, yielded in turn to a less intense maximum at 264 mμ (ε 20,300). (Found: C, 66·8; H, 6·85. $C_{27}H_{32}O_8$ requires C, 66·90; H, 6·65%). The corresponding acetate (C34) was purified by preparative t.l.c. but did not crystallise $\nu_{max.}^{CCl_4}$ 1742, 1715 $cm.^{-1}$, $\lambda_{max.}$ 287 mμ (ε 30,700) in alkaline ethanolic solution changing upon acidification to $\lambda_{max.}$ 264 mμ (ε 20,000). (Found: C, 65·85; H, 6·65. $C_{29}H_{34}O_9$ requires C, 66·14; H, 6·5%).

Eupha-7,9(11)-diene-3-one (C21)

Eupha-7,9(11)-diene-3-ol, obtained from euphol by standard reactions,[23] was oxidised with Jones reagent to eupha-7,9(11)-diene-3-one m.p. 114-115° (from MeOH). (Found: C, 82·8; H, 11·3. $C_{30}H_{48}O.\frac{1}{2}H_2O$ requires C, 83·1; H, 11·3%).

Characterisation of the Known Compounds

β-Sitosterol

The solid obtained with benzene-petroleum ether (1:3), crystallised from methanol, m.p. 136-137°, m.m.p. with authentic β-sitosterol undepressed.

Mexicanolide (C2) and Cedrolide (C5)

The solid mixture (40 gm.) on crystallisation from methanol gave a solid m.p. 265-268°, m.m.p. and n.m.r. identical with cedrolide.

The later crops after repeated fractional crystallisation gave crystals m.p. 227-230°, m.m.p. and n.m.r. identical with mexicanolide.

Methyl Angolensate (C6)

The fraction eluted with benzene-light petroleum (1:1) gave by preparative t.l.c. and crystallisation from methanol, a crystalline solid m.p. 197-199°, m.m.p. and n.m.r. identical with methyl angolensate.

Gedunin (C4)

The crystalline material eluted with benzene-light petroleum (1:1) gave on recrystallisation from ether-light petroleum, crystals m.p. 208-210°, $[\alpha]_D$ +45°, n.m.r. and physical constants identical with gedunin.

Carapin (C32)

The fraction eluted with benzene-light petroleum (1:1) gave , by preparative t.l.c. and crystallisation from methanol, a crystalline solid m.p. 123-126°, $[\alpha]_D$ +63°. In the u.v. the neutral solution in ethanol showed end absorption which was supplanted by an intense new maximum at 288 mμ when two drops of 4N NaOH were added to the u.v. cell and this, on acidification, changed to a less intense maximum at 265 mμ. The n.m.r. showed the characteristic peak for the vinyl proton (H-15) at τ 4·22 (d), J = 1·5 c/sec.

Carapin, dissolved in chloroform was kept in contact with acid, basic, neutral alumina for 24 hr. The n.m.r. was recorded before and after treatment with alumina but no isomerisation of carapin to mexicanolide was observed.

Table I

Chemical Shifts (τ) and Coupling Constants (c/sec. in parentheses) of protons in odoratol and its derivatives

Compound	Position of Protons					
	3	7	11	22	23	24
C9	6·57(bs)[+]	4·75(bs)	–	6·10(s)	–	6·17(s)
C10	6·56(bs)	4·75(bs)	–	6·19(d) (11)	5·84(d) (11)	–
C11	–	4·68(bs)	–	6·11(s)	–	6·18(s)
C12	–	4·68(bs)	–	6·16(d)	5·81(d)	–
C14	–	4·76(bs)	–	6·06(bd)[*]	4·96(t)	5·21(d)
C15	6·57(bs)	–	–	6·37(bd)	6·04(t)	6·19(d)
C17	–	4·69(bs)	–	6·11(bd) (6)	5·52(t) (6,7)	5·80(d) (7)
C18	–	–	–	6·11(bd)	5·53(t)	5·82(d)
C19	–	4·63(t), 4·78(t)		6·10(bd)	5·53(t)	5·80(d)
C22	5·39(bs)	4·82(bs)	–	6·06(bd) (6)	4·98(t) (6,7)	5·23(d) (7)
C23	6·58(bs)	4·78(bs)	–	6·12(bd) (6)	5·55(q) (6,7)	5·83(d) (7)
C24	6·75(m)	–	–	6·07(bd)	5·51(t)	5·77(d)
C25	5·35(bs)	–	–	6·00(bd) (6)	4·92(t) (6,7)	5·17(d) (7)
C26	5·33(bs)	4·69(t), 4·80(t)		5·99(bd)	4·92(t)	5·16(d)
C27	5·49(t)	4·75(bs)	–	6·01(bd)	4·91(t)	5·14(d)
C28	5·50(t)	4·77(bs)	–	6·13(bd)	4·98(q)	5·05(d)
C29	5·52(t)	4·74(bs)	–	5·95(bd) (6)	4·74(t) (6,5)	5·02(d) (5)
C30	6·52(bs)	4·70(bs)	–	6·06 – 6·36(m)		
C31	5·32(bs)	4·74(bs)	–	6·12(bd) (6)	4·96(q) (6,3)	5·03(d) (3)

[+] Broadened singlet. [*] Broadened doublet.

Table II

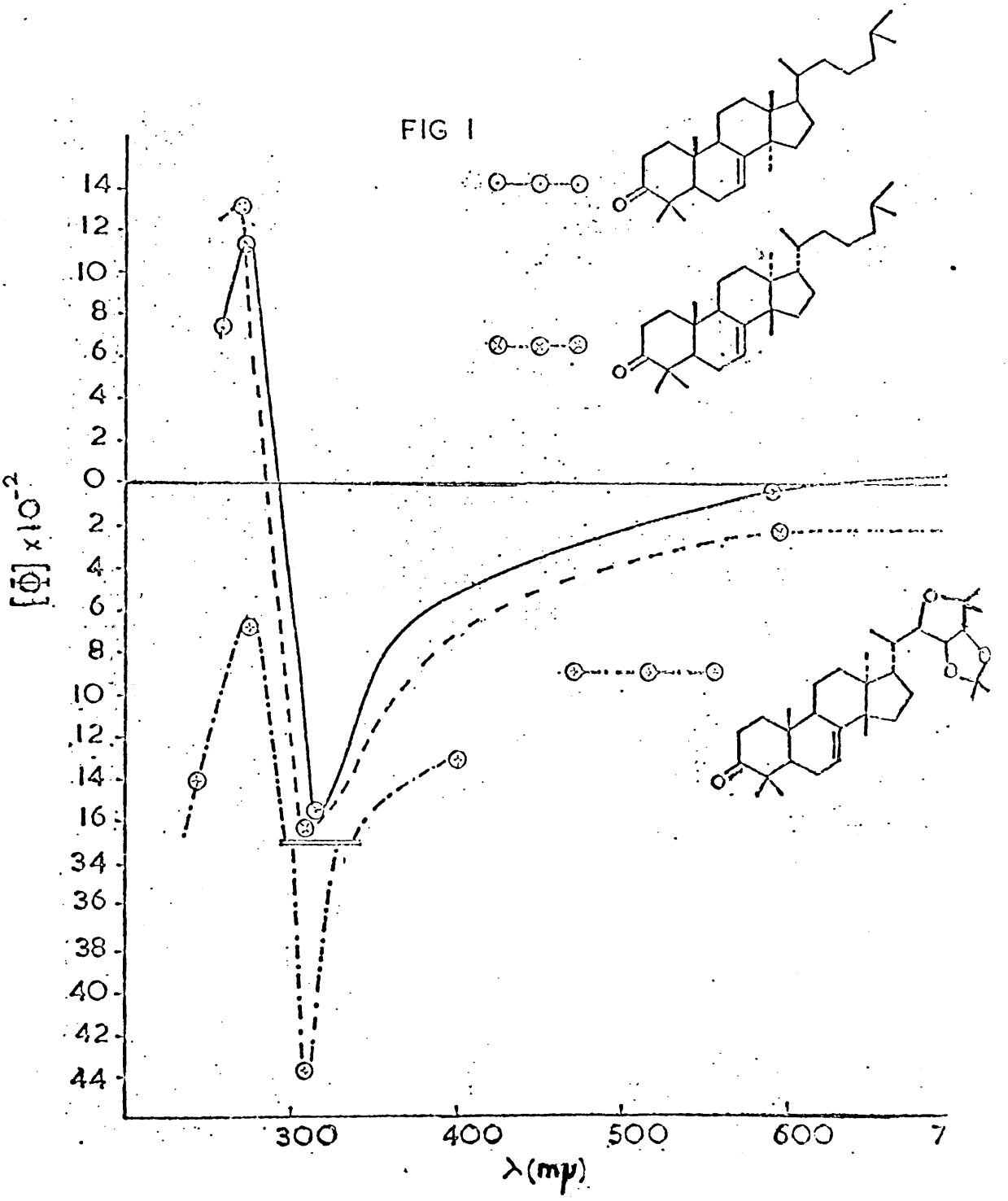
N.m.r. Spectra (τ values) of

	6-Hydroxy Carapin	6-Acetoxy Carapin	Carapin
Methyl groups	8·56, 8·84, 8·9, 8·98	8·76, 8·81 (2) 8·94	8·86 (2), 8·98, 9·12
Acetate	-	8·2	-
H-6	5·4 (bs)	4·35 (bs)	-
Methyl ester	6·18	6·2	6·29
H-15	4·2 (d) J = 1·5 c/sec.	4·15 (d) J = 1·5 c/sec.	4·2 (d) J = 1·5 c/sec.
H-17	4·96	4·92	4·93
Furans	2·56, 3·6	2·55, 3·6	2·58, 3·61

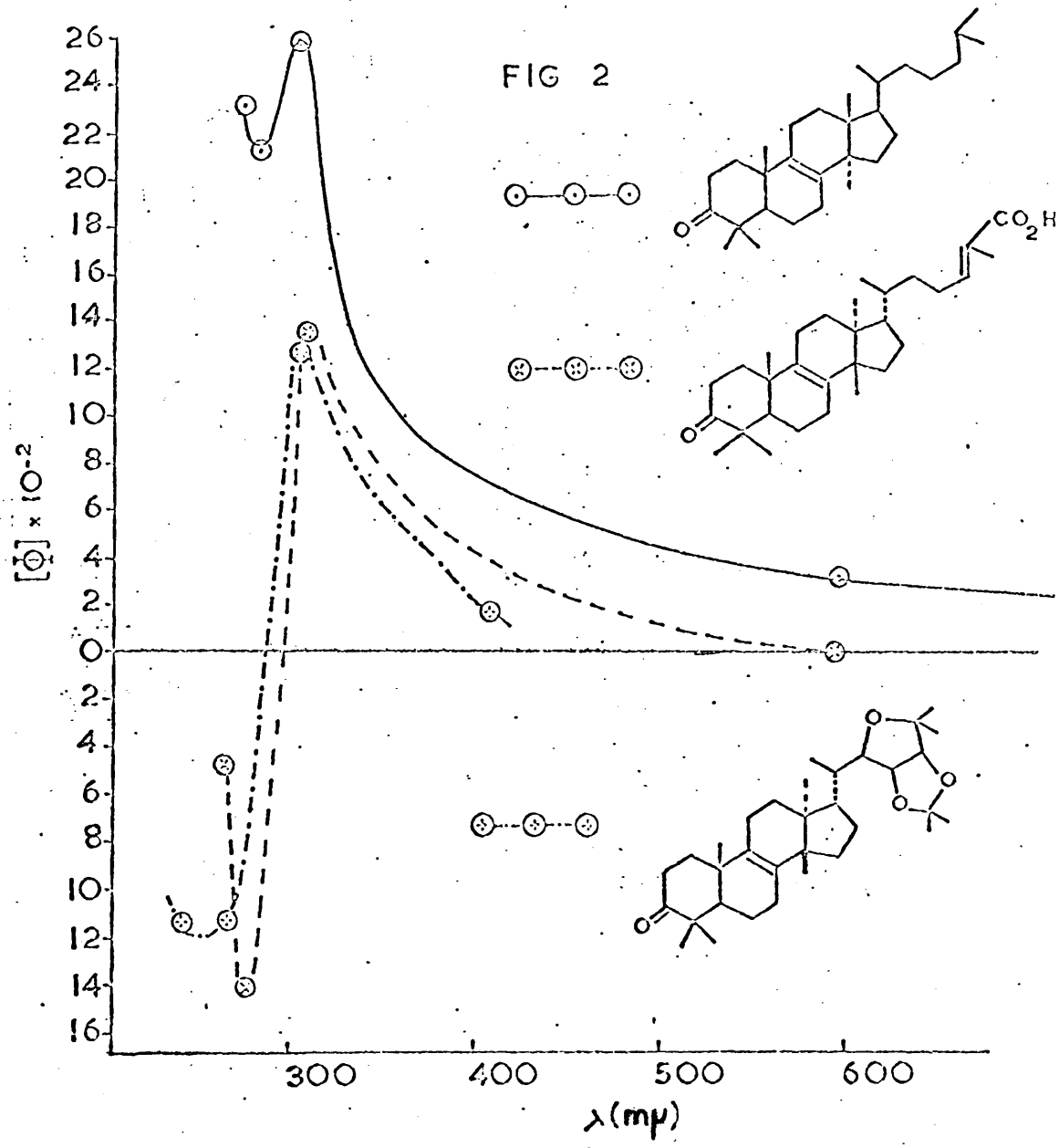
Table III

N.m.r. Spectra (τ values) of

	Tetranor-triterpenoid I	Tetranor-triterpenoid II
Methyl groups	8·89, 8·84, 9·05 9·21	8·86, 8·92, 9·04, 9·21
Methyl ester	6·34	6·38
H-17	4·82	4·82
H-15	3·45 (s)	3·45 (s)
Furans H-21, H-22	2·5, 3·5	2·5, 3·5

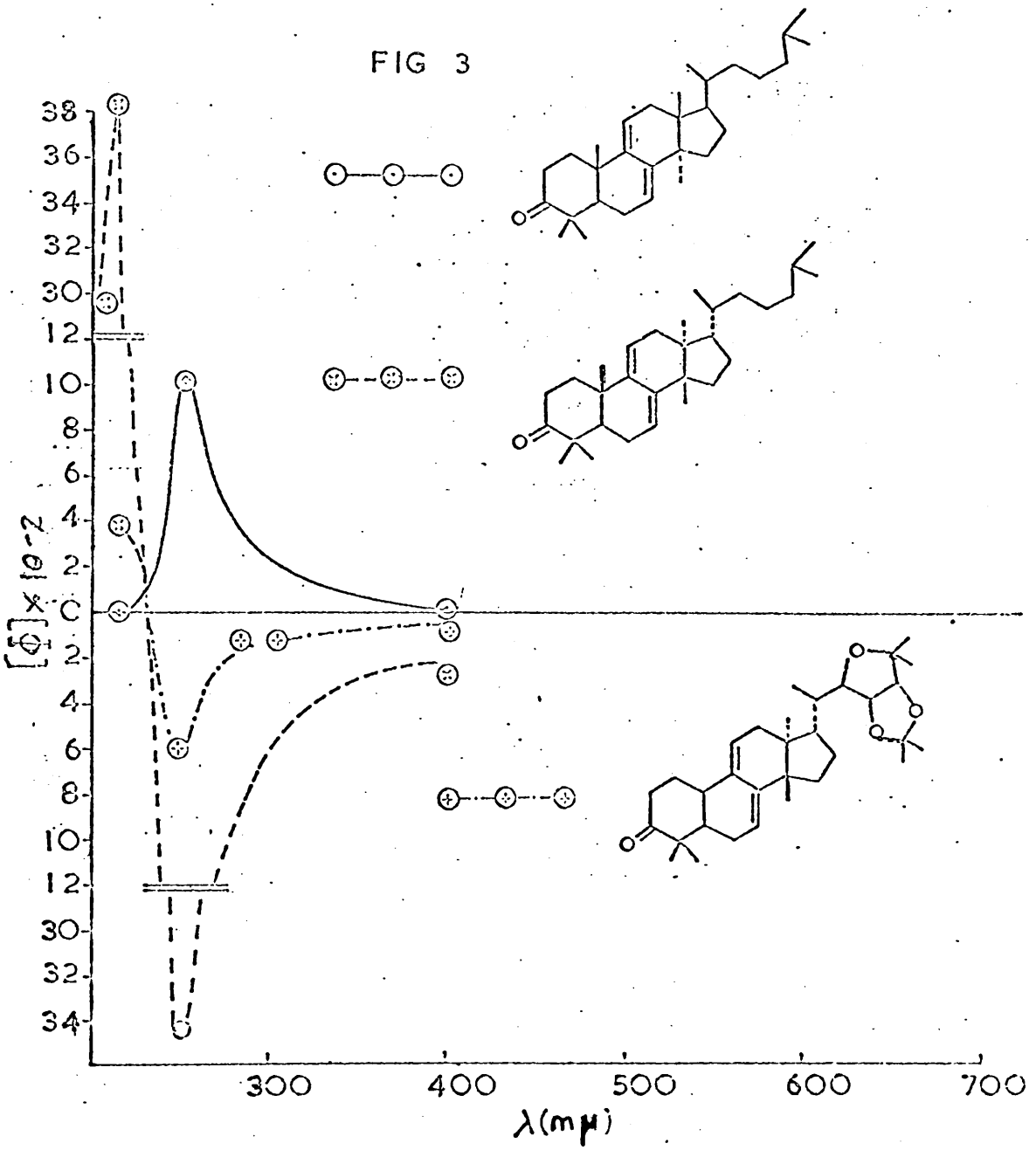


O.R.D. Curves of Lanost-7-en-3-one,
Euph-7-en-3-one and Odoratone Acetonide.

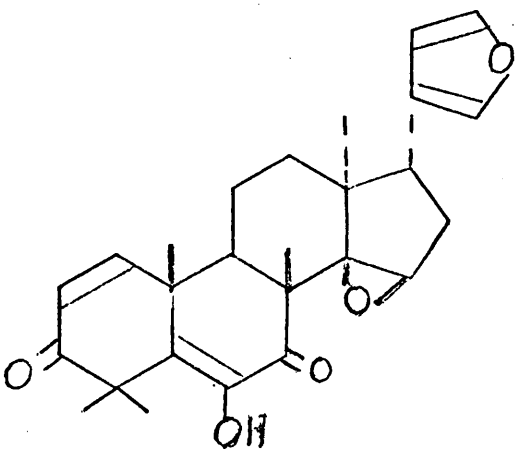


O.R.D. Curves of Lanost-8-en-3-one, Isomasticadienoic Acid and the $\Delta^{8(9)}$-Isomer of Odoratone Acetonide.

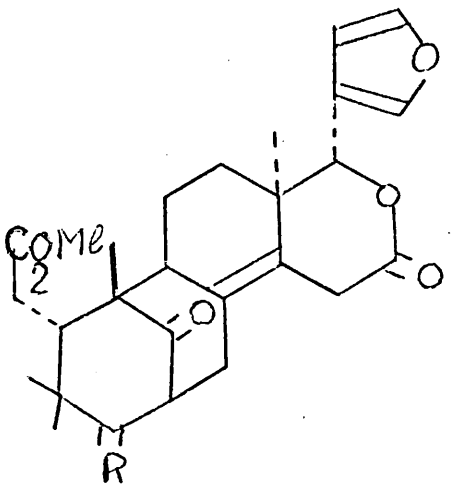
FIG 3



O.R.D. Curves of Lanosta-7,9(11)-diene-3-one, Eupha-7,9(11)-dien-3-one and 9(11)-Dehydro-odoratone Acetonide.

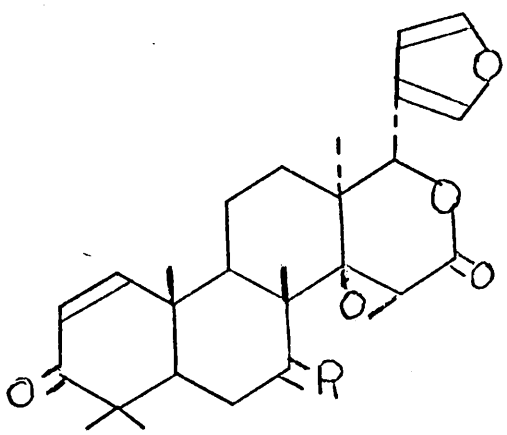


C1



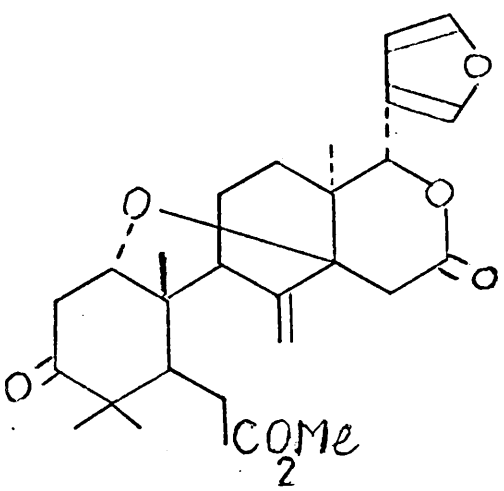
C2 R = O

C3 R = βOAc, H

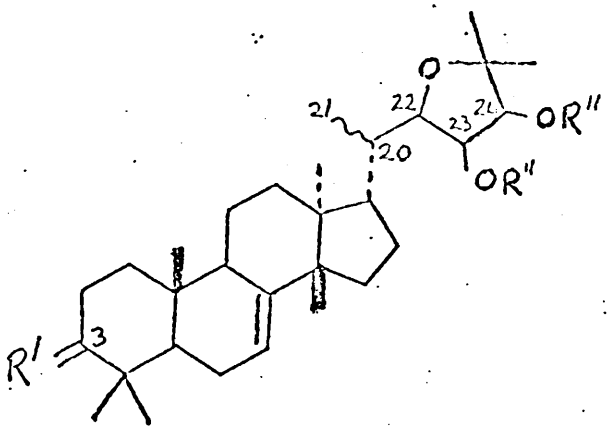


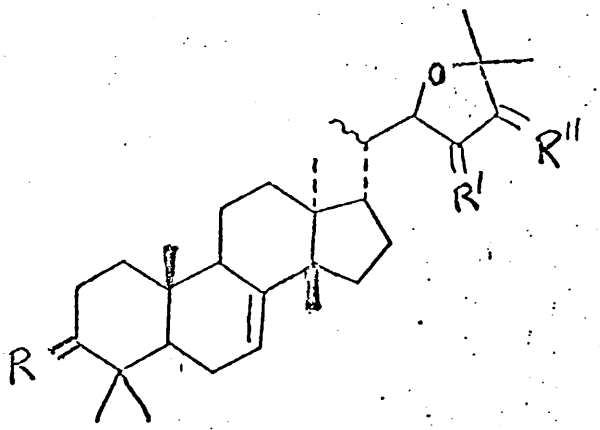
C4 R = αOAc, H

C5 R = O



C6





C7 R'=H,αOH; R''=H

C8 R'=O; R''=H

C14 R'=O; R''=Ac

C17 R'=O; R''+R''= $\rangle C(CH_3)_2$

C22 R'=H,αOAc; R''=Ac

C23 R'=H,αOH; R''+R''= $\rangle C(CH_3)_2$

C27 R'=H,βOAc; R''=Ac

C28 24 epi-(C27)

C29 23 epi-(C27)

C30 24 epi-(C7)

C31 24 epi-(C22)

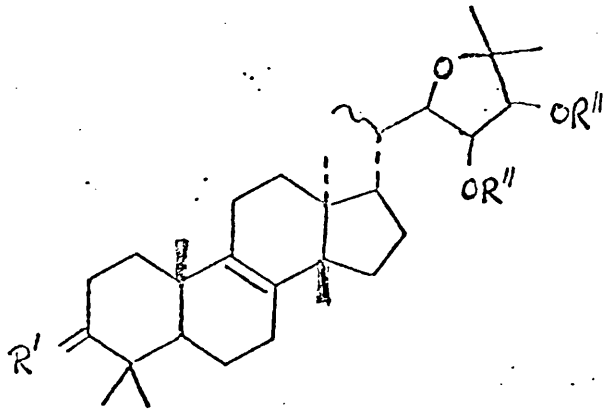
C9 R=H,αOH; R'=O; R''=H,OH

C10 R=H,αOH; R'=H,OH; R''=O

C11 R=R'=O; R''=H,OH

C12 R=R''=O; R'=H,OH

C13 Δ^{22}-(C12)

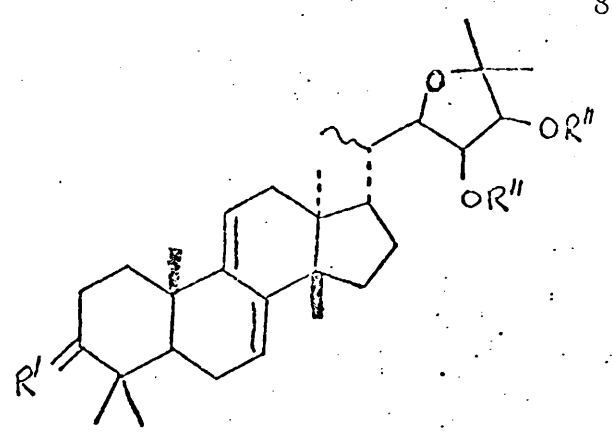


C15 R'=H, αOH; R''=H

C18 R'=O; R''+R''= $>C(CH_3)_2$

C24 R'=H, αOH; R''+R''= $>C(CH_3)_2$

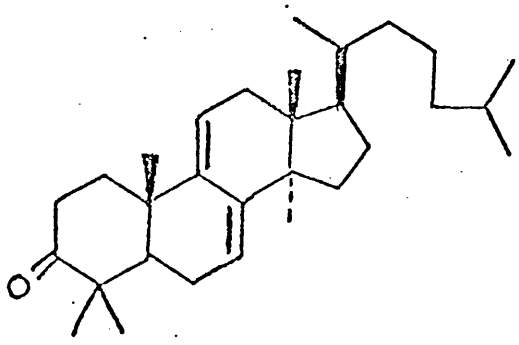
C25 R'=H, αOAc; R''=Ac



C16 R'=H, αOH; R''=H

C19 R'=O; R''+R''= $>C(CH_3)_2$

C26 R'=H, αOAc; R''=Ac



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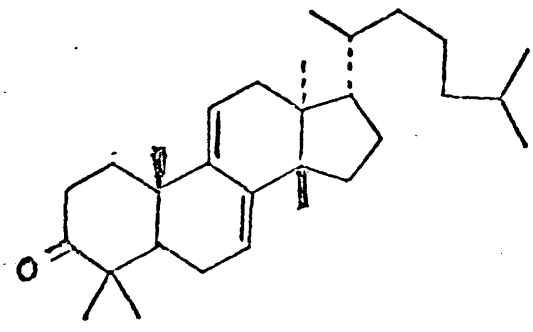
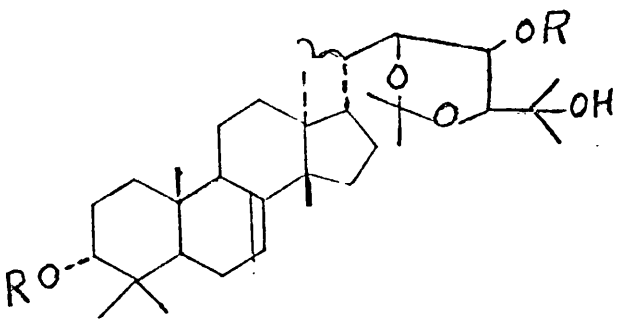
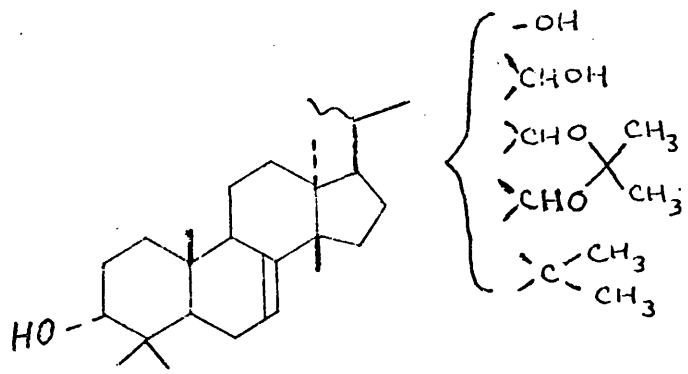


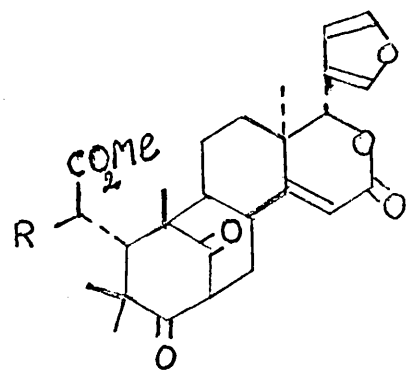
C21



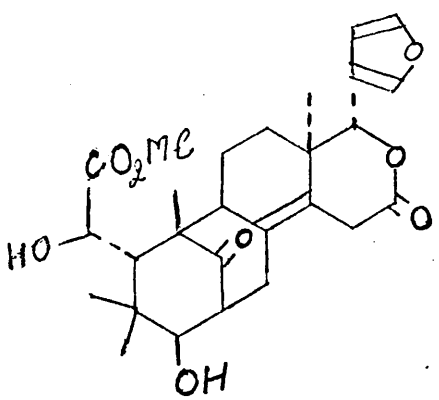
C35 R = H
C36 R = Ac



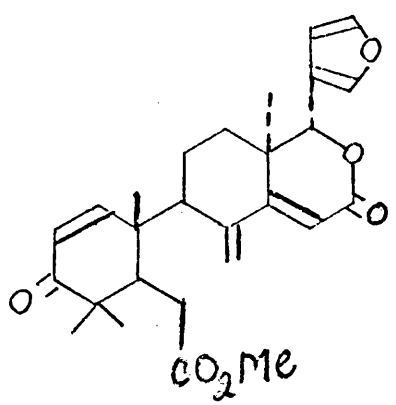
C37



C32 R = H
C33 R = OH
C34 R = OAc



C38



C39

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CHAPTER IV

FURTHER CONSTITUENTS OF

NIM OIL (MELIA AZADIRACHTA)

INTRODUCTION

The constituents of Melia azadirachta (neem) have interested many workers because of its reputation in the Ayurvedic system of medicine. Earlier investigations resulted in the isolation from bud oil (Nim oil),[1] root bark[2] and trunk bark[3] of nimbin (D1), nimbinin (D2) and nimbidin. The structure of nimbin and nimbinin were elucidated by chemical and n.m.r. studies.[4,5,6,7,8] Nimbin is of interest because it is the first tetranortriterpenoid with ring C cleaved. A second member of this class, salannin (D3), was isolated from the same source.[9]

Recently the biogenetically significant series, epoxyazadiradione (nimbinin) (D2), azadiradione (D4) and azadirone (D5), was obtained from nim oil together with meliantriol (D6), gedunin and 7-deacetyl gedunin.[10,11] Meliantriol has been found to possess antifeeding activity against locusts. Nimbolide (D7), a tetranortriterpenoid closely related to nimbin, has been reported[12] from the fresh leaves of Melia azadirachta.

DISCUSSION

In the course of our investigations in the Meliaceae family we isolated[13] from nim oil in very small yield (0·0005%) a new tetranortriterpenoid, meldenin (D8), which may be a nimbin[7] precursor. Meldenin (D8), $C_{28}H_{38}O_5$, m.p. 240-244°, had absorption in the i.r. attributable to a cyclohexanone, acetate and hydroxyl group (1716, 1747, and 3587 $cm.^{-1}$ respectively). Its n.m.r. spectrum revealed the presence of a β-substituted furan (τ 2·62, 2·74, 3·70, 1H each), five tertiary methyl groups (τ 8·72, 8·78, 8·90, 9·12, 9·17, 3H each), a trisubstituted double bond (τ 4·42, 1H), a secondary hydroxyl group (τ 5·91, 1H, doublet, J ≒ 2 c/sec.) and a secondary acetate group (τ 7·86, 3H; τ 4·68, 1H, quartet, J = 2, 12 c/sec.). The sequence $\rangle CH.CHOAc.CHOR-\overset{|}{C}-$ in nimbin (D1) was duplicated here and was readily confirmed by double resonance experiments. Thus irradiation at H-7 (τ 5·91) caused the collapse of the H-6 quartet to a sharp doublet (J = 12 c/sec.) and H-7 appeared as a sharp singlet on irradiation at H-6. The ketonic carbonyl was placed at C-3 on the basis of o.r.d. measurements,

$[\phi]_{400}$ +1805°, $[\phi]_{303}$ +9300°, $[\phi]_{269}$ ±0°, $[\phi]_{239}$ +4130° (see fig I), which are consistent with those of a 3-keto-8β-methyl triterpenoid[19] although the amplitude is unusually large. The above evidence lead to the structure (D8) for meldenin as the most acceptable biogenetically. The presence of an oxygenation pattern in ring B identical to that in nimbin (D1) suggested that meldenin may be converted into nimbin in the plant by further modification including cleavage of ring C and eventual closure of the C-7 hydroxyl group to C-15.

Nim oil also yielded another furanoid which, crystallised from ether-light petroleum, had m.p. 198-200°, $[\alpha]_D$ +39°, $\nu_{max.}^{CCl_4}$ 1750 (αβ-epoxycyclopentanone), 1736 (acetate) and 1680 $cm.^{-1}$ (cyclohexenone). Its n.m.r. spectrum showed peaks at τ 9·0 (2), 8·98, 8·85 and 8·82 (tertiary methyl groups), 8·0 ($C\underline{H}_3COO$-), 5·3 (broad singlet, $>C\underline{H}OAc$), 6·65 (singlet, H-15), 6·14 (singlet, H-17), 2·88 and 4·15 (doublets, J = 10 c/sec., H-1 and H-2), 2·5 (2) and 3·78 (β-substituted furan). From its spectroscopic data this compound appeared to be identical with nimbinin (D2), m.p. 202-204°, $[\alpha]_D$ +45°, the constitution of which was recently determined[8] by Narayanan and his colleagues.

Nimbinin may be identical with epoxyazadiradione,[10] m.p. 199-200° $[\alpha]_D$ -75°, although there is a discrepancy in the recorded specific rotations. The chemical shift values for the enone protons in nimbinin (τ 2·88, 4·15, J = 10 c/sec.) removed the residual uncertainty[8,10] as to the orientation of the enone in ring A. These values accorded[14] with those of a Δ^1-3-ketone rather than a Δ^2-1-ketone. This conclusion was reinforced by comparison of the o.r.d. curves of dihydronimbinin (D9), ($[\Phi]_{400}$ -725°; $[\Phi]_{322}$ -5060°; $[\Phi]_{286}$ +12300°; $[\Phi]_{266}$ +12300°) and grandifolione[15] (D10), ($[\Phi]_{400}$ -560°; $[\Phi]_{311}$ -8070°; $[\Phi]_{278}$ + 8610°; $[\Phi]_{249}$ +6130°) (see fig. II). A small positive contribution from the 3-ketone is apparent in the former.

From the polar fractions of nim oil deacetylnimbin (D15), $C_{28}H_{34}O_8$, m.p. 212-213° was isolated, This had not been previously reported from natural sources. Its structure was easily demonstrated by its conversion to nimbin and the diketone (D16).

The polar fraction also yielded a mixture of stearic and palmitic acid amides (D17) and (D18). These could not be resolved by normal chromatographic methods and were eventually separated by g.l.c. using a 1% SE 30 column. GC-MS comparison with authentic samples readily confirmed their identity.

From salannin mother liquors we obtained a compound $C_{34}H_{44}O_{10}$, m.p. 244-246°, $[\alpha]_D$ +126° which had an n.m.r. spectrum remarkably similar to that of salannin (see table I), except that the resonances arising from the β-furan moiety in salannin were replaced by those of a vinyl proton at τ 2·75 (1H, broad singlet) and a methylene group at τ 5·30 (2H, broad singlet). This together with the i.r. absorption at 1765 $cm.^{-1}$ (αβ-unsaturated γ-lactone) lead to the structure (D11) for this compound. Further support for the proposed structure (D11) came from catalytic reduction of the butenolide which yielded a mixture of two isomers with $\nu_{max.}^{CCl_4}$ 1780 $cm.^{-1}$ (γ-lactone). In the n.m.r. spectrum of the product, the signal for the vinyl proton at τ 2·75 was absent and the methylene group which appeared at τ 5·3 in (D11) had shifted upfield.

From nimbin mother liquors we isolated the corresponding lactone (D12) $C_{30}H_{36}O_{10}$, m.p. 184-186°, $[\alpha]_D$ +88°, $\nu_{max.}^{CCl_4}$ 1765 cm.$^{-1}$ (αβ-unsaturated γ-lactone). The n.m.r. spectrum resembled nimbin (see table II) except that the resonances arising from the furan protons were replaced by a vinyl proton at τ 2·75 (broad singlet) and methylene group at τ 5·3 (broad singlet). Catalytic reduction of (D12) again resulted in a mixture of isomers with $\nu_{max.}^{CCl_4}$ 1780 cm.$^{-1}$ (γ-lactone). On the above evidence the structure (D12) was assigned to this compound.

Since the two butenolides (D11) and (D12) could not be detected in fresh nim oil it was reasonable to assume that they arose from salannin and nimbin by the action of oxygen and light on the furan ring. Sörm and his colleagues[16] observed this type of process in a number of furanoids e.g., furanoeremophilane (D13) and petsalbine (D14). When nimbin (or salannin) was kept in a variety of solvents in presence of air and normal daylight for a few days a complex mixture resulted. Eventually the butenolide (D12) was isolated from a sample of nimbin which was kept in chloroform solution

under an atmosphere of nitrogen containing a trace of air for four days in the light.

Partial Synthesis of Nimbolide

Nimbolide (D7) was isolated from fresh leaves of Melia azadirachta by Ekong.[12] The partial synthesis of nimbolide from nimbin was achieved as described below:-

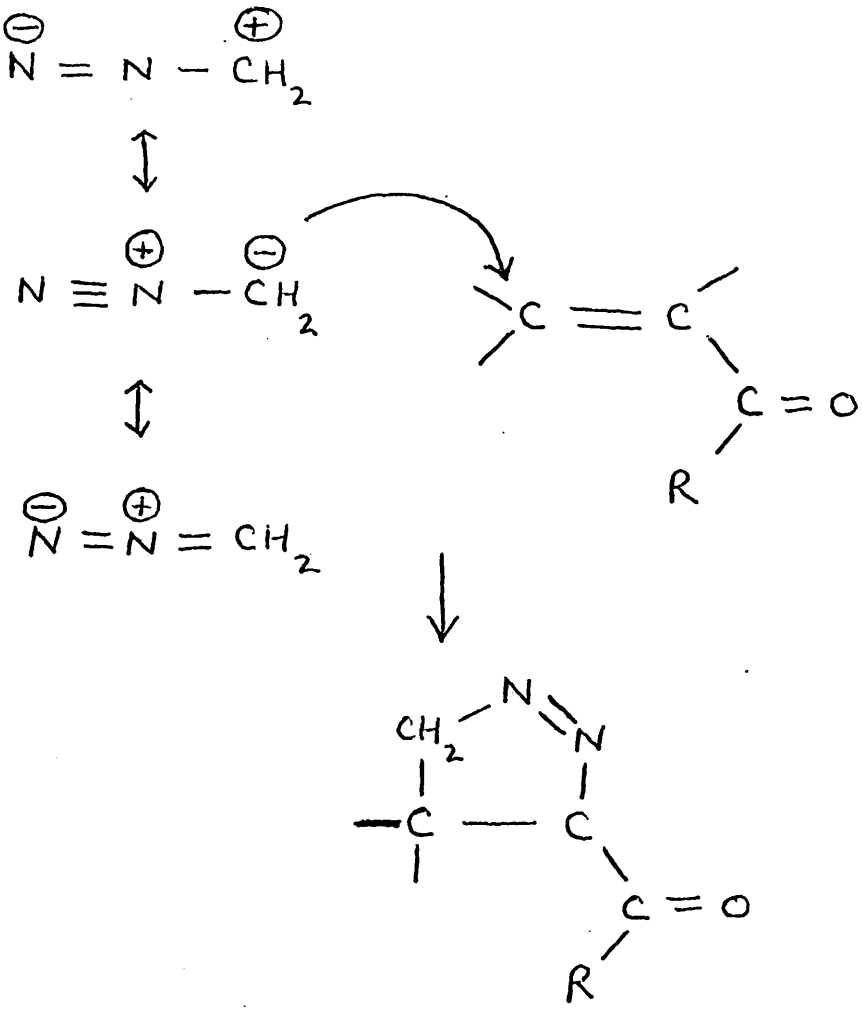
It was reported[17] that under mild hydrolysis conditions nimbin gave the monocarboxylic acid, nimbinic acid (D19). The structure (D19) accorded with our observations that nimbinic acid did not decarboxylate on heating in vacuo at 200° (cf. nimbic acid[7] (D20)) and did not yield any lactonic material on heating with acetic anhydride in pyridine. In contrast the dicarboxylic acid, nimbic acid (D20), gave a good yield of the γ-lactone (D21), when treated with acetic anhydride in pyridine on the steam bath. Methylation of (D21) with diazomethane afforded two main products whose ratio varied with the time of reaction and excess of diazomethane. The first was nimbolide (D7), $C_{27}H_{30}O_7$,

m.p. 243-244°, $[\alpha]_D$ +202°, $\nu_{max.}^{CCl_4}$ 1790 (γ-lactone), 1735 (methyl ester), 1690 $cm.^{-1}$ (enone). In the n.m.r. spectrum it had peaks at τ 9·03, 8·67, 8·57 (tertiary methyl groups), 8·35 (vinyl methyl group), 6·51 ($-CO_2Me$), 5·4 (quartet, J = 12, 3 c/sec.; H-6), 5·76 (doublet, J = 3 c/sec.; H-7), 4·49 (broad singlet, H-15), 2·7 and 4·08 (doublets, J = 10 c/sec.; H-3 and H-2). These physical and spectroscopic properties accorded with the published values.[12] *

The more polar methylation product was nimbolide pyrazoline (D22), $C_{28}H_{32}O_7N_2$, m.p. 212-214°, $[\alpha]_D$ +172° $\nu_{max.}^{CCl_4}$ 1790 (γ-lactone), 1740 (methyl ester) and 1700 $cm.^{-1}$ (cyclohexanone). Its n.m.r. spectrum had peaks at τ 8·8, 8·72, 8·6 (tertiary methyl groups), 8·36 (vinyl methyl group), 6·56 ($-CO_2Me$), 5·37 (quartet, J = 12, 3 c/sec.; H-6), 5·73 (doublet, J = 3 c/sec.; H-7), 4·5 (broad singlet, H-15).

It is known that even under very mild conditions diazomethane can add to the double bond of αβ-unsaturated ketones to give pyrazoline derivatives.[18]

* A direct comparison with authentic nimbolide was also carried out through the courtesy of Professor D.E.U. Ekong, Ibadan, Nigeria.



Nimbin did not react under the same conditions. Presumably the driving force for the reaction in the case of nimbolide is the relief of the additional strain in ring A resulting from the formation of the lactone ring.

EXPERIMENTAL

Chromatography of Nim Oil

Nim oil (3 kg.) was dissolved in light petroleum and chromatographed on grade III alumina (9 kg.). The column was eluted with light petroleum to remove most of the fats and then in turn with chloroform and methanol. The fractions were tested for the presence of furanoids by t.l.c. using Ehrlich's reagent. The light petroleum and methanol fractions gave negative results and were set aside.

The residue (480 gm.) from the chloroform fraction was defatted by dissolving it in methanol-water (4:1) and extracting several times with light petroleum. Removal of the methanol gave a semi-solid material (300 gm.) which was chromatographed on grade III alumina (9 kg.). Light petroleum and benzene-light petroleum (1:1) eluted fractions containing nimbinin (D2) and then meldenin (D8), benzene and benzene chloroform (19:1) eluted nimbin (D1) and salannin (D3), and chloroform eluted 6-deacetylnimbin (D15) and a mixture of palmitic (D17) and stearic (D18) acid amides.

Nimbinin (D2)

The fractions containing nimbinin were combined and purified by preparative t.l.c. (chloroform as solvent). Nimbinin crystallised from ether-light petroleum in needles (yield 0·0045%) m.p. 196-198°, $[\alpha]_D$ +39°, $\nu_{max.}^{CCl_4}$ 1750 (αβ-epoxycyclopentanone) 1736 (acetate) and 1680 cm.$^{-1}$ (cyclohexenone). Dihydronimbinin (D9) was prepared in the usual way[20] by sodium borohydride reduction followed by Jones oxidation. It was purified by preparative t.l.c. and, crystallised from ether-light petroleum, had m.p. 196-198° $\nu_{max.}^{CCl_4}$ 1750 (αβ-epoxycyclopentanone), 1736 (acetate) and 1710 cm.$^{-1}$ (cyclohexanone).

Meldenin (D8).

Meldenin (D8) was isolated in small yield from the fractions following nimbinin by preparative t.l.c. It crystallised from ether-light petroleum in needles m.p. 240-244°, $\nu_{max.}^{CCl_4}$ 1716 (cyclohexanone), 1747 (acetate) and 3587 cm.$^{-1}$ (hydroxyl). Mass spectrometric molecular weight 454·27179. $C_{28}H_{38}O_5$ requires 454·27191.

Nimbin (D1) and Salannin (D3)

The earlier fractions eluted with benzene were crystallised from methanol to yield nimbin m.p. 202-204° (yield 0·2%).

The later fractions in this range, on crystallisation from ethyl acetate-light petroleum, gave salannin m.p. 168-170° (yield 0·9%).

Deacetylnimbin (D15)

The more polar fractions gave, by preparative t.l.c., deacetylnimbin (300 mg.) which crystallised from ether-light petroleum and had m.p. 212-213°, $\nu_{max.}^{CCl_4}$ 1690 (αβ-unsaturated ketone) 1740 (methyl ester) and 3556 $cm.^{-1}$ (hydroxyl). The n.m.r. spectrum resembled that of nimbin except that the characteristic acetate signal at τ 7·98 was absent and the quartet for H-6 had shifted upfield from τ 4·75 to τ 6·5. 6-Deacetylnimbin was converted by acetylation into nimbin m.p. 202-204° and by oxidation with Jones reagent into the corresponding dione (D16) m.p. 155-157°.

Palmitic (D17) and Stearic (D18) Acid Amides

The later fractions eluted with chloroform deposited crystalline material (400 mg.) on standing. It was recrystallised from methanol and had m.p. 98-102°, $\nu_{max.}^{CCl_4}$ 3536, 3414 and 1696 $cm.^{-1}$ (free primary amide), τ 4·35 (broad signal; $-CONH_2$).

The amide gave a single spot on t.l.c. but showed two peaks of equal intensity on g.l.c. on a 1% SE 30 column. These were checked with authentic samples of palmitic and stearic acid amides by the serial dilution technique and there was no change in the profile of the chromatogram. When run on GC-MS, the two peaks m/e 225 and 288 respectively (palmitic acid amide 225 and stearic acid amide 288) and their mass spectra were identical with those of the respective authentic reference compounds.

Butenolide (D11) from Salannin

Salannin mother liquors (3·5 gm.) on preparative t.l.c. gave a more polar compound, the butenolide (D11) (130 mg.) which, crystallised from ether-light petroleum, had m.p. 244-246°, $[\alpha]_D$ +126° $\nu_{max.}^{CCl_4}$ 1710 (tiglate ester), 1743 (acetate and methyl ester), 1765 cm.$^{-1}$ (αβ-unsaturated γ-lactone). (Found: C, 66·75; H, 7·4. $C_{34}H_{44}O_{10}$ requires C, 66·5; H, 7·35%.)

Hydrogenation of the butenolide (D11)

The butenolide (30 mg.) and 10% palladium charcoal (8 mg.) in ethyl acetate (3 ml.) were stirred in the presence of hydrogen for 8 hr., when the uptake of hydrogen ceased. The catalyst was filtered and the solvent removed. The residue did not crystallise and showed on a chromatoplate two closely moving spots $\nu_{max.}^{CCl_4}$ 1740 (acetate and esters) and 1780 cm.$^{-1}$ (γ-lactone).

Butenolide (D12) from Nimbin

The mother liquors of nimbin (2 gm.) on preparative t.l.c. yielded a more polar compound, the butenolide (D12) (80 mg.) which crystallised from ether-light petroleum in rods m.p. 184-186°, $[\alpha]_D$ +88°, $\nu_{max.}^{CCl_4}$ 1690 (cyclohexenone), 1740 (acetate and ester) and 1765 cm.$^{-1}$ ($\alpha\beta$-unsaturated γ-lactone). (Found: C, 64·8; H, 6·55. $C_{30}H_{36}O_{10}$ requires C, 64·75; H, 6·5%.)

Hydrogenation of the butenolide (D12)

The butenolide (20 mg.) was hydrogenated as above. The product, which did not crystallise, again showed two closely moving spots on t.l.c. $\nu_{max.}^{CCl_4}$ 1715 (cyclohexanone) 1740 (acetate and ester) and 1780 cm.$^{-1}$ (γ-lactone).

Autoxidation of Nimbin

Samples of nimbin (30 mg.) in different solvents (5 ml.) (chloroform, carbon tetrachloride, benzene, ethyl acetate and methanol) in presence of air, oxygen-free nitrogen or nitrogen containing traces of air were kept in the light or in the dark for two weeks. Every two days the samples were checked on a chromatoplate. It was observed that those kept in the dark remained unchanged over a considerable period of time while those kept in light in presence of air rapidly became complex mixtures containing no unchanged nimbin. After four days in chloroform solution, in the presence of the air-nitrogen mixture in the light, nimbin was partially transformed into the butenolide (D12) (4 mg.) which was separated by preparative t.l.c. It crystallised from ether-light petroleum and had m.p. 183-186°, m.m.p. undepressed with authentic sample, identical i.r. and n.m.r.

Preparation of Nimbinic Acid (D19)

Nimbin (200 mg.) was dissolved in methanol (6 ml.) and benzene (2 ml.) and 10% aqueous potassium hydroxide (0·7 ml.) added. The reaction mixture was stirred for 4 hr. at 20° and separated into acidic and neutral material in the usual way. The acid fraction on crystallisation from aqueous methanol gave nimbinic acid (180 mg.) m.p. 258-260°.

Attempted Lactonisation of Nimbinic Acid

Nimbinic acid (30 mg.) was dissolved in dry pyridine (0·25 ml.) and acetic anhydride (0·25 ml.) and the reaction mixture heated on the steam bath for 5 hr. It was worked up in the usual way and the product (two spots) examined in the i.r. No lactonic carbonyl absorption was detected.

Attempted Decarboxylation of Nimbinic Acid

Nimbinic acid (5 mg.) was heated at 200° in a sublimation block at a pressure of 5 mm. Hg. After 5 hr. only the unchanged acid was recovered.

Preparation of Nimbic Acid (D20)

Nimbin (400 mg.) was treated with 10% methanolic potassium hydroxide (5 ml.) and allowed to stand for 2 hr. at 20°. When the reaction mixture was acidified with 6N hydrochloric acid, nimbic acid (360 mg.) crystallised out. It was filtered off, washed with water and recrystallised from aqueous methanol m.p. 160-161°.

Preparation of Nimbolide (D7)

Nimbic acid (100 mg.) was dissolved in dry pyridine (1 ml.) and acetic anhydride (1 ml.). The reaction mixture was heated on the steam bath for 3 hr. The residue after removal of the solvents was treated with an ethereal solution of diazomethane and separated into two components by preparative t.l.c.

Nimbolide (20 mg.), the less polar product, crystallised from ether-light petroleum and had m.p. 243-244°, $[\alpha]_D$ +202°, $\nu_{max.}^{CCl_4}$ 1790 (γ-lactone), 1735 (methyl ester) and 1680 $cm.^{-1}$ (cyclohexenone). (Found: C, 69·65; H, 6·65. $C_{27}H_{30}O_7$ requires C, 69·5; H, 6·6%.)

Nimbolide pyrazoline (D22) (10 mg.), the more polar product, crystallised from ethyl acetate-light petroleum and had m.p. 212-214°, $[\alpha]_D$ +172°, $\nu_{max.}^{CCl_4}$ 1790 (γ-lactone), 1740 (methyl ester), and 1710 $cm.^{-1}$ (cyclohexanone), $\lambda_{max.}$ 308 (ε 3403), 216 mμ (ε 4927). (Found: C, 65·8; H, 6·4; N, 5·4. $C_{28}H_{32}O_7N_2$ requires C, 66·1; H, 6·4; N, 5·5%.)

By increasing the quantity of diazomethane and the reaction time, the yield of the pyrazoline derivative was increased. Nimbin did not react with diazomethane under these conditions.

Table I

N.m.r. Spectra (τ values) of

	Butenolide (D11)	Salannin
Methyl groups	9·1, 8·9, 8·82, 8·35 (d) J = 1·5 c/sec.	9·0, 8·77, 8·68, 8·31(d) J = 1·5 c/sec.
Methyl ester	6·72	6·72
Acetate	8·18	8·02
Tiglate ester	8·18–8·28 (m), 3·0 (m)	8·0–8·25 (m), 3·0 (m)
Furans	-	2·7, 3·67
Olefinic	2·75 (bs)	-
Methylene groups	5·3 (bs)	

Table II

N.m.r. Spectra (τ values) of

	Butenolide (D12)	Nimbin
Methyl groups	8·74, 8·66 (2), 8·33 (d) J = 2 c/sec.	8·73, 8·66 (2), 8·33 (d) J = 2 c/sec.
Acetate	7·98	7·98
Methyl ester	6·38, 6·28	6·38, 6·28
H-2 and H-3	3·68 and 4·15 (d) J = 10 c/sec.	3·68 and 4·15 (d) J = 10 c/sec.
Furan	-	2·65, 3·65
Olefinic	2·75 (bs)	-
Methylene group	5·3 (bs)	-

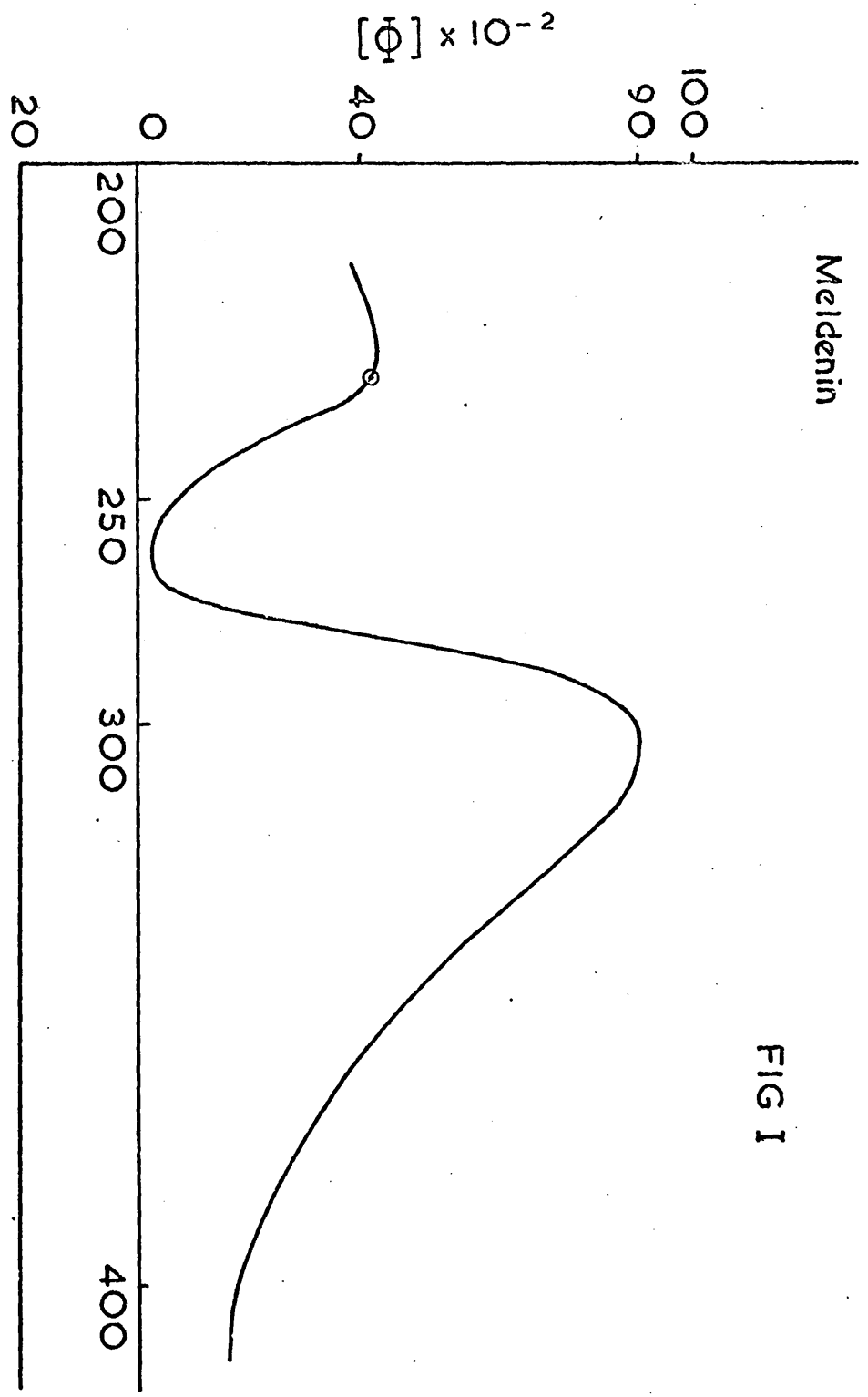


FIG I

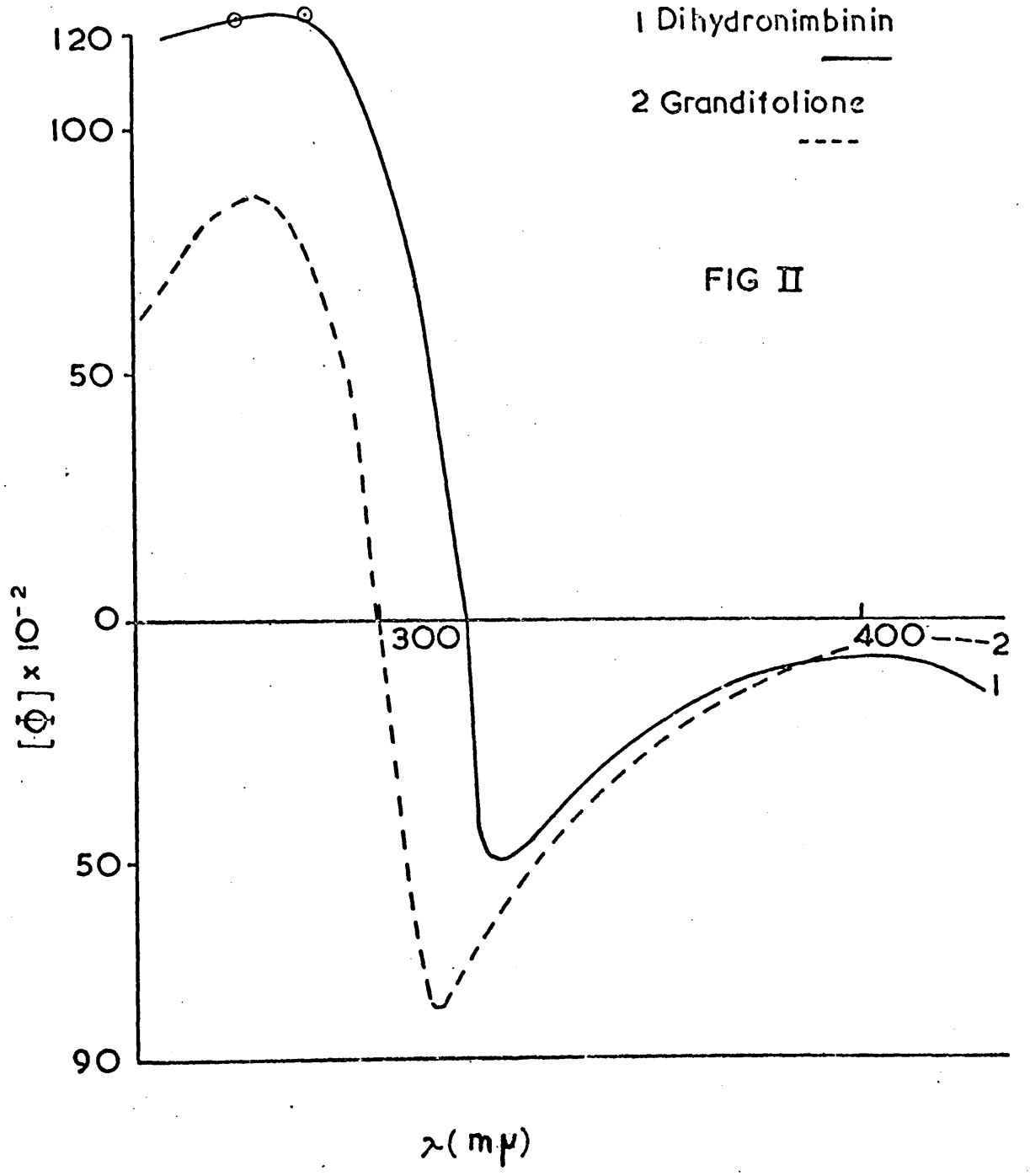
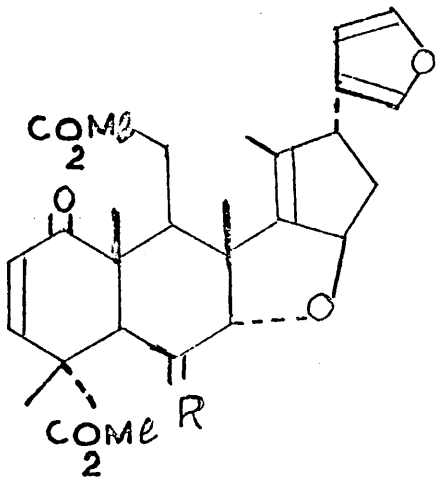

1 Dihydronimbinin
2 Granditolione
120
100
50
0
50
90
300
400
2
1
[ϕ] x 10⁻²
λ(mμ)

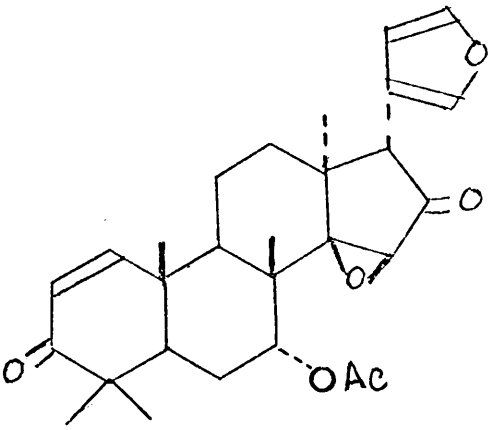
FIG II



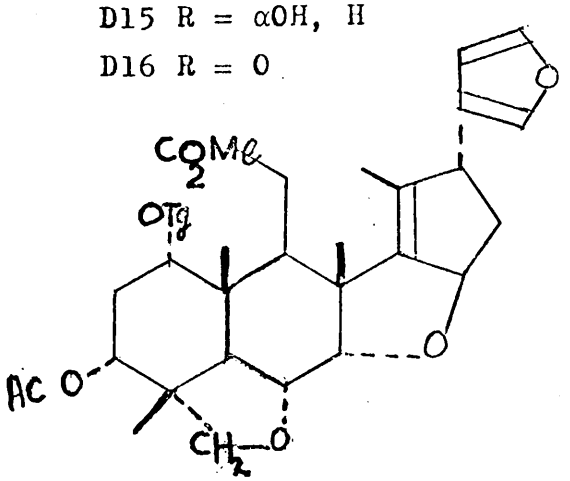
D1 R = αOAc, H

D15 R = αOH, H

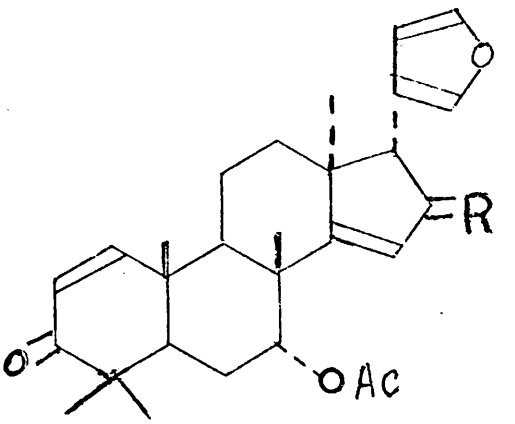
D16 R = O



D2

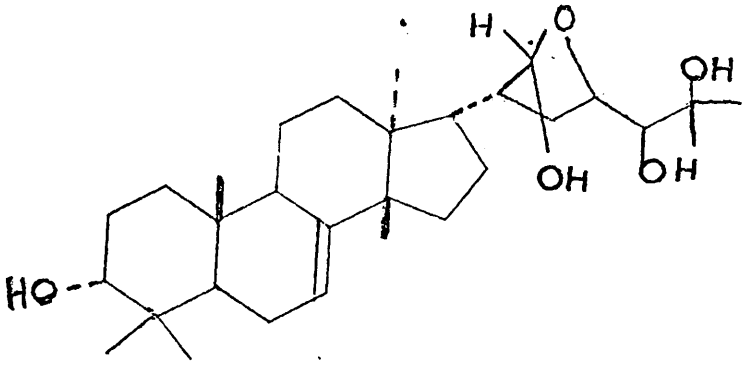


D3

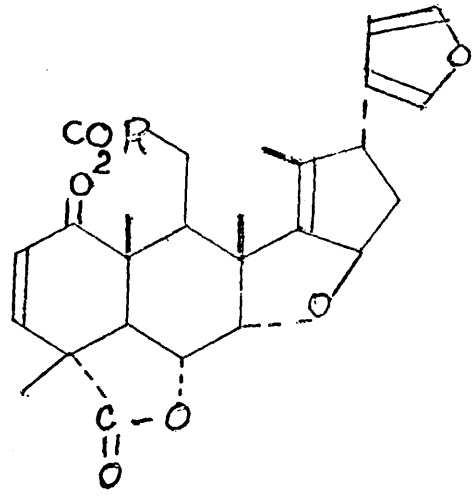


D4 R = O

D5 R = 2H

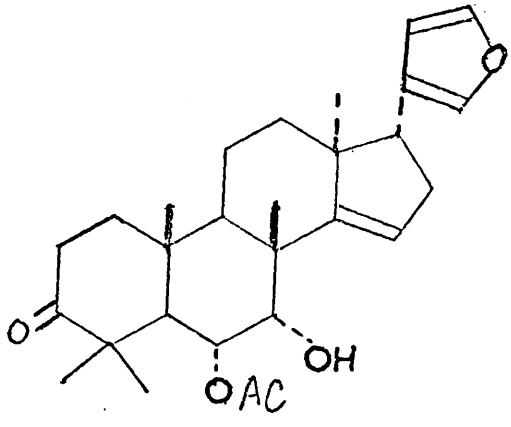


D6

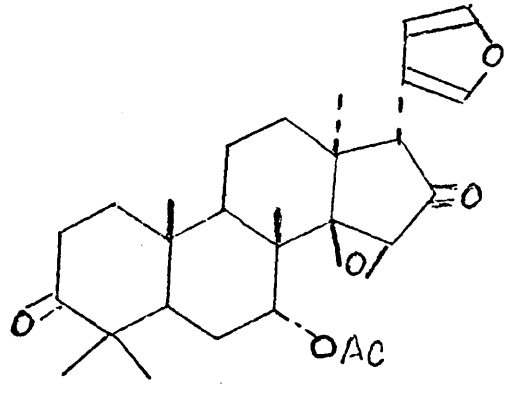


D7 R = Me

D21 R = H



D8



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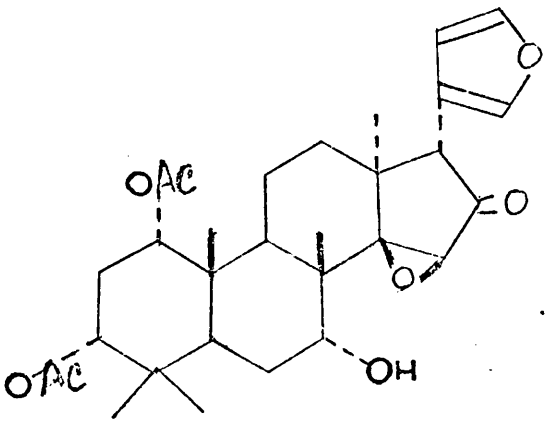
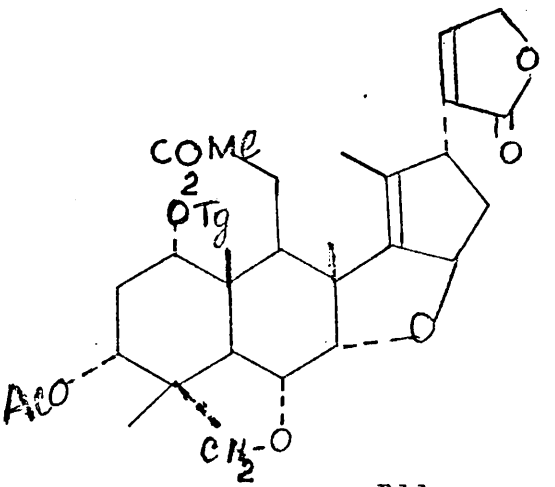
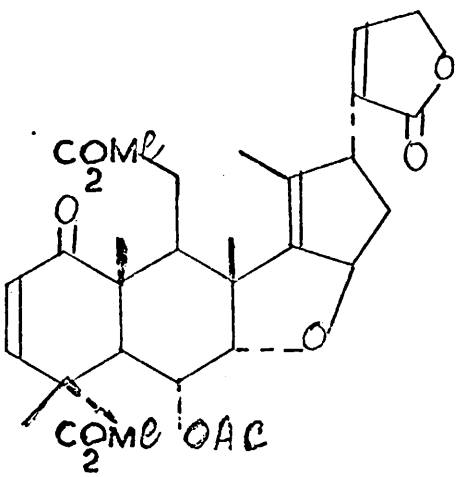


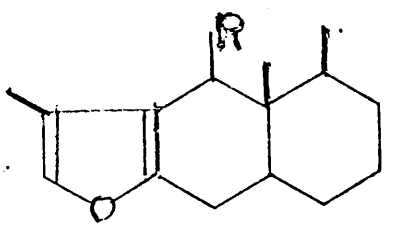
D10



D11

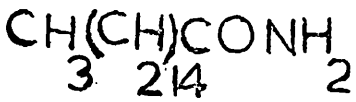


D12

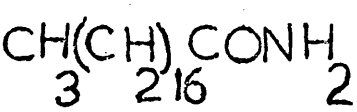


D13 R = H

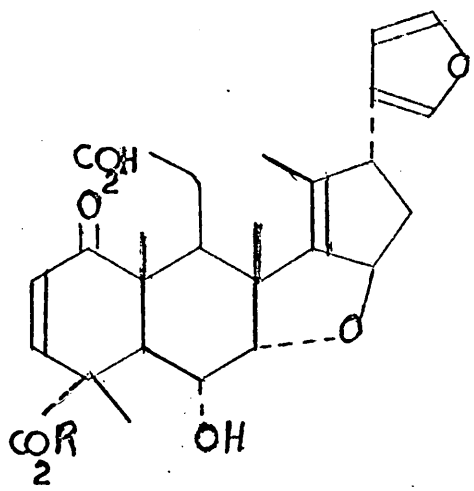
D14 R = αOH



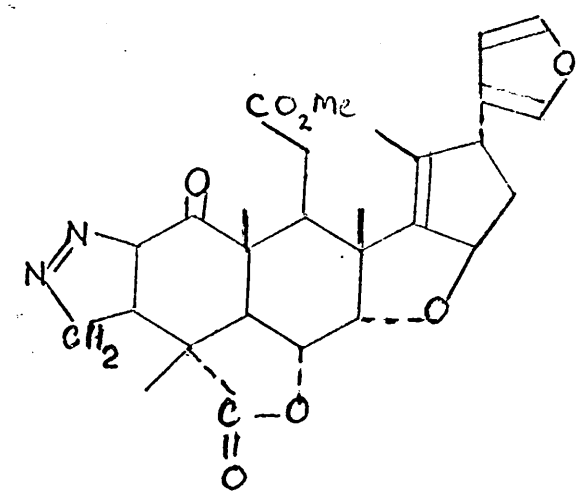
D17



D18



D19 R = Me
D20 R = H



D22

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CHAPTER V

EXAMINATION OF NINE SPECIES

OF THE MELIACEAE FAMILY

INTRODUCTION

From the previous chapters of this thesis it is apparent that trees of the family Meliaceae provide a good source of tetranor and related tetracyclic triterpenoids. The heartwoods and seeds of a number of members of the Meliaceae family were examined and the results are reported in this section. The trees involved are:-

Swietenia macrophylla, *S. mahagoni*,
Lovoa brownii and *L. kalineana*,
Chukrasia tabularis, *Khaya ivorensis*,
Soyamida febrifuga, *Cedrela sureni* and
C. serrata.

Swietenia macrophylla and S. mahagoni

(a) Seeds

From the seeds of S. macrophylla, two tetranor-triterpenoids swietenine (E1) and swietenolide (E2) have been reported[1,2] and their structures elucidated.[3,4] In an attempt to isolate some of the minor components we reinvestigated the chloroform extract of the defatted seeds of S. macrophylla by use of preparative t.l.c. on silica gel. The extract separated into four major bands. The compounds from second and fourth bands were identified as swietenine and swietenolide respectively. The material from the first band ran as a single spot on a chromatoplate but its n.m.r. spectrum showed it to be a mixture. The methyl region between τ 8·4-9·1 revealed the presence of at least seven methyl groups and the spectrum also showed signals arising from methoxyl and acetyl groups. The extractive from the third band was also found to be a mixture with strong methoxyl and acetyl peaks in the n.m.r. These mixtures could not be resolved and were therefore hydrolysed with methanolic sodium hydroxide and the products separated by t.l.c. The mixture from the first band gave swietenine (E1)

(0·03%), swietenolide (E2) (0·03%) and isoswietenolide (E3) (0·012%), suggesting that the original extract contained the corresponding mono and diacetates.

The products from the third band were found to be the 3β-alcohol (E4) (0·025%) previously obtained by reduction of mexicanolide,[5] swietenolide (0·05%) and isoswietenolide (0·018%). These were presumably present in the original extract as the corresponding 3- or 6-acetates.

Experimental

Defatted milled seeds (400 gm.) of S. macrophylla were extracted with chloroform in a soxhlet. The residue, after removal of the solvent, was obtained as an amorphous powder (20 gm.). A portion (6 gm.) of this powder was chromatographed on 0·5 mm. thick silica gel plates (1000 x 20 cm.) in chloroform containing 2% methanol. The material separated into four major bands which were extracted in the usual way. The material from band I (2 gm.), band III (0·86 gm.) did not crystallise.

The residue from band II (0·54 gm.) crystallised from ether-light petroleum in rods m.p. 270-274°, m.m.p. with swietenine 270-274°, n.m.r. spectrum identical with that of swietenine.

The residue from band IV (1·1 gm.) crystallised from methanol in rods m.p. 220-221°, undepressed on admixture with swietenolide, n.m.r. spectrum identical with that of swietenolide.

Hydrolysis of Band I

The mixture from band I (1 gm.) was dissolved in 2% methanolic potassium hydroxide (10 ml.) and allowed to stand at room temperature for 2 hr. The reaction mixture was acidified with 6N hydrochloric acid, diluted with water and extracted with chloroform. The solvent was removed *in vacuo* and the residue chromatographed on 0·5 mm. silica gel plates. Four bands were obtained and extracted as usual. The residue from the first band did not crystallise but the residue from the second crystallised from ether-light petroleum m.p. 272-274°, m.m.p. undepressed with swietenine and identical n.m.r.

The residue from the third, crystallised from methanol, had m.p. 221-224°, m.m.p. undepressed with authentic swietenolide, identical n.m.r.

The residue from the fourth band, crystallised from methanol, had m.p. 213-216°, m.m.p. with isoswietenolide undepressed, identical n.m.r.

Hydrolysis of Band III

The mixture (0·25 gm.) was hydrolysed as above and separated by preparative t.l.c. The material from first band crystallised from ether-light petroleum in prisms m.p. 122-125° and 193-196°, m.m.p. undepressed and n.m.r. identical with the 3β-alcohol (E4) obtained from mexicanolide.[5]

The residue from the second and third bands crystallised from methanol and had m.p. 221-224° and 213-216° respectively. They were identified as swietenolide and isoswietenolide respectively by m.m.p. and n.m.r. spectra.

(b) Heartwoods.

Since the seeds of S. macrophylla and S. mahagoni had proved to be a good source of tetranortriterpenoids, it was decided to investigate the heartwoods. Previously the isolation of cycloeucalenol (E5) from the light petroleum extract of the heartwood of S. mahagoni had been reported.[6]

The ethyl acetate extract of the heartwood of the trees was examined and showed four major spots and a few minor spots on t.l.c., only the minor spots gave a positive reaction to Ehrlich's reagent. Chromatography of the chloroform soluble portion of the extract on grade V alumina and further purification of the fractions by preparative t.l.c. yielded cycloeucalenol (E5) (0·012%) and 24-methylenecycloartenol (E6) (0·01%). These were found to be present free and as esters in the wood, and their identity was established by physical and spectroscopic methods. In the n.m.r. spectrum the cyclopropyl protons appeared as an AB quartet (J_{AB} = 4 c/sec.) at τ 9·7 and 9·96, in cycloeucalenol and at τ 9·62 and 9·86 in 24-methylenecycloartenol. Cycloeucalenol and 24-methylenecycloartenol have previously been reported from rice bran oil[7,8] and from Tristania conferta.[9]

Experimental

Powdered heartwood of S. macrophylla (1 kg.) was extracted in a Soxhlet with ethyl acetate and solvent removed in vacuo. The semi-solid extract obtained was exhausted with hot chloroform and the chloroform soluble material examined on a chromatoplate. It showed four spots. A portion (20 gm.) was chromatographed on grade V neutral alumina (400 gm.), light petroleum eluted fatty material, benzene-light petroleum (1:4) a mixture of cycloeucalenol and 24-methylenecycloartenol, benzene-light petroleum (1:1) β-sitosterol and chloroform-methanol more polar compounds which could not be crystallised.

The mixture of cycloeucalenol and 24-methylene-cycloartenol was separated by preparative t.l.c. in chloroform benzene (4:1). The more polar component, cycloeucalenol, crystallised from methanol, had m.p. 139-140°. The corresponding acetate, prepared in the usual way and crystallised from methanol, had m.p. 109-110° (lit.,[10] cycloeucalenol m.p. 140°, acetate m.p. 110°C).

The less polar component, 24-methylenecycloartenol had m.p. 118-120° (ex methanol). The derived acetate crystallised from methanol had m.p. 113-114° (lit.,[10] 24-methylenecycloartenol m.p. 121-122°, acetate 116-117°).

The residue from benzene-light petroleum (1:1) crystallised from methanol had m.p. 136-137° undepressed on admixture with β-sitosterol.

Saponification of the fatty portion

The fatty portion (6 gm.) eluted with light petroleum was dissolved in 10% alcoholic potassium hydroxide (25 ml.) and refluxed on the steam bath for 4 hr. The residue obtained after the usual work-up yielded *inter alia* cycloeucalenol and 24-methylenecycloartenol.

The ethyl acetate extract of *S. mahagoni* heartwood was also processed as above and yielded similar results.

Lovoa brownii and L. Kalineana

Lovoa brownii and L. kalineana are native to South Africa. Since no chemical studies on these trees have been reported, the ethyl acetate extracts of the heartwoods were examined. On t.l.c. these showed a pattern very similar to S. mahagony and S. macrophylla extracts but were completely negative to Ehrlich's reagent. The extracts were chromatographed as above and yielded only cycloeucalenol, 24-methylenecycloartenol and β-sitosterol.

Chukrasia tabularis

Chukrasia tabularis is distributed in the tropical parts of Asia. The ethyl acetate extract of the heartwood was investigated. The chloroform insoluble part yielded two known compounds, the flavanone ampelopsin[11] (E7) and the flavone myricetin[11] (E8). The chloroform soluble fraction consisted of a complex mixture of furanoid compounds (positive to Ehrlich's reagent) which could not be resolved despite repeated use of preparative t.l.c.

Experimental

Powdered heartwood of C. tabularis (4 kg.) was extracted in a soxhlet with ethyl acetate. Removal of solvent gave a semi-solid extract (3·5%) which was extracted with hot chloroform several times.

Ampelopsin (E7)

The chloroform insoluble solid was dissolved in hot methanol and allowed to stand. Ampelopsin crystallised out and had m.p. 340-344° (decomp.),* (yield 1·0%). The following derivatives were prepared in the usual way: hexa-acetate m.p. 174-176°, tetramethyl ether m.p. 168-170°, pentamethyl ether m.p. 192-193°, (lit.,[11] ampelopsin m.p. 245-246°, hexa-acetate 174-175°, tetra-methyl ether m.p. 168°, pentamethyl ether m.p. 194-195°).

* transition point at 245°.

Myricetin (E8)

The mother liquors of ampelopsin on concentration and standing deposited myricetin as needles m.p. 345-355° $\lambda_{max.}$ 278 and 255 mμ. The derived acetate had m.p. 210-211° (lit.,[11] myricetin m.p. 350°, acetate 212-213°).

The chloroform soluble portion showed a series of spots on t.l.c. which gave positive Ehrlich's test. Repeated column and preparative t.l.c. failed to isolate any pure compound.

Khaya ivorensis

This is the commonest species of the genus Khaya and the earlier investigations of the tree revealed the presence of khivorin (E9) and methyl angolensate (E10).[12] In a search for minor constituents we examined the chloroform soluble portion of the ethyl acetate extract of the heartwood. This was chromatographed on grade V neutral alumina and yielded only the known compounds khivorin, methyl angolensate and 6-hydroxy methyl angolensate.[13]

Soyamida febrifuga

Soyamida febrifuga is native of India. The chloroform soluble portion of the ethyl acetate extract of the heartwood was examined on t.l.c. and showed a number of diffuse spots, none of which gave a positive test with Ehrlich's reagent. The extract was chromatographed but only mixtures were obtained.

Cedrela species

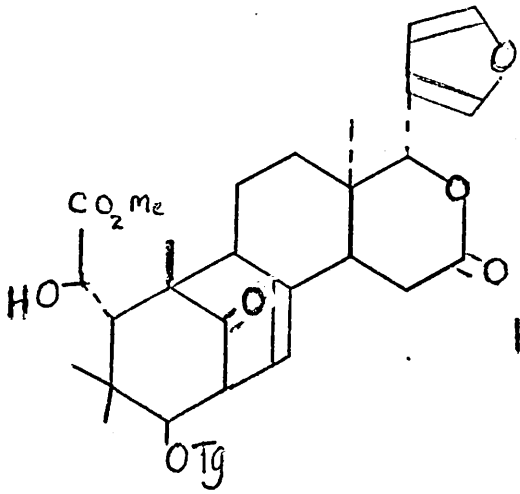
Cedrela species are distributed in the tropical parts of the world and several of these namely Cedrela mexicana, C. toona, C. odorata and C. glaziovii have yielded (vide supra) a very large number of triterpenoids. This prompted us to examine two further members of this genus, C. sureni and C. serrata.

C. sureni

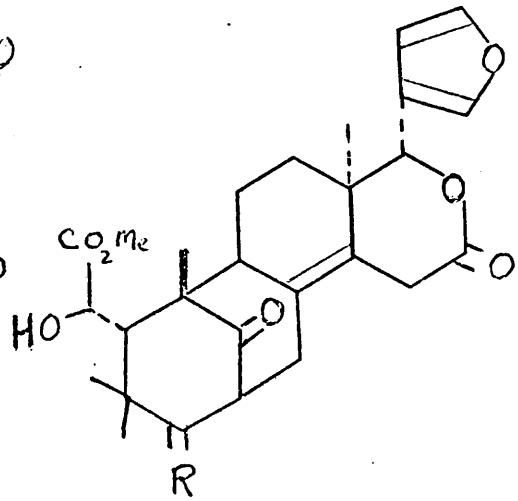
The chloroform soluble part of the ethyl acetate extract of the heartwood was diluted with methanol and allowed to stand. Cedrelone[14] (E11) crystallised out in high yield (0·1%). The residue from the mother liquors consisted of a mobile oil which probably contained mainly sesquiterpenoids (cf. C. toona[15,16,17]).

C. serrata

The heartwood of this tree gave a fairly mobile oil which gave no indication of the presence of furanoid material on t.l.c.

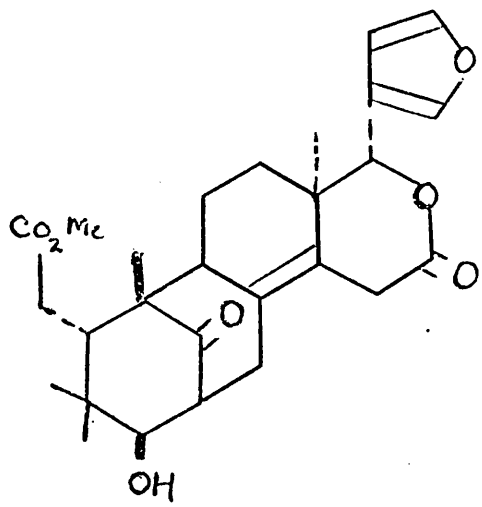


E1

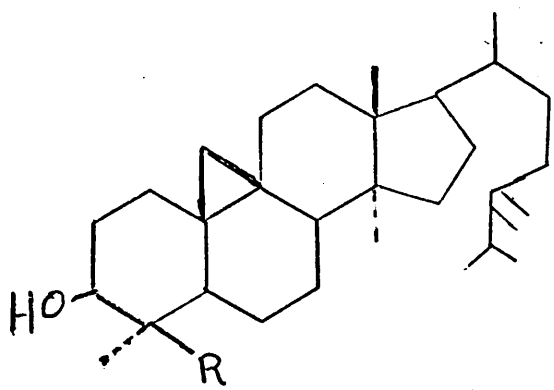


E2 R = βOH, H

E3 R = αOH, H

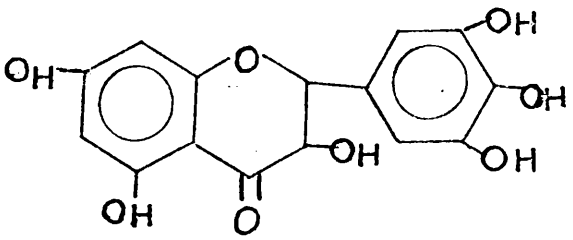


E4

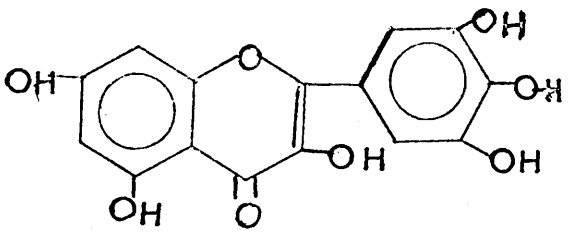


E5 R = H

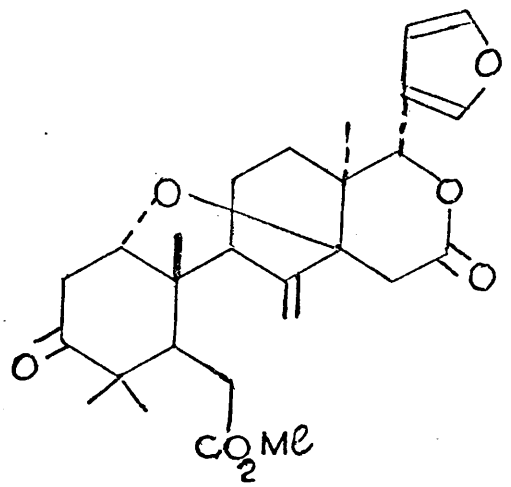
E6 R = Me



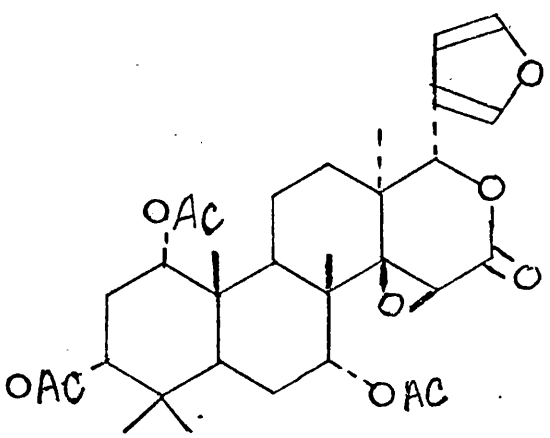
E7



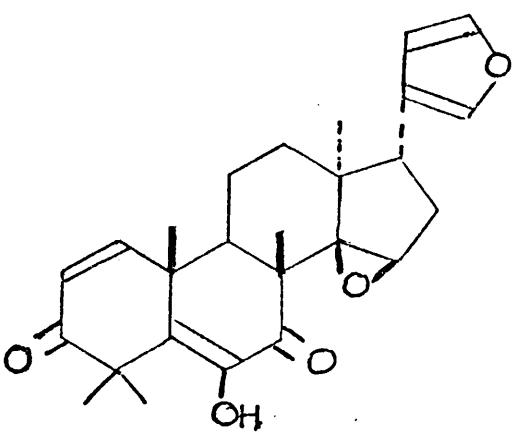
E8



E10



E9



E11

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