A THESIS

ENTITLED

'STUDIES IN THE TERPENOID FIELD'

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By ALEXANDER E. HARDING. B.Sc.

Chemistry Department

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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CONTENTS

SECTION 3 PRIEURIANIN, A COMPLEX TETRANORTRITERPENOID FROM TRICHILIA PRIEURIANA

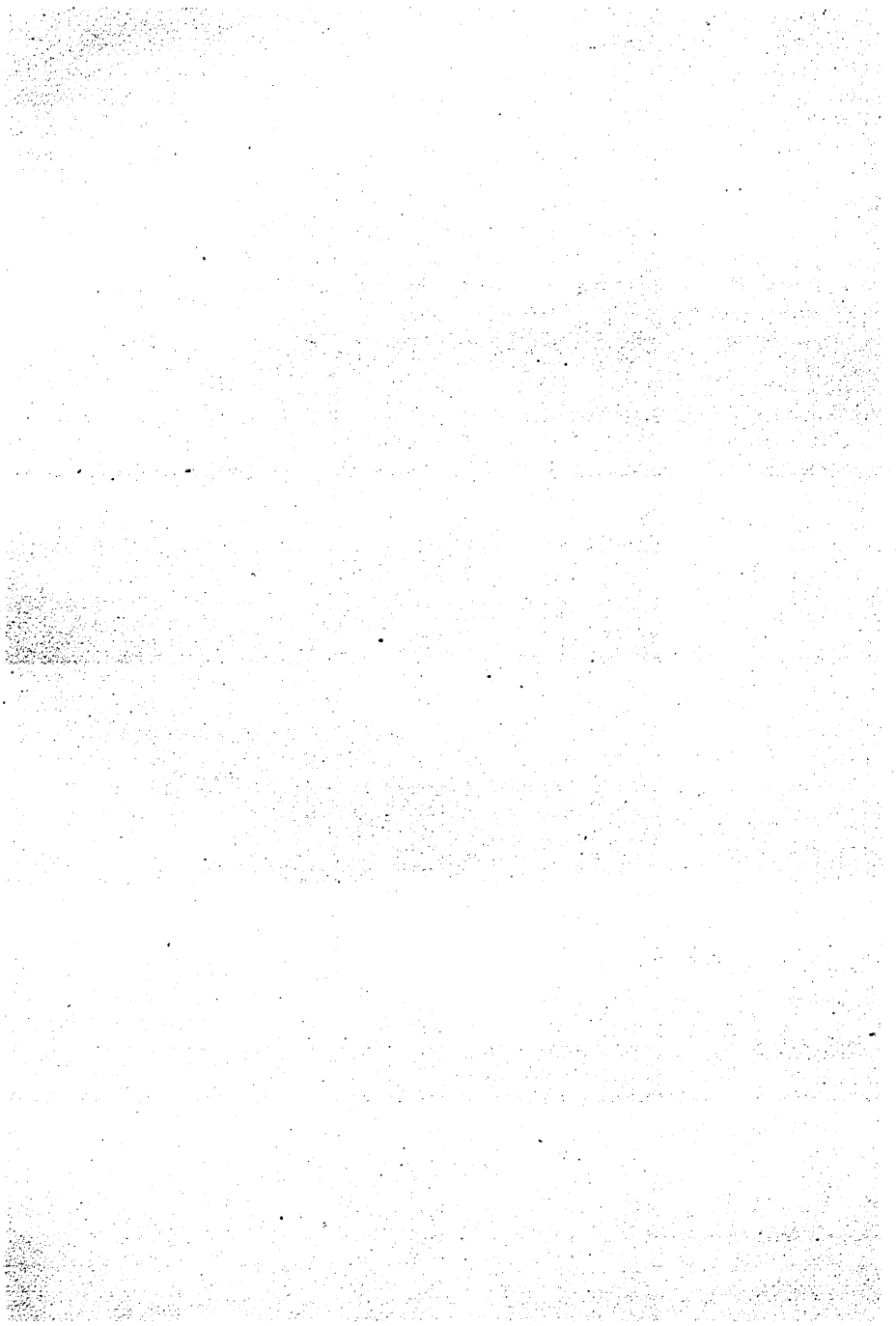
SUMMARY

This thesis is divided into three sections. Section one concerns an investigation of the liverwort Gymnomitrion obtusum (Lindb) Pears (Hepaticae) which proved to be a rich source of sesquiterpenoids with a novel (5, 3, 1) undecane carbon skeleton. Eight new sesquiterpenoids were isolated including the alcohol gymnomitrol, which is the major constituent. The chemical and spectroscopic properties of gymnomitrol and its congeners, which led to the structural elucidation, are described, together with some interesting reactions and more detailed discussions of the mass spectra and lanthanide ion induced shifts in the nuclear magnetic resonance spectra. The absolute configuration of gymnomitrol has been determined by circular dichroism and a plausible biogenesis proposed. The initial stages of a potential synthetic route to this new skeletal type are reported. The discussion of the chemistry of gymnomitrol is prefaced by a brief review of the literature of the Hepaticae.

In an extension of the previously reported work on the constituents of Erythroxylon monogynum Roxb., which is discussed briefly in the introduction, section two describes the isolation and structural elucidation of seven new diterpenoids from the rootwood of Erythroxylon australe. The structures of these new stachene - and erythroxydiol X - related diterpenoids were assigned on the basis of chemical and spectroscopic evidence and interrelation with known compounds. It is of interest that all the new compounds have an oxygen substituent at C-1. In addition six known diterpenoids were also isolated.

In section three the chemistry of some highly complex tetranor-triterpenoids is reviewed. The light petroleum extract of the wood of Trichilia prieuriana (Meliaceae) gave a crystalline substance named

prieurianin, which appears to be a member of this group. Despite a considerable amount of data on prieurianin, including p.m.r. and ^{13}C.m.r., its structure has still not been established. A suitable derivative for X-ray crystal structure analysis has been prepared and is being examined.



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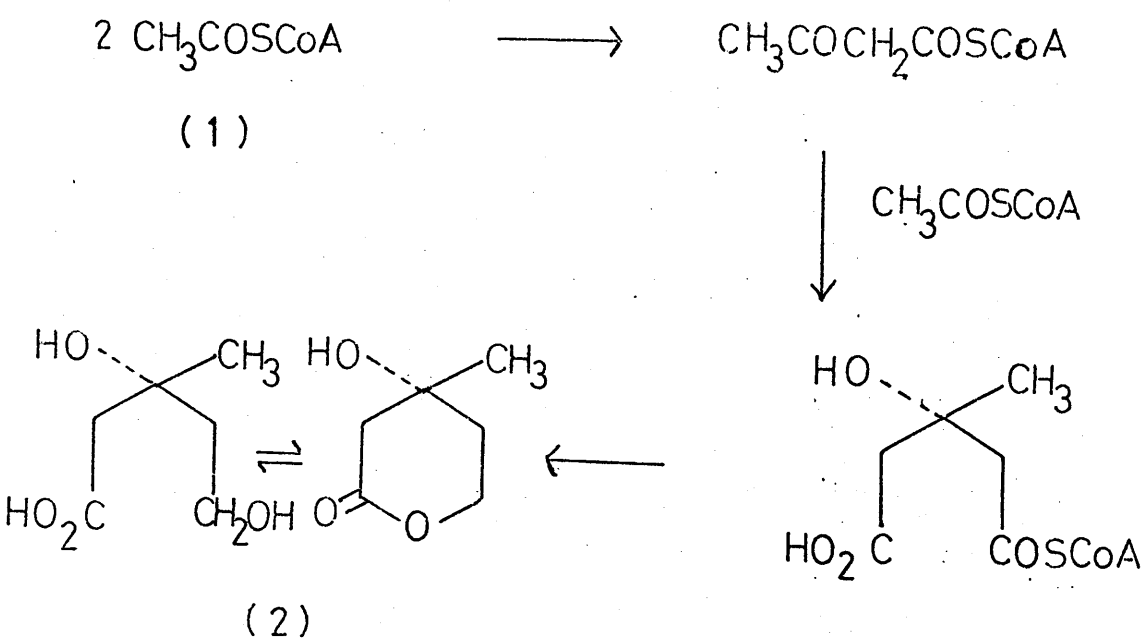

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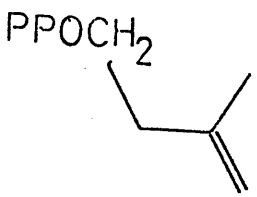

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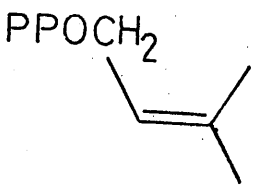

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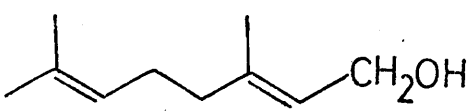

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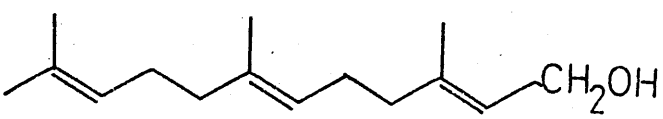

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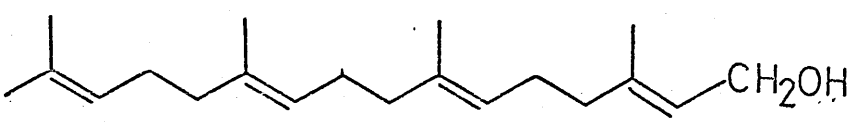

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GENERAL INTRODUCTION

The study of natural products in general, and terpenoids in particular, has long been a subject of fascination for organic chemists. The wealth of information derived from studying the diverse structural types and biosynthetic pathways has played a significant role in the development of general chemical concepts. Structural elucidation, with its inherent tendency to diverge, has provided many interesting inroads into the chemistry of alicyclic compounds.

Although the biogenetic origin of the terpenoids has been subjected to intense scrutiny since last century [1], it was not until 1921 that Ruzicka formulated the Isoprene rule. The majority of natural terpenes can be built up, on paper, from "isoprene units", but exceptions were soon noted. It was the recognition that even these irregular structures can arise by rational rearrangements of regular polyisoprenes that provided the basis for the Biogenetic Isoprene Rule [2]. This rule rationalises the biosynthesis of the "abnormal" terpenoids as proceeding via mechanistically feasible rearrangements of the "regular" polyisoprenoids.

The recognition of acetic acid, in the form of acetyl co-enzyme A (1), as the fundamental biogenetic progenitor of all terpenoids is now well established. By a sequence of claisen-like condensations, acetyl co-enzyme A (1) gives rise to mevalonic acid (2) [3], the intermediate precursor of the isoprene unit (scheme 1). Subsequent condensations of the "active isoprenes" [4], isopentenyl pyrophosphate (3) and dimethylallyl pyrophosphate (4) from the corresponding pyrophosphates of geraniol (5), farnesol (6) and geranyl geraniol (7).

The acyclic precursor of the triterpenoids, squalene (8) has long been known to derive from two farnesyl units joined "head to head". The work of Cornforth, Popjak, and co-workers [5, 6, 7] established the

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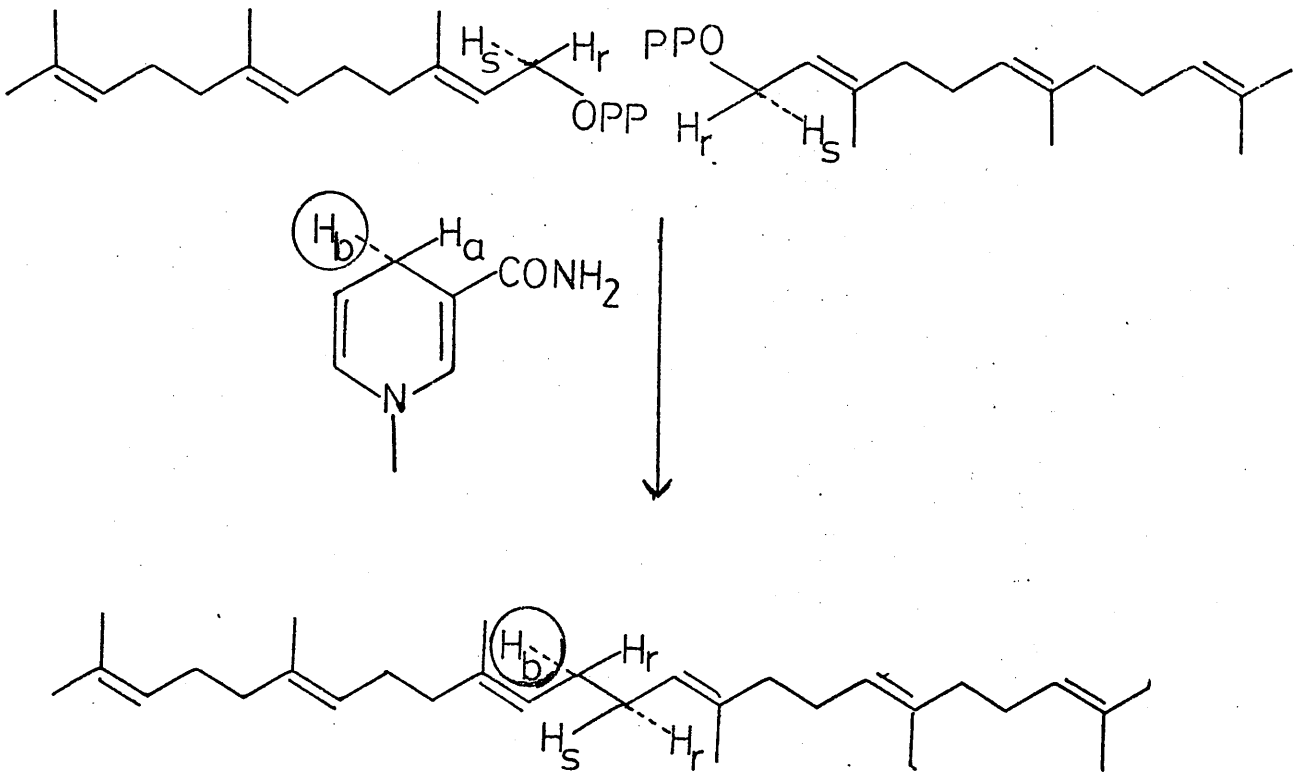

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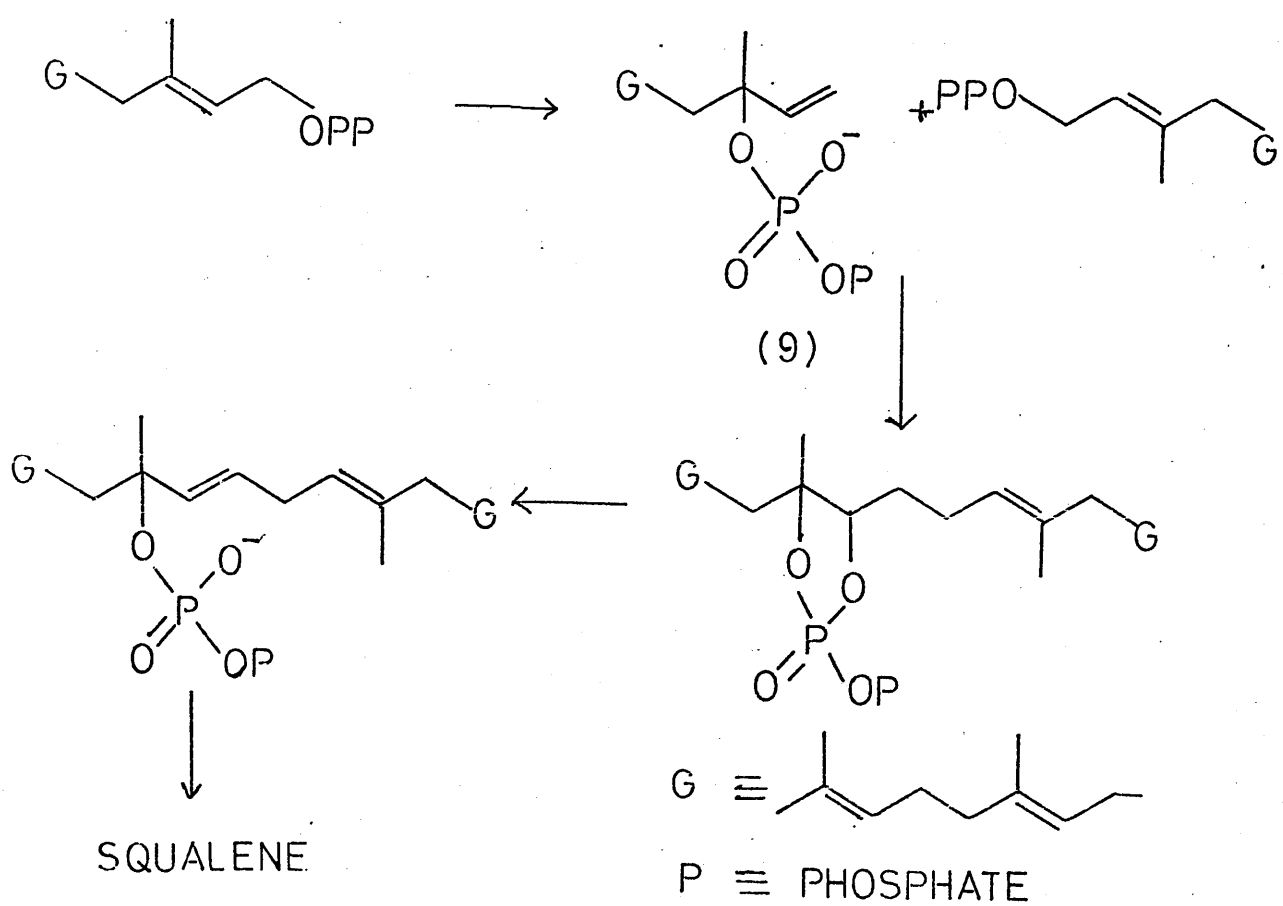

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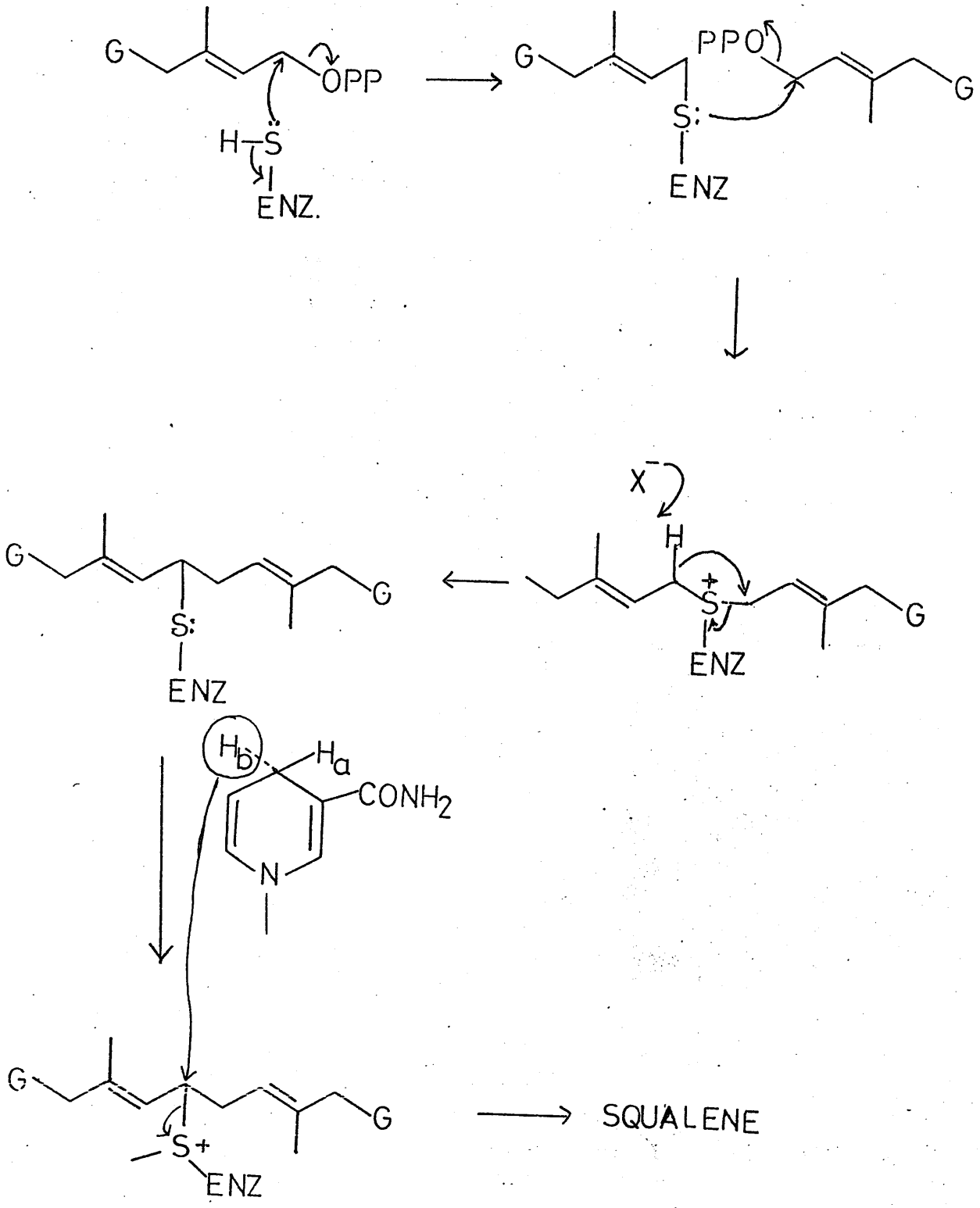
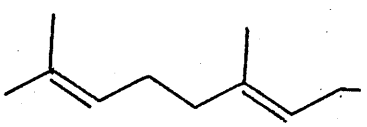


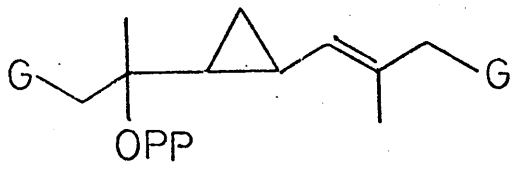
G ≡ 

P ≡ PHOSPHATE

stereochemistry of the processes by which mevalonic acid (2) is converted to squalene. In particular, the use of specifically deuteriated and tritiated farnesyl pyrophosphate revealed the stereochemical result of the linkage of farnesyl units by the enzymes of a rat-liver homogenate (scheme 2). The process produces an overall inversion of stereochemistry of the hydrogen atoms attached to the prochiral [8] carbon atom C-1 of one farnesyl residue and retention of stereochemistry of H_R attached to C-1 of the second farnesyl group while H_S is lost and stereospecifically replaced by a hydrogen atom (H_B) from the co-enzyme NADPH. The symmetrical molecule squalene (8) is therefore formed by an asymmetric process. The enzyme, trans - farnesyl pyrophosphate - squalene synthetase has been isolated and purified[9].

Two main mechanisms were proposed to account for the stereochemical features of the coupling process. The first (scheme 3) [10] involves the isomerisation of one farnesyl unit to a nerolidyl derivative with a terminal methylene group which is capable of nucleophilic attack on the second farnesyl moiety with inversion of stereochemistry. The pyrophosphate group can then be removed by reductive cleavage with hydride transfer from NADPH. This mechanism resembles that by which farnesyl pyrophosphate is formed from isopentenyl pyrophosphate and dimethylallyl pyrophosphate.

The second mechanism (scheme 4) involves displacement of the pyrophosphate group of one farnesyl unit by an enzymic sulphydryl group with inversion of stereochemistry [11]. Nucleophilic attack on the second farnesyl unit followed by rearrangement and reductive cleavage of the squalene - sulphur bond can furnish squalene. This type of mechanism has been demonstrated in a chemical synthesis of squalene from farnesol [12]. However, it has been shown that these mechanisms do not operate in rat and yeast enzyme systems.



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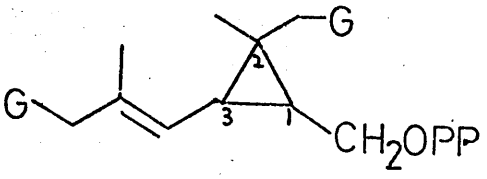

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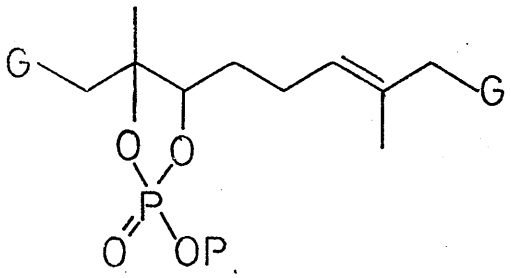

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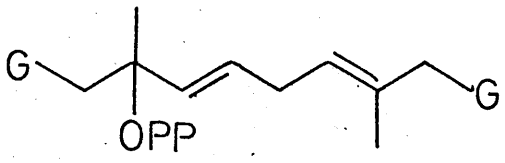

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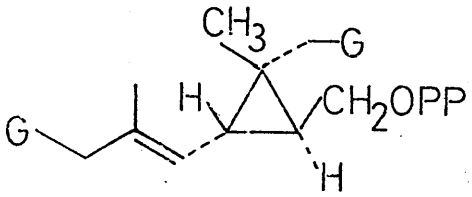

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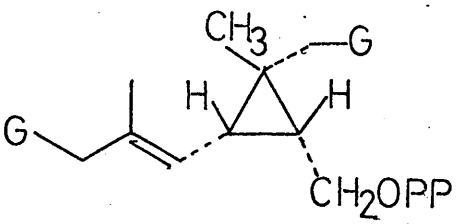

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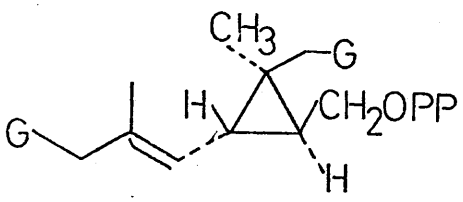

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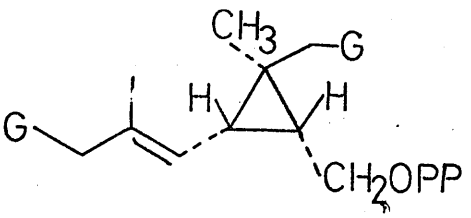

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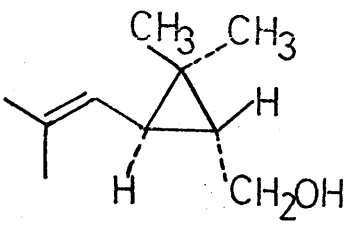

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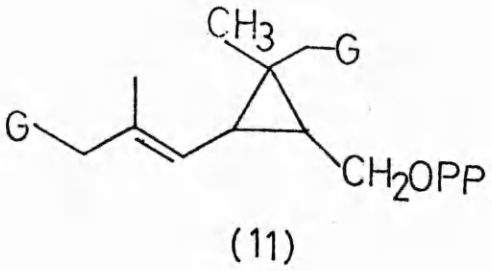
Contrary to the mechanism proposed in scheme (3) it has been demonstrated [13] that free nerolidyl pyrophosphate (9) is not an intermediate in the biosynthesis of squalene. However, in the absence of co-factor NADPH, a new intermediate, "presqualene pyrophosphate", is formed. This compound was first isolated by Rilling [14] and was shown to consist of two fifteen-carbon units and one pyrophosphate group. This intermediate retains only three of the four C-1 hydrogens of the initial farnesyl groups (as does squalene) and can be converted to squalene, in the same enzyme system, on addition of NADPH [14]. Four structures (10) to (13) have been proposed [14, 15, 16, 17] for presqualene pyrophosphate.

The structure (11), proposed by Rilling [15] has been confirmed by degradation [18] and by unambiguous synthesis [19-22]. Of the eight possible stereoisomers (14 - 17 and optical antipodes) only one is biologically active. This was assigned by Rilling [21], as the (1S, 2S, 3S) isomer (14) but recent results [23] have challenged this assignment. It was first demonstrated by Popjak et al [24] that presqualene pyrophosphate is optically active and gives a plain positive optical rotatory dispersion curve as far as 280 n.m., similar to that recorded for presqualene alcohol. Rilling et al [21] correlated the absolute configuration of presqualene alcohol and chrysanthemum alchol (18). The latter alcohol (18) was prepared from natural chrysanthemum monocarboxcylic acid, the absolute stereochemistry of which is known to be 1R, 3R [27]. The workers stated [21] that whereas both presqualene alcohol and chrysanthemum alcohol are dextrorotatory, presqualene alcohol gives a plain positive CD curve while chrysanthemum alcohol gives a similar but negative curve. They therefore concluded that the absolute configuration of presqualene alcohol was enantiomeric to that of chrysanthemum alcohol (i.e. absolute stereochemistry as in 14).

This comparison is, however, unsatisfactory in two respects. First, presqualene alcohol contains three asymmetric centres, while



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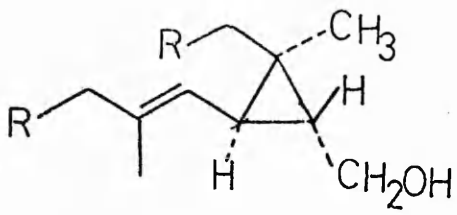

(19) R ≡ GERANYL

(21) R ≡ FARNESYL

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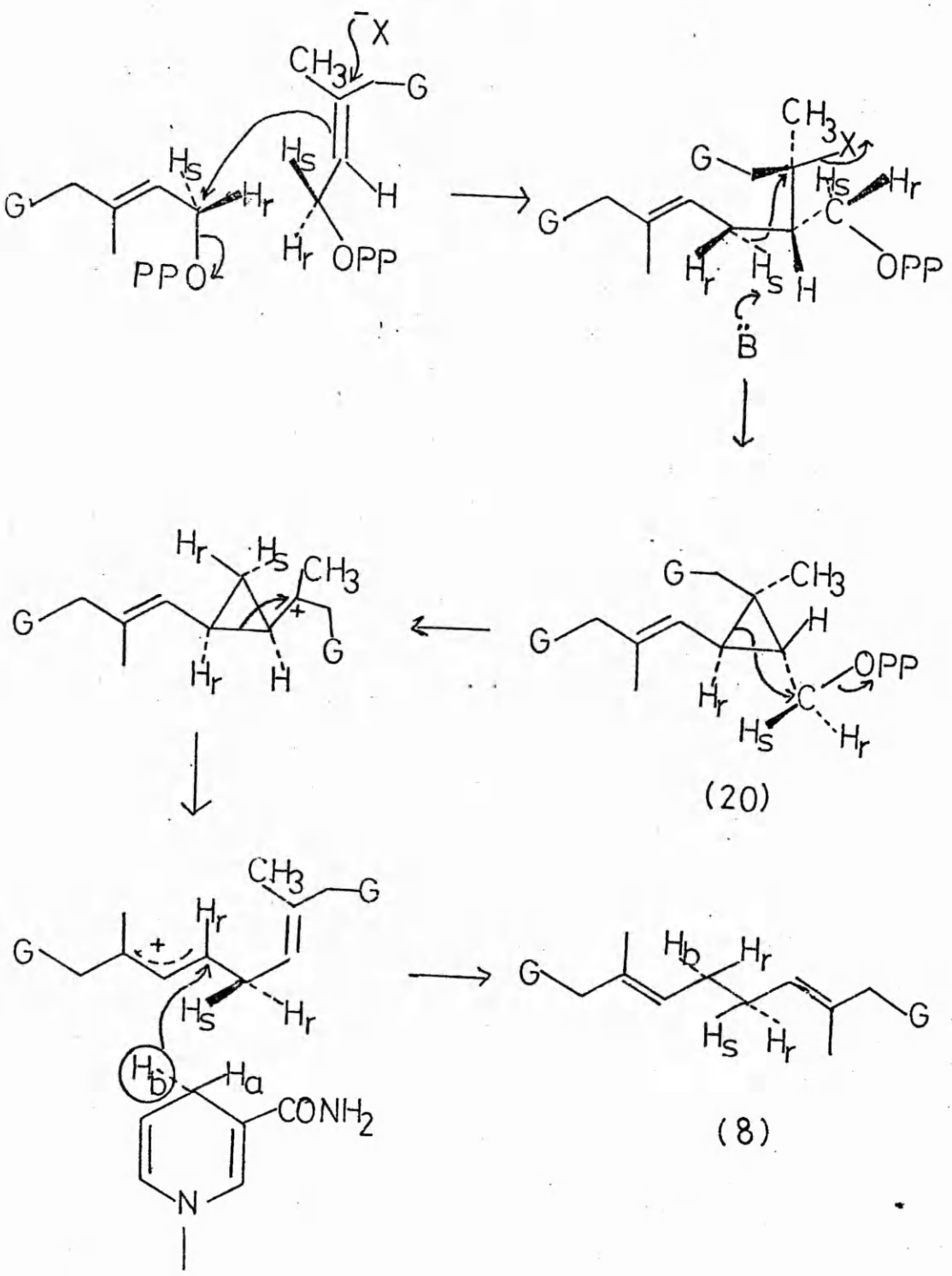

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(8)

trans - (R) - chrysanthemum alcohol contains only two. Second, the effect of the two large branched-chain olefinic substituents in presqualene alcohol on its optical properties is very difficult to predict. Another approach to this problem was, therefore, needed.

The absolute configuration of cyclic secondary alcohols and glycols has been successfully determined by Nakanishi and his colleagues [25] using the Cotton effect of the derived benzoates. It appears [26] that benzoates of asymmetric primary alcohols also exhibit the characteristic Cotton effect provided the optical centre is not too far removed from the hydroxy group. Popjak [23] used this method to make a comparison of the optical properties of the benzoates of presqualene alcohol and trans - (R) - chrysanthemum alcohol and their degradation products. These results imply that the absolute configuration of presqualene alcohol at positions 1 and 3 in (11) is the same as in the natural chrysanthemic acid, i.e. R, R [27]. Since the position of the methyl group on the cyclopropane ring was deduced to be syn to the carbinol carbon [18], it follows that the absolute configuration at the third asymmetric centre in presqualene alcohol is also R. The absolute stereochemistry of presqualene alcohol is therefore, (1R, 2R, 3R) (19). In view of these results scheme (5) is proposed to account for the stereochemistry in the conversion of farnesyl pyrophosphate, via (20) to squalene.

The C_{40} prenylogue of presqualene alcohol (19) prephytoene alcohol (21), has been synthesised [28]. Both natural and synthetic prephytoene alcohol were converted into carotenoids on incubation with Mycobacteria [29].

REFERENCES

1. G. Williams, Chem. News, 1860, 2, 206.
2. L. Ruzicka, Experentia, 1953, 9, 357.
3. K. Folkers, D.E. Wolf, C.H. Hofmann, P.E. Aldrick, H.R. Skeggs, and L.D. Wright, J. Amer. Chem. Soc., 1956, 78, 4499.
4. a) A.H. Phillips, T.T. Tchen, and K. Bloch, Fed. Proc., 1958, 17, 289 : b) F. Lynen, H. Eggerer, U. Heening, and I. Kessel, Angew. Chemie, 1958, 70, 738.
5. L.J. Mulheison, P.J. Ramm, Chem. Soc. Reviews, 1972, 1, 259.
6. R.B. Clayton, Quart. Rev., 1965, 19, 168 and references cited therein.
7. a) J.W. Cornforth and G. Popjak, Biochem. J., 1966, 101, 553 ; b) J.W. Cornforth, Quart.Rev., 1969, 23, 125.
8. K.R. Hanson, J. Amer. Chem. Soc., 1966, 88, 2731.
9. I. Schechter and K. Bloch, J. Biol. Chem., 1971, 246, 7690.
10. G. Popjak, De W.S. Goodman, J.W. Cornforth, R.H. Cornforth, and R. Ryhage, J. Biol. Chem., 1961, 236, 1934.
11. J.W. Cornforth, R.H. Cornforth, C. Donninger, and G. Popjak, Proc. Roy. Soc., 1966, B163, 492.
12. a) G.M. Blackburn, W.D. Ollis, C. Smith, and J.O. Sutherland, Chem. Comm., 1969, 99 ; b) J.E. Baldwin, R.E. Hackler, and D.P. Kelly, J. Amer. Chem. Soc., 1968, 90, 4758.
13. S.S. Sofer and H.C. Rilling, J. Lipid Res., 1969, 10, 183.
14. H.C. Rilling, J. Biol. Chem., 1966, 241, 3233.
15. W.W. Epstein and H.C. Rilling, J. Biol. Chem., 1970, 245, 4597.
16. G. Popjak, J. Edmond, K. Clifford and V. Williams, J. Biol. Chem., 1969, 244, 1897.
17. H. Wasner and F. Lynen, F.E.B.S. Letters, 1970, 12, 54.

18. J. Edmond, G. Popjak, S. Wong, and V.P. Williams, J. Biol. Chem., 1971, 246, 6254.

19. R.V.M. Campbell, L. Crombie, and G. Pattenden, Chem. Comm., 1971, 218.

20. L.J. Altman, R.C. Kowerski, and H.C. Rilling, J. Amer. Chem. Soc., 1971, 93, 1782.

21. H.C. Rilling, C.D. Poulter, W.W. Epstein, and B. Larsen, J. Amer. Chem. Soc., 1971, 93, 1783.

22. R.M. Coates and W.H. Robinson, J. Amer. Chem. Soc., 1971, 93, 1785.

23. G. Popjak, J. Edmond, and Siu-May Wong, J. Amer. Chem. Soc., 1973, 95, 2713.

24. G. Popjak, J. Edmond, K. Clifford, and V. Williams, J. Biol. Chem., 1969, 244, 1897.

25. N. Harada and K. Nakanishi, Accounts Chem. Res., 1972, 5, 257, and references cited therein.

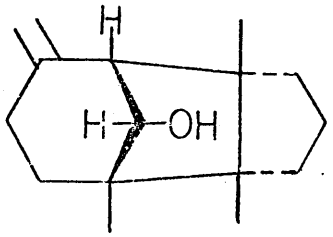
26. N. Harada and K. Nakanishi, J. Amer. Chem. Soc., 1968, 90, 7351.

27. L. Crombie and M. Elliot, Fortschr. Chem. Org. Naturst., 1961, 19, 120.

28. L. Crombie, D.A.R. Findley and D.A. Whiting, Chem Comm., 1972, 1045.

29. L.J. Altman, L. Ash, R.E. Kowerski, W.W. Epstein, B.R. Larsen, H.C. Rilling, F. Musico and D.E. Gregonis, J. Amer. Chem. Soc., 1972, 94, 3257.

SECTION 1



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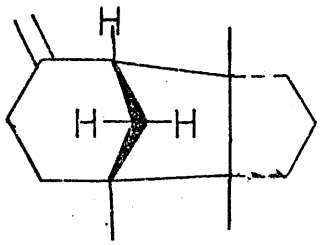

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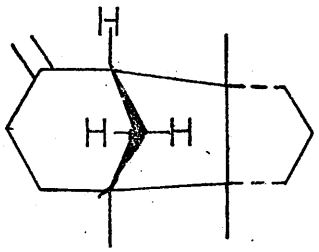

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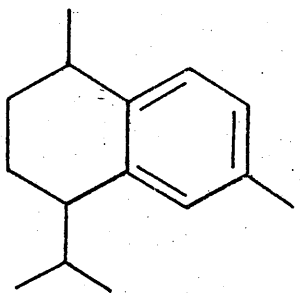

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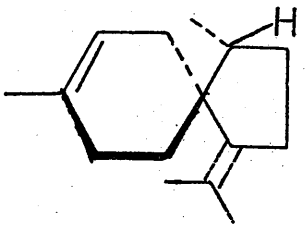

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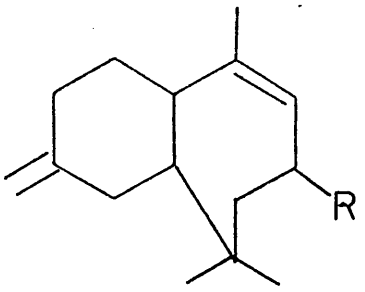
CONSTITUENTS OF GYMNOMITRION OBTUSUM

INTRODUCTION

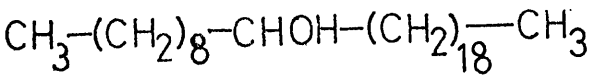
The liverworts (Hepaticae) are taxonomically placed between vascular plants and thallophytes. They are shade-loving plants and, in general, can tolerate complete darkness for a brief period of time without apparently affecting their enzyme activities [1]. Although the first chemical investigation of the essential oil from liverworts was made by Muller in 1905 [2], knowledge of the chemical composition of liverworts has remained rather poor until recent years. This was mainly due [3] to the difficulty in collecting a sufficient amount of the plant material and dissatisfaction with their botanical homogeneity.

Recently, however, systematic investigation of the chemical constituents of a number of liverwort species has resulted in the discovery of some interesting structures. Work in this laboratory on the constituents of Gymnomitrion obtusum (Lindb) Pears yielded a number of sesquiterpenoids with a novel tricyclic skeleton. The structural elucidation of these compounds is presented later. The publication [4] of the structures of gymnomitrol (1) and the parent hydrocarbon gymnomitrene (2) led to the rationalisation of much work which had been done in other laboratories. Thus Andersen et al [5] were able to resolve the problem of the structures of β - barbatene (≡ gymnomitrene) (2) and ∝ - barbatene (≡ isogymnomitrene) (3), isolated from a number of liverworts of the genus Barbilophozia. Other components of these liverworts include various alkanes, calamenene (4) and ∝ - alaskene (5) [6]. Screening of other liverwort species [5, 7] has shown that these hydrocarbons, (2) and (3), occur widely throughout the



(6) R ≡ H

(7) R ≡ OH



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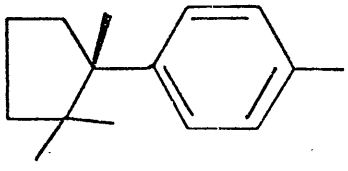

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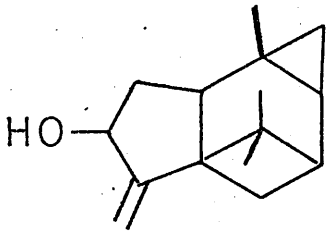

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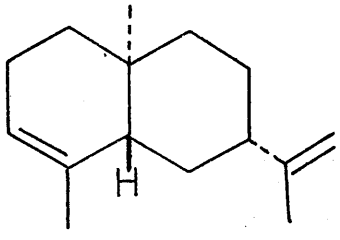

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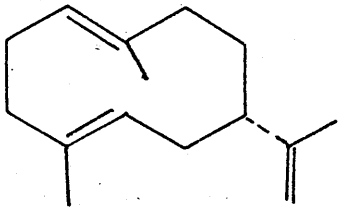

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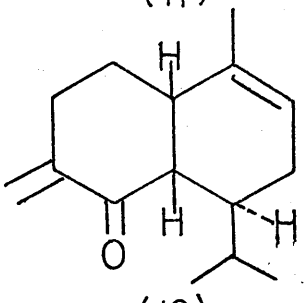

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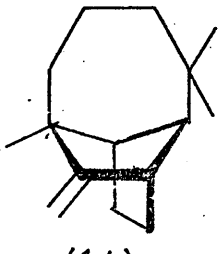

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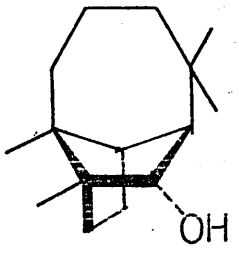

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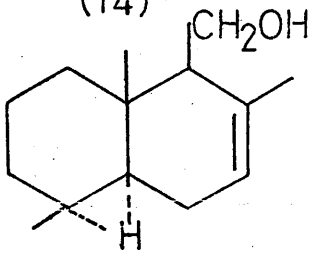

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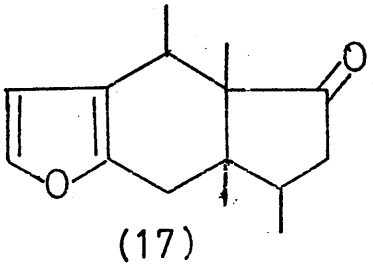


(17)

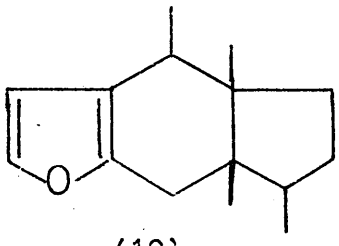
Hepaticae. Gymnomitrene (2) is by far the more prevalent.

Matsuo *et al* [8] have isolated bazzanene (6) and bazzanenol (7) from *Bazzania pompeana* (Lac.) Mitt. These two compounds represent a new skeletal type in the sesquiterpenoid field and their biogenesis may be formally considered to proceed *via* a bisabolene or related intermediate. In the course of the investigation of the methanol extract of this liverwort, a secondary alcohol, nonacosan - 10 - ol (8) and the benzenoid sesquiterpene hydrocarbon cuparene (9) were also found [9]. Bazzanene and gymnomitrene have also been isolated from *B. trilobata* [7].

The major component of the liverwort *Mylia taylorii* (Hook) Gray is the interesting tetracyclic alcohol, myliol (10). This liverwort also apparently contains sesquiterpene hydrocarbons which, as yet [11], have not been identified.

ent - α - Seliene (11), from *Chiloscyphus polyanthus* (L.) Corda [12] is the first enantiomeric selinene to be discovered in a plant source although (-) - germacrene - A (12) and some related selinenes have been isolated from marine invertebrates [13]. This is the third case of liverworts yielding previously unknown enantiomers of sesquiterpenes common in higher plants (*Frullania dilatata* produces an enantiomeric santonin - related lactone [14]; *Scapania* species produce (-) - longifolene and (-) - longiborneol [15]). *C. Polyanthus* also yielded [16] the novel sesquiterpenoid chiloscyphone (13). Matsuo *et al* [17] have found longifolene (14), longiborneol (15) and drimenol (16) in liverwort sources.

The main constituent of the extract of *Ancura pinguis* (L.) Dum. is pinguisone which on the basis of chemical degradation and n.m.r. spectroscopy is formulated [18] as (17). Although the constitution of pinguisone (17) merits is inclusion in the sesquiterpenoid class, it is not easy to envisage a rational biogenesis from farnesyl pyrophosphate.



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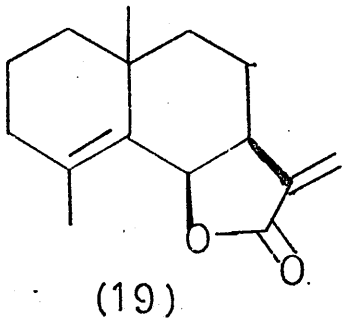

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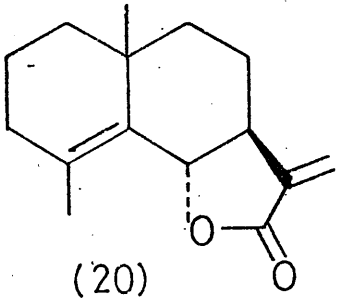

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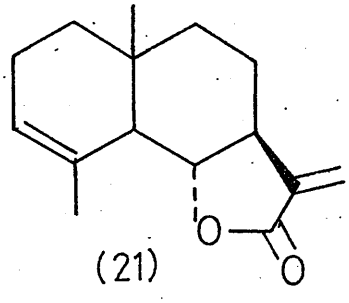

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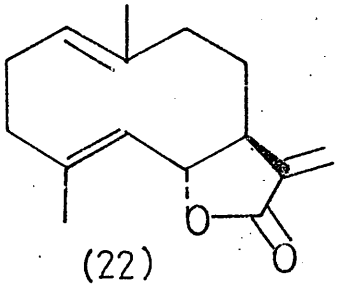

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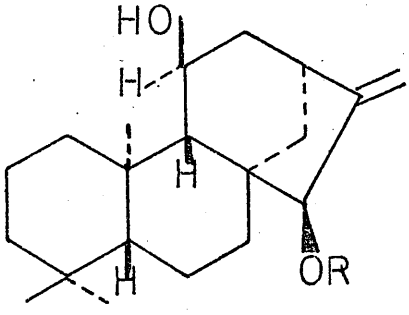
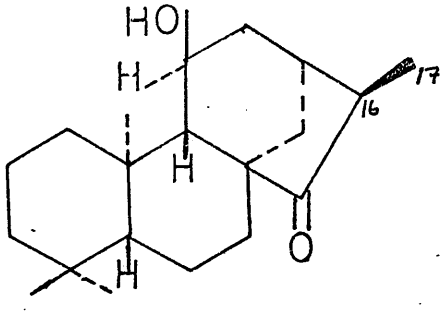


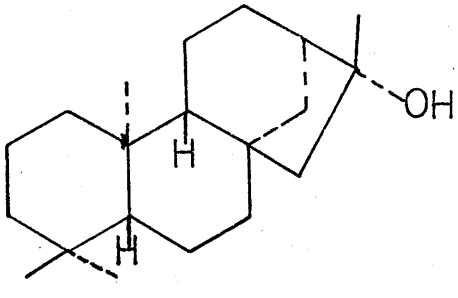
(23) R ≡ Ac

(24) R ≡ H



(25)

(26) 16,17-DIDEHYDRO



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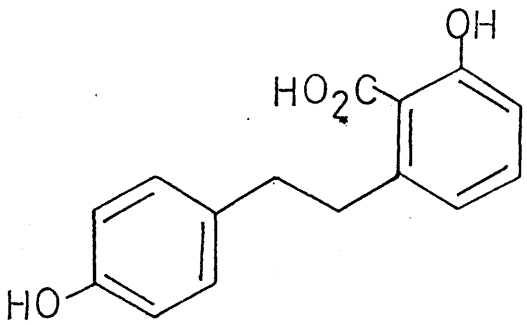

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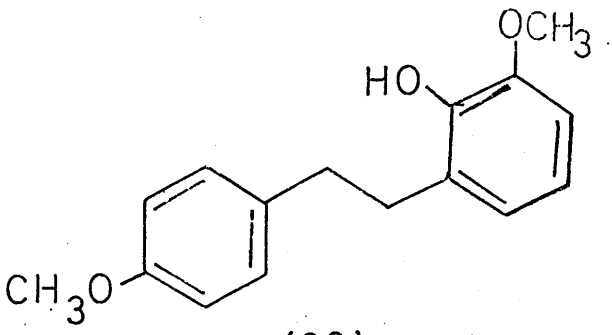

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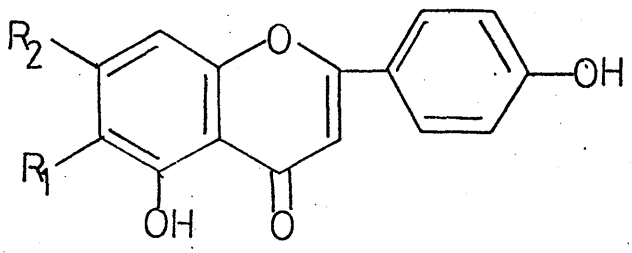
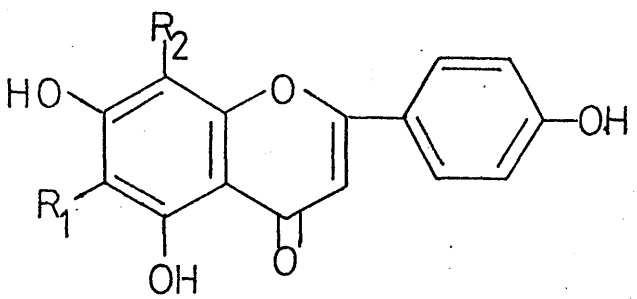


(30) $R_1 \equiv R_2 \equiv$ GLUCOSYL

(31) $R_1 \equiv$ GLUCOSYL , $R_2 \equiv$ OH



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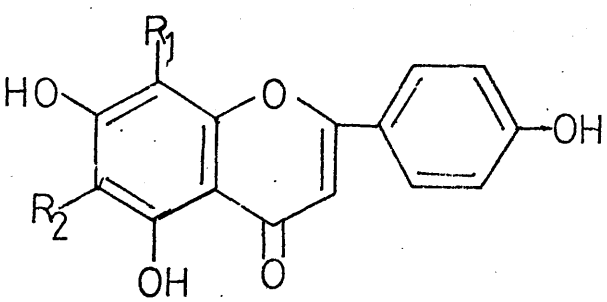
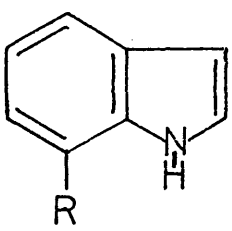


(33)

$R_1, R_2 \equiv$ SUGAR RESIDUES

$R_1 \neq R_2$



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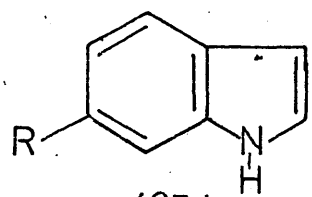


(35)

R ≡ $-CH_2CH=C(CH_3)_2$

Deoxopinguisone (18) was isolated [19] from the liverwort Ptilidium ciliare and its structure established by comparison with synthetic deoxopinguisone (18) prepared from authentic pinguisone (17). These authors [20] and Huneck et al [21] have reported on the gas.chromatographic analysis of sesquiterpene hydrocarbons from various species of liverworts.

The liverwort Frullania tamarisci yielded four isomeric sesquiterpenoid lactones, which were indentified [22] as the simple eudesmanolides (19), (20), (21), and costunolide (22). The allergenic activity of this liverwort is due to the eudesmanolide (19) [23]. Investigations of the liverwort Solenostoma triste afforded [24] four new 11 β - hydroxylated ent-kaurene derivatives (23-26). (-) - 16α - Hydroxykaurane (27) occurs [25] in the liverworts Anthelia julacea (L.) Dum and Anthelia jurantzkana (Limpr.) Trev.

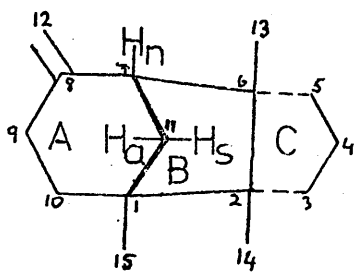
In addition to terpenoids other classes of compounds are found in liverworts. Nine fatty acid methyl esters were obtained [26] from the steam-volatile fractions of Pellia fabbroniana. In total, these methyl esters account for 75% of the steam-volatile substances with methyl palmitate (60%) the major constituent. Hydroxylated or methoxylated stilbenes exist predominantly in higher plants, mainly conifers [27]. However lunularic acid (28), the common endogenous growth inhibitor of Lunularia cruciata was detected in other species of liverwort [28]. The dihydrostilbene from Pellia epiphylla [29] (L.) Dum. has been formulated as (29).

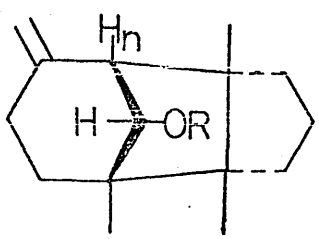
Some flavanoid compounds also occur in liverworts. The ethanol extract of Madotheca platyphylla (L.) Dum. contains [30] the flavanoid glycosides (30) and (31). Two interconvertible, isomeric 6, 8-di-C-glycosides of 5, 7, 4 [1] - trihydroxyflavone (32) and (33) were isolated [31] from the liverwort Hymenophytum flabellatum. The main components of the liverwort Ricardia sinuata (Hook.) Trev., are two isomeric indole derivatives (34) and (35) [32].

TABLE 1

Naturally Occurring Compounds of g. obtusum

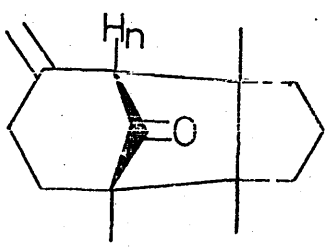
Compound	Structure	% Total Extract
gymnomitrol	(1)	3.6
gymnomitryl acetate	(36)	1.3
gymnomitrene	(2)	0.26
diacetate	(50)	0.36
epoxyacetate	(42)	0.25
epoxydiacetate	(49)	1.1
hydroxyacetate	(45)	0.25
hydroxydiacetate	(78)	0.9





(1) R ≡ H

(36) R ≡ Ac



(37)

DISCUSSION

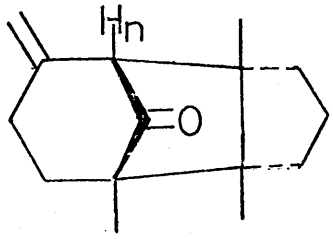
a) REACTIONS

The liverwort gymnomitrion obtusum (Lindb) Pears (Hepaticae) which was collected near the summits of Ben Lawers and Ben Ime proved to be a rich source of sesquiterpenoids with a novel tricyclic carbon skeleton. The eight new, naturally occurring compounds are summarised in table (1).

The major component of the extract is an alcohol, gymnomitrol (1), whose structure was elucidated in the following manner. Gymnomitrol, molecular formula $C_{15}H_{24}O$, has in its infra-red spectrum absorptions at 3625 (free hydroxy group), 1640 (C = C) and 890 cm^{-1} ($C=CH_2$). Thus it is tricyclic. The nuclear magnetic resonance spectrum shows three tertiary methyls, (δ0·93, 1·07, 1·21), one CHOH proton as a sharp singlet at δ3·70, and an exomethylene at δ4·63, 4·65 (sharp singlets). The one remaining feature of the spectrum is a singlet at δ2·32. This appears to be an allylic proton and double irradiation experiments with degassed solutions showed that it is coupled to the carbinol proton. There was no increase in integrated area. This proton, labelled H_n, proved to be very informative and its changes in chemical shift and intensity were followed as the functional groups were modified.

Acetylation of gymnomitrol gave the corresponding acetate (36) which also occurs naturally. Acetylation was slow at room temperature and required heating on a steam-bath before the reaction would go. This is in keeping with the hindered nature of the alcohol. The alcohol was regenerated by base hydrolysis.

Oxidation of gymnomitrol (1) afforded the ketone gymnomitrone (37), which has an i.r. carbonyl frequency at 1745 cm^{-1} (cyclopentanone). Hence the hydroxy group of gymnomitrol is in a five membered ring.



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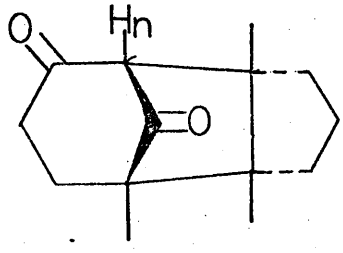

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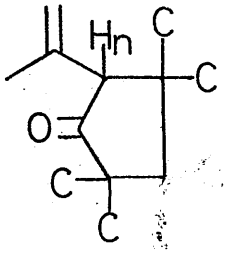
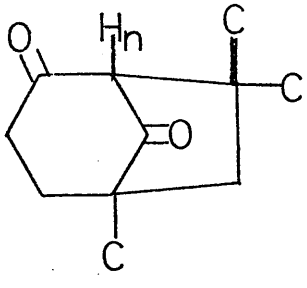
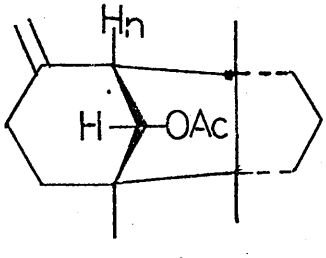


PART STRUCTURE A



PART STRUCTURE B



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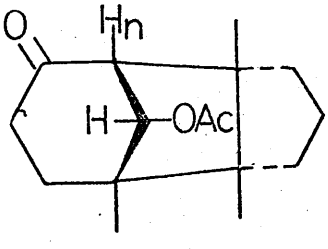

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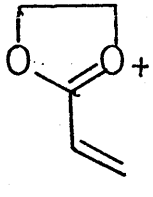


m/e 99

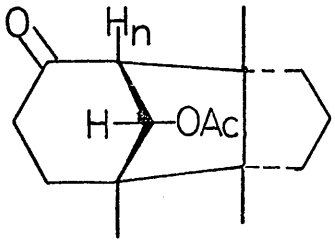
FRAGMENT C

H_n is deshielded (δ2·32 to 2·63) and sharper, confirming the loss of coupling to the original carbinol proton. Reduction of gymnomitrone (37) gave back only the original alcohol with no evidence of any epimeric alcohol. On the above evidence the part structure (A) can be built up.

The lack of coupling of H_n and the original carbinol proton requires substituents on the adjacent positions. The placing of the double bond at this point is dependent on the chemical shift of H_n (allylic). Further evidence was obtained as follows.

Ozonolysis of gymnomitrone (37) afforded a nor-diketone (38) which has i.r. frequencies at 1748 and 1710 cm^{-1}. H_n is further deshielded to δ3·00 thus supporting the suggestion that it should be placed as shown. It appears that (38) is, in fact, a β - diketone with one hydrogen (H_n) attached to the central carbon atom. However, the diketone (38) showed no signs of enolising in the u.v. even on addition of alkali. The obvious rationalisation of this is that the two carbonyl groups form part of a bridged bicyclic system. The most obvious choice is a bicyclo (3, 2, 1) octane system as in part structure (B). The i.r. is in agreement with this but can also fit a seven membered ring or higher. However, the fifteen carbons of the sesquiterpenoid can be accommodated on the basis of the bicyclo-octane system. At this point a start was made in assigning stereochemistry. The dihedral angle between the carbinol proton and H_n when the hydroxy group is over the five membered ring is almost 90° and therefore explains the low coupling constant. In the epimeric alcohol a J value of approximately $4H_Z$ would be expected [33] (see p. 32).

Evidence for the six-membered portion of the bicyclo system was obtained in several ways. First, ozonolysis of gymnomitryl acetate (36) gave the nor-ketoacetate (39) whose ethylene ketal has a base peak in the mass spectrum at m/e 99 corresponding to the fragment (C) [34].



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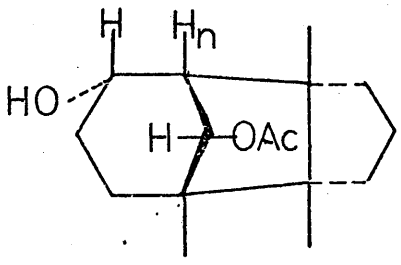

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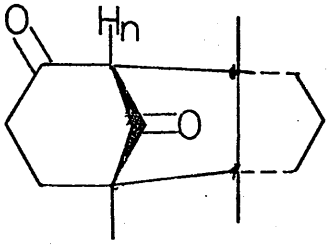

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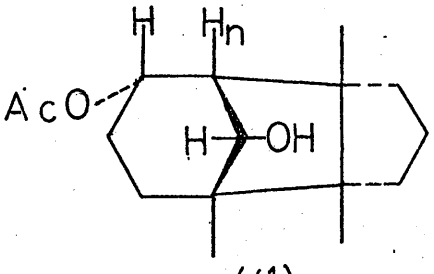

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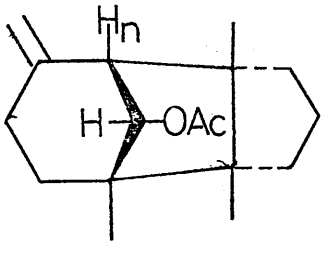

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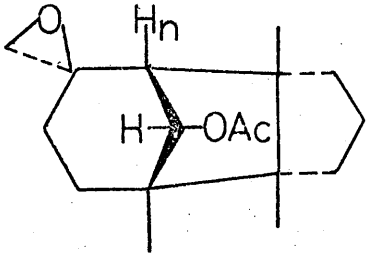

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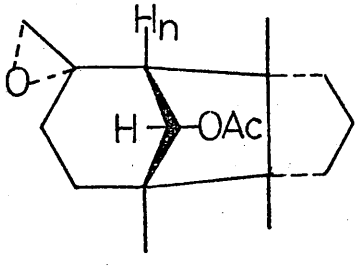

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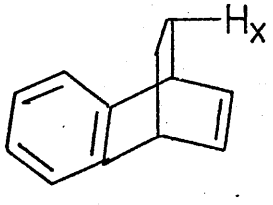

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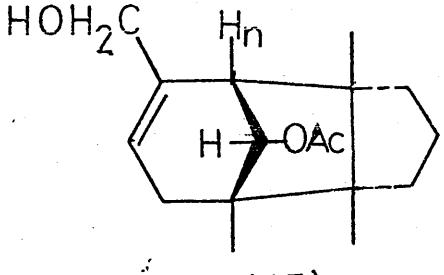
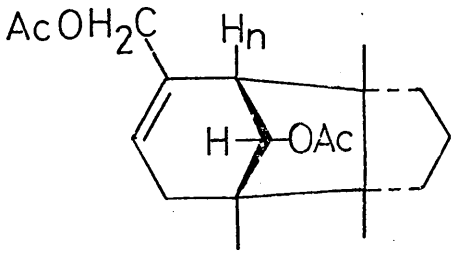


(45)

Reduction of the nor-ketoacetate (39) with sodium borohydride resulted in the formation of only one alcohol (40) - the endo isomer. The new carbinol proton appears as a multiplet in the n.m.r. consistent with the presence of three neighbouring hydrogens. H_n is not visible in the normal spectrum but on addition of Eu $(dpm)_3$ it moves downfield as a doublet (J 3 H_Z). Decoupling confirmed the assignment. This puts beyond doubt the allylic and bridgehead nature of H_n. An additional feature of the shifted spectrum is the relatively small shifts of the methyl groups. In the Eu $(dpm)_3$ spectrum of the isomeric hydroxyacetate (41) much larger shifts of the methyl groups are observed. This compound (41) was obtained by borohydride reduction of the nor-diketone (38) followed by selective acetylation. The Eu $(dpm)_3$ results will be discussed in more detail later.

Secondly, one of the other naturally occurring compounds is epoxygymnomitryl acetate (42) which was readily prepared from gymnomitryl acetate (36) with m-chloroperbenzoic acid. The major product of this reaction is identical with the natural epoxygymnomitryl acetate and arises from the expected exo attack of the peracid. The minor product is the α-isomer (43) which has the CHOAc proton as a slightly broadened singlet at δ4·87. The CHOAc signal of the β-epoxide (42) appears at δ5·26 as a slightly broadened singlet. This deshielding in the β-isomer presumably arises from the proximity of the CHOAc proton to the ether oxygen. A similar effect has been reported [35] for H_x in the endo and exo-epoxides of benzobicyclo [2, 2, 2] octene (44). These observations support the previous assignment of the stereochemistry of the bridge hydroxy group in gymnomitrol. Both epoxides (42) and (43) rearrange in acid to the allylic primary alcohol (45) whose n.m.r. has the expected signals, δ4·00 (broad singlet, 2H, $W_{\frac{1}{2}}$ 6·5 H_z, C= C-CH_2OH), 5·44 (Broad



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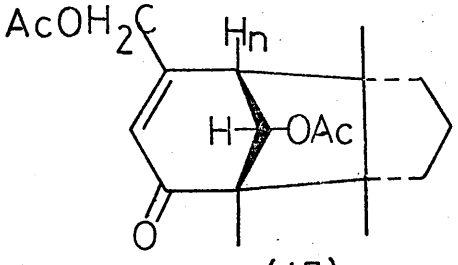

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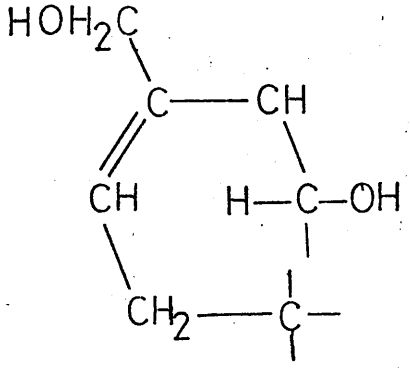
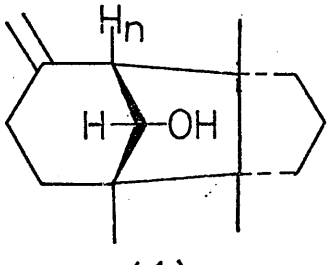


PART STRUCTURE D



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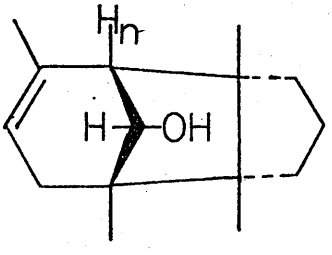

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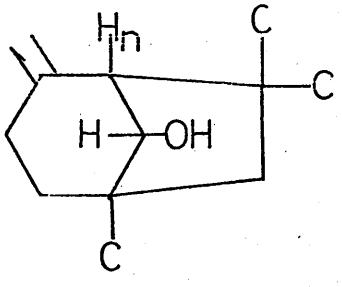
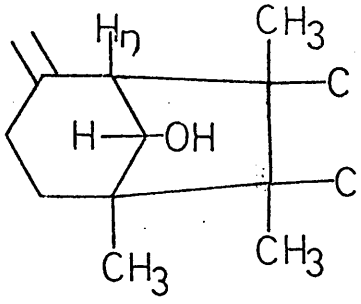


PART STRUCTURE E

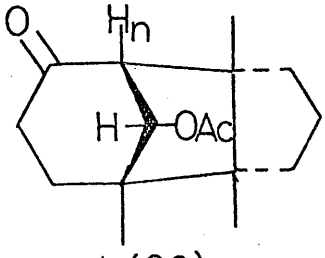
singlet, $W_{\frac{1}{2}}$ 7 H_z, C = C<u>H</u>). The corresponding diacetate (46) was oxidised by sodium chromate to the enone (47), λmax 240 n.m. (ϵ10,000). The n.m.r. of the enone has a remarkable lack of protons below δ1·7 (apart from <u>H_n</u> at δ2·45) indicating the fully substituted nature of the other position <u>α</u> to the carbonyl group and confirms the part structure (D). One of the methyl groups in the diacetate (46) is deshielded to δ1·25 on formation of the enone (47).

Thirdly, the same information is available from gymnomitrol (1) itself. On treatment with acid, either dry HCl gas in chloroform or dilute sulphuric acid in methanol, gymnomitrol (1) was transformed into isogymnomitrol (48). This transformation was also carried out by hydrogenation of gymnomitrol (1) at atmospheric pressure over palladium-charcoal catlyst. The n.m.r. spectrum of isogymnomitrol (48) clearly shows the presence of a vinyl methyl group, δ1·62, and a vinyl proton, δ5·05. Irradiation at the vinyl methyl frequency leaves the vinyl proton as a clean triplet (J 3 H_z) and simultaneously reveals the allylic methylene as a double AB system. Thus the next carbon atom to the double bond is fully substituted. The part structure (E) can now be constructed for gymnomitrol and it remains to place the three tertiary methyls and one additional ring.

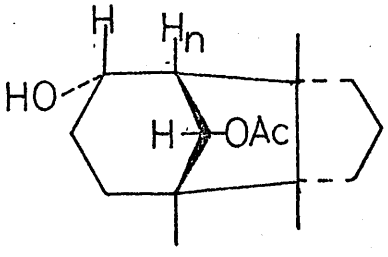
The Eu $(dpm)_3$ shifted spectrum of gymnomitrol (1) provides useful information. In fact it was a major tool in arriving at the final structure. Relative to a carbinol proton shift of approximately 4 p.p.m., <u>H_n</u> moves downfield by approximately 2·5 p.p.m., and all three methyl groups move down by approximately the same amount ($\sim$2 p.p.m.). The fact that all three methyls show nearly the same downfield shift suggest empirically that they are all nearly the same distance from the oxygen function and close to it. These requirements are best met by placing the methyls as



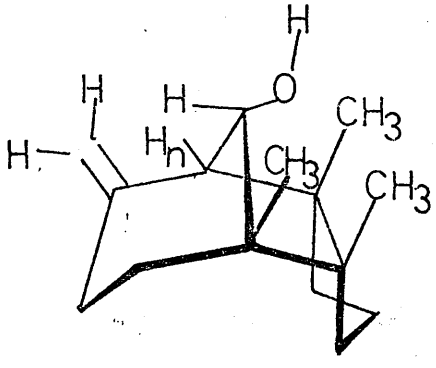
PART STRUCTURE F



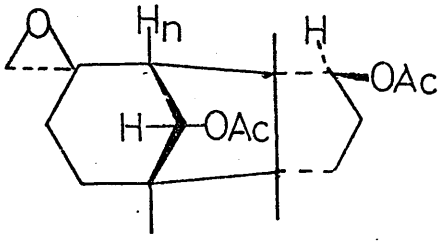
(39)



(40)



(1)



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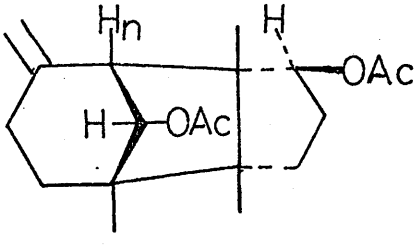

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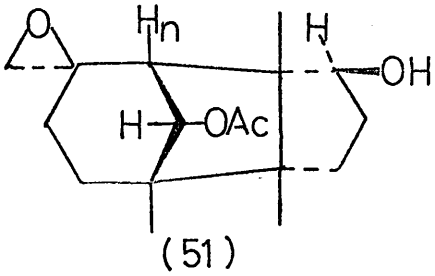

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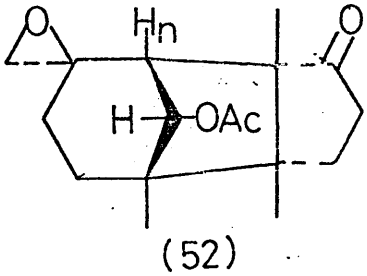
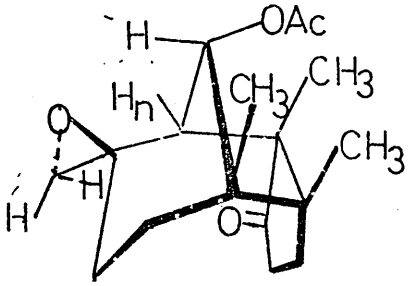


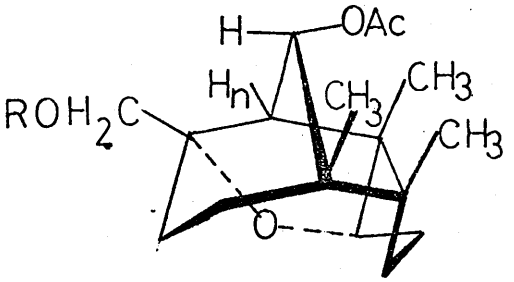
(52)

shown in part structure (F). The possibility of a gem-dimethyl group is excluded on this basis. This structure also accommodates the Eu $(dpm)_3$ shifts observed in the alcohol (40) derived from the nor-ketoacetate (39) (see later). The hindered nature of the hydroxy group in gymnomitrol (1) is satisfactorily accounted for by the close proximity (~3 Å) of the centre of rotation of the three tertiary methyl groups. Having arrived at part structure (F), we had no alternative but to complete the structure by making a second five membered ring. Thus gymnomitrol has the structure (1) with a new tricyclic skeleton. Confirmatory evidence for the second five membered ring was obtained in the following way.

Some minor components isolated from the extract contain an oxygen substituent in ring C-, namely the epoxy-diacetate (49) and the exomethylene diacetate (50). The latter was converted into the epoxydiacetate (49) on treatment with m-chloroper benzoic acid. Selective hydrolysis of the "new" acetate in the epoxydiacetate (49) afforded the hydroxy-epoxyacetate (51). This hydrolysis is possible because of the hindered nature of the bridge acetate. Collin's oxidation[36] of (51) yielded a compound (52) whose i.r. exhibits a broad carbonyl absorption at 1735 cm^{-1} with shoulders at 1745 and 1755 cm^{-1}. This excludes a cyclohexanone and therefore ring C is a cyclopentanone. In the hydroxyacetate (51) the carbinol proton is a quartet (J 6, 11 H_Z) and is placed β on the basis of Eu $(dpm)_3$ shifts. Relative to a shift of 6 p.p.m. of the carbinol proton, one methyl group shifts by 2·7 p.p.m. whereas the other two move by only 1·50 and 0·7 p.p.m. respectively. H_n moves downfield by approximately 2 p.p.m. One half of the epoxide AB system moves down by 0·7 p.p.m. while the other actually moves upfield by 0·15 p.p.m. The angle term in the lanthanide shift expression [37] is obviously the controlling factor. These results are satisfactorily accounted for by placing the hydroxy group as shown. A change in position or stereochemistry would lead to different

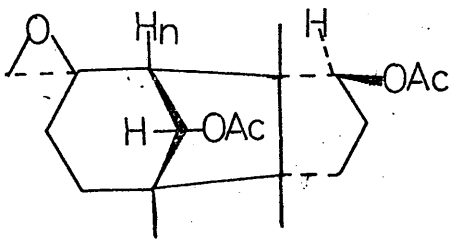


(52)



(53) R ≡ H

(54) R ≡ Ac



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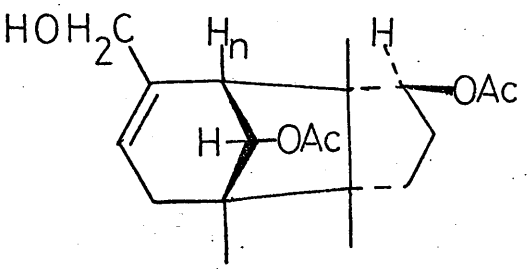

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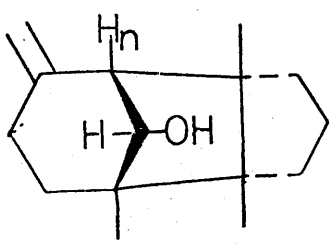

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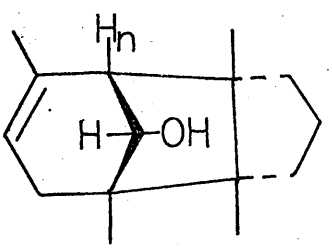

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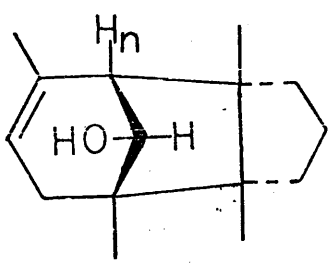

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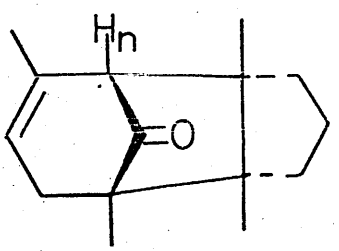


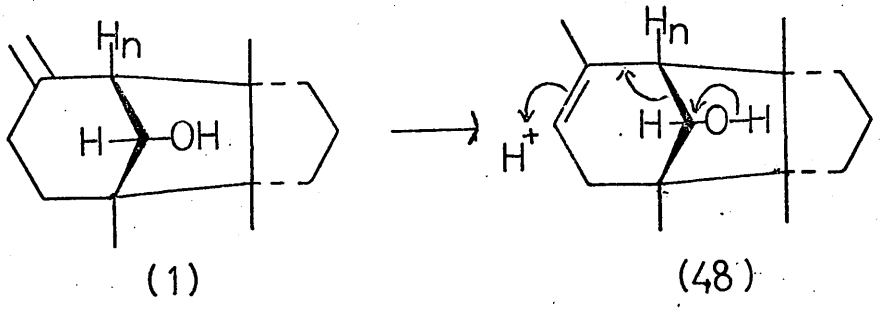
(56)

shifts (see later).

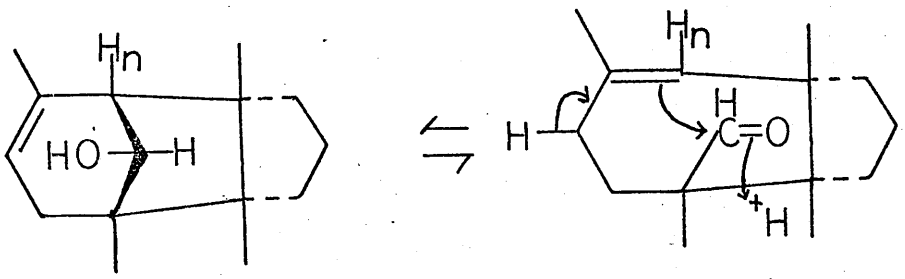
Proof of the stereochemistry of the hydroxy group in (51) was expected to come from the preparation of the epimeric alcohol by sodium borohydride reduction of the cyclopentanone (52). Reduction should occur from the exo-face. In the event reduction was slow and required a large excess of borohydride. The product was not the expected alcohol. The n.m.r. of the product shows a primary hydroxy group at δ3·41 (broad singlet) unsplit by neighbouring protons, loss of the epoxide and a new -CH-O- proton at δ4·08 as a triplet (J 3 H_Z). This compound readily formed an acetate (δ3·86, 4·06, AB quartet (J_{AB} 11 H_Z), CH_2OAc), with no hydroxyl absorption in the i.r. These data suggest that the reduction product is the ether (53) (acetate 54). It probably arises by intramolecular opening of the epoxide by the newly formed endo- hydroxy group. The formation of the ether provides valuable confirmatory evidence for the structure of the epoxy-diacetate (49) and indeed for gymnomitrol (1) itself. Treatment of (49) with dilute acid yielded the hydroxydiacetate (78) identical in all respects with natural material.

Another interesting reaction in this series is the isomerisation of gymnomitrol (1) under the influence of HCl gas in chloroform. In addition to isogymnomitrol (48) which has already been described a second product was formed. This second product (55) is isomeric and in its n.m.r. has a vinyl proton, δ5·47 (b.s., $W_{\frac{1}{2}}$ 8 H_Z), a vinyl methyl, δ1·67 (t., J 2 H_Z), a carbinol proton δ3·86 (d., J 4·2 H_Z), and a doublet (J 4·2 H_Z, H_n) at δ1·94. Double irradiation clearly established the relationship between H_n and the carbinol proton and indicates that this compound is the epimeric bridge alcohol (55). The magnitude of the coupling constant is in agreement withthe new dihedral angle between H_n and the carbinol proton. Oxidation of the alcohol (55) gave the ketone (56), isogymnomitrone, which

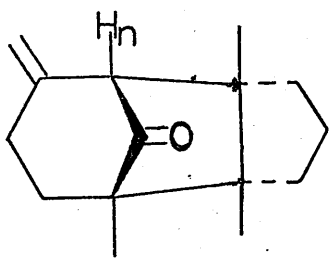
SCHEME 1



(1) (48)



(55) (58)



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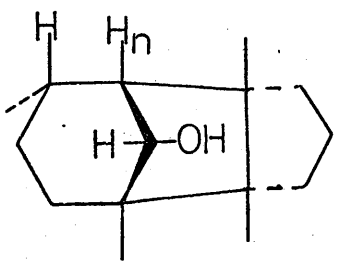

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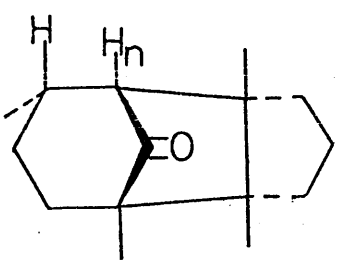

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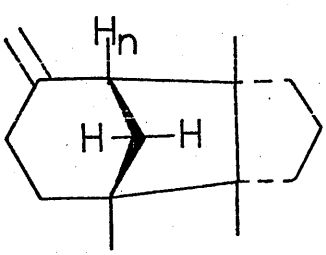


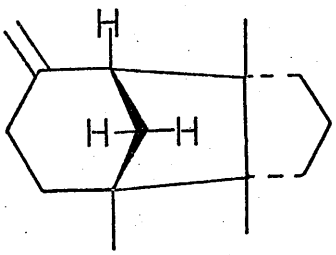
(2)

is identical with the ketone prepared by Jones' oxidation of isogymnomitrol (48). This ketone could also be prepared by treatment of gymnomitrone (37) with HCl gas in chloroform.

Since dihydrogymnomitrol (57) was stable to the above reaction conditions it appears that the double bond in gymnomitrol (1) is involved in the epimerisation. The yield of epimeric alcohol (55) from either gymnomitrol (1) or isogymnomitrol (48) increased with the length of the reaction time (equilibration?). A plausible mechanism for the epimerisation could involve a type of retro-Prins process (see scheme 1). A postulated intermediate is the aldehyde (58) but attempts to trap this compound as its thioacetal failed. In principle aldehyde (58) can cyclise to give both epimeric alcohols (48) and (55). The epimeric alcohol (55), however, is obviously the thermodynamically more stable alcohol since it lacks the severe steric interactions between the hydroxy group and the tertiary methyls which destabilises gymnomitrol (1) and isogymnomitrol (48). It is not surprising therefore that the equilibration strongly favours the epimeric alcohol (55).

Hydrogenation of gymnomitrol(1) at room temperature over Adam's catalyst gave the saturated alcohol, dihydrogymnomitrol (57) whilst hydrogenation using palladium - charcoal as catalyst yielded isogymnomitrol (48) as the sole product. The hydrogenation is stereo-specific and results in the new secondary methyl having the α-configuration. Jones' oxidation of dihydrogymnomitrol (57) gave dihydrogymnomitrone (59) in good yield. The circular dichroism spectrum of this compound proved to be useful in determining absolute configuration (see p. 50).

Some difficulty was encountered in the attempt to convert gymnomitrol (1) or gymnomitrone (37) into the parent hydrocarbon gymnomitrene (2). This interconversion was made all the more necessary



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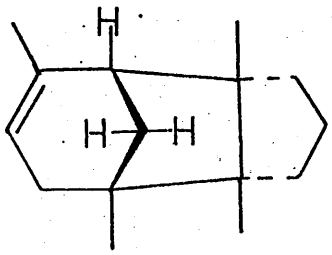

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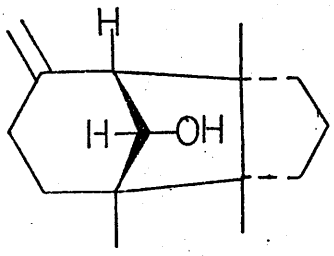

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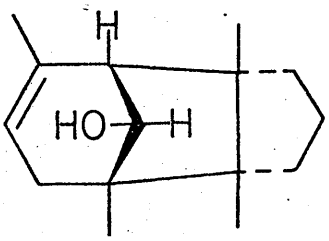

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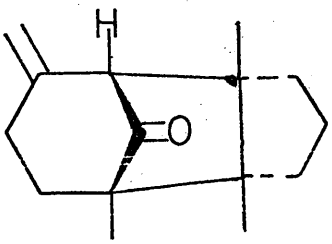

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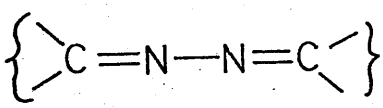


GYMNOMITRONE AZINE

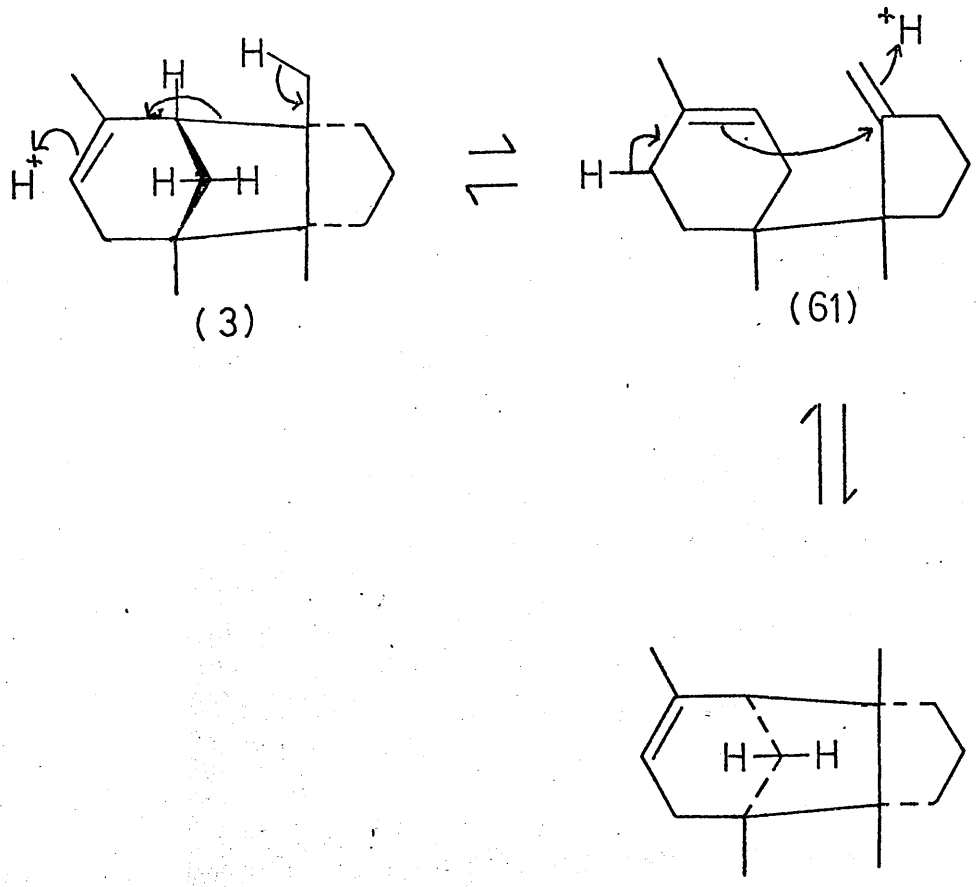
by the report [5], which followed our preliminary communication [4], that hydrocarbons with the same spectroscopic characteristics as gymnomitrene (2) and isogymnomitrene (3) had been isolated from at least twelve different species of liverwort. Isogymnomitrene (3) was not detected in the extract of gymnomitrion obtusum but was easily prepared by acid treatment of gymnomitrene (2).

Gymnomitrol (1) failed to react with p-toluene sulphonyl chloride in dry pyridine even under forcing conditions. This is not surprising in view of the hindered nature of the hydroxy group. This restriction does not apply to the epimeric alcohol (55) which readily formed a tosylate at room temperature overnight. Subsequent treatment of the tosylate with lithium aluminium hydride in refluxing tetrahydrofuran gave only the alcohol (55). There was no sign of hydrocarbon in the reaction product. The complete dominance of the S - O cleavage reaction over the C - O cleavage can also be explained by the steric hindrance caused by the tertiary methyl groups. Any attempt by solvated hydride ion to attack from the side opposite to the leaving tosylate group is made extremely difficult.

The Wolff-Kishner reduction of the ketone (37) was carried out under a variety of conditions but no recognisable hydrocarbon product was obtained. A strange product was isolated on several occasions, $C_{30}H_{44}N_2$, 0·94, 0·99, 1·10 (Tertiary methyls), 1·82, 2·02 (methyls?), 3·24 (s, C$\underline{H}$O-), 4·68,(t, J 3 H_Z, C=C$\underline{H}_2$). This product decomposed on standing and may be gymnomitrone azine.

Success was eventually achieved by Clemmensen reduction of gymnomitrone (37) which gave isogymnomitrene (3) identical with an authentic sample prepared by acid isomerisation of gymnomitrene (2). However, another isomeric hydrocarbon, whose structure remains a mystery,

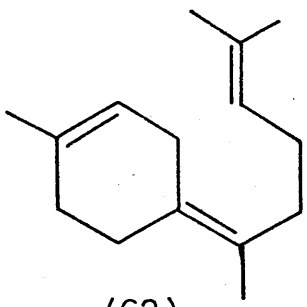
SCHEME 2



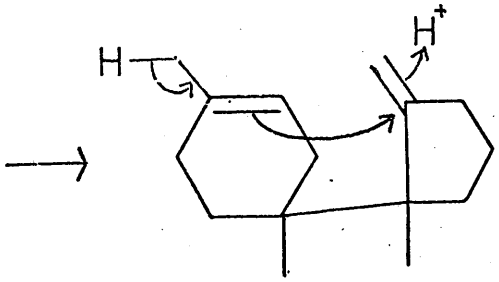
(3) (61) (60)

was formed in this reaction $C_{15}H_{24}$, δ0·82, 0·99, 1·10 (tertiary methyls) 1·64 (C=C-CH$_3$), 5·01 (d, J 2·5 H$_Z$, C=CH). This hydrocarbon and isogymnomitrene (3) are only separable by g.l.c. on 2½% SE-30 capillary column using a linear temperature programme of 100 - 220° at 2° per minute or by preparative t.l.c. using silica impregnated with 30% silver nitrate and petrol as solvent. The mass spectra of the new hydrocarbon and isogymnomitrene (3) are similar and suggests that these two hydrocarbons bear a close structural resemblence. The formation of one possible structure (60) is shown in scheme (2).

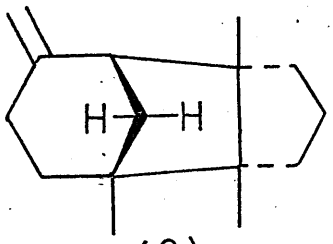
SCHEME 3



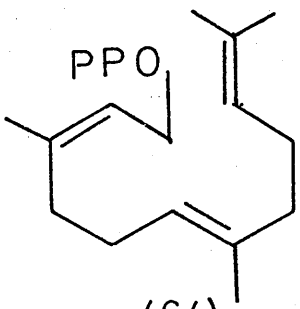
(62)



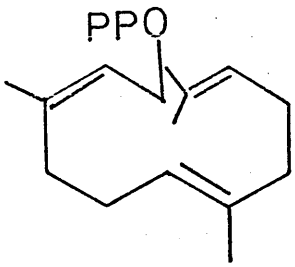
(61)



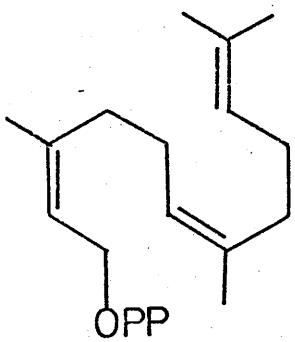
(2)



(64)

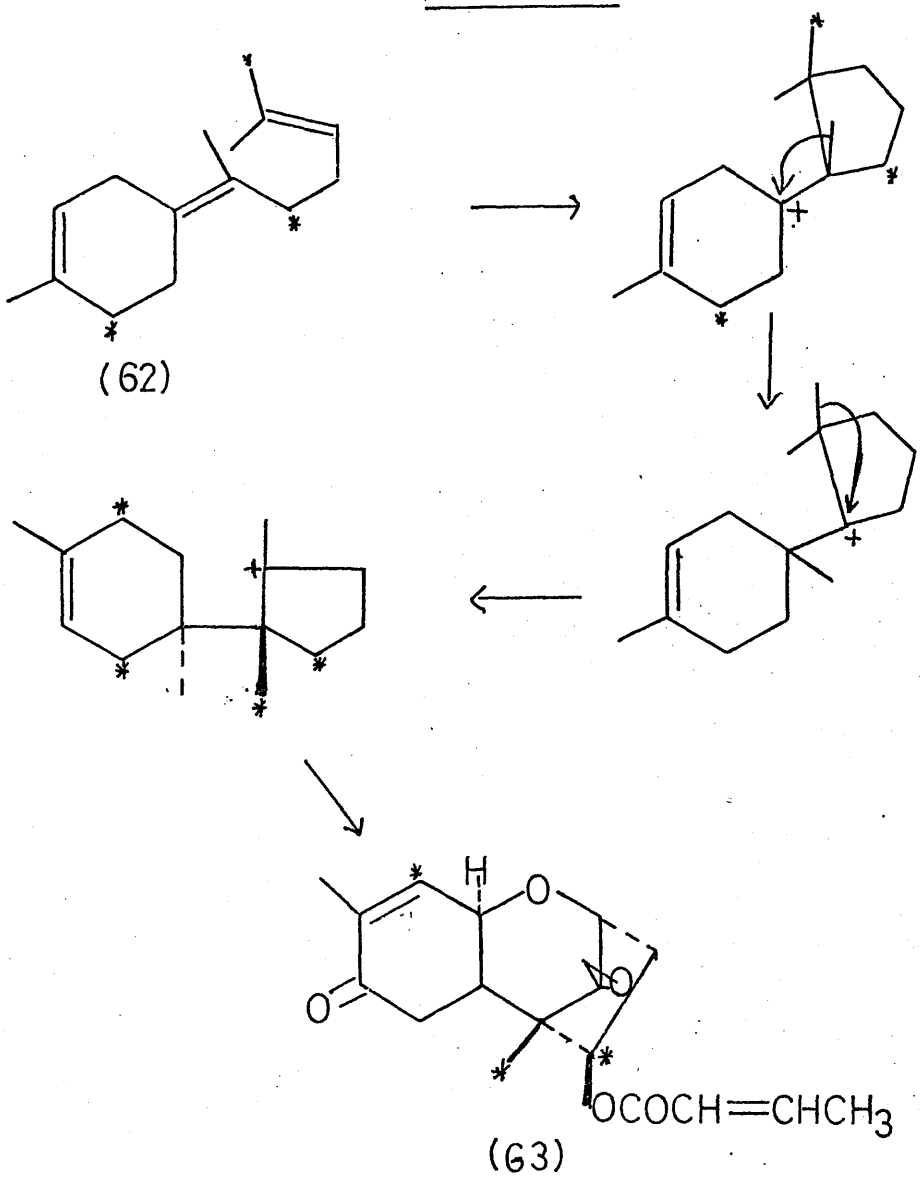


(65)

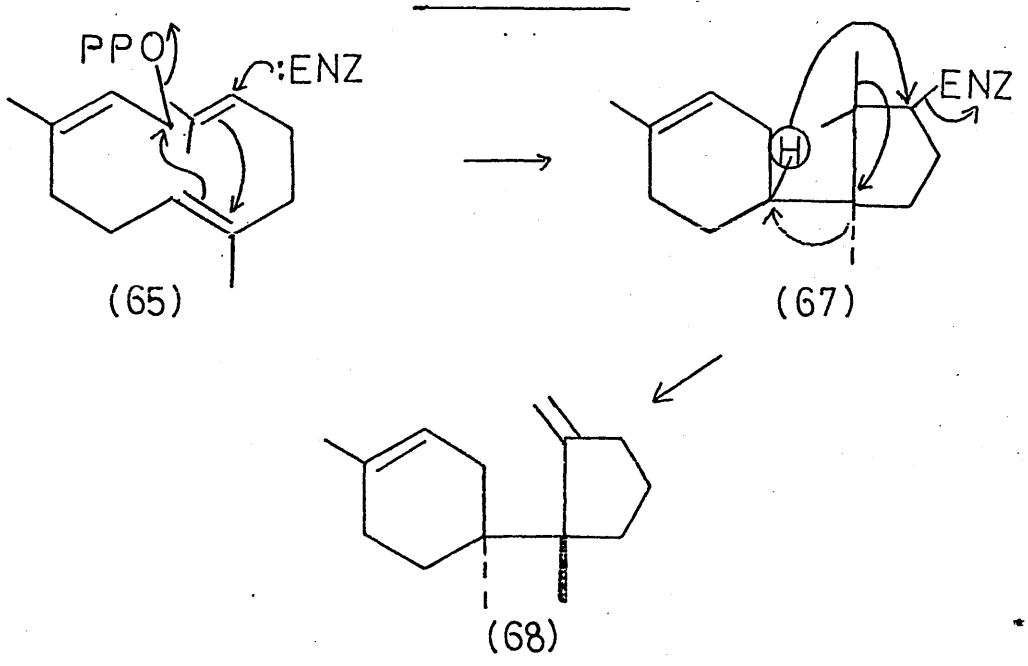


(66)

SCHEME 4



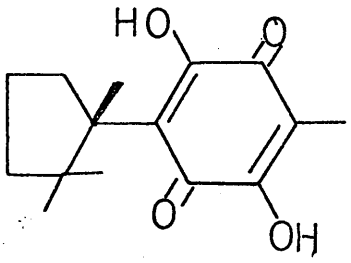
SCHEME 5



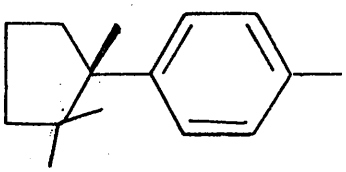
b) BIOGENESIS

A plausible biogenetic derivation for the new gymnomitrene skeleton (2) can be drawn from γ-bisabolene (62) via the trichodiene intermediate (61) as shown in scheme (3). This biogenesis is closely related to that of the trichothecane antibiotics, which has recently been the subject of considerable investigations. Results obtained by various research groups have shown that the complex structures (cf. 63) of the group are derived from three molecules of mevalonic acid [38, 39, 40, 41] and that farnesyl pyrophosphate (64) [39, 42] is an intermediate in this process. An early postulate [43, 44] to account for the stereochemistry and labelling pattern found in trichothecin (63) involves a boat-type folding of the side chain in γ-bisabolene (62) followed by two 1, 2 -- methyl migrations (see scheme 4). The tracer experiments conducted by Jones and Lowe [38] using (1 - C) - acetate and (2 - C) - mevalonate conclusively demonstrated that the 1, 2 methyl shifts do occur.

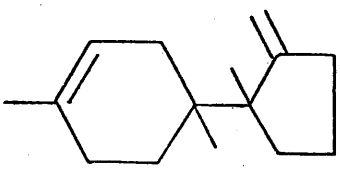
By elegant use of doubly labelled precursors, Hanson et al [45] have clearly shown that γ-bisabolene (62) is not an intermediate in the biosynthesis of trichothecin (63). There are two possible ways of folding the farnesyl pyrophosphate chain (65 and 66) to afford (on cyclisation) the trichothecane skeleton. Hanson and his co-workers used two approaches to this problem. First feeding experiments with suitably double labelled mevalonic acid and appropriate degradations gave the number and location of the labels incorporated into trichothecin. Second, the use of specifically labelled geranyl and farnesyl pyrophosphates made it possible to determine from which prenyl unit (distal, central or terminal) a particular hydrogen originated. This approach allowed the detection of unsuspected hydride shifts, and resulted in the proposal of a new biosynthetic scheme (see scheme 5). This involves a concerted



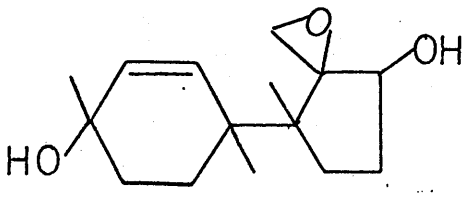
(69)



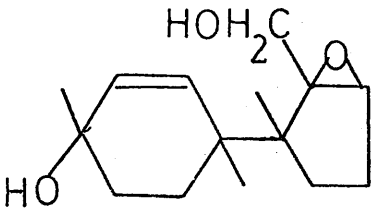
(70)



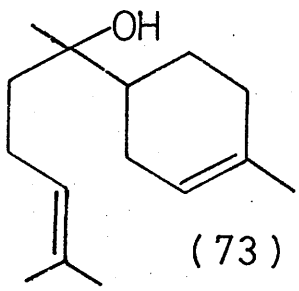
(68)



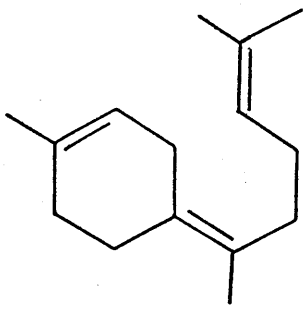
(71)



(72)



(73)



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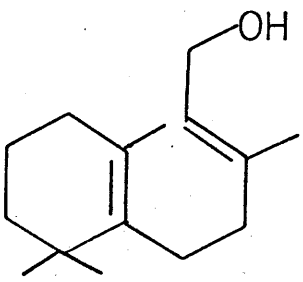

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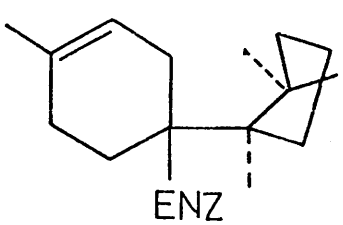

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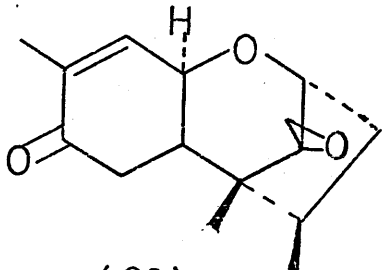
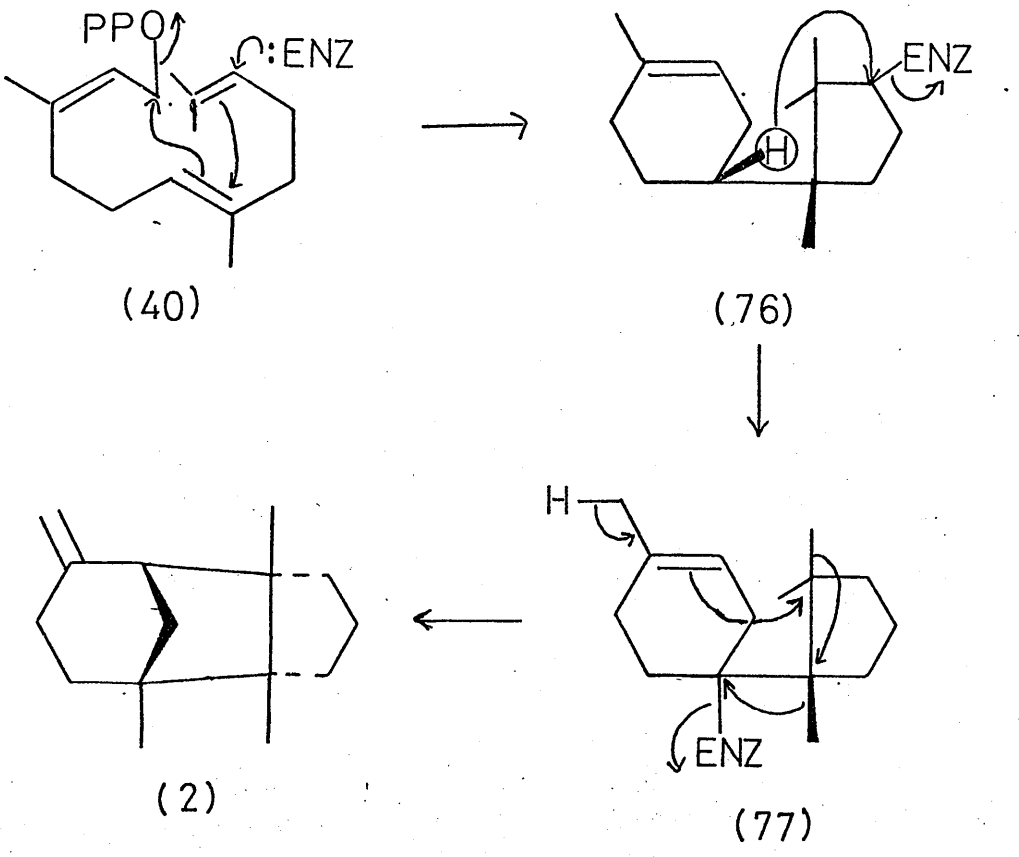


(63)

$OCOCH{=}CHCH_3$

SCHEME 6

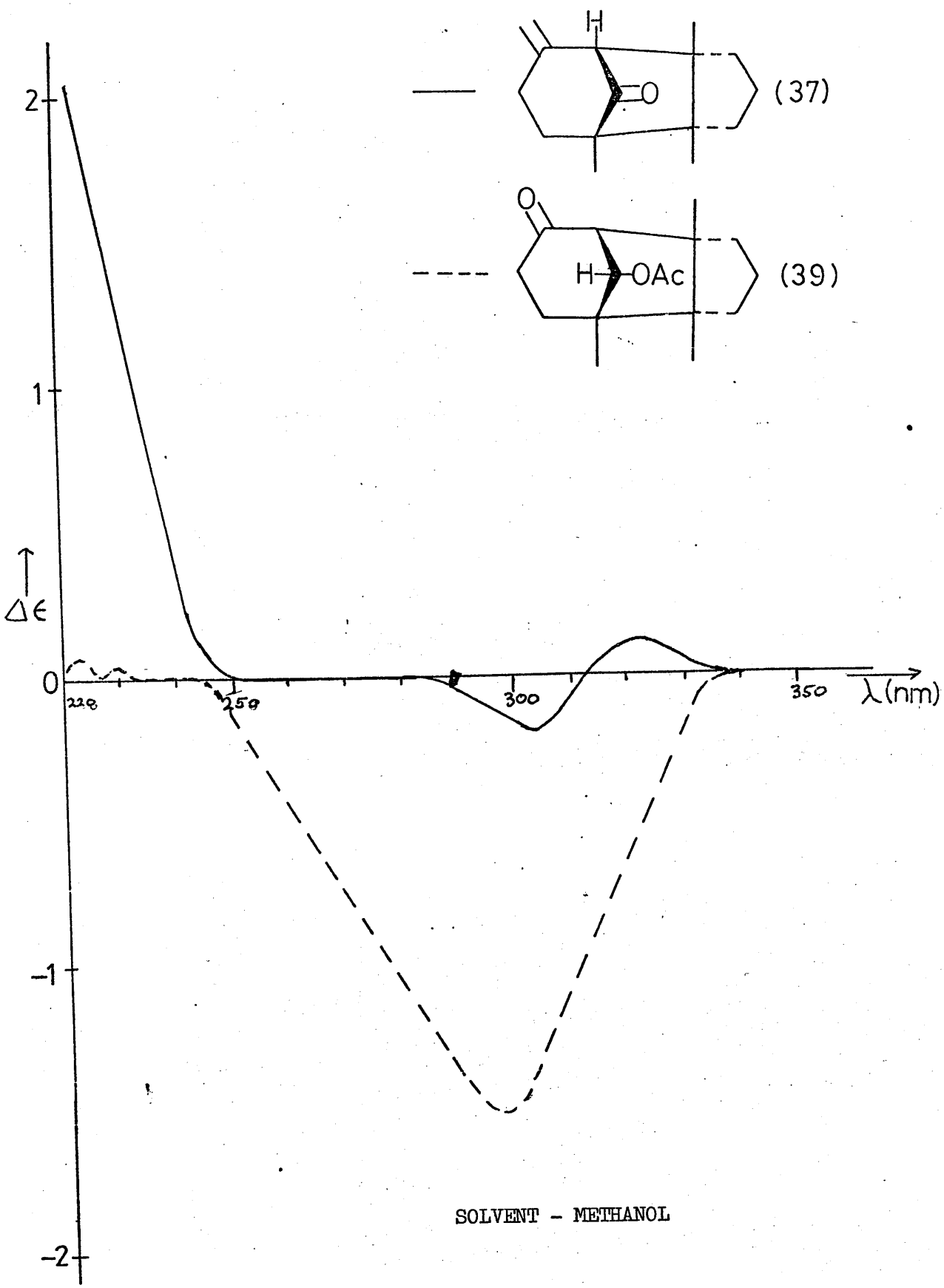


(40) (76) (77) (2)

cyclisation sequence (65) to (67) in which a hydrogen transfer occurs in the enzyme displacement step. In the case of helicobasidin (69) the intermediacy of γ-bisabolene has also been excluded [46]. Here an elimination may lead to a cuparene (70) type intermediate whilst in the trichothecane series the hydrogen transfer in (67) can act, not only as the enzyme displacement step, but also as the initiator for the methyl group rearrangements, leading to trichodiene (68) or related intermediate. Trichodiene (68) and trichodiol (71) have been isolated [47] from Trichothecium roseum, and trichodiol - A (72) shown to be an artefact produced from (71) by alkali saponification. The incorporation of trichodiene (68) into trichothecin (63) has been confirmed [48]. A more recent investigation [49] of the precursor activity of α-bisabolol (73), γ-bisabolene (62) and monocyclo-farnesol (74) indicates that these compounds are not intermediates in the biosynthesis of trichothecin (63). These authors suggest that the biosynthetic route from farnesyl pyrophosphate to trichothecin (63) proceeds directly to the intermediate (75).

In view of these results the biosynthesis of the gymnomitrene skeleton (2) might be better considered to derive from an enzyme-bound farnesyl pyrophosphate which on concerted cyclisation leads to a trichodiene-type intermediate (77) and then to gymnomitrene (2) (see scheme 6). The stereochemistry of the methyl groups in gymnomitrene (2) requires that the intermediates (76) and (77) be double bond isomers of trichodiene species (67) and (68) proposed by Hanson in the biosynthesis of trichothecin (63).

FIGURE 1



SOLVENT - METHANOL

c) DETERMINATION OF ABSOLUTE CONFIGURATION

The remaining problem in the gymnomitrol series was the determination of the absolute configuration. A Prentice-Hall model of gymnomitrone (37) suggested that its circular dichroism spectrum would be helpful in determining the absolute configuration. However, the spectrum of gymnomitrone (37) is bisignate and of low amplitude. The c.d. curve of the nor-ketoacetate (39) is, however, very informative (see figure 1). The fairly strong negative Cotton effect indicates that the absolute configuration is as shown. The octant diagram for (39) is shown in figure (2).

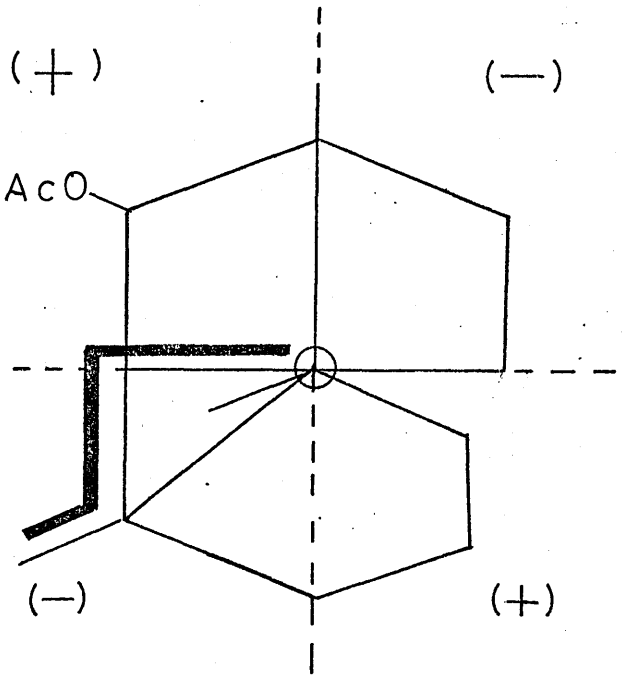
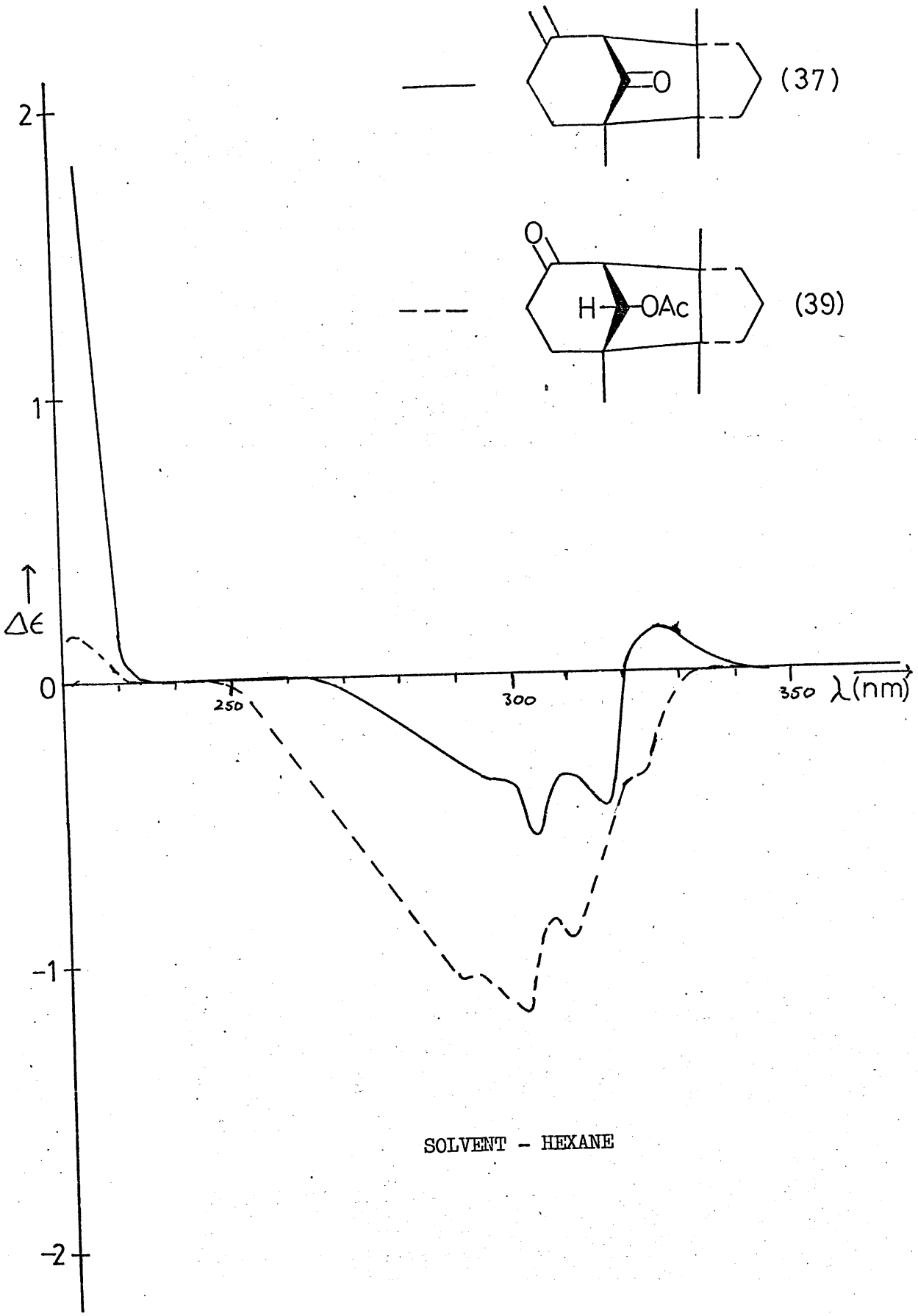


Figure (2) : Rear octant Diagram of the nor-ketoacetate (39).

Klyne [50] suggested that the zig-zag pattern shown in heavy lines would be the dominant one with a small positive contribution coming from the acetoxy group. This contribution can be estimated at about +0·2 in view of Snatzke's [51] work on adamantanones.

A closer examination of the c.d. curve for gymnomitrone (37) shows, as well as the small bisignate curve in the n-π^* region, a large positive band for which the maximum had not been reached at 222 n.m. Klyne [50]

FIGURE 3



suggested an anology of gymnomitrone (37) with ent-cholest-5-en-3-one since the geometry of the two chromophores is similar. However the c.d. curve of the steroidal ketone is very strong and positive (λmax 300 n.m.). This may be explained by the quite different positions of the alkyl groups. The c.d. curve of gymnomitrone (37) run in hexane as solvent shows some fine structure (see figure 3).

In an effort to obtain further information about the interaction between the $\beta\gamma$-double bond and the carbonyl group we turned to isogymnomitrone (57). Isogymnomitrone (57) shows a large negative Cotton effect at 298 n.m. The geometry of the chromophore in isogymnomitrone (57) is very close to that pictured in the classical Moscowitz [52] paper discussing $\beta\gamma$-unsaturated carbonyl interaction. This paper suggests that the composite carbonyl-carbon-π system of $\beta\gamma$-unsaturated ketones merits treatment as an inherently dissymmetric chromophore instead of as an assymmetrically perturbed intrisically symmetrical chromophore. The amplitude of the Cotton effect in $\beta\gamma$-unsaturated ketones is critically dependent on the relative disposition of the carbonyl group and the double bond. These characteristic high amplitudes are typical of the conformation of the enantiomeric chromophores associated with dissymetric $\beta\gamma$-unsaturated ketones (see figure 5).

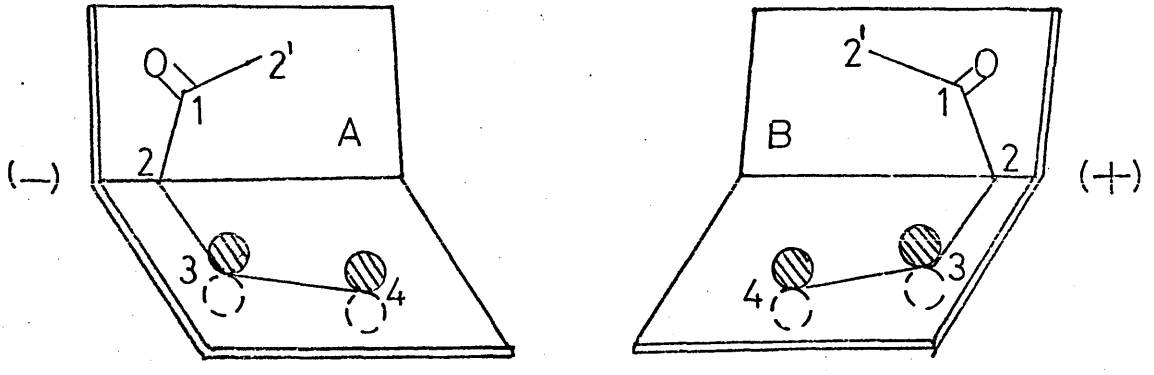
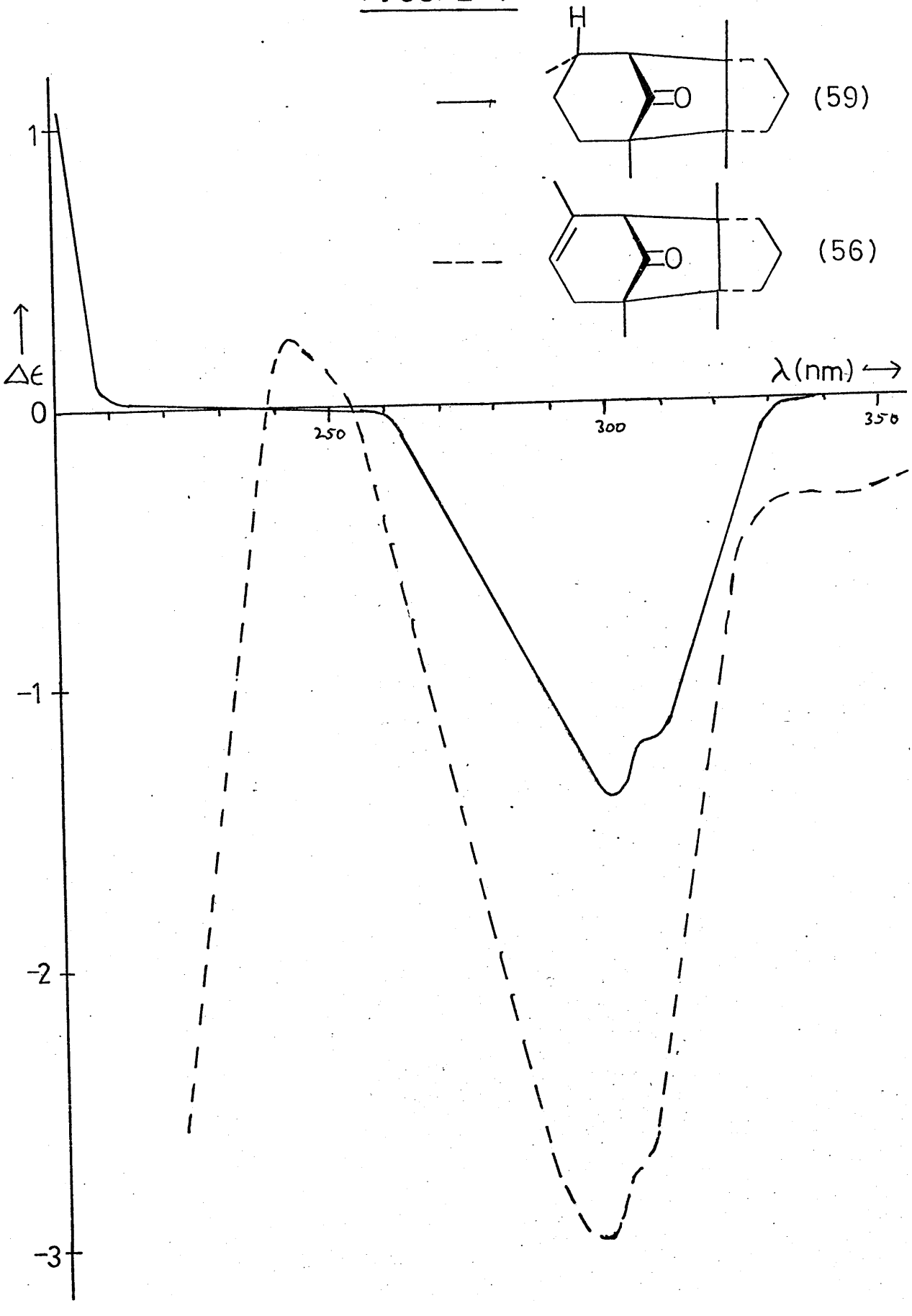


Figure (5) – Enantiomeric Chromophores associated with dissymmetric $\beta\gamma$-unsaturated ketones.

FIGURE 4



SOLVENT - HEXANE

The arrangement $C_2' - \overset{O}{\overset{\|}{C}}_1 - C_2 - C_3 - C_4$ therefore assumes one of two enantiomeric conformations, one giving rise to a negative (A) and the other to a position (B) Cotton effect. The large negative curve recorded for isogymnomitrone (56) (figure 4) suggests an absolute stereochemistry which is completely in accord with that postulated from the c.d. curve of the nor-ketoacetate (39).

The c.d. curve of dihydrogymnomitrone (59) i.e. the ketone with the exomethylene group stereospecifically reduced shows a fairly strong negative Cotton effect (figure 4). This is in good agreement with the octant diagram shown in figure (6).

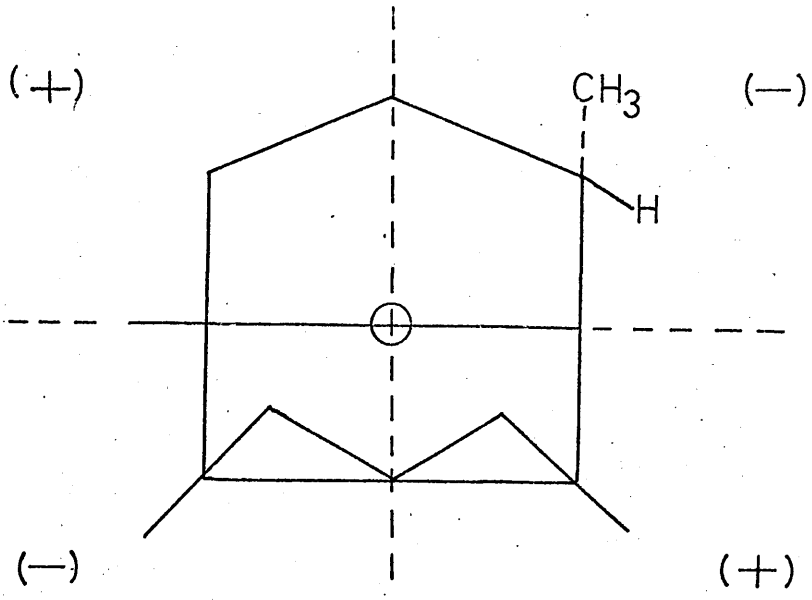


Figure (6) - Rear octant diagram of Dihydrogymnomitrone (59)

d) EUROPIUM SHIFTED SPECTRA

Since the first report by Hinckley [53] of the use of lanthanide shift reagents for structure determination a great number of publications have appeared on this subject. Considerable evidence now exists to support the view that chemical shifts induced in the p.m.r. spectra of organic aliphatic ligands by paramagnetic lanthanide ions are predominantly pseudocontact in nature [53-56]. Because of this, lanthanide induced shifts ($\Delta\delta$ - values [54]) can be calculated using the McConnell - Robertson expression [37] for the pseudocontact shift, i.e.

$$\Delta\delta = \frac{K\,(3\cos^2\theta - 1)}{r^3}$$

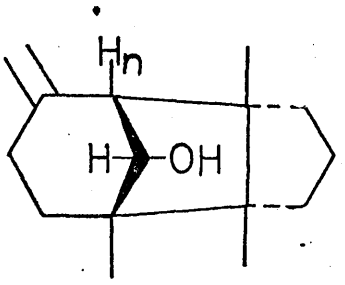
where r is the distance from the metal atom to the proton in question,

θ is the angle made by the r vector and the principal magnetic axis of the complex,

and K is a constant characteristic of the magnetic susceptibility of the metal.

Attempts to use this expression quantitatively [54, 57-59] have been hampered by the uncertainty in the precise metal ion location within the complex. These uncertainties lead early workers to arbitrarily measure r from the functional group heteroatom or some assumed lanthanide ion location [53, 54, 58, 59]. The lack of X-ray structural information on lanthanide complexes prompted Demarco *et al* [60] to investigate approximate, time-averaged, solution geometries for complexes formed by the interaction of both Eu $(fod)_3$ and Eu $(dpm)_3$ with model ligands.

Demarco[60] found that the Eu^{3+} ion co-ordinates in a time-averaged *cis* configuration to the functional group's vicinal methine proton.



(1)

TABLE 2

Molar Ratio	Carbinol Proton δ	Tertiary Methyls δ	H_n δ	Exomethylene Protons δ
0	3.70	0.93, 1.07, 1.21	2.32	4.63, 4.65
0.2	8.07	2.84, 2.81, 2.65	4.80	4.90, 4.87
Δδ	4.37	approx. 1.5-1.9	2.48	0.27, 0.22

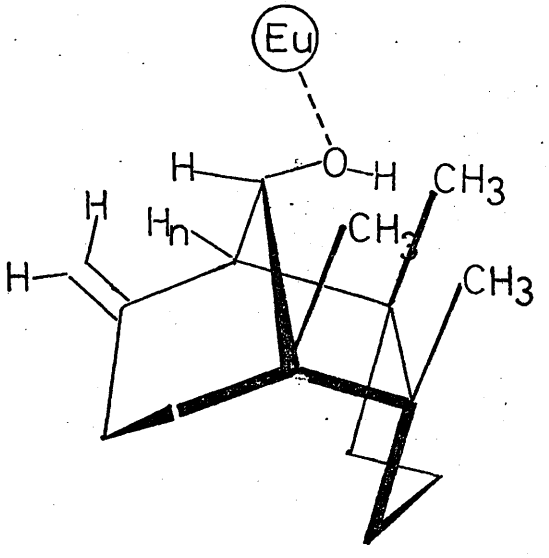


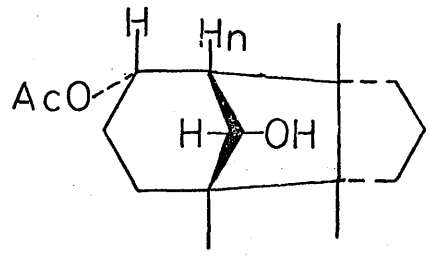
FIGURE 7 - GYMNOMITROL (1) - EUROPIUM COMPLEX

FOOTNOTE : Diagrams are taken from Prentice-Hall models and the europium ion sited according to the work of Demarco et al [60].

Therefore, in the presence of europium, rotating functionalities either adopt preferred conformations with their lone pairs oriented cis to the adjacent vicinal methine proton or Eu^{3+} binds to the ligand only when the lone pair is oriented in this direction. This observation contradicts the earlier, more qualitative, finding of Hinckley [57] (i.e., that Eu^{3+} binds in a trans configuration to the hydroxyl vicinal proton) derived on the basis of the $1/r^3$ model.

As has been previously stated it would have been very difficult to assign a structure to gymnomitrol (1) without the use of europium shifted spectra. The shifts measured for gymnomitrol (1) with an approximately 0·2 M concentration of Eu $(dpm)_3$ are shown in table (2). For a carbinol proton shift of over 4 ppm. the three tertiary methyls all move down by between 1·5 and 1·9 p.p.m., H_n by nearly 2·5 p.p.m., while the exomethylene protons only move down by a very small amount ($\sim$ 0·25 p.p.m.). This indicates that the europium ion must lie close to and roughly equidistant from the three tertiary methyl groups. The large shift of the singlet H_n means that it also must lie close to the europium ion but this is expected since it has been shown that H_n is on the neighbouring carbon to the carbon bearing the hydroxy group. It would appear reasonable to situate the europium ion at approximately 2Å from the hydroxy oxygen atom in the direction shown (see figure 7). This position for the europium ion can satisfactorily explain the observed shifts.

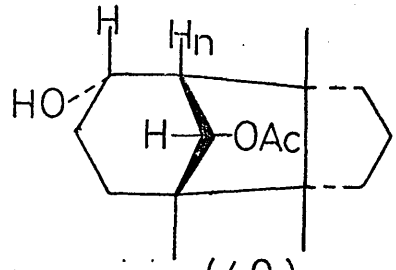
The nor-hydroxyacetate (41) shows in its n.m.r. spectrum in addition to three tertiary methyls and an acetate methyl, a sharp singlet at δ3·69 (CHOH) and a multiplet (CHOAc) at δ5·0. H_n is not clearly visible in the normal spectrum but is easily seen in the europium shifted spectrum (see table 3). Double irradiation experiments on the latter demonstrated the coupling between H_n (doublet J 3 H_Z) and the CHOAc proton. All three



(41)

TABLE 3

Molar Ratio	Tertiary Methyls δ	Acetate Methyl δ	H_n δ	CHOH δ	CHOAc δ
0	0.94, 1.10, 1.12	2.02	~8.1	3.69	5.0
0.2	1.56, 1.61, 1.92	2.88	3.52	5.29	6.58
0.4	2.29, 2.42, 2.72	3.20	5.13	7.07	8.27
Δδ (0.4)	approx. 1.1 - 1.6	1.18	~3.2	3.38	3.27



(40)

TABLE 4

Molar Ratio	Tertiary Methyls δ	Acetate Methyl δ	H_n δ	CHOH δ	CHOAc δ
0	0.83, 1.05, 1.12	2.02	not clearly seen	4.13	4.59
0.2	1.37, 1.61, 1.89	2.99	4.19	7.10	6.28
0.4	1.85, 2.07, 2.50	3.82	6.22	9.70	8.02
Δδ (0.4)	approx. 1.0 - 1.4	1.79		5.57	3.43

methyls show relatively large induced shifts with one methyl in particular moving downfield slightly faster than the others. The carbinol proton, the CHOAc proton, and H_n all appear to move down by approximately the same amount. This indicates that the europium ion is complexing equally well with the acetate as it is with the hydroxy group. This is possibly due to the hindered nature of the hydroxy group. Another interesting feature of the normal n.m.r. spectrum of (41) is a one proton multiplet centered at δ2·6 which is probably due to an endo-proton on ring C being deshielded by the acetoxy group.

The isomeric hydroxy acetate (40) has the expected signals in the n.m.r. at δ4·59 (s, CHOAc) and δ4·13 (m., CHOH). The shifts induced by Eu $(fod)_3$ are recorded in table (4). Decoupling again demonstrated the coupling between the carbinol proton and H_n (doublet, J 3 H_Z). The relative shifts of the methyl groups are less than in the case of gymnomitrol (1) itself and one tertiary methyl moves downfield faster than the other two. The model in figure (8) has the correct geometry to accommodate these shifts.

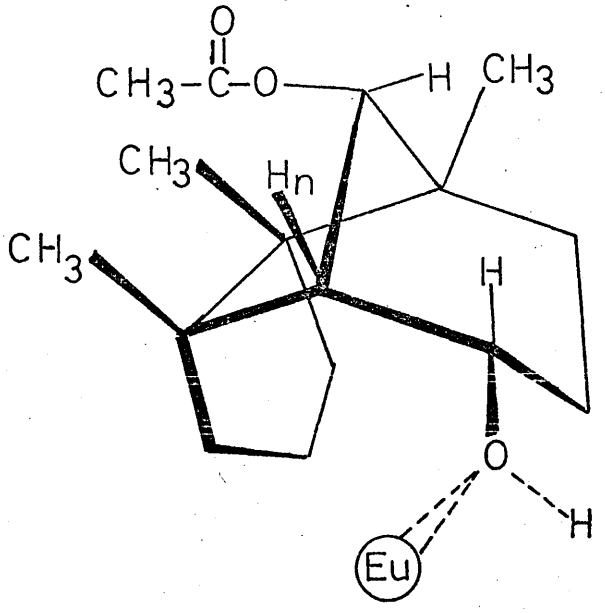
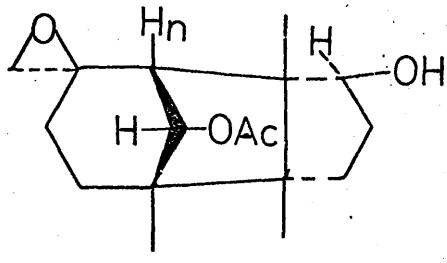


Figure (8) – The hydroxyacetate (40) – europium complex.



(51)

TABLE 5

Molar Ratio	Tertiary Methyls δ	Acetate Methyl δ	Epoxide AB Quartet δ	CHOH δ	CHOAc δ
0	0.91, 1.08, 1.11	2.06	2.68, 2.77	4.53, 5.63	5.23
0.12	0.97, 1.22, 1.38	2.11	2.67, 2.84	5.23	5.35
0.25	1.16, 1.57, 2.25	2.25	2.66, 3.03	7.20	5.66
0.5	1.53, 2.18, 3.81	2.51	2.45, 3.39	10.5	5.23
$\Delta\delta$ (0.5)	approx. 0.4-2.8	0.45	-0.13, +0.62	approx. 10.0	1.0

The lanthanide ion shifted spectrum of the epoxy-hydroxyacetate (51) is very interesting since it shows an upfield shift of one of the epoxide protons. In the normal n.m.r. spectrum the epoxide protons appeared at δ2·68, 2·77 as an AB quartet (J_{AB} 4 H_Z), the carbinol proton at δ4·53, 4·63 as a quartet (J 11, 6 H_Z) and the >C<u>H</u>OAc proton at δ5·23 as a sharp singlet. On increasing the concentration of the Eu $(dpm)_3$ the methyl signals separate, one methyl in particular moving downfield very rapidly and overtaking the acetate methyl which shows only a small shift (~0·45 p.p.m.) (table 5). Relative to a carbinol proton shift of about 6 p.p.m. the >C<u>H</u>OAc proton is deshielded by approximately 1 p.p.m. This indicates that the europium ion complexes largely with the hydroxy group and not with the acetate group (c.f. (41) p. 53). The magnitude of these shifts indicates that the position of the europium ion must be very close to one methyl and quite far removed from the acetate methyl and one other tertiary methyl. The position of the europium ion in figure (9) satisfies these requirements. The angle between the principal magnetic axis of the complex and one of the epoxide protons <u>H_Z</u> is slightly greater than 54·7°. This means that the term (3 $\cos^2\theta$ - 1) in the McConnell-Robertson expression [37] now becomes negative and therefore the proton <u>H_Z</u> will experience and upfield shift.

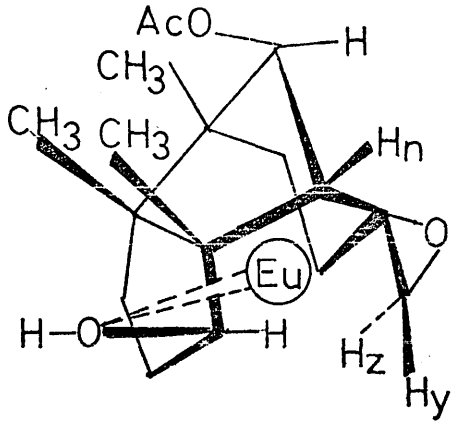
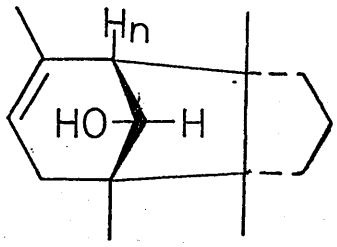


Figure (9) - Epoxy-hydroxyacetate (51) - Europium complex.



(55)

TABLE 6

Molar Ratio	Tertiary Methyls δ	Vinyl Methyl δ	Vinyl Proton δ	H_n δ	CHOH δ
0	0.86, 0.88, 1.04	1.67	5.47	1.94	3.86
0.1	0.98, 1.14, 1.27	1.81	5.76	2.44	5.76
0.3	2.57, 2.62, 5.64	3.72	6.70	8.25	9.05
$\Delta\delta$ (0.3)	approx. 1.5 - 4.7	2.05	1.23	6.31	5.19

In spite of the difficulties with line broadening, the epimeric alcohol (55) gave large shifts with a 0·3 M ratio of Eu $(dpm)_3$. The shifts of the carbinol proton, $\underline{H_n}$ and onetertiary methyl are particularly large, while the other two methyls move almost together with a more moderate shift (see table 6). Figure (10) with the europium ion placed above the bridge carbon atom, i.e. <u>syn</u> to the carbinol proton explains these observations.

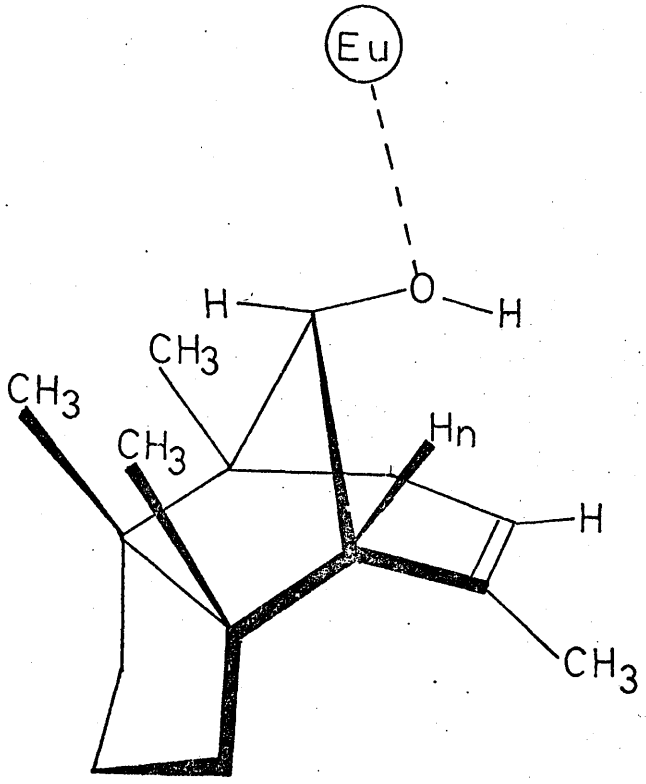
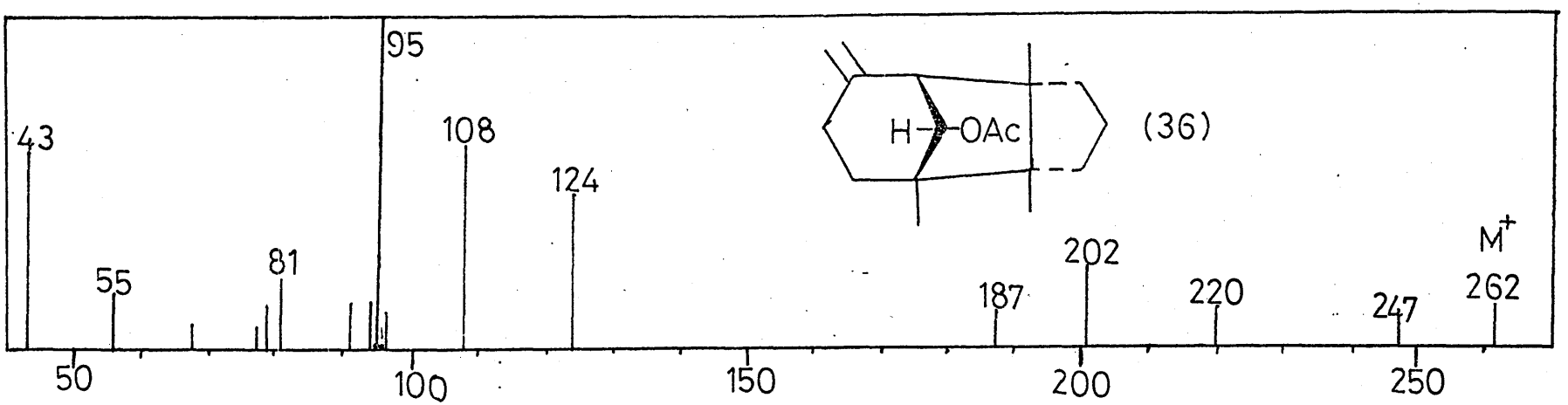
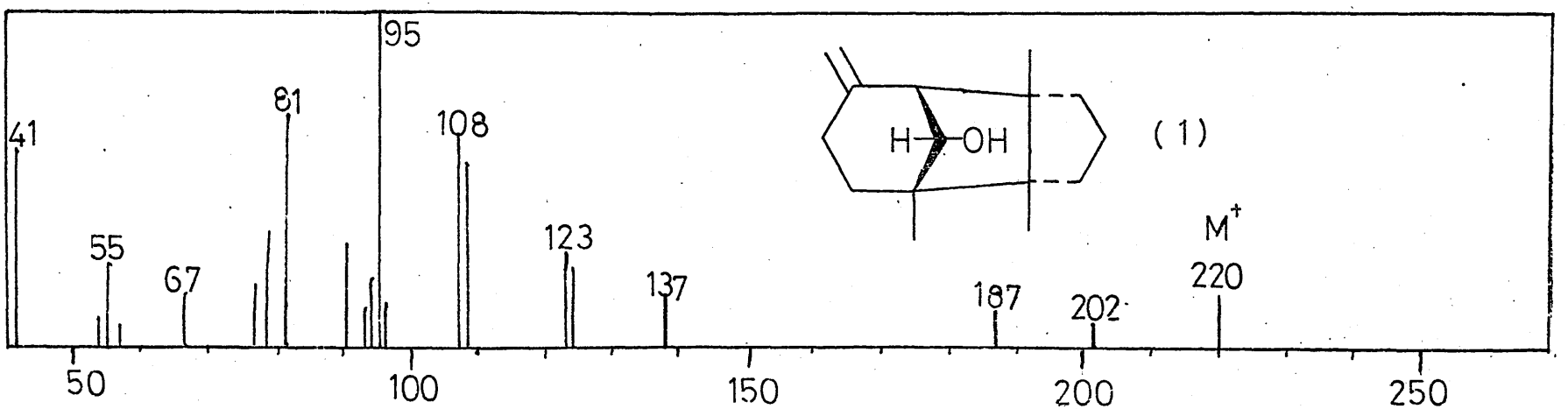


Figure (10) - The Epimeric alcohol (55) - Europium Complex.

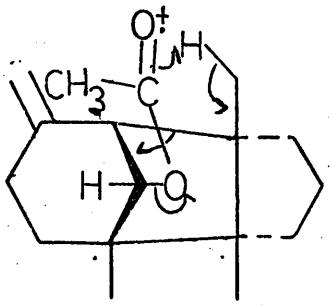
It is of interest to compare these results with the case of gymnomitrol (1) itself where all three tertiary methyl groups move downfield by almost the same amount.

FIGURE 11


95
43
108
124
H—OAc
(36)
55
81
187
202
220
247
M+
262
50
100
150
200
250

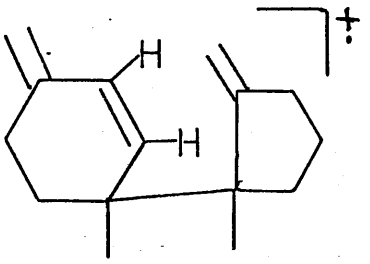

95
41
81
108
H—OH
(1)
55
67
123
137
187
202
M+
220
50
100
150
200
250

SCHEME 7



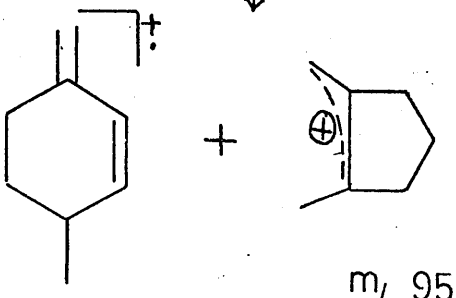
(36)

m/e 264



m/e 204 + m/e 60

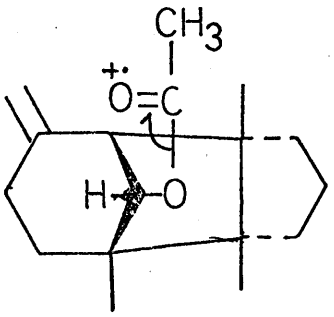
+H



(80)

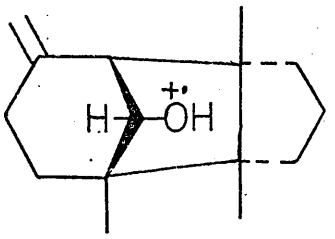
m/e 108

m/e 95



m/e 264

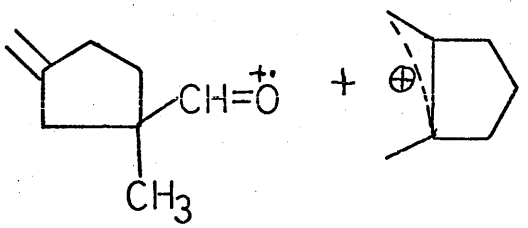
+H



+ O≡CCH3

m/e 43

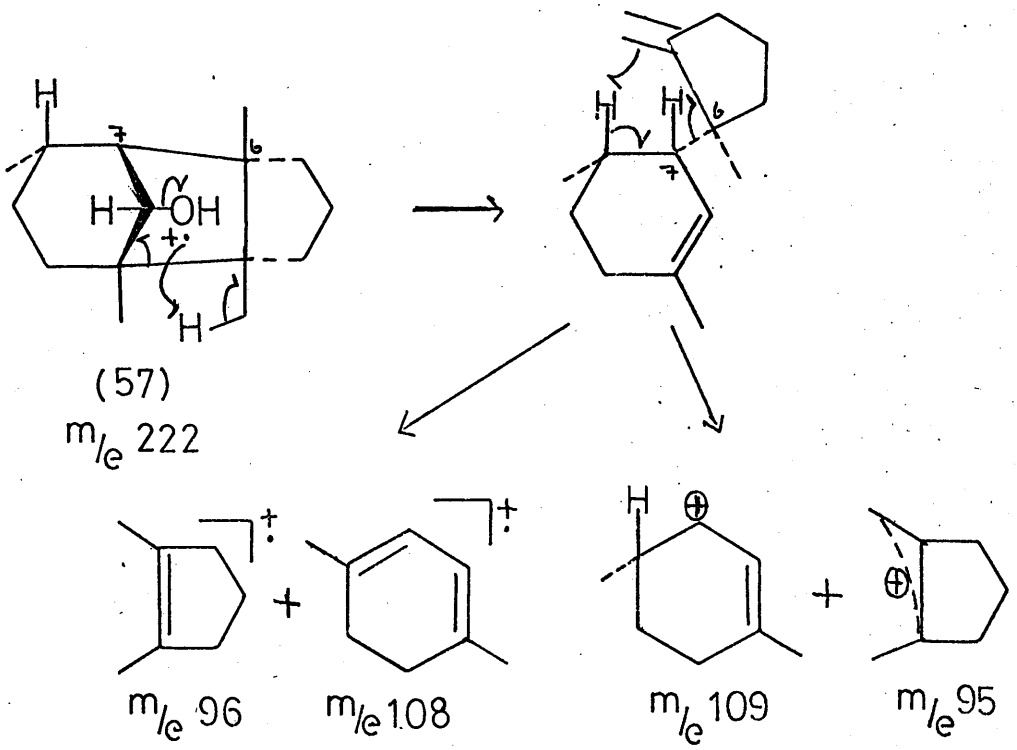
m/e 220



m/e 124

m/e 95

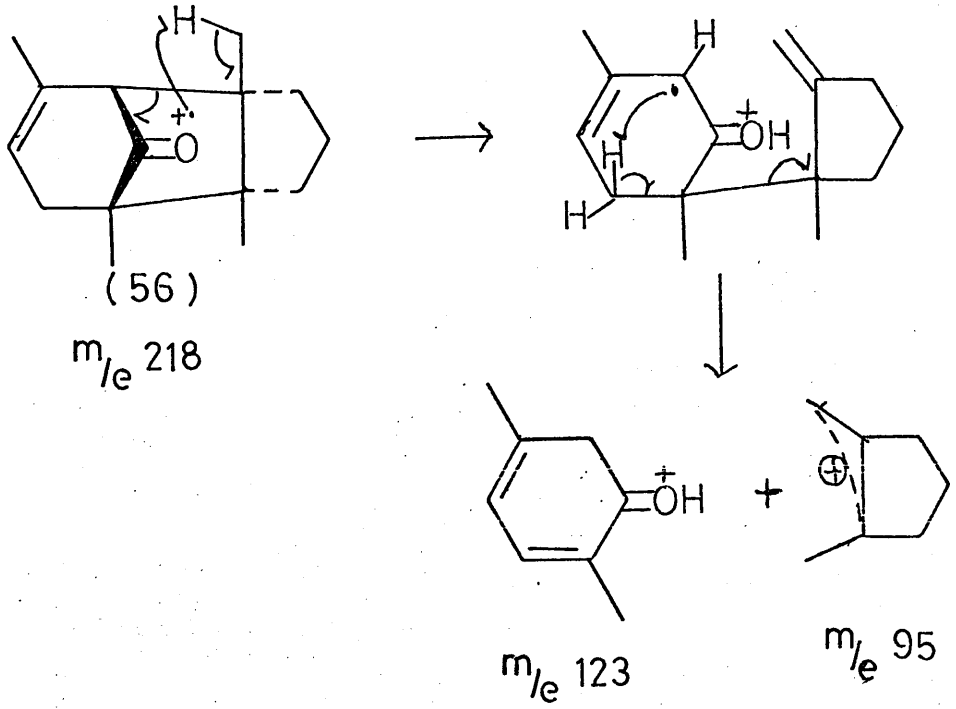
SCHEME 8



(57)
m/e 222

m/e 96 m/e 108 m/e 109 m/e 95

SCHEME 9



(56)
m/e 218

m/e 123 m/e 95

e) MASS SPECTRA

So far little mention has been made of the use of mass spectroscopy in the study of gymnomitrol (1) and its related compounds. However, this technique provided valuable confirmatory evidence for the previous structural assignments. In many of the spectra the base peak is $^{m}/e$ 95 and high resolution mass measurement indicated the constitution $C_7 H_{11}^+$. Line diagrams of the spectra of gymnomitryl acetate (36) and gymnomitrol are shown in figure (11). The acetoxy group in (36) is undoubtedly directing the fragmentation due to is proximity to the tertiary methyls. A fragmentation pathway, which accounts for the main features of the spectrum, is shown in scheme (7). The spectrum of gymnomitrol (1) shows, in addition to the base peak $^{m}/e$ 95, $^{m}/e$ 81 which is composed of two fragments in the ratio of approximately 10:1. The major component has the formula $C_6 H_9^+$ and probably arises by loss of a methylene unit from the base peak to give fragment (79) ;

Fragment (79) $^{m}/e$ 81

The minor component has the formula $C_5 H_5 O^+$. In addition gymnomitrol has a major fragment at $^{m}/e$ 108 ($C_8 H_{12}^+$) which is assigned the structure (80) in scheme (7). This fragmentation is more favourable in the case of dihydrogymnomitrol (57) and leads to the peak $^{m}/e$ 109 (see scheme 8) as the base peak. The next most intense peak in the spectrum is $^{m}/e$ 96.

In contrast to the very simple spectra obtained from gymnomitrone (37) and dihydrogymnomitrone (59), which show $^{m}/e$ 95 as the only major peak, isogymnomitrone (56) has its base peak at $^{m}/e$ 123. A proposed pathway for this breakdown is shown in scheme (9). It appears that the endo-cyclic

FIGURE 12

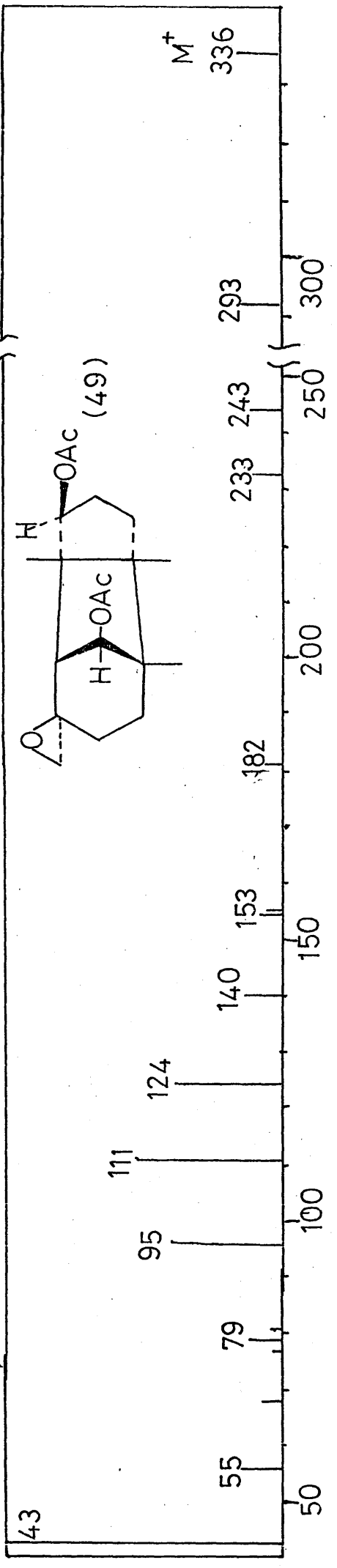
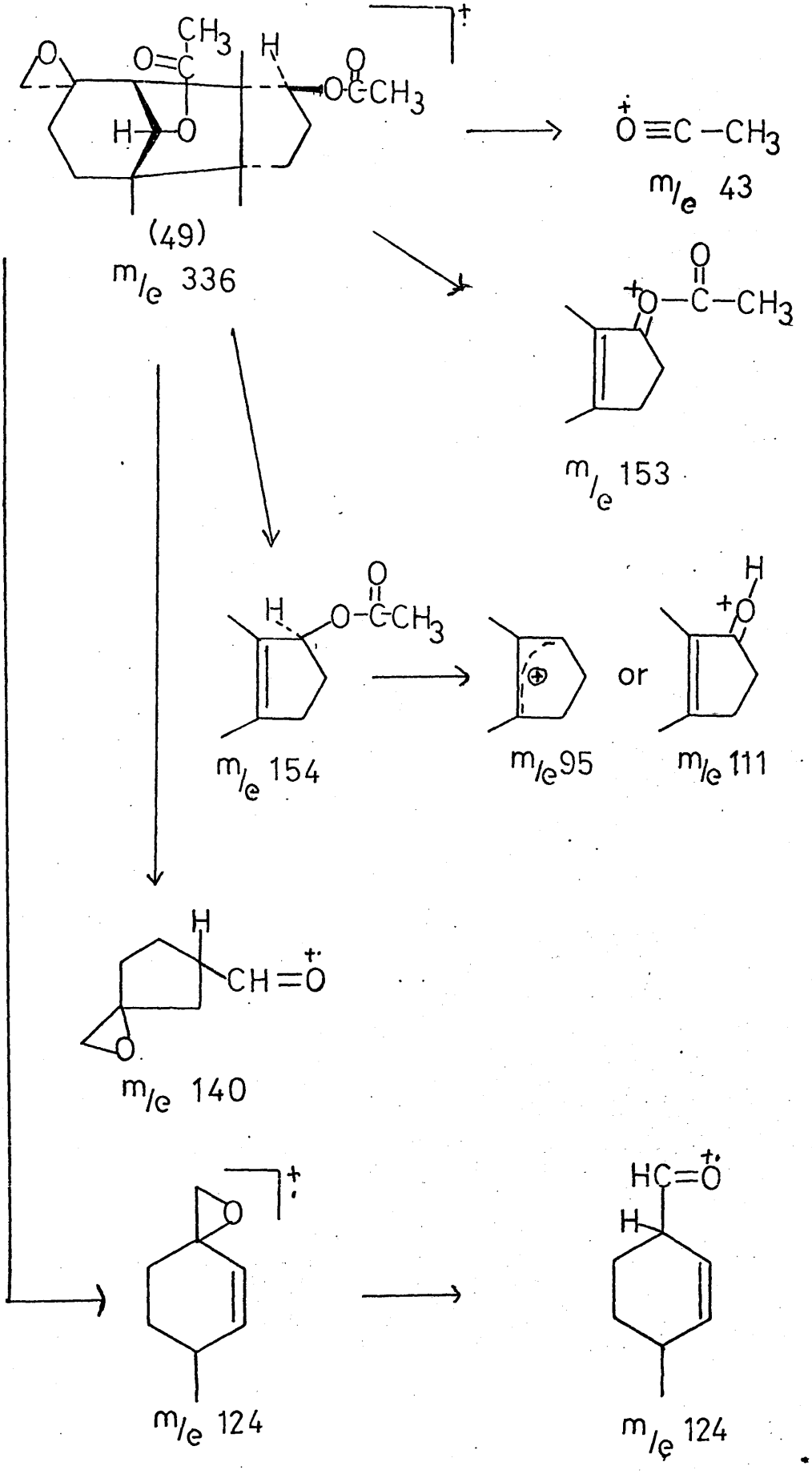


TABLE 7

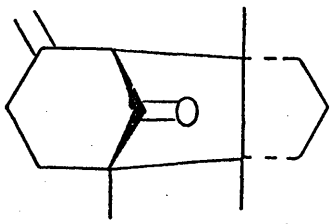
Mass measurements for (49)

m/e	Formula
111	$C_7H_{11}O^+$
95 (major component)	$C_7H_{11}^+$
95 (minor component)	$C_6H_7O^+$
124	$C_8H_{12}O^+$

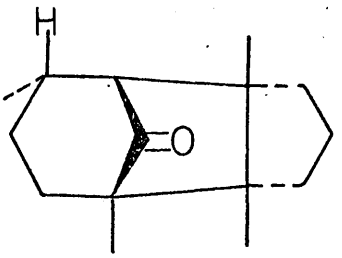
SCHEME 10



double bond is influencing the fragmentation.

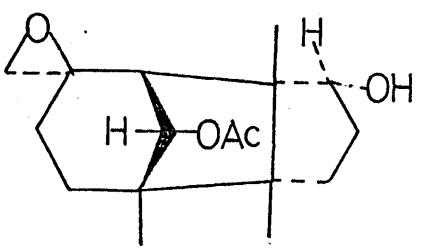


(37)

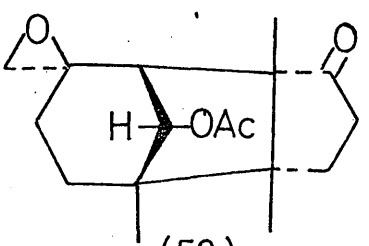


(59)

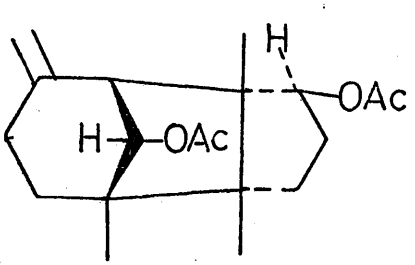
A more complex spectrum was obtained from the epoxydiacetate (49) (see figure 12). By far the most intense peak is $^{m}/e$ 43 which is due to the presence of the acetoxy groups. The other major peaks were mass measured and the results are shown in table (7). These data can be accommodated by the fragmentation pattern proposed in scheme (10). The presence of the intense $^{m}/e$ 111 indicates that this "extra" oxygen function of the epoxy diacetate is indeed located on ring C. The other compounds with an oxygen function in ring C, i.e. (51), (52), (50) and (78), give mass spectra which are very similar to the spectrum of (49).



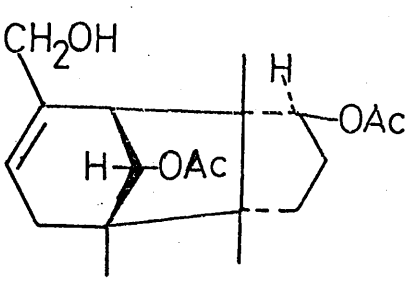
(51)



(52)

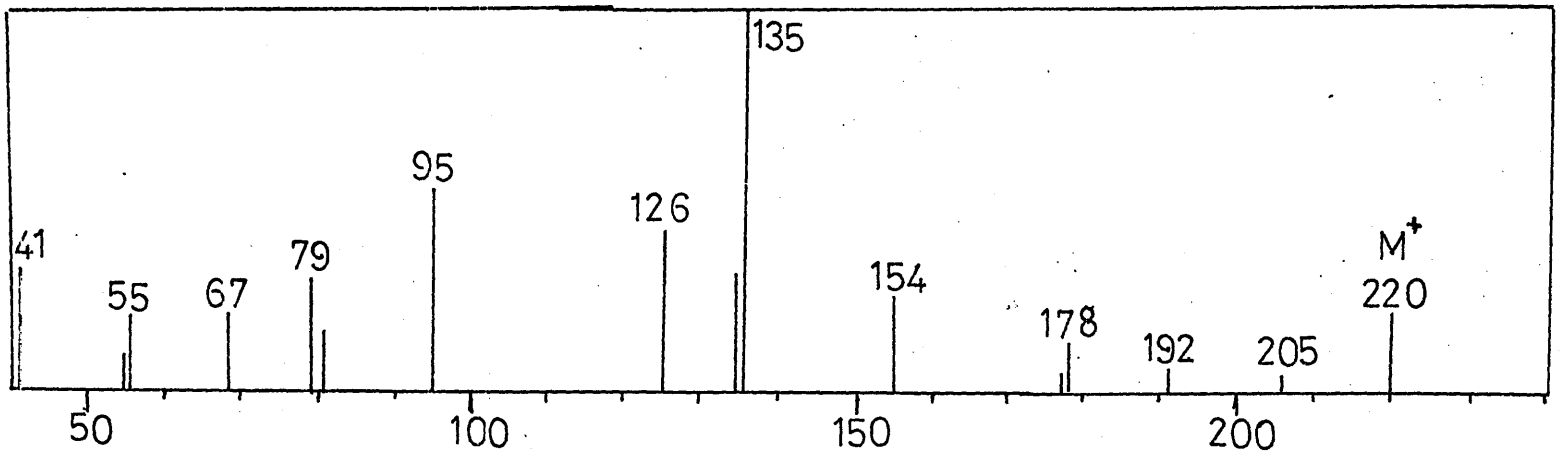


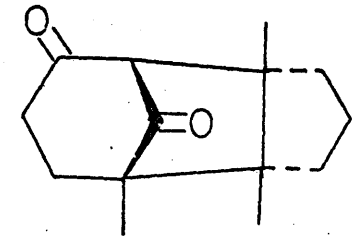
(50)



(78)

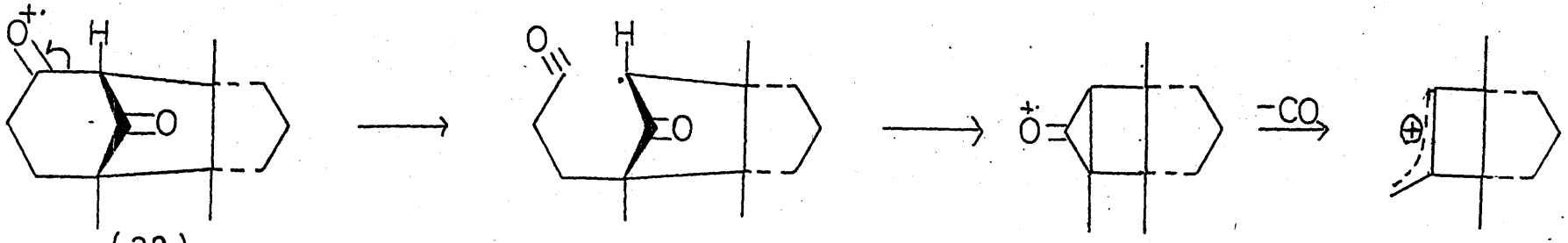
FIGURE 13





(38)

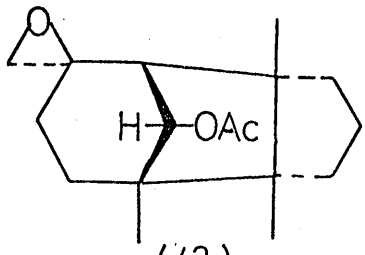
SCHEME 11



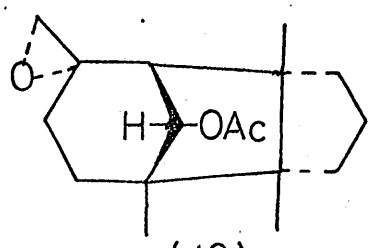
(38)
m/e 220

m/e 135

In contrast the epoxy monoacetates (43) and (42) show only a very weak peak of $^{m}/e$ 111. This suggests that $^{m}/e$ 111 is an intense peak only when there is an oxygen substituent in ring C. (43) and (42) have their base peak at $^{m}/e$ 43 with an almost equally intense $^{m}/e$ 95.

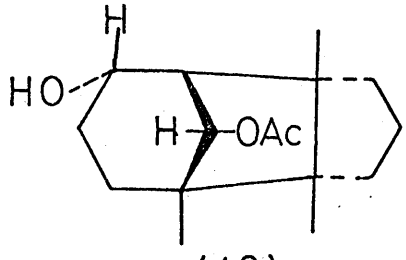


(42)

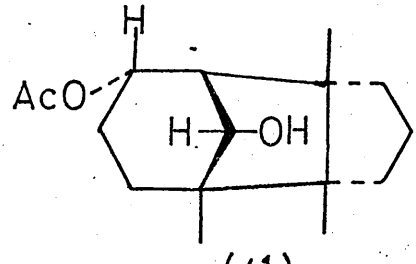


(43)

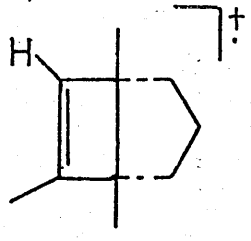
The nor-diketone (38) has its base peak at $^{m}/e$ 135 (see figure 13). This peak can arise as shown in scheme (11) and involves cleavage α to the cyclohexanone carbonyl followed by loss of carbon monoxide. This type of fragmentation process also occurs in the spectrum of the nor-hydroxy acetate (40) and to a lesser extent in the isomeric compound (41) and leads to a fragment at $^{m}/e$ 136 (81).



(40)



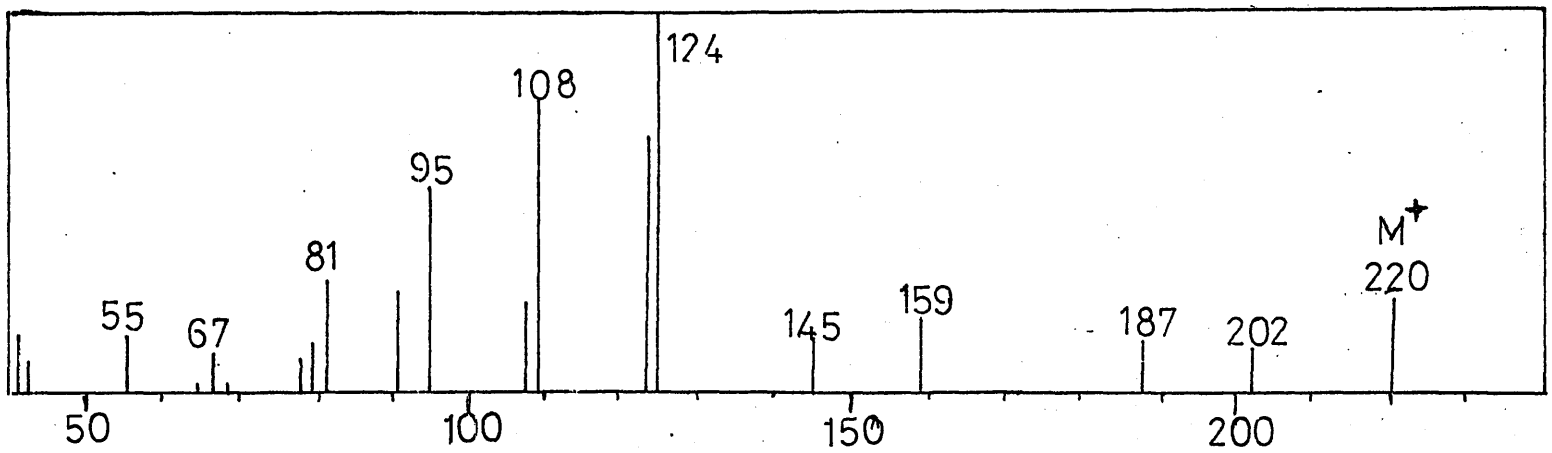
(41)

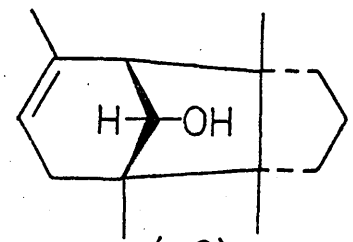


m/e 136

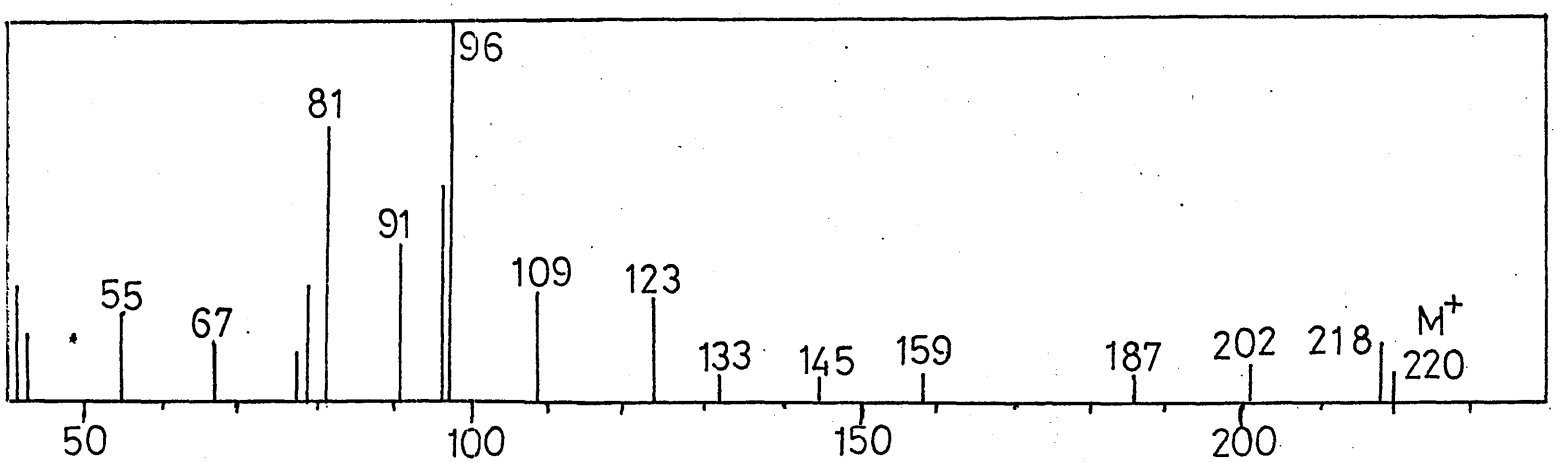
(81)

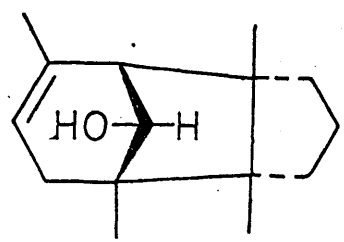
FIGURE 14





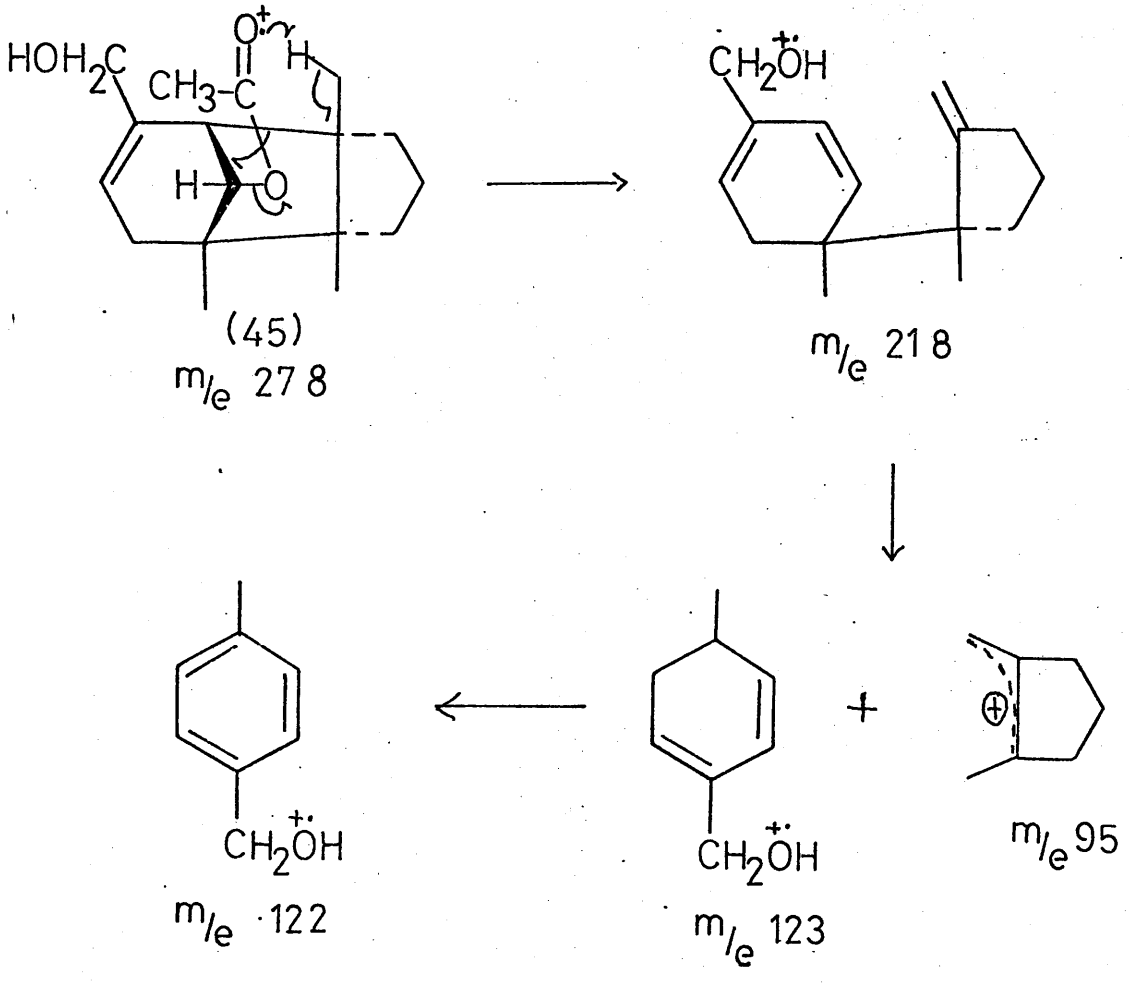
(48)



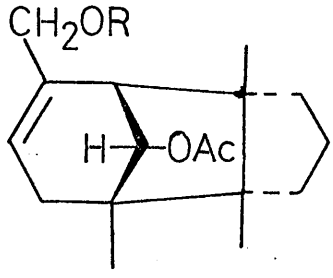


(55)

SCHEME 12

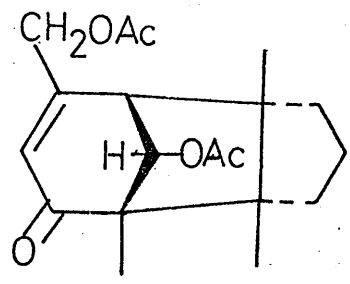

HOH2C
CH3-C
O+
H
H
O
(45)
m/e 278
CH2OH
m/e 218
CH2OH
m/e 122
CH2OH
m/e 123
+
m/e 95

The range of compounds with an endocyclic double bond in ring A give interesting mass spectra. As shown in figure (14), isogymnomitrol (48) and the epimeric alcohol (55) give different mass spectra. The base peak for isogymnomitrol (48) is at $^{m}/e$ 124 and together with $^{m}/e$ 109, 123 can be assigned to ring A fragments. The normal ring C fragments, $^{m}/e$ 95 and $^{m}/e$ 81 can arise as described earlier (see p. 63). The epimeric alcohol has its base peak at $^{m}/e$ 96 with the typical ring A fragments of relatively low intensity. The effect of having the hydroxy group anti to the tertiary methyls (13 and 14) is to prevent proton abstraction, by the hydroxy group, from one of these methyls. This results in the ring C fragment having one hydrogen more than normal and gives rise to $^{m}/e$ 96.



(45) R ≡ H

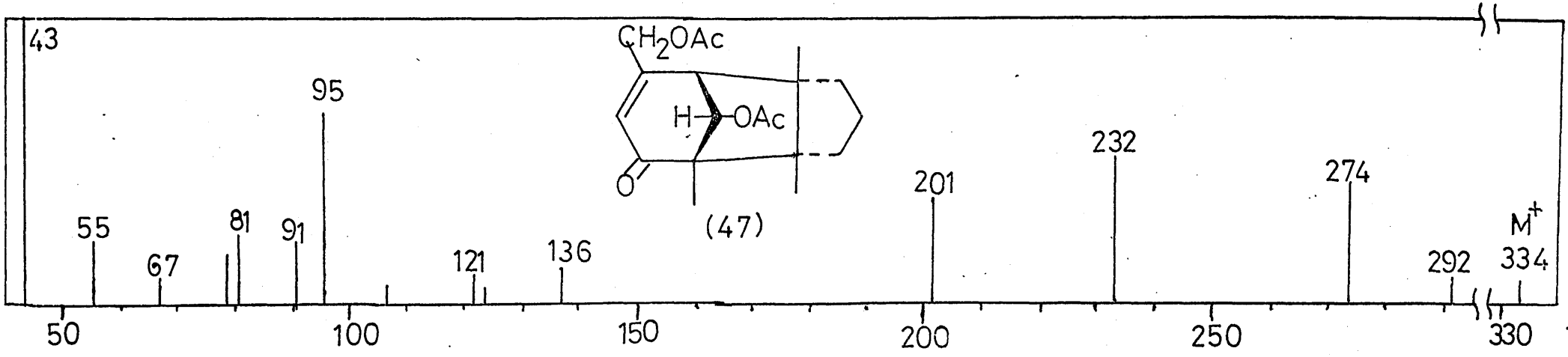
(46) R ≡ Ac



(47)

The hydroxyacetate (45) has its base peak at $^{m}/e$ 218 and, in addition to the expected ring C fragments, an intense $^{m}/e$ 122. These can arise by opening of the bicyclo ring system with loss of the acetoxy group as shown in scheme (12). By comparison the diacetate (46) (base peak $^{m}/e$ 43) has a less intense $^{m}/e$ 218. The derived enone (47) gives a different breakdown pattern. The base peak, is as expected, at $^{m}/e$ 43 followed by $^{m}/e$ 95. However two new peaks at $^{m}/e$ 232 and $^{m}/e$ 201 appear in this

FIGURE 15



SCHEME 13

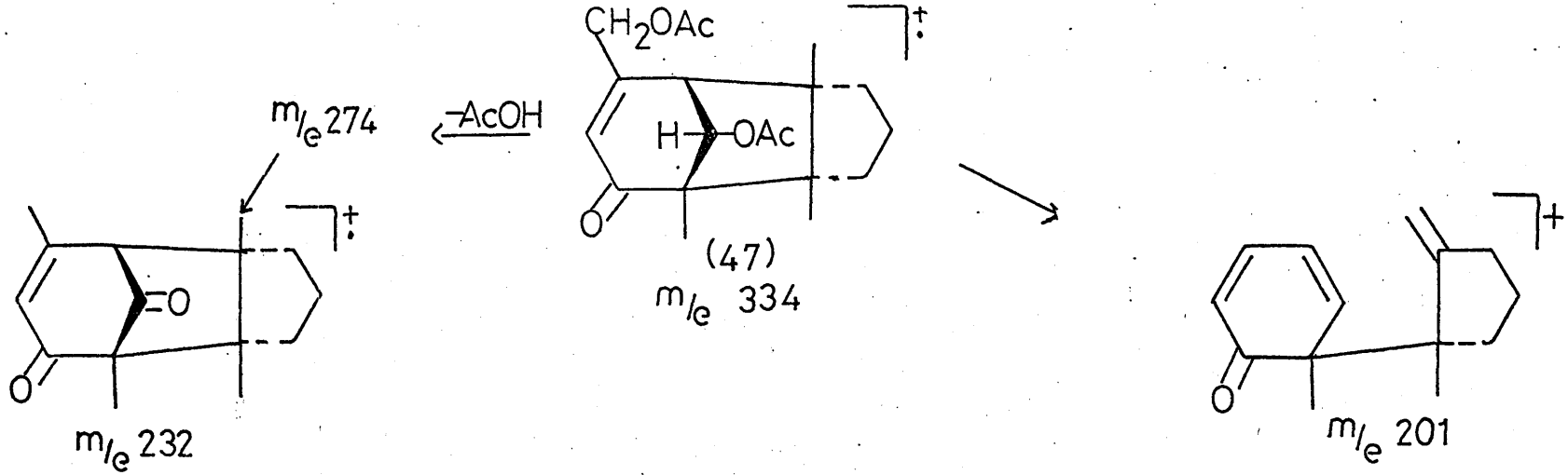
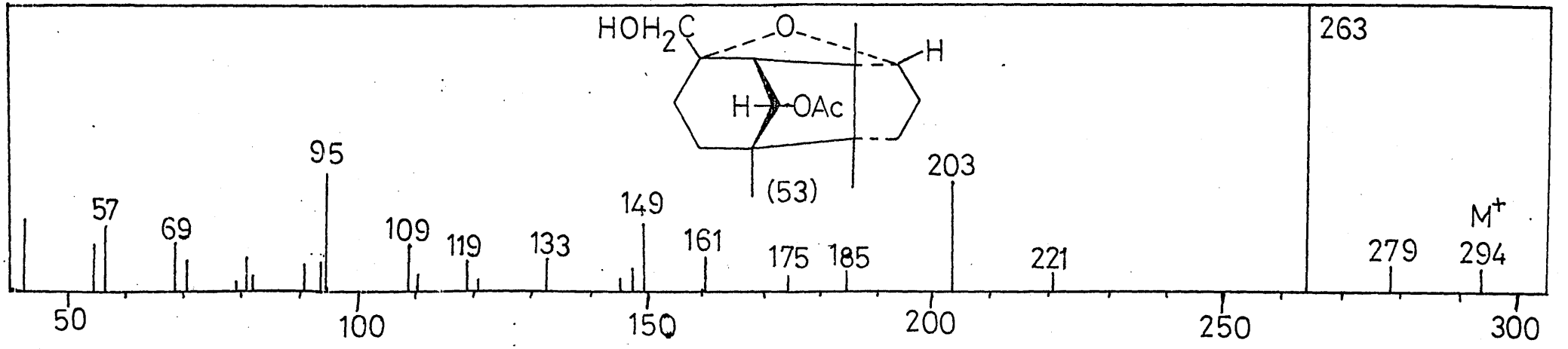
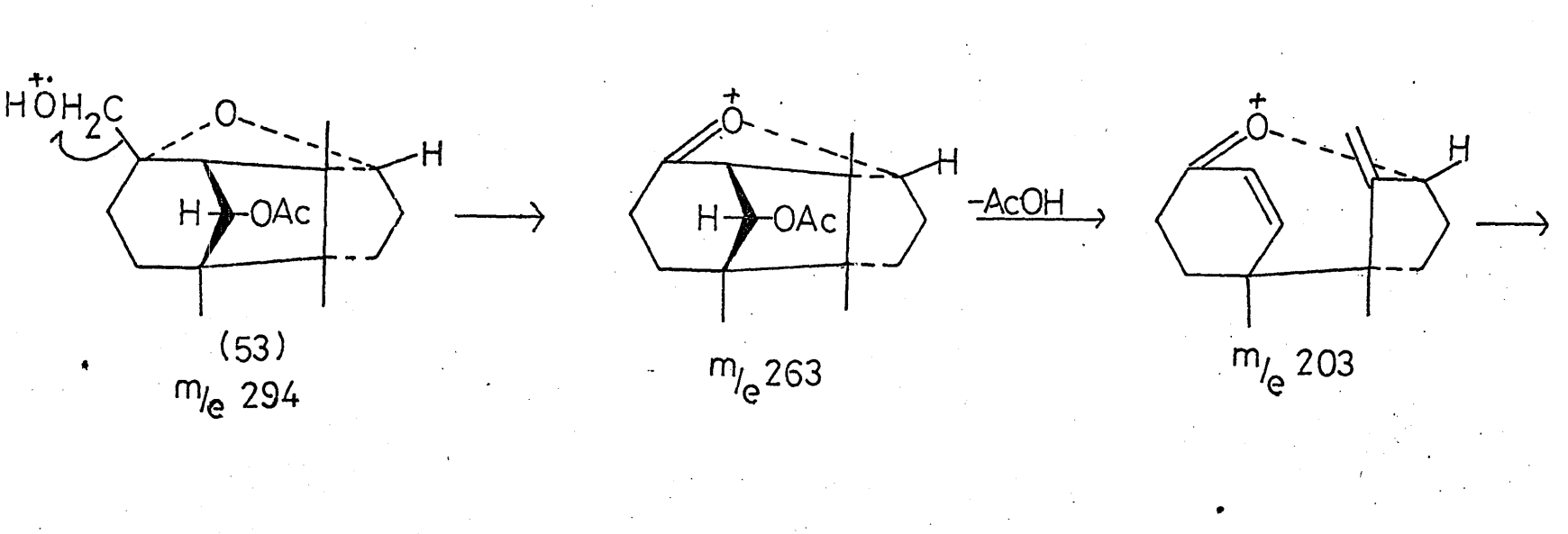


FIGURE 16

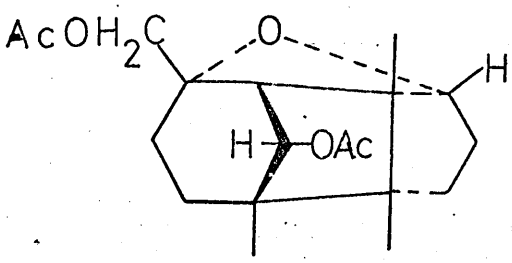


SCHEME 14



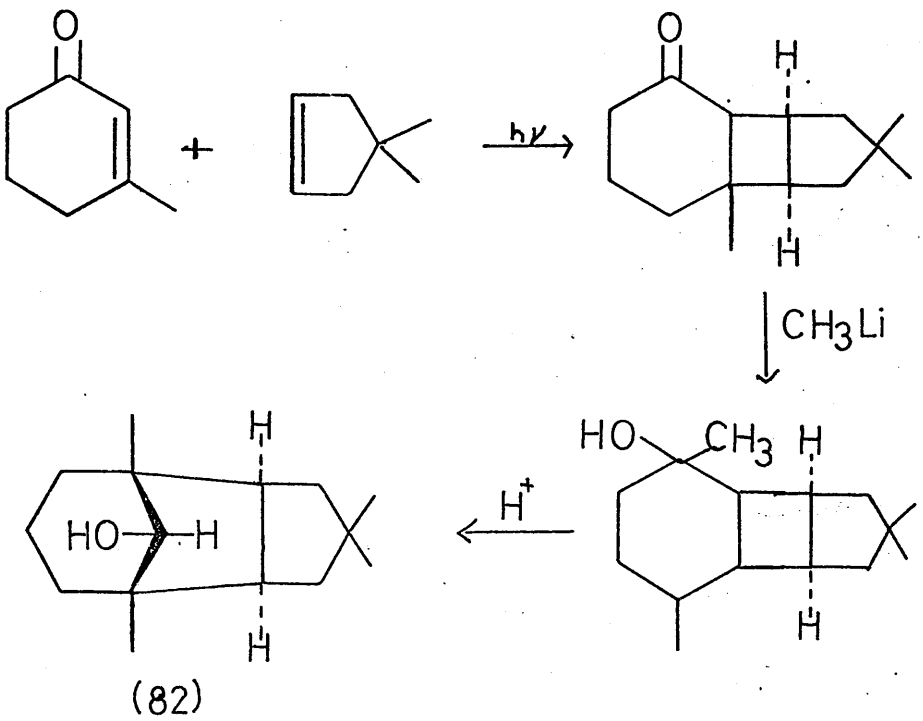
spectrum (see figure 15). Loss of acetic acid from the parent ($^{m}/e$ 334) followed by a further loss of 42 mass units gives $^{m}/e$ 232. The other new fragment, $^{m}/e$ 201, can arise by loss of the bridge acetoxy group as in (45) followed by cleavage of the primary acetoxy group (see scheme 13).

The mass spectra of the hydroxy ether (53) and the corresponding acetate (54) are very similar (see figure 16). Both have very small parents and have their base peak at $^{m}/e$ 263. A postulated breakdown pattern for the hydroxy ether (53) is shown in scheme (14). Cleavage of the primary hydroxy group gives rise to the base peak which can then lose acetic acid in the manner shown to give $^{m}/e$ 203.



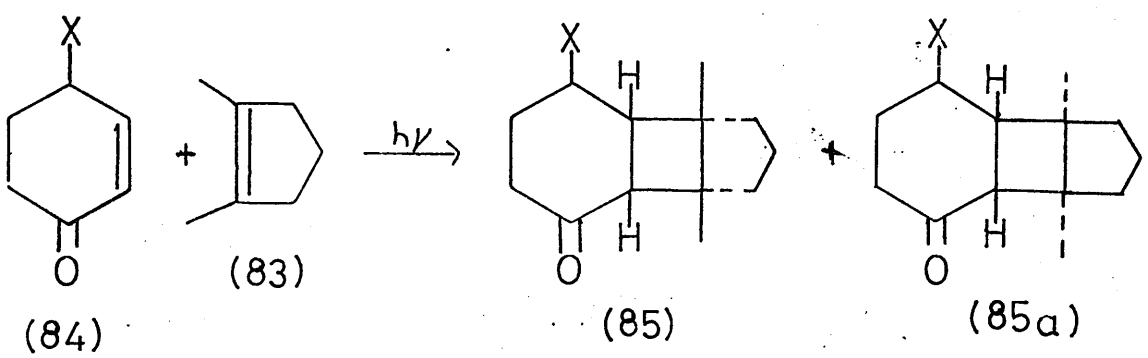
(54)

SCHEME 15

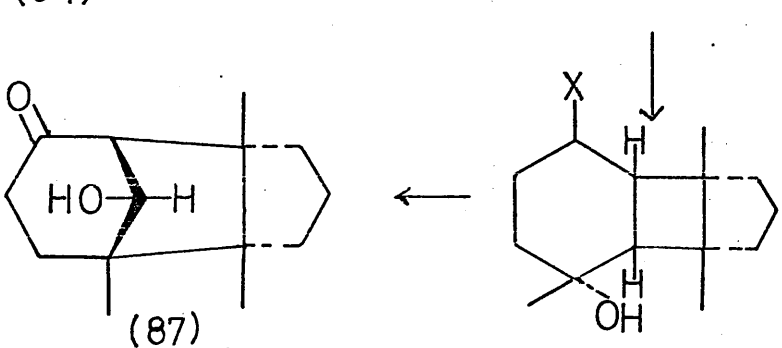


(82)

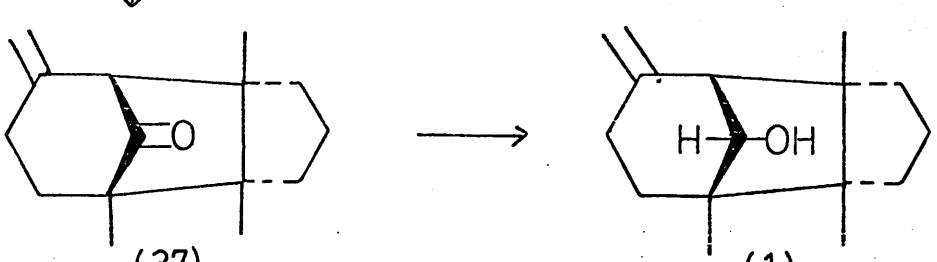
SCHEME 16



(84) (83) (85) (85a)

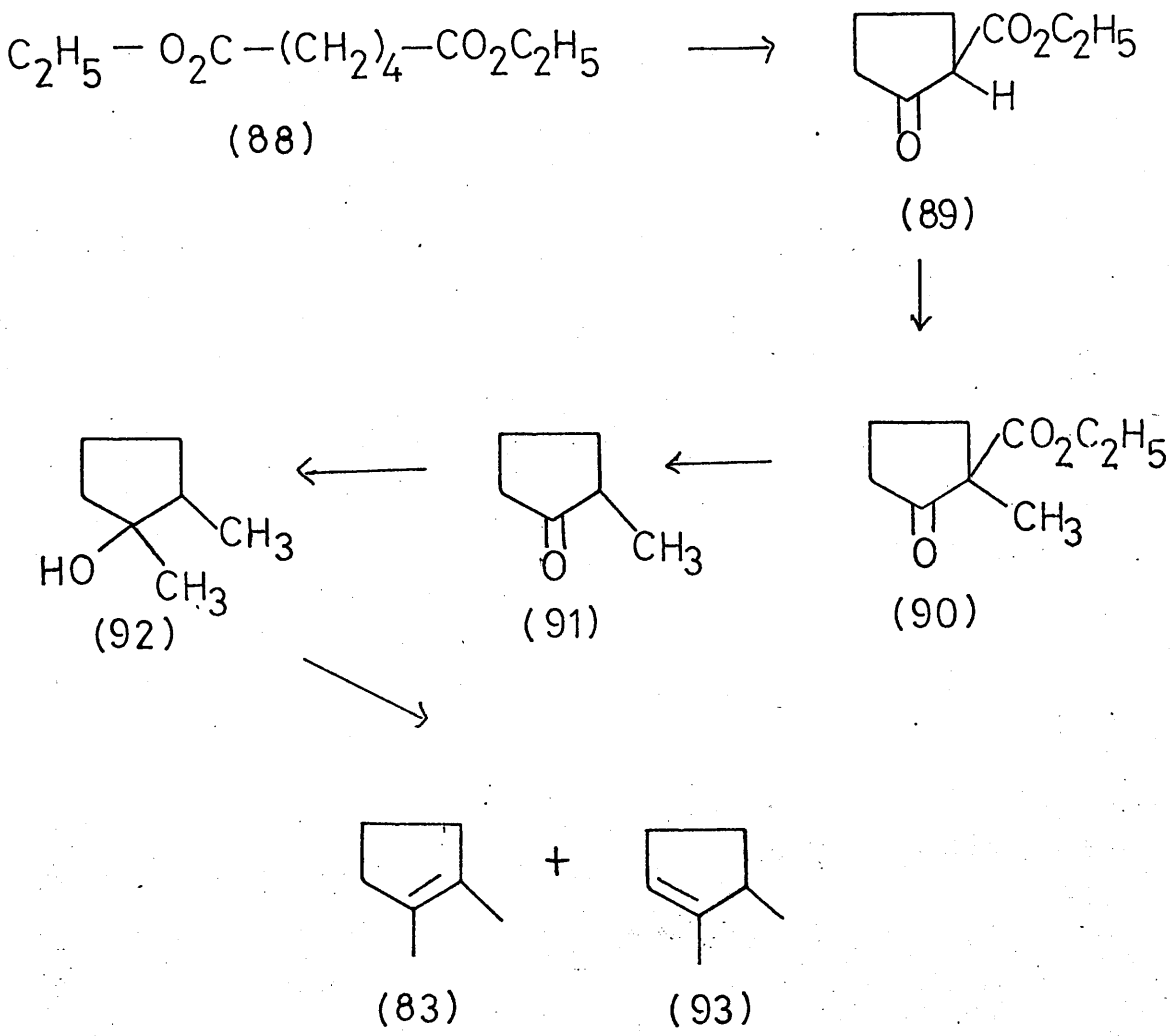


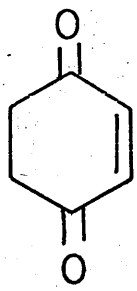
(86) (87)



(37) (1)

SCHEME 17


C2H5 – O2C – (CH2)4 – CO2C2H5
(88)
CO2C2H5
H
O
(89)
CO2C2H5
CH3
O
(90)
CH3
O
(91)
CH3
HO
CH3
(92)
+
(83)
(93)


O
O

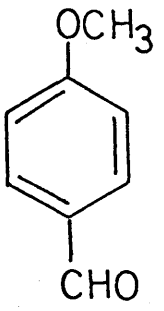
(94)

f) A SYNTHETIC APPROACH TO GYMNOMITROL (1)

The Corey [61] synthesis of α-caryophyllene alcohol (82) (see scheme 15) follows a route which appears to be adaptable to the synthesis of gymnomitrol (1). We envisaged a synthetic sequence for gymnomitrol (see scheme 16) starting from 1, 2 dimethylcyclopentene (83) and a cyclohex-2-en-1-one bearing a substituent at C-4 (e.g. X in 84) which is capable of being transformed, at a later stage, into a ketone. A ($\pi 2_s + \pi 2_s$) photoaddition between the enone and the olefin gives in theory [62], the cyclobutane adducts (85) and (85a). Reduction of the carbonyl group of (85) with methyl lithium to the tertiary alcohol (86), acid rearrangement and regeneration of the protected ketone leads to (87). A normal Wittig reaction followed by oxidation of the alcohol gives gymnomitrone (37) which can be easily converted to gymnomitrol (1) under conditions described previously.

The olefin (83) was prepared from diethyl adipate (88) with 2-methyl cyclopentanone as intermediate. Dieckmann condensation [63] of diethyl adipate (88) with sodium in toluene gave 2-carboxyethyl cyclopentanone (89). Addition of methyl iodide to the reaction mixture yielded 2-methyl, 2-carboxyethyl cyclopentanone (90) which on acid hydrolysis and subsequent thermal decarboxylation furnished 2-methylcyclopentanone (91) in 68·5% overall yield. Treatment of 2-methylcyclopentanone (91) with methyl magnesium iodide under normal Grignard conditions gave 1, 2, -dimethyl-cyclopentan-1-ol (92) which dehydrated readily on distillation. The 1, 2 -dimethylcyclopentene thus obtained was found to be a mixture of two isomers (83) and (93) with the tetrasubstituted olefin (83) predominating (ca. 91% by g.l.c.). This reaction sequence is summarised in scheme (17).

Preparation of a cyclohexenone with a suitable substituent at C-4 is more difficult. Cyclohexa-2-en-1, 4-dione (94) has been prepared [64] in



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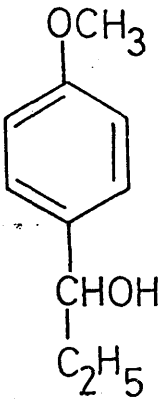
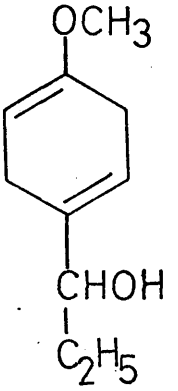
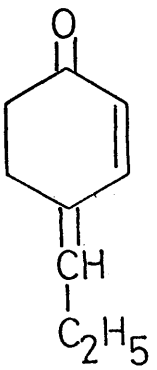


(96)



(97)



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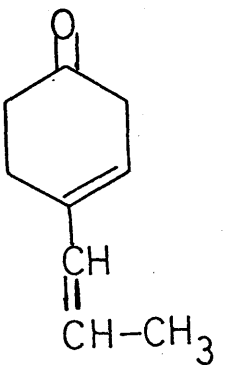

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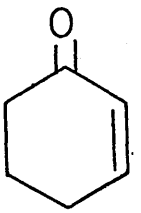
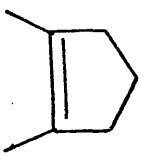
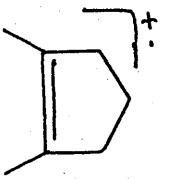


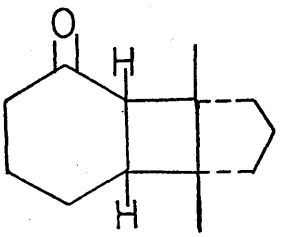
(100)



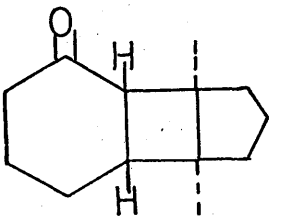
(83)



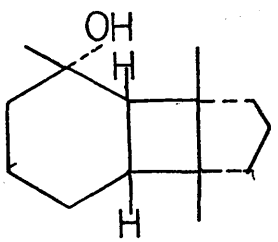
m/e 96



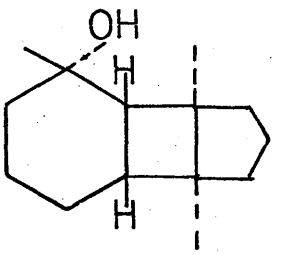
(101)



(102)



(103)



(104)

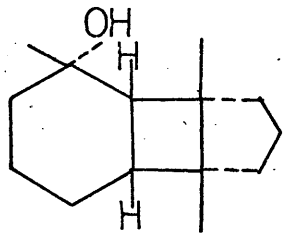
low yield but is unstable at room temperature. Our attention then turned to 4-alkylidene cyclohexenones. These compounds equilibrate on standing at room temperature to give, depending on substituents, various proportions of conjugated and unconjugated dienones. The preparation of compounds of this type was described by Inhoffen et al [65]. Anisaldehyde (95) on treatment with ethylmagnesium bromide gave the secondary alcohol (96) in high yield. Birch reduction [66] of (96) with sodium in liquid ammonia, followed by acid hydrolysis of the vinyl ether (97) gave a mixture of the dienones (98) and (99) which equilibrated on standing (55% of (98) : 45% of (99)). If this mixture of (98) and (99) is refluxed for 15 minutes in 3M sulphuric acid the conjugated dienone (98) accounts for 87% of the total material. However, there is a return to the former ratio on standing.

A pilot photolysis was carried out using cyclohexenone (100) and 1, 2 -dimethylcyclopentene (83), but even after six days irradiation the yield of addition products was disappointingly low (ca. 5%). However, the cyclo adducts (101) and (102) were separated by preparative t.l.c. on silver nitrate impregnated silica. The high volatility of these compounds made purification very difficult. The compound (101), ν max (liq. film) 1712 cm^{-1} has two tertiary methyl signals at δ 0·91 and 1·01 in its n.m.r. spectrum and the base peak at $^{m}/e$ 96 in its mass spectrum. The isomeric compound (102) has similar i.r. and mass spectra but has its methyl resonances at δ 0·91, 0·96.

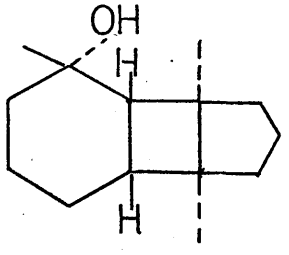
Treatment of (101) and (102) with methyl lithium gave the corresponding tertiary alcohols (103) and (104) in high yield. Examination of models indicates that the nucleophilic attack would be in the sense shown. The Eu $(dpm)_3$ shifted n.m.r. spectra confirmed the assignment of the structures (103) and (104). These results are shown in table (8). The shifted spectrum of (103) shows one tertiary methyl group moving rapidly downfield

TABLE 8

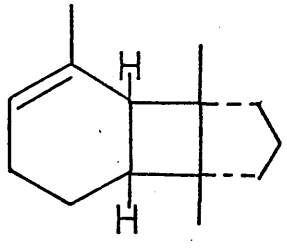
Molar ratio Eu (dpm)$_3$	Compound (103) δ Tertiary methyls	Compound (104) δ Tertiary methyls
0	0.89, 0.94, 1.27	1.21, 1.24, 1.32
approx. 0.2	2.05, 1.18, 1.16	2.76, 2.06, 2.06
approx. 0.4	3.82, 1.66, 1.49	5.15, 3.43, 3.23



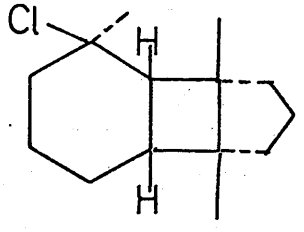
(103)



(104)



(105)



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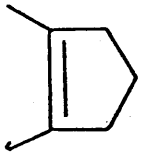
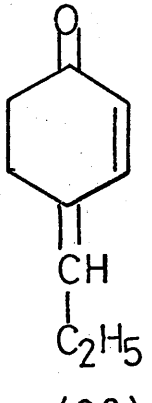


(83)



(98)

with the other two methyls showing only moderate shifts. As the concentration of Eu $(dpm)_3$ is increased the separation between these two tertiary methyls gradually increases. In the case of the isomeric compound (104) all three methyls move quickly downfield with one methyl in particular showing a very large shift. The i.r. spectra, ν_{max} (liq. film) 3,480, and mass spectra (base peak $^{m}/_{e}$ 95) of these compounds are very similar.

Treatment of the tertiary alcohol (103), i.e. the isomer with the required stereochemistry for gymnomitrol, with 40% aqueous sulphuric acid in tetrahydrofuran gave elimination instead of the expected rearrangement. The product obtained in high yield was the olefin (105) which has in its n.m.r. spectrum two tertiary methyls at δ 0·85 and 0·88 and a vinyl methyl, δ 1·22. In addition there is a very broad singlet at δ 5·58 due to the olefinic proton. This olefin (105) appears to be stable to treatment with HCl gas in chloroform. Models indicate that the stereochemistry of the tertiary hydroxy group in (103) is inverted with respect to Corey's compound [61] (see scheme 15, p. 75) and is well set up for elimination rather than the desired rearrangement. Attempts under various conditions to rearrange the alcohol (103) proved unsuccessful. A possible solution to this problem, may be to invert the stereochemistry of the hydroxy group using carbon tetrachloride and triphenylphosphine [67] to give the tertiary chloride (106).

At this point the photolysis was repeated using the olefin (83) and the enone (98) whose preparation is described above. Again the reaction was very slow and gave a very low yield. Lack of time prevented further investigation. A recent report [68] has appeared on the nickel (0) catalysed (2 + 2) cross-addition of olefins. Although the reaction is formally envisaged as a thermally forbidden $(_{\pi}2_{s} + _{\pi}2_{s})$ process [69] the intervention of a metallocyclic intermediate [70] in the metal catalysed (2 + 2) cycloaddition

reaction makes the reaction thermally allowed. The use of nickel (0) catalyst may, therefore, prove to be a useful alternative to the poor yield photochemical step.

EXPERIMENTAL

GENERAL

All melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded, by Mr. J. Gall and Mr. A. Haetzmann, on a Varian HA-100 or T-60 spectrometer using tetramethylsilane as an internal reference in deuterochloroform.

Ultraviolet absorption spectra were measured in ethanol solution using a Unicam S.P. 800 spectrometer. Infra-red solution spectra were recorded by Mrs. F. Lawrie and staff, on a Unicam S.P. 100 double beam spectrometer or on a Perkin-Elmer 225 instrument using carbon tetrachloride as solvent, unless otherwise stated.

Mass spectra were routinely determined by Mr. A. Ritchie on an A.E.I. - G.E.C. M.S. 12 mass spectrometer, high resolution spectra being obtained on an A.E.I. M.S. 902s instrument. Micro-analyses were carried out by Mr. J.M.L. Cameron, B.Sc. and Miss F. Cowan and staff. Optical rotations were measured in chloroform solution. Circular dichroism and optical rotatory dispersion spectra were recorded by Prof. W. Klyne and Dr. P.M. Scopes, Westfield College, London.

Chromatographic separations were effected using commercial "Woelm" alumina for column separations and Merck's "Kieselgel G" for both analytical and preparative thin layer chromatography.

Light petroleum refers to the fraction of boiling point 40 - 60°.

EXTRACTION

Powdered *g. obtusum* (100g) was extracted with hot chloroform in a

Soxhlet apparatus and the crude extract (22g) chromatographed over basic alumina (grade H) using ether and gradually increasing concentrations of ethyl acetate in ether. The column was finally washed with ethyl acetate and methanol.

Preparative t.l.c. of the early hydrocarbon fraction over silver nitrate-impregnated silica gave gymnomitrene (2) (60 mg), sublimed as an oil (60°, 0·01 mmHg), $[\alpha]_D$ - 26° (C4·97); νmax (CCl_4) 3070, 1641, 890 cm^{-1}, δ0·83, 0·89, 1·02 (tertiary methyls), 4·58 b.s. (2H, C=C$\underline{H}_2$), [Found : C, 88·26 ; H, 12·07. $C_{15}H_{24}$ requires C, 88·16 ; H, 11·84%].

The main component of the extract was gymnomitrol (1) (800 mg) which was purified by preparative t.l.c. and sublimed (50°, 0·01 mmHg) as crystals, m.p. 114 - 116°, $[\alpha]_D$ + 7° (C2·28); νmax (CCl_4) 3628 (sharp, free OH), 3070, 1641, 888 cm^{-1}, δ0·97, 1·07, 1·21 (tertiary methyls), 2·32 (s, $\underline{H}_n$), 3·70 (s, C$\underline{H}$OH), 4·63 and 4·65 (s, C=C$\underline{H}_2$), [Found : C, 81·52 ; H, 10·94. $C_{15}H_{24}O$ requires C, 81·76 ; H, 10·98%].

Gymnomitryl acetate (36) (300 mg) which was also isolated from the extract sublimed (75°, 0·01 mmHg) as crystals, m.p. 64 - 65°, $[\alpha]_D$ + 19° (C4·25) ; νmax (CCl_4) 3070, 1740, 1641, 891 cm^{-1}, δ0·85, 1·04, 1·12 (tertiary methyls), 2·03 ($C\underline{H}_3CO_2$-), 2·37 (s, $\underline{H}_n$), 4·68 (s, C$\underline{H}$OAc), 4·71 and 4·76 (C=C$\underline{H}_2$), [Found : C, 77·91 ; H, 10·06. $C_{17}H_{26}O_2$ requires C, 77·82 ; H, 9·92%].

From the intermediate fractions of the extract the epoxy diacetate (49) (250mg) was separated by t.l.c. and recrystallised from cold petrol as needles, m.p. 155 - 156°, $[\alpha]_d$ - 60° (C0·58) ; νmax (CCl_4) 1730 cm^{-1}, δ0·92, 1·07, 1·15 (tertiary methyls), 2·03, 2·05 ($C\underline{H}_3CO_2$-), 1·36 (s, $\underline{H}_n$), 2·76 (s, 2H, $C\underline{H}_2$<O>C), 5·28 (s, C$\underline{H}$OAc), 5·55, 5·65 (q., J11, $6H_Z$, CHOAc), [Found : C, 67·64; H, 8·31. $C_{19}H_{28}O_5$ requires C, 67·83 ; H, 8·39%]. A minor component of these fractions was the diacetate (50)

(80 mg) which sublimed (110°, 0·01mmHg) as an oil, $[\alpha]_D$ + 14° (C2·84) ; νmax (CCl_4) 3075, 1739, 1645, 897 cm^{-1}, δ0·87, 1·08, 1·12 (tertiary methyls), 1·99, 2·06 ($C\underline{H}_3$ CO_2-), 2·44 (s, $\underline{H}_n$), 4·82 (partially obscured singlet, C$\underline{H}$OAc), 5·03, 5·13 (q, J 11, $6H_Z$, C$\underline{H}$OAc), 4·82, 4·78 (C=C$\underline{H}_2$), [Found : C, 71·39 ; H, 9·06. C_{19} H_{28} O_4 requires C, 71·22 ; H, 8·81%]. The epoxy-acetate (42) (60 mg) was separated by preparative t.l.c. and sublimed (110°, 0·01 mmHg) as crystals, m.p. 149-150°, $[\alpha]_D$ - 27° (C2·25) ; νmax (CCl_4) 1749 cm^{-1}, δ0·92, 1·09, 1·16 (tertiary methyls), 2·04 (CH_3 CO_2-), 2·65 (s, 2H, C$\overset{O}{\frown}$C$\underline{H}_2$), 5·26 (s, C$\underline{H}$OAc), [Found : C, 73·39 ; H, 9·59. C_{17} H_{26} O_3 requires C, 73·34 ; H, 9·41%].

The more polar fractions of the extract yielded the hydroxyacetate (45) (60 mg) which, after preparative t.l.c., was sublimed (100°, 0·005 mmHg) as an oil, $[\alpha]_D$ + 52° (C 14·85), νmax (CCl_4) 3615 (free OH, increases on dilution), 3500 (very broad, bonded OH, disappears on dilution), 1749, 1735 (double acetate absorption, no change on dilution), δ0·94, 1·05, 1·14 (tertiary methyls), 2·07 ($C\underline{H}_3$ CO_2-), 4·00 (b.s., $W_{\frac{1}{2}}$ $6·5H_Z$, 2H, $C\underline{H}_2$-OH), 5·02 (s, C$\underline{H}$OAc), 5·44 (b.s., $W_{\frac{1}{2}}$ $7H_Z$, C=C$\underline{H}$-), [Found : C, 73·15 ; H, 9·46. C_{17} H_{26} O_3 requires C, 73·4 ; H, 9·41%]. The major product from the polar fractions was the hydroxydiacetate (78) (200 mg), which sublimed (160°, 0·05 mmHg) as an oil, $[\alpha]_D$ + 35° (c 0·85) ; νmax (CCl_4) 3620 (broad, free OH), 3550 (very broad, bonded OH) 1735 cm^{-1}., δ0·94, 1·10 (6H) (tertiary methyls), 2·03, 2·09 ($C\underline{H}_3$ CO_2-), 2·17 (s, $\underline{H}_n$), 4·03 (b.s., $W_{\frac{1}{2}}$ $7H_Z$, 2H, $C\underline{H}_2$OH), 5·07 (s, C$\underline{H}$OAc), 4·09, 5·21 (q., J11, $6H_Z$, C$\underline{H}$OAc), 5·56 (b.s., $W_{\frac{1}{2}}$ $7H_Z$, C=C$\underline{H}$-), [Found : C, 67·76 ; H, 8·35. C_{19} H_{25} O_5 requires C, 67·83 ; H, 8·39%].

OXIDATION OF GYMNOMITROL (1)

Gymnomitrol (1) (50 mg) in dry acetone (5 ml) was treated with a slight excess of 6N Jones' reagent at room temperature for five minutes. Normal work-up and prep. t.l.c. gave gymnomitrone (37) (46 mg) which sublimed (85°, 0·01 mmHg) as crystals, m.p. 84-85°, νmax (CCl_4) 3080, 1745 (5m. ring ketone), 1639, 892 cm^{-1}, δ0·82, 0·91, 0·92 (tertiary methyls), 2.63 (s, H_n), 4·74, 4·77 (C=CH_2), [Found : C, 82·64 ; H, 10·07. $C_{15}H_{22}O$ requires C, 82·51 ; H, 10·16%]. Sodium borohydride reduction of the ketone (37) gave exclusively gymnomitrol (1).

OZONOLYSIS OF GYMNOMITRONE (37)

The ketone, (37), (40 mg) in ethyl acetate (5 ml) was cooled to -70° and ozone passed through the solution for 15 minutes. The resultant blue solution was allowed to stand at -70° for one hour after which the solvent was removed at room temperature by blowing with nitrogen. Aqueous methanol was added and the reaction mixture stirred overnight at room temperature. Normal extraction procedure followed by prep. t.l.c. and sublimation (95°, 0·01 mmHg) furnished the nor-diketone (38) (35 mg) as a non-crystalline solid, νmax (CCl_4) 1749 (5m. ring ketone), 1710 (6m. ring ketone), δ0·91, 1·02, 1·09 (tertiary methyls), 3·00 (s, H_n), [Found : C, 76·57 ; H, 9·25. $C_{14}H_{20}O_2$ requires C, 76·32 ; H, 9·15%].

THE NOR-HYDROXY ACETATE (41)

The nor-diketone (38) (30 mg) in methanol (10 ml) was treated with excess sodium borohydride for ½ hour at room temperature. Normal work-up

yielded a product which was acetylated in pyridine (2 ml) and acetic anhydride (2 ml) at 20° overnight. Purification and sublimation (120°, 0·01 mmHg) gave the nor-hydroxy acetate (41) as crystals, m.p. 108-109°, ν_{max} (CCl_4) 3625 (sharp, free OH, increases on dilution), 3500 (very broad, bonded OH, decreases on dilution), 1739, 1729 (double carbonyl absorption, no change on dilution), δ0·94, 1·10, 1·12 (tertiary methyls), 2·02 (CH_3CO_2-), 5·69 (s, CHOH), 5·0 (m., CHOAc), [Found : C, 72·08 ; H, 9·93. $C_{16}H_{26}O_3$ requires C, 72·14 ; H, 9·84%].

ACETYLATION OF GYMNOMITROL (1)

Gymnomitrol (50 mg), in pyridine (2 ml) and acetic anhydride (2 ml), was heated on a steam bath for 4 hours. Normal work-up procedure followed by prep. t.l.c. yielded gymnomitryl acetate (36) (46 mg) identical in all respects to the naturally occurring acetate.

OZONOLYSIS OF GYMNOMITRYL ACETATE (36)

The acetate (36) (40 mg) was ozonised under the same conditions as described previously for the ketone (37) to give the nor-ketoacetate (39) (34 mg) which sublimed (110°, 0·02 mmHg) as an oil, ν_{max} (CCl_4) 1749 (acetate), 1718 (6m. ketone), δ0·96, 1·06, 1·16 (tertiary methyls), 2·04 (CH_3CO_2-), 2·45 (s, H_n), 5·04 (s, CHOAc), [Found : C, 72·74 ; H, 9·05. $C_{16}H_{24}O_3$ requires C, 72·69 ; H, 9·15%].

Sodium borohydride reduction of the nor-ketoacetate (39) afforded the nor-hydroxyacetate (40) which sublimed (120°, 0·02 mmHg) as crystals, m.p. 135-136°, ν_{max} (CCl_4) 3610 (free OH), 3500 (very broad bonded OH), 1740 (shoulder), 1725 (sharp, acetate), δ0·83, 1·05, 1·12 (tertiary methyls),

2·03 ($C\underline{H}_3\ CO_2$-), 4·13 (m, C<u>H</u>OH), 4·59 (s, C<u>H</u>OAc), [Fcund : C, 72·25 ; H, 10·09. $C_{16}\ H_{26}\ O_3$ requires C, 72·14 ; H, 9·84%].

TREATMENT OF GYMNOMITROL (1) WITH ACID

Dry HCl gas was bubbled for 1 hour through a solution of gymnomitrol (1) (50 mg) in analar chloroform (15 ml). The reaction mixture was washed with water and the chloroform layer dried, and evaporated under reduced pressure. Preparative t.l.c. afforded two products. The minor product, isogymnomitrol (48) (18 mg) was sublimed (80°, 0·04 mmHg) as needles, m.p. 82-84°, ν max (CCl_4), 3625 (sharp, free OH), 1669, δ0·92, 0·99, 1·15 (tertiary methyls), 1·47 (s, disappears on addition of D_2O, O<u>H</u>), 1·62 (vinyl methyl), 1·76 (s, $\underline{H}_n$), 3·97 (s, C<u>H</u>OH), 5·05 (b.s., $W_{\frac{1}{2}}$ 9H_Z, C=C<u>H</u>), [Found : C,81·75 ; H, 11·12. $C_{15}\ H_{24}\ O$ requires C, 81·76 ; H, 10·98%].

The major product was the epimeric alcohol (55) (29 mg) and sublimed (80°, 0·01 mmHg) as oily crystals, m.p. 57-59°, ν max (CCl_4) 3630 (sharp, free OH), 3570 (sharp, bonded OH), δ0·86, 0·88, 1·04 (tertiary methyl), 1·67 (t, J2H_Z, vinyl methyl), 1·94 (d, J4·2H_Z, $\underline{H}_n$). 3·86 (d, J4·2H_Z, C<u>H</u>OH), 5·47 (b.s., $W_{\frac{1}{2}}$ 8H_Z, C=C<u>H</u>), [Found : C, 81·69 ; H, 11·14. $C_{15}\ H_{24}\ O$ requires C, 81·76 ; H, 10·98%].

Gymnomitrol (1) (10 ml) was stirred for 3 hours in methanol (10ml) and 3<u>M</u> H_2SO_4 (3 ml) to give isogymnomitrol (48) (7 mg) and the epimeric alcohol (55) (2 mg). Treatment of isogymnomitrol (48) with HCl gas in chloroform gave the epimeric alcohol (55) as the major product with a small amount of recovered starting material.

PREPARATION OF ISOGYMNOMITRONE (56)

Jones' oxidation of isogymnomitrol (48) (20 mg) gave the ketone, isogymnomitrone (56) (17 mg) which sublimed (100°, 0·01 mmHg) as an oil, ν max (CCl_4) 1746 (5m. ring ketone), δ0·80, 0·94 (6H) (tertiary methyls), 1·69 (t, J2H_z vinyl methyl), 2·02 (s, H_n) 5·32 (b.s., $W_{\frac{1}{2}}$ 7H_z, C=C$\underline{H}$), [Found : C, 82·79 ; H, 10·38. $C_{15}H_{22}O$ requires C, 82·51 ; H, 10·16%].

This ketone (56) can also be prepared by Jones' oxidation of the epimeric alcohol (55) or by acid isomerisation of gymnomitrone (37).

HYDROGENATION OF GYMNOMITROL (1)

Hydrogenation of gymnomitrol (1) (20 mg) at atmospheric pressure for 1½ hours over Adam's catalyst in ethyl acetate gave the saturated alcohol, dihydrogymnomitrol (57) (18 mg), which sublimed (80°, 0·02 mmHg) as crystals, m.p. 72-73°, ν max (CCl_4) 3625 (sharp, free OH), 3470 (very broad, bonded OH), δ0·91, 1·09, 1·21 (tertiary methyls), 1·10 (d, J7H_z, secondary methyl), 3·68 (s, C$\underline{H}$OH), [Found : C, 81·19 ; H, 11·91. $C_{15}H_{26}O$ requires C, 81·02 ; H, 11·79%].

Using palladium- charcoal as catalyst the hydrogenation gave only isogymnomitrol (48). Only starting material was recovered on treatment of dihydrogymnomitrol (57) with HCl gas in chloroform.

DIHYDROGYMNOMITRONE (59)

Dihydrogymnomitrol (57) (15 mg) was oxidised in the usual manner with Jones' reagent to give dihydrogymnomitrone (59) (13 mg) which sublimed (90°, 0·01 mmHg), as an oil, ν max (CCl_4) 1740 cm^{-1}, δ0·78, 0·86, 0·92

(tertiary methyls), 1·14 (d, J7H_z, secondary methyl), (Found : C, 81·91 ; H, 11·07. $C_{15}H_{24}O$ requires C, 81·76 ; H, 10·98%).

EPOXIDATION OF GYMNOMITRYL ACETATE (36)

The acetate (36) (100 mg) in analar chloroform (10 ml) was stirred at room temperature for one hour with m-chloroperbenzoic acid (110 mg). After filtration through a short column of basic alumina, evaporation of the solvent and prep. t.l.c. yielded two products.

The more polar product (76 mg) was sublimed (110°, 0·01 mmHg), to give the β-epoxyacetate (42) identical in all respects with that found in the extract. The less polar product (19 mg) was sublimed (110°, 0·01 mmHg) as an oil to give the α-epoxyacetate (43), $[\alpha]_D$ + 107° (c 0·42), ν max (CCl_4) 1745, 1731 (double acetate absorption), δ0·92, 1·08, 1·11 (tertiary methyls), 1·41 (s, H_n), 2·61 (s, 2H, C$\overset{O}{\diagup\diagdown}CH_2$), 4·87 (s, CHOAc), [Found : C, 73·48 ; H, 9·62. $C_{17}H_{26}O_3$ requires C, 73·34 ; H, 9·41%].

THE HYDROXYACETATE (45)

The epoxyacetate (42) (30 mg) in aqueous methanol (10 ml) was treated with dilute sulphuric acid (1 ml) and stirred at room temperature for 2 hours. Work-up gave the hydroxyacetate (45) (28 mg) identical in all respects with the naturally occuring material. The derived diacetate (46) sublimed 115°, 0·01 mmHg) as an oil, ν max (CCl_4) 1745 cm^{-1}, δ0·92, 1·04, 1·13 (tertiary methyls), 2·06 (6H) (CH_3CO_2-), 4·44 (2H, b.s., $W_{\frac{1}{2}}$ 4·5H_z, C=C-CH_2-OAc), 5·01 (s, CHOAc), 5·49 (b.s., $W_{\frac{1}{2}}$ 7H_z, C=CH), [Found : C, 71·31 ; H, 8·83. $C_{19}H_{28}O_4$ requires C, 71·22 ; H, 8·81%].

ALLYLIC OXIDATION OF THE DIACETATE (46)

The diacetate (46) (12 mg) in acetic acid (1.3 ml) and acetic anhydride (0.7 ml) was stirred at 30° overnight with anhydrous sodium chromate (10 mg). After cooling and addition of water the crude product was extracted with chloroform. Purification by prep. t.l.c. yielded the enone (47) (8 mg) which sublimed (120°, 0·015 mmHg) as an oil, λ max 240 n.m., (ϵ 10,000), ν max (CCl_4) 1751 (acetate), 1680 (enone), 1645 cm^{-1}, δ1·08, 1·12, 1·25 (tertiary methyls), 2·08, 2·13 (CH_3CO_2-), 2·45 (s, H_n), 4·77 (d, 2H, J2H_Z, C=C-CH_2-OAc), 4·85 (s, CHOAc), 6·06 (b.s., $W_{\frac{1}{2}}$ 4H_Z, C=CH), [Found : C, 68·46 ; H, 8·07. $C_{19}H_{26}O_5$ requires C, 68·24 ; H, 7·84%].

INTERCONVERSION OF THE EPOXYDIACETATE (49) AND THE HYDROXYDIACETATE (78)

The epoxy-diacetate (49) (10 mg) was treated with dilute sulphuric acid (1 ml) in methanol (5 ml) for 1 hour at room temperature. The product obtained was identical in all respects with the naturally occuring hydroxydiacetate (78).

HYDROLYSIS OF THE EPOXY-DIACETATE (49)

To the epoxy-diacetate (49) (50 mg) in methanol (5 ml) and water (3 ml), potassium carbonate (100 mg) was added and the reaction mixture refluxed for 1 hour. After cooling, normal extraction and purification procedures gave the epoxy-hydroxyacetate (51) (38 mg) which sublimed (140°, 0·005 mmHg) as needles, m.p. 122-123°, ν max (CCl_4) 3625 (free OH, increases on dilution), 3490 (very broad, disappears on dilution), 1749, 1735 (double acetate absorption, no change on dilution) δ0·91, 1·08, 1·11 (tertiary methyls), 2·06 (CH_3-CO_2-),

2·77, 2·68 (ABq., J_{AB} $4H_Z$ C$\overset{O}{\diagup\!\diagdown}C\underline{H}_2$), 4·53, 4·63 (q (after D_2O exchange), J11, $6H_Z$, C$\underline{H}$OH), 5·23 (s, C$\underline{H}$OAc), [Found : C, 69·26 ; H, 8.99. $C_{17}H_{26}O_4$ requires C, 69·36 ; H, 8·90%].

FORMATION OF THE EPOXY-KETOACETATE (52)

Chromium trioxide (60 mg) was added to a stirred solution of pyridine (95 mg) in acid free methylene chloride (1·5 ml). The flask was stoppered with a drying tube and the deep burgandy solution was stirred for 15 minutes at room temperature. A solution of the alcohol (51) (30 mg) in a small volume of methylene chloride was then added in one portion. A tarry black deposit separated immediately and after stirring for an additional 15 minutes at 20°, the solution was decanted from the residue. The latter was washed twice with methylene chloride and the combined methylene chloride solutions were washed with saturated sodium bicarbonate solution, water and then dried. After evaporation of the solvent, prep. t.l.c. furnished the epoxy-ketoacetate (52) (29 mg) which sublimed (140°, 0·01 mmHg) as plates, m.p. 161-162°, νmax (CCl_4) 1735 with shoulders at 1745 and 1755 cm^{-1}. δ0·96, 1·25, 1·26 (tertiary methyls), 2·06 ($C\underline{H}_3CO_2$-), 2·65, 2·83 (ABq, J_{AB} $4H_Z$, C$\overset{O}{\diagup\!\diagdown}C\underline{H}_2$), 5·25 (s, C$\underline{H}$OAc), [Found : C, 69·99 ; H, 8·35. $C_{17}H_{26}O_4$ requires C, 69·83; H, 8.27%].

SODIUM BOROHYDRIDE REDUCTION OF THE EPOXY-KETOACETATE (52)

The epoxy-ketoacetate (52) (20 mg) in methanol (5 ml) was stirred for 4 hours at 20°. Sodium borohydride was added periodically during this time. Normal work-up followed by prep. t.l.c. gave recovered starting material (52) (6 mg) and the hydroxy ether (53) (11 mg), ν max (CCl_4) 3590, 3460,

1739 cm^{-1} (acetate), δ0·92, 1·06, 1·16 (tertiary methyls), 1·61 (s, disappears with D_2O, O<u>H</u>), 2·03 (C<u>H</u>$_3CO_2$-), 3·41 (s, (after D_2O exchange) $W_{\frac{1}{2}}$ $3H_Z$, 2H, C<u>H</u>$_2$OH), 4·08 (t, J $3H_Z$, C<u>H</u>-O), 5·04 (s, C<u>H</u>-OAc).

The derived acetate (54) sublimed (130°, 0·01 mmHg) as an oil, ν max (CCl_4), 1744 cm^{-1}, δ0·92, 1·07, 1·15 (tertiary methyls), 2·05, 2·08 (C<u>H</u>$_3CO_2$-), 3·86, 4·06 (ABq, J_{AB} 11H_Z, C<u>H</u>$_2$ OAc), 4·03 (partially obscured t, J $3H_Z$, C<u>H</u>-O), 5·08 (s, C<u>H</u>OAc), [Found : C, 67·96 ; H, 8·57. $C_{19}H_{28}O_5$ requires C, 67·83 ; H, 8·39%].

<u>ISOGYMNOMITRENE (3)</u>

Gymnomitrene (2) (10 mg) was treated with dry HCl gas in chloroform, as described previously. Subsequent preparative t.l.c. gave <u>isogymnomitrene</u> (3) which sublimed (60°, 0·01 mmHg) as an oil, νmax (CCl_4) 3070, 1620, δ0·86, 0·91, 1·01 (tertiary methyls), 1·67 (t, J $2H_Z$, C=C-C<u>H</u>$_3$), 5·17 (b.s., $W_{\frac{1}{2}}$ $8H_Z$, C=C<u>H</u>), [Found : M^+ (mass spectrometry), 204 · 1879. $C_{15}H_{24}$ requires M, 204 · 1878].

<u>WOLFF-KISHNER REDUCTION OF GYMNOMITRONE</u>

Gymnomitrone (37) (50 mg), triethylene glycol (5 ml), potassium hydroxide (100 mg), hydrazine hydrate (0·5 ml) were heated at 200° for two hours. Normal work-up gave two products, the least polar of which was starting material (37) (15 mg). The more polar material (23 mg) sublimed (220°, 0·01 mmHg) as an glass, δ0·94 0·99, 1·10 (tertiary methyls), 1·82, 2·02 (s, methyls?), 3·24, (s,), 4·68 (t, C=C<u>H</u>$_2$), [Found : M^+ (mass spectrometry) 432 · 3493. $C_{30}H_{44}N_2$ requires M, 432 · 3504].

CLEMMENSEN REDUCTION OF GYMNOMITRONE (37)

Amalgamated zinc dust was prepared by shaking zinc powder (300 mg), mercuric chloride (20 mg), conc. HCl (0·15 ml) and water (2 ml) for 5 minutes at room temperature. The aqueous solution was decanted off and the amalgamated zinc covered with conc. HCl (2·5 ml) and water (3 ml). Gymnomitrone (37) (100 mg) in ethanol (2 ml) was added and the reflux started immediately. After 4 hours reflux, extraction with ether, two hydrocarbon products were obtained. These products were separable by G.L.C. ($2\frac{1}{2}$% SE-30 capillary column, L.T.P. 100-220° at 2° per minute) or by prep. t.l.c. using silica impregnated with 30% silver nitrate. The least polar hydrocarbon (38 mg) was identified as isogymnomitrene (3). The slightly more polar hydrocarbon (60) (41 mg) has, δ0·82, 0·99, 1·10 (tertiary methyls), 1·64 (C=C-C$\underline{H}_3$), 5·01 (b.d., J 2·5H_Z, C=C$\underline{H}$), [Found : M^+ (mass spectrometry) 204 · 187218. $C_{15}H_{24}$ requires, M, 204 · 187790].

ATTEMPTED INTERCONVERSION OF THE EPIMERIC ALCOHOL (55) WITH ISOGYMNOMITRENE (3)

The alcohol (55) (20 mg) in dry pyridine (2 ml) was treated with a slight excess of p-toluene sulphonyl chloride at room temperature overnight to give the corresponding tosylate (18 mg). Reduction of the tosylate with excess lithium aluminium hydride in dry ether (5 ml) under reflux for 3 hours gave only unchanged tosylate. When the reaction was repeated using dry tetrahydrofuran as solvent under reflux for 4 hours the alcohol (55) was the only detectable product.

2-METHYL, 2-CARBOXYETHYLCYCLOPENTANONE (90)

Sodium (14•0 g) in toluene (724 ml) were heated until the sodium melted and the mixture stirred vigorously to break the sodium into small pieces. Diethyl adipate (101 g) was then added and reflux continued for two hours. The sodium salt of 2-carboxyethylcyclopentan-1-one was present under the spongy mass. Toluene and ethanol (125 mls), which formed during the reaction were distilled off. Another 75 ml of toluene were added and the distillation continued until the total volume of distillate reached 200 ml.

The reaction was then cooled in an ice-bath and the methyl iodide (160 g) added. After the addition was complete the mixture was heated under reflux for 16 hours. Addition of water to dissolve the sodium iodide, separation of the toluene layer, and the normal drying procedure gave 2-methyl, 2-carboxyethylcyclopentanone (90) (70 g), b.p. 105° (10 mm Hg) (litt [63] 105-106°, under 14 mm Hg).

2-METHYLCYCLOPENTANONE (91)

2-methyl, 2-carboxyethylcyclopentanone (90) (68 g) in dilute hydrochloric acid (250 ml) were refluxed for 16 hours. Extraction with ether, normal drying procedure and distillation gave 2-methyl cyclopentanone (91) (31 g), b.p. 139-141° at 760 mm (litt [63] 138-140° at 760 mm.).

1, 2 -DIMETHYLCYCLOPENTENE (83)

The Grignard reagent was prepared in the normal manner at -5° with magnesium turnings (14•4 g) methyl iodide (85•2 g) and anhydrous ether (250 ml). After the Grignard reagent had formed the reaction mixture was

kept below -5° and 2-methylcyclopentanone (91) (25 g) in ether (250 ml) added with stirring over $2\frac{1}{2}$ hours. The reaction mixture was allowed to come to room temperature andstirring continued for a further 2 hours. Cautious addition of dilute acid and ice followed by extraction with ether gave a crude product which on distillation at $80-86^{\circ}$, 760 mm. dehydrated to give the olefins (83) and (93) (17·6 g). Further distillation gave 1, 2 dimethylcyclopent-1-ene (83) (91% pure by g.l.c.), b.p. 106° (litt [71] $105·8^{\circ}$, 760 mm.).

ANISALDEHYDE (95) WITH ETHYLMAGNESIUM BROMIDE

Normal Grignard procedure using ethyl bromide (20 g), magnesum turnings (5 g), anisaldehyde (20 g) and dry ether (400 ml) at -5° gave the secondary alcohol (96) (20 gm). The crude product was washed four times with sodium metabisulphite to remove excess anisaldehyde.

BIRCH REDUCTION OF THE SECONDARY ALCOHOL (96)

The alcohol (96) (20 g) in absolute alcohol (40 ml) were added over 20 minutes to a stirred solution of sodium (25 g) in ammonia (750 ml). Thirty minutes after the addition was complete, absolute alcohol (30 ml) was added and the ammonia allowed to boil off overnight at room temperature. Water (750 ml) was then added and the products extracted with methylene chloride and washed and dried in the usual manner.

The Birch reduction product (16 g) was refluxed in methanol (260 ml) with 2N sulphuric acid (150 ml) for 15 minutes. Extraction with ether etc. gave a product which was predominantly the conjugated dienone, 4-propyliden-cyclohex-2-en-1-one (98) (15 g) [65].

PHOTOLYSIS OF CYCLOHEXANONE AND 1, 2 -DIMETHYLCYCLOPENTENE

Cyclohexenone (100) (1 g) and 1, 2 -dimethylcyclopentene (83) (1 g) in pentane (100 ml), under an Argon atmosphere, were photolysed at room temperature using an Hanovia type 1L photochemical reactor fitted with a medium pressure mercury arc lamp. The reaction was monitored by g.l.c. and after six days the reaction had gone to about 6% completion. Preparative t.l.c. afforded two isomers (101) and (102) (200 mg) in approximately equal amounts. The ketone (101) has ν max (liq film) 1712 cm^{-1}, δ 0·91, 1·01 (tertiary methyls), [Found : M^+ (mass spectroscopy), 192. $C_{13}H_{20}O$, requires M, 192]. The isomeric ketone (102) has νmax (liq. film) 1712 cm^{-1}, δ 0·91, 0·96 (tertiary methyls), [Found : M^+ (mass spectroscopy), 192. $C_{13}H_{20}O$, requires M, 192].

METHYL LITHIUM REDUCTION OF KETONES (101) and (102)

The ketones (101) and (102) (100 mg) were treated separately with a small excess of methyl lithium in dry ether under a nitrogen atmosphere for 15 minutes. Cautious addition of water followed by normal isolation procedure gave the tertiary alcohols (103) and (104) (96 mg). The alcohol (103) has ν max (liq. film) 3,480 (bonded OH), δ 0·89, 0·94 (tertiary methyls), 1·27 (carbinol methyl), [Found : M^+ (mass spectroscopy), 208. $C_{14}H_{24}O_1$ requires M, 208].

The isomeric alcohol (104) has ν max (liq. film) 3,480 (bonded OH), δ 1·21, 1·24 (tertiary methyl), 1·32 (carbinol methyl), [Found : M^+ (mass spectroscopy), 208. $C_{14}H_{24}O_1$ requires M, 208].

ACID TREATMENT OF ALCOHOL (103)

The alcohol (103) (30 mg) in tetrahydrofuran (5 ml) was treated with 40% aqueous sulphuric acid (5 ml) at room temperature for three hours. Normal work up yielded the olefin (105) (22 mg), δ 0·85, 9·88 (tertiary methyls), 1·22 (vinyl methyl), 5·58 (C=C<u>H</u>-), [Found : M^+ (mass spectrometry) 190. $C_{14}H_{22}$ requires M, 190].

PHOTOLYSIS OF 4-PROPYLIDENE-CYCLOHEX-2-EN-1-ONE (98) and 1, 2 -DIMETHYL-CYCLOPENTENE (83)

This photolysis was carried out as before using dienone (98) (10 g) and the olefin (83) (10 g). After six days irradiation, preparative t.l.c. afforded a mixture of four (g.l.c.) isomeric cycloaddition products (400 mg) which were not investigated further.

REFERENCES

1. K.R. Rao, N. Malakondaiah, and K.V.R. Ramana, Z. Pflanzenphysiol., 1969, 61, 168.
2. K. Muller, Hoppe-Seylers Z. Physiol. Chem., 1905, 45, 299.
3. R. Hegnauer, Chemotaxonomie der Planzen, 1962, Band 1, 176.
4. J.D. Connolly, A.E. Harding, and (in part) I.M.S. Thornton, J.C.S. Chem. Comm., 1972, 1320.
5. a) N.H. Andersen, C.R. Costin, C.M. Kramer, Y. Ohta and S. Huneck, Phytochemistry, in press ; b) N.H. Andersen, private communication ; c) N.H. Anderson and S. Huneck, Phytochemistry, in press.
6. N.H. Andersen and D.D. Byrdal, Tetrahedron Letters, 1972, 899.
7. P. Stafford, B.Sc. Thesis, University of Glasgow, 1973.
8. a) A. Matsuo, Tetrahedron, 1971, 27, 2757 ; b) S. Hayashi, A. Matsuo and T. Matsuura, Experientia, 1969, 25, 1139 ; c) S. Hayashi, A. Matsuo, Experientia, 1970, 26, 347.
9. A. Matsuo, M. Nakayama, Y. Ishida and S. Hayaski, Z. Naturforsch, 1971, 26b, 32.
10. V. Benesova, P. Ledmera, V. Herout and F. Sorn, Tetrahedron Letters, 1971, 2679.
11. V. Benesova, Hoang Minh Chau, and V. Herout, Phytochemistry, 1973, 12, 211.
12. N.H. Andersen, B. Shunk and C.R. Costin, Experientia, in press.
13. A.J. Weinheimer, W.W. Youngblood, P.H. Washecheck, T.K.B. Karns and L.S. Ciereszko, Tetrahedron Letters, 1970, 497.
14. H. Knoche, G. Ourisson, G.W. Perold, J. Foussereau and J. Maleville, Science, 1969, 166, 239.

15. S. Huneck and E. Klein, Phytochemistry, 1967, 6, 383.

16. A. Matsuo, Tetrahedron, 1972, 28, 1203, and references cited therein.

17. A. Matsuo, M. Nakayama, and S. Hayashi, Phytochemistry, 1971, 10, 430.

18. V. Benesova, Z. Samek, V. Herout and F. Sorn, Coll. Czech. Chem. Commun., 1969, 34, 582.

19. S.M. Krutov, Z. Samek, V. Benesova and V. Herout, Phytochemistry, 1973, 12, 1405.

20. V. Benesova, V. Herout and F. Sorn, Coll. Czech. Chem. Commun., 1969, 34, 1810.

21. S. Huneck, R. Grolle, and O. Velve, J. Hattori Bot. Lab., 1972, 36, 93.

22. J.D. Connolly and I.M.S. Thornton, Phytochemistry, 1973, 12, 631.

23. H. Knoche, G. Ourisson, G.W. Perold, J. Fossereau, and J. Maleville, Science, 1969, 166, 239.

24. J.D. Connolly and I.M.S. Thornton, J.C.S. Perkin I, 1973, 736.

25. S. Huneck and O. Velve, Z. Naturforch, 1970, 25b, 227.

26. A. Matsuo, M. Nakayama and S. Hayashi, Phytochemistry, 1971, 10, 430.

27. H. Erdtman, Tappi, 1949, 32, 303.

28. a) R.J. Pryce, Planta (Berl), 1971, 97, 354 b) S. Huneck and R.J. Pryce, Z. Naturforsch, 1971, 26b, 738.

29. V. Benesova and V. Herout, Coll. Czech. Chem. Commun., 1970, 35, 1926.

30. N.A. Tjukavkina, V. Benesova and V. Herout, Coll. Czech. Chem. Commun., 1970, 35, 1306.

31. K.R. Markham, L.J. Porter and B.G. Brehm, Phytochemistry, 1969, 8, 2193.

32. V. Benesova, Z. Samek, V. Herout and F. Sonn, Coll. Czech. Chem. Commun., 1969, 34, 1807.

33. M. Karplus, J. Amer. Chem. Soc., 1963, 85, 2870.

34. I.M.S. Thornton, Ph.D. Thesis, University of Glasgow, 1971.

35. K. Tori, K. Kitahonoki, Y. Takano, H. Tanida, and T. Tsuji, Tetrahedron Letters, 1964, 559.

36. R. Ratcliffe and R. Rodehorst, J. Org. Chem., 1970, 35, 4000.

37. H.M. McConnell, and R.E. Robertson, J. Chem. Phys., 1958, 29, 1361.

38. E.R.H. Jones and G. Lowe, J. Chem. Soc., 1960, 3959.

39. B.A. Achilladelis, P.M. Adams, and J.R. Hanson, Chem. Comm., 1970, 511.

40. R. Achini, B. Muller and Ch. Tamm, Chem. Comm., 1971, 404.

41. P.M. Adams and J.R. Hanson, Chem. Comm., 1971, 1414.

42. B.A. Achilladelis and J.R. Hanson, Chem. and Ind., 1967, 1643.

43. W.O. Godtfredsen and S. Vangedal, Acta. Chem. Scand., 1965, 19, 1088.

44. W. Parker, J.S. Roberts, and R. Ramage, Quart. Rev., 1967, 21, 331.

45. B.A. Achillandelis, P.M. Adams and J.R. Hanson, J.C.S. Perkin I, 1972, 1425.

46. S. Nozoe, M. Morisaki, and H. Matsumoto, Chem. Comm., 1970, 926.

47. S. Nozoe and Y. Machida, Tetrahedron, 1972, 28, 5105.

48. Y. Machida and S. Nozoe, Tetrahedron Letters, 1972, 1969.

49. I.J. Forrester and T. Money, Canad. J. Chem., 1972, 50, 3310.

50. W. Klyne, private communication.

51. G. Snatzke and G. Eckhardt, Tetrahedron, 1970, 26, 1143.

52. A. Moscowitz, K. Mislow, M.A.W. Glass and C. Djerassi, J. Amer. Chem. Soc., 1962, 84, 1945.

53. C.C. Hinkley, M.R. Klotz and F. Patil, J. Amer. Chem. Soc., 1971, 93, 2417 and references cited therein.

54. P.V. Demarco, T.K. Elzey, R.B. Lewis and E. Wenkert, J. Amer. Chem. Soc., 1970, 92, 5734.

55. J. Brigs, F.A. Hart and C.P. Moss, Chem. Comm., 1970, 1506.

56. B.L. Shapiro, J.R. Hlubucek, G.R. Sullivan and L.F. Johnson, J. Amer. Chem. Soc., 1971, 93, 3281.

57. C.C. Hinkley, J. Amer. Chem. Soc., 1969, 91, 5160.

58. G.H. Wahl and M.R. Peterson, Chem. Comm., 1970, 1167.

59. R.F. Frazer and Y.Y. Wigfield, Chem. Comm., 1970, 1471.

60. P.V. Demarco, B.J. Cerimele, R.W. Crane and A.L. Thakkar, Tetrahedron Letters, 1972, 3539.

61. E.J. Corey and S. Nozoe, J. Amer. Chem. Soc., 1964, 86, 1652.

62. R.M. Bowman, C. Calvo, J.J. McCullough, P.W. Rasmussen, and F.F. Snyder, J. Org. Chem., 1972, 37, 2084, and references cited therein.

63. L. Nicole and L. Berlinguet, Canad. J. Chem., 1962, 40, 353.

64. E.W. Garbisch, Jr., J. Amer. Chem. Soc., 1965, 87, 4971.

65. H.H. Inhoffen, D. Kampe and W. Milkouski, Annalen der Chemie 1964, Bd 674, 28.

66. A.J. Birch, Quart. Rev., 1950, 69.

67. J.B. Lee and I.M. Downie, Tetrahedron, 1967, 23, 359.

68. R. Noyori, T. Ishigami, N. Hayashi and H. Takaya, J. Amer. Chem. Soc., 1973, 95, 1674.

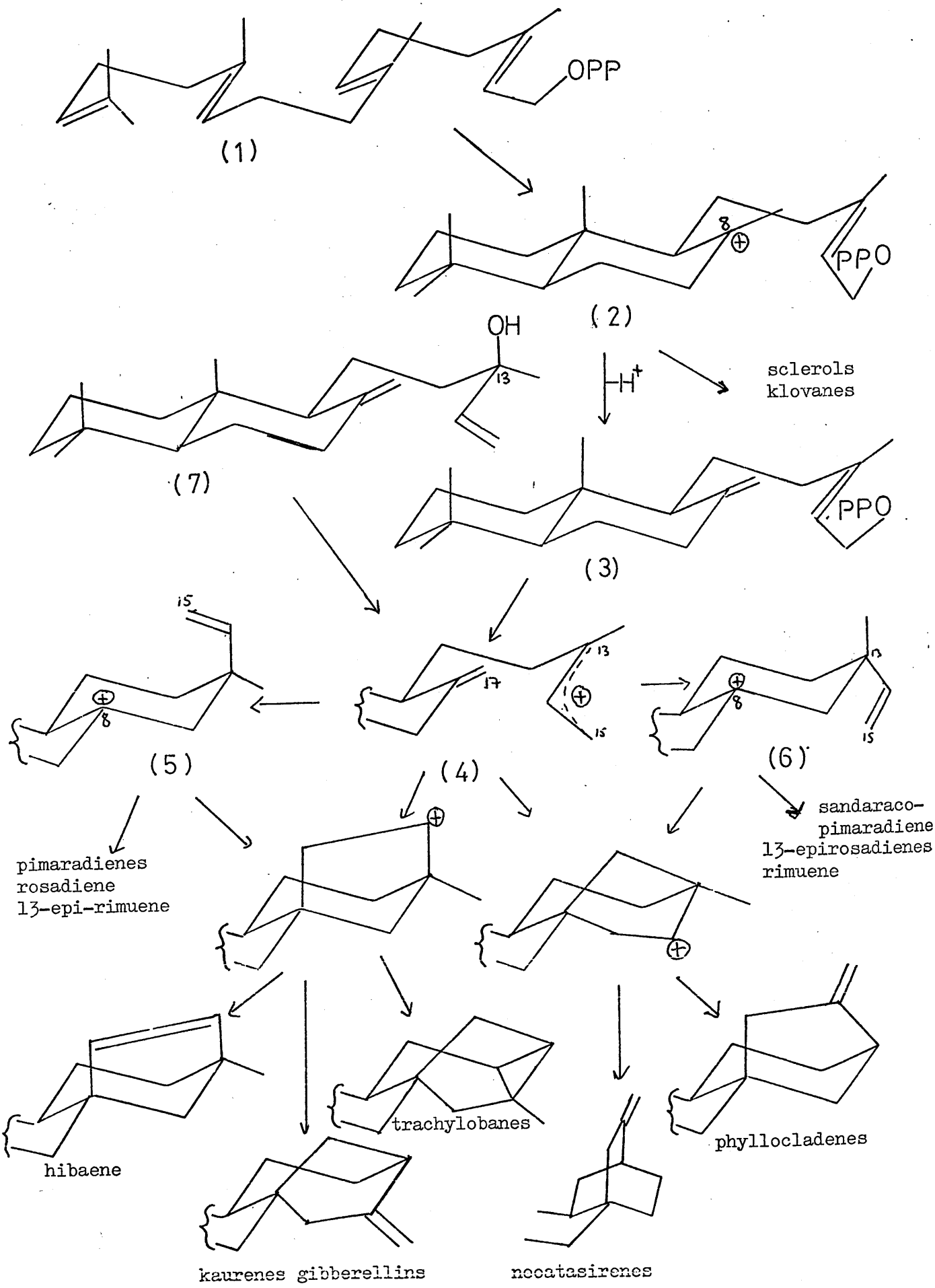
69. R.B. Woodward and R. Hoffmann, Angew. Chemie., 1969, 81, 797.

70. A.R. Fraser, P.H. Bird, S.A. Bezman, J.R. Shapley, R. White, and J.A. Osborn, J. Amer. Chem. Soc., 1973, 95, 597.

71. S. Siegel and B. Dmuchovsky, J. Amer. Chem. Soc., 1964, 86, 2192.

SECTION 2

SCHEME 1


OPP
(1)
8
PPO
(2)
OH
13
H+
sclerols
klovanes
(7)
PPO
(3)
15
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13
17
15
(5)
(4)
(6)
pimaradienes
rosadiene
13-epi-rimuene
sandaraco-
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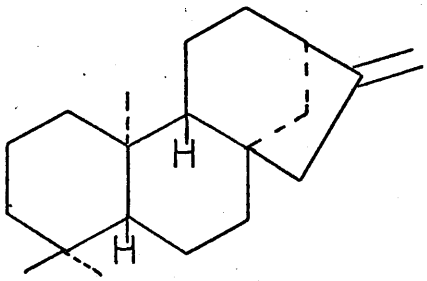

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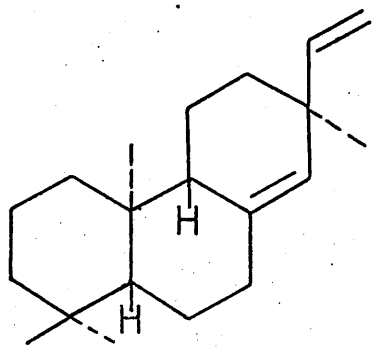

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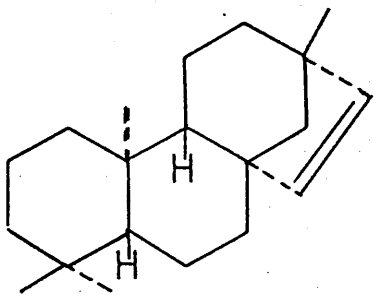

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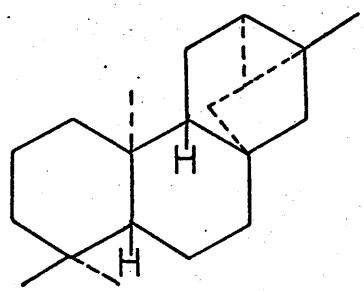


(−) −(11)

CONSTITUENTS OF ERYTHROXYLON AUSTRALE [1]

INTRODUCTION

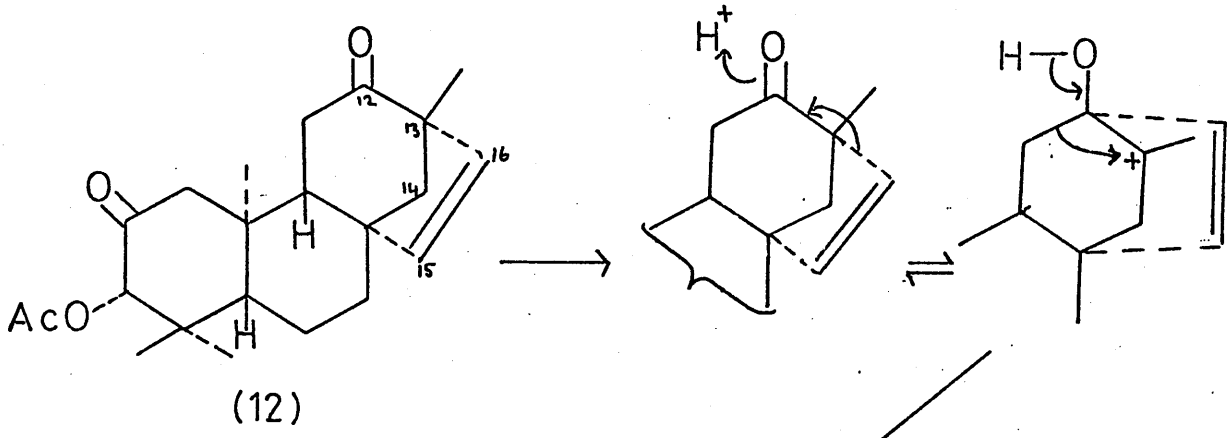
The great majority of naturally occurring tetracyclic diterpenoids fall into two main classes in which the ethano bridge forming the D ring spans the 8 and either the 12 or 13 positions of the perhydrophenanthrene nucleus [2]. The initial step in the biogenesis of the polycyclic diterpenes is the proton initiated cyclisation of geranyl geranyl pyrophosphate (1) to the bicyclic C-8 carbonium ion (2) (scheme 1). The hydration, neutralisation and rearrangement of (2) gives rise to sclerols, manools and backbone rearranged labdenes. A large number of mineral acid catalysed cyclisations of 1, 5- dienes [3] portray the essential features of this reaction, i.e. concerted cyclisations proceeding to cyclohexyl systems by trans-anti-parallel addition to double bonds. In addition this course of events is supported by the enzymatic transformation of (1) to copalyl - PP (ent-3) by Gibberella fugikuroi [4]. This bicyclic compound is probably a stabilised intermediate in the biogenesis of more highly cyclised diterpenes since it is transformed into (-) - kaurene (8) by soluble enzyme preparations from wild cucumber and G. fugikuroi and to a mixture of ent-sandaracopimaradiene (9), hibaene (10), kaurene (8) and trachylobane (11) in a soluble enzyme fraction from castor bean seedlings. Attempts have been made to inter-convert these diterpenoid skeletons under a variety of acidic conditions [5-8].

A double 1, 2- rearrangement of a 12-oxo-beyer-15-ene system (12)[9] across the 12, 13 - single bond has been confirmed [10] by X-ray crystal

Footnote

ent-15-beyerene ≡ (+) stachene ≡ (+) hibaene.

SCHEME 2



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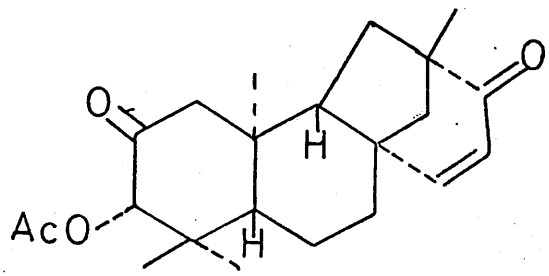

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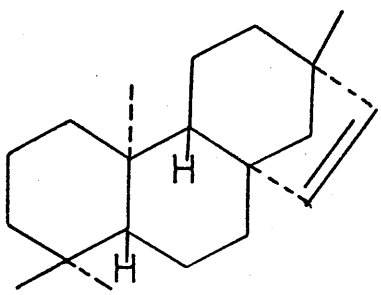

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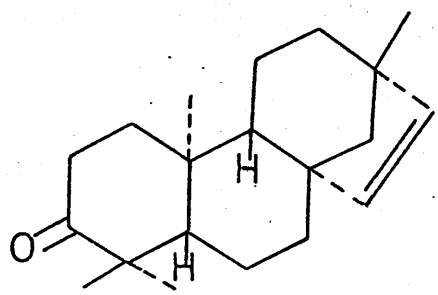

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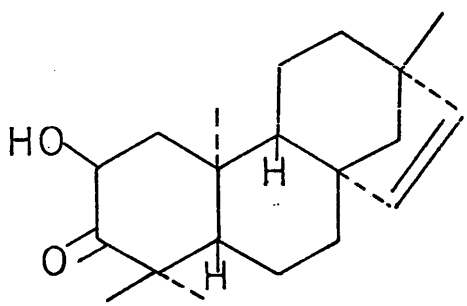

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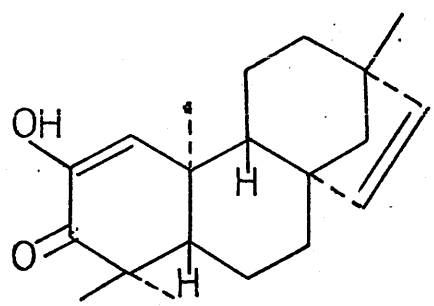
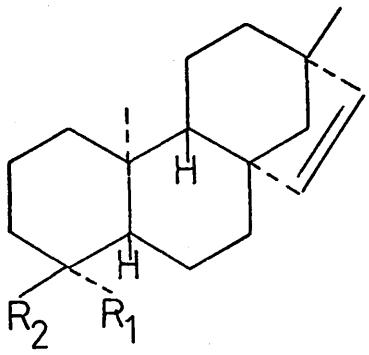


(16)

structure analysis. This rearrangement is reversible after reduction of the double bond and probably proceeds as indicated in scheme (2), the final product (13) being stabilised by the conjugation of the two unsaturated functions.

In 1954, Gupta and Muthana [11] reported the isolation of a diterpene and a diterpenoid alcohol from the trunkwood of Erythroxylon monogynum Roxb., a small tree endemic to certain areas of East India and Ceylon. This was the first of many publications on the extractives of E. monogynum (vide infra). Re-investigation of this plant yielded a host of new compounds. The diterpene was later shown to be (+) stachene ((+)-hibaene) (10)[12]. Its enantiomer, (-) hibaene, was isolated from Thujopsis dolobrata [13]. Several stachene - related diterpenoids have been obtained from Spirostachys africana [14]. These include stach-15-en-3-one (14), the α-ketol (2-hydroxystach-15-en-3-one) (15), and the diosphenol (2-hydroxy stach -1, 15, - dien-3-one) (16). It was originally proposed [15] that stach-15-en-3-one (14) should have a syn - backbone with a β - oriented ring D. On the basis of a biogenetic analysis Scott et al predicted [16] (as was later substantiated) that stach-15-en-3-one (14) would have the trans-anti-trans backbone although they retained the β - oriented ring D. However n.m.r. spectra in this series [17, 18] revealed a shielding effect by the ring D double bond, on the angular methyl group attached to C-10. This indicates that the methyl group and the double bond lie on the same side of the molecule, but as stach-15-en-3-one (14) had been assigned [14, 19] an antipodal A/B fusion on the basis of o.r.d. measurements and chemical correlations this requires an α - oriented ring D.

Hanson [20] used a direct correlation between the stachene series and the kaurene series, in which the stereochemistry had been established [21, 22] to prove that in fact ring D does have the α - configuration, and also



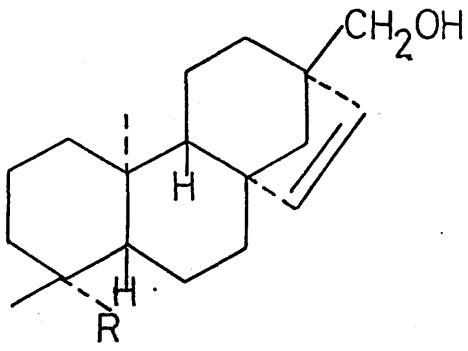
(17) $R_1 = CH_2OH$; $R_2 = CH_3$

(18) $R_1 = CH_2OAc$; $R_2 = CH_3$

(19) $R_1 = CHO$; $R_2 = CH_3$

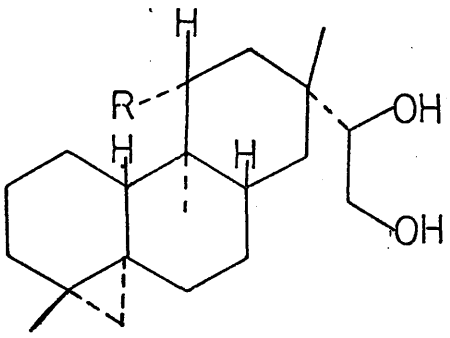
(27) $R_1 = CH_3$; $R_2 = OH$

(28) $R_1 = OH$; $R_2 = CH_3$



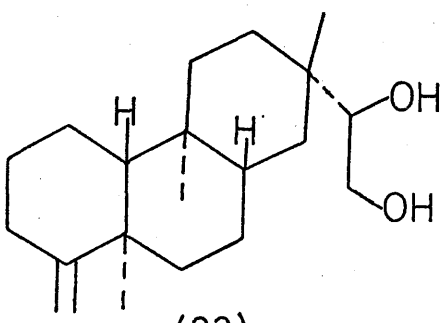
(20) $R = CH_3$

(21) $R = CH_2OH$



(22) R = H

(26) R = OH



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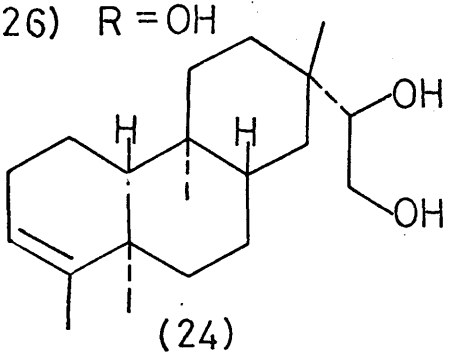

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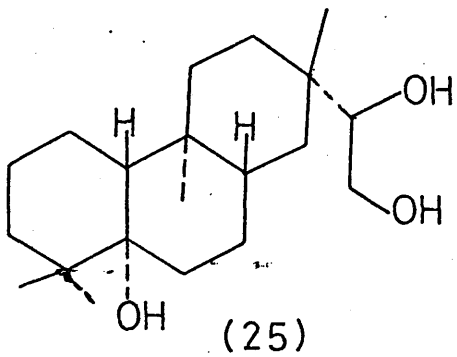

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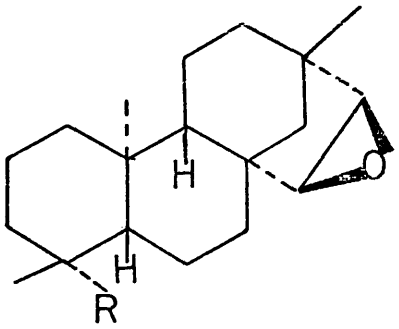
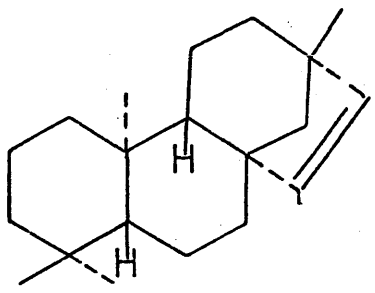


(29) $R = CH_3$

(30) $R = CH_2OH$

(31) $R = CH_2OAc$



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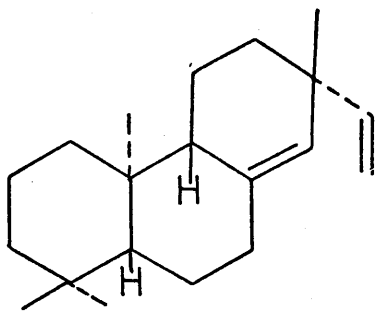

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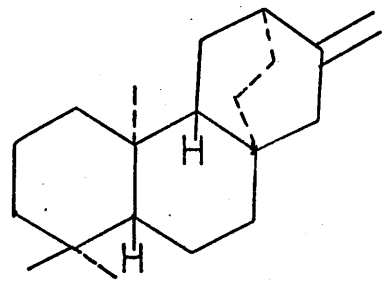

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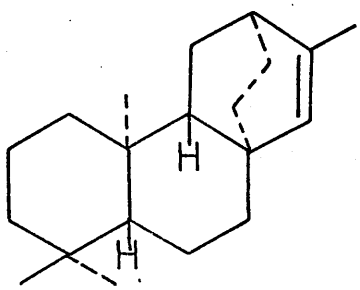

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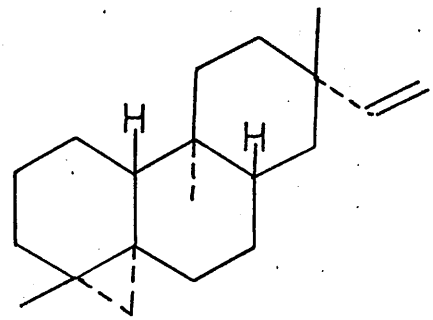

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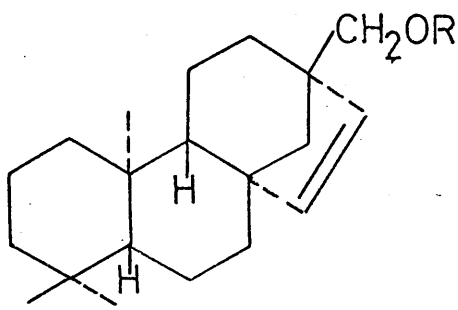
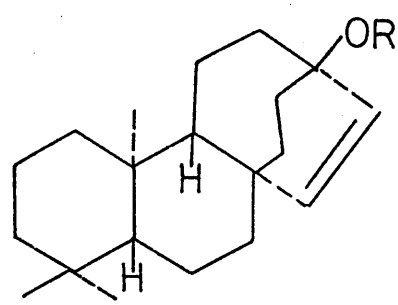


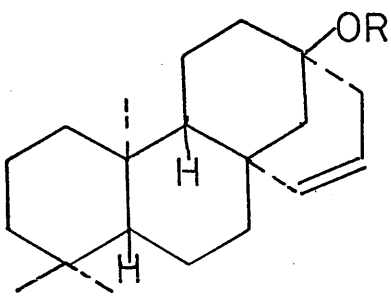
(36) R=Ts

(37) DIHYDRO—(36)



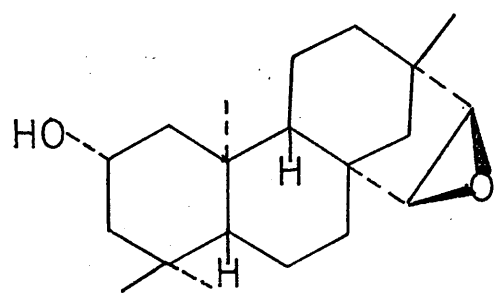
(38) R=Ac

(40) DIHYDRO—(38)



(39) R=Ac

(41) DIHYDRO—(39)



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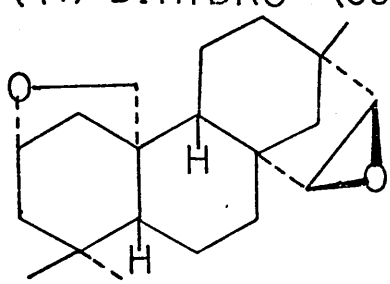

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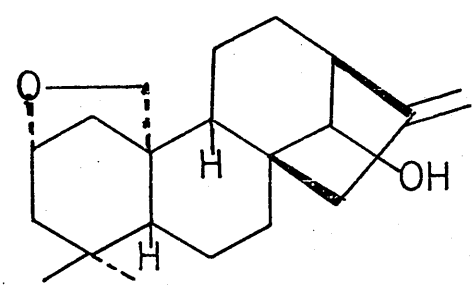


(44)

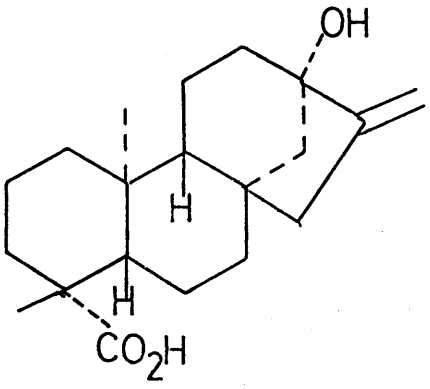
confirmed the stereochemistry proposed [23] by Murray and McCrindle for erythroxylol A (17).

The structures of further diterpenoid constituents of the heartwood of E. monogynum were established in a series of papers [12, 15, 23-30]. Erythroxylol A (monogynol) (17) was shown to be a primary alcohol and to posses the stachene skeleton. The n.m.r. spectra of erythroxylol A (17) and the derived acetate (18) and aldehyde (19) suggests that the CH_2OH substituent is axially oriented at C-4. The closely related erythroxylol B (20) and erythroxydiol A (hydroxymonogynol) (21) were also isolated [12, 15].

Other diterpenoids obtained include the erythroxydiols, X (devadrool) (22), Y (allodevadrool) (23), and Z (24) [26, 27], and the erythroxytriols P (25) and Q (26) [28]. Triol Q (26) is a mono-hydroxylated diol-X in which the additional hydroxy function is secondary and attached to C-11. Two norditerpenoid tertiary alcohols (4β-hydroxy-18-norhibaene (27) and the 4α-epimer (28)) and the epoxides, stachene epoxide (29), erythroxylol A epoxide (30) and the corresponding acetate (31) were also identified [25, 29]. The hydrocarbon fraction of E. monogynum contains [30] (+) - stachene (10) (74%), (-) - pimaradiene (32) (6%), (-) - atisirene (33) (5%), (-) - isoatisirene (34) (8%), and (+) - devadarene (35) (7%).

Other work on the compounds from E. monogynum include detailed studies of the oxidation [31] of some primary and secondary alcohols, and the solvolysis [32], in buffered acetic acid, of the toluene-p-sulphonates (36) and (37) of erythroxylol B and its dihydro derivative. The latter results in the formation of approximately equal amounts of two bridgehead acetates (38) and (39) from (36), and (40) and (41) from (37).

Beyer-15,-en-2α, 3α and 3β alcohols have been prepared [33] and their stereochemistry assigned. 2α-Hydroxybeyer-15, 16-epoxide (42) undergoes reaction with lead tetra-acetate to form a 2-20 ether (43) which may be



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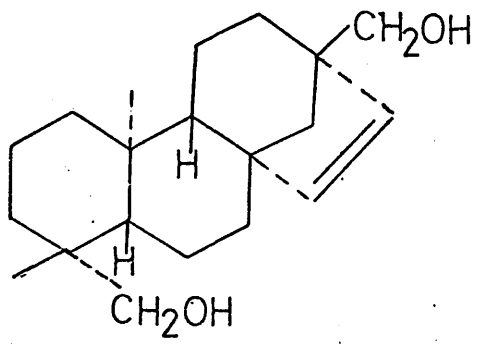

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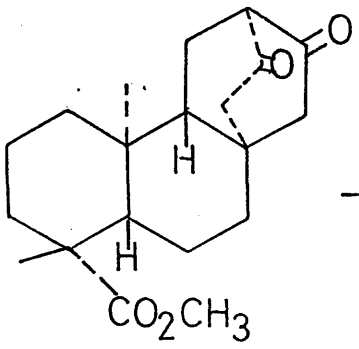

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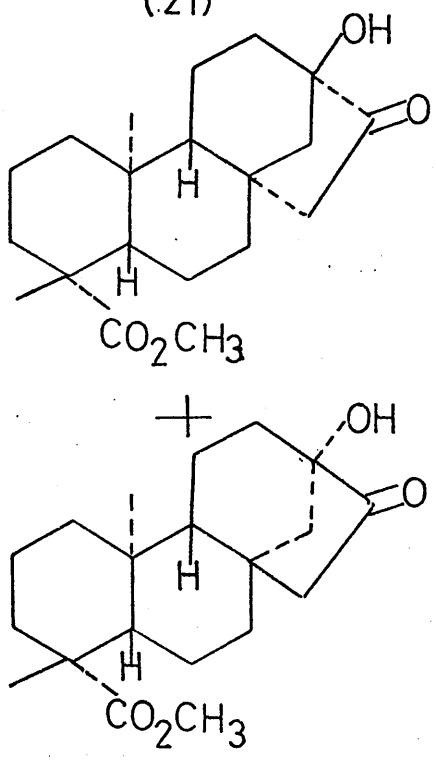
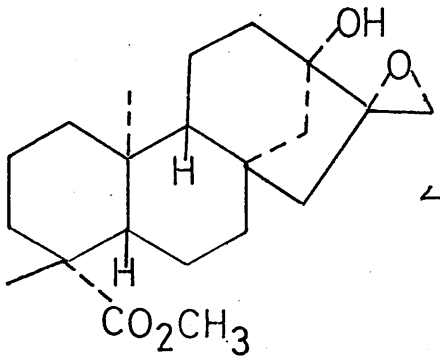


(47)



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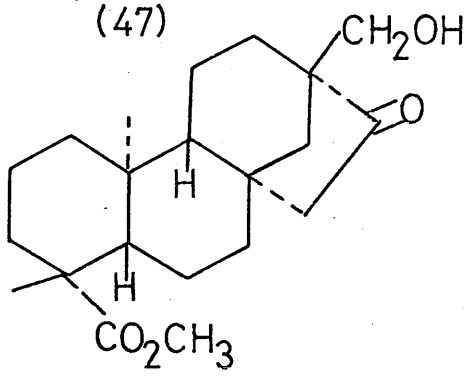

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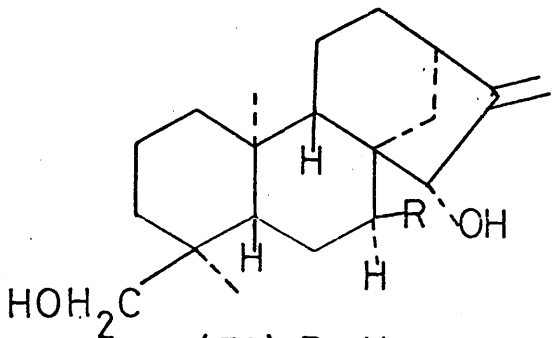
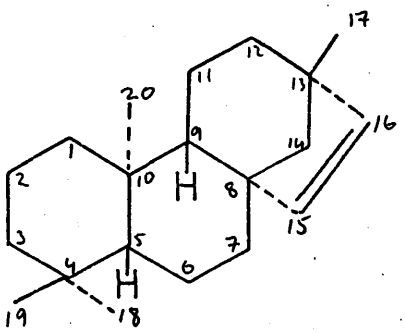


(50) R = H

(51) R = OH

rearranged to a kaurenoid compound (44) with BF_3.

(±) Steviol (45) and erythroxydiol A (21), have been synthesised [34] using two interesting rearrangement reactions ((46) to (47) and (48) to (49) respectively). The partial syntheses of ent-kaur-16-en-15β, 18-diol (candidiol) (50) and ent-kaur-16-en-7α, 15β, 18-triol (51) have been reported [35].



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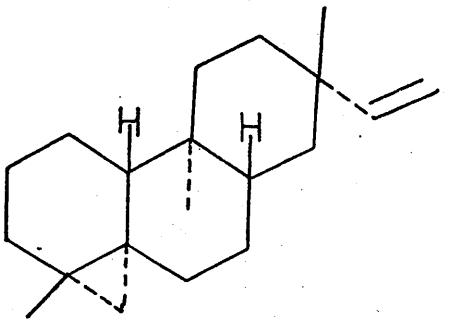

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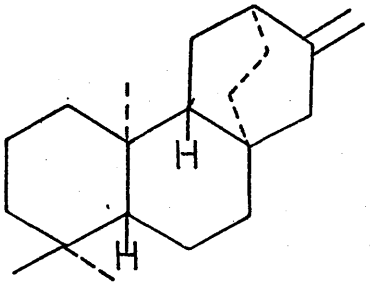

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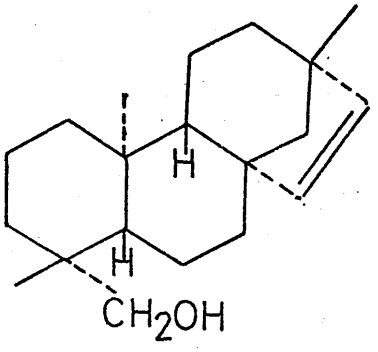

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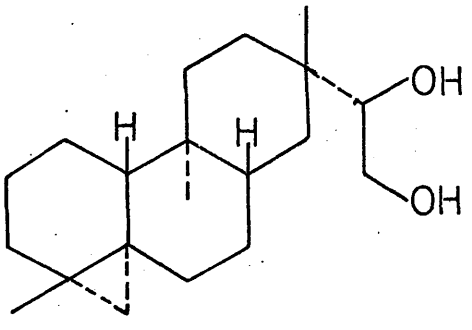

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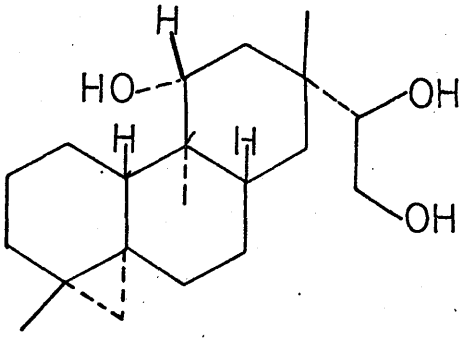

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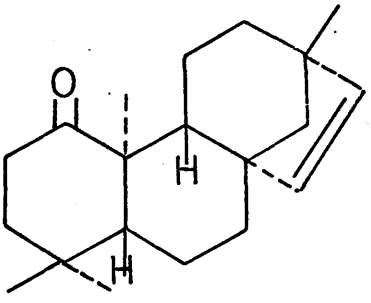

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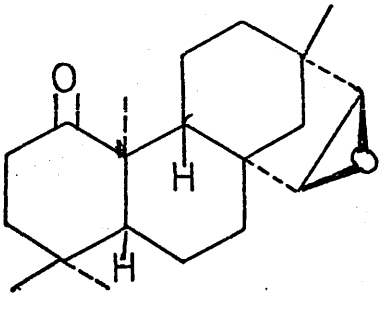


(53)

TABLE 1

New Naturally occurring Compounds of E. australe

Compound	Structure	% Total Extract
Ketone	(52)	1.3
α-ketol	(54)	6.7
Diosphenol	(55)	1.2
Keto-alcohol	(56)	0.11
Epoxyketone	(53)	0.1
Isopropylidene acetal of the alcohol	(65)	0.13
Isopropylidene acetal of the hydroxyacetate	(70)	0.038

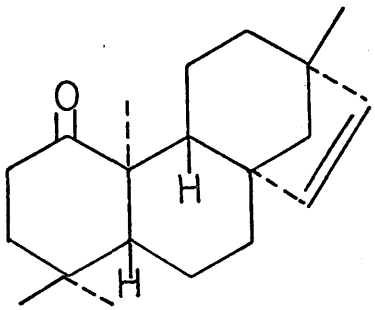
DISCUSSION

a) REACTIONS

In an extension of the previously reported [12, 15, 23-30] work on the light petroleum extract of E. monogynum, which proved to be a prolific source of diterpenoids, the ethyl acetate extract of powdered rootwood of E. australe was examined. From this extract seven new diterpenoids (see table 1), whose structures are described below, and the following known compounds, stachene (10) [12], devadarene (35) [30], atisirene (33) [30], erythroxylol A (17) [23], erythroxydiol X (22) [26, 27] and erythroxytriol Q (26) [28] were isolated.

It is obvious from the n.m.r. spectra (see experimental section) that five of the new compounds, the ketone (52), the epoxy-ketone (53), the α-ketol (54), the diosphenol (55), and the keto-alcohol (56), are related to stachene (10). The isolation of the ketone (52), the α-ketol (54) and the diosphenol (55) is interesting since Spirostachys africana [14] has been reported to give stach-15-en-3-one, 2-hydroxystach-15-en-3-one, and the diosphenol, 2-hydroxystach-1, 15-dien-3-one. It was soon apparent, however, that the compounds from E. australe, while possessing a stachene (10) skeleton, differed from the S. africana series in the position of the functional groups.

The ketone (52), stach-15-en-1-one, $C_{20}H_{30}O$, m.p. 77-78°, $[\alpha]_D$ - 114°, has in its i.r. spectrum a band at 1709 cm^{-1} attributed to a cyclohexanone and a symmetrical doublet at 1380, 1363 cm^{-1} indicating a geminal dimethyl group. In its n.m.r. spectrum the ketone (52) shows peaks due to four tertiary methyl groups and an AB quartet typical [12] of the protons on the cis-disubstituted double bond. Epoxidation of stach-15-en-1-one with m-chloroperbenzoic acid smoothly formed, in high hield, the keto-epoxide (53),



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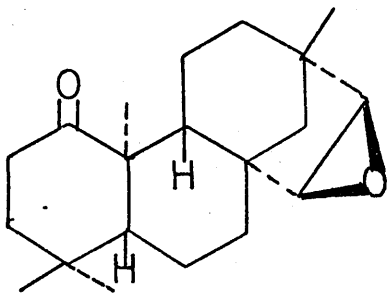

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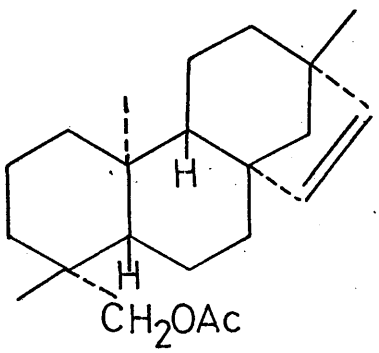

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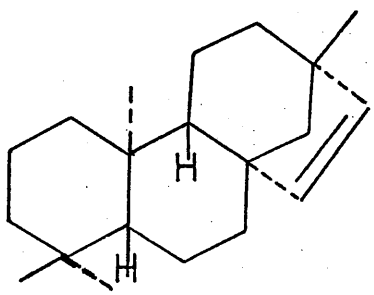

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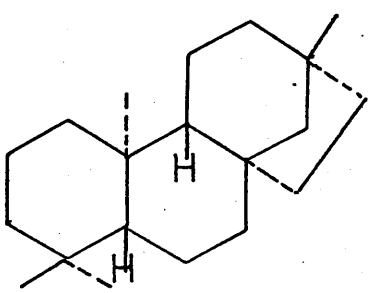

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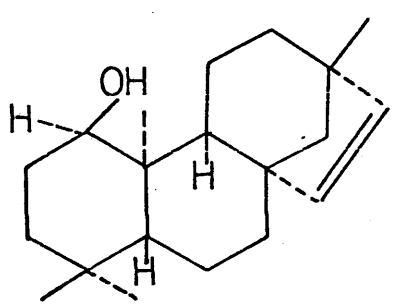

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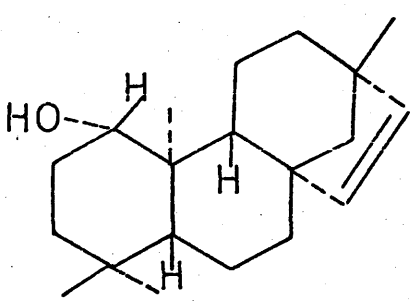
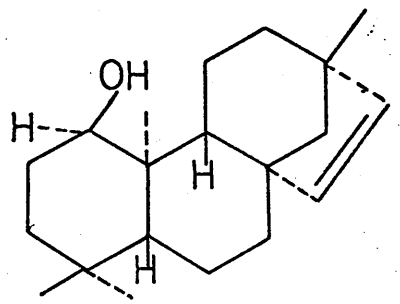


(59)

$C_{20}H_{30}O_2$, m.p. 131-132°, $[\alpha]_D$ -58°, identical with the keto-epoxide isolated from the extract. Since the cis-disubstituted double bond is very susceptible [29] to oxidation this keto-epoxide may well be an artefact. The n.m.r. of the keto-epoxide (53) has tertiary methyl resonances at δ0·96, 1·03, 1·09, 1·29, an AB quartet at δ3·38, 3·07 (J_{AB} $4H_Z$) due to the protons attached to the epoxide ring. The i.r. spectrum shows peaks at 842 and 1709 cm^{-1}. The spectroscopic evidence indicates, therefore, a cis-disubstituted epoxide. It has been shown [29] that epoxidation of erythroxylol A acetate (18) with m-chloroperbenzoic acid gives the β-epoxide by oxidative attack on the less hindered side of the double bond.

Wolff-Kishner reduction of the ketone (52) yielded a mixture of stachene (10) and stachane (57). Stachene (10) was identified by its n.m.r. spectrum which shows tertiary methyl resonances at δ0·73, 0·82, 0·85, and 0·98 and the olefinic AB quartet at δ5·41, 5·67 (J $5·5H_Z$), and by direct comparison with an authentic sample on g.l.c. Stachane (57) was also identified by its n.m.r. spectrum (methyl resonances at δ0·79 (3H), 0·82 (3H), 0·94 (6H)) and g.l.c. comparison. The presence of the stachene nucleus is therefore confirmed.

In a separate attempt to remove the ketonic carbonyl group the ketone (52) was reduced with sodium borohydride to the alcohol (58), stach-15-en-1β-ol. A small amount of the epimeric alcohol, stach-15-en-1α-ol (59) was also formed. Assignments of the configuration of the hydroxy group in these alcohols is made on the basis of the carbinol proton signal in the n.m.r. Stach-15-en-1β-ol (58) has, in its n.m.r. spectrum, the carbinol proton at δ3·6 (m, $W_{\frac{1}{2}}$ $7H_Z$) thus confirming the β (axial) nature of the hydroxy group. The carbinol proton of stach-15-en-1α-ol (59) appears as a triplet (J $7·5H_Z$). Because of the α configuration of the methyl group attached to C-10 it would be expected that reduction would occur from the



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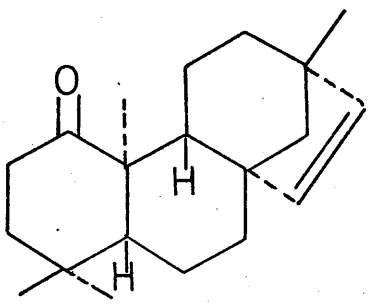

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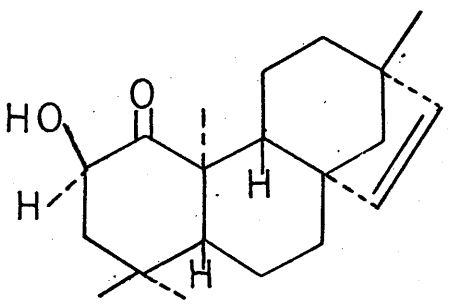
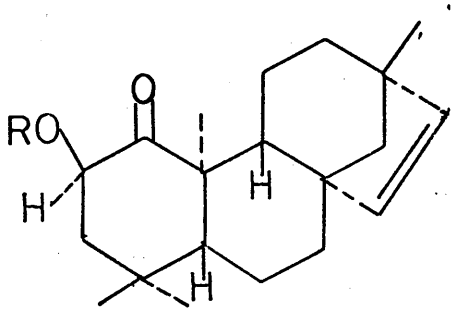


(54)

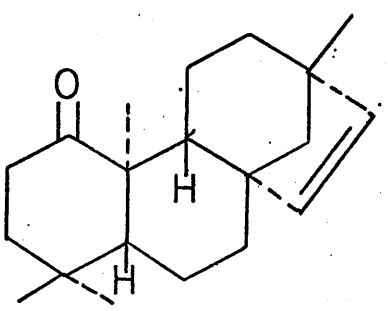
β-face of ring A leading to the α hydroxy compound as the major product. In fact, since the product is almost exclusively stach-15-en-1β-ol, there must be twisting of ring A to allow hydride attack from the apparantly more hindered α-face. Treatment of stach-15-en-1β-ol (58) with p-toluene sulphonyl chloride in dry pyridine gave the corresponding tosylate in good yield. The tosylate was then reduced with lithium aluminium hydride in refluxing tetrahydrofuran. However, the reaction proceeded solely by way of sulphur-oxygen cleavage instead of the desired carbon-oxygen cleavage. The nucleophilic attack of the solvated hydride ion at the sulphur atom may be due to steric hindrance of attack at C-1 because of the presence of the angular methyl group attached to C-10. This supports the placing of the carbonyl group at position 1 in (52). Confirmation of this assignment was obtained from o.r.d. evidence and by direct interrelation of (52) with the α ketol (54) (see later).

The α ketol (54) 2β-hydroxystach-15-en-1-one, $C_{20}H_{30}O_2$, m.p. 109-110° $[\alpha]_D$ + 220° has in its i.r. spectrum absorptions at 3470 (sharp, bonded OH), 1705 cm^{-1}, (cyclohexanone). The n.m.r. spectrum shows a doublet at δ5·8 (J $4H_Z$ disappears on addition of D_2O, OH) and a broad multiplet δ4·5 (q, after addition of D_2O J_{obs} 12·5, $5H_Z$, CHOH). The above n.m.r. evidence indicates the presence of the grouping - CH_2 - CH(OH) - CO - which can only be accommodated in ring A of a stachene skeleton. Since the α ketol (54) can be transformed via tosylation, lithium aluminium hydride reduction, and subsequent oxidation to the ketone (52) and since it differs from the known α-ketol [14] (2-hydroxystach-15-en-3-one) isolated from Spirostachys africana it follows that the carbonyl group must be at C-1. The ketone (52) must therefore be stach-15-en-1-one, and the α-ketol (54), 2β-hydroxystach-15-en-1-one. The equatorial (β) nature of the hydroxy substituent follows from the half-band width (17·5 H_Z) of the CHOH proton.

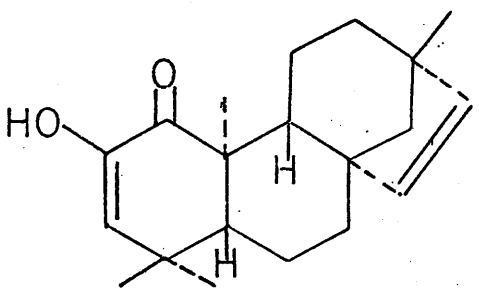


(54) R=H

(76) R=Ac



(52)



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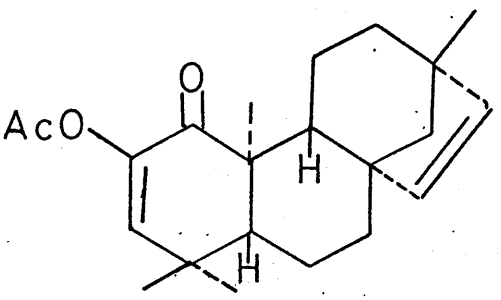

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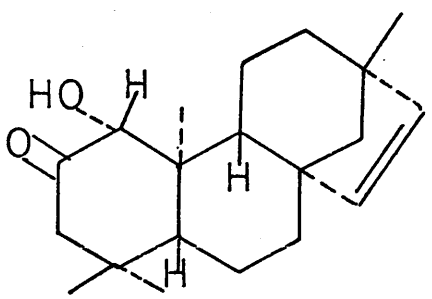

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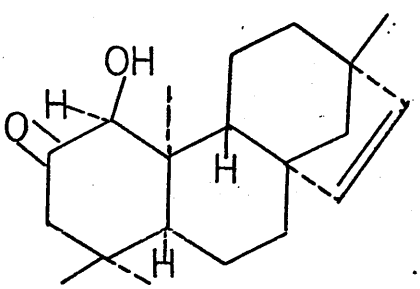
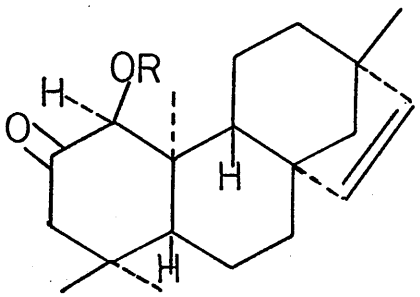


(62)

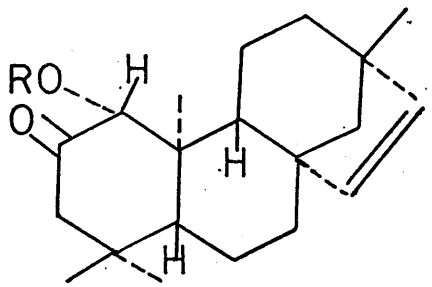
The diosphenol (55) present in the extract is very similar in polarity to the ketone (52). This problem was easily resolved by acetylation of the mixture, t.l.c. separation of the ketone and diosphenol acetate (60) and saponification of the acetate. The diosphenol, 2-hydroxystach-2, 15-dien-1-one, thus obtained, has m.p. 108-109°, $[\alpha]_D$ - 11°, ν_{max} (CCl_4) 3440 (bonded OH), 1682, 1660 cm^{-1}, λ_{max} 270 n.m. (ϵ10,000) in neutral ethanol, changing to λ_{max} 315 n.m. (ϵ6000) on addition of one drop of 0·1 M-potassium hydroxide. In the n.m.r. spectrum the sharp singlet due to the C-3 olefinic proton is superimposed on the AB quartet arising from the C-15 and C-16 protons of the cis disubstituted double bond. In the corresponding acetate (60) the C-3 olefinic proton moves downfield to δ5·96.

The structure of the diosphenol was readily confirmed as (55) by preparing it from the α-ketol (54) by bismuth oxide oxidation using the conditions described by Rigby [36]. When the reaction was carried out under milder conditions a mixture of starting material, diosphenol (55), and a new ketol (61) was obtained. This suggests that the bismuth oxide is causing, at least in part, some equilibration of the original α-ketol. The same three compounds together with a more polar ketol (62) resulted from equilibration of (54) in refluxing 5% ethanolic potassium hydroxide for five hours. The structures of these two new ketols follow readily from their spectroscopic properties.

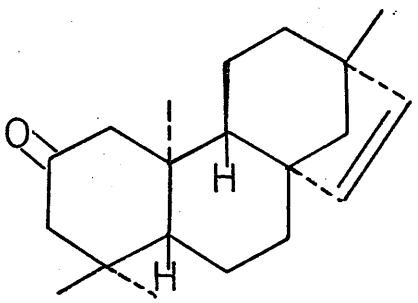
Thus the less polar compound (61), m.p. 104-106°, has, in the n.m.r. spectrum, a sharp singlet (after D_2O exchange) at δ3·89 and a singlet (2H) at δ2·34 (- $CH_2COCH(OH)$ -) indicating that it is a 1-hydroxystach-15-en-2-one. The configuration of the hydroxy group is α (equatorial) since it gives rise to an intra-molecularly bonded hydroxy-band at 3465 cm^{-1} (CCl_4 ; no change on dilution). The more polar ketol (62) m.p. 169-170°, has the same gross structure (δ3·59 (1H, s) and 2·95, 2·11 (ABq, J 12H_Z)) and is therefore



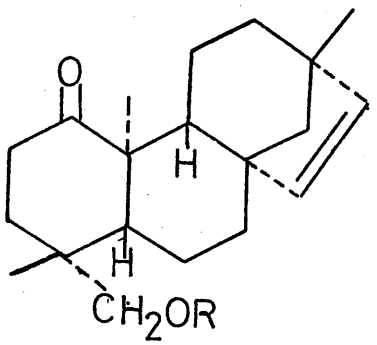
(62) R=H
(78) R=Ac



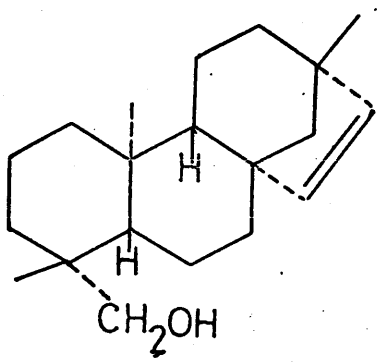
(61) R=H
(77) R=Ac



(63)



(56) R=H
(64) R=Ac

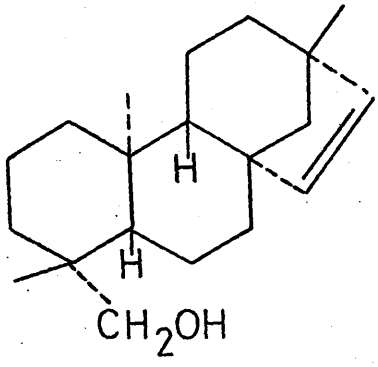


(17)

1β-hydroxystach-15-en-2-one. The i.r. spectrum shows that the hydroxy group is intermolecularly bonded (ν_{max} (CCl_4) 3620 and 3440 cm^{-1} (change in relative intensities on dilution)). Further evidence for the placing of the carbonyl group at C-2 in (61) and (62) comes from the respective o.r.d. curves (see later). When exposed separately to the equilibrating conditions these two ketols yielded a similar mixture to that described above.

Confirmation of the placing of the carbonyl group at C-2 in the ketol (61) was obtained in the following way. 1α-Hydroxystach-15-en-2-one (61) on treatment with p-toluene sulphonyl chloride smoothly formed the corresponding tosylate which was then reduced with chromous chloride using a modification of the procedure of Rosenkranz and Djerassi [37] to give stach-15-en-2-one (63) identical in all respects (n.m.r., m.p., and o.r.d.) with that prepared by Hanson [20].

From the later fractions of the extract the keto-β-alcohol (56), 18-hydroxystach-15-en-1-one, $C_{20}H_{30}O_2$, m.p. 105-106°, $[\alpha]_D$ - 31° was isolated. The n.m.r. spectrum shows three tertiary methyl resonances and the typical olefinic AB quartet. The presence of a primary hydroxy group is indicated by a second AB quartet (δ3·84, 3·63 (J 11 H_Z)). The chemical shift of this signal suggests [12] that it is axially oriented at C-4 and this was later confirmed (vide infra). The carbonyl group is placed at C-1 on the basis of o.r.d. evidence (see later). The i.r. spectrum of (56) has a free hydroxyl absorption at 3630 cm^{-1}, a weak broad band at 3480 cm^{-1}, which may be due to some intermolecular hydrogen bonding, and a carbonyl band at 1709 cm^{-1}. A feature missing from the i.r. of this compound is the symmetrical doublet at approximately 1380 cm^{-1} due to a geminal dimethyl group. The corresponding acetate (64) shows two carbonyl bands at 1743 and 1709 cm^{-1} respectively. The structure of (56) was confirmed as 18-hydroxystach-15-en-1-one by Huang Minlon reduction to erythroxylol A [12] (17),



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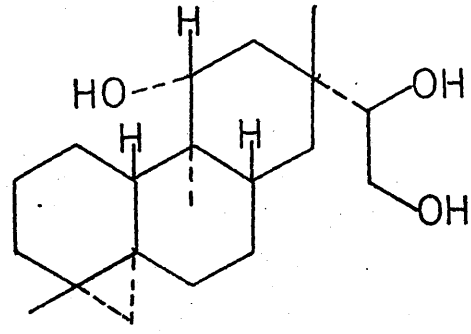
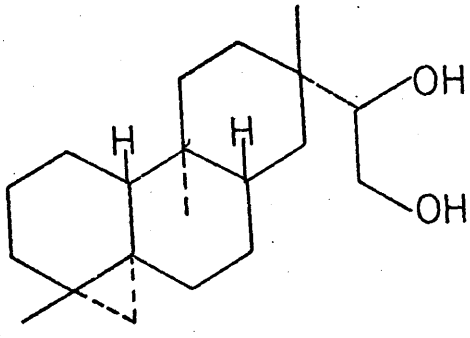
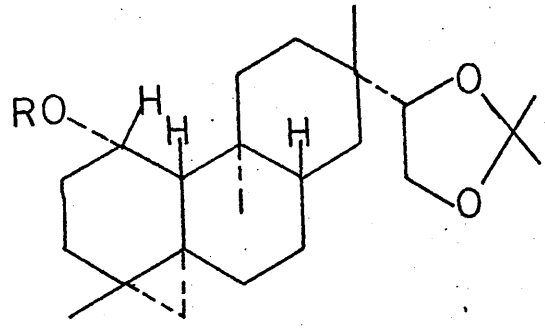


(26)

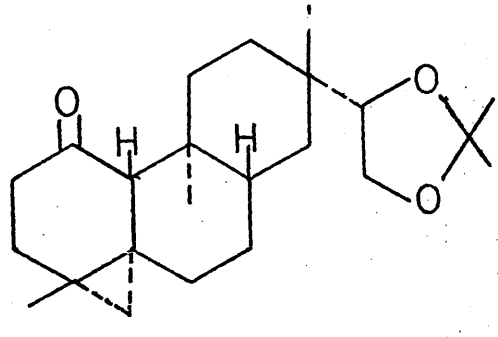


(22)



(65) R=H

(66) R=Ac



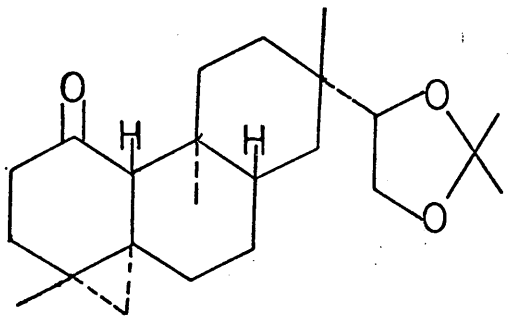
(67)

identical in all respects including absolute configuration with authentic material. A mixture of monohydric primary alcohols was also isolated from the extract. Complete separation was not attained but n.m.r. and t.l.c. analysis allowed identification of the major component as erythroxylol A (17).

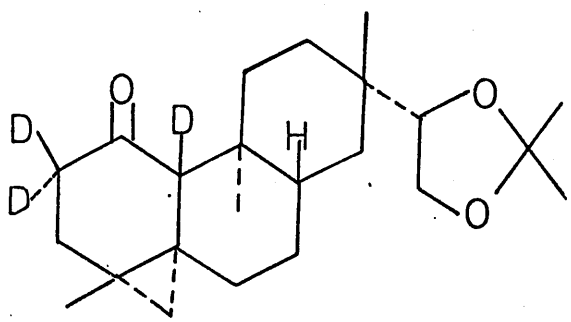
Examination of the more polar fractions of the extract was facilitated by the formation of isopropylidene acetals using acetone and anhydrous copper sulphate. In this way two new compounds were isolated in addition to the known erythroxydiol X [26, 27] (22) and erythroxytriol Q [28] (26). Both of the new compounds contain a cyclopropane ring (δ0·29 J_{AB} 4.5H_Z, one half of ABq)) and appear to be related to erythroxydiol X (22).

The first compound is the monohydroxylated erythroxydiol X isopropylidene acetal (65), m.p. 161-162°, $[\alpha]_D$ - 4·4°. The n.m.r. spectrum shows, in addition to three tertiary methyls and two isopropylidene acetal methyls, a broad singlet at δ4·22 ($W_{\frac{1}{2}}$ 8H_Z) due to the carbinol proton and one part of the cyclopropane quartet δ0·29 (J_{AB} 4·5H_Z). The i.r. spectrum of (65) shows a free hydroxy absorption at 3620 cm^{-1}. The corresponding acetate (66) has in its i.r. a carbonyl frequency at 1737 cm^{-1} and in its n.m.r. a broad singlet at δ5·22 ($W_{\frac{1}{2}}$ 8H_Z, C<u>H</u>OAc). The alcohol (65) failed to form a tosylate under normal conditions but was readily converted to the ketone (67), m.p. 103-104°, νmax (CCl_4) 1710 cm^{-1} on treatment with Jones' reagent. Sodium borohydride reduction of the ketone yielded exclusively the original alcohol (65). The highly hindered nature of the carbonyl group in the ketone (67) was demonstrated by the failure of the ketone to react under conditions normally used for Wolff-Kishner reduction, thioketalisation and enol acetylation.

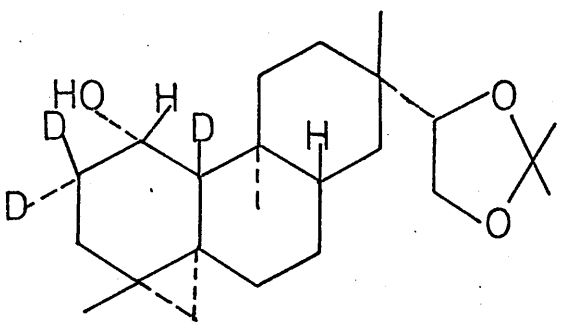
The ketone (67), however, incorporated three atoms of deuterium on exposure to sodium deuterioxide in dioxan-D_2O to furnish the



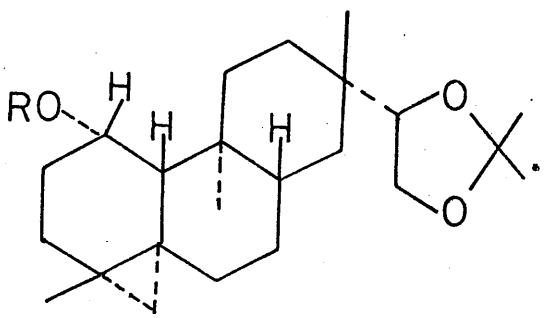
(67)



(68)

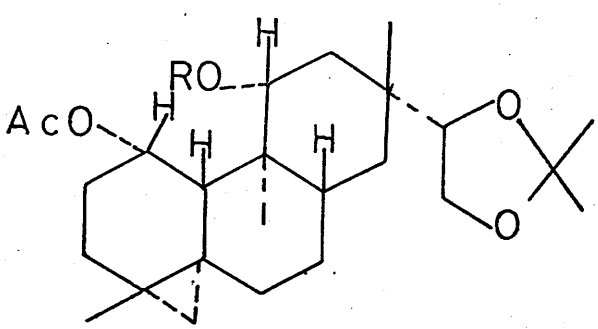


(69)



(65) R=H

(66) R=Ac



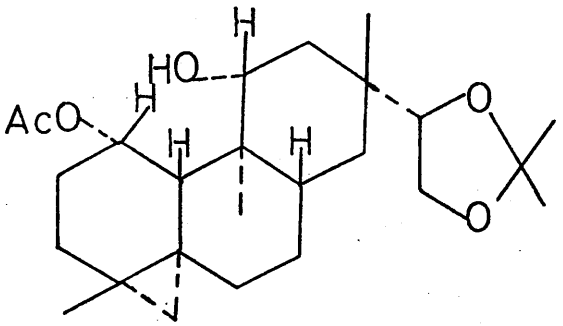
(70) R=H

(71) R=Ac

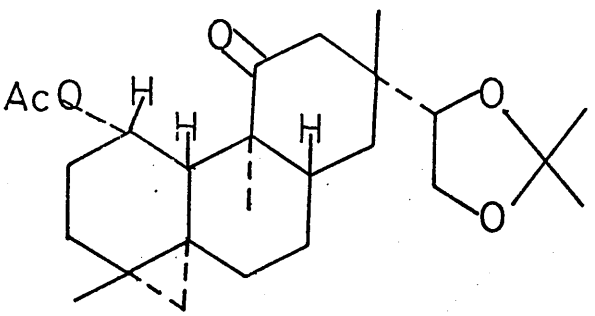
trideuterioketone (68). Sodium borohydride reduction of (68) gave the corresponding trideuterioalcohol (69) in which the CHOH resonance appears as a sharp singlet. These results can be accommodated by placing the original oxygen function at either C-1 or C-7. A decision in favour of the former was reached by use of the Eu $(dpm)_3$ shift reagent [38] (see later). In the shifted spectrum of the alcohol (65) and the acetate (66) the proton attached to C-10 moves downfield as a doublet (J 3·5 H_Z). This proton appears as a singlet in the shifted spectrum of the ketone (67) and is absent in the spectra of the trideuterioalcohol (69) and ketone (68). Thus the new alcohol (65) is 1α-hydroxyerythroxydiol X.

Confirmatory evidence was obtained from the mass spectrum of the ketone (67) which has the base peak at $^m/_e$ 101 (100%; isopropylidene acetal residue). The next most intense peak is at $^m/_e$ 136 (22%) and can arise by a McLafferty rearrangement involving the carbonyl group at C-1 and the α-proton at C-11 followed by cleavage of the C-6-C-7 bond (see scheme (7), p. 158). In the trideuterioketone this peak moves to $^m/_e$ 139.

The second new isopropylidene acetal is the hydroxyacetate (70), m.p. 186-187°, $[\alpha]_D$ - 26°. In its n.m.r. the C<u>H</u>OAc resonance (δ5·35 br. (s, $W_{\frac{1}{2}}$ 8 H_Z)) is very similar in shape and chemical shift to the corresponding signal in (66). The C<u>H</u>OH resonance is obscured by the acetal protons (δ3·8 - 3·5) but appears as a quartet (δ4·9 (J_{obs} 10, 6 H_Z)) in the diacetate (71), m.p. 175-176° (cf. erythroxytriol Q acetate [28] which has H-11 as a quartet (J 10·2, 6·6 H_Z) at δ4·83). The original C<u>H</u>OAc resonance in (70) experiences an upfield shift to δ4·81 on formation of the diacetate (71) suggesting the close proximity of the two oxygen functions. The i.r. spectrum of (70) shows absorptions at 3624 (sharp, free OH, no change on dilution), 3500 (very broad, bonded OH, disappears on dilution), 1735 cm^{-1} (broad, no change on dilution) thus indicating the absence of intramolecular bonding.



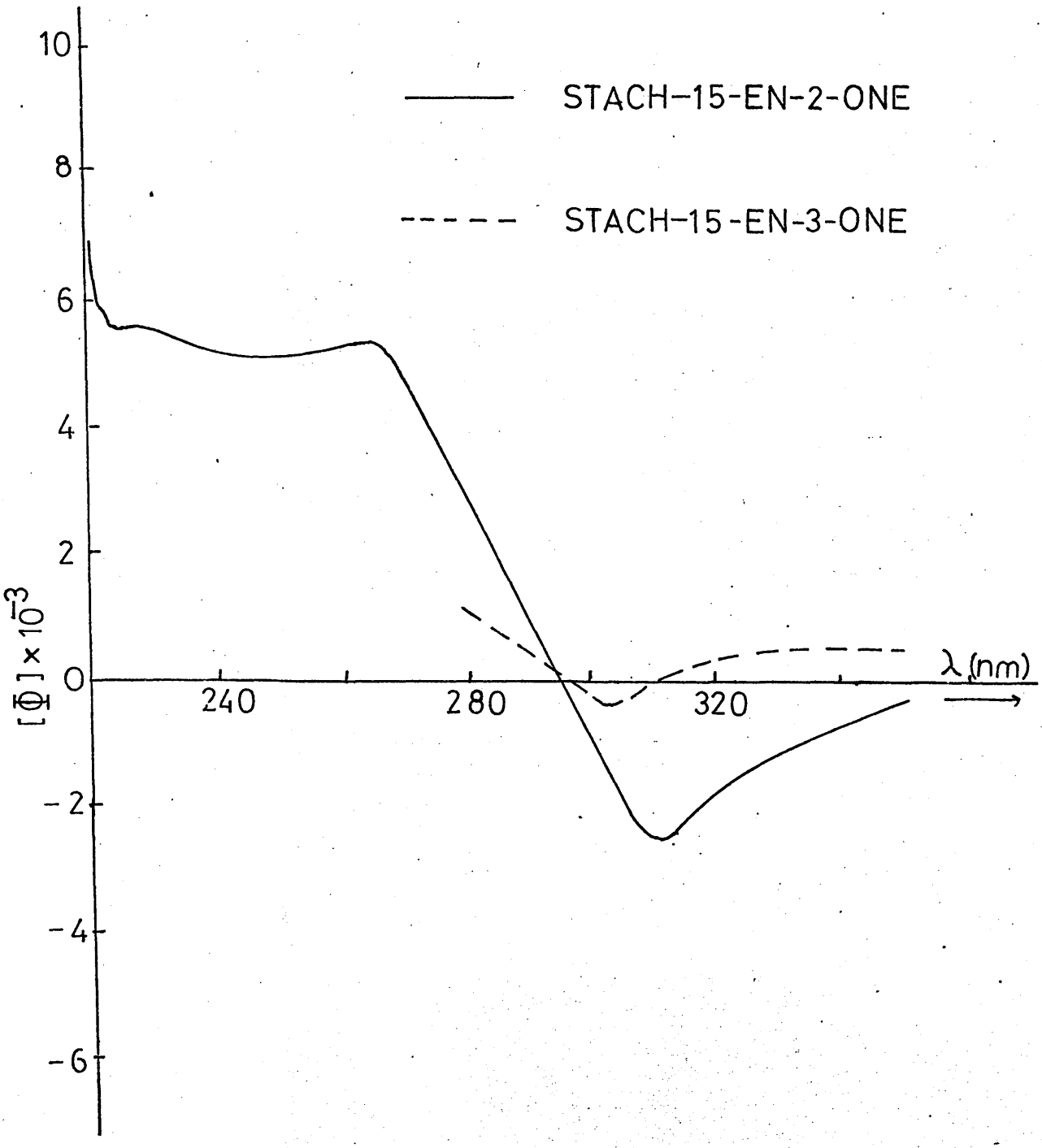
(70)



(72)

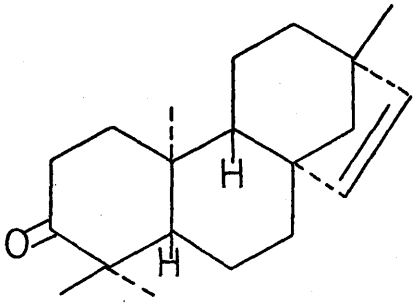
Oxidation of the hydroxyacetate (70) with Jones' reagent yielded the ketoacetate (72). In addition to the usual tertiary methyl signals, the n.m.r. shows one part of an AB quartet δ2·90 (J_{AB} 12 H_Z) indicating the presence of the part structure -CO-C$\underline{H}_2$-$\overset{|}{\underset{|}{C}}$-. Again there is a considerable change in the chemical shift of the C$\underline{H}$OAc resonance which moves downfield to δ5·85. The i.r. spectrum shows carbonyl absorptions at 1736 (acetate) and 1704 cm^{-1} (cyclohexanone). The relative positions of the two oxygen functions were readily settled by use of the benzene solvent shift method (see later) on the ketoacetate (72). In benzene relative to $CDCl_3$ the C$\underline{H}$OAc resonance is deshielded by 0·44 p.p.m. and must therefore lie in front [39, 40] of the carbonyl reference plane. This is only possible in an erythroxydiol X skeleton if the acetate and ketone are attached to C-1 and C-11. This result, in conjunction with the other n.m.r. data, confirms the structure of the hydroxyacetate (70) as 1$\underline{\alpha}$-acetoxy-11$\underline{\alpha}$-hydroxy erythroxydiol X.

FIGURE 1

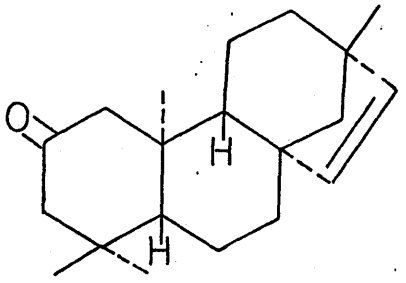

STACH-15-EN-2-ONE
STACH-15-EN-3-ONE
10
8
6
4
2
0
-2
-4
-6
240
280
320
λ (nm)
[Φ] x 10⁻³

b) OPTICAL ROTATORY DISPERSION AND CIRCULAR DICHROISM MEASUREMENTS

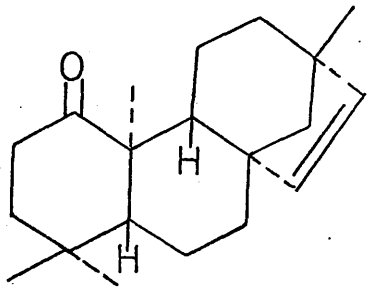
Stach-15-en-3-one (14) gives a weak positive Cotton effect [20] and stach-15-en-2-one (63) a strong negative effect [20] (figure 1).



(14)



(63)

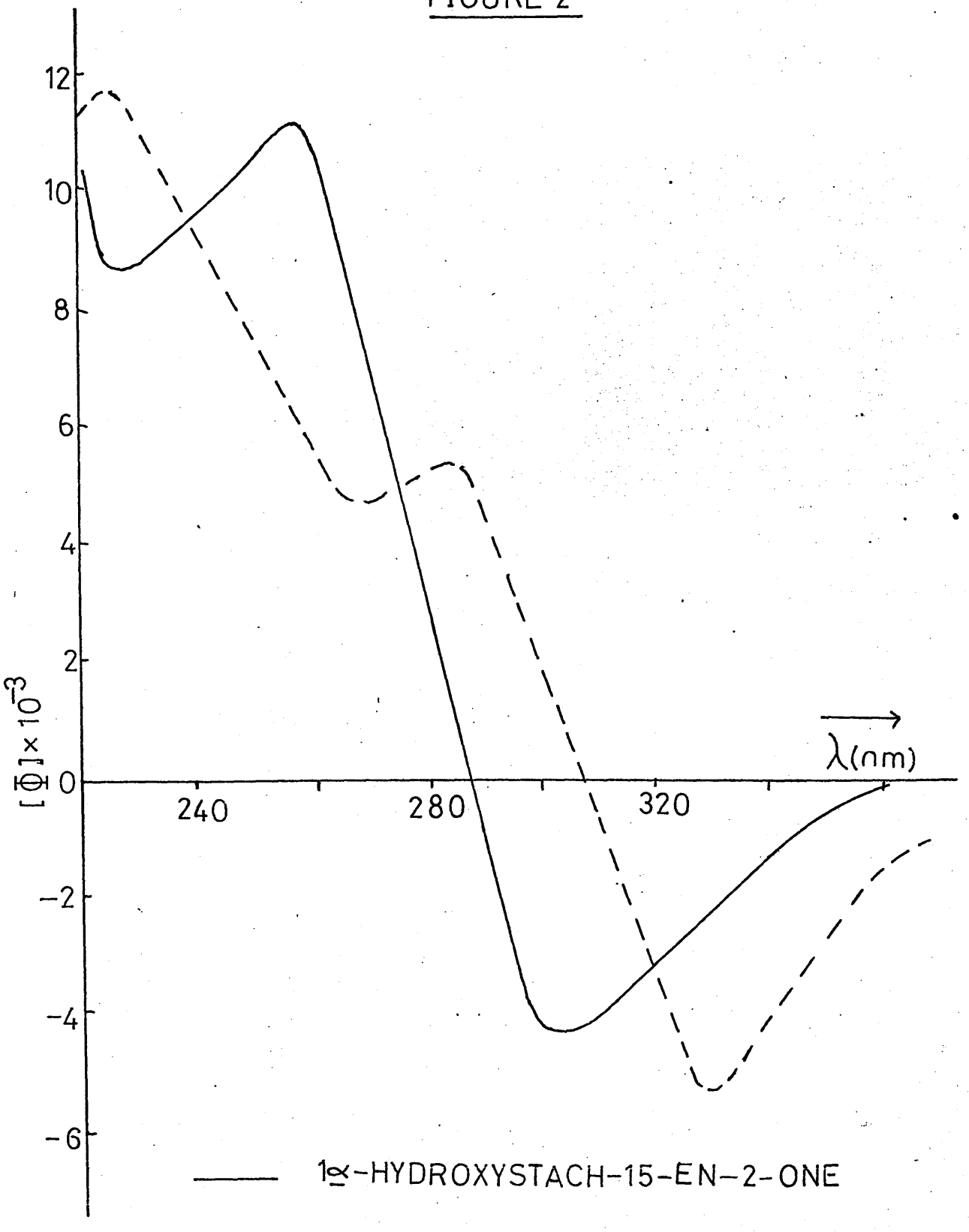


(52)

The ketone (52) shows a positive Cotton effect (figure 3). It is not immediately obvious from octant projections why this should be the case. However, if one assumes that the bulk of ring C, which lies in the front lower left octant, makes a greater contribution than the methyl attached to C-10 which is in the rear lower left octant, the experimental findings can be rationalised. The molecular amplitude (a), is defined as the difference between the molecular rotation at the extremum (peak or trough) of longer wavelength $(\Phi)_1$ and the molecular rotation at the extremum of shorter wavelength $(\Phi)_2$, divided by 100. Hence equation (1) expresses the molecular amplitude in hundreds of degrees.

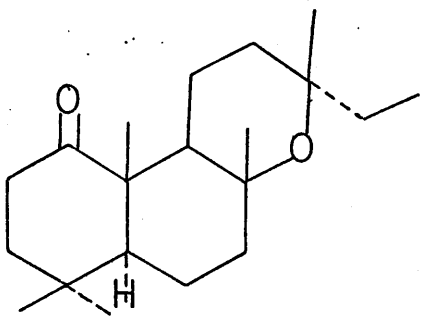
Eq. (1) $$a = \frac{(\Phi)_1 - (\Phi)_2}{100}$$

FIGURE 2


12
10
8
6
4
2
0
−2
−4
−6
[Φ]1×10⁻³
240
280
320
λ(nm)
1α-HYDROXYSTACH-15-EN-2-ONE
1β-HYDROXYSTACH-15-EN-2-ONE

For stach-15-en-1-one (52) the molecular amplitude a = +34·7. There are relatively few examples of 1-oxo-4, 4, -dimethyl steroids. However the compounds in table (2) indicate [41] that an amplitude of about -30 would be expected for a 1-oxo-4, 4, -dimethyl steroid with a 5α, 10β -methyl ring junction measured in methanol.

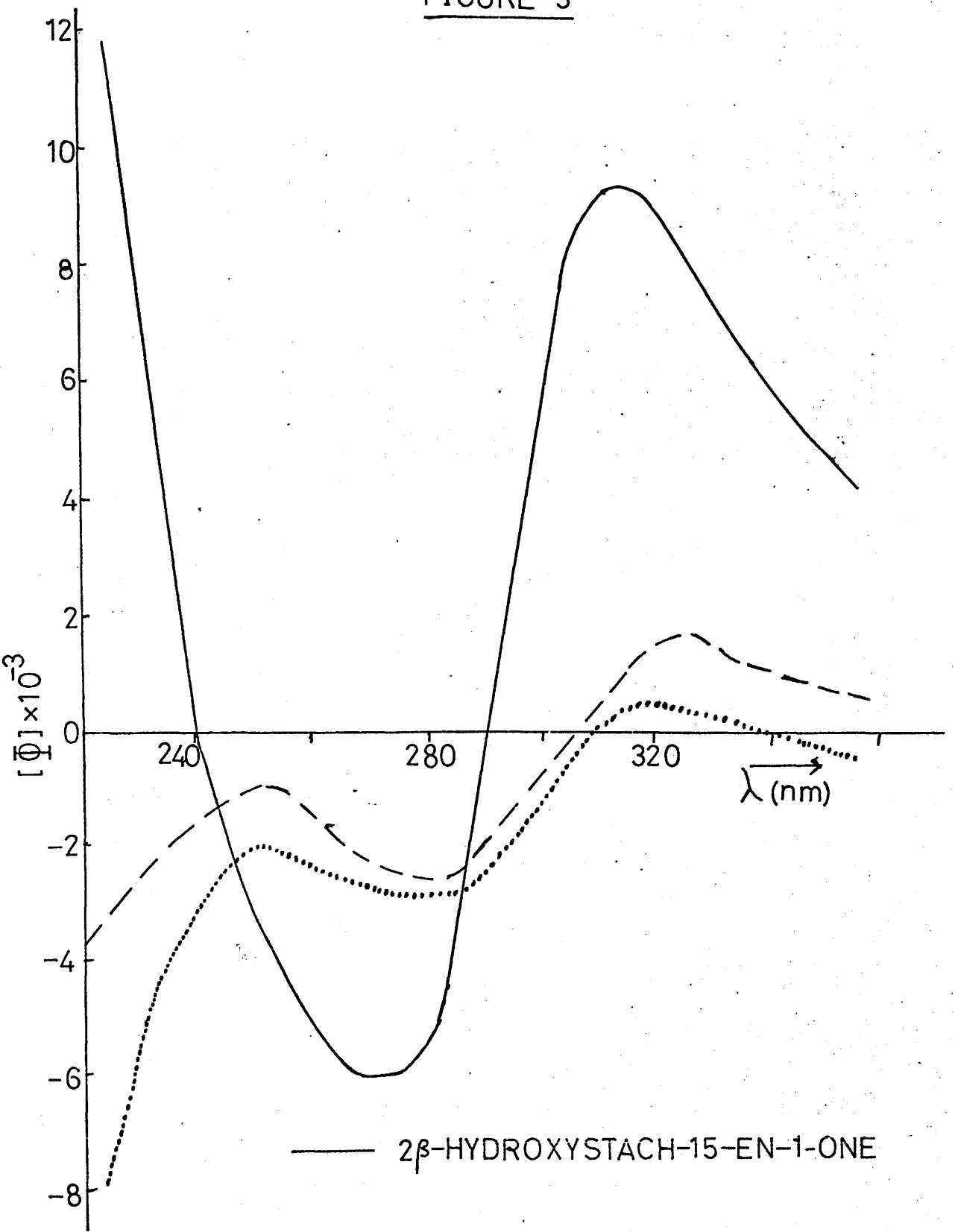
TABLE (2)

COMPOUND	SOLVENT	MOLECULAR AMPLITUDE
4, 4 -Dimethyl-5α-cholestane	MeOH	-37
4, 4 -Dimethyl-5α-cholestane	cyclohexane	-22
1-oxo-lanostane	MeOH	-36
1-oxo-lanostane	cyclohexane	-21
	MeOH	-25

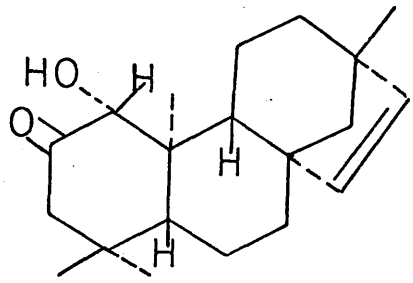
1-Ketomanoyl oxide [42]

The rearranged ketol (61), 1α-hydroxystach-15-en-2-one gives an o.r.d. curve (figure 2) which is very similar in shape to that of the corresponding 2-ketone thus confirming the position of the carbonyl group. In addition the ketol (61) shows an increase in amplitude and a hypsochromic shift of wavelength. This appears to be the normal effect of putting an equatorial hydroxy group α to a cyclohexanone [43]. The epimeric ketol (62) 1β-hydroxy stach-15-en-2-one also gives an o.r.d. curve (figure (2) which is similar to,

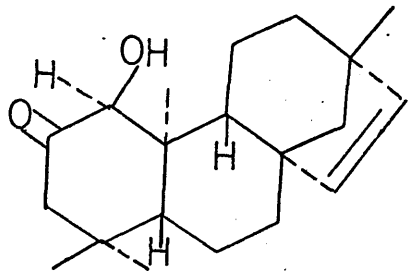
FIGURE 3


12
10
8
6
4
2
0
-2
-4
-6
-8
[Φ]×10⁻³
240
280
320
λ (nm)
2β-HYDROXYSTACH-15-EN-1-ONE
STACH-15-EN-1-ONE
18-ACETOXYSTACH-15-EN-1-ONE

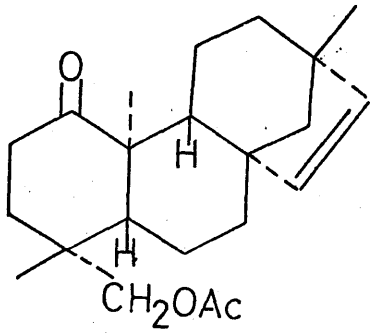
but more intense, than that of the ketone (63). In addition, this curve shows a bathochromic shift which is consistent [43] with the placing of an axial substituent next to a cyclohexanone.



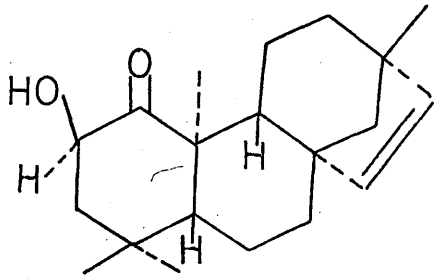
(61)



(62)



(64)

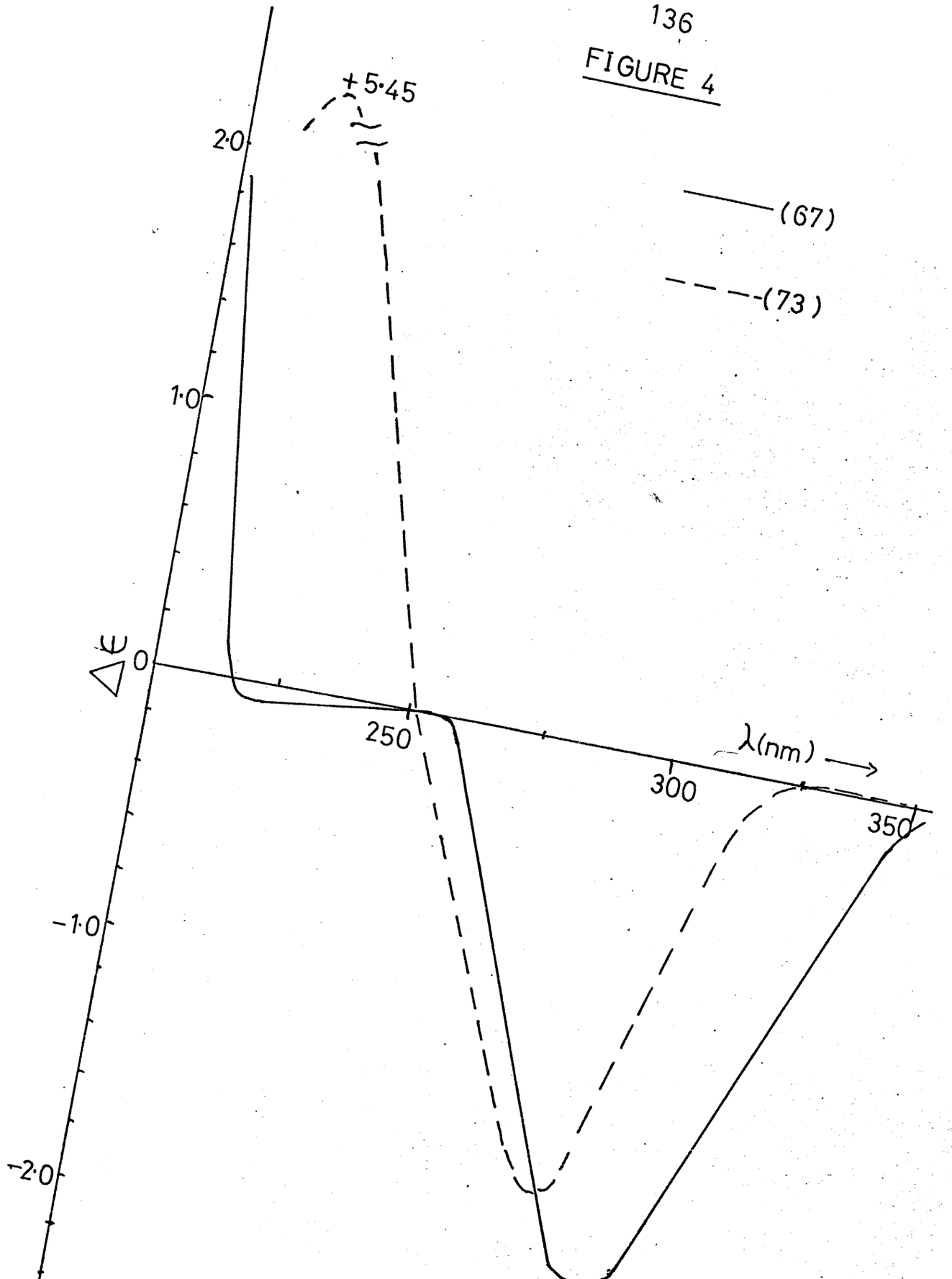


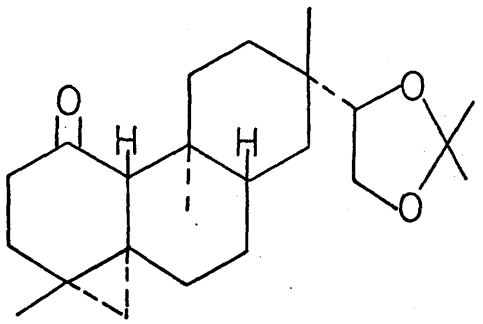
(54)

The o.r.d. of the keto-β-acetate (64) (figure 3) closely parallels that of the ketone (52) indicating that the carbonyl group is also at position 1. The ketol (54), 2β-hydroxystach-15-en-1-one gives an intense positive Cotton effect, in agreement with its proposed structure. Again there is an increase in amplitude and a hypsochromic shift of wavelength relative to the corresponding ketone (52).

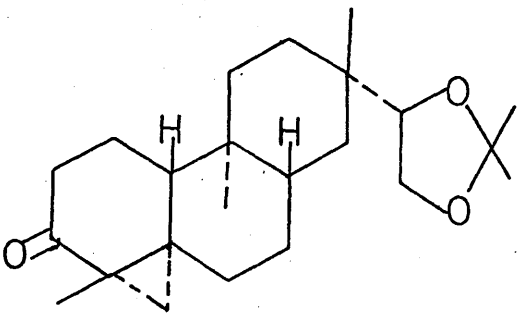
The c.d. curves of 1-keto and 3-ketoerythroxydiol X isopropylidene acetal (67) and (73) respectively were recorded in methanol (figure 4).

FIGURE 4



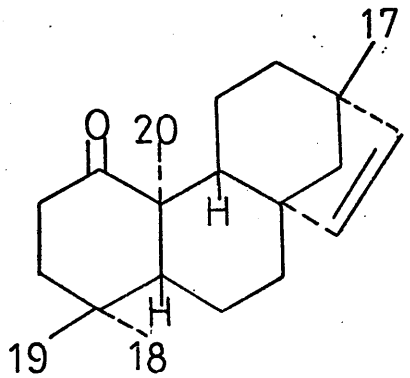


(67)

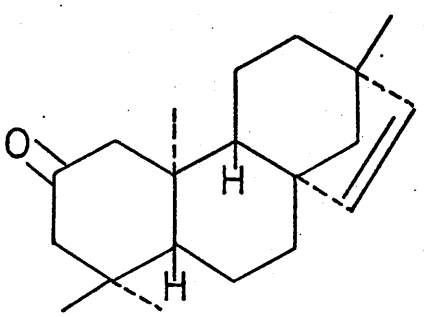


(73)

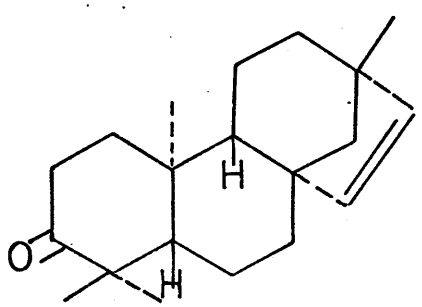
Both compounds give intense negative Cotton effects near 300 n.m. and 286 n.m. respectively with (73) giving a very large positive Cotton effect at 219 n.m. The negative Cotton effect in the case of (67) can be explained by some flattening of ring A due to the cyclopropane ring thus putting the methyl attached to C-9 and the major part of ring C into the rear lower left octant. In keeping with other findings [44] for the Cotton effect of α-cyclopropyl ketones the ketone (73) has a sign opposite to that predicted by the octant rule. Either delocalised electrons or the substantial conformational distortion in ring A may be responsible [44] for the abnormal Cotton effect.



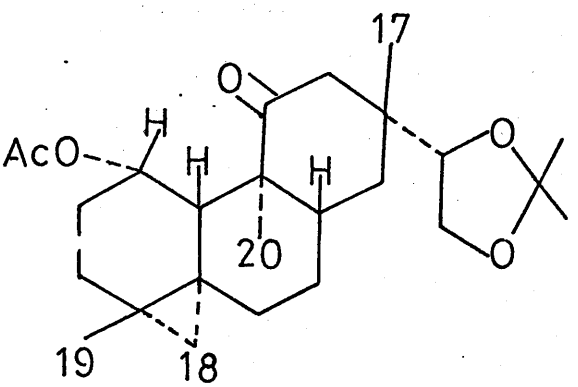
(52)



(63)



(14)



(72)

TABLE 3

Benzene Solvent Shifts of Methyl Groups

Methyl Group	δ_{CDCl_3}	$\delta_{C_6H_6}$	$\Delta = \delta_{CDCl_3} - \delta_{C_6H_6}$
Stach-15-en-1-one (52)			
C_{20}	0.94	0.70	+0.24
C_{18}	1.08	0.80	+0.28
C_{19}	1.13	0.97	+0.16
C_{17}	1.00	1.02	-0.02
Stach-15-en-2-one (63)			
C_{20}	0.75	0.60	+0.15
C_{18}	1.05	0.70	+0.35
C_{19}	0.88	0.69	+0.19
C_{17}	1.00	0.95	+0.05
Stach-15-en-3-one (14)			
C_{20}	0.92	0.68	+0.24
C_{18}	1.06	0.93	+0.13
C_{19}	1.10	1.13	-0.03
C_{17}	1.03	1.03	0.00

Footnote : The methyl shifts were followed using intermediate concentrations of benzene in deuterochloroform.

c) N.M.R. SHIFTED SPECTRA

1) Benzene Solvent Shifts of Methyl Groups

An investigation was made of the effect of addition of benzene on the positions of the methyl resonances in the n.m.r. of the deuterochloroform solutions of the ketones (52), (63), (14) and (72). Bhacca and Williams [39] and Connolly and McCrindle [40] have investigated benzene induced shifts in certain steroidal ketones and simpler ketones respectively and proposed the following empirical rule. If a reference plane (P) is drawn through the carbon atom of the carbonyl group at right-angles to the carbon-oxygen bond, then protons close to (P) show very small shifts ($\delta_{CDCl_3} - \delta_{C_6H_6}$), protons in front of (P), i.e. on the same side as the oxygen of the carbonyl are deshielded, while protons lying behind (P) are shielded.

The results of the methyl shift experiments with the ketones (52), (63) and (14) are shown in table (3) and are consistent with the placing of the ketonic carbonyl group at C-1 in (52), C-2 in (73) and C-3 in (14) respectively. In the case of stach-15-en-3-one (14) the C-20 methyl protons experience a shielding effect of 0·24 p.p.m. From Prentice-Hall models it can be seen that the C-20 protons lie well behind (P). The C-18 protons however lie just behind (P) and exhibit a smaller shielding effect of 0·13 p.p.m., while the C-19 methyl (i.e. the equatorial methyl) which lies very close to (P) but in front of it shows marginal deshielding. The C-17 methyl is unaffected.

Stach-15-en-1-one (52) also shows a large upfield shift for the C-20 protons (+0·24 p.p.m.) while the C-17 methyl exhibits a small deshielding effect (-0·02 p.p.m.) since it now lies in front of (P). It would be expected that the C-18 methyl which is axial would experience a larger upfield shift than the equatorial C-19 methyl. The values found are +0·28 p.p.m. (C-18 protons) and +0·16 p.p.m. (C-19 protons).

TABLE 4

Benzene Solvent Shifts of ketone (72)

Methyl Group	δ_{CDCl_3}	$\delta_{C_6D_6}$	$\Delta = \delta_{CDCl_3} - \delta_{C_6D_6}$
C_{17}	0.84	0.66	+0.18
C_{20}	1.07	0.95	+0.12
C_{19}	1.29	1.22	+0.07
Isopropylide acetal methyls	1.33 1.38	1.26 1.26	+0.07 +0.12
Acetate methyl	1.92	1.54	+0.38
C-12 proton (one half ABq)	2.90	2.83	+0.07
CH-OAc	5.82	6.26	-0.44
H-10 (d, J 4H_Z)	2.09	2.07	+0.02

In stach-15-en-2-one (63) one might expect equal benzene shifts for the C-18 and C-20 methyl groups on the basis of a normal chair cyclohexane model. The results show however that the C-18 methyl experiences a much larger shift (+0·35 p.p.m.) than the C-20 (+0·15 p.p.m.) or C-19 (+0·19 p.p.m.) methyls. This suggests a twisting of the cyclohexanone ring towards a boat conformation thus bringing the C-18 methyl closer to the carbonyl group. The C-17 methyl, as expected, is relatively unchanged (+0·05 p.p.m.).

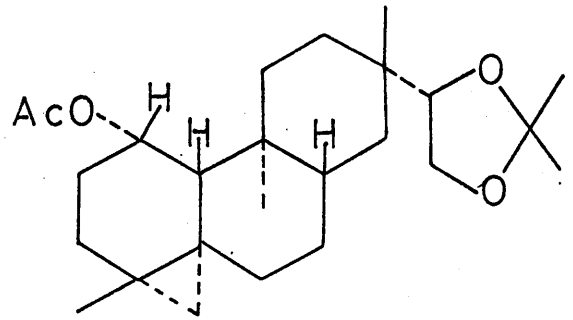
1α-Acetoxy-11-ketoerythroxydiol X isopropylidene acetal (72) shows a large upfield shift for the C-17 protons (+0·18 p.p.m.) and the C-20 protons (+0·12 p.p.m.) (table 4). Both these methyl groups lie behind the reference plane (P) and would be expected to show large shifts. The C-19 protons show a moderate upfield shift (+0·07 p.p.m.) consistent with their position behind, but close to, (P). A shift of approximately the same magnitude (+0·07 p.p.m.) occurs for one of the C-12 protons while a large shielding effect of 0·38 p.p.m. is recorded for the acetate methyl. The most interesting feature of the spectra is the large downfield shift (-0·44 p.p.m.) of the CHOAc proton. This dramatic deshielding can only be explained if the CHOAc proton lies in front of (P) and quite close to it and requires placing the carbonyl at C-11 with the acetate at C-1 in an

δ2·09 in $CDCl_3$ which shifts to δ2·07 in D_6-benzene. Decoupling experiments

δ2·09 in $CDCl_3$ which shifts to δ2·07 in D_6-benzene. Decoupling experiments showed that this proton is coupled to the CHOAc proton and therefore must be the C-10 proton. This assignment is consistent with the very small observed shift.

2) Europium Shifts

In a separate attempt to establish the location of the "extra" oxygen functions in the compounds (65) and (70) with the erythroxydiol X skeleton, recourse was made to the europium shifted spectra. The main object of these



(66)

TABLE 5

§

Molar Ratio	Tertiary Methyls	Isopropylidene Acetal Methyls	Acetate Methyl	C-10 H	C-11 ∝ H	Cyclopropane Protons	CHOAc
0	0.86, 0.88, 1.02	1.27, 1.33	1.93			0.30	5.22
0.2	0.99, 1.19, 1.44	1.55, 1.60	3.20	1.89 (d, J $4H_Z$)	8.0		7.30
0.4	1.14, 1.51, 2.34	2.12, 2.15	5.17	2.71	11.2	1.08 2.54 (J_{AB} $4.5H_Z$)	10.17
$\Delta\delta$ (0.4)	approx. 0.3-1.5	0.85, 0.82	3.24			0.78 2.24	4.95

experiments was the location of the C-10 proton which, with a C-1 oxygen substituent should be readily identified by double irradiation. As already stated (see p.125) deuterium labelling had shown the "extra" oxygen substituent in (65) to be located at either C-1 or C-7. Mass spectroscopic data suggests the former and this was confirmed by the Eu $(dpm)_3$ shifted spectra of the acetate (66), the alcohol (65) and the ketone (67).

The spectra obtained from the acetate (66) proved to be the most informative. This is possibly due to the weaker nature of the complex between an acetoxy group, rather than a hydroxy group, and an europium ion. Line broadening is marginal except in the signals which show very large shifts and therefore lie close to the europium ion. The shifts for the acetate (66) are recorded in Table (5). One tertiary methyl group (C-20) moves downfield quickly while the acetate methyl and the C<u>H</u>OAc proton show the expected [38] large shifts. Decoupling experiments easily identified the C-10 proton signal as a doublet (J $4H_Z$) which collapses to a singlet on irradiation at the C<u>H</u>OAc signal. With a 0•4<u>M</u> ratio of Eu $(dpm)_3$ it is easy to observe the signals for the cyclopropyl protons. The one with the larger shift (2•24 p.p.m.) is the proton <u>Hs</u> <u>syn</u> to the acetoxy group. Another proton which has a very large shift is the <u>α</u>-proton attached at C-11. Since the europium ion lies <u>syn</u> [45] to the C<u>H</u>OAc proton, the placing of the europium ion as shown in figure (5) satisfactorily accounts for the observed shifts.

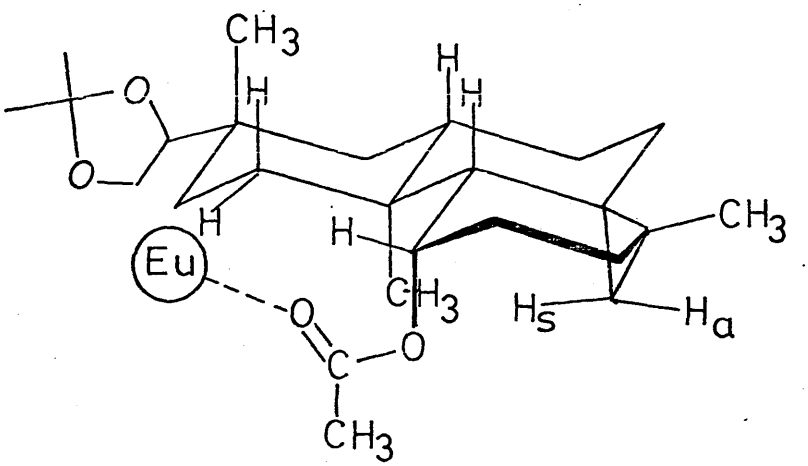
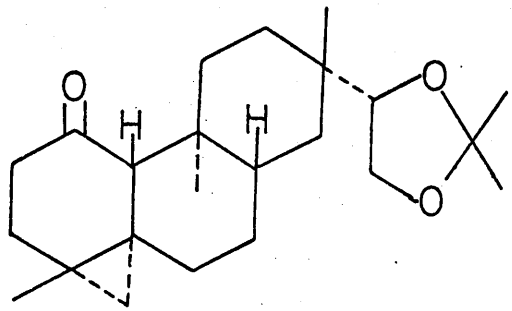


FIGURE 5

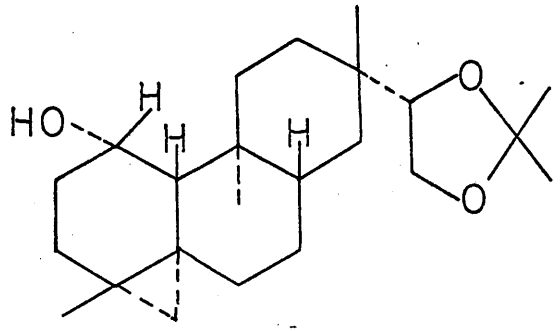


(67)

TABLE 6

δ

Molar Ratio	Tertiary Methyls	Isopropylidene Acetal Methyls	One part Cyclopropyl ABq	C-10 H	C-11 α H
0	0.87, 0.93, 1.06	1.28, 1.34	0.60		
0.5	1.43, 1.62, 2.98	1.95, 1.95	1.08	1.37	6.20
0.75	1.67, 1.85, 3.39	1.95, 1.95	1.40	3.87	7.85
$\Delta\delta_{(0.75)}$	approx. 0.7-2.4	0.67, 0.61	0.80		



(65)

TABLE 7

δ

Molar Ratio	Tertiary Methyls	Isopropylidene Acetal Methyls	One Part Cyclopropyl ABq	C-10 H	C- 2α H C-11α H	CHOH
0	0.88, 1.01, 1.03	1.34, 1.28	0.29			4.22
0.5	1.42, 2.08, 3.74	1.62, 1.62	1.95	3.88	6.25	5.39
$\Delta\delta$ (0.5)	approx. 0.4-2.8	0.28, 0.34	1.66			1.17

The shifted spectra of the ketone (67) also showed one methyl (C-20) moving downfield very quickly (see table 6). As expected the signal for H-10, now a singlet, also experiences large deshielding. This singlet is missing in the spectra of the trideuterioketone (68). From the position of the europium ion in figure (6) it would be expected that the α-proton attached to C-11 would show a large shift. This is indeed the case and it moves downfield as a broad doublet (J $14H_Z$). This signal is also present in the trideuterioketone (68).

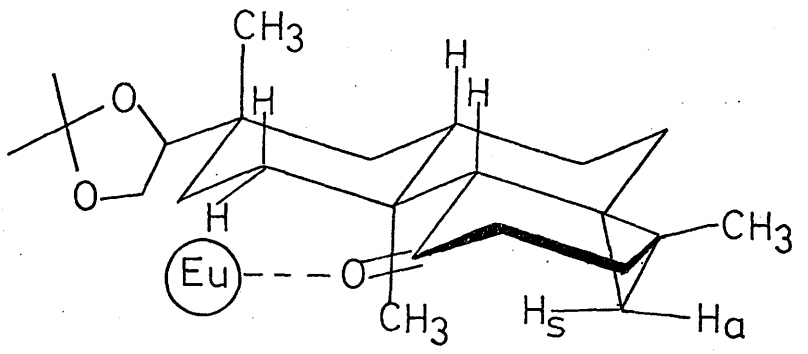
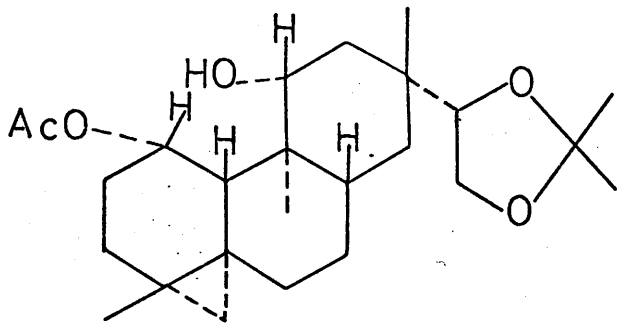


FIGURE 6

Similar results are obtained from the spectra of the alcohol (65) (table 7). The C-10 proton appears as a doublet (J $4H_Z$) coupled to the carbinol proton. This doublet is absent from the spectra of the trideuterioalcohol (69). The α-proton at C-11 also shows a large deshielding, but in this case is superimposed on another very broad signal, probably due to the C-2α proton. The latter signal is absent from the spectra of the trideuterioalcohol (69). A possible geometry for the complex is shown in figure (7).

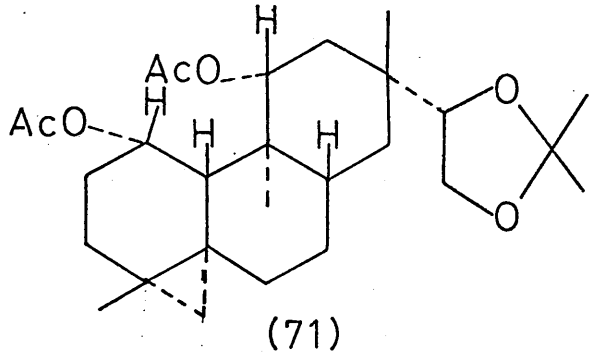


(70)

TABLE 8

δ

Molar Ratio	Tertiary Methyls	Isopropylidene Acetal Methyls	Acetate Methyl	CHOAc	CHOH
0	0.90, 0.96, 1.02	1.28, 1.35	1.91	5.35	3.6
0.4	0.78, 1.86, 4.46	1.43, 1.47	5.60	9.70	6.52
0.6	1.50, 1.58, 5.53	2.18, 2.18	7.50	14.20	10.1
$\Delta\delta_{(0.6)}$	approx. 1.6-4.5	0.90, 0.83	5.59	8.85	6.5



(71)

TABLE 9

δ

Molar Ratio	Tertiary Methyls	Isopropylidene Acetal Methyls	Acetate Methyls	CHOAc's	C-10 H
0	1.00, 1.08, 1.14	1.39, 1.32	2.00, 1.91	4.9, 4.81	
0.5	1.36, 1.49, 2.56	2.12, 2.22	3.44, 3.44	9.02, 8.20	3.00
$\Delta\delta_{(0.5)}$	approx. 0.36-1.5	0.73, 0.90	1.44, 1.53	4.12, 3.39	

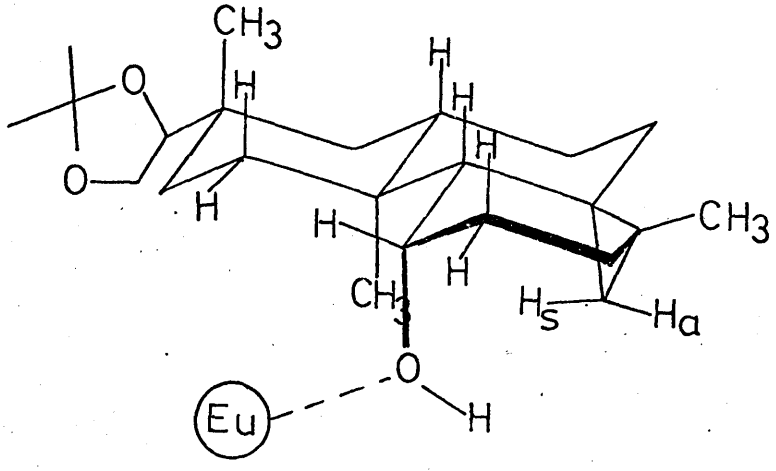


FIGURE 7

The second new isopropylidene acetal was assigned the structure (70) as described previously. Confirmatory evidence for the structure of 1α-acetoxy-11α - hydroxyerthroxydiol X isopropylidene acetal (70) was again obtained using the Eu $(dpm)_3$ shift reagent (table 8). The results obtained are very similar to those above, in that one methyl shows a large deshielding. The C-10 proton is difficult to detect in the case of the hydroxy acetate (70) but can be seen as a broad singlet ($W_{\frac{1}{2}}$ $7H_Z$) in the shifted spectra of the diacetate (71) (table 9). This proton sharpens considerably on irradiation at the CHOAc signal of C-1. Although the complexing strength does not appear to be as great here as in the case of the hydroxy acetate (70) the europium ion appears to be located in approximately the same place (see figure 8).

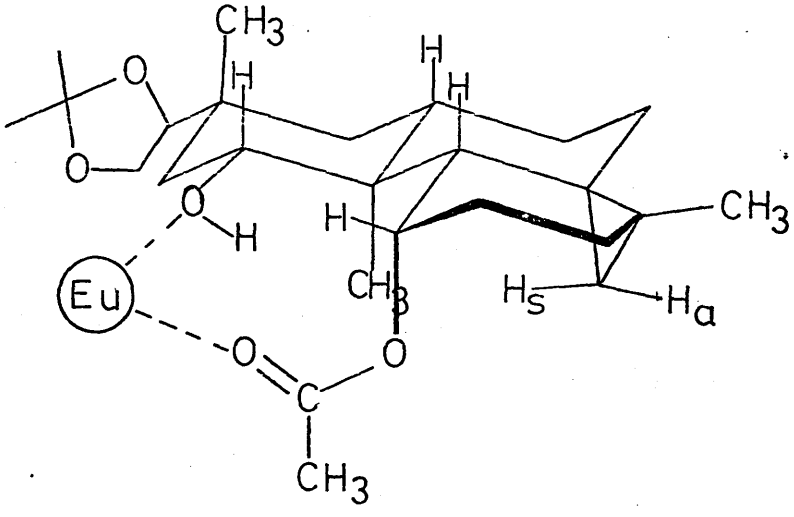
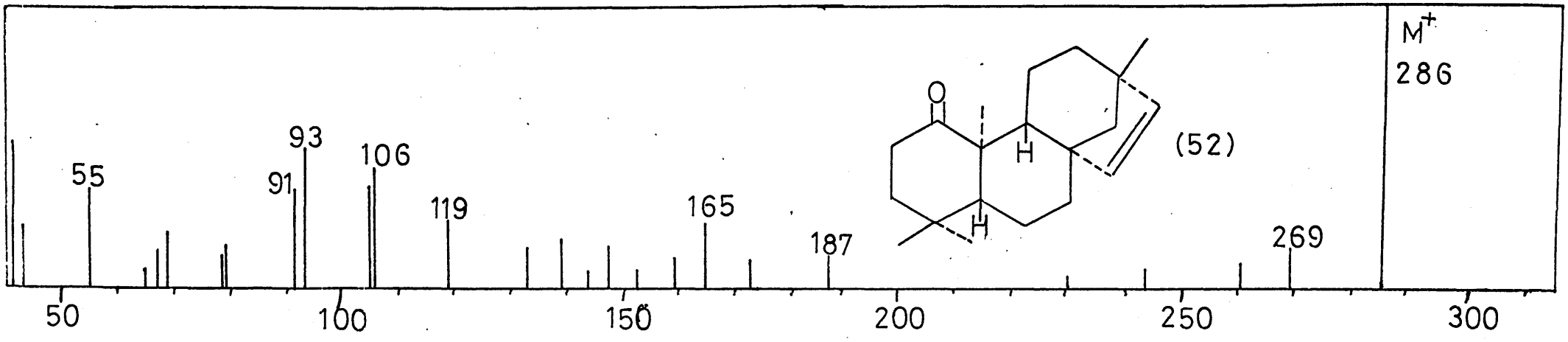


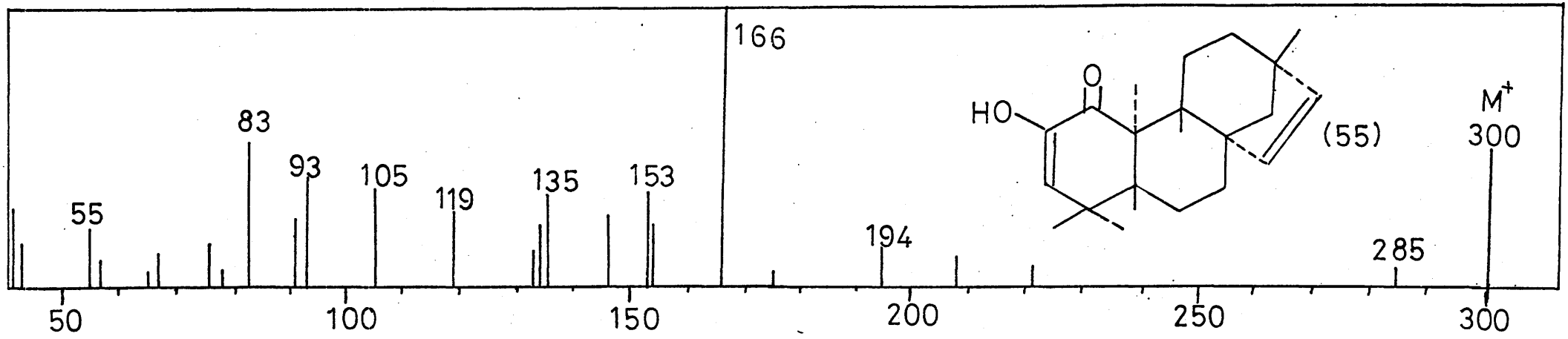
FIGURE 8

FIGURE 9



150

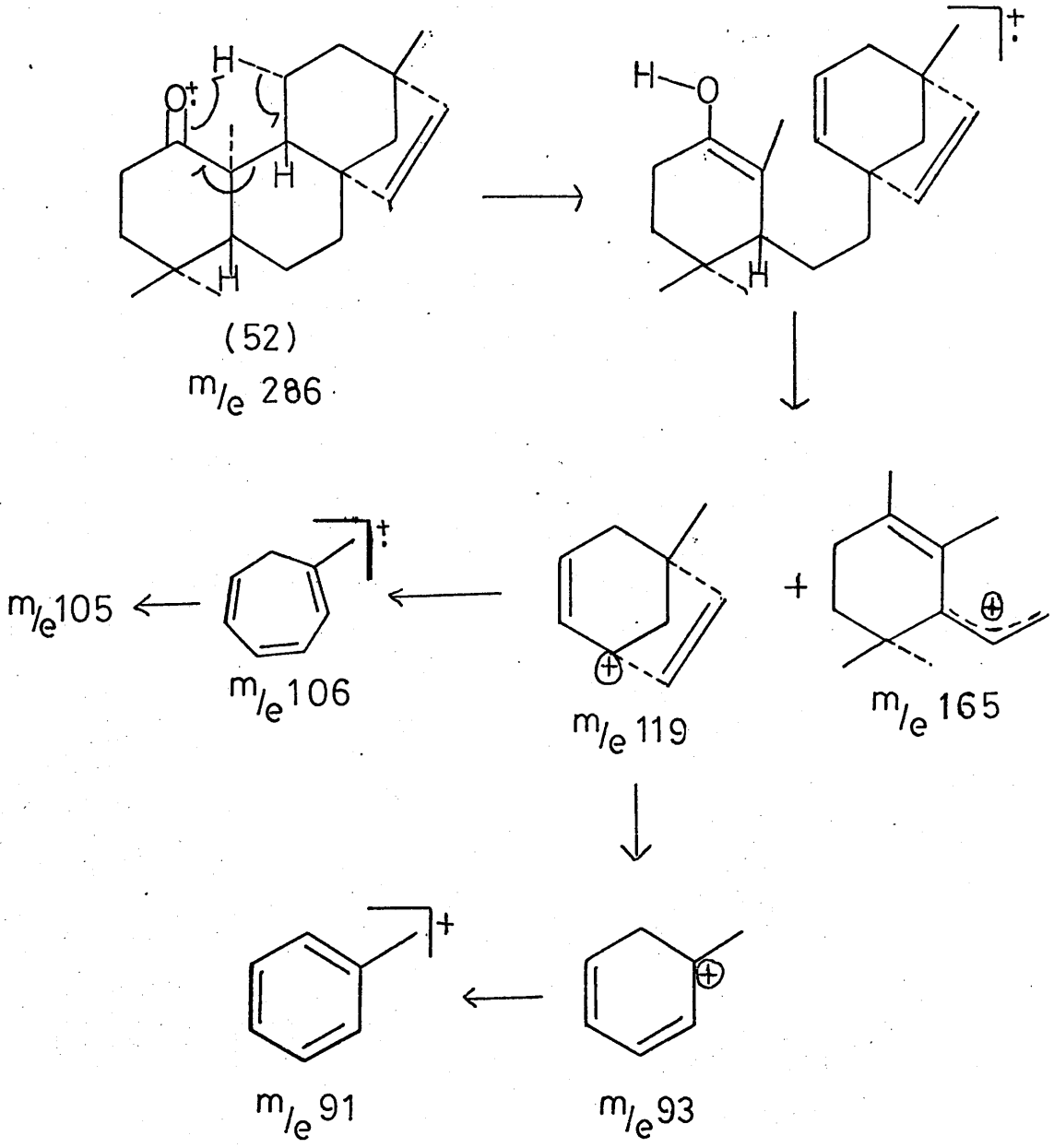
FIGURE 10



d) MASS SPECTRA

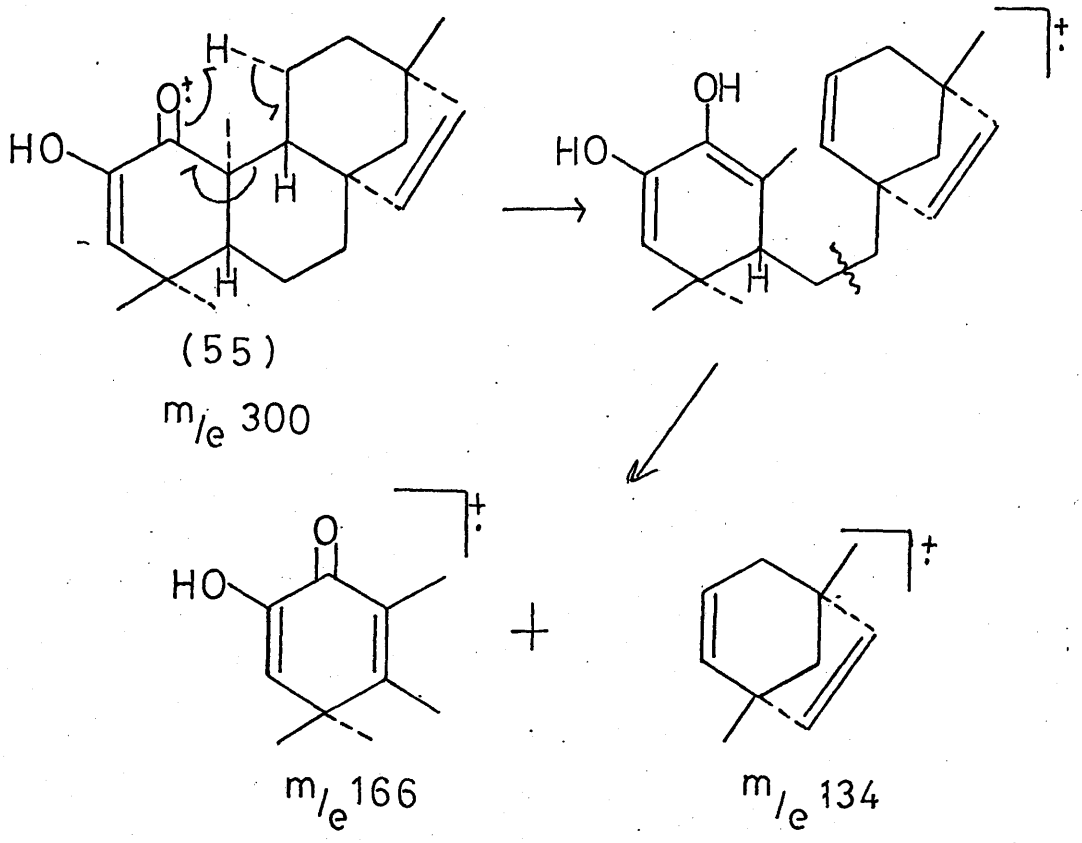
In the mass spectrum of the ketone (52) the base peak is the molecular ion $^{m}/_{e}$ 286. The spectrum is complicated but the most intense fragments are shown in figure (9). A McLafferty rearrangement involving the carbonyl group at C-1, and the α proton at C-11 is the main fragmentation process and subsequent cleavage of the remaining part of ring B gives ring A fragments and also fragments from rings C and D (see scheme 3).

SCHEME 3



In the spectrum of the diosphenol (55) (see figure 10) the McLafferty rearrangement followed by cleavage of the C-6 - C-7 bond leads to the base peak at $^{m}/e$ 166 (see scheme 4).

SCHEME 4



(55)

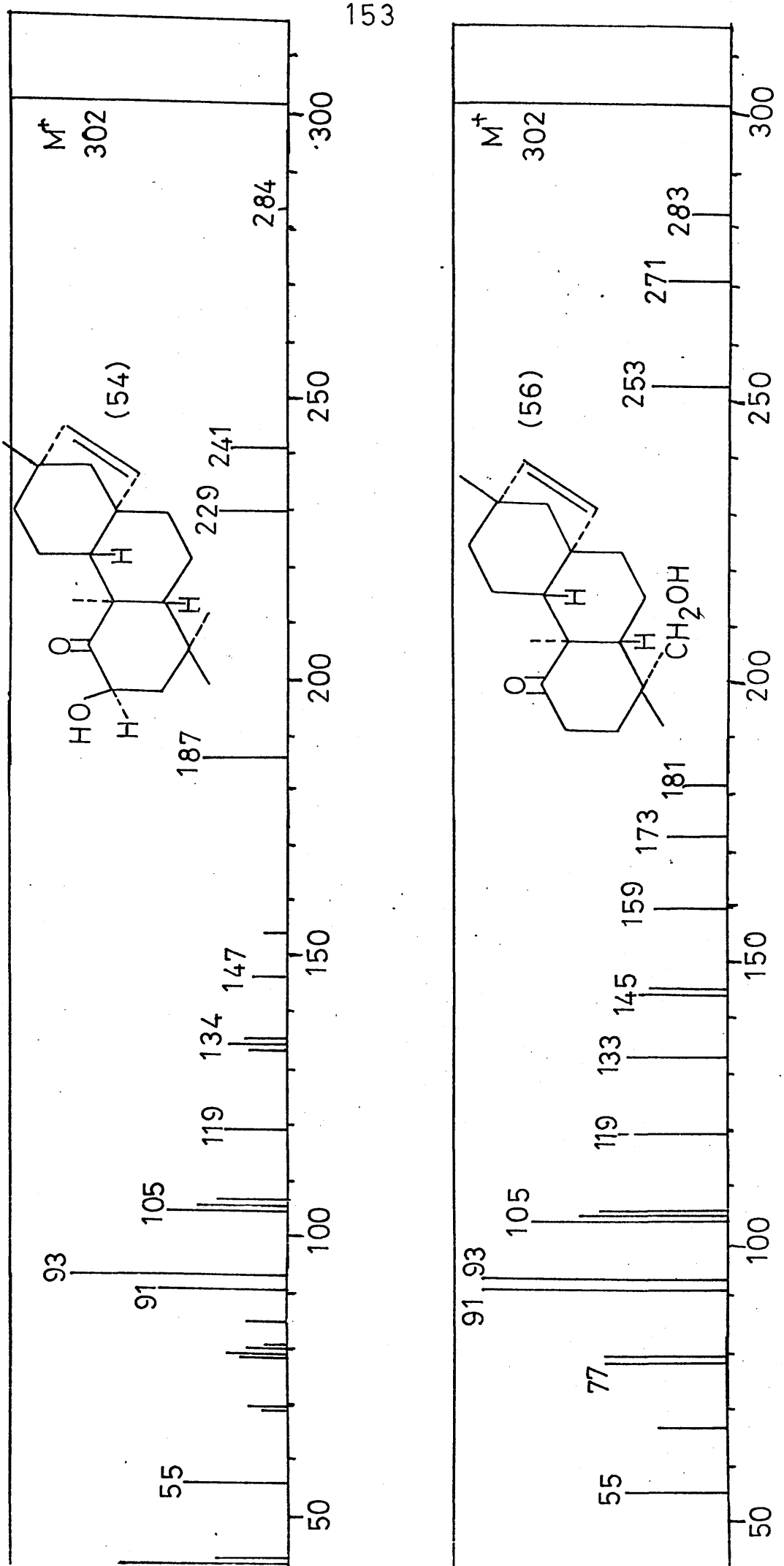
m/e 300

m/e 166

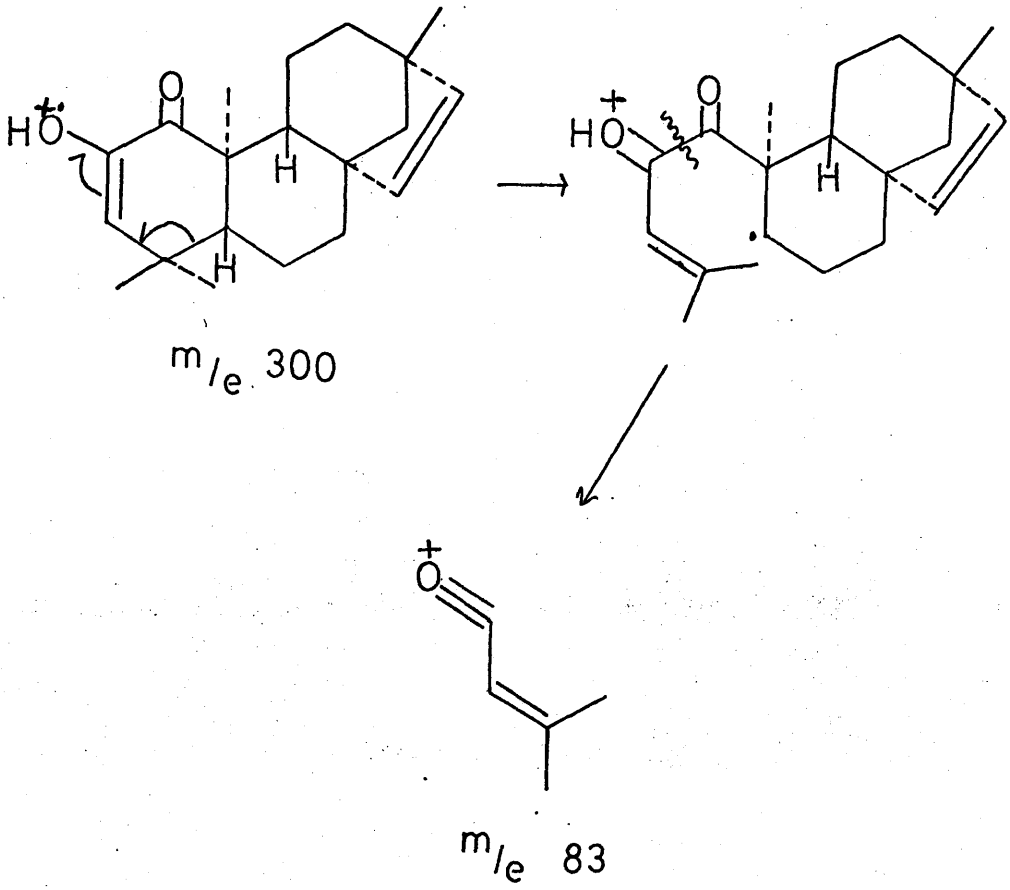
m/e 134

The base peak of the spectrum of the diosphenol acetate (60) is, as expected, at $^{m}/e$ 41 with the next most intense peaks at 43 and 83. The diosphenol itself also shows a large $^{m}/e$ 83 peak which probably arises by cleavage of the C-4 - C-5 bond and subsequent α-carbonyl cleavage as shown in scheme (5).

FIGURE 11


M+
302
(54)
HO
H
O
H
H
284
241
229
187
147
134
119
105
93
91
55
300
250
200
150
100
50
M+
302
(56)
CH2OH
H
O
H
283
271
253
181
173
159
145
133
119
105
93
91
77
55
300
250
200
150
100
50

SCHEME 5



The ketols (54) and (56) (see figure 11) both have their molecular ion as the base peak and many of the peaks present are also found in the mass spectrum of stach-15-en-1-one (52), especially the fragments arising from rings C and D. Two intense fragments in the spectrum of (54) and $^{m}/_{e}$ 229 and $^{m}/_{e}$ 187. A possible fragmentation pathway to account for these peaks is shown in scheme (6). The first step involves

elimination of H_2O followed by two possible cleavages of Ring A.

SCHEME 6

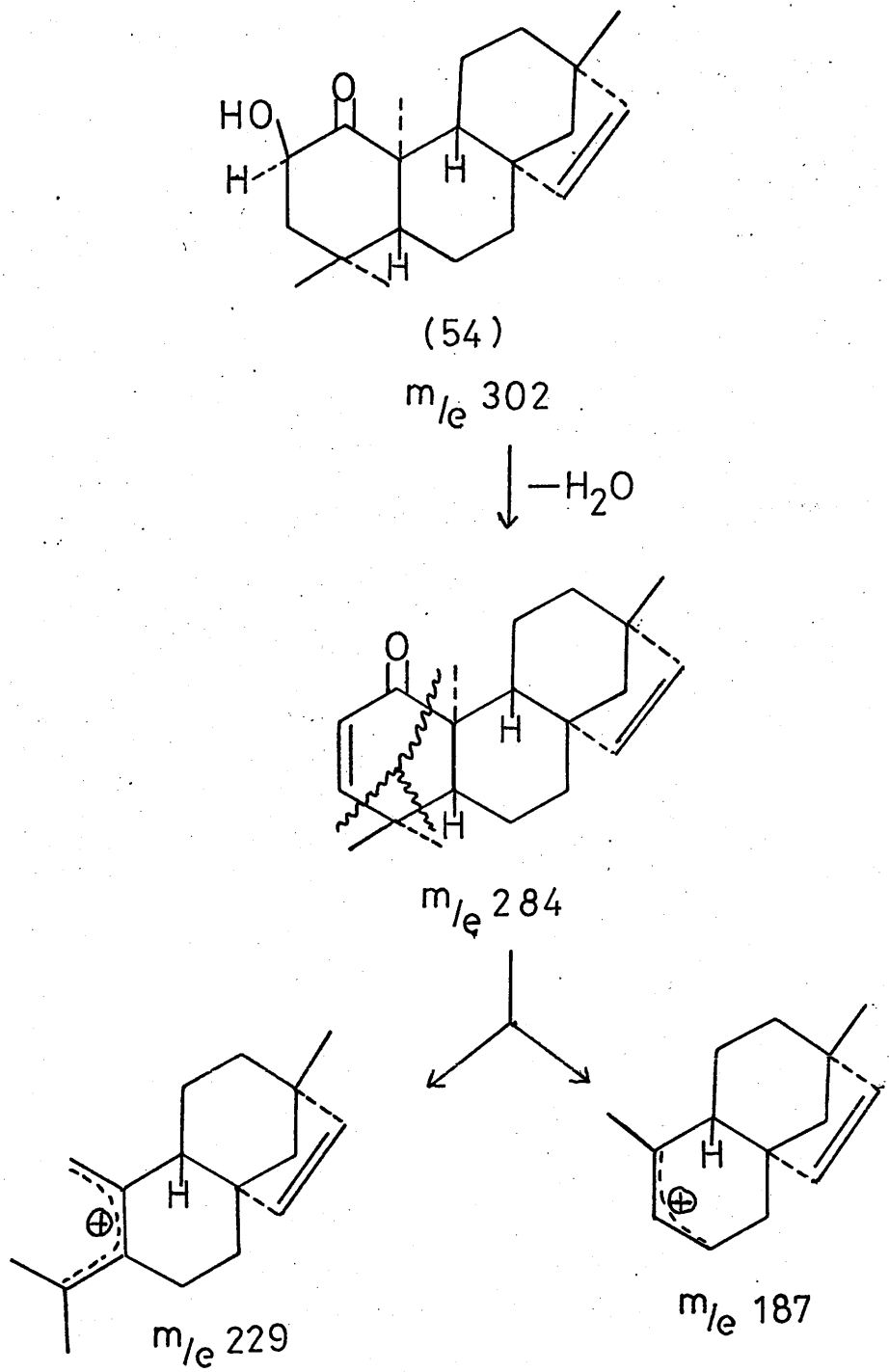


FIGURE 12

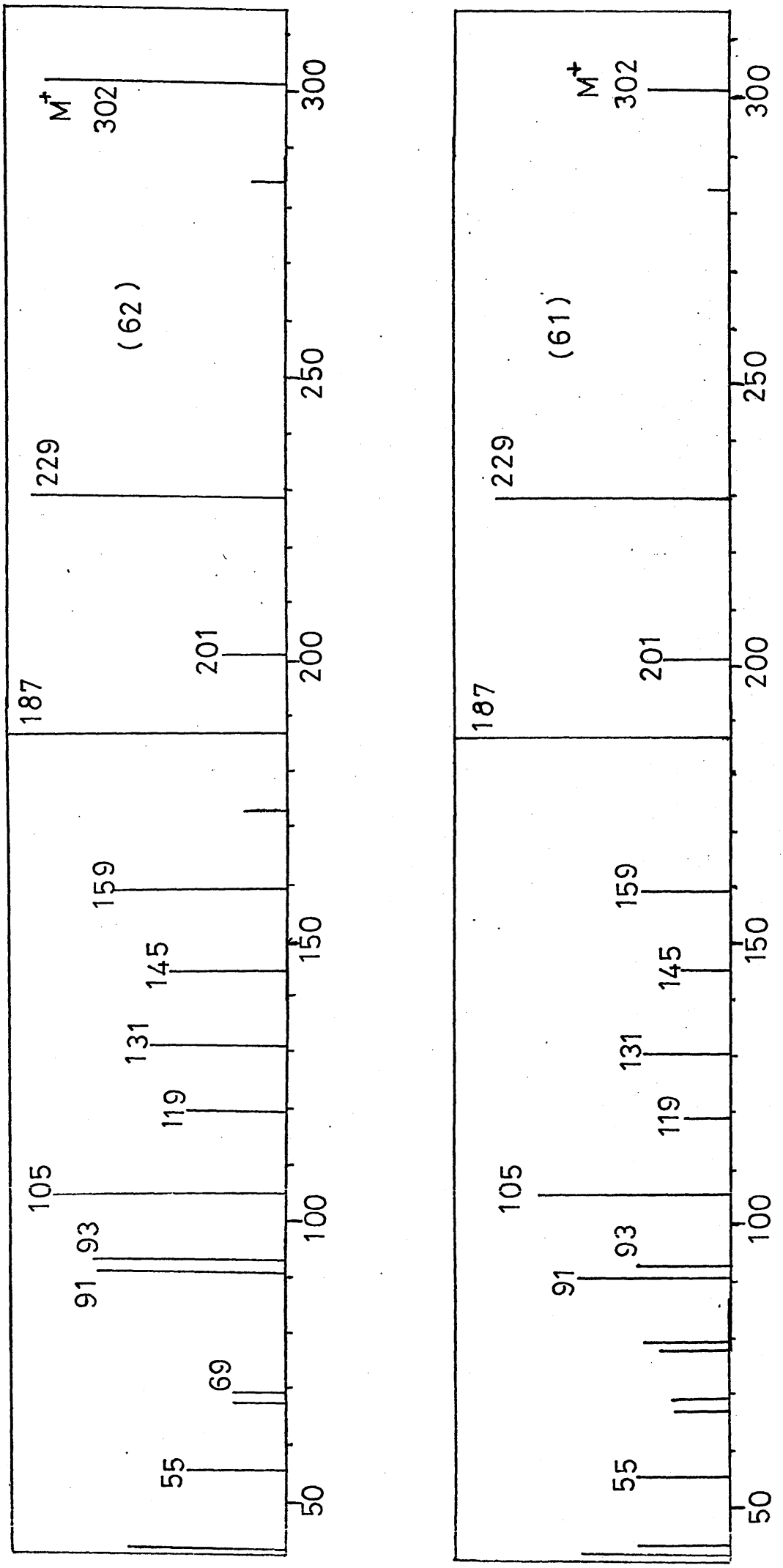
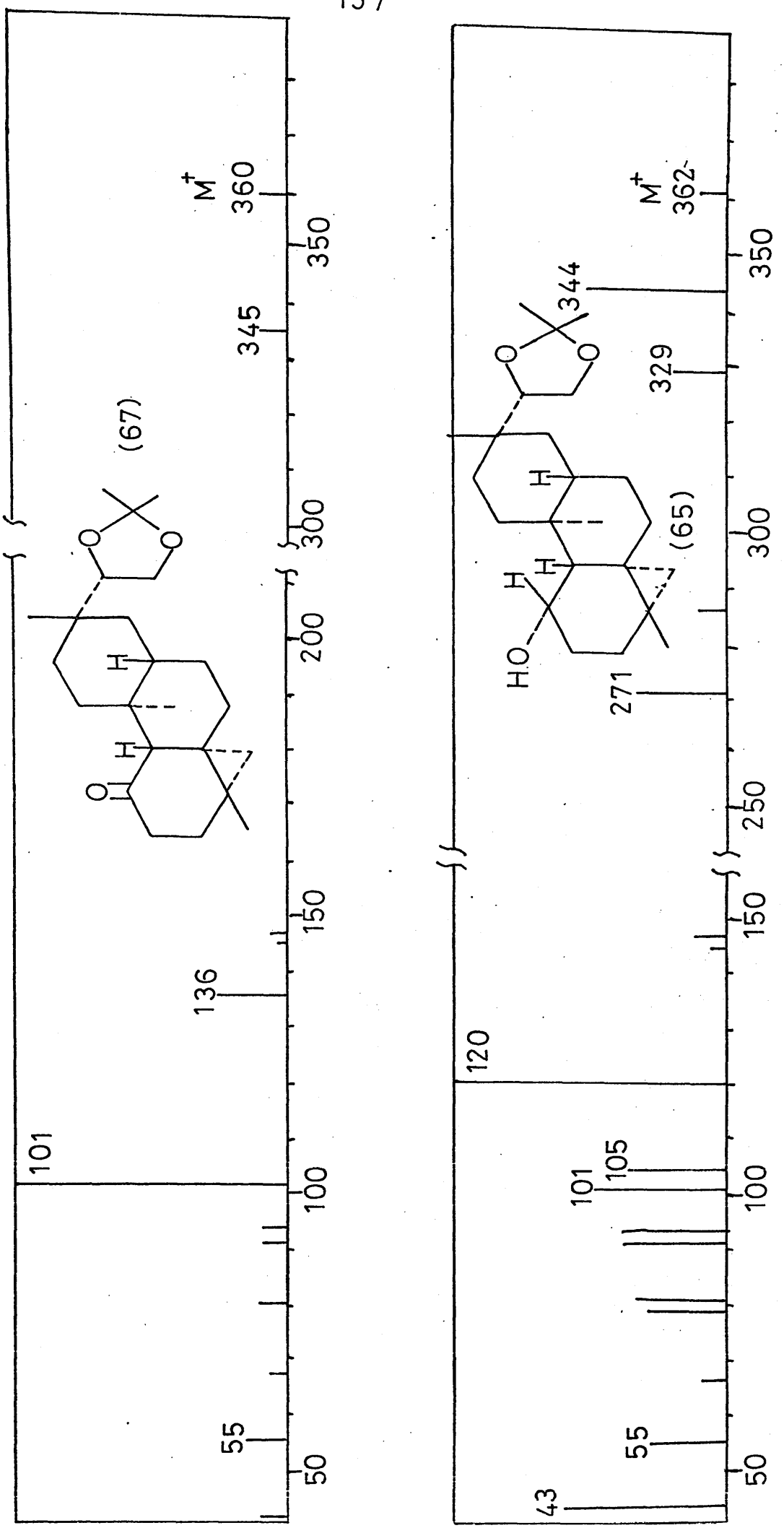
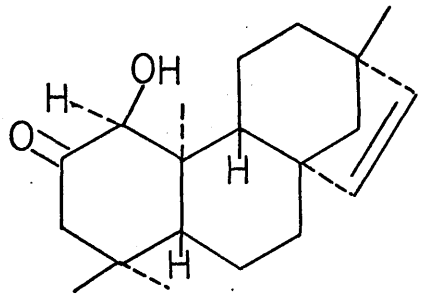

(62)
M+
302
229
201
187
159
145
131
119
105
93
91
69
55
50
100
150
200
250
300
(61)
M+
302
229
201
187
159
145
131
119
105
93
91
55
50
100
150
200
250
300

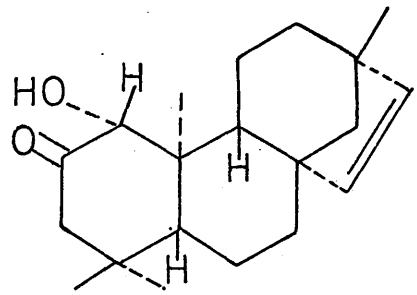
FIGURE 13


M+
360
350
345
300
200
150
136
101
100
55
50
(67)
O
O
H
H
O
M+
362
350
344
329
300
271
250
150
120
101
105
100
55
50
43
(65)
H
H
H
HO

The epimeric ketols (61) and (62) have nearly identical mass spectra (see figure 12) and give the same major fragments at $^{m}/e$ 187 (base peak) and $^{m}/e$ 229 as the ketol (54). The hydroxy group at C-1 appears to facilitate cleavage of ring A.



(62)



(61)

The compounds containing the erythroxydiol X skeleton show a different fragmentation pattern. 1-ketoerythroxydiol X isopropylidene acetal (67) has in it mass spectrum a base peak at $^{m}/e$ 101 (isopropylidene acetal residue). The next most intense peak is at $^{m}/e$ 136 (see figure 13) and can arise by a McLafferty rearrangement involving the carbonyl group at C-1 and the α-proton at C-11 followed by cleavage of the C-6 - C-7 bond as shown in scheme (7).

SCHEME 7

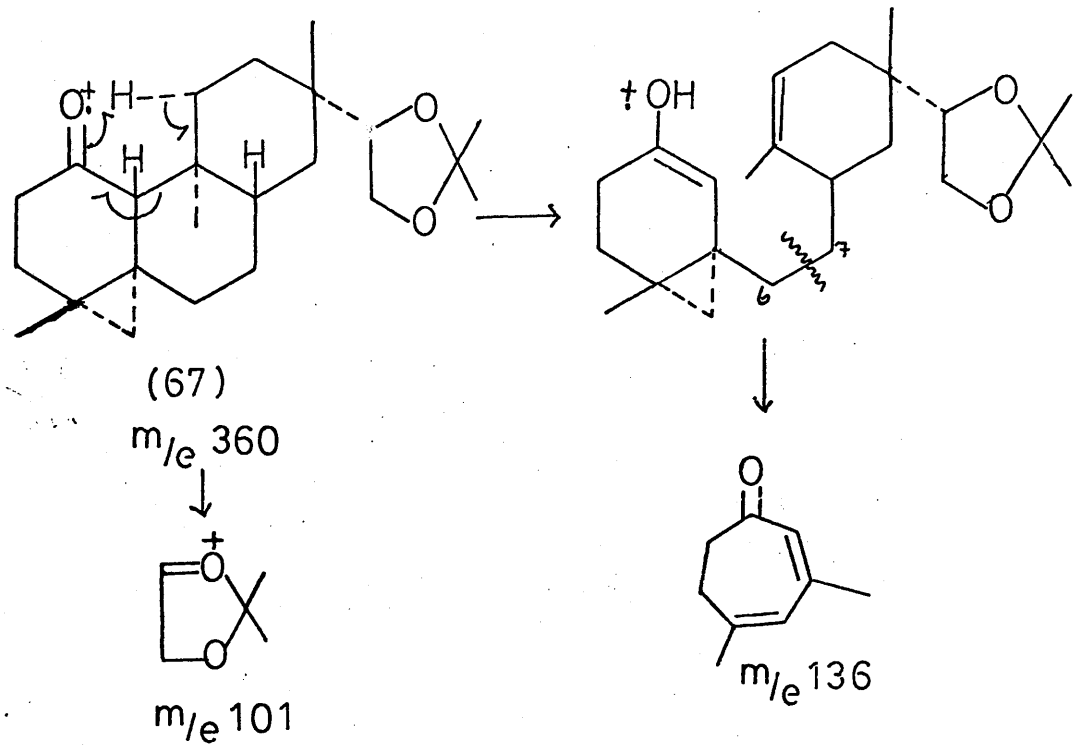
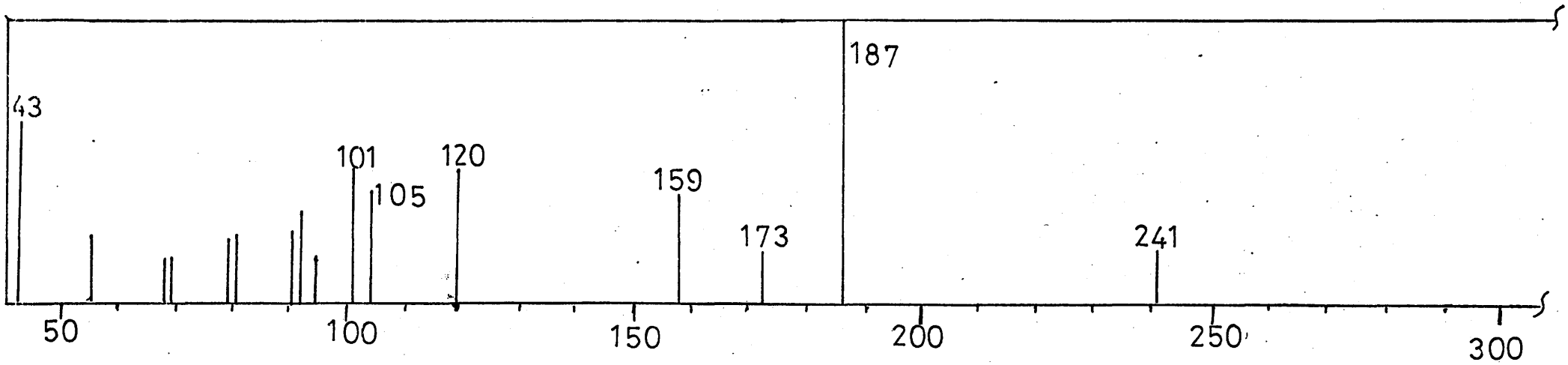
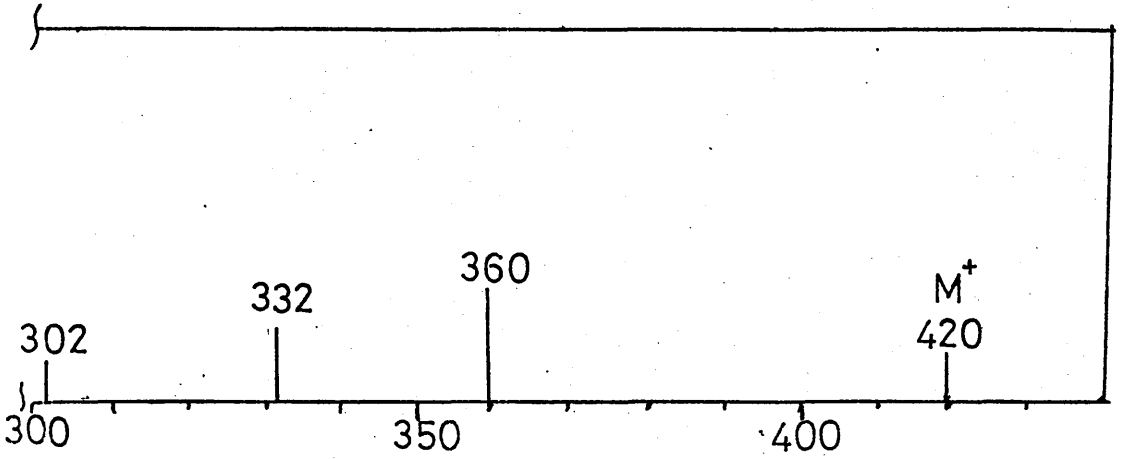
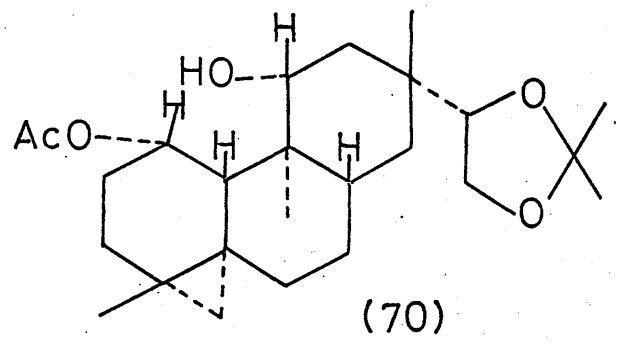


FIGURE 14



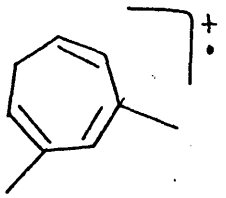




(70)

159

In the trideuterioketone (68) the peak at $^{m}/e$ 136 moves to $^{m}/e$ 139. Similarly in the case of the corresponding alcohol (65) this type of cleavage with loss of H_2O gives rise to $^{m}/e$ 120 (base peak) which, in the trideuterioalcohol, appears at $^{m}/e$ 123 and can therefore be attributed to the ion (74).

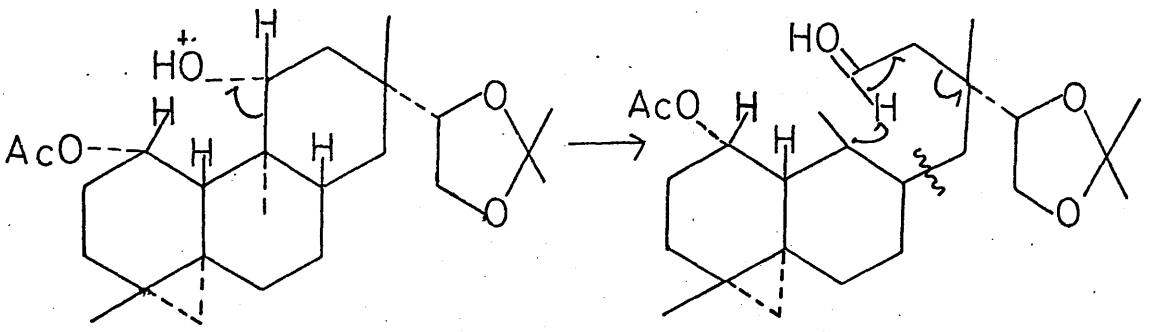


(74)

m/e 120

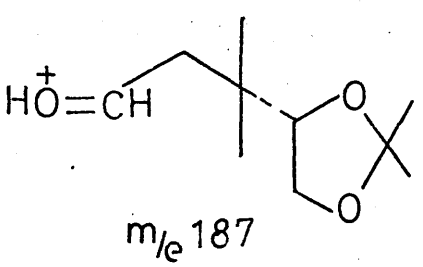
The mass spectrum of the hydroxyacetate (70) (see figure 14) is of interest since the base peak is not the isopropylidene acetal residue at $^{m}/e$ 101 but occurs jointly at $^{m}/e$ 43 and $^{m}/e$ 187 (see scheme 8). This is in accord with findings [46] for 11-oxygenated steroids where cleavage of the C-9 - C-11 bond dominates the breakdown pattern.

SCHEME 8

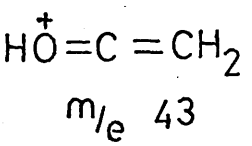


(70)

m/e 420



m/e 187



m/e 43

EXPERIMENTAL

EXTRACTION

Powdered root wood of E. australe was extracted with hot ethyl acetate in a Soxhlet apparatus and the crude extract (90 g.) chromatographed over silica gel using gradually increasing proportions of ethyl acetate in light petroleum. The column was finally washed with ethyl acetate and then methanol. Preparative t.l.c. of the early hydrocarbon fractions over silver nitrate - impregnated silica gave the known hydrocarbons, stachene [12] (10) (100 mg), devadarene [30] (35) (30 mg) and atisirene [30] (33) (150 mg) (identified by n.m.r. and g.l.c.). 2β-Hydroxystach-15-en-1-one (54) (6 g), was isolated by preparative t.l.c. and crystallised from light petroleum as needles, m.p. 109-110°, $[\alpha]_D$ + 220° (c 0·99); νmax (CCl_4) 3470 (sharp, bonded OH) and 1705 cm^{-1}, δ 0·94, 1·01, 1·08, 1·14 (tertiary methyls), 4·5 (q, J_{obs} 12·5 and $5H_Z$, after D_2O exchange, CHOH), 5·47 and 5·58 (olefinic AB_q, J_{AB} $5·5H_Z$), [Found : C, 79·54 ; H, 10·01. $C_{29}H_{30}O_2$ requires C, 79·4 ; H, 10·0%].

The ketone (52) and the diosphenol (55) were initially separated by multiple preparative t.l.c. A more convenient method of separation involved acetylation of the mixture followed by t.l.c. and saponification (see later). Stach-15-en-1-one (52) (1·2g) was recrystallised from methanol as prisms, m.p. 77-78° $[\alpha]_D$ - 114° (c1·88), νmax (CCl_4) 1709 cm^{-1}, δ 0·94, 1·00, 1·08, 1·13 (tertiary methyls), 5·47 and 5·58 (olefinic AB_q, J_{AB} $5·5H_Z$), [Found : C, 84·02 ; H, 10·60. $C_{20}H_{30}O$ requires C, 83·9 ; H, 10·6%]. 2-Hydroxystach-2-15-dien-1-one (55) (1·1g) was recrystallised from light petroleum as needles, m.p. 108-109°, $[\alpha]_D$ - 11° (c4·56), νmax (CCl_4) 3440, 1682 and 1660 cm^{-1}, λ max (Et OH) 270 n.m. (ϵ 10,000) changing to

λ max 315 n.m. (ϵ 6,000) on addition of 0·1M KOH, δ 1·04 and 1·14 (9H) (tertiary methyls), 5·64 and 5·50 (olefinic AB_q, J 5·5H_z), and 5·65 (s, 3-H), [Found : M^+ (mass spectrometry), 300 · 2091. $C_{20}H_{28}O_2$ requires M, 300 · 2091].

A minor product present in these fractions and separated by preparative t.l.c. was the epoxy-ketone (53) (100 mg) which was crystallised from light petroleum as needles, m.p. 131-132°, $[\alpha]_D$ - 36° (c 1·72), ν max (CCl_4) 1709 cm^{-1}, δ 0·96, 1·03, 1·09 and 1·29 (tertiary methyls), 3·38 and 3·07 (epoxide protons, J 4H_z), [Found : C, 79·33 ; H, 9·96. $C_{20}H_{30}O_2$ requires C, 79·42 ; H, 10.0%].

18-Hydroxystach-15-en-1-one (56) (100 mg), was isolated from the intermediate fractions and recrystallised from light petroleum as needles, m.p. 105-106°, $[\alpha]_D$ - 31° (c 1·93), ν max (CCl_4) 3630 (sharp, free OH), 3480 (bonded OH), and 1709 cm^{-1}, δ 1·00, 1·04, and 1·10 (tertiary methyls), 3·63 and 3·84 (AB_q, J_{AB}11H_z, CH_2OH), 5·47 and 5·62 (olefinic AB_q, J_{AB} 5·5H_z), [Found : M^+ (mass spectrometry), 302 · 2243. $C_{20}H_{30}O_2$ requires M, 302 · 2246]. Two further compounds present in these fractions were identified (m.p., t.l.c., and n.m.r.) as erythroxylol A[23] (17) (200 mg), and erythroxydiol X [26, 27] (22) (12 g).

The combined later fractions (1 g) were stirred with anhydrous copper sulphate (2 g) in acetone (50 ml) and the crude product subjected to preparative t.l.c. to yield erythroxydiol X isopropylidene acetal (780 mg) and erythroxytriol Q isopropylidene acetal (20 mg) (both identified [27, 28] by n.m.r. and comparison with authentic samples). 1α-Hydroxyerythroxydiol X isopropylidene acetal (65) (120 mg), was recrystallised from methanol as needles, m.p. 186-187°, $[\alpha]_D$ - 26° (c 1·86), ν max (CCl_4) 3624 (free OH), 3500 (bonded OH), and 1735 cm^{-1}, δ 0·29 (d, J_{AB}4·5H_z, one part of cyclopropane AB_q), 0·90, 0·96 and 1·02 (tertiary methyls), 1·35 and 1·28

(isopropylidene acetal methyls), 1·91 (CH_3CO_2), 3·6 (CHOH, obscured by acetal ABC system), and 5·35 br, (s, $W_{\frac{1}{2}}$ 8H_z, CHOAc), [Found : C, 71·15 ; H, 9·66. $C_{25}H_{40}O_5$ requires C, 71·39 ; H 9·59%].

EPOXIDATION OF STACH-15-EN-1-ONE (52)

The ketone (52) (20 mg) and m-chloroperbenzoic acid (25 mg) were dissolved in chloroform (4 ml) and left at room temperature for 1 hour. The reaction mixture was filtered through a short column of basic alumina and the epoxy-ketone (53) was purified by preparative t.l.c., and crystallised from light petroleum as needles (15 mg), m.p. 131-132°, $[\alpha]_D$ - 36° (c 1·5) (identical in all respects with natural material).

STACHAN-1-ONE (75)

The ketone (52) (15 mg) was hydrogenated over 10% palladium - charcoal catalyst in ethyl acetate (10 ml) for four hours. The product, stachan-1-one, was crystallised from light petroleum as needles (12 mg), m.p. 72-73°, ν_{max} (CCl_4) 1709 cm^{-1}, δ 0·96 (6H), 1·04 and 1·30 (tertiary methyls), [Found : C, 82·98 ; H, 10·98. $C_{20}H_{32}O$ requires C, 83·27 ; H, 11·18%].

WOLFF-KISHNER REDUCTION OF KETONE (52)

Ketone (52) (20 mg), hydrazine hydrate (0·3 ml), triethylene glycol (5 ml) and sodium hydroxide (100 mg) were heated at 200° for one and a half hours under a nitrogen atmosphere. The hydrocarbon fraction (12 mg) of the product was a mixture of stachene (10) and stachane (57) (n.m.r. and g.l.c. on 2½% SE-30 at 170° and 15% ApL at 240°).

STACH-15-EN-1β-OL (58)

Ketone (52) (78 mg) in methanol (5 ml) was reduced with excess sodium borohydride over four hours. The crude product was crystallised from light petroleum to yield stach-15-en-1β-ol (58) (65 mg), m.p. 82-84°, νmax (CCl_4) 3610 cm^{-1}, δ 0·79, 0·87, 0·92, and 1·02 (tertiary methyls), 3·6 (m, CHOH), and 5·70 and 5·46 (olefinic AB_q, $J_{AB}6H_Z$), [Found : M^+ (mass spectrometry), 288 · 2454. $C_{20}H_{32}O$ requires M, 288 · 2453]. A minor product from this reduction was stach-15-en-1α-ol (59) (9 mg), δ 0·83 (6H), 0·89, 1·02 (tertiary methyls), 3·35 (t, J $7H_Z$; -CHOH), 5·46, 5·68 (olefinic AB_q, J_{AB} $5·5H_Z$), M.Wt 288. Tosylation of stach-15-en-1β-ol (58) under normal conditions, followed by lithium aluminium hydride reduction resulted in recovery of the original alcohol.

ACETYLATION OF THE DIOSPHENOL (55)

The mixture of the ketone (52) and diosphenol (55) (175 mg) from the extract was acetylated under normal conditions. The product was separated by preparative t.l.c. to yield the ketone (52) (vide supra) and the diosphenol acetate (60)(57 mg) which was crystallised from light petroleum as fine needles, m.p. 162-163°, $[\alpha]_D$ - 43° (C 1·45), νmax (CCl_4) 1768 and 1700 cm^{-1}, δ 1·00, 1·11, 1·14, and 1·17 (tertiary methyls), 2·15 (CH_3CO_2), 5·68 and 5·53 (olefinic AB_q, J_{AB} $5·5H_Z$), and 5·96 (s, 3-H), [Found : C, 76·92 ; H, 8·88. $C_{22}H_{30}O_3$ requires C, 77·15 ; H, 8·83%]. Saponification of the acetate (60) (55 mg) in 1·5% ethanolic KOH (12 ml) under reflux for one hour gave the diosphenol (55) (52 mg), m.p. 108-109° (from light petroleum), identical with natural material.

2β-ACETOXYSTACH-15-EN-1-ONE (76)

The α-ketol (54) was acetylated under normal conditions and the product crystallised from light petroleum to give 2β-Acetoxy stach-15-en-1-one as needles, m.p. 112-113°, ν max (CCl_4) 1750 and 1728 cm^{-1}, δ 0·98 (6H), 1·04, and 1·06 (tertiary methyls), 2·15 (CH_3CO_2), 5·8 - 5·4 (superimposed CHOAc and olefinic AB_q), [Found : C, 76·60 ; H, 9·37. $C_{22}H_{32}O_3$ requires C, 76·70 ; H, 9·36%].

INTERCONVERSION OF THE α-KETOL (54) AND THE KETONE (52)

The α-ketol (54) (45 mg) was treated with toluene -β-sulphonyl chloride (0· 2 g) in dry pyridine (2 ml) at 20° for 60 hours. The crude tosylate was reduced with excess lithium aluminium hydride in refluxing ether for 4 hours and the product, in acetone (3 ml), subjected to oxidation with Jones' reagent at 20° for 3 minutes. Preparative t.l.c. afforded stach-15-en-1-one (52) (24 mg), identical with natural material.

INTERCONVERSION OF THE α-KETOL (54) AND THE DIOSPHENOL (55)

The α-ketol (54) (95 mg) in acetic acid (2 ml) was heated with an excess of bismuth oxide on a steam bath for 45 minutes. The solution was filtered, diluted with water, and extracted several times with benzene. The crude product (90 mg) was purified by preparative t.l.c. and crystallisation from light petroleum to give the diosphenol (55), m.p. 108-109° identical with natural material.

1α-HYDROXYSTACH-15-EN-2-ONE (61)

The α-ketol (54) (30 mg) in acetic acid (2 ml) was exposed to bismuth oxide at 100° for 15 minutes. The product was a mixture of three components which were separated by t.l.c. and identified as diosphenol (55) (10 mg), starting ketol (6 mg), and 1α-hydroxystach-15-en-2-one (61) (11 mg). The new ketol was crystallised from light petroleum as prisms, m.p. 104-105°, ν_{max} (CCl_4) 3465 and 1712 cm^{-1}, δ 0·66, 0·91, 0·99, and 1·07 (tertiary methyls), 3·89 (s, CHOH), and 5·57 and 5·45 (olefinic AB_q, J_{AB} 5·5 H_Z), [Found : C, 79·21; H, 9·96. $C_{20}H_{30}O_2$ requires C, 79·4; H, 10.0%]. The corresponding acetate (77) crystallised from light petroleum as needles, m.p. 151-152°, δ 0·85 (6H), 1·01, 1·18 (tertiary methyls), 2·12 (CH_3CO_2), 2·32 (one part AB_q, J_{AB} 6 H_Z, CO-CH_2-C-) 4·97 (s, CHOAc), 5·63 and 5·50 (olefinic AB_q, J_{AB} 5·5 H_Z), [Found : M^+ (mass spectrometry) 344 · 2352. $C_{22}H_{32}O_3$ requires M, 344 · 2351].

EQUILIBRATION OF THE KETOL (54) WITH BASE

The ketol (54) (60 mg) was refluxed for 5 hours in 5% ethanolic KOH (20 ml) under nitrogen. The product consisted of four compounds, separable by preparative t.l.c. These were the diosphenol (20 mg), starting material (16 mg), 1α-hydroxystach-15-en-2-one (61) (3 mg), and a more polar ketol (62) (13 mg) (1β-hydroxystach-15-en-2-one). The more polar ketol was recrystallised from chloroform - light petroleum as needles, m.p. 169-170°, ν_{max} (CCl_4) 3620, 3440 and 1720 cm^{-1}, δ 0·72, 0·87, 1·01 and 1·08 (tertiary methyls), 3·59 (s, CHOH), and 5·70 and 5·50 (olefinic AB_q, J_{AB} 5·5 H_Z), [Found : C, 79·32 ; H, 9·99. $C_{20}H_{30}O_2$ requires C, 79·4 ; H, 10.0%]. The derived acetate (78) was crystallised from methanol as needles, m.p. 150-151°, δ 0·75, 0·87, 0·99, 1·08 (tertiary methyls), 2·13 (CH_3CO_2-), 2·64 and 1·94

(AB_q, J_{AB} 12H_Z, CO-C$\underline{H}_2$-C(-)-), 4·49 (s, C$\underline{H}$OAc), 5·67 and 5·51 (olefinic AB_q, J_{AB} 5·5H_Z), [Found : M^+ (mass spectrometry), 344 · 2351. $C_{22}H_{32}O_2$ requires M, 344 · 2351].

STACH-15-EN-2-ONE (63)

1α-Hydroxystach-15-en-2-one (61) (26 mg) was converted into the corresponding tosylate under the usual conditions. To the tosylate (30 mg) in acetone (8 ml) and acetic acid (2 ml) was added excess of chromous chloride solution and the mixture was stirred under nitrogen at reflux temperature for 4 hours. The crude product was plated to remove unchanged tosylate. Stach-15-en-2-one (10 mg) was recrystallised from methanol and had m.p. 118-119° (lit, [20] 119-120°).

18-ACETOXYSTACH-15-EN-1-ONE (64)

The keto-alcohol (56) was acetylated under normal conditions to give 18-acetoxystach-15-en-1-one, m.p. 90-91° (from methanol), ν max (CCl_4) 1734 and 1709 cm^{-1}, δ 1·00, 1·03, and 1·12 (tertiary methyls), 2·07 ($C\underline{H}_3CO_2$-), 4·40 and 3·95 (AB_q, J_{AB} 11H_Z, C$\underline{H}_2$OAc), 5·83 and 5·48 (olefinic AB_q, J_{AB} 5·5H_Z), [Found : M^+ (mass spectrometry), 344 · 2344. $C_{22}H_{32}O_3$ requires M, 344 · 2351].

WOLFF-KISHNER REDUCTION OF THE KETO-ALCOHOL (56)

The keto-alcohol (70 mg), potassium hydroxide (200 mg), hydrazine hydrate (0·6 ml), and triethylene glycol (10 ml) were heated at 200° under nitrogen for 3 hours. Preparative t.l.c. of the product afforded

erythroxylol A (17) (55 mg) m.p. 119-120°, $[\alpha]_D$ + 40°, as needles from light petroleum (lit.[23] m.p. 119-120°, $[\alpha]_D$ + 39°).

1α-ACETOXYERYTHROXYDIOL X ISOPROPYLIDENE ACETAL (66)

1α-Hydroxyerythroxydiol X isopropylidene acetal (65) (30 mg) in dry pyridine (3 ml) and acetic anhydride (3 ml) was heated on the steam bath for 4 hours. Normal work-up followed by preparative t.l.c. gave the acetate (66) (28 mg), m.p. 138-139° (fine needles from methanol), νmax (CCl_4) 1737 cm^{-1}, δ 0·30 (one part cyclopropane AB_q, J_{AB} 4·5 H_Z), δ 0·86, 0·88 and 1·02 (tertiary methyls), 1·27 and 1·33 (isopropylidene acetal methyls), 1·93 (CH_3CO_2), 5·22 br (s, $W_{\frac{1}{2}}$ 8 H_Z, CHOAc), [Found : C, 74·08 ; H, 9·93. $C_{25}H_{40}O_4$ requires C, 74·21 ; H, 9·97%].

1 -KETOERYTHROXYDIOL X ISOPROPYLIDENE ACETAL (67)

The alcohol (65) (20 mg) in acetone (2 ml) was treated with Jones' reagent (3 drops) at room temperature for 3 minutes. Preparative t.l.c. afforded the ketone (67) (14 mg), m.p. 103-104° (plates from methanol), νmax (CCl_4) 1710 cm^{-1}, δ 0·25, 0·60 (cyclopropane AB_q J_{AB} 4·5 H_Z), 0·87, 0·93, and 1·06 (tertiary methyls), 1·28, 1·34 (isopropylidene acetal methyls), [Found : M^+ (mass spectrometry), 360 • 2670. $C_{23}H_{36}O_3$ requires M, 360 • 2664]. Sodium borohydride reduction of (67) gave back the original alcohol (65). The ketone (67) did not react under conditions normally used for a) Wolff-Kishner reduction, b) thioketalisation or c) enol acetylation.

DEUTERIUM EXCHANGE EXPERIMENTS

The ketone (67) (40 mg) was dissolved in dry dioxan (10 ml) and deuterium oxide (3 ml) under nitrogen. Sodium (100 mg) was added in small pieces and the reaction mixture stirred at 60° for 96 hours. The solvents were removed *in vacuo* and the residue was extracted with dry ether. Mass spectral analysis of the product showed 0 ^2H_o, 0 $^2H_1$, 10 $^2H_2$, 90 $^2H_3$ and 0% $^2H_4$.

The trideuterated ketone (68) (20 mg) was reduced with sodium borohydride to give the trideuterated alcohol (69), δ 4·21 (sharp s, C<u>H</u>OH). Mass spectral analysis showed 0 ^2H_o, 0 $^2H_1$, 12 $^2H_2$, 88 $^2H_3$ and 0% $^2H_4$.

1α, 11α-DIACETOXYERYTHROXYDIOL X ISOPROPYLIDENE ACETAL (71)

The hydroxy acetate (70) was acetylated under normal conditions to give the diacetate (71) which was crystallised from methanol as needles, m.p. 175-176°, νmax (CCl_4), 1732 cm^{-1}, δ 0·29 (one part cyclopropane AB_q, J_{AB}4·5H_Z), 1·00, 1·08, and 1·14 (tertiary methyls), 1·39, 1·32 (isopropylidene acetal methyls), 2·00, 1·93 ($C\underline{H}_3CO_2$), 4·9 (q, J_{obs} 10, 6H_Z, 11-H, C<u>H</u>OAc) and 4·81 br (s, $W_{\frac{1}{2}}$ 8H_Z, 1-H, C<u>H</u>OAc), [Found : C, 69·93 ; H, 9·22. $C_{27}H_{42}O_6$ requires C, 70·1 ; H, 9·15%].

1α-ACETOXY-11-KETOERYTHROXYDIOL X ISOPROPYLIDENE ACETAL (72)

Oxidation of the hydroxy-acetate (70) (10 mg) in acetone, with Jones' reagent at room temperature for 3 minutes afforded the acetoxy-ketone (72) as a gum, ν max (CCl_4) 1736 and 1704 cm^{-1}, δ 0·86, 1·07, 1·29 (tertiary methyls), 1·33, 1·38 (isopropylidene acetal methyls) 1·92 ($C\underline{H}_3CO_2$), 2·90

(one part of AB_q, J_{AB} 12H_Z, $COC\underline{H}_2$-C-), 5·85 br. (s, $W_{\frac{1}{2}}$ 8H_Z, 1-H, C$\underline{H}$OAc), [Found : M^+ (mass spectrometry), 418 · 2717. $C_{25}H_{38}O_5$ requires M, 418 · 2719].

REFERENCES

1. J.D. Connolly and A.E. Harding, J.C.S. Perkin I, 1972, 1996.
2. a) J.R. Hanson, "The Tetracyclic Diterpenes", Pergamon Press, London, 1968 ; b) R. McCrindle and K.H. Overton, Advan. Org. Chem., 1965, 5, 47.
3. W.S. Johnson, Accounts of Chemical Research, 1968, 1, 1 and references cited therin.
4. a) I.E. Graebe, D.T. Dennis, C.D. Upper and C.A. West, J. Biol. Chem., 1965, 240, 1847 ; b) I. Shechter and C.A. West, Ibid, 1969, 244, 3200 ; c) D.R. Robinson and C.A. West, Biochem., 1970, 9, 70, 80.
5. A.J. McAlees, R. McCrindle and R.D.H. Murray, Chem. and Ind., 1966, 240.
6. R.A. Appleton, A.J. McAlees, A. McCormick, R. McCrindle and R.D.H. Murray, J. Chem. Soc.(C), 1966, 2319.
7. R.M. Coates and E.F. Bertram, J. Org. Chem., 1971, 36, 3722.
8. S.F. Hall and A.C. Odhlschlager, Tetrahedron, 1972, 28, 3155.
9. K.H. Pegel, L.P.L. Piacenza, L. Phillips and E.S. Waight, Chem. Comm., 1971, 1346.
10. M. Laing, P. Sommerville, D. Hanouskova, K.H. Pegel, L.P.L. Piacenza, L. Phillips and E.S. Waight, Chem. Comm., 1972, 196.
11. R.C. Gupta and M.S. Muthana, J. Indian. Inst. Sci., 1954, 36A, 76, 122.
12. R. McCrindle, A. Martin andR.D.H. Murray, J. Chem. Soc. (C), 1968, 2349.
13. Y. Kitahara, A. Yoshikoshi, and S. Oida, Tetrahedron Letters, 1964, 1755, 1763.
14. W.H. Baarchers, D.H.S. Horn and Le Roy F. Johnston, J. Amer. Chem. Soc., 1962, 84, 4046.
15. A.H. Kapadi and Sukh Dev, Tetrahedron Letters, 1964, 1171, 1902.

16. A.I. Scott, F. McCapra, F. Comer, S.A. Sutherland, D.N. Young, G.A. Sim and G. Ferguson, Tetrahedron, 1964, 20, 1339.

17. P.R. Jefferies, R.S. Rosich and D.E. White, Tetrahedron Letters, 1963, 1793.

18. E. Wenkert, P.W. Jeffs and J.R. Mahajan, J. Amer. Chem. Soc., 1964, 86, 2218.

19. J.R. Hanson, Chem. and Ind., 1964, 1579.

20. J.R. Hanson, Tetrahedron, 1967, 23, 793.

21. C. Djerassi, P. Quitt, E. Mosettig, R.C. Lambie, P.S. Rutledge and L.H. Briggs, J. Amer. Chem. Soc., 1961, 83, 3720.

22. B.F. Cross, R.H.B. Galt, J.R. Hanson and W. Klyne, Tetrahedron Letters, 1962, 145.

23. R.D.H. Murray and R. McCrindle, Chem. and Ind., 1964, 500.

24. R. Soman and Sukh Dev, Tetrahedron Letters, 1964, 1181.

25. A.H. Kapadi and Sukh Dev, Tetrahedron Letters, 1964, 2751.

26. R. Soman, Sukh Dev, R. Misra and R.C. Pandey, Tetrahedron Letters, 1964, 3767.

27. J.D. Connolly, R. McCrindle, R.D.H. Murray, A.J. Renfrew, K.H. Overton and A. Melera, J. Chem. Soc. (C), 1966, 268.

28. J.D. Connolly, D.M. Gunn, R. McCrindle, R.D.H. Murray and K.H. Overton, J. Chem. Soc. (C), 1967, 668.

29. A. Martin and R.D.H. Murray, J. Chem. Soc. (C), 1968, 2529.

30 A.H. Kapadi, R.R. Sobti andSukh Dev, Tetrahedron Letters, 1965, 2729.

31. A. Martin, R.D.H. Murray, J. Chem. Soc. (C), 1970, 2023.

32. J.C. Fairlie, R. McCrindle and R.D.H. Murray, J. Chem. Soc. (C), 1969, 2115.

33. J.R. Hanson, Tetrahedron, 1970, 26, 2711.

34. K. Mori, Y. Nakahara and M. Matsui, Tetrahedron, 1972, 28, 3217.

35. F. Piozzi, P. Venturella, A. Bellino and M.L. Marino, J.C.S. Perkin I, 1973, 1164.

36. J. Rigby, J. Chem. Soc., 1951, 793.

37. G. Rosencranz, O. Mancera, J. Gatica and C. Djerassi, J. Amer. Chem. Soc., 1950, 72, 4077.

38. C.C. Hinkley, M.R. Klotz and F. Patil, J. Amer. Chem. Soc., 1971, 93, 2417.

39. M.S. Bhacca and D.H. Williams, Applications of N.M.R. Spectroscopy in Organic Chemistry. Illustrations from the Steroid Field, Holden-Day, San Francisco, 1964, 7 ; Tetrahedron Letters, 1964, 3127.

40. J.D. Connolly and R. McCrindle, Chem and Ind., 1965, 379.

41. P.M. Scopes, Private communication.

42. R. Hodges, Tetrahedron, 1961, 12, 215.

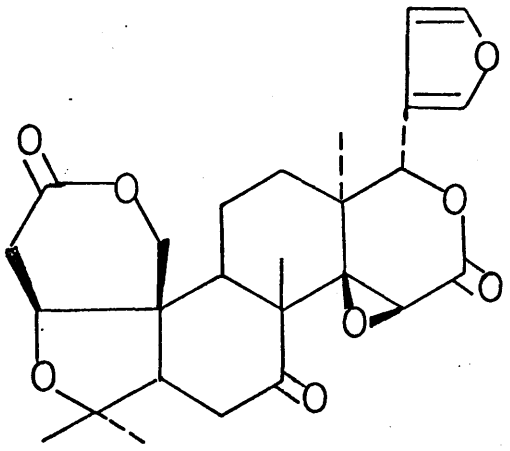
43. W. Klyne, Advan. Org. Chem., 1960, 1, 291.

44. a) M. Legrand, R. Viennet and J. Caumartin, Comptes Rendus, 1961, 253, 2378 ; b) P. Crabbe, Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry, Holden-Day, 1965, 166 and references cited therein.

45. P.V. Demarco, T.K. Elzey, R.B. Lewis and E. Wenkert, J. Amer. Chem. Soc., 1970, 92, 5734.

46. H. Obermann, M. Spiteller-Firedmann, and G. Spiteller, Tetrahedron, 1971, 27, 1093, 1101, 1737, 1747.

SECTION 3



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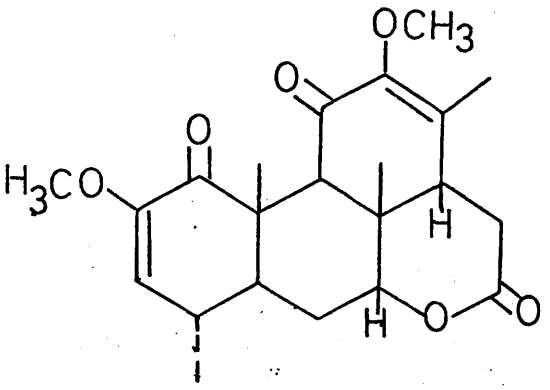

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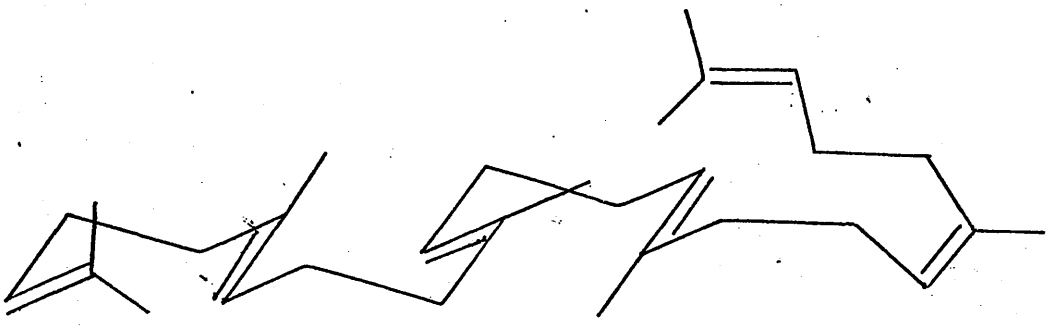

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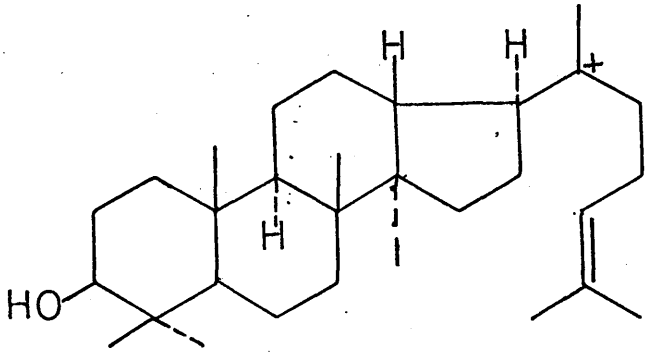

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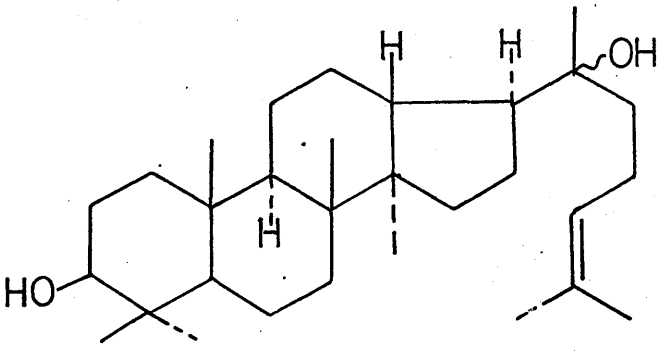

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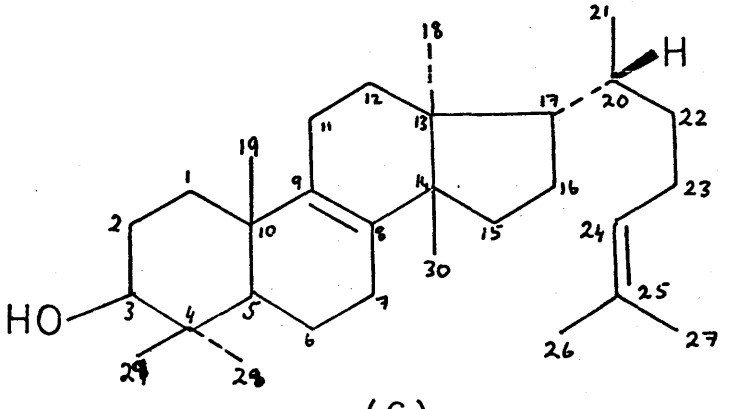

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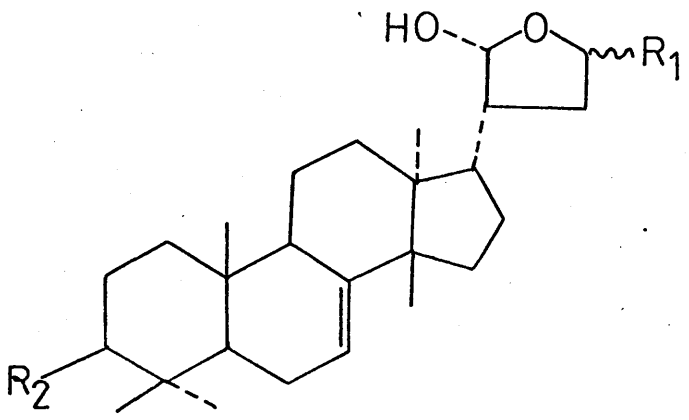
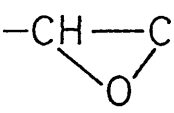
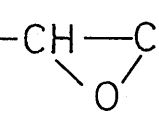
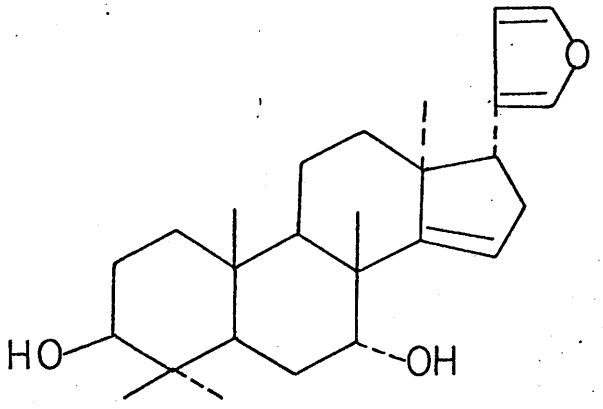


(7) $R_1 =$  ; $R_2 = \underline{\beta}$-OAc

(10) $R_1 = -CH=C(CH_3)_2$; $R_2 = \underline{\beta}$-OH

(11) $R_1 =$  ; $R_2 = =O$



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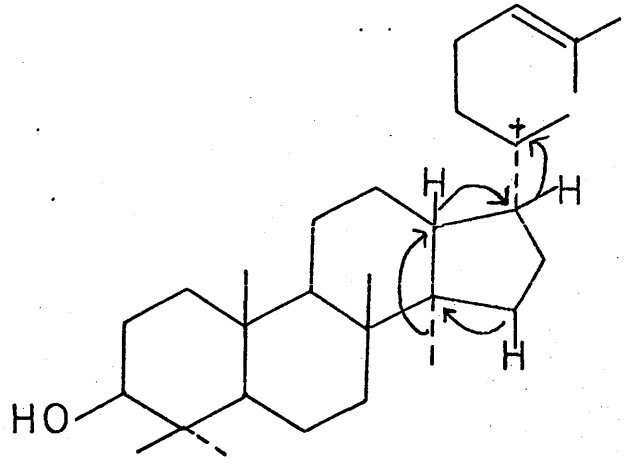


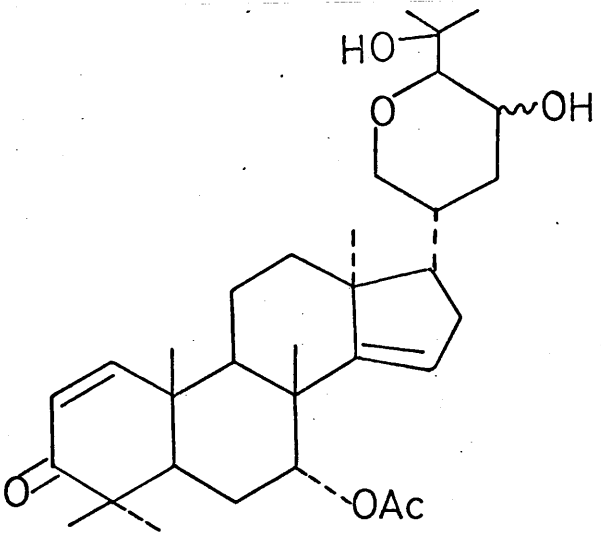
(9)

PRIEURIANIN, A COMPLEX TETRANORTRITERPENOID FROM TRICHILIA PRIEURIANA

INTRODUCTION

In 1960 the elucidation [1] of the structure of limonin (1) opened a new chapter in terpenoid chemistry. Two years later the structure of quassin (2) was established [2], followed by evidence [3-6] that both limonin (1) and quassin (2) share the same biosynthetic pathway.

Cyclisation of squalene in the chair, chair, chair, boat, conformation (3) gives rise to the carbonium ion (4) which can be hydrated to give dammarendiol (5) or undergo subsequent rearrangement to the euphol, (or its C-20 epimer, tirucallol) skeleton (6). Euphol (tirucallol) [7] (6) is the postulated biogenetic precursor of the limonoid and quassinoid group of triterpenoids. Thus euphol (6) suffers the loss of the four terminal side chain carbon atoms, by cleavage of the C-23 - C-24 bond and formation of the furan ring [8]. This is probably preceded by a skeletal rearrangement during which one methyl group migrates from C-14 to C-8, a step which may be initiated by epoxidation of the nuclear double bond of the Δ^7-isomer of euphol (i.e. the apo-euphol (tirucallol) rearrangement). These processes have been realised [9] in the laboratory in the conversion of turreanthin (7) into the diol (8) which can be regarded as the simplest limonoid. This rearranged carbon skeleton may also be considered [10, 11] to arise from the cationic intermediate ((9) arrows) resulting from the cyclisation of squalene, without the intervention of euphol (6). Since C-20 becomes trigonal in the course of this transformation it is uncertain whether limonoids derive from euphol (20 β H) or tirucallol (20 α H).



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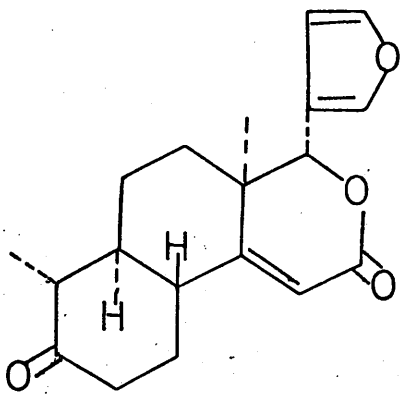

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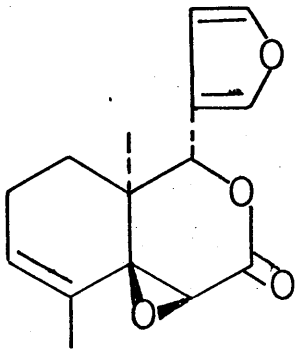

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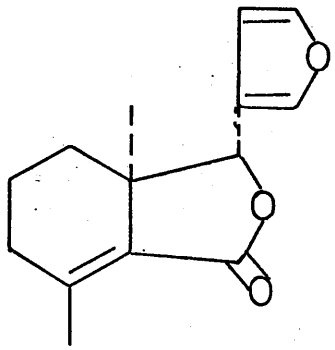

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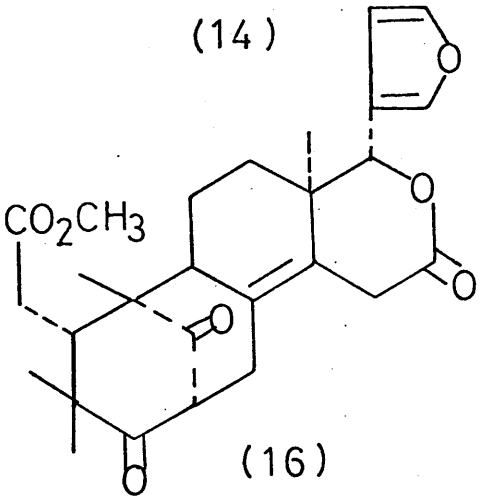

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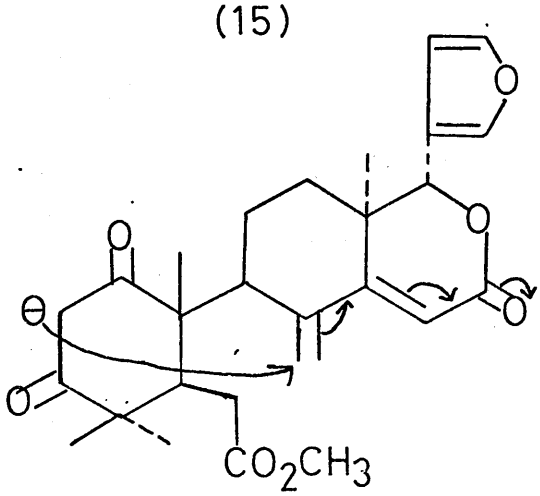

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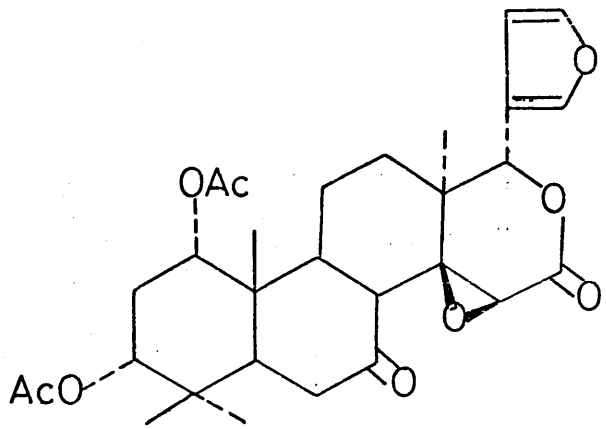

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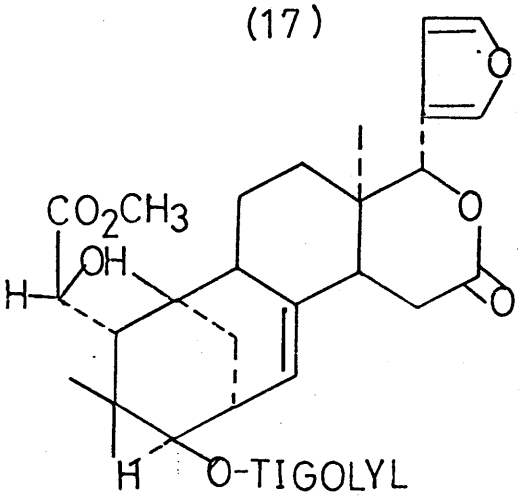
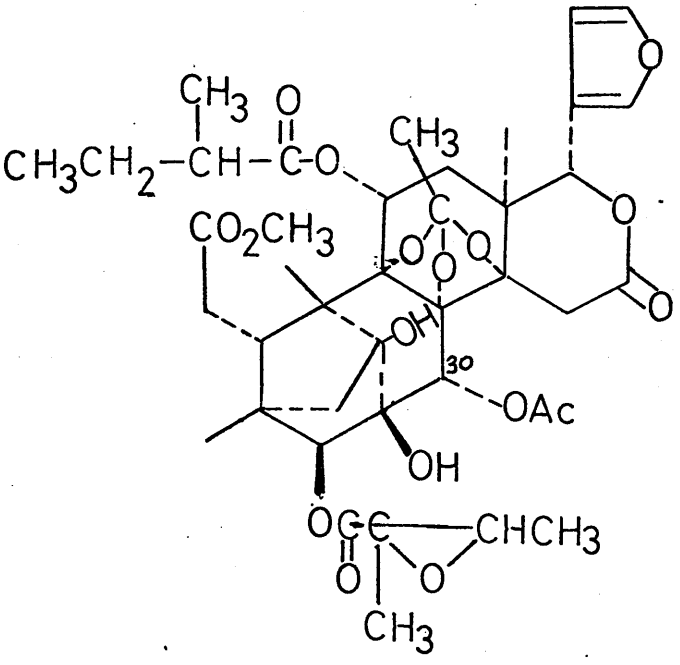


(19)

The isolation of C-30 compounds from Meliaceae, Rutaceae and Simaroubaceae with various stages of side-chain oxygenation lends support for this postulated biogenetic route. Flindissol (10) [12], turreanthin (7) [8], and melianone (11) [13] are all tirucallol derivatives with a potential furan ring in the side chain. Grandifoliolenone (12) [14] is particularly interesting since it was the first of a small group of naturally occurring apo-tirucallol derivatives. The C-20 quassinoids probably represent a later stage along the same biosynthetic pathway. Examples of further degradation have appeared with the isolation of odoratin (13) [15], calodendrolide (14) [16] and fraxillenone (15) [17]. Odoratin (13), from Cedrela odorata L. is biogenetically related to the bicyclononalide group of limonoids. The root bark of Calodendrum capense (Rutaceae) yielded [16] calodendrolide (14) the first naturally occurring C15 degraded limonoid. Fraxillenone (15) which was isolated [17] from several trees belonging to the Rutaceae and Meliaceae has been synthesised [18]. Several reviews dealing with the limonoids and quassinoids have appeared [10, 19-22]. These compounds are apparantly confined to the three families Meliaceae and Rutaceae (limonoids) and Simaroubaceae (quassinoids).

One of the most interesting of the modifications of the intact tetranortriterpenoid skeleton is the formation of the bicyclo (3.3.1) nonane system found in mexicanolide (16) (bicyclononalide group). In biogenetic terms, mexicanolide (16) can be derived from a precursor of the type (17) which can undergo intramolecular Michael addition. Two partial syntheses [23, 24] of mexicanolide (16) starting from 7-oxo-7-deacetoxykhivorin (18) [25] have been reported. Both these syntheses emulate the proposed biogenetic pathway by the transformation of an intact skeletal type (18), via ring-B cleaved derivatives into mexicanolide (16). The base induced fragmentations of some swietenine (19) derivatives have been studied [26].



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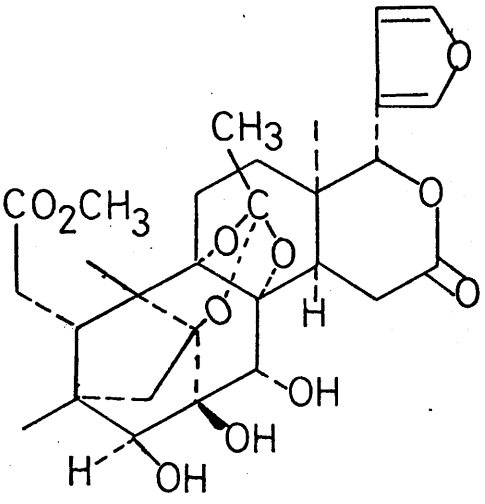

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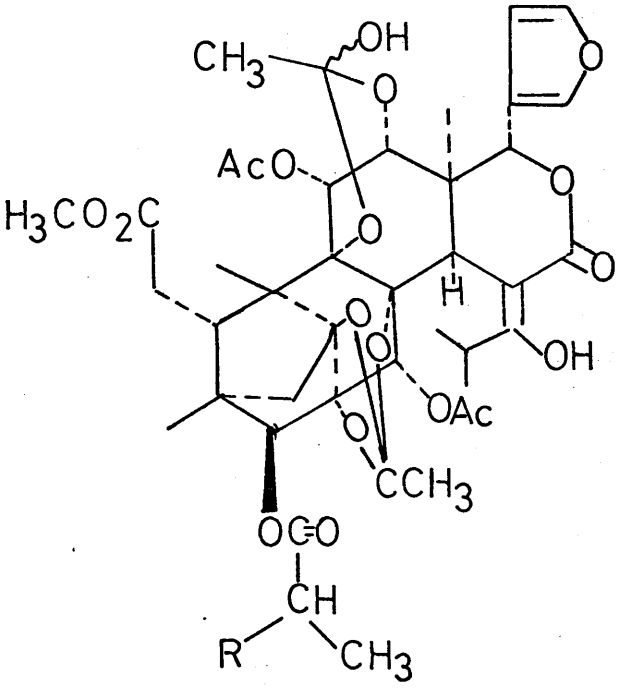
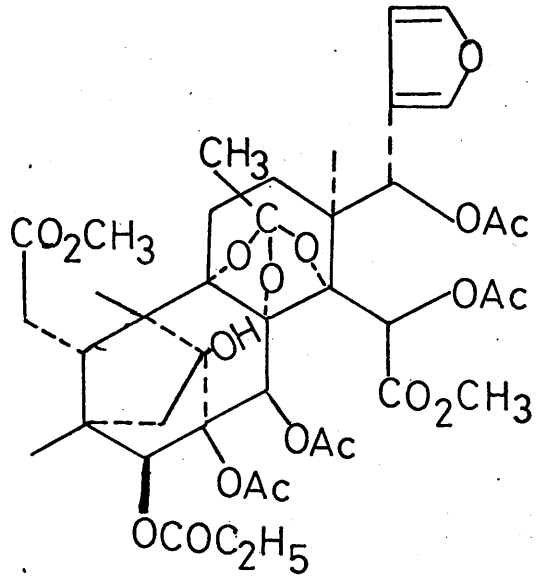
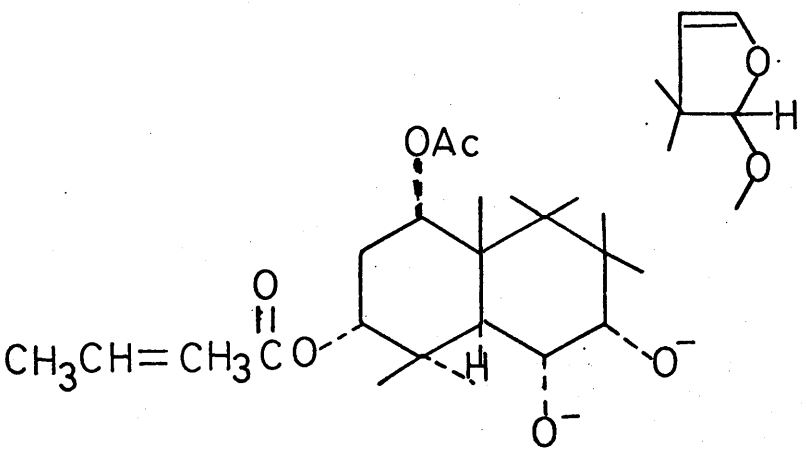


(22) R=C_2H_5

(23) R = CH_3


O
CH3
C
O
O
O
CO2CH3
OAc
OAc
OH
CO2CH3
OAc
OAc
OCOC2H5

(24)

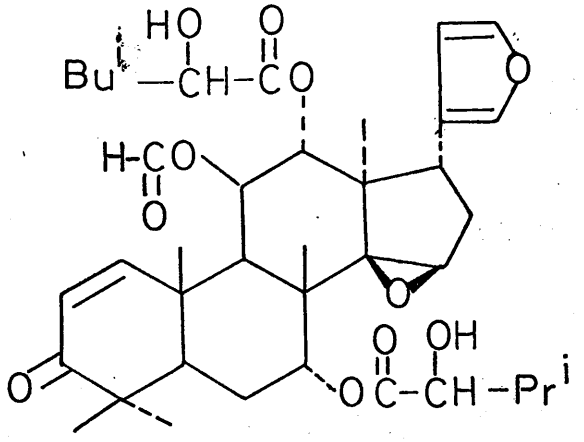

O
H
O
OAc
O
CH3CH=CH3CO
O
H
O−
O−

(25)

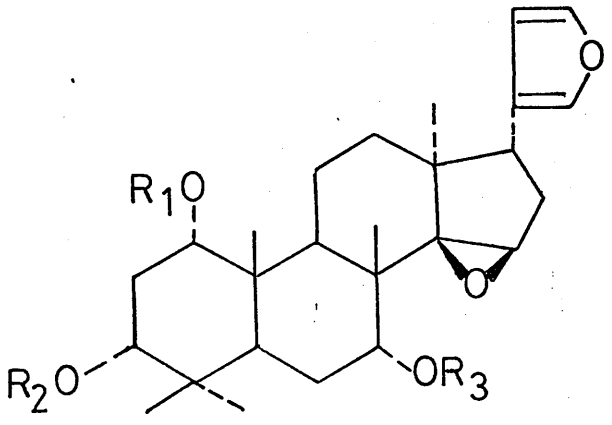
Recently some highly complex tetranortriterpenoids have been examined. Utilin, $C_{41}H_{52}O_{17}$, from the timber of Entandophragma utile, was shown [27] by X-ray crystal structure analysis to have the structure (20), with the interesting feature of bond formation between C-29 and C-1. Utilin has been interrelated [28] with entandophragmin, $C_{43}H_{50}O_{17}$. The most likely distribution of acyl groups in entandophragmin is as in utilin with an isobutyrate group replacing the acetate group at C-30.

Two basic constituents were isolated [29] from the bark of Entandophragma caudatum Sprague. Both these compounds gave, on alkaline hydrolysis, the same complex acid, $C_{28}H_{34}O_{11}$. The methyl ester of the latter was named phragmalin and on the basis of X-ray crystal structure analysis and spectroscopic evidence was assigned the structure (21). It was found [30] that bussein, a crystalline compound from the timber of Entandophragma bussei Harms. and E. caudatum Sprague [31] was a mixture of two components, i.e. bussein A (22) and bussein B (23). The C-29 methyl group forms an additional bridge from C-1 to C-4 as in utilin [27] and entandophragmin [28]. Other interesting features include the hemiorthoacetate group attached to C-9 and C-12 and the unique enolized β-keto-lactone system. The use of [13] C.m.r., together with p.m.r. allowed Taylor [32] to postulate structure (24) for procerin, a limonoid obtained from the bark of Carapa procera. Azadirachtin, $C_{35}H_{44}O_{16}$, isolated from the seeds of the Neem tree (azadirachta indica) was found [33] to be highly active in inhibiting the feeding response of the desert locust. This compound is a highly oxygenated triterpenoid with fourteen of the oxygen atoms deployed in five ester groups, three free hydroxy groups and a dihydrofuran ring. Morgan et al [33] have tentatively assigned the remaining oxygen atoms to two ether groups and have proposed part structure (25)

Prieurianin, the subject of this section was isolated from Trichilia



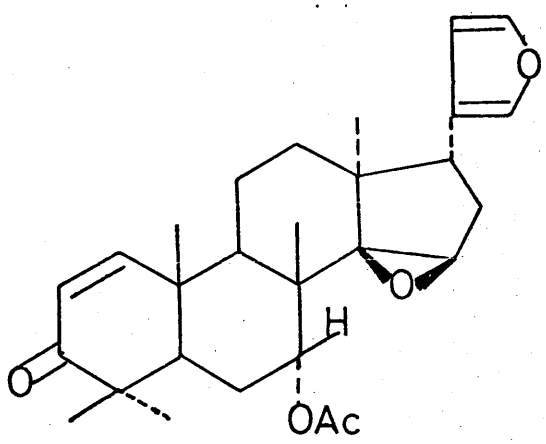
(26)



(27) $R_1 = R_2 = R_3 = Ac$

(28) $R_1 = H$; $R_2 = R_3 = Ac$

(29) $R_2 = H$; $R_1 = R_3 = Ac$



(30)

prieuriana. A number of other Trichilia species have been investigated. From the timber of Trichilia heudelotti Taylor and Okorie isolated [34] a number of related compounds exemplified by heudelottin (E) (26) [35] and dregeanin, a further member of the group of complex tetranortriterpenoids discussed above. The fruit of Trichilia havanensis Jacq. afforded [36] several compounds with the same carbon skeleton as the heudelottins. These include havanensin triacetate (27), havanensin 3, 7-diacetate (28), havanensin 1, 7-diacetate (29), and trichilenone acetate (30).

DISCUSSION

The light petroleum extract of the wood of Trichilia prieuriana (Meliaceae) yielded a crystalline substance, named prieurianin (31). This compound, m.p. 213-214°, λ max 212 n.m., has in its infra-red spectrum absorptions at 3560 (free OH), 3,370 (very intense, bonded OH), 1752, 1734 (ester carbonyls), 1712 (cyclohexanone). Prieurianin (31) was assigned the molecular formula $C_{38}H_{50}O_{16}$ by high resolution mass spectroscopy. The n.m.r. spectrum, even at 220 MH_Z is very complex, but shows a triplet at δ 0·78 (J 6·5H_Z, -C-CH_2-$\underline{CH_3}$), a secondary methyl at δ 0·87 (d, J 6·5H_Z) and one tertiary methyl as a singlet at δ 1·02. There are signals for two acetate groups at δ 2·08, 2·14 and a methyl ester, δ 3·71. The characteristic resonances of a β-substituted furan ring appear at δ 6·28, 7·22 and 7·40. The low field singlet at δ 7·81 is assigned to a formate proton while the singlets at δ 5·76 and 6·03 may be due to an exomethylene group. Other interesting aspects of the spectrum include an AB quartet at δ 2·33, 3·01 (J_{AB} 20 H_Z) which is further coupled to a triplet (J 10 H_Z) at δ 4·00. In addition there is a doublet (J 11 H_Z) at δ 4·15 and broad signals at δ 5·00 (1H, $W_{\frac{1}{2}}$ 30 H_Z), δ 5·58 (2H, $W_{\frac{1}{2}}$ 30 H_Z) and 6·18 (b.d., J 12 H_Z). The typical ring D δ lactone system is probably not present in prieurianin since there is no signal in the region δ 5-5·5 corresponding to H-17. It may be suggestive that other Trichilia species tend to favour limonoids with an intact ring D (see p.183).

The use of lanthanide shift reagent on the 100 MH_Z spectrum of prieurianin (31) together with double irradiation experiments demonstrated coupling between the doublet at δ 6·18 and the broad singlet at δ 5·58 ; the triplet at δ 4·00 and the AB quartet system. The presence of the grouping -C-CH_2-CH_3 was also established.

TABLE 1

^{13}C.m.r. Spectrum of Prieurianin

Signal p.p.m.	Multiplicity	Assignment
206.0	s	ketone
174.8	s	*COOCH$_3$
176.6	s	
170.1	s	Lactone or
169.5	s	ester carbonyl
168.3	s	groups
160.3	d	-OCOH
143.1	d	C21
140.7	d	C23
137.9	s	exomethylene C=CH$_2$
125.7	t	
122.9	s	C20
110.6	d	C22
74.7	d	C*H-O
84.6		
80.9		
74.5	s?	C*-O
73.9		
71.5	Multiplet of two superimposed signals?	
53.3	q	COOC*H$_3$
68.6	Partially resolved signal	
51.3	d	-CH<
49.7	s	C
47.0	s	C-C-C
		C
41.4	t	-CH$_2$-
38.0	t	-CH$_2$-
37.2	t	-CH$_2$-
35.2	d	-CH<
26.1	q	-CH$_3$
23.0	t	-CH$_2$-
21.1	q	-CH$_3$
20.6	q	-CH$_3$
15.2	q	-CH$_3$
12.9	q	-CH$_3$
11.5	t	-CH$_2$-

Acetylation of prieurianin at room temperature overnight with acetic anhydride and pyridine gave prieurianin acetate (32), m.p. 181-182°, $C_{40}H_{52}O_{17}$, which has, in the i.r. spectrum, absorptions at 3,370 (bonded OH), 1752, 1732 and 1712 cm⁻¹. The n.m.r. spectrum (220 MH_Z) of the acetate (32) shows the presence of three acetate methyls at δ 2·08 (3H), 2·12 (6H) and a sharp doublet at δ 4·60 (4 H_Z, C<u>H</u>OAc). Attempts to acetylate prieurianin further under a variety of conditions proved unsuccessful.

Recourse was then made to the ^{13} C.m.r. spectra of prieurianin and its acetate. The spectrum of prieurianin (31) (see table 1) shows the presence of a ketone, five singlets due to ester carbonyl carbons and one doublet which is assigned to the carbon atom of the formate carbonyl group. This requires that in addition to the groups described previously there are also two other ester groups in the molecule. In the C=C region of the spectrum, the four carbon atoms of the furan ring are easily assigned. In addition the singlet at 137·9 p.p.m. and the triplet at 125·7 p.p.m. are the carbons of the exomethylene moiety. The C-O region of the spectrum exhibits a doublet at 74·7 p.p.m. which is assigned to the carbon bearing the secondary hydroxy group. This is the hydroxy group which is acetylated on forming prieurianin acetate. The quartet at 53·3 p.p.m. is the methyl of the methyl ester group. Again there does not appear to be a signal due to C-17 which would be expected to appear as a doublet in the range 76-81 p.p.m. The high field region of the spectrum shows five quartets indicating the presence of five methyl groups. However, in this region there appear to be only two singlets, i.e. sp^3 carbon attached to four other carbons, which may be indicative of a highly cleaved ring system.

The ^{13} C.m.r. spectrum of prieurianin acetate (see table 2) again shows the presence of a ketone and a formate. The ester carbonyl carbon region shows four singlets clearly and another singlet at 170·1 p.p.m. partially

TABLE 2

^{13}C.m.r. of Prieurianin Acetate

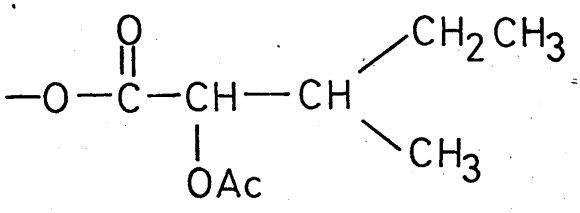
Signal p.p.m.	Multiplicity	Assignment
206.3	s	ketone
176.6	Partially resolved singlet	
170.2	s	
170.1	s	ester or lactone
169.6	s	carbonyl groups
168.9	s	including *$COOCH_3$
168.4	s	
160.8	d	-OCOH
143.1	d	C21
141.1	d	C23
137.8	s	C=CH_2
125.7	t	
122.8	s	C20
110.5	d	C22
82.4	Partially resolved singlet	
81.1	s	
78.4	s	-C-O
77.1	s	
75.9	Two superimposed triplets?	2 $-CH_2-O$
74.0	d	CH-O
72.1	d	
53.3	q	$COOC^*H_3$
68.6	Partially resolved signal	
55.8	Partially resolved signal	
51.3	d	-CH<
49.6	s	C
47.1	Partially resolved singlet	C-C-C
		C
41.5	t	$-CH_2-$
37.3	Triplet (partially resolved)	$-CH_2-$
36.0	t	$-CH_2-$
35.5	d	-CH<
32.9	t	$-CH_2-$
26.1	q	$-CH_3$
24.3	t	$-CH_2-$
21.1	q	$-CH_3$
20.6	q	$-CH_3$
15.5	q?	$-CH_3$
13.0	q	$-CH_3$
11.4	s?	-CH

obscured by a neighbouring, more intense, signal. In addition a partially resolved singlet at 176·5 p.p.m. is evident. These results suggest that there must be a lactone ring in the molecule. The four carbons of the furan ring, the triplet and singlet of the exomethylene group are also present. The signal at 75·8 p.p.m. is intense enough, even in the off-resonance decoupled spectrum to be assigned to two superimposed triplets. This indicates the presence of two primary oxygen functions. This region of the spectrum also exhibits two doublets and three sharp singlets. There is also a partially resolved signal at 68·6 p.p.m. which is also present in the spectrum of prieurianin. The methyl carbon of the methyl ester again appears as a quartet at 53·3 p.p.m. The multiplicity of some of the high field signals in the off-resonance decoupled spectrum is difficult to define. However there should be six methyl groups, excluding the methyl ester. Again only two singlets, one sharp and one partially resolved can be clearly seen along with a signal of undefined multiplicity at 11·4 p.p.m. To summarise, therefore, the functionalities which appear to be present in prieurianin include two acetates, one formate, one methyl ester, two other, as yet undefined, ester groups or lactones, one six membered ring ketone, one secondary hydroxy group, a tertiary hydroxy group, a furan ring and an exomethylene group. These groups account for the sixteen oxygen atoms present in prieurianin.

The hydrolysis of prieurianin under basic conditions proved to be an interesting reaction. When the reaction was carried out using 5% potassium hydroxide in methanol, followed by normal acetylation a crystalline compound (33) was obtained. This compound (33), m.p. 206-207°, $C_{37}H_{48}O_{14}$ has in its i.r. spectrum absorptions at 3,580 (free OH) and 1755 (very broad and intense). The n.m.r. spectrum has resonances at, δ 0·80 which is assigned to a tertiary methyl superimposed on a triplet (J 6·5 H_{zs}-C-CH_2-$C\underline{H}_3$), a doublet

at δ 0·89 (secondary methyl) and a singlet corresponding to a tertiary methyl at δ 1·07. There is also a three proton singlet at δ 1·54 which may be a vinyl methyl or an orthoacetate group. The question arises, where does the additional tertiary methyl group come from? The answer to this is possibly the key to solving the structure of prieurianin. Other features of the spectrum are two acetate methyls at δ 2·10, 2·14, a methyl ester δ 3·69, the characteristic β-substituted furan ring signals, the exomethylene proton singlets at δ 6·07 and 5·31 and the doublet at δ 4·74 (J $4H_Z$) which is also present in the spectrum of prieurianin acetate (32). Missing from the spectrum of (33) are the formate signal and the two very broad low field signals seen in prieurianin itself. The region around δ 4·0 in the spectrum of (33) is very complex and appears to consist of a broad singlet at δ 3·87 (1H, $W_{\frac{1}{2}}$ $7H_Z$), an AB quartet, δ 4·06, 4·27 (J_{AB} $12H_Z$), a triplet δ 4·02 (1H, J $4H_Z$) and a broad doublet δ 4·15 (1H, J $10H_Z$). The doublet at δ 5·84 (cf. δ 6·18 in prieurianin) now has a coupling constant of $7H_Z$. On going from prieurianin to (33) we appear to have lost the elements of formic acid, but since acetylation introduces a new secondary acetate (see above), then an acetate group must also be lost. The signal at δ 1·54 in the n.m.r. spectrum of (33) is more likely therefore to be a vinyl methyl than an orthoacetate group.

When the base hydrolysis of prieurianin was carried out using 5% potassium hydroxide in ethanol followed by acetylation, two compounds were obtained. First, the crystalline compound (34), m.p. 150-151°, $C_{38}H_{50}O_{14}$ has in the i.r. spectrum, 3628, 3580 (free OH), 3415 (very broad, bonded OH), 1785 (very broad), 1755, 1728 (shoulder)cm^{-1}. The n.m.r. spectrum (see experimental section) shows the presence of an ethyl ester instead of a methyl ester. This compound also has an ethyl group, a secondary methyl, two tertiary methyls and what appears to be a vinyl methyl. In addition



PART STRUCTURE A

TABLE 3

Double Irradiation Experiments on (35)

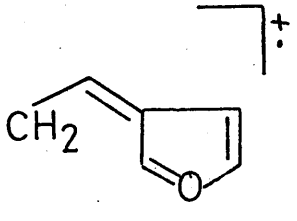
Signal	Assignment	Couplings
0.79 (t, J 6·5H_Z)	ethyl CH_3	G
0.80 (s.)	tertiary CH_3	
0.87 (d. J 6·5H_Z)	secondary CH_3	
1.02 (s)	tertiary CH_3	
1.22 (t, J 6·5H_Z)	$-O-CH_2\underline{CH_3}$	D
1.51 (s)	vinyl methyl?	
1.8 multiplet	methine or methylene	B, G
2.06, 2.13	acetate $C\underline{H_3}$	
2.33 (J_{AB} 20H_Z)	one part coupled AB system	E
2.8	various multiplets	C
3.25 (d, J 10H_Z)		F
3.56 (s, disappears on D_2O)	$O\underline{H}$	
3.79 (t, J 10H_Z)		E
4.05, 4.10 (q, J 6·5H_Z)	$-O-CH_2CH_3$	A, D, F
4.15	obscured multiplet	
4.13, 4.31 (ABq, J_{AB} 12H_Z)		
4.47 (t, J 6H_Z)		C
4.64 (d, J 4H_Z)	$CH-C\underline{H}-OAc$	B
6.21, 5.19	exomethylenes	
5.79 (d, J 7H_Z)		A
6.21, 7.33, 7.34	furan protons	

signals due to two acetate groups, the β-substituted furan ring and the exomethylene group are also present. This compound appears to be similar to (33) except that it has an ethyl ester instead of the methyl ester present in (33). Double irradiation experiments with (34) established the presence of the group shown in part structure A. The secondary acetate group in this ester side chain originates from the secondary hydroxy group in prieurianin itself.

Second, the non-crystalline compound (35) was also isolated. This compound has similar i.r. and mass spectra to those of (34) and an even more complex n.m.r. spectrum at 220 MH_Z (see experimental section). Double irradiation experiments established the presence of a number of mutually coupled signals. These results are summarised in table 3.

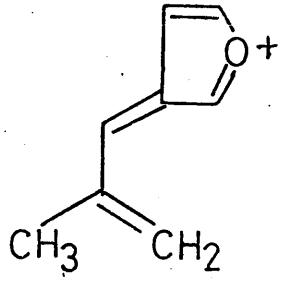
Hydrogenation of prieurianin gave the tetrahydro product (36) in which only the furan ring had been hydrogenated. Jones' oxidation of prieurianin at ice temperature gave the unstable ketone (37) which has in the i.r. spectrum absorptions at 3460 (broad, bonded OH), 1745, 1730, 1708. The n.m.r. spectrum of the ketone is very similar to that of prieurianin (see experimental section). This is further evidence for the presence of a tertiary hydroxy group in prieurianin.

The mass spectra of prieurianin (31) and its acetate (32) are shown in figures (1) and (2) respectively. The major peaks in the spectrum of prieurianin are $^{m}/e$ 94 and $^{m}/e$ 121, and can be assigned structures (38) and (39).



(38)

m/e 94



(39)

m/e 121

FIGURE 1

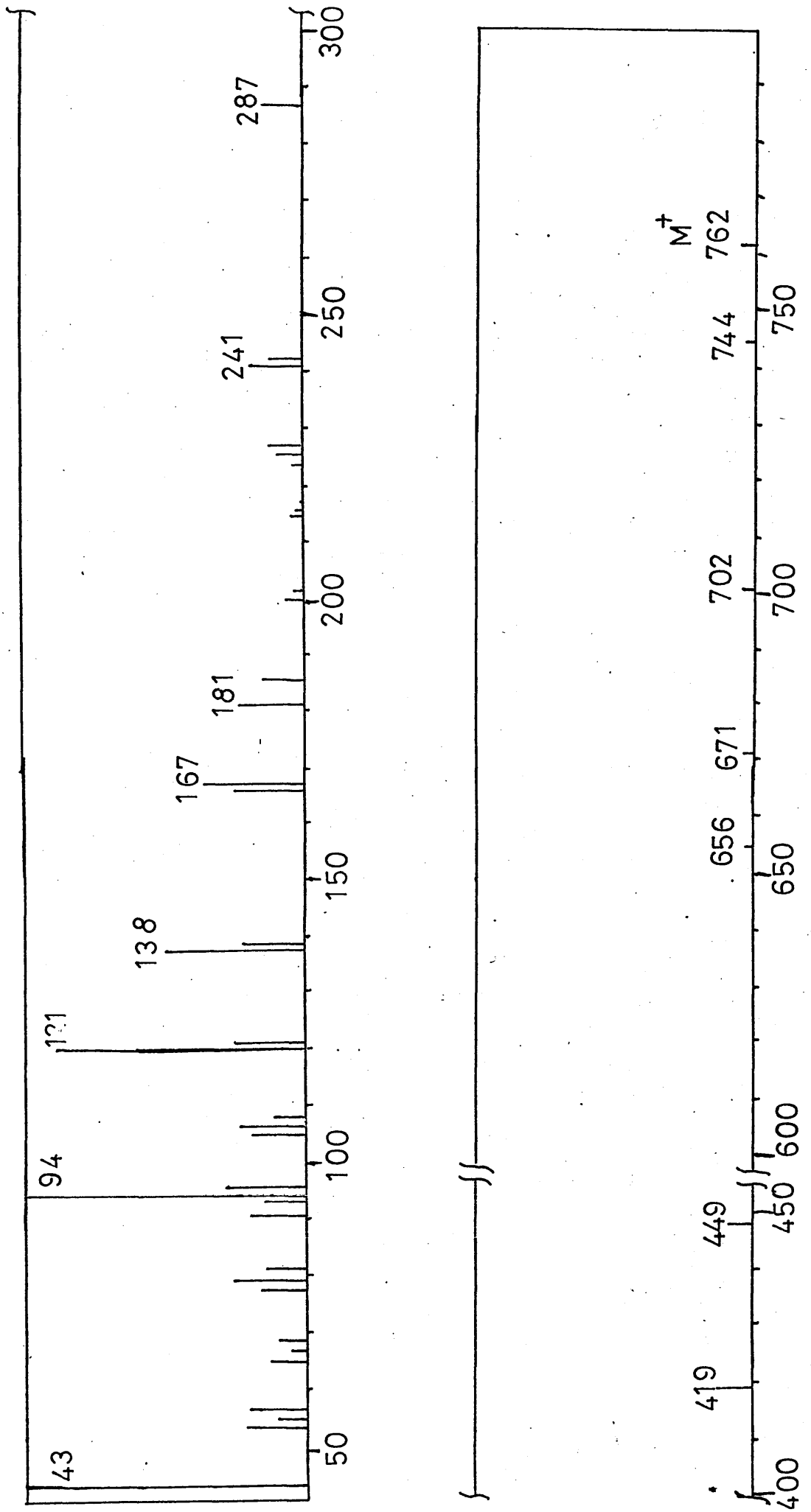
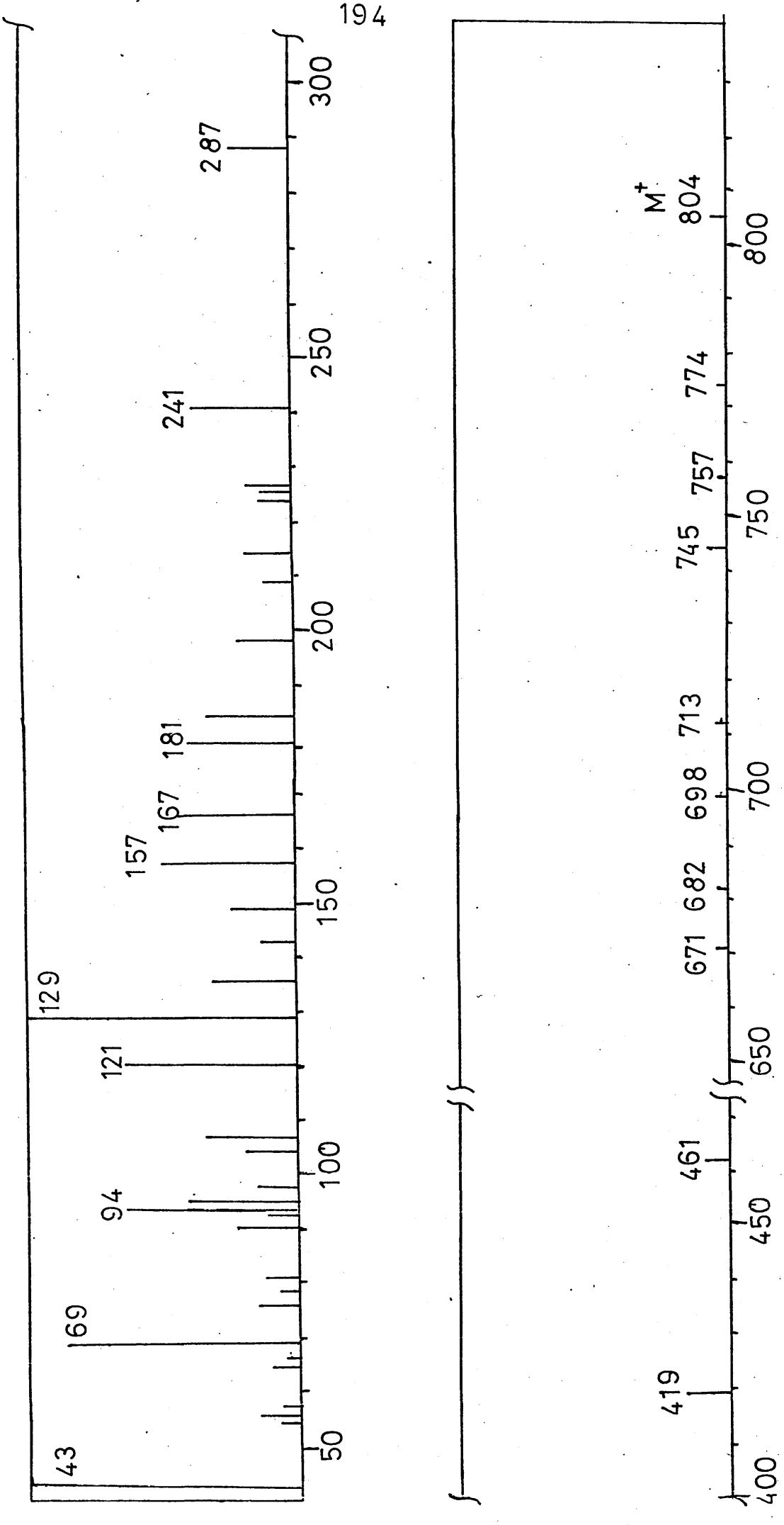
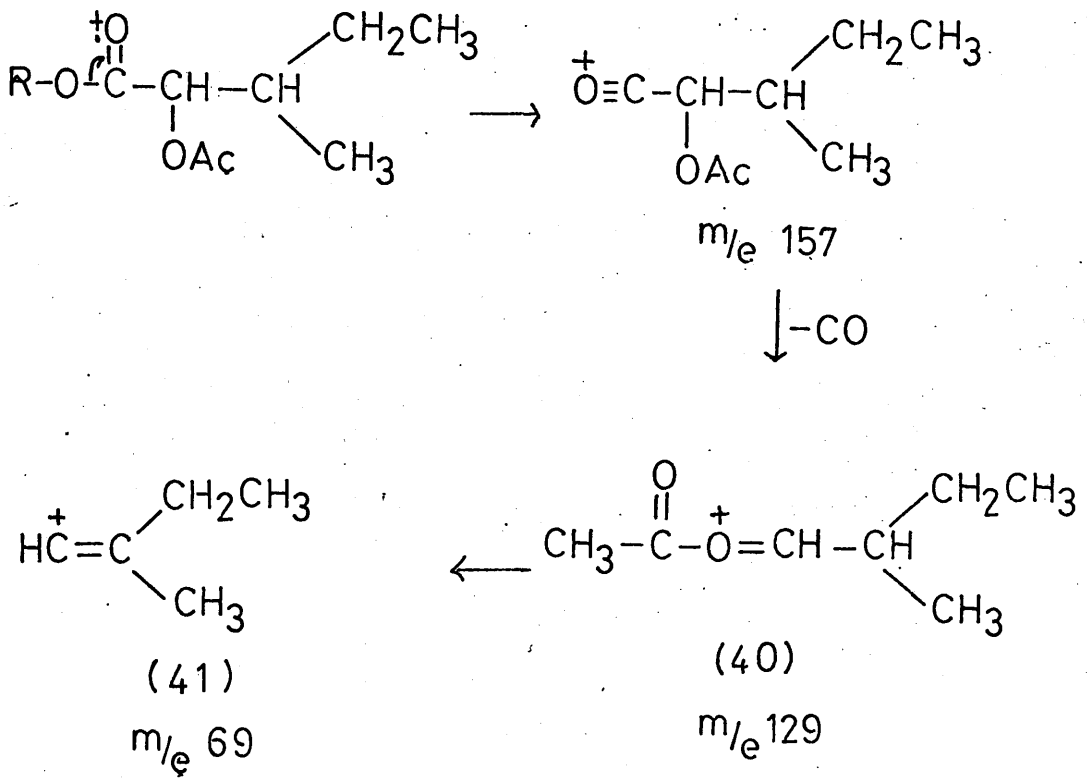

43
94
121
138
167
181
241
287
300
250
200
150
100
50
M+
762
744
750
702
700
671
656
650
600
449
450
419
400

FIGURE 2


43
69
94
121
129
157
167
181
241
287
50
100
150
200
250
300
400
419
450
461
650
671
682
698
700
713
745
750
757
774
800
804
M+

Both these peaks are very small in the mass spectrum of tetrahydroprieurianin (36). Limonoids with a δ-lactone ring D, e.g. phragmalin [29] give $^{m}/e$ 121 and $^{m}/e$ 95. This suggests that prieurianin does not contain a δ-lactone ring D. The peaks at $^{m}/e$ 129 and $^{m}/e$ 69 in the mass spectrum of prieurianin acetate (32) (figure 2) may derive from the acetoxy ester group to give fragments (40) and (41) (see scheme 1).

SCHEME 1



The mass spectra of the hydrolysis products (33) and (34) are shown in figures (3) and (4). Again the large peaks at $^{m}/e$ 129 and $^{m}/e$ 69 indicate the presence of the acetoxy ester group described above. It is noteworthy that $^{m}/e$ 181 and $^{m}/e$ 248 in the spectrum of (33) move to $^{m}/e$ 195 and $^{m}/e$ 262 respectively in the spectrum of (34). These fragments must

FIGURE 3

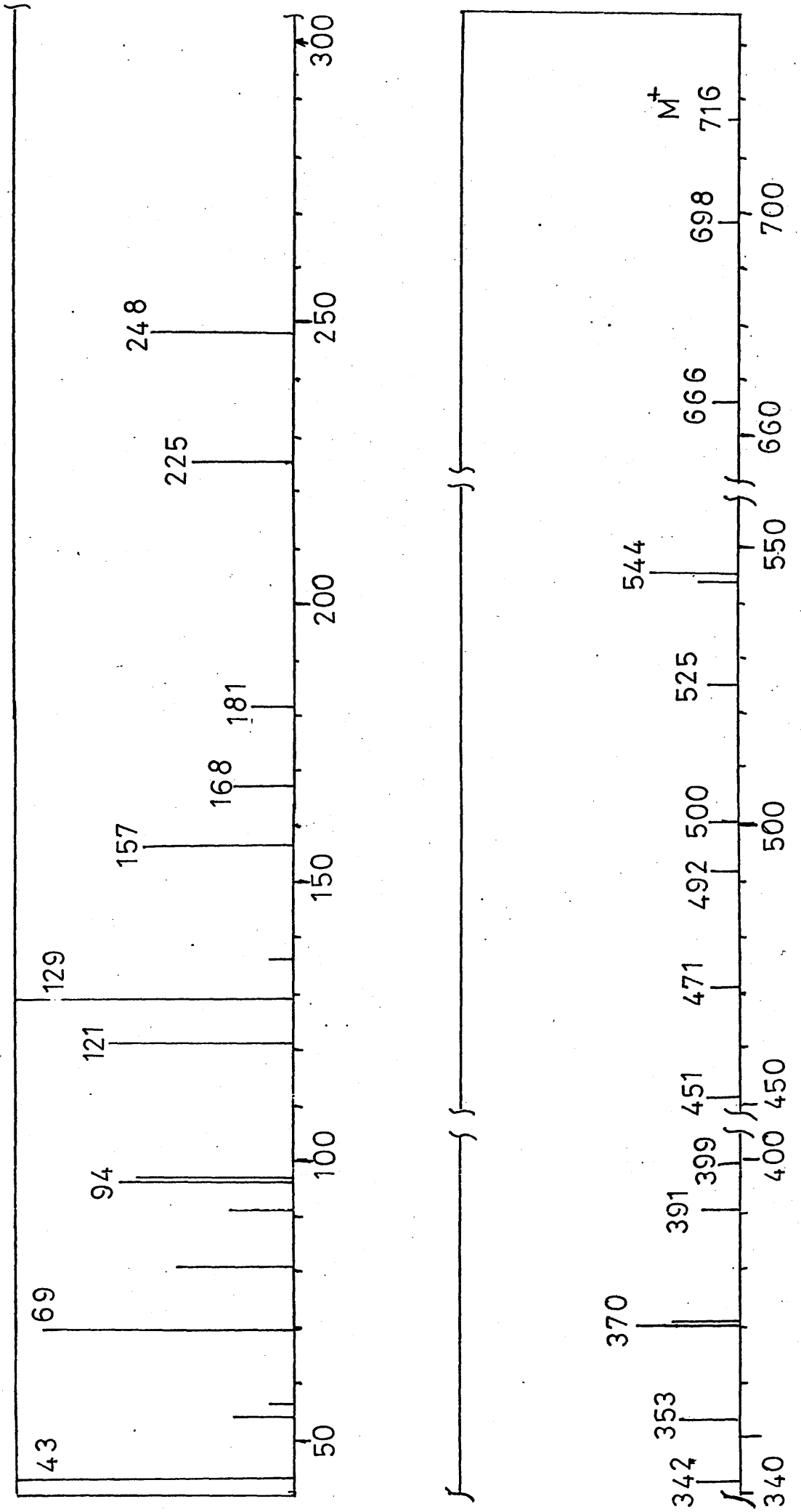
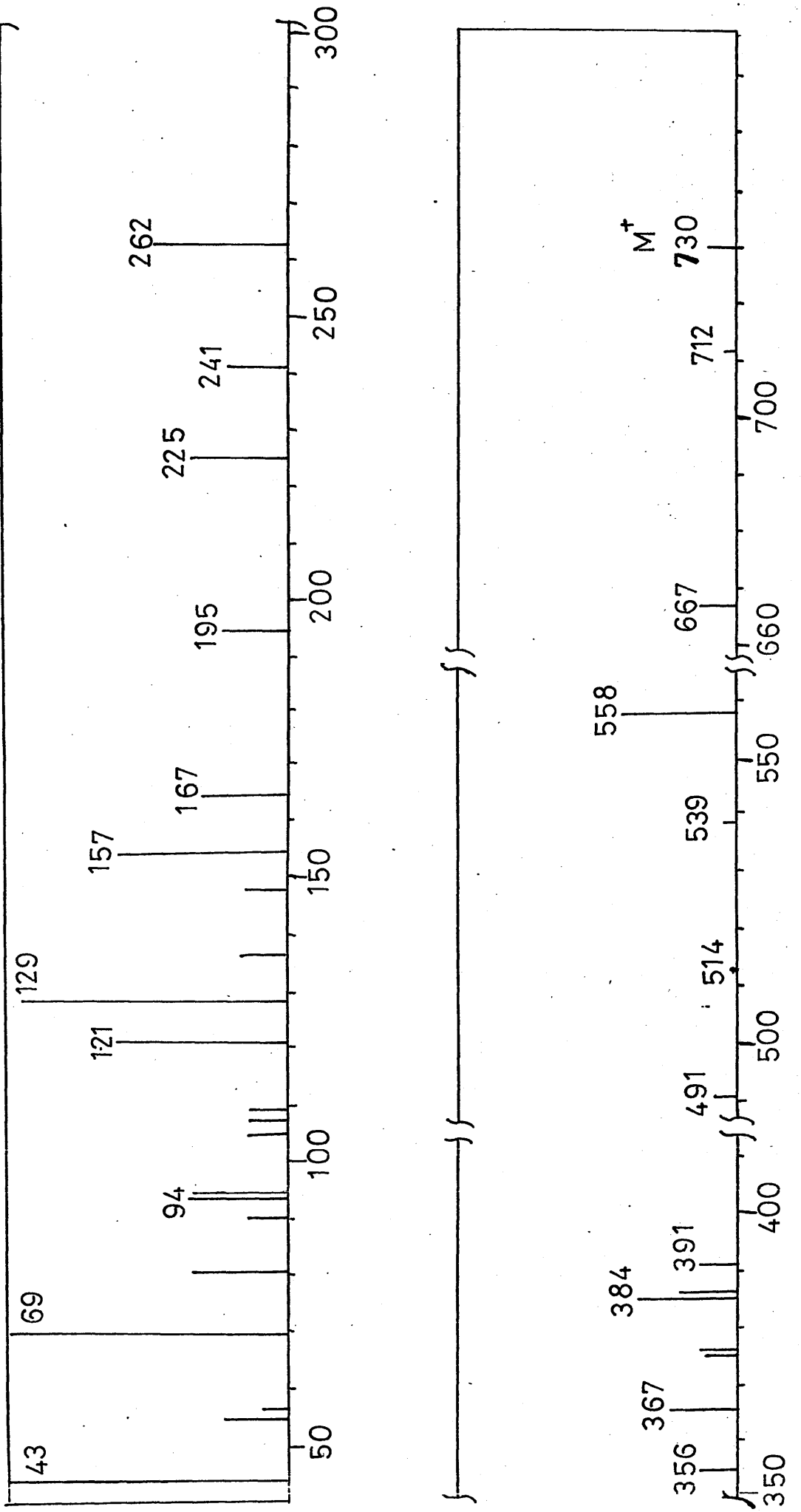

43
69
94
121
129
157
168
181
225
248
50
100
150
200
250
300
340
342
353
370
391
399
400
451
450
471
492
500
500
525
544
550
660
666
698
700
716
M+

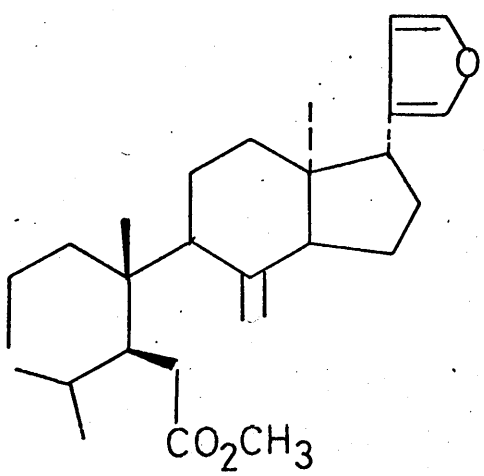
FIGURE 4


43
69
94
121
129
157
167
195
225
241
262
50
100
150
200
250
300
350
356
367
384
391
400
491
500
514
539
550
558
660
667
700
712
730
M+

therefore contain the methyl ester or ethyl ester group.

In view of the obvious complexity of the structure of prieurianin it was decided to prepare a suitable derivative for X-ray crystal structure analysis. The crystals of the p-bromobenzoate of prieurianin were of a reasonable size but lost solvent rapidly and became opaque. Prieurianin brosylate (42) however gave suitable crystals from methylene-chloride-light petroleum, m.p. 153-154°, $C_{44}H_{53}O_{18}$ S Br. The n.m.r. spectrum of this compound (see experimental section) is in agreement with the brosylate group being attached to the secondary hydroxy group of the side chain in prieurianin. The X-ray structure determination is at present in progress.

Despite the mass of available factual information about prieurianin we have been unable to draw a reasonable structure. It is possible that the carbon skeleton may be of the type (43). However the location of the functional groups and indeed the true nature of the carbon skeleton must at present remain a mystery.



(43)

EXPERIMENTAL

Powdered wood (34·5 kg) of Trichilia prieuriana was extracted with light petroleum. The crude extract (3 kg) precipitated on standing in petrol solution. The precipitate (8 g) was purified by preparative t.l.c. to give prieurianin (532 mg) which was recrystallised from methanol as prisms, m.p. 213-214°, λ max 212 n.m., ν max (CCl_4) 3560 (free OH), 3370 (bonded OH), 1752, 1734 (ester carbonyls) 1712 (cyclohexanone), δ 0·78 (t., J $6·5H_Z$, $-CH_2-CH_3$), 0·87 (d, J $6·5H_Z$, $>CHCH_3$), 1·02 (s, tertiary methyl), 2·08, 2·14 ($-OCOCH_3$), 2·33, 3·01 (ABq, J_{AB} $20H_Z$) further coupled (J $10H_Z$), 3·20 (b.d., J $12H_Z$), 3·71 (s, $-CO_2CH_3$), 4·00 (t, 2H, J $10H_Z$), 4·15 (d., 1H, J $11H_Z$), 5·00 (b.s., 1H, $W_{\frac{1}{2}}$ $30H_Z$), 5·58 (b.s., 2H, $W_{\frac{1}{2}}$ $30H_Z$), 5·76, 6·03 (s, $C=CH_2$), 6·18 (b.d., J $12H_Z$), 6·28 (β-furan proton), 7·22, 7·40 (α-furan protons), 7·81 (s, -OCOH), [Found : M^+ (mass spectroscopy), 762 · 31316. $C_{38}H_{50}O_{16}$ requires M, 762 · 30986].

Additional samples of prieurianinwere kindly gifted by Professor D.A.H. Taylor.

ACETYLATION OF PRIEURIANIN

Prieurianin (100 mg) in acetic anhydride (3 ml) and dry pyridine (2 ml) were set aside at room temperature overnight. Normal work-up procedure gave prieurianin acetate (96 mg) which was recrystallised from methanol as prisms, m.p. 181-182°, ν max (CCl_4) 3370 (bonded OH), 1752, 1732, 1712, δ 0·79 (t, J $6·5H_Z$, $-CH_2CH_3$), 0·84 (d, J $6·5H_Z$, $>CHCH_3$), 1·01 (s, tertiary methyl), 2·08, 2·12 ($-OCOCH_3$), 2·33, 3·01 (ABq, J_{AB} $20H_Z$) further coupled (J $10H_Z$), 3·20 (b.s., 1H), 3·71 ($-CO_2CH_3$), 3·98 (t, 2H, J $10H_Z$), 4·09 (d, 1H, J $11H_Z$), 4·60 (d, J $4H_Z$, $>CH-CHOAc$), 4·90 (v.b., 1H), 5·45 (b.s., 2H), 5·73, 6·01

(s, C=C$\underline{H}_2$), 6·14 (b.d., J 12H_Z), 6·30 (β furan proton), 7·38 (2H, α furan protons), 7·82 (s, -OCO$\underline{H}$), [Found : M^+ (mass spectroscopy), 804. $C_{40}H_{52}O_{17}$ requires M, 804].

OXIDATION OF PRIEURIANIN

Prieurianin (50 mg) was treated with a slight excess of Jones' reagent at ice temperature for five minutes. Addition of water and extraction into chloroform gave prieurianin ketone (37) (40 mg) as an unstable gum, νmax (CCl_4) 3460 (broad, bonded OH), 1745, 1730, 1708, δ 0·79 (t, J 6·5H_Z, -$CH_2C\underline{H}_3$), 0·93 (d, J 6·5H_Z, > CHC$\underline{H}_3$), 1·04 (tertiary methyl), 2·05, 2·12 (-OCOC$\underline{H}_3$), 3·72 (-$CO_2C\underline{H}_3$), 5·80, 6·27 (s, C=C$\underline{H}_2$), 6·18 (d, J 12H_Z), 6·27 (β furan proton), 7·24, 7·35 (α-furan protons), 7·91 (s, -OCO$\underline{H}$), [Found : M^+ (mass spectroscopy) 760, $C_{38}H_{48}O_{16}$ requires M, 760].

TREATMENT OF PRIEURIANIN WITH METHANOLIC KOH

Prieurianin (250 mg) in 5% methanolic potassium hydroxide (5 ml) were left at room temperature for one hour. Acidification, extraction and preparative t.l.c. afforded the major product which was immediately acetylated under conditions described previously. The product (33) (105 mg) was crystallised from methanol as prisms, m.p. 206-207°, νmax ($CHCl_3$), 3,580 (free OH), 1755 (very broad), δ 0·80 (tertiary methyl), 0·80 (t, J 6·5H_Z, -$CH_2C\underline{H}_3$), 0·89 (d., J 6·5H_Z, > CH-C$\underline{H}_3$), 1·07 (s, tertiary methyl), 1·54 (s, vinyl methyl?), 2·10, 2·14 (-OCOC$\underline{H}_3$), 2·89 one part of ABq (J_{AB} 20H_Z) further coupled (J 10H_Z), 3·45 (b.d., J 10H_Z superimposed on 1H singlet), 3·69 (-$CO_2C\underline{H}_3$) 3·87 (b.s., 1H, $W_{\frac{1}{2}}$ 7H_Z), 4·06, 4·27 (ABq, J_{AB} 12H_Z), 4·02 (t, 1H, J 4H_Z), 4·15 (b.d., 1H, J 10H_Z), 4·74 (d., J 4H_Z), 6·07, 5·31

(s, C=C$\underline{H}_2$), 5·84 (d., J 7H_Z), 6·22 (β furan proton), 7·28, 7·35 (α furan proton), [Found : M^+ (mass spectroscopy), 716 · 30490. $C_{37}H_{48}O_{14}$ requires M, 716 · 30438].

TREATMENT OF PRIEURIANIN WITH ETHANOLIC KOH

Prieurianin (250 mg) in 5% ethanolic potassium hydroxide (5 ml) was set aside at room temperature for 1 hour. The extraction and acetylation procedure described above yielded compound (34) (60 mg) which crystallised from methanol as prisms, m.p. 150-151°, νmax (CCl_4) 3628, 3580 (free OH), 3415 (very broad, bonded OH), 1785 (very broad), 1755, 1728 (shoulder), δ 0·85 (t, J 6·5H_Z -$CH_2C\underline{H}_3$) 0·85 (s, tertiary methyl), 0·93 (d, J 6·5 H_Z, >$CHC\underline{H}_3$), 1·11 (s, tertiary methyl), 1·34 (t, J 6·5H_Z -$CO_2CH_2C\underline{H}_3$), 1·60 (s, vinyl methyl?) 2·18, 2·19 (-OCOC$\underline{H}_3$), 3·02 one part of ABq (J_{AB} 20H_Z) further coupled (J 10H_Z), 3·58 (b.d., 1H, J 10H_Z), 3·58 (s, disappears on addition of D_2O, O$\underline{H}$), 3·99 (t, 1H, J 10H_Z), 4·18, 4·42 (ABq, J_{AB} 12H_Z), 4·27, 4·31 (q, J 6·5H_Z, -$CO_2C\underline{H}_2CH_3$), 4·89 (d., J 4H_Z, >CH-C$\underline{H}$OAc), 5·48, 6·22 (s, C=C$\underline{H}_2$), 6·01 (d, J 7H_Z), 6·39 (β furan proton), 7·45, 7·55 (α furan protons), [Found : M^+ (mass spectroscopy), 730. $C_{38}H_{50}O_{14}$ requires M, 730].

Preparative t.l.c. of the mother liquors from this crystallisation gave the non-crystalline compound (35) (32 mg), νmax (CCl_4) 3630, 3580 (bonded OH), 3410 (very broad bonded OH), 1785 (very broad), 1755, δ 0·79 (t, J 6·5H_Z, -$CH_2C\underline{H}_3$), 0·80 (s, tertiary methyl), 0·87 (d, J 6·5H_Z, $CHC\underline{H}_3$), 1·02 (s, tertiary methyl), 1·22 (t, J 6·5H_Z, -$CO_2CH_2C\underline{H}_3$), 1·51 (s, vinyl methyl), 1·8 (multiplet), 2·06, 2·13 (-OCOC$\underline{H}_3$), 2·33 one part of ABq (J_{AB} 20H_Z) further coupled (J 10H_Z), 2·8 (various multiplets), 3·25 (d, 1H, J 10H_Z), 3·56 (s, 1H, disappears on addition of D_2O, OH), 3·79 (t, 1H, J 10H_Z), 4·05, 4·10 (q, J 6·5H_Z, -$CO_2C\underline{H}_2CH_3$), 4·13, 4·31 (ABq, J_{AB} 12H_Z), 4·15 (multiplet

superimposed on previous signals), 4·47 (t, J $6H_Z$), 4·64 (d, J $4H_Z$ >CH-C<u>H</u>-OAc), 6·21, 5·19 (s, C=C<u>H</u>$_2$), 5·79 (d., J $7H_Z$), 6·21 (β furan proton), 7·33, 7·34 (α furan protons), [Found : M^+ (mass spectroscopy), 730. $C_{38}H_{50}O_{14}$ requires M, 730].

PRIEURIANIN BROSYLATE

Prieurianin (50 mg) in dry pyridine (2 ml) and a small excess of p-bromobenzene sulphonyl chloride were stirred at room temperature overnight. Addition of water and normal extraction procedures gave prieurianin brosylate (42) (45 mg) which was recrystallised from methylene chloride - light petroleum as prisms, m.p. 153-154°, νmax (K.Br.) 3420 (broad, bonded OH), 1748, 1730, 1578 cm^{-1}, δ 0·70 (t, J $6·5H_Z$, $-CH_2C\underline{H}_3$), 0·73 (d, J $6·5H_Z$, $CHC\underline{H}_3$) 0·98 (s, tertiary methyl), 2·04, 2·09 ($-OCOC\underline{H}_3$), 3·69 ($-CO_2C\underline{H}_3$), 4·36 (d, J $4H_Z$, >CH-C<u>H</u>-O-Brosylate), 5·72, 6·02 (s, C=C<u>H</u>$_2$), 6·15 (d, J $12H_Z$), 6·31 (β furan proton), 7·30, 7·47 (α furan protons), 7·80 (s, -OCO<u>H</u>), 7·73, 7·94 (ABq, J_{AB} $10H_Z$, Brosylate protons), [Found : C, 53·90 ; H, 5·30. $C_{44}H_{53}O_{18}SBr$ requires C, 53·82 ; H, 5·44%].

REFERENCES

1. D. Arigoni, D.H.R. Barton, E.J. Corey, O. Jeger, L. Caglioti, Sukh Dev, P.G. Ferrini, E.R. Glazier; A. Melera, S.K. Bradham, K. Schaffner, S. Sternhell, J.F. Templeton and S. Tobinga, Experientia, 1960, 16, 41.
2. a) Z. Valenta, S. Papadopoulos and C. Podesva, Tetrahedron, 1961, 15, 100 ; b) Z. Valenta, A.H. Gray, D.E. Orr, S. Papadopoulos and C. Podesva, Tetrahedron, 1962, 18, 1433.
3. J. Polonsky, C. Fouquey and A. Gaudermer, Bull. Soc. Chim. Fr., 1964, 1818 and 1827.
4. T.G. Halsall and R.T. Aplin, in Progress in the Chemistry of Organic Natural Products (ed. L. Zechmeister), Springer-Verlag, New York, 1964, 167.
5. J. B.' son Bredenberg, Chem. and Ind., 1964, 73.
6. D.L. Dreyer, Experientia, 1964, 20, 297.
7. R.M. Gascoigne and J.J.H. Simes, Quart. Rev., 1955, 9, 328.
8. C.W.L. Bevan, D.E.V. Ekong, T.G. Halsall and P. Toft, J. Chem. Soc. (C), 1967, 820.
9. J.G. St. C. Buchanan and T.G. Halsall, J. Chem. Soc. (C), 1970, 2280.
10. G.P. Moss, Planta Med., Suppl., 1966, 86.
11. G.P. Cotterrell, T.G. Halsall and M.J. Wriglesworth, Chem. Comm., 1967, 1121.
12. A.J. Birch, D.J. Collins, Sultan Muhammed and J.P. Turnbull, J. Chem. Soc., 1963, 2762.
13. D. Lavie, Mahendra K. Jain and I. Kirson, J. Chem. Soc. (C), 1967, 1347.
14. J.D. Connolly and R. McCrindle, J. Chem. Soc. (C), 1971, 1715.
15. W.R. Chan, D.R. Taylor and R.T. Aplin, Tetrahedron, 1972, 28, 431.
16. J.M. Cassady and Chiung-Sheue Lui, J.C.S. Chem. Comm., 1972, 86.

17. M. Pailer, G. Schaden, G. Spiteller and W. Fenzyl, Monatsh., 1965, 96, 1324.

18. Y. Fukuyama, T. Tokoroyama and T. Kubota, Tetrahedron Letters, 1973, 3401.

19. J.D. Connolly, K.H. Overton and J. Polonsky, Progress in Phytochemistry, Interscience, 1970, 2, 385.

20. D.L. Dreyer, Progress in the Chemistry of Organic Natural Products (Ed. L. Zechmeister), Springer-Verlag, New York, 1968, 190.

21. J. Polonsky, Planta Med., Suppl., 1966, 107.

22. G. Ourisson, P. Crabbe and O. Rodig, Tetracyclic Triterpenes, Holden-Day, San Francisco, 1964.

23. J.D. Connolly, I.M.S. Thornton and D.A.H. Taylor, Chem. Comm., 1971, 17; J.C.S. Perkin I, in press.

24. M.E. Obasi, J.I. Okogun and D.E.V. Ekong, J.C.S. Perkin I, 1972, 1943.

25. J.D. Connolly, K.L. Handa, R. McCrindle and K.H. Overton, J. Chem. Soc. (C), 1968, 2227.

26. J.D. Connolly, R. Henderson, R. McCrindle, K.H. Overton and N.S. Bhacca, J.C.S. Perkin I, 1973, 865.

27. H.R. Harrison, O.J.R. Hodder, C.W.L. Bevan, D.A.H. Taylor and T.G. Halsall, Chem. Comm., 1970, 1388.

28. E.O. Arene, C.W.L. Bevan, J.W. Powell, D.A.H. Taylor and K. Wragg, Chem. Comm., 1966, 627.

29. R.R. Arndt and W.H. Baarschers, Tetrahedron, 1972, 28, 2333.

30. R. Hanni and Ch. Tamm, Chem. Comm., 1972, 1253.

31. a) D.A.H. Taylor, J. Chem. Soc. (C), 1965, 3495 ; b) G.A. Adesida and D.A.H. Taylor, Phytochemistry, 1967, 6, 1429.

32. D.A.H. Taylor, unpublished work.

33. J.H. Butterworth, E.D. Morgan and G.R. Percy, J.C.S. Perkin I, 1972, 2445.

34. D.A. Okorie and D.A.H. Taylor, J. Chem. Soc. (C), 1968, 1828.

35. D.A. Okorie and D.A.H. Taylor, J.C.S. Perkin I, 1972, 1488.

36. W.R. Chan, J.A. Gibbs and D.R. Taylor, J.C.S. Perkin I, 1973, 1047.