A thesis entitled

"BIOGENETIC-TYPE SYNTHESES IN THE ROSANE SERIES"

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SUMMARY

The diterpenoid mould metabolites, rosenonolactone (29) and deoxyrosenonolactone (46), have been synthesised from isocupressic acid (128) by a route patterned on their known biogenetic pathway. The key step in the proposed synthetic sequence, conversion of a bicyclic labdane to a tricyclic rosane, was first perfected on model labdadienols which lack the C-19 carboxyl group necessary for subsequent lactone formation. On brief acid treatment the labdadienols (70), (91), (92) and (93) were cyclised to pimaradienes (87) and (88). The mechanistic and stereochemical details of this cyclisation were investigated. Upon prolonged acid treatment the initially formed pimaradienes were converted to the desired rosadienes (111) and (112).

Using the experience gained in the model series, isocupressic acid was similarly transformed into the acid (134) which had the correct C-4, C-8, C-9 and C-13 configurations required for a synthesis of the metabolites. The remaining problems of lactonisation, and, in the case of rosenonolactone, introduction of a C-7 carbonyl group, were jointly overcome by a method which employed the epoxide (149) as a key intermediate.

Information on the hydrocarbon products resulting from acid rearrangement of the pimaradienes (87) and (88) was obtained from a combined gas chromatographic-mass spectral analysis using deuterated derivatives. No tetracyclic structures were detected and the principal product was shown to have the 'mixed' rosa-abietadiene skeleton (217).

CONTENTS

I INTRODUCTION

1 Biogenesis and Structural Relationships of the Diterpenoids

The terpenoids have long commanded the attention of chemists and, for over a century now, have been the subject of a prodigious amount of unabating research. These investigations have not been without reward, for the wealth of resultant information has benefited all areas of organic chemistry, from biochemical to physical, synthetic to analytical. In retrospect, the study of terpenoids and steroids, perhaps more so than that of any other natural products, has helped establish fundamental concepts of conformation and reactivity, and has drawn together apparently diverse topics such as carbonium ions and stereospecificity in enzymatic reactions.

With the gradual accumulation of known terpenoid structures, it became clear to the early chemists that the basic skeletons were constructed according to a definite plan. This tenet, that momoterpenoid structures could be dissected into two 'isoprene' units, was extended by Ruzicka in 1921 who pointed out that the carbon skeletons not only of the monoterpenoids but also of the higher terpenoids could be envisaged as composed of isoprene units, joined in a regular

'head to tail' manner or an irregular 'tail to tail' order. The original Isoprene Rule, purely empirical in character, was refined by Ruzicka in 1953 to become his Biogenetic Isoprene Rule. The essentials of the rule are that the isoprene units are derived from isopentenyl pyrophosphate (1) and the isomeric dimethylallyl pyrophosphate (2). Sequential condensation of dimethylallyl pyrophosphate with isopentenyl pyrophosphate units then gives the pyrophosphates of geraniol (3), farnesol (4), geranylgeraniol (5), geranylfarnesol (6) and, by coupling of two farnesol units, squalene (7). These acyclic units [or the corresponding tertiary alcohols, for example geranyllinalool (8)] are the precursors of the mono-, sesqui-, di-, sester- and tri- terpenoids. These are formed by cationic, olefin cyclisations, often followed by secondary rearrangements which are governed by acceptable reaction mechanisms.

Important extensions to the above rule by Eschenmoser[2] and Stork[3] made it possible to interpret the stereochemistry of the triterpenoids and, indeed, to predict it, according to the following postulates:-

(1) The acyclic precursor (squalene) is in the all-trans configuration and is prefolded into a well-defined succession of potential chair or boat cyclohexane rings.

(2) Cyclisation then occurs by trans- antiparallel 1,2-additions to the olefinic bonds.

(3) If subsequent rearrangements occur, such as 1,2-hydride or methyl shifts, ring contractions or ring enlargements, the groups affected will be trans and coplanar. Furthermore, the observed stereospecificity of cyclisation and secondary rearrangement would agree with a non-stop process, no intermediate being formed by formal neutralisation of the cationic charge.

Originally applied to the triterpenoids, the biogenetic theories have been extended, with remarkable success, to cover the other classes of terpenoids. Several excellent reviews exist.[4]

Ruzicka[1] in 1953 had observed that many diterpenoids were based on the labdane skeleton (9), which could be derived from a geranylgeraniol precursor (5). Thus, proton induced cyclisation of geranylgeraniol pyrophosphate (5) or geranyllinalool pyrophospate (8) in conformation (11) should, according to the principle of the Biogenetic Isoprene Rule,

lead to a labdadienol of the correct configuration (10). This corresponds to the A/B ring fusion of the triterpenoids. Only the 'normal' 10β methyl stereochemistry has been depicted for (10) but many diterpenoids exist with the antipodal (10α methyl) stereochemistry, for example polyalthic acid (12).[5]

The pimarane skeleton (13) can then be considered as arising from cyclisation of a labdane precursor such as (9) by ionization of the pyrophosphate and participation of the 8,17 double bond to form ring C. Simple 1,2 methyl and hydride shifts, of the type already discussed, can then be invoked to derive other structural types such as rosane. Wenkert,[6] in 1955, extended the biogenetic schemes for the diterpenoids even further to include the tetra- and penta-cyclic classes. These could be derived from a pimarene carbonium ion precursor such as (13) by interaction of the 15,16 double bond with the cationic C-8 centre. The tetra- and penta- cyclic structures could then formally be derived from the non-classical homonortricyclonium ion (14) (Fig. 2).

It should be mentioned that this representation is merely a convenient way, using the analogy of the norbornyl series, to summarise the skeletal rearrangements involved. Indeed it has been observed[7] that, since the evidence is now

building up against face-protonated nortricyclene intermediates in norbornyl rearrangements, the face-protonated trachylobane type intermediate (14) might better be represented by two bridged, hydrogen-shift isomers (15) and (16).

Furthermore, the pimarene cation (13) can exist as two C-13 epimers, so each tetra- and penta cyclic structure should in principle have a (C-8, C-13) epimer. Only the (+) kaurene - (+) phyllocladene isomeric pair are known at present, but others have been synthesised in anticipation of their eventual discovery in nature.

A general scheme, therefore, can be set out in Figure 2, to show how most of the structural types of diterpenoids can be related back to a common 'trans-anti' labdane precursor which cyclises to a pimarane type. The further diversification in structure is due to 'trivial' transformations, such as oxygenations, reductions and ring cleavages, which do not contravene the basic biogenetic postulates.

Nevertheless, a small number of diterpenoids were found which appeared to be constructed from a labdane precursor with a trans- syn backbone (17), requiring the geranyl-

geraniol to be cyclised from a prefolded chair-boat conformation (18). These included eperuic acid (19), cafestol (20), rimuene (21) and gibberellic acid (28). However, the stereochemistries were eventually revised[8] and are now fully in accord with the biogenetic schemes.

Although most diterpenoids are based on the labdane alcohol precursor (10), there are three small groups presumably formed from geranylgeranyl pyrophosphate (5) prefolded in the different conformation (22). Cyclisation yields the monocyclic diterpenoid cembrene (23) and its oxygenated relatives.[9] Further cyclisation can then lead in the first place to the bicyclic verticillol (24)[10] and then on to the taxicins, such as taxicin-I (25).[11]

Satisfactory as the biogenetic schemes were in rationalising the structure and stereochemistry of the diterpenoids, there was no direct biochemical evidence such as there had been for squalene and the triterpenoids. The successful incorporation of 'labelled' C^{13} or C^{14} acetic acid into geraniol,[12] squalene,[13] cholesterol;[14,15] the isolation of mevalonic acid (27)[16] and the demonstration that mevalonate can replace acetate in cholesterol biosynthesis,[17] had been some of the decisive experiments in showing that mevalonic acid (27), itself derived from

condensations of acetyl co-enzyme A (26), was the progenitor of the isoprene unit. The now well-understood scheme[4] is shown in Figure 3.

Yet, the first direct evidence for the isoprenoid nature of the diterpenoids came only in 1958, with the successful incorporation by Birch[18] of both 1-^{14}C-acetate and 2-^{14}C-mevalonic acid lactone into gibberellic acid (28) and rosenonolactone (29), simultaneous with Arigoni's 2-^{14}C-mevalonic acid work on rosenonolactone.[19] The distribution of radioactive labels, as shown in Figure 4, agrees completely with the postulated biogenetic origins of these metabolites.

More recently, the mould metabolite pleuromutilin (30) was confirmed to be a 'disguised' diterpenoid.[20,21]

The major portion of this thesis is concerned with the synthesis of the rosane group of diterpenoids and investigation of their biogenetic relationship to the labdanes and pimaranes. Except for the hydrocarbon rimuene (21), all diterpenoids with the rosane skeleton are oxygenated and are metabolites of the mould Tricothecium roseum. There now follows a brief review of the elucidation of their structures, followed by an account of the latest knowledge of their biogenesis.

2 The Metabolites of *Tricothecium Roseum*

(a) Structural Studies

The metabolites of the mould *T. roseum* were first investigated by Freeman and Morrison[22-24] and by Michael,[25] who isolated a related series of diterpenoids which they named rosein I, II and III. They also obtained the sesquiterpenoid tricothecin, shown later by Godtfredsen and Vangedal[26] to have the structure (31). Much of the structural work has come from the Liverpool school who, in a series of papers,[27-31] established the constitution and absolute configuration of rosein I and II, renamed rosenonolactone and rosololactone respectively.

The major diterpenoid metabolite Rosenonolactone,[27,28,30] $C_{20}H_{28}O_3$, m.p. 214°C, $[\alpha]_D$ -107.5°, had infra-red absorption at 1786, 1724 cm^{-1}, indicative of a γ-lactone and a cyclohexanone grouping. The uptake of only 1 mole of hydrogen on catalytic hydrogenation, coupled with the ozonolytic cleavage to formaldehyde and a nor acid $C_{19}H_{26}O_4$, confirmed that rosenonolactone was a tricyclic keto-lactone with a vinylidene group. Using classical chemical and degradative methods, Whalley's group,[28,30] deduced the gross structure and the relative configuration at all the asymmetric centres, except C-13, to be as represented in (32).

The absolute configuration was determined[32,33] by converting rosenonolactone to the keto-acid (33) which, on treatment with alkali, was degraded to the keto-diacid (34), still retaining the same absolute stereochemical relationship of rosenonolactone ring - A. This initially-formed, cis keto-diacid was epimerised on base treatment to the more stable trans isomer (35). The absolute configuration of (35) was found to be as shown, since its O.R.D. curve was comparable to (-)trans-2-methyl-2-carboxy-6-ketocyclohexyl-propionic acid (36) whose absolute stereochemistry is known.[34] Hence rosenonolactone has the absolute configuration (37) for C-4, C-5, C-10, and, therefore, has the absolute stereo-chemistry as drawn in (32), except for the unsolved C-13 configuration.

The elusive C-13 configuration was finally established by relating rosenonolactone to rosololactone, another metabolite isolated from the mould, whose constitution and configuration were established by X-ray analysis.

Rosololactone,[29] $C_{20}H_{30}O_3$, m.p. 186°C, $[\alpha]_D$ + 6.3° was shown by Whalley[29] to be a hydroxy-lactone possessing a vinylidene group, closely related to rosenonolactone itself. He located

the hydroxyl group β to the lactone terminus and the two structures (38) and (39) were put forward as candidates. However, the structure and absolute stereochemistry was determined by Scott[35] and his co-workers who obtained the X-ray structure of 15,16 dibromorosololactone as (40). Hence rosololactone had the absolute stereochemistry (41), with an axial 6β hydroxyl grouping. Furthermore, they obtained a direct correlation between rosenonolactone and rosololactone by formation of the same diosphenol (42) from each lactone. Thus rosenonolactone has the overall absolute stereochemistry shown in (32). Chemical confirmation[31] of the absolute configuration at C-13 in rosenonolactone was afforded by degrading (+)-5-ethyl-2,5-dimethylcyclohexanone (43), derived from ring C of dihydrorosenonolactone (44), into (+)-3-ethyl-3-methyladipic acid (45), identical with a synthetic specimen.

Deoxyrosenonolactone m.p. 115-116°C, $C_{20}H_{30}O_2$, $[\alpha]_D$ + 57°, was first isolated from T. roseum by Arigoni.[32] The structure (46) was assigned to it since the dihydro derivative (47) was identical with the desulphurisation product of rosenonolactone -7 ethylene mercaptal.

Isorosenolic acid $C_{20}H_{30}O_3$, m.p. 193°C, $[\alpha]_D$ 0°, a minor metabolite from T. roseum, isolated by Scott,[36] is the only example without the γ-lactone group, although it possesses the functionality to form one. It was assigned the structure (48) from a combination of spectral and chemical studies, including its conversion to the diene-acid (49) also obtained from deoxyrosenonolactone (46).

A more recently isolated metabolite was 6-β Hydroxy-rosenonolactone or 'Lactone α',[37] m.p. 180-181°C, $[\alpha]_D$ -162°, identified as (50)[37,38] on the basis of its chemical and spectral properties, and by its conversion into the diosphenol (51) also obtained from rosenonolactone (32).

Rosein III m.p. 221°C, $[\alpha]_D$ -124°, although isolated in 1948, is still unformulated. Unpublished evidence from the Glasgow group[39] suggests it to be a hydroxylated rosenonolactone, isomeric with lactone α. However, Birch's group[40] have assigned a tentative structure (52), unpublished as yet. Work on elucidation is still in progress.

More recently, corroboration of the structural assignments of the metabolites has come from the O.R.D. studies of Klyne's group[41] who found that the O.R.D. curves of rosenono-

lactone, rosololactone, deoxyrosenonolactone and many of their related compounds are in agreement with their published structures and the predictions of the lactone sector rule.

(b) Biosynthetic Studies

With the virtual completion of the structural investigation of the metabolites, attention in recent years has been redirected towards elucidation of the biosynthetic pathways. It had been realised that the rosenonolactone system could be derived by the formal process (53) → (54) → (55) → (56) → (29), as shown in Figure 5, where the basic changes are cyclisation of the bicyclic labdane (54), accompanied by hydride shift from C-9 to C-8, methyl migration from C-10 to C-9 and lactonisation of a C-19 carboxyl group on to C-10. Convincing demonstration of the basic correctness of these assumptions was first obtained, as already described, by Birch[18] and Arigoni[19] when rosenonolactone incorporated four molecules of [2-^{14}C] mevalonic acid lactone to give the labelling pattern (29a) and eight molecules of [1-^{14}C] acetate to give the labelling pattern (29b). The degradation scheme to ascertain the labelling pattern was essentially that employed during the early structural work.[28]

The observation that [2-^{14}C] mevalonate introduced a radioactive carbon at the methyl group on C-4 but not at the lactonic carbon (C-19) was interpreted by both groups of workers to mean that the acyclic precursor must have the gem-dimethyl unsymmetrically labelled and that cyclisation to the first two rings is a concerted one.

At this stage many mechanistic details were still unsolved, namely:-

(1) Can other post-mevalonoid precursors, in particular a labdadienol such as (54), be incorporated into the rosane metabolites?

(2) Is the whole (55) $\longrightarrow$ (56) rearrangement process concerted, or are there any neutral intermediates, for example dienes or hydroxy compounds, formed by discharge of a C-8, C-9 or C-10 carbonium ion?

(3) At what stage in the biosynthesis does the oxygenation occur at C-7, C-19 etc.?

Many of these details have now been clarified by a series of recent papers from Hanson's group.[42-44] Thus, feeding experiments[42] on T. roseum showed that [1-^{14}C] geranyl pyrophosphate (3) was successfully incorporated into rosenonolactone (0.15%) and rosololactone (41) (0.34%). This result, the first instance of the incorporation of geranyl

pyrophosphate into a diterpenoid, is not unexpected since it is known as a specific precursor in squalene biosynthesis.[45] Similarly, [1-^{14}C] farnesyl pyrophosphate (4) was identified as a precursor for rosenonolactone and rosololactone by its observed incorporation,[42] into these metabolites, of 0.19 and 0.17% respectively, again the first demonstration of intervention of farnesyl pyrophosphate in diterpenoid biosynthesis. Final evidence that a C_{20} acylic precursor was involved in the biosynthesis of the tricyclic diterpenoids came with the successful incorporation of geranylgeraniol (53) itself.[43] Thus, geranylgeraniol was tritiated at C-1 by oxidation to the aldehyde and reduction with sodium borotritiide. The tritiated alcohol was solubilized in Tween 80 and fed to T. roseum to yield rosenonolactone with an incorporation of 0.09%. In addition, the radioactivity was shown to be located at C-16 in the vinylidene group, since ozonolysis and isolation of the formaldehyde as its dimedone derivative led to recovery of 96% of the radioactivity from C-16.

With the completion of work on the earlier acyclic stages of the biosynthesis, investigations turned towards the later biosynthetic stages, in particular the formation of ring C from a probable bicyclic intermediate. Direct

evidence for the intermediacy of a bicyclic labdane such as (54) was obtained[42] by the successful incorporation into rosenonolactone (0.16%) and rosololactone (0.13%) of the tritiated labdadienol (57), prepared from its parent alcohol by oxidising with manganese dioxide then reducing with sodium borotritiide. Ozonolysis of rosenonolactone confirmed the radioactivity to be in the vinylidene group.

Information on the rearrangement stage (55) to (56) was obtained by incorporation studies using tritiated mevalonic acid precursors. Thus rosenonolactone derived from doubly-labelled 4(R)-[4-^{3}H, 2-^{14}C] mevalonate was found, by degradation studies, to have retained tritium at C-5 and C-8. Hence the C-8 tritium must originally have migrated from C-9 to C-8, and so excludes the intervention of a neutral intermediate with an 8,9 double bond.[43,44] Hanson also discounts a $\Delta^{8(14)}$ pimaradiene as an intermediate, from the lack of incorporation into rosenonolactone of the tritiated pimaradiene (58),[42] although a negative result could perhaps be due to other causes.

On the assumption, then, of a concerted C-9 to C-8 hydride, C-10 to C-9 methyl shift, the key problem is the fate of the C-10 carbonium ion (59). A concerted C-10 to C-9 methyl migration with lactonisation of the C-19 carboxyl on to

C-10 is mechanistically improbable since the migrating methyl group is cis to the lactone ring (conformation 54). The only other reasonable course open to the C-10 carbonium ion (59) would be loss of a C-1 proton, loss of the C-5 proton or the more deep-seated process involving C-5 to C-10 hydride shift followed by loss of a C-6 proton, to give, respectively, the Δ^{1}-(60), $\Delta^{5(10)}$-(61), or Δ^{5}-(62) diene intermediates. These dienes could then be reprotonated with concomitant lactonisation on to the forming C-10 carbonium ion (59). However, in a series of labelling experiments with tritiated mevalonic acid, Hanson[44] has isolated rosenonolactone biosynthesised from (a) doubly-labelled 4(R)-[4-^{3}H, 2-^{14}C] mevalonate, from which tritium atoms were found to be located at C-8 and C-5 positions in the nucleus; (b) [5-$^{3}H_{2}$, 2-^{14}C] mevalonate, from which two tritium atoms were located at C-6; and (c) [2-$^{3}H_{2}$, 2-^{14}C] mevalonate which gave rosenonolactone with two tritium atoms at C-1. The location of the tritium atoms used the standard degradations of the original structural work,[28] or simple expedients like enolisation of carbonyl groups to cause loss of tritium at α carbon positions. The results clearly show that the protons at C-1, C-5 and C-6 are mevalonate-derived, hence any unsaturated diene intermediate involving C-1, C-5, C-6, C-10 cannot take part in the later

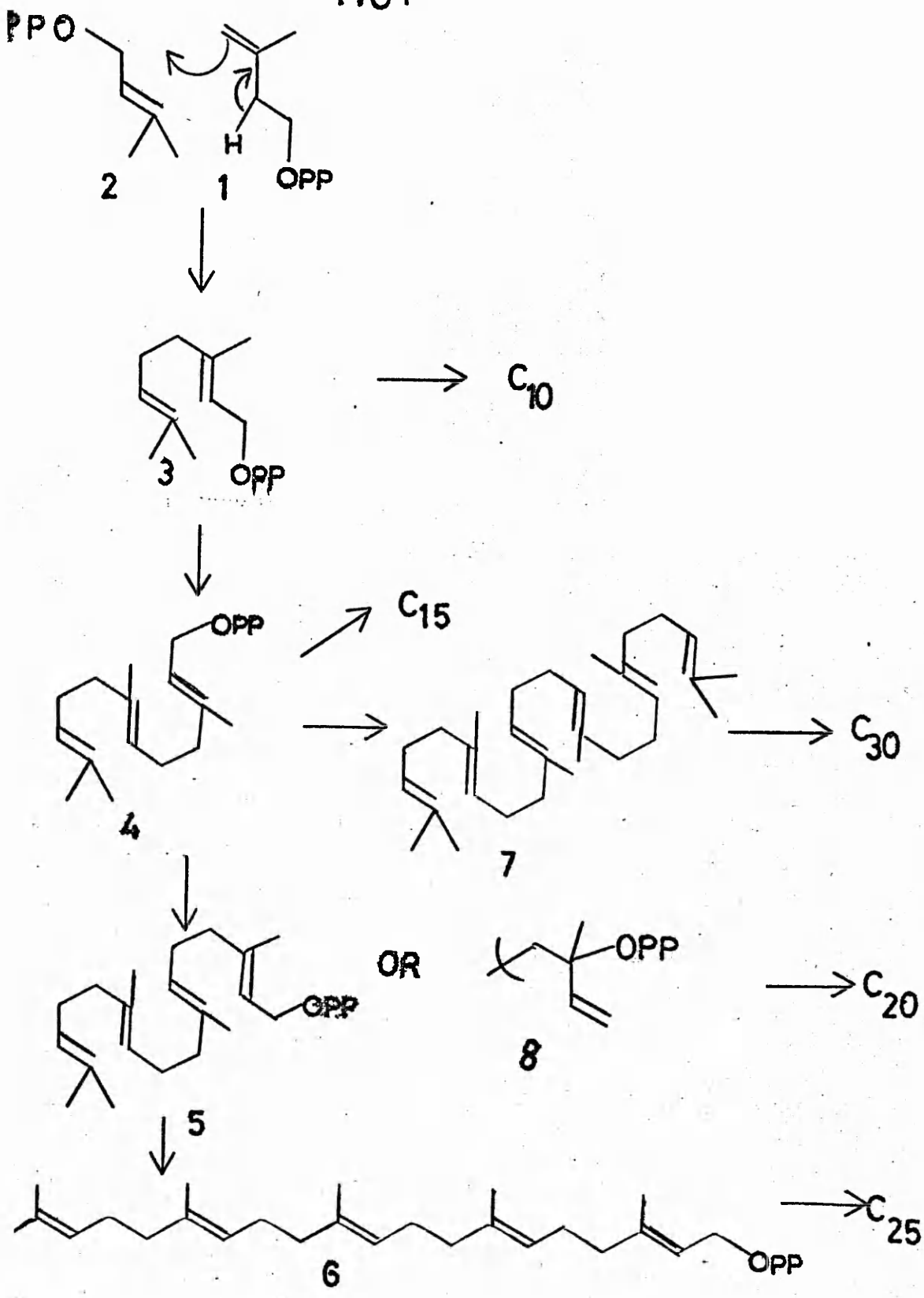
stages of rosenonolactone biosynthesis. (Unless the enzyme's basic site which removes the proton to give these intermediates delivers back the very same proton, prior to lactonisation - a dubious process?). On the basis of these new results, coupled with the unlikely possibility of a concerted cis lactonisation, a mechanism has been postulated that an α-oriented C-10 - enzyme or C-10 - hydroxyl bond is formed as the methyl group migrates from C-10 to C-9. This bond is then displaced with inversion when the lactone ring is formed.

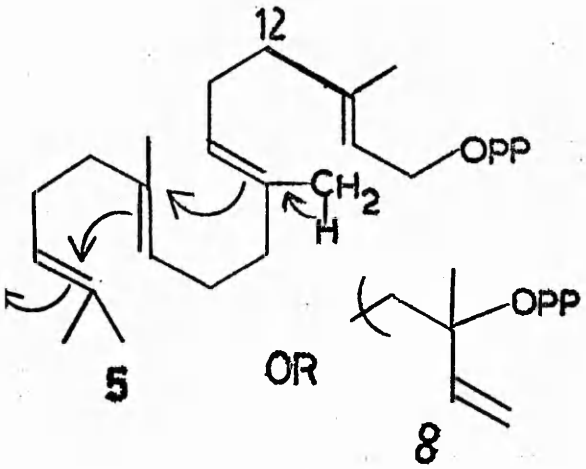
It is of interest to recall that this device of 'double inversion' at a centre via an enzyme (or nucleophile) bond has been suggested by Cornforth,[48] to account for the observed retention of tritium in farnesol or squalene biosynthesised from (4R)-[4-^{3}H] mevalonate. The result - that only the hydrogen corresponding to the 4S hydrogen in mevalonate is consistently lost during the stages where pyrophosphate is displaced to form a new carbon-carbon bond, coupled with the known inversion of configuration, on displacement of pyrophosphate - is best explained by the two stage scheme in Figure 6, where a nucleophilic group X adds to the existing double bond and then eliminates HX to form a new one.

Some light has also been shed on the final question concerning the sequence of oxygenation in the rosane metabolites.

The co-occurrence of small amounts of deoxyrosenonolactone (46) alongside rosenonolactone (29) and rosololactone (41) had suggested that (46) might be the precursor of the more oxygenated metabolites. Accordingly, T. roseum, fed with [2-^{14}C] mevalonate, was harvested at regular intervals so that the label distribution of the metabolites could be measured at different times.[42] It was observed that the maximum label in deoxyrosenonolactone appeared before that in rosenonolactone or rosololactone, suggesting a continuous channelling of mevalonate through the metabolite. More direct evidence,[43] however, was the successful incorporation of labelled deoxyrosenonolactone (46), itself prepared from (4R) - [4-^{3}H, 2-^{14}C] mevalonate, into rosenonolactone and rosololactone with incorporations of 3.7 and 1.1% respectively. Therefore, the enzymatic oxidation at C-6 or C-7 seems to be the final biosynthetic step.

FIG 1





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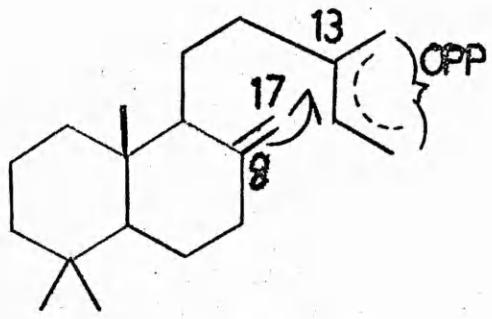

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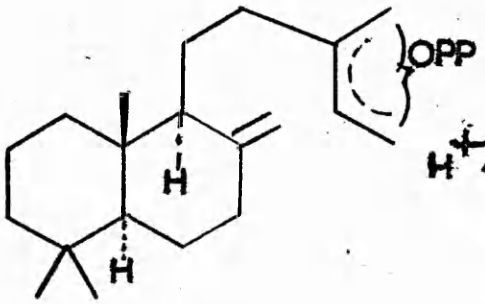

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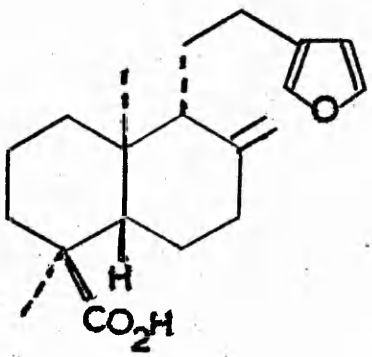

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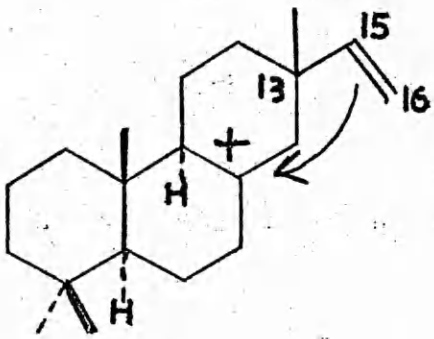

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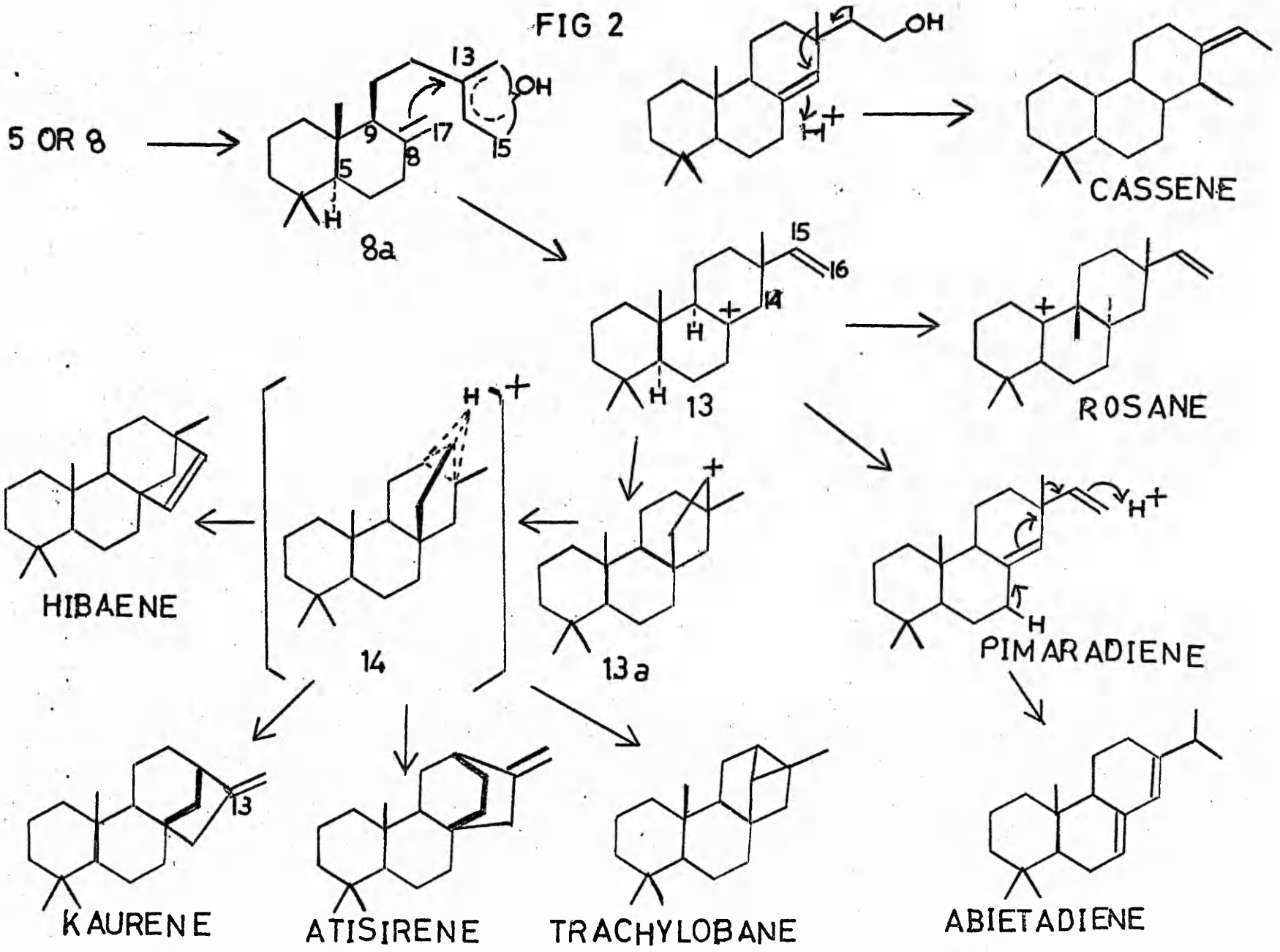
FIG 2


5 OR 8
8a
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TRACHYLOBANE
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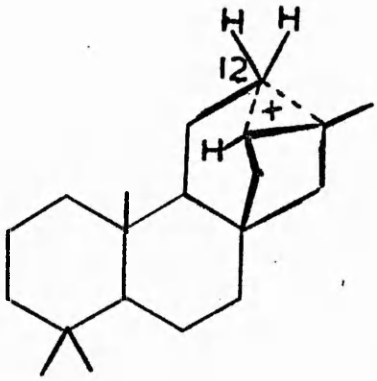

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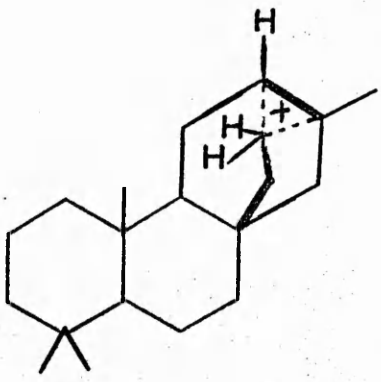

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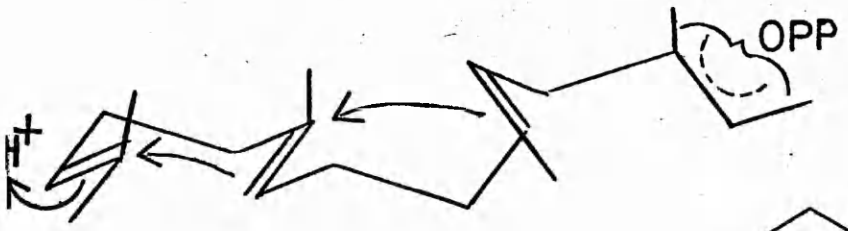

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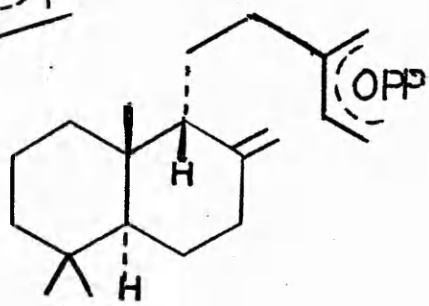

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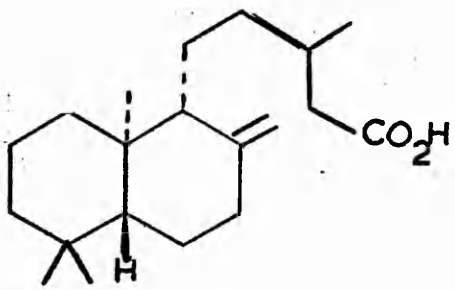

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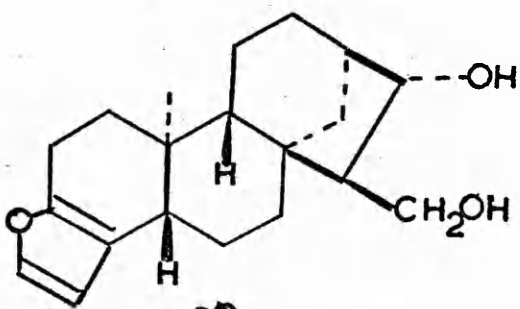

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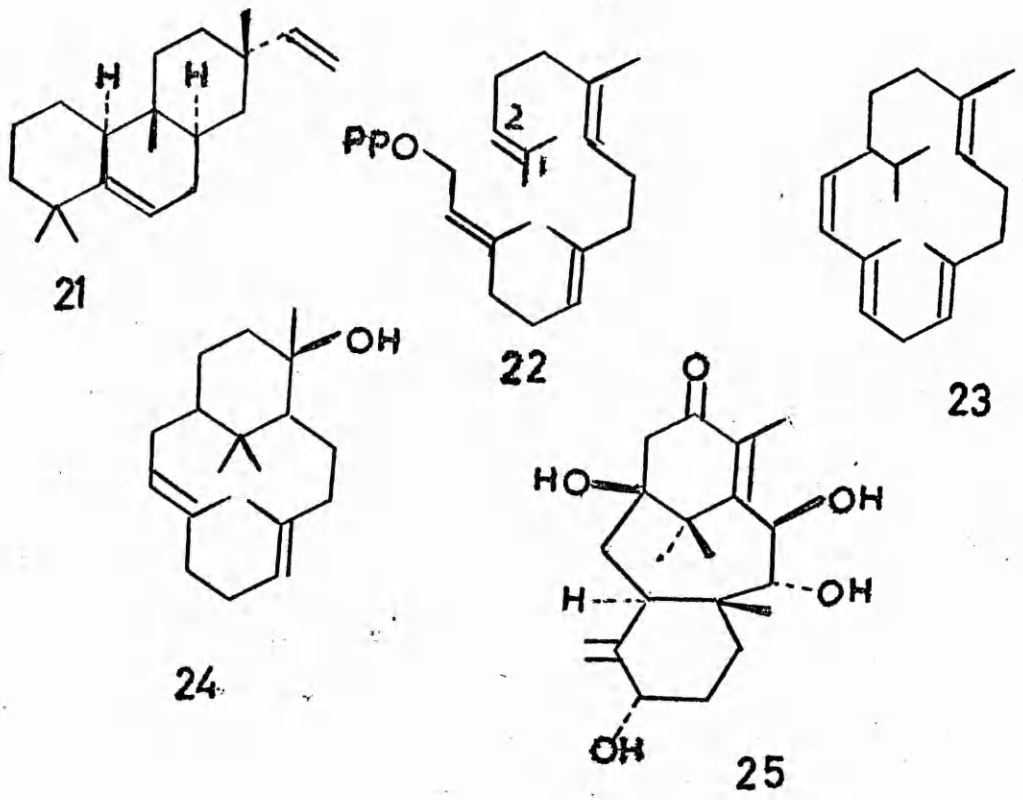

H
H
21
PPO
2
1
22
23
OH
24
HO
OH
H
OH
OH
25

FIG 3

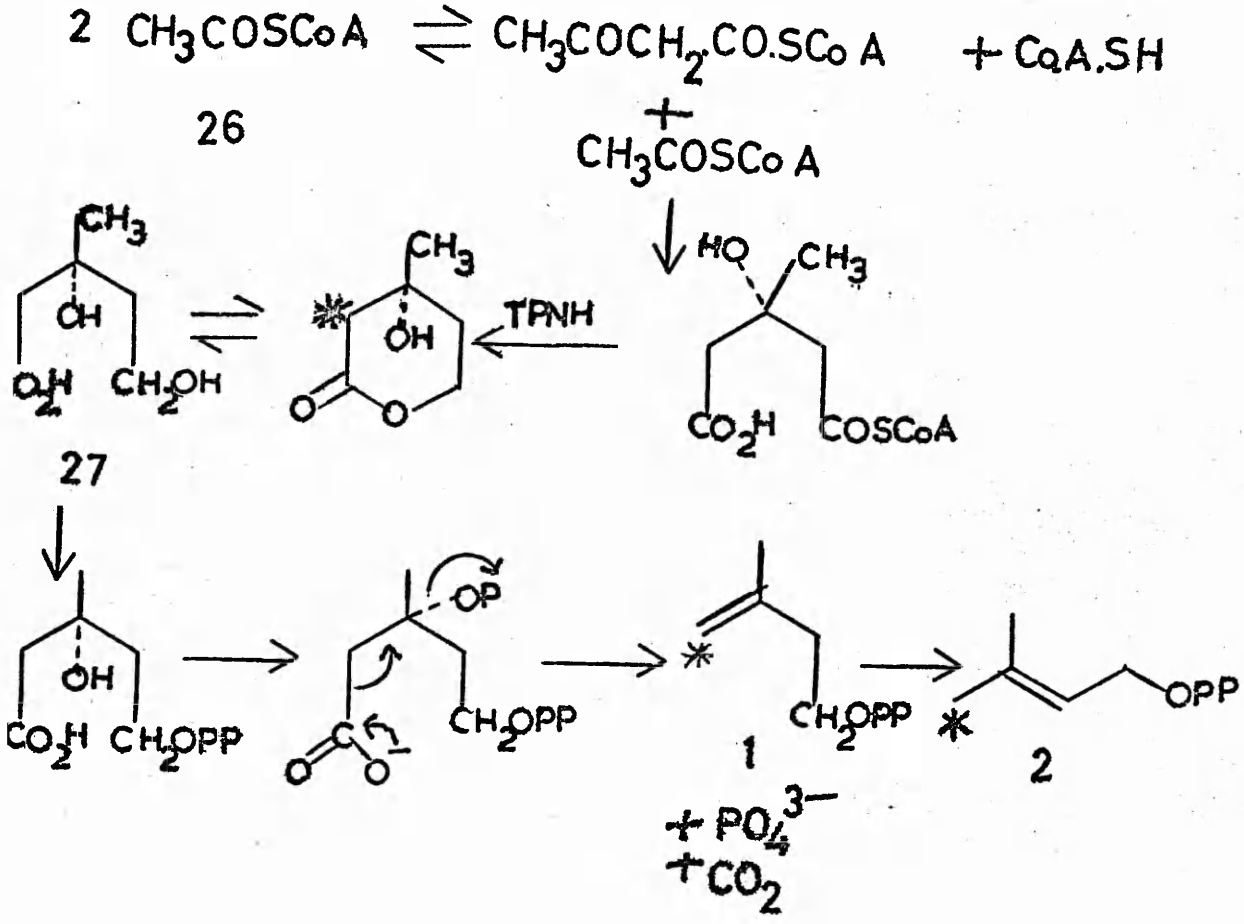
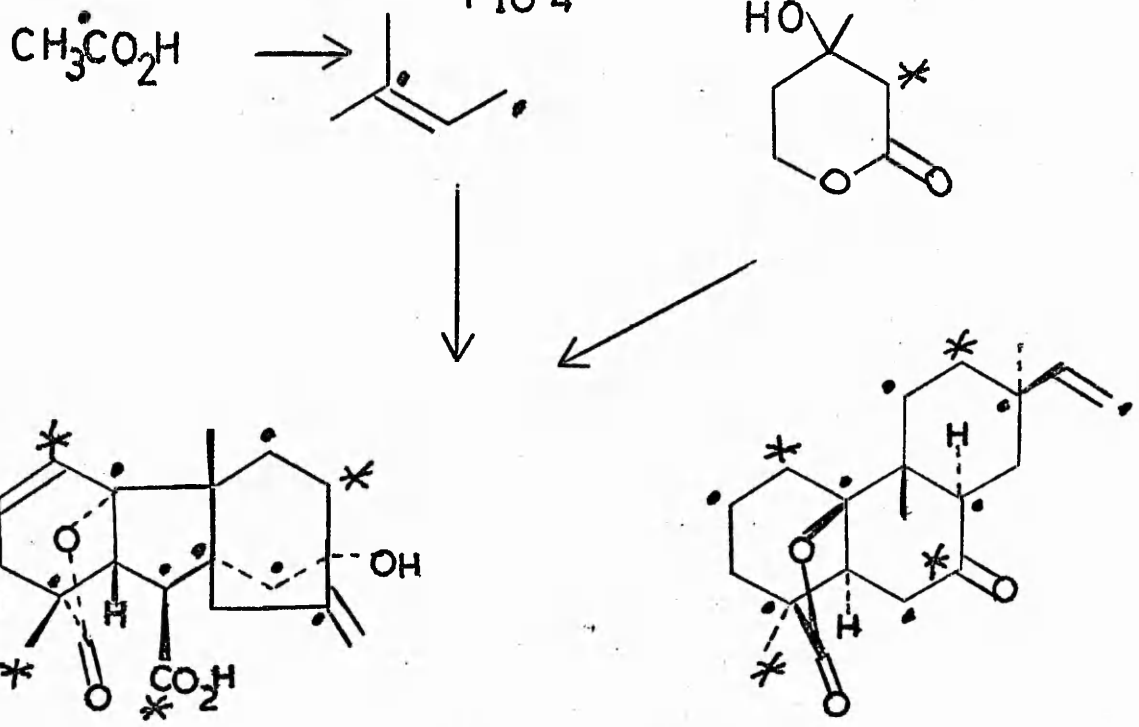

2 CH3COSCoA
26
CH3COCH2CO.SCo A
+ CoA.SH
+
CH3COSCo A
CH3
OH
CO2H
CH2OH
27
TPNH
HO
CH3
CO2H
COSCoA
OH
CO2H
CH2OPP
OP
CH2OPP
CH2OPP
1
+ PO4 3−
+ CO2
OPP
2

FIG 4



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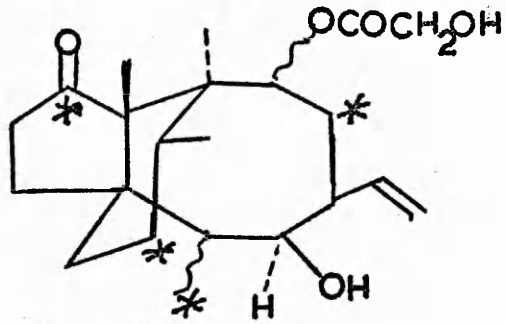

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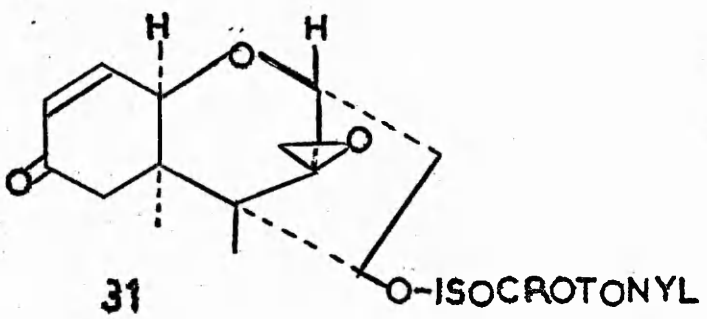

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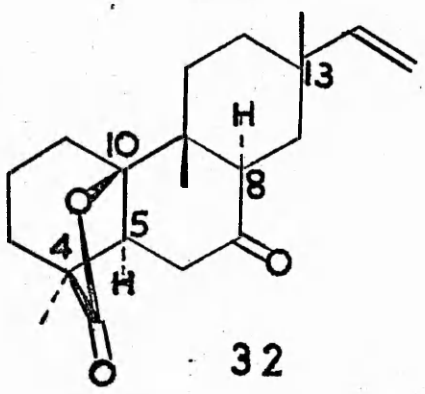

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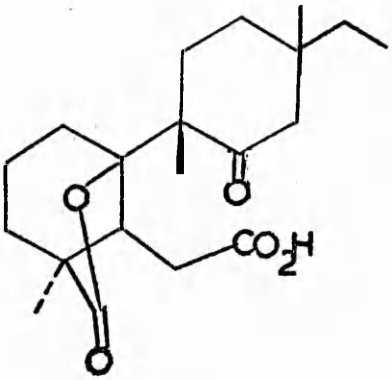

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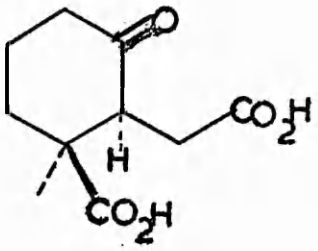

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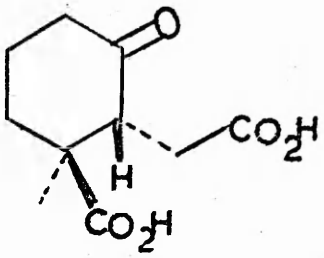

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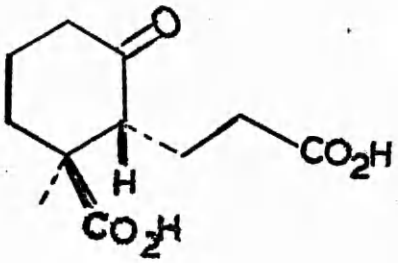

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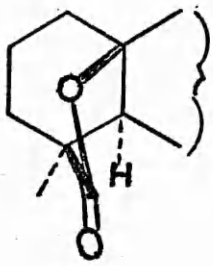

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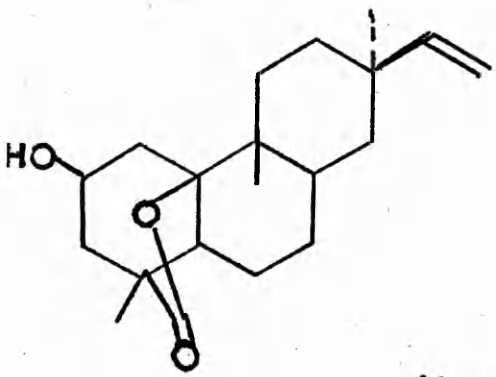

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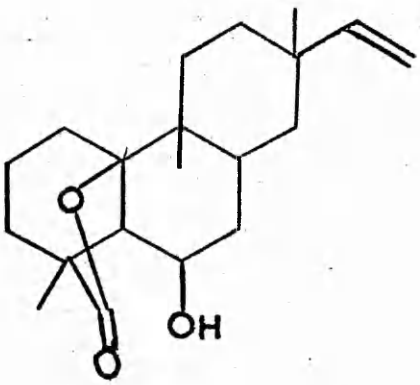

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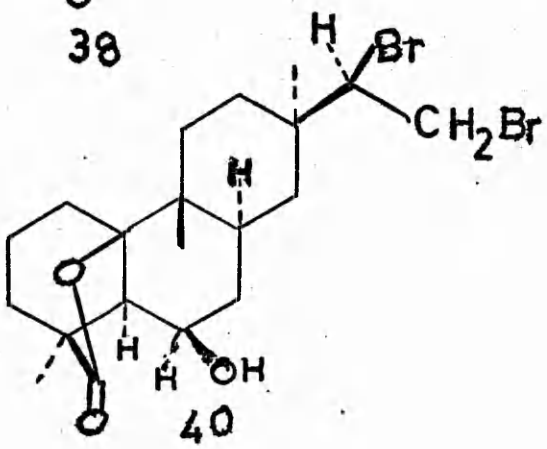

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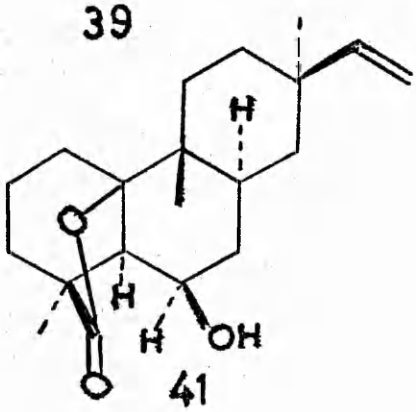

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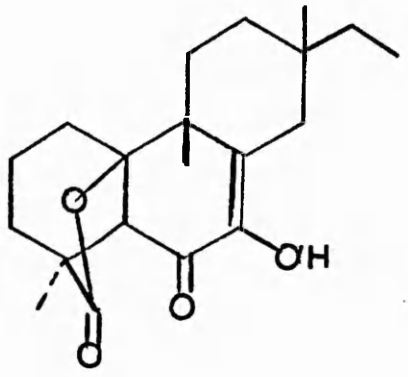

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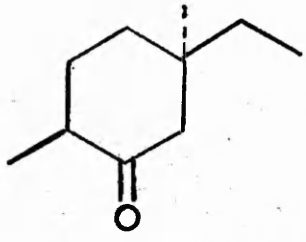

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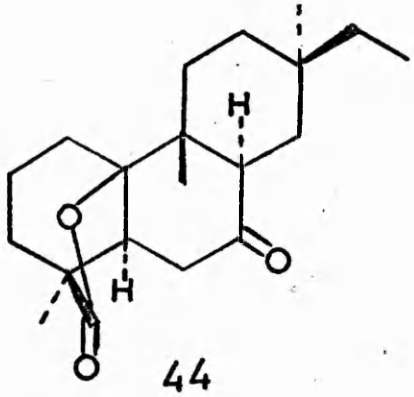

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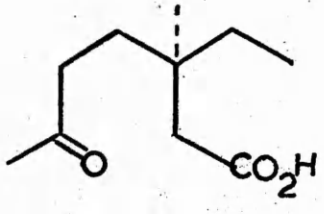

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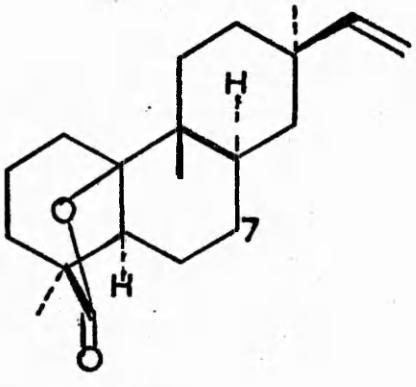

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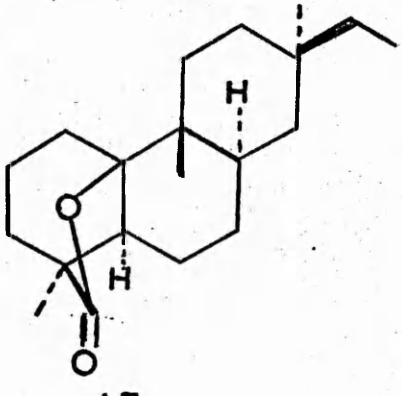

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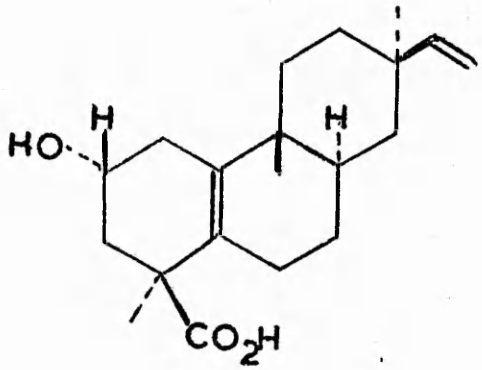

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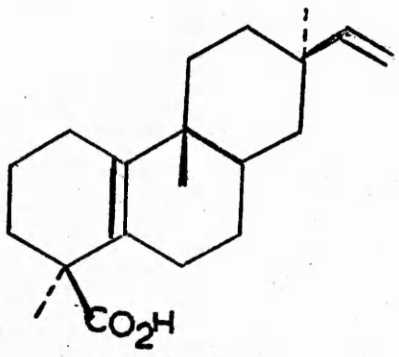

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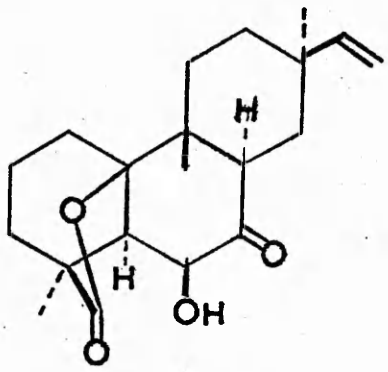

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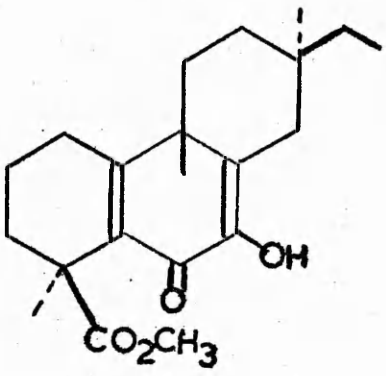

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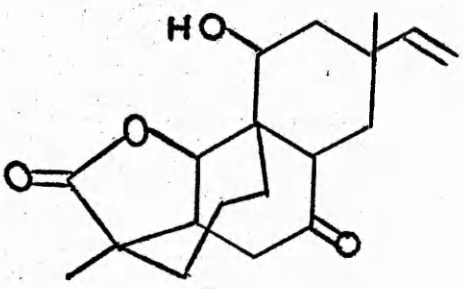

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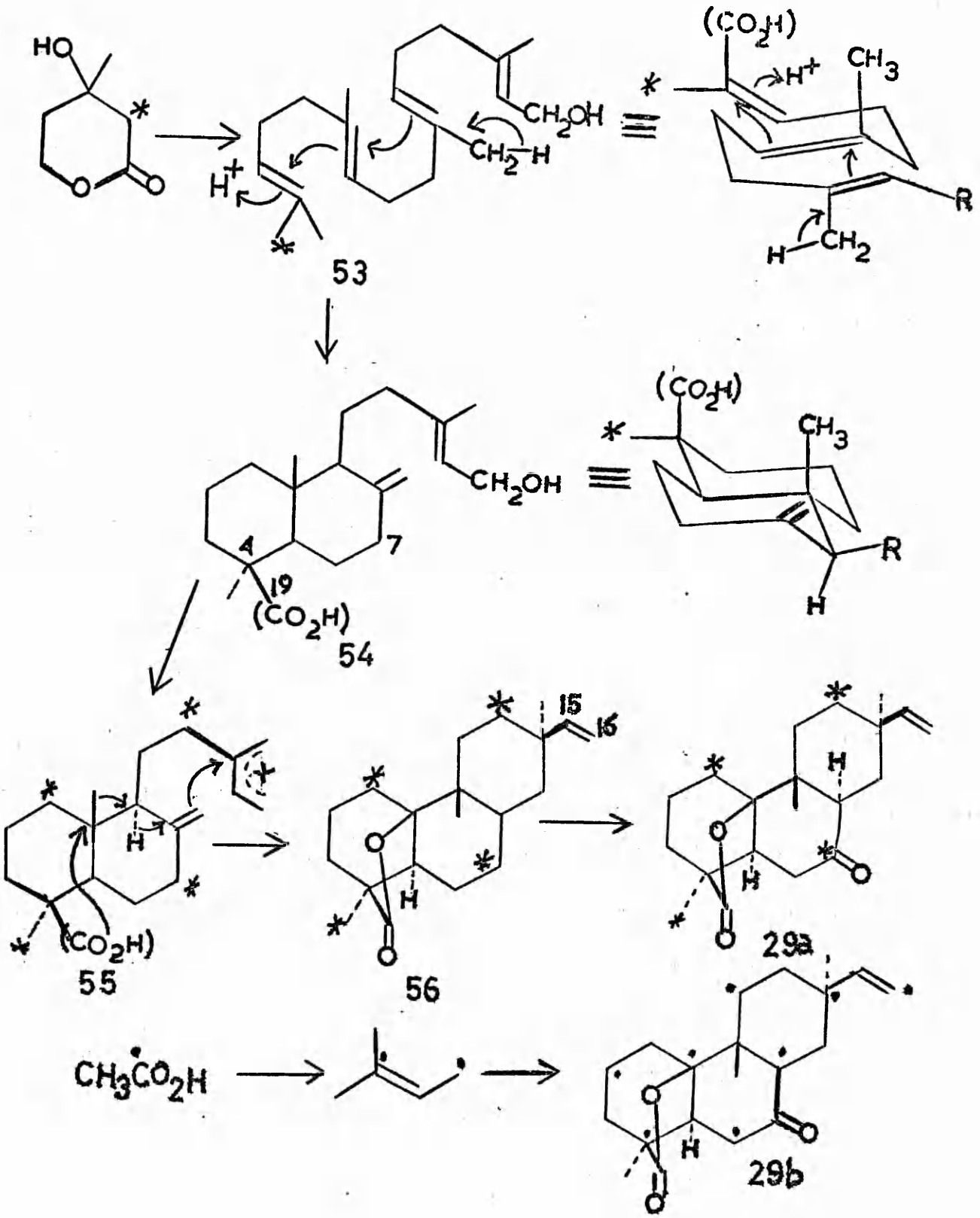
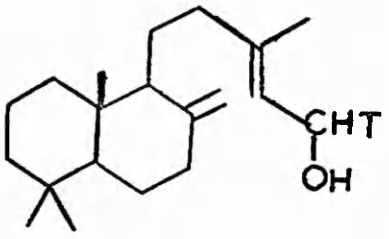
FIG 5


HO
CH2OH
CH2H
H+
(CO2H)
CH3
R
H
CH2
53
CH2OH
4
7
19
(CO2H)
54
55
15
16
56
29a
CH3CO2H
29b



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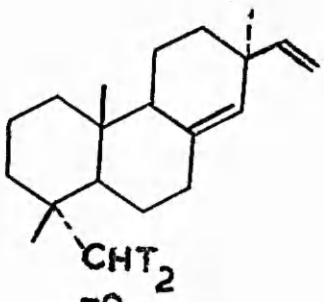

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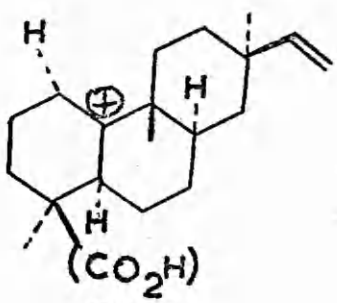

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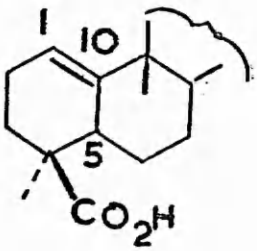

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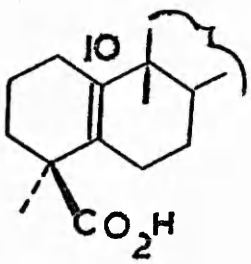

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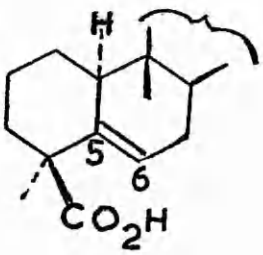

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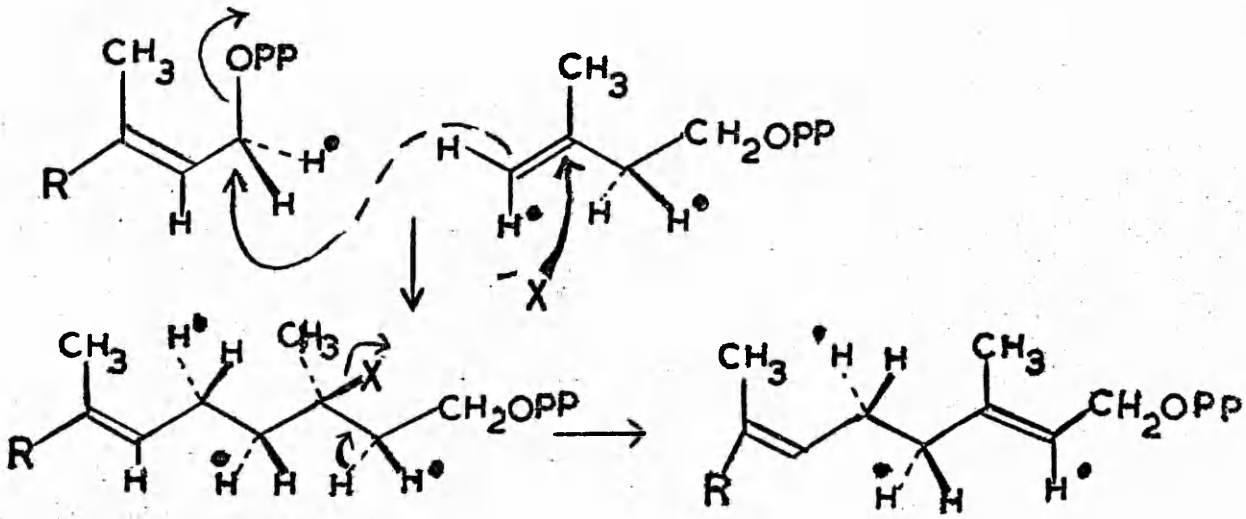
FIG 6



II SYNTHESIS OF ROSENONOLACTONE AND ITS CONGENERS

1 Synthetic Plan

The final solution of a natural product structure invariably acts as a stimulus for a synthetic attempt. Sometimes the syntheses serves to confirm the correctness of a structure, often they are solely an exercise in application of existing chemical knowledge. Certainly the terpenoids have inspired a host of syntheses whose range and ingenuity were a reflection of the challenging problems involved. Rosenonolactone (29) and the related rosane metabolites are of sufficient structural complexity to provide a severe synthetic test. Although the tricyclic ring system of the rosane skeleton would appear to invite a synthesis based on a phenanthrene type starting unit, it was decided, in this project, to depart from the more orthodox routes and attempt a synthesis of rosenonolactone and its relatives by a method patterned on the path whereby it is biosynthesised in nature. This biogenetically-patterned route is complementary to, but perhaps aesthetically more satisfying than, orthodox classical approaches. In addition, if the metabolites can be successfully synthesised by a series of transformations which simulate the known biogenetic pathway, then this lends indirect support to the proposed *in vivo* pathway.

From the *in vivo* studies previously described it is now well established that rosenonolactone and its relatives arise from cyclisation of a labdane precursor by the sequence outlined in Figure 5. Therefore, three key steps can immediately be defined in a biogenetically-patterned route:- (a) Cyclisation of a labdane, probably stopping at an intermediate pimarane skeleton. (b) Rearrangement of a pimarane to a rosane; however, it may well be that steps (a) and (b) are not experimentally separable. (c) Lactonisation at the rosane stage using a suitably oxygenated C-19 function. In order to separate these three major problems, it was decided to divide the work into two sections. In the first part a detailed study was undertaken to determine if the labdane to pimarane and pimarane to rosane rearrangements could be accomplished *in vitro*. The intention of this study, based on model labdane systems which lack oxygenation at C-19, was not only to derive the optimum conditions for cyclisation and backbone rearrangement but to explore the mechanism and stereochemical outcome of the cyclisation step, and to investigate the products, if any, of undesired skeletal rearrangements. In the second part, the knowledge gained from these investigations was applied to the synthesis of the rosenonolactone group; for the same labdane - rosane rearrangement can be performed on a suitable

C-19 oxygenated labdane to give a rosane whose functionality should allow elaboration into rosenonolactone.

At the outset of this work there was no published record of a synthetic attempt on rosenonolactone. Recently, however, Ireland and Mander,[46] have reported their efforts towards this end. As in the elegant synthesis of (d,l) rimuene and (d,l) -13-epirimuene,[47] the starting material was the tricyclic enone (64). The synthetic problem, nevertheless, is more formidable, for not only do the C-13 methyl and vinyl substituents have to be introduced in the epirimuene configuration (a harder task than getting the rimuene C-13 configuration[47]), but a carbonyl group has to be inserted at the relatively inaccessible C-7 position, a β-oriented lactone bridge constructed terminating at C-10, and the backbone stereochemistry ensured to be trans-syn-trans (although the presence of an epimerisable C-8 centre should facilitate this). In a nine stage sequence of 3% overall yield, they have arrived at the acetoxylactone (65) which has the functionality for introduction of the C-13 methyl and vinyl groups; furthermore, the triple chair conformation (66), resulting from the trans-syn-cis geometry, makes possible the oxygenation at C-7 by a transannular photolytic reaction involving the favourably placed, C-13 α oxygen. These final steps remain to be performed.

The problems listed above are, of course, not exclusively those of Ireland and Mander's, but must be faced and overcome in any synthetic attempt on rosenonolactone. However, the inherent advantage of a biogenetically-patterned route is that much of the ring fusion stereochemistry is automatically taken care of by the stereospecificity of the cyclisation and rearrangement processes.

Furthermore, the labdane to pimarane cyclisation step should theoretically furnish two C-13 epimers, so the rosenonolactone C-13 configuration can be obtained merely by selecting the appropriate epimer at the early pimarane stage in the synthetic scheme. These details are fully discussed later.

2 REARRANGEMENT OF LABDADIENOLS INTO PIMARA- AND ROSA- DIENES

Discussion

As outlined in the introduction, the purpose of this first section of the project was to examine the feasibility of deriving the rosane system from the labdane, in order to prepare the way for the eventual synthesis of rosenonolactone. Accordingly, the work was performed on model labdane compounds, such as manool (70), which lack oxygenation at C-19.

In the biogenetic transformation of a labdane to a pimarane skeleton the bicyclic precursor is believed to be either the primary labdadienol pyrophosphate (67) or the tertiary allylic isomer (68). The tricyclic skeletons are envisaged as arising from either of these precursors by ionization of the pyrophosphate followed by attack of the 8,17-exomethylene group, (as indicated by arrows), at the developing C-13 cationic centre. It is not known if this is a concerted process in vivo or whether the first step is departure of the biological leaving group to furnish the discrete carbonium ion intermediate (69). Any synthetic scheme, therefore, which purports to simulate the biogenetic pathway must start from a structure like (67) or (68), where the leaving group

(- OR) may be a hydroxyl or a wide variety of ester functions.

The first line of approach embarked upon was an investigation into the use of various esters as the leaving group and the type of solvolytic conditions necessary to induce ionization. As a purely speculative initial attempt, the diterpenoid manool (70) was reacted with phosphorous oxychloride in pyridine in the hopes that the intermediate chlorophosphite ester (71) might undergo the desired ionization - cyclisation at the expense of the usual 1,2-elimination reaction. Although no precedent could be found for such a cyclisation using phosphorous oxychloride on olefinic alcohols, the superficial resemblance of a chlorophosphite group to the 'biological' phosphate esters proved an irresistible invitation. However, the product from the dehydration was shown by t.l.c. and g.l.c. (SE30; ApL) to be a mixture of three isomeric olefins in the ratio of 7:2:9 (on g.l.c.). Their characterisation was made unnecessary by the timely work of Carman and Dennis[49] who identified them (in their g.l.c. order) as sclarene (72), cis-biformene (73) and trans-biformene (74). Hence cyclisation had failed, but in compensation had made available the three trienes as reference compounds for subsequent experiments.

Since sulphonate or carboxylate esters cannot ordinarily be prepared from tertiary alcohols such an manool (70) attention

was turned to the allylic primary alcohol system (68) which should furnish the same cation (69) on solvolysis. The only readily available compound possessing this functionality was agathadiol (75) which, unfortunately, also has a hydroxyl group at C-19. Prepared from hydride reduction of dimethyl agathate (76) according to Enzell,[50] the diol, m.p. 106°C, had i.r. 3643 cm^{-1} (free primary hydroxyl; all i.r. spectra are in carbon tetrachloride unless otherwise stated) and the n.m.r.[51] showed an AB quartet at τ 6.62, 6.28 (J 12 Hz) (C-19 hydroxymethyl group); the vinyl 14-H and the C-15 methylene gave a triplet and doublet (J 7 Hz) at τ 4.62, 5.88 respectively. Agathadiol was chosen for exploratory experiments simply because of its ready availability. It was of course realised that the presence of a second hydroxyl group at C-19 would introduce complications during the preparation of the esters. Admittedly, this extra hydroxyl group, although still primary, is in the 4β-axial orientation with its consequent 1,3-diaxial interaction with the methyl group on C-10. This congested environment therefore, should provide considerable steric hindrance to reaction at C-19 and so should allow a certain measure of preferential esterification of the more accessible allylic hydroxyl in the side chain. Sulphonate esters of allylic alcohols are known[52] to be difficult to make, or to be

very reactive when eventually obtained; hence it was no surprise that all attempts to make the allylic p-toluenesulphonate or p-bromobenzenesulphonate of agathadiol failed completely. Treatment of agathadiol with the respective acid chlorides in pyridine afforded, in both instances, a complex mixture of products. These products presumably arise from the allylic sulphonate reacting further under the preparative conditions themselves, the possibility of esterification at C-19 being an additional complication.

Success was eventually obtained by the preparation of the less reactive p-nitrobenzoate, Thus, reaction of agathadiol (75) for three days with a slight excess of p-nitrobenzoyl chloride in pyridine gave three products, separable by t.l.c. The major product, $C_{27}H_{37}O_5N$, m.p. 107-110°C was the desired monoester (77) as shown by its n.m.r. spectrum which had a singlet at τ 1.78 (four aromatic protons) with the H-14 triplet and C-15 methylene doublet shifted downfield to τ 4.53, 5.10 respectively (J 7 Hz). The rest of the spectrum was practically the same as the starting diol. The second product, $C_{27}H_{37}O_5N$, was confirmed to be the 'wrong' monoester (78) by the presence in its n.m.r. spectrum of a singlet at τ 1.76 (four aromatic H) and by the downfield shift of the C-19 methylene AB quartet to τ 5.83, 5.46 (J 11.5 Hz). The third, least polar, product,

m.p. 203-206°C, $C_{34}H_{40}O_8N_2$, is presumably the diester (79) but was not investigated further. From the relative proportions of the two p-nitrobenzoates (77) and (78) it was estimated that the allylic hydroxyl at C-15 was reacting four times as fast as its 'competitor' at C-19 in ring A. This rate difference gives a semi-quantitative estimate of the steric crowding around the two reactions sites.

With the allylic p-nitrobenzoate (77) finally in hand, solvolysis experiments were attempted as follows:- The ester (77) was refluxed in a solution of acetone-water (9:1), the course of the reaction being monitored by examining portions at regular time intervals. However, the p-nitrobenzoate was found to be completely unreacted, even, after two weeks. This observation is surprising in view of Sneen and Rosenberg's reported successful solvolysis[53] of allylic p-nitrobenzoate systems in solvents such as aqueous dioxan, aqueous acetone and methanol. Mild acetolysis conditions were tried next by shaking the ester (77) for two days in acetic acid buffered with sodium acetate, but the product after work up was again shown to be unreacted starting material. Acetolysis at 60°C for 20 hours furnished products which, after acetylation with acetic anhydride and pyridine, were identified to be mainly agathadiol diacetate (80), since the g.l.c. (SE30) and the

n.m.r. spectrum [τ 7.99, 7.97 ($-OCH_3$ singlets), 5.41 (doublet, C-15 methylene), 4.68 (triplet, H-14); AB quartet (J 12 Hz) at 6.14, 5.77 (C-19 methylene)] were identical with an authentic sample of diacetate prepared from acetylation of agathadiol. This result means that direct replacement of p-nitrobenzoate by acetate is taking place in preference to the desired solvolysis-cyclisation. Since solvolysis to an allylic carbonium ion such as (69) would be expected to yield at least some allylic tertiary acetate, this suggests that either a bimolecular nucleophilic substitution is taking place (unlikely in allylic systems?) or, perhaps more likely, ester exchange is occurring by an acyl-oxygen cleavage mechanism. Prolonged acetolysis of (77) at 60°C for 150 hours gave products which, after acetylation, were shown by g.l.c. to contain approximately 10% of three more volatile components. The molecular weight 330 (from g.c.m.s.) indicated each of the three components to be monoacetate, $C_{22}H_{34}O_2$. Hence, as the acetate must be that on C-19, the three products are either bicyclic trienes resulting from a simple elimination of the side chain, C-15 ester function or else they are tricyclic products derived from an ionization-cyclisation mechanism. More forcing acetolysis conditions, 100°C for 15 hours only succeeded in raising the proportion of the above three compounds to a mere 20%. The low yields of

these products, even assuming that they are indeed tricyclic structures, was a severe deterrent to a synthetic approach of this type. Therefore, the use of ester solvolysis as a means of cyclising labdadienols did not appear to be a fruitful approach and was not pursued any further.

In search of an alternative method of inducing cyclisation via allylic cation (69) it was considered that the allylic alcohol (70) or its primary hydroxyl isomer are themselves sufficiently reactive to ionize on protonation. There is ample precedent in the literature not only for such an ionization but also for ring formation, if there is a suitably placed olefinic bond to interact with the allylic cation. Thus, Prelog and Watanabe[54] have transformed linalool (81) into terpineol (82) with sulphuric acid. More recently Johnson and his co-workers[55] have achieved excellent results from formic acid cyclisation studies on dienols such as (83), which was stereoselectively converted into the bicyclic alcohol (84). Even more reassuring for the present project has been the work of Dolby and Iwamoto[56] who, by means of formic acid, have cyclised the dienol (85) into the bicyclic diene (86). The compound (85) used in this example is a very good model for a labdadienol like manool (70) and suggests not only that the desired labdane cyclisation

ought to be facile but also that the initial product may be a pimaradiene system with an 8,9 tetrasubstituted double bond.

In fact, the acid-catalysed cyclisation of manool (70) itself had already been investigated by Bory and Asselineau[57] in 1961. These workers isolated in small yield a tricyclic hydrocarbon which was formulated as 8,15-isopimaradiene (87). Due to the lack of physical tools like n.m.r. and g.l.c., however, they presented no real evidence, other than hydrogenation studies and optical rotations, to support their conclusions.

The chances, therefore, of cyclising manool (70) seemed good, but, rather than enter on such a specific project, it was decided that a more wide-ranging and comprehensive investigation was really required, to look into the mechanistic and stereochemical details of the cyclisation step. As already mentioned, the tricyclic structures possess a C-13 asymmetric centre which is newly created during the formation of ring C. In principle therefore, there can be formed two C-13 epimers, such as 8,15-isopimaradiene (87) (β methyl, α vinyl) and 8,15-pimaradiene (88) (α methyl, β vinyl), the relative proportions of which will depend on the mechanism of reaction and on the stereochemistry of the starting labdadienol. The presence of an asymmetric C-13 centre in manool will, if cyclisation is concerted with ionization, result in preferential formation of one tricyclic epimer. In particular the pimaradine C-13

configuration (88) should result exclusively from manool, provided that participation of the 8,17 double bond assists the departure of the oxygen function and that the transition state is as shown in (90). Similarly, C-13 epimanool (91) should yield the epimeric isopimaradiene (87). Although the cyclisation process may be concerted *in vivo*, it seemed probable that the *in vitro* reaction would be a two-stage process involving initial ionization to the mesomeric allylic cation (69). Now if this cation intermediate is unstable, there may still be some asymmetric induction possible, provided that the 8,17 double bond attacks the cation on the face opposite to that of the departed group. However, if the intermediate cation (69) is relatively long-lived then its rotational isomer (69a), the first formed from manool of conformation (70), can be converted to (69b) by rotation of the 12,13 single bond. Subsequent cyclisation would yield mixtures of the two possible C-13 epimers.

The allylic cation (69) ought also to be produced by acid treatment of the allylic primary alcohol system, of which there is a cis geometric isomer (92) and a trans isomer (93). It is more difficult to analyse the stereochemical outcome from cyclisation of these alcohols (92) and (93) than for manool and C-13 epimanool, but it is reasonable to expect that, in a concerted reaction, the cis and trans alcohols would furnish different ratio of tricyclic C-13 epimers. Hence,

details of the cyclisation mechanism can be derived by performing the reaction on the four labdadienols - manool, C-13 epimanool, and the primary alcohols (92) and (93). Should the same product ratio of epimers arise from all four alcohols then the cyclisation cannot be concerted with ionization. Both manool and epimanool[58] * occur naturally but the two primary alcohols had to be prepared as follows:-

Sclareol (94) was reacted with chromic acid according to the method of Stoll and Commarmont[59]. The aldehyde fraction resulting from isomerisation-oxidation (isolated by means of its bisulphite complex) was shown by t.l.c. to consist of two closely running compounds, in the ratio of 1:1. The infra-red absorption (liquid film) at 3500 (OH); 1670, 1630 cm^{-1} (α-β unsaturated aldehyde) confirmed the oily product to be a mixture of the cis-trans allylic isomers (95), reported previously by Bory and Lederer.[60] Without separation into its constituents, the aldehyde mixture (55% yield from sclareol) was reduced with lithium aluminium hydride in ether to furnish a colourless oil, shown on t.l.c. to be two closely running polar compounds. The i.r. spectrum (liquid film), 3400 (OH), 1665 cm^{-1} (C=C) confirmed the product as the expected cis-trans isomeric diols (96). It had been feared that hydride

*Epimanool was kindly supplied by Dr. J.S. Mills, London.

reduction of the unsaturated aldehydes might cause considerable amounts of undesired C=C bond reduction by a 1,4 addition process. Fortunately, no such products could be detected, even when a large excess of hydride was employed.

The individual diols could be separated only with difficulty, so it was decided to separate and characterise them as the acetates. Thus, the mixture (96) was reacted overnight with acetic anhydride in pyridine. The resultant yellow oil, shown by t.l.c. (two close spots) and i.r. 1735, 1245 cm^{-1} (liquid film), to be mainly the two isomeric monoacetates (97) and (98), was chromatographed over neutral alumina using petroleum ether-benzene gradient elution. Isolated first was the less polar isomer $C_{22}H_{38}O_3$, later to be assigned the cis geometry (97). Dilute solution (0.002 M in CCl_4) i.r. absorption at 1739, 1237 cm^{-1} indicated the presence of an acetate, while bands at 3608 (free tertiary OH) and 3557 (bonded OH) suggested there was an equilibrium proportion of the molecules in which hydrogen bonding occurs between the C-8 hydroxyl and the side chain acetate grouping. The n.m.r. spectrum confirmed the gross structural features with quaternary methyl signals at τ 9.22 (two), 9.13, 8.86; τ 8.22 (vinylic methyl), 7.99 (OAc). However, H-14 and the C-15 methylene group did not produce the triplet-doublet AX_2 spectrum as had been observed with agatha-

diol (75) and its derivatives. Instead an ABX spectrum had resulted in which the H-14 signal (X part) was approximately a triplet at τ 4.70 with the C-15 methylene (AB part) as a symmetric 8-line spectrum at τ 5.35. These conclusions were corroborated by double irradiation experiments, where the H-14 signal collapsed to a broad singlet on irradiating at τ 5.35 and the C-15 methylene signals became a broadened AB quartet on irradiating at τ 4.70. A possible reason for the non-equivalence of the C-15 methylene protons is that the hydrogen bonding between the hydroxyl at C-8 and the acetate group will restrict the number of conformations which the side chain can adopt and so place each C-15 proton in slightly different environments.

The more polar isomer, later to be assigned the trans geometry (98), was also eluted as an oil $C_{22}H_{38}O_3$. Dilute solution i.r. (0.002 M CCl_4) again demonstrated the presence of intramolecular hydrogen bonding, with bands at 3614 (free OH), 3544 (bonded OH) and 1742, 1234 cm^{-1} (acetate). Singlets (3H) in the n.m.r. at τ 9.22 (two), 9.14, 8.88 (quaternary methyls); τ 8.30 (vinyl methyl) and 7.98 (OAc) were consistent with the structure (98). Unlike the isomer (97) H-14 and the C-15 methylene had a simple AX_2 spectrum with a triplet at τ 4.65 (J 7 Hz) and a doublet (J 7 Hz) at τ 5.43.

The occurrence of non equivalence of the C-15 methylene only in the less polar acetate was the first tentative indication that it might be the cis geometric isomer (97). The correct geometries, however, were finally assigned to the acetates by the method of Bates and Gale.[61] According to this method, the n.m.r. chemical shift of the vinylic methyl group in compounds with a trisubstituted bond of type (101) (X = OH or OAc) depends on the geometry of the double bond, the methyl signal in the trans double bond isomers appearing consistently at about 0.07 ppm higher field than its cis counterpart. By application of this method to the two acetates the less polar isomer (vinylic methyl τ 8.22) must be the cis compound (97), and the more polar isomer (vinylic methyl τ 8.30) must have the trans geometry (98).

In fact, the stereochemical assignment did not just rely on this specific example; for, in the subsequent transformations, described below, of acetates (97) and (98) into labdadienols (92) and (93) respectively, it was found that all the newly obtained labdane compounds conformed to the rule; that is, the vinylic methyl resonances in the cis series always came at lower field than the methyls in their trans isomers. These compounds and the chemical shifts of their vinylic methyl groups are listed in Table I. Also included as reference compounds of known trans geometry[62,63]

are agathadiol (75) and agathadiol diacetate (80).

The final proof of the correctness of the stereochemical assignments came from hydrolysis of the acetates to their corresponding diols. Thus, a small portion of (97) was treated with 5% ethanolic potassium hydroxide, overnight at room temperature, to yield the diol (99) as an oil; whereas similar hydrolysis of (98) gave the diol (100) as a solid, m.p. 127°C. These two diols have previously been prepared by Fetizon and his co-workers[62] who have assigned the cis geometry to their oily diol and the trans geometry to their diol of m.p. 129°C.

Dehydration of the cis hydroxy acetate (97) with phosphorous oxychloride in pyridine gave a crude oily product which appeared to be homogeneous on 'ordinary' t.l.c. but was found on silver nitrate t.l.c. to consist of a major component (approximately 85%) and two more mobile components (15%). The major product must be the exomethylene compound (102) for the n.m.r. of the total product indicated that the C-8 quaternary methyl singlet at τ 8.86 was missing and was replaced by two broad singlets (approx. 1 H each) at τ 5.15, 5.44. It had been expected that the major double bond isomer from dehydration of the C-8 hydroxyl would be the $\Delta^{8(17)}$ exomethylene compound because the 8α equatorial orientation

of the hydroxyl makes impossible a trans antiplanar elimination with the C-7 or C-9 protons. In fact the proportion of exomethylene product agrees well with the work of Carman and Dennis[64] who dehydrated the analogous alcohol, abienol (108) and obtained 90% of $\Delta^{8(17)}$ exomethylene product, the remaining 10% being a mixture of Δ^{7} and Δ^{8} olefin isomers. Separation of the three double bond isomers from dehydration of (97) was achieved by preparative t.l.c. (silver nitrate).

The least polar component, an oil, was identified to be the Δ^{8} isomer (103) from its n.m.r. spectrum which, in addition to three quaternary methyls, an acetate methyl and an AX_2 spectrum for H-14 and the C-15 methylene group, showed the presence of two vinylic methyls at τ 8.40, 8.20, yet no vinyl absorption other than H-14. Isolated in small amounts was an oily product which was identified as the Δ^{7} isomer (104). The evidence comes solely from the n.m.r. spectrum which shows two vinylic methyls at τ 8.27, 8.22 and a broadened olefinic singlet (1 H) at τ 4.58. The rest of the n.m.r. had the signals expected from the structure.

Hydrolysis of the major allylic acetate (102) was performed under mild conditions by leaving it for 10 hours with a saturated solution of solid sodium bicarbonate in 1%

aqueous methanol. This yielded the cis allylic alcohol (92) as an oil, $C_{20}H_{34}O$, $[\alpha]_D$ + 5.1°. The n.m.r. spectrum was completely consistent with the structure, showing (in addition to three quaternary methyls, an acetate methyl and the AX_2 spectrum of H-14 and the C-15 methylene), singlets at τ 8.28 (3H, vinylic methyl); 8.4 (1H, hydroxyl); 5.45, 5.14 (1H each, exomethylene).

With the completion of the synthesis of the cis primary alcohol (92) the dehydration and hydrolysis steps described above were repeated in the trans series, as follows:- Dehydration of the trans hydroxy acetate (98) with phosphorous oxychloride in pyridine gave an oil, three components on silver nitrate t.l.c., which was shown to be predominantly the exomethylene olefin isomer (105) by the presence in its n.m.r. spectrum of broad singlets at τ 5.49, 5.18 (approximately 1H each). The remainder of the spectrum was in agreement with the expected structure. By careful use of silver nitrate t.l.c., it was agin possible to separate the above dehydration product into its three constituent double bond isomers.

The least polar of the three isomers, isolated in small amounts, was assigned the Δ^8 structure (106) because its n.m.r. spectrum revealed the presence of two vinylic methyls

at τ 8.43 and 8.28 together with the absence of olefinic signals (other than H-14). The second minor product isolated was expected to be the Δ^7 olefin isomer (107) and this was duly confirmed by the n.m.r. signals at τ 8.29 (6H singlet, vinylic methyls) and τ 4.55 (broadened singlet, H-7 proton).

Acetate (105), the major product, was characterised only after hydrolysis to its parent alcohol. Thus treatment of (105) with sodium bicarbonate solution, as described previously for its cis counterpart (102), resulted in clean conversion to the trans allylic alcohol (193) - an apparently known compound which Ohloff[65] had obtained but had not rigorously established its double bond geometry. The optical rotation of (93), $[\alpha]_D$ +27.0°, agrees with the reported value of 33.2° and the n.m.r. [3H singlets at τ 9.33, 9.21, 9.15; 8.34 (vinylic methyl); τ 5.49, 5.18 (broad singlets, 1H); AX_2 spectrum J 7 Hz, at 4.62 (triplet) and 5.88 (doublet)] was in complete harmony with the structure.

Acid Cyclisation of the Labdadienols

The completion of the synthesis of the primary alcohols (92) and (93) meant that the four labdadienols were now available for cyclisation experiments. Despite the popularity of formic acid as a dehydration catalyst in the cases cited

earlier, it was decided to employ the acetic-sulphuric acid system favoured by Bory and Asselineau[57]. Thus manool was reacted for 3 hours at 50°C with acetic acid-sulphuric acid-water (83:7:10). After work up the crude products were found on t.l.c. to fall into three distinct types:- (a) a hydrocarbon fraction (60%) (b) a more polar fraction (30%) which was later shown to be acetates (c) the most polar fraction (10%) presumed to be hydroxylic material.

At this stage it was decided to concentrate on the hydrocarbon fraction, although the acetate fraction probably contains valuable, cyclisation products. Silver nitrate t.l.c. of the separated hydrocarbon fraction revealed that it consisted mainly of two very close spots in approximately equal amounts. More quantitative information came from the g.l.c. (three columns; SE30, P.E.G.A. and ApL) which showed that 95% of the hydrocarbon fraction consisted of two components in the ratio of 1.3:1 (the g.l.c. trace is shown in Figure 7a). The remaining 5% of the hydrocarbon fraction was distributed over at least five peaks.

Isolated by preparative silver nitrate t.l.c. the major, more polar component was a solid, m.p. 50°C, confirmed to be $C_{20}H_{32}$ by its mass spectral molecular weight 272 (from g.c.m.s.). The presence in its n.m.r. spectrum of four

quaternary methyl singlets at τ 9.17, 9.12, 9.05 (two) and a characteristic ABC spectrum of a vinylidene group (multiplet, 3H, τ 5.35-3.95) was conclusive proof that the compound was a tricyclic diene with the remaining double bond in a tetrasubstituted position in the ring (no other olefinic signals in the n.m.r.).

The minor of the two components (less polar on t.l.c. but greater retained on g.l.c.) was isolated as an oil, $C_{20}H_{32}$ (from the g.c.m.s. molecular weight 272). The n.m.r. spectrum had quaternary methyl singlets (3H) at τ 9.17, 9.12, 9.09, 9.06 and a vinylic group ABC multiplet (3H) from τ 5.3-3.9. This, coupled with the absence of any other olefinic resonance again established that the compound was a tricyclic diene with a tetrasubstituted double bond in the nucleus. Evidence that the two isolated components were possibly related as epimers came from their mass spectral fragmentation patterns which were practically identical, except for the minor intensity differences. Only the C-13 epimeric pimaradiene structures (87) and (88) or the rosadienes (111) and (112) can accommodate the spectral evidence.

Definite proof that the major diene component was, in fact, $\Delta^{8,15}$-isopimaradiene (87) came from its comparison with an authentic sample of (87), m.p. 52-53°C, prepared from

isopimaric acid (109) by the method of Ireland and co-workers.[66] The two samples were found to be indistinguishable in g.l.c. (three columns), silver nitrate t.l.c., n.m.r. mass spectral[83] properties. Similarly, the minor diene component was identified as the C-13 epimer, $\Delta^{8,15}$ pimaradiene (88) since it was found identical in all properties (g.l.c., t.l.c., n.m.r., mass spectrum) with an authentic sample of (88) prepared[66] from pimaric acid (110).

Manool, therefore, has been smoothly cyclised into a mixture (1.3:1) of $\Delta^{8,15}$ isopimaradiene (87) and $\Delta^{8,15}$ pimaradiene (88) upon short-time acid treatment. The high proportion (95%) of these two tricyclic compounds means that either the simple dehydration of manool to the bicyclic trienes, sclarene (72), cis-biformene (73) and trans-biformene (74) is not an important process or, more likely, the triene intermediates so formed are unstable to the reaction conditions and eventually cyclise via reprotonation. (The simple experimental check of this - acid treatment of the trienes - was not performed). Screening of the hydrocarbon products, by combined use of t.l.c., g.l.c. and g.c.m.s., did in fact reveal three small peaks identical to the trienes in g.l.c. and mass spectrum, but the amounts (less than 5%) were quite negligible.

In a continuation of the cyclisation studies, C-13 epimanool (91) was subjected to the same acetic-sulphuric acid conditions as manool (three hours at 50°C). Examination of the hydrocarbon fraction by g.l.c. and g.c.m.s. showed that it had exactly the same product composition as the reaction products from manool:- that is, (87) and (88) in the ratio 1.3:1. Similarly, the remaining two labdadienols (92) and (93) were individually treated with acetic-sulphuric acid for five hours at 50°C. After work up and isolation of the hydrocarbon fractions, a g.l.c. and g.c.m.s. examination revealed that the product composition from dehydration of the cis alcohol (92) was identical to that from the trans alcohol (93). Furthermore, the olefin products from the two primary alcohols were essentially the same as those from the two tertiary alcohols, again consisting mainly of (87) and (88) in the ratio 1.3:1. The minor differences between the products from the primary alcohol series and the tertiary series might be ascribed to the slightly different reaction time employed, (5 hours for the primary series, 3 hours for tertiary) since it was naively assumed that allylic primary hydroxyls would ionize more slowly than their allylic tertiary isomers.

Therefore, since all four labdadienols cyclise to give the same ratios of pimaradiene C-13 epimers, it follows that

cyclisation cannot be concerted with the formation of the cationic centre at C-13. Instead, a stepwise process must be operating with the first stage being the ionization to the stable allylic cation (69). The observed 1.3:1 ratio of the pimaradiene epimers (instead of a 1:1 ratio) is probably a reflection of the relative energies of the cyclisation transition states leading to the two epimers. On the assumption that the transition states resemble the final diene products in having a ring C chair conformation, the results would be consistent with expectation; for $\Delta^{8,15}$ pimaradiene (88), the minor reaction product, has an axial β vinyl group, as shown in conformation (114), and might be marginally less stable than its epimer (87) which has the smaller methyl group in the axial β position as shown in conformation (115).

Rearrangement of Pimaradienes to Rosadienes

Although no rosadiene structures resulted from the rearrangements discussed so far, this was not altogether surprising in view of the mild acid conditions employed. The next objective, therefore, was to find conditions which would transform the initially formed pimaradienes into the rosadiene system. Fortunately for our project, this should

be feasible on thermodynamic gounds. Thus, the pimaradiene skeleton, as shown in (116), has a large 1,3-diaxial interaction between the β oriented methyl groups at C-4 and C-10. However, the pimarane to rosane transformation requires a C-10 to C-9 methyl migration, so the release of strain from the 1,3 diaxial interaction should provide a driving force for the desired backbone rearrangement.

The basic C-10 to C-9 methyl shift has already been demonstrated to occur in the chemistry of the resin acids. Wenkert[67] and Le-Van-Thoi[68] showed that sulphuric acid treatment of (117) and (118), the Δ^8 isomers of dihydroisopimaric and dihydropimaric acid respectively, gave the γ lactones (119) and (120), (the 5β-H stereochemistry undoubtedly being derived from lactonisation of a 5,10 olefin intermediate). By acid opening of an 8,9 epoxide, Herz and Wahlborg[69] rearranged 8,9 epoxyabietanoic acid (121) to the 5,10 olefin (122). A reaction of this type has some potential for a synthesis of rosenonolactone, since the hydroxyl at C-8 should aid the introduction of a carbonyl group at C-7.

Nevertheless it was decided, as a first approach, to attempt the pimaradiene-rosadiene conversion merely by using a longer reaction time in the same acid solvent as before. Accordingly, manool (70) was reacted with acetic-sulphuric acid at 50°C and the hydrocarbon products analysed at varying

time intervals by means of t.l.c. and g.c.m.s. The initially formed pimaradienes were found to be slowly transformed into a more complex mixture of isomeric hydrocarbons, at least 8 peaks on g.l.c. after 150 hours. The analysis of the problem was made easier by isolating the two pimaradienes (87) and (88) from the 3 hour reaction and subjecting them individually to the same acid conditions. The recombined g.l.c. traces from both series did, in essence, reconstitute the original manool 150 hours reaction mixture.

Reaction of $\Delta^{8,15}$ isopimaradiene (87) for 150 hours as above gave a hydrocarbon fraction which showed four main products all of molecular weight 272 (g.c.m.s.). The g.l.c. trace is reproduced in Figure 7b. After considerable difficulty, the major product (50%) was isolated by preparative silver nitrate t.l.c. as an oil. The presence in its n.m.r. of four quaternary methyl singlets at τ 9.16, 9.07 (three) and a vinylidene multiplet (3H) from τ 5.3-3.9 was unambiguous proof that it must be the $\Delta^{5(10),15}$-rosadiene (111). As further confirmation, it was found to be indistinguishable (g.l.c., t.l.c., g.c.m.s.) from the component of shorter g.l.c. retention time obtained[70] when rimuene is exposed to moist chloroform-hydrogen chloride.

The longer retained of the two products of rimuene iso-

merisation has the same g.l.c. retention time as rimuene itself, but the mass spectrum (g.c.m.s.) indicated it to be mainly rimuene with at least one other component in admixture (perhaps a $\Delta^{1(10)}$ isomer). In fact, 'screening' of the isopimaradiene rearrangement products by g.c.m.s. revealed this very peak (10%), with identical g.l.c. and mass spectrum to 'impure rimuene'. Although not isolated, and identified only by g.c.m.s.; g.l.c., the presence of rimuene should be expected if there is an equilibrium mixture of 5,6; 5,10 and 1,10 double bond isomers.

With the isolation of the rosadiene (111) (50% g.l.c.) and the identification of rimuene (21) (10% on g.l.c.) from the C-13 β methyl series, attention was directed towards further transformations on $\Delta^{8,15}$-pimaradiene (88) which has the correct C-13 configuration required for rosenonolactone synthesis. On prolonged acetic-sulphuric acid treatment, 150 hours at 50°C, (88) gave a hydrocarbon fraction of four main peaks on g.l.c. (Figure 7c). One of the peaks, approximately 30% on g.l.c., was identified as the $\Delta^{5(10),15}$-rosadiene (112) since it was indistinguishable in g.l.c. (retention data for the pimara- and rosa- dienes are in Table 2) and g.c.m.s. from its antipode (113) obtained[71] from erythroxydiol Y. Again it was noted that (112) and (113)

had mass spectra almost identical with the epimer (111). Unfortunately all attempts to isolate the rosadiene (112) failed because its t.l.c. mobility was the same as that of the other major products in the mixture.

In summary, therefore, the four labdadienols - manool, epimanool, (92) and (93) - have been cyclised *in vitro* to give, firstly, the pimaradiene epimers (87) and (88), which were further transformed to, *inter alia*, the rosadienes (111), (21) and (112). Thus the way has been paved for a synthesis of rosenonolactone and its relatives. A consideration of the many unidentified rearrangement products forms a different part of this thesis.

Since the completion of this work other groups have reported successful *in vitro* cyclisation of manool:- Wenkert[72] and Edwards,[73] independently have cyclised manool with formic acid to obtain the pimaradiene epimeric mixture in the same ratio as observed here. Furthermore, Wenkert has extended the rearrangement to the rosadiene (111) and rimuene (21), by prolonged formic acid treatment of (87).

EXPERIMENTAL

All melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

Ultra-violet spectra (U.V.) were obtained on a Unicam S.P.800 recording spectrophotometer, using absolute ethanol as solvent.

Routine infra-red spectra (i.r.) were recorded on a Perkin-Elmer 257 spectrophotometer. Unless otherwise stated, the spectra were run as 0.03M carbon tetrachloride solutions. Only the strong or significant peaks are quoted. The high resolution infra-red spectra were recorded on a Unicam S.P.100 double-beam spectrophotometer.

Proton magnetic resonance spectra (n.m.r.) were determined at 60 MHz. on the Perkin-Elmer R.10 or Varian T.60 spectrometers, and at 100 MHz on the Varian H.A.100 instrument. All samples were run in deuterochloroform, with tetramethylsilane as an internal reference.

Gas-liquid chromatography (g.l.c.) was performed on Pye Argon and Perkin-Elmer F.11 chromatographs.

Gas-liquid chromatography mass spectral analyses (g.c.m.s.) of mixtures were carried out on the L.K.B. 9000 spectrometer.

Mass spectra were obtained with an A.E.I., M.S.12 spectrometer and high reolution spectra on the A.E.I., M.S.9 instrument.

Microanalyses were carried out by Mr. J.M.L. Cameron, B.Sc., and his staff.

Merck 'Kieselgel G' was used for both analytical and preparative thin layer chromatography (t.l.c.). Woelm alumina, deactivated to the appropriate Brockmann grade,[113] was employed for column chromatography.

Unless otherwise stated, 'light petroleum' refers to petroleum ether, b.p. 60-80°.

All solutions were dried over anhydrous sodium (or magnesium) sulphate.

Effect of phosphorous oxychloride on manool (70)

Manool (420 mg) was reacted for 24 hours at room temperature with a solution of freshly-distilled phosphorous oxychloride (3 ml) in dry pyridine (10 ml). After addition of ice water and extraction with ether, the ether extract was washed with dilute hydrochloric acid, water, brine and dried over magnesium sulphate. Removal of the solvent afforded a pale yellow oil (152 mg). Silver nitrate t.l.c. showed the oil to consist of three olefin isomers and g.l.c. (SE30; XE 60; ApL) revealed three peaks present in the ratio of 7:2:9 (g.l.c. order). These were identified[49] as sclarene (72), cis-biformene (73) and trans-biformene (74) respectively.

G.l.c. retention times:- (72) 21.6, (73) 27.8, (74) 31.0 min. relative to the n-alkanes, C-18, 12.7, C-19 21.2, C-20 35.6 min.

p-Nitrobenzoate of agathadiol (75)

Agathadiol was obtained, as described by Enzell,[50] from reduction of dimethyl agathate (76). The diol (447 mg) was reacted with p-nitrobenzoyl chloride (296 mg, 10% excess) in dry pyridine (5 ml) at 20°C for 3 days. Water was added and the product extracted into ether. The ether was washed

till neutral, dried and removed to yield an oily white solid which, after preparative t.l.c. furnished the following compounds:-

(a) Unreacted diol (186 mg).

(b) The 'wrong' monoester (78) (36 mg) with n.m.r. τ 9.26, 8.91, 8.31 (singlets, 3H); 5.83, 5.46 (AB system, 2H, J 11.5 Hz); 5.85 (doublet, 2H, J 7Hz); 4.60 (triplet, 1H, J 7Hz); 5.45, 5.14 (broad singlets, 1H each); 1.76 (singlet, 4H).

(c) The desired monoester (77) (101 mg) m.p. 107-110°C. n.m.r. τ 9.34, 9.05, 8.21 (singlets, 3H); 6.60, 6.29 (AB system, 2H, J 11.5Hz); 5.10 (doublet, 2H, J 7Hz); 4.53 (triplet, 1H, J 7Hz); 5.48, 5.16 (broad singlets, 1H each); 1.78 (singlet 4H). (Found: C, 71.01; H, 8.27; N, 3.27. $C_{27}H_{37}O_5N$ requires C, 71.18, H, 8.19; N, 3.07%).

(d) The least polar products (26 mg) m.p. 203-206°C is presumably diester (79). (Found: C, 66.91; H, 6.45. $C_{34}H_{40}O_8N_2$ requires C, 67.53; H, 6.67%).

Attempts to make the allylic p-toluenesulphonate or p-bromobenzenesulphonate of the diol, by the method analogous to above, failed - complex mixtures being formed in both cases.

Solvolysis of allylic p-nitrobenzoate (77)

Method A: The ester (77) (50 mg) was refluxed in acetone-water (9:1) for 2 weeks. After removal of solvent, the ester was shown by t.l.c. to be completely unchanged.

Method B: The ester (77) (160 mg) was shaken for 2 days in acetic acid (6 ml, anhydrous) buffered with fused sodium acetate (32 mg). Work up, by addition of water and extraction into ether which was washed with water, brine and dried, returned unchanged starting material. Acetolysis at 60°C for 20 hr. gave products which, after treatment with acetic anhydride and pyridine, were mainly agathadiol diacetate[50] (80), by n.m.r. and g.l.c. (1% SE30, 200°C) comparison of an authentic sample. Prolonged, 150 hrs., reaction gave rise to small amounts (10%) of three, more volatile components:- g.l.c. retention times, after acetylation, 12.7, 15.25, 16.9 minutes relative to diacetate (80) at 51.7 min. and C-22, C-26, C-27 n-alkanes at 10.7, 53.25, 77.5 min. respectively (1% SE30 190°C). Molecular Weights (g.c.m.s.) 330. i.e. $C_{22}H_{34}O_2$. Acetolysis at 100°C for 15 hours increased the proportion of these products to 20%.

Isomerisation-oxidation of sclareol (94)

Sclareol (10 g) was oxidised with chromic acid, according to the method of Stoll and Commarmont,[59] to give a crude green oil (7.25g, 72%). This oil was purified by dissolving in ether (8 ml) and ethanol (4 ml) then shaking for several hours with 40% aqueous sodium bisulphite solution (200 ml), after which the non-aldehydes were extracted into ether. The aldehydes were regenerated by shaking with 36% sodium hydroxide (200 ml) and extracted with ether. The ether was washed with water till neutral, brine, dried over anhydrous magnesium sulphate and the solvent removed *in vacuo* to give a yellow oil (5.5 g; 55%). I.r. (liq. film) 3500, 1670, 1630, 695 cm^{-1}. T.l.c. confirmed this to be a 1:1 mixture of cis- trans allylic aldehyde isomers (95).[60]

Reduction of allylic aldehydes (95)

To a stirred suspension of lithium aluminium hydride (900 mg, 40% excess) in anhydrous ether (15 ml), at -10°C, was slowly added the above aldehyde mixture (10 g) in anhydrous ether (15 ml). The suspension was stirred at this temperature for 10 minutes then worked up by careful addition of water till a white granular precipitate was obtained. Filtration and evaporation of the clear,

supernatant ether solution gave a yellow oil (9.25 g). Column chromatography on 400 g of neutral grade III alumina, eluting throughout with benzene:chloroform (1:1), yielded the mixture of isomeric diols (96) as a colourless oil (7.65 g, 76%). I.r. (liq. film) 3400 (OH), 1665 (C=C), 780 cm^{-1}. Reductions using a larger hydride excess still gave the same results, with little, undesired C=C reduction.

Separation of the diol isomers (96) as their acetates

The above diol mixture (7.65 g) was left overnight at room temperature in a solution of acetic anhydride (15 ml) and dry pyridine (15 ml). Water was added and the aqueous phase extracted with ether. The ether was washed with dilute hydrochloric acid, water till neutral, brine and dried. Removal of solvent gave a pale yellow oil (7.67 g, 96%). I.r. (liq. film) 1735, 1245 (acetate), 3500-3550 (OH), 1665 cm^{-1} (C=C). This was shown by t.l.c. to consist mainly of the expected acetates (97) and (98). Column chromatography of the mixture on 500 g of neutral grade III alumina, using petroleum ether-benzene gradient elution, gave the more mobile cis isomer (97) (1.16 g) as an oil, b.p. approx. 170°C/ 0.03 mm Hg; i.r. (0.002M in CCl_4) 3608, 3557; 1739, 1237 cm^{-1}. G.l.c. (1% SE30 175°C) showed only one pure peak: n.m.r. 3H

singlets at τ 9.22 (two), 9.13, 8.86, 8.22 (vinyl CH_3), 7.99; τ 8.30 (singlet,1H, removed on D_2O exchange), 4.70 (triplet,1H, becomes broadened singlet on irradiation at τ 5.35), 5.3-5.6 (symmetric 8 line multiplet,2H, becomes a broadened AB quartet on irradiation at τ 4.70). The above two signal regions, both of which sharpen on irradiation at τ 8.22, analyse quite well for an ABX system with approximate values:- $J_{AX} = J_{BX} = 7Hz$, $J_{AB} = 13Hz$, $\delta AB = 20Hz$ (at 100 M Hz). (Found: C, 75.18; H, 10.82. $C_{22}H_{38}O_3$ requires C, 75.38; H, 10.93%).

The less mobile trans isomer (98) was eluted as an oil (930 mg), b.p. approx 200°C/0.04 mm Hg; i.r. (0.002 M in CCl_4) 3614, 3544 with shoulder at 3559; 1742, 1234 cm^{-1}. N.m.r. 3H singlets at τ 9.22 (two) 9.14, 8.88, 8.30 (vinyl CH_3), 7.98; τ 5.43 (doublet,2H, J 7Hz, becomes a broadened singlet on double irradiation at τ 4.65), 4.65 (triplet,1H, J 7Hz, becomes a broadened singlet on irradiation at τ 5.43). (Found: C, 74.94; H, 10.83. $C_{22}H_{38}O_3$ requires C, 75.38; H, 10.93%).

The argument, based on n.m.r. evidence, for the assigned stereochemistry of acetates (97) and (98) is discussed elsewhere.

Hydrolysis of the acetate (97)

The acetate (10 mg) was treated with 5% ethanolic potassium hydroxide at room temperature for 24 hours. Work up was by removal *in vacuo* of the bulk of the ethanol, addition of water and extraction into ether. The ether solution was dried and the solvent removed to give the oily diol (99) (9 mg), shown to be one pure compound on t.l.c. This known compound[62] is reported as an oil.

Hydrolysis of the acetate (98)

Treatment of this acetate, as above, gave the crystalline diol (100) (8 mg), m.p. 127°C. The literature value[62] 129°C is further proof of the correct stereochemical assignment of the two above acetates.

Dehydration of the acetate (97)

The acetate (1.1 g) was kept in a mixture of freshly distilled phosphorous oxychloride (3 ml.) and dry pyridine (5 ml) for 24 hours at 20°C. Water was added and extraction into ether, followed by the usual work up, gave an oil (900 mg) which was homogeneous on ordinary t.l.c. but was shown, on Silver Nitrate-Silica, to comprise of a major component (85%) and two, more mobile, olefin isomers. N.m.r.

of the crude mixture indicated it to be predominantly exo-methylene:- 3H singlets at τ 9.32, 9.20, 9.14, 8.25, 7.98; τ 4.65 (triplet,1H, J 7Hz), 5.15, 5.44 (broad singlets,1H each), 5.50 (doublet,2H). Preparative t.l.c. on 15% Silver Nitrate-Silica afforded the most mobile isomer, with probable structure (103) from its n.m.r. 3H singlets at τ 9.17, 9.12, 9.06; 8.40, 8.20, 7.98; τ 5.41 (doublet, 2H, J 7Hz), 4.64 (triplet, 1H, J 7Hz). Small amounts of the other minor isomer, presumably (104) were isolated. N.m.r. 3H singlets at τ 9.26, 9.13 (two), 8.27, 8.22, 7.98; τ 5.43 (doublet, 2H, J 7Hz), 4.64 (triplet, 1H, J 7Hz) superimposed on 4.58 (broad singlet,1H).

The major product isolated was the exomethylene isomer (102), characterised below by hydrolysis to the parent alcohol.

Hydrolysis of the acetate (102)

The allylic acetate (102) was left overnight at 20°C in a saturated solution of solid sodium bicarbonate in 1% aqueous methanol (10 ml). The methanol was removed, water added and the aqueous phase extracted with chloroform. Normal processing of the chloroform extract gave the cis allylic alcohol (92) as an oil (44 mg) $[\alpha]_D$ + 5.1°C (C = 1.47 $CHCl_3$) n.m.r. 3H singlets at τ 9.33, 9.21, 9.15, 8.28; τ 8.4 (broad,

1H, lost on D_2O exchange), 5.96 (doublet, 2H, J 7Hz), 5.45, 5.14 (broad singlets, 1H each), 4.59 (triplet,1H, J 7Hz). (Found: C, 82.41; H, 11.83. $C_{20}H_{34}O$ requires C, 82.69; H, 11.80%).

Dehydration of the acetate (98)

Dehydration of this acetate (900 mg) as described before gave an oil (600 mg) whose n.m.r. showed considerable exo-methylene character:- 3H singlets at τ 9.31, 9.19, 9.12, 8.29, 7.94; τ 5.42 (doublet, 2H, J 7Hz); 5.49, 5.18 (broad singlets, 1H each), 4.67 (triplet, 1H, J 7Hz).

T.l.c. separation gave the minor products (106) [n.m.r. 3H singlets at τ 9.17, 9.12, 9.06, 8.43, 8.28, 7.96; τ 5.40 (doublet,2H, H 7Hz), 4.62 (triplet, 1H, J 7Hz)] and (107) [n.m.r. 3H singlets at τ 9.24, 9.12 (two), 8.29 (two), 7.96; τ 5.41 (doublet, 2H, J 7Hz), 4.62 (triplet, 1H, J 7Hz), 4.55 (singlet, 1H)].

The major dehydration product (105), [g.l.c. retention time 16.4 min. relative to cis acetate (102) 15.4 min. on 1% SE30 column at 190°C] was about 85% of the mixture. It was characterised after hydrolysis to the alcohol.

Hydrolysis of the acetate (105)

Hydrolysis of the allylic acetate (70 mg), as before, gave the known allylic alcohol (93) as an oil (66 mg). $[\alpha]_D$ +27.0 (C=1.15 $CHCl_3$) (Lit. value[65] + 33.2°). N.m.r. 3H singlets at τ 9.33, 9.21, 9.15, 8.34; τ 5.88 (doublet, 2H, J 7Hz); 5.49, 5.18 (broad singlets, 1H each), 4.62 (triplet, 1H, J 7Hz).

Acid isomerisation of rimuene (21)

Rimuene (2 mg) was kept for 24 hours at 20°C in a chloroform-hydrogen chloride solution (5 ml), prepared by bubbling hydrogen chloride gas into moist chloroform for a few minutes. After dilution with more chloroform, the solution was washed with water till neutral, dried and evaporated. G.l.c. (1% SE30, 140°C) showed two peaks:- 5,10 isomer (111)[70] 12.2 min and 'impure rimuene'[70] at 13.3 min. with C-18, C-19, C-20 n-alkanes at 10, 16.3, 26.8 min. and rimuene at 13.3 min.

Acid-catalysed cyclisation of manool (70)

(a) Short reaction time:- Manool ($[\alpha]_D$ +29°, homogeneous on g.l.c.) (2 g) was treated for 3 hours at 50°C with a solution of acetic acid - water - sulphuric acid (83:10:7, by volumne).[57] Water was added and the products extracted with ether. The ether extract was washed with water till neutral, brine, dried and the solvent removed to give a brown oil which, on t.l.c., was found to contain hydrocarbons (60%), acetates (30%) and some hydroxylic material (10%). The isolated hydrocarbon fraction (1.1 g) was shown on g.l.c. (1% SE30, 5% ApL, 10% P.E.G.A. columns) and t.l.c. ($AgNO_3$-SiO_2) to consist of at least 95% of two compounds, in the ratio of 1.3:1 as shown in Figura 7a.

Preparative t.l.c. on 20% $AgNO_3$-SiO_2 plates gave the major, more polar component as a solid, m.p. 50°C (from methanol); M (from g.c.m.s.) 272 i.e. $C_{20}H_{32}$. N.m.r. 3H singlets at τ 9.17, 9.12, 9.05 (two); 5.35-3.95 (multiplet, 3H, vinyl ABC system). This was shown to be the $\Delta^{8,15}$-isopimaradiene (87) since it was identical in g.l.c., t.l.c., n.m.r. and Mass. Spec. (g.c.m.s.) with an authentic sample prepared[66] from isopimaric acid (109).

The less polar product, but with greater g.l.c. retention time, was an oil, M (g.c.m.s.) 272, n.m.r. τ 9.17, 9.12, 9.09,

9.06 (singlets,3H); 5.3-3.9 (multiplet,3H). This was identical by g.l.c., t.l.c., n.m.r. and Mass Spec. with an authentic sample of $\Delta^{8,15}$-pimaradiene (88), prepared[66] from pimaric acid (110).

Of the remaining small peaks on g.l.c. (at least 5, totalling < 5%), three had the same retention times as the known bicyclic trienes, cis-biformene (73), trans-biformene (74) and sclarene (72) prepared[49] by dehydration of manool.

(b) Prolonged Reaction time:- The above reaction was allowed to continue and its progress was monitored by g.l.c. at regular intervals. The two pimaradiene C-13 epimers were found to be slowly transformed into a complex mixture of at least 8 peaks. After 150 hours, a major, early running peak was observed with identical g.l.c. and Mass Spec. (g.c.m.s.) properties to the previously described rosadiene (111).

Prolonged acid treatment of $\Delta^{8,15}$-isopimaradiene (87)

The diene (87) (200 mg), from the above 3 hour experiment, gave 4 major hydrocarbon products (Figure 7b) of molecular weight 272 when subjected to 150 hours of acetic-sulphuric acid treatment. The major product (50%), isolated by preparative t.l.c. (20% $AgNO_3$-SiO_2) as an oil (40 mg), had

n.m.r. singlets at τ 9.16 (3H), 9.07 (9H); 5.3-3.9 (vinylic multiplet, 3H). It was identical in g.l.c., t.l.c. and mass spectrum with the rosadiene (111) prepared from isomerisation of rimuene.

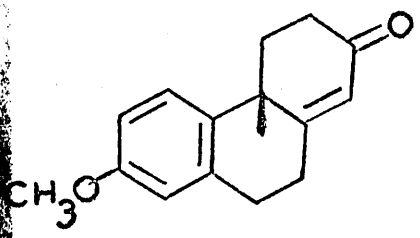
A minor product (10%), not isolated, had identical g.l.c. and g.c.m.s. to the peak of longer retention time when rimuene (21) was isomerised as already described.

The rosadiene (111) could also be isolated directly from the prolonged rearrangement of manool, without recourse to separating the initially formed pimaradiene C-13 epimers (87) and (88).

Prolonged acid treatment of $\Delta^{8,15}$-pimaradiene (88)

The hydrocarbon fraction from the 150 hour acid treatment of diene (88) contained at least 4 g.l.c. peaks (Figure 7c). One of the peaks (30%) was the rosadiene epimer (112), indistinguishable on g.l.c. and g.c.m.s. from its antipode (113), obtained[71] from erythroxydiol Y. The diene (112) could not be isolated as its t.l.c. mobility was too similar to the other products in the mixture.

Combination of the g.l.c. trace of these rearrangement products with the trace of the products from epimer (87) gave a good reproduction of the 150 hour, total rearrange-



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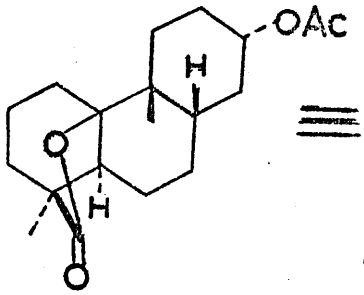

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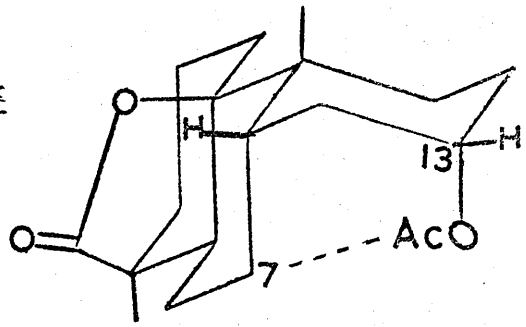

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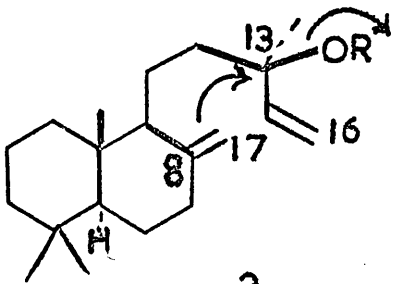
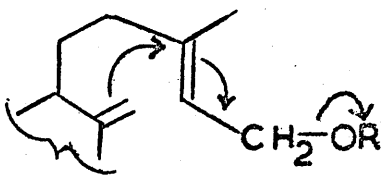
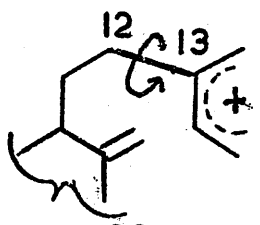


67 R = $P_2O_4^{3-}$
70 R = H
71 R = $POCl_2$



68 R = $P_2O_4^{3-}$



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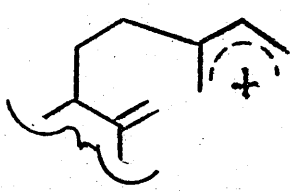

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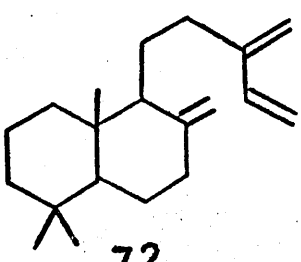

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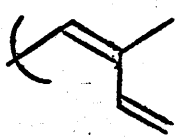

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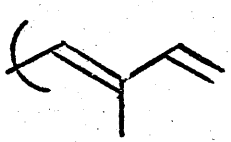

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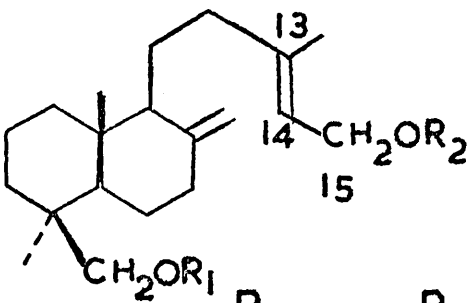
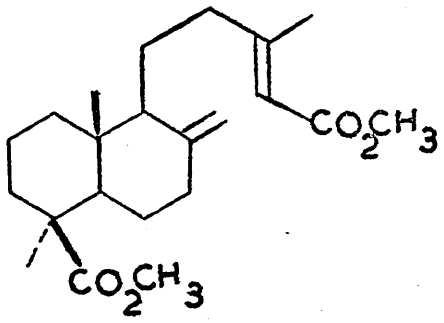


	R_1	R_2
75	H	H
77	H	$OC.C_6H_4NO_2$-P
78	$OC.C_6H_4NO_2$-P	H
79	$OC.C_6H_4NO_2$-P	$OC.C_6H_4NO_2$-P
80	Ac	Ac



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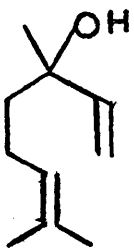

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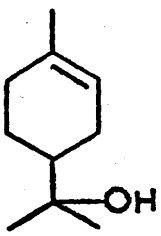

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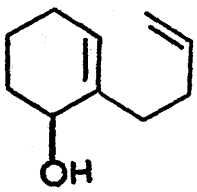

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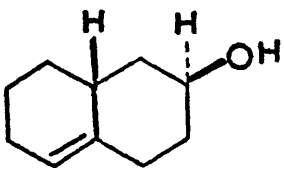

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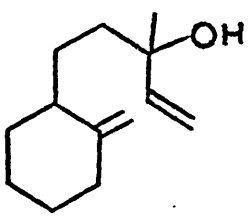

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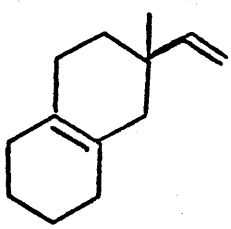

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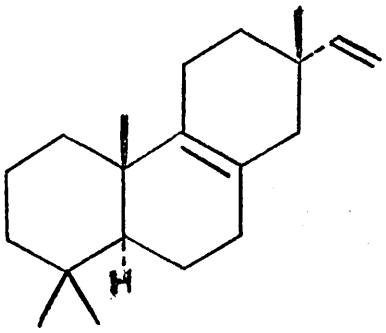

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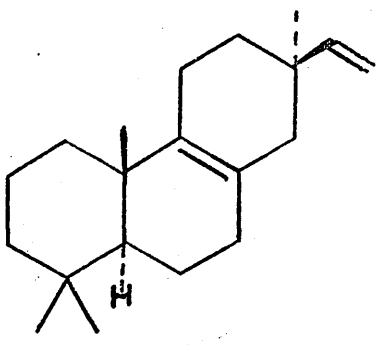

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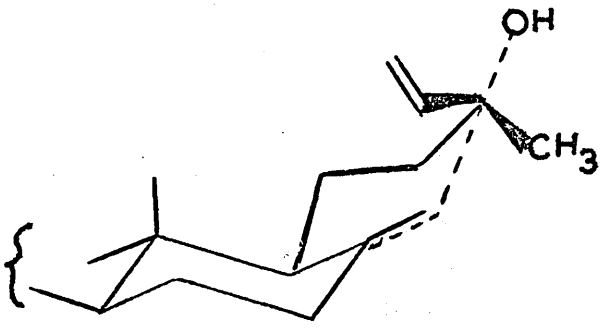

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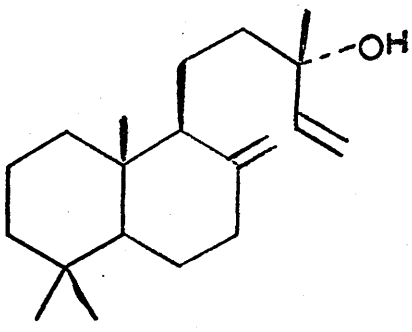

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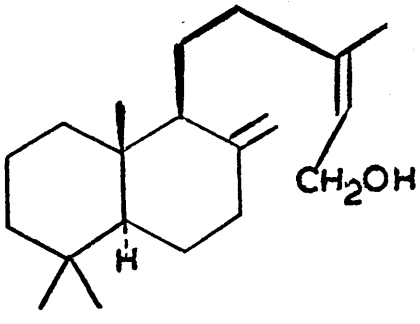

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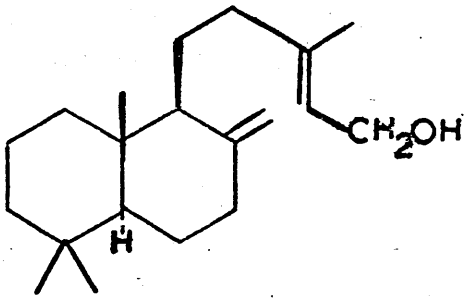

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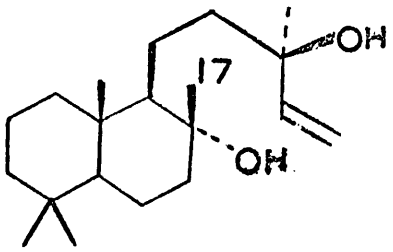

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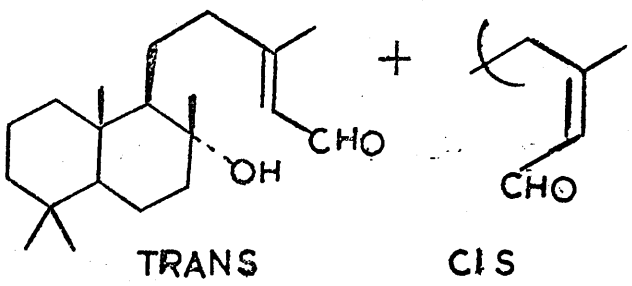
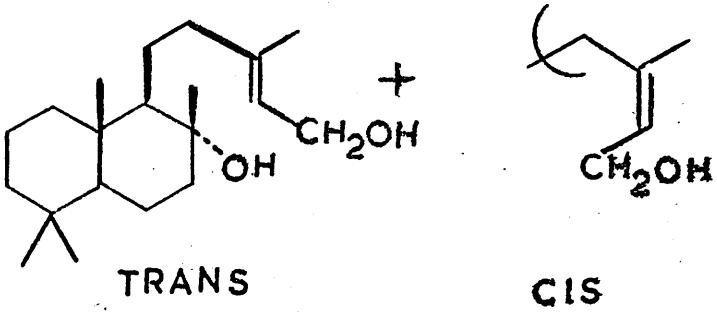


TRANS CIS

95



TRANS CIS

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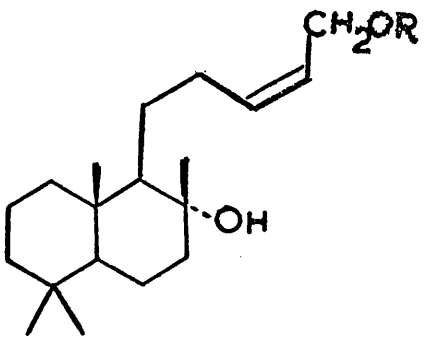
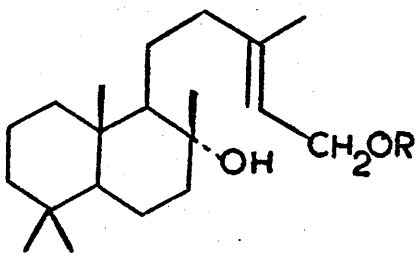
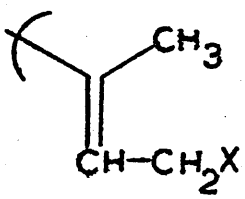


97 R= Ac
99 R= H



98 R = Ac
100 R= H



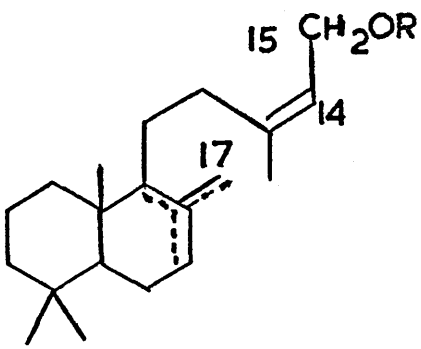
101

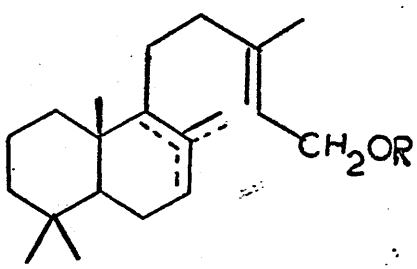
TABLE 1

Chemical Shift (τ) of Vinylic CH_3

		cis C=C	trans C=C
	97	8.22	
	98		8.30
	102*	8.25	
	104*		8.29
Agathadiol diacetate	80		8.31
	92	8.28	
	93		8.34
Agathadiol	75		8.35

*approx. 85% pure





102 R=Ac, $\Delta^{8(17)}$

103 R=Ac, Δ^{8}

104 R=Ac, Δ^{7}

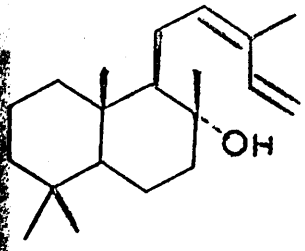
92 R=H, $\Delta^{8(17)}$

105 R=Ac, $\Delta^{8(17)}$

106 R=Ac, Δ^{8}

107 R=Ac, Δ^{7}

93 R=H, $\Delta^{8(17)}$



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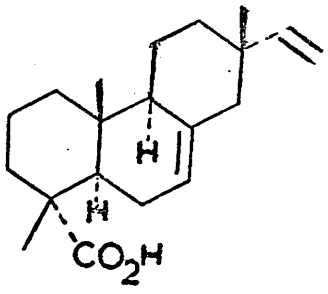

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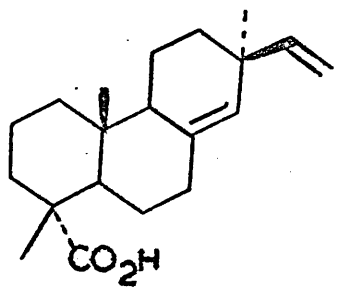

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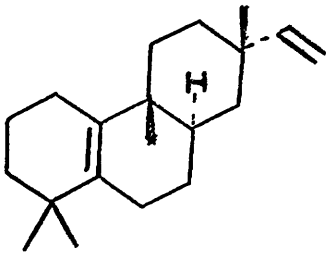
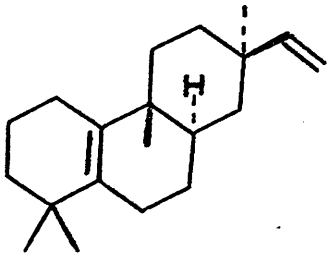


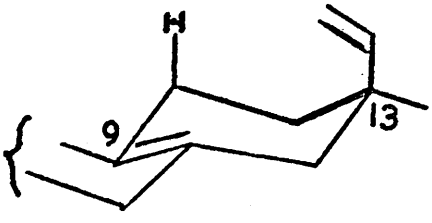
111

21 Δ^5 ISOMER



112

113 ANTIPODE



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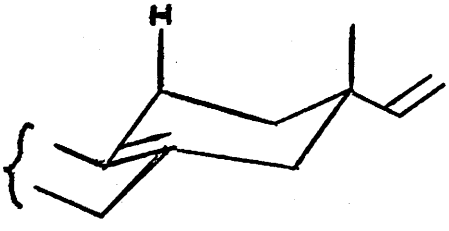

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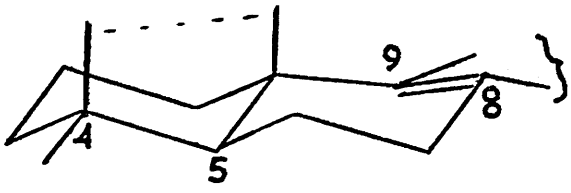

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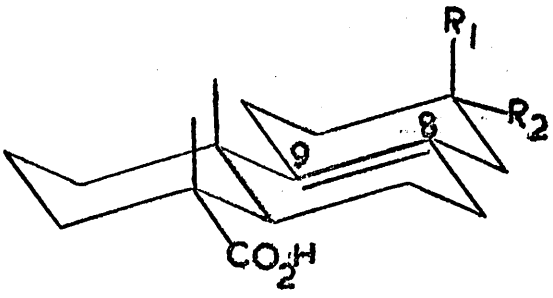
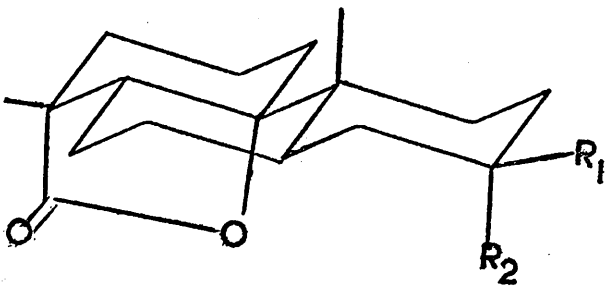


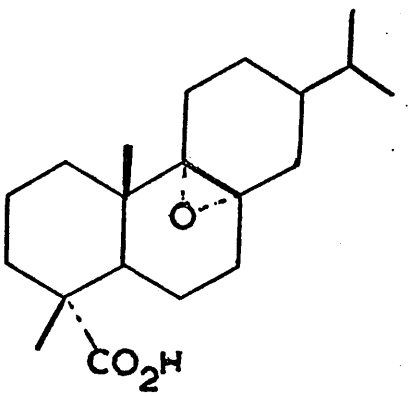


117 R_1=Me, R_2=Et

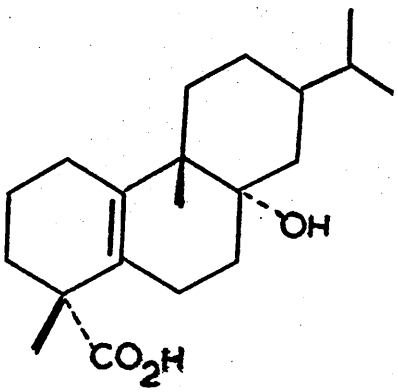
118 R_1=Et, R_2=Me

119 R_1=Me R_2=Et

120 R_1=Et, R_2=Me



121



122

FIG 7

GLC TRACES (1% SE 30 140°C)

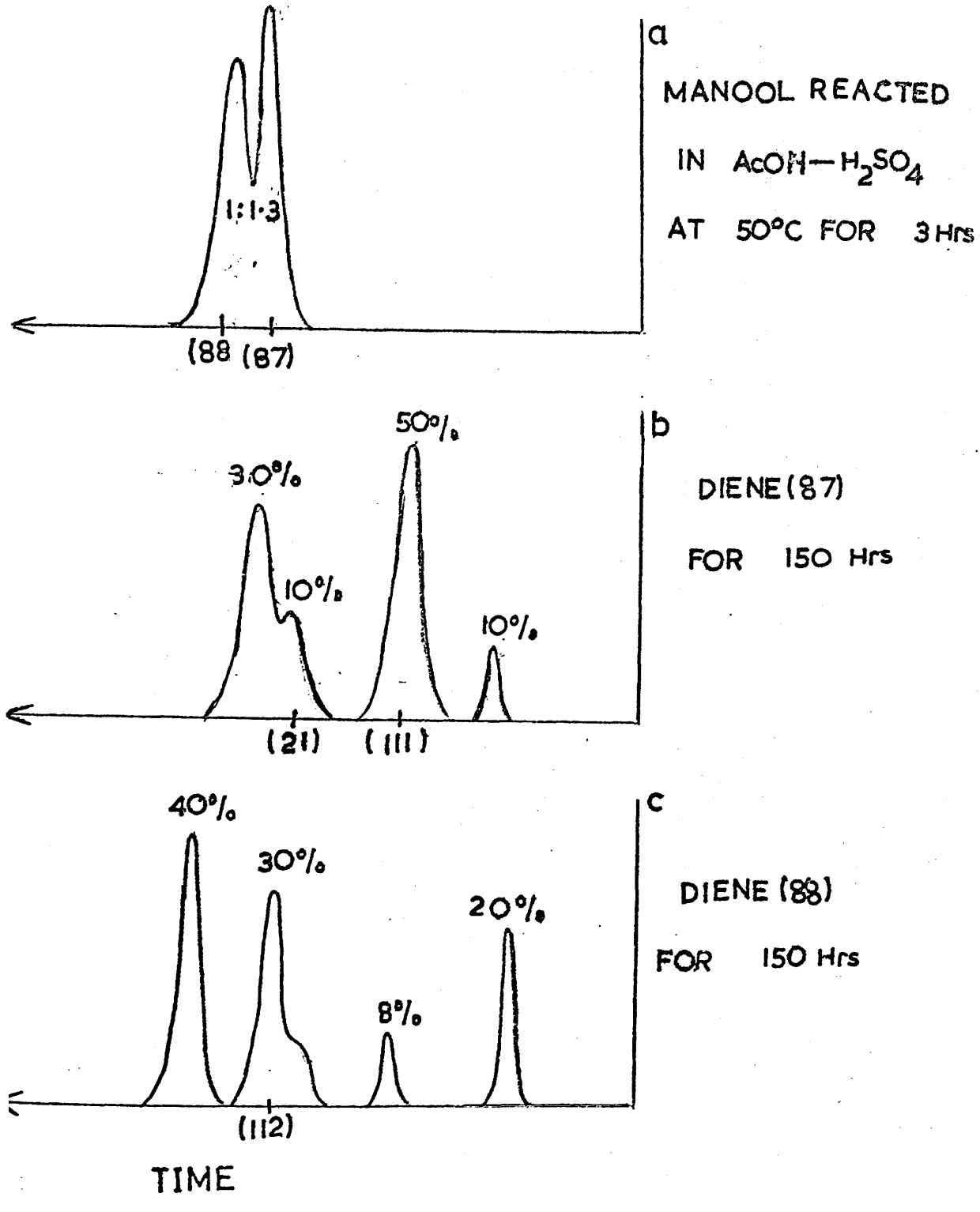


TABLE 2

Relative G.L.C. Retention Times

		1% SE30 140°C	10% P.E.G.A. 140°C
Isopimaradiene	(203)	506	287
Isopimaradiene Δ^8 isomer	(87)	365	161
Pimaradiene	(208)	416	190
Pimaradiene Δ^8 isomer	(88)	402	186
The rosadiene	(111)	310	133
Rimuene	(21)	338	154
ent-rosadiene	(113)	358	150
Dolabradiene	(176)	457	288
n $C_{18}H_{38}$		257	030
n $C_{19}H_{40}$		415	
n $C_{20}H_{42}$		673	072
Stachene	(184)	390	
iso-neoatisirene	(197)	417	
isophyllocladene	(195)	442	
Isokaurene(186), isoatisirene	(188)	486	
neoatisirene	(196)	532	
phyllocladene	(194)	535	
kaurene (195), atisirene	(187)	570	
From Fig. 16:- Peak A		289	
B		320	
C		352	
D		393	
E	(217)	433	

ment mixture from manool. The g.l.c. data for all the pimaradiene and rosadienes are given in Table 2.

Acid catalysed cyclisation of C-13 epimanool (91)

C-13 epimanool ($[\alpha]_D$ + 52.4°, Lit. value[58] + 51°) was exposed to the same acid conditions as for manool. The hydrocarbon products after 3 hours had g.l.c. and g.c.m.s. composition identical with the products from the corresponding reaction on manool; the products of further reaction were also identical.

Acid catalysed cyclisation of primary alcohol (92)

The alcohol (92) (15 mg) was kept at 50°C in the acetic sulphuric acid solvent (3 ml) for 5 hours. The g.l.c. composition of the isolated hydrocarbon fraction (7 mg) again was essentially the same as the early cyclisation products of manool, i.e. mainly dienes (87) and (88) in the ratio of 1.3:1.

Acid catalysed cyclisation of primary alcohol (93)

Upon similar acid treatment, allylic alcohol (93) gave products identical with those of its cis isomer (92).

3. SYNTHESIS OF ROSENONOLACTONE AND DEOXYROSENONOLACTONE

Discussion

Having established from studies in the model series that labdadienols can be rearranged into rosadienes, it is evident that this transformation can form the basis of a biogenetic-type synthesis of the rosane metabolites, if due provision is made for the introduction of the oxygen functionality, in particular, the γ lactone group. The most logical way to allow for the eventual formation of a lactone is to start with a labdane system which is oxygenated at C-19. With this approach in mind, the chosen starting point for the syntheses was isocupressic acid (128). Assuming the labdane to rosane rearrangement on this new substrate proceeds as before, then the rosa-5(10), 15-diene-19-oic acid (134) should be the end product and its β axial carboxylic group at C-4 has the potential for lactonisation on to the 5,10 double bond. The first stage of the work, therefore, concerns the transformation of isocupressic acid into the rosadienoic acid (134).

Although isocupressic acid has been found by Mangoni and Bellardini[74] to occur naturally in the resin of Cupressus sempervirens, it was found much more expedient to prepare it

from agathic acid (123) which is present in abundance (45%) among the resin acids from Manila copal. Two main procedures had previously been employed to isolate agathic acid from the copal:- Ruzicka and Hosking[75] (who first obtained the acid) and, later, Enzell[50] used a method which involved a series of selective extractions with ammonium carbonate, to obtain the acid in 13% yield. More recently Carman[76] isolated the acid by chromatography of the resin acids themselves on a charcoal-kieselguhr column, using methanol as eluant. In our hands, however, both these methods proved less than satisfactory, so it was decided to isolate agathic acid as its dimethyl ester (124).

Accordingly, finely powdered Manila copal (the acid fraction accounts for about 95% of the resin) was methylated with diazomethane. A t.l.c. and g.l.c. (SE30) examination of the resultant yellow oil revealed the presence of one major component (approx. 45%). Column chromatography on neutral alumina, with benzene-petroleum ether gradient elution afforded the major component as an oil, molecular weight 362 (mass spectrum), confirmed to be dimethyl agathate (124)[50] by its i.r. absorption (liquid film): 1645 (C=C), 1730-1710 cm^{-1} (broad, strong, C=O) and its n.m.r. spectrum: 3H singlets at τ 9.48, 8.82; 7.85 (vinyl CH_3); 6.39, 6.32 (OCH_3);

broadened singlets (1H) at τ 5.50, 5.12 (exomethylene) and 4.36 (H-14).

Dimethyl agathate (124) can, in principle, be related to methyl isocupressate (127) by reduction of the side chain methyl ester grouping to a primary hydroxyl. However, this innocuous single step proved surprisingly troublesome, the root cause of the difficulty being the presence of two methyl ester groups in the same molecule. A method was therefore required which would selectively reduce the C-15 side chain ester and leave the C-19 ester unaffected. Our stratagem was to capitalise on the greater steric hindrance to reaction at the C-19 carbonyl centre, on the reasoning that the C-19 carboxymethyl is in a fairly congested environment, (due mainly to the 1,3 diaxial interaction with the methyl group on C-10), and should react more slowly than the competing C-15 carboxymethyl in the side chain. Experimental vindication for these views comes from the previous observations that agathadiol (75) formed a p-nitrobenzoate four times faster at the side chain site than at C-19.

Our first reagent for selective reduction was lithium aluminium hydride (LAH). Enzell[50] has, in fact, fully reduced dimethyl agathate (124) to agathadiol (75) with an excess of this reagent; an indication, perhaps, that the

hydride species is 'too small' to achieve much selectivity. Nevertheless, it was felt worthwhile to repeat this reduction using the bare minimum of LAH. Thus, the diester (124) was reacted at room temperature for 24 hours with a 25% excess of LAH (based on one ester group). Alas, the products were shown by t.l.c. to be unreacted diester (70%) agathadiol (15%) and several other products of intermediate polarity.

In an effort to take greater advantage of the steric hindrance factor in reduction, the next reagent used was the bulkier lithium tri-t-butoxyaluminohydride (LTBA), a reagent which is generally unreactive to esters.[77] Dimethyl agathate was refluxed for 40 hrs in tetrahydrofuran with a large excess of LTBA. The major product, isolated as an oil by preparative t.l.c., was indeed a hydroxy ester, but it was identified as (129), the 13,14 dihydro compound which had previously been obtained[78] by Bouveault-Blanc reduction of dimethyl agathate. The assignment of this structure came from n.m.r. where the vinylic methyl (C-16), the H-14 olefin signal and one of the $-OCH_3$ singlets had disappeared, to be replaced by signals at τ 9.11 (3H doublet, J 6 Hz, C-16 methyl); 6.40 (2H triplet, J 7 Hz, C-15 methylene). Hence, the selective reduction of the side chain ester group was accomplished but was marred by the undesired reduction of the α,β C=C bond.

Rather than pursue the above promising approach by varying the reaction parameters or the nature of the hydride species, it was decided to attempt the reduction by a two-stage process:- Firstly, the side chain ester group would be selectively hydrolysed to an acid function which, after conversion to an activated derivative, could then be reduced to the alcohol by reagents known to be 'safe' for esters. The argument for the likelihood of selective hydrolysis is based on the same steric grounds as before, but in this case there is ample literature evidence that axial C-19 carboxymethyl groups in diterpenoids with axial methyls at C-10 are exceedingly inert to base. Methyl isocupressate (127), for example, was found to be unchanged after 6 hours reflux with ethanolic potassium hydroxide[74] and methyl podocarpate (148)[79] required 'Wolff-Kishner conditions' for hydrolysis. With these reassurances, therefore, dimethyl agathate was refluxed for 2 hours with 5% ethanolic potassium hydroxide. The resultant acid fraction was almost homogeneous on t.l.c. Further purification by preparative t.l.c. afforded methyl agathate (125) as an oil $C_{21}H_{32}O_4$. Assigment of structure came from the i.r. bands at 1730 (ester); 3600-2500, 1693, 1640 cm^{-1} (α,β unsaturated acid) and from the n.m.r. spectrum, where one methoxyl singlet (3H) had been replaced by a carboxyl

proton signal at τ 0.53 (broad singlet), the rest of the spectrum being similar to dimethyl agathate.

Reaction of this ester-acid (125) with triethylamine and ethyl chloroformate at -10°C in tetrahydrofuran furnished a crude yellow oil, one major spot on t.l.c. From its i.r. absoprtion (liquid film) at 1795 (shoulder 1775), 1720, 1635 cm^{-1} this compound was confirmed to be mainly the mixed anhydride (126).

Without further purification or characterisation, the above anhydride was reacted for one hour at 20°C with a large excess of sodium borohydride in ethanol. The major product, isolated as an oil from column chromatography on basic alumina, had i.r. absorption indicative of an unsaturated hydroxy ester; 3620, 1725, 3075, 1645 cm^{-1}. It was established to be methyl isocupressate (127) from comparison of its n.m.r. spectrum with that of an authentic specimen.[74]* Slightly better yields were obtained when the crude reaction mixture containing the anhydride was reacted with borohydride *in situ*,[80] no doubt due to the avoidance of competing ethanolysis reactions.

The successful selective reduction of the carboxyl group via the mixed anhydride meant that methyl isocupressate

* Sample kindly supplied by Professor Mangoni

was now freely available from dimethyl agathate (30% overall yield). If desired, methyl isocupressate could probably be hydrolysed to the parent acid (128) by the lithium-ammonia method of Wenkert and Jackson.[81] However, it was much more convenient to carry out the cyclisation-rearrangement studies on the ester itself since the products will be sufficiently volatile and non-polar to allow g.l.c. and t.l.c. examination. Furthermore, once the final C-10 to C-9 methyl shift is accomplished, giving the $\Delta^{5(10)15}$-rosadiene ester (133), then hydrolysis of the carboxymethyl ceases to become a problem as the 1,3 diaxial interaction no longer exists.

Acid Cyclisation of Methyl Isocupressate (127)

The conditions employed were the same as those used to cyclise the model labdadienols to pimaradienes. Thus, methyl isocupressate was reacted for 3 hours at 50°C with acetic-sulphuric acid.[57] Isolated by t.l.c., the olefinic-ester fraction, was revealed by silver nitrate t.l.c. and g.l.c. (SE30; P.E.G.A.) to consist almost entirely of two components in the same 1.3:1 ratio as had been observed with pimaradienes (87) and (88). After considerable difficulty the two products were separated by silver nitrate, t.l.c. The major product, molecular weight 316 ($C_{21}H_{32}O_2$); was established as a tricyclic

methyl ester from the presence in its n.m.r. spectrum of singlets (3H) at τ 9.22, 9.02, 8.80; 6.36 (OCH_3) and a vinyl multiplet (3H) from τ 5.4-3.8. By analogy with the results in the hydrocarbon model series, this major product must have the isopimaradiene C-13 configuration (87) and was therefore formulated as (130). Moreover, as in the model series, it had the shorter g.l.c. retention time of the two epimeric products. The close similarity of the n.m.r. vinyl signal pattern for (87) and (130) further substantiated the above configurational assignment.

The less volatile, minor product (molecular weight 316) was another tricyclic hydrocarbon, as evidenced by its n.m.r.; τ 9.21, 9.07, 8.80, 6.37 (singlets 3H); 5.3-3.7 (vinyl multiplet, 3H). Equivalence of its mass spectral cracking pattern with that of (130) and the close similarity of its n.m.r. vinyl signal pattern with that of its hydrocarbon analogue (88), established beyond doubt that it was the C-13 epimer (131). It was of interest to note that the mass spectral cracking patterns of (130) and (131) were broadly similar to but not identical with those of their C-4 epimers (144) and (146). These were prepared from isopimaric acid (145) and pimaric acid (147) respectively, according to Edwards and Howe.[84]

At this early stage in the synthesis, therefore, the problem of getting the correct rosenonolactone C-13 configuration was overcome by selection of the pimaradiene ester (131) for subsequent transformations.

Further Rearrangement of the Pimaradiene-esters

Experience with the model hydrocarbon series had shown that pimaradienes were converted to rosadienes under more forcing acid conditions. Before embarking on the same rearrangements in the oxygenated compounds, it was decided to have available for comparative purposes, an authentic sample of the rosadiene-ester (133), the hoped-for end product of the rearrangement. This was prepared as follows:- Deoxyrosenonolactone (136) was refluxed for four hours with ethanolic hydrochloric acid[28] to yield the known diene-acid (134),[82] m.p. 138-140°C. Methylation of the acid with diazomethane gave the diene-ester (133), molecular weight 316 from g.c.m.s. ($C_{21}H_{32}O_2$), n.m.r. signals at τ 9.09, 8.97, 8.76, 6.38 (3H singlets); 5.4-3.9 (vinyl, 3H).

Using (133) as a reference compound, an exploratory rearrangement study (acetic-sulphuric acid, 60°C) was carried out on the pimaradiene ester (131). The course of the reaction

was monitored by analysis of the products (g.l.c., g.c.m.s., silver nitrate t.l.c.) after 20, 40 and 85 hours. To our dismay, a very complex mixture was formed from which very little of (133) could be detected, despite exhaustive 'screening' on several g.l.c. columns.

The failure to achieve the desired transformation to (133) was somewhat perplexing in view of our success in the model series. In order, therefore, to exonerate the acid solvent used, the 'wrong' pimaradiene ester (130) was subjected to the same acid treatment. Although a complex mixture was also formed in this epimeric series, the rosadiene ester (132) (identified on later isolation) was indeed found present in 45% yield (g.l.c) after an optimum period of 40 hours. At this stage of reaction there were at least 10 other unknown peaks, of which 3 were quite substantial; prolonging the reaction past this point only caused a decrease in the proportion of (132).

With the realisation that the acetic-sulphuric acid system, which had served us well in the model reactions, would permit the preparation only of the rosadiene ester having the wrong C-13 configuration (132), a different acid system was now sought. The one finally chosen was formic acid-chloro-

form (1:1) which Wenkert has employed with considerable success.[72] Accordingly, the individual pimaradiene esters (130) and (131) were refluxed in formic acid-chloroform, the course of the two reactions being monitored by removal of aliquots after 20, 40 and 85 hrs.for examination by silver nitrate t.l.c., g.l.c. and g.c.m.s.

In the 'wrong' series, the diene (130) was observed to be cleanly transformed into its rosane isomer (132), reaching 70% conversion (g.l.c.) after 85 hours. At this 85 hr. stage the remaining products were mainly unreacted (130) (5%) and an unknown component (20%) which was not pursued.

Success was also obtained in the 'rosenonolactone' series. After 85 hours formic acid treatment, (131) had given a mixture of 5 peaks on g.l.c., of which one was the rosadiene (133) in 25% yield. Except for the major peak (50%) which was unreacted (131), no other products were identified.

When it came to the preparative scale isolation of (132) and (133), the happy discovery was made that there was no need to separate the two pimaradiene epimers (130) and (131) prior to rearrangement. Such was the unique t.l.c. mobility of both (132) and (133), that they could be isolated

direct from reaction of the pimaradiene epimeric mixture; the sole restriction was that (132) had to be isolated before 50 hours otherwise it was contaminated (on t.l.c.) by a minor product from the opposite C-13 series. Accordingly, after 45 hours reaction of the pimaradiene epimeric mixture, the rosadiene (132) was isolated as an oil, molecular weight 316 ($C_{21}H_{32}O_2$), and its gross structure confirmed from n.m.r. signals at τ 9.07, 9.05, 8.77, 6.38 (3H singlets); 5.3-3.9 (vinyl).

Rosadiene (133), the epimer required for rosenonlactone synthesis, was also isolated by silver nitrate t.l.c. and found to be identical in t.l.c., g.l.c. (SE30, P.E.G.A.), mass spectrum (g.c.m.s.) and n.m.r. to the authentic specimen prepared from deoxyrosenonolactone. As expected, its mass spectral cracking pattern was essentially identical with that of its C-13 epimer.

With the C-10 to C-9 methyl migration relieving the steric overcrowding at the C-19 carboxymethyl, the hydrolysis of (133) became a straightforward matter: the ester was refluxed for 22 hours in a solution of 2% ethanolic potassium hydroxide to furnish an acid whose m.p. 138-140°C, i.r. (3300-2700, 1690 cm^{-1}) and n.m.r. [3H singlets at τ 9.10, 8.99, 8.73; 5.3-3.9 (vinyl multiplet)] were identical with those

of authentic (134) prepared from lactone opening[28] of deoxyrosenonolactone (136).

From this point onwards in the synthesis, deoxyrosenonolactone was used as the source of the acid (134).

Lactonisation Studies on the Acids (134) and (139)

While the prospects of synthesising rosenonolactone (135) itself still looked remote, it did not escape our notice that the acid (134) was only one stage away from the simpler mould metabolite deoxyrosenonolactone (136). On mechanistic grounds an acid-catalysed lactonisation of the olefin-acid (134) would appear to be the ideal method; for a concerted process, involving the protonation of the 5,10 double bond on the α face simultaneously with attack at C-10 of the β axial carboxyl, would automatically lead to the required 5α-H stereochemistry. Nevertheless, Whalley and his group[28] had already investigated the lactonisation of the dihydro acid (139) and failed in this apparently simple objective: On treatment of (139) with ice-cold sulphuric acid, they isolated two γ lactones which they called allohydroxyrosanoic lactone, m.p. 138°C, $[\alpha]_D$ + 26.7°, and neohydroxyrosanoic lactone (the minor product) m.p. 123°C, $[\alpha]_D$ -19°. Neither of these lactones was identical with dihydrodeoxyrosenonolactone (138) and structures have still to be assigned to them.

Despite this pessimistic background, lactonisation attempts were still persevered with,* using a variety of acids. Accordingly, the diene-acid (134) was subjected to the following acid conditions:-

(1) Concentrated sulphuric acid at -10°C for 45 min. and 0°C for 60 min.

(2) Various concentrations of hydrochloric acid in dioxan for 1 hour at 0°C, 20°C and reflux temperature for 15 min.

(3) Hydrochloric acid-dioxan-water systems reacted as above.

In all above cases no lactonisation was found to occur, the main products were unreacted acid, or, occasionally, decarboxylated hydrocarbon material.

In the next approach (134) was refluxed for 36 hours with a saturated solution of p-toluenesulphonic acid in benzene. The crude products were chromatographed to give a non polar fraction (presumably decarboxylated material) and, as a minor product, a γ lactone, m.p. 174°C, molecular weight 302 $C_{20}H_{30}O_2$), i.r. 1780 cm^{-1}. This lactone was deemed to be the rearranged abietane compound (141) from the following arguments:- The n.m.r. spectrum showed that the vinyl multiplet and a quaternary methyl singlet were missing, having been replaced

*These experiments were first performed by Dr.A.J. Allison.

by a doublet at τ 9.02 (6H, J 7Hz) belonging to an isopropyl group, and an olefin multiplet at τ 4.63 (1H). This clearly indicates that ring C has an abietane structure, derived, undoubtedly, from protonation of the vinyl group followed by a C-13 to C-15 methyl migration. Absence of any low field H-C-O signals was evidence that the γ lactone terminates at the C-10 rather than the C-6 position.

Justification for the 5β-H stereochemistry came from the lactonisation results with the simpler dihydro-acid (139), a system in which the complication of abietane rearrangement cannot occur. On treatment with p-toluenesulphonic acid as before, (139) yielded two lactones; neither was found (g.l.c.) to be dihydrodeoxyrosenonolactone. The major component (cf Whalley's minor lactone),isolated by repeated crystallisation from petroleum ether, m.p. 124-125°C, molecular weight 302 ($C_{20}H_{30}O_2$), was shown to be a γ lactone by its i.r. absorption at 1778 cm^{-1}. From the absence of low field n.m.r. signals the lactone terminus must be again C-10 and the structure was tentatively formulated as (140). Although no O.R.D. measurements were performed, the close similarity of optical rotations for (140) and (141), (-21° and -22° resp.), suggested that they have the same stereochemistry except for the ring C abietane rearrangement. Taken together with the markedly different

rotation of +51° for dihydrodeoxyrosenonolactone (138)[28] and the knowledge[41] that the sign of the Cotton effect in lactones depends on the immediate environment of the lactone chromophore, then this is strong support for both (140) and (141) having the unnatural 5β-H configuration.

Both these lactones can scarcely have arisen by concerted lactonisation of the acids, but most likely have come from β protonisation at C-5 to yield a C-10 cationic intermediate. Unfavourable as this stepwise process may appear, the 5β-H lactones must, nevertheless, be inexorably formed under the equilibrating conditions employed, if they are the thermodynamically most stable products. Such a claim could, in fact, be made: for the 5β-H lactones have a ring B chair conformation (142), whereas lactones in the 5α series have ring B constrained to a boat conformation (143), with the consequent eclipsing interaction of C-6, C-7 methylenes.

During the lactonisation, one cannot discount the more deep-seated process of methyl migration from C-9 to C-10, giving 8,9 olefin intermediates. If this were so then there must be some doubt about the C-8 stereochemistry in (140) and (141).

Despite the failure of the lactonisation experiments described above, it had all along been clear to us that such a

route was specific for deoxyrosenonolactone, and could not lead to a synthesis of the oxygenated metabolites such as rosenonolactone (135) or rosololactone (41). In general terms, an approach to these metabolites must involve, as a first step, oxidation of the diene-acid (134) to a derivative which can - (a) help the lactonisation to occur and (b) 'direct' the oxygenation to the correct ring positions.

The major problem attending the synthesis of our main target, rosenonolactone, henceforth was the introduction of the ketone grouping at the inaccessible C-7 position which is two carbon atoms remote from the nearest reactive group, the 5,10 double bond. The most likely source of such a C-7 ketone was thought to be a hydration of a Δ^6 olefin precursor or an allylic oxidation on a Δ^5 olefin system such as (153). Of these two alternatives, the latter looked more promising, not only because a Δ^5 olefin should be more easily obtained than a Δ^6, but because such a Δ^5 intermediate could also be used for the synthesis of deoxyrosenonolactone (136) and possibly rosololactone (41). Our intermediate goal, therefore, was the construction of a Δ^5-ene-lactone system (153). However, due to a temporary shortage of deoxyrosenonolactone and, hence, of the rosadiene acid (134), the preparation of the ene-lactone was first perfected on the 15,16 dihydro

acid (139). This acid was prepared, according to Whalley,[28] by lactone opening of dihydrodeoxyrosenonolactone (138) which, in turn, was available from the much more plentiful rosenonolactone (135), by Raney nickel desulphurisation of its 7-ethylene mercaptal (137).[32]

The key step in conversion of the acid (139) to the Δ^5-ene-lactone (154) was the initial formation of a 5,10-α-epoxide (150), since, on Lewis-acid treatment, opening of the epoxide ring might be expected to induce attack of the C-19 carboxyl group at the developing cationic C-10 centre to yield the hydroxy lactone (152). Thus treatment of (139) for 30 hours with an excess of m-chloroperbenzoic acid in chloroform furnished a crude epoxy-acid, i.r. 3300-2500, 1700 cm^{-1}, fairly homogeneous on t.l.c.

Although not isolated and characterised, this intermediate epoxy acid was presumed to be the 5,10-α epoxy acid (150), since approach of reagent to the 5,10 olefinic bond is much easier from the α face than from the β face which is 'guarded' by the β axial methyl and carboxyl groups at C-9 and C-4 respectively. Such α geometry is, indeed, ideal for our task, as the transoid relationship of the epoxide to the carboxyl should be conducive to a concerted lactonisation-epoxide opening step. Accordingly, the intermediate epoxide (150) was

reacted at 0°C for 5 minutes with boron trifluoride etherate in benzene, After preparative t.l.c.. there was isolated in 45% yield a solid, m.p. 176-177°C, $C_{20}H_{30}O_3$, whose i.r. absorption at 3610 (free tertiary OH), 1775 cm^{-1} (γ lactone) and n.m.r. spectrum [singlets, 3H each, at τ 9.18, 9.09, 9.01; OH at τ 8.3 (broad)] were completely consistent with the hydroxy-lactone structure (152).

Dehydration of (152) was cleanly effected by reacting it at 0°C for 1 hour with thionyl chloride in pyridine. The oily product (i.r. 1782 cm^{-1}) was confirmed to be the Δ^5-ene-lactone (154) by the identity on t.l.c. and g.l.c. with the dehydration product[39] from dihydrorosololactone (41; 15,16 dihydro). The smooth formation of (154) was totally in accord with a 5α axial configuration for the OH group, since this would then permit an anti periplanar elimination with the 6β hydrogen.

Having developed a route to the Δ^5-ene-lactone system in the model dihydro series, the steps were repeated on the diene acid (134). The only complication we envisaged, undesired epoxidation of the vinyl 15,16 double bond, was not considered serious, since the tetrasubstituted 5,10 double bond ought to undergo electrophilic attack much faster. Indeed so, for treatment of (134) with m-chloroperbenzoic acid and boron tri-

fluoride, as described above for (139), furnished, in 40% yield, a solid, m.p. 182-184°C, $C_{20}H_{30}O_3$, which was assigned the hydroxy-lactone structure (151) from its i.r., (3610, 1778 cm^{-1}), and n.m.r. spectra, [singlets at τ 9.07 (3H), 9.02 (6H); 5.4-3.9 (vinyl H)].

On thionyl chloride treatment of (151), the sole product formed was the oily diene-lactone (153), i.r. 1782 cm^{-1}. Conclusive proof of the structure again came from its identity on t.l.c., g.l.c., n.m.r. (olefin triplet, J 3 Hz, τ 4.70) with an authentic specimen, prepared[39] by dehydration of rosolo-lactone (41).

With the diene-lactone (153) now in hand, the final requirements for a synthesis of rosenonolactone and deoxyrose-nonolactone were assessed as follows:-

(1) Rosenonolactone (135) is derivable from (153) by a formal two-stage process - allylic oxidation of the 5,6 olefin bond to give the enone (155), followed by reduction of the α,β double bond of the enone to yield the C-7 ketone.

(2) Although the allylic oxidation step is expected to be facile, the 'stumbling-block' in the synthesis is the reduction of the 5-6 bond of the enone (155), for there is also present in the molecule an easily-reduced 15,16 vinyl-idene group.

(3) There are two basic ways of overcoming the above reduction problem:- (a) Use of a selective reduction method which will saturate the 5-6 C=C bond of the enone, yet not reduce the vinylidene group (nor the lactone). (b) Use of a relatively non-selective reduction method, for example catalytic hydrogenation, but to counterbalance this by protecting the vinylidene group with a suitable derivative. Such a derivative must be resistant to the reduction conditions, and must also be removable, so as to regenerate the vinylidene group.

(4) Deoxyrosenonolactone (136) is only one formal step away from (153), but the problem still remains of selectively reducing the 5,6 double bond. In this case hydrogenation is the only realistic reduction method, so there is no alternative but to protect the vinylidene group.

(5) In reduction of the Δ^5 double bond, there is an extra problem of ensuring the correct 5α-H, configuration in the product. (This will be fully discussed at the appropriate time).

The best course of action, we decided, was to first try for a 'quick' synthesis of rosenonolactone by a specific reduction method which would obviate the need for vinyl group protection. It was felt that the key reduction step of enone (155) to ketone (135) could be done by lithium-liquid ammonia, since there were encouraging reports that such a reagent was harmless for simple olefins[85] and lactones,[86] under the mild conditions needed for enone reductions.

In anticipation that the enone (155) would eventually be obtained from oxidation of (153), it was, in fact, prematurely (and conveniently) prepared from dehydrogenation of rosenonolactone itself, in order to make it readily available for reduction experiments. Hence, rosenonolactone was refluxed for 24 hours with excess selenium dioxide in 94% dioxan-water, to yield, after preparative t.l.c., a solid, $C_{20}H_{26}O_3$, m.p. 136-138°C. Enone-lactone functionality was shown present by the U.V. maximum at 235 n.m. (C=C-C=O) and i.r. absorption at 1790 (lactone); 1680, 1632 cm^{-1} (enone). The n.m.r. olefin singlet at τ 4.21 (1H) has the chemical shift expected of a proton α to carbonyl: therefore the enone was assigned the Δ^5 structure (155), [rather than $\Delta^{8(14)}$.)

Surprisingly enough, all attempts to prepare (155) by dehydration of the metabolite 'lactone α' (50)[37] were unsuccess-

ful, returning unchanged starting material each time. This is perhaps due to the hindered nature of the 6β axial hydroxyl, although dehydration of α hydroxy ketones have been observed to be sluggish reactions.[103]

Lithium-Ammonia Reductions of the Enone (155)

To check that the γ lactone system will survive the reduction, rosenonolactone itself was reacted with lithium-liquid ammonia-ether for 6 minutes. The products, at least two polar spots on t.l.c., showed no ketone absorption in the i.r. (as expected), but no lactone bands either. However, Jones[87] oxidation of the crude hydroxy products regenerated fairly pure rosenonolactone. Hence, the γ lactone suffers some form of reduction with lithium-ammonia, but it can be regenerated by chromic anhydride oxidation.

In the reduction of enone (155), our analysis of the mechanism gave us cause to expect the correct stereochemical outcome, that is formation of rosenonolactone, and not its 5β-H epimer:- The currently favoured rationalisation of the stereochemistry of enone reduction in fused ring systems is that of Stork and Darling[88] and Robinson.[89] Briefly summarised, this states that, after the addition of the first electron to the β position of the conjugate enone, the so-formed enolate

anion-radical adopts its most stable conformation, while still preserving maximum overlap (i.e. planarity) of the four p orbitals of the delocalised system. The stereochemistry of reduction depends, therefore, on the direction of protonation at the B carbon of this anion-radical, and this is considered to be axial; that is, the new C-H bond will have an axial orientation in the ring containing the enolate radical.

Applied to the reduction of our enone system (155), this means that the intermediate radical-anion probably has the conformation shown partly in (157) where ring B is a flattened half-chair. Axial protonation could then come, as shown, from the less hindered α face to yield the correct C-5 configuration.

So much for prophecy: When the enone (155) was reacted with lithium-ammonia, the crude product was a complex mixture of polar streaky spots on t.l.c., i.r. 3600-3100, 1700 cm^{-1} (weak). Reoxidation of the crude product yielded neither rosenonolactone (135) nor the enone (155), but instead gave a mixture (85:15) of two acids (t.l.c.), i.r. 1722 (C=O), 1705 cm^{-1} (CO_2H). On diazomethane treatment, this acid mixture gave a keto-ester, i.r. 1735, 1722 cm^{-1}. Although not definitely proven, the likely structure for the keto-ester is (159). Therefore the major product from lithium-ammonia reduction (and reoxidation with CrO_3) is probably the product of lactone

cleavage (158), which can be envisaged as coming from the enolate anion-radical by the 1,2 elimination process shown in (160). Such reductive eliminations of allylic oxygen groups are on record,[90] and, indeed, we were fully aware of this at the outset of the experiment. However, we had considered this process unlikely in our system, since the C-O bond of the lactone terminus is 'equatorial' to ring B and hence, almost perpendicular to the p orbitals of the enolate radical-anion. Elimination, thus, should be less favourable, since the optimum electronic requirements - anti periplanarity of C-O bond and the C-5 p orbital - can not be obtained.

With the appreciation that the undesirable elimination process (160) is competing successfully with protonation (157), it was considered that some rosenonolactone might yet be salvaged if a proton source were present during reduction, to 'trap' the intermediate anion-radical. Accordingly the enone (155) was reacted with lithium-ammonia-tertiary butanol, under the same conditions as before. The products (weak lactone i.r. bond at 1785 cm^{-1}) contained a small amount of material, not isolated, which was identical with rosenonolactone on t.lc. and g.l.c. However, this was insignificant (less than 5%) in comparison with lactone-cleavage products, so this approach was not further pursued.

In a final gamble to selectively reduce the enone, (155) was reacted for 24 hours with a large excess of sodium borohydride in ethanol. The resultant crude product (several polar spots on t.l.c.) gave back unchanged enone, on Jones oxidation, with no trace of rosenonolactone. Hence the hoped-for C=C reduction of the enone had not taken place. Although 1,4 additions in the borohydride reduction of enones are not uncommon, the percentage of attack at the β carbon was found to decrease with increasing steric hindrance[101] at that carbon. This could account for the lack of C=C reduction in (155).

The failure of the above reduction attempts now left us with no alternative but to employ a vinylidene protecting group for the synthesis of both rosenono- and deoxyrosenonolactones. It was felt that the project could be most efficiently carried out in the following four stages:-

(1) Catalytic hydrogenation trials on the Δ^5 olefin system, using the unprotected diene-lactone (153) as a model, in order to find the optimum conditions and establish the stereochemical outcome (at C-5).

(2) Similar hydrogenation experiments on the enone system, using (155) as a model.

(3) Search for a suitable protecting group which is (a) easily prepared (b) resistant to hydrogenation conditions (c) removable.

(4) With the knowledge acquired above, the protecting group would then be inserted at the early hydroxy lactone stage (151).

Trial Hydrogenations of the 5,6 Double Bond

Using (153) as a model, hydrogenations were carried out with the following catalysts:- palladium-charcoal, rhodium-charcoal, platinum oxide, rhodium/platinum (3:1) mixed oxide[91] and tris(triphenylphosphine) rhodium chloride in benzene. With each catalyst a series of different solvents was used, ranging from ethyl acetate to acetic-hydrochloric acid, and the course of the hydrogenation was monitored at different time intervals by means of t.l.c. and g.l.c. The following results were obtained:-

Rapid reduction of the side chain 15,16 double bond took place with all catalysts, to yield the dihydro compound (154), identical with an authentic specimen. However, the 5,6 double bond was reduced only with great difficulty - undoubtedly because of its highly hindered position in the nucleus.

The reduction of the 5,6 olefin bond was further complicated by extensive hydrogenolysis of the lactone grouping, (as evidenced by much polar, acidic material on t.l.c.).

Hydrogenolytic cleavage of a C-O bond allylic to an olefin is a well known phenomenon,[93] and our lactone system - with the C-10 terminus allylic to the 5,6 double bond - was no exception.

In addition, catalyst-solvent systems found active enough to reduce the 5,6 olefin bond (rhodium-platinum in acetic acid, platinum in ethyl acetate, palladium-charcoal in ethanol) were found to cause a proportionate increase in hydrogenolysis. Therefore, a compromise had to be reached, and the best results obtained were with rhodium-platinum (3:1) in acetic acid:- 30% reduction, 30% unreacted and 30% hydrogenolysis after 30 hours' treatment.

Fortunately for our cause, the clean product of reduction was (138), identified on t.l.c. and g.l.c. (Table 4) with authentic dihydrodeoxyrosenonolactone. The stereospecific formation of this 'natural' 5α-H configuration is in accord with addition of hydrogen from the less hindered α face of the molecule.

Trial Hydrogenations of the 5-ene-7-one System

Early hydrogenations of the model (155) gave irreproducible results due to catalyst poisoning by residual traces of selenium [from the preparation of (155)]. This was removed

by chromic acid[94] treatment.

On hydrogenation for 2 hours with palladium-charcoal in ethyl acetate (our best solvent system), the enone (155) was smoothly converted to the 15,16 dihydro derivative (156);[30] i.r. 1790, 1682 cm^{-1}.

On more prolonged hydrogenation of (156), the 5,6 double bond slowly reduced. After 27 hours, a product (40% on t.l.c.) was isolated, which was identified as dihydrorosenonolactone (161), by comparison of its t.l.c., g.l.c. and i.r. (1785, 1722 cm^{-1}) with an authentic specimen prepared from rosenonolactone,[27] The remaining 60% consisted of hydrogenolysis products. Hence, reduction of the enone was less troublesome than that of (153), although it still gave the correct C-5 configuration in the product.

Protection of the 15,16 Double Bond

Using rosenonolactone as a model, the first derivative tried was the epoxide (162),[39] m.p. 173-175°C, n.m.r. τ 7.3 (multiplet 3H). In order to check that the epoxide group could be removed, (162) was subjected to a reductive-removal procedure[95] by reacting for 5 hours at 20°C with zinc and sodium iodide in buffered acetic acid. The crude products contained 60% rosenonolactone (t.l.c. and g.l.c. comparison)

with 15% each of two hydroxy compounds. Hence epoxidation can be reversed.

Reaction of the enone (155) with m-chloroperbenzoic acid, in the usual way, yielded the epoxide (163), $C_{20}H_{26}O_4$, i.r. 1792, 1682 cm^{-1}; n.m.r. signals at τ 7.3 (multiplet, 3H), 4.19 (singlet, 1H).

This stability of the epoxide grouping to hydrogenation was tested by attempting to reduce the enone epoxide (163) to rosenonolactone epoxide (162). However, after only 1 hour of reduction of (163) in palladium-charcoal-ethanol there was formed two closely similar polar compounds (t.l.c.). On separation, these two products had the same spectra:- i.r. (chloroform) 1780 (lactone), 1678 cm^{-1} (enone); n.m.r. τ 4.24 (enone singlet). The replacement of the epoxide signals by a lower field multiplet (3H) at τ 6.2 - 6.6 was compelling evidence that the products are epimeric (or isomeric) hydroxy compounds, from cleavage of the epoxide ring. The epoxide, therefore, cannot be used as a protecting group. Epoxides are, of course, known to be hydrogenolysed under fairly mild conditions,[93] but, at the time of their preparation we had not anticipated the hydrogenation of the 5,6 bond to be so difficult.

Our next olefinic derivative, the bromohydrin-acetate, was prepared, not by the usual addition of acetyl hypobromite,[97]

but by formation of an intermediate bromohydrin. Thus rose-nonolactone was reacted for 24 hours in acetone-water (8:1) with a slight excess of N-bromosuccinimide.[102] Chromatography of the crude products yielded an inseparable mixture of two isomeric bromohydrins (1:1 ratio on t.l.c.), with i.r. absorption (chloroform) at 3565, 3410, 1770, 1722 cm^{-1} and n.m.r. signals at τ 7.25 (OH), 5.9-6.1 (multiplet, 3H).

Acetylation of this 'bromohydrin' in the normal way furnished the 'bromohydrin-acetate', as an amorphous solid, $C_{22}H_{31}O_5Br$; i.r. (chloroform) 1770 (lactone), 1745 (acetate), 1723 cm^{-1} (ketone). The n.m.r. showed the presence of three quaternary methyls, and an acetate singlet at τ 7.89; but the three proton multiplet at τ 5.9 - 6.1 (H-15 and the C-16 methylene) could not be usefully interpreted. Although this product shows two close spots on t.l.c., the n.m.r. methyl signals are still sharp, suggesting, perhaps, that the product is mainly a mixture of C-15 epimers and not C-15, C-16 positional isomers. The reaction of terminal olefins with N-bromosuccinimide in moist dimethyl sulphoxide[107] and the analogous reactions with N-bromosuccinimide in alcohols or acetic acid[98] have been found to give predominantly (about 80%) the positional isomer with terminal bromine - as to be expected from the Markovnikov addition of bromine cation.

Hence, all the four possible isomers,(two positional isomers, each of which has two C-15 epimers), should be present in our case,but mostly the two with terminal bromine. For convenience, therefore, the bromohydrin and bromohydrin-acetate have been formulated as (164) and (165) respectively (C-15 epimers). Besides, since the protecting group is later removed, further investigation of its structure was unnecessary; all bromohydrin-acetates in the synthetic scheme were treated as single compounds, although they were all amorphous solid mixtures.

On catalytic hydrogenation with palladium-charcoal in ethyl acetate, (165), was recovered unchanged after 18 hours - sufficient proof of its stability to reduction. Confident, moreover, that the protecting group could be removed by zinc reduction, we were now prepared to complete the synthesis of the metabolites.

In the same way as described above, hydroxy-lactone (151) was transformed into the bromohydrin-acetate derivative (168), $C_{22}H_{33}O_5Br$ [i.r. ($CHCl_3$) 3600, 1763, 1745 cm^{-1}; n.m.r. signals at τ 7.91 (3H singlet), 5.2 - 6.2 (3H multiplet)].

On thionyl chloride-pyridine treatment (168) was cleanly dehydrated to the Δ^5 olefin (170). Confirmation of its structure came from its i.r. ($CHCl_3$) bands at 1760, 1740 cm^{-1} and the n.m.r. olefin signal at τ 4.74 (narrow triplet, H-6).

Using the experience gained in the model series, the olefin (170) was hydrogenated for 13 hours with rhodium-platinum (3:1) mixed oxide in acetic acid. To our dismay the reaction had not gone too cleanly and t.l.c. showed four products. Nevertheless, a minor product (20%) was isolated whose spectral properties [i.r. ($CHCl_3$) 1755, 1745 cm^{-1}; n.m.r. τ 7.89 (OAc), 5.2 - 6.2 (multiplet, 3H)] were consistent with the deoxyrosenonolactone bromohydrin-acetate structure (166). Conclusive proof of this assignment came from comparison with an authentic sample of (166), prepared directly from deoxyrosenonolactone. The major product (45%) from the reduction had i.r. absorption ($CHCl_3$) at 1756 (lactone) and 1735 cm^{-1} (OAc), but the mass spectral molecular weight 362 ($C_{22}H_{34}O_4$) showed the absence of bromine. In the n.m.r., a triplet (2H, J8 Hz) at τ 5.85 can only be from a C-16 acetate system, so the structure is, therefore, (171). This result immediately casts doubt on our formulation for the bromohydrin-acetate structure. Perhaps bromine is attached largely to C-15, with acetate at C-16?

Deoxyrosenonolactone bromohydrin-acetate (166) was refluxed in ethanol, for 2 hours, with freshly-prepared zinc-copper couple.[99] After preparative t.l.c., there was obtained a solid, m.p. 114-116°C, $[\alpha]_D$ + 54°, which was found to be

identical in t.l.c., g.l.c., i.r., and n.m.r. with an authentic specimen of deoxyrosenonolactone (literature[32] m.p. 115-116°C, $[\alpha]_D$ + 57°).

Our remaining task was to transform the olefin (170) into rosenonolactone:- The olefin was reacted for 15 hours at 40°C with anhydrous sodium chromate,[100] in acetic acid-acetic anhydride (2:1), to furnish the enone (169), [i.r. ($CHCl_3$) 1780, 1742, 1680 cm^{-1}; U.V. 234 n.m.; n.m.r. τ 4.23 (singlet 1H)], identical with an authentic sample prepared directly from (155). The ease of this oxidation (80%) was just as we had anticipated, for C-7 is the only allylic methylene position in the molecule.

Catalytic reduction of the enone (169) proved a slow process - as our experience in the model series had led us to expect. Nevertheless, after 70 hrs. with palladium-charcoal in ethyl acetate, a product (50% on t.l.c.) was isolated and found to be identical (t.l.c., i.r., n.m.r.), with an authentic specimen of rosenonolactone bromohydrin-acetate (165), prepared from rosenonolactone as previously described. Of the remaining products, 40% was unreacted enone.

Treatment of (165) with the zinc-copper couple, as described previously, caused facile removal of the protecting group, to afford a solid, m.p. 210-212°C, $[\alpha]_D$ -121°,

(literature[28] m.p. 208°C, $[\alpha]_D$ -116°), identical in t.l.c., g.l.c., i.r. and n.m.r. with an authentic sample of rosenonolactone. The biogenetically patterned synthesis of rosenono- and deoxyrosenono- lactone was complete.

It should be stated that the above work does not yet constitute a formal total synthesis of the metabolites, since a synthesis of isocupressic acid (128), or of its allylic isomer cupressic acid (173),[74] has still to be recorded. However, a synthesis of cupressic acid from the readily available intermediate (175)[104] should present little difficulty, considering that the analogous transformation of the enone (174) into manool (172) is already possible.[105,106]

EXPERIMENTAL

Metabolites of Tricothecium roseum

20 Litres of Czapek-Dox and corn steep liquor[22] were seeded with spores of T. roseum*. The mould was grown for 40 days as a surface culture, and the broth decanted from the mycelium. Both the broth and mycelium were treated with a little chloroform to ensure sterilisation.

The dried, powdered mycelium was extracted with chloroform to yield, after removal of solvent, a brown gum (8 g). Column chromatography on 450 g of alumina (Neutral Grade III), using light petroleum-benzene-chloroform gradient elution, furnished deoxyrosenonolactone (136) (344 mg)[32] m.p. 114-116° from ether, followed by rosenonolactone (135)[27,28] (2.1 g) m.p. 210-212° from ethanol.

The broth was neutralised to pH 7 by hydrochloric acid and extracted with methylene chloride. Drying and removal of the solvent gave a brown gum (6 g). Preparative t.l.c. of this gum (2.32 g) on ten (1000 x 200 x 0.5 mm) plates, using ethyl acetate-chloroform (1:9) as solvent, yielded 'lactone α' (50)[37,38] (280 mg) m.p. 178-180° from ether. Also isolated in smaller amounts (80 mg) was 'lactone β'[39] (the previously described rosein III[22,24]) m.p. 221°C from ether.

The isolation from the extracts of other metabolites, known or unknown, was not attempted.

*A strain supplied by Dr.J.R. Hanson, University of Sussex.

Dimethyl agathate (124)

Finely powdered Manila copal (50 g) was treated with an excess of diazomethane in the usual way. The resultant pale yellow oil (52 g) was shown by t.l.c. and g.l.c. (1% SE30 200°C) to contain about 45% of dimethyl agathate. Column chromatography on Grade III neutral alumina (2.51 kg), with benzene-light petroleum gradient elution, provided dimethyl agathate (124) as a colourless oil (9 g), homogeneous on t.l.c. and g.l.c. I.r. (liq. film) 3420, 3086, 3015; 1730-1710 (broad, strong), 1645, 886, 675 cm^{-1}.

U.V. max. 206 n.m. (ε 9,500), 221 n.m. (ε 11,200). Mass spec. M.wt. 362. $C_{22}H_{34}O_4$ requires 362. Fragmentation pattern is identical with that in literature.[114] N.m.r. 3H singlets at τ 9.48, 8.82, 7.85 (broadened), 6.39, 6.32; 5.50, 5.12 (broad singlets, 1H each), 4.36 (broad singlet, 1H). G.l.c. retention time (on 1% SE30, 175°C) 36.9 min relative to C-20, 22, 24 n-alkanes at 5.9, 13.15, 29.45 min.

Agathadiol (75)

Reduction of dimethyl agathate (124) (1.06 g) with excess lithium aluminium hydride gave the known[50] agathadiol (75) (814 mg), m.p. 106°C. I.r. (0.002M CCl_4) 3643 (shoulder at 3625), 3082, 1666, 1643, 1025, 894 cm^{-1}. N.m.r., τ 9.37, 9.05,

8.35 (singlets,3H), 5.88 (doublet, 2H, J 7Hz), 4.62 (triplet, 1H, J 7Hz); 6.62, 6.28 (AB quartet, 2H, J 12Hz); 5.49, 5.16 (broad singlets, 1H each), agrees well with lit. values.[51]

Agathadiol diacetate (80)

After reaction in the usual way with acetic anhydride and pyridine, agathadiol (45 mg) gave the diacetate (80) (47 mg) with n.m.r. τ 9.30, 9.03, 8.31, 7.99, 7.97 (singlets, 3H); 6.14, 5.77 (AB quartet, 2H, J 12Hz); 5.45, 5.16 (broad singlets, 1H each), 5.41 (doublet, 2H, J 7Hz), 4.68 (triplet, 1H, J 7Hz).

Attempted selective reduction of dimethyl agathate (124)

Method A: Lithium aluminium hydride (LAH).

Dimethyl agathate (103 mg, 0.284 m.moles) was refluxed for 24 hours with a solution of LAH (6.8 mg, 0.178 m.moles, 1.25 x weight needed for one ester group)in dry ether (5 ml). After recovery of material, t.l.c. showed the products to be unreacted dimethyl agathate (approx. 70%), agathadiol (15%) and several other products of intermediate polarity.

Method B: Lithium tri-t-butoxyaluminohydride (LTBA)

A stirred suspension of dimethyl agathate (96 mg, 0.265

m.moles)and LTBA[77] (1.35 g, 5.3 m.moles) in dry tetrahydrofuran (10 ml) was refluxed for 40 hours. After addition of water, the solution was filtered off, dried and evaporated. Preparative t.l.c. of the products furnished unreacted starting material (10 mg) and the known[78] primary alcohol (129) (38 mg) as an oil. N.m.r. τ 9.49, 8.85, 6.46 (singlets, 3H); 9.11 (doublet, 3H, J 6Hz); 5.50, 5.15 (broad singlets, 1H each), 6.40 (triplet, 2H, J 7Hz); absence of vinyl H at τ 4.60.

Methyl agathate (125)

Dimethyl agathate (124) (18 g) was refluxed for 2 hours in ethanol (120 ml), water (20 ml) and potassium hydroxide (6 g). Most of the ethanol was distilled off, water added and the neutral material extracted with ether. Acidification of the basic, aqueous phase to pH5 regenerated the acids which were also extracted with ether. Processing of the two extracts gave the unreacted starting material (528 mg) and the ester-acid (125) (14.12 g) as a fairly pure colourless oil. A portion, further purified by t.l.c., had i.r. 3600-2500 (broad), 1730, 1693, 1640, 890 cm^{-1}. N.m.r. τ 9.48, 8.82, 7.85, 6.38 (singlets, 3H); 5.50, 5.14 (broad singlets, 1H each), 4.35 (broadened singlet, 1H), 0.53 (broad singlet, 1H). (Found: C, 72.69; H, 8.97. $C_{21}H_{32}O_4$ requires C, 72.38; H, 9.26%).

Methyl isocupressate (127)

To a solution of methyl agathate (125) (7.13 g, 20.4 m.moles) in dry tetrahydrofuran (50 ml), stirred at -10°C under nitrogen, was slowly added to a solution of redistilled triethylamine (2.1 g, 20.08 m.moles) in tetrahydrofuran (20 ml), followed, 10 minutes later, by redistilled ethyl-chloroformate (2.3 g, 21.0 m.moles) in tetrahydrofuran (20 ml). The mixture was stirred for 2 hours at -10°C, filtered free of the precipitated amine salt and the solvent removed to give a crude, yellow oil whose t.l.c. and i.r. spectrum - 1795 (shoulder 1775), 1720, 1635 cm^{-1} for liq. film - showed it to be mainly the mixed anhydride (126).

The above oil in ethanol (80 ml) was stirred for 1 hour at 20°C with sodium borohydride (1.6 g, 42 m.moles). Water was added and the products extracted with ether. The recovered material (5.90 g) was chromatographed on a column of 350 g grade III basic alumina to yield methyl isocupress-ate (127) as an oil (3.03 g), t.l.c. homogeneous. I.r. 3620, 3075, 1725, 1645, 1152, 890 cm^{-1}, n.m.r. τ 9.48, 8.81, 8.32, 6.39 (singlets, 3H); 5.81 (doublet, 2H, J 7Hz), 4.60 (triplet, 1H, J 7Hz), 5.46, 5.12 (broad singlets, 1H each). The n.m.r. spectrum was identical with that of an authentic specimen of the natural product.[74]

Slightly better yields were obtained if the crude reaction products, containing the anhydride, were reduced *in situ* with sodium borohydride.[80]

The diene-acid (134)

Deoxyrosenonolactone (136) (20 mg) was refluxed for 4 hours in ethanol (5 ml) and aqueous hydrochloric acid[28] (2M, 5 ml). The solution was diluted with ethyl acetate, washed with sodium bicarbonate solution, water, brine and dried. Removal of solvent afforded the acid[82] (134) (19 mg), m.p. 138-140°C (plates from aqueous methanol). I.r. 3300-2700 (broad); 3080, 1640, 915; 1742 (weak), 1690 cm^{-1} (strong), n.m.r. τ 9.10, 8.99, 8.73 (singlets, 3H); 3.9-5.3 (multiplet, 3H). (Found: C, 79.35; H, 9.92. $C_{20}H_{30}O_2$ requires C, 79.42; H, 10.00%).

The methyl ester (133)

Methylation of acid (134) (15 mg) by diazomethane, in the usual way, gave the ester(133) as an oil (16 mg), $[\alpha]_D$ -120° (C = 1.75 $CHCl_3$). N.m.r. τ 9.09, 8.97, 8.76, 6.38 (singlets,3H); 3.9-5.4 (multiplet,3H). M 316 (g.c.m.s.). $C_{21}H_{32}O_2$ requires 316. Identical g.l.c. retention time (1% SE30 1752) with the ester (130).

Acid-cyclisation of methyl isocupressate (127)

(a) Short reaction time

Methyl isocupressate (490 mg) was left at 50°C for 3 hours in the acetic-sulphuric acid[57] medium (6 ml) used previously for manool (70). After recovery of material, the olefin-ester fraction (245 mg) was isolated by preparative t.l.c., and was shown by g.l.c. (several columns) and silver nitrate t.l.c. to be two components in the ratio 1.3:1. Preparative t.l.c. (20% $AgNO_3$-SiO_2) gave the major component, tricyclic ester (130) as an oil (46 mg). M 316 from g.c.m.s. $C_{21}H_{32}O_2$ requires 316. N.m.r. τ 9.22, 9.02, 8.80, 6.36 (singlets, 3H); 5.4-3.8 (vinyl multiplet, 3H). The vinyl signals were very similar to those of the diene (87).

The more polar, less volatile, C-13 epimer (131), isolated as an oil (32 mg), had molecular weight 316 ($C_{21}H_{32}O_2$) and the g.c.m.s. was the same as (130). The mass spectra for both (130) and (131) were broadly similar to but not identical with those of their C-4 epimers (144) and (146)[84] respectively. N.m.r. τ 9.21, 9.07, 8.80, 6.37 (singlets, 3H); 5.3-3.7 (vinyl multiplet, 3H); the vinyl signal pattern was similar to that of the pimaradiene (88). G.l.c. retention times on 1% SE30, 175°C:- (130) 8.50; (131) 9.29, with C-20, C-22 n-alkanes at 5.30, 11.95 min. respectively.

Exploratory Rearrangements Experiments.

(a) Prolonged acid treatment of the diene-esters (130) and (131).

The above diene-esters (5 mg each) were individually heated at 60°C with acetic-sulphuric acid (1.0 ml), the course of the rearrangement being monitored by working up aliquots after 20, 40 and 85 hours reaction and analysing the products by g.l.c., g.c.m.s. and silver nitrate t.l.c.

The diene-ester (131) was found to give a complex spread of unknown products, among which very little of the rosane derivative (133) could be detected.

The diene-ester (130) was found to be transformed in 45% yield (by g.l.c) to its rosane skeletal isomer (132) (identified on subsequent isolation) after an optimum period of 40 hours. At least 10 other unknown peaks (3 quite substantial) were present in the complex mixture and the proportion of (132) decreased with further reaction.

(b) Formic acid treatment of the diene-esters (130) and (131).

The individual diene-esters (5 mg each) were refluxed with (1:1) formic acid-chloroform (1 ml). The course of the reactions was followed by removing aliquots at 20, 40 and 85 hours, quenching in dilute sodium bicarbonate, extraction

into ether and analysis of the diene fraction by t.l.c., g.l.c. g.c.m.s.

The diene-ester (130) was found to be cleanly converted into its rosane isomer (132) (70% after 85 hours). The remaining products were mainly an unknown component (20%) and unreacted starting material (130) (5%).

On 85 hours formic acid treatment the epimeric diene-ester (131) gave at least 5 peaks on g.l.c. - including the rosane skeletal isomer (133) (25%) and unreacted starting material (131) (50%).

Inspection of the t.l.c. R.F. values (silver nitrate) for all the rearrangement products from both C-13 epimeric series showed that the rosadiene ester (133) was completely 'remote' from every other product and should therefore be isolable from direct reaction of the unseparated pimaradiene epimers (130) and (131). The rosadiene ester (132) had a unique t.l.c.: R.F. until 50 hours reaction, but, after this time, a product of the same t.l.c. mobility was slowly forming in the opposite C-13 series.

The rosadiene-ester (132)

On the basis of the results of the above trial experiments, the unseparated epimer mixture (130) and (131) (285 mg) was refluxed with formic acid-chloroform (25 ml) for 45 hours. Recovery, followed by preparative, silver nitrate t.l.c., gave the diene-ester (132) as an oil (17 mg). M 316 from g.c.m.s. ($C_{21}H_{32}O_2$). N.m.r. τ 9.07, 9.05, 8.77, 6.38 (singlets, 3H); 3.9-5.3 (multiplet, 3H). G.l.c. on 1% SE30 175°C; 6.75 with C-20, C-22 n-alkanes at 5.30, 11.95 min. respectively. Some starting material (122 mg) was recovered.

The rosadiene-ester (133)

Epimer mixture (130), (131) (122 mg) was reacted as above for 90 hours. Work up and silver nitrate t.l.c. yielded a diene-ester (9 mg) which had identical t.l.c., g.l.c., mass spectrum (g.c.m.s.) and n.m.r. to the diene-ester (133), prepared, as described previously, from deoxyrosenonolactone (136). The mass spectrum of (133) was almost identical to that of its C-13 epimer (132), Table 3 summarises the g.l.c. retention data for the pimara- and rosa- diene esters.

Hydrolysis of the ester (133)

The ester (20 mg) was refluxed for 22 hours in a solution (7 ml) of ethanolic potassium hydroxide (2g KOH: 90 ml EtOH: 10 ml H_2O). The total recovered material (16 mg) still had 15% unhydrolysed methyl ester. Preparative t.l.c. provided an acid (10 mg) with identical m.p., i.r., n.m.r. with those of the acid (134), obtained by the lactone opening of deoxyrosenonolactone.

Rosenonolactone 7-ethylene mercaptal (137)

Rosenonolactone (135) (400 mg) was left at 20°C for 12 hours in ethane dithiol (3 ml) and boron trifluoride etherate (0.5 ml). Dilute sodium bicarbonate was added and the products extracted into ethyl acetate which was washed with water, brine and dried. Removal of solvent and residual ethane dithiol *in vacuo*, followed by preparative t.l.c., furnished the thioketal[32] (137) (290 mg) as a white solid m.p. 202-203°C (from EtOH). I.r. 1778, 1635, 960, 940, 910 cm^{-1}. N.m.r. τ 9.03, 8.96, 8.90 (singlets, 3H); 7.1-6.5 (multiplet, 4H); 5.2-3.9 (vinyl multiplet, 3H). (Found: C, 67.63; H, 8.13. $C_{22}H_{32}O_2S_2$ requires C, 67.32; H, 8.22%).

Dihydrodeoxyrosenonolactone (138)

The above thioketal (200 mg) in dioxan (8 ml) and ethanol (12 ml) was refluxed for 24 hours with Raney nickel catalyst (2g, grade W-4). Filtration through celite and removal of solvent gave fairly pure dihydrodeoxyrosenonolactone[32](138) (150 mg). m.p. 98-99°C (Lit. value[28] 101°C) I.r. 1774, 960, 940 cm^{-1}. T.l.c., g.l.c. (see Table 4) and n.m.r., τ 9.16, 9.04, 8.93 (singlets, 3H), were identical with those of an authentic specimen prepared[39] from hydrogenation of deoxyrosenonolactone (136) over palladium-charcoal.

The olefin-acid (139)

The above lactone (80 mg) was treated with ethanolic hydrochloric acid, exactly as described before for deoxyrosenonolactone, to give the known[28] olefin-acid (139) (75 mg); i.r. 3300-2500, 1695 cm^{-1}.

Acid-catalysed lactonisation of the diene-acid (134) *

The acid (20 mg) was refluxed for 36 hours with a saturated solution of p-toluenesulphonic acid in benzene (10 ml). Ether was added and the solution washed thoroughly with water. After drying and removal of solvent, the crude products were chromatographed to give a non-polar fraction (10 mg; 1 major, 2 minor peaks on g.l.c.) and the lactone (141) (5 mg) m.p. 174°C. $[\alpha]_D$ -22° (C = 0.13 $CHCl_3$). I.r. 1780 cm^{-1}. Mass spectral m. wt. 302 ($C_{20}H_{30}O_2$). N.m.r. τ 9.05, 8.87 (singlets, 3H each); 9.02 (doublet, 6H, J 7Hz); 4.63 (multiplet, 1H).

The following lactonisation methods had proved unsuccessful, giving mainly unchanged starting material.

1. Concentrated sulphuric acid[28] at -10°C for 45 min. and at 0°C for 60 min.
2. Various combinations of concentrated hydrochloric acid in dioxan, e.g. 0.1 ml of each per mg of acid (134), at 0°C for 1 hour, 20°C for 1 hour and reflux temp. for 15 minutes.
3. Hydrochloric acid-dioxan-water combination (1:1:1) as above.

*First carried out by Dr. A.J. Allison

Acid-catalysed lactonisation of the olefin-acid (139)*

On treatment, as above, with p-toluenesulphonic acid in benzene, the acid(139) (25 mg) furnished two lactones, neither of which was dihydrodeoxyrosenonolactone. The major component (140) (5 mg) was obtained by crystallisation from ice-cold, light petroleum m.p. 124-125°C. $[\alpha]_D$ -21° (C = 0.31 $CHCl_3$). Mass spectral m.wt. 304 ($C_{20}H_{32}O_2$); i.r. 1778, 954 cm^{-1}. N.m.r. τ 9.10, 9.01, 8.85 (singlets, 3H).

The m.p. and optical rotation agree with the previously reported lactone[28,30] of undetermined structure.

Epoxidation of olefin-acid (139)

The acid (45 mg) was left for 30 hours, in the dark, with m-chloroperbenzoic acid (80 mg, excess) in chloroform (2 ml). After addition of ether, the solution was washed with 10% aqueous sodium sulphite, water, brine and dried. The crude intermediate epoxy-acid (150) (42 mg), obtained on removal of solvent, had i.r. 3300-2500, 1700 (strong), 1780 cm^{-1} (medium). This was, without further characterisation, rearranged with boron trifluoride.

* First carried out by Dr. A. J. Allison

The hydroxy-lactone (152)

The above epoxide (42 mg) was left at 0°C for 5 minutes with freshly-distilled boron trifluoride etherate (0.2 ml) in benzene (5 ml). After addition of water and extraction into ether, the normal recovery and preparative t.l.c. furnished the hydroxy-lactone (152) (20 mg). m.p. 176-177°C (rods from light petroleum). I.r. (8 mg/ml CCl_4) 3610 (free OH), 3550-3350 (intermolec. bonded OH) 1775, 960, 948 cm^{-1}. N.m.r. τ 9.18, 9.09, 9.01 (singlets, 3H); 8.3 (broad, 1H, lost on D_2O exchange). (Found: C, 75.09; H, 10.19. $C_{20}H_{32}O_3$ requires C, 74.96; H, 10.06%).

Epoxidation of diene-acid (134)

The acid (190 mg) in chloroform (3 ml) was left for 24 hours, at 20°C in the dark, with m-chloroperbenzoic acid (150 mg, 10% excess). The solvent was removed *in vacuo* and the resultant crude mixture, containing intermediate epoxy-acid (149) was treated with boron trifluoride as below.

The hydroxy-lactone (151)

Rearrangement of the above mixture with boron trifluoride, as described before on the dihydro analogue (150), gave, after t.l.c. the hydroxy-lactone (151) (62 mg) m.p. 182-184°C (rods from light petroleum). I.r. (5 mg/ml CCl_4) 3610 (free OH); 1778; 3080, 1640, 915; 960, 950 cm^{-1}. N.m.r. singlets at τ 9.07 (3H), 9.02 (6H); 5.4-3.9 (multiplet, 3H). (Found: C, 75.33; H, 9.53. $C_{20}H_{30}O_3$ requires C, 75.43; H, 9.50%).

Dehydration of hydroxy-lactone (152)

Hydroxy-lactone (152) (5 mg) was reacted at 0°C for 1 hour with freshly-distilled thionyl chloride (0.2 ml) in dry pyridine (0.5 ml). Water was added and the solution extracted with ether. Usual recovery of material gave the cleanly-formed Δ^5-ene-lactone (154) as an oil (4.5 mg); i.r. 1782 cm^{-1}; identical on silver nitrate t.l.c. and g.l.c. (see Table 4) with the dehydration product[39] of dihydrorosololactone (41; 15,16 dihydro).

Dehydration attempts with phosphorous oxychloride and pyridine, 8 hours at room temperature gave unreacted material. When repeated, at reflux for 2 hours, a complex mixture of products was formed.

Dehydration of hydroxy-lactone (151)

The lactone (151) (15 mg) was dehydrated with thionyl chloride, as for its dihydro derivative above, to give the oily diene-lactone (153) (14 mg), i.r. 1782 cm^{-1}. N.m.r. τ 9.17, 8.99, 8.80 (singlets, 3H); 5.3-3.9 (multiplet, 3H); 4.70 (triplet, 1H, J 3Hz). The n.m.r., silver nitrate t.l.c. and the g.l.c. (see Table 4) were identical with those of the dehydration product[39] of rosololactone (41).

Oxidation of rosenonolactone to the enone (155)

Rosenonolactone (135) (100 mg) was refluxed for 24 hours with selenium dioxide (300 mg, excess) in a 12 ml. solution of dioxan-water (94:6). The solution was diluted with ether, filtered, washed with water, brine and dried. Removal of solvent, followed by preparative t.l.c., yielded the enone (155) (45 mg), m.p. 136-138°C (long needles from acetone-light petroleum). Homogeneous on t.l.c. and g.l.c. (Table 4). Mass spectral m.wt. 314. I.r. 3080, 1790, 1682, 1638 cm^{-1}. U.V. max. 235 n.m. N.m.r. τ 9.07, 8.99, 8.68 (singlets, 3H); 5.3-3.9 (multiplet, 3H); 4.21 (singlet, 1H). (Found: C, 76.06; H, 8.16. $C_{20}H_{26}O_3$ requires C, 76.40; H, 8.34%).

Attempted dehydration of 'lactone α' (50)

Lactone α (7 mg) was subjected to the following dehydration conditions:-

(a) Thionyl chloride (0.4 ml) in pyridine (0.5 ml) for 1 hour at 0°C.

(b) As above for 21 hours at R.T.

(c) Phosphorous oxychloride (0.2 ml) in pyridine (0.5 ml) for 32 hours at R.T.

In each case, the products from work up were shown on t.l.c. to be unreacted lactone α. The U.V. (end absorption only) confirmed that the desired dehydration to enone (155) had not taken place.

Action of lithium-ammonia on rosenonolactone (135)

To a solution of lithium (30 mg) in liquid ammonia (15 ml), cooled in an acetone-Dry-Ice bath, was added rosenonolactone (10 mg) in dry ether (10 ml). After 6 minutes stirring, solid ammonium chloride was added, the ammonia allowed to evaporate and the crude products recovered by dissolving in ether and filtering off. T.l.c. showed the starting material to be replaced by at least two very polar compounds. I.r. 3620 (free OH), 3550-3300 cm^{-1} (bonded OH); no ketone nor lactone carbonyl bands.

Reoxidation of the crude products, by the method of Jones,[87] gave a reasonably clean product which was identical on t.l.c., g.l.c. (Table 4) and i.r. (1782, 1722 cm^{-1}) with starting rosenonolactone.

Action of lithium-ammonia on the enone (155)

On treatment with lithium-ammonia, exactly as described above, the enone (155) (10 mg) gave a mixture of polar, streaky spots on t.l.c. I.r. 3600-3100 (bonded OH), 1700 cm^{-1} (weak, broad).

Jones reoxidation of this mixture gave no rosenonolactone nor any enone (155); the product [(possible structure (158)] was two streaky, acidic spots on t.l.c., the major component approx. 85%; i.r. 1722, 1705 cm^{-1}.

Methylation of this acid with diazomethane gave a methyl ester, possibly (159), less polar than rosenonolactone, with i.r. 1735, 1722 cm^{-1}.

Action of lithium-ammonia-tertiary butanol on the enone (155)

To a solution of the enone (10 mg), tert. butanol (400 mg), anhydrous ether (10 ml) and liquid ammonia (10 ml), cooled to -80°C, was added lithium metal (30 mg). The dark

blue solution was stirred for 3 minutes before working up in the way described before.

After reoxidation by Jones reagent the same major component (158 ?) was again obtained, but there was also approx. 5%, not isolated, of a lactone identical on t.l.c., g.l.c. with rosenonolactone (135); the infra-red of the mixture had a weak 1785 cm^{-1} lactone absorption.

Action of sodium borohydride on the enone (155)

The enone (155) (7 mg) was reacted at 20°C with sodium borohydride (100 mg, excess) in ethanol (3 ml) for 24 hours. Addition of water, extraction into ether, washing with water, drying and removal of solvent furnished a crude solid (5 mg) which had several polar spots on t.l.c. Jones oxidation of this solid returned the starting enone (155), but no rosenonolactone, as seen by t.l.c. and g.l.c.

Studies on Hydrogenation of the 5,6 double bond

Use of the diene-lactone (153) as a model:

Trial hydrogenations were carried out on the diene-lactone, using the following catalysts - 10% palladium-charcoal, platinum oxide, 5% rhodium-charcoal, tris(triphenylphos-

phine) rhodium chloride[92] in benzene and rhodium/platinum (3:1) mixed oxide.[91] Different reaction times and several solvents, e.g. ethyl acetate, ethanol, ethanol with 3% triethylamine, ethanol-acetic acid, acetic acid, acetic-hydrochloric acid, were used for each catalyst. Typical conditions were diene (3 mg); catalyst (10 mg); solvent (6ml) for 2-100 hours. T.l.c. and g.l.c. (Table 4) analysis showed the following results:-

1) All catalysts quickly reduced the side chain 15,16 double bond to give the ene-lactone (154), but reduction of the 5,6 double bond was more difficult.

2) Catalysts powerful enough to reduce the 5,6 double bond on prolonged action, e.g. Pd-C in ethanol, PtO_2 in ethyl acetate, Rh/Pt in acetic acid, also caused much hydrogenolysis (polar acidic material on t.l.c.).

3) Use of more polar, acidic solvents enhanced the catalyst activity but also increased the proportion of hydrogenolysis.

4) Best compromise conditions were (a) Pd-C in EtOH for 12 hours, with 10% reduction, 90% hydrogenolysis. (b) PtO_2 in ethyl acetate for 17 hours, with 10% reduction, 20% unreacted, 70% hydrogenolysis. (c) Rh/Pt in acetic acid for 30 hours, with 30% reduction, 30% unreacted, 30% hydrogenolysis.

(d) Rh/Pt in acetic - 1% concentrated hydrochloric acid for 2 hours, with 30% reduction, 70% hydrogenolysis.

5) The reduction product so obtained was identical on t.l.c. and g.l.c. (1% SE30, 175°C) with authentic dihydrodeoxy-rosenonolactone (138).

Hydrogenation studies on the 5-ene-7-one system

Use of the enone (155) as a model:

Residual selenium traces, causing inconsistent results, were removed from enone (155) as follows[94]:- The enone (70 mg) was stirred for 15 hours at R.T. with chromium trioxide (250 mg) in a mixture of benzene (7 ml), water (5 ml) and acetic acid (1 ml). Separation, washing and drying of the benzene phase, followed by removal of the solvent, gave back the purified enone (70 mg).

Short-time hydrogenation:

The enone (10 mg) was hydrogenated for 2 hours in ethyl acetate (5 ml) with 30% palladium-charcoal (5 mg). Filtration through glass paper and removal of solvent gave the 15,16 dihydro-enone[30](156); i.r. 1790, 1682 cm^{-1}; t.l.c. and g.l.c. homogeneous (Table 4).

Prolonged hydrogenation:

The above dihydro-enone (156) (35 mg) was slowly hydrogenated further with 30% palladium-charcoal (20 mg) in ethyl acetate (6 ml). After 27 hours a product (161) was isolated (40% on t.l.c.) and shown by t.l.c., g.l.c. (SE30, QF1, P.E.G.A ., DC 710 columns; Table 4) and i.r. (1785, 1722 cm^{-1}) to be identical with a specimen of dihydrorose-nonolactone prepared by palladium-charcoal reduction of rose-nonolactone.[27] The remaining material was hydrogenolysis products - at least three streaky, polar spots on t.l.c.

Epoxidation of rosenonolactone

Prepared as described by Renfrew[39], rosenonolactone epoxide (162) had m.p. 173-175°C. Mass spectral m.wt. 332 ($C_{20}H_{28}O_4$); i.r. ($CHCl_3$) 1770, 1715 cm^{-1}; epoxide signals in n.m.r. at τ 7.3 (close multiplet, 3H).

Reductive removal[95] of epoxide from (162)

The epoxide (162) (23 mg) was stirred at 20°C with sodium iodide (60 mg), sodium acetate (40 mg) and zinc dust (16 mg) in 2 ml of acetic acid-water (80:1). After 5 hours the solution was filtered, diluted with ethyl acetate, washed

thoroughly with water and dried. The recovered material was shown by t.l.c. and g.l.c. to be rosenonolactone (60%) with about 15% each of two polar, hydroxy compounds.

Epoxidation of the enone (155)

The enone (155) (70 mg) was left at R.T. for 19 hours with m-chloroperbenzoic acid (50 mg, 10% excess) in chloroform (2 ml). The solution was then neutralised with solid calcium hydroxide[96] and filtered. Preparative t.l.c. of the recovered material gave the enone epoxide (163) (56 mg), melting range 184-195°C, i.r. 1792, 1682 cm^{-1}; n.m.r. τ 9.06, 9.03, 8.69 (singlets,3H); 7.3 (close multiplet, 3H); 4.19 (singlet, 1H). Mass spectral m.wt. 330 ($C_{20}H_{26}O_4$).

Catalytic hydrogenation of the enone-epoxide (163)

The epoxide (22mg) was hydrogenated for 1 hour with 10% palladium-charcoal (25 mg) in ethanol (8 ml). Filtration through glass paper, removal of solvent and preparative t.l.c. furnished two, more polar components (9 mg each); of similar t.l.c. mobility. Their i.r. and n.m.r. spectra were indistinguishable:- i.r. ($CHCl_3$) 1780 (lactone), 1678 cm^{-1} (enone), n.m.r. singlets at τ 9.07 (6H), 8.71 (3H), 4.24 (1H); 6.2-6.6 (multiplet, 5 peaks, 3H).

Rosenonolactone bromohydrin (164)

Rosenonolactone (135) (70 mg) was stirred at R.T. with N-bromosuccinimide[102] (50 mg, 20% excess) in 3 ml of acetone-water (8:1). After 24 hours the acetone was removed in vacuo, water added and the aqueous solution extracted with chloroform, then with ether. Recombination of the washed, dried extracts, followed by preparative t.l.c., yielded the bromohydrin (164) (46 mg), two inseparable isomers on t.l.c. (approx. 1:1 ratio). I.r. ($CHCl_3$) 3565, 3410, 1770, 1722 cm^{-1}. N.m.r. τ 9.07, 8.98, 8.90 (singlets, 3H); 7.25 (singlet, 1H, lost on D_2O exchange); 5.9-6.1 (multiplet, 3H).

This bromohydrin was characterised as the acetate (165).

Rosenonolactone bromohydrin-acetate (165).

The above bromohydrin (30 mg) was left overnight at R.T. with acetic anhydride (1 ml) and pyridine (1 ml). Usual work up gave the bromohydrin-acetate (165) (31 mg) as an amorphous solid, two close spots on t.l.c. I.r. ($CHCl_3$) 1770, 1745, 1723 cm^{-1}. N.m.r. τ 9.06, 8.96, 8.88, 7.89 (singlets, 3H); 5.0-6.2 (multiplet, 3H).
Molecular ion (Mass spectrum) was a doublet, m/e 454.1366 and 456.1328; $C_{22}H_{31}O_5{}^{79}Br$ requires 454.1355; $C_{22}H_{31}O_5{}^{81}Br$ requires 456.1336.

An alternative, more direct preparation of bromohydrin-acetate (165) was performed using acetyl hypobromite,[97] but the yield (30% approx.) was lower.

Stability of bromohydrin-acetate (165) to hydrogenation

The bromohydrin-acetate (3 mg) was hydrogenated for 18 hours with 30% palladium-charcoal (4 mg) in ethyl acetate (5 ml). The product was shown on t.l.c. to be unaffected starting material.

The bromohydrin (164) was also, shown to be undamaged by these conditions.

Bromohydrin-acetate of the enone (155)

The enone (155) (124 mg) was treated with N-bromo-succunimide as described previously. The crude reaction products were acetylated, without isolation of the intermediate bromohydrin, and the bromohydrin-acetate (169) was obtained by preparative t.l.c. as an amorphous solid (130 mg), two close spots on t.l.c. I.r. ($CHCl_3$) 1780, 1742, 1680 cm^{-1}. U.V. max. 234 n.m. N.m.r. τ 9.07, 8.91, 8.68, 7.89 (singlets, 3H); 5.2-6.2 (multiplet, 3H); 4.23 (singlet, 1H). (Found; C, 58.12; H, 6.74. $C_{22}H_{29}O_5Br$ requires C, 58.32; H, 6.47%).

Deoxyrosenonolactone bromohydrin-acetate (166)

By the method above, deoxyrosenonolactone (136) (66 mg) was converted to the bromohydrin-acetate (166) (26 mg), an amorphous solid showing two t.l.c. components. I.r. ($CHCl_3$) 1755, 1745 cm^{-1}. N.m.r. τ 9.03, 8.95, 8.92, 7.89 (singlets, 3H); 5.2-6.2 (multiplet, 3H). Molecular ion (Mass spectrum) was a doublet, m/e 440.1557 and 442.1540; $C_{22}H_{33}O_4{}^{79}Br$ requires 440.1563; $C_{22}H_{33}O_4{}^{81}Br$ requires 442.1543.

In addition to unreacted deoxyrosenonolactone (17 mg), another product (20 mg) isolated was the dibromide (167), with i.r. ($CHCl_3$) 1755 cm^{-1}. N.m.r. τ 9.03, 8.97, 8.93 (singlets,3H); 5.8-6.8 (multiplet, 3H). The mass spectral molecular ion was a 1:2:1 triplet at 462 $\pm$ 2, consistent with $C_{20}H_{30}O_2Br_2$.

The bromohydrin-acetate (168)

Hydroxy-lactone (151) (40 mg) was converted to its bromohydrin-acetate (168) (26 mg) in the same way as above. I.r. ($CHCl_3$) 3600, 1763, 1745 cm^{-1}. N.m.r. τ 9.07, 9.02, 8.95, 7.91 (singlets, 3H); 5.2-6.2 (multiplet, 3H). Molecular ion (Mass spectrum) was a doublet, m/e 456.1503

and 458.1482; $C_{22}H_{33}O_5{}^{79}Br$ requires 456.1512; $C_{22}H_{33}O_5{}^{81}Br$ requires 458.1492.

The two isomeric components of (168) could, in this case, be resolved by preparative t.l.c., but the i.r. spectra of the separated components were indistinguishable from each other and from the mixture (168). Hence, all subsequent reactions were performed on the total material.

Dehydration of (168)

The bromohydrin-acetate (168) (24 mg) was left for 1 hr. at 0°C with thionyl chloride (0.4 ml) and pyridine (0.5 ml). After the normal work up it was found that there was clean conversion to the olefin (170) (21 mg), an amorphous solid. I.r. ($CHCl_3$) 1760, 1740 cm^{-1}. N.m.r. τ 9.17, 8.97, 8.80, 7.90 (singlets, 3H); 5.2-6.2 (multiplet, 3H); 4.74 (narrow triplet, 1H).

Molecular ion (Mass spectrum) was a doublet, m/e 438.1401 and 440.1388; $C_{22}H_{31}O_4{}^{79}Br$ requires 438.1406; $C_{22}H_{31}O_4{}^{81}Br$ requires 440.1386.

Catalytic hydrogenation of the Δ^5-olefin (170)

Use was made of the results from the hydrogenation of the model olefin (153). Accordingly, the olefin (170) (21 mg) was hydrogenated for 13 hours with 60 mg of rhodium-platinum (3:1) mixed oxide in acetic acid (7 ml). Recovery by filtering, diluting with water and extracting with ethyl acetate, yielded a mixture of 4 components on t.l.c.

A minor component (4 mg, 20%), isolated by preparative t.l.c., was deoxyrosenonolactone bromohydrin-acetate (166), identical on t.l.c., i.r., n.m.r. with an authentic sample prepared from deoxyrosenonolactone as described previously.

The major product isolated (8 mg) was the acetate of probable structure (171): i.r. ($CHCl_3$) 1756, 1735 cm^{-1}. N.m.r. 3H singlets at τ 9.05, 9.01, 8.91; 7.99 (OAc); triplet at τ 5.85 (2H, J 8Hz). Mass spectral m.wt. 362 ($C_{22}H_{34}O_4$).

Allylic oxidation of the Δ^5-olefin (170)

The olefin (170) (10 mg) was left at 40°C for 15 hours with anhydrous sodium chromate[100] (8 mg) in 5 ml of a solution of acetic acid-acetic anhydride (2:1). Water was

added and the solution extracted with ether. The products, recovered as usual from the ether phase, were freed of residual acetic acid by azeotroping with benzene. The enone bromohydrin-acetate (169) (8 mg), isolated by preparative t.l.c., was identical in i.r., U.V., n.m.r., t.l.c. with an authentic specimen prepared, as described before, from the enone (155).

Catalytic hydrogenation of the enone (169)

The enone (169) (67 mg) was hydrogenated for 70 hours with 30% palladium-charcoal (60 mg) in ethyl acetate (7 ml). After filtration and removal of solvent, the main products were shown on t.l.c. to be the unreacted enone (40%) and rosenonolactone bromohydrin-acetate (165) (50%, two close spots). Preparative t.l.c. yielded 10 mg of (165), identical in i.r., n.m.r. and t.l.c. with an authentic specimen prepared directly from rosenonolactone (135). There was surprisingly little hydrogenolysis products compared to the model reductions on enone (155).

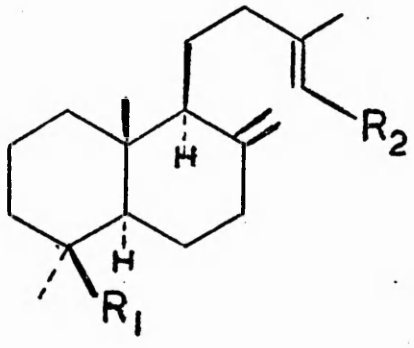
Removal of bromohydrin-acetate group from (166)

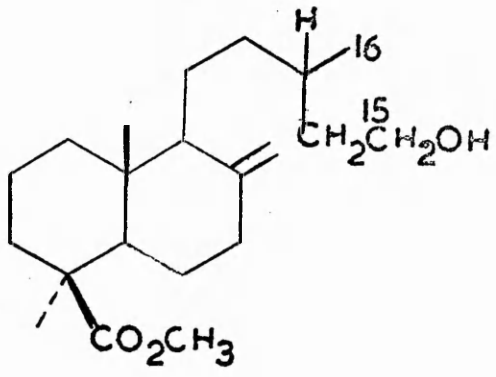
The zinc-copper couple[99] was prepared by shaking zinc dust (10 g) and hydrated copper sulphate (10.2 g) for 3 hours in water (70 ml). The residue was washed several times by shaking and decantation, with water then with ethanol.

The bromohydrin-acetate (166) (16 mg) was stirred under reflux with freshly-prepared zinc-copper couple (300 mg) in ethanol (10 ml). After 2 hours the solution was filtered hot. Removal of the ethanol gave a residue which was dissolved in ether, washed with water, brine and dried. Preparative t.l.c. of the recovered material yielded deoxyrosenonolactone (136) (12 mg) m.p. 114-116°C (prisms from ether), $[\alpha]_D$ + 54° (C = 0.68 $CHCl_3$). (Lit.[32] values 115-116°C, $[\alpha]_D$ + 57°). I.r. 3080, 1778, 1640, 946 cm^{-1}. N.m.r. singlets at τ 9.04 (6H), 8.93 (3H); τ 3.9-5.3 (multiplet, 3H). The t.l.c., g.l.c. (1% SE30; Table 4), i.r. and n.m.r. were identical with those of an authentic specimen of deoxyrosenonolactone.

Removal of bromohydrin-acetate group from (165)

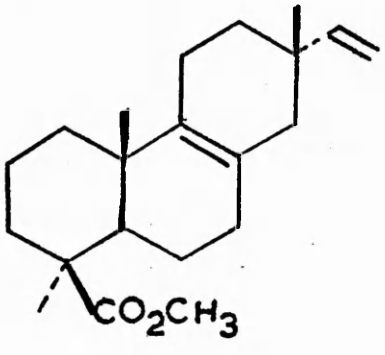
Rosenonolactone bromohydrin-acetate (165) (25 mg) was treated with the zinc-copper couple as above to give rosenonolactone (135) (14 mg); m.p. 210-212°C, $[\alpha]_D$ -121° C = 1.7 $CHCl_3$) (Lit.[28] m.p. 208°, $[\alpha]_D$ -116°). I.r. 3080,1782, 1722, 1640 cm^{-1}. N.m.r. singlets at τ 9.05, (6H), 8.90 (3H); τ 3.9-5.2 (multiplet, 3H). The t.l.c., g.l.c. (1% SE30; Table 4), i.r. and n.m.r. were identical with those of an authentic specimen of rosenonolactone.





129

	R_1	R_2
123	CO_2H	CO_2H
124	CO_2CH_3	CO_2CH_3
125	CO_2CH_3	CO_2H
126	CO_2CH_3	$COOCOOCH_3$
127	CO_2CH_3	CH_2OH
128	CO_2H	CH_2OH
75	CH_2OH	CH_2OH
80	CH_2OAc	CH_2OAc



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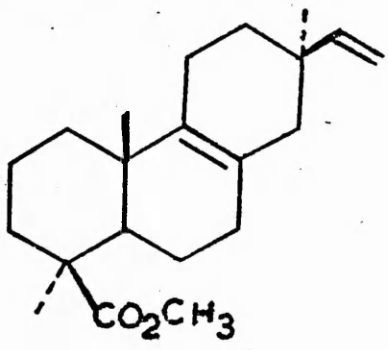

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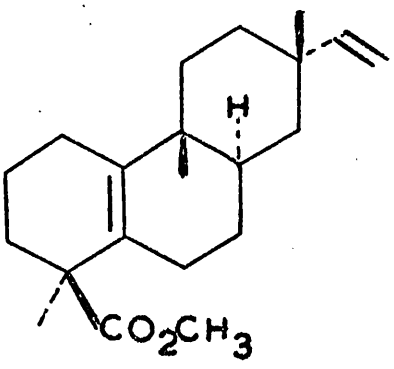

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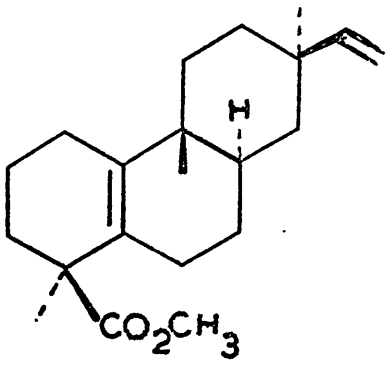

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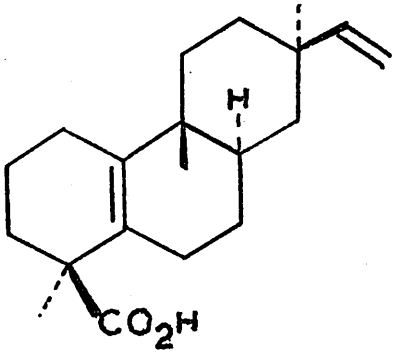

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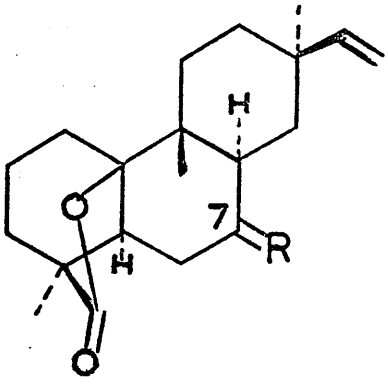
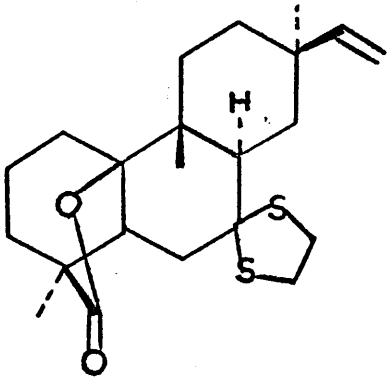


135 R = O

136 $R = H_2$



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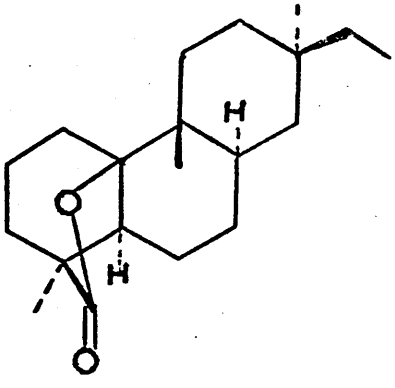

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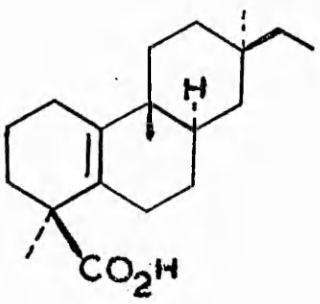
TABLE 3

Relative G.L.C. Retention Times

	1% SE30 175°C	10% P.E.G.A. 175°C
Pimaradiene ester (130)	850	2090
Pimaradiene ester (131)	929	2335
Rosadiene ester (132)	745	1810
Rosadiene ester (133)	929	
Methyl isopimarate (145)	1350	
Methyl isopimarate Δ^8 isomer (144)	945	
Methyl pimarate (147)	1090	
Methyl pimarate Δ^8 isomer (146)	1060	
n $C_{20}H_{42}$	530	165
n $C_{22}H_{46}$	1195	335
n $C_{24}H_{50}$		635



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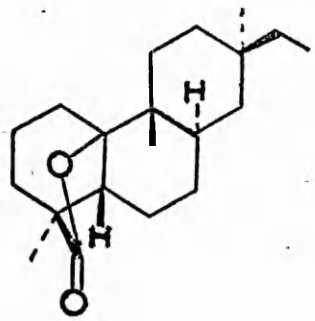

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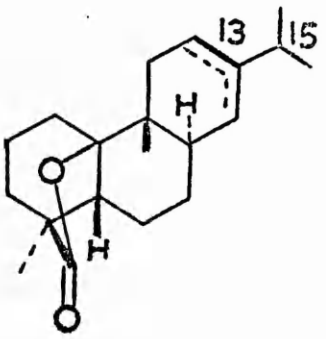

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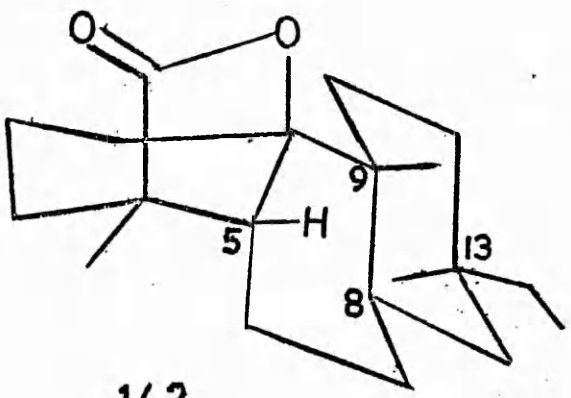

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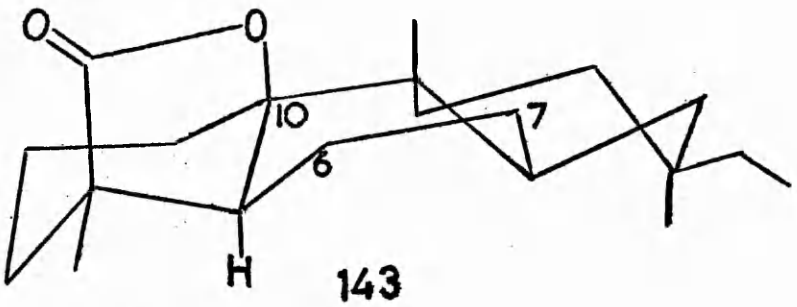

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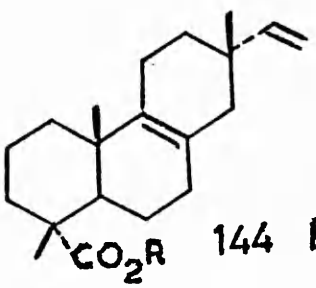
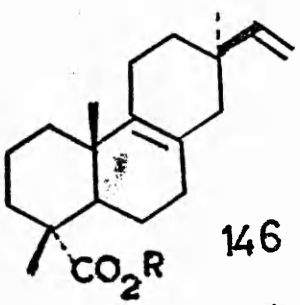


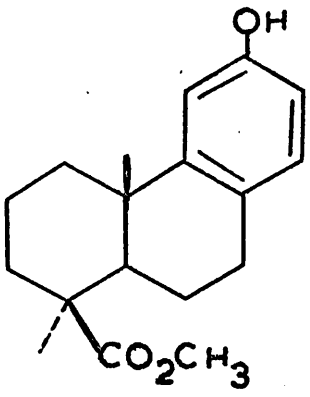
144 R= CH_3

145 R=H; Δ^7



146 R=CH_3

147 R=H; $\Delta^{8(14)}$



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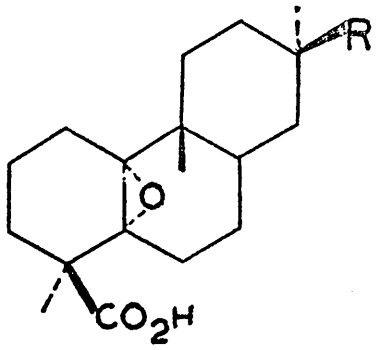
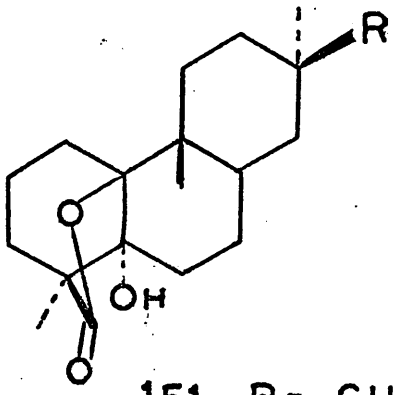


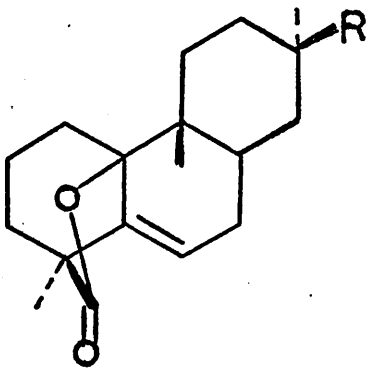
149 R = $CH{=}CH_2$

150 R = Et



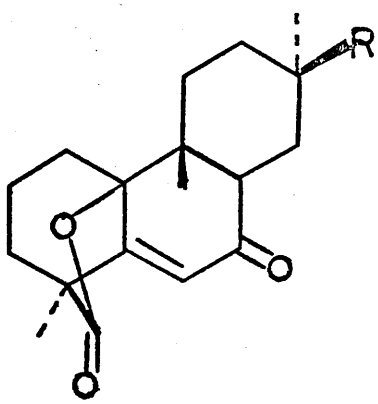
151 R = $CH{=}CH_2$

152 R = Et



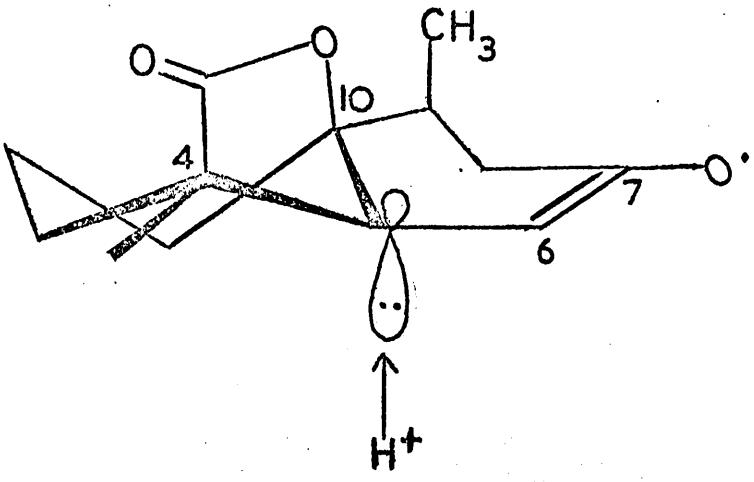
153 R = $CH{=}CH_2$

154 R = Et



155 R = $CH{=}CH_2$

156 R = Et



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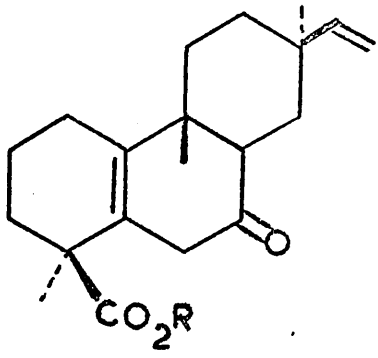
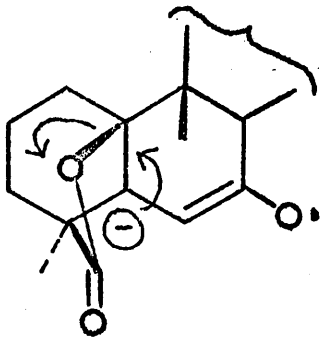


158 R = H

159 R = CH_3



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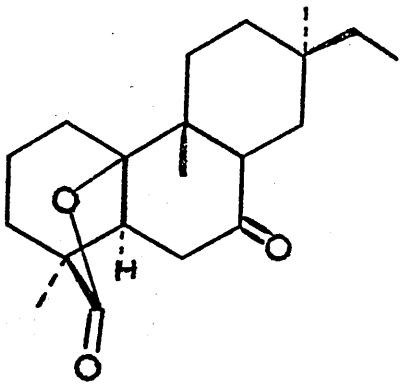

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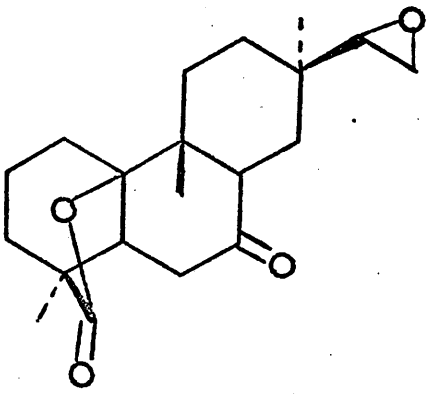

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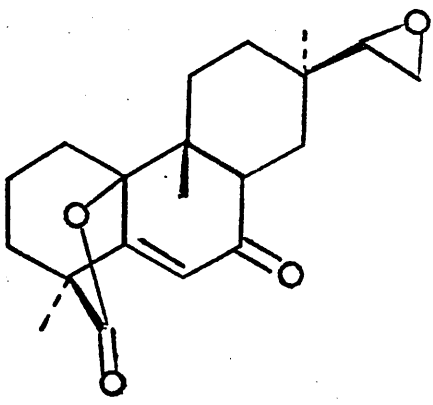
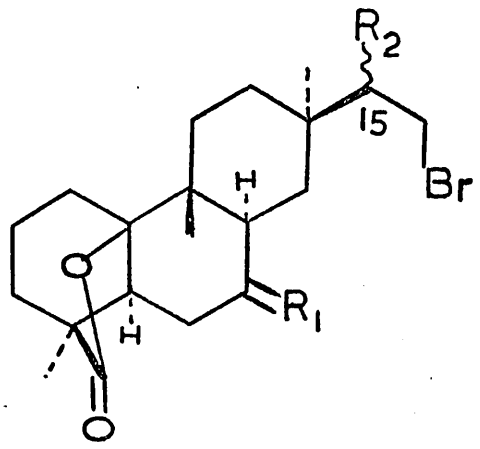


163

TABLE 4

Relative G.L.C. Retention Times

		1% SE30 175°C	1% SE30 185°C
Rosenonolactone	(135)	500	471
" 15,16 dihydro	(161)	544	520
Enone	(155)	363	360
" 15,16 dihydro	(156)		388
Deoxyrosenonolactone	(136)	297	296
" 15,16 dihydro	(138)	328	
Δ^5 olefin	(153)		228
" 15,16 dihydro	(154)	244	254
n $C_{20}H_{42}$		100	100
n $C_{22}H_{46}$		224	208
n $C_{23}H_{48}$			303
n $C_{24}H_{50}$		523	438

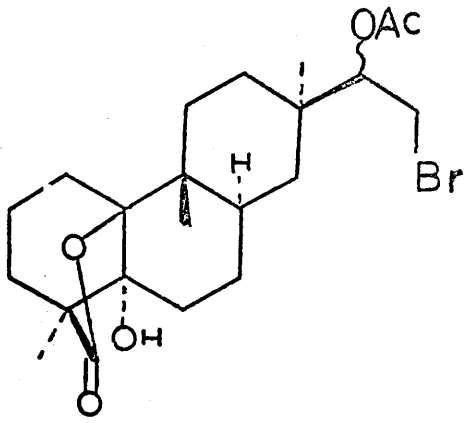


164 $R_1 = O$, $R_2 = OH$

165 $R_1 = O$, $R_2 = OAc$

166 $R_1 = H_2$, $R_2 = OAc$

167 $R_1 = H_2$, $R_2 = Br$



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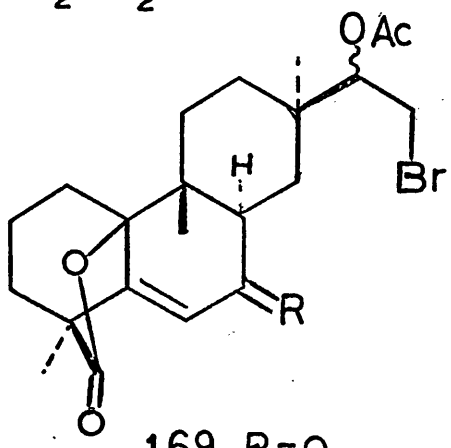
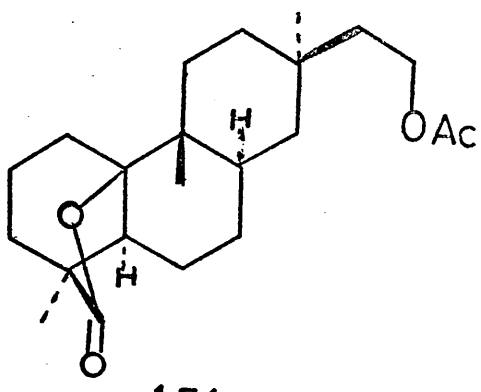
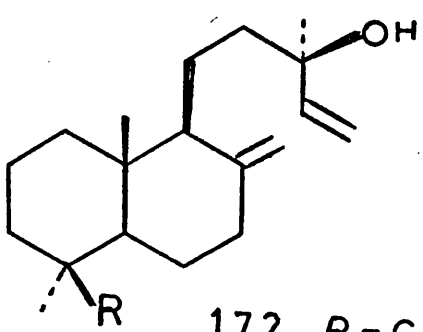


169 R=O

170 $R = H_2$

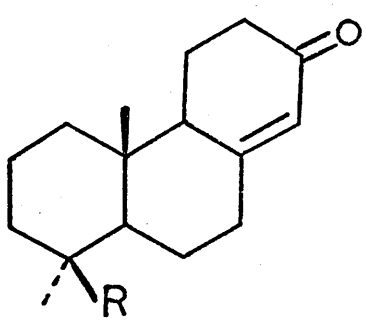


171



172 $R = CH_3$

173 $R = CO_2H$



174 $R = CH_3$

175 $R = CO_2H$

III OTHER HYDROCARBONS ARISING FROM REARRANGEMENT OF THE PIMARA-8,15-DIENES

DISCUSSION

Our earlier studies had shown that the initial products from the acid-catalysed cyclisation of labdadienols were the two epimeric pimaradienes (87) and (88). Under prolonged acid conditions the epimer (87) was further transformed to the rosadiene (111) (50% on g.l.c.; Figure 7b) as one of four peaks on g.l.c. Similarly, (88) gave a mixture of four main components on g.l.c., one of which was identified as the rosadiene (112) (30% on g.l.c.; Figure 7c). At the time, only the rosadienes were relevant to the synthetic project; however, it was now considered worthwhile to re-examine the rearrangement products from the two pimaradienes with the aim of identifying some of the other structural types present.

In the knowledge that the rearrangments proceed via carbonium ions, one can make certain reasonable predictions about the nature of the products: quite possibly they will belong to the known diterpenoid skeletal types, since, as outlined before in Figure 2, the olefinic cation (13) may perhaps occupy a central position in the postulated scheme

observed, admittedly on oxygenated derivatives. Thus, Wenkert and Chamberlin[67] had found that sulphuric acid treatment of both pimaric acid (205) and isopimaric acid (200) yielded abietic acid (178) in addition to the γ and δ abietic lactones, (179) and (180). This also shows that the C-13 to C-15 methyl migration takes place irrespective of the C-13 configuration, despite earlier arguments[6] that migration of a quasi-axial methyl should be easier than a quasi-equatorial one. The formation of the rearranged lactone (141) from our attempts to lactonise the diene-acid (134) was further evidence of the facile nature of the C-13 to C-15 methyl shift.

(c) If protonation of the 8,9 double bond were to give a C-8 cation then this could interact with the 15,16 vinyl group to yield, after further rearrangement, the tetra- and pentacyclic hydrocarbons. When $\Delta^{8,15}$-pimaradiene (88) is the starting epimer, then, by a process outlined in Figure 8, the products would be stachene (184), kaurene (185), isokaurene (186), atisirene (187), isoatisirene (188) and trachylobane (189). When the starting epimer is $\Delta^{8,15}$-isopimaradiene (87), ring C must first adopt a boat conformation (190) before the vinyl group can interact with the C-8 cation; the products, as shown in Figure 9, would be the (C-8, C-13) isomeric series—isohibaene (193), phyllocladene (194), isophyllocladene (195),

neoatisirene (196), iso-neoatisirene (197), and isotrachylobane (198).

Although many cases have now been recorded of acid-catalysed interconversion of the tetracyclic skeletons themselves, the *in vitro* transformation of a pimaradiene to a tetracyclic system has yet to be performed. Recently Wenkert[72] and Edwards[73] have isolated the tetracyclic compound, 14α-hibyl formate (198) in 10% yield from the formolysis of the bicyclic alcohol manool (70). Since the major products were the pimaradiene epimers (87) and (88), this, therefore, suggested that (198) was arising from the pimaradiene (88) by the 'biogenetic' pathway shown in Figure 10. However, labelling studies[108,109] have now shown the mechanism to be as in Figure 11, where, in an unusual first step, the exo-methylene double bond of the labdane structure captures the allylic cation at the terminal C-16 position to give the cyclooctenyl cation (199). The hibyl system is subsequently derived from this cation by a transannular reaction, followed by a 1,2 alkyl shift.

With this background information - that only the rosadiene and abietadiene structures have so far been observed from rearrangement of pimaradienes - a reinvestigation of the products from acetic-sulphuric acid treatment of (87) and

(88) was undertaken, using two methods of analysis:-

(1) Direct g.l.c. and g.c.m.s. comparison of the products with authentic olefin structures.

(2) Use of specifically deuterated pimaradiene substrates to aid the mass spectral identification of new structural types. From an analytical viewpoint, the most informative kind of labelled substrates will be those derivatives in which the deuterium is at widely differing sites in the molecule. Accordingly, isopimaradiene (203), 18-d_1-isopimaradiene (204) and 16-d_2-isopimaradiene (212) were prepared from isopimaric acid (200), as described below. Although these compounds have a Δ^7 double bond it is known[72] to be easily isomerised on acid treatment, giving mainly the desired Δ^8 isomer.

Isopimaric acid (200) was reduced with lithium aluminium hydride in ether to yield isopimarinol (201).[66] The conversion of isopimarinol to isopimaradiene by the method of Ireland[66] (oxidation to the aldehyde and Wolff-Kishner reduction of its semicarbazone) could have been adapted to prepare a dideuterated derivative, by performing the Wolff-Kishner step in the presence of deuterated solvent. However, it was felt that reduction of the tosylate (202) by lithium aluminium deuteride would be a 'cleaner' method - specifically introducing only one deuterium at C-18 to give the derivative (204).

Isopimarinol tosylate (202) was prepared by treatment of isopimarinol, for two days at 0°C, with an excess of p-toluenesulphonyl chloride in pyridine. Confirmation of the tosylate functionality came from the characteristic i.r. absorption at 1370, 1190, 1180 cm^{-1} and from the presence in the n.m.r. spectrum of an AB quartet, J 10 Hz, at τ 6.45, 6.35 (C-18 methylene) and an AA^1 BB^1 quartet, J 8 Hz, at τ 2.65, 2.21 (four aromatic H).

Although the tosylate is derived from a primary alcohol, it was still a 'neopentyl' system and reduction was expected to be fairly slow. Hence the conditions necessary for reduction were first established, with lithium alumnium hydride (LAH) itself:- After refluxing for 80 hours in dioxan with excess LAH, the tosylate was shown on t.l.c. to have been converted into a hydrocarbon fraction and a more polar component (later identified as isopimarinol). The separated hydrocarbon fraction, one single peak (95%) on g.l.c., was identified as isopimaradiene (203) since its i.r., g.l.c. (data in Table 2), mass spectrum (molecular weight 272) and n.m.r. were indistinguishable from those of an authentic sample prepared from isopimaric acid by Ireland's method.[66] Similarly, the tosylate (202) was reduced with lithium alumnium deuteride to give 18-d_1-isopimaradiene (204)

(90% pure), indistinguishable on g.l.c. from isopimaradiene. The mass spectrum (g.c.m.s., molecular weight 273) showed the material to be almost exclusively monodeuterated.

The preparation of 16-d_2-isopimaradiene (212) required the cleavage of the vinylidene group to the nor-aldehyde, followed by addition of a CD_2 unit from a Wittig reaction. Thus, isopimaradiene was reacted for 48 hours at 20°C with osmium tetroxide in dry dioxan to yield, after preparative t.l.c., the diol (210),[110] presumably two C-15 epimers. Without further characterisation, the diol was cleaved by stirring for 2 hours with periodic acid in ether. The cleanly formed product was confirmed to be isopimaradiene-16-nor-aldehyde (211)[110] from its i.r. absorption at 2690 and 1731 cm^{-1}.

The Wittig reaction between the aldehyde and trideuteromethyl triphenylphosphonium iodide (prepared from equivalent amounts of triphenylphosphine and methyl iodide-d_3) needs a basic catalyst. Our first choice, potassium t-butoxide in t-butanol,[111] was quite ill-conceived; for, although the condensation went smoothly, the product was found to be undeuterated isopimaradiene (203), showing that all deuterium had been lost from the 'ylid reagent by exchange with the alcohol proton source.

Success was eventually obtained using n-butyl lithium as base. The aldehyde (211) was reacted for 16 hours in ether-tetrahydrofuran with a mixture of the Wittig salt and n-butyl lithium. After chromatography, the dideutero-diene (212) was isolated as an oil, indistinguishable on g.l.c. from isopimaradiene. Evidence for its structure came from the n.m.r. spectrum which was identical with that of isopimaradiene, except that the high field, AB part of the vinylidene ABX multiplet was missing and the X part had now become a broad singlet at τ 4.20. This is consistent with the $CH{=}CD_2$ side chain grouping. The mass spectral molecular weight of 274 (g.c.m.s.) confirmed the compound to be dideuterated, although the small peak at m/e 273 (15% of 274) is presumably due to some d_1-species. (This might be arising by exchange on the g.c.m.s. column).

With the completed preparation of isopimaradiene (203) and the deuterated derivatives, (204) and (212), the whole procedure was repeated on the C-13 epimeric series. Thus, pimaric acid (205) was reduced with lithium aluminium hydride to pimarinol (206),[66] which, on treatment with p-toluenesulphonyl chloride, afforded the tosylate (207), identified by its i.r. (1370, 1185, 1175 cm^{-1}) and n.m.r. spectra (AB quartet, J 9Hz, τ 6.48, 6.28; AA' BB' quartet

J 8Hz, τ 2.69, 2.27). Upon prolonged reduction with lithium alumnium hydride, as described previously, the tosylate (207) was converted into pimaradiene (208), (95% pure on g.l.c.), identical in all respects with an authentic specimen obtained[66] from pimaric acid. When the tosylate was reduced with lithium aluminium deuteride, 18-d_1-pimaradiene (209) was obtained, 95% pure on g.l.c. It was shown by g.c.m.s. (molecular weight 273) to be completely monodeuterated.

Treatment of pimaradiene (208) with osmium tetroxide in dioxan furnished, after chromatography, the diol (213) - presumably two C-15 epimers. Without further characterisation the diol was reacted with periodic acid, as described before, to yield pimaradiene-16-nor-aldehyde (214); i.r. 2700, 1748 cm^{-1}.

On reaction with the Wittig salt, using n-butyl lithium as base, the aldehyde was cleanly converted into 16-d_2-pimaradiene (215), identical g.l.c. retention time to pimaradiene itself. Assignment of the structure (215) came from the n.m.r. which was the same as for pimaradiene, except that the vinyl ABX multiplet had been replaced by a broad olefinic signal at τ 4.28 (H-15). The mass spectrum again showed there to be small amounts (15%) of a d_1-species (m/e 273).

The availability of the three isopimaradienes (undeuterated, 18-d_1, 16-d_2) and the three pimaradienes (undeuterated, 18-d_1, 16-d_2) presented us with an excellent opportunity to verify the postulated mass spectral fragmentation mechanism of these hydrocarbons. Recently, Fetizon and his group[83] have examined the mass spectra of many tricyclic diterpenoid olefins, including isopimaradiene (203) and pimaradiene (208). However, their published cracking patterns are based mainly on analogy with work in the triterpenoid field or else on the use of oxygenated relatives - less reliable than studies with deuterated derivatives.

Reproduced in Figure 12 are the three main fragmentation pathways of pimaradiene (208) giving peak A (m/e 137), peak B (m/e 136) and peak C (m/e 148), where peaks A and B are derived from ring-A fragments of the diterpenoid and peak C comes from ring-C. The mass spectra of the deuterated pimaradienes (Figure 13) were found to be in complete harmony with the above spectral analysis:- Thus, in the 18-d_1 compound (209), peaks A and B were displaced to m/e 138 and 137 respectively, with peak C being unchanged; whereas, in the 16-d_2 compound (215), peak C was displaced to m/e 150, yet both A and B peaks were unchanged from m/e 137 and 136 respectively.

The postulated fragmentation pathways of isopimaradiene (203) are outlined in Figure 14, the principal peaks being F (m/e 124), F-15 (m/e 109), C (m/e 148) and C-15 (m/e 133). The first two peaks arise from ring-A in the skeleton and the last two come from ring-C. Once again the spectra of the deuterated isopimaradienes (Figure 15) were in full accord with the mass spectral analysis:- In the 18-d_1 species (204) peaks F and F-15 were displaced to m/e 125 and 110 respectively, leaving peaks C and C-15 unaffected. However, in the 16-d_2 compound (212), peaks C and C-15 were now displaced to m/e 150 and 135 respectively, whereas peaks F and F-15 were still as in isopimaradiene.

Acid rearrangement of the pimaradienes and isopimaradienes

Before the rearrangements proper it was necessary to first check that the 7,8 double bond of isopimaradiene (203) and the 8,14 double bond of pimaradiene (208) would quickly isomerise, as expected, into the 8,9 position, under the acid conditions employed. Accordingly, isopimaradiene and pimaradiene were each reacted for twelve hours at room temperature with the same acetic-sulphuric acid solvent used previously. Under these mild conditions the dienes were shown by g.l.c. to be 95% transformed into their 8,9

double bond isomers, (87) and (88) respectively. Hence it can be safely concluded that any products of prolonged acid reaction must have arisen from rearrangement of the 8,9 olefinic compounds (87) or (88).

The three isopimaradienes - parent (203), monodeuterated (204) and dideuterated (212) - were individually reacted with acetic-sulphuric acid at 60°C for 75 hours. G.l.c. analysis of the isolated hydrocarbon fractions revealed that the product composition was the same from all three starting derivatives, but it was markedly different from that obtained in our earlier studies during the synthesis of the rosane metabolites. The new g.l.c. trace, reproduced in Figure 16, contained 5 main peaks with the longest retained component (peak E) accounting for approximately 50% of the whole hydrocarbon fraction. On closer examination it was observed that no unreacted $\Delta^{8,15}$-isopimaradiene (87) remained and that the rosadiene (111) - previously the major product from isomerisation of (187) - was now present only in about 10% amount (peak B).

The failure to reproduce the same hydrocarbon mixture as we had obtained before is probably due to the different reaction conditions employed:- that is, a temperature of 60°C compared to the previous 50°C (although a shorter

reaction time - 75 hours as opposed to 150 hours - should partly compensate for this). Confirmation of our suspicions that our latest rearrangement conditions were more vigorous than before, and, hence, had caused some further change in the rosadiene (111) itself, came from examination of a portion of the reaction mixture after only 47 hours reaction. Although the same 5 peaks were present with peak E (50%) still the major component, the rosadiene (111) was found to be in 30% abundance at that stage. Nevertheless, it was decided to adhere to the new experimental conditions and attempt to identify some of the structural types present.

Reaction of the three pimaradienes - parent (208), monodeuterated (209) and dideuterated (215) - at 60°C for 75 hours also furnished hydrocarbon products of different composition from those of the earlier studies. The latest g.l.c. trace, reproduced in Figure 17, contained 6 main peaks of which only two could be recognised (g.l.c., g.c.m.s.) from before:- These were peaks D and F, which corresponded respectively to the least retained (20%) and most retained (40%) components - both unidentified - of our previous g.l.c. trace (Figure 7c).

To our surprise, however, 5 of the peaks from this latest rearrangement (all except the shortest retained component F) had the same g.l.c. retention times as the products from the epimeric isopimaradiene series described above. Although the similarity of the g.l.c. traces could perhaps be explained by 'accidental equivalence' of peaks, the observation, that the most retained component (peak E) is the major product (50%) in both epimeric series, strongly suggests that, at least, this major component from both series is one and the same compound. This, therefore, provided a clue to the nature of the major product; for the simplest way in which two C-13 epimers can react to yield the same compound is by a process in which the asymmetry at C-13 is destroyed. Such a rearrangement is the pimaradiene - abietadiene transformation, where the C-13 to C-15 methyl shift leaves C-13 as a planar, trigonal centre. Abietadiene structures, however, are not the only possibility, since backbone rearrangement to rosadiene systems, or even further, can occur independently of the abietadiene rearrangement in the side chain. Hence, 'mixed' rosa-abietadiene structures could also be expected present.

Our first approach in identifying any rearrangement products was to 'screen' the two hydrocarbon mixtures for the presence of any tetracyclic compounds. We were fortunate in having available, for direct g.l.c. comparison, the following authentic tetracyclic compounds - stachene (184), kaurene (185), isokaurene (186), phyllocladene (194), isophyllocladene (195), neoatisirene (196) and iso-neoatisirene (197). The additional knowledge,[115] that kaurene is inseparable on g.l.c. from atisirene (187) and isokaurene from isoatisirene (188), meant that nine out of the ten possible tetracyclic compounds [isohibaene (193) was the sole exception] could be compared on g.l.c. with the rearrangement products.

However, these tetracyclic compounds were found to be completely absent from the products of rearrangement. As can be seen from the g.l.c. data in Table 2, the tetracyclic compounds generally have much longer retention times (SE30) than the products of acid treatment of pimara- or isopimara- diene. Only the least retained tetracyclics - stachene and iso-neoatisirene - had retention times even remotely near those of the final two peaks

(D and E) in the rearrangement products, yet their mass spectra (g.c.m.s.) were completely different.

The somewhat surprising absence of tetracyclic hydrocarbon structures [and presumably of the pentacyclic trachylobanes (189) and (198)] compelled us to concentrate our attention on the tricyclic classes. Except for the pimara- and rosa- dienes, the only other compound available for direct comparison was dolabradiene (176). However no peak was found to correspond to dolabradiene on g.l.c. and, furthermore, its mass spectrum[83] was quite unlike any of these of the rearrangement products. In any event, dolabradiene was discovered to be unstable to the acetic-sulphuric acid conditions used:- After 50 hours at 50°C it had been transformed into two main products - the rosadiene (111) and a product which, by analogy with the acid isomerisation of erythroxydiol Y acetonide (216),[71] must be the Δ^3 double bond isomer of dolabradiene. This Δ^3 compound could not be detected present (g.l.c.) in the rearrangement products from isopimaradiene.

So far, the direct g.l.c. comparison of the rearrangement products with authentic samples of

'anticipated structures' had mainly served to establish what structural types were absent. Valuable as this negative evidence was, it now became necessary to try to identify some of the products from their mass spectral characteristics and the mass spectra of the deuterated derivatives. Unfortunately the 16-d_2 derivatives, (212) and (215), did not prove to be entirely satisfactory, since much of the deuterium was lost during the subsequent acetic-sulphuric acid treatment - undoubtedly from reversible protonation of the vinylidene double bond. The average deuterium content in the hydrocarbon products from rearrangement of (212) or (215) was:- 50% d_0, 25% d_1 and 25% d_2; [notable exceptions were the major products (peak E) from (212) and (215), which had lost all deuterium from C-16].

It was decided to confine our attentions to peaks E in the rearrangement products, for the following reasons:-

1) This peak is by far the most abundant compound from rearrangement in both C-13 series.
2) The identity in g.l.c. retention time and mass spectrum of the major peaks E from both C-13 series strongly suggested that they were the same compound.

3) A rather poor resolution was obtained on the g.c.m.s. column, with the result that E was one of the few peaks sufficiently well separated to guarantee meaningful mass spectra.

4) Unlike the other rearrangement products which had 'indifferent' mass spectra - that is, no dominant fragmentation process was occurring - peak E had a unique spectrum, completely dissimilar to that of any known tricyclic diterpenoid.

Reproduced in Figure 18 is the mass spectrum of the major product E, when obtained from acid treatment of pimaradiene (or isopimaradiene); below it is the spectrum of E when the 18-d_1 species (209) was the substrate. (The spectrum of E derived from the 16-d_2 compounds was identical with that of E derived from their undeuterated parents, since all the deuterium was lost during reaction). The undeuterated spectrum showed 4 main peaks:- m/e 176 (100%), m/e 161 (80%), m/e 149 (39%) and m/e 133 (40%). The general 'cleanness' of the spectrum indicated that one particularly favourable fragmentation mechanism was operating at the expense of any other. Since the base peak, m/e 176, has even mass this must have arisen by a double cleavage process - most probably a retro-Diels-Alder reaction.

In the monodeuterated (18-d_1) spectrum the base peak was displaced to m/e 177; hence this ion fragment must be derived from rings A and B.

The above facts can best be accommodated by assigning E the rosa-abietadiene structure (217), with 5,10 and 12,13 double bonds. Such a structure would be expected to undergo a facile retro-Diels-Alder fragmentation, shown in Figure 19, to give the ion (219), m/e 176. Ample precedent exists for such a fragmentation process; indeed, it is widely recognised to be the principal breakdown mode of Δ^{12} pentacyclic triterpenoids[116] and of Δ^{12} diterpenoids such as (218).[83]

It is necessary to invoke the rosane part-structure for rings A and B, since the above retro-Diels-Alder process can not occur if the second double bond is in the 7,8; 8,9; 9,11 or 8,14 positions of a 'pure' abietadiene skeleton. Although the allocation of the second double bond to the tetrasubstituted 5,10 position could be justified on stability grounds alone, the rest of the mass spectrum was completely compatible with such a structure:- Thus the large peak (80%) at m/e 161 - loss of a methyl group from the ion m/e 176 - becomes a 1:1 doublet at m/e 161, 162 in the 18-d_1 deuterated spectrum. If the loss of any

one of the three remaining methyl groups were equally probable, this would have given a 1:2 doublet; however, with structure (219) for ion m/e 176, the loss of the two quaternary methyls from the allylic C-4 position should be much easier than the vinylic methyl from C-9. Furthermore, the ion fragment m/e 149 (becomes 150 on deuteration) could have arisen by the reasonable, alternative process shown in Figure 19.

The structure (217) for the major rearrangement product is very acceptable, not only as it explains why the same compound was obtained from both C-13 epimeric series, but because there is now much evidence for the facile nature of both rosane and abietane skeletal rearrangements. However, one can not completely exclude the Δ^{13} double bond isomer of (217) as a possibility, since double bond migration is known to occur after ionisation in the mass spectrometer.[117,118] Although (217) is the only example of a 'mixed' rosa-abietadiene hydrocarbon, this skeleton has been obtained before in an oxygenated form: for example, the γ lactone (179) from acid treatment of pimaric and isopimaric acid;[67] or the γ lactone (141) obtained from our attempts to convert the acid (134) into deoxyrosenonolactone. A second alternative to structure (217) - the 13,15 double

bond isomer - was discounted since no evidence for such an exocyclic double bond could be found in the n.m.r. of the analogous compound (141).

In summary, therefore, prolonged acetic-sulphuric acid treatment of the pimara-8,15-dienes has given the diene (217) as the major hydrocarbon product. Double bond isomers of this skeleton may account for some of the other unidentified products. Conspicuously absent were the tetracyclic diterpenes, although the pimaradienes are considered the biogenetic precursors of such compounds. However, only the hydrocarbon fraction was investigated in the course of this work and it may well be that the acetate fraction from rearrangement is a rich source of other structural types.

EXPERIMENTAL

Reduction of isopimaric acid (200)

Isopimaric acid (855 mg) was refluxed for 6 hours with excess lithium aluminium hydride in anhydrous ether (15 ml). Work up in the usual fashion gave the known[66] isopimarinol (201) as an oil (830 mg). I.r. (liq. film) 3400, 1640, 1050, 1010, 922, 875, 845, 830, 690 cm^{-1}. Homogeneous on t.l.c.

Reduction of pimaric acid (205)

Treated as above, pimaric acid (500 mg) gave[66] pimarinol (206) as an oil (467 mg), t.l.c. pure. N.m.r. τ 9.21, 9.20, 9.01 (singlets, 3H); 6.84, 6.58 (AB quartet, 2H, J 11Hz), 4-5.3 (multiplet, 3H), 4.84 (singlet, 1H).

Isopimarinol tosylate (202)

Isopimarinol (455 mg) was left for 2 days at 0°C with recrystallised p-toluenesulphonyl chloride (465 mg, 50% excess) in dry pyridine (5 ml). The solution was added to ice-cold hydrochloric acid solution (50 ml, 1M) and then extracted with ethyl acetate. This extract was washed with dilute sodium bicarbonate, thoroughly with water, brine and

dried. Removal of solvent gave the tosylate (202) as a glassy solid (667 mg), quite pure on t.l.c. I.r. 3070, 3030, 1635, 1370, 1180, 1190 cm^{-1}. N.m.r. 3H singlets at τ 9.18 (two), 9.14, 7.59; 6.45, 6.35 (AB quartet, 2H, J 10Hz), 5.3-3.9 (multiplet, 3H), 4.8 (broad singlet, 1H); 2.65, 2.21 (AA' BB' quartet, 4H, J 8Hz). (Found: C, 73.15; H, 8.66. $C_{27}H_{38}O_3S$ requires C, 73.27; H, 8.65%).

Pimarinol tosylate (207)

By the method above, pimarinol (467 mg) gave the tosylate (207) as an oily, semi-solid (702 mg), pure on t.l.c.; i.r. 1370, 1185, 1175, and 960 cm^{-1}. N.m.r. τ 9.27, 9.20, 9.03 (singlets, 3H each); 6.48, 6.28 (AB quartet, 2H, J 9Hz), 5.4-4.0 (multiplet, 3H), 4.90 (broad singlet, 1H); 2.69, 2.27 (AA' BB' quartet, 4H, J 8Hz). (Found: C, 72.81; H, 8.39. $C_{27}H_{38}O_3S$ requires C, 73.27; H, 8.65%).

Isopimaradiene (203)

The tosylate (202) (150 mg) was refluxed with excess lithium aluminium hydride in dry dioxan (8 ml) for 80 hours. The minimum required amount of water was carefully

added and the solution filtered from the gelatinous, white precipitate, which was thoroughly extracted with hot solvent. Removal of solvent and preparative t.l.c. gave isopimaradiene (203) as an oil (40 mg); i.r. 3080, 1640, 910 cm^{-1}; n.m.r. 3H singlets at τ 9.16 (three), 9.09; 4.64 (singlet, 1H), 3.9-5.3 (multiplet, 3H). M 272 from g.c.m.s. It was 95% pure on g.l.c. (1% SE30, 140°C) and the mass spectrum n.m.r. and g.l.c. (Table 2) retention time were identical with an authentic sample prepared by the already-mentioned alternative route[66] from isopimaric acid.

18-Deutero-isopimaradiene (204)

Treated with lithium aluminium deuteride ($LiAlD_4$) in the same way as above, the tosylate (202) (150 mg) gave the deuterated diene (204) (30 mg). This was identical on g.l.c. (90% pure) with authentic isopimaradiene and g.c.m.s. gave M 273, with essentially 100% deuterium incorporation.

Pimaradiene (208)

Pimarinol tosylate (207) (214 mg) was treated with lithium aluminium hydride, as above, to yield pimaradiene (208) as an oil (67 mg), 95% pure on g.l.c. (1% SE30, 140°C). The g.l.c. retention time (Table 2), the g.c.m.s. and the n.m.r. [τ 9.25, 9.15, 9.12, 9.00 (singlets, 3H), 4-5.3 (multiplet, 3H), 4.88 (broadened singlet, 1H)] were the same as those of an authentic specimen prepared by the alternative method[66] from pimaric acid.

18-Deutero-pimaradiene (209)

Lithium aluminium deuteride on the tosylate (207) (224 mg) gave the deuterated diene (209) (80 mg). This also was identical on g.l.c. (95% pure) with authentic pimaradiene and had M 273 from g.c.m.s. The deuteration was practically quantitative.

Osmylation of isopimaradiene (203)

Following the literature method,[110] isopimaradiene (245 mg, 0.90 m.moles) was reacted for 48 hours at 20°C with osmium tetroxide (230 mg, 0.90 m.moles) in dry dioxan (7 ml). After bubbling in hydrogen sulphide for a few minutes, the solution was filtered, the residue being

washed with chloroform. On removal of solvent, the crude products (213 mg, mainly diol) were chromatographed to give the known[110] diol (210) as a semi-solid (90 mg), t.l.c. homogeneous. This intermediate was used without further characterisation.

Isopimaradiene-16-nor-aldehyde (211)

The above diol mixture (58 mg) was stirred for 2 hours at 20°C with periodic acid (40 mg, 10% excess) in dry ether (5 ml). The ether was filtered off from the precipitated iodic acid, washed with water, 10% sodium bicarbonate solution, brine, dried and evaporated to yield the known[110] aldehyde (211) (49 mg), pure on t.l.c. I.r. 2690, 1731 cm^{-1}.

Osmylation of pimaradiene (208)

Using the method described previously, pimaradiene (482 mg) was converted to the diol mixture (213) (315 mg), homogeneous on t.l.c.

Pimaradiene-16-nor-aldehyde (214)

The diol mixture (213) (95 mg) was cleaved by periodic acid, exactly as described before, to give the nor-aldehyde (214) (76 mg), pure on t.l.c. I.r. 2700, 1748 cm^{-1}.

The Wittig reaction on the nor-aldehyde (211)

Trideuteromethyl triphenylphophonium iodide, $CD_3P(C_6H_5)_3I$, was, first of all, prepared by mixing equivalent amounts of triphenyl phosphine (2.22 g) and trideuteromethyl iodide, CD_3I (1.22 g) in benzene (5 ml). After 2 days the white, ionic precipitate was filtered off, washed with hot benzene and dried *in vacuo*.

Method A:- Using potassium tert.butoxide as base.[111]

To a suspension of the Wittig salt (286 mg, 0.7 m.moles) in dry ether (8 ml), stirred under nitrogen at room temperature, was added a 0.8 M solution of potassium tert.butoxide in tert.butanol (0.84 ml, 0.67 m.moles). The creamy, yellow solution was stirred for 15 minutes before addition of the aldehyde (211) (48 mg, 0.175 m.moles) in dry ether (10 ml). After stirring overnight more ether was added and the solution washed with water, brine and dried. Evaporation of solvent and preparative t.l.c. gave

an oil (36 mg) which had identical g.l.c. (1 pure peak), n.m.r., g.c.m.s. (M 272) and i.r. with isopimaradiene (203) itself.

Method B:- n-butyl lithium as base.

To a stirred suspension of the Wittig salt (320 mg, 0.79 m.moles) in dry ether (3 ml), under nitrogen at room temperature, was added a solution of n-butyllithium in hexane (0.34 ml of 2.35 M, 0.79 m.moles), followed, 30 minutes later, by the aldehyde (211) (72 mg, 0.262 m.moles) in dry tetrahydrofuran (10 ml.). After 16 hours the ether was replaced by tetrahydrofuran and the reaction mixture refluxed for 4 hours; acetone (2 ml) was added before a final 30 minute reflux. Recovery, in the usual way, and preparative t.l.c. gave the dideutero-diene (212) as an oil (40 mg) indistinguishable on t.l.c. and g.l.c. (1 clean peak) from isopimaradiene. The g.c.m.s. gave M 274. In the n.m.r., the high field, AB, part of the vinyl (τ 3.9-5.3) ABX region was missing and the X part multiplet had become a broad singlet at 4.20 τ. The remainder of the spectrum was identical with isopimaradiene (203).

The Wittig reaction on the nor-aldehyde (214)

Method A:- Using the potassium tert.butoxide, as described previously, the aldehyde (214) (66 mg) gave a diene (54 mg) as an oil. This had t.l.c., g.l.c. (1 pure peak), g.c.m.s. (M 272), i.r. and n.m.r. identical with pimaradiene (208), itself.

Method B:- Using the n-butyllithium method above, the aldehyde (214) (36 mg) yielded the dideutero compound (215) as an oil (18 mg), indistinguishable on t.l.c. and g.l.c. (1 pure peak) from pimaradiene. G.c.m.s. gave M 274 and the n.m.r. vinyl group multiplet had been replaced by a singlet at τ 4.28 (broad, 1H). The remainder of the n.m.r. was the same as that of pimaradiene.

Effect of acid on dolabradiene (176)

Dolabradiene (5 mg) was reacted for 50 hours at 50°C with 1 ml of acetic acid - sulphuric acid - water (83:7:10).[57] After the usual work up, the hydrocarbon fraction was found to consist of two main g.l.c. components - the rosadiene (111), identical on g.l.c. with an authentic sample, and a component presumed[71] to be the Δ^3 isomer of dolabradiene. G.l.c. retention times on 1% SE30, 140°C: (176) 19.52 min., Δ^3 isomer 17.36 min., n $C_{18}H_{38}$ 10.64 min.

Rearrangement of deuterated pimara- and isopimara-dienes

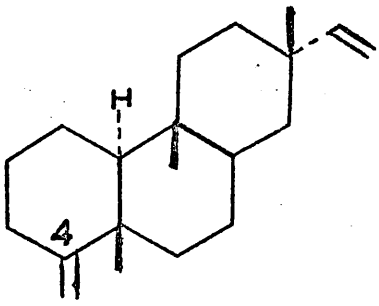
(a) Acetic-sulphuric acid on isopimaradiene (203): The diene (2 mg) was treated with the usual acetic-sulphuric acid solvent for 12 hours at room temperature. After work up and g.l.c. analysis, the hydrocarbon product was shown to consist 95% of the Δ^8 isomer (87), with 5% unreacted isopimaradiene.

(b) Pimaradiene (208), on similar treatment to above, was 95% transformed into the Δ^8 isomer (88).

(c) The three isopimaradienes - parent diene (203), monodeuterated (204), dideuterated (212) - and the three pimaradienes - (208), (209), (215) - were reacted with acetic-sulphuric acid (5 mg diene per 0.5 ml of reagent) at 60°C for 75 hours. The hydrocarbon products from the isopimaradiene series showed 5 major peaks on g.l.c. (1% SE30 140°C Table 2) as shown in Figure 16. The pimaradiene series furnished 6 main hydrocarbon products, as in Figure 17.

G.l.c. comparison of the rearrangement products with authentic samples of dolabradiene, rosadiene (111), and all the tetracyclic hydrocarbons except isohibaene (193), was carried out in conjunction with a mass spectral analysis. The principal findings were that all tetracyclic structures

were absent, and that the major rearrangement product - common to both series - was the rosa-abietadiene (217). The full details are in the discussion section.



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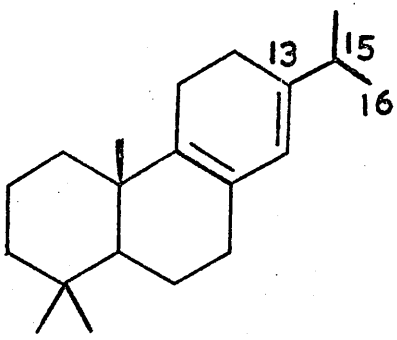

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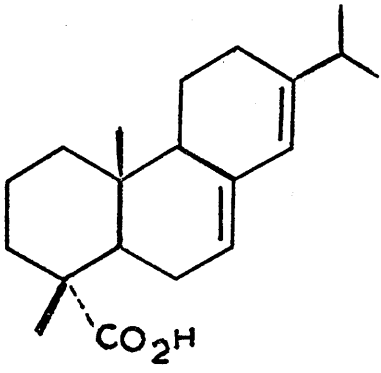

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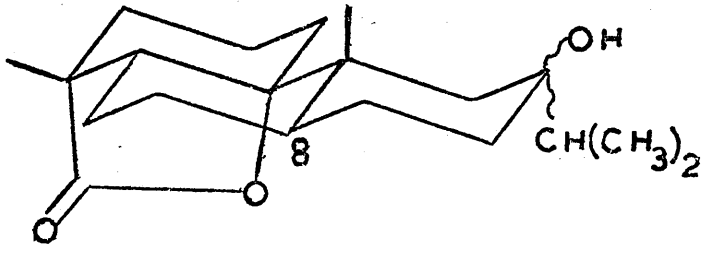

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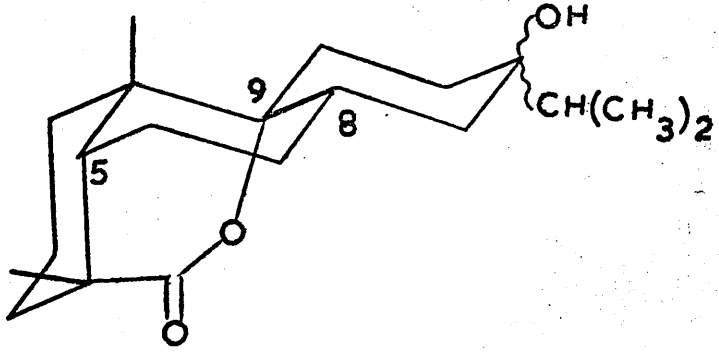

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FIG 8

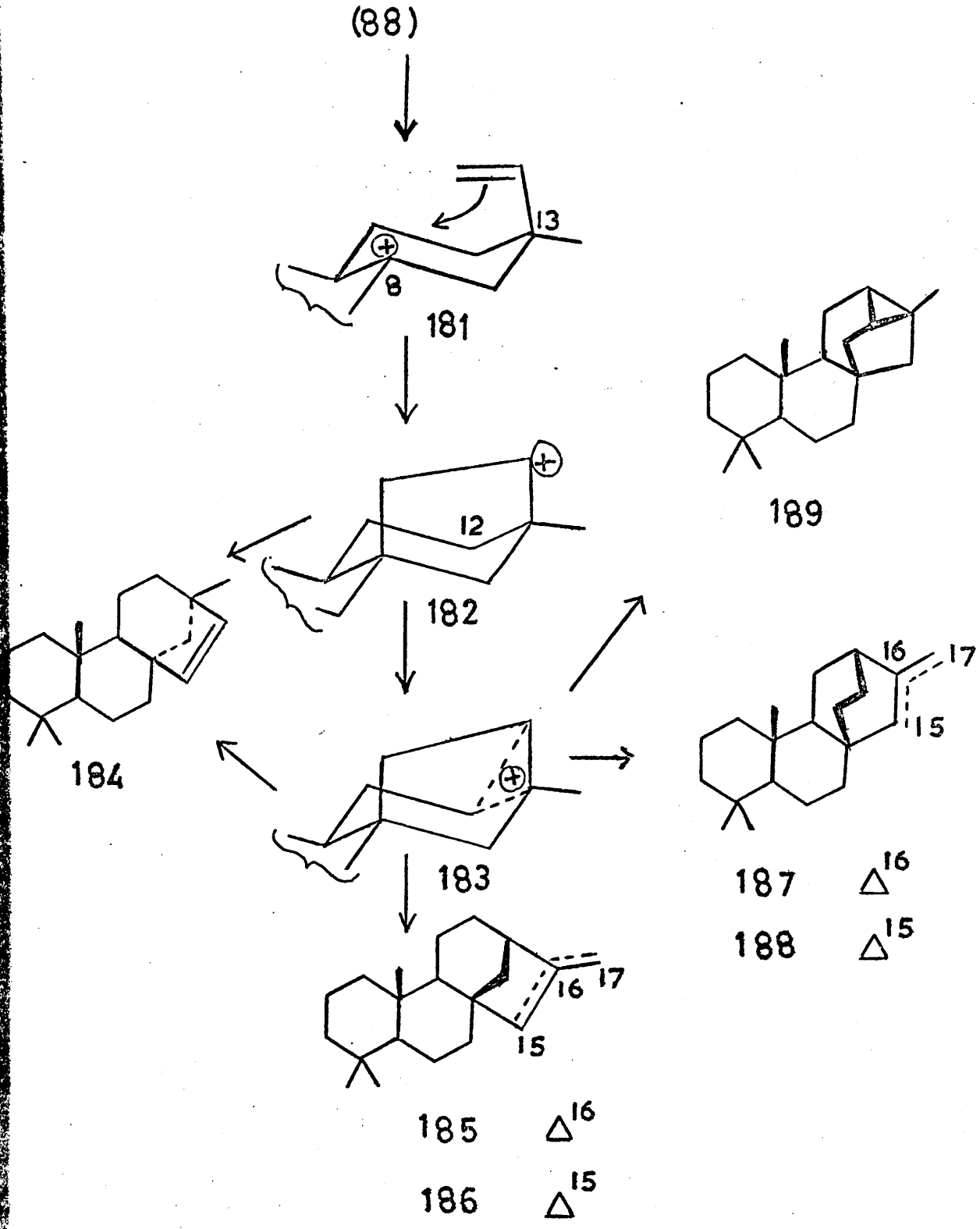
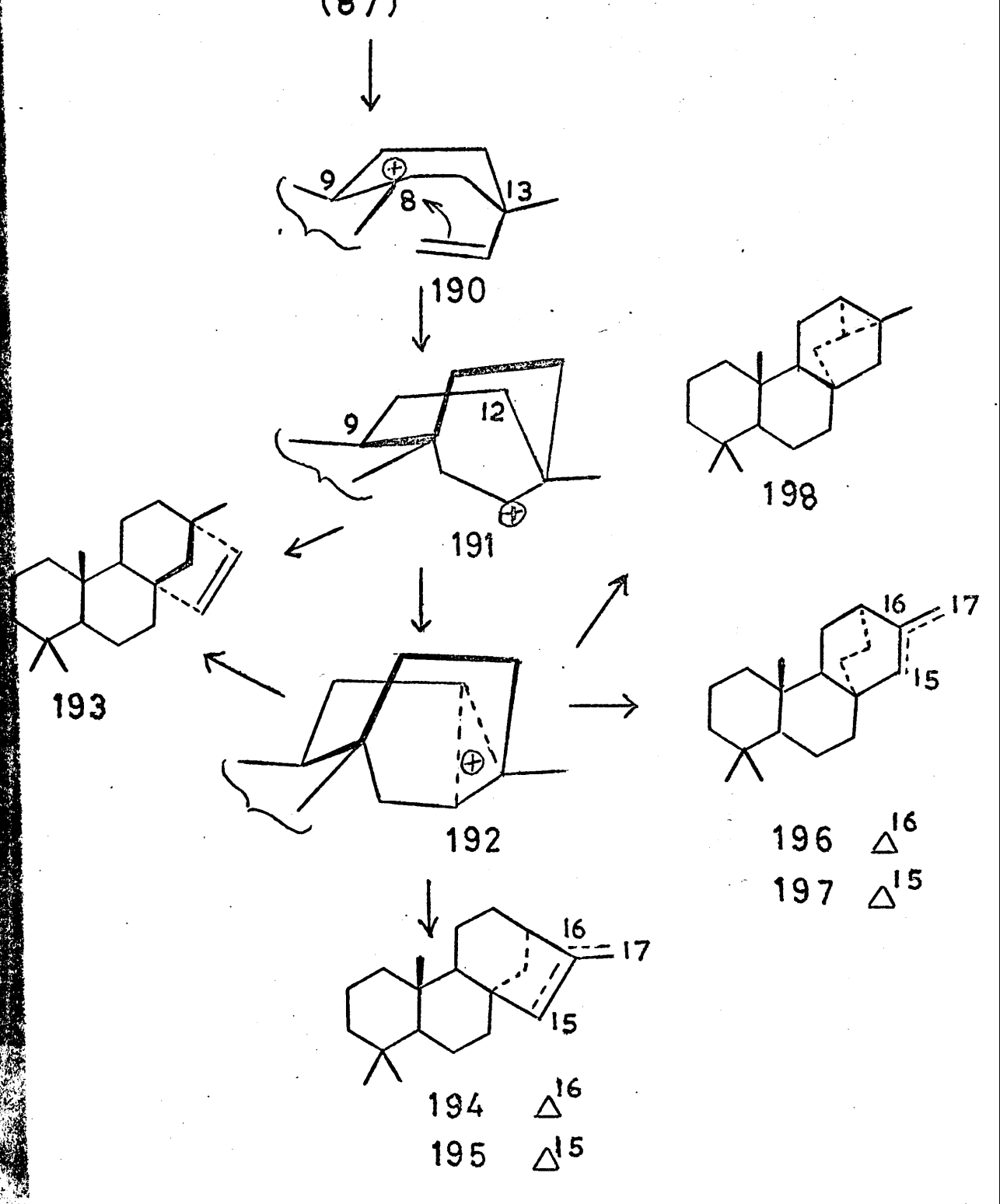

(88)
13
8
181
189
12
182
184
16
17
15
183
187 Δ16
188 Δ15
16
17
15
185 Δ16
186 Δ15

FIG 9


(87)
9
8
13
190
9
12
191
198
193
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17
15
192
196 Δ16
197 Δ15
16
17
15
194 Δ16
195 Δ15

FIG 10

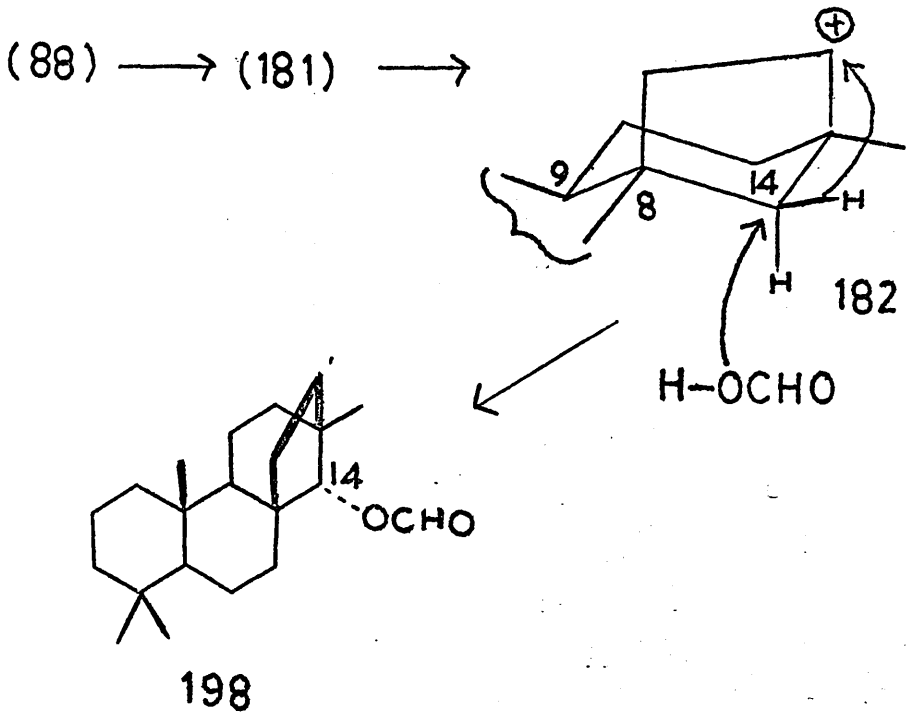
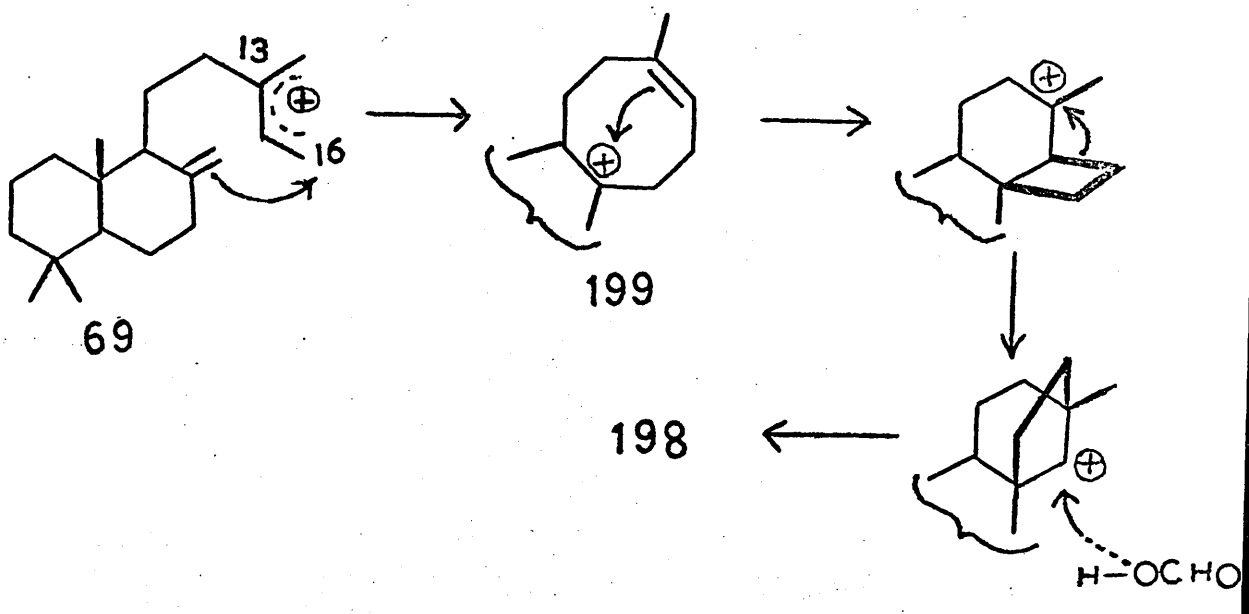
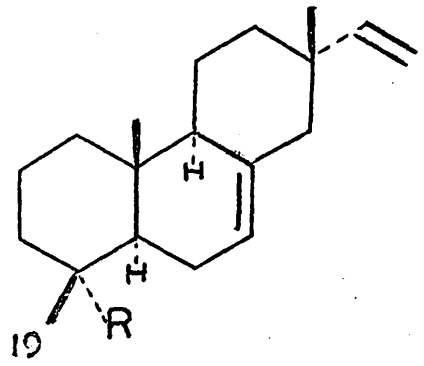
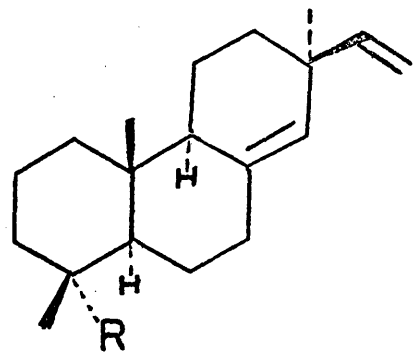


FIG 11

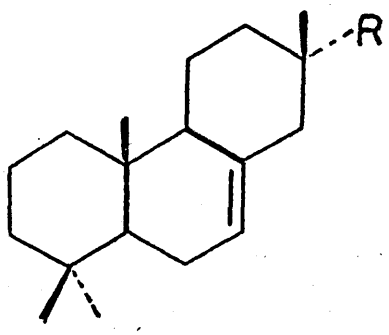




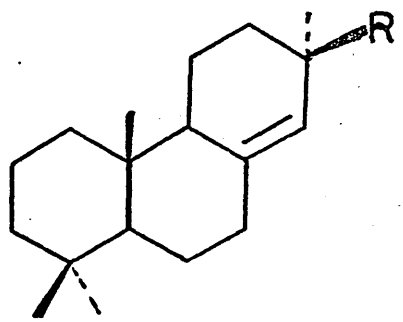
R	
200	CO_2H
201	CH_2OH
202	$CH_2OSO_2C_6H_4-CH_3P$
203	CH_3
204	CH_2D



R	
205	CO_2H
206	CH_2OH
207	$CH_2OSO_2C_6H_4-CH_3P$
208	CH_3
209	CH_2D

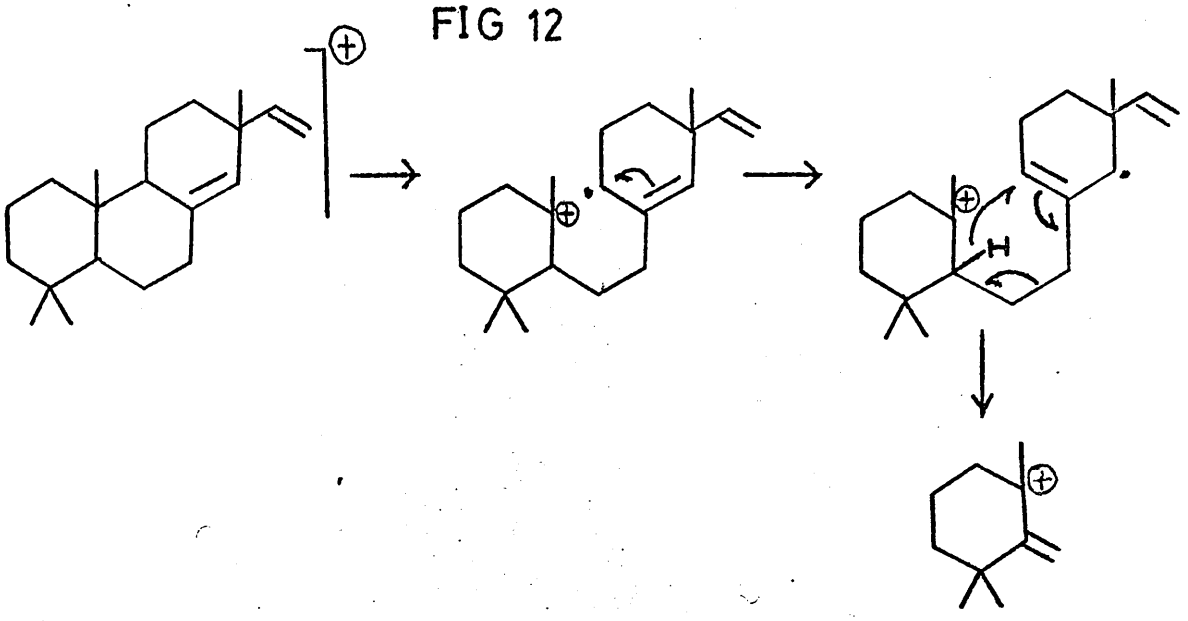


R	
210	$CH(OH).CH_2OH$
211	CHO
212	$CH=CD_2$

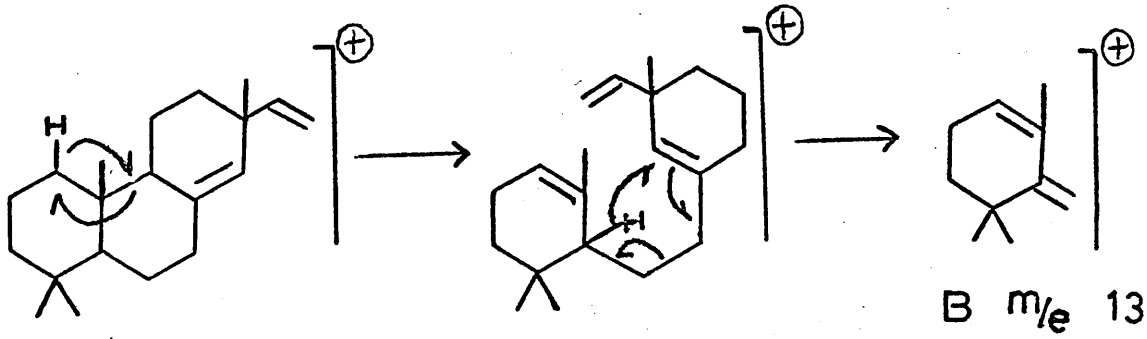


R	
213	$CH(OH).CH_2OH$
214	CHO
215	$CH=CD_2$

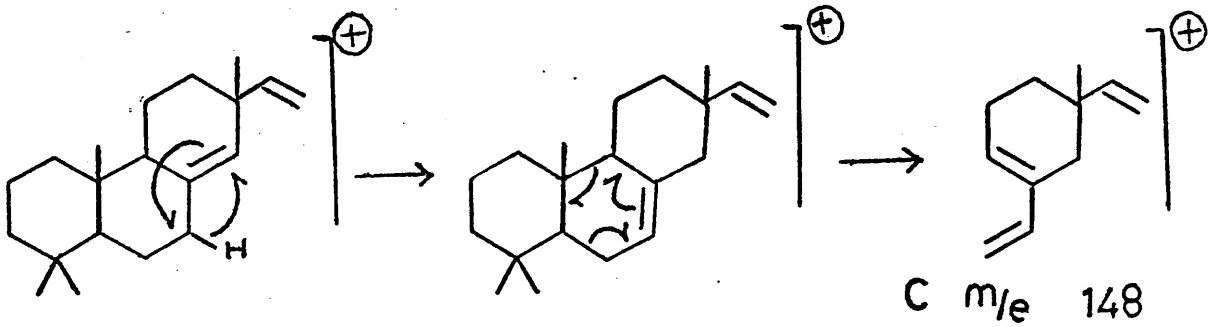
FIG 12



A m/e 137



B m/e 136



C m/e 148

FIG 14

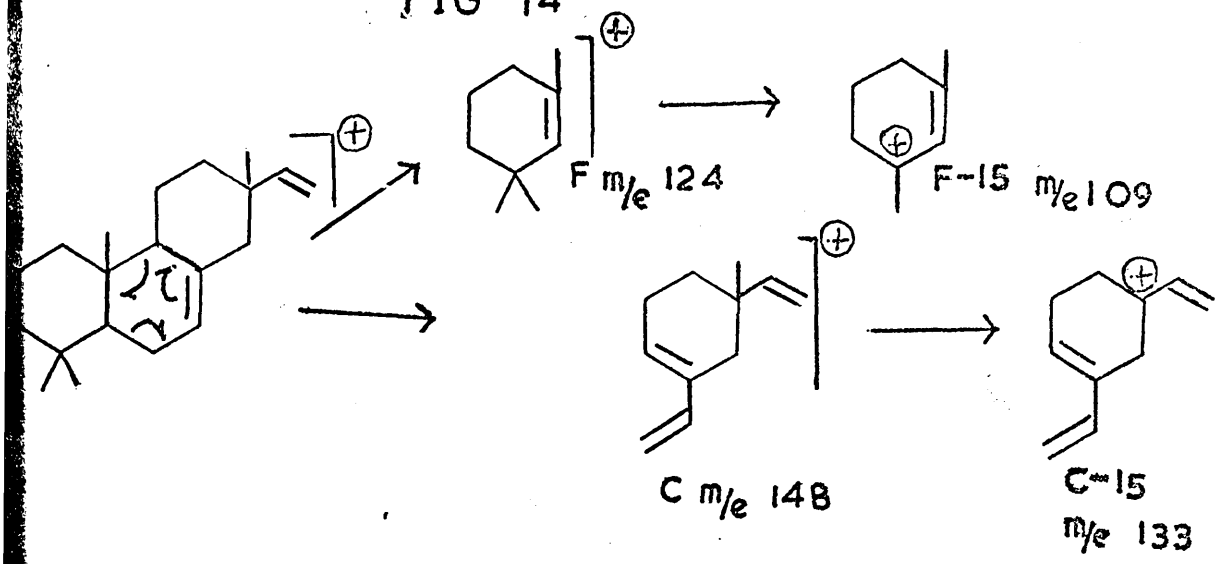
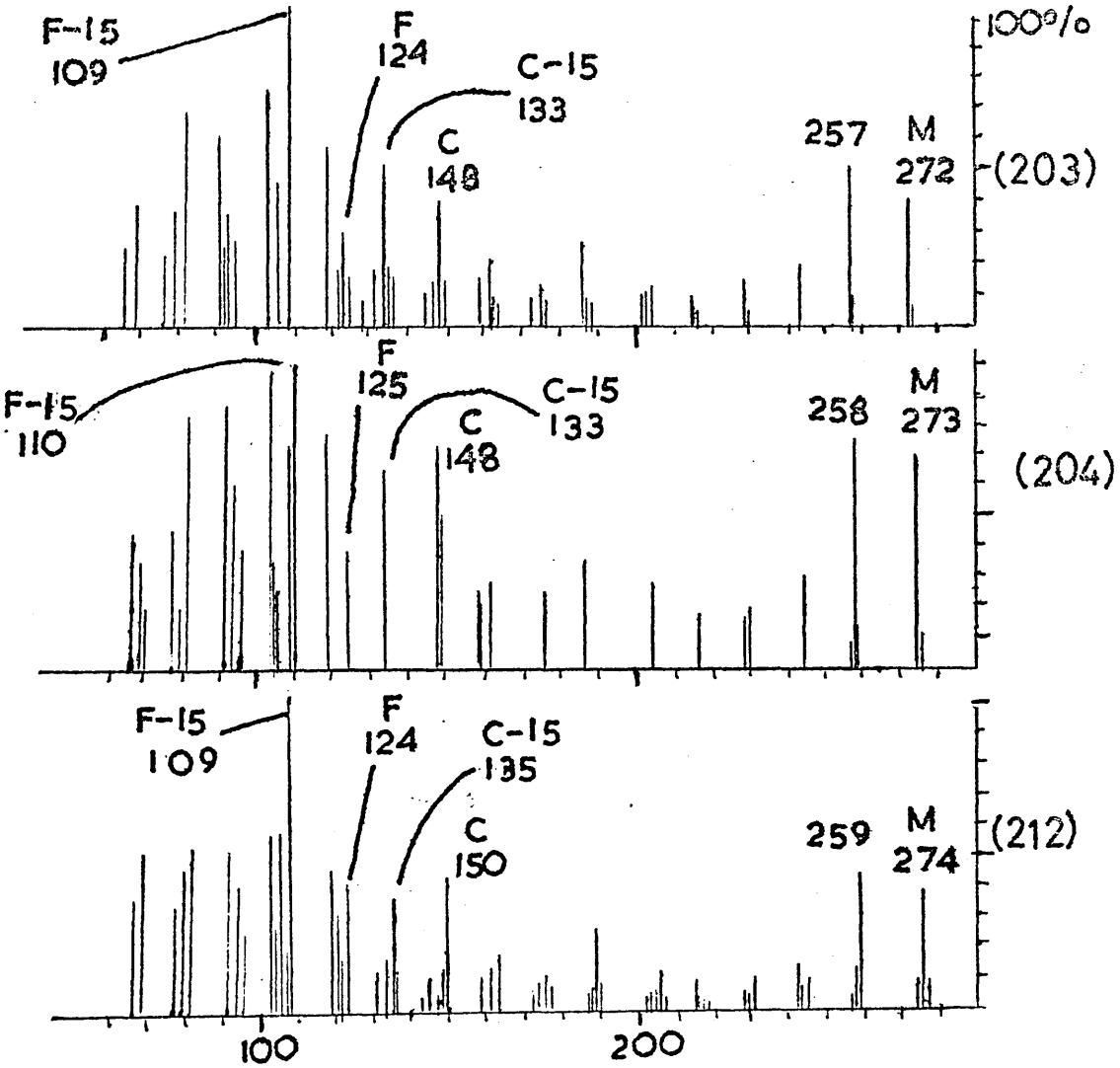


FIG 15

MASS SPECTRA OF ISOPIMARADIENES



G.L.C. TRACES (1% SE 30 140°C)

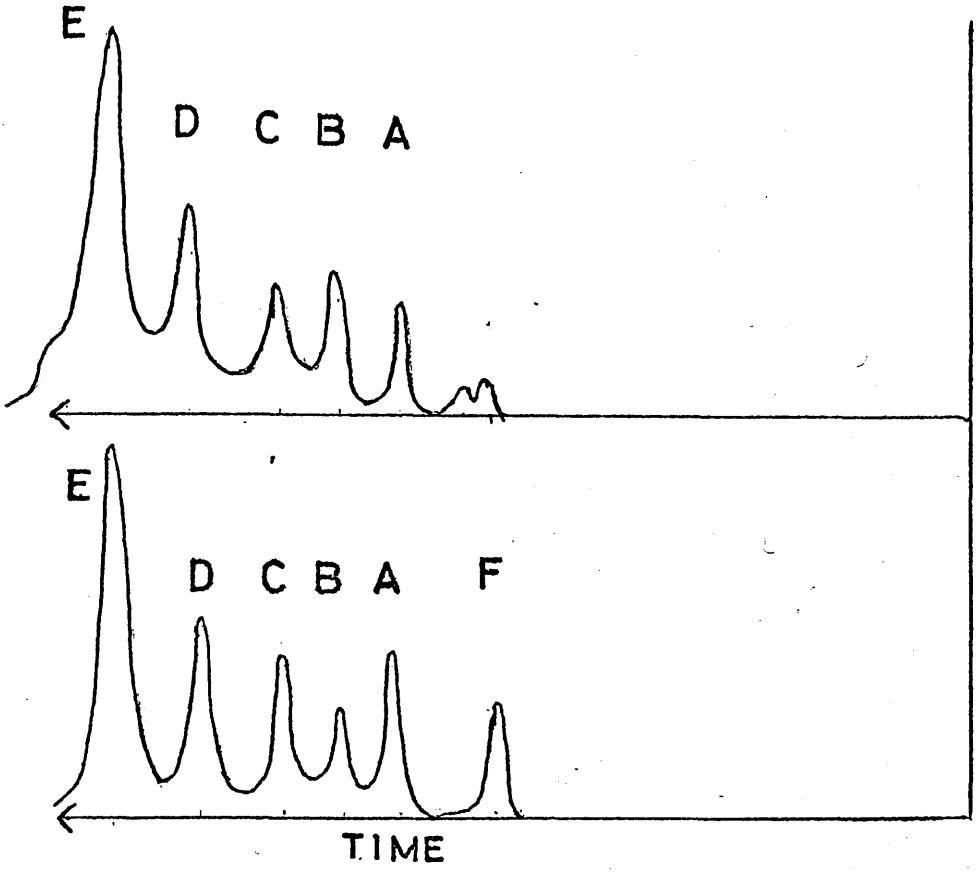
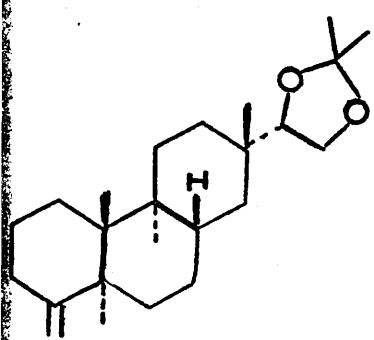


FIG 16

FIG 17



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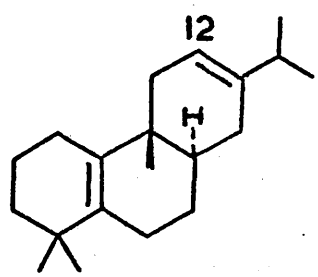

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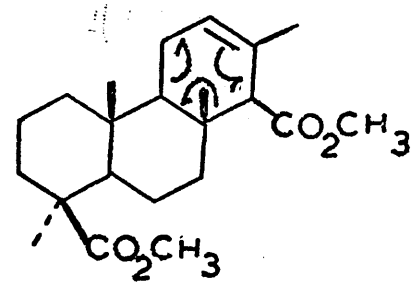


218

FIG 18

MASS SPECTRA OF PEAK E

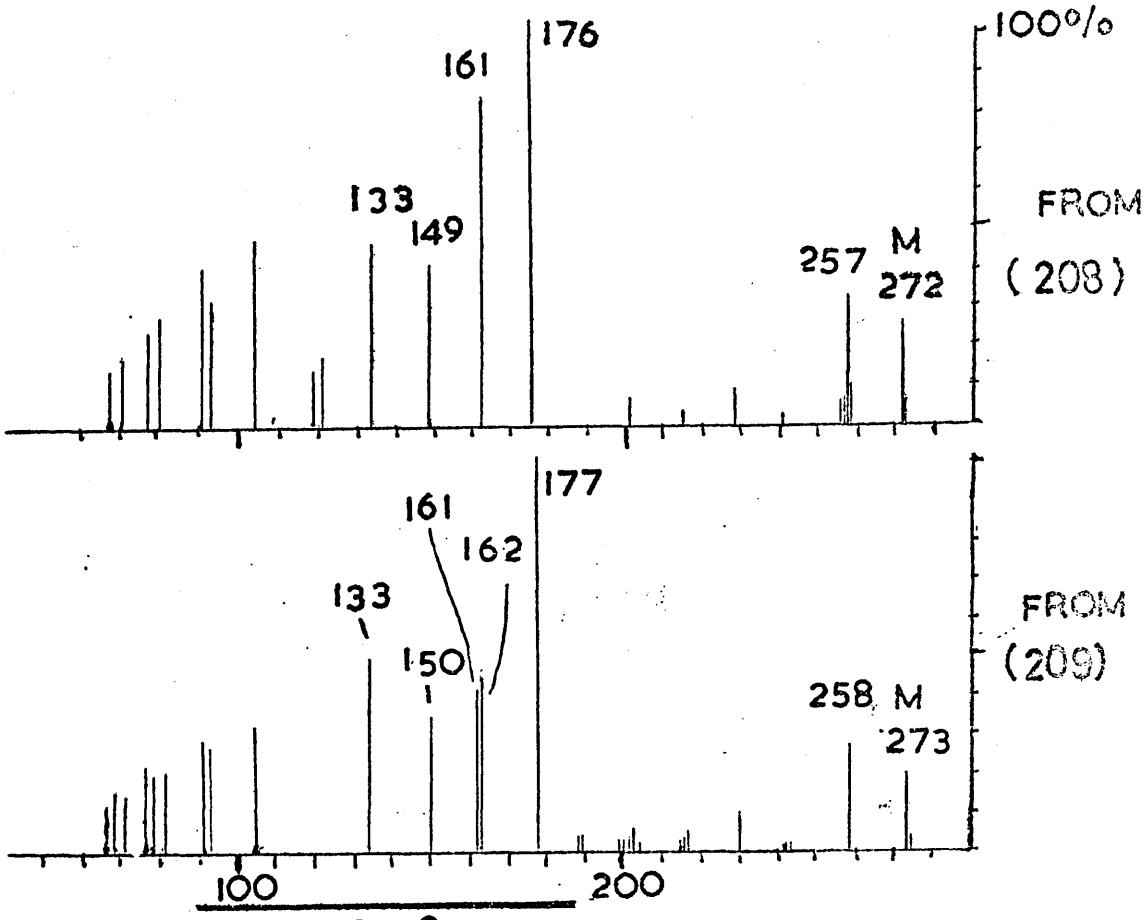
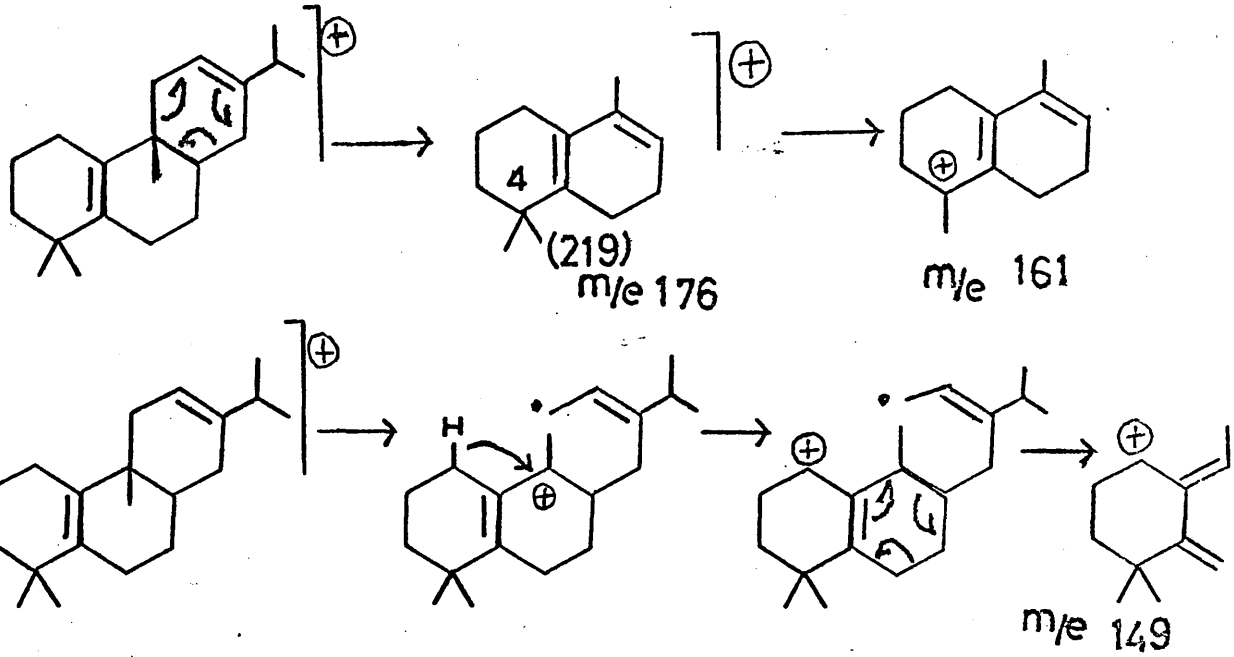


FIG 19



REFERENCES

1. L. Ruzicka, Experientia, 1953, 9, 357.
2. A. Eschenmoser, L. Ruzicka, O. Jeger and D. Arigoni, Helv. Chim. Acta., 1955, 38, 1890.
3. G. Stork and A.W. Burgstahler, J. Amer. Chem. Soc., 1955, 77, 5068.
4. See inter alia J.H. Richards and J.B. Hendrickson, 'Biosynthesis of Terpenes, Steroids and Acetogenins', Benjamin, New York, 1964; P. Bernfield, 'Biogenesis of Natural Compounds', Pergamon Press, Oxford, 1963.
5. K.W. Gopinath, T.R. Govindachari, P.C. Parthasarathy and N. Viswanathan, Helv.Chim.Acta., 1961, 44, 1040.
6. E. Wenkert, Chem. and Ind., 1955, 282.
7. R.M. Coates and E.F. Bertram, Chem. Comm., 1969, 797.
8. Reviewed in The 'Chemistry of the Cyclic Diterpenoids', p.67, Vol. 5 of 'Advances in Organic Chemistry', Interscience, 1965.
9. W.G. Dauben, W.F. Thiessen and P.R. Resnick, J. Amer. Chem. Soc., 1962, 84, 2015.
10. H. Grotman, T. Norio, M. Sumimito and A. Morrison, Tetrahedron Letters, 1964, 3879.

11. M. Dukes, D.H. Eyre, J.W. Harrison, and B. Lythgoe, Tetrahedron Letters, 1965, 4765.

12. K. Bloch, Vitamins and Hormones, 1957, 15, 119.

13. R.G. Langdon and K. Bloch, J. Biol. Chem., 1953, 200, 129.

14. J.W. Cornforth, L.Y. Gore and G. Popjak, Biochem. J., 1957, 65, 94.

15. J.W. Cornforth, G.D. Hunter and G. Popjak, Biochem. J., 1953, 54, 597.

16. K. Folkers, D.E. Wolf, C.H. Hofmann, P.E. Aldrich, H.R. Skeggs and L.D. Wright, J. Amer. Chem. Soc., 1956, 73, 4499.

17. P.A. Tavormina, M.H. Gibbs and J.W. Huff, J. Amer. Chem. Soc., 1956, 73, 4498; 6210.

18. A.J. Birch, R.W. Rickards, H. Smith, A. Harris and W.B. Whalley, Tetrahedron, 1959, 7, 241.

19. J.J. Britt and D. Arigoni, Proc. Chem. Soc., 1958, 224.

20. D. Arigoni, Gazz. Chim. Ital., 1962, 92, 884.

21. A.J. Birch, D.W. Cameron, C.W. Holzapfel and R.W. Rickards, Chem. and Ind., 1963, 374.

22. G.G. Freeman and R.I. Morrison, Biochem. J., 1948, 43, (XXIII).

23. G.G. Freeman and R.I. Morrison, Biochem. J, 1949, 44, 1.

24. G.G. Freeman, R.I. Morrison, and S.E. Michael, Biochem. J., 1949, 45, 191.

25. S.E. Michael, Biochem. J., 1949, 43, XXIV.

26. W.O. Godtfredsen and S. Vangedal, Proc. Chem. Soc., 1964, 188.

27. A. Robertson, W.R. Smithies, and E. Tittensor, J. Chem. Soc., 1958, 879.

28. A. Harris, A. Robertson, and W.B. Whalley, J.Chem.Soc., 1958, 1799.

29. A. Harris, A. Robertson and W.B. Whalley, J.Chem.Soc., 1958, 1807.

30. G.A. Ellestad, B. Green, A. Harris, W.B. Whalley, and H. Smith, J. Chem. Soc., 1965, 7246.

31. M.R. Cox, G. Ellestad, A.J. Hannaford, I.R. Wallwork, and W.B. Whalley, J. Chem. Soc., 1965, 7257.

32. W.B. Whalley, B. Green, D. Arigoni, J.J. Britt, and C. Djerassi, J. Amer. Chem. Soc., 1959, 81, 5520.

33. C. Djerassi, B. Green, W.B. Whalley, and C.G. De Grazia, J. Chem. Soc., (C), 1966, 624.

34. H. Inhoffen, S. Schutz, P. Rossberg, O. Berges, K.H. Nordsiek, H. Plenio, and E. Horoldt, Ber., 1958, 91, 2626.

35. A.I. Scott, S.A. Sutherland, D.W. Young, L. Guglielmetti, D. Arigoni, and G.A. Sim, Proc. Chem. Soc., 1964, 19.

36. A.I. Scott, D.W. Young, S.A. Hutchinson, and N.S. Bhacca, Tetrahedron Letters, 1964, 849.

37. A.J. Allison, J.D. Connolly, and K.H. Overton, J. Chem. Soc., (C), 1968, 2122.

38. C.W. Holzapfel and P,S. Steyn, Tetrahedron, 1968, 24, 3321.

39. A.J. Renfrew, Ph.D. Thesis, Glasgow, 1966.

40. A.R. Jones, Ph.D. Thesis, Manchester, 1966.

41. C.D. De Grazia, W. Klyne, P.M. Scopes, D.R. Sparrow, and W.B. Whalley, J. Chem. Soc. (C), 1966, 896.

42. B. Achilladelis and J.R. Hanson, Phytochemistry, 1968, 7, 589.

43 B. Achilladelis and J.R. Hanson, Tetrahedron Letters, 1968, 4397.

44. B. Achilladelis and J.R. Hanson, Chem. Comm., 1969, 488.

45. F. Lynen, H. Agranoff, H. Eggerer, V. Henning, and K. Moslein, Angew. Chem., 1959, 71, 657.

46. R.E. Ireland and L.N. Mander, J. Org. Chem., 1969, 34, 142.

47. R.E. Ireland and L.N. Mander, J. Org. Chem., 1967, 32, 689.

48. J.W. Cornforth, 'Exploration of Enzyme Mechanisms by Asymmetric Labelling', Quart. Rev., 1968, 23, 125.

49. R.M. Carman and N. Dennis, Austral. J. Chem., 1967, 20, 157.

50. C. Enzell, Acta. Chem. Scand., 1961, 15, 1303.

51. R.M. Carman and N. Dennis, Austral. J. Chem., 1964, 17, 395.

52. P.S. Kalsi, C.B. Sharma, and L.S. Bhatia, Perfumery and Essential Oil Record, 1967, 58, 84.

53. R.A. Sneen and A.M. Rosenberg, J. Amer. Chem. Soc., 1961, 83, 895; 900.

54. V. Prelog and E. Watanabe, Annalen, 1957, 603, 1.

55. W.S. Johnson, W.H. Lunn, and K. Fitzi, J. Amer. Chem., Soc., 1964, 86, 1972.

56. L.J. Dolby and R.H. Iwamoto, J. Org. Chem., 1965, 30, 2420.

57. S. Bory and C. Asselineau, Bull. Soc. Chim. France, 1961, 1355.

58. J.W. Rowe and J.H. Scroggins, J. Org. Chem., 1964, 29, 1554.

59. M. Stoll and A. Commarmont, Helv. Chim. Acta,, 1949, 32, 1354.

60. S. Bory and E. Lederer, Croat.Chem.Acta., 1957, 29, 157.

61. R.B. Gates and D.M. Gale, J. Amer. Chem. Soc., 1960, 82, 5749.

62. C. Asselineau, S. Bory, M. Fetizon,and P. Laszlo, Bull. Soc. Chim. France, 1961, 1429.

63. S. Bory, M. Fetizon, and P. Laszlo, Bull. Soc. Chim. France, 1963, 2310.

64. R.M. Carman and N. Dennis, Austral. J. Chem., 1967, 20, 157.

65. G. Ohloff, Annalen., 1958, 617, 134.

66. R.E. Ireland and P.W. Schiess, J.Org.Chem.,1963, 28, 6; R.F. Church and R.E. Ireland, ibid, p.17.

67. E. Wenkert and J.W. Chamberlin, J. Amer. Chem. Soc., 1959, 81, 688.

68. Le-Van-Thoi and J. Ourgaud, Bull. Soc. Chim. France, 1956, 202 and 205.

69. W. Herz and H.J. Wahlborg, J. Org. Chem., 1965, 30, 1881.

70. J.D. Connolly, R. McCrindle, R.D.H. Murray, and K.H. Overton, J. Chem. Soc., (C), 1966, 273.

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

72. E. Wenkert and Z. Kumazawa, Chem. Comm., 1968, 140.

73. O.E. Edwards and R.S. Rosich, Can.J.Chem., 1968, 46, 1113.

74. M. Mangoni and L. Bellardini, Gazz. Chim. Ital., 1964, 94, 1108.

75. L. Ruzicka and J.R. Hosking, Annalen, 1929, 469, 147.

76. R.M. Carman, Austral. J. Chem., 1964, 17, 393.

77. H.C. Brown and R.F. McFarlin, J. Amer. Chem. Soc., 1958, 80, 5372.

78. L. Ruzicka and J.R. Hosking, Helv. Chim. Acta., 1931, 14, 203.

79. W.P. Campbell and D. Todd, J. Amer. Chem. Soc., 1942, 64, 928.

80. Y.G. Perron, L.B. Crast, J.M. Essery, R.R. Frazer, J.C. Godfrey, C.T. Holdrege, W.F. Minor, M.E. Neubert, R.A. Partyka, L.C. Cheney, J.Med.Chem., 1964, 7, 483.

81. E. Wenkert and B.G. Jackson, J. Amer. Chem. Soc., 1958, 80, 217.

82. D.W. Young, Ph.D. Thesis, Glasgow, 1963.

83. H.E. Audier, S. Bory, M. Fetizon, and Nguyen-Trong Anh, Bull Soc. Chim. France, 1966, 4002.

84. O.E. Edwards and R. Howe, Can. J. Chem., 1959, 37, 760.

85. A.P. Krapcho and M.E. Nadel, J. Amer. Chem. Soc., 1964. 86, 1096.

86. J.A. Cella, E.A. Brown, and R.R. Burtner, J. Org. Chem., 1959, 24, 743.

87. A. Bowers, T.G. Halsall, E.R.H. Jones, and A.J. Leinin, J. Chem. Soc., 1953, 2548.

88. G. Stork and S.D. Darling, J. Amer. Chem. Soc., 1964, 86, 1761.

89. M.J.T. Robinson, Tetrahedron, 1965, 21, 2475.

90. C. Amendolla, G. Rosenkranz, and F. Sondheimer, J. Chem. Soc., 1954, 1226.

91. S. Nishimura, Bull. Soc. Chim. Jap., 1961, 34, 32.

92. A.J. Birch and K.A.M. Walker, J. Chem. Soc. (C), 1966, 1894.

93. R.L. Augustine, 'Catalytic Hydrogenation', Edward Arnold, London, 1965.

94. B.F. McKenzie, V.R. Mattox, L.L. Engel, and E.C. Kendall, J. Biol. Chem., 1948, 173, 271.

95. B. Berkoz, A.D. Cross, M.E. Adame, H. Carpio, and A. Bowers, J. Org. Chem., 1963, 28, 1976.

96. H.B. Henbest and B. Nicholls, J. Chem. Soc., 1957, 4608.

97. S. G. Levine and M.E. Wall, J. Amer. Chem. Soc., 1959, 81, 2826.

98. A. Jovtscheff, H. Reinheckel, K. Haage, and R. Pomakowa, Monatsh., 1966, 97, 1620.

99. J. Elks, G.H. Phillips, T. Walker, and L.J. Wyman, J. Chem. Soc., 1956, 4330.

100. C.W. Marshall, R.E. Ray, I. Laos, and B. Riegel, J. Amer. Chem. Soc., 1957, 79, 6308.

101. W.R. Jackson and A. Zurqiyah, J. Chem. Soc., 1965, 5280.

102. F. Puschel and C. Kaiser, Ber., 1964, 97, 2903.

103. T. Horawina, D. Ksiezny, J. Vaedtke, M. Iwaszkiewicz, and W. Lewenstein, Chem. Abs., 1966, 65, 18654g.

104. K. Mori and M. Matsui, Tetrahedron, 1966, 22, 879.

105. D.B. Bigley, J.A. Barltrop, and N.A.J. Rogers, J. Chem. Soc., 1960, 4613.

106. G. Buchi and K. Biemann, Croat. Chem. Acta., 1957, 29, 163.

107. D.R. Dalton, V.P. Dutta and D.C. Jones, J. Amer. Chem. Soc., 1968, 5498.

108. O.E. Edwards and B.S. Mootoo, Can. J. Chem., 1969, 47, 1189.

109. Jean-Louis Fourrey, J. Polonsky and E. Wenkert, Chem. Comm., 1969, 714.

110. R.E. Ireland and J. Newbould, J. Org. Chem., 1963, 28, 23.

111. R.A. Bell, R.E. Ireland, and R.A. Partyka, J. Org. Chem., 1966, 31, 2530.

112. Y. Kitahara and A. Yoshikoshi, Tetrahedron Letters, 1964, 1755.

113. H. Brockmann and H. Schodder, Ber, 1941, 74, 73.

114. C. Enzell and R. Ryhage, Arkiv. Kemi, 1965, 23, 367.

115. A.J. McAlees, Ph.D. Thesis, Glasgow, 1967.

116. For references see H. Budzikiewicz, C. Djerassi and D.H. Williams, 'Structure Elucidation of Natural Products by Mass Spectrometry', Vol.II, Holden-Day,1964.

117. D.S. Weinberg and C. Djerassi, J. Org. Chem., 1966, 31, 115.

118. B. Willhalm and A.F. Thomas, Helv. Chim. Acta., 1967, 50, 383.