A Thesis

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"Natural Product Studies in the Stilbene and Terpene Series"

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by

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Part I.

A Synthesis of Polyhydroxystilbenes.

ACKNOWLEDGMENTS.

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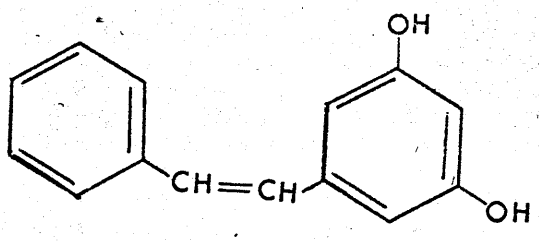
Thanks are also due to Professor H. Erdtman for kindly supplying an authentic sample of pinosylvin and pinosylvin diacetate.

INTRODUCTION.

Of the many woods in commercial use, some have been found to be more resistant to decay and insect attack than others, and it has been suggested[1] that such resistance results from the presence of substances toxic to the fungi which normally initiate the decomposition processes. In general, this durability seems to be restricted to the so-called heartwood of the tree.

The physiology of heartwood formation has not been studied in much detail. Heartwood is dead tissue which does not take part in the transport of water and is therefore much drier than sapwood and much more difficult to impregnate. (As a rule it is also darker in colour than sapwood, or becomes coloured on exposure to the atmosphere or light, due probably to the oxidation of various chromogens such as phenols, tannin, leucoanthocyanins, etc.). It seems quite natural that many plants during evolution should acquire an ability to protect the dead, and therefore especially vulnerable, heartwood by means of suitable preservatives excreted into the dead wood, or bark, as metabolic end-products.

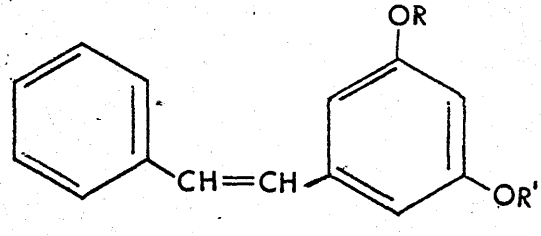
This postulated connection between a high degree of durability and the presence of some kind of fungal inhibitor was substantiated by the isolation, from



(1)

entirely unrelated genera of coniferous and dicotyledonous trees, of a family of fungal-inhibitory stilbenes, all of which, so far, have been shown to be substituted 3',5'-dihydroxystilbenes (1; 5-styryl-resorcinols), containing free or methylated hydroxyl groups.

However, important as such compounds may be in nature as protectors against disease, they also have value from a taxonomic point of view. Not all plant properties are significant in botanical classifications, and more attention is paid by taxonomists to the minor characteristics and constituents, since they are usually inherited by succeeding generations without undergoing much change. Comparatively recent specializations possess little taxonomic importance, and thus heartwood constituents are of greater significance in this respect than those of more highly specialized organs, since it is reasonable to suppose that once a good preservative was established, there would be no necessity for further improvements, and so the ability to form such a preservative would be handed down from species to species. Minor changes may have occurred; hydroxyl groups may have been introduced, or methylated, or dehydrations may have taken place, but the fundamental structure remains unaltered.



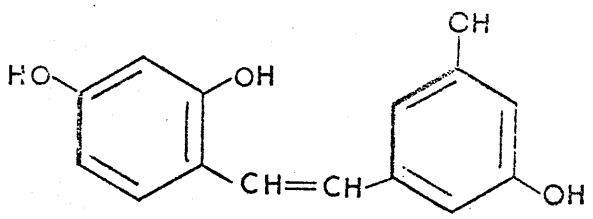
(2) R = R' = H

(3) R = H; R' = CH_3

(4) R = R' = CH_3

It was in an attempt to correlate chemical and botanical classifications that a systematic study of pine heartwoods was initiated by Erdtman[2] and subsequently carried on by Lindstedt[3], an investigation which originated primarily because of the outstanding economic importance of these conifers. Analysis of the phenolic content of the heartwood of forty-eight Pinus species showed the presence of a number of related phenols and flavones, the principal constituents being pinosylvin (2) or its mono-methyl ether (3). These compounds are responsible for the high resistance of the wood to attack by fungi (by inhibition of spore germination) as evidenced by tests carried out in vitro on a variety of such organisms. Erdtman and his associates have shown that while either pinosylvin or its methyl ether is present in the heartwood of the majority of Pinus species, neither is to be found in any other genus of conifer. However, the discovery of other stilbenes, possessing a hydroxy substituted nucleus, in entirely unrelated genera of dicotyledons[4] suggests that these polyhydroxystilbenes may also prove characteristic of certain hardwood genera.

The wood of the Osage orange (Toxylon pomiferum) was found to be remarkably resistant to decay, a fact



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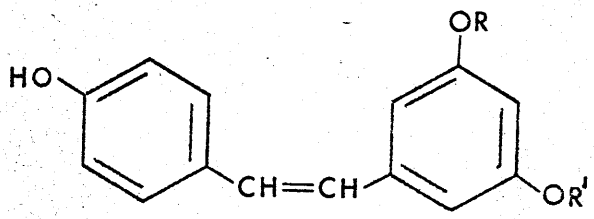
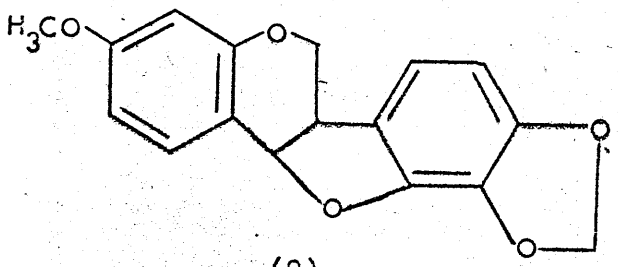


(6) R = R' = H

(7) R = R' = CH_3



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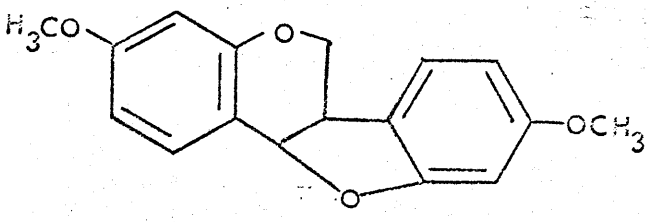
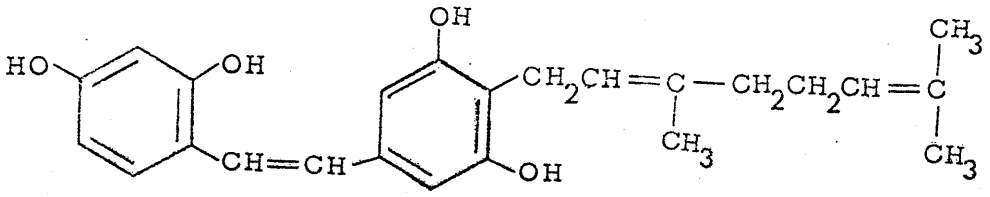


(9)

subsequently explained by the isolation of hydroxy-resveratrol (5; 2,4,3',5'-tetrahydroxystilbene), previously isolated from wild hellebore root by Takaoka[5,6] and shown to be toxic to five out of thirteen organisms tested. Resveratrol (6; 4,3',5'-trihydroxystilbene) was also isolated from wild hellebore root[7].

An examination of the heartwood of Pterocarpus dalbergioides, P. macrocarpus, P. soyauxii, and P. tinctorius revealed the general occurrence of pterostilbene (7), pterocarpin (8) and homopterocarpin (9), compounds previously isolated from red sandalwood by McGookin, Robertson and Whalley[8], and Späth and Schläger[9]. Experiments carried out at the D.S.I.R. Forest Products Research Laboratory showed pterostilbene to be strongly toxic to the brown rot fungus, whereas pterocarpin and homopterocarpin do not affect the growth of this wood rotting organism. Thus the presence of pterostilbene in the aforementioned Pterocarpus species is at least a contributing factor to the fungal resistant qualities of their heartwoods.

"Iroko" timber from the African tree Chlorophora excelsa (Benth and Hook f), although widely used and long noted for its resistance to fungal and insect attack, did not occasion any chemical investigation regarding its



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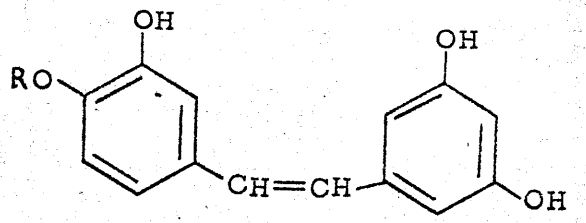
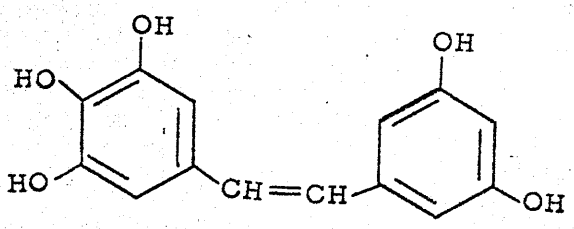


(11) R = H

(12) R = CH_3



(13)

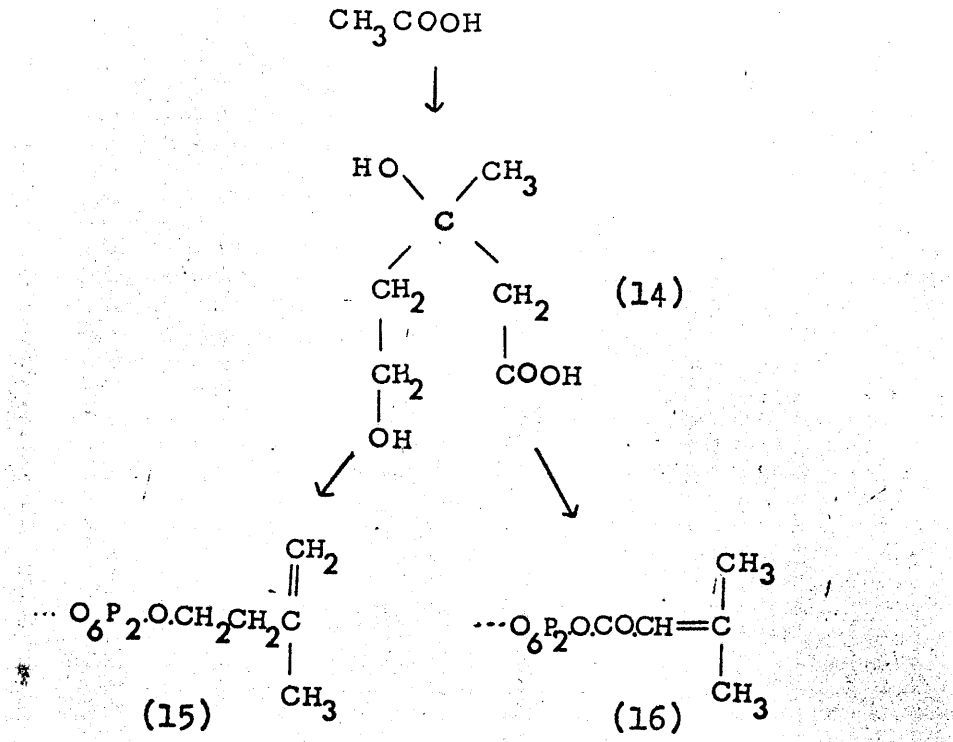
durability until 1949 when F.E. King and M.F. Grundon[10] isolated from it chlorophorin (10), another pinosylvin phenol, having the same hydroxyl pattern as hydroxy-resveratrol (5). In 1956, the most fully substituted naturally occurring stilbenes so far encountered were isolated[11] from the heartwood of the South American trees Vouacapoua macropetala and V. americana. Both contained 3,4,3',5'-tetrahydroxystilbene (11), and present also in V. macropetala was 3,4,5,3',5'-pentahydroxystilbene (13). Rhapontigenin (12), the monomethyl ether of (11), was isolated[12] as a glucoside in Turkish rhubarb root (Rhus pontifica).

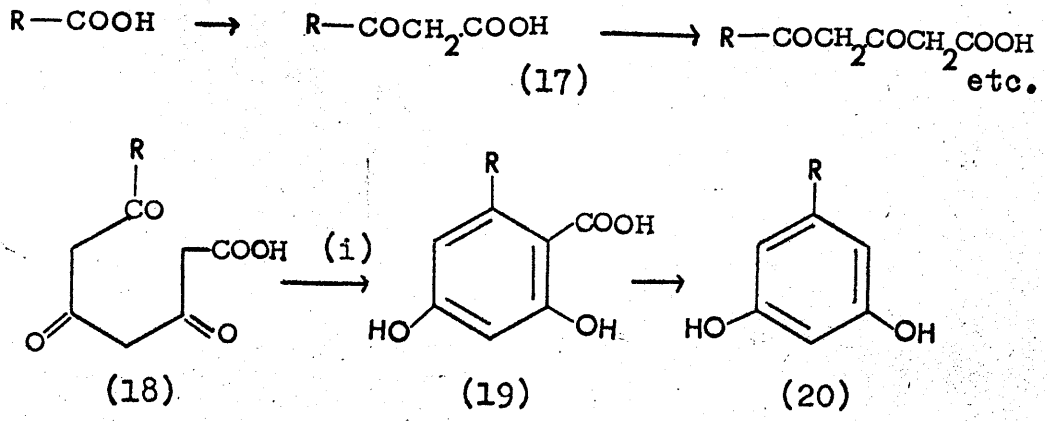
The fact that all the phenolic compounds so far isolated from the heartwood of these unrelated species are derivatives of 3',5'-dihydroxystilbene (1) would seem to denote the existence of a common biosynthetic origin.

The discoveries in the last decade of the importance of acetic acid, in the form of its thioester, acetyl coenzyme-A, as a building unit in cellular synthesis have simplified a number of difficulties concerning biosynthetic pathways. Three distinct synthetic routes in which "acetate" is involved can be distinguished:-

I. The condensation of acetate fragments into the branched chain compound mevalonic acid (14) and the ultimate

Flowsheet 1.


CH3COOH
HO
CH3
C
CH2
CH2
CH2
COOH
OH
(14)
CH2
O6P2O.CH2CH2C
CH3
(15)
CH3
O6P2O.CO.CH=C
CH3
(16)


R—COOH
R—COCH2COOH
(17)
R—COCH2COCH2COOH
etc.
R
CO
COOH
(i)
O
O
(18)
R
COOH
HO
OH
(19)
R
HO
OH
(20)

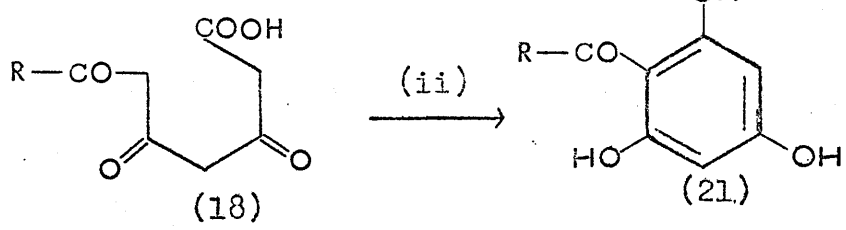
conversion of this into such building units as (15) - the origin of steroids, carotenoids and terpenes - and (16) (flowsheet I).

II. The linear condensation of acetate fragments into chains, with or without ultimate cyclization into benzene, naphthalene or anthracene derivatives.

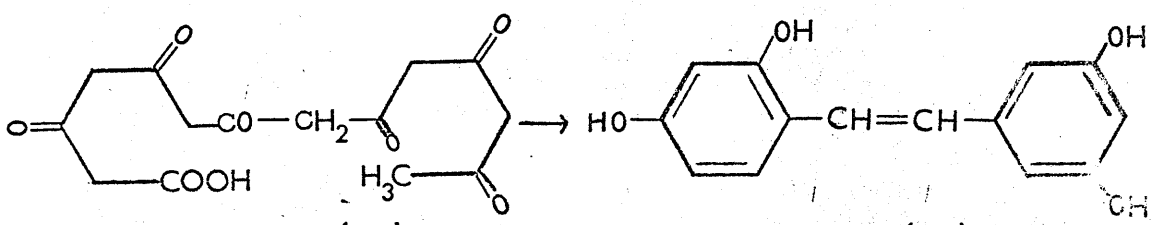
III. The addition of two-carbon units to a precursor which may or may not be acetate derived. This route is most commonly recognized in the addition of - $CH_2 \cdot CO$ - units to a carboxyl group (17).

Birch and Donovan[13] have suggested routes II and III as a general hypothesis for the plant biosynthesis of natural product molecules containing orcinol or phloroglucinol nuclei. In the simplest formulation, continuous condensation of two-carbon units (such as unreduced acetate) can lead to the formation of a β-polyketo-fatty acid chain (18). Cyclization of an intermediate of this type (probably as its coenzyme-A ester) can take place in several ways to give phenolic substances:

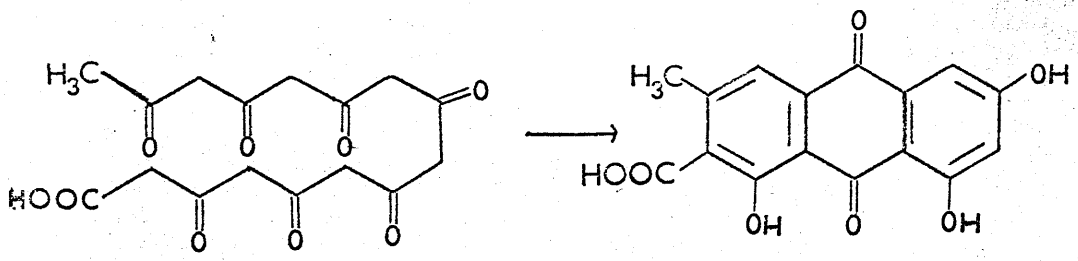
(i) Aldol condensations, giving rise to orcinol derivatives, (19) and (20).



(18) (21)



(22) (23)



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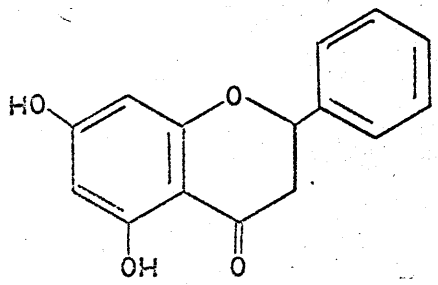

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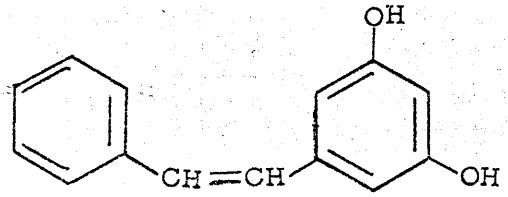


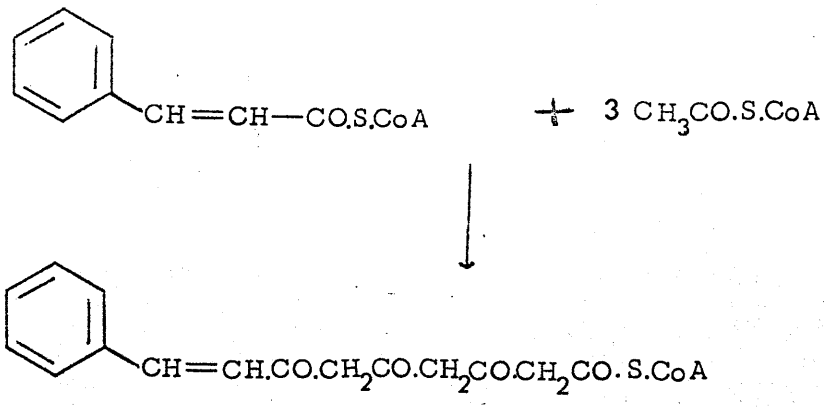
(2)

(ii) Claisen condensations, which lead to phloroglucinol-type compounds (21).

This general biosynthetic scheme is supported by the fact that there are many examples of compounds of the type (19), (20) and (21) to be found in nature. It may be that the β-polyketo precursors are in fact formed in nature as enzyme-substrate complexes, and that they cyclize spontaneously on release from the enzyme surface. With longer chain β-diketones, more complex reactions may occur (23, 24), and if the starting acid was other than a fatty acid of the type RCOOH, e.g. an acid of the type Ar.CH = CH-COOH, the scheme could be extended to account for the origin of more complex molecules.

The heartwood of nearly one hundred pine species is known to contain, in association, two groups of compounds; one based on pinosylvin (2), and the other on 5,7-dihydroxyflavone (25), the individual components of each group differing only in degree of methylation and oxidation level. The fundamental carbon skeleton of each of these two groups could be derived from a common precursor by the routes already indicated if the starting acid were cinnamic acid (i.e. a phenylpropane derived unit), and the chain generated by three

Flowsheet 11.



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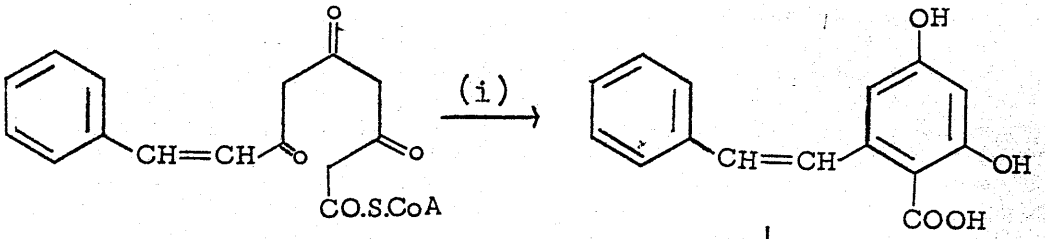

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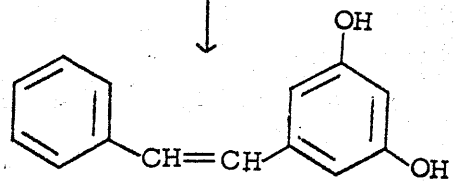
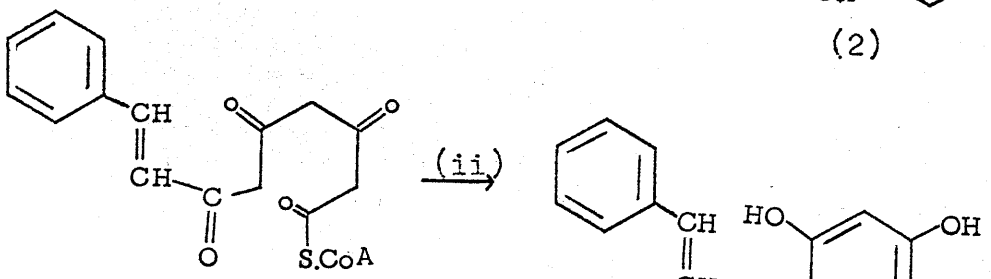
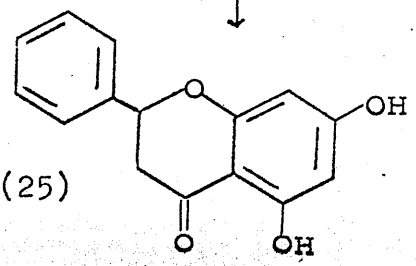


(2)



(26)



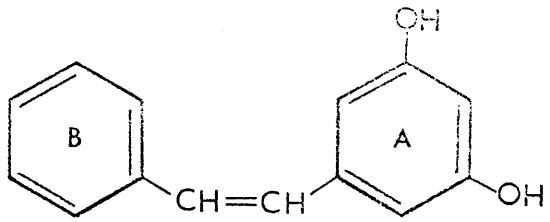
(25)

successive additions of effective acetate units to each cinnamyl coenzyme-A ester (flowsheet II). The β-keto intermediate (26) can now undergo cyclization as before:-

(i) Aldol condensation giving the substituted stilbenes (2) and

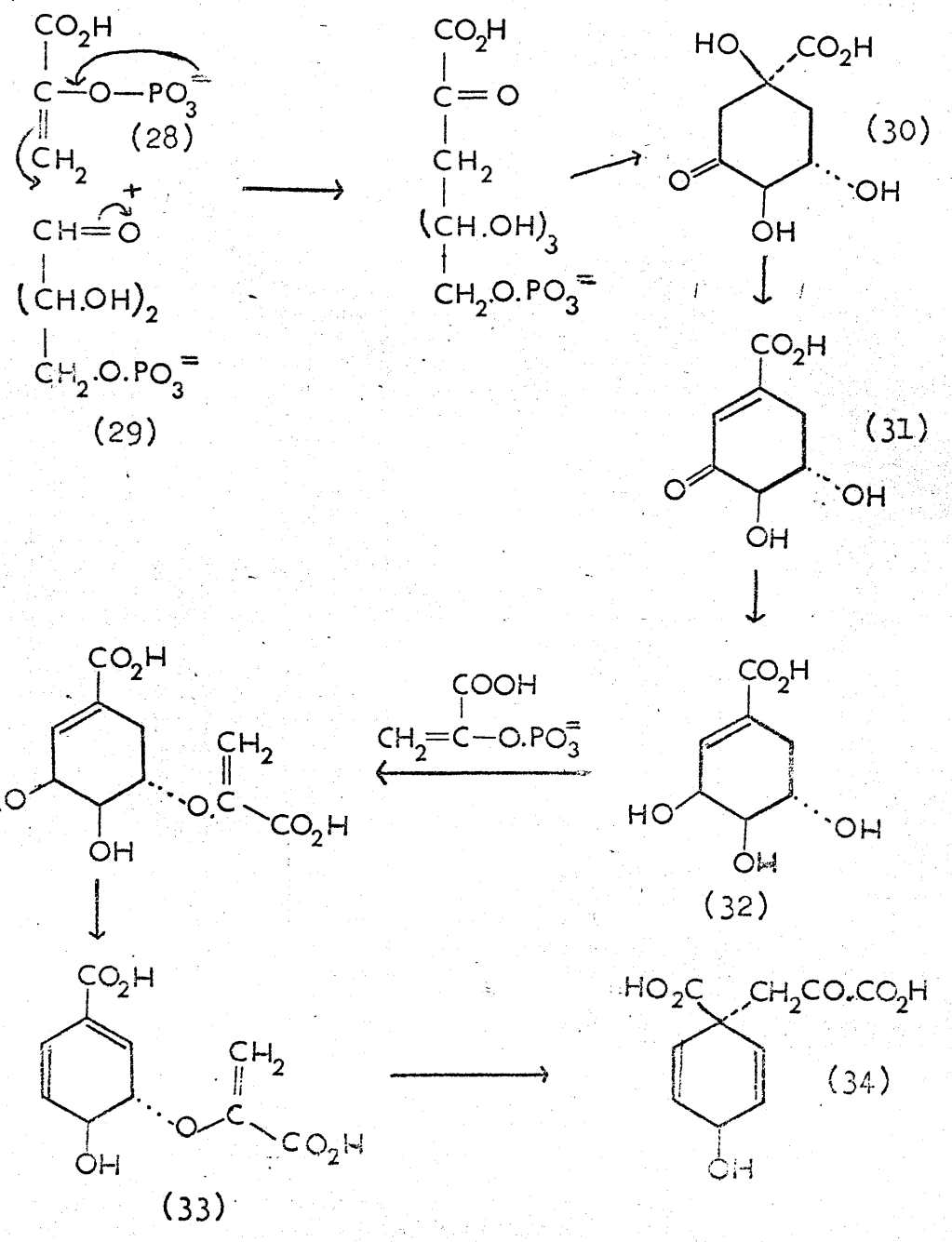
(ii) Claisen-type condensation leading to the flavone type of compound (25).

The formation of resveratrol (5; 23) by the condensation and ring closure of (22) as mentioned above is but one possible route to this compound. The occurrence in nature of other stilbenes with different hydroxylation patterns indicates an alternative method of biosynthesis in which one ring is phenylpropane derived, and the other acetate derived. It has been suggested that one (the resorcinol-A)[13 - 16] or both (A and B)[17] aromatic rings of naturally occurring hydroxystilbenes (27) are biosynthetically derived from acetate units. In the first case, it was considered that the remaining benzene ring was produced via the shikimic acid pathway, and more recent work[18] involving tracer experiments has confirmed the view that ring B does indeed originate from shikimic acid, and the A ring from acetate units.



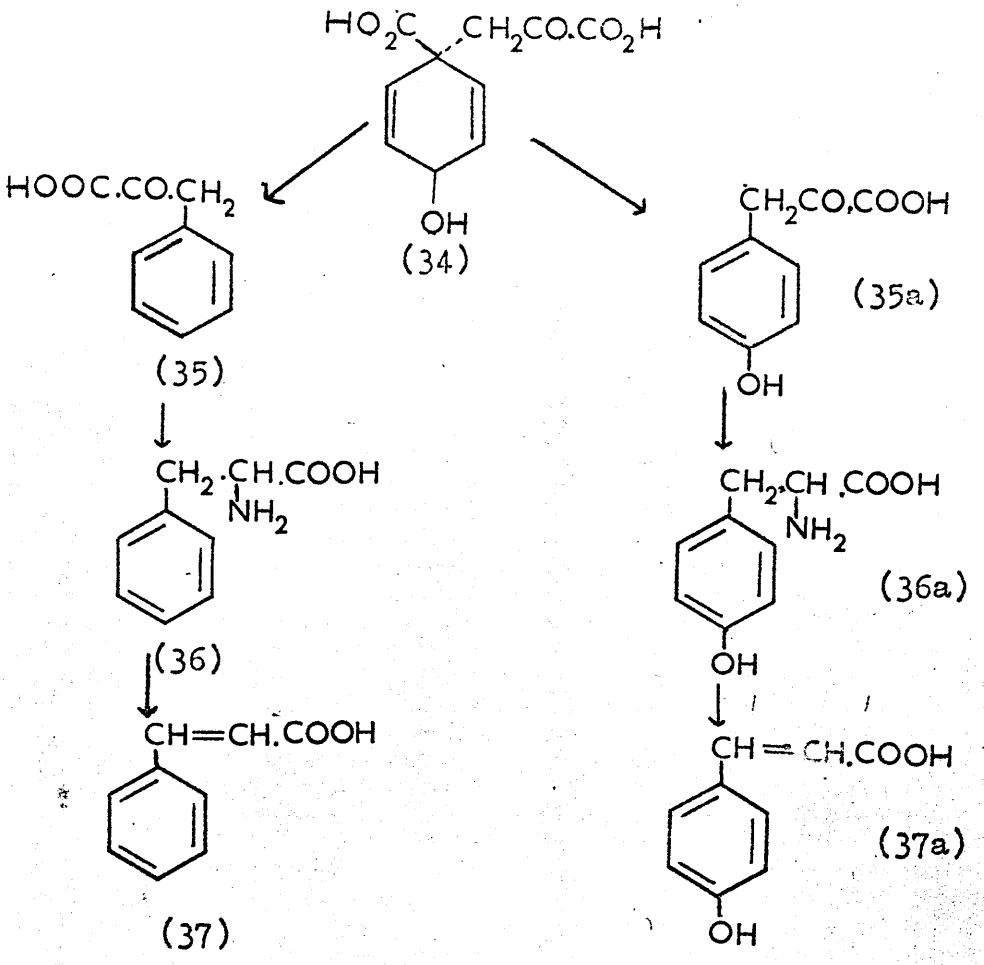
(27)

Flowsheet III.



Shikimic acid is the key intermediate in the biosynthesis of aromatic ring compounds from carbohydrate precursors,[19 - 22] the well-known phenylpropanoid ($C_6 - C_3$) units being derived from shikimic acid by addition of a three-carbon unit. Extracts have been obtained from micro-organisms (E. coli mutants) which are capable of carrying out the combination of phosphoenolpyruvate (28) with erythrose-4-phosphate (29) to give shikimic acid (32), (by way of dehydroquinic (30) and dehydroshikimic (31) acids) (flowsheet III). Further condensation of phosphorylated shikimic acid with (28) results in prephenic acid (34) via chorismic acid[23] (33). This latter acid (33) is a labile compound which can be converted both enzymatically and chemically into various intermediates in the biosynthesis of aromatic compounds[24]. It rearranges, on mild heating under alkaline conditions, into prephenic acid (34) and a model of (33) shows that the $C_{(3)}$ of the enolpyruvic acid side chain lies in close proximity to $C_{(1)}$ of the ring, which would presumably facilitate such a conversion to (34).

With the formation of prephenic acid (34), the pathway branches and the hydroxyl can either be lost via dehydration to phenylpyruvic acid (35) or retained



Flowsheet IV.

$CH_3CO.S.CoA + CO_2 \longrightarrow CH_2(COOH).CO.S.CoA$ (38)

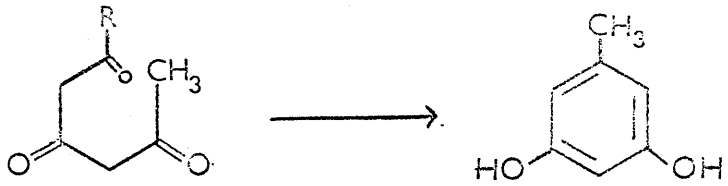
$R.CO.S.CoA + CH_2(COOH).CO.S.CoA$ (38) $\longrightarrow R.CO.CH(COOH).CO.S.CoA$

$R.CO.CH(COOH).CO.S.CoA \xrightarrow{-CO_2} R.CO.CH_2.CO.S.CoA$

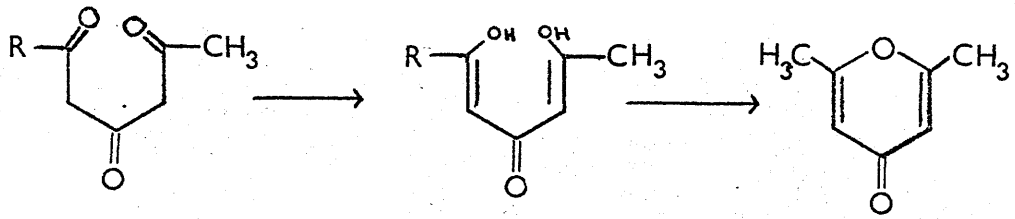
$R.CO.CH_2.CO.S.CoA + 2\ CH_2(COOH).CO.S.CoA \longrightarrow R(CO.CH_2)_3.CO.S.CoA$ (18)

to give p-hydroxyphenylpyruvic acid (35a). Each of these products can undergo transamination, giving phenylalanine (36) and tyrosine (36a) respectively. Cinnamic acid (37) is obtained by enzymatic deamination[25] of (36), and p-hydroxycinnamic acid (37a) - an intermediate in the biosynthesis of the coumarins - can arise either by an analogous deamination of tyrosine[26] (36a) or by hydroxylation of cinnamic acid itself. Tracer experiments[27] (using generally labelled shikimic acid) have disposed of the possibility that an important pathway to the cinnamic acids is by extension of a substituted benzoic acid by the addition of a two-carbon unit.

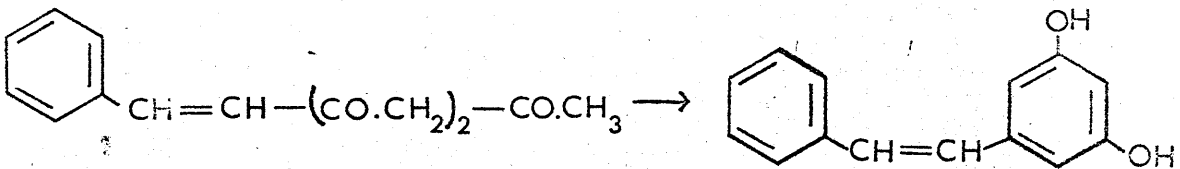
Recent work[28] on fatty acid biosynthesis has shown that the unit actually involved in the chain extension is malonyl coenzyme-A (38), formed by the carboxylation of acetyl coenzyme-A ester, an essential primary step in the biosynthesis (flowsheet IV). However, this does not significantly alter the above biogenetic scheme, although it is interesting to speculate that the actual cyclization which occurs may be influenced by the nature of the long chain β-keto intermediate, which could be either an acyl-malonyl derivative



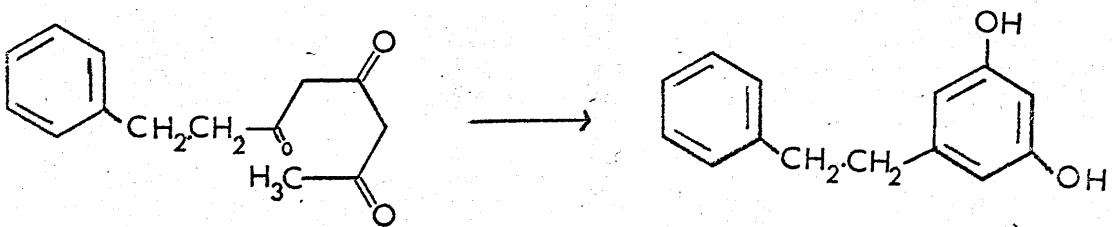
(18) R = CH_3 (20)



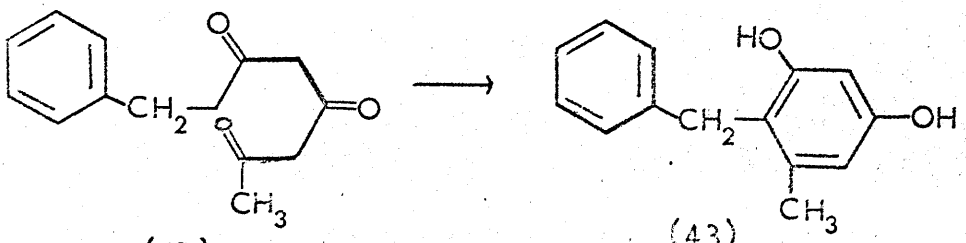
(18) R = CH_3 (39)



(40) (2)



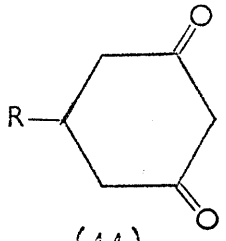
(41) (42)



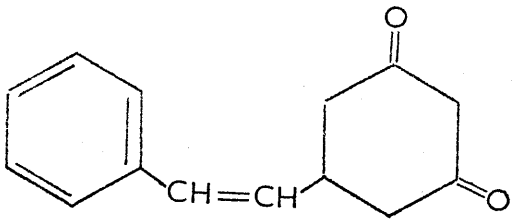
(41) (43)

R.CO.CH(COOH).CO.SCo.A., or an acyl-acetyl derivative, $R.CO.CH_2.CO.SCo.A.$

Theories and hypotheses must be substantiated by laboratory experiments, and as early as 1893, J.N. Collie[29] had observed the formation of orcinol (although in poor yields) (20) and of the related 2,6-dimethyl-4-pyrone (39; dehydracetic acid) by intramolecular aldol condensation of heptane-2,4,6-trione (18; diacetylacetone). Birch[30] synthesized the two β -polyketones, 8-phenyloct-7-ene-2,4,6-trione (40) and its corresponding dihydro-derivative (41). The former (40) might have been expected to undergo cyclization, via an aldol condensation, to give pinosylvin (2). However, treatment of trione (40) under a variety of conditions gave no indication whatever of the production of pinosylvin. A closer analogy to Collie's conversion of the heptane trione (18; $R = CH_3$) to orcinol would be cyclization of trione (41) to dihydro-pinosylvin (42). This is also a compound of some biosynthetic interest since it occurs with pinosylvin derivatives in pine heartwoods[31]. Birch finally accomplished this latter cyclization to (42), obtaining as a by-product 4-benzyl-5-methyl-resorcinol (43), produced by the alternative intramolecular aldol condensation of trione (41).

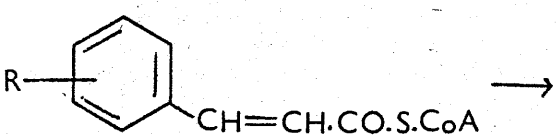


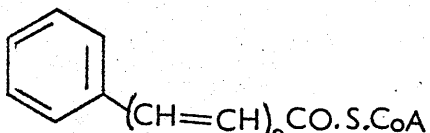
(44)



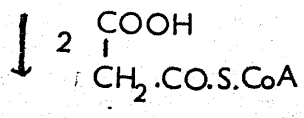
(45)

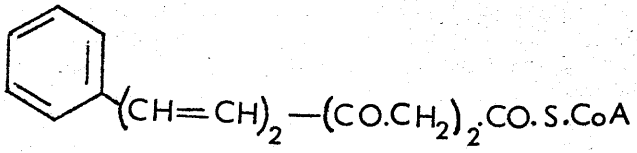
Flowsheet V.



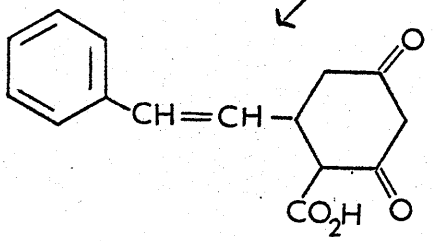


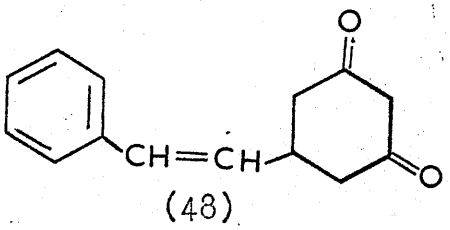
(46)



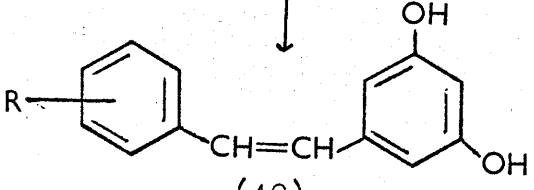


(47)





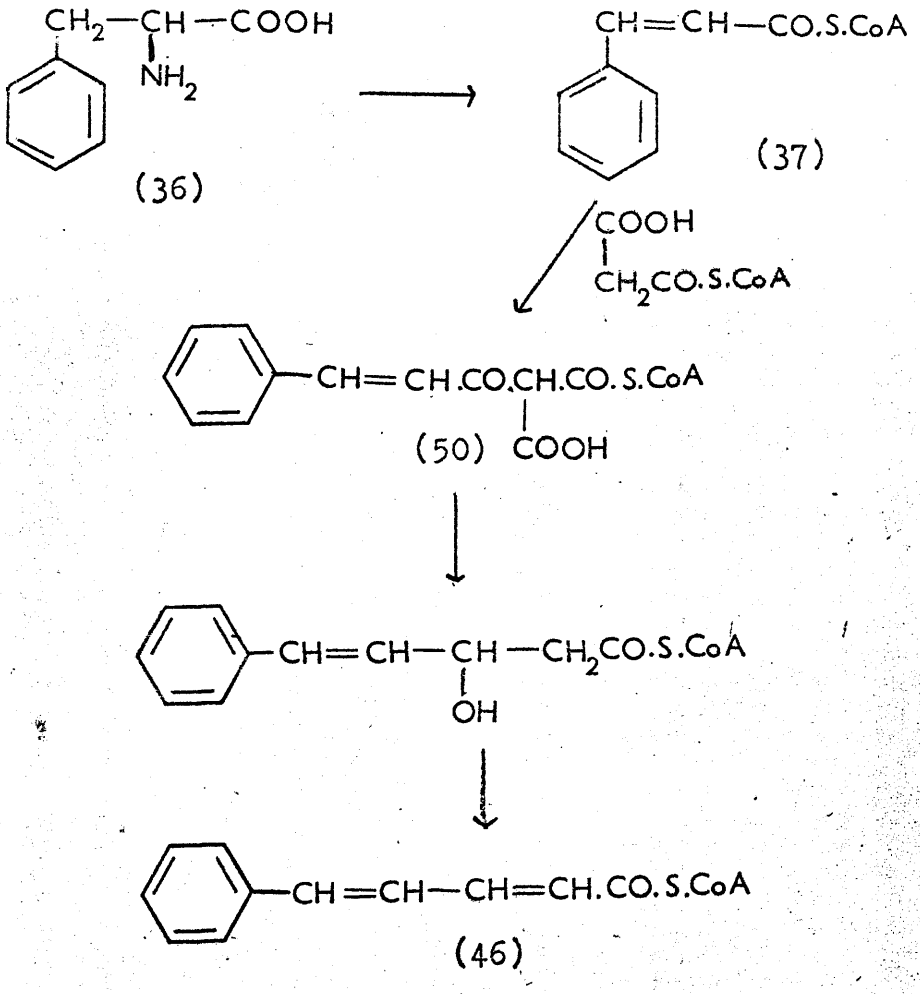
(48)

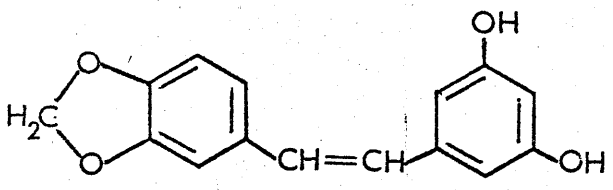


(49)

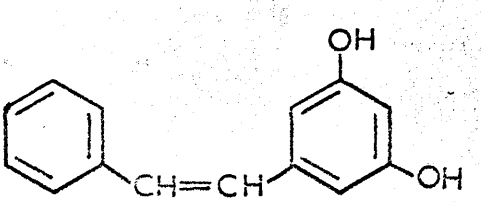
In the present work, the dehydrogenation of dihydroresorcinols (44) was studied, in particular the dehydrogenation of 5-styrylcyclohexane-1,3-dione (45), as this would provide a convenient route to pinosylvin and its analogues. It is of interest to note that an alternative biogenesis of "pinosylvin-type" compounds can be formulated (flowsheet V), with the cyclohexane-1,3-dione (45) as an intermediate. Thus, if the cinnamic acid is first converted to its homologous coenzyme-A ester (46; styrylacrylic acid), it may add on two units of malonyl coenzyme-A to give the ester (47). This latter can now only cyclize to a 5-styryl-cyclohexane-1,3-dione (48), and by dehydrogenation, may give the corresponding appropriately substituted pinosylvin (49).

The vital step in this scheme is that the starting ester (46) already contains two double bonds, and therefore, in the final "active" species (47) cyclization, via an internal Michael condensation, can only follow one path. Furthermore, the formation of the styrylcyclohexane dione (48) as an intermediate may be the driving force of the reaction. The starting ester (46) could, of course, arise from one of the intermediates in the original scheme. Deamination of


CH2—CH—COOH
NH2
(36)
CH=CH—CO.S.CoA
(37)
COOH
CH2CO.S.CoA
CH=CH.CO.CH.CO.S.CoA
(50) COOH
CH=CH—CH—CH2CO.S.CoA
OH
CH=CH—CH=CH.CO.S.CoA
(46)


OH
H2C
O
O
CH=CH
OH

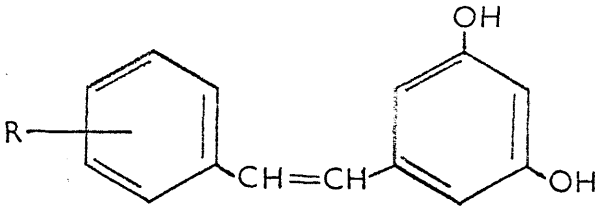
(51)


OH
CH=CH
OH

(2)

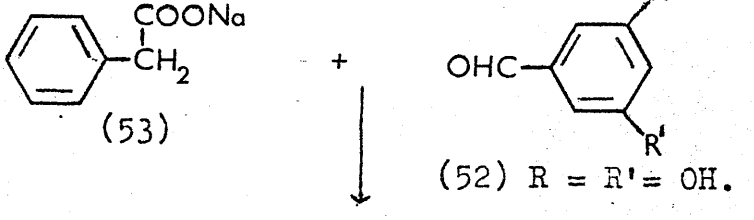
phenylalanine (36) produces cinnamic acid (37), from which ester (46) could be obtained by addition of a malonyl coenzyme-A ester unit, with subsequent decarboxylation, reduction and dehydration of the intermediate product (50).

A laboratory synthesis based on such a scheme was successfully established in the course of the present study, and both pinosylvin (2) and the 3,4-methylenedioxy derivative (51) of 3,4,3',5'-tetrahydroxystilbene (11) were prepared. Tracer experiments based on this scheme might well be of interest as regards its status as a possible biosynthetic pathway. The object of the present work was, in fact, to establish a general method of synthesis for these polyhydroxystilbenes since they are of interest from academic and taxonomic view-points. They may also have a considerable potential use as fungicides.



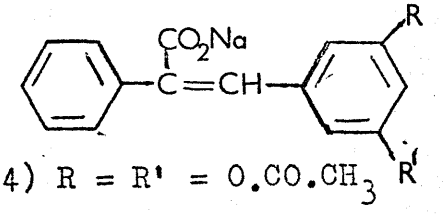
(1)

Flowsheet VI.

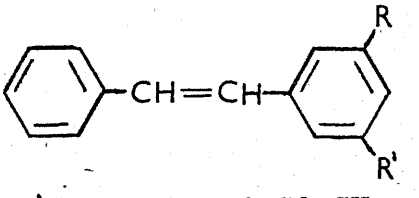


(53)

(52) R = R' = OH.



(54) R = R' = O.CO.CH_3

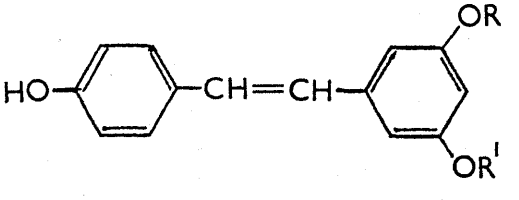


(55) R = R' = O.CO.CH_3

(2) R = R' = OH.

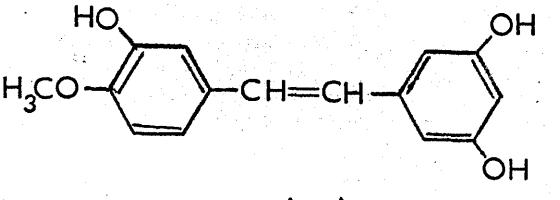
(3) R = OH; R' = O.CH_3

(4) R = R' = O.CH_3



(6) R = R' = H.

(7) R = R' = CH_3



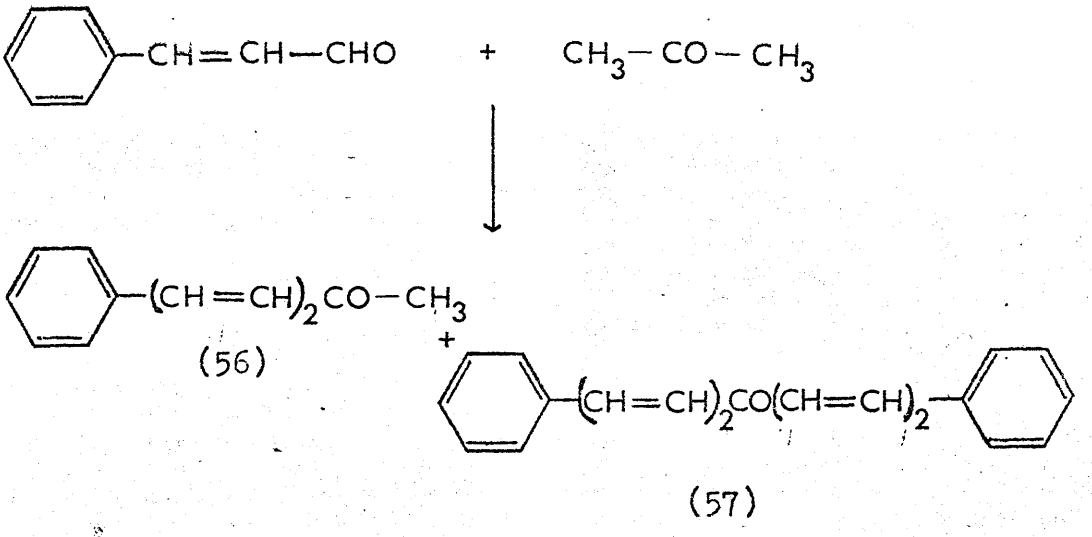
(12)

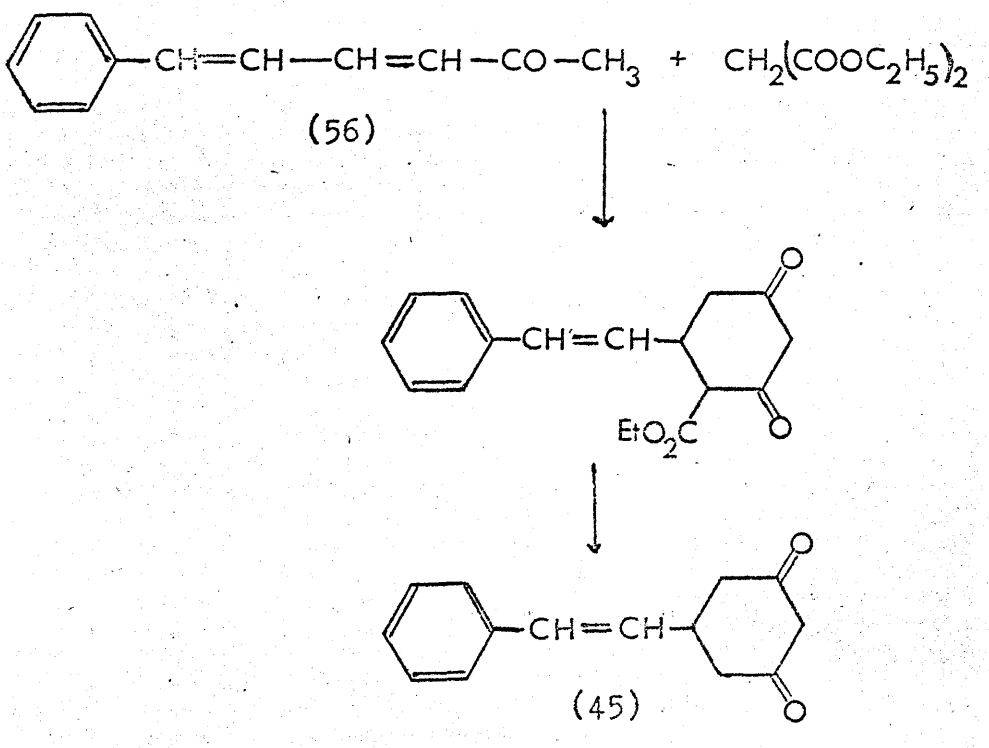
DISCUSSION.

The known naturally occurring polyhydroxystilbenes are substituted 5-styrylresorcinols[32](1). All previous attempts to synthesise this class of compounds have been characterized by rather drastic reaction conditions and have been of limited applicability. The general method employed in such attempts may be illustrated by the recorded synthesis[33] of 3',5'-dihydroxystilbene (2; pinosylvin) itself (flowsheet VI).

3,5-dihydroxybenzaldehyde (52) was condensed with sodium phenylacetate (53) in the presence of acetic anhydride by a Perkin reaction to give the corresponding stilbene carboxylic acid (54) in 46% yield. This latter was then subjected to a drastic high temperature decarboxylation (copper powder in quinoline solution at 240°), and the resulting oily mixture of cis- and trans-3',5'-diacetoxystilbene (55) hydrolysed with base, acid isomerised, and distilled under vacuum to produce the required trans-3',5'-dihydroxystilbene (2). To date, this method has been successful only in the preparation of pinosylvin[33] (2) and its mono-[34] (3) and di-methyl[35] (4) ethers, resveratrol[36] (6), pterostilbene[37] (7) and rhapontigenin[38] (12). It has the serious disadvantage of the vigorous decarboxylation conditions which result

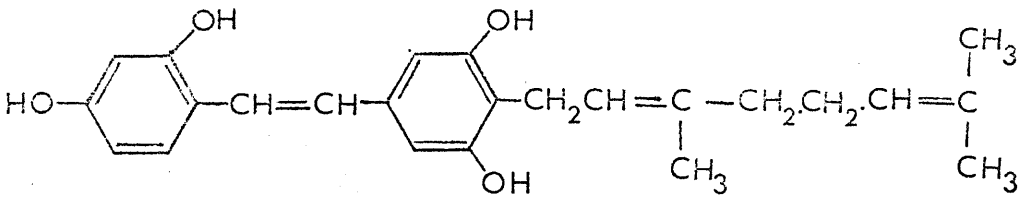
Flowsheet VII.



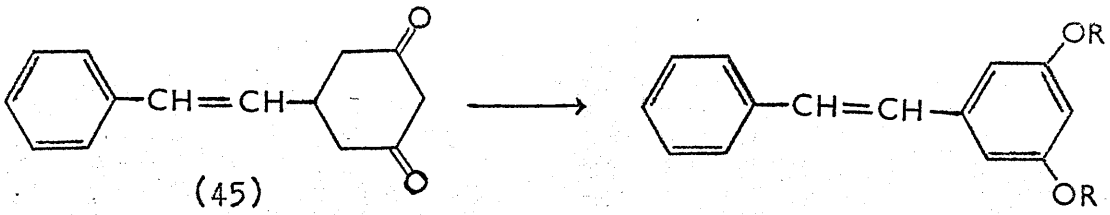


in an oily mixture of cis- and trans- stilbenes. Isomerization to the more stable desired trans-stilbene was achieved by acid in the cases of pterostilbene[37], its methyl ether and pinosylvin, and by thermal rearrangement for pinosylvin dimethyl ether. However, yields were low in all cases, only 24% of the oily mixture (55) being pure pinosylvin.

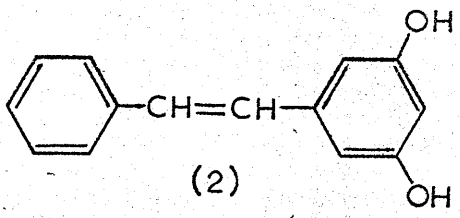
The present alternative route to pinosylvin was suggested by the ready availability of 5-styrylcyclohexane-1,3-dione (45). This latter can be prepared[39] by the reaction of cinnamaldehyde with acetone (flowsheet VII) followed by Michael addition of diethyl malonate to the resulting cinnamylideneacetone (56). Subsequent Claisen ring closure, with hydrolysis and decarboxylation, yields the required dione. The advantages of this scheme lie in the ready accessibility of the starting materials and also in the fact that reaction conditions are mild. The intermediate styrylcyclohexane-1,3-dione (45) is quite stable and contains two carbonyl groups which are conveniently situated for conversion to the required meta-positioned phenolic groupings. The two carbonyls also confer reactivity on the methylene group between them in the nucleus, thereby making it a suitable site for the introduction of a side-chain such as is found, for



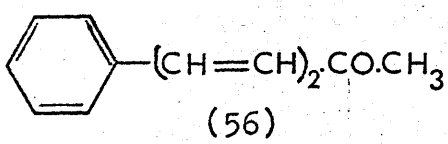
(10)



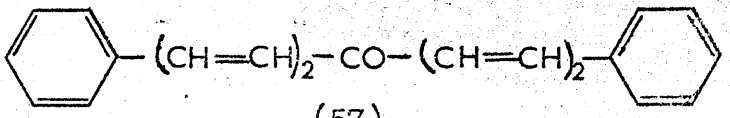
(45) (58) R = CO.CH$_3$



(2)



(56)



(57)

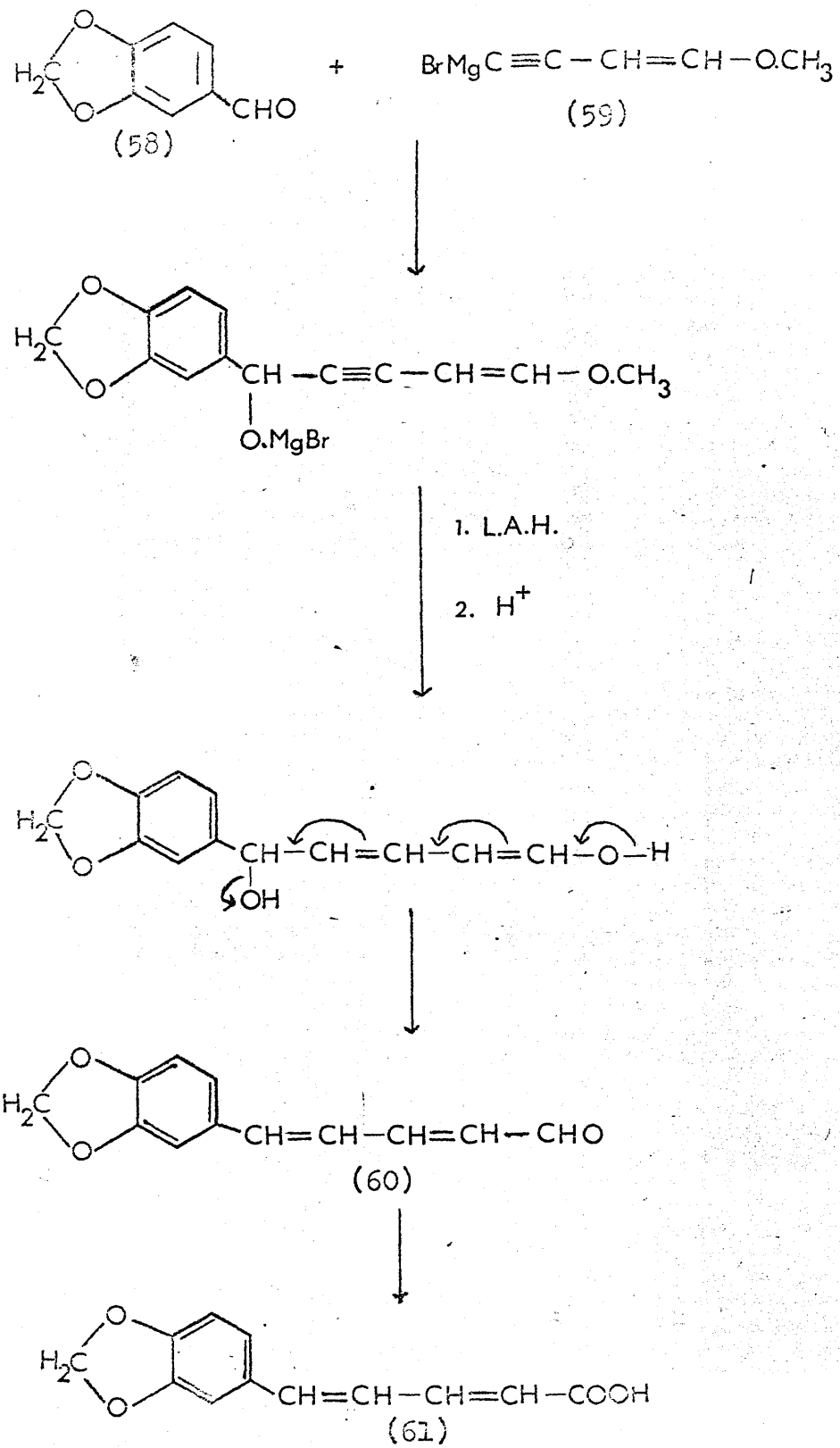
example, in chlorophorin (10).

It occurred to us that a simple dehydrogenation of the intermediate styrylcyclohexane-1,3-dione (45) should lead directly to pinosylvin (2). Dehydrogenation of such compounds to resorcinols had indeed already been effected by a variety of methods; anhydrous ferric chloride directly on the dione[40], sulphur or selenium on the dione enol ether[41] and palladium - calcium carbonate on the dione dibromide[42].

In the present work we found that 5-styrylcyclohexane-1,3-dione (45) was smoothly transformed in high yield (72%) to pinosylvin diacetate (58) on treatment with palladised charcoal in refluxing acetic anhydride. Base hydrolysis then converted the diacetate to the parent pinosylvin, identical in all respects with a sample derived from natural sources.

To extend this synthesis to more highly substituted 3',5'-dihydroxystilbenes with substituents in ring B, the correspondingly substituted cinnamylideneacetone would obviously be required as starting material. The method employed in the pinosylvin synthesis (flowsheet VII) was considered. However, despite the fact that the reaction conditions are mild, there is a serious disadvantage to

Flowsheet VIII.

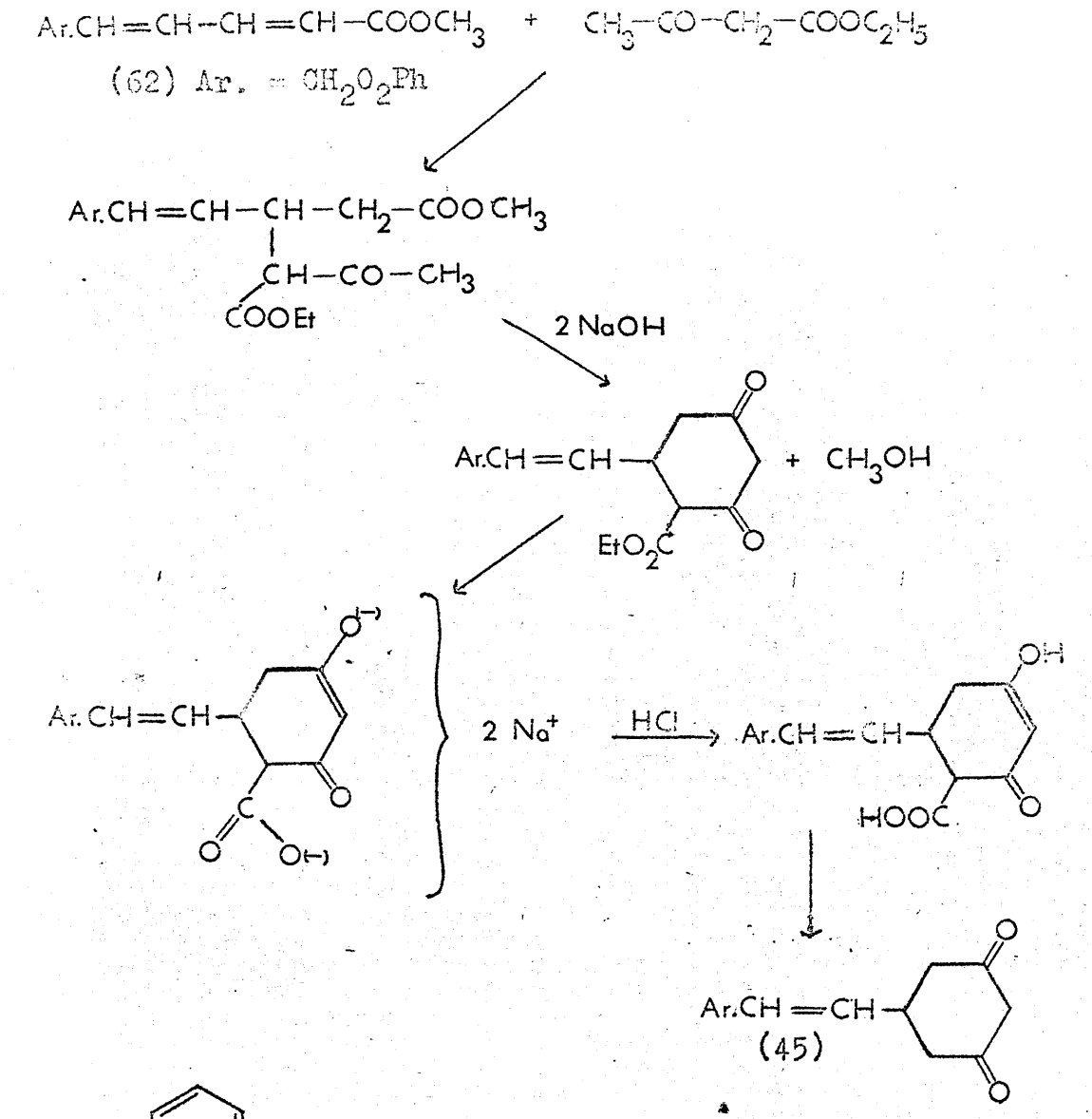


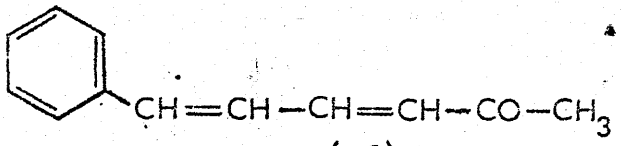
this synthetic route. The initial condensation gives rise to a mixture of two products, namely a monoarylidene-acetone (56) and a bis-compound (57). The separation of these two is difficult and so the overall yield is low. It was therefore necessary to devise an alternative route to these compounds, preferably using the fairly readily available substituted benzaldehydes as starting material.

Since pure piperonal (58) was commercially obtainable it was chosen as a trial compound for these studies. It was converted to the corresponding 3,4-methylenedioxy-cinnamylideneacetic acid (61; piperic acid) by a method which, it was hoped, would possess considerable generality. This involved (flowsheet VIII) a Grignard addition of 1-methoxybut-1-ene-3-yne magnesium bromide (59) to piperonal (58) with subsequent lithium aluminium hydride reduction of the triple bond, followed by acid catalysed rearrangement to the conjugated 3,4-methylenedioxy-cinnamylideneacetaldehyde (60). Oxidation of this latter with silver oxide gave piperic acid (61). The overall yield of acid from piperonal was good (89%) and the product was shown to be identical with an authentic sample of piperic acid by comparison of melting points, mixed melting points, infrared and ultraviolet spectra.

Theoretically, the required styrylcyclohexane-1,3-

Flowsheet IX.





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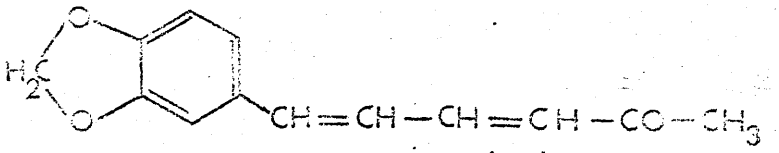


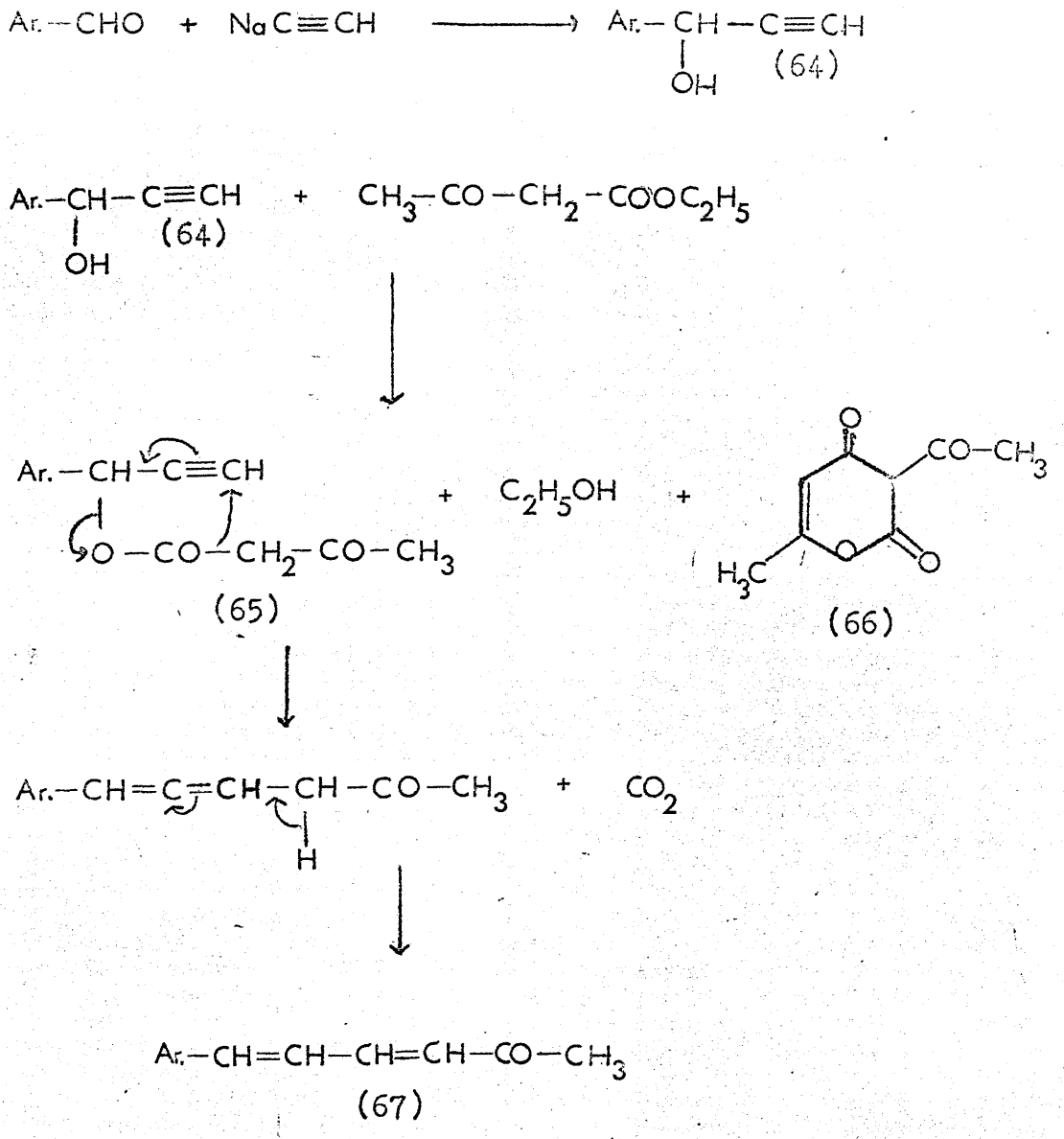
(63)

diones should now be obtainable via a Michael condensation of the appropriate cinnamylideneacetic acid, probably as its methyl ester (62), and ethyl aceteacetate, with subsequent ring closure, hydrolysis and decarboxylation (flowsheet IX). This reaction was attempted several times but without success.

It was then decided to attempt this condensation using the corresponding methyl ketone. However, conversion of piperic acid to the desired methyl ketone (63; methysticone) proved unexpectedly difficult. The reaction of lithium methyl with the acid[43], cadmium dimethyl with the acid chloride[44] and treatment of the corresponding diazoketone with hydrogen iodide[45] all gave surprisingly poor results.

In view of this disappointing lack of success, an alternative approach to the required substituted cinnamylideneacetones was attempted. It had been briefly reported in a patent[46] that pyrolysis of the acetoacetate of phenylethynylcarbinol (65; Ar. = Ph) gives cinnamylideneacetone (56) (presumably via the Cope-like mechanism indicated in flowsheet X). This reaction was therefore investigated and various modifications were employed to improve the yields of the product. The technique was finally perfected into what should prove

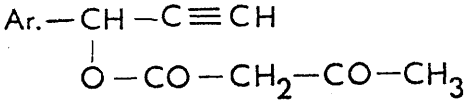
Flowsheet X.


Ar.—CHO + NaC≡CH
Ar.—CH—C≡CH
OH
(64)
Ar.—CH—C≡CH
OH
(64)
CH3—CO—CH2—COOC2H5
Ar.—CH—C≡CH
O—CO—CH2—CO—CH3
(65)
+ C2H5OH +
CO—CH3
H3C
O
(66)
Ar.—CH=C=CH—CH—CO—CH3
H
+ CO2
Ar.—CH=CH—CH=CH—CO—CH3
(67)

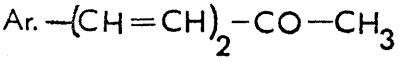
to be a smooth general method for the preparation of this class of compounds i.e. the naturally occurring polyhydroxy-stilbenes.

The required acetylenic carbinol (64) was formed (flowsheet X) from an appropriately substituted aromatic aldehyde by reaction with sodium acetylide in liquid ammonia[47], and then heated under reflux for several hours with acetoacetic ester in anhydrous toluene. The ethanol formed in the reaction (flowsheet X) was distilled off with the toluene, but the volume in the reaction flask was kept constant by a continuous dropwise addition of more pure toluene, thereby hoping to minimise the intermolecular condensation of acetoacetic ester to dehydracetic acid (66; formed as a by-product) by increased dilution. The progress of the alcoholysis was followed by examining the refractive index of aliquots of the distillate. When this reached a constant value, close to that of pure toluene, it was assumed that the ester exchange was complete and the remaining toluene was then replaced, again by slow distillation, with decalin.

According to the patent[46], the ethynylcarbinolacetoacetate (65) was rearranged to the corresponding arylideneacetone (67) by heating to 170° over six hours. However,



(65)



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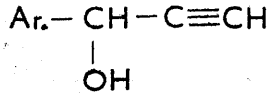
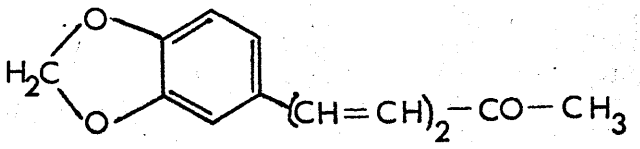
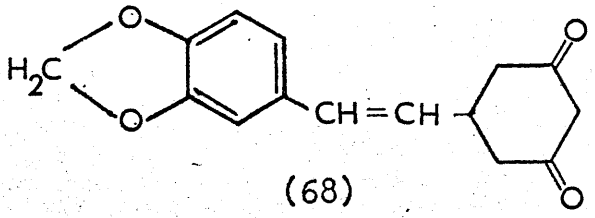


(64) Ar. = CH_2O_2Ph



(63)



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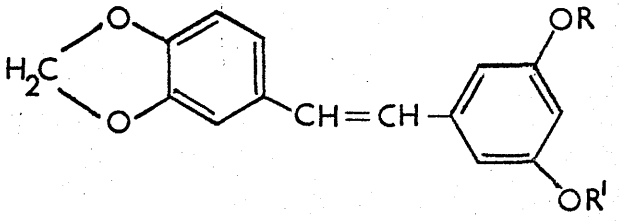
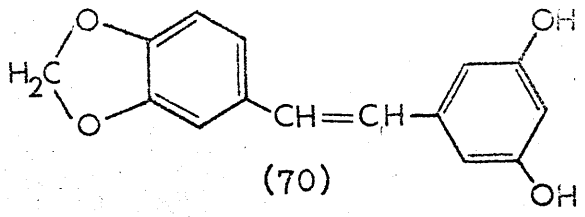


(69) R = R' = $CO.CH_3$

(70) R = R' = H

it was found to be extremely difficult to maintain this temperature for such a prolonged period; overheating gave rise to charring and polymerisation, while with insufficient heat the reaction did not attain completion. This problem was finally overcome by using the high boiling decalin as solvent for the rearrangement process. This vital modification avoided the extensive pyrolysis previously encountered and furnished the desired product direct from reaction mixture in good yield and in a pure form.

When this finally improved process was applied to the acetylenic carbinol derived from piperonal (64; Ar = CH_2O_2Ph) the crystalline 3,4-methylenedioxycinnamylideneacetone (63) was readily obtained in fair yield. This was converted to the 3,4-methylenedioxystyrylcyclohexane-1,3-dione (68) by condensation with diethyl malonate. Dehydrogenation of this dione by the palladised charcoal-acetic anhydride technique resulted in a smooth transformation to the diacetate (69) which was then converted to the parent phenol (70) by base hydrolysis. It was hoped to cleave the methylenedioxy ring in this product to afford the naturally occurring 3,4,3',5'-tetrahydroxystilbene (11) or rhapontigenin (12), the monomethyl



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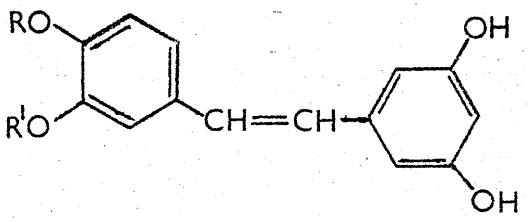
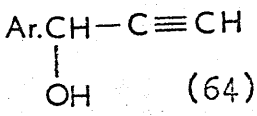
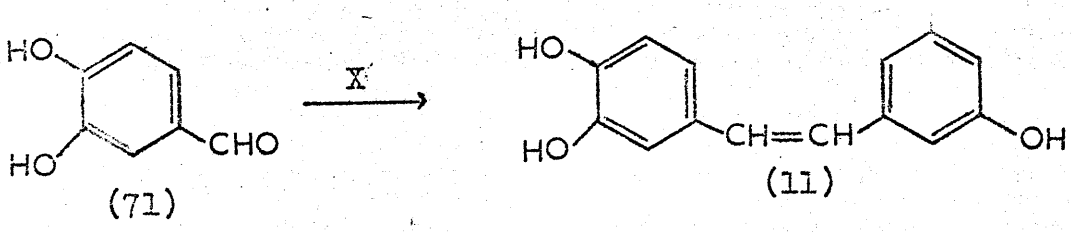


(11) R = R' = H

(12) R = CH_3; R' = H

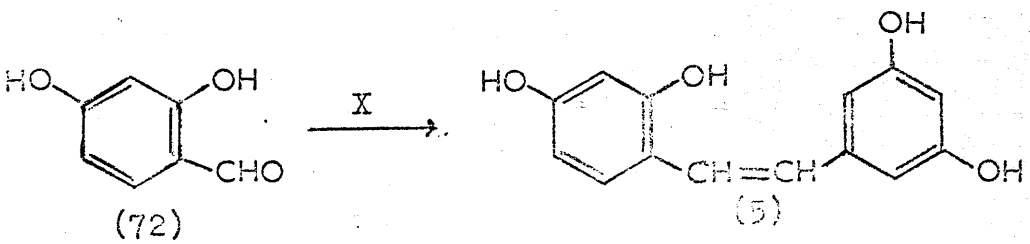


(64) Ar. = Ph.



(71)

(11)



(72)

(5)

ether of (11), but lack of time prevented this. Pinosylvin itself was also obtained via this process, starting from the corresponding ethynylcarbinol (64; Ar = Ph.).

It was also hoped to further illustrate the general applicability of this synthesis by preparing tetrahydroxystilbene (11) directly from protocatechualdehyde (71), and hydroxyresveratrol (5) from β-resorcylaldehyde (72), the free hydroxyl groups in each starting aldehyde being suitably protected, either as the tetrahydropyranyl ethers or as a carbomethoxy derivative. However, owing to circumstances, it was not possible to continue with this work and so these projected syntheses were not attempted. The work has, however, established a general synthetic route of some practicality to the substituted 3',5'-dihydroxystilbenes from the corresponding "ring-B substituted" aldehydes.

EXPERIMENTAL.

All infrared spectra were determined as nujol mulls unless otherwise stated, and were run on a Perkin-Elmer 137 spectrophotometer.

Ultraviolet spectra were measured in absolute ethanol solution with a Unicam S.P. 500 spectrophotometer.

All melting points are uncorrected and were determined on a Kofler block.

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

Pinosylvin Diacetate (58).

5-Styrylcyclohexane-1,3-dione[57] (662 mg.), palladised charcoal (650 mg. of 10%) and acetic anhydride (10 ml.) were heated under reflux for six hours. When cool, the reaction mixture was poured into water (30 ml.), allowed to stand for twelve hours, and the catalyst removed by filtration. The filtrate was extracted with ether (3 x 50 ml.), and the combined extracts washed with N sodium hydroxide solution (3 x 20 ml.), water (2 x 20 ml.), brine, and finally dried over anhydrous magnesium sulphate. The residue obtained on removal of the ether crystallized to give crude pinosylvin

diacetate (660 mg.) A specimen on recrystallization from methanol gave colourless prisms of m.p. 99-101°, alone or admixed with an authentic sample. (Found: C 72·91; H 5·57. $C_{18}H_{16}O_4$ requires C 72·96; H 5·44%). ν max. 1768 (acetyl carbonyl), 1603 (double bond), 1580 (aromatic unsaturation), 963 (trans double bond), 912 (O-Ph) cm^{-1}. λ max. 228 (ε 16,150), 295 (ε 28,000), 308 (ε 28,000), shoulder at 342 mμ (ε 12,800).

Pinosylvin (2).

The pinosylvin diacetate (307 mg.) was dissolved in methanol (10 ml.) and water (6 ml.) and sodium hydroxide (580 mg.) added. The mixture was heated on a steam-bath until all the alkali had dissolved, the solution changing from colourless to yellow to reddish brown. It was then allowed to stand at room temperature for four hours. Dilution with water, acidification to congo red paper with 6N hydrochloric acid, and extraction with ether gave a crude product which crystallized as plates from benzene to give pinosylvin (150 mg. 70%) m.p. 155-157°, alone or admixed with an authentic sample. (Found: C 79·5; H 6·02. $C_{14}H_{12}O_2$ requires C 79·22; H 5·70%). ν max. 3350 cm^{-1} (hydroxyl), no carbonyl absorption. λ max. 300 (ε 26,500), 310 mμ (ε 26,500).

Preparation of Piperic Acid (61) from Piperonal.

1-Methoxybut-1-ene-3-yne (26 gm.) in 175 ml. of tetrahydrofuran was added to a solution of ethyl magnesium-bromide, also in tetrahydrofuran, the temperature being maintained at about 40°. The mixture was stirred for an hour at room temperature, cooled with ice-water, and to it was added (over a twenty minute period) a solution of piperonal (37·5 gm.) in 50 ml. of tetrahydrofuran. The reaction was then allowed to stir overnight at room temperature under an atmosphere of nitrogen. The mixture was again cooled and treated with 16 cc. of absolute ethanol. Twenty minutes later, solid lithium aluminium hydride (9 gm.) was added in small portions over a 20-30 minute interval. The mixture was again stirred overnight under an atmosphere of nitrogen. Treatment with ethyl acetate (12 ml.) and water (56 ml.) together with 6N sulphuric acid destroyed the excess lithium aluminium hydride and at the same time hydrolysed the Grignard complex to a hydroxyl, which then eliminated to complete the conjugation. The aqueous and organic layers were separated, the latter being extracted with ether. The combined organic layers were washed with bicarbonate solution, brine, and dried over anhydrous sodium sulphate. The product, 3,4-methylenedioxycinnamylideneacetaldehyde, was not isolated, and the 'silver oxide' oxidation was

carried out "in situ". The theoretical yield of aldehyde was calculated, and to the alcoholic (20 ml.) solution was added double molecular quantities of silver nitrate (89 gm.) solution. Three molar quantities of 0·5N sodium hydroxide solution were added dropwise over 40 minutes with stirring and in a cooling mixture. The reaction mixture was allowed to stand overnight and then filtered through celite to remove metallic silver. The filtrate, an alkaline solution of the sodium salt of piperic acid together with any unchanged aldehyde, was extracted with ether and the piperic acid precipitated from the aqueous solution by acidification with hydrochloric acid. The precipitate was filtered and dried, the total yield of crude acid being 50 gm. (89%). The acid gave yellow needles from ethanol, m.p. (in a sealed capillary) 224° alone and when admixed with an authentic sample of the natural product. (Found: C 65·91; H 4·70. $C_{12}H_{10}O_4$ requires C 66·05; H 4·62%).

Methyl ester of piperic acid (62).

The acid (2·5 gm.) was refluxed with 25 gm. of a 2% methanolic-hydrogen chloride solution for six hours. The crude product recrystallized from methanol as yellow plates m.p. 146° (lit.[50] m.p. 146°). (Found: C 67·42; H 5·24. $C_{13}H_{12}O_4$ requires C 67·23; H 5·21%). This

ester was also prepared by treatment of the acid, in ether, with an excess of ethereal diazomethane.

Attempted Michael Condensation of Piperic Acid Methyl Ester with Ethyl Acetoacetate.

Sodium (0·5 gm; 1 atom) was dissolved in 40 cc. of freshly distilled anhydrous ethanol and to the solution was added methyl piperate (5 gm; 1 mole) and acetoacetic ester (3 gm; 1 mole), the mixture being heated on a steam-bath for three hours. A solution of sodium hydroxide (2 gm; 2 mole) in water (10 cc) was then added, and the whole refluxed for a further three hours. The reaction mixture was acidified to litmus with an aqueous-concentrated hydrochloric acid (2: 1) solution, and the alcohol removed by distillation, a periodic check being made to ensure that the solution remained acidic. The reaction mixture was further acidified to congo red paper with more of the previously used acid solution, cooled in ice, and the solid which was precipitated filtered and washed with a little water. However, instead of the expected styrylcyclohexane-1,3-dione, infrared and ultraviolet spectra, together with a mixed melting point, indicated that the solid product was in fact unchanged piperic acid.

Action of Lithium Methyl on Piperic Acid.

A solution of lithium methyl (0·06–0·07 molar) was prepared[43] and added to a stirred solution of piperic acid (5·6 gm.) in tetrahydrofuran. The mixture was refluxed for 30 minutes in an atmosphere of nitrogen, cooled to room temperature and then water slowly added to destroy any excess methyl lithium. The aqueous and organic layers were separated, the former being extracted several times with ether and the combined organic layers were washed with brine and dried over anhydrous magnesium sulphate. Evaporation of the organic solvents gave 2·2 gm. of crude methyl ketone (40%), but there was a considerable amount of unchanged piperic acid (3 gm.) recovered on acidification of the aqueous layer.

Action of Cadmium Dimethyl on Piperic Acid Chloride.

Piperic acid chloride (5 gm; 0·027 mole), obtained by refluxing the acid for two hours with an excess of redistilled thionyl chloride, was dissolved in anhydrous benzene (200 cc.) and the solution added to a cooled, stirred suspension of cadmium dimethyl[44], also in benzene. When addition of the acid chloride was complete, the mixture was refluxed for two hours and then diluted with water. The aqueous and organic layers were separated, the former being extracted three times with benzene.

The combined organic extracts were washed with bicarbonate solution, brine, and dried over anhydrous magnesium sulphate. However, removal of the benzene gave an intractable, black tarry product.

Hydrogen Iodide on the Diazoketone from Piperic Acid.

Piperic acid chloride, obtained by treating the acid (1·12 gm.), in benzene, with excess oxalyl chloride, was dissolved in benzene (5 ml.) and added slowly to an ethereal solution of diazomethane. After standing for several hours, the excess diazomethane was removed with the organic solvents by distillation under reduced pressure and the resulting crystalline solid dissolved in chloroform. Hydrogen iodide (1 ml. of 55%) was added to this chloroform solution and the mixture shaken in an ice-bath. Bubbling indicated the expected evolution of nitrogen. When the gas evolution ceased, the reaction mixture was diluted with water, washed several times with thiosulphate solution, brine, and then the chloroform layer dried over anhydrous magnesium sulphate. The product was again a sticky black tar, and although several attempts were made to purify it by chromatography, no success was achieved.

3,4-Methylenedioxycinnamylideneacetone (63).

3,4-Methylenedioxyphenylethynylcarbinol, prepared by the method of Clapperton and MacGregor[47], (38 gm. of

m.p. 33-35°), redistilled ethyl acetoacetate (42 gm.) and anhydrous toluene (200 ml.) were subjected to slow distillation in a stream of nitrogen. Pure toluene was added periodically to keep the volume in the distillation flask constant. When the refractive index of the distillate reached a steady value, decalin (180 ml.) was added dropwise and the distillation continued until the internal temperature in the reaction flask reached 170° and all the toluene had been removed. The whole was then heated under reflux for a further six hours. Crude 3,4-methylenedioxycinnamylideneacetone (25 gm; 55%) crystallized out of the decalin solution when it was cooled. A specimen crystallized from ethanol as pale yellow needles m.p. 89-90° (lit.[51] m.p. 89-90°). (Found: C 72·36; H 6·02. $C_{13}H_{12}O_2$ requires C 72·21; H 5·6%) ν max 1650 (dienone), 1490 and 1250 cm.$^{-1}$ (methylenedioxy group).

3,4-Methylenedioxystyrylcyclohexane-1,3-dione (68).

3,4-Methylenedioxycinnamylideneacetone (9·8 gm.) and redistilled diethyl malonate (7 ml.) were added to a solution of sodium (1·07 gm.) in anhydrous ethanol and the mixture heated under reflux for 2¾ hours. A solution of sodium hydroxide (3·7 gm.) in water (15 cc.) was added and the whole refluxed for a further 2½ hours and then

allowed to stand for 12 hours. The reaction mixture was acidified to litmus with dilute hydrochloric acid and the alcohol removed by distillation. When cool, the solution was acidified to congo red paper with more dilute hydrochloric acid, and the precipitate removed by filtration. The crude material (6·8 gm; 56%) crystallised from ethyl acetate to give 3,4-methylenedioxystyrylcyclohexane-1,3-dione as yellow prisms m.p. 179 - 181°. (Found: C 70·9; H 5·68. $C_{15}H_{14}O_4$ requires C 69·8; H 5·5%). ν max. 1603, 1550, 1490 and 1250 cm^{-1}. $\lambda_{max.}^{EtOH}$ 260 mμ (ε 24,800); $\lambda_{max.}^{EtOH/NaOH}$ 273 mμ (ε 29,200), 285 mμ (ε 27,800).

Dehydrogenation of 3,4-Methylenedioxystyrylcyclohexane-1,3-dione.

3,4-Methylenedioxystyrylcyclohexane-1,3-dione (1·9 gm.) was dehydrogenated by refluxing with palladised charcoal (1·07 gm. of 10%) in acetic anhydride (20 ml.) for six hours. The product, isolated as previously described, was chromatographed on silica gel, using benzene:chloroform (3:1) as eluent. The major fraction (1·2 gm; 47%) crystallized from methanol to give the diacetate (69) as white prisms m.p. 134-135°. (Found: C 66·89; H 4·64. $C_{19}H_{16}O_3$ requires C 67·05; H 4·75%). ν max. 1762 (acetyl carbonyl), 1603 (double bond), 1250 and 1490

cm^{-1} (methylenedioxy group). λ max. 293 (ε 12,650), 302 (ε 14,100), and 330 m μ (ε 20,300).

3,4-Methylenedioxy-3',5'-dihydroxystilbene (70).

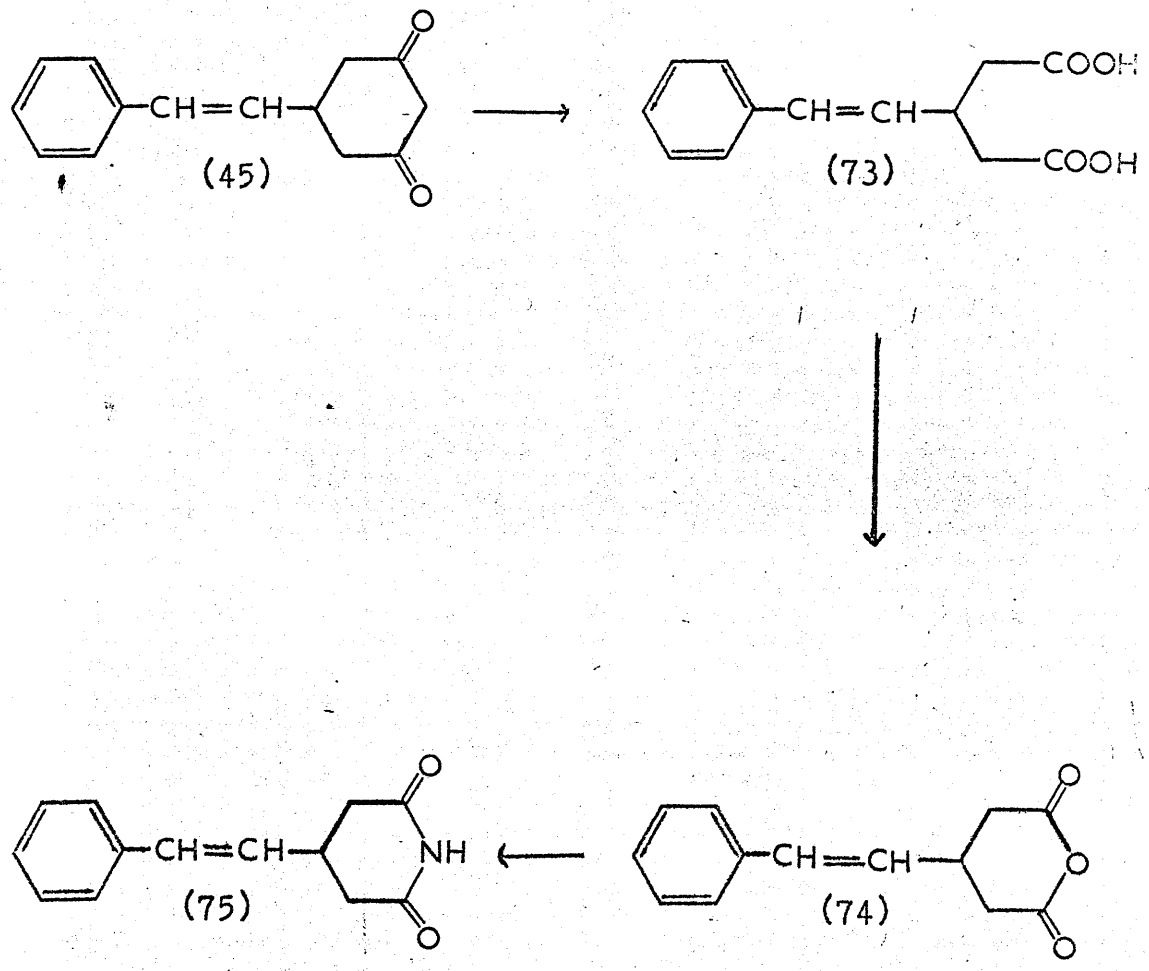
The diacetate, prepared above (0·2 gm.), dissolved in methanol (20 ml.) and water (1 ml.) was hydrolysed with sodium hydroxide (0·48 gm.) as before. The crude dark brown product (0·13 gm. 85%) on sublimation in high vacuum gave the 3,4-methylenedioxy derivative of 3,4,3',5'-tetrahydroxystilbene as white prisms, m.p. 171 - 173°. (Found: C 70·2; H 5·05. $C_{15}H_{12}O_4$ requires C 70·3; H 4·72%). ν max. 3,300-3400 (hydroxyl), 1603 (double bond), 1490 and 1250 (methylenedioxy group), 935 (trans double bond) cm^{-1}. λ max. 229 (ε 21,600), 290 (ε 17,550), 302 (ε 20,600), 328 m μ (ε 27,200).

The availability of styrylcyclohexane-1,3-dione prompted the examination of its conversion to styryl-glutarimide, a structure analogous to the cycloheximide group of antibiotics. This transformation was effected in the following way (flowsheet XI):-

2-Styryl-glutaric acid (73).

Styrylcyclohexane-1,3-dione (1·75 gm.) was dissolved in tertiary butanol (175 ml.). To this solution was

Flowsheet XI.



added sodium periodate (14 gm.) in water (50 ml.) and the mixture stirred overnight at room temperature. The precipitated sodium iodide was removed by filtration, washed with ether, and the aqueous/organic filtrate extracted with ether to remove any unchanged dione and then with sodium bicarbonate solution to remove the free acid. The acid (0·58 gm.) was precipitated from the bicarbonate with concentrated sulphuric acid and recrystallized from benzene to give plates m.p. 134 - 135°[48]. (Found: C 66·79; H 6·03. $C_{13}H_{14}O_4$ requires C 66·65; H 6·02%).

2-Styryl-glutaric anhydride (74).

The acid prepared above (0·56 gm.) was boiled with five times its weight of acetic anhydride for six hours. The crude product (0·497 gm.), obtained on evaporation of the acetic anhydride under reduced pressure, had a melting point of 135-138° (lit.[49] m.p. 138°, sinters at 135°).

2-Styryl-glutarimide (75).

The anhydride (0·126 gm.) was heated in a Wood's metal bath to about 125 - 135° and anhydrous ammonia bubbled through it for an hour. The mixture was heated for a further hour (205 - 215°) and allowed to cool. The residue was purified by sublimation, and crystallized as

needles from acetone, n.p. 175·5 - 179° with sublimation at 148°. (Found: C 72·33; H 6·04; N 6·76. $C_{13}H_{13}NO_2$ requires C 72·54; H 6·09; N 6·51%).

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Part II.

Studies in the Diterpenoid Series.

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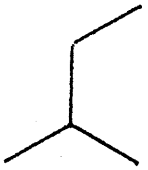

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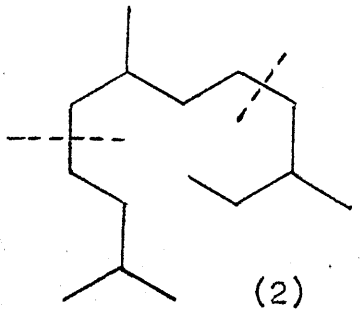

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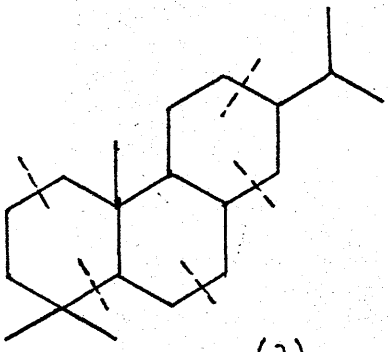

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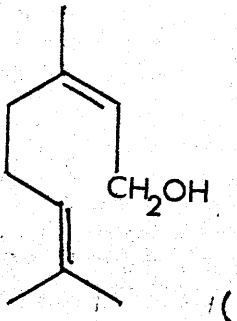

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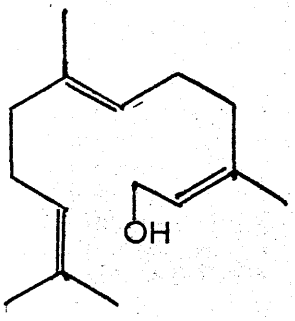

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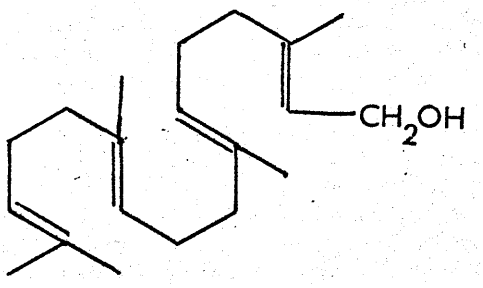

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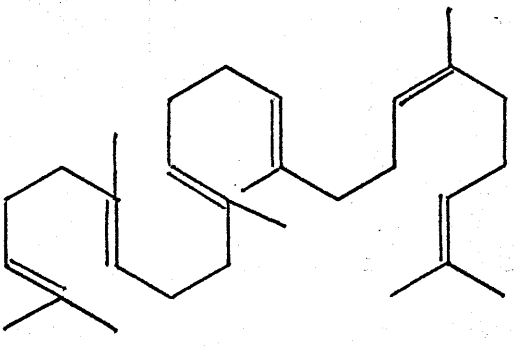
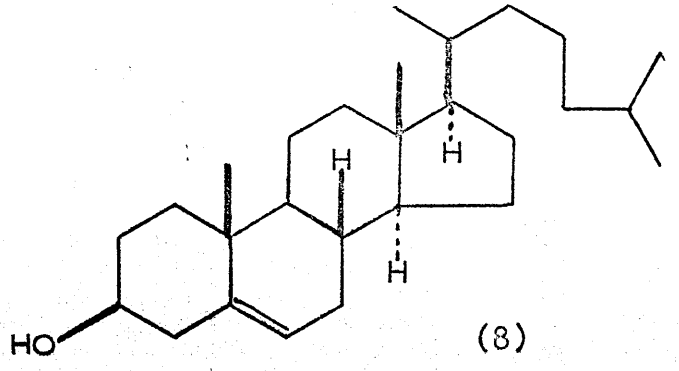


(7)

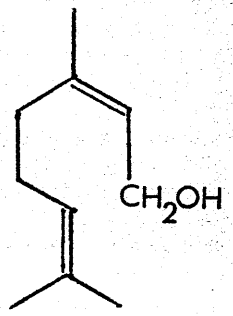
INTRODUCTION.

Early work in the monoterpene field[1] had indicated that the structures of several members of this class of compounds appeared to be derived from the condensation of two five-carbon fragments (1). With the subsequent elucidation of more complex terpenoid structures, this simple rule that terpene skeletons are, in principle, divisible into isopentane units was still found to apply. These C_5 building units can be linked together either in a regular head-to-tail fashion, as is found in the carbon skeleton of farnesol (2) and those of the sesquiterpenes derived from it, or irregularly as in the case of the diterpenoid skeleton of abietic acid (3). The fact that this five-carbon isopentane (or isoprene) unit apparently represents the single structural feature linking the many varied terpenoid groups would seem to imply a common biochemical origin for these compounds.

The structural complexities of this class of natural products were simplified by the ennunciation of Ruzicka's Biogenetic Isoprene Rule[2]. This proposed that all terpenoids can be derived from the condensation of isopentane units into a number of simple acyclic intermediates such as geraniol (4), farnesol (5), geranyl geraniol (6) and squalene (7). These then undergo cyclizations and



(8)



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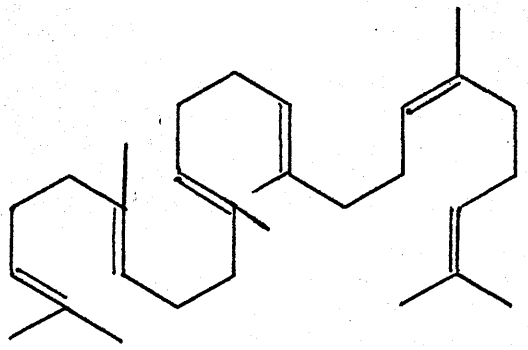
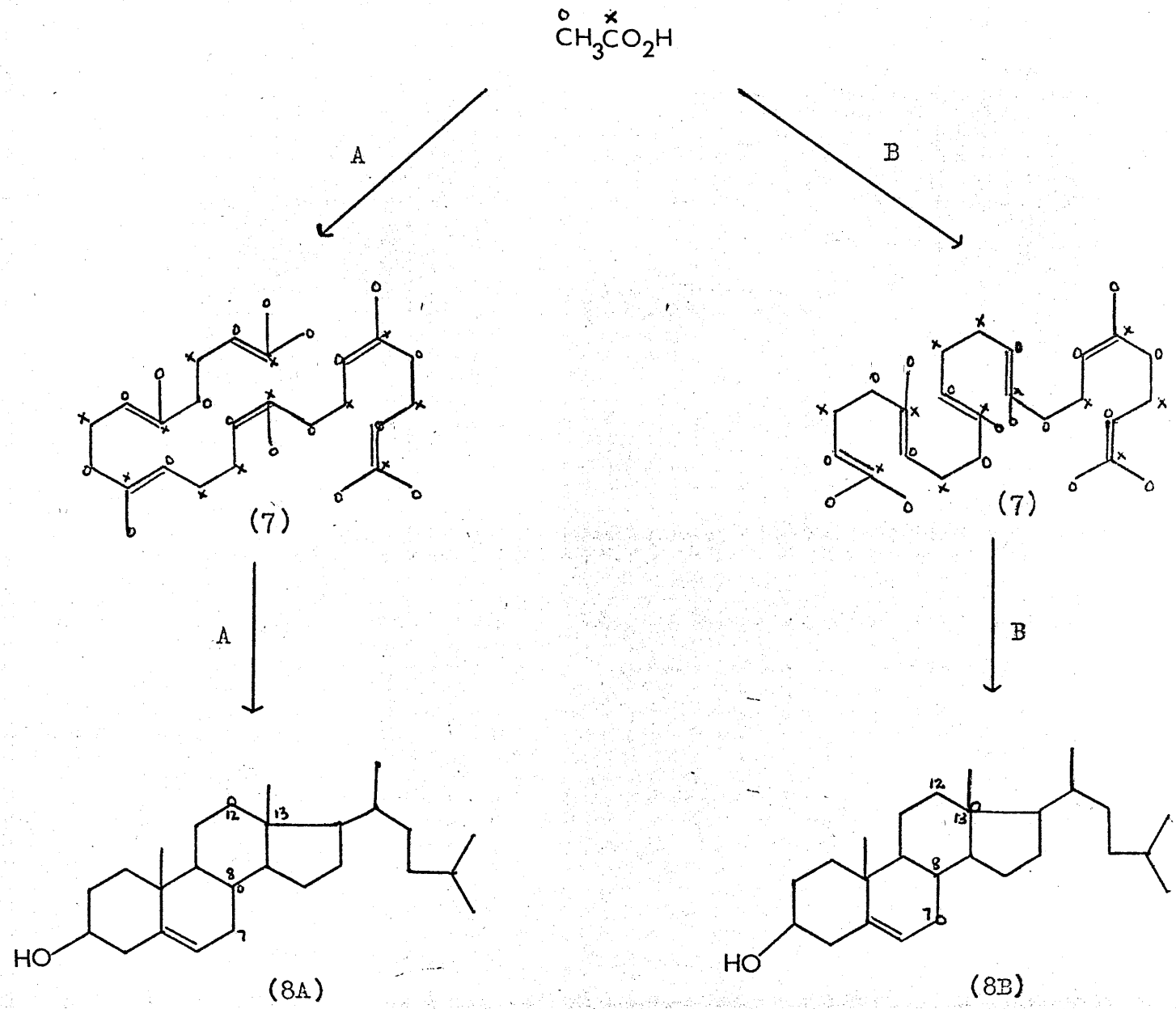


(7)

rearrangements (where appropriate) by accepted reaction mechanisms to give the individual members of the classes of mono-, sesqui-, di- and triterpenoids (and steroids) respectively. The ennunciation of this Biogenetic Isoprene Rule not only served to outline possible biogenetic pathways to the terpenoids but also extended, in any one case, the number of carbon skeletons which could be postulated on the basis of a simple linkage of isopentane units.

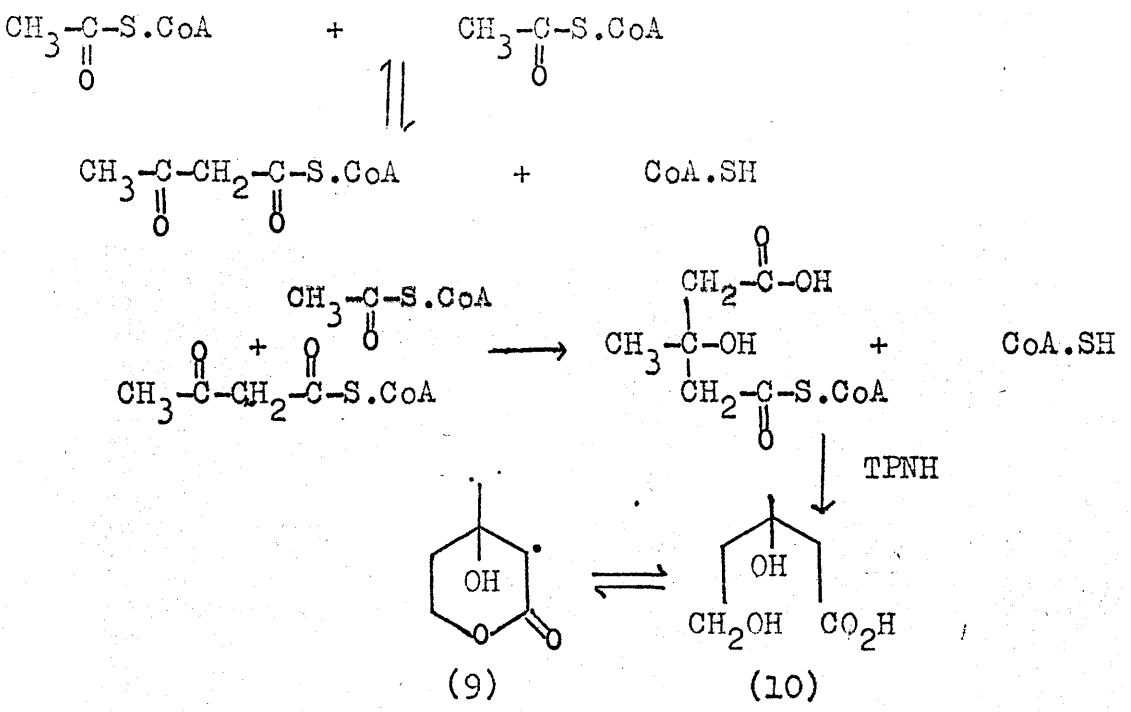
The nature of the pathways and mechanisms involved in the biogenesis of terpenoid compounds can be considered in three distinct stages. First, there is the problem of the origin of the actual isopentane building units. Second, the manner in which these units, once formed, condense to the postulated acyclic precursors of the Isoprene Rule. Finally, the mechanisms involved in the cyclization and rearrangements of these precursors, or their equivalents, to the individual terpenoids.

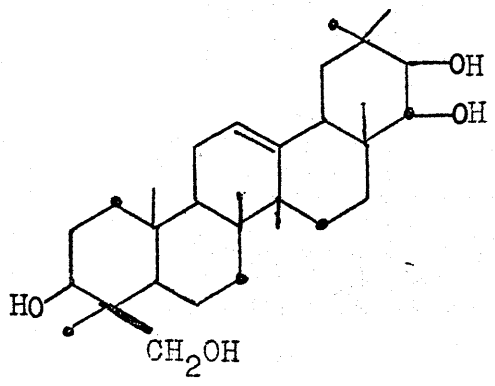
The importance of acetic acid, in the form of its coenzyme-A ester, as a building unit in cellular synthesis has been recognized for some time[3,4]. In particular, it is well-known as a carbon source for a number of steroids and terpenoids e.g. cholesterol[5](8), geraniol[6](4) and squalene[7](7). The synthesis of cholesterol from acetic


°CH3C*O2H
A
B
(7)
(7)
A
B
HO
12
13
8
7
(8A)
HO
12
13
8
7
(8B)

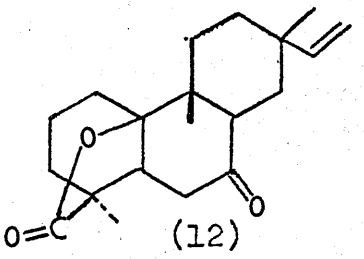
acid labelled on the methyl and carboxyl carbon atoms[3] has been more fully studied than that of any other "terpenoid". It was Sir Robert Robinson[8] who first suggested a probable biogenetic relationship between squalene and the steroids by postulating a cyclization and demethylation of squalene (7) which would lead to cholesterol (8A) as indicated (route A). A later alternative scheme (B) was advanced by Woodward and Bloch[9]. However, a steroid molecule synthesised from labelled acetate, would have an isotope distribution as shown. A comparison of the two schemes A and B indicates that in the cholesterol so formed the carbon atoms at positions 7, 8, 12 and 13 are derived from different acetate carbons. However, the more recent degradative studies[9,10] on an appropriately labelled cholesterol have completely elucidated the order of every acetic acid residue in the steroid molecule and shown it to be in complete agreement with the original hypothesis of Woodward and Bloch[9]. The sequence of labelled carbon atoms in the product is also consistent with a derivation based on a linkage of isoprenoid units. However, although it was now obvious that the acetate molecules condensed to five-carbon fragments, the exact nature and mode of formation of these isopentane units from acetate caused considerable difficulty until Folkers isolated β-hydroxy-β-methyl-δ-valerolactone[11] (9), the lactone of

Flowsheet 1.

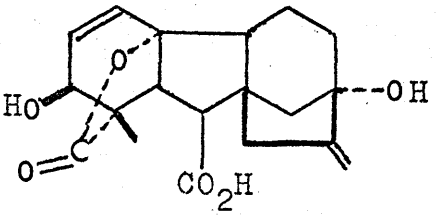




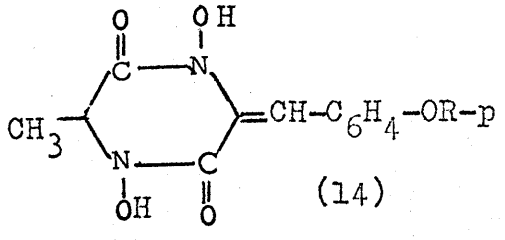
(11)



(12)

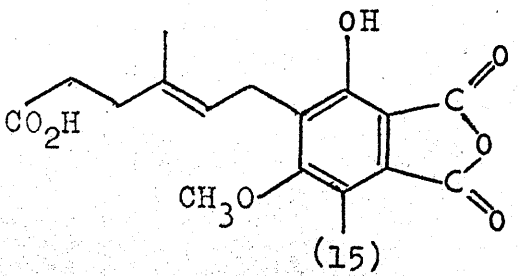


(13)



(14)

R = $CH_2C(=CH.CH_3)C(CH_2)_2$ with CH and $C-(CH_3)_2$

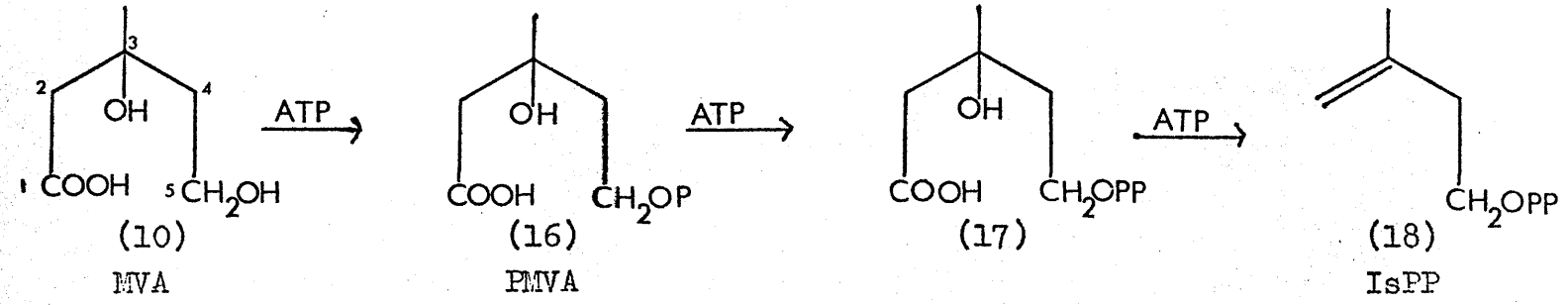


(15)

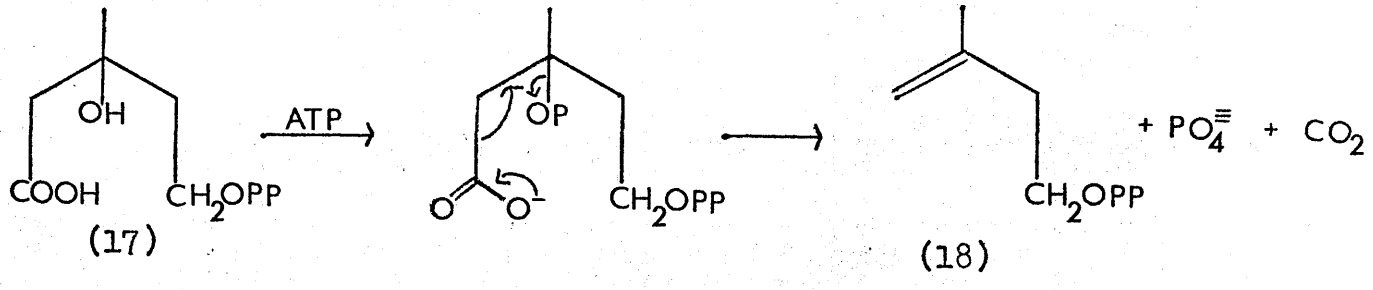
mevalonic acid (10; MVA) and it was shown[12,13] that it could replace acetate in the biosynthesis of cholesterol. The extent[13] to which mevalonic acid was incorporated into cholesterol suggested that this molecule is itself directly involved without prior cleavage to smaller units. The acid can be shown[14] to be derived from acetyl coenzyme-A ester molecules via a series of Claisen-type condensations (flowsheet 1).

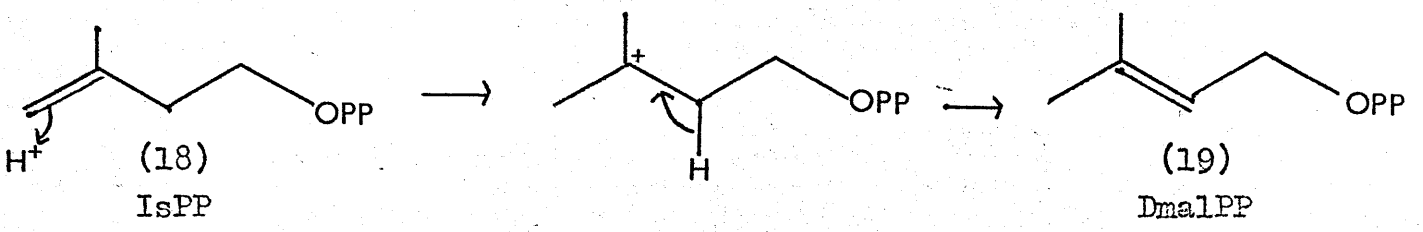
The importance of mevalonic acid in terpenoid biogenesis has been emphasized and supported by the biochemical studies of Birch and Arigoni. These workers and their collegues have demonstrated the incorporation of 2-^{14}C-MVA into soyasapogenol-A[15] (11), rosenonolactone[16,17] (12), gibberellic acid[18] (13), mycelianamide[19] (14) and mycophenolic acid[19] (15). This work confirmed the view of Tavormina[13] that at some stage in its incorporation, the mevalonic acid decarboxylates to a five-carbon unit and the carboxyl carbon atom does not appear in the product. The exact nature of the isopentane (isoprene) unit involved in terpenoid biogenesis and its evolvement from MVA was elucidated by Lynen[25,28] and Bloch[26]. They also indicated the mechanism by which the "active" isoprene undergoes condensation to yield the farnesol and geraniol precursors postulated by Ruzicka[2] (flowsheet 11).

Flowsheet II.



Flowsheet III.

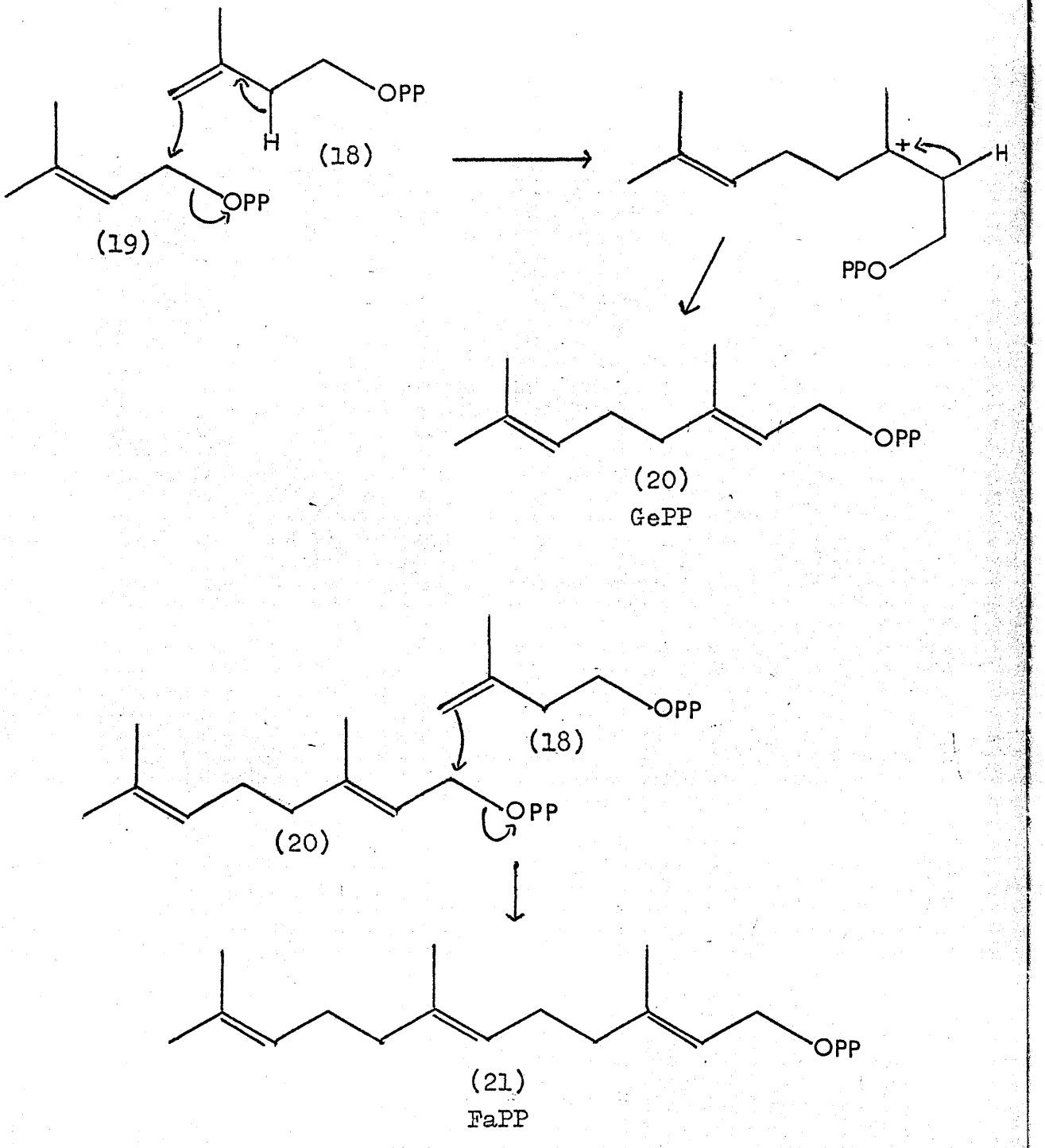




The action of adenosine triphosphate (ATP) on mevalonic acid (10) produces first of all mevalonic acid-5-phosphate (16; PMVA); further phosphorylation of this yields mevalonic acid-5-pyrophosphate (17). Both these compounds still contain the carboxyl group of MVA since they can be formed from both 1-^{14}C and 2-^{14}C-mevalonic acid. However, prolonged action of ATP and yeast extracts on PMVA (16) yields not only mevalonic acid-5-pyrophosphate (17) but also a new compound which lacks the carboxyl group of MVA. The formation of this new compound, which was shown[20,21,22] to be isopentenyl pyrophosphate (18; IsPP), from the pyrophosphate of mevalonic acid (17) involves both decarboxylation and dehydration since, although the process is ATP dependent[22], no additional phosphate group is introduced. In addition, the mechanisms of the dehydration and decarboxylation processes must be concerted (flowsheet 111) since there is no deuterium transferred to an intra-chain carbon atom when squalene is synthesized from mevalonic acid in heavy water.

Isopentenyl pyrophosphate (18; IsPP) undergoes enzymatic isomerization to dimethylallyl pyrophosphate (19; DmalPP) in the presence of isopentenol pyrophosphate isomerase[23], and it is these two compounds (IsPP and DmalPP) which must now be regarded as the "active isoprene"

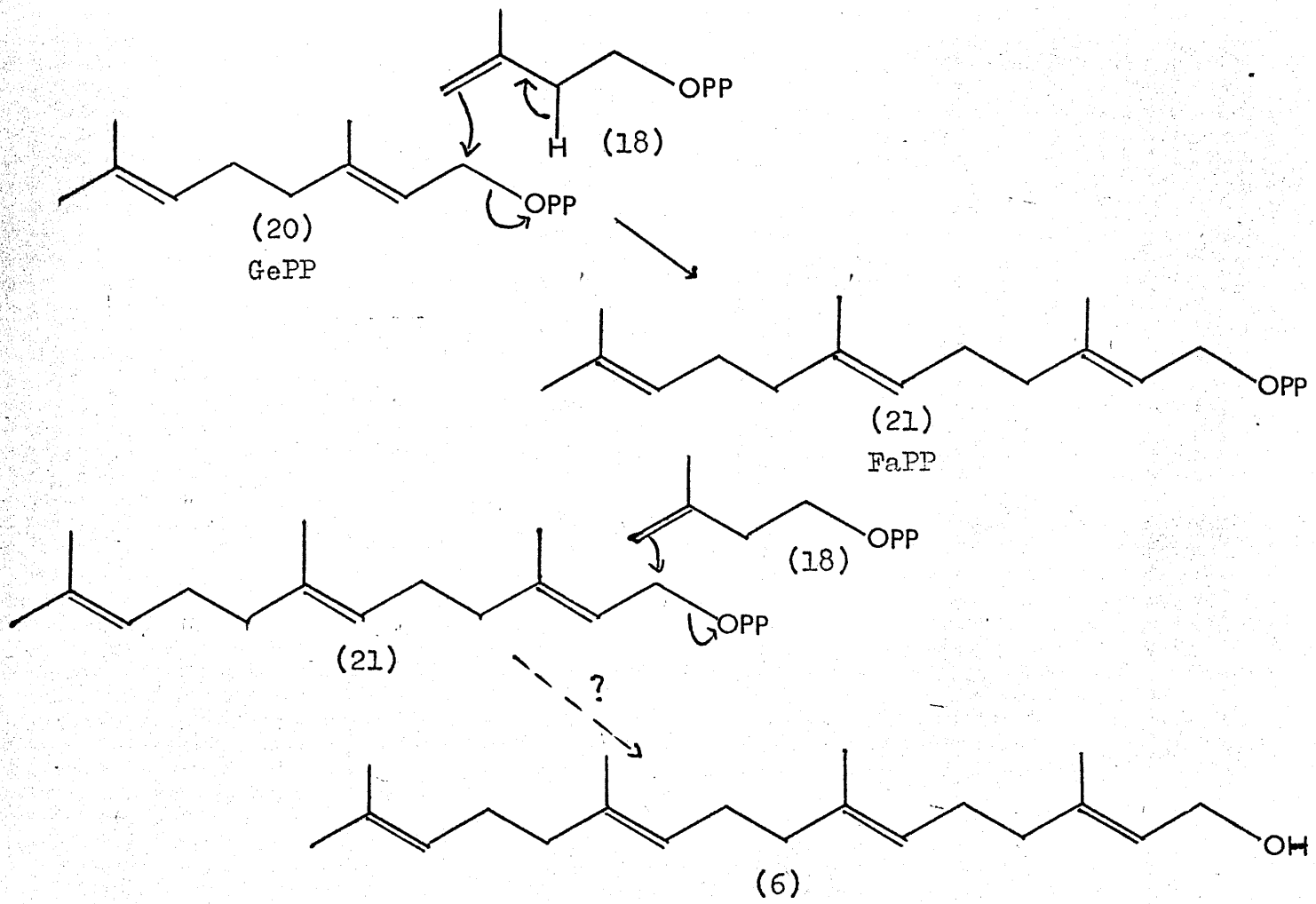
Flowsheet IV.



precursors of the terpenoids.

Isopentenyl pyrophosphate, labelled with ^{14}C on C-1, has been enzymatically converted into squalene and cholesterol[20]. In experiments using crude yeast extract from which TPNH (the reduced form of triphospho-pyridine nucleotide essential for the reductive dimerization of farnesyl residues) had been omitted, an acid-labile allylic pyrophosphate was isolated and shown to be farnesyl pyrophosphate[20,21] (21; FaPP). The biosynthesis of this latter compound involves more than a simple self-condensation of dimethylallyl pyrophosphate since it has been shown[24,25] that synthesizing systems containing only one or other of the "active isoprenes", i.e. either IsPP or DmalPP but not both together, fail to produce the farnesyl pyrophosphate. The synthesis of FaPP does in fact require[24] an initial condensation (flowsheet 1V) between isopentenyl pyrophosphate (18; IsPP) and its isomerized form (19; DmallPP) to give geranyl pyrophosphate (20; GePP).

Lynen[20,24] and Bloch[26] have postulated that this condensation involves the formation of an allylic carbonium ion from heterolytic cleavage of (19) and this can alkylate the activated double bond of IsPP (18). Subsequent loss of a proton furnishes the geranyl pyrophosphate (20). This product is also an allylic pyrophosphate and can now

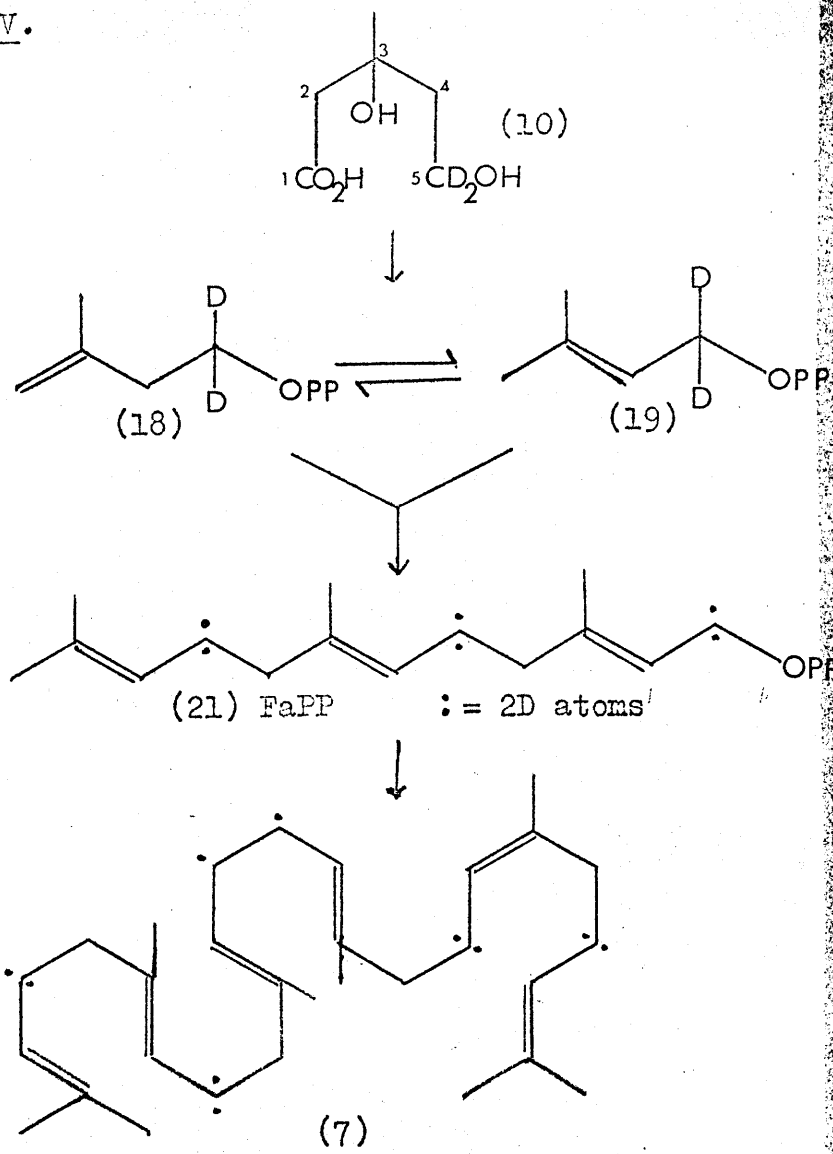

OPP
H
(18)
OPP
(20)
GePP
OPP
(21)
FaPP
OPP
(18)
OPP
(21)
?
OH
(6)

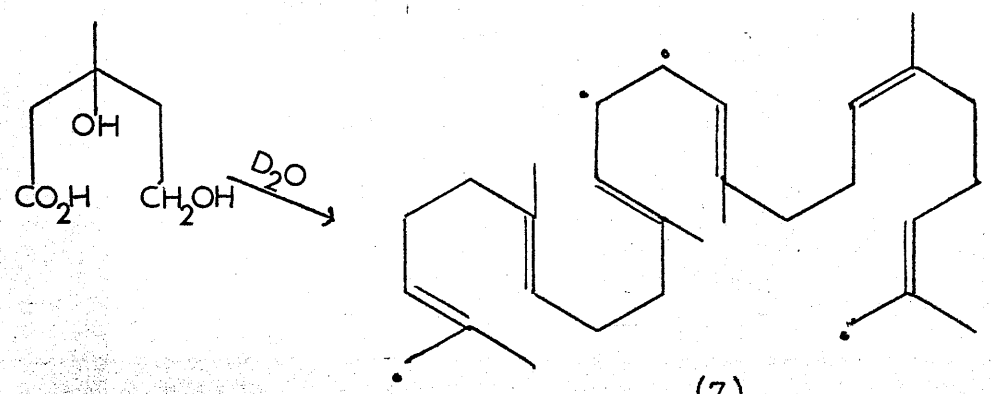
similarly alkylate another molecule of IsPP, this second condensation resulting in farnesyl pyrophosphate (21). However, the existence of a carbonium ion has not been proved and the proton elimination and alkylation may well be concerted. Further condensation of farnesyl pyrophosphate (21; FaPP) with a third molecule of isopentenyl pyrophosphate (18; IsPP) to give geranylgeraniol (6), the postulated isoprenoid precursor of the diterpenes, has not as yet been demonstrated experimentally.

Support for the Biogenetic Isoprene Rule had thus reached the point of indicating how the isoprene units are first formed and then condense to give Ruzicka's postulated acyclic isoprenoid intermediates. The next extension of the Rule to be considered was the manner in which these simple precursors undergo cyclization and rearrangement to the many and varied terpenoid types. The greater part of this work has been concerned almost exclusively with the transformation of farnesyl pyrophosphate into cholesterol, via squalene and lanosterol, but the results can be extrapolated to the as yet more obscure areas of terpenoid biogenesis.

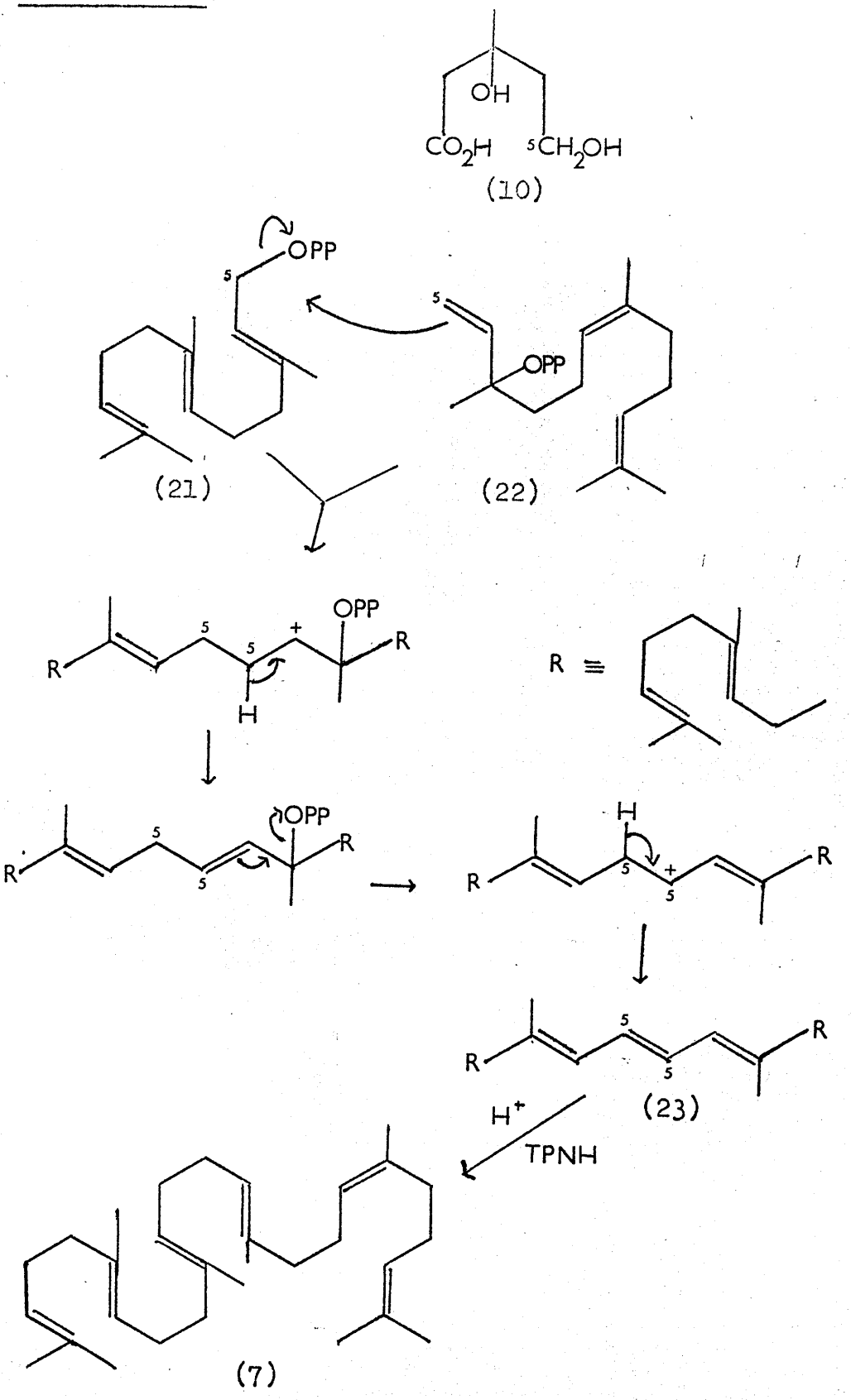
The condensation of farnesyl pyrophosphate molecules to squalene requires the presence of TPNH. Any proposed mechanism for this process must be consistent with two

Flowsheet V.





Flowsheet VI.

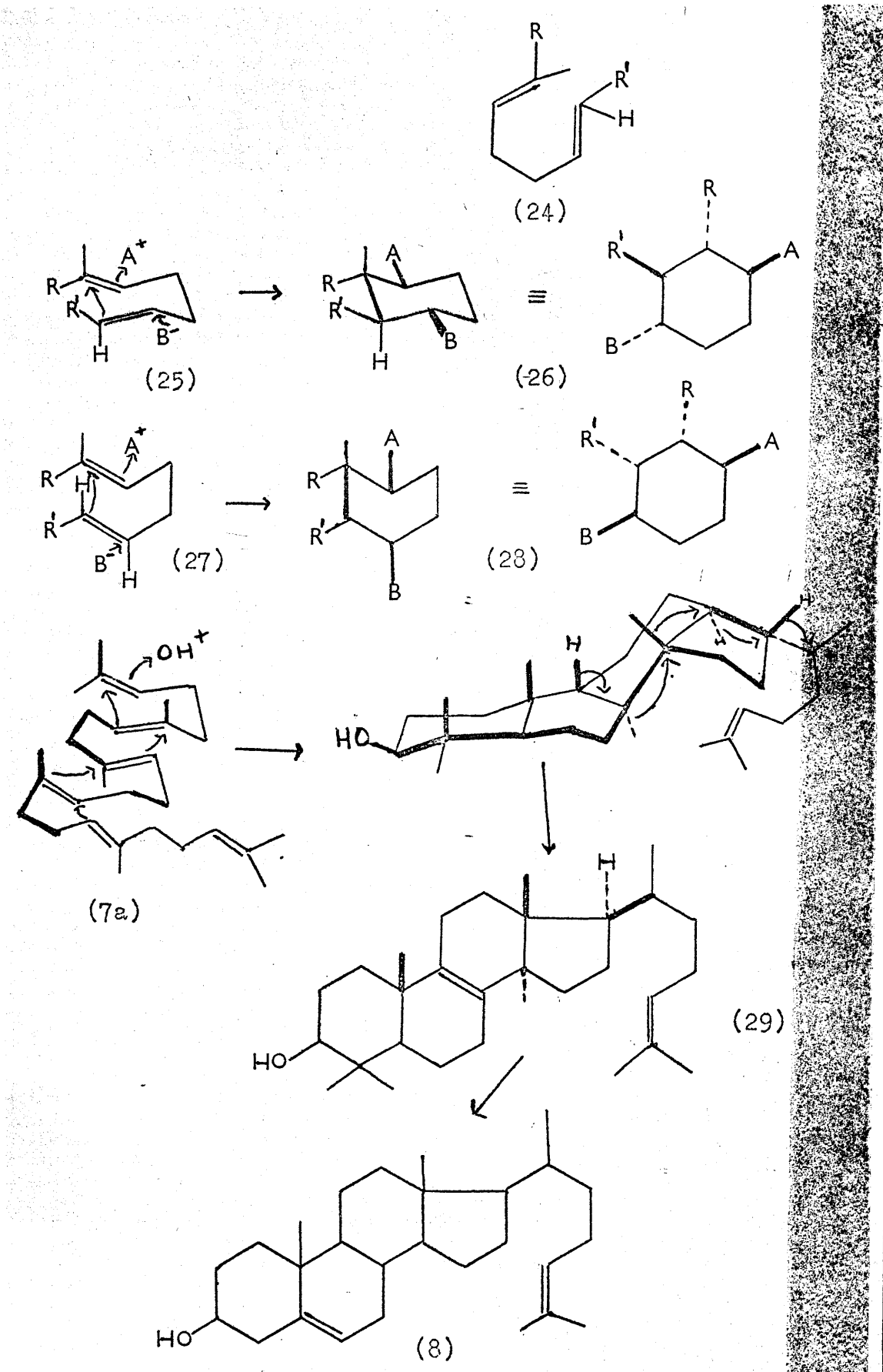


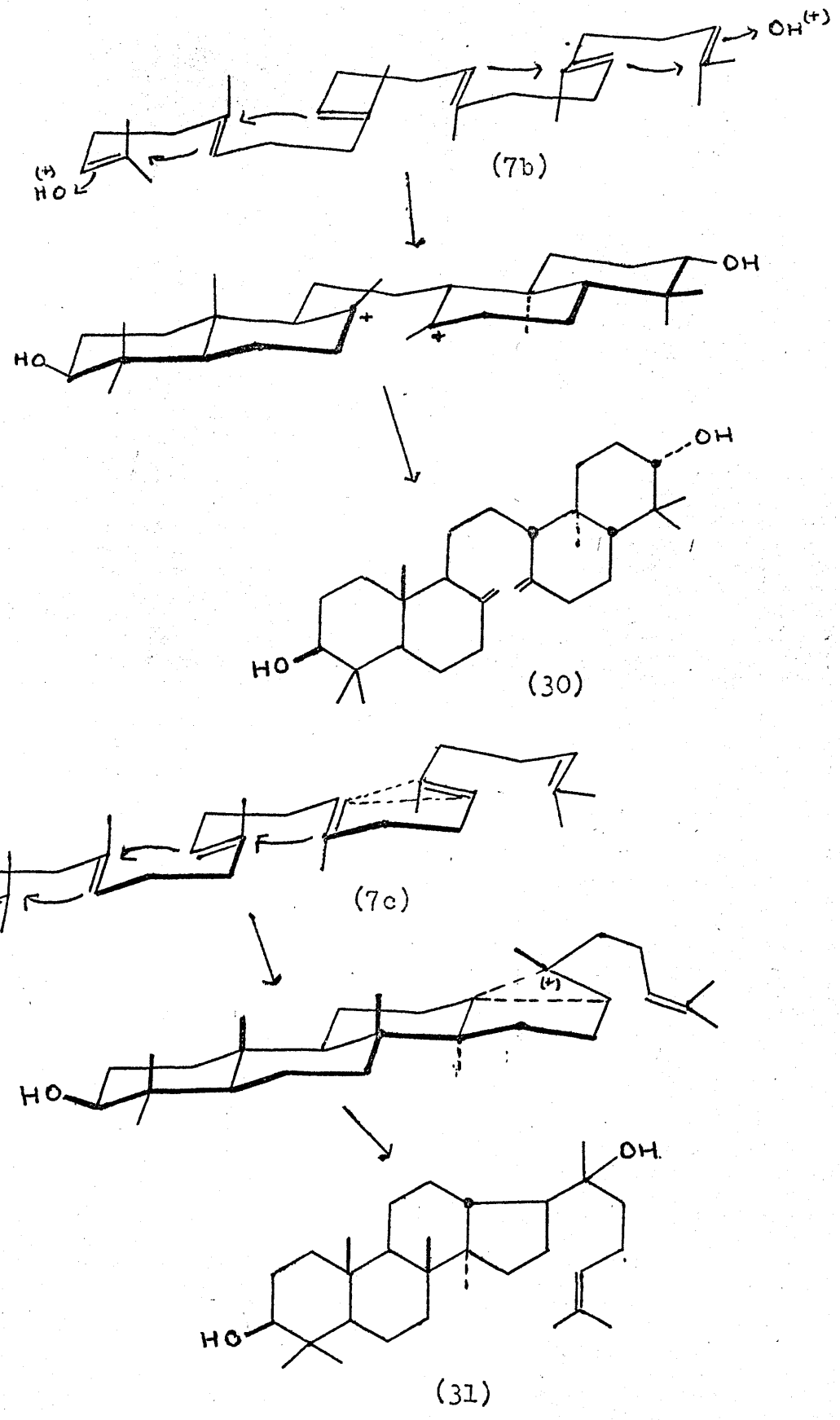
facts, namely that on condensation of six molecules of 5-D_2-mevalonic acid (10) to give one molecule of squalene (7), only ten of the possible twelve deuterium atoms are incorporated into the final product (flowsheet V), the two missing deuterium atoms being lost from the central two carbon atoms of squalene; also, the synthesis of squalene from mevalonic acid in heavy water results in the uptake of four deuterium atoms from the solvent, two of which were found on the terminal isopropyl groups and two on the central two carbon atoms of the product. These facts have been accounted for in the mechanism proposed by Cornforth and Popjak[29] for the biogenesis of squalene (flowsheet VI). This mechanism postulates that farnesyl pyrophosphate (21) undergoes condensation not with another molecule of itself but rather with an allylic isomer nerolidol pyrophosphate[30] (22). Since such a condensation gives rise to squalene (7) via dehydrosqualene (23) all of the above facts can be explained since this requires that two of the protons originally bound to C-5 of mevalonic acid be eliminated and subsequently replaced by two others. When the synthesis is carried out in heavy water therefore, the replacements may be deuterium atoms.

Ruzicka's initial form[2] of the Biogenetic Isoprene Rule made no comment regarding stereochemistry. This

was remedied by a later extension to the Rule[31] which indicated that the configuration of the product terpenoid is dependent on the conformation adopted by the cyclizing molecule. Ruzicka's proposed scheme for the biogenesis of all the known cyclic triterpenoids and steroids from cyclization of a suitably orientated squalene molecule was based on certain well-defined assumptions regarding the course of acid-catalysed cyclizations and rearrangements. The principal requirement concerns the addition, elimination and rearrangement processes and necessitates that they proceed by stereospecific trans-anti planar mechanisms - which result in all the newly formed bonds being parallel.

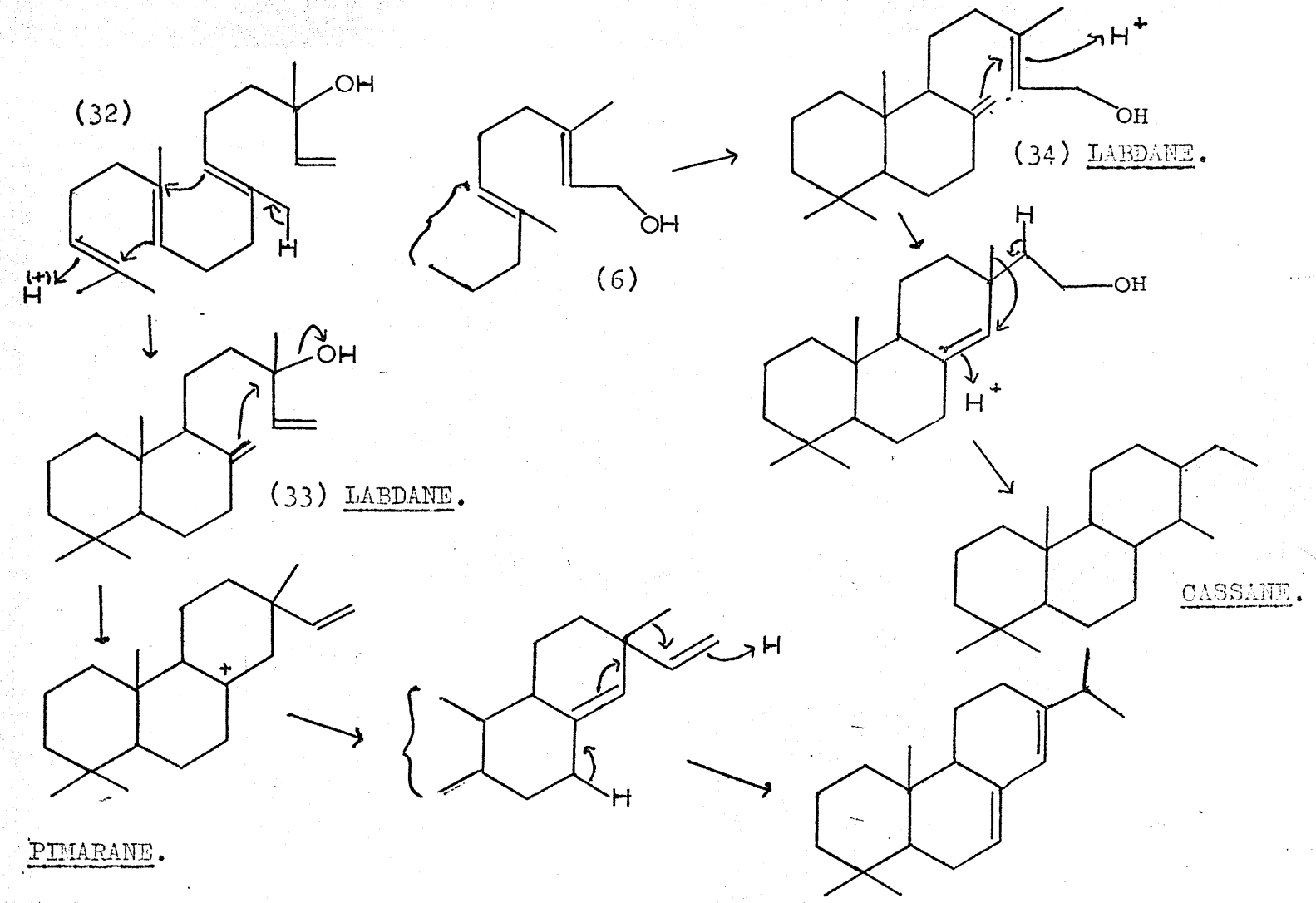
The conformation of the cyclizing molecule is important in that the initial polarization by a cationoid species can be assisted by the π electrons of the isolated double bonds provided the chain is folded so as to allow maximum overlap of the π orbitals. If the molecule is so orientated, cyclization is fully concerted and without formation of intermediate carbonium ions. Evidence for the synchronous nature of the cyclization and its initiation (in the case of squalene) through the medium of OH^+ or its equivalent has been obtained from tracer studies carried out on the cyclization of squalene to lanosterol[32,33].


R
R'
H
(24)
A+
B
(25)
A
B
≡
A
(26)
(27)
(28)
OH+
HO
H
(7a)
(29)
(8)


OH(+)
(+)
HO
(7b)
OH
HO
(30)
F
(7c)
(+)
HO
(31)

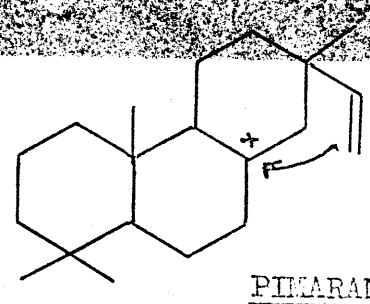
These assumptions can be illustrated by considering the simple isoprenoid substituted hexa-1,5-diene (24). This can undergo acid-catalysed cyclization either in a chair conformation (25) to give a trans-anti-trans product (26) or in a boat conformation (27) to a trans-syn-trans system (28).

By analogy with these cyclizations occurring by a concerted trans-anti planar mechanism, natural squalene, which undergoes transformation to lanosterol, has all its double bonds in the trans-configuration, but the same isomer can give rise to all the basic triterpene skeletons depending on the conformation adopted by the molecule prior to cyclization. Lanosterol (29), which is converted to cholesterol (8) with oxidative loss of three carbon atoms[34], arises from cyclization of the chair-boat-chair-boat conformation of squalene (7a). The repetitive form of the carbon skeleton of onocerin (30) on the other hand indicates a derivation from squalene with simultaneous cyclization of both ends of the all-trans molecule, these ends being folded in the chair-chair conformation (7b). The dammarene diols (31), which differ from each other only in the configuration of the long side chain, are produced by cyclization of squalene folded in the chair-chair-chair-boat conformational sequence (7c), followed

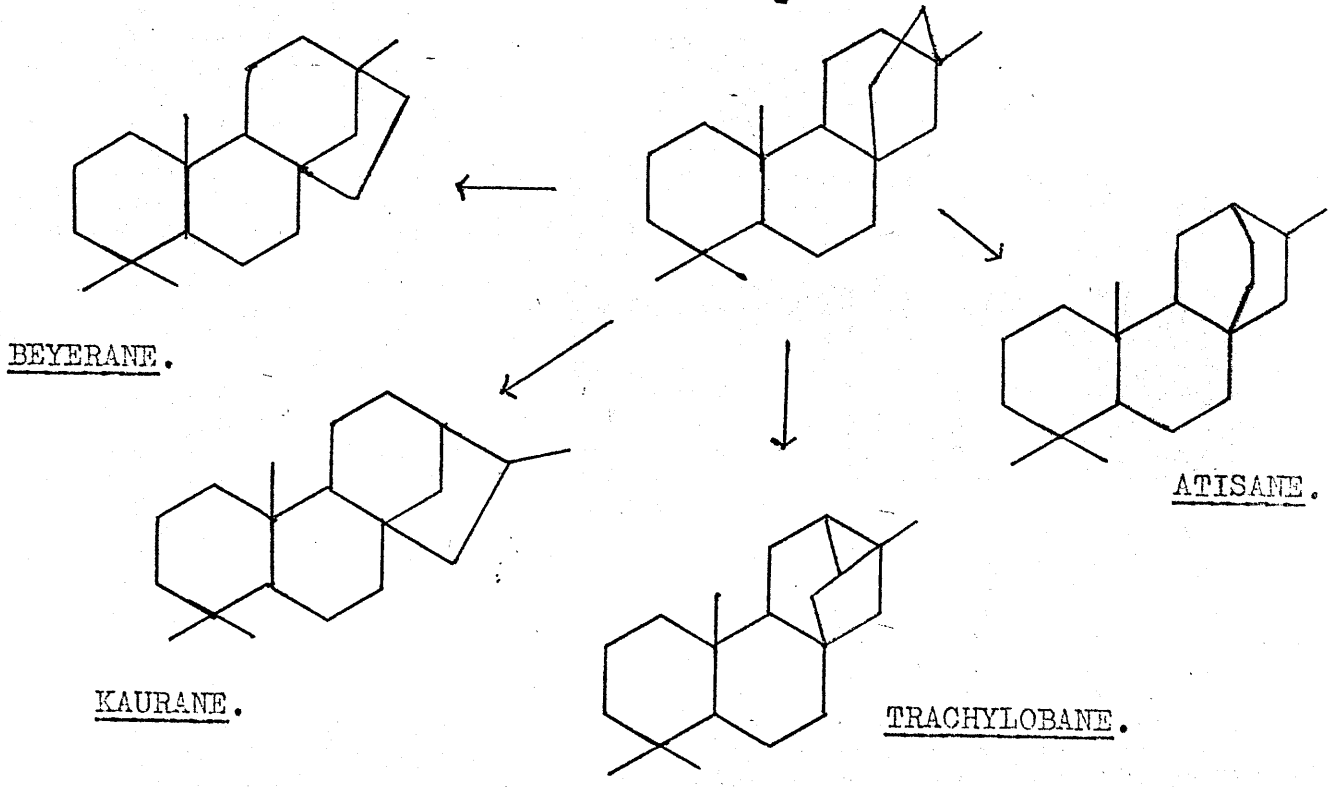


ABIETANE.

DITERPENOID CARBON SKELETONS.



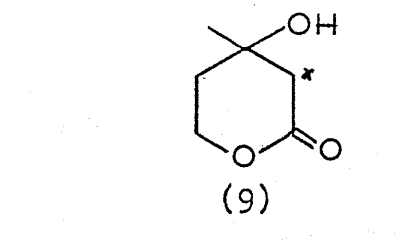
PIMARANE.



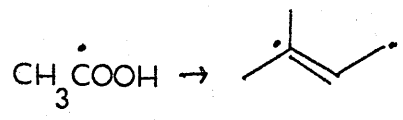
by antiplanar addition of OH^-. However, all triterpenoids (and steroids) derivable from squalene, regardless of the initial conformation of the squalene molecule, have the same configuration in the C-10 position viz., a C-10 (β)-methyl. No examples have as yet been found of triterpenoids (or steroids) with an "unnatural" C-10 (α)-methyl group.

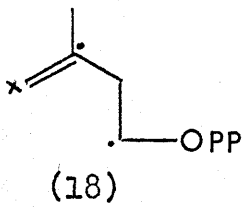
According to the Biogenetic Isoprene Rule, all the varied skeletal types of the diterpenoids are derivable from the cyclization and subsequent rearrangement of geranylgeraniol (6) or the related geranyl linalool system (32), this latter compound having been found to occur naturally[35]. Thus the eight main types of carbon skeleton comprising the di-, tri-, tetra- and pentacyclic diterpenoids can be derived, in principle, from these related precursors via the isomeric, allylic bicyclic alcohols (33) and (34) of the labdane group. No detailed biochemical studies comparable to those dealing with the formation and cyclization of squalene to cholesterol have been undertaken for this group of compounds. Nevertheless, it can be shown that even those diterpenes with the most apparently complex and highly rearranged structures can be derived, on paper at least, from these simple precursors. The structure of pleuromutilin[36] (35) provides a good

Flowsheet VII.



(9)





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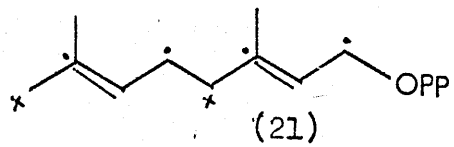

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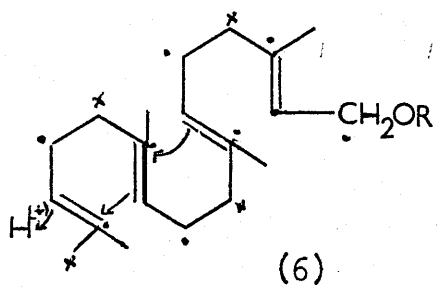

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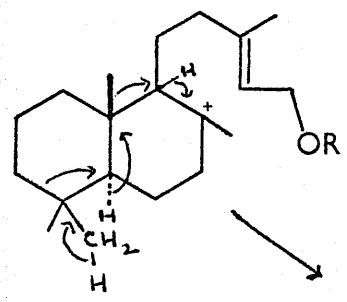

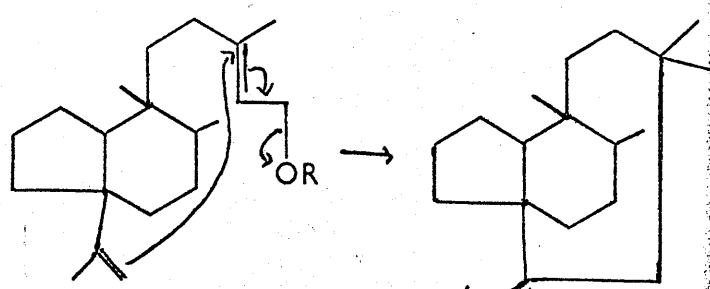

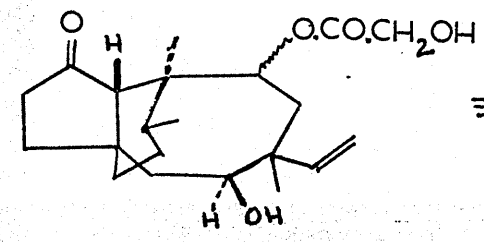

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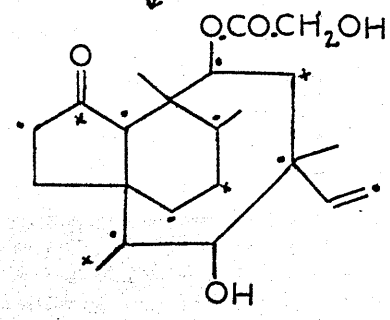

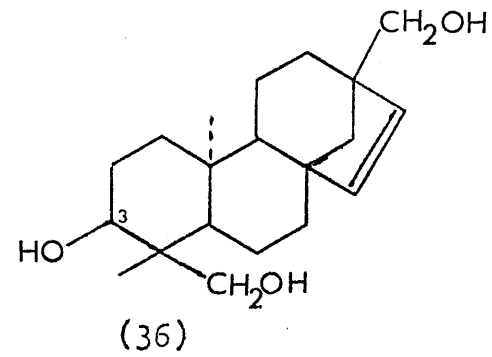

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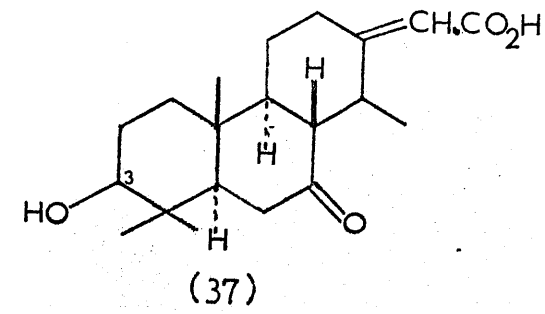

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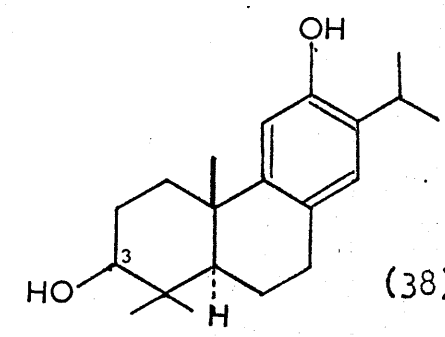

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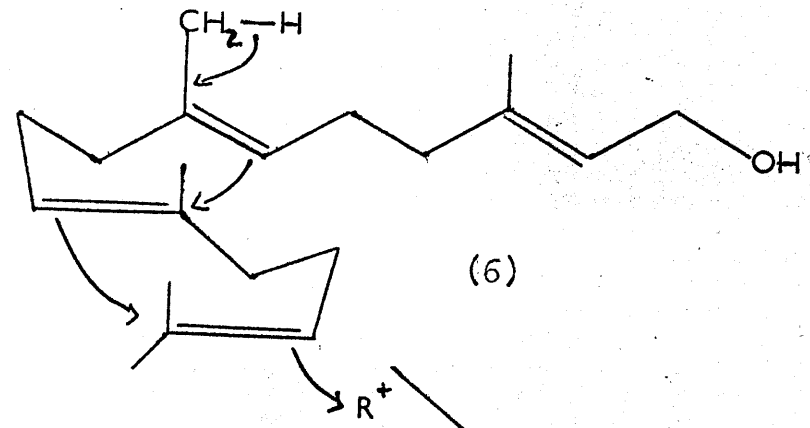

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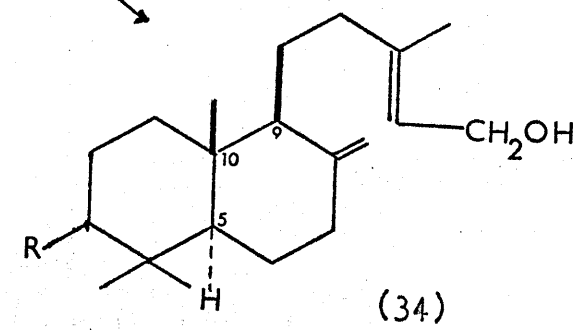
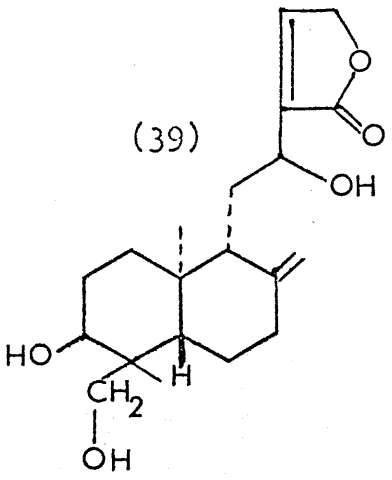


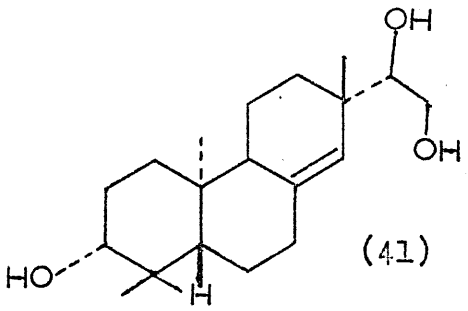
(34)

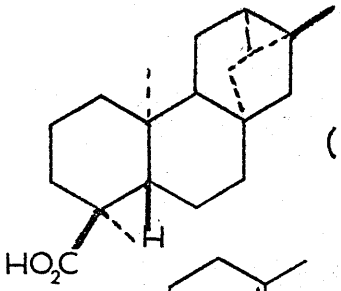
illustration of this point, and the biochemical studies undertaken so far[37,38], using labelled mevalonolactone and acetic acid, indicate that its biogenesis follows the route shown in flowsheet VII.

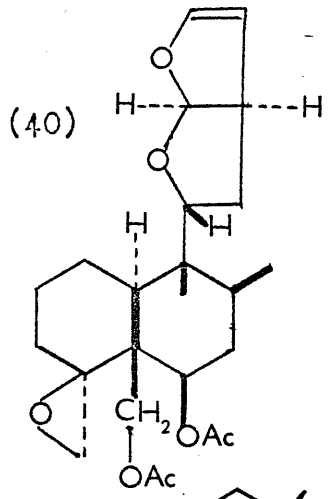
It is of interest to note that in the biosynthesis of diterpenoids, the initiating electrophile is usually a proton, whereas in the case of triterpenes and steroids from squalene, OH^+ or its equivalent is postulated. There are exceptions to both cases and several diterpenes are known which carry oxygen in ring A - mostly at position C-3 as would be expected on biogenetic grounds. Three examples of such oxygenated diterpenes are beyerol (36), cassaic acid (37) and hinokiol (38).

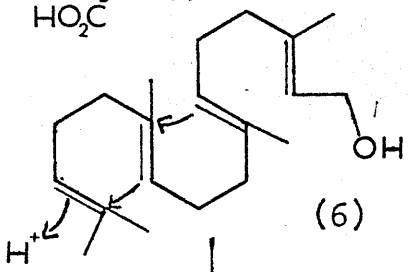
As the present work is concerned with a member of the labdane group of diterpenoids, the derivation of this skeleton from the postulated geranylgeraniol precursor will now be considered in some detail. The stereochemical implications of the previously discussed cyclization of squalene via a series of concerted antiparallel 1,2-additions would appear to be applicable to the cyclization of geranyl geraniol (6) (or its equivalent geranyl linalool), since in all the authenticated cases, the stereochemistry at positions 5, 9 and 10 is in accordance with this type of cyclization. Although antipodal compounds are found

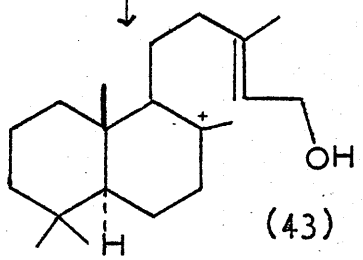

(39)
O
O
OH
HO
H
CH2
OH

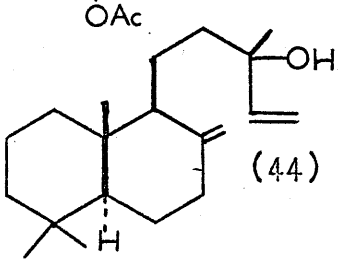

OH
OH
HO
H
(41)

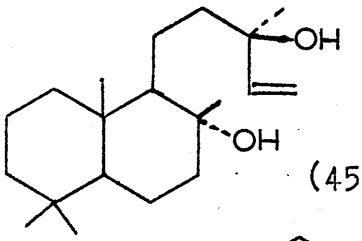

(42)
HO2C
H

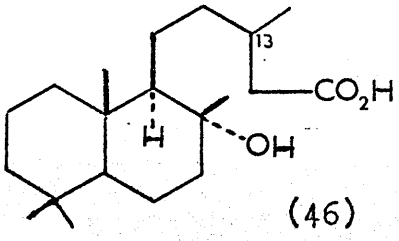

(40)
H
H
O
O
H
H
CH2
OAc
OAc

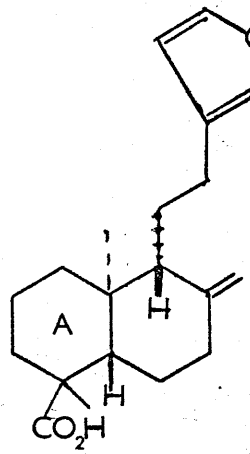

OH
H+
(6)

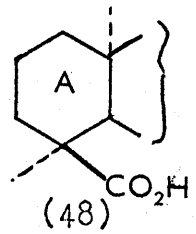

OH
H
(43)

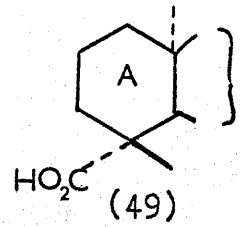

OH
(44)
H


OH
OH
(45)


13
CO2H
H
OH
(46)

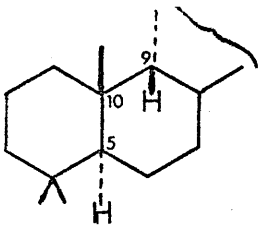

O
A
H
CO2H


A
CO2H
(48)


A
HO2C
(49)

in the diterpenoid group i.e. having an "unnatural" C-10 (α) methyl group as opposed to the "normal" C-10 (β) configuration, the absolute configuration remains the same. All the diterpenoids have been shown[39] to have the trans-anti-trans arrangement predicted by the Biogenetic Isoprene Rule. Diterpenoids so far found with this "unnatural" C-10 stereochemistry include andrographolide (39), clerodin (40), darutigenol (41) - the only known pimaranoid diterpene with a C-10 (α) methyl, beyerol (36) and trachylobanic acid (42).

Protonation of the double bond in the 3,4-position of the geranyl geraniol precursor (6) initiates concerted cyclization in a trans-anti planar fashion to a trans-decalin carbonium ion (43). This species can then be neutralized via solvent attack, proton loss or Wagner-Meerwein 1,2-shifts of hydrogen or methyl groups, and these processes, which may or may not be accompanied by an allylic rearrangement of the α, β -unsaturated primary alcohol, give rise to the skeletons of all the members of the labdane group. These include manool (44), sclareol (45), labdanolic acid (46), the rearranged labdane clerodin (40), polyalthic (48) and daniellic (49) acids, the last two compounds having the terminal four carbon atoms of the side chain form a β -substituted furan ring.



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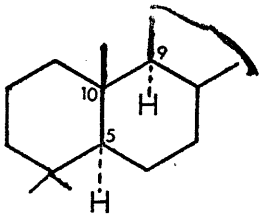

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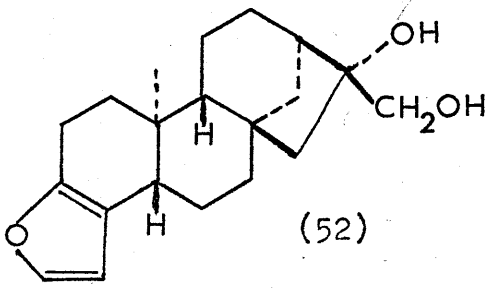

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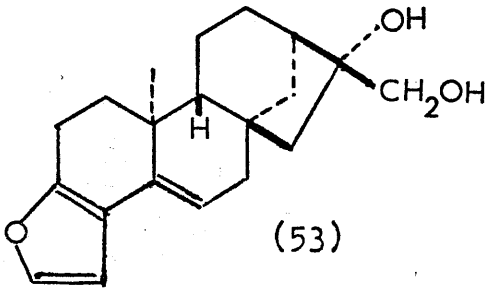

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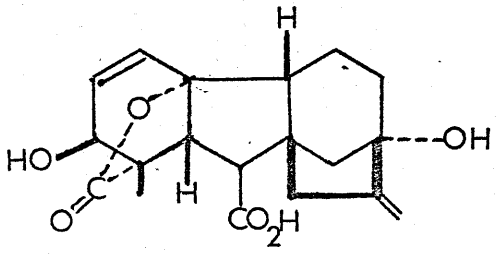

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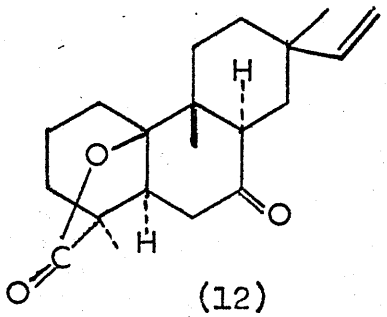

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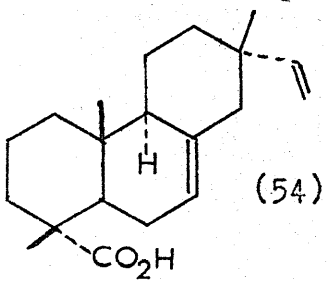

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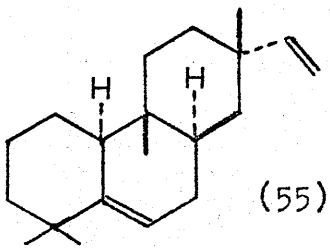

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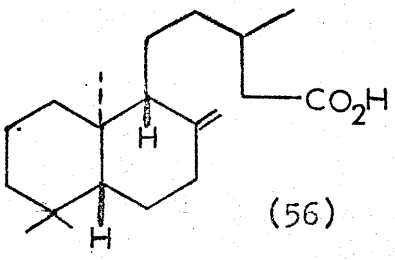
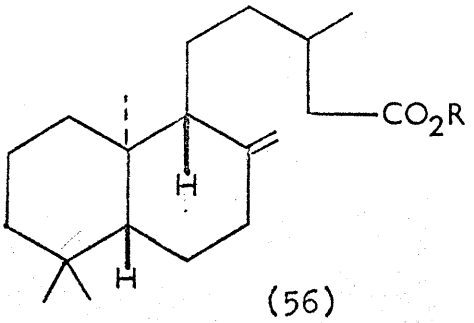


(56)

It would therefore appear probable on the basis of the Biogenetic Isoprene Rule and the mass of evidence accumulated in its support that the C-5/C-10/C-9 backbone in all of the diterpenoids would have a trans-anti configuration. However, this assumption was called in question by the apparent existence of a small group of diterpenoids, the proposed configuration at positions 5, 9 and 10 in each of which resulted in a trans-syn (50) rather than the trans-anti (51) arrangement. Of the compounds included in this group, cafestol (52) and kahweol (53)[40], gibberellic acid (13)[41], rosenonolactone (12) and its derivatives[42], isopimaric acid (54)[43,44] and, most recently, rimuene (55)[45] have now been shown to follow the orthodox pattern. In fact, up to the present time, eperuic acid (56) has remained the only obstacle to the complete stereochemical uniformity of the diterpene family[39] in accordance with the Biogenetic Isoprene Rule.

However, in the course of the present work, it has been shown that the previously proposed[46] and subsequently accepted[47] stereochemistry of eperuic acid cannot be supported either on the basis of the previously recorded[42] or additional evidence now obtained. This therefore disposes of the last known "anomaly" to Ruzicka's Isoprene Rule.



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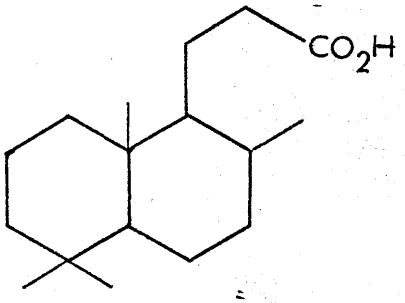

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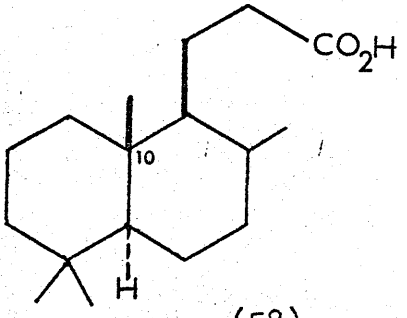

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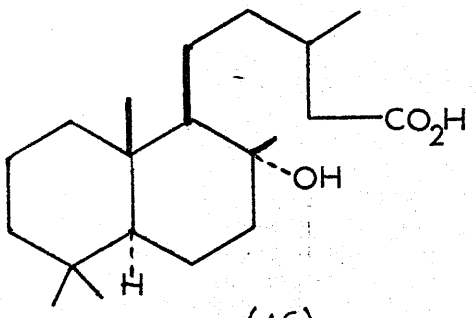

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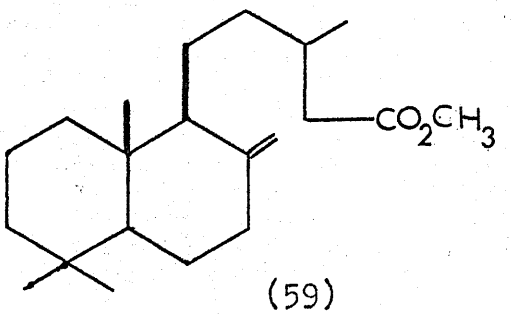


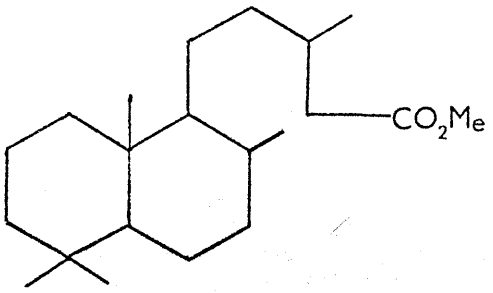
(59)

DISCUSSION.

1. The Stereochemistry of Eperuic Acid.

Eperuic acid (56; R=H), the chief constituent of an oleo-resin derived from the wallaba tree of British Guiana, was isolated and its structure elucidated by King and Jones[48]. They made no assumptions regarding the stereochemistry of this compound beyond the fact that the C - 17 acid (57) obtained from Barbier - Wieland degradation of the side chain (m.p. 134-135°; $[\alpha]_D$ - 29.9°) appeared to be antipodal with that of the same structure obtained from ambrein[49] (58; m.p. 136-137°; $[\alpha]_D$ + 33°).

Their work on the structure and stereochemistry of labdanolic acid, a constituent of gum labdanum, led Cocker and Halsall[50] to structure (46), or a stereoisomer, for this acid. King and Jones[48] had indicated that methyl eperuate (56; R = CH_3) was a stereoisomer antipodal to the dehydration product (59) from methyl labdanolate since a comparison of the rotations of these two compounds (- 28·2° and + 27° respectively) showed that they were not identical. Comparison of the C-17 acid (57) from labdanolic acid with that from marrubiin and ambrein proved that the rings in labdanolic acid are trans - fused and have the same absolute configuration at C-10 as ambrein and the di- and triterpenes i.e. an angular C-10 (β)



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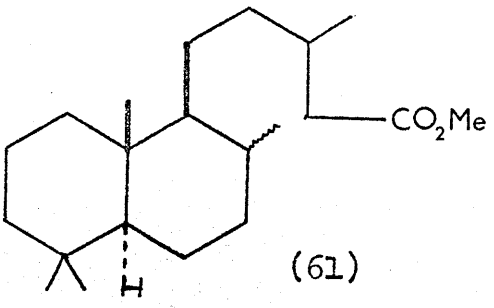

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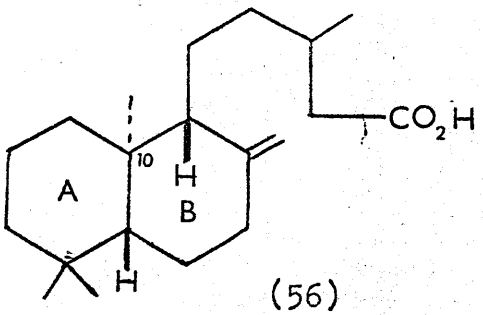

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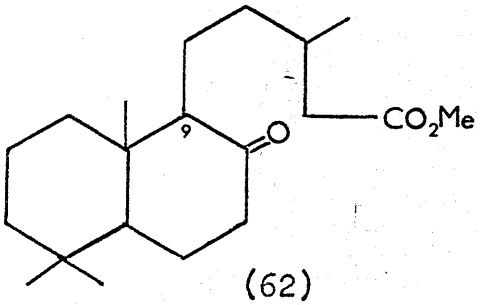

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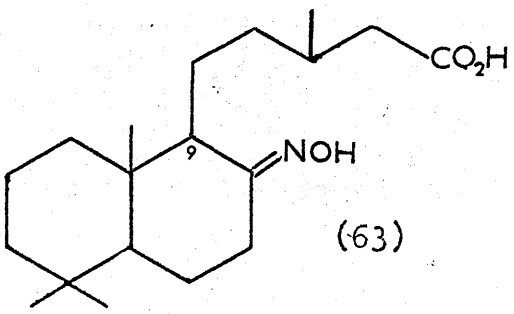
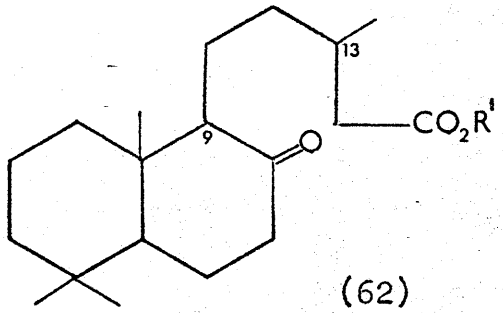


(63)

methyl group. The β - configuration of the C-9 side chain in labdanolic acid was established by comparing the hydrogenation product (60) from dehydrolabdanolate with methyl dihydrocativate[51] (61), the two being identical.

A comparison of the physical data of degradation products from labdanolic acid with the corresponding products from eperuic acid for which King and Jones[48] had proposed identical structures (exclusive of stereochemistry), brought Cocker and Halsall[50] to the apparently startling conclusion that eperuic acid has a carbon skeleton (56) in which rings A and B are antipodal to those of all the other then known di- and triterpenes and steroids. This was the first encountered example of the existence of two naturally occurring antipodes in the diterpenoid series as all the members of this group had been thought to possess the C-10 (β) angular methyl group of the steroids. However, the optical rotatory dispersion curves of the keto esters (62) from the two series of acids were found[46] to be near but not exact mirror images, as would be required of antipodes. This discrepancy, coupled with the more serious divergence of the melting points of the corresponding acid oximes (63) of the two series (eperuic 223°, labdanolic 190°) was attributed to a configurational difference at C-9.

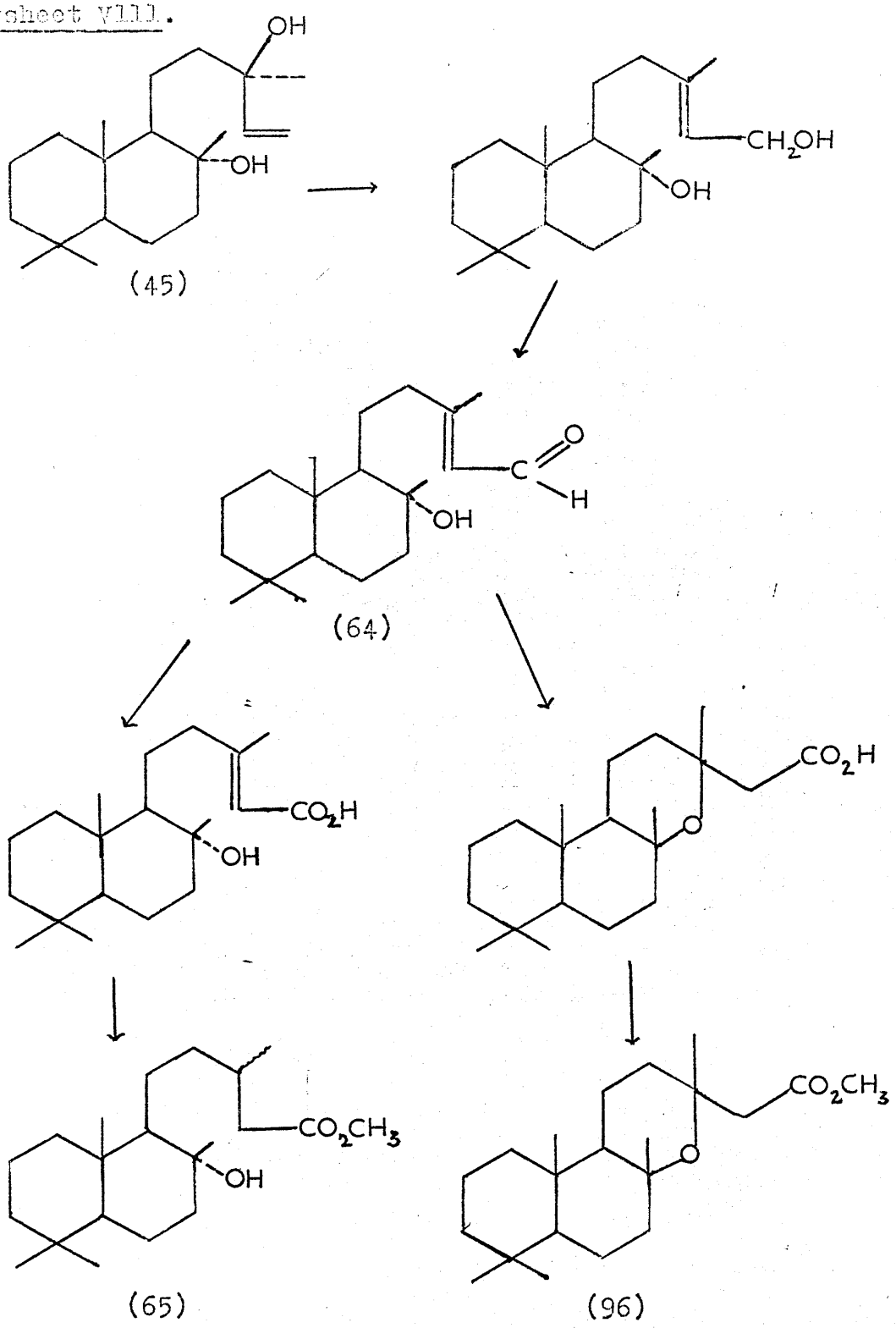


(62)

On the evidence which existed, there does not appear to have been any strong reason for choosing C-9 rather than C-13, the other relevant asymmetric centre, as the site of a possible configurational difference, especially when a comparison of the physical constants quoted by Cocker and Halsall[50] suggests that the two series become more closely antipodal as soon as the C-13 asymmetry is removed by Barbier - Wieland degradation of the side-chain (although this point was not made by these authors[50]). Also, assignment of such a configurational difference to C-9 implies that the side-chain at this position is axially (β)- attached, giving an anomolous (on the basis of the Biogenetic Isoprene Rule) trans - syn backbone. Such an assumption is also brought into question by an examination of the hydrolysis conditions employed[48,50] to obtain the two keto acids (62; R' = H) prior to oxime formation (methanolic 2N potassium hydroxide under reflux for one and two hours respectively) since such conditions must result in the side chain of both ketones (and oximes) existing in the more stable equatorial configuration. Therefore, any stereochemical difference between the two oximes, derived from the labdanolic and eperuic acid series respectively, must be contained in the configuration at C-13.

There was already in the literature[52] a method for

Flowsheet VIII.



(45)

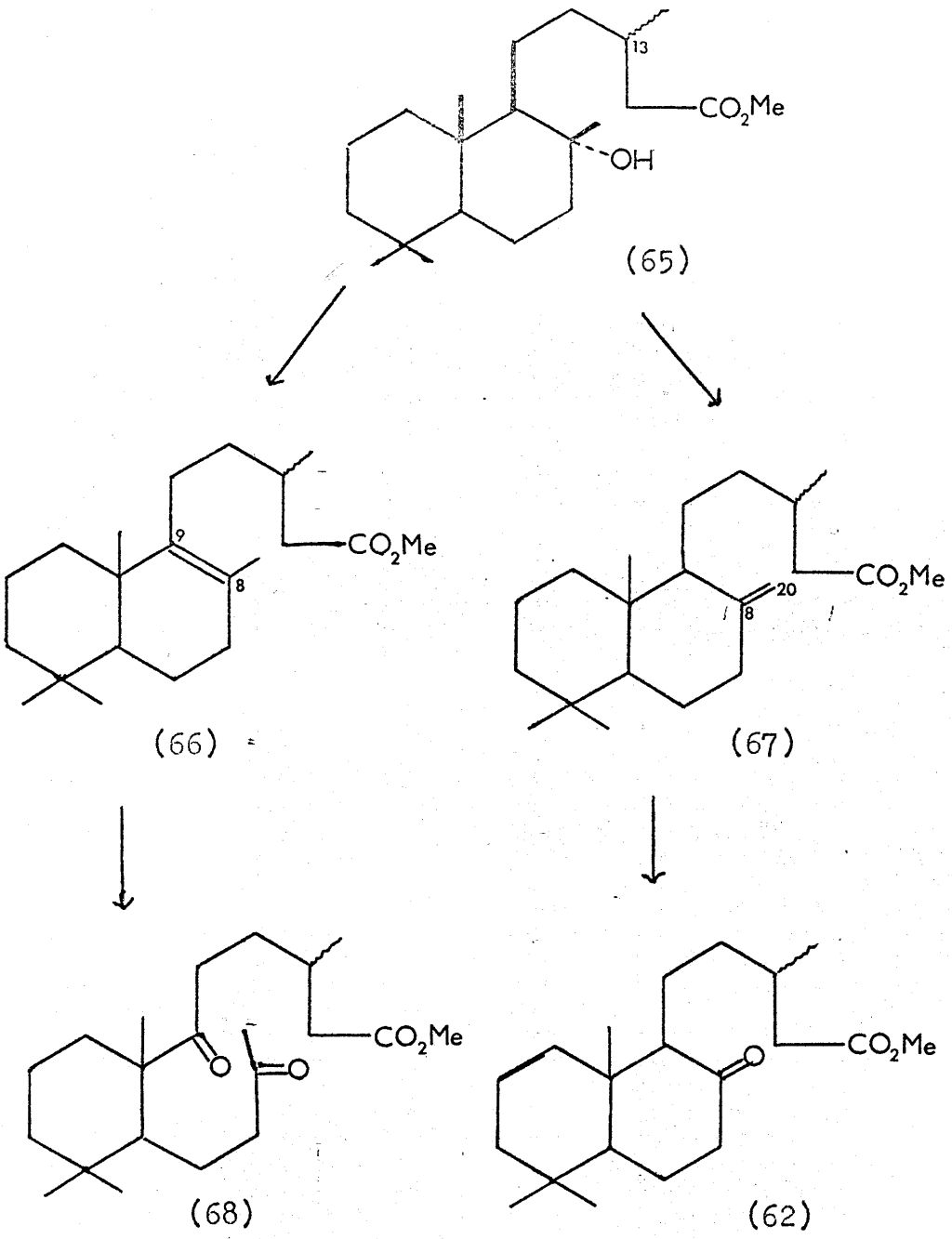
(64)

(65)

(96)

the preparation of both labdanolic and 13-epilabdanolic acids (46) by oxidative rearrangement of sclareol (45). The conformation of the side chain at C-9 in eperuic acid would be established unequivocably if the keto acid oxime (63) from the 13-epi acid proved to be completely antipodal to the corresponding derivative from eperuic acid. The present work demonstrates that eperuic (56) and dehydro-13-epilabdanolic (59) acids are completely antipodal. The C-9 side chain in eperuic acid therefore has an (α) configuration, resulting in a C-5/C-9/C-10 backbone with a normal trans - anti stereochemistry.

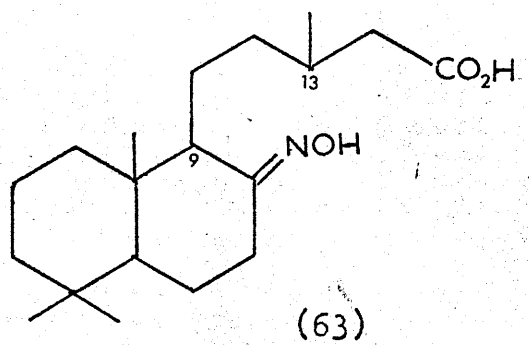
Sclareol (45), in acetic acid solution, was oxidatively rearranged with chromic acid[52] and the resulting mixture of allylic aldehydes (64) subjected to silver oxide oxidation to the corresponding acids (flowsheet VIII). Esterification and hydrogenation gave a mixture of the saturated C-13 epimeric labdanolate esters (65) which were separated by careful gradient elution chromatography over activated alumina. However, although methyl 13-epilabdanolate was obtained pure and in good yield, it was not possible to obtain methyl labdanolate uncontaminated by the 13-epimer even after repeated gradient elution chromatography since the two compounds have almost identical polarities. Thus the methyl


13
CO2Me
OH
(65)
9
8
CO2Me
(66)
20
8
CO2Me
(67)
CO2Me
O
O
(68)
CO2Me
O
(62)

labdanolate employed in this present study was obtained from natural gum labdanum[53]. Dehydration of the two epimers with phosphorus oxychloride in pyridine[50] afforded in each case a mixture of methyl labd-8,9-(66) and labd-8,20-enoates (67) containing 58% and 72% respectively of the exocyclic olefin from the 13-normal and 13-epi esters, the percentage of exocyclic isomer in each mixture being determined by a quantitative infrared comparison of the band at 890 cm^{-1} with the corresponding band in 3-methylene-cholestane. It is of interest to note that whereas Cocker and Halsall[50] obtained only the exocyclic isomer on dehydration, in the present work both were obtained from each ester as indicated by infrared spectra, gas-liquid chromatography, the production of two ketones on ozonolysis and two diols on osmylation, the mixtures in each case being separated and the components identified.

Each olefin mixture was ozonized[50] and the required mono-keto ester (62) separated by chromatography. The corresponding keto-ester from natural methyl eperuate kindly supplied by Professor G. Jones (the specimen used was a similar mixture of double-bond isomers containing 32% of the exomethylene isomer) was similarly obtained.

Comparison of the three keto esters showed no detectable difference (apart from sign) in their rotatory



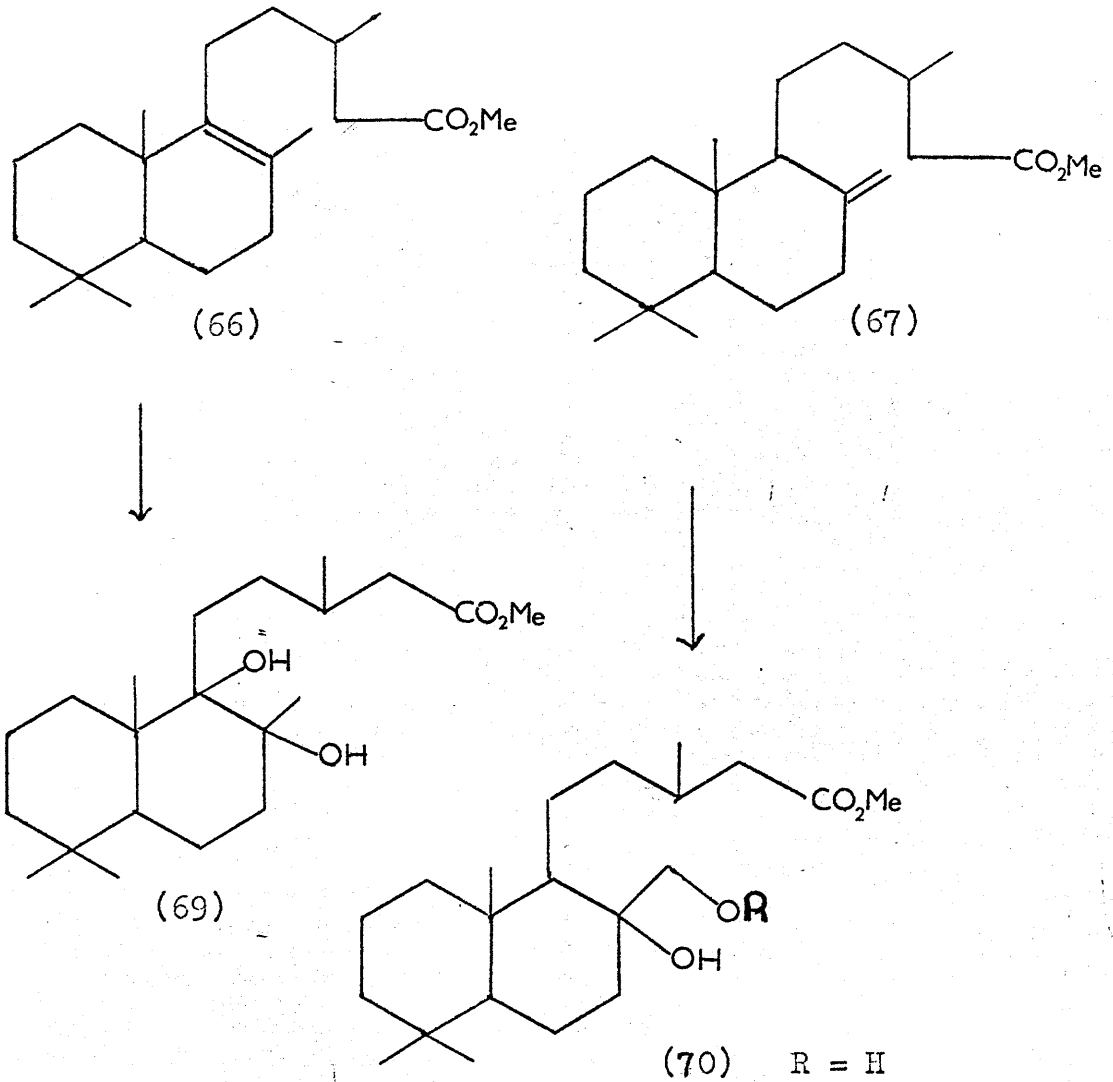
(63)

dispersion or circular dichroism, and there were only minor differences in the infrared solution spectrum of the ketone derived from methyl labdanolate when compared with the other two, which were identical. However, a comparison of the three corresponding acid oximes (63) proved more decisive. Eperuic acid and 13-epilabdanolic acid gave oximes of m.p. 224-226°, 223-226° and optical rotation - 82° and + 87° (pyridine) respectively. Their infrared (KCl disc) spectra and X-ray single crystal rotation photographs were identical, but differed from those of the oxime, m.p. 188-190°, $[\alpha]_D$ + 53° (pyridine) from labdanolic acid.

The keto esters from labdanolic and eperuic acids are therefore antipodal except for the configuration at C-13, which is identical in both cases.

There still remained the rather remote possibility that inversion at C-9 may have occurred during the formation of the keto ester (or the oxime) from eperuic acid and that therefore this acid is epimeric with the antipode of dehydrolabdanolic acid at C-9 as well as at C-13. However, this unlikely situation was disposed of by an examination of the three diol ester mixtures obtained from osmylation of methyl eperuate and the dehydration products from methyl labdanolate and methyl 13-epilabanolate.

Flowsheet IV.



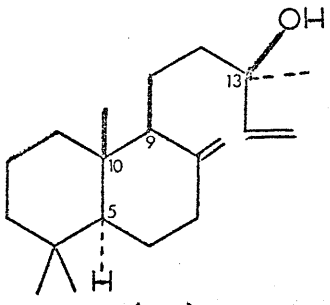
(70) R = H

(71) R = $CO.CH_3$

(72) R = $C_6H_4.NO_2$

Reaction of the mixture of olefinic esters already described with osmium tetroxide in pyridine afforded in each of the three cases a mixture of products (69; 70) from which the required primary-tertiary diol ester (70) was separated by chromatography over activated alumina. Its constitution was supported by elementary analysis, an infrared solution spectrum, the formation, under mild conditions, of an (oily) monoacetate (71) and mono-p-nitrobenzoate (72) and the presence in the nuclear magnetic resonance spectrum of a two-proton singlet at 6·5 arising from the equivalent methylene protons of the primary alcohol. The diol esters from eperuic acid (m.p. 88-90°, $[\alpha]_{546}$ + 4°) and 13-epilabdanolic acid (m.p. 88-90°, $[\alpha]_{546}$ - 5°) had identical infrared solution spectra and X-ray powder photographs but differed from those of the diol ester (m.p. 77-79°, $[\alpha]_{546}$ - 20°) from labdanolic acid.

It is therefore established that eperuic acid has the normal trans - anti configuration exhibited by all the known diterpenoids of established structure in accordance with the Biogenetic Isoprene Rule.



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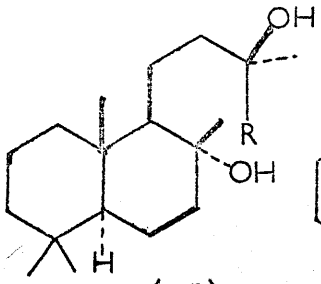

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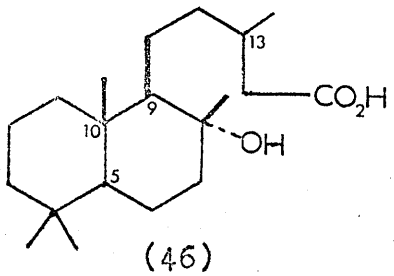

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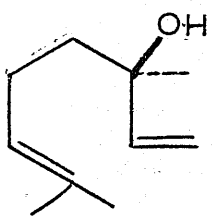

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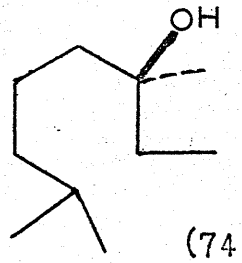

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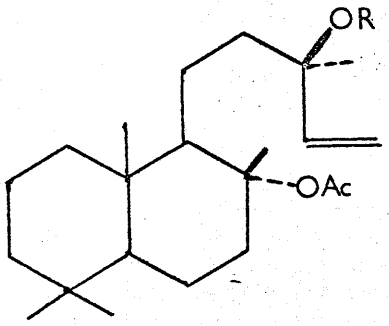

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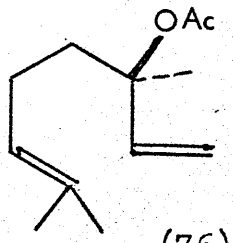


(76)

2. The C-13 Configuration in Labdanolic Acid.

The absolute configuration at C-13 in the parent members of the labdane group of diterpenoids, manool (44), sclareol (45) and labdanolic acid (46) have been the subject of much recent interest[54-58]. However, it would appear, as will emerge from the following discussion, that attempts to determine the stereochemistry at C-13 based on indirect methods of configurational analysis can lead to considerable uncertainty and confusion.

By their conversion of sclareol into manool, Buchi and Bieman[54] were drawn to the conclusion that these two diterpenes have the same configuration at positions 5, 9, 10 and 13. They arrived at the absolute configuration of C-13 in these two compounds by comparing molecular rotation differences in the sclareol series with the corresponding values in the (-)-linalool series, as indicated:-

1. Sclareol (45; R = $CH{=}CH_2$) Dihydrosclareol (45; R = CH_2CH_3)

-9° $0{\cdot}0^\circ$ $\Delta = +\ 9{\cdot}0^\circ$

(-)-Linalool (73) (-)-Tetrahydrolinalool (74)

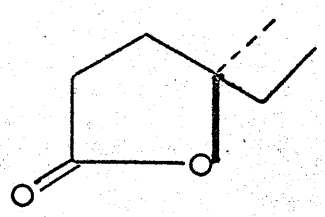
$-22{\cdot}6^\circ$ $-0{\cdot}87^\circ$ $\Delta = +\ 21{\cdot}7^\circ$

2. Sclareol monoacetate (75; R=H) Sclareol diacetate (75; R=Ac)

-124° $-106{\cdot}8^\circ$ $\Delta = +\ 17{\cdot}2^\circ$

(-)-Linalool (73) Linalylacetate (76)

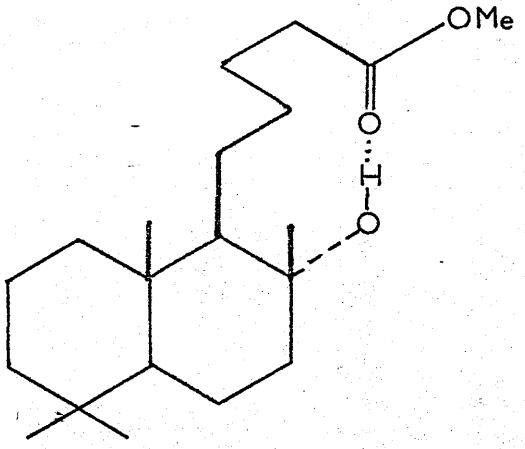
$-22{\cdot}6^\circ$ $-13{\cdot}0^\circ$ $\Delta = +\ 9{\cdot}4^\circ$



(77)

These two comparisons indicated that (-)-linalool and C-13 in sclareol have the same absolute configuration and since an (S)-configuration had already been assigned to (-)-linalool by Prelog and Watanabe[55], this same stereochemistry was now given to both manool and sclareol. Later work[57] resulted in changing the absolute configuration of (-)-linalool from (S) to (R) and a consequent change in the assignment of C-13 stereochemistry in manool and sclareol became necessary. The C-13 (R) configuration in sclareol was finally conclusively established by degradation of both sclareol and laevo-rotatory (R)-linalool to the same optically active lactone[58] (77). Barltrop, Bigley and Rogers[56,59] also arrived at this conclusion that (-)-linalool and C-13 in sclareol and manool had an identical (R)-configuration on the basis of molecular rotation differences in the three series.

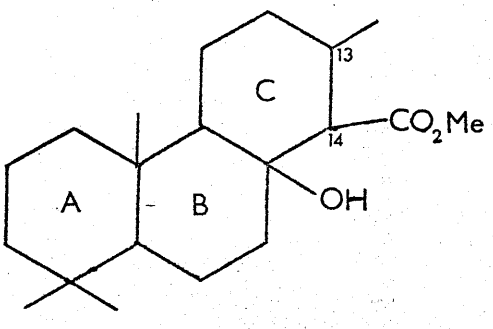
However, their attempt in the same publication[56] to establish the C-13 configuration in labdanolic acid was based on certain erroneous assumptions which invalidate their conclusions and leave the C-13 configuration in labdanolic acid undecided. The structure of labdanolic acid and the stereochemistry at all the asymmetric centres except C-13 had already been firmly established[50]. Lederer[52] had previously assigned an (R)-configuration



(78)

to C-13 in methyl labdanolate based on a study of molecular rotation differences, and Barltrop, Bigley and Rogers[56] were led to reinvestigate this work since they had found molecular rotation data to be inconclusive in the presence of hydrogen bonding. They prepared[56] methyl labdanolate and methyl 13-epilabdanolate and since they claimed to observe no hydrogen bonding in either compound, assumed Lederer's assignment to be correct. Barltrop and his collegues quote a single sharp band at 3540 $cm.^{-1}$ in the carbon disulphide solution spectra of methyl labdanolate and its 13-epimer as evidence for the absence of intramolecular hydrogen bonding, but it would appear more likely that this band indicates exactly the opposite i.e. that both epimeric esters can form an intramolecular hydrogen bond. In fact it has been demonstrated in the course of the present work that both methyl labdanolate[60] and its 13-epimer do possess an intramolecular hydrogen bond. Infrared spectra of both these latter compounds, in carbon disulphide and carbon tetrachloride solutions, had bands at (approximately) 3600 (free hydroxyl), 3550 (bonded hydroxyl) and 1740 $cm.^{-1}$ The "closure" of the ten-membered ring involved in this bonding is sterically unexceptionable and can be represented by (78).

Thus, the stereochemistry of C-13 in labdanolic



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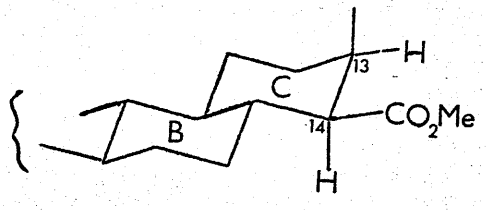
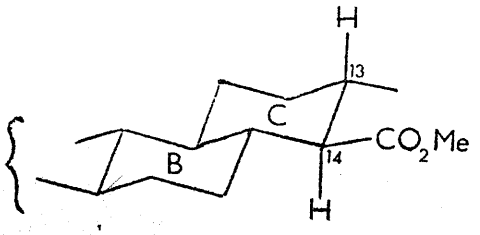


(80)

acid is again open to question and an independent and unambiguous determination of the C-13 configuration in labdanolic acid (and hence of eperuic acid) was therefore undertaken.

The classical method for determining the configuration at an asymmetric centre involved degradation of the molecule to a simple, known, optically active compound which still contains the original asymmetric centre. However, in this case there appeared to be a quicker and more direct solution to the problem, based on the cyclic hydroxy esters (79), the compound from methyl eperuate having already been prepared by King and Jones[48].

It has been generally established[61] for a wide variety of rigid six-membered ring systems that axial ring protons absorb at higher field than do their epimeric equatorial counterparts. The coupling constant for vicinal protons depends on the dihedral angle between the protons and has a maximum value of about 16 c.p.S. for a diaxial coupling (i.e. a dihedral angle of 180°). In the case of the cyclic hydroxy-esters (79), assuming the carbomethoxyl group is in the more stable equatorial configuration and ring C has a chair conformation, a C-13 (β) methyl group (80) would result in an axial-equatorial H-H coupling between C-13 and C-14 and the alternative



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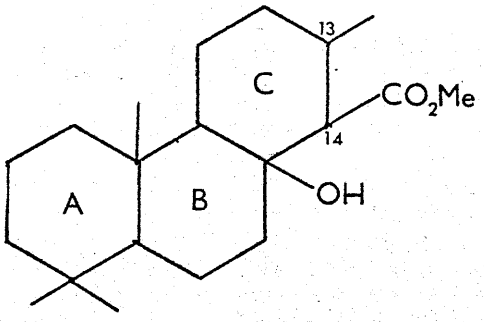

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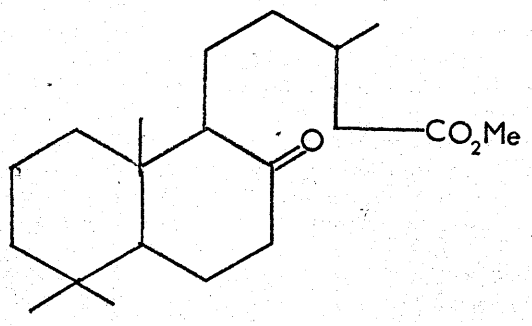
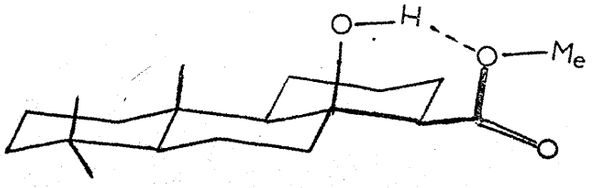


(62)

C-13 (α) methyl (81) would produce the larger diaxial coupling constant. Also, since the C-14 proton is adjacent to a carbomethoxyl group it should be more easily identifiable by being shifted downfield.

Thus, it was with this in view that the cyclic hydroxy-esters (79) were prepared from eperuic, labdanolic and 13-epilabdanolic acids, the previously described mono-keto ester (62) from each series being treated with alcohol-free sodium methoxide in toluene[48]. The quoted yield[48] of cyclized material was low, being only 24% and several attempts were made to establish an improved technique for the cyclization. The keto-esters were reacted with sodamide and lithium amide suspensions in liquid ammonia respectively, but T.L.C. examination of the reaction products from both methods indicated a considerable mixture of products together with unchanged starting material. Also, these alternative methods had the additional disadvantage that the strongly basic reaction conditions were sufficient to hydrolyse the carbomethoxyl group, making re-esterification of the mixture necessary after the work-up. However, by careful addition of the keto-ester in anhydrous toluene to stirred sodium methoxide, from which all the alcohol had been scrupulously removed, it was possible to obtain yields of up to 60% of crystalline cyclized product.



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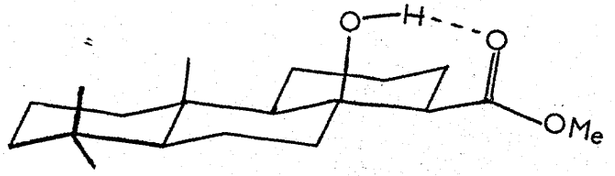
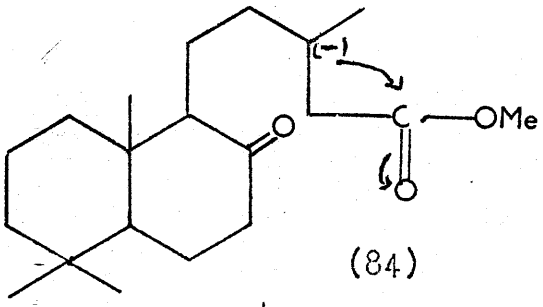


(83)

Startlingly, a comparison of the cyclic esters from methyl labdanolate and 13-epimethylabdanolate indicated that the two compounds were identical! Their infrared and nuclear magnetic resonance spectra were indistinguishable; they had melting points of 202-203° and 201-202° respectively, undepressed on admixture; optical rotations (-30° and - 31° respectively) were also identical. The infrared spectra in carbon tetrachloride solution indicated the expected hydrogen bonding and had bands at 3622 (free hydroxyl), 3582 and 3524 $cm.^{-1}$ (bonded hydroxyl). The carbonyl region of the infrared spectrum showed a large bonded ester band at 1713 $cm.^{-1}$ with the smaller, free ester band at 1743 $cm.^{-1}$ The two bonded hydroxyl bands can be attributed to conformations (82) and (83) respectively, the latter being the more intense. The 60 megacycle nuclear magnetic resonance spectrum had peaks at τ 6·3 (3 proton singlet, methyl ester) and τ 7.0 (1 proton singlet) which disappeared on equilibration with deuterium oxide. Also there was no indication of the C-14 proton having been moved downfield.

The discovery that these two derivatives from the epimeric acids appeared to be identical was disturbing, but even more so was the melting point 202-203° (184-5°[48]) and rotation - 26° of the corresponding cyclized keto ester



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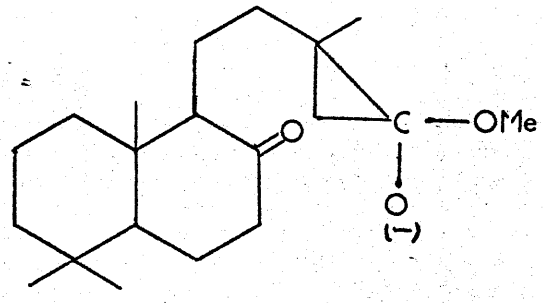
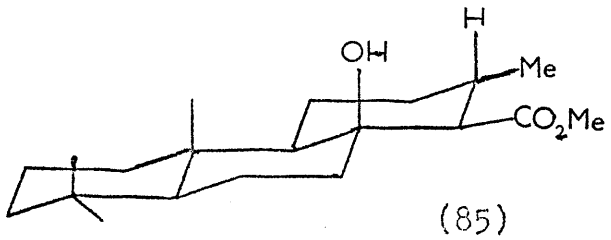


from methyl eperuate, since this was expected to be completely antipodal to the corresponding compound from methyl 13-epilabdanolate.

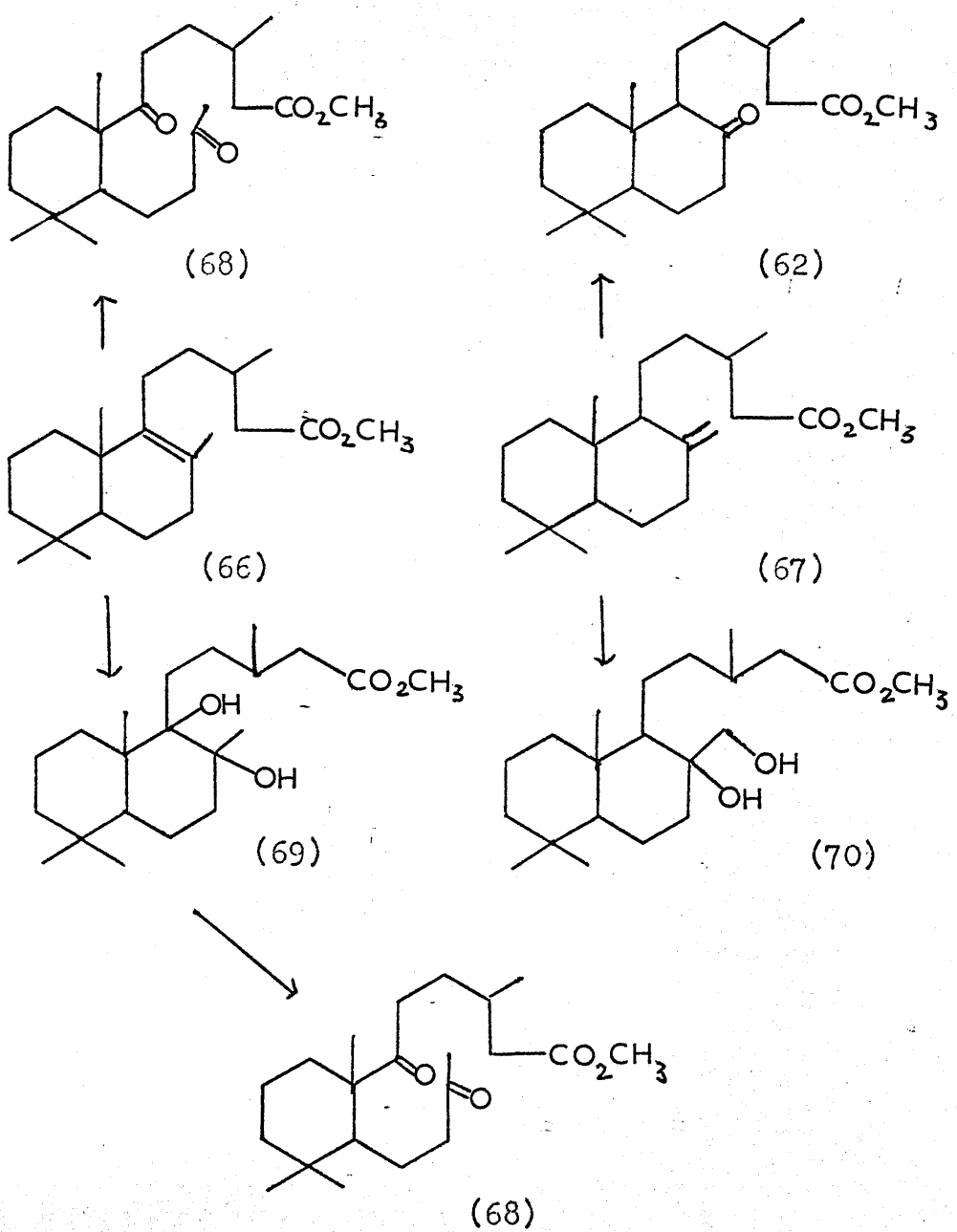
To eliminate the possibility of any confusion in the previous handling of the three samples, the preparations were carefully repeated using natural methyl eperuate, methyl labdanolate from gum labdanum and methyl 13-epilabdanolate from the oxidative rearrangement of sclareol. The appropriate keto esters were formed in each case and carefully recyclized, but again the melting points and optical rotations were identical (200-202°, $[\alpha]_D$ - 20°; 201-203°, $[\alpha]_D$ - 29°; 204°, $[\alpha]_D$ - 30° respectively). X-ray single crystal rotation photographs of the derivatives from methyl labdanolate and methyl 13-epilabdanolate were indistinguishable, and it would therefore appear that epimerization of C-13 in one of the isomers was taking place during the cyclization.

Recent work[62] has demonstrated that hydrogen atoms beta to a carbonyl can be abstracted by alkali and that reprotonation can proceed via an inversion of configuration. The anion so formed in this case (84) could be stabilized as indicated. However, such a stabilization would depend on the polarizability of the C = O and also (and perhaps more important) on the extent to which the carbonyl carbon



(85)

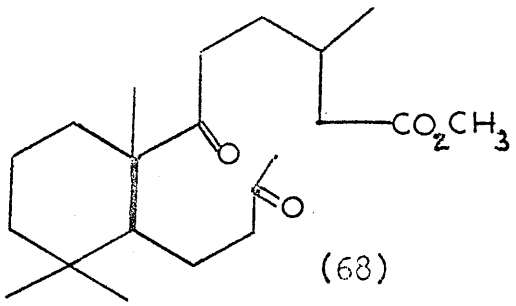
Flowsheet X.



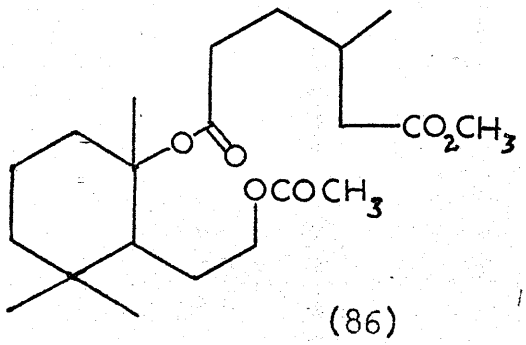
atom can sustain a positive charge. This is obviously less in the case of an ester than with a ketone. If such a situation could be assumed to exist here then reprotonation would furnish the most stable configuration at C-13, namely an axial proton and an equatorially (α)-attached methyl group (85).

The failure of this apparently straightforward approach to the absolute configuration at C-13 in the labdanolic and eperuic acid series led us to consider more orthodox routes to an unambiguous assignment of configuration. Cleavage of the side chain at C-9 to give an optically active (β)-methyl adipic acid which still retains the original asymmetric centre at C-13 was therefore chosen as an alternative approach to this problem.

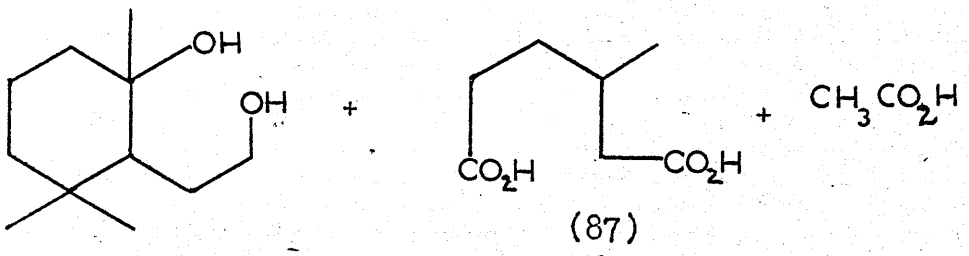
The first attempt to achieve such a cleavage involved a Baeyer - Villiger oxidation of the diketone (68) obtained from the previously described ozonolysis of double bond isomers which comprise natural methyl eperuate. The required diketone was separated from the ozonolysis mixture by chromatography over activated alumina and compared with the product obtained from osmylation of the same isomeric mixture, with subsequent lead tetraacetate cleavage of the ditertiary diol (69; Flowsheet X). Comparison of the two compounds indicated that they were identical. They



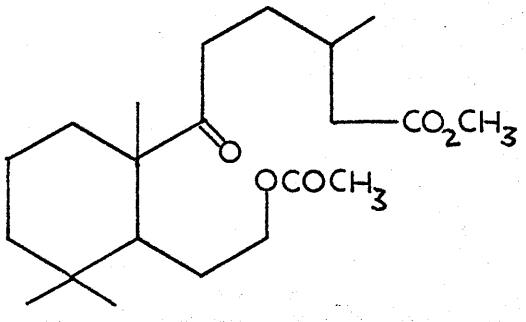
(68)



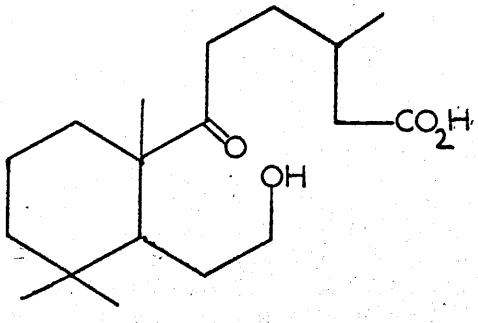
(86)



(87)



(88)



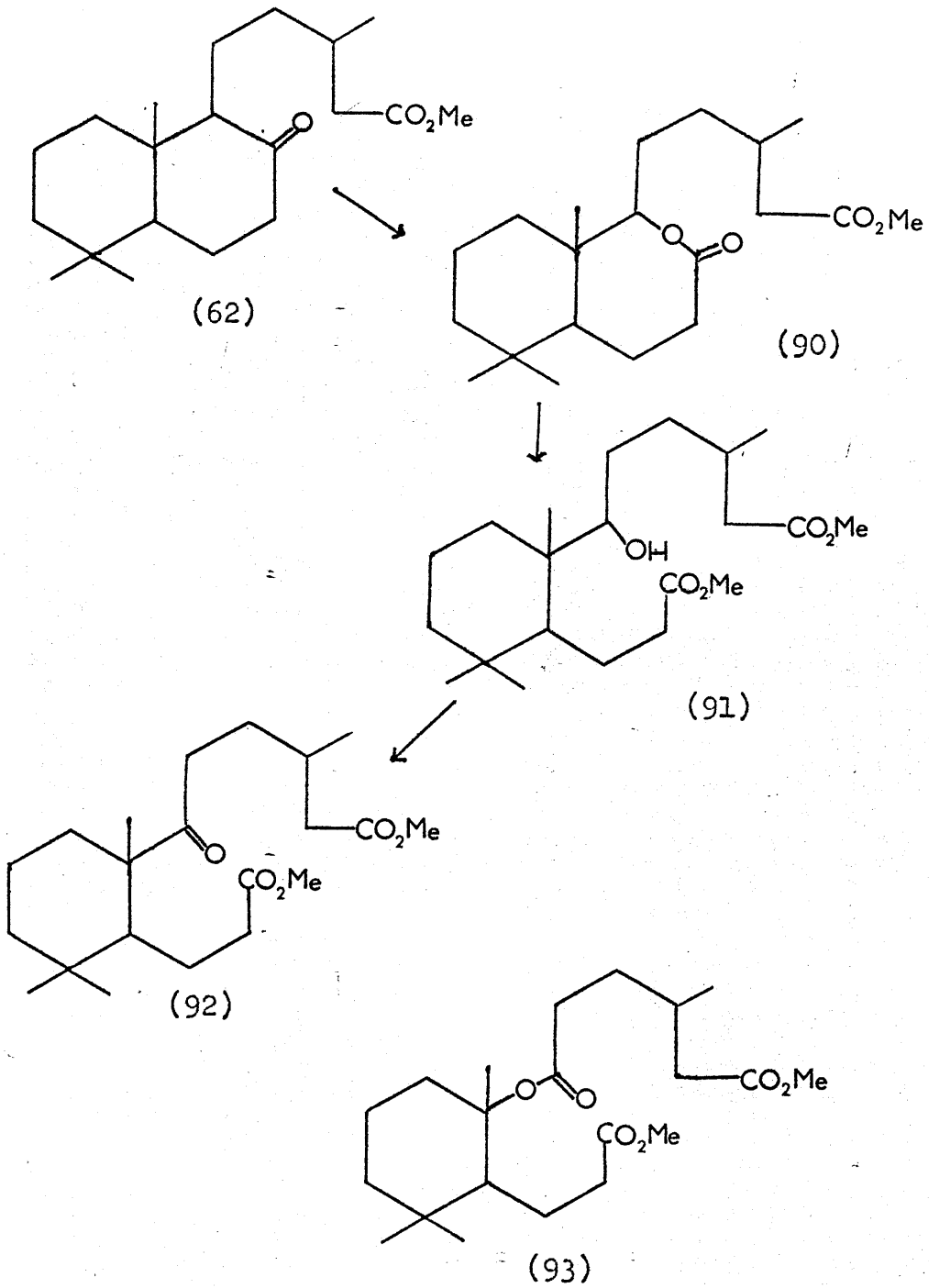
(89)

both appeared as a single spot with the same R_f value on T.L.C. Gas-liquid chromatographic examination of the two compounds, individually and as a mixture, indicated that they had the same retention time (36.5 minutes on 10% A.P.L. at 225°). Their infrared and nuclear magnetic resonance spectra were also identical.

It was hoped that alkaline hydrolysis of the expected Baeyer - Villiger product (86) would furnish an optically active β-methyl adipic acid (87). However, despite several attempts under different reaction conditions, using both m-chloroperbenzoic and trifluoroperacetic[63] acids, apparently only the partially oxidized keto-acetoxy ester (88) was obtained as evidenced by the survival of ketonic carbonyl absorption in the infrared spectrum ($\nu_{max.}^{film}$ 1740, 1700 $cm.^{-1}$) (but see below). Hydrolysis of this compound gave an acid fraction, presumably (89) since a nuclear magnetic resonance spectrum compared with that of authentic material indicated that it was not the desired β-methyl adipic acid.

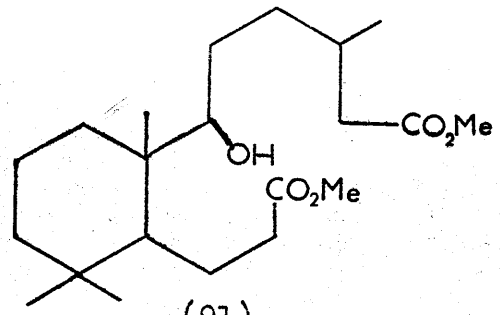
An alternative scheme (flowsheet XI) for the side chain cleavage started from the previously described mono-keto ester (62) from methyl 13-epilabdanolate. Treatment of this compound with a solution of trifluoroperacetic acid in methylene chloride[63] gave a single product which was not the starting material (T.L.C.). The infrared

Flowsheet XI.

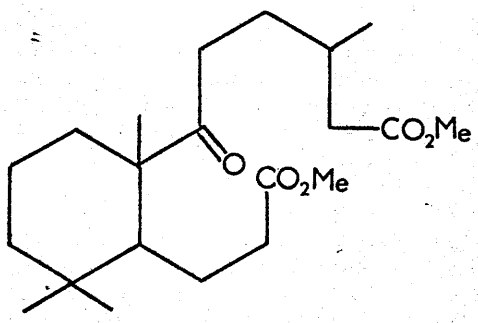


solution spectrum had $\nu_{max.}^{CCl_4}$ 1740 $cm.^{-1}$ and no absorption at 1712 $cm.^{-1}$ (cyclohexanone). The product was therefore assumed to be the lactone (90). Alkaline hydrolysis of this lactone, followed by re-esterification of the acid fraction with diazomethane, gave the hydroxy-diester (91), also a single product on T.L.C. The constitution of this latter compound was confirmed by elementary analysis. The infrared (carbon tetrachloride) solution spectrum had bands at 3635, 3600 and 3500 $cm.^{-1}$, with a broad carbonyl absorption at 1740 $cm.^{-1}$ The nuclear magnetic resonance spectrum had a six-proton doublet (the two methyl esters) at τ 6·4 and a diffuse one-proton singlet at τ 6·9, which disappeared on equilibration with deuterium oxide.

Several attempts were made to oxidize this alcohol (91) to the corresponding carbonyl compound (92), using both chromium trioxide in pyridine[64] and Jones reagent[65], but in all cases the product was seen (T.L.C.) to contain some unoxidized alcohol. The mixture was separated by chromatography over activated alumina and the keto-diester (92) treated with trifluoroperacetic acid[63]. It was hoped to obtain the triester (93) from this Baeyer - Villiger oxidation but several attempts gave back unchanged starting material (T.L.C. and $\nu_{max.}^{film}$ 1740, 1700 $cm.^{-1}$).



(91)

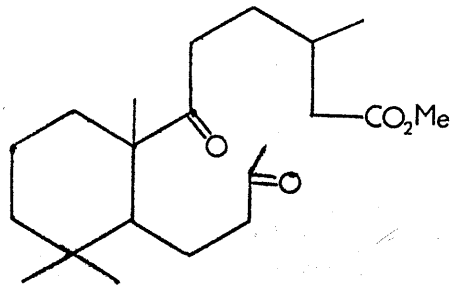


(92)

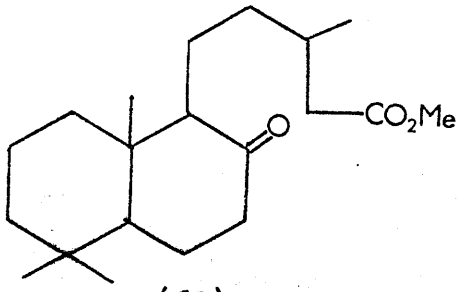
The difficulty experienced in obtaining results with this last Baeyer - Villiger reaction caused some concern and as a last resort it was decided to use an oxidation procedure from the classical era of terpene chemistry, namely nitric acid oxidation.

The first of these oxidations attempted involved heating the product mixture (91 and 92) from the Sarett oxidation[64] with concentrated nitric acid at steam-bath temperature in the presence of a catalytic amount of vanadium pentoxide. Comparison of the re-esterified product mixture with an authentic sample of methyl β - methyl adipate by gas-liquid chromatography indicated the presence of the required ester, but only as a minor constituent of a considerable mixture of products. Since it therefore appeared, from the large number of other products present in the mixture, that heating with concentrated nitric acid was too drastic, various other oxidation experiments were carried out using milder conditions, e.g. dilute nitric acid in the cold and with heating, concentrated nitric acid at room temperature, alkaline potassium permanganate in the cold and with heating - but none of these subsequent reactions gave any trace of the required product, as indicated by gas-liquid chromatography.

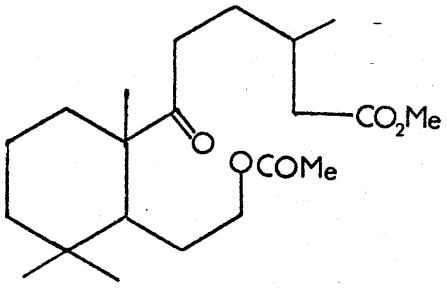
Flowsheet XII.



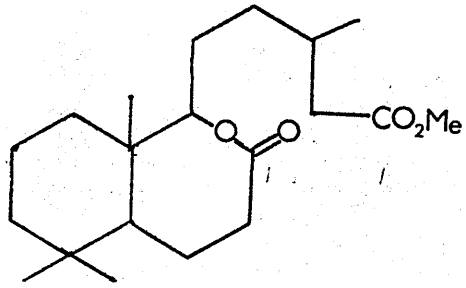
(68)



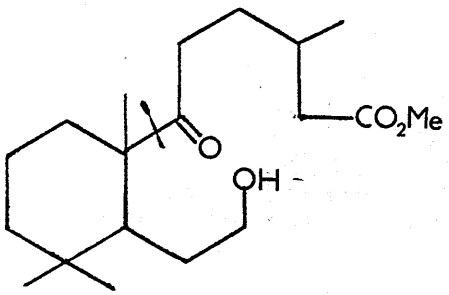
(62)



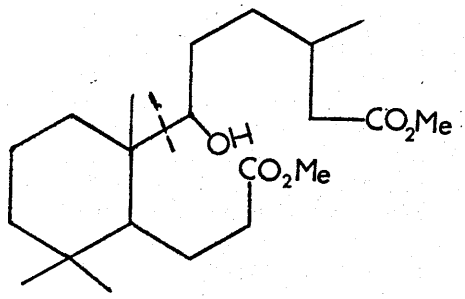
(88)



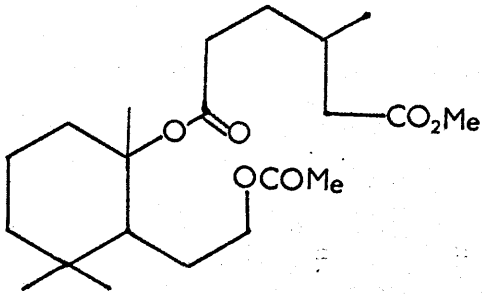
(90)



(89)



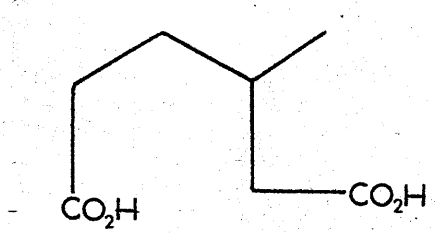
(91)



(86)

In order to obtain more starting material for oxidation, the mixture of double bond isomers comprising methyl eperuate was ozonized and the resultant mixture of ketones (62; 68) treated with a solution of trifluoro-peracetic acid without prior separation. It was considered that Baeyer - Villiger oxidation of such a mixture (flowsheet XII) would, with subsequent hydrolysis (to open the lactone 90) and re-esterification of the acid fraction, yield a mixture of the compounds (89) and (91). Since both of these compounds contain a side chain suitable for oxidative cleavage, separation of the components of the mixture at a previous stage is not mandatory.

However, this time the Baeyer - Villiger reaction conditions were slightly different from all the previous attempts, the ketonic material remaining in contact with the peracid solution, in the cold, for at least forty-eight hours before refluxing for a further six hours. Gas-liquid chromatography of the hydrolysed and re-esterified product mixture, compared with an authentic sample, indicated the presence of methyl β -methyl adipate. Thus, under these conditions, Baeyer - Villiger oxidation of the diketone (68) must have produced the acetoxy-diester (86).



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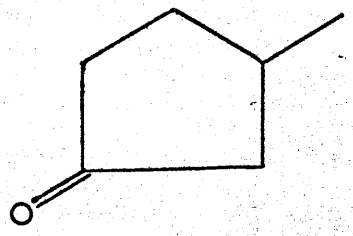
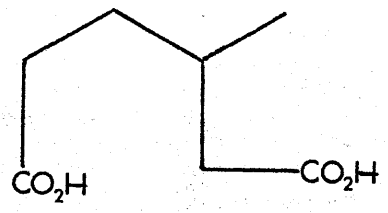


(94)

It is reported[66] that (+) β -methyl adipic acid gives, when distilled at 180° (1 mm.), the colourless acid m.p. 85-89° and it appeared that this might provide a suitable method of purification since chromatography of the ester mixture obtained from the Baeyer - Villiger oxidation had met with little success. However, it was found that distillation of an authentic sample of (+) β - methyl adipic acid under the recorded conditions of temperature and pressure (in a sublimation apparatus) did not in fact give either the unchanged acid, the anhydride or even 3-methyl cyclopentanone,[68] by a comparison of the infrared spectrum of the sublimation product with those of authentic samples of acid and cyclopentanone. Thus, since it was obvious that the separation of β -methyl adipic acid from the product mixture would be difficult an alternative method of isolating the optically active material was required.

β -Methyl adipic acid had been cyclized[66,67] to the corresponding 3-methylcyclopentanone (94) by distillation from barium hydroxide at 300°. This was first of all attempted on an authentic sample of the β -methyl adipic acid and an infrared solution spectrum of the product compared with that of an authentic sample of 3-methyl-cyclopentanone. Both had a single sharp carbonyl band



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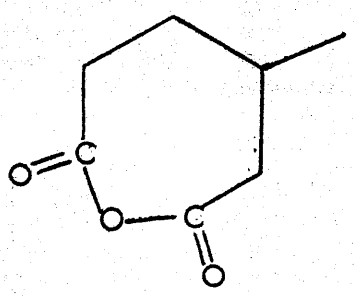


(95)

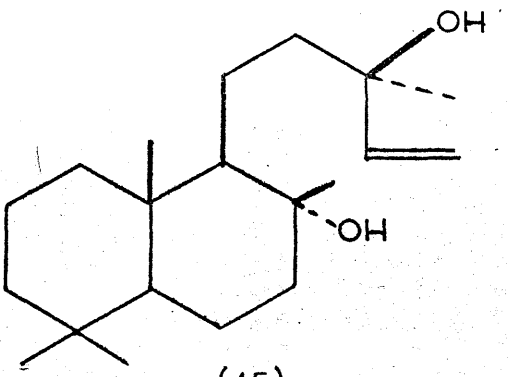
at 1740 $cm.^{-1}$ This cyclization was repeated on the mixture of products from the oxidation of eperuic acid, using a fraction rich in β-methyl adipic acid obtained by chromatography. An infrared solution spectrum of the product did show the cyclopentanone carbonyl at 1740 $cm.^{-1}$, but contaminants could not be removed by distillation.

An alternative method[69] for cyclization of the dicarboxylic acid involved refluxing with acetic anhydride and pyridine followed by decarboxylation of the resultant anhydride (95). This was achieved with an authentic sample of β-methyl adipic acid but only a minute amount (as judged by infrared solution spectrum of the carbonyl region) of impure ketone was obtained from the eperuic acid oxidation product.

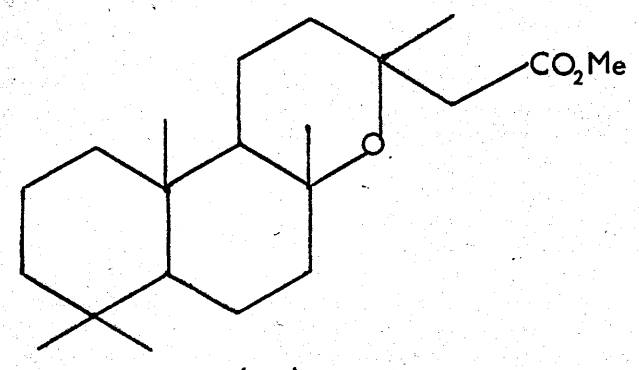
Since the determination of the absolute configuration of C-13 in the labdanolic and eperuic acid series depended on obtaining from this mixture a specimen of an optically active compound having a specific rotation whose magnitude would leave no doubt as to its origin and the cyclopentanone could not be separated from the attendant impurities by distillation, its conversion to the dibenzylidene derivative was considered. This is a nicely crystalline compound[66] m.p. 150·5-152·5°; $[\alpha]_D$ - 53·5°, and indeed was easily prepared from an authentic sample of

3-methylcyclopentanone. However attempts to prepare this derivative from our minute amounts of impure 3-methyl-cyclopentanone were unsuccessful.

Lack of time prevented any attempt being made to isolate the required β-methyl adipic acid via preparative scale gas-liquid chromatography on the ester mixture, although this would now appear to be a profitable approach to the problem.



(45)



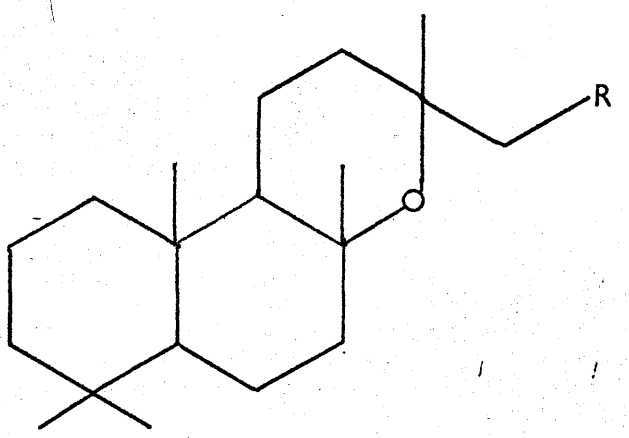
(96)

3. Some Oxidation Products of Sclareol.

In the course of the oxidation of sclareol to obtain the labdanolic and 13-epilabdanolic acids required for the work in section 1 a number of secondary products was obtained. Since these have not been previously discussed in the literature, there now follows a brief discussion of their chemistry.

Oxidative rearrangement of sclareol (45) with chromic acid in acetic acid solution gives rise to a mixture of three main products (cf. flowsheet VIII), the two previously discussed epimeric labdanolic acids and a saturated ether to which Bory and Lederer[52], without firm support, assigned the structure (96). We now supply evidence which confirms Lederer's structure.

Elementary analysis of compound (96) supported Lederer's proposed structure and this was further strengthened by examination of infrared and nuclear magnetic resonance spectra. The infrared (carbon tetrachloride) solution spectrum had a single sharp ester carbonyl band at 1740 $cm.^{-1}$ and no absorption in the hydroxyl region. This spectrum also had $\nu_{max}^{CCl_4}$ 1177, 1098 and 1076 $cm.^{-1}$, bands characteristic of a tetrahydropyran ring. The 60 megacycle nuclear magnetic resonance spectrum had peaks at τ 6·4 (a three-proton



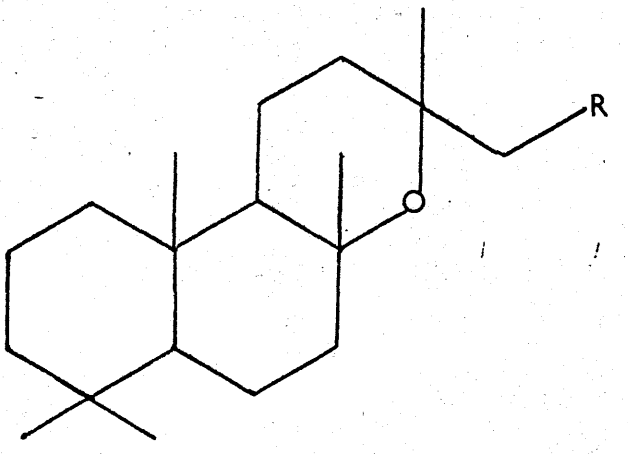
(96) R = CO_2CH_3

(97) R = CO_2H

(98) R = CH_2OH

singlet, corresponding to the methyl ester); τ 7·7 (a two-proton singlet due to the CH_2 group alpha to carbomethoxyl); τ 8·7 and 8·76 (two singlets, probably corresponding to the methyl groups at C-8 and C-13, though it cannot be stated which frequency corresponds to which methyl group); τ 9·14, 9·2, 9·24 (the three singlets due to the quaternary methyl at C-10 and the gem-dimethyl at C-4). A mass spectroscopic molecular weight determination (336) agreed with the molecular weight calculated for structure (96).

Further evidence for structure (96) was obtained from its hydrolysis to the corresponding acid (97) and lithium aluminium hydride reduction to the primary alcohol (98). This latter compound analysed correctly for $C_{20}H_{36}O_2$ and a solution spectrum had no carbonyl absorption but $\nu_{max.}^{CCl_4}$ 3630, 3490 $cm.^{-1}$, the hydroxyl presumably being bonded to the ether oxygen. The nuclear magnetic resonance spectrum had peaks at τ 6·4 (a two-proton triplet arising from coupling of the hydroxyl proton with the alpha CH_2 group); τ 6·8 (one proton, a diffuse singlet which disappears on equilibration with deuterium oxide); τ 8·48 and 8·74 (two singlets corresponding to six protons, probably those of the methyl groups at C-8 and C-13); τ 9·15, 9·22 and 9·25,



(96) R = CO_2CH_3

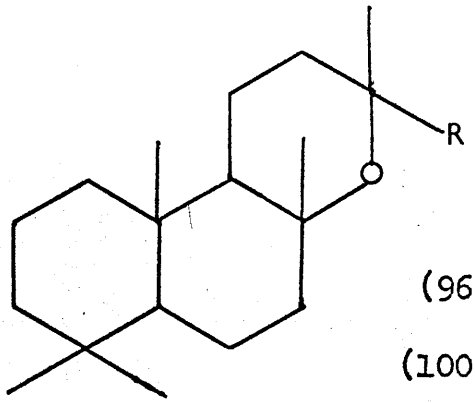
(98) R = CH_2OH

(99) R = CH_2Cl

three singlets arising from the remaining three methyl groups.

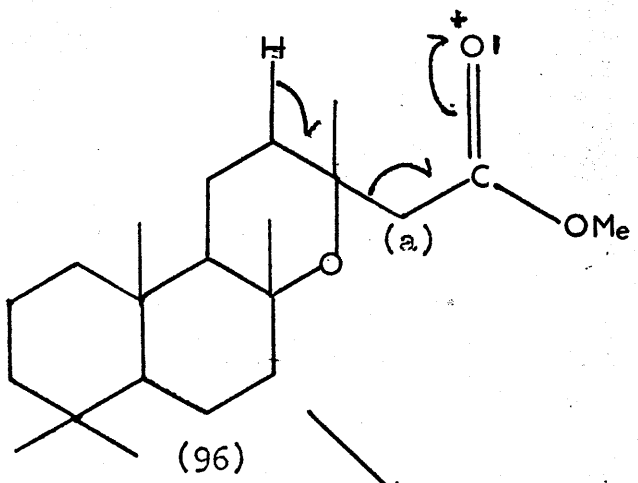
An attempt was made to dehydrate this alcohol, by refluxing with phosphorus oxychloride in pyridine, in order to convert it to either manoyl oxide or 13-epimanoyl oxide. However, the reaction product was in fact the chloride (99) on the basis of elementary analysis, positive Beilstein reaction, no unsaturation in the infrared solution spectrum and no vinyl protons in the nuclear magnetic resonance spectrum. The infrared spectrum showed only CH_2 absorption and the ether bands at $\nu_{max}^{CCl_4}$ 1120, 1098 and 1076 $cm.^{-1}$ The nuclear magnetic resonance spectrum showed a two-proton triplet centred at τ 6·25. After completion of this work, Fetizon[70] published a comparison of alcohol (98), obtained from reduction of ester (96), with the product from hydroboration of 13-epimanoyl oxide, which suggests that (98) has the same stereochemistry at C-13 as manoyl oxide.

In the course of separating the mixture of epimeric labdanolates (65) from ether (96) by gradient elution chromatography over activated alumina, it was noticed that there were several other minor constituents present in the fractions containing the ether (96). These were



(96) R = $CH_2CO_2CH_3$

(100) R = CO_2CH_3



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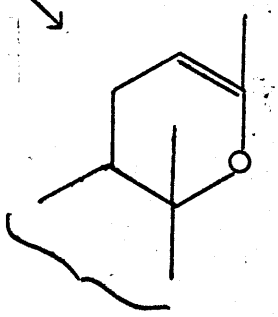
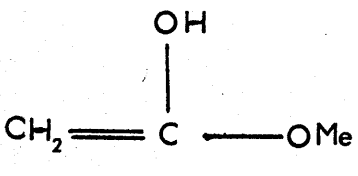


m/e 262

+

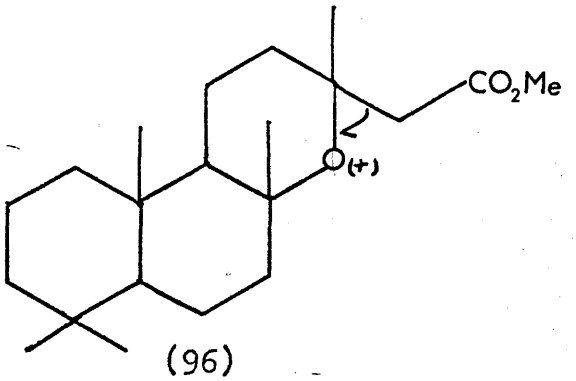


m/e 74

separated from the ether by thick plate chromatography in a solvent system of ethyl acetate-light petroleum (1: 9). Only two were present in sufficient abundance for a structure determination, and it is of interest to note that the presence of the expected 13-epimer of ether (96) was not observed.

The next component to be examined was shown to be the nor-ester (100) of ether (96). This was confirmed by elementary analysis, an infrared spectrum similar to that of (96) and a nuclear magnetic resonance spectrum which was similar to that of ether (96) except that it lacked the two-proton singlet at τ 7.7.

A mass spectroscopic molecular weight determination corresponded to the calculated molecular weight (322) for structure (100). However, a comparison of the mass spectra obtained by us from compounds (96) and its nor-ester (100) with Fetizon's recently published[70] spectrum of (96) indicated some outstanding differences between the spectra of the two, presumably identical, ethers (96). Our mass spectrum of this ether (96) has a large peak at $^{m}/_{e}$ 74 which can be postulated as corresponding to the side chain (a) together with one additional proton. This breakdown can be represented as shown and is corroborated by a small peak at $^{m}/_{e}$ 262. Fetizon on the other hand



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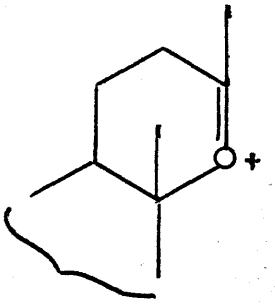
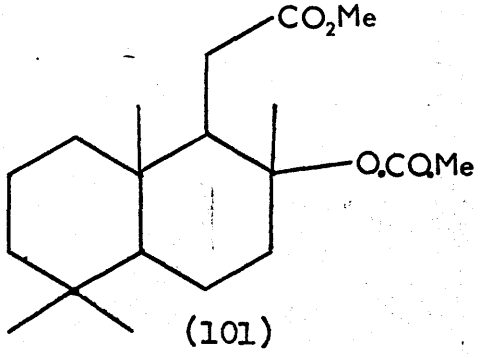


m/e 263

+ • CH_2—CO_2Me

m/e 73

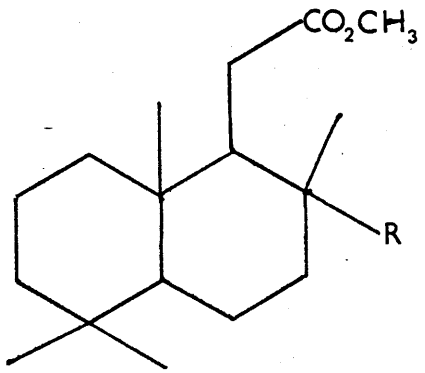


(101)

postulates a loss of 73 mass units to explain the ion at $^{m}/_{e}$ 263 in his spectrum, the suggested fragmentation being as indicated. However, his spectrum does not seem to contain a corresponding peak at $^{m}/_{e}$ 73!

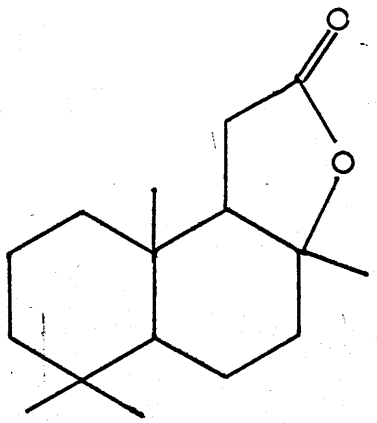
There are other differences between the two spectra; our mass spectrum has ions at $^{m}/_{e}$ 244 and 191 whereas the corresponding peaks in Fetizon's spectrum have $^{m}/_{e}$ 245 and 192; also the relative abundances of the ions which are common to both spectra are not identical. It was at first thought that the two ethers were in fact C-13 epimers but this possibility was discounted since Fetizon's ether (96) is stated to be that previously isolated by Bory and Lederer[52] m.p. 107-111°; $[\alpha]_D$ + 18°. Our ether (96) has m.p. 107-109°, $[\alpha]_D$ + 18°. It is unlikely that instrumental differences or even sampling techniques could produce such discrepancies.

The only other compound to be isolated in a workable amount from the mixture was thought, on the basis of an elementary analysis and a nuclear magnetic resonance spectrum, to have the structure (101). This latter had peaks at τ 6·4 (three-proton singlet, methyl ester); τ 7·78 (three-proton singlet, acetate CH_3); τ 8·2 (three-proton singlet, C-8 methyl group); τ 9·13, 9·18, 9·22 (three singlets, methyl groups). The infrared



(101) R = O.CO.CH_3

(102) R = OH



(103)

(carbon tetrachloride) solution spectrum had no hydroxyl absorption and $\nu_{max}^{CCl_4}$ 1740, 1735 and 1240 $cm.^{-1}$ This postulated acetoxy-ester structure (101) was confirmed by a mass spectroscopic molecular weight determination in which the ion of highest $^{m}/_{e}$ value corresponded to a mass number of 264. This agrees with a loss of acetic acid from the postulated structure (calculated molecular weight 324) and is confirmed by a fairly abundant peak at $^{m}/_{e}$ 60.

The acetoxy-ester (101) was hydrolysed with aqueous methanolic potassium hydroxide and re-esterified with diazomethane. It was seen from T.L.C. that the product was a mixture of two compounds which were at first thought to be the expected hydroxy ester (102) and unchanged starting material (101) since the less polar component had an R_f value similar to that of the original acetate (T.L.C.). Attempts to separate the constituents of the mixture by chromatography over activated neutral alumina produced a single (T.L.C.) pure crystalline compound. Rechromatography of the residue yielded a further crop of this crystalline material (m.p. 123-4°, $[\alpha]_D$ + 42°, $\nu_{max}^{CCl_4}$ 1780 $cm.^{-1}$) which was identified as the lactone (103; m.p. 123-4°, $[\alpha]_D$ + 46°) previously obtained[71] from the products of permanganate oxidation of sclareol. Thus the mixture obtained from hydrolysis and re-

esterification of (101) must have been the expected hydroxy-ester (102) together with a little of the lactone (103), chromatography over alumina, which was probably slightly basic, increasing the yield of the lactone.

EXPERIMENTAL.

All melting points were determined on a Kofler block and are uncorrected. All boiling points are uncorrected.

Infrared solution and KCl disc spectra were recorded linearly in $cm.^{-1}$ as percentage transmission by Mrs. F. Lawrie with a Unicam S.P. 100 double-beam spectrophotometer equipped with an S.P. 130 sodium chloride prism-grating double monochromator operated under dry-air conditions. The wave-number scale was calibrated against water vapour (3656-2000 $cm.^{-1}$) and acetone (2000-650 $cm.^{-1}$). Some infrared spectra were recorded on a Perkin-Elmer 237 spectrophotometer.

Proton magnetic resonance spectra were recorded by Dr. J.D. Connolly and Mr. J. Gall with a Perkin-Elmer 60 megacycle spectrometer, using tetramethylsilane as internal reference in carbon tetrachloride solutions of samples.

Woelm alumina, deactivated to the appropriate Brockmann grade[72] was used for column chromatography. Thin layer chromatcplates (T.L.C.) were prepared[73] from Merck's 'Kieselgel G'.

Analytical gas-liquid phase chromatograms were run on a Pye-Argon chromatograph with a Strontium-90 Ionization

detector.

Mass spectral molecular weights were determined with an A.E.I. M.S.9 mass spectrometer by Mr. T. Bryce, B.Sc., and Miss J. Wilkie.

Optical rotations were measured in chloroform unless otherwise stated.

The light petroleum used was of b.p. 60-80° unless stated to the contrary.

Methyl Labdanolate and Methyl 13-epilabdanolate.

Sclareol (45) was oxidized with sodium dichromate according to the method of Stoll and Commarmont[74]. The aldehydic product (58% yield) was further oxidized with silver oxide[52], the total acid product (98% yield) esterified with diazomethane and hydrogenated in ethanolic solution in the presence of platinum oxide.

The mixture of saturated esters (24·5 g.) was then adsorbed on activated alumina (neutral, grade III, 1·8 kg.) and displaced by gradient elution [2 l. of benzene: light petroleum (2: 3) added to 4 l. of (1: 9) followed by 3 l. of (3: 2) and 3 l. of (3: 1)]. Fractions 9 to 60 (10·3 g.) gave the ester (96), m.p. (from methanol) 107-109°, $[\alpha]_D$ + 18° (C 2·05), 109-111°; + 18° [52]. Fractions 140 to 184 (6·35 g.) gave methyl 13-epilabdanolate, m.p. (from

light petroleum) 71-74°, $[\alpha]_D$ + 3° (C 1·28) [74-75°; + 2°][52]. The infrared spectrum, as a KCl disc, was identical with the recorded spectrum[52]. Subsequent fractions gave methyl labdanolate contaminated with methyl 13-epilabdanolate (shown by comparison of infrared spectra) which could not be satisfactorily separated by repeated chromatography to give the required quantity of methyl labdanolate. The methyl labdanolate used m.p. 69-72°, $[\alpha]_D$ - 7° (c 2·12) [72-74°; - 7°][52] was extracted[53] from gun labdanum.

Dehydration of Methyl Labdanolate and Methyl 13-epi-labdanolate.

The two hydroxy-esters were dehydrated with phosphoryl chloride in pyridine[50] at 20°; The products had $[\alpha]_D$ + 21° [+ 27°][50] (c 2·09) from methyl labdanolate and - 22° (c 4·46) from methyl 13-epilabdanolate. Gas-liquid chromatography (0·5% A.P.L./80-100 Embacel; 175°; 40 ml. Argon/min.) showed the presence in both cases of two products of retention times 8 and 9·5 min. Quantitative infrared comparison of the band at 890 $cm.^{-1}$ (CS_2; exo-methylene) in each mixture with the corresponding band in 3-methylene cholestane gave its content as 72% (13-epi) 58% (13-normal).

Ozonolysis of the Dehydration Products.

The olefin mixtures obtained above were ozonised in ethyl acetate at - 70° [50]. The ketonic product containing two major components (T.L.C.) was chromatographed over activated alumina (neutral, grade 111). The fractions containing the least polar component (T.L.C.) were examined for homogeneity by gas-liquid chromatography (10% A.P.L.; 225°; 40 ml. Argon/min.; retention time 24 mins.). This component showed $\nu_{max.}^{CCl_4}$ 1712 (cyclohexane), 1739 (methyl ester) $cm.^{-1}$. Hydrolysis[50] furnished 20-nor-8-oxolabdan-15-oic acid, and 20-nor-8-oxolabdan-13-epi-15-oic acid which were characterised as the oximes as follows:-

From labdanolic acid, prisms from ethanol, m.p. 188-190°, $[\alpha]_D$ + 52° (pyridine, c 0·84).

From 13-epi-labdanolic acid, needles from ethanol, m.p. 222-226°, $[\alpha]_D$ + 87° (pyridine, c 0·72).

Natural methyl eperuate, n_D^{25} 1·4960 $[\alpha]_D$ - 22°, was shown by gas-liquid chromatography (0·5% A.P.L.; 175°; 40 ml. Argon/min.) to consist of a mixture of two olefins whose retention times were identical with those of the previously described dehydration products. Quantitative infrared estimation (see above) showed the presence of 32% exocyclic olefin. Ozonolysis, separation of the products as above (G.L.C., T.L.C. and I.R. comparisons

match the product from methyl 13-epilabdanolate) and hydrolysis furnished the oily enantio-20-nor-8-oxo-13-labdan-15-oic acid, characterised as the oxime (needles from ethanol) m.p. 224-226°; $[\alpha]_D$ - 82° (pyridine, c 1·18).

Methyl 8ξ, 20ξ-dihydroxylabdan-15-oate.

The olefin mixture (499 mg.), obtained from dehydration of methyl labdanolate (with phosphoryl chloride in pyridine) (see above), in anhydrous ether (10 ml.) and pyridine (10 ml.) and osmium tetroxide (500 mg.) were kept in the dark for two days, and the mixture of osmates decomposed with hydrogen sulphide. The gummy mixture of diol esters (550 mg.; two components by T.L.C.) was separated by chromatography over activated alumina (grade II neutral, deactivated with 7·5% of 10% acetic acid; 30 g.)

Elution with benzene-light petroleum (1: 1) afforded methyl 8ξ, 9ξ-dihydroxylabdan-15-oate (108 mg.; $\nu_{max.}$ 3580, 1735 $cm.^{-1}$) which could not be induced to crystallise. Benzene then eluted the second product, methyl 8ξ, 20ξ-dihydroxylabdan-15-oate (270 mg.) prisms from ice-cold light petroleum (40-60°), m.p. 77-79°, $[\alpha]^{21}_{546}$ - 20° (pyridine, C 1·11) (Found: C 70·98; H 10·78. $C_{21}H_{38}O_4$ requires C 71·14; H 10·8%). $\nu^{CCl_4}_{max.}$ 3644, 3615 (free hydroxyl); 3570 (bonded hydroxyl); 1740 (ester) $cm.^{-1}$ Singlet (2H) at τ 6·5 (in $CDCl_3$ with T.M.S. as internal

standard). In a later osmylation, the osmate esters were decomposed by stirring the reaction mixture, at room temperature, with sodium bisulphite solution and aqueous pyridine until a clear orange solution was obtained[75]. The aqueous solution was then extracted with chloroform to give the gummy mixture of diol esters.

Acetylation of Methyl-8ζ, 20ζ-dihydroxylabdan-15-oate.

This primary-tertiary diol (39 mg.) was converted quantitatively (T.L.C.) into an oily monoacetate by dissolving in pyridine and leaving overnight at room temperature with an equal weight of acetic anhydride. The oily product had $\nu_{max.}^{film}$ 3550, 1735, 1240 $cm.^{-1}$

Benzoylation of Methyl-8ζ, 20ζ-dihydroxylabdan-15-oate.

The primary-tertiary diol (100 mg.) was dissolved in pyridine, recrystallized p-nitrobenzoyl chloride (330 mg.) added and the mixture allowed to stand overnight at room temperature. Conversion to an oily mono p-nitrobenzoate was quantitative (T.L.C.), the single product having $\nu_{max.}^{film}$ 3600, 1720, 1280 and 1120 $cm.^{-1}$

This primary-tertiary diol ester also formed an oily acetonide quantitatively (T.L.C.) by stirring with excess acetone in the presence of anhydrous copper sulphate at room temperature for four to five hours.

Methyl 8ξ, 20ξ-dihydroxy-13-epilabdan-15-oate.

Prepared from the dehydration product of methyl 13-epilabdanolate by osmylation as above, this afforded fine needles from ice-cold light petroleum (40-60°), m.p. 88-90° $[\alpha]_{546}^{21}$ - 5° (pyridine, c 2·22) (Found: C 71·47; H 10·17. $C_{21}H_{38}O_4$ requires C 71·14; H 10·8%). I.R., T.L.C. and N.M.R. properties showed only very minor differences from the corresponding diol emanating from methyl labdanolate.

Antipode of Methyl 8ξ, 20ξ-dihydroxy-13-epilabdan-15-oate.

Prepared from natural methyl eperuate by osmylation as above, this afforded fine needles from ice-cold light petroleum (40-60°) m.p. 88-90°, $[\alpha]_{546}^{21}$ + 4° (pyridine, c 1·80).

Reduction of this diol with lithium aluminium hydride afforded an oily triol (one product by T.L.C.; absence of $\nu_{max.}$ 1700-1800 $cm.^{-1}$). The derived p-nitrobenzoate and α-naphthylurethane were likewise non-crystalline.

The dihydroxy acid obtained by alkaline hydrolysis and its cyclohexylamine salt could not be crystallised.

Alternative Dehydration of Methyl 13-epilabdanolate.

This was attempted with the object of obtaining only the exomethylene isomer[76]. Methyl 13-epilabdanolate

(300 mg.) was heated under reflux for twelve hours with anhydrous copper sulphate (10 g.) in anhydrous benzene (40 ml.). However, the single product (T.L.C.) appeared to be methyl labd-8-en-15-oate since an infrared spectrum showed no absorption at 3500 or 890 $cm.^{-1}$ $\nu_{max.}^{film}$ 1740 $cm.^{-1}$

Cyclization of Methyl 20-nor-8-oxolabdan-15-oate (62).

The keto ester (360 mg.) was cyclized in the presence of alcohol-free sodium methoxide according to the method of King and Jones[48]. The crystalline product (122 mg.) recrystallized from benzene/light petroleum to give colourless needles which sublimed to prisms at 170° and had a m.p. of 202-203°. (Found: C 74·66; H 10·82. $C_{20}H_{34}O_3$ requires C 74·49; H 10·63%). $[\alpha]_D$ - 30° (c 0·78). $\nu_{max.}^{CCl_4}$ 3622, 3582, 3524, 1743, 1714 $cm.^{-1}$ Nuclear magnetic resonance spectrum had bands at τ 6·3 (3H, singlet); τ 7 (IH diffuse singlet); τ 9 (3H, singlet); τ 9·19 (6H singlet); 9·27 (3H singlet).

Cyclization of Methyl 20-nor-8-oxo-13-epilabdan-15-oate.

The keto ester from methyl 13-epilabdanolate (543 mg.) was cyclized as above[48] and the product (325 mg.), when recrystallized from benzene/light petroleum, also gave colourless needles which changed to prisms in the range 165-175°, and had m.p. 204°. (Found: C 74·35; H 10·77. $C_{20}H_{34}O_3$ requires C 74·49; H 10·63%). $[\alpha]_D$ - 31° (c 0·62).

$\nu_{max.}^{CCl_4}$ 3622, 3582, 3524, 1743, 1713 $cm.^{-1}$

Alternative Methods for Cyclization of Keto Esters (62).

(1) A solution of the keto ester from methyl eperuate (50 mg.) in anhydrous ether was added to a suspension of sodamide in liquid ammonia. When the addition was complete, the ammonia was replaced by anhydrous ether and the mixture stirred under reflux for two hours. Excess sodamide was destroyed by careful addition of an aqueous hydrochloric acid solution and the mixture extracted with ether. The washed and dried ether extracts gave acidic material (T.L.C. and I.R.) which on re-esterification with an ethereal solution of diazomethane gave a mixture of products, including unchanged starting material. Chromatography of the product mixture (58 mg.) over activated acidic alumina (grade III, 7 g.), eluting with benzene: light petroleum (1: 4), gave 3 mg. of crystalline product, m.p. 198-200°.

(2) The cyclization of methyl eperuate keto ester was also attempted using lithium metal in liquid ammonia, but again a considerable mixture of products was obtained. These methods of cyclization were therefore abandoned.

Cleavage of Methyl 8,9-dihydroxylabdan-15-oate.

(a) With sodium periodate.

(i) To the ditertiary diol methyl 8,9-dihydroxylabdan-15-oate (55 mg.) dissolved in methanol was added a solution

of sodium periodate (163 mg.) in water. The milky white suspension was then left at 0^{o} for one hour. However, an infrared spectrum and T.L.C. of the product obtained on extraction with chloroform indicated that it was unchanged starting material.

(ii) The process was repeated using an increased amount of periodate (240 mg.) and the mixture shaken for one hour at room temperature. Again a quantitative yield of ditertiary diol was obtained.

(iii) To the diol was added a further 240 mg. of sodium periodate in aqueous solution and the mixture shaken at room temperature for forty-four hours, but again the product was mainly unchanged starting material.

(b) With lead tetra-acetate.

The diol (50 mg.) dissolved in acetic acid was left in the dark with excess lead tetraacetate for thirty-six hours. The mixture was then diluted with water, extracted with chloroform and the organic layer washed with brine, bicarbonate solution, brine, dried and the solvent evaporated to give an oily diketone identical (by I.R., G.L.C., N.M.R.) with that obtained from ozonolysis of methyl eperuate. The diketones from the two sources were examined for homogeneity by gas-liquid chromatography

(10% A.P.L. at 225°, 45 ml. argon/minute) and both had a retention time of 36·5 minutes. $\nu_{max.}$ 1740, 1720, 1710 $cm.^{-1}$. Both nuclear magnetic resonance spectra had peaks at τ 6·4 (3H, singlet, methyl ester); τ 8·0 (3H, singlet, methyl ketone); τ 8·77, 8·88, 9·1 (4 methyl groups).

Baeyer - Villiger Oxidation of the Diketone (68).

(a) With m-chloroperbenzoic acid.

(i) To the dione (53 mg.) dissolved in analar chloroform was added excess m-chloroperbenzoic acid and the mixture stirred overnight at room temperature. Excess peracid was destroyed by the addition of a 10% solution of sodium sulphite until no further reaction was obtained with starch iodide paper. Washing with a saturated solution of sodium bicarbonate was not sufficient to extract all the remaining m-chlorobenzoic acid and this was removed from the product mixture by filtration through a short column of activated alumina (grade 1V, acid), eluting with benzene-light petroleum (1: 1). A single (T.L.C.) oily product was obtained which appeared to be the acetoxy-keto ester (88). $\nu^{film}_{max.}$ 1740, 1700 $cm.^{-1}$ A nuclear magnetic resonance spectrum showed τ 6·4 (3H, singlet, methyl ester CH_3); τ 8·05 (3H, singlet, acetate CH_3); τ 8·8 (3H, singlet, CH_3); τ 9·03 (9H, 3 methyl groups).

(ii) The Baeyer - Villiger reaction was repeated on a further 213 mg. of diketone and stirred for sixty hours at room temperature. The single product (T.L.C.) obtained from chromatography (80 mg.) was hydrolysed with methanolic potassium hydroxide solution. The nuclear magnetic resonance spectrum of the acid fraction was examined and found to be quite different from that of an authentic sample of (+) β -methyl adipic acid.

(b) With trifluoroperacetic acid.

A solution of trifluoroperacetic acid in methylene chloride was added dropwise to a cooled stirred suspension of diketone (72 mg.) and anhydrous disodium hydrogen phosphate (200 mg.) in methylene chloride. The mixture was heated under reflux for $2\frac{1}{2}$ hours, diluted with water, the aqueous layer separated and extracted several times with methylene chloride. The combined organic layers were washed with a saturated solution of sodium bicarbonate, brine, dried over anhydrous sodium sulphate and the solvent evaporated. The oily product (80 mg.) was chromatographed over activated alumina (grade IV, acid, 10 g.). Elution with 30% benzene-light petroleum gave a single pure compound (T.L.C.) which appeared to be identical with that already obtained from Baeyer - Villiger oxidation with m-chloroperbenzoic acid (T.L.C. and $\nu_{max.}^{film}$ 1700 $cm.^{-1}$).

Baeyer - Villiger Oxidation of Methyl 20-nor-8-oxo-13-epilabdan-15-oate (62).

The monoketone, methyl 20-nor-8-oxo-13-epilabdan-15-oate (207 mg.), was treated with trifluoroperacetic acid as described above for the diketone (68). The product was chromatographed on activated alumina (grade IV, acid) and the required lactone (one spot on T.L.C.) eluted with 10% benzene-light petroleum, $\nu_{max.}^{CCl_4}$ 1740 $cm.^{-1}$ (ester and ε lactone). No ketonic absorption at 1700 $cm.^{-1}$.

Hydrolysis of the Lactone (90).

The lactone-ester (152 mg.) was refluxed on a steam-bath for twenty minutes with sodium hydroxide (200 mg.) in aqueous methanol. The acid fraction was re-esterified with diazomethane to give the hydroxy diester (91). $\nu_{max.}^{CCl_4}$ 3635, 3600, 3500 (free and bonded hydroxyl) 1740 $cm.^{-1}$ (broad carbonyl). The nuclear magnetic resonance spectrum had τ 6·4 (6H doublet); τ 6·9 (1H, diffuse singlet); τ 7·87 (4H, singlet, CH_2 alpha to carbomethoxyls); τ 9·1 (singlet, 4 methyl groups). The viscous hydroxy diester was distilled (160° at 0·15 mm.) and analysed. (Found: C 68·37; H 10·24. $C_{21}H_{38}O_5$ requires C 68·07; H 10·34%).

Oxidation of the Hydroxy diester (91).

(i) Chromium trioxide (150 mg.) was added to the hydroxy diester dissolved in pyridine and the mixture

left overnight at room temperature. Excess chromium trioxide was destroyed by addition of methanol and the chromate complex decomposed by water. The aqueous solution was extracted with ether, washed with dilute sulphuric acid (to remove the pyridine), brine, and dried over anhydrous sodium sulphate. Evaporation of the ether gave 150 mg. oily product which was seen from T.L.C. to contain unchanged starting material.

(2) The hydroxy-diester (150 mg.) was dissolved in acetone and treated with twenty drops of Jones Reagent[64,65] (6N chromic acid + 12N sulphuric acid) at ice-bath temperature, with stirring, for ten minutes. The mixture was diluted with water and extracted with ether. The gummy product was again a mixture of hydroxy (91) and keto (92) diesters (T.L.C.). The components of the mixture were separated by chromatography over activated acidic alumina (grade IV), the desired keto-diester (92) being eluted with benzene-light petroleum (1:9). $\nu_{max.}^{film}$ 1740, 1700 $cm.^{-1}$. Nuclear magnetic resonance spectrum had τ 6·38 (6H, sharp doublet); τ 8·73, 8·79 (two singlets, probably methyl groups); τ 9·05 (singlet, gem-dimethyl).

Baeyer - Villiger oxidation of the keto-diester (92).

(i) The keto diester (93 mg.) was treated with trifluoroperacetic acid as previously described, the reaction

mixture being heated under reflux for two hours. However, T.L.C. and an infrared spectrum of the product indicated unchanged starting material. $\nu_{max.}^{film}$ 1740, 1700 $cm.^{-1}$,

(ii) The process was repeated on 70 mg. ketone, using a large excess of peracid and the mixture refluxed overnight, but the unchanged starting material was again obtained (68 mg.)

The remaining keto di ester was divided into two portions and the Baeyer - Villiger oxidation attempted again.

(iii) One portion of the keto diester (50 mg.) dissolved in methylene chloride was refluxed with excess m-chloro-perbenzoic acid for twelve hours.

(iv) The second portion of keto diester (20 mg.) was refluxed with a solution of trifluoroperacetic acid in methylene chloride for forty hours.

However the infrared spectra of the products from both these attempts still showed carbonyl absorption at 1700 $cm.^{-1}$, and there was apparently no change by T.L.C.

Nitric Acid Oxidation of the C-9 side chain.

(i) A mixture of the hydroxy- (91) and keto-diesters (92) (200 mg., obtained from incomplete Sarett and Jones oxidations of the hydroxylic material) was heated on a steam-bath for twelve minutes (three to four minutes under

reflux) with concentrated nitric acid (2·6 ml.) and a catalytic amount of vanadium pentoxide. The reaction mixture was cooled, poured on to ice and extracted with ether. The organic extracts were washed with brine, dried over anhydrous sodium sulphate and the acidic product obtained (T.L.C.) treated with excess of an ethereal solution of diazomethane prior to examination by gas-liquid chromatography (10% P.E.G.A., 125°, 35 ml. Argon/minute). Comparison with an authentic sample of (+) methyl-β-methyl adipate (retention time 10 mins.) indicated the presence of a small amount of the required ester in a considerable mixture of oxidation products. An attempt was made to remove some of these other products by chromatography of the ester mixture (200 mg.) over activated acidic alumina (grade IV, 20 g.), eluting with benzene-light petroleum (1: 9) the fractions being examined by gas-liquid chromatography. Those fractions (30 mg.) containing methyl-β-methyl adipate were filtered through a second column of activated acidic alumina (grade III, 20 g.) eluting with light petroleum since they still contained a considerable number of contaminants. However, this proved unsuccessful since the impurities were also eluted in petrol.

An attempt to purify the acid according to the method of Eisenbraun[66] by distillation of the hydrolysed ester

mixture at 180° and 0·5 mm. pressure was unsuccessful and it was discovered that distillation of authentic (+) β - methyl adipic acid under the same conditions did not yield the pure, unchanged acid either.

(ii) The ketonic-hydroxylic mixture of diesters mentioned above (13 mg.) was stirred at room temperature with concentrated nitric acid and vanadium pentoxide for two hours.

(iii) To pure hydroxy-diester (91; 12 mg.) dissolved in a little analar dioxan at ice-bath temperature was added 65% nitric acid (3 ml.) and a catalytic amount of vanadium pentoxide. The mixture was then allowed to warm up to room temperature and stirred for thirty minutes.

(iv) The mixture of ketonic and hydroxylic diesters (18 mg.) was heated under reflux for one hour with 65% nitric acid (5 ml.) in the presence of vanadium pentoxide.

None of the products from the above experiments contained β -methyl adipic acid, as seen when the esterified mixture was compared by gas-liquid chromatography with an authentic sample.

Alkaline Potassium Permanganate oxidation of the C-9 side chain.

(i) To pure hydroxy diester (91; 40 mg.) dissolved

in a little analar dioxan was added, with stirring and at ice-bath temperature, 5% aqueous-alkaline potassium permanganate solution (2 ml.). The mixture was stirred at room temperature for one hour, diluted with water and extracted with ether to remove any neutral material. The aqueous solution was then acidified with dilute sulphuric acid, re-extracted with ether, the ether extracts washed with brine and dried over anhydrous sodium sulphate. The acidic (T.L.C.) product obtained on evaporation of the solvent was esterified with diazomethane and examined by gas-liquid chromatography as before. However, the product was almost entirely unchanged hydroxy diester and contained no methyl-β-methyl adipate.

(ii) The unchanged product from above was heated with more of the alkaline permanganate solution (3·5 ml.) for thirty minutes on a steam bath, but no methyl-β-methyl adipate was obtained.

Baeyer - Villiger Oxidation of the Mixture of Ketones (62 and 68).

The Baeyer - Villiger reaction was carried out on the mixture of mono- and diketone obtained from ozonolysis of methyl eperuate as previously described.

Hydrogen peroxide (2 ml. of 90% plus) and trifluoro-

peracetic anhydride (12 ml.) were introduced, with stirring, into dry methylene chloride (25 ml.) at ice-bath temperature. The suspension was then placed in the deep freeze for several hours (at least four) until the mixture became homogeneous. The solution of peracid was added dropwise, with cooling, to a stirred suspension of the ketone mixture (2·6 g.) and disodium hydrogen phosphate in anhydrous methylene chloride (100 ml.). When the addition was complete, the mixture was placed in the deep freeze and left, with only an occasional shake, for forty-eight hours, and then heated under reflux for a further six hours. It was cooled, the inorganic salts dissolved in water and the aqueous mixture left for several hours to ensure hydrolysis of any excess anhydride which had contaminated previous similar experiments. The reaction was worked up as before, the product being hydrolysed by refluxing for thirty minutes with sodium hydroxide (2·5 g.) in methanol (30 ml.) and a few drops of water.

The acidic fraction from the hydrolysis was re-esterified with an ethereal solution of diazomethane and the resultant ester mixture (1·76 g.) examined by gas-liquid chromatography. Comparison with an authentic sample of (+) methyl-β-methyl adipate indicated the presence of the required ester in a considerable mixture of products. This

mixture was filtered, in light petroleum, through a column of activated acidic alumina (grade IV, 60 g.), the fractions being examined by gas-liquid chromatography. However, it was not possible to obtain the methyl-β-methyl adipate pure and free from contaminants.

Cyclization of Authentic β-Methyl Adipic Acid to 3-Methylcyclopentanone.

(1) An authentic sample of (+) β-methyl adipic acid (14 mg.) was distilled with finely ground crystalline barium hydroxide in a Wood's metal bath according to the method of Thorpe and Kon[67]. The distillate was extracted with ether, the organic extracts washed with brine, dried and the infrared (carbon tetrachloride) solution spectrum of the product compared with that of authentic 3-methylcyclopentanone. The two spectra were identical, having single sharp carbonyl bands at 1740 $cm.^{-1}$.

(2) To β-methyl adipic acid (38 mg.) was added 1 ml. of a solution of pyridine (0·5 ml.) in acetic anhydride (5 ml.) and the mixture refluxed for two hours. It was then diluted with water and extracted with ether. The organic extracts, after washing with bicarbonate and brine, gave an oily product which appeared to be the anhydride (95) from the infrared solution spectrum; $\nu_{max.}^{CCl_4}$ 1820, 1760 $cm.^{-1}$.

This oily material was distilled in a sublimation tube at atmospheric pressure and the product (a mixture of cyclopentanone and acid, $\nu_{max.}^{CCl_4}$ 1740, 1700 cm.$^{-1}$ with a broad hydroxyl region) washed with a saturated solution of sodium carbonate. The solution spectrum of this purified material showed a sharp single band at 1740 cm.$^{-1}$ and was therefore assumed to be 3-methylcyclopentanone.

Cyclization of Impure β-Methyl Adipic Acid to 3-Methyl-cyclopentanone.

(1) A sample of the ester mixture (96 mg.) containing methyl-β-methyl adipate was hydrolysed and the crude acidic material distilled with finely ground crystalline barium hydroxide in a Wood's metal bath. The oily product obtained from the very small amount of distillate had $\nu_{max.}^{CCl_4}$ 1740 (cyclopentanone), 1700 cm.$^{-1}$, the latter being the stronger band. Distillation of this product did not improve its purity.

(2) Another sample of impure methyl-β-methyl adipate (200 mg.) was hydrolysed and the crude acid product refluxed for two hours with 1 ml. of a solution of pyridine (0·5 ml.) in acetic anhydride (5 ml.). On cooling, the reaction mixture was diluted with water, washed with saturated sodium carbonate solution, brine, dilute hydrochloric acid, brine and finally dried over anhydrous sodium

sulphate. The dark brown gummy residue obtained on evaporation of the solvent was sublimed in a cold finger apparatus. The distillate appeared to be mainly acetic anhydride but the infrared spectrum, in carbon tetrachloride solution, did show a small band at 1740 $cm.^{-1}$.

All attempts to obtain the dibenzylidene derivative from these two impure products were unsuccessful.

Oxidation Products of Sclareol.

The main products from the chromic acid oxidation of sclareol (apart from the epimeric esters of labdanolic acid) were separated by thick plate chromatography, using ethyl-acetate-light petroleum (1: 9) as solvent system.

Ether (96).

This was a white crystalline solid, plates from methanol m.p. 107-109°; $[\alpha]_D$ + 18° (c 2·05) [109-111°; + 18°]^{52}. (Found: C 75·28; H 10·74. $C_{21}H_{36}O_3$ requires C 74·95; H 10·78%).

$\nu_{max.}^{CCl_4}$ 1740; 1117, 1098 and 1076 $cm.^{-1}$ (ether ring). Nuclear magnetic resonance spectrum had peaks at τ 6·4 (3H, singlet, methyl ester CH_3); τ 7·7 (2H, singlet, CH_2 alpha to carbomethoxyl); τ 8·7, 8·76 (two methyl groups); τ 9·14, 9·2, 9·24 (a gem-dimethyl and a quaternary CH_3). Mass spectroscopic molecular weight 336 (calculated, 336).

Hydrolysis of Compound (96).

The ester (84 mg.) was refluxed for two hours with potassium hydroxide (200 mg.), methanol (2 ml.) and a drop of water. The crude product (97) recrystallized from light petroleum as colourless needles, m.p. 114-116° (116-8°)[70]. (Found: C 74·74; H 10·49. $C_{20}H_{34}O_3$ requires C 74·49; H 10·63%).

Lithium Aluminium Hydride Reduction of (96).

To the ester (125 mg.) dissolved in anhydrous ether (10 ml.) was added an excess of lithium aluminium hydride and the mixture left overnight at 0°. The excess lithium aluminium hydride was destroyed by careful dropwise addition, with cooling, of a saturated solution of sodium sulphate. The oily product (107 mg.) had no carbonyl absorption and $\nu_{max.}^{CCl_4}$ 3630, 3490 $cm.^{-1}$.
The alcohol (97) was distilled (140° at 0·05 mm.) and analysed. (Found: C 77·52; H 11·52. $C_{20}H_{36}O_2$ requires C 77·86; H 11·76%).
The nuclear magnetic resonance spectrum had peaks at τ 6·4 (2H, triplet); τ 6·8 (1H, diffuse singlet); τ 8·48, 8·74 (6H, two methyl groups); τ 9·15, 9·22, 9·25 (three methyl groups).

Attempted Dehydration of Alcohol (98).

The alcohol (100 mg.) dissolved in anhydrous,

redistilled pyridine (3 ml.) was treated with phosphorus oxychloride (0·5 ml.). The mixture was heated under reflux for three hours and left standing overnight. Evaporation to dryness, dilution with water and extraction with ethyl acetate gave 40 mg. of an oily product which moved as one spot on a chromatoplate and was much less polar than the starting alcohol. There was no unsaturation in the infrared spectrum and no vinyl protons in the nuclear magnetic resonance spectrum. The compound gave a positive Beilstein reaction. (Found: C 73·75; H 10·55. $C_{20}H_{35}Cl$ O requires C 73·5; H 10·72%).

Compound (100).

This was shown to be the nor-ester of compound (96). It distilled at 120° (0·05 mm.). (Found: C 73·69; H 10·72. $C_{20}H_{34}O_3$ requires C 74·49; H 10·63%). Mass spectroscopic molecular weight 322 (calculated, 322). $\nu_{max.}^{CCl_4}$ 1740 $cm.^{-1}$; 1120, 1098 and 1076 $cm.^{-1}$. Nuclear magnetic resonance spectrum had peaks at τ 6·3 (3H, singlet, methyl ester); τ 8·74 (singlet, methyl group); τ 8·8 (3H, singlet, methyl group); τ 9·13 and 9·23 (9H, two singlets).

Compound (101).

Crystallized from petroleum ether (40-60°) as colourless needles m.p. 69-72°C. (Found: C 70·58;

H 10·00. $C_{19}H_{32}O_4$ requires C 70·33; H 9·94%).
$\nu_{max.}^{CCl_4}$ 1735, 1730, 1240 $cm.^{-1}$.
Nuclear magnetic resonance spectrum had peaks at τ 6·4 (3H, singlet, methyl ester); τ 7·78 (3H, singlet, acetate CH_3); τ 8·2 (3H, singlet, C-8 methyl); τ 9·12, 9·18, 9·22 (three singlets, methyl groups).

Hydrolysis of compound (101).

The acetoxy-ester (34 mg.) was refluxed for two hours with potassium hydroxide (200 mg.) in aqueous methanol (2ml.). The acidic product was esterfied with diazomethane and chromatographed over activated neutral alumina (grade III, 12 g.). Crystalline material (14 mg.) was eluted with benzene-light petroleum (1: 1·5) and rechromatography of the residue produced a further 6 mg. of this compound, m.p. (from light petroleum) 122-124°; $[\alpha]_D$ + 42° (c 0·38). [123-4°; + 46°]71. $\nu_{max.}^{CCl_4}$ 1780 $cm.^{-1}$.

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