S T U D I E S I N T E R P E N O I D

B I O S Y N T H E S I S

A thesis submitted by

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A B S T R A C T

The various types of sesquiterpenoids are discussed in terms of their structural and biogenetic relationships. Possible biosynthetic pathways are proposed for all types, and some existing proposals are modified or extended. Emphasis is placed on the small number of different initial cyclisations of farnesyl pyrophosphate required to explain the biogenesis of the various skeletal types, and the key role of intermediates of the bisabolene and germacrane types is stressed.

The published experimental studies on sesquiterpenoid biogenesis are reviewed and discussed.

Experimental work on the biosynthesis in Artemisia maritima of the sesquiterpenoid bitter principle, santonin, is described. On the basis of the incorporations obtained, it is necessary to postulate two pathways for the later oxidative steps in its biosynthesis. These differ at the stage at which the first hydroxylation occurs. The incorporation of the ten-membered ring compound, 11,12-dihydrocostunolide suggests that this lactone is cyclised to a decalin system in the biosynthetic pathway.

The incorporation of mevalonic acid lactone into santonin is shown to have occurred with scrambling of

the label. Farnesyl pyrophosphate was not incorporated, while a very low incorporation of farnesol is recorded.

The occurrence of 1,2-dihydrosantonin in Artemisia stellariana is proved.

The isolation of the triterpenoid bitter principle, limonin, from the roots of Dictamnus albus is described. A low incorporation of mevalonic acid lactone into limonin is recorded.

CONTENTS

ACKNOWLEDGEMENTS 7

SECTION 1 - REVIEW OF SESQUITERPENOID STRUCTURAL TYPES AND THEIR PROBABLE BIOGENESIS

INTRODUCTION 9

OCCURRENCE AND FUNCTION OF SESQUITERPENOIDS ... 10

IMPORTANT BIOSYNTHETIC MECHANISMS
- Eliminations 11
- Cyclisations 11
- Reactions of carboxyl groups 13
- Oxidation and reduction 13
- Hydroxylation and dehydration 14

FARNESYL PYROPHOSPHATE 16

ACYCLIC SESQUITERPENOIDS 17

TEN-MEMBERED RING SESQUITERPENOIDS - GERMACRANES
- Introduction 20
- Lactonic germacranes - Germacranolides ... 25
- Linderane group 30
- Miscellaneous 31

EUDESMANE AND RELATED SESQUITERPENOIDS
- Introduction 32
- Eudesmanes without lactone, furan or 6,7,11-cyclopropane rings 37
- Lactonic eudesmanes - Eudesmanolides ... 45
- Furanoeudesmanes 49
- Agarofuran group 51
- Eudesmanes with 6,7,11-cyclopropane rings ... 53
- Eremophilane group 54
- Valeranone type 59
- Agarospirol type 60

HYDROAZULENIC SESQUITERPENOIDS
- Introduction 63
- Guaianes without lactone, 6,7,11-cyclopropane or oxide rings 66
- Lactonic guaianes - Guaianolides 68
- Pseudoguaianolides 70
- Kessane group 74
- Valerene type 75
- Guaianes with 6,7,11-cyclopropane rings ... 76
- Tricyclic guaianes 77

SESQUITERPENOIDS REGARDED AS DERIVED FROM BISABOLENE TYPE INTERMEDIATES
Introduction 80
Bisabolene type 80
Cadinene type 82
Helminthosporal group 86
Acorone and cedrene groups 88
Trichothecin group 88

ELEVEN-MEMBERED RING SESQUITERPENOIDS AND OTHERS REGARDED AS DERIVED THEREFROM
Humulene group 91
Illudins and marasmic acid 92

SESQUITERPENOIDS FOR WHICH SEVERAL POSSIBLE BIOSYNTHETIC ROUTES ARE AVAILABLE
Copaene, longifolene, santalene and bergamotene groups 94
Himachalene group and allohimachalol 100
Widdrol type including thujopsene and other cyclopropanes 101
Chamigrene, cuprene, laurene and aplysin types 102

BICYCLOFARNESOL GROUP 104

MISCELLANEOUS SMALL GROUPS
Maturin and cacalol group 106
Elemene group 107
Carotane type 108
Tutin group 109

CONCLUSIONS 110

SECTION 2 - REVIEW OF THE PUBLISHED EXPERIMENTAL INVESTIGATIONS INTO SESQUITERPENOID BIOSYNTHESIS

FARNESYL PYROPHOSPHATE 112
IPOMEAMARONE 112
HELMINTHOSPORAL 115
CARYOPHYLLENE 117
ILLUDINS AND MARASMIC ACID 117
LONGIFOLENE 119
CAROTOL 120
GOSSYPOL 120

SECTION 3 - BRIEF REVIEW OF THE ISOLATION AND DETERMINATION OF STRUCTURE OF SANTONIN

EXTRACTION AND ISOLATION OF SANTONIN 123
GROSS STRUCTURE 124
STEREOCHEMISTRY 126
TOTAL SYNTHESIS 127
CONCLUSION 129

SECTION 4 - DISCUSSION OF EXPERIMENTAL WORK CARRIED OUT ON THE BIOSYNTHESIS OF SANTONIN

PRELIMINARY WORK 131

FIRST SEASON
- Introduction 132
- Preparation of the lactonic possible precursors 134
- Preparation of the acidic possible precursors 139
- Radioactivity determinations 140
- Synthesis of the labelled compounds for feeding experiments 143
- Feeding Experiments 147

SECOND SEASON
- 11,12-Dihydrocostunolide 153
- Further eudesmanolide possible precursors ... 156
- Labelling of precursors for second season's feedings 161
- Feeding experiments 162
- Degradation of the santonin from the mevalonate feeding 167

THIRD SEASON
- Preliminary considerations 171
- Farnesyl pyrophosphate 172
- Attempted syntheses of hydroxyketo lactone (401) 172
- Douglanine 175
- Germacrene 176
- Tetrahydrosantonins 177
- Labelling of precursors for third season's feedings 179
- Feeding experiments 179

EXAMINATION OF OTHER SPECIES OF ARTEMISIA 182

LIMONIN 183

SECTION 5 - EXPERIMENTAL DETAILS

GENERAL REMARKS 185
PRELIMINARY EXPERIMENTS 188
FIRST SEASON 189
SECOND SEASON 204
THIRD SEASON 222
EXTRACTIONS OF OTHER ARTEMISIA SPECIES 235
LIMONIN WORK 235
TABLES OF FEEDING EXPERIMENTS AND N.M.R. DATA ... 237

SECTION 6 - REFERENCES

REFERENCES 247

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John A. Whittle,

September, 1967.

SECTION 1

REVIEW OF SESQUITERPENOID STRUCTURAL TYPES AND THEIR PROBABLE BIOGENESIS

INTRODUCTION

This section begins with a brief review of the occurrence and function of sesquiterpenoids, and discussions of certain important biosynthetic mechanisms. There follows a group by group description of the various skeletal types, classified by structural and biosynthetic considerations. The ten-membered ring and eudesmane sesquiterpenoids are considered in greater detail than other types, because of their relevance to the experimental work discussed in Section 4 of this thesis.

Throughout the thesis, the term sesquiterpene is applied only to compounds of molecular formula $C_{15}H_{24}$. All related natural products are described as sesquiterpenoids. The numbering system used for each type is shown diagrammatically near the beginning of its section.

Sesquiterpenoids have been reviewed[1,2] as have some aspects of their biogenesis.[3,4,5]

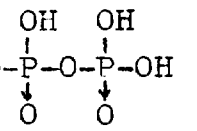
OCCURRENCE AND FUNCTION OF SESQUITERPENOIDS

Sesquiterpenoids have been found in almost all forms of living matter, but occur principally in higher plants. They exhibit a rich variety of functional groups and ring systems. Apart from the lactones[6] for which the family Compositae is noted, furans, epoxides and cyclopropane rings occur frequently in the four hundred or so known sesquiterpenoids. Less common are larger ring oxides, aldehydes, and even acetylenes.

Most sesquiterpenoids (farnesyl pyrophosphate is an important exception) are secondary metabolites, and many are produced only during certain phases of the life cycle of the organism. It is not known what function sesquiterpenoids, or indeed, any secondary metabolites fulfil, but it has been suggested[7] that they are formed in order to dispose of accumulations of certain intermediates. It seems probable that, when conditions are perfectly balanced, only replication activities take place. When, however, conditions are unbalanced, as must often occur in nature, some intermediates may accumulate. If these intermediates are not to interfere with the synthesis of essential (primary) metabolites, then they must be removed or deactivated.

IMPORTANT BIOSYNTHETIC MECHANISMS

Eliminations.

Pyrophosphate () is a good leaving group, and facilitates many biological elimination reactions. The substrate hydroxyl groups are enzymatically phosphorylated with ATP. In some instances, monophosphate is eliminated.

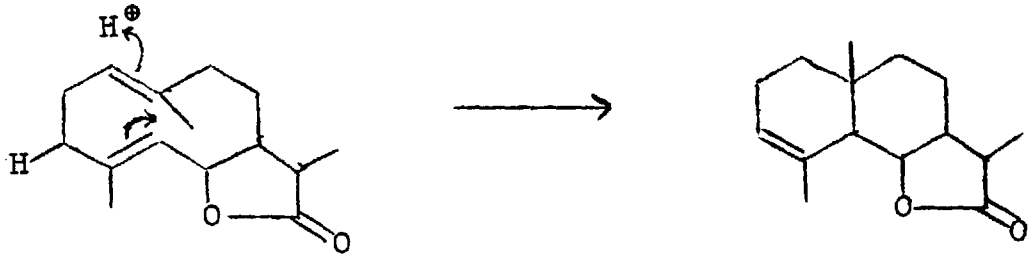
Cyclisations.

A cyclisation is basically an intramolecular elimination reaction. Almost all cyclic sesquiterpenoid skeletons can reasonably be regarded as derived from one of a limited number of cyclised farnesol derivatives. In most of these cases, the initial cyclisation results in the elimination of pyrophosphate, and the reaction may be considered as the biological equivalent of an intramolecular condensation.

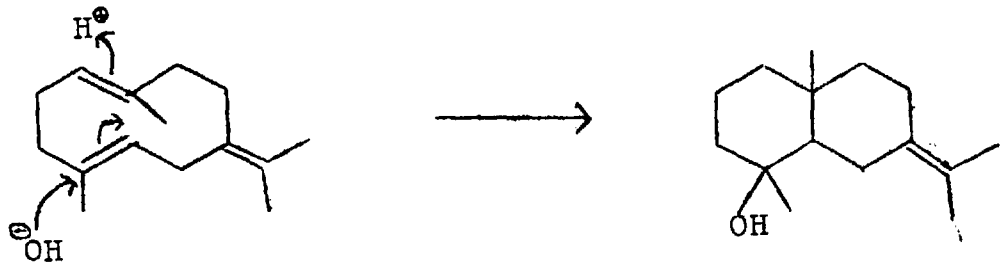
In many cases, further rings are formed by isomerisations - i.e. the electrons for the new bond are derived from a double bond. Such cyclisations, in a concerted form, are important in the pathway from squalene to the triterpenoids and steroids.

Chemically, such cyclisations can be induced in

a non-hydroxylic acidic medium to yield the unsaturated compound, as in the cyclisation of 11,12-dihydro-costunolide : (see page 165)



In a hydroxylic medium, the product is the corresponding hydroxy compound, as in the cyclisation of germacrene:[8]



It will be convenient in the rest of this section to discuss these cyclisations in terms of the form:

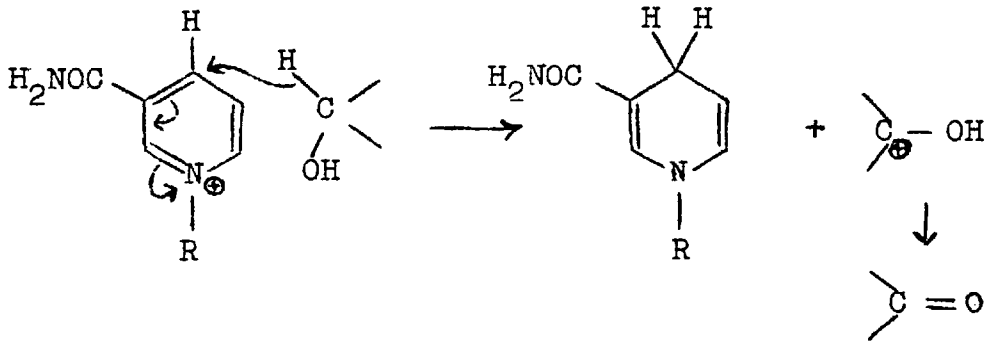
This is not intended to imply the existence of the carbonium ion as a discrete entity, but as a convenient indication that it is possible to have a double bond or a hydroxyl group at that centre. It may be, of course, that the positive centre is a point of attachment to the enzyme.

Reactions of Carboxyl Groups.

Many reactions of carboxylic acids take place through the reactive thiol ester formed by the carboxyl group and the thiol group of co-enzyme A (usually represented as CoA - SH). "Condensation" of an activated methylene group with the carbonyl of a thiol ester is a common biosynthetic process.

Oxidation and Reduction.

Biological oxidation (and reduction) is often achieved by means of nicotinamide adenine dinucleotide (NAD), or its phosphate (NADP). The reaction involves the transfer of a hydride ion from the substrate to the nicotinamide, forming the reduced co-enzyme NADH (or NADPH). The reverse of this process results in reduction of the substrate.



Other co-enzymes, notably flavin nucleotides are also involved in biological oxidation/reduction processes.

Hydroxylation and Dehydration.

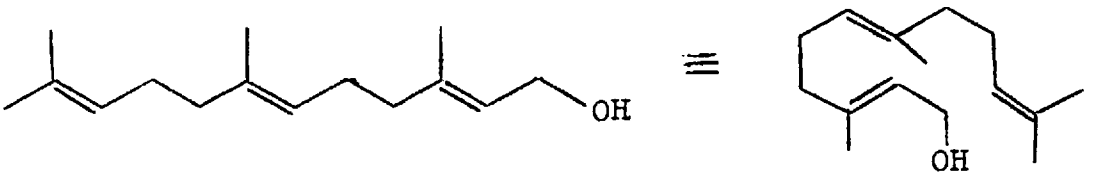
Direct hydroxylation of saturated (or aromatic) carbon atoms is encountered frequently in biological systems, and is used commercially in the micro-biological hydroxylation of some steroids. Little is known about the mechanism of hydroxylation, except that it appears that the oxygen atom is derived from atmospheric oxygen. It has been suggested[9] that the active hydroxylating agent is a hydroperoxy flavin.

A direct hydroxylation at an unsaturated carbon atom should in principle give an enol, and thence the corresponding ketone, although there is no evidence for such a process. It seems, however, difficult to suggest a reasonable mechanism for biological epoxidation without invoking a similar process.

The dehydration of a hydroxy compound (presumably *via* a mono- or pyrophosphate) to produce a double bond is a well established biosynthetic step. The reverse of this process, the hydration of a double bond, is rather less firmly established. It has been proposed[10] as a step in the breakdown of unsaturated fatty acids, but more work is required before it can be regarded as established.

FARNESYL PYROPHOSPHATE

Farnesol (1) was first recognised by Ruzicka[3] as the biogenetic precursor of all sesquiterpenoids. The natural alcohol, the gross structure of which was determined by Kerschbaum[11] as early as 1913, was shown by Weedon and his co-workers[12] to be the all trans isomer. While it is often assumed that farnesol (as its pyrophosphate) is cyclised directly to produce all the various cyclic skeletal types, it must be noted



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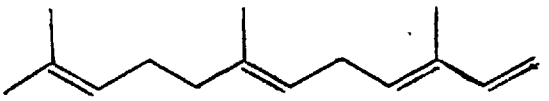

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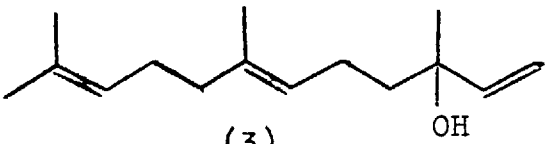


(3)

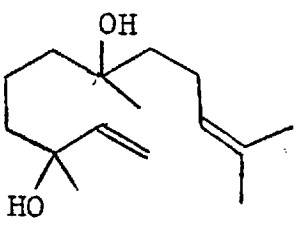
that farnesene (2), nerolidol (3), or indeed farnesol (as the alcohol) could possibly be involved in some cases. The detailed biosynthetic pathway to farnesyl pyrophosphate is described in Section 2. In some cases, e.g. bisabolene type, it appears that the initial cyclisation involves a cis-trans-farnesyl pyrophosphate, or alternatively nerolidol.

ACYCLIC SESQUITERPENOIDS

The expression cyclic is used throughout this section to describe an alicyclic ring containing more than three carbon atoms. Rings containing hetero-atoms - lactones, oxides, furans, etc. - are excluded from this term, as are cyclopropane rings, all of which are more conveniently treated as functional groups.

The most important acyclic sesquiterpenoids are farnesol (1) and nerolidol (3), both of which occur widely.[13] Examples of other types shown in Chart I are caparrapidiol (4)[14] and caparrapitriol (5)[14] isolated from *Ocotea caparrapí*; dendrolasin (6)[15] from the ant *Lasius* (*Dendrolasius*) *fuliginosus*, and also from the wood oil of *Torreya nucifera*,[16] the source of torreyal (7) and neotorreyol (8); ngione (9)[17] from *Eremophila freelingii* and *E. latrobei*; ipomeamarone,[18] a stereoisomer of ngione, isolated from black rotted sweet potato (*Ceratocystis fimbriata* on *Myoporum acuminatum*); freelingyne (10)[19] from *Eremophila freelingii*; and α-sinesal (11)[20] and β-sinesal (12)[21] from *Citrus sinensis*.

The sinesals are noteworthy in that the n.m.r. spectra suggest that the central double bond is



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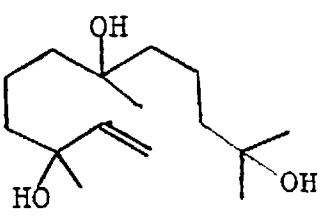

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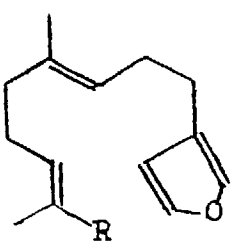
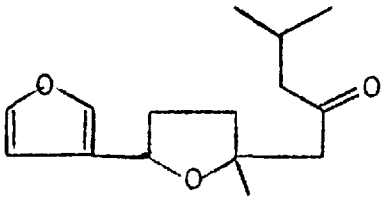


(6) R = H
(7) R = CHO
(8) R = CH_2OH



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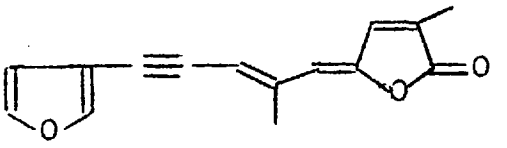

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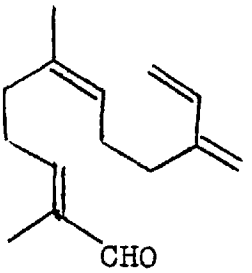

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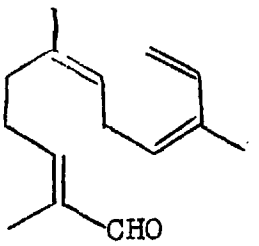


(12)

CHART I

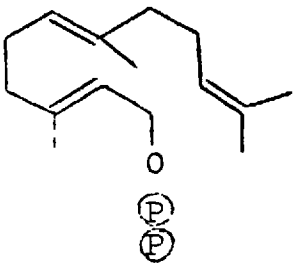
cis,[21] in contrast to farnesol. Most of the modifications to farnesol required to produce these compounds are standard or trivial, although the acetylene requires comment. Freelingyne (10) was in fact the first acetylenic terpenoid to be

recognised, although acetylenic polyenes have been known for some time. The acetylene group is presumably formed by the elimination of pyrophosphate from an enol pyrophosphate, although there is some experimental support for the dehydrogenation of an olefin in acetylenic fatty acid biosynthesis.[22]

TEN-MEMBERED RING SESQUITERPENOIDS - GERMACRANES

Introduction.

Although no ten-membered ring sesquiterpenoids had been recognised as such at the time, Ruzicka[3] in 1953 suggested that the ion (16) was a possible intermediate in the biosynthetic pathways to the eudesmane (19) and guaiane (20) skeletons. The idea received theoretical



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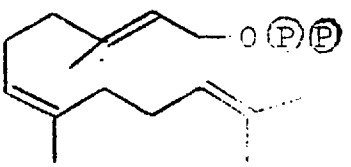

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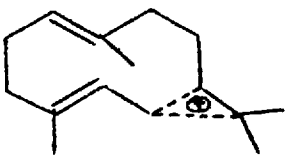

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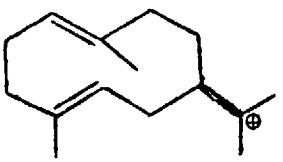

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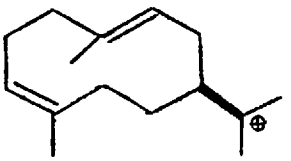

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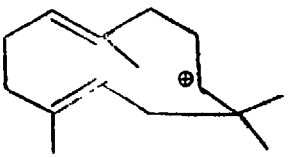

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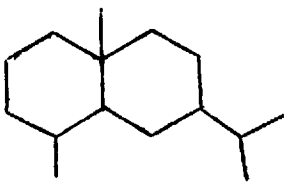

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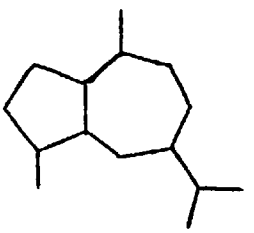
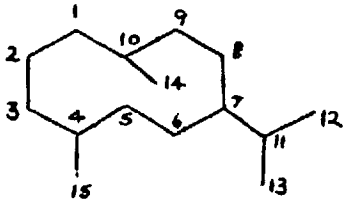


(20)

support from Barton and de Mayo[23] and Šorm,[24] and the first experimental support is reported in the incorporation of 11,12-dihydrocostunolide into santonin in this thesis. (Section 4).

Farnesyl pyrophosphate (or farnesol, etc.) may cyclise directly to the ion (16), or alternatively through the non-classical carbonium ion (15), which can also be considered as the precursor of the eleven-membered ring ion (18) leading to the humulene group. (See page 91).

Ion (17) is also a possibility to be considered, and differs from ion (16) only in the stereochemistry of one double bond, and potentially at C-7. (Numbering is indicated in (21)). The isomerisation



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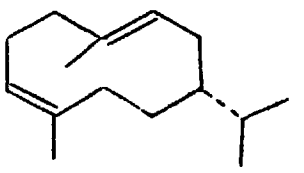

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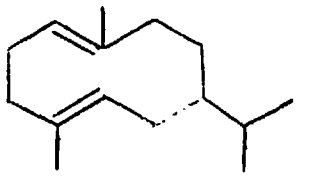
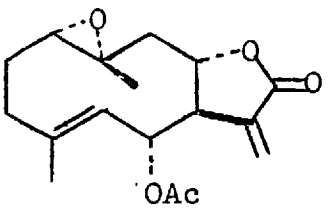


(23)

of one of the double bonds in ion (16) from trans to cis, and its rewriting in the form (22) results in the configuration at C-7 apparently inverting. On the other hand, cyclisation of the trans-cis-farnesyl

pyrophosphate (14) results in the normal (β) configuration at C-7, if one accepts that cyclisation in biological systems of this type always proceeds in the same manner. Hendrickson[5] has suggested that trans-trans-farnesyl pyrophosphate is involved in almost all the cyclisations producing nine-, ten-, and eleven-membered ring sesquiterpenoids. It is convenient, following general practice[25] to write all germacranes in the form (16), even when the geometry of the double bonds and/or the stereochemistry of the side chain at C-7 are unknown. No examples of structures derived from either (17) or (22) have been convincingly demonstrated.

The first ten-membered ring sesquiterpenoid to be recognised as such (Barton et al.,[26] 1957) was pyrethrosin (24), the active insecticide principle



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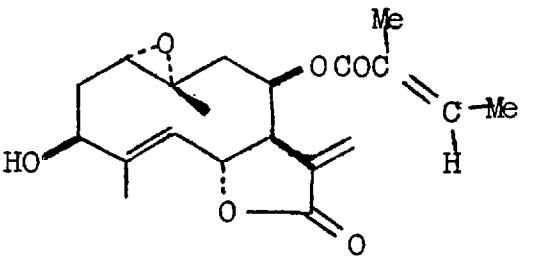
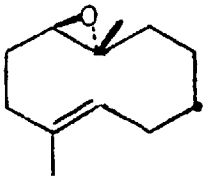


(25)

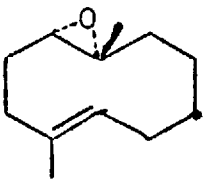
from Chrysanthemum cinerariaefolium. The remaining stereochemical questions were resolved by a later X-ray

diffraction study.[27]

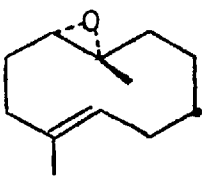
It is important at this point to note the difficulties encountered in representing the stereochemical details of ten-membered ring compounds. Many authors use the form (23), despite the somewhat unusual appearance of the trans-1(10) double bond. Examination of the available X-ray structural details for pyrethrosin (24)[27] and heliangine (25),[28] and of molecular models indicates that the 10-methyl group projects into the centre of the ten-membered ring, and is in close proximity to (above in the usual representation) the vinyl hydrogen on C-5. For this reason,



(26)



(27)

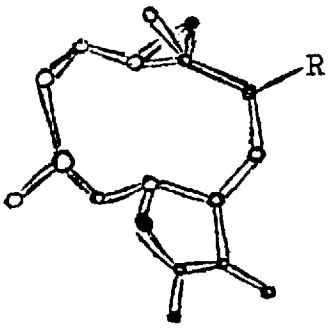


(28)

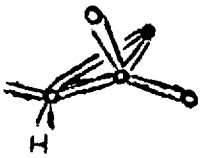
the structures of the germacranes are written in the sterically more accurate form (16), now finding increasing favour in the literature, throughout this thesis.

The difficulties caused by the (potential) flexibility of the ten-membered rings are highlighted

by consideration of the stereochemistry of the epoxide in heliangine and pyrethrosin. The epoxide in heliangine is represented as in partial formula (26) by the X-ray workers.[28] The stereoformula is shown in (29), with the epoxide portion in detail in (29b).



(29a)



(29b)

Examination of models and the stereoformula suggests that partial formula (27) is an equally valid representation, while (28) is probably the best in two dimensions. The hydrogen atom on the epoxide bearing carbon (C-1) projects down and into the ring, and the C-1 C—O bond projects down and out from the ring. Although it is possible to consider the

epoxides as trans-epoxides, as brought out by form (26), it is probably unjustifiable to apply normal cyclohexane conformational terms to ten-membered rings without further qualification.

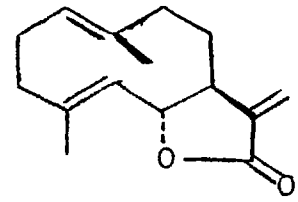
Thus, the stereochemistries shown for the epoxide of pyrethrosin by Rogers[27] (partial formula (27)) and Iriuchijima and Tamura[29] (partial formula (26)), and for heliangine by Nishikawa et al.[28] (partial formula (26)) are identical. The R,S convention[30] allows unambiguous description of the epoxide asymmetric centres - both C-1 and C-10 are R.

The structures of many other ten-membered ring sesquiterpenoids have been elucidated, notably by the groups headed by Šorm in Prague, and Bhattacharyya in Poona. Šorm and Dolejš[31] have reviewed the germacranolides.

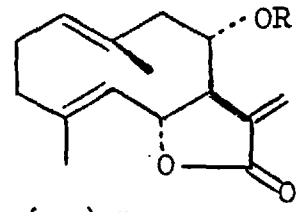
Lactonic germacranes - Germacranolides.

Chart II lists most of the important 6,13-germacranolides, the structures of which have been determined. Costunolide (30),[32,33,34] the principal sesquiterpenoid of costus oil (Saussurea lappa), and also found in Artemisia balchanorum, has been thoroughly investigated. Presumably arising

CHART II

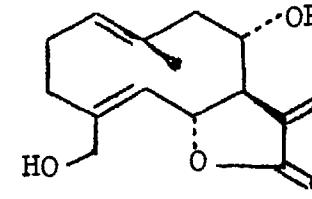


(30)

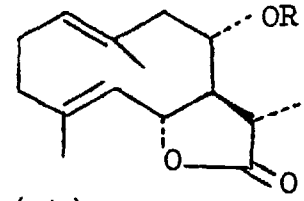


(31) R = H

(32) R = CO.C(=CHCH$_2$OH)CH$_2$OH

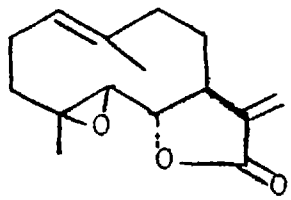


(33) R = COCHMe, CH$_2$OH

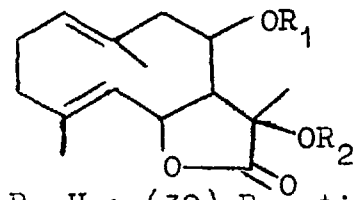


(34) R = H

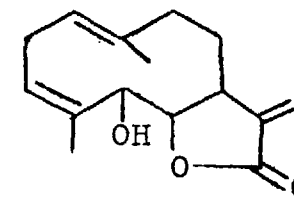
(35) R = Ac



(36)



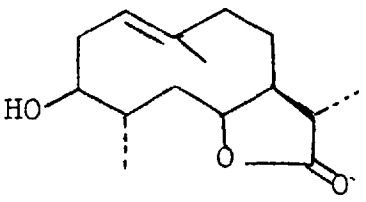
(38) R_1=R_2=H ; (39) R_1 = tiglate, R_2 = Ac.



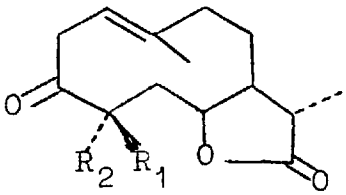
(37)

biosynthetically from costunolide as indicated on Chart II are, from Artemisia balchanorum, hydroxycostunolide (31);[35] balchanolide (34)[36] and hydroxybalchanolide (38);[36] parthenolide (36)[37] from Chrysanthemum parthenium; chamissonin (37)[38] from Ambrosia chamissonis; acetylbalchanolide (35)[39] from Achillea millefolium; arctiopicrin (33)[40] from Arctium minus; eupatoriopicrin (32)[41] from Eupatorium cannabinum; and laserolide (39)[42] from Laser latifolium. Heliangine (25) has already been discussed.

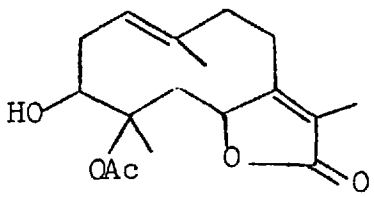
Four germacranolides in which the 4(5)-double bond has been hydrogenated are hydroxypelanolide (40);[43]



(40)



(41) R_1 = H; R_2 = Me
(42) R_1 = Me; R_2 = H

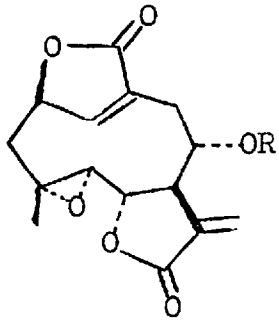


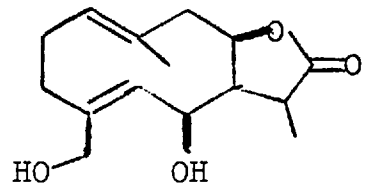
(43)

and ketopelanolide A (41)[43] and B (42)[43] from Artemisia absinthin, and, from Geigeria africana, gafrinin, for which structure (43) has been proposed.[44]

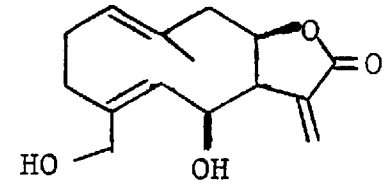
Germacranolides with lactones closed to C-8 include the already mentioned pyrethrosin (24);

elephantin (44)[25,45] and elephantopin (45),[45] two esters occurring in Elephantopus elatus; salonitolide (46)[46,47] and salonitenolide (47)[47] from Centaurea salonitana; and scabiolide (48)[40] and cnicin (49)[48], also from Centaurea species. The absolute configuration of elephantol (50), the hydroxy-8,13-lactone obtained by hydrolysis of elephantin and elephantopin,





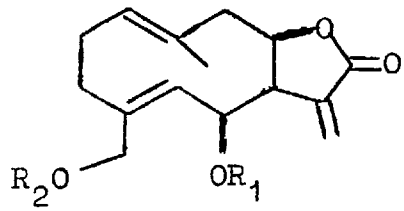
(46)



(47)

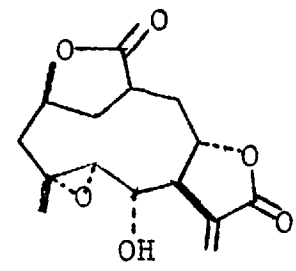
(44) R = COCH=CMe$_2$

(45) R = COC(Me)=CH$_2$



(48) R_1 = $COCH_2OH$; R_2 = Ac

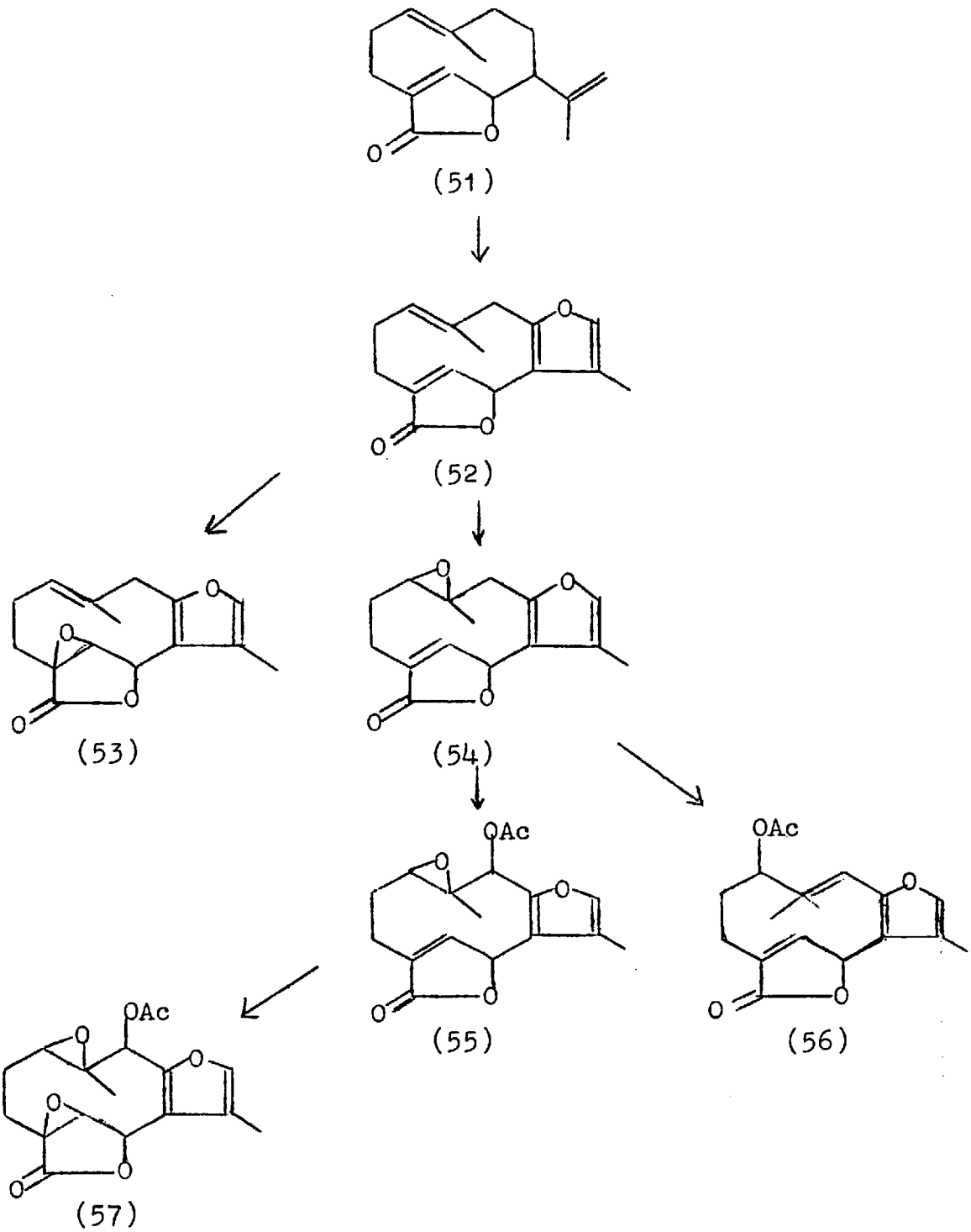
(49) R_1 = $COC(=CHCH_2OH)CH_2OH$; R_2 = H



(50)

has been determined by an X-ray diffraction study.[45]

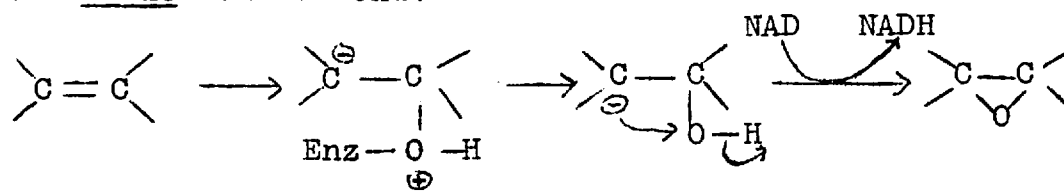
CHART III



Linderane group.

Chart III depicts the unusual furano-6,15-lactones. Linderolactone (52)[49] and linderane (53)[50] were first isolated from Lindera strychnifolia, but have since also been found[51] in Neolitsea zeylanica along with neolinderane (54),[52] zeylanicine (55),[52] zeylanine (56)[52] and zeylanidine (57).[52] The related aristolactone (51)[53] has been isolated from Aristolachia species. Unfortunately, no stereochemical studies have yet been reported for this group.

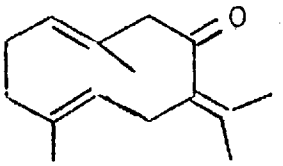
The co-existence in the same plant of various epoxides and the unsaturated compounds from which, biogenetically, they must be presumed to arise is interesting, and probably provides the best evidence at present available to support the general hypothesis. Mechanistically, it seems reasonable to suppose that the epoxide arises from an enzymatic process similar to that involved in hydroxylation at a saturated carbon. The stereochemistry of the epoxides in pyrethrosin, heliangine and elephantol is consistent with a "peracid" ($^{\oplus}OH$) attack on the underside of the trans-double bond.



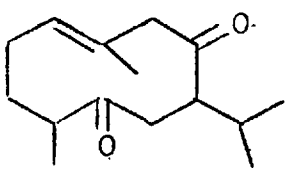
It is pertinent to mention here that epoxides may be involved in the cyclisations of ten-membered rings. The product of a cyclisation of a double bond and an epoxide is, of course, an alcohol. It would then be necessary to postulate that the double bonds which seem frequently to be the products of biological cyclisations, are formed by dehydration. There is experimental evidence supporting the involvement of an epoxide of squalene in the cyclisation to lanosterol[54] and β-amyrin,[55] both *in vivo* and *in vitro*.

Miscellaneous Germacranes.

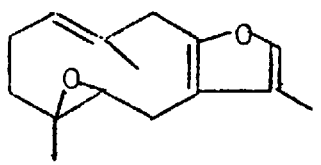
There remain to be considered three non-lactonic germacranes: germacrone (58)[56] from *Geranium macrorhizum*, and curdione (59)[57] and zederone (60),[58] both isolated



(58)



(59)



(60)

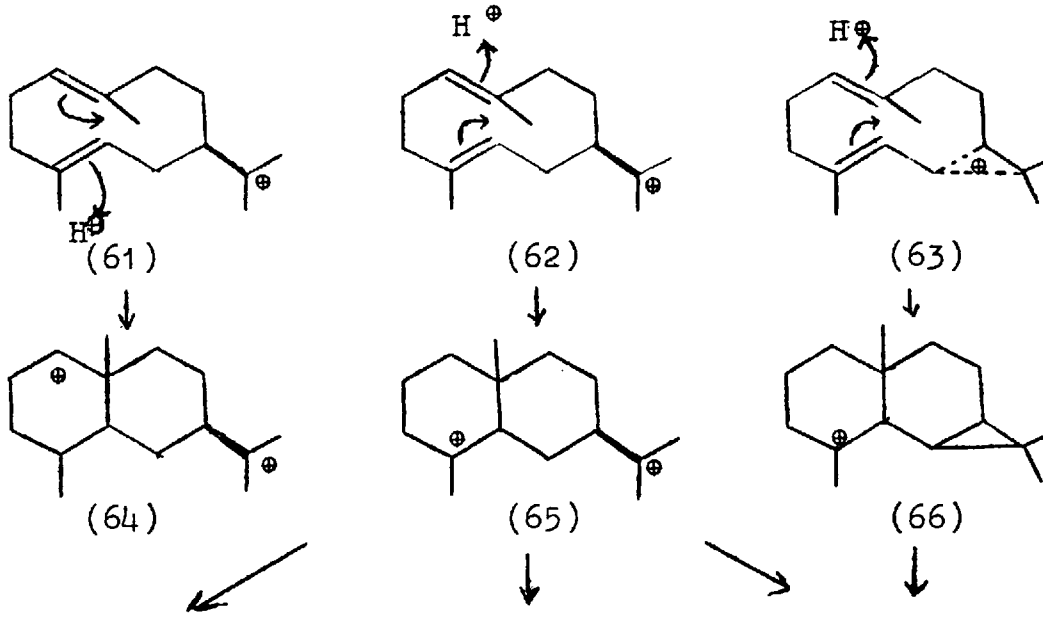
from *Curcuma zedoaria*. These compounds do not exhibit any new biosynthetically important features.

EUDESMANE AND RELATED SESQUITERPENOIDS

Introduction.

Chart IV summarises the processes considered to be probable biosynthetic pathways to the eudesmanes (derived from (65)), eremophilanes (derived from (71)), "valeranone" type (72), and "agarospirol" type (73). In writing di-cations such as (65), it is not intended to imply that such species occur in natural systems - see page 13.

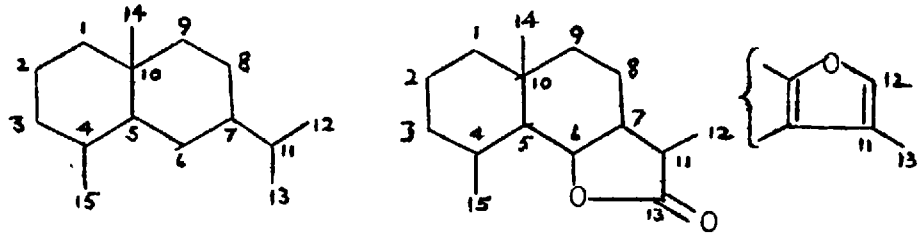
An examination of the known germacrane sesquiterpenoids (above) suggests that the lactone and furan rings may be formed before the cyclisation of the ten-membered ring. The occurrence of the linderene group (see furanoeudesmanes below) along with the linderolactone group of germacranolides in the same species supports this hypothesis. It is less easy to suggest whether the cyclopropane ring is formed before cyclisation, or *via* the non-classical carbonium ion (60), or by another mechanism. These three functions, if indeed the cyclopropane ring is formed before cyclisation, are probably the only ones commonly introduced before cyclisation.

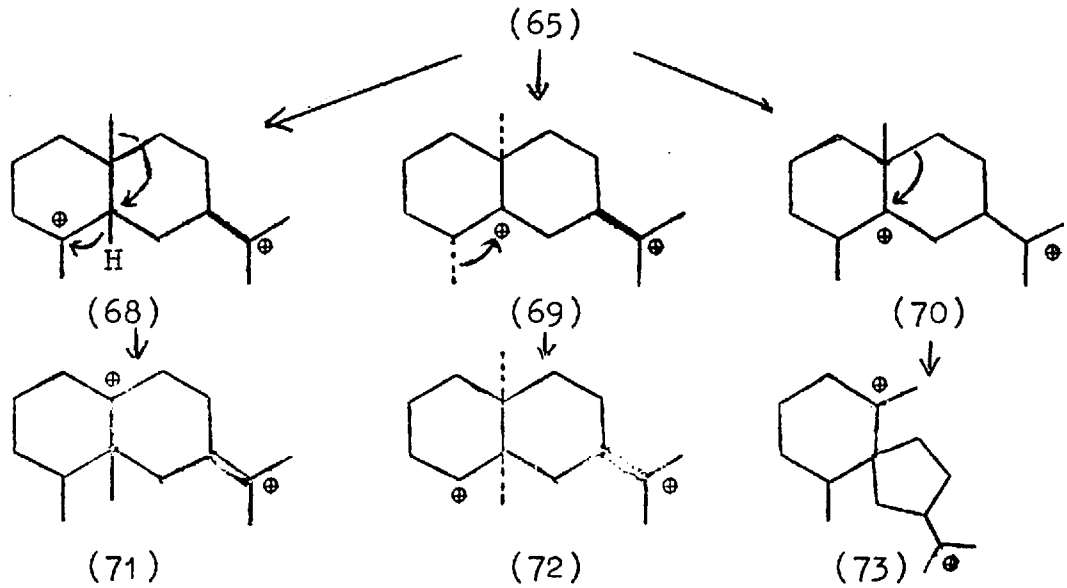


Non-lactonic etc. eudesmanes.

Eudesmane lactones, furans.

Cyclopropanyl eudesmanes.





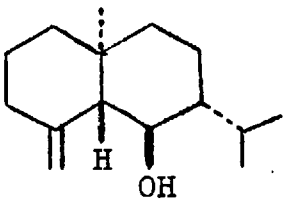
Eremophilane type. Valeranone type. Agarospirol type.

Cyclisation of ion (62) to ion (65) as shown results in the basic eudesmane structure. It may be argued that cyclisation of ion (61) to ion (64) is equally possible. However, virtually all eudesmane sesquiterpenoids known have either oxygen functions or double bonds involving C-4, and very few similar functions involving C-1. This points to the involvement of the ion (65) rather than ion (64). Additionally, ion (64) is a secondary carbonium ion, while (65) is a more stable tertiary ion. Even allowing that, *in vivo*, the ions are probably not formed as such, the stability considerations may well still apply to incipient positive centres in the transformations.

Models indicate two important conformations for structures similar to (62). In one of these, viewing the molecule as shown, with an equitorial (β) side chain at C-7, the 10-methyl group is above the vinyl hydrogen on C-5 (as found in the previously mentioned X-ray studies of pyrethrosin and heliangine). Cyclisation then leads to the *trans*-fused eudesmane structure with the 10β-methyl/5α-hydrogen configuration. The other important conformation, with the 10-methyl group below the C-5 vinyl hydrogen would lead to a

trans-fused system with a 10α-methyl. This raises an interesting point concerning the stereochemistry at C-7. Where there is a 10α-methyl configuration, and several such examples have been adequately proved, it is possible to envisage either configuration at C-7. The "C-10 methyl group below the C-5 vinyl hydrogen" conformation will presumably still lead to a β-side chain at C-7. On the other hand, an α-side chain will result from the mirror image of the "C-10 methyl above the C-5 vinyl hydrogen" conformation.

Unfortunately, the eudesmanes for which the 10α-methyl configuration has been proposed do not include any in which the stereochemistry at C-7 can be regarded as beyond doubt. For example, it has been proposed[59] that laevojunenol is (74), the antipode of



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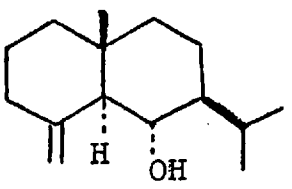
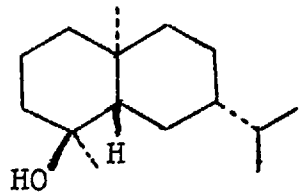


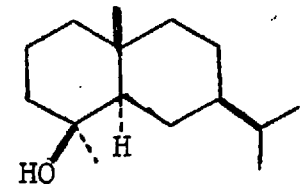
(75)

junenol (75), but further investigation is desirable. A similar proposal[60] that intermediol (structure (76)

proposed) and neointermediol (77) from respectively Indian, and Malayan and East African plants of



(76)



(77)

species Bothriochloa intermedia, are antipodal (except at C-4) appears doubtful, for structure (76) has been synthesised[62] and found not to be identical with the natural compound.

Several of the eremophilane group do have a 5α-methyl configuration, and a 7β-side chain, although the methyl group has been the subject of a 1,2-shift in these compounds. It may well be that the 7-side chain is β-orientated in almost all germacrane, eudesmane, hydroazulene and related sesquiterpenoids. α-Ferulene (see page 59) is possibly an important exception.

Eudesmanes without lactone, furan or 6,7,11-cyclopropane groups.

Many of these "simple" eudesmanes are derived directly from ion (65) by varying combinations of two processes:-

1. Loss of a proton to give a double bond. The protons on carbons 3,5,15, 7,12 and 13 are all available, allowing three possible double bonds at C-4, and two at C-11. (C-12 and C-13 are equivalent.)
2. Addition of a hydroxyl at C-4 (two isomers possible) and/or C-11.

There are fifteen possible combinations, with 10β-methyl and, where applicable, 5α-hydrogen configuration, and those known (nine in all) are given in Table 1.

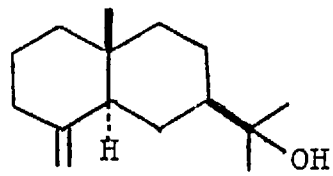
TABLE 1

At C-4	At C-11	Name	Genus	Ref.
3(4) d.b.	7(11) d.b.	selina-3,7(11)-diene	Humulus	63
3(4) d.b.	11(12) d.b.	α-selinene	Acorus	64
3(4) d.b.	11-OH	α-eudesmol	Eucalyptus	65
4(5) d.b.	11-OH	γ-eudesmol	Calitropsis	66
4(15) d.b.	7(11) d.b.	γ-selinene	Humulus	63
4(15) d.b.	11(12)d.b.	β-selinene	Apium	67,68
4(15) d.b.	11-OH	β-eudesmol	Eucalyptus	68,69
4α-OH	11(12)d.b.	selin-11-en-4α-ol	Podocarpus	62
4α-OH	11-OH	cryptomeridiol	Cryptomeria	70

d.b. = double bond. Sources given are principal sources.

It will be seen that none of the compounds with a 4β-hydroxyl group are known, and only one with a 4(5)-double bond. Frequently, eudesmanes with 3(4)- and 4(15)-double bonds occur together in almost inseparable mixtures.

β-Eudesmol (78) occurs in Zingiber officinale along with a closely related alcohol. The separation of these compounds is so difficult that the mixture was originally thought to be a single substance called zingiberol. It has been proposed[71] that the



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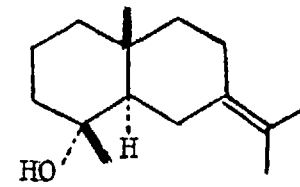
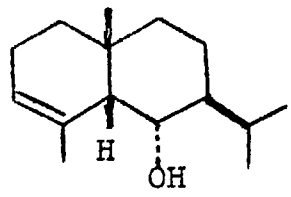


(79)

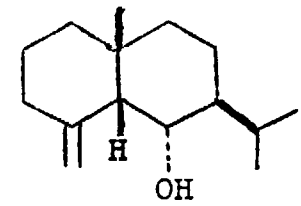
other alcohol is cis-fused (10β-methyl/5β-hydrogen) β-eudesmol, but there seems good reason to doubt the validity of the n.m.r. considerations on which this is based, as they were also used to suggest a similar cis-fused structure for juniper camphor, since proved[62] to be the trans-fused structure (79).

As for the other eudesmanes for which cis-fused

structures have been proposed, there seems little doubt that those for α-verbesinol (80)[72] and



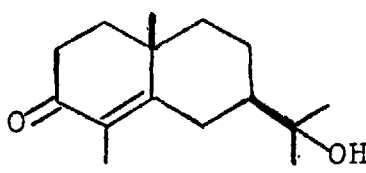
(80)



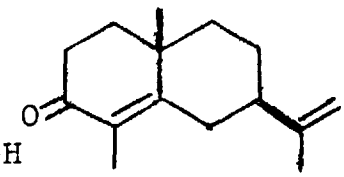
(81)

β-verbesinol (81)[72] are incorrect. Hydrogenation of derivatives of these compounds yields trans-fused compounds. Occidentalol and the chamaecynone group are discussed later.

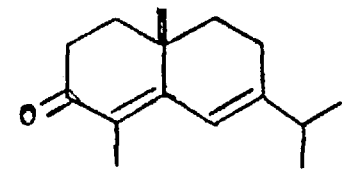
Carissone (82)[73] from Carissa species, and α-cyperone (83)[74] and β-cyperone (84)[74] from Cyperus



(82)



(83)

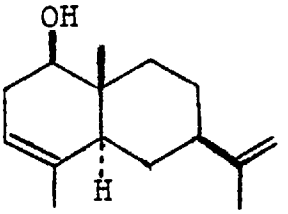


(84)

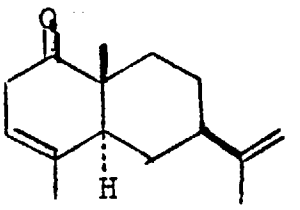
rotundus are important eudesmanes, to which others have been related. The stereochemistries of carissone and the cyperones have been related to β-eudesmol (78),

which has itself been related to steroids.[69]

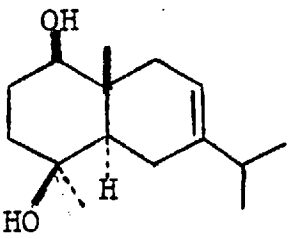
There are four eudesmanes with an oxygen function at C-1. Dictyopterol (85)[75] and the corresponding



(85)



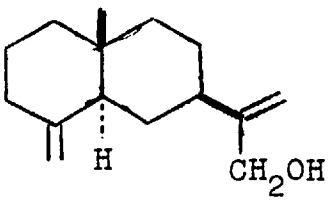
(86)



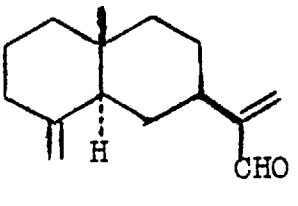
(87)

ketone, dictyopterone (86)[75] have been isolated from different species of Dictyopteris. The alcohol is a mixture of the 3(4)- and 4(15)-double bond compounds. Oplodiol (87)[76] from Oplopanax japonicus is unusual in being the only known eudesmane with a 4β-hydroxyl group.

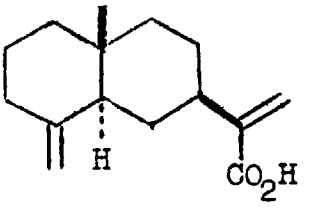
Relevant to discussions on the biogenesis of the lactone function are three eudesmanes which differ only in the oxidation level at C-13, α-costol (88),[77]



(88)

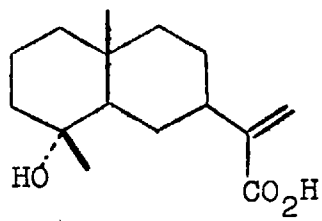


(89)

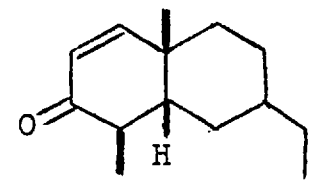


(90)

costal (89)[78] and costic acid (90).[79] The structures of all three have been confirmed by synthesis.[68] α-Costol occurs in Saussurea lappa (costus oil), along with costic acid, and in Chamaecyparis formosensis. Costal, which contains the inseparable 3(4)-double bond isomer, has been found in Thujopsis dolabrata. It seems likely that (88) to (89) to (90) are examples of the first steps in the biosynthesis of the lactone function so frequently encountered in sesquiterpenoids. Ilicic acid (91)[80] from



(91)

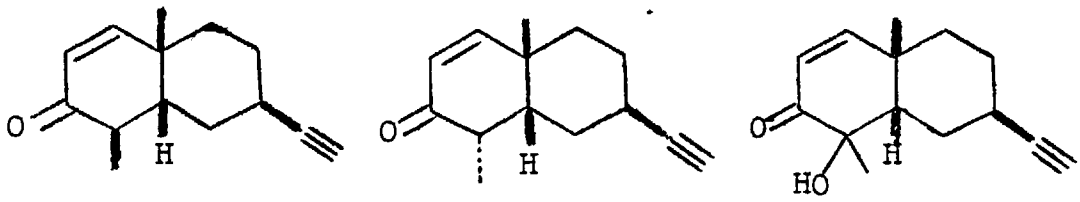


(92)

Ambrosia ilicifolia is another case in which the 13-methyl has been oxidised.

Three interesting acetylenic, and a related olefinic, nor-sesquiterpenoids have been isolated from Chamaecyparis formosensis by Nozoe, Cheng and Toda.[81] They are dihydroisochamaecynone (92), isochamaecynone (93), chamaecynone (94) and

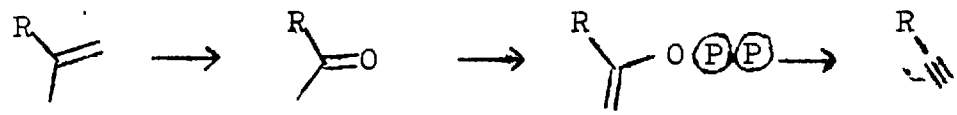
hydroxyisochamaecynone (95). The synthesis of chamaecynone[82] from santonin has confirmed that the



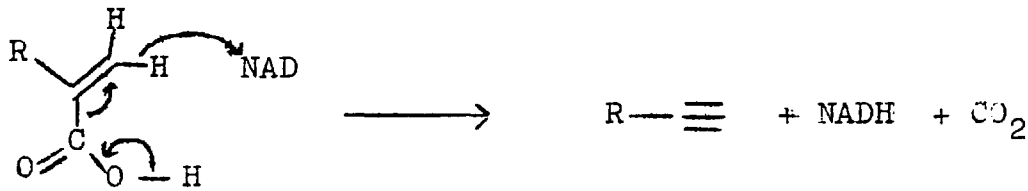
(93) (94) (95)

ring fusion is cis.

These acetylenes pose an interesting biosynthetic problem, for the mechanism postulated to explain the acetylene in polyene compounds (page 19) is not convincing in this case. While it is possible that the side chain is degraded to the ketone, and thence through the enol pyrophosphate to the acetylene:-



it seems more attractive to postulate the involvement of an eliminative decarboxylation:-

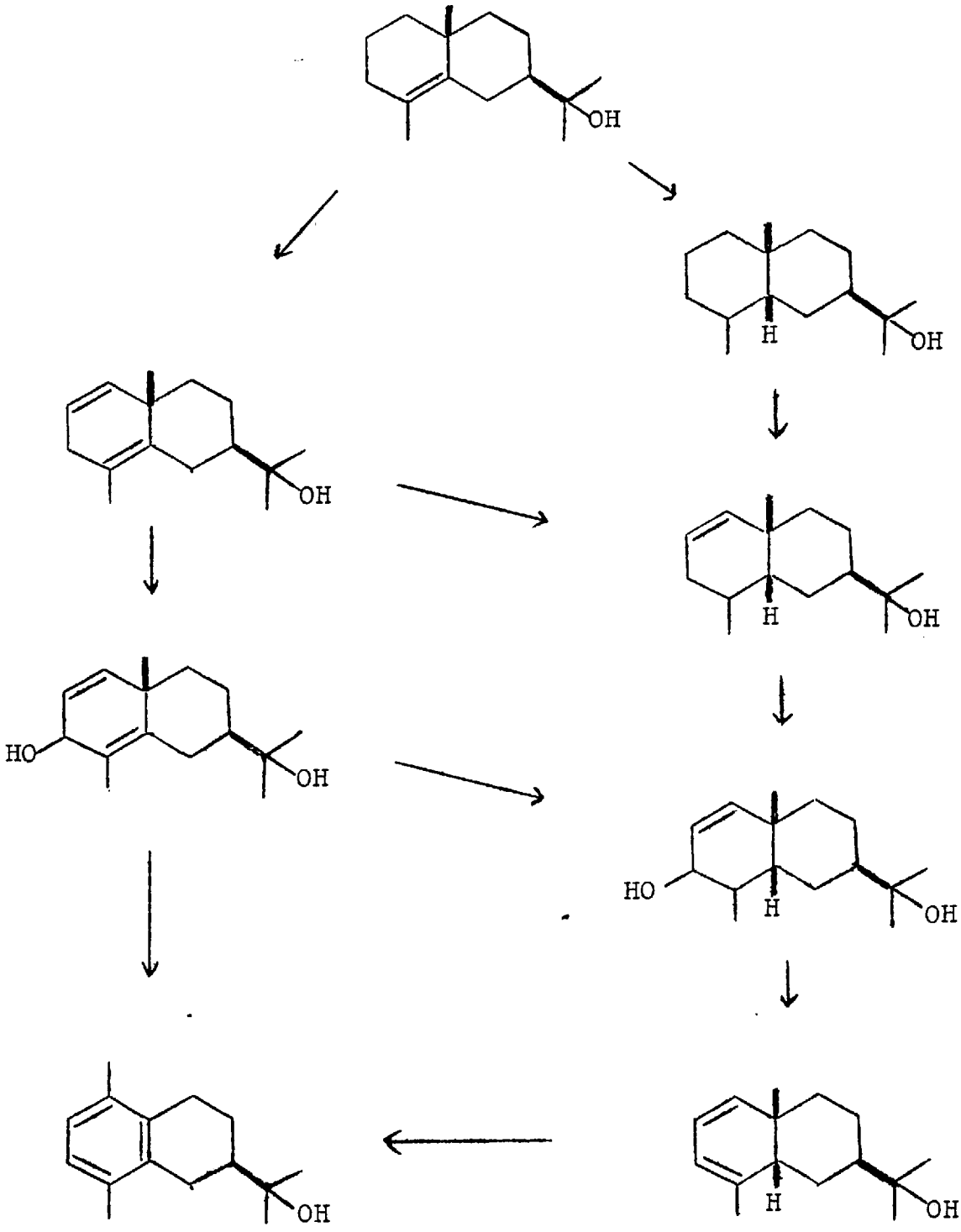


Another possibility is the dehydrogenation of an olefin, such as dihydroisochamaecynone (92), although there is as yet only limited precedent[22] for such a reaction. α-Costol (88), the αβ-unsaturated alcohol, has been found in the same species.

The occurrence of the cis-fused decalin system in these nor-sesquiterpenoids may indicate that they are formed by a pathway involving hydrogenation of a 4(5)-double bond. It certainly does not seem plausible that cyclisation of a trans-trans-germacrane could produce a cis-fused system. It has, however, been suggested[83] that cyclisation of a cis-trans-germacrane would yield a cis-fused decalin.

The cis-fused system of occidentalol (97)[84] has been less rigorously established, although again hydrogenation of a 4(5)-double bond is possible. Occidentalol is, of course, a dihydrobenzene derivative, and as such would be expected to aromatise readily with shift of the angular methyl to the 1-position. Occidol (96)[85] is found in the same species (Thuja occidentalis), but may be an artifact. Chart V shows several reasonable biosynthetic schemes for the two compounds.

CHART V

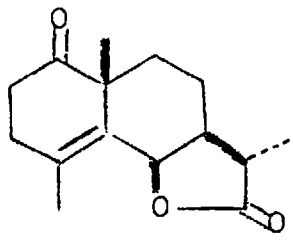


(96) (97)

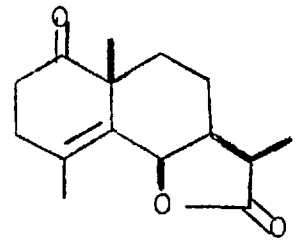
Lactonic Eudesmanes - Eudesmanolides.

The known eudesmanolides are tabulated in Charts VI (6,13-lactones with 6α-oxygen), VII (6,13-lactones with 6β-oxygen) and VIII (8,13-lactones, all of which have 8β-oxygen). All these compounds have the trans-fused 10β-methyl/5α-hydrogen configuration, and all have the 7β-side chain.

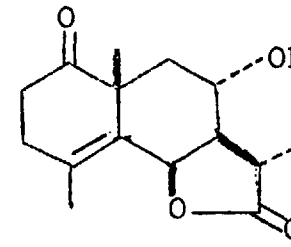
CHART VII



(109)
Desoxypseudo-santonin
A. maritima[98]



(110)
Finitin
A. finitin[98,99]

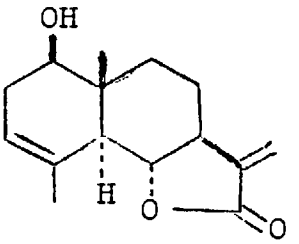


(111)
Pseudosantonin.
A. maritima[100]

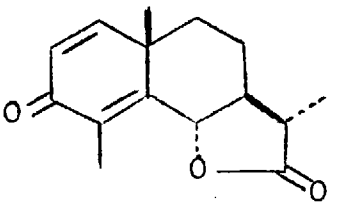
Artemisin (104), β-santonin (103), pseudo-santonin (111) and desoxypseudosantonin (109) are all found in small quantities as a by-product of the commercial extraction of santonin from Artemisia maritima. The biosynthesis of santonin is discussed in Section 4.

The actual mechanism of lactone formation has, unfortunately not been investigated, and is considered briefly here. The formation of the

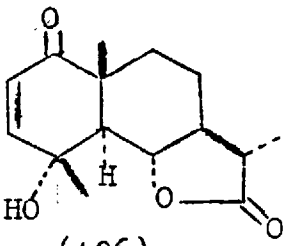
CHART VI



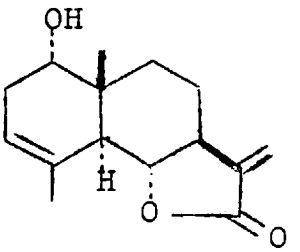
(98)
Santamarine[86]
Chrysanthemum parthenium



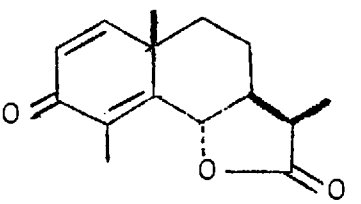
(102)
α-Santonin[89,90]
A. maritima
and others



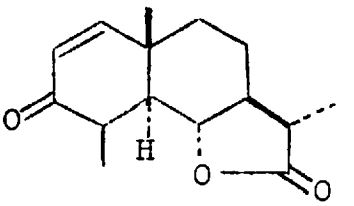
(106)
Vulgarin[94,95,96]
A. vulgaris
and others



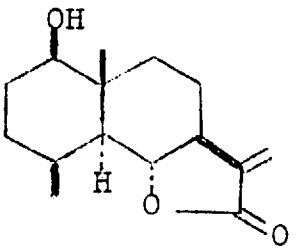
(99)
Douglanine[87]
A. douglasiana



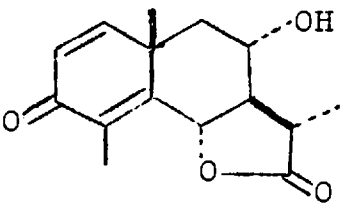
(103)
β-Santonin[91]
A. maritima
and others



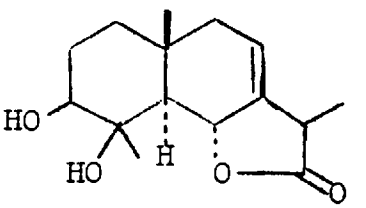
(107)
Monogynin[97]
A. monogyna



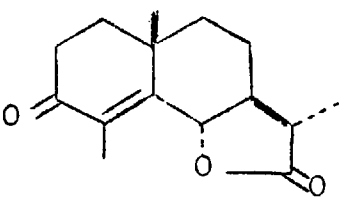
(100)
Balchanin[88]
A. balchanorum



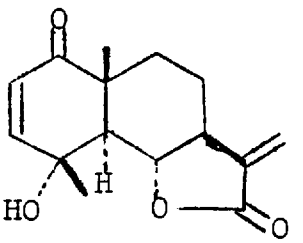
(104)
Artemisin[92]
A. maritima



(108)
Mibulactone[97]
A. monogyna
and A. taurica

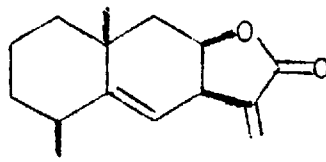


(101)
Dihydrosantonin
A. maritima



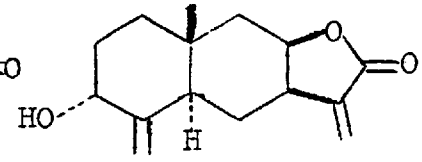
(105)
Arglanine[93]
A. douglasiana

CHART VIII



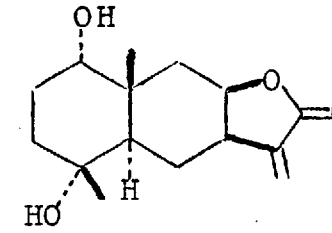
(112)

Alantolactone[101,102]
Inula helenium



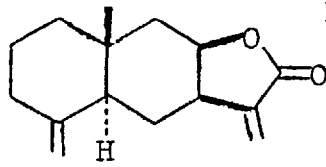
(116)

Isotelekin[102,105]
Telekia speciosa



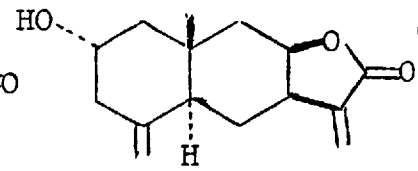
(120)

Microcephalin[108]
Iva axillaris and
I. microcephala



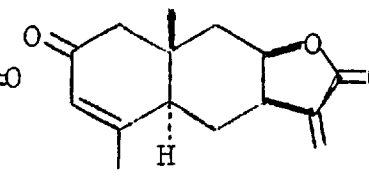
(113)

Isoalantolactone[103,104]
Inula helenium and
I. racemosa



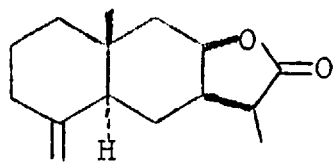
(117)

Ivalin[106]
Iva microcephala
and Iva imbricata



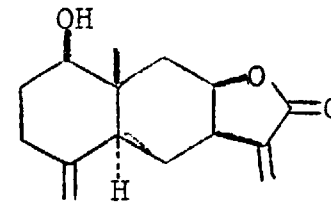
(121)

Pinnatifidin[109]
Helenium
pinnatifidum



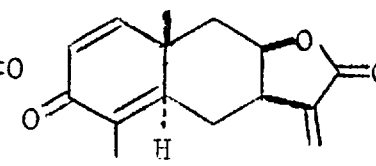
(114)

Dihydroisoalantolactone[103]
Inula helenium and
I. racemosa



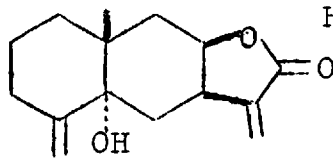
(118)

Asperilin[107]
Iva asperifolia
and I. texensis



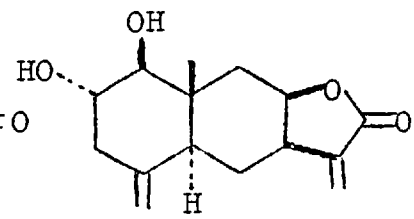
(122)

Yomogin[110]
Artemisa
princeps



(115)

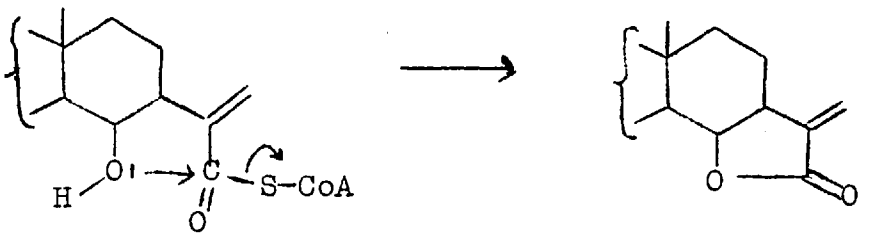
Telekin[102,105]
Telekia speciosa



(119)

Ivasperin[107]
Iva asperifolia
and I. texensis

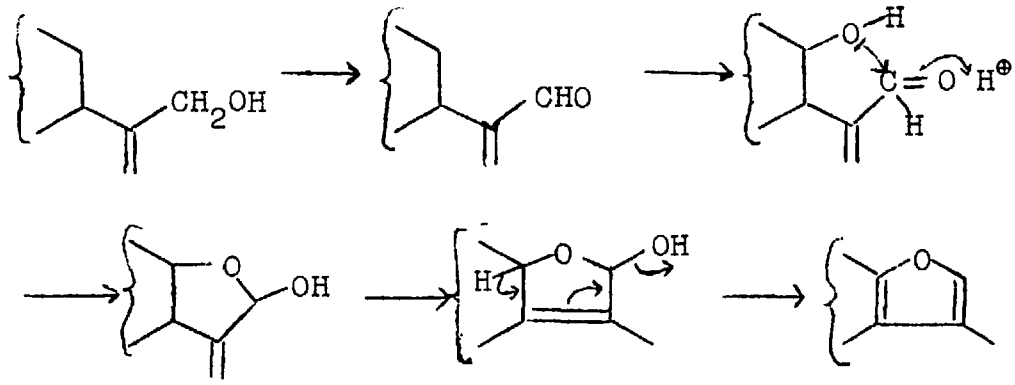
αβ-unsaturated carboxylic acid was discussed on page 41. Presumably, lactone formation takes place with the hydroxyl group at either C-6 or C-8 already present, although direct attack on the carbon atom has been suggested.[111] The mechanism of this lactonisation may involve an activated ester (e.g. thiol ester of Co A), or may even be spontaneous.



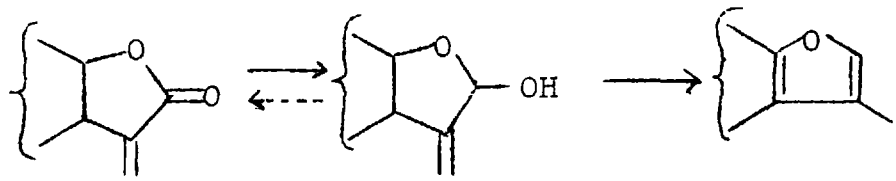
The possibility of attack of a carboxylate anion on either a double bond or a phosphate ester at C-6 or C-8 cannot be ruled out, but seems less likely. Very few eudesmanolides (or guaianolides) with a double bond involving C-6 are known. The phosphate ester mechanism is less easily dismissed, but it seems unlikely that the necessary nucleophilic oxygen can be present at biological pHs. It is also possible that the lactone is formed from a furan, and this is discussed in the following sub-section.

Furanoeudesmanes.

It is possible that the furan ring is formed in a manner similar to the lactone. One possibility involves formation and dehydration of a hemi acetal.

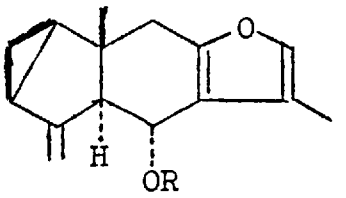


Alternatively, the αβ-unsaturated lactone may be reduced to give the hemi-acetal.

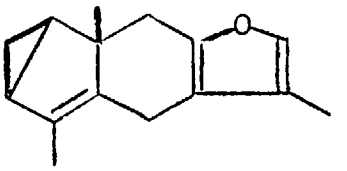


It is, of course, also possible that the hemi-acetal is an intermediate common to the formation of both the αβ-unsaturated lactone and the furan, and indeed, the reverse of the above sequence constitutes a possible biosynthetic route from a furan to a lactone. Mention has already been made of the probability of the furan ring being formed before cyclisation of the ten-membered ring.

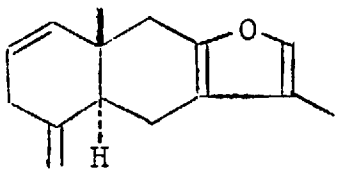
Lindera strychnifolia is the source of four of the five known furanoeudesmanes - linderene (123)[112] and its acetate (124),[112] isolinderoxide (125)[113] and lindestrene (126).[112,114] The other is



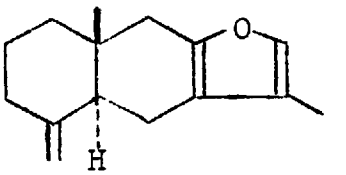
(123) R = H
(124) R = Ac



(125)



(126)

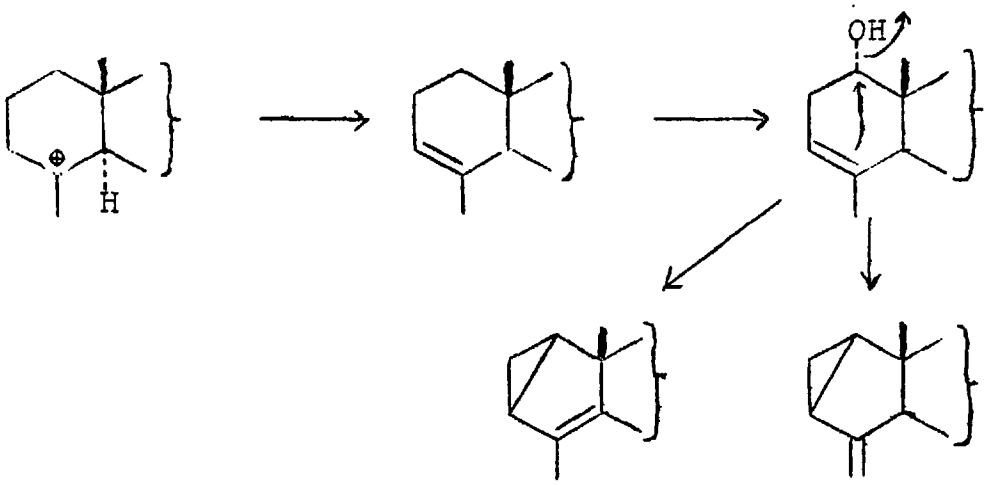


(127)

atractylon (127)[115] from Atractylis species.

The origin of the cyclopropane ring in linderene and isolinderoxide is interesting, for there is no readily available activated centre as in maaliol and maaliene (below). It may well be that the cyclopropane is formed by expulsion of a hydroxyl (or pyrophosphate) at C-1. Position 1 is

sometimes hydroxylated, and such a proposal would also conveniently explain the formation (by dehydration) of linestrene.

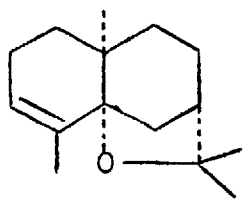


The agarofuran group.

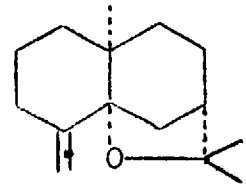
This group of six sesquiterpenoids, occurring[116] in fungus infected agar wood oil (Aquillaria agallocha) contain the interesting tetrahydrofuran system. The structures are shown in Chart IX.

The stereochemistries shown are those tentatively suggested by Bhattacharyya,[116] and while the 14α-methyl has been established by interrelation with δ-selinene, the configuration at C-5 and C-7 rests only on the o.r.d. of norketoagarofuran (133). The failure of the octant rule in certain similar oxides has recently been reported,[117] and it is tempting to suggest that the C-5 and C-7 configurations are both β - it is not

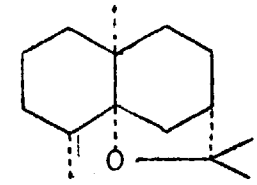
CHART IX



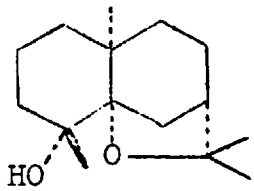
(128)
α-Agarofuran



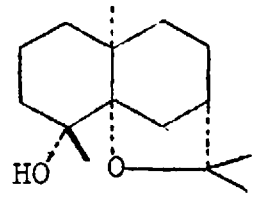
(129)
β-Agarofuran



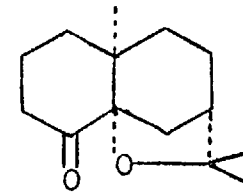
(130)
Dihydroagarofuran



(131)
4-Hydroxydihydroagarofuran

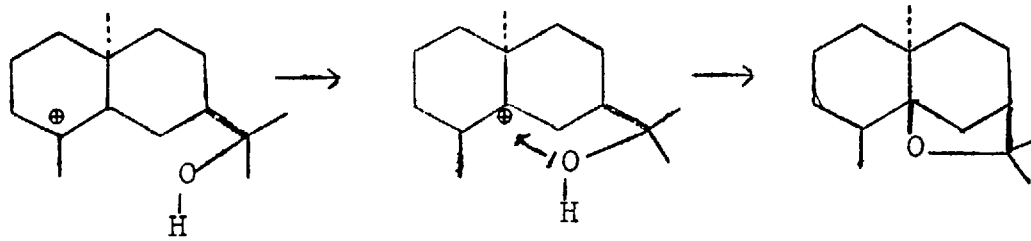


(132)
3,4-Dihydroxydihydroagarofuran

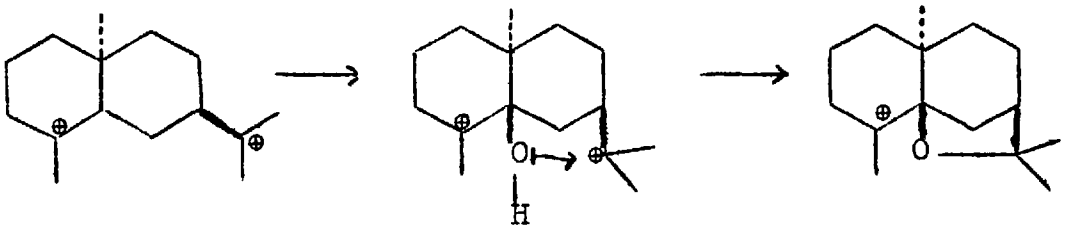


(133)
Norketoagarofuran

possible for the C-5 and C-7 substituents to be <u>trans</u> to one another. Biosynthetically, if the C-5 and C-7 configurations are both β, then a possible pathway merely involves the attack of the 11-hydroxyl on a carbonium ion at C-5.



On the other hand, it may be that position 5 is hydroxylated, and the oxide ring formed by the attack of that hydroxyl on a carbonium ion or incipient carbonium ion at C-11. This latter scheme is supported by the fact that most the agarofurans have either a hydroxyl group or a double bond involving C-4, making it unlikely that the C-4 "ion" rearranges to the C-5 "ion" as required by the first scheme.

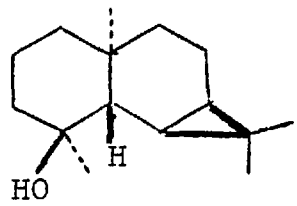


Eudesmanes with 6,7,11-cyclopropane rings.

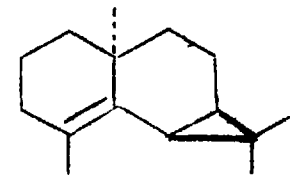
Referring to Chart IV, it will be seen that two possibilities arise for the formation of the cyclopropane ring. Loss of a proton from position 6 of the non-classical carbonium ion (63) results in the cyclopropane directly, and, indeed, the overall reaction bears similarities to carbene attack on a double bond. It is also possible, however, that an intermediate derived from ion (65), such as one with a 4(5)-double

bond activating the 6-position, loses a proton from C-6 to yield the cyclopropane.

Both known compounds of this group, maaliol (134)[118] and β-maaliene (135)[119] have a 10α-methyl group. If it is assumed that the cyclopropane ring is formed before cyclisation of the ten-membered ring, then it may be that the constraint imposed on the conformation is such as to favour cyclisation to yield the 10α-methyl. Examination of models supports this proposal.



(134)

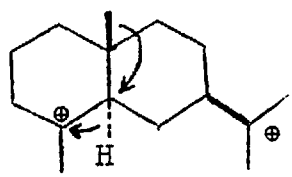


(135)

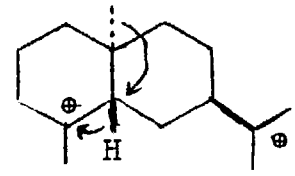
Eremophilane group.

The eremophilane group divides conveniently into two sub-groups, differing in the configuration of the methyl groups. One sub-group has the "normal" 4β,5β-methyl configuration (137), derived from the "normal" eudesmane precursor (136) by the 1,2-shifts shown, and the other, the "epimeric" 4α,5α-methyl configuration (139)

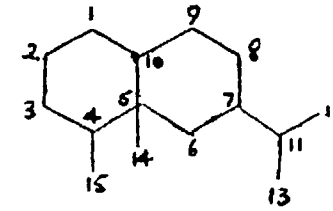
from the "epimeric" eudesmane (138).



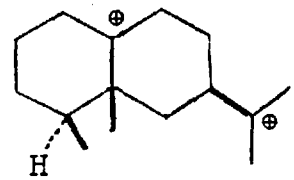
(136)



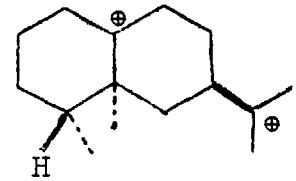
(138)



(140)



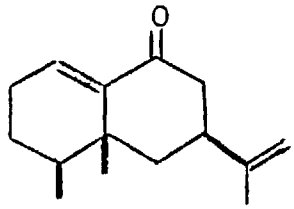
(137)



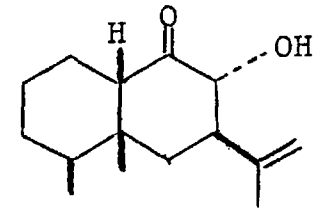
(139)

Many eremophilanes have 1(10)- or 9(10)-double bonds derived from the positive centre (or incipient positive centre) produced at C-10 by the 1,2-shifts. It is of interest to note that, in cases where C-10 is saturated - all are "normal" eremophilanes - the C-10 hydrogen is β, i.e. ring fusion is cis. Such compounds can arise by capture of a hydride ion at C-10, or by hydrogenation of a double bond. Many "normal" eremophilanes have unsaturation at C-7, but in the few exceptions, and in all compounds of the "epimeric" series, the C-7 side chain is in the β-configuration.

Two typical eremophilanes from Eremophila mitchelli, the source of many of the "normal" series, are eremophilone (141)[120,121] and dihydrohydroxy-eremophilene (142).[122] Other fruitful sources of the

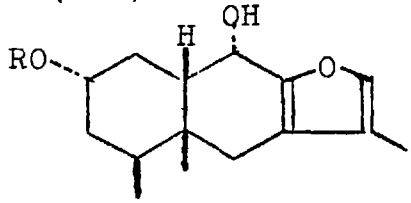


(141)

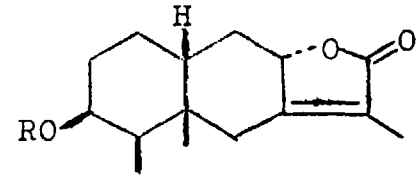


(142)

"normal" series are Petasites officinalis (≡ P. hybridus), P. albus and Ligularia fischeri. Many are furans, but there are also a few αβ-unsaturated γ-lactones, all closed to C-8. Typical of this group are furanopetasin (143)[123,124] and petasitolide A (144)[124,125] and B (145).[124,125] Interesting



(143) R = COCMe=CHMe (cis)

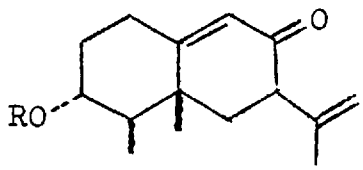


(144) R = COCMe=CHMe (cis)
(145) R = COCMe=CHMe (trans)
(147) R = COCMe=CHSMe (cis)
(148) R = COCMe=CHSMe (trans)

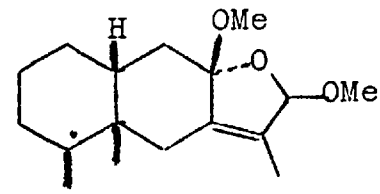
is S-petasin (146)[124] with a sulphur containing ester, which in its cis- and trans-forms occurs also in

S-petasitolide A (147)[124,125] and B (148).[124,125]

A interesting modification to the lactone or furan is shown by dimethoxydihydrofuranoeremophilane (149).[126]



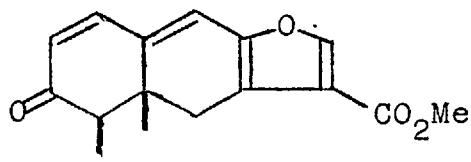
(146) R = COCMe=CHSMe (cis)



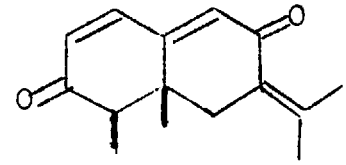
(149)

This may well be an example in which dehydration of the hemi-acetal (see page 49) to form the furan has been blocked by methylation of the hydroxyl on C-12.

Two eremophilanes with extended conjugation, warburgin (150)[127] and warburgiadione (151)[128] have



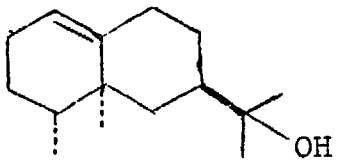
(150)



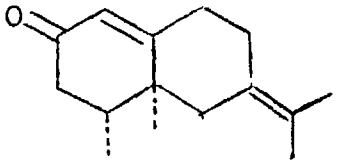
(151)

been isolated from Warburgia ugandensis.

"Epimeric" eremophilanes can be neatly further classified into two sections. The first section, which can be simply and directly derived from ion (139) includes valerianol (152)[129] and α-vetivone (153).

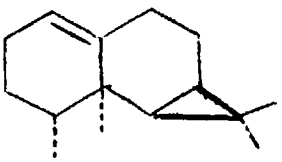


(152)

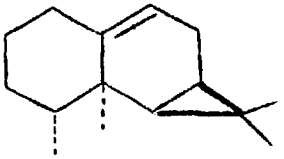


(153)

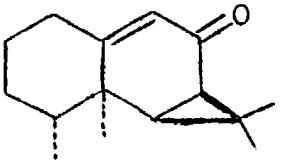
The latter was long thought to be similar to the previously accepted structure of β-vetivone (see page 61), but the correct structure (153) was deduced almost simultaneously by de Mayo[130] and Marshall,[131] who subsequently synthesised it.[132] The second section comprises five eremophilanes with cyclopropane rings.



(154)



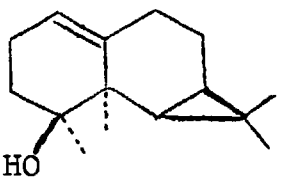
(155)



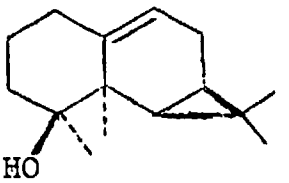
(156)

These are β-gurjunene (154)[100,133] and 9-aristolene (155),[133] aristolone (156)[133] and calarenol (157).[134]

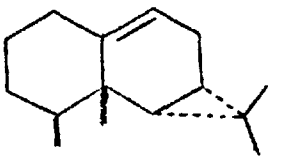
A mixture of β-gurjunene and 9-aristolene occurring in Acorus calamus was initially thought to be a single compound called calarene. Calarenol from Nardostachys



(157a)



(157b)



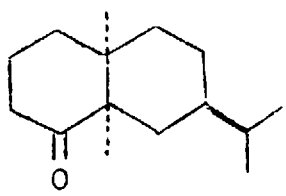
(158)

jatamansi (Chinese spikenard oil), one of the other sources of "calarene", is similarly a mixture of the two double bond isomers, which have, however, not been separated in this case.

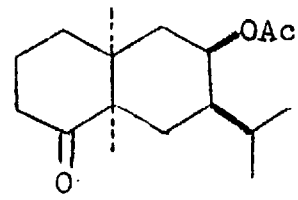
α-Ferulene (158)[135] from Ferula communis is reported to be the antipode of 9-aristolene, and is thus of great interest, having a 7α-side chain.

Valeranone type.

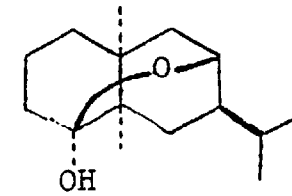
This group, presumably formed as indicated in formulae (69) and (72) in Chart IV, is found in various species of Valeriana. The progression from valeranone (159)[136,137,138] through fauronyl acetate (160),[139] or the free hydroxy compound, to the hemi-ketal,



(159)



(160)

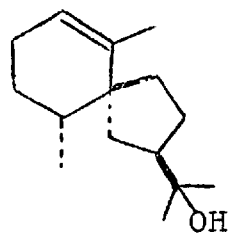


(161)

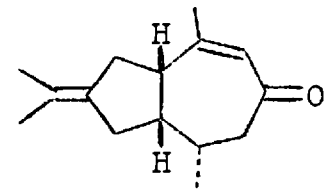
cryptofauronyl (161);[139,140] is an obvious biosynthetic possibility.

Agarospirol type.

Until recently, agarospirol (162)[141] from fungus infected Aquilaria agallocha (the source of the agaro-



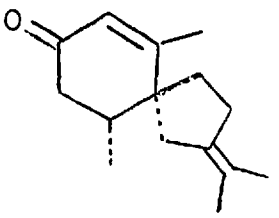
(162)



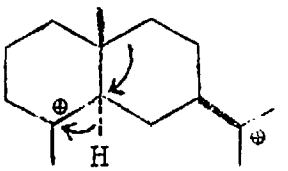
(163)

furans) was the only known spiro sesquiterpenoid derived from a eudesmane precursor. Marshall, however, recently synthesised[142] the previously accepted structure (163) for β-vetivone,[143,144] and

found that it was not identical with the natural product. By the stereospecific synthesis,[145] he proved that β-vetivone is in fact the spiro compound (164). This requires revision of the structure of



(164)



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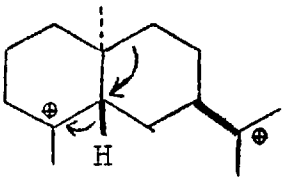

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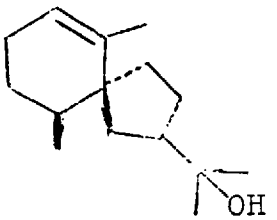

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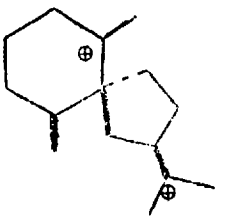

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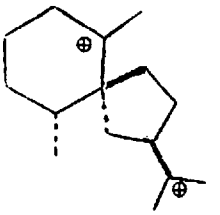


(169)

hinesol[146,147] to the spiro structure (165). The close relationship between these spiro compounds and the eudesmanes is shown by the occurrence together in Atractylodes lancea of hinesol and eudesmol (atractylol).[147] Various other sesquiterpenoids from Vetiveria zizanioides have been assigned structures based on the wrong β-vetivone structure (158) on the basis of correlation with it, and require reconsideration, or

in some cases, further investigation.

Hinesol (165) is formed from the "normal" 10β-methyl eudesmane as shown (166) to (167), while agarospirol and β-vetivone appear to be formed from the 10α-methyl eudesmane as shown (168) to (169). The stereochemistry shown for agarospirol has not been rigorously established, but there is some evidence for a 7β-side chain. The stereochemistry of the side chain in hinesol is unknown.

THE HYDROAZULENIC SESQUITERPENOIDS

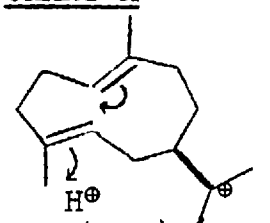
Introduction.

It is ironic that the first "hydroazulenic" sesquiterpenoid structure "elucidated" was β-vetivone, which has recently been shown (see page 61) to be a spiro compound of the agarospirol type. Work on the hydroazulenic sesquiterpenoids really began with the early work[148] on the structures of the azulenes produced by steam distillation of acid solutions of the natural products.

At the present time, the hydroazulenic group is the largest group of sesquiterpenoids, but this may reflect the extensive and systematic investigations of Herz and his co-workers. Unfortunately, there are still many unsolved stereochemical problems in this group, and, for the most part, only the gross structures can be used for classification. Further subclassification will no doubt be necessary when more stereochemical details are elucidated.

Chart X depicts the probable biosynthetic processes leading to the hydroazulenic sesquiterpenoids. As with the eudesmanes, it is possible to envisage

CHART X



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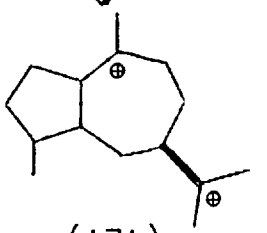
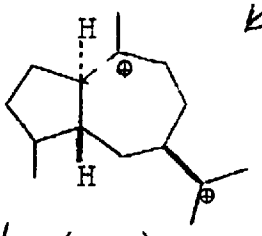
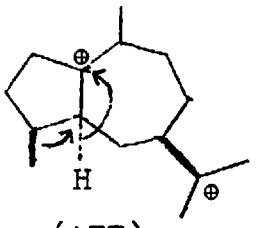


(171)

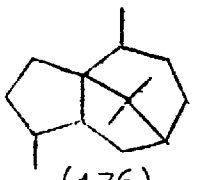


(173)



(177)

6,13 & 8,13 lactones.



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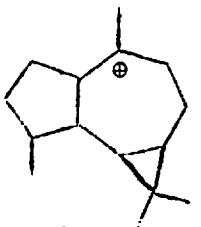

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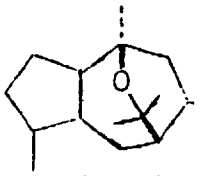
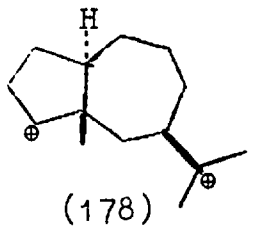
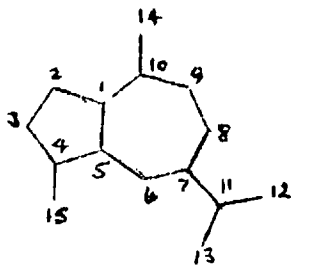


(175)



(178)

6,13 and 8,13 lactones.



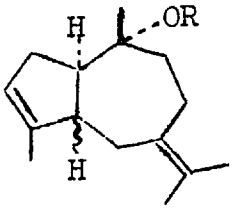
(172)

cyclisation both in the manner shown (170) to (171), and in the opposite direction to produce an (incipient) positive centre at C-4. Although it is not as clear cut as with the eudesmanes, it appears that most guaianes (compounds derived from ion (173)) have unsaturation or a hydroxyl group at C-10. Rather fewer have similar evidence of a possible positive centre at C-4. The pseudo-guaianolides (lactones derived from (178)) are more reasonably explicable on the basis of a C-10 positive centre.

The first sub-division to be made is between the guaiane type (173), and the pseudoguaiane type (178), in which a 1,2-methyl shift has occurred. All the pseudoguaianes are in fact lactones, closed to C-6 or C-8, suggesting that the shifts occur after lactone formation. The guaiane group is conveniently sub-divided into lactones (guaianolides), 6,7,11-cyclopropanes (derived from (174)), the kessanes (from (175)), and the tricyclic systems, of which skeleton (176) is one of several.

Guaianes without lactone, 6,7,11-cyclopropane or oxide rings.

Remarkably few - less than ten - non lactonic, etc. sesquiterpenoids directly derived from ion (173) have been isolated. Typical are partheniol (179),[149]



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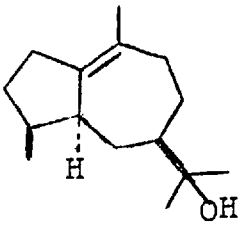
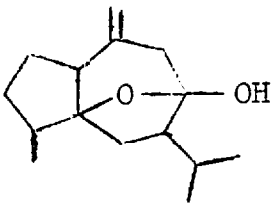
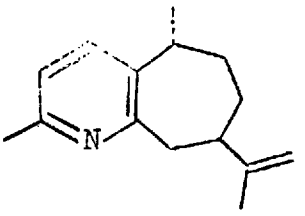


(180)

which occurs in Parthenium argentatum as its cinnamate, and bulnesol (180).[150] One of several sesquiterpenoid hemiketals now known is curcumol (181)[151]



(181)

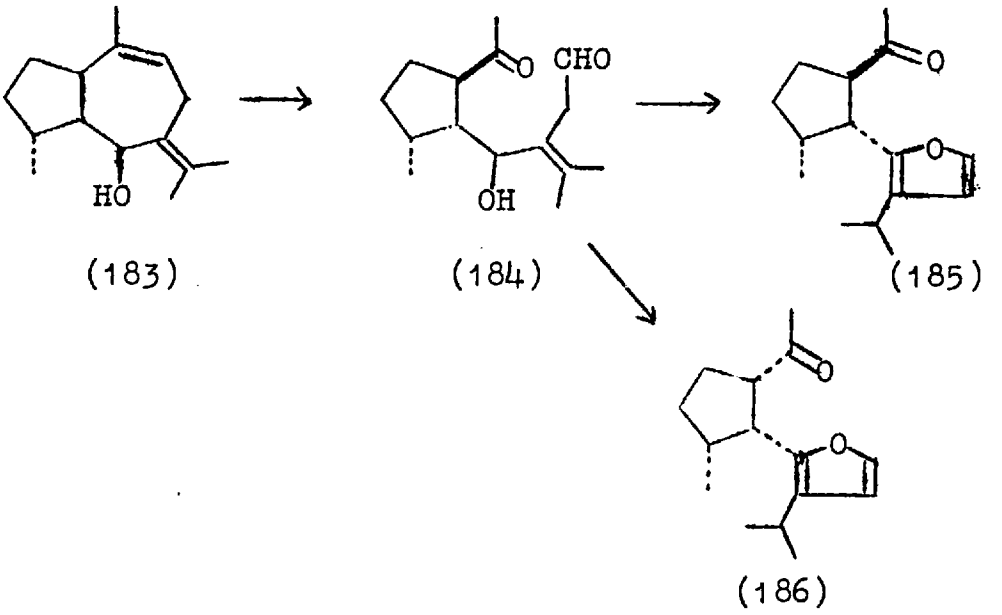


(182)

from Curcuma zedoaria. Epiguaiapyridine (182)[152] is presumably derived from an intermediate of the simple guaiane type by cleavage and recyclisation

with incorporation of nitrogen.

Geranium bourbon oil (probably Pelargonium roseum and others) is the source of two interesting modified guaianes. Furopelargone A (185)[153] and furopelargone B (186)[153] are probably formed by cleavage of an



(183) (184) (185)

(186)

intermediate similar to (183), and subsequent furan formation. α-Bourbonene (187)[154] and β-bourbonene

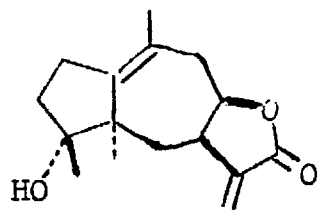
(187) (188)

(188)[154] contain an unusual tricyclic ring system.

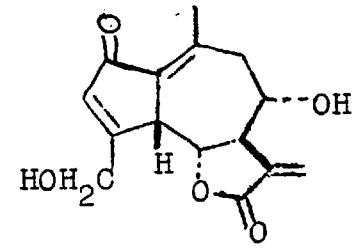
Lactonic Guaianes - Guaianolides.

The guaianolides were reviewed in detail by Šorm and Dolejš,[31] but even since that time, many more structures have been elucidated, and several modified in the light of further investigations.

Very few generalisations can be made about the guaianolides. 6,13-Lactones frequently have the 3-en-2-one system in the five-membered ring. Very few stereochemical details are available for these compounds. It happens that all those rigorously established have a 6α-hydroxyl (or lactone oxygen) and/or an 8β-hydroxyl (or lactone oxygen), but it would be unjustified to more than suggest that this is possibly the normal configuration. Examples of guaianolides are pseudoivalin (189)[155] and



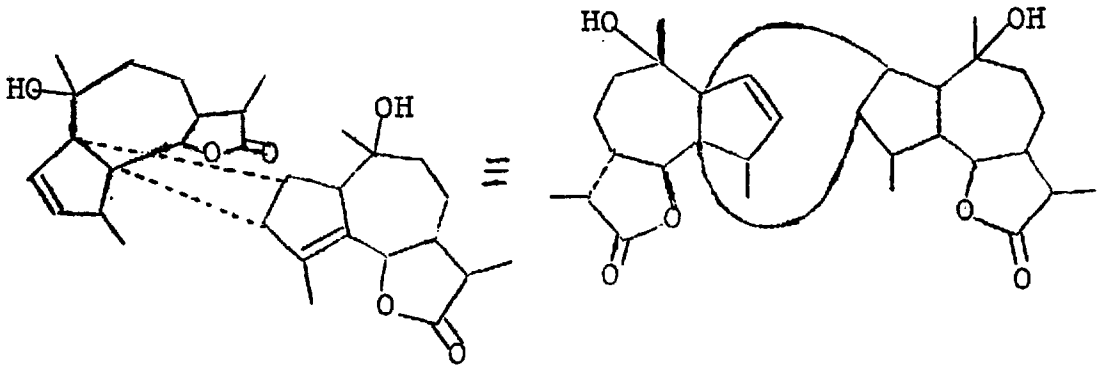
(189)



(190)

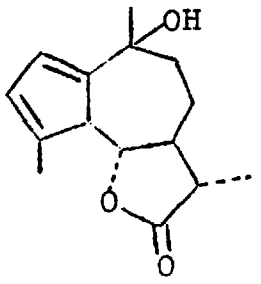
lactucin (190),[156] which occurs in the wild lettuce, Lactuca virosa, as its p-hydroxyphenylacetate.

The "dimeric" sesquiterpenoid absinthin (191)[157] is undoubtedly derived from normal guaianolide

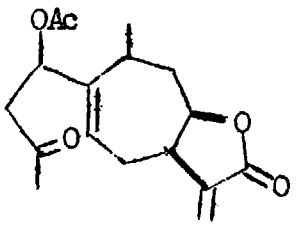


(191a) (191b)

precursors. The evidence for the details of the fusion of the two hydroazulenic moeities is tenuous, and it may be that absinthin is, in fact, a dimer of artabsin (192),[158] also found in Artemisia absinthium. A Diels-Alder dimerisation similar to that of cyclopentadiene would result in the fusion being between positions 2 and 5 of one residue and 1 and 4 of the other.



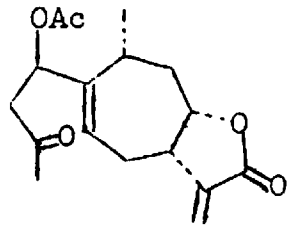
(192)



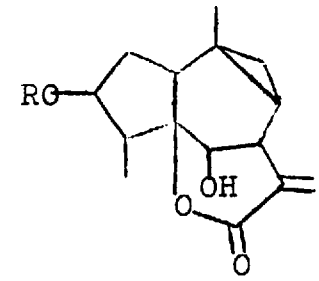
(193)

Xanthinin (193),[159] from Xanthium pennsylvanicum, is an example of a guaianolide in which the 4,5 bond has been cleaved. A stereoisomer of xanthinin is xanthumin, for which the stereochemistry shown in (194) has been proposed,[160] but there is little supporting evidence.

Zaluzanin A (195)[161] and zaluzanin B (196)[161] have an interesting variation on the usual lactone. It is probable that the 5-position is hydroxylated, and the 5,13-δ-lactone the formed. The cyclopropane ring could arise by the attack of a carbanion at C-10 on a hydroxyl group (or pyrophosphate) at C-8.



(194)



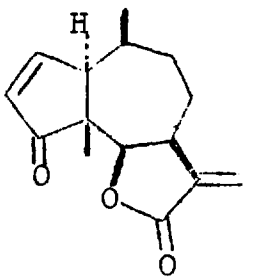
(195) R = H
(196) R = Ac

Pseudoguaianolides.

The pseudoguaianolides (structures related to (178)) can be regarded as being derived in nature from an intermediate such as (177) by the 1,2-shifts

shown in Chart X. In principle, they could be derived from intermediate (171) by concerted 1,2-shifts. This process, however, produces the configuration in which the 10-methyl group is trans to the C-1 hydrogen, whereas there exist many sesquiterpenoids of this type in which the 10-methyl group is cis to the C-1 hydrogen (both α). The stereochemistries of many of this group have been elucidated, principally by Herz and his co-workers.

The occurrence of the pseudoguaianolides is restricted to a few genera. The genus Ambrosia produces both 6β,13- and 8β,13-lactones, all of which have the 10β-methyl configuration. Examples are ambrosin (197)[162] and coronopilin (198).[163]



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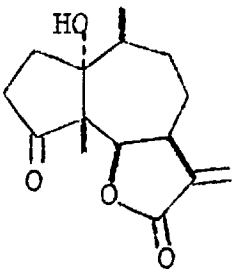
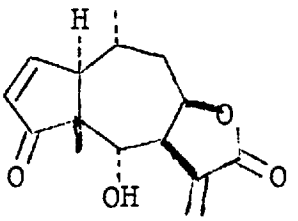


(198)

The genus Helenium is the source of several 8,13-lactones with 10α-methyl groups. Both 8α-

and 8β-lactone oxygen configurations occur. Typical are helanalin (199),[164] bigelovin (200)[165] and



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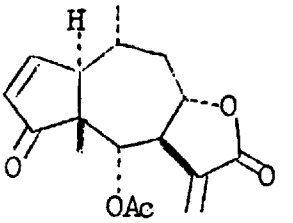

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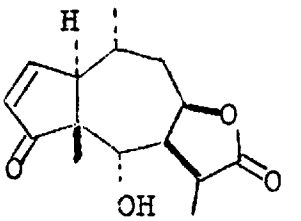
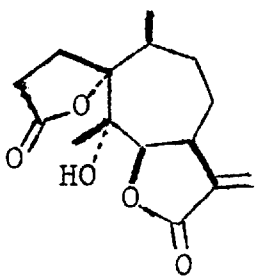


(201)

mexicanin C (201).[166]

The genus Gaillardia produces a number of lactones, some of which have also been isolated from Helenium species. Unfortunately, the stereochemistry at C-10 has not been determined for any of the several lactones unique to Gaillardia species.

Some modified pseudoguaianolides are known. From specimens of Ambrosia psilostachya not containing the usual coronopilin (198), psilostachyin (202)[167] has



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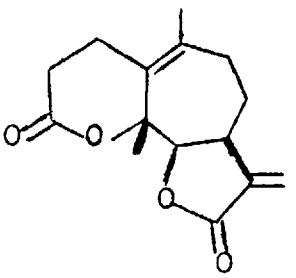
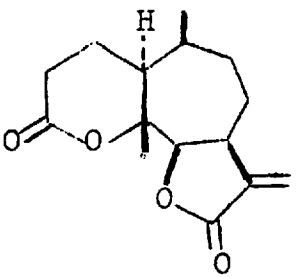


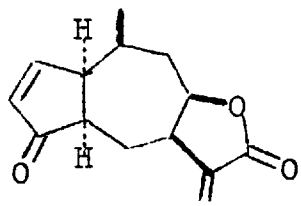
(203)



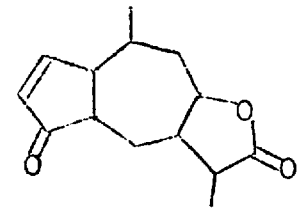
(204)

been isolated. This is the product of a biological Baeyer-Villiger reaction on coronopilin, followed by opening of the lactone so formed (as in psilostachyin B (203)[168] and psilostachyin C (204)[169] found in the same plants), and relactonisation on the C-1 hydroxyl. Chemically, psilostachyin can be obtained by the action of peracetic acid on coronopilin.[167]

Among the many mexicanin pseudoguaianolides (so called after Helenium mexicanum), are mexicanin E (205)[170] and its dihydro derivative (206).[171]



(205)



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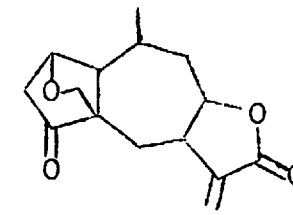
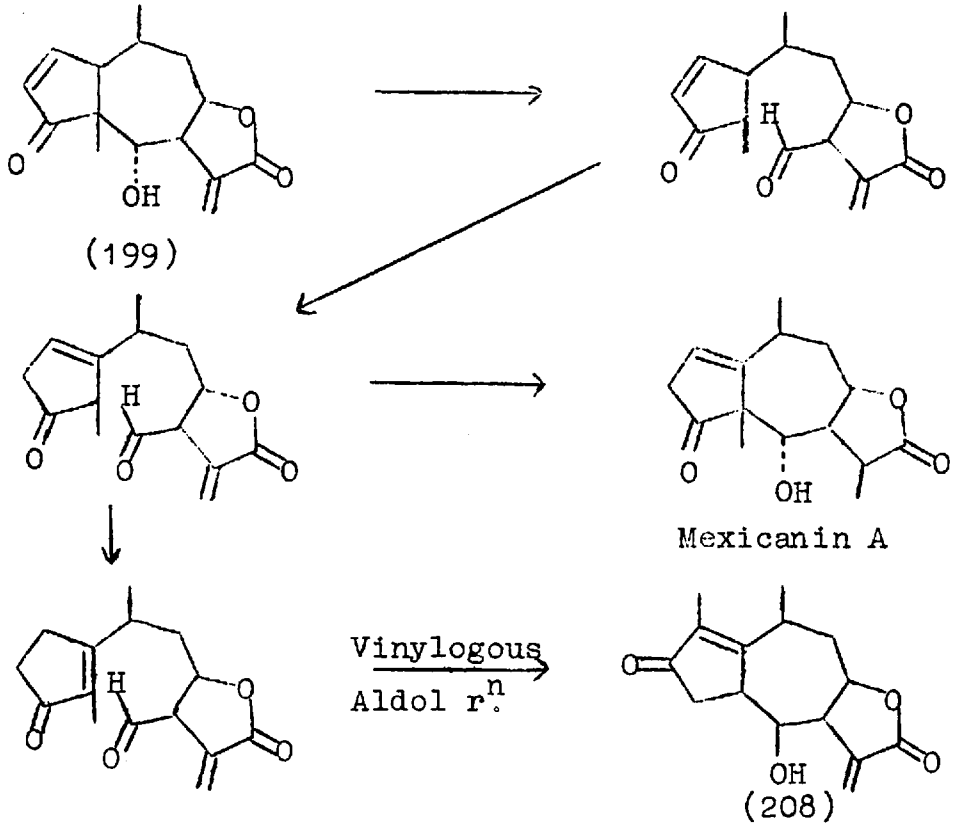


(207)

In view of the exclusive occurrence of pseudoguaianolides in Helenium, these 15-nor sesquiterpenoids must be considered as degraded pseudoguaianolides. Mexicanin H (207),[172] with a five membered oxide (tetrahydrofuran) involving C-15 may be indicative of the initial stages of an oxidative removal of C-15 via a β-keto acid.

A final, somewhat anomolous compound is neohelanalin (208),[164,173] also known as mexicanin D, obtained from Helenium and Balduina species. The conversion from helanalin (199) to neohelanalin has been achieved under mildly acidic conditions,[164]

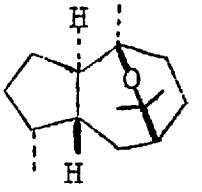


and the illustrated mechanism is a possibility for both the chemical and biological conversion.

Kessane group.

Japanese valerian species produce a series of guaianes, the parent compound of which is

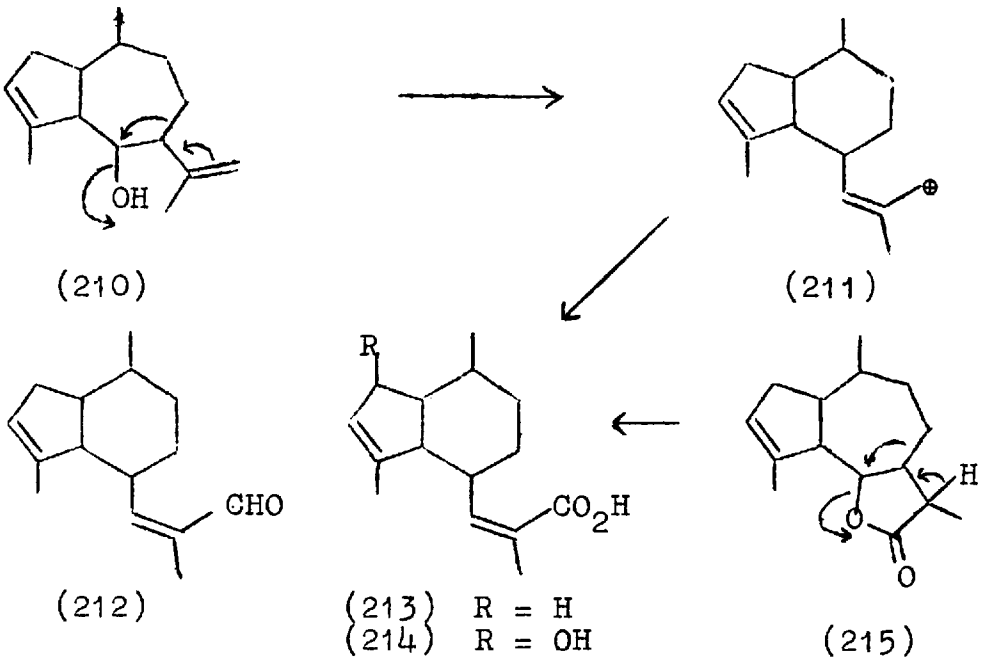
kessane (209).[174] An obvious biosynthetic pathway involves the attack of an 11-hydroxyl on the C-10 carbonium iom formed by cyclisation of a ten-membered ring.



(209)

Valerene type.

A modification to the guaiane skeleton, probably similar to that indicated (210) to (211), produces

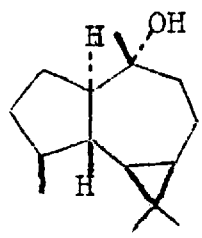


(210) (211) (212) (213) R = H (214) R = OH (215)

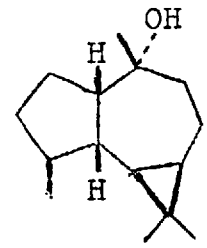
three sesquiterpenoids from Valeriana officinalis. These are valerenal (212),[175] valerenic acid (213)[176] and valerenolic acid (214).[177] Büchi[176] has suggested a biosynthetic scheme involving rearrangement of a lactone (215). It would be unusual, however, for an aldehyde such as valerenal to be formed from an acid, rather than vice versa.

Guaianes with 6,7,11-cyclopropane rings.

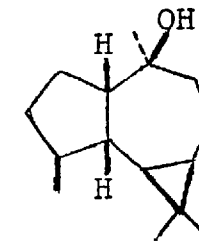
A series of 6,7,11-cyclopropanyl guaianes derived from (174) (Chart X), all with β-configuration of the cyclopropane, has been isolated, principally, but not exclusively, from Eucalyptus species, especially E. globulus. Both 10α- and 10β-methyl configurations occur, as do both cis- and trans-fused ring systems. Examples are globulol (216),[178] ledol (217)[178] and viridiflorol (218).[178]



(216)



(217)



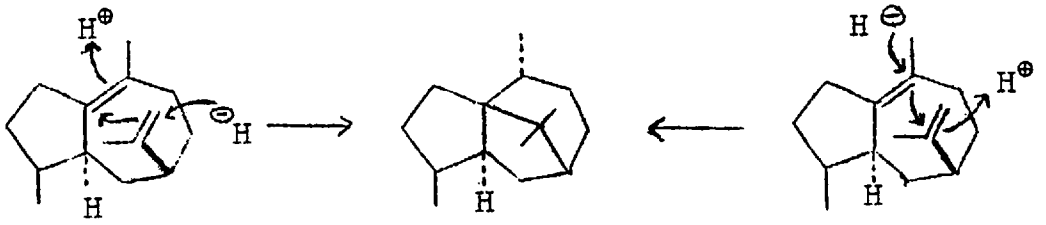
(218)

Biosynthetically, these present few features not previously discussed, save perhaps further consideration of the cis-fused examples. It is possible that, if the cyclopropane ring is formed before cyclisation of the ten-membered ring intermediate, then the conformations which the molecule can adopt are such as to result in either cis- or trans-fused systems. A examination of models suggests that, given the β-configuration of the cyclopropane ring, then the three known fusions are feasible, but the 1α,5α-configuration, which does not occur in any known sesquiterpenoid of this type, does not appear to arise from any of the reasonable conformations.

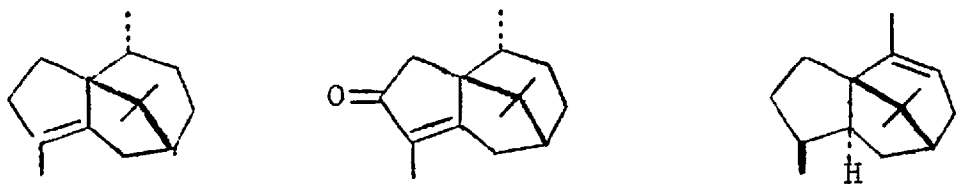
Tricyclic guaianes.

Three separate skeletons are considered under this heading. The first, and most straightforward, is the cyperene type. This is typified by cyperene (219)[179] and cyperotundone (220)[180] (also known as cyperenone, isopatchouleneone and articulone). The biogenesis of this type, which is found in Cyperus species, especially Cyperus rotundus, can be considered as a cyclisation of one of

the types:



or by hydrogenation of an intermediate similar to α-patchoulene (221),[181] from Pogostemon patchouli.

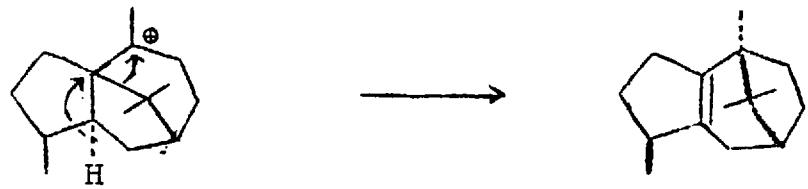


(219) (220) (221)

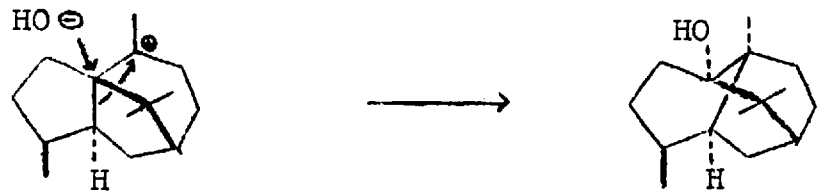
All the cyperene type from Cyperus have a 4(5)-double bond, but α-patchoulene has the expected trans-ring fusion.

All three different skeletons are exhibited by sesquiterpenoids from Pogostemon patchouli. α-Patchoulene has already been mentioned as a cyperene type. β-Patchoulene (223)[181,182] is perhaps derived

from an α-patchoulene type of intermediate (222) by the 1,2-shift shown, with loss of a proton to form a double bond. An alternative 1,2-shift (224)



(222) (223)



(224) (225)

yields patchouli alcohol (225).[181,183] Patchouli alcohol in fact contains a tricyclic decalin system, but must surely be formed in a manner similar to α- and β-patchoulene.

SESQUITERPENOIDS REGARDED AS DERIVED FROM BISABOLENE TYPE INTERMEDIATES

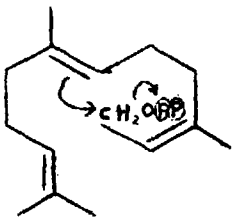
Introduction.

Two biosynthetic routes to bisabolene (228)[184] seem possible. As shown on Chart XI, cis-trans-farnesyl pyrophosphate (226) leads, via ion (227) to bisabolene (228a), while the less likely cis-cis-farnesyl pyrophosphate (229) leads via ion (230) to bisabolene (228b). Structures (228a) and (228b) are not identical, but are extremely similar chemically. In terms of the suggested biosynthetic routes, the difference is only significant if the intermediate ions (227) and (230) retain the stereochemistry at C-1 and C-10. There are very few bisabolenes known in which the 1(10)-double bond is still present, and the only way to distinguish the structures may be by an X-ray diffraction study, possibly of a silver nitrate adduct.

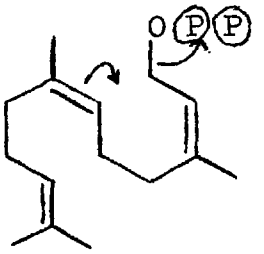
Bisabolene type.

Most bisabolene sesquiterpenoids are derived directly from bisabolene (228), with only the usual variations involving double bonds and hydroxyl groups.

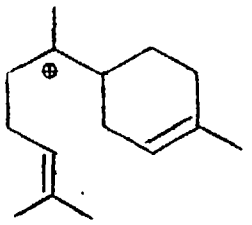
CHART XI



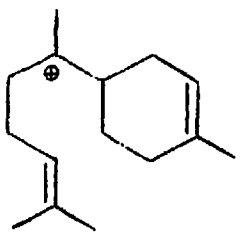
(226)



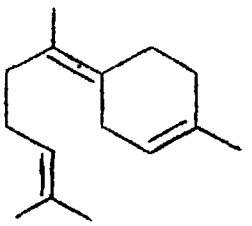
(229)



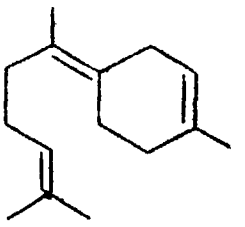
(227)



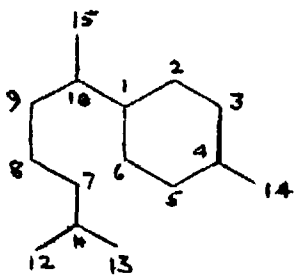
(230)



(228a)

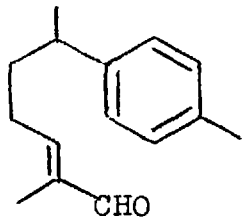


(228b)

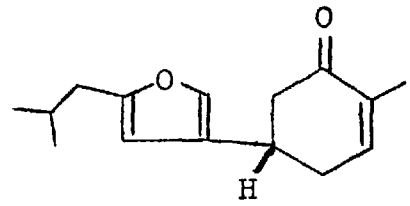


(231)

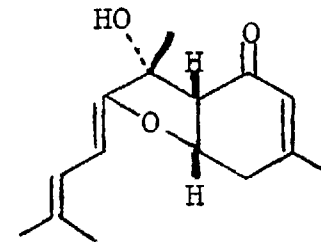
In some natural products, the alicyclic ring has been dehydrogenated to an aromatic ring, as in nuciferal (232).[185] A furan ring occurs in bilobanone (233),[186]



(232)



(233)

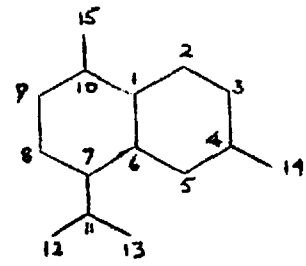


(234)

and an oxide ring in bisabolangelone (234).[187]

Cadinene type.

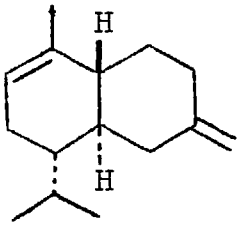
The cadinene group of sesquiterpenoids, based on the skeleton (235) is sometimes referred to as the cadalene group, after the aromatic hydrocarbon produced by dehydrogenation of its members. The group displays less stereospecificity than any other group



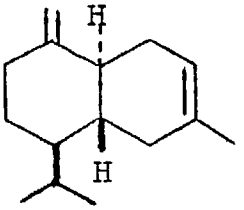
(235)

of sesquiterpenoids, and generalisations are impossible.

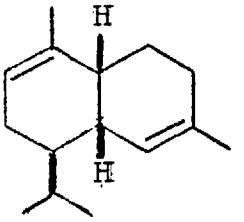
The review by Herout and Sýkora[188] illustrates well the many stereochemistries involved. Typical are γ_1-cadinene (236),[188] γ_2-cadinene (237)[189] and



(236)



(237)

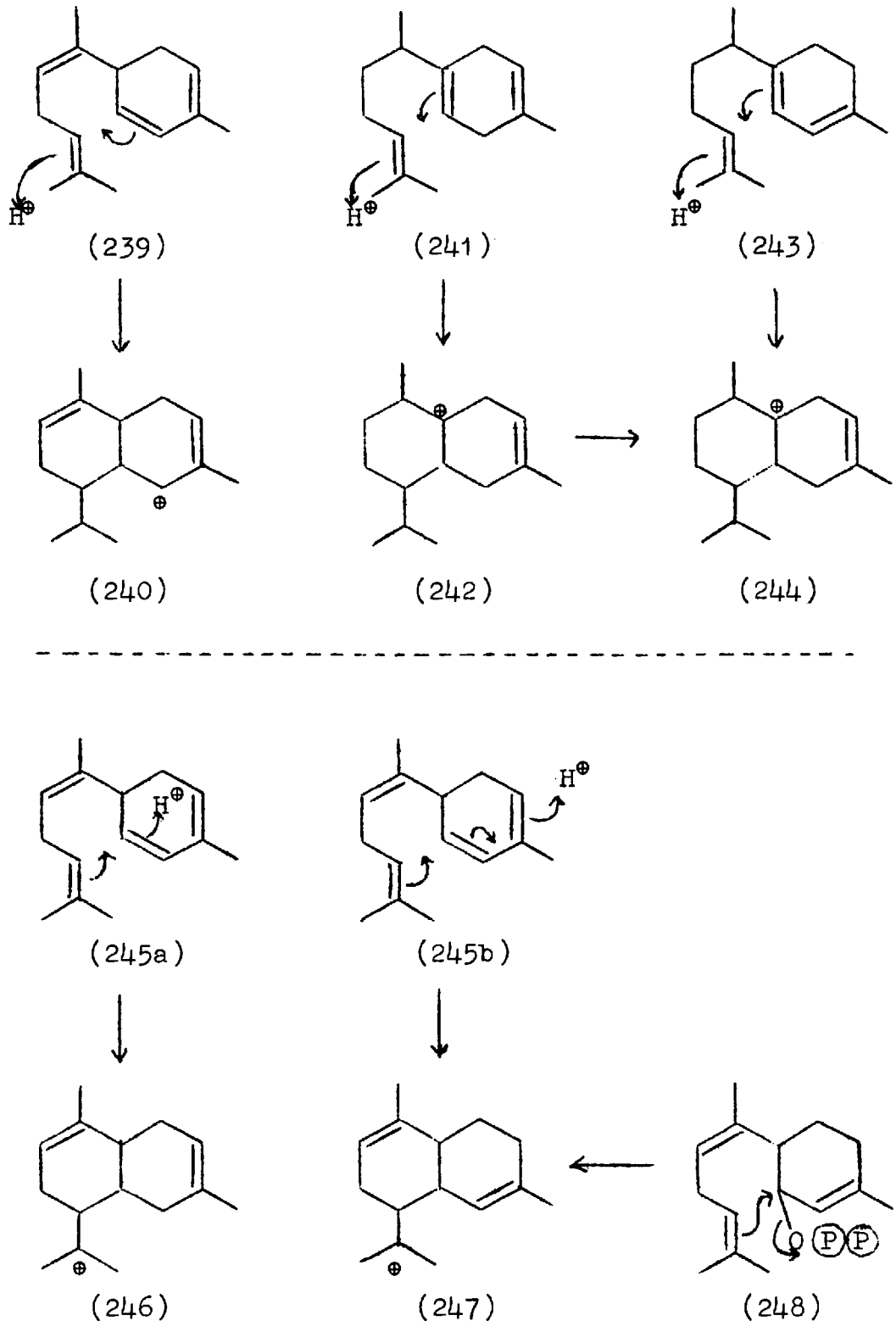


(238)

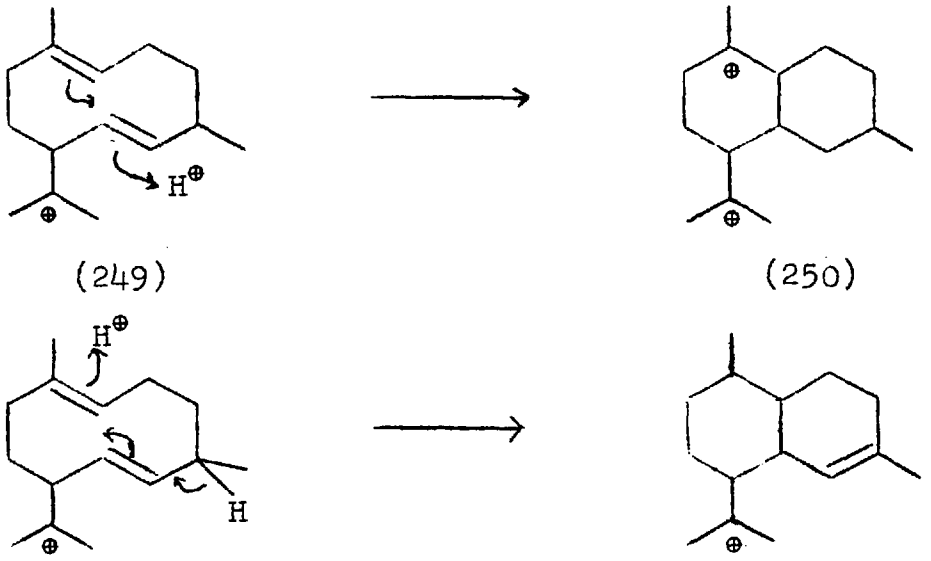
α-muurolene (238).[190]

Almost all the group have unsaturation (or a hydroxyl group) at both C-4 and C-10. Of the possible cyclisations of bisabolene derivatives shown in Chart XII, those from (241) and (243) give a positive centre at C-1. This does not readily explain why all the hydroxyl groups and double bonds involve C-10, but it may be that there are compelling reasons for the C-1 carbonium ion rearranging to C-10. An alternative and attractive possibility is the cyclisation of intermediate (245) or (248) to either intermediate (246) or (247), or both. Nevertheless, it seems strange that there are no cadinenes in which there is unsaturation or an oxygen function at C-11.

CHART XII



As suggested by the usual numbering (235), it is possible to consider the cadinenes as formed from a ten-membered ring intermediate as shown in the transformations (249) to (250) and (251) to (252).

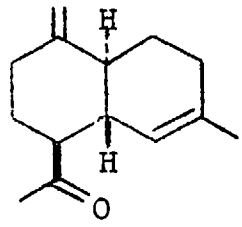


(249) (250)

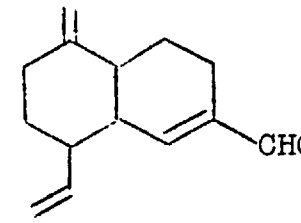
(251) (252)

The resulting intermediates do not explain the almost universal occurrance of unsaturation or oxygenation at both C-4 and C-10 as readily as the bisabolene scheme leading to intermediates (246) or (247). It is also significant that bisabolene and cadinene types of sesquiterpenoids often occur in related species, and sometimes together.

Some sesquiterpenoids have slightly modified cadinene skeletons. These include the two 12-nor compounds, khusitone (253)[191] and khusilal (254),[192]

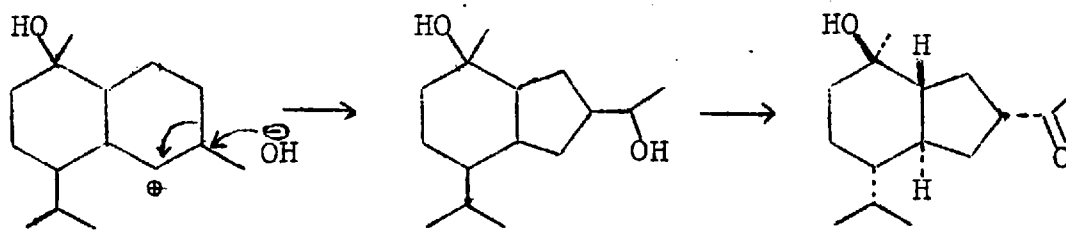


(253)



(254)

both from Vetiveria zizanioides, a prolific source of many different types of sesquiterpenoids. Oplopanone (256)[193] may well be derived from a



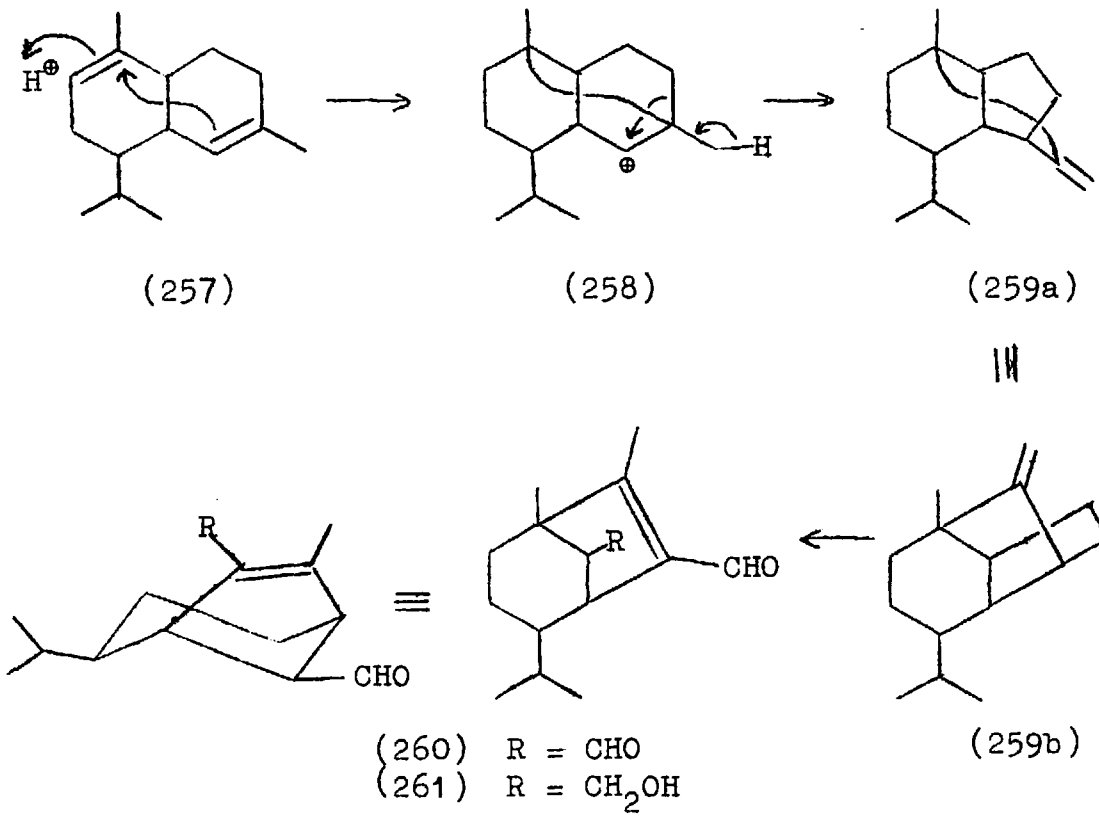
(255) (256)

cadinene such as (255) by the shift indicated, although other schemes can be envisaged.

Helminthosporal group.

Three interesting natural products have been isolated from Helminthosporium sativum (recently

CHART XIII



(257) (258) (259a) ⇌ (259b)

(260) R = CHO
(261) R = CH_2OH

reclassified as Bipolaris sorokinawa). De Mayo[194] isolated helminthosporal (260), the absolute stereochemistry of which was determined by Corey[195] in the course of a synthesis. The natural product appears to be a masked aldehyde, probably similar to an acetal. Sativene (259) was predicted as a biosynthetic intermediate by de Mayo, and subsequently isolated[196] from the same source, as was helminthosporol (261) by other workers.[197] A biosynthetic route similar to that shown in Chart XIII was proposed by de Mayo, and some

experimental support for this scheme is described in Section 2.

Acorone and cedrene groups.

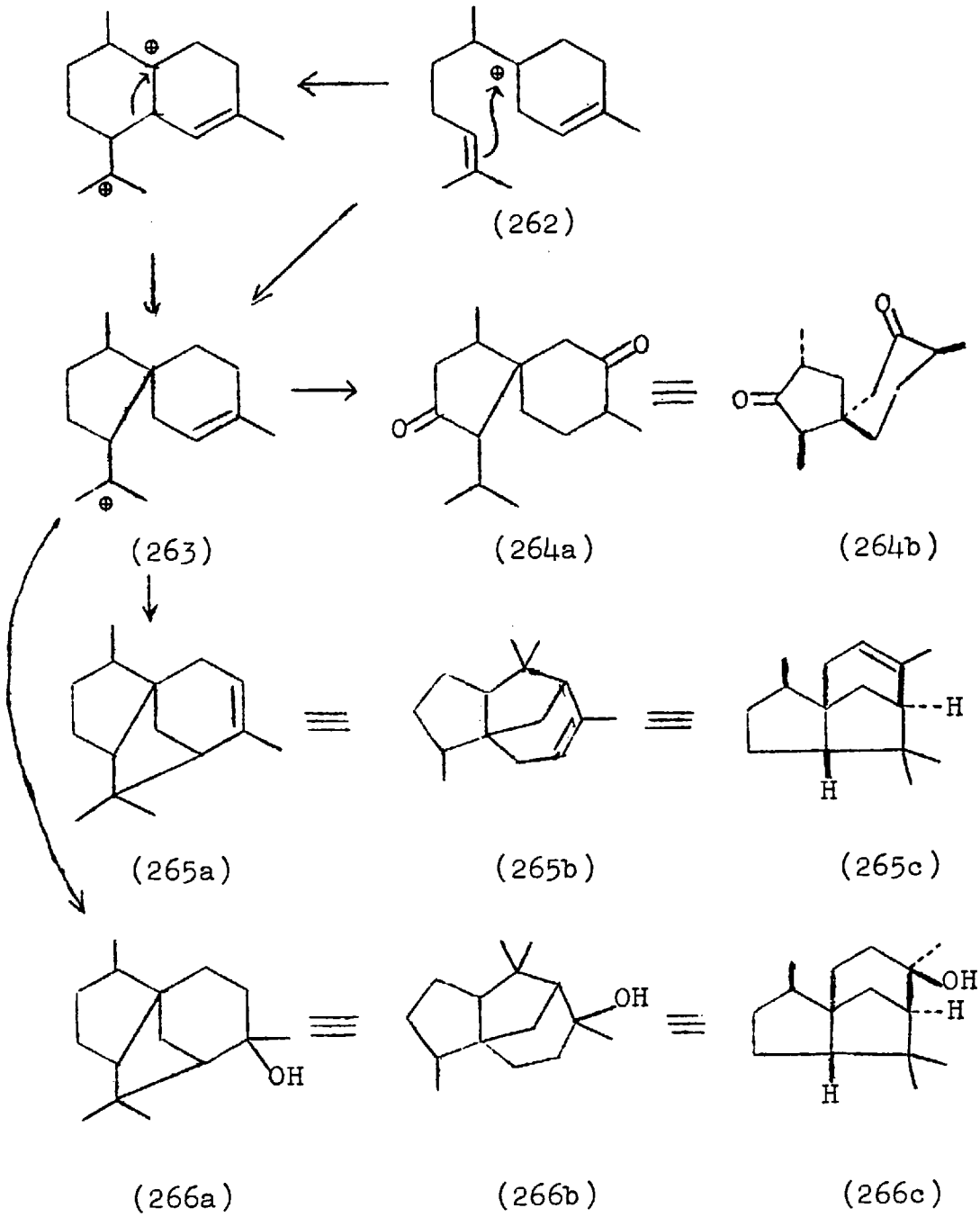
Acorus calamus (sweet flag oil) is the source of a small group of very closely related spiro compounds. The relative configuration of acorone (264),[198] a typical member of the group has been determined by X-ray studies.[199]

An attractive hypothesis for the biosynthesis of both the acorone and cedrene groups is shown in Chart XIV. The tricyclic system of the cedrane skeleton was first recognised in cedrene (265)[200,201,202] and cedrol (266).[201] These compounds, from Juniperus virginiana, have been known for a considerable time - cedrol was first isolated[203] in 1841. A few other examples of this skeleton have since come to light.

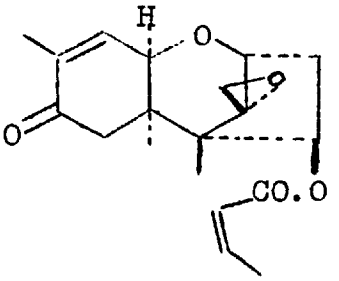
Trichothecin group.

This interesting group of mould metabolites, typified by trichothecin (267)[204,205] and verrucarin A (269)[205,206] and isolated from various Trichothecium, Myrothecium and Fusarium species apparently bears little

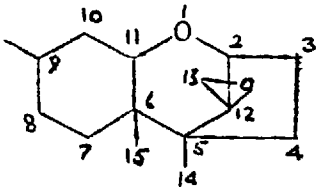
CHART XIV



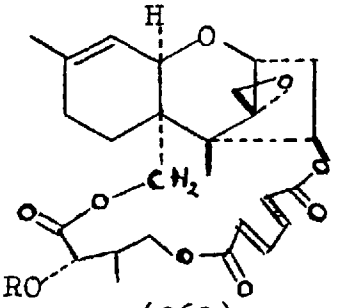
resemblance to a bisabolene type. Nevertheless,



(267)



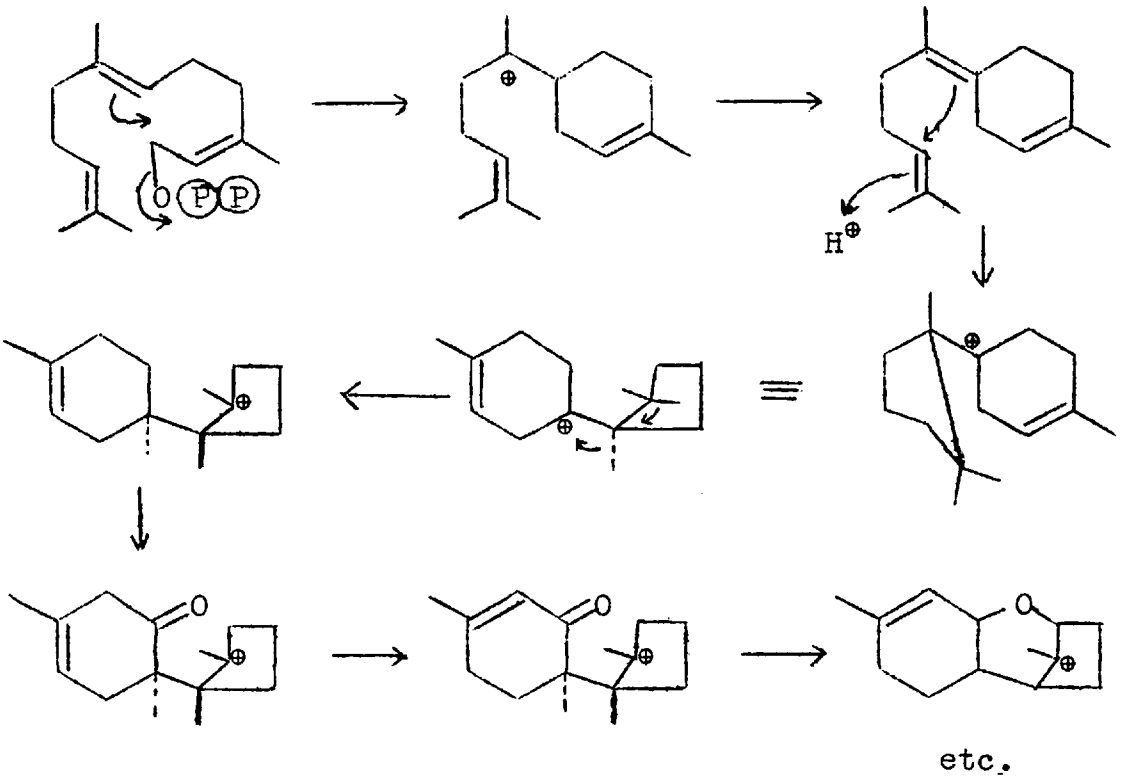
(268)



(269)

evidence is available (see Section 2) to support, not necessarily to the exclusion of others, the scheme shown in Chart XV.

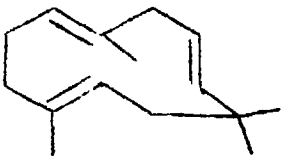
CHART XV



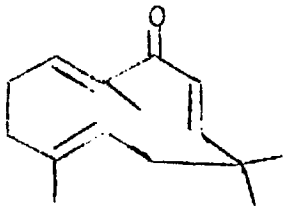
ELEVEN-MEMBERED RING SESQUITERPENOIDS AND OTHERS REGARDED AS DERIVED THEREFROM

Humulene group.

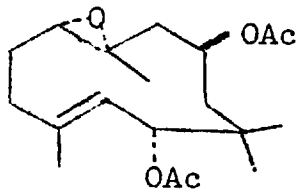
The parent compound of this group is humulene (270), which occurs in hops (Humulus lupulus) and widely elsewhere. There was initially confusion over the position of the "central" double bond, and only recently has an X-ray study[207] confirmed that the structure is (270) with all the double bonds trans.



(270a)



(271)

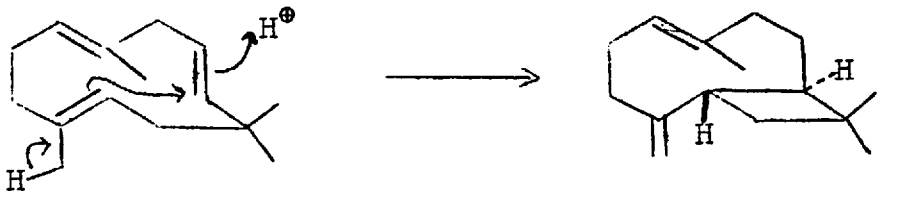


(272)

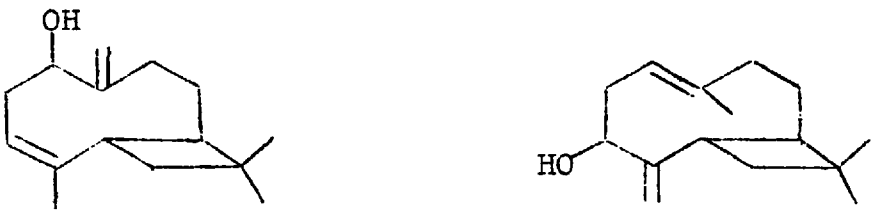
Other eleven-membered ring sesquiterpenoids are zerumbone (271)[208] and caucalol diacetate (272).[209]

Caryophyllene (273),[210] from oil of cloves among many other sources, was the subject of much early work before the final details of the structure were determined by X-ray studies.[211] Biosynthetically, it would appear to be produced by direct cyclisation

of humulene (270b). The same carbon skeleton is exhibited by α-betulenol (274)[212] and β-betulenol (275),[212]



(270b) (273)



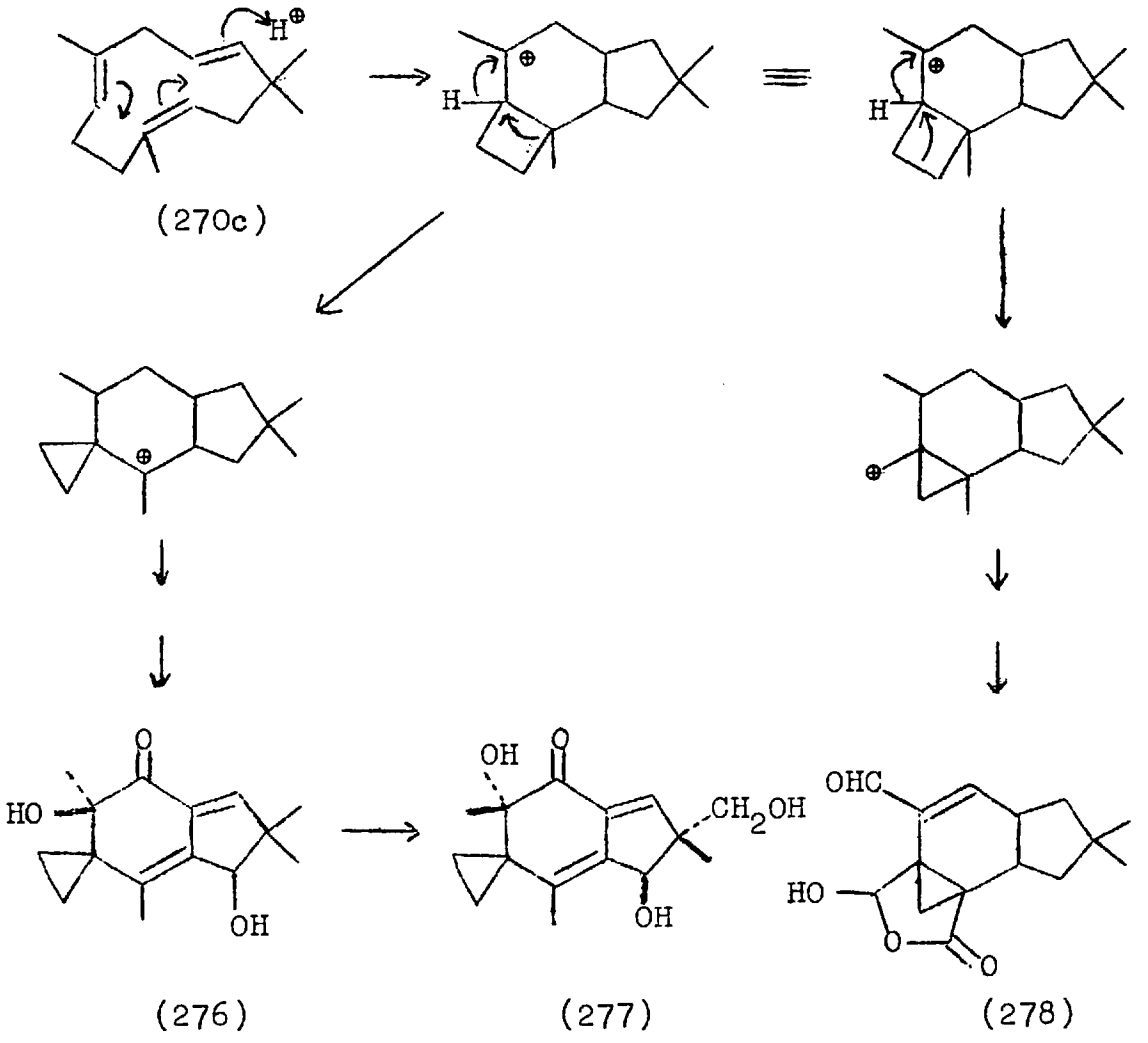
(274) (275)

isolated from white birch buds (Betula alba), which also contain large amounts of caryophyllene monoxide.

Illudins and Marasmic Acid.

Chart XVI gives details of the proposed[213,214] biosynthetic pathways to this group, starting from humulene (270c). Illudin M (276)[213] and illudin S (277)[213,215] have been isolated from the bioluminescent

CHART XVI



mushroom Clitocybe illudens, and illudin S also occurs (lampterol) in Lampteromyces japonicus. Some limited experimental evidence is available (see Section 2) in support of the biogenetic scheme shown for marasmic acid (278),[214] from the mould Marasmius conigenus.

SESQUITERPENOIDS FOR WHICH SEVERAL POSSIBLE BIOSYNTHETIC ROUTES ARE AVAILABLE

Introduction.

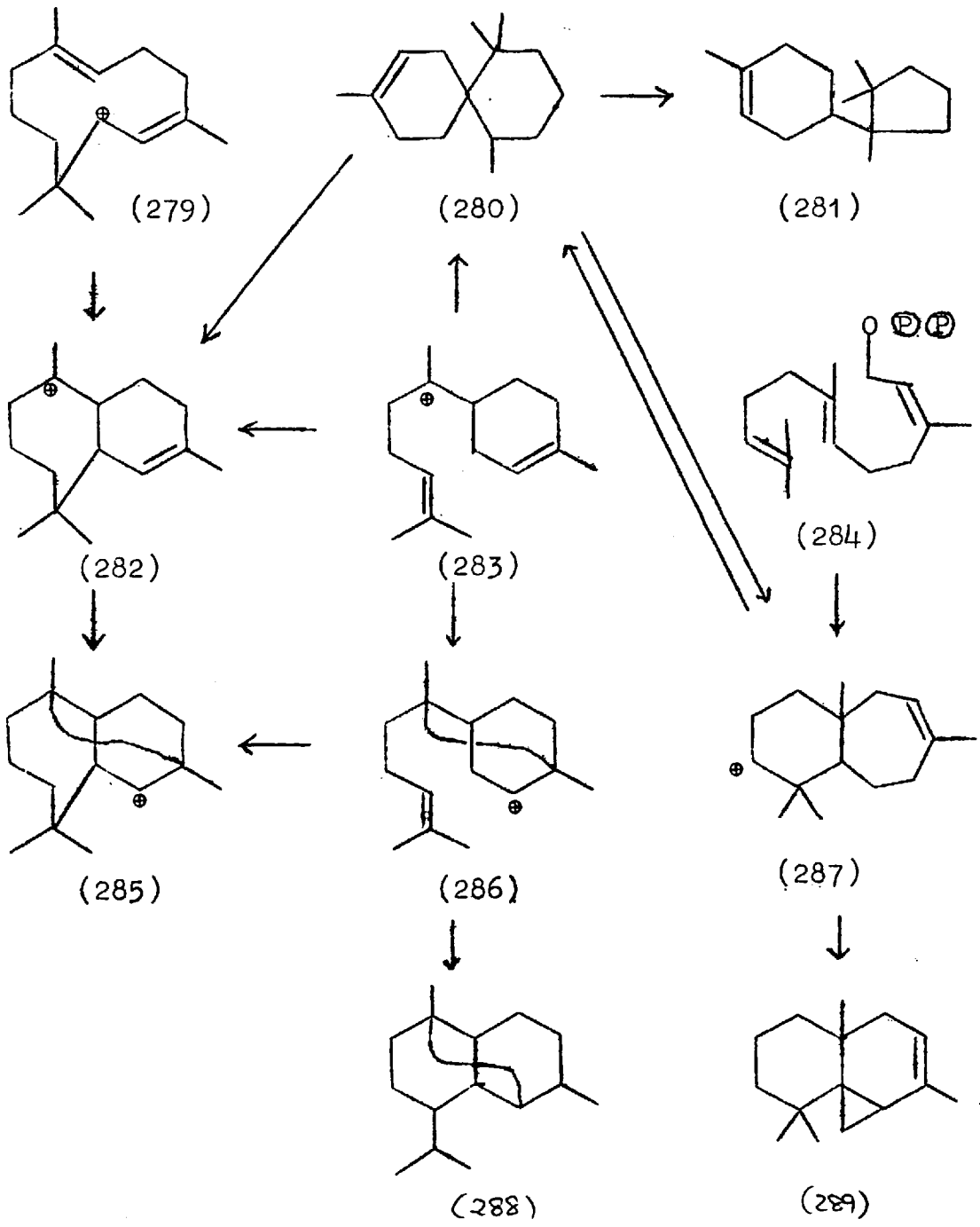
A series of sesquiterpenoid groups are considered together here, as several possible biosynthetic pathways, involving three different initial cyclisations of farnesyl pyrophosphate, seem equally reasonable. The more direct of these pathways are illustrated in Chart XVII. Those sesquiterpenoids which are regarded as uniquely derived from the humulene precursor (279) and from the bisabolene precursor (283) have already been considered.

Copaene, longifolene, santalene and bergamotene groups.

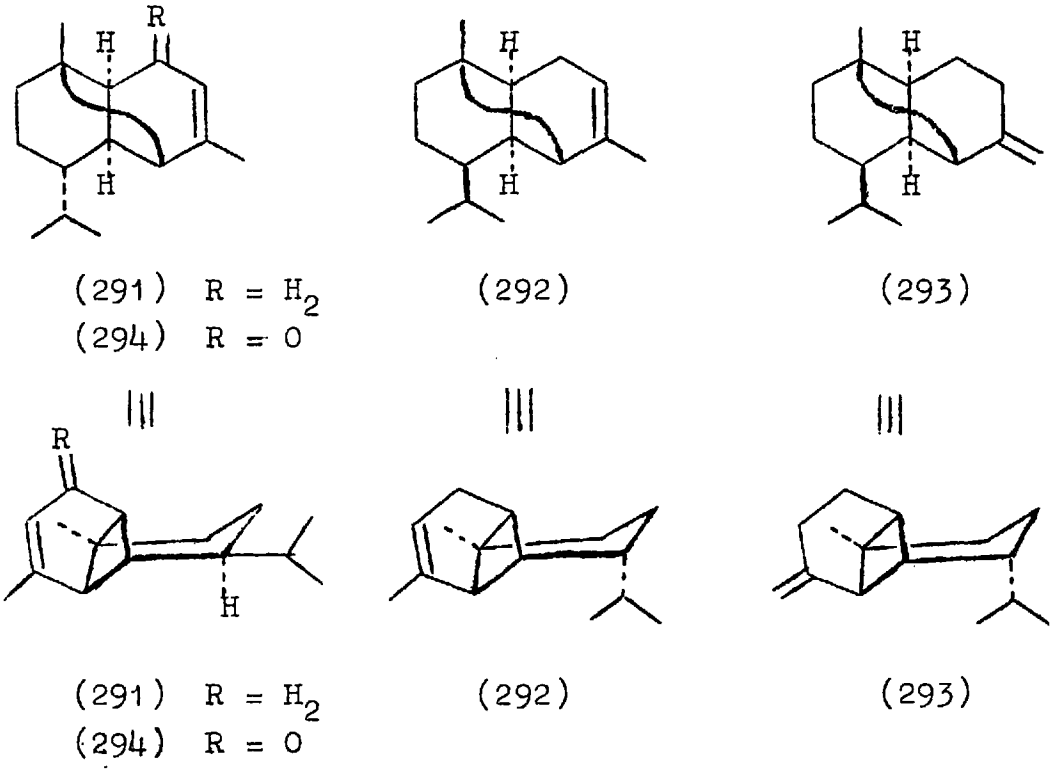
As usually written, these groups have little resemblance to each other. When, however, they are written in the forms shown in Chart XVII - copaene type (288), longifolene type (285) and santalene type (286), with the bergamotene type (305) a variant of the last mentioned - the similarities become apparent.

The four known copaene type sesquiterpenoids are

CHART XVII



copaene (291);[216,217] α-ylangene (292);[218]
β-ylangene (293)[219] and mustakone (294).[218]

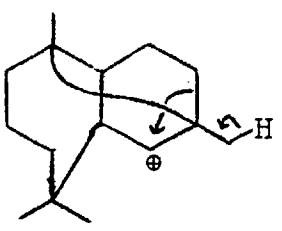


(291) R = H_2 (292) (293)
(294) R = O

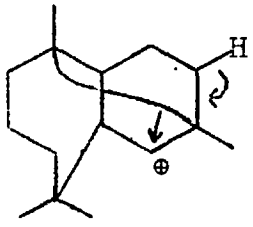
(291) R = H_2 (292) (293)
(294) R = O

In addition to the two possibilities shown in Chart XVII for the biogenesis of this group, there is the obvious, and in some ways attractive, possibility of cyclisation of a cadinene derivative. There has been some dispute as to the stereochemistry of the ylangenes, but it seems that the only difference between their structure and that of copaene is the configuration of the isopropyl group.

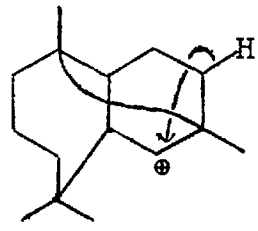
Three sesquiterpenoid hydrocarbons, found in various Pinus species. must arise from an intermediate similar to (285). The rearrangements necessary to produce the three compounds are shown in Chart XVIII.



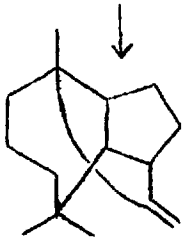
(285a)

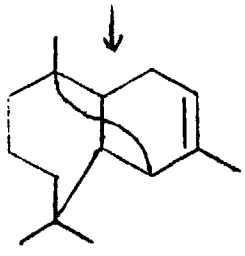


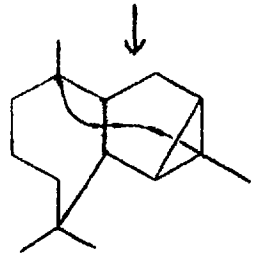
(285b)

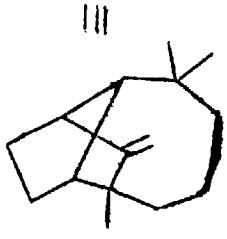


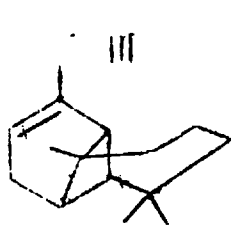
(285c)

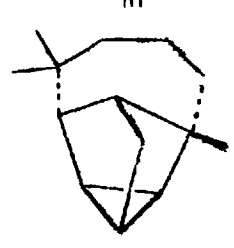






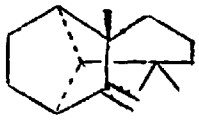






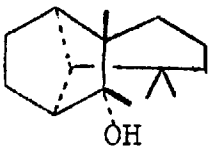
(296)

(297)

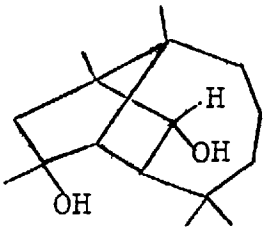


(295)

It has generally been assumed that the intermediate (285) is formed from an eleven-membered ring compound such as (279), but there is no reason for preferring this scheme over one involving the bisabolene intermediate (283). Indeed, the santalenes and bergamotenes can be reasonably derived only from a bisabolene intermediate, and provide perfect analogies for the other processes required to produce the longifolene and longicyclene types. Bisabolene and cadinene sesquiterpenoids are found in trees of genera related to Pinus. Longifolene (295)[220] and α-longipinene (296)[221] are the products of the two possible 1,2-shifts, while longicyclene (297)[222] is formed by direct cyclisation. The alcohols, longiborneol (298)[223] and, from a mould, culmorin (299)[224] are similar.



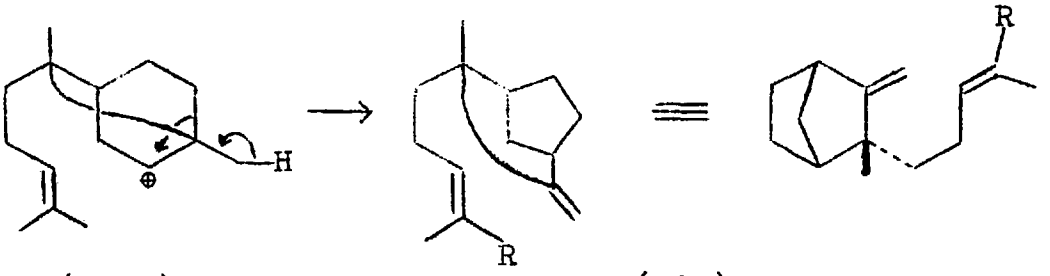
(298)



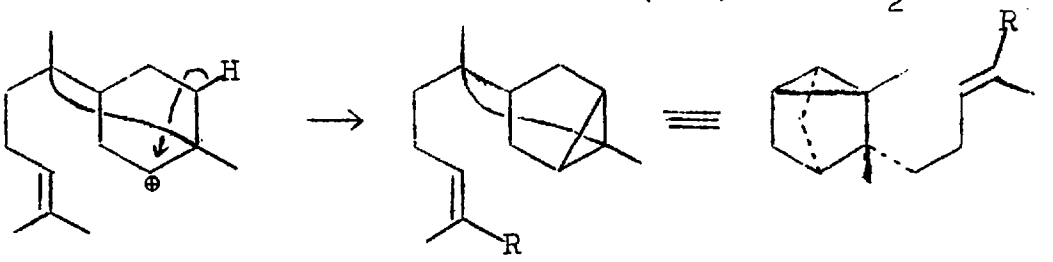
(299)

Ruzicka[4] pointed out that the santalenes obey the isoprene rule, and can be derived (in theory) directly from farnesol. Such a biogenesis, however, involves

cyclisation onto a methyl group. It has been suggested[225] that an intermediate derived from ion (283) - i.e. an ion such as (300) - is involved.



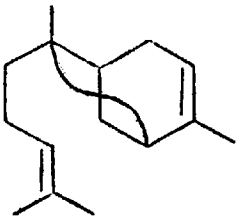
(300a) (301) R = Me; (302) R = CH_2OH



(300b) (303) R = Me; (304) R = CH_2OH

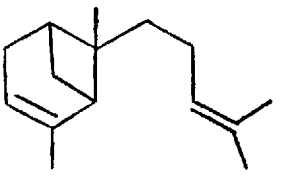
β-Santalene (301)[226] and the alcohol β-santalol (302)[226] are analogous to longifolene, while α-santalene (303)[226] and the corresponding α-santalol (304)[226] are analogous to longicyclene.

The structure of α-bergamotene[227,228] is said to be (305), although full details have yet to be published. The β-isomer has structure (306).[228] These compounds are presumably derived directly from ion (283).

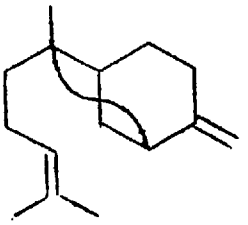


(305a)

≡

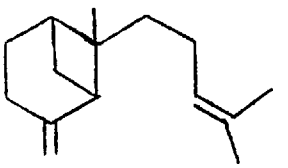


(305b)



(306a)

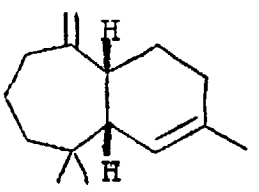
≡



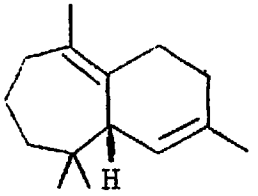
(306b)

Himachalene group and allohimachalol.

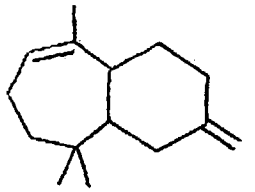
A series of hydrocarbons and alcohols derived from intermediate (282) has been isolated from Cedrus deodara and Schisandra fruits. The hydrocarbons are α-himachalene (307),[229] β-himachalene (308),[229] γ-himachalene (309)[230] and the tricyclic cyclo-himachalene (310).[230] The alcohol allohimachalol (312)[231] is presumably derived from the "normal" himachalene



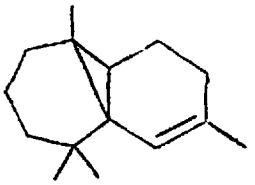
(307)



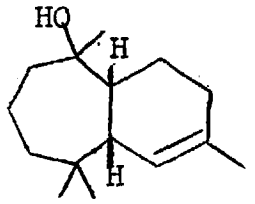
(308)



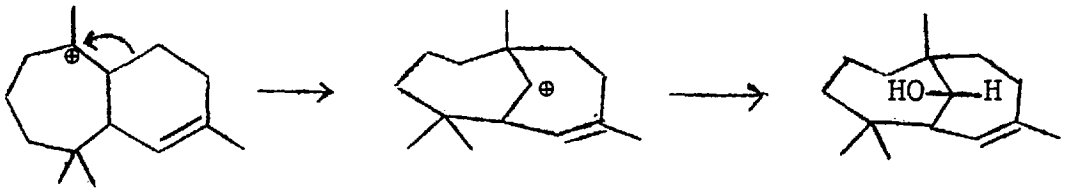
(309)



(310)



(311)



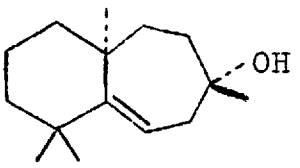
(282) (312)

skeleton by the 1,2-shift indicated. The "normal" himachalol is (311).[229]

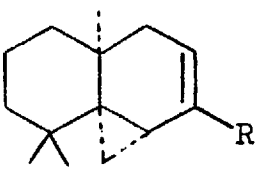
Widdrol type, including thujopsene and other cyclopropanes.

The basic widdrol skeleton (287) may be derived directly from the spiro chamigrene intermediate (280)

or by direct cyclisation of farnesyl pyrophosphate (284). Widdrol (313)[232] and its α-epoxide, from Widdringtonia species are the only sesquiterpenoids with this skeleton, although it occurs with a cyclopropane ring in thujopsene (314)[233] and related compounds such as



(313)

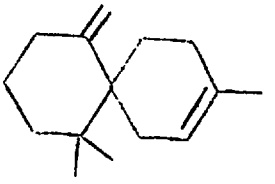


(314) R = Me
(315) R = CO_2H

hinokiic acid (315).

Chamigrene, cuprene, laurene and aplysin types.

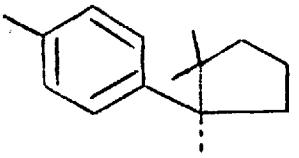
Chamigrene (316)[234] provides a link between the widdrol skeleton, and the cuprene, laurene and aplysin



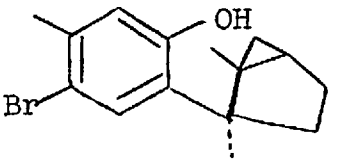
(316)

types, although other possible pathways appear in

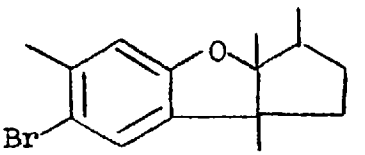
Chart XVII. The subsequent steps in the biosynthesis may proceed through cuparene (317)[235] (cuprene type), laurinterol (318)[236] (laurene type) to aplysin (319).[237]



(317)



(318)

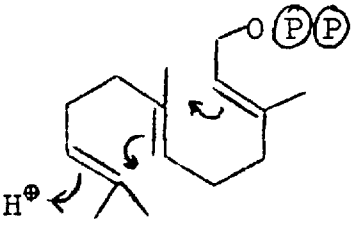


(319)

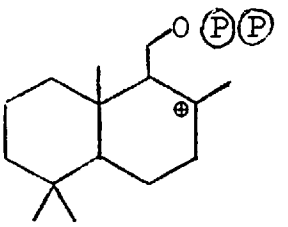
On the other hand, the aplysin group is produced by Aplysia species (mollusc), and the cuprene type by trees such as Chamaecyparis widdringtonia and Thujopsis dolobrata (also containing thujopsene). It may be that aplysin is more closely related to the trichothecin group than to the cuprene and laurene (from trees of Laurencia species) groups.

BICYCLOFARNESOL GROUP

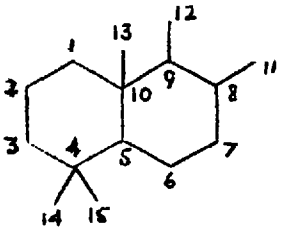
The cyclisation which produces the bicyclofarnesol skeleton (321) is unusual in not involving the



(320)



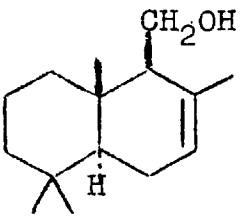
(321)



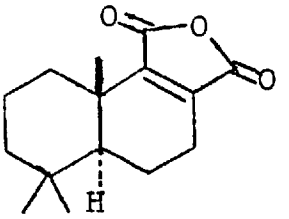
pyrophosphate group of farnesyl pyrophosphate. Cyclisation in the manner shown is preferred over the alternative producing a carbonium ion at C-3, as almost all natural products of this type have unsaturation at C-8, or groups reasonably derived from a positive charge at that centre. The same AB ring system occurs in many diterpenes.

The sesquiterpenoids of this group fall neatly into two antipodal sub-groups. Compounds such as drimenol (323)[238,239] and winterin (324)[239] with the normal triterpenoid (e.g. lanosterol) configuration, have been isolated from Drimys and Polygonum species. The absence of oxygenation at C-3 in most of these is biosynthetically interesting. On the assumption

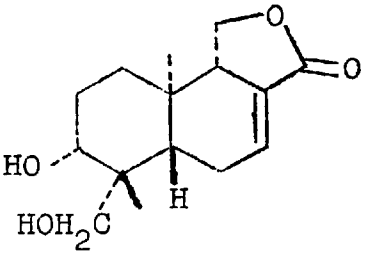
that the direction of electron flow in the cyclisation is such as to require capture of a positively charged species at C-3, as in the currently accepted theory of the cyclisation of squalene to the triterpenoid skeleton, the occurrence of compounds without an oxygen function at C-3 supports the proposal[240] that the initial product of squalene cyclisation is a



(323)



(324)



(325)

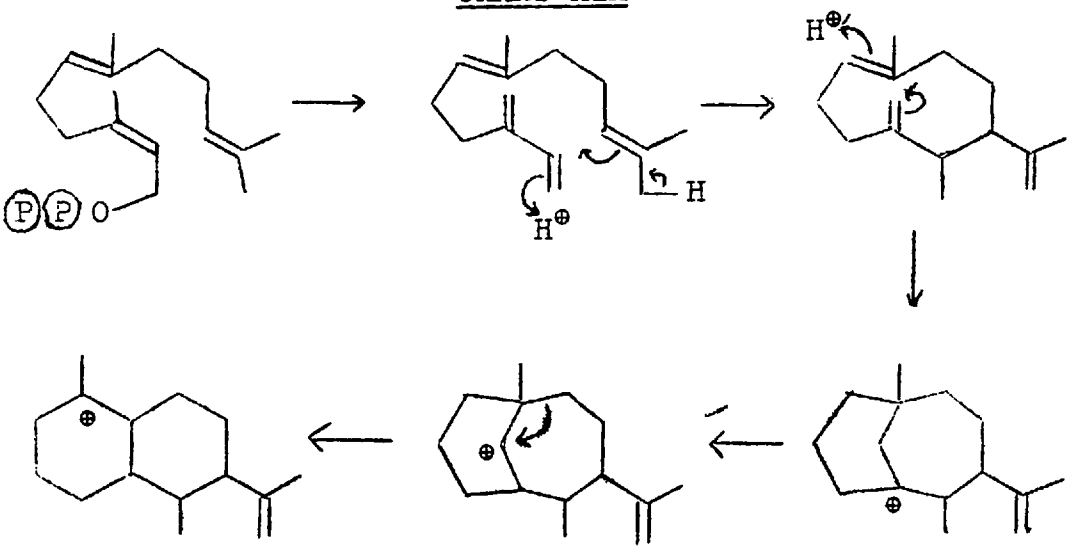
hydrocarbon which is then hydroxylated, rather than capture of the biological equivalent of $OH^{\oplus}$. All the bicyclofarnesol sesquiterpenoids with the antipodal stereochemistry, such as iresin (325),[241] one of a number of similar compounds found in Iresine celesioides, do have an α-hydroxyl or keto group at C-3.

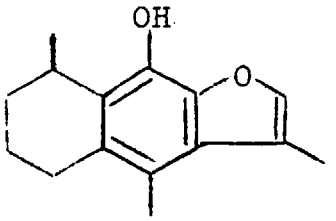
MISCELLANEOUS SMALL GROUPS

Maturin and cacalol group.

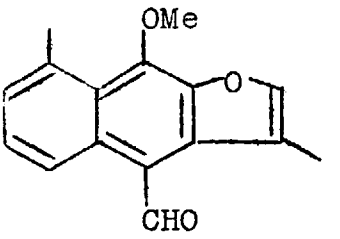
Chart XIX shows a possible sequence leading to compounds such as cacalol (326)[242] and maturinin (327),[243]

CHART XIX





(326)

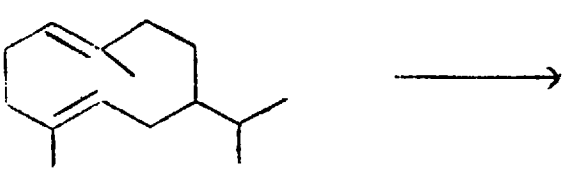


(327)

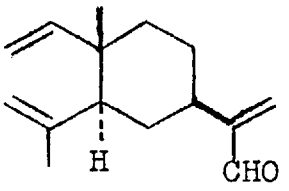
a small group of which occurs in Cacalia decomposita. There are, however, many unsatisfactory features in this scheme, and, indeed, it may even be that these compounds are not sesquiterpenoids, but are acetate and malonate derived.

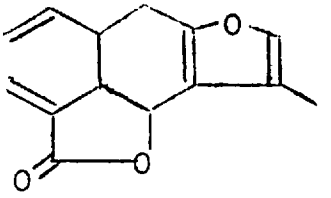
Elemene group.

A Cope reaction on ten-membered ring compounds such as (328) yields compounds of the type (329). A straightforward example of this group is elemenal (330),[78] while a more complex one is isolinderalactone (331).[244] With this group, there must always be doubt



(328) (329)



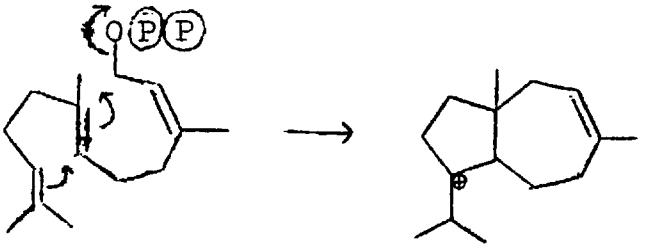


(330) (331)

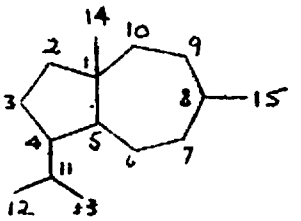
as to whether the compound isolated is an artifact.

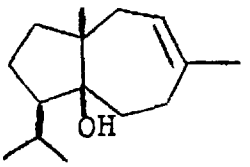
Carotane type.

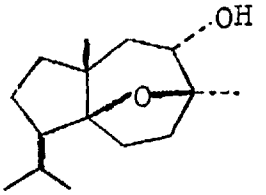
Cyclisation of farnesyl pyrophosphate (332) in the manner indicated, whether concerted or stepwise, results in sesquiterpenoids such as carotol (334)[245] and daucol (335),[245] isolated from carrot seed (Daucus



(332) (333)





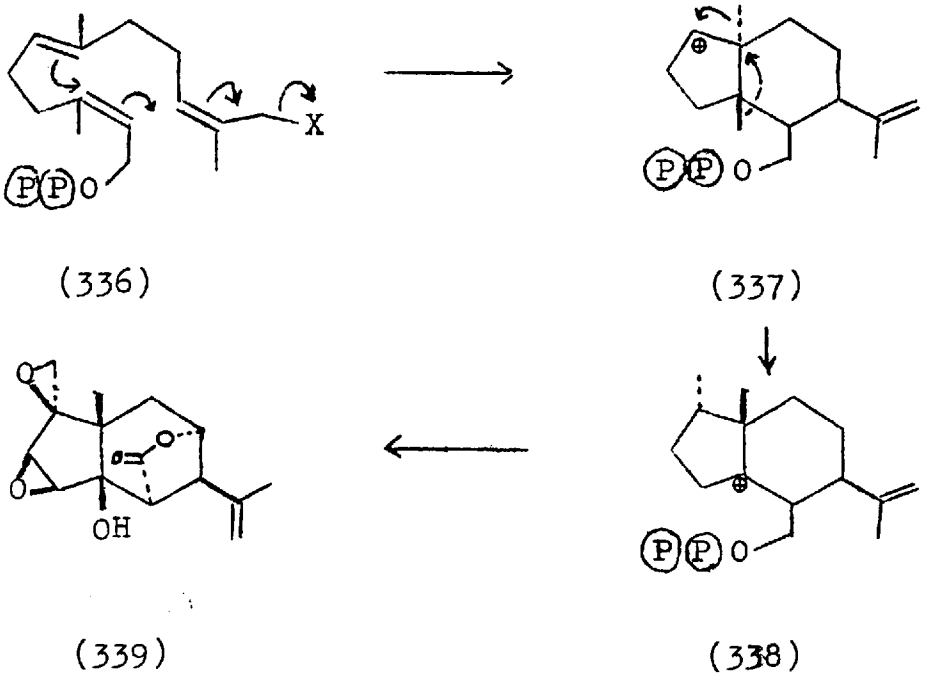


(334) (335)

carota). Some experimental work, reviewed in Section 2, supports the proposed route.

Tutin group.

Four compounds isolated from Hyaenanche globusa (= Toxicodendrum capanse) have basic skeletons derived from (338), and typified by tutin (339).[246]



(336) (337)

(339) (338)

A biogenetic scheme, outlined in structures (336) to (339) resembles that proposed for maturin and cacalol in some respects.

CONCLUSIONS

It will be seen from the preceeding discussions that the biogenesis of sesquiterpenoids can be considered in terms of remarkably few different initial cyclisations of farnesyl pyrophosphate. Particularly noteworthy are the key roles of the ten-membered ring and bisabolene types of intermediates.

SECTION 2

REVIEW OF THE PUBLISHED EXPERIMENTAL INVESTIGATIONS INTO SESQUITERPENOID BIOSYNTHESIS

FARNESYL PYROPHOSPHATE

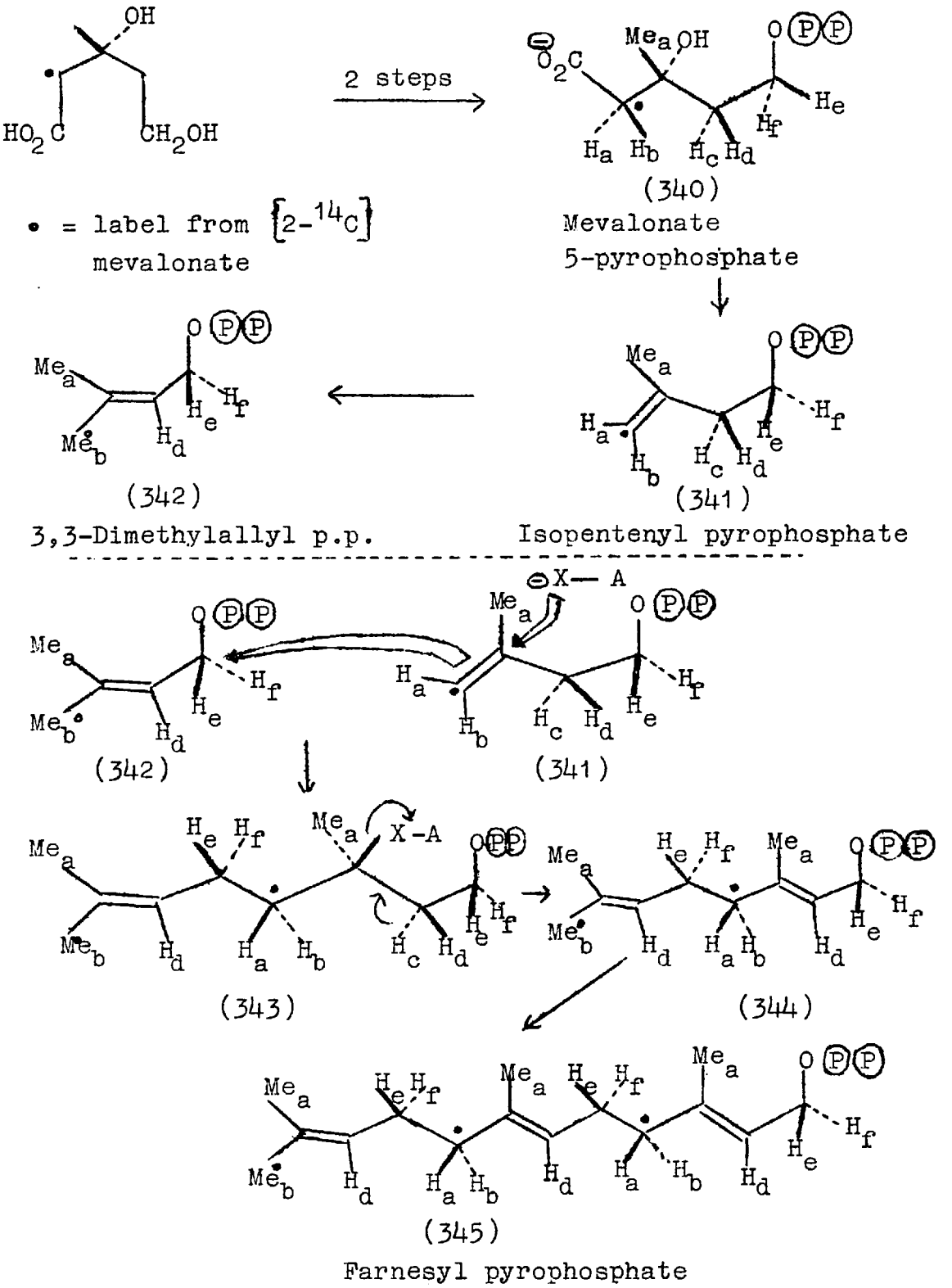
The biosynthetic pathway to farnesyl pyrophosphate has been elucidated in great stereochemical detail by the outstanding investigations of Cornforth and Popják. These workers summarised their findings in their CIBA medal lecture,[247] and the details are shown in Chart XX. Doubly labelled mevalonic acids (tritium or deuterium and ^{14}C) were extensively used in this work.

One step in the scheme shown requires further explanation. The condensation of the isopentenyl (341) and 3,3-dimethylallyl (342) pyrophosphates to give geranyl pyrophosphate (344) is shown in two steps. As the authors remark, it is tempting to consider the process as a concerted one step process, not involving any species such as $AX^{\ominus}$. The additional step is postulated in order to avoid a mechanism involving the addition and withdrawl of electrons from the same side of the double bond.

IPOMEAMARONE

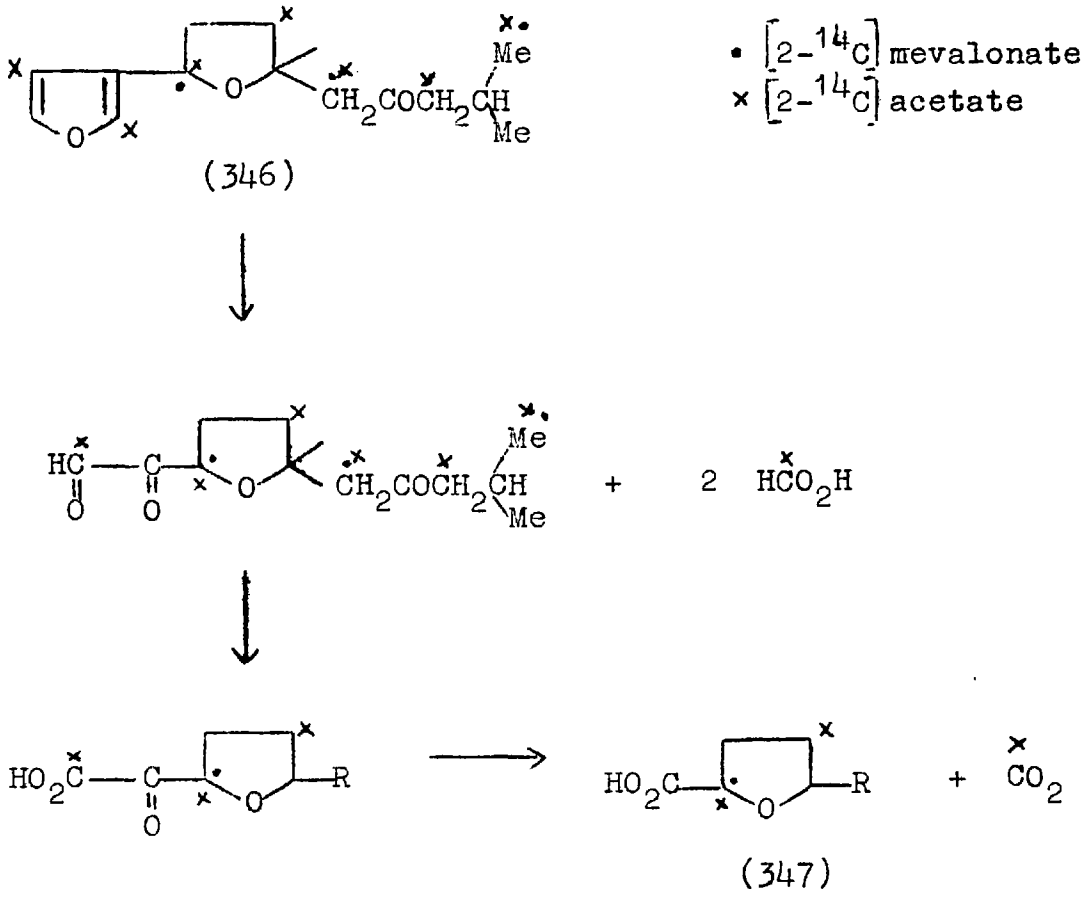
[2-^{14}C]Acetate and [2-^{14}C]mevalonate have been fed to black rotted sweet potato (Ceratocystis fimbriata on Myoporum acuminatum) and incorporations of 11.6 %

CHART XX


2 steps
(340)
Mevalonate 5-pyrophosphate
• = label from [2-14C] mevalonate
(342)
3,3-Dimethylallyl p.p.
(341)
Isopentenyl pyrophosphate
(343)
(344)
(345)
Farnesyl pyrophosphate

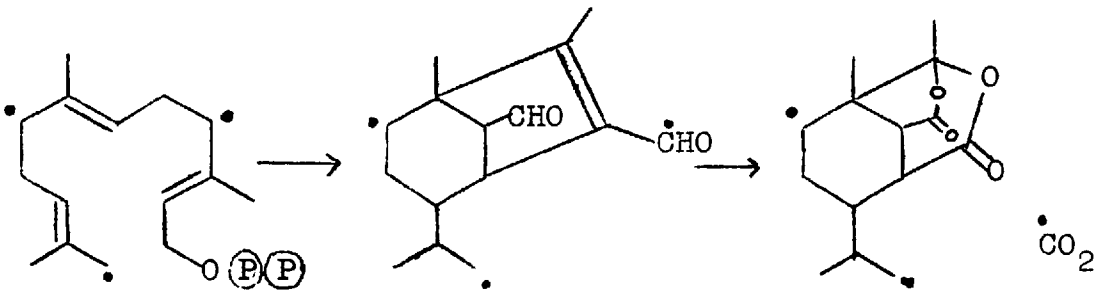
and 48 % respectively into ipomeamarone (9)[248] obtained. The expected labelling patterns are shown in (346). Oxidative degradation (Chart XXI) to the acid (347), carbon dioxide and formic acid proved that one of the labels in the acetate fed ipomeamarone was, as expected, in the β-position of the furan ring, and that another of the labels was in one of the α-positions of the furan.

CHART XXI



HELMINTHOSPORAL

De Mayo and his co-workers[249] have fed [2-^{14}C] mevalonate into helminthosporal. Chart XIII (page 87) details the proposed pathway. [2-^{14}C]mevalonate would then be expected to lead to the labelling pattern shown for farnesyl pyrophosphate (348) and hence helminthosporal (349). Ozonolysis of the active helminthosporal proved that, as predicted,



(348) (349) (350)

one third of the activity was contained in the aldehyde carbon of the αβ-unsaturated aldehyde group.

TRICHOTHECIN GROUP

Coincident with the initial structure determination, Jones and Lowe[250] published details of the incorporation of [2-^{14}C]mevalonate into trichothecin, and of a subsequent degradation. The data have required reinterpretation in terms of the revised

structure (352)[205] of trichothecin. Chart XXII (see also Chart XV, page 90) shows the degradative evidence which leads to the labelling pattern shown in (352) being confirmed. The figures shown in the Chart are relative molar activities. The degradation locates two of the labels unambiguously. The other label must be located either at the indicated carbon (C-10) or

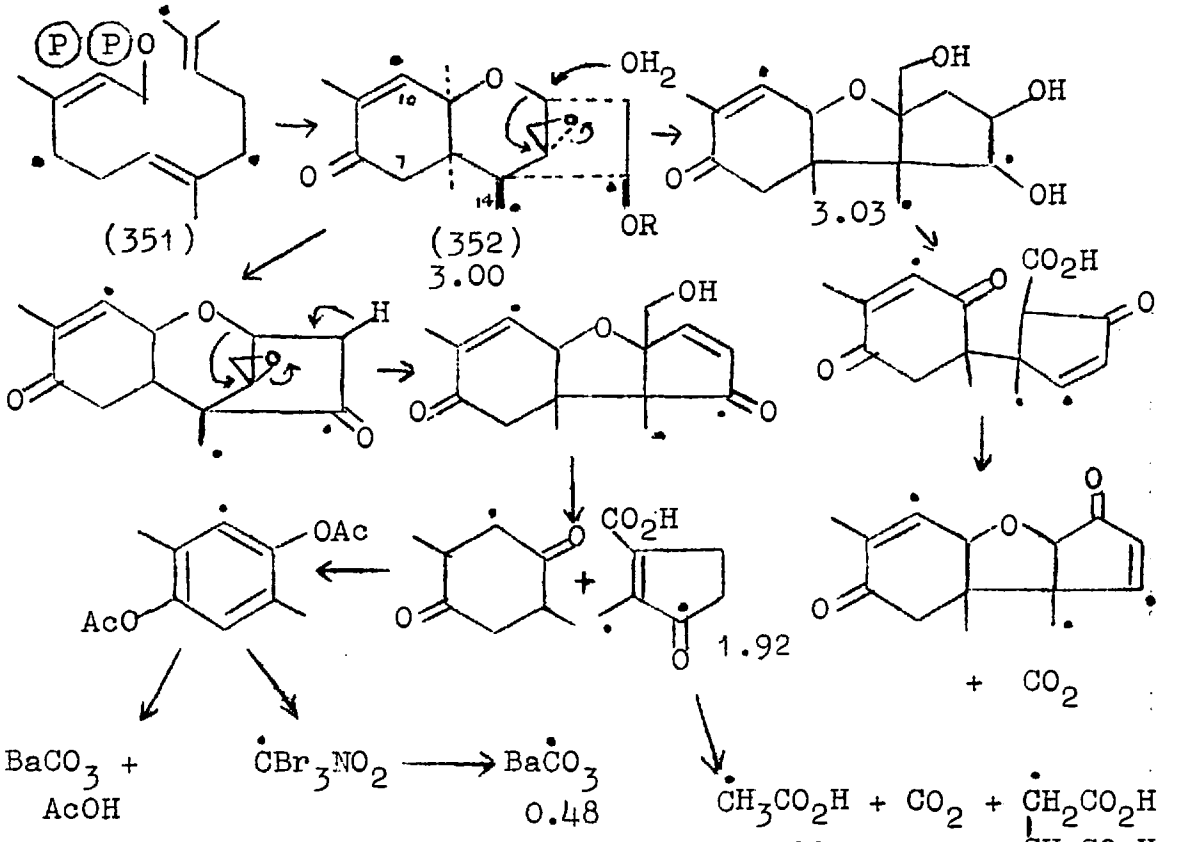


CHART XXII

at C-7, but the latter is extremely unlikely. The label of the 14-methyl group confirms that this methyl is derived from the gem dimethyl of farnesyl pyrophosphate.

Biosynthetic studies[251] with mevalonate and [illegible] acetate into verrucarin A (269), and with mevalonate into diacetoxy scirpenol (353)[252] do not add significantly to the information obtained from the trichothecin work. The isocrotonyl ester of trichothecin (269) is derived from acetate, as, no doubt, are the other complex esters typical of this group of compounds.

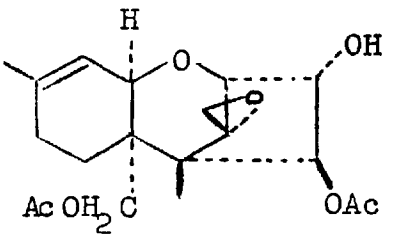
CARYOPHYLLENE

A 0.03 % incorporation of [2-^{14}C]mevalonate into caryophyllene (270) in Nepata catoria has been reported,[253] apparently as a by-product of work on another natural product elaborated by the plant. No degradation was reported.

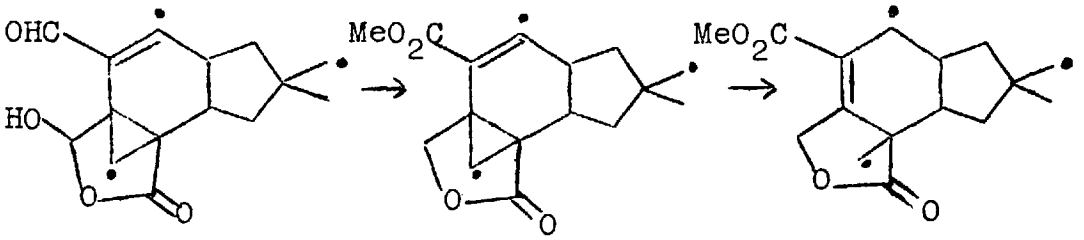
ILLUDINS AND MARASMIC ACID

Chart XVI (page 93) depicts the proposed pathway to these sesquiterpenoids. De Mayo[214] obtained an incorporation of about 0.3 % with [2-^{14}C] mevalonate. Kuhn-Roth degradation of the marasmic acid (354) proved that, when allowance was made for the yield of acetic acid and the fact that only half of it came from the labelled carbon, 33 % of the activity was located in one of the methyl groups

of the gem dimethyl. The marasmic acid was degraded by a Cannizzaro reaction, followed by hydrogenolysis



(353)



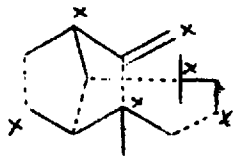
(354) (355) (356)

to the ester (356). Kuhn-Roth degradation of this compound showed that a total of 55 % of the activity was located in the gem dimethyl carbon, plus the cyclopropane CH_2 carbon (or the adjacent carbon). Presumably some scrambling of the label reduced the expected 66 % to 55 %. Nevertheless, the position of these two labels provides strong support for de Mayo's proposed biosynthetic scheme.

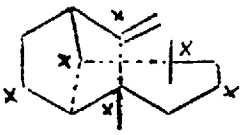
[2-^{14}C]Mevalonate has also been incorporated[213] into illudin M (276) and S (277), but no degradation was undertaken.

LONGIFOLENE

It is possible to construct the longifolene skeleton (357) from three isopentenyl units without rearrangement. The labelling pattern to be expected from the incorporation of [1-^{14}C] mevalonate on such a scheme is shown in (357a). The routes from farnesyl pyrophosphate shown in Charts XVII (page 95) and XVIII (page 97) result in the labelling pattern shown



(357a)



(357b)

in (357b). Sanderman and Bruns[254] have recorded the incorporation of [1-^{14}C] mevalonate into longifolene, and proved by ozonolysis that the carbon of the exomethylene is inactive, thus eliminating the direct route.

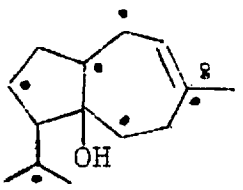
CAROTOL

[1-^{14}C] Acetate would, on the scheme set out on page 108, result in the labelling pattern indicated in

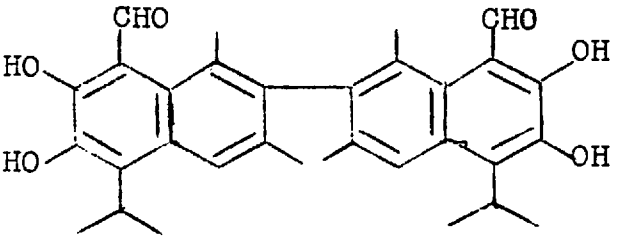
formula (358) in carotol. Součeк[255] has degraded [1-^{14}C] acetatefed carotol, and proved that one of the six labels is located at C-8. This provided further evidence for the structure of carotol, and eliminates any biosynthetic schemes involving ten-membered ring intermediates.

GOSSYPOL

An interesting and thorough investigation of the biosynthesis of the cotton seed pigment, gossypol (359)



(358)



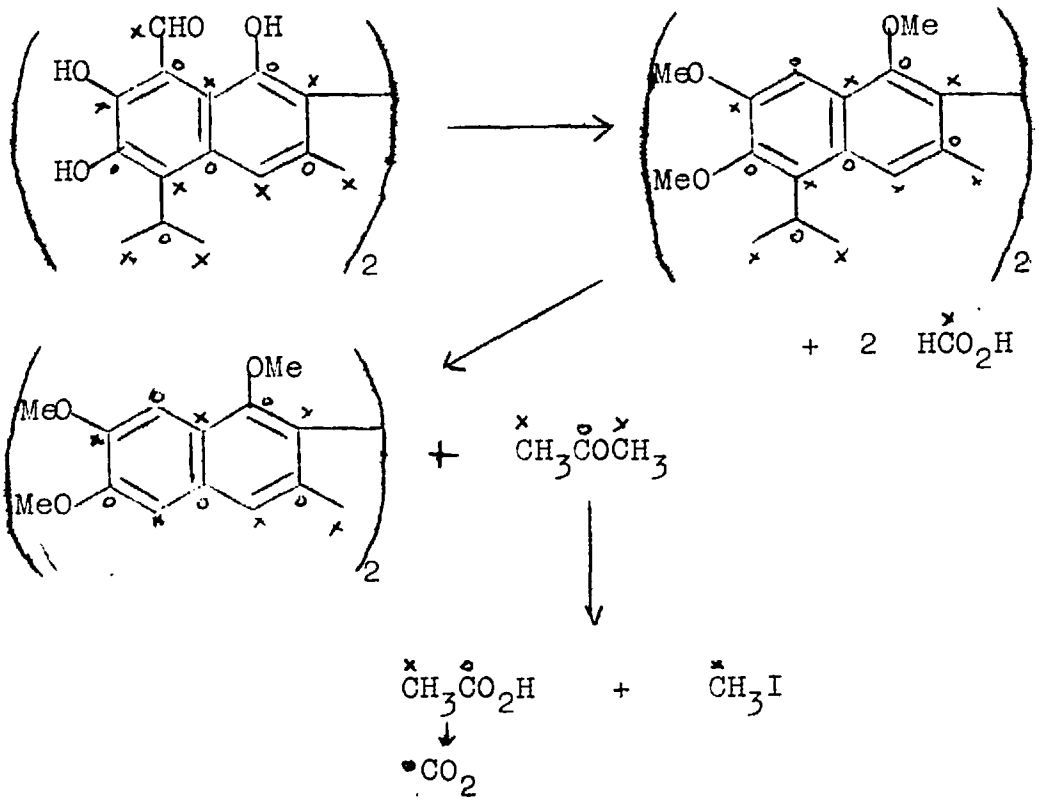
(359)

has been reported by Heinstein et al.[256] While some of the experiments were carried out on intact plants, others were carried out on a homogenate. [2-^{14}C] farnesyl pyrophosphate, [2-^{14}C] mevalonate, and both [1-^{14}C] and [2-^{14}C] acetate were found to be incorporated, mevalonate to the extent of 22 %.

Chart XXIII depicts the labelling patterns expected in the acetate feedings, together with an outline of the degradation used to establish the

positions of the labels in the aldehyde and isopropyl groups.

CHART XXIII



× [2-^{14}C]acetate label.
o [1-^{14}C]acetate label.

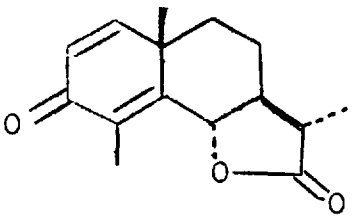
These results confirm that gossypol is indeed a bis-sesquiterpenoid, formed by the phenol coupling two aromatic C_{15} intermediates of the cadinene type.

SECTION 3

BRIEF REVIEW OF THE ISOLATION AND DETERMINATION OF THE STRUCTURE OF SANTONIN

EXTRACTION AND ISOLATION OF SANTONIN

Santonin (l-santonin or α-santonin) (360) occurs extensively in various species of the genus Artemisia. The bitterness of wormwood, an Artemisia species, has long been known, and is mentioned in the Bible,[257] and by Shakespeare.[258] Santonin, the bitter principle involved, subsequently found extensive use in medicine as an anthelmintic until it was replaced by more modern drugs. Its metabolism by humans has not been studied,



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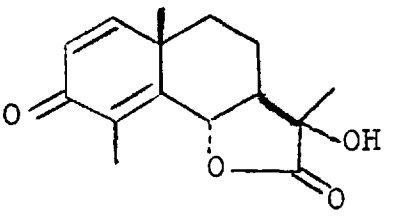


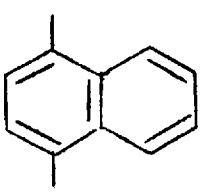
(361)

although it has been shown[259] that it is excreted by dogs as α-hydroxysantonin (361).[260]

The demand for santonin for medicinal use stimulated much research into the best sources and extraction procedures. It was first isolated from wormseed in 1830 by Kahler.[261] Commercial extraction is (or was) carried out principally in Pakistan and Japan, usually from Artemisia maritima or A. kurramensis.

GROSS STRUCTURE

The early investigations into the structure have been described in detail.[262] These investigations soon proved that santonin was an unsaturated ketolactone. A series of experiments by Cannizzaro[263] using zinc dust distillation yielded 1,4-dimethylnaphthalene (362)



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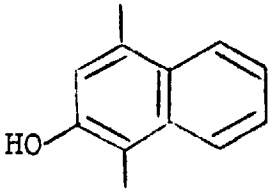

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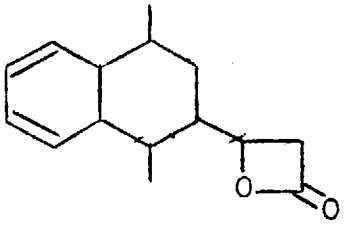
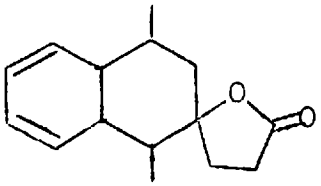


(364)

and some 1,4-dimethylnaphth-2-ol (363). Cannizzaro's structures (364) and (365) were eliminated by Gucci's experiments[264] on santonin oximes. Later, Gucci and Cannizzaro[265] proposed structure (366). Andreocci,[266]



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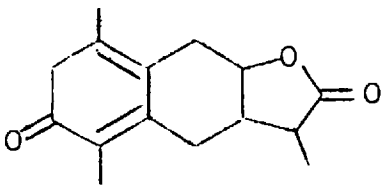

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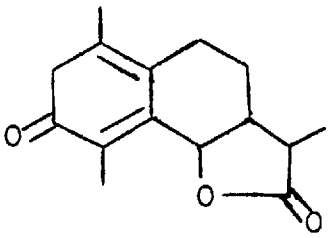
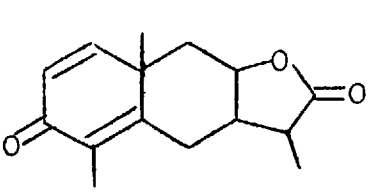


(367)

in supporting this structure pointed out that structure (367) equally fitted the facts. It will

be seen that structure (367), when written in the phenolic form, is that of desmotroposantonin. The lack of phenolic properties in santonin itself, coupled with easy formation of a phenol when santonin is treated with acid led Clemo and his co-workers[267] to the realisation that santonin contained a methyl group at the ring junction, and to their proposal of structure (368). This was supported by a synthesis of santonous acid (369), but proof of the lactone closure was still wanting. Shortly after, Clemo[268] synthesised desmotroposantonin (370), proving that



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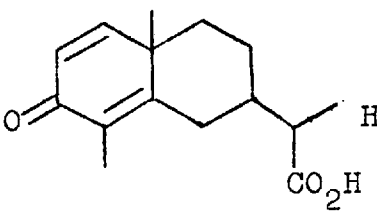

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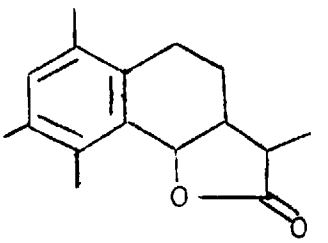
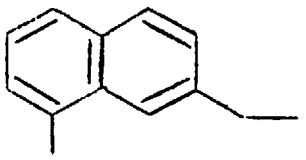


(370)

the lactone was closed to C-6. Further support for the skeleton was provided at almost the same time by the identification of 1-methyl-7-ethylnaphthalene (371) as the dehydrogenation product of hexahydrosantonin (372).[269]



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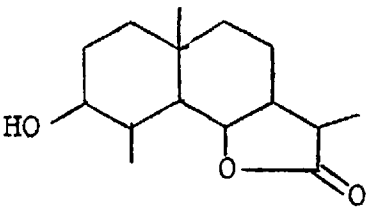


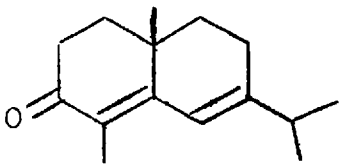
(372)

STEREOCHEMISTRY

The stereochemistry of santonin and related eudesmanes has been reviewed by Cocker and McMurry.[270]

The stereochemistry of the C-7 side chain was determined during the course of Abe and co-workers' total synthesis[271] of santonin as the more stable equitorial β-configuration.

The stereochemistry at C-10 was determined by correlation with β-cyperone (373) by Bruderer, Arigoni



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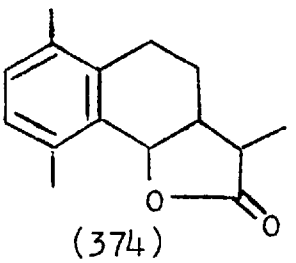


(374)

and Jeger.[272]

The detailed reactions of the desmotroposantonins

and hyposantonins (374)[273] led to the conclusion that the side chain at C-7 and the 6-oxygen were trans, thus establishing the 6α-oxygen configuration.

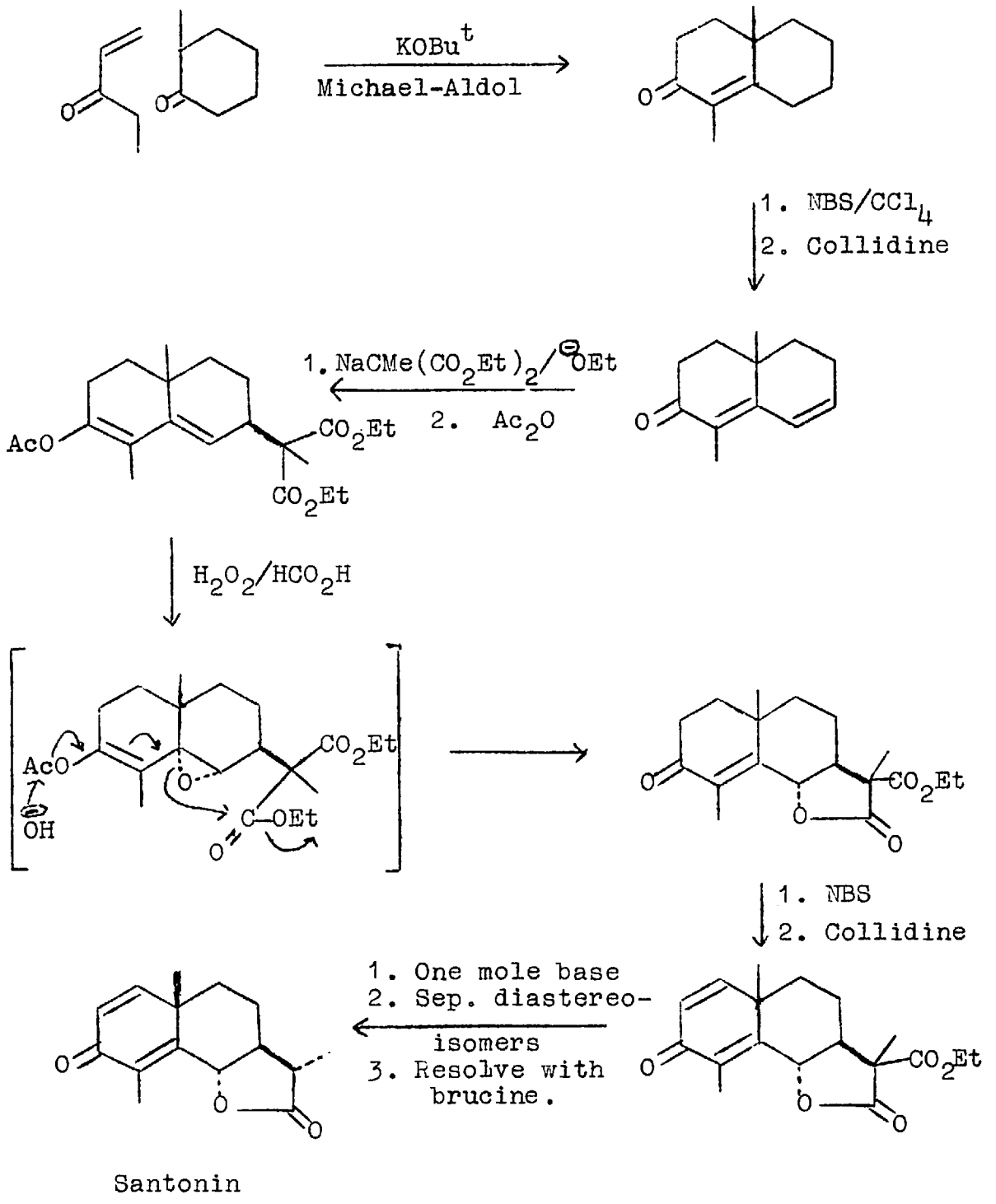
The stereochemistry at C-11 was initially assigned with a β-methyl group on the basis of various stability considerations. Chemical evidence led to doubts being cast on this assignment, and on the basis of chemical[90] and X-ray diffraction[89] studies, the structure was revised to (360). As a result of this, the configuration shown at C-11 in santonin (and compounds which were correlated with it) in papers published prior to 1962 must be amended.

TOTAL SYNTHESIS

The first and most remarkable claim[274] to have achieved, without recourse to asymmetric reagents or syntheses, a stereospecific total synthesis of santonin was immediately challenged,[275] and later shown to be false.

One of the routes employed by Abe and his co-workers[271] is detailed in Chart XXIV. The configurations at C-6 and C-7 were stereospecifically created during the synthesis, but the C-1 and C-11 centres were obtained in racemic form. After separation of the

CHART XXIV



Santonin

diastereoisomers, the final resolution was achieved using brucine.

CONCLUSION

It must be noted that the chemistry of santonin has been investigated to an extent probably unparallelled in any other natural product, with the possible exception of cholesterol. Much of the recent work, especially the stereochemical inter-relations of the santonin derivatives is due to Cocker and McMurry, Dublin.

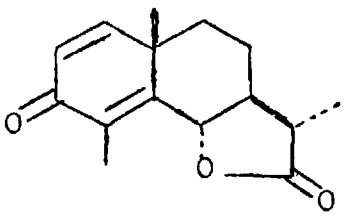
SECTION 4

DISCUSSION OF EXPERIMENTAL WORK CARRIED OUT

ON THE BIOSYNTHESIS OF SANTONIN

PRELIMINARY WORK

The suitability of the available strain of Artemisia maritima as a source of santonin was of fundamental importance to the project. From the extensive literature, and extraction procedure[276] was selected and modified for initial experiments. The aerial parts of one Artemisia maritima plant* were harvested and air dried overnight. The plant material was then crushed in liquid nitrogen, and extracted with cold benzene by stirring for ca. 60 hours. The filtered benzene extract was evaporated to yield a deep green gum. Column chromatography gave 5 mg. of impure santonin. On further purification, 3 mg. of reasonably pure santonin (375) was obtained. This amount proved too small to crystallise,



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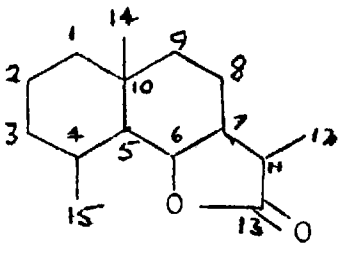


(375a)

but its t.l.c., u.v. and i.r. properties were identical with those of authentic santonin. Various other

* Supplied by Royal Botanic Gardens, Edinburgh.

species of Artemisia were investigated as they became available, but no santonin was detected in any of them.

The plant from which the santonin was isolated was harvested in October. Extensive work connected with the commercial extraction[277] has shown that the santonin content of a plant increases steadily during the period of bud formation (normally July to late August in London), and rapidly decreases when the flowers open. It was reasonable, therefore, to suppose that, during the appropriate period, the santonin content of the plant would be considerably greater. It was decided that feedings should be carried out towards the end of the period of bud formation, in order that the santonin content of the plant would be maximal.

FIRST SEASON

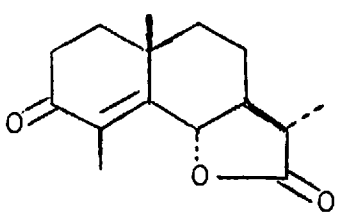
Introduction.

As no successful experimental investigations had previously been carried out on santonin or any related sesquiterpenoid, there was no available indication of a probable route, other than that which could be deduced from considerations of the structures of related natural products, especially those found in

Artemisia species. The only practical starting points for the syntheses of higher precursors were the commercially available santonin, and certain other sesquiterpenoids. There is a report[278] in the literature of the failure of [^{14}C]acetate to be incorporated into santonin in a hydroponic culture of A. maritima.

It was decided to attempt first to determine whether the lactone function was formed at a late stage in the biosynthetic pathway. Various considerations, such as the structures of other known eudesmanolides and possible cyclisation mechanisms led to the selection of 1,2-dihydrosantonin (376), 6,11β(H)-eudesm-4-en-6,13-olide (377) and 5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378) as possible lactonic precursors. Possible acidic precursors considered were 3-oxo-11β(H)-eudesm-1,4-diene-13-oic acid (379), 3-oxo-11β(H)-eudesm-4-en-13-oic acid (380) and 11β(H)-eudesm-4-en-13-oic acid (382).

The nomenclature and numbering adopted here (375a) and throughout the remainder of this thesis are those due to Cahn and Cocker.[279] Where certain simple derivatives of santonin and other sesquiterpenoids have been named in the literature as such (e.g. 1,2-dihydrosantonin), these names are retained in this discussion, although



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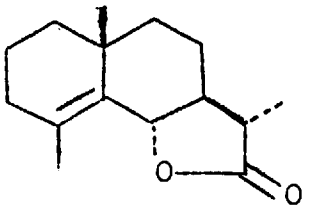
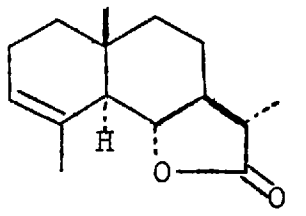
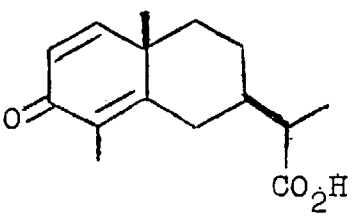


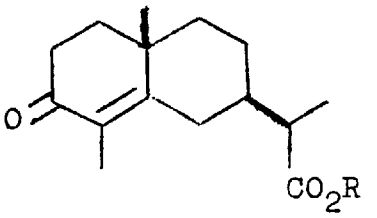
(377)



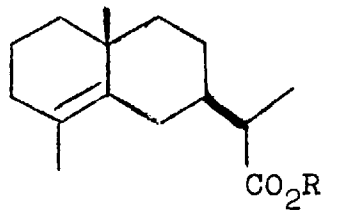
(378)



(379)



(380) R = H
(381) R = Me



(382) R = H
(383) R = Me

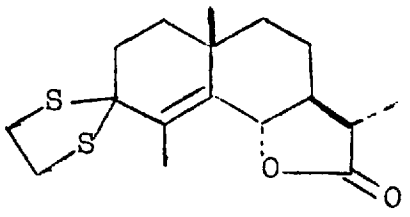
the "systematic" name is also given on first mention in the experimental section.

Preparation of the lactonic possible precursors.

1,2-Dihydrosantonin (376) was prepared by hydrogenation of santonin over Raney nickel, as described by Barton.[280] The structure was readily confirmed by the absence of vinyl protons in the n.m.r. spectrum.

The ethylene thioketal of 1,2-dihydrosantonin (384) was then prepared by treating 1,2-dihydrosantonin with

ethane dithiol and boron trifluoride in glacial acetic acid for one hour. The crystalline thioketal showed the expected spectral properties. Desulphurisation with Raney nickel in refluxing ethanol, however, proceeded with partial concomitant hydrogenation of the double bond. It must be that sufficient hydrogen was absorbed



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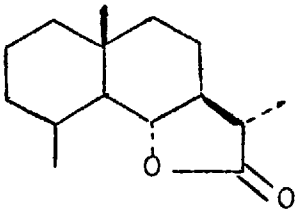
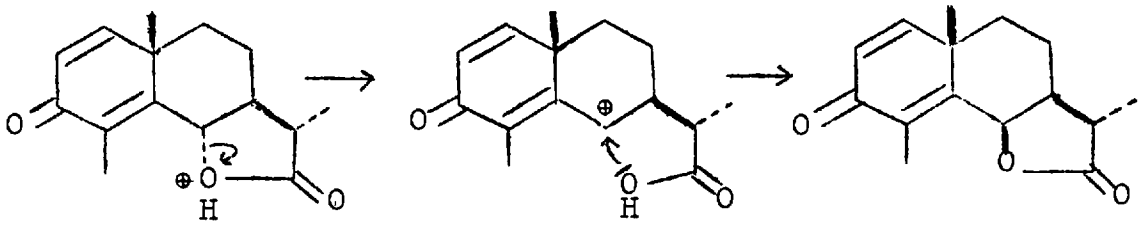


(385)

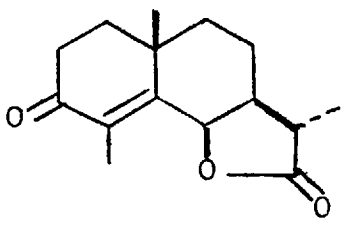
on the Raney nickel to effect partial hydrogenation. Attempts were made to avoid this problem by deactivating the Raney nickel in refluxing acetone, and by change of solvent, but to no avail. Attention was then turned on the problem of separating the saturated compound (385) from the desired product. This was achieved by preparative t.l.c. on Silica Gel GF_{254} impregnated with silver nitrate.[281] By repeated chromatography, a small but adequate quantity of the Δ^4-lactone (377) was obtained.

The use of boron trifluoride in the preparation of

the thioketal made it desirable to prove that C-6 had not been epimerised. The so called "6-epi" (i.e. 6β-oxygen) configuration is the more stable, and is known to be produced by the action of acid on santonin. The mechanism of such epimerisation presumably involves the opening of the lactone with alkyl - oxygen cleavage, and subsequent relactonisation to give the thermodynamically more stable form.



1,2-Dihydro-6-epi-santonin (386) was prepared by treatment of 1,2-dihydrosantonin with 5 % hydrochloric



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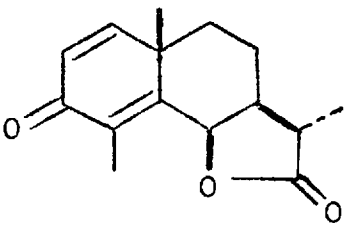


(387)

acid gas in dimethylformamide[282] at 95° for six hours.

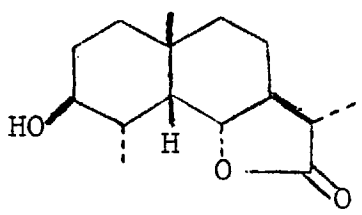
The crude product was purified by chromatography on alumina, and crystallised. The yield was rather disappointing, but the same compound (t.l.c., i.r., u.v. and mixed m.p.) was prepared in 70 % yield by hydrogenation of 6-epi-santonin (387),[282] a supply of which was available.

Treatment of the 1,2-dihydro-6-epi-santonin with ethane dithiol and boron trifluoride produced a new substance, presumably the thioketal, but attempts to crystallise it were unsuccessful.

The n.m.r. spectra of the santonins of the normal and 6-epi series were sufficiently different to allow assignment of the stereochemistry at that centre. In santonin and 1,2-dihydrosantonin, the 6-proton appears as a broad doublet, $J = 9$ c/s, centred at τ 5.20 and τ 5.62 respectively. In the corresponding 6-epi compounds, the signal is a sharp doublet, $J = 5$ c/s, at τ 4.37 and τ 4.57 respectively. Also, in the normal, but not in the 6-epi series, the 4-methyl group is homoallylically coupled[283] ($J = 1$ c/s) to the 6-proton. Detection of this splitting requires a spectrum run under good instrumental conditions on a reasonably concentrated solution. The n.m.r. spectrum of the thioketal (384) showed the 6-proton signal as a broad doublet at τ 5.45, $J = 8.5$ c/s, and the 4-methyl

absorption at τ 7.91 (J = 1.5 c/s). Similarly, the 6-proton of the Δ^4-compound (377) appeared as a broad doublet at τ 5.38, J = 7 c/s, confirming that the configuration at C-6 is unchanged.

5α(H),6,11β(H)-Eudesm-3-en-6,13-olide (Δ^3-lactone) (378) was prepared from santonin as described by Cocker and McMurry.[284] A solution of santonin in glacial acetic acid was hydrogenated over Adams catalyst to give a mixture of the alcohols (388) and (389). The alcohols



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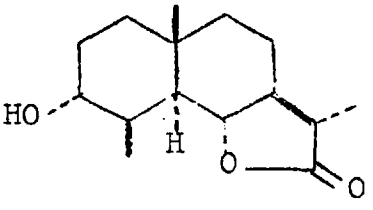

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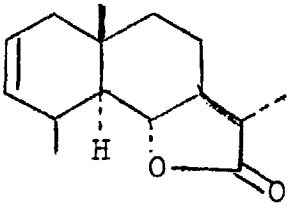


(390)

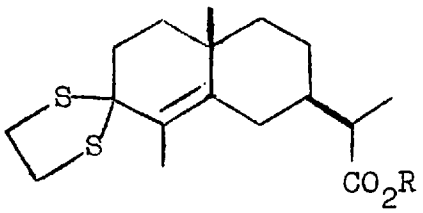
were separated - the <u>cis</u>-fused alcohol (388) is virtually insoluble in ether - and the <u>trans</u>-fused alcohol (389) dehydrated with phosphorus oxychloride in pyridine, to yield a mixture of the Δ^2-lactone (390), and the desired Δ^3-lactone (378). These were separated either by preparative t.l.c. on silver nitrate impregnated silica gel, or by laborious column chromatography on alumina. The overall yield from santonin was 15 %.

Preparation of the acidic possible precursors.

The 6-desoxysantoninic acid (379) was prepared in low yield by reduction of santonin in aqueous acetone with chromous chloride in hydrochloric acid.[285] Neither the acid, nor its methyl ester could be crystallised, so the compound was characterised as its p-phenylphenacyl ester.

The 1,2-dihydro-6-desoxysantoninic acid (380) was conveniently prepared in good yield by Birch reduction of santonin.[272]

Attempts to prepare the 11β(H)-eudesm-4-en-13-oic acid (382) from the unsaturated keto acid (380) by a method similar to that employed in the preparation of the analogous Δ^4-lactone (377) were not persued to a successful conclusion. Preparation of the thioketal (391) proceeded normally, yielding an oil, which was



(391) R = H
(392) R = Me

methylated and purified by chromatography on alumina. The same oil (t.l.c.) was obtained from the methyl

ester (381). The pure methyl ester (392) was heated under reflux with Raney nickel for six hours to produce, in moderate yield, the methyl ester (383) of the desired product. This ester showed the expected n.m.r. and i.r. spectral properties, but could not be crystallised. Preliminary attempts to hydrolyse the ester with potassium hydroxide in dioxan/water mixtures were not successful, probably because the methyl ester is somewhat hindered. Further work on the preparation of the Δ^4-acid had to be postponed until after the first season's feedings. The results of these feedings indicated potentially more profitable lines to persue, and the hydrolysis was abandoned.

Radioactivity determinations.

The actual assay of radioactive compounds was carried out in a coincidence type of liquid scintillation counter, using a commercial xylene-based liquid scintillator. The efficiency of the system was about 25 % for tritium, and about 80 % for ^{14}C. The reproducibility of the instrument was stated to be around 3 %. The background count rate was approximately one count per second (c.p.s.), and maximum assayable rate approximately 1,000 c.p.s. Some of the later

experiments were assayed on an improved counter, with a efficiency of about 35 % for tritium, with a background of about 0.3 c.p.s. Whenever possible, the amounts of compounds to be assayed were chosen such that the count due to the compound was at least equal to that due to background. Occasionally, it was necessary to count at somewhat lower levels, but such results must consequently be treated with more caution.

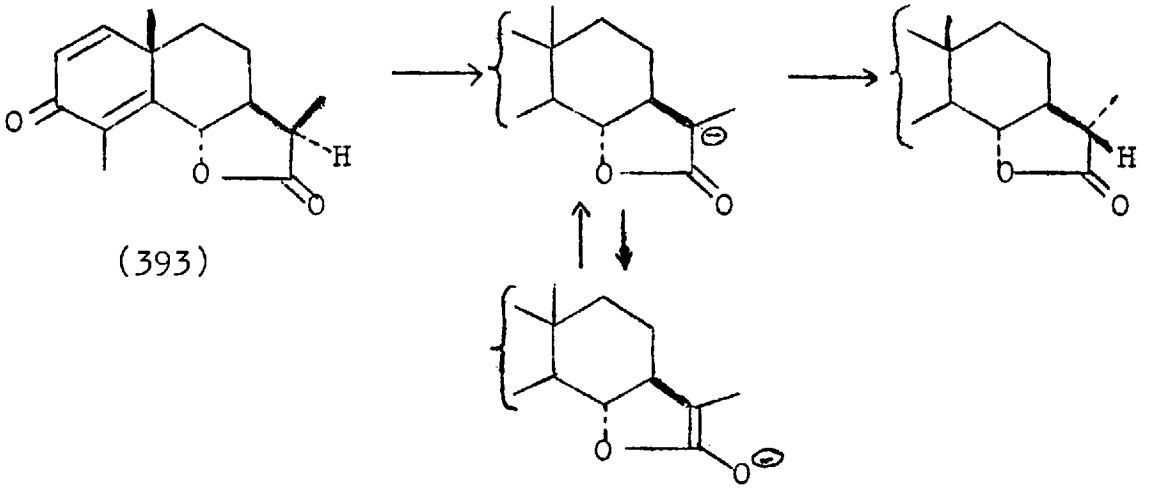
A further complication was added to the radiochemical assay by the phenomenon of quenching. Some compounds, when dissolved in liquid scintillator, affect the emission of light by the scintillator. The net result of the change is to reduce the efficiency of the counter at the usual settings for the isotope concerned. The concentration required to produce significant quenching varies from compound to compound, but, as a general rule, coloured compounds quench to an extent which renders them unusable for the purposes of assay. Quenching is easily detected by adding an appropriate quantity of the substance under test to an already counted tube containing the usual volume of scintillator and a convenient amount of a radioactive standard. A significant drop in count rate indicates the presence of quenching.

Santonin was found to quench to a significant extent at concentrations greater than about 0.5 mg. per 1.2 ml. (the usual volume) of liquid scintillator. It was first determined that the quenching factor Q (observed count/theoretical count) was independent of the activity over the relevant range - 30 c.p.s. to 600 c.p.s. The quenching factors for a series of solutions containing between 0.44 and 1.15 mg. santonin per 1.2 ml. scintillator were determined. The higher figure represents almost the maximum solubility of santonin in the liquid scintillator. A plot of log Q versus concentration of santonin was satisfactorily linear, when due regard was paid to the errors involved in the weighings. This graph was used to correct the activity in all cases where more than 0.5 mg. of santonin was assayed.

In the cases where it was necessary to assay other compounds (usually highly radioactive precursors), concentrations were kept below 0.5 mg. per 1.2 ml. whenever possible, as qualitative experiments showed that most compounds related to santonin exhibited similar quenching. Compounds of high activity were assayed at very small concentrations, generally 0.01 mg. per 1.2 ml. or less, by 100-fold volumetric dilution.

Synthesis of the labelled compounds for feeding experiments.

α-Santonin (375) is apparently thermodynamically more stable than β-santonin (393), as the latter can be converted to α-santonin with potassium carbonate in refluxing xylene.[286] The epimerisation presumably involves loss of and subsequent capture of a proton at C-11.



(393)

Attempts to label the 11-position of santonin by exchange using labelled water must, perforce, be beset by difficulties caused by the use of aqueous base. Such systems open the lactones, yielding the anion of the hydroxy acid. The carboxylate anion must effectively prevent any anion formation at C-11. Exchange at C-11 must depend on the presence of at least small concentrations of the closed form. This analysis indicated the advisability of using only a catalytic quantity of base, and certainly less that one molar

equivalent.

Experiments were carried out on santonin using fully deuterated potassium deuteroxide (conveniently prepared from potassium t-butoxide and deuterium oxide). It was found that ca. ½ molar equivalent of base in a dioxan/deuterium oxide (ca. 5:1) mixture resulted in complete exchange of the 11-proton on heating in a sealed tube at 100° for about 24 hours. It was later found that heating for longer periods did not substantially alter the result, or cause significant decomposition. Weaker bases such as triethylamine and pyridine were ineffective. About 75 % or more of the santonin was recovered as α-santonin m.p. 171-172°, and the remainder as a higher melting mixture of α- and β-santonins.

The extent of deuteration was determined by n.m.r. spectroscopy. While the 11-proton signal cannot be distinguished, the 11-methyl group is split by that proton. Thus, complete deuteration resulted in the collapse of the doublet due to the 11-methyl group to a singlet. Some splitting is observed, as the deuteron does couple, but the coupling constant is about one sixth of that for a proton.

Tritiated santonin ([11-^{3}H]santonin) was prepared several times using tritiated water under conditions

otherwise identical to those employed in the successful deuteration, but the maximum exchange ever achieved was ca. 60 % of the theoretical, based on the stated (approximate) activity of the water. It seems likely that this was due to an isotope effect or effects, although the magnitude is larger than normally encountered. Any such isotope effect was absent in the deuteration experiment, which was carried out on a fully deuterated system.

The specific activity of the santonin was determined by one hundred fold dilution of a small quantity with inactive santonin, and crystallisation of the diluted material to constant activity. The active santonin was recrystallised until two successive dilutions were in agreement.

[11-^{3}H]1,2-dihydrosantonin was prepared from tritiated santonin as described for the inactive compound. Purification was achieved by repeated preparative t.l.c. on silica, using a solvent system which had been proved to separate santonin from 1,2-dihydrosantonin. A check was made to confirm that no unchanged tritiated santonin had been carried through the purification procedure. A small weighed quantity of [11-^{3}H]1,2-dihydrosantonin was added to a

convenient weighed quantity of inactive santonin, and the santonin separated from the dihydrosantonin by the same preparative t.l.c. procedure. When the santonin was assayed, it was found that there was less than 0.004 % tritiated santonin in the tritiated dihydrosantonin. This figure was taken into account when assessing the (lack of) incorporation of 1,2-dihydrosantonin into santonin in the plant.

The Δ^4-lactone (377) and Δ^3-lactone (378) were each tritiated in the 11-position by exchange with potassium hydroxide in tritiated water/dioxan. The tritiated compounds were purified by t.l.c., crystallised, and the m.p.s checked. As the m.p.s were unchanged, it is extremely unlikely that any epimerisation at C-11 could have occurred.

The 6-desoxysantoninic acid (379) was tritiated in the 2-position by direct exchange with a high concentration of base in tritiated water/dioxan[287] at 100°. N.m.r. studies[287] on santonin deuterated under similar conditions showed no vinyl proton signal from the C-2 "proton", indicating complete deuteration at C-2. The acid was separated from neutral and basic impurities, and finally purified by chromatography on silica plates.

[11-^{3}H]1,2-Dihydro-6-desoxysantoninic acid (380) was prepared from tritiated santonin as described for the inactive compound, after a trial run with "dilute" tritiated santonin to ascertain how much of the label was washed out by the procedure. Ca. 75 % of the label was retained.

Feeding experiments.

For feeding, lactonic compounds were dissolved in an excess of sodium hydroxide (with as little heating as possible), forming the corresponding sodium salts. The solutions were then neutralised to pH 7 with hydrochloric acid to yield the sodium salt of the hydroxy acid in a sodium chloride solution. The acidic compounds were similarly solublised as their sodium salts.

The use of warm alkali to dissolve the lactones necessitated a check that this procedure did not wash out the label. Some dilute tritiated santonin was treated with warm sodium hydroxide solution until it dissolved, and then for three hours more. The mixture was allowed to cool, left for 12 hours, and then neutralised to pH 7. After seven days, the santonin was recovered by acidification, and purified. On assay,

the santonin was found to have retained at least 86 % of the label. Thus, conditions more severe than those actually employed in dissolving the lactones actually used in the feeding experiments, washed out less than 15 % of the tritium. It was not possible to obtain the equivalent figure for the compounds fed, but it seems certain that the loss of label was less than 15 %. The loss of label in this manner was ignored in the incorporation calculations.

Each solution for feeding - generally containing about 5 mg. of the precursor in up to 10 ml. solution - was fed in late August to an Artemisia maritima plant using the wick technique.[288] Uptake of the solution and washings took about three days. The plants were allowed to grow for a further three days, and then harvested. The santonin (ca. 30 mg. from an average plant) was extracted as previously described, and purified by preparative t.l.c. and crystallisation to constant activity.

The results of the feeding experiments are summarised in Table 2. In the two cases where an incorporation was found, the remaining santonin from the plant was converted to its oxime, crystallised to constant activity and assayed. This served to check

TABLE 2

Precursor	Activity 10^{-2} μC/mM. Santonin	Oxime	After wash out	Incorporation
1,2-dihydrosantonin (376)	Nil	-	-	Nil
6,11β(H)-eudesm-4-en-6,13-olide (377)	3.51	-	-	0.017_7 %
5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378)	3.10	3.28	Nil	0.030_7 %
6-desoxysantoninic acid (379)	Nil	-	-	Nil
1,2-dihydro-6-desoxy-santoninic acid (380)	Nil	-	-	Nil
[2-^{14}C] mevalonic acid lactone	Nil	-	-	Nil

that the activity was not caused by an impurity co-crystallising with santonin.

Experiments with dilute tritiated santonin proved that the label could be completely washed out by treating santonin, or its oxime, with base under the exchange conditions, using, of course, isotopically normal water. The remaining santonin oxime from each incorporation was treated under these conditions, repurified, and reassayed.

The incorporations recorded in the table are low but clear cut, at least for the Δ^3-lactone. The attempted oxime preparation from the Δ^4-lactone fed

santonin failed through paucity of material. All the oxime had been consumed before the activity had become constant.

Mevalonic acid is undoubtedly a precursor of farnesyl pyrophosphate, and hence santonin. It was, therefore, both surprising and disturbing that [2-^{14}C] mevalonate was not incorporated. The incorporations recorded for the Δ^3- and Δ^4-lactones are very low, and it may be that there are barriers effectively preventing almost all of the precursor from reaching the site of synthesis. Mevalonic acid is the presumed precursor of all terpenoids, not merely the sesquiterpenoids. If the plant were synthesising large amounts of other terpenoids and carotenoids at the same time as santonin, then it follows that much of the mevalonic acid which did reach the correct site would have been used in the synthesis of terpenoids other than santonin. In vivo, mevalonic acid is almost certainly not present as a lactone, and this may have been a further disadvantage. A further discussion appears under the second season's feedings.

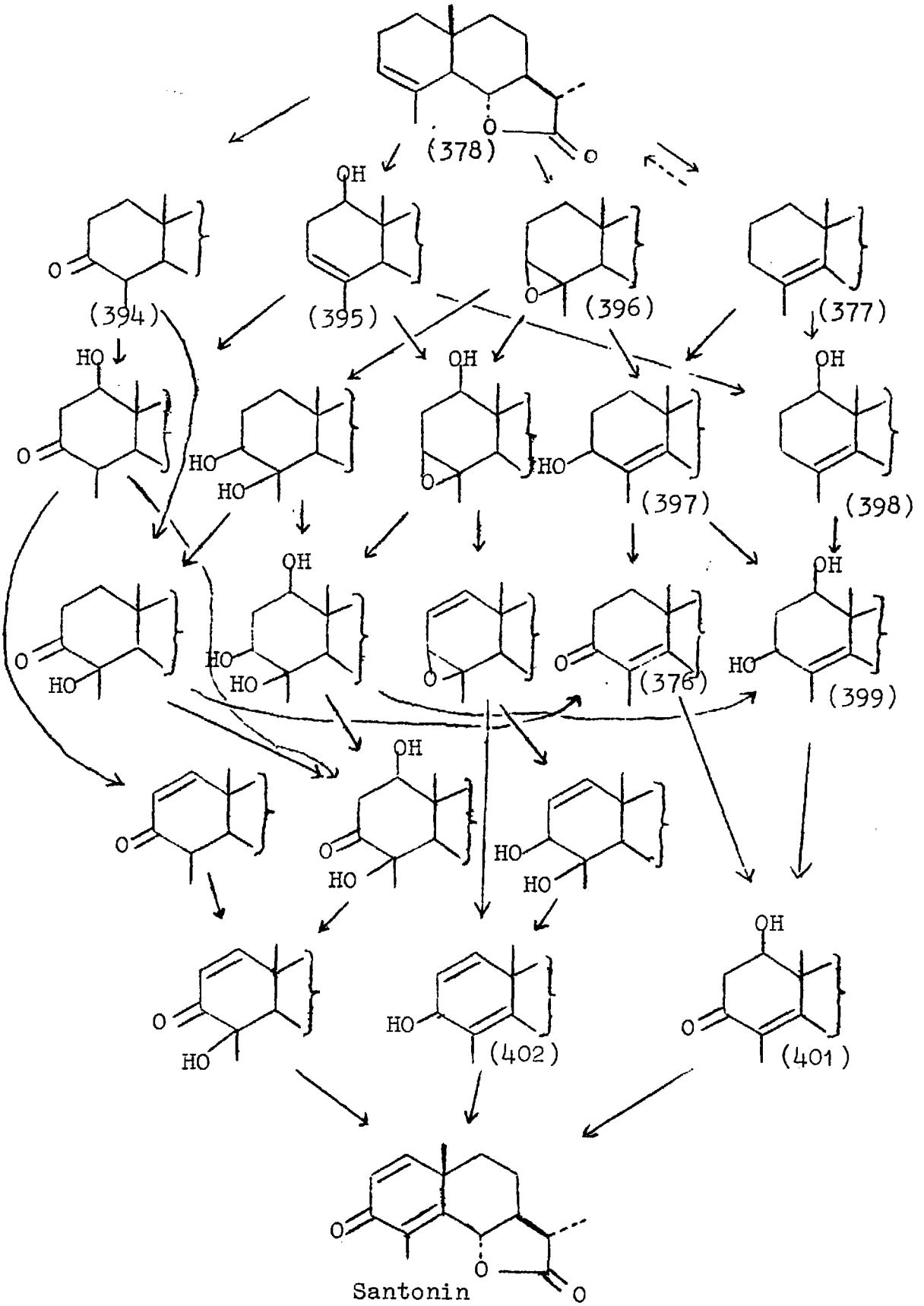
While it must be borne in mind that a nil incorporation is not necessarily a significant result, the non-incorporation of 1,2-dihydrosantonin was

nevertheless surprising, for this compound was on the most plausible biosynthetic route. Most possible pathways from the Δ^3-lactone to santonin are shown on Chart XXV, on which, for convenience, 1,2-dihydro-santonin has been included.

In arriving at Chart XXV, certain principles have been adopted. Double bonds are considered to be formed by dehydration of hydroxy compounds, and hydroxylation is considered to occur only at carbons 1, 3 and 4. In naturally occurring eudesmanolides, these are the only centres at which hydroxyl or keto groups normally occur. An over-riding general principle is that each step must be a step towards santonin. This is almost equivalent to saying that the oxidation level must never decrease.

Certain of the illustrated pathways are less attractive than others. For example, there must be some doubt as to the biological feasibility of the transformation of a double bond to a ketone, as shown in compound (374) being converted to compound (390).

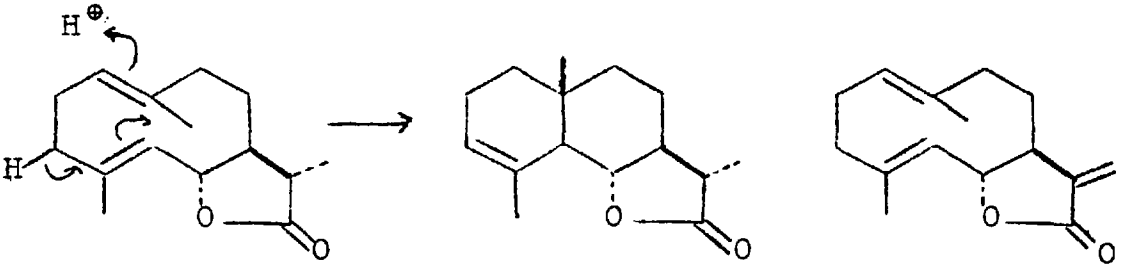
CHART XXV



SECOND SEASON

11,12-Dihydrocostunolide.

The incorporation of the Δ^3-lactone (378) immediately suggested that 11,12-dihydrocostunolide (403)

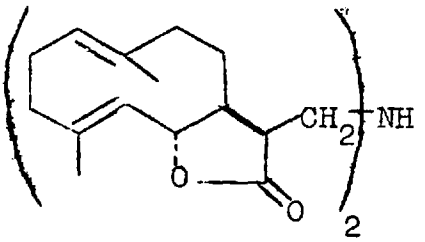


(403) (378) (404)

was the preceeding precursor. The cyclisation of 11,12-dihydrocostunolide (403) proceeds smoothly *in vitro* under mild acidic conditions.[32] Dr. S.C. Bhattacharyya, Poona, India kindly placed a very generous quantity of costunolide (404) at our disposal. Selective hydrogenation[32] of the 11-double bond of costunolide over palladium on charcoal proved unexpectedly tricky. Attempted hydrogenation over 1 % palladium on charcoal resulted in the recovery of costunolide, while over 5 % palladium, almost complete hydrogenolysis of the lactone to acidic products occurred. An older batch of 5 % palladium catalyst, probably somewhat deactivated, allowed selective

hydrogenation of the 11-double bond, although substantial quantities of acidic hydrogenolysis products were also obtained.

All attempts to introduce a functional group into the costunolide molecule at C-12 (in order to permit labelling of and recovery of costunolide) were unsuccessful. The first attempts were based on the expectation that a nitrone attached to position 12 would allow exchange at that position under the action of base. Hydroxylamine reacted with costunolide to give a discrete product, but this was not persued, as the i.r. spectrum indicated that the attack had been at the lactone carbonyl rather than the 12-position. Phenyl hydrazine did not react at all under similar conditions. Bhattacharyya has described[33] the preparation of an adduct (405) of two moles of costunolide with one mole of ammonia. By adding a



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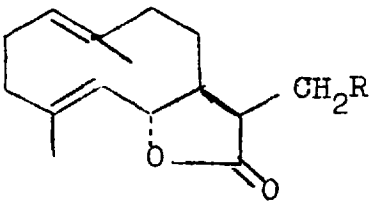


(406) R = NH_2
(407) R = N=CH-Ph

solution of costunolide in absolute ethanol slowly to

an excess of a saturated solution of dry ammonia in ethanol at 0^{o}, a new substance was obtained as a glass, and purified by preparative t.l.c. The n.m.r. and i.r. spectra of the substance were consistent with the structure (406). In particular, the n.m.r. signal assigned to NH protons integrated as two protons, and disappeared on shaking with deuterium oxide. The 2:1 adduct (405) would show only "one half" of an NH proton.

It was hoped to condense the amine (406) with either benzaldehyde or p-nitrobenzaldehyde to form the Schiff's base (407) or equivalent. It was also hoped that such a compound would exchange in tritiated water with base, and that its N-tosylate would spontaneously eliminate to regenerate the costunolide. In marked contrast to the reaction between β-phenylethylamine and benzaldehyde,[289] which occurred on mixing equal molar quantities of the reactants in the absence of a solvent, the costunolide amine (406) was inert to both benzaldehyde and p-nitrobenzaldehyde.

Further eudesmanolide possible precursors.

The incorporation of both the Δ^{3}-lactone (378) and the Δ^{4}-lactone (377) would tend to support a pathway

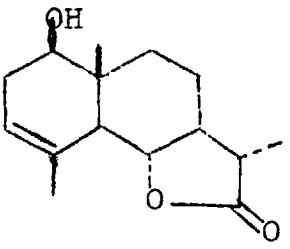
involving the isomerisation of the trisubstituted 3-double bond to the tetrasubstituted 4-double bond. It was, however, considered desirable at this stage to assume that it was at least a possibility that these double bond isomers were interconvertable, and also that there could be more than one pathway involved.

The next stage in the investigation was, then, to endeavour to determine the actual pathway or pathways involved out of the numerous possibilities depicted in Chart XXV. Attention was first focussed on the four compounds (394), (395), (396) and (377) directly derived from the Δ^3-lactone (378). The Δ^4-lactone (377) had already been shown to be incorporated.

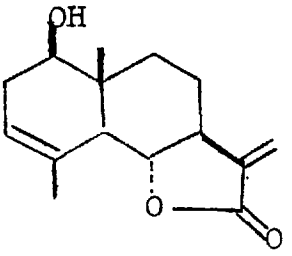
Of the other compounds, the ketone (394) presented no difficulty, as the chemistry of the tetrahydrosantonins has been thoroughly investigated.[284] The number of plants available, was, however, limited, and feeding of tetrahydrosantonin was deferred until the third season in order to allow other feedings considered more likely to be successful.

The hydroxylactone (395) with the 1β-hydroxyl is 11,12-dihydrosantamarine (408). Santamarine (409) has been isolated [86] from Chrysanthemum parthenium by Romo de Vivar, who kindly provided us with a generous sample.

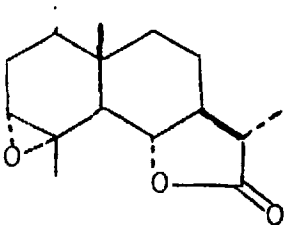
Despite a report in the literature[86] to the contrary, santamarine was smoothly selectively hydrogenated over 1 % palladium on charcoal to 11,12-dihydro-santamarine (408). It is of interest to note that



(408)



(409)



(410)

the santamarine was contaminated by a small quantity of what appeared (from its n.m.r. spectrum) to be 11,12-dihydrosantamarine. The 1-epimer of dihydro-santamarine is discussed with the third season's feedings.

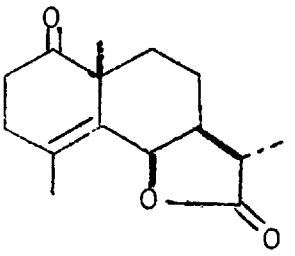
The epoxylactone (396) was readily prepared by the action of monoperphthalic acid on the Δ^3-lactone (378). Only one epimer was obtained, and this must be the α-epoxide (410), for attack of the peracid on the β-face of the molecule would be severely hindered by the 10-methyl group.

In view of the incorporation of the Δ^4-lactone (377), it was decided to try and synthesise the hydroxy-lactones (397) and (398).

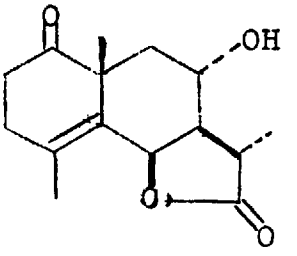
The allylic hydroxy lactone (397) was obtained by sodium borohydride reduction of 1,2-dihydrosantonin (376). Not unexpectedly, it was rather readily oxidised in air back to the αβ-unsaturated ketone. The rate of aerial oxidation was not so rapid as to preclude feeding of the compound, especially since the keto compound had already been shown not to be incorporated. There was some evidence in the n.m.r. spectrum (especially in the signal due to the C-3 proton) that more than one isomer was present, although one isomer greatly predominated. If the reduction were subject to thermodynamic control, the expected alcohol would be the equitorial β-alcohol. Kinetic control, in which hydride attack is from the least hindered face, would also produce the β-alcohol, for attack on the β-face must be hindered by the angular methyl. It was therefore concluded that the reduction yielded principally the β-alcohol, and, indeed, the crystalline acetate of the product appeared to be one epimer only.

The other hydroxy lactone (398) is the 6-epimer of the hydroxy lactone produced by the reduction of desoxypseudosantonin (411). There was some dispute in the literature[100,290] about the configuration at C-6

in pseudosantonin (412) and desoxypseudosantonin (411). Desoxypseudosantonin was isolated[98] from the mother liquors from pseudosantonin purification, while pseudosantonin was itself isolated from the mother



(411)

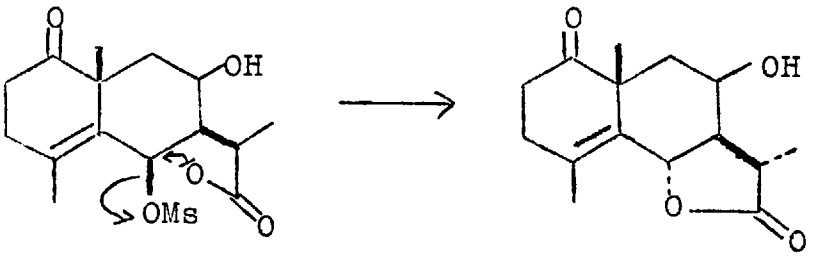


(412)

liquors of santonin purification. A substantial quantity of pseudosantonin was available, and t.l.c. immediately indicated that it contained ca. 10 % of a much less polar substance. The less polar compound (ca. 0.5 g.) was obtained by column chromatography, and as expected, proved to be desoxypseudosantonin. The n.m.r. spectra of these pseudosantonins clearly showed that they belonged to the 6-epi series.

Epimerisation of the 6-epi-lactone back to the normal 6α-configuration has never been reported. Various experiments with this object in mind were carried out using the more plentiful pseudosantonin. It was thought that, if the 6-mesylate could be formed, then the carboxylate anion would carry out an internal

S_N2 reaction, producing the "normal" 6-lactone configuration.



Unfortunately, treatment of pseudosantonin with mesyl chloride in sodium hydroxide (Schotten-Baumann reaction) yielded a substance having the expected spectral properties of the 8-mesylate of pseudosantonin, but still with the 6-epi configuration. The 6-hydroxyl group was apparently too hindered to react.

A further possibility was that the reduction of a 6-ketone with sodium borohydride would yield the thermodynamically more stable 6α-hydroxyl group. On the other hand, it was also possible that the angular methyl group would prevent the attack of the hydride from the β-face. While the sodium salt of santoninic acid (from santonin) was oxidised by chromium trioxide in acetic acid to give the 6-ketone,[291] pseudosantonin was not. This is undoubtedly a consequence of the much more favourable, and therefore faster, lactone formation in pseudosantonin. The oxidation could only

be successful if the 6-hydroxyl oxidised significantly faster than the rate of lactonisation. Pseudo-santonin was not oxidised by Sarett reagent (chromium trioxide-pyridine complex), probably because pyridine is not a strong enough nucleophile to open the lactone.

The dienol-lactone (402) was produced by sodium borohydride reduction of santonin in isopropanol, but predictably, it was very readily aerially oxidised back to the dienone, santonin. It was, therefore, impossible to feed this compound.

Labelling of precursors for second season's feedings.

11,12-Dihydrocostunolide (403) was labelled by hydrogenation of costunolide (404) with tritiated hydrogen, conveniently generated by the action of lithium on tritiated water, to yield [11,12-$^3H_2$] 11,12-dihydrocostunolide.

The hydroxy lactone (397) was prepared by reduction of [11-^3H]1,2-dihydrosantonin as described for the inactive compound.

[11,12-$^3H_2$] 11,12-Dihydrosantamarine (408) was obtained by hydrogenation of santamarine with tritiated hydrogen.

The tritiated epoxylactone (410) was prepared from [11-^{3}H] Δ^3-lactone (378).

The feeding programme for the second season also included repeat feedings of the Δ^3-lactone (378) and Δ^4-lactone (377), 1,2-dihydrosantonin (as a control for the hydroxy lactone (397)), and mevalonic acid lactone.

Feeding experiments.

The feeding experiments were carried out in the same manner as the previous season's, but somewhat earlier in the season - early July. The plants were, however, much further advanced at this time than in the preceeding and succeeding seasons. The results are tabulated in Table 3.

The incorporation of 11,12-dihydrocostunolide (403) was probably the most significant result obtained in the investigation. It must first be stated that the incorporation was very low. It was not, however, grossly different from the other incorporations obtained. Comparison with the figures obtained for the 1,2-dihydrosantonin (376) and 3,4-epoxy lactone (410) (see experimental section) indicated that any random labelling was at a very low level.

TABLE 3

Precursor	Activity 10^{-2} μC/mM. Santonin	Oxime	After wash out	Incorporation
11,12-dihydrocostunolide (403)	4.62	3.66	1.10	0.004_2 %
3-hydroxy-6,11β(H)-eudesm-4-en-6,13-olide (397)	3.57	2.98	0.0	0.007_5 %
11,12-dihydrosantamarine (408)	7.80	-	-	0.009_2 %
3,4-epoxy-5α(H),6,11β(H)-eudesman-6,13-olide (410)	Nil	-	-	Nil
5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378)	6.38	-	-	0.015_0 %
6,11β(H)-eudesm-4-en-6,13-olide (377)	3.01	3.00	0.0	0.009_0 %
1,2-dihydrosantonin (376)	Nil	-	-	Nil
[2-^{14}C]mevalonic acid lactone	1.48	-	-	0.011_4 %

When the label was washed out of the 11-position of the oxime from the dihydrocostunolide feeding experiment, considerably more than the expected 50 % of the label was lost. This necessitated a check to ascertain the ratio of the labels in the 11- and 12-positions of the dihydrocostunolide which had been fed. Unfortunately, 11,12-dihydrocostunolide is not adequately stable to acid or oxygen to enable the necessary chemistry to be performed

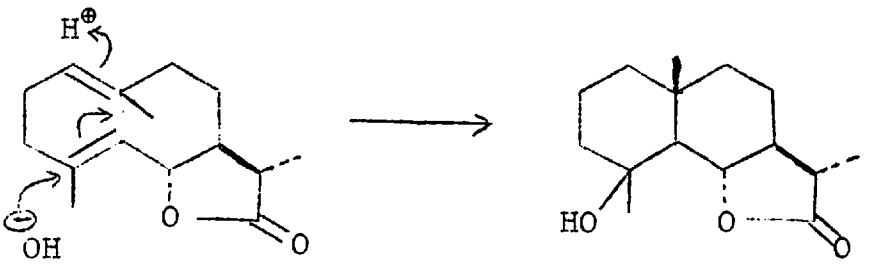
on the limited material available. Additionally, its behaviour towards hot alkali has not been investigated. Ample [11,12-$^3H_2$]11,12-dihydrosantamarine was available, however, and a control experiment was carried out on it.

A small quantity of the labelled 11,12-dihydrosantamarine was diluted with inactive material to yield 11,12-dihydrosantamarine with a convenient 400 c.p.s./mg. activity. Ca. 50 mg. of this dihydrosantamarine was treated with base in a sealed tube using quantities and conditions similar to those used in the wash-out experiments. On work up, a small quantity of a readily crystalline substance m.p. 60-61° was obtained. (11,12-Dihydrosantamarine is a reluctantly crystalline compound m.p. 124-127°.) This new substance showed no significant differences from 11,12-dihydrosantamarine in its i.r. spectral properties. It was therefore concluded that the new substance was probably the 11-epimer of 11,12-dihydrosantamarine. In view of the formation of a significant quantity of β-santonin in the exchange labelling of α-santonin, it would not be surprising if some of the 11-epimer of 11,12-dihydrosantamarine was produced under similar conditions. It is unfortunate that there was insufficient material

to characterise the new substance fully. The substance was assayed, and found to have lost about 80 % of the label. This suggested that there was indeed, much more than 50 % of the label in the 11-position in the compounds labelled by hydrogenation of an 11,12-exomethylene. The most likely explanation of this is that the hydrogenation occurs by a stepwise mechanism, and that only one of the steps is subject to an isotope effect, or that the isotope effects in the two steps work in opposite senses. A hydrogen atom from the hydroxylic solvent may be the source of the 12-hydrogen in the above cases.

One of the obvious possible "decomposition" products of dihydrocostunolide *in vitro* is the Δ^3-lactone (378). As this latter compound has been shown to be incorporated into santonin, it was necessary to check that cyclisation did not occur during the feeding procedures. A small quantity of [11,12-$^3H_2$]11,12-dihydrocostunolide was dissolved in base, neutralised to pH 7, and allowed to stand for two weeks. Inactive Δ^3-lactone (378) was then added to the solution. Repeated chloroform extraction of a neutral solution of the sodium salt of the hydroxy acid from the Δ^3-lactone was shown to extract the organic acid, which presumably

lactonised spontaneously. The 11,12-dihydrocostunolide/ Δ^3-lactone sodium salts were extracted from the neutral solution, and the Δ^3-lactone reisolated and purified by t.l.c. The appropriate band was extracted from the silica, the solution evaporated, and the Δ^3-lactone assayed. It was found to be almost completely inactive, thus eliminating the possibility that the incorporation of the dihydrocostunolide was due to cyclisation of the 11,12-dihydrocostunolide before it was absorbed by the plant. Chemical cyclisation to the hydroxy lactone (413) is



(413)

also a possibility which ought to be considered, but it seems more likely that a cyclisation in base would proceed through to the unsaturated compound.

The quantity of santonin obtained from the feeding of dihydrosantamarine was unfortunately insufficient to permit the preparation and purification of the oxime. The feeding was repeated in the third season.

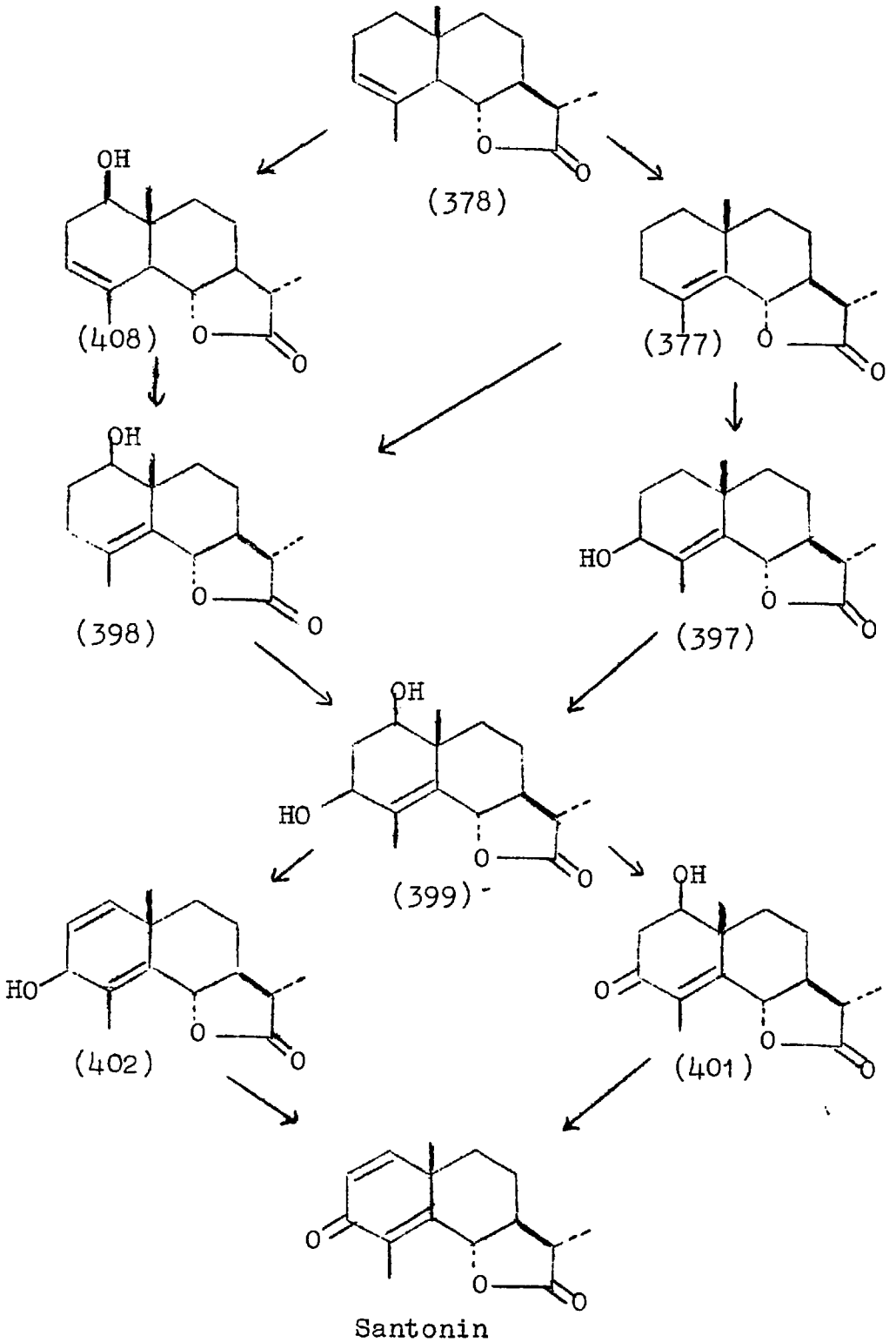
The incorporation of dihydrosantamarine, although not confirmed at this stage, and of the hydroxy lactone (397) requires the postulation of two pathways leading to santonin, as shown on Chart XXVI. Excluding less likely reactions such as hydrations of double bonds and epoxidations, Chart XXVI represents the remaining possible routes.

Both the Δ^3- and Δ^4-lactones were again incorporated, but the incorporations are both considerably lower than those recorded in the first season. The quantity of precursor fed in the second season was considerably greater than in the first season, and was probably more than the plant was able to use. Nevertheless, the lower incorporations did result in more radioactivity in the santonin, as much more activity was fed. This made the establishment of the incorporations easier and more convincing.

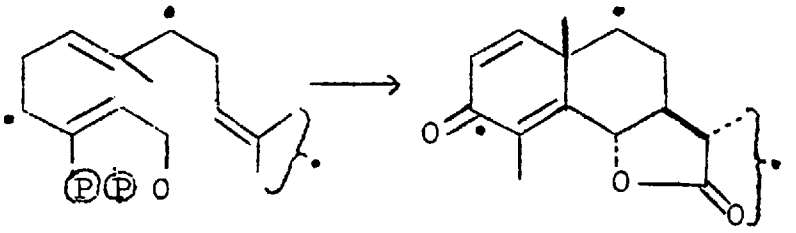
Degradation of the santonin from the mevalonate feeding.

The incorporation of the [2-^{14}C]mevalonate into santonin provided an opportunity to attempt to gain some information on the cyclisation of farnesyl pyrophosphate. Two different cyclisations, depicted in (414) and (415) can be envisaged. The positions of the labels in the

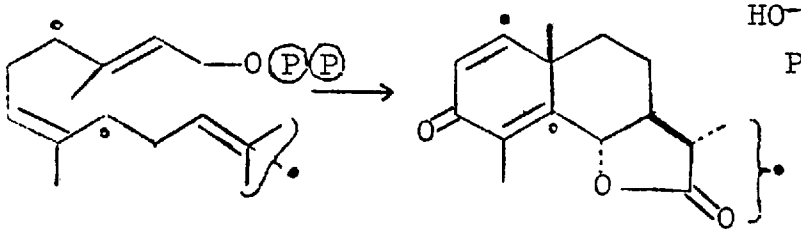
CHART XXVI



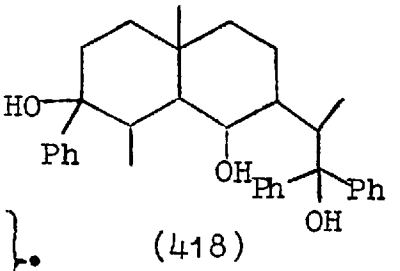
derived santonin are shown in (416) and (417). It may be expected that the label in the gem dimethyl is



(414) (416)



(415) (417)



(418)

located in the methyl group <u>trans</u> to the chain of the farnesyl pyrophosphate, as found in the steroid soyasapogenol A,[292] but it must be borne in mind that it is possible the isopropyl group in a ten-membered ring intermediate rotates freely.

Santonin was hydrogenated over Adams catalyst to give tetrahydrosantonins (two isomers). Treatment of the tetrahydrosantonins with phenyl lithium in benzene[293] gave substance (418). The crude product (418) was

vigorously oxidised with chromium trioxide in dilute sulphuric acid, and benzoic acid (containing C-3 of santonin) and benzophenone (containing C-13) obtained and purified. This procedure was then repeated on the remaining santonin from the mevalonate feeding, but about twice the theoretical quantity of benzoic acid was obtained. A control experiment showed that some of the extra benzoic acid was formed by the phenyl lithium solution, and the rest by oxidation of biphenyl, a by-product in the preparation of the phenyl lithium. When correction was made for the extra benzoic acid, the activity in C-3 of the mevalonate fed santonin was found to be about one fifteenth of the total. The activity in the benzoic acid was less accurately determined, but was about one twelfth of the total. An attempt to isolate the acetic acid from the Kuhn-Roth type of degradation produced a small quantity, which was converted to the p-phenyl phenacyl derivative, and aasayed. It was virtually inactive.

Thus, it must be concluded that the mevalonate was not incorporated as such, but was degraded by the plant. The activity in the santonin was the result of some of the breakdown products entering the biosynthetic pathways. The acetic acid in the degradation should have contained $\frac{6}{15}$ of the activity, but possibly the oxidation was not vigorous enough to degrade the C-Me groups.

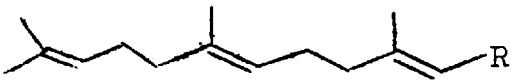
THIRD SEASON

Preliminary considerations.

The failure of mevalonic acid to be incorporated specifically led to consideration of feeding farnesyl pyrophosphate (and farnesol), labelled on the carbon bearing the oxygen.

The elimination of the remaining ambiguities in Chart XXVI required feeding of compounds (398), (399), (401) and (402). Of these, unsuccessful attempts to prepare the hydroxy lactone (398) have already been described, and the feeding of the dienol lactone (402) has already been shown to be impracticable. It was thus decided to attempt to synthesise the closely related dihydroxy lactone (399) and hydroxyketo lactone (401).

The preparation of two other possible precursors, germacrene and dihydrodouglanine became possible during the course of the year, and are described later. The two trans-fused tetrahydrosantonins, the preparation of which had been postponed in the second season was undertaken for the third season.

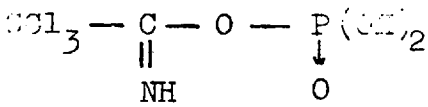


(419) R = CH_2OH
(420) R = CHO

Farnesyl pyrophosphate.

Farnesol (419) was oxidised in good yield to farnesal (420) by manganese dioxide, and reduced back to farnesol by sodium borohydride, thus enabling labelling with tritiated sodium borohydride. The g.l.c. of both the authentic (believed synthetic) and regenerated farnesol showed two peaks in similar ratio. The larger of the two peaks was almost certainly the trans-trans-isomer.

Farnesyl pyrophosphate was obtained in excellent yield by phosphorylation[294] with di-(triethylamine) hydrogen phosphate and trichloro-acetonitrile[295] in acetonitrile. The effective phosphorylating agent is

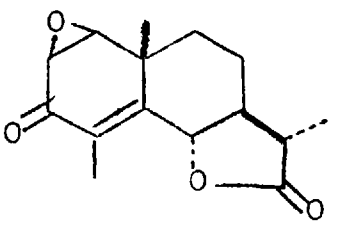


Attempted syntheses of hydroxyketo lactone (401).

The basic problem in the synthesis of the hydroxy-keto lactone (401), and the related dihydroxy lactone (399) was to introduce at C-1, a function capable of being converted to an alcohol. The available desoxypseudosantonin (411) has the wrong configuration at C-6, and has defied efforts to epimerise that centre. (See page 159) Thus, unfortunately, the problem in hand could not be solved by allylic oxidation of the 1-hydroxy-4-en system.

The first approach to the synthesis was to endeavour to selectively epoxidise the 1-double bond of santonin. The claim that prolonged treatment of santonin with hot perbenzoic acid[296] produced the 1,2-epoxide (421) has been disproved.[297] The product is in fact the 4,5-epoxide as would be predicted on electronic grounds.

Alkaline hydrogen peroxide (i.e. $^{\ominus}OOH$) did not react with santonin. It appeared that decomposition of the hydroperoxy anion was more rapid than attack on the double bond. Peracids attack isolated double bonds more readily than conjugated ones. Hence, an attempt was made to prepare the acetate (422). This proved



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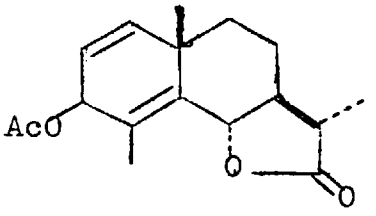

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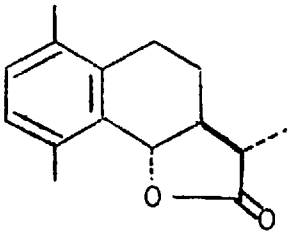


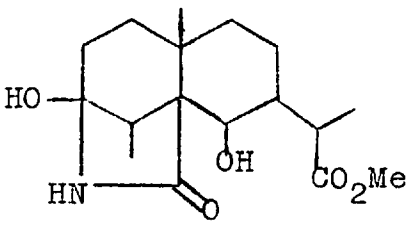
(423)

unexpectedly troublesome. Reduction of santonin with sodium borohydride in isopropanol at room temperature yielded, after acetylation, a compound with a strong acetate absorption in the i.r., but lacking lactonic carbonyl absorption. This was presumably the

6,13-diacetate of santoninic acid. A second attempt this time using methanol as solvent, produced, after acetylation, a complex mixture of acetates, and a highly crystalline aromatic compound, which was proved to be hyposantonin (423). This was, no doubt, produced by a methyl shift on acidifying the dienol acetate (422) after acetylation. Two of the acetates were also aromatic, but were unstable and non-crystalline, so no further work was performed on them. When the reduction and acetylation were repeated, avoiding acidification, an oily acetate with the spectral properties to be expected of the dienol acetate (422) was obtained. This substance could not be induced to react with monoperphthalic acid, and further work on it was discontinued.

When santonin in dry methanol was heated under reflux with potassium cyanide, a substance containing a cyanide group was obtained. The substance, however, possessed neither ketonic nor lactonic carbonyl absorptions in the i.r. N.m.r. and i.r. spectral evidence pointed to it being a hydroxy lactam methyl ester (424), similar to the compound (425) described by Theobald[298] as the product of cyanide addition to an αβ-unsaturated ketone. It was ascertained that the

three i.r. bands due to the dienone in santonin all disappeared at the same rate during the reaction with cyanide, indicating that the first addition was the rate determining step. It was, therefore, not possible to stop the reaction after the adddition of one cyanide group only.



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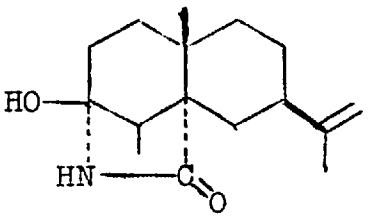


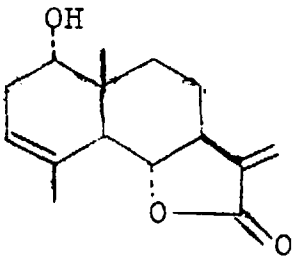
(425)

Attempts to add azide, cyanate and thiocyanate in a similar manner, and also in aprotic solvents such as dimethyl sulphoxide were unsuccessful.

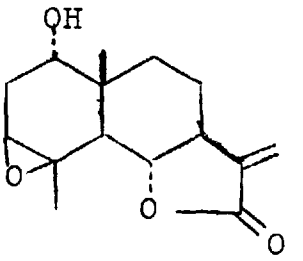
Douglanine.

Shortly after the incorporation of 11,12-dihydro-santamarine had been shown, the 1-epimer of santamarine, known as douglanine (426) was isolated[87] from Artemisia douglassiana. Professor T.A. Geissman kindly supplied a quantity of crude douglanine. This, however, had substantially decomposed. From the n.m.r. and i.r. spectra, it was clear that the substance was closely related to douglanine, but that it lacked the signal

expected from the vinyl proton on C-3. Also, the signal due to the C-4 methyl group was at τ 8.5 (sharp singlet) instead of at τ 8.1. It was thought that the compound was probably the 3,4-epoxide of douglanine (427). It was smoothly hydrogenated over 1 % palladium on charcoal to give the 11,12-dihydro derivative (428). Further work is in progress.



(426)



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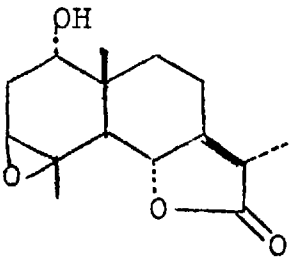
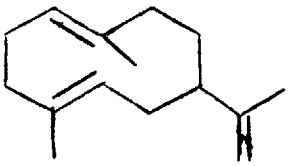


(428)

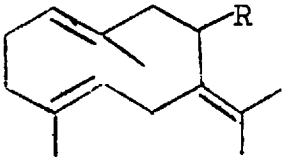
Germacrene.

In order to explore further the biosynthetic pathway to santonin between farnesyl pyrophosphate and 11,12-dihydrocostunolide, triene (429) was a desirable one to feed. The availability[8] of the isomer (430), known as germacrene, or germacratriene, prompted its preparation for a feeding experiment. The triene has been shown, by an X-ray study of the silver nitrate adduct[299] to be the all trans isomer. Germacrone (431),

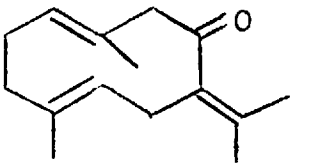
kindly supplied by Dr. J.K. Sutherland, was reduced with sodium borohydride in pyridine to the alcohol (432),



(429)



(430) R = H
(432) R = OH
(433) R = OAc

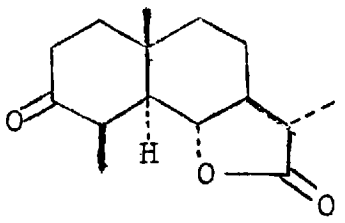


(431)

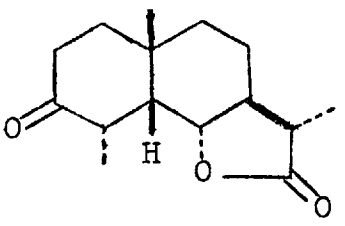
which was acetylated. The acetate (433) was reduced with lithium in liquid ammonia to yield the triene (430).

Tetrahydrosantonins.

Hydrogenation[284] of santonin with 5 % palladium on charcoal produced a mixture of the cis- and trans-fused tetrahydrosantonins (434) and (435). This mixture was



(434)



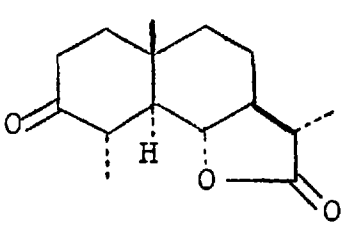
(435)

separated into two parts.

The first part was purified by crystallation to

give 3-oxo-4,5α(H),6,11β(H)-eudesman-6,13-olide (434), still slightly contaminated with the cis-fused isomer (435).

The second part was dissolved in sodium hydroxide solution, a process which epimerised the 4-methyl group, acidified and reisolated. Crystallisation afforded 3-oxo-5α(H),4,6,11β(H)-eudesman-6,13-olide (436),



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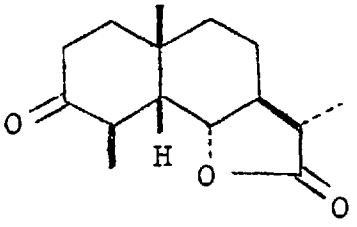

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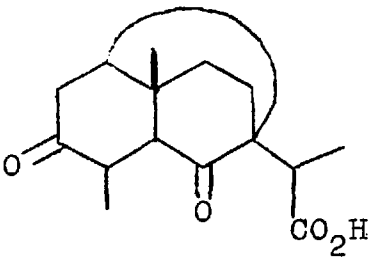


(438)

still slightly contaminated with the cis-fused isomer (437).

It seemed extremely unlikely that the cis-fused tetrahydrosantonins could possibly be involved in the biosynthesis of santonin, so these were considered acceptable impurities. In the event of either of the tetrahydrosantonins which were fed having been incorporated, it would obviously have been necessary to purify rigorously the trans-fused compound and repeat the experiment.

Labelling of the precursors for the third season's feedings.

Farnesol (419) (and hence farnesyl pyrophosphate) was labelled by reduction of farnesal (420) to farnesol with tritiated sodium borohydride.

The "dihydrodouglanine epoxide" (427) was labelled by hydrogenation of "douglanine epoxide" with tritiated hydrogen.

Germacrene (430) was labelled at the 8-position by reduction of germacrone with tritiated sodium borohydride, and subsequent lithium/ammonia reduction of the acetate.

The labelled [11-^{3}H]tetrahydrosantonins were prepared from [11-^{3}H]santonin.

Feeding experiments.

Three of the precursors for the third season's feedings could not be fed as sodium salts of hydroxy acids, as in the previous two seasons' experiments. Germacrene and farnesol are not lactones, and the tetrahydrosantonin (434) would be epimerised at C-4 on attempting to open the lactone with base. The germacrene and farnesol were each divided into two portions. One portion was injected directly into a plant stem, and the other portion emulsified with Tween 80 in water, and fed to another stem of the same plant through a wick, as usual.

TABLE 4

Precursor	Activity 10^{-2} μC/mM. Santonin	Oxime	After wash out	Incorporation
Farnesol (419)	2.25	3.07	-	0.0002_5 %
Farnesyl pyrophosphate	Nil	-	-	Nil
Germacrene (430)	Nil	-	-	Nil
3-oxo-4,5α(H),6,11β(H)-eudesman-6,13-olide (434)	Nil	-	-	Nil
3-oxo-5α(H),4,6,11β(H)-eudesman-6,13-olide (436)	Nil	-	-	Nil
"3,4-epoxy-11,12-dihydrodouglanine (428)	Nil	-	-	Nil
11,12-dihydrosantamarine (408)	3.60	3.03	-	0.005_5 %

The tetrahydrosantonin (434) was fed entirely as an emulsion. The farnesyl pyrophosphate was, of course, fed as an aqueous solution.

The results of the feeding experiments are shown in Table 4. The incorporation of farnesol is below that normally regarded as significant, even bearing in mind the low incorporations obtained with other precursors. Firstly, it must be stated that the actual incorporation is at least twice the "simple" incorporation, as one half of the label is lost in the biosynthetic process, assuming that the label finished up at C-6. Secondly, although it

is believed that the farnesol fed was largely the trans-trans-isomer, there was some of another isomer. Nevertheless, it may well be that the farnesol was degraded, and the tritium distributed throughout the biosynthetic processes of the plant.

An attempt was made to was out the label (if at C-6) in the farnesol fed santonin. The santonin was treated with concentrated potassium hydroxide under reflux for one hour, and the santonic acid $(438)^{300}$ isolated. Unfortunately, there was insufficient material to allow recrystallisation to constant activity.

The 11,12-dihydrosantamarine incorporation was confirmed, but there was unusual difficulty in purifying to constant activity. Although this was achieved for the oxime, there was insufficient material remaining after wash out of the label at C-11 to enable the process to be repeated.

It would be interesting to try and feed the isomer of germacrene with the 11(12)-double bond, as this seems a more likely precursor.

The non-incorporation of farnesyl pyrophosphate (and mevalonate in the earlier seasons) may indicate that cell barriers effectively prevent such precursors reaching the appropriate site.

Unfortunately, the third season's results did not eliminate any of the pathways shown in Chart XXVI.

EXAMINATION OF OTHER SPECIES OF ARTEMISIA

Artemisia stellariana.

One plant of the species Artemisia stellariana, harvested during bud formation, was extracted as described for Artemisia maritima. (See page 131). No santonin was detected in a preliminary t.l.c. examination, but a substance inseparable (t.l.c.) from 1,2-dihydrosantonin was observed. By preparative t.l.c., 5.6 mg. of this substance were obtained. The i.r. and u.v. spectra were identical with those of 1,2-dihydrosantonin. As 1,2-dihydrosantonin does not readily crystallise, the substance from the plant was converted to its semi-carbazone, and a mixed m.p. with authentic material confirmed that the substance was indeed 1,2-dihydrosantonin.

A re-examination of the plant after flowering revealed no lactonic chromophoric substances.

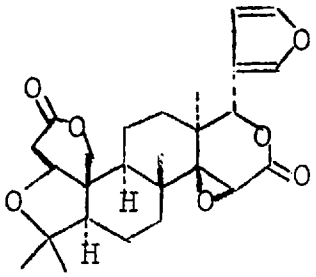
1,2-Dihydrosantonin has been suggested[301] as a minor component of Artemisia maritima.

Other Artemisia species.

Plants of the species A. vulgaris, A. vulgaris variegata, A. abrotanum, A. lactiflora, and A. palmeri were examined, but no lactones were found in any.

LIMONIN

Certain preliminary work was carried out to find a system for the investigation of the biosynthesis of limonin (439). A two year old grapefruit tree (Citrus



(439)

decumana) was harvested an extracted with acetone. T.l.c. examination of the extract using Erlich's reagent as spray showed the presence of many apparently furanoid compounds, but no limonin.

An extraction of Dictamnus albus with alcohol showed the presence of limonin in the roots of this species. 4.1 mg. pure limonin were obtained from one plant. Its identity was proved by mixed m.p., and the correspondance of the i.r. spectra of isolated and authentic limonin.

[2-^{14}C]DL-Mevalonic acid lactone was fed to a Dictamnus albus plant during May, 1966, and the limonin isolated by dilution with carrier material, but the limonin was completely inactive. When the experiment was repeated during August, 1966, and incorporation of 0.001 % was obtained. No further work on the system was undertaken.

SECTION 5

EXPERIMENTAL DETAILS

GENERAL REMARKS

Unless otherwise stated, the following conditions etc. apply to all experiments reported in this section.

1. Thin-layer chromatography (t.l.c.) was carried out using Kieselgel GF_{254}, and the spots detected by fluorescence under low pressure ultra-violet light (maximum emission at 254 mμ) and /or by staining with iodine vapour. Detection of compounds on silver nitrate impregnated plates was by spraying with 0.1 % solution of dichloro-R-fluorescin in 1:1 aqueous ethanol.[281]

2. Column chromatography was carried out on alumina graded according to Brockman.

3. Infrared (i.r.) spectra were recorded on a Unicam SP.200 spectrophotometer. All maxima are quoted in wave numbers (cm^{-1}).

4. Ultraviolet (u.v.) spectra were recorded on a Unicam SP.700 spectrophotometer. Maxima are quoted in mμ, and extinction coefficients in terms of log ε.

5. Nuclear magnetic resonance (n.m.r.) spectra were recorded (on a Varian A.60) in deuterochloroform solution. Chemical shifts are quoted in τ values. Most of the

n.m.r. data are presented in tabular form in Tables 11 and 12 at the end of this section.

6. Sodium and magnesium sulphate were the drying agents normally used.

7. Petroleum ether refers to the fraction of b.p. 60-80°, unless otherwise noted.

8. All radioactive compounds were assayed by liquid scintillation counting, using an IDL 6012 coincidence counter. 50 mm x 10 mm glass tubes were used, with 1.2 ml. Nuclear Enterprises NE.213 liquid scintillator. All were counted for five suitable periods of time, and the results averaged. Where significant, allowance was made for backgroud count rate. The efficiency of the counter was determined from time to time with standard $[^{3}H]$ and $[^{14}C]$ hexadecanes, supplied by United Kingdom AEA, Radiochemical Centre, Amersham, Bucks. C = curie (i.e. 3.7×10^{10} disintegrations per second); mC = millicurie (10^{-3}curie); μC = microcurie (10^{-6} curie); c.p.s. = counts per second. The detailed figures and calculations for the assays are given in tables 5 to 10 at the end of this section. Those activities (in c.p.s.) marked with an asterisk * were determined on the improved counter. See pages 140 and 141. Radioactive decay (the half-life of

tritium is 12.26 years) was neglected, as all experiments were completed within six months.

9. The standard feeding procedure was as follows: The compound to be fed was dissolved in sodium hydroxide solution (5N, 5 ml.) with as little heating as possible, and neutralised to pH 7 with hydrochloric acid. The solution was fed through wicks of untreated cotton (one per stem) to one Artemisia maritima plant. Immediately preceeding and during the feeding, the plants were deprived of other sources of water. When all the test solution had been taken up, distilled water was added to wash in any remaining compound. This process took between three and six days. The plant was then allowed to grow normally (with watering) for three to five days, and harvested.

10. The standard extraction procedure was as follows: The aerial parts of the plant were allowed to dry in air for up to 24 hours, and then powdered in liquid nitrogen. The powered plant material was extracted in cold benzene (ca. 1 l.) for at least 48 hrs., and often for several weeks. The benzene solution was filtered, evaporated, and the crude santonin obtained by preparative t.l.c. (1:1 benzene/

ethyl acetate). The santonin was diluted, if necessary, to 30 mg. with inactive material, purified on two further plates, and crystallised to constant activity. This usually required six crystallisations. Only the last two assays are recorded in the tables. Less than 0.1 c.p.s./mg. is reported as a nil activity, except in special cases. An indication of the maximum incorporation which would thus escape detection is recorded in the tables.

PRELIMINARY EXPERIMENTS

Initial extraction of santonin from Artemisia maritima.

One Artemisia maritima plant was harvested during October, 1964, and allowed to dry in air overnight. The aerial parts of the plant were powdered in liquid nitrogen, and stirred for 72 hours in benzene (ca. 1 l.). The suspension was filtered, and the benzene removed under reduced pressure to yield a green gum (ca. 300 mg.). This gum was treated with petroleum ether to remove fats etc., and the residue chromatographed on alumina (Grade I, 3.7 g.). Gradient elution was employed, and the santonin (5 mg.) was eluted with 100 % chloroform. This was purified to yield pure santonin (ca. 3 mg.). This small quantity

could not be induced to crystallise, but was shown to be santonin by the identity of its t.l.c. properties, and i.r. and u.v. spectra, with those of authentic santonin.

FIRST SEASON

Properties of santonin.

Santonin (375) is a highly crystalline white solid, m.p. 171-172°; $[\alpha]_D^{20}$ + 172° ($CHCl_3$); $\nu_{max}^{CHCl_3}$ 1775 cm^{-1} (lactone), 1665, 1640, 1620 cm^{-1} (dienone); λ_{max}^{EtOH} 240 mμ (log ε = 4.02).

1,2-Dihydrosantonin (376) (3-oxo-5α(H),6,11β(H)-eudesm-4-en-6,13-olide).

Santonin (9.73 g.) in dry benzene (500 ml.) was hydrogenated at room temperature and pressure over Raney Nickel W-2 (ca. 7.5 g.). Uptake ceased after one equivalent of hydrogen had been consumed. The solution was filtered and evaporated. Crystallisation from di-isopropyl ether/ 80-100° petroleum spirit yielded 1,2-dihydrosantonin (376) (6.77 g.; 72 % yield) as colourless plates, m.p. 101-102° (lit.[280] 101-102°); λ_{max}^{EtOH} 245 mμ (log ε = 4.17); $\nu_{max}^{CHCl_3}$ 1770 cm^{-1} (lactone), 1665, 1625 cm^{-1} (αβ-unsaturated ketone).

Ethylene thioketal of 1,2-dihydrosantonin (384).

1,2-Dihydrosantonin (376) (62 mg.) in glacial acetic acid (ca. 1 ml.) was treated with ethane dithiol (0.3 ml.) and boron trifluoride etherate (0.3 ml.) at room temperature for 1 hr. The mixture was diluted with water, neutralised with sodium carbonate solution, and extracted with chloroform. The dried chloroform solution was evaporated under reduced pressure and the crude thioketal purified by filtration through an alumina (Grade I) column. The ethylene thioketal of 1,2-dihydrosantonin (384) was crystallised from ethanol as colourless plates (61.5 mg.; 80 % yield) m.p. 164.5-165.5°; λ_{max}^{EtOH} 266 mμ (log ε = 3.38) and end absorption; $[\alpha]_D^{26}$ = + 3.4° (c = 1.0, EtOH). (Found: C, 63.22; H, 7.42; S, 19.91. $C_{17}H_{24}O_2S_2$ requires C, 62.95; H, 7.46; S, 19.76 %).

Desulphurisation of thioketal (384) with Raney Nickel.

A mixture of thioketal (384) (124 mg.) and Raney Nickel W-2 (ca. 1.8 g.) in absolute ethanol (5 ml.) was heated under reflux for 4 hrs., cooled, filtered, and evaporated under reduced pressure. The product was taken up in chloroform, filtered through alumina (Grade V) to remove acidic by-products, and chromatographed on a preparative t.l.c. plate (1:1 ether/

petroleum ether) to yield a mixture of 5α(H),6,11β(H)-eudesm-4-en-6,13-olide (377) and 5α(H),6,11β(H)-eudesman-6,13-olide (385). These were separated by chromatography on Silica Gel GF_{254} plates impregnated with silver nitrate (1:1 ether/petroleum ether). Only the desired Δ^4-lactone (377) (37 mg.; 41 % yield) was obtained pure, as colourless plates, m.p. 90-91°; $[\alpha]_D^{24}$ + 27.4° (c = 1.3; $CHCl_3$); $\nu_{max}^{CHCl_3}$ 1760 cm^{-1} (lactone). (Found: C, 76.81; H, 9.36. $C_{15}H_{22}O_2$ requires C, 76.88; H, 9.46 %).

The reaction was repeated with varying amounts of Raney nickel W-2, deactivated for varying periods by heating under reflux in acetone, but no catalyst could be found to desulphurise without partial hydrogenation. Change in solvent for the desulphurisation was also ineffective.

1,2-Dihydro-6-epi-santonin (386) (3-oxo-5α(H),6,11β(H)-eudesm-4-en-6,13-olide).

(a). 1,2-Dihydrosantonin (376) (2.06 g.) was heated with a 5 % solution of hydrochloric acid gas in dry dimethylformamide (20 ml.) on a steam bath for 6 hrs. Water was added, and the solution extracted with chloroform. The chloroform extract was washed with water, sodium bicarbonate solution, water again,

and dried. The crude product, obtained by evaporation under reduced pressure, was chromatographed on alumina (Grade I), and crystallised from di-isopropyl ether to yield 1,2-dihydro-6-epi-santonin (386) as colourless plates (ca. 300 mg.) m.p. 123.5-124.5° (lit.[282] 127°); $\nu_{max}^{CHCl_3}$ 1765 cm^{-1} (lactone); 1670 cm^{-1} (αβ-unsaturated ketone); λ_{max}^{EtOH} 244.5 mμ (log ε = 4.18).

(b). 6-Epi-santonin (387) was available from previous santonin work at Imperial College, m.p. 104-105° (lit.[282] 105°); $\nu_{max}^{CHCl_3}$ 1770 cm^{-1} (lactone), 1665, 1640 cm^{-1} (dienone).

6-Epi-santonin (387) (541 mg.) in dry benzene solution was hydrogenated over Raney nickel W-2 at room temperature and pressure. Uptake ceased after one equivalent of hydrogen had been consumed. The solution was filtered and evaporated, and the 1,2-dihydro-6-epi-santonin (386) crystallised from di-isopropyl ether to yield colourless plates (370 mg.; 68 % yield) m.p. 124-124.5°, identical with that obtained in (a) above.

Thioketal of 1,2-dihydro-6-epi-santonin.

1,2-Dihydro-6-epi-santonin (386) (97 mg.) in glacial acetic acid (0.5 ml.) was treated with ethane dithiol (0.25 ml.) and boron trifluoride (0.25 ml.) at

room temperature for 45 mins. Work up produced an oil (ca. 50 mg.), which could not be induced to crystallise. No further work was carried out on it.

Preparation of 5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378).

This compound was prepared by slight modifications of the method of Cocker and McMurry.[284] Santonin (375) (10 g.) in glacial acetic acid (300 ml.) was hydrogenated over Adams catalyst (370 mg.). The solution was filtered, evaporated under reduced pressure, and the oil left overnight at 0° to solidify. The cis-fused hexahydro derivative, 3β-hydroxy-4,5,6,11β(H)-eudesman-6,13-olide (388) was removed by treatment of the mixture with ether, and filtration. The filtrate was evaporated, and the 3α-hydroxy-4,5α(H),6,11β(H)-eudesman-6,13-olide (389) (6.3 g.) obtained as plates m.p. 109-110° (lit.[284] 108-110°) from ethanol; $\nu_{max}^{CHCl_3}$ 3500 cm^{-1} (OH), 1765 cm^{-1} (lactone).

The hydroxy lactone (389) (6.3 g.) in pyridine (64 ml.) was treated with phosphorus oxychloride (9.5 ml.) at room temperature overnight. Distilled water was added dropwise, and a white precipitate thrown down. On standing, this precipitate changed to an oil. The mixture was acidified with conc. hydrochloric acid and extracted with chloroform. The chloroform solution

was dried over calcium chloride, and evaporated to yield the crude product (6.1 g.). After preliminary purification on silica plates (1:1 ether/petroleum ether), a portion of the mixture of the Δ^2- and Δ^3-lactones was separated on silver nitrate impregnated t.l.c. plates to yield 5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378) (38.8 mg.; 16.4 % yield) as colourless plates (from ethanol) m.p. 138-139° (lit.[284] 136-137°); ν_{max}^{Nujol} 1765 cm.$^{-1}$ (lactone).

Chromous chloride reduction of santonin.

Santonin (0.989 g.) in acetone (25 ml.) was treated with chromous chloride solution (1M in 1M HCl; 45 ml.) under dry nitrogen at room temperature overnight. The solution was extracted with chloroform, and the acid taken into sodium bicarbonate solution. The solution was acidified, extracted, and the 3-oxo-11β(H)-eudesm-1,4-diene-13-oic acid (379) obtained as an oil (218 mg.; 25 % yield); $\nu_{max}^{CCl_4}$ 3505 cm.$^{-1}$ (free OH of acid), 1750, 1705 cm.$^{-1}$ (hydrogen bonded and free carbonyl of acid), 1660, 1635, 1615 cm.$^{-1}$ (dienone); λ_{max}^{EtOH} 240 mμ (log ε = 4.00), with shoulder 263 mμ (log ε = 3.87); p-phenyl phenacyl derivative as plates from ethanol m.p. 137-138°; $[\alpha]_D^{25}$ + 34° (c = 1.0, EtOH); (Found: C, 78.46; H, 6.57. $C_{29}H_{30}O_4$ requires C,78.70; H, 6.83%).

Lithium/ammonia reduction of santonin.

Santonin was reduced as described by Bruderer, Arigoni and Jeger[272] with lithium in liquid ammonia. 1,2-Dihydro-6-desoxy-santoninic acid (380) (3-oxo-11β(H)-eudesm-4-en-13-oic acid) was obtained in 63 % yield, m.p. 122-124°; λ_{max}^{EtOH} 250 mμ (log ε = 4.18); $\nu_{max}^{CHCl_3}$ 1705 cm.$^{-1}$ (acid), 1660, 1615 cm.$^{-1}$ (αβ-unsaturated ketone).

Attempts to prepare the 11β(H),-eudesm-4-en-13-oic acid (382).

1,2-Dihydro-6-desoxy-santoninic acid (380) (51 mg.) was treated with ethane dithiol and boron trifluoride etherate as described for the thioketal of 1,2-dihydro-santonin (see page 190). Similar work up produced a viscous oil (44 mg.), which was chromatographed on Silica. Attempts to desulphurise the thioketal with Raney nickel W-2 were unsuccessful. A small quantity of the thioketal was methylated with diazomethane, and purified by preparative t.l.c. (1:1 ether/petroleum ether). The n.m.r. of the methyl ester thioketal (383) was consistent with the structure, but it was clear that the material was not quite pure. On desulphurisation with Raney nickel W-2, the signal due to the thioketal disappeared, while the signal due to

the 4-methyl group indicated that the 4-double bond had not been hydrogenated. Hydrolysis of the ester function of the desulphurised material could not, however, be achieved. In most cases, the methyl ester was recovered, but, using more vigorous basic conditions, extensive decomposition occurred. Further work on the preparation was discontinued.

Quenching effect of santonin in liquid scintillator.

[^{3}H]Hexadecane standard (ca. 20 mg.) was placed in a scintillation tube, liquid scintillator (1.2 ml.) added, and the tube counted in the usual manner. Santonin (ca. 1 mg.) was added, allowed to dissolve, and the tube recounted. It was found that the count had significantly dropped. Accordingly, a correction curve was prepared. Convenient quantities of the hexadecane standard were accurately weighed into scintillation tubes. Into these tubes was weighed accurately, a range of weights of inactive santonin. Liquid scintillator (1.2 ml.) was added to each, and the tubes counted. Quenching factors (Q), equal to $\frac{\text{actual count}}{\text{true count}}$, where "true count" is the count to be expected from the standard in the absence of quenching, were calculated.

Wt. standard (mg.)	19.49	9.82	8.72	12.52
Wt. santonin (mg.)	0.60	0.89	0.44	1.13
True count (c.p.s.)	320	161	143	205
Actual count (c.p.s.)	301	138	140	170
Quenching factor (Q)	0.940	0.856	0.977	0.828
Log Q	$\bar{1}.973$	$\bar{1}.932$	$\bar{1}.990$	$\bar{1}.918$

A plot of log Q versus weight santonin was a good fit to a straight line. This line did not pass through the origin (log Q = 0, Q = 1, weight santonin = 0) as expected. It seems probable that the relationship is not linear below 0.5 mg. santonin, below which the correction is in any case negligable. Errors in weighing quantities less than 0.5 mg. were probably greater than those introduced through neglect of quenching.

A check was made to ascertain that the quenching factor was independent of activity. To prove this, it was necessary to prove that Q was constant over a suitable range of activity, for a given weight of santonin. As $Q = \frac{\text{actual count}}{\text{true count}}$, and the true count is directly proportional to the weight of standard, it was sufficient to prove that the $\frac{\text{actual count}}{\text{wt. standard}}$ was constant over a range of weights of standard.

Wt. standard (mg.)	37.60	18.39	1.80
Wt. santonin (mg.)	0.94	0.94	0.93
Actual count (c.p.s.)	572	279	27.3
$\frac{\text{Actual count}}{\text{Wt. standard}}$	15.2	15.2	15.1

The figures confirm that Q was independent of the activity.

Labelling of santonin by exchange.

Santonin (104 mg.) in dry dioxan (ca. 2 ml.) and deuterium oxide (0.2 ml.) was treated with KOD produced from potassium t-butoxide (23 mg.) at 100° in a sealed tube for 24 hrs. The tube was opened, and the santonin re-isolated, yielding [11-D]santonin (75 mg.) m.p. 171-172°. The absence of any splitting of the 11-methyl signal in the n.m.r. spectrum confirmed that the deuteration was practically 100 %. Santonin was labelled with tritium in a similar manner. The [11-^{3}H]santonin was crystallised, and ca. 1 mg. quantitatively diluted approximately 100-fold with ianctive santonin. The dilute santonin was re-crystallised and assayed. The [11-^{3}H]santonin was recrystallised, and the dilution repeated. The activity of the first batch was 3.43×10^4 c.p.s./mg. = 3.51 μC/mg. = 0.87 mC/mM. The stated approximate

activity of the tritiated water was 3.60 mC/mM. Thus there was only 49 % of the theoretical exchange. Further batches of santonin were tritiated in the same manner as required, and up to 60 % exchange observed.

Preparation and feeding of [11-^{3}H]1,2-dihydrosantonin (376).

[11-^{3}H]1,2-Dihydrosantonin was prepared by hydrogenation of [11-^{3}H]santonin as described on page 189. The [11-^{3}H]1,2-dihydrosantonin was carefully purified by three successive preparative t.l.c. plates (1:1 ether/petroleum ether) and assayed by 100-fold volumetric dilution of a small amount.

[11-^{3}H]1,2-Dihydrosantonin (0.92 mg.; 4.67 x 10^4 c.p.s./mg.) was added to inactive santonin (9.03 mg.), and the santonin recovered and purified by preparative t.l.c. on three successive plates (1:1 ether/petroleum ether). The santonin was crystallised (ethanol) three times and assayed. The activity of the santonin was 0.184 c.p.s./mg. Thus the maximum percentage of [11-^{3}H]santonin in the [11-^{3}H]1,2-dihydrosantonin was 0.004_0 %. This is therefore a figure below which any incorporation of 1,2-dihydrosantonin must be disregarded.

The [11-^{3}H]1,2-dihydrosantonin (376) was fed to

an Artemisia maritima plant in the usual way.
The results (see Tables 5 and 6 at end of this section) indicated that the incorporation was below 0.008 %.

Tritiation and feeding of 6,11β(H)-eudesm-4-en-6,13-olide.

[11-^{3}H]6,11β(H)-Eudesm-4-en-6,13-olide (377) was prepared by a direct exchange with tritiated water in dioxan/tritiated water as described for santonin on page 198.

The results of the feeding experiments are given in Tables 5 and 6. Incorporation : 0.017 %.

After assaying the santonin from the feeding, the remaining material was converted to the oxime in the usual way, and the oxime purified by preparative t.l.c. (1:1 ether/petroleum ether) and crystallised. There was unfortunately insufficent material to enable crystallisation of the oxime to constant activity.

Tritiation and feeding of 5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378).

[11-^{3}H]5α(H),6,11β(H)-Eudesm-3-en-6,13-olide (378) was prepared by direct exchange with tritiated water as described for santonin on page 198.

The results of the feeding experiment are given

in Tables 5 and 6. Percentage incorporation 0.030_7 %.

The remaining santonin was converted to the oxime in the usual way, purified by preparative t.l.c. (1:1 ether/petroleum ether), and crystallised to constant activity.

The figures are recorded in Tables 5 and 6.

The remaining oxime was treated with base under the exchange conditions (page 198), reisolated, repurified, and assayed. It was found to be completely inactive, indicating that all the label in the oxime was at position 11, and that no scrambling of the label had occurred.

Tritiation and feeding of 6-desoxy-santoninic acid (379).

6-Desoxy-santoninic acid (379) (49 mg.) was dissolved in tritiated water (3 drops), and potassium t-butoxide (54 mg.) added. The tube was sealed, heated at 100° for 4 days, cooled, opened, and the contents acidified. The acid solution was extracted with chloroform , and the product extracted into sodium bicarbonate. The sodium bicarbonate solution was acidified and extracted with chloroform. The dried chloroform extract was evaporated, and the crude [2-^{3}H]6-desoxy-santoninic acid (379) purified by preparative t.l.c. to give an oil (ca. 35 mg.)

The oil was assayed and fed in the usual way. The results are shown in Tables 5 and 6. No incorporation.

Tritiation and feeding of 1,2-dihydro-6-desoxy-santoninic acid (380).

[11-^{3}H]1,2-Dihydro-6-desoxy-santoninic acid (380) was prepared by lithium/liquid ammonia reduction of [11-^{3}H] santonin as described on page 195. A trial preparation was carried out using dilute [11-^{3}H]santonin (343 c.p.s./mg.). The isolated 1,2-dihydro-6-desoxy-santoninic acid was purified and assayed, using the santonin quenching correction as an approximation. The activity was 250 c.p.s./mg., indicating that 74 % of the label remained. The preparation was repeated with "hot" [11-^{3}H]santonin, and the acid (380) purified and fed. The results are shown in Tables 5 and 6. No incorporation.

Feeding of [2-^{14}C]mevalonic acid lactone.

This radiochemical was purchased from UK AEA, Radiochemical Centre, Amersham, Bucks. Specific activity was 5.01 mC/mM. The compound was fed as an aqueous solution in the usual way. The results are shown in Tables 5 and 6. No incorporation was detected.

Assessment of wash out of tritium under the conditions used in bringing the lactones into solution.

Santonin (5 mg.; 373 c.p.s./mg.) was dissolved in sodium hydroxide solution (ca. 5 ml.; 5N) and heated for ca. 5 hrs. at 60°. The solution was left overnight, neutralised to pH 7 with hydrochloric acid, and allowed to stand for ten days. The solution was rendered weakly acidic, the santonin recovered, purified and assayed. The activity was 319 c.p.s./mg. Thus, 86 % of the tritium remained.

Santonin oxime.

(a). Formation. Santonin (15 mg.), hydroxylamine hydrochloride (30 mg.) and anhydrous sodium acetate (60 mg.) were dissolved in water (0.3 ml.) and heated for about 1 hr. on a steam bath. The oxime was extracted with chloroform, purified by preparative t.l.c. and crystallised from ethanol m.p. 218-220° (lit.[1] 218°).

(b). Wash out of tritium in 11-position. Low activity [11-^{3}H]santonin oxime was prepared from tritiated santonin. The activity of the santonin was 409 c.p.s./mg. = 10.2 μC/mM, and that of the oxime 405 c.p.s./mg. = 10.9 μC/mM, confirming that the preparation of the oxime did not wash out any of the

tritium.

The santonin oxime (8 mg.) in dry dioxan (0.3 ml.), water (0.4 ml.) and potassium hydroxide (from 2.8 mg. potassium t-butoxide) were heated in a sealed tube at 100° for 72 hrs. The tube was opened, and the oxime recovered and crystallised. It was assayed, and found to be completely inactive.

(c). Quenching. A quick examination, as described for santonin on page 196 showed that santonin oxime quenched scintillation to approximately the same degree as santonin. A quantitative experiment was not carried out, as most assays were on less than 0.5 mg. oxime. In other cases, the equivalent correction for santonin was applied as a reasonable approximation.

SECOND SEASON

Costunolide (404).

The costunolide used was a gift from Dr. S,C. Bhattacharyya, National Chemical Laboratory, Poona, India. The compound is somewhat unstable at room temperature, and was stored at 0°. When required pure, costunolide was purified by preparative t.l.c. (1:1 ether/petroleum ether) immediately before use.

Properties: m.p. 109-111° (decomp.) (lit.[32] 109-111° (decomp.)); $\nu_{max}^{CHCl_3}$ 1755 $cm.^{-1}$ (αβ-unsaturated lactone); 1665 $cm.^{-1}$ (double bonds).

Hydrogenation of costunolide (404).

Costunolide (404) (525 mg.) in ethanol (ca. 45 ml.) was hydrogenated over 5 % Pd/C (25 mg.). Uptake ceased after 45 mins. (40 ml.; 23°, 741 mm.). The solution was filtered and evaporated, but the crude product could not be dissolved in dry ether.[32] The 11,12-dihydrocostunolide (403) was purified by preparative t.l.c. (1:1 ether/petroleum ether) and crystallised, with extreme difficulty, from ether/petroleum ether to yield plates (173 mg.) m.p. 76-77° (lit.[32] 77-78°); $\nu_{max}^{CHCl_3}$ 1760 $cm.^{-1}$ (lactone), 1665 $cm.^{-1}$ (double bonds).

1 % Pd/C was found to be ineffective as catalyst, whereas the first batch of 5 % Pd/C used (fresh) was so active that almost all the costunolide was hydrogenolysed to acidic products.

Attempts to add hydroxylamine and phenyl hydrazine to costunolide (404).

(a). Hydroxylamine. A mixture of costunolide (404) (58 mg.), hydroxylamine hydrochloride (50 mg.) in absolute ethanol (ca. 2 ml.) and pyridine (1 drop) was heated

under reflux. T.l.c. indicated that a reaction product was being formed. After 30 mins., the mixture was evaporated under reduced pressure, and the product obtained by preparative t.l.c. The i.r. spectrum of the crude product showed maxima at 3550 and 3325 $cm.^{-1}$, but only weak lactonic carbonyl absorption at 1760 $cm.^{-1}$

(b). Hydroxylamine at lower temperature. Costunolide (404) (60 mg.), hydroxylamine hydrochloride (50 mg.) in methanol (2 ml.) containing pyridine (3 drops) were heated at 55° under dry nitrogen for 6 hrs. Work up as above yielded a small quantity of a substance differing (t.l.c.) from costunolide. This compound proved to be unstable to oxygen, and, therefore, was immediately taken up in methanol (ca. 5 ml.), benzaldehyde (0.2 ml.) added, and the mixture heated gently for 30 mins. No change in the reactants (t.l.c., i.r.) was detected.

(c). Phenyl hydrazine. Costunolide (404) (78 mg.) was dissolved in pyridine (0.3 ml.) and phenyl hydrazine hydrochloride (162 mg.) added. The mixture was allowed to stand for 6 hrs. The mixture was poured into water, and extracted with chloroform. The extract was dried and evaporated. T.l.c. indicated two products, but these were present in extremely small quantities.

Preparation of 12-aminocostunolide.

Costunolide (404) (109 mg.) in ethanol (min. vol.) was slowly added to a cooled saturated ethanolic solution of ammonia (ca. 20 ml.) and left at 0° for 2 days. The ammonia and ethanol were removed at room temperature under reduced pressure to give a pale yellow gum, which on evaporation at 0.1 mm. formed a glass (120 mg.), 12-aminocostunolide (406). It was purified by preparative t.l.c. ν_{max}^{Nujol} 3400 $cm.^{-1}$ (NH), 1760 $cm.^{-1}$ (lactone), 1665 $cm.^{-1}$ (double bonds).

Attempted reactions with 12-aminocostunolide (406).

(a). Benzaldehyde/pyridine. To 12-amino-costunolide (406) (23 mg.) in dry pyridine (3 drops) was added redistilled benzaldehyde (3 drops), and the mixture allowed to stand for 20 hrs. The starting material was recovered almost quantitatively.

The experiment was repeated at 60°, but there was no evidence of any reaction.

The experiment was repeated using a solvent, methanol, heated under reflux. No benzeldehyde remained after 10 hrs., but its disappearance was apparently due to its self condensation. The ¢2-amino-costunolide was recovered almost quantitatively.

The last mentioned experiment was repeated,

replacing the pyridine with N/10 sodium hydroxide solution (ca. 1 ml.). The †2-aminocostunolide was recovered quantitatively.

(b). Model reactions using β-phenyl ethylamine.

(i). β-Phenyl ethylamine (126 mg.) and benzaldehyde (163 mg.) in pyridine (0.5 ml.) was allowed to stand at room temperature for 72 hrs., but starting materials were quantitatively recovered.

(ii). β-Phenyl ethylamine (113 mg.) and benzaldehyde (126 mg.) was treated with sodium hydroxide (0.5 ml.; 4N) and sufficient methanol to keep the mixture as one phase added. After 12 hrs. at room temperature, there was no indication (t.l.c.) of any reaction.

(iii). A mixture of benzaldehyde (267 mg.) and β-phenyl ethylamine (290 mg.) was allowed to stand at room temperature for 30 mins. Complete reaction had occurred, and the water produced was removed on an oil pump. The product was recrystallised (ethanol) m.p. 72-73° (lit.[289] ca. 70°).

(iv). p-Nitro-benzaldehyde reacted equally readily with β-phenyl ethylamine under the same conditions as (iii).

(c). Attempted reaction of 12-aminocostunolide (406) with benzaldehyde and p-nitrobenzaldehyde in the absence of a solvent.

(i). Benzaldehyde (31.3 mg.) was mixed with 12-aminocostunolide (406) (69.4 mg.), but the amine was not sufficiently soluble in benzaldehyde. Sufficient chloroform was added to ensure that the mixture formed one phase. Most of the chloroform was removed under reduced pressure to leave a viscous oil. After some hours at room temperature (t.l.c. indicated no reaction), the mixture was heated on the steam bath for one hour. On work up, the costunolide amine was recovered almost quantitatively.

(ii). 12-Aminocostunolide (406)(45.9 mg.) and p-nitrobenzaldehyde (40.6 mg.) were dissolved in the minimum volume of chloroform. Most of the chloroform was then evaporated, and the viscous oil heated overnight on a steam bath. On work up, the amine (406) was recovered almost quantitatively.

Santamarine (409).

The santamarine (409) used was kindly made available by Dr. A. Romo de Vivar, Mexico. Properties: m.p. 134-136° (lit.[86] 134-136°); $\nu_{max}^{CHCl_3}$ 3400 $cm.^{-1}$(OH), 1760 $cm.^{-1}$ (lactone), 1665, 1630 $cm.^{-1}$ (double bonds).

Hydrogenation of santamarine (409).

Santamarine (409) (28 mg.) in absolute ethanol (ca. 4 ml.) was hydrogenated over 1 % Pd/C (8 mg.). Uptake ceased after ca. 2.8 ml (calc. for one equivalent 2.74 ml.). The solution was filtered, and evaporated under reduced pressure to yield an oil (27 mg.). Crystallisation (with difficulty) from ether/petroleum ether yielded 11,12-dihydrosantamarine (408) (1β-hydroxy-5α(H),6,11β(H)-eudesm-3-en-6,13-olide) yielded colourless plates m.p. 124-127° (lit.[86] 124-125°); $\nu_{max}^{liq.}$ 3500 $cm.^{-1}$ (OH), 1765 $cm.^{-1}$ (lactone).

Epoxidation of 5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378).

5α(H),6,11β(H)-Eudesm-3-en-6,13-olide (378) (Δ^3-lactone) (30.2 mg.) was treated with monoperphthalic acid in chloroform (ca. 7 ml.; 0.6 N) at 0° for ca. 1hr. Water was added, and the mixture extracted with chloroform. The choroform solution was dried and evaporated. The crude epoxide was purified by preparative t.l.c. on alumina (1:1 ether/petroleum ether), and crystallised (ethanol) to yield colourless plates (5.2 mg.) m.p. 146-148° of 3,4-epoxy-5α(H),6,11β(H)-eudesman-6,13-olide (410). $\nu_{max}^{CHCl_3}$ 1765 $cm.^{-1}$ (lactone).

Sodium borohydride reduction of 1,2-dihydrosantonin (376).

1,2-Dihydrosantonin (345 mg.) in dry isopropanol (ca. 10 ml.) was treated with an excess of sodium borohydride at 0° overnight. The 3-hydroxy-6,11β(H)-eudesm-4-en-6,13-olide (397) was obtained in the usual manner as an oil (330 mg.). The oil was unstable to oxygen, slowly reverting to 1,2-dihydrosantonin. A small quantity was acetylated with acetic anhydride and pyridine at 0° to yield colourless plates (ethanol) m.p. 204-205°. Alcohol: $\nu_{max}^{CHCl_3}$ 3600 cm.$^{-1}$ (OH), 1765 cm.$^{-1}$ (lactone), 1610 cm.$^{-1}$ (double bond); Acetate: $\nu_{max}^{CHCl_3}$ 1765 cm.$^{-1}$ (lactone), 1720 cm.$^{-1}$ (acetate).

Pseudosantonin (412) (8α-hydroxy-1-oxo-6,11β(H)-eudesm-4-en-6,13-olide).

Crude pseudosantonin (412) (6.10 g.) was chromatographed on alumina (Grade III, 150 g.) to give pure pseudosantonin (4.53 g.) as plates (ethanol) m.p. 185-186° (lit.[302] 183-186°), and desoxypseudosantonin (411) (1-oxo-6,11β(H)-eudesm-4-en-6,13-olide) (0.59 g.), also as plates (ethanol) m.p. 98-98.5° (lit.[100] 101-102°). Pseudosantonin : $\nu_{max}^{CHCl_3}$ 3450 cm.$^{-1}$ (OH), 1765 cm.$^{-1}$ (lactone), 1710 cm.$^{-1}$ (ketone). Desoxypseudosantonin: $\nu_{max}^{CHCl_3}$ 1760 cm.$^{-1}$ (lactone), 1710 cm.$^{-1}$ (ketone).

Experiments with pseudosantonin (412).

(a). Mesylation. Pseudosantonin (412) (50 mg.) was dissolved in sodium hydroxide solution (0.5 ml.; N/10), and cooled to 0°. Redistilled methane sulphonyl chloride (ca. 0.3 ml.) was added, and the mixture shaken vigorously for 36 hrs. The product of this reaction showed the n.m.r. signal typical of the normal 6β-lactonic oxygen, and also a mesyl group, presumably the 8-mesylate.

(b). Attempted oxidation of the hydroxy acid from pseudosantonin with chromium trioxide in acetic acid. Pseudosantonin (412) (29.9 mg.) was treated with one molar equivalent of potassium hydroxide in water, and the solution neutralised to pH 7, extracted, and evaporated. The hydroxy acid was treated with a solution of chromium trioxide in acetic acid containing 5 % water. The product did not show any αβ-unsaturated ketone absorption in the i.r. or u.v.

(c). Attempted oxidation with Sarett reagent. Pseudosantonin (412) (30 mg.) was treated with one molar equivalent of potassium hydroxide in water, and evaporated. The residue was taken up in pyridine, and slowly added to Sarett reagent, chromium trioxide (40 mg.) in pyridine (0.3 ml.). The mixture was left at 0° for

24 hrs., and at 25° for 8 hrs. Ice was added, and the reaction mixture worked up to yield a complex mixture (32.4 mg.). The i.r. and u.v. spectra showed no indication of an αβ-unsaturated ketone.

(d). Alkaline manganesedioxide. Pseudosantonin (412) (205 mg.) was dissolved in sodium hydroxide solution (ca. 10 ml.; N/10) and activated manganese dioxide (ca. 2.5 g.) added. The mixture was heated under reflux overnight. Work up produced a complex mixture, which showed no signs of containing an αβ-unsaturated keto group.

Sodium borohydride reduction of santonin.

Santonin in dry isopropanol/methanol (1:1) was reduced with excess of sodium borohydride at room temperature in the usual way, to yield an alcohol, the i.r. spectrum of which was consistent with it being the dienol. After standing in air for 30 mins., the dienol had almost quantitatively reverted to santonin.

Preparation and feeding of $[11,12\text{-}^{3}H_2]$11,12-dihydrocostunolide (403)

Costunolide was hydrogenated with tritiated hydrogen (produced by the action of lithium on tritiated water), and the $[11,12\text{-}^{3}H_2]$ 11,12-dihydrocostun-

olide (403) purified by t.l.c. and assayed. The results, and the results of the feeding are given in Tables 7 and 8. The incorporation was 0.004_2 %.

The santonin remaining was converted to the oxime, assayed, and the remaining oxime treated under the exchange condition to wash out the label at C-11. The washed out oxime had a specific activity of 30.1 % of the activity of the untreated oxime.

Preparation and feeding of [11-^{3}H] 3-hydroxy-6,11β(H)-eudesm-4-en-6,13-olide (397).

The title compound (397) was prepared by the reduction of [11-^{3}H] 1,2-dihydrosantonin as described on page 211. The tritiated alcohol (397) was purified by t.l.c. immediately before use for feeding. The results of the assay and feeding are given in Tables 7 and 8. The incorporation was 0.007_5 %.

The remaining santonin was converted to the oxime, assayed, and the remaining oxime treated under the exchange conditions. After wash out the oxime was completely inactive, indicating that all the label had been located at C-11.

Preparation and feeding of [11,12-$^3H_2$]11,12-dihydrosantamarine (408).

[11,12-$^3H_2$]11,12-Dihydrosantamarine (408) was prepared by hydrogenation of santamarine (409) with tritiated hydrogen, and purified by preparative t.l.c. (1:1 benzene/ethyl acetate). The results of the assay and feeding are recorded in Tables 7 and 8. The incorporation was 0.009_2 %. There was insufficient material to enable to oxime to be crystallised to constant activity.

Preparation and feeding of 3,4-epoxy-5α(H),6,11β(H)-eudesman-6,13-olide (410), tritiated at 11.

[11-^3H]3,4-Epoxy-5α(H),6,11β(H)-eudesman-6,13-olide (410) was prepared by epoxidation of [11-^3H]Δ^3-lactone (378), as described for the inactive compound on page 210. The assay and feeding results are tabulated in Tables 7 and 8. There was no incorporation.

Repeat feeding of [11-^3H]5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378).

See page 200 and Tables 7 and 8. The incorporation was 0.015_0 %. The oxime preparation and wash out were not repeated.

Repeat feeding of [11-^{3}H] 6,11β(H)-eudesm-4-en-6,13-olide (377).

See page 200 and Tables 7 and 8. The incorporation was 0.009_0 %. The remaining santonin was converted to the oxime, purified, assayed, and then washed out under the exchange conditions. The washed out oxime was completely inactive, indicating that all the label had been at the 11-position.

Repeat feeding of [11-^{3}H] 1,2-dihydrosantonin (376).

See page 199, and Tables 7 and 8. There was no incorporation.

Repeat feeding of [2-^{14}C] mevalonic acid lactone.

See page 202 and Tables 7 and 8. The incorporation was 0.011_4 %.

Control wash out of [11,12-$^3H_2$] 11,12-dihydrosantamarine.

Dilute [11,12-$^3H_2$] 11,12-dihydrosantamarine (408) (50 mg.) in dioxan (1 ml.), water (0.5 ml.) was heated with potassium hydroxide (from 15 mg. potassium t-butoxide) at 100° in a sealed tube for 48 hrs. The tube was opened, the contents acidified and extracted. The extract was dried, evaporated, and the residue chromatographed on a t.l.c. plate to yield colourless plates (5 mg.) m.p. 60-61°. The i.r. spectrum of this

substance was identical with that of 11,12-dihydrosantamarine to all intents and purposes. It was assumed that this was the 11-epimer of 11,12-dihydrosantamarine. The results of the assays (Table 8) showed that the specific activity of this substance was 21 % of the specific activity of the 11,12-dihydrosantamarine, suggesting that much more than 50 % of the label was in the 11-position.

Check that procedure used in feeding 11,12-dihydrocostunolide (403) did not cyclise any to the Δ^3-lactone (378).

It was first determined that 11,12-dihydrocostunolide (403) was well separated from Δ^3-lactone (378) by t.l.c. (1:1 ether/petroleum ether).

[11,12-$^3H_2$]11,12-dihydrocostunolide (1 mg.; 9.01×10^4 c.p.s./mg. = 10.μC/mg. = 2.39 mC/mM) was converted to the corresponding sodium salt of the hydroxy acid by the procedure used in the feeding experiments. The solution was then left exposed to air and light for 14 days. The sodium salt of the hydroxy acid derived from Δ^3-lactone (378) (prepared from 3.4 mg. of the lactone) was added, and the organic components extracted from the mixture with chloroform (several extractions). The extracts were

combined, dried, and evaporated, during which procedure the lactones reformed. The residue was chromatographed on a t.l.c. plate (1:1 ether/petroleum ether), and the band corresponding to the Δ^3-lactone (378) extracted from the plate with chloroform. The extract was evaporated to yield the Δ^3-lactone (ca. 2 mg.) which was assayed without further purification. The total activity was 0.34 c.p.s. Thus 1 mg. of [11,12-$^3H_2$] 11,12-dihydrocostunolide could produce a total of 0.34 c.p.s. = 0.26×10^{-4} μC. This represents a percentage conversion of the 11,12-dihydrocostunolide to the Δ^3-lactone of 0.0002_5 %. Thus the incorporation of 11,12-dihydrocostunolide (0.004_2 %) was well above anything which could have been caused by the cyclisation of the 11,12-dihydrocostunolide to the Δ^3-lactone during the feeding procedures.

Degradation of santonin from the feeding of [2-^{14}C]mevalonic acid lactone.

Santonin from the mevalonate feeding (16.1 mg.; 1.74 c.p.s./mg. = 6.03×10^{-5} μC/mg. = 1.48×10^{-3} μC/mM) was diluted with inactive santonin (14.6 mg.) to total 30.7 mg. (0.911 c.p.s./mg. = 3.16×10^{-5} μC/mg. = 0.777×10^{-3} μC/mM). This santonin (30.7 mg.) in ethanol (ca. 2 ml.) was hydrogenated over Adams

catalyst (13.2 mg.) until uptake ceased, to yield a mixture of tetrahydrosantonins. To the tetrahydrosantonins in benzene (10 ml.) was added phenyl lithium in ether (15 ml.; 1M) under nitrogen. The ether was distilled off, and the mixture refluxed for 6 hrs. Water was carefully added, the solution carefully acidified with dilute hydrochloric acid, and extracted with chloroform. The chloroform extract was dried and evaporated. The residue showed no carbonyl absorption in the i.r.

The residue, in dilute sulphuric acid (5 ml.; 10 %) was treated with chromium trioxide (8 g.) in sulphuric acid (12 ml.; 10 %). The mixture was heated under reflux for ca. 15 mins., and then slowly distilled for 6 hrs. in a flow of air into sodium hydroxide solution. Distilled water was added to maintain the volume.

The sodium hydroxide solution was extracted with ether, the ether extract dried and evaporated to yield a mixture of biphenyl and benzophenone. The sodium hydroxide solution was acidified and extracted to yield the acids.

The biphenyl and benzophenone were separated by preparative t.l.c. (10 % ether in petroleum ether) and

the benzophenone (25.0 mg.) converted to its oxime, purified by t.l.c. and crystallised m.p. 142-3°.

The acids were dissolved in ether, xylene (2 ml.) added, and the mixture distilled into sodium hydroxide. The volatile acetic acid was thereby distilled into the sodium hydroxide solution, leaving the benzoic acid in the flask. The benzoic acid was sublimed three times, and crystallised (ethanol/water) to give pure benzoic acid (49.9 mg.) as plates, m.p. 121°.

Blank experiments. (a). A blank experiment, under identical conditions, but omitting the tetrahydrosantonin afforded benzoic acid (30.5 mg.) and a trace (less than 1 mg.) of benzophenone.

(b). With rigorous exclusion of carbon dioxide during the preparation of the phenyl lithium, the yield of benzoic acid was reduced to 7.0 mg. It appeared that biphenyl was being slowly oxidised to benzoic acid.

(c). Repeating (b), but separating the biphenyl from the desired intermediate triol before oxidation, yielded no benzoic acid.

(d). Repeating (c), but including tetrahydrosantonin (9.5 mg.) yielded benzoic acid (7.3 mg.) and benzophenone (ca. 6 mg.).

Radiochemical assay.

The activity of the benzoic acid was 0.0325* c.p.s./mg. Total activity in benzoic acid is 49.9 x 0.0325 c.p.s. = 1.62* c.p.s. = 0.609 x 10^{-4} μC. Assuming that the benzoic acid in the degradation was produced quantitatively, plus the extra, then this total represents 1/16 or 6.3 % of the activity in the santonin.

The activity of the benzophenone oxime was 0.090* c.p.s./mg. = 3.36 x 10^{-6} μC/mg. = 0.71 x 10^{-4} μC/mM. The molar activity of the benzophenone oxime is then 1/12 or 9.2 % of the molar activity of the santonin from which it was obtained.

An attempt was made to assay the acetic acid produced in the degradation by converting it to its p-phenyl phenacyl derivative, but only 3.8 mg. m.p. 109° was obtained. This was essentially inactive.

THIRD SEASON

Oxidation of farnesol to farnesal (420).

To farnesol (419) (876 mg.) in dry petroleum ether was added activated manganese dioxide (ca. 5 g.) and the mixture shaken overnight at room temperature. The mixture was filtered, and the filtrate evaporated to yield crude farnesal (420). The farnesal (420) was purified by preparative t.l.c. to yield an oil (756 mg.). The compound was not stable, apparently polymerising on standing. ν_{max}^{liq} 1680, 1665 $cm.^{-1}$ (αβ-unsaturated aldehyde.

Reduction of farnesal (420) to farnesol (419).

Farnesal (420) (30 mg.) in dry ether/methanol (2:1, 10 ml.) was treated with sodium borohydride (ca. 20 mg.) at 0° for four days. Water, and then dilute hydrochloric acid was added, and the mixture extracted with dichloromethane. The extract was dried and evaporated to yield farnesol (26 mg.). This was purified by preparative t.l.c. (5 % ethyl acetate in benzene). The farnesol was identical (i.r.,t.l.c.) with authentic farnesol. The g.l.c. showed a mixture of two isomers, very similar to the authentic farnesol. ν_{max}^{liq} 3500 $cm.^{-1}$ (OH), 1660 $cm.^{-1}$ (double bonds), 1000 $cm.^{-1}$ (C - O stretch of alcohol).

Phosphorylation of farnesol.

The di(triethylamine) salt of phosphoric acid was prepared by dissolving phosphorus pentoxide (7.1 g.) in water (ca. 50 ml.) and redistilled triethylamine (20 g.) added. The solution was evaporated on a steam bath under reduced pressure overnight to yield the required phosphate as an extremely viscous (at room temperature) colourless gum.

Trichloro-acetonitrile was prepared by the method of McBee et al.[295]

To farnesol (419) (58 mg.) in trichloro-acetonitrile (149 mg.) were added portions of a saturated solution of di(triethylamine) hydrogen phosphate in acetonitrile. A total of ca. 15 ml. was added over three days. Water and ether were added, and the aqueous layer separated and evaporated to ca. 5 ml. Cyclohexylamine (ca. 2 ml.) was added, and the small quantity of precipitated farnesyl monophosphate removed by filtration. Lithium chloride (ca. 1 g.) was added to the filtrate, and the precipitated farnesyl pyrophosphate obtained by centrifugation. The farnesyl pyrophosphate was dried in vacuo to give 109 mg. (101 % yield - slight contamination with lithium chloride).

Ascending paper chromatography of the farnesyl pyrophosphate on Whatman No. 1 (6:3:1 n-propanol:

0.880 ammonia: water) showed only one spot with [illegible] phosphate detecting spray,[303] R_f = 0.60 (lit.[294] 0.74 (ascending method?).

Attempts to add a functional group selectively to the 1-position of santonin.

(a). Hydrogen peroxide in methanol with pyridine. Santonin (1.45 g.) in methanol (110 ml.) was cooled to 0°, and hydrogen peroxide solution (26 ml.; 30 %) and pyridine (8 ml.) added. The mixture was left at 0° for 24 hrs. and then at 25° for several days, but santonin was quantitatively recovered.

(b). Hydrogen peroxide in methanol with N/10 sodium hydroxide. Santonin (2.46 g.) in methanol (25 ml.) was treated with sodium hydroxide (ca. 2 ml.; N/10) and hydrogen peroxide (10 ml.; 30 %) added in portions (0.2 ml.) over three days. Work up yielded only unchanged santonin.

(c). Hydrogen peroxide in methanol with 5N sodium hydroxide. Santonin (1.50 g.) in methanol (100 ml.) was treated with hydrogen peroxide (26 ml.; 30 %) and sodium hydroxide solution (10 ml.; 5N) at 5-10°. The mixture was allowed to stand at 0° overnight. Work up yielded only unchanged santonin.

(d). Reduction and acetylation of santonin. First attempt. Santonin (2 g.) in isopropanol (50 ml.) was treated with an excess of sodium borohydride at 50° for 15 mins, and at room temperature overnight. The alcohols obtained by the usual work up were acetylated with acetic anhydride in pyridine. Chromatography yielded as principal product (ca. 1 g.), an oil, the i.r. spectrum of which showed intense maxima at 1730 and 1240 $cm.^{-1}$ (acetate), and also maxima at 1660, 1630 and 1610 $cm.^{-1}$ (dienone), but no lactone carbonyl.

(e). Reduction and acetylation. Second attempt. Santonin (20 g.) in dry methanol (ca. 400 ml.) was treated with sodium borohydride (10 g.) and heated to 50° for two hours. The mixture was allowed to stand overnight at room temperature, diluted with water, and acidified. The crude product obtained by extraction was acetylated with acetic anhydride and pyridine at 0° overnight. Water and then dilute hydrochloric acid were added, and the products obtained in the usual way. The mixture of crude "acetates" was chromatographed on alumina (500 g.; Grade I). Three major products were obtained. (i). A highly crystalline colourless solid as plates (benzene/petroleum ether) m.p. 149-151° (ca. 3 g.). This proved to be hyposantonin lit.[264] m.p. 152°. (ii), and (iii). These two

acetates (total 12 g.) were not completely separated on the column. A small portion was purified by preparative t.l.c. (1:1 benzene/ethyl acetate). The substances were not stable, undergoing considerable decomposition while being extracted from the plate. The n.m.r. spectra of the somewhat impure substances indicated that they each contained two aromatic protons (τ 3.05), and an acetate (τ 7.9). They were not further investigated.

(f). Reduction and acetylation without acidification. Santonin (1 g.) in dry distilled ethanol (50 ml.) and ether (50 ml.) was treated with an excess of sodium borohydride at room temperature overnight. Water was added, and the solution extracted three times with dichloromethane. The extracts were combined, dried, evaporated, and acetylated with acetic anhydride in pyridine at 0° overnight. The acetate, obtained by removal of the excess of reagents by an oil pump, was purified by preparative t.l.c. (25 % ethyl acetate in benzene) to yield an oil (ca. 800 mg.). ν_{max}^{liq} 1775 $cm.^{-1}$ (lactone), 1730 $cm.^{-1}$ and 1240 $cm.^{-1}$ (acetate), 1665 $cm.^{-1}$ (double bonds).

(g). Attempted epoxidation of the acetate from (f). The acetate from (f), presumably (422), (100 mg.), in chloroform was treated with monoperphthalic acid

(3 ml.; 0.6N) at room temperature overnight. On work up, the acetate was almost quantitatively recovered. The experiment was repeated, heating the mixture under reflux for 0.5 hr. Work up yielded rather less than quantitative recovery of the acetate. The rest of the product was, however, a complex mixture of highly polar substances.

(h). Attempts to add potassium cyanide, potassium cyanate, potassium thiocyanate and sodium azide.

(i). Potassium cyanide. Santonin (1.08 g.) and potassium cyanide (0.388 g.) in dry methanol (ca. 50 ml.) was heated under reflux for 6 hrs. The mixture was acified (with care) and extracted. The crude product was purified by preparative t.l.c. (30 % ethyl acetate in benzene) to yield a viscous oil (116 mg.) which could not be induced to crystallise. $\nu_{max}^{CHCl_3}$ 3550 $cm.^{-1}$ (OH and NH ?), 1770 $cm.^{-1}$ (lactone), 1725 $cm.^{-1}$ (lactam and methyl ester). This was presumed to be (424).

A repeat of this experiment, taking aliquots every 15 mins. showed that the three i.r. bands due to the dienone in santonin all disappeared at the same rate.

Potassium cyanide and ammonium chloride in dimethylformamide did not react with santonin.

(ii). Potassium cyanate. Santonin was heated under reflux with a twofold excess of potassium cyanate in each

of dry methanol, dry dimethylsulphoxide and dry pyridine. In all cases, santonin was almost quantitatively recovered.

(iii). Potassium thiocyanate. Experiment (ii) was repeated using potassium thiocyanate in place of potassium cyanate, but with the same result.

(iv). Sodium azide. Experiment (ii) was repeated using sodium azide in place of potassium cyanate, and with suitable precautions, but with the same result.

Preparation of germacrene (430).

Germacrone. Gift from Dr. J.K. Sutherland. m.p. 54-55° (lit.[304] 55-56°); ν_{max}^{Nujol} 1680, 1655 $cm.^{-1}$ ($\alpha\beta$-unsaturated ketone).

Reduction of germacrone (434). Germacrone (431) (220 mg.) in pyridine (1 ml.) was stirred for 18 hrs. at 70° with sodium borohydride (30 mg.). Ether (20 ml.) was added, and the ether solution washed with hydrochloric acid (3 x 10 ml.; 2N), water, sodium bicarbonate, and water again. The dried ethereal solution was evaporated, and purified by preparative t.l.c. (5 % ethyl acetate in benzene) to yield germacrol (432) as an oil (180 mg.). ν_{max}^{liq} 3500 $cm.^{-1}$ (OH), 1625 $cm.^{-1}$ (double bonds).

Acetylation of germacrol (432). Freshly prepared germacrol (432) (180 mg.) in pyridine (2 ml.) was allowed to stand overnight at room temperature with acetic anhydride (ca. 0.5 ml.) and pyridine (excess). After most of the pyridine and acetic anhydride had been removed on an oil pump, the residue was taken up in chloroform, and washed with water. The chloroform solution was dried and evaporated to yield germacrol acetate (433) (153 mg.) as an oil; $\nu_{max}^{liq.}$ 1735, 1245 $cm.^{-1}$ (acetate), 1660 $cm.^{-1}$ (double bond).

Lithium/ammonia reduction of germacrol acetate (433). Lithium (ca. 0.5 g.) was added to a solution of germacrol acetate (433) (130 mg.) in dry distilled liquid ammonia (20 ml.). After about 30 mins., a blue colour appeared, indicating that the reduction was complete. Ether (30 ml.) was added, followed by ammonium chloride, and the ammonia allowed to evaporate. Water was carefully added, and then dilute hydrochloric acid (2N). The ether layer was separated and dried. Evaporation at room temperature yielded crude germacrene (430), which was immediately taken up in petroleum ether, and filtered through a short alumina (Grade V) column. The pure germacrene (430) (95 mg.) was stored at - 45° under dry, oxygen free nitrogen. $\nu_{max}^{liq.}$ 1650 $cm.^{-1}$ (double bond).

Tetrahydrosantonins.

Santonin (50 mg.) in acetone (5 ml.) was hydrogenated over 10 % Pd/C (10.0 mg.) to give a mixture (49 mg.) of 3-oxo-4,5α(H),6,11β(H)-eudesman-6,13-olide (434) and 3-oxo-4,5,6,11β(H)-eudesman-6,13-olide (435). This mixture was divided into two approximately equal parts.

(i). The first part was crystallised from ethanol (three times) to yield plates of 3-oxo-4,5α(H),6,11β(H)-eudesman-6,13-olide (431) m.p. 140-147° (lit.[284] 144°) slightly contaminated with 3-oxo-4,5,6,11β(H)-eudesman-6,13-olide (435).

(ii). The second part of the mixture was taken up in sodium hydroxide (5 ml.; 2N), acidified and re-isolated. The product was crystallised three times from ethanol to yield 3-oxo-5α(H),4,6,11β(H)-eudesman-6,13-olide (436) as plates m.p. 147-153° (lit.[284] 154-155°) slightly contaminated with 3-oxo-4α(H),5,6,11β(H)-eudesman-6,13-olide (437).

Douglanine (426).

The douglanine available was a gift from Professor T.A. Geissman, Los Angeles. It had, unfortunately substantially decomposed. Its m.p. was 178-186° (lit.[87] 115-117°); ν_{max}^{Nujol} 3500 cm^{-1} (OH),

1770, 1680 $cm.^{-1}$ (αβ-unsaturated lactone). The n.m.r. spectrum (see Table 11) showed no vinyl proton at τ 4.7, and the 4-methyl signal as a sharp singlet at τ 8.5. It appeared that the compound was, in fact, largely 3,4-epoxy-douglanine (427) (3,4-epoxy-1α-hydroxy-5α(H),6,11β(H)-eudesm-3-en-6,13-olide).

Hydrogenation of "3,4-epoxy-douglanine" (427).

"3,4-Epoxy-douglanine" (427) (14.2 mg.) in ethanol (5 ml.) was hydrogenated over 1 % Pd/C (5.3 mg.). Uptake ceased after 1.32 ml. (760 mm., 25°). The dihydroderivative was filtered, and evaporated under reduced pressure. The "3,4-epoxy-11,12-dihydro-douglanine" (428) (12 mg.) was obtained by crystallisation from ethanol as plates, m.p. 180-182°; ν_{max}^{Nujol} 3520 $cm.^{-1}$ (OH), 1775 $cm.^{-1}$ (lactone). Further work on the compound is in progress.

Preparation and feeding of [1-^{3}H]farnesol (419).

Farnesol (420) was reduced with tritiated sodium borohydride (194 mC/mM) as described previously on page 222 for the inactive compound to yield [1-^{3}H]farnesol. This was purified by t.l.c. and

assayed. The results, and those of the feeding experiment are recorded in Tables 9 and 10. The incorporation was 0.0002_5 %.

Half of the remaining santonin was converted to the oxime, purified and assayed. An attempt was made to wash the label out of the other half of the remaining santonin (see below).

Preparation and feeding of [1-^{3}H]farnesyl pyrophosphate.

[1-^{3}H]Farnesyl pyrophosphate was prepared from [1-^{3}H]farnesol (419) as described on page 223. The feeding results are detailed in Tables 9 and 10. There was no incorporation.

Preparation and feeding of [8-^{3}H]germacrene (430).

[8-^{3}H]Germacrene was prepared for germacrone (431) by reduction with tritiated sodium borohydride (194 mC/mM), acetylation and lithium/ammonia reduction, as previously described on page 228 and 229. The results of the assay, and of the feeding experiment are given in Tables 9 and 10. There was no incorporation.

Preparation and feeding of [11-^{3}H]4,5α(H),6,11β(H)-eudesman-6,13-olide (434).

[11-^{3}H]4,5α(H),6,11β(H)-eudesman-6,13-olide (434) was prepared by hydrogenation of [11-^{3}H]santonin as previously described on page 230. The results of the assay and feeding experiment are detailed in Tables 9 and 10. There was no incorporation.

Preparation and feeding of [11-^{3}H]5α(H),4,6,11β(H)-eudesman-6,13-olide (436).

[11-^{3}H]5α(H),4,6,11β(H)-eudesman-6,13-olide (436) was prepared by hydrogenation of [11-^{3}H]santonin as previously described on page 230. The results of the assay, and the feeding experiment are given in Tables 9 and 10. There was no incorporation.

Preparation and feeding of [11,12-^{3}H$_2$]"3,4-epoxy-11,12 dihydrodouglanine" (428).

[11,12-^{3}H$_2$]"3,4-Epoxy-11,12-dihydrodouglanine (428) was prepared by hydrogenation of "3,4-epoxy-11,12-douglanine" (427) with tritiated hydrogen, as previously described on page 230. The results of the assay and feeding experiment are recorded in Tables 9 and 10. There was no incorporation.

Repeat feeding of [11,12-$^{3}H_{2}$]11,12-dihydrosantamarine (458).

See page 215 and Tables 9 and 10. On this occasion, there was sufficient santonin remaining to permit its conversion to the oxime, and purification of the oxime to constant activity. There was, however, insufficient oxime remaining to permit the oxime from an attempted wash out to be crystallised to constant activity. The incorporation was 0.005_5 %.

Attempted wash out of the label in the farnesol fed santonin.

The remaining santonin from the feeding of farnesol (10 mg.) was heated in a sealed tube with 3 drops potassium hydroxide solution (1.1 g. in 2.5 ml. water) at 100° for two hours. The tube was cooled, opened, and the santonic acid obtained by extraction. The crude santonic acid (438) was purified by crystallisation from benzene/petroleum ether to yield plates m.p. 164-167° (lit.[300] 165-168°). Unfortunately, there was insufficient material to allow recrystallisation to constant activity.

EXTRACTIONS OF OTHER ARTEMISIA SPECIES

Artemisia stellariana.

A plant of the species Artemisia maritima was extracted in the usual way. By preparative t.l.c. (1:1 ether/petroleum ether), crude 1,2-dihydrosantonin (376) (8 mg.) was obtained, and purified to yield an oil (5.6 mg.). This was converted to its semicarbazone, and crystallised, m.p. 230-233° (decomp.) Authentic 1,2-dihydrosantonin semicarbazone m.p. 228-232° (decomp.). Mixed m.p. 227-230° (decomp.). The i.r. spectra of both the 1,2-dihydrosantonin and the semicarbazone were identical with those of authentic material.

Other species.

Plants of the species A. vulgaris variegata, A. vulgaris, A. lactiflora, A. palmeri and A. abrotanum were examined, but no lactones were found.

LIMONIN WORK

Extraction of Citrus decumana.

In April 1965, one plant of the species Citrus decumana (grapefruit; 2 yrs. old) was harvested, crushed in liquid nitrogen, and extracted with acetone.

Although some furans were detected using p-dimethylamino-benzaldehyde (Erlich reagent) as a spray in t.l.c. experiments, no limonin was detected.

Extraction of Dictamnus albus.

A plant of the species Dictamnus albus was extracted with acetone as described above. By preparative t.l.c. (1:1 chloroform/ethyl acetate) crude limonin (6 mg.) was obtained. This was crystallised from dichloromethane/ isopropanol to yield pure limonin (4.1 mg.) as needles m.p. 283-285°. Authentic limonin m.p. 292-294°. Mixed m.p. 286-291°. The i.r. spectrum (Nujol) was identical with that of authentic limonin; ν_{max}^{Nujol} 1755 $cm.^{-1}$ (lactones), 1715 $cm.^{-1}$ (ketones).

Feeding of [2-^{14}C]mevalonic acid lactone to Dictamnus albus.

One plant of this species was fed over three weeks with 10 μC of [2-^{14}C]mevalonic acid lactone by the wick technique in June 1966. The plant was extracted after three weeks, and the limonin (3 mg.) isolated from the roots. On assay, the results were:

(a) 0.631 c.p.s./0.65 mg. = 0.97 c.p.s./mg.

(b) 0.533 c.p.s./0.57 mg. = 0.94 c.p.s./mg.

Mean 0.95 c.p.s./mg. = 3.21×10^{-5} μC/mg. = 1.45×10^{-2} μC/mM.

Total activity recovered 9.63×10^{-5} μC. Incorporation of [2-^{14}C]-DL mevalonic acid lactone = 0.0009_6 %.

TABLE 5 FIRST SEASON'S FEEDINGS

Precursor	Precursor fed				Santonin recovered			Incorp-oration %
	Wt. mg.	Activity 10^{-4} cps/mg.	Activity μC/mg.	Total activ. fed. μC	Wt.* mg.	Activ. 10^{-4} μC/mg.	Total activ. 10^{-3} μC	
[11-^{3}H] 1,2-dihydro-santonin (376)	4.4	4.67	4.93	20.3	42.3	Nil	Nil	(<0.0008)
[11-^{3}H] 6,11β(H)-eudesm-4-en-6,13-olide (377)	4.6	4.61	4.87	21.5	26.9	1.43	3.85	0.017_7
[11-^{3}H] 5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378)	4.2	2.59	2.74	11.2	27.3	1.26	3.44	0.030_7
[2-^{3}H] 6-desoxy-santoninic acid (379)	4.9	2.85	3.00	14.7	27.0	Nil	Nil	(<0.002)
[11-^{3}H] 1,2-dihydro-6-desoxy-santoninic acid (380)	8.2	3.00	3.22	26.3	10.0	Nil	Nil	(<0.0004)
[2-^{14}C] mevalonic acid lactone (DL)	0.26	-	38.7	10.0	125	Nil	Nil	(<0.0003)

* including dilution, if any.

TABLE 6 FIRST SEASON RADIOCHEMICAL ASSAY RESULTS.

Experiment and Substance	Wt. mg.	Obs. cps	Q	True cps	Effic-iency	Activity		
						cps/mg.	10^{-4} μC/mg.	10^{-2} μC/mM
[11-^{3}H] 6,11β(H)-eudesm-4-en-6,13-olide (377) feeding.								
Santonin (a)	0.72$_5$	0.93	0.91	1.02	26.2%	1.41		
Santonin (b)	0.74^5	0.90	0.90	1.00	26.2%	1.36		
Mean of (a) and (b)					26.2%	1.38	1.43	3.51
[11-^{3}H] 5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378) feeding.								
Santonin (a)	0.70	0.77	0.91$_5$	0.83$_5$	26.2%	1.20		
Santonin (b)	0.75	0.85	0.90^5	0.94^5	26.2%	1.25		
Mean of (a) & (b)					26.2%	1.22	1.26	3.10
Oxime (2.7 mg. diluted with 4.0 mg.)	0.85	0.38	0.88	0.43	26.2%	0.51	0.52	1.36
Equivalent activity of undiluted oxime.							1.25	3.28
Oxime after wash out.	-	Nil	-	-	-	-	Nil	Nil

TABLE 7 SECOND SEASON'S FEEDINGS

Precursor	Precursor fed				Santonin recovered			Incorporation %
	Wt. mg.	Activity 10^{4} cps/mg.	Activity µC/mg.	Total activ. fed. µC	Wt.* mg.	Activ. 10^{-4} µC/mg.	Total activ. 10^{-3} µC	
[11,12-$^{3}H_2$] 11,12-dihydrocostunolide (403)	14	9.01	10.2	140	31.2	1.87	5.86	0.004_2
[11-^{3}H] 3-hydroxy-6,11β(H)-eudesm-4-en-6,13-olide (397)	20	2.45	2.81	56.2	29.0	1.46	[illegible].23	0.007_5
[11,12-$^{3}H_2$] 11,12-dihydrosantamarine (408)	15	6.13	7.03	101	29.1	3.16	9.15	0.009_2
[11-^{3}H] 3,4-epoxy-5α(H)-6,11β(H)-eudesm-3-en-6,13-olide (410)	14	1.41	1.62	22.4	6.1	Nil	Nil	(<0.0003)
[11-^{3}H] 5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378)	25	2.05	2.35	59.0	34.2	2.59	8.85	0.015_0
[11-^{3}H] 6,11β(H)-eudesm-4-en-6,13-olide (377)	16	2.21	2.53	40.5	29.6	1.23	3.64	0.009_0
[11-^{3}H] 1,2-dihydrosantonin (376)	24.1	5.21	6.00	144	32.7	Nil	Nil	(<0.0004)
[2-^{14}C] mevalonic acid lactone (DL)	0.40	-	38.7	15.0	28.4	0.603	1.72	0.011_4

* includes dilution, if any.

TABLE 8 SECOND SEASON RADIOCHEMICAL ASSAY RESULTS.

Experiment and substance	Wt. mg.	Obs. cps	Q	True cps	Efficiency	Activity cps/mg.	Activity 10^{-4} µC/mg.	Activity 10^{-2} µC/mM
[11,12-$^3H_2$] 11,12-dihydrocostunolide (403) feeding.								
Santonin (a)	0.650	1.00	0.93	1.08	23.6%	1.66		
Santonin (b)	0.675	1.00	0.92	1.10	23.6%	1.63		
Mean of (a) & (b)					23.6%	1.64	1.87	4.62
Santonin oxime	0.220	0.400*	1.00	0.400*	35.1%	1.82*	1.40	3.66
Santonin oxime after wash out (a)	0.380	0.202*	1.00	0.202*	35.1%	0.53*		
Santonin oxime after wash out (b)	0.140	0.080*	1.00	0.080*	35.1%	0.57*		
Mean of (a) & (b)					35.1%	0.55*	0.421	1.10
[11-^3H] 3-hydroxy-6,11β(H) eudesm-4-en-6,13-olide (397) feeding.								
Santonin (a)	0.610	0.70	0.94	0.75	23.6%	1.22		
Santonin (b)	0.775	0.91	0.90	1.02	23.6%	1.31		
Mean of (a) & (b)					23.6%	1.27	1.46	3.57
Santonin oxime	0.720	0.97*	0.91	1.07*	35.1%	1.50*	1.16	2.98
Santonin oxime after wash out.	-	Nil	-	-	-	-	Nil	Nil
[11,12-$^3H_2$] 11,12-dihydrosantamarine (408) feeding.								
Santonin (a)	0.605	1.61	0.94	1.71	23.6%	2.83		
Santonin (b)	0.425	1.11	0.98	1.13	23.6%	2.66		
Mean of (a) & (b)					23.6%	2.75	3.16	7.80

* assayed on the improved counter.

TABLE 8 (CONTINUED) SECOND SEASON RADIOCHEMICAL ASSAY RESULTS

Experiment and substance	Wt. mg.	Obs. cps	Q	True cps	Effic-iency	Activity cps/mg.	Activity 10^{-4} µC/mg.	Activity 10^{-2} µC/mM
[11-^{3}H]5α(H),6,11β(H)-eudesm-3-en-6,13-olide (378) feeding.								
Santonin (a)	0.750	1.50	0.90	1.67	23.6%	2.23		
Santonin (b)	0.550	1.20	0.95	1.26	23.6%	2.29		
Mean of (a) & (b)					23.6%	2.26	2.59	6.38
[11-^{3}H]6,11β(H)-eudesm-4-en-6,13-olide (377) feeding.								
Santonin (a)	0.875	1.27*	0.87	1.46*	35.1%	1.66*		
Santonin (b)	0.615	0.87*	0.93_5	0.93*	35.1%	1.52*		
Mean of (a) & (b)					35.1%	1.59*	1.23	3.01
Santonin oxime	0.720	0.97*	0.91	1.07*	35.1%	1.49*	1.15	3.00
After wash out	-	Nil	-	-	-	-	Nil	Nil
[2-^{14}C]mevalonate feeding								
Santonin (a)	1.015	1.49	0.84	1.77	78%	1.74		
Santonin (b)	0.695	1.10	0.91	1.21	78%	1.74		
Mean of (a) & (b)					78%	1.74	0.603	1.48
Control wash out of [11,12-^{3}H]11,12-dihydro santamarine (408)								
Before wash out (a)	0.485	212*	0.97	219*	35.1%	452*		
Before wash out (b)	0.675	282*	0.92	307*	35.1%	453*		
Mean of (a) & (b)					35.1%	452*	347	862
After wash out (a)	0.560	52.5*	0.95	54.9*	35.1%	98.0*		
After wash out (b)	0.250	23.5*	1.00	23.5*	35.1%	94.2*		
Mean of (a) & (b)					35.1%	96*	73.5	184

* assayed on the improved counter.

TABLE 9 THIRD SEASON'S FEEDINGS

Precursor	Precursor fed				Santonin recovered			Incorporation %
	Wt. mg.	Activity 10^4 cps/mg.	Activity µC/mg.	Total activ. fed µC	Wt.* mg.	Activ. 10^{-4} µC/mg.	Total activ. 10^{-3} µC	
[1-^{3}H] farnesol (419)	5.4	251	193	1040	28.6	0.916	2.61	0.0002_5
[1-^{3}H] farnesyl pyrophosphate	6.5	149	115	747	38.7	Nil	Nil	(<0.00005)
[8-^{3}H] germacrene (430)	3.3	5 1	45.5	150.2	38.7	Nil	Nil	(<0.0005)
[11-^{3}H] 3-oxo-4,5α(H),6,11β(H)-eudesman-6,13-olide (434)	3.4	3.39	2.61	8.87	26.6	Nil	Nil	(<0.005)
[11-^{3}H] 3-oxo-5α(H),4,6,11β(H)-eudesman-6,13-olide (436)	3.0	3.34	2.57	7.71	34.3	Nil	Nil	(<0.005)
[11-^{3}H] "3,4-epoxy-11,12-dihydrodouglanine" (428)	6.6	14.0	10.78	71.2	40.5	Nil	Nil	(<0.0005)
[11,12-$^3H_2$] 11,12-dihydrosantamarine (408)	12.3	8.37	6.44	79.2	29.8	1.46	4.35	0.005_5

* including dilution, if any.
All assays in c.p.s. in this table were recorded on the improved counter.

TABLE 10 THIRD SEASON RADIOCHEMICAL ASSAY RESULTS

Experiment and substance	Wt. mg.	Obs. cps	Q	True cps	Efficiency	Activity cps/mg.	Activity 10^{-4} μC/mg.	Activity 10^{-2} μC/mM
[1-^{3}H] farnesol (419) feeding.								
Santonin (a)	0.910	0.897*	0.86	1.04*	35.1%	1.14*		
Santonin (b)	0.790	0.873*	0.89	0.979*	35.1%	1.24*		
Mean of (a) & (b)					35.1%	1.19*	0.916	2.25
Santonin oxime	0.765	1.060*	0.89	1.19*	35.1%	1.53*	1.18	3.07
[11,12-$^3H_2$] 11,12-dihydro-santamarine (408) feeding.								
Santonin (a)	0.825	1.409*	0.88	1.59*	35.1%	1.93*		
Santonin (b)	1.020	1.596*	0.84	1.90*	35.1%	1.86*		
Mean of (a) & (b)						1.90*	1.46	3.60
Santonin oxime	0.860	1.122*	0.88_5	1.38	35.1%	1.61*	1.23	3.03

* assayed on the improved counter.

TABLE 11 N.M.R. DATA FOR THE EUDESMANE TYPE COMPOUNDS

Compound	Methyl group on						Other protons
	C-4		C-10		C-11		
	τ	J	τ	J	τ	J	
santonin (375)	7.87d	1	8.63s	-	8.66d	7½	C-1 3.76d J=10; C-2 3.27d J=10 C-6 5.18d J=10.
1,2-dihydrosantonin (376)	8.05d	½	8.69s	-	8.79d	7	C-6 5.31d J=10.
thioketal of 1,2-dihydrosantonin (384)	7.90d	1½	8.85s	-	8.78d	7	C-6 5.45d J=8½; thioketal 6.69s.
6,11β(H)-eudesm-4-en-6,13-olide (377)	8.20d	1	8.89s	-	8.81d	7	C-6 5.51d J=7.
3-hydroxy-6,11β(H)-eudesm-4-en-6,13-olide (397)	8.05d	1	8.82s	-	8.79d	7	C-6 5.4d J=8.
6-episantonin (387)	7.93s	-	8.71s	-	8.60d	8	C-6 4.38d J=4½; C-1 3.74d J=10 C-2 3.13d J=10.
1,2-dihydro-6-epi-santonin (386)	8.11s	-	8.76s	-	8.63d	8	C-6 4.57d J=5.
pseudosantonin (412)	8.06s	-	8.75s	-	8.61d	8	C-6 4.47d J=6.
desoxypseudo-santonin (411)	8.08s	-	8.75s	-	8.65d	8	C-6 4.60d J=6.
5α(H),611β(H)-eudesm-3-en-6,13-olide (378)	8.20s	-	9.08s	-	8.79d	7	C-3 4.60s.

TABLE 11 (CONTINUED) N.M.R. DATA FOR THE EUDESMANE TYPE COMPOUNDS

Compound	Methyl group on						Other protons
	C-4		C-10		C-11		
	τ	J	τ	J	τ	J	
santamarine (409)	8.13s	-	9.10 9.18	-	3.92d 4.57d	3	C-6 5.14d J=6; C-3 4.67s; C-10 methyl split.
11,12-dihydro-santamarine (408)	8.18s	-	9.10 9.18	-	8.80d	6	C-3 4.67s; C-6 5.14d J=8½; C-10 methyl split.
3,4-epoxy-5α(H),6,11β(H)-eudesman-6,13-olide (410)	8.55s	-	9.08s	-	8.78d	6	C-3 7.1m.
"3,4-epoxy-douglanine" (427)	8.51s	-	9.14s	-	3.98d 4.65d	4	C-6 6.19d J=10.
"3,4-epxoy-11,12-dihydrodouglanine"(428)	8.50s	-	9.11s	-	8.78d	6	C-6 6.2.
6-desoxysantoninic acid (379)	8.13s	-	8.80s	-	8.77d	7	C-1 3.76d J=10; C-2 3.24d J=10.
1,2-dihydro-6-desoxysantoninic acid (380)	8.25s	-	8.82s	-	8.76d	6	
3α-hydroxy-4,5α(H),6,11β(H)-eudesman-6,13-olide(397)	8.98d	8	8.92s	-	8.76d	7	
hyposantonin (423)	7.59s	-	-		8.72d	6½	C-6 5.0d J=9; 1-Methyl 7.85s.

TABLE 12 N.M.R. DATA FOR THE GERMACRANE TYPE COMPOUNDS

Compound	Methyl group/exomethylene on						Other protons
	C-4		C-10		C-11		
	τ	J	τ	J	τ	J	
Costunolide (404)	8.29s	-	8.57s	-	3.76d 4.48d	4	C-1 5.32d J=9; C-5 5.38d J=9; C-6 5.16m.
11,12-dihydrocost-unolide (403)	8.29s	-	8.58d	1	8.75d	$6\frac{1}{2}$	C-1 5.33d J=6; C-5 5.40d J=6; C-6 5.20m.
12-aminocostunolide (406)	8.28s	-	8.53s	-	?	-	C-1 5.28m; C-5 5.33m; NH 6.9 (2 protons).
germacrone (431)	7.88s	-	8.28d	3	8.40s 8.59s	- -	C-1 5.18m; C-5 5.30m.
germacrol (432)	8.10d	$\frac{1}{2}$	8.68s	-	8.25s 8.35s	- -	C-1 and C-5 indistinct.
germacrene (430)	7.95m	-	8.48s	-	8.30s		C-1 4.8m; C-5 5.4m.

s singlet; d doublet; m multiplet.

SECTION 6

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