0033-4545/79/0401-0837 $02.00/0

BIOGENETIC CONCEPTS IN TERPENE STRUCTURE ELUCIDATION

Sukh Dev

Malti-Chem Research Centre, Nandesari, Vadodara, India

Abstract —— In the development of Natural Products Chemistry,bio-
genetic considerations have played an important and useful role. In
this article, the author cites examples from his work in the area of
Terpene Chemistry to highlight the usefulness of these concepts. To
illustrate their application in structure elucidation, an example each
from sesquiterpene, diterpene, triterpene and sesterterpene chemistry
has been described. Role of these concepts in the search for new
structural types, congeners and precursors has been brought out. The
usefulness of Absolute Stereochemistry Biogenetic Rule has been
pointed out.

INTRODUCTION

Classically, structure elucidation was the prime motivation for the study of
a natural product, whether biologically active or just an academic curiosity.
Degradation, transformations, synthesis —— all, formed an integral part of
this excercise. Many such studies, which were oftenprotracted, constitute
landmarks in Natural Products Chemistry, and stand testimony to the patience,
ingenuity and experimental skill of these workers.

With the induction of powerful spectroscopic methods and newer, more effective
separation techniques, coupled with newer advances in Organic Chemistry theory
and practice, the above situation has continuously changed since 1950, and
during the next one-and a-half to two decades underwent complete metamorphosis.
Structure determination has now become vastly simple and, often routine.
Complex molecules, when crystalline, are invariably handled by X-ray crysta-
llography. These developments have affected the activity in this area in two
ways. Firstly, more and more effort is being expended on biologically signi-
ficant molecules, whether for drug use or for understanding biochemical proce-
sses or ecological interactions; structure elucidation being just a secondary
objective. Some of these aspects were just not possible to study earlier as
the available research tools were not refined enough to tackle them. Secondly,
a given raw material is now searched for compounds of a given class, as thoro-
ughly as possible, so as to arrive at what may be called biogenetic patterns
or profiles and hopefully look for new, novel skeletal types; often the mate-
rial under study is so selected as to maximise the chances for the discovery
of new structural variants. Such investigations, though purely academic, are
still being actively pursued and have vastly enriched our knowledge of the
so-called secondary metabolites. The full impact of this activity is best
appreciated, when one considers the present state of knowledge, say in the
area of sesquiterpenoids, by way of illustration. Thus, for example,
Simonsen's classical work, The Terpenes , vol. III (Ref. 1), published in
1951, records gross structures of some 35 sesquiterpenoids, belonging to
twelve different skeletal types. Of these, structures of no less than ten
compounds had to be re-formulated later and, stereochemical details could not
be given even for a single sesquiterpene. In contrast, at present, at least
1400 well-characterised sesquiterpenoids, falling into over one hundred
distinct skeletal types are known and, for a vast majority of these complete
stereochemical details are available!

In this proliferation of our knowledge of the secondary metabolites, biogene-
tic concepts have often played a useful role and it is the purpose of this
lecture to highlight this aspect by citing examples from Terpenoid Chemistry.

As is customary in such lectures, I shall restrict myself to work carried
out in our own laboratory, though I am keenly aware that many more beautiful
examples can be cited from work of other investigators (Ref. 2).

Biogenetic concepts can assist workers in the area of Natural Products
Chemistry in several ways:
1. Structure elucidation
2. Searching for congeners and precursors
3. Looking for new structural types
4. Absolute stereochemistry
5. Synthesis (Biomimetic)

In this lecture, I shall restrict myself to the first four aspects, which
broadly come under 'Structure Elucidation', the theme for to-day's lecture.
Of these four facets, I propose to treat the first in some detail.

STRUCTURE ELUCIDATION

I have selected one example each from the area of sesquiterpene, diterpene,
triterpene and sesterterpene chemistry.

Allohimachalol

The essential oil from the wood of Himalayan deodar (Cedrus deodara Loud.)
contains ∿4% of a sesquiterpene alcohol, m.p. 85-86° $[\alpha]_D$ + 37.4° (CHCl$_3$,
c 3.3%), which we have termed allohimachalol (Ref. 3).   It analyses for
C$_{15}$H$_{26}$O (M$^+$, m/e 222), is clearly mono-olefinic (IR: OH 3400, 1020 cm^{-1};
C=CH 1660, 853 cm^{-1}. Quantitative hydrogenation to C$_{15}$H$_{28}$O, m.p. 102-103°,
giving no color with TNM) and hence bicyclic. Secondary nature of the
hydroxyl is obvious from the PMR spectrum, which also discloses other stru-
ctural features: three tert. methyls (3H singlets at 0.75, 0.82 and 1.00 ppm),
one vinylic methyl (3H, bs, 1.76 ppm), CHOH (1H, d x d, 3.09 ppm, J$_1$=5.0Hz,
J$_2$=10.0 Hz), C=CH (1H, bd, 5.22 ppm, J=5Hz).

On pyridine-CrO$_3$ oxidation, allohimachalol gave an olefinic ketone, C$_{15}$H$_{24}$O,
in which the olefinic linkage and carbonyl function are not conjugated
(λ_{max} 290 nm, ϵ 27). From the IR carbonyl stretch (1699 cm^{-1}) of this ketone,
it is also obvious that the original OH must be located on a 6-membered or a
higher ring. The above structural features appeared to be incompatible with
the then known bicyclic sesquiterpene frameworks and it is concluded that,
in all probability, a new type in sesquiterpenoids is involved. For arriving
at a most logical working structure for this alcohol, recourse was made to
biogenetic considerations. The essential oil of Cedrus deodara contains,
besides this alcohol and bisabolane-based sesquiterpenoids, longiborneol (2)
and a new class of sesquiterpenes, represented by α-himachalene (3), β-hima-
chalene (4) and himachalol (5), structures of which, we had just then eluci-
dated (Refs. 3,4). It was considered reasonable that the species 1, the
possible biogenetic progenitor of himachalenes (Ref. 4), longiborneol and
related compounds (Ref. 5), could as well be the precursor for allohimachalol,
as a suitable 1,2-shift, in principle, would lead to structures (Fig. 1) with
the required structural features.

The interrelationship of allohimachalol with himachalenes on the lines sugge-
sted in Fig. 1, could be demonstrated in a rather simple and straightforward
manner. Allohimachalol tosylate (m.p. 56-57°) on solvolysis in aq. dioxane,
in presence of Li$_2$CO$_3$, furnished a product which was shown to consist of
α-himachalene (3; 3-4%), β-himachalene (4; 15-20%), himachalol(5; 30-35%) and
allohimachalol (40-45%) (Fig. 2). This limits the possible structures of
allohimachalol to 6, 7 and 8.

A distinction between the above structures could be made by the sequence of
reactions depicted in Fig. 3. Allohimachalone was osmylated and the osmate
ester decomposed with hydrogen sulphide to give a crystalline keto-diol
(m.p. 102-103°), which on cleavage with sodium metaperiodate furnished a
diketo aldehyde. The latter was oxidised with CrO$_3$ and the product esteri-
fied to furnish a diketo methyl ester, which must have one of the structures
9, 10 or 11. The diketo ester (as well as the diketo aldehyde) failed to
give any color with aq. or alc. FeCl$_3$, a finding which argues against its
formulation as 11. This is also supported by its PMR spectrum, which displays
signals for 5H located between 2.5-3.1 ppm, and assignable to methylene and

(3) (4) (5)

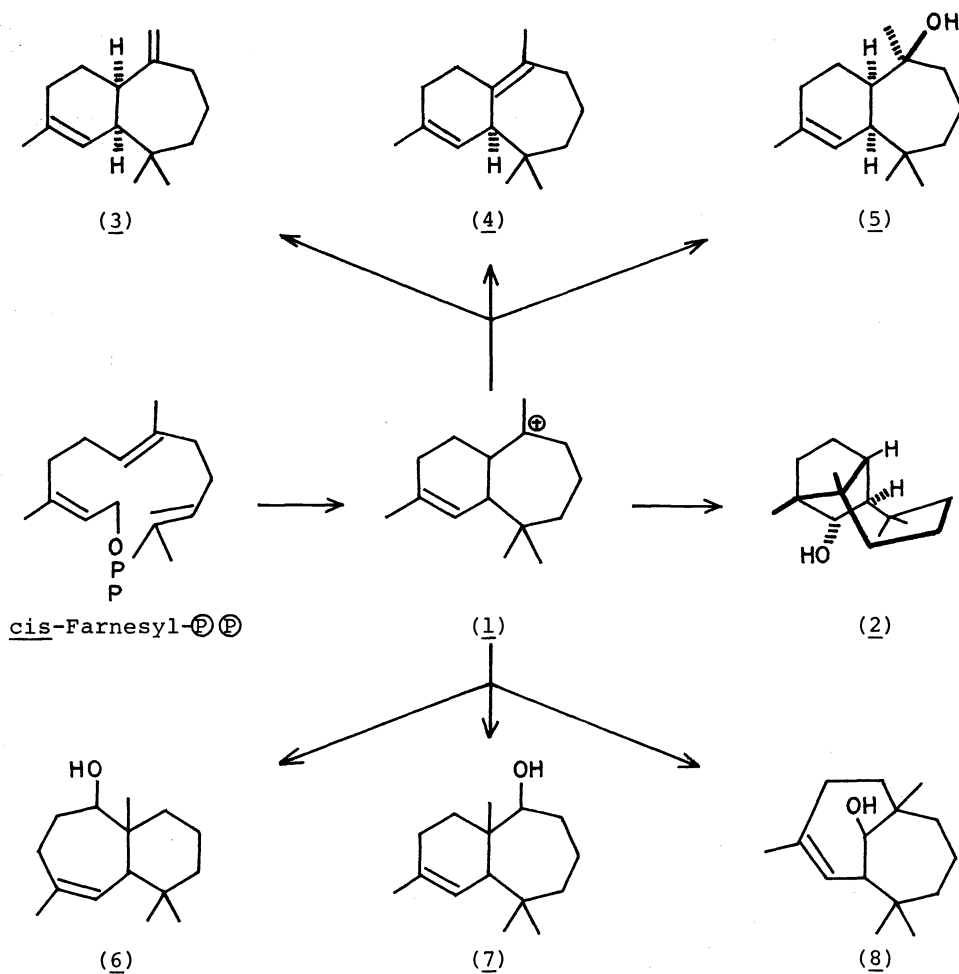cis-Farnesyl-⑫⑫ (1) (2)

(6) (7) (8)

Fig. 1

methine protons α to C=O function; 11 has only three such protons. A distinction between 9 (a 1,4-diketone) and 10 (a 1,5-diketone) could be made as follows: The diketo methyl ester on being heated with phosphorus pentasulphide
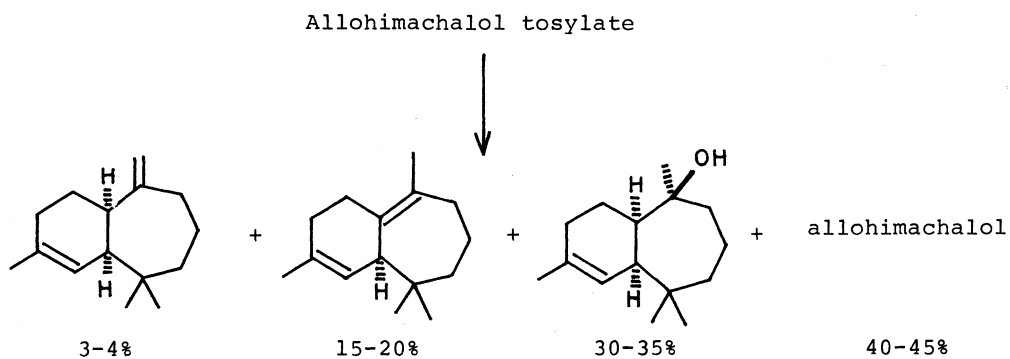
Allohimachalol tosylate

3-4%   15-20%   30-35%   40-45%

+   +   +   allohimachalol

Fig. 2

gave in 50% yield a product, characterised as the thiophene derivative 12
(mixture of C-1 epimers; 4:1): M^+, m/e 280; UV absorption (λ_{max} 221-223 nm,
ϵ 8500); IR (COOMe 1735, 1155 cm^{-1}; thiophene 1222, 1028, 788 cm^{-1}); PMR
(major isomer: three tertiary methyls at 0.62, 0.92 and 1.15 ppm; Ar-Me, s,
2.27 ppm; COOMe, s, 3.57 ppm; CH-COOMe, s, 2.9 ppm; thiophene ring protons,
essentially s, 5.78 ppm). This conversion to a thiophene is consistent only
with the formulation of the diketo ester as 9 and, consequently allohimachalol
must be represented by the gross structure 6 (Ref. 6).

The derivation (Ref. 6) of the stereochemistry of allohimachalol on the basis
of solvolysis of its tosylate to (+)-himachalol, a compound with established
(Ref. 3) absolute stereochemistry (5), cannot be taken as entirely free from
pitfalls (Ref. 7), except for the absolute configuration at C-1 (13). X-ray
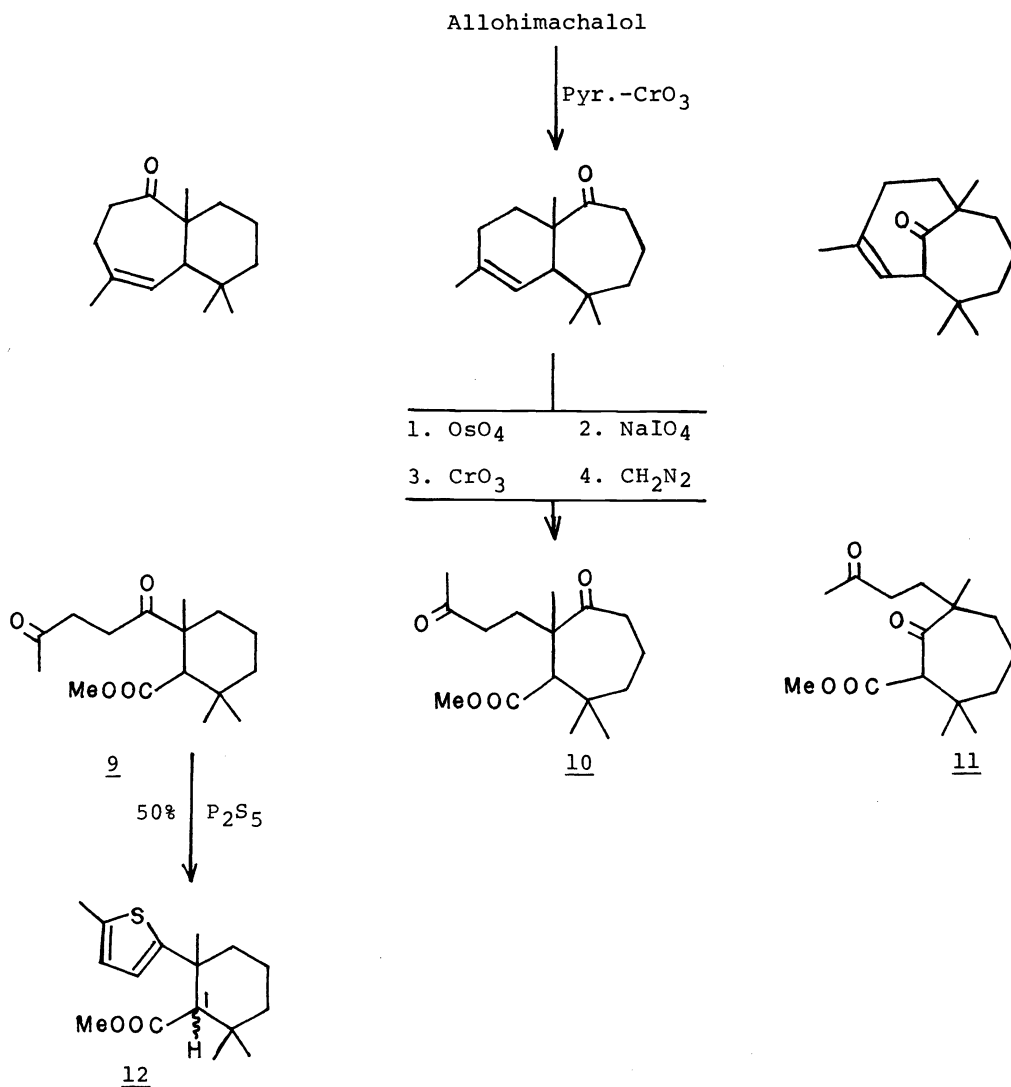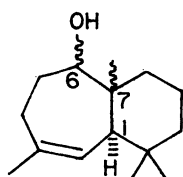diffraction analysis of allohimachalol is under study.

Allohimachalol

Pyr.-CrO$_3$

1. OsO$_4$ 2. NaIO$_4$

3. CrO$_3$ 4. CH$_2$N$_2$

9

10

11

50% P$_2$S$_5$

12

Fig. 3

OH

6   7

H

<u>13</u>

Hardwickiic acid
<u>Hardwickia pinnata</u> Roxb. is a large handsome three (often 100 ft. high and
with a trunk of 4-8 ft diameter) growing wildly in the evergreen forests of
Western Ghats of India. The tree, when tapped, yields in large quantities,
a dark oleoresin, which is a complex blend of sesquiterpenes and diterpenes.
The major diterpene constituent is an acid, m.p. 106-107°, which we have
named, hardwickiic acid.

Hardwickiic acid analyses for $C_{20}H_{28}O_3$, and its spectral data (UV, IR, PMR),
clearly shows the following structural features:

two $\overset{\displaystyle C}{\underset{\displaystyle C}{C-C}}-Me$;   one   —CH—Me

H
H

H

COOH   ;
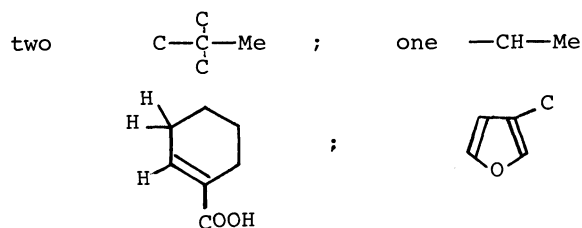
From quantitative hydrogenation studies, it was concluded that hardwickiic
acid has a total of three C=C linkages. Usual carbon valency computation,
next, showed that hardwickiic acid must be bicarbocyclic.

$\oplus$H CH_2OPP → CH_2OPP

<u>14</u>

H
H

<u>16</u>

normal bicyclic
diterpenoids
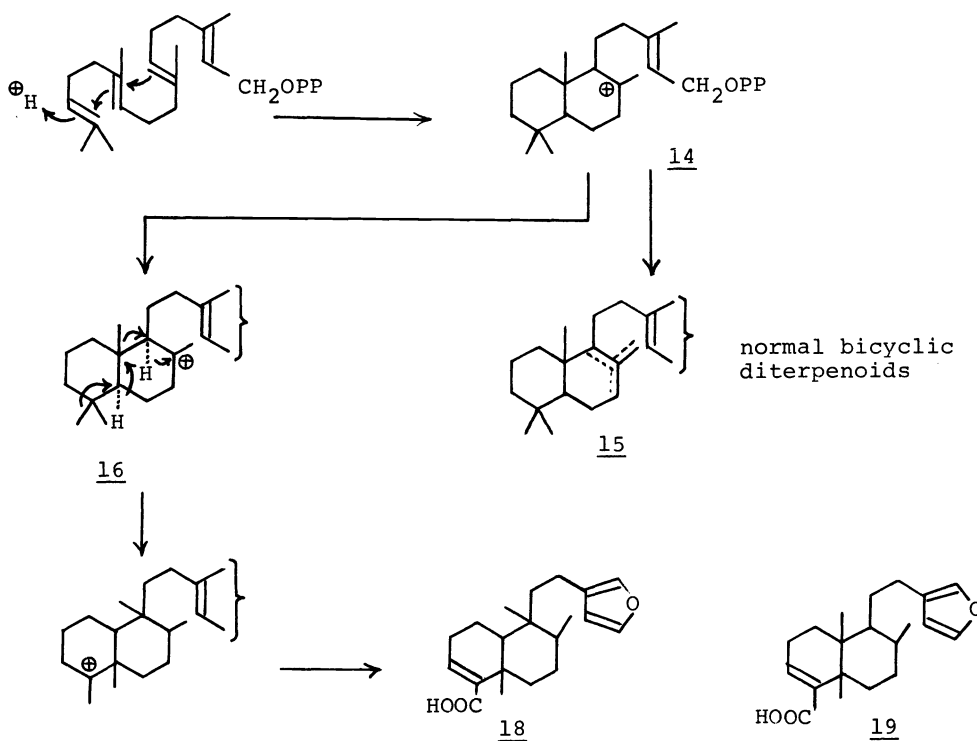
<u>15</u>

HOOC   <u>18</u>   HOOC   <u>19</u>

Fig. 4

On catalytic dehydrogenation over 10% Pd-C, this acid yielded a 3:2 mixture of 1,2-dimethyl- and 1,2,5-trimethylnaphthalene.

From the number of Me groups (free and 'functionalised'), it is clear that hardwickiic acid is a bicarbocyclic diterpenoid. While looking for a suitable framework for the various structural features, it became clear that both, a secondary Me and an $\alpha\beta$-unsaturated carboxyl function, cannot be accommodated in a normal bicyclic diterpene framework: (15; Fig. 4) with furan in the side-chain. This dictates a rearranged bicyclic skeleton for the new acid, and, gross structure 18 which can be generated from the ion 14 (Fig. 4), the accepted biogenetic progenitor (Ref. 8) for many bicyclic diterpene types, appeared most appropriate, as it not only meets all the structural require-ments of hardwickiic acid, but would also account for the preferential for-mation of 1,2-dimethylnaphthalene during dehydrogenation. Another possibility (19), which can also be accommodated in the Biogenetic Isoprene Rule, is considered less likely in view of the dehydrogenation results.

To prove unequivocally the gross structure 18, it is essential to adduce sound evidence for (i) presence of Me groups at C-5 and C-9, and (ii) relative dis-position of the functionalities. This was achieved as follows (Fig. 5):
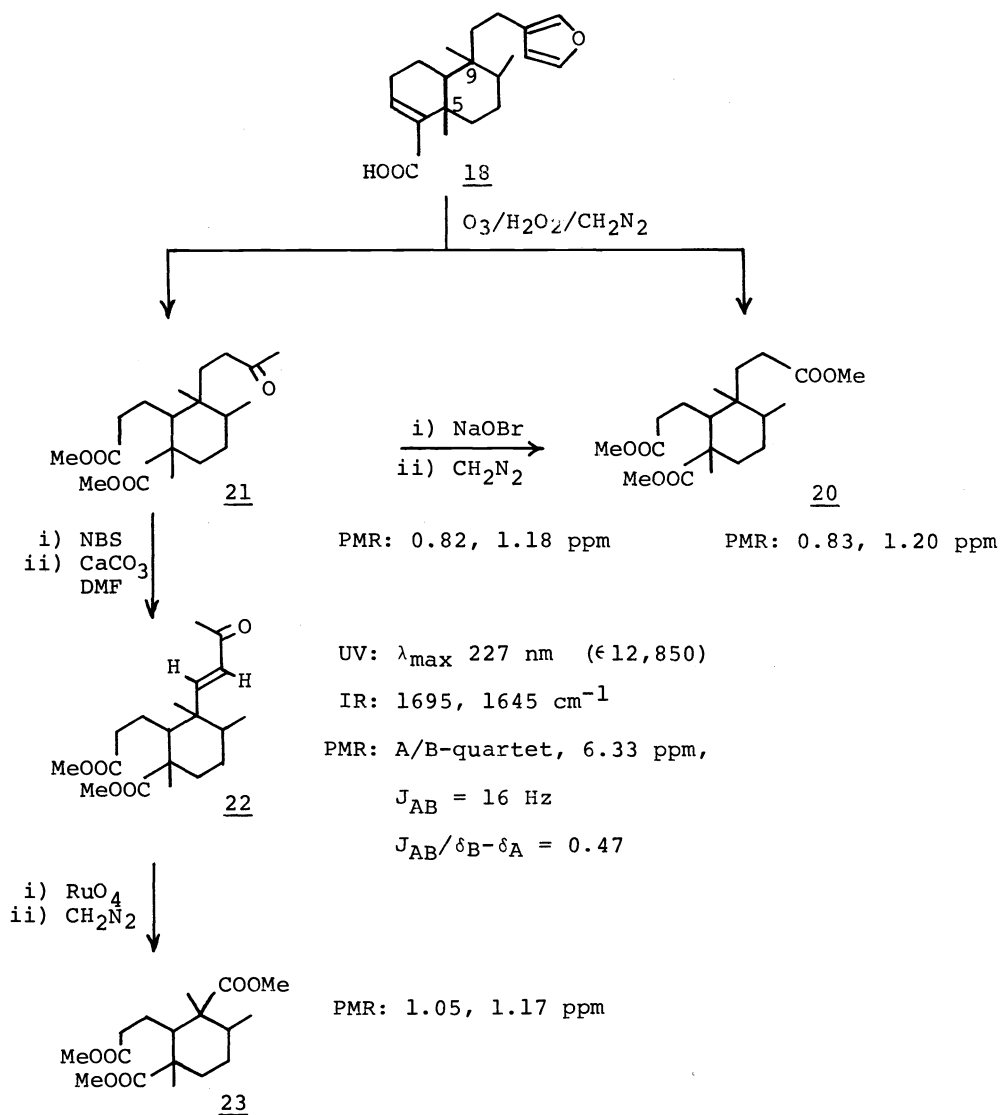

$O_3/H_2O_2/CH_2N_2$

21

i) NBS
ii) CaCO_3
   DMF

i) NaOBr
ii) CH_2N_2

20

PMR: 0.82, 1.18 ppm

PMR: 0.83, 1.20 ppm

22

UV: λ_{max} 227 nm (ϵ 12,850)

IR: 1695, 1645 cm^{-1}

PMR: A/B-quartet, 6.33 ppm,

J_{AB} = 16 Hz

$J_{AB}/\delta_B - \delta_A$ = 0.47

i) RuO_4
ii) CH_2N_2

23

PMR: 1.05, 1.17 ppm

Fig. 5

Ozonolysis of hardwickiic acid, followed by oxidative (H_2O_2) work-up, yielded two major products (as Me esters). The analytical and spectroscopic data (IR, PMR) of these compounds are entirely consistent with their formulation as 20 and 21, derivable from structure 18 for hardwickiic acid. Both 20 and 21 show one of the tertiary methyls downfield (at δ 1.20 and 1.18 ppm respectively) as required for a methyl group α to the carbomethoxy function, thus supporting the presence of a tertiary methyl at C-5 in 18. Compound 21 came handy in locating the position of the second tertiary methyl. Bromination of 21 with NBS or Br_2-Et_2O complex, furnished a bromo derivative, which on dehydrohalogenation with $CaCO_3$-DMF, gave an αβ-unsaturated ketone: λ_{max} 227 nm (ε 12,850); IR, C=C—C=O 1695, 1645 cm^{-1}. The PMR spectrum of this unsaturated ketone shows two olefinic protons as an AB-quartet centred at 6.33 ppm
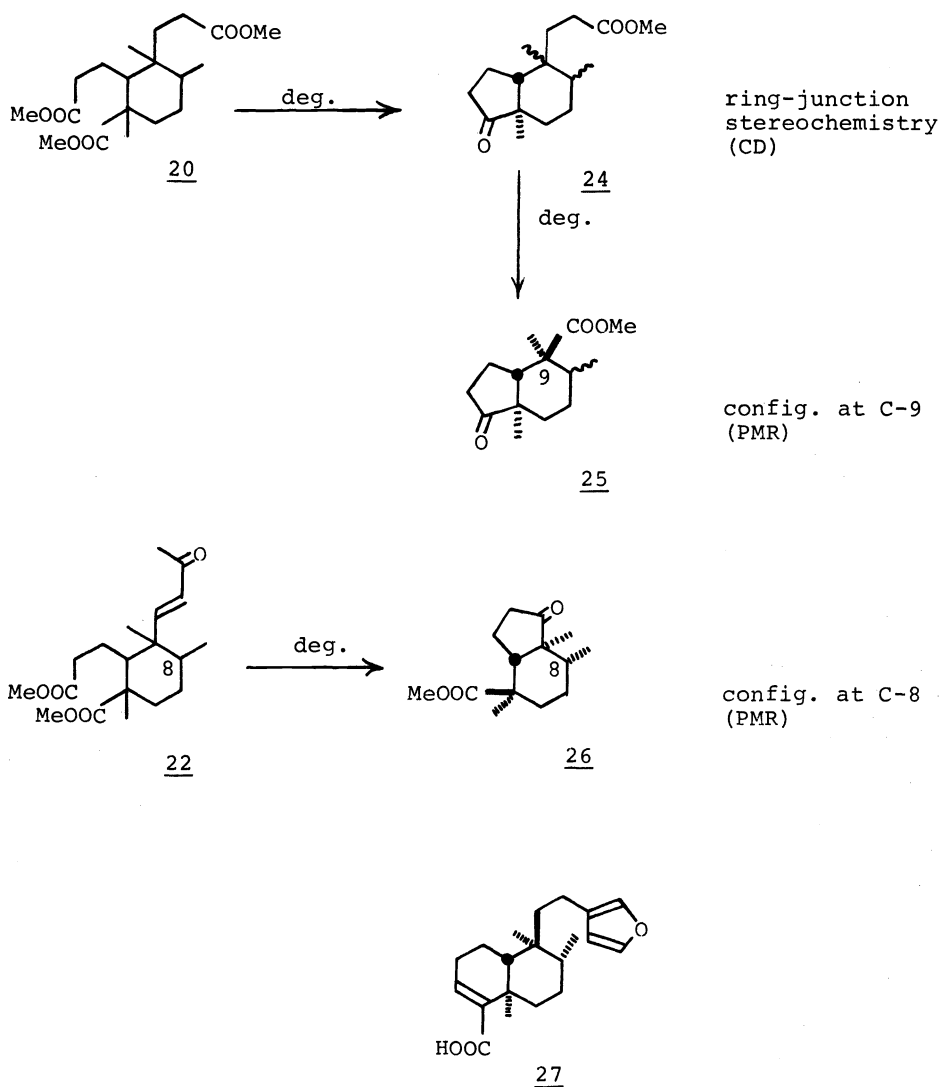

Fig. 6

(J_{AB} = 16 Hz), a finding consistent only with fully substituted C-9, as shown in 22. Further degradation of 22 gave 23 (Fig. 5), which as required by its formulation shows both the tertiary methyls downfield (1.05, 1.17 ppm).

Further work was designed to establish the absolute stereochemistry at each
of the chiral centres, but will not be discussed in any detail, as this will
amount to a major diversion from the general theme of the lecture. It will
suffice to say that suitable degradations led to key compounds 24, 25 and 26,
the CD curves or PMR spectral characteristics of which enabled derivation of
the relevant stereochemistry (Fig. 6) and finally helped us to arrive at the
absolute stereostructure 27 for (-)-hardwickiic acid (Ref. 9). (-)-Hardwickiic
acid, thus, became the first simple member of the rearranged labdanes, repre-
sented at that time (Ref. 10) by such highly oxygenated compounds as clerodin,
cascarillin and columbin. It is also the first diterpene with ent-clerodane
absolute stereochemistry, and has since served as the central reference compo-
und for interrelating the absolute stereochemistry of a number of other natu-
rally occurring diterpenoids (Ref. 11).

Once the absolute stereostructure of (-)-hardwickiic acid could be established,
structures of various other related diterpenoids occurring in the oleoresin
of Hardwickia pinnata were readily elucidated (Fig. 7).

(—)-Kolavic acid   (—)-Kolavenic acid   (—)-Kolavenolic acid

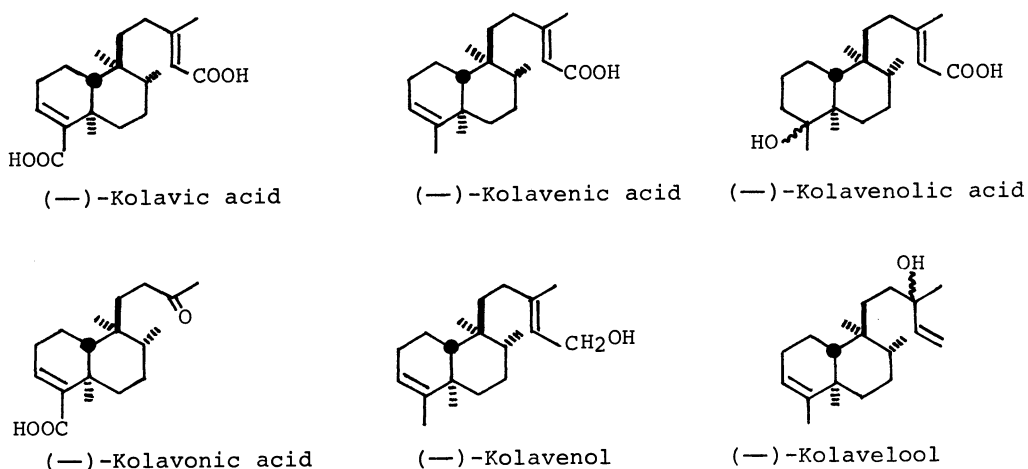(—)-Kolavonic acid   (—)-Kolavenol   (—)-Kolavelool

Fig. 7

Malabaricol
Ailanthus malabarica DC is a lofty tree, endemic to the Western Ghats of
India. When an incision is made on the trunk, a light brown oleoresin flows
out, which soon sets to a viscous mass. This material is an article of
commerce in India and is much valued as an ingredient of agar-battis ('joss
sticks'). The resinous exudate has been found to contain several triter-
penoids belonging to a novel skeletal type. The major component has been
named malabaricol.

Malabaricol, m.p. 68-69,5°, {α}$_D$ + 36.1° (CHCl$_3$, c 1%) analyses for C$_{30}$H$_{50}$O$_3$
(M$^+$, m/e=458), and on the basis of spectral characteristics, quantitative
hydrogenation and acetylation experiments, must possess the following
structural units:
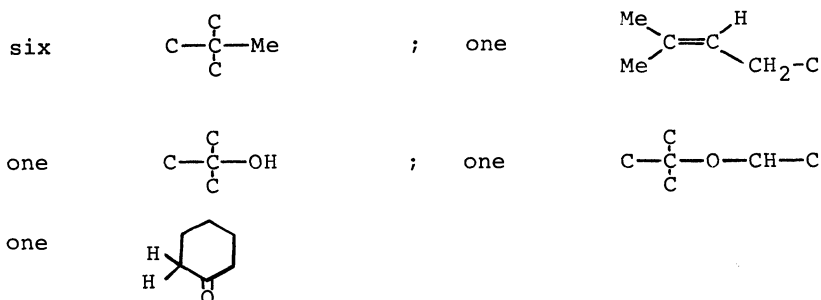
Thus, malabaricol is clearly a triterpene and must be either tetracarbocyclic with an acyclic ether linkage or tricarbocyclic with a cyclic ether function.

When exposed to Jones reagent, malabaricol furnished in good yield a product (m.p. 145-146°), characterised as an octanor-γ-lactone, $C_{22}H_{34}O_3$ (M$^+$, m/e = 346: C═O 1775 cm^{-1}). From a consideration of the PMR spectrum of this lactone and possible mechanism of this degradation (Fig. 8), part structure (28) appeared appropriate for malabaricol. This formulation received support, when
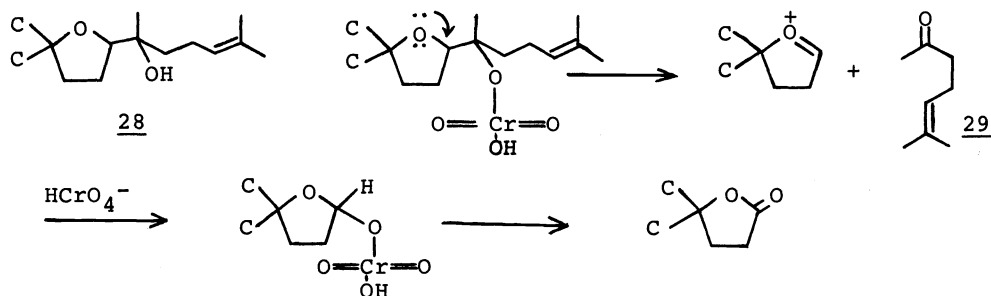

Fig. 8

the second oxidation product could be isolated and identified as methylhept-enone (29). This degradation clearly formulates the ether linkage of mala-baricol in a ring and hence, this compound must be tricarbocyclic. This con-clusion was most thrilling, as at that time only two tricarbocyclic triterpenes, ambrein (30) and ebelin lactone (31) (Ref. 12) were known and clearly, these carbon skeletons were not appropriate to accommodate the part structure 28.

30

31
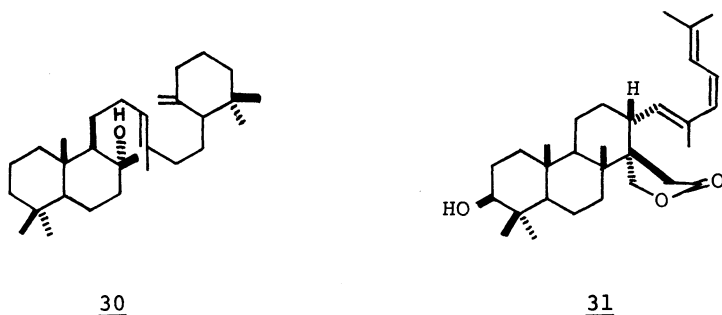
While examining (Fig. 9), theoretically, the possible modes of cyclization of squalene (32), the well-established precursor of triterpenoids and steroids, to arrive at a tricyclic system suitable for accommodating the structural features of malabaricol, it was noted that if ring C is closed Markownikoff-wise (33), rather than the usual anti-Markownikoff-wise (34) so far observed for all tetra- and pentacyclic triterpenoids, the resulting species 33 is eminently suited for incorporating the part-structure 28 (cf. 35), to finally give 36, as a possible structure for malabaricol. Participation of a suitably located hydroxyl group during an electrophilic attack on an ethylenic linkage, as depicted in 35, is a well-precedented chemical reaction (Fig. 10).
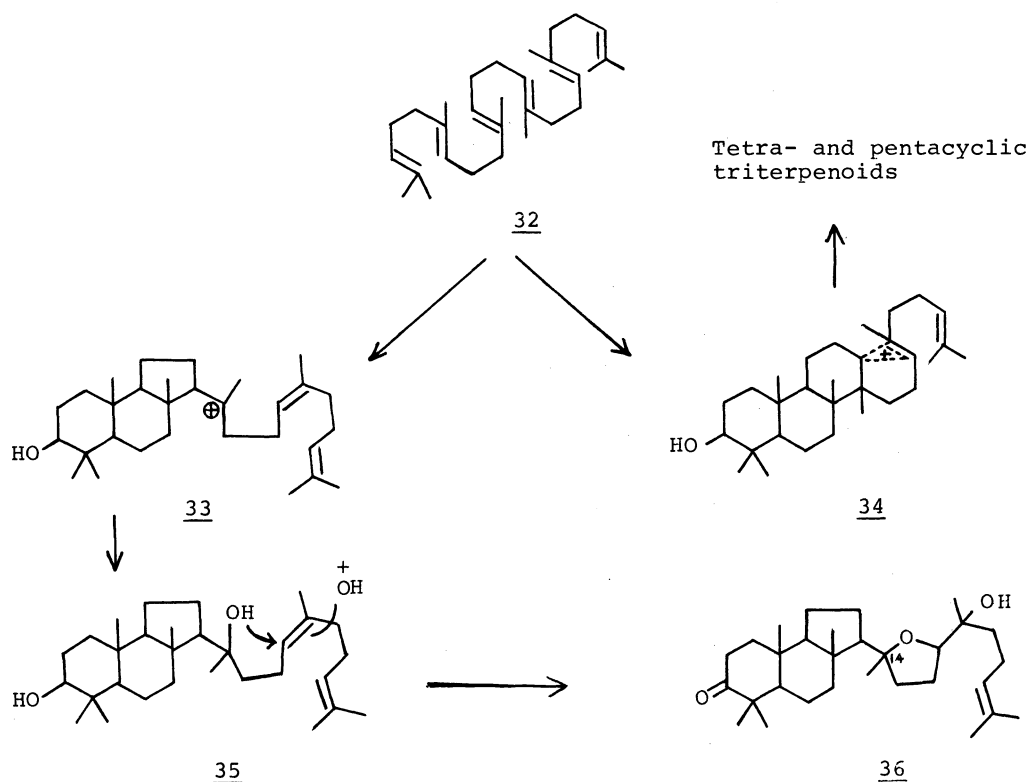
Fig. 9

Formulation **36** for malabaricol appeared to gain support from its mass spectral fragmentation: If correctly represented by 36, it should show, on electron impact, the characteristic α-fission of α-substituted tetrahydrofurans (Ref. 15) and indeed this is the case (Fig. 11).
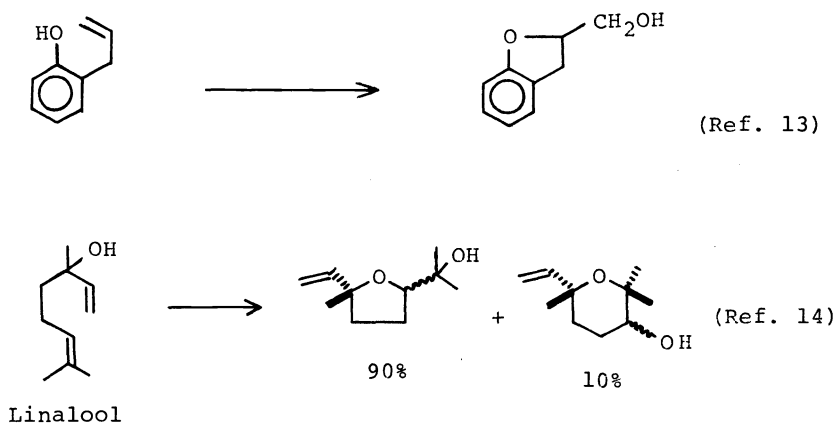

(Ref. 13)

(Ref. 14)

Linalool

Fig. 10

Since, clear-cut proof for the side-chain structure of malabaricol has already been obtained, to place the gross structure **36** beyond any reasonable doubt, sound evidence must be adduced for (i) the location of a tertiary methyl at C-14, and (ii) for the size of ring C. Once, the size of ring C is established

and since, that of another one is already known to be six-membered, that of third one would follow, while taking into consideration other structural features. The above twin objectives were met by a suitable degradation
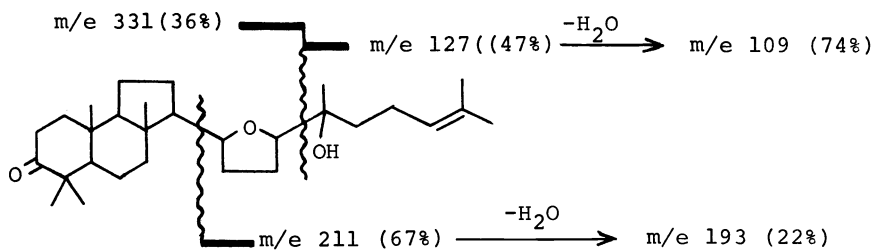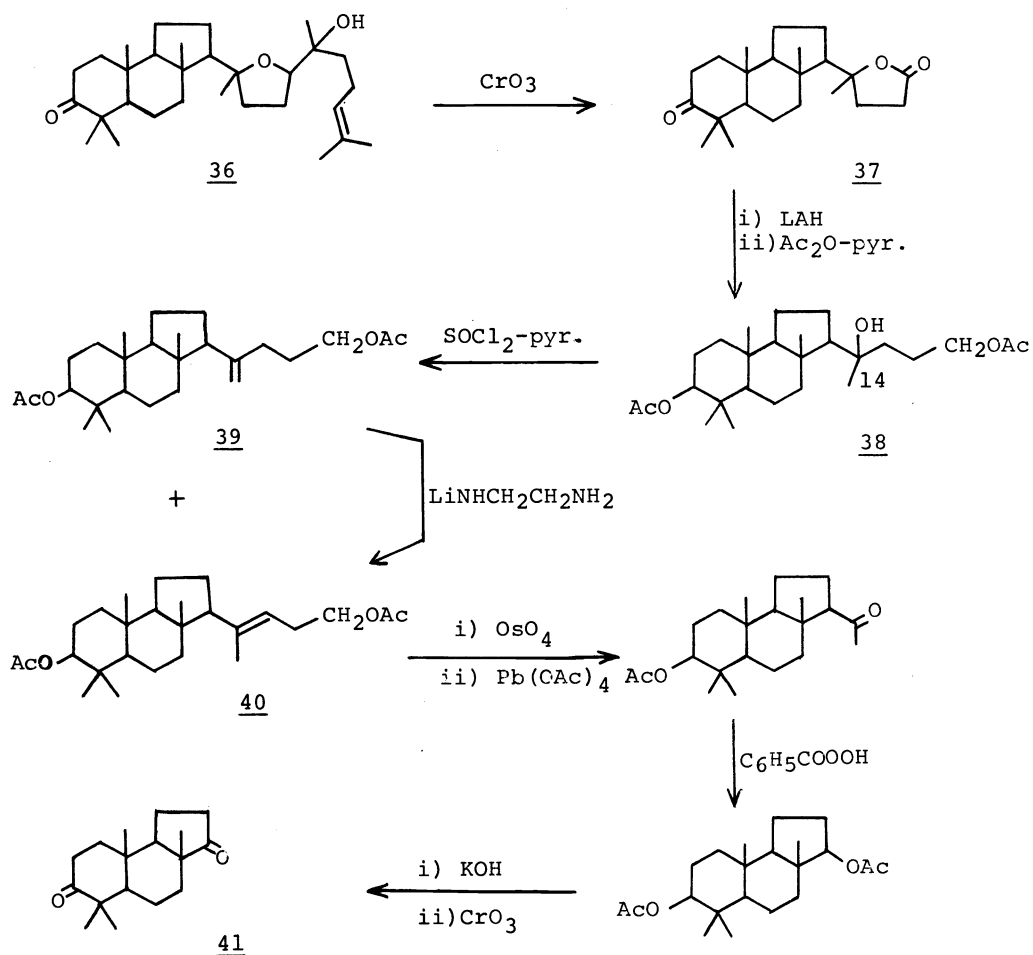
m/e 331(36%) ▬▬▬┐
                 │▬▬ m/e 127((47%) ──$-H_2O$──➤ m/e 109 (74%)

▬▬▬ m/e 211 (67%) ──$-H_2O$──➤ m/e 193 (22%)

Fig. 11

(Fig. 12) of octanor-γ-lactone, which on the basis of structure __36__ for mala-
baricol, can be represented by __37__. LAH reduction of __37__ gave a triol, which

__36__   ──CrO$_3$──➤   __37__

i) LAH
ii) Ac$_2$O-pyr.

__39__ ◀──SOCl$_2$-pyr.── __38__ (14)

+

LiNHCH$_2$CH$_2$NH$_2$

__40__   ──i) OsO$_4$── ──ii) Pb(OAc)$_4$──➤

C$_6$H$_5$COOOH

__41__ ◀── i) KOH ii) CrO$_3$ ──

IR: C═O 1703, 1738 cm^{-1}

Fig. 12

on acetylation furnished the expected hydroxydiacetate 38. This on dehydra-
tion furnished essentially a mixture of two olefins, which could be separated
and recognised on the basis of spectral data (IR, PMR) as 39 and 40; olefin
structure in 39 and 40 confirm the presence of a tertiary methyl at C-14 in
38 and consequently in 36. Isomerization of the total olefin mixture with
LiNHCH$_2$CH$_2$NH$_2$ gave essentially 40, which was degraded as shown in Fig. 12 to
a diketone, m.p. 64-65° having all the PMR and Mass spectral requirements of
41 and showing in the infrared (CCl$_4$) two carbonyl absorptions at 1703 (six-
membered) and 1738 cm^{-1} (five-membered) and of equal intensity. This degra-
dation, thus clearly defines the size of ring C as five-membered and conse-
quently the third ring must be six-membered (Ref. 16).

The data presented so far, is fully consistent with the formulation 36 for
malabaricol, derived in the first instant on the basis of biogenetic consi-
derations. To get chemical evidence for the remaining features (viz. rings
A and B and the four tertiary methyls) and elucidate its absolute stereo-
chemistry, a direct chemical correlation of malabaricol with (+)-ambreinolide
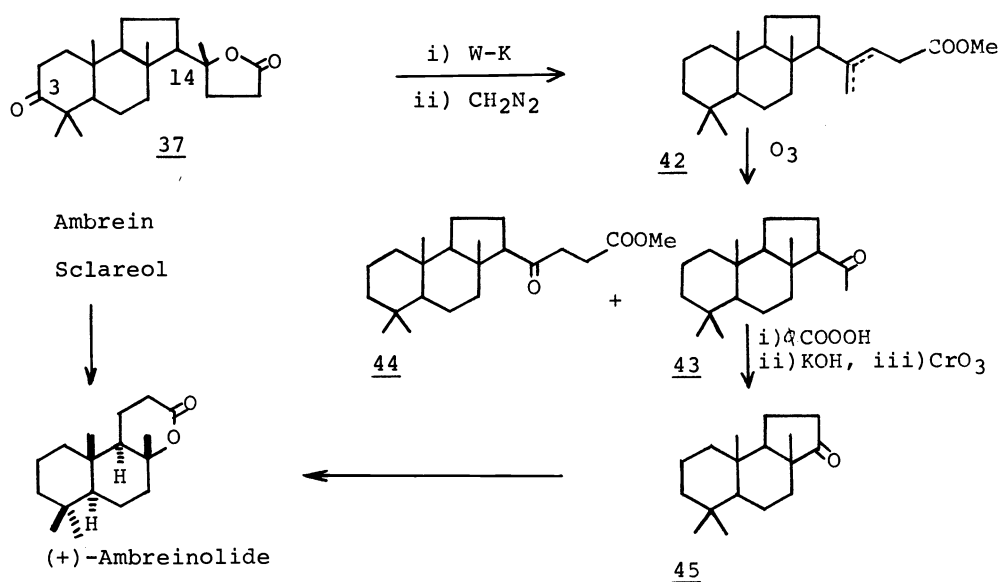was carried out (Fig. 13). Octanor-γ-lactone (37), under the conditions of

Fig. 13

Wolff-Kishner reduction (215-220°, 24 hr) underwent both reduction of the
C-3 carbonyl and dehydration at C-14 tertiary alcohol (derived from the lactone
opening under the alkaline reaction conditions) to furnish, after esterifica-
tion, the mixture 42. This on ozonolysis gave two products 43, 44, which
were separated and the methyl ketone (43) converted to the cyclopentanone 45
(m.p. 98-98.5°) as shown in Fig. 13. Prolonged exposure of 45 to perbenzoic
acid yielded a product (m.p. 142-143°; {α}$_D$ + 28°, CHCl$_3$) identified (mixed
m.p., TLC, IR) as (+)-ambreinolide (Ref. 17).
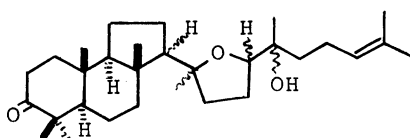
46

The above degradations, not only rigorously define the gross structure of mala-
baricol as 36, but also elucidate its absolute stereochemistry at C-5, C-8,
C-9 and C-10, as depicted in 46. In order to unravel the configurations at

the remaining chiral centres, ^{13}C-NMR studies have been undertaken, but so far, without any conclusive results. Likewise, preparation of crystals, suitable for X-ray diffraction analysis, of malabaricol or its derivatives, has been so far unsuccessful.

Once the structure of malabaricol could be established, the structures of various other related triterpenoids present in the oleoresin could be clarified with facility (Fig. 14) (Ref. 18).
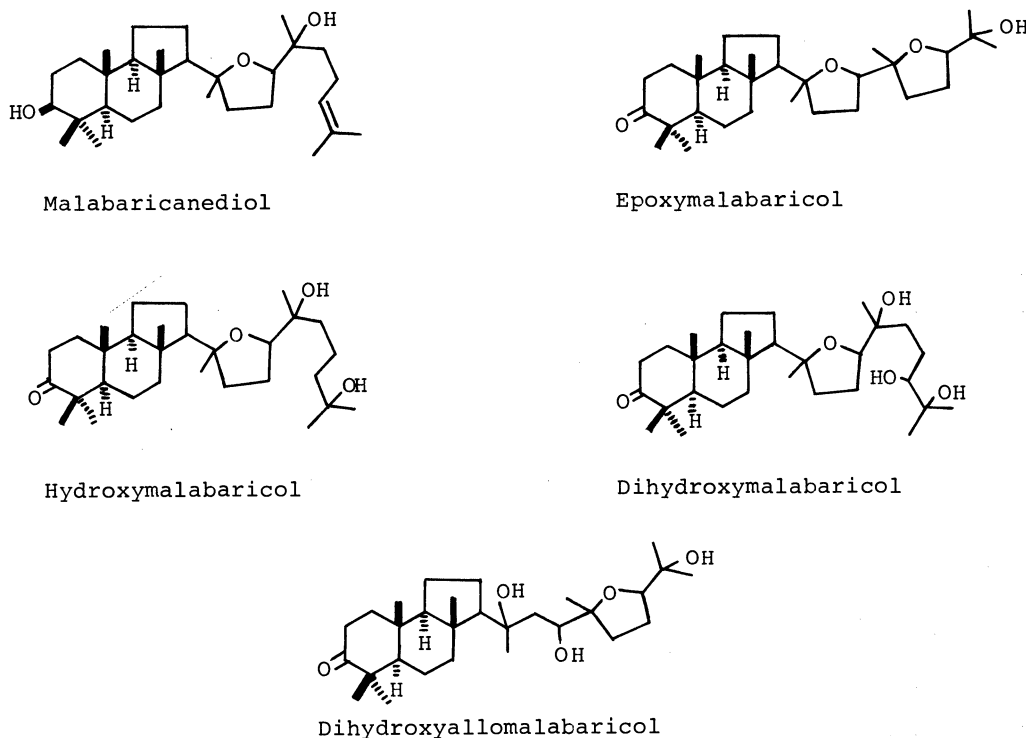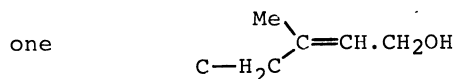

Malabaricanediol

Epoxymalabaricol

Hydroxymalabaricol

Dihydroxymalabaricol

Dihydroxyallomalabaricol

Fig. 14

Cheilanthatriol

The fern, Cheilanthes farinosa Kaulf, on extraction with petroleum ether, yields a crystalline triol, m.p. 182-183°, $\{\alpha\}_D$ + 30.4° (CHCl$_3$, c 1%), which we have named cheilanthatriol.

The compound analysis for $C_{25}H_{44}O_3$, and on the basis of spectral characteristics, acetylation experiments and quantitative hydrogenation data, must possess the following structural features:

five

$$C-\underset{\underset{C}{|}}{\overset{\overset{C}{|}}{C}}-Me$$

three   hydroxyls, one each prim,, sec. and tert.

one

$$C-H_2C\overset{Me}{\underset{}{\diagdown}}C=CH.CH_2OH$$

<u>47</u>

Thus, cheilanthatriol is only mono-olefinic and being $C_{25}H_{41}(OH)_3$, must be tricarbocyclic.

The structural features revealed so far, make it clear that cheilanthatriol must be a sesterterpene and not a degraded triterpene. This conclusion became all the more interesting on realization that the part structure 47 would require cyclization of geranylfarnesol, the most plausible immediate precursor of sesterterpenes, to begin at the isopropylidene end and not at the usual allylic alcohol terminus, as had been observed in the case of all sesterter-penes known till then. Conceivably, the cyclization mode 48 (Fig. 15), so
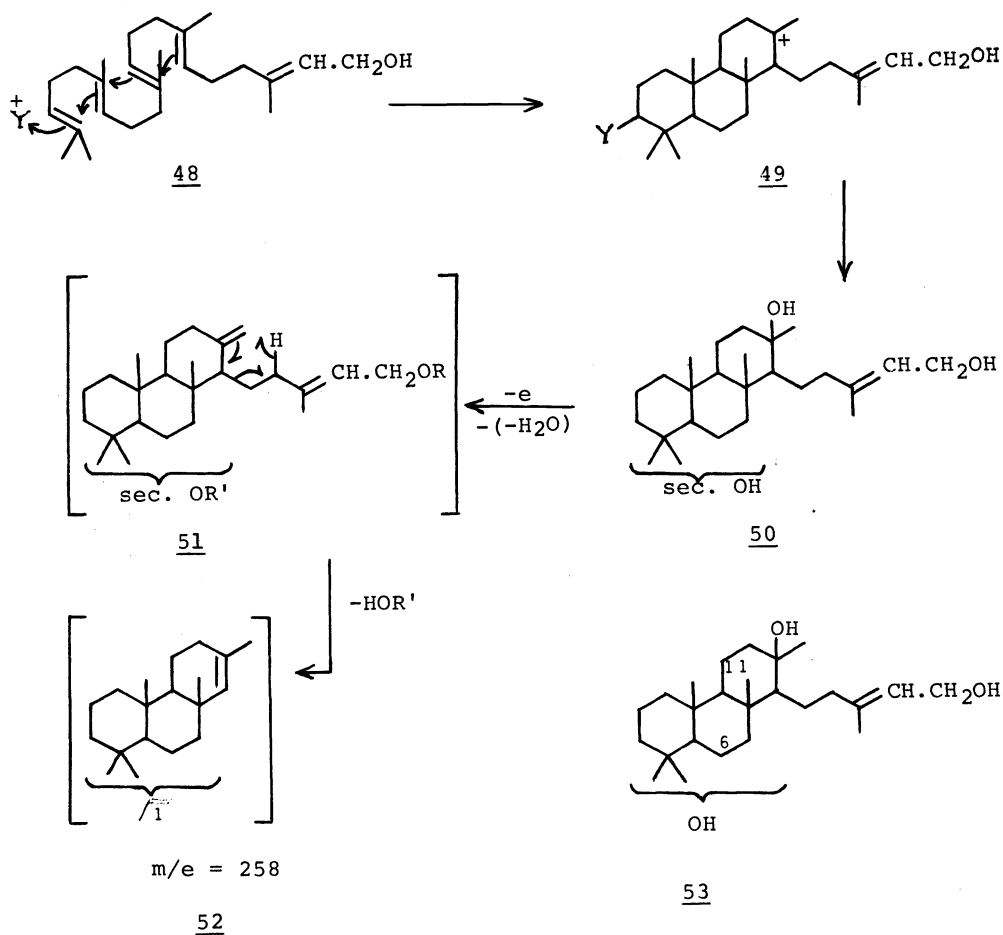

Fig. 15

characteristic of many diterpenoids, would generate skeleton 49, which appe-ared quite appropriate for incorporating various structural features of cheilanthatriol (e.g. 50). Some support for this was forthcoming from the results of electron impact. Cheilanthatriol, its monoacetate and diacetate, all fragment to the same ion at m/e, = 258 (base peak in the case of mono- and diacetate and, 65% intensity in the case of triol). This is readily rationalised in terms of fragmentations depicted in Fig. 15 (51, 52).

The above fragmentation also clearly shows that the secondary hydroxyl must be located on a ring. Furthermore, in cheilanthatriol and many of its deriva-tives, the CHOR signal appears as a triplet of doublets with $J_1=J_2 \approx 11$ Hz and $J_3 \approx 4$ Hz, clearly suggesting two axial-axial and one axial-equatorial coupl-ings. On this basis, it is considered most likely that $Y^+ = H^+$ and that the most likely position for the secondary hydroxyl is C-6 or C-11. Evidence in favour of hydroxyl at C-6, was obtained as follows: On catalytic hydrogena-tion (PtO$_2$/AcOH), cheilanthatriol consumed ~2 mole equivalents of H$_2$ to

furnish, as the major product, a saturated diol, $C_{25}H_{46}O_2$ (M^+, m/e = 378, in
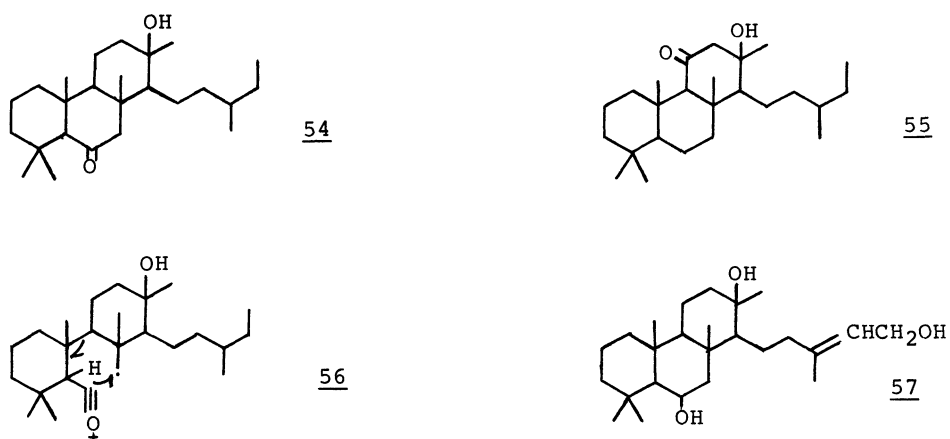
which the primary hydroxyl had been lost. This on Sarett oxidation furnished
a keto alcohol, which can be formulated as 54 or 55 on the basis of structure
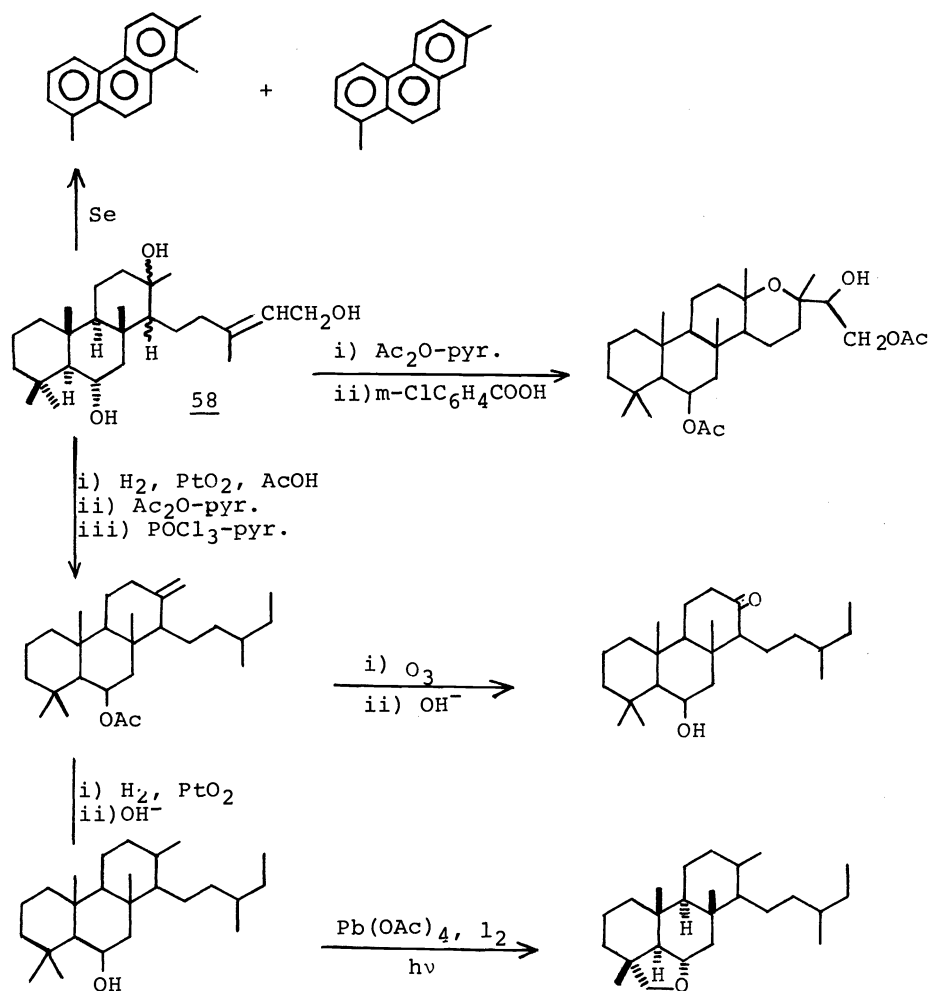

Fig. 16

53 for cheilanthatriol. Since, this keto alcohol was found to be stable in
alkaline medium, possibly 55 can be eliminated. Structure 54 is also clearly
supported by its mass fragmentation, showing base peak at m/e 151, which is
readily understood in terms of 56. Thus, structure 57 can be taken as a very
appropriate working structure for cheilanthatriol (Ref. 19).

Suitable degradations, transformations as outlined in Fig. 16 (Ref. 18) were
next carried out and these not only fully confirm the gross structure 57, but
also suggest a trans-anti-trans backbone for the tricyclic system and confirm
α-configuration for the secondary hydroxyl (58).

A ¹³C-NMR study carried out in collaboration with Prof. G. Lukacs (Ref. 20)
has helped in elucidating its stereochemistry as shown in 59.
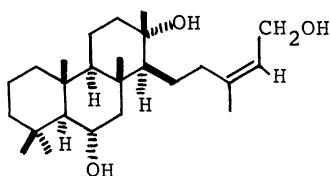
59

CONGENERS AND PRECURSORS

Conceptually, a metabolite could arise from a given precursor by a one-step or
a multi-step process; the term one-step implying that only the final product
leaves the enzyme surface. If a multi-step sequence is operative, then pools
of intermediate compounds, acting as substrates in subsequent steps, must exist
in the living tissue at a given time, the effective pool representing the
balance of feed-in and utilization processes occurring side by side, though
the balance could, conceivably be vanishingly minute (Ref. 21). Thus, if the
genesis of a given compound proceeds by a multi-step process then pools of
intermediates must exist and their isolation/detection can be of both chemical
and biogenetic interest.

Allodevadarool   Devadarool   Hydroxydevadarool
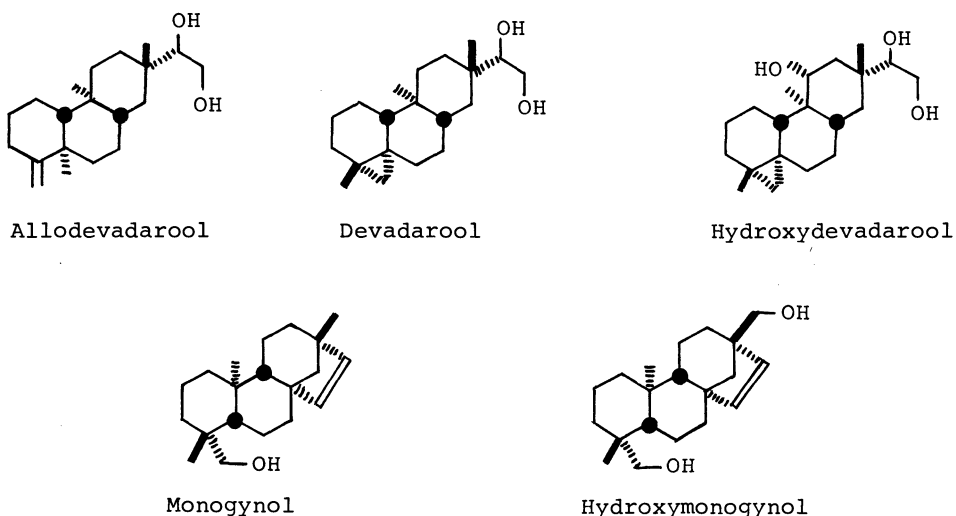
Monogynol   Hydroxymonogynol

Fig. 17

Though, it would be difficult to say per se which compound is formed by a
multi-step sequence, certain situations are obvious. Thus, it should be
considered reasonable to say that a majority of oxygenated terpenoids (except
of course, the 3-OH function in triterpenoids and some related diterpenoids,
and certain tertiary terpene alcohols which represent one mode of stabilization

of the parent cation) arise by a subsequent oxidation step(s) from the parent immediate precursor and hence, given suitable means, it should be possible to isolate or detect such precursors.

The wood of <u>Erythroxylon monogynum</u> elaborates (Refs. 22, 23) a number of oxygenated diterpenoids (Fig. 17). A detailed investigation of the hydrocarbon fraction (constituting ∿1% of the total diterpenoids) and other minor constituents of this wood was specifically undertaken (Refs. 18, 21) to locate the hydrocarbon precursors of these oxygenated diterpenes, and other suspected intermediates. This study resulted in the isolation of devadarene (<u>60</u>), (+)-hibaene (<u>61</u>), (-)-pimaradiene (<u>62</u>), atisirene (<u>63</u>), isoatisirene (<u>64</u>), (-)-copalol (<u>65</u>), and (-)-kauranol (<u>66</u>); besides, another group of investigators (Ref. 24) reported the presence of <u>67</u> (erthyroxytriol-P) (Fig. 18). As can be seen, not only the hydrocarbons corresponding to monogynol and devadarool could be isolated, but also (-)-pimaradiene (<u>62</u>) and (-)-copalyl alcohol (<u>65</u>).

                    <u>60</u>   <u>61</u>   <u>62</u>

                    <u>63</u>   <u>64</u>   <u>65</u>

                            <u>66</u>   <u>67</u>
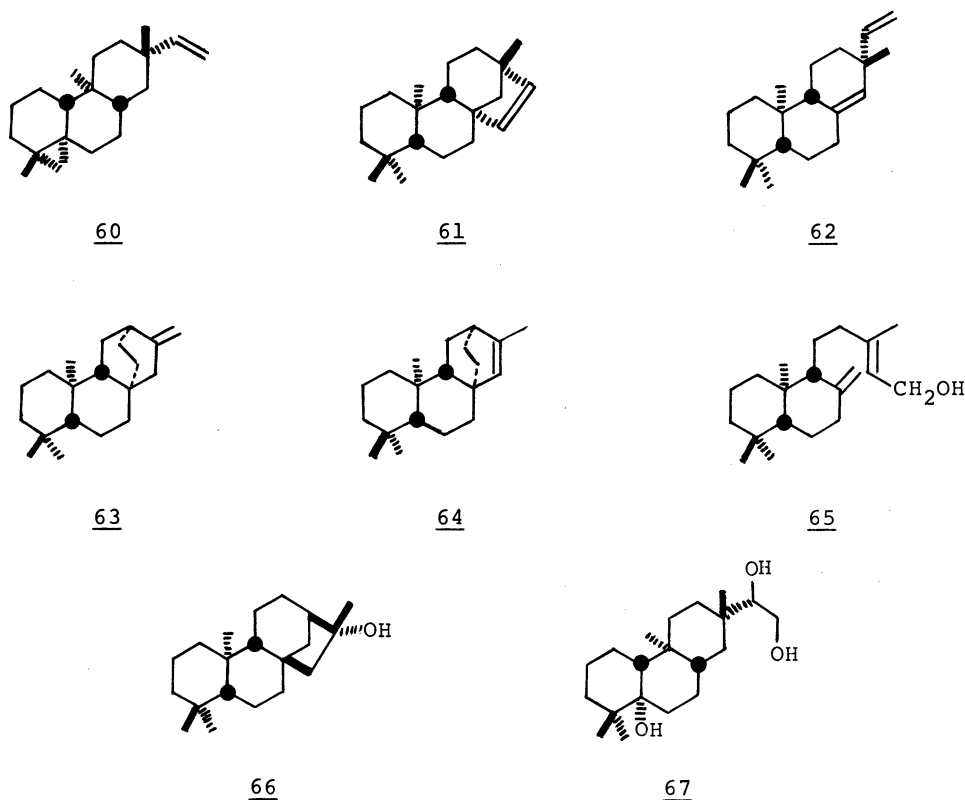
Fig. 18

The isolation of the above two compounds (<u>62</u>, <u>65</u>) was especially satisfying as would become clear from the following.

From the currently accepted (Ref. 25) biosynthetic pathways to the tricyclic diterpenes of pimarane and isopimarane type, and the tetracyclic diterpenoids, it is clear that the bicyclic labdadienol pyrophosphate (<u>68</u>) is an obligatory, discrete intermediate (Fig. 19) and hence the isolation (Ref. 26) of the corresponding alcohol, copalol (<u>65</u>) is significant. In view of the present discussion, it is surprising that the bicyclic alcohol <u>65</u> or its antipode have not been isolated from natural sources often, as one would have expected it to co-occur freely with pimarane and tetracyclic diterpenoids. As a matter of fact, apparently there is only one other reference to its occurrence in nature (Ref. 27). These remarks would apply equally well to the various acyclic terpenoids precursors, which have a highly restricted distribution (apparent!), with the exception of geraniol/linalool. Perhaps the situation

is similar to that obtaining for cholestrol before 1958 (Ref. 28) when it was
considered as a typical animal tissue sterol, but is now known to be very
widely distributed in the plant kingdom (Ref. 29)!

Though, pimaradiene is not considered an obligatory intermediate in the bio-
synthesis of tetracyclic diterpenoids such as kaurene (Refs. 25, 30), it may
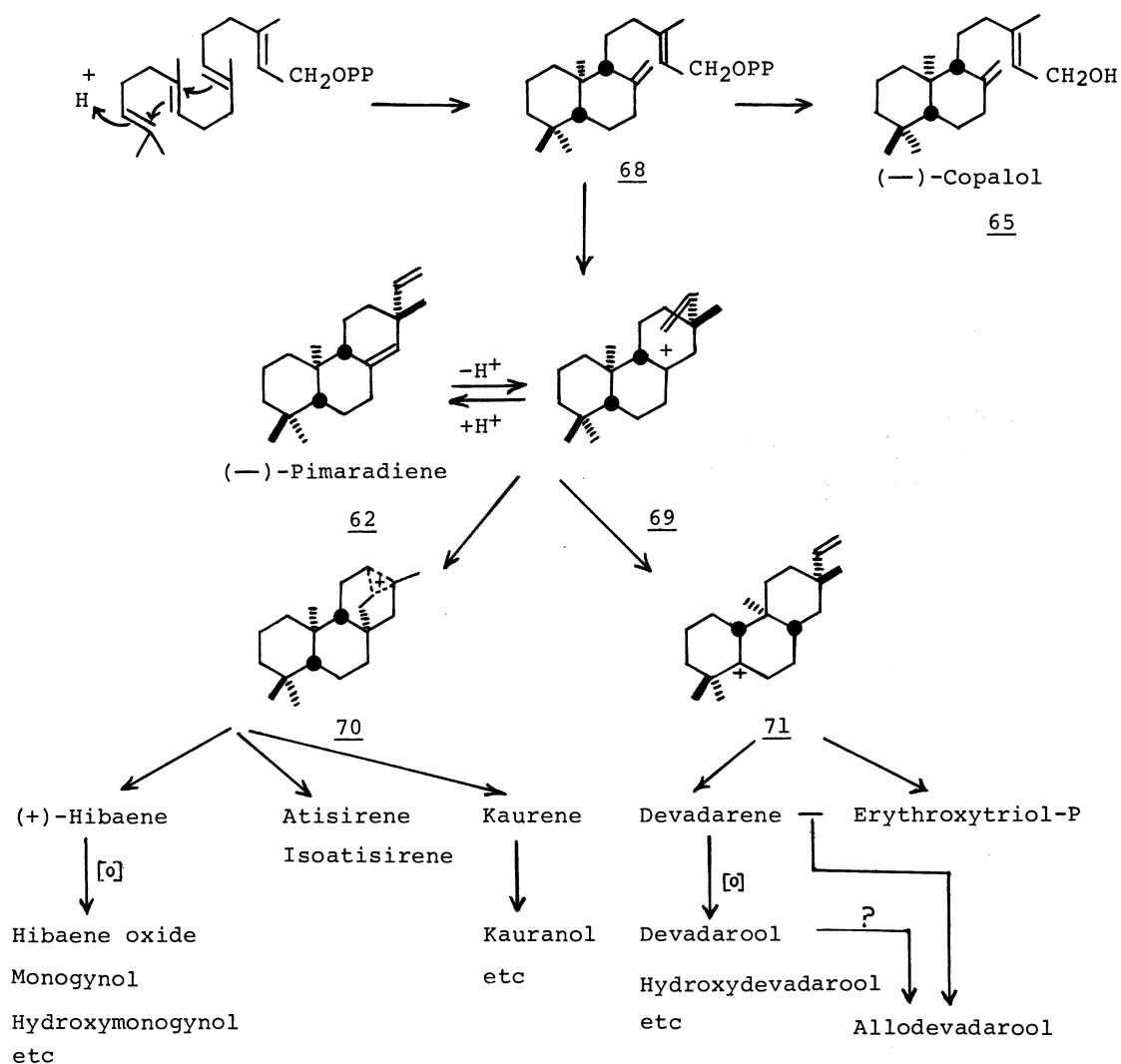be noted (Fig. 19) with reference to the diterpenoids of E. monogynum, that

Fig. 19

both hibaene (61) and devadarene (60) cannot be formed in a single enzymatic
step from the ion 69, thus necessitating intervention of pimaradiene, unless
direct cyclization of 68 to 70/71 by separate enzymes is envisaged. The iso-
lation of pimaradiene would suggest a pathway as shown in Fig. 19.

The isolation of hibaene (61), atisirene (63), isoatisirene (64) and kauranol
(66) is also significant, as it has been postulated (Ref. 25) that the ion 70
arising directly from the cation 69 is the common progenitor of these tetra-
cyclic systems. The co-occurrence of all these types in E. monogynum would
suggest a common origin, such as their formation from a solvated cation 70,
still bound to the enzyme, by a non-specific reaction, rather than the involve-
ment of distinct enzyme systems for each type. A study aimed at proportio-
nality relationship between the percentages of these structural types in the
plant during different seasons should help clarify this aspect (Ref. 31).

NEW STRUCTURAL TYPES

Biogenetic concepts can prove useful in a search for new structural variants, which have so vividly enriched the structural mosaic of different classes of terpenoids.

Camphene (72) and tricyclene (74) invariably co-occur. Till 1963 no tetra-cyclic sesquiterpene had been isolated from nature. It was thought that longifolene (73), which is the next higher isoprenologue of camphene, might be co-occurring with a tetracyclic sesquiterpene having the same relationship

72	73	74	75

with it as camphene has with tricyclene. This possibility got reinforced, when we looked at the results, of the action of perbenzoic acid on longifolene, which we had on hand. Further investigations led to the isolation of longi-cyclene(75), the first tetracyclic sesquiterpene (Ref. 32).

To many organic chemists, before 1965, the absence of C_{25}-terpenoids from the general family of terpenes was most intriguing and it will not be surprising if this provided the motivation for many to specifically look for this class, many members of which are now known (Ref. 33).

ABSOLUTE STEREOCHEMISTRY

With the exception of triterpenoids and possibly sesterterpenes, antipodal configurations for other terpenoids are known to occur in nature and hence for these classes of compounds, the absolute stereochemistry must also be defined.

It is obvious that if a series of compounds has been generated in a sequential manner, as discussed earlier, the absolute stereochemistry of these compounds would have a direct relationship with the first chiral precursor. For example, the absolute stereochemistry of the various diterpene metabolites of Erythroxylon monogynum is indeed as would be expected on the basis of chira-lity of copalol (65), (Fig. 19). A number of other biogenetic profiles of mono-, sesqui- and diterpene constituents of various plant species have been examined from this point of view and invariably the absolute stereochemistry of the constituents has the expected relationship. This led us in 1965, (Ref. 21) to propose, what may be called Absolute Stereochemistry Biogenetic Rule:

        The absolute stereochemistry of various constituents of a
        given class of chiral natural products in a given tissue must
        have the same (or derivable therefrom) absolute stereochemistry
        at a common reference point (unless each compound is formed
        by a one-step process, which is considered less likely).

It should be pointed out that earlier Birch (Ref. 34) while discussing the biosynthesis of monoterpenes, examined pairs of compounds of same configura-tion to deduce sequential conversions.

Exceptions to the above rule, though very rare are however known. For example, (+)-α-pinene commonly occurs in admixture with (-)-β-pinene of opposite abso-lute configuration (Refs. 32, 35). Oxystigma oxyphyllum (Ref. 36) as well as Agathis australis (Ref. 37) have been found to elaborate diterpenes of both the normal and antipodal series.

R.R. Sobti and R. Soman. I would also like to take this opportunity to gratefully acknowledge the valuable collaboration of Prof. A. Zaman and Prof. G. Lukacs on cheilanthatriol.

REFERENCES

1. J.L. Simonsen and D.H.R. Barton, The Terpenes, vol. III, University Press, Cambridge (1951).
2. See e.g.: Sukh Dev in Some Recent Developments in the Chemistry of Natural Products (Editors: S. Rangaswami and N.V. Subba Rao), pp. 308-324, Prentice-Hall of India, New Delhi (1972).
3. S.C. Bisarya and Sukh Dev, Tetrahedron 24, 3861 (1968)
4. T.C. Joseph and Sukh Dev, Tetrahedron 24, 3809, 3841 (1968).
5. G. Ourisson, Bull. Soc. Chim. 895 (1955); J.B. Hendrickson, Tetrahedron 7 83 (1959).
6. S.C. Bisarya and Sukh Dev, Tetrahedron 24, 3869 (1968).
7. Cf. e.g.: J.A. McMillan, J.C. Paul, U.R. Nayak and Sukh Dev, Tetrahedron Letters 419 (1974).
8. See e.g.: J.H. Richards and J.B. Hendrickson, The Biosynthesis of Steroids, Terpenes and Acetogenins, p. 240, Benjamin, New York (1964).
9. R. Misra, R.C. Pandey and Sukh Dev, Tetrahedron Letters 2681 (1968); Tetrahedron, in press.
10. See e.g.: R. McCrindle and K.H. Overton in Rodd's Chemistry of Carbon Compounds (Editor: S. Coffey), vol. IIc, p. 377, Elsevier, Amsterdam (1969).
11. See e.g.: G. Ferguson, W.C. Marsh, R. McCrindle and E. Nakamura, J. Chem. Soc. Chem. Comm. 299 (1975).
12. See e.g.: R. McCrindle and K.H. Overton in Rodd's Chemistry of Carbon Compounds (Editor: S. Coffey), vol. IIc, p. 406, Elsevier, Amsterdam (1969).
13. S.W. Tinsley, J. Org. Chem. 24, 1197 (1957).
14. D. Felix, A. Melera, J. Seibl and E. Kovats, Helv. Chim. Acta 46 1513 (1963).
15. H. Budzikiewicz, C.Djerassi and D.H. Williams, Structure Elucidation of Natural Products by Mass Spectrometry, vol. II, p. 270, Holden-Day, San Francisco (1964).
16. A. Chawla and Sukh Dev, Tetrahedron Letters 4837 (1967).
17. R.R. Sobti and Sukh Dev, Tetrahedron Letters 2215 (1968).
18. Unpublished results from this Laboratory.
19. H. Khan, A. Zaman, G.L. Chetty, A.S. Gupta and Sukh Dev, Tetrahedron Letters 4443 (1971).
20. A.S. Gupta, Sukh Dev, M. Sangare, B. Septe and G. Lukacs, Bull. Soc. Chim. Fr.1879 (1976).
21. A.H. Kapadi, R.R. Sobti and Sukh Dev, Tetrahedron Letters 2729 (1965).
22. A.H. Kapadi and Sukh Dev, Tetrahedron Letters 1171, 2751 (1964).
23. R. Soman, Sukh Dev, R. Misra and R.C. Pandey, Tetrahedron Letters 3767 (1964).
24. J.D. Connolly, D.M. Gunn, R. McCrindle, R.D.H. Murray and K.H. Overton, Tetrahedron Letters 2109 (1966).
25. See e.g. J.R. Hanson, Fortschr. Chem. Org. Naturstoffe 29, 395 (1971).
26. R. Soman, Ph.D. Thesis (Kerala University, 1966), p. 171.
27. E.N. Schmidt and V.A. Pentegova, Izv. Sib. Otd. Acad. Nauk SSSR, Ser. Khim Nauk 84 (1966); Chem. Abstr. 67, 32822 (1967).
28. K. Tsuda, S. Akagi, Y. Kishida, R. Hayatsu and K. Sakai, Chem. Pharm. Bull. 6, 724 (1958).
29. E. Heftmann in Progress in Phytochemistry (Editors: L. Reinhold, J.B. Harborne and T. Swain), vol. 4, p. 258, Pergamon, Oxford (1977).
30. R. Evans and J.R. Hanson, J. Chem. Soc. Perkin I 2382 (1972).
31. See e.g.: E. Zavarin, Phytochemistry 9, 1049 (1970).
32. U.R. Nayak and Sukh Dev, Tetrahedron 24, 4099 (1968).
33. See e.g.: G.A. Cordell in Progress in Phytochemistry (Editors: L. Reinhold, J.B. Harborne and T. Swain), vol. 4, p. 209, Pergmon, Oxford (1977).
34. A.J. Birch in Chemical Plant Taxonomy (Editor: T. Swain), p. 156, Academic Press, New York (1963).
35. D.V. Banthorpe, B.V. Charlwood and M.J.O. Francis, Chem. Rev. 72, 124, 126 (1972).
36. C.W.L. Bevan, D.E.U. Ekong and J.I. Okogun, J. Chem. Soc. C. 1067 (1968).
37. R.T. Aplin, R.C. Cambie and P.S. Rutledge, Phytochemistry 2, 205 (1963).