BIOGENETIC CONCEPTS IN TERPENE STRUCTURE ELUCIDATION

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Abstract — In the development of Natural Products Chemistry, biogenetic 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 exercise. Many such studies, which were often protracted, 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 crystallography. These developments have affected the activity in this area in two ways. Firstly, more and more effort is being expended on biologically significant molecules, whether for drug use or for understanding biochemical processes 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 thoroughly 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 material 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, biogenetic 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° (α)D + 37.4° (CHCl₃, e 3.3%), which we have termed allohimachalol (Ref. 3). It analyses for C₁₅H₂₆O (M⁺, m/e 222), is clearly mono-olefinic (IR: OH 3400, 1020 cm⁻¹; C=CH 1660, 853 cm⁻¹). Quantitative hydrogenation to C₁₅H₂₈O, 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 structural 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, 3J=5.0Hz, 2J=0. Hz), C=CH (1H, bd, 5.22 ppm, J=5Hz).

On pyridine-CrO₃ oxidation, allohimachalol gave an olefinic ketone, C₁₅H₂₄O, in which the olefinic linkage and carbonyl function are not conjugated (λmax 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-himachalene (4) and himachalol (5), structures of which, we had just then elucidated (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 suggested 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 Li₂CO₃, 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 CrO₃ and the product esterified 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. FeCl₃, 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
methine protons 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

**Fig. 1**

Allohimachalol tosylate

**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 (λmax 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.

Fig. 3
Hardwickiic acid

*Hardwickia pinnata* Roxb. is a large handsome tree (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:

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.
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 α-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 requirements of hardwickiic acid, but would also account for the preferential formation 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 disposition of the functionalities. This was achieved as follows (Fig. 5):

Fig. 5

![Diagram](Image)
Ozonolysis of hardwickiic acid, followed by oxidative (H₂O₂) 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 Br₂-Et₂O complex, furnished a bromo derivative, which on dehydrohalogenation with CaCO₃-DMF, gave an α,β-unsaturated ketone: λmax 227 nm (ε 12,850); IR, C=O-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

(JAB = 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, represented 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 compound for interrelating the absolute stereochemistry of a number of other naturally 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).

![Chemical structures]

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 triterpenoids belonging to a novel skeletal type. The major component has been named malabaricol.

Malabaricol, m.p. 68-69.5°, (α)D + 36.1° (CHCl3, c 1%) analyses for C30H50O3 (M+, m/e=458), and on the basis of spectral characteristics, quantitative hydrogenation and acetylation experiments, must possess the following structural units:

- six \( \text{C} \rightarrow \text{C} \rightarrow \text{Me} \);
- one \( \text{Me} \rightarrow \text{C} \rightarrow \text{C} \rightarrow \text{H} \);
- one \( \text{Me} \rightarrow \text{C} \rightarrow \text{C} \rightarrow \text{O} \rightarrow \text{CH} \rightarrow \text{C} \);
- one \( \text{H} \rightarrow \text{C} \rightarrow \text{O} \rightarrow \text{CH} \rightarrow \text{C} \).
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 the second oxidation product could be isolated and identified as methylheptenone (29). This degradation clearly formulates the ether linkage of malabaricol in a ring and hence, this compound must be tricarbocyclic. This conclusion 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.

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-precended chemical reaction (Fig. 10).
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).

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 of octanor γ-lactone, which on the basis of structure 36 for malaricol, can be represented by 37. LAH reduction of 37 gave a triol, which

\[
\text{Fig. 11}
\]

(Fig. 12) of octanor γ-lactone, which on the basis of structure 36 for malaricol, can be represented by 37. LAH reduction of 37 gave a triol, which
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
LiNHCH₂CH₂NH₂ 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 (CCl₄) 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

![Chemical diagrams]

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°, CHCl₃) identified (mixed
m.p., TLC, IR) as (+)-ambreinolide (Ref. 17).

![Chemical diagrams]

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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-6,
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).

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 $\text{C} - \text{C} - \text{Me}$
- three hydroxyls, one each prim., sec. and tert.
- one $\text{Me} - \text{C} - \text{CH}_2\text{OH}$

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 isopropylenide end and not at the usual allylic alcohol terminus, as had been observed in the case of all sesterterpenes known till then. Conceivably, the cyclization mode 48 (Fig. 15), so

![Diagram 48](image)

Fig. 15

characteristic of many diterpenoids, would generate skeleton 49, which appeared 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 derivatives, the CHOR signal appears as a triplet of doublets with \( J_1 = J_2 = 11 \text{ Hz} \) and \( J_3 = 4 \text{ Hz} \), clearly suggesting two axial-axial and one axial-equatorial couplings. On this basis, it is considered most likely that \( Y^+ = \text{H}^+ \) 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 hydrogenation (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
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/z 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.

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.

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 *Erythroxylon monogynum* 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 (60), (+)-hibaene (61), (-)-pimaradiene (62), atisirene (63), isoatisirene (64), (-)-copalol (65), and (-)-kauranol (66); besides, another group of investigators (Ref. 24) reported the presence of 67 (erythroxytriol-P) (Fig. 18). As can be seen, not only the hydrocarbons corresponding to monogynol and devadarool could be isolated, but also (-)-pimaradiene (62) and (-)-copalyl alcohol (65).

The isolation of the above two compounds (62, 65) 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 (68) is an obligatory, discrete intermediate (Fig. 19) and hence the isolation (Ref. 26) of the corresponding alcohol, copalol (65) is significant. In view of the present discussion, it is surprising that the bicyclic alcohol 65 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 cholesterol 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 biosynthesis of tetracyclic diterpenoids such as kaurene (Refs. 25, 30), it may be noted (Fig. 19) with reference to the diterpenoids of *E. monogynum*, that

![Diagram of diterpenoid structures](image)

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 isolation 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 tetracyclic 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 involvement of distinct enzyme systems for each type. A study aimed at proportionality 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 (2a) and tricyclene (74) invariably co-occur. Till 1963 no tetracyclic 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 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 longicyclene (75), the first tetracyclic sesquiterpene (Ref. 32).

To many organic chemists, before 1965, the absence of C_{13}-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 chirality 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 configuration 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 absolute 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.

Acknowledgement—The work I have described has resulted essentially from the investigations carried out by my students for their Doctoral Dissertations of different Indian Universities. I would like to acknowledge their skillful and dedicated efforts by expressing my sincere thanks to all of them: S.C. Bisarya, A. Chawla, G.L. Chetty, A.S. Gupta, R. Misra, U.R. Nayak, P.C. Pandey,
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.

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