Revisited after 50 Years: The 'Stereochemical Interpretation of the Biogenetic Isoprene Rule for the Triterpenes'

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In memoriam Leopold Ruzicka and *Oskar Jeger*

1. Introduction. – In the December issue of *Helvetica Chimica Acta*, 1955, we published, together with *Leopold Ruzicka*[1]) and *Oskar Jeger*[2]), a paper entitled '*Eine stereochemische Interpretation der biogenetischen Isoprenregel bei den Triterpenen*' [3], of which *John W. Cornforth* in 1961 wrote that it '*might be termed the apotheosis of the isoprene rule*' [4]. In conjunction with a related publication by *Stork* and *Burgstahler* [5], which also appeared in 1955, the paper had a decisive influence on research in the fields of structure determination, biomimetic chemical synthesis, and biosynthesis of polycyclic triterpenoids and steroids in the decades that followed its publication. Today, half a century later, interest in the paper still seems to persist, so that, for example, a young organic chemist, *Jeffrey Johnston* [6], on his way to write a representative review on biomimetic carbocyclization to terpenes and steroids, recently inquired whether an English translation of the 1955 *Helvetica Chimica Acta* paper might exist. The answer, quite luckily, happened to be yes, since, about five years ago, *Erik Sorensen* at *Scripps* had persuaded *Lucy Stark*, one of his Ph.D. students, to produce just such a translation of the paper that had been written in an era when major chemistry departments in the US still required their Ph.D students to be capable of reading chemical literature in German. It is this coincidence, besides the fact that, now, after half a century, X-ray analyses of squalene and squalene oxide cyclases have provided experimental evidence [7][8] for the essential correctness of the paper's central postulates, that led us, the two surviving authors of the 1955 paper, to consider revisiting it in the light of contemporary knowledge of the chemistry and biochemistry of this family of natural products and 'celebrating', so to say, the paper's hemi-centennial by publishing the English translation in the December issue of the same journal in which the German original had appeared exactly 50 years earlier. The proposal found the enthusiastic support of Dr. *M. Volkan Kısakürek*, the Editor-in-Chief, and so we present here an edited version of *Lucy Stark*'s faithful English translation of the 1955 paper, together with facsimile reproductions of the original schemes and figures, the single alteration being the sequential numbering of the footnotes which, in the original paper, were grouped pagewise. The presentation is accompanied by a commentary which, first, puts the content of the paper into a historical perspective, then comments on some aspects of the paper's terminology, pinpoints in retrospect its essentials, discusses its relationship to

[1]) *Leopold Ruzicka* (1887–1976), for biographical information, see [1].
[2]) *Oskar Jeger* (1917–2002), for biographical information, see [2].

the 1955 paper of *Stork* and *Burgstahler*, and, finally, attempts a critical analysis of the paper's extensions, corrections, and corroborations that have come up during the last 50 years from the work of other researchers (for reviews, see [9]).

226. On Triterpenes

190th Communication[1])

A Stereochemical Interpretation of the Biogenetic Isoprene Rule for the Triterpenes

by **A. Eschenmoser**, **L. Ruzicka**, **O. Jeger**, and **D. Arigoni**

(13. X. 55.)

Helvetica Chimica Acta **1955**, *38*, 1890 – 1904

Translated on the suggestion of *Erik Sorensen* by **Lucy M. Stark** (*The Scripps Research Institute*), July 2000, and edited by *A. E.* and *D. A.,* Spring 2005

The biosynthesis of cholesterol from acetic acid, first reported by *K. Bloch* and *D. Rittenberg*[2]), has been suggested[3]) to proceed *via* squalene as a hypothetical intermediate. Next to the experimental results of biochemical studies, it was the constitutional formula of lanosterol disclosed shortly before[4]) that constituted an essential contribution to that suggestion, since, in the context of the squalene hypothesis, the formula of lanosterol shows an even closer relationship to squalene than does that of cholesterol. Therefore, not only squalene, but also lanosterol, could be assumed to be a hypothetical intermediate of the biosynthesis of cholesterol from acetic acid. Recent experimental results concerning squalene[5]) as well as lanosterol[6]) can be considered to heavily support these views.

Two years ago, we pointed out that not only lanosterol, but all pentacyclic triterpene compounds known at that time, can be formally derived from squalene[7]). This fact, as well as the possibility of formulating analogous relationships in the series of mono-, sesqui-, and diterpenes, led to the promulgation of the '*biogenetic isoprene rule*'. There are two meanings to this rule: *i*), it includes a general hypothetical scheme for the biogenesis of the terpenes and, *ii*), it delineates a comprehensive working hypothesis for research

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[1]) 189th Communication: *Helv. Chim. Acta* **1955**, *38*, 1857.

[2]) K. Bloch, D. Rittenberg, *J. Biol. Chem*. **1945**, *159*, 45; see also the review on the biogenesis of cholesterol, J. W. Cornforth, *Rev. Pure Appl. Chem.* **1954**, *4*, 275.

[3]) R. B. Woodward, K. Bloch, *J. Am. Chem. Soc.* **1953**, *75*, 2023; see also W. G. Dauben, S. Abraham, S. Hotta, I. L. Chaikoff, H. L. Bradlow, A. H. Soloway, *J. Am. Chem. Soc.* **1953**, *75*, 3038.

[4]) W. Voser, M. V. Mijovic, H. Heusser, O. Jeger, L. Ruzicka, *Helv. Chim. Acta* **1952**, *35*, 2414.

[5]) J. W. Cornforth, G. Popjak, I. Youhotsky Gore, '*Further Studies on the Biosynthesis of Cholesterol and Squalene*'; lecture delivered at the internal symposium on the biochemistry of lipids, University of Gent, July 28, 1955.

[6]) We thank *K. Bloch* for this private communication.

[7]) L. Ruzicka, A. Eschenmoser, H. Heusser, *Experientia* **1953**, *9*, 362.

on the constitution of these natural products[8]) in that it assigns, among all stuctural possibilities for a given compound, an *a priori* higher probability to those structures that can be derived from the aliphatic precursors geraniol, farnesol, geranyl-geraniol, and squalene through formal cyclizations according to the mechanistic rules of organic chemistry. To what extent the notions about the biosynthesis of the cyclic terpenes expressed in this rule correspond to reality can, in the end, be decided only by experiment. Yet beyond this, one has to appreciate that contemporary research on the constitution of terpenes has reached such an advanced stage that the relationships deduced from a large number of established structural formulae can no longer be viewed merely as formal regularities in the architecture of carbon skeletons, but must be considered as relevant hypotheses for biogenetic research.

Basically, the biogenetic isoprene rule is nothing else than an extension of the classical isoprene rule to such terpenoid compounds whose carbon skeletons cannot be formally resolved into isoprene units. In the two years which have elapsed since the publication of the biogenetic isoprene rule, the number of terpenoid compounds, whose carbon skeletons cannot be formally dissected into isoprene units, has been growing continuously, especially in the area of triterpenes. To lanosterol and those C_{30} and C_{31} compounds that are derived from lanosterol[9]) have been added compounds of the euphol[10]) and tirucallol[11]) type as well as friedelin[12])[13]). It is a highly remarkable fact that – if one ignores the C_{31} triterpenoids for the moment – all carbon skeletons of cyclic triterpenes known hitherto fit into the scheme of the squalene hypothesis. Completely analogous is the situation in the area of the sesqui- and diterpenes, so that the biogenetic isoprene rule has so far proved successful without exception in its function to assist the determination of the constitution of cyclic terpenoid compounds.

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[8]) In this paper, the term '*structural formula*' is used throughout in the sense of *Kekule*'s structure theory. The term '*constitutional formula*' is meant to include configuration.

[9]) See the summarizing article of E. R. H. Jones, T. G. Halsall, in 'Fortschritte der Chemie organischer Naturstoffe', Vol. 12, 1955, p. 44.

[10]) K. Christen, M. Dünnenberger, C. B. Roth, H. Heusser, O. Jeger, *Helv. Chim. Acta* **1952**, *35*, 1756; D. H. R. Barton, J. F. McGhie, M. K. Pradhan, S. A. Knight, *J. Soc. Chem. Ind.* **1954**, 1325; *J. Chem. Soc.* **1955**, 876; D. Arigoni, R. Viterbo, M. Dünnenberger, O. Jeger, L. Ruzicka, *Helv. Chim. Acta* **1954**, *37*, 2306; E. Ménard, H. Wyler, A. Hiestand, D. Arigoni, O. Jeger, L. Ruzicka, *Helv. Chim. Acta* **1955**, *38*, 1517.

[11]) D. Arigoni, H. Wyler, O. Jeger, *Helv. Chim. Acta* **1954**, *37*, 1553; D. Arigoni, O Jeger, L. Ruzicka, *Helv. Chim. Acta* **1955**, *38*, 222; E. Ménard, H. Wyler, A. Hiestand, D. Arigoni, O. Jeger, L. Ruzicka, *Helv. Chim. Acta* **1955**, *38*, 1517.

[12]) E. J. Corey, J. J. Ursprung, *J. Am. Chem. Soc.* **1955**, *77*, 3667, 3668; H. Dutler, O. Jeger, L. Ruzicka, *Helv. Chim. Acta* **1955**, *38*, 1268; G. Brownlie, F. S. Spring, R. Stevenson, W. S. Strachan, *J. Soc. Chem. Ind.* **1955**, 686, 1156; G. Ourisson, T. Takahashi, *J. Soc. Chem. Ind.* **1955**, 1155.

[13]) All known triterpenes that have been found thus far occur in plants, except lanosterol. In this context, it is of special interest that squalene has been isolated also from plant material (see T. Thorbjarnarson, J. C. Drummond, *Analyst* **1935**, *60*, 23).

The mechanistic reaction schemes on which the biogenetic isoprene rule is based were previously exploited only for the derivation of structural formulae[14]). Up till now, it had not been examined whether they are also useful for the rationalization of configuration. A special challenge to deal with this question derives from the constitutional elucidation of the triterpenes of the lanosterol-, euphol- and tirucallol-type, which differ amongst themselves only with regard to the spatial positioning of the Me groups at C(13) and C(14) as well as the configuration at C(17) and C(20). In this paper, therefore, an attempt is made to give a comprehensive stereochemical interpretation of the squalene hypothesis encompassing all triterpenoid compounds.

It stands to reason that such an attempt can only be made on the basis of supplementary assumptions that may seem arbitrary in light of the present state of knowledge. The most important of these assumptions is the applicability of the rules governing the steric course of acid-catalyzed cyclizations. Therefore, these rules are shortly discussed here in a special chapter.

Steric Course of Acid-Catalyzed Cyclizations

Only little is actually known about the *mechanism* of acid-catalyzed cyclizations of the type discussed here. At this time, therefore, only surmises can be made about the temporal course of such reactions as well as about the structure of intermediates and/or transition states. With regard to the *steric course* of such reactions, there are some useful leads available from a few studies[15]) that are mostly of preparative nature; for the rest, one is forced to apply the experimentally more extensively supported views on the steric course of electrophilic addition reactions that are isoelectronic to cyclization reactions.

It must be assumed that a number of mechanisms are available to the acid-catalyzed cyclization *in vitro*, depending on reaction conditions and constitutional factors. Of these, two *extreme* types can be distinguished on the basis of their (in part) different stereochemical outcome. Whereas for the first limiting case a *potentially* nonstereospecific reaction course proceeding *via classical* carbocations is to be assumed, the second case is distinguished by a stereospecificity that cannot be explained as the consequence of sterically preferred addition steps (*e.g.*, determined through one-sided steric hindrance) to transient classical (planar and symmetrically solvated) carbocations. The steric result of this type of reaction course is, however, compatible with the general scheme of *antiparallel addition to C=C bonds*, a scheme that, on the one hand, is experimentally supported for

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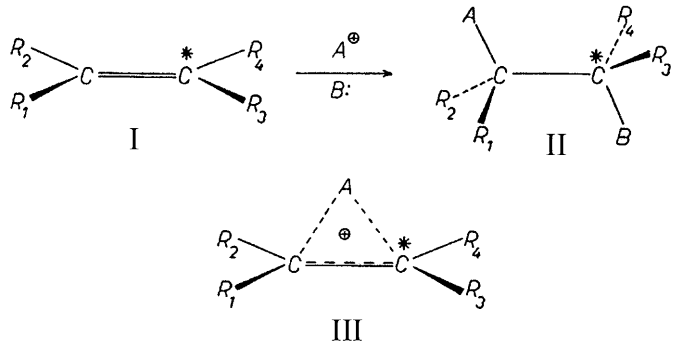
[14]) See *Footnote 8*.

[15]) R. P. Lindstead *et al.*, *J. Chem. Soc.* **1936**, 470; **1937**, 1136, 1140; G. Stork, H. Conroy, *J. Am. Chem. Soc.* **1951**, *73*, 4748; R. A. Barnes, *J. Am. Chem. Soc.* **1953**, *75*, 3004; R. Helg, H. Schinz, *Helv. Chim. Acta* **1952**, *35*, 2408; G. Gamboni, H. Schinz, A. Eschenmoser, *Helv. Chim. Acta* **1954**, *37*, 964, as well as results of *A. Frey, A. Nechvatal, P. Stadler* from our laboratory (to be published soon). *Added in Proof*: see also the most recent paper by G. Stork, A. W. Burgstahler, *J. Am. Chem. Soc.* **1955**, *77*, 5068.

other electrophilic addition reactions[16]) and, on the other hand, is theoretically justified by stereoelectronic considerations[17]). This principle of antiparallel addition entails the viewpoint essential for the derivation of the steric course of cyclizations of the second type, namely, that in the reaction's configuration-determining stage, the four centers participating in the addition process (A–C–C*–B in Scheme 1) remain arranged in a '*reaction plane*' that is perpendicular to the original double-bond plane. Such a reaction course defines a primary constellation II of the addition product that is distinguished by the antiparallel positioning of the two addition elements.

As for the mechanism of cyclization reactions that proceed according to the scheme of antiparallel addition, one of the possibilities for a closer description of the reaction course consists in the assumption of *configurationally stable*, *cationic intermediates* of type III[18]). Without delving into details as to the justification of such a formulation, in the present paper we write such *nonclassical* ions by convention in all those cases where the discussion demands a pictorial representation of a reaction plane or, for example, of attack trajectories of incoming bases.

Scheme 1



If one presupposes that a cyclization process of type IV → V proceeds in accordance with I → II, then the following prediction can be made with regards to its steric course (Scheme 2): among the possible constellations of the cyclizing aliphatic precursor, only constellations VI and VII, denoted as *chair* and *boat*

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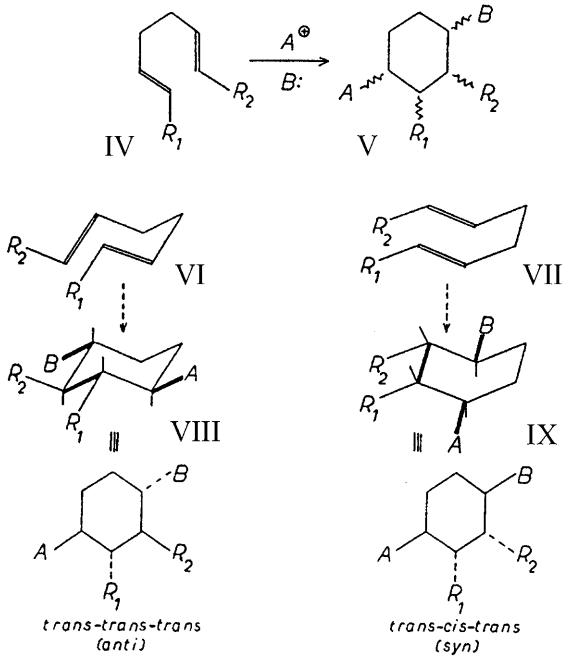
[16]) See G. H. Alt, D. H. R. Barton, *J. Chem. Soc.* **1954**, 4284.

[17]) For the principle of such reasoning, see E. J. Corey, *Experientia* **1953**, *9*, 329; *J. Am. Chem. Soc.* **1954**, *76*, 175.

[18]) In recent time, various authors (*D. J. Cram*, *M. J. S. Dewar*, *C. K. Ingold*, *H. J. Lucas*, *V. Prelog*, *J. D. Roberts*, *S. Winstein*, and others) have postulated the involvement of such nonclassical (enchimeric, synarthetic) ions or complexes in a variety of reactions that are believed to proceed *via* cationic intermediates. See also the ref. of *R. A. Barnes* in *Footnote 15*.

Scheme 2



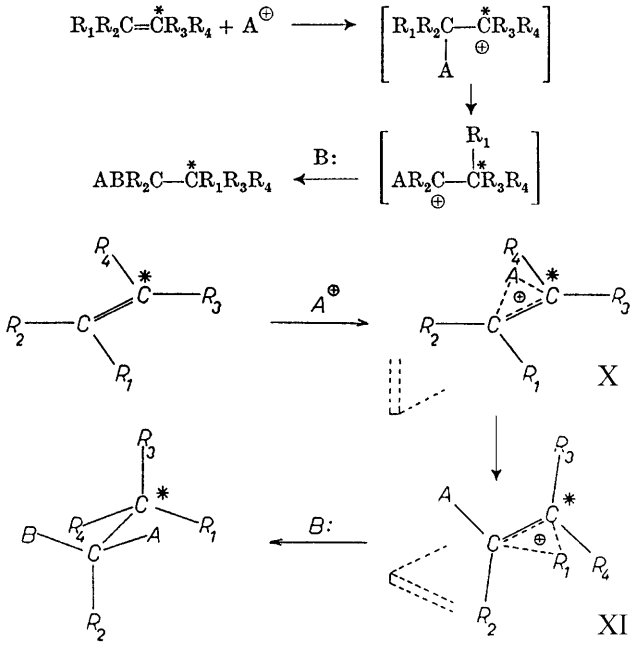
foldings, respectively, meet the necessary requirement of the general scheme[19]). In the corresponding cyclization products VIII and IX, the added residues A and B must occupy the *equatorial* position; in this constellation, they are lying antiparallel to the C–C bond formed by the ring closure, as demanded for two consecutive antiparallel addition reactions. With respect to the relative configuration of the four substituents A, R_1, R_2, and B, a cyclization *via* the chair conformation leads to the *trans-trans-trans* configuration, and one *via* the boat-conformation to the *trans-cis-trans* arrangement. From the energetic point of view, the cyclization *via* the chair conformation is, of course, to be taken as the favored one. It is to be emphasized that of *eight* possible configurations of a cyclic product of structure type V, only *two* are expected to result when a *trans-trans* diene of type IV is cyclizing according to the assumptions mentioned above.

In view of the relationship discussed in the next chapter, the question of the steric course of a reaction sequence of the type depicted in Scheme 3 must be briefly analyzed. If such a reaction proceeds *via* classical carbocations, a

[19]) This simplified procedure for deriving the steric cyclization course does not imply that a molecule must pre-exist in one of the mentioned constellations in order for cyclization to occur. What it assumes is that the geometry of a cationic intermediate (*e.g.*, III) corresponds to the geometry of the original double bonds. In the two constellations VI and VII, the two double-bond planes are thought to be roughly parallel to each other.

potentially nonstereospecific course must be reckoned with. Assuming, however, that the addition of A^+ and the subsequent rearrangement of R_1 are coupled and follow the principle of antiparallel addition, then, for stereoelectronic reasons, a sterically unambiguous course can be postulated. In Scheme 3, this course is formulated *via* nonclassical cations as intermediates[20]).

Scheme 3



Derivation of the Constitutional Formulae of Cyclic Triterpenes from Squalene

As described in what follows, the constitutional formulae of the cyclic triterpenes can be derived from the formula of squalene in a uniform manner if one starts from the following *arbitrary* premises:

1. The cyclizations are to proceed starting from a squalene, all of whose double bonds have the *trans*-configuration[21]).

2. The cyclizations are to proceed in *specific constellations* of the squalene chain (that is, a particular sequence of chair or boat foldings).

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[20]) The scheme also indicates the spatial relationship relative to each other of the reaction planes (determined by the centers A, C, and C*, and R, C, and C*, resp.) in the intermediates X and XI.

[21]) Natural squalene isolated from animal sources has all-*trans*-configuration according to N. Nicolaides, F. Laves, *J. Am. Chem. Soc.* **1954**, *76*, 2596.

3. The cyclizations are to proceed without exception according to the scheme of *cationoid antiparallel additions. Wagner–Meerwein* rearrangements and 1,2-eliminations are to occur only when the stereochemical prerequisites are optimally fulfilled.

4. All transformations of squalene into the final C_{30} products are *nonstop-reactions*, that is, no stable compounds, which could form through saturation (hydration, H-elimination) of the cyclizing molecule's positive charge, should appear as intermediates.

Scheme 4 lists the derivations of the constitutional formulae of the polycyclic triterpenes euphol[22]), tirucallol[22]), lupeol[23]), taraxasterol[24]), germanicol[25]), β-amyrin[26]), taraxerol[27]), and friedelin[28]). It turns out that, within the framework of the stated premises, the structures as well as the configurations of all these natural products can be derived in a consistent fashion if one starts from the constellation XII of the squalene chain (chair-chair-chair-*boat*). The choice to use the acid OH^+ as cyclization-initiator seems justified by the remarkable fact that almost all known tetra- and pentacyclic triterpenes have an oxygen functionality in position 3, indeed in most cases an equatorial OH group[29]).

In its first phase, the cylization process forms the characteristic *trans-anti-trans-anti-trans*-arrangement of rings A, B, C, and D, and leads to an intermediate of type XIII[30]), from which rearrangements in two different directions are possible. In applying the reaction principle exemplified in example X → XI (Scheme 3), a stereochemically unambiguous rearrangement to intermediate XIV can be formulated; the transition is connected with a constellational change of ring D from boat to the chair that corresponds to the *trans*-ring junction of rings C and D. If, on the other hand, an analogous rearrangement starting from XIII

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[22]) See *Footnotes 10* and *11*.

[23]) T. R. Ames, T. G. Halsall, E. R. H. Jones, *J. Chem. Soc.* **1951**, 450.

[24]) T. R. Ames, J. L. Beton, T. Bowers, T. G. Halsall, E. R. H. Jones, *J. Chem. Soc.* **1954**, 1905; see also P. Dietrich, O. Jeger, *Helv. Chim. Acta* **1950**, *33*, 711.

[25]) D. H. R. Barton, C. J. W. Brooks, *J. Chem. Soc.* **1951**, 257; S. David, *Bull. Soc. Chim. Fr.* **1949**, 155.

[26]) R. D. Haworth, *Annu. Rep. Prog. Chem.* **1937**, *34*, 327; B. Bischof, O. Jeger, L. Ruzicka, *Helv. Chim. Acta* **1949**, *32*, 1944.

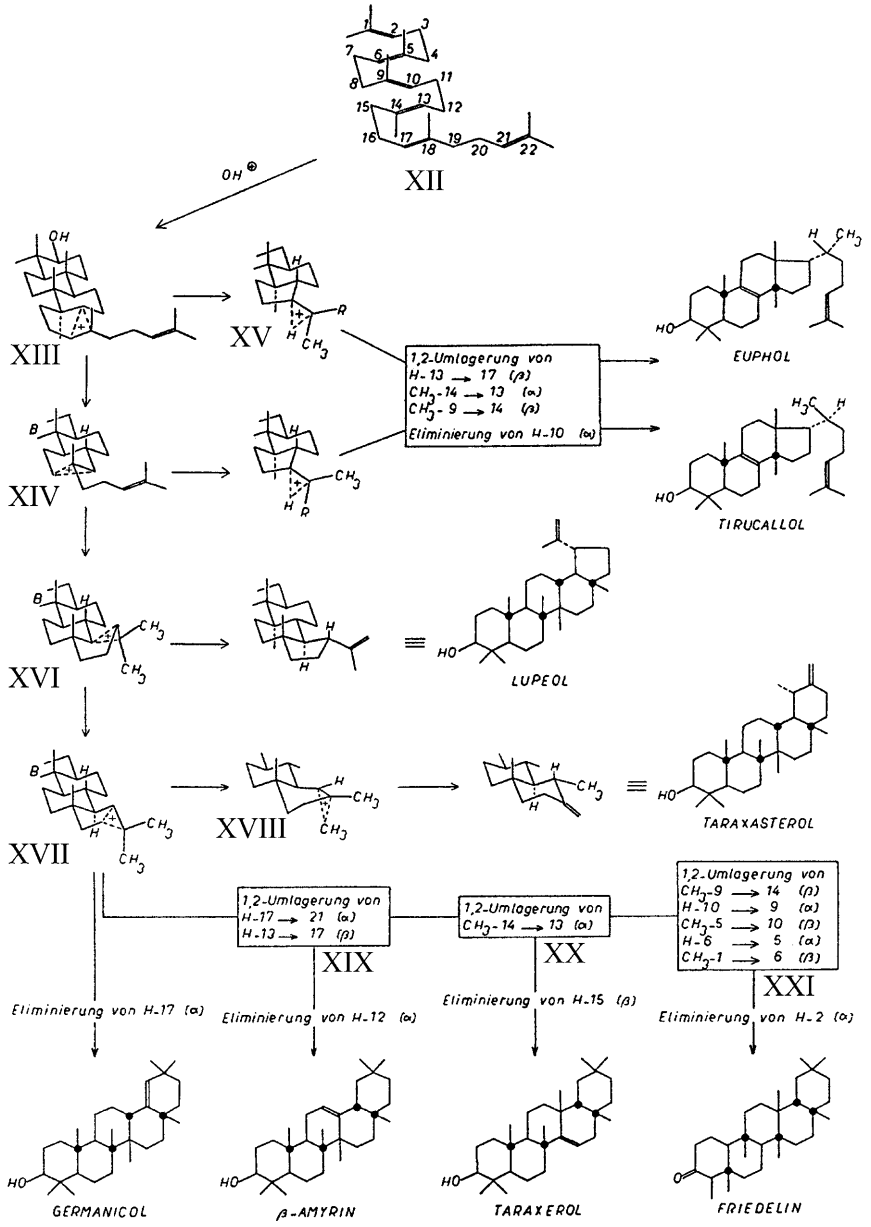
[27]) J. M. Beaton, F. S. Spring, R. Stevenson, J. L. Stewart, *J. Chem. Soc.* **1955**, 2131; see also E. Koller, A. Hiestand, P. Dietrich, O. Jeger, *Helv. Chim. Acta* **1950**, *33*, 1050; K. Takeda, *J. Pharm. Soc. Jpn.* **1943**, *63*, 197.

[28]) See citations in *Footnote 12*.

[29]) An exception is, for example, the unsaturated pentacyclic hydrocarbon taraxeren (see T. Bruun, *Acta Chem. Scand.* **1954**, *8*, 1291. The OH groups at C(3) of polyporenic acid A and elemadienolic acid assume the axial α-position. Importantly, in nature these compounds occur together with corresponding derivatives that possess a carbonyl group at this position.

[30]) The formulation XII → XIII involves an *anti-Markovnikov* cyclization with regard to the formation of ring C. Cyclizations of such type are known (see, *e.g.*, R. P. Linstead *et al.*, *J. Chem. Soc.* **1937**, 1136).

Scheme 4


XII
OH⊕
XIII
XV
XIV
XVI
XVII
XVIII
XIX
XX
XXI
1,2-Umlagerung von
H-13 → 17 (β)
CH3-14 → 13 (α)
CH3-9 → 14 (β)
Eliminierung von H-10 (α)
EUPHOL
TIRUCALLOL
LUPEOL
TARAXASTEROL
1,2-Umlagerung von
H-17 → 21 (α)
H-13 → 17 (β)
1,2-Umlagerung von
CH3-14 → 13 (α)
1,2-Umlagerung von
CH3-9 → 14 (β)
H-10 → 9 (α)
CH3-5 → 10 (β)
H-6 → 5 (α)
CH3-1 → 6 (β)
Eliminierung von H-17 (α)
Eliminierung von H-12 (α)
Eliminierung von H-15 (β)
Eliminierung von H-2 (α)
GERMANICOL
β-AMYRIN
TARAXEROL
FRIEDELIN

and involving the α-H-atom at C(17)[31]) instead of the CH_2(16) is considered, we arrive in a stereochemically equally unambiguous manner to intermediate XV, which leads to the stereochemically correct constitutional formula of euphol as the end product of a sequence of rearrangements that follow the scheme of antiparallel additions throughout. A reaction sequence formulated in a precisely analogous manner leads from XIV to the stereochemically correct formula of tirucallol[32]).

By adding a further cyclization step to XIV, one arrives at the series of pentacyclic compounds, provided that one carries out the ring closure XIV → XVI in the boat folding of ring E. Directly derivable from XVI is the formula of lupeol with its *anti-trans*-arrangement of all five rings. Alternatively, a rearrangement of type XIII → XIV leads, again under constellational change of ring E, to the important central intermediate XVII. From this intermediate, triterpenes of the β-amyrin type can consistently be derived by strict adherence to the scheme of the antiparallel 1,2-rearrangement and elimination. It is to be pointed out that the *cis*-ring junction of rings D and E, characteristic of all triterpenes related to β-amyrin, appears as a direct consequence of the 1,2-shift of the β-H from position 13 to position 17.

The intermediate XVII yields, after elimination of the H_α–C(17), germanicol. On the other hand, XVII connects through the 1,2-migrations shown in Scheme 4 to the intermediate XIX, which leads to β-amyrin. Out of XIX, taraxerol is formed *via* XX. Finally, the intermediate XXI ensues from XX by a series of rearrangements, the former yielding *friedelin*[33]) after H-elimination.

Finally, a simple 1,2-transfer of a methyl group from C(22) to C(21) in XVII leads to intermediate XVIII. According to the example X → XI, this methyl transfer unequivocally has to involve the α-methyl group. Out of XVIII follows the formula of taraxasterol.

As the parent compound of an important group of pentacyclic triterpenes, α-amyrin requires a special discussion. Although today the structural *Ruzicka*–

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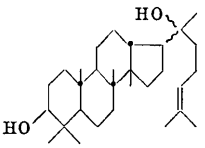
[31]) The C-atoms are numbered according to their formal connection to those of squalene, if not stated otherwise (see Scheme 4).

[32]) In the context of the biogenetic isoprene rule, it is of special relevance that recently *J. S. Mills* found two new triterpene diols which are stereoisomeric at C(20) (see J. S. Mills, A. E. A. Werner, *J. Chem. Soc.* **1955**, 3132) and, in the meantime, elucidated their structure according to the following formula.

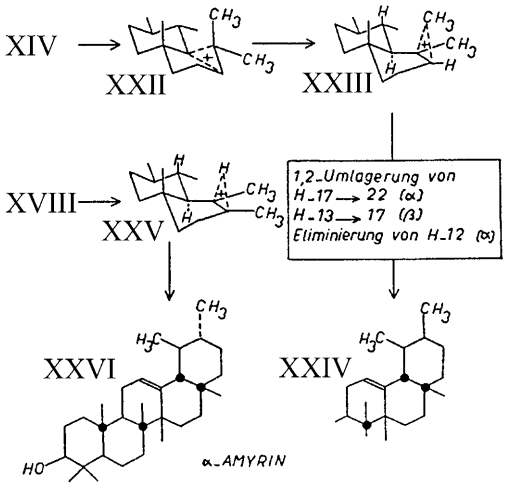


We thank Dr. *Mills* (the National Gallery, London) for his kind permission to mention his unpublished results in the present paper.

[33]) The possible biogenetic relationship between friedelin and β-amyrin depicted in Scheme 4 was first pointed out by *E. J. Corey* and *J. J. Ursprung*[12]). See also the ref. of *H. Dutler*, *O. Jeger*, and *L. Ruzicka* in *Footnote 12*.

Jeger formula[34]) for this compound can be viewed as established[35]), questions referring to the relative configuration of the H-atom at C(18)[36]) as well as of the carbon centers bearing the two methyl groups in ring E remain. Within the framework of presuppositions taken to be valid here, there are two possibilities for the derivation of this part of the α-amyrin formula (Scheme 5). The first possibility uses a ring E cyclization in the chair folding from XIV to XXII and subsequently a sequence of 1,2-rearrangements *via* XXIII similar to those presented above for the β-amyrin series, leading to the constitutional formula XXIV for α-amyrin. The second possibility involves an essentially analogous sequence of rearrangements to give the precursor XVIII of taraxasterol, whence we arrive *via* XXV to the stereo-formula XXVI for α-amyrin, the same as the one suggested by *E. J. Corey* and *J. J. Ursprung*[37]). The α-amyrin formulae resulting from these two pathways differ only in the configuration of the methyl group at C(20)[36]). A common characteristic of both formulae and one that emerges in the present context in an unambigous fashion is the *cis*-junction of rings D and E.

Scheme 5



What finally remains is the question regarding a stereochemical interpretation of the *Woodward–Bloch* cyclization scheme from squalene to lanosterol. The latter is the only natural product among those discussed here for which the

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[34]) A. Meisels, O. Jeger, L. Ruzicka, *Helv. Chim. Acta* **1949**, *32*, 1075; A. Meisels, R. Rüegg, O. Jeger, L. Ruzicka, *Helv. Chim. Acta* **1955**, *38*, 1298.

[35]) In the meantime, *A. Melera* in our laboratory succeeded in providing a further proof for this formula.

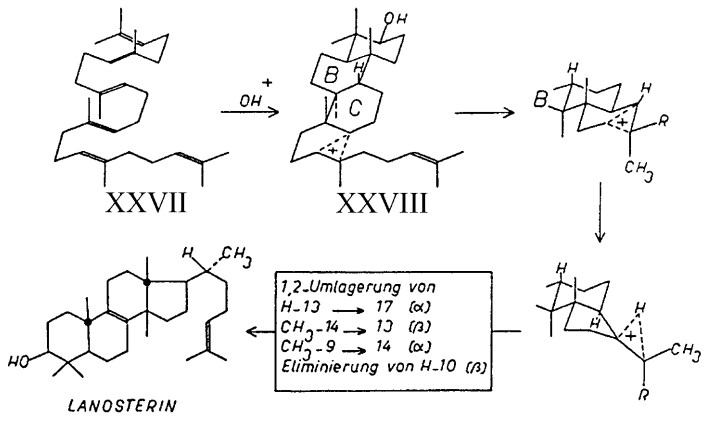
[36]) Ursane numbering.

[37]) E. J. Corey, J. J. Ursprung, *Chem. Ind.* **1954**, 1387.

assumption of a direct biogenetic connection with squalene rests on experimental data[38]) and, therefore, does not merely represent pure speculation.

It has become clear that, under strict adherence to the presuppositions followed so far, the lanosterol formula *cannot* be derived by starting from the primary squalene folding XII that had to be chosen for the rest of the triterpenes[39]). The only possible solution seems to be the one illustrated in Scheme 6, involving the chair-*boat*-chair-boat folding XXVII, from which a reaction sequence (corresponding exactly to the one considered for tirucallol) leads *via* XXVIII and further intermediates unambiguously to the stereoformula of lanosterol.

Scheme 6



The delineations depicted in Schemes 4 – 6 refer without exception to tetra- and pentacyclic compounds that can be viewed as the parent representatives of special groups of triterpenes. These representatives are distinguished by their common 'oxidation level', which corresponds – apart from the OH group at C(3) – to that of squalene[40]). The numerous triterpenes that have additional oxygen functionalities on their carbon skeleton need not be discussed here, as the possibility of their origin through subsequent biological oxidations of corresponding parent member seems obvious.

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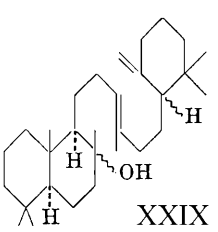
[38]) See *Footnote 6* and also the results of *W. G. Dauben* and *J. H. Richards* (*Chem. Ind.* **1955**, 94) referring to the biosynthesis of eburicolic acid (lanosterol type) from acetic acid in Polyporus sulphureus.

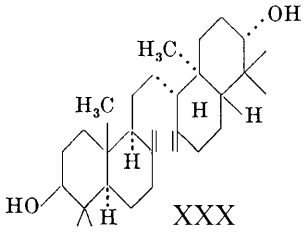
[39]) This is also the case when the possibility of 1,3-rearrangements (see *Footnote 7*) is taken into account. If, however, we were to drop presupposition 4 (see above), according to which the transition squalene → lanosterol is supposed to be a nonstop process, then the possibility would arise to derive the lanosterol formula, also from the squalene folding XII, by including a 1,3-methyl shift from C(9) to C(13).

[40]) Σ (carbon rings + double bonds and potential double bonds, resp.) = 6.

For completeness, the formulae for the two cyclic triterpenes ambreine[41]) (XXIX) and onocerine[42]) (XXX) are also depicted here. The biogenetic connection of these compounds with squalene does not require further comment.



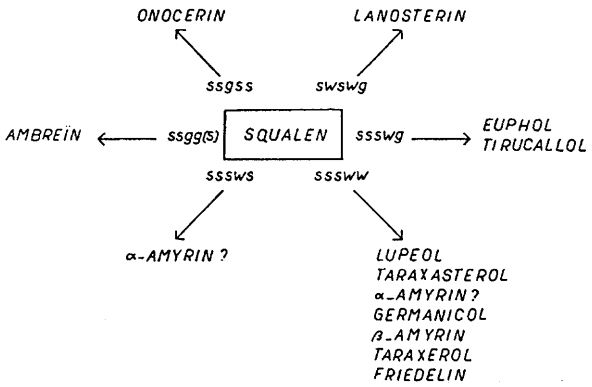
XXIX



XXX

In the light of the ideas propounded here, the constitutional diversity of the natural triterpenes can be interpreted to originate partly from the *constellational* diversity the squalene chain has access to when it undergoes cyclization during the formation of these compounds. Thereby, the squalene chain may be only partially folded and remain partially stretched, as, for example, for the steroid-type tetracyclic triterpenes (lanosterol, euphol, and tirucallol). Scheme 7 gives a compilation of all the cyclizations discussed above, each with an annotation referring to its requisite type of squalene folding[43]).

Scheme 7



One might imagine that fixing particular foldings of the squalene chain represents a specific role of the enzymatic systems that are involved in the biological cyclization. Such a process would imply a considerable increase in free

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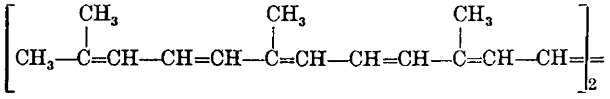
[41]) L. Ruzicka, F. Lardon, *Helv. Chim. Acta* **1946**, *29*, 912; E. Lederer, F. Marx, D. Mercier, G. Perot, *Helv. Chim. Acta* **1946**, *29*, 1354; O. Jeger, O. Dürst, L. Ruzicka, *Helv. Chim. Acta* **1947**, *30*, 1859; E. Lederer, D. Mercier, *Experientia* **1947**, *3*, 188.

[42]) D. H. R. Barton, K. H. Overton, *J. Chem. Soc.* **1955**, 2639.

[43]) s = Chair folding; w = boat folding; g = stretched conformation of the squalene chain.

energy[44]) by the squalene molecule and could be equated with its activation towards cyclization[45]). It can be assumed that the cyclization of an optimally folded squalene chain would require only a small amount of free activation energy for embarking on its highly exothermic course. From this point of view, it would be understandable that such cyclizations, requiring, after all, strong acids as catalysts *in vitro*[46]), are possible at all under the mild physiological reaction conditions *in vivo*.

On the other hand, the envisioned foldings of the squalene might well be coupled with the enzymatic synthesis of squalene. A possibility that would appear plausible in every respect could be a squalene formation that proceeds *via* a decadehydrosqualene as immediate precursor, with five CH=CH bonds in the *cis*-configuration originating in a synthesis of the polyisoprene chain from C_5 units that are on a higher oxidation level, such as *β*,*β*-dimethylacrylic acid[47]). Enzymatic hydrogenation of these five double bonds leads directly to a folded squalene (XII), that either cyclizes or unfolds, and thereby becomes accessible to isolation. Such a decadehydrosqualene might play the role of *G. Popjak*'s[48]) postulated unknown intermediate of cholesterol biosynthesis, that can reversibly turn into squalene, but in contrast to the latter lies in the direct line from acetic acid to cholesterol.



Decadehydrosqualene

Reverting to the stereochemical interpretation of the squalene hypothesis presented above, it can be stated in summary that, within the framework of a defined system of arbitrary assumptions, a reaction scheme can be designed that connects in a consistent manner the formula of squalene with the structure and configuration of the parent members of all families of polycyclic triterpenes known today.

Finally, the question that refers to the objective meaning of this scheme with regard to the problem of triterpene biogenesis is a question about the justification of the assumptions on which the scheme is based; it may seem unnecessary to emphasize that, according to what we know today, there is no guarantee for their

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[44]) Such an increase would mainly refer to a change in the entropy term of the free energy.

[45]) Compare, *e.g.*, the enormous influence of spatially fixed and suitably positioned double bonds on the rate of the formation of cationic intermediates in the solvolysis reactions of certain toluene sulfonates (S. Winstein, M. Shatavsky, C. Norton, R. B. Woodward, *J. Am. Chem. Soc.* **1955**, *77*, 4183.

[46]) The *in vitro* cyclization of squalene to 'tetracyclosqualene' requires conditions such as prolonged heating with 98% formic acid (see I. M. Heilbron, E. D. Kamm, W. M. Owens, *J. Chem. Soc.* **1926**, 1630).

[47]) See *A. Eschenmoser*, Habilitationsschrift ETH, 1955. Experiments aiming at a synthesis of corresponding poly-yne derivatives are under way in our laboratory.

[48]) G. Popjak, *Arch. Biochem. Biophys.* **1954**, *48*, 102.

correctness. *For the time being, the justification of these assumptions can be seen exclusively in the fact that on their basis a complete and consistent derivation of the structure and the configuration of the triterpenes can be constructed. This result may be regarded as solid support for the squalene hypothesis as well as a contribution of research on constitution to research on the biogenesis of triterpenes.*

Summary

The biogenetic isoprene rule in its application to the triterpenes is discussed from a stereochemical standpoint. On the basis of a well-defined system of *arbitrary* assumptions, a scheme has been developed leading from squalene to the formulae of the basic representatives of all known cyclic triterpene groups – *i.e.*, euphol, tirucallol, lupeol, taraxasterol, germanicol, β-amyrin, taraxerol, friedelin, α-amyrin, lanosterol – in their full structural and configurational detail. This result is considered to support the squalene hypothesis of the biogenesis of cyclic triterpenes.

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2. Historical Perspective. – The content of the 1955 *Helvetica Chimica Acta* paper is deeply rooted in the momentous scientific work of *Leopold Ruzicka* and his school on mono-, sesqui-, di-, and triterpenes, carried out at the ETH in Zurich from the 1920s up to the mid 1950s. One of the sources of *Ruzicka*'s dominance in terpene chemistry during that period was the pragmatic way he applied the so-called isoprene rule, according to which the constitutional formulae of terpenes are to be '*composed of isoprene units*', in his research on terpenes. Originally recognized by *Otto Wallach* [10] as constituting a compositional regularity connecting the skeletal formula of isoprene with the skeletal formulae of monoterpenes and (tentatively) sesquiterpenes, in *Ruzicka*'s work the rule gained the status of a general working hypothesis in the form of a qualifying formula-selection criterion in chemical structure elucidation, valid for all terpenoid natural products [11].

In the early 1950s, this isoprene rule, up to then applied in a rigorously *formal* way, underwent a sudden metamorphosis into a general *mechanistic* hypothesis of the biosynthesis of terpenoids; the classical (empirical) isoprene rule evolved into the '*biogenetic isoprene rule*' [12]. As such, it became a conceptual tool to assist not only structure determination in the terpene field, but also to promote research on the biosynthesis of terpenoids. The impetus to this change – in as far the developments at ETH-Zurich were concerned – came from two directions. One of them had its roots in the work

of *Hans Schinz*[3]) who, in the late 1940s and early 1950s, had been systematically exploring the scope of the classical pseudoirone–irone cyclization [13] by studying the reaction products of this type of acid-catalyzed ring formation for a variety of synthetic aliphatic mono- and sesquiterpenoid model compounds [14]. It was the good fortune of working as a student in the *Schinz* laboratory that brought *A. E.* into contact with this specific research topic [15]. It led him in his diploma work to become involved in the mechanistic aspects of the reaction and, in the context of his thesis work [16], perceive its role as a process that, conceptually, connects the chemical formula of the aliphatic sesquiterpene farnesol with the constitutional formulae of the entire family of sesquiterpenes. He found that the formulae of all then known polycyclic sesquiterpenes of known or still unknown chemical structure could be derived from farnesol (or its equivalent farnesene) by hypothetical acid-catalyzed polyolefin cyclizations, realizing that all those sesquiterpene formulae described in the literature that failed to comply with this postulate had to be, and were corrected [16][4]). In the wake of his thesis, he also recognized that such a *mechanistic* formula-selection criterion for the elucidation of terpene structures was not restricted to the sesquiterpene series, but also applicable to the polycyclic di- and triterpenes known at the time. It turned out that the constitutional formulae of the family of tetra- and pentacyclic triterpenes are derivable from the formula of squalene by postulating that cationoid squalene cyclizations can connect with complex sequences of cationoid *Wagner–Meerwein* rearrangements and hydride shifts leading to the constitutionally diverse polycyclic triterpenes. Essential part of

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[3]) *Hans Schinz* (1899–1990), born in Oberrieden (canton Zurich), studied chemistry at the ETH-Zurich and became one of the first doctoral students (1922–1924) of *Leopold Ruzicka* at a time when the latter was appointed *Titular Professor* under *Hermann Staudinger* at the ETH (1923). Through the close connection of *Ruzicka* with the Geneva perfume company *Chuit & Naef* (later *Firmenich & Cie*), *Schinz* remained associated with *Ruzicka* as a research chemist of *Firmenich* company while working with him at the ETH on the chemistry of monoterpenes. During World War II, he was granted the right to do independent, yet still *Firmenich*-oriented, research with students, officially *Ruzicka*'s students, who had done their diploma in natural sciences rather than in chemistry. In 1942, *Schinz* isolated and determined the constitution of lavandulol, a monoterpenoid constituent of lavender oil possessing an irregular isoprene skeleton. In the later 1940s and early 1950s, he was engaged in synthesizing nonnatural aliphatic monoterpenoid alcohols as potential fragrances, and in studies on the constitutional course of acid-catalyzed cyclizations in the mono- and sesquiterpene series. He retired from his activity at ETH in the early 1960s. In 1955, *Hans Schinz* received the *Fritsche* Award (an award for outstanding achievements in analysis, structure elucidation, and chemical synthesis of essential oils, isolates, flavors, and related substances, predecessor of the *Ernest Guenther* Award in the Chemistry of Natural Products) of the American Chemical Society.

[4]) As early as 1922, *Ruzicka* and *Stoll* [17] had recognized that the C skeletons of (as he wrote) '*most of*' the sesquiterpenes known at the time (farnesol, nerolidol, bisabolene, selinene, eudesmol) are composed of a regular (head-to-tail) chain of three isoprene units '*as Nature produces it in farnesol*'. Even though he subsequently observed [18] that nerolidol is transformed to bisabolene by treatment with acid in analogy to the already known conversion of linalool to terpineol in the monoterpene series, he continued until the early 1950s to interprete structural relationships among terpenes strictly in the spirit of the classical isoprene rule. A statement such as the one found in [19b], according to which *Ruzicka* had recognized '*the role of farnesol as the universal precursor of sesquiterpenes in the 1920s*' is historically not correct. For instance, in his work on sesquiterpenes *Ruzicka*, as late as 1935, proposed formulae for β-caryophyllene which, while all in accord with the classical isoprene rule, did not show any relationship with the formula of farnesol. The same is true of *Ruzicka*'s 1936 proposals for the structure of the tricyclic sesquiterpene cedrene (for a discussion and documentation, see [20]).

this proposal was the novel concept of *oxidative* initiation of squalene cyclization, as opposed to its initiation by protons [16][12b][5]). Contrary to what it may appear today, these schemes which, for the first time, established a detailed *mechanistic* connection between squalene and polycyclic triterpenes, were actually far from obvious at that time, be it only, for instance, for the need to overcome the mental barrier against the assumption that a cationic squalene cyclization might violate the venerable *Markovnikov* rule[6]).

The other direction of research at the ETH that chaperoned the metamorphosis of the isoprene rule was the outcome of the structure determination of lanosterol. During World War II and beyond, *Ruzicka* in his assault upon the complex structures of polycyclic triterpenes was fortunate to be able to rely on the highly competent assistance of his former student *Oskar Jeger* in the supervision of generations of (officially) *Ruzicka* doctoral students. It was under *Jeger*'s engagement that the experimental efforts of the ETH school in triterpene research reached, in 1952, their climax: the elucidation of the chemical structure of lanosterol by methods of chemical degradation [22][7]). Surprisingly, and thus far unheard of in the triterpene family, the chemical formula of this tetracyclic triterpene from animal sources did *not* obey the classical isoprene rule. Moreover, and most revealingly, it displayed an unmistakably close relationship to the chemical formula of cholesterol.

Knowledge of the lanosterol structure was bound to have an immediate impact on the interpretation of *Konrad Bloch*'s pioneering biochemical work on the biosynthesis of cholesterol from acetic acid. *Bloch*'s experimental evidence, together with the emergence of the lanosterol formula, allowed *Woodward* and *Bloch* [24] at Harvard to rush into print the correct way of relating the formula of squalene to that of cholesterol (as opposed to an earlier, mechanistically mysterious proposal by *Robert Robinson* [25]),

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[5]) The concept of *oxidative* initiation of polyene cyclization [16] in its application to the enzymic cyclization of squalene was perceived as occurring by an oxidant that can act as potential hydroxy cation [12b]. According to *Bloch* [21], the proposal inspired the experiments on the biosynthesis of cholesterol in the presence of $^{18}O_2$ that demonstrated the oxidative (as opposed to aqueous) origin of cholesterol's OH group at C(3).

[6]) For a chemobiographical essay on *Ruzicka* and the history of the metamorphosis of the classical isoprene rule into the biogenetic isoprene rule, see [20].

[7]) Somewhat ironically, *Jeger* and *Ruzicka*'s main competitor in the race for the structure of lanosterol by chemical degradation, *D. H. R. Barton*, fell victim to the classical isoprene rule by preferring to interpret his own experimental results in terms of a wrong formula for lanosterol, misled by the conviction that the chemical formula of lanosterol has to comply with the classical isoprene rule [23]. In this context, it is worthwhile noting that *Wagner–Meerwein* rearrangements, as implied by the biogenetic isoprene rule *may*, but do *not have* to destroy compliance of a terpene formula with the classical isoprene rule. Another and, at the same time, successful competitor was the crystallographer *A. McL. Mathieson* in far-off Melbourne, who discovered the correct structure of lanosterol by X-ray analysis of lanostenol iodoacetate and published it in a preliminary communication in *Nature* [22b], that appeared about three months before the ETH paper did in *Helvetica Chimica Acta* [22a]. Nevertheless, the impact of the ETH paper on organic natural-product chemistry turned out to be far greater than that of the *Mathieson* paper (*e.g.*, neither the *Woodward–Bloch* paper of 1953 or the *Experientia* paper of 1953 did mention the *Mathieson* paper, nor did – regrettably – our own 1955 *Helvetica Chimica Acta* paper). This, in retrospect, is to be seen as an expression of the mental reservation natural-product chemists originally had against X-ray structures at the time of the historic transition when X-ray analysis began to replace chemical degradation as a tool of structure determination in organic natural-product chemistry, initiating the decline of the method of chemical degradation.

implying that the biosynthesis of cholesterol from squalene proceeds *via* lanosterol[8]). Soon afterwards, decisive experimental evidence for this hypothesis was brought forward by *Tchen* and *Bloch* [32], and *Clayton* and *Bloch* [33].

For the septuagenarian *Ruzicka*, to witness how in the early 1950s the answers to some of the basic questions he had been pondering over decades, fell into place all at once, was indeed the culmination of his lifework as terpene and steroid chemist: lanosterol emerging as the biosynthetic link between '*his*' triterpenes and '*his*' steroids, the lanosterol formula unmistakably revealing a limit of '*his*' classical isoprene rule, and yet this very rule resurrecting in a new form as a general working hypothesis for the biosynthesis of terpenoids while remaining what it had been all along, namely, a powerful tool in the service of the structure determination of terpenoids, though now much more powerfully so. *Ruzicka*'s enthusiasm and deep satisfaction about these developments emanates from the article he wrote following his lecture at the IUPAC conference in Stockholm in summer 1953 '*The Isoprene Rule and the Biogenesis of Terpenic Compounds*' [12]. There, he reviewed his life work on the determination of the structure of terpenes by chemical methods and, importantly, used the concluding chapter [12a] of the article as a forum for presenting the new ideas that had emerged within his school in consequence of the postulates put forward in *A.E.*'s doctoral thesis [16]. In this chapter, the mechanistic pathways that connect cyclic mono-, sesqui-, di-, and triterpenes with their respective aliphatic precursors geraniol, farnesol, geranylgeraniol, and squalene appeared for the first time in print. With his authority as the leading terpene chemist of his time, *Ruzicka* proclaimed the postulates underlying these relationships to con-

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[8]) The history of the biological relationship of squalene to cholesterol is worth remembering. The relationship was first alluded to in 1926, *i.e.*, at a time in which the structure of the two compounds had not yet been assigned, by *Channon* and *Marrian* [26], who detected upon feeding of the hydrocarbon to rats a 100% increase of cholesterol in the liver of the animals. In an attempt to overlap, at least partially, the formulae of the two compounds, *Vanghelovici* suggested in 1927 [27] that the problem of the still undecided location of the angular Me group at the C-D ring junction of cholesterol should be settled in favor of C(14) (modern numbering) for biogenetic reasons. Shortly afterwards, *Robinson* pointed out that there was overwhelming *chemical* evidence for placing the critical Me group at C(14) and that one should not '*…allow the biogenetic tail to wag the chemical dog*' [25]. In the same paper, he proposed an alternative way of overlapping the two molecules, one that was supposed to avoid the necessity of a rearrangement of Me groups, yet was no less mysterious from a mechanistic point of view. In this context, it is of interest to point out that the formal kind of mechanistic view implicitly underlying the *Woodward–Bloch* proposal had been foreshadowed in two largely ignored 1935 papers of *Bryant* [28] who, in criticizing *Robinson*'s proposal, suggested a carotinoid as alternative precursor of cholesterol. While this proposal was in error, the '*mechanistic*' thinking in the second paper was remarkably ahead of its time in suggesting that a biological cyclization of a carotenoid might proceed along the lines *Ruzicka* [29] had considered for the formation of tetracyclosqualene in acid-catalyzed cyclization of squalene of *Heilbron et al.* [30]. Ten years later, *Ruzicka* in his *Nobel* lecture [11] documented his old belief that cholesterol derives from a polycyclic triterpene by drawing the constitutional formula of a remarkable cholesterol-like triterpene that differed from lanosterol only in the position of a single angular Me group and the position of the (endocyclic) double bond (besides lacking lanosterol's side chain double bond; see also [20]). Finally, in the year of the formulation of the biogenetic isoprene rule, *Mondon* [31], in a paper also reviewing the history of the problem proposed an '*iso-squalene*' containing the head-to-head junction between a geranyl and a geranylgeranyl residue instead two farnesyl residues as the $C_{30}H_{50}$ precursor of cholesterol. The proposal was supposed to allow for a straightforward cyclization pathway leading directly to the backbone of cholesterol. In retrospect, the proposal may serve as a documentation of the kind of mental barrier that had to be overcome in deducing the *anti-Markovnikov* cyclization–rearrangement scheme that is part of the biogenetic isoprene rule.

stitute the '*biogenetic isoprene rule*', destined to replace the '*empirical*' isoprene rule of the past.

The biogenetic isoprene rule of 1953 demanded that a constitutional formula assigned to a terpene must be derivable from the formula of its aliphatic precursor terpene by specific reaction schemes that include (hypothetically biogenic) protonatively or oxidatively initiated cationic carbocyclizations, *Wagner–Meerwein* rearrangements or hydride shifts. Not only was it now possible (as well as mandatory !) to check by '*retro-biosynthetic*' reasoning whether a given terpene formula concurred with the rule, it also became feasible to apply the rule in the inverse mode and conceive by '*forward-biosynthetic*' reasoning alternative formulae that might equally be in accordance with existing chemical degradation and spectroscopic evidence. The first published terpene formulae that fell victim to such a combined '*top-down*' and '*bottom-up*' screening were those of the classical and (for that time) complex sesquiterpenes, β-caryophyllene, clovene, humulene (α-caryophyllene), cedrene, elemol, and lanceol [16][12b][9]). It is difficult to overestimate how many of the innumerable new sesqui-, di-, or triterpenes discovered during the last 50 years have profited from the predicting and discriminating power of the biogenetic isoprene rule in the context of their structure determination[10]).

In 1953, the number of tetra- and pentacyclic triterpenes of known chemical constitution was quite small: ambreine, β- and α-amyrin, germanicol, lupeol, and lanosterol. Yet a host of additional ones were under intense investigation in the *Jeger–Ruzicka* laboratory, in hot competition with other research groups. Again, it was a piece of good fortune that *D. A.* was accepted in 1951 as Ph.D. student in the *Jeger* group and became engaged in 1954 in the structure elucidation of the two important lanosterol diastereoisomers euphol [42] and tirucallol [43]. Since the 1953 formulation of the biogenetic isoprene rule had dealt only with the *constitution* of terpenes, the very existence of this amazing trio of tetracyclic triterpenes, differing only in the *configuration* of their C skeletons, clearly called for an extension of the mechanistic schemes of the biogenetic isoprene rule for the triterpenes to include their configuration.

At the ETH, the prospects for such an extension happened to be near optimal; not only had triterpene stereochemistry made great strides in that year, but also *A. E.*'s studies on the stereochemical course and mechanism of the cationoid carbocyclization reaction had moved forward conceptually, as well as experimentally, by work on model systems taken up in the meantime (for *Stork*'s contribution in this context, see below).

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[9]) For the confusing role the classical isoprene rule had played in the numerous earlier attempts to formulate the chemical structures of β-caryophyllene and cedrene, see [20]. The correct chemical formula for β-caryophyllene was independently proposed in 1951 by three groups [16][34][35]. The proposal from ETH was based on what two years later became propounded as the biogenetic isoprene rule and was complemented by two experimental studies that corroborated the proposal [36][37]. Final structural proof for β-caryophyllene was provided by *Barton et al.* in 1952 [38], and for cedrene independently by *Plattner et al.* [39], and by *Stork* and *Breslow* [40] in 1953.

[10]) *De lege*, the biogenetic isoprene rule refers to the '*prototype representatives*' among the cyclic mono-, sesqui-, di-, and triterpenoids, *i.e.*, to cyclic terpenes the structure of which is on the same oxidation level as the terpenoid precursor (in the triterpene series allowing for either squalene or oxidosqualene). The large majority of naturally occurring terpenoids have molecular structures that result from secondary (mostly oxidative) modifications of the prototype structures. According to a recent survey of *Matsuda* and co-workers [9f], there are nearly 100 different prototypes in the triterpene series and, according to *Cane* [19a][41], *ca.* 300 different sesquiterpene C skeletons known today.

With *Barton*'s manifesto on the role of molecular conformations [44] in the background, the hidden stereoelectronic message of the ETH-born '*Fürst–Plattner* rule' [45][11]), in conjunction with the notion of polyolefine cyclizations proceeding *via* cationoid π-complexes (or, to use an equivalent metaphor, three-membered ring nonclassical carbocations) [16], gave rise to the postulate, according to which the stereochemical outcome of (nonstereorandom) carbocyclizations ought to comply with a principle that demands all four reaction centers participating in a polar addition process involving a double bond to remain in a common plane from the beginning to the end of the reaction (principle of 'antiparallel addition'). Moreover, analysis of the *conformational* aspects of polyene cyclization in the context of these studies led to the recognition of the *chair/boat dichotomy* in the stereochemical course of the reaction: the configuration of the cyclization product will be co-determined by whether the central ring-forming step proceed *via* a *chair* or, alternatively, a *boat* folding of the di-olefin backbone unit. The deeper importance of this concept, however, remained still unrecognized at that time, since it was presumed that all polyene cyclizations (nonenzymic as well as enzymic) would adopt, by energetic reasons, the chair folding.

Applying these concepts to hypothetical cyclizations of all-*trans*-polyisoprenologs under the presumption that the ring-forming steps proceed *via* the chair folding implied that the ring systems in the cyclization products would have the *trans-anti-trans* configuration, the type of stereochemistry characteristic of the natural polycyclic triterpenes and steroids. This hypothetical relationship between the stereochemistry of cationoid polyene cyclization and that of polycyclic triterpenoids was first advocated in a paper published in 1954 [46]. There it was reported that acid-catalyzed cyclization of the two diastereoisomeric 3-demethylgeranic acids proceeds stereospecifically to yield the two correspondingly diastereoisomeric cyclic β-hydroxycarboxylic acids, in agreement with cyclization *via* a chair folding of the di-olefin chain and antiparallel addition to the disubstituted double bond. This model study, carried out in the *Schinz* laboratory to which *A.E.* still maintained an association, paved the way for a more-extended model study on three (of the four possible) diastereoisomeric 3-demethylfarnesic acids [47]. The aim of this study was to demonstrate the presumed preference of (*in vitro*) carbocyclizations to proceed *via* chair folding. This was, in fact, what the study eventually demonstrated, at least with respect to formation of the second ring of the bicyclic reaction product, even though the study failed to show, under the conditions used, the expected stereospecificity of cyclization with respect to the central (trisubstituted) olefinic bond of the model substrate.

It was the chemical structure of euphol, established in 1954 at the ETH [42] and independently at the Birckbeck College [48], that was then to play a central role in

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[11]) '*Fürst–Plattner* rule': Ring opening reactions by nucleophiles involving endocyclic steroidal epoxides in six-membered rings proceed in such a way that the reaction products will contain both resulting substitutents in *diaxial* (as against diequatorial) position. In the context of the notion that acid-catalyzed polyene cyclization proceed *via* nonclassical carbocations as intermediates [16], this remarkably consistent behavior of epoxides was taken to indicate that their reactions are not simply *trans*-openings of expoxide rings, but rather processes in which the four reaction centers involved in the S_N2 reaction (proceeding by inversion) lie in a common plane throughout the entire ring-opening process. The strict regio- and stereospecificity of the reactions that gave rise to the '*Fürst–Plattner* rule' provided one of the earliest pieces of stereochemical evidence for the postulate of *antiparallel addition* to olefinic C=C bonds.

the context of the stereochemical interpretation of the biogenetic rule for the triterpenes. The relative configurational relationship between the euphol A/B- and C/D-*trans*-ring junction was clearly deducible by assuming chair-chair-chair folding of the first three ring-forming units in an enzyme-catalyzed oxidative cyclization of all-*trans*-squalene, followed by a cascade of H and Me shifts, in provoking contrast to the quest for rationalizing the inverse configurational relationship between the corresponding *trans*-ring-junctions in the constitutionally identical lanosterol. It was at an Institutskolloquium at the ETH in late 1954[12]) that *D. A.*, after presenting his work on the structure elucidation of euphol, raised the possibility of interpreting this configurational difference by suggesting that the enzymic cyclization of squalene to the first three rings of lanosterol could proceed *via* a chair-*boat*-chair folding, as opposed to the corresponding chair-*chair*-chair folding in the case of euphol. This proposal – convincing as it seemed, once it had been made – boosted our confidence in the existence of a comprehensive solution of the problem, one that would cover all stereochemical details of all rings in all tetra- and pentacyclic triterpenes.

The final key to this ambitious goal was a mechanistic hypothesis by *A. E.*, conceived specifically to delineate the stereoelectronically preferred course of stereospecific *Wagner–Meerwein* rearrangements or 1,2-H shifts connecting a given nonclassical carbocation of defined configuration with a constitutionally isomeric nonclassical carbocation of again defined configuration, a stereochemical problem with no known precedent at the time[13]). Armed now with a complete set of mechanistic postulates, together with the principle of enzymic control on the chair/boat folding dichotomy (see Schemes 2 and 3 in the 1955 *Helvetica Chimica Acta* paper), we were fortunate in arriving eventually at a comprehensive and mechanistically consistent scheme that encompassed the constitution as well as configuration of all cyclic triterpenoids known in 1955. In restropect, this accomplishment appears to have become possible by the lucky temporal and spatial coincidence of complementary knowledge, competence, and ambition of two young, enthusiastic students of *Ruzicka*, who had the good fortune to come of age scientifically in an academic environment as exciting as that created at the ETH by that extraordinary natural product chemist.

3. Comments on the Terminology and the Content of the 1955 Paper. – In commenting on the paper from today's vantage point, what first needs clarification is the 1955 terminology regarding some aspects of the stereochemistry and the mechanism of the organic and (hypothetical) enzymic reactions discussed in the paper. Unfortunately, the terms '*structural formula*' and '*constitutional formula*' were used in a way (see the

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[12]) October 29, 1954, at a time when the other of the present authors happened to be serving in the Swiss army.

[13]) See Scheme 3 in [3]. The stereoelectronic reasoning behind this postulate required a *concerted* transition leading from one nonclassical carbocation into its constitutionally rearranged counterpart to choose the path that corresponds to an S_N-type *inversion* by the moving substituent as nucleophile, implying a 60° rotation around the π-bond axis, as against the 120° rotation resulting in *retention*. If the overall process were to proceed as an overall *substitution* (reaction of an electrophile with the double bond to form a carbocation, 1,2-rearrangement of this cation, and reforming an olefin by proton elemination) instead of *addition*, then the very same stereoelectronic reasoning predicted that such an (electrophilic) substitution will occur with *retention.* This is in agreement with experimental evidence on the stereochemistry of S_E-substitutions at double bonds that has become available later (for examples, see [49]).

paper's Footnote 5) that will mislead the contemporary reader. The term '*constitutional formula*' implied not only what we mean today by '*constitution*', but also the configuration (relative and absolute), whereas '*structural formula*' was meant to imply only what today is '*constitution*' (atom connectedness). This use of the term '*constitution*' in the 1955 paper appears today especially confusing by the title of the main chapter '*Derivation of the Constitutional Formulae of Cyclic Triterpenes from Squalene*', whereas the main message of the chapter concerns the *configuration* of the triterpenes (besides their constitution).

Another term that requires clarification is '*constellation*'. The word rings a bell to remind us of the very beginnings of stereochemical reasoning beyond configuration in organic chemistry. In his 1948 Centenary Lecture on the chemistry of medium-sized rings [50], *Vlado Prelog* used the term '*constellation*' for what *Derek Barton* in his 1950 landmark paper [44] called '*conformation*'[14]). The *Prelog–Barton* agreement that canonized the use of '*conformation*' in place of '*constellation*' came after 1955, and this explains why an ETH paper written before that agreement had to use '*constellation*' for what has been referred to as '*conformation*' ever since.

Finally, there is a term pointedly used in the 1955 paper that demands a detailed comment in view of the serious misinterpretations it has given rise to (perhaps partly because the paper had been written in German) in the subsequent chemical and biochemical literature: the term '*nonstop reaction*'[15]). It stands for the central point of the fourth of the basic premises adopted as framework of mechanistic constraints within which the relations between squalene, and the constitution and configuration of the cyclic triterpenes were to be formulated. The four premises were set up primarily to achieve internal consistency in delineating these relations, and were meant only in the second place to represent actual working hypotheses for the biosynthesis of the cyclic triterpenes. We clearly remember our need to coin a new term in this context in order to avoid the already existing term '*concerted reaction*' (proceeding *via* a single transitions state) for (enzymic) reactions, which we firmly believed *not* to be concerted processes, even though their stereochemical outcome might be indistinguishable from that one would expect, if the reactions were '*concerted*' in the organic chemist's sense. As used in the 1955 *Helvetica Chimica Acta* paper, the term '*nonstop reaction*' referred to a process in which '*no stable compounds that could arise through saturation (hydration, H-elimination) of the cyclizing molecule's positive charge should be formed as intermediate*' and clearly did *not* imply that no reaction intermediates (in the sense of a text book reaction-coordinate/energy representation) would be formed; on the con-

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[14]) *Barton*'s choice of the term '*conformation*' rests on a 1929 paper of *Haworth* [51] and *Prelog*'s term '*constellation*' relates back to *K. Freudenberg*'s classical treatise '*Stereochemistry*' of 1933, in which *F. Ebel* authored a chapter on '*isomers*' deriving from rotation about C–C bonds [52]. In two articles written in 1956, one by *Barton* [53a] and the other by *Prelog* [53b] in *Todd*'s '*Perspectives in Organic Chemistry*' (dedicated to *Robert Robinson* on the occasion of his 70th birthday), the two authors were still clinging to their choices.

[15]) To quote (anonymously) but a few examples of persistent misunderstanding: *a*) '*the idea has been widely entertained that the cyclization step is concerted (nonstop), i.e., without the intermediacy of mono-, bi-, or tricyclic carbocations*'; *b*) '*the important conclusion that the transformation of squalene to lanosterol is a concerted or nonstop process*'; *c*) '*all steps on the route from squalene to the final products would proceed in a nonstop fashion. However the concertedness of the overall ring-forming process is a matter of debate*'; *d*) '*molecular modeling and experiment have cast doubts on the seminal hypothesis that cyclization is a concerted process*'.

trary, the reaction schemes contained in the paper clearly illustrate the assumption of whole sequences of such intermediates in the form of *nonclassical* carbocations, taken to act *nonclassically* in order to conserve the stereochemical information (configurational and conformational) of the ring-forming units throughout the entire cyclization process, and demanding stereochemical unambiguity at each reaction step.

When we read the 1955 paper today, its final conclusion as stated in the paper's summary, according to which the results '*support the squalene hypothesis of the biogenesis of the cyclic triterpenes*', may seem like a rather cautious and deliberately modest way of assessing the paper's significance. In reality, at the time, the opinion of the four authors about the paper was quite different; they were standing in awe, so to say, before the innate chemical harmony and consistency of these astonishing relationships which seamlessly connected the formula of squalene with *all* basic representatives of the families of polycyclic triterpenes known at the time in their full constitutional and configurational detail. Probably never before had there been such an opportunity, namely, without any knowledge whatsoever about the actual enzymes involved, to imagine intimate (hypothetical) workings of (hypothetical) enzymes on a common substrate to such a degree of structural and mechanistic resolution on the basis of the constitutional and configurational information expressed in the chemical structures of a family of natural products, and of translating the structural diversity of that family into a corresponding mechanistic diversity of (hypothetical) biosynthetic pathways. Out of the four basic premises that made this possible, the two that refer to the *conformational control* of the squalene transformations and on the *stereoelectronic control* of the reaction steps undergone by the cationic intermediates were preeminent. They were at the very heart of the reasoning in as far as they gave rise to the insight that the stereochemical puzzle posed by the triad of diastereoisomeric triterpenes (lanosterol, euphol, and tirucallol) finds a convincing solution in the central postulate that the squalene molecule on its way to lanosterol cyclizes in the chair-*boat*-chair conformation (with regard to the first three rings), whereas the pathway *via* chair-*chair*-chair conformation produces the two diastereoisomers of lanosterol, as well as the triterpenes of the lupeol and amyrin family. Finally, these two premises were preeminent because the two main aspects of the analysis that were eventually considered to provide the strongest and most-stringent support for the significance of the overall hypothesis – mechanistic consistency and completeness – could *not* be attained except by strict adherence to those two central premises.

During the two years since 1953, when the constitutional version of the biogenetic isoprene rule covering the mono-, sesqui- di-, and triterpenes had been enunciated, the chemical structures of seven additional polycyclic triterpenes had become known[16]). In 1955, the successful derivation of the constitution and configuration of 13 such polycyclic triterpenes had convincingly consolidated the validity of the basic postulates of the biogenetic isoprene rule not only for the triterpenes, but for all other terpene families as well[17]).

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[16]) Among them, the most important newcomers, besides euphol and tirucallol, were *E. J. Corey*'s friedelin [54] and *J. S. Mills*' dammarene diol [55], see Footnote 32 in the English translation of [3].

[17]) In its attempt to add stereochemistry to the biogenetic isoprene rule, the 1955 *Helvetica Chimica Acta* paper was restricted on purpose to the cyclic triterpenes not only because at that time this family of terpenoids

It seems proper for a perspective on the 1955 paper to finally touch on the paper's cautious comment on the mechanistic role of the enzymes in squalene cyclizations, especially since, in recent years, this question has gained momentum in current fields of biostructural research, such as protein crystallography [7][8] and computational chemistry [58]. In the organic chemistry of the early 1950s, the phenomenon of the acceleration of electrophilic dissociation processes at reaction centers of nucleophilic substitutions through *anchimeric* assistance by neighboring C=C bonds (through-space bonding to the incipient carbocation center) was already known [59]. By connecting this phenomenon to the squalene problem, it was argued in the 1955 paper that a squalene folded by an enzyme towards the shape of its cyclization products should be expected to cyclize *per se* faster than a squalene in solution that could be assumed to exist mainly in the thermodynamically more-stable stretched conformation. Therefore, the enzyme-induced folding of the squalene molecule should be a process that amounts to an activation of the squalene substrate towards cyclization. In the light of recent theoretical treatments of this aspect of the squalene cyclization problem [58], that early conceptual glimpse into the role of the cyclases has certainly not become obsolete[18]).

4. The 1955 Paper of *Stork* and *Burgstahler*. – Whereas the 1955 *Helvetica Chimica Acta* paper has its roots in decades of work on the structure determination of terpenes by the *Ruzicka* school and, more directly, in the theoretical developments that induced the metamorphosis of the empirical into the biogenetic isoprene rule, the 1955 *J. Am. Chem. Soc.* paper entitled '*The Stereochemistry of Polyene Cyclizations*' by *Stork* and *Burgstahler* [5][19]) originated in *Stork*'s early and, for that time, pioneering interest in the stereochemical course of organic reactions in the context of organic natural-products total synthesis. This foresighted focussing on an aspect of synthetic chemistry that, up to the late 1940s, had been almost completely ignored by the practitioners of organic natural-products synthesis, led *Stork* to become aware of the significance of *Linstead*'s early studies on the formation of decalin derivatives by acid-catalyzed 1,5-diene cyclizations [60], especially of the formation of almost exclusively *cis*-decalin derivatives in the cyclization of a 1-methylbut-2-enylcyclohexene substrate under mild conditions. A paper published in 1951 together with *Conroy* [61] in the context of a problem in alka-

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happened to be in the limelight of attention as a consequence of its biogenetic relationship to cholesterol, but also because the diversity, volume, and depth of the stereochemical information available in this series was much greater than, for instance, in the sesquiterpene series. It was *J. B. Hendrickson* [56] who, a few years later, first applied and extended the stereochemical analysis to the (outwardly simpler, but actually more complex) family of sesquiterpenoids. The problem of stereochemistry in sesquiterpene biosynthesis was taken up experimentally by *D. A.* [57] and later by *Cane* [9c][19].

[18]) How loose the ground for a discussion of the role of the (unknown) enzymes in the (hypothetical) cyclizations to the polycyclic triterpenes actually was at that time, is documented by the existence, in the 1955 paper, of the paragraph on a possible role of a decadehydrosqualene, a paragraph plainly obsolete today.

[19]) *Stork*'s paper has the priority of submission as well as publication date (March 17 and October 5, *vs.* October 13 and December 15 for the *Helvetica Chimica Acta* paper). At the time of submission of the latter, the ETH authors had no knowledge of the *J. Am. Chem. Soc.* paper (in 1955, a *J. Am. Chem. Soc.* issue would not reach the shores of Europe before about a month after its publication); however, they mention the publication of the *J. Am. Chem. Soc.* paper in a footnote *Added in Proof* (see Footnote 15 in the translated paper).

loid synthesis is the first documentation in print of *Stork*'s special attention to stereoselection in polyene cyclization. This paper gives an explicit mechanistic interpretation of *Linstead*'s case of *cis*-diastereoselection by postulating it to be the result of a *concerted* trans-*addition* of the initiating proton and the exocyclic C=C bond to the substrate's endocyclic olefinic bond. Although this paper does not refer in any way to terpenes, it contains the nucleus of *Stork*'s central mechanistic idea on the stereochemistry of 1,5-diene cyclizations that eventually led to his 1955 *J. Am. Chem. Soc.* paper.

The first documentation of *Stork*'s early involvement in the problem of polyene cyclization stereochemistry exists in the form of an abstract of a seminar given at the Harvard Chemistry Department on March 14, 1950[20]). There, *Stork* summarized *Linstead*'s cyclizations, interpreted the preference for formation of a *cis*-decalin as the result of a '*concerted*' *trans*-addition to the substrate's endocyclic C=C bond, extended this view to hypothetical cyclizations of terpenoid 2,6,10-trienes, and suggested that, if such a triene cyclization were to proceed by a 'concerted' mechanism, the resulting decalin derivatives would have the *trans*-configuration. The last sentence in the abstract, '*the significance of this conclusion will be discussed*', points to what *Stork* today considers an essential part of his message delivered in that seminar, namely, the possible relationship between such cyclization stereochemistry and the ring-junction stereochemistry of polycyclic triterpenoids[21]). What also belongs to the nonprinted preliminaries of *Stork*'s 1955 *J. Am. Chem. Soc.* paper is *Burgstahler*'s 1952 thesis [62], in which an extensive attempt was made to experimentally corroborate by model studies *Stork*'s views on the the stereochemical course of terpenoid polyene cyclizations.

Outwardly, the discussion of the experimental results of the *Burgstahler* thesis constitutes the major part of the 1955 *J. Am. Chem. Soc.* paper. Yet it may appear as if the publication of these results had to provide the opportunity to finally put into print the major message of *Stork*'s 1950 seminar concerning the basic hypothesis on the stereochemistry of polyene cyclizations, and of the relationship between this stereochemistry, and that of polycyclic triterpenoids and steroids[22]). This message needed to be and was indeed updated in the light of what had been published by others since then, namely, the chemical structure of lanosterol [22], the paper of *Woodward* and *Bloch* [24], and the 1953 chapter in *Experientia* [12b] on the biogenetic isoprene rule in which the concept of the oxidative cyclization–rearrangment cascades connecting in mechanistic detail the constitution of squalene with that of polycyclic triterpenes had been published. *Stork* in his 1955 paper took up that mechanistic scheme, extended it with

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[20]) *A. E.* thanks Prof. *Stork* for providing a facsimile copy of the abstract of this seminar as well as for an extended recent e-mail exchange in which the two correspondents jointly attempted to reconstruct the events of the early fifties.

[21]) In a presentation analogous to that appearing in *Woodward* and *Bloch*'s 1953 paper [24], *Stork*'s seminar abstract depicts a terpenoid 2,6,10-triene formula as a hypothetical cyclization substrate. No allusion to an oxidative initiation of the cyclization is made. It is fair to add that pondering about squalene cyclization by using this formula perspective was at that time equally done at the ETH [15][16] (see also *Footnote 8*) and probably elsewhere, however (up to 1953/54 [46]) *without* referring to stereochemistry.

[22]) The proposal of such a relationship between the stereochemistry of polyene cyclization and the stereochemistry of polycyclic triterpenes was put into print for the first time in the 1954 *Helvetica Chimica Acta* paper [46]. To the best of *A. E.*'s recollection, at that time the authors of the paper had no knowledge of either *Stork*'s 1950 Harvard seminar, or *Burgstahler*'s 1952 thesis.

a configurational notation, and, importantly, recognized the necessity of differentiating between two configurationally distinct pathways, one leading to the lupeol-type of triterpenes, the other to lanosterol and (implicitely) cholesterol. As solution to this problem, he proposed that the configuration of lupeol is compatible with an altogether '*concerted*' course of the oxidative squalene cyclization, whereas the pathway to lanosterol requires the flow of '*concerted*' cyclizations to become interrupted after the formation of ring B by an intermediate that behaves as a classical carbocation. In the light of the 1955 *Helvetica Chimica Acta* paper, this proposal amounts to a substitute for the ETH solution of the problem of lanosterol's stereochemical uniqueness, based on the concept of the chair-boat dichotomy in polyene cyclization. The basic concept, according to which the stereochemical outcome of a 1,5-diene cyclization depends on whether the reaction proceeds *via* chair or boat folding, was not part of *Stork*'s approach to the problem[23]).

As already indicated above, the major part of the 1955 *J. Am. Chem. Soc.* paper was devoted to a detailed description and extensive discussion of the experimental work that had been undertaken '*to determine to what extent concerted polyene closures to* trans-*hydronapthalene systems could be carried out experimentally*'. Prime model substrates for their attempt were *trans-trans*-farnesic acid and its mono-cyclized isomers containing their endocyclic C=C bond in the tetrasubstituted position and the exocyclic (conjugated) C=C bond in the *trans*- and the *cis*-arrangement, respectively. In timing and content, the work more or less paralleled the systematic investigations of *Caliezi* and *Schinz* [14], on farnesic acid cyclization, as well as to some extent work of *Lederer* and co-workers [63], except that these groups did not address the stereochemical problem involved. Later, it became clear [64], through the work carried out at the ETH during 1954–1957 on the stereochemical course of the acid-catalyzed cyclization of three (out of four) diastereoisomeric 3-demethylfarnesic acids [47][65], that a major part of the conclusions on the stereochemistry of cyclizations in the farnesic acid series, as described in the 1955 *J. Am. Chem. Soc.* paper, was invalid, because of an error in a configurational assignment[24]). Nevertheless, in retrospect *Stork*'s study on the cyclization stereochemistry in the farnesic acid series is to be seen as a pioneering experimental effort to shed light on the stereochemistry of a reaction type that, since the beginning of the 1950s, was considered by him to be of central biological relevance. With the benefit of hindsight, it may be said today that the attempt to draw conclusions on the degree to which *in vitro* cyclizations of the farnesic acids proceed as concerted reactions by determining the configuration of the products was bound to be abortive, because later evidence has indicated that the stereochemical outcome of such cyclizations, carried out under the conditions used at the time, was most probably the result of steric, rather than stereoelectronic, reaction control [47][64][65]. In these genuinly terpenoid model substrates, in which the C=C bonds bear the full number of Me substituents, a *trans*-decalin configuration in the cyclization products can result not only from a '*con-*

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[23]) As evidenced on p. 7 of *Burgsthaler*'s thesis [62], depicting a 1,5,9-triene cyclization process as occurring *via* a chair-chair conformation had been familiar to the authors of the 1955 *J. Am. Chem. Soc.* paper, even though they do not make use of it in the paper.

[24]) All three isomeric *rac*-bicyclofarnesic acids assigned in [5] to be *cis*-decalin derivatives were subsequently shown to be *trans*-decalin derivatives [64].

certed' reaction path, but also from a stepwise reaction in which intermediate carbocations react under steric control. The same is presumably true also for the second model series described in the 1955 *J. Am. Chem. Soc.* paper, the farnesylacetic and the monocyclofarnesylacetic acid(s).

About a decade after the 1955 Columbia and ETH papers had appeared, *W. S. Johnson* at Stanford started his pragmatic, comprehensive, and brilliantly successful work on the reaction type of acid-catalyzed polyene cyclization as a tool in organic synthesis. It was in his hands that research on the constitutional as well as stereochemical potential of this type of reaction for organic synthesis came to fruition and reached its climax. His earlier writings [66] clearly attest the inspiration he felt to have received for this project from *Stork*'s pioneering assault on the stereochemical problem of polyene cyclization, and from the work done at ETH in this field. It was *Johnson* who started to propagate in this context the term '*Stork–Eschenmoser* hypothesis' [66][67], a short-hand term that has irreversibly entered the literature and, over the years, became more and more identified with the messages contained in the two 1955 papers. The present authors have always felt that this identification makes the short-hand term too short-handed in the sense that both the ambition and the content of the 1955 *Helvetica Chimica Acta* paper went far beyond what the term was supposed to imply. Originally, the '*Stork–Eschenmoser* hypothesis' may have meant (in modernized terms) the following: when an acid-catalyzed polyene cyclization proceeds under stereoelectronic control (either as a '*concerted*' process or, what is stereochemically equivalent, as a stepwise process *via* carbocationic intermediates that retain stereochemical information), then the stereochemical outcome will correspond to stereospecific *trans*-additions at C=C bonds. When extrapolated to the cyclization of all-*trans*-polyolefinic isoprenoids, such a steric course produces the *trans-anti-trans*-configuration characteristic of the structure of the natural polycyclic terpenoids and steroids. *Johnson*'s short-hand term may be said to point correctly to the fact that the two named chemists recognized this crucial relationship between the stereochemistry of polyene cyclization and that of polycyclic triterpenoids independently of each other. As far it is known, *Stork* was the first to have thought and spoken about it, while his colleagues at ETH happened to precede him in print[25]).

5. An Updated Evaluation of the 1955 *Helvetica Chimica Acta* Paper. – In the 50 years which have elapsed since the original ETH paper was first committed to print, the number of triterpene prototypes derivable from squalene has increased dramatically from 13 to *ca.* 100 [9f], and an impressive and still expanding body of chemical and biochemical experimental evidence has accumulated concerning the detailed mode of biological formation of these compounds (for recent reviews, see [9]). Among the milestone contributions which accelerated the progress in this area one should mention: *i*) the discovery of mevalonic acid by *Folkers* and co-workers [68][26]) and the demonstra-

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[25]) *Johnson* was doubtlessly informed about the 1950 Harvard seminar, yet apparently had not been aware of the 1954 *Helvetica Chimica Acta* paper [46] (as judged from the citations in his writings).

[26]) The same compound, dubbed '*hiochic acid*', had been isolated shortly before as a growth factor for *Hiochi* bacteria from the broth of *Aspergillus oryzae* by *Tamura* [69], who was misled by a positive iodoform reaction (!) in assigning to it the structure of a 3,5-dihydroxyhexanoic acid.

tion of its central role in the biosynthesis of cholesterol [70], followed by the determination of its absolute configuration [71], which provided the background for the complete deciphering of the cryptic stereochemistry of the biochemical sequence which leads from mevalolactone to IPP and DMAPP [72]; *ii*) the advent, in the early 1970s, of ^{2}H- and ^{13}C-NMR techniques, which allowed incorporation studies with stable isotopes freed of the necessity of carrying out lengthy and substance consuming degradative work for localization of the label in the product(s) of the reaction; *iii*) the biotechnological revolution which, starting in the late 1980s, enabled the cloning and sequencing of specific cyclase genes and the expression and purification of the encoded proteins as well as the creation of mutants for mechanistic studies; *iv*) the late and surprising discovery of an alternative, mevalonate-independent pathway of biological access to IPP and DMAPP, the two universal C_5-units of terpene biosynthesis (for a comprehensive review, see [9h]). In this section, we shall attempt, in the light of these advances, a critical evaluation of the extent to which the postulates and predictions of the 1955 *Helvetica Chimica Acta* paper have withstood the test of time.

Because of the pivotal role played by lanosterol as a precursor of the biologically important cholesterol, details of its biosynthesis have been scrutinized with particular intensity over the years, and it seems appropriate to give precedence to this compound in our analysis (*cf.* Scheme 6 in [3]). Shortly after the publication of the *Helvetica Chimica Acta* paper, *Tchen* and *Bloch* submittetd two of its basic tenets to experimental tests and demonstrated that no deuterium is incorporated into lanosterol when the incubation of squalene with a crude enzyme system is carried out in D_2O, and that the OH group of the product is derived from molecular O_2 rather then from the solvent [73]. While not excluding for the cyclization reaction the formation of stable intermediates due to the *reversible* quenching of ionic entities with a nucleophile postulated later on by *Cornforth* [72], the first result is in keeping with the postulate of a nonstop reaction inasmuch as it rules out the operation of intermediate deprotonation/protonation steps[27]). *Bloch*'s substantiation of the concept of an oxidative cyclization prompted *Corey et al.* and *van Tamelen et al.* to investigate the role of 2,3-oxidosqualene as an intermediate in the pathway leading from squalene to lanosterol; experiments with the racemic compound were crowned with success [76a][76b]. It was left for *Barton et al*. to demonstrate that the natural occuring isomer has the (expected) (3*S*)-configuration [77]. A list of triterpenes known to be derived from oxidosqualene is available from a recent review article [9f]. Meanwhile, FAD-dependent enzymes catalyzing the epoxidation reaction have been isolated from yeast, mammalian sources, and, more recently, higher plants (*cf.* [78] and refs. cit. therein).

In the paper by *Woodward* and *Bloch* [24], the question of the detailed rearrangement(s) required for the generation of lanosterol from squalene was left open but for the statement that '*one* or more *methyl migration is necessary at some stage for the construction of the quaternary center at C13*'. In the 1955 ETH paper, a choice was met in favor of a double 1,2- *vs.* a single 1,3-Me migration on the basis of the observation that

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[27]) As pointed out by *van Tamelen* [74], the argument does not apply to the H-atom involved in the concluding proton elimination step. However, the later observation that the critical H-atom is retained in the biosynthesis of cycloartenol, a substitute of lanosterol used in higher plants for the production of phytosterols, militates against this possibility [75a][75b].

the former, but not the latter, was well precedented in triterpene chemistry. Interlocking experimental verification for the validity of this choice was eventually presented by *Bloch* and co-workers [79] and by *Popjak*, *Cornforth*, and co-workers [80]. To date, no evidence has been obtained to support the feasibility of cationic 1,3-transfer of alkyl groups in chemical systems. Proof that the rearrangement cascade which leads to lanosterol is initiated by a double 1,2-hydride shift has been provided by *Barton et al.* [81].

The first indirect but unequivocal confirmation for the correctness of the postulated chair-boat-chair folding of the (oxido)squalene precursor on its way to a nonrearranged tetracyclic ionic intermediate (nowaday refered to as a protosteryl cation) in the formation of lanosterol was obtained in 1965 by the chemical elucidation of the structure of fusidic acid, **1** [82], soon corroborated by the results of the X-ray investigation of an appropriate derivative [83][28]). The subsequent isolation from *Cephalosporium caerulens* of the two isomeric triterpene alcohols **2** [86] and **3** [86b] retaining the intact protosterol C framework fitted even more neatly the 1955 scheme, but a closer look at their structure should have alerted the community of adepts in this area of research that at least one detail of the original picture might require revision. The conformation of the tetrasubstituted C=C bond of **2** defines the frozen conformation of the protosteryl cation from which it is derived by loss of the H-atom at C(17), and this, together with the presumed (20*R*)-configuration of **3** (supported later by the ability of a microsomal preparation from the fungus to catalyze the parallel formation of lanosterol and **2** from oxidosqualene [86b]), is easily explained by assuming a 17β-configuration of the side chain of **4**, a detail which is at variance with the 17α-configuration postulated in the original scheme. This encoded message escaped everybody's attention (including, alas, the younger author of the present paper). As a consequence, the original credo remained unchallenged for 22 additional years, until in an important piece of work *Corey* and *Virgil* eventually provided at first strong support and then incontrovertible evidence for the 17β-configuration of the protosteryl cation **4** [87][88]. In short, incubation of an oxa analogon of the natural substrate with a yeast lysate containing lanosterol synthase as the sole oxidosqualene cyclase led by quenching of the enzymically generated oxonium ion to the formation of compound **5** [87], whose structure was secured by correlation with a synthetic sample of known configuration. This result was next complemented by a second experiment [88], in which a modified form of the natural substrate containing an additional strategically placed double bond was similarly quenched upon incubation with the enzyme to provide a protosterol derivative unequivocally identified by comparison with an authentic specimen obtained by chem-

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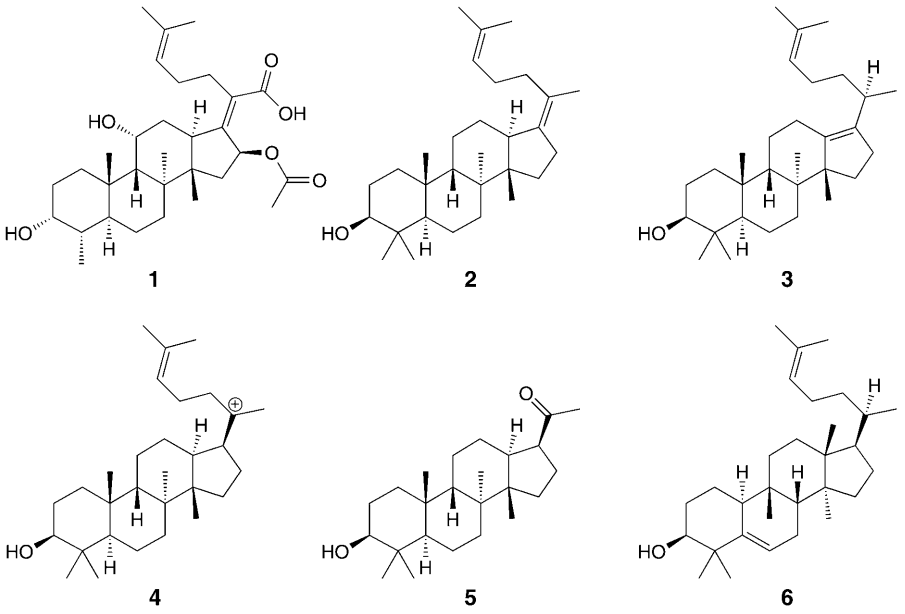
[28]) This work paved the way for the structural elucidation of two closely related compounds, cephalosporin P_1 and helvolic acid [84a][84b]. Historians of science will be interested to learn that crystals of the latter compound and its methyl ester had been submitted in 1943 (presumably by a member of the *Chain–Florey* group) to *Dorothy Crowfoot* for structural studies. After a preliminary cristallographic characterization [85], the problem was assigned a low priority and set aside (private communication of *D. Crowfoot Hodgkin* to *D. A.*, 1964). How the chemical community would have reacted to an early disclosure of the structure of this compound, and which impact it might have exerted on the ongoing studies of cholesterol (and triterpene) biosynthesis remains a matter of speculation. The story is nevertheless a good example for the haphazard nature of the rules which govern the rate of scientific progress.



ical synthesis[29]). In terms of the terminology of the 1955 ETH paper, this result requires that in the cyclization process which leads from (oxido)squalene to lanosterol ring D is formed from a chair rather than from the postulated boat folding of the aliphatic precursor; moreover the double 1,2-hydride shift which initiates the backbone rearrangement cascade is now seen to represent a *syn*-process, in clear violation of the rule according to which successive 1,2-rearrangements require an antiperiplanar rearrangement of the rearranging groups. A similar *syn*-rearrangement was implicitly required for the formation of the *cis*-B/C ring junction of the oxidosqualene derived cucurbitadienol **6** [89], and an analogous precedent had already been detected as early as 1968 during studies on the biosynthesis of the diterpene pleuromutilin [90]. Such deviations from the original credo are best interpreted *a posteriori* by assuming, *for this part of the biosynthetic sequence*, the involvement of (classical) carbocations deprived of stereochemical memory, made unnecessary by the fact that the stereochemical information for the multiple 1,2-rearrangements is already engraved in the ionic product of the cyclization steps.

In recent years, genes encoding lanosterol synthases have been cloned from many sources, and the corresponding enzymes exploited intensively for mechanistic studies involving substrate analogues as well as potential inhibitors (for extensive reviews,

[29]) We note in passing that the structure of the products resulting from these successful quenching experiments disposed at last of an alternative biosynthetic proposal by *van Tamelen et al.* [74], in which the first-formed C(20)-centered tetracyclic cation on the way to lanosterol had an unusual spirane partial structure. This somewhat cumbersome looking scheme, compatible with all the experimental evidence available at the time, was, in the main, devised to circumvent the '*psychological barrier*' of accepting an *anti-Markovnikov* mechanism for the formation of ring C.

see [9f][9e]). A milestone of this research is represented by the crystallization and subsequent X-rays investigation of the enzyme from human sources [8b]. In the context of the present discussion, it is important to note that, in the complex formed by saturation of the enzyme with lanosterol, the side chain of the latter adopts the stretched conformation predicted by the lack of participation of its isopropylidene unit in the cyclization process.

All the known triterpene prototypes, for which hypothetical pathways of biological access were outlined in Scheme 4 of [3], had been isolated from plant sources. The first experimental verification that formation of such compounds was governed by the same rules which controlled the biosynthesis of lanosterol came from a study, in which it was shown that (2-^{14}C)mevalonic acid was efficiently incorporated by germinating soya beans into soyasapogenols, a set of oxygenated derivatives of β-amyrin [91]. The fortuitous presence of a 1,3-diol group in ring A of these compounds could be exploited for a simple degradation, which indicated that no radioactivity of the labeled material was associated with the axially oriented CH_2OH group at C(4), whence it was concluded that *i*) the radioactive label of the precursor had been incorporated stereospecifically into the terminal (*E*)-Me groups of the (then putative) squalene intermediate[30]) and *ii*) formation of the A ring of the prototype triterpene was indeed requiring a chair folding of the aliphatic substrate.

A critical hindsight evaluation of Scheme 4 must begin with the case of tirucallol. This compound differs from lanosterol in the antipodal configuration of the four stereogenic centers C(13), C(14), C(17), and C(20); in view of the necessary revision discussed before for their formation in the lanosterol case, the problem can now be easily solved by postulating an antipodal chair folding of the oxidosqualene precursor in the formation of ring D of tirucallol. We note in passing that, within the rules of the game, the same chair conformation is a prerequisite for the formation of a large number of triterpenes not yet known in 1955 [9f]. Euphol differs from tirucallol only in its (20*R*)-configuration and, in compliance with the 1955 proposal, this difference can now be easily correlated with the conformational difference (boat *vs.* chair) in the fold of the precursor segment involved in the formation of ring D of the two diastereoisomers[31]).

Because of the complications associated with the postulate that two successive rearrangements of the main chain of the precursor are necessary for the biological formation of β-amyrin, studies on the biosynthesis of this compound have attracted for decades the attention of several research groups. Rewardingly, the actual situation can be summarized by the statement that every attempt to falsify the 1955 scheme has consistantly met with failure. The fragmented experimental evidence which has accumulated over the years has been reviewed [9e]. For completeness, let us recall that the specific origin implied by the Zurich scheme for the Me groups at C(20) of β-amyrin has been

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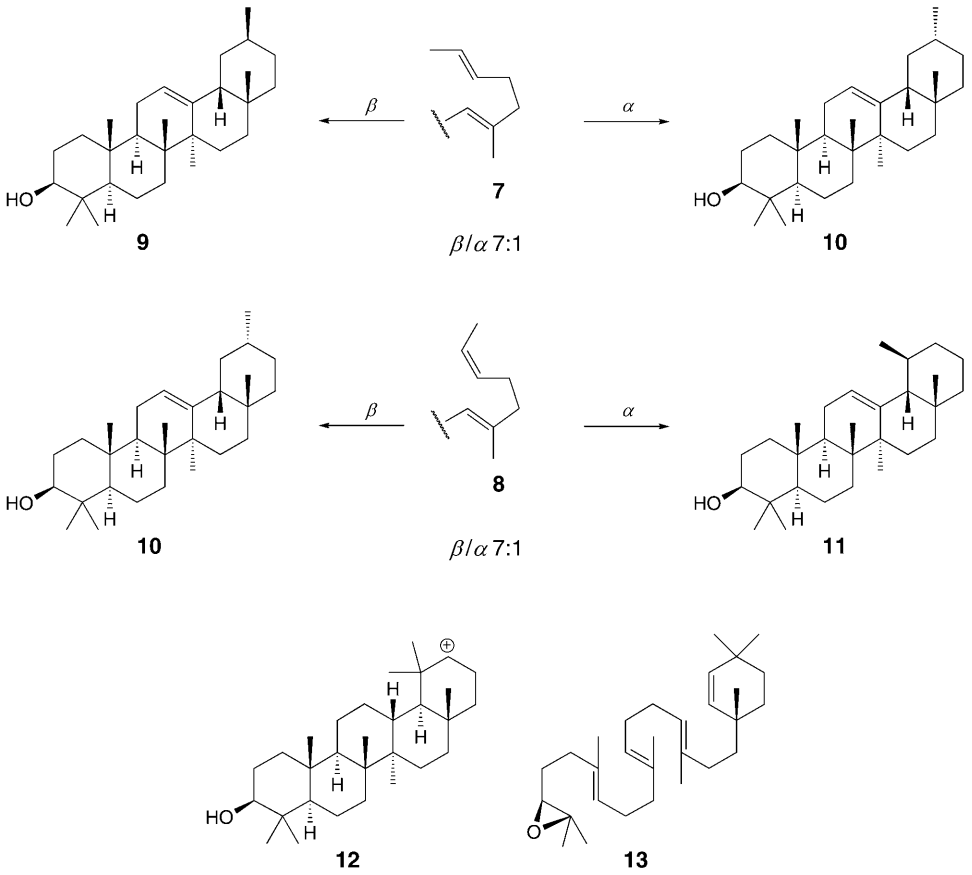
[30]) Later work disclosed that this Me group is generated in the (reversible) isomerization which intercoverts IPP and DMAPP, the two universal C_5-building blocks of terpenes (for a recent review, see [92]). The lack of fidelity of the corresponding enzyme has been made responsible for the partial scrambling of label occasionally observed in experiments with labeled substrates, but knowledge of the above relationship has nevertheless been resorted to routinely for the solution of specific problems in studies of terpene biosynthesis.

[31]) To date, no evidence is available for the existence of a (20*S*)-lanosterol counterpart of euphol.

verified in two different ways *i*) feeding of CD_3-labeled mevalolactone to an extract from *Pisum sativum* resulted in the production of a specimen of β-amyrin carrying a CD_3-group in α-position at C(20) [93] (as confirmed by the partial synthesis of an authentic specimen [94] and expected on the basis of the correlation discussed in *Footnote 30*), and *ii*) feeding of the (*E*)- and (*Z*)-noroxidosqualenes **7** and **8** generated the diastereoisomeric nor-β-amyrins **9** and **10**, respectively [95]. Following the demonstration that the pea seedling β-amyrin cyclase is capable of cyclizing the bisnor form of oxidosqualene to 20-bisnoramyrin, *Corey* and *Gross* proposed that the normal cyclization process might involve the intermediacy of the ion **12** [96], and thus bypass the second ring rearrangement invoked by the Zurich postulate (*cf.* XVI → XVII in Scheme 4 of [3]). Such a scheme is at variance with the results of subsequent investigations, in which it was shown by the use of doubly ^{13}C-labeled acetic acid that formation of the E-ring of α-amyrin is associated with a reshuffling of the initial bond sequence of the ring-forming chain [97]. We round up this paragraph by noting that recent efforts by *Matsuda* and co-workers with the purified lupeol synthase of *Arabidopsis thaliana* have confirmed the boat-shape folding postulated for the formation of ring D during lupeol (and hence β-amyrin) biosynthesis by quenching of the ionic intermediate and identification of the product, a stereoisomer of **5**, in experiments carried out with an oxa analogon of the natural substrate [98] along the lines pioneered by *Corey* and *Virgil* for the lanosterol case.

At the time of the writing of the *Helvetica Chimica Acta* paper, a number of questions concerning the structure of ring E in α-amyrin were still open. The remaining gaps were filled first through the identification of a monocyclic degradation product, which confirmed the six-membered nature of this ring and allowed the determination of the (20*R*)-configuration of the starting material [99], and soon thereafter by a partial synthesis of the triterpene from glycerrhetic acid [100], which confirmed the correctness of the *Corey–Ursprung* proposal (*cf.* XXVI in Scheme 5 of [3]). According to the sequence proposed in 1955, formation of this structure proceeds from the same ionic intermediate (XVIII in [3]), which is involved in the biosynthesis of β-amyrin and requires a specific 1,2-migration of its axially oriented α-Me group at C(20). The mechanism for the formation of ring E was shown to match the one already demonstrated in the β-amyrin case [97], and a clear-cut validation for the proposed origin of the Me group at C(19) was provided by showing that incubation of the (*Z*)-nor form **8** of oxidosqualene with a *Pisum sativum* extract, known to cyclize the normal substrate to a *ca.* 7:1 mixture of β- and α-amyrin (*Scheme*), resulted in the formation of 30-nor-α-amyrin, **11**, while under the same conditions the (*E*)-nor compound **7** was cyclized to 19-nor-α-amyrin **10** [95] (indistinguishable from the compound generated from **7** by the β-amyrin sequence!). Support for the suggestion that biosynthesis of both amyrins proceeds through the same germanicyl cation (though not necessarily bound to the same enzyme) was obtained by showing that upon feeding to the *P. sativum* system one of the components of the mixture of (tritiated) stereoisomers represented by **13** underwent cyclization to give a *ca.* 2:1 mixture of β- and α-amyrin [101]. One additional feature of α-amyrin biosynthesis deserves specific mention: interlocking evidence obtained for two different biological systems has revealed that, whereas in the case of β-amyrin formation, the process is terminated by the (expected) *anti*-elimination of the axial H_α–C(12), the final step in the formation of α-amyrin involves the anom-

Scheme

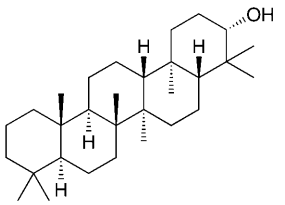


alous specific *syn*-elimination of an H-atom from the equatorial 12β-position [102a][102b]. This additional violation of the antiplanarity rule is most probably a consequence of the unduely large steric hindrance exerted by the Me group at C(19) on the back-side approach of the necessary base.

In 1955, a single example of naturally occurring tetra- or pentacyclic 3-desoxy-triterpenes was on record [3]. In the meantime, the number of such compounds has grown explosively in the literature and the recent review of *Matsuda* and co-workers [9f] accounts for no less than 57 representatives, including a number of new prototypes, isolated from bacterial sources, protozoa, and lower plants, such as ferns and mosses, but occasionally also from angiosperms. They all share a squalenoid origin, and their formation is mediated by squalene cyclases (SCs) which are capable to initiate the cyclization by direct protonation of the hydrocarbon substrate. As a result of the unbending and pioneering efforts of the *Poralla* group, research in this specific area of terpene biosynthesis culminated in the isolation, purification, and crystallization of the squalene-hopene cyclase, and thus paved the way for a succesful X-ray investigation,

which revealed for the first time the intricate structural details of a triterpene cyclase [7]. Later, this work was complemented by the analysis of a complex formed by saturation of the enzyme with its inhibitor 2-azasqualene, which provided welcome ocular evidence for the advanced folding of the natural substrate in the active groove of the protein [8][32]). While biosynthesis of the desoxytriterpenes turned out, not surprisingly, to be governed by the same rules which, in their slightly updated version, are by now known to control the formation of the 3-hydroxy counterparts, two specific sets of results deserve appropriate mention in the context of our discussion.

In an early investigation on the biosynthesis of tetrahymanol (**14**), in the protozoan *T. pyriformis*, the *Caspi* group demonstrated that during the cyclization process of the squalene precursor in D_2O label from the solvent is incorporated exclusively in the 3β-position of the product, and that the OH group in the other terminal ring of **14** is derived from a H_2O molecule (rather than from dioxygen) and thus represent the outcome of an (*anti-Markovnikov*) addition to the last double bond of the cyclizing chain [104]. The beautiful simplicity of this case, with its evident requirement for an all-chair folding of the aliphatic substrate, is a good testimonial for the general validity of the major postulates of the 1955 proposal. Subsequent work by *Ourisson, Rohmer*, and co-workers established that the tetrahymanol synthase can cyclize the naturally occurring (3*S*)-form of oxidosqualene to a 3β-hydroxy derivative of **14**, and, more surprisingly, that the (3*R*)-enantiomer is also accepted as a substrate by the enzyme and processed to the 3α-epimer of the previous diol [105]. As cleanly demonstrated in experiments with specifically ^{13}C-labeled samples of the two oxidosqualenes, cyclization of the unnatural enantiomer is achieved by binding of the substrate in a ring A boat rather



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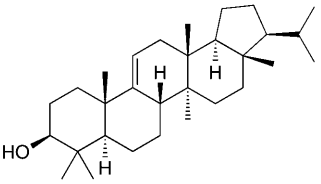

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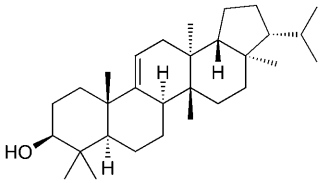

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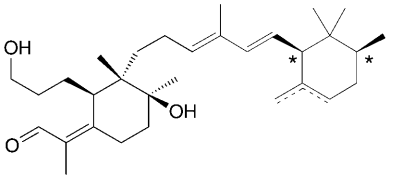

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[32]) In the language of the authors, the observed folding was stated to agree with the '*common text book presentation*'. The record was graciously put straight by *Poralla* [103] in his lucid commentary of this work.

than in the normal chair conformation, *i.e.*, in the conformation which is avoided in the cyclization of the normal hydrocarbon substrate[33]).

6. Concluding Remarks. – The prerequisite of an appropriate folding of the aliphatic precursor postulated in 1955 for the specific generation of tetra- and pentacyclic triterpenes has met with wide acceptance, and a critical perusal of the exhaustive article of *Matsuda* and co-workers [9f] confirms that all the new prototypes discovered eversince comply in a rewarding manner with the general scheme. Accordingly, diversity in the formation of polycyclic ring systems is governed, in the first place, by the different sequences of chair and boat local conformations imposed by the enzyme on the aliphatic precursor. Even when taking into account the fact that the (relative) conformation of the precursor segment corresponding to ring A of all polycyclic triterpenes is irrelevant for the formation of a specific cyclization product, analysis of the material collected by *Matsuda* and co-workers [9f] indicates that the potential of the residual four-digit signature has most probably not been exploited exhaustively in the course of evolution. Some trends are nevertheless clearly recognizable. Bacterial SCs seems to be able to accept exclusively a *c-c-c-c-x*[34]) folding signature of the precursor; a major evolutionary jump occurred when aerobic organisms learned how to deal with the *c-b-c-c-s* folding pattern of oxidosqualene, thus paving the way which eventually led, *via* lanosterol, to cholesterol and hence to the modern panoply of steroidal compounds. Up to date, no case of the exploitation of a *x-x-b-x-x* signature is on record, whereas variations of the last two signature digits are widespread among lower and higher plants.

The 1955 postulate according to which formation of polycyclic triterpenes takes place in a nonstop reaction sequence (as discussed in detail in a precedent section of this paper) was challenged in 1968 by *Cornforth* [72], who suggested that at least some of the ionic intermediates in the cyclization sequence may be quenched *reversibly* by a nonspecified nucleophilic group X, so as to allow interimistically large conformational changes of the cyclizing chain, stated not to be compatible with the operation of a nonstop mechanism. Attempts to substantiate this idea in feeding experiments testing the possible intermediacy of dammarandiol and lupandiol in the biosynthesis of β-amyrin have met with failure [81]. Lack of positive evidence reinforces the belief that the expected exothermicity of the quenching step is hardly reconcilable with its postulated reversibility.

One specific feature of lanosterol biosynthesis, the apparent violation of the venerable *Markovnikov* rule implied in the formation of ring C, has been a matter of considerable and still ongoing debate. Similar 'anomalies' turned out to be a recurrent benchmark in the biosynthesis of a large majority of all known tetra- and pentacyclic triterpenes; a typical example is provided by the unrearranged squalenoid structure of tetrahymanol (**14**), formation of which requires three such violations of the rule. Using

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[33]) When the problem of the (3*S*)-selectivity of yeast lanosterol cyclase was revisited by *Corey et al.* [106], it was found that the unnatural (3*R*)-enantiomer was accepted by the enzyme and processed to 3-epilanosterol, albeit only in a rate ratio of 1:50 in comparison with the natural substrate.

[34]) Local conformations of the cyclizing substrate are denotated as follows: *b* = boat *c* = chair *s* = stretched *x* = undefined.

a scheme involving exclusively classical carbocations, *Corey* has suggested that formation of the ring C of lanosterol occurs *via* the intermediary formation of a tertiary cyclopentylcarbinylcation, which *then* rearranges (apparently in an up-hill reaction) to the required secondary cyclohexyl ion, and claimed that this proposal was supported by the experimental outcome of different trapping experiments (for a summary of this work, *cf.* [9e]). Similar arguments have then been adopted in order to explain two such 'anomalies' in the biosynthesis of hopene [107]. *Rajamani* and *Gao* [58], later joined by *Wendt* [9g], have cast doubts on the biological significance of results obtained by using substrate analoga or specific enzyme mutants, and suggested, on the basis of computational model studies, a scheme which bypasses the pitfall of the the five-membered intermediate by invoking a concerted mechanism for the *simultaneous* formation of rings C, D, and E in the biosynthesis of hopene. The 1955 proposal concerning the participation of nonclassical delocalized cationic intermediates remains, in our opinion, an alternative pragmatic way to approach the problem. In addition, the involvement of these intermediates continues to provide a realistic mean for rationalizing in a systematic way the stereochemical outcome of the rearrangement steps which result in a disruption of the original order of atom contiguity. Inspection of the structure of the final products reveals that such rearrangements *invariably* require a boat conformation of the cyclizing segment; the first formed boat-shaped intermediate is then transformed, with stereochemical control ensuring the appropriate configurational transmission, to a chair-shaped intermediate in a process which will benefit from the energetically favorable boat-to-chair conversion. Interaction with a distal double bond may provide further assistance, but is clearly not an essential requirement, as evident in the formation of ring E in β-amyrin, and demonstrated for the formation of ring D of the same compound by the β-amyrin-synthase-catalyzed cyclization of dihydrooxidosqualene to the tetracyclic dihydrobaccharenol [108].

A second and well-exploited opportunity for further diversification in the generation of triterpene prototypes is provided by the set of successive 1,2-rearrangements of hydride and Me groups, which is often appended to the cyclization process proper; in the most spectacular case, the biosynthesis of friedelin, the sequence consists of nine consecutive steps. In a majority of cases, the migration and elimination steps involve a pairwise *anti*-orientation of the participating groups, but evidence discussed in detail in the preceding section is now available to show that a *syn*-orientation of these groups is occasionally tolerated; this requires an adequate amendment of the related discordant note in the 1955 paper. In the case of lanosterol biosynthesis, it has been shown that the rearrangement sequence of the biological process can be easily reproduced under nonenzymic conditions [87b], probably as a consequence of the driving force exerted by the boat shape of ring B in the nonrearranged precursor. This parallelism, however, turns out to be an exception rather than a rule, as demonstrated in many cases by the *inverse* direction of the rearrangement reactions observed as the outcome of acid catalyzed *in vitro* experiments. In other words, the biological sequence of post-cyclization 1,2-shifts is in most cases an up-hill process, which as such does not justify the widespread use of the expression '*reaction cascade*'. Strategic placement of the basic group required for the regiospecificity of the concluding deprotonation step is certainly important, but, in addition, the enzyme must be able to cope with the up-hill problem, and probably manages to do so by differential binding of the ionic intermediates. In this connection,

it might be rewarding to investigate the potential ability of the enzyme to overshoot reversibly the point of deprotonation.

In view of the important role played by the lanosterol/tirucallol dichotomy as a stimulus for the elaboration of the 1955 paper, it seems appropriate to point out that such a relationship is not any longer a unique case. Subsequent developments have confirmed that insertion of a *b*-digit in the second position of the conformational blueprint is always linked with an inversion in the helicity sense of the bits encoded by the successive digits. A beautiful illustration of this principle is provided by the structures of isoarborinol (**15**) [109] and fernenol (**16**) [110], which differ in the antipodal configuration of six out of the nine stereogenic centers as a consequence of a *b* to *c* switch in the second position of their conformational blueprint. In addition, the existence of these diastereoisomers certifies that specific regions of the two cyclases encompassing up to two thirds of their catalytic grooves are capable of accomodating extended antipodal bits, first of the cyclization substrate and then of the final rearranged products. This remarkable flexibility of the two enzymes makes it the more surprising that, in sharp contrast with the situation wich prevails in the biosynthesis of mono-, sesqui-, and diterpenes, no *chiral* choice of substrate conformation is tolerated by any of the known SCs and OSCs in the first cyclization step of triterpene biosynthesis. But for one anomalous case mentioned below, there is until now no evidence of breach in the empirical observation of the initial homochirality of all known triterpenes, exemplified by the constant absolute configuration of the two stereogenic centers in the A/B ring junction of, say, lanosterol and lupeol. The anomalous case concerns a set of two unusual oxidosqualene-derived diastereoisomeric triterpenes from the rhizomes of *Iris* species, which display an antipodal configuration of the stereogenic centers marked with an asterisk in formula **17**, reflected in the variable enantiomeric purity of the volatile odoriferous compounds (known as irones) derived from the triterpene precursors by oxidative degradation [111]. It is important to note that the oxidized moieties of the two compounds represent the outcome of a rearrangement–fragmentation process of a bicyclic ionic endowed with the usual absolute configuration of its A/B ring junction, whereas the nonstereoselective reaction which leads to the antipodal forms of ring E requires the stoichiometric participation of the electrophilic Me group of (*S*)-adenosyl-methionine in the cyclization step, a feature which clearly differentiates the responsible enzyme from all other known members of the CS and OCS family. The existence of antipodal forms of terpene prototypes is, of course, not impossible, but the bulk of the collected evidence certainly makes it higly improbable. Alleged identification of members of the antipodal set is occasionally reported in the literature for structurally complex natural products considered to be derived from triterpene prototypes through lengthy sequences of steps involving oxidative degradation, fragmentation, as well as creation of new C,C bonds. The evidence offered is at best questionable, and the authors seem to be unaware of the far reaching biological implications of their claims. In the absence of rigorous proof, editors and referees should exert stricter control of the situation.

We are indebted to *Erik Sorensen* and *Lucy Stark*, without whose initiative we would hardly have engaged in the present revisitation of the 1955 *Helvetica Chimica Acta* paper. We feel obliged to *M. Volkan Kısakürek* for his encouragement and support, which proved essential for the completion of the project. The critical help and control of our colleague *Jack Dunitz* in linguistic and other matters is greatly appreciated. Thanks are due to

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