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The evolution of terpenes to sterols

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Abstract - Molecular fossils of polyterpenoids are major constituents of the organic matter of sediments. They contain bacterial lipids, more or less modified structurally, from several classical families (steroids, carotenoids), but also from groups (hopanoids, bisphytanoids) which have first been isolated in sediments, and have only later been recognized as bacterial lipids or postulated as such (tricyclopolyprenoids, isoarborinol, novel archaebacterial tetraterpenoids). The recognition in these terpenoids of common structural features (dimensions, rigidity, amphiphilicity) and of a common functional role (membrane reinforcement), proved both in vivo and in vitro, leads to the hypothesis of a phylogenetic derivation of the various groups, by progressive enrolment of new enzymatic systems.

INTRODUCTION

This essay presents conclusions drawn from apparently unrelated results established in particular by my associates in Strasbourg. Of these, I must single out Yoichi Nakatani, first met at IUPAC 64 in Kyoto. I am also greatly indebted to my former coworkers, present friends, and highly successful colleagues, Pierre Albrecht and Michel Rohmer: they and their coworkers have not only contributed most of the hard facts (over more than 200 man-years !) but have also helped me to interpret them, not too softly I hope, and have allowed me to use freely some of their recent independent work. Of my other coworkers, named in the references, I must mention in particular Bertrand Chappe, Alain Milon, Jörg Saar and Geneviève Wolff, who, more than others, have had to bridge the gap between our goals and our experience, in fields new to all of us.

In previous IUPAC Symposia, in New Delhi (ref. 1) and in Varna (ref. 2), I have described some of the unexpectedly general results accruing from our study of the organic constituents of sediments. We could have been satiated with our discoveries of the most abundant family of complex molecules on Earth, the hopanoids, of the wide occurrence and diversity of bacterial biohopanoids, of the molecular fossils of archaebacterial lipids, of the general mechanisms of maturation in sediments (ref. 3). The urge to transcend these factual novelties into a much wider, though potentially debatable, framework has been, as I have already admitted, my attempt at responding to the nagging question of Marie-Claire Dillenseger: "So what ? And what now ?".

I accept the risk of being chastised for my unspeakable immodesty, but I wish to present this contribution as pursuing the series of general papers by Ruzicka, who had dubbed me his "Statthalter für Frankreich": the series starting with the proposal of the structural isoprenic rule (ref. 4), continuing with that of the biogenetic isoprenic rule (ref. 5), and culminating with that of the stereochemical isoprenic rule (ref. 6). My ambition is to give now a "phylogenetic isoprenic rule" (ref. 7), allowing us to recognize, in the multitude of terpenic substances, those which are really important from the point of view of their function and of their place in biochemical evolution.

Our main theme will be that one central role of terpenes, in all phyla of living organisms, is to participate in the formation and reinforcement of biomembranes. From the simplest, possibly abiotic, polyterpenes up to cholesterol, the phylogenetic progression is punctuated by the progressive enrolement of new enzymatic systems. The very last stages of this evolution have been discussed by W.R.Nes (ref. 8) and mostly by K.Bloch (ref. 9).

Like its organismic counterparts, the molecular phylogeny thus proposed draws from fossil evidence but, paradoxically, this sheds light on extant organisms, not, like organismic palaeontology, on foregone worlds, as the sediments available for study are all relatively "young" (less than 1.5 billion years !), and the molecular fossils they contain are essentially derived from microorganisms identical with or akin to those at present at work.

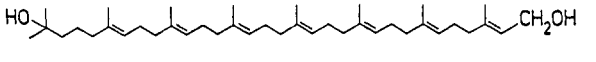
THE PROPOSED PHYLOGENY

We have very recently published an extensive review of our work and views (Ref.10), and a repetition would be unjustified. We shall therefore only summarize our conclusions, asking the reader to consult that reference if interested.
Membranes are formed by the self-assembly, in water, of amphiphilic molecules bearing a suitably large hydrophilic head-group such as (usually) a substituted phosphoglycerol or even a phosphate. The lipophilic tail cannot be too short (solubility in water), nor too long (self-assembly impossible). The consequence is that the lipid thickness of all known biomembranes is about 40 A, which corresponds to about 40 C-C bonds. Most membranes appear furthermore to be stable enough only when reinforced by rigid inserts, and we have proposed an operational rule stating that these inserts had to be amphiphilic, and either one-headed, rigid and about 20 x 6 x 6 A large, or α,Ω-two-headed, rigid or not, and about 40 x 6 x 6 A large (Ref. 7).

We postulate that the most primitive lipids participating in the elaboration of membranes were exclusively polyterpenoids, formed by only one biosynthetic reaction: the condensation of isopentenol. The fundamental reaction which leads to these polyprenols is simply the condensation of a carbenium ion with a double bond to give a new carbenium ion:

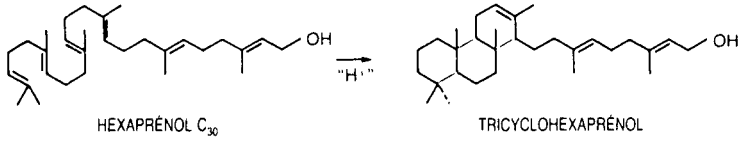
$$C^{+} + C{=}{=}C \longrightarrow C{-}C{-}C^{+}.$$

The chemistry required is therefore only acid catalysis; it could have been brought about e.g. by clays before these were relayed by a simple enzymatic system. Condensation of four or five C_5 units, giving geranyl-geraniol (tetraprenol), could lead spontaneously to a phase separation and to the formation of vesicles if this C_{20} alcohol were linked to a suitable polar head; a simple di-tetraprenyl phosphate would be a possibility. Another possibility for membrane formation/reinforcement would be the continuation of the isopentenol condensation up to the stage of octaprenol, with additional acid-catalyzed hydration at the Ω-position. So far, no organism is known to contain such simple polyterpene lipids in its membranes, but of course the corresponding saturated di-phytanyl ether lecithins of Archaebacteria are quite similar; yet, they require the recruitment of another enzymatic step for the saturation of the double bonds, and we postulate that more primitive organisms will be found, containing, with suitable head-groups, tetraprenol and octaprenediol.



Octaprene-diol (Synth., Ref. 11)

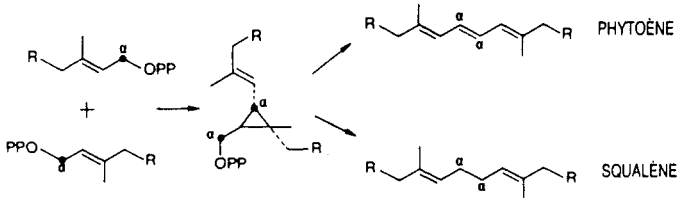
The chemistry involved so far is of course chemically analogous to the corresponding cyclization to a six-membered ring. Thus, formation from hexaprenol of tricyclohexaprenol



HEXAPRÉNOL C_{30} TRICYCLOHEXAPRÉNOL

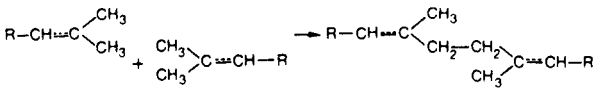
would not require recruitment of a novel enzyme, but only small changes in the relative position of some of the groups in the active site; in fact, such a cyclization could well also be effected abiotically, with clays. Tricyclohexaprenol is still not known in Nature, but its molecular fossils are abundant in many sediments, and it has been synthesized (Ref. 12). Its molecular dimensions, amphiphilicity and partial rigidity would make it an excellent cholesterol surrogate.

The next step in the phylogeny would require enrolement of a novel enzymatic reaction to ensure coupling of polyprenol chains. Two modes of couplings are known: either by head-to-head dimerization (leading from farnesol to squalene, or from geranyl-geraniol to prephytoene), or by tail-to-tail linkage (leading to the bis-phytanyl ethers of the Archae bacteria). The head-to-head coupling has been well studied; it appears that, in its two versions, it follows similar and complex steps, and that the two enzymatic systems involved are not strictly substrate-selective, accepting as they do the other substrate; there again, they could be "identical" enzymes, with some minor change resulting from a mutation.



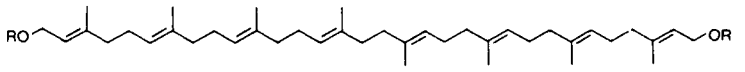
Head-to-head dimerizations of farnesol and octaprenol

Nothing at all, by contrast, is known about the biochemistry of this second coupling, which involves the elimination of two hydrogen atoms. These dimerizations lead therefore to two



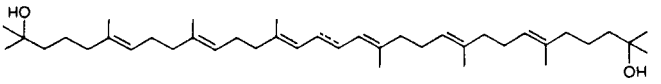
Tail-to-tail duplication

different branches, one present in Archaebacteria (where we could expect to find also the unsaturated precursors, such as bis-geranyl-geraniol, of the known bis-phytanyl lipids),



Bisgeranyl-geraniol
(Synt., Ref. 11)

and one presenting itself two variants: the formation of squalene (also present as such in Archaebacteria), and that of the precursors of carotenoids; we have postulated that these



"Pre-carotenoid"
(Synth., Ref.11)

incompletely desaturated pre-carotenoids would be found as phylogenetic precursors of the α,Ω-dihydroxylated carotenoids which we have now shown conclusively to be bacterial membrane reinforcers (Ref. 13).

Next comes the cyclization of squalene (analogous to that of prephytoene to β-carotene derivatives). This is of course very similar to the cyclization, mentioned above, of hexaprenol, with a major difference however (Table 1): instead of being all-Markovnikof, it leads to hopanoids only through two anti-Markovnikof ring closures. We have assumed that this cyclization is carried out by the "same" enzyme, with a minor mutation, as schematically suggested on Table I.

This "near identity" is indeed made quite probable by the remarkable fact that squalene-hopene cyclases show little substrate specificity, as they can accept and cyclize not only squalene, but also polyprenols, even as short as farnesol. We postulate that the formation of tetrahymanol is again the result of a minor mutation, involving a small change in the relative position of one of the amino-acids in the cyclase. This is an example of a conclusion amenable to test, once the structures of squalene-cyclases will be determined.

The next major change comes with the formation of squalene epoxide, which involves molecular oxygen. Once formed, this substance would not escape cyclization, as we have shown that the squalene cyclases of microorganisms can accept as well squalene epoxides (R and S) as substrate; however, the simple products of cyclization could not be used as cholesterol surrogates, as they would be diols, not easily inserted in membranes. Instead of hydration of the terminal carbenium ion, rearrangement must occur, and the smallest change would be to go from hopanoids to isoarborinol, a hybrid between hopanoids and tetracyclic triterpenes, and a constituent of sediments which we have postulated to be potentially a

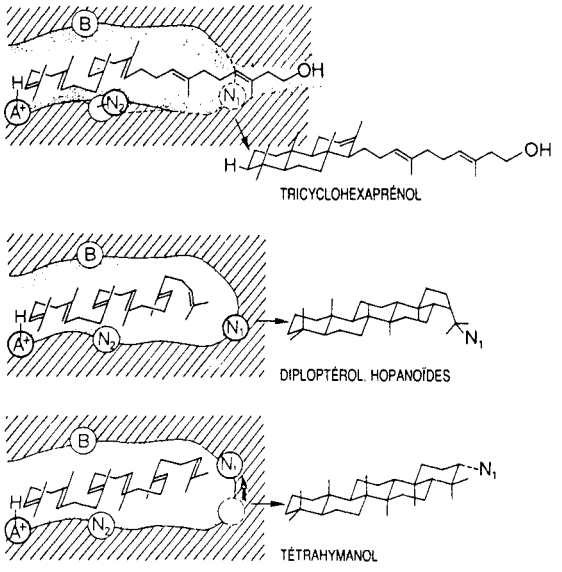


Table 1

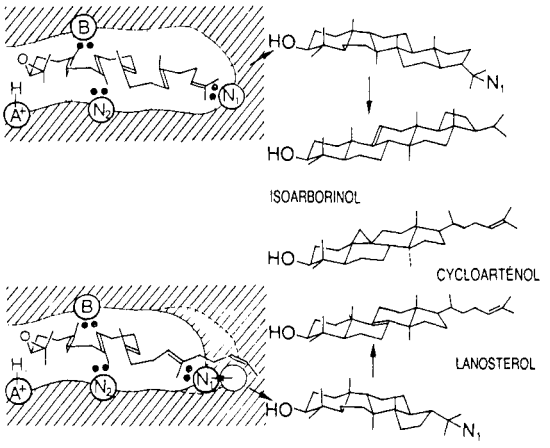


Table 2

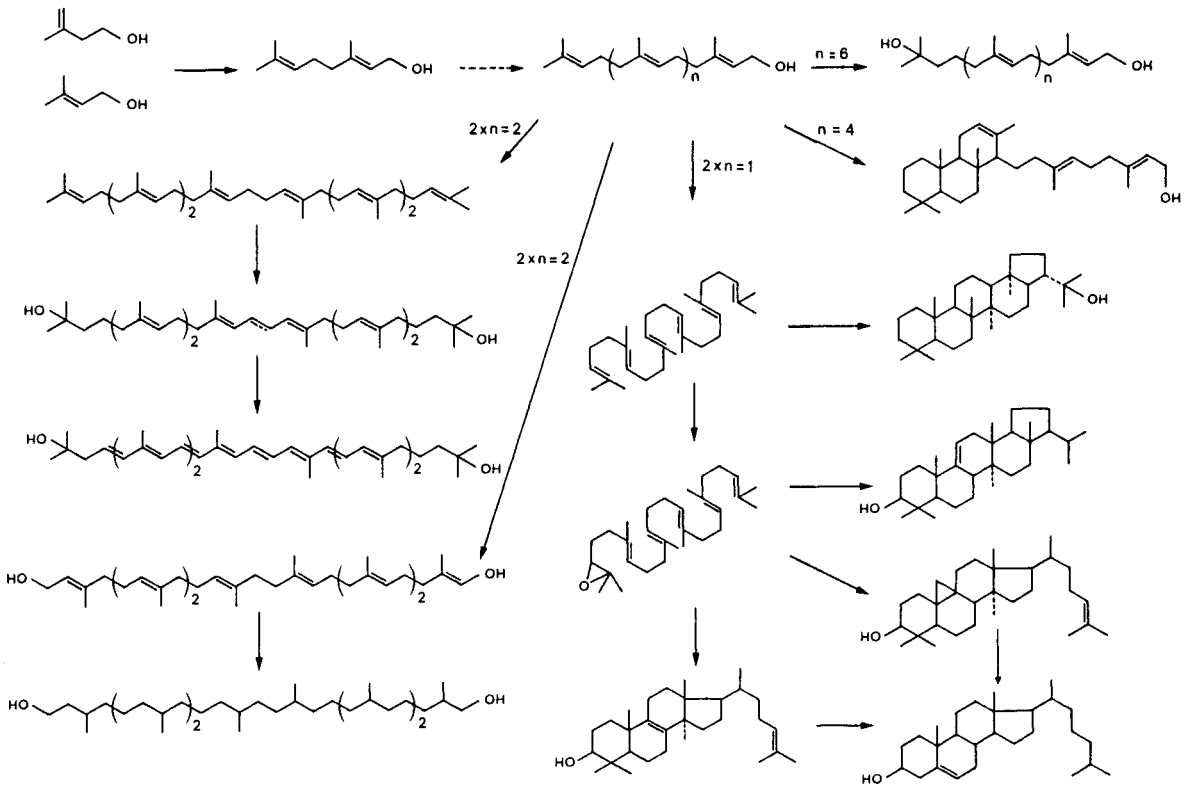


Table 3

bacterial membrane reinforcer. The next change in the cyclase could lead to cycloartenol, a major membrane component in some amoebas, and the biosynthetic precursor of sterols in plants. The small change from cycloartenol to lanosterol appears to block (for reasons still unknown) the possibility of reinforcing membranes; however, oxidative degradation mechanisms already introduced for the catabolism of cycloartenol would convert lanosterol to the sterols proper of animals or fungi (Table 2). It it is thus possible that the dichotomy of cycloartenol- and lanosterol-organisms, long appearing to be only one of the many unnecessary complications of Nature, probably reflects an evolutionary trend, cycloartenol being first itself a cholesterol surrogate, then a cholesterol biosynthetic precursor, and only later being superseded by cholesterol ex-lanosterol.This is summarized on Table 3.

In short, a series of minimal changes operating on very few enzymatic systems could explain the whole series of known cholesterol surrogates, and suggests "missing links", which are in two cases (tricyclohexaprenol and isoarborinol) in agreement with fossil evidence.

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