

Evolution of Terpenes: A Hypothetical Phylogeny from Clays to Cholesterol

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Appointed by *L. Ruzicka* in 1972 as his «Stammhalter für Frankreich», I am particularly proud to present some «Sonn-tagschemie» based on solid week-day work, and giving some very general perspective on the significance of terpenes. This is based on the extensive work of *P. Albrecht* (Strasbourg), of *M. Rohmer* (Mulhouse), of *Y. Nakatani* (Strasbourg), and their coworkers.

Cholesterol (1) is a universal constituent of eukaryotic membranes, which it stabi-

lizes by insertion between the phospholipid molecules, by its orientation induced by its amphiphilic nature, and by the rigidity imparted by its polycyclic structure to neighbouring phospholipids by cooperative van der Waals forces. Following our discovery of their molecular fossils, ubiquitous in sediments, we have shown that «hopanoids» like bacteriohopanetetrol (2) and its many relatives, quite widespread in bacteria, form a novel and hitherto unsuspected bacterial lipid family. While «geo-



hopanoids» are probably, due to their ubiquity, the most abundant complex molecules on earth (mostly underground!), at least some of the «biohopanoids» are structural equivalents of sterols (amphi-

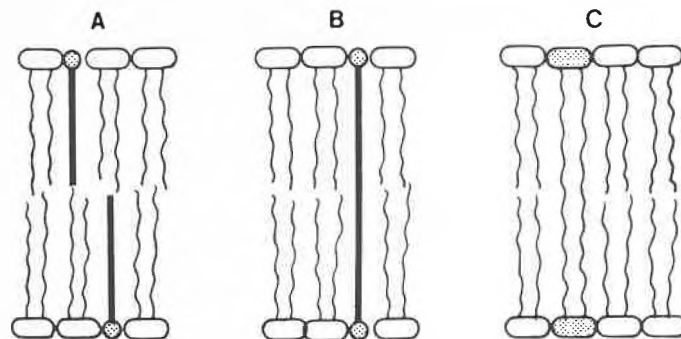
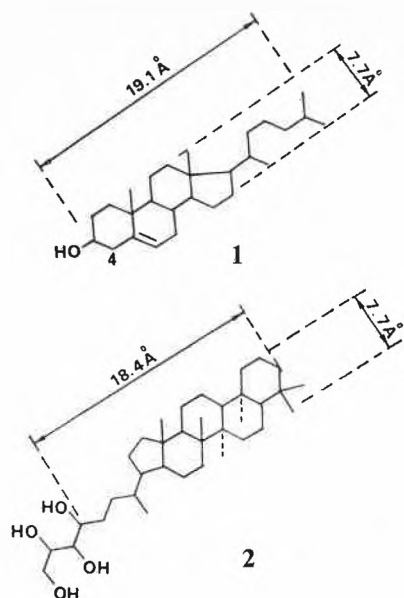
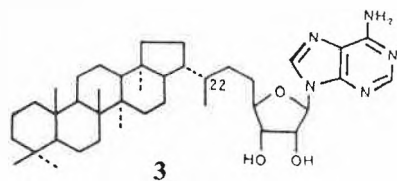


Fig. 1. Schematic representation of the insertion, into phospholipid bilayers, (A) of cholesterol or hopanoids, adapted to one half of the bilayer, as «pegs»; (B) of α, ω -dihydroxylated carotenoids, as trans-membrane «rivets»; (C) of transmembrane phospholipidic diethers in archaebacterial membranes.

philic character, dimensions, rigidity), and functional equivalents, both *in vivo* (*Tetrahymena*) and *in vitro* (monolayers, vesicles). It is possible that some of the most complex biohopanoids, like the adenosylhopane (3) of *Neunlist* and *Rohmer*, are rather «steroids» of bacteria, i.e. that they display some more elaborate function than a mechanical one.



Our hypothesis of the further equivalence of α, ω -dihydroxylated bacterial carotenoids with cholesterol (as «rivets» instead of «pegs») (Fig. 1), indicated by *Razin's* experiments *in vivo*, has now been largely substantiated *in vitro* by us with vesicles.

Other known microbial lipids are also of terpenic nature:

- the archaebacterial phytanyl and bis-phytanyl ethers (Fig. 2), which exemplify both bilayers and transmembrane lipids, and for which we know molecular fossils (some of which are «orphan» lipids);
- tricyclohexaprenol (4), the postulated parent of tricyclic polyterpenes abundant in sediments;
- maybe isoarborinol (5), found in many sediments (but also in a few plants), a hybrid of hopane and lanosterol, which we *postulate* to be of bacterial origin (from aerobic bacteria);
- cycloartenol (6), which is not only the biosynthetic precursor of cholesterol in plants as *P. Benveniste* had shown 20 years ago, but also a cholesterol sur-

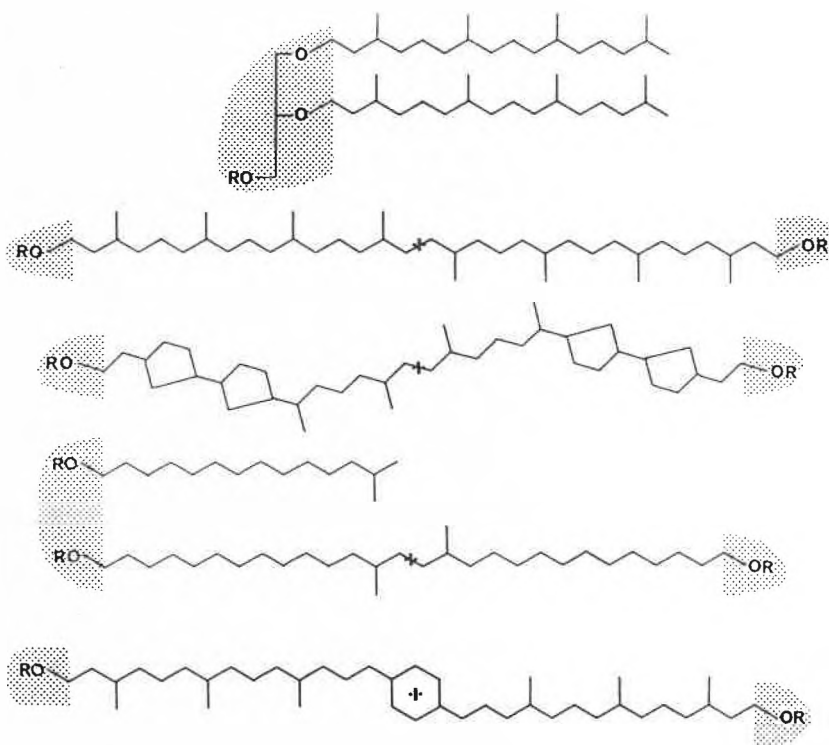


Fig. 2. Archaeobacterial lipids. In the first three structures, the corresponding molecular fossils are known both intact, or as hydrocarbons (with the polar heads, in gray, missing). The bottom three structures are known only as the corresponding hydrocarbons; the complete structures shown (with the gray polar heads) are putative, and represent «orphan» lipids.

rogate in some amoebas (*Rohmer* and *Raedersdorff*);

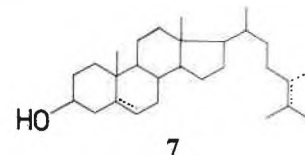
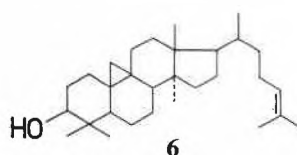
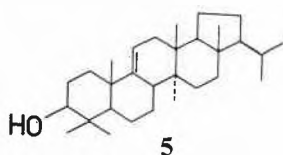
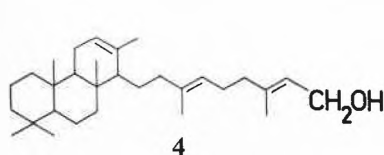
- 4 α -methyl sterols (7), biosynthetic precursors of cholesterol, but sterol surrogates in Dinoflagellates and in *Methylococcus*, and which provide abundant molecular fossils.

Thus, a general function of polyterpenes appears to be, in all phylums of living organisms, membrane reinforcement. We consider this as the fundamental role of

terpenes, the other ones (hormones, pheromones, defense substances, participation in photosystems, etc.) being «secondary».

The series of structures mentioned could be derived, by small changes in substrate or in product specificity, from a very limited number of enzymatic systems:

- the universal one leading to the linear polyterpenes, essentially by one reaction of the type $C^{\oplus} + C=C \rightarrow C-C-C^{\oplus}$, i.e. head-to-tail condensation;



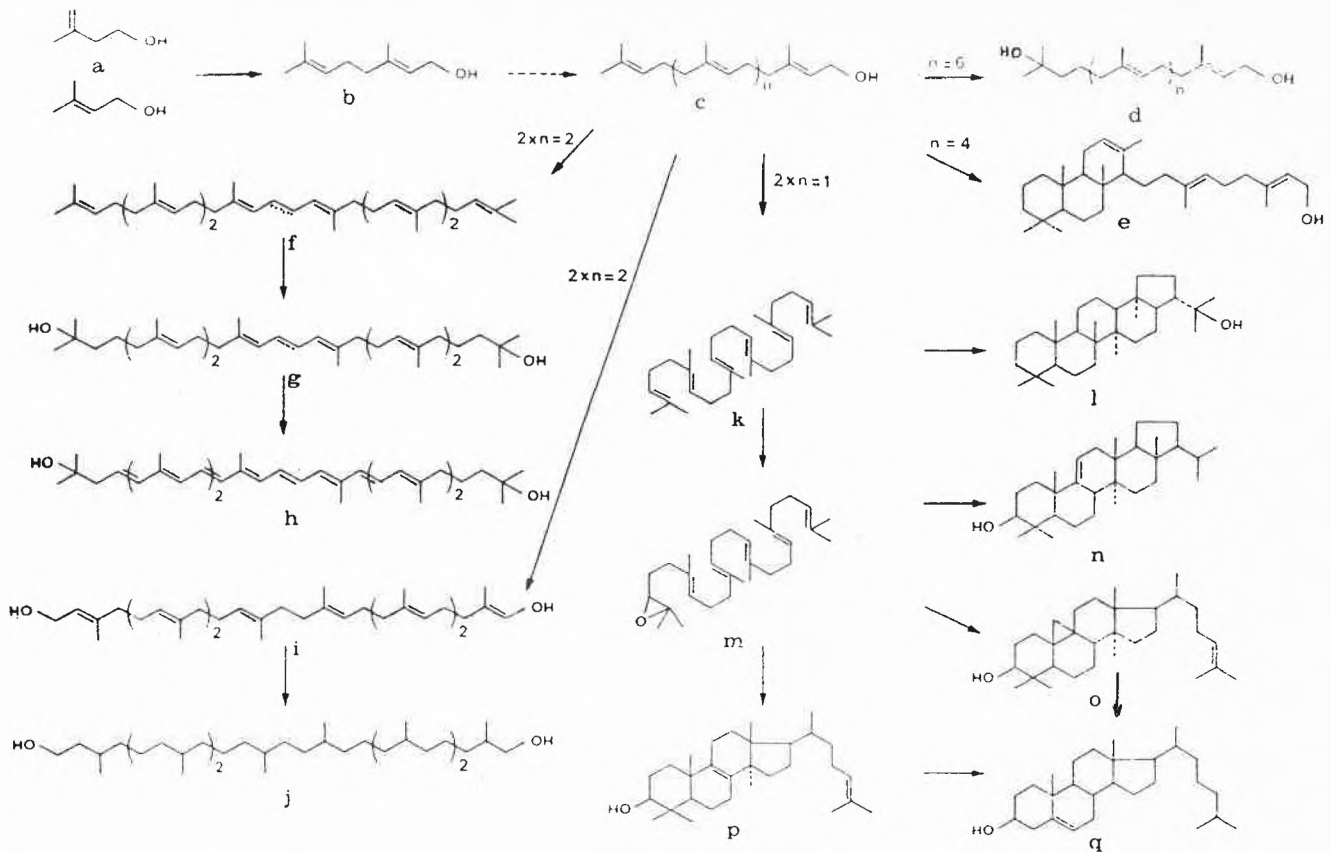


Fig. 3. A hypothetical biosynthetic/phylogenetic scheme for membrane lipids equivalent to cholesterol (q). a-c: polyterpene biosynthesis; d: postulated hydrated polyterpenol; e: tricyclohexaprenol – d and e require only the intervention of the fundamental mechanism of polyterpene biosynthesis: carbenium ion attack by C=C or H₂O (head-to-tail condensation); f: phytoene; g: postulated pre-carotenoid diol; h: «dihydroxyspheroidene», a carotenoid diol; i: pre-archaeobacterial bisphytanol; j: archaeobacterial bisphytanol, which requires a third enzymatic step: the head-to-head condensation; k: squalene; l: hopane derivatives – g and l require a second fundamental enzymatic step (tail-to-tail condensation); m: squalene epoxide; n: isoarborinol; o: cycloartenol; p: lanosterol; q: cholesterol. The formation of m requires the novel enzymatic epoxidation; the next stages require novel enzymatic constraints for the cyclization.

– a variant of the first one, leading to cyclization, or/and to hydration;
 – one leading to head-to-head dimerization (2 C₁₅ → squalene; 2 C₂₀ → carotenoids), through essentially identical intermediates and with a moderate substrate specificity;

– and one leading to tail-to-tail dimerization, in archaeobacteria, through an entirely unknown biochemistry.
 The progressive sophistication of these enzymes, away from simple («clay-catalyzed-like») reactions to the most subtle ones, leads one to postulate the phyloge-

netic sequence of Fig. 3, in which the dihydroxy-hydrocarotenoids, the corresponding unsaturated bisphytanediols (or derivatives such as phosphates), tricyclohexaprenol, and isoarborinol remain to be found in membrane lipids.

Zwischenreden – Zwiesprachen

Vor dem Referat über den letzten Vortrag beim «Leopold Ruzicka Centennial Symposium» von Professor Eigen sollen einige Passagen aus der Einführung durch Professor Eschenmoser stehen, welche anhand einiger Faksimile-Bilder das eminente Interesse bezeugen, das Ruzicka für die Eigenschen Arbeiten zur Evolution des Lebens aufgebracht hat. Die Antwort Eigens spiegelt die Stimmung bei dieser Veranstaltung wider.



Albert Eschenmoser:

... Manche von uns wissen, dass Leopold Ruzicka ein EIGEN-FAN war. Deshalb freuen wir uns besonders, beim heutigen Anlass Professor Eigen hier bei uns zu haben ... Er hat sich mit einem Fundamentalproblem der Naturwissenschaften, dem Problem der Selbstorganisation der Materie befasst und gehört zu den wenigen Wissenschaftlern, die das Weltbild unserer Zeit beeinflusst haben ... Ich möchte ihm ein kleines Geschenk machen: Auf dem ersten Faksimile (Bild 1) sieht man die Umschlagseite des Hefts der «Naturwissenschaften» vom Oktober 1971, in welchem

EIGEN DIE NATURWISSENSCHAFTEN

HEFT 10 OKTOBER 1971

1

18. Jahrgang, (67)

Self-organization of Matter and the Evolution of Biological Macromolecules

Manfred Eigen, Max-Planck-Institut für Biophysikalische Chemie, Karl-Friedrich-Hofmeister-Institut, Göttingen-Nikolausberg

Table of contents listing various scientific topics and their corresponding page numbers.

I. Introduction

1.1. Cause and Effect

The question about the origin of life often appears as a question about "cause and effect". Physical theories of macroscopic processes usually involve answers to such questions...

which even in its simplest form always appears to be associated with complex mechanisms (i.e. multicomponent systems) such as the living cell.

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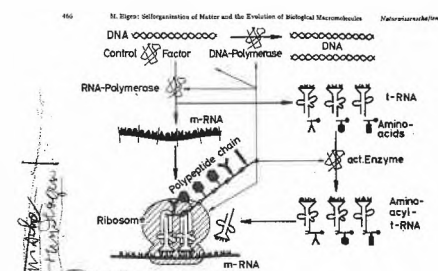


Table 1. Genetic code and amino acid codon assignments in their respective codons. This important conceptual table sets the stage for the subsequent discussion of the genetic code.

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Eigen eine seiner grundlegenden Arbeiten publiziert. Es handelt sich um das persönliche Exemplar von Ruzicka mit authentischen Anmerkungen in Ruzickascher Handschrift - ein Dokument dafür, wie intensiv man in Zürich die Eigenschen Arbeiten gelesen hat.

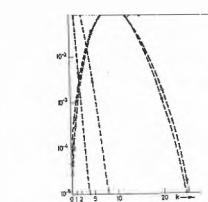


Fig. 4. Probability distributions for the occurrence of error output, O_n , according to Eq. (5) in Table 5. The parameters are: $\mu = 0.02$, $\sigma_1 = 0.000$, $\sigma_2 = 0.1$, $\lambda = 4$, $q_1 = 0.990$, $q_2 = 1$, $\sigma_3 = 0.000$, $\sigma_4 = 1$.

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to the accuracy of elementary digit recognition. The quantity, as defined by the variation of the μ and σ parameters, enters only as a logarithmic term, and hence it will be of restrictive influence only for small variations of μ and σ (i.e. $\mu, \sigma \ll 1$, and $\sigma, \mu \ll 1$).

11.6. Kinetics of Selection The phenomenological equations for both constraints always represent systems of nonlinear differential equations. Explicit solutions, of course, depend on the special form of the equations as determined by the particular reaction mechanism.

Table of contents for the 'VIII. Conclusion' section, listing sub-sections like 'VIII.1. Limits of Theory' and 'VIII.2. What the Theory Does Explain'.

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VIII. Conclusion VIII.1. Limits of Theory What the Theory Does Explain It is the general principle of selection and evolution at the molecular level, based on the primary reaction between the free-radical, thermodynamic theory of reaction states. Evolution appears to be an inevitable event, given the presence of certain matter with specified autocatalytic properties and under the maintenance of the thermized energy flow necessary to compensate for the steady production of entropy. The theory provides a comprehensive basis for the evolution of laboratory microorganisms and evolution.

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Manfred Eigen, introduction was conceived after the recognition of molecular and atomic models of biological evolution. The theory was developed in the form of a book, 'The Self-Organization of Matter and the Evolution of Biological Macromolecules', which was published in 1971. The book is a comprehensive treatment of the subject, covering the physical and chemical aspects of the theory.

für Sie ein schönes Erlebnis sein, die Hand Ruzicka in Ihren eigenen Publikationen festgehalten zu sehen.



Manfred Eigen

... Darauf will ich mit einem Gleichnis antworten, das ich einen Tag zuvor von Ephraim Katzir gehört habe.

Da war ein Mann in Israel, der züchtete Hühner, und eines Tages gingen die Hühner an zu sterben. Er ging zum Rabbi und sagte: "Rabbi, meine Hühner sterben." Da sagte dieser: "Füttere sie mit Reis." Das tat er, doch nach einer Woche kam er wieder und sagte: "Rabbi, meine Hühner sterben immer noch." Da sagte der Rabbi: "Nimm Mais." Nach einer Woche kam er wieder: "Meine Hühner sterben immer noch." Die Antwort: "Mein Sohn, nimm Käse." Und als er nach einer Woche wiederkam: "Rabbi, meine Hühner sterben", da fragte der Rabbi: "Wieviele Hühner hast Du noch?" "Ich habe noch zwei." "Wie schlimm", sagte der Rabbi, - "und ich habe noch so viele gute Theorien."

Zu einer Chemischen Gesellschaft zu kommen und einen Vortrag mit dem Titel «Die physikalische Basis der Evolution» anzukündigen, hört sich wie eine Provokation an, und wahrscheinlich hat es damals Ruzicka so empfunden, als ich auf der Birmensdorf-Konferenz 1971 über dieses Thema sprach, und er sehr erregt am Ende des Vortrages aufstand und sagte: «Es hat meiner ganzen Lebensarbeit bedurft, um mich zu überzeugen, dass das Leben Chemie ist, und nun kommen Sie daher und wollen uns erzählen, es sei Physik.»

Matter and the Evolution of Biological Macromolecules» (Bild 2) las Ruzicka offensichtlich mit Hilfe eines Wörterbuchs Englisch-Deutsch; er wollte sich auch wirklich gar nichts entgehen lassen. Die nächste Seite (Bild 3) veranschaulicht Leopold Ruzicka studiert Manfred Eigen, eine Auseinandersetzung im echten Sinne des Wortes. Wie die Abhandlung mehr mathematisch wird (Bild 4), werden die Ruzickaschen Notizen eher zaghaft. Beim Abschnitt Conclusion (Bild 5) wird's wieder spannend, insbesondere was die heftigen Unterstreichungen betrifft: Limits of Theory: What the theory does explain - What the theory may explain = What the theory will never explain. Diese Seite charakterisiert nicht nur Ruzicka, sondern besonders deutlich auch unseren Referenten, d.h. wie souverän er mit Theorie und der Forschung umgeht. Noch im Literaturverzeichnis (Bild 6) findet man die Ruzickaschen Spuren praktisch bis zum letzten Wort ... Ich glaube, Herr Eigen, es muss