Pure & Appl. Chem., Vol.51, pp.709-729.
Pergamon Press Ltd. 1979. Printed in Great Britain.
©IUPAC
0033-4545/79/0401-0709 $02.00/0

THE HOPANOIDS

PALAEOCHEMISTRY AND BIOCHEMISTRY OF A GROUP OF NATURAL PRODUCTS

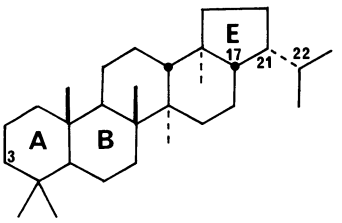
Guy OURISSON, Pierre ALBRECHT and Michel ROHMER

Institut de Chimie, Université Louis Pasteur, 1 rue Blaise Pascal, STRASBOURG - France.

Abstract - More than 100 individual derivatives of hopane have been isolated from sedimentary organic matter of most varied origin. They are ubiquitous molecular fossils derived from cellular constituents of micro-organisms. Hopanoids are shown to be widely distributed among Bacteria and Cyanobacteria (blue-green algae). Their function in cells is probably equivalent to that of sterols in the eucaryote cell : to act as optimizers of the fluid lipid membranes. They are even probably phylogenetic precursors of sterols. In procaryotes containing no hopanoids, other mechanisms of fluidity control of biomembranes are probably operating ; they imply acyclic di- or tetraterpenes (carotenoids or isomers), the molecular fossils of which are also present in sediments. These terpenes may in turn be phylogenetic precursors of hopanoids.

INTRODUCTION

The purpose of this review is to summarize both present knowledge and current hypotheses on a family of triterpenes, the *hopanoids*. A few derivatives of the skeleton of *hopane* had been known for some 20 years, in scattered plants.



Hopane

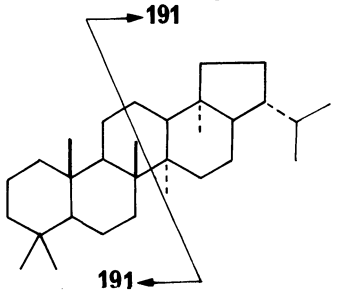
For instance, the Devon-Scott Index (1), which lists some 750 naturally occurring triterpenes, mentions only 26 hopanoids *sensu stricto* and 12 more substances with rearranged hopane skeletons. Furthermore, these triterpenes were known only from a few groups of living organisms : some tropical trees, some grasses, some lichens, and several ferns. It was therefore a surprise, during the last decade, to find hopanoids to be in fact vastly more widely distributed, and to be one of the major families of biolipids present on Earth. They are found in many microörganisms, and constitute the only group of triterpenoids to have produced ubiquitous molecular fossils ; furthermore, they show many characteristics suggesting that they may have been phylogenetic precursors of the sterols.

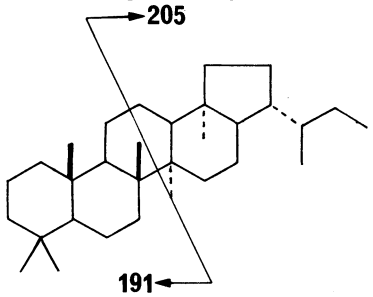
We shall approach hopanoids from the geochemical angle, describing first their molecular fossils. We shall then review their occurrences in presently living organisms and shall give our views on their probable biochemical function and on their evolutionary significance.

PALAEOCHEMISTRY OF THE HOPANOIDS

Methodology :

The organic matter of sediments, whether dispersed like in clays or concentrated like in coals or petroleums, is extremely complex (2). Suitable analytical procedures (3) and precautions against contamination (4) make it possible to separate this mixture by column and thin-layer chromatographies in groups of similar polarity (*e.g.* saturated hydrocarbons, olefins, aromatic hydrocarbons, carbonyl derivatives, alcohols, acids,...). Inclusion in urea, thiourea and molecular sieves can then be used to obtain sub-groups of similar molecular cross-sections. Each of these sub-groups (Cf. Table 1) can then be analyzed by computer-assisted gas chromatography/mass spectrometry. The mass spectrum of hopane derivatives is indeed very informative : hopane itself is fragmented in particular into two major fragments of





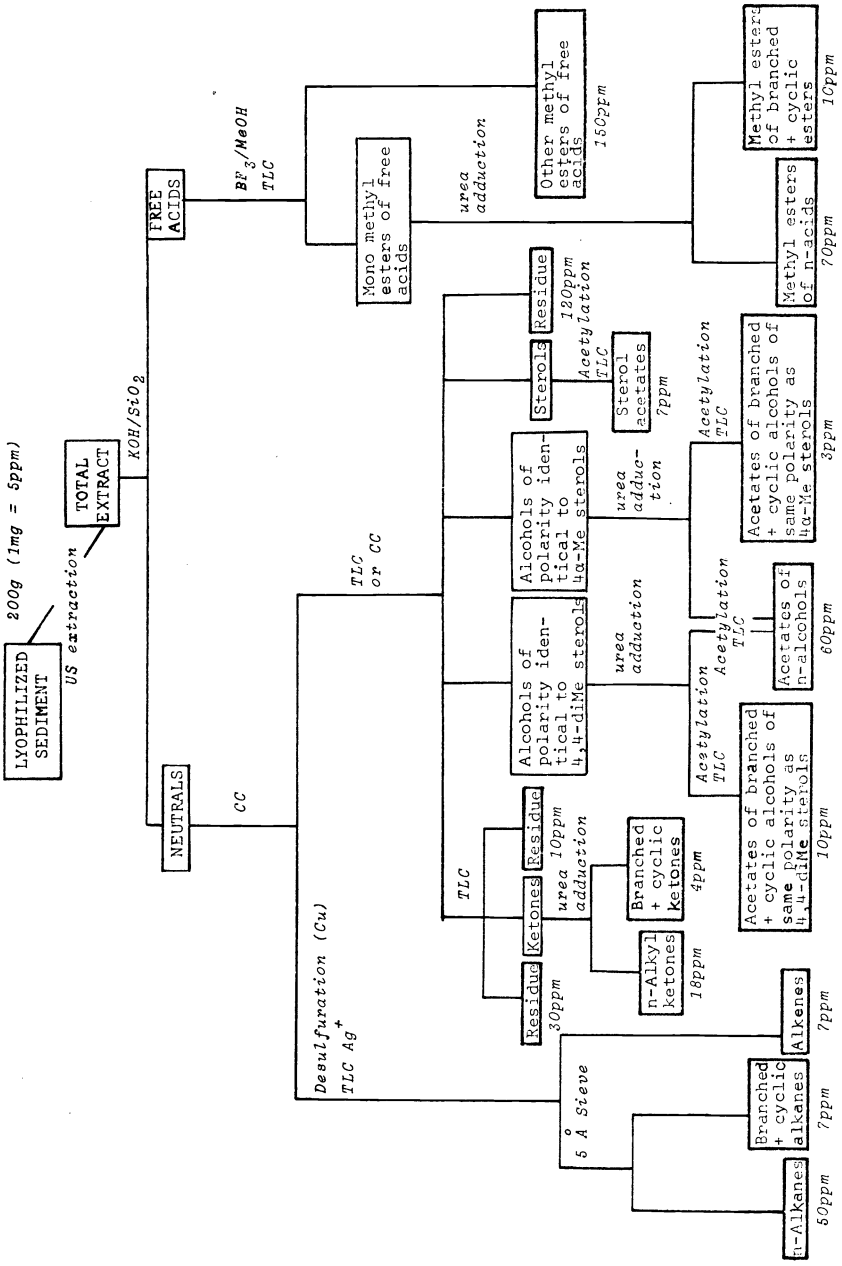
identical m/e = 191 ; modified hopanoids display the same fragmentation in ring C, giving two fragments of different masses. Furthermore, the relative intensities of the fragments due to rings A + B and to rings D + E can be directly correlated with the cis or trans nature of the D/E ring junction (5). It is thus possible to deduce, from the main fragmentations, a plausible structure and stereochemistry for the substance corresponding to a given peak of the gas chromatogram. Synthesis, usually from closely related precursors, but sometimes total, enables one to submit the hypothetical structures to test, in particular by comparison of mass spectra and co-injection on highly selective columns. In some cases, it has been possible to obtain more direct evidence, by nmr (6,7) or X-ray crystallography (7,8) ; it is this last method which has initially provided compelling evidence for the presence of hopane derivatives in geolipids (8).

RESULTS

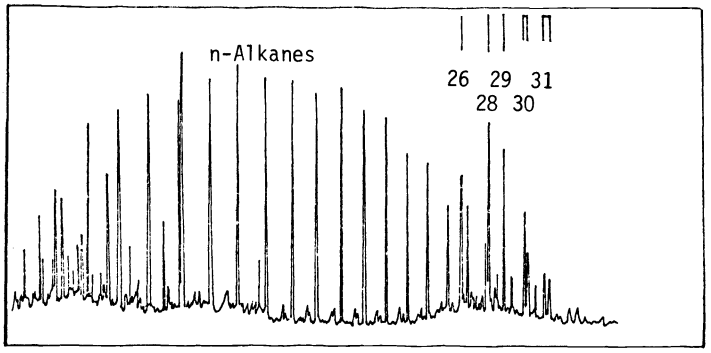
We reproduce below four examples of distributions observed, selected from many hundreds established in Strasbourg : the hydrocarbons of a Lorraine coal and of an Aquitaine crude oil, those of a polluted coastal mud (showing the immense help provided by the computer, in the form of the fragmentogram m/e 191, which is a "hopanogram"), and the acids of the Messel shale. In

Typical separation procedure :

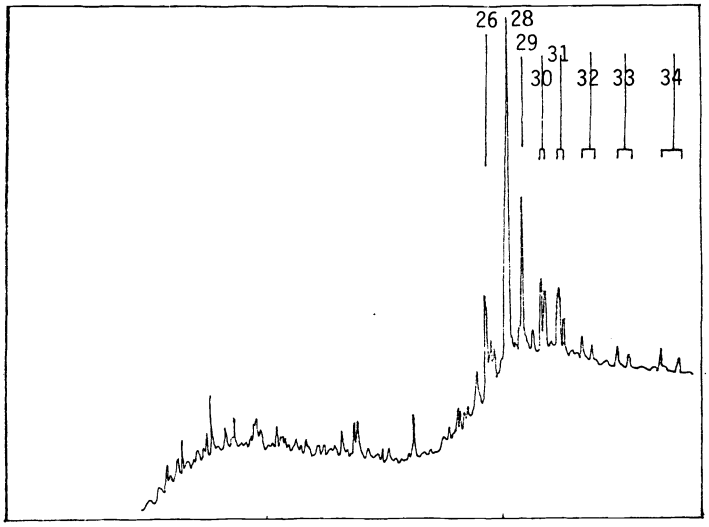
(Ref.18)



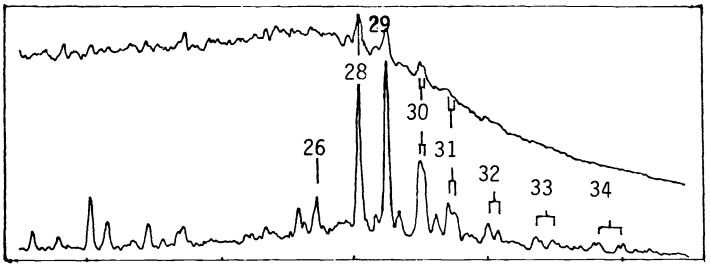
US: Ultra-sound; CC: column chromatography (SiO_2); TLC: thin layer chromatography.



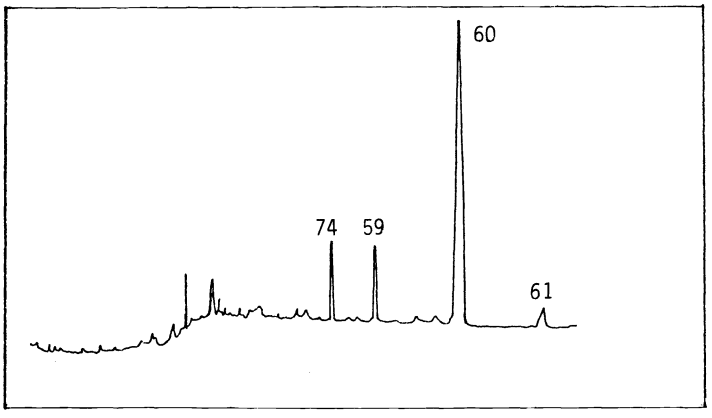
Total hydrocarbons from a Lorraine coal (275.10^6 yrs)



Branched and cyclic alkanes from an Aquitaine crude oil



Branched and cyclic alkanes from a coastal mud (Le Havre). *The upper trace is the total ion current, the lower one the fragmentogram 191.*



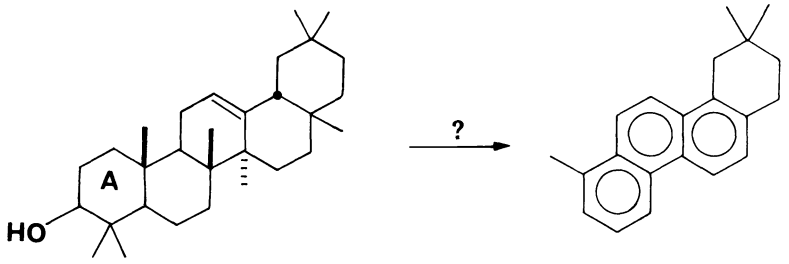
Total acids (as methyl esters) from the Messel shale (50.10^6yrs)

NUMBERS REFER TO THE FORMULAS OF THE TABLES (1 to 78).

each case, the identification of the peaks indicated is established beyond any doubt, including stereochemistry, which is related to maturation.

Every sample of sedimentary organic matter studied so far contains at least some members of the hopane family. This includes the widest variety of samples, comprising as well young muds, lake or sea sediments, petroleums, lignites, shales or coals, etc. Ages range from a few to 5 x 10^8 yrs. Contents vary from just detectable traces to nearly 50 % of the whole extract. Usually, the hopanoids are a very complex mixture, with little predominance of any substance ; but in one case, that of an Australian lignite, one C_{32} hopanoid acid forms some 90 % of the total acidic fraction, and represents some 0.05 % of the total rock !

Structures are extremely varied. The Tables summarize those known to-day : hydrocarbons of various classes, aldehydes and ketones, alcohols or acids. Even though some classes have not been examined (*e.g.* amines, sulfur derivatives, heterocycles), *more than one hundred hopanoids* have already been identified in sediments. This is in sharp contrast to the very limited number and the restricted distribution of molecular fossils of *other* series of triterpenes. Thus, only one other substance of this complexity appears to present a similar generality of distribution : it is an aromatic hydrocarbon, most probably derived from β-amyrin by loss of ring A. Nothing is

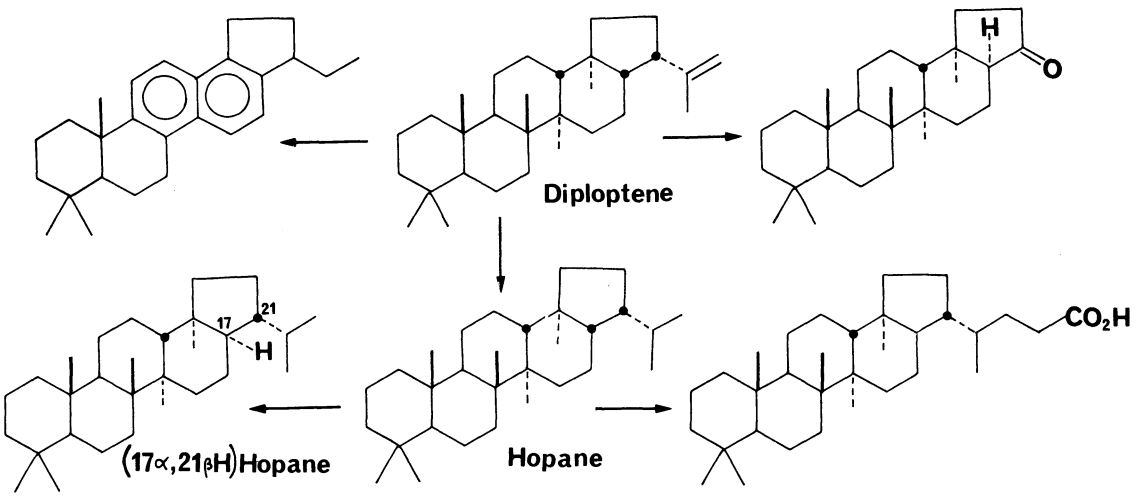


β-Amyrin

known yet of its origin (9).

The structures of sedimentary hopanoids, once known, led to the traditional problem of palaeontology : the reconstruction, from fossils, of the organisms (in this case, of the molecules) of origin . It is evident that such complex substances must have originated from very similar molecules, and that these in turn can provide information on the living organisms which had contained them. A complex structure contains more information than a simple one because it is so much less "probable". It cannot just have "happened". Furthermore, redundancy (like here, with the variety of closely related structures of isolated substances) increases the weight of the evidence - but, in this case, the exquisite structural variations also provide information on the nature of the reactions having taken place in geological conditions, during *diagenesis*.

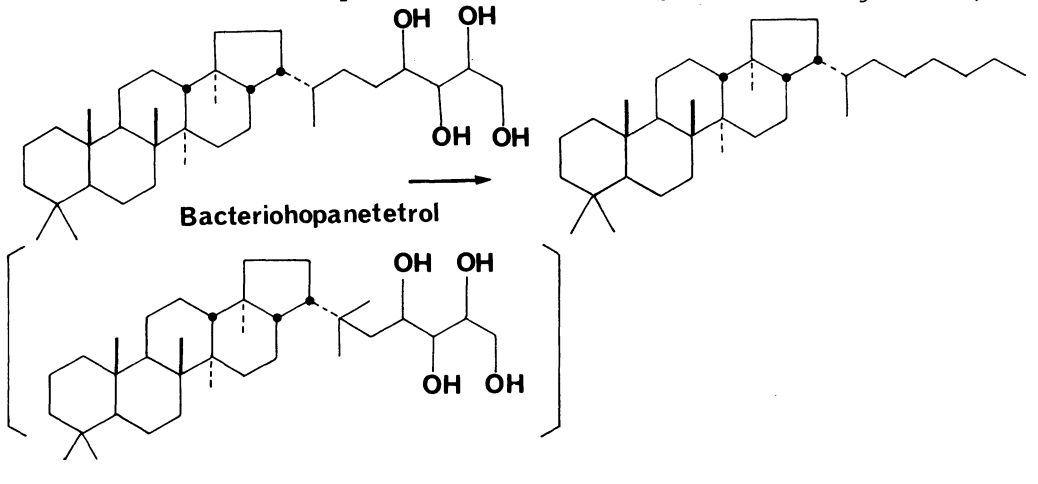
Hopanoids with 30 or fewer carbon atoms could of course easily be interpreted (10), in broad terms, as the products of the diagenesis of C_{30} hopanoids, for instance of *diploptene*, known in several organisms living to-day. It is easy to accept that diagenesis can transform C_{30} hopanoids into hopane itself,



or into a partially aromatized C_{26} hydrocarbon. However, the structure of the C_{32} acid already mentioned is obviously a much more important piece of information, *either* for the knowledge of the diagenetic mechanisms (can they also lead to the formation of new C-C bonds ?) *or* for the definition of the organisms having produced the precursors of the molecular fossils observed.

In fact, many sediments contain *extended hopanoids*, ranging up to but not higher than C_{36} (11). Of the supplementary carbon atoms, one to five at most are present as one n-alkyl group attached to the side-chain (which leads to the frequent presence of two diastereomers at C-22). The other supplementary carbon atom is in the ring system (and is rarely present).

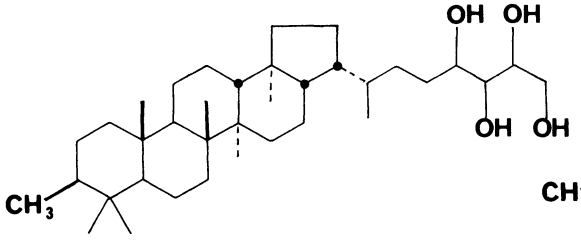
At the time of these observations, no living organism was known, containing additional carbon atoms on any pentacyclic triterpene derivative ; this might have meant that these extended hopanoids were metabolites of extinct organisms. However, their presence even in relatively young sediments gave more weight to another hypothesis : that we were in fact dealing with fossils related to metabolites of present-day organisms, but *not yet known*. This would have been the same situation that had occurred with "living fossils" such as the Crossopterigian fish *Latimeria chalumneae*, closely related to well-known Devonian fossils, but found only some thirty years ago not to be extinct. Our *molecular coelacanths* (12) are obviously derived from substances related to the "bacteriohopanetetrol" isolated, just at the right time, from



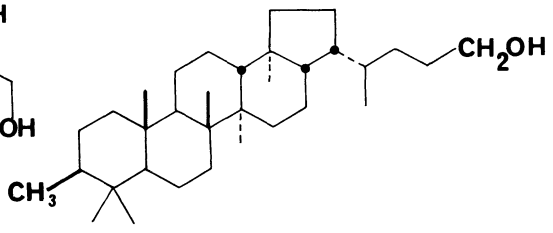
Acetobacter xylinum, and for which two hypothetical structures had been suggested (13). Our proof of the correct structure for the fossil bacteriohopane (11) led us to conclude that the correct structure for the tetrol *must* be the one indicated, despite the authors' preference for the one in brackets ; this hypothesis was proved by a direct correlation (14).

Bacteriohopane derivatives have so far been isolated only either from sediments, or from micro-organisms, where they are very frequent (12,13,15,16). This is a convincing argument in favour of the microbiological origin of at least part of the organic matter of all sediments.

An additional argument comes from the isolation of hopane derivatives carrying an additional nuclear methyl group, first from micro-organisms (17), and



Acetobacter xylinum



Baltic sea sediment

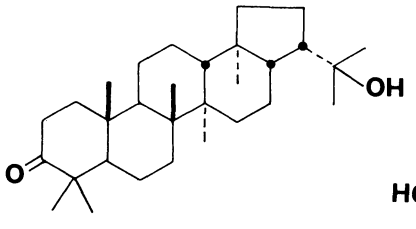
soon thereafter from a Baltic Sea sediment (18).

PRESENT DISTRIBUTION OF HOPANOIDS

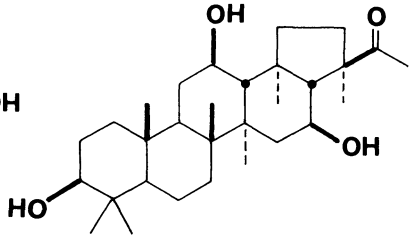
Hopanoids, in contemporary organisms, form two distinct groups.

The earliest ones to have been found were, like all other naturally occurring pentacyclic triterpenes, oxygenated at C-3. They are 3β-alcohols or 3-ketones, localized in resins or genins of saponins, and found only in a few families of higher plants (1).

We shall only mention two typical structures, that of hydroxyhopanone, and that of the much more heavily oxygenated genin of a saponin, spergulagenin A. The first one is a constituent of resins, and therefore, like the second one, a secretion product. They are obviously products of cyclization of squalene



Hydroxyhopanone

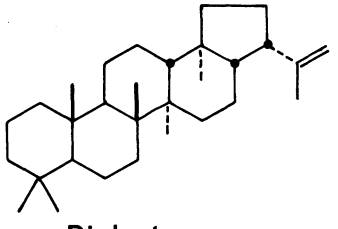


Spergulagenin - A

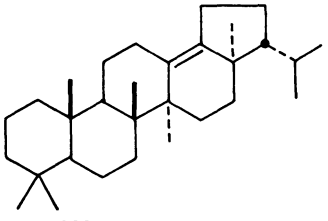
epoxide to hopane-3β,22-diol, and of further oxidations. Their scattered distribution, as well as their usual presence in complex mixtures, point to the absence of any specific intracellular role.

The second group of hopanoids in present living organisms are devoid of the 3β-hydroxyl or 3-oxo group characteristic of all the other groups of triterpenoids. These 3-deoxyhopanoids are found in two large taxonomic groups of living organisms : ferns or allied plants, and micro-organisms.

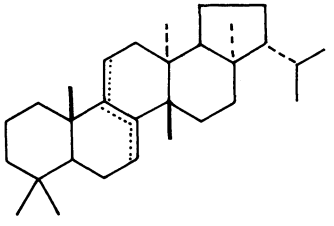
Ferns, or at least the order of Filicales, constitute a large store of hopane derivatives, and of rearranged structures (19). Some mosses and lichens also have been reported to contain hopanoids. Fern triterpenes include mostly hydrocarbons, and in particular the remarkable series ranging from diploptene to filicene, comprising all the intermediate stages of the complete back-bone



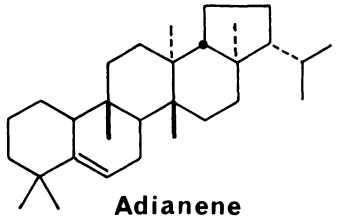
Diploptene



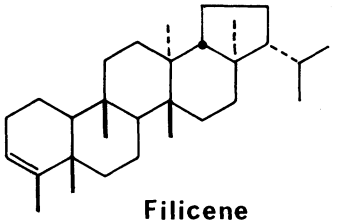
Wallichiene



Fernenes

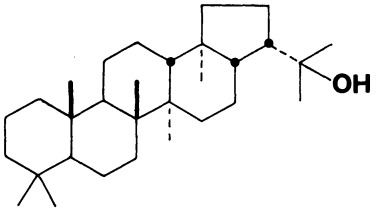


Adianene

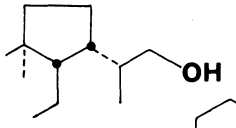


Filicene

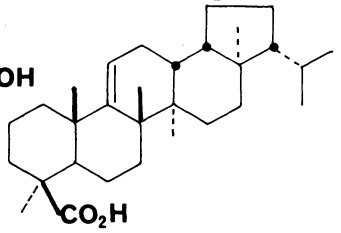
rearrangement of diploptene. To these, are to be added oxygenated derivatives, *e.g.* diplopterol or its isomer neriifoliol, or davallic acid. Leucotylin is, like several other lichen constituents, more heavily oxygenated.



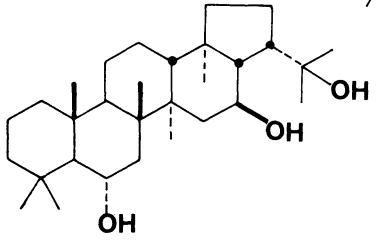
Diplopterol



Neriifoliol



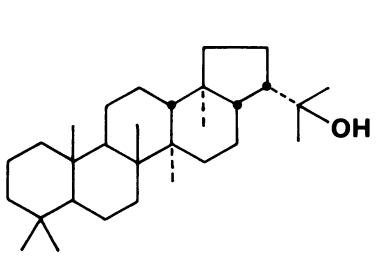
Davallic acid



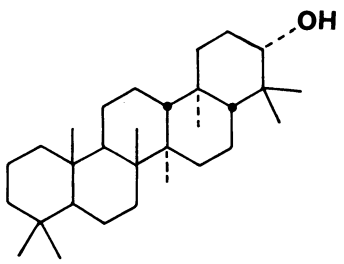
Leucotylin

The function of these substances is not defined. We consider most of them, excluding diplopterol and neriifoliol, as secretion products, *i.e.* products of the cell metabolism not directly used by the cell for its internal workings. This point will be discussed later.

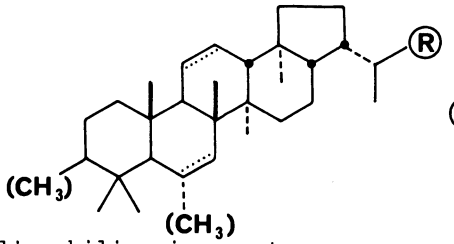
The hopanoids of microorganisms form, by contrast, a very homogeneous group, of obvious cellular importance. They comprise, beside diplopterol and its isomer tetrahymanol, a variety of bacteriohopane derivatives, all with a strongly dissymetric structure from the point of view of lyophilicity. The



Diplopterol



Tetrahymanol



R = $CH_2-CH_2-CHOH-CHOH-CHOH-CH_2OH$

$CH_2-CHOH-CHOH-CHOH-CHOH-CH_2OH$

$CH_2-CHOH-CH_2-CO-CHOH-CH_2OH$

etc...

lipophilic ring system may or may not bear one, or two, double bonds, and/or one additional methyl group ; the hydrophilic side-chain contains up to 5 hydroxyl groups on an n-C_5 chain.

Their distribution is by far not yet delineated. The Table summarizes some of our data. It shows that, in several groups of bacteria, the presence of hopanoids is constant, in amounts sufficient to enable them to play a structural role. In other well segregated groups, they are constantly absent. The taxonomic significance of many other data is not yet clear, and will be reserved for another discussion.

In only *two organisms*, hopanoids are found simultaneously with sterols : in the protozoon, *Tetrahymena pyriformis* (but there, diplopterol and tetrahymanol are found only when it is grown on a sterol-free diet (20)), and in *Methylococcus capsulatus* (but there, no sterols proper but 4α-methyl sterols are present (21)).

POSSIBLE FUNCTION OF HOPANOIDS

We have proposed that hopanoids of the amphiphilic type found in prokaryotes play a structural role in the cells. They have molecular dimensions similar to those of sterols, and a similar lyophilic dissymetry. They should therefore be able to replace sterols in their structural role of inserts into the phospholipid part of biomembranes (16)*. This is what appears to have been demonstrated with tetrahymanol ; in the sterol-free form of *Tetrahymena pyriformis*, tetrahymanol is localized in the membrane, which adjusts slightly the composition of its n-acyl chains (by increasing unsaturation) to accomodate the new, bulkier inserts (22). For other hopanoids, no direct evidence is available yet, and experiments with model biomembranes, possessing n-acyl lipids adjusted to fit better around the hopane skeleton (*i.e.* branched-chain acyl lipids), have not been reported.

* see Note at the end of the manuscript.

Distribution of Hopanoids in Procaryotes (16, 29)

	Hopanoids not detected	Hopanoids present
Cyanobacteria	*Synechococcus sp.* *Spirulina sp.*	*Anabaena sp.* *Nostoc sp.* (2 strains) *Synechocystis sp.*(2 strains)
Purple sulfur bacteria	*Chromatium sp.* ☆ *Amoebobacter sp.* ☆ *Thiocapsa sp.* ☆	
Green sulfur bacteria	*Chlorobium* (2 strains) ☆	
Purple non sulfur bacteria		6 strains of *Rhodopseudomonas* ☆ *Rhodospirillum* ☆ *Rhodomicrobium* ☆
Methylotrophs		7 strains of *Methylococcus* *Methylomonas* *Methylocystis* *Methylosinus*
Other bacteria	*Thiobacillus* (2 strains)	***Hyphomicrobium sp.*** *Nitrosomonas europaea* *Pseudomonas cepacia*
	Pseudomonas fluorescens *Ps. aeruginosa* *Ps. stutzeri* ***Ps. maltophilia*** *Ps. diminuta* *Xanthomonas campestris* *Rhizobium lupini* *Agrobacterium tumefaciens* *Caulobacter crescentus*	
		Azotobacter vinelandii *Acetobacter* (12 strains, covering 9 species)
	Moraxella (2 strains) *Escherichia coli* *Proteus vulgaris*	
	Bacillus subtilis +	*Bacillus acidocaldarius* +
	Sporosarcina lutea + *Clostridium paraputrificum* ☆+ *Streptococcus faecalis* ☆+ *Micrococcus luteus* + *Micromonospora sp.*+ *Actinoplanes brasiliensis* +	*Streptomyces chartreusi* +
	Desulfovibrio desulfuricans ☆	
Archaebacteria	*Methanobacterium thermoautotrophicum* ☆ *Halobacterium cutirubrum* *Sulfolobus acidocaldarius* *Thermoplasma acidophilum*	

Notes: a) Hopanoids present comprise diploptene, diplopterol and bacteriohopane-polyols (the latter usually preponderant).

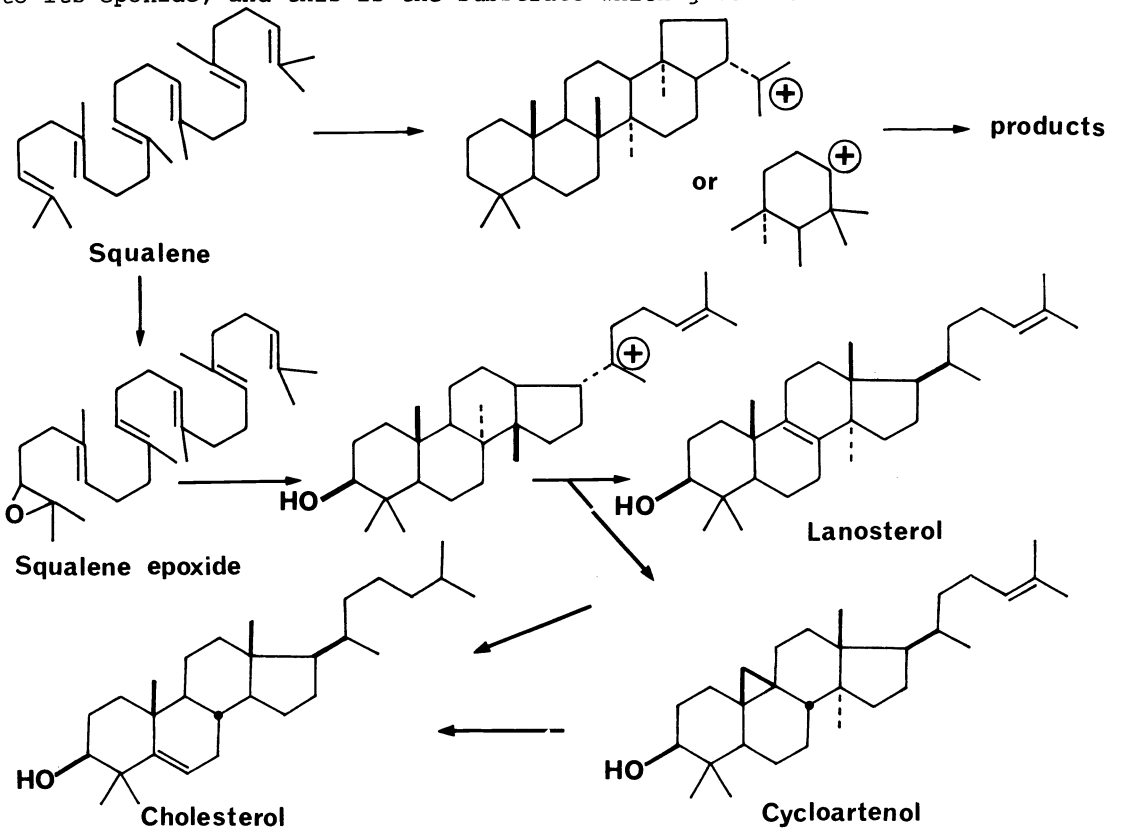
b) The strains studied by us are all defined by a proper collection number available on request, and will be reported in a later publication, together with the quantitative data.

+ Gram-positive bacteria; the others are Gram-negative (*Methanobacterium* variable)

☆ Grown anaerobically; the others are grown aerobically

"PRIMITIVENESS" OF THE HOPANOIDS, POTENTIAL PHYLOGENETIC PRECURSORS OF STEROLS

The hopanoids share in common with sterols to be biogenetically derived from the C_{30} acyclic triterpene precursor, squalene. Whereas squalene is directly cyclized to tetrahymanol (23) or fern hydrocarbons (24), it is first oxidized to its epoxide, and this is the substrate which gives sterols *via* lanosterol



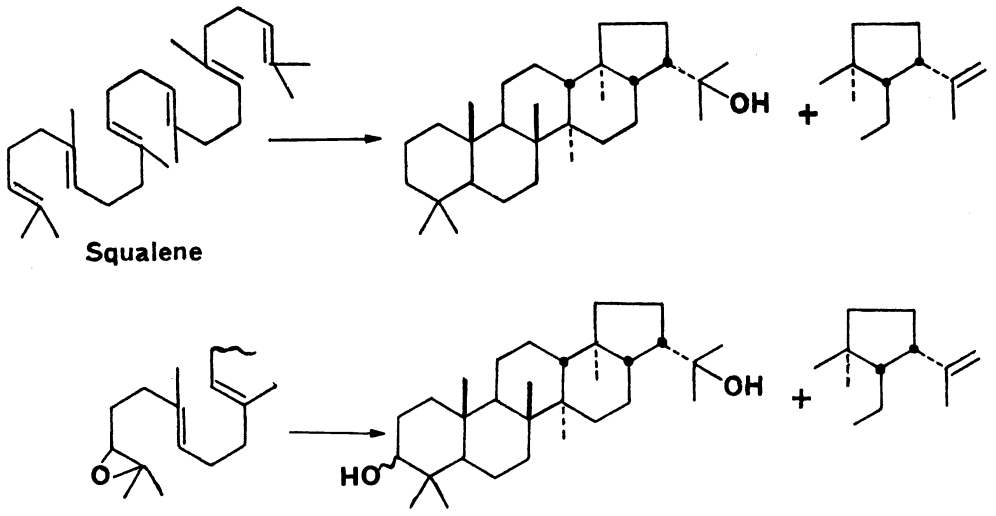
(in fungi and vertebrates) or *via* cycloartenol (in green plants) (25). The cyclization of squalene into hopane derivatives is more "primitive" by a number of criteria.

1- It leads to the amphiphilic products, diplopterol or tetrahymanol, by an anaerobic reaction, a *hydration* of the substrate. Thus, the OH group of the products derives from water, whereas, in cholesterol, it comes from air, and therefore has become available only after the onset of photosynthesis, if one accepts that the primitive atmosphere was oxygen-free (27).

2- The squalene-hopanoid cyclization is "simple", in that it is not accompanied by the extensive rearrangement necessary for sterol biosynthesis.

3- This cyclization is also "simple", in that it entails folding the squalene skeleton into an all-prechair conformation, whereas, for sterols, the conformation of squalene must partially approximate the more energetic boat form.

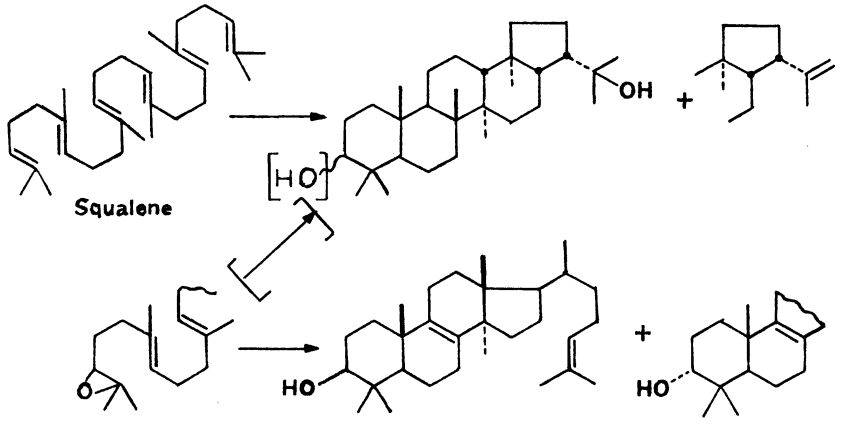
4- An enzymatic system cyclizing squalene to diplopterol in *Acetobacter rancens* has been found to display little substrate specificity : it accepts as well squalene itself, as both its R- and S-epoxides (which give the 3β- and 3α-



hydroxy derivatives of diplopterol)

All these primitive characteristics may imply that cyclization of squalene to functionally active amphiphilic molecules may have occurred already at a pre-aerobiotic stage of biochemical evolution.

Furthermore, it takes little molecular evolution to orient squalene towards sterols rather than triterpenes : the existence of a specific squalene oxidase, and a slight modification of the squalene cyclase to a specific squalene oxidocyclase , leading to the appropriate isomeric triterpene skeleton, that of lanosterol ; and finally a few oxidative degradation steps, removing the methyl groups. By sheer luck, it appears that one of the micro-organisms we have investigated illustrates beautifully how these evolutionary steps might have happened : a cell-free system derived from *Methylococcus capsulatus* cyclizes squalene to diploptene but squalene (R,S)-epoxide to a mixture of 3β- and 3α-lanosterols and of 3β- and 3α-hydroxydiploptenes ; the further oxidative steps to a 4α-methyl sterol are not carried out *in vitro* (29). In



this case, obviously, there are two distinct cyclases. The one operating on squalene has "become" specific to this substrate and does not operate on squalene epoxide (contrary to the case of *Acetobacter* mentioned above).

The other one operates on squalene epoxide but, unlike squalene oxidocyclases of higher organisms, also affects squalene (29). However, whereas the squalene oxidocyclases of higher organisms are specific for the cyclization of the 3S-epoxide only, the squalene oxidocyclase of *Methylococcus* has "not yet become" stereospecific, and cyclizes both the R- and S-epoxides. One can envision an evolutionary mechanism with, for example, the following sequence :

a- one enzyme, aspecific, acting on the only available substrate, say squalene.

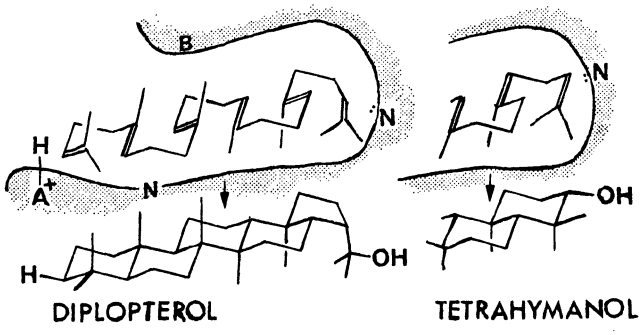
b- one enzyme, becoming specific to that substrate.

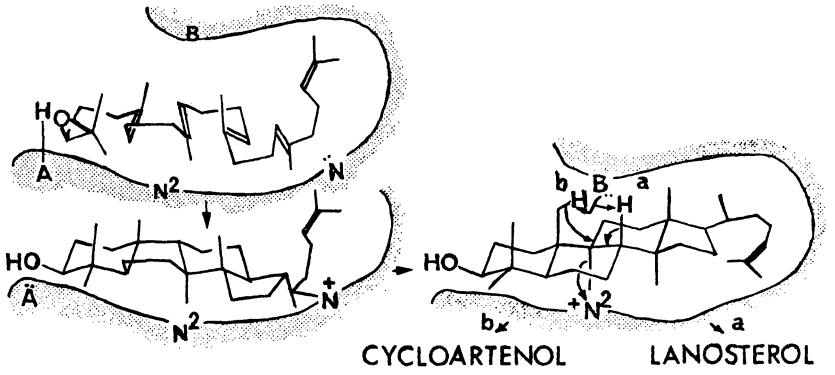
c- several isoenzymes, specific to the same substrate.

d- one of these isoenzymes becoming operative to squalene epoxide, once this new substrate becomes available (by a stereospecific epoxidation) but not yet stereospecific towards R or S epoxides ; the other isoenzymes, acting on squalene only, subsist.

e- the initial isoenzyme(s), specific to squalene, becoming obsolete once the function played by their products (*e.g.* tetrahymanol, diplopterol, etc.) is better carried out by sterols, formed by a completely stereospecific enzyme.

Acetobacter would be at step a, *Methylococcus* at step d, and plants, fungi or vertebrates at step e. In *Methylococcus*, the specificity of products observed furthermore implies compartments in the cell.
Let us finally remark that the gross nature of the active groups of the various cyclases implied is similar. The figure shows two schematic repre-



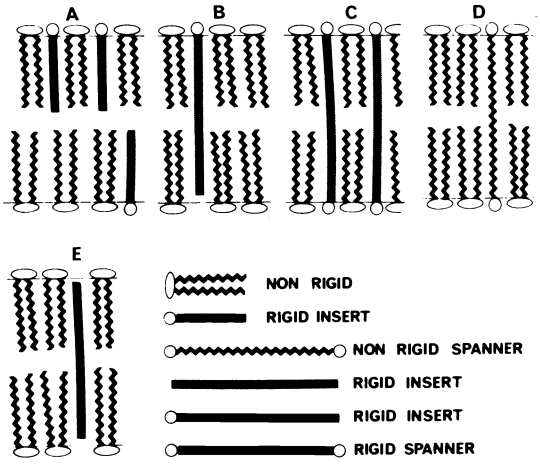


sentations of the requirements for the production of a hopane, and of a lanosterol derivative. The same acidic and nucleophilic + basic groups are found, at distances differing by a few Å only (30).

SUBSTITUTES OF HOPANOIDS AND OF STEROLS

Not every microorganism contains hopanoids. Many groups are devoid both of them and of sterols. Their function, if important for the life of a cell, must in these cases be fulfilled by other constituents. In fact, little is known of the structures of biomembranes of most taxonomic groups of micro-organisms, and in many cases there is simply no reason to assume that they conform to the usual model accepted for the eukaryotic membranes, and extended above for the hopanoid-containing ones.

It appears however that, at least in some cases, one can discern that related mechanisms may be at play to stabilize a double-layer membrane formed of amphiphilic partners, with a *ca.* 15 Å long hydrocarbon chain. Mechanisms A to E are either documented or at least plausible. Mechanism A is the classical one, with rigid amphiphilic inserts (sterols or hopanoids), about 15 Å long, oriented parallel to the lipidic chains and increasing the rigidity of the fluid lipid matrix with which they interact. Mechanism B or C would be a variant, with rigid amphiphilic inserts about 30 Å long, *i.e.* able to span completely across the double-layer. They could possess either one, or two hydrophilic terminal groups, and are probably exemplified by the many C_{40}-C_{50} carotenoids found in bacteria, and carrying, about 30 carbons apart, two hydroxylic groups or two glucosides - or, at one end only, one such group. Some typical structures are shown next page; many others are known.

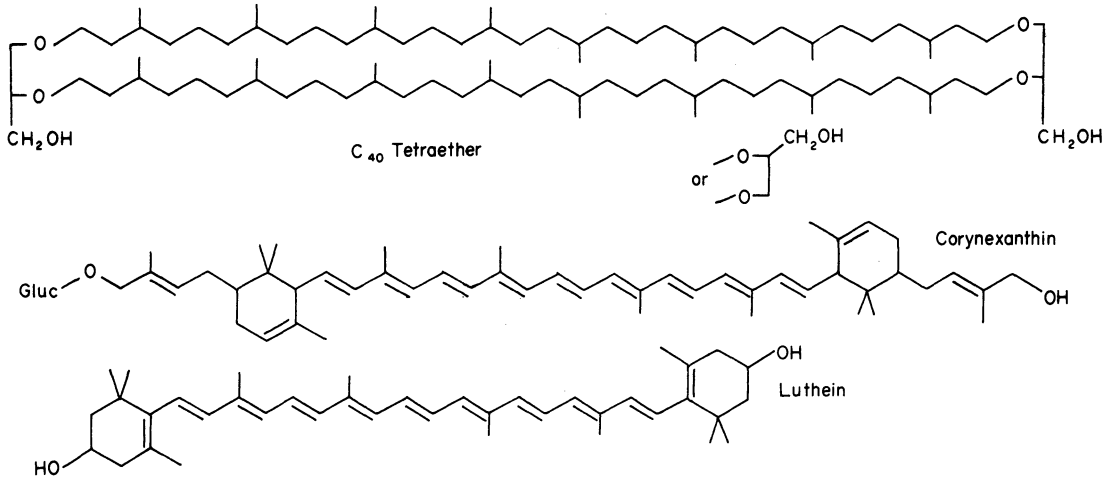


The localisation of carotenoids in bacterial membranes has been proved in one case (31) and it has been shown that, in one case, they can indeed stabilize the membranes (32)*. However, in other cases, the production of carotenoids is too large for them to be only structural elements of the membranes, or their structures are not suitable for such a role. Also, it is not at all certain that their orientation in the membranes is that implied by the drawings - and this applies also to mechanism E, which would necessitate a

* see Note at the end of the manuscript.

rigid carotenoid *hydrocarbon* to be oriented parallel to the lipid chains.

Mechanism D appears at first sight to be a variant of mechanism C : a chain of about 30 C would span across the membrane, linked at each end to a hydrophilic head group. However, in this case, the chain would not be rigid, and its effect on the double-layer would come from its being kept taut by the necessary inclusion of both head-groups in the aqueous phase. One such case appears to be present in the lipids of extreme thermo-acidophilic micro-organisms, the *Thermoplasma*, considered as part of a group of primitive bacteria, the archaebacteria. These contain, on the one hand glyceryl *ethers* of dihydrodiphytol (33) and of its unsaturated derivatives (32), and on the other hand the remarkable very large ring ethers shown (35) formed from a dimer of dihydrophytol, the structure of which we accept here as formed by ω,ω'-dimeri-



zation (in the reverse sense to carotenoids). This remarkable combination of partners having the same cross-section, and lengths in the exact ratio 1:2, represents probably rather ideally mechanism D. It is not only potentially operative in micro-organisms as extreme as that in which it has been studied. We have in fact obtained independent evidence for the presence of ethers of a C_{40} diol (or alcohol) of skeleton identical with that of *Thermoplasma*, by isolating the corresponding hydrocarbon from the insoluble organic matter (the "kerogen") of a lacustrine eocene shale (Messel, Germany). The procedure followed happened to have been identical to that later used for the bacterial lipids, so that a complete identification of the hydrocarbons from the fossil source and from presently living micro-organisms was possible (36). In the case of the Messel shale, geology excludes any episodes of extreme acidity, high temperature or high salinity, typical of the ecological niches of many present-day archaebacteria, but methanogenic bacteria, also part of this primitive group, may well have played a role in the diagenesis of the shale.

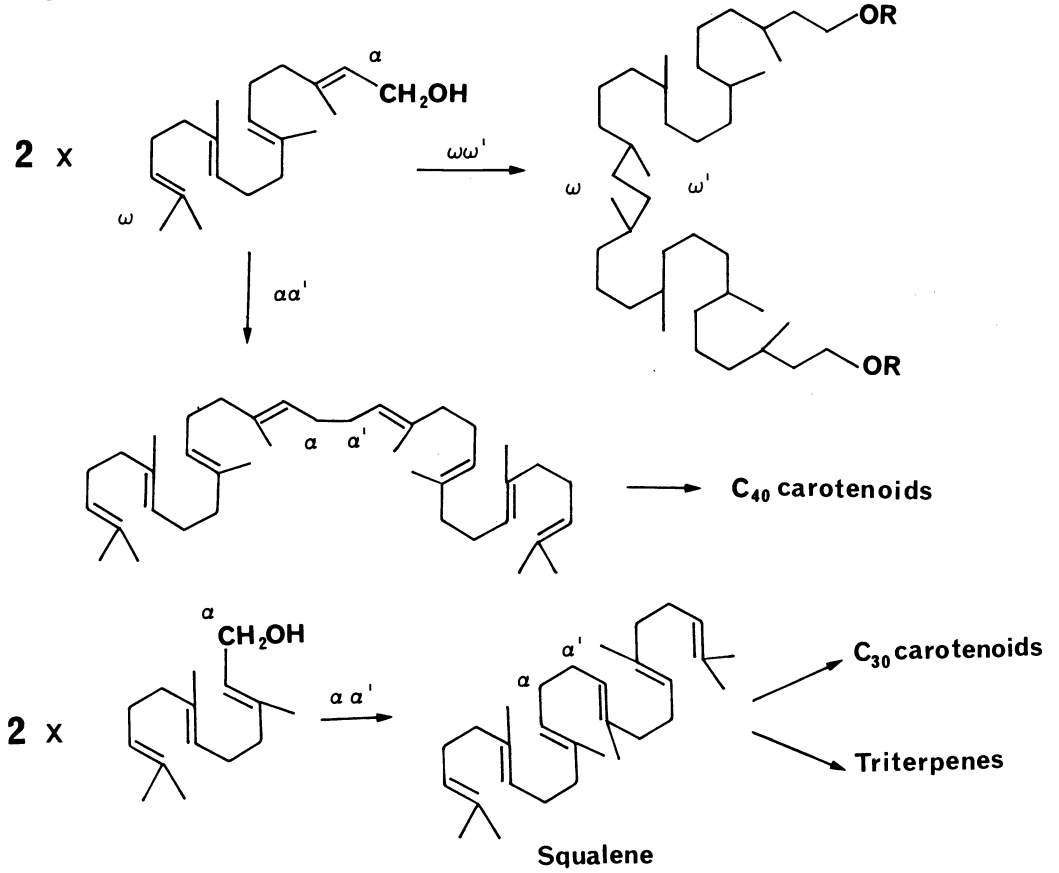
PHYLOGENETIC DERIVATION OF HOPANOIDS

It appears that, in organisms presumed to be the most primitive known to-day, the lipid part of membranes is of type D, with partners deriving both from the biosynthesis of acyclic diterpenes, followed by a) reduction - and, for

the C_{40} partners, b) dimerization by a mechanism which we still fail to understand. This is probably the most primitive type of stabilized bio-membrane, in view of its biosynthetic homogeneity.

The dimerization of the C_{20} precursor of dihydrophytol, geranyl-geraniol, can also occur by the α,α'-ends, to yield phytoene, the precursor of all carotenoids, which can therefore be considered as variants of the ω,ω'-dimer of mechanism D.

The precise sequence of steps operating to dimerize geranyl-geraniol into



phytoene is identical to that leading from farnesol, a required intermediate of the biosynthesis of geranyl-geraniol, to squalene. In bacteria, C_{30} carotenoids derived from squalene are known ; they simply represent products of the same mechanism operating on a simpler substrate than for the usual C_{40} carotenoids (37).

We thus see that carotenoids may have preceded, in the biochemical evolution, the substances derived from squalene, and in particular the hopanoids, and later the sterols.

We realize of course that such a hypothetical scheme, based on the available scant and scattered data, can be put to test in many ways - if not directly in the sense of a reconstruction of biochemical evolution. We propose it here, with all the limitations it still has, in the hope that it will provide a frame-work for later work by others and by ourselves.

One point remains to be briefly discussed : the presence of 3-deoxyhopanoids in ferns and a few "lower" plants. Provisionally, we entertain the (testable) hypothesis that they may originate in some of the organelles of the cells, maybe in the chloroplasts. If this were to be confirmed, it would be in perfect agreement with the theory of the "capture", which assumes that chloroplasts were initially free-living cyanobacteria, captured by a larger amoeboid pre-eucaryotic cell and having become obligatory symbionts (38). In the case of ferns, this would then imply that, contrary to other green plants and to *Tetrahymena*, production of hopanoids is not repressed by the sterols produced by other parts of the cell. This is only one example of experimental work suggested to test some of the hypotheses presented here.

Acknowledgements : We acknowledge very helpful discussions about a very preliminary form of the ideas presented here, with Professor Konrad Bloch and with Professor Roger Y. Stanier. Subsequent extension has owed much to many individuals, especially in discussions following oral presentations of our progressively more comprehensive hypothetical scheme.

Our work on Organic Geochemistry has been generously supported by the ELF company, the Compagnie Française des Pétroles, the Institut Français du Pétrole ; this, and our work in microbial biochemistry, form part of our activities supported by the Centre National de Recherche Scientifique, and by grants from Hoffmann-La Roche (Basle), Roure-Bertrand (Grasse) and Rhône-Poulenc (Paris).

Cet article est dédié au Professeur Edgar LEDERER, à l'occasion de son soixante-dixième anniversaire, en témoignage de sincère admiration et de fidèle reconnaissance pour la confiance qu'il a témoignée, et communiquée, à l'un de nous (G.O.) au début de la carrière de celui-ci.

Note added after the first submission of this paper to the Scientific Editor

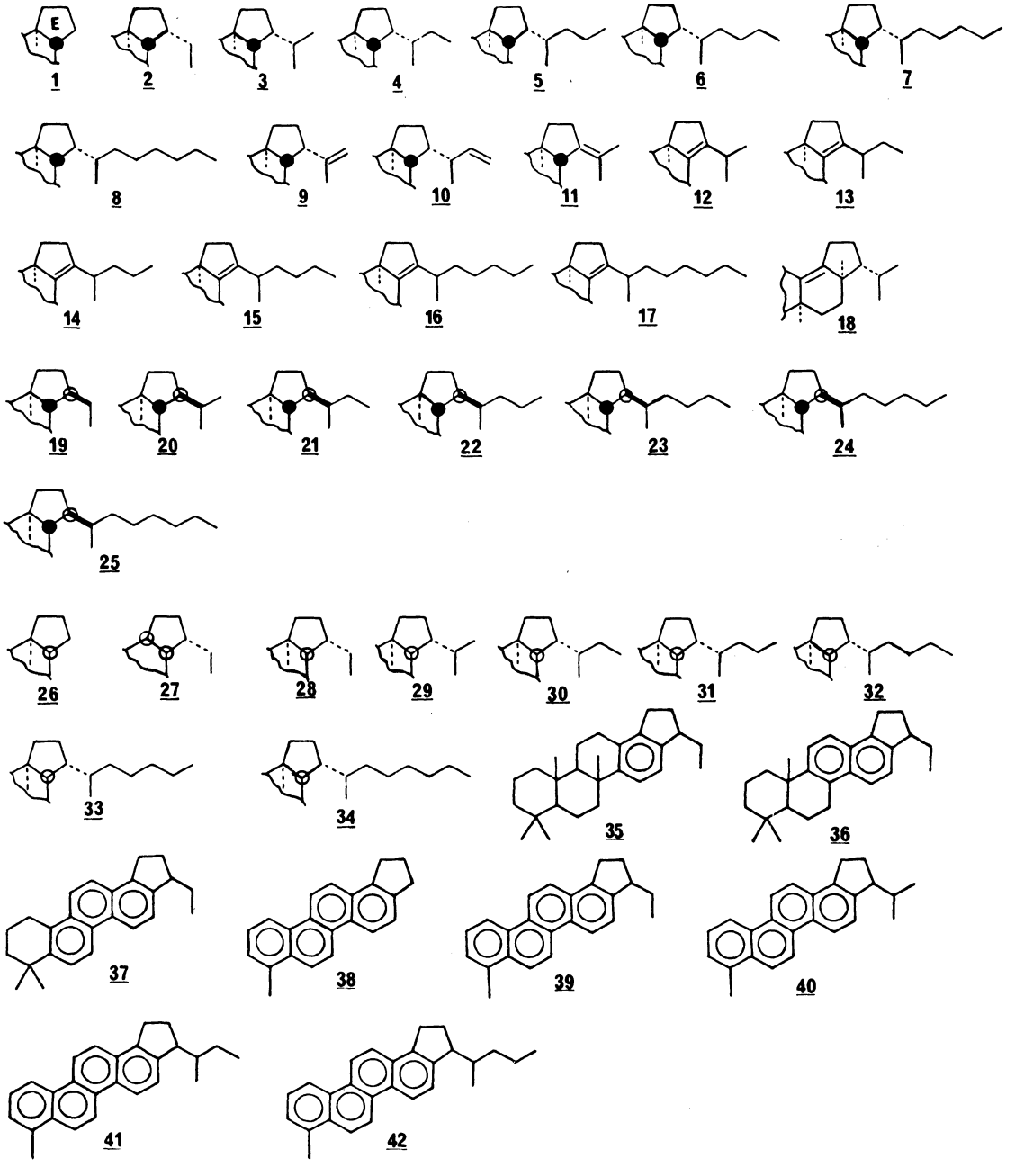
We have become aware of the paper by Nes (39), which also proposes that sterols may be replaced, in some Procaryotes, by sterol-like molecules such as Tetrahymanol. However, we feel this is the only convergence between Nes' views and ours. One of us (G.O.) has attended a lecture by Dr Nes on this subject and, aware of the dangers linked with cryptomnesia, acknowledges that this lecture may have led to his initial latent interest in the evolution of biomembranes.

Manuscript typed by Denise Voegel; drawings by Marie-Claire Demézy.

H O P A N O I D S

isolated from sediments :

TABLE I : HYDROCARBONS

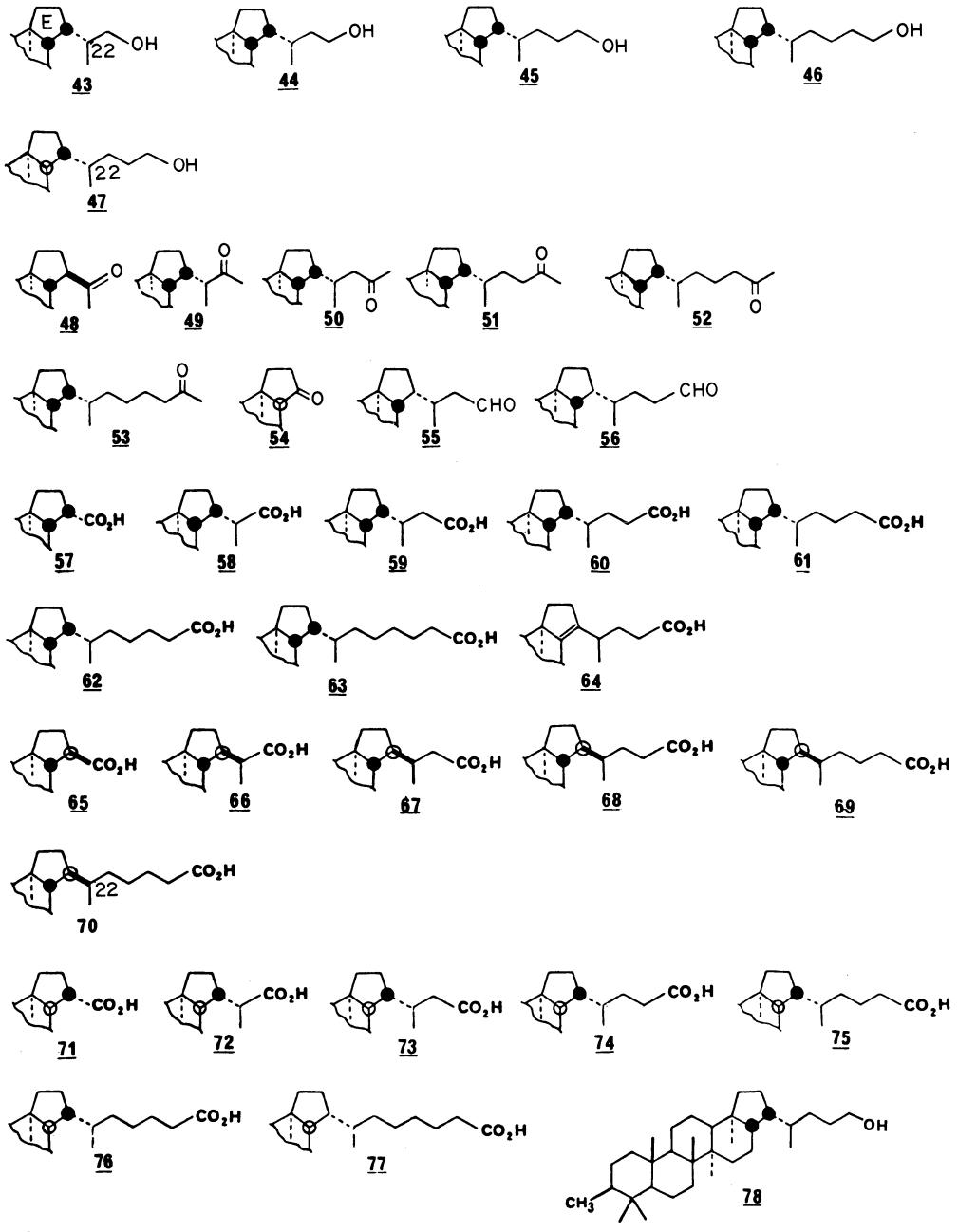


Refs. 2, 5, 6, 7, 8, 9, 11, 18.

H O P A N O I D S

isolated from sediments :

TABLE II : OXYGENATED DERIVATIVES



Refs. 2, 5, 18.

Tables I and II: C-22 diastereomers (R, or S, or both in matured sediments) for substances 4-8, 10, 13-17, 21-25, 30-34, 41-47, 49-53, 55-56, 59-64, 67-70, 73-78.

REFERENCES

1. T.K. DEVON and A.I. SCOTT, Handbook of Naturally Occurring Compounds, Vol. II : Terpenes, Academic Press, New York (1972).
2. E.g. a) G. EGLINTON and M.T.J. MURPHY, Eds., Organic Geochemistry ; Methods and Results, Springer Verlag, Heidelberg (1969).
   b) Advances in Organic Geochemistry : a series of yearly monographs, various publishers.
3. E.g. P. ALBRECHT and G. OURISSON, Angew. Chem. Int. Ed. Engl., 10, 209 (1971).
4. P. ALBRECHT and G. OURISSON, Tetrahedron, 28, 2880 (1972).
5. A. VAN DORSSELAER, P. ALBRECHT and G. OURISSON, Bull. Soc. Chim. France, 165 (1976).
   A. VAN DORSSELAER, Dr. Sc. Thesis, Université Louis Pasteur, Strasbourg (1975).
6. In the case of hop-17(21)-ene, isolated from the Messel shale.
   A. ENSMINGER, Dr. Sc. Thesis, Université Louis Pasteur, Strasbourg (1977).
7. W. SEIFERT, J.M. MOLDOWAN, G.W. SMITH and E.V. WHITEHEAD, Nature, 271, 436 (1978).
8. I.R. HILLS and E.V. WHITEHEAD, ibid., 209, 977 (1966).
9. C. SPYCKERELLE, A.C. GREINER, P. ALBRECHT and G. OURISSON, J. Chem. Research, (S), 330 ; (M), 3746 (1977).
10. P. ARPINO, P. ALBRECHT and G. OURISSON, in Advances in Organic Geochemistry, 1971, p. 173, Pergamon Press, Oxford (1972).
11. A. VAN DORSSELAER, P. ALBRECHT and G. OURISSON, Bull. Soc. Chim. France, 165 (1977).
12. ANON. (alias G. OURISSON), Images de la Chimie, 1977, Ed.du CNRS, Paris, 45 (1978).
13. a) H.J. FÖRSTER, K. BIEMANN, W.G. HAIGH, N.H. TATTRIE and J.R. COLVIN, Biochem. J., 135, 133 (1973).
    b) T.A. LANGWORTHY and W.R. MAYBERRY, Biochem. Biophys. Acta, 431, 570 (1976).
14. M. ROHMER and G. OURISSON, Tetrahedron Letters, 3633 (1976).
15. *Id.*, *ibid.*, 3641 (1976).
16. M. ROHMER, P. BOUVIER and G. OURISSON, Proc. Nat.Acad.Sc.USA, in the press.
17. M. ROHMER and G. OURISSON, Tetrahedron Lett., 3637 (1976).
18. M. DASTILLUNG, Dr. Sc. Thesis, Université Louis Pasteur, Strasbourg (1976).
19. G. BERTI and F. BOTTARI, Progress in Phytochemistry, 1, 589 (1969).
20. R.L. CONNER, J.R. LANDREY, C.H. BARNS and F.B. MALLORY, J. Protozool., 15, 600 (1968).

21. a) C.W. BIRD, C.M. LYNCH, F.J. PIRT, W.W. REID, C.J.W. BROOKS and B.S. MIDDLEDITCH, Nature, 230, 473 (1971).
b) Structures defined by P. BOUVIER, M. ROHMER, P. BENVENISTE and G. OURISSON, Biochem. J., 159, 267 (1976).

22. K.A. FERGUSON, F.M. DAVIS, R.L. CONNER, J.R. LANDREY and F.B. MALLORY, J. Biol. Chem., 250, 6998 (1975).

23. J.M. ZANDER, J.B. GREIG and E. CASPI, *ibid.*, 245, 1247 (1970).

24. D.H.R. BARTON and G.P. MOSS, Chem. Com., 261 (1966).

25. L.J. GOAD, in T.W. GOODWIN, Ed., Natural Substances Formed Biologically from Mevalonic Acid, p. 45, Academic Press, London (1971).

26. K. BLOCH, in A. KORNBERG, B.L. HORECKER, L. CORNUDELLA and J. ORO, Eds., Reflections on Biochemistry, p. 143, Pergamon Press, Oxford (1976).

27. Photodissociation may have led to pre-biotic formation of molecular oxygen. For a recent discussion, see K.M.TOWE, Nature, 271, 657 (1978).

28. C.ANDING, M.ROHMER and G.OURISSON, J.Amer.Chem.Soc., 98, 1274 (1976).

29. P. BOUVIER, Dr. Sc. Thesis, Université Louis Pasteur, Strasbourg (1978).

30. R. ANTON, Dr. Sc. Thesis, Université Louis Pasteur, Strasbourg (1974).

31. a) S.C. KUSHWAHA, M. KATES and W.G. MARTIN, Can. J. Biochem., 128, 751 (1975).
b) M. ANVAR, T. HASAN KHAN, J. PREBBLE and P.F. ZAGALSKY, Nature, 270, 538 (1977).

32. L. HUANG and A. HAN, Biochim. Biophys. Acta, 352, 361 (1974).

33. M. KATES, in Ether Lipids, Chemistry and Biology, F.S. SNYDER, Ed., p.351, Academic Press, New York (1972).

34. M. DE ROSA, S. DE ROSA, and A. GAMBACORTA, Phytochemistry, 15, 1995 (1976).

35. a) M. DE ROSA, S. DE ROSA, A. GAMBACORTA, L. MINALE and J. BU'LOCK, *ibid.*, 16, 1961 (1977).
b) T.A. LANGWORTHY and W.R. MAYBERRY, Soc. Gen. Microbiol. Proc., 3, 165 (1976).

36. W. MICHAELIS and P. ALBRECHT, unpublished results.

37. The dimerization of geranyl-geraniol to phytoene and that of farnesol to squalene involve a similar cyclopropane-alcohol. However, this is opened with concomitant dehydration in the first case, and reduction in the second. The precursor of bacterial C_{30} carotenoids appears not to be known. It would be of considerable interest to define whther it is squalene or 12(13)-dehydrosqualene.

38. A.F.W. SCHIMPER, Bot. Z., 41, 105 (1883). For a recent discussion, see M.A. RAGAN and D.J. CHAPMAN, A Biochemical Phylogeny of the Protists, Academic Press, New York (1978).

39. W.R. NES, Lipids, 9, 596 (1974).