Prog. Lipid Res. Vol. 27, pp. 153–175, 1988
Printed in Great Britain.

0163-7827/88/$0.00 + 0.50

THE LIPIDS OF ARCHAEBACTERIA

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CONTENTS

I. INTRODUCTION

Living organisms are assumed to stem from the early divergence of a putative progenote into three primary kingdoms: eubacteria, archaebacteria and eukaryotes. The archaebacteria have been recognized as a phylogenetically separate group of microorganisms for only a few years, although various species belonging to this new kingdom have been well studied for some time and described as eubacteria.[77,78]

The archaebacteria group was initially defined by Woese and collaborators on the basis of partial ribosomal RNA 16S sequence comparison;[31] this successively has been well supported by a great body of other general biochemical features, such as RNA polymerase, transfer RNA, 5S ribosomal RNA, translation system, cell wall and lipids.[76,80] Some characteristics of the archaebacteria are similar to those of the eubacteria, such as small size and structural simplicity, absence of a nuclear membrane and low deoxyribonucleic acid content. However, they differ from them and in most other aspects are more closely related to the eukaryotes, such as in the nature of their translation system, the sequence of their 5S RNA and that of ribosomal proteins, presence of histone-like proteins, etc.[31,34,57,59,67,76,78,80] A recent cross-hybridization experiment with total ribosomal RNA and DNA from various archaebacteria, eukaryotes and eubacteria has shown the archaebacteria to be a monophyletic holophyletic group, as different from eubacteria as they are from eukaryotes.

Archaebacteria are quite interesting organisms from the point of view of the early evolution of life, since they were probably the dominant forms of life in the primaeval biosphere.[2,31,77,78] In fact, many aspects of their metabolism seem to be suitable for the

ecological parameters which characterized the early history of life on earth. In this respect, the archaebacteria are very important as they give us some indication of the early events in the evolution of cells, thus contributing to a better understanding of the universal ancestor.

All archaebacteria classified thus far are confined within a few ecological niches,[80] such as very high temperature and low pH, very high salt concentration or anaerobic conditions. The initial archaebacterial phylogeny was, in fact, structured essentially along three phenotypic lines: halophiles which require saturated NaCl habitats, methanogens which thrive in habitats where very low redox potential occurs, and thermophiles confined to habitats at temperatures between 50° and 110°C.[31,80] Although archaebacterial taxonomy is complex and controversial, the new recent isolates all conform essentially to one of the three original types.[1,40,41,80,82]

However, recent studies by Yang *et al.*[82] based on the comparison of complete 16S ribosomal RNA sequences confirm, refine and extend earlier archaebacterial phylogeny. Thus, the archaebacteria could now be divided into two major branches: the 'sulfur-dependent' thermophilic microorganisms, and the methanogens, which comprise also the extreme halophiles and two thermophilic species: *Thermoplasma acidophilum* and *Thermococcus celer*, although the latter could be considered to represent a separate third division.[79,80,82,84,87]

The first group comprises species closely related and phenotypically quite similar among themselves. In fact, they have similar metabolic patterns, are thermophilic, and are strict or facultative anaerobes, with the exception of the *Sulfolobus* species, which are aerobes[29,30,60,79,80,82,84,87] The species of the second group, in contrast, have more varied characteristics; in fact, this division comprises all archaebacterial phenotypes: methanogens, halophiles, and thermophiles.[80,82]

According to Woese and colleagues, the thermoacidophilic archaebacterial phenotype, which appears in both the major groups, seems to be the ancestral archaebacterial type.[79] It is interesting to note that species of the *Pyrodictium* genus belonging to the thermophilic phenotype of archaebacteria have represented, up until now, the only purified microorganisms able to grow optimally at 105°C and to tolerate up to 110°C.[63] Baross and Deming[3] have reported the existence of microorganisms at 250°C, but at the moment cultures able to survive at this temperature are not available.

Archaebacteria are characterized by a wide metabolic diversity and a high degree of morphological variability, roughly comparable to that found in eubacteria. This group includes aerobes, anaerobes, autotrophs, heterotrophs, thermophiles, acidophiles, phototrophs, cocci, rods, disk-shaped and pleiomorphic forms. They range in size from 0.2 μm to 10 μm, some are motiles while others are not; most have rigid or plastic walls, but there are also wall-less species.[18,64,80]

Upon comparing molecular components of archaebacteria with prokaryotes and eukaryotes, one is struck by the extensive chemical differences observed in the lipids, especially in comparison with the other components, whose essential chemical features appear to be preserved. In fact, the structure of membrane lipids remains one of the major distinctions between all archaebacteria and other organisms.

All the hitherto identified membrane lipids of the archaebacteria are characterized by unusual structural features, which can be considered to be a specific and useful taxonomic marker of these microorganisms.[16,29,45,80] Unlike the eubacterial and eukaryotic lipids, which are based on ester linkages formed by condensation of alcohols and fatty acids, archaebacterial lipids are mainly isopranyl glycerol ethers. These molecules are obtained by the condensation of glycerol (or more complex polyols) with isoprenoid alcohols of 20, 25 or 40 carbon atoms. Moreover, it is worth noting that the C-2 configuration of the glycerol in archaebacterial lipids is the opposite of the conventional diglyceride configuration.

In fact, the glycerol ethers of archaebacteria contain a 2,3-di-*O*-*sn*-glycerol moiety, while the naturally occurring glycerophosphatides or diacyl glycerols are known to have an *sn*-1,2-glycerol stereochemistry.[16,45,80]

The uniqueness of archaebacterial lipid structure also suggests important differences in their topology within the membrane structure.[23,80] We may wonder how lipids with such different chemical compositions can manage to perform similar functions, and why that chemical variability is required for life.

Recently special interest has been directed toward the detection in ancient geological deposits of molecules related to natural compounds occurring in archaebacteria, in particular to their unusual lipids.[18,35] Even though the organisms from which these molecular fossils derive are barely identifiable, the evidence directly demonstrates the considerable antiquity of the archaebacteria, which have existed for at least one billion years, and possibly even for more than three billion years.[80]

Many reviews[16,18,38,45] on archaebacterial lipids have been published, but they have been restricted mostly to the unusual isoprenoid ether lipids, and up until now a general overview of other classes of lipid molecules such as quinones has not been available. Moreover, a wealth of information has been obtained, more recently, concerning structural definition, biosynthesis and distribution of archaebacterial lipids. Therefore, it seems to be of interest to give a general survey of both classes of lipids, isoprenoid ethers and quinones, found in the archaebacteria, focusing our attention on three different aspects: structural identity of such unusual molecules, their spectroscopic properties and distribution among archaebacteria.

II. ISOPRENOID GLYCEROLIPID: STRUCTURAL TYPES, SPECTROSCOPIC PROPERTIES AND DISTRIBUTION IN ARCHAEBACTERIA

Although some authors report traces of fatty acids detected in lipid preparations of some archaebacteria,[80] ether glycerol lipids are the main components of all archaebacteria investigated so far.[16,38,45,60,64,80] Isopranyl glycerol ethers represent the hydrophobic pattern of the archaebacterial complex lipids, whereas mainly fatty acid ester linked glycerol lipids are found in the eukaryotes and eubacteria.

In 1962 Kates and coworkers first found in the membrane of the extreme halophiles lipids based mainly on ethers of glycerol and C_{20} saturated isoprenoid alcohols.[38] Subsequently, lipids were found in all other archaebacteria which were based on ether linkage between glycerol, with 2,3-*sn*-configuration, or other polyol(s) and isopranoid alcohols with 20, 25 or 40 carbon atoms.[16,45,80]

The complex lipids of archaebacteria are mainly based on two classes of isoprenoid ether core lipids that are classified as diethers and tetraethers. These compounds can be easily obtained by acid or alkaline hydrolysis of complex lipids.[16,45,80]

A. *Structural Types*

1. *Diethers*

The diethers found in archaebacteria are reported in Fig. 1.

Figure 1a shows the 2,3-di-*O*-phytanyl-*sn*-glycerol formed by condensation, via ether linkage, between two C_{20} isopranoid alcohols and a glycerol molecule. The three chiral centres of the isopranoid chain have the 3R, 7R, 11R configuration. The diether is dextrorotatory, and has an *sn*-2,3-glycerol configuration, opposite to that of the naturally occurring diacylglycerols.[16,38,45,80]

Diethers b and c (Fig. 1) differ from the 2,3-di-*O*-phytanyl-*sn*-glycerol in the nature of alkyl residues; in fact, in b, a sesterterpanyl chain substitutes the phytanyl residue at C2 of the glycerol, while in c, two sesterterpanyl alcohols are present.[16,18]

The diether d (Fig. 1) is a macrocyclic glycerol diether with a 36-member ring, which originates from the condensation of a glycerol, in the 2,3-*sn*-configuration, with the 3,7,11,15,18,22,26,30-octamethyldotriacontane-1,32-diol.[10]

The diether e (Fig. 1) is a tetritol-diphytanyl-diether in which the α and β OH groups of the tetritol form ether linkages with two C_{20} isopranoid alcohols identical to those in

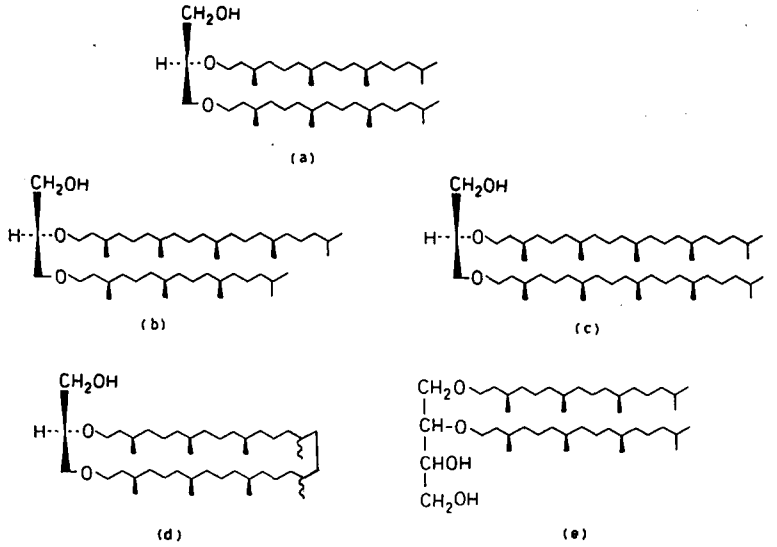


FIG. 1. Isoprenoid diethers basic components of some complex lipids of archaebacteria. a: 2,3-di-*O*-phytanyl-*sn*-glycerol; b: 2-*O*-sesterpanyl-3-*O*-phytanyl-*sn*-glycerol; c: 2,3-di-*O*-sesterpanyl-*sn*-glycerol; d: macrocyclic diether; e: tetritol-dyphytanyl-diether.

the glycerol diether a (Fig. 1). So far, this tetritol diether represents the only example among archaebacteria of a core lipid component in which the glycerol is absent.[20]

2. *Tetraethers*

Two series of tetraethers with unprecedented molecular architecture have been characterized as core lipids in archaebacteria. While classic lipids, in fact, are monopolar amphipatic molecules, these tetraethers are bipolar amphipatic molecules characterized by the presence of two (equivalent or not) polar heads, linked by two C_{40} alkyl components which are practically twice the average length of the aliphatic components of classic ester lipids.

The more common structural type is the glycerol tetraether a (Fig. 2), consisting of a 72-member ring with 18 stereocentres, formed by two *sn*-2,3-glycerol moieties bridged through ether linkages by two isopranoid C_{40} diols formally derived from head to head linkage of two *O*-phytanyl residues. In the other members of this class, generally named glycerol-dialkyl-glycerol-tetraethers (GDGT), the aliphatic component (3R,7R,11R,15S,18S,22R,26R,30R)-3,7,11,15,18,22,26,30-octamethyldotriacontane[35a] contains up to four cyclopentane rings (Fig. 2b–i), originated by the formation of C—C bonds between methyls in 3,7,26,30 and methylenes 6,10,23,27, respectively.[16,45,80]

The GDGT are all dextrorotatory, indicating that both glycerols have the *sn*-2,3-stereoconfiguration and consequently, the primary —OH groups of the two opposed glycerols are in the *trans* configuration. Moreover, it is interesting to note the high degree of structural specificity of GDGT; in fact, from the 5 known C_{40} isoprenoids, differently cyclized (two of which are not symmetrical end-to-end), there are 27 possible pairs, of which only 9 occur in the GDGT. In tetraethers a–e (Fig. 2), the two identical C_{40} isoprenoids are antiparallel, while in the series f–i, with two differently cyclized C_{40} isoprenoids, the more cyclized end of the C_{40} chain is specifically linked to the primary carbinol of the glycerol.[22,24]

The second class of tetraethers (Fig. 2a′–i′) is similar to GDGT, with a more complex branched nonitol replacing one of the glycerols. These tetraethers, simply named glycerol-dialkyl-nonitol tetraethers (GDNT), show the same structural and stereochemical constraints previously reported for GDGT. In particular, it is worth noting that the presence of two different polar heads increases the number of possible C_{40} isoprenoid pairs to 45, of which only 9 (Fig. 2a′–i′) occur in lipids of archaebacteria.[24]

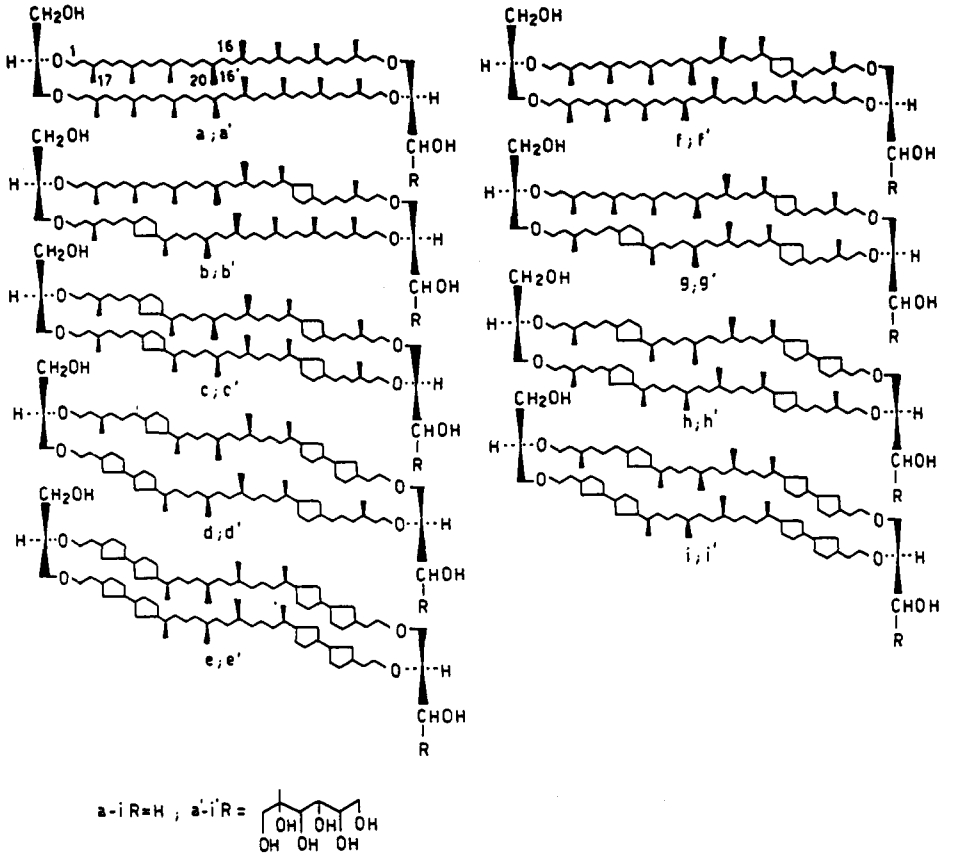


FIG. 2. Isoprenoid tetraethers basic components of some complex lipids in archaebacteria. a–i: Glycerol-dialkyl-glycerol tetraethers (GDGT); a'–i': Glycerol-dialkyl-nonitol tetraethers (GDNT).

3. *Minor Isoprenoid Ethers Found in Archaebacteria Lipids*

In addition to the principal structural types described before, two minor compounds (Fig. 3a,b) are found as core lipids in archaebacteria.

Figure 3a shows the 3-*O*-phytanyl-*sn*-glycerol in which one phytanyl residue etherifies specifically the carbinol 3 of the glycerol residue.[20] In Fig. 3b is reported the structure of the glycerol-trialkyl-glycerol tetraether based upon two glycerols linked by four ether bonds with two molecules of (3R,7R,11R)-3,7,11,15-tetramethylesadecane-1-ol and one of (3R,7R,11R,15S,18S,22R,26R,30R)-3,7,11,15,18,22,26,30-octamethyldotriacontane-1,32-diol.[24]

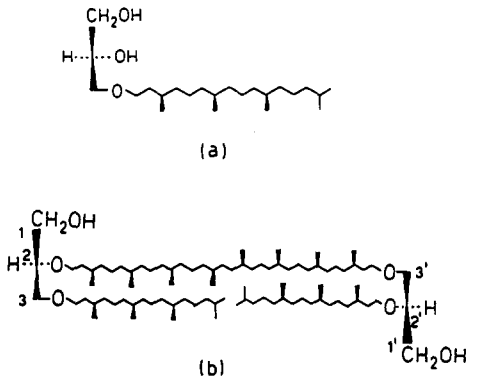


FIG. 3. Isoprenoid ethers, minor components of some lipids of archaebacteria. a: 3-*O*-phytanyl-*sn*-glycerol; b: glycerol-trialkyl-glycerol tetraether.

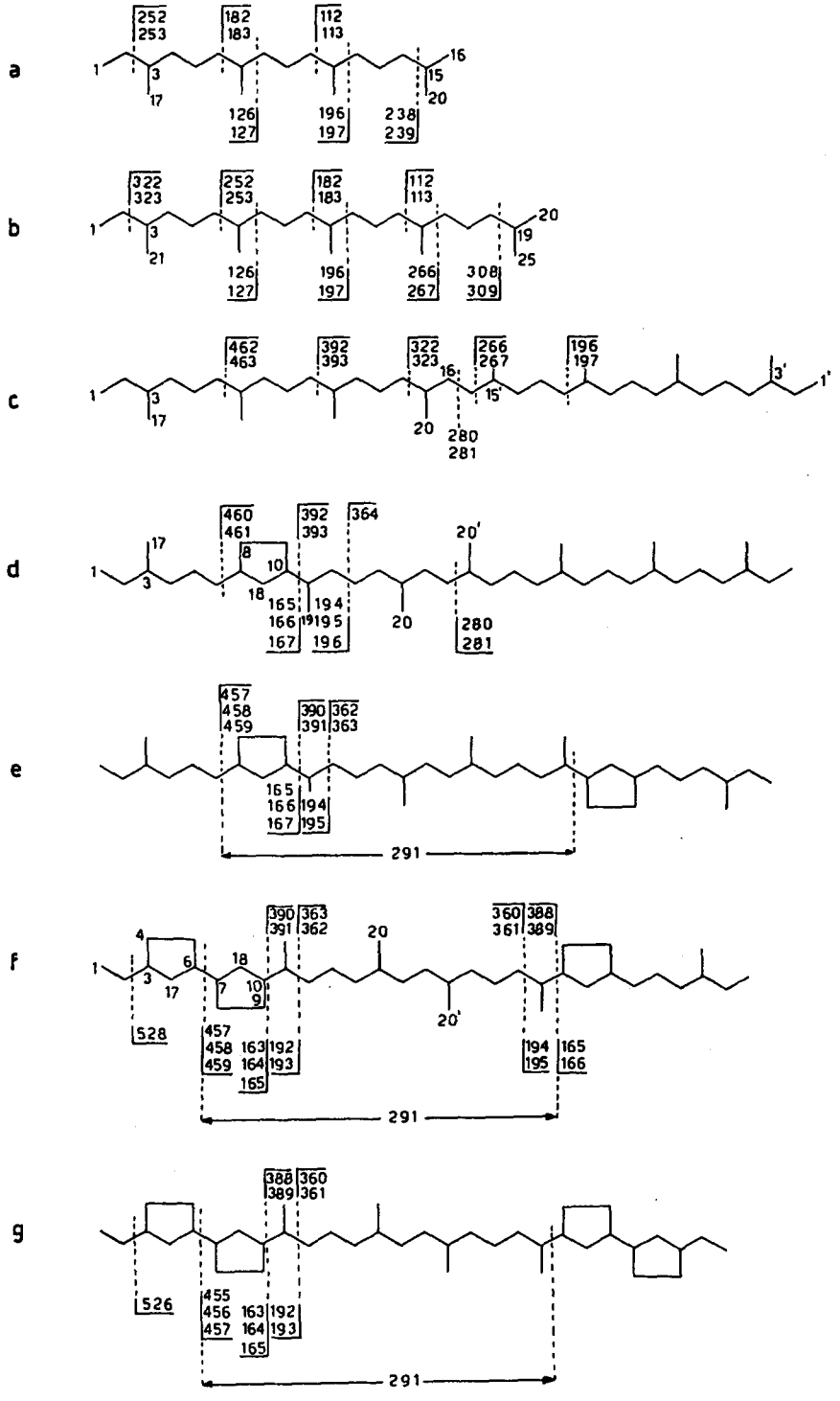


FIG. 4. Electron impact MS fragmentation patterns of C_{20}, C_{25} and C_{40} hydrocarbons obtained by HI-$LiAlH_4$ degradation of ether core lipids of archaebacteria.

B. *Spectroscopy Properties*

1. *Mass Spectrometry (MS)*

Skeletal characterization of ether core lipids of archaebacteria is mainly based on MS studies. Figure 4 shows the fragmentation pattern of C_{20}, C_{25} and C_{40} hydrocarbons,

obtained by degradation of ether core lipids with HI to give C_{20}, and C_{25} mono-iodides and C_{40} di-iodides, which were converted to the corresponding C_{20}, C_{25} and C_{40} hydrocarbons[17,26,38] by reduction with $LiAlH_4$.

Hydrocarbons with deuterium on terminal carbons, useful in assigning certain ions in the mass spectra of C_{40} isoprenoid chains, are prepared by using $LiAl^2H_4$.[13] The electron impact–mass spectra (EI–MS) of $C_{20}H_{42}$ (M^+ m/e 282) and of $C_{25}H_{52}$ (M^+ m/e 352) hydrocarbons, characterized by two series of cleavages, both with and without H transfer to the uncharged fragment α to =CHMe groups, indicate the presence of a regular 'head-to-tail' structure in both isoprenoids. The EI–MS of the acyclic hydrocarbon $C_{40}H_{82}$ (M^+ m/e 562, $^2H_2$ analogue 564)[13] can be rationalized in terms of (i) a series of cleavages, both with and without H transfer to the uncharged fragment, α to =CHMe groups and removing successively 7,5,5,4 and 5C atoms, finally generating the base peaks at m/e 196/197, and (ii) a median cleavage (again with or without H transfer) β to two central =CHMe groups, giving fragments at m/e 280/281. The two types of fragmentation are independent, since in the chemical ionization spectra only the molecular ion and the base peak from series (i) appear. All the fragmentation peaks are shifted by 1 mass unit in the spectra of $^2H_2$-hydrocarbon. The EI–MS of the monocyclic hydrocarbon $C_{40}H_{80}$ (m/e 560 and m/e 562 in the $^2H_2$-hydrocarbon)[13] shows significant differences. While the molecular ion and the first major fragments at m/e 460/461 have one formal unsaturation, the next fragments at m/e 392/393 do not. Cleavage at the same bond also generates a group of strong peaks at m/e 165/167 (base peak 166). There are corresponding cleavages apparently at an adjacent bond, differing by 28 mass units; the central cleavage gives only fragments of m/e 280/281, presumably because the other moiety, for which peaks at m/e 278/279 would be expected, generates instead the smaller fragments at m/e 166 and 194. As before, all the fragmentation peaks are shifted by 1 mass unit in the spectrum of $^2H_2$-hydrocarbons. For the bicyclic hydrocarbon $C_{40}H_{78}$, the molecular ion m/e 558 (560 in the $^2H_2$-hydrocarbon)[13] and the fragments at m/e 457/459, 390/391 and 362/363 (all shifted by 1 mass in the spectra of the $^2H_2$-hydrocarbon) show a further deficit of two mass units, when compared with the data for monocyclic hydrocarbon, with the important counterparts at m/e 165/166 and 194/195 appearing as before. The next major fragment in the spectrum has m/e 291, corresponding to a C_{21} fragment with two formal unsaturations. This peak is not shifted in the spectrum of the $^2H_1$-hydrocarbon; it is, therefore, formed by cleavage at each end of the molecule and retains only one of the two rings originally present. The EI–MS of the tricyclic hydrocarbon $C_{40}H_{76}$ (m/e 556)[12] is similar to that of the bicyclic hydrocarbon, but fragments at m/e 388/389, 360/361, 192/193, 163/164 show a further deficit of two mass units, when compared with the data for the bicyclic hydrocarbon. This evidence and the fragment at m/e 528 allow us to localize the third cyclopentane ring on the isoprenoid skeleton. The structure of tetracyclic hydrocarbon (m/e 554)[12] is supported by entirely similar data, with significant differences arising from the symmetry of its structure.

Figure 5 shows the fragmentation pattern of acetate derivatives of isoprenoid diethers a, b and c of Fig. 1. EI–MS of acetylated glycerol diether derivatives does not give a molecular ion, but shows peaks, respectively, at m/e 635 (C_{20}, C_{20} glycerol diether acetate a, Fig. 5), 705 (C_{20}, C_{25} glycerol diether acetate b, Fig. 5) and 775 (C_{25}, C_{25} glycerol diether acetate c, Fig. 5) corresponding to the loss of the CH_3CO_2 group. At m/e values higher than 250, there are three critical peaks associated with the loss of aliphatic chains linked at the α- or β-glycerol carbons. In particular, in the C_{20}, C_{25} glycerol diether, peaks at m/e 453 and 467 are related to the loss of CH_2—O—$C_{20}H_{41}$ and O—$C_{20}H_{41}$, respectively, and the former shows that the C_{20} residue is located on the α-glycerol carbon, while the peak at m/e 397, associated with the loss of O—$C_{25}H_{51}$, places this residue on the β-carbon. The absence of a peak of significant size at m/e 383 in the diether b shows that these locations are unique.

Different MS ionization methods, in addition to EI, can be used efficiently to define structures of higher molecular weight ether isoprenoids, such as the GDGT and GDNT (Fig. 2),[24] and of polar complex lipids of archaebacteria. In particular, field desorption

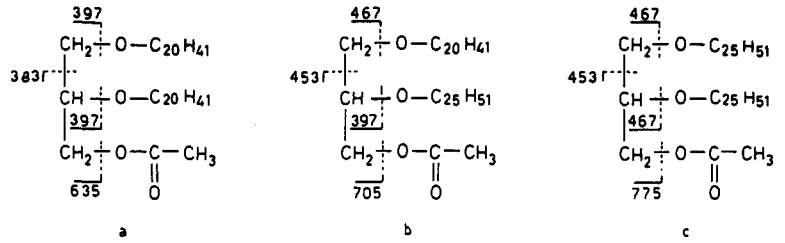


FIG. 5. Electron impact MS fragmentation patterns of acetate derivatives of the isoprenoid diethers based on C_{20} and C_{25} isoprenoid chains.

(FD) and fast atom bombardment (FAB) techniques have been employed to elucidate the structures of phospholipids of some species of halophilic archaebacteria.[19,47,51]

2. *NMR Spectroscopy*

^{13}C-NMR spectroscopy is, much more than ^{1}H-NMR, an important diagnostic tool used to define the structures of isoprenoid ethers, the backbone of complex archaebacteria lipids. Table 1 lists the chemical shifts, multiplicities and assignments of the natural abundance ^{13}C-NMR spectra of the hydrocarbons a–g of Fig. 4, obtained by cleavage of the isoprenoid ethers with HI and reduction with $LiAlH_4$.

The assignments[11,12] are based on chemical shift rules,[50,65] comparisons with appropriate model compounds[5] and observed multiplicities, combined with additional spectral data, obtained for the corresponding alcohols. In particular, spectra for samples of C_{40} hydrocarbons c–g (Fig. 4), labelled with ^{13}C from both 1-^{13}C and 2-^{13}C acetate provide, on a biogenetic basis, additional discriminatory data for ^{13}C assignments. The ^{13}C-NMR spectra of C_{20} and C_{25} isoprenoid (a, b, Fig. 4, Table 1) show only 15 resolved signals, because so many of the carbon atoms are either strictly, or effectively, equivalent. The assignments are fully consistent with a regular head-to-tail isoprenoidic structure. The ^{13}C-NMR spectrum of acyclic hydrocarbon c, Fig. 4, for the symmetry of its structure,

TABLE 1. ^{13}C Chemical Shifts (from TMS) and Multiplicities of the Hydrocarbons Obtained by HI-$LiAlH_4$ Degradation of Ether Core Lipids of Archaebacteria

Carbon* No.	a	b	c	d	e	f	g
1,1′	11.32 (*q*)	11.32 (*q*)	11.32 (*q*)	11.32 (*q*)	11.32 (*q*)	12.40 (*q*); 11.32 (*q*)	12.40 (*q*)
2,2′	29.71 (*t*)	29.71 (*t*)	29.71 (*t*)	29.71 (*t*)	29.71 (*t*)	29.34 (*t*); 29.71 (*t*)	29.34 (*t*)
3,3′	34.73 (*d*)	34.73 (*d*)	34.73 (*d*)	34.73 (*d*)	34.73 (*d*)	41.73 (*d*); 34.73 (*d*)	41.73 (*d*)
4,4′	37.02 (*t*)	37.02 (*t*)	37.02 (*t*)	36.91 (*t*); 37.02 (*t*)	36.91 (*t*)	31.46 (*t*); 36.91 (*t*)	31.46 (*t*)
5,5′	24.35 (*t*)	24.35 (*t*)	24.35 (*t*)	25.87 (*t*); 24.35 (*t*)	25.87 (*t*)	30.47 (*t*); 25.87 (*t*)	30.47 (*t*)
6,6′	37.46 (*t*)	37.46 (*t*)	37.46 (*t*)	37.19 (*t*); 37.46 (*t*)	37.19 (*t*)	46.43 (*d*); 37.19 (*t*)	46.43 (*d*)
7,7′	32.85 (*d*)	32.85 (*d*)	32.85 (*d*)	39.13 (*d*); 32.85 (*d*)	39.13 (*d*)	45.60 (*d*); 39.13 (*d*)	45.60 (*d*)
8,8′	37.46 (*t*)	37.46 (*t*)	37.46 (*t*)	33.37 (*t*); 37.46 (*t*)	33.37 (*t*)	32.52 (*t*); 33.37 (*t*)	32.52 (*t*)
9,9′	24.53 (*t*)	24.53 (*t*)	24.53 (*t*)	31.28 (*t*); 24.53 (*t*)	31.28 (*t*)	31.45 (*t*); 31.28 (*t*)	31.45 (*t*)
10,10′	37.46 (*t*)	37.46 (*t*)	37.46 (*t*)	44.89 (*d*); 37.46 (*t*)	44.89 (*d*)	45.15 (*d*); 44.89 (*d*)	45.15 (*d*)
11,11′	32.85 (*d*)	32.85 (*d*)	32.85 (*d*)	38.29 (*d*); 32.85 (*d*)	38.29 (*d*)	38.29 (*d*)	38.29 (*d*)
12,12′	37.46 (*t*)	37.46 (*t*)	37.46 (*t*)	35.77 (*t*); 37.46 (*t*)	35.77 (*t*)	35.77 (*t*)	35.77 (*t*)
13,13′	24.79 (*t*)	24.53 (*t*)	24.53 (*t*)	24.53 (*t*)	24.53 (*t*)	24.53 (*t*)	24.53 (*t*)
14,14′	39.34 (*t*)	37.46 (*t*)	37.60 (*t*)	37.60 (*t*)	37.60 (*t*)	37.60 (*t*)	37.60 (*t*)
15,15′	28.02 (*d*)	32.85 (*d*)	33.12 (*d*)	33.12 (*d*)	33.12 (*d*)	33.12 (*d*)	33.12 (*d*)
16,16′	22.68 (*q*)	37.46 (*t*)	34.35 (*t*)	34.35 (*t*)	34.35 (*t*)	34.35 (*t*)	34.35 (*t*)
17,17′	19.41 (*q*)	24.79 (*t*)	19.41 (*q*)	19.41 (*q*)	19.41 (*q*)	39.20 (*t*); 19.41 (*q*)	39.20 (*t*)
18,18′	19.80 (*q*)	39.34 (*t*)	19.80 (*q*)	36.00 (*t*); 19.80 (*q*)	36.00 (*t*)	34.74 (*t*); 36.00 (*t*)	34.74 (*t*)
19,19′	19.80 (*q*)	28.02 (*d*)	19.80 (*q*)	17.75 (*q*); 19.80 (*q*)	17.75 (*q*)	17.75 (*q*)	17.75 (*q*)
20,20′	22.68 (*q*)	22.68 (*q*)	19.80 (*q*)	19.80 (*q*)	19.80 (*q*)	19.80 (*q*)	19.80 (*q*)
21	—	19.41 (*q*)	—	—	—	—	—
22	—	19.80 (*q*)	—	—	—	—	—
23	—	19.80 (*q*)	—	—	—	—	—
24	—	19.80 (*q*)	—	—	—	—	—
25	—	22.68 (*q*)	—	—	—	—	—

*For structures and numbering, see Fig. 4a–g.

shows only 14 signals (Table 1). The assignments are fully consistent with the 16,16′-biphytanyl structure and, in particular, they confirm the unusual head-to-head central linkage. Thus, the chemical shifts of the pairs 16,16′ and 14,14′ differ from those of the biogenetically-related pairs 6,6′; 8,8′; 10,10′ and 12,12′ by -3.11 ($-\gamma_2+\delta_2$ effects) and $+0.14$ ($+\varepsilon_2$ effect),[50,65] respectively; that of the pair 15,15′ differs from that of the biogenetically-related pairs 7,7′ and 11,11′ by $+0.27$ ($+\delta_3+\varepsilon_3$ effects).[50,65] This establishes the structure of the head-to-head region of the molecule. In addition, the spectrum shows three resolved methyl signals, two at δ11.32 and δ19.41 (1,1′ and 17,17′, respectively), confirming the existence of two free tails, and one at δ 19.80 (18,18′, 19,19′, 20,20′).

The ^{13}C-NMR spectrum of the bicyclic hydrocarbon e (Fig. 4) shows just 20 resolved signals (Table 1), as its symmetrical C_{40} structure requires. Of the four methyl signals, three are also present in the spectrum of acyclic hydrocarbon c (Fig. 4) and are ascribed to carbons 1,1′, 17,17′ and 20,20′, while the fourth at δ 17.75 is assigned to the carbons 19,19′, which are β to the cyclopentane rings. Chemical shifts of carbons distant from the cyclopentane rings are the same as in the acyclic C_{40} hydrocarbon; assignments for those near or in the rings have been calculated from data for model compounds,[5] for both 1,3-*cis*- and 1,3-*trans*-stereochemistry. These calculations confirm the location of the two rings and unambiguously indicate the *trans* stereochemistry in each. The mutual stereochemistry of the two rings, of course, remains unknown. The ^{13}C-NMR spectrum of the tetracyclic hydrocarbon g (Fig. 4) shows 20 resolved signals (Table 1), as its symmetrical structure requires. Of the three methyl signals, two are also present in the spectrum of the bicyclic hydrocarbon e (Fig. 4) and are ascribed to carbons 19,19′ and 20,20′, while the third at δ12.40 is assigned to the carbons 1,1′, which are β to the cyclopentane rings. Chemical shifts of carbons 11–16, 11′–16′ are the same as in the bicyclic C_{40} hydrocarbon; assignments for those in the four rings have been calculated from data for model compounds.[5]

In the ^{13}C-NMR spectrum of the C_{40} hydrocarbon d (Fig. 4), the two sets of signals given by c and e C_{40} hydrocarbons (Fig. 4) are both observed (Table 1), confirming the

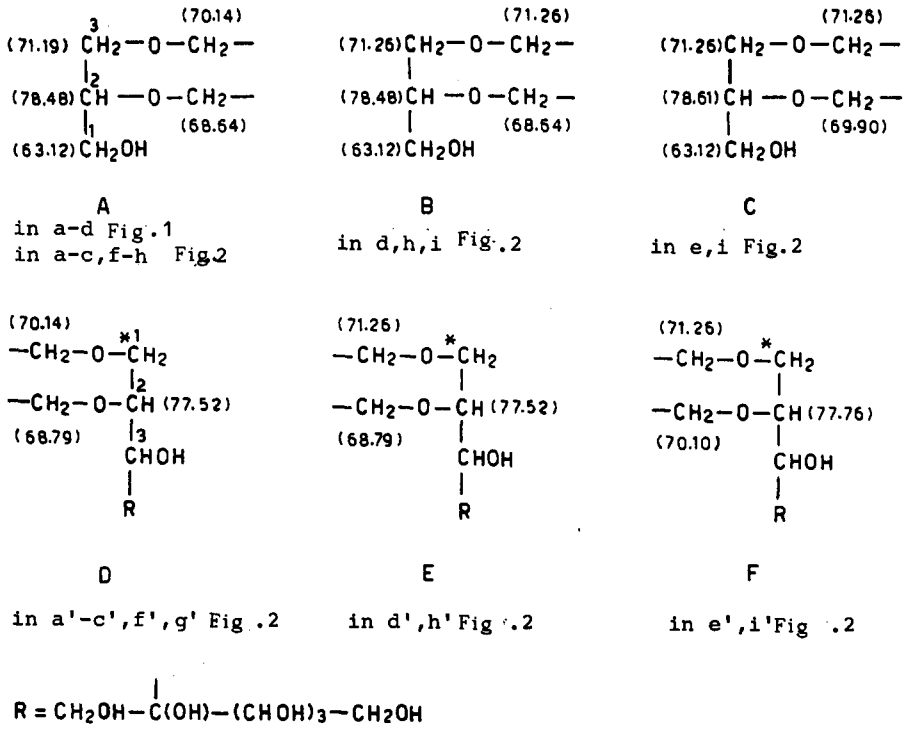


FIG. 6. Partial structures of isoprenoid ethers, basic components of complex lipids of archaebacteria. In brackets are reported ^{13}C-NMR values in δ. For structure, see Figs 1 and 2. *Overlapped signals.

asymmetric monocyclic structure assigned to it. Similarly, the ^{13}C-NMR spectrum of the C_{40} tricyclic hydrocarbon f (Fig. 4) shows the signals given by e and g C_{40} hydrocarbons.

^{13}C-NMR chemical shifts assignments for the oxygen-bearing carbons in the isoprenoid ethers are given in Fig. 6. Of particular interest are ^{13}C-NMR chemical shifts of these carbons in GDGT and GDNT tetraethers seen in Fig. 2; in fact, from these data, it is possible to obtain information on the orientation of the asymmetric C_{40} isoprenoid d and f (Fig. 4) in the macrocycle. In partial structures A–C (Fig. 6), the chemical shift of C-1 of the isoprenic chain is higher by 1.4–1.5 units when it is linked to the primary carbinol of glycerol and higher by 1.1–1.2 units when it is in a chain with a ring at C(3); moreover, the chemical shifts of C(3) and C(2) of the glycerol are higher (by 0.07 and 0.13 units, respectively) when they are linked to an isoprenoid with a cyclopentane ring at C(3) of the aliphatic chain. From the data reported in partial structures B and C (Fig. 6), it is possible, therefore, to define that the 'bicyclic end' of the tricyclic C_{40} chain in the GDGT d, h and i (Fig. 2) is linked to the primary carbinol of the glycerol; the orientation of monocyclic C_{40} isoprenoid in the GDGT b, f and g (Fig. 2) follows by simple analogy. With similar considerations, confirmatory evidence of GDNT structures (Fig. 2) are derived from ^{13}C-NMR chemical shifts for the oxygen-bearing carbons. In particular, for GDNT i′ (Fig. 2) containing one tricyclic and one tetracyclic C_{40} chain, the only signals observed were those whose chemical shifts are given in partial structure B and F (Fig. 6), from which it follows that the 'bicyclic end' of the tricyclic C_{40} isoprenoid is linked by an ether bond at the C(1) of the nonitol. This structural condition also occurred in GDNT h′ (Fig. 2) containing one bicyclic and one tricyclic C_{40} chain. In fact, for this tetraether, the only signals observed are those whose chemical shifts are given in partial structures A and E (Fig. 6). The structures assigned to remaining GDNT (Fig. 2) follow by simple analogy.

3. *Infrared Spectroscopy*

Isoprenoid ether lipids, which are the backbone of complex lipids of archaebacteria, have infrared spectra showing the presence of OH group(s) (3580 cm^{-1}), long-chain isoprenoids (2920, 2850, 1465, 1385–1375 cm^{-1}), ether C—O—C groups (1110 cm^{-1}), and primary alcoholic C—O groups (1040 cm^{-1}). No bands indicative of C=C and C=O double bond are present.

C. *Distribution of Structural Types Among Archaebacteria*

The structural types of lipids found in the archaebacteria show a less structural variety as compared to glycerolipids occurring in eubacteria and in eukaryotes. The occurrence of isopranoid ether lipids, the relative ratio of diethers and tetraethers, and the nature of the isopranoid chain could be useful tools both in the identification and in the taxonomy of archaebacteria.

The distribution of ether core lipids in archaebacteria analyzed to date is quite consistent with generic groupings derived by 16S rRNA, and, in some cases, could aid in distinguishing among the archaebacterial groups.[16,33,45]

Tables 2, 3 and 4 show the distribution of the ether core lipids among the three phenotypes of the archaebacteria: halophiles, methanogens and thermophiles.

All the archaebacteria analyzed so far seem to have at least traces of 2,3-di-*O*-phytanyl-*sn*-glycerol (Fig. 1a), which could be considered a universal core lipid in such types of microorganisms. It may be present as a major component depending on the type of archaebacteria;[80] 2,3-di-*O*-phytanyl-*sn*-glycerol represents, in fact, 100% of the ether core lipids in the majority of neutral halophiles[18,45,80] and in some coccoid forms belonging to methanogens and thermophiles.[10,33,45] In contrast, the tetraethers occur in the methanogen phenotype and in particular in thermophiles, and with few exceptions they constitute nearly all of the core lipids present.[18,45,80]

TABLE 2. Distribution of Isopranyl Ether Lipids Among Halophilic Archaebacteria

Microorganisms	Compound(*)	References
Halobacterium cutirubrum	1a	56, 80
H. salinarium	1a	56, 80
H. volcanii	1a	56, 80
H. halobium	1a	56, 80
H. saccharovorum	1a	56, 80
H. trapanicum	1a	56, 80
H. simoncinii	1a	80
H. marismortui	1a	56, 80
H. sodomense	1a	56
H. vallismortis	1a	56
H. mediterranei	1a	56, 80
H. halobium NCMB 777	1a; 1b	56
H. salinarium NCMB 786	1a; 1b	56
H. trapanicum NCMB 784	1a; 1b	56
Halococcus morrhuae	1a	80
H. morrhuae NCMB 749	1a; 1b	56, 72
Haloarcula sinaiensis	1a	37, 73
H. hispanica	1a	37, 73
H. californiae	1a	37, 73
Haloferax gibbonsii	1a	37, 73
Natronobacterium magadii	1a; 1b	56, 71, 72, 80
N. pharaonis	1a; 1b	56, 71, 72, 80
N. pharaonis NCMB 2191	1a; 1b; 1c	71
N. gregoryi	1a; 1b; 1c	56, 71, 72, 80
Natronococcus occultus	1a; 1b	56, 72, 80
SP-8	1a; 1b; 1c	25

*1a = 2,3-di-*O*-phytanyl-*sn*-glycerol.
1b = 2-*O*-sesterterpanyl-3-*O*-phytanyl-*sn*-glycerol.
1c = 2,3-di-*O*-sesterterpanyl-*sn*-glycerol.

1. *Halophiles*

The extreme halophiles (Table 2) possess lipids based on the diethers a, b and c (Fig. 1).[25,37,56,71–73,80] In the neutral halophiles, the 2,3-di-*O*-phytanyl-*sn*-glycerol is the most abundant or the only type of ether core lipid present. In the extremely alkaliphilic red halophiles living at pH 10,[72] and in a few strains of neutral halophiles,[56] in addition to diether a (Fig. 1), the structural types b and c (Fig. 1) are also present, in which one or both phytanyl chain(s) are substituted by sesterterpanyl residue(s).[56,72] The phytanyl-sesterterpanyl-diether b (Fig. 1), present in a high percentage (up to 80% of total ether core lipids)[71] in the alkaliphilic red halophiles, also occurs in trace amounts in some methanogens belonging to *Methanosarcina* and *Methanolobus* species.[20,33]

2. *Methanogens*

As reported in Table 3, microorganisms belonging to the genera *Methanobacterium*, *Methanobrevibacterium*, *Methanospirillum*, *Methanomicrobium*, *Methanoplanus*, *Methanogenium*, *Methanolobus* and *Methanothermus* generally have lipids based essentially on diether a (Fig. 1) and on tetraether a (Fig. 2).[18,33,45,49,53,80] In contrast, the *Methanotrix* and *Methanococcus* genera have lipids based essentially on diethers, such as 2,3-di-*O*-phytanyl-*sn*-glycerol[33] and diether d (Fig. 1), the latter occurring as the major component, specifically in *Methanoccus jannaschii*.[10]

The *Methanosarcina* genus has a wide range of structural types, in that the lipids are based on eight different ether core lipids. In particular, it is interesting to note that this genus represents, at the moment, the only example of mesophilic archaebacteria having tetraethers with cyclized C_{40} aliphatic chains.[20] Moreover, the presence of both 3-*O*-phytanyl-*sn*-glycerol (Fig. 3a) and tetritol diether (Fig. 1e) seems to be typical of such a genus. Lastly, it is worth noting that *Methanolobus tindarius* has a core lipid pattern similar to that of the *Methanosarcina* genus, giving further indications of a possible close phylogenetic relationship between these two genera.[33,80]

TABLE 3. Distribution of Isopranyl Ether Lipids Among Methanogenic Archaebacteria

Microorganisms	Compound*	References
Methanobacterium bryantii	1a; 2a	33
M. formicicum	1a; 2a	33
M. thermoautotrophicum	1a; 2a	33, 80
M. ruminantium M-1	1a; 2a	80
M. ruminantium PS	1a; 2a	80
M. strain M.O.H.	1a; 2a	80
Methanobrevibacter smithii	1a; 2a	33
M. ruminantium	1a; 2a	33
M. arboriphilicus	1a; 2a	33
Methanospirillum hungatii	1a; 2a	33, 80
M. strain AZ	1a; 2a	80
Methanomicrobium mobilis	1a; 2a; unidentified	33
Methanoplanus limicola	1a; 2a	33
Methanogenium cariaci	1a; 2a	33
M. marisnigri	1a; 2a	33
M. thermophilicum	1a; 2a	33
Methanolobus tindarius	1a; 1b; 2a(trace); 3a	33
*Methanothermus fervidus strain V*245	1a; 2a; unidentified	80
M. fervidus	2a; 3a	33
M. sociabilis	1a; 2a; 2b; unidentified	49
Methanothrix sohngenii	1a	80
Methanococcus strain PS	1a	80
M. methylutens	1a; unidentified	80
M. vannielii	1a; 2a(trace)	33, 80
M. voltae	1a	33
M. thermolithotrophicus	1a	33
M. jannaschii	1a; 1d; 2a	10, 80
Methanosarcina barkeri	1a; 1b; 1e; 2c; 2d; 2h; 3a	20, 33, 80
M. mazei	1a; 1b; 1e; 2a; 3a	33

*1a = 2,3-di-*O*-phytanyl-*sn*-glycerol; 1b = 2-*O*-sesterterpanyl-3-*O*-phytanyl-*sn*-glycerol; 3a = 3-*O*-phytanyl-*sn*-glycerol; 1d = macrocyclic diether; 1e = tetritol-diphytanyl-diether; 2a = glycerol-dialkyl-glycerol-tetraether (GDGT); 2c,2d,2h = GDGT with four, five and six cyclopentane per molecule.

3. *Thermophiles*

Table 4 reports the distribution of isopranyl ether lipids among thermophilic archaebacteria. Lipids of this phenotype are mainly based on tetraethers with exception of *Pyrococcus woesei*, *Thermococcus celer* and AN1 isolate, whose lipids are based only on diphytanyl diether a (Fig. 1) (De Rosa and Gambacorta personal communication and Ref. 27). On the basis of this evidence, it is difficult to draw up general rules concerning membrane stabilization at high temperatures and the presence of bipolar tetraether lipids, in that *P. woesei*, *T. celer* and AN1 isolate are all extreme thermophiles but have lipids based on monopolar isoprenoid diether. Moreover, the lipid composition confirms a close phylogenetic relationship among these three species of thermophilic archaebacteria, as suggested by molecular analysis,[84] justifying the introduction of a separate order of *Thermococcales*, which could represent a third major division of the thermophilic archaebacteria between the *Thermoproteales* and *Sulfolobales*.[80,84]

Sulfolobales display the widest lipid spectrum occurring in archaebacteria at the level both of polar end structure and of isopranoid chain variety. They have lipids based essentially on tetraethers of GDGT and GDNT type (Fig. 2).[16,45,80] In particular, the GDNT, found only in the *Sulfolobales*,[16,80] seems to be a specific taxonomic marker for this order. The recently isolated *Desulfurolobus*,[87] assigned to the *Sulfolobaceae* family, confirms this general rule. In fact, also in this new genus, lipids are mainly based on GDGT and GDNT (De Rosa and Gambacorta, personal communication). On the basis of these considerations, the genus *Acidianus*, which also presents lipids based on GDNT,[60] could be ascribed to the *Sulfolobales* order.

The value of diethers/tetraethers ratio (from 0 to 0.1) and of GDNT/GDGT ratio (from 1.3 to 5), and the degree of cyclization of C_{40} isoprenoid chains in the *Sulfolobales*, vary according to the species and depend upon growth conditions (temperature, heterotrophy,

TABLE 4. Distribution of Isopranyl Ether Lipids Among Thermophilic Archaebacteria

Microorganisms	Compound(‡)	References
Thermoplasma acidophilum	1a; GDGT^{w}	68,80
Sulfolobus solfataricus	1a; 2a–i; 2a′–i′; 3b	18, 45
S. acidocaldarius	1a(trace); GDGT†; GDNT†	80
Acidianus brierleyi		
aerobically grown	1a*; GDGT†; GDNT†	45, z, 80
anaerobically grown	1a; tetraethers°	45
Acidianus infernus	isopranyl ether lipids§	60
Desulfurolobus ambivalens		
aerobically grown	1a*; 2a–c, f–h; 2c′, g′	45, z, 87
anaerobically grown	1a*; 2c, g; 2c′	45, z, 87
Thermoproteus tenax	1a; 2a–c, f–h	68, 80, 85
T. neutrophilus	1a; tetraethers°	45
Desulfurococcus mobilis	1a*; 2a	47
D. mucosus	1a; tetraethers°	80
Pyrodictium occultum	1a; tetraethers°; unidentified	80
Thermodiscus maritimus	n.d.	—
Thtermophilum pendens	1a; tetraethers°	83
T. librum	1a*; tetraethers°	45
Staphylothermus marinus F1	1a; GDGT^{x}; unidentified	30
Pyrococcus furiosus Vc1	isopranyl ether lipids§	29
P. woesei	1a	45, 84, z
Thermoccocus celer	1a	27
isolate ANI	1a	45, 84, z
Archaeglobus VC16	n.d.	1

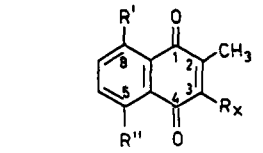
‡1a = 2,3,di-*O*-phytanyl-*sn*-glycerol; 2a–i = glycerol dialkyl-glycerol tetraethers (GDGT); 2a′–i′ = glycerol dialkyl-nonitol tetraethers (GDNT); 3b = glycerol-trialkyl-glycerol-tetra-ether.
^{w} = based on acyclic, monocyclic and bicyclic C_{40} chains.
† = based on acyclic to tetracyclic C_{40} chains.
§ = no data on the structure are available.
° = no data on C_{40} cyclization and GDGT, GDNT presence.
^{x} = no data on C_{40} cyclization.
n.d. = no data are available.
* = in trace amount.
z = De Rosa and Gambacorta, personal communication.

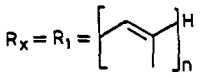
autotrophy, anaerobiosis, aerobiosis) (De Rosa and Gambacorta, personal communication and Refs 15, 16, 32, 42–45, 60, 80). For example, in *Sulfolobus solfataricus* grown at different temperatures, the degree of cyclization of C_{40} isopranoid in the tetraethers, calculated according to the formula [1 × % monocyclic + 2 × % bicyclic + 3 × % tricyclic + 4 × % tetracyclic × 10^{-2}] is 1.9 at low temperature and 2.5 at high temperature.[58]

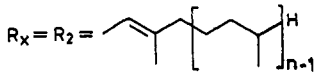
As found in *S. solfataricus*, and also in *Thermoplasma*, the degree of cyclization is influenced by the temperature of growth; in fact, the ratio of acylic/monocyclic/bicyclic chain is 25/50/24 for the cells grown at 60°C, and 62/37/1 for the cells grown at 40°C.[43] Likewise, in *Desulfurolobus ambivalens*, the lipid composition is strongly affected by the presence or the absence of oxygen during growth. In fact, in aerobic conditions, lipids are mainly based on tetraethers a–c, f–h, c′ and g′ (Fig. 2), while in anaerobic conditions tetraethers c, g, c′ (Fig. 2) are the most representative ether core lipids (De Rosa and Gambacorta, personal communication and Ref. 87).

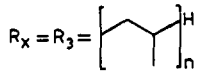
III. ISOPRENOID QUINONE: STRUCTURAL TYPES IN ARCHAEBACTERIA AND THEIR TAXONOMIC IMPLICATIONS

Isoprenoid quinones are constituents of bacterial plasma membranes[54] and play important roles in electron transport, oxidative phosphorylation and active transport[4,28,55,66,81] Although the physiological importance of isoprenoid quinones has been known for a long time, it is only within the last few years that their importance in microbial taxonomy has been fully realized.[7,9,61,75] In this article, we report the current knowledge on such a class of compounds in archaebacteria.









	n	R_x
MK-8	8	R_1
MK-7	7	R_1
MK-6	6	R_1
MK-5	5	R_1
MK-4	4	R_1
MK-8 (H_2)	8	R_1^*
MK-7 (H_2)	7	R_1^*
MK-6 ($6H_2$)	6	R_3
MK-5 ($5H_2$)	5	R_3
MK-4 ($4H_2$)	4	R_3
MK-6 ($5H_2$)	6	R_2
MK-5 ($4H_2$)	5	R_2
MK-4 ($3H_2$)	4	R_2
TK-7	7	R_1
TK-6 ($6H_2$)	6	R_3
TK-6 ($5H_2$)	6	R_2

MK-type R' and R" = H.

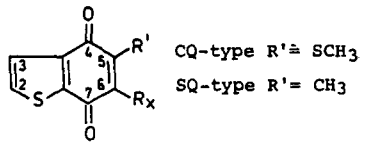
TK-type R' and R" = H and CH_3 (not necessarily respectively).

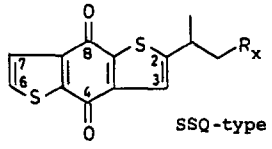
R_1^* = R_1 with one double bond hydrogenated.

FIG. 7. Structure of naphthoquinones occurring in archaebacteria.

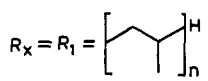
A. *Structural Types*

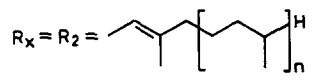
Two major structural groups of isoprenoid quinones occur in archaebacteria, the naphthoquinones and a novel group of benzothiophenquinones. Naphthoquinones can be divided into two main types based on their chromophore structure (Fig. 7, MK and TK type). Both 2-methyl-3-polyprenyl-1,4 naphthoquinones (Fig. 7, MK type) and 2,5- or





SSQ-type





	n	R_x
CQ-6 ($6H_2$)	6	R_1
CQ-6 ($5H_2$)	6	R_2
CQ-6 ($4H_2$)	6	R_2^*
CQ-6 ($3H_2$)	6	R_2^*
CQ-5 ($5H_2$)	5	R_1
CQ-4 ($4H_2$)	4	R_1
SQ-6 ($6H_2$)	6	R_1
SQ-5 ($5H_2$)	5	R_1
SQ-4 ($4H_2$)	4	R_1
SQ-3 ($3H_2$)	3	R_1
SQ-6 ($5H_2$)	5	R_2
SSQ-5 ($5H_2$)	5	R_1

R_2^* = R_2 with one or two additional isoprenoidic double bond.

FIG. 8. Structure of benzothiophenquinones occurring in *Sulfolobales*.

TABLE 5. Ultraviolet and Visible Absorption Characteristics of Quinones of Archaebacteria (in brackets are reported the values of log)

Compound[a]	Solvent	λ_{max}(nm)	Ref.
MK type	isooctane	242, 248, 260, 269, 326	20
TK type	not indicated	242, 247, 264, 275, 335	69
TK-7	isooctane	242, 248, 259, 269, 325	6
CQ-6($6H_2$)	methanol	241(4.11), 282(3.90), 333(3.70), 471(3.07)	14, 21
CQ-6($6H_2$)	isooctane	237, 272, 278, 322	6
CQ-6($6H_2$)	hexane	239, 273, 280, 320, 456	70
SQ-6($6H_2$)	hexane	225, 270, 279, 321	70
SSQ-5($5H_2$)	chloroform	250, 297, 346	74

a = For structures, see Figs 7 and 8.

8-dimethyl-3-polyprenyl-1,4 naphthoquinones (Fig. 7, TK type) are present in archaebacteria, with varying degrees of length and of saturation of the C-3 polyprenyl side chain.

The second major class of archaebacterial isoprenoid quinones are the benzothiophenquinones, of which there are three main types: the 6-polyprenyl-5-methylthiobenzo[b]thiophene-4,7-quinones (Fig. 8, CQ type), the 6-polyprenyl-5 methylbenzo[b]thiophene-4,7-quinones (Fig. 8, SQ type) and the 2-polyprenylbenzo[1,2-b; 4,5-b']dithiophene-4,8-quinone (Fig. 8, SSQ type).

The benzothiophenquinones represent the major or sole components of the quinone fraction in all species belonging to the *Sulfolobus* genus,[8,48,74] and different isoprenologs (Fig. 8) differ in the number of double bonds of the aliphatic chain.

B. *Spectroscopic Properties*

1. *Ultraviolet and Visible Spectroscopy*

In Table 5 are reported the ultraviolet and visible absorption characteristics of quinones occurring in archaebacteria. Both MK and TK type exhibit qualitatively identical ultaviolet spectra, with five absorption maxima (λmax) at 242, 248, 260, 269 and 326 nm (Table 5). Absorption bands at 242, 248, and 238 nm are due to the benzenoid contributions, whereas bands at 260 and 269 nm are due to quinone absorption. The unusual benzothiophenquinones of CQ, SQ and SSQ types (Fig. 8) range in color from red orange for the CQ type, to yellow, for SQ and SSQ types. These quinones exhibit characteristic ultraviolet spectra (Table 5) quite different from that of any of the other already described bacterial isoprenoid quinones. It is particularly noteworthy that, in CQ-type quinones, absorption at 283 nm is of diagnostic value to distinguish between the two isomeric possibilities 5-isopropyl-6-methylthiobenzo[b]thiophen-4,7-quinone and 5-methylthio-6-isopropylbenzo[b]thiophen-4,7-quinone, the natural isomer.[14]

2. *Mass Spectrometry*

Mass spectrometry is one of the most efficient methods for determining the isoprenoid quinone structure, providing information on molecular weight, the nature of the ring system and the length and degree of saturation of the isoprenyl side chain.[62] In Fig. 9 are reported the most important and structurally diagnostic electron-impact mass fragments occurring in the MS spectra of naphthoquinones of MK and TK types occurring in archaebacteria. Loss of the isoprenoid side chain originates fragments at m/z 186 and 187, for MK type, and at m/z 200 and 201, for TK type. The presence of a double bond insaturation on the α isoprenic unit (Fig. 7, $R_x = R_2$) originates characteristic fragments at m/z 225, for the MK type, and 239, for the TK type.[69]

In Fig. 10 are reported the most important electron-impact mass fragments occurring in the MS spectra of benzothiophenquinones of SQ, CQ and SSQ types. Loss of the isoprenoid side chain originates fragments at m/z 192 and 193, for SQ type, and at m/z 224 and 225, for CQ type. Also, in these quinones, the presence of unsaturation on the

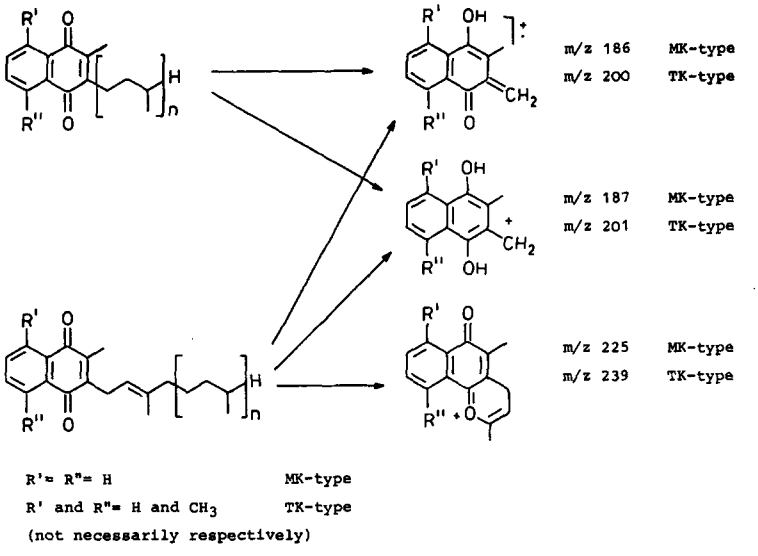


FIG. 9. Structure of mass fragments of naphthoquinones of MK and TK types occurring in the archaebacteria.

isoprenic unit (Fig. 8, $R_x = R_2$) originates characteristic fragments at m/z 231, for the SQ type, and 263 for the CQ type.[14,70] Electron-impact MS spectrum of SSQ type quinone is quite different from that of CQ and SQ types, showing an intense peak at m/z 247 due to α-cleavage at the methyl of the first isoprene unit.[74] Figure 11 shows the electron-impact MS spectra of different types of archaebacterial quinones.

3. *NMR Spectroscopy*

In addition to MS data, ^1H and ^{13}C NMR spectroscopy give supporting evidence for chemical structures of archaebacterial quinones. Table 6 shows chemical shifts for the ^1H-NMR spectra of naphthoquinones of MK and TK types. In particular, the ^1H-NMR

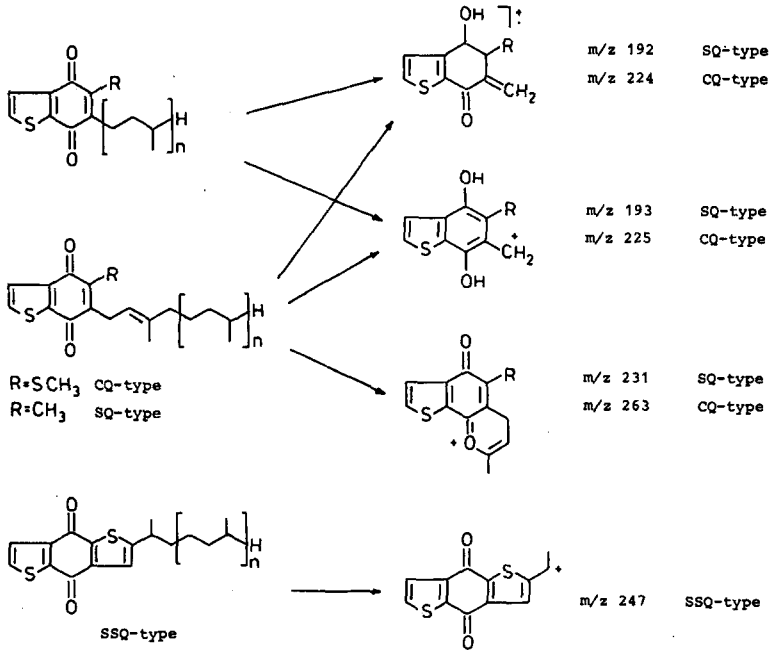


FIG. 10. Structure of mass fragments of benzothiophenquinones of SQ, CQ and SSQ types.

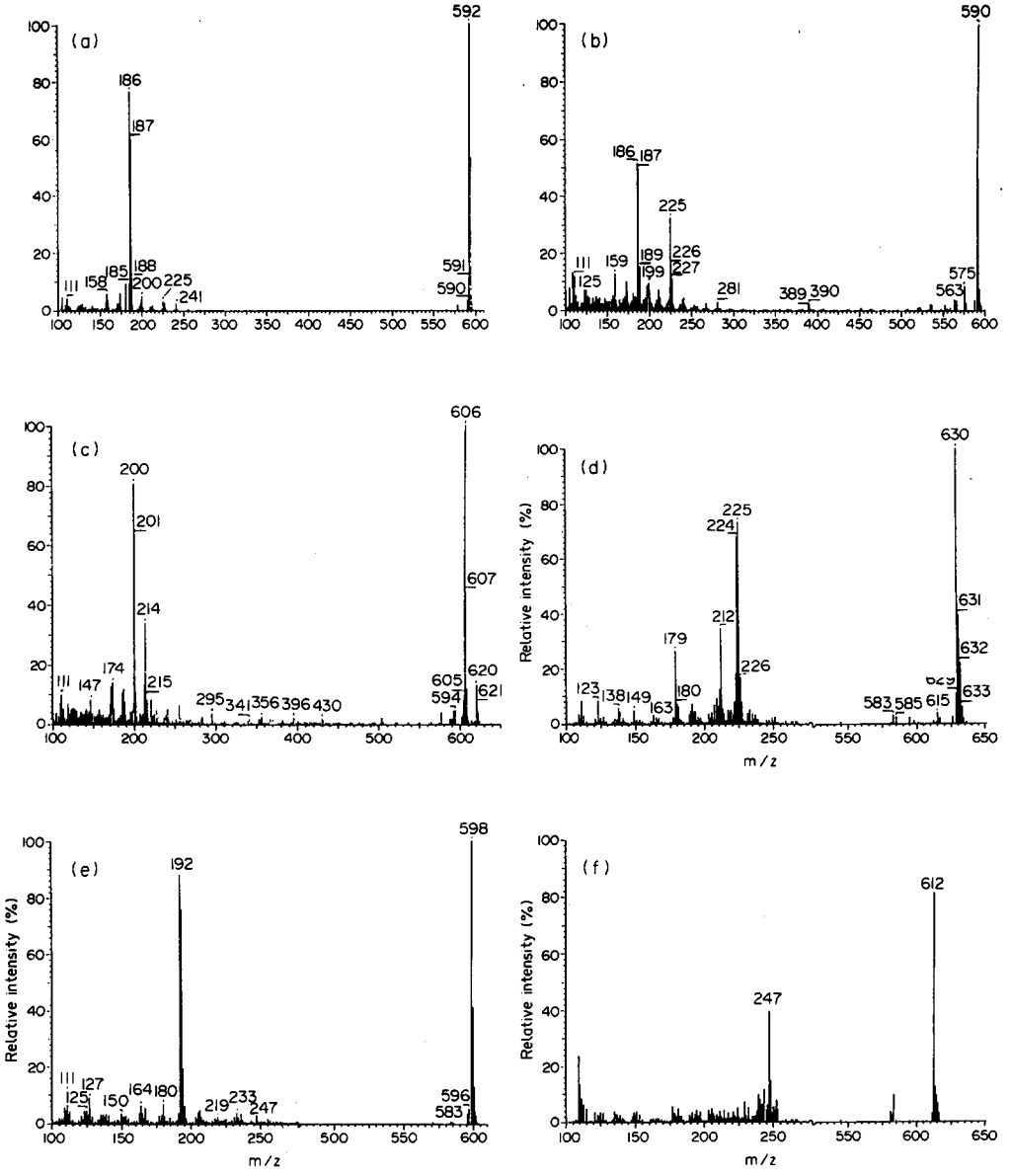


FIG. 11. Electron impact MS spectra at 70 eV of: a: MK-6($6H_2$), Mw 592; b: MK-6($5H_2$), Mw 590; c: TK-6($6H_2$), thermoplasmaquinone, Mw 606; d: CQ-6($6H_2$), Caldariellaquinone, Mw 630; e: SQ-6($6H_2$), Sulfolobusquinone, Mw 598; f: SSQ-5(H_2) Mw 612.

spectrum of TK type quinones is diagnostic for the presence of the methyl groups in a peri-position on the benzenoid ring system (C-5 or C-8). In fact, a complex absorption is observed in the δ7.4–8.0 region due to the presence of three aromatic protons. The methyl group causes a shielding of the three aromatic protons with the ortho proton, the most shielded. The doublet at δ8.0 (J = 7.5 Hz) corresponds to a peri-proton (i.e. H in position C-5 and/or C-8), whereas the doublet at 7.45 (J = 7.5 Hz) and triplet at 7.52 (J = 7.5 Hz) correspond to aromatic protons at C-6 and C-7 (not necessarily respectively). The aromatic methyl group in the peri-position (C-5 or C-8) originates a strong triplet at δ2.73.[6,69] Detailed NMR studies on benzothiophenquinones of CQ, SQ and SSQ types, based on multipulse one-dimensional and two-dimensional techniques,[48] are summarized in Table 7. In this complete NMR study, the signal multiplicities of CQ-6 ($6H_2$) have been determined from DEPT experiments, one-bond carbon–proton connectivities have been obtained by two-dimensional carbon–proton shift correlation via $^1J_{CH}$ and chemical shifts

TABLE 6. ^{1}H-NMR Data of Naphthoquinones of MK and TK Types Occurring in Archaebacteria* (for structures see Fig. 7)

Compound* Proton signal	MK-6($6H_2$)	MK-6($5H_2$)	TK-6($6H_2$)
	Chemical shifts (ppm)		
Aromatic protons (C-5 and/or C-8)	8.07	8.07	8.00
Aromatic protons (C-6 and/or C-7)	7.68	7.68	7.52–7.45
Benzenoid CH_3	—	—	2.73
Quinonoid CH_3	2.19	2.19	2.16
CH_2 next to quinone	2.62	3.37	2.59
CH_2-CH= (α isoprene unit)	—	5.10	—
CH=$CH(CH_3)$—CH_2 (α isoprene unit)	—	2.00	—
CH_3— (α isoprene unit)	0.87	1.77	0.87
CH_2 and CH— sat.	1.1–1.4	1.1–1.4	1.1–1.4
CH_3 sat.	0.85	0.85	0.85

of aromatic and quinoid carbons have been fully assigned from two- and three-bond carbon–proton couplings, as available from a gated decoupled ^{13}C-NMR spectrum.

Further, a carbon–proton shift correlation and a selective polarization transfer for the thiophene ring protons at $\delta = 7.45$ and 7.35 have been performed, in order to support all assignments of the quaternary carbons C-4′ and C-9′ (Table 7). ^{13}C-NMR signals of quinones SQ-6($6H_2$) and SSQ-5($5H_2$) have been assigned by comparison with those of CQ-6($6H_2$). In this work, it is also reported that T_1 values of ^{13}C nuclei in the isopranyl side chain of CQ-6($6H_2$) increase more significantly towards the periphery, thus confirming the assignments of the closely crowded CH_2 and CH signals of the chain in the ^{13}C NMR spectrum. Since signals in SQ-6($6H_2$) and SSQ-6($6H_2$) quinones occur at identical positions, the presence of a hydrocarbon side chain similar to that of CQ-6($6H_2$) quinone, has been assumed.

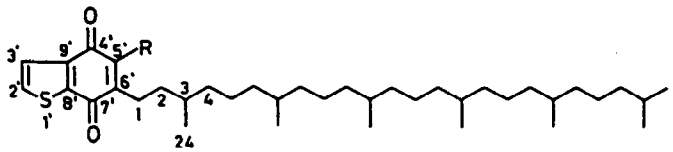
C. *Distribution of Isoprenoid Quinones in Archaebacteria*

Up until now, studies on the isoprenoid quinone content in archaebacteria have been restricted to halophilic and thermophilic species (Gambacorta *et al.*, personal communication, Refs 6–9, 14, 21, 36, 39, 46, 52, 69, 70, 74), while detailed studies on the quinone content of the methanogens have not been performed. The only data available on this last group of archaebacteria are of Zeikus and colleagues,[82a] reporting the absence of both menaquinones and ubiquinones in *Methanobacterium thermoautotrophicum*. The distribution of isoprenoid quinones in halophilic archaebacteria,[9,39,52] as reported in Table 8, is quite homogeneous. In fact, MK-8 and MK-8(H_2) constitute the major isoprenoid quinones in all microorganisms of this group examined so far, while minor components of quinone pool are generally MK-7 and MK-7(H_2). The presence of dihydrogenated menaquinones in the halophilic archaebacteria is unusual. Partially saturated menaquinones have been described previously only in gram-positive eubacteria.[7]

From the point of view of quinone distribution, thermophilic archaebacteria could be divided into two groups: microorganisms with naphthoquinone isoprenoids and microorganisms with benzothiophenquinones.

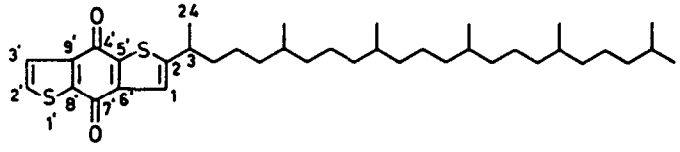
The first group (Table 8) comprises the sulfur reducing thermophilic archaebacterium *Thermoproteus tenax*,[69] which MK isoprenologs with 4 and 6 saturated isoprene units, while minor components are MK-5($5H_2$), MK-6($5H_2$), MK-5($4H_2$), MK-4($3H_2$), TK-6($6H_2$) and TK-5($5H_2$), and *Thermoplasma acidophilum*,[6,8,36,46] which have only unsaturated isoprenologs of the MK type, with 7 to 5 isoprene units, and TK-7 quinone, named Thermoplasmaquinone (2,5 or 8-dimethyl-3-heptaprenyl-1,4-naphthoquinone). Belonging to the second group are all the species of *Sulfolobus*, *Desulfurolobus* and *Acidianus* genera studied up until now, whose quinones (Table 8) are all based on a benzothiophene nucleus. These quinones, in analogy with lipid composition, indicate a close relationship among the three genera. The major quinone in the species *Sulfolobus solfataricus*, *Sulfolobus acidocaldarius* and *Acidianus brierleyi* is CQ($6H_2$), named Caldariellaquinone (6-(3,7,11,15,19,23-

TABLE 7. NMR Data for Quinones from *Sulfolobus solfataricus*



R=SCH$_3$ CQ-6(6H$_2$)

R=CH$_3$ SQ-6(6H$_2$)



SSQ-5(5H$_2$)

C	NT_1	CQ-6(6H$_2$) δ_c	J_{CH}	$^{2,3}J_{CH}$	CQ-6(6H$_2$) δ_H	SQ-6(6H$_2$) δ_C	SQ-6(6H$_2$) δ_H	SSQ-5(5H$_2$) δ_C	SSQ-5(5H$_2$) δ_H
	s	ppm	HZ		ppm				
2′		132.4 D	187.5	d7.5 (3′-H)	7.45	132.6	7.50	133.2	7.58
3′		126.3 D	175.0	d5.0 (2′-H)	7.35	126.1	7.43	126.7	7.54
4′		177.4 S	—	—	—	182.0	—	182.6	—
5′		145.4 S	—	—	—	141.2	—	143.1[b]	—
6′		149.8 S	—	t5.0 (1-H$_2$)	—	147.1	—	148.3[b]	—
7′		176.8 S	—	d7.5 (3′-H) d5.0 (2′-H)	—	181.0	—	181.9	—
8′		143.2 S	—	d10.0 (2′-H) d5.0 (3′-H)	—	143.3	—	144.7[c]	—
9′		141.5 S	—	n.r.	—	141.4	—	143.0[c]	—
1	0.5	26.7 T	126 ± 1	n.r.	2.75	29.6	2.49	122.3 D	7.24
2	0.54	35.6 T	126 ± 1	n.r.	—	35.7	—	133.4 S	—
3	0.5	33.3 D	126 ± 1	n.r.	—	33.3	—	29.8	2.98
4	0.66	36.9 T	126 ± 1	n.r.	0.95 or 1.25	37.0	0.98 or 1.27	37.0	0.96 or 1.26
5, 9, 13, 17	0.74	24.4 T	126 ± 1	n.r.	1.15; 1.34	24.5	1.14; 1.30	24.6	1.10; 1.28
6, 8, 10, 12, 14, 16, 18, 20	0.84	37.4 T	126 ± 1	n.r.	0.95; 1.25	37.4	0.98; 1.20	37.6	0.98; 1.20
7, 11, 15, 19	0.3[d]	32.8 D	126 ± 1	n.r.	1.32	32.8	1.28	32.8	1.26
21	1.66	24.8 T	126 ± 1	n.r.	—	24.8	—	24.9	—
22	2.34	39.3 T	126 ± 1	n.r.	1.05	39.4	1.04	39.5	1.03
23	2.36	27.9 D	126 ± 1	n.r.	[a]	28.0	—	28.1	—
24	2.25	19.5 Q	126 ± 1	n.r.	0.90	19.5	0.90	19.8	1.30
25, 26, 27, 28	2.73	19.8 Q	126 ± 1	n.r.	0.75	19.8	0.78	19.9	0.76
29, 30	5.01	22.6 Q 22.7	126 ± 1	n.r.	0.75	22.6 22.7	0.78	22.7 22.8	0.76
S-CH$_3$	6.0	17.9 Q	126 ± 1	n.r.	2.52				
CH$_3$						14.1	2.05		

[a]No correlation observed.
[b,c]Assignment interchangeable.
[d]Not exactly measurable because of signal overcrowding.
n.r. not resolved.

TABLE 8. Distribution of Isoprenoid Quinones in Archaebacteria

Microorganisms	Major Isoprenolog(s)	Minor Components	Ref.
Halobacterium cutirubrum	MK-8		39
H. cutirubrum	MK-8	MK-8(H_2), MK-7(H_2), MK-7	9
H. halobium	MK-8		39
H. halobium NCMB 736, NCMB 764, NCMB 777, and NCMB 2080	MK-8, MK-8(H_2)	MK-7(H_2), MK-7	9
H. saccharovorum	MK-8, MK-8(H_2)		9
H. salinarium	MK-8		39
H. salinarium	MK-8, MK-8(H_2)	MK-7(7H_2), MK-7	9
H. simoncinii subsp. neapolitanum	MK-8, MK-8(H_2)	MK-7(H_2), MK-7	9
H. trapanicum	MK-8, MK-8(H_2)	MK-7(H_2), MK-7	9
H. volcanii	MK-8	MK-8(H_2)	52
H. volcanii	MK-8, MK-8(H_2)	MK-7(H_2), MK-7	9
H. species	MK-8, MK-8(H_2)	MK-7(H_2), MK-7	9
Halococcus morrhuae	MK-8, MK-8(H_2)	MK-7(H_2), MK-7	9
Amoebacter morrhuae	MK-8		39
Paracococcus haloxanthus	MK-8		39
Sarcina morrhuae	MK-8, MK-8(H_2)	MK-7(H_2), MK-7	9
Sarcina litoralis	MK-8(H_2)	MK-8, MK-7(H_2), MK-7	9
Sarcina sreenivasani	MK-8(H_2)	MK-8, MK-7(H_2), MK-7	9
Alkalophilic halophiles SP-1 SP-3 and MS3	MK-8, MK-8(H_2)	MK-7(H_2), MK-7	9
Thermoplasma acidophilum	MK-7		36, 46
T. acidophilum	MK-7, TK-7	MK-6, MK-5	6, 8
Thermoproteus tenax	MK-6(6H_2), MK-4(4H_2)	MK-5(5H_2), MK-6(5H_2), MK-5(4H_2), MK-4(3H_2), TK-6(6H_2), TK-5(5H_2)	69
Sulfolobus solfataricus	CQ-6(6H_2)	SQ-6(6H_2), SSQ-5(5H_2)	14, 21, 48, 74
S. solfataricus	CQ-6(6H_2)	CQ-6(5H_2), CQ-5(5H_2) CQ-4(4H_2)	8
S. acidocaldarius	CQ-6(6H_2)	CQ-6(5H_2), CQ-5(5H_2), CQ-4(4H_2)	8
S. acidocaldarius	CQ-6(6H_2)	CQ-6(5H_2), CQ-6(4H_2) CQ-6(3H_2), SQ-6(6H_2)	70
Acidianus brierleyi	CQ-6(6H_2)	CQ-6(5H_2), CQ-5(5H_2), CQ-4(4H_2)	8
Desulfurolobus ambivalens anaerobically grown	CQ-6(6H_2), SQ-6(6H_2)	SQ-5(5H_2), SQ-4(4H_2), SQ-3(3H_2), MK-6(6H_2), CQ-6(5H_2), SQ-6(5H_2)	70
D. ambivalens anaerobically grown	SQ-6(6H_2)		†
D. ambivalens aerobically grown	CQ-6(6H_2), SQ-6(6H_2)	SSQ-5(5H_2)	†

†Gambacorta *et al.*, personal communication.

hexamethyltetracosyl)-5-methylthiobenzo[b]thiophen-4,7-quinone), while minor components are CQ isoprenologs with 6 to 4 isoprene units of different degree of unsaturation.[8,14,21,48,70,74] In the species *S. solfataricus* and *S. acidocaldarius*, the SQ-6(6H_2) quinone, named Sulfolobusquinone (5-methyl-6-(3,7,11,15,19,23-hexamethyltetracosyl)-5-methylbenzo[b]-thiophen-4,7-quinone), is also present.[8,48]

In trace amounts in *S. solfataricus* has been identified the SSQ-5(5H_2) quinone 2-(1,5,9,13,17,21-hexamethyldocosyl)benzo[1,2-b,4,5′-b′]dithiophen-4,8-quinone)[48,74] probably metabolically related to CQ-6(6H_2) quinone, the major quinone present in this micro-organism. The quinone content of *Desulfurolobus ambivalens* is strongly affected by growth conditions (Gambacorta *et al.*, personal communication, and Ref. 70). This microorganism is unique for its ability to perform both oxidation and reduction of elementary sulfur, when grown in the presence or absence of oxygen.[86] In aerobically grown bacteria (Gambacorta *et al.*, personal communication), CQ-6(6H_2) represents 83% of the quinone pool, while SQ-6(6H_2) is 16%. In contrast, in anaerobically grown microorganisms (Gambacorta *et al.*, personal communication), SQ-6(6H_2) is the only quinone present. On this last piece of data, conflicting evidence is given by Thurl and colleagues,[70] who report in anaerobically grown *D. ambivalens* the presence of equivalent amounts of both CQ-6(6H_2) and SQ-6(6H_2). Also, the total quinone content in this microorganism seems to be affected by growth conditions (Gambacorta *et al.*, personal communication); in fact, while the quinone

fraction represents 0.16% of lyophilized cells in aerobically grown cultures, in the absence of oxygen the quinone content drops to 0.05%.

The presence of these novel sulfur-containing isoprenoid quinones in the genera *Sulfolobus*, *Desulfurolobus* and *Acidianus* is very interesting in the context of the physiology and metabolism of these sulfur-dependent archaebacteria. Concrete evidence concerning their roles is not yet available.

In other anaerobic species of thermophilic archaebacteria, such as *Thermococcus celer*, *Desulfurococcus mucosus*, *Desulfurococcus mobilis*,[70] the absence of detectable amounts of quinones has been reported. The lack of, or a lower, quinone content in anaerobic species of archaebacteria is in keeping with the situation most commonly, but not invariably, encountered in other strictly anaerobic eubacteria.

In conclusion, although the data on the distribution of the isoprenoid quinone structural types of archaebacteria are still fragmentary at present, the information available indicates that a correlation among classifications based on other criteria and the type of isoprenoid quinones found in archaebacterial cells could be formulated. In this respect, a systematic investigation on the distribution of the structural types of respiratory quinones in archaebacteria can provide extra material for elucidating the complex taxonomic relationships among members of this recently discovered evolutive line of prokaryotes.

IV. CONCLUSIONS

It was merely 10 years ago that Woese[77] presented the hypothesis that the archaebacteria might represent a third evolutionary lifeline on Earth. This idea catalyzed an intense research activity which still involves microbiologists, molecular biologists, chemists, and biochemists. Today, the discovery and characterization of new archaebacteria are now being reported in the scientific literature with increasing frequency, rendering precise definitions within a taxonomical framework an arduous task.

As is evident in this article, the ether lipids and unusual quinones found in archaebacteria up until now can certainly be considered excellent chemical markers of this evolutive line. Obviously, more significant taxonomical contributions will come about only when a structural investigation of these molecules is extended systematically to include the definition of the complex lipid structures.

In any case, whatever the interesting role that these molecules might have in the taxonomy of archaebacteria, the fact remains that in the field of lipid chemistry and biochemistry, these microorganisms and their lipids constitute one of the most promising research fields today. Up until now, in fact, in spite of the great variety of molecular topology, lipid biogenesis has been characterized in all living organisms by recurrent structures such as ester linkages, fatty acids and 1,2-*sn*-glycerol stereochemistry. The discovery of ether isoprenoid lipids in archaebacteria has shattered this dogma, by showing that even at a level of lipid biogenesis, Nature has selected various molecular populations capable of efficiently carrying out the multiple and delicate roles of lipids, under adverse environmental conditions.

As reported in this article, much has been accomplished concerning the chemical structure of lipids in archaebacteria; very little is known, however, about their relative biosynthetic, enzymologic and functional aspects. The near future should bring fresh knowledge to these themes, in order to adequately define the evolutionary process at the molecular level of this new class of natural molecules.

Acknowledgements—The authors are indebted to Drs A. Basso and A. Trincone for their help in compiling the review and to Mr. R. Turco for drawing the figures.

(*Received 17 November 1987*)

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