# Polar Lipids of Archaebacteria in Sediments and Petroleums

Abstract. *Glycerol tetraethers with head-to-head isoprenoid 40-carbon chains that are typical of archaebacteria, in particular of methanogens, were identified in the polar lipids of sediments and petroleums. These structures are at least partially preserved in the subsurface beyond the stage of petroleum formation. Their identification provides further evidence that a significant part of geological organic matter derives from the lipids of membranes of microorganisms.*

The polar lipids of archaebacteria are mainly composed of diethers and tetraethers of glycerol or other polyols (*1–3*). Phytane ($C_{20}$) constitutes the lipid part in the diethers, whereas the tetraethers are macrocycles containing two $C_{40}$ acyclic head-to-head isoprenoid chains and related mono- to tetracyclic compounds (*1*). The tetraethers may play the same roles in the membranes of these organisms as sterols do in eukaryotic membranes to improve the mechanical properties (*4, 5*). We have shown by chemical degradation that such molecular entities were present as "molecular fossils" of archaebacteria in the insoluble organic matter (kerogen) of an Eocene oil shale (Messel, Germany) (*6, 7*). We also identified novel ether-bound branched $C_{15}$ and $C_{30}$ alkanes which may have originated in the same way from the same type of organisms (*7, 8*).

We have now examined the polar fractions of several recent and ancient sediments and petroleums of various origins and ages (*9*) by an approach similar to that used in our kerogen study. We found that polar ether lipids typical of archaebacteria are still present and preserved in varying degrees in these geological samples.

The least polar fractions of the sediments obtained by extraction in a mixture of toluene and methanol (3:1) and of the crude oils were chromatographed over silica gel and exhaustively eluted with chloroform. Subsequent elution with acetone and methanol yielded the more polar fraction which was hydrolyzed with methanolic HCl (*10*). The resulting mixture was again chromatographed by $SiO_2$ thin-layer chromatography (hexane/ether/acetic acid, 60:40:1) (*10*), and a band covering the polarity range of di- and tetraethers was separated. The latter fraction was then treated with boron tribromide (*7*) or trichloride (*1*) for cleavage of the ether bonds. The bromides or chlorides were separated on a silica gel column by elution with chloroform. Glycerol and the more polar polyols were eluted with chloroform/methanol (7:3 and 1:1, respectively) and were acetylated (acetic anhydride and pyridine) (*3*). The bromides and chlorides were then reduced to the corresponding alkanes with lithium aluminium hydride or deuteride. The *n*-alkanes were separated from the total alkanes by inclusion in 5-Å molecular sieves. The alkane fractions were further analyzed by glass capillary column gas chromatography (CCGC) and CCGC coupled with computerized mass spectrometry (LKB 9000 S; Varian CH 7A) (*7*).

The total alkanes ranged from traces (Grenade oil) up to 0.6 percent of the total extract or crude oil and comprised varying proportions of *n*-alkanes. In the sediment from the Cariaco Trench, for example, *n*-alkanes were virtually absent, the bulk of the hydrocarbons being composed of $C_{40}$ head-to-head isoprenoid components typical of archaebacteria (Fig. 1). Most of the compounds (0, 1, 2, and $C_{30}$ in Figs. 1 and 2) were identified, as in our work on kerogen (*6–8*), by comparison with synthesized reference compounds—$C_{30}$ and 0 (*11*)—and a $C_{40}$ mixture obtained from the lipids of archaebacteria of the *Caldariella* group (*1*) (Figs. 2 and 3). The shorter chains (isopentadecane and phytane) were generally minor and originally present as terminal monoethers (*12*), whereas the $C_{30}$ and $C_{40}$ compounds were derived from α, ω-diethers, as shown by mass spectrometry on the deuterated alkanes (*13*). Mass spectral data indicated that our mixtures contained structural isomers of the compounds labeled 1 (1′ from Messel shale) and 2 (2′ and 2″ from Black Sea, Cariaco Trench, Angola Basin, and Gulf of California samples) (Fig. 3) which could not be identified. In most samples the acyclic $C_{40}$ (biphytane) predominates; this is not surprising since this compound is typical of the ether lipids of methanogens (*2, 14*) and since most of the studied environments are confined and anoxic. However, the bicyclic compound 2 was almost equally as important as biphytane in the Cariaco Trench sediment, and it was the only $C_{40}$ component detected in the terrestrial Mahakam oil which originates from more open environments.

Although the more cyclized (three and four rings) compounds present in the lipids of the thermoacidophilic *Caldariella* group (*1*) could not be detected in our samples, the presence of the mono- and bicyclic components, 1 and 2, was noteworthy, since so far only the acyclic biphytane, 0, had been identified in methanogens (*2, 14*), which are the most likely of the archaebacteria to inhabit the studied environments. This could well mean that these compounds represent still unrecognized constituents of some methanogenic species (*15*). The varying distributions observed in our samples (Fig. 3) probably reflect a present or extinct archaebacterial activity in the respective environments and may therefore be useful as indicators of recent and paleoenvironments.

Our results provide further evidence of the widespread occurrence of archaebacterial lipids in geological organic mat-

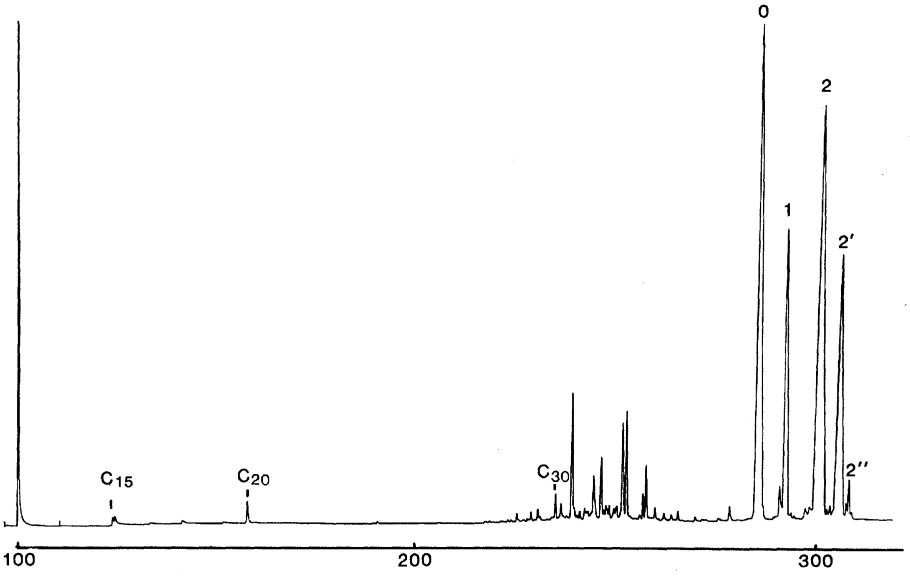

Fig. 1. Gas chromatogram of the alkanes obtained by selective degradation of polar lipids of a recent sediment from the Cariaco Trench, off Venezuela. (Column, SE-30; 25 m by 0.3 mm; 100° to 300°C; gradient 4°C per minute.) Peaks are identified in Fig. 2; 2′ and 2″ are isomers of 2.

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ter (*7–9, 16*) and show that the polar lipids of these organisms are at least partially preserved in the sediments even through an advanced degree of maturation, beyond the stage of petroleum generation. This reflects the stability of these lipids, in particular of the ether linkage in geologic conditions. The presence of the tetraether 4 was even more strongly established by the identification of glycerol (as its triacetate in the solution obtained after the treatment with boron trichloride) in amounts compatible with those of the $C_{40}$ isoprenoid alkanes. Evidence obtained by mass spectrometry, in particular on a sample of the tetraethers of glycerol from *Caldariella*, further confirmed by comparison that the tetraethers were indeed present as such in the Cariaco Trench sediment. Hydrophilic groups may still be attached to these ethers, as indicated by the presence of significant amounts of phosphate after the hydrochloric acid hydrolysis. The specific nature of these groups as well as that of the unidentified $C_{40}$ mono- and bicyclic alkanes, is not yet known.

In a later stage of maturation in the subsurface these ethers may lead to the head-to-head isoprenoid alkane series which has been reported in several petroleums (*17, 18*). This possibility is strengthened by the concomitant presence of the acyclic $C_{40}$ diphytane in the alkanes of the North Sea oil, as well as in the hydrocarbons obtained from the boron trichloride treatment of its polar fractions, which shows that the geochemical transformation of the ethers into the hydrocarbons is not yet completed.

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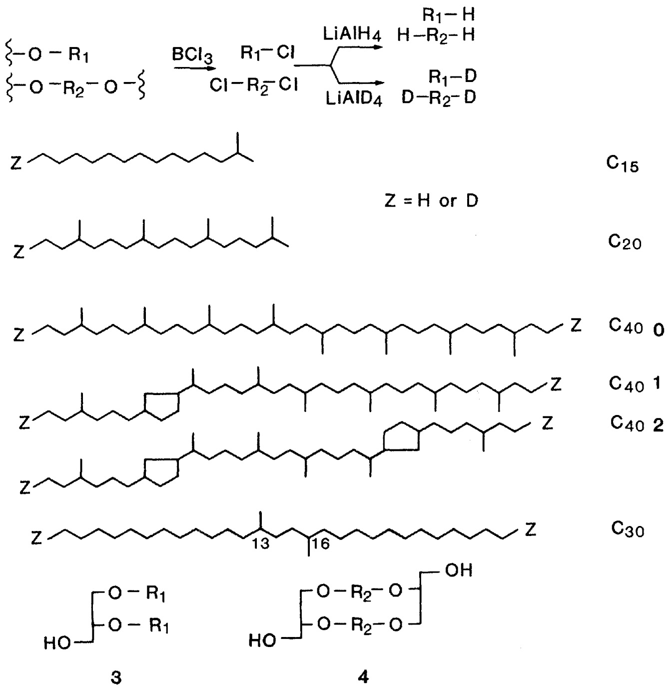

Fig. 2. Branched and isoprenoid alkanes ($C_{15}$ to $C_{40}$) obtained by selective degradation (top scheme) of polar lipids of sediments and petroleums, from archaebacterial precursors such as the di- and tetraethers of glycerol 3 and 4. $C_{15}$, 2-methyltetradecane; $C_{20}$, phytane; $C_{30}$, 13,16-dimethyloctacosane; biphytane, 3,7,11,15, 18, 22, 26, 30 - octamethyldotriacontane; $R_1$, $C_{15}$ or $C_{20}$; $R_2$, $C_{30}$ or $C_{40}$ (0, 1, and 2); and *D*, deuterium.

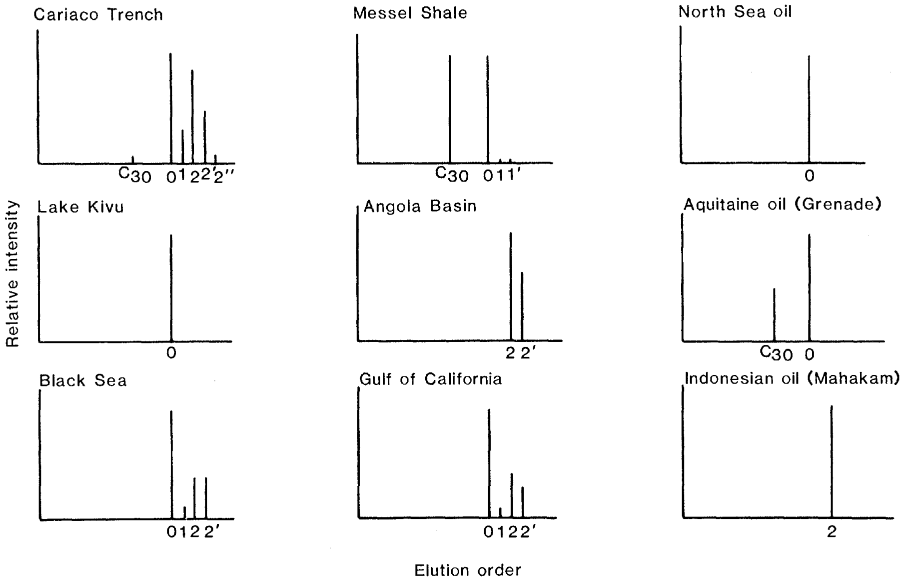

Fig. 3. Relative distribution of archaebacterial alkanes ($C_{30}$ and $C_{40}$) obtained from polar lipids of recent and ancient sediments and from petroleums (*9*), as deduced from the gas chromatograms. See Fig. 2 for the corresponding structures; 1′, 2′, and 2″ are isomers of 1 and 2.

### References and Notes

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19. We further thank the Société Nationale Elf Aquitaine, the Institut Français du Pétrole, and the Compagnie Française des Pétroles for financial support and supply of samples; M. De Rosa (Naples) for reference mixtures of the tetraethers and hydrocarbons from *Caldariella*, and helpful discussions; G. Ourisson for his interest and encouragement. Supported by the Centre National de la Recherche Scientifique, France, and the Deutsche Forschungsgemeinschaft.

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