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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSIS OF NEUTRAL LIPIDS FROM METHANOGENIC AND THERMOACIDOPHILIC BACTERIA

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SUMMARY

The neutral lipids of nine methanogenic and three thermoacidophilic bacteria were analyzed. The major compounds are acyclic isoprenoids in the range of C_{15} to C_{30}. The compounds were identified by means of their mass spectral fragmentation pattern and/or by co-chromatography. A high similarity is seen between the isoprenoid content of the examined bacteria and the distribution of these compounds in ancient sediments and petroleum.

INTRODUCTION

The analysis of organic constituents in ancient sedimentary rocks and petroleum is of great interest in the study of biological processes which occurred on the Earth millions of years ago. Among the various classes of compounds originating from biosynthetic sequences, are proteins (amino acids), nucleic acids, organic pigments, carbohydrates and lipids. However, not all of these substances can serve as biological markers because they are not capable of retaining their chemical integrity in the geological environment. Saturated hydrocarbons are among the most stable bioorganic compounds[1] and they can be expected to survive over long periods of time, possibly as long as the age of the Earth. Thus it is not surprising that alkanes, isoalkanes, anteisoalkanes and acyclic isoprenoids are found as common organic constituents in ancient sediments and petroleum[2]. Isoprenoids ranging from C_{10}–C_{40} have been identified in petroleum and in sediments of various geological periods[3–14], with phytane (2,6,10,14-tetramethylhexadecane) and pristane (2,6,10,14-tetramethylpentadecane) as their major components[2].

Despite the well documented evidence of these compounds in the geosphere the possible biological source of the isoprenoids remains still unclear. Pristane has been isolated from plants[15] and from marine organisms[16,17]. The phytol side-chain of chlorophyll is generally believed to be the common biological precursor for the

C_{18}, C_{19} and C_{20} isoprenoids[18–20]. For the formation of the lower isoprenoids a complex series of chemical reactions has been proposed, including diagenesis (oxidations, reductions, decarboxylations) and maturation (thermal cracking processes)[5,20,21]. Halophilic bacteria have been identified as a source for phytane in Dead Sea sediments[22]. These organisms contain lipids consisting of phytanyl glycerol ethers[23], as well as substantial amounts of squalene[24]. Another report describes the occurrence of head to head linked isoprenoid hydrocarbons in petroleum, suggesting thermoacidophilic bacteria as a possible source[25]. In a recent study carried out in our laboratories[26,27], we isolated C_{30}, C_{25} and C_{20} isoprenoids as major neutral lipid constituents of thermoacidophilic and methanogenic bacteria. The minor constituents consisted of isoprenoids in the range from C_{14} to C_{20}. These bacteria diverge distinctly from the rest of the classified bacteria, and they have been designated as Archaebacteria, representing a different phylogenetic group[28,29]. The properties of some of these bacteria, to exist at extreme temperatures in a reducing environment, are suggestive that they may have been abundant in Archean times. Thus the neutral lipid composition of Archaebacteria may be directly related to the occurrence of isoprenoids in ancient sediments or petroleum. In this study we present mass spectral evidence for a series of previously not reported isoprenoids, from thermoacidophilic and methanogenic bacteria and we will discuss the geochemical significance of these findings.

EXPERIMENTAL

Lyophilized cells of *Methanobacterium thermoautotrophicum*, *Methanobacterium* strain M.O.H., *Methanobacterium* strain AZ, *Methanobacterium ruminantium* PS, *Methanobacterium ruminantium* M-1, *Methanospirillum hungatii*, *Methanococcus vannielii*, *Methanococcus* strain PS and *Methanosarcina barkeri* were prepared at the University of Illinois by W. E. Balch and obtained from R. S. Wolfe. *M. thermoautotrophicum* and the remaining above methanogens were grown and harvested as previously described[30,31]. Similar methods were applied to *Thermoplasma acidophilum*, *Sulfolobus acidocaldarius* and a *Sulfolobus* species[32,33]. The extraction procedures for the total lipids of all methanogenic and thermoacidophilic bacteria have been reported earlier[26,27]. The total lipid extracts were fractionated on silicic acid columns (Unisil, 326 mesh) with *n*-hexane, benzene and chloroform to remove the non-polar, neutral lipids. All investigations described in this report were conducted on the hexane and benzene extracts of the non-polar lipid fractions.

Gas chromatography (GC) was done on a F&M 5750 gas–liquid chromatograph or a Varian Aerograph 2000, both equipped with flame-ionization detectors. Depending on the volatility and other characteristics of the extracted lipids, analyses were carried out using four different GC columns: 1.8 m × 6 mm stainless-steel column packed with 5% SE-30 on 80–100 Gas-Chrom Q; 1.8 m × 3 mm stainless-steel column packed with 10% SP 2330 on 100–120 Chromosorb W AW; 1.8 m × 6 mm glass column packed with 5% QFI and 5% OV-17 on 80-100 Gas-Chrom Q; 10 m × 0.2 mm glass capillary column coated with OV-101. Mass spectra were recorded with an LKB gas–liquid chromatograph–mass spectrometer combination (seperator: single jet, ion source: 240°, 70 eV, 60 μA).

RESULTS AND DISCUSSION

The methyl branched hydrocarbons extracted from the microorganisms are listed in Table I. The genealogical and biochemical significance of some of these findings has been reported elsewhere[26,27]. In the following lines we will discuss the mass spectral evidence showing that the isoprenoids in sediments and petroleum could have been directly synthesized by methanogens, thermoacidophiles, halobacteria and other related microorganisms.

The mass spectrum of the $C_{15}H_{32}$ isoprenoid hydrocarbon, isolated from *Thermoplasma*, is shown in Fig. 1. The saturated isoprenoid skeleton undergoes bond cleavages at positions adjacent to a methyl group. The charge remains usually at the secondary carbon unless the primary carbon fragment consists of a long chain. Thus the major fragments at *m/e* 113, 127 and 183 indicate a regular head to tail structure. The mass spectrum is identical to a C_{15}-isoprenoid isolated from the Windy Knoll bitumen (Derbyshire, Great Britain) and later identified as 2,6,10-trimethyldodecane[34]. For similar reasons a regular head to tail structure is assumed for the $C_{16}H_{34}$-isoprenoid (Fig. 2). A C_{16}-isoprenoid monoene, isolated from *Sulfolobus*, was found to have also the regular head to tail skeleton. In addition a C_{16}-isoalkane was isolated from *Thermoplasma* (Fig. 3b). The mass spectrum of this compound is compared with a C_{16}-isoalkane standard (2-methylpentadecane) (Fig. 3a).

The mass spectrum of a C_{17}-isoprenoid isolated from a *Sulfolobus* species is shown in Fig. 4. There are indications that this isoprenoid has a regular head to tail structure corresponding to a 2,6,10-trimethyltetradecane.

McCarthy *et al.*[35] found that a fragment consisting of a long straight carbon chain will have an intensified even numbered peak (C_nH_{2n}). Such a "doublet" can be noted in Fig. 4 at *m/e* 84, 85. Fragment ions at *m/e* 113, 155 and 183 support the regular head to tail structure. The mass spectrum recorded in Fig. 4 is very similar to those reported for C_{17}-isoprenoids isolated from petroleum and sediments[5,7,8,34]. A C_{17}-isoalkane was identified in the neutral lipids content of *Thermoplasma* (Fig. 3d). Its mass spectrum is compared to a standard C_{17}-isoalkane (Fig. 3c).

The GC retention values and the mass spectra of the C_{18}-isoprenoids isolated from *Sulfolobus* and *Thermoplasma* were identical. A similar compound obtained from *M. vannielii* had different mass spectral characteristics. The fragmentation pattern of the standard norpristane and of the C_{18}-isoprenoids from *Thermoplasma* and *Sulfolobus* are shown in Fig. 5a–c. The doublet at *m/e* 98 and 99, indicating the presence of a long straight chain and the major fragments at *m/e* 113, 169 and 183, are consistent with the structure of 2,6,10-trimethylpentadecane. The fragmentation patterns presented in Fig. 5a–c are very similar to those reported in the literature for C_{18}-isoprenoids isolated from shales and oils[5,8].

Pristane (2,6,10,14-tetramethylpentadecane) was detected in the neutral lipid content of *Thermoplasma*, *Sulfolobus*, *M. vannielii* and *M.* strain PS. The identification of the C_{19}-isoprenoids was done by comparison of GC retention and mass spectral data with those of authentic pristane. In addition mass spectral evidence for a C_{19}-isoalkane from *Thermoplasma* was obtained (Fig. 3e). Phytane ($C_{20}H_{42}$) was detected in the extracts of *Methanobacterium* strain M.o.H. and *Thermoplasma*. The structures were substantiated by co-chromatography and mass spectral fragmentation patterns. Phytane was tentatively identified in *Sulfolobus*.

TABLE I

METHYL-BRANCHED HYDROCARBONS (%) IN METHANOGENS, *THERMOPLASMA*, AND *SULFOLOBUS*

The percent composition of isopranyl and isoprenyl lipids is listed in relation to the total lipid content from the combined hexane and benzene fractions. (a) The remaining neutral lipids consist of a series of *n*-alkanes (C_{19}–C_{32}); (b) the remaining neutral lipids consist of *n*-alkanes and other compounds not listed in this table.

Molecular ion	*M. strain AZ (a)*	*M. ruminantium PS*	*M. ruminantium M-1*	*M. thermoautotrophicum*	*M. strain M.o.H.*	*M. vannielii*	*M. strain PS*	*M. hungatii*	*M. barkeri*	*Thermoplasma acidophilum (b)*	*Sulfolobus acidocaldarius (b)*	*Sulfolobus species (b)*
$C_{18}H_{32}$										3.8		
$C_{16}H_{34}$										5.4		
$C_{16}H_{32}$											<0.1	
$C_{19}H_{30}$										1.5		
$C_{17}H_{36}$										<0.1		1.0
$C_{17}H_{34}$										<0.1		
$C_{18}H_{38}$						0.8				2.4	<0.1	1.6
$C_{18}H_{36}$						1.0						
$C_{18}H_{34}$						1.2						
$C_{19}H_{40}$					<0.1	0.7	0.5			2.6	0.5	6.4
$C_{19}H_{38}$											0.3	
$C_{20}H_{42}$	<0.1				5.8					0.8	1.2	15.1
$C_{20}H_{40}$	<0.1				4.0					1.9	<0.1	

$C_{20}H_{38}$					2.8	0.3					<0.1	0.5
$C_{20}H_{36}$					1.5	1.0					<0.1	
$C_{20}H_{34}$	<0.1				1.0	1.3				3.1		
$C_{21}H_{44}$												5.0
$C_{24}H_{50}$											2.1	
$C_{24}H_{48}$											0.5	
$C_{25}H_{52}$									39.5		6.8	
$C_{25}H_{50}$				15.0					26.3		3.4	
$C_{25}H_{48}$				<0.1					13.5			
$C_{25}H_{46}$		0.2				0.8	0.8		13.0			
$C_{25}H_{44}$						7.0						
$C_{25}H_{42}$		2.0				16.5				7.2		
$C_{26}H_{54}$											5.1	
$C_{28}H_{58}$											2.7	
$C_{28}H_{56}$											1.9	
$C_{29}H_{58}$											7.1	
$C_{30}H_{62}$				<0.1							8.6	
$C_{30}H_{60}$			3.0	<0.1							1.3	
$C_{30}H_{58}$			6.2	2.5							0.4	
$C_{30}H_{56}$		0.6	9.3	7.5			18.1					
$C_{30}H_{54}$		3.0	16.0	20.0	3.7	0.3	52.0	1.8				
$C_{30}H_{52}$		12.0	22.5	27.4	10.6	4.1	13.2	10.0				
$C_{30}H_{50}$	64.0	82.0	41.5	24.9	69.0	64.0	2.5	88.0		34.0		

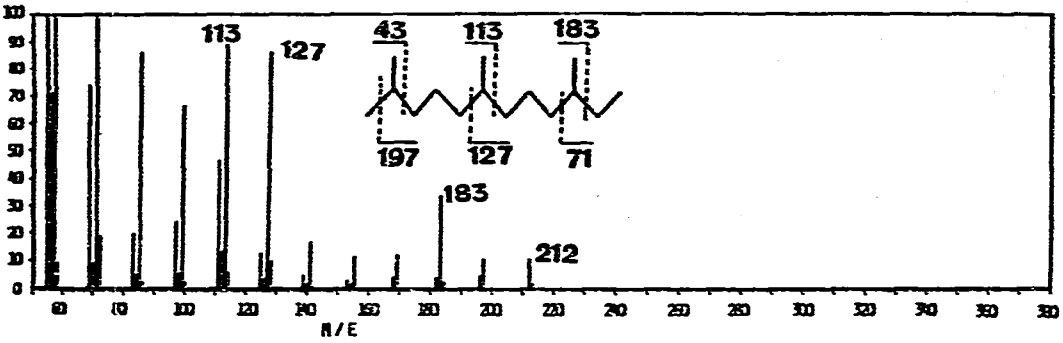


Fig. 1. Mass spectrum of a C_{15}-isoprenoid isolated from *Thermoplasma*.

The mass spectra of the $C_{20}H_{40}$ isoprenoid monoenes isolated from *Sulfolobus* and *Methanobacterium* strain M.o.H. were identical. The spectrum of *Methanobacterium* strain M.o.H. showed major peaks at *m/e* 56, 70, 126, 140, 198, 210 and 280. Although the fragmentation pattern is consistent with a phytane skeleton, a change in the position of the terminal methyl group will probably generate a very similar mass spectrum. A variety of other unsaturated (or cyclic) C_{20}-isoprenoids were isolated. Fragmentation patterns as well as retention data suggest that they are methyl branched, however a structural identification from mass spectral data alone is not possible.

A C_{21}-isoprenoid was isolated from *Sulfolobus* and its mass spectrum is shown in Fig. 6. McCarthy *et al.*[36] characterized a C_{21}-isoprenoid, isolated from Precambrian sediments, using two synthetic standard compounds, 2,6,10,14-tetramethylheptadecane (head to tail), and 2,6,10,15-tetramethylheptadecane (tail to tail). The mass spectra of the two isoprenoids are very similar, except that the head to tail structure showed a fragment at *m/e* 253, which cannot be formed from the tail to tail structure and thus was absent in the mass spectrum of 2,6,10,15-tetramethylheptadecane. On the basis of the *m/e* 253 peak which is clearly present in the mass spectrum of the C_{21}-isoprenoid from *Sulfolobus* we suggest 2,6,10,14-tetramethylheptadecane as structure, which was incidentally the same one assigned to the C_{21}-isoprenoid isolated from Precambrian sediments.

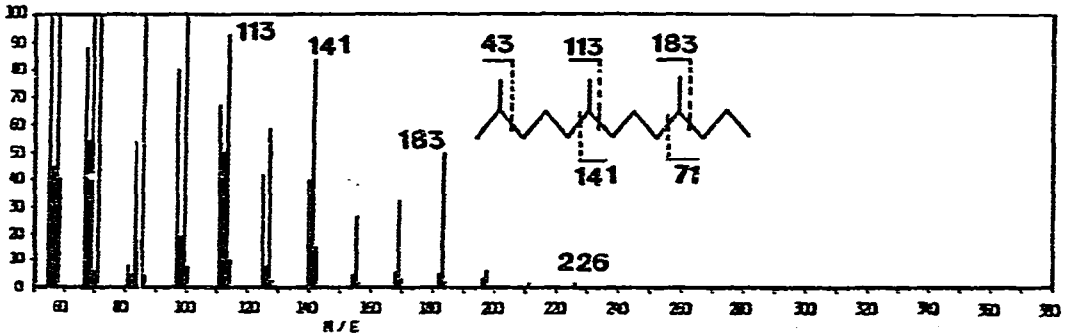


Fig. 2. Mass spectrum of a C_{16}-isoprenoid isolated from *Thermoplasma*.

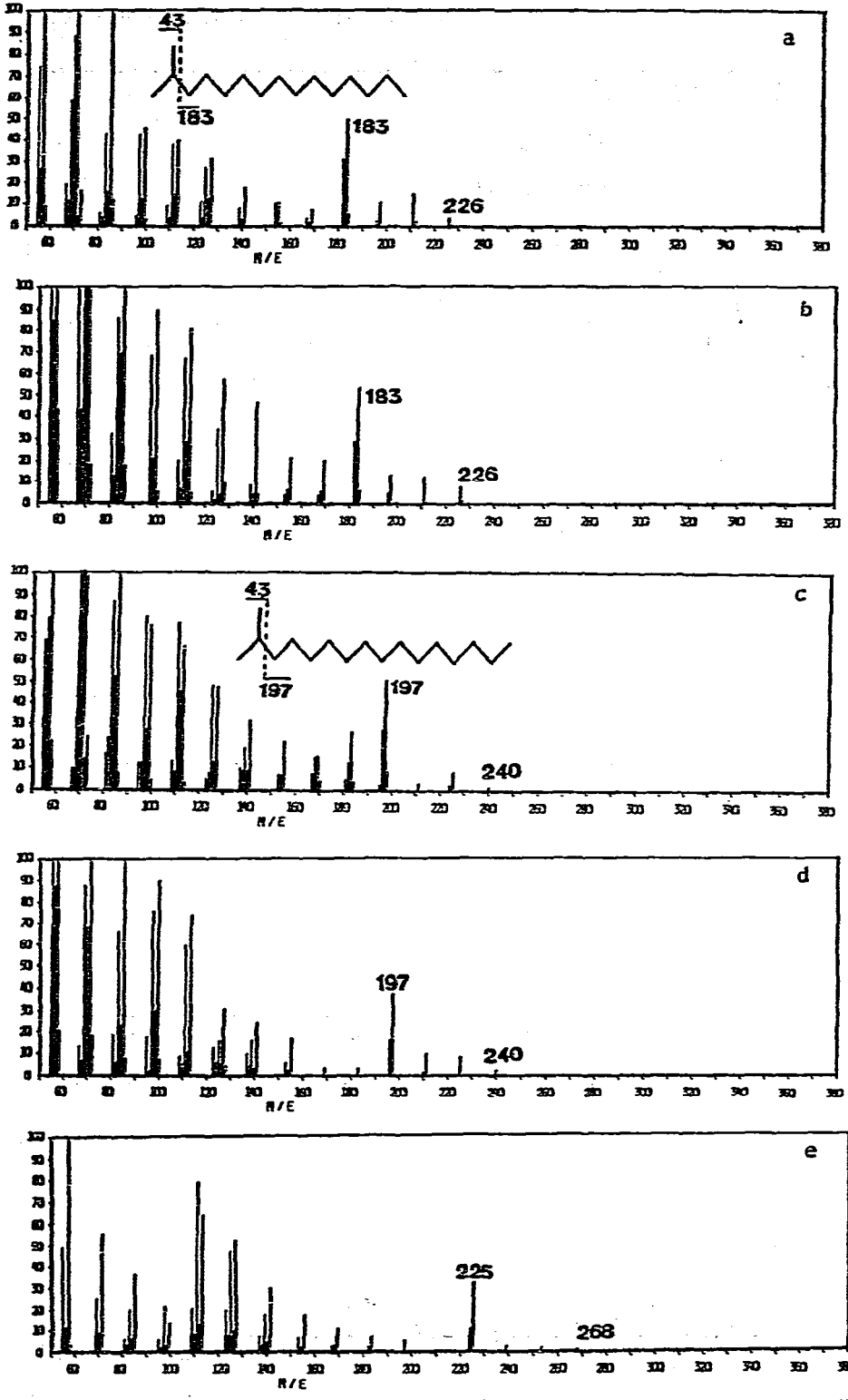


Fig. 3. Mass spectra of (a) a standard C_{16}-isoalkane (2-methylpentadecane); (b) a C_{16}-isoalkane isolated from *Thermoplasma*; (c) a standard C_{17}-isoalkane (2-methylhexadecane); (d) a C_{17}-isoalkane isolated from *Thermoplasma* and (e) a C_{15}-isoalkane isolated from *Thermoplasma*.

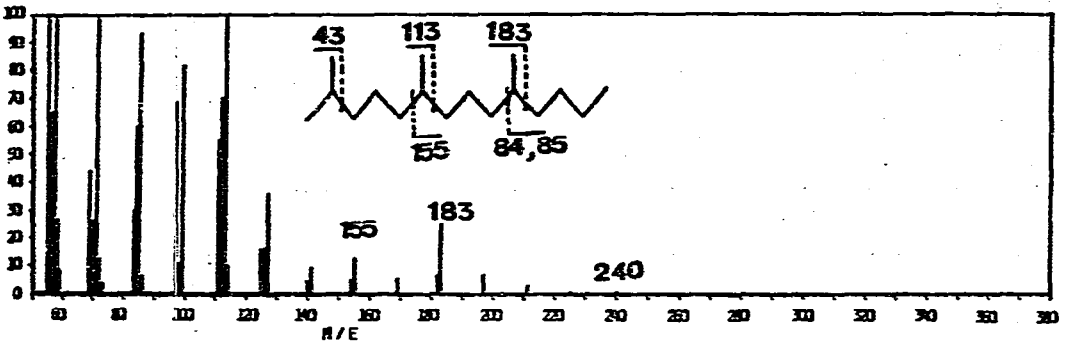


Fig. 4. Mass spectrum of a C_{17}-isoprenoid isolated from a *Sulfolobus* species.

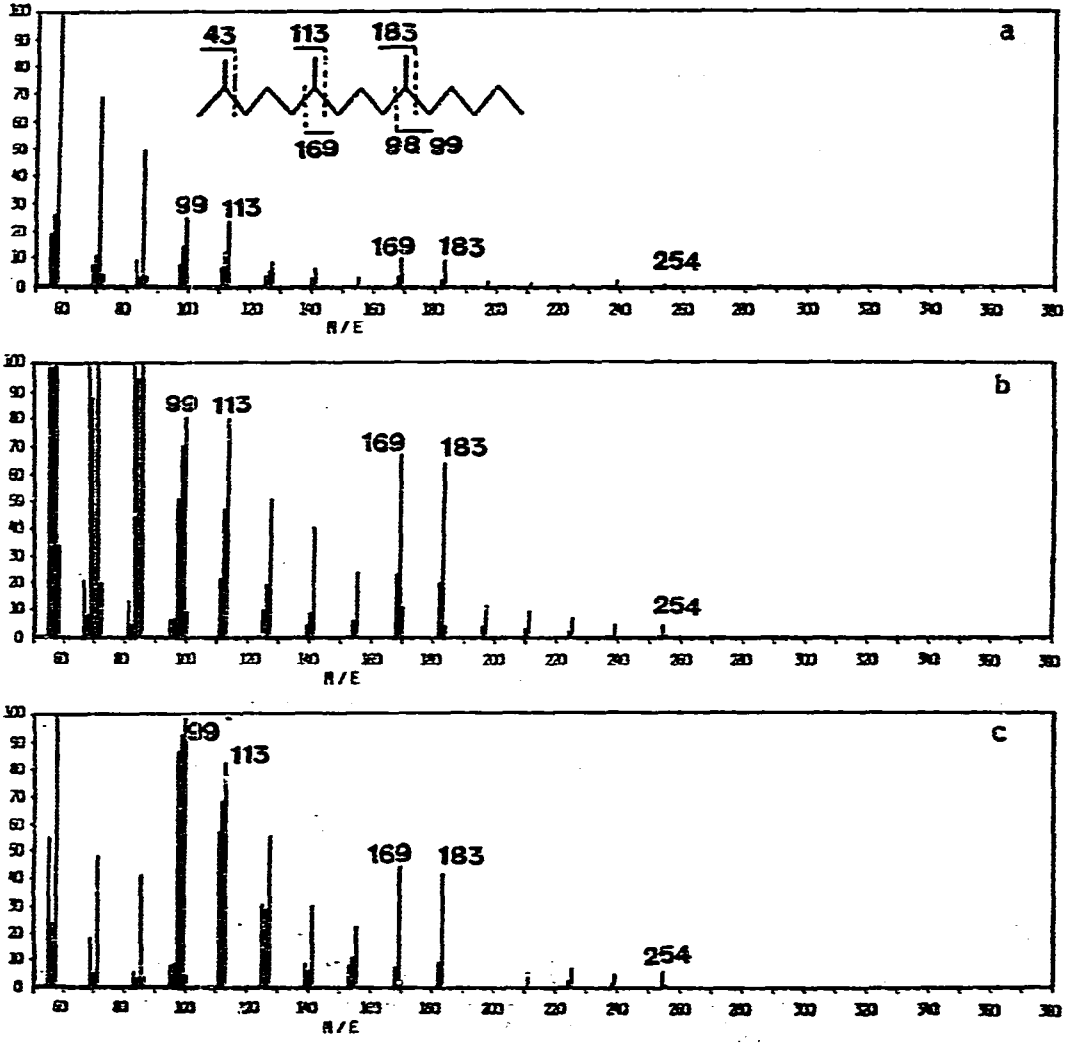


Fig. 5. Mass spectra of (a) a standard C_{18}-isoprenoid (norpristane); (b) a C_{18}-isoprenoid isolated from *Thermoplasma* and (c) a C_{18}-isoprenoid isolated from *Sulfolobus*.

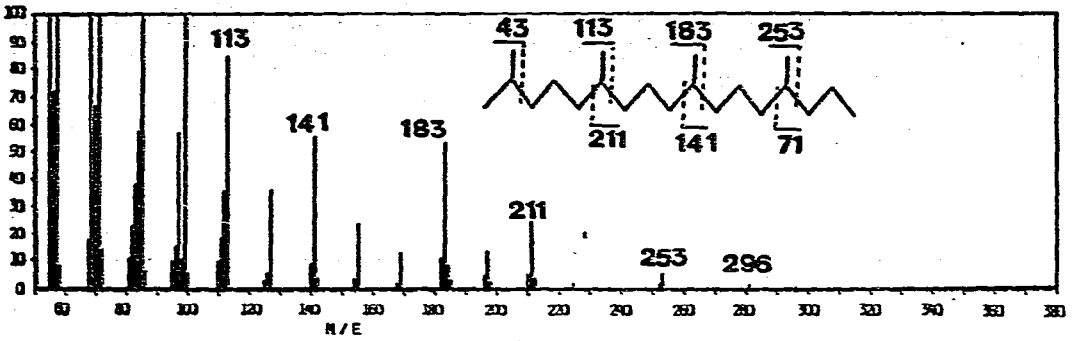


Fig. 6. Mass spectrum of a C_{21}-isoprenoid isolated from *Sulfolobus*.

The mass spectrum of the C_{24}-isoprenoid isolated from *Sulfolobus* is presented in Fig. 7. Its fragmentation pattern is practically identical to those reported in the literature for a regular C_{24}-isoprenoid[8,12]. Prominent peaks at *m/e* 113, 183 and 253 indicate the regular head to tail structure. This assumption is further substantiated by the absence of a doublet at *m/e* 85, 84, which would be observed if the isoprenoid had a tail to tail condensation site.

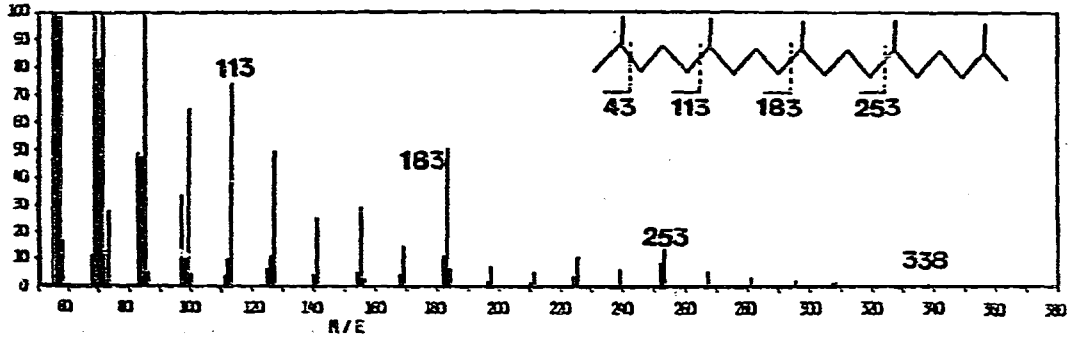
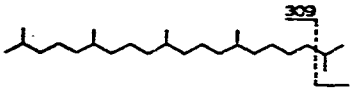
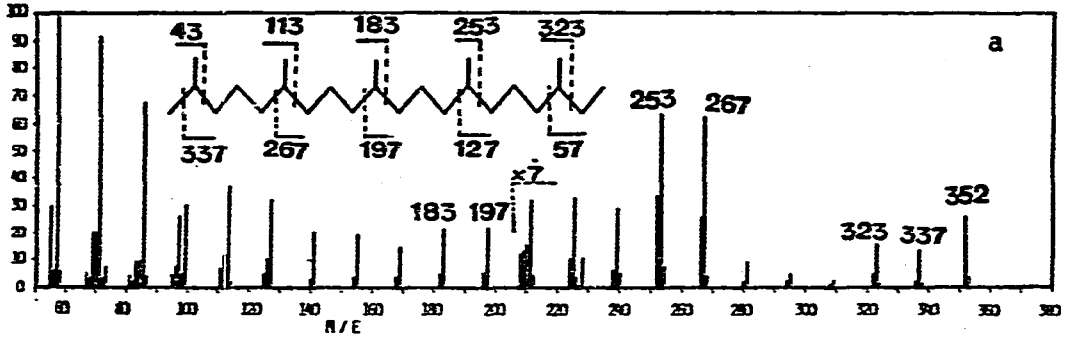


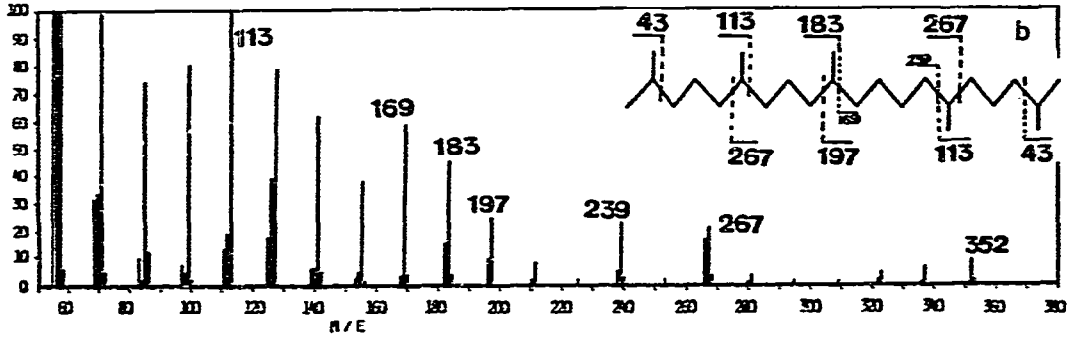
Fig. 7. Mass spectrum of a C_{24}-isoprenoid isolated from *Sulfolobus*.

The mass spectra of the C_{25}-isoprenoid of *M. thermoautotrophicum* and *M. barkeri* are practically identical and are shown in Fig. 8b and c. Their fragmentation pattern is quite different from C_{25}-isoprenoids isolated from *Sulfolobus* (Fig. 8a). The major fragments of the *Sulfolobus* isoprenoid at *m/e* 113, 127, 183, 197, 253, 267, 323 and 337 seem to indicate the presence of a regular head to tail structure. A tail to tail condensation as in 2,6,10,15,19-pentamethylcosane can be ruled out because these compounds cannot produce an *m/e* 253 peak. Spyckerelle *et al.*[10] demonstrated that 2,6,10,14,18-pentamethylcosane (regular head to tail) and 2,6,10,14,19-pentamethylcosane (tail to tail) have quite similar mass spectra and recommended cochromatography for their positive identification. A close examination of the reported mass spectrum of 2,6,10,14,19-pentamethylcosane[10] shows a major fragment at *m/e* 309, which is missing in the fragmentation pattern of the regular head to tail structure of 2,6,10,14,18-pentamethylcosane. After bond cleavage at a methyl branch, the charge usually remains on the secondary carbon, however if the primary carbon

chain is long enough, the charge can stay on the primary fragment. This exactly is observed in the mass spectrum of 2,6,10,14,19-pentamethylcosane where the *m/e* 309 fragment consists of a sufficiently long chain.







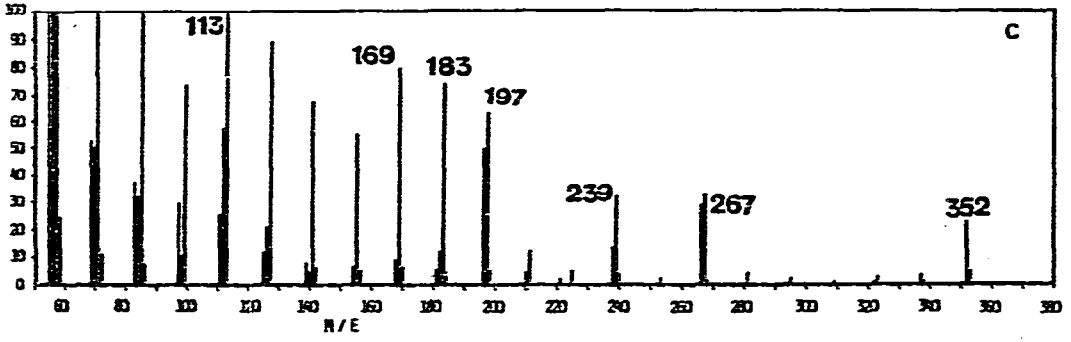


Fig. 8. Mass spectra of (a) a C_{25}-isoprenoid isolated from *Sulfolobus*; (b) a C_{25}-isoprenoid isolated from *Thermoautotrophicum* and (c) a C_{25}-isoprenoid isolated from *M. barkeri*.

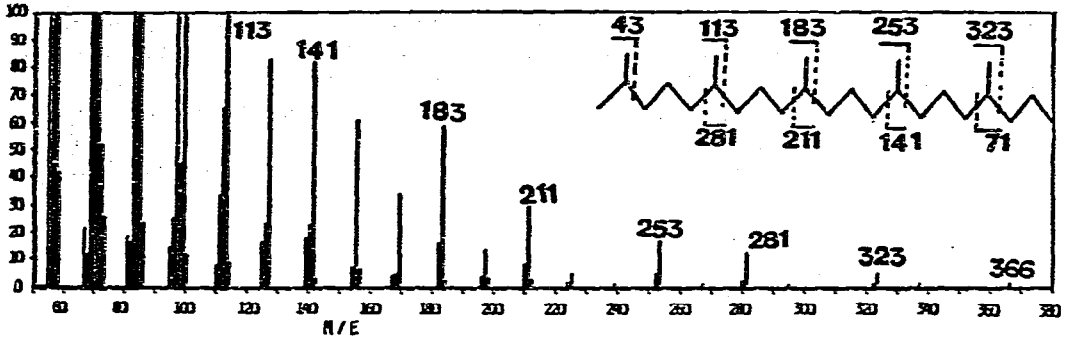


Fig. 9. Mass spectrum of a C_{26}-isoprenoid isolated from *Sulfolobus*.

Since the C_{25}-isoprenoid from *Sulfolobus* does not have an intense fragment at *m/e* 309 we suggest a regular head to tail structure for the compound. The structure of 2,6,10,15,19-pentamethylcosane was assigned to the fragmentation pattern of the C_{25}-isoprenoids in Fig. 8b and c. Such a compound is expected to produce major fragments at *m/e* 113, 183, 197 and 267. The additional intense peaks at *m/e* 169 and 239 are due to bond cleavage at the tail to tail condensation site. This generates a long primary carbon chain, which holds the positive charge. Cleavage at C-10 produced a secondary carbon fragment at *m/e* 183 and a primary carbon fragment at *m/e* 169, whereas cleavage at C-15 results in a secondary carbon fargment at *m/e* 113 and a primary at *m/e* 239. The doublet formation at *m/e* 196, 197 and *m/e* 266, 267, which is characteristic for fragments with long straight chains, substantiates the above described fragmentation mechanism. The monounsaturated C_{25}-isoprenoid from *M. barkeri* has a strong molecular ion peak at *m/e* 350 and shows intense fragment ions at *m/e* 126, 196, 210 and 280. These data suggest a 2,6,10,15,19-pentamethylcosane skeleton for the C_{25}-isoprenoid monoene, which is consistent with the structure of the corresponding saturated compound.

The mass spectrum of the C_{26}-isoprenoid, isolated from *Sulfolobus*, is presented in Fig. 9. On the basis of the intense peaks at *m/e* 113, 141, 183, 211, 253, 281, 323 and the molecular ion at 366 the spectrum supported a regular head to tail structure. The C_{28}-isoprenoid (Fig. 10) is also believed to have the regular head to tail

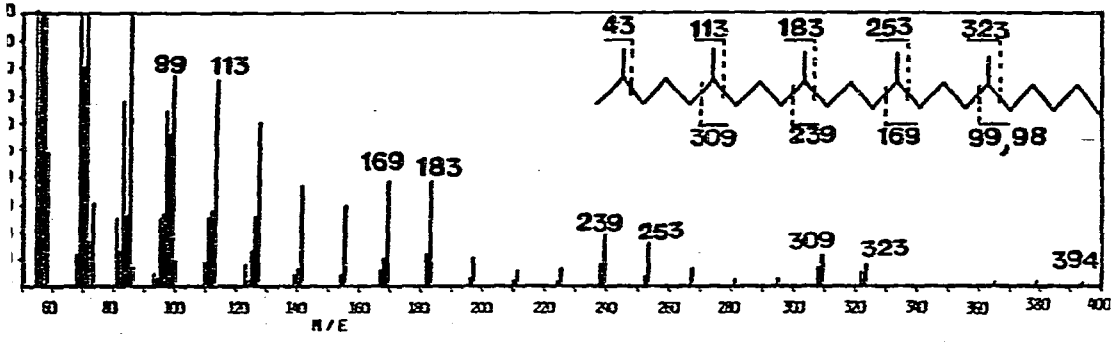


Fig. 10. Mass spectrum of a C_{28}-isoprenoid isolated from *Sulfolobus*.

skeleton. The mass spectrum exhibits the corresponding major fragments, and shows a doublet at *m/e* 98, 99. The doublet, indicating the presence of a straight chain, can only be produced by the regular head to tail skeleton, thus eliminating any irregular structure. For the saturated C_{29}-isoprenoid from *Sulfolobus* we have only chromatographic evidence, however a mass spectrum of the corresponding C_{29}-monoene was obtained. It showed intense peaks at *m/e* 56, 126, 196, 266, 336 and 406 which are an indication for a regular head to tail structure.

From the large number of mass spectra obtained for various C_{30}-isoprenoids we selected a few for discussion. In Fig. 11a the mass spectrum of the C_{30}-isoprenoid from *Sulfolobus* is presented. The fragmentation supports a regular head to tail structure. On the basis of arguments developed earlier for the regular C_{25}-isoprenoid (Fig. 8a), a 2,6,10,14,18,23-hexamethyltetracosane skeleton can be ruled out since a peak at *m/e* 379 (M—43) is not observed. Thus a regular head to tail structure seems likely for the C_{30}-isoprenoid. The mass spectrum of the C_{30}-isoprenoid monoene, isolated from the same source, shows a strong molecular ion peak at *m/e* 420 and major fragments at *m/e* 126, 140, 196, 210, 252, 266, 280 and 336 suggesting also a 2,6,10,14,18,23-hexamethyltetracosane skeleton.

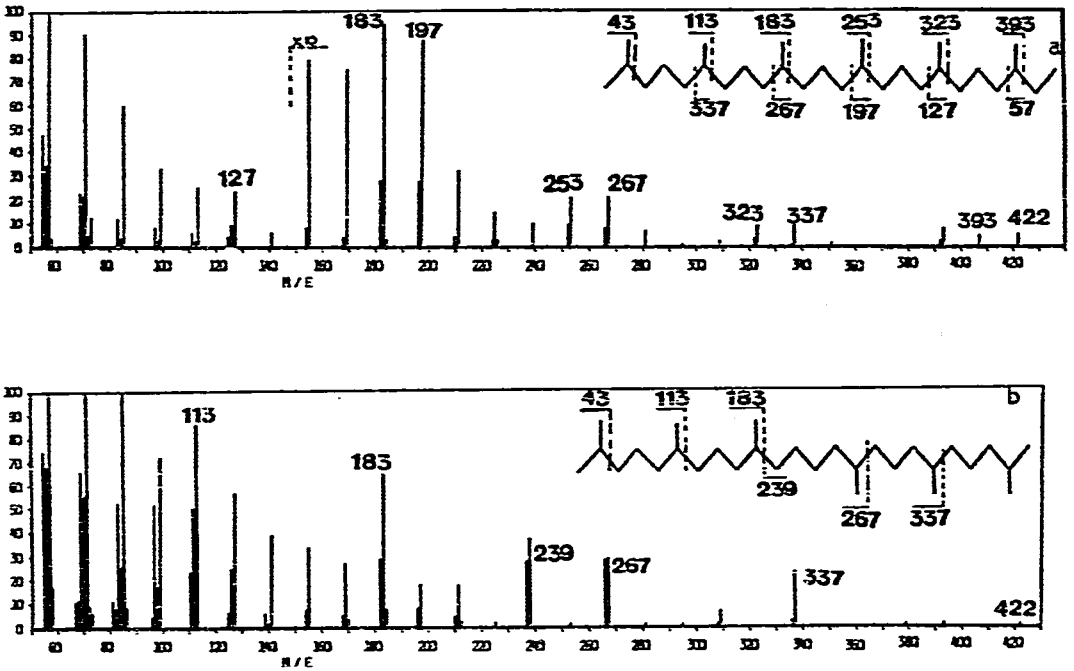


Fig. 11. Mass spectra of (a) a C_{30}-isoprenoid isolated from *Sulfolobus* and (b) a C_{30}-isoprenoid (squalane).

The mass spectrum of squalane (2,6,10,15,19,23-hexamethyltetracosane) is shown in Fig. 11b. The compound exhibits the expected fragmentation pattern with major peaks at *m/e* 113, 183, 276 and 337. The intense peak at *m/e* 239 is obtained because of the favored formation of the primary carbon chain fragment. The peaks at

m/e 266 and 238 are intensified as a result of the long straight carbon chain of these fragments. The mass spectrum of the corresponding C_{30}-isoprenoid monoene isolated from *M. ruminantium* M-1 shows peaks at *m/e* 126, 196, 280, 350 and 420, indicative for a 2,6,10,15,19,23-hexamethyltetracosane.

There is solid evidence that the phytol side chain of chlorophyll is a biological precursor to phytane, one of the abundant isoprenoids found in sediments and petroleum[18–20]. The formation of the lower isoprenoids from phytol is believed to occur through a series of oxidation, reduction or cracking mechanisms, which might have been prevalent in the geological environment. Although it is known from diagenesis experiments that microbial activity or high subsurface temperatures can alter the chemical integrity of compounds such as phytol[11,37], there is no experimental evidence which assesses the role of these factors on the distribution of isoprenoids in sediments and petroleum. Our present study shows that the majority of these isoprenoids are present as neutral lipids or intermediate metabolites in the examined microorganisms.

In Table II, they are compared with a series of isoprenoids, known to be present in ancient geological samples (up to $2.7 \cdot 10^9$ years). The isoprenoids of both groups are in the same carbon range. All compounds listed in Table II have a regular head to tail structure except for squalane (or squalene) which have resulted from a tail to tail condensation of two farnesane residues.

TABLE II

C_{15}–C_{30} ISOPRENOIDS FOUND IN ANCIENT OIL SHALES AND PETROLEUM, AND THEIR OCCURRENCE IN METHANOGENS AND THERMOACIDOPHILES

Squalane has been identified only in *M. thermoautotrophicum*, however, most methanogenic bacteria contain squalene and various squalene analogs differing only in degree of hydrogenation.

Isoprenoid	*Geological sample*	*Isoprenoid*	*Microorganism*
C_{15}–C_{20}	Green River shale[40]	C_{15}–C_{20}	*Thermoplasma*
C_{15}–C_{21}	Nonesuch shale[5,36,41]	C_{16}, C_{18}–C_{20}	*Sulfolobus*
C_{15}, C_{18}–C_{21}	Antrim shale[5,36]	C_{17}–C_{21}	*Sulfolobus* species
C_{18}–C_{21}	Soudan shale	C_{18}, C_{19}	*M. vannielii*
		C_{19}, C_{20}	*M.* M.o.H.
		C_{20}	*M.* PS; *M.* AZ
C_{21}–C_{25}, C_{30} (Squalane)	African cretaceaus shale[10]	C_{21}	*Sulfolobus* species
		C_{24}, C_{25}	*Sulfolobus*
		C_{30} (Squalene)	Methanogens
C_{22}–C_{25}	Bell Creek crude oil[8]	C_{24}, C_{25}	*Sulfolobus*
C_{24}, C_{25}, C_{28} C_{30}	Costa Rican seep oil[12]	C_{24}, C_{25}, C_{28} C_{30}	*Sulfolobus*
C_{30} (Squalane)	Nigerian petroleum[38]	C_{30} (Squalene)	Methanogens

Squalane, a major isoprenoid in most methanogenic bacteria (Table I) has been isolated in its fully hydrogenated form (squalane) from Nigerian petroleum[38]. Squalane is one of the few isoprenoids in sediments or petroleum known to have a tail to tail skeleton, a structure which is also prevalent in the C_{25} and C_{30}-isoprenoids of the methanogens, so far analyzed. The corresponding C_{30}-isoprenoid with the

regular head to tail skeleton was reported in Bell Creek crude oil[8]. We isolated a structurally similar compound from *Sulfolobus*. The identified isoprenoids from *Sulfolobus* (C_{16}–C_{30}) seem to possess all the regular head to tail skeleton; there was no evidence for the irregular tail to tail structure. Of further interest is the isolation of a C_{17}-isoprenoid from a *Sulfolobus* species, as well as from ancient sediments[7–11,25]. This hydrocarbon is difficult to generate from phytane in a diagenetic pathway because it involves the bond cleavage of two carbon–carbon bonds.

As minor components, several isoalkanes have been isolated from *Thermoplasma* (iso-C_{16}, C_{17}, C_{19}). These compounds which are also known to occur in algae[39] have been detected in various sediments[5,36]. Besides the branched hydrocarbons, which are the major constitutents of the examined microorganisms, several other groups of substances were detected. Various samples contained a sequence of methyl branched fatty acids. Cyclic terpenoids have been identified in two samples, as well as an entire series of methyl branched alkylbenzenes.

CONCLUSION

Our present study shows that the majority of isoprenoids detected in sediments and petroleum are also present as cellular products or intermediate metabolites in methanogens and in specific thermoacidophiles. The fact that these microorganisms may have dominated the biosphere in the early history of our planet suggests that many isoprenoids isolated from ancient geological samples may actually have been synthesized by microorganisms such as described here. It also suggests that current anaerobic environments, such as for instance the geopressurized natural gas zones of the Gulf of Mexico, and other special environments, are the abode of these unique and interesting microorganisms currently called Archaebacteria.

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