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A polyunsaturated irregular acyclic C_{25} isoprenoid in a methanogenic archaeon

Jaap S. Sinninghe Damsté[1*], Stefan Schouten[1], N. Herbert van Vliet[1], Robert Huber[2] and Jan A.J. Geenevasen[3]

[1] Netherlands Institute for Sea Research (NIOZ), Department of Marine Biogeochemistry and Toxicology,
P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands.

[2] Lehrstuhl für Mikrobiologie, University of Regensburg, Universitätstrasse 31, D-93053 Regensburg, Germany.

[3] University of Amsterdam, Faculty of Chemistry, Department of Organic Chemistry,
Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands.

Abstract: All *trans* 2,6,10,15,19-pentamethylicosa-2,6,14,18-tetraene **1** has been isolated from the methanogenic archaeon *Methanosarcina mazei* and identified by ^1H and ^{13}C NMR spectroscopy and mass spectrometry. © 1997 Elsevier Science Ltd.

Recently, we determined the lipid composition of a suite of methanogenic archaea.[1] Two out of six methanogenic archaea contained relatively high amounts of unsaturated alkenes containing 3-5 double bonds and had the irregular tail-to-tail isoprenoid 2,6,10,15,19-pentamethylicosane (**2**) skeleton as determined by hydrogenation and mass spectral analysis.[2] To establish the positions of the four double bonds of the most abundant unsaturated irregular C_{25} isoprenoid in *Methanosarcina mazei*, it was isolated. Cell masses of the *M. mazei* isolate G1 (Culture no. DSM 3338) were grown at 37°C under stirring at pH 6.9 in a 100 l fermenter pressurised with 200 kPa N_2/CO_2 (80:20; v/v). For cultivation, modified M3-medium[3], supplemented with methanol (0.5%, final concentration) was used. Cells were harvested by continous centrifugation, yielding 4.65 g of freeze-dried biomass. The biomass was ultrasonically extracted with dichloromethane/methanol (1:1, v/v; 3x) and dichloromethane (3x) yielding 180 mg of total extract, which was separated by column chromatography and

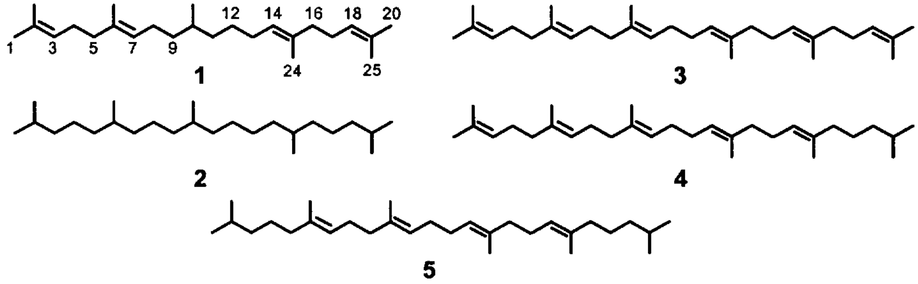

Fig. 1: Structures of compounds.

subsequent preparative thin layer chromatography of the apolar fraction using hexane as developer. This yielded a fraction (2.7 mg) consisting of 90% of **1** (other 10% consisted of a complex mixture of hydrocarbons, none representing more than 1% of the total fraction). This was analysed by high field ^1H and ^{13}C NMR (Table 1), which led to complete assignment of proton and carbon chemical shifts. The ^1H NMR spectrum revealed the presence of 4 olefinic H, 12 allylic H, 6 olefinic CH_3 and 1 aliphatic CH_3, 7 aliphatic protons in CH_2 and CH groups. Carbon multiplicities were established by APT and DEPT spectra and revealed that **1** contains 25 carbon atoms with 4 olefinic C, 4 olefinic and 1 aliphatic CH, 9 CH_2 and 7 CH_3 units. ^1H-^1H COSY, normal and inverse one and multiple (^2J and ^3J) bond ^1H-^{13}C correlations (e.g. Fig. 2c) were used to assign chemical shifts. These assignments, in combination with the known irregular isoprenoid carbon skeleton of **1**[1], established that the double bonds are at positions 2, 6, 14 and 18. Allylic and homoallylic long-range couplings observed in the ^1H-^1H COSY spectrum (Fig. 2a) established the structure of **1**. The structural assignment was further confirmed by NOE experiments. These experiments also unequivocally proved the stereochemistry to be all *trans* by the indicated NOE interactions (Fig. 2b), no NOE interactions being observed between H-3 and H-21, H-7 and H-22, H-14 and H-24, H-18 and H-25, H-4 and H-1 and H-17 and H-20.

Although pentamethylicosenes have been tentatively identified in various methanogenic archaea[4-5], this is the first rigorous identification of a polyunsaturated irregular C_{25} isoprenoid in such archaea. The occurrence of the fully saturated analogue **2** in the methanogenic archaeon *M. barkeri* has been unambigiously demonstrated.[5-6] The occurrence of **1** fits with the fact that thermophilic archaea generally only biosynthesize

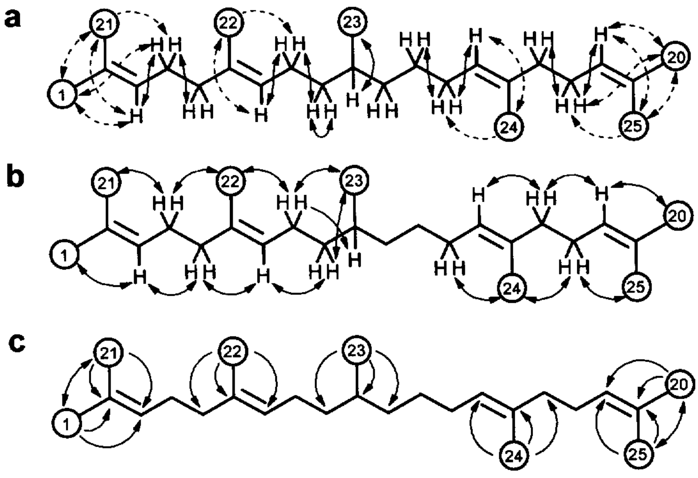

Fig. 2: (a) Connectivities from a COSY experiment (stippled lines indicate long-range allylic and homoallylic couplings), (b) long-distance NOE interactions of methyl groups (C-1, C-20, C-21, C-22, C-23, C-24 and C-25), olefinic CH groups and allylic and one aliphatic (C-9) CH_2 groups and (c) connectivities from an inverse long range (^2J and ^3J) ^1H-^{13}C correlation experiment.

squalene **3**, whereas the methanogenic archaea contain a more diverse suite of isoprenoid hydrocarbons.[7] Interestingly, halophilic archaea contain, in addition to squalene, its reduced counterparts (e.g. **4-5**)[8], probably biosynthesized from partial reduction of squalene. This suggests that the novel isoprenoid **1** is biosynthesized in an analogous fashion through the tail-to-tail coupling of farnesyl pyrophosphate and geranyl pyrophosphate and subsequent reduction of the central double bond, although in the biosynthesis of **4** and **5** not the central

Table 1. ^{1}H and ^{13}C Data of **1**.

C-number	H-shift	C-shift			
		CH_3	CH_2	CH	C
1	1.68 (m, 3H)	25.69			
2	-				131.24
3	5.09 (m, 1H)			124.42	
4	2.06 (m, 2H)		26.74		
5	1.98 (m, 2H)		39.75		
6	-				134.75[c]
7	5.12 (m, 1H)			124.85[b]	
8	1.97 (m, 2H)		28.23		
9	1.13 (m, 1H), 1.33 (m, 1H)		37.09		
10	1.40 (m, 1H)			32.33	
11	c. 1.26 (m, 2H)		36.63		
12	c. 1.32 (m, 2H)		27.28		
13	1.96 (m, 2H)		25.46		
14	5.12 (m, 1H)			124.96[b]	
15	-				134.56[c]
16	1.98 (m, 2H)		39.75		
17	2.06 (m, 2H)		26.74		
18	5.09 (m, 1H)			124.42	
19	-				131.24
20	1.68 (m, 3H)	25.69			
21	1.60 (m, 3H)	17.68			
22	1.59 (m, 3H)	15.93[a]			
23	0.86 (d, 3H, J=6.5 Hz)	19.63			
24	1.59 (m, 3H)	15.98[a]			
25	1.60 (m, 3H)	17.68			

[a,b,c] assignments may be interchanged

double bond(s) but the external double bond(s) are hydrogenated. This also suggests that the pentamethylicosene isomer with 5 double bonds identified in *Methanolobus bombayensis*[1] possesses its double bonds at positions 2, 6, 10, 14 and 18 and those with 0-3 double bonds identified in various methanogenic archaea[1,4-6] are further biosynthetic reduction products of **1**.

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