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CONFIRMATION OF 2,6,10,15,19-PENTAMETHYLEICOSANE IN METHANOGENIC BACTERIA AND SEDIMENTS.

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Abstract: A C_{25} alkane (1) proposed previously to occur in Methanobacterium barkeri and M.thermoautotrophicum[1] and to be a sedimentary biological marker for methanogenic bacteria[9], has been synthesised and its occurrence confirmed.

Recently there has been considerable interest in the lipid content of a distinct phylogenetic group of microorganisms known as the Archaebacteria[1-5]. These organisms, distinguishable[6-8] from other bacteria by the presence of characteristic ribosomal RNA and absence of muramic acid in the cell wall, contain a number of isoprenoid hydrocarbons and ethers apparently as their principal lipids[1-5]. Amongst these the C_{25} alkane 2,6,10,15,19-pentamethyleicosane (1) has so far been tentatively identified only in Methanobacterium barkeri and Methanobacterium thermoautotrophicum, although several related alkenes have also been detected in three other species of methanogens[1]. Alkane (1) has recently been proposed as a biological marker for methanogenic activity in marine sediments of different ages[9]. Possibly, diagenetic reduction of the alkenes could also give rise to (1) in sediments where M.barkeri and/or M.thermoautotrophicum were/are not major lipid contributors. Alternatively, the ratio of alkanes to alkenes may change in relation to the growth stage.

Structural assignment of (1) has to date been inferred only from interpretation of electron impact (EI) mass spectra[1,9,10], which has been proposed to distinguish it from 2,6,10,14,18-pentamethyleicosane (2) found in sedimentary rocks[11,12], petroleums[13] and Sulfolobus acidocaldarius[1], a thermoacidophilic bacterium. This differentiation is based upon the presence of a minor ion at m/z 253 (% ≈ m/z 239, 267) in the spectrum of the "regular" isoprenoid (2), an ion not observed in the spectrum of (1) which has a linkage formed from a tail-to-tail condensation of isoprene units[1].

We report here the synthesis of (1) and confirm its presence in a recent marine sediment. The synthetic scheme is outlined in Figure 1. Two isomeric C_{25} alkanes (2,3) were also synthesised for comparison, using methods described previously[12].

3RS,7-dimethyloctanal (4) was condensed with 1-bromo-3RS,7RS,11-trimethyldodecane (5) in a Grignard reaction. Dehydration of the resulting 2,6RS,11RS,15RS,19-pentamethyleicosan-8RS-ol (6) gave initially two major isomeric C_{25} monoenes (GC-MS analysis), inseparable by silver nitrate t.l.c. and which could not be hydrogenated at ambient temperature and pressure. The alkenes could be isomerised fully, however, on a silica column ($AgNO_3$; 10%) to two double bond isomers (7) which gave the required alkane (1) upon hydrogenation. The double bond positions of the alkenes (7) were determined by cis-hydroxylation with OsO_4, followed by GC-MS analysis of their 1,2-di-trimethylsilyl ethers[14,15]. The initial alkene products of dehydration could not be hydroxylated with OsO_4, suggesting (with the t.l.c. and hydrogenation behaviour) that the double bonds are more hindered.

The EI spectrum of (1) was identical to that reported previously[1,10]. The rationalisation of the spectrum made by Holzer et al.[1] is therefore confirmed and it is clear that the compound observed in methanogens and in marine sediments from recent to Lower Cretaceous age is indeed (1).

The ^{13}C NMR spectrum of (1) is consistent with the NMR additive rules for branched acyclic hydrocarbons[16] and with the spectrum of a standard,2,6R,10S,14-tetramethylpentadecane (meso-pristane), the assignments below being based upon this agreement. The spectrum was also readily distinguishable from those of (2) and (3) and hence would provide proof of the presence of (1) in methanogens or sediments, if sufficient amounts could be isolated pure from either source.

Alkane (1) was tentatively assigned earlier in a diatomaceous ooze from Walvis Bay, off Namibia[10]. We have confirmed this identification by comparison of the EI spectra of both the synthesised compound and the unknown by GC-MS and by coinjection on two GC stationary phases (20m x 0.2mm OV-1, 120-270°C at 6°C/min; 100m x 0.24mm DEGS/PEGS 3/1, 50-135°C at 4°C/min.). The four pairs of enantiomers of the synthesised compound (1) were partly resolved into a broad 1:2:1 triplet under the latter GC conditions which separate diastereoisomers of other isoprenoid alkanes[17], whereas the Walvis Bay component showed only a single peak coeluting with the last peak of the triplet under the same conditions (Figure 2). This result indicates that in vivo synthesis of (1) is stereospecific (one or two enantiomers), as demonstrated for certain other isoprenoids in Archaebacteria[18].

The isomeric alkanes (1), (2) and (3) could not be separated when coinjected on a number of GC phases (OV-1, OV-73, Carbowax 20M, Apiezon L; 20m x 0.2mm) under both isothermal and slow temperature-programmed conditions. Indeed, they could not be completely separated when examined under the conditions which partly separated their diastereoisomers (DEGS/PEGS). As a consequence, GC-MS remains the only effective method of identifying these compounds in biological and geological mixtures[1,9]. However, this is not an ideal method of distinguishing the alkanes since (2) (m/z 253 present) would obscure the presence of (1) in a mixture of both.

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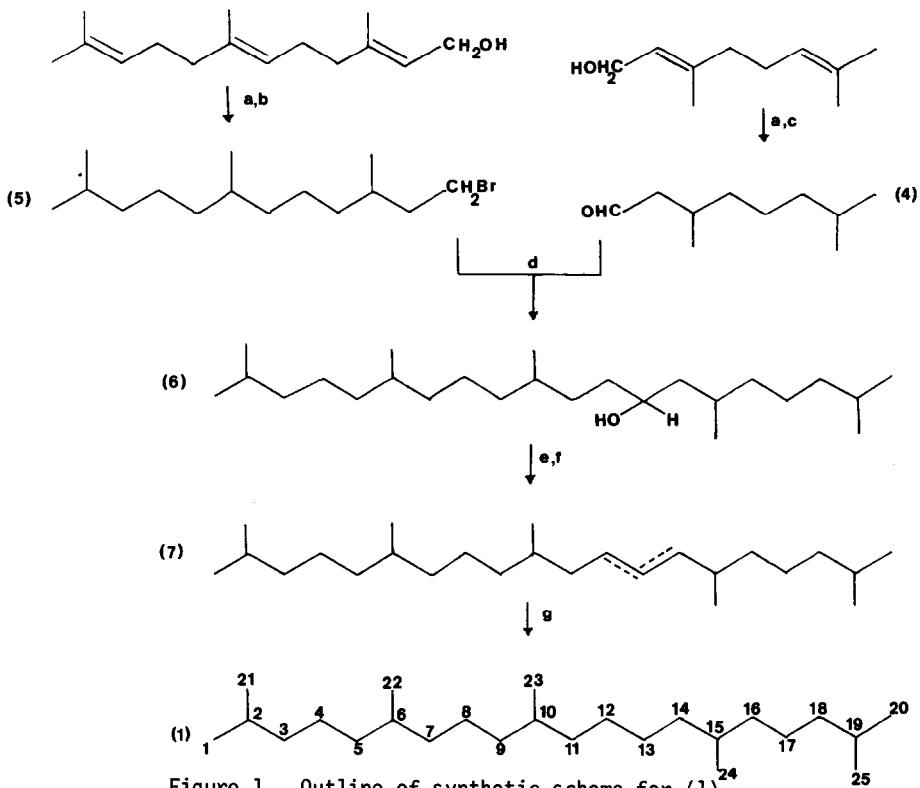
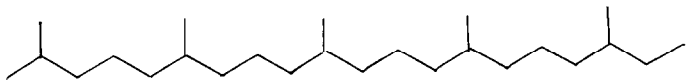


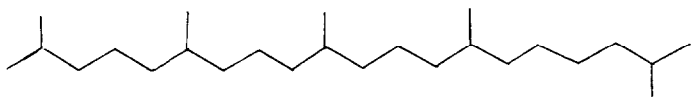
Figure 1. Outline of synthetic scheme for (1)

a. H_2/Pt/C, EtOH; b. CBr_4, PPh_3; c. $(COCl)_2$, DMSO; d. Mg, THF; e. $POCl_3$, pyr.; f. SiO_2, 10% $AgNO_3$; g. H_2/PtO_2, AcOH/$HClO_4$. Where stereochemistry is undefined, both configurations are present.

(2)



(3)



2,6RS,11RS,15RS,19-pentamethyleicosan-8RS-ol. Oily. ν (CCl_4); 3600 (sharp); δ ppm ^{1}H (200 MH_z, $CDCl_3$) 0.87 (21H, d, Me), 1.15-1.59 (29H, m, methylene), 3.68 (1H, m, CHOH); as the trimethylsilyl ether - m/z (40eV) 425 (M^+-15, 2%), 313 (M^+-C_9H_{19}, 60%), 229 (M^+-$C_{15}H_{31}$, 100%). 2,6RS,10RS,15RS,19-pentamethyleicosane. Oily. (Found: C, 85.6; H, 15.0. $C_{25}H_{52}$ requires C, 85.2; H, 14.8). δ ppm ^{1}H (200MH_z, $CDCl_3$) 0.84 (6H, d, Me), 0.86 (15H, d, Me), 1.11-1.56 (31H, m, methylene); δ ppm ^{13}C (90 MH_z, $CDCl_3$), 19.73 (C-22, C-23, C-24), 22.62, 22.72 (C-1, C-20, C-21, C-25), 24.48 (C-8), 24.80 (C-4, C-17), 27.43 (C-12, C-13), 27.99 (C-2, C-19), 32.80 (C-6, C-10, C-15), 37.15, 37.35, 37.41 (C-5, C-7, C-9, C-11, C-14, C-16), 39.40 (C-3, C-18); m/z (40eV) 352 (M^+; < 1%), 337 (M^+-15, 1%), 267 (M^+-C_6H_{13}, 2%), 239 (M^+-C_8H_{17}, 3%), 57(100%).

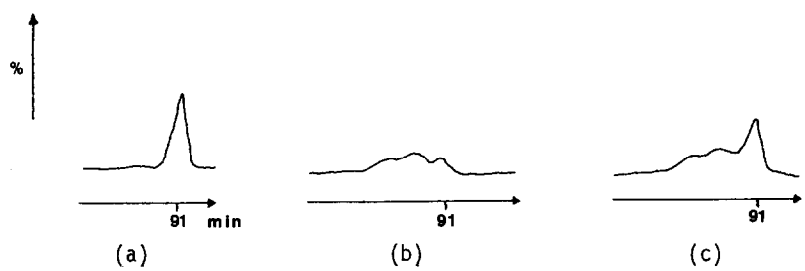


Figure 2. Partial gas chromatograms of (a) Walvis Bay (1); (b) all isomer (1); (c) a + b coinjected. DEGS/PEGS 3/1, for conditions see text.

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