Newly discovered non-isoprenoid glycerol dialkyl glycerol tetraether lipids in sediments

Jaap S. Sinninghe Damsté,*[a] Ellen C. Hopmans,[a] Richard D. Pancost,[a] Stefan Schouten[a] and Jan A. J. Geenevasen[b]

[a] *Netherlands Institute for Sea Research (NIOZ), Department of Marine Biogeochemistry and Toxicology, PO Box 59, 1790 AB Den Burg, Texel, The Netherlands. E-mail: damste@nioz.nl*

[b] *University of Amsterdam, Faculty of Chemistry, Organic Chemistry Unit, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands*

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Newly discovered non-isoprenoid dialkyl diglycerol tetraethers containing 13,16-di- or 5,13,16-trimethyloctacosanyl moieties have been identified in peats and coastal marine and lake sediments by HPLC–MS and high-field NMR spectroscopy.

Hyperthermophilic archaea thrive at temperatures > 60 °C and their ecological occurrence is, therefore, restricted to extreme environments such as waters near volcanic areas.[1] To meet the requirements posed by these hostile environments,[2] the membranes of these archaea (the third kingdom of life) are predominantly composed of isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs),[3] which are mainly comprised of acyclic (**1**)† or cyclic biphytane core lipids. Recently we reported a new technique for the direct analysis of intact GDGTs in extracts of archaeal cell material and sediments using high performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry.[4] Initial results indicated that GDGTs are widespread in low temperature environments, in contrast to the previous belief that GDGTs are restricted to hyperthermophilic archaea. Using this technique we also encountered unidentified GDGTs in extracts of peats. Here we report the identification of these abundant GDGTs as unprecedented, non-isoprenoid GDGTs.

The total ion current chromatogram of the HPLC–MS analysis revealed unknown components in the peat extracts, which possessed $[M + H]^+$ ions of 1050, 1036 and 1022 in order of increasing elution time (Fig. 1). Characteristic losses of $[M+H]^+$-18 and $[M+H]^+$-74 corresponding to loss of water and of a glycerol moiety (as $C_3H_6O_2$)[4] suggested these components to be GDGTs. Cleavage of ether bonds[5] with HI and subsequent reduction of the formed iodides with $LiAlH_4$, performed on a fraction of an extract of the Holocene Bargerveen peat (SE Drenthe, the Netherlands) containing these newly discovered GDGTs, produced two dominant branched hydrocarbons. The first eluting hydrocarbon was identified as 13,16-dimethyloctacosane by GC–MS analysis and co-elution with an authentic standard.[6] The second eluting isomer is a C_{31} alkane; its mass spectral characteristics indicate also a C_{28} linear alkyl chain with methyl branching at C-13, C-16 and C-5. Its relative retention time is consistent with this structure; the experimental Kovats index of 2912 compares favourably with the one calculated[7] (RI = 2914). The iodides formed upon HI treatment were also treated with $NaSCH_3$,[5] resulting in the formation of the α,ω-diSMe derivatives of the C_{30} and C_{31} branched alkanes. This established the number and positions by which these

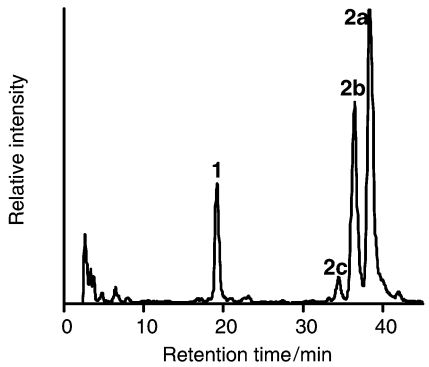
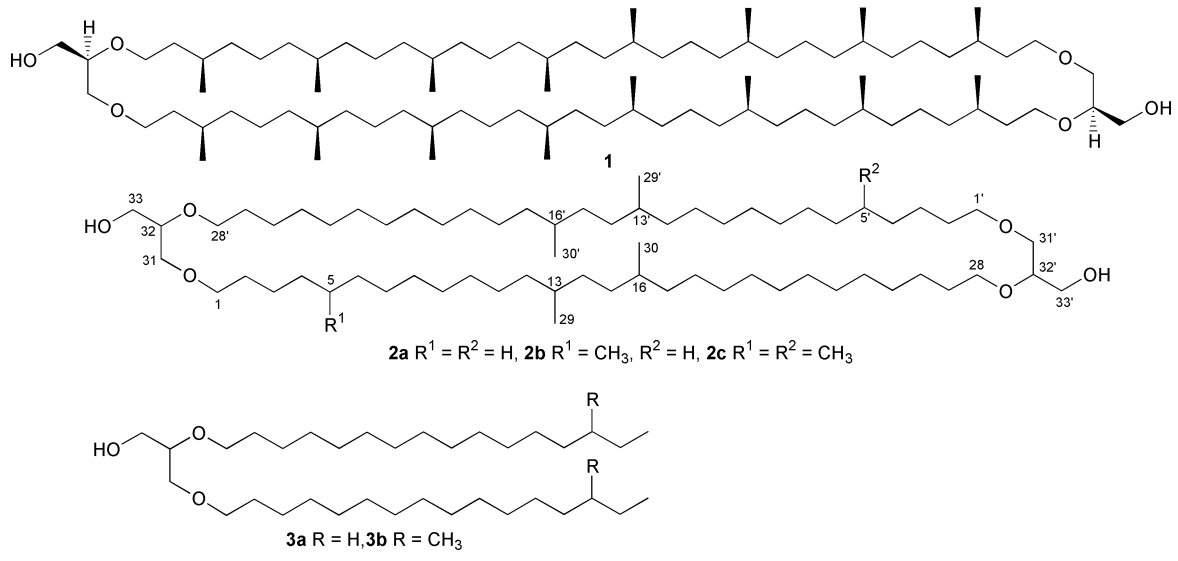


Fig. 1 Total ion current chromatogram of an extract of the Bargerveen peat revealing the abundance of GDGTs **1** and **2a**–**c**.



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skeletons are ether-bound. These data suggest that the new GDGTs comprise diether-linked di- or trimethyl branched C_{28} linear chains (**2a**–**c**), which fits with the determined molecular weights and indicates elemental compositions of $C_{66+n}H_{132+2n}O_6$ (n = 0–2).

These structural assignments were confirmed by high field ^{1}H and ^{13}C NMR‡ of GDGTs **2a** and **2b**, which were isolated from the peat extract by HPLC on a semi-preparative NH_2 Econosphere 10 × 250 mm column (Alltech) eluted with a linear gradient from 1 to 2% propanol in hexane in 55 min, yielding 1.6 and 1.1 mg per component, respectively. Both the ^{1}H and ^{13}C NMR gave substantial evidence for the presence of ether-bound glycerol units in **2a** and **2b**. The ^{13}C shifts of the carbon atoms of the glycerol moieties and the ether-bound CH_2 units of the alkyl moieties are in good agreement with those reported for GDGT **1**[3b] and commercially available 1,2-dihexadecylglycerol (**3a**). The remaining signals in the ^{13}C spectrum of **2a**, apart from a large suite of secondary C-atoms, indicated only 4 equivalent primary and 4 equivalent tertiary C-atoms, consistent with the proposed structure. The ^{13}C spectrum of **2b** is somewhat more complicated due to the additional methyl group in one of the branched alkyl chains. The shifts of the C-atoms around this additional methyl group are in good agreement with the proposed position for branching. A TOCSY experiment with **2b** indicated that the additional methyl group is present in the *O*-bound alkyl chain attached at the C-1 position of the glycerol moiety through a correlation between H-1 and the protons of the methyl group at C-5.

We have thus established a new type of GDGT not composed of isoprenoid but of branched alkyl core lipids. The new tetraethers all consist of a 64-membered ring with 6–8 stereocentres. Although the newly discovered GDGTs have been isolated from peat, they also occur in lake and coastal marine sediments. Their close structural similarity to **1** suggests that **2a**–**c** are also core membrane lipids, although they have not yet been identified in organisms. Branched dialkyl glycerol diethers (*e.g.* **3b**) have been reported in a thermophilic bacterium[8] and it may be that the GDGTs **2** represent 'dimers' of such lipids, in the same way as **1** is a dimer of the isoprenoid dialkyl glycerol ether, *sn*-2,3-diphytanylglycerol diether. This suggests that bacteria are more likely producers of these membrane lipids than archaea.

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Notes and references

† Antiparallel arrangement of glycerol units is indicated and assumed for **2** but it has been shown for **1** that the parallel arrangement occurs in approximately equal amounts.[9]

‡ NMR data for **2a** and **2b** at 600 MHz for ^{1}H in $CDCl_3$, COSY, TOCSY, HMBC: δ 3.71 (2H, m, H-33, 33′), 3.62 (4H, m, H-28, 28′, H′-33, 33′), 3.52 (6H, m, H′-28, 28′, H-31, 32, 31′, 32′), 3.49 (2H, m, H′-31, 31′), 3.43 (4H, m, H-1, 1′), 2.17 (2H, OH), 1.58 (4H, m, H-27, 27′), 1.56 (4H, m, H-2, 2′), 1.39 (1H, m, H-5, **2b**), 1.33 (4H, m, H-13, 16, 13′, 16′), 1.10 (~16H, m, H-12, 14, 15, 17, 12′, 14′, 15′, 17′ and 4 and 6 for **2b**), 0.845 (3H, d, J = 7 Hz, H-34, **2b**), 0.84 (12H, d, J = 7 Hz, H-29, 29′, 30, 30′). ^{13}C (125 MHz), APT: δ 78.38 (C-32, 32′), 71.78 (C-1, 1′), 71.10 (C-31, 31′), 70.41 (C-28, 28′), 63.06 (C-33, 33′), 37.14 (C-12, 17, 12′, 17′), 36.94, 36.77 (C-4, 6, **2b**), 34.35 (C-14, 15, 14′, 15′), 33.04 (C-13, 16, 13′, 16′), 32.66 (C-5, **2b**), 30.04, 29.96 (C-2, 10, 19, 27, 2′, 10′, 19′, 27′; C-8, **2b**), ~29.7 (all other C's), 29.39 (C-25, 4′, 25′; C-4, **2a**), 27.03 (C-11, 18, 11′, 18′; C-7, **2b**), 26.06 (C-26, 3′, 26′; C-3, **2a**), 22.65 (C-3, **2b**), 19.79 (C-29, 30, 29′, 30′), 19.75 (CH_3 at C-5, **2b**).

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