Lipid Structures in the Caldariella Group of Extreme Thermoacidophile Bacteria

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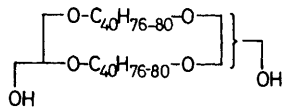
Summary The major lipids of thermoacidophile bacteria of the Caldariella series are based on macrocyclic tetraethers comprising two C_{40} ω,ω'-biphytanyl residues, with up to four cyclopentane rings, and either two glycerols or one glycerol and one branched-chain nonitol; in the corresponding complex lipids, polar residues are attached either to the nonitol or to one of the two glycerols.

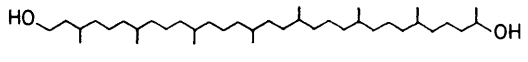
THE Caldariella series of extreme thermoacidophile bacteria,[1,2] which includes[3] the isolates designated *Thermoplasma*[4] and *Sulfolobus*,[5] has several unique chemical features,[6,7] one of the most striking being the complete absence of ester lipids.[1,3] The unique nature of the Caldariella lipids is closely connected with the special requirement that in these organisms the cell membranes are fully functional at temperatures of up to 90 °C and external pH as low as 0·5. Here we report data on lipid structures in the MT-4 strain,[1] which grows optimally at 87 °C, pH 3·5. Lyophilized cells afford a 6·8% yield of chloroform-soluble lipids which on acid cleavage (HCl in aq. MeOH, 6 h reflux) give two major products, R_f *ca.* 0·95 and 0·35 respectively (on silica gel t.l.c. in 9:1 $CHCl_3$–MeOH).

The less polar product, previously described by us as a cyclic glycerol alkyl diether[8,9] and by Langworthy *et al.* as a dialkyl glycerol diether,[10] is a mixture of cyclic diglycerol dialkyl tetraethers (**1**), as shown by the molecular weight (vapour pressure osmometry: found, $M=1290$; diacetate, found, $M=1350$) and cleavage with BCl_3 to equimolar amounts of glycerol and the C_{40} dichlorides described previously.[8,9] A similar structural revision has now been proposed by Langworthy.[11] Detailed chemical and ^{1}H-n.m.r. evidence,[12] supported by ^{13}C-n.m.r. and biosynthetic data,[13] establishes that in all Caldariella isolates[9] the bifunctional alkyl chains in the diglycerol tetraethers correspond to the three $\omega\omega'$-biphytanyl diols (**2**)—(**4**). In lower-temperature forms the acyclic C_{40} component (**2**) predominates, and in MT-4 the main C_{40} component of (**1**) is the bicyclic (**4**). This type of tetraether, with regular diterpene units linked head-to-head and with a 72-membered ring, is structurally and biosynthetically unique.

The more polar product from lipid hydrolysis has the part-structure (**5**), molecular weight (found) 1490; it is cleaved by BCl_3 to a 1:2:1 mixture of glycerol, C_{40} dichlorides, and a nonitol $C_9H_{20}O_9$, the first instance of such a polyol in nature. The nonitol has $[\alpha]_D^{20}$ − 8·7° (in H_2O) and the largest mass spectral ion is at m^+/e 255 (M^+ − OH). ^{13}C-N.m.r. data for its nona-acetate (Ac_2O–C_5H_5N, 2 h reflux) suggest a branched chain with three methylene and five methine groups, and one quaternary carbon atom; ^{1}H-n.m.r. spectroscopy confirms this and decouplings establish the part-structure (**6**). As for (**1**),[9,12] the C_{40} components of this lipid are most conveniently recovered as the corresponding alkanes, found to be $C_{40}H_{78}$, $C_{40}H_{76}$, and $C_{40}H_{74}$ (all saturated). The first of these is identical, by g.l.c.-mass spectra, with the bicyclic alkane from (**1**),



(1)



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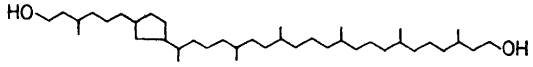

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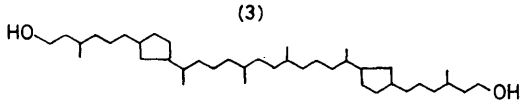

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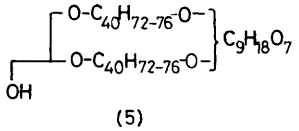

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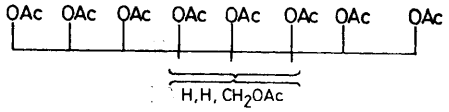

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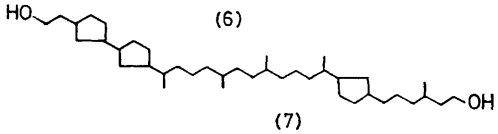
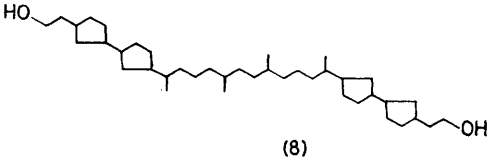


(7)



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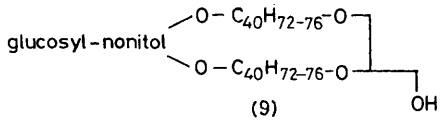

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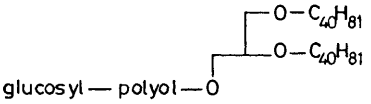

(10)

cf. diol (**4**); by mass spectrometry and ^{1}H- and ^{13}C-n.m.r. spectroscopy, the second and third alkanes correspond to the tri- and tetra-cyclic analogues (**7**) and (**8**) respectively. Thus this second type of lipid resembles (**1**) but the alkyl chains are more frequently cyclized and one glycerol is replaced by the nonitol. On present data, ether linkages could also exist within the nonitol unit.

If the complex lipids are fractionated before cleavage, the tetraether (**5**) is recovered from, *inter alia*, the most abundant glycolipid (75% of the total glycolipid in MT-4), which from ^{13}C-n.m.r. data carries one glucosyl unit attached to the nonitol, the glycerol CH_2OH being free (structure **9**). This glycolipid is presumably the same as that from *Sulfolobus* previously formulated[14] as (**10**), with a C_7 or C_8 polyol ether-linked to glycerol and with monofunctional C_{40} chains. The required revision of this structure can be extended to that of other complex lipids described from *Thermoplasma* and *Sulfolobus*; on re-examination of the data[10,14,15] we suggest that all these lipids are based upon either (**1**) or (**5**), with the further conclusion that in most if not all cases there is only one polar residue and that this is attached either to one of the two glycerol units in (**1**) or to the nonitol in (**5**). This is an important consideration because, since the long alkyl chains of the tetraethers must extend right through the membranes in which they occur, one of the two polar ends of each complex lipid molecule comes from the inner face of the membrane and one from the outer. Thus the chemical and functional asymmetry of the membrane is uniquely represented in the individual molecules of the complex lipids.

Lipids based on the glycerol–nonitol tetraether (**5**) are more conspicuous in the higher-temperature isolates such as MT-4, and since they contain the tri- and tetra-cyclic C_{40} components this emphasizes the trend, already remarked,[9] towards increased cyclization of C_{40} components at higher temperatures. Cyclization, by incorporating three freely-rotating carbon–carbon bonds from the open chain into each rigid cyclopentane ring, decreases the rotational possibilities in the alkyl chains and thus, we suppose, helps to maintain membrane integrity against thermal disruption.

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