Cyclic Diether Lipids from Very Thermophilic Acidophilic Bacteria

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Summary In acidophilic bacteria growing at up to 89°, the lipids are based on cyclic glycerol diethers [part-structure (I)]; the contribution of this structure-type to membrane stability is considered.

We have studied the bacterium designated *Sulfolobus acidocaldarius* by Brock *et al.*,[1] and, in more detail, organism MT-3 which is one of our own isolates, very close to *S. acidocaldarius* except in its DNA composition.[2] Both are acidophilic (pH optima 2·0—5·0) and very thermophilic (up to 89° in the MT series) and grow on simple media. Their lipids are derived from a unique type of cyclic glycerol diether whose structural features probably contribute to the extraordinary properties of the lipid-based membrane of these bacteria.

Lyophilized cells afford 6—8% extractable lipid (comprising 70% polar phospholipids, 10% glycolipids, 20% neutral lipids) with no trace of ester $>$ C=O absorption in the i.r. spectra. Acid cleavage (HCl in aq. MeOH; 6 h reflux) gives as the major neutral product a mixture of three saturated cyclic glycerol diethers, $C_{43}H_{82-86}O_3$.† This mixture has so far only been resolved by g.l.c. of derived hydrocarbons (see below) but over 75% of the mixture from MT-3 is the diether $C_{43}H_{82}O_3$ for which part-structure (I) is here proposed. The diether is dextrorotatory ($[\alpha]_D^{20}$ + 7·5° in $CHCl_3$) and hence[3] has the *sn*-2,3- or L-α,β-configuration, opposite to that in natural glycerol diesters. It incorporates mevalonate selectively (in parallel experiments, 3·2% incorporation from [2-^{14}C]mevalonolactone, 1·8% incorporation from [1-^{14}C]acetate), forms a mono-acetate, and is cleaved by BCl_3–$CHCl_3$ to a 1 : 1 mixture of glycerol and the (predominant) dichloride $C_{40}H_{76}Cl_2$. Alternatively cleavage with HI (24 h reflux) gives di-iodide $C_{40}H_{76}I_2$, converted conventionally into diacetate $C_{41}H_{82}O_4$, diene $C_{40}H_{74}$, and alkane $C_{40}H_{78}$.

From i.r. and n.m.r. data the predominant diene has two similar CH=CH–CHMe– groups, and in addition four saturated $>$ CHMe and one tertiary $\geqslant$CMe group; from its composition it must be bicyclic. The minor components must similarly be monocyclic (+2H) and non-cyclic (+4H).

Kates and his co-workers[4] showed that extreme obligate halophile bacteria (which have some ultrastructural resemblance to the organisms we have studied) have characteristic ether lipids based on 2,3-di-*O*-phytanyl-*sn*-glycerol. In our diether the one isoprenoid C_{40} unit is clearly attached to oxygen at two 'tails,' but it has only 7 Me groups, and we suggest it is formed by head-to-head linkage of two *O*-phytanyl chains,‡ with further cyclizations in the major component. Support and some clarification, leading to part-structure (I), comes from mass spectra, particularly of the alkanes, in which conspicuous cleavages (of C_7 and C_{19} fragments) are assigned to the bonds [marked

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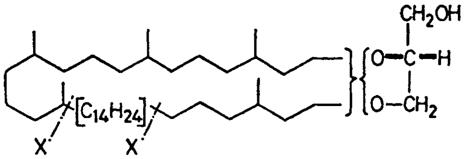
† All formulae are given for the predominant component of mixtures, from microanalysis and low-resolution mass spectra.

‡ *Added in Proof:* surprisingly, 'head' and 'tail' are used ambiguously, but most authors use 'head' to denote the branched end of an isoprene unit, as here. For clarification, the above minor alkane $C_{46}H_{82}$ is thought to be 1,1′-biphytanyl, *ie.* 3,7,11,15,18,22,26,30-octamethyldotricontane, an isomer of perhydrolycopene.

X in (I)] at which open-chain sections are linked to the cyclic residue.



(1)

Structural studies on (I) continue, problems of large-scale growth of these bacteria being a limiting factor. It is reported[5] that *Thermoplasma acidophila*, which we believe is closely related to the MT bacteria, similarly has ether-based lipids from which mevalonate-derived alkanes $C_{40}H_{80}$ and $C_{40}H_{82}$ were obtained, and we suggest that similar cyclic diethers are their source.

Meanwhile the structure of (I) and its congeners already suggests important features from the standpoint of membrane structure. In complex lipids derived from (I) the hydrophobic residue is a macrocyclic loop with projecting Me groups, made still more irregular when the residue is partly cyclic; its packing in bilayers will be quite unlike that of the regular hydrocarbon chains of normal acyl lipids. In bilayer membranes based upon conventional acyl phospholipids it is clear that biological function depends upon the maintenance of a'fluid' state, and in such bilayers the presence of, *e.g.*, cholesterol promotes fluidity under conditions in which the lipids would otherwise crystallise, while maintaining the bilayer integrity under conditions when the membrane would otherwise break up.[6] As a tentative explanation of the striking thermostability of the membrane in the bacteria we have studied, we suggest that in lipids based on structures such as (I) the 'cholesterol effect' is, as it were, built-in. We would further suggest that in the membrane lipids of the somewhat less extreme acidothermophile *Bacillus acidocaldarius*, the high proportion of ω-cyclohexyl-substituted acyl lipids[7] has a similar biophysical function.

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[2] A full description of the MT series of isolates is in preparation.

[3] M. Faure, J. Marechal, and J. Troestler, *Compt. rend.*, 1963, **257**, 2187.

[4] *E.g.* M. Kates, E. E. Park, B. Palameta, and C. N. Joo, *Canad. J. Biochem.*, 1971, **49**, 275.

[5] T. A. Langworthy, P. F. Smith, and W. A. Mayberry, *J. Bact.*, 1972, **112**, 1193.

[6] *E.g.*, S. Rottem, V. P. Cirillo, B. de Kruyff, M. Shinitzky, and S. Razen, *Biochim. Biophys. Acta*, 1973, **323**, 509; D. Papahadjopoulos, M. Cowden, and H. Kimelberg, *ibid.*, 1973, **330**, 8.

[7] M. de Rosa, A. Gambacorta, L. Minale, and J. D. Bu'Lock, *Chem. Comm.*, 1971, 1334; *J. Bact.*, 1974, **117**, 212.