

THE CALDARIELLA GROUP OF EXTREME THERMOACIDOPHILE BACTERIA: DIRECT COMPARISON OF LIPIDS IN SULFOLOBUS, THERMOPLASMA, AND THE MT STRAINS

M. DE ROSA and A. GAMBACORTA

CNR Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico, via Toiano 2, Arco Felice, Napoli, Italy

and

J. D. BU'LOCK

Weizmann Microbial Chemistry Laboratory, The University, Manchester M13 9PL, England

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Abstract— It is shown that the lipids from 5 extreme thermoacidophile bacteria of the Caldariella group—2 isolates of *Sulfolobus acidocaldarius*, one of *Thermoplasma acidophila*, and 2 of the MT series—are all based on the same type of cyclic diether combining glycerol and one of a series of very unusual C_{40} isoprenoid diols. The relative proportions of the different C_{40} components in each isolate have been determined.

INTRODUCTION

The name Caldariella has been proposed [1] to denote a form/habitat group of extreme thermoacidophile organisms, of small size and simple structure, which includes the monotypic genera *Thermoplasma* [2] and *Sulfolobus* [3] and a series of *Sulfolobus*-like organisms we have designated MT [4]. Though the DNA compositions (%GC) of these organisms cover a wide range, in most other respects they seem very closely related. In our first proposal we noted that the structurally unique lipids which we had found in the MT series might be an important common 'marker' for the whole Caldariella assembly.

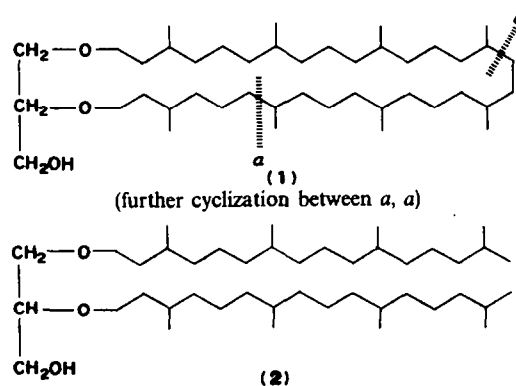
The lipids of MT organisms are based on *sn*-2,3-glycerol combined as a cyclic diether with a saturated C_{40} isoprenoid residue which is itself made up from two phytanyl chains linked head-to-head. The C_{40} component can itself be acyclic, monocyclic, or bicyclic [5]. In our own preliminary study of a *Sulfolobus* isolate, we obtained what appeared to be the same lipid type, while published data [6] on the lipids of *Thermoplasma acidophilum* suggested major structural similarities. Subsequently, however, Langworthy *et al.* [7] described the lipids of a different strain of *Sulfolobus acidocaldarius* and gave further details of the *Thermoplasma* lipids, interpreting their data in a rather different manner; from their account it followed that either these organisms showed less resemblance to the MT organisms than we had supposed, or that one or other set of structural interpretations was incorrect.

To resolve the matter, Dr. Langworthy very kindly gave further details in correspondence and provided samples of the glycerol diethers from his *Sulfolobus* and

Thermoplasma isolates. This has allowed us to make the requisite comparison directly and critically, and to show that the lipids of the MT strains, of two different *Sulfolobus* strains, and of *Thermoplasma*, are all based on the same cyclic diethers, differing only in the proportions of the three C_{40} components.

DISCUSSION

Detailed spectroscopic studies, which will be described elsewhere, show that the simplest of the cyclic diethers from these lipids has the structure 1 (Fig. 1); this is the component which predominates in *T. acidophila* and gives rise to the hydrocarbon $C_{40}H_{82}$ (see Table 1). In other diethers, cyclizations (as indicated in Fig. 1) have occurred within the C_{40} component. This is the type of cyclic diether structure assigned by us to the diethers



from MT organisms [5], whereas for the lipids from *Sulfolobus acidocaldarius* Langworthy *et al.* had deduced a different structure with 2 separate C_{40} monoether residues [7]. Without pursuing a multiplicity of chemical data, crucial evidence for the cyclic diether structure can be drawn from the PMR spectra and MS data and, in particular, from the ratio of the C -methyl protons (24 in the acyclic C_{40} residue, cf. structure 1; 21 in the mono- and bicyclic C_{40} components) to the protons of CH_2 groups at which ether oxygens (or halogen) are attached (of which there are 4). Minor observational discrepancies between our data and those of Langworthy *et al.* [6,7] have been resolved by our recent work.

The structures of 1 and the accompanying diethers are remarkable and unique, and the fact that these diethers constitute the structural basis of all the membrane lipids in the organisms examined strengthens the case for considering "Caldariella" as a valid classificatory assembly, within which, however, significant variation can be found. Just as the designation *Sulfolobus acidocaldarius* now appears to have been applied to organisms displaying detailed differences in physiology [8] and immunochemistry [9], so the detailed lipid composition is now seen to vary both within *S. acidocaldarius* and between this and *Thermoplasma acidophilum* and the MT organisms (Table 1). Both the MT strains, and *S. acidocaldarius* culture SL, have high proportions of the bicyclic C_{40} component; *S. acidocaldarius* SM has roughly equal proportions of the three different C_{40} chains, and in *T. acidophilum* the acyclic C_{40} component, as in 1, predominates.

From the comparative viewpoint the chemical and biosynthetic relationship between Caldariella group and the extreme halophiles such as *Halobacter cutirubrum* is very suggestive. The latter have characteristic lipids [10] based on *sn*-2,3-di-*O*-phytanylglycerol, 2 (Fig. 1), only differing from 1 in that in the latter the two C_{20} chains are joined (uniquely, by a head-to-head link). There are also significant similarities in the structures of the complex lipids in these 2 groups [7,10]. The idea that the extreme halophiles and the extreme thermoacidophiles might share certain episodes in their evolutionary history—even though on other grounds both groups would be considered to be polyphyletic—is not without interest.

RESULTS

Total lipids. By soxhlet extraction of freeze-dried cells both the MT strains and *S. acidocaldarius* SM afforded 5–10% of total lipid which could be fractionated to give 70% polar phospholipids, 10% glycolipids, and 20% neutral lipids; all fractions afforded the same C_{40} components in each case and were not further investigated by us (but cf. Langworthy *et al.* [7] for *S. acidocaldarius* SL). The IR spectra of the total lipids [4] show complete absence of ester links (no peak at 1700 cm^{-1}) and a strong signal at 1100 cm^{-1} due to ether links, as in *T. acidophilum* [6] and *S. acidocaldarius* SL [7].

All five diether samples, gave, in different proportions, the same three spots on TLC, which were separated for elementary analysis: $C_{43}H_{82}O_3$ (R_f 0.43) (trace only from *T. acidophilum*); $C_{43}H_{84}O_3$ (R_f 0.49); $C_{43}H_{86}O_3$ (trace only from *S. acidocaldarius* SL) (R_f 0.55). The diether mixtures were dextrorotatory, $[\alpha]_D^{20} + 7.5^\circ$; the IR spectra all show peaks corresponding to hydroxyl (3450

cm^{-1}), ether (1115 cm^{-1}), carbinol (1045 cm^{-1}) and alkyl (1375 , 1460 , 2860 , 2929 and 2960 cm^{-1}) groups but no $C=C$ or $C=O$ absorption. The PMR spectra likewise all show the absence of unsaturation and all show signals integrating for 9 protons ($H-C-O$) at $\delta = 3.40$ (multiplet) and for 20.6–23.0 CMe protons (depending on the sample) at $\delta = 0.87$ (multiplet). These data are in critical accord with the structures discussed below.

Dihalides. All 5 diether samples gave similar mixtures of dichlorides, analyzing for $C_{40}H_{76.80}Cl_2$ and giving mass spectra with the same molecular ions (at M^+/ϵ 626, 628, 630) in different mutual proportions. All MS also showed fragments corresponding to losses of HCl and 2HCl. Similarly the di-iodides, $C_{40}H_{76.80}I_2$, gave molecular ions at M^+/ϵ 810, 812, and 814 and fragments corresponding to HI and 2HI losses. The PMR spectra of all di-iodide and dichloride samples showed signals integrating for 4 protons, (CH_2-CH_2-X) at $\delta = 3.52$ ($X = Cl$) or $\delta = 3.12$ ($X = I$) and for 21–23 CMe protons at $\delta = 0.88$. Again, these data are in critical accord with the structures proposed.

Hydrocarbons. The derived hydrocarbons are the most suitable materials for establishing both the detailed identity and the mutual proportions of the C_{40} components in the lipid samples; the three hydrocarbons were shown to be $C_{40}H_{82}$, $C_{40}H_{80}$, and $C_{40}H_{78}$ (respectively acyclic, monocyclic, and bicyclic, since fully saturated) by combined GLC–MS measurements, and the comparative data for the five samples are summarized in Table 1. In addition to these three hydrocarbons, the MT-3 and MT-4 lipids also afford a fourth (tricyclic) hydrocarbon $C_{40}H_{76}$ (equivalent chain length 38.2 on SE-30, 38.3 on OV-1) as a minor component (6% in MT-3, 12% in MT-4). The other three components in all 5 samples had identical retention times on both GLC columns used, and gave identical fragmentations in the GLC–MS runs.

EXPERIMENTAL

General—Isolation and culture methods for the MT strains have been fully described [4]; in the present work we used the strains MT-3 and MT-4, grown on the standard medium and at the optimum temperatures which are 75° and 87° , respectively. Our own culture of *Sulfolobus acidocaldarius*, designated SM, derived from strain 98/3 (provided by Dr. J. A. Mosser) was grown at 70° on the same medium [3]. Lipid extraction was as previously described [4]. Growth conditions and lipid workup used by Langworthy *et al.* for *Thermoplasma acidophilum* (59° , pH 2.0) and for *Sulfolobus acidocaldarius* (also originating from strain 98/3 but here designated SL) (70° , pH 3.0) were as described [6,7]; we received samples of the worked-up diethers (see below).

Isolation and conversion of the diethers. The total lipid extract from the freeze-dried cells was treated with methanolic HCl (conc. HCl–MeOH, 6:94) for 6 hr under reflux; the hydrolyzate was dil with an equal vol H_2O and extracted several times with *n*-hexane. The hexane extract was chromatographed on Sil gel (Merck Kieselgel, 75 230 mesh) in light petrol (bp $40-70^\circ$) with increasing proportions of Et_2O , and the glycerol diethers were recovered from the 50% ether/petrol fraction. When required, TLC to separate individual diethers was effected on Sil gel plates (Merck F.254) in $CHCl_3$ – Et_2O , 9:1. The C_{40} dichlorides were obtained by treatment of the diether mixture (5 mg) with BCl_3 in $CHCl_3$ (1:1 w/w, 2 ml) at room temp. for 12 hr. After removal of solvent and partition between MeOH and light petrol, the dichlorides (from the light petrol phase) were purified by TLC in *n*-hexane. C_{40} di-iodides were obtained by treating the diether (10 mg) with HI (57% 1 ml) for 24 hr under reflux; the product, taken up

Table 1. GLC analysis of derived C₄₀ hydrocarbons from Caldariella lipids

Component	Equivalent chain length*		(% total hydrocarbons in mixture)*				
	on SE-30	on OV-1	MT-3	MT-4	S. acido- caldarius, SM	S. acido- caldarius, SL	T. acidophilum
C ₄₀ H ₈₂ (acyclic)	35.0	35.3	3.6	2.2	29.9	trace	65.0
C ₄₀ H ₈₀ (monocyclic)	36.4	36.4	18.2	8.6	32.3	14.4	32.5
C ₄₀ H ₇₈ (bicyclic)	37.6	37.7	71.6	70.8	37.8	85.6	2.5

* See Experimental.

in light petrol, was washed successively with H₂O, saturated aq K₂CO₃, 50% (w/w), aq sodium thiosulphate, H₂O, and aq MeOH (9:1 v/v), and the di-iodides then chromatographed on a Sil gel column in *n*-hexane.

C₄₀ hydrocarbons. These were obtained from the di-iodides by treatment with excess LiAlH₄ in diethyl ether; after conventional work-up the hydrocarbons were purified by chromatography on Sil gel in *n*-hexane.

Instrumental details. MS were determined on the AEI MS-30 instrument at 70 eV, which for combination with GLC was attached to a Pye-Unicam 104 instrument fitted with a glass column (5 mm × 1.5 m) packed with 1% OV on silanized Chromosorb W (100–120 mesh) operating at 220–310° (6° min⁻¹) with He carrier at 60 ml min⁻¹. For GLC of the C₄₀ hydrocarbons we used the Carlo Erba Fractovap GV, with 4 mm × 2.0 m columns packed with either 1% OV-1 or 1% SE-30 on silanized Chromosorb W (100–120 mesh) operating at 275° with N₂ carrier at 1.6 kg cm⁻² and 60 ml min⁻¹; the relative proportions of the different components were determined from the peak areas, and the equivalent chain lengths by reference to logarithmic plots of the retention time vs chain length for a C₂₀–C₄₀ *n*-paraffin mixture. PMR spectra were measured at 100 MHz on a Varian XL-100 using CCl₄ solns with SiMe₄ internal standard; IR spectra in liquid film or CHCl₃ soln; optical rotations, [α]_D²⁰, in CHCl₃ soln using a Perkin-Elmer 141 polarimeter; elementary analyses using a Perkin-Elmer 240 analyzer.

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