ELSEVIER

Biochimica et Biophysica Acta 1345 (1997) 56–64

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Identification of β-L-gulose as the sugar moiety of the main polar lipid *Thermoplasma acidophilum*

Marianne Swain, Jean-Robert Brisson, G. Dennis Sprott *, Fredrick P. Cooper, Grishchandra B. Patel

Institute for Biological Sciences, National Research Council of Canada, 100 Sussex Drive, Ottawa, Ont. K1A 0R6 Canada

Received 22 July 1996; revised 15 October 1996; accepted 18 October 1996

Abstract

The main polar lipid (MPL) of *Thermoplasma acidophilum* has been purified and its structure determined. NMR, mass spectrometry, and capillary gas chromatography-mass spectrometry experiments have shown that the previously unidentified sugar moiety of MPL is the rare sugar L-gulose. MPL is thus a tetraether lipid with cyclopentane rings and head groups of phosphoglycerol, as previously reported, and β-L-gulopyranose. Further, MPL is also the dominant lipid found in lipid extracts from another species of the *Thermoplasma* genus, *T. volcanium*, suggesting that L-gulose may represent a dominant sugar moiety of the polar lipids biosynthesized by this archaeobacterial genus. Minor phospholipids were tentatively identified as diether and hydroxydiether analogs of phosphatidylglycerol, and phosphatidylinositol.

Keywords: Main polar lipid; Structure; β-L-Gulose; Tetraether lipid; (*Thermoplasma acidophilum*)

1. Introduction

Thermoplasma acidophilum, an archaeobacterium originally isolated from a self-heating coal refuse pile, grows optimally at a pH of 1–2 and 55–59°C [1]. Because the bacterium appears to lack a cell wall, the naked membrane must have adapted well to withstand the harsh conditions required for growth. It has been known for some time that about 90% of the polar membrane lipids of *T. acidophilum* occur as caldarchaeol tetraethers with varying numbers of cyclopentane rings, and the remainder as archaeol diethers [2]. The polar lipids represent about 82% of the total lipids, and consist of at least 6 glycolipids and at least 7 phosphorous-containing lipids [3]. Oddly, with the exception of a minor tetraether lipid characterized by an extended 25 sugar chain [4], detailed structural studies of these lipids have not been done. Even the main polar lipid (MPL), accounting for about 46% of the total polar lipids [5], is incompletely characterized. MPL has been reported to be a tetraether lipid with $sn-3$-glycerophosphate as one head group, and an unidentified sugar [2], widely assumed to be glucose [6], as the other.

Structural studies have become of special importance, because compared to conventional liposomes those prepared from the polar lipids of *T. acidophilum* are remarkably heat stable [7], and are phagocytosed to a greater extent by macrophage cultures [8]. Here, we isolate MPL and determine by two dimensional NMR, and other experiments, that the

* Corresponding author. Fax: +1 (613) 9411327; E-mail: dennis.sprott@nrc.ca

0005-2760/97/$17.00 Crown copyright © 1997 Published by Elsevier Science B.V.
PII S0005-2760(96)00163-4

previously unknown sugar of MPL is the rare sugar L-gulose. Further, we show for the first time that hydroxydiether lipids are present as minor lipid components in lipid extracts of *T. acidophilum*.

2. Materials and methods

2.1. Materials

Silica-gel G-25 plates (0.25 mm) were purchased from Brinkmann Instruments (Mississauga, Canada). Reacti-Vials™ were from Pierce (Rockford, IL, USA). (*R*)-(−)-2-butanol, D-gulose and L-gulose were from Sigma-Aldrich (Mississauga, Canada). The capillary column for gas chromatography was obtained from Terochem (Mississauga, Canada), and deuterated solvents from Isotec (OH, USA).

2.2. Archaeobacteria and growth

Thermoplasma acidophilum 122-1B3 (ATCC 27658) was grown aerobically in 75 l or 250 l fermenters, in DSM medium 158 (pH 2, 55°C) [9] containing 0.2% yeast extract. Further yeast extract supplementation, each of 0.2% (w/v), was done at 12 and 29 h following inoculation. Cells were harvested in the late logarithmic/early stationary growth phase (ca. 48 h) and frozen at −20°C until required.

Thermoplasma volcanium GSS 1 (DSM 4299, obtained from Dr. K. Jarrell, Queen's University, Kingston, Ontario, Canada) was grown aerobically (pH 2, 55°C) in DSM 158 medium (500 ml/2 l Erlenmeyer flask) containing 0.2% (w/v) yeast extract and 150 μg/ml vancomycin. Further supplements of 0.2% and 0.1% yeast extract, respectively, were made at 24 and 48 h of the 64 h incubation (120 rpm). Cells in the late logarithmic/early stationary growth phase were harvested and stored at −20°C.

2.3. Isolation of MPL

Lipids were extracted from frozen-thawed cell paste by the neutral Bligh and Dyer method described previously [10]. When required, the total polar lipids (TPL) in the total lipid extract (TLE) were separated from the neutral lipids by acetone precipitation, as described [7]. MPL was isolated by preparative thin-layer chromatography of the TLE using silica gel plates developed with $CHCl_3/CH_3OH$/acetic acid/H_2O (85:22.5:10:4). Chromatography was repeated 2- to 3-times until purity was established by negative-ion fast atom bombardment (FAB) MS analysis. Paramagnetic ions were removed prior to NMR spectroscopy by treating with EDTA at acidic pH [10].

2.4. Physical measurements

Optical rotation was performed with 4.5 mg MPL/ml $CHCl_3$ as solvent, using a Perkin-Elmer 243 polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were acquired at 300 K with Bruker AMX-500 and AMX-600 instruments operating at proton frequencies of 500 or 600 MHz, respectively. For NMR, 4.5 mg MPL was dissolved in 0.8 ml benzene-d_6/CD_3OD (7:1, v/v). One-dimensional and two-dimensional techniques were employed (COSY, ROESY, HMQC = heteronuclear multiple quantum coherence [11]. Chemical shifts are relative to tetramethylsilane (0.0 ppm). Negative-ion FAB MS spectra were recorded with a Jeol JMS-AX 505H instrument and triethanolamine matrix; negative-ion electrospray MS was performed with a Fisons VG Quattro instrument.

2.5. D/L-configuration of gulose

Glycolipids (0.5–1 mg of pure MPL or total lipid extract) dissolved in $CHCl_3/CH_3OH$ (2:1, v/v) were transferred to 5 ml Reacti-Vials™ and taken to dryness with a N_2 stream. Hydrolysis was achieved by heating at 70°C for 2 h in 2 ml of 2.5% methanolic HCl. 0.2 ml of water and 2 ml of petroleum ether were added and the top phase discarded. Ether extraction was repeated thrice. The bottom phase was taken to dryness under N_2, and demethylated in 1 ml of 1 N aqueous HCl at 100°C for 3 h. The dried sample, along with appropriate sugar standards were placed in vacuo with P_2O_5 for 2 h. Samples received 0.2 ml of (R)-(−)-2-butanol plus 10 μl of trifluoroacetic acid, and incubated for 16 h at 100°C. The dried samples were acetylated by adding 0.3 ml of acetic anhydride and incubating at 105°C for 3 h. Samples were dried, dissolved in $CHCl_3$, and injected into a Varian Saturn II GC/MS using a 30 m, 0.25 mm ID fused silica capillary column, programmed from 180°C to 290°C

at 4°C/min. The carrier was helium at a flow rate of 1.0 ml/min. Each peak was analyzed by MS to distinguish furanose from pyranose forms. Retention times of pyranose and furanose sugar derivatives were compared to those of D- and L-gulose, and D- and L-glucose standards to determine the D/L configuration [12] and to confirm the identity of the unknown sugar.

3. Results

3.1. Identity of the sugar moiety of MPL

A negative electrospray MS spectrum of the total polar lipids extracted from *T. acidophilum* confirmed that the MPL lipid peak of m/z 1616.8 consists of a tetraether lipid core, a hexose sugar, and a glycerophosphate (theoretical monoisotopic m/z of 1616.4 for $[M\text{-}H]^-$, with no cyclopentane rings). The cluster of resolved signals in this region suggested that a series of MPL structures were present, each distinguished by the number of cyclopentane rings varying from 0 to 5 per molecule, where the dominant form would correspond to 2 rings per molecule. The theoretical monoisotopic m/z for $[M\text{-}H]^-$ of the dicyclopentane form is 1612.4, close to the obtained m/z of 1612.7 (Fig. 1A). When the TPL of *T. acidophilum* was subjected to ^{13}C-NMR spectroscopy only one strong anomeric carbon signal was observed (Fig. 1B), showing that only one type of dominating hexose was present in the MPL lipids. Other MS signals of much lower intensity and differing from the m/z of MPL by 1 and 2 hexose units (not shown in this spectrum), together with the occurrence of minor NMR, signals in the anomeric region, suggested the possibility of additional sugar moieties in the higher molecular weight phosphoglycolipids.

The most dominant lipid band, located with iodine vapour following TLC, was isolated and used in further studies to identify the unknown hexose moiety. Negative-ion FAB MS confirmed the purity of MPL and established that $[M\text{-}H]^-$ of the isolated lipid was m/z of 1612.6.

NMR assignments for the unknown sugar moiety of purified MPL are shown in the COSY, ROESY and HMQC spectra (Figs. 2–4) and in Table 1. In the COSY spectrum (Fig. 2), all the ^{1}H resonances of the sugar moiety could be located and assigned. All the resonances except for the two H-6 protons were isolated and proton coupling constants measured from the 1-D spectrum. The large $J_{1,2}$ coupling of 8 Hz indicated the β configuration at C-l, with H-1, and H-2, trans to each other. The small coupling of 3.5 Hz for $J_{2,3}$ and $J_{3,4}$ indicated that these protons were gauche to each other. The small coupling of 1 Hz for $J_{4,5}$ is similar to the one found for galactopyranose where H-4 is equatorial and H-5 is axial. The rotamer distribution about the C-5 and C-6 bond could not be determined since H-6_s and H-$6'_s$. overlapped, and their couplings with H-5_s could not be determined. Hence, the J_{HH} coupling constants indicated that the sugar moiety was a β-gulopyranoside, with H-1, H-2

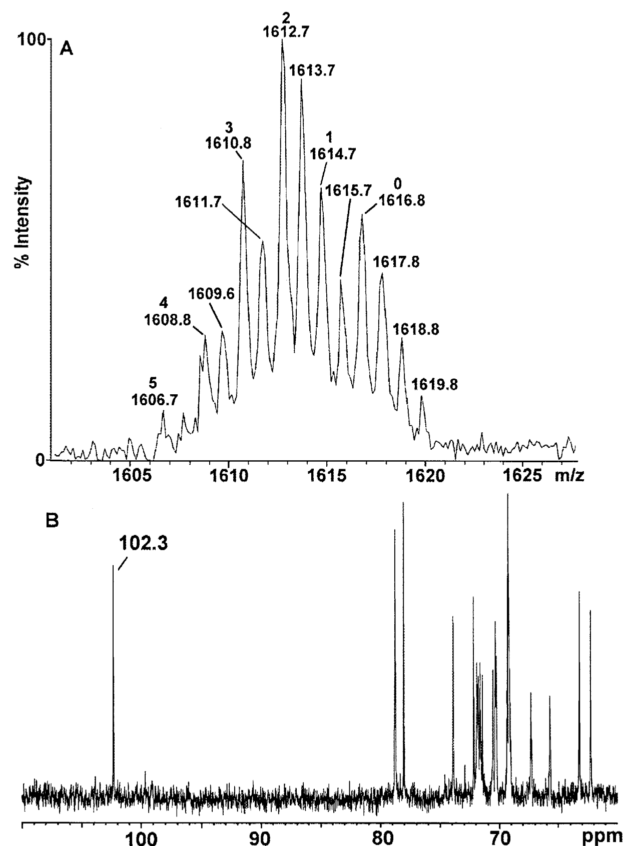

Fig. 1. Negative electrospray MS (panel A) showing an expanded view of the $[M\text{-}H]^-$ ions, and ^{13}C-NMR (panel B) of a total polar lipid extract of *T. acidophilum*. In panel A, the monoisotopic component for each of the cyclopentane ring forms of MPL is indicated. Tetramethylsilane was used as the NMR reference.

and H-5 axial and H-3 and H-4 equatorial in the pyranose ring. In the ROESY spectrum (Fig. 3) the large NOE between H-1_s and H-5_s confirmed that H-1_s and H-5_s were trans diaxial and that the anomeric configuration was β. The absence of the NOE between H-1_s and H-3_s, which is observed for β-glucopyranose, indicated that H-3_s was now equatorial, as confirmed from the J_{HH} coupling constants and the observed NOE of H-3_s with H-2_s and H-4_s. The NOE of H-1_s and H-$1'_g$ indicated the glycosidic linkage.

From the HMQC spectrum the ^{13}C resonances of the sugar moiety were then assigned (Fig. 4). The ^{13}C spectrum is shown as a DEPT spectrum with the CH_2 signals negative, to distinguish more clearly signals from the lipid moiety, whose assignments are compatible with the literature data [12,13]. The ^{13}C chemical shifts of the sugar moiety were similar to those previously reported for β-gulose [14].

Hence, the complete ^1H- and ^{13}C-NMR analysis of the sugar moiety is fully consistent with the identification of the unknown sugar as β-gulopyranoside.

To confirm the assignment of the unknown sugar as gulose and to determine which stereoisomer is present in MPL, butylated derivatives were formed of the released sugar moiety and compared with gulose and glucose standards. Derivatives separating on the capillary column were analyzed by MS. In addition to peaks at m/z 331 and 57 diagnostic for acetylated butyl hexosides, furanosides could be distinguished by prominent peaks at m/z 143, 169 and 259 from pyranosides with peaks at m/z 98, 115, and 182. Acetylated butyl glycosides prepared using (R)-(−)-2-butanol will result in 2 peaks for the pyranose (α and β) and 2 more if the furanose is generated (α and β). This procedure confirmed that the unknown sugar is gulose (and not glucose), and established it to be the L-stereoisomer (Table 2).

Because of the dominance of MPL in total polar

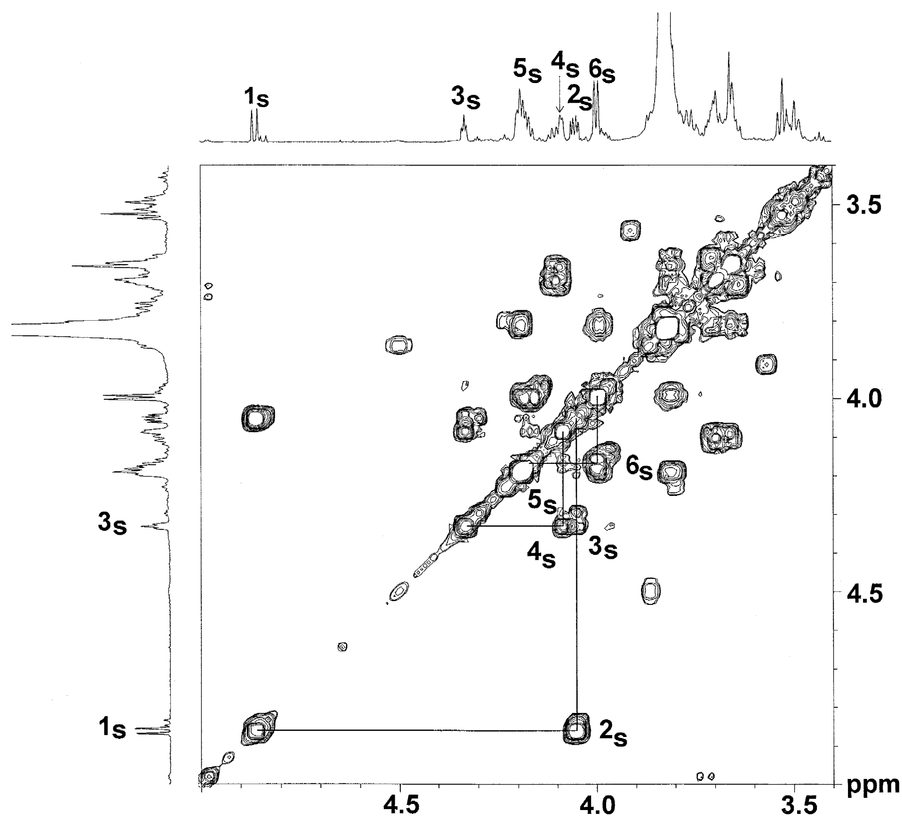

Fig. 2. COSY spectrum along with the ^1H spectrum showing the head group region of purified MPL from *T. acidophilum*. X_s, carbon position of the unknown sugar of MPL.

lipid extracts, it was possible to readily compare the sugar residue released by hydrolysis from the MPL lipids of *T. acidophilum* and *T. volcanium*, without purification of the lipid extracts. Only one major sugar was identified, which was L-Gul$_p$ in both *Thermoplasma* species (Table 2).

3.2. Phytanyl chain of MPL

While a series of MPL lipids differing only in the number of cyclopentane rings appear present in lipid extracts of *T. acidophilum* (Fig. 1), the main MPL lipid isolated had a m/z for $[M\text{-}H]^-$ of 1612.6, indicative of 2 cyclopentane rings. ^{13}C-NMR confirmed the presence of 1 ring per chain based on the detailed assignments given by DeRosa and Gambacorta [12]. The observed shifts of signals of MPL due to the position and number of rings is contrasted to a tetraether lipid lacking rings in Fig. 5.

3.3. Structure of MPL

All of the data obtained for MPL indicate the structure to be a tetraether with 2 cyclopentane rings, and with $sn-3$-glycerophosphate and β-L-Gul$_p$ as head groups (Fig. 6). Optical rotation data further support the proposed structure with a theoretical molecular rotation of $+218°$ (data available only for a tetraether without cyclopentane rings), and an observed value of $+161°$ for the dicyclopentane ring form of MPL (Table 3).

3.4. Minor diether lipids of T. acidophilum

Total polar lipid extracts from *T. acidophilum* were subjected to TLC, and a wide band enriched for diether lipids was isolated. Negative-ion FAB MS of this sub-fraction produced fragment ions characteristic of phosphodiether (m/z 731.6) and phosphohy-

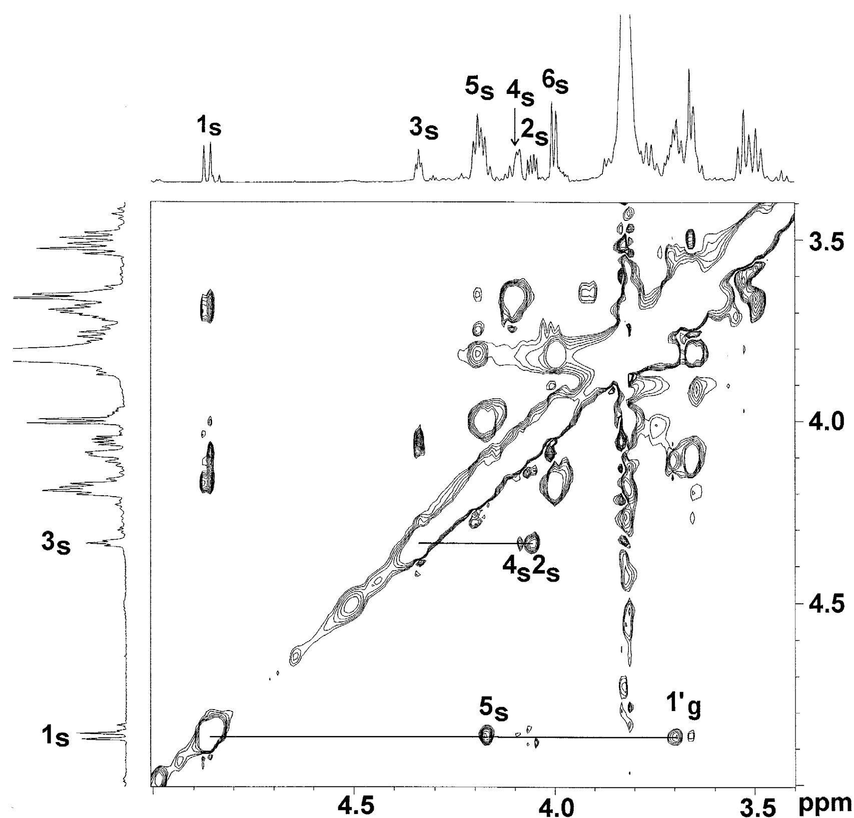

Fig. 3. ROESY spectrum along with the ^{1}H spectrum showing the head group region of purified MPL from *T. acidophilum*. X_s, carbon position of the unknown sugar of MPL; X_g, carbon position of glycerol, see Fig. 6.

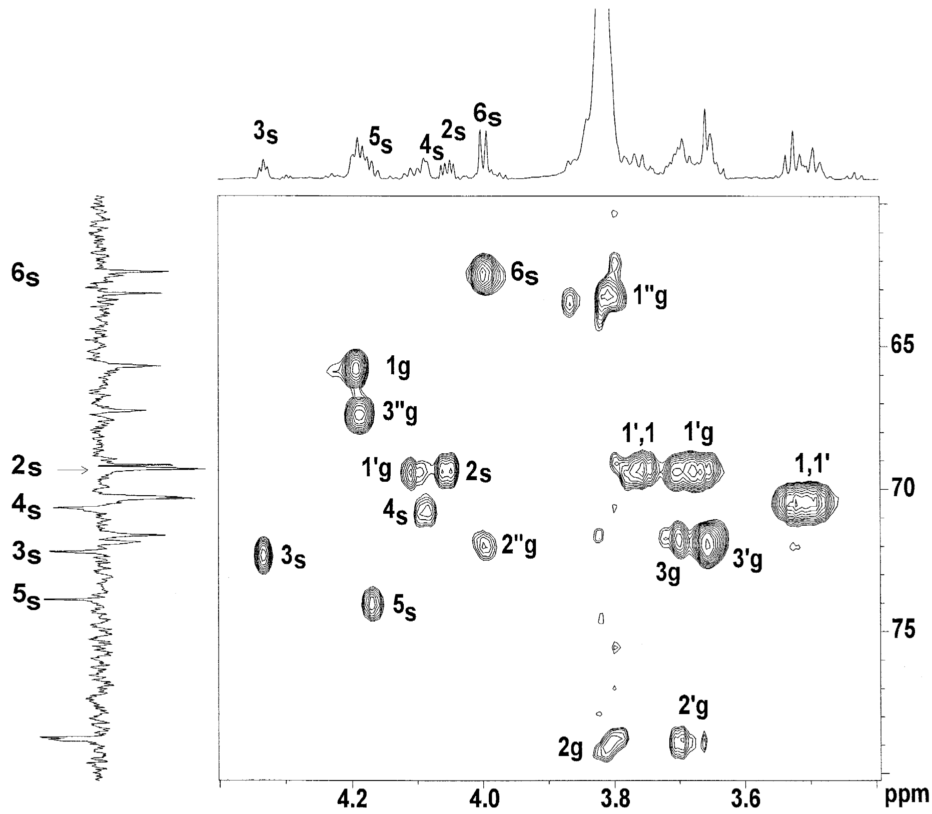

Fig. 4. HMQC spectrum of the head group region of the MPL purified from *T. acidophilum* along with the DEPT spectrum and ^{1}H spectrum. X_s, carbon position of the unknown sugar of MPL; X_g carbon position of glycerol, see Fig. 6.

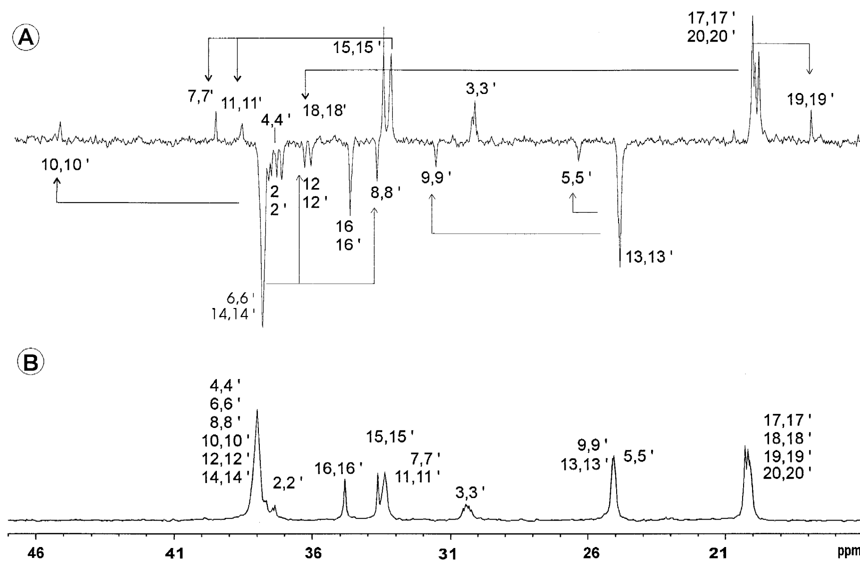

Fig. 5. ^{13}C DEPT spectrum of the phytanyl chain region of MPL isolated from *T. acidophilum* (panel A), and comparison to a similar spectrum of a tetraether lipid lacking cyclopentane rings from *Methanobrevibacter smithii* (panel B).

Table 1
Chemical shifts [a] and coupling constants $J_{H,H}$ for the sugar moiety of MPL from *T. acidophilum*

Sugar resonance	^{1}H Chemical shifts (ppm)	$J_{H,H}$ Coupling (Hz)	Sugar resonance	^{13}C Chemical shifts (ppm)	
H-1	4.864	8	C-1	102.25	102.6 [c]
H-2	4.056	8, 3.5	C-2	69.18	69.1
H-3	4.337	3.5, 3.5	C-3	72.18	72.3
H-4	4.091	3.5, 1	C-4	70.67	70.5
H-5	4.17	– [b]	C-5	73.86	74.9
H-6	4.001	– [b]	C-6	62.40	62.1
H-6′	4.001	– [b]			

[a] Chemical shifts relative to tetramethylsilane.
[b] $J_{H,H}$ coupling between H-5 and H-6/H-6′ could not be determined because H-6 and H-6′ have the same chemical shift.
[c] Data for β-methylgulopyranoside from Tipson and Horton [14].

Table 2
Retention time (min) of butylated glycoside derivatives of standard sugars and the sugar moiety of glycolipids following lipid hydrolysis

Peak	MPL [a]	TPL [b]	TPL [c]	L-Gulose	D-Gulose	L-Glucose	D-Glucose
1	11.58(*p*) [d]	11.55(*p*)	11.55(*p*)	11.55(*p*)	11.59(*p*)	11.53(*p*)	11.56(*p*)
2	12.24(*p*)	12.22(*p*)	12.24(*p*)	12.24(*p*)	12.07(*p*)	12.04(*f*)	12.03(*f*)
3	12.39(*f*)	12.37(*f*)	12.39(*f*)	12.39(*f*)	12.35(*f*)	12.08(*f*)	12.06(*p*)
4	12.54(*f*)	12.53(*f*)	12.53(*f*)	12.54(*f*)	13.06(*f*)	12.16(*p*)	12.19(*f*)

[a] MPL, main polar lipid of *T. acidophilum*.
[b] TPL, total polar lipids of *T. acidophilum*.
[c] TPL, total polar lipids of *T. volcanium*.
[d] Furanosides (*f*) were distinguished by prominent masses at 143, 169 and 259 *m/z* from pyranosides (*p*) with masses at 98, 115 and 182 *m/z*.

droxydiether (*m/z* 747.6) lipids previously described in certain other archaeobacteria [18,19]. Further, the molecular ions [M-H]$^{-}$ observed were characteristic of those produced by *Methanosarcina* species [20] but not found previously in *Thermoplasma*; namely, *m/z* 805.5 diether phosphatidylglycerol, 821.6 hydroxydiether phosphatidylglycerol, 893.6 diether phosphatidylinositol, and 909.6 hydroxydiether phosphatidylinositol (Fig. 7).

3.5. Comparison between the lipids of *Thermoplasma volcanium* and *Thermoplasma acidophilum*

Comparison between negative-ion FAB MS spectra of total lipid extracts from *T. volcanium* and *T. acidophilum* revealed that the *m/z* of the lipid species were essentially identical, with the possible exception of the number of cyclopentane rings in the dominant form of MPL (Fig. 8). Minor peaks were

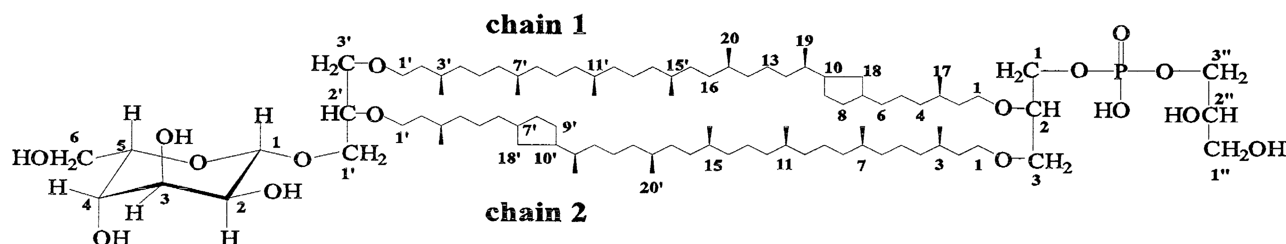

Fig. 6. The proposed structure of MPL incorporates data from NMR, MS and optical rotation. MPL is a tetraether lipid ($C_{95}H_{189}O_{16}P$), the dominant form having 1 cyclopentane ring per chain, with *sn* − 3-glycerophosphate as one head group, and β-L-gulopyranoside as the other. Glycerol carbons are designated throughout as X_g; sugar signals as X_s.

Table 3
Molecular rotations (M_D) of MPL purified from *T. acidophilum*, and related compounds

Compound [a]	Solvent	M_D (degrees)	Reference
MPL (2 rings)	$CHCl_3$	+161.4	This study
D_s-PG ($sn-1$-$sn-3'$ isomer)	$CHCl_3$	+28	[15]
D_s core lipid	$CHCl_3$	+55	[15]
T_s core lipid (no rings)	$CHCl_3$	+110	[16]
α-methyl-L-Gulopyranoside $\cdot H_2O$	H_2O	−196	[17]
β-methyl-L-Gulopyranoside	H_2O	+135	[17]
MPL (no rings)	$CHCl_3$	+218 [b]	calculated

[a] D_s, diether/archaeol; T_s, tetraether/caldarchaeol.
[b] 28 + 55 + 135.

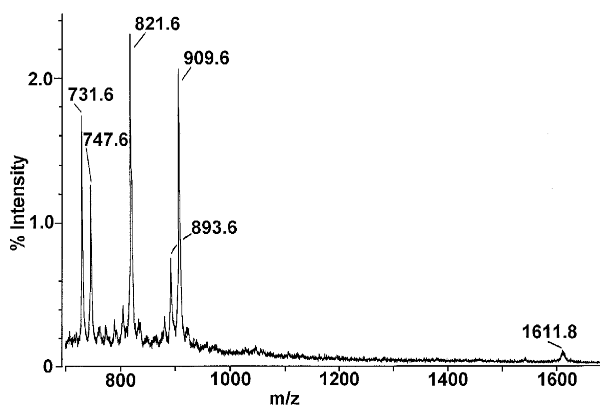

Fig. 7. Negative-ion FAB MS spectrum of a diether lipid fraction isolated by TLC separation of the total polar lipid extract from *T. acidophilum*.

observed differing from MPL in m/z by the addition of one or two hexose moieties. Comparison of the average m/z for $[M-H]^-$ of diglycosyl (calculated 1779.6 for no rings; obtained 1773.7) and triglycosyl (calculated 1941.6 for no rings; obtained 1935.9) variations of MPL-like lipids suggested an average of 3 cyclopentane rings per molecule (Fig. 8A). Certain of the minor diether lipids observed in enriched sub-fractions are not detected in these analyses.

4. Discussion

The previously unknown sugar of the main polar lipid in *Thermoplasma acidophilum*, and *T. volcanium*, is established to be a β-L-gulopyranose residue. This sugar has been found only occasionally in nature, and in small amount. The only example which we are aware of describing the biological occurrence of the L-stereoisomer is α-L-gulopyranose found as a component of the antitumor glycopeptide, bleomycin, produced by *Streptomyces* [21]. D-Gulose is found in the extracellular polysaccharide of *Caulobacter crescentus* [22], and in the cardiac glycoside antiarigenin [23]. Minor amounts of gulose were found in the glycoprotein of a green alga [24] and in an exoglycolipid of *Chlamydia trachomatis* [25]. In the case of both strains tested here, L-gulopyranoside is nearly molar equivalent to the total polar lipids present in the cytoplasmic membrane of *Thermoplasma*.

The ^{13}C spectrum of the total polar lipids of *T. acidophilum* showed only one dominant anomeric carbon, corresponding to the C-1 of β-gulopyranoside, indicating that Gul is the only sugar residue present in the MPL lipid fraction. However, some heterogeneity is introduced into the MPL structure by the varying numbers of cyclopentane rings, and this is known to vary with growth temperature [2]. Electrospray MS indicated that the most abundant form of

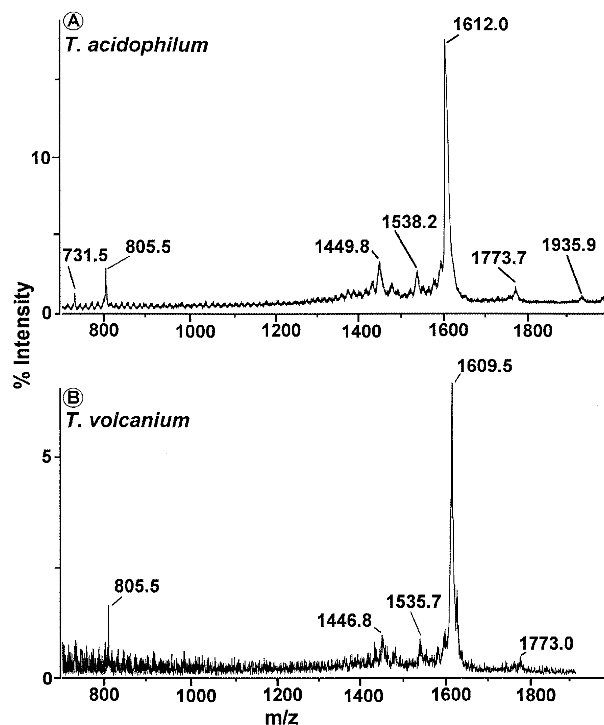

Fig. 8. Comparison between negative-ion FAB MS spectra of total lipid extracts from *T. acidophilum* and *T. volcanium*.

MPL contained 2 rings/molecule, when *T. acidophilum* was grown as in this study.

Minor archaeol diether lipids extracted from *T. acidophilum* are dominated by a peak of m/z 805.5 in negative-ion FAB MS spectra of total lipid extracts. The phosphatidylglycerol diether lipid commonly found in archaeobacterial lipid extracts may be anticipated to account for this peak, partly because of the dominance of $sn-3$-glycerophosphate as one of the two head groups of the MPL tetraether. However, peaks tentatively assigned to smaller amounts of phosphatidylinositol diether, and the hydroxydiether variations of these head groups was unexpected. Hydroxydiethers were discovered as major diether variants in *Methanosaeta concilii* [18] and *Methanosarcina* species [19], but these have not been observed before in a thermoacidophilic archaeobacterium. Their suspected occurrence as minor components in *T. acidophilum* suggests a possible role as metabolic intermediates.

Thermoplasma volcanium produces the same dominant lipid as *T. acidophilum*, suggesting the possibility that this unusual sugar may be a taxonomic characteristic of the genus.

Acknowledgements

Valuable discussions with Dr. Malcolm Perry are acknowledged. Thanks are expressed to Chantal Dicaire for performing the computer graphics.

References

[1] Darland, G., Brock, T.D., Samsonoff, W. and Conti, S.F. (1970) Science 170, 1416–1418.
[2] Langworthy, T.A., Tornabene, T.G. and Holzer, G. (1982) Zbl. Bakt. Hyg. I. Abt. Orig. C 3, 228–244.
[3] Langworthy, T.A. (1985) The Bacteria 8, 459–497.
[4] Smith, P.F. (1980) Biochim. Biophys. Acta 619, 367–373.
[5] Langworthy, T.A., Smith, P.F. and Mayberry, W.R. (1972) J. Bacteriol. 112, 1193–1200.
[6] Freisleben, H.-J., Henkel, L., Gutermann, R., Rudolph, P., John, G., Steinberg, B., Winter, S. and Ring, K. (1994) Appl. Microbiol. Biotechnol. 40, 745–752.
[7] Choquet, C.G., Patel, G.B. and Sprott, G.D. (1996) Can. J. Microbiol. 42, 183–186.
[8] Tolson, D.L., Latta, R.K., Patel, G.B. and Sprott, G.D. (1996) J. Liposome Res. 6, in press.
[9] Catalogue of Strains (1989) DSM-German Collection of Microorganisms and Cell Cultures.
[10] Sprott, G.D., Ferrante, G. and Ekiel, I. (1994) Biochim. Biophys. Acta 1214, 234–242.
[11] Bax, A. and Summers, M.F. (1986) J. Am. Chem. Soc. 108, 2093–2094.
[12] DeRosa, M. and Gambacorta, A. (1988) Prog. Lipid Res. 27, 153–175.
[13] Ekiel, I., Smith, I.C.P. and Sprott, G.D. (1983) J. Bacteriol. 156, 316–326.
[14] Book, K. and Pedersen, C. (1983) in Advances in Carbohydrate Chemistry and Biochemistry (Tipson, R.S. and Horton, D., eds.), Vol. 41, pp. 27–66, Academic Press, New York.
[15] Kates, M. (1978) Prog. Chem. Fats Other Lipids 15, 301–342.
[16] Kushwaha, S.C., Kates, M., Sprott, G.D. and Smith, I.C.P. (1981) Blochim. Biophys. Acta 664, 156–173.
[17] Isbell, H.S. (1932) Bur. Standards J. Res. 8, 1–8.
[18] Ferrante, G., Ekiel, I., Patel, G.B. and Sprott, G.D. (1988) Biochim. Biophys. Acta 963, 173–182.
[19] Sprott, G.D., Ekiel, I. and Dicaire, C.J. (1990) J. Biol. Chem. 265, 13735–13740.
[20] Sprott, G.D., Dicaire, C.J. and Patel, G.B. (1994) Can. J. Microbiol. 40, 837–843.
[21] Oshitari, T., Tomita, M. and Kobayashi, S. (1994) Tetrahedron Lett. 35, 6493–6494.
[22] Ravenscroft, N., Walker, S.G., Dutton, G.G.S. and Smit, J. (1991) J. Bacteriol. 173, 5677–5684.
[23] Wagner, H., Habermeier, H. and Schulten, H.-R. (1984) Helv. Chim. Acta 67, 54–63.
[24] Mengele, R. and Sumper, M. (1992) FEBS Lett. 298, 14–16.
[25] Stuart, E.S., Tirrell, S.M. and MacDonald, A.B. (1987) Immunology 61, 527–533.