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EFFECTS OF TEMPERATURE ON ETHER LIPID COMPOSITION OF *CALDARIELLA ACIDOPHILA*

MARIO DE ROSA*, ENRICO ESPOSITO, AGATA GAMBACORTA*, BARBARA NICOLAUS* and JOHN D. BU'LOCK†

*Laboratorio per la Chimica M.I.B. del C.N.R., Via Toiano n. 2, Arco Felice, Naples, Italy; †Department of Chemistry, University of Manchester, U.K.

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Key Word Index—*Caldariella*; archaebacteria; thermoacidophiles; lipids; ether lipids; complex lipids; temperature effects.

Abstract—The composition of the ether lipids of a strain of *Caldariella acidophila*, with respect to the different numbers of cyclizations of the biphytanyl components, is shown to differ between the various complex lipid classes, but the degree of cyclization increases systematically with the growth temperature in the range 75–89°. The problem of distinguishing adaptive from phyletic features in archaebacterial lipids is considered.

INTRODUCTION

The preceding paper [1] describes the complex lipid composition of *Caldariella acidophila*, taken as a typical member of the *Caldariella* group of extreme thermoacidophiles [2–4] and discusses some aspects of the relationships between these organisms and other representatives of the archaebacteria [5]. For the first time, in the light of those relationships, it has become possible to consider what features of the *Caldariella* group are adaptive, and specifically related to the extreme habitats from which they have been isolated, rather than phyletic, being determined by their archaebacterial ancestry.

For example, the absence of peptidoglycan cell walls and the assembly of the cell membrane from ether lipids in *Caldariella* [2, 3] are now seen as general archaebacterial features [5], and not as adaptive to extreme thermal acid environments. Equally, the predominance of ether lipids based on 16,16′-biphytanyl chains is seen as phyletic since it is also found in several species of *Methanobacterium* and *Methanospirillum* [6, 7]. On the other hand, some of the more detailed lipid features of *Caldariella* may qualify for consideration as adaptive since their occurrence is less widespread; these include [1] the tendency to cyclizations within the biphytanyl chains and the presence of calditol glycerol tetraethers.

The MT-4 strain of *C. acidophila* can be grown at temperatures between 75 and 89° with reasonable facility, and, as already indicated [1], the mutual proportions of the different complex lipids which make up the cell membrane do not differ significantly in cells grown at different temperatures in this range. However, within each complex lipid, the mutal proportions of the differently cyclized biphytanyl components [1] do vary systematically, as is described in the present communication.

RESULTS

The composition of the C_{40} component mixtures in the different complex lipids of *C. acidophila* strain MT-4 growing at 75–89° is presented in Table 1. The data can be combined by calculating the 'average cyclization' (analogously to average unsaturation) of the biphytanyls for each lipid at each temperature, and these values are shown in Table 2.

The analytical data show firstly that there are significant differences in the degree of cyclization in the different complex lipids, secondly that there is a systematic effect of temperature on the degree of cyclization, and thirdly that the magnitude of the temperature effect differs in different complex lipids.

As the growth temperature is increased, all the lipids show increasing proportions of the tri- and tetracyclic biphytanyls, largely at the expense of the acyclic and monocyclic; this effect is seen in each lipid and in the total lipid data, which also show that in this strain the bicyclic biphytanyl is always the major component. The calditol-containing lipids, **4**, **6** and **8**, all show more extensive cyclization than the diglycerol lipids, **3**, **5** and **7**. An observation of particular significance is that the temperature effect is minimal in lipid (**5**), (diglycerol tetraether) ← phosphoinositol, the biphytanyl composition of which remains relatively constant over the whole temperature range.

DISCUSSION

Comparing *C. acidophila* with those other archaebacteria whose membrane lipids have been characterized, we can note, firstly, that the biphytanyl lipids are absent in the relevant extreme halophiles such as *Halobacterium* where the membrane is based on diphytanyl glycerol [9]. Amongst the methanogens this is also true of the *Methanococcus*,

Table 1. Biphytanyls (%) in complex lipids **3**–**8** of *C. acidophila* grown at 75–89°

	Biphytanyl									
	$C_{40}H_{82}$	$C_{40}H_{80}$	$C_{40}H_{78}$	$C_{40}H_{76}$	$C_{40}H_{74}$	$C_{40}H_{82}$	$C_{40}H_{80}$	$C_{40}H_{78}$	$C_{40}H_{76}$	$C_{40}H_{74}$
Temp.			**3**		Lipid			**4**		
75°	3	17	54	21	5	0	5	68	26	1
80°	2	14	52	26	6	0	4	68	25	3
85°	0	7	49	32	12	0	2	47	38	12
89°	0	4	46	37	13	0	1	46	40	13
Temp.			**5**		Lipid			**6**		
75°	10	18	47	17	7	4	14	63	17	2
80°	9	18	46	18	8	2	14	59	20	4
85°	8	17	46	20	8	1	11	47	31	8
89°	8	13	45	24	10	0	1	44	40	14
Temp.			**7**		Lipid			**8**		
75°	11	26	46	13	4	5	21	56	17	1
80°	10	21	45	19	5	3	16	55	22	3
85°	5	16	43	27	7	2	12	55	25	6
89°	1	13	42	34	9	0	7	48	34	11

Methanosarcina group [7]. However, in *Methanobacterium* and *Methanospirillum* the phytanyl diethers and biphytanyl tetraethers are found together [6, 7]. Allegedly, the latter are based only on the acyclic biphytanyl type (**9**), and the proportion of the biphytanyl tetraethers is not related to the wide-ranging temperature optima of these methanogens [6]. Among the thermoacidophiles of the *Caldariella* group, only the biphytanyl tetraether lipids are found, but here there are differences in the degree of cyclization, which do run parallel to the temperature optima of the different isolates. Thus in *Thermoplasma*, grown at 59°, and *Sulfolobus*, grown at 70°, the proportions of acyclic: monocyclic: bicyclic C_{40} components in the diglycerol lipids are 65:32:2 and 30:32:38, respectively [4]. This suggests that the extent of cyclizations in the biphytanyls could qualify as a temperature-related adaptive feature; the present data support this view by showing the phenotypic aspect of this same variation.

Membranes based on the tetraethers **1** and **2** are necessarily monolayer structures, formally analogous to the conventional bilayer but with pairs of covalently-linked hydrophobic chains extending right through the membrane. Such a membrane already possesses considerable added stiffness and rigidity, e.g. in its containing effect on membrane proteins. However, because of its biosynthesis [10] it cannot be modified in adaptation to higher temperature environments by the same means as are open to eubacteria with bilayer membranes based on glycerol diesters, increases in chain-length [11] and/or the incorporation of specially bulky end-groups in the fatty acids [12]. The primary effects of such changes are to raise the transition temperatures ('melting points') of the layered assembly, and the same effect can be achieved in the tetraether structure by introducing the observed cyclizations into the biphytanyl chains. Each such cyclization in the chain considerably decreases the available modes of flexing and of rotation. Though the mechanism of this cyclization is unknown, chemical arguments based on detailed tetraether structures [13] suggest that it occurs at a middle stage in tetraether synthesis, e.g. in an intermediate diglycerol dibisgeranylgeranyl tetraether; the present work establishes the temperature response of the relevant enzyme system.

Table 2. Average cyclization* of C_{40} components in *C. acidophila* complex lipids

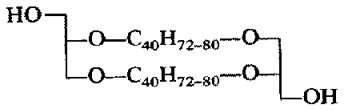
Lipid	Temperature 75°	80°	85°	89°
3	2.09	2.20	2.51	2.61
4	2.23	2.26	2.60	2.66
5	1.94	1.97	2.04	2.16
6	1.99	2.10	2.34	2.68
7	1.74	1.89	2.15	2.38
8	1.88	2.06	2.20	2.48
Total lipids	1.94	2.10	2.31	2.52

*[% Monocyclic + 2 × % bicyclic + 3 × % tricyclic + 4 × % tetracyclic] × 10^{-2}.

The varying degrees of cyclization in the different complex lipids are interesting but not at present explicable. Those containing calditol are more cyclized; those containing phosphoinositol are less cyclized, and the diglycerol tetraether phosphoinositol (**5**) shows minimum temperature effects. Whether the phosphoinositol lipids have a special biosynthetic or membrane function remains to be ascertained.

EXPERIMENTAL

The cultures of *C. acidophila* were grown, and the lipids worked up as already described [1]. Analysis of the C_{40} components in the individual lipid categories was approached by way of the parent diglycerol tetraethers (**1**) and calditol glycerol tetraethers (**2**) as follows. *Neutral lipids.* Not analysed. *Glycolipids.* The (diglycerol tetraether)-galactose-glucose (**3**) and the (calditol glycerol tetraether)-glucoside (**4**) were not separated as such, but hydrolysed with HCl–MeOH



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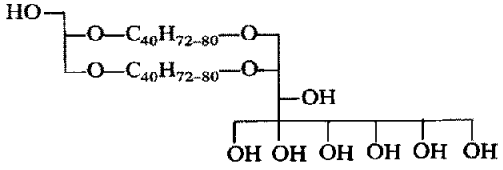

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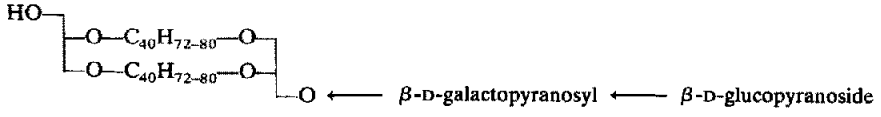

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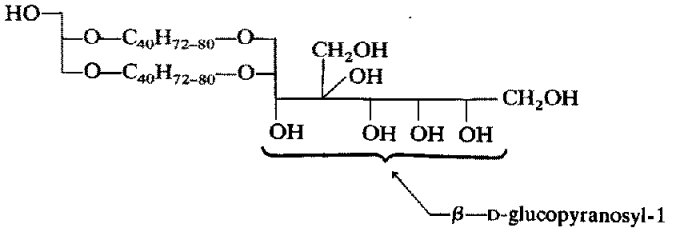

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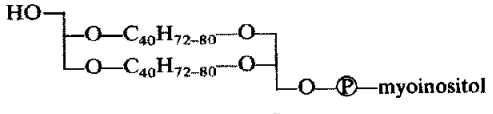


5

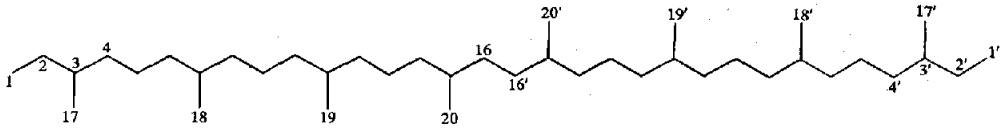
4 ← sulphate
6

3 ← phosphoinositol
7

4 ← phosphoinositol
8

to a mixture of diglycerol tetraethers and calditol glycerol tetraethers, which were then separated by TLC on Si gel in $CHCl_3$–MeOH (9:1) (R_f 0.9 and 0.5, respectively). *(Diglycerol tetraether)-phosphoinositol* (**5**) was separated and hydrolysed [1] to the parent diglycerol tetraether, and recovered by TLC as above.

(Calditol glycerol tetraether)-glucoside sulphate (**6**) was separated and hydrolysed [1] to the parent calditol glycerol tetraether and recovered by TLC as above. [*(Diglycerol tetraether)-galactose-glucose)]-phosphoinositol* (**7**) *and* [*(calditol glycerol tetraether)-glucoside]-phosphoinositol* (**8**) were not separated as such, but after hydrolysis, as for **3** and **4** above. With each complex lipid type thus converted into the component tetraether **1** or **2**, the latter were cleaved with HI and the C_{40} di-iodides converted into the C_{40} hydrocarbons by treatment with excess $LiAlH_4$ as previously described [4].



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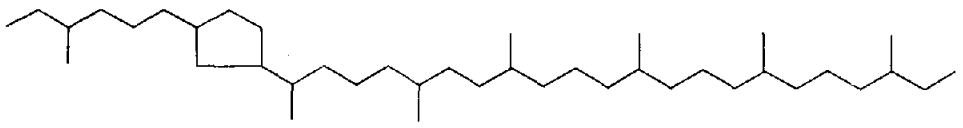

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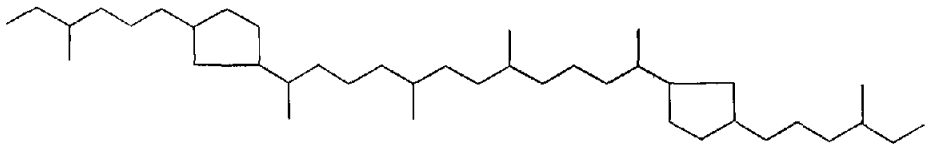

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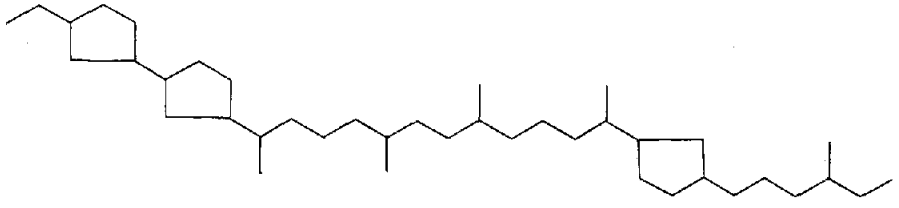

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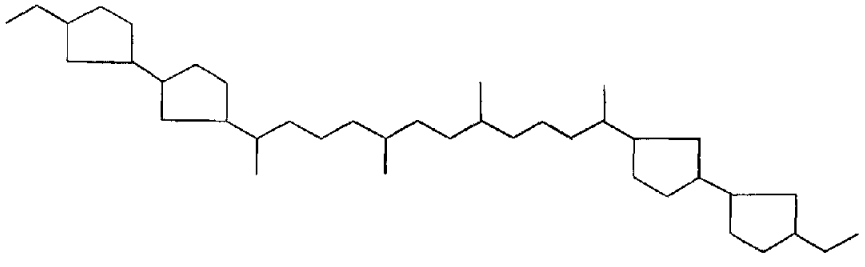

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The C_{40} hydrocarbons thus obtained are [8] $C_{40}H_{82}$ (acyclic) (**9**), $C_{40}H_{80}$ (monocyclic) (**10**), $C_{40}H_{78}$ (bicyclic) (**11**), $C_{40}H_{76}$ (tryciclic) (**12**), and $C_{40}H_{74}$ (tetracyclic) (**13**); the mixtures of these were analysed quantitatively by GLC as previously described [4, 8].

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