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STRUCTURE OF CALDITOL, A NEW BRANCHED-CHAIN NONITOL, AND OF THE DERIVED TETRAETHER LIPIDS IN THERMOACIDOPHILE ARCHAEBACTERIA OF THE *CALDARIELLA* GROUP

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Key Word Index—*Caldariella* group; archaebacteria; thermoacidophile; ether lipids; calditol; biphytanyl.

Abstract—A second category of membrane lipids in extreme thermoacidophile archaebacteria of the *Caldariella* group is based on the same type of macrocyclic tetraether, incorporating two 16,16′-biphytanyl chains, as those described earlier, but only one of the hydrophilic components is glycerol; the second hydrophilic component is calditol, a unique branched-chain nonitol. It is also shown that in the biphytanyl chains there can be up to 4 cyclopentane rings whose location is demonstrated.

INTRODUCTION

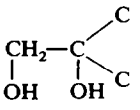
The *Caldariella* group of microorganisms [1–3] comprises extreme thermoacidophile representatives of the recently recognized [4, 5] archaebacteria. In the isolates we have studied [1, 2] the membrane is built up from lipids of varying complexity which on hydrolysis afford two main types of component. Both of these are macrocyclic tetraethers in which the two lipophilic portions are C_{40} residues with the 16,16′-biphytanyl skeleton [6]. In one type, the hydrophilic portions are two glycerol units and for these structure **1** has been established: in the same work three of the C_{40} components were fully characterized as having structures **2, 3** or **4** [7].

In the present paper we show that the second type of lipid is based on tetraethers with structure **5** in which the hydrophilic portions are glycerol and a unique branched-chain nonitol, for which the trivial name calditol is appropriate. Additional examples of the biphytanyl components are shown to be further cyclized variations of the basic 16,16′-biphytanyl-structure, namely **7** and **8**.

RESULTS

Calditol glycerol tetraethers (**5**)

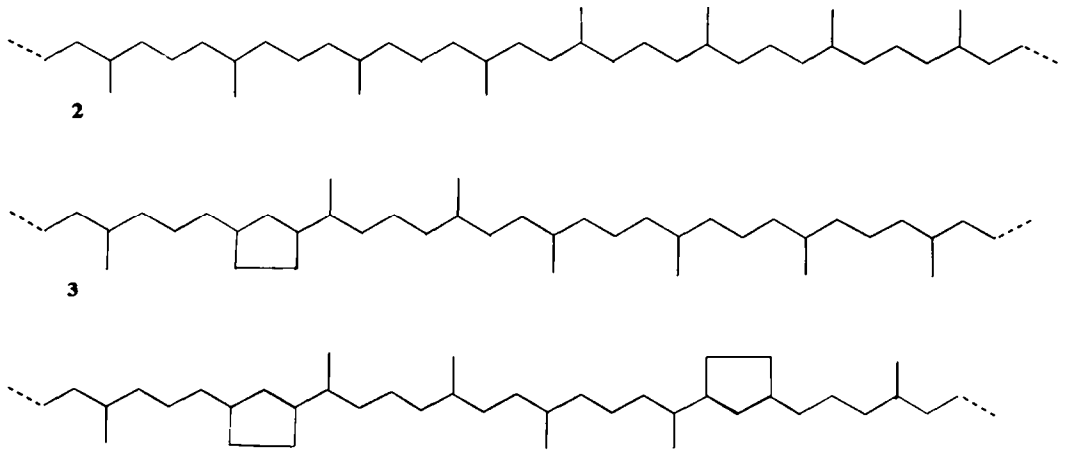
By TLC of the hydrolysate from freeze-dried cells of *Caldariella acidophila* [6] the tetraether lipids are readily separated into the diglycerol tetraethers (**1**) and the more polar calditol glycerol tetraethers. The latter have MW 1490 (by vapour pressure osmometry in $CHCl_3$; $C_{92}H_{168-176}O_{12}$ requires 1464–1472), and the ^{1}H NMR spectrum shows H—C—O and H—O protons ($\delta \sim 3.5$) and H—C protons (δ 0.7–1.8) in *ca* 30:140 ratio. Acetylation readily gives a hepta-acetyl derivative still showing HO absorption in the IR, and under more vigorous conditions the hydroxyl-free octa-acetyl derivative. The ^{1}H NMR spectra of both derivatives (respectively 21 and 24 O·COC$\underline{H}_3$ at δ 2.05) show the presence of three —C$\underline{H}_2$OAc groups, two with further couplings which give broad signals at δ 4.15 and one giving a sharp AB quartet with no further couplings, which appears at δ 4.3 in the hepta-acetate and δ 4.7 in the octa-actate. This indicates that the polyol moiety of **5** is branched and has the partial structure:



The calditol tetraethers are cleaved by BCl_3 to glycerol, calditol and C_{40} dichlorides (molar ratio 1:1.01:2.05); for the products of this and of periodate cleavage, see below.

Structure of calditol

The free polyol (**6**) is obtained by BCl_3 cleavage of **5** but neither the MS (highest peak $M^+ - OH$, $C_9H_{19}O_8$;



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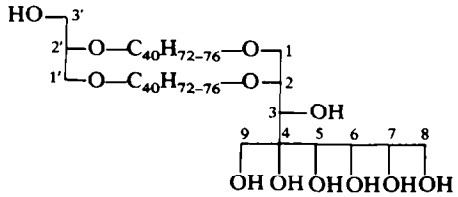

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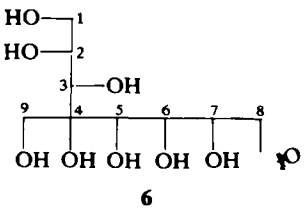

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fragments C_8, C_6 and C_4) nor the ^{1}H NMR spectrum are informative. Acetylation readily gives the octa-acetate and the nona-acetate with more difficulty, and the ^{1}H NMR spectra of these show features corresponding to those noted above for the hepta- and octa-acetyl derivatives of **5**. Decoupling data from these spectra establish partial structures in calditol as:

$CH_2OH{\cdot}CHOH—$
$CH_2OH{\cdot}CHOH{\cdot}CHOH—$
$CH_2OH{\cdot}C(OH)<$

Prolonged periodate oxidation of **5**, followed by $NaBH_4$ reduction, gives a small amount of material not chromatographically distinguishable from the diglycerol tetraethers (**1**) and, as the major product (also found after shorter periodate treatment), a glycerol hexitol tetraether which is assigned structure **9** (from which the structures of calditol (**6**) and of **5** follow). This product forms a penta-acetyl derivative and the ^{1}H NMR spectrum of this shows the presence of three —CH_2OAc groups; the —CH_2—OAc signal of one (δ 4.15) is coupled to adjacent —CHO— (δ 3.50), the others (δ 4.45 and 4.55) are independent, simple AB quartets. The tetraether (**9**) is cleaved by BCl_3 to glycerol, the C_{40} dichlorides and a branched-chain hexitol (**11**); the ^{1}H NMR spectrum of the hexa-acetyl derivative of this hexitol has been fully assigned (see Experimental).

The partial periodate cleavage of **5**, followed by $NaBH_4$ reduction (see above) also affords a glycerol hexitol tetraether isomeric with **9** which we have shown is the *n*-hexitol derivative (**10**); however the characterization of **10** does not advance determination of the calditol structure and it is not detailed here.

Structure of the C_{40} chains

Our samples of the calditol glycerol tetraether lipids proved to contain, in addition to known C_{40} components [6, 7], two new components with 3 and 4 unsaturation equivalents, respectively. Their characterization as **7** and **8** was by procedures already described [6, 7] in establishing structures **2–4** for the biphytanyl components of **1**; the C_{40} dichlorides from the BCl_3 cleavage of **5** were converted into the corresponding [1, 1′-$^2H_2$]-labelled hydrocarbons for GC–MS study and into the corresponding 1,1′-diols (**17**) and (**18**) for ^{1}H NMR study using the Eu shift method.

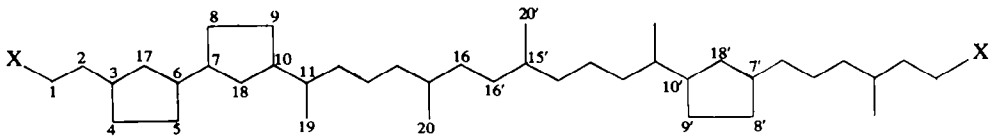
The MS of the tricyclic hydrocarbon $C_{40}H_{76}$ shows the diagnostic fragmentations represented in **13**.

The ^{1}H NMR spectrum of the corresponding tricyclic diacetate (**15**) shows signals for 5 CHMe groups (3H, δ 0.83; 6H, δ 0.94; 6H, δ 0.96). That of the tricyclic diol (**17**) is partly summarized in the partial structures (**14**) together with δ values and the δ increments (in parentheses) observed with the Eu shift reagent.

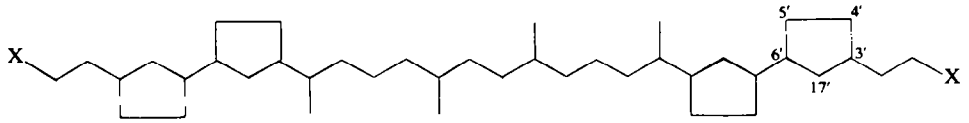
These data establish the location of the third ring in **7**. Structure **8** for the tetracyclic biphytanyl component is supported by entirely similar data to these, with small but significant differences arising from the symmetry of its structure.

^{13}C NMR studies

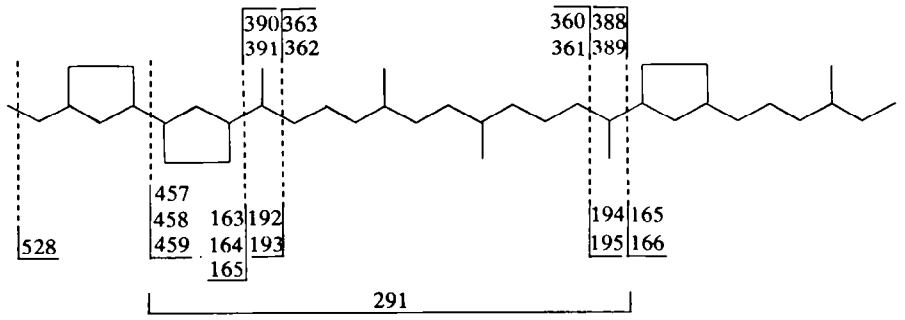
As in our previous study of the diglycerol tetraethers [8], it has been possible to make virtually complete assignments of the ^{13}C NMR spectra of newly isolated C_{40} hydrocarbons, diols, and diacetates which fully confirm structures **7** and **8**, and also of the derivatives and degradation products of **5** relevant to the structure of calditol. These assignments are summarized in Tables 1–5.



7 X = H
15 X = OAc
17 X = OH



8 X = H
16 X = OAc
18 X = OH



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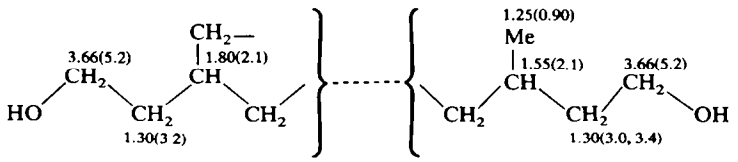

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Table 1. ^{13}C chemical shifts (from TMS, ±0.02) and multiplicities of the C_{40} hydrocarbons **7** and **8**

Carbon No.	7	8
1,1′	12.40 (*q*); 11.32 (*q*)	12.40 (*q*)
2,2′	29.34 (*t*); 29.71 (*t*)	29.34 (*t*)
3,3′	41.73 (*d*); 34.73 (*d*)	41.73 (*d*)
4,4′	31.46 (*t*); 36.91 (*t*)	31.46 (*t*)
5,5′	30.47 (*t*); 25.87 (*t*)	30.47 (*t*)
6,6′	46.43 (*d*); 37.19 (*t*)	46.43 (*d*)
7,7′	45.60 (*d*); 39.13 (*d*)	45.60 (*d*)
8,8′	32.52 (*t*); 33.37 (*t*)	32.52 (*t*)
9,9′	31.45 (*t*); 31.28 (*t*)	31.45 (*t*)
10,10′	45.15 (*d*); 44.89 (*d*)	45.15 (*d*)
11,11′	38.29 (*d*)	38.29 (*d*)
12,12′	35.77 (*t*)	35.77 (*t*)
13,13′	24.53 (*t*)	24.53 (*t*)
14,14′	37.60 (*t*)	37.60 (*t*)
15,15′	33.12 (*d*)	33.12 (*d*)
16,16′	34.35 (*t*)	34.35 (*t*)
17,17′	39.20 (*t*) ; 19.41 (*q*)	39.20 (*t*)
18, 18′	34.74 (*t*); 36.0 (*t*)	34.74 (*t*)
19,19′	17.75 (*q*)	17.75 (*q*)
20,20′	19.80 (*q*)	19.80 (*q*)

Table 2. Chemical shifts and lanthanide-induced shifts for tricyclic diol **17** with Eu(fod-d_9)$_3$ at reagent–diol ratio (1:1)

Carbon No.		1:1	
1,1′	62.20; 61.22	6.03;	5.43
2,2′	39.73; 40.10	0.96;	0.76
3,3′	36.55; 29.67	0.78;	0.74
17,17′	39.51; 19.70	0.60;	0.57
4,4′	31.90; 37.52	0.58;	0.52
5,5′	30.47; 25.87	0.31;	0.33
6,6′	46.43; 37.19	0.31;	0.22
7,7′	45.60; 39.13	0.19;	0.12
18,18′	34.74; 36.00	0.12;	<0.02
8,8′	32.52; 33.37	0.13;	<0.02
9,9′	31.45; 31.28	0.10;	<0.02
10,10′	45.15; 44.89	<0.02	
Rest	—	<0.02	

Table 3. Acetylation effect for tricyclic diol **17**

Carbon No.	δ
1,1′	+1.88
2,2′	−4.54
3,3′	+0.25
4,4′	−0.20; −0.33
17,17′	−0.20; −0.13

Table 4. ^{13}C chemical shifts (from TMS, ±0.02) and multiplicities for C—O carbons of the calditol–glycerol tetraethers (**5**) and of its oxidative degradation products **9** and **10** as fully acetylated derivatives

Carbon No.*	**5**	**9**	**10**
1′		70.60 (*t*)	
2′		76.60 (*d*)	
3′		64.10 (*t*)	
1	71.06 (*t*)	70.98 (*t*)	71.20 (*t*)
2	76.60 (*d*)	77.77 (*d*)	77.80 (*d*)
3	85.04 (*d*)	77.96 (*d*)	77.00 (*d*)
4	87.20 (*s*)	81.96 (*s*)	69.20† (*d*)
5	80.40† (*d*)	61.86† (*t*)	70.38† (*d*)
6	73.20† (*d*)		62.26 (*t*)
7	73.08† (*d*)		
8	64.10 (*t*)		
9	58.80 (*t*)	61.74† (*t*)	

* For numbering see formulae **5**, **9** and **10**.
† These signals are not unequivocally assigned.

Table 5. ^{13}C chemical shifts (from TMS, ±0.02) and multiplicities for polyols **6**, **11** and **12** as fully acetylated derivatives

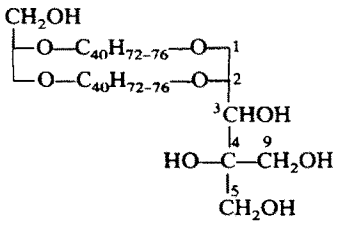
Carbon No.*	**6**	**11**	**12**
1	70.0 (*t*)	70.61 (*t*)	70.86 (*t*)
2	70.0 (*d*)	70.28 (*d*)	70.32 (*d*)
3	85.40 (*d*)	78.43 (*d*)	77.11 (*d*)
4	87.11 (*s*)	81.83 (*s*)	69.78† (*d*)
5	80.30† (*d*)	61.83† (*t*)	69.48† (*d*)
6	73.10† (*d*)		62.23 (*t*)
7	72.90†		
8	62.40 (*t*)		
9	58.80 (*t*)	61.61† (*t*)	

* For numbering see formulae **6**, **11** and **12**.
† The signals are not unequivocally assigned.

As for the previously characterized C_{40} components [8], the assignments are based on chemical shift rules, comparisons with appropriate model compounds, selective proton decouplings, and the observed multiplicities in partly-decoupled spectra, together with lanthanide and acetylation shift data for the diols; the assignments are, of course, very close to those for the compounds characterized earlier.

In addition, spectra for samples incorporating ^{13}C from both acetate-[1-^{13}C] and acetate-[2-^{13}C] were again available and provided additional discriminatory data; as in the simpler biphytanyls [8], the C_{40} chain shows the expected pattern of labelling from the two species of acetate-[^{13}C] corresponding to biosynthesis via two regular geranylgeranyl chains which undergo very unusual head-to-head coupling followed by cyclizations. In each labelling experiment the enrichments at the labelled positions were all in the range 3.1–3.8 times natural abundance, compared with 0.90–1.06 times at the unlabelled positions, and are not reported in detail since they merely confirm our earlier data [8].

Assignments of the ^{13}C NMR spectra of the calditol moiety of **5**, **9**, **10**, of calditol itself (**6**), and its degradation products **11** and **12** as fully acetylated derivatives, have similarly been made and are shown in Tables 4 and 5; neither the calditol nor the glycerol moiety of **5** was significantly enriched in the acetate-[^{13}C]-labelling experiments. The ^{13}C NMR assignments fully confirm the assigned structures, in particular the position of branching in calditol and the location of the two ether links on the calditol residue in **5**.



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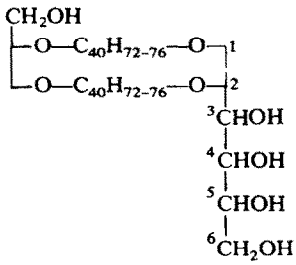

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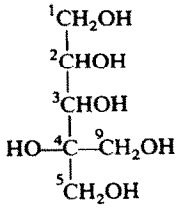

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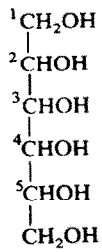

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DISCUSSION

The present work completes the skeletal characterization of the principal components of the membrane lipids of *Caldariella acidophila* as being the diglycerol tetraethers (**1**) and the calditol glycerol tetraethers (**5**), with their constituent biphytanyl components having structures **2**, **3**, **4**, **7** and **8**. With important quantitative

variations, this picture also occurs in the lipids of *Sulfolobus* and *Thermoplasma* [9] species which, for general reasons [1], we have classed as members of the *Caldariella* group. For example, those components of the *Sulfolobus* complex lipids described by Langworthy [10] as containing an ether-linked (glycerol–polyol) unit, are here characterized as calditol glycerol tetraether derivatives. Similarly the complex lipid component in *Thermoplasma* described by Langworthy *et al.* [11] as a glycerolphosphoryl derivative of an 'unidentified' glycosyl-diglycerol tetraether is possibly a calditol-containing tetraether lipid.

The C_{40} components in all these members of the *Caldariella* group belong to the same series of biphytanyls, **2**, **3**, **4**, **7** and **8**, but the proportions of the cyclized components from different strains vary considerably ([9] and unpublished data).

Until quite recently, the closest parallel to the lipids based on **1** and **5** was the series of membrane lipids in Halobacteria, based on 2,3-diphytanylglycerol diether [12]. However, the demonstration that (on quite different grounds) the *Caldariella* series of thermoacidophiles, the Halobacter series of halophiles, and the wide range of methanogenic bacteria all belong to a distinct and major taxon, the archaebacteria *sensu* Woese [4, 5], has stimulated the investigation of lipids in the methanogens.

Two recent independent studies [13, 14] have shown that while all 10 methanogens so far investigated contain diphytanyl glycerol diether lipids similar to those of the halophiles, 8 of the strains also contain significant or even predominant amounts of diglycerol di(biphytanyl) tetraethers, i.e. of **1**. Thus it is no longer possible to view the predominance either of ether lipids in general or of the biphytanyl lipids in particular as features directly related to evolutionary adaptations to extreme (saline, thermal or acid) environments. The reports on methanogen lipids [13, 14] are to date rather preliminary, but from published data it is apparent that they contain little (or no) calditol and that the only type of biphytanyl component so far found is the simple 'acyclic' (**2**).

The lipid structures we have defined raise a number of stereochemical and biosynthetic problems; data bearing on some of these aspects will be presented in forthcoming publications.

EXPERIMENTAL

Isolation and culture methods for the MT strains of *Caldariella* were described [2], using the MT-4 strain grown at 87°. Instrumental methods (^{1}H NMR, ^{13}C NMR, MS and GC–MS were as previously described [7, 8].

Glycerol–dialkyl–calditol tetraethers. The glycerol–dialkyl–calditol tetraether mixture (**5**), was obtained as previously described [6, 9]. The mixture that represents 2.5–3% of the lyophilized cells, had MW 1490 ($C_{92}H_{168-176}O_{12}$ requires: 1464–1472) by vapour pressure osmometry in $CHCl_3$; $[\alpha]_D$ − 4.8° (*c* 1.0 in $CHCl_3$); ν_m cm^{-1}: 3450, 2960, 2929, 2860, 1460, 1375, 1115, 1045; δ 3.5 (30–32 H, *br*, H—C—O, —OH), 0.7–1.8 (*ca* 140, aliphatic proton).

Acetylation reaction. (a) The acetylation of **5** (50 mg) with Ac_2O (5 ml) and Py (0.5 ml) at room temp. for 24 hr gave a partially acetylated product, single spot on TLC, R_f 0.3 in $CHCl_3$—Et_2O (9:1). The compound had $[\alpha]_D$ − 15.2° (*c* 1.0 in $CHCl_3$); ν_m cm^{-1}: 3450, 1745, 1235, 1115, 1045; δ 5.50 (2H, *br*, CH—O—Ac), 5.2 (1H, *br*, CH—OAc), 4.3 (2H, AB*q* (δA − δB = 26 Hz; *J* = 13 Hz), (—C—CH$_2$—O—Ac)), 4.15 (4H, *br*, glycerol CH$_2$—O—Ac + CH$_2$OAc), 3.8 (1H, *br*, CH-OAc), 3.5 (14H, *br*, CH—O), 2.05 (21 H, overlapped *s*, CH_3CO). (b) The mixture of **5** (50 mg) acetylated with Ac_2O (5 ml) and Py (0.5 ml) at reflux for 6 hr gave a fully acetylated compound, single spot in TLC, R_f 0.8 in $CHCl_3$—Et_2O (9:1). The compound had $[\alpha]_D$ + 2.3° (*c* 2.0 in $CHCl_3$); ν_m cm^{-1}: 1745, 1235, 1115; δ 5.5 (3H, *br*, CH—O—Ac), 4.70 (2H, AB*q* (δA − δB = 38 Hz; *J* = 13 Hz), >C—CH$_2$—OAc), 4.15 (5H, *br*, glycerol CH$_2$—OAc + CH$_2$OAc and CH—OAc), 3.5 (14 H, *br*, CH—O); 2.05 (24 H, overlapped *s* CH$_3$—CO).

Cleavage of tetraethers with BCl_3. the glycerol–dialkyl–calditol tetraether mixture (**5**) (1 g) was treated with BCl_3 (10 ml in 10 ml $CHCl_3$ at 18° for 12 hr); the reaction mixture was evapd under N_2 and chromatographed; $CHCl_3$ eluted the C_{40} dichlorides (840 mg), $CHCl_3$—MeOH (7:3) eluted glycerol (60 mg) and $CHCl_3$—MeOH (1:1) eluted calditol (180 mg) (mol ratio C_{40} dichlorides–glycerol–calditol, 2.05:1:1.01). Calditol (**6**), obtained as above, (R_f 0.3 in $CHCl_3$—MeOH (2:3)), has $[\alpha]_D$ − 8.72° (*c* 1.3 in H_2O); ν_m cm^{-1}: 3450, 1045; δ (CD_3OD) 3.5–4.1 *br*; *m/e* 255.11150 ($C_9H_{19}O_3$ requires: 255.10997; M$^+$ − OH, 1%), 223.08196 ($C_8H_{15}O_7$ requires: 223.08175; 1); 205 (1); 187.06008 ($C_8H_{11}O_5$ requires: 187.06064; 1); 181.06830 ($C_6H_{13}O_6$ requires 181.07120; 1); 169.04930 ($C_8H_9O_4$ requires: 169.05008; 1); 164.06958 ($C_6H_{12}O_5$ requires: 164.06847; 2); 147.06570 ($C_6H_4O_4$ requires: 147.06573; 100); 114 (6); 103.03910 ($C_4H_7O_3$ requires: 103.03951; 100); 85 (56); 73 (50).

Calditol acetylation. (a) Calditol (**6**) (50 mg) acetylated with Ac_2O (5 ml) and Py (0.5 ml) at room temp. for 24 hr gave a partially acetylated product, single spot in TLC, R_f 0.4 with petrol–Et_2O (1:4); $[\alpha]_D$ + 6.80° (*c* 1.0, $CHCl_3$); ν_m cm^{-1}: 3450, 1745, 1235, 1045; δ 5.3 (4H, complex multiplet, CH—OAc), 4.25 (2H, AB*q* (δA − δB = 26 Hz: *J* = 13 Hz), >C—CH$_2$—O—Ac). 4.2 (2H, *t*, CH$_2$OAc), 3.70 (3H, complex *m*, CH$_2$OAc, CH—OAc), 2.05 (24 H, overlapped *s*, CH$_3$—CO); δ (C_6D_6): 5.7 (3H, complex *m*, CHOAc), 5.25 (1H, *m*, CHOAc), 4.5 (2H, AB*q* (δA − δB = 40 Hz; *J* = 13 Hz), >C—CH$_2$—O—Ac), 4.25 (2H, *o*, AB part of an ABX. system (δA − δB = 38 Hz, J_{AB} = 13, J_{AX} = 6, J_{BX} = 4 Hz), —CH—CH$_2$—OAc), 3.8 (3H, complex *m*, CH—OAc and CH$_2$—OAc), 1.8 (24 H, overlapped *s* CH$_3$—CO). (b) Calditol (**6**) (50 mg) acetylated with Ac_2O (5 ml) and Py (0.5 ml) at reflux for 6 hr gave a fully acetylated product, single spot in TLC, R_f 0.4 with petrol–Et_2O (3:7); $[\alpha]_D$ − 5.48° (*c* 2.4, $CHCl_3$); ν_m cm^{-1}: 1745, 1235, 1045; δ 5.45 (3H, complex *m*, CHOAc), 5.3 (1H, *m*, CHOAc), 4.7 (2H, AB*q* (δA − δB = 40 Hz, *J* = 13 Hz), >C—CH$_2$—OAc), 4.2 (3H, complex *m*, CH$_2$—OAc and CH—OAc), 3.8 (2H, *d*, (*J* = 5 Hz), CH$_2$OAc), 2.05 (27 H, overlapped *s*, CH$_3$—CO); *m/e* 549 (M$^+$ − MeCOO − MeCO; 2%), 488 (5), 444 (14), 402 (1), 385 (5), 372 (15), 343 (26), 330 (2), 326 (6), 284 (3), 271 (20), 211 (35), 169 (32), 168 (14), 160 (55), 159 (100), 152 (15), 145 (30).

$NaIO_4$ *degradation of glycerol–dialkyl–calditol tetraethers.* The glycerol–dialkyl–calditol tetraether mixture (**5**) (1 g) dissolved in $CHCl_3$—MeOH (1:1) (60 ml) was treated with 8% aq. $NaIO_4$ (10 ml) with stirring for 12 hr. Excess reagent was destroyed with glycerol and the oxidation products recovered by extraction with $CHCl_3$. This mixture, dried under N_2, was dissolved in $CHCl_3$—MeOH (3:7) (20 ml) and treated with excess $NaBH_4$ for 3 hr with stirring. Excess borohydride was destroyed with N HCl and the reduction products (920 mg), extracted with $CHCl_3$, were resolved by column chromatography. $CHCl_3$—MeOH (49:1) eluted **9** (370 mg) and

$CHCl_3$—MeOH (96:4) eluted **10** (350 mg). Products **9** (300 mg) and **10** (300 mg) were acetylated with Ac_2O (5 ml) and Py (0.5 ml) at reflux for 6 hr.

The acetylated derivative of **9** has TLC R_f 0.5, $CHCl_3$–MeOH (95:5), ν_m cm^{-1}: 1745, 1235, 1115; δ 4.55 (2H, ABq ($\delta A - \delta B = 18$ Hz, $J = 13$ Hz), —C<u>H</u>$_2$—OAc), 4.45 (2H, ABq ($\delta A - \delta B = 18$ Hz, $J = 13$ Hz) —C<u>H</u>$_2$—OAc), 4.15 (3H, complex *m*, glycerol C<u>H</u>$_2$—OAc plus C<u>H</u>—OAc), 3.5 (14 H, *br*, C<u>H</u>—O plus C<u>H</u>$_2$—O), 2.05 (15 H, overlapped *s*, C<u>H</u>$_3$CO), 0.7–1.8 (*ca* 140 aliphatic protons). The acetylated derivative of **10** has TLC R_f 0.45, $CHCl_3$–MeOH (19:1), ν_m cm^{-1}: 1745, 1235, 1115; δ 5.28 (2H, *br*, C<u>H</u>—OAc); 4.4 (2H, *o*, AB part of ABX system ($\delta A - \delta B = 28$ Hz, $J_{AB} = 13$, $J_{AX} = 6$, $J_{BX} = 4$ Hz), C<u>H</u>$_2$OAc), 4.15 (3H, *br*, glycerol-C<u>H</u>$_2$—OAc plus C<u>H</u>—OAc), 3.50 (14 H, *br*, C<u>H</u>—O plus C<u>H</u>$_2$O), 2.05 (15 H, overlapped *s*, C<u>H</u>$_3$—CO), 0.7–1.8 (*ca* 140 aliphatic protons).

Cleavage of **9** *and* **10** *with* BCl_3. **9** or **10** (300 mg) was treated with BCl_3 (5 ml in 5 ml $CHCl_3$ at 18° for 12 hr); the reaction mixture was evapd under N_2 and chromatographed. In both cases $CHCl_3$ eluted the C_{40} dichlorides (250 mg), $CHCl_3$—MeOH (7:3) eluted glycerol (15 mg) and $CHCl_3$—MeOH (3:2) eluted **11** or **12** (30 mg). Compound **11** gave the following MS fragmentation: *m/e* 149 ($M^+ - MeOH_2$, 78%), 133 (28), 103 (85), 85 (19), 74 (73), 73 (100), 61 (85), 57 (53), 56 (53), 43 (83). The MS of **12** was identical to that of **11**. **11** and **12** (30 mg) were separately acetylated with Ac_2O (2 ml) and Py (0.2 ml) for 6 hr at reflux. The fully acetylated product of **11** and **12** was purified by prep-TLC eluted with Et_2O–petrol (4:1) R_f, in both cases, 0.5.

Fully acetylated **11** has ν_m cm^{-1}: 1745, 1235, 1045; δ 5.12 (1H, complex *m*, C<u>H</u>OAc), 4.52 (2H, ABq ($\delta A - \delta B =$ 22 Hz, $J = 13$ Hz), >C—C<u>H</u>$_2$—OAc), 4.45 (2H, ABq ($\delta A - \delta B = 22$ Hz, $J = 13$ Hz), 4.18 (1H, *m*, C<u>H</u>OAc), 3.75 (2H, *o*, AB part of ABX system ($\delta A - \delta B = 24$ Hz, $J_{AB} = 13$, $J_{AX} = 6$, $J_{BX} = 4$ Hz), C<u>H</u>$_2$OAc), 2.05 (18H, overlapped *s*, C<u>H</u>$_3$—CO); *m/e* 419 ($M^+ - 15$, 1%) 405 (2), 375 ($M^+ - CH_3COO$, 2), 333 ($M^+ - (MeCOO + MeCO)$ 2), 303 ($M^+ - (MeCOO + MeCO + CH_2O)$ 5), 261 (43), 244 (3), 231 (15), 201 (3), 159 (100). Fully acetylated **12** has ν_m cm^{-1}: 1745, 1235, 1045; δ 5.15 (2H, complex, *m*, C<u>H</u>—OAc) 4.6–4 (4H, complex *m*, (C<u>H</u>$_2$OAc) + (2C<u>H</u>OAc)), 3.75 (2H, complex *m*, C<u>H</u>$_2$OAc), 2.05 (18H, overlapped *s*, C<u>H</u>$_3$—CO); *m/e* 419 ($M^+ - 15$, 1%) 405 (1), 375 (($M^+ - MeCOO$), 5), 333 ($M^+ - (MeCOO + MeCO)$ 33), 303 ($M^+ - (CH_3COO + CH_3CO + CH_2O)$ 13), 261 (8), 244 (1), 231 (3), 201 (5), 159 (100).

C_{40} *hydrocarbons*. Cleavage of the glycerol–dialkylcalditol tetraethers (**5**) with HI followed by $LiAlH_4$ reduction gave the mixed C_{40} hydrocarbons as previously described [9]. On GC–MS this mixture gave 3 peaks; $C_{40}H_{78}$ previously described [7]; $C_{40}H_{76}$ (**7**), *m/e* 556 (M^+, 1%), 528 (1), 459 (1), 458 (1), 457 (1), 391 (1), 390 (1), 389 (1), 388 (1) 363 (1), 362 (1), 361 (1), 360 (1), 291 (4), 195 (16), 194 (25), 193 (50), 192 (10), 166 (25), 165 (100), 164 (40), 163 (32); $C_{40}H_{74}$ (**8**), *m/e* 554 (M^+, 1%), 526 (1), 457 (1), 456 (1), 455 (1), 389 (1), 388 (1), 361 (2), 360 (1), 291 (2), 193 (45), 192 (11), 165 (100), 164 (25), 163 (35).

C_{40} *diacetates and diols*. The mixed di-iodides (2 g) were converted to the C_{40} diacetates and these were separated by prep-GLC as previously reported [7]. Three C_{40} diacetates were recovered, bicyclic (500 mg), tricyclic (270 mg) and tetracyclic (50 mg) (ν_m cm^{-1}; 1745, 1235, 1030 for all three). The bicyclic diacetate was identical to the bicyclic diacetate previously described [7] in the diglycerol tetraether. Tricyclic C_{40} diacetate (15), *m/e* 672 (M^+, 2%), 612 ($M^+ - HOAc$, 2), 583 ($M^+ - (HOAc + C_2H_5)$, 2) 552 ($M^+ - 2HOAc$, 2), 515 ($M^+ - (HOAc + C_7H_{13})$, 1), 513 ($M^+ - HOAc + C_7H_{15}$), 1), 455 ($M^+ - (2HOAc + C_7H_{13})$, 1), 453 ($M^+ - (2HOAc + C_7H_{15})$, 1), 291 (5), 223 (40), 221 (20), 165 (100), 163 (80); $\delta(C_6D_6)$ 3.96 (4H, two overlapped *t*, ($J = 7$ Hz), CH_2—C<u>H</u>$_2$-OAc), 1.92 (6H, *s*, C<u>H</u>$_3$—CO), 1.80 (*ca* 6H, *b*, ring C<u>H</u>), 1.55 (*ca* 5H, *b*, C<u>H</u>Me), 0.96 (6H, *d* ($J = 5.5$ Hz), CH—C<u>H</u>$_3$ (20, 20′)), 0.94 [6H, *d* ($J = 5.5$ Hz), CH—CH_3 (19, 19′)), 0.83 (3H, *d* ($J = 5.5$ Hz), CH—C<u>H</u>$_3$ (17′)). Tetracyclic C_{40} diacetate (**16**), *m/e* 670 (M^+, 2%), 610 ($M^+ - HOAc$, 2), 581 ($M^+ - (HOAc + C_2H_5)$, 3), 550 ($M^+ - HOAc$, 2), 513 ($M^+ - HOAc + C_7H_{13}$), 2), 453 ($M^+ - (2HOAc + C_7H_{13})$, 3), 291 (5), 221 (30), 163 (100); δ (C_6D_6): 3.95 (4H, *t* ($J = 7$ Hz), CH_2—C<u>H</u>$_2$—OAc), 1.92 (6H, *s*, C<u>H</u>$_3$CO), 1.8 (*ca*. 8H, *b*, ring C<u>H</u>), 1.55 (*ca* 4H, *b*, C<u>H</u>Me), 0.96 (6H, *d*, ($J = 5.5$ Hz), CH—C<u>H</u>$_3$ (20,20′)), 0.94 (6H, *d* ($J = 5.5$ Hz), CH—C<u>H</u>$_3$ (19,19′)). The separate diacetates were saponified (10% aq. KOH, 6 hr reflux); the hydrolysate was diluted with H_2O and extracted several times with Et_2O to afford, separately, the C_{40} diols **17** and **18**. For ^1H NMR and Eu shift data, see Results.

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