A New 15,16-Dimethyl-30-glyceryloxytriacontanoic Acid from Lipids of *Thermotoga maritima*

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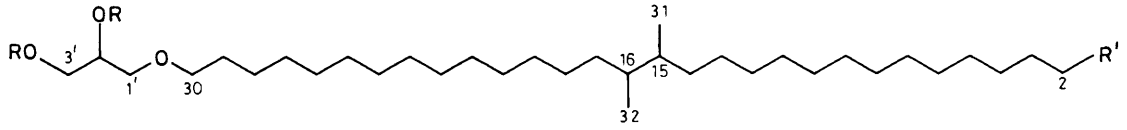
The lipids of the thermophilic anaerobic eubacterium *Thermotoga maritima* are mainly based on: (a) n-C_{14}, n-C_{16}, and n-C_{18} fatty acids; (b) 15,16-dimethyltriacontanedioic acid (diabolic acid); (c) a new compound identified by chemical and degradative evidence as 15,16-dimethyl-30-glyceryloxytriacontanoic acid.

Here we report data on lipid structures of *Thermotoga maritima*, a new anaerobic eubacterium growing at temperatures up to 90 °C, isolated from geothermally heated sea floors in Italy and the Azores.[1] Lyophilized cells afforded a 7–8% yield of lipids, which were refluxed with methanol/HCl (9 : 1) for 6 h. The $CHCl_3$-soluble fraction of the methanolysis mixture was chromatographed on a silica-gel column. Light petroleum/Et_2O (99 : 1) eluted a mixture of fatty acid methyl esters (*ca.* 36% of the $CHCl_3$-soluble fraction) identified by g.l.c. as methyl myristate (12%), palmitate (86%), and stearate (1%); light petroleum/Et_2O (98 : 2) eluted a long-chain dicarboxylic dimethyl ester (*ca.* 33% of the $CHCl_3$-soluble fraction); $CHCl_3$ eluted a novel compound (**1a**) (*ca.* 30%) and trace amounts of an identified compound; $CHCl_3$/MeOH (80 : 20) gave a more polar, and as yet unidentified compound (1%).

The long chain dicarboxylic dimethyl ester [R_f *ca.* 0.4 on t.l.c., silica gel in light petroleum/Et_2O (98 : 2)] was identified as dimethyl 15,16-dimethyltriacontanedioate (trivial name, diabolic acid) by a comparison of its physical properties {$[\alpha]_D$, ^1H and ^{13}C n.m.r. electron-impact mass spectrometry (E.I.M.S.)} with those reported in the literature.[2]

The new compound (**1a**) [R_f *ca.* 0.5 on t.l.c., silica gel in $CHCl_3$/MeOH (95 : 5)] was acetylated with Ac_2O/pyridine (9 : 1) at 60 °C overnight. The acetylated derivative (**1b**) [R_f 0.95 on t.l.c. silica gel, $CHCl_3$/MeOH (95 : 5)] was purified by silica gel column chromatography with $CHCl_3$ as eluant. The E.I.M.S. of diacetate (**1b**), besides M^+ at *m/z* 668 (0.1%), includes diagnostically important peaks at *m/z* 637 ($M^+ - CH_3O$; 3.1%), 609 ($M^+ - OCOCH_3$; 4.1), 608 ($M^+ - AcOH$; 4.1), 595 ($M^+ - CH_2OAc$; 0.9), 565 ($M^+ - AcOH$ and Ac; 7.2), 548 ($M^+ - 2AcOH$; 4.3), 535 ($M^+ - CH_2OAc$ and



(1)a; R = H, R' = CO_2Me
b; R = Ac, R' = CO_2Me
c; R = H, R' = CH_2OH

AcOH; 6.0), 523 (M^+ − CH(OAc)-CH_2OAc; 7.1), 493 [M^+ − $C_7H_{11}O_5$; C(30)–O cleavage; 3.9], 491 (M^+ − CH_3OH and CHOAc–CH_2OAc; 4.0), 479 [M^+− $C_8H_{13}O_5$; C(29)–C(30) cleavage; 1.7], 477 [M^+ − CH_3OH and $C_7H_{11}O_4$; C(1′)–O cleavage; 1.7], 461 [M^+ − CH_3OH and $C_7H_{11}O_5$; C(30)–O cleavage; 8.7], 339 [M^+ − AcOH and $C_{17}H_{33}O_2$; C(15)–C(16) cleavage; 32.9], 159 [loss of $C_{33}H_{65}O_3$; C(1′)–O cleavage; 100], thus localizing the ether linkage at the 1′-position of glycerol and *vic*-dimethyl branching at the 15-16 position of the C_{30} aliphatic chain.

The ^1H n.m.r. spectrum ($CDCl_3$) of (**1a**) shows in addition to the CH_3O signal (δ 3.67) significant resonances at δ 3.88 (1 H, br.m, H-2′), 3.72 (2 H, br.d, H-3′), 3.53 (2H, d, H-1′), 3.46 (2 H, t, H-30), 2.30 (2 H, t, H-2), 0.73 (6 H, d, H-31 and H-32). The ^1H n.m.r. spectrum of (**1b**) shows singlets due to two acetyl groups at δ 2.06 and 2.08, a doublet at δ 3.54 due to the 1′-protons, and an ABX system centred at δ 4.25 and 5.18 due to the 3′ and 2′ protons, respectively.

The ^{13}C n.m.r. data of (**1a**) fully confirm the 15,16-dimethyl-30-glyceryloxytriacontanoic acid structure. In particular the steric compression shift of methyl resonance to higher field (^{13}C n.m.r.: δ 14.5), also observed for diabolic acid,[2] indicates a higher probability of *gauche* environment for these methyl groups. Further details on *vic*-dimethyl branching were obtained by chemical degradation of (**1a**). In this procedure (**1a**) was reduced to (**1c**) with $NaAlH_2(OCH_2CH_2OCH_3)_2$[3] and then converted by HI/$LiAlH_4$ degradation[4] in the parent C_{32} hydrocarbon. This was identified as 15,16-dimethyltriacontane by comparison with E.I.M.S. data previously reported.[2]

Full characterization of complex lipids is in progress.

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