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A mixed ladderane/*n*-alkyl glycerol diether membrane lipid in an anaerobic ammonium-oxidizing bacterium

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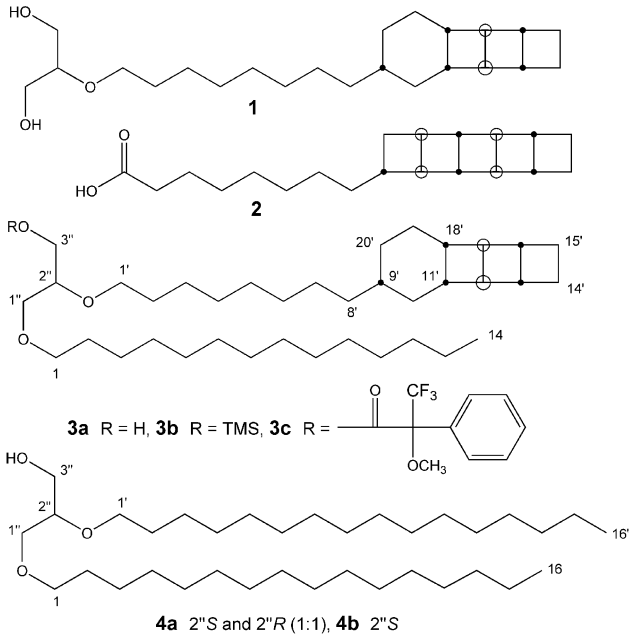
A novel glycerol diether containing ladderane and tetradecyl moieties has been identified in an anaerobic ammonium-oxidizing bacterium by GC/MS and high-field NMR spectroscopy.

Recently, we have reported on the occurrence of novel, so-called ladderane membrane lipids (*e.g.* **1** and **2**) in bacteria capable of anaerobic ammonium oxidation (anammox) belonging to the order of the *Planctomycetales*.[1] These unique natural products are intriguing from a chemical point of view since they are composed of 3–5 linearly concatenated cyclobutane rings, forming highly strained moieties. In the anammox bacteria they fulfil a role as lipids of the membrane of the intracellular organelle, the anammoxosome. In this cell compartment, the anaerobic oxidation of ammonium with nitrite takes place with hydrazine as the mutagenic intermediate.[2] Membranes composed of ladderane lipids are much more dense than conventional membranes and are, therefore, thought to be used to contain hydrazine within the anammoxosome.[1] Here, we report on a new type of ladderane biolipid, which is biosynthesized by a novel genus of anammox bacteria (*Scalindua* sp.) occurring in the biomass of an oxygen-limited wastewater treatment reactor.



Lipid analysis, by GC/MS, of the biomass of a wastewater treatment plant in Pitsea (UK), in which the microbial community was comprised for 20% of two species belonging to a newly proposed genus of anammox bacteria, revealed the presence in relatively high amounts of a dialkyl glycerol diether **3a**, containing one [3]-ladderane moiety.[3] Its mass spectrum (as TMS ether derivative, **3b**)† revealed a molecular weight of 632, fragments for a dialkyl glycerol ether (*m*/*z* 130, 131, 133)[4] and characteristic fragment ions (*m*/*z* 273, 289, 315, 404, 405) also observed in the mass spectrum of the [3]-ladderane moiety-containing 2-alkyl glycerol monoether **1**. In combination with the molecular weight this indicated the presence of an ether-bound $C_{14}H_{29}$ moiety and an overall formula of $C_{37}H_{68}O_3$.

The structural assignment was confirmed by high-field NMR‡ of **3a**, which was isolated (0.5 mg; 85% pure by GC) from a large batch of biomass (*ca.* 30 g dry weight) from the reactor by a combination of column chromatography using alumina and silica, preparative thin layer chromatography and preparative HPLC. Its proton spectrum was quite similar to that of **1**,[1] revealing the rather characteristic signals at 1.8–2.8 ppm of the protons of the linearly concatenated cyclobutane moieties and the signals at 3.4–3.8 ppm of the dialkyl diether glycerol moiety, which were virtually identical to those observed for the commercially available 1,2-di-*O*-dihexadecyl-*rac*-glycerol (**4a**)§. Both spectra also contain a triplet at 0.88 ppm, arising from the terminal carbon atom of the *n*-alkyl chain(s). The ^{13}C NMR spectrum of **3a** is consistent with its proposed structure; all signals have almost identical chemical shifts and multiplicities to those of the corresponding carbon atoms in the structurally related components **1**[1] and **4a**§. This experiment confirmed that the ether-bound alkyl moiety of **3a** is a tetradecyl moiety.

The stereochemistry of C-2″ of the glycerol unit was elucidated by ^{19}F NMR spectroscopy of the Mosher esters[5] of the mixed ladderane/alkyl glycerol diether (**3a**) and of 1,2-di-*O*-dihexadecyl-*rac*-glycerol (**4a**) and 1,2-di-*O*-dihexadecyl-*sn*-glycerol (**4b**). Reaction of the *S*- and *R*-Mosher acid chlorides with glycerol diether **3a** gave signals at similar chemical shifts for the CF_3 group as with 1,2-*O*-dihexadecyl-*sn*-glycerol (Table 1). Since it is unlikely that the [3]-ladderane moiety in **3c** will affect the resonance of the CF_3 group of the Mosher derivative, it is likely that the stereochemistry of C-2″ of the glycerol unit of **3c** is the same as that of **4b**, namely 2″*S*, in line with the general stereochemistry of bacterial membrane lipids. The experiment also establishes that **3a** occurs as one enantiomer, as would be expected for a natural product produced by enzymatic reactions.

We have, thus, established a new type of bacterial glycerol diether containing an uncommon ladderane moiety. In contrast to the ladderane lipids reported previously,[1] **3a** combines the unique characteristics of anammox membrane lipids (*i.e.* the ladderane moiety) with much more common traits of bacterial membranes (*i.e. n*-alkyl chains). However, glycerol dialkyl diether membrane lipids are rather unique bacterial products[1,6] and the role this new type of ladderane lipid plays in the physiology of the anammox reaction catalyzed by the *Scalindua* sp. remains to be investigated and has to await cultivation of this novel species of anammox bacteria.

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Table 1 Chemical shift (in ppm relative to $CFCl_3$) of the CF_3 group of the Mosher products of the reaction of diethers **3a**, **4a** and **4b** with the *S*- and *R*-Mosher acid chlorides

	3a	**4a**	**4b**
S-Mosher acid chloride	−73.166 (100%)	−73.106 (50%), −73.170 (50%)	−73.170 (97%), −73.106 (3%)[b]
R-Mosher acid chloride	−73.107 (100%)	n.d.[a]	−73.106 (97%), −73.172 (3%)[b]

[a] n.d. = not determined. [b] Caused by a minor impurity in the commercially available **4b**.

Notes and references

† Mass spectral data for **3b** (EI 70 eV): 632 (M^+, 10%), 405 (8%), 404 (8%), 315 (55%), 289 (9%), 285 (16%), 273 (20%), 133 (43%), 131 (100%), 130 (68%), 117 (50%), 103 (68%).

‡ NMR data for **3a** at 600 MHz: ^{1}H in $CDCl_3$, COSY. δ 3.72 (m, 1 H, H-3″), 3.63 (m, 1 H, H′-3″), 3.61 (dt, J = 9.5 & 6.3 Hz, 1 H, H-1′), 3.53 (t, J = 8.6 Hz, 1 H, H-1″), 3.52 (bd, J = 9.3 Hz, 1 H, H′-1′), 3.51 (m, 1 H, H-2″), 3.47 (dd, J = 8.6 & 4.8 Hz, 1 H, H′-1″), 3.44 (m, 2 H, H-1), 2.74 (bd, J = 7.1 Hz, 1 H, H-13′), 2.64 (m, 1 H, H-16′), 2.51 (m, 1 H, H-14′), 2.44 (bs, 1 H, H-17), 2.42 (m, 1 H, H-15′), 2.31 (bd, 1 H, H-12′), 2.29 (m, 1 H, H-18′), 2.22 (m, 1 H, H-11′), 1.96 (dddd, 1 H, H′-15′), 1.86 (dddd, 1 H, H′-14′), 1.76 (m, 1 H, H-10′), 1.58 (m, 2 H, H-2), 1.56 (m, 2 H, H-2′), 1.52 (m, 1 H, H-20′), 1.51 (m, 2 H), 1.30–1.20 (other H's), 1.11 (m, 1 H, H′-20′), 1.04 (m, 1 H, H′-10′), 0.88 (t, 3 H, H-14). ^{13}C (150 MHz), HSQC: δ 78.5 (C-2″), 72.1 (C-1), 71.2 (C-1″), 70.7 (C-1′), 63.5 (C-3″), 49.7 (C-12′), 47.8 (C-17′), 42.6 (C-16′), 41.9 (C-13′), 38.5 (C-8′), 38.3 (C-11′), 38.1 (C-18′), 34.4 (C-10′), 32.8 (C-9′), 32.2 (C-12), 30.5 (C-2), 30.0 (C-2′), ~29.7 (all other C's), 28.5 (C-20′), 27.1 (C-6), 26.4 (C-3, C-3′, C-14′), 25.8 (C-15′, C-19′), 23.1 (C-13), 14.4 (C-14).

§ NMR data for **4a** at 500 MHz: ^{1}H in $CDCl_3$ δ 3.72 (m, 1 H, H-3″), 3.63 (m, 1 H, H′-3″), 3.61 (dt, J = 9.3 & 6.7 Hz, 1 H, H-1′), 3.44 (m, 2 H, H-1), 2.18 (bt, 1 H, OH), 1.58 (m, 2 H, H-2′), 1.56 (m, 2 H, H-2), 1.26 (m, remaining H), 0.88 (t, J = 7.0 Hz, 6 H, H-16, H-16′). ^{13}C (125 MHz), APT: δ 78.38 (C-2″), 71.78 (C-1), 71.10 (C-1″), 70.41 (C-1′), 63.06 (C-3″), 31.92 (C-14, C-14′), 30.07 (C-2 or C-2′), ~29.7 (all other C's), 29.47 (C-2′ or C-2), 26.09 (C-3, C-3′), 22.68 (C-15, C-15′), 14.10 (C-16, C-16′).

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