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A dialkylcyclohexadienecarbinol from the bacterium *Zymomonas mobilis*, a novel type of potential membrane lipid

Anna-Irini Koukkou,* **Eugenia Douka and Constantin Drainas**

University of Ioannina, Department of Chemistry, 45110 Ioannina, Greece

Catherine Pale-Grosdemange and Michel Rohmer

Université Louis Pasteur/CNRS, Institut Le Bel, 4 rue Blaise Pascal, 67070 Strasbourg Cedex, France

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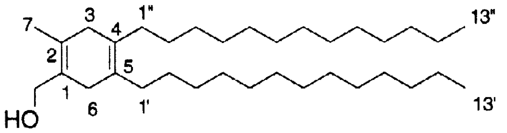
Abstract. (2-methyl-4,5-ditridecyl-1,4-cyclohexadienyl)methanol has been isolated from the lipidic fraction of the ethanologenic bacterium *Zymomonas mobilis*. The amphiphilic feature of this novel lipid is compatible with interactions within a phospholipid bilayers and suggests a function in membrane metabolism of the bacterium. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Zymomonas mobilis is an efficient ethanologenic Gram-negative bacterium, converting in anaerobic conditions glucose into ethanol with high yields. This bacterium is capable of tolerating high ethanol concentrations (up to 13%, w/v) as well as high sugar concentrations (currently 20% and even 30-40% for most strains).[1] Membrane lipids are most probably involved in these unusual features. Indeed, *Z. mobilis* is the best hopanoid producer amongst bacteria.[2a-d] These membrane stabilizing triterpenoids[3a-c] are present in such high concentrations (up to 30 mg/g, freeze-dried cells, *i.e.* one order of magnitude higher than in other hopanoid producing bacteria) that they are thought to be able to counterbalance the destabilizing action of ethanol, whatever its concentration in the culture medium.[2c] In a search for a better understanding of this unusual tolerance towards membrane unfriendly growth conditions, a screening of all lipid compounds, including the minor ones, was started in this bacterium, allowing the detection of (2-methyl-4,5-ditridecyl-1,4-cyclohexadienyl)methanol.[4]



The ^{1}H-NMR spectrum of this novel compound showed signals due to two primary methyl groups at δ 0.87 (t, *J* 7.7 Hz), 22 aliphatic methylene groups at δ 1.25 (broad s) and two methylene groups at δ 1.68 (quintuplet, *J* 7.7 Hz) and 1.69 (quintuplet, *J* 7.7 Hz), indicating the presence of two aliphatic chains. Further, two methylene groups at δ 2.61 and 2.77 appeared as two pseudo-triplets (*J* 7.7 Hz). Their chemical shifts corresponded to those of methylene protons of a cyclohexa-1,4-diene.[5] The ^{1}H-NMR spectrum showed also an olefinic methyl singlet (δ 2.53) and a doublet for methylene protons on a carbon bearing an OH group (δ 4.71, *J* 4.5 Hz), indicating coupling to only an hydroxyl proton (δ 4.46, t, *J* 4.5 Hz). The presence of the hydroxy group was confirmed by deuterium exchange and by the band at 3433 cm^{-1} on the IR spectrum. In the ^{1}H/^{1}H COSY spectrum, the protons of the two cyclohexadiene methylene groups at δ 2.61 and 2.67 were

Fax: +30 (651) 47832; e-mail: akukku@cc.uoi.gr

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only coupled with the methylene groups of the aliphatic chains, respectively at δ 1.68 and 1.69. Furthermore the carbon atoms found in a DEPT experiment corresponded to three methyl, 27 methylene and four quaternary carbons. On the MS spectrum, the base peak at *m/z* 320 corresponded to a McLafferty rearrangement with elimination of a neutral olefin molecule ($M^{+\cdot}$- $C_{12}H_{24}$). This unique signal indicated the presence of two identical tridecyl chains on the cyclohexadiene double-bond(s). Moreover, a fragment at *m/z* 457 corresponded to $M^{+\cdot}$- CH_2OH. From these data and those of the ^{13}C NMR spectrum,[6] the structure of the novel lipid was suggested to be a cyclohexa-1,4-diene substituted on the quaternary carbons by one methyl group, one CH_2OH group and two tridecyl chains. In order to confirm the localisation of the two alkyl chains, long-range heteronuclear multiple bond (HMBC) $^1H/^{13}C$ correlations were performed. The HMBC spectrum showed long-range couplings between the methylene proton of the CH_2OH group with carbons C-1, C-2, C-5 and C-7 and between the C-7 methyl protons with carbons C-1, C-2, C-4 and CH_2OH indicating that the two tridecyl chains were located at C-5 and C-4, and that the novel compound was (2-methyl-4,5-ditridecyl-1,4-cyclohexadienyl)methanol.

The dialkylcyclohexyl carbinol from *Z. mobilis* is an amphiphilic compound. Its structural features are compatible with interactions within the phospholid bilayer of a biological membrane. Indeed, this compound mimics a monocyclic cholesterol analogue.[7] i) The primary hydroxy group is capable of forming hydrogen bonds with the aqueous phase outside the membrane or with the hydrophilic membrane surface (*e.g.* polar heads of phospholipids, polar carbohydrate derived moieties of hopanoids). ii) The C_{13} alkyl chain are capable of van der Waals interactions with the hydrophobic fatty acid chains from phospholipids. iii) The average length of this lipid is similar to those of usual fatty acids and of the ω-cyclohexyl fatty acid chains found in the *Alicyclobacillus* spp. hopanoids[8] allowing its insertion in the phospholipid bilayer. The lipid identified in the present report (125 μg/g, lyophilized cells) has been completely overlooked in previous studies on *Z. mobilis* lipids.[9] It remains to check now whether such a compound, or similar ones, can be found in other bacteria, to study its effect on phospholipid membranes and to elucidate its physiological role.

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References and Notes

1. Bringer-Meyer, S.; Sahm, H., *FEMS Microbiol. Rev.*, **1988**, *54*, 131-142.
2. (a) Rohmer, M.; Bouvier-Navé, P.; Ourisson, G., *Biochem. J.*, **1984**, *130*, 1137-1150. (b) Flesch, G.; Rohmer, M., *Biochem. J.*, **1989**, *262*, 673-675. (c) Sutter, B., *Ph D Thesis*, Université de Haute Alsace, Mulhouse, France, **1991**. (d) Hermans, M.A.F.; Neuss, B.; Sahm, H., *J. Bacteriol.*, **1991**, *173*, 5592-5595.
3. (a) Rohmer, M.; Bouvier, P.; Ourisson, G., *Proc. Natl. Acad. Sci. USA*, **1979**, *76*, 847-851. (b) Ourisson, G.; Rohmer, M.; Poralla, K., *Annu. Rev. Microbiol.*, **1987**, *41*, 301-333. (c) Ourisson, G.; Rohmer, M., *Acc. Chem. Res.*, **1992**, *25*, 403-407, and references cited therein.
4. (a) *Z. mobilis* ATCC 10988 was grown as previously described and harvested during the stationary phase of growth.[4b] Freeze-dried cells (40g, from a 120 l culture) were extracted with $CHCl_3/CH_3OH$ (2:1).[2a] The non-polar lipids were isolated by chromatography on a silica gel column (CH_2Cl_2). They were further separated by TLC on silica gel (hexane/ether/acetic acid, 7:3:1). The fraction with R_f 0.32 was scrapped off, recovered from silica gel using CH_2Cl_2, and a final TLC (CH_2Cl_2, R_f=0.27, 5 mg) yielded the novel compound. (b) Galani, I.; Drainas, C.; Typas, M.A., *Biotech. Lett.*, **1985**, *9*, 673-678.
5. NMR-Spektroskopie. Eine Einführung in die Protonenresonanz-Spektroskopie und ihre Anwendungen in der Chemie. H. Günther, Georg Thieme Verlag, Stuttgart, New York, **1983**, p. 317.
6. The ^1H and ^{13}C NMR assignments were supported by supplementary experiments including DEPT and $^1H/^1H$ COSY and $^1H/^{13}C$ HMBC correlations. ^1H-NMR (500 MHz, $CDCl_3$): δ = 0.87 (6H, t, *J* 7.7, 13' and 13"-H_3), 1.25 (22H, broad s, 2' to 12' and 2" to 12"- H_2), 1.68 (2H, quintuplet, *J* 7.7, 1"-H_2), 1.69 (2H, quintuplet, *J* 7.7, 1'-H_2), 2.53 (3H, s, 7-H_3), 2.61 (2H, t, *J* 7.7, 6-H_2), 2.77 (2H, t, *J* 7.7, 3-H_2), 4.46 (1H, t, *J* 4.5, OH), 4.71 (2H, d, *J* 4.5, CH_2OH). ^{13}C-NMR (50 MHz, $CDCl_3$): δ = 14.17 (C-13' and 13"), 21.11 (C-7), 22.74 to 29.73 (C-1' to C-11' and C-1" to C-11"), 31.97 (C-12' and 12"), 32.87 (C-6), 34.18 (C-3), 60.53 (CH_2OH), 146.94 (C-1), 149.29 (C-2), 150.38 (C-5), 150.80 (C-4). GC-MS (70 eV): *m/z* (%) = 488 (M^+, 10), 473 (M^+ - CH_3, 2), 457 (M^+ - CH_2OH, 15), 320 (McLafferty rearrangement, 100), 289 (18), 151 (5). IR ($CHCl_3$): $\nu_{max} = 3433\ cm^{-1}$.
7. Demel, R.A.; de Kruyff, B., *Biochim. Biophys. Acta*, **1976**, *457*, 109-132.
8. Poralla, K.; Kannenberg, E.; Blume, A., *FEBS Lett.*, **1980**, *113*, 107-110.
9. (a) Tornabene, T.G.; Holzer, G.; Bittner, A.S.; Grohmann, K., *Can. J. Microbiol.*, **1982**, *28*, 1107-1118. (b) Barrow, K.D.; Collins, G.J.; Rogers, P.L.; Smith, G.M., *Biochim. Biophys. Acta*, **1983**, *753*, 324-330.