Tetrahymanol from the phototrophic bacterium *Rhodopseudomonas palustris*: first report of a gammacerane triterpene from a prokaryote

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Tetrahymanol, a rare triterpene from the gammacerane series, previously detected only in a few scattered eukaryotic taxa, has been isolated for the first time from the phototrophic bacterium *Rhodopseudomonas palustris*. This extends the significance of geochemical markers from this series, which are abundant in marine sediments and palaeohypersaline environments.

Introduction

Rhodopseudomonas palustris is capable of synthesizing triterpenoids of the hopane series, a widespread family of prokaryotic membrane constituents (Rohmer *et al.*, 1984). Thus diploptene (II), diplopterol (III) and aminobacteriohopanetriol (IV) (Fig. 1) have been reported as major triterpenoids from this bacterium (Rohmer *et al.*, 1984; Neunlist *et al.*, 1988; Neunlist & Rohmer, 1988; Flesch & Rohmer, 1988). In this communication, we report now the isolation and identification from this bacterium of tetrahymanol (I), a

compound biogenetically related to the hopanoids. This rare triterpene has previously been found in a few scattered eukaryotic taxa, but is widespread as molecular fossil in marine sediments and palaeohypersaline environments (ten Haven *et al.*, 1989, and references therein).

Methods

Analytical methods and growth of *Rhodopseudomonas palustris* DSM 123 were as previously described (Rohmer *et al.*, 1984; Neunlist *et al.*, 1988). NMR spectra were recorded in CDCl₃ solution on a Bruker W400 spectrometer using CHCl₃ (δ =7.260 p.p.m.) as internal

Fig. 1. Triterpenoids from *Rhodopseudomonas palustris*: I, tetrahymanol; II, diploptene; III, diplopterol; IV, aminobacteriohopanetriol.

standard for ^1H -NMR and CDCl_3 ($\delta=77.0$ p.p.m.) for ^{13}C -NMR. Freeze-dried cells (15 g) were extracted with cyclohexane under reflux (5×500 ml, 1 h). Combined extracts were evaporated to dryness and separated by TLC using methylene chloride as solvent system (two migrations). Two bands corresponding to diplopterol (II) ($R_F = 0.25$) and tetrahymanol (I) ($R_F = 0.20$, 0.4 mg per g freeze-dried cells) were scraped off and studied further. Tetrahymanol was acetylated (Rohmer *et al.*, 1984), and its acetate purified by TLC using cyclohexane/ethyl acetate (98:2, v/v) as solvent system, in which $R_F = 0.25$. Tetrahymanone was obtained by CrO_3 /pyridine oxidation in methylene chloride (Ratcliffe & Rodehorst, 1970) and separated by TLC using methylene chloride as eluent, in which $R_F = 0.35$.

Analytical Data

Tetrahymanol

^1H -NMR (400 MHz), δ (p.p.m.): 0.757 (3H, s, 29-H), 0.787 (3H, s, 24-H), 0.809 (6H, s, 25- and 28-H), 0.841 (3H, s, 23-H), 0.951 and 0.959 ($2 \times 3\text{H}$, 2s, 26- and 27-H), 0.967 (3H, s, 30-H), 3.19 (1H, dd, J 5.0 Hz, 11.5 Hz, 21 β -H).

^{13}C -NMR (100 MHz), δ (p.p.m.): 15.4 (C-29), 16.0 (C-25, C-28), 16.5 (C-26, C-27), 18.4 (C-11), 18.7 (C-2, C-6), 21.1 and 21.3 (C-12, C-16), 21.6 (C-24), 27.4 (C-20), 28.0 (C-30), 33.1 (C-7, C-17), 33.3 (C-4), 33.4 (C-23), 37.1 (C-18), 37.3 (C-10), 38.7 (C-19), 38.9 (C-22), 40.3 (C-11), 41.7 and 41.9 (C-8, C-14), 42.1 (C-3), 50.2 (C-13), 50.3 (C-9), 55.1 (C-17), 56.2 (C-5), 79.1 (C-21). Multiplicity of the signals was determined by DEPT (distortionless enhancement by polarization transfer) experiments. Carbon atoms are numbered as those of the hopane skeleton.

Tetrahymanol acetate

Mass spectrum (GC-MS), m/z (%): 470 (M^+ , 20%), 455 (M^+-CH_3 , 4%), 410 (M^+-AcOH , 11%), 395 ($\text{M}^+-\text{AcOH}-\text{CH}_3$, 8%), 367 (2%), 249 (ring C cleavage, 15%), 191 (ring C cleavage, 100%), 189 (249-AcOH, 49%).

Tetrahymanone

Mass spectrum (GC/MS), m/z (%): 426 (M^+ , 29%), 411 (M^+-CH_3 , 13%), 370 (1%), 232 (5%), 205 (ring C cleavage, 31%), 191 (ring C cleavage, 100%).

Results and Discussion

Tetrahymanol (I) isolated from *R. palustris* was identified by direct comparison of its analytical data (^1H -NMR, ^{13}C -NMR of the alcohol, GC and GC-MS of the alcohol, its acetate and the corresponding ketone) with those of reference compounds previously isolated from the ciliate *Tetrahymena pyriformis* (Bouvier *et al.*, 1980; Raederstorff & Rohmer, 1988) or synthesized from tetrahymanol, and with those reported in the literature (Wittstruck & Caspi, 1977; Wilkins *et al.*, 1987). This compound is identical with paluol, a long-unidentified triterpene isolated from the same bacterium by Liaaen-Jensen (1962) and for which all ancient data have been

now completed with ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopy.

This is the first report of the occurrence of a gammacerane-type triterpene in a prokaryote. Phylogenetically, tetrahymanol and hopanoids are closely related. They can be considered as primitive triterpenoids, since their biosynthesis does not require molecular oxygen and both series derive from a direct proton-induced, enzyme-catalysed cyclization of squalene. All other triterpene series found in eukaryotes and bearing an oxygenated function at C-3 derive from the cyclization of (3S)-squalene oxide, and their biosynthesis requires molecular oxygen and more complex enzymic reactions, often including transpositions (Rohmer *et al.*, 1979; Ourisson *et al.*, 1987, and references therein).

Until now tetrahymanol has been found only in ciliates of the genus *Tetrahymena* (and often together with small amounts of its isomer from the hopane series, diplopterol) (Mallory *et al.*, 1963; Elliott, 1973), in the fern *Oleandra wallichii* (Zander *et al.*, 1969) and in cultures of the anaerobic rumen fungus *Piromonas communis* (Kemp *et al.*, 1984). However, the abundance and ubiquity of gammacerane and tetrahymanol in marine sediments and palaeohypersaline environments led us to postulate that tetrahymanol should be far more widespread in living organisms (ten Haven *et al.*, 1989, and references therein). Thus the discovery of tetrahymanol in *Rhodospseudomonas palustris* extends the significance of the gammacerane series as molecular fossil biomarkers, and might be the first record of a long list of prokaryotes capable of synthesizing such compounds.

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References

- BOUVIER, P., BERGER, Y., ROHMER, M. & OURISSON, G. (1980). Non-specific biosynthesis of gammacerane derivatives by a cell-free system from the protozoan *Tetrahymena pyriformis*. *European Journal of Biochemistry* **112**, 549–556.
- ELLIOTT, A. M. (1973). Life cycle and distribution of *Tetrahymena*. In *Biology of Tetrahymena*, pp. 256–286. Edited by A. M. Elliott. Stroudsburg: Dowden, Hutchinson & Ross.
- FLESCHE, G. & ROHMER, M. (1988). Prokaryotic hopanoids: the biosynthesis of the bacteriohopane skeleton. Formation of isoprenic units from two distinct acetate pools and a novel type of carbon/carbon linkage between a triterpene and D-ribose. *European Journal of Biochemistry* **175**, 405–411.
- TEN HAVEN, H. L., ROHMER, M., RULLKÖTTER, J. & BISSERET, P. (1989). Tetrahymanol, the most likely precursor of gammacerane, occurs ubiquitously in marine sediments. *Geochimica et Cosmochimica Acta* **53**, 3073–3079.

- KEMP, P., LANDER, D. J. & ORPIN, C. G. (1984). The lipids of the rumen fungus *Piromonas communis*. *Journal of General Microbiology* **130**, 27–37.
- LIAAEN-JENSEN, S. (1962). The constitution of some bacterial carotenoids and their bearing on biosynthetic problems. *Det Kongelige Norske Videnskabers Selskabs Skrifter* **8**, 180–182.
- MALLORY, F. B., GORDON, J. T. & CONNER, R. L. (1963). The isolation of a pentacyclic triterpenoid alcohol from a protozoon. *Journal of the American Chemical Society* **85**, 1362–1363.
- NEUNLIST, S. & ROHMER, M. (1988). A convenient route to an acetylenic C₃₅ hopanoid and the absolute configuration of the side-chain of aminobacteriohopanetriol. *Journal of the Chemical Society, Chemical Communications* 830–832.
- NEUNLIST, S., BISSERET, P. & ROHMER, M. (1988). The hopanoids of the purple non-sulfur bacteria *Rhodopseudomonas palustris* and *Rhodopseudomonas acidophila* and the absolute configuration of bacteriohopanetetrol. *European Journal of Biochemistry* **171**, 242–252.
- OURISSON, G., ROHMER, M. & PORALLA, K. (1987). Prokaryotic hopanoids and other sterol surrogates. *Annual Review of Microbiology* **41**, 301–333.
- RAEDERSTORFF, D. & ROHMER, M. (1988). Polyterpenoids as cholesterol and tetrahymanol surrogates in the ciliate *Tetrahymena pyriformis*. *Biochimica et Biophysica Acta* **960**, 190–199.
- RATCLIFFE, R. & RODEHORST, R. (1970). Improved procedure for oxidations with chromium trioxide–pyridinium complex. *Journal of Organic Chemistry* **35**, 4000–4001.
- ROHMER, M., BOUVIER, P. & OURISSON, G. (1979). Molecular evolution of biomembranes: structural equivalents and phylogenetic precursors of sterols. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 847–851.
- ROHMER, M., BOUVIER-NAVE, P. & OURISSON, G. (1984). Distribution of hopanoid triterpenes in prokaryotes. *Journal of General Microbiology* **130**, 1137–1157.
- WILKINS, A. L., BIRD, P. W. & JAGER, P. M. (1987). Carbon-13 NMR of some triterpenes of the hopane group. *Magnetic Resonance in Chemistry* **25**, 503–507.
- WITTSTRUCK, T. A. & CASPI, E. (1977). Assignment of the methyl resonances in the ¹H n.m.r. of tetrahymanol and tetrahymanone. *Journal of Chemical Research (S)*, 180–181.
- ZANDER, J. M., CASPI, E., PANDEY, G. N. & MITRA, C. R. (1969). The presence of tetrahymanol in *Oleandra wallichii*. *Phytochemistry* **8**, 2265–2267.