Biochem. J. (1985) 231, 635–639 (Printed in Great Britain)

Novel hopanoids from the methylotrophic bacteria *Methylococcus capsulatus* and *Methylomonas methanica*

(22*S*)-35-aminobacteriohopane-30,31,32,33,34-pentol and (22*S*)-35-amino-3β-methylbacteriohopane-30,31,32,33,34-pentol

Serge NEUNLIST and Michel ROHMER*
École Nationale Supérieure de Chime de Mulhouse, 3 rue Alfred Werner, 68093 Mulhouse Cedex, France

The major hopanoid of the methylotrophic bacteria *Methylococcus capsulatus* and *Methylomonas methanica* was identified by spectroscopic methods as (22*S*)-35-aminobacteriohopane-30,31,32,33,34-pentol. Minor companions were, in both bacteria, 35-aminobacteriohopane-31,32,33,34-tetrol and in *Methylomonas methanica*, 35-aminobacteriohopane-32,33,34-triol. In *Methylococcus capsulatus* the aminopentol and the aminotetrol were accompanied by their homologues possessing an extra methyl group at C-3. Bacterial hopanoids with a functionalized C-30 carbon atom such as these two new aminopentols are possible precursors of widespread C_{29} hopanoid chemical fossils.

Hopanoids are typical prokaryotic triterpenoids [1,2]. The most widespread members of this family are derived from the C_{35} bacteriohopane skeleton formed by the C_{30} pentacyclic nucleus linked to a C_5 n-alkyl polyhydroxylated unit [2]. These bacteriohopanepolyols are very often linked through the C-35 hydroxy group to various polar moieties [3,4]. This C-35 hydroxy group is replaced in some bacteria, such as *Rhodomicrobium vannielli* or *Methylosinus trichosporium*, by an amino group [5,6]. Since most of the analysed methylotrophs are good hopanoid producers [2], we have completed our investigation of this group and determined the structures of the native hopanoids from *Methylococcus capsulatus* and *Methylomonas methanica*. In a preliminary report we have shown that our usual side-chain-degradation procedure involving H_5IO_6 cleavage followed by $NaBH_4$ reduction [2] released, in the case of *Methylococcus capsulatus* and several *Methylomonas* species, mainly hopanoids with a C_3 side chain: (22*S*)-hopan-29-ol and (22*S*)-3β-methylhopan-29-ol [7]. Obtaining these reaction products is rather unusual since in most cases C_{32} alcohols are obtained as major products by this procedure [2]. We describe in this paper the full structure of the hopanoids from which they arise: (22*S*)-35-aminobacteriohopane-30,31,32,33,34-pentol and (22*S*)-35-amino-3β-methylbacteriophane-30,31,32,33,34-pentol.

MATERIALS AND METHODS

General methods

The analytical procedures have been described previously [5]. Attribution of the ^{1}H-n.m.r. signals was confirmed by decoupling experiments and two dimensional proton/proton correlation (c.o.s.y.). Tentative assignment of the ^{13}C-n.m.r. signals was obtained by comparison with published data [8,9] and with spectra of bacteriophane derivatives isolated in our laboratory [3,5,6] and by utilization of a J-modulated spin-echo technique (MULT).

Methylococcus capsulatus (N.C.I.B. 11132) and *Methylomonas methanica* (N.C.I.B. 11130) were grown on methane as described previously [10]. The chloroform/methanol extract of the freeze-dried cells (2–5 g, depending on the experiment) was acetylated and separated by flash chromatography with ethyl acetate as eluent [5,11]. The fractions containing hopanoids were combined and further separated by t.l.c. (chloroform/methanol, 19:1, v/v) giving, in the case of *Methylococcus capsulatus*, a mixture of peracetylated hopanoids (I–IV) (R_F 0.50, 5.5 mg/g dry wt.). In one experiment when larger amounts of the hexa-acetates of (I) (Fig. 1) and (II) were prepared, a penta-acetate of (I) and (II) with a free hydroxy group at C-30 was obtained (R_F 0.40). Peracetylated hopanoids (I–IV, Fig. 1) were separated one from each other by reverse-phase h.p.l.c. on a Waters C_{18} μ-Bondapak column (3.9 mm × 300 mm) using methanol/water (92:8, v/v, 1.5 ml/min) as eluent and giving peracetylated (I) (3.2 mg/g), (II) (1.5 mg/g), (III) (0.4 mg/g) and (IV) (0.3 mg/g). In the case of *Methylomonas methanica* the mixture of peracetylated hopanoids [(I), (III) and (V), Fig. 1] obtained by t.l.c. was also separated by h.p.l.c. giving pure peracetylated (I) (2.5 mg/g), (III) (1.1 mg/g) and (V) (0.2 mg/g).

Hexa-acetate of (22*S*)-35-aminobacteriohopane-30,31,32,33,34-pentol (I)

The ^{1}H-n.m.r. [400 MHz, (^{2}H)chloroform] characteristics were as follows: δ (p.p.m.) = 0.643 (3H, s, 18α-CH_3), 0.788 (3H, s, 4β-CH_3), 0.809 (3H, s, 4α-CH_3), 0.844 (3H, s, 10β-CH_3), 0.926 and 0.932 (2 × 3H, 2s, 8β- and 14α-CH_3), 0.947 (3H, d, J = 6.5 Hz, 22-CH_3), 1.957 (3H, s, CH_3CONH-), 2.081 (3H, s, CH_3CO_2-), 2.086 (3H, s, CH_3CO_2-), 2.112 (3H, s, CH_3CO_2-), 2.113 (3H, s, CH_3CO_2-). 2.125 (3H, s, CH_3CO_2-), 3.34 (1H, ddd, $J_{35a,35b}$ = 15 Hz, $J_{34,35a}$ = 7 Hz, $J_{35a,NH}$ = 6 Hz, 35-H_a), 3.64 (1 H, ddd, $J_{35a,35b}$ = 15 Hz, $J_{35b,NH}$ = 6 Hz,

Abbreviation used: c.o.s.y., two-dimensional proton/proton correlation.
* To whom correspondence and reprint requests should be addressed.

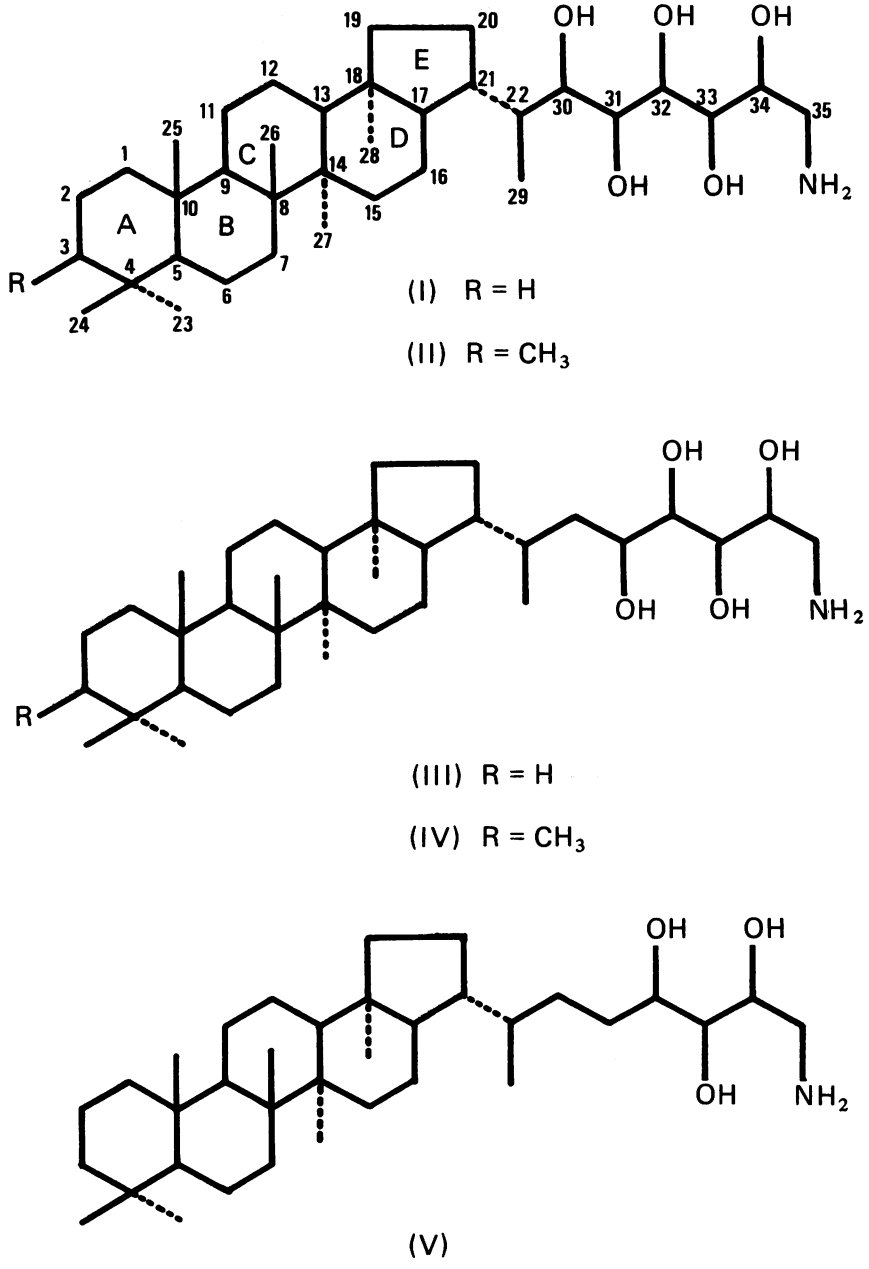

Fig. 1. Bacteriophane derivatives from *Methylococcus capsulatus* [(I), (II), (III) and (IV)] and *Methylomonas methanica* [(I), (III) and (V)]

$J_{34,35b} = 4$ Hz, 35-H_b), 5.11 (1H, dt, $J_{34,35a} = 7$ Hz, $J_{34,35b} = J_{33,34} = 4$ Hz, 34-H), multiplet centred at 5.24 (3H, m, 30-H, 31-H and 32-H), 5.33 (1H, dd, $J_{32,33} = 6.5$ Hz, $J_{33,34} = 4$ Hz, 33-H), 5.71 (1H, t, $J_{35a,NH} = J_{35b,NH} = 6$ Hz, CH_3CONH-, exchangeable with ^2H_2O). The structure of the multiplet centred at 5.24 p.p.m. was elucidated by using an LAOCN3 algorithm, which permitted estimation of chemical shifts and coupling constants: δ (p.p.m.) = 5.23 (1H, dd, $J_{30,31} = 8$ Hz, $J_{31,32} = 3.5$ Hz, 31-H), 5.24 (1H, dd, $J_{30,31} = 8$ Hz, $J_{22,30} = 2$ Hz, 30-H), 5.26 (1 H, dd, $J_{32,33} = 7$ Hz, $J_{31,32} = 3.5$ Hz, 32-H).

The ^{13}C-n.m.r. [50 MHz, (^{2}H)chloroform] characteristics were as follows: δ (p.p.m.) = 14.1 (C-29), 15.8 and 15.9 (C-25 and C-28), 16.4 and 16.6 (C-26 and C-27), 18.7 (C-2 and C-6), 20.8 (C-11 and 5 CH_3CO_2-), 21.6 (C-24), 23.1 (C-16 and CH_3CONH-), 24.0 (C-12), 28.2 (C-20), 33.3 (C-7), 33.4 (C-4 and C-23), 33.7 (C-15), 37.5 (C-10), 38.2 (C-22), 38.9 (C-35), 40.4 (C-1), 41.5 (C-8 or C-14), 41.6 (C-19), 41.8 (C-8 and C-14), 42.2 (C-3), 42.9 (C-21), 44.6 (C-18), 49.3 (C-13), 50.5 (C-9), 54.0 (C-17), 56.2 (C-5), 69.8, 70.3, 70.6, 71.1 and 72.4 (C-30, C-31, C-32, C-33 and C-34), 169.7 ($2CH_3CO$-), 169.8 (CH_3CO-), 170.0 (CH_3CO-), 170.2 (CH_3CO-), 170.4 (CH_3CO-).

The mass-spectrum (direct inlet, 70 eV) characteristics were as follows: m/z = 829 (M^+, 95%), 814 ($M^+ - Me$, 5%), 787 (6%), 769 ($M^+ - AcOH$, 20%), 754 ($M^+ - AcOH - Me$, 11%), 727 (4%), 712 (3%), 709 (3%), 694 (8%), 608 (*ring C cleavage* [12], 5%), 589 (16%), 548 (608-*AcOH*, 20%), 529 (23%), 488 (608-*2AcOH*, 15%), 469

(9%), 428 (11%), 409 (12%), 402 (40%), 369 (*M^+–side chain*, 29%), 368 (*M^+ – side chain-H*, 55%), 367 (*M^+ – side chain-2H*, 63%), 342 (60%), 191 (*ring C cleavage* [12], 100%).

The mass-spectrum (chemical ionization using NH_3 as reactant gas) characteristics were as follows: m/z = 847 ($M+NH_4^+$, 5%), 830 ($M+H^+$, 100%), 548 (4%), 530 (6%), 369 (*M-side chain*, 40%), 191 (*ring C cleavage*, 62%).

Penta-acetate of (22*S*)-35-aminobacteriohopane-30,31,32,33,34-pentol (I)

The hydroxy group at C-30 is free in this derivative. The ^{1}H-n.m.r. [200 MHz, (^{2}H)chloroform], characteristics were as follows: δ (p.p.m.) = 0.647 (3H, s, 18α-CH_3), 0.789 (3H, s, 4β-CH_3), 0.812 (3H, s, 4α-CH_3), 0.844 (3H, s, 10β-CH_3), 0.886 (3H, d, J = 6.5 Hz, 22-CH_3), 0.930 and 0.948 (2 × 3H, 2s, 8β- and 14α-CH_3), 1.976 (3H, s, CH_3CONH-), 2.060 (3H, s, CH_3CO_2-), 2.070 (3H, s, CH_3CO_2-), 2.115 (3H, s, CH_3CO_2-), 2.131 (3H, s, CH_3CO_2-), 3.38 (1H, ddd, $J_{35a,35b}$ = 15 Hz, $J_{35a,NH}$ = 6.5 Hz, $J_{34,35a}$ = 6 Hz, 35-H_a), 3.79 (1H, ddd, $J_{35a,35b}$ = 15 Hz, $J_{35b,NH}$ = 6.5 Hz, $J_{34,35b}$ = 3 Hz, 35-H_b), 4.00 (1H, broad d, $J_{30,31}$ = 9.5 Hz, 30-H), 5.09 (1H, dd, $J_{30,31}$ = 9.5 Hz, $J_{31,32}$ = 2.5 Hz, 31-H), 5.23 (1H, ddd, $J_{34,35a}$ = 6.5 Hz, $J_{34,35b}$ = 3.5 Hz, $J_{33,34}$ = 3 Hz, 34-H), 5.46 (1H, dd, $J_{32,33}$ = 7 Hz, $J_{31,32}$ = 3 Hz, 32-H), 5.55 (1H, dd, $J_{32,33}$ = 7 Hz, $J_{33,34}$ = 3 Hz, 33-H), 5.68 (1 H, t, $J_{35a,NH}$ = $J_{35b,NH}$ = 6.5 Hz, CH_5CONH^-, exchangeable with ^2H_2O).

The mass-spectrum (direct inlet, 70 eV) characteristics were as follows: m/z = 787 (M^+, 33%), 769 (M^+-H_2O, 38%), 754 (M^+-H_2O-*ME*, 4%), 727 (M^+–*AcOH*, 8%), 712 (M^+–*AcOH*–*Me*, 10%), 694 (4%), 549 (8%), 548 (*ring C cleavage-H_2O*, 7%), 529 (9%), 506 (*ring C cleavage –AcOH*, 9%), 446 (506-*AcOH*, 7%), 422 (10%), 409 (14%) 390 (*cleavage between C-22 and C-30*, 100%), 369 (M^+–*side-chain*, 18%), 368 (M^+–*side-chain-H*, 26%), 367 (M^+–*side-chain-2H*, 26%), 191 (*ring C cleavage*, 38%).

Hexa-acetate of (22*S*)-35-amino-3β-methylbacteriohopane-30,31,32, 33,34-pentol (II)

The ^{1}H-n.m.r. [200 MHz, (^{2}H)chloroform] characteristics were as follows: δ (p.p.m.) = 0.623 (3H, s, 18α-CH_3), 0.633 (3H, s, 4α-CH_3), 0.766 (3H, s, 4β-CH_3), 0.803 (3H, d, J = 6.5 Hz, 3β-CH_3), 0.853 (3H, s, 10β-CH_3), 0.923 (6H, s, 8β-and 14α-CH_3), 0.940 (3H, d, J = 6.5 Hz, 22-CH_3), 1.951 (3H, s, CH_3CONH-), 2.084 (6H, s, CH_3CO_2-), 2.111 (6H, s, CH_3CO_2-), 2.123 (3H, s, CH_3CO_2-), 3.34 (1H, ddd, $J_{35a,35b}$ = 15 Hz, $J_{34,35a}$ = 7 Hz, $J_{35a,NH}$ = 6 Hz, 35-H_a), 3.63 (1H, ddd, $J_{35a,35b}$ = 15 Hz, $J_{35b,NH}$ = 6 Hz, $J_{34,35b}$ = 3.5 Hz, 35-H_b), 5.10 (1 H, dt, $J_{34,35a}$ = $J_{33,34}$ = 7 Hz, $J_{34,35b}$ = 3.5 Hz, 34-H), 5.23 (3H, m, 30-H, 31-H and 32-H), 5.72 (1H, t, $J_{35a,NH}$ = $J_{35b,NH}$ = 6 Hz, CH_3CONH-, exchangeable with ^2H_2O).

Penta-acetate of 35-amino-3β-methylbacteriophopane-31,32,33,34-tetrol (IV)

The ^{1}H-n.m.r. [200 MHz, (^{2}H)chloroform] characteristics were as follows: δ (p.p.m.) = 0.627 (6H, broad s, 18α-and 4α-CH_3), 0.773 (3H, s, 4β-CH_3), 0.806 (3H, d, J = 7 Hz, 3β-CH_3), 0.857 (3H, s, 10β-CH_3), 0.909 (1H, d, J = 6.5 Hz, 22-CH_3), 0.923 and 0.938 (2 × 3H, 2s, 8β- and 14α-CH_3), 1.966 (3H, s, CH_3-CONH-), 2.041 (3H, s, CH_3-CO_2-), 2.086 (3H, s, CH_3-CO_2-), 2.109 (3H, s, CH_3CO_2-), 2.131 (3H, s, CH_3CO_2-), 3.38 (1H, m, 35-H_a), 3.68 (1H, m, 35-H_b), 5.21 (4H, m, 31-H, 32-H, 33-H and 34-H), 5.70 (1H, m, CH_3CONH-).

RESULTS

The structures of the pentacyclic nucleus of hopanoids (I) and (II) as well as the stereochemistry of the chiral centre at C-22 were determined by direct comparison of the derivatives obtained by H_5IO_6 oxidation followed by $NaBH_4$ reduction with the synthetic hopanoids (22*S*)-hopan-29-ol and (22*S*)-3β-methylhopan-29-ol [7,13]. This correlation showed that the C-22 configuration of aminopentols (I) and (II) is 22*S*, i.e. identical with that of bacteriohopanetetrol, which was assumed to be 22*R* on the basis of spectroscopic data [14]. The full structure of their side chain was deduced from spectroscopic data. The singlets of six methyl groups appeared in the 2 p.p.m. region of the ^{1}H-n.m.r. spectrum of peracetylated hopanoid (I) corresponding to the presence of six acetyl groups. Furthermore, in the ^{13}C-n.m.r. spectrum, five signals at 69.8, 70.3, 70.6, 71.1 and 72.4 p.p.m., corresponding to carbon atoms bearing an acetoxy group and one signal at 38.9 p.p.m., corresponding to a carbon atom bearing an acetamido group, confirmed the structure of a hexa-acetate. The presence of this acetamido group at C-35 was also suggested by the signal of this C-35 carbon atom (δ = 38.9 p.p.m.), which resonates at higher field than the C-35 carbon atom of tetra-acetoxybacteriohopane (δ = 62.1 p.p.m.) [3], and at a field similar to that of the C-35 carbon atom of peracetylated aminotriol (V) (δ = 39.2 p.p.m.) [5,6]. The localization of the acetamido group at C-35 could also be deduced from the ^{1}H-n.m.r. of peracetylated compounds (I) and (II). The signals of the two C-25 protons appeared at fields (3.34 and 3.64 p.p.m.) similar to those of the C-35 protons of the tetra-acetate of (V) (3.37 and 3.69 p.p.m.) or the penta-acetate of (III) (3.37 and 3.68 p.p.m. [5,6], and at much higher field than those of tetra-acetoxybacteriohopane (4.14 and 4.39 p.p.m.) [3]. Finally the presence of the acetamido group was revealed by the presence of the triplet at 5.71 p.p.m. corresponding to the nitrogen-linked proton, which is exchangeable in the presence of ^2H_2O. The electron-impact mass spectrum of peracetylated hopanoid (I) was consistent with a hexa-acetate structure with a molecular ion at m/z 829. The molecular mass was confirmed by chemical ionization, with NH_3 as reactant gas and giving ions at m/z 847 ($M+NH_4^+$) and 830 ($M+H^+$). The typical fragmentation of the hopane skeleton was observed: ring C cleavage, giving fragments at m/z 191 and 608, and loss of the side chain at m/z 369 [12]. As in the mass spectra of all hopanoids possessing an oxygen atom at C-30 [13], two other ions were observed at m/z 368 and 367 corresponding to the loss of the side chain and of one or two protons.

Full confirmation of the structure of aminopentol (I) was obtained from the ^{1}H-n.m.r. spectrum and the mass spectrum of a penta-acetate of (I), accidentally obtained in small amounts by incomplete acetylation. The spectroscopic data showed clearly that the C-30 hydroxy group was not acetylated: the proton at C-30 resonates at much higher field (δ = 4.00 p.p.m.) than does the C-30

proton of the hexa-acetate of (I) ($\delta = 5.24$ p.p.m.). Furthermore the base peak of the mass spectrum of the penta-acetate of (I) at m/z 390 corresponds to a cleavage between the C-22 and C-30 carbon atoms induced by the free hydroxy group. The complete structure of the side chain of this penta-acetate of (I) could be unambiguously deduced from the ^{1}H-n.m.r. spectrum and from c.o.s.y.; the replacement of the C-30 acetoxy group by a free hydroxy group resulted in a fully resolved spectrum.

The minor hopanoids (III) and (IV) from *Methylococcus capsulatus* and (III) and (V) from *Methylomonas methanica* were identified by comparison of the ^{1}H-n.m.r. spectra of their peracetylated derivatives with those of reference hopanoids [5,6]. The extra methyl group of hopanoid (IV) from *Methylococcus capsulatus* was localized at C-3β by comparison of the ^{1}H-n.m.r. spectrum from the penta-acetate of (IV) with those of synthetic 3β-methylhopanoids [13].

DISCUSSION

The isolation of the two new aminopentols (I) and (II) from the methylotrophic bacteria *Methylococcus capsulatus* and *Methylomonas methanica* completes the series of the bacteriohopane-derived aminopolyols. The aminotriol (V) was already isolated from two purple non-sulphur bacteria, *Rhodomicrobium vannielii* [5] and *Rhodopseudomonas palustris* (S. Neunlist & M. Rohmer, unpublished work), and from the methylotroph *Methylosinus trichosporium* [6] and the aminotetrol (III) as the major hopanoid of the latter bacterium [6]. It seems that aminopentol (I) or other similar hopanoids with functionalized C-30 and C-31 carbon atoms are characteristic of the two known *Methylococcus capsulatus* strains and of all analysed type-I methylotrophs, since hopan-29-ol is always released by our standard $H_5IO_6/NaBH_4$ side-chain-degradation procedure [2].

The amounts of peracetylated aminopolyols isolated from both *Methylococcus capsulatus* (5.5 mg/g) and *Methylomonas methanica* (3.8 mg/g) were nearly in accordance with the quantities of primary alcohols obtained by $H_5IO_6/NaBH_4$ treatment of the crude bacterial chloroform/methanol extract [2], showing that no complex hopanoids are present in large amounts in these bacteria. Only free aminopolyols were detected. Neither free polyols, nor derivatives in which a hopanoid is linked to a polar moiety [3–5,15], could be detected. However, one minor hopanoid remains still to be detected in *Methylococcus capsulatus*. Indeed, after $H_5IO_6/NaBH_4$ treatment, diplopterol is accompanied by small amounts (10–50 μg/g dry wt.) of (22*R*)-adiantol [13]. This hopanoid is only present after the $H_5IO_6/NaBH_4$ degradation procedure, and because of the too-low concentration, the native hopanoid releasing this alcohol could not be isolated.

In most sediments, the hopanoid fractions contain strikingly large amounts of derivatives possessing an ethyl side chain. This preferred cleavage cannot easily be explained by chemical maturation of the known bacterial hopanoids. Since this would be an obvious process if hopan-29-ol were present in some prokaryotes, we have postulated the existence of this alcohol as bacterial lipid [16].

It seems now more likely that a C-30 functionalized bacteriohopane derivative such as the aminopentol (I) isolated from widely distributed methylotrophs could be the precursor of the C_{29} chemical fossils, since a similar process [17] would give a C_{31} hydrocarbon [18] from bacteriohopanetetrol and a C_{29} hydrocarbon from aminobacteriohopanepentol (I).

Methylococcus capsulatus occupies a peculiar position among prokaryotes, since it contains 4α-methylsterols [19,20] derived from the cyclization of squalene epoxide as well as hopanoids derived from a direct cyclization of squalene [8]. The presence of sterol precursors is an unique feature from a bacterium, since only one other prokaryote, the gliding bacterium *Nannocystis exedens*, has been unambiguously shown to be able to synthesize sterols *de novo* [21]. We have postulated a few years ago that hopanoids play in prokaryotic membranes the reinforcer role normally played by sterols in eukaryotic membranes [1,22]. This assertion is now supported by several sets of experimental data obtained on membrane models or on biological systems [23–28]. The presence, in *Methylococcus capsulatus*, of two different kinds of potential membrane reinforcers, 4α-methylsterols and aminobacteriohopanepolyols, rises interesting questions on the respective role and intracellular localization of both triterpenoid families.

We thank Dr. H. Strub and Mrs. E. Krempp for the n.m.r. measurements and the Centre National de la Recherche Scientifique (Unité Associée 135 and Action Thématique Programmée 'Microbiologie 1983') for financial support.

REFERENCES

1. Rohmer, M., Bouvier, P. & Ourisson, G. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 847–951
2. Rohmer, M., Bouvier-Navé, P. & Ourisson, G. (1984) J. Gen. Microbiol. **130**, 1137–1150
3. Renoux, J. M. (1984) Thèse de Docteur-Ingénieur, Université de Haute Alsace, Mulhouse
4. Langworthy, T. A., Mayberry, W. R. & Smith, P. F. (1976) Biochim. Biophys. Acta **431**, 550–569
5. Neunlist, S., Holst, O. & Rohmer, M. (1985) Eur. J. Biochem. **147**, 561–568
6. Neunlist, S. & Rohmer, M. (1985) J. Gen. Microbiol., in the press
7. Zundel, M. & Rohmer, M. (1985) FEMS Microbiol. Lett. **28**, 61–64
8. Wenkert, E., Baddley, G. B., Burfitt, I. R. & Moreno, L. N. (1978) Org. Magn. Reson. **11**, 337–343
9. Bauer, P. E., Dunlop, N. K., Arrenayidis, S. & Watt, D. S. (1983) J. Org. Chem. **48**, 4493–4497
10. Rohmer, M., Bouvier, P. & Ourisson, G. (1980) Eur. J. Biochem. **112**, 557–560
11. Still, W. C., Kahn, M. & Mitra, A. (1978) J. Org. Chem. **43**, 2933–2942
12. Budzickiewicz, H., Wilson, J. M. & Djerassi, C. (1963) J. Am. Chem. Soc. **85**, 3688–3699
13. Zundel, M. & Rohmer, M. (1985) Eur. J. Biochem. **150**, 23–27
14. Rohmer, M. & Ourisson, G. (1976) Tetrahedron Lett. 3633–3636
15. Neunlist, S. & Rohmer, M. (1985) Biochem. J. **228**, 769–771
16. Ourisson, G., Albrecht, P. & Rohmer, M. (1982) Trends Biochem. Sci. **7**, 236–239
17. Van Dorsselaer, A. (1975) Thèse d'Etat, Université Louis Pasteur, Strasbourg
18. Ensminger, A., Albrecht, A., Ourisson, G., Kimble, B. J., Maxwell, J. R. & Eglinton, G. (1972) Tetrahedron Lett. 3681–3684

19. Bird, C. W., Lynch, C. M., Pirt, F. J., Reid, W. W., Brooks, C. J. W. & Middleditch, B. S. (1971) Nature (London) **230**, 473
20. Bouvier, P., Rohmer, M., Benveniste, P. & Ourisson, G. (1976) Biochem. J. **159**, 267–271
21. Kohl, W., Gloe, A. & Reichenbach, H. (1983) J. Gen. Microbiol. **129**, 1629–1635
22. Ourisson, G. & Rohmer, M. (1982) Curr. Top. Membr. Transp. **17**, 153–172
23. Poralla, K., Kannenberg, E. & Blume, A. (1980) FEBS Lett. **113**, 107–110
24. Bisseret, P., Wolff, G., Albrecht, A. M., Tanaka, T., Nakatani, Y. & Ourisson, G. (1983) Biochem. Biophys. Res. Commun. **110**, 320–324
25. Benz, R., Hallmann, D., Poralla, K. & Eibl, H. (1983) Chem. Phys. Lipids **34**, 7–24
26. Kannenberg, E., Blume, A., McElhaney, R. N. & Poralla, K. (1983) Biochim. Biophys. Acta **733**, 111–116
27. Poralla, K., Härtner, D. & Kannenberg, E. (1984) FEMS Microbiol. Lett. **23**, 253–256
28. Kannenberg, E., Blume, A., Geckeler, K. & Poralla, K. (1985) Biochim. Biophys. Acta **814**, 179–185

Received 16 July 1985; accepted 8 August 1985