A novel hopanoid, 30-(5'-adenosyl)hopane, from the purple non-sulphur bacterium *Rhodopseudomonas acidophila*, with possible DNA interactions

Serge NEUNLIST and Michel ROHMER*

École Nationale Supérieure de Chimie de Mulhouse, 3 Rue Alfred Werner, 68093 Mulhouse, France

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A novel hopanoid, 30-(5'-adenosyl)hopane, was isolated from the purple non-sulphur bacterium *Rhodopseudomonas acidophila* and identified. The significance of this triterpenoid in terms of bacteriohopanepolyol biosynthesis, membrane reinforcement and possible interactions with nucleic acids is discussed.

The wide distribution of hopanoids was first revealed by the ubiquity of their chemical fossils in the organic matter of sediments (Ourisson *et al.*, 1979). Bacteriohopanepolyols are very widespread among prokaryotes belonging to many taxonomic groups (Rohmer *et al.*, 1979, 1984). In most cases these polyols are accompanied by larger amounts of derivatives possessing various polar moieties linked to their C-35 hydroxy or amino group: glucosamine derivatives (Langworthy *et al.*, 1976; Renoux, 1984), methylcyclopentylaminocyclitols (Renoux, 1984) or α -amino acids (Neunlist *et al.*, 1984, 1985). In an attempt to discover further such new complex hopanoids, to test the generality of their presence and to detect missing links in the bacteriohopanepolyol biosynthetic pathway, we analysed the hopanoids of *Rhodopseudomonas acidophila*, a good hopanoid producer (up to 2 mg/g dry wt.; Rohmer *et al.*, 1984). In the present paper we report the identification of a new class of prokaryotic triterpenoids, possessing a C–C linkage between the hopane side chain and the C-5' carbon atom of the ribose of adenosine. The presence of such a compound, capable of interaction with bacterial DNA, might reflect another role for hopanoids in addition to the predicted (Rohmer *et al.*, 1979) and already experimentally supported role of membrane reinforcers (Poralla *et al.*, 1980, 1984; Bisserset *et al.*, 1983; Benz *et al.*, 1983; Kannenberg *et al.*, 1983).

freeze-dried cells of *Rhodopseudomonas acidophila* DSM 145 was acetylated and separated by t.l.c. on silica gel by using ethyl acetate as eluent, this giving the triacetate of compound (I) (R_F 0.30; 1 mg/g dry wt.), as well as other hopanoids whose structures remain to be investigated. Acetylated compound (I) was further purified by t.l.c. [with chloroform/methanol (19:1, v/v); R_F 0.40] and identified by spectroscopic methods and comparison of the obtained data with those of adenosine (II) tetra-acetate.

Triacetate of 30-(5'-adenosyl)hopane (I)

The ^1H -n.m.r. (200 MHz; C^2HCl_3) characteristics were as follows: δ (p.p.m.) = 0.678 (3H, s, $18\alpha\text{-CH}_3$), 0.789 (3H, s, $4\beta\text{-CH}_3$), 0.810 (3H, s, $4\alpha\text{-CH}_3$), 0.845 (3H, s, $10\beta\text{-CH}_3$), 0.933 (3H, d, $J = 4\text{ Hz}$, 22R-CH_3), 0.942 (6H, s, $8\beta\text{-CH}_3$ and $14\alpha\text{-CH}_3$), 2.063 (3H, s, CH_3CO_2^-), 2.152 (3H, s,

Materials and methods

General methods

All analytical procedures and the separation scheme have been described previously (Neunlist *et al.*, 1985). The chloroform/methanol extract of

* To whom all correspondence should be addressed.

CH_3CO_2^-), 2.633 (3H, s, $\text{CH}_3\text{CONH}-$), 4.18 (1H, td, $J_{4',5'} = 8\text{ Hz}$, $J_{3',4'} = 4\text{ Hz}$, 4'-H), 5.43 (1H, dd, $J_{2,3} = 5.5\text{ Hz}$, $J_{3,4'} = 4\text{ Hz}$, 3'-H), 5.95 (1H, t, $J_{1,2} = J_{2,3} = 5.5\text{ Hz}$, 2'-H), 6.14 (1H, d, $J_{1,2} = 5.5\text{ Hz}$, 1'-H), 8.08 and 8.69 ($2 \times 1\text{H}$, 2s, 2-H and 8-H of *N*-acetyladenine), and 8.63 (1H, s, exchangeable with $^2\text{H}_2\text{O}$, $-\text{NHCO}-$).

The mass-spectrum (direct inlet, 70 eV) characteristics were as follows: $m/z = 787$ (M^+ , 27%), 772 ($M^+ - \text{CH}_3$, 1%), 610 ($M^+ - N$ -acetyladenine-H, 12%), 595 [ring-C cleavage (Budzikiewicz *et al.*, 1963), 4%], 491 (4%), 405 (3%), 391 (7%), 390 (7%), 389 (13%), 367 ($M^+ - \text{side chain} - 2\text{H}$, 7%), 191 [ring-C cleavage (Budzikiewicz *et al.*, 1963), 15%] and 178 (*N*-acetyladenine-H, 100%).

The u.v.-spectrum [λ_{max} , nm (ϵ_{max} , $\text{M}^{-1} \cdot \text{cm}^{-1}$)] characteristics were as follows: in methanol, 210 (22500) and 270 (12000); in chloroform, 235 (6500) and 270 (13000).

The c.d. spectrum (in methanol) gave $[\theta] = -6900 \text{ degrees} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$ (265 nm).

Tetra-acetate of adenosine (II)

The ^1H -n.m.r. (80 MHz; C^2HCl_3) characteristics were as follows: δ (p.p.m.) = 2.09 (3H, s, CH_3CO_2^-), 2.12 (3H, s, CH_3CO_2^-), 2.16 (3H, s, CH_3CO_2^-), 2.64 (3H, s, $\text{CH}_3\text{CONH}-$), 4.43 (3H, m, 4'-H and 5'-H), 5.68 (1H, dd, $J_{2,3} = 5.5\text{ Hz}$, $J_{3,4'} = 4\text{ Hz}$, 3'-H), 5.96 (1H, t, $J_{1,2} = J_{2,3} = 5.5\text{ Hz}$, 2'-H), 6.22 (1H, d, $J_{1,2} = 5.5\text{ Hz}$, 1'-H), 8.14 and 8.69 ($2 \times 1\text{H}$, 2s, 2-H and 8-H of *N*-acetyladenine), and 8.64 (1H, s, exchangeable with $^2\text{H}_2\text{O}$, $-\text{NHCO}-$).

The u.v.-spectrum [λ_{max} , nm (ϵ_{max} , $\text{M}^{-1} \cdot \text{cm}^{-1}$)] characteristics were as follows: in methanol, 207 (23200) and 265 (12800).

The c.d. spectrum (in methanol) gave $[\theta] = -1300 \text{ degrees} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$ (281 nm).

Results

The methyl region of the ^1H -n.m.r. spectrum of acetylated 30-(5'-adenosyl)hopane is typical of a bacterial hopanoid (Rohmer & Ourisson, 1976). Furthermore, after H_2IO_6 oxidation followed by NaBH_4 reduction of the total chloroform/methanol extract from *R. acidophila*, only the C_{32} hopanoid primary alcohol, with a presumed 22*R* configuration (Rohmer & Ourisson, 1976), is released (Rohmer *et al.*, 1984); this means that all bacteriohopane derivatives of this bacterium have the same configuration at C-22. The presence of the adenosyl moiety is clearly indicated by the u.v. and ^1H -n.m.r. spectra of acetylated hopanoid (I), which are nearly identical with those of adenosine (II) tetra-acetate. The c.d. spectra of acetylated compound (I) and adenosine (II) tetra-acetate both exhibit a similar negative Cotton effect at 265 and

281 nm. As the sign of this Cotton effect is determined by the configuration at C-1' of the nucleoside (Miles *et al.*, 1967, 1968; Nishimura *et al.*, 1968), it can reasonably be assumed that the configuration at C-1' of 30-(5'-adenosyl)hopane is identical with the configuration at C-1' of adenosine (II). Furthermore the chemical shifts and the coupling constants of the signals of the side chain from acetylated adenosine (II) show that the relative configurations at the C-1', C-2', C-3' and C-4' chiral centres of 30-(5'-adenosyl)hopane (I) are respectively identical with those at C-1', C-2', C-3' and C-4' in adenosine (II). Thus the absolute configuration of the side chain of 30-(5'-adenosyl)hopane (I) is that of a D-ribofuranoside. The presence of the C-C linkage between the C-30 carbon atom of the hopane skeleton and the C-5' carbon atom of adenosine is indicated by the signal of the C-4' proton in the ^1H -n.m.r. spectrum of acetylated 30-(5'-adenosyl)hopane (I): this proton is coupled to the proton at C-3' ($J = 3\text{ Hz}$) and to the two protons at C-5' ($J = 8\text{ Hz}$), the signals of which are masked in the methylene and methine region.

Discussion

From a biogenetic point of view, 30-(5'-adenosyl)hopane (I) could arise from a nucleophilic attack by the double bond of hop-22(29)-ene on the C-5' carbon atom of a suitable adenosyl donor. A similar reaction is, for instance, involved in *S*-adenosylmethionine biosynthesis. The adenosyl donor might be ADP or ATP or even *S*-adenosylmethionine, which is already a methyl donor in methylation reactions and after decarboxylation a propylamine donor in polyamine biosynthesis.

Two different roles, which are not mutually exclusive, are possible for C-adenosylhopane. On the one hand it might be a biosynthetic precursor for bacteriohopane derivatives that is accumulated by *R. acidophila* and might have been overlooked in other bacteria because of the small amounts usually found for biosynthetic intermediates. Therefore we propose a numbering of the side chain of adenosylhopane that is in accordance with our previous numbering of the bacteriohopane skeleton, the C-30, C-5', C-4', C-3', C-2' and C-1' carbon atoms of adenosylhopane being respectively the equivalents of the C-30, C-31, C-32, C-33, C-34 and C-35 carbon atoms of a bacteriohopane derivative (Neunlist *et al.*, 1984, 1985; Neunlist & Rohmer, 1985; Renoux, 1984). On the other hand, it might be a structural element of membranes. Because of structural and biogenetic similitudes between sterols and hopanoids, we have postulated that hopanoids act as membrane reinforcers in prokaryotes, much like cholesterol in eukaryotic

membranes (Rohmer *et al.*, 1979). This assertion has already received experimental support with membrane models (Poralla *et al.*, 1980; Bisseret *et al.*, 1983; Benz *et al.*, 1983; Kannenberg *et al.*, 1983) and biological systems (Conner *et al.*, 1968; Kannenberg & Poralla, 1982; Poralla *et al.*, 1984). This structural role is compatible with the structures of almost all known hopanoids. However, a compound such as 30-(5'-adenosyl)hopane is capable of two different kinds of interactions. Through its planar lipophilic nucleus it can be anchored in a phospholipid bilayer by van der Waal's interactions with the n-acyl chains; through its adenine moiety it can be linked by hydrogen-bonding to thymine from a single-stranded region of DNA and by stacking interactions with other bases. Since bacterial DNA is frequently linked to membrane systems (Ryter, 1968; Hirschbein & Guillen, 1982), a hopanoid capable of interactions with both cytoplasmic membrane and DNA could in some cases be the link between the two entities.

This is the first report of triterpenoids potentially capable of direct interactions with prokaryotic nucleic acids. This surprising new aspect of triterpenoid biochemistry points to possible new roles for hopanoids. As structural constituents of membranes they are sterol equivalents, but they are perhaps also steroid equivalents and share an effector role in metabolic regulations.

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