Structural Elucidation of Two Hopanoids from the Photosynthetic Bacterium *Rhodomicrobium vannielii*

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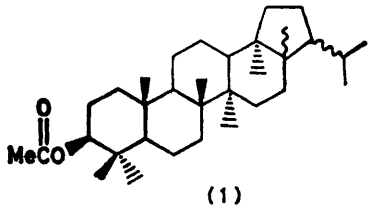
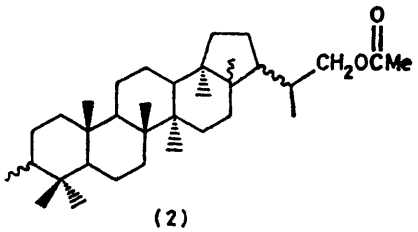
Summary Two hopanoids, 3β-hydroxy-17-methylhopane and 29-hydroxy-3,17-dimethylhopane, have been isolated from *Rhodomicrobium vannielii* and identified by g.c.–m.s. and n.m.r. spectroscopy.

Two pentacyclic triterpenoids of the hopane series have been isolated from the anaerobic photosynthetic bacterium, *Rhodomicrobium vannielii* and identified as 3β-hydroxy-17-methylhopane and 29-hydroxy-3,17-dimethylhopane. *R. vannielii* (ATCC No. 17100) was grown anaerobically in nitrogen (99·998% N_2) on sparged, inorganic sulphide media;[1] anaerobiosis was determined by both oxygen electrode and Winkler titrimetric analysis. The cells (62 g dry wt.) were repeatedly extracted in chloroform–ethanol (2:1) followed by acetone. The extract was chromatographed on a silica gel column and eluted with ethyl acetate–light petroleum (15:85). The chlorophyll-free eluate was saponified (15% KOH in MeOH) and chromatographed on thin layer plates (silica gel G) using dichloromethane as eluant. The zone corresponding to the lanosterol standard (R_f = 0·2) was reclaimed and the acetate derivatives formed. Capillary g.l.c.–mass spectrometry indicated the presence of two components, designated compounds (a) and (b). The mass spectrum of compound (a), identified as 3β-acetoxy-17-methylhopane (**1**), included the major fragment ions: *m/e* 484 (M^+, 11%), 424 [$M^+ - CH_3CO_2H$, 15%), 409 [$M^+ - CH_3CO_2H - CH_3$, 9%), 381 [$M^+ - CH_3CO_2H - (CH_3)_2CH$, 6%], 205 (rings D + E, 100%), 191 (rings D + E − CH_3, 62%), 189 (rings A + B − CH_3CO_2H, 82%), and 163 [rings D + E − $(CH_3)_2CH$, 11%].



(1)



(2)

The ^1H n.m.r. methyl assignments of compound (a) (free hydroxy- and acetate-derivatives) were based upon comparisons with various hopane standards.[2–5] The chemical shifts (δ) of the methyl groups (singlets) from tetramethyl-

silane (TMS) in $CDCl_3$ (200 MHz) were assigned as follows (acetate derivative): 0·83 (9H, 4α, 4β, and 10β), 0·95 (3H, 8β), 0·94 (3H, 14α), 0·78 (3H, 18α), and 0·75 (3H, 17α or β); (free hydroxy-compound): 0·96 (3H, 4α), 0·75 (6H, 4β and 17α or β), 0·84 (3H, 10β), 0·95 (3H, 8β), 0·93 (3H, 14α), and 0·78 (3H, 18α). The position of the hydroxy-group and the 4α, 4β, and 10β methyl assignments were confirmed by n.m.r. spectra taken with incremental additions of $Eu(fod)_3$ [$Eu(fod)_3 = Eu(C_{10}H_{10}F_7O_2)_3$].[6] Based on differences in the induced chemical shifts, the hydroxy-group of compound (a) was determined to be at the 3β position. The ^1H multiplets centred at δ 3·2 and 4·4 in the free hydroxy- and acetate-derivatives, respectively, are characteristic of the C-3 proton when a 3β-hydroxy-group is present.[3,4,7]

The mass spectrum of compound (b), identified as 29-acetoxy-3,17-dimethylhopane (**2**), displayed ions typical of an acetylated hopane with two extra methyl groups on the ring skeleton; principal ions: *m/e* 498 (M^+, 5%), 483 ($M^+ - CH_3$, 3%), 438 ($M^+ - CH_3CO_2H$, 10%), 423 ($M^+ - CH_3CO_2H - CH_3$, 10%), 395 (M^+ — side-chain $C_3H_6OCOCH_3$, 3%), 263 (rings D + E, 15%), 205 (rings A + B, 100%), and 203 (rings D + E — side-chain $C_3H_6OCOCH_3$, 75%). The presence of the ion *m/e* 395, and the absence of an ion *m/e* 383, precluded a C_4H_8OAc side-chain, which would have first fragmented, giving rise to an ion *m/e* 438, followed by the loss of C_4H_7, thereby producing a peak at *m/e* 383.

The n.m.r. spectrum of compound (b) indicated the presence of ten methyl groups; they have not been assigned owing to the lack of published n.m.r. data for 3,17-dimethylhopanes.

This communication is the first report of a C-3 hydroxylated pentacyclic triterpenoid isolated from a prokaryote. In aerobic eukaryotes it has been demonstrated that the oxygen moiety of the C-3 hydroxy-group in tetracyclic triterpenes (sterols) is derived from molecular oxygen *via* the intermediate squalene epoxide.[8] A similar mechanism has been postulated for the biosynthesis of C-3 hydroxylated pentacyclic triterpenes isolated from higher plants.[9] Evidence for a non-oxidative cyclization of squalene has been well documented for the biosynthesis of pentacyclic triterpenes in several aerobic organisms including *Tetrahymena pyriformis*,[10,11] *Polypodium vulgare*,[12] and *Acetobacter rancens*.[13] It has been demonstrated in *T. pyriformis* that the hydroxy-groups of 22-hydroxyhopane and tetrahymanol are derived from water and not molecular oxygen.[11,14] We can only speculate as to the origin of the hydroxy-group on 3β-hydroxy-17-methylhopane of *R. vannielii*; hydroxylation may follow cyclization of squalene. Evidence for enzyme systems capable of introducing a hydroxy-group at C-3 has been presented, but in every instance hydroxylation has occurred after cyclization.[15] Rohmer and Ourisson have proposed that the C-3 methyl group of triterpenes of *Acetobacter rancens* and *A. xylinum* is derived from *S*-adenosyl methionine and is introduced either prior to or during squalene cyclization.[16] The presence of these two hopanes in an anaerobic photosynthetic bacterium poses a number of interesting questions[17] about the mechanism and evolution of squalene cyclization.

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