Occurrence of Triterpenoids in Methanol-oxidizing Bacteria. 2-Methyl-22-hydroxyhopane from *Corynebacterium*

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A novel pentacyclic triterpenoid was isolated from a *Corynebacterium sp.* and identified by ^{13}C n.m.r. spectroscopy as 2-methyl-22-hydroxyhopane.

Isoprenoid compounds are widely distributed and some of them play important roles in the cells of living organisms.[1] However, little is known about isoprenoids in methanol-oxidizing bacteria. We now report the isolation and structure determination of a novel C_{31} pentacyclic triterpenoid from a prokaryotic organism.

The bacterium was a facultative methylotrophic strain *Corynebacterium* sp. XG, grown in a fermentor with FB2 medium containing 2% methanol (v/v).[2] The cells were extracted with methanol and the extract was saponified with 3% aqueous potassium hydroxide. The unsaponified fraction was obtained by extraction with light petroleum. Separation and purification were performed by preparative h.p.l.c. using a Lichroprep RP8 column. The major component, $C_{31}H_{54}O$, was isolated with light petroleum–acetone (97 : 3) as eluant (0.17% of cell dry weight): M^+, m/z 442, m.p. 184—186 °C, $[\alpha]_D^{29}$ +0.64°.

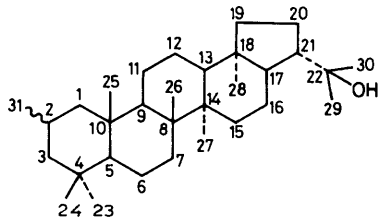
I.r., ^1H and ^{13}C n.m.r., and mass spectrometry indicated that the compound was 2-methyl-22-hydroxyhopane (2-methyldiplopterol). The mass spectrum is very similar to spectra of most hopanoid compounds[3,4] and its 250 MHz ^1H n.m.r. spectrum is consistent with a 22-hydroxyhopane structure:[5,6] δ 0.78 (s, 3H), 0.85 (s, 3H), 0.89 (s, 3H), 0.90 (s, 3H), and 0.96 (s, 6H). We conclude that $C_{31}H_{54}O$ is a methyl homologue of hopan-22-ol.

The position of the methyl group was determined by ^{13}C n.m.r. spectroscopy, using a Bruker AM 200 spectrometer operating at 200 MHz in the Fourier-transform mode using a DEPT sequence.[7] The substitution profile of the carbon centres was determined; shift assignments (Table 1) relied on

Table 1. ^{13}C N.m.r. data.†

Carbon	(1)	Hopan-22-ol
C-1	45.4	40.2
C-2	24.6	18.6
C-3	49.9	42
C-4	32.5	33
C-5	51.3[a]	56
C-6	20.0[b]	18.6
C-7	32.7	32.9
C-8	42.1[c]	41.6
C-9	50.5	50.4
C-10	38.0	37.2
C-11	22.1[b]	20.8
C-12	24.6	23.8
C-13	49.0	49.5
C-14	42.2[c]	41.8
C-15	34.6	34.2
C-16	22.1	21.7
C-17	54.2	53.8
C-18	44.3	43.9
C-19	41.5	43.4
C-20	26.6	26.5
C-21	51.1[a]	50.9
C-22	73.9	73.5
C-23	31.1[d]	33.2
C-24	21.8	21.4
C-25	17	15.6
C-26	16.3	16.4
C-27	16.3	16.5
C-28	23.2	22.7
C-29	29.0	28.7
C-30	30.9[d]	30.8
C-31	26.1	—

† δ Values in p.p.m. downfield from $SiMe_4$. Values bearing the same superscript may be interchanged. Shifts for hopan-22-ol are inferred from data in ref. 8.



(1)

analogy with data for triterpenoids related to hopane[8] and additivity parameters for the prediction of ^{13}C chemical shifts in *trans*-decalin systems (Table 2).[9]

Table 2. Incremental calculation of the shifts for C-1, C-2, and C-3, with axial and equatorial orientations of the 2-methyl group.

Carbon		δ (expt.)	δ (calc.)
C-1	Me (*eq.*)	45.4	46.5
	Me (*ax.*)		43.5
C-2	Me (*eq.*)	24.6	25.7
	Me (*ax.*)		29.1
C-3	Me (*eq.*)	49.9	50.9
	Me (*ax.*)		48.0

The data in Table 1 show large shifts for the carbon atoms originally at δ *ca.* 42 in hopan-22-ol, which may be explained by the deshielding effect of the additional methyl group. On this basis the only position for this group is the 2-position of the hopane skeleton. This hypothesis was confirmed by calculation of C-1, C-2, and C-3 shifts by Beierbeck's method[9] (Table 2).

We conclude that the compound isolated is 2-methyl-22-hydroxyhopane (**1**) with the methyl group probably in the equatorial conformation. This type of methylated hopan-22-ol was first isolated by Natori *et al.*[10] from *Pseudomonas* without a precise determination of the position of the methyl group.

This work presents, to the best of our knowledge, the first example of a product biogenetically derived from squalene substituted in the 2-position. Some isolated hopanoid derivatives may have the same methyl position.[11]

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