Biosynthesis of Poriferasterol in *Ochromonas malhamensis*: ^{13}C NMR Assignment of the Isopropyl Methyl Groups of 2-Methylpentan-3-ol

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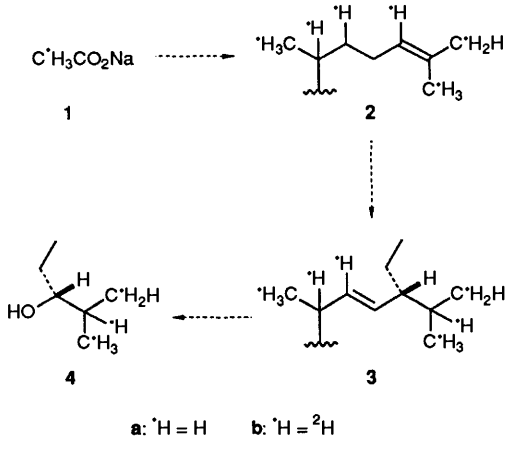
2-Methylpentan-3-ol stereospecifically labelled with deuterium on one of the isopropyl methyl groups has been synthesized, thus allowing the unequivocal assignment of their ^{13}C NMR resonances. ^{13}C NMR assignments for these groups proposed earlier are shown to be incorrect. Involvement in the biosynthesis of the 24β-ethylsterol poriferasterol in the crysophyte *Ochromonas malhamensis* is discussed.

In the course of work on the stereochemistry of the mechanism for introduction of the 24-alkyl group into the phytosterol side chain,[1] we have studied the stereochemistry of the hydride migration from C-24 to C-25 in the biosynthesis of poriferasterol **3a** in the crysophyte *Ochromonas malhamensis*.[1d,e] The problem was approached through a biosynthetic experiment in

which *Ochromonas malhamensis* was grown in the presence of sodium [2-$^2H_3$]acetate **1b** to yield the C_{29} sterol **3b** (see Scheme 1).



Scheme 1

On the basis of an NMR analysis of the hexadeuteriated 2-methylpentan-3-ol **4b**, obtained from **3b** by chemical degradation we established that migration of the hydrogen atom (a deuterium in our case) from C-24 to C-25 engages the *Si*-face of the 24(25)-double bond of the $\Delta^{24(25)}$-sterol intermediate **2b**.[1d,e] This analysis was based on the ^{13}C NMR assignments for the isopropyl methyl groups of 2-methylpentan-3-ol **4a** proposed in the literature.[2]

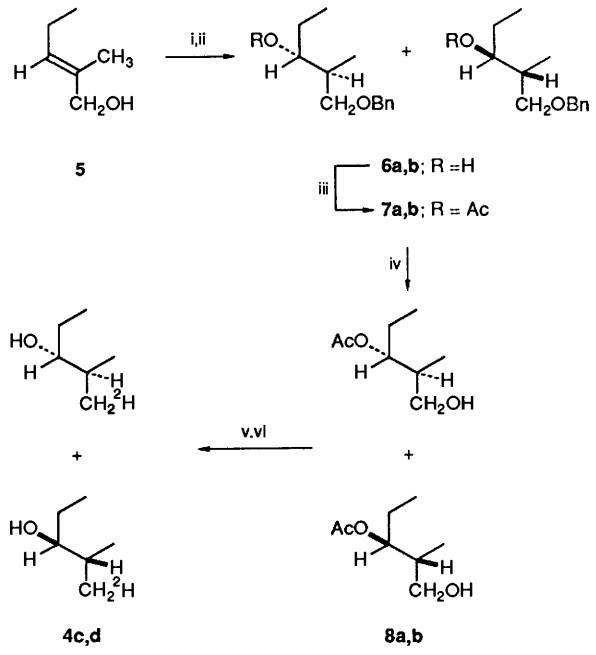
In this way the 2*S* configuration for the deuteriated alcohol **5b**—and hence the 25*S* configuration for the sterol **4b**—were established. Moreover, as a consequence of this biosynthetic result we assigned the ^{13}C resonances of C-26 and C-27 in the NMR spectrum of poriferasterol and of clionasteryl acetate.[3]

However, a later paper by Horibe *et al.*[4] reversed our assignments. A reexamination of our work indicated that the discrepancy between the conclusions of Horibe and our own could be only a consequence of a wrong assignment[2] for the ^{13}C NMR resonances of the diastereotopic methyls of 2-methylpentan-3-ol. An unequivocal assignment of these resonances, therefore, appeared necessary; here we report the chemical synthesis of 2-methylpentan-3-ol stereospecifically labelled with deuterium on one of the isopropyl methyl groups; this allows an unequivocal assignment of the methyl chemical shifts in the ^{13}C NMR spectrum.

As a first step (Scheme 2), (*E*)-2-methylpent-2-en-1-ol[5] **5** was submitted to benzylation, followed by hydroboration to yield a racemic mixture of the 3-alcohols (2*S*,3*R*)-**6a** and (2*R*,3*S*)-**6b**,* in which the C-2 stereochemistry was deduced from the established *cis*-addition mechanism of hydroboration, together with 30% (by NMR analysis) of the tertiary 2-alcohol. Direct acetylation of the hydroboration mixture, followed by chromatographic separation (light petroleum–ethyl acetate, 9:1) gave the pure 3-acetates **7a** and **7b**. Hydrogenolysis of the benzyl group in **7** was achieved using 10% Pd/C as the catalyst in ethyl acetate: the desired 1-hydroxy 3-acetates **8a** and **8b**, which were contaminated by *ca.* 10% of the acetyl migration product 3-hydroxy 1-acetate, were obtained pure by chromatography (light petroleum–ethyl acetate, 8:2).

Mesylation, reduction of the mesylate with $LiAl^2H_4$ in ether, and work-up with the procedure designed in ref. 1*e*, afforded a $CDCl_3$ solution of the desired alcohol stereospecifically deuteriated at C-1, *i.e.* the racemic (2*S*,3*R*)- and (2*R*,3*S*)-[1-^{2}H]-2-methylpentan-3-ol **4c** and **4d**.

The synthetic sequence just described ensures that the relative configuration at C-2 and C-3 of the deuteriated alcohol is dictated by the *E* configuration of the starting alkene; *i.e.* it is a racemic mixture of only two stereoisomers (2*S*,3*R*) and (2*R*,3*S*).



Scheme 2 *Reagents and conditions:* i, NaH, BnBr, THF, room temp., 4 h; ii, BH_3/THF, room temp., 18 h then H_2O_2/NaOH, 0 °C, 1 h; iii, Ac_2O, pyridine, room temp., 18 h; iv, H_2, Pd/C, AcOEt, 18 h; v, MsCl, Et_3N, CH_2Cl_2, 0 °C then room temp., 1 h; vi, $LiAl^2H_4$, Et_2O, room temp., 2 h

Table 1 ^{13}C NMR resonances[a] of compounds **4a** and **4c,d**

C	4a	4c,d
1	17.05	16.75[b]
1′	18.88	18.86
2	33.03	32.96
3	78.20	78.20
4	26.91	26.91
5	10.25	10.25

[a] Relative to $CDCl_3$ (77.00 ppm). [b] Triplet, *J* 19.1 Hz.

The proton decoupled ^{13}C NMR spectrum of **4c,d** showed (Table 1) the expected singlets for all the carbon atoms, with the exception of a triplet at 16.75 ppm (*J* 19.1 Hz) which could, therefore, be easily attributed to the carbon atom bearing the deuterium atom. The two isopropyl methyl groups in unlabelled **4a** resonate at 17.05 and 18.88 ppm; the triplet at 16.75 ppm in

* All the new compounds have elemental analyses consistent with the proposed structures. NMR data (solvent $CDCl_3$):† **6a,b**, δ_H(200 MHz) 0.92 (3 H, d, $J_{1'.2}$ 7.5, 1′-H_3), 0.98 (3 H, t, $J_{4.5}$ 7.5, 5-H_3), 1.44 (1 H, ddq, $J_{3.4a}$ 7.5, $J_{4a.4b}$ 14.5, 4-H_a), 1.61 (1 H, ddq, $J_{3.4b}$ 3.5, 4-H_b), 1.88 (1 H, dddq, $J_{2.3}$ 7.5, $J_{1a.2}$ 7.5, $J_{1b.2}$ 4.5, 2-H), 3.48 (1 H, dddd, $J_{3.OH}$ 3.5, 3-H), 3.49 (1 H, dd, $J_{1a.1b}$ 9.0, 1-H_a), 3.62 (1 H, dd, 1-H_b), 3.31 (1 H, br d, OH), 4.53 (2 H, s, CH_2Ph) and 7.20–7.40 (5 H, m, Ph); δ_C(50.3 MHz, relative to $CDCl_3$, δ_C 77.00) 9.51, 13.89, 27.44, 37.83, 73.36, 75.00, 77.10, 127.53, 127.63, 128.34 and 137.77; **7a,b**: δ_H(200 MHz) 0.90 (3 H, t, $J_{4.5}$ 7.5, 5-H_3), 0.99 (3 H, d, $J_{1'.2}$ 7.0, 1′-H_3), 1.40–1.80 (2 H, m, 4-H_2), 2.03 (3 H, s, $COCH_3$), 2.10 (1 H, m, 2-H), 3.31 (1 H, dd, $J_{1a.2}$ 7.0, $J_{1a.1b}$ 9.5, 1-H_a), 3.49 (1 H, dd, $J_{1b.2}$ 5.5, 1-H_b), 4.50 (2 H, s, CH_2Ph), 4.89 (1 H, ddd, $J_{3.4a}$ 8.0, $J_{3.4b}$ 4.5, $J_{2.3}$ 6.0, 3-H) and 7.20–7.40 (5 H, m, Ph); δ_C(50.3 MHz, relative to $CDCl_3$, δ_C 77.00) 9.35, 13.35, 20.72, 23.64, 36.33, 71.84, 72.74, 76.39, 127.19, 127.30, 128.03, 138.29, 170.39; **8a,b**: δ_H(200 MHz) 0.89 (3 H, t, $J_{4.5}$ 7.5, 5-H_3), 0.98 (3 H, d, $J_{1'.2}$ 7.0, 1′-H_3), 1.40–1.90 (3 H, m, 2-H and 4-H_2), 2.08 (3 H, s, $COCH_3$), 2.26 (1 H, m, OH), 3.51 (2 H, m, 1-H_2) and 4.79 (1 H, ddd, $J_{3.4b}$ 8.0, $J_{3.4b}$ 4.0, $J_{2.3}$ 8, 3-H); δ_C(50.3 MHz, relative to $CDCl_3$, δ_C 77.00) 9.36, 13.11, 20.79, 23.95, 38.56, 63.92, 76.72 and 171.46.

† *J* Values in Hz.

4c,d corresponds to the signal at 17.05 ppm, which is shifted to higher fields owing to the α isotopic shift. Thus, the signal at 17.05 can be assigned to the *pro-S* methyl group of the unlabelled 3*R* alcohol and to the *pro-R* methyl group of the unlabelled 3*S* alcohol. Conversely, the signal at 18.88 ppm is due to the *pro-R* methyl of the 3*R* alcohol and to the *pro-S* methyl of the 3*S* alcohol.

These assignments on which we based our biosynthetic investigations,[1d,e] are the reverse of those reported.[2] Consequently, in agreement with the results of Horibe *et al.*,[4] the assignment of C-26 and C-27 in the ^{13}C NMR spectrum of poriferasterol and clionasterol, previously reported by us,[3] have to be reversed. Moreover, the migration of the hydrogen atom from C-24 to C-25 during the biosynthesis of poriferasterol **3a** in the crysophyte *O. malhamensis* actually engages the *Re*-face of the 24(25) double bond of the intermediate **2a**.

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