Stereochemistry of Hydride Migration from C-24 to C-25 in the Biosynthesis of Poriferasterol in *Ochromonas malhamensis*

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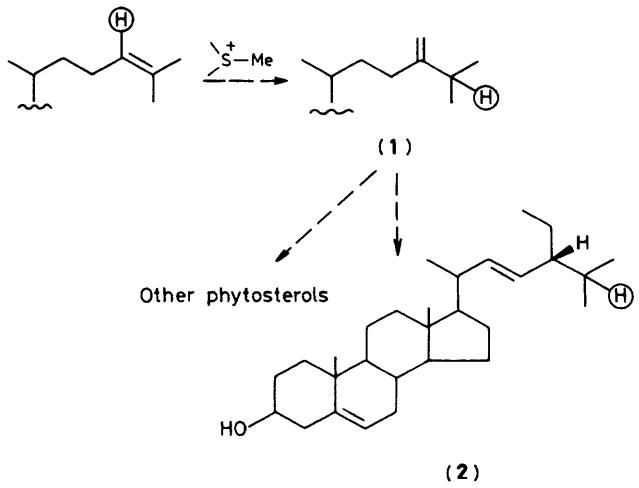
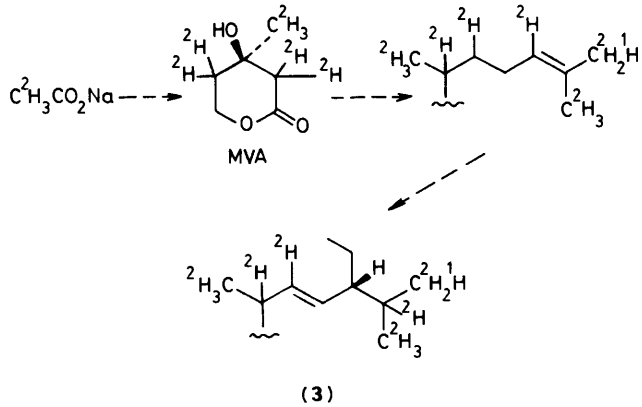
^{2}H-N.m.r. analysis of (2*S*,3*R*)-2-([$^2H_2$]methyl)-[1,1,1,2-$^2H_4$]pentan-3-ol obtained from [$^2H_{22}$]poriferasterol, biosynthesized in the Chrysophyte *Ochromonas malhamensis* from $C^2H_3CO_2Na$, shows that the hydride migration from C-24 to C-25 occurs on the *si*-face of the Δ^{24}-precursor double bond.

The mechanism of the transmethylation reaction through which phytosterols are formed from a Δ^{24}-sterol precursor implies, in most cases, the formation of a 24-methylene intermediate (**1**) (Scheme 1).

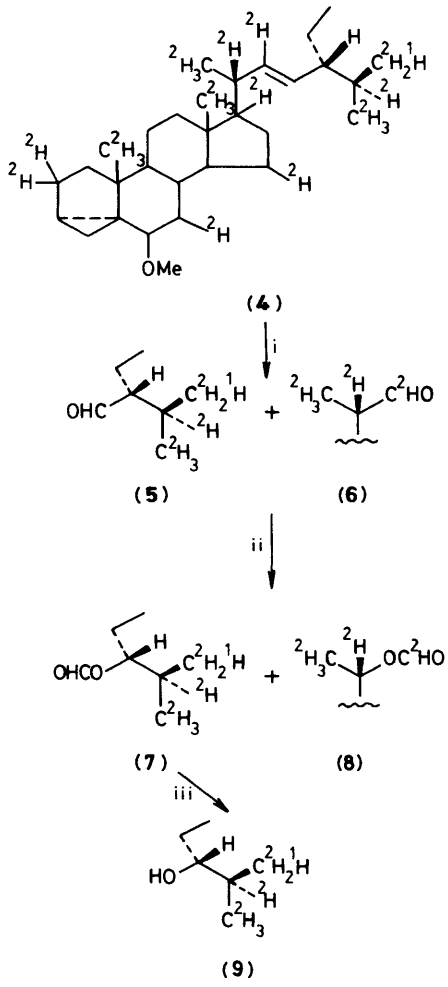
An important feature of this process is the 1,2-hydride migration from C-24 to C-25; the stereochemistry of this migration has been studied in the higher plants (*e.g.* the biosynthesis of isofucosterol in *Pinus pinea*),[1] in which the H-24 → 25 migration occurs on the *si*-face of the 24-double bond, and in fungi (*e.g.* the biosynthesis of ergosterol in *Claviceps paspali*),[2] in which the migration occurs on the *re*-face. This major difference in the sterol biosynthesis requires the elucidation of this stereochemical aspect in other species.



Scheme 1



Scheme 2



Scheme 3. *Reagents:* i, O_3; ii, Baeyer–Villager, *m*-chloroperbenzoic acid; iii, $LiAlH_4$.

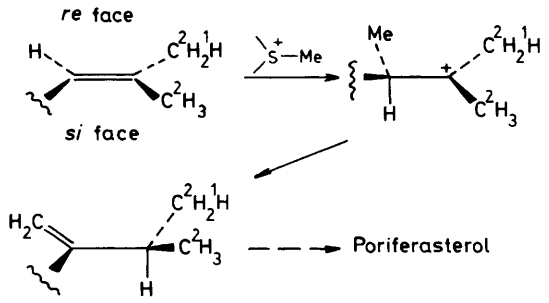
Moreover, as the stereochemistry of the hydride migration from C-24 to C-25 is strictly linked to the side of attack of *S*-adenosylmethionine,[1,2] the knowledge of this stereochemistry would throw further light on the topological relationship between the substrate and the active site of the enzyme operating in the methylation.

Accordingly, we decided to study this stereochemical problem in the Chrysophyte *Ochromonas malhamensis* which, being a phytoflagellate, is quite different from higher plants and fungi. Its main sterol, the C_{29} phytosterol poriferasterol (**2**) is formed, as with isofucosterol and ergosterol, *via* (**1**) and hence migration of the hydride from C-24 to C-25 is involved.[3]

Our approach was based on the fact that, using $C^2H_3CO_2H$ as the precursor, the biosynthesized sterol (**3**) (Scheme 2) should have, according to its biosynthetic origin, three deuterium atoms in one of the two isopropyl methyl groups (which originates from C-6 of mevalonic acid, MVA) and two deuterium atoms in the other one (which originates from C-2 of MVA), so allowing the stereospecific analysis of the poriferasterol isopropyl group.

O. malhamensis (933/1A, Cambridge Culture Collection), grown in the presence of 1% glucose and 0.05% sodium [2-$^2H_3$]acetate (99% ^2H, Merck, Sharp and Dohme) at 27 °C in the light for 4 days afforded[4] [$^2H_{22}$]poriferasterol (**3**) (2.23 g from 48 l of shaken culture[5]).

The C-25 configuration of the biosynthetic [$^2H_{22}$]poriferasterol (**3**) was determined by studying the C(24)—C(29) fragment of (**3**) according to Scheme 3: the 22,23-double bond of [$^2H_{22}$]poriferasterol methyl ether (**4**) was cleaved by ozonolysis in CH_2Cl_2–pyridine (1%) and the reaction mixture, containing the aldehydes (**5**) and (**6**), was directly submitted to Baeyer–Villiger oxidation to afford the formates (**7**) and (**8**). The volatile phase from the reaction mixture, containing the formate (**7**), was reduced with $LiAlH_4$, after change of the solvent from CH_2Cl_2 to Et_2O, to yield (2*S*,3*R*)-2-([$^2H_2$]methyl)[1,1,1,2-$^2H_4$]pentan-3-ol (**9**).



Scheme 4

The proton decoupled ^2H n.m.r. spectrum of (**9**) in the presence of tris dipivaloylmethanatoeuropium(III) [Eu(dpm)$_3$] (molar ratio 2.3 : 1) showed two resonances at 5.89 and 6.29 p.p.m. for the prochiral methyl groups, which were sufficiently resolved for reliable integration to be possible. As the above signals were in a 3 : 2 ratio, we assigned the upfield signal to C^2H_3 and the downfield signal to $C^2H_2{}^1H$.

This assignment was confirmed by the broadening only of the low field resonance ($\Delta\nu_{\frac{1}{2}}$ 2.34 → 4.30 Hz) in the ^2H n.m.r. spectrum of the same sample, run in the ^1H-coupled mode.

Taking into account the conformational studies by Williamson *et al.*[6] on aliphatic alcohols, in which the ^{13}C resonances of the *pro-R* and *pro-S* methyl groups of 2-methylpentan-3-ol are assigned, we were able to correlate the ^{13}C resonances with the ones due to the methyl groups in the ^2H n.m.r. spectrum. This was accomplished by running selective ^1H-irradiations of the two prochiral methyl resonances during ^{13}C n.m.r. observations of a sample of 2-methylpentan-3-ol after the addition of increasing concentrations of Eu(dpm)$_3$.

Consequently, the low-field signal ($C^2H_2{}^1H$ in the ^2H spectrum) is due to the *pro-R* methyl and the high field signal (C^2H_3 in the ^2H spectrum) to the *pro-S* methyl. These data clearly indicate that, in poriferasterol biosynthesized by *O. malhamensis*, the isopropyl methyl group coming from C-2 of MVA occupies the *pro-R* position, whereas the methyl coming from C-6 occupies the *pro-S* one. Owing to the known *pro-E* orientation of the isopropylidene methyl from C-2 of MVA in the Δ^{24}-sterol precursor, it follows that the 1,2-hydride migration engages the *si*-face of the substrate double bond (Scheme 4), as was found in *P. pinea*.

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