Origin of the 28-*pro-R* and 28-*pro-S* Hydrogens in the Biosynthesis of Poriferasterol in *Ochromonas malhamensis*

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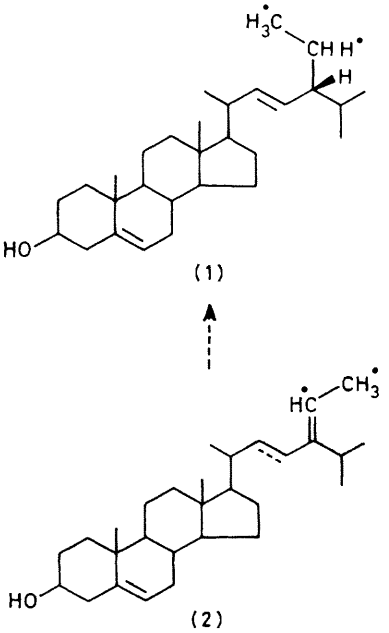
Summary It is shown, by ^2H n.m.r. spectroscopy, that in the biosynthesis of poriferasterol in *Ochromonas malhamensis* the hydrogen atom at C-28 arising from *S*-adenosylmethionine assumes the 28-*pro-S* position, whereas the hydrogen atom coming from the reducing system assumes the 28-*pro-R* position.

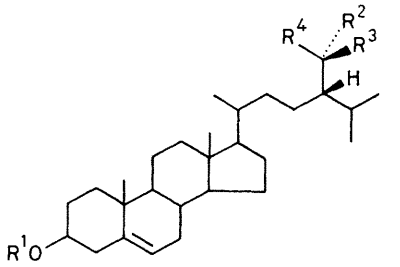
MOST C_{29} phyto-sterols are characterized by an ethyl-group at C-24, which arises by a double transmethylation from *S*-adenosylmethionine;[1-3] even though the mechanism of this process has been extensively studied,[4-9] its stereochemical aspects have been less-well investigated. It is difficult to determine the stereochemistry of the hydrogen atoms at C-28 by the usual tracer techniques (*i.e.* tritium) owing to the lack of chemical and/or biological reactions allowing stereospecific analysis of the side chain. A more promising approach to the solution of these problems seems to be the ^2H n.m.r. technique, provided that there is a reasonable ^2H enrichment in the biosynthetic sample and that the diastereotopic hydrogens at C-28 have different chemical shifts.

We now report the application of this technique to the study of the stereochemistry of the hydrogen atoms at C-28 in the biosynthesis of poriferasterol (**1**), the major[10] sterol of the chrysophyte alga *Ochromonas malhamensis*.

The formation of this sterol in *Ochromonas malhamensis* occurs *via* an intermediate with a 24(28)-double bond (**2**),[4] the ethylidene-group of which, arising from *S*-adenosylmethionine, is subsequently reduced to the 24-ethyl-group (Scheme 1). This mechanism implies that poriferasterol which is biosynthesized in *Ochromonas malhamensis* from L-[*methyl*-$^2H_3$]methionine will contain a ^2H atom at C-28 arising from methionine and a ^1H atom introduced by the $\Delta^{24(28)}$-reductase present in the alga. To determine the stereochemistry of the above atoms by ^2H n.m.r. spectroscopy, we had to know the chemical shift values of the diastereotopic 28-H_R and 28-H_S hydrogens. These were measured on model compounds (24*S*,28*S*)-[28-^2H]- and (24*S*,28*R*)-[28-^2H]-3β-acetoxystigmast-5-ene (**3**) and (**4**), which were synthesized as follows: (24*S*)-6β-methoxy-3α,5-cyclostigmastan-28-one (**5**)[11] was reduced with $LiAl^2H_4$ and the diastereoisomeric deuteriated alcohols (**6**) and (**7**) obtained were separated by repeated preparative t.l.c. and their configurations at C-28 assigned[11] by Horeau's procedure. The alcohols (**6**) and (**7**) were then transformed into (**3**) and (**4**) by $LiEt_3BH$ reduction of the mesylates[11] and by acetolysis.[12]

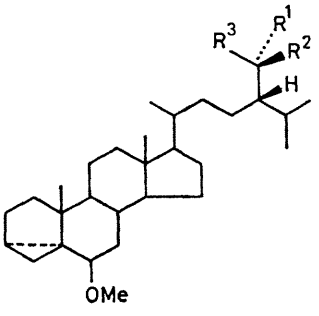


SCHEME. H• = Hydrogen from methionine.



(**3**) R^1 = Ac; R^2 = ^2H; R^3 = H; R^4 = Me
(**4**) R^1 = Ac; R^2 = H; R^3 = ^2H; R^4 = Me
(**8**) R^1 = Ac; R^2 = ^2H; R^3 = H; R^4 = C^2H_3; Δ^{22}
(**9**) R^1 = R^3 = H; R^2 = ^2H; R^4 = C^2H_3; Δ^{22}
(**11**) R^1 = Ac; R^2 = ^2H; R^3 = H; R^4 = C^2H_3

^2H N.m.r. analysis of the above model compounds (see Table) showed a difference between the chemical shifts of ^2H_R and ^2H_S sufficiently large to determine the position assumed by the 28-^2H atom in the biosynthetic poriferasterol. To obtain this compound, *Ochromonas mal-*



(**5**) R^1, R^2 = O; R^3 = Me
(**6**) R^1 = OH; R^2 = ^2H; R^3 = Me
(**7**) R^1 = ^2H; R^2 = OH; R^3 = Me
(**10**) R^1 = ^2H; R^2 = H; R^3 = C^2H_3; Δ^{22}

hamensis, 933/1A, Cambridge Culture Collection, was cultured at 27 °C in 20 ml batches[13] in the presence of L-[*methyl*-$^2H_3$]methionine (99% ^2H)[4] with continuous shaking. After two days the cells from ten flasks were centrifuged and saponified with 10% KOH in 80% aq. EtOH under reflux for 1 h. The unsaponifiable material (560 mg), extracted with diethyl ether, was chromatographed on silica gel to yield 93 mg of crude sterol fraction; acetylation and purification on 25% $AgNO_3/SiO_2$ yielded 65 mg of deuteriated poriferasteryl acetate (**8**) (checked by g.l.c., SE-30 2·5%, T_c 225 °C and OV-17 1%, T_c 260 °C, 98% pure). Hydrolysis of the compound (**8**) with methanolic KOH and crystallization from methanol afforded 57 mg of deuteriated poriferasterol (**9**) which showed, by mass spectrometry, the presence of the expected four deuterium atoms in the side chain (34·7% $^2H_0$, 22·9% $^2H_1$, 24·9% $^2H_3$, 17·5% $^2H_4$, 40·4% ^2H enrichment at C-28).

The tetradeuteriated (**9**) was transformed, using the method of Steele and Mosettig,[14] into (24*R*)-[$^2H_4$]6β-methoxy-3α,5-cyclostigmast-22-ene (**10**). Hydrogenation with 10% Pd on carbon and rearrangement with zinc acetate in boiling acetic acid[12] afforded 32 mg of tetradeuteriated (24*S*)-3β-acetoxystigmast-5-ene (**11**), the ^1H n.m.r. spectrum of which was indistinguishable from that of an authentic sample. The ^2H n.m.r. spectrum of the tetradeuteriated compound (**11**) (see Table) showed two peaks, the area ratio of which was 3 : 1; the former peak, at 0·81 p.p.m., corresponded to the trideuteriated C-29 methyl-group and the latter, corresponding to a single deuterium atom, was found at 1·13 p.p.m., a value essentially identical with that of (24*S*,28*S*)-[28-^2H]-3β-acetoxystigmast-5-ene (**3**).

TABLE.

Compound	Chemical shifts values in p.p.m.[a] 28-^2H	29-C^2H_3
(**3**)	1·19	—
(**4**)	1·33	—
(**11**)	1·13	0·81

[a] 'Resolution-enhanced' ^2H-n.m.r. spectra recorded on a Varian XL-200 instrument at 30·7 MHz in $CHCl_3$ solution using $CDCl_3$ at 7·24 p.p.m. as the internal standard.

These results indicate that in the biosynthesis of poriferasterol in *Ochromonas malhamensis* the hydrogen at C-28 arising from *S*-adenosylmethionine assumes the 28-*pro*-*S* position, whereas the hydrogen coming from the reducing system assumes the 28-*pro*-*R* position. Moreover, the absence of any detectable signal due to the 28-*R* deuterium, coupled with the known stereospecificity of the biological reduction processes, strongly suggests that only one of the *E*- and *Z*-24(28)-ethylidene sterols, which is transformed into poriferasterol in *Ochromonas malhamensis*,[15] is the real biosynthetic intermediate.

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