Biosynthesis of the C_{29}-Phytosterol Side-chain: Evidence that the Same Stereochemistry at C-25 can Originate from Different Mechanisms

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It has been demonstrated that in poriferasterol, biosynthesized in *Ochromonas malhamensis*, the hydrogen atom originally present at C-24 of the Δ^{24}-precursor is located at C-25.

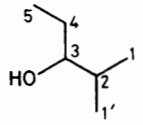
The biosynthesis of the phytosterol side-chain from a Δ^{24}-precursor (**1**) involves either one or two transmethylation processes, in which *S*-adenosylmethionine (SAM) is operating,[1] in order to obtain C_{28} or C_{29} alkyl sterols respectively (Scheme 1). Most phytosterols are formed through the intermediacy of a 24-methylene compound (**2**), which originates, after the methylation of the 24-double bond, by 1,2-hydride migration from C-24 to C-25, and proton loss from C-28. In principle, the configuration at C-25, which arises from the hydride migration, can be retained in the ultimate biosynthetic product or can be inverted as a consequence of mechanistic steps which could involve the C-25 centre.

The data in the literature concerning the configuration at C-25 shows lack of consistency: in some cases the C-26 (*pro-R*) methyl group is derived from C-2 of mevalonic acid (MVA)[1c,d,2] (all the C_{29} phytosterols studied so far belong to this group), while in other cases (all the C_{28} phytosterols except dihydrobrassicasterol isolated from *Physalis peruviana* callus[3]) the same *pro-R* methyl is derived from C-6 of MVA.[1b,2d,3]

Recently a Japanese group has isolated a C_{28} and a C_{29} phytosterol from tissue cultures of *Trichosanthes kirilowii*; 24-methylenecycloartanol (**2a**) and 22-dihydrochondrillasterol (**4b**), respectively. These are sequentially formed along the same biosynthetic pathway, and have opposite stereochemistry at C-25.[2d] It has been proposed that (**4b**) is derived from (**2a**) and that the opposite stereochemistry at C-25 of (**4b**) is due to back migration of the hydride from C-25 to C-24, followed by the formation of a 25(26)-double bond which is eventually reduced in order to yield (**4b**) with opposite stereochemistry at C-25 with respect to (**2a**).

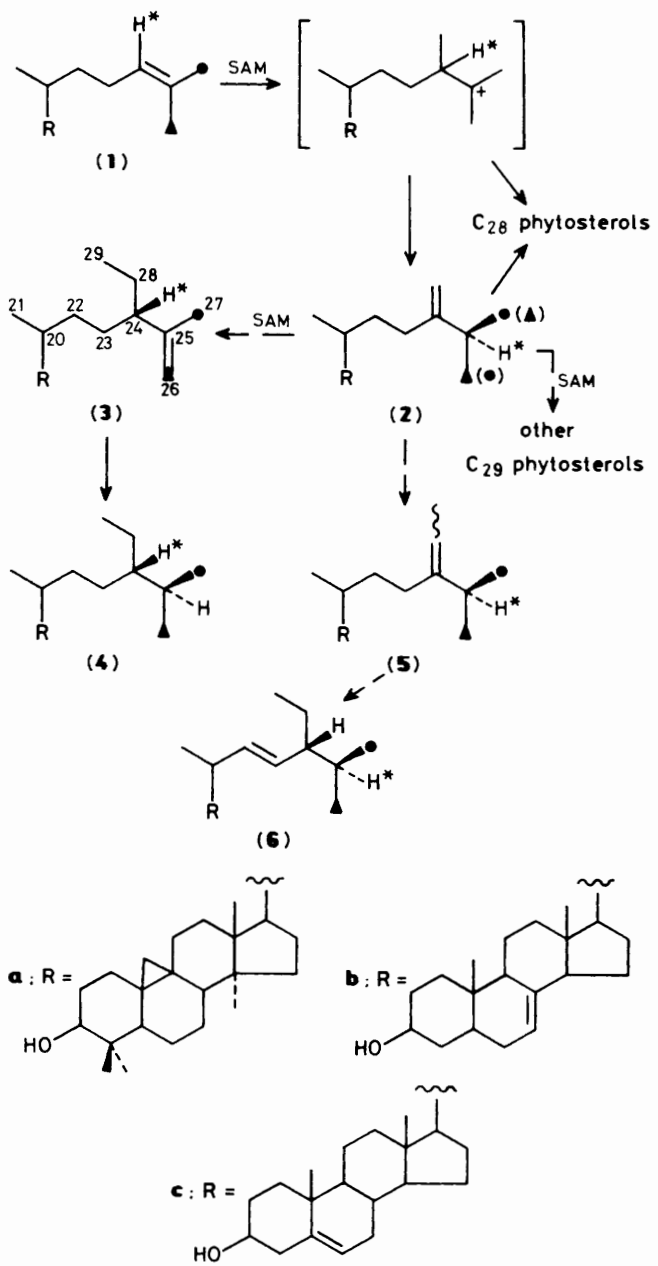
This hypothesis was supported by the observation that the deuterium label, derived from C-24 of the Δ^{24}-precursor, was present at C-24 rather than C-25 in the product and also the occurrence of a $\Delta^{25(26)}$-C_{29} sterol (**3b**) in *Trichosanthes kirilowii* callus. Thus in the above case the configuration at C-25 of dihydrochondrillasterol (**4b**) is linked to the stereochemistry of the saturation of the 25(26)-double bond.

Table 1. ^1H and ^2H n.m.r. data (Varian XL-200 spectrometer) for 2-methylpentan-3-ol (**7**) and (2*S*,3*R*)-2-[$^2H_2$]methyl[1,1,1,2,-$^2H_4$]pentan-3-ol (**8**).



	1	1′	2	3	4a	4b	5	OH
^1H n.m.r. of (**7**) ($CDCl_3$)	0.89[a]	0.90[b]	1.65[c]	3.36[d]	1.38[e]	1.51[f]	0.94[g]	1.34[h]
^2H n.m.r. of (**8**) ($CHCl_3$)		0.89	1.63	—	—	—	—	—

[a] d, *J* 6.7 Hz; [b] d, *J* 6.9 Hz; [c] dqq, *J* 5.0, 6.7, and 6.9 Hz; [d] ddd, *J* 5.0, 8.5, and 4.0 Hz; [e] ddq, *J* 8.5, 14.0, and 7.4 Hz; [f] ddq, *J* 4.0, 14.0, and 7.4 Hz; [g] t, *J* 7.4 Hz; [h] bs.



Scheme 1. ● From C-2 of MVA; ▲ from C-6 of MVA.

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Some years ago we reported a study of the biosynthesis of poriferasterol (**6c**)[1c,d]‡ in *O. malhamensis* establishing the origin of the isopropyl methyl groups of the side-chain and assigning to C-25 a configuration which was the same as that reported for dihydrochondrillasterol (**4b**). Here we report that the mechanism suggested for the biosynthesis of (**4b**) does not apply to the case of poriferasterol of *O. malhamensis*.

In 1972 Smith *et al.*[5] demonstrated, through incorporation experiments with [2-^{14}C,(4*R*)-4-^{3}H] MVA, that the tritium atom present in the C(23)—C(29) fragment of the side-chain of poriferasterol biosynthesized in *O. malhamensis* was not located at C-24, suggesting a 1,2-shift to the C-25 position.

We now give a definitive demonstration that this is indeed the case and that the hydrogen atom which was present at C-24 of the Δ^{24}-precursor is effectively located at C-25 in poriferasterol (**6c**). In fact, on analysis of the ^{2}H n.m.r. spectrum of the hexadeuteriated 2-methyl-pentan-3-ol (**8**) obtained by chemical degradation[1c,d] of the deuteriated poriferasterol biosynthesized in *O. malhamensis* from $C^{2}H_3CO_2Na$, we detected two signals only in the spectrum, whose chemical shifts are reported in the Table, together with the ^{1}H chemical shifts of a cold sample of 2-methyl-pentan-3-ol (**7**). The Table clearly shows that a deuterium atom is present at C-2 of the alcohol, which corresponds to the C-25 position of poriferasterol.

So, in poriferasterol obtained from *O. malhamensis* the back shift of hydride does not take place; this fact suggests that the C-25 carbon atom is not involved in further stereochemical events following the first 1,2-hydride shift and that the configuration at C-25 at the end of the biosynthetic pathway reflects the stereochemistry of the first approach of SAM to the 24-double bond of the precursor.[1b—d]

In this respect the methylation of the Δ^{24} precursor is thought to happen with opposite stereochemistry in *O. malhamensis* and in *T. kirilowii*.

‡ It is known that (**6c**) is formed in *O. malhamensis* from (**2**) through the intermediacy of a 24(28)-ethylidenesterol (**5c**).[4]

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References

1 (a) L. J. Goad, J. R. Lenton, F. F. Knapp, and T. W. Goodwin, *Lipids*, 1974, **9**, 582; (b) D. Arigoni, Ciba Foundation Symposium 60, 1978, 243; (c) F. Nicotra, F. Ronchetti, G. Russo, L. Toma, P. Gariboldi, and B. M. Ranzi, *J. Chem. Soc., Chem. Commun.*, 1984, 383; (d) F. Nicotra, F. Ronchetti, G. Russo, L. Toma, P. Gariboldi, and B. M. Ranzi, *J. Chem. Soc., Perkin Trans. 1*, 1985, 521.

2 (a) F. Nicotra, F. Ronchetti, G. Russo, G. Lugaro, and M. Casellato, *J. Chem. Soc., Perkin Trans. 1*, 1981, 498; (b) S. Seo, A. Uomori, Y. Yoshimura, and K. Takeda, *J. Am. Chem. Soc.*, 1983, **105**, 6343; (c) S. Seo, U. Sankawa, H. Seto, A. Uomori, Y. Yoshimura, Y. Ebizuka, H. Noguchi, and K. Takeda, *J. Chem. Soc., Chem. Commun.*, 1986, 1139; (d) S. Seo, A. Uomori, Y. Yoshimura, U. Sankawa, Y. Ebizuka, H. Seto, and K. Takeda, *J. Chem. Soc., Chem. Commun.*, 1987, 1876.

3 S. Seo, A. Uomori, Y. Yoshimura, and K. Takeda, *J. Chem. Soc., Chem. Commun.*, 1984, 1174.

4 (a) F. F. Knapp, L. J. Goad, and T. W. Goodwin, *Phytochemistry*, 1977, **16**, 1683; (b) F. Nicotra, B. M. Ranzi, F. Ronchetti, G. Russo, and L. Toma, *J. Chem. Soc., Chem. Commun.*, 1980, 752.

5 A. R. H. Smith, L. J. Goad, and T. W. Goodwin, *Phytochemistry*, 1972, **11**, 2775.