The Stereochemistry of Hydrogen Elimination at C-6 and C-23 in Phytosterol Biosynthesis by *Ochromonas malhamensis*

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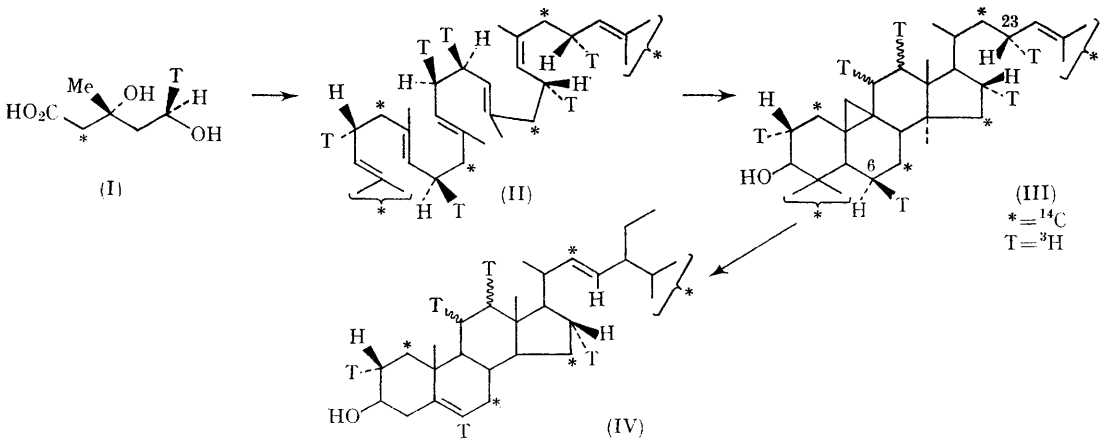
THE mechanism of introduction of double bonds into the sterol molecule is currently under investigation[1-3] and it has been established that the introduction of the Δ^5-double bond in cholesterol involves a *cis*-elimination of the 5α- and 6α-hydrogen atoms.[1,2] We have reported[4] the stereochemistry of hydrogen removal from C-22 during introduction of the *trans*-Δ^{22}-double bond into poriferasterol by *Ochromonas malhamensis*, and now report the stereochemistry of hydrogen elimination from C-6 and C-23 of poriferasterol, biosynthesised by this same organism.

Enzymic conversion[5] of (3*R*)-[2-^{14}C-(5*R*)-5-$^3H_1$]-mevalonic acid (MVA, I) into squalene (II) will result in stereospecific labelling with tritium as shown. Cyclisation of this squalene (II) by the accepted mechanism will give cycloartenol (III), the postulated phytosterol precursor,[6] with stereospecific tritium labelling at C-6 and C-23. The incorporation of this substrate into poriferasterol will therefore permit investigation of the stereochemistry of hydrogen elimination at C-6 and C-23 during introduction of the Δ^5- and Δ^{22}-double bonds into poriferasterol.

O. malhamensis was cultured as described previously[7] but with the addition of (3*R*)-[2-^{14}C-(5*R*)-5-$^3H_1$]-MVA† (5·0 μc of ^{14}C). The cells were harvested after 5 days and squalene (698 decomp./min. of ^{14}C; ^{3}H : ^{14}C = 12·60) and poriferasterol were isolated from the non-saponifiable lipid in the usual manner.[7] Carrier poriferasterol (200 mg.) was added and the sterol crystallised (m.p. 154°) to constant specific activity (4150 decomp./min. of ^{14}C/mg.; ^{3}H : ^{14}C = 12·88; corrected ^{3}H : ^{14}C atomic ratio‡ = 5·11 : 5). A portion of the poriferasterol (100 mg.) in diethyl ether (40 ml.) was stirred for 2 hr. at room temperature with chromic acid solution (2 ml., prepared by dissolving 1 g. $K_2Cr_2O_7$ in 6·0 ml. of water and 1 ml. of conc. H_2SO_4). The resulting (24*S*)-ethylcholesta-4,22-diene-3,6-dione, m.p. 151—153°, was isolated and purified by crystallisation (4085 decomp./min. of ^{14}C/mg., ^{3}H : ^{14}C = 10·66; corrected ^{3}H : ^{14}C atomic ratio = 4·23 : 5). The observed decrease in the ^{3}H : ^{14}C ratio on formation of the C-6 ketone shows that a tritium atom was located at this position in poriferasterol (IV) and consequently the 6α-hydrogen must have been eliminated during introduction of the Δ^5-double bond. This agrees with previous reports for cholesterol biosynthesis in an animal system.[1,2]

The corrected ^{3}H : ^{14}C atomic ratio for the poriferasterol (5·11 : 5), coupled with the above results, indicate that tritium had probably been



† The (3*R*)-[5*R*-5-$^3H_1$]-MVA used in this work was synthesised in our Department (R. J. H. Williams, G. Britton, J M. Charlton, and T. W. Goodwin, *Biochem. J.*, 1967, **104**, 767).

‡ The corrected ^{3}H : ^{14}C atomic ratios were calculated on the basis of the squalene ^{3}H : ^{14}C ratio, *i.e.* the squalene ^{3}H : ^{14}C ratio (12·60) represents a ^{3}H : ^{14}C atomic ratio of 6 : 6.

lost from C-23 upon introduction of the *trans*-Δ^{22}-double bond. This was confirmed by ozonolysis of a second portion of poriferasterol (100 mg., 705 decomp./min. of ^{14}C/mg., $^{3}H:^{14}C = 12{\cdot}93$) and isolation of the side-chain fragment, 2-ethyl-3-methylbutanal, as its dimedone derivative. The dimedone compound, m.p. 128—130°, contained 148 decomp./min. of ^{14}C/mg. but was virtually devoid of tritium ($^{3}H:^{14}C = 0{\cdot}66$; corrected $^{3}H:^{14}C$ atomic ratio = 0·05 : 1). This result therefore establishes that the 23-*pro-R* hydrogen is eliminated during introduction of the *trans*-Δ^{22}-double bond into poriferasterol by *O. malhamensis*. We have already shown that the 22-*pro-R* hydrogen is eliminated in the elaboration of this sterol.[4] The results thus indicate that the mechanism of introduction of the Δ^{22}-double bond of plant sterols is similar to that operative in the formation of unsaturated fatty acids.[8] In contrast, our preliminary work[9] with fungi indicates that it is the 22-*pro-S* and 23-*pro-S* hydrogens which are eliminated during ergosterol biosynthesis.

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