The Stereochemistry of Hydrogen Elimination at C(7) and C(22) in Phytosterol Biosynthesis by *Ochromonas malhamensis*

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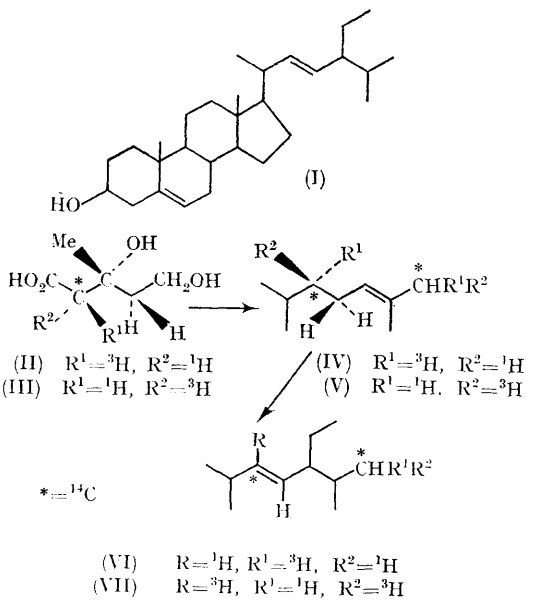
A NUMBER of phytosterols are characterised by a Δ^{22} bond in the side-chain.[1] Little is known about the mechanism of formation of the Δ^{22} bond but, as would be expected, a hydrogen atom is eliminated from C(23) during introduction of this double bond into ergosterol.[2] We now report the use of 3*R*-[2-^{14}C-(2*R*)-2-$^{3}H_1$]mevalonic acid [MVA, (II)] and 3*R*-[2-^{14}C-(2*S*)-2-$^{3}H_1$]MVA (III) to study the stereospecific hydrogen eliminations at C(7) and C(22) during the biosynthesis of poriferasterol (I) by *Ochromonas malhamensis*.

Two cultures of *O. malhamensis* were grown as previously described[3] but with the addition to the growth medium of either 3*R*-[2-^{14}C-(2*R*)-2-$^{3}H_1$]MVA (II) (2 μc of ^{14}C; ^{3}H : ^{14}C = 8·58) or 3*R*-[2-^{14}C-(2*S*)-2-$^{3}H_1$]MVA (III) (2·0 μc of ^{14}C; ^{3}H : ^{14}C = 8·59). The cells from the two experiments were harvested after six days and treated in an identical manner. The non-saponifiable lipid was chromatographed on silica gel thin layers and the sterol band eluted. Poriferasterol (100 mg.) was added to a portion of the radioactive sterol and crystallised to constant specific activity. Acetylation, followed by oxidation[4] gave 3β-acetoxy-24*S*-ethylcholesta-5,22-dien-7-one, m.p. 188—191°, purified by chromatography and crystallisation. A second portion of the radioactive poriferasterol, after addition of carrier sterol, was converted into



24-ethyl-cholesta-4,22-dien-3-one by Oppenauer oxidation.[5] Ozonolysis then gave 3-oxobisnor-4-cholen-22-al, m.p. 154—156° which was finally converted into 3-oxobisnor-4-cholenic acid, m.p.

266—269° (decomp.) by treatment with chromic acid.[6] The results are given in the Table.

The decrease in the $^3H:^{14}C$ ratio observed when the poriferasterol biosynthesised from 3*R*-[2-^{14}C-(2*R*)-2-$^3H_1$]MVA (II) was converted into 3β-acetoxy 24*S*-ethylcholesta-5,22-dien-7-one demonstrates the biosynthetic retention of the C(7) hydrogen derived from the 2-pro*R* hydrogen[7] of MVA. By contrast, the much smaller fall in the $^3H:^{14}C$ ratio upon formation of the 7-ketone, when 3*R*-[2-^{14}C-(2*S*)-2-$^3H_1$]MVA is the poriferasterol precursor, shows the biosynthetic loss of the 7β-hydrogen derived from the 2-pro*S* hydrogen of MVA. This is in complete accord with results obtained for cholesterol biosynthesis in liver.[8]

Incorporation o 3*R*-[2-^{14}C-(2*R*)-2-$^3H_1$]MVA *and* 3*R*-[2-^{14}C-(2*S*)-2-$^3H_1$]MVA *into poriferasterol in* Ochromonas malhamensis

	3*R*-[2-^{14}C-(2*R*)-2-$^3H_1$]MVA		3*R*-[2-^{14}C-(2*S*)-2-$^3H_1$]MVA	
	Specific activity d.p.m. of ^{14}C/mg.	$^3H:^{14}C$ ratio	Specific activity d.p.m. of ^{14}C/mg.	$^3H:^{14}C$ ratio
Poriferasterol	1728	4·65	1840	3·80
3β-Acetoxy-24*S*-ethylcholesta-5,22-dien-7-one	1510	3·90	1670	3·66
24-Ethylcholesta-4,22-dien-3-one	—	4·68	—	3·88
3-Oxobisnor-4-cholen-22-al	1715	4·45	1790	3·63
3-Oxobisnor-4-cholenic acid	1685	4·00	1885	2·21

Incorporation of (II; 2*R*) or (III; 2*S*) into the side-chain of the poriferasterol precursor sterol will give tritium at C(22) in the configuration shown in (IV; 22*R*) and (V; 22*S*) respectively. The large drop in the $^3H:^{14}C$ ratio upon oxidation of the 3-oxobisnor-4-cholen-22-al obtained from the 3*R*-[2-^{14}C-(2*S*)-2-$^3H_1$]MVA (III) culture proves that a tritium atom was located at C(22) and therefore that the C(22) proton of poriferasterol was derived from the 2-pro*S* hydrogen of MVA [(V) → (VII)]. Oxidation of the 3-oxobisnor-4-cholen-22-al obtained by ozonolysis of the poriferasterol from the 3*R*-[2-^{14}C-(2*R*)-2-$^3H_1$]MVA (III) incubation resulted in only a relatively small decrease in the $^3H:^{14}C$ ratio, thus showing the presence of only a small amount of tritium at C(22) of the poriferasterol. The two results demonstrate that the C(22) hydrogen originating from the 2-pro*R* hydrogen of MVA [*i.e.* the C(22) pro*R* hydrogen] is lost in the introduction of the Δ^{22} bond into poriferasterol [(IV) → (VI)] by *O. malhamensis*. The retention of some tritium at C(22) from the [2*R*-2-$^3H_1$]MVA and at C7 from the [2*S*-2-$^3H_1$]-MVA is probably due to loss of stereospecificity caused by the reversibility of the isopentenyl pyrosphosphate–dimethyl allyl pyrophosphate enzymic isomerisation.[9] This equilibration would also explain the unexpectedly large drop in the $^3H:^{14}C$ ratios observed upon conversion of the doubly labelled mevalonates into sterol. These findings will be discussed in more detail elsewhere.

We thank the S.R.C. and Unilever for financial support.

(*Received, June 4th,* 1968; *Com.* 721.)

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