Biosynthesis of Plant Sterols: Stereochemistry of Hydrogen Elimination at C-7 in α-Spinasterol

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Summary It is indicated that the 7β-hydrogen of lanosterol derived from the 2-pro*S* hydrogen of mevalonic acid (MVA) is removed in the biosynthesis of α-spinasterol.

IT has been shown previously[1] that the biosynthesis of cholesterol in a rat liver preparation involves a 5,7-diene intermediate and that during the formation of this intermediate the 7β-hydrogen of lanosterol is eliminated. The 7β-hydrogen of lanosterol was shown also to be lost when the biosynthesis of poriferasterol was investigated in *Ochromonas malhamensis*.[2] However, in the yeast homogenate, the biosynthesis of C-27 cholesteryl analogues takes place by the elimination of the 7α-hydrogen *via* the formation of a Δ^7-olefin.[3]

Since α-spinasterol (I) has a Δ^7 bond, we evaluated the mechanism of the formation of this double bond in this phytosterol.

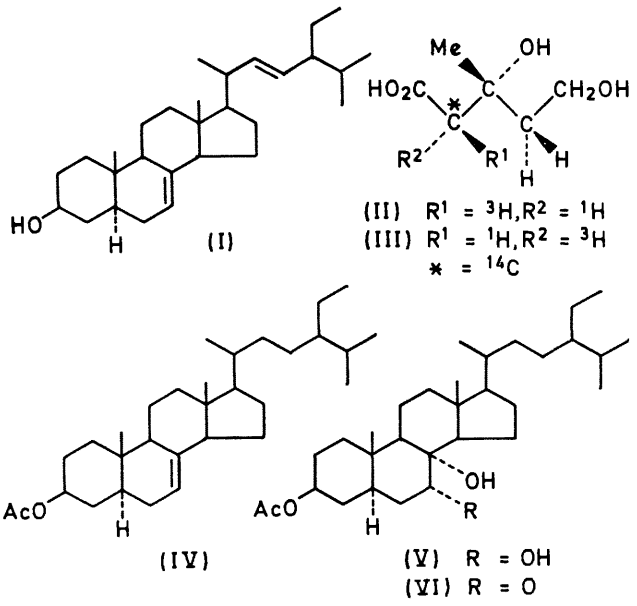
3*R*-[2-^{14}C-(2*R*)-2-$^3H_1$]MVA (II) (^{3}H : ^{14}C ratio 9·37, 50 μc of ^{14}C) or 3*R*-[2-^{14}C-(2*S*)-2-$^3H_1$]MVA (III) (^{3}H : ^{14}C ratio 9·74, 50 μc of ^{14}C) was administered to the *Camellia sinensis* plant.[4] The plant was harvested after five weeks and α-spinasterol (I) was isolated from the non-saponifiable lipid.[4] The carrier α-spinasterol was added and the sterol crystallized to constant specific activity and ^{3}H : ^{14}C ratio.

The ^{3}H : ^{14}C ratio obtained by the incorporation of [2*R*-2-^{14}C-2-^{3}H]mevalonic acid was 7·27 (atomic ratio 3·82 : 5), indicating the presence of four tritium atoms. The ^{3}H : ^{14}C ratio obtained by the incorporation of [2*S*-2-^{14}C-2-^{3}H]mevalonic acid was 5·90 (atomic ratio 3·03 : 5), indicating the presence of three tritium atoms.

The α-spinasterol biosynthesized from [2*S*-2-^{14}C-2-^{3}H]-MVA (III) was first acetylated and then hydrogenated over platinum oxide catalyst to give 3β-acetoxy-5α(*H*)-stigmast-7-ene[5] (IV) (^{3}H : ^{14}C 5·78, atomic ratio 2·96 : 5). The reaction of (IV) with osmium tetroxide in pyridine[6] yielded 3β-acetoxy-7α,8α-dihydroxy-5α(*H*)-stigmastane (V) (^{3}H : ^{14}C ratio 5·75, atomic ratio 2·95 : 5). Oxidation of (V) (chromium trioxide–pyridine) gave 3β-acetoxy-8α-hydroxy-5α(*H*)-stigmastan-7-one (VI) without the loss of tritium (^{3}H : ^{14}C ratio 6·30, atomic ratio 3·23 : 5). This proves the absence of tritium atom at the C-7 position in the biosynthesized α-spinasterol isolated from *Camellia sinensis*. It also indicates that the 7β-hydrogen of lanosterol derived from the 2-pro*S* hydrogen of MVA is removed in the formation of this phytosterol. Since the biosynthesized α-spinasterol derived from [2*R*-2-^{3}H]MVA has four tritium atoms, it is inferred that there is no tritium at 22*R*. This supports the earlier observation[2] that the C-22 hydrogen originating from the 2-pro*R* hydrogen of MVA is lost in the introduction of the Δ^{22} bond.

The ^{3}H : ^{14}C *ratios of the products obtained after the administration of* 3*R*-[2-^{14}C-(2*R*)-2-$^3H_1$]MVA (^{3}H : ^{14}C *ratio* 9·37) *and* 3*R*-[2-^{14}C-(2*S*)-2-$^3H_1$]MVA (^{3}H : ^{14}C *ratio* 9·74) *to a* Camellia sinensis *plant*

	3*R*-[2-^{14}C-(2*R*)-2-$^3H_1$]MVA ^{3}H : ^{14}C Ratio	3*R*-[2-^{14}C-(2*S*)-2-$^3H_1$]MVA ^{3}H : ^{14}C Ratio
α-Spinasterol	7·27	5·90
3β-Acetoxy-5α(*H*)-stigmast-7-ene		5·78
3β-Acetoxy-7α,8α-dihydroxy-5α(*H*)-stigmastane		5·75
3β-Acetoxy-8α-hydroxy-5α(*H*)-stigmastan-7-one		6·30



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