Short Communications

The Stereochemistry of Hydrogen Elimination at C-7, C-22 and C-23 during the Conversion of Cholesterol (Cholest-5-en-3 β -ol) into Cholesta-5,7,22-trien-3 β -ol by *Tetrahymena pyriformis*

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The stereochemical course of the introduction of double bonds during sterol biosynthesis has recently been the subject of extensive investigations (Akhtar & Marsh, 1967; Canonica *et al.* 1968*a,b*; Caspi, Greig, Ramm & Varma, 1968*a*; Dewhurst & Akhtar, 1967; Gibbons, Goad & Goodwin, 1968*a*; Paliokas & Schroepfer, 1968). In our laboratory we have been particularly interested in the stereochemistry of hydrogen elimination at C-22 and C-23 during the formation of the *trans*- Δ^{22} bond in algal and fungal sterols (Bimpson, Goad & Goodwin, 1969; Smith, Goad & Goodwin, 1968*a,b*). A report by Mallory, Conner, Landrey & Iyengar (1968) demonstrated that the protozoan *Tetrahymena pyriformis* can absorb cholesterol (cholest-5-en-3 β -ol) from the medium and convert it into cholesta-5,7,22-trien-3 β -ol. We have therefore extended our studies to determine the stereochemistry of hydrogen eliminations from C-22 and C-23 during elaboration of the sterol Δ^{22} bond by this organism.

Materials and methods. Cholesterol labelled stereospecifically with tritium was prepared biosynthetically. (3*R*)-[2- 14 C,(2*R*)-2- 3 H₁]MVA,* (3*R*)-[2- 14 C,(2*S*)-2- 3 H₁]MVA and (3*R*)-[2- 14 C,(5*R*)-5- 3 H₁]MVA were separately incubated with a rat liver homogenate (Bucher & McGarrahan, 1955) and the radioactive squalene and cholesterol were isolated. The cholesterol was purified by t.l.c. on silica gel followed by t.l.c. on silica gel impregnated with AgNO₃ and finally crystallized to constant specific radioactivity after addition of pure carrier cholesterol. The samples of cholesterol had the 3 H/ 14 C ratios given in Table 1. They were labelled with tritium as follows: (a) from (3*R*)-[2- 14 C,(2*R*)-2- 3 H₁]MVA at the 1 β -, 7 β -, 15-, 22*R*- and 26-positions; (b) from (3*R*)-[2- 14 C,(2*S*)-2- 3 H₁]MVA at the 1 α -, 22*S*- and 26-positions (Cannonica *et al.* 1968*a,b*; Caspi *et al.* 1968*a*; Gibbons, Goad & Goodwin, 1968*a,b*); (c) from (3*R*)-[2- 14 C,(5*R*)-5- 3 H₁]MVA at the 2 α -, 6-, 11 $\alpha\beta$ -, 12 $\alpha\beta$ -, 16 α - and 23*S*-positions (Akhtar, Rahmitula, Watkinson, Wilton & Munday, 1969; Goad, Gibbons, Bolger, Rees & Goodwin, 1969). The configurations at C-22 and C-23 are based on

* Abbreviation: MVA, mevalonate.

the assumption that these carbon atoms retain the same configurations as in the parent squalene (Popják & Cornforth, 1966). In all cases the cholesterol was labelled with 14 C at positions 1, 7, 15, 22 and 26.

T. pyriformis was grown at 28° in several flasks, each containing 200 ml. of a defined medium (Caspi, Greig & Zander, 1968*b*) but with the addition of one of the species of labelled cholesterol (30 mg. total at a final concentration of 10 μ g. of cholesterol/ml. of culture medium). After 2–3 days the cells were harvested and the non-saponifiable lipids obtained. Cholesta-5,7,22-trien-3 β -ol was isolated by t.l.c. on silica gel and silica gel impregnated with AgNO₃, crystallized to constant specific radioactivity and characterized by m.p., i.r. and mass spectrometry.

Results and discussion. The results obtained are given in Table 1. The cholesta-5,7,22-trien-3 β -ol produced from the cholesterol derived from [2- 14 C,(2*R*)-2- 3 H₁]MVA showed a loss of approximately two tritium atoms, whereas conversion of the cholesterol derived from [2- 14 C,(2*S*)-2- 3 H₁]MVA resulted in very little loss of tritium. These results, which are complementary, indicate that the 7 β - and 22-*pro-R*-hydrogen atoms are eliminated from the cholesterol. Two incubations with cholesterol labelled from [2- 14 C,(5*R*)-5- 3 H₁]MVA were performed. In the first experiment there was a loss of only about 65% of the expected amount of tritium. In the second experiment, where the cholesta-5,7,22-trien-3 β -ol was purified further through the acetate, there was a loss of one tritium atom in accord with the elimination of the 23-*pro-S*-hydrogen atom of cholesterol. We are at present unable to account satisfactorily for the first result, but possibly there was contamination by the corresponding 22,23-dihydro compound. It is notable that an apparent loss of only half a tritium atom from C-23 was reported in the formation of stigmast-22-en-3 β -ol from 24-methylene-[23- 3 H₂]lanost-8-en-3 β -ol by the slime mould *Dictyostelium discoideum* (Ellouz & Lenfant, 1969).

In summary, the present results indicate that the metabolism of cholesterol by *T. pyriformis* results in the *cis* removal of the 7 β - and 8 β -hydrogen atoms

Table 1. Conversion of cholesterol biosynthesized from various stereospecifically labelled species of MVA into cholesta-5,7,22-trien-3 β -ol by *T. pyriformis*

Initial substrate	Product	³ H/ ¹⁴ C ratio	³ H/ ¹⁴ C atomic ratio	¹⁴ C specific radioactivity (c.p.m./mg.)
[2- ¹⁴ C,(2 <i>R</i>)-2- ³ H ₁]MVA	Squalene*	5.58:1	6:6	—
	Cholesterol*	5.33:1	4.77:5	675
	Cholesta-5,7,22-trien-3 β -ol†	3.50:1	3.14:5	665
[2- ¹⁴ C,(2 <i>S</i>)-2- ³ H ₁]MVA	Squalene*	5.36:1	6:6	—
	Cholesterol*	3.50:1	3.28:5	280
	Cholesta-5,7,22-trien-3 β -ol†	3.22:1	3.02:5	266
[2- ¹⁴ C,(5 <i>R</i>)-5- ³ H ₁]MVA	Cholesterol*	10.15:1	6:5	181
	Cholesta-5,7,22-trien-3 β -ol (2)††	8.53:1	5.05:5	172
	Cholesta-5,7,22-trien-3 β -ol (1)†	9.07:1	5.36:5	—

* Biosynthesized by rat liver from the respective species of MVA.

† Isolated from *T. pyriformis* after incubation with the appropriate sample of cholesterol.

†† Purified as the acetate.

to give the Δ^7 bond. A similar *cis* elimination of the 7 β - and 8 β -hydrogen atoms has been found in the conversion of cholestan-3 β -ol into cholest-7-en-3 β -ol by the cockroach (Clayton & Edwards, 1963). The mechanism of Δ^{22} bond formation in *T. pyriformis* apparently results in the loss of the 22-*pro-R*- and 23-*pro-S*-hydrogen atoms of cholesterol. This stereochemistry is the same as that observed for poriferasterol biosynthesis in the alga *Ochromonas malhamensis* (Smith *et al.* 1968*a,b*), but is opposite to that found in ergosterol production by the mould *Aspergillus fumigatus* (Bimpson *et al.* 1969).

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