The Origin of Lanosta-7,24-dienol in Rat Skin

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Results from this and other laboratories (Gaylor, 1966; Rahman, Sharpless, Spencer & Clayton, 1970; Hornby & Boyd, 1971) suggest that the Δ^8 -to- Δ^7 isomerization step of cholesterol biosynthesis takes place at the 4α -monomethyl- C_{28} stage in rat liver. Goad (1970) has pointed out that this isomerase specificity makes the presence of lanosta-7,24-dienol (III) in rat skin (Gaylor, 1963) anomalous, and he has suggested two possible explanations. Either (a) the skin isomerase has a specificity different from that of the liver enzyme and can accommodate a C_{30} substrate, or (b) there is an alternative mode of cyclization and rearrangement of squalene oxide in skin leading directly to the Δ^7 -compound. These possibilities have now been distinguished by a double-labelling experiment with a $[2-^{14}C, 4R-4-^3H]$ mevalonate substrate as shown in Scheme 1.

Adult rats were killed with ether and skinned. The skin was shaved, scraped clean of subcutaneous fat and cut into small pieces (about 1 cm^2), which were frozen in liquid N_2 . The frozen pieces were added to 3 vol. of Gaylor's medium (Gaylor, Delwiche, Brady & Green, 1966) and the mixture was homogenized at 4°C in a top-drive blender for three periods of 20 s each. The homogenate was centrifuged to give an 18000g supernatant. $[2-^{14}C, 4R-4-^3H]$ Mevalonic acid (The Radiochemical Centre, Amersham, Bucks., U.K.) was incubated for 2 h at 37°C with this supernatant in the presence of glucose 6-phosphate (2.2 mM), glucose 6-phosphate dehydrogenase (0.04 unit/ml), NADP $^+$ (0.22 mM), NAD $^+$ (0.9 mM), fructose 1,6-diphosphate (3.0 mM), ATP (2.0 mM), magnesium chloride (4.0 mM) and 2-mercaptoethylamine (8.0 mM). The thiol was added to stop the biosynthesis at the

Scheme 1. Possible pathways of lanosta-7,24-dienol biogenesis in rat skin. ● denotes ^{14}C . Numbers in parentheses denote theoretical $^{14}C/^3H$ ratios. Pathway (a) represents the known cyclization of squalene oxide to lanosterol (II), followed by isomerization; pathway (b) represents direct formation of lanosta-7,24-dien- β -ol (III) by way of the hypothetical carbonium ion (I). The $^{14}C/^3H$ ratio found confirms pathway (b).

C₃₀ stage (Hornby, Onajobi & Boyd, 1970). T.l.c. of the non-saponifiable product gave a C₃₀ fraction (1% incorporation from [2-¹⁴C]mevalonate). This fraction was acetylated and hydrogenated (platinum dioxide, ethyl acetate) to give a mixture of Δ^7 - and Δ^8 -lanostenyl acetate, to which was added a non-radioactive mixture of the same compounds as carrier (Gaylor, 1963). Column chromatography at 4°C on alumina [Merck; basic activity 1, treated with 10% (v/v) of 10% (w/v) silver nitrate solution] gave in turn pure samples of Δ^8 -lanostenyl acetate (m.p. 117–118°C; lit. m.p. 118–121°C) and Δ^7 -lanostenyl acetate (m.p. 147–148.5°C; lit. m.p. 148–149°C). Fractions from the column were monitored by g.l.c. (1% SE-30; 240°C) and counting of ¹⁴C and ³H radioactivities was by liquid scintillation with efficiencies of 59% and 47% respectively. A minimum of 1000 counts from the less abundant radioisotope was accumulated. In the results shown below, which are the mean of two separate experiments, ¹⁴C/³H ratios have been normalized to a value of 1.0 for mevalonate.

[2-¹⁴C,4R-4-³H]Mevalonate (¹⁴C/³H ratio 1.0:1, atomic ratio 6:6.0) gave Δ^8 -lanostenyl acetate (1.22:1, 6:4.9) and Δ^7 -lanostenyl acetate (1.0:1, 6:6.0). Lanosta-7,9(11)-dienyl acetate (1.35:1, 6:4.5) was derived from the Δ^7 -compound by epoxidation with *m*-chloroperbenzoic acid followed by treatment with hydrochloric acid in methanol-tetrahydrofuran (Fried, Brown & Applebaum, 1965). These results show that no tritium is lost during formation of the Δ^7 -compound from mevalonate. The presence of tritium at C-9 in

this compound is confirmed by its loss on formation of the $\Delta^{7,9(11)}$ -diene. From these studies we conclude that the biosynthesis of lanosta-7,24-dienol (III) in rat skin may occur by a mechanism different from that proposed for the formation of lanosterol (II) in liver. The difference could be attributed to a second mode of cyclization and rearrangement of squalene oxide in which hydrogen elimination from the hypothetical carbonium ion intermediate (I) was from C-7 (pathway b). Alternative fates of squalene oxide are common in higher plants. The biogenetic origin of ambrein in ambergris is unknown, so this is the first time that evidence for a second pathway in mammalian tissues has been presented.

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