ENZYMATIC CONVERSION OF 32-OXYGENATED Δ^7-LANOSTEROL DERIVATIVES AND OF $\Delta^{8(14)}$-4,4-DIMETHYL-CHOLESTENOL TO CHOLESTEROL[1]

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In spite of the wealth of information regarding the identity of the intermediates involved in the biosynthesis of cholesterol, (Frantz and Schroepfer, 1967) some gaps remain, among these the precise sequence of steps involved in the oxidative demethylation of lanosterol. Evidence available to-date suggests (Olson et al., 1957) that the sequence $-CH_2OH \rightarrow -CHO \rightarrow -COOH$ is involved, followed by loss of CO_2 from the requisite carboxylic acid, activated by the 3-keto group in the case of C-30 and C-31, and the 8,9-double bond in the case of C-32. In none of these cases, however, have the proposed intermediates been isolated, nor have they been tested as substrates in a cholesterol-synthesizing enzyme system.

We have been particularly interested in the 32-demethylation sequence, which, according to Gautschi and Bloch (1957, 1958) proceeds with retention of the double bond in the 8,9-position. This is contrary to the well known thermal decarboxylation reaction of β,γ-unsaturated acids, which proceeds with shift of the double bond, and would therefore be expected to lead to a $\Delta^{8(14)}$-stenol.

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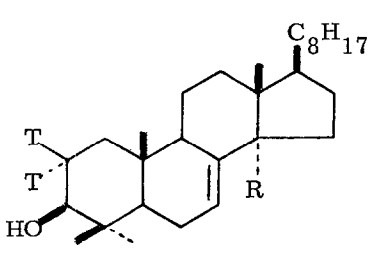
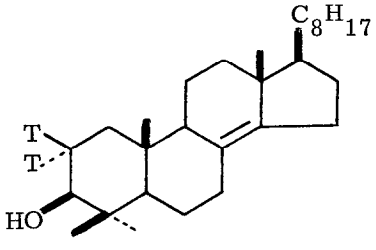
In view of the demonstrated equivalence of Δ^8- and Δ^7-stenols as substrates convertible into cholesterol (Dempsey, 1965; Gaylor, 1963; Gaylor et al., 1966) we have employed the 32-oxygenated Δ^7-stenol derivatives I and II as well as the $\Delta^{8(14)}$-stenol III, bearing a ^{3}H-label at C-2 (vide infra) recently synthesized in our laboratory, (Fried and Brown, 1966, 1967) for studies of the detailed mechanism of the 32-demethylation reaction. We wish to report at this time on the conversion of these 3 substrates to cholesterol with an efficiency equal to or greater than that found with Δ^8-lanostenol.



I R = CH_2OH
II R = CHO



III

The sterols (17.5 to 69μg in 0.2 ml of propylene glycol) were incubated aerobically with rat liver homogenates (Bucher and McGarrahan, 1956) and added NAD for three hours, and the nonsaponifiables extracted with ether. Cold cholesterol was added, the mixture converted to 5,6-dibromocholesterol and the latter crystallized to constant specific activity. For radio-g.l.c. the nonsaponifiable fraction was acetylated, and the acetates chromatographed on a 6 ft column of 2% SE-30 on Diatoport S at 250° provided with a stream splitter. One half of the emerging gas (He) was passed through a flame ionization detector the other through narrow collection tubes for counting of radioactivity.

The labeled sterols were prepared as follows: A solution of 20 mg

of Δ^7-lanostene-3-one-32-al (m.p. 133-136°) and 50 mg of KOH in 1.5 ml of dioxane and 0.5 ml of ^{3}HOH (20 mc/mmole) was heated at 70° under He for 30 min. After extraction a solution of the tritiated material in 2.38 ml of dioxane was selectively reduced (Fried and Brown, 1967) with 1.33 ml of a solution of 50 mg of KBH_4 in 10 ml of dioxane-water 3:2 for 1 hr at 25°. TLC on silicagel gave 13.3 mg of II, which was recrystallized to constant specific activity (1.08×10^7 dpm/mg), m.p. 131-134°, rep. (Fried and Brown, 1967) 131-133°. The diol I was prepared from the above keto aldehyde with excess KBH_4, and crystallized to constant specific activity (1.00×10^7 dpm/mg), m.p. 202-204°, rep. (Fried and Brown, 1967) 204-205°. Tritiated III was prepared by pyrolysis of II at 250° under He for 30 min (Fried and Brown, 1967).

The results are summarized in table 1, and representative radio gas chromatograms (experiments 6 and 7) are shown in Fig. 1 and 2. With the exception of experiment 1 where the substrate concentration of the Δ^8-lanostenol control proved too high, all experiments showed conversions to cholesterol of upward of 40%, indicating that these substrates may either be on the biosynthetic pathway or are sufficiently close in structure to natural substrates to qualify as models for further mechanistic studies. Our experiments with III were prompted by the observation (see Fig. 1) that incubates with both I and II gave rise, in addition to the major product, cholesterol, to a peak amounting to 15% of the total eluted radioactivity, which traveled with the acetate of III. The radioactivity under that peak increased to 30% when a washed microsomal suspension (105,000 g + NAD, O_2) was used instead of the whole homogenate. In this latter case the only other labeled material found was the aldehyde II. The possibile identity of this accumulating sterol with III is further suggested

TABLE 1

ENZYMATIC CONVERSION OF I, II and III TO CHOLESTEROL

Exp.	Compound added	Cpm	µg	Cpm in nonsaponifiable	Cpm in cholesterol dibromide	% conversion via dibromide	% conversion by radio g.l.c.
1	Δ^8-Lanostenol	300,000	69	178,600	24,900	14%	
2	"	150,600	35	81,000	30,500	38%	
3	I	159,200	32	67,500	41,100	61%	
4	I	79,600	16	36,300	23,600	65%	
5	II	150,100	35	80,800	41,500	51%	
6	II	75,000	17.5	36,300	24,600	68%	84%[a]
7	III	76,000	17.5	32,200	17,800	55%	70%[a]
8[b]	III	76,000	17.5			—	0%[c]
9[d]	III	76,000	17.5			—	0%[c]

a) $\frac{\text{Cpm under cholesterol acetate peak}}{\text{Total cpm collected}}$; 22% of the total radioactivity collected was associated with the III acetate peak.

b) Washed microsomal suspension prepared by 105,000 g centrifugation of Bucher homogenate for 30 min. at 0-5°, 2 mg NAD added, suspended in phosphate buffer at pH 7.4.

c) All the radioactivity was recovered under the peak for the acetate of III.

d) Same as experiments 1-8 except that He was substituted for O_2.

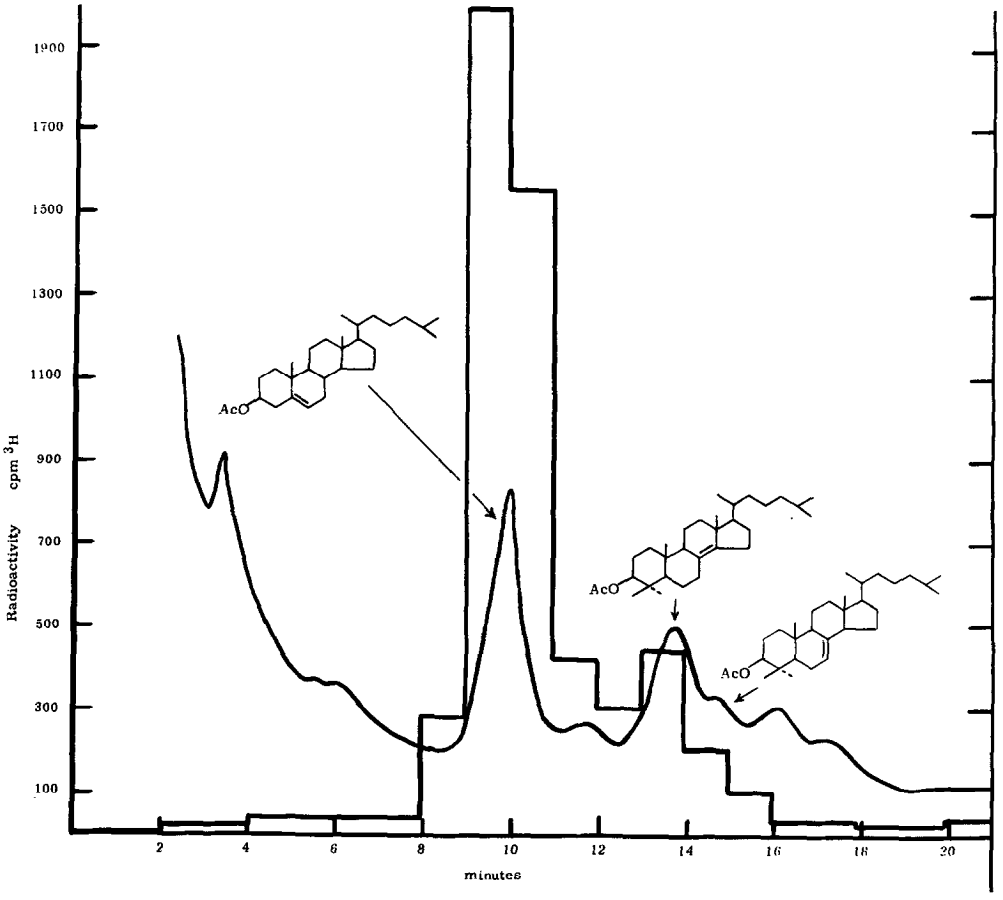


Fig. 1. Radio chromatogram of nonsaponifiable fraction from incubation of 2-^{3}H-Δ^7-lanostene-3β-ol-32-al (II) with rat liver homogenate (Exp. 6) to which cold standards have been added. Curve represents output from flame ionization detector.

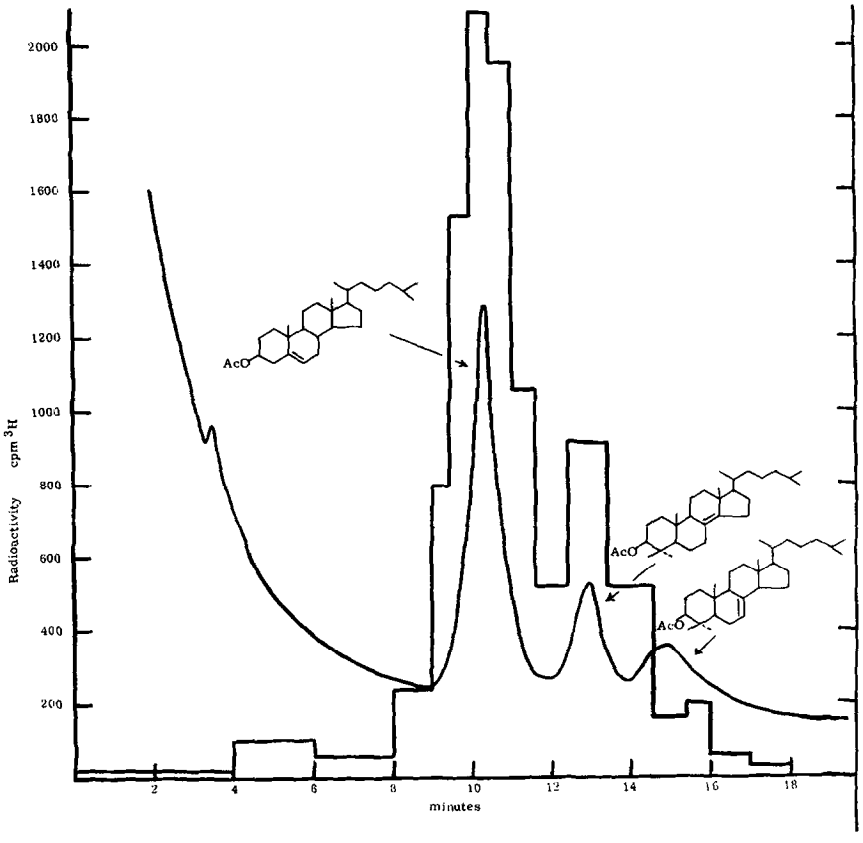


Fig. 2. Same as above using 2-^{3}H-$\Delta^{8(14)}$-4,4-dimethylcholestene-3β-ol (III) as substrate (Exp. 7).

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by the finding (experiment 8) that authentic III is likewise stable under these conditions. Assessment of the significance of III in the overall biosynthetic process must await further investigation.

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