

# The Pathway for the Removal of the 15 $\alpha$ -Methyl Group of Lanosterol

## The Role of Lanost-8-ene-3 $\beta$ ,32-diol in Cholesterol Biosynthesis<sup>1</sup>

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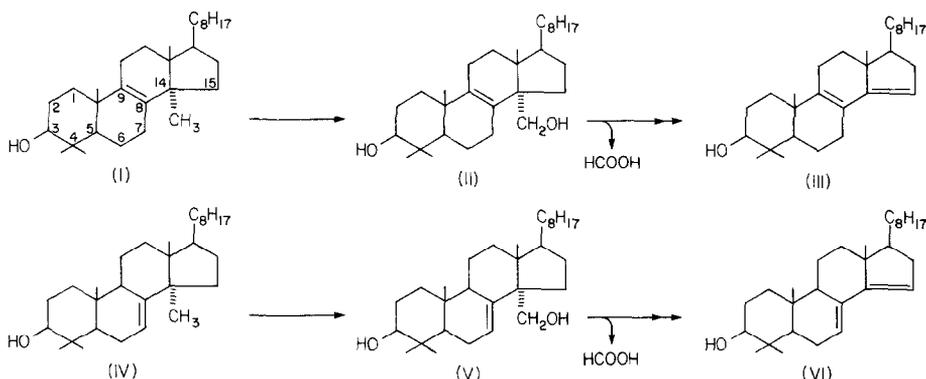
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[9 $\alpha$ -<sup>3</sup>H]Lanost-7-ene-3 $\beta$ -ol is synthesized and is shown to be demethylated by a rat liver homogenate to give 4,4'-dimethylcholesta-7,14-dien-3 $\beta$ -ol. [32-<sup>3</sup>H]Lanost-8-ene-3 $\beta$ ,32-diol is synthesized and is shown to be demethylated by a rat liver microsomal preparation to give 4,4'-dimethylcholesta-8,14-dien-3 $\beta$ -ol with the release of C-32 as formic acid.

### INTRODUCTION

Since Bloch and his co-workers (1) first proposed a biosynthetic route for the conversion of lanosterol into cholesterol, the one facet of this pathway that has been the subject of much scientific scrutiny and modification is that concerned with the removal of the 14 $\alpha$ -methyl group. Not only has it now been shown that the demethylation process involves the loss of the 15 $\alpha$  hydrogen and the formation of the  $\Delta^{8,14}$ -diene system (2-10), but also it has recently been demonstrated that this methyl group is lost as formic acid (11, 12), and not carbon dioxide, as originally proposed by Bloch (1). The modified pathway is shown in Scheme 1. The involvement of formic acid was



SCHEME 1.

<sup>1</sup> Dedicated to the memory of Professor S. Morris Kupchan, an outstanding scholar and a master practitioner of the art of structure determination.

established in experiments using lanost-7-ene-3 $\beta$ ,32-diol (V) in which the 3 $\alpha$  and 32 positions were labelled with tritium. The metabolism of this compound by rat liver microsomes gave formic acid and 4,4'-dimethylcholesta-7,14-dien-3 $\beta$ -ol (VI) which were both radioactive (11, 12). Although there is considerable indirect evidence to suggest that the  $\Delta^7$ -isomer in the lanosterol series is metabolized in the liver by the same pathway as the naturally occurring  $\Delta^8$ -lanosterol, a more rigorous proof of the pathways outlined in Scheme 1 was required. In particular the pathways would require the demonstration that the corresponding  $\Delta^8$ -isomer, lanost-8-ene-3 $\beta$ ,32-diol (II), is dealkylated to 4,4'-dimethylcholesta-8,14-dien-3 $\beta$ -ol (III) with the release of C-32 as formic acid. In addition, the demonstration that  $\Delta^7$ -lanosterol itself (IV), like the  $\Delta^7$ -diol (V), is also converted to the 7,14-diene intermediate would complete the evidence in support of Scheme 1.

We describe below the preparation of radioactive  $\Delta^7$ -lanosterol and lanost-8-ene-3 $\beta$ ,32-diol and their metabolism by rat liver enzyme preparations.

## RESULTS AND DISCUSSION

### *The Metabolism of Lanost-7-ene-3 $\beta$ -ol (IV) to give 4,4'-dimethylcholesta-7,14-dien-3 $\beta$ -ol (VI)*

[9 $\alpha$ -<sup>3</sup>H]Lanost-7-ene-3 $\beta$ -ol (IV) was prepared by the isomerization of dihydrolanosterol under acid conditions in the presence of tritiated water followed by exhaustive thin-layer chromatography to separate the two isomers. [9 $\alpha$ -<sup>3</sup>H]Lanost-7-ene-3 $\beta$ -ol was incubated in the presence or absence of a "trap" of 4,4'-dimethylcholesta-7,14-dien-3 $\beta$ -ol (VI) with a 10,000g<sub>av</sub> supernatant of rat liver homogenate. The presence of the nonradioactive diene (VI) has a significant effect on the metabolism of (IV) into cholesterol (Table 1) since the biosynthesis decreases from the already low level of 1.3 to 0.07% in the presence of the diene with a resulting increase in the amount of radioactivity trapped in the diene.

TABLE I

THE METABOLISM OF [9 $\alpha$ -<sup>3</sup>H]LANOST-7-ENE-3 $\beta$ -OL (IV) INTO 4,4'-DIMETHYLCHOLESTA-7,14-DIEN-3 $\beta$ -OL (VI) AND CHOLESTEROL BY A 10 000 g<sub>av</sub> SUPERNATANT OF RAT LIVER HOMOGENATE<sup>a</sup>

Substrate	4,4'-Dimethylcholesta-7,14-dien-3 $\beta$ -ol (VI) added as "trap"	Percentage conversion	
		Cholesterol	4,4'-Dimethylcholesta-7,14-dien-3 $\beta$ -ol
[9 $\alpha$ - <sup>3</sup> H]Lanost-8-ene-3 $\beta$ -ol (10 $\mu$ g; 1.75 $\times$ 10 <sup>5</sup> dpm)	None	15.6	—
[9 $\alpha$ - <sup>3</sup> H]Lanost-7-ene-3 $\beta$ -ol (10 $\mu$ g; 6 $\times$ 10 <sup>5</sup> dpm)	None	1.3	—
	1 mg	0.07	—
	None	—	3.2
	1 mg	—	11.5

<sup>a</sup> Incubations (10 ml) were carried out aerobically for 30 min at 37°C. The sterols were extracted and separated on 10% AgNO<sub>3</sub> impregnated tlc plates developed in chloroform: diethyl ether:acetic acid (97:2.5:0.5, v/v/v). The cholesterol was isolated as its dibromide derivative.

The radioactive 4,4'-dimethylcholesta-7,14-dien-3 $\beta$ -ol (VI) that was trapped was authenticated as the 7,14-diene rather than the 8,14-diene (III) by the following procedure. The radioactive material was acetylated and co-crystallized with authentic carrier 4,4'-dimethylcholesta-7,14-dien-3 $\beta$ -yl acetate (149,000 dpm/mmol), which was then converted to the specific derivative 4,4'-dimethylcholest-8(14)-ene-3 $\beta$ ,7 $\epsilon$ ,15 $\epsilon$ -triol (156,000 dpm/mmol) by oxidation with perphthalic acid followed by alkaline hydrolysis. This procedure resulted in complete retention of the radioactivity and provides conclusive proof that the  $\Delta^7$ -isomer of lanostenol is demethylated to give the 7,14-diene (VI).

#### Preparation of Lanost-8-ene-3 $\beta$ ,32-diol

Treatment of 3 $\beta$ -acetoxy-7 $\alpha$ ,32-epoxy-5 $\alpha$ -lanostane (13) with pyridine hydrochloride in refluxing acetic anhydride, followed by base hydrolysis of the resulting acetates, afforded a mixture of diols. These were separated by repeated preparative layer chromatography. The first component had mp 190–192°C,  $[\alpha]_D -29^\circ$ , and contained two olefinic protons which appeared in the nmr spectrum as an AB quartet. The nmr data together with the characteristic negative rotation suggested that this compound was 5 $\alpha$ -lanost-6-ene-3 $\beta$ ,32-diol. The second product was the known 5 $\alpha$ -lanost-7-ene-3 $\beta$ ,32-diol (V). The final diol had mp 159–161°C,  $[\alpha]_D +43^\circ$ . The nmr spectrum contained no signal for an olefinic proton, and the positions of the resonances for the quaternary methyl groups were in good agreement with those calculated (14) for 5 $\alpha$ -lanost-8-ene-3 $\beta$ ,32-diol (see Table 2). This structure was confirmed by molecular rotation considerations. Thus, the molecular rotations,  $M_D$ , of  $\Delta^7$ - and  $\Delta^8$ -5 $\alpha$ -lanosten-3 $\beta$ -ol are +43° and +247°, respectively. When combined with the observed  $M_D$  for 5 $\alpha$ -lanost-7-ene-3 $\beta$ ,32-diol (+58°), these figures predict a molecular rotation for 5 $\alpha$ -lanost-8-ene-3 $\beta$ ,32-diol of +262°. This is in acceptable agreement with the observed value of +191°. That the  $\Delta^8$  isomer is formed by isomerization of the  $\Delta^7$  isomer under the reaction conditions used to cleave the ether was confirmed in a separate experiment starting with pure 5 $\alpha$ -lanost-7-ene-3 $\beta$ ,32-diol.

Lanost-8-ene-3 $\beta$ ,32-diol (II) was oxidized with a suspension of chromium trioxide in pyridine to yield a mixture of various oxidized species. This mixture was allowed to

TABLE 2  
RESOURCES FOR THE QUATERNARY METHYL GROUPS IN THE NMR SPECTRUM OF  
5 $\alpha$ -LANOST-8-ENE-3 $\beta$ ,32-DIOL

Compound	Resonance of CH <sub>3</sub> Group ( $\tau$ )				
	C-30	C-31	C-19	C-18	C-32
Lanost-7-ene-3 $\beta$ -ol <sup>a</sup>	9.01	9.11	9.12	9.35	9.01
Lanost-7-ene-3 $\beta$ ,32-diol	9.01	9.09	9.09	9.28	—
Lanost-8-ene-3 $\beta$ -ol	9.01	9.19	9.00	9.31	9.12
Lanost-8-ene-3 $\beta$ ,32-diol (calculated)	9.01	9.17	8.97	9.24	—
Lanost-8-ene-3 $\beta$ ,32-diol (found)	9.00	9.17	8.96	9.29	—

<sup>a</sup> On the basis of independent data the assignments given here for C-19 and C-32 are reversed relative to those given by Hemmert *et al.* (15).

react first with a limiting amount of  $\text{NaB}^3\text{H}_4$  of high specific radioactivity and then with an excess of unlabelled  $\text{NaBH}_4$  to complete the reduction process. The resulting  $\Delta^8$ -diol (II) should contain tritium at C-3 and C-32, thus providing a derivative suitable for the examination of the fate of the C-32 as well as the steroid nucleus during the projected biosynthetic experiments. The estimated distribution of radioactivity between C-3 and C-32 in the  $[3,32\text{-}^3\text{H}]\text{-}\Delta^8$ -diol was about 4 : 1, respectively, and this suggested that in the first stage of the treatment with  $\text{NaB}^3\text{H}_4$  the reaction had occurred predominantly at C-3. It should be borne in mind that a knowledge of the precise distribution of radioactivity between the two positions, though desirable, is not absolutely obligatory for the interpretation of the projected biosynthetic experiments.

*Metabolism of Lanost-8-ene-3 $\beta$ ,32-diol (II) to 4,4'-Dimethylcholesta-8,14-dien-3 $\beta$ -ol (III) and formic acid*

The  $[3,32\text{-}^3\text{H}]\text{-}\Delta^8$ -diol was now incubated in the presence of a "trap" of 4,4'-dimethylcholesta-8,14-dien-3 $\beta$ -ol (III) with a microsomal preparation of rat liver homogenate for 30 min. The 4,4'-dimethylcholesta-8,14-dien-3 $\beta$ -ol that was isolated from the biosynthetic experiments contained about 36% of the radioactivity originally incubated. The material was acetylated and recrystallized with authentic 4,4'-dimethylcholesta-8,14-dien-3 $\beta$ -yl acetate to give 97% retention of radioactivity over four crystallizations. In order to evaluate whether in the trapping experiment the corresponding 7,14-diene (VI) was also formed, a portion of the labelled biosynthetic diene was diluted with 4,4'-dimethylcholesta-7,14-dien-3 $\beta$ -ol and converted to the characteristic 4,4'-dimethylcholest-8(14)-ene-3,7 $\epsilon$ ,15 $\epsilon$ -triol. This material was found to be completely devoid of any radioactivity. The experiment therefore proves that 4,4'-dimethylcholesta-8,14-dien-3 $\beta$ -ol was exclusively formed from the  $\Delta^8$ -3,32-diol (Scheme 1).

An incubation conducted in parallel with the one described above was performed for the recovery of the total acid volatile fraction. This sample was found to contain 13% of the radioactivity originally incubated. The all important distribution of the radioactivity between water and formic acid was next determined, and it was found

TABLE 3

THE MECHANISM OF  $[3\alpha,32\text{-}^3\text{H}]\text{LANOST-8-ENE-3}\beta,32\text{-DIOL}$  AND  $[32\text{-}^3\text{H}]\text{LANOST-7-ENE-3}\beta,32\text{-DIOL}$  BY RAT LIVER MICROSOMAL PREPARATION<sup>a</sup>

Substrate	Percentage of acid volatile radioactivity recovered	
	Formic acid	Water
$[3\alpha,32\text{-}^3\text{H}]\text{Lanost-8-ene-3}\beta,32\text{-diol}$ (100 $\mu\text{g}$ ; $1.2 \times 10^6$ dpm)	42	58
$[32\text{-}^3\text{H}]\text{Lanost-7-ene-3}\beta,32\text{-diol}$ (100 $\mu\text{g}$ ; $5.3 \times 10^5$ dpm)	48	52

<sup>a</sup> Incubations (5 ml) were carried out aerobically with an unwashed microsomal preparation at 37°C for 30 min. The isolation of the formic acid is described in the Experimental section.

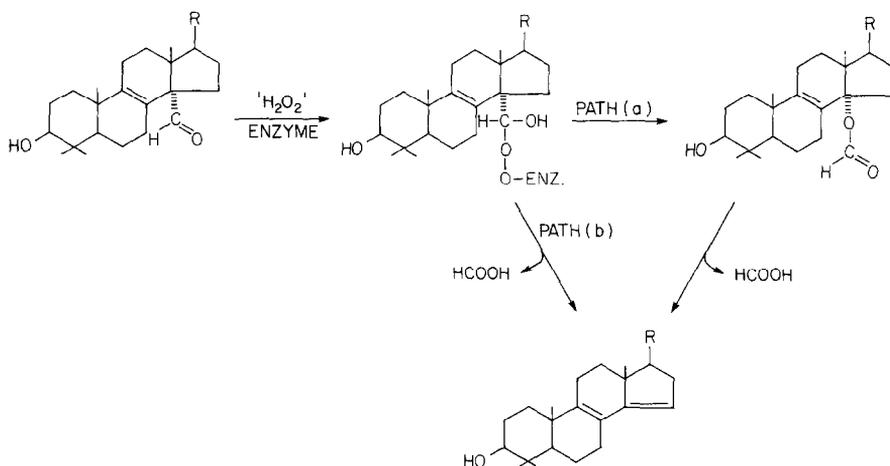
that these two components contain 58 and 42%, respectively, of the radioactivity present in the acid-volatile fraction. The distribution of radioactivity was comparable to the one obtained when in another parallel incubation the acid-volatile fraction from the metabolism of  $[32\text{-}^3\text{H}]\Delta^8\text{-}3,32\text{-diol}$  was analysed (Table 3).

A sample of sodium formate (49 800 dpm/mmol) obtained from the incubation of  $[3,32\text{-}^3\text{H}]\Delta^8\text{-}3,32\text{-diol}$  (**II**) was converted to the specific derivative *p*-bromophenacyl formate (44 500 dpm/mmol) by refluxing with *p*-bromophenacyl bromide. The conversion into the derivative was attended by the retention of about 90% of the original radioactivity, thus confirming the presence of tritium in the formic acid.

The above results establish that the metabolism of lanost-8-ene- $3\beta,32$ -diol (**II**) by rat liver microsomes results in the formation of 4,4'-dimethylcholesta-8,14-dien- $3\beta$ -ol in high yield and that in this process the C-32 is released as formic acid.

### GENERAL DISCUSSION

The evidence presented above and in other communications (2-12, 21) provides direct experimental support for the pathways outlined in Scheme 1. The removal of the  $14\alpha$ -methyl group from the sterol nucleus as formic acid would require that the 32-aldehyde be the intermediate in which cleavage of the C-14-C-32 bond occurs. Although the precise mechanism for the removal of this carbon atom is not known, it was initially anticipated that a mechanism involving a leaving group at the C-15 position coupled with a nucleophilic attack on the aldehyde might be a strong possibility. However, the more recent observations (16) concerning the analogous C-19 demethylation in the biosynthesis of oestrogens highlights one of the alternative mechanisms. It has been observed that in oestrogen biosynthesis the incubation of the 19-aldehyde intermediate with a placental microsomal preparation in the presence of  $^{18}\text{O}_2$  gas resulted in incorporation of  $^{18}\text{O}$  into the isolated formic acid. This result is most



SCHEME 2. Path (a): via Baeyer-Villiger process. Path (b): elimination via a concerted process. Whether in the first step it is an Enz-dioxygen or Enz-monoxygen species that interacts with the substrate is a matter of conjecture at the present time. However, it is merely for the sake of mechanistic simplicity that an Enz-dioxygen species has been used above.

simply explained in terms of a mechanism involving a further oxygen insertion onto the C-19 aldehyde substrate to give a peroxy-enzyme intermediate which on elimination, possibly via a Baeyer-Villiger-type process, results in the formation of the diene and the release of formic acid. A similar mechanism can be drawn for the loss of the C-32 methyl group of lanosterol, and this interpretation is shown in Scheme 2. It should be noted that in this mechanism the 15 $\alpha$ -hydrogen will be lost as a proton.

## EXPERIMENTAL

Materials and methods not mentioned in detail below have been described previously (3, 4).

### *Rat Liver Preparations and Incubation Procedures*

(I) *Crude rat liver homogenate.* Male Wistar albino rats (100–150 g) were killed by cervical dislocation and the excised livers were homogenized in cold (1–4°C) 0.1 M potassium phosphate buffer, pH 7.3, containing 30 mM nicotinamide and 4 mM MgCl<sub>2</sub> (1.25 ml/g of liver). The homogenization consisted of 60 strokes in a loose-fitting piston-barrel-type homogenizer. Cell debris and mitochondria were sedimented by centrifugation at 10 000g for 15 min at 1–4°C, after which the supernatant was filtered through glass wool to give the crude rat liver homogenate employed in the incubations.

(II) *Subcellular fractions.* The crude homogenate was centrifuged for 60 min at 105 000g. The 105 000g supernatant was then decanted off and the remaining microsomal pellet was rinsed, resuspended in the same volume of buffer from which it had sedimented, and recentrifuged for 60 min at 105 000g. The supernatant was then decanted off and the microsomal pellet was rinsed with cold buffer and resuspended in the same volume of buffer from which it had sedimented. This preparation was used in the washed microsomal incubations.

All operations in the above preparations were performed at 1–4°C.

(III) *Incubation procedures.* Sterols were dissolved in acetone (1 ml) containing Tween 80 (10 mg). The acetone was then removed with a stream of N<sub>2</sub> and the residue was emulsified in water (0.5 ml) prior to the addition of the rat liver preparation. The crude rat liver homogenates were supplemented with glucose 6-phosphate (1.0 mM) and glutathione (1.0 mM). For the microsomal preparations any NADPH generator added contained glucose 6-phosphate (4  $\mu$ mol) and glucose 6-phosphate dehydrogenase (0.6 units) per micromole of NADP. Incubations were performed in 50-ml Erlenmeyer flasks at 37°C with gentle shaking. Aerobic conditions were obtained by incubating in unstoppered flasks.

### *Cleavage of 3 $\beta$ -Acetoxy-7 $\alpha$ ,32-epoxy-5 $\alpha$ -lanostane*

3 $\beta$ -Acetoxy-7 $\alpha$ ,32-epoxy-5 $\alpha$ -lanostane (13) (0.53 g) and pyridine hydrochloride (0.54 g) were heated in refluxing acetic anhydride (100 ml) for 15 h. The mixture was cooled, poured into ice-water, and extracted with diethyl ether. The combined extracts were washed with 2 N sodium hydroxide solution and water, dried, and evaporated. The residue was dissolved in ethanol (20 ml) containing 5% potassium hydroxide and heated under reflux for 1 h. The mixture was poured into water and extracted with diethyl ether. The combined extracts were washed with water, dried, and evaporated.

Preparative layer chromatography on silver nitrate-impregnated silica gel eluting with chloroform–diethyl ether–acetic acid (97:2.5:0.5, v:v:v) readily afforded a less polar and a more polar fraction. The latter yielded material (60 mg, 12%) mp (from acetone) 190–192°C,  $[\alpha]_D -29^\circ$  (*c* 0.6 in chloroform),  $\tau$  (deuteriochloroform) 4.16 and 4.34 (each 1H, *d*, *J* = 12 Hz, 6- and 7-H), 5.83 (1H, *d*, *J* = 12 Hz, 32-H), and 6.4–6.9 (2H, *m*, 3 $\alpha$ - and 32-H) (found: C, 80.8; H, 12.0. C<sub>30</sub>H<sub>52</sub>O<sub>2</sub> requires C, 81.0; H, 11.8%). This compound was evidently 5 $\alpha$ -lanost-6-ene-3 $\beta$ ,32-diol. The less polar fraction from the above chromatography was further separated by plc on silica gel eluting with the same solvent system. This afforded 5 $\alpha$ -lanost-8-ene-3 $\beta$ ,32-diol (32 mg, 7%), mp (from acetone) 159–161°C,  $[\alpha]_D +43^\circ$  (*c* 0.7 in chloroform),  $M_D +191^\circ$ ,  $\tau$  6.37 (1H, *d*, *J* = 11 Hz, 32-H), 6.73 (1H, *m*, 3 $\alpha$ -H), 6.80 (1H, *d*, *J* = 11 Hz, 32-H), 8.96, 9.00, 9.17, and 9.29 (each 3H, *s*, see Table 2 for assignments) (found C, 81.00; H, 12.00. C<sub>30</sub>H<sub>52</sub>O<sub>2</sub> requires C, 81.0; H, 11.8%), and 5 $\alpha$ -lanost-7-ene-3 $\beta$ ,32-diol (44 mg, 9%), mp (from acetone) 207–209°C,  $[\alpha]_D +13.5^\circ$  (*c* 0.7 in chloroform) (lit. (17) mp 201–203°C,  $[\alpha]_D +12^\circ$ ).

A sample of lanost-8-ene-3 $\beta$ ,32-diol was subjected to combined GC/MS and the fragmentation pattern was compared to that for lanost-7-ene-3 $\beta$ ,32-diol. The two mass spectra were nearly identical, neither gave a molecular ion, and the base peak was at *m/e* 414 due to loss of the 14 $\alpha$ -CH<sub>2</sub>OH with a back transfer of a hydrogen. The  $\Delta^8$ -isomer gave additional small peaks at *m/e* 426 and 392 not present in the  $\Delta^7$  isomer. This great similarity of mass spectra has also been seen with  $\Delta^8$ - and  $\Delta^7$ -dihydrolanosterol (18).

#### Preparation of [3 $\alpha$ ,32-<sup>3</sup>H]Lanost-8-ene-3 $\beta$ ,32-diol (II)

Three consecutive purifications of the diol mixture on silica gel GF<sub>254</sub> tlc plate developed in light petroleum (60–80°C): acetone (8:2, v/v) yielded pure lanost-8-ene-3 $\beta$ ,32-diol (50 mg), which was dissolved in pyridine (1.5 ml). To this was added a suspension of CrO<sub>3</sub> (50 mg) in pyridine (1.25 ml) and the mixture was left 12 h. The product was extracted with diethyl ether (3 × 20 ml) and the pooled extracts were washed with 0.01 *M* HCl (6 × 15 ml) to remove all traces of pyridine. After further washing with saturated NaHCO<sub>3</sub> solution, water, and saturated NaCl solution and drying (anhydrous Na<sub>2</sub>SO<sub>4</sub>), the solvent was removed under reduced pressure. The solid residue was purified on silica gel GF<sub>254</sub> tlc plates developed in the solvent systems above.

The lanost-8-ene-3,32-dione fraction was dissolved in methanol (3 ml) and left for 3 h with NaB<sup>3</sup>H<sub>4</sub> (1 mg; 1 mCi). After this time excess NaBH<sub>4</sub> was added and the reaction mixture was left to stand for a further 3 h. Water was then added and the precipitated product was extracted with diethyl ether in the usual manner. Purification on silica gel GF<sub>254</sub> tlc plates developed by the solvent systems above gave [3 $\alpha$ ,32-<sup>3</sup>H]-lanost-8-ene-3 $\beta$ ,32-diol (II) (25 mg, sp act 1.26 × 10<sup>6</sup> dpm/100 $\mu$ g).

#### Preparation of [9 $\alpha$ -<sup>3</sup>H]Lanost-7-ene-3 $\beta$ -ol (IV)

An extract of sheep wool fat was acetylated to yield a crude 1:1 mixture of lanosteryl and dihydrolanosteryl acetates, which was crystallized four times from acetone until pure. The purified acetate mixture (1 g) was dissolved in glacial acetic acid (70 ml) and the solution was run into a hydrogenation flask containing Adams Catalyst (22 mg)

prereduced with hydrogen. After hydrogenating at room temperature and 1 atm pressure until no more hydrogen was taken up, the mixture was filtered through Kieselguhr and the filtrate was evaporated to dryness. The acetate was dissolved in diethyl ether (40 ml) and refluxed with lithium aluminium hydride (300 mg) for 30 min to yield chromatographically pure dihydrolanosterol. After refluxing this in pyridine (5 ml) and benzoyl chloride (0.5 ml) for 1 h the dihydrolanosteryl benzoate was precipitated with water, filtered off, and crystallized 3× from ether–methanol.

The dihydrolanosteryl benzoate (50 mg) was dissolved in dry chloroform (3 ml) saturated with HCl gas and allowed to isomerize in the presence of tritiated water (10  $\mu$ l; 50 mCi) at room temperature for 2 h in a stoppered flask. After removal of the solvent under reduced pressure the mixture of  $\Delta^7$ - and  $\Delta^8$ -dihydrolanosterol benzoate was applied to four silica gel GF<sub>254</sub> tlc plates (200  $\mu$ m thick, 40 cm long) and developed (9×) in petroleum ether (bp 40–60°): acetone (300:1, v/v). A scan of the radioactivity revealed a gradual separation of the less polar  $\Delta^8$  isomer from the  $\Delta^7$  isomer as the chromatography progressed. The two bands were separately eluted and rechromatographed until pure. Hydrolysis of the benzoate with 10% methanolic-KOH yielded [ $9\alpha$ -<sup>3</sup>H]lanost-7-en-3 $\beta$ -ol (IV) (12.3 mg;  $6.0 \times 10^7$  dpm/mg) and [ $7$ -<sup>3</sup>H]lanost-8-ene-3 $\beta$ -ol (I) (11.6 mg;  $1.75 \times 10^7$  dpm/mg).

*Chemical Conversion of 4,4'-Dimethylcholesta-7,14,-dien-3 $\beta$ -yl Acetate to 4,4'-Dimethylcholesta-8[14]-ene-3 $\beta$ ,7 $\epsilon$ ,15 $\epsilon$ -triol (19)*

4,4'-Dimethylcholesta-7,14-dien-3 $\beta$ -yl acetate (55 mg) was dissolved in anhydrous diethyl ether (2.3 ml) and treated with a solution of perphthalic acid (25.5 mg) in anhydrous diethyl ether (0.8 ml). The reaction mixture was left to stand at room temperature for 45 h, after which it was extracted with diethyl ether. The extracts were washed with 0.1 M NaOH, water, and saturated NaCl solution and dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>). The ether was then removed under reduced pressure and the residue was dissolved in a solution of KOH (215 mg) in 95% ethanol (7.5 ml) and refluxed for 3 h. The ethanol was subsequently removed under reduced pressure and the product was washed with water, a little ice-cold methanol, and dried. Crystallization (3×) from chloroform gave pure 4,4'-dimethylcholest-8(14)-ene-3 $\beta$ ,7 $\epsilon$ ,15 $\epsilon$ -triol (20.8 mg, mp 240°C).

*Analysis of Incubations for Radioactive Formic Acid*

Incubations were terminated by the addition of orthophosphoric acid (0.5 ml, sp gr 1.75). The mixture was then lyophilized and the volatile distillate was collected in a liquid nitrogen trap. The volume of the distillate was measured and a portion (0.1 ml) was counted for radioactivity to determine the amount of tritiated formic acid and tritiated water present. The acid-volatile distillate was neutralized with 0.1 M NaOH, using phenolphthalein as an indicator, to form the nonvolatile sodium salt of formic acid. The mixture was again lyophilized and water (2 ml) was added to the residual sodium formate. Nonradioactive sodium formate (100 mg) was added and the sodium formate was crystallized from methanol and diethyl ether.

*Chemical Conversion of Sodium Formate to p-Bromophenacyl Formate (20)*

Sodium formate (68 mg) was dissolved in water (1 ml) and treated with p-bromophenacyl bromide (277 mg) in ethanol (3 ml) and the mixture was refluxed for 30 min.

The ethanol was then removed under reduced pressure at room temperature and the product was extracted with diethyl ether. The ether extract was washed with water and saturated NaCl solution, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness under reduced pressure at room temperature. The residue was applied to a silica gel GF<sub>254</sub> tlc plate and developed in benzene : ethyl acetate (95.5). The band corresponding to *p*-bromophenacyl formate (*R<sub>f</sub>* 0.43) was eluted and crystallized (2×) from dry ethanol. Yield = 82 mg.

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