On the Mechanism of the Hydrogen Chloride Catalysed Isomerization of 5α-Cholest-8(14)-en-3β-ol to 5α,17β-Cholest-14-en-3β-ol: Fate of the 17α-Hydrogen Atom

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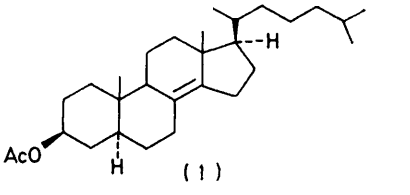
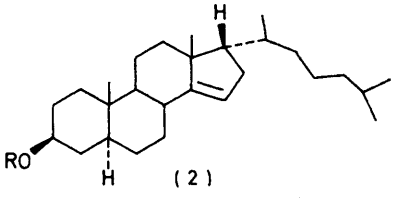
(4*R*)-[^{3}H,2-^{14}C]Mevalonic acid was incubated with a rat liver homogenate, in the presence of the inhibitor AY–9944, to yield [$^3H_3$.$^{14}C_5$]cholesta-5,7-dien-3β-ol, which was reduced (Li–NH_3) to [17α,20,24*R*-$^3H_3$.$^{14}C_5$]-5α-cholest-7-en-3β-ol. [$^3H_3$.$^{14}C_5$]-5α-Cholest-7-en-3β-ol was isomerized (platinum–acetic acid–hydrogen) and the resulting [$^3H_3$.$^{14}C_5$]-5α-cholest-8(14)-en-3β-ol was acetylated. The acetate was treated with hydrogen chloride in chloroform at −78° followed by aqueous $NaHCO_3$ to give [20,24*R*-$^3H_2$.$^{14}C_5$]-5α,17β-cholest-14-en-3β-yl acetate. The isomerization proceeded with the loss of *ca.* 0.9 atom of tritium. The mechanism of the hydrogen chloride catalysed isomerization is discussed.

RECENTLY, we [1] and Fiecchi *et al.*[2] have reported that treatment of a chloroform or ether solution of 5α-



(1)



(2)

a; R = Ac
b; R = H
c; *p*-BrC_6H_4CO

cholest-8(14)-en-3β-yl acetate (1) with dry hydrogen chloride at −60 or −78°, followed by the exposure of the product to base, resulted in the nearly quantitative formation of 5α,17β-cholest-14(15)-en-3β-yl acetate (2a). By omitting the treatment with base (triethylamine–methanol), Fiecchi *et al.*[2] obtained 14β-chloro-5α,17β-cholestan-3β-yl acetate (4a) [as (4) except that the stereochemistry at C-17 is reversed]. In contrast, when the reaction was carried out under similar conditions, but at a higher temperature (−30°), the main product obtained was 5α-cholest-14(15)-en-3β-yl acetate.[3]

In the context of studies of the biosynthesis of sterols having a 17α-side chain (17β-H), the possibility was considered that the elaboration of the 17α-side chain [4-6] of the natural products may proceed in principle by mechanisms similar to that operating in the hydrogen chloride isomerization at −78°. With this in mind, we undertook a study of the mechanism of the transformation of (1) by hydrogen chloride in chloroform at −78° to sterols with a 17α-side chain (2).

Fiecchi *et al.*[2] have proposed a mechanism for the 17β ⟶ 17α isomerization of the side chain. They suggested that the reaction may proceed *via* (3) which acquires HCl to yield chloride (4). Loss of the 14β-chlorine atom of (4) would yield the cation (5) which could collapse to the $\Delta^{13(17)}$-14-spiro-intermediate (6a). Addition of a 17β-proton and a 14β-chlorine to (6a) followed by the elimination of HCl would then yield (2). It should be stressed, however, that the 17β-analogue (4a) could be formed through the addition of HCl either to the proposed spiro-intermediate (6a), or to the product (2).

Although the proposed mechanism appears reasonable, several others seem equally possible. For example, the 13β-methyl of cation (5) could migrate to the 14β-position with concomitant loss of the 17α-hydrogen atom to give the intermediate olefin (6b). Protonation of (6b) at the 17β-position and reversal of the methyl group shift to C-13, followed by the elimination of a 15-hydrogen atom, would yield (2). The drawback of this mechanism is that the 17β protonation and the methyl migration (14β ⟶ 13β) both occur on the same face of the molecule. Such a mechanism would, therefore, contradict one of the accepted rules of Wagner–Meerwein rearrangements requiring an anti-parallel orientation of migrating groups. A better interpretation is provided if the loss of the chlorine in (4) could involve the cleavage of the C-13–C-17 bond to yield the $\Delta^{13(14)}$-17-carbonium ion (7). Stabilization of the carbonium ion by the attack of electrons of the double bond on the β-face would result in the inversion of the configuration of the side chain and yield, finally, (2).

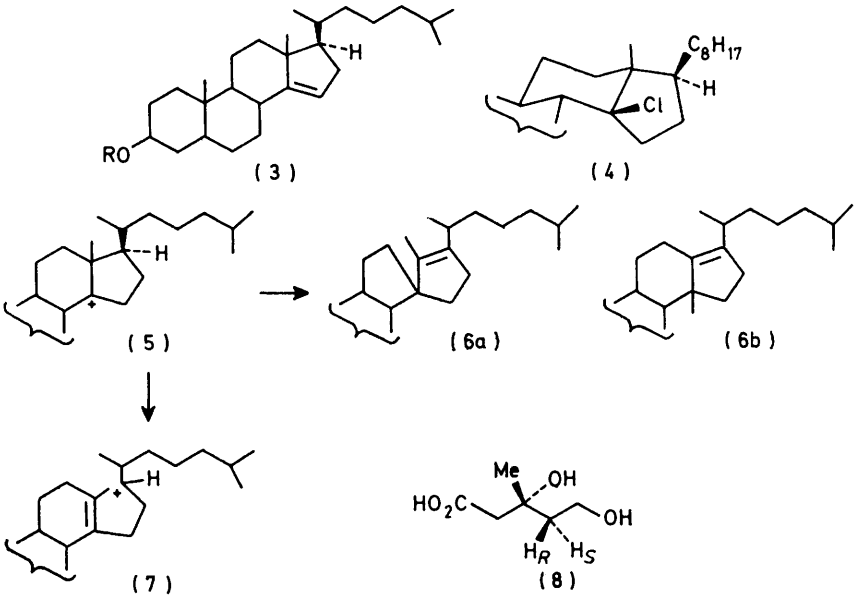
The possibility could also be considered that in carbonium ion (5), three clockwise 1,2 shifts of ring D hydrogen atoms [17α ⟶ 16β ⟶ 15α ⟶ (14^+)] occur, ultimately resulting in the formation of a new carbonium ion at C-17. Reversal of the process now involving the counter-clockwise migration of [15α ⟶ 16β ⟶ (17^+)] hydrogen atoms and then formation of the Δ^{14}-product would result in inversion of configuration at C-17. Obviously, in this case the hydrogen (tritium) atom initially present at the 17α-position would not be lost and would be retained at the 16α-position. In the cases of the intermediacy of olefins (6a and b) the 17α-H(^{3}H) atom would be lost. Finally, should the mechanism proceed *via* (7), the 17α-H(^{3}H) atom would be retained and assume the 17β-configuration in (2).

RESULTS AND DISCUSSION

Cornforth *et al.*[7] found that the biosynthesis of squalene from mevalonic acid (MVA) (8) proceeded with the loss of six 4-*pro-S* hydrogen atoms and retention of six 4-*pro-R* hydrogen atoms. Subsequently, we have established that, in accord with mechanistic expectations, cholesterol (9a) biosynthesized from (4*R*)-[4-^3H]MVA retained three atoms[8,9] of tritium. The isotopic hydrogen atoms were located at the 17α, 20-*pro-R*, and 24-*pro-R* positions.[8,9] It was demonstrated that when MVA was incubated with a rat liver enzyme preparation in the presence of the inhibitor AY-9944,* the biosynthesis of cholesterol was arrested and resulted in the accumulation[10,11] of cholesta-5,7-dien-3β-ol (9b) in the medium. The dienol (9b) is a convenient starting material for the synthesis of the required 5α-cholest-8(14)-en-3β-ol and it is labelled with tritium exactly like cholesterol. Consequently we carried out the biosynthesis of the [$^3H_3$]dienol (9b), and converted it into (12).

A mixture of (3*R*,4*R*)-[4-^{3}H]- and (3*S*,4*S*)-[4-^{3}H]-MVA lactone (*ca.* 200 μCi) and (3*RS*)-[2-^{14}C]MVA lactone (*ca.* 20 μCi) was prepared, and a portion was converted to the diphenylmethylamide for determination of the ^3H : ^{14}C ratio [^3H : ^{14}C 9.23; atomic ratio (a.r.) ^3H : ^{14}C 1 : 1]. The remainder was incubated, as previously described,[11] with the supernatant from a rat liver homogenate prepared with buffers containing 5×10^{-5}M-AY-9944 centrifuged at 1×10^4. Following treatment with base, the nonsaponifiable products were recovered with hexane. From previous experience, we knew that the mixture of the nonsaponifiable products consisted mainly of labelled 7,8-didehydrocholesterol, accompanied by unlabelled cholesterol. Therefore, the crude nonsaponifiable residue was reduced with lithium in liquid ammonia to a mixture containing predominantly cholesterol and 5α-cholest-7-en-3β-ol.

The residue[11] was not fractionated, but instead was oxidized with Jones reagent to a mixture of cholest-4-ene-3,6-dione and 5α-cholest-7-en-3-one, easily resolvable by preparative t.l.c. The latter (^3H : ^{14}C 6.01)was then reduced with sodium borohydride to 5α-cholest-7-en-3β-ol (10a) (^3H : ^{14}C 5.64, a.r. ^3H : ^{14}C 3.06 : 5). Recrystallization of a sample of (10a), diluted with an inactive carrier, resulted in only a very small change in the ^3H : ^{14}C ratio. We considered it of importance to further ascertain the radiochemical purity of (10a). Consequently, a portion of the derived acetate (10b) was

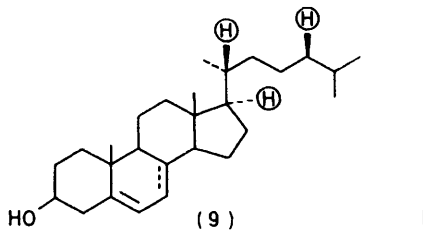


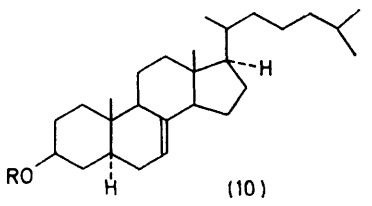
treated with osmium tetraoxide, and the resulting glycol (11a)[12] was converted to diacetate (11b). The sequence of transformations (10a) ⟶ (10b) ⟶ (11a) ⟶ (11b) proceeded without loss of tritium and the ^3H : ^{14}C ratios remained essentially unchanged (^3H : ^{14}C 5.79; a.r. ^3H : ^{14}C 3.13). The bulk of [$^3H_3$,$^{14}C_5$]cholest-7-en-3β-ol (10a) was then dissolved in acetic acid and shaken with platinum oxide under hydrogen. The obtained [$^3H_3$,$^{14}C_5$]cholest-8(14)-en-3β-ol (12a) was purified and counted as the acetate (12b) (^3H : ^{14}C 5.71; a.r. ^3H : ^{14}C 3.09 : 5). A chloroform solution of (12b) was cooled to −78° and treated with dry HCl to yield, after exposure to aqueous $NaHCO_3$, non-crystalline (2a) (^3H : ^{14}C 4.02; a.r. ^3H : ^{14}C 2.18 : 5). The acetate (2a) was saponified to the alcohol (2b) (glass; ^3H : ^{14}C 4.09), which, in turn, was converted into the crystalline *p*-bromobenzoate (2c) (^3H : ^{14}C 4.04). To confirm the homogeneity of (2c), it was treated with *m*-chloroperbenzoic acid to yield a homogeneous epoxide (13), (^3H : ^{14}C 4.03). The stereochemistry of the chromatographically and spectroscopically (n.m.r.) homogeneous epoxide has not been determined. Thus, it is evident that throughout the described transformations, the ^3H : ^{14}C ratios of the products remained essentially the same as that of (2a).

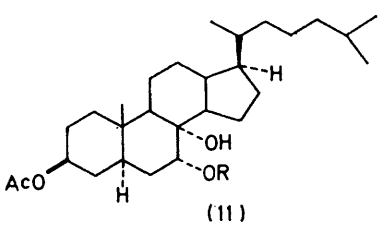
* AY-9944 = *trans*-1,4-*bis*-(2-chlorobenzylaminoethyl)cyclohexane dihydrochloride.

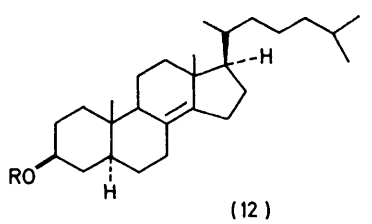
The results of this study indicate that the isomerization of the steroidal side-chain from the 17β (1) to the 17α (2) orientation on reaction with hydrogen chloride in chloroform at −78°, followed by treatment with base, proceeded with significant loss of tritium. Based on the ^{3}H : ^{14}C atomic ratios of (10a), (11b), and (12b), we arrive at an average atomic ratio (^{3}H : ^{14}C 3.09 : 5) for the starting material used in the isomerization experiments. Similarly, from the atomic ratios of (2a—c) and (13), we calculated the average atomic ratio (^{3}H : ^{14}C 2.19) for the Δ^{14}-17β isomerization product. It follows that the isomerization proceeded with the average loss of *ca.* 29% of the tritium initially present in the starting material. This is equivalent to the statistical loss of *ca.* 0.9 atom of tritium per molecule of the starting material. This conclusion is based on the fact that the 7,8-didehydrocholesterol (9b) biosynthesized from (4*R*)-[4-^{3}H,2-^{14}C]MVA contains three atoms of tritium at the 17α, 20-*pro-R*, and 24-*pro-R* positions. From *X*-ray investigations,[1] it is known that the stereochemistry at C-20 of the isomerization product (2) and of the starting material (1) is the same. Hence, (2) must have retained a tritium atom at C-20. The 24*R* tritium atom is remote from the site of reaction and, therefore, must also be present in (2). Consequently, it can be concluded that the isomerization of (1) to (2) proceeded with the loss of the 17α-hydrogen (tritium) atom of (1). These results are in accord with the view that the reaction has proceeded *via* intermediates of the type of (6a) or considerably less likely (6b). In view of the isolation of a spiro-compound [13] of the type (6a), the pathway *via* a 12(14)-spiro intermediate seems more plausible.

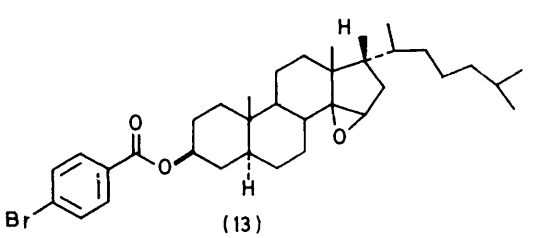
The apparent retention in (2) of *ca.* 0.1 atom of tritium initially present at C-17α of (1) may be the result of cumulative experimental errors. Alternatively, it may be indicative of the operation of one or several competing mechanisms. Indeed, as indicated earlier, the operation of the sequence (5) ⟶ (7) ⟶ (2), or of the 1,2 hydrogen shift mechanism, would result in the retention in (2) of the 17α-tritium atom of (1). However, if such a minor pathway is operative, it is of little significance as compared with the pathway(s) involving the loss of the C-17 hydrogen.











EXPERIMENTAL

I.r. spectra were taken in $CHCl_3$ solution on a Perkin-Elmer 237 instrument. N.m.r. spectra were taken for $CDCl_3$ solutions on Varian EM-360 or Varian HA-100 instruments. Mass spectra were taken on a Nuclide 12-90-G mass spectrometer with a Nuclide DA/CS I.2 data acquisition system. Samples for liquid scintillation

counting were dissolved in New England Nuclear Aquasol (10 ml) and counted on a Nuclear Chicago Mark II automatic liquid scintillation counter. Female Sprague–Dawley rats (150—200 g) used in these studies were obtained from Charles River Breeding Laboratories. (3*RS*)-[2-^{14}C]Mevalonic acid lactone and (3*R*,4*R*)- and (3*S*,4*S*)-[4-^{3}H]mevalonic acid lactone were obtained from Amersham–Searle Corporation. Silica gel (Merck HF 254 + 366) was used for t.l.c.; where indicated, 15% $AgNO_3$ (w/w) was added to silica gel before preparation of plates. AY-9944 was a gift from Dr. D. Dvornik, Ayerst Laboratories, Montreal. M.p.s were taken on a hot stage apparatus and are corrected.

Preparation of Precursor.—(3*R*,4*R*)- and (3*S*,4*S*)-[4-^{3}H]-Mevalonic acid lactone (*ca.* 200 μCi; 25 mCi mmol^{-1}) and (3*RS*)-[2-^{14}C]mevalonic acid lactone (*ca.* 20 μCi; 22 mCi mmol^{-1}) were dissolved in benzene (25 ml). A portion of the solution (0.125 ml) was removed and mixed with unlabelled mevalonic acid lactone (250 mg), and converted in the usual way [14] into mevalonic acid diphenylmethylamide (126 mg). The amide was crystallized from ethyl acetate–hexane and counted (Table).

^{3}H : ^{14}C Ratios of the biosynthesized 5α-cholest-7-en-3β-ol (10a) and its transformation products

Compound and crystallization	^{3}H: ^{14}C Ratio Atomic Isotopic	Experimental	Theoretical
(4*R*)-[4-^{3}H, 2-^{14}C]MVA lactone diphenylmethylamide			
1	9.38		
2	9.23		
3	9.23		1 : 1
Cholest-4-ene-3,6-dione	6.00	3.25 : 5	3 : 5
5α-Cholest-7-en-3-one	6.01	3.25 : 5	3 : 5
5α-Cholest-7-en-3β-ol (10a)			
Crude	5.83		
1	5.76		
2	5.76		
3	5.64	3.06 : 5	3 : 5
3β,7α-Diacetoxy-8α-hydroxy-5α-cholestane (11b)			
1	5.88		
2	5.86		
3	5.81		
4	5.79	3.13 : 5	3 : 5
5α,Cholest-8(14)-en-3β-yl acetate, (12b)			
1	5.85		
2	5.80		
3	5.71	3.09 : 5	3 : 5
5α,17β-Cholest-14-en-3β-yl acetate (2a)	4.02	2.18 : 5	2 : 5
5α,17β-Cholest-14-en-3β-ol (2b)	4.09	2.22 : 5	2 : 5
5α,17β-Cholest-14-en-3β-yl-*p*-bromobenzoate (2c)			
1	4.08		
2	4.06		
3	4.04	2.19 : 5	2 : 5
13,14-Epoxy-5α,17β-cholestan-3β-yl *p*-bromobenzoate (13)			
1	4.04		
2	4.03	2.18 : 5	2 : 5

The [^{3}H,^{14}C]MVA lactone solution was equally distributed among four 50 ml Erlenmeyer flasks. To each was added 0.1M-KOH (2 drops) in ethanol and, after 1 h at room temperature, the solvent was removed in a gentle stream of nitrogen.

Incubation of (3R,4R)- *and* (3S,4S)-[4-^{3}H,2-^{14}C]-*Mevalonic Acid with a Rat Liver Preparation.*—Rat liver (20 g) from four rats was homogenized in 0.05M-phosphate buffer (2.5 ml per g of liver), pH 7.4, containing nicotinamide (0.03M), $MgCl_2$ (0.004M), and AY-9944 (2×10^{-4}M), using a loose-fitting glass Potter–Elvejhem homogenizer. The homogenate was centrifuged at 1×10^4 g for 20 min at 4°. The lipid layer was removed by aspiration, and the homogenate distributed equally in the four 50 ml Erlenmeyer flasks, containing [^{3}H,^{14}C]mevalonic acid. The flasks were then incubated aerobically at 37° in a Dubnoff shaking incubator for 4 h. After 1, 2, and 3 h, to each flask was added 1.25 ml of a stock solution containing NADP (24 mg), NADH (23 mg), ATP (138 mg), and ascorbic acid (138 mg) in the above buffer solution (15 ml).

Isolation of [17α,20,24R-$^{3}H_3$,$^{14}C_5$]*Cholest-7-en-3β-ol* (10a).—At the end of the incubation, the homogenates from the four flasks were combined, and refluxed with 10% KOH in ethanol (100 ml) for 4 h under nitrogen in the dark. After standing at 25° overnight, the solution was extracted with hexane (4 × 100 ml) and the extract was washed, dried (Na_2SO_4), and evaporated. The crude product contained 9.46×10^6 d.p.m. ^{14}C. This was then reduced with lithium in liquid ammonia and the recovered product was oxidized with Jones' reagent, as previously described.[11] From the resultant mixture, cholest-4-ene-3,6-dione (2.72×10^5 d.p.m. ^{14}C) and cholest-7-en-3-one (3.0×10^6 d.p.m. ^{14}C) were isolated by preparative t.l.c. The 7-en-3-one was then reduced with sodium borohydride,[11] and following t.l.c. purification [solvent, ethyl acetate–hexane (1 : 3)] 5α-cholest-7-en-3β-ol (10a) (2.04×10^6 d.p.m. ^{14}C) was obtained. Approximately one-half of this material was diluted with unlabelled cholest-7-en-3β-ol (800 mg), and a portion of this mixture was recrystallized from $CHCl_3$–MeOH and counted (Table).

[$^{3}H_5$,$^{14}C_5$]-3β,7α-*Diacetoxy-5α-cholestan-8α-ol* (11b).—A portion (*ca.* 5% of total) of the undiluted (10a) was mixed with unlabelled cholest-7-en-3β-ol (350 mg) and acetylated (acetic anhydride–pyridine) to give, following t.l.c. [solvent, ethyl acetate–hexane (3 : 7)], (10b) (360 mg). A mixture of the acetate, ether (25 ml), pyridine (3 ml), and osmium tetraoxide (250 mg) was stored at room temperature for 42 h in the dark.[12] Water (20 ml), pyridine (15 ml), and sodium hydrogensulphite (1.2 g) were added and the mixture was stirred for 25 min. The solid was removed by filtration through Celite and the filtrate was extracted with $CHCl_3$ (5 × 50 ml). The extract was washed with water, saturated NaCl, dried (Na_2SO_4), and evaporated. The major product 3β-acetoxy-5α-cholestane-7α,8α-diol (11a) (40 mg) was isolated by preparative t.l.c. [ethyl acetate–hexane (3 : 2); R_F 0.3], and was identical with an authentic specimen.[12] The diol (11a) was acetylated in the usual manner and the acetate (11b) was recrystallized from $CHCl_3$–MeOH (needles), m.p. 169—170°; $\nu_{max.}$ 3 580, 1 720, and 1 250 cm^{-1}; δ 0.72 (3 H, s, 13-Me), 0.95 (3 H, s, 10-Me), 2.05 (3 H, s, OAc), 2.06 (3 H, s, OAc), 4.7br (1 H, m, 3α-H), and 5.20 (1 H, dd, J_1 8, J_2 7 Hz, 7α-H). The acetate (11b) was counted (Table).

[17α,20,24R-$^{3}H_3$,$^{14}C_5$]-5α-*Cholest*-8(14)-*en*-3β-*yl Acetate* (1).—A mixture of the 5α-cholest-7-en-3β-ol (10a) (780 mg), platinum oxide (50 mg), and acetic acid (12 ml) was stirred in an atmosphere of hydrogen (2 h). Then chloroform (50 ml) was added, the solid was removed by filtration and the filtrate was evaporated. The obtained alcohol was acetylated to give (1). A sample of (1) was recrystallized from $CHCl_3$–MeOH and counted.

[20,24-$^{3}H_2$,$^{14}C_5$]-5α,17β-*Cholest*-14-*en*-3β-*yl Acetate* (2a).—[$^{3}H_3$,$^{14}C_5$]-5α-Cholest-8(14)-en-3β-yl acetate (1) (200 mg) in

$CHCl_3$ (2.5 ml) was treated with a stream of hydrogen chloride gas (*ca.* 10 ml min^{-1}, dried by passage through concentrated H_2SO_4 and $CaCl_2$) at −78° for 7 h. Then nitrogen was bubbled through the solution for 20 min and saturated $NaHCO_3$ (50 ml) and ether (50 ml) were added. The mixture was stirred for 12 h at room temperature, extracted with ether, and the extract was washed (saturated NaCl), dried (Na_2SO_4), and evaporated. The residue was fractionated by argentation t.l.c. [solvent, ether–light petroleum (b.p. 35—70°) (1 : 19)] and the major product (2a) (R_F 0.4) was isolated as a glass (107 mg), δ 0.83 (3 H, s), 0.90 (3 H, s), 0.92 (3 H, s), 1.12 (3 H, s), 2.02 (3 H, s, OAc), 4.78br (1 H, s, $W_{1/2}$ *ca.* 20 Hz, 3α-H), and 5.11br (1 H, s, $W_{1/2}$ 5 Hz, 14-H).

[$^3H_2$,$^{14}C_5$]-5α,17β-*Cholest*-14-*en*-3β-*yl* p-*Bromobenzoate* (2c).—The acetate (2a) (75 mg) was dissolved in THF (0.5 ml) and treated with 1M-KOH in MeOH (10 ml) at room temperature for 24 h. The mixture was diluted with H_2O, extracted with ether and processed in the usual manner. The product was purified by preparative t.l.c. [solvent, ethyl acetate–hexane (1 : 4)] to give the alcohol (2b) (63 mg) as a glass, δ 0.82 (3 H, s), 0.85 (3 H, s), 0.90 (3 H, s), 1.10 (3 H, s), 3.56 (1 H, m, 3β-H), and 5.08br (1 H, s, $W_{1/2}$ 5 Hz, 14-H).

The alcohol (2b) was treated with *p*-bromobenzoyl chloride (141 mg) in dry pyridine (3 ml) at room temperature for 36 h. The mixture was diluted with water, extracted with ether, and the extract washed with water, 1M-HCl, saturated Na_2CO_3, saturated NaCl, dried (Na_2SO_4), and evaporated. The product was purified by preparative t.l.c. [solvent, ethyl acetate–hexane (1 : 19)] to yield (2c) (100 mg). Crystallization ($CHCl_3$–MeOH) gave (2c) (needles), m.p. 100—101°; $\nu_{max.}$ 1 710, 1 275, and 1 220 cm^{-1}; δ 0.77 (3 H, s), 0.82 (3 H, s), 0.84 (3 H, s), 1.04 (3 H, s), 4.80br (1 H, m, $W_{1/2}$ *ca.* 20 Hz), and 5.02br (1 H, s, $W_{1/2}$ 5 Hz).

[$^3H_2$,$^{14}C_5$]-14,15-*Epoxy*-5α,17β-*cholestan*-3β-*yl* p-*Bromobenzoate* (13).—The *p*-bromobenzoate (2c) (36 mg) in $CHCl_3$ (5 ml) was treated with *m*-chloroperbenzoic acid (30 mg) at room temperature for 6 h. The solvent was evaporated and the epoxide (13) was crystallized from $CHCl_3$–MeOH (needles), m.p. 120—121°, $\nu_{max.}$ 1 710, 1 275, 1 120, and 1 102 cm^{-1}; δ 0.84 (3 H, s), 0.90 (3 H, s), 0.92 (3 H, s), 1.12 (3 H, s), 3.33br (1 H, s, $W_{1/2}$ 3 Hz), 4.90br (1 H, m, $W_{1/2}$ 20 Hz), and 7.56 and 7.88 (4 H, AB q, J_{AB} 8 Hz).

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