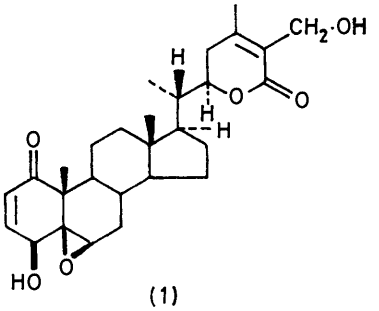
Synthetic Studies of Withanolides. Part I.[1] Synthesis of 5,6β-Epoxy-4β-hydroxy-5β-cholest-2-en-1-one and Related Compounds

By **Masaji Ishiguro, Akira Kajikawa, Toshio Haruyama, Yoshiko Ogura, Mari Okubayashi, Masuo Morisaki,** and **Nobuo Ikekawa,*** Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology, Meguro-ku, Tokyo, Japan

The title compounds, which have functionalities in rings A and B similar to those of withaferin A(1), have been synthesized through the key intermediates cholesta-2,5-dien-1-one (10) and -2,4-dien-1-one (26). Electrophilic reactions of the steroidal 2,4-dien-1-one system were observed to occur stereoselectively on the β-side of the 4,5-double bond.

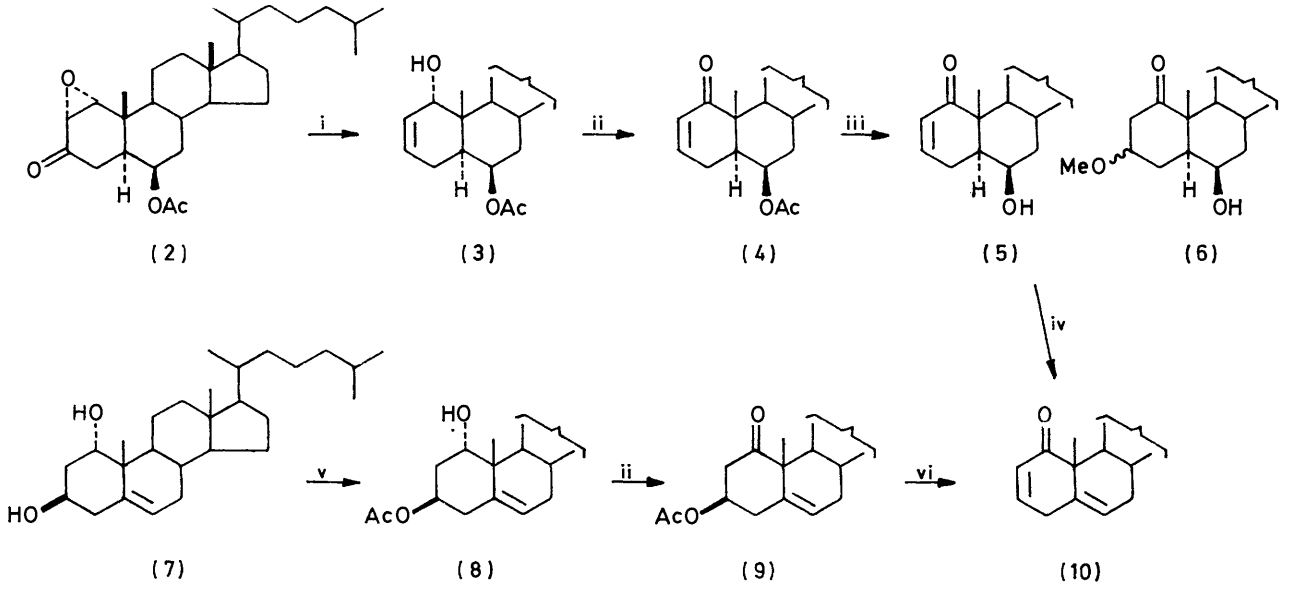
We have briefly reported[1] a stereoselective synthesis of 5,6β-epoxy-4β-hydroxy-5β-cholest-2-en-1-one (25),



(1)

which has the same functionalities in rings A and B as withaferin A (1), a cytotoxic principle found in some species of Solanaceae.[2,3] An inhibitory effect of (25) against the growth of Sarcoma 180 ascites tumour[4] prompted us to synthesize analogous compounds for elucidating the structure–activity relationship. The present paper gives details of the synthesis of (25) and its analogues (16), (18), (20), (22), (39), and (40), all of which contain an α-hydroxy-epoxide system connected to the cholest-2-en-1-one unit. Another congener (30) has been prepared by the method of Weissenberg *et al.*[5]

The key intermediates were cholesta-2,4-dien-1-one (26)[6] and its isomer, cholesta-2,5-dien-1-one (10). The latter was prepared by two alternative routes. 6β-Acetoxy-1α,2α-epoxy-5α-cholestan-3-one (2)[7] was reduced with hydrazine to give the 2-en-1α-ol (3), which was oxidized with Jones reagent, affording the ketone (4) in 45% yield from (2). Alkaline hydrolysis of (4) in methanol induced a Michael-type addition yielding the methanol adduct (6), in addition to the expected alcohol (5). Changing the solvent to dioxan, however, gave the alcohol (5) as the sole product. Dehydration of (5) with



Scheme 1 Reagents: i, $NH_2{\cdot}NH_2$; ii, Jones reagent; iii, KOH; iv, $POCl_3$; v, Ac_2O–AcOH; vi, NaOH

[1] Preliminary report, M. Ishiguro, A. Kajikawa, T. Haruyama, M. Morisaki, and N. Ikekawa, *Tetrahedron Letters*, 1974, 1421. The present paper is Part XX of the series 'Studies on Steroids;' Part XIX, M. Morisaki, N. Koizumi, N. Ikekawa, T. Takeshita, and S. Ishimoto, *J.C.S. Perkin I*, 1975, 1421.

[2] S. M. Kupchan, R. W. Doskotch, P. Bollinger, A. T. McPhail, G. A. Sim, and J. A. S. Renauld, *J. Amer. Chem. Soc.*, 1965, **87**, 5805.

[3] D. Lavie, E. Glotter, and Y. Shovo, *J. Chem. Soc.*, 1965, 7517.

[4] Details of biological activities will be described elsewhere.

[5] M. Weissenberg, E. Glotter, and D. Lavie, *Tetrahedron Letters*, 1974, 3063.

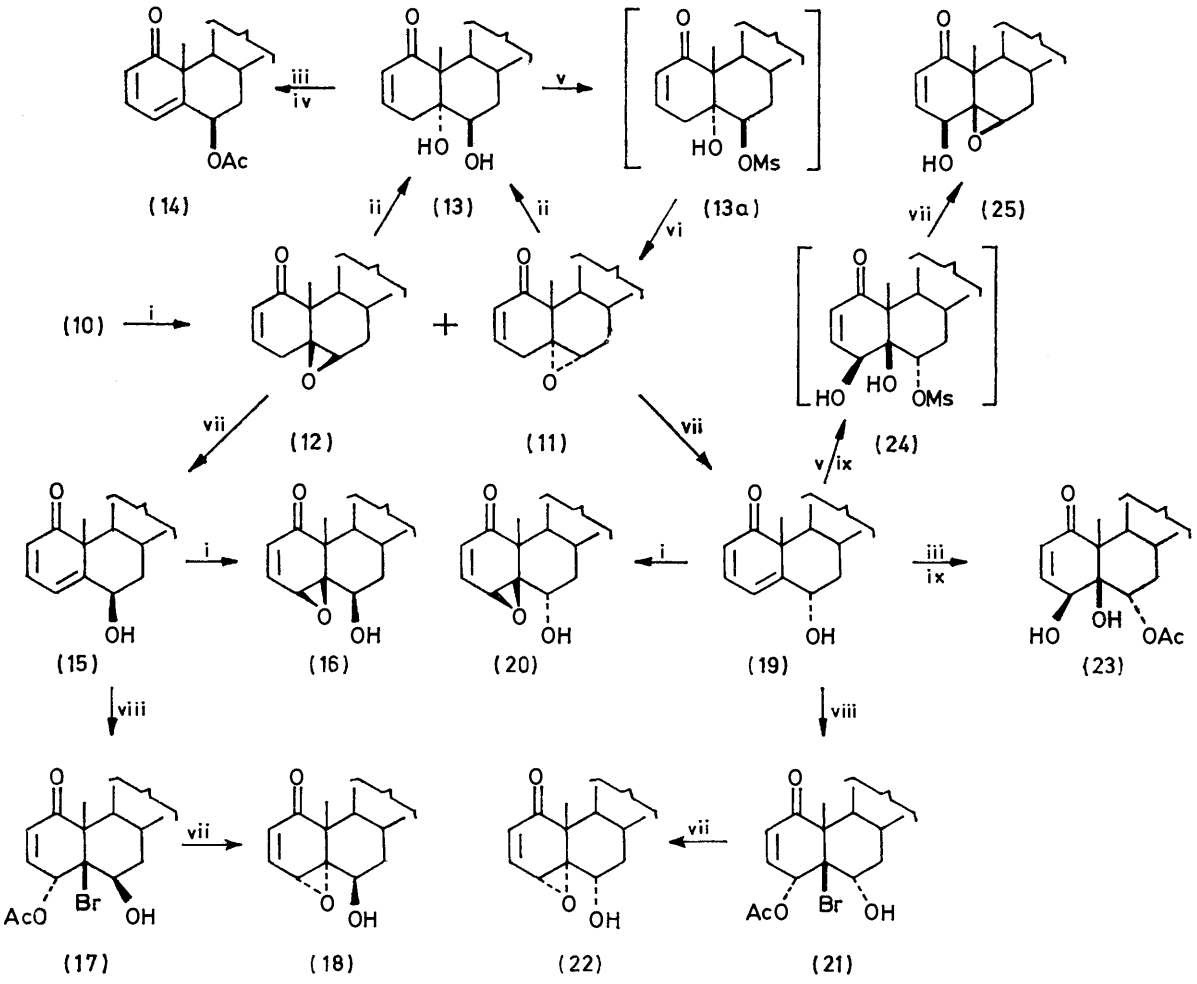
[6] H. Izawa, M. Morisaki, and K. Tsuda, *Chem. and Pharm. Bull. (Japan)*, 1966, **14**, 873.

[7] B. Pelc and E. Kodicek, *J. Chem. Soc. (C)*, 1970, 1624.

phosphoryl chloride in pyridine led to the dienone (10) in 90% yield.

The second synthesis of (10) started from 1α-hydroxycholesterol (7), the preparation of which has been studied by many groups because of its usefulness as an immediate synthetic precursor of an active form of vitamin D_3, 1α-hydroxycholecalciferol.[8] The less hindered 3β-hydroxy-group in (7) was selectively acetylated with acetic anhydride in acetic acid, giving the monoacetate (8) in 60% yield (39% of starting material was recovered). Jones oxidation of (8) afforded the ketone (9), and the latter was treated with 1.5% sodium hydroxide in aqueous dioxan, resulting in elimination of acetic acid to give the dienone (10) [90% yield from (8)].

Epoxidation of the dienone (10) with *m*-chloroperbenzoic acid afforded a mixture of two epoxides (11) and (12) in the ratio 2 : 1 and these were separated by silica gel column chromatography. Their n.m.r. data (Table) were not adequate for assignment of the respective structures. Treatment of both epoxides (11) and (12) with 3% perchloric acid in tetrahydrofuran gave the same single glycol (13), suggesting a *trans*-diaxial ring opening. The configuration of the C-6 hydroxy-group in (13) was established as β by its transformation into the 6β-acetoxydienone (14) by acetylation followed by dehydration.



SCHEME 2 Reagents: i, *m*-CPBA; ii, $HClO_4$; iii, Ac_2O; iv, $SOCl_2$; v, MsCl, vi, $NaHCO_3$; vii, NaOH; viii, NBA–AcOLi; ix, OsO_4

The structure of (14) was confirmed by an alternative preparation from the enone (4), involving allylic bromination with *N*-bromosuccinimide and subsequent dehydrobromination with calcium carbonate. On the other hand, the methanesulphonate of the glycol (13a) could be reconverted into the more polar major epoxide (11) by treatment with sodium hydrogen carbonate in pyridine. From those chemical transformations, the major product of epoxidation of (10) was determined to be the 5α,6α-epoxide (11); the 5β,6β-epoxide structure (12) was assigned to the less polar minor product.

The 5β,6β-epoxide is reported to be the predominant

[8] M. Morisaki, K. Bannai, and N. Ikekawa, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 1853; D. H. R. Barton, R. H. Hesse, M. M. Pechet, and E. Rizzardo, *J. Amer. Chem. Soc.*, 1973, **95**, 2748; C. Kaneko, S. Yamada, S. Sugimoto, M. Ishikawa, T. Sasaki, and T. Suda, *Tetrahedron Letters*, 1973, 2339; A. Fürst, L. Labler, W. Meier, and K-H. Pfoertner, *Helv. Chim. Acta*, 1973, **56**, 1708; M. N. Mitra, A. W. Norman, and W. H. Okamura, *J. Org. Chem.*, 1974, **39**, 2931.

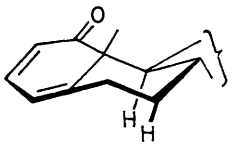
product of epoxidation by peroxy-acid of withanolide L, which contains a 2,5,14-trien-1-one system.[9]

When the epoxides (12) and (11) were refluxed with dioxan containing 15% sodium hydroxide,[10] the dienone alcohols (15) and (19), respectively, were obtained in nearly quantitative yield. Compound (15) was identified with the hydrolysis product of the acetoxy-dienone (14), confirming its 6β-hydroxy-configuration. This suggests, in turn, retention of configuration at C-6 of each epoxide during base-catalysed ring openings.[11] The configurations of (15) and (19) were supported by n.m.r. analysis. Thus, the 10-methyl signal of (15) appeared at lower field ($\Delta\delta$ *ca.* 0.19 p.p.m.) than that of (19), indicating the presence of a hydroxy-group in a 1,3-diaxial relation. Furthermore, the half-height widths of the C-6 proton signals of (15) (5 Hz) and (19) (14 Hz) were compatible with their quasi-equatorial and axial nature, respectively.

Oxidation of compound (15) or (19) with *m*-chloroperbenzoic acid occurred stereoselectively to afford a single epoxide, (16) or (20) respectively. The homogeneity of the products was shown by t.l.c. and n.m.r. spectra. In order to ascertain their stereochemistry, we attempted to prepare the stereoisomeric epoxides. For this purpose, the dienone (15) was treated with *N*-bromoacetamide–lithium acetate in acetic acid.[12] In this reaction also, the electrophile (*i.e.* Br^+) seemed to approach the 4,5-double bond preferentially from one side, since a single bromoacetate (17) was obtained in 70% yield. The β-orientation of bromine function was inferred from the 10-methyl n.m.r. signal, which appeared at low field (δ 1.62). Compound (17) was converted into the epoxide (18) by treatment with 10% sodium hydroxide in dimethylformamide. Similarly the dienone (19) was transformed, *via* the bromoacetate (21), into the epoxide (22).

Configurational assignments of the epoxides (16), (18), (20), and (22) were based on a comparative n.m.r. analysis (Table). The C-4 proton signal in each case appeared as a doublet of doublets, suggesting a quasi-equatorial orientation, since only this conformation is compatible with W-type coupling with the C-2 proton. The C-10 methyl signal of the β-epoxide (16) appeared at δ 1.51, to low field of that of the α-isomer (18) (δ 1.37); this may be ascribable to deshielding by the 1-oxo-function of (16). Analogously, compound (20) showed a significant lower field shift in comparison with its counterpart (22). Furthermore, the C-4 proton of the acetyl derivative of (18) resonated at δ 3.70, owing to deshielding by the acetyl carbonyl group. The corresponding signal of the β-epoxide (16) acetate appeared at δ 3.30.

Although participation[13] by the neighbouring 6β-hydroxy-group may assist in controlling the stereochemical course of the epoxidation of (15), the stereoselective 4β,5β-attack seemed to be intrinsic in electrophilic reactions of the 2,4-dien-1-one system. Thus, the 6α-hydroxy-analogue (19), as well as cholesta-2,4-dien-1-one (26), showed a similar stereoselectivity in peroxy-acid oxidation, bromoacetoxylation, and osmium tetraoxide oxidation (see later). Complete rationalization is difficult at present, but a molecular model indicates that the 2,4-diene-1-one system has a conformation similar to that of the 1,4-dien-3-one system,[14] in which rings A and B may assume a quasi-*cis* and thence the 10-methyl group a quasiequatorial conformation [see (A)]. In those situations, α-side approach of reagents to the 4,5-double bond may suffer from steric interaction with the 7α- and 9α-hydrogen atoms.



(A)

Oxidation of the hydroxy-dienone (19) acetate with osmium tetraoxide provided the glycol (23) in 95% yield. The n.m.r. signal at δ 1.27 due to the 10-methyl group was shifted to δ 1.38 by changing the solvent from $CDCl_3$ to C_6D_6, and the C-4 proton signal at δ 4.80 showed coupling with the allylic C-2 proton (*J* 2 Hz). These data strongly indicated a 4β,5β-structure for the glycol (23). In view of this information, a similar oxidation was carried out on the dienone (19) methanesulphonate, leading to the 4β,5β-glycol (24). This was directly treated with 15% sodium hydroxide in pyridine to afford the epoxide (25) in 85% yield from (19). The relevant n.m.r. signals (Table) showed perfect agreement with those of withaferin A(1), providing an additional proof of structure.

The remaining two analogues (39) and (40) were prepared from cholesta-2,4-dien-1-one (26).[6] A stereoselective epoxidation of the dienone (26) with *m*-chloroperbenzoic acid gave the 4β,5β-epoxide (27) in 80% yield. The isomeric 4α,5α-epoxide (29) was also synthesized through bromoacetoxylation of (26), in a manner similar to that described above. Their stereochemistry was deduced from analogous considerations of n.m.r. spectra (Table), as for the structural assignments of compounds (16), (18), (20), and (22).

When the β-epoxide (27) was treated with 60% perchloric acid in tetrahydrofuran, the 4α,5β-diol was obtained in 70% yield, and was characterized as its acetate (31). The presence of the 4α-acetoxy-group was evidenced by conversion into the 4α-acetoxy-dienone (33). Thus, dehydration of (31) with thionyl chloride in pyridine afforded three products. One of them (30%) was identified as the enol acetate (32) from spectroscopic

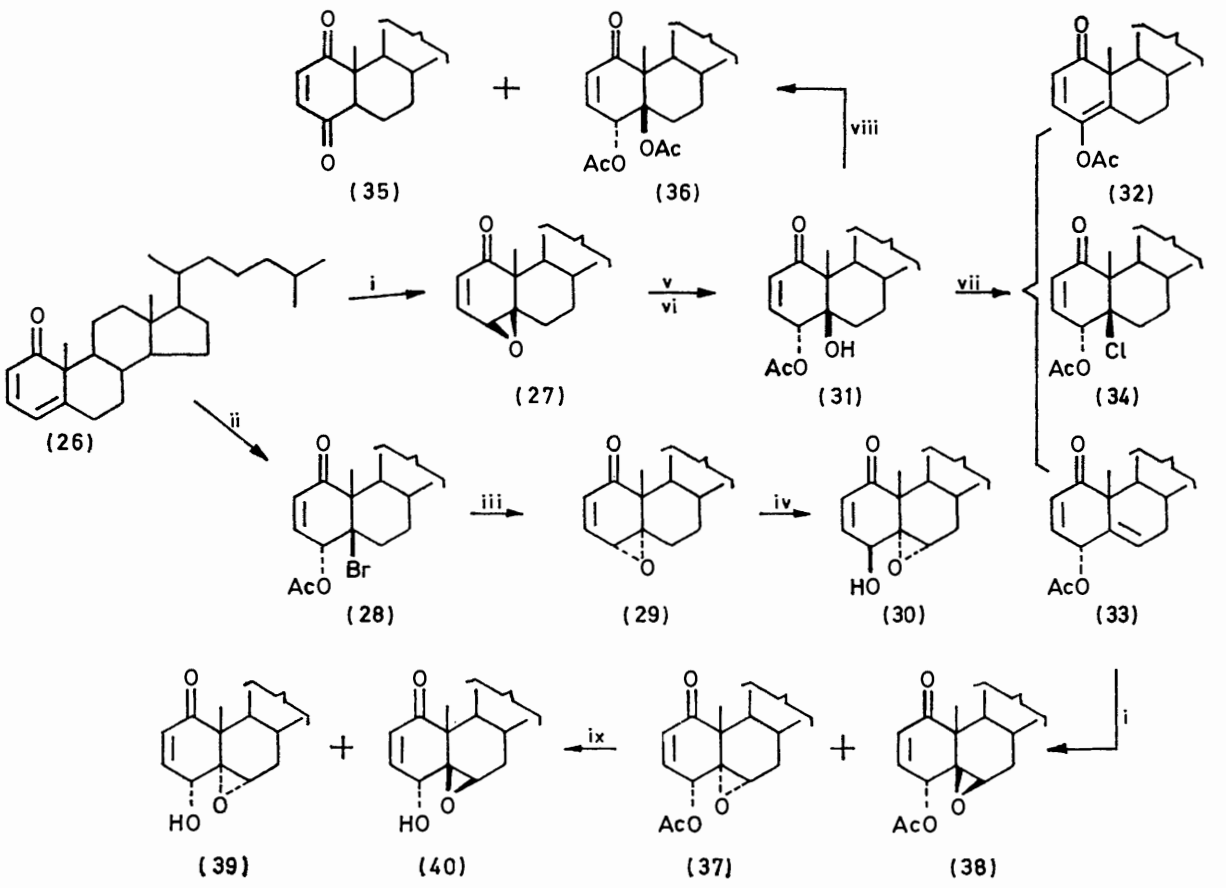
[9] E. Glotter, A. Abraham, and D. Lavie, *Tetrahedron*, 1973, **29**, 1353.
[10] R. Tschesche, H. Schwang, H. W. Fehlhaber, and G. Snatzke, *Tetrahedron*, 1966, **22**, 1129.
[11] D. H. R. Barton and Y. Houminer, *J.C.S. Perkin I*, 1972, 919.
[12] S. G. Levine and M. E. Wall, *J. Amer. Chem. Soc.*, 1959, **81**, 2826.
[13] H. B. Henbest and R. N. L. Wilson, *J. Chem. Soc.*, 1957, 1958.
[14] R. Bucourt, *Topics Stereochem.*, 1974, **8**, 159.

evidence (Experimental section), and the other (50%) was assigned structure (33), showing spin coupling between the 2- and 4β-protons (J 2 Hz) and also between the 3- and 4β-protons (J 2 Hz). The third product (34) (15%) attempted with phophorus pentaoxide in benzene; this gave the 1,4-dioxo-compound (35) [15] (12%) and the diacetoxy-compound (36) (30%). The n.m.r. spectrum of (36) revealed two acetyl methyl signals at δ 1.95 and

^{1}H N.m.r. (δ values; J and $W_{\frac{1}{2}}$ in Hz)

Compound	2-H	3-H	4-H	$J_{2,3}$	$J_{2,4}$	$J_{3,4}$	6-H	$W_{\frac{1}{2}}$6-H	10-Me
(16)	6.02	7.02	3.27	10	2	4	3.58	5	1.51
(18)	5.97	6.93	3.33	10	2	4	3.33	5	1.37
(20)	6.03	7.05	3.72	10	2	4	4.20	12	1.30
(22)	6.00	6.92	3.75	10	2	4	3.95	12	1.18
(27)	6.00	7.08	3.25	10	2	4			1.33
(29)	5.95	6.90	3.20	10	2	4			1.26
(1) [a]	6.20	6.93	3.74	10	0	6	3.20	4	1.40
(25)	6.20	6.93	3.74	10	0	6	3.20	4	1.40
(30)	5.97	6.68	3.70	10	0	4	3.23		1.50
(40)	6.01	6.82	4.69	10	2	2	3.66	4	1.25
(39)	5.97	6.67	4.69	10	2	2	3.97		1.35
(12)	6.00	6.80	2.95 [b]	10	2	2 and 5	3.07		1.23
(11)	5.94	6.68	3.05 [b]	10	2	2 and 5	3.02		1.33

[a] We thank Dr. S. M. Kupchan for the spectrum of withaferin A (1). [b] Only the 4β-proton signal was discerned; the 4α-H signal was hidden in the methylene envelope.



SCHEME 3 Reagents: i, *m*-CPBA; ii, NBA–AcOLi; iii, NaOH; iv, $HClO_4$, Ac_2O, $SOCl_2$, *m*-CPBA, and NaOH; v, $HClO_4$; vi, Ac_2O; vii, $POCl_3$; viii, P_2O_5; ix, NaOH

might be produced in an S_Ni fashion; its structure was deduced from its n.m.r. spectrum which is almost identical with that of the 5β-bromo-analogue (28). An unusual reaction was observed when dehydration of (31) was 2.18 and a signal corresponding to an equatorial proton (at C-4), α to the acetoxy-group (δ 6.33, $J_{3,4}$ 4 Hz).

[15] E. Glotter, M. Weissenberg, and D. Lavie, *Tetrahedron*, 1970, **26**, 3857.

Further corroboration came from identification with the product of acetylation of (31) with acetic anhydride–toluene-*p*-sulphonic acid.

The stereochemical course of the acid-catalysing ring opening of the epoxide (27) seemed to be controlled mainly by steric factors: hydroxide anion attacks the less hindered C-4 in S_N2 fashion, rather than a tertiary C-5 carbocation. A similar situation was observed in bromoacetoxylation of the dienones (15), (19), and (26), where the intermediate bromonium ions appeared to be attacked by acetate ion regioselectively at C-4 in the *anti* sense.

Oxidation of the dienone acetate (33) with *m*-chloroperbenzoic acid afforded a mixture of two epoxides (37) and (38) in the ratio 3 : 1. Subsequent saponification yielded the corresponding alcohols (39) and (40). Their stereochemistry was deduced by comparing n.m.r. data (Table) with those of the 4-deoxy-analogues (11) and (12). The signals due to the 6-proton in the β-epoxides (12) and (40) were doublets, whereas the corresponding signals of the α-epoxides (11) and (39) were broad singlets. Furthermore the β-epoxides (12) and (40) showed 10-methyl signals at significantly higher field (Δδ *ca.* 0.1 p.p.m.) than the α-epoxides (11) and (39).

EXPERIMENTAL

M.p.s were determined with a hot-stage microscope. I.r. spectra were taken for KBr discs with a Hitachi ESI-G_2 spectrometer and optical rotations for solutions in chloroform with a JASCO-DIP-S polarimeter. U.v. spectra were recorded by use of a Hitachi ESP-3T apparatus, with ethanol as solvent. ^{1}H N.m.r. spectra were run with a Varian T60 or JEOL JNM-4H-100 spectrometer ([^{2}H]chloroform as solvent and Me_4Si as internal reference). Mass spectra were determined with an LKB-9000S or Hitachi RMU-7L instrument at 70 eV.

Column chromatography was normally effected with Wako silica gel C-200. Preparative t.l.c. was carried out on 20 × 20 cm plates, precoated with Merck silica gel F_{254} (2 mm thick). Organic extracts were dried over Na_2SO_4. 'The usual work-up' refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying, filtration, and evaporation under vacuum. Ether refers to diethyl ether, THF to tetrahydrofuran, DMF to dimethylformamide, NBA to *N*-bromoacetamide, and *m*-CPBA to *m*-chloroperbenzoic acid.

5α-Cholest-2-ene-1α,6β-diol 6-Acetate (3).—The epoxy-acetate (2) (24.7 g, 54 mmol) in propan-2-ol (520 ml) was refluxed with hydrazine hydrate (130 ml) and acetic acid (6.5 ml). The usual work-up, with ethyl acetate for extraction, gave a crude product (32 g). Chromatography on silica (300 g) [benzene–hexane (4 : 1)] afforded the *allylic alcohol* (3) (12 g, 48%), m.p. 108—110° (from methanol) $[\alpha]_D$ +74° (*c* 1.72), $\nu_{max.}$ 3 350, 1 735, 1 640, and 1 240 cm^{-1}, δ 2.05 (3 H, s, Ac), 3.45 (1 H, s, 1β-H), 5.05 (1 H, m, 6α-H), and 5.75 (2 H, s, 2- and 3-H) (Found: C, 78.5; H, 11.0. $C_{29}H_{48}O_3$ requires C, 78.3; H, 10.9%).

6β-Acetoxy-5α-cholest-2-en-1-one (4).—The allylic alcohol (3) (50 mg, 0.11 mmol) in acetone (2 ml) was treated with Jones reagent until the mixture turned orange-brown. The usual work-up (ether for extraction) provided the crystalline *enone* (4) (50 mg), m.p. 94—94.5° (from methanol), $[\alpha]_D$ +74° (*c* 1.55), $\lambda_{max.}$ 227 nm, $\nu_{max.}$ 1 735, 1 680, and 1 250 cm^{-1}, δ 2.05 (3 H, s, Ac), 5.05 (1 H, m, 6α-H), 5.69 (1 H, m, 2-H), and 6.63 (1 H, m, 3-H) (Found: C, 78.4; H, 10.45. $C_{29}H_{46}O_3$ requires C, 78.7; H, 10.45%).

Cholest-5-ene-1α,3β-diol 3-Acetate (8).—1α-Hydroxycholesterol (7) (316 mg, 0.79 mmol) was stirred with acetic acid (1.1 ml) and acetic anhydride (0.5 ml, 5.3 mmol) at 80 °C for 4 h. The usual work-up (ether for extraction) gave a crude product (343 mg). Chromatography on silica (17 g) [benzene–ether (19 : 1)] gave the *monoacetate* (8) (206 mg, 59%), m.p. 170—172° (from methanol), δ 1.02 (3 H, s, 10-Me), 2.0 (3 H, s, Ac), 3.85 (1 H, m, 1β-H), 5.00 (1 H, m, 3α-H), and 5.58 (1 H, m, 6-H). Elution with benzene–ether (1 : 1) afforded unchanged (7) (123 mg).

6β-Hydroxy-5α-cholest-2-en-1-one (5).—The enone (4) (5 g, 11.3 mmol) in dioxan (100 ml) was refluxed with aqueous 15% sodium hydroxide (34 ml) for 12 h. The usual work-up (ether for extraction) afforded the *hydroxy-enone* (5) (4.5 g), m.p. 145—147.5° (from methanol), $[\alpha]_D$ +69° (*c* 0.91), $\nu_{max.}$ 3 420 and 1 660 cm^{-1}, δ 1.23 (3 H, s, 10-Me), 3.95 (1 H, m, 6α-H), 5.80 (1 H, dd, *J* 10 and 3 Hz, 2-H), and 6.80 (1 H, m, 3-H) (Found: M^+, 400.3295. $C_{27}H_{44}O_2$ requires *M*, 400.3341).

3β-Acetoxycholest-5-en-1-one (9).—The 3β-monoacetate (8) (206 mg, 0.465 mmol) in acetone (10 ml) was treated with Jones reagent. The usual work-up (ether for extraction) gave the crystalline *ketone* (9) (196 mg), m.p. 142—144° (from methanol), δ 1.28 (3 H, s, 10-Me), 2.05 (3 H, s, Ac), 5.0 (1 H, m, 3α-H), and 5.58 (1 H, m, 6-H) (Found: M^+, 442.3502. $C_{29}H_{46}O_3$ requires *M*, 442.3449).

Cholesta-2,5-dien-1-one (10).—(*a*) The 6β-hydroxy-enone (5) (1 g, 2.5 mmol) in pyridine (20 ml) at 0 °C was treated with phosphoryl chloride (1.1 ml) for 3 h. The usual work-up (ether for extraction) gave the *dienone* (10) (910 mg), m.p. 98.5—100° (from methanol), $[\alpha]_D$ −18° (*c* 1.49), δ 1.22 (3 H, s, 10-Me), 2.95 (1 H, m, 4α-H), 3.20 (1 H, m, 4β-H), 5.60 (1 H, m, 6-H), 5.85 (1 H, dq, *J* 10.3 and 2 Hz, 2-H), and 6.75 (1 H, dq, *J* 10.5 and 3 Hz, 3-H) (Found: M^+, 382.322. $C_{27}H_{42}O$ requires *M*, 382.323).

(*b*) The 3β-acetoxy enone (9) (60 mg, 0.316 mmol) in dioxan (5 ml) containing aqueous 15% sodium hydroxide (0.5 ml) was stirred at room temperature for 6 h. The usual work-up (ether for extraction) gave the dienone (10) (55 mg).

5,6α- and 5,6β-Epoxy-5α- and -5β-cholest-2-en-1-ones (11) *and* (12).—The 2,5-dienone (10) (4.08 g, 10.6 mmol) in chloroform (187 ml) was treated with *m*-CPBA for 3 h. The usual work-up provided a crude product (4.58 g) containing two epoxides. Chromatography on silica (180 g) [benzene–hexane (4 : 1)] gave the *5,6β-epoxide* (12) (1.27 g, 30%), m.p. 113—115° (from methanol), $[\alpha]_D$ +45° (*c* 1.51) (Found: C, 81.45; H, 10.8. $C_{27}H_{42}O_2$ requires C, 81.35; H, 10.6%). Further elution with benzene–hexane (9 : 1) gave the *5,6α-epoxide* (11) (2.40 g, 60.5%), m.p. 123—124° (from methanol).

5,6β-Dihydroxy-5α-cholest-2-en-1-one (13).—The 5,6α-epoxide (11) (200 mg, 0.50 mmol) in THF (27 ml) was stirred with aqueous 3% perchloric acid (10.7 ml) at room temperature for 5 h. The usual work-up (ether for extraction) gave the crystalline *diol* (13) (178 mg), m.p. 141.5—143.5° (from hexane–acetone), $[\alpha]_D$ +37° (*c* 1.53), δ 1.32 (3 H, s, 10-Me), 3.30 (1 H, dt, *J* 20 and 3 Hz, 4β-H), 3.64 (1 H, m, 6α-H), 5.88 (1 H, dd, *J* 10 and 3 Hz, 2-H), and 6.60 (1 H, dq, *J* 10 and 3 Hz, 3-H) (Found: M^+, 416.3248. $C_{27}H_{44}O_3$ requires *M*,

416.3288). By the same method, the same diol (154 mg) was obtained from the 5,6β-*epoxide* (12) (154 mg).

6β-*Acetoxycholesta*-2,4-*dien*-1-*one* (14).—(*a*) A solution (3 ml) in pyridine of 6β-acetoxy-5-hydroxy-5α-cholest-2-en-1-one (44 mg, 0.1 mole), obtained by acetylation (acetic anhydride–pyridine, room temperature, overnight) of the 5α,6β-diol (13), was treated with thionyl chloride (0.09 ml) at 0 °C for 20 min. The usual work-up (ether for extraction) gave the crystalline *dienone* (14) (40 mg), m.p. 103—104.5° (from methanol) $\lambda_{max.}$ 310 nm, $\nu_{max.}$ 1 735, 1 650, 1 620, and 1 240 cm^{-1}, δ 2.0 (3 H, s, Ac), 5.52 (1 H, m, 6α-H), 6.03 (1 H, d, *J* 10 Hz, 2-H), 6.3 (1 H, d, *J* 8 Hz, 4-H), and 6.9 (1 H, dd, *J* 10 and 8 Hz, 3-H) (Found: M^+ — AcOH, 380.3096. $C_{27}H_{30}O$ requires *M* — AcOH, 380.3077).

(*b*) The 6β-acetoxy-enone (4) (1 g, 2.49 mmol) in carbon tetrachloride (15 ml) was stirred with *N*-bromosuccinimide (600 mg, 3.37 mmol) in sunlight for 3 h. After filtration, the solvent was evaporated off under vacuum. Chromatography on silica (50 g) [hexane–benzene (1 : 5)] gave the 4ξ-bromide (950 mg, 73.5%), m.p. 140—140.5° (from methanol) $\nu_{max.}$ 1 740, 1 680, and 1 240 cm^{-1}. The 4ξ-bromide (950 mg, 1.83 mmol) in dimethylacetamide (2 ml) was added to a refluxing suspension of calcium carbonate (1 g) in dimethylacetamide (10 ml), and the mixture was refluxed for 30 min. The usual work-up (ether for extraction) gave a crude product (0.75 g). Chromatography on silica (24 g) [benzene–ether (9 : 1)] afforded the dienone (14) (550 mg, 68%).

6β-*Hydroxycholesta*-2,4-*dien*-1-*one* (15).—The 5β,6β-epoxy-enone (12) (1.26 g, 2.79 mmol) in dioxan (22 ml) was refluxed with aqueous 15% sodium hydroxide (2.52 ml) for 4 h. The usual work-up (ether for extraction) provided the crystalline *dienone* (15) (1.27 g), m.p. 165—167° (from methanol), $[\alpha]_D$ −146° (*c* 1.55), $\lambda_{max.}$ 312 nm, $\nu_{max.}$ 3 475, 1 650, and 1 565 cm^{-1}, δ 1.42 (3 H, s, 10-Me), 4.6 (1 H, m, 6α-H), 5.9 (1 H, d, *J* 9.6 Hz, 2-H), 6.11 (1 H, d, *J* 6 Hz, 4-H), 6.9 (1 H, dd, *J* 9.6 and 6 Hz, 3-H) (Found: M^+, 398.3132. $C_{27}H_{42}O_2$ requires *M*. 398.3182).

4β,5-*Epoxy*-6β-*hydroxy*-5β-*cholest*-2-*en*-1-*one* (16).—The 6β-hydroxy-dienone (15) (0.9 g, 2.26 mmol) in chloroform (40 ml) was treated with *m*-CPBA (505 mg, 2.49 mmol) at room temperature for 20 h. The usual work-up (chloroform for extraction) gave the crystalline *epoxide* (16) (963 mg), m.p. 182.5—184.5° (from methanol), $[\alpha]_D$ +11° (*c* 1.55), $\lambda_{max.}$ 242 nm, $\nu_{max.}$ 3 500 and 1 650 cm^{-1} (Found: C, 78.45; H, 10.35. $C_{27}H_{42}O_3$ requires C, 78.2; H, 10.2%).

4α-*Acetoxy*-5-*bromo*-6β-*hydroxy*-5β-*cholest*-2-*en*-1-*one* (17). A solution of the 6β-hydroxy-dienone (15) (220 mg, 0.53 mmol) in acetic acid (8 m.) containing lithium acetate (800 mg, 12.1 mmol) and NBA (80 mg, 0.58 mmol) was stirred at room temperature for 30 min. Water was added and the precipitate (261 mg) was filtered off and crystallized from methanol, to afford the *bromo-acetate* (17), m.p. 182.5—183°, $[\alpha]_D$ −128° (*c* 1.55), $\lambda_{max.}$ 220 nm, $\nu_{max.}$ 3 400, 1 740, 1 665, and 1 230 cm^{-1}, δ 1.62 (3 H, s, 10-Me), 2.17 (3 H, s, Ac), 4.0 (1 H, m, 6α-H), 6.13 (1 H, d, *J* 10 Hz, 2-H), 6.18 (1 H, d, *J* 4 Hz, 4β-H), and 6.70 (1 H, dd, *J* 10 and 4 Hz, 3-H), *m/e* 536 and 538 (M^+ and $M^+ + 2$).

4α,5-*Epoxy*-6β-*hydroxy*-5α-*cholest*-2-*en*-1-*one* (18).—The bromo-acetate (17) (180 mg, 0.355 mmol) in DMF (10.8 ml) was treated with aqueous 10% sodium hydroxide at room temperature for 30 min. The usual work-up (chloroform for extraction) afforded a crude product (140 mg). Chromatography on silica (6 g) [benzene–acetone (100 : 1)] gave the α-*epoxide* (18) (70 mg, 48%), m.p. 184—187° (from hexane–acetone), $[\alpha]_D$ +57° (*c* 1.49) (Found: M^+, 414.3200. $C_{27}H_{42}O_3$ requires *M*, 414.3132).

6β-*Hydroxycholesta*-2,4-*dien*-1-*one* (19).—The 5α,6α-epoxy-enone (11) (2.40 g, 5.28 mmol) in dioxan (42 ml) was refluxed with aqueous 15% sodium hydroxide (4.8 ml) for 4 h. The usual work-up (ether for extraction) gave the crystalline *dienone* (19) (2.27 g), m.p. 156—157° (from methanol), $[\alpha]_D$ −438° (*c* 1.56), $\lambda_{max.}$ 320 nm, δ 1.23 (3 H, s, 10-Me), 4.53 (1 H, m, 6β-H), 6.00 (1 H, d, *J* 10 Hz, 2-H), 6.37 (1 H, d, *J* 6 Hz, 4-H), and 7.03 (1 H, dd, *J* 10 and 6 Hz, 3-H) (Found: C, 81.35; H, 10.6. $C_{27}H_{42}O_2$ requires C, 81.35; H, 10.6%).

4β,5-*Epoxy*-6α-*hydroxy*-5β-*cholest*-2-*en*-1-*one* (20).—The 6α-hydroxy-dienone (19) (500 mg, 1.26 mmol) in chloroform (25 ml) was treated with *m*-CPBA (310 mg, 1.52 mmol) at room temperature for 20 h. The usual work-up (chloroform for extraction) afforded the crystalline *epoxide* (20) (512 mg), m.p. 150—151° (from methanol), $[\alpha]_D$ +3° (*c* 1.59) (Found: C, 77.9; H, 10.3. $C_{27}H_{42}O_3$ requires C, 78.2; H, 10.2%).

4α-*Acetoxy*-5-*bromo*-6α-*hydroxy*-5β-*cholest*-2-*en*-1-*one* (21).—A solution of the 6α-hydroxy-dienone (19) (660 mg, 1.56 mmol) in acetic acid (24 ml) containing lithium acetate (2.4 g) and NBA (240 mg, 1.74 mmol) was stirred at room temperature for 40 min. The usual work-up (di-isopropyl ether for extraction) gave a crude product (851 mg). Chromatography on silica (34 g) [benzene–ether (10 : 1)] afforded the *bromo-acetate* (21) (573 mg, 67.5% oil), $\nu_{max.}$ 3 600, 1 735, 1 665, and 1 220 cm^{-1}, δ 1.41 (3 H, s, 10-Me), 2.10 (3 H, s, Ac), 4.40 (1 H, m, 6β-H), 6.05 (1 H, d, *J* 10 Hz, 2-Hz, 2-H), 6.20 (1 H, d, *J* 5 Hz, 4-H), and 6.66 (1 H dd, *J* 10 and 5 Hz, 3-H).

4α,5-*Epoxy*-6α-*hydroxy*-5α-*cholest*-2-*en*-1-*one* (22).—The bromo-acetate (21) (145 mg, 0.313 mmol) in pyridine (5.8 ml) was treated with N-sodium hydroxide (1.5 ml) at room temperature for 30 min. The usual work-up (di-isopropyl ether for extraction) gave a crude product (113 mg). Chromatography on silica (4.5 g) [benzene–ether (50 : 1)] afforded the α-*epoxide* (22) (72 mg, 60%), m.p. 181—183° (from hexane–acetone), $[\alpha]_D$ +66° (*c* 1.51) (Found: M^+, 414.3067. $C_{27}H_{42}O_3$ requires *M*, 414.3132).

6α-*Acetoxy*-4β,5-*dihydroxy*-5β-*cholest*-2-*en*-1-*one* (23).—The 6α-acetoxy-dienone (140 mg, 0.315 mmol) [prepared from (19) by treatment with acetic anhydride–pyridine] in ether (2 ml) was treated with pyridine (0.1 ml) and osmium tetraoxide (0.34 mmol) at room temperature for 30 h. The ether was evaporated off and the residue treated with pyridine (2 ml) and saturated aqueous sodium hydrogen sulphite (2 ml) at room temperature for 30 min. The usual work-up (ether for extraction) gave the 4,5-*diol* (23) (140 mg), m.p. 171—172° (from methanol), δ 1.27 (3 H, s, 10-Me), 2.10 (3 H, s, Ac), 5.10 (1 H, m, 4α-H), 5.20 (1 H, m, 6β-H), 6.00 (1 H, dd, *J* 10 and 2 Hz, 2-H), and 6.57 (1 H, dd, *J* 10 and 2 Hz, 3-H), δ (C_6H_6) 1.37 (3 H, s, 10-Me), 1.67 (3 H, s, Ac), 4.80 (1 H, m, 4α-H), 5.20 (1 H, m, 6β-H), 5.93 (1 H, dd, *J* 10 and 2 Hz, 2-H), and 6.32 (1 H, dd, *J* 10 and 2 Hz, 3-H).

5,6β-*Epoxy*-4β-*hydroxy*-5β-*cholest*-2-*en*-1-*one* (25).—The 6α-hydroxy-dienone (19) (113 mg, 0.28 mmol) in pyridine (3.8 ml) was treated with methanesulphonyl chloride (0.04 ml) at 0 °C overnight. The usual work-up (ether for extraction), performed below room temperature, gave the 6α-mesylate (137 mg). This product in ether (2 ml) was treated with osmium tetraoxide (76 mg, 0.30 mmol) and pyridine (0.07 ml) at 0 °C overnight. Ether was removed at room temperature. Pyridine (2 ml) and saturated aqueous

sodium hydrogen sulphite were added to the residue, and the mixture was stirred at 0 °C for 30 min. The usual work-up (ether for extraction) afforded the diol (128 mg). This diol in pyridine (1.2 ml) was treated with aqueous 15% sodium hydroxide (0.075 ml) at 80°C for 15 min. The usual work-up (ether for extraction) provided the *epoxide* (25) (92 mg), m.p. 220.5—223.5° (from hexane–acetone), $[\alpha]_D$ +39° (*c* 1.60) (Found: M^+, 414.3128. $C_{27}H_{42}O_3$ requires *M*, 414.3132).

4β,5-*Epoxy*-5β-*cholest*-2-*en*-1-*one* (27).—The 2,4-dienone (26) (75 mg, 0.188 mmol) in chloroform (3.7 ml) was treated with *m*-CPBA (41.2 mg, 0.203 mmol) at room temperature for 20 h. The usual work-up gave the crystalline *epoxide* (27) (75 mg), m.p. 155.5—158° (from methanol), $[\alpha]_D$ +10° (*c* 1.50), $\nu_{max.}$ 1 660 cm^{-1} (Found: C, 81.5; H, 10.7. $C_{27}H_{42}O_2$ requires C, 81.35; H, 10.6%).

4α-*Acetoxy*-5-*bromo*-5β-*cholest*-2-*en*-1-*one* (28).—The 2,4-dienone (26) (550 mg, 1.38 mmol) in acetic acid (20 ml) was treated with lithium acetate (2 g, 30.3 mmol) and NBA (200 mg, 1.45 mmol) at room temperature for 30 min. The usual work-up (di-isopropyl ether for extraction) gave a crude product (750 mg). Chromatography on silica (30 g) [hexane–benzene (1 : 1)] afforded the bromo-acetate (28) (500 mg, 69.5%), m.p. 98—107° (from methanol), $[\alpha]_D$ −132° (*c* 1.55), δ 1.44 (3 H, s, 10-Me), 2.16 (3 H, s, Ac), 6.08 (1 H, d, *J* 10 Hz, 2-H), 6.18 (1 H, d, *J* 5 Hz, 4β-H), and 6.63 (1 H, dd, *J* 10 and 5 Hz, 3-H).

4α,5-*Epoxy*-5α-*cholest*-2-*en*-1-*one* (29).—The bromo-acetate (28) (370 mg, 0.71 mmol) in DMF (22 ml) was treated with aqueous 10% sodium hydroxide (1.1 ml) at room temperature for 1 h. The usual work-up (di-isopropyl ether for extraction) afforded a crude product (271 mg). Chromatography on silica (11 g) [benzene–hexane (3 : 2)] provided the α-*epoxide* (29) (155 mg, 55%), m.p. 91—92° (from methanol), $[\alpha]_D$ +72° (*c* 1.55) (Found: C, 81.5; H, 10.85. $C_{27}H_{42}O_2$ requires C, 81.35; H, 10.6%).

5α,6-*Epoxy*-4β-*hydroxy*-5α-*cholest*-2-*en*-1-*one* (30).—The 4α,5-epoxy-enone (29) (60 mg, 0.15 mmol) in THF (4.5 ml) was treated with aqueous 60% perchloric acid (0.11 ml) at room temperature for 3 h. The usual work-up (chloroform) afforded the diol (56 mg), which was acetylated with acetic anhydride–pyridine. The resulting 4β-acetate (57 mg) was treated with thionyl chloride (0.05 ml) in pyridine (2.5 ml) at 0 °C for 1 h. The usual work-up (ether) provided the 2,5-dienone (48 mg), δ 0.7 (3 H, s, 13-Me), 1.37 (3 H, s, 10-Me), 2.07 (3 H, s, Ac), 5.77 (1 H, d, *J* 5 Hz, 4α-H), 6.03 (1 H, d, *J* 10 Hz, 2-H), 6.12 (1 H, m, 6-H), and 6.75 (1 H, dd, *J* 10 and 5 Hz, 3-H).

The 4β-acetoxy-dienone (48 mg) in chloroform (2.2 ml) was treated with *m*-CPBA (30 mg) at room temperature for 24 h. Preparative t.l.c. [benzene–ether (9 : 1)] of the crude product (57 mg) afforded 4β-acetoxy-5,6α-epoxy-5α-cholest-2-en-1-one (24 mg), δ 0.63 (3 H, s, 13-Me), 1.45 (3 H, s, 10-Me), 2.10 (3 H, s, Ac), 3.27 (1 H, d, *J* 4 Hz, 6β-H), 4.80 (1 H, d, *J* 5 Hz, 4α-H), 6.00 (1 H, d *J* 10 Hz, 2-H), and 6.60 (1 H, dd, *J* 10 and 5 Hz, 3-H), and starting material (20 mg). (In contrast to the results of Weissenberg *et al.*,[5] the isomeric 5β,6-epoxide was not detected.) This epoxide (24 mg) was treated with aqueous 10% sodium hydroxide (0.06 ml) in dimethylformamide (1.2 ml) at room temperature for 1 h. The usual work-up (ether) afforded the crystalline alcohol (30) (19 mg) (for n.m.r. data see Table).

4α-*Acetoxy*-5-*hydroxy*-5β-*cholest*-2-*en*-1-*one* (31).—The 4β,5β-epoxy-enone (27) (800 mg, 2.01 mmol) in THF (60 ml) was stirred with aqueous 60% perchloric acid (1.47 ml) at room temperature for 16 h. The usual work-up (ether for extraction) afforded the diol (820 mg). Acetylation (acetic anhydride–pyridine, room temperature, overnight) yielded the crystalline monoacetate (31) (842 mg), m.p. 169.5—173° (from methanol), $\nu_{max.}$ 3 450, 1 720, 1 670, and 1 260 cm^{-1}, δ 1.22 (3 H, s, 10-Me), 2.17 (3 H, s, Ac), 5.53 (1 H, d, *J* 4 Hz, 4β-H), 6.07 (1 H, d, *J* 10 Hz, 2-H), and 6.60 (1 H, dd, *J* 10 and 4 Hz, 3-H).

Dehydration of the Monoacetate (31) *with Thionyl Chloride.*—The monoacetate (31) (100 mg, 0.218 mmol) in pyridine (5 ml) was treated with thionyl chloride (0.06 ml) at 0 °C for 1 h. The usual work-up (ether for extraction) provided a crude product, showing three spots on t.l.c. Each compound was isolated by preparative t.l.c: 4α-*acetoxy*-5-*chloro*-5β-*cholest*-2-*en*-1-*one* (34) (upper band; 16 mg, 15.4%; oil), δ 1.39 (3 H, s, 10-Me), 2.18 (3 H, s, Ac), 5.97 (1 H, d, *J* 5 Hz, 4β-H), 6.10 (1 H, d, *J* 10 Hz, 2-H), and 6.64 (1 H, dd, *J* 10 and 5 Hz, 3-H) (Found: M^+, 476.3014. $C_{29}H_{45}ClO_3$ requires *M*, 476.3057); 4α-*acetoxycholesta*-2,5-*dien*-1-*one* (33) (middle band; 32 mg, 33.3%), m.p. 106—107° (from methanol), $\nu_{max.}$ 1 740, 1 680, and 1 660 cm^{-1}, δ 1.30 (3 H, s, 10-Me), 2.20 (3 H, s, Ac), 5.67 (1 H, m, 6-H), 5.90 (1 H, dd, *J* 10 and 2 Hz, 2-H), 6.20 (1 H, m, 4β-H), and 6.58 (1 H, dd, *J* 10 and 2 Hz, 3-H) (Found: C, 78.6; H, 9.95. $C_{29}H_{44}O_3$ requires C, 79.05; H, 10.05%); 4α-*acetoxycholesta*-2,4-*dien*-1-*one* (32) (lower band; 19 mg, 19.8%; oil), $\lambda_{max.}$ 322 nm, δ 1.30 (3 H, s, 10-Me), 2.22 (3 H, s, Ac), 5.98 (1 H, d, *J* 10 Hz, 2-H), and 6.77 (1 H, d, *J* 10 Hz, 3-H) (Found: M^+, 440.3260. $C_{29}H_{44}O_3$ requires *M*, 440.3290).

Dehydration of the Monoacetate (31) *with Phosphorus Pentaoxide.*—The monoacetate (31) (133 mg, 0.3 mmol) in dry benzene (6 ml) was treated with phosphorus pentaoxide (210 mg, 14.4 mmol) at room temperature for 3 h. The usual work-up (ether for extraction) gave a crude product (98 mg), showing three spots on t.l.c. Chromatography on silica (7 g) [benzene–ether (5 : 1)] afforded *cholest*-2-*ene*-1,4-*dione* (35) (14 mg, 12.3%), $\nu_{max.}$ 1 680 cm^{-1}, $\lambda_{max.}$ 225 nm, δ 1.25 (3 H, s, 10-Me) and 6.62 (2 H, s, 2- and 3-H). Further elution with benzene–ether (5 : 1) gave the *diacetate* (36) (47 mg, 29.1%), δ 1.25 (3 H, s, 10-Me), 1.95 (3 H, s, Ac), 2.18 (3 H, s, Ac), 6.07 (1 H, d, *J* 10 Hz, 2-H), 6.33 (1 H, d, *J* 4 Hz, 4β-H), and 6.67 (1 H, dd, *J* 10 and 4 Hz, 3-H) (Found: M^+, 500.3440. $C_{31}H_{48}O_5$ requires *M*, 500.3501). Continued elution with benzene–ether (5 : 1) afforded starting material (31) (27 mg).

4α-*Acetoxy*-5,6α-*epoxy*-5α- *and* 5β-*cholest*-2-*en*-1-*ones* (37) *and* (38).—The 2,5-dienone acetate (33) (107 mg, 0.24 mmol) in chloroform (5 ml) was treated with *m*-CPBA (63 mg, 0.31 mmol) at room temperature for 6 h. The usual work-up afforded a crude product showing two spots on t.l.c. Each compound was isolated by preparative t.l.c. [benzene–ether (10 : 1)]: the β-epoxide (38) (20 mg, 18.3%), m.p. 77—81° (from ether), δ 1.27 (3 H, s, 10-Me), 2.08 (3 H, s, Ac), 3.52 (1 H, d, *J* 2 Hz, 6α-H), 5.75 (1 H, m, 4β-H), 6.05 (1 H, dd, *J* 10 and 2.5 Hz, 2-H), and 6.65 (1 H, dd, *J* 10 and 2 Hz, 3-H); the α-epoxide (37) (75 mg, 68.5%), m.p. 161—165° (from n-hexane), δ 1.40 (3 H, s, 10-Me), 2.11 (3 H, s, Ac), 3.13 (1 H, d, *J* 5 Hz, 6β-H), 6.01 (2 H, m, 2- and 4β-H), and 6.55 (1 H, dd, *J* 10 and 2 Hz, 3-H).

5,6α-*Epoxy*-4α-*hydroxy*-5α-*cholest*-2-*en*-1-*one* (39).—The 5α,6α-epoxy-acetate (37) (30 mg, 0.066 mmol) in THF (1.5 ml) was treated with 2N-potassium hydroxide (0.21 ml) at room temperature overnight. The usual work-up (ether for extraction) gave a crude product (29 mg) which was purified by preparative t.l.c. [benzene–acetone (8 : 2)] to

afford the *epoxy-alcohol* (39), m.p. 195—200° (from ether) (Found: M^+, 414.3120. $C_{27}H_{42}O_3$ requires M, 414.3132).

5,6β-*Epoxy*-4α-*hydroxy*-5β-*cholest*-2-*en*-1-*one* (40)—Similar treatment of the 5,6β-epoxide (38) (10 mg, 0.022 mmol) gave a crude product (10 mg) which was purified by preparative t.l.c. [benzene–acetone (8 : 2)] to afford the *alcohol* (40), m.p. 200—209° (from ether) (Found: M^+, 414.3080. $C_{27}H_{42}O_3$ requires M, 414.3132).

This work was supported by a research grant from the Ministry of Health and Welfare.

[5/875 *Received*, 9*th May*, 1975]