1-Oxo-steroids. Part 2.[1] Model Studies for the Synthesis of the Withanolides

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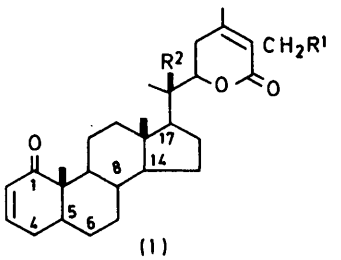
Several cholestane derivatives possessing rings A and B with the same substitution pattern as in the naturally occurring steroidal lactones of the withanolide group have been synthesised: 4β-hydroxycholesta-2,5-dien-1-one (10), 5,6β-epoxy-4β-hydroxy-5β-cholest-2-en-1-one (12), cholesta-2,5-dien-1-one (19), and 5,6β-epoxy-5β-cholest-2-en-1-one (21). Several other compounds in which rings A and B have a substitution pattern not yet encountered among the natural withanolides have also been prepared: 5,6α-epoxy-4β-hydroxy-5α-cholest-2-en-1-one (11), 5,6α-epoxy-5α-cholest-2-en-1-one (20), and 4α-acetoxy-5-hydroxy-5β-cholest-2-en-1-one (28).

We have recently presented preliminary accounts [2] of the synthesis of the AB ring system in withaferin A (1a) [3] and in other steroidal lactones of the withanolide group (1b—i).[4] Withaferin A possesses bacteriostatic [5] and cytotoxic [6] activity on experimental tumours in mice, as well as immunosuppressive properties; [7] withanolide E (1d) also has cytotoxic activity.[8] The biological activity of other withanolides is now being investigated. We have been exploring the possibility of synthesising these compounds from readily available steroids; concurrently with our work, at least two other groups [9,10] have independently investigated synthetic approaches to withaferin A (1a).

We now present a detailed account of the construction of several cholestane † derivatives in which the substitution pattern of rings A and B represents the following four



(1)

a; 4β-OH, 5β,6β-epoxy, 17α-H; R^1 = OH, R^2 = H
b; 4β-OH, 5β,6β-epoxy, 17α-H; R^1 = H, R^2 = OH
c; 4β-OH, 5,6-didehydro 17α-OH, R^1 = R^2 = H
d; 5β,6β-epoxy, 14α-OH, 17β-OH; R^1 = H, R^2 = OH
e; 5β,6β-epoxy, 17α-H; R^1 = OH, R^2 = H
f; 5,6,8,14-tetradehydro, 17α-H; R^1 = H, R^2 = OH
g; 5,6,8,14-tetradehydro, 17α-H; R^1 = R^2 = OH
h; 5,6,8,14-tetradehydro, 17α-OH; R^1 = H, R^2 = OH
i, 5,6-didehydro,14α-OH, 17β-OH; R^1 = H, R^2 = OH

structural types encountered in the withanolides: 2,5-dien-1-one, present *inter alia* in withanolides G, H, and J [11]

† The synthesis and biological activity of the related androstane and pregnane derivatives [2] will be reported elsewhere.

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[9] P. Tsui, *Diss. Abs.* (*B*), 1971, **32**, 2615 (*Chem. Abs.*, 1972, **76**, 86,000).
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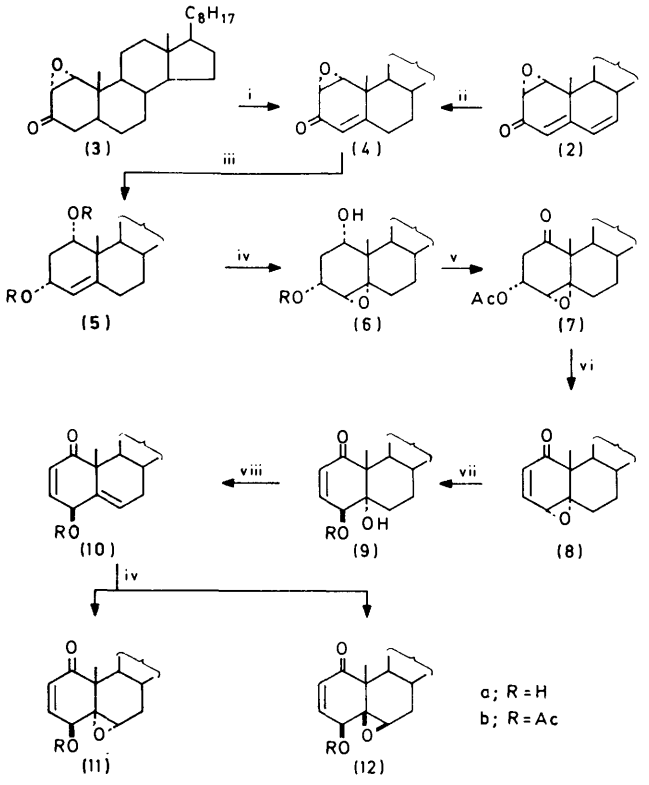
[11] E. Glotter, I. Kirson, A. Abraham, and D. Lavie, *Tetrahedron*, 1973, **29**, 1353.

and F [12] (1f—i), and in physalins B and C; [13] 4β-hydroxy-2,5-dien-1-one, present in withanolide O (1c); [14] 5β,6β-epoxy-2-en-1-one, present in withanolide E (1d) [12] and in jaborosalactone A (1e); [15] and 5β,6β-epoxy-4β-hydroxy-2-en-1-one, present *inter alia* in withaferin A (1a) [3] and in withanolide D (1b).[16]

The sequence of reactions designed for the synthesis



Reagents: i, SeO_2; ii, H_2 (Pd–$CaCO_3$); iii, $LiAlH_4$; iv, $PhCO_3H$; v, CrO_3; vi, Al_2O_3; vii, H_2SO_4–AcOH; viii, $SOCl_2$

of rings A and B in withaferin A (1a) begins with 1α,2α-epoxycholest-4-en-3-one (4), prepared by catalytic hydrogenation of 1α,2α-epoxycholesta-4,6-dien-3-one (2), obtained [1] in turn in two steps from cholesterol; alternatively, compound (4) was prepared by dehydrogenation with selenium dioxide [17] of 1α,2α-epoxy-5α-cholestan-3-one (3).[18] Reduction of compound (4) with lithium aluminium hydride afforded, in high yield, the diol (5a). The configurational assignment at C(3) is based on the similarity between the 3-H and 4-H n.m.r. signals in this compound and in cholest-4-en-3α-ol,[19] and supported by further reactions described below. Stereospecific epoxidation of compound (5a) gave 4α,5-epoxy-5α-cholestane-1α,3α-diol (6a). In addition to its role in the elaboration of the functionality of withaferin A, the α-oriented epoxide system in compound (6a) increases the hindrance to the approach of a reagent to the 1α-hydroxy-group, thus allowing the selective acetylation of the 3α-hydroxy-group (6b). The oxidation of the remaining 1α-hydroxy-group proceeded smoothly to give the ketone (7), in the n.m.r. spectrum of which the 10-methyl signal is shifted downfield and shows a positive aromatic solvent-induced shift (ASIS) [$\Delta(CDCl_3 - C_6D_6)$ +13.5 Hz]. The *trans*-junction of rings A and B is thus firmly established. The axial 3α-acetate was eliminated on alumina to give 4α,5-epoxy-5α-cholest-2-en-1-one (8) [in 70% overall yield from (3)], characterised in the n.m.r. spectrum by three sets of double doublets for 2-, 3-, and 4-H. Acid-catalysed opening of the epoxide ring in (8), followed by acetylation, gave the corresponding 4β-acetate (9b) in the n.m.r. spectrum of which the 2-, 3-, and 4-H signals showed the same pattern as the signals for the corresponding protons in withaferin A (1a). The orientation of the substituents at C-4 and C-5 was confirmed by a positive ASIS (+6 Hz), a significant downfield shift of the 10-methyl signal (1,3-diaxial interaction with the 4β-acetate), and the lack of measurable allylic coupling between 2-H and 4-H. Treatment of the diol acetate (9b) with thionyl chloride in pyridine afforded quantitatively the corresponding 5-ene (10b).

A satisfactory mild procedure for the removal of the acetate group in (10b) is barium methoxide catalysed transesterification,[20] commonly used in carbohydrate chemistry, which afforded quantitatively the corresponding allylic alcohol (10a). Attempted hydrolysis of the acetate (10b) with methanolic potassium hydroxide led to a mixture of compound (10a) and a saturated 3-methoxy-derivative (such a Michael-type addition of the solvent to a 2-en-1-one has already been encountered during the determination of the structure of withaferin A[3,21]). The substitution pattern of rings A and B in (10a) is the one present in withanolide O (1c), isolated in minute amounts from *Withania somnifera*, chemotype I.[14]

Epoxidation of compound (10a) gives stereospecifically the *cis*-epoxy-alcohol (12a) in which rings A and B are similar to those present in withaferin A (1a), withanolide D (1b), and several other, related compounds. The axial orientation of the 4β-hydroxy-group, in conjunction with the large negative ASIS (−13 Hz) of the 10-methyl n.m.r. signal indicate that ring A in compound (12a) has the same boat conformation as in withaferin A.[22,23]

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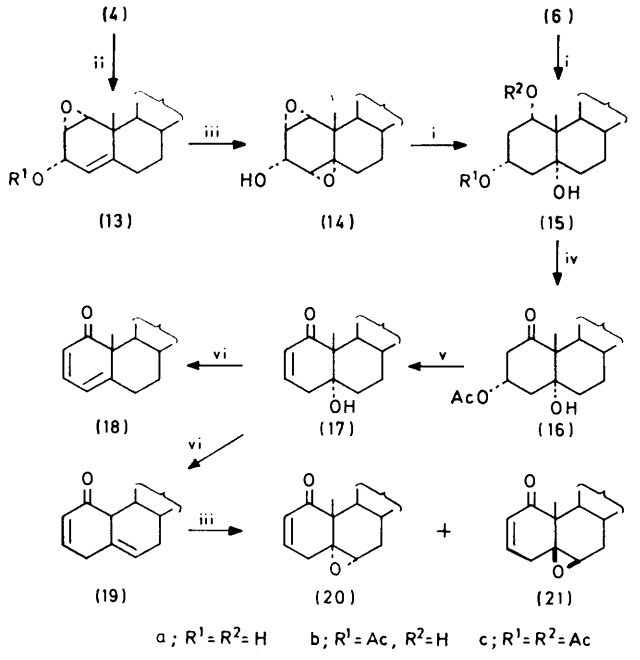
The allylic acetate (10b) gave with perbenzoic acid a crystalline mixture of the 5α,6α- (11b) and the 5β,6β-epoxy-acetate (12b) in the ratio 2 : 1, separated by chromatography. Upon treatment with barium methoxide, compound (11b) afforded the pure epoxy-alcohol (11a); the 6β-H n.m.r. signal of this compound is a doublet with J 4.5 Hz, in contrast to the 6α-H signal of compound (12a), which is a doublet with J 2.5 Hz.[24] The positive ASIS of the 10-methyl signal of compound (11a) (+5 Hz) confirms as well the *trans*-junction of rings A and B.

The next task was to devise a sequence of reactions leading to cholestane derivatives unsubstituted at C-4, which simulate rings A and B in withanolides E (1d) and G (1f) and other, related compounds. The key intermediate was 5α-cholestane-1α,3α,5-triol (15a), obtained by reduction of compound (6) with lithium aluminium hydride. It was also obtained according to a procedure



Reagents: i, $LiAlH_4$; ii, $NaBH_4$; iii, $PhCO_3H$; iv, CrO_3; v, Al_2O_3; vi, $SOCl_2$

developed in the androstane series:[17] compound (4) was reduced with sodium borohydride to the 3α-hydroxy-derivative (13), which gave stereospecifically with perbenzoic acid the diepoxy-alcohol (14), further reduced with lithium aluminium hydride to compound (15a). The structure assigned to the unsaturated epoxy-alcohol (13) was confirmed by its reduction with lithium aluminium hydride to cholest-4-ene-1α,3α-diol (5a), alternatively obtained by reduction of compound (4). The stereospecific hydride reduction of the carbonyl group in compound (4) to a quasiaxial hydroxy-group in compounds (5a) and (13a) is in contrast with the results obtained in a related compound possessing an exocyclic double bond (1α,2α-epoxy-4-methylene-5α-cholestan-3-one), giving exclusively the corresponding 3β-hydroxy-derivative.[25]

Acetylation of the triol (15a) with acetic anhydride–pyridine at room temperature afforded a mixture of the 3α-monoacetate (15b) and the diacetate (15c); the monoacetate (15b) alone was obtained by performing the acetylation in chloroform solution (as for the acetylation of the 3β-hydroxy-group in steroidal 1β,3β,5β-triols from natural sources [26]). Oxidation of compound (15b) gave 3α-acetoxy-5-hydroxy-5α-cholestan-1-one (16), in which the *trans-junction* of rings A and B was confirmed by a positive ASIS (+17.5 Hz) of the 10-methyl signal. Elimination of the axial 3α-acetoxy-group proceeded smoothly on alumina, leading to 5-hydroxy-5α-cholest-2-en-1-one (17), $\lambda_{max.}$ 225 nm; the n.m.r. signals of the vinylic 2-H and 3-H in this compound are similar to those exhibited by 5α-cholest-2-en-1-one.[27]

Dehydration of compound (17) with thionyl chloride in pyridine resulted in a 2 : 1 mixture of the dienones (18) and (19), which were easily separated by chromatography. The major component (18), $\lambda_{max.}$ 324 nm, was identical with cholesta-2,4-dien-1-one obtained by dehydrobromination of 4α- and 4β-bromo-5α-cholest-2-en-1-one.[28] The cholesta-2,5-dien-1-one structure assigned to the minor component (19) is based on its u.v. absorption, $\lambda_{max.}$ 222 nm, and the similarity of its n.m.r. spectrum (low-field region) to those of withanolide G and related compounds (1f—i). The tendency to extend the conjugation of the 2-en-1-one system in compound (17) drives the reaction towards preferential elimination of the 4β-H, thus yielding the conjugated dienone (18) as the major product.

Treatment of cholesta-2,5-dien-1-one (19) with perbenzoic acid gave a 2 : 1 mixture of the corresponding 5α,6α- and 5β,6β-epoxides (20) and (21). After chromatographic separation, the two epoxides were identified by n.m.r. (doublet, J 5 Hz, for the 6β-H in the α-epoxide, and doublet J 2 Hz, for the 6α-H in the β-epoxide). Furthermore the ASIS of the 10-methyl signal of (20) is positive (+17 Hz), whereas that of compound (21) is negative (−4 Hz). Compound (21) has the same substitution pattern of rings A and B as withanolide E (1d) and jaborosalactone A (1e).

Similar reactions were performed with 5β-cholestane-1β,3β,5-triol (24a) as key intermediate. The compound was obtained by epoxidation with peroxy-acid of 5β-cholest-1-ene-3β,5-diol (22) [29] to 1β,2β-epoxy-5β-cholestane-3β,5-diol (23) and subsequent reduction with lithium aluminium hydride. Slightly different procedures have previously been used to obtain similar compounds in the androstane series.[17,30] Selective acetylation of the triol

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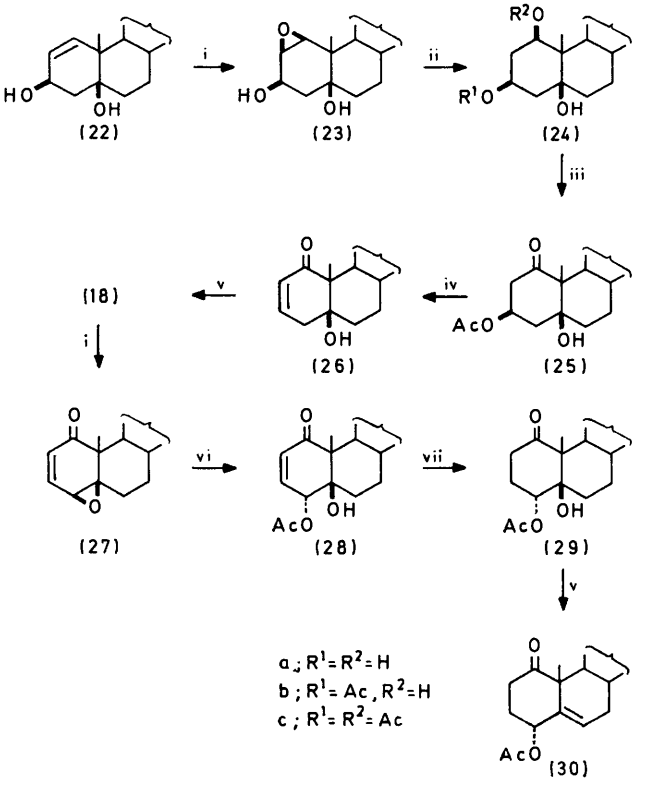
(24a) afforded the 3-monoacetate (24b), which was oxidised (25) and subjected to elimination on alumina to give 5-hydroxy-5β-cholest-2-en-1-one (26). The *cis*-junction of rings A and B was unequivocally established



Reagents: i, $PhCO_3H$; ii, $LiAlH_4$; iii, CrO_3; iv, Al_2O_3; v, $SOCl_2$; vi, H_2SO_4–AcOH; vii, H_2

by the influence of the 5β-hydroxy-group on the pyridine-induced shift [31] of the 10-methyl signal [$\Delta(CDCl_3 - C_5D_5N)$ −21 Hz; in the isomeric 5α-hydroxy-derivative (17) this shift is only −3 Hz]. Dehydration with thionyl chloride, under the same conditions as for compound (17), yielded only the conjugated dienone (18), which, on treatment with perbenzoic acid, gave stereospecifically the 4β,5β-epoxy-derivative (27). Acid-catalysed opening of the epoxide ring in the latter, followed by acetylation, gave 4α-acetoxy-5-hydroxy-5β-cholest-2-en-1-one (28); the *cis*-junction of rings A and B was confirmed by the negative ASIS (−8 Hz) of the 10-methyl n.m.r. signal. Although the route (18) ⟶ (27) ⟶ (28) was not useful for the synthesis of withanolide-like compounds, we report several observations concerning the direction of the elimination of a 5β-hydroxy-group by thionyl chloride–pyridine reagent. In 4β-acetoxy-5β-hydroxy-1-oxo-steroids with or without a 2,3-double bond (derivatives of kitigenin [32] or of withaferin A[3]) the reaction proceeds exclusively with elimination of the 4α-H (*trans*-diaxial relationship) to give the corresponding 4-enol acetates. In 4α-acetoxy-5-hydroxy-5β-cholestan-1-one (29), obtained by catalytic hydrogenation of compound (28b), the elimination proceeds smoothly to give the 5-ene (30). Complications arise in compound (28b) in which such a *trans*-diequatorial elimination leading to a 5-ene is counterbalanced by the tendency to extend the conjugation of the 2-en-1-one, which should necessarily involve elimination of the 4β-H (*cis* with respect to the 5β-hydroxy-group). In our hands, the dehydration of compound (28b) gave a complex mixture which was not further investigated. However, Ikekawa and his co-workers[10b] succeeded in fractionating the crude product of such a reaction, and obtained 4α-acetoxycholesta-2,5-dien-1-one and 4α-acetoxycholesta-2,4-dien-1-one as major components.

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EXPERIMENTAL

M.p.s were taken with a Fisher–Johns apparatus. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter and refer to solutions in chloroform. I.r. spectra were recorded on a Perkin-Elmer Infracord 137 spectrophotometer and refer to solutions in chloroform; u.v. spectra were recorded with a Cary 14 instrument for solutions in ethanol; n.m.r. spectra were determined with a Varian A-60 instrument for *ca.* 5% solutions in deuteriochloroform. T.l.c. was carried out on chromatoplates of silica gel G (Merck) and spots were developed with iodine vapour. For column chromatography, neutral alumina (Woelm, activity III) was used, unless otherwise specified. Mass spectra were taken under the direction of Dr. Z. Zaretskii with an Atlas CH4 instrument. Analyses were performed in the microanalytical laboratory of the Weizmann Institute, under the direction of Mr. R. Heller.

1α,2α-*Epoxycholest*-4-*en*-3-*one* (4).—(a) *By catalytic hydrogenation of* 1α,2α-*epoxycholesta*-4,6-*dien*-3-*one* (2). A solution of compound (2) [1] (250 mg) in benzene (15 ml) was hydrogenated over 5% Pd–$CaCO_3$ (375 mg) at room temperature and atmospheric pressure. The reaction was discontinued after the absorption of 1 mol. equiv. and the crystalline product, which was homogeneous on t.l.c., was recrystallised from methanol to give, almost quantitatively, compound (4), m.p. 118—120 °C, $[\alpha]_D$ +204° (*c* 1.0) [lit.,[33] m.p. 99—100 °C (from ether), $[\alpha]_D$ +180°; according to the described [33] procedure, compound (4) is obtained in low yield].

(b) *By dehydrogenation with selenium dioxide of* 1α,2α-*epoxycholestan*-3-*one* (3). This was carried out by an adaptation of the procedure developed in the androstane [17] series. A solution of compound (3) [18] (3.6 g) in t-butyl alcohol (150 ml) containing acetic acid (15 ml) and selenium dioxide (3.6 g) was heated to reflux for 24 h, then cooled, filtered, concentrated to small volume, and diluted with water; the product was extracted with chloroform. The extract was washed with dilute aqueous sodium hydrogen carbonate and the crude product crystallised from hexane (yield 2.8 g, 78%). Filtration through alumina and recrystallisation from methanol afforded the pure compound (4), m.p. 118—120 °C.

Cholest-4-*ene*-1α,3α-*diol* (5a).—A solution of compound (4) (250 mg) in dry tetrahydrofuran (15 ml) was added drop-

N.m.r. data *

Compd.	1-H	2-H	3-H	4-H	6-H	(C10)Me	C(13)Me	OAc
(4)	3.54d (4)	3.41dd (4; 2)		5.68m ($W_{\frac{1}{2}}$ 4)		1.25s	0.74s	
(5a)	3.87m ($W_{\frac{1}{2}}$ 6)		4.15m ($W_{\frac{1}{2}}$ 10)	5.53d (5)		0.94s	0.69s	
(5b)	4.88t (3)		5.22m ($W_{\frac{1}{2}}$ 10)	5.48d (5)		1.04s	0.67s	2.01; 2.05
(6a)	3.58m ($W_{\frac{1}{2}}$ 5)		4.28m ($W_{\frac{1}{2}}$ 12)	3.32d (4)		0.92s	0.70s	
(6b)	3.48m		5.33m ($W_{\frac{1}{2}}$ 12)	3.33d (4)		0.95s	0.69s	2.11
(7)			5.62dt (7; 1)	3.23d (1)		1.20s [+13.5]	0.69s	2.10
(8)		5.97dd (10;1.5)	6.95dd (10; 4)	3.25dd (4; 1.5)		1.18s [+11.5]	0.71s	
(9b)		5.98d (10)	6.51dd (10; 4.5)	5.20d (4.5)		1.29s [+6.0]	0.68s	2.13
(10a)		5.99d (10)	6.82dd (10; 4.5)	4.64d (4.5)	6.0m	1.45s	0.71s	
(10b)		6.01dd (10; <1)	6.73dd (10; 4.5)	5.78dd (4.5; <1)	6.1m ($W_{\frac{1}{2}}$ 7)	1.38s [−1.0]	0.70s	2.09
(11a)		6.02d (10)	6.73dd (10; 4.5)	3.71d (4.5)	3.26d (4.5)	1.52s [+5.0]	0.65s	
(11b)		6.07d (10)	6.67dd (10; 4.5)	4.83d (4.5)	3.30d (4.5)	1.47s	0.65s	2.13
(12a)		6.22d (10)	6.96dd (10; 6)	3.75d (6)	3.23d (2.5)	1.40s [−13.0]	0.67s	
(12b)		6.30d (10)	7.10dd (10; 6)	4.69d (6)	3.24d (2.5)	1.40s	0.65s	2.05
(13a)	3.28d (4.5)	3.54dd (4.5; 2.5)	4.37m ($W_{\frac{1}{2}}$ 6)	5.13m ($W_{\frac{1}{2}}$ 5)		1.06s	0.70s	
(13b)	3.27d (4.5)	3.57dd (4.5; 2.5)	5.62m ($W_{\frac{1}{2}}$ 6)	5.06m ($W_{\frac{1}{2}}$ 5)		1.09s	0.69s	2.14
(14a)	3.12d (4)		4.24m ($W_{\frac{1}{2}}$ 6)	3.30dd (4; 2)		1.11s	0.71s	
(14b)	3.12d (4)	3.30dd (4; 2.5)	5.47t (2.5)	3.30d (2.5)		1.15s	0.71s	2.19
(15b)	3.81m ($W_{\frac{1}{2}}$ 7)		5.20m ($W_{\frac{1}{2}}$ 8)			0.87s	0.67s	2.05
(15c)	4.98m ($W_{\frac{1}{2}}$ 7)		5.22m ($W_{\frac{1}{2}}$ 8)			0.97s	0.65s	2.03; 2.09
(16)			5.54m ($W_{\frac{1}{2}}$ 11)			1.26s [+17.5]	0.66s	2.04
(17)		5.87dq (10; 2; <1)	6.54dq (10; 5; 2)			1.17s [+12.0]	0.67s	
(18)		5.99d (10)	6.98dd (10; 6)	6.01d (6)		1.27s [+2.0]	0.71s	
(19)		5.90dq (10; 2.5; <1)	6.80dq (10; 4.5; 2.5)		5.61m ($W_{\frac{1}{2}}$ 10]	1.23s [+6.5]	0.70s	
(20)		5.94dq (10; 1.5; <1)	6.72dq (10; 5; 2)		3.05d (5)	1.33s [+17.0]	0.64s	
(21)		6.03dd (10; 3)	6.82m		3.12d (2)	1.24s [−4.0]	0.67s	
(23)	3.40d (4)	3.55t (4)	4.29m ($W_{\frac{1}{2}}$ 15)			1.20s	0.67s	
(24b)	3.95m ($W_{\frac{1}{2}}$ 10)		5.26m ($W_{\frac{1}{2}}$ 9)			1.24s	0.67s	2.06
(24c)	5.30m (narrow)		5.30m (narrow)			1.10s	0.67s	2.06; 2.09
(25)			5.58m ($W_{\frac{1}{2}}$ 8)			1.17s	0.64s	2.05
(26)		6.02dq (10; 4; 2)	6.92m			1.18s	0.67s	
(27)		6.0dd (10; 1.5)	7.08dd (10; 4)	3.24dd (4; 1.5)		1.33s [−5.0]	0.67s	
(28)		6.04d (10)	6.60dd (10; 4.5)	5.51d (4.5)		1.20s [−8.0]	0.66s	2.17
(29)				5.05 ($W_{\frac{1}{2}}$ 6)		1.13s	0.65s	2.18
(30)				5.6m (broad)	5.72m (narrow)	1.29s	0.68s	2.13s

* Recorded at 60 MHz; solvent $CDCl_3$; δ values; coupling constants or signal widths ($W_{\frac{1}{2}}$/Hz) in parentheses; solvent shifts [Δ($CDCl_3$ − C_6D_6)/Hz] in square brackets.

wise to a stirred slurry of lithium aluminium hydride (250 mg) in the same solvent. After 3 h at reflux temperature, the mixture was worked up with ethyl acetate and saturated aqueous sodium sulphate. The crude product (245 mg) was suitable for the next step. A *sample* obtained by chromatography [elution with hexane–ether (8 : 2)] had m.p. 138—140° (from methanol), $[\alpha]_D$ +127° (*c* 0.3) (Found: C, 80.2; H, 11.3%; M^+, 402. $C_{27}H_{46}O_2$ requires C, 80.55; H, 11.5%; *M*, 402.6). The *diacetate* (5b) was prepared by treatment with acetic anhydride and pyridine for 60 h at room temperature; m.p. 162—163 °C (from dichloromethane–methanol), $[\alpha]_D$ +186° (*c* 0.3), M^+ 486.

4α,5-*Epoxy*-5α-*cholestane*-1α,3α-*diol* (6a).—To a solution of compound (5a) (1.4 g) in dry benzene (20 ml), a solution of perbenzoic acid (50% excess)in benzene was added. After 24 h at room temperature, the solution was washed with aqueous sodium carbonate and with water, then dried and evaporated. The crude product (6a) (1.4 g) was homogeneous on t.l.c. and crystallised from acetone–hexane; m.p. 167—169°, $[\alpha]_D$ +69° (*c* 0.4), M^+ 418. The 3-*monoacetate* (6b) was prepared with acetic anhydride and pyridine, overnight at room temperature; m.p. 134.5—135 °C (from methanol), $[\alpha]_D$ +134.5° (*c* 0.6) (Found: C, 75.7; H, 10.4%; M^+, 460. $C_{29}H_{48}O_4$ requires C, 75.6; H, 10.5%; *M*, 460.6).

3α-*Acetoxy*-4α,5-*epoxy*-5α-*cholestan*-1-*one* (7).—A solution of compound (5b) (100 mg) in acetone (15 ml) was treated with Jones reagent [34] for 10 min at 10 °C. The excess of reagent was destroyed with methanol, most of the solvent was removed, and the product was extracted with ether; the extract was washed with water, dried, and evaporated. The *residue* (100 mg) was homogeneous on t.l.c.; m.p. 112—113 °C (from methanol), $[\alpha]_D$ +107.5° (*c* 0.3) (Found: C, 75.8; H, 9.95%; M^+, 458. $C_{29}H_{46}O_4$ requires C, 75.95; H, 10.1%; *M*, 458.6).

4α,5-*Epoxy*-5α-*cholest*-2-*en*-1-*one* (8).—A solution of compound (7) (180 mg) in benzene was stored overnight on a column of alumina (10 g) in hexane. Elution with chloroform gave an oil (145 mg) which crystallised from acetone–hexane; m.p. 156—158°, $[\alpha]_D$ +104° (*c* 0.3), M^+ 398.

4β-*Acetoxy*-5-*hydroxy*-5α-*cholest*-2-*en*-1-*one* (9b).—To a solution of compound (8) (1 g) in acetone (80 ml) and glacial acetic acid (32 ml), 9 : 1 acetic acid–sulphuric acid (16 ml) was added. After 3 h at room temperature ice–water was added and the product was extracted with ether. The extract was washed with water and with aqueous sodium hydrogen carbonate. The solvent was removed and the crude product was acetylated under the usual conditions. The crude acetate was chromatographed; elution with hexane–ether (4 : 6) gave the *product* (9b) (840 mg) m.p. 125—126 °C (from methanol), $[\alpha]_D$ +175° (*c* 0.5) (Found: C, 75.7; H, 9.95%; M^+, 458. $C_{29}H_{46}O_4$ requires C, 75.95; H, 10.1%; *M*, 458.6).

4β-*Acetoxycholesta*-2,5-*dien*-1-*one* (10b).—To an ice-cold solution of compound (9b) (560 mg) in dry pyridine (25 ml), an ice-cold solution of freshly distilled thionyl chloride (2.5 ml) in pyridine (10 ml) was added. After 1 h at 0 °C the solution was poured onto ice and the product was extracted with ether. The extract was washed with dilute hydrochloric acid and with water, dried, and evaporated. The residue (475 mg) was homogeneous on t.l.c.; m.p. 90—92 °C (from ethanol), $[\alpha]_D$ +59.5° (*c* 0.4), M^+ 440.

4β-*Hydroxycholesta*-2,5-*dien*-1-*one* (10a).—A solution of compound (10b) (315 mg) in dry chloroform (50 ml) containing absolute methanol (2 ml) was treated with N-barium methoxide in methanol (0.15 ml). After 24 h at 0 °C the solution was neutralised by swirling with a few beads of Dowex resin (H^+), the solvent was removed, and the product (285 mg) was crystallised from ethanol; m.p. 125—127°, $[\alpha]_D$ +61.5° (*c* 0.4), M^+ 398.

5,6α-*Epoxy*-4β-*hydroxy*-5α-*cholest*-2-*en*-1-*one* (11a).—Compound (10b) (475 mg) in benzene (25 ml) was treated with perbenzoic acid as described for (5a), to give a mixture of the 5α,6α- and 5β,6β-epoxides [(11b) and (12b)] in the ratio 2 : 1 (by n.m.r.). Following repeated chromatography on silica gel, the pure α-*epoxide* (11b) was obtained; m.p. 88—90° (from methanol), $[\alpha]_D$ +113° (*c* 0.7) (Found: C, 76.1; H, H, 9.85%; M^+, 456. $C_{29}H_{44}O_4$ requires C, 76.25; H, 9.7%; *M*, 456.6).) The alcohol (11a) was obtained by treatment with barium methoxide as described for (10a). Chromatography on silica gel [elution with hexane–ether (3 : 7)] afforded the pure alcohol (11a), m.p. 185—187 °C (from methanol), $[\alpha]_D$ +102° (*c* 0.5), M^+ 414. The β-epoxide could not be obtained in pure form from this preparation.

5,6β-*Epoxy*-4β-*hydroxy*-5β-*cholest*-2-*en*-1-*one* (12a).—Compound (10a) (230 mg) in benzene (30 ml) was treated with perbenzoic acid as described above, to give (12a) (230 mg), homogeneous on t.l.c., m.p. 223—225 °C (from acetone–hexane), $[\alpha]_D$ +40° (*c* 0.6), M^+ 414. The acetate (12b) could not be induced to crystallise.

1α,2α-*Epoxycholest*-4-*en*-3α-*ol* (13a).—To a stirred solution of compound (4) (2 g) in methanol (250 ml), sodium borohydride (2 g) was added over a few min. After 2 h at room temperature the solution was neutralised with dilute hydrochloric acid, most of the solvent was removed, and the product was extracted with ether, washed, and dried. The crude product (2 g) was used as such for the following step. A sample prepared by chromatography [elution with hexane–ether (1 : 1)] had m.p. 113—115 °C (from acetone–hexane), $[\alpha]_D$ +75.5° (*c* 0.3) (Found: C, 80.75; H, 10.9%; M^+, 400. $C_{27}H_{44}O_2$ requires C, 80.95; H, 11.0%; *M*, 400.6). The *acetate* (13b) had m.p. 113—115 °C (from methanol), $[\alpha]_D$ +75° (*c* 0.5) (Found: C, 78.5; H, 10.3%; M^+, 442. $C_{29}H_{46}O_3$ requires C, 78.7; H, 10.45%; *M*, 442.6).

1α,2α:4α,5-*Diepoxy*-5α-*cholestan*-3α-*ol* (14a).—Compound (13a) (275 mg) in benzene (10 ml) was epoxidized as described above. The *product* (14a) (285 mg) was homogeneous on t.l.c.; m.p. 185—187 °C (from methanol), $[\alpha]_D$ +52.5° (*c* 0.9) (Found: C, 77.6; H, 10.8%; M^+, 416. $C_{27}H_{44}O_3$ requires C, 77.8; H, 10.65%; *M*, 416.6). The *acetate* (14b) had m.p. 151—153 °C (from methanol), $[\alpha]_D$ +58.5° (*c* 0.6) (Found: C, 75.95; H, 9.9%; M^+, 458. $C_{29}H_{46}O_4$ requires C, 75.95; H, 10.1%; *M*, 458.6).

5α-*Cholestane*-1α,3α,5-*triol* (15a).—Compound (14a) (300 mg) was reduced with lithium aluminium hydride in dry tetrahydrofuran as described for (4a). The crude *product* (295 mg) was homogeneous on t.l.c.; m.p. 217—219° (from methanol–chloroform), $[\alpha]_D$ +33° (*c* 0.5) (Found: C, 76.85; H, 11.4. $C_{27}H_{48}O_3$ requires C, 77.1; H, 11.5%). The 3-*monoacetate* (15b) was obtained by treatment of (15a) (3 g) in chloroform solution (200 ml) with acetic anhydride (30 ml) and pyridine (30 ml) for 5 days at room temperature. The crude product (3.2 g) crystallised from methanol; m.p. 156—158 °C, $[\alpha]_D$ +28° (*c* 0.5) (Found: C, 75.3; H, 11.0%; M^+, 462. $C_{29}H_{50}O_4$ requires C, 75.3; H, 10.9%; *M*, 462.6), The *diacetate* (15c) was obtained along with the monoacetate (15b) by treatment of (15a) (500 mg) with acetic anhydride

[34] K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 1946, 39.

(8 ml) and pyridine (15 ml) for 2 h at reflux. Chromatography on alumina [elution with hexane–ether (4 : 1)] yielded (15c) (440 mg), m.p. 117—119 °C (ethanol), $[\alpha]_D$ +36.5° (*c* 1.0) (Found: C, 73.7; H, 10.25%; M^+, 504. $C_{31}H_{52}O_5$ requires C, 73.75; H, 10.4%; *M*, 504.7). Further elution with hexane–ether (1 : 1) gave the monoacetate (15b) (115 mg).

3α-*Acetoxy*-5-*hydroxy*-5α-*cholestan*-1-*one* (16).—A solution of the monoacetate (15b) (110 mg) in acetone (35 ml) was oxidised with Jones reagent as described for (6). The crude *product* (109 mg) was homogeneous on t.l.c.; m.p. 133—134.5 °C (from ethanol with a few drops of water), $[\alpha]_D$ +66.5° (*c* 0.6) (Found: C, 75.5; H, 10.4%; M^+, 460. $C_{29}H_{48}O_4$ requires C, 75.6; H, 10.5% *M*, 460.6).

5α-*Hydroxycholest*-2-*en*-1-*one* (17).—A solution of compound (16) (100 mg) in benzene was stored overnight on a column of alumina (Alcoa F_{20}; 10 g) in hexane. Elution with chloroform gave *compound* (17) (80 mg), m.p. 132—133 °C (from methanol), $[\alpha]_D$ +78.5° (*c* 0.4) (Found: C, 81.1; H, 10.9%; M^+, 400. $C_{27}H_{44}O_2$ requires C, 80.95; H, 11.05%; *M*, 400.6).

Cholesta-2,4-*dien*-1-*one* (18) *and Cholesta*-2,5-*dien*-1-*one* (19).—Compound (17) (500 mg) was. treated with thionyl chloride in pyridine as described for (9b), to give a mixture of (18) and (19) in the ratio 2 : 1 (by n.m.r.). Chromatography on silica gel [elution with hexane–ether (9.6 : 0.4)] gave *compound* (19) (120 mg), m.p. 102—103 °C (from ethanol), $[\alpha]_D$ −26.5° (*c* 0.8), $\lambda_{max.}$ 222 nm (ε 8 800) (Found: C, 84.8; H, 11.05%; M^+, 382. $C_{27}H_{42}O$ requires C, 84.75; H, 11.05%, *M*, 382.6). Further elution gave a mixture, followed by pure (18) (280 mg), m.p. 99—100 °C (from methanol), $\lambda_{max.}$ 322 nm (ε 4 900), identical with a sample prepared according to ref. 28.

5,6α-*Epoxy*-5α-*cholest*-2-*en*-1-*one* (20) *and* 5,6β-*Epoxy*-5β-*cholest*-2-*en*-1-*one* (21).—Epoxidation of compound (19) (90 mg) was performed as above, to give a mixture of (20) and (21) in the ratio 2 : 1 (by n.m.r.). Chromatography on silica gel [elution with hexane–ether (9 : 1)] gave compound (21) (19 mg), m.p. 114—115 °C (from methanol), $[\alpha]_D$ +26.5° (*c* 0.2), M^+ 398. Further elution gave a mixture followed by pure (20) (45 mg), m.p. 121—123° (from methanol), $[\alpha]_D$ +37° (*c* 0.3), M^+ 398.

5β-*Cholest*-1-*ene*-3β,5-*diol* (22).[29]—The compound was prepared by reduction with lithium aluminium hydride of 4β,5-epoxy-5β-cholest-1-en-3-one.[29] The latter was obtained by treatment with perbenzoic acid [29,35] of cholesta-1,4-dien-3-one or by dehydrogenation with selenium dioxide [35] of 4β,5-epoxy-5β-cholestan-3-one.

1β,2β-*Epoxy*-5β-*cholestane*-3β,5-*diol* (23).—Epoxidation of compound (22) (100 mg) was performed as above. The crude *product* (98 mg) was homogeneous on t.l.c.; m.p. 174—176 °C (from methanol–chloroform), $[\alpha]_D$ +67° (*c* 0.8) (Found: C, 77.3; H, 11.05. $C_{27}H_{46}O_3$ requires C, 77.45; H, 11.1%).

5β-*Cholestane*-1β,3β,5-*triol* (24a).—Compound (23) (200 mg) was reduced with lithium aluminium hydride as described for (4a) to give the *triol* (24a) (200 mg), m.p. 220—222 °C (from methanol–chloroform), $[\alpha]_D$ +24° (*c* 0.4) (Found: C, 77.25; H, 11.4. $C_{27}H_{48}O_3$ requires C, 77.1; H, 11.5%). The 3-*monoacetate* (24b) was prepared from (24a) (1 g) in chloroform solution (30 ml) with acetic anhydride (10 ml) and pyridine (10 ml) for 4 days at 0 °C. Chromatography of the crude product [elution with hexane–ether (1 : 1)] gave the diacetate (24c) (90 mg), which could not be induced to crystallise, followed by the monoacetate (24b) (485 mg), m.p. 168—170° (from ethanol with a few drops of water), $[\alpha]_D$ +14.5° (*c* 0.7) (Found: C, 75.5; H, 10.65%; M^+, 462. $C_{29}H_{50}O_4$ requires C, 75.3; H, 10.9%; *M*, 462.6.)

3β-*Acetoxy*-5-*hydroxy*-5β-*cholestan*-1-*one* (25).—Oxidation of compound (24b) (100 mg) with Jones reagent as described above afforded the ketone (25) (98 mg), m.p. 156—158 °C (from acetone–hexane), $[\alpha]_D$ −32° (*c* 0.4) M^+ 460.

5β-*Hydroxycholest*-2-*en*-1-*one* (26).—A solution of compound (25) (100 mg) in benzene was stored overnight on a column of alumina (Alcoa F_{20}) in hexane. Elution with chloroform gave *compound* (26) (85 mg), m.p. 194—196 °C (from methanol–chloroform), $[\alpha]_D$ +11° (*c* 1.0), $\lambda_{max.}$ 225 nm (ε 8 300) (Found: C, 80.95; H, 10.9%; M^+, 400. $C_{27}H_{44}O_2$ requires C, 80.95; H, 11.05%; *M*, 400.6).

Dehydration of the Alcohol (26).—Compound (26) (100 mg) was treated with thionyl chloride in pyridine as described for (17). Cholesta-2,4-dien-1-one (18) was obtained nearly quantitatively; m.p. and mixed m.p. 99—100 °C.

4β,5-*Epoxy*-5β-*cholest*-2-*en*-1-*one* (27).—Compound (18) (100 mg) was treated with perbenzoic acid as described above to give the *epoxide* (27) (95 mg), homogeneous on t.l.c. Two crystallisations from methanol afforded a sample with m.p. 155—157 °C, $[\alpha]_D$ +13° (*c* 0.45) (Found: C, 81.45; H, 10.7%; M^+, 398. $C_{27}H_{42}O_2$ requires C, 81.35; H, 10.6; *M*, 398.6).

4α-*Acetoxy*-5-*hydroxy*-5β-*cholest*-2-*en*-1-*one* (28).—The reaction was carried out with compound (27) (600 mg) under the same conditions as for (9b). The crude product was acetylated and chromatographed on alumina. Elution with hexane–ether (6.5 : 3.5) gave *compound* (28) (330 mg), m.p. 170—172 °C (from aqueous methanol), $[\alpha]_D$ −101° (*c* 1.0) (Found: C, 75.8; H, 10.0%; M^+, 458. $C_{29}H_{46}O_4$ requires C, 75.95; H, 10.1; *M*, 458.6).

4α-*Acetoxy*-5-*hydroxy*-5β-*cholestan*-1-*one* (29).—Compound (28) (100 mg), in ethanol (15 ml) was hydrogenated over 10% Pd–$CaCO_3$ until absorption ceased. The product (85 mg) was homogeneous on t.l.c.; m.p. 180—182° (from methanol), $[\alpha]_D$ −90° (*c* 0.15), M^+ 460. Treatment with thionyl chloride in pyridine as described for compound (8b) afforded 4α-acetoxycholest-5-en-1-one (30) (60 mg), which was homogeneous on t.l.c. The product was characterised only by its n.m.r. spectrum.

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