19-HYDROXYSTEROIDS SUBSTITUTED IN POSITION 6*

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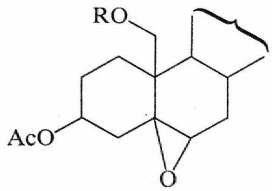
Cleavage of the 5,6-epoxides of the 19-hydroxylated steroids has been studied. Syntheses of 6,19-dihydroxycholest-4-en-3-ones epimeric at $C_{(6)}$ are described and their structures established by spectral means.

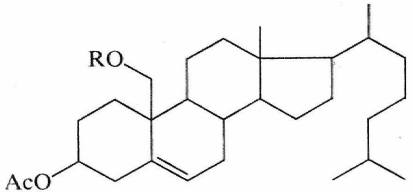
In the course of our studies of steroids with potential biological activities 19-nor-steroids carrying substituents in position 6 became of interest. The key compounds for syntheses of such derivatives represent the ketones *XXI* and *XXXII*. In this paper we present the model experiments in the cholestane series which led to the syntheses of the desired ketones. In connection with this work the stereochemistry of the epoxide ring cleavage in the epoxides *IV* to *IX* as well as possibilities of protection of hydroxyls in tetrols *X* and *XXIV* had to be studied in detail.

Epoxidation of the 5,6-double bond in 19-hydroxylated steroids has been described in the literature and always both epimers were isolated. It has been shown[1,2] that the stereochemistry of the epoxidation largely depends on the character of the substituent at $C_{(19)}$ and the configuration of the epoxide ring has been established by spectral means. Our starting compounds were the known epoxides *IV*, *V*, *VII* and *VIII* and the newly prepared benzoates *VI* and *IX*. They were prepared by perphthalic acid oxidation of the benzoate[3] *III*. Cleavage of the epoxide ring has been carried out with perchloric acid in acetone–water. The β-epoxides (compounds *IV* to *VI*) afforded products of the *trans*-diaxial opening, *i.e.* alcohols *XI* to *XIII*. In accordance with the the literature[4] the α-epoxides (compounds *VII* to *IX*) yield the 5β,6α-diols (compounds *XXIV*, *XXVI*, and *XXVIII*). This anomalous behaviour has been attributed[4] to the participation of the 19-acyloxy group in the epoxide ring fission. This presumption seems to be supported by the fact that the 19-methoxy-5α,6α-epoxides open normally to the 5α,6β-diols, as observed in our Laboratory[5]. However, unexpectedly, the 19-hydroxy epoxide *VII* afforded in our hands again the anomalous 5β,6α-disubstituted derivative *XXIV*; this reaction proceeds much slower than cleavage of the 19-acyloxy derivatives.

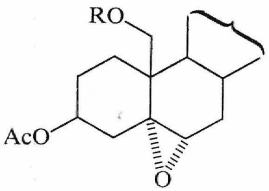
* Part CCX in the series On Steroids: Part CCIX: J. Chem. Soc., Perkin Trans. 1, in press.



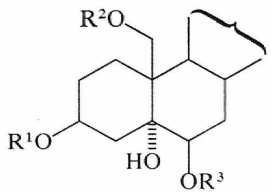
IV, R = H
V, R = Ac
VI, R = C_6H_5CO



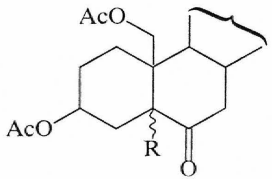
I, R = H
II, R = Ac
III, R = C_6H_5CO



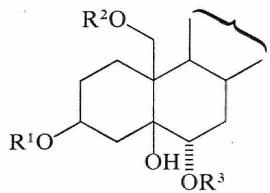
VII, R = H
VIII, R = Ac
IX, R = C_6H_5CO



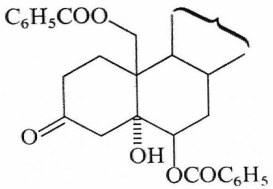
X, $R^1 = R^2 = R^3 = H$
XI, R^1 = Ac, $R^2 = C_6H_5CO$, $R^3 = H$
XII, R^1 = Ac, $R^2 = R^3 = H$
XIII, $R^1 = R^2$ = Ac, $R^3 = H$
XIV, $R^1 = R^2 = R^3$ = Ac
XV, $R^1 = H$, $R^2 = R^3 = C_6H_5CO$
XVI, R^1 = Ac, $R^2 = R^3 = C_6H_5CO$
XVII, $R^1 = R^2$ = Ac, $R^3 = C_6H_5CO$



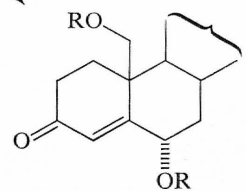
XVIII, R = ⁞⁞⁞OH
XIX, R = —OH



XXIV, $R^1 = R^2 = R^3 = H$
XXV, $R^1 = R^2 = R^3$ = Ac
XXVI, $R^1 = R^2$ = Ac, $R^3 = H$
XXVII, $R^1 = R^2$ = Ac, $R^3 = C_6H_5CO$
XXVIII, R^1 = Ac, $R^2 = C_6H_5CO$, $R^3 = H$
XXIX, R^1 = Ac, $R^2 = R^3 = C_6H_5CO$
XXX, R^1 = H, $R^2 = R^3 = C_6H_5CO$

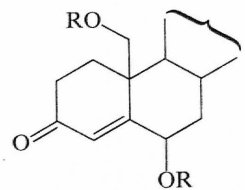


XXXI



XXXII, R = H
XXXIII, R = Ac
XXXIV, R = C_6H_5CO

XX



XXI, R = H
XXII, R = Ac
XXIII, R = C_6H_5CO

In further experiments a series of esters of the tetrols *X* and *XXIV* was prepared and their partial hydrolyses were studied in order to synthesize the properly protected derivatives desired for further steps. Diacetates *XIII* and *XXVI* were then oxidized by Jones reagent to the ketones *XVIII* and *XIX*. Stereochemistry at $C_{(5)}$ in these ketones follows from spectral evidence: The width of the multiplet of the $C_{(3)}$ proton in the ketone *XVIII* is about 30 Hz (3α axial) whereas in the epimer *XIX* this value is about 14 Hz (3α equatorial). Also CD data are in agreement with the predictions obtained from octant projections: Negative shift is expected for the 5α-epimer *XVIII* ($\Delta\varepsilon_{284}$ − − 5·33) in relation to the 5β-epimer *XIX* ($\Delta\varepsilon_{300}$ − 2·47). Oxidation of the dibenzoates *XV* and *XXX* afforded the ketones *XX* and *XXXI* which on reflux with glacial acetic acid gave the desired unsaturated ketones *XXIII* and *XXXIV*. They were characterized also as the diols *XXI* and *XXXII* and diacetates *XXII* and *XXXIII*.

EXPERIMENTAL

Melting points were determined on a Kofler block. Optical measurements were carried out in chloroform with an error of ±2°. The infrared spectra were recorded on the Zeiss UR 20 spectrometer in tetrachloromethane. The ^{1}H-NMR spectra were recorded on the Varian HA-100 instrument in deuteriochloroform and corrected to tetramethylsilane (7·25 ppm). The chemical shift is given in ppm. The mass spectra were recorded on the mass spectrometer AEI MS 902. The UV spectra were recorded on the CF 4 spectrometer in ethanol. The identity of samples prepared by different routes was checked by mixture melting point determination, by thin layer chromatography (TLC) and by spectral evidence. Usual working up of a solution implies washing the solution with 5% aqueous hydrochloric acid, water, 5% aqueous potassium hydrogen carbonate solution, water, drying with sodium sulphate, and evaporation of the solvent *in vacuo*. Ligroin refers to the fraction of b.p. 40–60°C.

5,6α-Epoxy-5α-cholestane-3β,19-diol 3-Acetate 19-Benzoate (*IX*)

a) *From 5-cholestene-3β,19-diol 3-acetate 19-benzoate* (III): The olefin[3] *III* (6·4 g) in ether (80 ml) was treated with perphthalic acid (6 g) in ether (150 ml) and allowed to stand at room temperature for 3 days. The mixture was diluted with ether, the excess peracid was extracted with 5% sodium carbonate, and the ethereal solution was dried and ether removed. The residue was chromatographed on a silica gel column (500 g) in benzene. Fractions with the lipophilic component were combined, and the solvent removed to yield 1·62 g of the α-epoxide *IX* which resisted all attempts at crystallization. $[\alpha]_D^{20}$ −56° (*c* 1·68). IR spectrum: 1030, 1038, 1245, 1738 (acetate), 1272, 1729 cm^{-1} (benzoate). For $C_{36}H_{52}O_5$ (564·8) calculated: 76·56% C, 9·28% H; found: 76·40% C, 9·35% H.

b) *From 5,6α-epoxy-5α-cholestane-3β,19-diol 3-monoacetate* (VII): The alcohol[2] *VII* (4 g) in pyridine (30 ml) was treated with benzoyl chloride (6 ml) and set aside for 20 h. The mixture was decomposed with ice and water, the product extracted into ether, and the ethereal solution was worked up. The oily residue (5·1 g) was pure on TLC and identical with the compound prepared as under *a*).

5,6β-Epoxy-5β-cholestane-3β,19-diol 3-Acetate 19-Benzoate (*VI*)

a) *From 5-cholestene-3β,19-diol 3-acetate 19-benzoate* (III): Elution of the chromatography from the foregoing experiment with the same solvent and working up of the corresponding fractions yielded the oily β-epoxide *VI* (160 mg), $[\alpha]_D^{20}$ −1·6° (*c* 1·59). For $C_{36}H_{52}O_5$ (564·8) calculated: 76·56% C, 9·28% H; found: 76·20% C, 9·05% H.

b) *From 5,6β-epoxy-5β-cholestane-3β,19-diol 3-monoacetate* (IV): The alcohol[1] *IV* (1·5 g) in pyridine (12 ml) was treated with benzoyl chloride and allowed to stand at room temperature for 18 h. The excess chloride was decomposed with ice, and the product isolated with ether. The ethereal solution was worked up and the solvent was distilled off. Yield 2·3 g of the oily diester *VI*, identical with the compound prepared by epoxidation of the olefin *III*.

5α-Cholestane-3β,5,6β,19-tetraol (*X*)

a) *From 5α-cholestane-3β,5,6β,19-tetraol 3,19-diacetate* (XIII): The diacetate *XIII* (500 mg) in methanol (50 ml) was refluxed with a solution of potassium carbonate (1 g) in water (10 ml) for 2 hours. The mixture was diluted with water, methanol was distilled off under reduced pressure, and the product was taken into ethyl acetate. The organic layer was washed with water, solvent was removed, and the residue was crystallized from ethyl acetate to yield 300 mg of the tetrol *X*, m.p. 227—228°C, $[\alpha]_D^{20}$ 0° (*c* 1·19 in pyridine). For $C_{27}H_{48}O_4$ (436·6) calculated: 74·26% C, 11·08% H; found: 74·19% C, 11·03% H.

b) *From 5α-cholestane-3β,5,6β,19-tetraol 3-monoacetate* (XIII): The monoacetate *XII* (200 mg) was hydrolysed with potassium carbonate (200 mg) in methanol as described in the previous experiment. Working up and crystallization from ethyl acetate gave 105 mg of the tetrol *X*, m.p. 225—227°C, identical with the sample prepared as under *a*).

c) *From 5α-cholestane-3β,5,6β,19-tetraol 3,6,19-triacetate* (XIV): The triacetate *XIV* (200 mg) was hydrolysed with potassium carbonate (400 mg) in methanol (20 ml) and water (6 ml) as described above. Similar working up and crystallization from ethyl acetate yielded 95 mg of the tetrol *X*, m.p. 227—228°C, identical with the authentic sample.

5α-Cholestane-3β,5,6β,19-tetraol 3-Monoacetate 19-Monobenzoate (*XI*)

The epoxide *VI* (5·1 g) in acetone (130 ml) and water (10 ml) was treated with perchloric acid (70%; 6·5 ml) and set aside for 20 hours. The acid was neutralized sodium bicarbonate, and the solvents were removed under reduced pressure. The residue was extracted with ether, the ethereal solution was dried, and ether removed. The crude product (4·2 g) was chromatographed on silica gel (500 g) in benzene–ether (3 : 1) to yield 3·36 g of the oily diol *XI*, $[\alpha]_D^{20}$ 0° (*c* 1·44). For $C_{36}H_{54}O_6$ (582·8) calculated: 74·19% C, 9·34% H; found: 73·91% C, 9·25% H.

5α-Cholestane-3β,5,6β,19-tetraol 3-Monoacetate (*XII*)

a) *From 5,6β-epoxy-5β-cholestane-3β,19-diol 3-monoacetate* (IV): The epoxide[1] *IV* (1 g) in acetone (50 ml) was treated with 9% perchloric acid (3 ml) and allowed to stand at room temperature for 2 h. The mixture was worked up as described in the previous experiment, and the product was chromatographed over silica gel (80 g) in benzene–ether (4 : 1). Fractions with the required product were worked up, and the crystalline residue (810 mg) was crystallized from methanol to yield 500 mg of the acetate *XII*, m.p. 229—231°C, $[\alpha]_D^{20}$ −14° (*c* 1·45). For $C_{29}H_{50}O_5$ (478·7) calculated: 72·76% C, 10·53% H; found: 72·89% C, 10·40% H.

b) *From* 5,6β-*epoxy*-5β-*cholestane*-3β,19-*diol* 3,19-*diacetate* (V): The epoxide[1] *V* (5 g) in acetone (200 ml) was treated with perchloric acid (5 ml; 9%) and after 30 h at room temperature the product was isolated as described in the previous experiment. The residue was chromatographed on a silica gel column (500 g) in benzene–ether (4 : 1). Fractions with the polar component were worked up to yield after crystallization from methanol 1·56 g of the monoacetate *XII*, m.p. 228–229°C, $[\alpha]_D^{20}$ −14° (*c* 1·27), identical with the derivative prepared as under *a*).

5α-Cholestane-3β,5,6β,19-tetraol 3,19-Diacetate (*XIII*)

a) *From* 5,6β-*epoxy*-5β-*cholestane*-3β,19-*diol* 3,19-*diacetate* (V): Fractions with the lipophilic component from the foregoing experiment were worked up to yield 2·63 g of the non-crystalline diacetate *XIII*, $[\alpha]_D^{20}$ −22° (*c* 1·55). Mass spectrum: M^+ 520. IR spectrum: 1732, 1710, 1265, 1242 (acetate), 3630, 3460 cm^{-1} (hydroxyl). ^{1}H-NMR spectrum: 0·67 (s, 18-H), 0·86 (d, J = 6 Hz, 26-H and 27-H), 0·90 (d, J = 5·5 Hz, 21-H), 1·92 (s, 5α and 6β OH), 2·01 (s, acetate), 2·055 (s, acetate), 3·54 (broad s, W = 12 Hz, 6α-H), 4·44 and 4·66 (two d, J = 13 Hz, 19-H), 5·16 (broad mt, W = 30 Hz, 3α-H). For $C_{31}H_{52}O_6$ (520·7) calculated 71·50% C, 10·07% H; found: 71·35% C, 9·89% H.

b) *From* 5,6α-*epoxy*-5α-*cholestane*-3β,19-*diol* 3,19-diacetate (VIII): The epoxide[1] *VIII* (6·5 g) in acetone (300 ml) was treated with perchloric acid (7 ml; 70%) in water (16 ml) and allowed to stand at room temperature for 2 h. The product was isolated as described for the benzoate *XI* and chromatographed on a silica gel column (500 g) in benzene–ether (2 : 1). Working up of the corresponding fractions afforded 150 mg of the diacetate *XIII*, $[\alpha]_D^{20}$ −25° (*c* 1·63), identical with the compound prepared in the previous experiment.

c) *From* 5α-*cholestane*-3β,5,6β,19-*tetraol* 3-*monoacetate* (XII): The acetate *XII* (400 mg) in pyridine (2 ml) was acetylated with acetic anhydride (1·5 ml) for 20 h at room temperature. The mixture was decomposed with ice and water, the product taken into ether, and the ethereal solution was worked up. The residue was chromatographed on a silica gel column (40 g) in benzene–ether (9 : 1). Fractions with the polar component afforded after working up 70 mg of the diacetate *XIII*, $[\alpha]_D^{20}$ −24° (*c* 1·31), identical with the samples prepared as under *a*).

5α-Cholestane-3β,5,6β,19-tetraol 3,6,19-Triacetate (*XIV*)

a) *From* 5α-*cholestane*-3β,5,6β,19-*tetraol* 3-*monoacetate* (XII): Fractions with the lipophilic component from the chromatography of the foregoing experiment afforded after working up 245 mg of the triacetate *XIV*, m.p. 93–95°C (methanol), $[\alpha]_D^{20}$ −37° (*c* 1·12). For $C_{33}H_{54}O_7$ (562·8) calculated: 70·43% C, 9·67% H; found: 70·38% C, 9·50% H.

b) *From* 5α-*cholestane*-3β,5,6β,19-*tetraol* 3,19-*diacetate* (XIII): The diol *XIII* (300 mg) was acetylated with acetic anhydride (1·5 ml) in pyridine (2 ml) for 20 h at room temperature. The product was isolated with ether, usual working up afforded a crude product which was purified by column chromatography on silica gel (30 g) in benzene–ether (7 : 3). Crystallization from methanol yielded 160 mg of the triacetate *XIII*, m.p. 94–95°C, $[\alpha]_D^{20}$ −39° (*c* 1·90), identical with the compound prepared as under *a*).

5α-Cholestane-3β,5,6β,19-tetraol 6,19-Dibenzoate (*XV*)

The triester *XVI* (1·2 g) in ether (40 ml) was treated with a solution of potassium hydroxide (600 mg) in methanol (100 ml) and allowed to stand at 0°C for 4 h. The mixture was diluted with water, the product extracted into ether, and the residue after evaporation of ether (800 mg)

was chromatographed over silica gel (40 g) in benzene–ether (4 : 1). The corresponding fractions afforded after working up and crystallization from methanol 720 mg of the alcohol *XV*, m.p. 125—126°C, $[\alpha]_D^{20}$ +5° (*c* 1·26). For $C_{41}H_{56}O_6$ (644·9) calculated: 76·35% C, 8·75% H; found: 76·20% C, 8·80% H.

5α-Cholestane-3β,5,6β,19-tetraol 3-Monoacetate 6,19-Dibenzoate (*XVI*)

a) *From 5α-cholestane-3β,5,6β,19-tetraol 3-monoacetate* (XII): The acetate *XII* (1·6 g) in pyridine (10 ml) was treated with benzoyl chloride (2 ml) and allowed to stand at room temperature for 20 h. Usual working up yielded a crude product which was chromatographed over silica gel (150 g) in benzene–ether (9 : 1). Fractions with the pure triester were worked up and the product was crystallized from ligroin to yield 1·2 g of the triester *XVI*, m.p. 97—99°C, $[\alpha]_D^{20}$ −28° (*c* 1·56). For $C_{43}H_{58}O_7$ (686·9) calculated: 75·18% C, 8·51% H; found: 74·90% C, 8·40% H.

b) *From 5α-cholestane-3β,5,6β,19-tetraol 3-monoacetate 19-monobenzoate* (XI): The diol *XI* (3·3 g) in pyridine (20 ml) was esterified with benzoyl chloride (4·4) as described in the previous experiment. Chromatography over silica gel (300 g) in the same solvent mixture and crystallization from ligroin yielded 2·9 g of the triester *XVI*, m.p. 98—100°C, $[\alpha]_D^{20}$ −26° (*c* 1·10), identical with the product from the foregoing reaction.

5α-Cholestane-3β,5,6β,19-tetraol 3,19-Diacetate 6-Monobenzoate (*XVII*)

The diacetate *XIII* (1·5 g) in pyridine (8 ml) was esterified with benzoyl chloride (1·5 ml) 20 h at room temperature. Usual working up gave crude product which was purified by column chromatography on silica gel (100 g) in benzene. Yield 1·5 g of the noncrystalline triester *XVII*, $[\alpha]_D^{20}$ −56° (*c* 1·36). IR spectrum: 3 595 (hydroxyl), 1 722, 1 270 (benzoate), 1 736, 1 241, 1 030 cm^{-1} acetate). For $C_{38}H_{56}O_7$ (624·8) calculated: 73·04% C, 9·03% H; found: 73·30% C, 8·98% H.

3β,5,19-Trihydroxy-5α-cholestan-6-one 3,19-Diacetate (*XVIII*)

The diol *XIII* (600 mg) in acetone (8 ml) was oxidized with excess Jones' reagent. After 10 minutes at room temperature the excess reagent was removed with methanol, the reaction mixture was diluted with water, and the product isolated with ether. The ethereal solution was worked up, and ether distilled off. The residue was crystallized from methanol to yield 370 mg of the ketone *XVIII*, m.p. 136—138°C, $[\alpha]_D^{20}$ −55° (*c* 1·90). Mass spectrum: M^+ 518. IR spectrum: 3 587, 3 480, 3 420, 3 380 (hydroxyl), 1 745, 1 740, 1 233 (acetate), 1 720, 1 711 cm^{-1} (carbonyl). CD spectrum: $\Delta\varepsilon_{300}$ −2·47. ^{1}H-NMR spectrum: 0·655 (s, 18-H), 0·865 (d, J = 6 Hz, 26-H and 27-H), 0·908 (d, J = 6 Hz, 21-H), 1·965 (s, acetate), 2·00 (s, acetate), 4·09 and 4·32 (two d, J = 12·5Hz, 19-H), 5·09 (broad m, W = 30 Hz, 3α-H). For $C_{31}H_{50}O_6$ (518·7) calculated: 71·78% C, 9·72% H; found: 71·83% C, 9·76% H.

3β,5,19-Trihydroxy-5β-cholestan-6-one 3,19-Diacetate (*XIX*)

T he diol *XXVI* (1 g) in acetone (20 ml) was oxidized with Jones' reagent as described in the previous experiment. Similar working up gave crude product which was chromatographed over silica gel (150 g) in benzene–ether (9 : 1). Fractions with the desired ketone were combined and the solvents removed to yield 400 mg of the noncrystalline ketone *XIX*, $[\alpha]_D^{20}$ −26·8° (*c* 2·09). Mass spectrum: M^+ 518. IR spectrum: 3 469 (hydroxyl, bonded), 1 738, 1 750 (acetate), 1 711 cm^{-1} (carbonyl). CD spectrum: $\Delta\varepsilon_{284}$ −5·33. ^{1}H-NMR spectrum: 0·667 (s, 18-H), 0·868 (d,

$J = 6{\cdot}3$, 26-H and 27-H), 0·917 (d, $J = 6$ Hz, 21-H), 1·905 (s, acetate), 2·045 (s, acetate), 3·94 and 4·21 (two d, $J = 11{\cdot}5$ Hz, 19-H), 5·05 (m, $W = 14$ Hz, 3α-H). For $C_{31}H_{50}O_6$ (518·7) calculated: 71·78% C, 9·72% H; found: 71·49% C, 9·60% H.

5,6β,19-Trihydroxy-5α-cholestan-3-one 6,19-Dibenzoate (*XX*)

The diol *XV* (800 mg) in acetone (15 ml) was oxidized with Jones' reagent at room temperature for 10 min. Methanol was added, and the product isolated with ether. Working up and evaporation of ether gave crude product which was chromatographed on a silica gel column (40 g) in benzene. The corresponding fractions yielded 700 mg of the noncrystalline ketone *XX*, $[\alpha]_D^{20}$ −2° (*c* 1·39). CD spectrum: $\Delta\varepsilon_{287}$ +1·21. For $C_{41}H_{54}O_6$ (642·8) calculated: 76·59% C, 8·46% H; found: 76·41% C, 8·30% H.

6β,19-Dihydroxycholest-4-en-3-one (*XXI*)

The dibenzoate *XXIII* (100 mg) in methanol (50 ml) was treated with potassium hydroxide (280 mg) in methanol (15 ml) and allowed to stand at room temperature of 4 h. The excess alkali was removed with acetic acid, the solvents were distilled off under reduced pressure, the residue was diluted with water, and the product taken into ether. The etheral solution was worked up, and the residue after evaporation of ether was crystallized from ethyl acetate to yield 60 mg of the diol *XXI*, m.p. 188—190°C, $[\alpha]_D^{20}$ +50° (*c* 1·59). IR spectrum: 3610, 1031 (hydroxyl), 1678, 1645 cm^{-1} (carbonyl). For $C_{27}H_{44}O_3$ (416·6) calculated: 77·83% C, 10·65% H; found: 77·65% C, 10·49% H.

6β,19-Dihydroxycholest-4-en-3-one 6,19-Diacetate (*XXII*)

The diol *XXI* (100 mg) in pyridine (0·5 ml) was acetylated with acetic anhydride (0·3 ml) for 20 h at room temperature. The mixture was decomposed with ice and water, and the product was taken into ether. The ethereal solution was worked up, and the residue, after evaporation of ether was crystallized from methanol to yield 65 mg of the diacetate *XXII*, m.p. 151—153°C, $[\alpha]_D^{20}$ +69° (*c* 1·51). For $C_{31}H_{48}O_5$ (500·7) calculated: 74·36% C, 9·66% H; found: 74·54% C, 9·53% H.

6β,19-Dihydroxycholest-4-en-3-one 6,19-Dibenzoate (*XXIII*)

a) *From* 5,6β,19-*trihydroxy*-5α-*cholestan*-3-*one* 6,19-*dibenzoate* (XX): The alcohol *XX* (550 mg) in acetic acid (10 ml) was heated at 100°C for 7 h. Acetic acid was removed *in vacuo* the residue was treated with water, and the product extracted into ether. The extract was washed with a sodium hydrogen carbonate solution, water, dried, and ether removed. The crude product (500 mg) was chromatographed on a silica gel column (50 g) in benzene. The corresponding fractions afforded after working up and crystallization from methanol 300 mg of the unsaturated ketone *XXIII*, m.p. 140—141°C, $[\alpha]_D^{20}$ +17° (*c* 1·64). IR spectrum: 1725, 1271 (benzoate), 1690, 1628 cm^{-1} (carbonyl). For $C_{41}H_{52}O_5$ (624·8) calculated: 78·80% C, 8·38% H; found: 78·78% C, 8·43% H.

b) *From* 6β,19-*dihydroxycholest*-4-*en*-3-*one* (XXI): The diol *XXI* (80 mg) in pyridine (2 ml) was treated with benzoyl chloride (0·5 ml) and allowed to stand at room temperature for 20 h. The mixture was decomposed with ice and water, the product taken into ether, and the ethereal solution was worked up. The residue was crystallized from methanol to yield 60 mg of the di-

benzoate *XXIII*, m.p. 140–141°C, $[\alpha]_D^{20}$ +13° (*c* 1·4), identical with the sample prepared as under *a*).

5β-Cholestane-3β,5,6α,19-tetraol (*XXIV*)

a) *From* 5,6α-*epoxy*-5α-*cholestane*-3β,19-*diol* 3-*monoacetate* (VII): The epoxide *VII* (150 mg) in acetone (6 ml) was treated with perchloric acid (0·5 ml; 20%) and allowed to stand at room temperature for 48 h. The excess acid was neutralized with sodium bicarbonate, solvents were removed *in vacuo*, and the product was extracted with ethyl acetate. The crude product (110 mg) after evaporation of the solvent was crystallized from ethyl acetate to yield 60 mg of the tetrol *XXIV*, m.p. 223–225°C, $[\alpha]_D^{20}$ +43° (*c* 1·08 in pyridine). For $C_{27}H_{48}O_4$ (436·6) calculated: 74·26% C, 11·08% H; found: 74·05% C, 10·90% H.

b) *From* 5β-*cholestane*-3β,5,6α,19-*tetraol* 3,19- *diacetate* (XXVI): The diacetate *XXVI* (340 mg) in methanol (40 ml) was treated with a solution of potassium carbonate (700 mg) in water (7 ml) and refluxed for 2 h. Methanol was distilled off under reduced pressure, the residue was treated with water, and the product collected by succion. The crystals were dissolved in ethyl acetate, the solution was washed with water, dried, and solvent removed. The residue was crystallized from ethyl acetate to yield 190 mg of the diacetate *XXIV*, m.p. 224–226°, $[\alpha]_D^{20}$ +46° (*c* 1·25 in pyridine), identical with the sample prepared as under *a*).

5β-Cholestane-3β,5,6α,19-tetraol 3,6,19-Triacetate (*XXV*)

The diacetate *XXVI* (130 mg) in pyridine (2 ml) was acetylated with acetic anhydride (0·8 ml) for 18 hours at room temperature. Usual working up and crystallization from ligroin gave 90 mg of the triacetate *XXV*, m.p. 62–65°C, $[\alpha]_D^{20}$ +48° (*c* 1·53). For $C_{33}H_{54}O_7$ (562·8) calculated: 70·43% C, 9·67% H; found: 70·39% C, 9·38% H.

5β-Cholestane-3β,5,6α,19-tetraol 3,19-Diacetate (*XXVI*)

The epoxide *VIII* (6·5 g) in acetone (300 ml) was treated with perchloric acid (25 ml; 20%) and allowed to stand at room temperature for two hours. The mixture was diluted with water, the product taken into ether, and the ethereal, solution was washed with potassium hydrogen carbonate, dried, and ether removed. The residue was chromatographed over silica gel (500 g) in benzene–ether (3 : 2). Fractions with the desired product were worked up and the crystalline residue was crystallized from ligroin to yield 5·8 g of the diol *XXVI*, m.p. 101–103°C, $[\alpha]_D^{20}$ +39° (*c* 1·83). Mass spectrum: M^+ 520, IR spectrum: 3582 (hydroxyl-bonded), 1748, 1240, 1225 cm^{-1} (acetate). ^{1}H-NMR spectrum: 0·656 (s, 18-H), 0·863 (d, J = 6·2 Hz, 26-H and and 27-H), 0·896 (d, J = 5·7 Hz, 21-H), 2·045 (s, acetate), 2·085 (s, acetate), 3·86 (d of doublets, $J_{6,7}$ = 11·5 + 4·5 Hz, 6-H), 4·34 (s, 19-H), 5·28 (m, W = 14 Hz 3α-H). For $C_{31}H_{52}O_6$ (520·7) calculated: 71·50% C, 10·07% H; found: 71·64% C, 9·91% H.

5β-Cholestane-3β,5,6α,19-tetraol 3,19-Diacetate 6-Monobenzoate (*XXVII*)

The diol *XXVI* (1·9 g) in pyridine (10 ml) was treated with benzoyl chloride (2 ml) for 20 h at room temperature. The mixture was decomposed with ice, the product isolated with ether, and the ethereal solution was worked up as usual. The crude product was chromatographed on a silica gel column (200 g) in benzene–ether (4 : 1) and crystallized from methanol. Yield 1·8 g of the triester *XXVII*, m.p. 122–124°C, $[\alpha]_D^{20}$ +65° (*c* 1·25). For $C_{38}H_{56}O_7$ (624·8) calculated: 73·04% C, 9·03% H; found: 72·78% C, 8·89% H.

5β-Cholestane-3β,5,6α,19-tetraol 3-Monoacetate 19-Monobenzoate (*XXVIII*)

The epoxide *IX* (1·6 g) in acetone (40 ml) was treated with perchloric acid (5 ml; 40%). After 90 min at room temperature the mixture was diluted with water, and the product taken into ether. The ethereal solution was washed with a sodium hydrogen carbonate solution, water, dried, and ether distilled off. The crude product (1·7 g) was chromatographed on a silica gel column (100 g) in benzene–ether (2 : 1). Fractions with the desired product were combined and solvents removed to yield 560 mg of the noncrystalline diester *XXVIII*, $[\alpha]_D^{20}$ +43° (*c* 1·34). For $C_{36}H_{54}O_6$ (582·8) calculated: 74·19% C, 9·34% H; found: 73·89% C, 9·16% H.

5β-Cholestane-3β,5,6α,19-tetraol 3-Monoacetate 6,19-Dibenzoate (*XXIX*)

The diol *XXVIII* (3·4 g) in pyridine (20 ml) was treated with benzoyl chloride (5 ml). After 20 h at room temperature the mixture was decomposed with ice and the product isolated with ether. Usual working up gave an oil which was purified by column chromatography on silica gel(300g) in benzene–ether (25 : 1). The corresponding fractions were worked up to yield 3·9 g of the noncrystalline dibenzoate *XXIX*, $[\alpha]_D^{20}$ +77° (*c* 1·29). ^{1}H-NMR spectrum: 0·54 (s, 18-H), 0·86 (d, J = 6·5 Hz, 26-H and 27-H), 0·88 (d, J = 6 Hz, 21-H), 2·065 (s, acetate), 2·31 (broad m, 4-H), 4·59 (d, J_{gem} = 12 Hz, 19-H), 5·33 (m, 3α-H), 5·60 (d of doublets, $J_{6,7}$ = 12 Hz, 6-H), 7·30–7·62 and 7·95–8·17 (m, aromatic protons). For $C_{43}H_{58}O_7$ (686·9) calculated: 75·18% C, 8·51% H; found: 75·10% C, 8·49% H.

5β-Cholestane-3β,5,6α,19-tetraol 6,19-Dibenzoate (*XXX*)

The triester *XXIX* (1·2 g) was dissolved in methanol (40 ml) and treated with a solution of potassium hydroxide (250 mg) in methanol (15 ml). After 7 min at 0°C the mixture was diluted with water, and the product taken into ether. The ethereal solution was washed with water, dried, and the residue after evaporation of ether was crystallized from chloroform–methanol to yield 260 mg of a pure product. The mother liquors afforded after chromatography over silica gel (50 g) in benzene–ether (7 : 1) and crystallization from the same solvent mixture additional 190 mg of the pure product. Total yield 450 mg of the dibenzoate *XXX*, m.p. 201–203°C, $[\alpha]_D^{20}$ +59° (*c* 1·39). Mass spectrum: M^+ 644. IR spectrum: 3490 (hydroxyl bonded), 1718, 1712, 1275 cm^{-1} (benzoate). For $C_{41}H_{56}O_6$ (644·9) calculated: 76·35% C, 8·75% H; found: 76·08% C, 8·47% H.

5,6α,19-Trihydroxy-5β-cholestan-3-one 6,19-Dibenzoate (*XXXI*)

The diol *XXX* (2·5 g) in acetone (250 ml) was treated with excess Jones' reagent and allowed to stand at room temperature for 10 min. Methanol was added to destroy the excess reagent, the mixture was diluted with water, and the product was taken into ether. The ethereal solution was worked up and the crude product was chromatographed on a silica gel column (200 g) in benzene–ether (49 : 1). The corresponding fractions afforded after working up and crystallization from methanol 2 g of the ketone *XXXI*, m.p. 195–196°C, $[\alpha]_D^{20}$ +81° (*c* 1·30). CD spectrum: $\Delta\varepsilon_{288}$ +0·45. For $C_{41}H_{54}O_6$ (642·8) calculated: 76·59% C, 8·46% H; found: 76·58% C, 8·52% H.

6α,19-Dihydroxycholest-4-en-3-one (*XXXII*)

The dibenzoate *XXXIV* (1·4 g) in ether (50 ml) and methanol (40 ml) was treated with a solution of potassium hydroxide (4 g) in methanol (80 ml) and allowed to stand in a nitrogen atmosphere for 45 min at 0°C. The excess alkali was removed with acetic acid, the mixture was diluted with

water, and the product extracted with ether. The extract was washed with a sodium hydrogen carbonate solution, dried, and ether removed. Chromatography over silica gel (100 g) in benzene–ether (1 : 1) and crystallization from ethyl acetate yielded 480 mg of the diol *XXXII*, m.p. 187–189°C, $[\alpha]_D^{20}$ +78° (*c* 1·50). IR spectrum: 3625, 3605, 1076, 1044 (hydroxyl), 1660, 1645 cm^{-1} (carbonyl). For $C_{27}H_{44}O_3$ (416·6) calculated: 77·83% C, 10·65% H; found: 77·89% C, 10·46% H.

6α,19-Dihydroxycholest-4-en-3-one 6,19-Diacetate (*XXXIII*)

The diol *XXXII* (100 mg) was acetylated with acetic anhydride (0·6 ml) in pyridine (2 ml) for 20 h at room temperature. Usual working up afforded 90 mg of noncrystalline diacetate *XXXIII*, $[\alpha]_D^{20}$ +100° (*c* 1·06). For $C_{31}H_{48}O_5$ (500·7) calculated: 74·36% C, 9·66% H; found: 74·20% C, 9·51% H.

6α,19-Dihydroxycholest-4-en-3-one 6,19-Dibenzoate (*XXXIV*)

a) *From* 5,6α,19-*trihydroxy*-5β-*cholestan*-3-*one* 6,19-*dibenzoate* (XXXI): The ketone *XXXI* (2 g) in acetic acid (40 ml) was heated to 100°C for 7 h. Acetic acid was distilled off *in vacuo*, the residue was treated with water, and the product was taken into ether. The ethereal solution was washed with a sodium hydrogen carbonate solution, dried, and ether removed. The crude product was chromatographed over silica gel (100 g) in benzene. The corresponding fractions afforded after working up 1·6 g of the noncrystalline unsaturated ketone *XXXIV*. $[\alpha]_D^{20}$ +169° (*c* 1·37). IR spectrum: 1728, 1270 (benzoate), 1690, 1682, 1629 cm^{-1} (carbonyl). For $C_{41}H_{52}O_5$ (624·8) calculated: 78·80% C, 8·38% H; found: 78·69% C, 8·21% H.

b) *From* 6α,19-*dihydrocholest*-4-*en*-3-*one* (XXXII): The diol *XXXII* (100 mg) in pyridine (2 ml) was treated with benzoyl chloride (0·9 ml) and allowed to stand at room temperature for 20 h. Usual working up and chromatography over silica gel (10 g) in ligroin–ether (9 : 1) afforded 80 mg of the diester *XXXIV*, $[\alpha]_D^{20}$ +171° (*c* 1·34), identical with the compound prepared as under *a*).

The analyses were carried out in the Analytical Laboratory of this Institute by Mrs E. Sýkorová and Mrs E. Šípová under the direction of Dr J. Horáček. The infrared and UV spectra were recorded by Mr P. Formánek under the direction of Dr J. Smolíková. The mass spectra were recorded by Dr J. Kohoutová under the direction of Dr J. Dolejš. ^{1}H-NMR *spectra were recorded by Dr M. Synáčková.*

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