362. *Studies in the Sterol Group. Part XLIX. 7-Substituted Cholesterol Derivatives and their Stereochemistry (Part II). Esters of the Epimeric 7-Hydroxycholesterols.*

By H. B. Henbest and E. R. H. Jones.

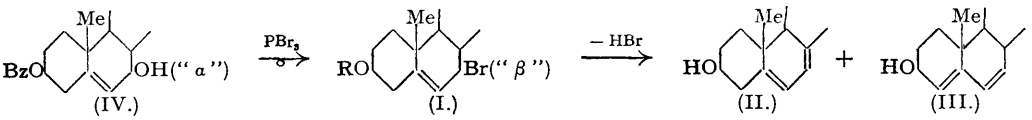
Further evidence for the formulation of the *N*-bromosuccinimide bromination product of cholesteryl acetate as "β"-7-bromocholesteryl acetate (I) has been adduced from the study of replacement reactions of the 7-bromine atom. Partial alkaline hydrolysis leads to the production of the isomeric 7-hydroxy-Δ^5- and 5-hydroxy-Δ^6-compounds (XIX and XX), presumably *via* a mesomeric carbonium cation (XVIII). This same intermediate is also apparently involved in certain reactions of the 5-hydroxy-Δ^6-compounds in which 7-substituted cholesterol derivatives are produced.

The stereochemical picture has been largely completed by the preparation of several new esters of the epimeric "α"- and "β"-7-hydroxycholesterols by these reactions and also by partial acetylation of the 3 : 7-diols (*e.g.*, XXI) and partial hydrolysis of the corresponding diacetates (*e.g.*, XXIII).

When cholesteryl acetate is treated with *N*-bromosuccinimide, a bromo-acetate is produced (Part XLVII, this vol., p. 1783) which has been formulated as "β"-7-bromocholesteryl acetate (I; R = Ac);* analogous compounds are similarly produced by the bromination of cholesteryl

* The significance of the "α" and "β" convention is explained in Part XLVIII.

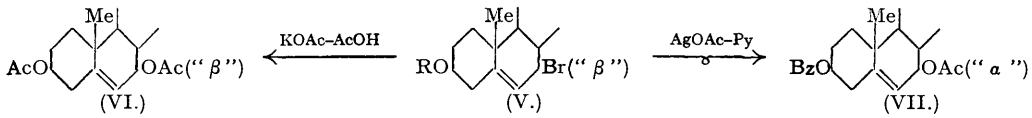
benzoate and cholesteryl chloride. Some evidence in support of the 7-bromo-formulæ for these products has been adduced from the formation of esters of 7-dehydrocholesterol (II) on dehydrobromination with certain tertiary bases, but the value of this evidence is appreciably diminished by the fact that esters of cholesta-4 : 6-dienol (III) are invariably produced simultaneously.



In Part XLVIII (preceding paper) rather more direct proof has been obtained since it has been shown that treatment of the well-authenticated " α "-7-hydroxy-benzoate (IV) (Eckhardt, *Ber.*, **1938**, **71**, 469) with phosphorus tribromide in ether yields a bromo-compound (I; R = Bz) identical with that produced by the *N*-bromosuccinimide reaction.

It seemed possible that further useful evidence for the formulation of these bromination products as belonging to the " β "-7-bromo-series would be forthcoming from a study of replacement reactions of the labile bromine atom. Moreover, the preparation of a variety of 7-substituted cholesterol derivatives in this and in other ways was of considerable interest and offered the possibility of amplifying and consolidating the general statement made in Part XLVIII that the " α "-7-substituted cholesterol derivatives are dextrorotatory while those of the " β "-7-series are uniformly lævorotatory.

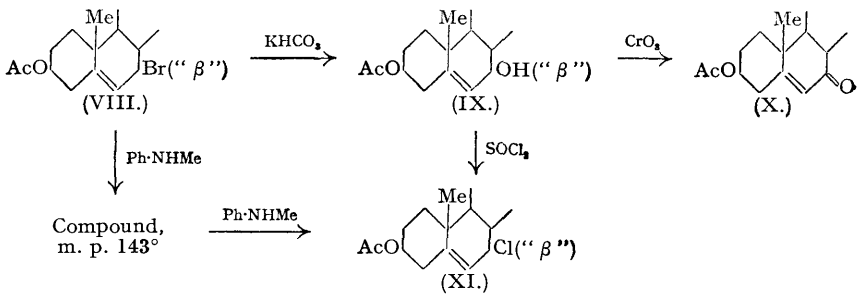
Replacement of the 7-Bromine Atom by Acyloxy-groups.—The 7-bromine atom was readily replaced when the " β "-7-bromo-acetate (V; R = Ac) was treated with potassium acetate in acetic acid at 20°, the main isolable product (30% yield) being the known lævorotatory



" β "-7-acetoxycholesteryl acetate (VI) prepared by Barr, Heilbron, Parry, and Spring (*J.*, **1936**, 1437) by acetylation of the " β "-diol. (This diol had been obtained by these authors by hydrolysis of the product resulting from the oxidation of cholesteryl hydrogen phthalate with permanganate.)

The " β "-7-*formoxy*-compound was obtained similarly but in much better yield, and its structure was confirmed by hydrolysis to the " β "-diol. These replacement reactions thus proceed apparently without appreciable inversion of configuration at C_7, but when the " β "-7-bromo-benzoate (V; R = Bz) was treated with silver acetate in pyridine at 20° the main product was the dextrorotatory " α "-7-acetoxycholesteryl benzoate (VII), identical with material made from the " α "-diol by the procedure devised by Eckhardt (*Ber.*, **1938**, **71**, 469).

Partial Hydrolysis of " β "-7-Bromo-compounds.—So reactive is the bromine atom in 7-bromo-compounds such as (V; R = Ac or Bz) that selective hydrolysis involving only the bromine atom is possible. With potassium hydrogen carbonate in aqueous dioxan, or better with silver hydroxide in the same medium, the bromo-acetate (VIII) gave a solid product which could not

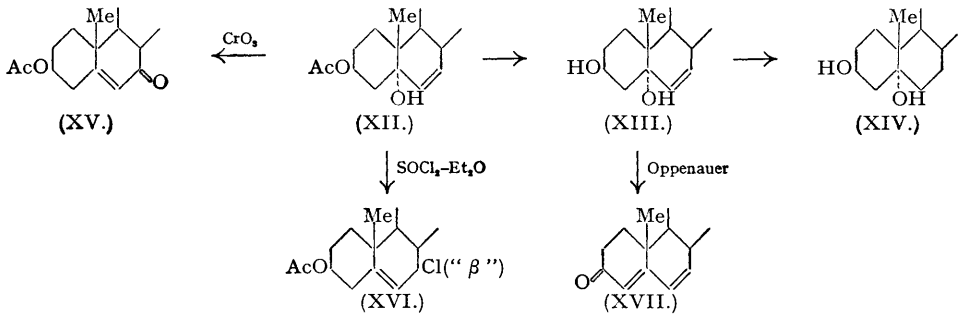


be purified by crystallisation. By chromatography, however, two pure compounds were isolated; one of these (m. p. 143°) is discussed in detail below; the other, lævorotatory like the starting material, was shown to be " β "-7-*hydroxycholesteryl acetate* (IX). Acetylation yielded the known " β "-7-acetoxy-compound, and oxidation with chromic acid produced 7-keto-

cholesteryl acetate (X). Benzoylation gave the hitherto unknown " β "-7-*benzoyloxycholesteryl acetate*. Chlorination with thionyl chloride in ether at 20° gave, as already described in the previous paper, the " β "-7-chloro-acetate (XI), and this was correlated with the corresponding bromo-compound (VIII) by formation of identical products by reaction with methylaniline (forthcoming publication).

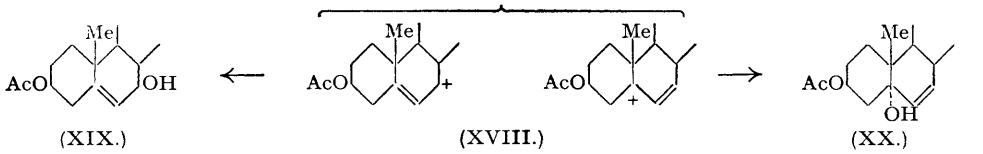
Partial hydrolysis of the " β "-7-bromo-benzoate similarly yielded lævorotatory " β "-7-*hydroxycholesteryl benzoate* (benzoylated to the known dibenzoate), together with a second compound (m. p. 175°) corresponding to the second product (m. p. 143°) obtained by hydrolysis of the bromo-acetate.

The compound of m. p. 143° was found to be isomeric with the " β "-7-hydroxy-acetate (IX), and it seemed at first that it might be the epimeric and hitherto undescribed " α "-7-hydroxycholesteryl acetate. This was rendered extremely improbable when it proved to be lævorotatory, and quite impossible when it was found that it could neither be acetylated nor benzoylated. Its formulation as 3(β)-*acetoxycholest*-6-*en*-5(α)-*ol* (XII), however, was largely



proved by hydrogenation of the corresponding *diol* (XIII) to cholestane-3(β) : 5(α)-diol (XIV), which had been prepared previously by hydrogenation of α-cholesterol oxide (Plattner, Petrzilker, and Lang, *Helv. Chim. Acta*, 1944, **27**, 1872). Benzoylation of the Δ^6-diol (XIII) yielded the product, m. p. 175°, obtained by partial hydrolysis of the " β "-7-bromo-benzoate.

The formation of the isomeric hydroxy-acetates (XIX and XX) by the partial hydrolysis reaction suggests that it proceeds by an S_N1 mechanism (cf. review by Hughes, *J.*, 1946, 974),



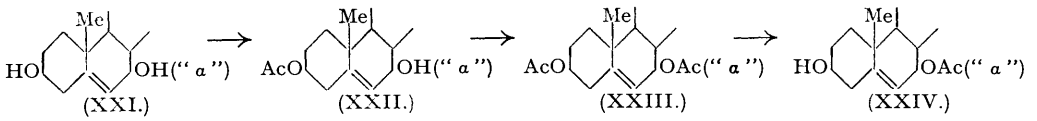
the first stage being the formation of the mesomeric carbonium ion (XVIII) which is then converted into either the 5- or the 7-hydroxy-compound (XIX and XX). On this basis the mechanism of this hydrolysis reaction is closely parallel to that of the elimination reaction which occurs when the 7-bromo-compounds are treated with certain tertiary bases; it has been suggested (Part XLVII) that this is of the E_1 type, separation of the bromide ion leaving the mesomeric carbonium ion (XVIII) which, by loss of a proton, can give rise to either the $\Delta^{5:7}$- or $\Delta^{4:6}$-cholestadienyl acetates. It may also be noted that the diol (XX) has the C_5 hydroxyl group (α)-orientated, the hydroxyl ion apparently combining with (XVIII) to give the more stable *trans*-decalin arrangement of rings A and B.

Some other reactions of the new diol monoacetate (XII) were investigated. Oxidation with chromic acid was slow, much slower than with the 7-hydroxy-compound, but it gave the same product, *viz.*, 7-ketocholesteryl acetate (XV), doubtless *via* an acid-induced rearrangement to the 7-hydroxy-isomer. With thionyl chloride in ether at 20° " β "-7-chlorocholesteryl acetate (XVI) was obtained, presumably *via* the carbonium cation (XVIII), and it may be pointed out that once again the more stable " β "-7-halogeno-compound is formed (cf. Part XLVIII, preceding paper). As was anticipated, Oppenauer oxidation of the diol (XIII) yielded cholesta-4 : 6-dienone, isolated as its semicarbazone.

Only two cholest-6-ene-3(β) : 5-diols, differing in configuration at C_5 (*i.e.*, α and β), can possibly exist, and two such " diols " are already described in the literature. The reactions described for

these two substances are consistent with the formulations suggested, but for neither has any rigorous structural proof been obtained, for example, by hydrogenation. The structures of these two compounds have now been completely elucidated, and this work is described in Part L (following paper).

Partial Acetylation of " α "-7-Hydroxycholesterol and Partial Hydrolysis of the " α "- and " β "-7-Acetoxycholesteryl Acetates.—Various experiments have been carried out in order to prepare 7-hydroxycholesterol derivatives required to complete the series of stereoisomers. Monobenzoylation of " α "-7-hydroxycholesterol (XXI) with benzoic anhydride in pyridine gives a good yield of the 3-monobenzoate (Eckhardt, *loc. cit.*), but, when acetylation of the same diol with the theoretical amount of acetic anhydride in pyridine solution was attempted, only a 5% yield of " α "-7-*hydroxycholesteryl acetate* (XXII) was obtained. This yield could doubtless be improved by using chromatographic methods rather than crystallisation to isolate the monoacetate. [Other workers have experienced difficulty in effecting partial acetylation at the C_3 position (Reichstein and Sorken, *Helv. Chim. Acta*, 1942, **25**, 797; Plattner and Heusser,



ibid., 1944, **27**, 748.] The structure of (XXII) was confirmed by its smooth oxidation to 7-ketocholesteryl acetate (XV) and by acetylation to the known diacetate (XXIII).

Wintersteiner and Ruigh (*J. Amer. Chem. Soc.*, 1942, **64**, 1177, 2453) carried out partial hydrolyses on the epimeric " α "- and " β "-7-benzoyloxycholesteryl benzoates, thus obtaining the two epimeric 7-monobenzoates. The two diacetates (*e.g.*, XXIII) have now been partially hydrolysed yielding " α "-7-*acetoxycholesterol* (XXIV) and " β "-7-*acetoxycholesterol* respectively. Benzoylation of the former gave the " α "-7-acetoxycholesteryl benzoate described by Eckhardt (*loc. cit.*) while the latter gave the new " β "-7-*acetoxycholesteryl benzoate*.

In Part XLVII (*loc. cit.*) attention was directed to a recent publication by Buisman, Stevens, and Vliet (*Rec. Trav. chim.*, 1947, **66**, 83) containing " preliminary results " which to a limited extent are identical with those described in this and the preceding two papers. The work of the Dutch authors on the bromination of cholesteryl esters and the subsequent conversion of the brominated products into 7-dehydrocholesterol has already been commented on (*loc. cit.*), and here certain replacement reactions which Buisman *et al.* have reported are discussed. The Dutch workers were unable to isolate pure bromo-compounds from the *N*-bromosuccinimide brominations, and consequently the few experiments which they carried out on the crude products lose much of their significance. By treatment of " α "-7-hydroxycholesteryl benzoate (IV) with phosphorus tribromide they obtained the same bromo-compound (I; R = Bz), m. p. 140° (which we term " β "-7-bromocholesteryl benzoate), as was obtained by us using the same procedure (Part XLVIII) and also by the bromination of cholesteryl benzoate with *N*-bromosuccinimide (Part XLVII). Buisman *et al.* found that during chromatography on alumina this bromo-compound underwent hydrolysis to " β "-7-hydroxycholesteryl benzoate, m. p. 165—166°, identical with one of the products (m. p. 167—168°) reported in this paper as resulting from hydrolysis with potassium hydrogen carbonate in aqueous dioxan. They also describe an isomeric bromo-benzoate (m. p. 132—133°) obtained by treating the " β "-7-hydroxycholesteryl benzoate (above) with phosphorus tribromide. However, in the absence of optical rotation data and evidence from substitution reactions it is by no means certain that this substance is the hitherto undescribed " α "-7-bromocholesteryl benzoate, as Buisman *et al.* claim.

Experimental.

Replacement of the " β "-7-Bromine Atom by Acyloxy-groups.

" β "-7-*Acetoxycholesteryl Acetate* (VI.).—Potassium acetate (5 g.) was dissolved in acetic acid (200 c.c.) by gentle warming and then cooled to 20°. Powdered " β "-7-bromocholesteryl acetate (9 g.) was added, and the solution was kept at room temperature for 24 hours. The steroid was isolated with ether; recrystallisation from methanol and then from aqueous acetone gave " β "-7-acetoxycholesteryl acetate (2·2 g.), m. p. and mixed m. p. with an authentic sample, 122°; $[\alpha]_D^{18°}$ − 176° (*c*, 0·63) {Barr *et al.*, *J.*, 1936, 1437, give m. p. 121—122°; $[\alpha]_D^{19°}$ − 174·6° (*c*, 0·86)}. A similar yield of the diacetate was obtained by using pyridine acetate instead of potassium acetate.

" β "-7-*Formoxycholesteryl Acetate.*—Solutions of " β "-7-bromocholesteryl acetate (2 g.) in ether (20 c.c.), and sodium formate (2 g.) in formic acid (20 c.c.), were mixed, and kept at room temperature for 24 hours. Ether was added, and most of the formic acid was removed by washing with water and the last traces with sodium hydrogen carbonate solution. Removal of the ether and two crystallisations

of the residue from methanol gave "β"-7-*formoxycholesteryl acetate* (1·15 g.) as small prisms, m. p. 98°; $[\alpha]_D^{20°}$ — 139° (*c*, 1·07) (Found: C, 76·0; H, 10·2. $C_{30}H_{48}O_4$ requires C, 76·25; H, 10·2%).

Hydrolysis of "β"-7-*Formoxycholesteryl Acetate*.—A solution of potassium hydroxide (1 g.) in water (5 c.c.) was added to a suspension of "β"-7-formoxycholesteryl acetate (2 g.) in methanol (100 c.c.), and the mixture was refluxed for 1 hour. The steroid rapidly dissolved. Cooling to room temperature gave a product (1·55 g.), m. p. 178°. Recrystallisation from methanol yielded "β"-7-hydroxycholesterol, m. p. (and mixed) 185°; $[\alpha]_D^{17°}$ — 86·6° (*c*, 1·21) {Barr *et. al* (*loc. cit.*) give m. p. 185°, $[\alpha]_D^{19°}$ —86·4° (*c*, 0·98)}. The mother liquor from the reaction mixture was diluted with a little water to precipitate a further quantity of this material, and after this had been removed by filtration the remaining steroid was isolated *via* ether. The product was benzoylated with benzoyl chloride (3 c.c.) in pyridine (10 c.c.) for 48 hours at room temperature. Isolation of the product *via* ether, followed by recrystallisation from acetone, gave "α"-7-benzoyloxycholesteryl benzoate (0·25 g.), m. p. (and mixed m. p.) 172°; $[\alpha]_D^{16°}$ + 97·5° (*c*, 0·93).

"α"-7-*Acetoxycholesteryl Benzoate* (VII).—"β"-7-Bromocholesteryl benzoate (0·5 g.) was added to a solution of silver acetate (1 g.) in dry pyridine (10 c.c.) which was kept at room temperature for 24 hours. Isolation of the steroid *via* ether, followed by 3 crystallisations from methanol–benzene (3 : 1), gave "α"-7-acetoxycholesteryl benzoate (0·17 g.), m. p. (and mixed m. p. with an authentic sample) 153°.

Partial Hydrolysis of "β"-7-*Bromocholesteryl Acetate* (VIII).—The bromo-acetate (20 g.) dissolved in dioxan (250 c.c.) was stirred vigorously with a solution of potassium hydrogen carbonate (10 g.) in water (50 c.c.) for 20 hours. The product was isolated with ether, and after removal of the last traces of dioxan under reduced pressure the residue (18·5 g.) was dissolved in benzene (100 c.c.) and adsorbed on a 90 × 2·7 cm. column of Birlec alumina. Development with benzene (5·6 l.) gave (after rejection of some gummy material) a solid product which was crystallised from methanol to give long needles of 3(β)-*acetoxycholest*-6-*en*-5(α)-*ol* (XII) (2·8 g.), m. p. 143°; $[\alpha]_D^{20°}$ — 22·1° (*c*, 0·77) (Found: C, 78·4; H, 10·7. $C_{29}H_{48}O_3$ requires C, 78·3; H, 10·9%). Elution with benzene–ether (3 l.; 9 : 1) yielded another solid product, which was crystallised from aqueous methanol to give flat needles of "β"-7-*hydroxycholesteryl acetate* (IX) (3·85 g.), m. p. 139°; $[\alpha]_D^{20°}$ — 87·5° (*c*, 0·73) (Found: C, 78·4; H, 10·8. $C_{29}H_{48}O_3$ requires C, 78·3; H, 10·9%).

Reactions of "β"-7-*Hydroxycholesteryl Acetate* (IX).

"β"-7-*Acetoxycholesteryl Acetate*.—The hydroxy-acetate (100 mg.) was treated at room temperature for 40 hours with acetic anhydride (1·5 c.c.) in pyridine (3 c.c.). Isolation *via* ether gave "β"-7-acetoxycholesteryl acetate (72 mg.) as needles from aqueous acetone, m. p. (and mixed m. p. with an authentic specimen) 122°.

7-*Ketocholesteryl Acetate* (X).—The hydroxy-acetate (100 mg.) was oxidised with a solution of chromic acid (30 mg.) in acetic acid (6 c.c.) at room temperature during 2 hours. Dilution with water gave 7-ketocholesteryl acetate (76 mg.), m. p. (and mixed m. p. with an authentic sample) 158°. Light absorption: Maximum, 2360 A.; $\epsilon = 13{,}000$.

"β"-7-*Benzoyloxycholesteryl Acetate*.—The hydroxy-acetate (200 mg.) was treated with benzoyl chloride (2 c.c.) and pyridine (5 c.c.) at room temperature for 2 hours. The steroid isolated *via* ether crystallised after being kept in methanol solution at 0° for several days. Two recrystallisations from methanol gave "β"-7-*benzoyloxycholesteryl acetate* (103 mg.) as needles, m. p. 113°; $[\alpha]_D^{20°}$ — 176° (*c*, 0·62) (Found: C, 78·8; H, 9·5. $C_{36}H_{52}O_4$ requires C, 78·8; H, 9·55%).

Partial Hydrolysis of "β"-7-*Bromocholesteryl Benzoate*.—The bromo-benzoate (4·5 g.) was dissolved in warm dioxan (150 c.c.) and stirred with a solution of potassium hydrogen carbonate (2·5 g.) in water (10 c.c.) for 48 hours at 50°. The product, isolated with ether, was then adsorbed from benzene (25 c.c.) on a 30 × 1·6 cm. column of activated alumina. Development with benzene–ether (1 l.; 9 : 1) gave, after crystallisation from acetone, 3(β)-benzoyloxycholest-6-en-5(α)-ol (1·2 g.), m. p. 175°, undepressed on mixing with a specimen prepared (as described below) by benzoylation of the diol obtained by hydrolysis of the monoacetate.

Elution with ether yielded "β"-7-*hydroxycholesteryl benzoate* (1·85 g.) which separated from acetone as granular prisms, m. p. 167—168°, $[\alpha]_D^{18°}$ — 50·5° (*c*, 1·32) (Found: C, 80·4; H, 9·85. $C_{34}H_{50}O_3$ requires C, 80·6; H, 9·95%). Benzoylation of this hydroxy-benzoate produced "β"-7-benzoyloxycholesteryl benzoate, m. p. (and mixed m. p. with an authentic specimen) 152°.

Reactions of 3(β)-*Acetoxycholest*-6-*en*-5(α)-*ol* (XII).

Cholest-6-*ene*-3(β) : 5(α)-*diol* (XIII).—3(β)-Acetoxycholest-6-en-5(α)-ol (300 mg.) was refluxed in a solution of potassium hydroxide (200 mg.) in methanol (25 c.c.) for 15 minutes. The product obtained by dilution with water was crystallised from methanol to give *cholest*-6-*ene*-3(β) : 5(α)-*diol* (265 mg.), m. p. 181°; $[\alpha]_D^{20°}$ — 16·4° (*c*, 0·67) (Found: C, 80·5; H, 11·4. $C_{27}H_{46}O_2$ requires C, 80·5; H, 11·5%). Benzoylation of the diol (70 mg.) with benzoyl chloride (1 c.c.) and pyridine (2 c.c.) gave, after 2 crystallisations from acetone, 3(β)-*benzoyloxycholest*-6-*en*-5(α)-*ol* (47 mg.) as needles, m. p. 175°; $[\alpha]_D^{20°}$ — 26·2° (*c*, 1·22) (Found: C, 80·5; H, 10·0. $C_{34}H_{50}O_3$ requires C, 80·6; H, 9·95%).

Cholestane-3(β) : 5(α)-*diol*(XIV).—The diol (100 mg.) in alcohol (30 c.c.) was shaken with hydrogen for 4 hours in the presence of platinic oxide (20 mg.). After the catalyst had been filtered off and the solvent removed under reduced pressure, the residual solid, which gave only a very weak Lifschutz test, was crystallised from methanol to give cholestane-3(β) : 5(α)-diol (77 mg.) as felted needles, m. p. 224°; $[\alpha]_D^{19°}$ + 21·2° (*c*, 0·80) {Plattner, Petrzilka, and Lang, *Helv. Chim. Acta*, 1944, **27**, 513, give m. p. 224·5°; $[\alpha]_D$ + 20·6° (*c*, 0·48)}.

Acetylation with acetic anhydride and pyridine at room temperature gave 3(β)-acetoxycholestan-5(α)-ol, m. p. 184°, after one crystallisation from aqueous methanol (Plattner *et al.*, *loc. cit.*, give m. p. 185°).

Oxidation to 7-*Ketocholesteryl Acetate* (XV).—A solution of 3(β)-acetoxycholest-6-en-5(α)-ol (100 mg.) in acetic acid (3 c.c.) was treated with a solution of chromic acid (30 mg.) in acetic acid (3 c.c.). Oxidation

was slow, and the mixture was left overnight. The steroid was isolated *via* ether, and the product crystallised from methanol to give 7-ketocholesteryl acetate (55 mg.), m. p. (and mixed m. p. with authentic material) 157°. Light absorption : Maximum, 2370 A.; $\epsilon = 13{,}500$.

" β "-7-*Chlorocholesteryl Acetate* (XVI).—3(β)-Acetoxycholest-6-en-5(α)-ol (100 mg.) was dissolved in dry ether (5 c.c.) and cooled to 0°. Thionyl chloride (0·2 c.c.) was added and the solution was allowed to warm up to room temperature during 1 hour. Solvent was removed under reduced pressure and the residue was twice crystallised from dry acetone to give " β "-7-chlorocholesteryl acetate (Part XLVIII, *loc. cit.*) as needles (48 mg.), m. p. (and mixed m. p.) 113—114°.

Oppenauer Oxidation of Cholest-6-ene-3(β) : 5(α)-diol.—A mixture of the diol (200 mg.), freshly sublimed aluminium *tert.*-butoxide (300 mg.), dry benzene (10 c.c.), and dry acetone (5 c.c.) was refluxed for 24 hours. After addition of water the steroid was isolated *via* ether, and the crude gummy product had light absorption (Maxima, 2820 and 2890 A.; $\epsilon = 19{,}500$ and 19,000) which indicated the presence of about 65% of cholesta-4 : 6-dien-3-one (XVII). A light petroleum (b. p. 40—60°) solution of this product was passed through a short column of activated alumina in order to remove hydroxylic impurities, and the eluate (after removal of the solvent under reduced pressure) was treated with semicarbazide acetate. The crude semicarbazone so obtained was crystallised from methanol to give cholesta-4 : 6-dien-3-one semicarbazone (88 mg.), m. p. (and mixed m. p. with an authentic sample) 235—236° (decomp.). Light absorption : Maximum, 3040 A.; $\epsilon = 35{,}000$. (Wintersteiner and Ruigh, *J. Amer. Chem. Soc.*, 1942, **64**, 2453, give for the light absorption in dioxan : Maximum, 3050 A.; $\epsilon = 46{,}000$.)

Preparation and Reactions of " α "-7-Hydroxycholesteryl Acetate (XXII).

" α "-7-*Hydroxycholesteryl Acetate* (XXII).—" α "-7-Hydroxycholesterol (10 g.; Windaus, Lettré, and Schenck, *loc. cit.*) was dissolved in dry pyridine (40 c.c.) by gentle warming, and the solution was then cooled to 0°. Acetic anhydride (2·5 g.) and dry pyridine (10 c.c.) were added during 5 minutes at 0°, and the mixture was kept at 0° for 4 days after which the steroid was isolated *via* ether. The gelatinous residue was stirred with warm light petroleum (200 c.c.; b. p. 40—60°) and filtered from unchanged diol (5·2 g.). After evaporation of the solvent under reduced pressure, the residue was dissolved in acetic acid (100 c.c.) by gentle warming and then cooled to 10°. The crystalline product (m. p. 97°) was twice crystallised from methanol–water (9 : 1) to give " α "-7-*hydroxycholesteryl acetate* (0·53 g.) as fine filamentous needles, m. p. 110—111°; $[\alpha]_D^{16°} - 5{\cdot}0°$ (*c*, 1·0), or as plates from light petroleum (b. p. 40—60°) (Found : C, 78·05; H, 10·75. $C_{29}H_{48}O_3$ requires C, 78·3; H, 10·9%). Further acetylation with acetic anhydride and pyridine at room temperature gave " α "-7-acetoxycholesteryl acetate (XXIII), m. p. (and mixed m. p. with an authentic sample) 100°.

[Throughout this work the lower-melting form of this diacetate was always obtained. Windaus and Schenck (U.S.P. 2,098,985) state that the two forms have m. p.s 98—100° and 106·5—107·5°.] The hydroxy-acetate (100 mg.) was treated with benzoyl chloride and pyridine at room temperature for 2 hours. Isolation with ether, followed by crystallisation from methanol–benzene (4 : 1), gave long needles of " α "-7-*benzoyloxycholesteryl acetate* (81 mg.), m. p. 166°; $[\alpha]_D^{17°} + 82°$ (*c*, 0·86) (Found : C, 79·2; H, 9·55. $C_{36}H_{52}O_4$ requires C, 78·8; H, 9·55%).

7-*Ketocholesteryl Acetate* (XV).—" α "-7-Hydroxycholesteryl acetate (50 mg.) was suspended in acetic acid (3 c.c.); a solution of chromic acid (30 mg.) in acetic acid (3 c.c.) was added, and gentle warming rapidly completed the reaction. Dilution with water, followed by crystallisation from acetone–methanol, gave 7-ketocholesteryl acetate (32 mg.), m. p. (and mixed m. p. with an authentic sample) 158°.

Partial Hydrolyses of the " α "- and " β "-7-Acetoxycholesteryl Acetates.

" α "-7-*Acetoxycholesterol* (XXIV).—11·2 C.c. of a solution of potassium hydroxide (2·8 g.) in ethanol (250 c.c.) were added to a solution of " α "-7-acetoxycholesteryl acetate (1·1 g.; prepared by acetylation of " α "-7-hydroxycholesterol; Windaus, Lettré, and Schenck, *loc. cit.*) in benzene (5 c.c.) and ethanol (10 c.c.), and the mixture was kept at room temperature for 72 hours. Isolation with ether followed by 2 crystallisations from aqueous methanol gave " α "-7-*acetoxycholesterol* (0·62 g.) as fluffy needles, m. p. 83—84°; $[\alpha]_D^{19°} + 72{\cdot}5°$ (*c*, 1·07) (Found : C, 78·0; H, 10·7. $C_{29}H_{48}O_3$ requires C, 78·3; H, 10·9%).

Benzoylation of this product gave " α "-7-acetoxycholesteryl benzoate, m. p. (and mixed m. p.) 153°. (Sample prepared by the method of Eckhardt, *loc. cit.*)

" β "-7-*Acetoxycholesterol.*—12·5 C.c. of a solution of potassium hydroxide (2·8 g.) in ethanol (250 c.c.) were added to a solution of " β "-7-acetoxycholesteryl acetate (1·21 g.; prepared from " β "-7-bromocholesteryl acetate and potassium acetate) in benzene (10 c.c.) and ethanol (10 c.c.), and the mixture was kept at room temperature for 72 hours. The steroid isolated *via* ether did not crystallise until a methanol solution was left at 0° for 6 days. Recrystallisation from aqueous methanol gave " β "-7-*acetoxycholesterol* (0·63 g.) as long needles, m. p. 143—144° (softening at 140°); $[\alpha]_D^{17°} -193°$ (*c*, 1·25) (Found : C, 78·6; H, 10·8. $C_{29}H_{48}O_3$ requires C, 78·3; H, 10·9%).

A solution of this compound (200 mg.) in pyridine (3 c.c.) and benzoyl chloride (1 c.c.) was kept at room temperature for 24 hours. Isolation of the steroid *via* ether followed by crystallisation from acetone–methanol (1 : 1) gave " β "-7-*acetoxycholesteryl benzoate* as silky needles, m. p. 132°; $[\alpha]_D^{17°} - 121°$ (*c*, 0·79) (Found : C, 78·85; H, 9·4. $C_{36}H_{52}O_4$ requires C, 78·8; H, 9·55%). An identical acetoxybenzoate, m. p. (and mixed) 132°, was obtained by acetylation of the " β "-7-hydroxycholesteryl benzoate produced (see above) by partial hydrolysis of the " β "-7-bromo-benzoate.

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