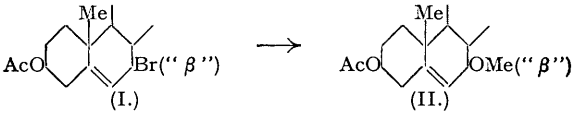
363. *Studies in the Sterol Group. Part L. 7-Substituted Cholesterol Derivatives and their Stereochemistry (Part III). 7-Alkoxycholesterol Derivatives.*

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

7-Methoxy- and 7-ethoxy-cholesterol derivatives have been prepared from the 7-bromo-compounds. Some of the ethers appear to be formed *via* the " β "-7-hydroxy-compound which has been shown to undergo alkylation very readily in alcoholic solutions in the presence of acids, even acetic acid. As a result of this discovery it has been demonstrated that two compounds hitherto believed to be epimeric cholest-6-ene-3(β) : 5-diols are in fact " β "-7-methoxy- and " β "-7-ethoxy-cholesterols.

The optical rotatory powers of all the known 7-substituted cholesterol derivatives have been collated and discussed.

In continuation of our study of the replacement reactions of 7-halogeno-cholesterol derivatives, it has been found that the introduction of 7-alkoxyl groups by replacement reactions takes place with great ease. Although " β "-7-bromocholesteryl acetate (I) (produced by the action of *N*-bromosuccinimide on cholesteryl acetate; Part XLVII, this vol., p. 1783) reacted rapidly with warm alcohols, only resinous products were obtained. When the bromo-compound was treated with silver nitrate in aqueous dioxan a resinous product was again isolated by ether extraction, but in solution in methyl alcohol this rapidly deposited a crystalline solid which was eventually



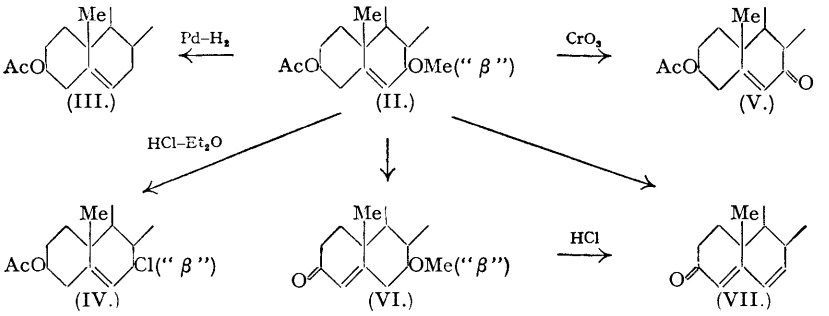
(I.) (II.)

shown to be " β "-7-*methoxycholesteryl acetate* (II), m. p. 111°. The same compound was produced when the 7-bromo-compound was treated with a limited amount of potassium hydroxide in aqueous dioxan, the crude resinous product becoming crystalline on treatment with methyl alcohol. The rôle of the alcohol in these reactions was not immediately obvious; the compound, m. p. 111°, was at first considered to be the then unknown " β "-7-hydroxycholesteryl acetate, and later, 5-hydroxy-3-acetoxycholest-6-ene, when it was discovered that the third oxygen atom could not be esterified. A zero Zerewitinoff value and the absence of any coloration with tetranitromethane rendered even the second choice untenable. These results seemed to indicate a formulation such as 5 : 7-epoxycholestanyl acetate, and it was not until hydrogenation and Oppenauer oxidation experiments had been carried out that the possibility of the presence of an alkoxyl group was seriously considered. Carbon and hydrogen determinations on such substances appear to be unreliable (see below) because of the possibility of methane formation, but a Zeisel determination revealed the methoxyl group. The failure of the tetranitromethane reaction was paralleled by the behaviour of the closely related " α "-7-hydroxycholesterol (Hardegger, Ruzicka, and Stein, *Helv. Chim. Acta*, 1943, **26**, 2220), and this has now been shown to be general with all the 7-hydroxycholesterol derivatives prepared during the course of this work (see preceding papers).

The structure of the methyl ether was confirmed by hydrogenation, oxidation, and other reactions. Hydrogenation in acetic acid solution with a palladium catalyst gave cholesteryl acetate (III) (probably contaminated with a little cholestanyl acetate); similar reductive removal of 7-substituents in cholesterol derivatives (see Part XLVIII, this vol., p. 1788) has been observed by Barr, Heilbron, Parry, and Spring (*J.*, 1936, 1437) and Wintersteiner and Ruigh (*J. Amer. Chem. Soc.*, 1942, **64**, 2453). In the previous cases completely hydrogenated (cholestanol) derivatives were obtained; the isolation of cholesteryl acetate in the present example clearly indicates the sequence of events, the activated 7-substituent being removed before the saturation of the activating ethylenic linkage.

The ether grouping was found to be readily replaced in the presence of acidic reagents, hydrogen chloride in ether yielded " β "-7-chlorocholesteryl acetate (IV), and treatment with acetic acid containing a trace of sulphuric acid gave the corresponding 7-acetoxy-compound. A somewhat analogous reaction ensued when the methoxy-compound was oxidised in acetic acid

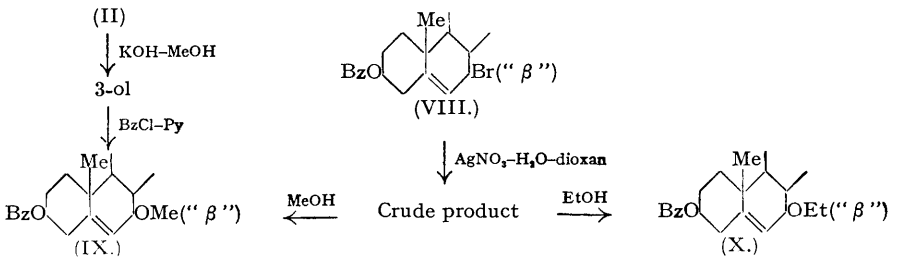
solution with chromic acid; oxidation proceeded only very slowly, but the product was 7-ketocholesteryl acetate (V) :



After this work had been completed a structurally similar unsaturated methoxy-steroid exhibiting closely analogous reactions was described by Kendall and his co-workers (*J. Biol. Chem.*, 1946, **164**, 569). This 12-methoxy-$\Delta^{9:11}$-cholenate readily undergoes methoxyl-group fission with acidic reagents and is slowly oxidised by chromic acid to the 12-keto-compound.

Oxidation of the " β "-7-*methoxycholesterol* under the usual Oppenauer conditions proceeded in the expected manner and gave " β "-7-*methoxycholest*-4-*en*-3-*one* (VI) together with some cholesta-4 : 6-dienone (VII). The formation of the dienone from the methoxy-ketone (VI) takes place smoothly under the influence of hydrogen chloride in ethereal solution.

It was thus established that a 7-methoxy-compound had been prepared by the rather unusual procedure employed, *i.e.*, the methoxyl group having been introduced as a result of crystallising the crude product from methanol; the following further experiments led to the elucidation of the reactions involved. The bromo-benzoate (VIII) was mixed with a solution of silver nitrate

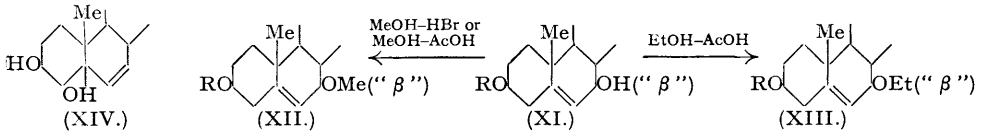


in aqueous dioxan; silver bromide was precipitated almost immediately. When the non-crystalline product, isolated with ether, was treated with methyl alcohol it gave " β "-7-*methoxycholesteryl benzoate* (IX) (identical with the material prepared by benzoylation of the alcohol obtained from the methoxy-acetate). On the other hand, treatment with ethyl alcohol yielded the corresponding " β "-7-*ethoxycholesteryl benzoate* (X). These results suggested the possibility that the crude product was " β "-7-hydroxycholesteryl acetate (XI; R = Ac) and that this intermediate was then etherified by the alcohol in the presence of nitric acid from some 7-nitrate, or hydrogen bromide, the latter being produced from residual bromo-compound which had escaped hydrolysis. This hypothesis was confirmed by the fact that when a solution of " β "-7-hydroxycholesteryl acetate (XI; R = Ac) in methanol was treated with a trace of hydrogen bromide the methoxy-acetate (XII; R = Ac) soon crystallised out.

Further confirmation of the hypothesis was forthcoming from experiments in which " β "-7-hydroxycholesterol (XI; R = H) was subjected to the action of alcohols in the presence of acetic acid as the acidic catalyst. With methyl alcohol " β "-7-methoxycholesterol (XII; R = H) was obtained, whereas the use of ethyl alcohol gave " β "-7-*ethoxycholesterol* (XIII; R = H).

In the methyl alcohol experiments a small quantity of an isomeric methyl ether was isolated from the mother liquors as its dextrorotatory benzoate. This is regarded as " α "-7-*methoxycholesteryl benzoate* since the formation of some of the " α "-7-ether would be expected in view of the known tendency of " β "-7-hydroxycholesterol to form an equilibrium mixture with the " α "-isomer under the influence of acetic acid (Ruzicka, Prelog, and Tagmann, *Helv. Chim.*

Acta, 1944, **27**, 1149). Additional evidence for the structure proposed for this isomeric ether was obtained when the derived sterol, " α "-7-*methoxycholesterol*, was oxidised by the Oppenauer method, the light absorption of the crude product indicating the presence of some 30% of an αβ-unsaturated ketone and a similar amount of cholesta-4 : 6-dienone.



It was mentioned in the preceding paper, in which the preparation and complete characterisation of cholest-6-ene-3(β) : 5(α)-diol (XIV) were described, that only two such 3(β)-diols could possibly exist [*i.e.* 5(α) and 5(β)] and that two such diols were already known. As an outcome of our work on the 7-alkoxycholesterol derivatives we have been able to clear up this difficulty by showing that neither of these substances is a diol or even a 3 : 5-dihydroxy-derivative (cf. Henbest and Jones, *Nature*, 1946, **158**, 950).

Wintersteiner and Bergström (*J. Biol. Chem.*, 1941, **141**, 597; 1942, **143**, 503) obtained what they believed to be a cholest-6-ene-3(β) : 5-diol (XIV) by heating under reflux a solution of " β "-7-hydroxycholesterol in ethyl alcohol containing 10% of acetic acid. They observed that their product gave cholesta-4 : 6-dienone on oxidation by the Oppenauer method with aluminium phenoxide, but they were surprised to find that they could not hydrogenate their " diol " to cholestane-3 : 5-diol [a reaction that is readily achieved with the true Δ^6-diol (preceding paper)]. It is now clear that Wintersteiner and Bergström were dealing with " β "-7-ethoxycholesterol, and this has been confirmed by its preparation by their method and also from a 7-bromo-compound. In addition, Dr. Wintersteiner very kindly sent us a sample of the benzoate of his product, and no depression was observed in a mixed m. p. determination.

During the course of their extensive and remarkably thorough investigations on the steroid content of various organ extracts, Prelog, Ruzicka, and Stein (*Helv. Chim. Acta*, 1943, **26**, 2222) isolated a compound from pig spleen which they provisionally formulated as cholest-6-ene-3(β) : 5-diol (XIV). It seemed as if their substance was the 5-epimer of the Wintersteiner and Bergström " diol ", and in agreement with this conception they found that on Oppenauer oxidation (with aluminium phenoxide) cholesta-4 : 6-dienone was formed. The constants of the " diol " and its derivatives obtained by the Swiss workers were observed to be identical with those of our " β "-7-methoxycholesterol and its derivatives, and in mixed m. p. determinations (kindly carried out by Dr. Prelog on the sterol and its acetate and benzoate) no depressions were oberved. Carbon and hydrogen determinations on these alkoxy-sterols and their derivatives are unreliable unless special precautions are taken, and this doubtless explains why both the Swiss and the American workers were led to draw incorrect deductions as to the structures of their compounds.

It is practically certain that the " β "-7-methoxycholesterol isolated by Prelog, Ruzicka, and Stein (*loc. cit.*) was not present in the original organ extract. One of the stages in the treatment of the pig-spleen material involved the separation of ketonic from non-ketonic substances by means of the Girard reagent which was used in methyl-alcoholic solution containing acetic acid. As has already been mentioned, under these conditions " β "-7-hydroxycholesterol, which is known to occur in animal tissues (Wintersteiner and Ritzmann, *J. Biol. Chem.*, 1940, **136**, 697; Hardegger, Ruzicka, and Tagmann, *Helv. Chim. Acta*, 1943, **26**, 2205), would be converted into the 7-methoxy-compound. This has been shown to be so in the presence or absence of the Girard reagent. The conclusion that the methoxycholesterol was an artefact is further strengthened by the fact that in the two cases cited above, where the " β "-7-hydroxycholesterol was isolated from natural sources, the Girard separation was not employed, the diol being isolated directly by chromatographic methods.

A 7-phenyl ether was obtained by interaction of " β "-7-bromocholesteryl acetate and potassium phenoxide in methyl alcohol, the product, formed presumably as the result of an inversion at C_7, being the dextrorotatory " α "-7-*phenoxycholesteryl acetate*.

The optical rotatory powers of all the known 7-substituted cholesterol derivatives (with the exception of certain amino-compounds which will be discussed in a later publication) are given in Table I.

In the steroid series, optical rotation differences between epimers are usually small, and Table I further emphasises the point (see Part XLVIII, *loc. cit.*) that for 7-epimers in the

cholesterol series these differences are remarkably large, sufficiently and so consistently large as to justify the conclusion (see Part XLVIII, *loc. cit.*) that sign of rotation can be used to assign compounds to the " α "- or the " β "-series.

The use of the method of molecular rotation differences in the steroid series has proved of value in relation to certain structural problems (Barton, *J.*, 1945, 813; 1946, **512**, 1116). This method has been used in order to compare the 7-substituted cholest-5-enols described in this and previous papers with the unsubstituted Δ^5-stenols. The molecular rotation differences

TABLE I.

Optical rotatory powers ($[\alpha]_D$) *of 7-substituted cholesterol derivatives.*

	Cholesterol. −26°.		Cholesteryl acetate. −43°.		Cholesteryl benzoate. −17°.	
	" α."	" β."	" α."	" β."	" α."	" β."
7-Hydroxy-	+ 7°	− 87°	− 5°	− 88°	+ 14°	− 51°
7-Acetoxy-	+ 73	−193	+52	−176	+ 76	−121
7-Benzoyloxy-	+111	−201	+82	−176	+ 94	−106
7-Methoxy-	+ 23	−127	+18	−124	+ 39	− 34
7-Chloro-	—	—	—	−165	+ 61	−119
7-Bromo	—	—	—	−245	—	−172
7-Iodo-	—	—	—	−362	—	−270
7-Phenoxy-	+111	—	+83	—	+114	—

(M.R.D.) are given in Table II where Δ_1 is the M.R.D. between the 3(β)-sterol and its 3-acetate and Δ_2 is the M.R.D. between the sterol and its benzoate, and these values are compared with the standard values. Only the Δ_1 and Δ_2 values of the hydroxy- and methoxy-compounds approximate to the differences for the unsubstituted compounds, large deviations being apparent in all the other cases. These deviations are attributable in part to the size of the C_7 substituents, but also to the polarisable nature of the groups associated with the anomalies. Barton and Cox (*Nature*, 1947, **159**, 470) have shown that acetyl, benzoyl, and other groups, even in the 17-position, can cause anomalies in M.R.D.s for the 3-position.

TABLE II.

Molecular rotation differences for 7-substituted cholesterol derivatives.

	Δ_1 (× 10^{-2}) ($[M]_D$ of 3-acetate − $[M]_D$ of 3-ol).	Δ_2 (× 10^{-2}) ($[M]_D$ of 3-benzoate − $[M]_D$ of 3-ol).
" α "-7-Hydroxy-	− 38°	+ 54°
" β "-7-Hydroxy-	− 41	+ 92
" α "-7-Acetoxy-	− 72	+ 92
" β "-7-Acetoxy-	+ 2	−195
" α "-7-Benzoyloxy-	−113	+ 48
" β "-7-Benzoyloxy-	+ 54	+372
" α "-7-Methoxy-	− 12	+106
" β "-7-Methoxy-	− 39	+ 92
" α "-7-Phenoxy-	−100	+132
Average values for Δ^5-stenols (Barton and Cox, *loc. cit.*)...	− 34	+ 80

EXPERIMENTAL.

" β "-7-*Methoxycholesteryl Acetate* (II).—(*a*) A solution of silver nitrate (7·5 g.) in water (50 c.c.) was added all at once with stirring to a solution of " β "-7-bromocholesteryl acetate (15 g.) in dioxan (200 c.c.). There was an immediate precipitation of silver bromide. After 5 minutes, ether (500 c.c.) was added, and the ethereal solution was then decanted from the silver salts, washed with distilled water, and dried (Na_2SO_4). After the ether had been removed under reduced pressure, the residue was dissolved in methanol (150 c.c.) and water (5 c.c.) and left overnight. The crystalline product which separated from the strongly acidic solution was recrystallised from acetone to give a product (7·6 g.), m. p. 109°. Further recrystallisation from acetone or acetone–methanol (1 : 1) gave " β "-7-*methoxycholesteryl acetate* as rectangular needles, m. p. 111°, $[\alpha]_D^{20°}$ − 124° (*c*, 1·05) (Found: C, 78·35; H, 10·8; OMe, 6·4. $C_{30}H_{50}O_3$ requires C, 78·55; H, 11·0; OMe, 6·75%). {Prelog, Ruzicka, and Stein, *loc. cit.* give m. p. 110—111°, $[\alpha]_D^{20°}$ − 118° (*c*, 0·56) for this compound, which they suggested might be the 3-acetate of cholest-6-ene-3(β) : 5-diol.}

(*b*) A solution of " β "-7-bromocholesteryl acetate (3 g.) in ether (30 c.c.) was added to a solution of potassium hydroxide (0·34 g.) in methanol (30 c.c.). Potassium bromide soon began to separate, and after the mixture had been kept at 25° for 15 minutes, the steroid was isolated with ether. Two recrystallisations from methanol gave slightly impure " β "-7-methoxycholesteryl acetate (1·4 g.), m. p. 109°, undepressed on admixture with a specimen prepared as in (*a*). A similar yield of the methoxy-compound was obtained by substitution of potassium benzoate for the potassium hydroxide. The yield in either case was somewhat improved (1·7 g.) by filtering a light petroleum (b. p. 40—60°)

solution of the crude product through a short column of activated alumina, followed by crystallisation from methanol.

" β "-7-*Methoxycholesterol and* " β "-7-*Methoxycholesteryl Benzoate* (IX).—(*a*) " β "-7-Methoxycholesteryl acetate (1 g.) was refluxed in methanol solution (50 c.c.) containing potassium hydroxide (0·5 g.) for 30 minutes. Water (5 c.c.) was then added, and on cooling, 0·91 g. of product, m. p. 158°, was deposited. Recrystallisation from methanol gave " β "-7-*methoxycholesterol* (0·85 g.) in long needles, m. p. 158—159°, $[\alpha]_D^{20°}$ − 127° (*c*, 0·79) (Found: C, 80·4; H, 11·5. $C_{28}H_{48}O_2$ requires C, 80·7; H, 11·6%) {Prelog, Ruzicka, and Stein,* *loc. cit.*, give m. p. 156°, $[\alpha]_D^{19°}$ − 132° (*c*, 0·59)}.

Benzoylation of this product with benzoyl chloride in pyridine in the usual manner gave " β "-7-*methoxycholesteryl benzoate* as needles from acetone, m. p. 136°, $[\alpha]_D^{20°}$ − 84·3° (*c*, 0·83) (Found: C, 80·6; H, 10·1. $C_{35}H_{52}O_3$ requires C, 80·7; H, 10·1%) {Prelog, Ruzicka, and Stein,* *loc. cit.*, give m. p. 134—135°, $[\alpha]_D^{20}$ − 79° (*c*, 0·68)}.

When a solution of silver nitrate (150 mg.) in water (1 c.c.) was added to a solution of " β "-7-bromocholesteryl benzoate (150 mg.) in dioxan (7·5 c.c.), there was an immediate precipitation of silver bromide. After 2 minutes ether was added, and the solution was decanted from silver bromide, dried (Na_2SO_4), and evaporated under reduced pressure. The residual gum was dissolved in warm methanol; this solution soon deposited solid material which after two recrystallisations from acetone gave " β "-7-methoxycholesteryl benzoate (56 mg.), m. p. (and mixed m. p.) 135°.

(*b*) A solution of " β "-7-hydroxycholesterol (2 g.) in methanol (90 c.c.) and acetic acid (10 c.c.) was heated under reflux for 5 hours. The steroid was isolated *via* ether, and after 3 crystallisations gave long needles of " β "-7-methoxycholesterol (0·64 g.), m. p. 158°, undepressed on admixture with a specimen prepared by method (*a*). (Benzoylation gave " β "-7-methoxcholesteryl benzoate, m. p. and mixed m. p. 136°.) The mother liquor from the first crystallisation (above) was diluted with water, and the steroid isolated *via* ether. This product was treated with benzoyl chloride in pyridine at room temperature; isolation *via* ether followed by two crystallisations from acetone–benzene (3 : 1) gave " α "-7-*methoxycholesteryl benzoate* (0·35 g.), m. p. 151°, $[\alpha]_D^{16°}$ + 38·6° (*c*, 1·15) (Found: C, 80·9; H, 10·1; OMe, 6·3. $C_{35}H_{52}O_3$ requires C, 80·7; H, 10·1; OMe, 6·0%).

" α "-7-*Methoxycholesterol.*—" α "-7-Methoxycholesteryl benzoate (280 mg.) in benzene (5 c.c.) was refluxed with a solution of potassium hydroxide (100 mg.) in ethanol (5 c.c.) for 4 hours. Isolation in the usual way gave " α "-7-*methoxycholesterol* (125 mg.) as long needles after two crystallisations from aqueous methanol, m. p. 146—147°, $[\alpha]_D^{16°}$ + 22·9° (*c*, 0·87) (Found: C, 80·4; H, 11·5. $C_{28}H_{48}O_2$ requires C, 80·7; H, 11·6%). Acetylation of the sterol with acetic anhydride in pyridine gave " α "-7-*methoxycholesteryl acetate* as needles from methanol, m. p. 93—94°, $[\alpha]_D^{16°}$ + 18·2° (*c*, 1·20) (Found: C, 78·5; H, 10·8. $C_{30}H_{50}O_3$ requires C, 78·55; H, 11·0%).

Oppenauer oxidation of " α "-7-methoxycholesterol with acetone in the presence of aluminium *tert.*-butoxide gave a gum, with the following light absorption: Maximum, 2450 A.; $\epsilon = 5000$ corresponding to about 30% of " α "-7-methoxycholest-4-en-3-one. Maximum: 2810 A.; $\epsilon = 9000$ corresponding to about 30% of cholesta-4 : 6-dien-3-one.

" β "-7-*Ethoxycholesterol* (XIII; R = H).—This compound was prepared from " β "-7-hydroxycholesterol by using the procedure of Bergström and Wintersteiner (*loc. cit.*). The " β "-7-*ethoxycholesterol* crystallised from methanol in small plates, m. p. 138°, $[\alpha]_D^{13°}$ − 132° (*c*, 1·27) (Found: C, 81·0; H, 11·85; OEt, 10·1. $C_{29}H_{50}O_2$ requires C, 80·9; H, 11·7; OEt, 10·45%). {Bergström and Wintersteiner give m. p. 139–140°, $[\alpha]_D^{24°}$ − 134°, for this compound, which they believed to be cholest-6-ene-3(β) : 5-diol.}

" β "-7-*Ethoxycholesteryl Benzoate* (X).—The preparation of the 7-methoxy-benzoate from the bromo-compound, described above, was repeated except that the crude gum was dissolved in ethanol instead of methanol. Two recrystallisations from ethanol gave " β "-7-*ethoxycholesteryl benzoate* (25 mg.), m. p. 116°, undepressed on admixture with a sample prepared by Bergström and Wintersteiner (*J. Biol. Chem.*, 1941, **141**, 597), $[\alpha]_D^{17°}$ − 88·0° (*c*, 1·00). Bergström and Wintersteiner describe this compound as the 3-monobenzoate of cholest-6-ene-3(β) : 5-diol, but their analytical data are in excellent agreement with the " β "-7-ethoxycholesteryl benzoate formulation.

" β "-7-*Chlorocholesteryl Acetate* (IV).—" β "-7-Methoxycholesteryl acetate (250 mg.) was dissolved in ethereal hydrogen chloride (10 c.c.; half-saturated solution) at 0°, and kept at 0° overnight. After removal of the solvent under reduced pressure, the residue was twice recrystallised from dry acetone to give " β "-7-chlorocholesteryl acetate (95 mg.), m. p. 114°, undepressed on admixture with the product, m. p. 114°, obtained from " β "-7-hydroxycholesteryl acetate by treatment with thionyl chloride.

" β "-7-*Acetoxycholesteryl Acetate.*—The 7-methoxy-compound (250 mg.) was dissolved in acetic acid (20 c.c.) and cooled to 10°. Acetic acid (5 c.c.) containing 1 drop of concentrated sulphuric acid was added, and the solution kept at 10° for 5 minutes. Isolation of the steroid *via* ether gave a gum that solidified in contact with methanol. Crystallisation from methanol gave " β "-7-acetoxycholesteryl acetate (105 mg.), m. p. 122°, undepressed on admixture with an authentic sample. Hydrolysis and subsequent benzoylation of the diacetate gave " β "-7-benzoyloxycholesteryl benzoate, m. p. 152°, undepressed on admixture with an authentic sample.

7-*Ketocholesteryl Acetate* (V).—A solution of " β "-7-methoxycholesteryl acetate (300 mg.) in acetic acid (10 c.c.) containing chromic acid (100 mg.) was kept at room temperature for 24 hours. The steroid was then isolated with ether, and the solid product, dissolved in light petroleum (b. p. 40—60°), chromatographed on an 18 × 1·2 cm. column of activated alumina. The eluate obtained by development with light petroleum (b. p. 40—60°) contained unchanged starting material (105 mg.). Development with benzene, evaporation, and two crystallisations of the residual solid from acetone gave 7-ketocholesteryl acetate (95 mg.), m. p. 157—158°, undepressed on admixture with an authentic sample.

Hydrogenation of " β "-7-*Methoxycholesteryl Acetate.*—A solution of the methoxy-acetate (1 g.) in " AnalaR " acetic acid (50 c.c.) was shaken with hydrogen and freshly prepared palladium black (100 mg.); after 18 hours, 64 c.c.(1·2 mols.) of hydrogen had been absorbed. The steroid was isolated

* These authors believed these compounds might be cholest-6-ene-3-(β) : 5-diol and its 3-monobenzoate respectively.

with ether, and the semi-solid product was chromatographed on a 30 × 1·6 cm. column of activated alumina, the chromatogram being developed with light petroleum (b. p. 40—60°) and the eluate collected in 50 c.c. portions. The first two fractions gave a small amount of gum; fractions 3 and 4 yielded material (0·58 g.) of m. p. 111—112°, $[\alpha]_D^{20°}$ − 38·9° (*c*, 0·46), after one crystallisation from acetone–methanol (1 : 1), which was not altered by further recrystallisation. It gave a yellow colour with tetranitromethane, and was subsequently identified as impure cholesteryl acetate (m. p. 114°, $[\alpha]_D^{20°}$ − 42·5°), probably contaminated with some cholestanyl acetate {m. p. 110—111°, $[\alpha]_D^{20°}$ − 13·5° (*c*, 0·89)}, since by hydrolysis and crystallisation it yielded pure cholesterol.

" β "-7-*Methoxycholest*-4-*en*-3-*one* (VI).—A mixture of " β "-7-methoxycholesterol (3·5 g.), freshly sublimed aluminium *tert.*-butoxide (3 g.), dry benzene (70 c.c.), and dry acetone (40 c.c.) was gently refluxed for 24 hours with the exclusion of moisture. After addition of water, the steroid was isolated with ether. Most of the mesityl oxide present was removed in a high vacuum, the last traces being removed by adding a little xylene and again evaporating in a high vacuum. The resulting gum gave the following light absorption data: Maximum, 2420 A.; ϵ = 9500, corresponding to about 55% of " β "-7-methoxycholest-4-en-3-one. Maxima, 2810 and 2900 A.; ϵ = 9500 and 8500 respectively, corresponding to about 35% of cholesta-4 : 6-dien-3-one. The gum was dissolved in a mixture of acetone (10 c.c.) and methanol (30 c.c.), and scratched to induce crystallisation. The crude solid (m. p. *ca.* 125°) was twice crystallised from acetone–methanol (1 : 1) to give " β "-7-*methoxycholest*-4-*en*-3-*one* (1·15 g.) in small prisms, m. p. 143°, $[\alpha]_D^{20°}$ + 38·3° (*c*, 1·22) (Found: C, 80·95; H, 11·2. $C_{28}H_{46}O_2$ requires C, 81·1; H, 11·2%). Light absorption: Maximum, 2420 A.; ϵ = 16,000 (Jones and Wilkinson, *J.*, 1942, 391, for cholest-4-en-3-one give maximum, 2405 A.; ϵ = 18,000).

The 2 : 4-*dinitrophenylhydrazone* crystallised from benzene–alcohol (1 : 1) in vermilion needles, m. p. 196° (Found: C, 68·2; H, 8·2; N, 9·05. $C_{34}H_{50}O_5N_4$ requires C, 68·6; H, 8·5; N, 9·4%). Light absorption: Maximum, 3950 A.; ϵ = 31,000 (Jones and Wilkinson, *loc. cit.*, for cholest-4-enone 2 : 4-dinitrophenylhydrazone give maximum, 3910 A.; ϵ = 32,000).

Cholesta-4 : 6-*dien*-3-*one* (VII) *from* " β "-7-*Methoxycholest*-4-*en*-3-*one* (VI).—The ketone (100 mg.) was dissolved in half-saturated ethereal hydrogen chloride (5 c.c.) and kept overnight at room temperature. The solvent was removed under reduced pressure, and the residue was twice crystallised from methanol–acetone (3 : 1) to give cholesta-4 : 6-dien-3-one (52 mg.), m. p. and mixed m. p. 81°, $[\alpha]_D^{16°}$ + 37° (*c*, 1·07). Light absorption: Maxima, 2810 A. and 2900 A.; ϵ = 28,000 in each case (Wintersteiner and Ruigh, *J. Amer. Chem. Soc.*, 1942, **64**, 2453, give maximum, 2850 A.; ϵ = 26,000).

" β "-7-*Methoxycoprostan*-3-*one*.—" β "-7-Methoxycholest-4-en-3-one (500 mg.) dissolved in ethyl acetate (40 c.c.) was shaken with hydrogen and freshly prepared palladium black (50 mg.); approximately 1 mol. of hydrogen (28 c.c.) was absorbed within 15 minutes. The catalyst was filtered off, and the solvent removed under reduced pressure; the residual gum solidified in contact with acetone. Two recrystallisations from aqueous acetone (the product tended at first to form an emulsion) gave " β "-7-*methoxycoprostan*-3-*one* (310 mg.), m. p. 72—73°, $[\alpha]_D^{21°}$ + 22·7° (*c*, 1·03) (Found: C, 80·45; H, 11·3. $C_{28}H_{48}O_2$ requires C, 80·7; H, 11·6%). A further quantity (95 mg.), m. p. 71—72°, was obtained from the mother liquors.

" β "-7-Methoxycoprostan-3-one was recovered unchanged after being left in ethereal hydrogen chloride solution overnight (compare " β "-7-methoxycholest-4-en-3-one).

The ketone readily gave a 2 : 4-*dinitrophenylhydrazone* which separated from alcohol–benzene (3 : 1) as yellow leaflets, m. p. 158—159° (Found: C, 68·4; H, 8·75. $C_{34}H_{52}O_5N_4$ requires C, 68·4; H, 8·8%). Light absorption: Maximum, 3660 A.; ϵ = 26,500.

" α "-7-*Phenoxycholesteryl Acetate*.—" β "-7-Bromocholesteryl acetate (2 g.) dissolved in ether (20 c.c.) was added to a solution of phenol (2 g.) and potassium hydroxide (0·5 g.) in methanol (20 c.c.). The mixture was kept at 25° for 30 minutes, and the steroid was then isolated with ether, excess of phenol being removed by dilute aqueous sodium hydroxide solution. Crystallisation from acetone and then from acetone–methanol (3 : 1) gave " α "-7-*phenoxycholesteryl acetate* (1·03 g.) as fine matted needles, m. p. 154°, $[\alpha]_D^{20°}$ + 83° (*c*, 0·77) (Found: C, 80·8; H, 10·2. $C_{35}H_{52}O_3$ requires C, 80·7; H, 10·1%). Light absorption: Maximum, 2240 A.; ϵ = 15,500.

" α "-7-*Phenoxycholesterol*.—The acetate (1·1 g.) was dissolved in hot methanol (50 c.c.) and the least amount of benzene necessary to effect complete solution; potassium hydroxide (0·5 g.) in a little water was added, and the solution was refluxed for 15 minutes. The product (m. p. 139°) crystallised on cooling; recrystallisation from methanol containing a little ether gave " α "-7-*phenoxycholesterol* (0·88 g.) as micro-needles, m. p. 140°, $[\alpha]_D^{20°}$ + 111° (*c*, 0·53) (Found: C, 82·8; H, 10·25. $C_{33}H_{50}O_2$ requires C, 82·8; H, 10·5%). Benzoylation of this sterol gave " α "-7-*phenoxycholesteryl benzoate* as needles from acetone–benzene (4 : 1), m. p. 167° (clearing at 177°), $[\alpha]_D^{18°}$ + 114° (*c*, 0·72) (Found: C, 82·6; H, 9·1. $C_{40}H_{54}O_3$ requires C, 82·45; H, 9·35%).

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