289. *Some Reactions of α- and β-Cholesteryl Benzoate Oxides.*

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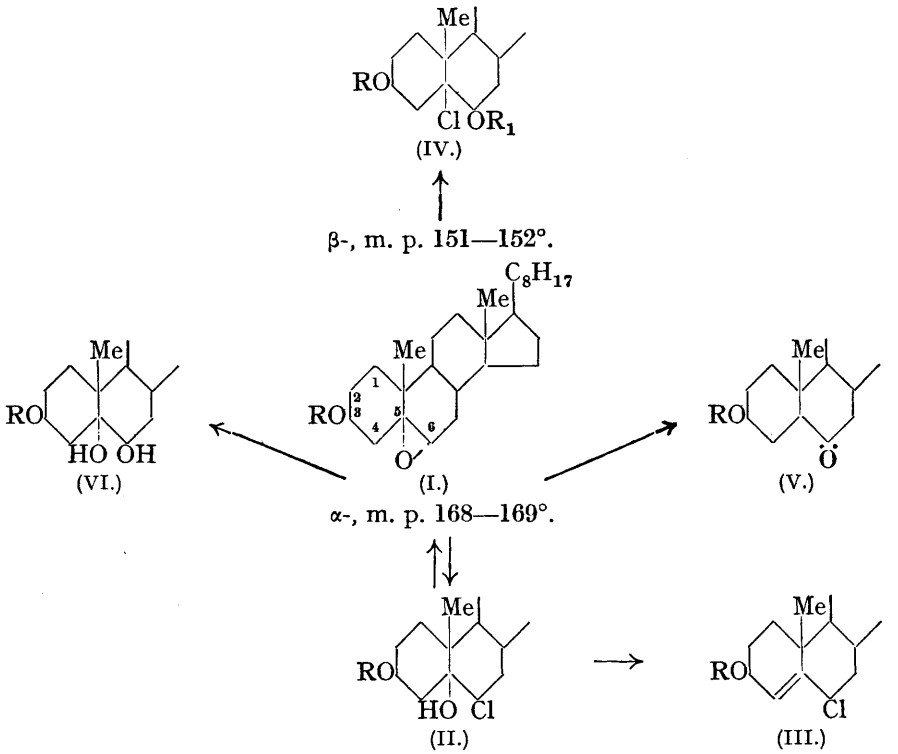
As a preliminary to a study of the dehydration of the oxides of cholesteryl esters, α- and β-*cholesteryl benzoate oxides* (I, R = COPh) have been prepared and some of their reactions investigated. Treatment of α-cholesteryl benzoate oxide with hydrochloric acid gives **6-*chloro*-5-*hydroxy*-3-*benzoyloxycholestane*** (II, R = COPh) which on dehydration yields **6-*chloro*-3-*benz*-Δ⁴-*cholestene*** (III, R = COPh). Similar treatment of the β-benzoate oxide give ***loro*-6-*hydroxy*-3-*benzoyloxycholestane*** (IV, R = COPh, R_1 = H). On treatment of α-cholesteryl benzoate oxide with either dehydrated alum or phosphoric oxide it is in part isomerised to 6-ketocholestanyl benzoate (V, R = COPh).

Treatment of cholesteryl benzoate with perbenzoic acid gives a mixture of α- and β-*cholesteryl benzoate oxides*, m. p.'s 168—169° and 151—152° respectively, characterised by hydrolysis to the corresponding cholesterol oxides. The yields of these benzoates are approximately 50% and 40%, respectively, whereas by an analogous method cholesteryl acetate affords a 75% yield of its β-oxide (Ruzicka and Bosshard, *Helv. Chim. Acta*, 1937, **20**, 244). An α-cholesteryl benzoate oxide, m. p. 181°, has been obtained by Lettré and Müller (*Ber.*, 1937, **70**, 1947) by the pyrolysis of the dibenzoate of 3 : 5 : 6-trihydroxycholestane; since repeated crystallisations of our product, m. p. 168—169°, failed to alter its melting point, we attempted to repeat their preparation of the α-benzoate oxide, using their method. The dibenzoate of 3 : 5 : 6-trihydroxycholestane has not been described in the literature. Treatment of the triol with benzoyl chloride and pyridine gave a gelatinous product containing halogen which could not be removed, and treatment with benzoic anhydride in pyridine gave a mixture of α-cholesteryl benzoate oxide, m. p. 168—169°, identical with the specimen prepared by the perbenzoic acid method, and 5 : 6-*dihydroxy*-3-*benzoyloxycholestane*, m. p. 222—223°, characterised by its hydrolysis to 3 : 5 : 6-trihydroxycholestane. The monobenzoate of the 3 : 5 : 6-triol is also obtained by the hydration of α-cholesteryl benzoate oxide with dilute sulphuric acid.

With benzoyl chloride and pyridine, α-cholesterol oxide (I, R = H) (Westphalen, *Ber.*, 1915, **48**, 1064; Ruzicka and Bosshard, *loc. cit.*) gives a compound $C_{34}H_{51}O_3Cl$, m. p. 202—203°, which is also obtained by treatment of α-cholesteryl benzoate oxide with either hydrogen chloride or benzoyl chloride. Of the two possible constitutions, *viz.*, (II, R = COPh) and (IV, R = COPh, R_1 = H), for this compound, the former (6-*chloro*-5-*hydroxy*-3-*benzoyloxycholestane*) is correct since the compound cannot be further acylated. On heating with quinoline, (II, R = COPh) yields α-cholesteryl benzoate oxide, m. p. 168—169°, and with thionyl chloride it gives **6-*chloro*-3-*benzoyloxy*-Δ⁴-*cholestene*** (III, R = COPh).

The oxide ring of β-cholesteryl benzoate oxide is similarly opened on treatment with hydrogen chloride, but in this case the isomeric **5-*chloro*-6-*hydroxy*-3-*benzoyloxycholestane*** (IV, R = COPh, R_1 = H) is obtained. It is characterised by further benzoylation to

5-chloro-3 : 6-dibenzoyloxycholestane (IV, R = R_1 = COPh), identical with a specimen prepared from 5-chloro-3 : 6-dihydroxycholestane (Windaus, *Z. physiol. Chem.*, 1921, **117**, 154; Lettré and Müller, *loc. cit.*). The dibenzoate (IV, R = R_1 = COPh) is also obtained from β-cholesterol oxide or β-cholesteryl benzoate oxide by treatment with benzoyl chloride. Thus a remarkable difference in the behaviour of β-cholesteryl benzoate



oxide and β-cholesteryl acetate oxide is to be noted, the latter on treatment with hydrogen chloride giving 6-chloro-5-hydroxy-3-acetoxycholestane (II, R = COMe) (Ruzicka and Bosshard, *loc. cit.*).

Fusion of α-cholesteryl benzoate oxide with dehydrated alum gives 6-ketocholestanyl benzoate, which is also obtained by treatment of the α-benzoate oxide with phosphoric oxide.

Experimental.

α- and β-Cholesteryl Benzoate Oxides.—Cholesteryl benzoate (60 g.) in dry chloroform (240 c.c.) was treated with a solution of perbenzoic acid in chloroform (840 c.c.; 0·4N) at 0°, the mixture kept at this temperature for 12 hours and then at 20° for 4 days. The solution was washed with 5% sodium carbonate solution, and dried (sodium sulphate). Removal of the chloroform under reduced pressure yielded a crystalline residue, m. p. 147—149°, which was recrystallised thrice from ethyl acetate, *α-cholesteryl benzoate oxide*, m. p. 168—169°, separating in prismatic needles (28 g.; yield 46%), $[\alpha]_D^{20°}$ — 31·3° (l = 1, c = 1·12 in chloroform). Three further crystallisations from the same solvent did not alter the m. p. It is sparingly soluble in ether and in alcohol but readily soluble in chloroform (Found : C, 80·3; H, 10·0. $C_{34}H_{50}O_3$ requires C, 80·6; H, 9·95%). Hydrolysis of this oxide (0·5 g.) was effected by heating under reflux with alcoholic potassium hydroxide (15 c.c.; 5%) for 2 hours. The hot solution was treated with water until faintly turbid, and after cooling, the crystalline mass was collected and recrystallised from methyl alcohol, α-cholesterol oxide separating in needles, m. p. 142°, showing no depression on admixture with a specimen prepared by the method of Ruzicka and Bosshard (*loc. cit.*).

Concentration of the combined ethyl acetate mother-liquors from the α-benzoate oxide

gave β-*cholesteryl benzoate oxide*, which, after several recrystallisations from ethyl acetate–methyl alcohol (3 : 1), separated in fine needles, m. p. 151—152°, unaltered by further crystallisation (23 g.; yield 38%), $[\alpha]_D^{20°}$ + 3·8° (l = 1, c = 2·3 in chloroform) (Found: C, 80·3; H, 9·9%). β-Cholesteryl benzoate oxide is not depressed in m. p. on admixture with cholesteryl benzoate (m. p. 150—151°). Hydrolysis of this β-oxide by the method described for the α-isomer gave β-cholesterol oxide as needles from methyl alcohol, m. p. 107—108°, not depressed on admixture with a specimen (m. p. 108°) prepared by hydrolysis of β-cholesteryl acetate oxide (Ruzicka and Bosshard, *loc. cit.*).

Benzoylation of 3 : 5 : 6-*Trihydroxycholestane*.—The triol (Pickard and Yates, J., 1908, **93**, 1678) (4 g.) in pyridine (20 c.c.) and benzoyl chloride (8 c.c.) were heated on the steam-bath for 1 hour. The mixture was poured into excess of aqueous sodium bicarbonate solution, and after standing for 30 minutes, the product was isolated by means of ether. Removal of the ether gave an oil which separated as a gel from a variety of hot solvents. On long standing in ethyl acetate–methyl alcohol, crystallisation slowly set in, giving a mass of felted needles, m. p. 120—150°; on attempted recrystallisation from the same solvent, gel formation recurred, there being no improvement in the m. p. of the final crystalline product, which contained an appreciable amount of chlorine.

5 : 6-*Dihydroxy*-3-*benzoyloxycholestane*.—(a) The triol (4 g.) in dry pyridine (20 c.c.) and benzoic anhydride (8 g.) were heated on the steam-bath for 9 hours. The mixture was poured into aqueous sodium carbonate solution and set aside for 48 hours. The separated solid was fractionated from ethyl acetate–methyl alcohol; the least soluble fraction, after several crystallisations from ethyl acetate, formed prismatic needles, m. p. 168—169°, not depressed in admixture with α-cholesteryl benzoate oxide. The more soluble fraction was crystallised from ethyl acetate giving 5 : 6-*dihydroxy*-3-*benzoyloxycholestane* as plates, m. p. 222—223° (Found: C, 77·8; H, 10·25; $C_{34}H_{52}O_4$ requires C, 77·8; H, 10·0%), $[\alpha]_D^{25°}$ − 4·9° (l = 1, c = 1·23 in chloroform). Hydrolysis of the monobenzoate (1 part) was effected by heating under reflux with methyl-alcoholic potassium hydroxide (3%; 50 parts) for 1 hour. The product separated from methyl alcohol as needles, m. p. 239°, showing no depression in admixture with 3 : 5 : 6-trihydroxycholestane.

(b) α-Cholesteryl benzoate oxide (3 g.) in benzene (60 c.c.) was heated under reflux for 2 hours with sulphuric acid (0·5 c.c.; 66%); the solution acquired a pink coloration. Water was added to the cold solution, the mixture extracted with ether, and the extract washed with sodium carbonate solution and dried (sodium sulphate). The resinous product obtained after removal of the ether was crystallised from ethyl acetate, 5 : 6-dihydroxy-3-benzoyloxycholestane separating in plates, m. p. 222—223°, showing no depression in admixture with the specimen described under (a).

6-*Chloro*-5-*hydroxy*-3-*benzoyloxycholestane* (II; R = COPh).—(a) α-Cholesterol oxide (4 g.) was heated on the steam-bath for 1 hour with benzoyl chloride (12 c.c.) and pyridine (16 c.c.). The mixture was poured into excess of sodium carbonate solution (5%) and, after standing for 30 minutes, extracted with ether. The product, isolated in the usual manner, was crystallised from ethyl acetate–methyl alcohol, from which the *benzoate* (3·5 g.) separated in plates, m. p. 202—203° (decomp.), $[\alpha]_D^{20°}$ −19·5° (l = 1, c = 2·3 in chloroform) (Found: C, 75·0; H, 9·3. $C_{34}H_{51}O_3Cl$ requires C, 75·2; H, 9·5%). It is sparingly soluble in alcohol but easily soluble in chloroform and moderately soluble in ethyl acetate and pyridine. It does not give a coloration with antimony trichloride in chloroform.

(b) α-Cholesteryl benzoate oxide (1 g.) in absolute methylated spirit (30 c.c.) and benzene (10 c.c.) was treated with hydrochloric acid (d 1·16; 2 c.c.), and the mixture heated under reflux for 30 minutes. The solid separating on cooling was crystallised from ethyl acetate–methyl alcohol, giving 6-chloro-5-hydroxy-3-benzoyloxycholestane as plates (0·6 g.), $[\alpha]_D^{20°}$ −19·2° (l = 1; c = 2·2 in chloroform), m. p. 202—203° (decomp.), showing no depression on admixture with the specimen prepared by method (a). The same product was obtained when α-cholesteryl benzoate oxide in chloroform was treated with dry hydrogen chloride.

α-*Cholesteryl Benzoate Oxide from* 6-*Chloro*-5-*hydroxy*-3-*benzoyloxycholestane*.—The foregoing benzoate (1 g.) in quinoline was heated to 180° during 10 minutes and maintained at this temperature for a further 7 minutes (long heating gives rise to unworkable resin). The solution was rapidly cooled, treated with excess sulphuric acid (5%), and extracted with ether. The extract was washed with sodium carbonate solution, and dried. Removal of the solvent gave a solid residue which was crystallised from ethyl acetate, giving prismatic needles (0·5 g.), $[\alpha]_D^{20°}$ − 29·05° (l = 1, c = 1·48 in chloroform), m. p. 168—169°, showing no depression on admixture with α-cholesteryl benzoate oxide.

6-Chloro-3-benzoyloxy-Δ⁴-cholestene (III, R = COPh).—6-Chloro-5-hydroxy-3-benzoyloxycholestane (1 g.) in dry pyridine (5 c.c.) was treated at 0° with thionyl chloride (0·2 c.c.) with stirring. After 5 minutes the mixture was poured into ice-water. The oil which first separated gradually solidified; it was collected, washed with water and methyl alcohol, and crystallised from ethyl acetate–methyl alcohol, *6-chloro-3-benzoyloxy-Δ⁴-cholestene* separating in prismatic needles (0·6 g.), m. p. 127—128°, $[\alpha]_D^{20°}$ −79·4° ($l = 1$, $c = 1·03$ in chloroform) [Found: C, 77·7; H, 9·4; *M*(Rieche), 455. $C_{34}H_{49}O_2Cl$ requires C, 77·7; H, 9·4%; *M*, 525]. It is moderately soluble in ethyl acetate, freely soluble in chloroform, but only sparingly soluble in alcohol. With the antimony trichloride reagent it gives an immediate intense blue coloration changing to violet on standing.

5-Chloro-6-hydroxy-3-benzoyloxycholestane (IV, R = COPh, R_1 = H).—β-Cholesteryl benzoate oxide (1 g.) in alcohol (35 c.c.) and benzene (5 c.c.) was treated with hydrochloric acid (2 c.c.; *d* 1·16) and heated under reflux for 30 minutes. The solution was diluted with water until turbid, and the solid separating on cooling was collected and crystallised five times from ethyl acetate, from which the *benzoate* separated in rectangular plates, m. p. 206—207° (decomp.), $[\alpha]_D^{20°} \pm 0°$ (Found: C, 75·1; H, 9·6. $C_{34}H_{51}O_3Cl$ requires C, 75·2; H, 9·5%). The same product was obtained when β-cholesteryl benzoate oxide in chloroform was treated with dry hydrogen chloride. When mixed with its 6-chloro-5-hydroxy-analogue, its m. p. is depressed to 170°.

5-Chloro-3 : 6-dibenzoyloxycholestane.—(a) The foregoing 3-monobenzoyloxy-compound (0·3 g.) was heated on the steam-bath for 1 hour with pyridine (2 c.c.) and benzoyl chloride (0·7 c.c.). The mixture was poured into sodium bicarbonate solution, and the product isolated by means of ether. After crystallisation from ethyl acetate–methyl alcohol and then from ethyl acetate, the dibenzoate separated in triangular prisms (0·2 g.), m. p. 184°, not depressed on admixture with a specimen, m. p. 184°, $[\alpha]_D^{20°}$ − 68·6° ($l = 1$, $c = 1·2$ in chloroform), prepared by the method of Lettré and Müller (*loc. cit.*) who record m. p. 181°.

(b) Treatment of β-cholesterol oxide with benzoyl chloride and pyridine under the conditions described above gave the same dibenzoate, m. p. and mixed m. p. 184°, in hard triangular prisms from ethyl acetate (Found: C, 76·1; H, 8·7. Calc. for $C_{41}H_{55}O_4Cl$: C, 76·05; H, 8·6%). The chloro-dibenzoate obtained by similar treatment of β-cholesteryl benzoate oxide separated in triangular prisms from ethyl acetate, m. p. 184°, $[\alpha]_D^{20°}$ − 68·0° ($l = 1$, $c = 1·22$ in chloroform). No depression in m. p. was observed in any case on mixing the various specimens of chloro-dibenzoate. That prepared by method (a) was further characterised by conversion into the enol-benzoate of 6-ketocholestanyl benzoate (Lettré and Müller, *loc. cit.*; Petrow, Rosenheim, and Starling, J., 1938, 677) by heating with quinoline; it separated from ethyl acetate–methyl alcohol in needles, m. p. 180—181°, not depressed on admixture with a specimen, m. p. 179—180°, kindly supplied by Dr. O. Rosenheim.

6-Ketocholestanyl Benzoate.—(a) α-Cholesteryl benzoate oxide (2 g.) in xylene (20 c.c.) was heated under reflux for 2 hours with phosphoric oxide (1 g.). The cold solution was decanted, diluted with ether, and washed with water. Removal of the solvent from the dried (sodium sulphate) solution gave a resin which was digested with a little ethyl acetate. On standing, a crystalline mass separated which, after three recrystallisations from ethyl acetate, gave small prisms (yield, 8%), m. p. 170—171°, clearing at 179°, the melt showing a fine play of colour (Found: C, 80·3; H, 10·2. Calc. for $C_{34}H_{50}O_3$: C, 80·6; H, 9·95%). The m. p. was depressed to 140° on admixture with α-cholesteryl benzoate oxide but was unchanged on admixture with a specimen of 6-ketocholestanyl benzoate (Windaus, *Ber.*, 1903, **36**, 3752; Petrow, Rosenheim, and Starling, *loc. cit.*), which had m. p. 170—171°, clearing at 179° and $[\alpha]_D^{20°}$ + 7·9° ($l = 1$, $c = 2·0$ in chloroform).

(b) A finely ground mixture of the α-benzoate oxide (1 g.) and anhydrous alum (1·5 g.) was heated to 180°/0·1 mm. for 30 minutes. The cooled mass was extracted with ether, and the product obtained on removal of the solvent crystallised from ethyl acetate, from which the above benzoate separated in prisms, m. p. 170—171° (clearing at 179°, with colour play) undepressed on admixture with an authentic specimen; yield, 10%; $[\alpha]_D^{20°}$ + 5·9° ($l = 1$, $c = 1·2$ in chloroform).

Our thanks are due to Imperial Chemical Industries, Ltd. (Dyestuffs Group), for the award of a scholarship which enabled one of us (G. S.) to participate in this investigation.

THE UNIVERSITY, MANCHESTER. [*Received, June 29th,* 1939.]