164. *β-Cholesterol Oxide.*

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As suggested by Hattori (*J. Pharm. Soc. Japan*, 1940, **60**, 334), the β-cholesterol oxide described by numerous investigators is a mixed crystal of α-cholesterol oxide and a new β-cholesterol oxide. Fission of α-cholesterol oxide and its derivatives with hydrochloric acid gives solely chlorohydrins of type (I); similar fission of β-cholesterol oxide derivatives gives only chlorohydrins of type (II).

Hydrolysis of the β-cholesteryl benzoate oxide, m. p. 151—152° (Spring and Swain, J., 1939, 1356) gives as major product the β-cholesterol oxide, m. p. 107—108°, $[\alpha]_D^{21^\circ} - 15^\circ$, described by several investigators (Westphalen, *Ber.*, 1915, **48**, 1064; Windaus, *Z. physiol. Chem.*, 1921, **117**, 146; Ruzicka and Bosshard, *Helv. Chim. Acta*, 1937, **20**, 244). From the mother-liquors of the compound, m. p. 107—108°, we have isolated an oxide, m. p. 131—132°, $[\alpha]_D^{18\cdot5^\circ} + 11\cdot5$, which has been further characterised by the preparation of its acetate, m. p. 111°, and *benzoate*, m. p. 172—173°. A comparison of the specific rotations of the three oxides suggests that the original β-oxide is in reality a 1 : 1-mixed crystal of α-cholesterol oxide and the new oxide; a similar relationship exists for the three acetate oxides. The specific rotations, $[\alpha]_D$, recorded below were measured in chloroform solution at temperatures between 18° and 23°:

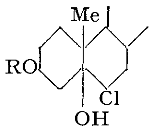
	α.	αβ (previously β).	β.
Cholesterol oxide	−43·3°	−15·0°	+11·5°
Cholesteryl acetate oxide	−43·8	−21·1	± 0
Cholesteryl benzoate oxide	−31·3	+ 3·6	+16

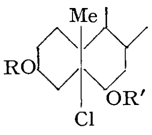
This relationship has been confirmed by the artificial preparation of a mixed crystal by the crystallisation of a mixture of equal parts of α-cholesterol oxide and the new oxide from methanol. This mixed crystal, m. p. 107—108°, $[\alpha]_D - 15\cdot4^\circ$, is indistinguishable from the original β-cholesterol oxide; the latter is designated αβ-cholesterol oxide and the new compound, β-cholesterol oxide. The optical rotations indicate that the previously described β-cholesteryl benzoate oxide is a 1 : 2-mixed crystal of the α-benzoate oxide and the new β-benzoate oxide. This relationship explains the isolation of β-cholesterol oxide in addition to αβ-cholesterol oxide when the benzoate mixed crystal is hydrolysed.

Hattori (*J. Pharm. Soc. Japan*, 1940, **60**, 334; *Chem. Abs.*, 1940, **34**, 7294) has described a β-cholesterol oxide, m. p. 136°, $[\alpha]_D - 10\cdot4^\circ$; this compound was obtained by treatment of 5-chloro-3 : 6-diacetoxycholestane with alcoholic potassium hydroxide. We have repeated the experiment and obtained β-cholesterol oxide, m. p. 131—132°, $[\alpha]_D + 10\cdot7^\circ$,* identical with the new oxide described above.

Treatment of α-cholesterol oxide and its benzoate with benzoyl chloride and pyridine, or of the α-benzoate oxide with hydrochloric acid gives 6-chloro-5-hydroxy-3-benzoyloxycholestane (I, R = COPh) (Spring and Swain, *loc cit.*). Subsequently, similar fissions of the α-oxide and its derivatives have been described, in each case leading to chlorohydrins of type (I) (Hattori, *loc. cit.*; Chakravorty and Levin, *J. Amer. Chem. Soc.*, 1942, **64**, 2317). The fission of αβ-cholesterol oxide and its derivatives, however, presents a more complicated picture. Ruzicka and Bosshard (*loc. cit.*) obtained (I, R = COMe) by treatment of the αβ-acetate oxide with hydrogen chloride. Spring and Swain (*loc. cit.*) observed the opposite type of fission when the αβ-oxide or its benzoate was treated with benzoyl chloride and pyridine, the product being (II, R = R′ = COPh), and also when the αβ-benzoate oxide was treated with hydrochloric acid, the product in this case being (II; R = COPh, R′ = H). Hattori (*loc. cit.*) observed both types of fission when αβ-cholesteryl acetate oxide was treated with hydrogen chloride, the chlorohydrin (II; R = COMe, R′ = H) being isolated in addition to (I, R = COMe). Finally, Chakravorty and Levin (*loc. cit.*) report that treatment of the αβ-oxide and of the αβ-acetate oxide with benzoyl chloride and pyridine gives (I, R = COPh) and (I, R = COMe) respectively; no chlorohydrin of type (II) was observed among their products. In view of this apparent confusion, we have examined the fission of both αβ-cholesterol oxide and its derivatives and that of the new β-cholesterol oxide and its derivatives.

(I.)





(II.)

As reported by Spring and Swain, we find that the αβ-oxide (20%) and the αβ-benzoate oxide (55%) both give the dibenzoate (II, R = R′ = COPh) when treated with benzoyl chloride and pyridine. In the former case, a second substance is formed which is probably (I, R = COPh). Again, in contrast to the observation of Chakravorty and Levin (*loc. cit.*), we find that treatment of αβ-cholesteryl acetate oxide with the same reagent gives 5-*chloro*-3-*acetoxy*-6-*benzoyloxycholestane* (II; R = COMe, R′ = COPh), m. p. 176°, as major isolable product together with a small quantity of 6-chloro-5-hydroxy-3-acetoxycholestane (I, R = COMe). The dual nature of the fission of αβ-cholesterol oxide derivatives was further confirmed in the case of the acetate, which, when treated with hydrogen chloride, gave 5-chloro-6-hydroxy-3-acetoxycholestane (II; R = COMe,

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* We have not had access to the original paper of Hattori; the sign of the rotation for the oxide quoted in the abstract may be a misprint.

R′ = H) in addition to the chlorohydrin (I, R = COMe) previously obtained by Ruzicka and Bosshard (compare Hattori, *loc. cit.*). Similar treatment of αβ-cholesterol oxide gives 5-chloro-3 : 6-dihydroxycholestane (II, R = R′ = H).

In agreement with the experiments of Hattori, we find that fission of derivatives of the β-oxide is simple, giving only chlorohydrins of type (II) in yields approximating to 90%. Thus with benzoyl chloride and pyridine the β-benzoate oxide gives the dibenzoate (II, R = R′ = COPh), similar treatment of the β-acetate oxide gives (II; R = COMe, R′ = COPh), and the β-acetate oxide with hydrogen chloride gives 5-chloro-6-hydroxy-3-acetoxycholestane (II; R = COMe, R′ = H). In all the cases examined, fission of αβ-cholesterol oxide derivatives gives chlorohydrins of type (II), the formation of which is to be attributed to the β-cholesterol oxide content of the original oxide. In some cases—where solubility relationships have been favourable—we have in addition obtained chlorohydrin derivatives of type (I), the formation of which is to be attributed to the α-cholesterol oxide content of the original oxide.

EXPERIMENTAL.

Specific rotations are measured in chloroform in a 1 dm. tube.

α- and αβ-Cholesteryl Benzoate Oxides.—Spring and Swain (*loc. cit.*) used perbenzoic acid for the preparation of these oxides. Whereas Chakravorty and Levin (*loc. cit.*) report that oxidation of cholesteryl benzoate with monoperphthalic acid gives only the α-benzoate oxide, we find that this reagent yields the same mixture of oxides as perbenzoic acid. The conditions employed were those described by the latter authors. The chloroform-soluble reaction product from cholesteryl benzoate (16·7 g.) was dissolved in boiling ethyl acetate (*ca.* 500 c.c.). The first crop of prismatic needles (9·5 g.; 56%) consisted of the α-benzoate oxide, m. p. 167—168°, which after one recrystallisation attained the constant m. p. 168—169°. Concentration of the original mother-liquor to 150 c.c. gave αβ-cholesteryl benzoate oxide, m. p. 150—151°, $[\alpha]_D^{20^\circ}$ + 3·6° (c = 1·4) (after one recrystallisation from ethyl acetate). By combining and concentrating mother-liquors, a total yield of 5·5 g. (32%) of the αβ-benzoate oxide was obtained. It was identical with the "β-cholesteryl benzoate oxide" of Spring and Swain.

β-Cholesterol Oxide.—(*a*) Hydrolysis of αβ-cholesteryl benzoate oxide (20 g.) as described by Spring and Swain (*loc. cit.*), followed by crystallisation of the product from methyl alcohol, gave a top crop of αβ-cholesterol oxide, which after four crystallisations from the same solvent formed needles, m. p. 107—108°, $[\alpha]_D^{20^\circ}$ − 15° (c = 1·1). Acetylation gave αβ-cholesteryl acetate oxide as plates from methyl alcohol, m. p. 111—112°, $[\alpha]_D^{19^\circ}$ − 21·1° (c = 1·6) (Chakravorty and Levin give $[\alpha]_D$ −12·7° for the αβ-oxide and −21·8° for the αβ-acetate oxide prepared from cholesteryl acetate).* The original methyl-alcoholic mother-liquor of the αβ-oxide deposited a crop of fine needles, which after two crystallisations from the same solvent gave β-cholesterol oxide (2 g.), m. p. 131—132°, $[\alpha]_D^{18\cdot5^\circ}$ + 11·5° (c = 1·4) (Found: C, 80·1; H, 11·3. Calc. for $C_{27}H_{46}O_2$: C, 80·6; H, 11·45%). The acetate separated from methanol in fine needles, m. p. 111°, $[\alpha]_D^{18^\circ}$ ± 0° (c = 1·5) (Found: C, 78·1; H, 10·8. Calc. for $C_{29}H_{48}O_3$: C, 78·3; H, 10·8%). The *benzoate*, prepared by means of benzoic anhydride and pyridine, separated from methanol in plates, m. p. 172—173°, $[\alpha]_D^{22^\circ}$ + 16° (c = 0·9) (Found: C, 80·7; H, 9·8. $C_{34}H_{50}O_3$ requires C, 80·6; H, 9·9%).

(*b*) 5-Chloro-3 : 6-diacetoxycholestane (m. p. 112—113°, 3 g.) was refluxed with alcoholic potassium hydroxide (10%; 60 c.c.) for 2 hours. The solution was diluted with water, and the product isolated by means of ether and crystallised from methanol, yielding β-cholesterol oxide (1·5 g.) in needles, m. p. 131—132°, $[\alpha]_D^{18\cdot5^\circ}$ + 10·7° (c = 1·4) (Found: C, 80·2; H, 11·3%). The m. p. of a mixture with the β-oxide described under (*a*) was undepressed. The acetate separated from methanol in long needles, m. p. 111° either alone or when mixed with the acetate described under (*a*); $[\alpha]_D^{21^\circ}$ ± 0° (c = 1·5) (Found: C, 78·3; H, 11·0%).

Oxide Fissions.—(A) *With benzoyl chloride and pyridine.* (a) The αβ-oxide (5 g.) in pyridine (20 c.c.) and benzoyl chloride (16 c.c.) was heated on the steam-bath for 2 hours. The product, isolated in the usual manner, was crystallised twice from ethyl acetate–methyl alcohol and twice from ethyl acetate to give 5-chloro-3 : 6-dibenzoyloxycholestane (1·0 g.) in hard prisms, m. p. 183—184°, undepressed by an authentic specimen, $[\alpha]_D^{21^\circ}$ −68·8° (c = 1·8) (Found: C, 75·8; H. 8·4. Calc. for $C_{41}H_{55}O_4Cl$: C, 76·05; H, 8·6%). The original mother-liquor gave a material which after six crystallisations from methyl alcohol formed needles, m. p. 193—194° (not constant); this was probably impure 6-chloro-5-hydroxy-3-benzoyloxycholestane (Spring and Swain. *loc. cit.*, give m. p. 202—203°); the m. p. of a mixture with the latter was not depressed.

(b) Similar treatment of αβ-cholesteryl benzoate oxide (2·0 g.) gave 5-chloro-3 : 6-dibenzoyloxycholestane (1·1 g.) in hard prisms from ethyl acetate, m. p. 183—184°, $[\alpha]_D^{20^\circ}$ − 67·9° (c = 1·55), undepressed in m. p. when mixed with an authentic specimen.

(c) Similar treatment of β-cholesteryl benzoate oxide (m. p. 172—173°, 0·2 g.) gave 5-chloro-3 : 6-dibenzoyloxycholestane (0·18 g.), m. p. 183—184° either alone or mixed with an authentic specimen; $[\alpha]_D^{21^\circ}$ − 68·2° (c = 1·2).

(d) Similar treatment of αβ-cholesteryl acetate oxide (2 g.), followed by crystallisation of the product from ethyl acetate–methanol, gave a first crop, which was twice recrystallised from the same solvent to give *5-chloro-3-acetoxy-6-benzoyloxycholestane* (0·5 g.) in needles, $[\alpha]_D^{22^\circ}$ − 75·8° (c = 0·7), m. p. 176°, depressed to 150—160° by 6-chloro-5-hydroxy-3-acetoxycholestane (Found: C, 74·0; H, 9·0. $C_{36}H_{53}O_4Cl$ requires C, 73·9; H, 9·1%). The mother-liquor of the first crop was evaporated, and the residue thrice crystallised from methyl alcohol to give felted needles of 6-chloro-5-hydroxy-3-acetoxycholestane (0·1 g), $[\alpha]_D^{18^\circ}$ − 27° (c = 1·0), m. p. 186—187°, undepressed by the specimen prepared as described below.

(e) Similar treatment of β-cholesteryl acetate oxide (0·3 g.) gave 5-chloro-3-acetoxy-6-benzoyloxycholestane (0·28 g.), $[\alpha]_D^{22^\circ}$ − 74·0° (c = 1·3), m. p. 176°, undepressed by the specimen described at (d).

(B) *With hydrogen chloride in chloroform.* (a) αβ-Cholesterol oxide (2 g.) in chloroform (100 c.c.) was treated with a stream of dry hydrogen chloride for 30 minutes. After 24 hours the separated solid was collected (1·0 g; m. p. 155—160°) and crystallised first from ethyl acetate–methanol and then from light petroleum (b. p. 60—80°), from which 5-chloro-3 : 6-dihydroxycholestane separated in needles, m. p. 171°, $[\alpha]_D^{19^\circ}$ − 22·5° (c = 1·0) (Found: C, 73·9; H, 10·7. Calc. for $C_{27}H_{47}O_2Cl$: C, 73·8; H, 10·7%). Acetylation gave the corresponding diacetate, which separated from methanol in needles, m. p. 112—113° (Found: C, 71·3; H, 9·8. Calc. for $C_{31}H_{51}O_4Cl$: C, 71·15; H, 9·8%).

(b) αβ-Cholesteryl acetate oxide (2 g.) was similarly treated in pure dry chloroform (15 c.c.) solution with dry hydrogen chloride for 30 minutes. After standing overnight, the solvent was removed, and the residue fractionally crystallised from ethyl acetate–methyl alcohol. The first crop formed felted needles which after two recrystallisations from the same solvent gave 6-chloro-5-hydroxy-3-acetoxycholestane in felted needles (0·7 g.), m. p. 186—187°, $[\alpha]_D^{18^\circ}$ − 26·7° (c = 1·1)

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* The solvent in which these measurements were made is not recorded.

(Found : C, 72·4; H, 10·15. Calc. for $C_{29}H_{49}O_3Cl$: C, 72·4; H, 10·2%) [Ruzicka and Bosshard, *loc. cit.*, give m. p. 191° (corr.) for this compound but do not record a specific rotation]. The second crop formed plates, which after two crystallisations from ethyl acetate–methyl alcohol gave 5-chloro-6-hydroxy-3-acetoxycholestane (0·15 g.), m. p. 190—191° (Found : C, 72·0; H, 10·4. Calc. for $C_{29}H_{49}O_3Cl$: C, 72·4; H. 10·4%). The m. p. was not altered by repeated crystallisations from this solvent; Hattori (*loc. cit.*) gives m. p. 198—200° for this substance. When benzoylated, it gave 5-chloro-3-acetoxy-6-benzoyloxycholestane as needles from ethyl acetate–methanol, $[\alpha]_{D}^{18\cdot5^\circ} - 73\cdot0^\circ$ ($c = 0\cdot5$), m. p. 176°, undepressed by the specimen described at A(d).

(c) Fission of β-cholesteryl acetate oxide (1·0 g.) as described at (a) gave 5-chloro-6-hydroxy-3-acetoxycholestane (0·8 g.) as plates from ethyl acetate–methyl alcohol, $[\alpha]_{D}^{22^\circ} - 24\cdot3^\circ$ ($c = 1\cdot3$), m. p. 190—191°, undepressed by the specimen prepared by method (a).

(C) *With alcoholic hydrochloric acid.* (a) αβ-Cholesterol oxide (2 g.) in absolute methylated spirit (60 c.c.) containing hydrochloric acid (*d* 1·16; 4 c.c.) was refluxed for 30 minutes. Water was added until the solution was turbid and the solid separating on cooling was crystallised from ethyl acetate–methanol. Recrystallisation from light petroleum (b. p. 60—80°) gave the 5-chloro-3 : 6-diol (0·5 g.) in needles, m. p. 170—171°, $[\alpha]_{D}^{19^\circ} - 22\cdot1^\circ$ ($c = 1\cdot2$). The diacetate formed needles, m. p. 112—113° either alone or when mixed with the diacetate prepared by method B(a).

(b) Similar treatment of αβ-cholesteryl acetate oxide (2 g.) gave the 5-chloro-3 : 6-diol (0·4 g.) in needles, m. p. 170—171°, $[\alpha]_{D}^{20^\circ} - 21\cdot5^\circ$ ($c = 1\cdot4$) (Found : C, 73·9; H, 10·4. Calc. for $C_{27}H_{47}O_2Cl$: C, 73·8; H, 10·7%). The dibenzoate formed hard prisms from ethyl acetate, m. p. 184°, undepressed by an authentic specimen. The three specimens of the 5-chloro-3 : 6-diol prepared by methods B(a), C(a) and C(b) showed no depression in m. p. when mixed with each other or with a specimen prepared by the method of Windaus (*loc. cit.*).

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