517. *The Application of the Method of Molecular-rotation Differences to Steroids. Part XII. Cholest-6-en-3β-ol.*

By D. H. R. Barton and W. J. Rosenfelder.

Pyrolysis of 3β-acetoxycholestan-7α-yl benzoate gives cholest-6-en-3β-yl acetate, *cholesta-2 : 6-diene*, and *cholest-2-en-7α-yl benzoate*. The first two of these products are also obtained by the pyrolysis of *3β-acetoxycholestan-6β-yl benzoate*. Pyrolysis of *3β-acetoxycholest-7β-yl benzoate* affords mixtures of cholesta-2 : 6- and -2 : 7-diene and of cholest-6- and -7-en-3β-yl acetate. These results disprove the claims of Plattner, Heusser, Troxler, and Segre (*Helv. Chim. Acta*, 1948, **31**, 852) to have prepared a pure cholest-7-en-3-ol by the first of the above-mentioned pyrolyses, and confirm the criticisms of their work which we have previously advanced on molecular-rotation grounds.

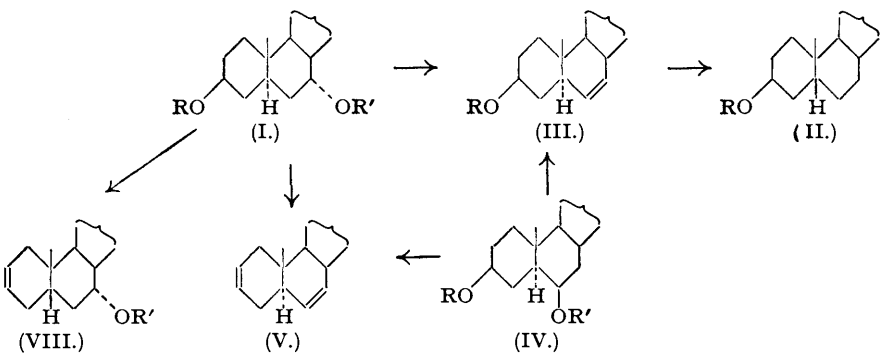
Plattner, Heusser, Troxler, and Segre (*Helv. Chim. Acta*, 1948, **31**, 852) recently described the preparation of an interesting new cholesten-3β-ol, the acetate of which was said to be formed by the pyrolysis of 3β-acetoxycholestan-7α-yl benzoate (I; R = Ac; R′ = Bz).* On the basis of chemical evidence, the most important aspects of which were oxidation by chromic acid to 7-ketocholestan-3β-yl acetate and rearrangement on attempted hydrogenation in acetic acid solution to a further new cholesten-3β-yl acetate, Plattner *et al.* considered that their compound was pure " cholest-7-en-3-ol." Now we had already (Barton, *J.*, 1945, 813; **1946**, 512; Barton and Cox, *J.*, 1948, 1354; this vol., p. 214) given what we considered to be a proof of the correctness of the formulation of " γ "-type stenols and stadienols as 7 : 8-unsaturated, and therefore the views of Plattner *et al.* could not be correct. Furthermore, although the chemical evidence given by Plattner *et al.* was adequate for, and consistent with, the formulation claimed, the molecular-rotation data recorded (see table) were anomalous. We shall not repeat the detailed discussion of this aspect already given elsewhere (Barton, *Angew. Chem.*, 1949, **61**, 57; Barton and Rosenfelder, *Helv. Chim. Acta*, 1949, **32**, 975), in which we fully, and critically, examined the relevant chemical evidence.

The rearranged stenyl acetate of Plattner *et al.* is comparable in its method of preparation to

* The justification for the steric configuration at the 7-position in this compound is discussed in an earlier paper (Barton, this vol., p. 2174).

the well known " α "-cholestenyl acetate [cholest-8(14)-en-3β-yl acetate]. We first examined the latter for heterogeneity (see Experimental), but, in agreement with previous views, could obtain no evidence therefor. Next we repeated the pyrolysis of 3β-acetoxycholestan-7α-yl benzoate and, by chromatography, isolated three substances. The main product was a cholestenyl acetate, with the same m. p. as that recorded by Plattner *et al.*, but with a different specific rotation ($[\alpha]_D$ −89° instead of −64°). Hydrolysis of this acetate furnished a cholestenol, again with the m. p. given by Plattner *et al.*, but with a very different rotation ($[\alpha]_D$ −93° instead of −16°). Benzoylation furnished the benzoate, m. p. 123—124°, $[\alpha]_D$ −75°, whilst oxidation gave the corresponding cholestenone, again with the m. p. recorded by Plattner *et al.*, but with a very different rotation ($[\alpha]_D$ about −78° instead of −34°). In marked contrast to the behaviour of Plattner's stenyl acetate, the cholestenyl acetate described here gave cholestanyl acetate (II; R = Ac), in almost quantitative yield, on catalytic hydrogenation *in both neutral and acetic acid solution*. This is what would be expected for cholest-6-en-3β-yl acetate (III; R = Ac) (cf. Henbest and Jones, *Nature*, 1946, **158**, 950; *J.*, 1948, 1792; Barton, Cox, and Holness, this vol., p. 1771), and having regard to the resistance to hydrogenation under these conditions shown by substances that are truly 7 : 8-unsaturated, we regard it as a proof of structure. This structure, was, however, confirmed by the preparation of the stenyl acetate, $[\alpha]_D$ −89°, with equal ease, by the pyrolysis of 3β-*acetoxycholestan*-6β-*yl benzoate* (IV; R = Ac; R′ = Bz). For larger-scale preparations this is the more convenient route.

The second substance obtained from the pyrolysis of 3β-acetoxycholestan-7α-yl benzoate was a diethylenic hydrocarbon, $C_{27}H_{44}$, shown to be *cholesta*-2 : 6-*diene* (V) on the basis of the following evidence. The hydrocarbon showed no absorption in the ultra-violet region and therefore the ethylenic linkages are not in conjugation. It was hydrogenated in neutral solution to give, almost quantitatively, cholestane. It cannot, therefore, contain a 4(5)-ethylenic linkage. Since it was also obtained by the pyrolysis of 3β-acetoxycholestan-6β-yl benzoate it must possess one ethylenic linkage at the 6(7)-position. It must, then, be either cholesta-3 : 6-diene (VI) or cholesta-2 : 6-diene. It was unchanged by anhydrous hydrogen chloride or caustic alkali under drastic conditions, which facts favour the second formulation. Finally, pyrolysis of cholestanyl benzoate (II; R = Bz) gave, not cholest-3-ene, but cholest-2-ene (VII) (*neo*cholestene).



The third substance obtained in the pyrolysis of 3β-acetoxycholestan-7α-yl benzoate appeared, from analysis, to be probably a cholestenyl benzoate and showed the appropriate absorption spectrum in the ultra-violet region. We regard it as somewhat impure *cholest*-2-*en*-7α-*yl benzoate* (VIII; R′ = Bz), for, on hydrogenation, alkaline hydrolysis, and chromic acid oxidation, it furnished cholestan-7-one.

All the fractions obtained in the pyrolyses of 3β-acetoxycholestan-7α-yl and -6β-yl benzoates gave *negative* Tortelli–Jaffé reactions, thus showing the absence of contamination by substances unsaturated at the 7(8)-position. On the other hand, pyrolysis of 3β-acetoxycholestan-7β-yl

benzoate afforded a mixture of about equal parts of cholest-6-en- and -7-en-3β-yl acetate (the latter isolated as the benzoate). At the same time a mixture of cholesta-2 : 6- and -2 : 7-diene was formed. All the fractions obtained in this pyrolysis gave *positive* Tortelli–Jaffé tests.

In an earlier article (Barton, this vol., p. 2174) we have shown how the pyrolyses so far described in the present paper are examples of *cis*-elimination. Not all pyrolytic eliminations are stereochemically specific in this way, and the pyrolytic elimination of strongly electron-attracting (or -repelling) substituents may constitute exceptions to our generalisation. Thus the pyrolysis of the potassium salt of the hydrogen sulphate of 3β-acetoxycholestan-6β-ol furnished, as main product, cholesteryl acetate with not more than 10% of cholest-6-en-3β-yl acetate. This *trans*-elimination implies that the reaction proceeds by an ionic mechanism. This, indeed, is proved by the discovery, by Natelson and Gottfried (*J. Amer. Chem. Soc.*, 1939, **61**, 971), that pyrolysis of potassium bornyl sulphate gives, not bornylene, but camphene. In contrast, the thermal decomposition of the methyl xanthate (*cis*-elimination) affords bornylene and some tricyclene, but *no* camphene (Tschugaeff, *Annalen*, 1912, **388**, 288; cf. Hückel, Tappe, and Legutke, *ibid.*, 1940, **543**, 191). As would be expected, we found that treatment of 3β-acetoxycholestan-6β-ol with phosphorus pentachloride afforded cholesteryl acetate (*trans*-elimination); thionyl chloride caused a double elimination and gave cholesterilene.

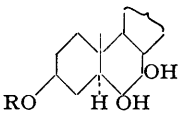
It is of interest to compare the molecular rotations established in this paper for cholest-6-en-3β-ol with those for stenols with comparable positions of the ethylenic linkage (see the table). The extent of " vicinal action " (Barton and Cox, *Nature*, 1947, **159**, 470; *J.*, 1948, 783) between the isolated 6(7)-ethylenic linkage and the 3-position is small for the three transformations summarised.

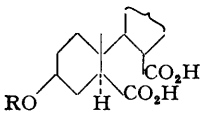
After the work described above had been brought to a successful conclusion we learnt that Dr. O. Wintersteiner of the Squibb Institute, New Brunswick, had been engaged on a similar project. Through the courtesy of Dr. Wintersteiner, whose friendly collaboration we greatly appreciate, it was agreed that a short summary of the results of the two investigations should be published simultaneously (Barton and Rosenfelder, *Nature*, 1949, **164**, 316; Wintersteiner and Moore, *ibid.*, p. 317; *J. Amer. Chem. Soc.*, in the press). On attempted repetition of the work of Plattner *et al.* (*loc. cit.*), Wintersteiner and Moore obtained cholest-6-en-3β-yl acetate with properties in excellent agreement with those recorded above and similarly observed its ready

	$[M_D]$ ($CHCl_3$).						
Substance.	Alcohol.	Acetate.	Benzoate.	Ketone.	Δ_1.	Δ_2.	Δ_3.
Cholestan-3β-ol * (standard) ...	+ 89°	+ 60°	+ 94°	+162°	− 29°	+ 5°	+ 73°
Cholest-5-en-3β-ol *	−154	−188	− 74	− 30	− 34	+80	+124
Cholest-6-en-3β-ol	−359	−381	−368	−300 †	− 22	− 9	+ 59
Ergost-7-en-3β-ol *	− 8	− 18	+ 10	+ 88	− 10	+18	+ 96
Plattner's stenol	− 62	−274	—	−131	−212(!)	—	− 69(!)

* Values from Part IV, *J.*, 1948, 783. The abbreviations used in the table are there explained.
† Approximate value.

hydrogenation to cholestanyl acetate. The position of the ethylenic linkage was elegantly proved by conversion of the unsaturated compound into the corresponding triol (IX; R = H) by treatment with osmium tetroxide. This triol gave a tri-*p*-nitrobenzoate (proof of *three* secondary hydroxyl groups). Contrary to Plattner *et al.* (*loc. cit.*) chromic acid oxidation gave the acetate (X; R = Ac) of the Windaus–Stein acid (*Ber.*, 1904, **37**, 3699; Shoppee, *J.*, 1948, 1032).

(IX.) 



(X.)

Wintersteiner and Moore also isolated cholesta-2 : 6-diene and cholest-2-en-7α-yl benzoate as by-products in the preparation of cholest-6-en-3β-yl acetate.

The conclusions to be drawn from the two investigations are in complete accord.

Experimental.

(M. p.s are uncorrected.)

The substances whose rotations are listed below were dried *in vacuo* before weighing, at 20° below their m. p.s, or at 120°, whichever was the lower temperature. All rotations are for the Na_D line and in chloroform solution. They were taken in a 1-dm. micro-tube, of capacity about 1 ml.

Standard chemical operations were performed as in Part IV (*J.*, 1948, 783) unless specified to the contrary. Unless stated to the contrary all chromatograms were carried out using Savory and Moore's standardised alumina. The light petroleum had b. p. 40—60°.

Micro-analyses are by Drs. Weiler and Strauss, Oxford.

Reactions at the 6-Position.

Hydrogenation of 3β-Acetoxycholestan-6-one.—(a) *In ethanol.* 800 Mg. of the acetoxy-ketone (for preparation see Part IV) were dissolved in 100 ml. of anhydrous ethanol and hydrogenated in presence of a platinum catalyst in the usual way until the theoretical volume of hydrogen had been taken up (6 hours). The crude reaction product had m. p. 184—185° and gave, after one recrystallisation from ethanol, cholestan-3β : 6β-diol, m. p. 188—189° (Plattner and Lang, *Helv. Chim. Acta*, 1944, **27**, 1872, give m. p. 189—190°).

(b) *In dioxan.* 300 Mg. of the acetoxy-ketone were dissolved in 30 ml. of dry dioxan and hydrogenated in presence of 150 mg. of pre-reduced platinum oxide catalyst. After 15 minutes 40 c.c. of hydrogen had been taken up (theory, 18 c.c.) and the reaction then ceased. Crystallisation of the product from methanol gave, almost quantitatively, cholestan-3β-yl acetate, m. p. and mixed m. p. with an authentic specimen, 109—110°.

(c) *In ethyl acetate.* 1·1 G. of the acetoxy-ketone were dissolved in 100 ml. of ethyl acetate, and 135 mg. of platinum oxide catalyst added. After hydrogenation in the usual way 91 c.c. of hydrogen had been absorbed (theory, 91 c.c.) after 2 hours. Uptake of hydrogen then ceased. The reaction product, after crystallisation from methanol, had m. p. 155—156°, unchanged on further crystallisation. To confirm its purity it was chromatographed over Birlec alumina.

Fraction no.	Eluent.	M. p. of fraction after crystn. from MeOH.
1	100 ml. of 50% C_6H_6–light petroleum	trace of oil
2	100 ml. ,, ,, ,,	—
3	100 ml. of 70% C_6H_6–light petroleum	trace, amorphous
4	100 ml. ,, ,, ,,	amorphous (small fraction)
5	50 ml. ,, ,, ,,	amorphous (small fraction)
6	80 ml. ,, ,, ,,	m. p. 154—155°
7	80 ml. ,, ,, ,,	m. p. 155—156 (large fraction)
8	80 ml. ,, ,, ,,	m. p. 155—156 (large fraction)
9	100 ml. ,, ,, ,,	m. p. 155—156
10	100 ml. ,, ,, ,,	m. p. 155—156
11	200 ml. of methanol	m. p. 155—156

Fractions 7 and 8 were combined and recrystallised repeatedly from methanol without any change in m. p., $[\alpha]_D$ −6° (*c*, 5·24). Fraction 11 also had $[\alpha]_D$ −6° (*c*, 5·32). For 3β-acetoxycholestan-6(β)-ol Plattner and Lang (*loc. cit.*) gave m. p. 141—142°, $[\alpha]_D$ −6°. The careful fractionation to which we have subjected our specimen of the 3β-acetate leaves little doubt as to purity. On alkaline hydrolysis our specimen gave, after recrystallisation from ethanol, cholestan-3β : 6β-diol, m. p. 188—189°, whilst on acetylation it afforded cholestan-3β : 6β-diol diacetate, m. p. 136—137°, after recrystallisation from ethyl acetate–methanol. For the diacetate Plattner and Lang (*loc. cit.*) record m. p. 136—137°.

Reactions of 3β-Acetoxycholestan-6β-ol.—(a) *With phosphorus pentachloride.* 90 Mg. of the acetoxy-alcohol in 3 ml. of dry benzene were rubbed with 225 mg. of phosphorus pentachloride in a mortar for 15 minutes. After being worked up in the usual way the product (negative Beilstein test) gave, on recrystallisation from ethyl acetate–methanol, slightly impure cholesteryl acetate m. p. and mixed m. p. 109—110°. It gave a strongly positive Liebermann–Burchard reaction of the same type as that shown by cholesterol.

(b) *With thionyl chloride.* 80 Mg. of the acetoxy-alcohol were treated with 3 ml. of thionyl chloride and set aside for 24 hours at room temperature. After removal of the thionyl chloride *in vacuo*, the residue could not be crystallised. It was dissolved in light petroleum and chromatographed on alumina. The main (hydrocarbon) fraction was very easily eluted and gave, after crystallisation from methanol, long needles (negative Beilstein test) of cholesterilene, m. p. 78—79°.

(c) *With benzoyl chloride.* 2·1 G. of the acetoxy-alcohol were benzoylated in the usual way. Crystallisation from methanol gave *3β-acetoxycholestan-6β-yl benzoate*, m. p. 118—119°, unchanged on repeated recrystallisation, $[\alpha]_D$ −39° (*c*, 12·19) (Found: C, 78·2; H, 9·7. $C_{36}H_{54}O_4$ requires C, 78·6; H, 9·9%).

(d) *With chlorosulphonic acid and pyridine.* 1·12 G. of the dry acetoxy-alcohol were dissolved in 5 ml. of dry ether, and 5 ml. of dry pyridine added. The solution was cooled to −60° and 1 ml. of redistilled chlorosulphonic acid, diluted with 5 ml. of dry ether, was slowly added at the same temperature. After being allowed to warm to room temperature the mixture was heated under reflux for 2 hours on the water-bath. The ether was removed *in vacuo*, and a little water was added and then excess of saturated potassium acetate solution. The white potassium salt thus precipitated was filtered off and washed with a little water. After being dried *in vacuo* it was used, without further purification, for pyrolysis experiments.

Pyrolysis of 3β-Acetoxycholestan-6β-yl benzoate.—(a) *By distillation.* 130 Mg. of the acetoxy-benzoate were distilled at 14 mm. at 220—340° (air-bath temperature) as described in detail below for the pyrolysis of 3β-acetoxycholestan-7α-yl benzoate. The distillate, which readily crystallised, was dissolved in methanol and titrated with standard potassium hydroxide (phenolphthalein) (Found: 61% of the theoretical amount of benzoic acid). The methanol solution was diluted with water and extracted with benzene. The benzene was removed, and the crystalline product dissolved in light petroleum and chromatographed. The first two fractions, eluted with light petroleum, were oily (hydrocarbon); the next three fractions, eluted with 50—60% C_6H_6–light petroleum, had, after crystallisation from

methanol, (i) m. p. 99—100°, (ii) m. p. 102—103°, and (iii) m. p. 103—104°. All these fractions gave a final bluish-violet shade in the Liebermann–Burchard reaction. Fractions (ii) and (iii) gave no depression in m. p. on admixture with cholest-6-en-3β-yl acetate (see below) and had $[\alpha]_D$ −87° (*c*, 0·79). On alkaline hydrolysis they afforded cholest-6-en-3β-ol, m. p. 114—115°, not depressed on admixture with cholest-6-en-3β-ol prepared from 3β-acetoxycholestan-7α-yl benzoate (see below).

(b) *By distillation through a heated tube.* 500 Mg. of the acetoxy-benzoate were slowly (during 1 hour) distilled in a stream of dry, oxygen-free nitrogen (about 2 l. per hour at normal pressure) through a glass tube held at 400° for 5″ of its length by heating with a short electric furnace. A pressure of 0·6 mm. was maintained in the apparatus during this distillation. The distillate was dissolved in methanol and titrated (see above) (Found: somewhat more than the theoretical amount of benzoic acid). Further working up as above and chromatography gave six fractions (out of seventeen) which contained appreciable amounts of steroid. The first two, eluted with light petroleum, were recrystallised from methanol, to give 55 mg. of cholesta-2 : 6-diene (see below), m. p. and mixed m. p. with the hydrocarbon obtained from 3β-acetoxycholestan-7α-yl benzoate (see below), 70—71°. The last four fractions, eluted with 50—60% benzene–light petroleum, had m. p. 96—97°, 103·5—104·5°, 103·5—104·5°, and 103·5—104·5°, respectively. On recrystallisation the m. p. of the first of these rose to 103·5—104·5°, the others were unchanged. The total yield of pure cholest-6-en-3β-yl acetate, m. p. 103·5—104·5°, was 120 mg.

Pyrolysis of the Potassium Sulphate of 3β-Acetoxycholestan-6β-ol.—The potassium salt prepared as above was pyrolysed in a retort at 270—300°/0·4 mm. during ½ hour. The oily distillate, which readily crystallised, was dissolved in light petroleum and chromatographed to give 16 fractions. The first three fractions (traces), m. p. ~98°, were rejected. Fractions 4—13 were recrystallised from methanol, to give 210 mg. of m. p. 109—110° (mixed m. p. with cholesteryl acetate, 108—109°), $[\alpha]_D$ −44° (*c*, 3·99). Fractions 15 and 16 had m. p. 96—97° and 95—96°, respectively; they were combined and recrystallised from methanol and then had m. p. 98—99°, $[\alpha]_D$ −75° (*c*, 0·96). From the mother-liquor of this crystallisation was obtained a material, m. p. 95—96°, $[\alpha]_D$ −54° (*c*, 1·23). Whilst the cholesteryl acetate isolated from fractions 4—13 gave a final olive-green colour in the Liebermann–Burchard reaction, fractions 15—16 gave a final bluish-violet shade. The product, m. p. 98—99°, from these fractions gave no depression of m. p. on admixture with cholest-6-en-3β-yl acetate (see below). Fractions 15 and 16 were combined and rechromatographed to give five fractions, all eluted with 8% C_6H_6–light petroleum: (i) m. p. 104—105° (final olive-green in the Liebermann–Burchard reaction), (ii) m. p. 103—104°, (iii) trace, m. p. 95—96°, (iv) m. p. 97—98°, and (v) a large fraction, m. p. 99—100° (all m. p.s after recrystallisation from methanol). Fraction (v) gave no depression of the m. p. on admixture with cholest-6-en-3β-yl acetate (see below) and had $[\alpha]_D$ −90° (*c*, 0·13). On recrystallisation from methanol it gave needles, m. p. 102—103°, not depressed on admixture with cholest-6-en-3β-yl acetate (see below) and giving the characteristic bluish-violet shade of the latter in the Liebermann–Burchard reaction; admixture with cholesteryl acetate depressed the m. p. to 95—96°.

Reactions at the 7-Position.

Preparation of 3β-Acetoxycholestan-7α-yl Benzoate.—7-Ketocholesteryl acetate was converted into 7-ketocholestanyl acetate as described in Part IV. The latter compound was hydrogenated in ethyl acetate–acetic acid solution, and the mixture of epimeric 7-alcohols separated by chromatography (Wintersteiner and Moore, *J. Amer. Chem. Soc.*, 1943, **65**, 1503). In our experience chromatography is essential in order to obtain a satisfactory yield of pure 3β-acetoxycholestan-7α-ol. This was benzoylated in the usual way. The reaction mixture was worked up by prolonged digestion with hot water, followed by extraction with ether. The crude 3β-acetoxycholestan-7α-yl benzoate was obtained as a pale yellow gum (cf. Plattner, Heusser, Troxler, and Segre, *loc. cit.*; Fieser, Fieser, and Chakravarti, *J. Amer. Chem. Soc.*, 1949, **71**, 2226).

Pyrolysis of 3β-Acetoxycholestan-7α-yl Benzoate.—The crude benzoate from 1·0 g. of pure 3β-acetoxycholestan-7α-ol was pyrolysed by distillation from a small flask at 250—350°/10—14 mm. (air-bath temperature). The distillation required 1 hour. The partly crystalline distillate was dissolved in methanol and titrated with standard potassium hydroxide (phenolphthalein) (Found: 115% of the theoretical amount of benzoic acid). A duplicate experiment with 1·5 g. of 3β-acetoxycholestan-7α-ol required 1½ hours for the distillation and gave 117% of the theoretical amount of benzoic acid. After removal of methanol *in vacuo* and extraction with benzene, etc., the reaction product was dissolved in light petroleum and chromatographed over alumina. A typical chromatogram, each fraction after one recrystallisation from methanol, is given on p. 2464.

Fractions 1 and 2 were examined further as detailed below (p. 2464). Fractions 8 and 9, which gave positive Liebermann–Burchard reactions, a very faint yellow colour with tetranitromethane and negative Tortelli–Jaffé reactions, were combined and recrystallised from methanol m. p. 131—132°, $[\alpha]_D$ −28° (*c*, 0·68). Further chromatography gave three fractions, (*a*) m. p. 131—132°, (*b*) m. p. 136—137°, $[\alpha]_D$ −31° (*c*, 0·72), and (*c*) m. p. 103—104°. Fraction (*b*) showed $\lambda_{max.}$ 228 mμ., $E^{1\%}_{1\,cm.}$ = 340 (in alcohol). It was very probably somewhat impure cholest-2-en-7α-yl benzoate (Found: C, 82·4; H, 10·3. Calc. for $C_{34}H_{50}O_2$: C, 83·25; H, 10·3%). On hydrogenation in ethyl acetate in presence of a platinum catalyst it afforded a saturated compound, m. p. 163—163·5°, hydrolysed by heating under reflux with potassium hydroxide in di-2-hydroxyethyl ether to cholestan-7α-ol. Oxidation of the last-mentioned compound with chromic acid furnished cholestan-7-one, purified by chromatography and recrystallised from methanol (plates), m. p. and mixed m. p. with an authentic specimen m. p. 114—115°. We are much indebted to Professor Sir Ian Heilbron, D.S.O., F.R.S., for the authentic specimen (see Heilbron, Shaw, and Spring, *Rec. Trav. chim.*, 1938, **57**, 529). The yield of cholestan-7-one was about 5 mg. from 15 mg. of the benzoate.

Fractions 10—17 inclusive were combined and recrystallised from ethyl acetate–methanol, to give pure cholest-6-en-3β-yl acetate, m. p. 103·5—104·5° (200 mg. from 1·0 g. of 3β-acetoxycholestan-7α-ol). Cholest-6-en-3β-yl acetate gave an immediate yellow colour with tetranitromethane. The Liebermann–

Fraction no.	Eluent.	M. p.
1	50 ml. of light petroleum	(not cryst.)
2	40 ml. ,, ,,	69—70°
3	30 ml. ,, ,,	
4	30 ml. ,, ,,	
5	70 ml. ,, ,,	(traces)
6	100 ml. ,, ,,	
7	100 ml.	(nil)
8	150 ml. of 2% benzene–light petroleum	(little) 131—132
9	80 ml. of 10% benzene–light petroleum	(little) 131—132
10	40 ml. ,, ,, ,,	101—102
11	60 ml. ,, ,, ,,	99—100
12	80 ml. ,, ,, ,,	100—101
13	100 ml. ,, ,, ,,	101—102
14	100 ml. ,, ,, ,,	100—101
15	100 ml. ,, ,, ,,	100—101
16	150 ml. ,, ,, ,,	99—100
17	150 ml. ,, ,, ,,	99—100
18	70 ml. of 30% benzene–light petroleum	(little) 99—100
19	250 ml. of benzene	(nil)

Fractions 10—18 gave negative Tortelli-Jaffé tests.

Burchard reaction was reddish-violet to violet-blue. The product had $[\alpha]_D$ −89° (*c*, 2·20), −89° (*c*, 2·13) $[M]_D$ −381°. Neither the m. p. nor the rotation was changed on recrystallisation. Alkaline hydrolysis afforded, after recrystallisation from methanol, cholest-6-en-3β-ol, m. p. 114—115°, $[\alpha]_D$ −92° (*c*, 1·37), −93° (*c*, 1·88), $[M]_D$ −359°, which afforded a *benzoate* (recrystallised from chloroform–methanol), m. p. 123—124°, $[\alpha]_D$ −74° (*c*, 1·10), −76° (*c*, 1·005), −75° (*c*, 0·73), $[M]_D$ −368° (Found: C, 83·05; H, 10·1. $C_{34}H_{50}O_2$ requires C, 83·25; H, 10·3%). The benzoate showed the same play of colours in the Liebermann–Burchard reaction as did the acetate. Oxidation of cholest-6-en-3β-ol by chromic acid and by the Oppenauer method afforded, in poor yield, cholest-6-en-3-one, purified by chromatography and recrystallisation from methanol, m. p. 116—117°, $[\alpha]_D$ −80° (*c*, 0·330), −76° (*c*, 0·221), $[M]_D$ approx. −300°.

Hydrogenation of Cholest-6-en-3β-yl Acetate.—(a) *In ethyl acetate.* 35 Mg. of cholest-6-en-3β-yl acetate were dissolved in 20 ml. of ethyl acetate and hydrogenated in the usual way for 12 hours in presence of a platinum catalyst. After removal of the catalyst by filtration and of the solvent *in vacuo*, the product was saturated both to tetranitromethane and to the Liebermann–Burchard reagent. The m. p., after one recrystallisation from methanol, was 107—108°, undepressed on admixture with authentic cholestan-3β-yl acetate of the same m. p., $[\alpha]_D$ +14° (*c*, 0·47). On alkaline hydrolysis the acetate furnished cholestan-3β-ol, m. p., after one recrystallisation from methanol, 137—138°, giving no depression on admixture with authentic cholestan-3β-ol.

(b) *In acetic acid.* 10 Mg. of cholest-6-en-3β-yl acetate were dissolved in 10 ml. of acetic acid (AnalaR) and hydrogenated in the usual way for 2 hours in presence of a platinum catalyst. After being worked up in the usual manner the product was saturated to tetranitromethane and to the Liebermann–Burchard reagent. It had m. p. 107—108° and gave no depression with pure cholestan-3β-yl acetate. Hydrolysis gave cholestan-3β-ol, not depressed in admixture with pure cholestan-3β-ol.

Cholesta-2 : 6-diene.—The hydrocarbon material eluted by light petroleum in fractions 1 and 2 of the chromatogram of the pyrolysis product of 3β-acetoxycholestan-7α-yl benzoate recorded above (p. 2463), was rechromatographed. It gave three fractions, m. p. 70—71° (after recrystallisation from methanol). All fractions gave immediate yellow colours with tetranitromethane and the same Liebermann–Burchard colour as cholest-6-en-3β-yl acetate, but negative Tortelli–Jaffé reactions. Further recrystallisation from methanol (very long needles) raised the m. p. of *cholesta-2 : 6-diene* to 71—71·5° and the product had $[\alpha]_D$ ±0° (*c*, 2·95) (Found: C, 87·8; H, 12·1. $C_{27}H_{44}$ requires C, 88·0; H, 12·0%).

The hydrocarbon was recovered unchanged after being heated under reflux in a methanol–dioxan solution of potassium hydroxide and after treatment in chloroform solution with dry hydrogen chloride. 50 Mg. of the hydrocarbon, 10 ml. of di-2-hydroxyethyl ether (b. p. 245°) and 300 mg. of potassium hydroxide were heated under reflux in an oil-bath for 1 hour. After being worked up in the usual way and chromatographed on alumina, unchanged cholesta-2 : 6-diene was recovered, m. p. and mixed m. p. 71—72°, $[\alpha]_D$ ±0° (*c*, 0·77—0·86).

The hydrocarbon fractions obtained in the pyrolysis of 3β-acetoxycholestan-6β-yl benzoate at 14 mm. (see above) were combined and rechromatographed. Elution with light petroleum and recrystallisation from methanol afforded cholesta-2 : 6-diene, m. p. and mixed m. p. with hydrocarbon obtained as above, 70·5—71·5°, $[\alpha]_D$ ±0° (*c*, 1·55).

Hydrogenation of Cholesta-2 : 6-diene.—30 Mg. of the hydrocarbon, dissolved in 10 ml. of ethyl acetate, were hydrogenated overnight with a platinum oxide catalyst. After removal of the catalyst by filtration and of the solvent *in vacuo*, the reaction product was saturated to tetranitromethane and to the Liebermann–Burchard reagent. Recrystallisation from ethyl acetate–methanol gave 25 mg. of cholestane, m. p. and mixed m. p. with authentic specimen (Part IV), 79—80°, $[\alpha]_D$ +25° (*c*, 0·91).

Pyrolysis of 3β-Acetoxycholestan-7β-yl Benzoate.—The gummy fractions eluted by 50—80% benzene–light petroleum during the chromatography of the hydrogenation product of 7-ketocholestanyl acetate (see above) were rechromatographed (17 fractions) and the six fractions eluted by 40—60% benzene–light petroleum, which still did not crystallise, were benzoylated in the usual way. After recrystallisation from methanol there was obtained a good yield of *3β-acetoxycholestan-7β-yl benzoate*, m. p. 156—157°, $[\alpha]_D$ +64° (*c*, 3·29), $[M]_D$ +352° (Found: C, 78·6; H, 9·7. $C_{36}H_{54}O_4$ requires C, 78·6; H, 9·9%). By use of the standard Δ values of Part IV (*J.*, 1948, 783) and the molecular rotations for

cholestan-3β : 7α- and -3β : 7β-diol and their dibenzoates given by Wintersteiner and Moore (*loc. cit.*), the calculated molecular rotation is +382°. The calculated molecular rotation for the corresponding -7(α)-yl benzoate is similarly +87°.

650 Mg. of acetoxy-benzoate, prepared as described above, were pyrolysed by distillation during about 1 hour at 1·5 mm. in a current of oxygen-free, dry nitrogen through a furnace maintained at 400° (see above). The benzoic acid eliminated was 91% of the theoretical. The product was chromatographed with the following results, each fraction being recrystallised once from methanol.

Fraction no.	Eluent.	M. p.	
1	30 ml. of light petroleum	64—65°	Positive Tortelli–Jaffé reaction. M. p. 65—66°; on admixture with cholesta-2 : 6-diene, m. p. 70—71°.
2	30 ml. ,, ,,	65—66	
3	60 ml. ,, ,,	64—65	
4	60 ml. ,, ,,	64—65	
5—12	30 ml. each of 1% increasing to 50% benzene–light petroleum	nil	
13	30 ml. of 60% benzene–light petroleum	trace, oily	
14	30 ml. ,, ,, ,,	trace, oily	
15	30 ml. ,, ,, ,,	100—101°	Positive Tortelli–Jaffé reaction.
16	30 ml. ,, ,, ,,	100—101	
17	30 ml. ,, ,, ,,	101—102	
18	50 ml. ,, ,, ,,	101—102	
19	50 ml. ,, ,, ,,	104—105	
20	50 ml. of 70% benzene–light petroleum	107—108	
21	50 ml. ,, ,, ,,	111—112	
22	50 ml. of 80% benzene–light petroleum	trace	

Fractions 1—4 were combined and recrystallised repeatedly from methanol without significant change in m. p.; $[\alpha]_D$ +11° (*c*, 0·68). They were clearly inseparable mixtures of cholesta-2 : 6- and -2 : 7-diene. Fractions 15—21 inclusive were shown to be mixtures of cholest-6-en-3β-yl acetate and cholest-7-en-3β-yl acetate. The composition of the mixtures was calculated, as indicated in the table below, from the observed rotations, on the assumption of $[\alpha]_D$ as −89° and ±0°, respectively, for the pure acetates.

			Calculated from $[\alpha]_D$.			
Fraction no.	Wt., mg.	$[\alpha]_D$.	6-en acetate, %.	7-en acetate, %.	6-en acetate, mg.	7-en acetate, mg.
15	44	−63°	71	29	31	13
16	37	−45	51	49	19	18
17—18	47	−36	40	60	19	28
19—20	49	−29	33	67	16	33
21	4	—	—	—	1 *	3 *

* Estimated.

Totals of 86 mg. (46%) of cholest-6-en-3β-yl acetate and of 95 mg. (52%) of cholest-7-en-3β-yl acetate were found. In order to confirm the presence of cholest-7-en-3β-ol, fractions 17—20 were combined, hydrolysed, benzoylated, and chromatographed into two fractions, m. p. 133—134° and 142—143°. The second of these was recrystallised four times from chloroform–methanol and then had m. p. 148—149°, $[\alpha]_D$ ±0° (*c*, 0·37). After three further crystallisations, cholest-7-en-3β-yl benzoate was obtained with m. p. and mixed m. p. with an authentic specimen (Part VII, *J.*, 1949, 214), 153—154°.

Pyrolysis of Cholestan-3β-yl Benzoate.—1 G. of cholestanyl benzoate was pyrolysed by distillation at 1·5 mm. during 1 hour in a current of dry, oxygen-free nitrogen through a furnace maintained at 400°. The benzoic acid eliminated was titrated and corresponded to 80% of the theoretical. The distillate was chromatographed to give, after recrystallisation from ethyl acetate–methanol (long needles), cholest-2-ene (*neo*cholestene), m. p. 67—68°, $[\alpha]_D$ +62° (*c*, 5·90), +63° (*c*, 4·90).

Fractionation of Cholest-8(14)-en-3β-ol and its Acetate.—5 G. of *iso*dehydrocholesteryl acetate (Part VII, *loc. cit.*), very kindly provided by Professor A. Windaus (of Göttingen) to whom our best thanks are due, was hydrogenated in ether–acetic acid solution with a platinum catalyst. After being worked up in the usual way and recrystallised once from ethyl acetate–methanol, pure "α"-cholestenyl acetate, m. p. 77—78°, was obtained in almost quantitative yield. Six recrystallisations did not change the m. p. (77·5—78°); $[\alpha]_D$, initially +10·4° (*c*, 17·1), was finally +9·9° (*c*, 6·8); $[M]_D$ was +43°. Hydrolysis of "α"-cholestenyl acetate, gave, after one recrystallisation from ethyl acetate–methanol, "α"-cholestenol, m. p. 118·5—119·5°. Six recrystallisations did not change the m. p.; the final $[\alpha]_D$ was +21·4° (*c*, 6·5). $[M]_D$ was +83°. The Δ_1 value for "α"-cholestenol was thus −40°, in exact agreement with that found previously (Part IV, *loc. cit.*) for "α"-ergostenol.

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