120. *Steroids and Related Compounds. Part II. The Dehydration of Cholestanetriol.*

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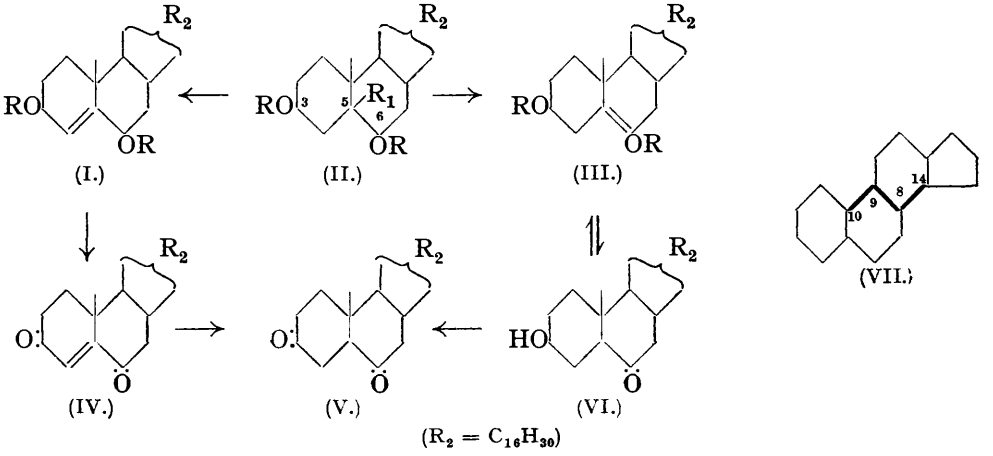
An unsaturated diol $C_{27}H_{46}O_2$ has been obtained by a Darzens dehydration of cholestane-3 : 5 : 6-triol diacetate, and its constitution as a Δ^4-cholestene-3 : 6-diol established. This constitution had previously been assigned to two isomeric diols of the same empirical composition (to be referred to as Lettré's and Westphalen's diol) which were obtained from the triol by different methods. It is now shown that Lettré's unsaturated diol $C_{27}H_{46}O_2$ is a saturated keto-alcohol identical with 6-ketocholestanol, $C_{27}H_{46}O_2$. Evidence in favour of the constitution of Westphalen's diol as 5-methyl-$\Delta^{8:9}$-norcholestene-3 : 6-diol has been obtained and it is concluded that only one of the four possible stereoisomerides of Δ^4-cholestene-3 : 6-diol is obtainable by the dehydration of cholestane-3 : 5 : 6-triol.

WHEN attempting to enforce acetylation of the tertiary hydroxyl of cholestane-3 : 5 : 6-triol diacetate (II; R = Ac, R_1 = OH) by treatment with acetic anhydride and sulphuric acid, Westphalen (*Ber.*, 1915, **48**, 1064; cf. Dunn, Heilbron, Phipers, Samant, and Spring, J., 1934, 1580) obtained the diacetate of an unsaturated diol $C_{27}H_{46}O_2$, to be referred to as Westphalen's diol. Lettré and Inhoffen (" Über Sterine u.s.w.," 1936, p. 33; cf. Petrow, J., 1937, 1077) ascribed to this diol the constitution of a Δ^4-cholestene-3 : 6-diol (I, R = H). Lettré and Müller (*Ber.*, 1937, **70**, 1947) were forced, however, to discard this view when

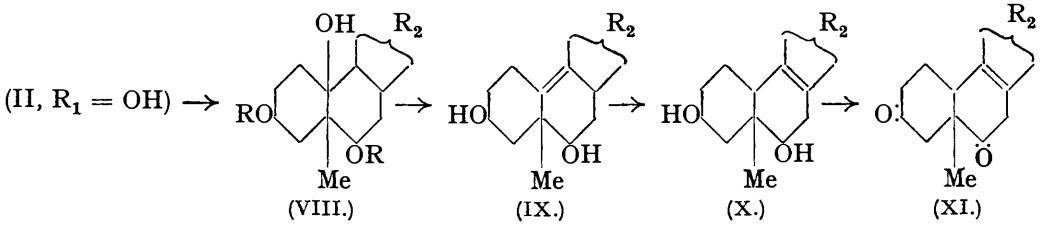


they found that on oxidation (at 4°) the diol did not yield the expected unsaturated diketone (IV), but a substance $C_{27}H_{46}O_3$ (?), m. p. 143°, which was not further characterised. They suggested that the diol might possibly have the constitution (IX).

We have now obtained, on oxidation at room temperature, an unsaturated diketone $C_{27}H_{46}O_2$, m. p. 106°, which was characterised by a *bis*-2 : 4-*dinitrophenylhydrazone*, m. p. 218°, and a *mono*-o-*tolylsemicarbazone*, m. p. 235°. Of the possible constitutions of the diketone, we favour (XI), 5-*methyl*-$\Delta^{8:9}$-*norcholestene*-3 : 6-*dione*, on the assumption that the positions of the functional groups are identical in the diketone and the diol. The evidence for the proposed constitutional formula is based on the following facts: (1) Westphalen's diol is not precipitated by digitonin, showing that the spatial position of the angular methyl group at C_{10} relative to the hydroxyl at C_3 has undergone a change during dehydration (cf. the non-precipitability of lumisterol, Dimroth, *Ber.*, 1936, **69**, 1123). This conclusion was strengthened when it was found that (2) the diol and its esters are strongly dextrorotatory: an increase of dextrorotatory power is known to follow changes in the relative position of the 10-methyl and the 3-hydroxyl group (cf. Callow and Young, *Proc. Roy. Soc.*, 1936, *A*, **890**, 194). Further, (3) the diol and its esters gave a typical green

Tortelli–Jaffé reaction with bromine (Häussler and Brauchli, *Helv. Chim. Acta*, 1929, **12**, 187), which is specific for ergosterol and steroids of analogous constitution. The ethylenic linkage of the diol may therefore be assumed to occupy a position between two quaternary carbon atoms in the ring system (C_8:C_9 or C_9:C_{10} in VII) analogous to that of the ethylenic linkage C_8:C_{14} in α-ergostenol, which is responsible for the Tortelli–Jaffé reaction (cf. Heilbron and Spring, *Biochem. J.*, 1930, **24**, 133). The diol also reacts readily with selenium dioxide in alcoholic solution, a characteristic of α-ergostenol, ergosterol and other steroids which give the Tortelli–Jaffé reaction (cf. Callow and Rosenheim, J., 1933, 387).

These observations lead to the conclusion that on dehydration a structural rearrangement, hitherto unrecorded, has occurred in the steroid molecule: the typical pinacol-alcoholic group $OH-\overset{5}{C}-\overset{10}{C}-CH_3$ present in cholestanetriol has undergone a retro-pinacolic rearrangement, as suggested by Lettré and Müller (*loc. cit.*), leading *via* (VIII) and (IX) to 5-methyl-$\Delta^{8:9}$-norcholestene-3 : 6-diol (X) and, on oxidation of this, to the diketone (XI). The migration of the ethylenic linkage C_9:C_{10} to C_8:C_9 during the reaction is assumed in analogy to that of $\Delta^{1:9}$-octalin to $\Delta^{9:10}$-octalin (Hückel and co-workers, *Annalen*, 1929, **474**, 121). Further, the C_8:C_9 linkage of the diol is resistant to catalytic hydrogenation, like the analogous linkage of hexadecahydrochrysene (Braun and Irmisch, *Ber.*, 1932, **65**, 883), and the diol in chloroform solution is not isomerised by treatment with hydrogen chloride.



The constitution of a Δ^4-cholestene-3 : 6-diol (I, R = H) was retained, however, by Lettré and Müller (*loc. cit.*) for a substance $C_{27}H_{46}O_2$, m. p. 137—138° (to be referred to as Lettré's diol), which was obtained as a dibenzoate by pyrolysis of 5-chlorocholestane-3 : 6-diol dibenzoate (II; R = COPh, R_1 = Cl). The latter dibenzoate was prepared by benzoylating the chlorohydrin of cholestane-3 : 5 : 6-triol (II, R = H, R_1 = Cl) (Windaus, *Z. physiol. Chem.*, 1921, **117**, 155). The new diol gave, according to the above authors, a violet Liebermann–Burchard reaction, presumably taken as evidence of unsaturation, but yielded on oxidation, somewhat unexpectedly, the saturated diketone (V) instead of the unsaturated Δ^4-cholestene-3 : 6-dione (IV).

We have confirmed the experimental results of the German authors, but are unable, on the following evidence, to accept their formulation of the substance $C_{27}H_{46}O_2$, m. p. 137—138°, as an unsaturated diol. Instead of the dibenzoate, m. p. 179°, from which it was derived, the " diol " yielded a monobenzoate, $C_{34}H_{50}O_3$, m. p. 179—180°, when treated in pyridine solution with benzoyl chloride in excess. A mixture of the two benzoates showed a depression of 25—30° in melting point. A monoacetate, $C_{29}H_{48}O_3$, m. p. 129—130°, resulted on acetylation. That the second oxygen atom of these esters functions as a carbonyl follows from the preparation of an acetoxy-*p*-nitrophenylhydrazone, m. p. 146—147°. Further, the " diol " $C_{27}H_{46}O_2$ yielded an *o-tolylsemicarbazone* and a *p*-nitrophenylhydrazone and was thus characterised as a keto-alcohol. The C_6 position of the new carbonyl group was the most likely one, and a comparison of the keto-alcohol and its derivatives with 6-ketocholestanol (Mauthner and Suida, *Monatsh.*, 1903, **24**, 654; Windaus, *Ber.*, 1903, **36**, 3755) proved their identity.

These results show that preferential elimination of chlorine at C_5 with hydrogen at C_6, rather than at C_4, has occurred in the pyrolysis of 5-chlorocholestane-3 : 6-diol dibenzoate, dehalogenation leading to an enol-benzoate, Δ^5-cholestene-3 : 6-diol dibenzoate (III, R = COPh). In confirmation of this interpretation of the reaction, it was found that the enol-benzoate is easily obtainable, in excellent yield, by refluxing 6-ketocholestanol with

benzoyl chloride in the presence of benzoic anhydride. The enol-benzoate yields on hydrolysis the keto-alcohol (VI), which is readily oxidised to cholestane-3 : 6-dione (V) (Windaus, *loc. cit.*; Lettré and Müller, *loc. cit.*).

The atypical violet Liebermann–Burchard reaction of the saturated keto-alcohol $C_{27}H_{46}O_2$ is clearly due to its ready enolisation, introducing the ethylenic linkage $C_5{\cdot}C_6$, which is also demonstrable by the yellow colour reaction with tetranitromethane. It was further found that all the saturated steroid ketones examined which contain a carbonyl group at C_6 gave a positive Liebermann–Burchard reaction (see Experimental).

It is thus proved that neither dehydration with sulphuric acid nor dehalogenation *via* the chlorohydrin gives rise to the formation of the expected Δ^4-cholestene-3 : 6-diol from cholestane-3 : 5 : 6-triol. The desired diol is easily obtained, however, in quantitative yield by dehydration of the di-esters of the triol with thionyl chloride in pyridine by Darzens' method (*Compt. rend.*, 1911, **152**, 601). The resulting esters yield on hydrolysis an unsaturated diol, $C_{27}H_{46}O_2$, m. p. 256—258°. Its constitution as a Δ^4-cholestene-3 : 6-diol (I, R = H) was proved by oxidation to Δ^4-cholestene-3 : 6-dione (IV), which was converted into the characteristic enol-ether by mineral acids in alcoholic solution. On reduction of the diol with platinum oxide, cholestane-3 : 6-diol was obtained, which yielded cholestane-3 : 6-dione (V) on oxidation.

Δ^4-Cholestene-3 : 6-diol so obtained was found to be identical with the diol, m. p. 258°, previously prepared from cholesteryl acetate by oxidation with selenium dioxide (Rosenheim and Starling, J., 1937, 377; cf. Butenandt and Hausmann, *Ber.*, 1937, **70**, 1154). Its alternative formation by the dehydration of cholestanetriol provides conclusive evidence for the interpretation of the selenium dioxide reaction, suggested by Butenandt and Hausmann (*loc. cit.*) : the primary oxidation product, *i.e.*, Δ^5-cholestene-3 : 4-diol, undergoes in the course of the reaction an allylic rearrangement to Δ^4-cholestene-3 : 6-diol. That this allylic isomerisation is a reversible one follows from the observation that dilute mineral acids readily convert the diol, m. p. 258°, into cholestenone, in the same way as the isomeric *cis*-Δ^5-cholestene-3 : 4-diol (Rosenheim and Starling, *loc. cit.*).

The interesting fact emerges from these results that the tertiary hydroxyl of cholestane-3 : 5 : 6-triol possesses an unexpected mobility, giving rise under different conditions to three isomerides $C_{27}H_{46}O_2$, of which only one is a Δ^4-cholestene-3 : 6-diol.

Experimental.

Dehydration of Cholestane-3 : 5 : 6-triol with Thionyl Chloride.—(*a*) To a solution of the cholestanetriol diacetate (2 g.) (Pickard and Yates, J., 1908, **93**, 1681) in anhydrous pyridine (10 c.c.), cooled in ice, thionyl chloride (0·4 c.c.) was added dropwise with shaking. The solution turned yellow and a white solid was formed, which was transferred into water after 5 minutes. After some hours the solid (1·8 g., m. p. 133—134°) was removed and washed with methyl alcohol. On crystallisation from methyl alcohol, colourless needles of Δ^4-cholestene-3 : 6-diol diacetate (I, R = Ac) were obtained, m. p. 135—136°, $[\alpha]_D^{19°}$ − 13·2° (*c*, 1·104 *) (Found : † C, 76·7; H, 10·3. Calc. for $C_{31}H_{50}O_4$: C, 76·5; H, 10·4%).

(*b*) Δ^4-Cholestene-3 : 6-diol 3-benzoate 6-acetate was obtained by the above procedure from 5-hydroxy-3-benzoyloxy-6-acetoxycholestane (5 g.) (Petrow, *loc. cit.*). The product, m. p. 127°, yielded, after crystallisation from spirit, prisms of the mixed ester, m. p. 128—129°, $[\alpha]_D^{20°}$ − 20·0° (*c*, 2·68) (Found : C, 78·6; H, 9·3. Calc. for $C_{36}H_{52}O_4$: C, 78·7; H, 9·6%).

On hydrolysis of the above esters with 5% methyl-alcoholic sodium methoxide Δ^4-cholestene-3 : 6-diol (I, R = H) was obtained, m. p. 257—258°, $[\alpha]_D^{20°}$ + 9·0° (*c*, 1·05 in pyridine) (Found : C, 80·5; H, 11·3. Calc. for $C_{27}H_{46}O_2$: C, 80·5; H, 11·5%). On benzoylation in pyridine the diol gave the dibenzoate, m. p. 180—181°, $[\alpha]_D^{20°}$ − 73·0° (*c*, 2·62).

The diol and its esters were identical (mixed m. p.'s, optical activity) with the diol, m. p. 258°, and its esters obtained by oxidation of cholesteryl acetate with selenium dioxide (Rosenheim and Starling, *loc. cit.*). The diol gave the typical blue colour reaction with trichloroacetic acid and the Lifschütz " oxycholesterol " reaction (*Ber.*, 1908, **41**, 252) with sulphuric acid in acetic acid solution. On warming with dilute mineral acids the diol was converted into cholestenone, identified as the 2 : 4-dinitrophenylhydrazone, m. p. 233—234°.

* The optical rotations were measured in a 4 dm. tube in chloroform solution.

† Micro-analyses by Dr. G. Weiler, Oxford.

Δ^4-*Cholestene*-3 : 6-*dione* (IV).—Kiliani's chromic acid mixture (12 c.c.) was added dropwise with mechanical stirring during 1½ hours to a suspension of the finely powdered diol (2 g.) in benzene (40 c.c.) and acetic acid (10 c.c.), and the stirring continued for a further 30 minutes. The benzene layer was separated, washed with water and dilute alkali solution, and dried over sodium sulphate. After removal of the solvent, the residue was rubbed with petrol. The insoluble colourless crystals, m. p. 122°, yielded, after recrystallisation from 85% alcohol, Δ^4-cholestene-3 : 6-dione, m. p. and mixed m. p. 122—123° (Found : C, 81·6; H, 10·6. Calc. for $C_{27}H_{42}O_2$: C, 81·3; H, 10·6%). When the unsaturated dione was refluxed with alcohol containing 4% of sulphuric acid, it yielded the enol-ether $C_{29}H_{46}O_2$, m. p. and mixed m. p. 165—166°.

Δ^4-*Cholestane*-3 : 6-*dione* (V).—The unsaturated diol (I, R = H) was catalytically reduced with platinum oxide (Rosenheim and Starling, *loc. cit.*). The product (38 mg.), m. p. 194—195°, was dissolved in acetic acid (0·5 c.c.) and oxidised with Kiliani's chromic acid mixture (0·12 c.c.). A few drops of alcohol were added to remove the excess of chromic acid, and the crystals, m. p. 168°, removed. On recrystallisation from methyl alcohol, needles of cholestane-3 : 6-dione (24 mg.), m. p. 169—170°, were obtained. The same substance resulted when Δ^4-cholestene-3 : 6-dione (IV) (0·1 g.) was reduced with zinc dust (0·1 g.) in acetic acid (2·5 c.c.). A mixture of the two substances with an authentic specimen melted at 169—170°.

Dehydration of Cholestane-3 : 5 : 6-*triol with Sulphuric Acid.*—The optical activity of Westphalen's diol and its esters is given in the following table :

	$[\alpha]_D^{18°}$	$[\alpha]_{5461}^{18°}$.	*c*.	α_{5461}/α_D.
Westphalen's diol	+ 118·8°	+ 143·6°	1·104	1·21
Diacetate	+ 83·9	+ 99·0	1·040	1·18
Dibenzoate	+ 109·4	+ 132·9	1·034	1·22

A solution of the diol in 96% alcohol, to which an excess of a 1% digitonin solution in 90% alcohol had been added, was heated on a steam-bath. The solution remained clear and no precipitate was produced on keeping at 0° for 24 hours and at room temperature for 7 days.

Bromine titration (by the pyridine sulphate dibromide method) indicated that substitution as well as addition of bromine occurred. After 1, 5, and 10 minutes at room temperature, 0·050 g. of the diol absorbed 40·8, 49·5, and 50·4 mg. of bromine, corresponding to 4·1, 4·9, and 5 atoms of bromine.

The Tortelli–Jaffé reaction, carried out by adding a 1% solution of bromine in acetic acid dropwise to a chloroform solution of the diol, gave an intense green colour. The sensitivity of the reaction was increased when bromine–acetic acid containing hydrobromic acid was used, a violet-blue colour (band at 5400—5800 A.) being at first produced, which changed to green on further addition of the reagent.

In a perbenzoic acid titration, after 24 hours at 0°, 0·500 g. of the diol consumed the equivalent of 18·1 mg. of oxygen, corresponding to 1·1 ethylenic linkages.

A solution of the diol diacetate (0·5 g.) in alcohol (10 c.c.) and acetic acid (10 c.c.) was shaken with palladised charcoal (0·25 g.) in hydrogen at room temperature for 3 hours No absorption of hydrogen occurred and the diol was recovered unchanged (mixed m. p.).

5-*Methyl*-$\Delta^{8:9}$-*norcholestene*-3 : 6-*dione* (XI).—A solution of the diol (1 g.) was shaken for 6 hours at room temperature with a solution of chromic acid (0·66 g.) in water (7 c.c.) and acetic acid (15 c.c.). The neutral fraction of the oxidation products was obtained by the usual technique; it crystallised from acetone–methyl alcohol in prismatic needles, m. p. 105—106°, $[\alpha]_D^{19°}$ − 45·7° (*c*, 0·952) (Found : C, 81·0; H, 10·3. $C_{27}H_{42}O_2$ requires C, 81·3; H, 10·6%). The Zerewitinoff value was nil. The *diketone* gave a yellow colour with tetranitromethane and showed no absorption above 2300 A. in 0·01% alcoholic solution. The *bis*-2 : 4-*dinitrophenylhydrazone*, prepared by warming a solution of the diketone (50 mg.) and 2 : 4-dinitrophenylhydrazine (40 mg.) in alcohol (8 c.c., containing 2 drops of concentrated hydrochloric acid) on a steam-bath until a voluminous yellow precipitate formed, separated from chloroform–alcohol in yellow micro-needles, m. p. 217—218° (Found : N, 14·2. $C_{39}H_{50}O_8N_8$ requires N, 14·4%). The *mono*-o-*tolylsemicarbazone* was obtained by warming a solution of the diketone (50 mg.) in alcohol (1 c.c.) with a solution of *o*-tolylsemicarbazide in alcohol (5 c.c., containing 2 drops of acetic acid) on a steam-bath. Colourless clusters of needles formed after 20 minutes, and the quantity increased on cooling. The semicarbazone, recrystallised from chloroform–alcohol, separated in needles, m. p. 234—235°, turning yellow at 228° (Found : C, 77·3; H, 9·0; N, 7·9. $C_{35}H_{51}O_2N_3$ requires C, 77·1; H, 9·4; N, 7·7%).

Dehalogenation of 5-Chlorocholestane-3 : 6-diol Dibenzoate.—Δ^5-Cholestene-3 : 6-diol dibenzoate (III, R = COPh) was prepared (a) according to Lettré and Müller (*loc. cit.*) by heating 5-chloro-3 : 6-dibenzoyloxycholestane (II; R = COPh, R_1 = Cl) (2 g.) for 2 hours at 210°/0·5 mm. Benzoic acid (0·24 g.) sublimed. The yellowish resinous residue crystallised on treatment with cold ether (10 c.c.), yielding a white solid (1·15 g.), m. p. 178—179°. On recrystallisation from ethyl acetate–methyl alcohol (1 : 1), prismatic needles of the enol-benzoate, m. p. 179—180°, were obtained.

(b) A solution of 3-ketocholestanol (0·5 g.) and benzoic anhydride (1 g.) in benzoyl chloride (5 c.c.) was refluxed for 1½ hours. Benzoyl chloride was removed in a vacuum, and the residue dissolved in ether (5 c.c.). Crystallisation of the enol-benzoate began on addition of methyl alcohol (5 c.c.) and was complete at 0° after a few hours. The white product (0·4 g.) was recrystallised from ethyl acetate–methyl alcohol and was identical with the specimen prepared by method (a); m. p. 179—180°, $[\alpha]_D^{19°}$ + 6·9°, $[\alpha]_{5461}^{19°}$ + 8·7° (*c*, 1·342); α_{5461}/α_D = 1·26 (Found : C, 80·3; H, 8·8. Calc. for $C_{41}H_{54}O_4$: C, 80·6; H, 8·9%). The enol-benzoate gave a yellow colour with tetranitromethane and a violet Liebermann–Burchard reaction.

6-Ketocholestanol (VI) (= Lettré's " diol ") was obtained from the above enol-benzoate (a) (0·5 g.) by hydrolysis with alcoholic potassium hydroxide. In agreement with the statement of the German authors, the product melted at 137—138°. The m. p. was raised, however, to 142—143° after two recrystallisations from 95% methyl alcohol and was not depressed by authentic 6-ketocholestanol prepared by the original method of Mauthner or Windaus (*loc. cit.*). The saturated keto-alcohol gave a violet Liebermann–Burchard reaction and was precipitated by digitonin (cf. Westphalen's diol above).

6-Keto-cholestanol (10 mg.) in alcohol (2 c.c.) containing 2 drops of acetic acid was heated with *o*-tolylsemicarbazide (8 mg.) on the steam-bath for ½ hour; the crystals (9·8 mg.) were collected after 24 hours, and recrystallised from spirit, *6-ketocholestanol-o-tolylsemicarbazone* separating in aggregates of fine needles, m. p. 223—224° (Found : C, 76·7; H, 9·9. $C_{35}H_{53}O_2N_3$ requires C, 76·7; H, 9·8%). The *p*-nitrophenylhydrazone, prepared by the usual technique, crystallised from methyl alcohol in yellow needles, m. p. and mixed m. p. 196—197°.

3-Ketocholestanyl Benzoate from the Enol-benzoate.—The enol-benzoate (0·3 g.), prepared by method (a), was hydrolysed as above, and the product (0·2 g.) benzoylated in pyridine solution with benzoyl chloride. After a few hours the well-formed crystals deposited were removed, washed with spirit, and recrystallised from acetone, yielding 3-ketocholestanyl benzoate (0·18 g.) in large transparent prisms, m. p. 179—180°, not depressed by a specimen prepared by the same method from 3-ketocholestanol (Windaus, *loc. cit.*, gives m. p. 173° for a specimen prepared by the Schotten–Baumann method) (Found : C, 80·4; H, 9·7. Calc. for $C_{34}H_{50}O_3$: C, 80·6; H, 9·9%). A mixture of the benzoate with the enol-benzoate (a) of the same m. p. began to melt at 150° and was fused at 155°.

3-Ketocholestanyl Acetate from the Enol-benzoate (a).—The product (0·4 g.) obtained on hydrolysis of the enol-benzoate was refluxed with acetic anhydride (4 c.c.) for ½ hour; the acetate produced crystallised in elongated prisms, m. p. 129—130°, not depressed by an authentic specimen (Found : C, 78·2; H, 10·7. Calc. for $C_{29}H_{48}O_3$: C, 78·3; H, 10·8%). The *p*-nitrophenylhydrazone separated in fine needles when a mixture of alcoholic solutions of the constituents was kept for 24 hours; it crystallised from methyl alcohol in yellow needles, m. p. 146—147°, unaltered on admixture with an authentic specimen (Found : C, 72·2; H, 9·0; N, 7·2. Calc. for $C_{35}H_{53}O_4N_3$: C, 72·5; H, 9·2; N, 7·3%).

Liebermann–Burchard Reaction of Saturated Steroid Ketones.—The following gave a violet colour, showing a band at 5500—6000 A.: cholestan-6-one, α-chlorocholestan-6-one, β-chlorocholestan-6-one, cholestanol-6-one (and its esters). Cholestane-3 : 6-dione gave a green colour but cholestan-3-one and coprostan-3-one remained colourless.

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