527. *The Application of the Method of Molecular Rotation Differences to Steroids. Part XVII.* The Action of Mercuric Acetate on* iso-*Dehydrocholesteryl* p-*Nitrobenzoate.*

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

When treated with mercuric acetate in chloroform–acetic acid and then hydrolysed by alkali, *iso*dehydrocholesteryl *p*-nitrobenzoate furnishes a mixture of cholesta-7 : 9(11)-diene-3β : 6α- and -3β : 6β-diols.

In an extensive study of the action of mercuric acetate and lead tetra-acetate on *iso*dehydrocholesteryl *p*-nitrobenzoate (I; R = *p*-NO_2·C_6H_4·CO) Windaus and his collaborators (*Annalen*, 1942, **552**, 135, 142) prepared a number of interesting labile derivatives. The main material produced by reaction with mercuric acetate and subsequent alkaline hydrolysis was a doubly unsaturated diol, $C_{27}H_{44}O_2$, m. p. 228°, $[\alpha]_D$ −49° (in chloroform), −51° (in pyridine), $\lambda_{max.}$ 248 mμ., $\varepsilon_{max.}$ 15,000 (in ether) [Calc.: $\lambda_{max.}$ 255 mμ. in ethanol, Fieser and Fieser's correction table ("Natural Products Related to Phenanthrene," Reinhold Publ. Corp., 3rd Edn., p. 184) being used]. This was assigned the constitution cholesta-7 : 9(11)-diene-3β : 6-diol (II; R = H) on the basis of the absorption spectrum, the structure of its precursor, and it ready dehydration by acetic anhydride to cholesta-5 : 7 : 9(11)- and -6 : 9(11) : 8(14)-trien-3β-yl acetates (III and IV respectively). In Part II of this series (Barton, *J.*, 1946, 512) this constitution was questioned, mainly on the grounds that the rotation recorded was too highly negative for a 6-hydroxylated cholesta-7 : 9(11)-dien-3β-ol and that the wave-length of the absorption maximum should have been closer to 242 mμ., the value for ergosterol D and 22-dihydroergosterol D (V; R = C_9H_{17} and C_9H_{19} respectively) (Barton and Cox, *J.*, 1949, 219).

Through the courtesy of Professor A. Windaus (Göttingen) who very kindly provided us with a supply of the difficultly accessible *iso*dehydrocholesteryl *p*-nitrobenzoate, it became possible to investigate this subject further. Our work has confirmed the homogeneity of the diol and its ease of diacylation, but there are discrepancies as regards the absorption spectrum ($\lambda_{max.}$ 245 mμ., $\varepsilon_{max.}$ 18,300 in *ethanol*) and rotation ($[\alpha]_D$ −41° in pyridine but +20° in chloroform). The revised physical constants remove our objections to the Windaus formulation which we now regard as correct. The relevant argument can be briefly summarised as follows. The absorption spectrum shows that the two double bonds must be in different rings. Of the various possibilities the $\Delta^{7:9(11)}$-formulation is adequate for the position of the maximum and follows from the structure of the starting material and the mechanism of the reaction (see below). That the diol is an allylic alcohol is shown by the smooth hydrogenolysis of its diacetate (cf. *inter al.*, Wintersteiner and Ruigh, *J. Amer. Chem. Soc.*, 1942, **64**, 2456; Henbest and Jones, *J.*, 1948, 1798) to give a 3 : 1 mixture of cholestanyl and "α"-cholestenyl [cholest-8(14)-enyl] acetates [(VI) and (VII) respectively] (cf. Windaus *et al.*, *loc. cit.*). The ease of diacylation excludes formulæ containing a tertiary hydroxyl group. The formation of the two trienes on dehydration (see above) confirms that the easily eliminated hydroxyl must be at $C_{(6)}$. As will be clear from the sequel the diol must have the β-configuration at $C_{(6)}$ and therefore is represented by (II; R = H).

In our hands benzoylation of the mother-liquors from the preparation of the cholesta-7 : 9(11)-diene-3β : 6β-diol gave the dibenzoate, m. p. 184—185°, $[\alpha]_D$ +144° (in chloroform), of a further isomeric diol. The latter was obtained from the dibenzoate by alkaline hydrolysis in the usual way and was further characterised as the diacetate, m. p. 126—127°, $[\alpha]_D$ +126° (in chloroform), $\lambda_{max.}$ 242 mμ., $\varepsilon_{max.}$ 19,500 (in ethanol). On catalytic hydrogenation in acetic acid in presence of platinum the diacetate gave cholest-8(14)-ene-3β : 6α-diol diacetate (VIII), m. p. 141—142°, $[\alpha]_D$ +28° (in chloroform). The constitution assigned to the latter is based on (*a*) its rotation and ultra-violet absorption spectrum (see Experimental), (*b*) its catalytic hydrogenation in acetic acid containing dissolved hydrogen chloride to cholestanyl acetate (VI), and (*c*) its conversion into cholestan-3β : 6α-diol diacetate (IX) on rearrangement of the double bond by hydrogen chloride in chloroform, followed by further hydrogenation. This formulation and the interpretation of the reactions are also based on sound analogy (*e.g.*, Fieser and Fieser, *op. cit.*, p. 240 *et seq.*). Isolation of this compound, coupled with the absorption

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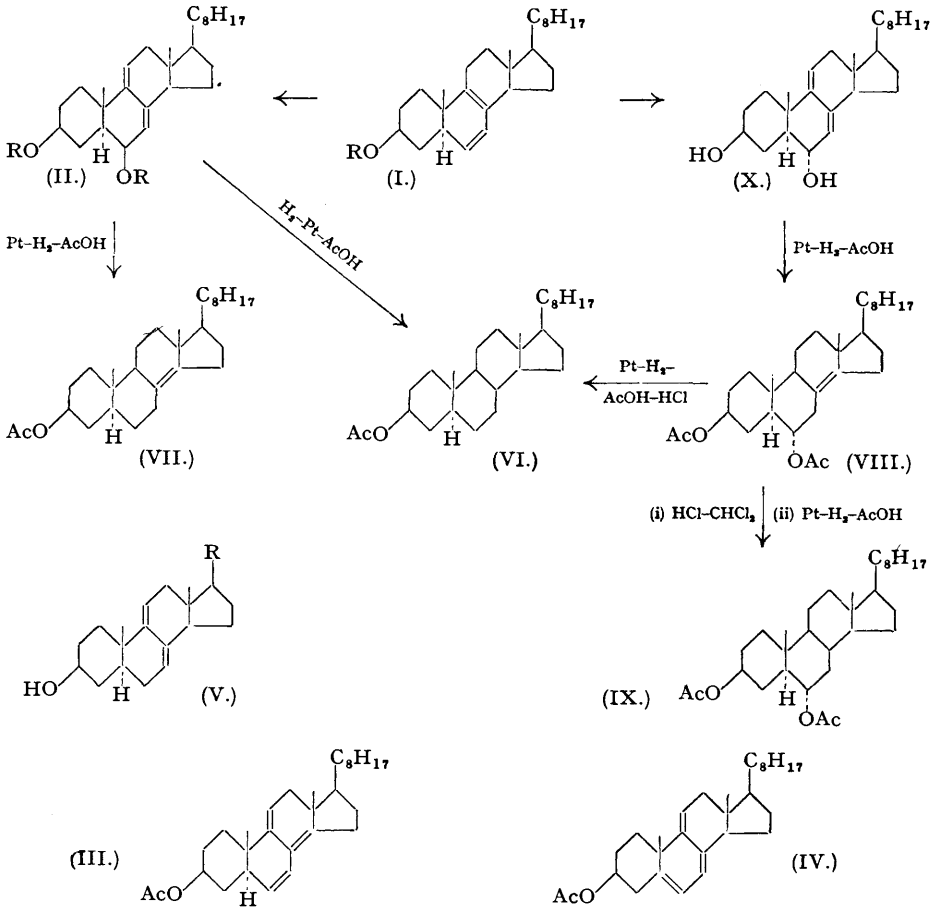
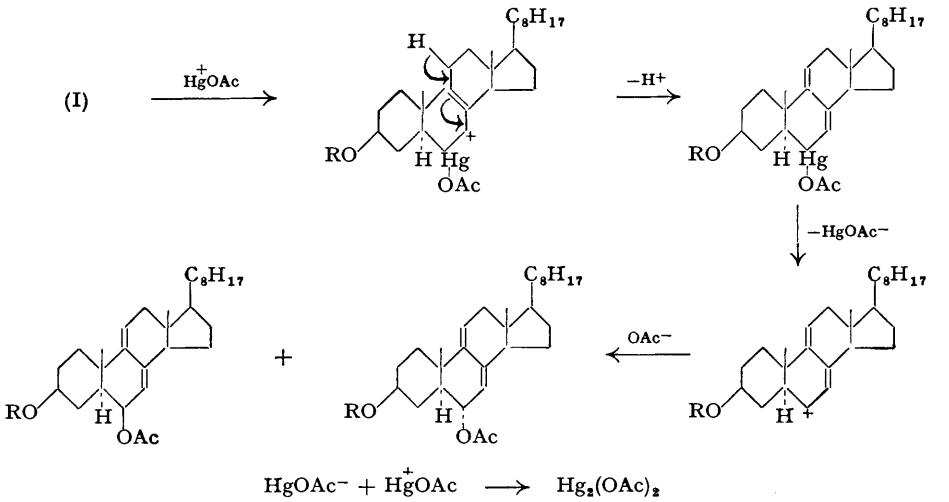
spectrum of the parent dienediol diacetate and the known structure of its precursor, shows that the dienediol must be cholesta-7 : 9(11)-diene-3β : 6α-diol (X).

Cholesta-7 : 9(11)-diene-3β : 6α-diol is very probably identical with an incompletely characterised diol obtained by Windaus *et al.* (*loc. cit.*) by the action of lead tetra-acetate on *iso*dehydrocholesteryl *p*-nitrobenzoate. In agreement with this view, Windaus *et al.* reported that on treatment with acetic anhydride their diol gave a mixture of (III) and (IV).



The mechanism of the action of mercuric acetate on *iso*dehydrocholesteryl *p*-nitrobenzoate appears worthy of brief mention. It would seem that this is best regarded as electrophilic attack of $\overset{+}{H}gOAc$ on the diene system, followed by loss of a proton. The organomercuri-complex thus formed could conceivably be the actual product of the reaction, the two cholesta-7 : 9(11)-dienediols resulting therefrom only by hydrolysis on working up. Against this theory is the fact that lithium aluminium hydride, which cleaves, for example, the Hg–C bond of diphenylmercury very smoothly, gives the same reaction products as the ordinary alkaline hydrolysis with no indication of the formation of cholesta-7 : 9(11)-dien-3β-ol (V; R = C_8H_{17}). The first stage of the reaction that can be studied (see Experimental) leads to the introduction of a non-acylatable group at $C_{(6)}$ which is, presumably, the acetoxy-group. The reaction sequence can be formulated as indicated below (R = *p*-NO_2·C_6H_4·CO throughout).

Windaus *et al.* (*loc. cit.*) reported the formation of a substance, $C_{34}H_{43}O_5N$, as a by-product of the action of mercuric acetate on *iso*dehydrocholesteryl *p*-nitrobenzoate. This compound was isolated before the usual alkaline hydrolysis. We have confirmed these findings and have shown that the newly introduced oxygen function cannot be acetylated readily or oxidised by



$$HgOAc^- + \overset{+}{H}gOAc \longrightarrow Hg_2(OAc)_2$$

chromic acid under very mild conditions. The infra-red absorption spectrum showed the hydroxyl band at 2·87 μ. and the compound is clearly a tertiary alcohol. Its constitution and mode of formation remain obscure.

Experimental.

M. p.s are uncorrected. Unless specified to the contrary rotations were determined in chloroform at room temperature 15—25°. For rotation measurements all specimens were dried *in vacuo* at 20° below their m. p.s or at 110°, whichever was the lower. Values of $[\alpha]_D$ have been approximated to the nearest degree.

Savory and Moore's alumina for chromatography was used in all cases. Light petroleum refers to the fraction of b. p. 40—60°.

In the text below the phrase " in the usual way " refers to dilution with water, extraction with ether, washing successively with aqueous potassium hydroxide solution, aqueous hydrochloric acid, and water, followed by evaporation of the ethereal solution *in vacuo*. Where necessary water was removed from the residue by azeotropic distillation *in vacuo* with benzene as entrainer.

Alkaline hydrolyses were effected by using several equivalents of potassium hydroxide and refluxing the reactants for 30—60 minutes in methanolic or dioxan–methanolic solution depending on the solubility requirements of the ester.

Absorption spectra were measured in ethanol by a Unicam S.P. 500 spectrophotometer.

Action of Mercuric Acetate on iso*Dehydrocholesteryl* p-*Nitrobenzoate.*—*iso*Dehydrocholesteryl *p*-nitrobenzoate was treated with mercuric acetate in chloroform–acetic acid according to the directions of Windaus, Riemann, and Zühlsdorff (*Annalen*, 1942, **552**, 135) except that it was found advantageous to leave the reactants for only 1 hour before working them up. As reported by Windaus, Riemann, and Zühlsdorff (*loc. cit.*) the crude reaction product, on treatment overnight with acetone, gave long pale yellow needles of a compound, $C_{34}H_{43}O_5N$, which, recrystallised from ethyl acetate, had m. p. 209—210°, $[\alpha]_D$ −121° (*c*, 2·11), $\lambda_{max.}$ 258 and 312 mμ., $\varepsilon_{max.}$ 16,000 and 13,500 respectively (Found : C, 74·8; H, 7·7. Calc. for $C_{34}H_{43}O_5N$: C, 74·8; H, 7·9%). After attempted acetylation (acetic anhydride–pyridine at room temperature overnight) or benzoylation (similar conditions but with benzoyl chloride) the compound was recovered unchanged. After treatment of the compound (67·5 mg.) in AnalaR benzene (2 ml.) with a solution (2·04 ml.) of AnalaR chromium trioxide (101·2 mg. in 25 ml.) in AnalaR acetic acid at 0° overnight, not all the chromic acid had been consumed. Working up in the usual way gave 34 mg. of unchanged starting material.

After removal of the *p*-nitrobenzoate referred to above unsuccessful attempts were made to crystallise the residual compounds. The vacuum-dried residue had $[\alpha]_D$ −14° (*c*, 1·24), $\lambda_{max.}$ 253 mμ., $\varepsilon_{max.}$ about 20,000. After attempted acetylation (as above) the rotation was unchanged {$[\alpha]_D$ −16° (*c*, 2·18)} and therefore the products at this stage of the reaction cannot have a free hydroxyl group at $C_{(6)}$.

Two methods of working up the mixed *p*-nitrobenzoates were adopted : (*a*) hydrolysis with methanolic potassium hydroxide according to the directions of Windaus, Riemann, and Zühlsdorff (*loc. cit.*); and (*b*) reduction by an excess of lithium aluminium hydride in ethereal solution (1 hour's reflux), followed by working up in the usual way. Both procedures gave the two diols referred to below, but the yield was better on following procedure (*a*).

In a quantitative experiment, *iso*dehydrocholesteryl *p*-nitrobenzoate (1·74 g.), treated with mercuric acetate (4·0 g.), gave mercurous acetate (2·16 g., 114% on the assumption of a 1 : 1 molar relationship).

This again indicates that soluble mercuriacetate complexes cannot be isolatable intermediates in the reaction.

Cholesta-7 : 9(11)-*diene*-3β : 6α-*diol*.—Isolated according to the procedure of Windaus *et al.* (*loc. cit.*), this compound had m. p. 203—204° (decomp.; slow heating), 226—227° (decomp.; if placed in the m. p. bath at 220°), $[\alpha]_D$ +20°, +17° (both *c*, 0·33; *l* = 2 dm.), −41° (*c*, 2·12) in pyridine. Cholesta-7 : 9(11)-diene-3β : 6β-diol is an unstable compound and decomposes spontaneously when kept in chloroform solution at room temperature. Treatment with acetic anhydride in pyridine solution at room temperature overnight and working up in the usual way gave the *diacetate*, m. p. 131—132° (from methanol), $[\alpha]_D$ −115° (*c*, 2·21), $\lambda_{max.}$ 245 mμ., $\varepsilon_{max.}$ 18,300 (Found : C, 77·1; H, 10·1. $C_{31}H_{48}O_4$ requires C, 76·8; H, 10·0%). Alkaline hydrolysis regenerated unchanged starting diol.

Cholesta-7 : 9(11)-diene-3β : 6β-diol and benzoyl chloride in pyridine overnight at room temperature gave, after working up in the usual way, the *dibenzoate*, m. p. 149—150° (from ethyl acetate–methanol), $[\alpha]_D$ −132° (*c*, 2·22) (Found : C, 80·7; H, 8·8. $C_{41}H_{52}O_4$ requires C, 80·8; H, 8·6%). Alkaline hydrolysis regenerated the starting diol.

Hydrogenation of Cholesta-7 : 9(11)-*diene*-3β : 6β-*diol Diacetate*.—Cholesta-7 : 9(11)-diene-3β : 6β-diol diacetate (40 mg.) in AnalaR acetic acid (20 ml.) was hydrogenated overnight in presence of a platinum catalyst. After being worked up in the usual way the crude reaction product had m. p. 95—96°, $[\alpha]_D$ +12° (*c*, 1·12). A mixture ($[\alpha]_D$ +13°) of three parts of cholestanyl acetate with one part of "α"-cholestenyl acetate had m. p. 94—95°, and was not depressed on admixture with the hydrogenation product. To confirm the composition of the latter 37 mg., dissolved in 5 ml. of carbon tetrachloride and 3 ml. of acetic anhydride, were treated at 10° with 5 drops of concentrated sulphuric acid according to Anderson and Nabenhauer's method (*J. Amer. Chem. Soc.*, 1924, **46**, 1957). After 20 minutes the mixture was diluted with water and worked up in the usual way. Chromatography over alumina, benzene being used as eluant, gave 28 mg. of cholestanyl acetate, m. p. 103—104° not depressed on admixture with authentic cholestanyl acetate (m. p. 107—108°).

Cholesta-7 : 9(11)-*diene*-3β : 6α-*diol*.—After separation of the cholesta-7 : 9(11)-diene-3β : 6β-diol as reported above the ethereal mother-liquors were evaporated *in vacuo* and the residue benzoylated (benzoyl chloride–pyridine overnight at room temperature). Working up in the usual way afforded *cholesta*-7 : 9(11)-*diene*-3β : 6α-*diol dibenzoate* which, recrystallised from chloroform–methanol, had m. p. 184—185°, $[\alpha]_D$ +144° (*c*, 2·58) (Found : C, 80·1; H, 8·1. $C_{41}H_{52}O_4$ requires C, 80·8; H, 8·6%).

Alkaline hydrolysis of this furnished cholesta-7 : 9(11)-diene-3β : 6α-diol; recrystallised from methanol, this had m. p. 194—195° (decomp.), $[\alpha]_D$ +94° (*c*, 0·55). Acetylation as above then gave the *diacetate*, m. p. 126—127° (from methanol), $[\alpha]_D$ +126° (*c*, 1·15), $\lambda_{max.}$ 242 mμ., $\varepsilon_{max.}$ 19,500 (Found : C, 76·5; H, 9·5. $C_{31}H_{48}O_4$ requires C, 76·8; H, 10·0%).

Windaus, Riemann, Rüggeberg, and Zühlsdorff (*Annalen*, 1942, **552**, 142) recorded m. p. 196° for this diol and isolated it as the bis-3 : 5-dinitrobenzoate. They did not characterise it further.

Cholest-8(14)-*ene*-3β : 6α-*diol*.—Cholesta-7 : 9(11)-diene-3β : 6α-diol dibenzoate (250 mg.) in 20 ml. of ether–AnalaR acetic acid was hydrogenated overnight over platinum. After working up in the usual way the product, once recrystallised from ethyl acetate–methanol, had m. p. 120—121°, $[\alpha]_D$ +25° (*c*, 2·75), +25° (*c*, 0·83). It was not investigated further but was hydrolysed in the usual way and the hydrolysis product acetylated (as above). The product was then chromatographed over alumina (6 fractions), to give *cholest*-8(14)-*ene*-3β : 6α-*diol diacetate*, eluted with 5% benzene–light petroleum; recrystallised from methanol, this had m. p. 141—142°, $[\alpha]_D$ +28° (*c*, 1·44) (Found : C, 76·5; H, 10·4. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%). It gave a yellow colour with tetranitromethane and showed $\lambda_{max.}$ 205 mμ., $\varepsilon_{max.}$ 8200, ε_{215} 4500. These absorption values are characteristic, within the limited range of possible double-bond positions, of olefinic unsaturation at 8(14) (private communication from Dr. H. B. Henbest, Manchester). The calculated rotation for cholest-8(14)-ene-3β : 6α-diol diacetate, based on $[\alpha]_D$ +38° for cholestane-3β : 6α-diol diacetate (see below) and the standard tables of Barton and Klyne (*Chem. and Ind.*, 1948, 755), is $[\alpha]_D$ +34°, in satisfactory agreement with the value found. The diacetate was also prepared by similar hydrogenation of cholesta-7 : 9(11)-diene-3β : 6α-diol diacetate.

Alkaline hydrolysis of cholest-8(14)-ene-3β : 6α-diol diacetate then gave the corresponding *diol* which, recrystallised from light petroleum, had m. p. 179—180°, $[\alpha]_D$ +24° (*c*, 1·43) (Found : C, 78·3; H, 11·3. $C_{27}H_{46}O_2$,0·5H_2O requires C, 78·7; H, 11·6%).

Benzoylation of the diol as above afforded the *dibenzoate*, m. p. 181—182° (from ethyl acetate–methanol), $[\alpha]_D$ +51° (*c*, 2·80) (Found : C, 81·7, 79·8; H, 8·5, 8·5. $C_{41}H_{54}O_4$ requires C, 80·6; H, 8·9%).

Hydrogenation of Cholest-8(14)-*ene*-3β : 6α-*diol Diacetate*.—(a) *Hydrogenation in acetic acid containing hydrogen chloride*. Cholest-8(14)-ene-3β : 6α-diol diacetate (25 mg.) in "AnalaR" acetic acid (20 ml.) containing a little hydrogen chloride gas was hydrogenated overnight in presence of a platinum catalyst. Working up in the usual way afforded cholestanyl acetate, m. p. 105—107°, $[\alpha]_D$ +16° (*c*, 0·68) in 0·5 dm. tube, on recrystallisation from methanol, undepressed in m. p. on admixture with an authentic specimen.

(b) *Hydrogenation after rearrangement*. Cholest-8(14)-ene-3β : 6α-diol diacetate (70 mg.), dissolved in chloroform (10 ml.), was treated with a stream of dry hydrogen chloride at room temperature for 30 minutes. After removal of the solvent *in vacuo* the residue was hydrogenated overnight in "AnalaR" acetic acid (20 ml.). The product was worked up in the usual way and the hydrogen chloride treatment and subsequent hydrogenation repeated twice more. If it is assumed that the equilibrium established by hydrogen chloride leads to equal amounts of 8(14)- and 14(15)-isomers (cf. Barton, Cox, and Holness, *J.*, 1949, 1771), then the repetitions of rearrangement and hydrogenation should have led to 87·5% of the required saturated product. To destroy any remaining unsaturated material the final hydrogenation

product was treated with chromium trioxide (30 mg.) in " AnalaR " acetic acid (10 ml.) on the water-bath for 30 minutes. After working up in the usual way the product was chromatographed over alumina (3 fractions), to give *cholestane-3β : 6α-diol diacetate*, eluted with 80% benzene–light petroleum and recrystallised from methanol; this had m. p. 55—56°, $[\alpha]_D$ +37° (*c*, 1·02), and gave no colour with tetranitromethane (Found : C, 76·1; H, 10·95. $C_{31}H_{52}O_4$ requires C, 76·2; H, 10·7%). The m. p. of this diacetate is very much lower than that (m. p. 107—108°, $[\alpha]_D$ +39°) recorded by Plattner and Lang (*Helv. Chim. Acta*, 1944, **27**, 1872). It appears that the diacetate prepared by us is a lower-melting form, for authentic specimens of cholestane-3β : 6α-diol diacetate were prepared (i) from cholestan-6-on-3β-yl acetate and (ii) from cholestane-3 : 6-dione, by reduction with sodium and *n*-propanol, followed by acetylation in pyridine overnight with acetic anhydride. In each case the crystal form (plates from methanol) was the same as that of the hydrogenation product and the m. p. (54—55°) and rotation {$[\alpha]_D$ +39° (*c*, 2·45)} were both in agreement. There was no depression in m. p. on admixture. To confirm the identity the hydrogenation product was hydrolysed to cholestane-3β : 6α-diol, m. p. 203—204° (from methanol) alone or mixed with authentic cholestane-3β : 6α-diol, m. p. 209—210°, prepared by alkaline hydrolysis of the authentic diacetate (see above). The m. p. of the diol is in agreement with that (m. p. 213—215°, corr.) recorded by Plattner and Lang (*loc. cit.*).

Reduction of Diphenylmercury by Lithium Aluminium Hydride.—Diphenylmercury (3·5 g.) in ether was treated with an excess of lithium aluminium hydride. Mercury immediately separated. After 0·5 hour's refluxing the solution was worked up and the mercury removed by filtration. Weight of mercury = 1·93 g. (Calc., 1·95 g.). The ethereal solution was washed with dilute sulphuric acid and water, and dried (Na_2SO_4). The ether was distilled off through a Vigreux column; the residue, recrystallised by freezing, had f. p. +2° undepressed by dry " AnalaR " benzene of f. p. +4·5°.

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