[CONTRIBUTION FROM SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

The Pyrolysis of 3(β)-Acetoxycholestan-7(α)-yl Benzoate. Δ^6-Cholestenol

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Some years ago we prepared from 3(β)-acetoxycholestan-7(α)-ol (I)[1] by treatment with *p*-toluenesulfonyl chloride in boiling pyridine and other dehydrating procedures a mixture of isomeric cholestenyl acetates ("crude γ-cholestenyl acetate") which could not be effectively separated into its components by fractional crystallization or chromatography.[2] Though generally slightly dextrorotatory ([α]D + 0.5° to + 9°), these products resembled in their properties fairly closely the γ(Δ^7)-cholestenyl acetate (m. p. 119°, [α]D 0°) which Schenk, *et al.*,[3] had obtained by the reduction of 7-dehydrocholesterol with sodium and ethanol. That the main constituent of "crude γ-cholestenyl acetate" was indeed Δ^7-cholestenyl acetate (III) was shown by its almost quantitative isomerization to α($\Delta^{8(14)}$)-cholestenyl acetate with hydrogen and platinum oxide in acetic acid, its conversion with osmic acid into a cholestane-3(β),7,8-triol which formed a diacetate, and by the identification of the product resulting from its reaction with two moles of perbenzoic acid as 3(β)-acetoxycholestan-7-ol-8,14-oxide.[2,4] Our work was repeated and confirmed in its essential features by Buser,[5] whose method of dehydration (phosphorus oxychloride in pyridine at room temperature) afforded a γ-cholestenyl acetate closely approximating in its properties that of Schenk, *et al.*[3]

More recently, in an attempt to improve the yields and degree of purity realizable by the methods mentioned above, Plattner, Heusser, Troxler and Segré[6] investigated the pyrolysis of the benzoate (II) and anthraquinone-β-carboxylate of 3(β)-acetoxycholestan-7(α)-ol. The distillates from both esters after chromatographic purification yielded an apparently homogeneous cholestenyl acetate m. p. 104–105° which differed from all the isomers hitherto described by its high levorotation ([α]D − 64°, chloroform). Hydrolysis to the free stenol (m. p. 118°) produced a pronounced rotation shift toward the positive side ([α]D − 16°). The acetate did not consume any hydrogen in the presence of platinum oxide and acetic acid, but was rearranged to an unidentified dextrorotatory isomer (m. p. 106°, [α]D + 14.8°), which gave melting point depression with the starting material as well as with cholestanyl acetate and $\Delta^{8(14)}$-cholestenyl acetate. Oxidation of the original levorotatory stenyl acetate with chromic acid afforded 3(β)-acetoxycholestan-7-one (IV) in good yield. From these findings the Swiss authors concluded that their new compound was pure Δ^7-cholestenyl acetate, and that the products prepared by the older methods were poorly defined mixtures of isomers.

We found it difficult to accept this view for the following reasons: There was little doubt from the chemical evidence cited further above that "crude γ-cholestenyl acetate" contained a very large proportion, perhaps 80 to 90%, but certainly over 60%, of the genuine Δ^7-isomer. Our preparations and those of Buser showed rotation values ranging between 0 and +9°. Assuming for the sake of argument that the Δ^7-compound present was the strongly levorotatory cholestenyl acetate described by the Swiss authors, the remainder of these preparations should clearly have consisted of an isomer (or a mixture of isomers) possessing very pronounced dextrorotatory power, with [α]D certainly greater than + 70°. However, no cholestenols or ergostenols answering that description are known. Moreover, extensive chromatographic fractionation of "crude γ-cholestenyl acetate" had failed to disclose the presence of either levorotatory products or such with an [α]D exceeding + 9°.[2]

These considerations let us suspect that the levorotatory stenol was a new isomer, most probably the hitherto undescribed Δ^6-cholestenol (VI). This supposition proved to be correct, as will appear from the evidence presented below.

Δ^6-Cholestenol.—On repeating the pyrolysis reaction on 3(β)-acetoxycholestan-7(α)-ol benzoate and chromatographing the distillate we obtained several crystalline fractions, one of which yielded on purification the levorotatory cholestenyl acetate m. p. 104–106° (V). However, [α]D of our purest preparation was considerably more negative (−88°) than the value reported by Plattner, *et al.* (−64°). The free cholestenol (VI) prepared by us melted at 114–119°, and after drying at 100° *in vacuo*, at 129–131° (Plattner, *et al.*, 115–116°, after sublimation at 110°, 118°); more serious is the marked discrepancy between the respective rotation figures (−81 and −16°). For further characterization the stenol was converted into its benzoate (m. p. 128–129°, [α]D − 74°), a derivative not described by the Swiss workers.

Contrary to the experience of Plattner, *et al.*, we found that the stenyl acetate on hydrogena-

(1) O. Wintersteiner and M. Moore, THIS JOURNAL, **65**, 1605 (1943). This compound was then arbitrarily designated 7β, but has since for various reasons been assigned the 7(α)-configuration: *cf.* P. A. Plattner and H. Heusser, *Helv. Chim. Acta*, **27**, 748 (1944); L. F. Fieser, M. Fieser and R. D. Chakravorti, ref. 9a; L. F. Fieser and M. Fieser, ref. 9b, p. 242.

(2) O. Wintersteiner and M. Moore, *ibid.*, **65**, 1507 (1943).

(3) F. Schenk, K. Buchholz and O. Wiese, *Ber.*, **69**, 2696 (1936).

(4) O. Wintersteiner and M. Moore, THIS JOURNAL, **65**, 1513 (1943).

(5) W. Buser, *Helv. Chim. Acta*, **30**, 1379 (1947).

(6) P. A. Plattner, H. Heusser, F. Troxler and A. Segré, *ibid.*, **31**, 852 (1948).

tion with platinum oxide in acetic acid rapidly consumed one mole of hydrogen, forming cholestanyl acetate in almost quantitative yield. The identity of the product was confirmed by hydrolysis to cholestan-3(β)-ol. This result not only excluded the presence in the starting product of significant amounts of Δ^7-cholestenyl acetate, but was clearly in accord with the behavior to be expected of a Δ^6-stenol.

Another point on which we find ourselves in disagreement with the Swiss authors concerns the behavior of the stenyl acetate toward osmic acid. They state in their paper without further elaboration that their product did not react with this agent. We, to the contrary, found that an osmic acid adduct is readily formed, at least when the reaction is carried out in the presence of pyridine.[7] The cholestanetriol (m. p. 209–210°) obtained from the crystalline complex by hydrolysis with sodium sulfite, on treatment with *p*-nitrobenzoyl chloride in pyridine, formed a tri-*p*-nitrobenzoate (m. p. 226–227°) in quantitative yield. The acetyl derivative (m. p. 84–87°) could not be effectively purified on account of its extreme solubility in organic solvents, but nevertheless gave a satisfactory analysis indicating a triacetate. Both hydroxyl groups introduced with osmic acid must therefore be secondary, leaving little doubt that we had in hand the expected cholestane-3(β),6,7-triol (VII).

The triol VII rapidly consumed one mole of periodic acid in aqueous-methanolic solution, but the resulting crystalline dialdehyde could not be obtained in analytically pure form. In this connection we made the interesting observation that secondary-tertiary *cis*-glycols of the steroid class such as cholestane-3(β),7,8-triol, cholestane-3(β),5(α),6(α)-triol, and equilin glycol (7,8-dihydroxyestrone) are completely inert toward periodic acid under the conditions used by us. On the other hand, the disecondary *cis*-glycols Δ^5-cholestene-3(β),4(β)-diol and methyl 3(α),11(α),12(α)-trihydroxycholanate were attacked at about the same rate as the triol VII. 3(α),11(β),12(β)-Trihydroxycholanic acid reacted slowly and incompletely, evidently on account of the hindered condition of the 11(β)-hydroxyl group. Since isolated double bonds except those common to, or linking, two rings can generally be hydroxylated with osmic acid, the behavior of the resulting *cis*-glycols toward periodic acid would seem to afford a diagnostic means for differentiating ring double bonds of the types $RHC{=}CR_1R_2$ and $RHC{=}CHR_1$ in alicyclic compounds.

We next reexamined the reaction of the stenyl acetate with chromic acid. For the sake of expediency a slightly impure sample ($[\alpha]D$ −77°) was used as the starting material, but this was thought permissible in view of the fact that Plattner, *et al.*, had obtained 3(β)-acetoxycholestan-7-one (IV) in good yield from a preparation with $[\alpha]D$ only −64°. As it happened we could not secure this or any other pure compound from the neutral fraction by direct crystallization. Chromatographic fractionation was then resorted to, but likewise failed to disclose the presence of the saturated 7-ketone. Instead we obtained four crystalline products, two of which are of structural interest and merit more detailed discussion here. One of these was 3(β)-acetoxy-Δ^5-cholesten-7-one (7-ketocholestenyl acetate, VIII), and the other a hitherto undescribed diacetoxy ketone (m. p. 164–165° $[\alpha]D$ −77°). The presence in the latter compound of an unconjugated keto group was inferred from the absorption spectrum which showed a single band at 295 mμ with $\epsilon = 90$. It should also be mentioned that both acetoxy groups were readily demonstrable in the acetyl determination according to Kuhn–Roth, because we consider this evidence for the location of the newly introduced acetoxy group on a secondary carbon atom. The tertiary acetoxy group at least in 3(β),5(α)-diacetoxycholestan-6-one (XI), a by-product obtained in the chromic acid oxidation of cholesteryl acetate, does not behave in this manner.[8] Only one acetyl group was found in this compound when the acetyl determination was conducted in the usual way, and special, more stringent conditions were required to render the tertiary acetoxy group demonstrable by this method. On this premise the new diacetoxy ketone could then be either a 3(β),6-diacetoxycholestan-7-one (IX), or a 3(β),7-diacetoxycholestan-6-one. Of these alternative structures IX is preferred on account of its obvious relationship to the simultaneously formed unsaturated 7-ketone VIII.

An attempt to convert the diacetoxy ketone to VIII under acidic conditions failed, but this does not necessarily argue against its formulation as IX, as the 6-acetoxy group may have the α-configuration, in which case facile elimination of acetic acid is not to be expected on account of its *cis*-orientation with respect to the hydrogen atom at C_5.

The formation from Δ^6-cholestenyl acetate (V) of the oxidation products VIII and IX—provided IX is the correct structure of the diacetoxy ketone—may be pictured as follows: The primary oxidative attack takes place at the double bond itself rather than at the allylic positions, a postulate which receives support from the fact that the acidic portion of the oxidation products consists largely of the 6||7-dicarboxylic acid (see below). The resulting 6,7-oxide X (or mixture of the two stereoisomeric oxides) is acetolyzed by the acetic acid used as solvent, yielding a 3(β),6(β)-diacetoxycholestan-7-ol which is oxidized to the ketone IX. Elimination of acetic acid from a part of IX finally yields the unsaturated ketone VIII. Alternatively it could be assumed

(7) R. Criegee, B. Marchand and H. Wannovius, *Ann. Chem.*, **550**, 99 (1942).

(8) F. Schenk, *Z. physiol. Chem.*, **243**, 119 (1936).

that both 6-epimeric diacetoxyketones of structure IX are produced via the two forms of the oxide X, and of these only the stable α-epimer (the isolated diacetoxyketone) survives, while the β-epimer undergoes *in toto* the elimination reaction leading to VIII.

In the considerations presented above the parts pertaining to the structure of the diacetoxy ketone and its role as a precursor of VIII are admittedly speculative. On the other hand, there is ample evidence at hand to support the postulate that the primary oxidation product is an oxide. This aspect of the chromic acid oxidation of unsaturated sterols has been recently discussed and stressed by Fieser and Fieser,[9] who propose formulations similar to the above to explain the formation of by-products which cannot arise by allylic oxidation. Thus in the case already referred to[8] the formation of 3(β),5(α)-diacetoxycholestan-6-one XI from cholesteryl acetate is envisaged as leading through the 5,6-(β)-oxide in a sequence quite analogous to V→X→IX.

A third crystalline substance isolated from the neutral fraction (m. p. 107–108°, $[\alpha]_D$ + 12°) showed the analytical composition $C_{29}H_{48}O_2$ of the starting material and hence is not an oxidation product. It cannot be impure Δ^7-cholestenyl acetate, as on catalytic hydrogenation it failed to yield the $\Delta^{8(14)}$-isomer. Lack of material prevented further investigation.

The acidic fraction from the chromic acid oxidation of Plattner's stenyl acetate consisted largely of a readily crystallizable dibasic acid (m. p. 212–213°) of the composition $C_{29}H_{48}O_6$, obviously the acetyl derivative XII of the 3(β)-hydroxycholestane-6||7-dicarboxylic acid XIII which was first described by Windaus and Stein[10] and recently reinvestigated by Shoppee.[11] This was confirmed by hydrolysis of the oxidation product to XIII and conversion of the latter to the characteristic lactone XIV.[12,11] Conclusive identification of the latter two compounds was achieved by comparison of their melting points and rotations with those of authentic specimens kindly supplied by Professor Shoppee.[13] This finding removes any vestige of doubt regarding the 6-7 position of the double bond in Plattner's stenol.

The fact that the Windaus–Stein acid XIII is readily convertible to the lactone XIV proves that the 3-hydroxyl group is oriented *trans* with respect to hydrogen atom at C_5, and consequently *cis* with respect to the 10-methyl group. Till recently it could not be asserted that the same relationship also holds for cholesterol from which XIII was prepared, because the method of Windaus and Stein,[10] involving as it does 3-chloro derivatives as intermediates, gave no assurance that the original configuration at C_3 had been preserved. However, Shoppee[11] in connection with his important studies on Walden inversions in the steroid class, has recently prepared the acid XIII from cholesterol by a procedure to which this objection does not apply, namely, via 7-ketocholestanyl acetate and 6,7-diketocholestanol, and has thus adduced the first direct proof for the β-configuration of the hydroxyl group in cholesterol and related steroids. Our finding that XIII is accessible also via Plattner's stenyl acetate—that is, by a variant of Shoppee's procedure in which 7-ketocholestanyl acetate is the last common intermediate—may be regarded as collateral evidence to Shoppee's demonstration. In a later paper Heusser, Segré and Plattner[14] mention an abortive attempt to bring about the above proof by oxidation of the levorotatory stenyl acetate considered by them the Δ^7-isomer, on the presumption that the oxidation product would be 3(β)-acetoxy-7||8-cholestan-8-one-7-carboxylic acid, and that this acid after hydrolysis would be convertible to a 7→3 lactone. Though the failure of these authors to arrive at an acid of this description can now be readily explained, it is not clear why the oxidation experiment in their hands did not yield the easily crystallizable acetoxy acid XII obtained by us.

Δ^2-Cholesten-7(α)-yl Benzoate and $\Delta^{2,6}$-Cholestadiene.—Besides Δ^6-cholestenyl acetate the chromatographic fractionation of the pyrolysis afforded two new compounds, namely, a cholestenyl benzoate (m. p. 137°, $[\alpha]_D$ −28°), and a cholestadiene (m. p. 72°, $[\alpha]_D$ −4°) in which the two double bonds are not conjugated. From the mode of formation of these products it could be surmised that the former compound was either Δ^2-cholestene-7(α)-benzoate (XVa) or Δ^3-cholestene-7(α)-benzoate (XVb), and the hydrocarbon either $\Delta^{2,6}$-cholestadiene (XIXa) or $\Delta^{3,6}$-cholestadiene (XIXb). The evidence presented below shows that in the cholestenyl benzoate the double bond occupies the 2–3 position as in XVa; from this it can be inferred that the hydrocarbon possesses the analogous 2,6-diene structure XIXa.

The structure of the cholestenyl benzoate was established as follows: Vigorous hydrolysis with alkali yielded the free stenol (XVI) (m. p. 80–85°, $[\alpha]_D$ +42°, possibly a partly epimerized product). The position of the functional group follows from the conversion of the stenol by hydrogenation and then chromic acid oxidation into a saturated ketone m. p. 115° which is undoubtedly cholestan-7-one.[15] The attempt to bring about the proof for the position of the double bond by converting the stenol XVI to the known cholestane-2||3-dicarboxylic acid was temporarily aban-

(9) (a) L. F. Fieser, M. Fieser and R. D. Chakravorti, THIS JOURNAL, **71**, 2226 (1949). (b) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Corporation, New York, N. Y., 1949, pp. 227 ff.

(10) A. Windaus and G. Stein, *Ber.*, **37**, 3699 (1904).

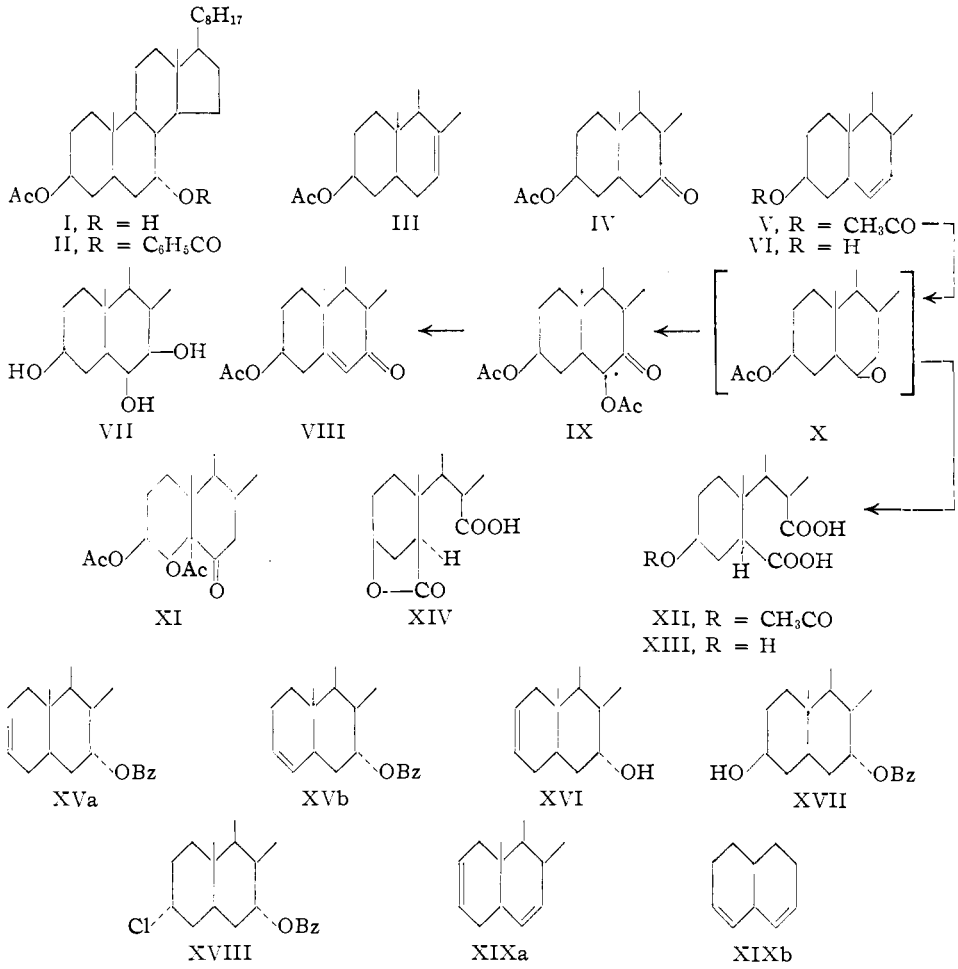
(11) C. W. Shoppee, *J. Chem. Soc.*, 1032 (1948).

(12) H. Lettré, *Ber.*, **68**, 766 (1935).

(13) We wish to express our sincere thanks to Prof. Shoppee, University College of Swansea, England, for the gift of these samples.

(14) H. Heusser, A. Segré and P. A. Plattner, *Helv. Chim. Acta*, **31**, 1183 (1948).

(15) A. Windaus, *Ber.*, **53**, 488 (1920).



doned on account of the small yield, as well as of certain difficulties encountered in the analytical characterization of the last intermediate, 7-ketocholestane-2||3-dicarboxylic acid. Instead, the new cholestenyl benzoate was prepared from the original starting product II by a procedure which left no doubt regarding the 2-3 position of the newly introduced double bond. Partial hydrolysis of II afforded 3(β)-hydroxycholestan-7-(α)-yl benzoate, XVII (m. p. 155°, $(\alpha)_D$ −12°), which was then converted with phosphorus oxychloride in pyridine or with phosphorus pentachloride in chloroform to 3(α)-chlorocholestan-7(α)-yl benzoate, XVIII (m. p. 169°, $[\alpha]_D$ −4°). The chloro derivative on treatment with potassium acetate in boiling *n*-valeric acid underwent dehydrohalogenation to a cholestenyl benzoate identical with the pyrolysis product. Since Shoppee[16] had shown that the same reaction when applied to either 3(α)- or 3(β)-chlorocholestane yields Δ^2-cholestene as the main product, it follows that the unsaturated 7-benzoxy compound must likewise have the Δ^2-structure (XVa).

The hydrocarbon m. p. 72° isolated from the pyrolysate was characterized as a cholestadiene by analysis and perbenzoic acid titration. The ultraviolet spectrum showed a weak band at 237 mμ (ethanol) indicating contamination with about 5% of a heteroannular conjugated diene, *i.e.*, either the $\Delta^{3,5}$- or the $\Delta^{4,6}$-isomer. Prolonged refluxing with hydrochloric acid in alcohol raised the extinction coefficient to a constant value about twice as high as the original, while on similar treatment in acetic acid the maximum merely became less sharply defined. We believe that the purified preparation consisted essentially of $\Delta^{2,6}$-cholestadiene (XIXa) contaminated with small amounts of the $\Delta^{3,5}$- or $\Delta^{4,6}$-isomer (giving rise to the original light absorption of 237 mμ) and also of $\Delta^{3,6}$-cholestadiene (XIXb) (to explain the increased absorption after heating with acid).

While this work was in progress we became aware of a paper by Barton and Rosenfelder[17] in which these authors advance cogent arguments for the correctness of the Δ^7-structure assigned to the known "γ"-stenols, and through consideration of the molecular rotation data are led to the

(16) C. W. Shoppee, *J. Chem. Soc.*, 1138 (1946).

(17) D. H. R. Barton and W. J. Rosenfelder, *Helv. Chim. Acta*, **32**, 975 (1949).

view that Plattner's stenol does not possess this structure. We subsequently learned that in the meantime Dr. Barton had likewise reinvestigated this substance and elucidated its true nature. With his kind coöperation arrangements were made for the simultaneous publication of preliminary notes reporting the results obtained in the two laboratories.[18] We also wish to express our appreciation to Dr. Barton for making available to us the manuscript of a paper giving the details of his experiments.[19] The data reported by the English authors for Δ^6-cholestenol and its derivatives are in excellent agreement with ours. Their proof for the position of the double bond is based on the facile conversion of the acetate to cholestanyl acetate on hydrogenation, and particularly on the important observation that the stenyl acetate can be prepared also by pyrolysis of 3(β)-acetoxycholestan-6(β)-yl benzoate. There is also substantial agreement between the two investigations in regard to the properties and structure of the cholestenyl benzoate and the cholestadiene formed as by-products in the pyrolysis of 3(β)-acetoxycholestan-7(α)-yl benzoate. The British investigators deduce the Δ^2-position of the double bond in these compounds from the fact that cholestan-3(β)-yl benzoate on pyrolysis yields Δ^2-cholestene.

Experimental

All melting points were taken in the capillary and are corrected. The solvent in the rotation measurements was chloroform unless indicated otherwise. A 1-decimeter polarimeter tube was used which could be filled through a lateral tubule and hence permitted measuring the experimental and solvent blank angles without changing the position of the end-plates between the two readings.

Pyrolysis of 3(β)-Acetoxycholestan-7(α)-yl Benzoate; Separation of Reaction Products.—The amorphous benzoate was prepared from 3(β)-acetoxycholestan-7(α)-ol (m. p. 114.5–116°)[1] and pyrolyzed in 1.5–3.0 g. portions as described by Plattner, *et al.*,[6] except that in some runs the heating and distillation were conducted at a somewhat higher pressure (18–20 mm. instead of 14 mm.). The neutral fraction obtained from the distillate was chromatographed on alumina in hexane solution. While Plattner, *et al.*,[6] recovered the stenyl acetate from the petroleum ether–benzene eluates (proportion not specified), we found that the three crystalline substances isolated by us were usually all eluted by hexane alone. We ascribe this to the fact that our chromatographed alumina had been washed with sulfuric acid or acetic acid to *p*H 4.6 and reactivated by heating at 150° for forty-eight hours, and hence was probably less active than the preparation used by Plattner, *et al.* The hydrocarbon was invariably found in the first few effluent portions, and was followed in rapid succession by Δ^2-cholesten-7(α)-yl benzoate and Δ^6-cholestenyl acetate. From the 1:5 benzene–hexane eluates, and often the later hexane eluates, amorphous but relatively pure starting material was recovered, sometimes to the extent of 60% of the original weight. The proportions in which the crystalline constituents were present varied a good deal from run to run, and in some experiments little or no hydrocarbon could be isolated in crystalline form. We also found it necessary in most cases to rechromatograph the fractions containing the Δ^6-stenyl acetate in order to obtain pure material. A representative experiment is described below.

The glassy benzoate obtained from 3 g. of 3(β)-acetoxycholestan-7(α)-ol was pyrolyzed according to Plattner, *et al.* (14 mm., heating with a free flame for five minutes just below boiling point, followed by distillation, which required twenty minutes). The neutral fraction from the distillate (2.9 g.) was dissolved in hexane (100 cc.), and the solution was passed through a column of acetic acid-washed alumina (25 × 400 mm.). Elution was effected by continued washing with hexane (800 cc.), followed by benzene–hexane 1:4 (240 cc.). The effluent was collected in 40-cc. portions. Except for fractions 1, 2, 6 and 7, all eluates were wholly or in part crystalline. Fractions 2–4, together 663 mg., melted around 60°, and on repeated recrystallization from ether–methanol and acetone yielded $\Delta^{2,6}$-cholestadiene m. p. 70–71°. Fraction 8 (65 mg.), conspicuous for its higher melting point (109–124°), needed only one recrystallization from methanol to give practically pure Δ^2-cholesten-7(α)-yl benzoate, m. p. 136–138°. The remaining hexane eluates 8–20 (together 665 mg.) showed unsharp melting points ranging between 80 and 100°. They were combined and recrystallized twice from methanol, yielding 393 mg. of still impure (m. p. 100–104°) Δ^6-cholestenyl acetate. As in other instances, this preparation was combined with similar material from other runs and rechromatographed. A similar but still less pure product was recovered by recrystallization of the 6 fractions eluted with benzene-hexane 1:4 (142 mg.). The material subsequently eluted with benzene–hexane 1:1 was not crystallizable.

In subsequent runs conducted in the same manner except that sulfuric acid-washed alumina was used, the crystalline products were eluted in the same order but much more rapidly, and the starting benzoate began to emerge already in the subsequent hexane eluates (from fraction 7 on), as shown in one instance by analysis (calcd. for $C_{36}H_{54}O_4$ (550.8): C, 78.5; H, 9.88. Found: C, 78.9; H, 9.93), and absorption spectrum ($\lambda_{max.}^{alc.}$ 230 mμ, ε = 12,000); $[\alpha]^{24}D$ −8.0° (*c*, 0.68).

Δ^6-Cholesten-3(β)-yl Acetate (V).—In the purification of this compound by recrystallization from methanol the rotation value rather than the melting point was relied upon as a criterion of purity, since we repeatedly observed that in recrystallized samples, including such derived from rechromatographed material, melting points close to that of the pure compound (104–106°) or even higher (107–109°) were associated with low rotation values (−70° to −84°). The impurity involved may be Δ^2-cholesten-7(α)-yl benzoate, or an isomeric cholestenyl acetate.[20] A carefully purified sample of the stenyl acetate (m. p. 104–106°) showed $[\alpha]^{24}D$ −88 ± 1° (*c*, 0.834).

Anal. Calcd. for $C_{29}H_{48}O_2$ (428.7): C, 81.25; H, 11.29. Found: C, 81.23; H, 11.23.

Δ^6-Cholesten-3(β)-ol (VI).—The acetate (150 mg.) was hydrolyzed by boiling in 10% methanolic potassium hydroxide solution (7.5 cc.) for one hour. The mixture was worked up in the usual way, and the crude stenol (133 mg.) was recrystallized several times from dry methanol, yielding rectangular plates of m. p. 114–119° (desiccator-dry). The analytical sample on drying at 100° 2 mm. lost 4.5% of its weight and then melted at 129–131°; $[\alpha]^{25}D$ −81 ± 1° (*c*, 0.951).

Anal. Calcd. for $C_{27}H_{46}O$ (386.6): C, 83.87; H, 11.99. Found: C, 83.75; H, 11.82.

Δ^6-Cholesten-3(β)-yl Benzoate.—The above preparation (49 mg.) was benzoylated in pyridine (0.5 cc.) with benzoyl chloride (0.05 cc.). After standing overnight at room temperature the benzoate was recovered in the usual manner (56 mg., m. p. 123–128°). Recrystallization from

(18) D. H. R. Barton and W. J. Rosenfelder, *Nature*, **164**, 316 (1949); O. Wintersteiner and M. Moore, *ibid.*, **164**, 317 (1949).

(19) D. H. R. Barton and W. J. Rosenfelder, *J. Chem. Soc.*, in press, 1949.

(20) L. F. Fieser, M. Fieser and R. D. Chakravorti (ref. 9a), conducting the pyrolysis reactions at 8 mm., obtained a product m. p. 113–114°, $[\alpha]^{25}D$ −31° in chloroform, which analyzed sharply for a cholestenyl acetate, and depressed the melting point of Δ^7-cholestenyl acetate prepared according to Buser.

ether–methanol 1:9 and then from methanol yielded whetstone shaped crystals melting at 128–129° after slight sintering at 124°, $[\alpha]^{25}D$ −74 ± 2° (*c*, 0.654).

Anal. Calcd. for $C_{34}H_{50}O_2$ (490.7): C, 83.20; H, 10.27. Found: C, 83.15; H, 10.21.

Catalytic Reduction.—A solution of Δ^6-cholestenyl acetate (223 mg.) in acetic acid (22 cc.) was shaken in a hydrogen atmosphere with platinum oxide (25 mg.) previously saturated with the gas. Within thirty minutes exactly one mole had been consumed (found 12.8 cc., calcd. 12.5 cc.) and no further uptake was observed. The residue obtained by evaporating the filtered solution was recrystallized thrice from methanol, yielding 134 mg. of cholestanyl acetate m. p. 108–109°, $[\alpha]^{25}D$ +15.1 ± 1° (*c*, 0.987).

Anal. Calcd. for $C_{29}H_{50}O_2$ (430.7): C, 80.78; H, 11.70. Found: C, 80.97; H, 11.56.

The cholestanol obtained by hydrolysis of the above preparation with hot methanolic potassium hydroxide melted at 139–141° (sample dried *in vacuo* at 110°); $[\alpha]^{25}D$ +23.5 ± 1° (*c*, 0.996).

Anal. Calcd. for $C_{27}H_{48}O$ (388.6): C, 83.44; H, 12.45. Found: C, 83.43; H, 12.19.

Cholestane-3(β),6,7-triol (VII).—A solution containing Δ^6-cholestenyl acetate (350 mg.) and osmium tetroxide (252 mg., 1.2 molar equivalents) in 15 cc. of dry ether and 0.25 cc. of pyridine[7] (4 molar equivalents) was allowed to stand in the dark at room temperature. Crystallization of the adduct commenced within fifteen minutes. After forty-eight hours the crystalline precipitate, consisting of clusters of dark brown needles, was filtered off and washed with ether (255 mg.). Two further crops, together 130 mg., were obtained by concentrating the mother liquor, and eventually adding a few drops of pyridine. The crystalline material was dissolved in ethanol (5 cc.) and an aqueous solution of sodium sulfite (503 mg. in 3 cc.) was added. The solution was refluxed for fifteen minutes, filtered while still hot from the resulting black precipitate, and after concentration *in vacuo* to a small volume, extracted with chloroform. The extract was washed several times with small volumes of water, dried, and evaporated. The residue (168 mg.), consisting of fine felted needles, was recrystallized repeatedly from ethyl acetate and then melted at 211.5–212.5°; $[\alpha]^{25}D$ +41 ± 1° (*c*, 1.00 in ethanol).

Anal. Calcd. for $C_{27}H_{48}O_3$ (420.7): C, 77.09; H, 11.50. Found: C, 77.41; H, 11.66.

The **triacetate** was prepared by allowing a solution of the triol (25 mg.) in pyridine (1.0 cc.) and acetic anhydride (0.5 cc.) to stand at room temperature for forty-eight hours. It was obtained as a colorless glass (27 mg.) which could not be crystallized and was therefore chromatographed in hexane solution on alumina. Nothing was eluted with hexane and benzene–hexane 1:4, but the first benzene–hexane 1:1 eluates yielded 17 mg. of material which crystallized on addition of a little methanol. The product was very soluble in all organic solvents including hexane, and hence could not be effectively purified. From warm acetone containing a small amount of water it was obtained as a gelatinous precipitate melting at 84–87°, $[\alpha]^{23}D$ + 24 ± 1° (*c*, 0.969).

Anal. Calcd. for $C_{33}H_{54}O_6$ (546.4): C, 72.46; H, 9.96. Found: C, 72.04; H, 9.81.

For the preparation of the **tri-*p*-nitrobenzoate** the triol (48.8 mg.) was treated with *p*-nitrobenzoyl chloride (161 mg.) and pyridine (3 cc.) at room temperature for forty-eight hours. The precipitate obtained on addition of ice water was centrifuged and washed several times with water (100.0 mg.). Crystallization from hot 95% ethanol (25 cc.) yielded 44 mg. of needles m. p. 221–224°. An additional crop (34 mg.) of the same melting point was obtained on concentrating the mother liquor. After recrystallization from absolute ethanol the compound showed a constant melting point of 226–227°, $[\alpha]^{25}D$ +180 ± 2° (*c*, 0.557).

Anal. Calcd. for $C_{48}H_{57}O_{12}N_3$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.24; H, 6.86; N, 5.05.

After small-scale experiments (see below) had shown that the triol consumed one mole of periodic acid, the preparation of the **6,7 dialdehyde** was attempted. The triol (61.4 mg.) was dissolved in methanol (50 cc.) and a 0.025 *M* aqueous solution of periodic acid (7.0 cc., 1.20 molar equivalent) and water (18 cc.) was added. Titration of a 5.0-cc. aliquot after four hours showed that 1.02 moles per mole of substance had been consumed. After removal of most of the methanol *in vacuo* and addition of water the product was recovered by. ether extraction. The gummy ether residue crystallized on addition of ethyl acetate. Careful recrystallization from the latter solvent with only slight warming gave 20 mg. of small rods which melted at 113–114° after sintering at 110°, but in the subsequent two recrystallizations from hexane the melting point progressively fell and became quite unsharp. The product thus obtained (m. p. 100–106°, $[\alpha]^{24}D$ +75 ± 2° (*c*, 0.531)) was analyzed after drying at 78° (2 mm.) (no weight loss).

Anal. Calcd. for $C_{27}H_{46}O_3$ (418.6): C, 77.46; H, 11.07. Found: C, 76.75; H, 11.14.

Behavior of Disecondary *cis*- and Secondary-tertiary *cis*-Steroid Glycols toward Periodic Acid.—Accurately weighed (10–13 mg.) samples of the glycols were dissolved in 15.0 cc. of methanol, whereupon 1.50 cc. of 0.0252 *M* periodic acid (HIO_4) solution and 3.5 cc. of water were added. A blank solution containing the reagent and the solvents in the same proportion was simultaneously prepared. Aliquots of 5.0 cc. were withdrawn from both solutions after 1.25, 4 and 22 hours, and after the addition of 0.85 cc. of 0.5 *N* sulfuric acid, 2 cc. of 12% sodium acetate, 10 cc. of water and 0.3 cc. of 50% potassium iodide titrated with 0.01 *N* sodium thiosulfate solution. The periodic acid consumption was calculated from the difference between the titration values of the blank and the experimental solution at each time interval. As a rule the 1.25-hour blank value slightly decreased in the following 2.45-hour period, but then remained constant. The mole per mole uptake at 1.25, 4 and 22 hours, respectively, was as follows:

Cholestane-3(β),6,7-triol (VII): 0.73, 0.95, 0.95; Δ^5-cholestene-3(β),4(β)-diol[21]: 0.74, 0.85, 0.85. Methyl 3(α),11-(α),12(α)-trihydroxycholanate[22,23]: 0.51, 0.86, 1.00; 3(α),11(β),12(β)-trihydroxycholanic acid (formerly designated 3(α),11(β),12(α))[22]: 0.19, 0.34, 0.58, 0.63 (last value determined on 4-cc. aliquot after forty-eight hours).

The following compounds consumed no periodic acid within twenty-two hours: cholestane-3(β),7,8-triol[1]; cholestane-3(β),5(α),6(α)-triol[24,25]; equilin glycol (7,8-dihydroxyestrone).[26]

Chromic Acid Oxidation of Δ^6-Cholestenyl Acetate.—The acetate (303 mg., 0.706 millimole) was dissolved in acetic acid (120 cc.) and a solution of chromium trioxide (281 mg., 6 atoms of oxygen per mole of substance) in a few drops of water and 6 cc. of acetic acid was added. After standing at 24° for sixty-five hours the excess chromic acid was reduced with methanol. The solution was concentrated *in vacuo* to a sirup and the residue separated into neutral and acidic fractions in the usual manner.

(a) **Neutral Fraction.**—The neutral fraction (170 mg.) was combined with similar material from another run (total 343 mg.), dissolved in benzene (10 cc.) and hexane (40 cc.), and chromatographed on a column of sulfuric acid-washed alumina (17 × 250 mm.). The effluent was collected in 60-cc. portions. Elution was effected with benzene–hexane 1:4 (fractions 1–4); benzene–hexane 1:1 (fractions 5–10); benzene (fractions 11–15); ether–ben-

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zene 1:4 (fractions 16–23); ether (fractions 24, 25). The crystalline fractions among the above were combined as follows: A, fractions 2 and 3, 50 mg. (m. p. 95–101°); B, fractions 5–8, 124 mg. (m. p. 145–151°); C, fractions 11 and 12, 74 mg. (m. p. 110–141°); D, fraction 24, 12 mg. (m. p. 130–154°). Product A on recrystallization from methanol yielded needles (30 mg.) showing a constant melting point of 107–108°; $[\alpha]^{24}D$ +13 ± 3° (*c*, 0.568).

Anal. Calcd. for $C_{29}H_{48}O_2$ (428.6): C, 81.25; H, 11.29. Found: C, 81.23; H, 11.58.

Since it seemed possible that this product might be impure Δ^7-cholestenyl acetate, a sample (25 mg.) was subjected to catalytic hydrogenation (acetic acid, platinum oxide) to see whether it could be isomerized to the easily identifiable $\Delta^{8(14)}$-cholestenyl acetate (m. p. 78°). However, this was not the case, as about one half mole of hydrogen was taken up, and the recovered material melted at 99–104° in the crude state, and 107–108° after two recrystallizations from methanol, $[\alpha]^{24}D$ +18 ± 2° (*c*, 0.389). The melting point was not depressed on admixture of cholestanyl acetate (m. p. 108–109°). The nature of the original substance, probably a mixture, thus remains obscure.

Product B after purification was identified as 3(β)-acetoxycholesten-7-one (7-ketocholesteryl acetate, VIII). After several recrystallizations from methanol it melted at 154–156° and gave no depression with an authentic specimen, m.p. 156–158°, $[\alpha]^{24}D$ −103 ± 1° (*c*, 0.838); $\lambda_{max.}^{alc.}$ 235 mμ, ε = 12,050.

Anal. Calcd. for $C_{29}H_{46}O_3$ (442.7): C, 78.68; H, 10.48. Found: C, 78.49; H, 10.36.

Product C consisted for the most part of the diacetoxycholestanone X. It was recrystallized twice from methanol, from which it formed hexagonal plates (19 mg.) melting at 164–165°; $[\alpha]^{23}D$ −77 ± 1° (*c*, 0.693); $\lambda_{max.}^{alc.}$ 295 mμ, ε = 90.

Anal. Calcd. for $C_{31}H_{50}O_5$ (502.7): C, 74.06; H, 10.03; CH_3CO, 17.1. Found: C, 73.87, 74.23; H, 10.29, 9.70; CH_3CO, 17.0.

A solution of 10 mg. of the diacetoxyketone in acetic acid (10 cc.) containing a drop of concentrated hydrochloric acid was heated on the water-bath for one hour. A 0.2-cc. aliquot was removed, and its contents recovered by ether extraction. The ultraviolet spectrum (in ethanol) showed a well-defined maximum at 260 mμ (ε = 360). By continuing the heating for five hours after another drop of hydrochloric acid had been added the extinction at 260 mμ was about doubled. The position of the maximum and its low intensity indicated that no appreciable amounts of 7-ketocholesteryl acetate were formed under these conditions. From the remainder of the solution some unchanged starting material was recovered.

The diacetoxyketone did not react with ethanolic 2,4-dinitrophenylhydrazine reagent in the cold or on warming, nor was there evidence of hydrazone formation after prolonged heating in acetic acid solution. That a neighboring acetoxy group may prevent this reaction also has been observed in the case of methyl 3(α),11(α)-diacetoxy-12-ketocholanate.[22]

The substance obtained from Product D on recrystallization from aqueous methanol (7 mg. of needles, m. p. 159–163°) was not further investigated.

(b) Acidic Fraction; 3(β)-Acetoxycholestane-6|7-dicarboxylic Acid (XII).—The acidic fraction (131 mg.) obtained in the above experiment crystallized on wetting with acetone. Recrystallization from acetone–water 1:1 yielded small rods (97 mg.) melting at 209–212°. The product was recrystallized once more from this solvent mixture, and finally from ethyl acetate–hexane (m. p. 212–213°, $[\alpha]^{24}D$ + 23° ± 2° (*c*, 0.519)).

Anal. Calcd. for $C_{29}H_{48}O_6$ (492.6): C, 70.69; H, 9.82; neut. equiv., 246.3. Found: C, 70.49; H, 9.79; neut. equiv., 240.

3(β)-Hydroxycholestane-6|7-dicarboxylic Acid (XIII).—A solution of the acetoxy acid (107 mg.) in 90% methanol containing 5% of potassium hydroxide (6 cc.) was allowed to stand at 24° for twenty-one hours. After the addition of water the methanol was removed *in vacuo*, and the hydrolysis product was recovered in the usual way by acidification to *p*H 2 and ether extraction. The partly crystalline residue of the washed and dried ether extract was subjected to the procedure used by Shoppee[11] for the removal of tenaciously held water from the Windaus–Stein acid (dissolving in anhydrous dioxane and drying by repeated azeotropic distillation with benzene). The dried product was recrystallized twice from acetone–pentane 1:10, from which it formed small rods melting at 224–225° with effervescence; $[\alpha]^{24}D$ +45.7 ± 2° (*c*, 0.552 in abs. ethanol).

Anal. Calcd. for $C_{27}H_{46}O_5$ (450.6): C, 71.96; H, 10.29. Found: C, 72.08; H, 10.22.

Shoppee[11] determined the melting point of the water-free 3(β)-hydroxycholestane-6|7-dicarboxylic acid on the Kofler block and found 241–242°. Windaus and Stein[10] give the melting point of the monohydrate as 239–240°, but in a footnote stress the necessity of rapid heating to attain this value. They ascribe the lower and variable melting points observed on slow heating to "anhydride" (*i. e.*, lactone) formation. The lower value found by us with our own preparation and with the three specimens supplied by Dr. Shoppee is probably to be explained on this basis. The latter samples differed in derivation as follows: Sample I was prepared by the Windaus–Stein procedure; Sample II, by Dr. Shoppee's own procedure via the 6,7-diketone; Sample III had been obtained in 1937, likewise via the 6,7-diketone, by Dr. E. R. H. Jones, but could then not be satisfactorily identified, as it melted at 225–226°; however, after drying by distillation with benzene in Dr. Shoppee's laboratory the melting point was 241–242° on the Kofler block (*cf.*[11]). In our hands Sample I melted at 213–216°, II at 221.5–224°, and III at 206–209°. The melting point of II was not depressed by admixture of our own sample melting at 223–225°. The rotation values determined on I ($[\alpha]^{25}D$ + 46.8 ± 2° (*c*, 0.543)) and III ($[\alpha]^{25}D$ +45.7 ± 2° (*c*, 0.639)) were in good agreement with our own (+45.7°).

For further identification the hydroxy dicarboxylic acid (37 mg.) was converted into the 6 → 3 lactonic acid XIV by short boiling with acetic anhydride.[12,11] The crude product crystallized on seeding with a specimen supplied by Dr. Shoppee. Repeated recrystallization from ether–pentane afforded needles melting at 204–207°. A mixture with the reference sample (m. p. 201–211.5°) showed the melting point of the latter, $[\alpha]^{25}D$ + 1.5 ± 1° (*c*, 0.907); reference sample: +4.9 ± 2° (*c*, 0.509).

Anal. Calcd. for $C_{27}H_{44}O_4$ (432.6): C, 74.96; H, 10.25. Found: C, 74.79; H, 10.51.

Δ^2-Cholesten-7(α)-yl Benzoate (XVa).—For preparation see first section, Experimental. The pure substance melted at 136–138°, $[\alpha]^{26}D$ −27.7° ± 1° (*c*, 0.750).

Anal. Calcd. for $C_{34}H_{50}O_2$ (490.7): C, 83.20; H, 10.27. Found: C, 83.10; H, 10.23.

Δ^2-Cholesten-7(α)-ol (XVI).—The above benzoate was completely resistant to hydrolysis with a 7 molar excess of sodium methylate in boiling methanol–benzene 1:1 (five hours). However, complete hydrolysis was effected when a solution containing 80 mg. in 20 cc. of 10% methanolic potassium hydroxide solution was boiled under reflux for four hours. After dilution with water and removal of the methanol *in vacuo* the product was recovered in the usual way by ether extraction (58 mg.). It was recrystallized from 85% methanol (needles, m. p. 71–80°). The sample for the analysis and rotation measurements was dried at 66° (2 mm.) for three hours (2.5% weight loss) and then melted at 80–85°; $[\alpha]^{24}D$ +41.7 ± 2° (*c*, 0.611); +41.6 ± 2° (*c*, 0.575). The large increase in dextrorotation consequent to hydrolysis ($\Delta[M]D$ +299°) seems anomalous.

Anal. Calcd. for $C_{27}H_{46}O$ (386.6): C, 83.87; H, 11.99. Found: C, 83.55; H, 11.92.

For the conversion to cholestan-7-one the stenol (30 mg.) was hydrogenated in acetic acid (1.5 cc.) in the presence of platinum oxide (uptake 1.96 cc., calcd. 1.87 cc.), and the resulting product was oxidized with chromic acid (1.3 atoms of O, two days at room temperature). The crude oxidation product (23 mg.) on recrystallization from methanol yielded pure cholestan-7-one, m. p. 113.5–115°; $[\alpha]^{24}D$ −47° ± 2° (*c*, 0.566); $\lambda_{max.}^{alc.}$ 292 mμ, ε = 40.

Anal. Calcd. for $C_{27}H_{46}O$ (386.6): C, 83.87; H, 11.99. Found: C, 83.77; H, 11.82.

3(β)-Hydroxycholestan-7(α)-yl Benzoate (XVII).—The partial hydrolysis of 3(β)-acetoxycholestan-7(α)-yl benzoate was effected with sodium methylate as previously described for the dibenzoate of 7″α″-hydroxycholesterol.[27] The amorphous benzoate (1 g.) was dissolved in benzene (20 cc.), and sodium methylate (700 mg.) dissolved in absolute methanol (35 cc.) was added. After standing at room temperature for eighteen hours the product was precipitated by the addition of water and extracted with ether. The residue of the washed and dried ether solution crystallized on triturating it with warm pentane, yielding 800 mg. of hairlike needles melting at 143–153°. The absorption spectrum ($\lambda_{max.}^{alc.}$ 230 mμ, ε = 11,000) showed that the hydrolysis had not affected the benzoxy group. A part of the material was recrystallized twice from warm hexane and then showed a constant melting point of 154–157° after slight sintering at 151°; $[\alpha]^{24}D$ −11.4 ± 1° (*c*, 1.091). The analytical sample was dried to constant weight at 110° (2 mm.).

Anal. Calcd. for $C_{34}H_{52}O_3$ (508.8): C, 80.26; H, 10.30. Found: C, 80.07; H, 10.56.

3(α)-Chlorocholestan-7(α)-yl Benzoate (XVIII).—A solution of the above crude preparation of XVII (m. p. 143–153°, 725 mg.) in anhydrous pyridine (14 cc.) and phosphorus oxychloride (2.5 cc.) was refluxed for two hours. After cooling and cautiously adding ice water the mixture was worked up by ether extraction. The ether extract was washed successively with dilute sulfuric acid, sodium bicarbonate solution and water, dried and evaporated. The crystalline residue was recrystallized by dissolving it in the minimum amount of ether and adding excess methanol. After several recrystallizations there were obtained 350 mg. of needles melting at 167.5–169°, $[\alpha]^{24}D$ −4.3 ± 1° (*c*, 1.020).

The same product was obtained by treating crude XVII (495 mg.) with phosphorus pentachloride (425 mg.) in the presence of calcium carbonate (500 mg.) in the manner described for the preparation of 3(β)-acetoxycholestanyl-7-chloride.[1] The crude product was recrystallized twice from ether–methanol, yielding 197 mg. of needles (m. p. 166–168°, undepressed by admixture of the sample prepared with phosphorus oxychloride).

Anal. Calcd. for $C_{34}H_{51}O_2Cl$ (527.2): C, 77.45; H, 9.75; Cl, 6.72. Found: C, 77.27; H, 10.08; Cl, 6.90.

For the dehydrohalogenation to XVa a mixture of the chloro derivative (100 mg.), anhydrous potassium acetate (1 gm.) and *n*-valeric acid (3 cc.) was heated at the reflux temperature for twenty-four hours.[16] After cooling 2 *N* sodium carbonate solution (10 cc.) and excess ether was added, and some black insoluble material which had formed during the reaction was removed by passing both phases through a suction filter. The ether phase, after repeated washings with sodium carbonate solution and water, was dried and evaporated. The residue, a yellow glass, was chromatographed in pentane solution on a column of sulfuric acid-washed alumina (10 × 160 mm.). Continued washing with pentane (collected in 40-cc. portions) eluted a small amount of oil, which was followed by two nicely crystalline fractions. These were combined (34 mg.) and recrystallized from ether–methanol (22 mg., m. p. 136–139°, not depressed on admixture of Δ²-cholestenyl benzoate from the pyrolysis reaction) $[\alpha]^{28}D$ −23.4 ± 1° (*c*, 1.073).

Anal. Calcd. for $C_{34}H_{50}O_2$ (490.7): C, 83.20; H, 10.27. Found: C, 83.16; H, 10.21.

Δ²,⁶-Cholestadiene.—For the isolation from the pyrolysis mixture see first section, Experimental. The analytical sample was recrystallized from absolute acetone, and after drying at 56° (2 mm.) melted at 70–72°; $[\alpha]^{25}D$ −3.7 ± 1° (*c*, 1.09).

Anal. Calcd. for $C_{27}H_{44}$ (368.6): C, 87.97; H, 12.03. Found: C, 87.93; H, 11.93.

The mole per mole perbenzoic acid consumption at 4° was 1.53 after six hours, and 2.04 after thirty hours.

The absorption spectrum in ethanol showed maxima at 237 mμ (ε = 925) and 305 mμ (ε = 70). A sample heated in 1 cc. of absolute ethanol containing 0.02 cc. of concentrated hydrochloric acid for six hours and then recovered by ether extraction showed ε 237 mμ = 1900, while the maximum at 305 mμ was no longer in evidence. There was no further increase in the extinction at 237 mμ when the heating was continued for four days. Boiling in acetic acid containing a drop of concentrated hydrochloric acid merely raised the extinction below 230 mμ, so that the former maximum at 237 mμ appeared now as a poorly defined shoulder (ε 237 mμ = 1050) on the end absorption part of the curve.

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Summary

It has been shown that the levorotatory cholestenyl acetate obtained by Plattner, *et al.*,[6] by pyrolysis of 3(β)-acetoxycholestane-7(α)-benzoate is Δ⁶-cholesten-3(β)-yl acetate, and not, as these authors contend, the Δ⁷-isomer. The salient points in the structure proof are (1) its conversion with osmic acid into a cholestane-triol in which all three hydroxyl groups are readily esterifiable and which therefore must be cholestane-3(β),6,7-triol, and (2) the fact that on oxidation with chromic acid it yields the acetyl derivative of the known 3(β)-hydroxycholestane-6||7-dicarboxylic acid.

The neutral products formed on oxidation with chromic acid contained 7-ketocholesteryl acetate and a diacetoxyketone which is probably 3(β),6-diacetoxycholestan-7-one. A reaction mechanism which envisages a 6,7-oxide as the primary oxidation product is proposed.

Two by-products formed in the pyrolysis reaction have been identified as Δ²-cholesten-7(α)-yl benzoate and Δ²,⁶-cholestadiene.

Incidentally it was observed that secondary–tertiary *cis*-glycols of the steroid class are not attacked by periodic acid under conditions under which di-secondary *cis*-glycols are readily oxidized by this agent.

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