bonate (1 g.) and dry chloroform (12 cc.) was cooled to 0° in a glass-stoppered flask. Freshly sublimed phosphorus pentachloride (850 mg.) was added in small portions to the contents over a period of one and three-fourths hours, while the flask was shaken mechanically in an ice-bath. The shaking was continued for one-half hour, when 15 cc. of a cold saturated sodium bicarbonate solution was added to the mixture and the shaking resumed for another one-half hour. The chloroform solution was separated, filtered, dried and evaporated. The slightly brownish crystalline residue (1.09 g.) was recrystallized several times from absolute ethanol, yielding needles (725 mg.) melting at 118–119°, $[\alpha]^{23}D$ −21.7° (−0.26°, 12.27 mg. in 1.025 cc., 1 dm.).

Anal. Calcd. for $C_{29}H_{49}O_2Cl$: C, 74.86; H, 10.62; Cl, 7.63. Found: C, 74.76; H, 10.37; Cl, 7.78.

Cholestanol-3(β) Chloride-7.—The acetate (100 mg.) was refluxed with 20% methanolic potassium hydroxide (5 cc.) for one hour, and the material recovered by ether extraction. By two recrystallizations from methanol long needles (56 mg.) melting at 170.5–171.5° were obtained; $[\alpha]^{22}D$ −19.8° (−0.248°, 12.5 mg. in 2 cc., 2 dm.).

Anal. Calcd. for $C_{27}H_{47}OCl$: C, 76.63; H, 11.20; Cl, 8.39. Found: C, 76.55; H, 10.99; Cl, 8.75.

The compound is precipitated by digitonin in 80% ethanol, yielding a crystalline digitonide melting in the crude state at 192–197°.

Boiling of the chloroacetoxy compound with 12% aqueous potassium hydroxide solution for seven hours eliminated only traces of hydrogen chloride. The reaction product was identical with that obtained with methanolic alkali, but the yield was less satisfactory.

Di-(3-(β)-acetoxycholestanol-7(α))-sulfurous Ester.—3(β)-Acetoxycholestanol-7(α) (527 mg.), dissolved in absolute ether (8 cc.), and calcium carbonate (2.5 g.) were shaken at ice temperature, while thionyl chloride (1.6 g.) was added in small portions over a period of three-fourth hour. The mixture was allowed to stand in the refrigerator overnight. It was then transferred to a separatory funnel containing crushed ice. More of the solvent was added, and the ether phase was thoroughly extracted with saturated bicarbonate solution and washed with water. The residue of the dried ether solution, dissolved in a few drops of ethanol, crystallized after several days in the refrigerator. The crystals were filtered, washed with a little cold alcohol (329 mg.). Several recrystallizations from pentane yielded rosets of white needles (76 mg.) melting at 131.5–133.5°.

Anal. Calcd. for $C_{58}H_{98}O_7S$: C, 74.14; H, 10.52; S, 3.42. Calcd. for $C_{58}H_{98}O_7S \cdot H_2O$: C, 72.72; H, 10.53; S, 3.35. Found: C, 73.20; H, 10.66; S, 3.75, 3.64.

21.6 mg. of the compound was refluxed with 2 cc. of 5% methanolic potassium hydroxide for one and one-half hours. The material recrystallized from methanol melted at 167–168° and gave no depression of the melting point in mixture with cholestanediol-3(β),7(α).

The microanalyses were carried out by Mr. J. F. Alicino.

Summary

The 7-epimeric 3(β)-acetoxycholestanols-7 have been prepared by catalytic reduction of 7-ketocholesteryl acetate or of 7-ketocholestanyl acetate. The corresponding diols and several of their derivatives are described. The epimeric compounds have been correlated sterically with other epimeric 7-hydroxysteroids by rotation data.

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[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

The Dehydration of the 7-Epimeric 3(β)-Acetoxycholestanols-7. Some Transformation Products of γ-Cholestenol

BY O. WINTERSTEINER AND MILDRED MOORE

For the extension of our studies on the autoxidation of sterols,[1] we needed the double bond isomers of cholesterol with ethylenic bonds in the positions 8–14, 14–15, 7–8 and 8–9 (α,β,γ, and δ-cholestenols, respectively). None of these isomers are easily accessible. The first three have been prepared so far only via 7-dehydrocholesterol,[2] which is difficult to obtain in pure form, and the preparation of which in the amounts needed by us is troublesome. The starting material for δ-cholestenol, in which we were particularly interested, is the practically inaccessible isodehydrocholesterol ($\Delta^{6,8-9}$-cholestandienol-3-(β)), a by-product in the industrial manufacture of 7-dehydrocholesterol.[3] An alternative route to this cholesterol isomer was suggested by the observation of Eck and Hollingsworth[4] that cholestanol-7 on treatment with anhydrous copper sulfate in boiling xylene containing a small amount of propionic acid is dehydrated to Δ^{8-9}-cholestene. When this reaction was applied to 3(β)-acetoxycholestanol-7(β)[5] it yielded crystalline products which, however, in spite of their homogeneous appearance proved to be mixtures,

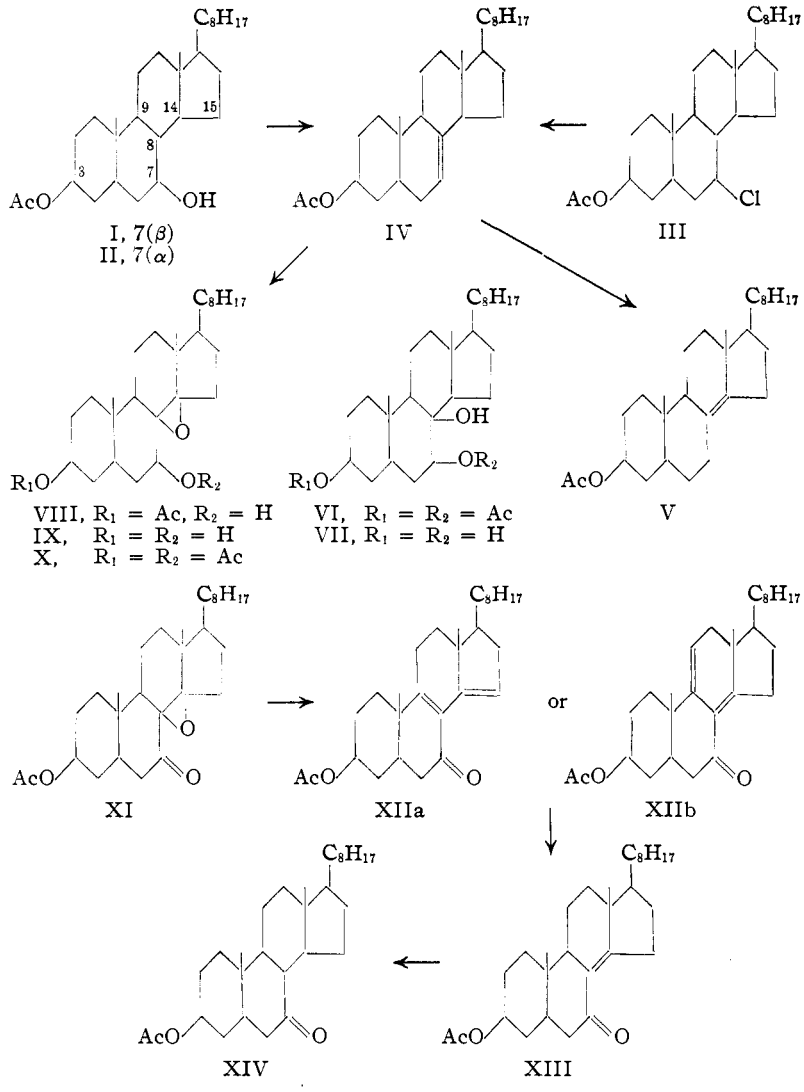
(1) Bergström and Wintersteiner, *J. Biol. Chem.*, **141**, 597 (1941); **145**, 327 (1942).

(2) Schenk, Buchholz and Wiese, *Ber.*, **69**, 2696 (1936).

(3) Windaus, Linsert and Eckhardt, *Ann.*, **534**, 22 (1938).

(4) Eck and Hollingsworth, THIS JOURNAL, **63**, 2986 (1941).

(5) Wintersteiner and Moore, *ibid.*, **65**, 1503 (1943).



practically inseparable by physical means, of isomeric cholestenyl acetates. The melting point (108–112°) and specific rotations (+5.0 to 6.5°) of the purified fractions ranged between those of γ-cholestenyl acetate (m. p. 119°, $[\alpha]^0D$) and δ-cholestenyl acetate (m. p. 108°, $[\alpha]D$ +13.8°). These properties excluded α-cholestenyl acetate (m. p. 78°) and β-cholestenyl acetate (m. p. 92°) as major components. As will be shown below, γ-cholestenyl acetate (IV) is the main constituent of the mixture. The dextrorotatory contaminant could not be identified, but on the basis of Eck and Hollingsworth's observations it seems likely that it is either δ- or α-cholestenyl acetate. Also, the presence of the yet undescribed Δ^6-cholestenyl acetate has to be reckoned with.

The epimeric 3(β)-acetoxycholestanol-7(α) (II) is much more resistant to dehydration by this method, most of the material remaining unchanged under conditions yielding a high proportion of dehydrated products from the 7-(β) epimer (I).

A variety of other direct and indirect methods of dehydration were subsequently explored, but whenever double bond formation occurred the crystallizable part of the reaction product consisted of isomeric mixtures of approximately the same properties as those obtained by the dehydration with copper sulfate and xylene (subsequently referred to as method (a)). The alternative procedures were: (b) treatment of 3-acetoxycholestanol-7(β) with toluenesulfonylchloride in boiling pyridine; (c) treatment of the same compound with phosphorus pentachloride in chloroform at 0°; (d) elimination of toluenesulfonic acid from 3-acetoxycholestanyl-7(α)-tosylate with pyridine and sodium iodide; (e) elimination of hydrogen chloride from 3-acetoxycholestanyl chloride-7(α) (III) with acetic acid and potassium acetate at 130°. Of these five procedures, reactions (b) and (e) gave the highest yields of crystallizable products (60–70%); method (b) was adopted for routine purposes as the starting material was more readily available, and the reaction product appeared to contain a higher proportion of γ-cholestenyl acetate than those from the other procedures. In fact it can serve as a substitute for the pure compound in further transformations. Material of this description is referred to in the following as "crude γ-cholestenyl acetate."

Both γ(Δ^{7-8})-cholestenol and δ(Δ^{8-9})-cholestenol are isomerized by palladium and hydrogen to α(Δ^{8-14})-cholestenol[2,3] which as such is resistant to hydrogenation, but can be converted with hydrogen chloride in chloroform to the reducible β(Δ^{14-15})-cholestenol.[2] When "crude γ-

cholestenyl acetate" from reactions (a) or (b) was subjected to the catalytic reaction, α-cholestenyl acetate (V), easily distinguishable from the other isomers by its low melting point (78°), was obtained in good yield. This result shows that the bulk of the starting material consisted of either γ or δ-cholestenyl acetate, but does not exclude the presence of small amounts of α-cholestenyl acetate itself, or of Δ^6-cholestenyl acetate. At any event, the method provides a practicable route to α- and β-cholestenol which is operatively simpler and more convenient than the route via 7-dehydrocholesterol.

When "crude γ-cholestenyl acetate" reacted with osmium tetroxide and the reaction product was reacetylated after reductive hydrolysis of the intermediate osmic acid ester, a cholestanetriol diacetate m. p. 169° (VI) was obtained. Hydrolysis yielded the free triol, m. p. 178° (VII). The formation of a cholestanetriol with two esterifiable hydroxyl groups proves that the major part of the starting product consisted of γ-cholestenyl acetate. δ-Cholestenyl acetate should be expected to yield with osmium tetroxide a ditertiary 8,9-glycol, or more likely an α,β-unsaturated tertiary alcohol, in analogy with the conversion of isoequilin A to Δ^{9-11}-8-hydroxyequilin.[6]

According to Schenk, Buchholz and Wiese,[2] γ-cholestenol on treatment with 2 moles of perbenzoic acid yields a compound $C_{27}H_{48}O_3$ (m. p. 192°) to which the authors assigned the structure of a cholestanetriol-3,7,8 (VII), since on acetylation it formed a diacetate (m. p. 165°). Under similar conditions δ-cholestenol is converted into an unsaturated diol, probably Δ^7-cholestenediol-3,9.[3]

When "crude γ-cholestenyl acetate" from reactions (a), (b) and (e) was treated with 2 moles of the reagent, a compound $C_{29}H_{48}O_4$ (m. p. 123°) was obtained. Neither its hydrolysis product (m. p. 187°), nor the diacetate (m. p. 163°) were identical with compounds VII and VI from the reaction with osmium tetroxide. Nor can we definitely affirm their identity with Schenk's products, as our compounds have somewhat lower melting points, and no rotation data are recorded in the paper of the German authors. Furthermore, the analyses of our hydrolysis product of m. p. 187° and its two derivatives are in better agreement with formulas demanding two hydrogen atoms less than a cholestanetriol, but this is true also of one of Schenk's analyses. Lastly, we observed that both moles of perbenzoic acid were invariably consumed in the reaction, whereas only one mole is required for the formation of a triol via the intermediate oxide. That the structure of our substance, at any rate, is represented not by VII but by IX was shown by the following transformations.

The monoacetate m. p. 123° (VIII) was oxidized with chromic acid to a monoketone (XI). XI on treatment with hydrochloric acid in alcohol gave rise to a doubly unsaturated ketone (XIIa or XIIb) which exhibited strong selective absorption in the ultraviolet region with a maximum at 297 mμ. Structure XIIb is in better accord with the absorption characteristics, though on chemical grounds XIIa deserves preference. At any event, the formation of two new double bonds in the purely hydrolytic reaction XI → XII proves that the non-esterifiable oxygen atom in VIII is present in form of an epoxide linkage or of a group representing an equivalent state of oxidation.

The proof for the 8,14-position of the epoxide group in VIII and XI rests on the formation of the latter from α-cholestenyl acetate by chromic acid oxidation, described in the following paper.

The formation of the dioloxide monoacetate VIII from γ-cholestenyl acetate evidently occurs by dehydration of an intermediate triol to a Δ^{8-14}-diol-3,7, the double bond of which then reacts with the second mole of perbenzoic acid to form the oxide ring. The search for such intermediates only resulted in the isolation of small amounts of an isomer of VIII melting at 146°, which on acetylation yielded a diacetate and therefore seems to be likewise derived from γ-cholestenol. The cause of the isomerism is probably steric (at C_7 or the epoxide group). When only one mole of perbenzoic acid was used, again only compound VIII could be isolated, but in much smaller amounts than were obtained in the reaction with 2 moles.

The conclusive proof for the position of the keto group in XI was brought about by stepwise reduction of the dienone XII to 7-keto-cholestanyl acetate. XII was hydrogenated in alcohol with palladium catalyst to the singly unsaturated ketone XIII, characterized by its absorption spectrum, which exhibits a single maximum at 262.5 mμ. The 8,14-position of the double

(6) Hirschmann and Wintersteiner, *J. Biol. Chem.*, **126**, 737 (1938).

bond is inferred from this location of the maximum on the basis of the generalizations advanced by Woodward.[7] Structure XIII represents an α,β,β-substituted ketone with a doubly exocyclic ethylenic bond and should absorb in the region around 257 mμ, while for a Δ^{8-9}-7-ketone, in which the double bond is equally substituted but not exocyclic to any ring, the maximum should lie around 247 mμ.[8] XIII was then hydrogenated in acetic acid with the same catalyst, whereby it was converted to a mixture of α-cholestenyl acetate and 7-ketocholestanyl acetate (XIV).

Experimental

Preparation of "Crude γ-Cholestenyl Acetate."—(a) 3(β)-Acetoxycholestanol-3(β) (1 g.) and anhydrous copper sulfate (1 g.) were refluxed in 5 cc. of xylene containing 0.03 cc. of propionic acid for five hours. After dilution with 10 cc. of hexane the solution was filtered and the filtrate was passed through a column of aluminum oxide (1.7 × 22 cm.). Continued washing with hexane (900 cc.) removed almost all of the material, without effecting any marked fractionation (m. p.'s 88–89° to 92–94°). Recrystallization from 18 cc. of ethyl acetate–methanol (1:5) yielded long shiny needles (680 mg., m. p. 93–95°). On continued recrystallization from the above mixture or from methanol alone, 250 mg. melting at 105–109° was obtained. The melting point could not be raised by further recrystallization, $[\alpha]^{28}D$ +5.0° (0.08°, 15.92 mg. in 2 cc., 2 dm.).[9] More material with similar properties could be recovered from the mother liquors. Other fractions with lower melting points (m. p. 91–92°, 72–80°) gave somewhat higher rotation values (+7 to 9°).

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.23; H, 11.29. Found: C, 81.42; H, 11.41.

52 mg. of the top fraction was hydrolyzed with hot methanolic potassium hydroxide. The melting point of the hydrolysis product (32 mg., needles from methanol) was 121–122°, and did not change on further crystallization; $[\alpha]^{28}D$ +9.4° (0.06°, 6.55 mg. in 1.025 cc., 1 dm.).

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.86; H, 12.00. Found: C, 83.51; H, 11.78.

Extension of the reaction time to eight hours resulted in a lower yield of crystallizable products.

When 3(β)-acetoxycholestanol-7(α) (102 mg.) was treated as described above with the proportionate amounts of reagents, the product on chromatographing yielded in the hexane washings 30 mg. of needles, m. p. 88–94°, recrystallized m. p. 90–107°. The rest of the material, eluted with ether, was identified as starting material (m. p. 72–74°).

(b). 3(β)-Acetoxycholestenol-7(β)(5 g.) and *p*-toluenesulfonyl chloride (5 g.) were refluxed in pure pyridine (50 cc.) for eight and one-half hours. The solvent was removed by distillation *in vacuo*, the residue taken up in ether, and the ether solution washed successively with several portions of dilute sulfuric acid, potassium carbonate solution and water. The crystalline residue from the dried and evaporated ether solution (4.7 g.) was dissolved in hexane and chromatographed in the usual manner on aluminum oxide (3.3 × 12.5 cm.). Elution was effected with hexane (700 cc.), hexane–benzene 1:1 (400 cc.), benzene (300 cc.) and ether (300 cc.), in 40-cc. portions. Only about 300 mg. was eluted by the last two solvents, proving that there was little starting material present in the mixture. That some fractionation of the remainder of the material had occurred was indicated by the rising melting points and decrease in dextrorotation as the elution progressed. Thus fractions eluted by the first 200 cc. of hexane, recrystallized together from methanol–ethyl acetate, yielded 862 mg. with m. p. 101–106°, $[\alpha]D$ +9.4°, while the crystals from the combined hexane–benzene eluates (529 mg.) melted at 102–112° and had an $[\alpha]D$ +5.5°. But extensive fractional crystallization of these and other fractions failed to afford any products with the characteristics of a pure compound. This may be illustrated by the behavior of the hexane–benzene fraction on successive recrystallizations: (1) m. p. 113–116.5°, $[\alpha]D$ +4.7°; (2) m. p. 115–116.5°, $[\alpha]D$ +0.5°; (3) m. p. 115–117°, $[\alpha]D$ +2.2°; (4) m. p. 115–117°, $[\alpha]D$ +4.5°. The melting point of the final product was strongly depressed by admixture of the starting compound. Hydrolysis yielded fine needles with a constant melting point of 122–123°, $[\alpha]D$ +6.5°, and reacetylation of the latter an acetate of improved melting point (118–119°), but still showing about the same dextrorotation (+4.2°) as before hydrolysis. Apparently the original purified product consisted essentially of γ-cholestenyl acetate contaminated with a small amount of a dextrorotatory isomer. (Schenk, *et al.*[2]: γ-cholestenyl acetate, m. p. 118–119°, $[\alpha]D$ 0; γ-cholestenol: m. p. 122–123°, $[\alpha]D$ 0.) Analyses of several other chromatographic fractions which were similarly purified also indicated that the presence of isomers was the sole cause of their inhomogeneity.

It was found later that fractionation of the reaction product could be dispensed with, the crude preparations being suitable for further transformations. These were obtained by dissolving the ether residue in hot ethyl acetate (2 cc. per g.) and adding 5 volumes of warm methanol. Needles, or occasionally plates which changed into needles on standing overnight, deposited on slow cooling. Refluxing of the mixture for twelve hours gave the best results, while heating on the steam-bath for twenty-four hours resulted in a lower yield. The yield from 5–10 g. batches was 55–65% of the theoretical, not including additional crops from the mother liquor. The melting points ranged from 98–106° to 104–112°, while the rotation values were much more uniform (+1.1 to 2.5°).

Treatment of such "crude γ-cholestenyl acetate" with copper sulfate and xylene according to (a) yielded slightly levorotatory material $[\alpha]^{22}D$ −1.9° of similar appearance and melting point as the starting product, showing that no isomerization to either of the dextrorotatory isomers α- or δ-cholestenyl acetate had occurred.

(c). 3(β)-Acetoxycholestanol-7(β) (540 mg.), dissolved in chloroform (6 cc.), was treated in the presence of dry

(7) Woodward, THIS JOURNAL, **64**, 76 (1942).

(8) *Cf.* the studies by Stavely and Bollenback, *ibid.*, **65**, 1285 1290 (1943), who have obtained ketones of both types in the ergosterol series.

(9) The solvent used in all rotation measurements reported in this paper was chloroform.

calcium carbonate (320 mg.) with phosphorus pentachloride (460 mg.) as described previously for the 7(α)-epimer.[5] The reaction product crystallized partly on addition of a small volume of ethanol and standing in the refrigerator (155 mg., m. p. 97–113°). By several recrystallizations from methanol 63 mg. of needles melting at 112–115° was obtained; $[\alpha]^{23}D$ +5.4° (+0.06°, 11.36 mg. in 1.025 cc., 1 dm.).

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.23; H, 11.29. Found: C, 81.18; H, 11.18.

(d). 3(β)-Acetoxycholestanyl-7(α)-tosylate (98 mg.) was heated in a bomb tube with 300 mg. of sodium iodide and 3 cc. of pyridine at 120° for twenty-four hours. After addition of water and some sodium thiosulfate, the reaction product was recovered in the usual way by ether extraction. By several recrystallizations from methanol needles melting at 113–115° were obtained. Admixture of a preparation of the same melting point obtained by method (b) caused no depression; $[\alpha]^{21}D$ +3.8° (+0.07°, 18.56 mg. in 2 cc., 2 dm.).

(e). 3(β)-Acetoxycholestanyl chloride-7(α) (1 g.) was refluxed for five hours in 21 cc. of glacial acetic acid containing 9 g. of potassium acetate. The mixture was poured into 250 cc. of ice-water and extracted with ether. The ether phase was washed twice with water, and the chloride ion in the combined aqueous solutions determined gravimetrically. Calcd.: Cl, 77.4 mg. Found: Cl, 75.9 mg. The ether phase, after washing with bicarbonate solution and water, on evaporation yielded a crystalline residue (931 mg.) which after recrystallization from ethyl acetate–methanol melted at 103–105°, $[\alpha]^{27}D$ +6.8°. Further purification gave 445 mg. of needles, m. p. 106–109°, $[\alpha]^{23}D$ +7.6°. The melting point was not depressed on admixture of similar material from reaction (b).

α-Cholestenyl Acetate from "Crude γ-Cholestenyl Acetate."—For the preparation of larger batches we found it advantageous to carry out the reaction in glacial acetic acid instead of in ethyl acetate, the solvent used by Schenk, *et al.*[2] This ensured completion of the isomerization in one to two hours and a somewhat better yield. For example, 5.5 g. of starting material made by method (b) was dissolved in glacial acetic acid (250 cc.) and shaken in hydrogen with previously reduced palladium catalyst (500 mg.) for two hours. The solvent was removed from the filtered solution by vacuum distillation and the residue recrystallized twice from 97% ethanol, yielding 3.28 g. of large rhombohedral plates, m. p. 76–77°, $[\alpha]D$ +9.3° (+0.08°, 8.865 mg. in 1.025 cc., 1 dm.). (Schenk, *et al.*[2]: m. p. 77–78°, $[\alpha]D$ +9.4°.)

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.23; H, 11.29. Found: C, 81.52; H, 11.24.

About the same yield was obtained from "crude γ-cholestenyl acetate" m. p. 104–109° made according to method (a).

Hydrolysis of the acetate yielded needles m. p. 120–120.5°; $[\alpha]^{21}D$ +22.9° (0.468°, 20.4 mg. in 2 cc., 2 dm.). Schenk, *et al.*[2] give for α-cholestenol: m. p. 119–120°, $[\alpha]D$ +20.36°.

Cholestanetriol-3(β),7,8-diacetate.—"Crude γ-cholestenyl acetate" m. p. 104–108° (1 g.) and osmium tetroxide (650 mg.) were dissolved in absolute ether. The solution was allowed to stand at room temperature for six days. The ether was removed by distillation, and the residue dissolved in 30 cc. of absolute alcohol. After addition of sodium sulfite (6 g.) and water (25 cc.) the solution was boiled for one and one-half hours. The black precipitate was filtered off and washed with 200 cc. of hot 95% ethanol. The filtrate was concentrated to about 20 cc. and, together with the precipitate formed sodium sulfite, poured into 200 cc. of cold water. The reaction product was recovered by ether extraction; the partly crystalline residue (1.18 g.) from the washed and dried ether solution was recrystallized from methanol, yielding 272 mg. of needles m. p. 104–110°, which were identified as starting material. The mother liquor material was acetylated in pyridine and chromatographed in the usual manner. A further small amount of starting product was eluted with hexane–benzene 4:1; the bulk of the material was recovered with benzene–ether 1:1 (532 mg.). On recrystallization from methanol fine long needles of the diacetate m. p. 168–169° were obtained; $[\alpha]D$ −39.8° (−0.99°, 24.9 mg. in 2 cc., 2 dm.).

Anal. Calcd. for $C_{31}H_{52}O_5$: C, 73.75; H, 10.41. Found: C, 73.79, 73.76; H, 10.36, 10.41.

Cholestanetriol-3(β),7,8.—The diacetate (61 mg.) was refluxed for forty-five minutes with 5% methanolic potassium hydroxide (4 cc.). The product was recovered by ether extraction (52 mg.). Repeated recrystallization from methanol yielded fine needles which sintered at 170° and melted at 176–178°; $[\alpha]^{21}D$ −12.9° (−0.139°, 10.8 mg. in 2 cc., 2 dm.). The compound was not precipitated by digitonin in 80% ethanol.

Anal. Calcd. for $C_{27}H_{48}O_3$: C, 77.08; H, 11.42. Found: C, 77.13; H, 11.43.

3(β)-Acetoxycholestanol-7-oxide-8,14.—"Crude γ-cholestenyl acetate" m. p. 106–111° from reaction (a) (52.2 mg., 0.122 mmole), treated with an excess of perbenzoic for eight days at 8°, consumed 0.254 milliatoms of O, corresponding to 2.08 double bonds.

The same preparation (504 mg., 1.18 mmole) was treated under the same conditions with perbenzoic acid (2.46 mmole) in 18 cc. of chloroform After eight days all of the active oxygen had been consumed. The solution was washed with potassium carbonate solution and water, dried and evaporated. The residue, which crystallized on addition of a little acetone, was recrystallized from a small amount of the same solvent (153 mg., m. p. 110–114°), and then several times from methanol, from which it formed characteristic triangular platelets, m. p. 122–123°; $[\alpha]^{23}D$ +6 1° (0.107°, 17.5 mg. in 2 cc., 2 dm.). Including some material recovered from the mother liquors the yield was about 120 mg. Chromatographing of the resinous fractions yielded an additional amount (57 mg.) as well as the isomer described below.

Anal. Calcd. for $C_{29}H_{48}O_4$: C, 75.59; H, 10.50. For $C_{29}H_{50}O_4$: C, 75.26; H, 10.90. Found: C, 75.87, 75.54, 75.49; H, 10.44, 10.13, 10.47.

In repeating the reaction on starting material obtained by methods (b) and (e), the melting point could not be raised by crystallization beyond 118°. The crystalline portion of the crude product was therefore chromatographed. Elution with hexane–ether 9:1 yielded the diol oxide monoacetate in almost pure state. The subsequent eluates (hexane–ether 4:1) contained a higher

melting product, which was purified by rechromatographing. It crystallized from 80% ethanol in clear-cut square platelets m. p. 145.5–146°; $[\alpha]^{23}D$ +27.6° (0.49°, 17.7 mg. in 2 cc., 2 dm.).

Anal. Calcd. for $C_{29}H_{48}O_4$: C, 75.59; H, 10.50. Found: C, 75.76, 75.50; H, 10.43, 10.46.

On acetylation in pyridine it formed a diacetate which crystallized from methanol in form of rosets and melted at 63–64° after sintering at 59°.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.04; H, 10.03. Found: C, 74.39; H, 10.06.

Cholestanediol-3(β),7-oxide-8,14-diacetate.—The monoacetate m. p. 123° (48 mg.) was dissolved in pyridine (2 cc.) and acetic anhydride (1 cc.) and the mixture allowed to stand for forty-eight hours at room temperature. The product, recovered by ether extraction, was recrystallized several times from methanol, from which it formed elongated plates, m. p. 162–163°; $[\alpha]^{23}D$ −11.9° (−0.264°, 22.2 mg. in 2 cc., 2 dm.).

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.04; H, 10.03. Found: C, 73.64, 73.93; H, 9.91, 10.03.

Cholestanediol-3(β),7-oxide-8,14 was obtained by hydrolysis of either the mono- or diacetate by boiling with 5% methanolic potassium hydroxide for one-half hour. The compound formed characteristic rhombohedral plates (m. p. 186–187°) when recrystallized from a small volume of acetone or methanol; $[\alpha]^{21}D$ +8.1° (0.156°, 19.2 mg. in 2 cc., 2 dm.).

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.44; H, 11.10. Found: C, 77.82; H, 11.18.

The dioloxide yielded a crystalline precipitate (dec. 225°) with digitonin in 80% ethanol.

3(β)-Acetoxycholestanone-7-oxide-8,14.—To 3(β)-acetoxycholestanol-7-oxide-8,14 (159 mg.) dissolved in glacial acetic (3 cc.) a solution of chromium trioxide (45.7 mg., 2 atoms O) in 1 cc. of 80% acetic acid was added. After standing at room temperature for twenty-four hours the mixture was worked up in the usual way. The neutral fraction (150 mg.) was recrystallized repeatedly from 80% ethanol from which the ketone deposited as rhombohedral plates melting at 139.5–140°; $[\alpha]^{22}D$ −75.7° (−1.33°, 17.6 mg. in 2 cc., 2 dm.). The ultraviolet spectrum showed only weak end absorption below 240 mμ.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.92; H, 10.11. Found: C, 76.05; H, 10.12.

Attempts to prepare the semicarbazone by various methods (treatment with semicarbazide acetate in boiling 80% alcohol for two hours, or at room temperature for several days, with or without pyridine) led to amorphous mixtures which were extremely soluble in absolute or dilute ethanol. However, a crystalline 2,4-dinitrophenylhydrazone readily was obtained. To 19 mg. in 0.5 cc. of ethanol a saturated solution of the reagent in absolute alcohol containing 1% hydrochloric acid (1.5 cc.) was added. After a few minutes orange-colored needles separated which were filtered off after one hour. The crude preparation melted at 225–227°, which is also the melting point of the dinitrophenylhydrazone of the dienone described in the next section. A mixture of both derivatives melted at the same temperature. The analysis likewise indicated that the formation of the hydrazone had been accompanied by hydrolysis of the epoxide ring.

Anal. Calcd. for $C_{35}H_{48}O_6N_4$: C, 67.70; H, 7.80; N, 9.03. For $C_{35}H_{50}O_7N_4$: C, 65.79; H, 7.89; N, 8.78. Found: C, 67.72; H, 7.56; N, 8.75.

The preparation was recrystallized from benzene–ethanol 1:2 and then melted at 235–236°. There was no substantial change in the analytical composition: C, 67.88; H, 7.80; N, 9.13.

3(β)-Acetoxycholestadienone-7.—The ketoxide XI (102 mg.) was refluxed for two hours in 4 cc. of ethanol containing 0.2 cc. of concentrated hydrochloric acid. The yellow solution was diluted with water, extracted with ether, and the latter washed with potassium carbonate solution and water. The crystalline residue was reacetylated with acetic anhydride and pyridine at room temperature (twenty-four hours). The product thus obtained melted over a wide range (80–125°) and was therefore chromatographed on a column of aluminum oxide (1.6 × 8.5 cm.) in the usual manner. The compound appeared in the eluate with 100% benzene, the subsequent washings with ether–benzene 9:1 containing the resinous contaminants. Several recrystallizations from methanol yielded long filamentous needles (32 mg.) which sintered at 163° and melted at 166°; $[\alpha]^{21}D$ −17.6° (0.30°, 17.0 mg. in 2 cc., 2 dm.). The absorption curve is characterized by a maximum at 297 mμ, ε = 4800, a minimum at 257 mμ (ε = 1500), and strong end absorption below 240 mμ (in ethanol). The analysis had to be carried out on freshly prepared material, otherwise the carbon values showed deficits of 0.5–0.6%, apparently due to autoxidation.

Anal. Calcd. for $C_{29}H_{44}O_3$: C, 79.03; H, 10.07. Found: C, 78.78; H, 10.25.

The **2,4-dinitrophenylhydrazone** formed orange-colored needles melting at 225–228°.

Anal. Calcd. for $C_{35}H_{48}O_6N_4$: N, 9.03. Found: N, 8.54.

3(β)-Acetoxy-Δ^{8-14}-cholestenone-7.—The dienone XII (128 mg.), dissolved in absolute ethanol (12 cc.), was shaken with palladium catalyst (50 mg.) in hydrogen until 1 mol. (7.0 cc.) had been consumed, which was the case in five minutes. The residue of the filtered and evaporated solution was recrystallized twice from methanol, from which it formed square platelets melting at 141.5–142.5°. The yield was practically quantitative; $[\alpha]^{21}D$ −62.2° (−1.125°, 18.1 mg. in 2 cc., 2 dm.). The absorption curve shows a well-defined maximum at 262.5 mμ, ε = 9500 (in ethanol).

Anal. Calcd. for $C_{29}H_{46}O_3$: C, 78.67; H, 10.48. Found: C, 78.85; H, 10.44.

The **2,4-dinitrophenylhydrazone** was obtained in the form of orange-colored needles melting at 210–211°.

The unsaturated ketone (33 mg.) was hydrogenated in glacial acetic (3 cc.) in the presence of palladium catalyst. The hydrogen uptake stopped after three hours with about 1.5 mol. consumed. The residue of the filtered solution appeared inhomogeneous and was chromatographed in the usual manner: 19.5 mg. of α-cholestenyl acetate, m. p. 76–78°, and 3.5 mg. of 7-ketocholestanol acetate, m. p. 148–149.5°, were obtained. Neither of the preparations depressed the melting point of authentic specimens.

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Summary

The dehydration of the 7-epimeric 3(β)-acetoxy-cholestanols-7 by a variety of direct and indirect methods has been studied. In all cases the dehydration products consisted of a crystalline mixture of isomeric cholestenyl acetates resistant to separation by physical means. However, by chemical methods it could be demonstrated that the preponderant constituent was γ(Δ^{7-8})-cholestenyl acetate.

The dehydration products could be converted in good yield to α(Δ^{8-14})-cholestenyl acetate. The dehydration reaction thus provides a practicable and simple route to this rare cholesterol isomer and, by further isomerization, to β(Δ^{14-15})-cholestenol.

The dehydration product yielded with osmium tetroxide a cholestanetriol-3(β),7,8, derived from γ-cholestenol. Reaction with 2 moles of perbenzoic acid resulted in the formation of a compound probably identical with that obtained under the same conditions by Schenk, *et al.*,[2] from γ-cholestenol and described by these authors as a cholestanetriol-3,7,8. It has been shown by a series of transformations involving oxidation of the 3-monoacetate to the 7-ketone, hydrolysis to a dienone and eventual reduction to 7-keto-cholestanyl acetate that this compound has the structure of a cholestanediol-3,7-oxide-8,14.

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Oxidation Products of α-Cholestenyl Acetate

BY O. WINTERSTEINER AND MILDRED MOORE

In the preceding paper[1] we reported that the treatment of γ(Δ^{7-8})-cholestenyl acetate with 2 moles of perbenzoic acid leads to the formation of a 3(β)-acetoxycholestanol-7-oxide-(8,14?) (I), in which only the position of the epoxide group remained to be determined. Oxidation of this compound yielded the corresponding 3(β)-acetoxycholestanone-7-oxide (II). The formation of ketoxides by chromic acid oxidation of steroids with a "bridge" double bond has been observed by Petrow[2] in the case of Westphalen's diol, and more recently in this Laboratory by Stavely and Bollenback in their detailed studies of this reaction on α-ergostenyl acetate,[3a] α-dihydroergosteryl acetate,[3b] and α-spinasteryl acetate.[3c] It occurred to us that the ketoxide obtained from γ-cholestenyl acetate might be also accessible by chromic acid oxidation of α-(Δ^{8-14})-cholestenyl acetate (III) since of the two possible positions 8,14 and 8,9 for the epoxide group the former appeared more probable. This expectation was realized. Among the five compounds which were isolated from the oxidation mixture, the one obtained in largest amounts was identical with II. The position of the epoxide group in I was thus fixed in 8,14.

The other oxidation products were: (1) an α,β-unsaturated ketone (IV) which is not identical with the Δ^{8-14}-7-ketone (V) previously prepared from II via a dienone,[1] and therefore can only be a 15-ketone. The ultraviolet spectrum exhibits a maximum at 259 mμ, a location which according to the generalizations of Woodward[4] is to be expected from an α,β,β-substituted ketone with a doubly exocyclic α,β-ethylenic bond. Only structures IV and V, with the double bond in the 8,14-position, fulfill this requirement. While V can be reduced catalytically with palladium in acetic acid to yield α-cholestenyl acetate and the saturated ketone, 7-ketocholestanyl acetate, IV is considerably more resistant to hydrogenation under these conditions. Hydrogenolysis of the keto group is the preferred reaction, and α-cholestenyl acetate, except for some unattacked starting material, is, therefore, the sole product.

(2) A ketoxide isomeric with II, which in analogy with IV has been assigned the 15-ketone structure VI. The presence of a ketoxide group follows from the fact that on hydrolysis with acid

(1) Wintersteiner and Moore, THIS JOURNAL, **65**, 1507 (1943).

(2) Petrow, *J. Chem. Soc.*, 998 (1939).

(3) Stavely and Bollenback, (a) THIS JOURNAL, **65**, 1285 (1943); (b) **65**, 1290 (1943); (c) **65**, 1600 (1943).

(4) Woodward, *ibid.*, **64**, 76 (1942).