We are greatly indebted to Dr. N. H. Coy of the Vitamin Laboratory of E. R. Squibb and Sons for the spectrographic measurements. The microanalyses were carried out by Mr. J. F. Alicino of this Laboratory.

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.

NEW BRUNSWICK, N. J. RECEIVED MARCH 27, 1943

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

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.

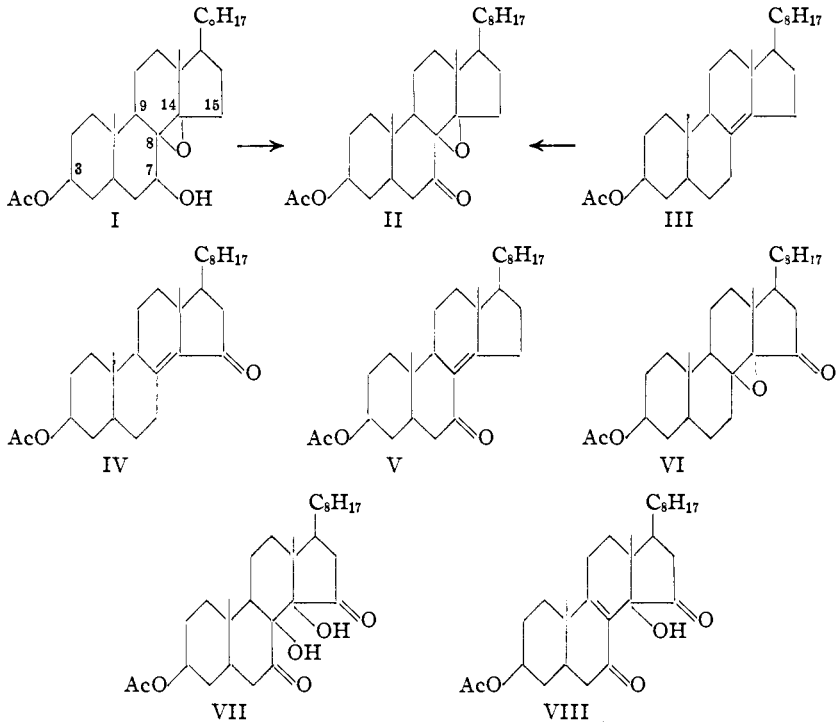
(2) A ketoxide isomeric with II, which in analogy with IV has been assigned the 15-ketone structure VI. The presence of a ketoxide group follows from the fact that on hydrolysis with acid

(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).



it yields a product with an absorption maximum at 319 mμ. Although this compound, which is highly autoxidizable, could not be isolated in pure form, it must be assumed from its absorption characteristics, and from the analogous behavior of II on treatment with acid,[1] that it is a dienone, and consequently its precursor a ketoxide. The possibility that the isomerism of the latter with II rests merely on a difference in the steric character of the epoxide ring is not excluded. However, on steric grounds it seems improbable that the other of the two theoretically possible forms of an 8,14-epoxide would be capable of existence, since the 7-keto group must interfere with oxide ring in at least one of these orientations.

(3) A compound $C_{29}H_{46}O_6$, which does not absorb light in the ultraviolet region, and for which we tentatively suggest formula VII.

(4) A compound $C_{29}H_{44}O_5$, which shows strong selective absorption around 254 mμ, and, therefore, must be an α,β-unsaturated ketone. The location of the band can best be reconciled[4] with the presence of an α,β,β-substituted unsaturated keto group in which the ethylenic bond is not exocyclic to any ring, *i. e.*, with a Δ^{8-9}-7-ketone structure as in formula VIII. The maximum also is near that (252 mμ) of a compound of the ergosterol series to which Stavely and Bollenback[3b] have assigned, on the basis of chemical evidence, the structure of a Δ^{8-9}-ergostenol-3-one-7. It is obvious that VIII could arise from VII by dehydration of the 8-hydroxyl group. We wish to emphasize that the structures suggested for VII and VIII should be considered provisional.

The supposition that a 15-keto group is present in compounds IV, VI, VII and VIII is strengthened by the fact that Stavely and Bollenback[3a] obtained from α-ergostenyl acetate a small amount of an oxidation product which was shown to be 3(β)-acetoxy-$\Delta^{8,14}$-ergostenedione-7,15, since it yielded a crystalline pyridazine derivative with hydrazine. Our failure to isolate the corresponding diketone from α-cholestenyl acetate may be due to the fact that we had to work with smaller amounts of starting material, and used a somewhat different method for separating the oxidation products. Stavely and Bollenback, on the other hand, did not obtain the compound corresponding to our compound VIII. In all other respects (yields, melting point and rotation trends, absorption spectra) the results in both series bear close resemblance. In Table I the melting points and specific rotations of the corresponding compounds of both series are tabulated. It can be seen that the specific rotations in the cholestane series are generally more positive than those on

TABLE I

A, Cholestane series; B, ergostane series[3a]

Name of compound in cholestane series	Melting point, °C. A	B	$[\alpha]_D$ A	B
3-(β)-acetoxy-Δ^{8-14}-cholestene, III	78	110	+ 9.3°	0
3-(β)-acetoxycholestanone-7, XIV in (1)	149	184	− 33.1°	− 36°
3-(β)-acetoxy-Δ^{8-14}-cholestenone-7, V	142.5	155	− 62.2°	− 65°
3-(β)-acetoxy-Δ^{8-14}-cholestenone-15, IV	135	170	+118°	+110°
3-(β)-acetoxycholestanone-7-oxide-8,14, II	140	134	− 75.3°	− 83°
3-(β)-acetoxydienone-7, XII in (1)	166	178	− 17.6°	− 22°
3-(β)-acetoxycholestanone-15-oxide-8,14, VI	181	210	+ 4.7°	− 6°
3-(β)-acetoxycholestanediol-8,14-dione-7,15	185	202	+ 73.5°	+ 69°

the ergostane series by a value approximating the difference between the specific rotations of α-cholestenyl acetate (+9.3) and α-ergostenyl acetate (0). It is also evident that the compounds which possess a keto group in position 7 only are characterized by markedly higher negative rotations than those designated as 15-ketones.

Experimental

α-Cholestenyl acetate (4.68 g.), prepared by the method described in the preceding paper,[1] was dissolved in glacial acetic acid (560 cc.) and benzene (190 cc.), and a solution of chromium trioxide (3.75 g.) in 90% acetic acid (32 cc.) was added with mechanical stirring over a period of one and one-half hours. After twenty hours of standing at room temperature most of the solvent was distilled off *in vacuo*. After addition of ether the oxidation products were separated in the usual way into acidic and neutral fractions. The neutral products were thoroughly dried *in vacuo* (4.41 g.), dissolved in hexane (50 cc.) and adsorbed on a column of aluminum oxide (2.5 × 41 cm.). Development and elution was effected with hexane (350 cc.), hexane–benzene 4:1 (1000 cc.) and 1:1 (1500 cc.), benzene (1000 cc.), benzene–ether 4:1 (2000 cc.) and 1:1 (1750 cc.), ether (500 cc.) and acetone (500 cc.). The eluates were collected and evaporated in 100-cc. portions, and the crystalline fractions combined on the basis of weight and melting point trends. Six combined fractions were thus obtained: A (177 mg.) and B (484 mg.) from hexane–benzene 1:1; C (211 mg.) from benzene; D (1265 mg.) from benzene–ether 4:1; E (149 mg.), followed without break by F (142 mg.) from benzene–ether 1:1. The rest of the eluted material, for the most part resinous, was not further examined.

Fraction A yielded a product of m. p. 98–102° which behaved like the cholestenyl acetate mixture from which the starting material was prepared, and probably represented a contaminant of the latter.

Fraction B yielded on two recrystallizations from methanol 290 mg. of **3(β)-acetoxy-Δ^{8-14}-cholestenone-15** (IV), large hexagonal plates m. p. 134–135°, $[\alpha]D$ +118° (2.27°, 19.2 mg. in 2 cc., 2 dm.),[5] ε 259 mμ 12,750 (in alcohol).

Anal. Calcd. for $C_{25}H_{46}O_3$: C, 78.67; H, 10.48. Found: C, 78.85; H, 10.45.

The **2,4-dinitrophenylhydrazone** formed yellow needles, m. p. 208–209°.

Anal. Calcd. for $C_{35}H_{50}O_6N_4$: N, 9.00. Found: N, 8.73.

On hydrolysis with boiling 5% methanolic potassium hydroxide **Δ^{8-14}-cholestenol-3(β)-one-15,** needles from 75% ethanol, m. p. 145–146°, was obtained. The compound yielded a crystalline digitonide in 90% ethanol.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.93: H, 11.08. Found: C, 80.60; H, 11.18.

The acetate IV (108 mg.) was dissolved in glacial acetic acid (15 cc.) and shaken with previously reduced palladium catalyst (20 mg.) in an atmosphere of hydrogen. Absorption of the gas ceased after two hours with only 0.3 mole consumed. After removal of the catalyst and solvent the procedure was repeated with new catalyst, till a total of 1.1 mole had been taken up. The reaction product was chromatographed in the usual manner on aluminum oxide and yielded on elution with hexane–benzene (9:1) 44 mg. of α-cholestenyl acetate which after recrystallization from alcohol melted at 76.5–78°. The rest of the material appeared in the hexane–benzene 1:1 eluates and was identified as starting material by its melting point and absorption spectrum.

Fraction C was recrystallized three times from methanol and then consisted of pure **3(β)-acetoxycholestanone-15-oxide-8,14, VI;** 98 mg. of rods melting at 180–181° was obtained, $[\alpha]^{22}D$ +4.7° (0.092°, 19.7 mg. in 2 cc., 2 dm.). The compound was not precipitated by 2,4-dinitrophenylhydrazine in 1% alcoholic hydrochloric acid.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.92 H, 10.11. Found: C, 75.88; H, 10.09.

The ketoxide (48 mg.) was refluxed for two hours with alcohol (5 cc.) containing 0.05 cc. concentrated hydrochloric acid. The reaction product was recovered by ether extraction, reacetylated in pyridine at room temperature and chromatographed on a small column. Only 22 mg. of crystalline material was obtained in one of the eluates with hexane–benzene 1:1. This product was inhomogeneous, and by repeated recrystallization from methanol only 3.3 mg. of small rosets m. p. 132–133.5° was eventually recovered. The absorption curve showed a well-defined maximum at 219 mμ, ε 6750, and a minimum at 261 mμ, ε 2000, followed by end absorption. (ε calculated on mol. wt. of a dienone.)

Fraction D on several recrystallizations from 80% methanol yielded 834 mg. of 3(β)-acetoxycholestanone-7-oxide-8,14 (II), m. p. 139.5–140.5°. The melting point was not depressed by admixture of a specimen prepared from the diol oxide I, $[\alpha]^{22}D$ −75.3° (−1.53°, 20.3 mg. in 2 cc., 2 dm.).

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.92; H, 10.11. Found: C, 75.50; H, 10.10.

The ketoxide (602 mg.) was refluxed for two hours in alcohol (24 cc.) containing concentrated hydrochloric acid (1.2 cc.). The reaction product was acetylated and purified in the manner previously described.[1] 204 mg. of 3-(β)-acetoxydienone-7 m. p. 163–166° was obtained. The absorption spectrum of this preparation was identical with that of the speicmen obtained by the alternative route.[1]

The material in fraction E was recrystallized twice from 80% ethanol and yielded 58 mg. of the compound tentatively assigned structure VII, elongated platelets melting at 184–185°: $[\alpha]^{22}D$ +73.5° (1.294°, 17.6 mg. in 2 cc., 2 dm.). The compound did not react with 2,4-dinitrophenylhydrazine under the usual conditions.

Anal. Calcd. for $C_{29}H_{46}O_6$: C, 70.97; H, 9.45. Found: C, 71.38; H, 9.34.

Fraction F did not yield homogeneous material on recrystallization and was therefore adsorbed from benzene solution on aluminum oxide (1.6 × 9 cm.). Benzene–ether 9:1 eluted most of the material. The later fractions obtained with this solvent mixture showed melting points higher than that of compound VII. These fractions were

(5) The solvent used in all specific rotation measurement was chloroform.

combined (49 mg.) and recrystallized several times from methanol. The pure compound, tentatively assigned structure VIII, formed rosets of elongated platelets melting at 218–219° (dec.); $[\alpha]^{24}D$ +143.5° (2.67°, 18.6 mg. in 2 cc., 2 dm.). The absorption curve showed a well-defined single maximum at 254 mμ, ε 10,400 (in ethanol).

Anal. Calcd. for $C_{28}H_{44}O_2$: C, 73.68; H, 9.38. Found: C, 73.50; H, 9.49.

The compound failed to react with 2,4-dinitrophenylhydrazine under the usual conditions.[6]

We are greatly indebted to Dr. N. H. Coy of the Vitamin Laboratory of E. R. Squibb and Sons for the spectrographic measurements. The microanalyses were carried out by Mr. J. F. Alicino of this Laboratory.

(6) The fact that compounds VI, VII and VIII which undoubtedly are ketones could not be derivatized with 2,4-dinitrophenylhydrazine must be ascribed to steric factors, that is, most probably to the presence of interfering groups in positions 8 and 14. However, it must be admitted that this explanation does not account for the failure of 3-(β)-acetoxy-Δ^{8-14}-ergostenedione-7,15 to yield a dinitrophenylhydrazone, although this compound readily reacts with hydrazine to form a pyridazine derivative.[5b]

Summary

Mild oxidation of $\alpha(\Delta^{8-14})$-cholestenyl acetate with chromic acid results in the formation of a number of ketonic compounds. Both methylene groups in α-position to the 8,14-double bond are attacked. The double bond itself either remains unchanged, or adds oxygen in form of an epoxide group or of tertiary hydroxyl groups. The compound formed in largest amount is the 7-keto-8,14-oxide previously obtained via the corresponding 7-hydroxy compound from $\gamma(\Delta^{7-8})$-cholestenyl acetate.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

The Action of Diazomethane upon Acyclic Sugar Derivatives. V.[1] Halogen Derivatives

BY M. L. WOLFROM AND ROBERT L. BROWN[2]

The conversion of a diazomethyl to a chloro- or bromomethyl group with halogen hydride was established by Curtius.[3] Such conversions have been effected[4] with 1-diazo-1-desoxy-*keto*-D-fructose tetraacetate and 1-diazo-1-desoxy-*keto*-D-glucoheptulose pentaacetate. In the present work, the optical rotations of these previously reported halogen derivatives have been remeasured and new values established in several cases. 1-Diazo-1-desoxy-*keto*-D-galaheptulose pentaacetate[1] (I) has now been converted to the 1-chloro and 1-bromo derivatives in the same manner. The 1-chloro compound exhibited dimorphism. The 1-iodo derivatives in these three sugar structures were then obtained from the chloro-compounds by halogen interchange[5] with sodium iodide in acetone.[6]

The measured physical constants obtained for the three 1-halo-derivatives of each of these acetylated *keto*-sugar structures are tabulated in Table I. The melting points show a decrease in the sequence Cl → Br → I for the D-fructose and D-glucoheptulose (D-gluco-D-sorbo-heptose) structures but exhibit an increase for D-galaheptulose (D-gala-L-fructo-heptose). The trend of molecular rotation sequence is reversed in the D-fructose

TABLE I

COMPARATIVE ROTATORY POWERS OF 1-HALO-*keto*-ACETATES

Substance	M. p., °C.	$[\alpha]^{21-28}D$ abs. $CHCl_3$	[M]
1-Chloro-*keto*-D-fructose tetaacetate[4]	77.5–78	+68°	+24,900
1-Bromo-*keto*-D-fructose tetraacetate	67–68	+65	+26,800
1-Iodo-*keto*-D-fructose tetraacetate	55–56	+63	+28,900
1-Chloro-*keto*-D-glucoheptulose pentaacetate	100–101	− 2.8	− 1,200
1-Bromo-*keto*-D-glucoheptulose pentaacetate	87–88	− 5.5	− 2,700
1-Iodo-*keto*-D-glucoheptulose pentaacetate	79–81[a] 89–90[a]	− 9.9	− 5,200
1-Chloro-*keto*-D-galaheptulose pentaacetate	101–102[a]	−33	−14,500
1-Bromo-*keto*-D-galaheptulose pentaacetate	124–125	−36	−17,400
1-Iodo-*keto*-D-galaheptulose pentaacetate	144–146	−45	−23,900

[a] Dimorphic.

(1) Previous publication in this series: M. L. Wolfrom, R. L. Brown and E. F. Evans, THIS JOURNAL, **65**, 1021 (1943).

(2) Du Pont Fellow, 1941-1942.

(3) (a) T. Curtius, *Ber.*, **16**, 754, 2230 (1883); (b) *J. prakt. Chem.*, [2] **38**, 396 (1888).

(4) M. L. Wolfrom, S. W. Waisbrot and R. L. Brown, THIS JOURNAL, **64**, 1701 (1942).

(5) W. H. Perkin and B. F. Duppa, *Ann.*, **112**, 125 (1859); P. van Romburgh, *Rec. trav. chim.*, **1**, 233 (1882).

(6) H. Finkelstein, *Ber.*, **43**, 1528 (1910).