[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Oxidation of Steroids. IV. Methyl $\Delta^{9(11)}$-Lithocholenate and Methyl 9α,11α-Oxidolithocholanate[1,2]

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This extension of previous studies[4-9] of the reactions of 9,11-dehydro and 9,11-oxido derivatives of cholanic acid and lithocholic acid was undertaken with the idea that a successful method of converting a 9,11-ethylene into an 11-keto derivative might be applied to intermediates obtainable from sources other than desoxycholic acid. The fact that no good method yet exists for the preparation of methyl $\Delta^{9(11)}$-lithocholenate (I) from desoxycholic acid thus is no matter for immediate concern. The unsaturated ester employed in the present work was prepared from 12-keto-$\Delta^{9(11)}$-lithocholenic acid or its 3-carbethoxy methyl ester[2c] by Wolff–Kishner reduction according to Huang-Minlon[10] and esterification in over-all yield of about 50%. As is generally true of α,β-unsaturated ketones, the Wolff–Kishner reaction in this instance[5,9] gives a mixture of $\Delta^{9(11)}$-lithocholenic acid, Δ^{11}-lithocholenic acid and lithocholic acid,[5,11] but a convenient method was found for isolation of the $\Delta^{9(11)}$-acid as the methyl ester.

Methyl $\Delta^{9(11)}$-lithocholenate (I) proved to be completely resistant to N-bromosuccinimide in aqueous acetone or even in aqueous *t*-butanol. Stavely[11a] has reported that N-bromoacetamide in aqueous acetone converts the 3-acetate of I into the bromohydrin, but we did not investigate the action of N-bromosuccinimide on this derivative. We previously reported[2b] that in aqueous acetone N-bromosuccinimide attacks the 5,6-double bond of cholesterol, and we have now found that methyl Δ^{11}-lithocholenate reacts under the same conditions to give the 11β,12α-dibromide and the 11β-hydroxy-12α-bromo derivative; the 3α-hydroxy compound thus reacts the way its 3-acetate reacts with N-bromoacetamide in aqueous *t*-butanol.[12] The behavior of methyl $\Delta^{9(11)}$-lithocholenate thus affords further evidence of the inert character of the 9,11-double bond. The resistance of the ester to oxidation at the alcoholic function by N-bromosuccinimide in aqueous *t*-butanol is abnormal, since 3α-hydroxyl groups usually are oxidized.[2c]

It was therefore surprising to find that the corresponding oxide, formulated as the 9α,11α-oxide (II) on the basis of evidence to be reported later, is oxidized to a dehydro compound by even milder reagents, namely, N-bromosuccinimide in aqueous acetone or potassium chromate in aqueous acetic acid. The latter reagent has been observed to oxidize 3α-hydroxysteroids in a few instances,[2c] but no case of oxidation by the former method is on record. The difference in molecular rotation between the alcohol II (M_D + 97 Di) and the dehydro compound (M_D + 16 Di) is considerably greater than that normally found between a 3α-hydroxy compound of the bile acid series and the corresponding ketone, as illustrated by the first six comparisons given in Table I (average Δ^{Ket} = +3; because of solvent differences the comparisons are not all strictly valid). However, the last three entries in the table show that the introduction of a 9,11-double bond gives rise to still greater optical anomalies, attributable to vicinal action. That the dehydro compound is indeed the 3-keto-9,11-oxide III was first inferred by Dr. Evelyn Wilson at the Merck Laboratories from infrared spectra taken before and after treatment with semicarbazide. We then isolated the semicarbazone of III, hydrogenated the 3-ketone to the original 3α-hydroxy compound (II, isolated as the acetate), and converted the 3-ketone by Wolff–Kishner reduction to 9α,11α-oxidocholanic acid.[4] That the 9,11-oxide bridge withstands alkali in triethylene glycol at 190° was established also by the observation that trial Wolff–Kishner reduction of II resulted merely in hydrolysis of the ester group.

TABLE I

MOLECULAR ROTATIONS[a]

3α-Hydroxy compound	M_D	3-Ketone, M_D	ΔKet
Methyl lithocholate	+113 Di[2c]	+124 Di[2c]	+11
Methyl 3α-hydroxy-11-keto-cholanate	+248 Al[13]	+243 Al[13]	5
Methyl 3α-hydroxy-12-keto-cholanate	+372 Di[2c]	+370 Di[2c]	2
3α-Hydroxy-7-ketocholanic acid[2c]	−109 Di[2c]	−105 Al[14]	4
Methyl Δ^{11}-lithocholenate	+150 Al[15]	+143 An[16]	8
Methyl 11α,12α-oxidolithocholanate	+144 An[15,17]	+131 An[15]	13
3α-Hydroxy-12-keto-$\Delta^{9(11)}$-cholenic acid	+440 Al[18]	+286 Chf[18]	154
3α,12α-Dihydroxy-$\Delta^{9(11)}$-cholenic acid	+406 Al[18]	+283 Chf[18]	123
Methyl 3α-hydroxy-12-methoxy-$\Delta^{9(11)}$-cholenate	+552 Chf[18]	+458 Chf[18]	94

[a] Di = dioxane, Al = CH_3OH or C_2H_5OH, An = acetone, Chf = chloroform.

The oxide bridge is also untouched by lithium aluminum hydride, which reduces both the alcohol-oxide II and the keto-oxide III to the oxidodiol IV, which readily forms a diacetate. We found

(1) This work was supported in part by grants from the U. S. Public Health Service, the Rockefeller Foundation and Research Corporation.

(2) Fieser and Rajagopalan, (a) Part I, THIS JOURNAL, **71**, 3935 (1949); (b) Part II, *ibid.*, **71**, 3938 (1949); (c) Part III, *ibid.*, **72**, 5530 (1950).

(3) Fellow of the National Cancer Institute.

(4) Alther and Reichstein, *Helv. Chim. Acta*, **26**, 492 (1943).

(5) Seebeck and Reichstein, *ibid.*, **26**, 536 (1943).

(6) Berner and Reichstein, *ibid.*, **29**, 1374 (1946).

(7) Sarett, *J. Biol. Chem.*, **162**, 591 (1946).

(8) Hicks and Wallis, *ibid.*, **162**, 641 (1946).

(9) Hicks, Bergs and Wallis, *ibid.*, **162**, 645 (1946).

(10) Huang-Minlon, THIS JOURNAL, **68**, 2487 (1946); **71**, 3301 (1949).

(11) See also Alther and Reichstein.[4]

(11a) Stavely, *Fed. Proc.*, **9**, 233 (1950).

(12) Ott and Reichstein, *Helv. Chim. Acta*, **26**, 1799 (1943).

(13) Turner, Mattox, Engel, McKenzie and Kendall, *J. Biol. Chem.*, **166**, 345 (1946).

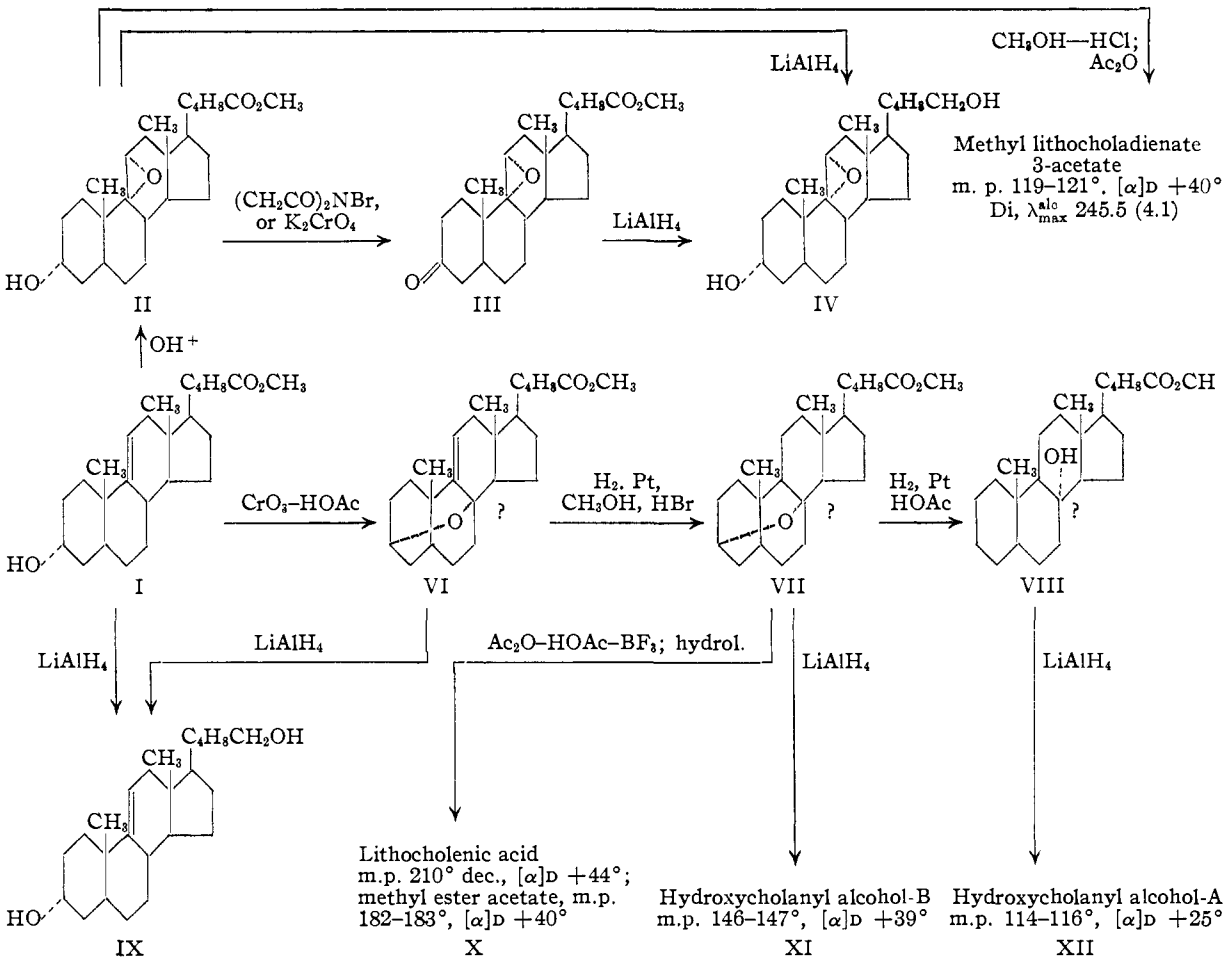
(14) Iwasaki, *Z. physiol. Chem.*, **244**, 181 (1936).

(15) Press and Reichstein, *Helv. Chim. Acta*, **25**, 878 (1936).

(16) Burckhardt and Reichstein, *ibid.*, **25**, 821 (1942).

(17) Engel, Mattox, McKenzie, McGuckin and Kendall, *J. Biol. Chem.*, **162**, 565 (1946).

(18) Turner, Mattox, Engel, McKenzie and Kendall, *ibid.*, **162**, 571 (1946).



that a 3,9-oxide bridge is also resistant to lithium aluminum hydride, for the product obtained from methyl 11-keto-3α,9α-oxidocholanate formed a monoacetate having a free hydroxyl group (infrared spectrum) and therefore is 11β-hydroxy-3α,9α-oxidocholanyl alcohol.

Only two of many processes tried resulted in an attack on the 9,11-oxide grouping. One was formation of an oxidation product $C_{25}H_{33}O_5$, a study of which will be reported later[18a]; the other consisted in methanolysis of methyl 3α-hydroxy-9α,11α-oxidocholanate (II), followed by acetylation. The product (V), obtained only in low yield, is a heteroannular diene of unestablished structure.

The observation that methyl $\Delta^{9(11)}$-lithocholenate (I) exhibits the abnormal property of resistance to oxidation to the 3-ketone by N-bromosuccinimide in aqueous *t*-butanol invited trial of the stronger reagent potassium chromate in aqueous acetic acid.[2c] Although Seebeck and Reichstein[5] showed that chromic acid oxidizes the 3-acetate of I to the 12-ketone, we found that potassium chromate does not attack the 3-acetate or 3-carbethoxy derivative. Potassium chromate does attack free methyl $\Delta^{9(11)}$-lithocholenate (I) and gives in 35% yield a dehydro compound of apparently novel type that is tentatively formulated as the $\Delta^{9(11)}$-3α,8α-oxide VI. The same compound is formed in 55% yield by the anhydrous procedure of oxidation with chromic anhydride in glacial acetic acid.[19] That the dehydro compound is not the 3-ketone is inferred from the following transformations. The substance absorbs one mole of hydrogen in methanol containing a trace of hydrobromic acid to give a product (VII) having no hydroxyl group (infrared); this cannot be a saturated 3-ketone because it is reduced by further hydrogenation in acetic acid to an ester (VIII) that contains a hydroxyl group (infrared) that is non-acylable. The substance formulated as the saturated oxide (VII) is further reducible with lithium aluminum hydride to a diol (XI) that forms a monoacetate containing a free hydroxyl group. An isomeric diol (XII) resulted from similar reduction of VIII. These diols are isomeric with that resulting from lithium aluminum hydride reduction of lithocholic acid, which, as expected, forms a diacetate.

The abnormal oxidation product is formulated as having a 9,11-double bond (VI) on the evidence that lithium aluminum hydride reduction gives an unsaturated diol (IX) identical with that obtained from methyl $\Delta^{9(11)}$-lithocholenate (I). The formation of a 3,8-oxide bridge by simultaneous attack at the 3-hydroxyl group and the bridgehead position C_8 adjacent to the double bond seems a reasonable hypothesis, but we have not yet been able

(18a) See Fieser, Heymann and Rajagopalan, THIS JOURNAL, 72, 2307 (1950).

(19) Fieser, *ibid.*, 70, 3237 (1948).

to adduce evidence of the position of the oxide bridge or the location and orientation of the tertiary hydroxyl groups in the diols XI and XII. Acetolysis of the saturated oxide VII gave a small amount of a mono-unsaturated product (X) that is resistant to hydrogenation or isomerization. Since proof of structure of this product would furnish evidence of the position of the oxide link in VI, known methods were applied to the synthesis of comparison samples of substances presumably having the structures of Δ^{7}- and $\Delta^{8(14)}$-lithocholenic acids. Neither substance corresponded to X, and hence it is possible that this is the Δ^{8}-isomer. However, our experiences with enic and dienic bile acids, to be reported separately, emphasize the difficulty in establishing homogeneity and identity in this series and we feel obliged to defer consideration of the structure X and the nature of the isomerism between the diols XI and XII until the various unsaturated derivatives can be more fully characterized.

Under the conditions of chromate oxidation leading to the abnormal product VI, methyl 12-keto-$\Delta^{9(11)}$-lithocholenate and methyl Δ^{11}-lithocholenate were recovered unchanged.

We are indebted to the Merck Laboratories for coöperation, supplies, and some of the analyses, to Dr. George Krsec for determining some of the rotations, and to Wei-Yuan Huang and Shirley Golden for technical assistance.

Experimental[20]

Methyl $\Delta^{9(11)}$-Lithocholenate (I).[5,21]—Wolff–Kishner reduction[10] of 116.4 g. of approximately 95% pure 12-keto-$\Delta^{9(11)}$-lithocholenic acid furnished by Merck & Co., Inc., was conducted with 75 cc. of 85% hydrazine hydrate, 60 g. of potassium hydroxide, and 600 cc. of triethylene glycol (refluxed 45 minutes after the initial vigorous reaction; distilled to b.p. 190°; refluxed at 190–195° for two hours). Dilution with 3.5 l. of water and acidification gave gelatinous acid but when ether (150–200 cc.) was stirred into the mixture the gel coagulated and soon gave granular material easily collected on the filter. A solution in excess methanol was clarified with charcoal, diluted to turbidity and left overnight, when crude $\Delta^{9(11)}$-lithocholenic acid crystallized; m.p. 180–186°, $[\alpha]^{25}D$ +47 ± 2°, yield 83.8 g.

Similar reduction of 150 g. of methyl 3α-carbethoxy-12-keto-$\Delta^{9(11)}$-cholenate[20] gave 79 g. of crude acid mixture, m.p. 174–180°. Dr. Hans Heymann later esterified this material with diazomethane, prepared the acetate (87 g.), chromatographed samples of the oxide derived from it and isolated derivatives corresponding to the following components in the original acid mixture: 30% lithocholic acid, 34–41% $\Delta^{9(11)}$-lithocholenic acid and 16% Δ^{11}-lithocholenic acid.

In the present work the crude lithocholenic acid (75 g.) was esterified with 350 cc. of 1% methanolic hydrogen chloride overnight at 25° and the hot filtered solution was diluted carefully with water. A small low-melting first fraction was rejected; dilution with a few cc. more water caused slow separation of methyl lithocholenate as colorless needles; m.p. 104–106°, $[\alpha]^{25}D$ +46 ± 2°, yield 55 g. (about 50% from ketolithocholenic acid; the mother liquor contains a mixture of substances).

Dr. Heymann later chromatographed a sample of oxide made from this ester and isolated 4% of the 11,12-oxide.

The 3-acetate methyl ester[5,21] crystallized fromm ethanol in prismatic needles, m.p. 137–138°, $[\alpha]^{25}D$ +60 ± 2°.

Anal. Calcd. for $C_{27}H_{42}O_4$ (430.61): C, 75.30; H, 9.85. Found: C, 75.27; H, 9.71.

(20) Melting points are uncorrected. All rotations were determined in dioxane.

(21) Mattox, Turner, Engel, McKenzie, McGuckin and Kendall, *J. Biol. Chem.*, **164**, 569 (1946).

Methyl 3-Carbethoxy-$\Delta^{9(11)}$-lithocholenate was prepared from 19 g. of methyl lithocholenate in 50 cc. of pyridine, treated gradually with cooling under the tap with 25 g. of ethyl cholorocarbonate. After standing overnight at 25° the product was precipitated with water (sintered at 144°, m.p. 147–149°). Crystallization from methanol gave prismatic needles; m.p. 149–151°, $[\alpha]^{25}D$ +57 ± 1°, yield 21.7 g. (96%).

Anal. Calcd. for $C_{28}H_{44}O_5$ (460.63): C, 73.03; H, 9.56. Found: C, 73.08; H, 9.46.

Methyl $\Delta^{9(11)}$-lithocholenate, like the 3-acetate and 3-carbethoxy derivative, is completely resistant to the action of N-bromosuccinimide in aqueous acetone, in aqueous *t*-butanol, or in aqueous pyridine. Attempts to hydroxylate the 9,11-double bond of methyl $\Delta^{9(11)}$-lithocholenate and of methyl 12-keto-$\Delta^{9(11)}$-lithocholenate (or their 3-acyl derivatives) with osmium tetroxide, hydrogen peroxide and osmium tetroxide, or performic acid were unsuccessful.

Methyl 3α-Hydroxy-9α,11α-oxidocholanate (II).—A solution of 51 g. of crude methyl $\Delta^{9(11)}$-lithocholenate in 1 l. of ether was treated with an ethereal solution of 40 g. (1.66 equiv.) of perphthalic acid and let stand for 5 days, when considerable phthalic acid had separated. The mixture was then refluxed for 32 hours and the solution filtered, washed neutral, dried, concentrated to about 250 cc. and diluted with 1 l. of petroleum ether (30–60°). Almost colorless prismatic needles of oxide (37 g.) separated overnight; m.p. 123–127°, remelting at 128–129°. Adsorption of 24 g. of product from benzene on acid-washed alumina and elution with 400-cc. portions of petroleum ether, petroleum ether–benzene, benzene and benzene–ether gave ten fractions, of which fractions 3–9 were combined and crystallized from aqueous acetone to give 21 g. of the 9,11-oxide, m.p. 130–132°, $[\alpha]^{25}D$ +24 ± 1°.

Anal. Calcd. for $C_{25}H_{40}O_4$ (404.57): C, 74.21; H, 9.97. Found: C, 74.51; H, 10.09.

The 3-acetate,[5,6,9] prepared by heating 1 g. of oxide with 3 cc. of pyridine and 1.5 g. of acetic anhydride for one-half hour on the steam-bath and crystallized twice from dilute methanol, was obtained as colorless needles (1 g.), m.p. 121–122°, $[\alpha]^{25}D$ +42 ± 2°, in agreement with Reichstein.[5,6]

Anal. Calcd. for $C_{27}H_{42}O_5$ (446.61): C, 72.61; H, 9.48. Found: C, 72.51; H, 10.09.

Free 9α,11α-oxidolithocholic acid resulted by hydrolysis of the methyl ester in a trial to see if the oxide ring would withstand conditions of the Wolff–Kishner reduction. The acid crystallized from aqueous acetone in needles, m.p. 200–201°, $[\alpha]^{22}D$ +24 ± 2°.[22]

The following attempts to cleave the 9,11-oxide bridge were unsuccessful and led only to total recovery of starting material: oxidation of the methyl ester acetate with N-bromoamides, potassium chromate, chromic acid or anhydrous chromic anhydride; periodic acid cleavage of the methyl ester acetate (perchloric acid caused extensive decomposition); acetolysis with acetic anhydride and varying amounts of pyridine; rearrangement with activated zinc, copper or nickel.

Methyl 3-Keto-9α,11α-oxidocholanate (III).—A solution of 2 g. of II in 60 cc. of pure acetone and 6 cc. of water was treated with 1 g. of N-bromosuccinimide and two drops of acetic acid, let stand overnight, and the solvent evaporated in a current of air. The residue was washed in ether with bicarbonate solution and water and the product recovered, washed with petroleum ether, and extracted with hot water to remove succinimide. The low-melting solid so obtained (1.8 g.) was chromatographed as above into eleven fractions. Fractions 4–7 on two crystallizations from methanol furnished 0.65 g. of keto–oxide as colorless plates, m.p. 129–130°, $[\alpha]^{25}D$ +4 ± 1°.

Anal. Calcd. for $C_{25}H_{38}O_4$ (402.55): C, 74.52; H, 9.52. Found: C, 74.34; H, 9.38.

Oxidation of the alcohol oxide with N-bromosuccinimide or N-bromoacetamide in 50% *t*-butanol gave crude material of better quality (m.p. 127–129°). Oxidation of 5 g. of II in acetic acid with aqueous potassium chromate at 25° (overnight) gave 2.8 g. of crude III, m.p. 113–119°, which when chromatographed yielded 2.1 g. of colorless plates, m.p. 129–130°, $[\alpha]^{25}D$ +4° (Found: C, 74.58; H, 9.40).

(22) Mattox, *et al.*,[21] report m.p. 199.5–200.5°, $[\alpha]D$ +36° (methanol).

Oxidation of 1 g. of II in 25 cc. of acetic acid with 0.5 g. of anhydrous chromic oxide (overnight at 25°) gave crude material, m.p. 122–124°; chromatography and recrystallization afforded 0.48 g. of plates, m.p. 129–130°, $[\alpha]^{25}D$ +3 ± 1° (Found: C, 74.42; H, 9.41).

The free acid crystallized from methanol in needles, m.p. 188–190°. The semicarbazone, prepared in methanol at 25° (18 hours), separated from methanol as prismatic needles, m.p. 209–210°, dec.

Anal. Calcd. for $C_{26}H_{41}O_4N_3$ (459.61): C, 67.95; H, 8.99. Found: C, 67.87; H, 8.77.

The keto-oxide resisted hydrogenation in the presence of Adams catalyst in methanol or methanol containing hydrobromic acid but in acetic acid solution took up one mole of hydrogen. Acetylation and chromatography afforded methyl 3α-acetoxy-9α,11α-oxidocholanate, m.p. 119–120°, $[\alpha]^{22}D$ +41 ± 2° (no depression with above sample). Wolff–Kishner reduction of III gave in 85% yield an acid corresponding in analysis to 9α,11α-oxidocholanic acid, m.p. 158–159° (aqueous acetone), $[\alpha]^{22}D$ +17 ± 1°. The methyl ester is described by Althor and Reichstein.[4]

Anal. Calcd. for $C_{24}H_{38}O_3$ (374.54): C, 76.96; H, 10.23. Found: C, 76.93; H, 10.42.

3α-Hydroxy-9α,11α-oxidocholanyl Alcohol (IV).—The keto-oxide III (600 mg.) in dry ether (100 cc.) was added in 1 hour to a refluxing, stirred solution of 1 g. of lithium aluminum hydride in 150 cc. of ether and the reaction was continued for 2 hours more. On recovery and crystallization from aqueous methanol the dialcohol was obtained as colorless needles; m.p. 184–185°, $[\alpha]^{25}D$ +27 ± 2°, yield 450 mg. An identical substance (mixed m.p., infrared spectra) was obtained by similar reduction of methyl 9α,11α-oxidolithocholanate (2 g.): m.p. 184–185°, $[\alpha]^{25}D$ +25 ± 1°, yield 1.6 g. The analyses refer to the two samples.

Anal. Calcd. for $C_{24}H_{40}O_3$ (376.56): C, 76.53; H, 10.70. Found: C, 76.39, 76.20; H, 10.42, 10.38.

The diacetate (identical samples from the two diol preparations), prepared with acetic anhydride and either a trace or a large excess of pyridine, separated from methanol in needles, m.p. 104–105°, $[\alpha]^{25}D$ +41 ± 2° (no infrared hydroxyl band).

Anal. Calcd. for $C_{28}H_{44}O_5$ (460.63): C, 72.83; H, 9.62. Found: C, 72.53, 72.68; H, 9.41, 9.53.

11β-Hydroxy-3α,9α-oxidocholanyl Alcohol.—Lithium aluminum hydride reduction of 600 mg. of methyl 11-keto-3α,9α-oxidocholanate[23] gave 410 mg. of the diol as a felt of soft needles from methanol; the crystals melted partially at 104–105° with loss of solvent and remelted at 154–155°. Evaporation twice with benzene and crystallization from benzene–ligroin and a trace of ether gave long, colorless needles, m.p. 155–156°, $[\alpha]^{25}D$ +63 ± 2°.

Anal. Calcd. for $C_{24}H_{40}O_3$ (376.56): C, 76.53; H, 10.70. Found: C, 76.25; H, 10.58.

Acetylation with acetic anhydride and pyridine for 1 hour on the steam-bath gave the monoacetate; needles from methanol, m.p. 130–131°, $[\alpha]^{25}D$ +57 ± 2°; infrared spectrum shows presence of a hydroxyl group.

Anal. Calcd. for $C_{26}H_{42}O_4$ (418.60): C, 74.61; H, 10.10. Found: C, 74.64; H, 9.95.

Oxidation Product $C_{25}H_{38}O_5$.—A solution of 1 g. of methyl 3α-hydroxy-9α,11α-oxidocholanate (II) in 40 cc. of acetic acid was treated gradually with a solution of 1 g. of chromic anhydride in 2 cc. of water and the mixture was left overnight at 25° and diluted. Addition of a little water caused separation of colorless needles, m.p. 110–115°. Purification by chromatography and crystallization from methanol afforded silky needles of variable melting point (*e.g.*, 119–120°, 114–118°), $[\alpha]^{22}D$ +96 ± 2° (infrared hydroxyl band).

Anal. Calcd. for $C_{25}H_{38}O_5$ (418.55): C, 71.66; H, 9.16. Found: C, 71.35; H, 9.41.

The acetate, obtained by the action of boiling acetic anhydride and a trace of pyridine, formed colorless needles from methanol, m.p. 144–146°, $[\alpha]^{21}D$ +96 ± 3° (no infrared band).

Anal. Calcd. for $C_{27}H_{40}O_6$ (460.59): C, 70.38; H, 8.77. Found: C, 70.64, 70.73; H, 8.71, 8.96.

(23) Kindly supplied by Dr. Max Tishler.

Methyl Lithocholadienate 3-Acetate (V).—A solution of 1 g. of methyl 3α-acetoxy-9α,11α-oxidocholanate (II) in 20 cc. of methanol containing 1 cc. of 36% hydrochloric acid was refluxed for three hours. Dilution with water gave a sticky oil, which was dried by evaporation with 50 cc. of methanol and then with 50 cc. of benzene. The material was reacetylated with acetic anhydride–pyridine; the acetate was oily but solidified when rubbed with methanol. Crystallization from aqueous methanol afforded almost colorless needles (low yield); m.p. 119–121°, $[\alpha]^{22}D$ +40 ± 2°, $\lambda_{max.}^{alc.}$ 245.5 mμ (log ε 4.1).

Anal. Calcd. for $C_{27}H_{40}O_4$ (428.59): C, 75.67; H, 9.64. Found: C, 75.43; H, 9.86.

Methyl 3α,8α-Oxido-$\Delta^{9(11)}$-cholenate (VI) (a) By Chromate Oxidation of Methyl $\Delta^{9(11)}$-Lithocholenate (I).—A solution of 2 g. of (I) in 95 cc. of acetic acid was treated gradually with 2 g. of potassium chromate in 4–5 cc. of water at 25° and the solution let stand for 13 hours. Dilution with water precipitated 1.25 g. of product, m.p. 114–117°. Repeated crystallization from methanol did not effect purification (m.p. 116–118°) and so 1 g. of crude product was adsorbed onto alumina from benzene and separated into ten fractions by elution with petroleum ether (fractions 1,2), petroleum ether–benzene (3–6), benzene (7), and benzene–ether (8–10). Fractions 8 and 9 were oily but solidified and afforded small amounts of starting material (I). Fractions 3–7 gave needles of the oxide, m.p. 119–120°, and crystallization from aqueous methanol afforded 0.7 g. (35%) of colorless needles, m.p. 119–120°, $[\alpha]^{25}D$ +36 ± 2°.

Anal. Calcd. for $C_{25}H_{38}O_3$ (386.55): C, 77.66; H, 9.91. Found: C, 77.64, 77.71; H, 10.13, 9.95.

The infrared spectrum (chloroform) showed no band for a hydroxyl group. The substance was recovered unchanged after treatment with cold methanolic hydrogen chloride for 36 hours and after being refluxed with periodic acid in aqueous acetone for 1 hour. Acetolysis with acetic anhydride–sulfuric acid led to extensive decomposition.

(b) **Oxidation with Anhydrous Chromic Anhydride.**—A solution of 4 g. of methyl $\Delta^{9(11)}$-lithocholenate in 100 cc. of acetic acid was treated gradually with 2 g. of anhydrous chromic anhydride with shaking and cooling in cold water. The mixture was let stand with occasional shaking for 12 to 14 hours, diluted with water and the product collected and washed (m.p. 114–117°). Crystallization from aqueous methanol gave 2.2 g. (55%) of needles, m.p. 117–119°, remelting at 118–120°, $[\alpha]^{25}D$ +37 ± 2°.

3α,8α-Oxido-$\Delta^{9(11)}$-cholenic acid, obtained by refluxing the ester with methanolic potassium hydroxide for one-half hour, separated from methanol in colorless needles, m.p. 158–161° dec., $[\alpha]^{25}D$ +38 ± 2°.

Anal. Calcd. for $C_{24}H_{36}O_3$ (372.53): C, 77.38; H, 9.74. Found: C, 77.62; H, 9.66.

Methyl 3α,8α-Oxidocholanate (VII).—A solution of 1 g. of methyl 3α,8α-oxido-$\Delta^{9(11)}$-cholenate (VI) in 40 cc. of methanol when shaken with 0.1 g. of Adams catalyst and hydrogen absorbed gas sufficiently merely to reduce the catalyst (17 cc. in ten minutes). After addition of a few drops of 48% hydrobromic acid one mole of hydrogen (55 cc.) was absorbed in 45 minutes and there was no further uptake. The saturated oxide was obtained from methanol as colorless plates, m.p. 117–118° (0.8 g.), and two recrystallizations gave material of constant m.p. 121–122°, $[\alpha]^{25}D$ +34 ± 2°; no infrared hydroxyl band.

Anal. Calcd. for $C_{25}H_{40}O_3$ (388.57): C, 77.27; H, 10.38. Found: C, 77.52, 77.45; H, 9.98, 10.18.

Free 3α,8α-oxidocholanic acid, resulting on saponification of the ester, separated from methanol in needles, m.p. 182–184°, $[\alpha]D$ +30 ± 2°.

Anal. Calcd. for $C_{24}H_{38}O_3$ (374.54): C, 76.94; H, 10.23. Found: C, 76.63; H, 9.94.

Methyl 8(?)-Hydroxycholanate (VIII).—When 0.4 g. of the saturated oxide VII in 10 cc. of acetic acid was hydrogenated in the presence of Adams catalyst (100 mg.) one mole of hydrogen was taken up in one-half hour and the reaction stopped. The product crystallized from aqueous methanol in plates, m.p. 103–104°, $[\alpha]^{22}D$ +25 ± 1°.

Anal. Calcd. for $C_{25}H_{42}O_3$ (390.59): C, 76.86; H, 10.83. Found: C, 76.61; H, 10,64. The infrared spec-

trum shows the presence of a hydroxyl group, but attempted acetylation led to recovery of the starting material.

The free acid crystallized from aqueous acetone in colorless plates, m.p. 143–145°, $[\alpha]^{22}D$ +25 ± 1°.

Anal. Calcd. for $C_{24}H_{40}O_3$ (376.56): C, 76.53; H, 10.65. Found: C, 76.71; H, 10.63.

8(?)-Hydroxycholanyl Alcohol-A (XII), obtained by lithium aluminum hydride reduction of VIII, separated from dilute methanol in colorless prismatic needles, m.p. 114–116°, $[\alpha]^{22}D$ +25 ± 1°.

Anal. Calcd. for $C_{24}H_{42}O_2$ (362.58): C, 79.50; H, 11.64. Found: C, 79.71; H, 11.75.

8(?)-Hydroxycholanyl Alcohol-B (XI).—Lithium aluminum hydride reduction of 250 mg. of methyl 3α,8α-oxidocholanate (VII) gave 200 mg. of this diol, which crystallized from aqueous methanol in colorless plates, m.p. 146–147°, $[\alpha]^{22}D$ +39 ± 2°.

Anal. Calcd. for $C_{24}H_{42}O_2$ (362.58): C, 79.50; H, 11.64. Found: C, 80.05; H, 11.41.

The monoacetate, prepared with acetic anhydride and either pyridine or boron fluoride etherate, separated from methanol in colorless needles, m.p. 58–60°. $[\alpha]^{22}D$ +35 ± 2°; infrared hydroxyl band.

Anal. Calcd. for $C_{26}H_{44}O_3$ (404.60): C, 77.16; H, 10.97. Found: C, 77.12; H, 10.62.

Lithocholanyl alcohol, obtained in excellent yield by lithium aluminum hydride reduction of lithocholic acid, crystallized from aqueous methanol in long needles, m.p. 176–177°, $[\alpha]^{22}D$ +35 ± 2°.

Anal. Calcd. for $C_{24}H_{42}O_2$ (362.58): C, 79.50; H, 11.64. Found: C, 79.69; H, 11.83.

The diacetate crystallized from methanol in plates, m.p. 56–58°, $[\alpha]^{22}D$ +44 ± 2°.

Anal. Calcd. for $C_{28}H_{46}O_4$ (446.65): C, 75.29; H, 10.40. Found: C, 75.15; H, 10.44.

$\Delta^{9(11)}$-Lithocholenyl Alcohol (IX).—Reduction with lithium aluminum hydride of 600 mg. of methyl $\Delta^{9(11)}$-lithocholenate (I) gave 430 mg. of the diol (a), m.p. 176–177°, $[\alpha]^{25}D$ +50 ± 2° and reduction of 1 g. of 3,8-oxido-$\Delta^{9(11)}$-lithocholenate gave 0.8 g. of an identical product (b), m.p. 176–177°, $[\alpha]^{25}D$ +51 ± 2°. Identity was established by mixed m.p. and infrared spectra comparisons of the diol and diacetate samples.

Anal. Calcd. for $C_{24}H_{40}O_2$ (360.56): C, 79.95; H, 11.18. Found (a,b): C, 80.05, 80.12; H, 10.88, 11.04.

The diacetate formed glistening plates from methanol, m.p. 87–88° (a,b), $[\alpha]^{25}D$ +58 ± 2°, +59 ± 2°.

Anal. Calcd. for $C_{28}H_{44}O_4$ (444.62): C, 75.61; H, 9.96. Found (a,b): C, 75.69, 75.52; H, 9.65, 9.65.

?-Lithocholenic Acid (X).—A solution of 500 mg. of methyl 3α,8α-oxidocholanate (VII) in 10 cc. of acetic acid was treated with 3 cc. of acetic anhydride and five drops of boron fluoride etherate and let stand at 25° for 12 hours. Addition of water and extraction with ether gave a gummy solid, which was adsorbed from petroleum ether onto 20 g. of alumina and eluted as above in twenty fractions. Fractions 7–10 (petroleum ether–benzene) gave starting material, m.p. and mixed m.p. 121–122°. Fractions 12–15 (chiefly benzene) gave, after recrystallization from aqueous methanol, 21 mg. of the **methyl ester acetate** as colorless plates, m.p. 182–183°, $[\alpha]^{21}D$ +40 ± 2°; faint Tortelli-Jaffé test similar to apocholic acid.

Anal. Calcd. for $C_{27}H_{42}O_4$ (430.61): C, 75.30; H, 9.82. Found: C, 75.33, 75.20; H, 9.89, 9.89.

The methyl ester acetate proved stable to hydrogenation or isomerization. A 50-mg. sample was added to a freshly reduced suspension of 200 mg. of palladium black in 25 cc. of acetic acid and the mixture shaken with hydrogen for 12 hours. The material recovered from the filtered and diluted solution melted at 178–180°, and recrystallization gave 40 mg. of needles or plates, m.p. 181–183°. $[\alpha]^{22}D$ +42 ± 2°, no depression with starting material, positive color with tetranitromethane.

The free hydroxycholenic acid separated from aqueous methanol in colorless needles that partially melted at 176–178°, solidified, and remelted at 200–210° dec., $[\alpha]^{21}D$ +44 ± 2°; strong test for unsaturation with tetranitromethane.

Anal. Calcd. for $C_{24}H_{38}O_3$ (374.54): C, 76.94; H, 10.23. Found: C, 77.17; H, 10.23.

Action of N-Bromosuccinimide on Methyl Δ^{11}-Lithocholenate.[15,17,24]—A solution of 900 mg. of the ester in 36 cc. of pure acetone and 4 cc. of water was treated with 190 mg. of recrystallized N-bromosuccinimide and two drops of acetic acid and let stand overnight. The solvent was removed in a current of air and an ethereal solution of the residue washed with water, bisulfite, alkali and water, dried and evaporated. The resulting oil was extracted repeatedly with hot petroleum ether to remove traces of methyl lithocholenate, and then taken up in acetone and the solution diluted carefully to faint turbidity. On standing a few hours in the cold, 200 mg. of colorless needles of **methyl 3α-hydroxy-11β,12α-dibromocholanate**,[17,25] m.p. 161–163°, separated. Recrystallization from aqueous methanol gave material, m.p. 168–169° dec., $[\alpha]^{25}D$ +54 ± 2°, that showed no depression on admixture with an authentic sample (m.p. 174–175° dec.) furnished by Dr. R. B. Turner.

Anal. Calcd. for $C_{25}H_{40}O_3Br_2$ (548.41): C, 54.75; H, 7.35. Found: C, 55.05; H, 7.49.

Slight further dilution of the mother liquor gave a small crop of halogen-positive needles. Further dilution gave an oil that, when acetylated with acetic anhydride–pyridine (90°), gave 100 mg. of crystalline **3α-acetoxy-11β-hydroxy-12α-bromocholanate**,[26] which separated from methanol in colorless needles, m.p. 188–190° dec., $[\alpha]^{25}D$ +76 ± 2°.

Anal. Calcd. for $C_{27}H_{43}O_5Br$ (527.53): C, 61.47; H, 8.22. Found: C, 61.43; H, 8.26.

Summary

Methyl 9α,11α-oxidolithocholate has the unusual property of being oxidizable to the 3-ketone with N-bromosuccinimide in aqueous acetone. The 9,11-oxide bridge is inert to conditions of the Wolff–Kishner reduction, to periodic acid, and to lithium aluminum hydride. The latter reagent likewise does not attack a 3α,9α-oxide bridge. Methanolysis of methyl 9α,11α-oxidolithocholate eliminates the oxide bridge and gives a diene.

In contrast to the oxide, methyl $\Delta^{9(11)}$-lithocholenate has the unusual property of being resistant to the oxidizing or other action of N-bromosuccinimide in aqueous *t*-butanol, in which solvent the reagent is more powerful than it is in aqueous acetone. However, stronger oxidizing agents (chromate in aqueous acetic acid, chromic anhydride in anhydrous acetic acid) oxidize methyl $\Delta^{9(11)}$-lithocholenate to a novel product tentatively formulated as a 9,11-unsaturated 3α,8α-oxide. As yet only the location of the double bond has been established. The oxide ring in both the unsaturated compound and its dihydride is opened by lithium aluminum hydride, and the oxide bridge of the dihydride is opened by hydrogenation in acetic acid.

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(24) The ester used (m.p. 105–106°) was prepared from acid kindly furnished by Dr. Richard B. Turner.

(25) Mattox, Turner, McKenzie, Engel and Kendall, *J. Biol. Chem.*, **173**, 283 (1948).

(26) Ott and Reichstein[12] report m.p. 196–198°, $[\alpha]D$ + 71°.