and 5 g. of 2-phenyl-Δ^2-cyclohexenone. The flask was stoppered and the mixture was swirled until it became homogeneous. After forty hours at room temperature the mixture was treated with 2 ml. of acetic acid and 25 ml. of water and the aqueous layer was extracted with ether. The product was separated from the excess malonic ester by fractional distillation; yield, 5.7–6.4 g.; b. p. 180–185° at 0.1 mm. The *trans*-diethyl 3-oxo-2-phenylcyclohexanemalonate (II) was analyzed in the form of its **semicarbazone**, which crystallized from methanol in small colorless rhombs; m. p. 154–155°.

Anal. Calcd. for $C_{20}H_{27}N_3O_5$: C, 61.7; H, 6.9. Found: C, 61.8; H, 6.7.

***trans*-3-Oxo-2-phenylcyclohexaneacetic Acid (III).**—A mixture of 2 g. of II, 15 ml. of acetic acid and 25 ml. of concentrated hydrochloric acid was refluxed in a nitrogen atmosphere for fourteen hours. Removal of the solvents under reduced pressure left a liquid which crystallized when scratched under benzene. The keto acid crystallized from benzene in small colorless prisms; yield, 1 g. (71%); m. p. 121–123°, raised to 124–125° by further recrystallization.

Anal. Calcd. for $C_{14}H_{16}O_3$: C, 72.4; H, 6.9. Found: C, 71.6; H, 6.9.

The **methyl ester** of III, which was prepared by refluxing 0.49 g. of the acid with 10 ml. of methanol and 1 ml. of concentrated sulfuric acid for three hours, was recrystallized from petroleum ether (60–75°); yield, 0.35 g.; m. p. 68–70°, raised to 71–73.5° by further recrystallization.

Anal. Calcd. for $C_{15}H_{18}O_3$: C, 73.2; H, 7.3. Found: C, 73.4; H, 7.4.

The **2,4-dinitrophenylhydrazone of the methyl ester** crystallized from methanol in yellow needles; m. p. 126–127°.

Anal. Calcd. for $C_{21}H_{22}N_4O_6$: C, 59.2; H, 5.2; N, 13.1. Found: C, 59.1; H, 5.2; N, 13.0.

***trans*-2-Phenylcyclohexaneacetic Acid (IV).**—A solution of 1 g. of diethyl-3-oxo-2-phenylcyclohexanemalonate in 25 ml. of toluene and a mixture of 20 g. of amalgamated zinc, 25 ml. of water and 35 ml. of concentrated hydrochloric acid were refluxed for thirty hours with frequent additions of hydrochloric acid. The reduced acid was extracted from the toluene layer with aqueous bicarbonate and precipitated from the aqueous solution by hydrochloric acid; weight, 0.38 g. (59%); m. p. 109–112°, raised to 112–112.5° by one recrystallization from aqueous methanol. Gutsche[6] has recently reported the literature on this compound.

(6) Gutsche, THIS JOURNAL, **70**, 4150 (1948).

Clemmensen reduction of 3-oxo-2-phenylcyclohexaneacetic acid by the same procedure gave a 61% yield of *trans*-2-phenylcyclohexaneacetic acid; m. p. 110.5–112°.

Treatment of 100 mg. of the acid with 3 ml. of concentrated sulfuric acid on a steam-bath for ten minutes[7] gave *trans*-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene, which crystallized from aqueous methanol in colorless needles; yield, 60 mg. (65%); m. p. 95.5–96.5°.

***cis*-2-Phenylcyclohexaneacetic Acid.**—A mixture of 4.35 g. of 2-phenylcyclohexanone, 5.66 g. of ethyl cyanoacetate, 0.39 g. of ammonium acetate, 0.6 g. of acetic acid, 0.1 g. of palladium-charcoal catalyst and 10 ml. of absolute ethanol was shaken in an atmosphere of hydrogen at room temperature for twenty-four hours. The procedure differed from that of Alexander and Cope[5] for other ketones only in the use of double the amounts of the non-ketonic reagents. After the addition of benzene to the filtered solution, followed by washing with water, acid and aqueous bicarbonate, the product was fractionated under reduced pressure; the fore-run yielded unchanged ketone which crystallized. The ethyl *cis*-2-phenylcyclohexanecyanoacetate was collected at 149–151° and 0.1 mm.; weight, 4.3 g. (64%). When the reaction was carried out for only sixteen hours, the yield dropped to 47%. The product had the correct analysis for nitrogen, but was low in carbon.

A mixture of 1 g. of the cyanoester, 20 ml. of acetic acid, 10 ml. of concentrated hydrochloric acid and 5 ml. of water was refluxed for fifteen hours, an additional 10 ml. of hydrochloric acid was added and the refluxing was continued for three hours. Addition of water and cooling precipitated 0.69 g. (85%) of *cis*-2-phenylcyclohexaneacetic acid; m. p. 165–168.5°. One recrystallization from aqueous methanol gave 0.59 g. (73%) of the acid with m. p. 168–170°. Hydrolysis of the cyanoester by hydrochloric acid alone has been reported to be unsatisfactory.[4]

Summary

The Michael addition of malonic ester to 2-phenyl-Δ^2-cyclohexenone gave *trans*-3-oxo-2-phenylcyclohexanemalonic ester from which *trans*-3-oxo-2-phenylcyclohexaneacetic acid and *trans*-2-phenylcyclohexaneacetic acid were prepared.

The reductive condensation of 2-phenylcyclohexanone with cyanoacetic ester to give ethyl *cis*-2-phenylcyclohexanecyanoacetate is described.

(7) Blumenfeld, *Ber.*, **74**, 524 (1941).

ANN ARBOR, MICHIGAN RECEIVED MAY 22, 1950

[CONTRIBUTION FROM THE CHEMICAL LABORATORY, HARVARD UNIVERSITY]

Oxidation of Steroids. III. Selective Oxidations and Acylations in the Bile Acid Series[1]

BY LOUIS F. FIESER AND SRINIVASA RAJAGOPALAN[2]

In previous papers we reported high selectivity in the oxidation of the 7α-hydroxyl group of cholic acid[3] and the 6β-hydroxyl group of cholestane-3β,5α,6β-triol[4] by use of N-bromosuccinimide in bicarbonate solution or in aqueous acetone, dioxane or methanol–ether. Later experiments have shown that the specificity is associated with the solvent as well as the oxidizing agent and that our comparison of the behavior of N-bromosuccinimide in any of the above solvents with that of N-bromoacetamide in aqueous *t*-butanol[5] was misleading, since the bromoamides are much more powerful oxidizing agents in *t*-butanol than in the other solvents. Thus all three alcoholic functions of cholic acid are oxidized rapidly by either N-bromosuccinimide or N-bromoacetamide in aqueous *t*-butanol (experiments by Renato Ettore to be published

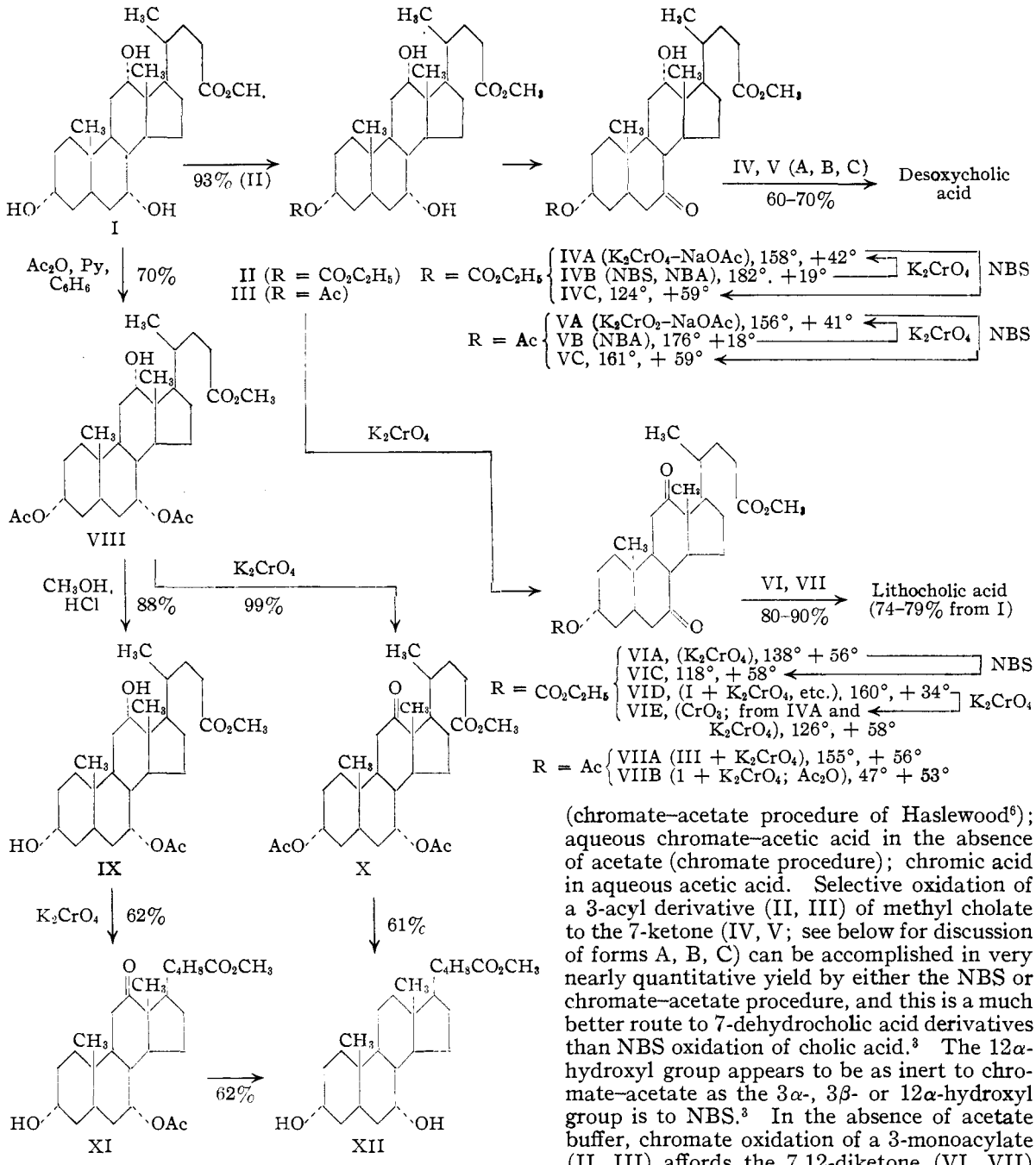
(1) This work was supported in part by grants from the U. S. Public Health Service, the Rockefeller Foundation and Research Corporation.

(2) Fellow of the National Cancer Institute.

(3) Fieser and Rajagopalan, THIS JOURNAL, **71**, 3935 (1949).

(4) Fieser and Rajagopalan, *ibid.*, **71**, 3938 (1949).

(5) Reich and Reichstein, *Helv. Chim. Acta*, **26**, 562 (1943).



later). The present investigation of selective oxidations and acylations was motivated by interest in obtaining a clearer definition of relative reactivities and in finding good preparative routes to key bile acid intermediates.

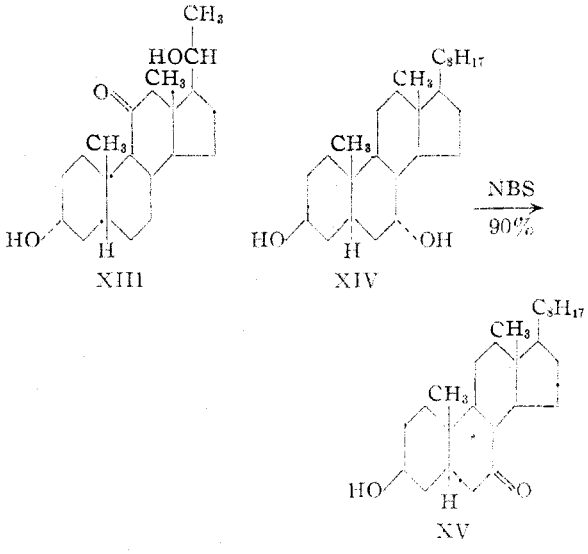
The oxidation procedures studied, all conducted at room temperature, may be listed in the following order of increasing intensity in attack of secondary alcoholic groups: N-bromosuccinimide (NBS) in aqueous acetone; aqueous potassium chromate added to a solution of the steroid in acetic acid heavily buffered with sodium acetate (chromate–acetate procedure of Haslewood[6]); aqueous chromate–acetic acid in the absence of acetate (chromate procedure); chromic acid in aqueous acetic acid. Selective oxidation of a 3-acyl derivative (II, III) of methyl cholate to the 7-ketone (IV, V; see below for discussion of forms A, B, C) can be accomplished in very nearly quantitative yield by either the NBS or chromate–acetate procedure, and this is a much better route to 7-dehydrocholic acid derivatives than NBS oxidation of cholic acid.[3] The 12α-hydroxyl group appears to be as inert to chromate–acetate as the 3α-, 3β- or 12α-hydroxyl group is to NBS.[3] In the absence of acetate buffer, chromate oxidation of a 3-monoacylate (II, III) affords the 7,12-diketone (VI, VII) in excellent yield when the reaction is allowed to continue for twelve to sixteen hours. In one instance the oxidation was interrupted after six to seven hours and the 7-monoketone was the sole product. We confirmed Borsche's[7] oxidation of methyl 3-carbethoxycholate to the 7,12-diketone (VI) with chromic acid, but the yield was less than half that obtained by the chromate procedure.

We explored the possibility of effecting chro-

(6) Haslewood, *Nature*, **150**, 211 (1942); *Biochem. J.*, **37**, 109 (1943).

(7) Borsche, *Ber.*, **57**, 1620 (1924).

mate oxidation at C_{12} in the presence of an unprotected 3α-hydroxyl group but found that the latter group, although much less reactive than a 12α-group, is attacked to such an extent that the reaction has limited preparative value. Thus chromate oxidation of methyl cholate followed by carbethoxylation afforded methyl 3α-carbethoxyoxy-7,12-diketocholanate (VI) in only 50% yield and some methyl dehydrocholate was isolated. Chromate oxidation of methyl cholate 7-acetate (IX) gave the 12-ketone (XI) in 62% yield. Similar oxidation of methyl desoxycholate, investigated as a possible shortcut in the synthesis of cortisone from desoxycholic acid, gave material found by chromatography to be a mixture of the desired methyl 3α-hydroxy-12-ketocholanate and methyl 3,12-diketocholanate in the ratio 3:1. The susceptibility of a hydroxyl at C_3 seems to vary with configuration at C_3 and the structure of the whole molecule. Thus chromate oxidation of pregnane-3α,20β-diol-11-one (XIII) afforded the 3,11,20-triketone in good

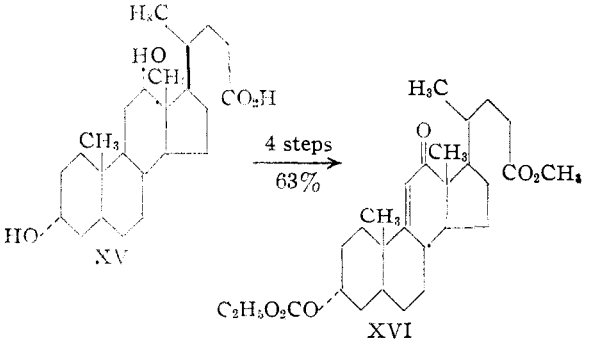


yield, but under the same conditions lithocholic acid was largely recovered unchanged (77%) and methyl lithocholate yielded only 12% of the corresponding 3-ketone. Chromate oxidation of cholestane-3β,7α-diol (XIV) gave cholestane-3,7-dione as the only isolated product, whereas, in line with our previous results, NBS oxidation of the diol XIV afforded cholestane-3β-ol-7-one (XV) in high yield. Oxidation of chenodesoxycholic acid with either chromate or NBS afforded 7-ketolithocholic acid in yields of 40–60%. In view of the susceptibility of the 3β-hydroxyl group of XIV to chromate, it was surprising to find that after a mixture of cholesterol and chromate in acetic acid–chloroform had stood for eighteen hours 92% of the sterol was recovered unchanged.

Two improvements in the preparation of partially acetylated derivatives of cholic acid are summarized in the first formula chart, namely, the conversion of methyl cholate into the 3,7-diacetate (VIII, 70% yield) and deacetylation of VIII to the 7-monoacetate (IX, 88% yield) by the action of methanol and dry hydrogen chloride. Good preparative routes are thus open to 12-ketochenodesoxycholic acid, X, and to chenodesoxycholic acid, XII (also to 7α-hydroxycholanic acid, to be reported later).

We previously reported[3] the acylation of methyl cholate with excess ethyl chlorocarbonate in pyridine according to Borsche[7] as affording the 3-carbethoxy derivative (II) in high yield; actually the yield of usable product is 93%, and an improved procedure gives methyl cholate in 91–93% yield. Carbethoxylation appears to be specific to the 3-position, at least as compared to positions 7 and 12, and affords a superior means of protecting the 3α-hydroxyl group. The further carbethoxy derivatives now investigated have excellent properties of crystallization and stability. The carbethoxy group at C_3 is not subject to hydrogenative fission; it is removed very easily by alkali. In several instances reported in the experimental part separation of a mixture has been greatly facilitated by carbethoxylation at C_3.

Methyl desoxycholate as frequently obtained is ill defined and low melting, but such samples readily afford the pure 3-carbethoxy derivative. A test was made of the applicability of this derivative in place of the benzoate for the preparation of 12-keto-Δ^9-lithocholenic acid from desoxycholic acid according to Kendall.[8] High melting methyl desoxycholate was obtained by an improved procedure and carbethoxylated, and chromate oxidation followed by desaturation with selenium dioxide gave the unsaturated ketone XVI of proper extinction coefficient in over-all yield of 63% from technical desoxycholic acid.



The citations under the formulas of the 7-ketones IV and V and the 7,12-diketones VI and VII indicate that from two to four forms corresponding to each structure were obtained, according to the method of oxidation employed. In each instance the forms differ considerably in melting point and rotation. In two instances

(8) McKenzie, Mattox, Engel and Kendall, *J. Biol. Chem.*, **173**, 271 (1948).

the B-form, obtained by NBS oxidation, is convertible into the A-form, the product of chromate oxidation, by treatment with chromate under the conditions of an oxidation. In three instances, the A-form yielded a new form on treatment with NBS. On Wolff–Kishner reduction according to Huang-Minlon[9] all six 7-ketones yielded desoxycholic acid and all six 7,12-diketones yielded lithocholic acid. The oxidations were repeated several times with consistent results, and the yields in both oxidations and interconversions were very high. Discussion of the phenomenon will be deferred until more information is at hand.

We are indebted to the Merck Laboratories for helpful coöperation, supplies, and for 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[10]

Methyl Cholate.—The following procedure is an improvement over that reported[3] because it affords the anhydrous ester directly: 200 g. of technical cholic acid (m. p. 194–195°) was dissolved in 600 cc. of methanol containing 6 g. of hydrogen chloride by warming on the steam-bath with exclusion of moisture, and the solution was heated for fifteen minutes and let stand at room temperature. Crystallization started after a few hours, or could be initiated, after two hours, by seeding with the anhydrous ester (m. p. 156°). In any case the mixture was left overnight, iced, and the product collected, washed with ether, and dried for several hours at 100°. The yield of satisfactory ester, m. p. 156–157°, was 190–193 g. (91–93%). Evaporation of the methanol mother liquor and ether washings, saponification of the residue with methanolic potassium hydroxide and crystallization from aqueous acetone gave 14.5 g. (7%) of cholic acid, m. p. 197–198° (needles, dried at 100°).

Methyl 3-carbethoxyoxy-7-keto-12α-hydroxycholanate. Form IVA.—A solution of 4 g. of crude methyl 3-carbethoxycholate (as prepared[3] in 93% yield, m. p. 173–175°, $[\alpha]^{25}D$ +39°) in 50 cc. of acetic acid containing 10 g. of sodium acetate trihydrate was treated gradually with 3 g. of potassium chromate in 8 cc. of water with shaking. After ten hours at 25° the solution was diluted and the product collected; yield 3.9 g. (97%), m. p. 150–158°. Crystallization from methanol gave hard, compact needles or rods, m. p. 157–158°, $[\alpha]^{25}D$ +41 ± 2°.

Anal. $C_{29}H_{44}O_7$ (492.63): C, 68.26; H, 9.00. Found: C, 67.93; H, 9.04.

The same substance (IVA) was also obtained (93% yield) by conducting the above oxidation with omission of sodium acetate but stopping the reaction after six to seven hours (if the period is extended the 7,12-diketone is formed).

Form IVB, prepared previously[3] by oxidation with N-bromoacetamide, was obtained as plates, m. p. 181–182°, $[\alpha]^{25}D$ +19 ± 1°. The same substance has now been obtained by oxidation with N-bromosuccinimide. Mixtures of IVA and IVB melted at 157–159°.

Conversion of IVB into IVA.—A solution of 1 g. of IVB (m. p. 182°) in 20 cc. of acetic acid containing 4 g. of sodium acetate trihydrate was treated with a solution of 1 g. of potassium chromate in 2–3 cc. of water. The solution was left at 25° overnight, diluted with water, and the solid collected, washed and crystallized from aqueous methanol to give prismatic needles, m. p. 157–158°, $[\alpha]^{25}D$ +41°; yield almost quantitative.

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

(10) Melting points are uncorrected. All rotations were determined in dioxane. Room temperature is assumed to be 25°.

Anal. $C_{29}H_{44}O_7$ (492.63): C, 68.26; H, 9.00. Found: C, 68.36; H, 8.82.

Conversion of IVA into IVC.—A solution of 1 g. of IVA (m. p. 158°) in 30 cc. of acetone and 8 cc. of water was treated with 1 g. of N-bromosuccinimide followed by 1 cc. of acetic acid and left at 25° overnight. Water precipitated a solid that crystallized from methanol as plates, m. p. 122–124°, $[\alpha]^{25}D$ +59 ± 2°; yield nearly quantitative.

Anal. $C_{29}H_{44}O_7$ (492.63): C, 68.26; H, 9.00. Found: C, 68.26; H, 8.75.

Wolff–Kishner reduction of IVA and IVC, as already described[3] for IVB, afforded desoxycholic acid in 60–70% yield. The infrared absorption spectra (chloroform) of IVA, B, and C were indistinguishable.

Methyl 3α-Acetoxy-7-keto-12α-hydroxycholanate.—**Form VA** was prepared by oxidation of 3 g. of methyl cholate 3-acetate[3] by the procedure described for IVA. Crystallization of the product from methanol afforded 2.9 g. (97%) of needles, m. p. 155–156, $[\alpha]^{29}D$ +41 ± 2°. **Form VB**, described earlier,[3] was obtained as platelets, m. p. 175–176°, $[\alpha]^{25}D$ +17 ±1°; a mixture of VA and VB melted at 155–166° and remelted at 163–173°. The conversions of VB into VA and of VA into VC were accomplished in excellent yield by the procedures described for the IV series. **Form VC** crystallized from methanol in micaceous plates, m. p. 160–161°, $[\alpha]^{25}D$ +59 ± 2°. As in the case of the 3-carbethoxy derivatives, forms VA, B and C gave desoxycholic acid in 60–70% yield on Wolff–Kishner reduction.

Anal. $C_{27}H_{42}O_5$ (462.61): C, 69.93; H, 9.15. Found: (VA) C, 70.20; H, 9.03; (VC) C, 69.91; H, 8.84.

Methyl 3α-Carbethoxyoxy-7,12-diketocholanate. Form VIA.—A solution of 10 g. of crude methyl 3-carbethoxycholate in 150 cc. of acetic acid was treated with 8 g. of potassium chromate in 25 cc. of water with occasional cooling under the tap. After twelve hours, extensive dilution with water precipitated 9.9 g. of crude diketone, m. p. 130–134°. Crystallization from methanol (water) afforded needles, m. p. 137–138°, $[\alpha]^{25}D$ +56 ± 2° (analysis below).

Conversion of VIA into form VIC was accomplished exactly as outlined for the transformation IVA → IVC; the substance separated from methanol in colorless plates, m. p. 117–118°, $[\alpha]^{25}D$ +58 ± 2°.

Form VID was obtained by gradually treating a solution of 20 g. of methyl cholate in 150 cc. of acetic acid with 20 g. of potassium chromate in 35 cc. of water with shaking and cooling under the tap. After standing at 25° for twelve to fourteen hours the mixture was diluted with a large volume of water. The resulting semisolid material was washed by kneading under water and taken up in excess boiling methanol; the solution on standing deposited 1 g. of methyl dehydrocholate, m. p. 239–240°, $[\alpha]^{25}D$ + 28°. The filtrate was evaporated to dryness and the residue taken up in 50 cc. of pyridine and treated gradually with 20 cc. of ethyl chlorocarbonate with shaking and cooling. Addition of water after five hours precipitated an oily product that solidified when rubbed with aqueous methanol. Crystallization from methanol afforded VID as colorless plates, m. p. 158–160°, $[\alpha]^{25}D$ +34 ± 2°; yield about 50%.

Conversion of VID into form VIE was done with chromate in acetic acid as described for the transformation of IVB into IVA and gave in 90% yield material separating from aqueous methanol in colorless needles, m. p. 125–126°, $[\alpha]^{25}D$ +57 ± 2°. An identical product, m. p. 125–126°, $[\alpha]^{25}D$ +59 ± 2°, was obtained in 44% yield by oxidation of methyl 3-carbethoxycholate with chromic acid as described by Borsche.[7] Form VIE also resulted in 92% yield from chromate oxidation of methyl 3α-carbethoxyoxy-7-keto-12α-hydroxycholanate (IVA).

Anal. $C_{28}H_{42}O_7$ (490.62): C, 68.55; H, 8.62. Found: (A) C, 68.82; H, 8.77; (C) C, 68.46; H, 8.44; (D) C, 68.71; H, 8.86; (E from D): C, 68.62; H, 8.52; (E, Borsche) C, 68.47; H, 8.47.

No appreciable differences were observable in infrared spectra of VIA, C, and D in chloroform. Wolff-Kishner reduction of 9 g. of VIA according to Huang-Minlon and crystallization of the product from aqueous acetone gave two crops of lithocholic acid: 4.75 g., m. p. 185–186°, $[\alpha]^{25}D$ +31 ± 2°; 0.85 g., m. p. 184–185°; total yield 81%. Forms VIC, D, and E also gave lithocholic acid in comparable yield.

Methyl 3α-Acetoxy-7,12-diketocholanate. Form VIIA.—Oxidation of 3 g. of methyl cholate 3-acetate[9] in 45 cc. of acetic acid with 2 g. of potassium chromate in 5 cc. of water at 25° for sixteen hours gave 2.7 g. of product that on one crystallization from methanol formed micaceous plates, m. p. 152–155°, $[\alpha]^{25}D$ +56 ± 2° (Grand and Reichstein[11]: m. p. 163–164°).

Anal. $C_{27}H_{40}O_6$ (460.59): C, 70.39; H, 8.76. Found: C, 70.17; H, 8.99.

Form VIIB was obtained starting with oxidation of 6 g. of methyl cholate in 90 cc. of acetic acid with 4 g. of potassium chromate in 10 cc. of water (gradual addition with cooling). After standing overnight the product was precipitated as a semisolid that was kneaded with water and dissolved in methanol. The solution slowly deposited 0.3 g. of colorless needles of **methyl dehydrocholate**, m. p. 229–230°; the recrystallized sample, m. p. 239–240°, $[\alpha]^{25}D$ +28 ± 2°, was identified by mixed m. p. determination.

Anal. $C_{25}H_{36}O_5$ (416.54): C, 72.08; H, 8.72. Found: C, 72.09; H, 8.90.

The oily residue recovered from the methanol mother liquor was heated with 10 cc. of acetic anhydride and 15 cc. of pyridine at 100° for one-half hour and the resulting 3-acetate (VIIB) crystallized from aqueous methanol. The substance formed prismatic needles, m. p. 145–147°, remelting at 145–146°, $[\alpha]^{25}D$ +53 ± 2°. A mixture of VIIA and B melted at 146–150°.

Anal. $C_{27}H_{40}O_6$ (460.59): C, 70.39; H, 8.76. Found: C, 69.99; H, 9.00.

Oxidation of Lithocholic Acid and Ester.—A mixture of 20 cc. of acetic acid containing 600 mg. of lithocholic acid (m. p. 184–185°) and 2 cc. of water containing 700 mg. of potassium chromate was let stand at 25° for twelve hours. The material precipitated with water on crystallization from acetone yielded a total of 460 mg. of lithocholic acid (m. p. and mixed m. p. 184–185°). In a similar experiment with methyl lithocholate chromatographic analysis indicated the product to be a mixture of unchanged ester and the corresponding 3-ketone in the ratio 88:12. The **methyl lithocholate** separated from methanol in colorless needles, m. p. 129–130°, $[\alpha]^{25}D$ +29 ± 2°.

Anal. $C_{25}H_{42}O_3$ (390.59): C, 76.78; H, 10.83. Found: C, 76.94; H, 10.59.

Methyl lithocholate 3-acetate crystallized from methanol in prismatic needles, m. p. 133–134°, $[\alpha]^{25}D$ +45 ± 2°.

Anal. $C_{27}H_{44}O_4$ (432.62): C, 74.94; H, 10.26. Found: C, 74.89; H, 9.96.

Methyl dehydrolithocholate separated from methanol in prismatic needles, m. p. 116–117°, $[\alpha]^{25}D$ +32 ± 2°.

Anal. $C_{25}H_{40}O_3$ (388.57): C, 77.20; H, 10.37. Found: C, 77.31; H, 10.16.

Methyl Cholate 3,7-Diacetate (VIII).—Previous procedures have afforded preparations of varying melting point in poor yield.[5,12–15] The following procedure gives consistently good yields of material corresponding to the highest melting preparation reported.[15]

A mixture of 63 g. of methyl cholate, 300 cc. of benzene, 75 cc. of pyridine and 75 cc. of acetic anhydride was let stand at 25° for one day and poured into a large excess of water. The benzene layer was separated, washed repeatedly with water and the solvent evaporated on the steam-bath. The residual solid was washed once with petroleum ether and one crystallization from methanol water) gave colorless needles, m. p. 185–187°; yield 53–54 g. (70–71%). Recrystallization raised the m. p. to 187–188°.

Methyl Cholate-7-acetate (IX).—Wieland and Kapitel[13] prepared this compound from the free acid and isolated it as an acetone solvate, m. p. 100–130°, remelting at 181°. The following procedure employs the ester diacetate and gives a solvent-free product.

A suspension of 25 g. of methyl cholate-3,7-diacetate (m. p. 185–187°) in 300 cc. of absolute methanol containing 2 g. of dry hydrogen chloride was shaken occasionally at room temperature for two hours, when a clear solution resulted. After three hours more, the solution was diluted with water; the resulting oil soon solidified to needles of a solvate melting with effervescence at 115–130°. The solvent was eliminated by evaporating a solution of the material in 100 cc. of benzene. Crystallization of the residue from benzene–ligroin afforded methyl cholate-7-acetate in the form of prismatic needles, m. p. 178–179°, $[\alpha]^{25}D$ +17 ± 2°; yield 18 g. (88%).

Anal. $C_{27}H_{44}O_6$ (464.62): C, 69.83; H, 9.49. Found: C, 69.88; H, 9.54. The material recovered from the mother liquors (3.6 g.) appeared to be a mixture (m. p. 169–173°).

Methyl 3α,7α-Diacetoxy-12-ketocholanate (X).—A solution of 25 g. of methyl cholate-3,7-diacetate (m. p. 185–186°) in 400 cc. of acetic acid was treated gradually with 16 g. of potassium chromate in 30 cc. of water, let stand at 25° for twelve hours, and diluted to distinct turbidity. The 12-ketone separated as almost colorless needles of high purity; m. p. 177–179°, $[\alpha]^{25}D$ +66 ± 1°; yield 24.8 g. (99%). The melting point checks with values reported.[13,14] The same product was obtained in lower yield (75%) when sodium acetate was employed in the oxidation mixture.

Methyl 3α-Hydroxy-7α-acetoxy-12-ketocholanate (XI).—Oxidation of 16 g. of methyl cholate 7-acetate in 300 cc. of acetic acid with 12 g. of potassium chromate in 30 cc. of water (25°, twelve hours) and crystallization of the product from aqueous methanol gave 14 g. of solvated material (low m. p.) that was dried by evaporation with 150 cc. of benzene and recrystallized from benzene–ligroin. This afforded 10 g. (62%) of needles, m. p. 181–182° (Wieland and Kapitel[13]; 184°), $[\alpha]^{25}D$ +61 ± 2°.

Anal. $C_{27}H_{42}O_6$ (462.61): C, 70.10; H, 9.10. Found: C, 70.06; H, 8.96.

Chenodesoxycholic Acid (XII) from XI.—A 9-g. batch of methyl 3α-hydroxy-7α-acetoxy-12-ketocholanate was reduced by the Huang-Minlon procedure[9] (5 cc. of 85% hydrazine hydrate, 4 g. of potassium hydroxide, 110 cc. of triethylene glycol). The crude product exhibited the unsatisfactory melting point characteristics encountered by Wieland and Kapitel[13] and was therefore processed as follows. The acid was dissolved in excess ether–benzene (3:1) and the solution washed with water, dried and evaporated. The residue, now largely insoluble in ether or benzene, was dried further by adding 150 cc. of benzene and evaporating the solvent. The residual solid was dissolved in hot ethyl acetate and the solution diluted with ether–petroleum ether (1:1) to distinct turbidity. After standing in the cold room for several hours the chenodesoxycholic acid that separated was collected and washed freely with dry ether. The yield of white, prismatic needles, m. p. 140–142° with effervescence, $[\alpha]^{25}D$ +11 ± 2°, was 4 g. (52%).

Anal. $C_{24}H_{40}O_4$ (392.56): C, 73.46; H, 10.25. Found: C, 73.31; H, 10.25.

The material recovered from the mother liquors was esterified with 75 cc. of 1% methanolic hydrogen chloride (eighteen hours at 25°) and the oily ester was acetylated with 4 cc. of acetic anhydride and 5 cc. of pyridine (twelve hours at 25°). The product, collected and dried by ether extraction, solidified in contact with cold methanol into

(11) Grand and Reichstein, *Helv. Chim. Acta*, **28**, 344 (1945).
(12) Borsche and Beske, *Z. physiol. Chem.*, **176**, 109 (1928).
(13) Wieland and Kapitel, *ibid.*, **212**, 269 (1932).
(14) Plattner and Heusser, *Helv. Chim. Acta*, **27**, 748 (1944).
(15) Berner, Lardon and Reichstein, *ibid.*, **30**, 1542 (1947).

colorless, prismatic needles, m. p. 93–95°; yield 0.9 g. (10%); total yield 62%. Recrystallization from methanol gave needles, m. p. 96–98°, $[\alpha]^{25}D$ +20 ± 2°.

Anal. $C_{29}H_{46}O_6$ (490.66): C, 71.01; H, 9.39. Found: C, 70.92, 70.88; H, 9.32, 9.59.

Chenodesoxycholic Acid (XI) from X.—Wolff-Kishner reduction of 24 g. of crude methyl 3α,7α-diacetoxy-12-ketocholanate (m. p. 177–179°) and processing of the product as above furnished 4.75 g. (23%) of pure chenodesoxycholic acid, m. p. 139–141°, and 9.6 g. (38%) of methyl chenodesoxycholate diacetate, m. p. 95–96°.

7-Ketolithocholic Acid from Chenodesoxycholic Acid.—7-Ketolithocholic acid has previously been obtained[16,17] by partial hydrogenation of dehydrochenodesoxycholic acid and by partial oxidation of chenodesoxycholic acid; Iwasaki[16] reports m. p. 203°, $[\alpha]^{20}D$ −27° (alcohol). Oxidation of 3 g. of chenodesoxycholic acid by the potassium chromate (2 g.) procedure gave a mixture that was fractionated from aqueous methanol. The crops melting between 190 and 198° on recrystallization from aqueous acetone afforded 1.8 g. (60%) of prismatic needles of 7-ketolithocholic acid, m. p. 202–203°, $[\alpha]^{25}D$ −28 ± 2°.

An oxidation of 2 g. of chenodesoxycholic acid in 45 cc. of acetone containing 5 cc. of water was conducted with 2 g. of N-bromosuccinimide for eleven hours at 25° and the reaction product was freed of excess reagent by repeated extraction with boiling water. Further processing as in the previous experiment gave 0.85 g. (42%) of 7-ketolithocholic acid, m. p. 199–201°, $[\alpha]^{25}D$ −26 ± 2°.

In a third experiment 2 g. of chenodesoxycholic acid in 100 cc. of 2% sodium bicarbonate solution was treated with 2 g. of N-bromosuccinimide (twelve hours, 25°). Processing as before gave 1 g. (50%) of 7-ketolithocholic acid, m. p. 202–203°, $[\alpha]^{25}D$ −28 ± 2°. Analyses of the three samples are as follows.

Anal. $C_{24}H_{38}O_4$ (390.54): C, 73.78; H, 9.81. Found: C, 73.68, 73.53, 73.78; H, 9.84, 10.01, 9.75.

Methyl 3-Carbethoxydesoxycholate.—A solution of 40 g. of crude methyl desoxycholate (m. p. 75–86°) prepared by fractionation of the product of esterification of technical acid according to Kendall in 200 cc. of pyridine was treated gradually with 32 g. of ethyl chlorocarbonate (3 moles) with cooling under the tap. After six hours at 25° the solution was diluted with water and neutralized with acetic acid. An oil separated and soon solidified; it was ground with water in a mortar, washed well, and crystallized from aqueous methanol to give 36 g. (79%) of colorless needles, m. p. 138–140°. On recrystallization, the derivative melted at 142–143°, $[\alpha]^{25}D$ +54 ± 2°.

Anal. $C_{28}H_{46}O_6$ (478.65): C, 69.97; H, 9.64. Found: C, 70.25; H, 9.71.

The 3-carbethoxy methyl ester was also obtained satisfactorily from the total product of esterification of desoxycholic acid.[18] A suspension of 40 g. of desoxycholic acid etherate, prepared as described[3] from total bile acids, in 120 cc. of methanol containing 1.2 cc. of 36% hydrochloric acid was warmed on the steam-bath and the resulting solution filtered from a trace of solid. The filtrate and washings were let stand at 25° overnight and diluted with 500 cc. of water containing 2.5 g. of sodium bicarbonate. The tan-colored methyl desoxycholate after being dried at 60° melted at 88–105° (40 g.); it was treated in 120 cc. of pyridine with 20 cc. of ethyl chlorocarbonate as above, and crystallization from aqueous methanol gave 28.2 g. (59%) of colorless methyl 3-carbethoxydesoxycholate, m. p. 137–140°.

Methyl 3-carbethoxy-12-ketocholanate was prepared by oxidation of 24 g. of methyl 3-carbethoxydesoxycholate in 400 cc. of acetic acid with 10 g. of potassium chromate in 30 cc. of water (overnight at 25°). The product separated from methanol in prismatic needles, m. p. 157–158°, $[\alpha]^{25}D$ +91 ± 1°; yield 22.4 g. (93%).

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

(17) Kaziro and Shimada, *ibid.*, **249**, 220 (1937).

(18) We are indebted to Dr. Richard L. Markus for this experiment.

Anal. $C_{28}H_{44}O_6$ (476.63): C, 70.55; H, 9.32. Found: C, 70.59; H, 9.00.

Another experiment was conducted in the same way except for the addition of a large amount of sodium acetate. The product obtained appeared to consist largely of unoxidized starting material, but the rotation, $[\alpha]^{25}D$ +68 ± 2°, suggests the presence of a certain amount of the 12-ketone.

Methyl 3α-carbethoxyoxy-12-keto-$\Delta^{9(11)}$-lithocholenate.—Initial batches of this substance prepared from methyl 3α-carbethoxyoxy-12-ketocholanate by selenium oxide oxidation as described below (4 g. gave 3.8 g., m. p. 157–159°) and from crude methyl 12-keto-$\Delta^{9(11)}$-lithocholenate (m. p. 113–115°, from a batch of 85% acid kindly supplied by Merck and Co., Inc.; 20 g. of ester gave 22 g. of product) had the following properties: m. p. 158–160°, 158–161°; $[\alpha]^{25}D$ +92 ± 2°, + 86 ± 1°, $\lambda^{alc.}_{max.}$ 239, 239 mμ (log ε 4.05, 4.1).

Anal. $C_{28}H_{42}O_6$ (474.62): C, 70.88; H, 8.86. Found: C, 71.01; H, 8.56.

The following procedure was worked out for the large-scale preparation of the substance from technical desoxycholic acid without purification of intermediates; it includes a method of preparing methyl desoxycholate more conveniently and in better yield than that of Kendall.[3]

Two hundred g. of technical desoxycholic acid, m. p. 168–169°, was dissolved in 400 cc. of absolute ethanol, the solution was let stand for twelve hours (no contaminants precipitated) and converted into the ether complex with 2 l. of ether by the process described.[3] The **desoxycholic acid etherate** was washed well with ether and dried at 110° for several hours (193 g.); it was heated with 600 cc. of methanol and 10 cc. of 36% hydrochloric acid on the steam bath until dissolved and the solution left overnight for crystallization. A part of the ester crystallized and the rest was recovered by treatment of the filtrate with an equal volume of water containing 20 g. of sodium bicarbonate. The **methyl desoxycholate** was washed with 50% methanol (1 l.) and dried for several hours at 60–70°; yield 192 g., m. p. 104–106°. This ester was treated in 600 cc. of pyridine with 200 g. of ethyl chlorocarbonate as described above and the **methyl 3-carbethoxydesoxycholate** was pulverized and washed with dilute hydrochloric acid and with water, dried superficially (m. p. 128–138°), and oxidized in 1800 cc. of acetic acid with 200 g. of potassium chromate in 200 cc. of water (overnight at 25°). Dilution with 2 l. of water precipitated **methyl 3α-carbethoxyoxy-12-ketocholanate**, which was washed with 1 l. of 50% acetic acid and with water and dried at 110° overnight; yield 201 g., m. p. 148–153°. A mixture of this material and 100 g. of selenium dioxide in 1 l. of acetic acid was refluxed in an oil-bath for eighteen hours, and the solution was filtered hot through a fluted funnel and diluted with water to distinct turbidity. The product crystallized and was washed with 1 l. of 50% acetic acid and then with water; and a solution of the lemon yellow solid in 1 l. of acetic acid was heated with 20 g. of Darco for one-half hour on the steam-bath, filtered hot, and the nearly boiling filtrate diluted with hot water to turbidity and let crystallize. The **methyl 3α-carbethoxyoxy-12-keto-$\Delta^{9(11)}$-lithocholenate**, collected and washed as before and thoroughly dried at 110°, was faintly yellow from selenium, m. p. 156–159°, $\lambda^{alc.}_{max.}$ 237–238 mμ (log ε 4.03); yield 153 g. (63% from technical desoxycholic acid).

Selenium was removed from a 69.5 g. batch[18] by treating it in 300 cc. of chloroform with a solution of 25 g. of potassium chromate in 250 cc. of acetic acid. The mixture was stirred at 25° for five hours, 1 l. of water was added, the chloroform layer was separated, and the aqueous layer extracted with 150 cc. more chloroform. The pooled chloroform extract was washed with water, bicarbonate solution, water again, dried and evaporated. The residual unsaturated ketone crystallized from aqueous acetic acid was nearly colorless, m. p. 157–160°, $\lambda^{alc.}_{max.}$ 237–238 mμ (4.08); yield 59.2 g. (85% recovery).

Oxidation of Methyl Desoxycholate.—A solution of 20 g. of methyl ester m. p. 75–86° in 200 cc. of acetic acid was

treated with 13 g. of potassium chromate in 25 cc. of water (fourteen hours at 25°). The washed and dried product, m. p. 114–117° (19.4–19.7 g.) gave well-formed needles from methanol, m. p. 117–119°, but was separated by chromatography into methyl 12-ketolithocholate and methyl dehydrodesoxycholate[12,19] (m. p. 129–130°, $[\alpha]^{25}D$ +92 ± 3°) in the ratio of 3:1.

Methyl 12-ketolithocholate[20,21] separated from methanol in prismatic needles, m. p. 111–112°, $[\alpha]^{25}D$ +92 ± 3°. The **methyl ester 3-acetate**[21,22] formed colorless plates from methanol, m. p. 145–147°, $[\alpha]^{25}D$ +100 ± 1°, apparently of a solvate.

Anal. $C_{27}H_{42}O_5 \cdot CH_3OH$ (478.65): C, 72.68; H, 10.03. Found: C, 73.01; H, 10.16. The **methyl ester 3-carbethoxy derivative**, m. p. 155–157°, $[\alpha]^{25}D$ + 92 ± 2°, showed no depression in m. p. with the sample described above.

Anal. $C_{28}H_{44}O_6$ (476:63): C, 70.55; H, 9.32. Found: C, 70.70; H, 9.37.

The **methyl ester 3-benzoate**,[5] crystallized from aqueous methanol, melted at 126–127°. Free **12-ketolithocholic acid**[20,23] as obtained by the action of methanolic potassium hydroxide on the methyl ester was solvated (m. p. 105° and upwards); distillation with benzene and crystallization from benzene–ether gave anhydrous acid, m. p. 162–163°.

Oxidation of Cholestane-3β,7α-diol.[24]—The starting material, obtained by hydrolysis of a sample of the 3-acetate kindly supplied by Dr. O. Wintersteiner, melted at 157–158°.

(a) A solution of 200 mg. of diol in 20 cc. of acetone and 2 cc. of water was treated with 300 mg. of N-bromosuccinimide and a drop of acetic acid. After thirteen hours at 25°, water was added and the solid product was washed with bicarbonate solution, extracted with hot water and crystallized from aqueous methanol. The product, **cholestane-3β-ol-7-one**,[25] separated as platelets, m. p. 155–157°, $[\alpha]^{25}D$ −16 ± 2°; yield 180 mg. (90%).

Anal. $C_{27}H_{46}O_2$ (402.64): C, 80.52; H, 11.52. Found: C, 80.70; H, 11.50.

(b) A solution of 200 mg. of the diol in 10 cc. of acetic acid was treated with 300 mg. of potassium chromate in 1 cc. of water (thirteen hours at 25°). The product, cholestane-3,7-dione,[25] crystallized from dilute methanol in needles, m. p. 175–178°, $[\alpha]^{25}D$ −19 ± 2°.

Anal. $C_{27}H_{44}O_2$ (400.62): C, 80.52; H, 11.22. Found: C, 80.92; H, 11.08.

Behavior of Cholesterol.—A mixture of 5 g. of cholesterol in 80 cc. of acetic acid, 5 g. of potassium chromate in 10 cc. of water and enough chloroform to produce a homogeneous solution was left for eighteen hours at 25° and the product precipitated with water. Crystallization from methanol gave 4.6 g. of unchanged cholesterol, m. p. 148–149°; acetate, m. p. 114–116° (both identified by mixed m. p.).

Oxidation of Pregnane-3α,20β-diol-11-one.—A 300 mg. sample of diolone (m. p. 229–230°) kindly supplied by Dr. L. H. Sarett in 10 cc. of acetic acid was treated with 300 mg. of potassium chromate in 1 cc. of water (ten hours at 25°). Addition of water precipitated 230 mg. of solid, m. p. 144–147°, and crystallization from methanol (water) furnished needles, m. p. 152–153°. A mixture of this with a sample of **pregnane-3,11,20-trione** (m. p. 157–158°) supplied by Dr. Sarett melted at 156–157°.

Anal. $C_{21}H_{30}O_3$ (330.45): C, 76.34; H, 9.14. Found: C, 75.98; H, 9.04.

Summary

Selective oxidation of a 3-acyl derivative of methyl cholate to the 7-ketone can be accomplished in high yield with either N-bromosuccinimide or by Haslewood's procedure (aqueous potassium chromate added to an acetic acid solution buffered with sodium acetate). Potassium chromate in unbuffered aqueous acetic acid oxidizes 3-acyl derivatives of methyl cholate smoothly to the 7,12-diketones. The behavior on chromate oxidation of steroids having a free 3-hydroxyl group is variable; some are oxidized readily, others to a small extent and others (cholesterol) not at all.

Improved techniques of selective oxidation and acylation have led to development of improved procedures for the preparation of methyl cholate, methyl cholate 3,7-diacetate, methyl cholate 7-acetate, chenodesoxycholic acid, methyl 3α,7α-diacetoxy-12-keto cholanate, methyl desoxycholate, methyl 3-carbethoxydesoxycholate and methyl 3α-carbethoxyoxy-12-keto-$\Delta^{9(11)}$-lithocholenate.

Carbethoxylation of bile acid derivatives is specific to the 3α-hydroxyl group, and in several instances 3-carbethoxy derivatives have proved advantageous as intermediates and in effecting separations and isolations.

7-Keto and 7,12-diketo derivatives of cholic acid have been isolated in several forms of unestablished nature.

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