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Seroflocculating Steroids. III.[1] Chloro and Other Bile Acid Derivatives[2]

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For study of their seroflocculating activity, a number of 3β-chloro bile acid derivatives were prepared by a new method involving replacement of a tosylate group in pyridine with pyridinium chloride. Several new acetates, tosylates and other related compounds are reported.

When the synthesis of ethyl 3β-chloro-11-cholenate (28)[4] by the method previously described[5] was attempted on a larger scale, the yield and quality of product were found to be considerably lowered. Various other direct methods for preparing halides also proved unsatisfactory for this compound. The presence of 28 among the products of a tosyl chloride reaction in pyridine described in the preceding paper[1] furnished a clue for a satisfactory new method for preparing the compound.

Since 28 obviously resulted from an intermediate tosylate, the commonly used reaction of a tosylate with lithium chloride in acetone (and in dioxane) was tried on the tosylate of ethyl 3α-hydroxy-11-cholenate. Intractable mixtures of unsaturated and chloro compound resulted. However, by substituting pyridine and pyridine hydrochloride for the solvent–halide system, a good quality chloro product was obtained in 51% yield. The yield is only moderate, and the superiority of the method lies in the purity of the product, which is very little contaminated by dienic impurity. A mixture of chloro compound containing more than traces of diene is separated only with much difficulty; the inferior products obtained in other halogenation methods are such mixtures. The reaction temperature is an important factor, as previous studies on replacement reactions would suggest,[6] and at elevated temperatures undesirable mixtures of chloro and dienic compounds are obtained. At temperatures between 78 and 90° the substitution reaction proceeds at a satisfactory rate, with little competition from the elimination reaction.

Methyl 3β-chlorocholanate, methyl and ethyl 3β-chloro-11-cholenate, methyl 3β-chloro-9(11)-cholenate and methyl 3β-chloro-12α-hydroxycholanate have all been prepared by this method, which we are studying further with the view of its use as a general method of preparing halides.[7]

For the preparation of ethyl 3β-chloro-9(11)-cholenate, because of the low melting points and poor crystallizing behavior of the corresponding 3α-hydroxy and 3α-tosyloxy compounds which are intermediates in the synthesis, conversion from the methyl ester is preferred. The sequence of reactions, part of which is also illustrative of other chloro compounds mentioned above, is shown.

Puzzling changes in melting point were encountered during crystallizations of several of the ethyl ester tosylates when methanol was used as solvent at 50°. Since the fractions continued to give correct analyses, replacement of tosylate group was not taking place, and ester interchange was not suspected, because numerous other ethyl esters in this related group of compounds can be crystallized unchanged from methanol. However, ester interchange did take place, as was evident when the supposed ethyl ester tosylate 24 was converted into methyl 3β-chloro-11-cholenate. Apparently a trace of *p*-toluenesulfonic acid resulting from hydrolysis of the 3-tosylate group (it is known that esters of the 3-hydroxy steroids are easily hydrolyzed) catalyzes the ester interchange. By adding *p*-toluenesulfonic acid to the refluxing methanol solution during crystallization, conversion of ethyl to methyl ester is fairly rapid and complete. The facile ester interchange of the bile acid esters might have been expected from the marked ease with which cholenic and substituted cholanic acids are esterified. For instance, 3α-hydroxy-11-cholenic acid is completely esterified in 40 minutes by methanol containing small amounts of aqueous hydrochloric acid at room temperature.[8] Yamasaki, Rosnati, Fieser and Fieser[9] recently encountered an ester interchange during the reduction of methyl 4β-bromodehydrolithocholate by sodium borohydride in the presence of ethanol. They also converted ethyl 3,4-dibromocholanate into the methyl ester by refluxing in methanol containing concentrated hydrochloric acid. Several of our esters underwent interchange similarly when refluxed, but a purer product usually resulted when the reaction was left at room temperature for 10 to 18 days. Ethyl 3β-chloro-11-cholenate (28) prepared in this way crystallized directly from the

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(4) Numbered according to the consecutive order in which the compounds are described in the Experimental sections of this (III) and the accompanying paper (IV).

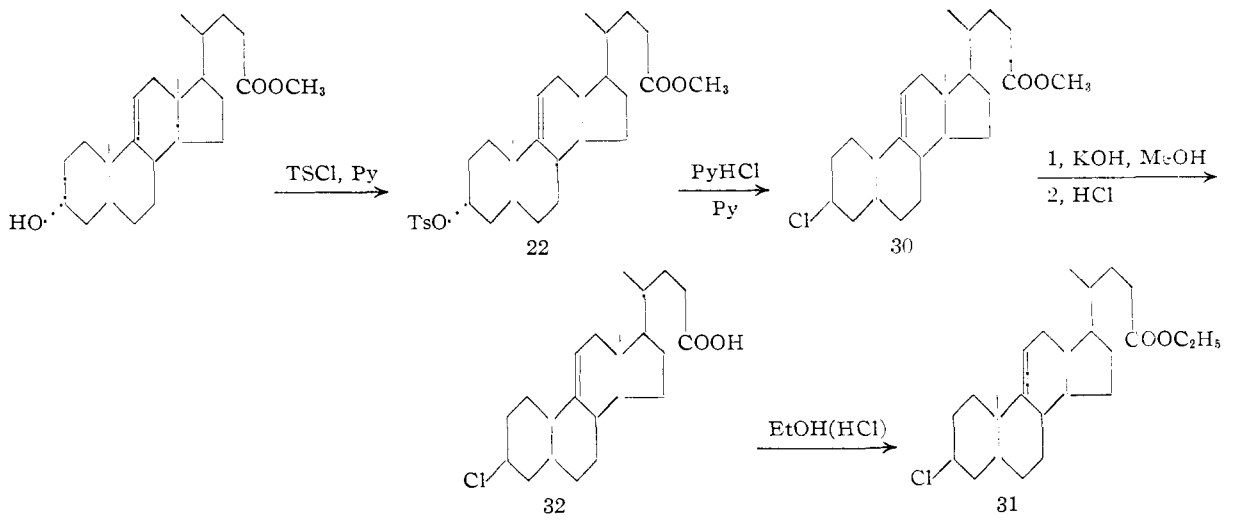
(5) Paper I of this series, THIS JOURNAL, **76**, 3213 (1954).

(6) E. D. Hughes and C. K. Ingold, *Trans. Faraday Soc.*, **37**, 657 (1941).

(7) Organic halides have been obtained often as a result of reaction between alcohols and tosyl (and mesyl) chlorides in pyridine [*e.g.*, C. R. Noller, C. A. Luchetti, E. M. Acton and R. A. Bernhard, THIS JOURNAL, **75**, 3851 (1953)], but the use of pyridine base and pyridinium halide on a tosylate appears to be novel. F. Drahowzal and D. Klamann [*Monatsh.*, **82**, 460 (1951)] record failure to obtain *n*-butyl and *n*-octyl chlorides from the tosylates under conditions similar to ours except for a considerably smaller proportion of pyridine, but they describe successful preparations of halides using pyridinium chloride without solvent and with carbon tetrachloride as solvent.

(8) L. L. Engel, V. R. Mattox, B. F. McKenzie, W. F. McGuckin and E. C. Kendall, *J. Biol. Chem.*, **162**, 565 (1946).

(9) K. Yamasaki, V. Rosnati, M. Fieser and L. F. Fieser, THIS JOURNAL, **77**, 3308 (1955).



reaction mixture in a state of highest purity in near quantitative yield. This ester interchange constitutes a good method for preparing higher esters of the bile acids from the often more readily available methyl esters.

The extreme ease of esterification of the C_{24}-bile acids is in contrast to the considerable resistance exhibited by the bisnorcholanic acids to this reaction. 3β-Hydroxy-5-bisnorcholenic acid forms the methyl and ethyl esters in poor yield even after several days of reflux with the respective alcohols. Perhaps for this reason, only the methyl ester prepared with diazomethane has been reported. The expected difference in the rates of reaction between the C_{20}-secondary and C_{23}-primary carboxyl groups is doubtless intensified considerably by the interference offered by the steroid nucleus.

Experimental[10,11]

ESTERS OF HYDROXY ACIDS. Esterification of Hydroxy Acids.—All of the C_{24}-acids were esterified readily by using the proportion of 1 g. of acid:10 ml. of absolute (methyl or ethyl) alcohol:0.1 ml. of concd. HCl. (The alcoholic solution is approximately 0.12 *N* in HCl.) With the 10:1 ratio of solvent to acid, the reaction is rapid and complete.

Methyl lithocholate (1),[12] was obtained from lithocholic acid in quantitative yield as large needles, m.p. 129–130°; recrystallized from methanol–water and dried at 100° (1 mm.) for 24 hr. for analysis, m.p. 125–127.5°, $[\alpha]_D$ +22°. *Anal.* Calcd. for $C_{25}H_{42}O_3$ (390.59): C, 76.87; H, 10.84. Found: C, 76.6; H, 10.8. This conflicts with the Reindel report[12] that the 125–127° melting product contains 1.5 moles of methanol as solvent of crystallization. Fieser and Ettore[13] reported m.p. 125–127°; Fieser and Rajagopalan,[14] 129–130°, $[\alpha]_D$ +29 ±2° (diox.).

Ethyl Lithocholate (2).[12] **By ester interchange** from 1 by refluxing 1.5 hr. in ethanol and concentrated hydrochloric acid; 74% of 92–95° melting product. Recrystallized from methanol–water, m.p. 94.5–96° (lit. m.p. 92–93°), $[\alpha]_D$ +28.8°*. *Anal.* Calcd. for $C_{26}H_{44}O_3$ (404.60): C, 77.18; H, 10.96. Found: C, 77.18; H, 10.84.

Methyl 12α-hydroxycholanate (3) was prepared according to Barnett and Reichstein,[15] m.p. 119.5–120.0° (lit. 120–121°), $[\alpha]_D$ +39°.

Ethyl 12α-hydroxycholanate (4) was prepared by the same method from ethyl 12α-hydroxy-3-cholenate (54)[16] in quantitative yield; crystallized from absolute ethanol, m.p. 135–136.5°, $[\alpha]_D$ +46°. *Anal.* Calcd. for $C_{26}H_{44}O_3$ (404.60): C, 77.2; H, 11.0. Found: C, 77.1; H, 10.9.

Compound 4 was also prepared by esterification of 12α-hydroxycholanic acid, m.p. 135–136.5°.

Ethyl 3α-hydroxy-9(11)-cholenate (5) was prepared by esterification of 3α-hydroxy-9(11)-cholenic acid,[17] m.p. 75.0–76.5°, $[\alpha]_D$ +35.6°. *Anal.* Calcd. for $C_{26}H_{42}O_3$ (402.60): C, 77.56; H, 10.52. Found: C, 77.25; H, 10.44.

Compound 5 also was obtained from methyl 3α-acetoxy-9(11)-cholenate (12) by treatment with ethanol and hydrochloric acid.

Methyl 3β-hydroxy-5-bisnorcholenate (6) was prepared by refluxing the corresponding acid in methanol and concd. hydrochloric acid for two days; 54% yield of product melting 140–144° out of ligroin (63–70°). A chromatographed sample for analysis melted at 142–143.7°, $[\alpha]_D$ −55.4°*. (This compound was previously prepared by use of diazomethane by Fernholz,[18] m.p. 140°.) *Anal.* Calcd. for $C_{23}H_{36}O_3$ (360.52): C, 76.62; H, 10.07. Found: C, 76.95; H. 9.97.

Ethyl 3β-Hydroxy-5-bisnorcholenate (7).—Two grams of 3β-hydroxy-5-bisnorcholenic acid (Nutritional Biochemical Corp.) in 150 ml. of absolute ethanol and 2 ml. of concd. sulfuric acid was refluxed for 45 hr. After usual processing a product melting at 162–164° was obtained in 34% yield, after recrystallization from ligroin (63–70°), $[\alpha]_D$ −65.6°. *Anal.* Calcd. for $C_{24}H_{38}O_3$(374.54): C, 76.96; H, 10.23. Found: C, 77.2; H, 10.5.

ACETATES.—The 3-acetates were made by room temperature acetylation using acetic anhydride and pyridine; the 12-acetates required heating to 100°, with the exception of those prepared using *p*-toluenesulfonic acid as catalyst (see compounds 18 and 19).

Ethyl 3α-acetoxycholanate (8)[12] was obtained from ethyl lithocholate, out of ethanol, m.p. 95–98° (lit. 90–91°), $[\alpha]_D$ +38.2°. *Anal.* Calcd. for $C_{28}H_{46}O_4$ (446.65): C, 75.29; H, 10.38. Found: C, 75.48; H, 10.56.

Methyl 12α-acetoxycholanate (9) from 3, heavy prisms out of methanol, m.p. 95.5–96.5°, $[\alpha]_D$ +69°. *Anal.* Calcd. for $C_{27}H_{44}O_4$ (432.62): C, 74.95; H, 10.25. Found: C, 75.03; H, 10.14.

Ethyl 12α-acetoxycholanate (10) from 4, out of absolute ethanol as short, thick prisms, m.p. 63–64°, $[\alpha]_D$ +69.9°.

(10) Microanalyses by the Microchemical Laboratory of New York University, and by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

(11) Melting points were taken on an electrical micro-hotstage and are uncorrected. Optical rotations were determined in 1–2% chloroform solutions at about 25°, except where noted, using a Schmidt and Haensch polarimeter, and values are accurate to ±1°. The values marked with an asterisk (*) were obtained with a Keston polarimeter attachment (Standard Polarimeter Co., 225 E. 54th Street, N. Y. C.) to a Beckman DU spectrophotometer with accuracy estimated to be better than ±2°.

(12) F. Reindel and A. Niederländer, *Ber.*, **68B**, 1969 (1935).

(13) L. F. Fieser and R. Ettore, THIS JOURNAL, **75**, 1700 (1953).

(14) L. F. Fieser and S. Rajagopalan, *ibid.*, **72**, 5530 (1950).

(15) J. Barnett and T. Reichstein, *Helv. Chim. Acta*, **21**, 926 (1938).

(16) Paper IV of this series, THIS JOURNAL, **79**, 2167 (1957).

(17) L. F. Fieser and S. Rajagopalan, *ibid.*, **73**, 121 (1951).

(18) E. Fernholz, *Ann.*, **507**, 128 (1933); A. Butenandt and G. Fleischer, *Ber.*, **70**, 96 (1937).

Anal. Calcd. for $C_{28}H_{46}O_4$ (446.65): C, 75.29; H, 10.38. Found: C, 75.33; H, 10.36.

Ethyl 3α-acetoxy-11-cholenate (11) from ethyl 3α-hydroxy-11-cholenate,[5] out of methanol as fine needles, m.p. 91.5–93.7°, $[\alpha]_D$ +42°. *Anal.* Calcd. for $C_{28}H_{44}O_4$ (444.63): C, 75.63; H, 9.97. Found: C, 75.6; H, 9.9.

Ethyl 3α-acetoxy-9(11)-cholenate (12) from 5, out of legroin (35–60°), m.p. 96.5–97.5°, $[\alpha]_D$ +64.8°. *Anal.* Calcd. for $C_{28}H_{44}O_4$ (444.63): C, 75.63; H, 9.97. Found: C, 75.44; H, 10.35.

Methyl 12α-acetoxy-3-cholenate (13) from 53,[16] flat prisms out of methanol, m.p. 107–108°, $[\alpha]_D$ +63.9°. *Anal.* Calcd. for $C_{27}H_{42}O_4$ (430.61): C, 75.31; H, 9.83. Found: C, 75.23; H, 9.72.

Ethyl 12α-acetoxy-3-cholenate (14) from 54,[16] out of acetone–H_2O (3:1), m.p. 86–87.5°, $[\alpha]_D$ +62°. *Anal.* Calcd. for $C_{28}H_{44}O_4$ (444.63): C, 75.63; H, 9.97. Found: C, 75.52; H, 10.12.

Methyl 3β-acetoxy-5-bisnorcholenate (15)[18] prepared by the action of diazomethane on 3β-acetoxy-5-bisnorcholenic acid (Nutritional Biochemicals Corp.) out of methanol in 76% yield, m.p. 153.5–156.5° (lit. m.p. 156–157°), $[\alpha]_D$ +63.4°*.

Ethyl 3β-acetoxy-5-bisnorcholenate (16) from 7 out of ligroin (35–60°), leaflets, which at 124–125° were transformed into needles, m.p. 129–130.5°, $[\alpha]_D$ +63.0°*. *Anal.*[19] Calcd. for $C_{26}H_{40}O_4$(416.58): C, 74.96; H, 9.68. Found: C, 75.13; H, 9.67.

Ethyl 3α,12α-diacetoxycholanate (17) was prepared from ethyl desoxycholate by refluxing in acetic anhydride and pyridine. All attempts at crystallization failed. This distilled as colorless oil in a molecular still at 0.07 mm., $[\alpha]_D$ +89.7° (*c* 2.05, chf); +98.2° (*c* 2.06, ethanol). *Anal.* Calcd. for $C_{30}H_{48}O_6$ (504.68): C, 71.39; H, 9.59. Found: C, 71.73, 71.86; H, 9.91, 9.93.

Methyl 3β-chloro-12α-acetoxycholanate (18) was obtained by the method recently reported[20] for difficultly acetylated hydroxyl groups, using acetic acid, acetic anhydride and *p*-toluenesulfonic acid at room temperature, as colorless needles when crystallized from methanol, m.p. 129–130°, $[\alpha]_D$ +56.8°*. *Anal.* Calcd. for $C_{27}H_{43}O_4Cl$(467.07): C, 69.43; H, 9.28; Cl, 7.59. Found: C, 69.32; H, 9.30; Cl, 7.53.

Ethyl 3β-chloro-12α-acetoxycholanate (19) was prepared as for 18, from 36, crystallizing from ethanol–water (3:1) as colorless needles, m.p. 82.0–83.5°, $[\alpha]_D$ +72.2°*. *Anal.* Calcd. for $C_{28}H_{45}O_4Cl$ (481.10): C, 69.89; H, 9.43; Cl, 7.37. Found: C, 70.00; H, 9.46; Cl, 7.35.

When prepared by the acetic anhydride and pyridine method at 100°, the product was poor.

TOSYLATES.—All are 3α-tosylates, prepared by room temperature addition of *p*-toluenesulfonyl chloride and pyridine to the 3-hydroxy compound. In general, yields were nearly quantitative. Where they were lower than expected, it was possible to increase the yield by first drying the hydroxy compound by an azeotropic distillation in benzene.

Methyl 3α-tosyloxycholanate (methyl lithocholate tosylate) (20)[21] from methyl lithocholate, out of methanol as lovely, light-refracting, thin, square plates, m.p. 120–121.5° (lit.[21] 107–109°), $[\alpha]_D$ +35°. *Anal.* Calcd. for $C_{32}H_{48}O_5S$ (544.75): C, 70.56; H, 8.88. Found: C, 70.6; H, 8.9.

Ethyl 3α-tosyloxy cholanate (21) by ester interchange of 20 in ethanol and HCl by 2-hr. reflux, a low yield of crystalline material out of hexane, m.p. 94.5–95.5°. *Anal.* Calcd. for $C_{33}H_{50}O_5S$ (558.8): C, 70.93; H, 9.02; S, 5.74. Found: C, 71.25; H, 9.17; S, 5.65, $[\alpha]_D$ +33.0°*.

Methyl 3α-tosyloxy-9(11)-cholenate (22)[21] was prepared by tosylation of methyl 3α-hydroxy-9(11)-cholenate at room temperature, out of methanol or acetone as colorless crystals, m.p. 112.5–114°, $[\alpha]_D$ +33° (lit. 111–112°). *Anal.* Calcd. for $C_{32}H_{46}O_5S$ (542.69): C, 70.82; H, 8.54; S, 5.90. Found: C, 70.60; H, 8.61; S, 5.77.

Methyl 3α-tosyloxy-11-cholenate (23) was prepared by (a) tosylation of methyl 3α-hydroxy-11-cholenate to give a 91% yield of lustrous plates, m.p. 103–107°, from methanol. Recrystallized from warm methanol (not refluxed), m.p. 104.5–107°, $[\alpha]_D$ +40°. *Anal.* Calcd. for $C_{32}H_{46}O_5S$ (542.69): C, 70.81; H, 8.54. Found: C, 70.9; H, 8.9. (b) **By ester interchange**: When the ethyl ester (24) was first prepared, crystallization from methanol at 50° brought out colorless crystals, the melting point of which on continued crystallization rose from 65 to 104.5°. That an ester interchange from the ethyl to methyl ester had taken place was proved by the conversion of the product to methyl 3β-chloro-11-cholenate (27).

Ethyl 3α-tosyloxy-11-cholenate (24) by tosylation of ethyl 3α-hydroxy-11-cholenate.[5] Well-shaped needles out of absolute ethanol; has double melting point 57.5–61° and 95–96.5°, $[\alpha]_D$ +30.2°*. *Anal.* Calcd. for $C_{33}H_{48}O_5S$ (556.77): C, 71.18; H, 8.69. Found: C, 71.1; H, 8.90.

Methyl 3α-tosyloxy-12α-hydroxycholanate (25) was prepared by tosylation of methyl desoxycholate according to Barnett and Reichstein.[15] The compound decomposes at slightly above its melting point, which varies between 139 and 150° depending on the rate and length of heating. When put on the m.p. block at 149°, melting was observed at 149–150°, the melt turning yellow and effervescing, $[\alpha]_D$ +40.4°. (Barnett and Reichstein reported a m.p. of 149°.) *Anal.* Calcd. for $C_{32}H_{48}O_6S$ (560.70): C, 68.54; H, 8.63. Found: C, 68.36; H, 8.89.

Ethyl 3α-tosyloxy-12α-hydroxycholanate (26) was obtained by tosylation at room temperature of ethyl desoxycholate (crystallized originally from ether but had been on the shelf for about one year) in poor yield. A second tosylation using ethyl desoxycholate that had first received a preliminary azeotropic distillation with benzene gave a nearly quantitative yield of product of melting point 98–100°. Recrystallization out of ether–Skellysolve F (1:1) gave good, colorless needles, m.p. 101–103.5°. Dissolved in benzene and diluted with ligroin (63–70°), long needles separated, m.p. 114–116.5°, $[\alpha]_D$ +38.8°. *Anal.* Calcd. for $C_{33}H_{50}O_6S$ (574.8): C, 69.00; H, 8.78; S, 5.59. Found: C, 68.81; H, 8.73; S, 5.5.

CHLORO COMPOUNDS.—The 3-chlorocholanic acid derivatives were made by the pyridine–pyridine hydrochloride method; the bisnorcholenic acid compounds were made with thionyl chloride. The pyridinium chloride method is described in detail only for the preparation of compound 27.

Methyl 3β-chloro-11-cholenate (27) was made by the pyridinium chloride method. The tosylate 23 (1.4 g.) was heated for 21 hr. at 90° in 12 ml. of pyridine containing 1.4 g. of pyridine hydrochloride. Chips of ice and water were added and the mixture was stirred and refrigerated. The solid which precipitated was shaken with cold 3% hydrochloric acid and filtered. After washing with water and drying, the solid was crystallized from methanol. A small amount of insoluble material was first removed and the clear methanol solution deposited thin flakes, 446 mg. (42.5%), m.p. 96–100°. Two crystallizations raised the m.p. to 100.5–102°, $[\alpha]_D$ +24.5°. (Another run starting with 400 mg. of tosylate, and heated at 90° for 11 hr. gave a 53.5% yield of comparable product.) *Anal.* Calcd. for $C_{24}H_{37}O_2Cl$ (407.02): C, 73.77; H, 9.66. Found: C, 73.6; H, 9.8.

Ethyl 3β-Chloro-11-cholenate (28).[5]—(a) By the pyridinium chloride method from the tosylate (24), from 95% ethanol in 51% yield, in long laths with hexagonal ends, m.p. 78.5–79.5°, $[\alpha]_D$ +25°. *Anal.* Calcd. for $C_{26}H_{41}O_2Cl$(421.05): C, 74.16; H, 9.82. Found: C, 73.9; H, 9.7.

(b) By esterification of the acid 29, long prismatic needles by slow crystallization out of dilute 95% ethanol solution, m.p. 78–79.5°.

3β-Chloro-11-cholenic acid (29) was obtained in nearly quantitative yield by hydrolysis of the methyl ester and was recrystallized from acetic acid as well-shaped elongated prisms, m.p. 167–170.5°, $[\alpha]_D$ +24.5°. *Anal.* Calcd. for $C_{24}H_{37}O_2Cl$ (393.00): C, 73.34; H, 9.49. Found: C, 73.7; H, 9.5.

Methyl 3β-chloro-9(11)-cholenate (30) was obtained by the pyridinium chloride method on the tosylate 22 at 76.5° for 26 hr., as crystals from methanol in 51.5% yield, m.p. 86.5–88°, $[\alpha]_D$ +31°. *Anal.* Calcd. for $C_{25}H_{39}O_2Cl$ (407.02): C, 73.75; H, 9.66. Found: C, 74.2; H, 9.9.

Ethyl 3β-chloro-9(11)-cholenate (31) by esterification of the acid 32. Recrystallization in 95% ethanol gave colorless prisms, m.p. 67–68°, $[\alpha]_D$ +33.0°. *Anal.* Calcd. for $C_{26}H_{41}O_2Cl$ (421.05): C, 74.16; H, 9.82. Found: C, 73.8; H, 10.0.

(19) Analyses by Oakwold Laboratories, Alexandria, Va.

(20) R. B. Turner, THIS JOURNAL, **74**, 4220 (1952); Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, *ibid.*, **74**, 5394 (1952); E. P. Oliveto, C. Gerold, L. Weber, H. E. Jorgensen, R. Rausser and E. B. Hershberg, *ibid.*, **75**, 5486 (1953).

(21) J. C. Babcock and L. F. Fieser, *ibid.*, **74**, 5474 (1952).

3β-Chloro-9(11)-cholenic acid (**32**), prepared by hydrolysis of its methyl ester, crystallized from ethanol, m.p. 150–153°, $[\alpha]_D$ +33.4°. *Anal.* Calcd. for $C_{24}H_{37}O_2Cl$ (393.00): C, 73.34; H, 9.49. Found: C, 73.4; H, 9.8.

Methyl 3β-chlorocholanate (**33**) by pyridinium chloride method on methyl lithocholate tosylate (20), in 80% yield of crude product melting about 80°. After recrystallization from methanol, well-shaped, elongated prisms of m.p. 86–87.5° were obtained, $[\alpha]_D$ +17°. *Anal.* Calcd. for $C_{25}H_{41}O_2Cl$ (409.03): C, 73.41; H, 10.1. Found: H, 73.1; H, 9.8.

3β-Chlorocholanic acid (**34**), prepared by hydrolysis of the methyl ester 33, separated in long needles, from acetone, m.p. 186–190°, $[\alpha]_D$ +14.3°. *Anal.* Calcd. for $C_{24}H_{39}O_2Cl$ (395.01): C, 72.97; H, 9.95; Cl, 8.98. Found: C, 73.22; H, 9.91; Cl, 9.07.

Methyl 3β-chloro-12α-hydroxycholanate (**35**), by pyridinium chloride on the tosylate 25, crystallized out of methanol as transparent, dense prisms, m.p. 141.5–143°, $[\alpha]_D$ +29.6°*. *Anal.* Calcd. for $C_{25}H_{41}O_3Cl$ (425.04): C, 70.64; H, 9.72; Cl, 8.34. Found: C, 70.81; H, 9.85; Cl, 8.28.

Ethyl 3β-chloro-12α-hydroxycholanate (**36**) by ester interchange on 35, by refluxing for 3.5 hr. with hydrochloric acid in ethanol, 86% yield of fine needles, m.p. 116.5–118.8°. Analytical sample out of ligroin (35–60°)–benzene (20:1), m.p. 117.5–118.8°, $[\alpha]_D$ +28.6°*. *Anal.* Calcd. for $C_{26}H_{43}O_3Cl$ (439.06): C, 71.12; H, 9.87; Cl, 8.08. Found: C, 71.05; H, 9.73; Cl, 7.96.

Methyl 3β-chloro-5-bisnorcholenate (**37**) by the action of thionyl chloride on the 3β-hydroxy compound, m.p. 130–133°, out of methanol in 79% yield, $[\alpha]_D$ −48°. *Anal.* Calcd. for $C_{23}H_{35}O_2Cl$ (378.97): C, 72.89; H, 9.31; Cl, 9.36. Found: C, 72.96; H, 9.01; Cl, 9.44.

Ethyl 3β-chloro-5-bisnorcholenate (**38**) was prepared in a room temperature reaction of thionyl chloride on the hydroxy ester 7 to give a crystalline product from ethanol, m.p. 120–123°, $[\alpha]_D$ −47.3°. *Anal.* Calcd. for $C_{24}H_{37}O_2Cl$ (393.00): C, 73.34; H, 9.49; Cl, 9.02. Found: C, 73.5; H, 9.5; Cl, 8.9.

OTHER COMPOUNDS.—**Ethyl 12α-benzoxycholanate** (**39**) by reaction of benzoyl chloride in pyridine on the 12-hydroxy compound 4, 4 hr. reflux. The oil, after processing and chromatography, did not crystallize; distilled at 0.1 mm., $[\alpha]_D$ +51°. *Anal.* Calcd. for $C_{33}H_{48}O_4$ (508.7): C, 77.91; H, 9.51. Found: C, 78.0; H, 9.6.

Ethyl 3α-benzoxy-12α-hydroxycholanate (**40**) was prepared by benzoylation in pyridine at room temperature; 75% of product, m.p. 103–114°. Recrystallization from methanol, m.p. 118.8–120.7°, $[\alpha]_D$ +51.9°*. *Anal.* Calcd. for $C_{33}H_{48}O_5$ (524.68): C, 75.53; H, 9.22. Found: C, 75.80; H, 9.28.

Ethyl 3α,12α-dibenzoxycholanate (**41**) from ethyl desoxycholate with benzoyl chloride and pyridine by 3 hr. reflux, crystalline product from ethanol, m.p. 139.2–141.4°, $[\alpha]_D$ +89.7°*. *Anal.* Calcd. for $C_{40}H_{52}O_6$ (628.80): C, 76.40; H, 8.33. Found: C, 76.43; H, 8.59.

Ethyl 3-ketocholanate (**42**)[22] by esterification of 3-ketocholanic acid,[23] crystallized out of ethanol–water as dense prisms, m.p. 94.5–95.5°, $[\alpha]_D$ +28.9°. *Anal.* Calcd. for $C_{26}H_{42}O_3$ (402.60): C, 77.56; H, 10.52. Found: C, 77.52; H, 10.70.

Methyl 3-keto-9(11)-cholenate (**43**)[21] was prepared from methyl 3α-hydroxy-9(11)-cholenate by the chromium trioxide–pyridine method[24] to give an 82% yield of colorless crystals, m.p. 117–120°, $[\alpha]_D$ +31.3° (lit. m.p. 117–119°, $[\alpha]_D$ +35°).

Methyl 3-keto-11-cholenate (**44**)[25] from chromium trioxide–pyridine oxidation of the corresponding 3α-hydroxy compound, out of isopropyl ether–acetone (9:1) giving transparent flat plates, m.p. 125–127° (lit. m.p. 125.5–126°), $[\alpha]_D$ +37.3°. *Anal.* Calcd. for $C_{25}H_{38}O_3$ (386.55): C, 77.67; H, 9.91. Found: C, 77.4; H, 10.2.

(22) This compound is mentioned among other carbonyl compounds [H. Reich, K. F. Crane and S. J. Sanfilippo, *J. Org. Chem.*, **18**, 822 (1953)] without indication of its source or properties.

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Seroflocculating Steroids. IV.[1] Unsaturated Bile Acid Esters[2]

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For study of their seroflocculating activity, methyl and ethyl 3,11-choladienate, methyl 3,9(11)-choladienate, methyl and ethyl 3-cholenate, methyl and ethyl 12α-hydroxy-3-cholenate were prepared by elimination of suitably constituted 3α-tosylates. Each elimination reaction appears to give a single (or highly predominant) olefinic product. The assignment of the Δ^3-structure to these compounds is based on the identity of our methyl cholenate with the product prepared previously by Fieser and Ettore and proved by them to be methyl 3-cholenate.

Our first attempts to prepare a pure ethyl choladienate for seroflocculation studies were by pyrolysis of diacyl derivatives of ethyl desoxycholate by the methods used by Wieland[4] and subsequently by Reichstein.[5] Solid products were obtained readily enough, but isolation of pure material by crystallization, or even by chromatography and crystallization, was tedious and gave low yield. For example, with ethyl 3α,12α-dibenzoxycholanate, the pyrolysis product yielded a minute amount of pure material only after chromatography and eight crystallizations. The diacyl methyl esters were no more amenable than the ethyl compounds.

The difficulty of isolation of pure product induced us to try an elimination reaction on a tosylate. Dehydrotosylation in a pyridine base which presumably takes place through a heterolytic mechanism might be expected to yield a different and possibly more tractable mixture than that resulting from pyrolysis. This method of producing unsaturation in steroids has been applied frequently,[6] but the

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