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Seroflocculating Steroids. V.[1] Reduction of the Bile Acid Side Chain[2,3]

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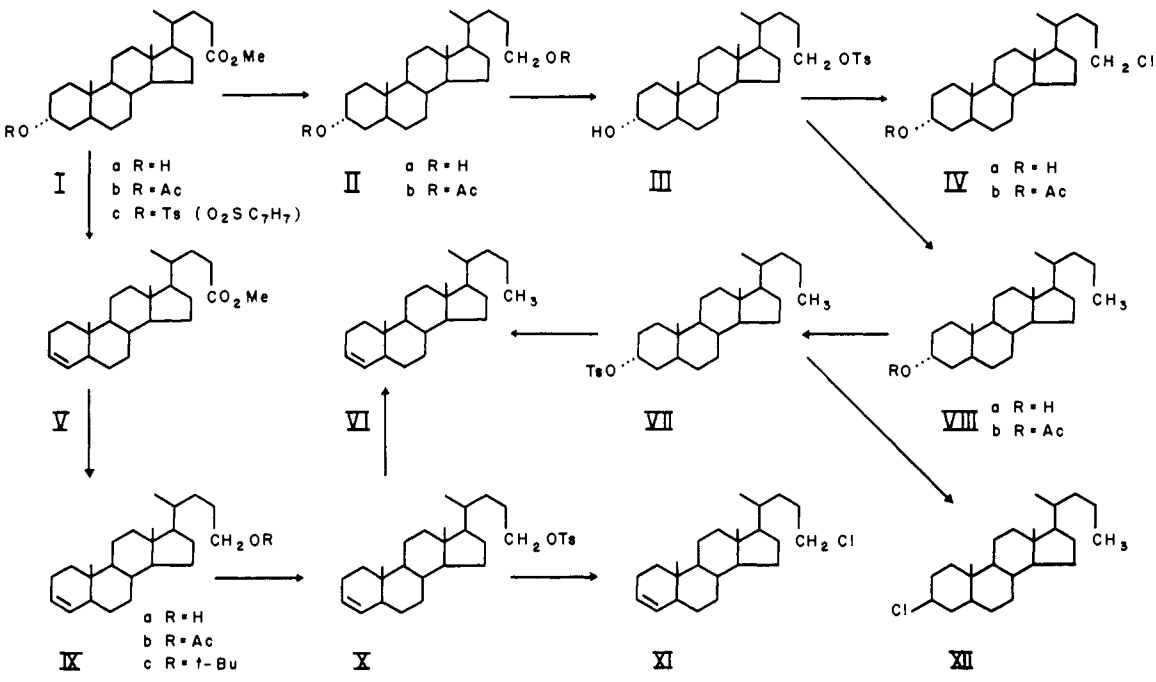
Conversion of the ω-carbomethoxy group to methyl in the presence of a C-3 hydroxyl group has been accomplished in the bile acid series by (1) reduction to $-CH_2OH$, (2) selective tosylation of the primary hydroxyl group and (3) reductive removal of the tosyloxy group. By this sequence of reactions methyl lithocholate was converted to 3α-cholanol. Several related chloro and unsaturated analogs were prepared. An epimerization was encountered in the conversion of a 3α-tosyloxy group to 3β-hydroxy on an alumina column.

In previous papers in this series dealing with derivatives of bile acids having the original ω-acid or ester grouping, the finding of several compounds of good activity in a seroflocculation reaction was reported.[4] Structural variations studied thus far consist of substitutional changes on the nucleus. The present work comprises a logical extension of this structure *vs.* activity study, namely, alteration of the side chain by reduction. Using as prototype the simplest of the bile acids, lithocholic acid, the proposed synthetic scheme is shown in Chart A whereby methyl lithocholate is reduced to the diol, which is selectively deoxylated (IIa→III→VIIIa).

CHART A



Numerous selective tosylations have been reported in the sapogenin series, the most pertinent of which distinguished between a primary, side chain hydroxyl group and a 3-hydroxyl group in the equatorial conformation.[5] It was necessary to alter the conditions slightly in applying this reaction to 3α,24-cholanediol.

24-Tosyloxy-3α-cholanol (III) was prepared in 26% yield by treating IIa with a 50% molar excess of *p*-toluenesulfonyl chloride in pyridine at 5°, and by working up the reaction mixture without allowing it to warm to room temperature. Chromatography of the total, crude product on Florisil gave three major fractions. The first was a chlorine-containing mixture of compounds, probably similar in part to those obtained by a room temperature tosylation of IIa which will be described later. The second fraction proved to be the desired monotosylate III, and the third represented a 23% recovery of IIa.

24-Tosyloxy-3α-cholanol (III) was reduced smoothly with lithium aluminum hydride in ether to 3α-cholanol (VIIIa). Dehydrotosylation of 3α-cholanyl tosylate (VII) in lutidine gave a 90%

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

(2) Presented before the division of Organic Chemistry, 132nd National A.C.S. Meeting, New York, Sept., 1957.

(3) This investigation was supported in part by grants (CS-9053, C-2249 and C-3407) from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

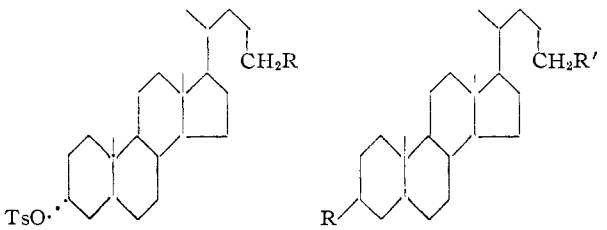
(4) F. C. Chang, *et al.*, THIS JOURNAL, **79**, 2161 (1957).

(5) I. Scheer, M. J. Thompson and E. Mosettig, *ibid.*, **78**, 4733 (1956). We wish to take this opportunity to thank the authors for informing us of their work prior to publication.

yield of 3-cholene (VI), and no evidence for the simultaneous production of the isomeric 2-cholene was obtained, in agreement with the apparent sole production of methyl 3-cholenate (V), by dehydrotosylation of methyl 3α-tosyloxycholanate (Ic).[1,6] An independent synthesis of 3-cholene was carried out by lithium aluminum hydride reduction of methyl 3-cholen-24-ol (IXa), and subsequent lithium aluminum hydride reduction of its tosylate X.

The monotosylate isomeric with III, 3α-tosyloxy-24-cholanol (XVI, Chart B), was isolated from a mixture of products obtained by a room temperature tosylation of the diol II. The total tosylation

CHART B



	R		R	R'		R	R'
XIII	Cl	XIVa	OH	Cl	XVIIIa	H	OH
XV	OTs	b	OAc	Cl	b	H	OAc
XVI	OH	XVIIa	OH	OH	c	H	OTs
		b	OAc	OAc	XX	H	Cl

product was separated by elution chromatography on alumina. Fraction A proved to be 24-chloro-3-cholene (XI), which was prepared also from 3-cholen-24-yl tosylate (X).[7] Fraction B was approximately a 50:50 mixture of 3-cholen-24-ol (IXa) and 24-chloro-3β-cholanol (XIVa), which was not separated by a second chromatographic treatment nor by recrystallization. Acetylation of the mixture, however, permitted easy separation of the two acetates (IXb and XIVb) chromatographically. The infrared spectrum of 24-chloro-3β-cholanyl acetate exhibits the three characteristic maxima in the 8μ region, in contrast to that of the 3α-acetate IVb, which shows only one.[8]

3α-Tosyloxy-24-cholanol (XVI) constituted fraction C; its structure was proved by its reduction with lithium aluminum hydride to 24-cholanol (XVIIIa), identical with authentic samples prepared by reduction of methyl cholanate and by catalytic hydrogenation of 3-cholen-24-ol (IXa). Fraction D was identified as 3β,24-cholanediol (XVIIa) by comparison with an authentic specimen prepared by reduction of methyl 3β-hydroxycholanate (XIXa). It is precipitated by digitonin whereas the 3α,24-diol is not, and its diacetate XVIIb exhibits the three characteristic infrared maxima at 8.00, 8.08 and 8.14 μ. Molecular rotation differences for the diols are in agreement with those predicted for alcohols epimeric at C-3.[9]

Fraction E is recovered 3α,24-cholanediol. This elution sequence, 3β,24-diol prior to 3α,24-diol, would be expected in view of the frequently more facile elution of axial *versus* equatorial hydroxy compounds.[10]

It is obvious that three reactions in addition to tosylation have taken place to produce the variety of products obtained. Chlorination at C-24 (by pyridinium chloride) occurred in the tosylation medium,[11] and dehydrotosylation[12,12a] and hydrolysis (with inversion in the case of the 3α-tosylates) took place on the alumina during the protracted chromatographic separation.[12a,13] Thus, the Δ^3-compounds XI and IXa resulted from dehydrotosylation and the 3β-hydroxy products XIVa and XVIIa from hydrolysis of 3α-tosyloxy intermediates. Similarly, the 24-hydroxy products IXa, XVI and XVIIa resulted from hydrolysis of a 24-tosyloxy intermediate. The precursor of XI and XIVa was the chlorotosylate XIII, and likewise the precursor of IXa, XVI and XVIIa was most logically the ditosylate XV.

24-Chlorocholane (XX) was prepared readily by the action of pyridinium chloride on 24-cholanyl tosylate (XVIIIc) in pyridine at room temperature. (24-Chloro-3-cholene was prepared similarly except that in this case the solvent was N,N-dimethylformamide.[14]) The facile conversion of these tosylates to the corresponding chloro derivatives indicates the greater reactivity of the 24-tosyloxy group over the 3α-tosyloxy group. Methyl 3α-tosyloxycholanate was recovered unchanged after standing in pyridine containing pyridinium chloride at room temperature for two weeks.[15] 3β-Chlorocholane (XII) was prepared similarly to the 24-chloro compounds, except that a temperature of 75° was used.

No ether-soluble product was obtained when 3-cholen-24-yl tosylate (X) was heated in lutidine in an effort to prepare 3,23-choladiene. Similarly the base potassium *t*-butoxide failed to dehydrotosylate X, but a 58% yield of the *t*-butyl ether IXc was obtained.

(6) The Δ^3 rather than the alternative Δ^2 assignment for these compounds is based on the demonstration (see ref. 1) that methyl 3-cholenate prepared by dehydrotosylation is identical to that prepared by the action of zinc on the methyl 3-hydroxy-4β-bromocholanates.

(7) F. C. Chang, *et al.*, THIS JOURNAL, **79**, 2164 (1957).

(8) R. N. Jones, *et al.*, *ibid.*, **73**, 3215 (1951).

(9) D. H. R. Barton and W. Klyne, *Chemistry & Industry*, 755 (1948).

(10) K. Savard, *Rec. Prog. Hor. Res.*, **9**, 185 (1954); R. V. Brooks, W. Klyne and E. Miller, *Biochem. J.*, **54**, 212 (1953); R. J. Bridgewater and C. W. Shoppee [*J. Chem. Soc.*, 1709 (1953)] found, however, that epicoprostanol (OH eq.) is eluted more easily than coprostanol (OH ax.).

(11) Evidence that this reaction takes place at room temperature is presented later in this paper. Furthermore, when the chromatographic separation is carried out rapidly, 24-chloro-3α-cholanyl tosylate (XIII) can be isolated (see Experimental).

(12) No dehydrotosylation was observed in any of the numerous room temperature tosylations we have carried out in this and previous work (ref. 7) where separation with alumina was not required. Dehydrotosylations of axial compounds are known [A. Ruff and T. Reichstein, *Helv. Chim. Acta*, **34**, 70 (1951); H. R. Nace, THIS JOURNAL, **74**, 5937 (1952); D. D. Evans and C. W. Shoppee, *J. Chem. Soc.*, 540 (1953)], but Evans and Shoppee state that 3α-coprostanyl tosylate is stable to neutral alumina.

(12a) ADDED IN PROOF.—Our attention has been called to prior work of R. J. W. Cremlyn and C. W. Shoppe (*J. Chem. Soc.*, 3515 (1954)) reporting the conversion of 7β-tosyloxycholestane into 7α-cholestanol and 7-cholestane on alkaline alumina.

(13) These deductions of the dehydrotosylation and epimerization reactions are confirmed in subsequent work being reported (paper VI, *Chemistry and Industry*, in press (1958)) in which a number of 3α-tosylates are hydrolyzed with inversion of configuration, with no uninverted product formed. The hydrolysis of a 24-tosylate on alumina is illustrated by the conversion of X to IXa (see Experimental).

(14) Further work indicates that dimethylformamide is superior to pyridine as the solvent in the pyridinium chloride reaction (ref. 7) even though DMF undergoes reaction with certain tosylates (paper VII, THIS JOURNAL, in press).

(15) Unpublished observation in this Laboratory.

Screening Results.—A number of compounds reported in this work were screened in a seroflocculation test which shows some promise as a diagnostic aid in the detection of cancer.[4] They are classified below in three groups according to the scheme already described; the groups are listed in order of decreasing activity.

	Group A
XIVb	24-Chloro-3β-cholanyl acetate
	Group B
IVb	24-Chloro-3α-cholanyl acetate
VI	3-Cholene
IXb	3-Cholen-24-yl acetate
IXc	24-*t*-Butoxy-3-cholene
XI	24-Chloro-3-cholene
XII	3β-Chlorocholane
XIXb	Methyl 3β-acetoxycholanate
	Group C
XIXa	Methyl 3β-hydroxycholanate
IIb	3α,24-Cholanediol diacetate
XVIIb	3β,24-Cholanediol diacetate
VIIIb	3α-Cholanyl acetate
XVIIIb	24-Cholanyl acetate
IXa + XIVa[16]	3-Cholen-24-ol and 24-chloro-3β-cholanol
XIII	24-Chloro-3α-cholanyl tosylate
XX	24-Cholorocholane

The number of compounds tested here is too small to permit drawing any major conclusions, but two points stand out clearly. Prior to this work all active compounds were methyl or ethyl esters of substituted cholanic and bisnorcholanic acids. Finding activity among this new group of compounds indicates that such a side chain ester group is not necessary for seroflocculating activity. This result gives added impetus to the search for additional seroflocculants in the other classes of steroids. Also, it is interesting to observe that, with the exception of 3-cholen-24-ol, all Δ^3-compounds are in group B regardless of side chain; methyl and ethyl 3-cholenate are also in this group.[4]

Acknowledgment.—We wish to thank Dr. D. H. Sprunt for his advice and encouragement, and to acknowledge the able technical assistance of Miss Anne Anderson. Also, we are indebted to Mr. E. W. Lard and Dr. M. T. Giachino of Grace Chemical Co. for the infrared data.

Experimental[17]

Selective Tosylation of 3α,24-Cholanediol.—3α,24-Cholanediol[18] (12.63 g., 34.8 mmoles) was dried by twice distilling from it a small amount of benzene, dissolved in 65 ml. of anhydrous pyridine and cooled to 4° in an ice-bath. This solution was stirred at 4–5° while a solution of *p*-toluenesulfonyl chloride (9.95 g., 52.2 mmoles) in 15 ml. of anhyd. pyridine was added dropwise over a period of 12 minutes. After an additional 30 minutes at 4–5°, the solution was poured on crushed ice and acidified by dropwise addition of concd. hydrochloric acid. The mixture was extracted with ether, and the ether solution was washed in succession with cold, aq. 10% sodium bicarbonate, aq. 10% hydrochloric acid and water, then dried over sodium sulfate. Evaporation left a viscous, liquid residue, 17.46 g., which was chromatographed as a cloudy solution in benzene on 380 g. of Florisil (Floridin Co., 60/100 mesh.). Elution with benzene gave fraction A, 4.96 g., positive Beilstein test. Fractional crystallization and a second chromatographic separation failed to yield any pure compounds from this mixture.

24-Tosyloxy-3α-cholanol (III).—Fraction B, 6.28 g. was eluted with 5% ethyl ether in benzene. Crystallization from ligroin[19]–methanol (20:1) gave 3.459 g. of tiny laths, m.p. 115–120°. An analytical sample crystallized from ligroin–acetone (10:1), m.p. 119–120.6°, $[\alpha]_D$ +21.3°; λ_{max}^{KBr} 3.50, 6.96, 7.45, 8.45, 8.56, 10.45, 10.64, 12.09, 12.35, 15.1 μ. An additional 1.24 g. was recovered from the mother liquor and from the tail fraction A.

Anal. Calcd. for $C_{31}H_{48}O_4S$: C, 72.05; H, 9.36; S, 6.20. Found: C, 71.77; H, 91.40; S, 6.26.

Fraction C, 2.94 g., was eluted with ethyl ether–benzene (1:1); recrystallization in ethyl acetate gave 3α,24-cholanediol (IIa), m.p. 173–176°.

3α-Cholanol (VIIIa).—24-Tosyloxy-3α-cholanol (III, 2.91 g., 5.64 mmoles) in 37 ml. of dry ether was added gradually to a stirred suspension of 324 mg. (8.5 mmoles) of lithium aluminum hydride in 37 ml. of dry ether. The mixture was stirred and refluxed 5 hr., then allowed to stand overnight at room temperature. Ethyl acetate (10 ml.), then aq. 10% HCl were added dropwise, the ether was evaporated, and the white solid that separated was filtered from the aq. layer and washed thoroughly with water. The dried product 1.99 g. (quant. yield) melted at 138–141°. Recrystallization from ethyl acetate gave very fine needles, m.p. 142.3–143.0°, $[\alpha]_D$ +29.8°.

Anal. Calcd. for $C_{24}H_{42}O$: C, 83.13; H, 12.22. Found: C, 83.04; H, 12.28.

The acetate VIIIb was prepared by conventional means and crystallized from methanol as plates, m.p. 106.2–107.3°.

Anal. Calcd. for $C_{26}H_{44}O_2$: C, 80.35; H, 11.41. Found: C, 80.00; H, 11.46.

The tosylate VII, prepared by room temperature tosylation,[7] crystallized from acetone–water (5:1) as plates, m.p. 147.1–148.0°, $[\alpha]_D$ +40.3°; $\lambda_{max}^{CS_2}$ 3.44, 3.53, 7.36, 8.44, 8.53, 10.50, 10.70, 10.86, 11.60, 11.89, 12.32, 15.0 μ.

Anal. Calcd. for $C_{31}H_{48}O_3S$: C, 74.35; H, 9.66; S, 6.40. Found: C, 74.29; H, 9.79; S, 6.40.

3-Cholene (VI) from 3α-Cholanyl Tosylate (VII).—A solution of 600 mg. (1.2 mmoles) of VII in 6 ml. of 2,6-lutidine was refluxed 3 hr., 40 min. On standing overnight at room temperature large crystals of lutidinium tosylate separated. They dissolved when crushed ice was added, and a white precipitate formed, which was filtered, washed with cold aq. 10% HCl, then water. Vacuum drying over KOH left 361 mg. of light gray solid, which was chromatographed from ligroin on alumina. One fraction was obtained, eluted by ligroin, 356 mg. (90%) melting at 69–74°. Recrystallization in benzene–methanol (1:10) gave large needles, m.p. 74.8–75.0°, $[\alpha]_D$ +18.6°.

Anal. Calcd. for $C_{24}H_{40}$: C, 87.73; H, 12.27. Found: C, 87.50; H, 12.33.

3-Cholene from 3-Cholen-24-yl Tosylate (X).—Reduction with lithium aluminum hydride by the method described above for 3α-cholanol gave an 87% yield of 3-cholene melting at 69.5–72.5°. Recrystallization from methanol–benzene (10:1) gave needles, m.p. 72.5–73.1°, m.p. not lowered on admixture with the sample prepared by dehydrotosylation.

3β-Chlorocholane (XII).—A solution of 385 mg. of VII and 0.39 g. of pyridinium chloride in 4 ml. of N,N-dimethylformamide was heated at 75° for 17 hr. Dilution of the solution with ice-water precipitated a white solid, which was filtered and washed thoroughly with water; the dried, crude product, 253 mg., melted at 78–88°. Two recrystallizations in acetone–water (20:1) raised the m.p. to above 90°. The analytical sample, obtained by chromatography

(16) Fraction B of the large scale chromatographic separation, approximately a 50:50 mixture of these two compounds.

(17) Microanalyses by Galbraith Microanalytical Laboratories, Knoxville, Tenn. Melting points were taken on an electrical micro-hot-stage and are uncorrected. Optical rotations were determined in 2% chloroform solutions, except where noted, at about 25°, using a Keston polarimeter attachment (Standard Polarimeter Co., 225 E. 54th St., New York, N. Y.) to a Beckman DU spectrophotometer, with accuracy estimated to be better than ±2°.

(18) L. F. Fieser and S. Rajagopalan, THIS JOURNAL, **73**, 118 (1951). Our preparation m. 177.0–178.6°, $[\alpha]_D$ +33.0° (dioxane); λ_{max}^{KBr} 3.12, 3.51, 6.96, 7.35, 9.55, 9.90 μ.

(19) The "ligroin" used in these experiments was Skellysolve B (Skelly Oil Co.), b.p. 63–70°, purified by sulfuric acid treatment and distillation.

on alumina, elution with ligroin and crystallization from ethanol, melted at 92.5–95.0°, $[\alpha]_D$ +20.9°.

Anal. Calcd. for $C_{24}H_{41}Cl$: C, 78.96; H, 11.32; Cl, 9.71. Found: C, 79.00; H, 11.21; Cl, 9.60.

3-Cholene-24-ol (IXa).—Lithium aluminum hydride reduction of methyl 3-cholenate[1] (V) gave a 97% yield of crude product, which on recrystallization in ethyl acetate (80% recovery) gave lustrous laths, m.p. 103–108°, and after a second recrystallization in ethyl acetate, m.p. 107.5–108.5°, $[\alpha]_D$ +18.6°.

Anal. Calcd. for $C_{24}H_{40}O$: C, 83.65; H, 11.70. Found: C, 83.90; H, 11.84.

The acetate IXb crystallized from acetone–water (4:1) as needles with slightly irregular edges, m.p. 68.5–69.2°, $[\alpha]_D$ +17.8°.

Anal. Calcd. for $C_{26}H_{42}O_2$: C, 80.77; H, 10.95. Found: C, 80.49; H, 11.18.

The Tosylate X.—3-Cholen-24-ol (IXa), 3.25 g., was dried by benzene distillation, dissolved in 15.3 ml. of anhyd. pyridine and the solution cooled to 12°. A solution of 5.83 g. of tosyl chloride in 8 ml. of anhyd. pyridine was added dropwise to the stirred reaction mixture. The temperature rose to 15° and was held there an additional 30 min., then crushed ice was added. A liquid separated which crystallized on seeding with 24-cholanyl tosylate (XVIIIc).

The crude product was filtered, washed with aq. 10% HCl, then water, and air-dried: 4.73 g., m.p. 98–108°. Crystallization in acetone–water (10:1) gave 3.40 g., m.p. 110–114.5°. An analytical sample crystallized from acetone–water (8:1) as platelets, m.p. 113.0–114.8°, $[\alpha]_D$ +15.3°.

Anal. Calcd. for $C_{31}H_{46}O_3S$: C, 74.65; H, 9.30; S, 6.43. Found: C, 74.65; H, 9.57; S, 6.29.

The tosylate was chromatographed on alumina (Fisher Alumina, Adsorption A-540) and left on the column for 24 hr. Elution with ligroin gave a few mg. of unidentified material, and elution with benzene gave **3-cholen-24-ol (IXa)**, yield 97%, m.p. and mixture m.p. 107.0–108.5°.

24-Chloro-3-cholene (XI).—A solution of 3-cholene-24-yl tosylate (X, 1.00 g., 2 mmoles) and 1.0 g. of pyridinium chloride in 10 ml. of N,N-dimethylformamide stood at room temperature for 40 hr. Dilution with ice and water, followed by thorough washing of the precipitate formed with water, gave 723 mg. (99%) of crude product, m.p. 60–69°. Two recrystallizations in acetone–water (7.5:1) raised the m.p. to 70.0–71.2°, $[\alpha]_D$ +20.0°.

Anal. Calcd. for $C_{24}H_{39}Cl$; C, 79.40; H, 10.83; Cl, 9.77. Found: C, 79.66; H, 10.75; Cl, 9.45.

24-*t*-Butoxy-3-cholene (IXc).—3-Cholen-24-yl tosylate (400 mg., 0.80 mmole) was added to a solution of 156 mg. of potassium in 15 ml. of dry *t*-butyl alcohol[20] and the mixture refluxed under nitrogen for 30 minutes. The cooled mixture was poured on ice, diluted to 70 ml. with water and extracted with ligroin. The ligroin layer was washed with water to neutrality, dried over Na_2SO_4 and evaporated, leaving 296 mg. of clear, viscous liquid. This was redissolved in ligroin and chromatographed on 9 g. of alumina. The first fraction, eluted by ligroin, weighed 184 mg. (57.5% yield); two recrystallizations from methanol gave irregular plates, m.p. 78.5–79.4°, $[\alpha]_D$ +16.4°.

Anal. Calcd. for $C_{28}H_{48}O$; C, 83.93; H, 12.08. Found: C, 84.14; H, 12.11.

The second chromatographic fraction, eluted by benzene and by ether, 85 mg., was crystallized from methanol–water and shown by mixed melting point to be identical with 3-cholen-24-ol (IXa).

24-Chloro-3α-cholanol (IVa).—A mixture of 487 mg. of 24-tosyloxy-3α-cholanol, 495 mg. of pyridinium chloride and 5 ml. of anhyd. pyridine stood at room temperature for 36 hr. The mixture was diluted to 10 ml. with crushed ice, filtered, and the crystalline precipitate washed with aq. 5% HCl, then water; yield, 193 mg. melting at 136–140°. Two recrystallizations in acetone–water raised the m.p. to 142.5–144.0°; the product which gave poor analyses was characterized as the acetate. Acidification of the original pyridine–water filtrate precipitated another 160 mg. of crude product.

(20) *Org. Syntheses*, **30**, 18 (1950).

The acetate IVb crystallized easily from acetone–water (5:1) as platelets, m.p. 140.9–142.3° (mixed with IVa melted at 120–130°), $[\alpha]_D$ +43.5°; λ_{max}^{KBr} 3.45, 3.53, 5.77, 6.90, 7.25, 7.35, 7.98, 9.77, 14.03 μ.

Anal. Calcd. for $C_{26}H_{43}O_2Cl$: C, 73.81; H, 10.24; Cl, 8.38. Found: C, 73.70; H, 10.02; Cl, 8.78.

24-Cholanol (XVIIIa) was obtained by catalytic hydrogenation (Adams catalyst) of 3-cholene-24-ol (IXa) and by lithium aluminum hydride reduction of cholanic acid or its methyl ester. It crystallizes nicely from ethyl acetate or from methanol–water (15:1) to give laths, m.p. 129.5–130.5° (m.p. varies substantially with rate of heating), $[\alpha]_D$ +25.7° (lit.[21] m.p. 130.5–132.5°, $[\alpha]_D$ +26.4° in alcohol).

The acetate XVIIIb crystallized from methanol as laths, m.p. 84.5–85.5°, $[\alpha]_D$ +22.4°.

Anal. Calcd. for $C_{26}H_{44}O_2$: C, 80.35; H, 11.41. Found: C, 80.29; H, 11.30.

The tosylate XVIIIc, prepared by tosylation at 12–15°, crystallized from acetone–water (25:4) as platelets, m.p. 99–100.5°, $[\alpha]_D$ +20.7°.

Anal. Calcd. for $C_{31}H_{48}O_3S$: C, 74.35; H, 9.66; S, 6.40. Found: C, 74.44; H, 9.88; S, 6.30.

Cholane.—Reduction of 24-cholanyl tosylate with lithium aluminum hydride by the method described for 3α-cholanol gave cholane in 78% yield, out of ethanol as plates, m.p. 89.2–90.7°, $[\alpha]_D$ +29.9° (lit.[22] m.p. 90°).

24-Chlorocholane (XX) was prepared from the corresponding tosylate by the method described for 24-chloro-3-cholene (XI) except that here the solvent was pyridine. The product crystallized out of acetone–water as platelets, m.p. 74.0–74.5° (lit.[21] m.p. 74.6°), $[\alpha]_D$ +26.8° (1.5 chf.).

Anal. Calcd. for $C_{24}H_{41}Cl$: C, 78.96; H, 11.32; Cl, 9.71. Found: C, 79.04; H, 11.47; Cl, 9.71.

Methyl 3β-Hydroxycholanate (XIXa).—Methyl 3-ketocholanate (3.76 g.) in 184 ml. of glacial acetic acid and 3.8 ml. of concd. hydrochloric acid was hydrogenated in the presence of platinum oxide according to Fieser and Ettorre.[23] The crude product, melting at 48–122°, was chromatographed from benzene on 100 g. of alumina. The first fraction, eluted with benzene, 0.899 g., was recrystallized in ethyl acetate to give **methyl 3β-acetoxycholanate (XIXb)**,[24] hexagons, m.p. 168.5–171.5°, $[\alpha]_D$ +19.1° (lit.[23] m.p. 165–167°, $[\alpha]_D$ +21.1°).

The second fraction, eluted with 2% ethanol in ether, 1.69 g., crystallized from acetone–water (20:1) as clear plates which became striated at 88–90°, melted and immediately resolidified at 105–106°, finally melted at 117.0–118.0°. Recrystallization in methanol gave **methyl 3β-hydroxycholanate (XIXa)**, laths, m.p. 117.1–118.0°, $[\alpha]_D$ +19.8° (lit.[23] m.p. 114–116°, $[\alpha]_D$ +21.3°).

3β,24-Cholanediol (XVIIa) was prepared by lithium aluminum hydride reduction of 195 mg. of methyl 3β-hydroxycholanate. An 87% yield of crude product, m.p. 149–153°, was obtained. Recrystallization in methanol gave needles, m.p. 150–151.5°.

Anal. Calcd. for $C_{24}H_{42}O_2$: C, 79.49; H, 11.68. Found: C, 79.91; H, 11.82.

Room Temperature Tosylation of 3α,24-Cholanediol.—3α,24-Cholanediol (10.00 g., 27.6 mmoles) was dried as before and dissolved in 87 ml. of anhyd. pyridine. Over a period of 2 hr. and 15 min. a solution of 26.20 g. (0.138 mole) of *p*-toluenesulfonyl chloride in 105 ml. of anhyd. pyridine was added dropwise. After standing an additional 2 hr. the solution was diluted with ice and extracted with ether. The ether solution was washed in succession with cold, aq. 10% hydrochloric acid, cold, aq. 10% sodium bicarbonate and cold water, then dried over sodium sulfate. Evaporation left a viscous, liquid residue, 13.20 g., which was chromatographed as a cloudy solution in ligroin–benzene (3:1) on 450 g. of alumina[25]; the separation took about 48 hours.

(21) F. Wessely and W. Swoboda, *Monatsh.*, **82**, 437 (1951).

(22) H. Wieland, *et al.*, *Z. physiol. Chem.*, **161**, 80 (1926).

(23) L. F. Fieser and R. Ettorre, THIS JOURNAL, **75**, 1700 (1953).

(24) Presumably formed by acetylation during hydrogenation. Not reported in ref. 23 because of the hydrolysis step.

(25) Harshaw Alumina, Activated, Chromatographic, powdered catalyst grade, Al-0109 P, Harshaw Scientific Co.

Fraction A, 0.40 g., eluted by ligroin–benzene (3:1), after several recrystallizations proved to be **24-chloro-3-cholene (XI)** m.p. 67–69°, m.p. not lowered on admixture with an authentic sample.

Fraction B, 2.13 g., eluted by 2% ethanol in ether, was a chlorine-containing mixture which would not separate by recrystallization or on a second chromatographic column. A portion of the mixture, 1.24 g., was acetylated with acetic anhydride and pyridine, and the crude product, 0.95 g., in ligroin solution was chromatographed on 28.5 g. of alumina. The first fraction, eluted by ligroin, 268 mg., crystallized out of acetone–water (4:1) to give needles of **3-cholen-24-yl acetate (IXb)**, m.p. 68.5–69.2°, m.p. not lowered on admixture with an authentic sample. After a small intermediate fraction a second major fraction was eluted with ligroin–benzene (1:1), 173 mg., which crystallized nicely by slow concentration of its acetone–water (5:1) solution, two recrystallizations giving **24-chloro-3β-cholanyl acetate (XIVb)**, platelets, m.p. 149–152°, mixed with IVb melted at 120–130°, $[\alpha]_D$ +18.8°; λ_{max}^{KBr} 3.47, 3.53, 5.78, 6.92, 7.29, 7.33, 7.66, 7.98, 8.08, 8.16(sh.), 8.63, 9.78, 10.15, 10.42, 14.01 μ.

Anal. Calcd. for $C_{26}H_{43}O_2Cl$: C, 73.81; H, 10.24; Cl, 8.38. Found: C, 73.61; H, 10.34; Cl, 8.31.

Fraction C, 2.32 g., eluted by 5% ethanol in ether, crystallized from acetone–water (4:1) to give **3α-tosyloxy-24-cholanol (XVI)** as platelets, 1.46 g., m.p. 125.8–128.3°, $[\alpha]_D$ +38.7°; λ_{max}^{KBr} 3.49, 6.95, 7.45, 8.45, 8.55, 10.52, 10.72, 10.85, 11.58, 11.95, 15.0 μ. The analytical sample, m.p. 127.5–129.5°, crystallized from methanol–water (10:1). Its mixture with the isomeric III melted at 103–118°.

Anal. Calcd. for $C_{31}H_{48}O_4S$: C, 72.05; H, 9.36; S, 6.20. Found: C, 72.13; H, 9.32; S, 6.05.

Fraction D, 1.69 g., also eluted by 5% ethanol in ether, but almost completely separated from fraction C, crystallized from acetone–water (4:1) as needles, **3β,24-cholanediol (XVIIa)**, m.p. 151–154°, m.p. not lowered on admixture with authentic sample, $[\alpha]_D$ +23.3°.

Anal. Calcd. for $C_{24}H_{42}O_2$: C, 79.49; H, 11.68; mol. wt., 362.6. Found: C, 79.50; H, 11.76; mol. wt., 354.

The diacetate XVIIIb crystallized out of methanol–water (20:1) as needles, m.p. 99–101°, $[\alpha]_D$ +13.3°; $\lambda_{max}^{CS_2}$ 3.45, 5.75, 8.00(sh.), 8.08, 8.14(sh.), 9.78 μ.

Anal. Calcd. for $C_{28}H_{46}O_4$: C, 75.29; H, 10.38. Found: C, 75.03; H, 10.33.

Fraction E, 0.60 g., eluted by 10% ethanol in ether, crystallized from ethyl acetate to give **3α,24-cholanediol (IIa)**, m.p. 176–178°.

Lithium Aluminum Hydride Reduction of 3α-Tosyloxy-24-cholanol (XVI).—Reduction of 1.42 g. of 3α-tosyloxy-24-cholanol by the method described above for 3α-cholanol gave a crude product, 1.14 g., which was chromatographed from ligroin–benzene (5:1) solution on 36 g. of alumina. The first fraction, 255 mg., eluted by ligroin–ether (5:1), crystallized from acetone–methanol or from ethanol as laths, m.p. 85.2–86.0°, m.p. not lowered on admixture with an authentic sample of **24-cholanyl acetate (XVIIIb)**.[26]

Anal. Calcd. for $C_{26}H_{44}O_2$: C, 80.35; H, 11.41. Found: C, 80.06; H, 11.53.

After a small intermediate fraction, the second major fraction, 296 mg., eluted by ligroin–ether (1:2) crystallized from methanol–water (20:1) as laths, m.p. 124.5–125.1°, m.p. unchanged on admixture with authentic **24-cholanol (XVIIIa)**, $[\alpha]_D$ +26° (95% EtOH).

After another small intermediate fraction, a final fraction consisting of 126 mg. of 3α,24-cholanediol (IIa) was obtained.

24-Chloro-3α-cholanyl Tosylate (XIII).—Another room temperature tosylation of 3α,24-cholanediol stood for 2 days. Working up the reaction mixture as described above, and rapidly chromatographing the neutral, ether-soluble portion on alumina gave a fraction, eluted by ether, m.p. 109–117°. Four recrystallizations from benzene–ligroin (30–60°) (1:10) gave platelets, m.p. 116.8–119.0°, in 10% yield; λ_{max}^{KBr} 3.49, 6.96, 7.45, 8.45, 8.55, 10.52, 10.72, 10.85, 11.60, 11.95, 15.0 μ. A comparison of the infrared spectra of this compound and those of III and XVI indicates that this is the 3α-tosylate. In addition, under the reaction conditions 3-chloro substitution does not take place, as mentioned earlier.

Anal. Calcd. for $C_{31}H_{47}O_3ClS$: C, 69.56; H, 8.85; Cl, 6.63; S, 5.99. Found: C, 69.28; H, 8.91; Cl, 6.60; S, 5.73.

(26) Probably formed by ester interchange between 24-cholanol and ethyl acetate during decomposition of excess $LiAlH_4$.

MEMPHIS, TENNESSEE

[CONTRIBUTION FROM THE RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Effects of Ionizing Radiation on Choline Chloride and its Analogs. II[1]

BY RICHARD M. LEMMON, PEGGY KWONG GORDON, MARGARET A. PARSONS AND FRANCO MAZZETTI

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The radiation sensitivities of crystalline choline chloride and nineteen crystalline analogs have been compared. The chloride is extremely radiation sensitive and appears to decompose by a free-radical chain mechanism. Choline bromide is about one-third as sensitive as the chloride; none of the other analogs show abnormal radiation instability. That choline chloride's susceptibility to radiation damage is a function of its crystal structure is shown by its contrasting stability in solution. Determinations were made of the radiation sensitivity of crystalline choline chloride at low temperature, in the presence of added iodide or iodine, and after repeated recrystallizations. The electron spin resonance signal from irradiated choline chloride also was observed.

An earlier report[2] described the abnormal sensitivity of crystalline choline chloride toward ionization radiation. *G* values (molecules decomposed/100 e.v.) as high as 1250 were found. In addition, the failure of six crystalline choline analogs to show similar abnormal behavior was recorded. The present work describes the efforts which have been made toward an understanding of choline chloride's radiation sensitivity. This work comprises studies on (1) the sensitivity of thirteen additional analogs; (2) the sensitivity of choline chloride in solution; and (3) the rates of decomposition observed when crystalline choline chloride is irradiated (a) at low temperatures, (b) in the presence of added iodide or iodine and (c) after repeated crystallization from different solvents. In addition, this report includes some observations on the electron spin resonance spectrum of irradiated choline chloride.

(1) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

(2) R. M. Lemmon, *et al.*, THIS JOURNAL, **77**, 4139 (1955).

Experimental

Preparation of Choline Analogs.—The thirteen additional analogs were prepared as follows: The choline salts (bromide, nitrate, sulfate, acetate and cyanide) were prepared from choline iodide by conversion to the quaternary base with Ag_2O, followed either by titration with the appropriate