(singlet), two ring hydrogens at 4.70 and 5.08, each as a doublet (J = 7 cps), and eight aromatic hydrogens centered at 2.70 (multiplet). The uv spectrum showed uv max (CH_3OH) 280 mμ mμ (ε 14,300) and 314 mμ (ε 9400).

Anal. Calcd for $C_{18}H_{11}O_4Cl$: C, 66.2; H, 3.4; Cl, 10.8. Found: C, 65.9; H, 3.7; Cl, 10.8.

1,3-Dichloro-2,4-bis(O-hydroxyphenyl)-1,3-cyclobutanecarboxylic Acid Ethyl Ester δ-Lactone (9).—To 30 ml of ethanol was added 0.5 g (0.0015 mol) of the head-to-tail 3-chlorocoumarin dimer and 0.3 ml of 12 N HCl. This mixture was heated at reflux until all of the material went into solution. The ethanol was evaporated and the residue recrystallized from benzene to give 0.40 g (71%) of a white solid, mp 191–193°. The infrared spectrum showed hydroxyl absorption at 3448 cm^{-1} and carbonyl absorption at 1754 and 1727 cm^{-1}. The nmr spectrum contained two cyclobutane hydrogens at τ 4.50 and 4.88 (singlets), two methylene hydrogens at 5.97 (quartet), three methyl hydrogens at 9.02 (triplet), and eight aromatic hydrogens at 2.70 (multiplet).

Anal. Calcd for $C_{20}H_{16}O_5Cl_2$: C, 59.0; H, 4.0; Cl, 17.4. Found: C, 58.9; H, 4.2; Cl, 17.5.

3,4′-Bicoumarin (10).—In a Pyrex test tube was placed 1.0 g (0.003 mol) of 3′-chloro-3′,4′-dihydro-3,4′-bicoumarin (8). The test tube was then heated at 250° in a Wood's metal bath for 20 min. After cooling, the solid material in the test tube was removed and recrystallized from benzene. Approximately 300 mg (34%) of a solid white precipitate, mp 255–257°, was obtained. The infrared spectrum showed strong absorption at 1724, 1608, 1449, and 1376 cm^{-1}. Th uv spectrum of the material showed uv max (CH_3OH) 282 mμ (ε 18,400) and 313 mμ (ε 13,900). The nmr spectrum contained one β-coumarin hydrogen at τ 2.08 (singlet), one α-coumarin hydrogen at 4.33 (singlet), and eight aromatic hydrogens centered at 3.03 (multiplet).

Anal. Calcd for $C_{18}H_{10}O_4$: C, 74.5; H, 3.5. Found: C, 74.9; H, 3.6.

Sensitized Irradiation of the Head-to-Tail 3-Chlorocoumarin Dimer (7).—A solution containing 0.70 g (0.002 mol) of the head-to-tail 3-chlorocoumarin dimer, 0.20 g (0.001 mol) of benzophenone, and 150 ml of dioxane was prepared and added to the outside vessel of the 450-W, medium-pressure mercury arc irradiation apparatus. After flushing with nitrogen, the solution was irradiated for 45 hr. The solvent was then removed on the rotating evaporator. The infrared spectrum of the residue indicated that no amount of 3′-chloro-3′,4′-dihydro-3,4′-bicoumarin (8) was formed.

3′-Chloro-3,4′-bicoumarin (14).—In 15 ml of tetrahydrofuran was dissolved 1.0 g (0.003 mol) of the head-to-tail 3-chlorocoumarin dimer. The mixture was then added to a suspension of 0.25 g (0.006 mol) of sodium hydride (54.7%) in 5 ml of tetrahydrofuran. The mixture was stirred for 1 hr and then 1 N hydrochloric acid added to neutralize any unreacted sodium hydride. A yellow oil formed which was extracted with chloroform and the chloroform solution dried over anhydrous sodium sulfate. The chloroform was stripped off and the residue recrystallized from benzene. A white solid precipitate was formed, 250 mg (28%), mp 256–258°. The infrared spectrum showed strong absorption at 1721, 1608, and 1447 cm^{-1}. The nmr spectrum contained one β-coumarin hydrogen at τ 2.08 (singlet) and eight aromatic hydrogens centered at 2.50 (multiplet). The uv spectrum showed uv max (CH_3OH) 284 mμ (ε 20,000) and 315 mμ (ε 15,100). The compound gave a molecular ion at m/e 324 (326).

Anal. Calcd for $C_{18}H_9O_4Cl$: C, 66.6; H, 2.8; Cl, 10.9. Found: C, 66.4; H, 2.9; Cl, 11.4.

α-Chloro-2,3-dihydro-3-o-hydroxybenzylidene-2-oxo-4H-2-benzopyran-$\Delta^{4,\alpha}$-acetic Acid Lactone (16).—In a Pyrex test tube was placed 1.0 g (0.003 mol) of the heat-to-tail 3-chlorocoumarin dimer. The test tube was then heated at 290° for 20 min in a Wood's metal bath. The evolution of hydrogen chloride was easily detected with litmus paper. After heating, the test tube was cooled and the contents recrystallized from benzene to yield 270 mg (30%) of a light tan solid, mp 279°. The infrared spectrum showed strong absorption at 1724, 1610, 1560, and 1485 cm^{-1}. The nmr spectrum contained one β-coumarin hydrogen at τ 2.12 (singlet) and eight aromatic hydrogens centered at 2.55 (multiplet). The uv spectrum showed uv max (CH_3OH) 291 mμ (ε 16,600) and 327 mμ (ε 18,800). The compound gave a molecular ion at m/e 324 (326).

Anal. Calcd for $C_{18}H_9O_4Cl$: C, 66.6; H, 2.8; Cl, 10.9. Found: C, 66.2; H, 3.3; Cl, 10.7.

Registry No.—**7**, 16666-82-3; **8**, 16666-83-4; **9**, 16666-84-5; **10**, 16666-85-6; **14**, 16666-86-7; **16**, 16666-87-8.

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Bile Acids. XXIV. Raney Nickel in the Preparation of Allocholanic Acids[1]

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Treatment of methyl polyhydroxy-5β-cholanoates, such as methyl cholate (I) or methyl chenodeoxycholate (XI), with Raney nickel in boiling p-cymene provided a mixture of 5α-cholanoates now separated and characterized as the 3,7-diketones (III and XIII), the 7-deoxy derivatives (II and XII), and the methyl 3-ketoallocholanoates (IV and XIV, respectively). Methyl deoxycholate (X) and methyl lithocholate (XVI) afforded the corresponding 3-keto-5α-cholanoates, II and XII, respectively; reduction provided the isomeric hydroxyallocholanoates, VIII and IX and XVII and XVIII. Treatment of methyl 7β-tritiocholate (XX) with Raney nickel gave tritiated IV (XXIII) and II (XXI); the amount of tritium retained in p-cymene was equivalent to that lost in the formation of the diketone III.

During a study of the metabolism of cholestanol-4-^{14}C in our laboratory[2-4] a new method of synthesis of allocholic acid[1c] and allochenodeoxycholic acid[5] was developed. The first step in this method provides a mixture of products after treatment of methyl cholate (I) or methyl chenodeoxycholate (XI) with Raney nickel in boiling p-cymene. After chromatography on alumina these mixtures yielded three major compounds from distinct fractions (A, B, and C) in each case.

(1) (a) This investigation was supported in part by the National Institutes of Health (Grant No. HE-07878 and AM-09992). (b) Presented in part at the 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966. (c) For Paper XXIII in this series, see M. N. Mitra and W. H. Elliott, *J. Org. Chem.*, **33**, 175 (1968). (d) The following abbreviations have been used: tlc, thin layer chromatography; plc, preparative layer chromatography; glpc, gas–liquid partition chromatography; R_t, retention time relative to methyl deoxycholate (methyl 3α,12α-dihydroxy-5β-cholanoate; absolute time = 29 min.).

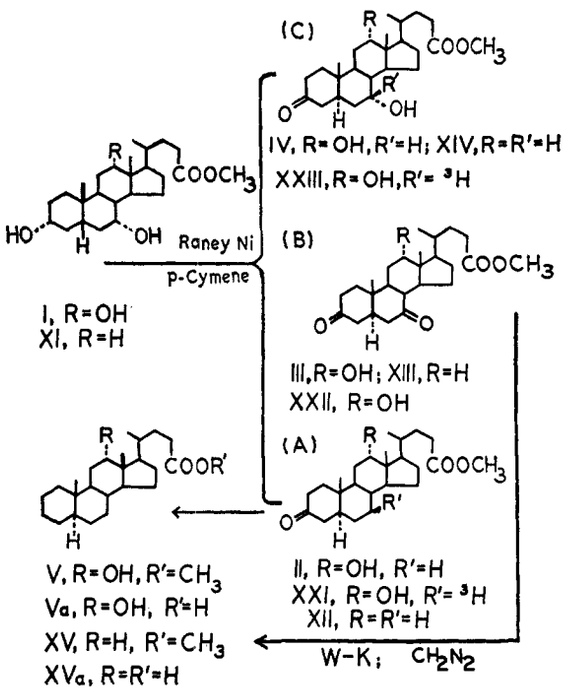
(2) H. J. Karavolas, W. H. Elliott, S. L. Hsia, E. A. Doisy, Jr., J. T. Matschiner, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.*, **240**, 1568 (1965).

(3) H. J. Karavolas and W. H. Elliott, "The Biliary System," W. Taylor, Ed., F. A. Davis Co., Philadelphia, Pa., 1965, pp 175–181.

(4) S. A. Ziller, Jr., and W. H. Elliott, *Fed. Proc.*, **25**, 221 (1966).

(5) S. A. Ziller, Jr., M. N. Mitra, and W. H. Elliott, *Chem. Ind.* (London), 999 (1967).

While the products from fractions C were identified as methyl 3-keto-7α,12α-dihydroxy-5α-cholanoate[1c] (IV) from I and methyl 3-keto-7α-hydroxy-5α-cholanoate[5] (XIV) from XI, the synthetic precursors of allocholic and allochenodeoxycholic acids, respectively, the products from fractions A and B have now been identified and found to be useful starting materials for the preparation of a number of allocholanic acids.



After treatment of methyl cholate (I) with freshly prepared Raney nickel in boiling *p*-cymene for 10 hr, separation of the nickel and *p*-cymene, and chromatography of the residue on alumina,[1c] fraction A was purified by plc on silica gel H to provide methyl 3-keto-12α-hydroxy-5α-cholanoate[6] (II) in 20–22% yield. II may also be prepared directly from methyl deoxycholate (X) in 45% yield by the above reaction.

After plc of fraction B on silica gel H methyl 3,7-diketo-12α-hydroxy-5α-cholanoate (III) was obtained in crystalline form in 18–20% yield. Reduction of either II or III by the Wolff–Kishner method (W–K) followed by diazomethylation provided the same known compound, methyl 12α-hydroxy-5α-cholanoate[1c] (V), whereas on oxidation with chromic acid II or III afforded methyl 3,12-diketo-5α-cholanoate[7] (VI) or methyl 3,7,12-triketo-5α-cholanoate[1c] (VIII), respectively. The above chemical degradation supports the structure of III.

The mass spectrum of III (Figure 1) exhibits a rather weak mass peak (*m/e* 418, 4.9%) and the fragments M − 18, 27.3%, M − 36, 10.5%, M − (18 + 31), 6.3%, M − 73, 7.3%, and M − (18 + 73), 6.6%; base peak, *m/e* 285, M − (18 + 115), is a fragment in which the C_5 side chain and a molecule of water have been lost from the parent ion. The mass spectrum of methyl 3,7-diketo-12α-hydroxy-5β-cholanoate is similar to that of III.

On reduction with sodium borohydride compound II yielded a mixture of methyl allodeoxycholate[6] (VIII) in 12% yield and its 3β isomer (IX) in 72% yield, which was conveniently separated by acetic acid partition chromatography.[8] Catalytic hydrogenation of II in the presence of platinum, however, gave 28% of VIII and 48% of IX.

In an analogous manner fraction A obtained by chromatographic separation of the mixture of products from treatment of methyl chenodeoxycholate (XI) with Raney nickel in boiling *p*-cymene was purified by plc on silica gel H; methyl 3-keto-5α-cholanoate[9] (XII) was obtained in 20–25% yield. Fraction B from XI gave methyl 3,7-diketo-5α-cholanoate (XIII) in 15–18% yield. The structure of XIII was established by physical and chemical means. Both XII and XIII on W–K reduction yielded allocholanic acid.[10] Oxidation of an authentic sample of methyl 3-keto-7α-hydroxy-5α-cholanoate[5] with chromic acid provided a compound identical with XIII. These observations along with mass spectral data confirmed the structure of XIII.

The mass spectrum of XIII (Figure 1) exhibits a strong mass peak (*m/e* 402, 56%), the usual fragments of keto bile acid esters [M − 18, M − 32, M − (18 + 31), M − 73], and the fragments *m/e* 287 [M − 115] and *m/e* 269 [M − (115 + 18)]. The major fragment, *m/e* 192, retains the carbonyl groups of rings A and B, and is apparently stabilized as the enolic triene. The spectrum is significantly different from that of methyl 3,7-diketo-5β-cholanoate or III.

Although the melting point of XIII (158–159°) is close to that of VI (162–163°), the specific rotations are different and are in agreement with calculated values as shown in Table I. The calculated values are based on a sample of methyl allocholanoate, $[\alpha]^{25}D$ +22 ± 1° (*c* 0.49, in chloroform) as reported[1c] and the values as tabulated by Klyne.[11,12]

TABLE I
MOLECULAR ROTATION OF METHYL DIKETO-5α-CHOLANOATES

Diketo-5α-cholanoate	MD, deg Calcd	MD, deg Found
3,12- (VI)	+423	+358
3,7- (XIII)	−80	−87
12α-Hydroxy-3,7- (III)	+13	0

Methyl 3-keto-5α-cholanoate (XII) was also obtained by treatment of methyl lithocholate (XVI) with Raney nickel in boiling *p*-cymene. On catalytic reduction of XII in the presence of platinum a mixture of methyl allolithocholate[13] (XVII) and its 3β isomer[14]

(6) H. Danielsson, A. Kallner, and J. Sjövall [*J. Biol. Chem.*, **238**, 3846 (1963)] prepared II from methyl 3-keto-12α-hydroxy-5β-cholanoate by treatment with Raney nickel in boiling *p*-cymene. They reported, for II, mp 134–136°, $[\alpha]D$ +56.8°; for VIII, mp 174–176°, $[\alpha]D$ +35.6°; for VIIIa, mp 214–215°, $[\alpha]D$ +42°; for IX, mp 137–138°, $[\alpha]D$ +41°; for IXa, mp 228°.

(7) A. F. Hofmann and E. H. Mosbach [*ibid.*, **239**, 2813 (1964)] reported mp 157–160° for VI.

(8) J. T. Matschiner, T. A. Mahowald, W. H. Elliott, E. A. Doisy, Jr., S. L. Hsia, and E. A. Doisy, *ibid.*, **235**, 771 (1957).

(9) H. Wieland, E. Dane, and C. Martius [*Z. Physiol. Chem.*, **215**, 15 (1933)] reported mp 114° and mp 184° for XII and XIIa, respectively.

(10) L. F. Fieser and Mary Fieser ("Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 54) reported mp 94° for XV and mp 173° and $[\alpha]D$ +22° for XVa.

(11) W. Klyne, "The Chemistry of Steroids," Methuen and Co. Ltd., London, 1960, p 55.

(12) D. H. R. Barton and W. Klyne, *Chem. Ind.* (London), 755 (1948).

(13) (a) L. Ruzicka, M. Oberlin, H. Wirz, and J. Mayer [*Helv. Chim. Acta*, **20** 1283 (1937)] reported mp 169–170° for XVII. (b) I. G. Anderson and G. A. D. Haslewood [*Biochem. J.*, **85**, 236 (1962)] reported mp 161–164° for XVII.

(14) A. Stoll and J. Renz [*Helv. Chim. Acta*, **24**, 1380 (1941)] reported mp 150–151° and $[\alpha]D$ +23° for XVIII.

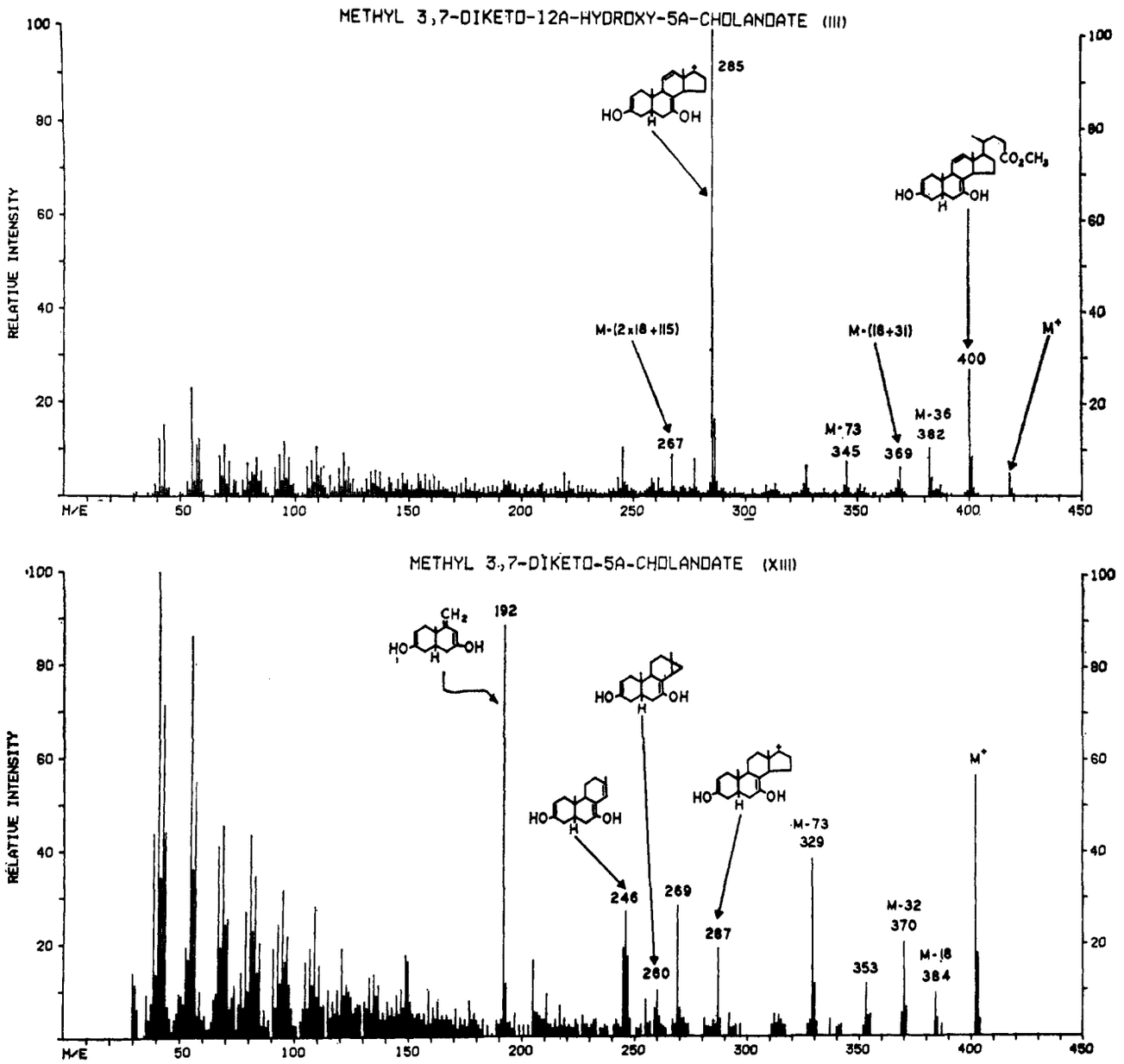


Figure 1.—Mass spectra of methyl 3,7-diketo-12α-hydroxy-5α-cholanoate (III) and methyl 3,7-diketo-5α-cholanoate (XIII) were determined with an LKB Model 9000 mass spectrometer with the direct probe at 75 and 105°, respectively. Other conditions were ion source, 270°; ionizing energy, 70 eV; ionizing current, 60 μA. The structures drawn for the fragment ions are purely formal.

(XVIII) was obtained in a ratio of approximately 1:2. The isomers XVII and XVIII were easily separated by plc.

As obtained, compounds II, III, XII, and XIII are always associated with the corresponding 5β isomers before purification by plc. The separation of 5α from 5β isomers is effected by taking advantage of their differences in R_f values with different concentrations of acetone in benzene (Table II).

TABLE II

R_f VALUES OF METHYL CHOLANOATES

	% acetone in benzene	5α	5β
3-Keto-12α-hydroxy (II)	20	0.49	0.40
3,7-Diketo-12α-hydroxy (III)	20	0.30	0.33
3-Keto (XII)	5	0.60	0.62
3,7-Diketo (XIII)	20	0.70	0.74

The allo (5α) compounds differed from the normal (5β) compounds in their behavior in glpc as indicated by their different relative retention times given in the Experimental Section.

After completion of the identification of the various compounds formed in the Raney nickel reaction with methyl cholate or methyl chenodeoxycholate, an investigation was undertaken to elucidate the mechanism by which elimination of the 7α-hydroxy group takes place in I or XI. Methyl cholate was preferentially oxidized at position 7 with N-bromosuccinimide using the method of Fieser and Rajagopalan.[15] After purification by plc methyl 7-keto-3α,12α-dihydroxy-5β-cholanoate[16] (XIX) was reduced with sodium borotri-

(15) L. F. Fieser and S. Rajagopalan, *J. Amer. Chem. Soc.*, **71**, 3935 (1949).

(16) I. Tanasescu, E. Ramontian, I. Ganea, and F. Hodosan, *Rev. Chim. Acad. Rep. Populaire Roumaine*, **2**, 157 (1957), cited in H. Van Belle, "Cholesterol, Bile Acids and Atherosclerosis," North-Holland Publishing Co., Amsterdam, 1965, p 43.

tide to provide methyl 3α,7α,12α-trihydroxy-7β-tritio-5β-cholanoate (XX). The mobility of XX agreed with I (R_f 0.23 in 50% acetone–benzene) as opposed to methyl 3α,7β,12α-trihydroxy-5β-cholanoate (R_f 0.16 in 50% acetone–benzene). Since approximately 5% of the tritium was retained after preferential oxidation of XX with N-bromosuccinimide at position 7, XX must contain about 95% of the tritium at the 7β position.

The tritiated methyl cholate (XX) was mixed with methyl cholate, heated with Raney nickel in *p*-cymene in the usual way, and the products XXI, XXII, and XXIII corresponding to II, III, and IV, respectively, were isolated in the pure state. From the specific activities of these products (Table III) it is clear that tritium has been retained completely in XXI and XXIII, and that the activity retained in XXII is essentially that introduced elsewhere in the molecule during reduction of XIX with borotritide.

TABLE III
RETENTION OF TRITIUM AFTER RANEY NICKEL REACTION

Compd	Specific activity, dpm/mg — First crystn	Specific activity, dpm/mg — Second crystn	% tritium
XX (calcd)	2.74×10^5		
XX	2.66×10^5	2.69×10^5	100
XXI	2.57×10^5	2.58×10^5	96
XXII	0.10×10^5	0.09×10^5	4
XXIII	2.65×10^5	2.60×10^5	98

From these results it is evident that III is not an intermediate in the formation of II or IV from methyl cholate (I); thus, the 7α-hydroxyl group may have been eliminated as water with an adjacent proton, followed by saturation of the double bond to provide XXI or II. *p*-Cymene recovered from the above reaction was found to retain about 20% of the total tritium present in XX (1.07×10^8 dpm). Further studies on the mechanism of the Raney nickel reaction are in progress.

Experimental Section

The procedures and conditions for the determination of melting points, optical rotations, infrared and mass spectrometry, and thin layer, preparative layer, and gas chromatography have been described.[1c] Radioactivity was measured in a Model 3314 Tricarb liquid scintillation spectrometer as reported;[17] counting efficiency was 20% as determined with tritiated water as internal standard.

Raney Nickel Catalyst.—W-2 catalyst was prepared from Raney catalyst powder (No. 2813, W. R. Grace and Co., Chattanooga, Tenn.) according to the method of Mozingo.[18]

Action of Raney Nickel on Methyl Cholate.—Dry purified methyl cholate (mp 156–157°, 10.0 g) was heated for 10 hr with freshly prepared Raney nickel catalyst (*ca.* 25 g) in refluxing *p*-cymene as reported.[1c] After removal of the catalyst and the solvent, the residue was chromatographed on neutral Woelm alumina deactivated with 12% water. Elution of the column and evaporation of the solvents provided the following three major fractions: (A) eluted with hexane–benzene, 2:1 (2.8 g); (B) with hexane–benzene, 1:1 (3.1 g); (C) with benzene and benzene–ethyl acetate, 9:1 (1.7 g).

Methyl 3-Keto-12α-hydroxy-5α-cholanoate[6] (II).—Fraction A was purified by plc with 20% acetone in benzene on 20 plates (20 cm × 40 cm) coated with silica gel H. The upper bands of solids on the plates corresponding to R_f 0.49 were removed and extracted with acetone. After evaporation of the solvent 2.2 g of crystalline residue was crystallized from acetone–hexane as stout needles[6] of II: mp 144–145°; $[\alpha]^{25}D$ +51.7 ± 0.5° (*c* 1.0); R_t 2.23 (R_t for methyl 3-keto-12α-hydroxy-5β-cholanoate 1.18); ir 3401, 1706, 1068, 1043, 958, 893, 852 cm^{-1}.

Methyl 3,7-Diketo-12α-hydroxy-5α-cholanoate (III).—Fraction B was purified by plc with 25% acetone in benzene in a similar manner. In this case the lower bands of solids on the plates were removed and extracted with acetone. After removal of the solvent 2.04 g of crystalline residue was crystallized from acetone–hexane to afford soft shining needles of III: mp 178°; $[\alpha]^{25}D$ 0 ± 0.5° (*c* 1.0); R_t 7.14 (R_t for methyl 3,7-diketo-12α-hydroxy-5β-cholanoate, 5.05); ir 3496, 3355, 1739, 1709, 1075, 952, 893 cm^{-1}.

Anal. Calcd for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 71.65; H, 9.16.

On hydrolysis with 5% potassium hydroxide III yielded the corresponding acid, 3,7-diketo-12α-hydroxy-5α-cholanoic acid: mp 222–223°; ir 3472, 1709, 1207, 1077, 952 cm^{-1}.

Anal. Calcd for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97. Found: C, 71.09; H, 9.06.

W-K Reduction of II.—Reduction of 100 mg of II was carried out[19] in a mixture of 1 ml of 85% hydrazine hydrate, 500 mg of potassium hydroxide, and 5 ml of triethylene glycol. The reduction product (98 mg) was crystallized from acetone–hexane as plates of 12α-hydroxy-5α-cholanoic acid (Va): mp 199°; $[\alpha]^{25}D$ +42.1 ± 0.5° (*c* 1.0); ir 3378, 1712, 1093, 1030, 884 cm^{-1} (lit.[1c] mp 199°; $[\alpha]^{25}D$ +42.2°).

Methylation of Va with diazomethane yielded needles of V: mp 118° (acetone–hexane); $[\alpha]^{25}D$ +41.5 ± 1° (*c* 1.0); R_t 0.37; ir 3436, 1736, 1160, 936, 886 cm^{-1} (lit.[1c] mp 118–119°; $[\alpha]^{25}D$ +41.6 ± 1°).

W-K Reduction of III.—Reduction of 34 mg of III was carried out as above. The product (30 mg) had the same R_f value as that obtained from II in analytical tlc with a mixture of isooctane, ethyl acetate, acetic acid (50:50:0.7). On crystallization from acetone–hexane shining plates of 12α-hydroxy-5α-cholanic acid[1c] (Va) were obtained: mp 198–199°; $[\alpha]^{25}D$ +42.3 ±0.5° (*c* 0.98). The infrared spectrum was comparable with that of a sample obtained by reduction of II.

On methylation with diazomethane, Va obtained from III gave the same methyl ester (V): mp 119°; R_t 0.37.

Oxidation of III.—A solution of III (62 mg) in 0.6 ml of acetic acid was oxidized with chromic anhydride (20 mg in 0.6 ml of acetic acid) for 2 hr at room temperature. The product (50 mg) was purified by plc with 20% acetone in benzene, and finally crystallized from acetone–hexane as fine needles of methyl 3,7,12-triketo-5α-cholanoate (VII): mp 198–199°; $[\alpha]^{25}D$ +29.0 ± 1° (*c* 1.0, in chloroform); R_t 9.24; ir 1736, 1706, 1282, 811 cm^{-1} (lit.[1c] mp 198–199°; $[\alpha]^{25}D$ +29.1 ± 1°).

Oxidation of II (50 mg) was accomplished with chromic acid as above. After crystallization from aqueous acetone shining flakes of methyl 3,12-diketo-5α-cholanoate[7] (VI) were obtained: mp 162–163°; $[\alpha]^{25}D$ +88.0 ± 1.5° (*c* 0.55); R_t 3.52 (R_t for methyl 3,12-diketo-5β-cholanoate 2.86); ir 3472, 1709, 1207, 1077, 952 cm^{-1}.

Action of Raney Nickel on Methyl Deoxycholate.—A mixture of methyl deoxycholate (X) (4.0 g, mp 82°), freshly prepared Raney nickel (*ca.* 10 g), and 60 ml of freshly distilled *p*-cymene was heated as before. After removal of the nickel and the solvent, the resulting solid residue (3.5 g) was chromatographed on Woelm alumina deactivated with 12% water. The column was eluted with successive quantities of hexane, mixtures of hexane and benzene, and benzene. The fraction eluted with hexane–benzene (2:1) yielded a residue (2.9 g) containing mainly methyl 3-keto-12α-hydroxy-5α-cholanoate as shown by analytical tlc. After purification by plc with 20% acetone in benzene and crystallization from acetone–hexane, stout needles of II were obtained:[6] mp 145–146°; $[\alpha]^{25}D$ +51.2 ± 0.5° (*c* 1.0); R_t 2.23.

Reduction of II with Sodium Borohydride.—II (100 mg) was dissolved in methanol (5 ml) and treated with powdered sodium borohydride (50 mg) for 0.5 hr at room temperature. The mixture was diluted with water and acidified, and the product was extracted with ether. After evaporation of the ether the residue (99.0 mg) was purified by acetic acid partition chromatography.[8] Methyl allodeoxycholate[6] (VIII) (16 mg) was eluted with hexane: shining needles (aqueous methanol): mp 177–178°; $[\alpha]^{25}D$ +43.3 ± 1° (*c* 0.48); R_t 1.07; ir 3058, 3378, 1730, 1028, 905 cm^{-1}. On hydrolysis with 5% methanolic potassium hydroxide VIII yielded allodeoxycholic acid[6] (VIIIa), mp 215–216°.

(17) P. D. Ray, E. A. Doisy, Jr., J. T. Matschiner, S. L. Hsia, W. H. Elliott, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.*, **236**, 3158 (1961).

(18) R. Mozingo in "Organic Syntheses," Coll. Vol. III, E. C. Horning, Ed., John Wiley and Sons, Inc., New York, N. Y., 1955, p 181.

(19) Huang-Minlon, *J. Amer. Chem. Soc.*, **71**, 3301 (1949).

On elution with 20% benzene in hexane, methyl 3β,12α-dihydroxy-5α-cholanoate[6] (IX) (78 mg) was obtained: shining flakes (aqueous methanol); mp 137–138° (sintering at 90°); $[\alpha]^{25}D$ +35.2 ± 1° (*c* 0.33); R_t 1.16; ir 3424, 3333, 1718, 1312, 1041, 902, 890 cm^{-1}. Hydrolysis of IX with 5% methanolic potassium hydroxide afforded 3β,12α-dihydroxyallocholanoic acid[6] (IXa), mp 231–232°.

Catalytic hydrogenation of II (100 mg) was carried out in the presence of Adams catalyst in glacial acetic acid containing a few drops of concentrated hydrochloric acid. After the reduction, methyl allodeoxycholate (VIII, 28 mg) and methyl 3β,12α-dihydroxy-5α-cholanoate (IX, 47 mg) were separated by acetic acid partition chromatography,[8] crystallized from aqueous methanol and identified in the usual manner.

Action of Raney Nickel on Methyl Chenodeoxycholate.—Methyl chenodeoxycholate[20] (mp 89–90°, 9.5 g) (XI) was heated for 10 hr in boiling *p*-cymene in the presence of freshly prepared Raney nickel (*ca.* 18 g) in the usual manner. After separation of the Raney nickel and *p*-cymene, the semisolid (8.21 g) was chromatographed over alumina deactivated with 12% water. Three major fractions were collected: (A) with hexane–benzene, 4:1 (3.0 g); (B) with hexane–benzene, 2:1 (2.0 g); and (C) with benzene and benzene–ethyl acetate, 9:1 (2.2 g).

Methyl 3-Keto-5α-cholanoate (XII).—Fraction A was purified by plc with 4% acetone in benzene and provided a crystalline residue of XII (2.3 g). Crystallization from aqueous methanol afforded shining plates of methyl 3-keto-5α-cholanoate[9] (XII): mp 113–114°; $[\alpha]^{25}D$ +40.5 ± 1° (*c* 0.99); R_t 1.06 (R_t for methyl 3-keto-5β-cholanoate 1.00); ir 1739, 1715, 1228, 985 cm^{-1}.

Hydrolysis of XII with 5% methanolic potassium hydroxide provided the corresponding 3-keto-allocholanoic acid:[9] mp 183–184°; $[\alpha]^{25}D$ +32.6 ± 0.5° (*c* 0.98).

Methyl 3,7-Diketo-5α-cholanoate (XIII).—Fraction B was purified by plc with 10% acetone in benzene to provide 1.6 g of crystalline residue. After crystallization from acetone–hexane stout needles of methyl 3,7-diketo-5α-cholanoate (XIII) were obtained: mp 160–161°; $[\alpha]^{25}D$ −21.6 ± 2.0 (*c* 1.0); R_t 3.96 (R_t for methyl 3,7-diketo-5β-cholanoate 2.90); ir 1736, 1706, 1319, 1162, 977, 906, 894 cm^{-1}.

Anal. Calcd for $C_{25}H_{38}O_4$: C, 74.59; H, 9.51; mol wt, 402. Found: C, 74.53; H, 9.40; mol wt, 402 (mass spectrometry).

W–K Reduction of XIII.—Compound XIII (100 mg) was reduced by the W–K method using 5 ml of triethylene glycol, 500 mg of potassium hydroxide, and 1 ml of 85% hydrazine hydrate. The reduction product (99 mg) on crystallization from aqueous acetone provided shining flakes of allocholanoic acid[10] (XVa): mp 172–173°; $[\alpha]^{25}D$ +22.2 ± 1° (*c* 0.50, in chloroform).

After methylation with diazomethane, purification of the product by plc in benzene, and crystallization from aqueous methanol, shining flakes of methyl allocholanoate[10] (XV) were obtained: mp 93°; $[\alpha]^{25}D$ +22.1 ± 1° (*c* 0.49, in chloroform).

W–K Reduction of XII.—Reduction of 100 mg of XII by the W–K method in the usual way yielded allocholanoic acid (XVa) identical with that obtained from XIII.

Oxidation of Methyl 3-Keto-7α-hydroxy-5α-cholanoate.[5]—Oxidation of XIV (50 mg) with chromic anhydride (50 mg) in acetic acid provided 40 mg of product which was crystallized from aqueous methanol as XIII: mp 162–163°; $[\alpha]^{25}D$ −21.6 ± 2.0° (*c* 1.0). The infrared spectrum was comparable with that of methyl 3,7-diketo-5α-cholanoate obtained directly from XI by the action of Raney nickel.

Catalytic Hydrogenation of XII.—Compound XII (108 mg) was hydrogenated in the presence of Adams catalyst in glacial acetic acid solution containing a few drops of hydrochloric acid. The product of reduction was purified by plc in 17% acetone in benzene. The upper band in plc corresponding to R_f 0.72 yielded methyl allolithocholate[13] (XVII) (24 mg), which was crystallized from aqueous methanol as shining flakes: mp 167–168°; $[\alpha]^{25}D$ +22.5 ± 1° (*c* 0.85, in chloroform); R_t 0.50 (R_t for methyl lithocholate 0.50); ir 3225, 1736, 1275, 1034,10 02, 956, 894 cm^{-1}.

The lower band in plc corresponding to R_f 0.57 yielded methyl 3β-hydroxy-5α-cholanoate[14] (XVIII, 47 mg) which formed needles from aqueous methanol: mp 150–151°; $[\alpha]^{25}D$ +24.3 ± 0.5° (*c* 1.0); R_t 0.53; ir 3494, 1712, 1297, 1051, 1002, 952, 893 cm^{-1}.

Action of Raney Nickel on Methyl Lithocholate.—After refluxing methyl lithocholate[21] (XVI) (1.0 g, mp 129–130°) in *p*-cymene with freshly prepared Raney nickel (*ca.* 3.0 g), and removal of the nickel and solvent, the crude reaction product (800 mg) was taken up in ether and washed with water. The residue obtained after removal of the ether was crystallized from aqueous methanol to provide shining plates of methyl 3-keto-5α-cholanoate[9] (XII) in 65% yield: mp 113–114°; R_t 1.06.

On hydrolysis of XII with 5% methanolic potassium hydroxide 3-ketoallocholanoic acid[9] was obtained, mp 183–184°.

Methyl 7-Keto-3α,12α-dihydroxy-5β-cholanoate (XIX).—Methyl cholate (200 mg) was oxidized with N-bromosuccinimide (175 mg) in a mixture of 25 ml of dioxane and 4 ml of water at room temperature for 1 hr.[15] The residue from oxidation (196 mg) was purified by plc with 35% acetone in benzene. The major product was crystallized from acetone–hexane as needles of methyl 7-keto-3α,12α-dihydroxy-5β-cholanoate[16] (XIX): mp 153–154°; $[\alpha]^{25}D$ +0.3 ± 1° (*c* 0.99); R_t 3.51.

Methyl Cholate-7β-^{3}H (XX).—To a solution of 56 mg of XIX in 5 ml of methanol was added a few milligrams of sodium borotritide (New England Nuclear Corp.). The mixture was allowed to stand at room temperature for 0.5 hr and was then diluted with water and acidified. The resulting precipitate was separated and purified by plc on silica gel H with 50% acetone in benzene. The residue (30 mg) was acetylated with acetic anhydride (2 ml) and pyridine (1 ml), crystallized, and hydrolyzed with methanolic potassium hydroxide to remove tritium bonded to oxygen. The tritiated cholic acid thus obtained was methylated with diazomethane, and the product (25.0 mg) again purified by plc with 50% acetone in benzene. After crystallization from a mixture of acetone and hexane, crystals of methyl cholate-^{3}H (XX) were obtained, mp 154–155°. After successive crystallizations from acetone–hexane, the specific activities of XX and of the mother residue were 5.27×10^7 and 5.20×10^7 dpm/mg, respectively.

A sample of XX (0.837 mg) was mixed with methyl cholate (I) and oxidized with N-bromosuccinimide as described before. The product was purified by plc and crystallized from acetone–hexane to provide methyl 7-keto-3α,12α-dihydroxy-5β-cholanoate, mp 154°. The specific activities of the diluted tritiated methyl cholate and the oxidized product were 5.1×10^5 and 0.28×10^5 dpm/mg, respectively. Thus, 5.5% of the tritium in XX was not located at position 7.

Action of Raney Nickel on Tritiated Methyl Cholate.—A mixture of 2.089 mg of XX and 399.3 mg of I (Table III) was heated in boiling *p*-cymene with freshly prepared Raney nickel for 10 hr and the reaction product (304 mg) separated into the major compounds XXI, XXII, and XXIII as described above. The purity of these compounds was checked by analytical tlc; the specific activities after successive crystallizations from acetone–hexane are given in Table III. *p*-Cymene was recovered by steam distillation, dehydrated thoroughly with anhydrous sodium sulfate, and radioactivity was determined from an aliquot in the scintillation counter; 0.21×10^8 dpm were found in the total *p*-cymene.

Registry No.—III, 16656-66-9; free acid of III, 16656-67-0; XIII, 16656-68-1.

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