point of our sample (mp 100–101°) to polymorphism, as our sample was chromatographically homogeneous (in particular, free from starting material and the isomeric IIIb) and had the expected nmr spectrum. All melting points are corrected and determined on a Kofler block.

Low-resolution mass spectra reported in Table I were obtained on an Hitachi RMU 6 mass spectrometer using 80-eV ionization energy with source and direct-inlet temperatures of 180–200°. The complete high-resolution mass spectrum of compound IV was obtained on a CEC-110 mass spectrometer. Individual exact ion masses were determined using the RMU 7 high-resolution instrument.

Registry No.—IIa, 18897-72-8; IIb, 18897-73-9; IIIa, 2760-91-0; IIIb, 2760-93-2; IVa, 18897-78-4; IVb, 17021-85-1; IVc, 18897-79-5; IVd, 18897-77-3; VIa, 18897-74-0; VIb, 18897-75-1.

Acknowledgment.—The assistance of Mr. W. R. Landis of the National Institutes of Health and of the Purdue University Mass Spectrometry Center is most gratefully acknowledged. We also thank Miss M. Pyles for her skillful assistance.

Intramolecular Catalysis in the Acetylation of Methyl Cholate[1]

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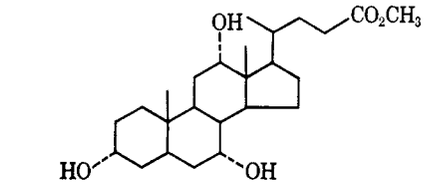
The hydroxyl groups of methyl cholate decrease in reactivity toward acetic anhydride in the order 3 > 12 > 7 when they are present in compounds free of intramolecular influences. In methyl cholate, however, the 7-hydroxyl is acetylated in preference to the 12-hydroxyl as a result of three interactions: (1) deactivation of the 12-hydroxyl by the side chain, (2) enhancement of 7-hydroxyl reactivity by a 3-acetoxy group, and (3) enhancement of 7-hydroxyl reactivity by the 12-hydroxyl. Preferential acetylation at the 7-hydroxyl occurs also in the absence of a 3-acetoxy group, as in methyl 3α,7α-dihydroxycholanate.

The order of reactivity of the hydroxyl groups of methyl cholate (1) toward acylating agents is established as 3 > 7 > 12, based in part on its acetylation to the 3,7-diacetate by acetic anhydride and pyridine.[2] On conformational grounds, however, the reverse order of reactivity for the 7- and 12-hydroxyls might have been predicted. From inspection of structure 2, it is evident that the 7-hydroxyl is surrounded axially by two hydrogens and a methylene group, while merely three hydrogens surround the 12-hydroxyl, giving rise to less steric inhibition. Some possible explanations for this anomaly include (a) an indirect route for acylation at C-7, (b) inhibition of reactivity of the 12α-hydroxyl, and (c) enhancement of 7α-hydroxyl group reactivity.



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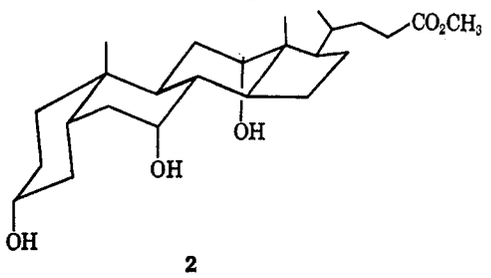

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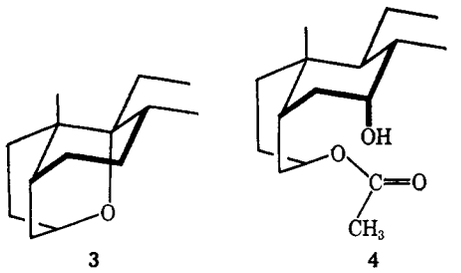

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An indirect route *via* acetyl migration from the 3 position is conceivable. When the A ring assumes a chair conformation (as in 2), the 3- and 7-hydroxyls are relatively remote. In the conformational equilibrium some portion of the molecules could exist in a boat conformation, however (as is required in the 3,9-oxide 3[3]), producing a structure 4 which could permit acetyl migration from the more reactive 3 position. Acetyl migration does *not* occur, however, under the conditions which produce the 3,7-diacetate of methyl cholate.[4]

In the work reported here we confirm the reactivity sequence predicted on conformational grounds, and examine the latter two explanations of the methyl cholate anomaly. Relative reactivities of 3α-, 7α-, and 12α-hydroxyl groups were assessed by comparing yields of acetate produced under identical conditions with methyl lithocholate (5), methyl 7α-hydroxycholanate (6), and 5β-pregnan-12α-ol (7). Inhibition of the reactivity of the 12-hydroxyl by the bile acid side chain was assessed by comparing 5β-pregnan-12α-ol (7) with methyl 12α-hydroxycholanate (8). The acetylation of methyl 7α-12α-dihydroxycholanate (9) was studied in order to determine whether a 3α-acetoxy group influences the course of the reaction, and acetylation yields of other bile acid derivatives were compared for the purpose of detecting enhancement of 7α-hydroxyl group reactivity, should it exist.

In order to test the prediction that the inherent relative reactivity is 3 > 12 > 7 when these three hydroxyl groups are free of influence by any other group in the molecule, we chose compounds in which

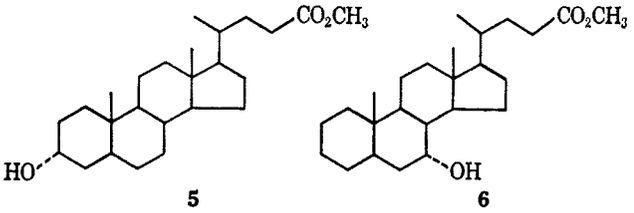
(1) Taken in part from the M.S. thesis of B. Orwig, Indiana University, Indianapolis, Ind., 1967.

(2) L. F. Fieser and S. Rajagopalan, *J. Amer. Chem. Soc.*, **72**, 5530 (1950).

(3) V. R. Mattox, *et al.*, *J. Biol. Chem.*, **164**, 569 (1946); R. B. Turner, *et al.*, *ibid.*, **166**, 345 (1946).

(4) R. T. Blickenstaff and B. Orwig, *J. Org. Chem.*, **32**, 815 (1967).

the side chain was either too far from (5 and 6) or too short to reach (7) the hydroxyl. So that the results



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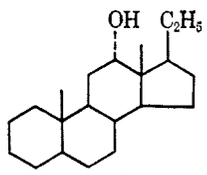

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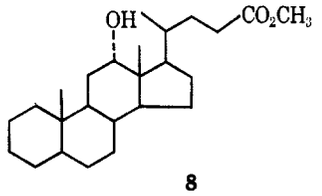

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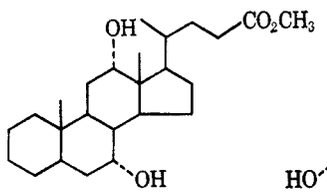

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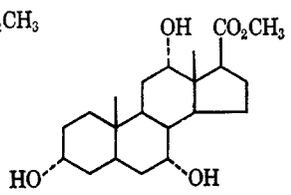

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would be pertinent to methyl cholate, the three monohydroxy steroids were treated with acetic anhydride and pyridine under conditions similar to those which convert methyl cholate into the 3,7-diacetate.[2] The yields presented in Table I confirm the sequence 3 > 12 > 7, illustrating the sensitivity of this reaction to neighboring conformational factors.

TABLE I

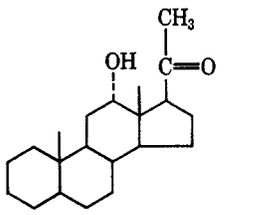
ACETYLATION OF HYDROXY STEROIDS WITH ACETIC ANHYDRIDE AND PYRIDINE[a]

Compd no.	Name	Yield of acetate, %
5	Methyl lithocholate	77
6	Methyl 7α-hydroxycholanate	3–7
7	5β-Pregnan-12α-ol	45–50
8	Methyl 12α-hydroxycholanate	5–8
9	Methyl 7α,12α-dihydroxycholanate	56
11	5β-Pregnan-12α-ol-20-one	18–21
12	12α-Cholanol	5–10
17	Methyl 3α-acetoxy-7α,12α-dihydroxycholanate	60–70
18	Methyl 3α-acetoxy-7α-hydroxycholanate	19

[a] Steroid (0.37 mmol), Ac_2O (0.1 ml), pyridine (0.1 ml), and benzene (0.84 ml), room temperature, 24 hr.

Inhibition of 12α-hydroxyl group reactivity by side-chain shielding has been evoked to explain 3,7 diacetylation of methyl cholate (1) in contrast to 3,12 diacetylation of methyl etiocholate (10).[5] In this comparison the difference in results could, however, have been attributed to a difference in reaction conditions. The diacetylation of methyl cholate was carried out with acetic anhydride and pyridine in benzene,[6] while the diacetylation of methyl etiocholate was achieved by letting a solution of the steroid in glacial acetic acid containing anhydrous HCl stand at room temperature for 5 days.[7,8] The mechanism for acid-catalyzed esterifications is well known,[9] but, even if one accepts that alcoholysis follows the same mechanism as hydrolysis of anhydrides,[10] it is unlikely that steric requirements in the two transition states (in the acetic acid and the acetic anhydride reactions) would be identical. Consequently, it seemed advisable to compare methyl 12α-hydroxycholanate (8) with 5β-pregnan-12α-ol-20-one (11), models for methyl cholate and methyl etiocholate, under the same conditions in order to eliminate any possible ambiguity.[11] The observation that the latter gives about four times as high a yield of acetate as methyl 12α-hydroxycholanate clearly implicates the side chain (Table I). 5β-Pregnan-12α-ol (7) gives an even higher yield, suggesting some indirect influence by the 20-carbonyl of 11.

The Fieser postulate, shielding by the bile acid side chain, is, thus, confirmed, although the detailed chemical nature of that shielding remains to be clarified. It is tempting to relate shielding to hydrogen bonding of the hydroxyl to the side chain, for Wall, *et al.*, found that the 12α-hydroxy compound 13, which cannot H bond intramolecularly, is easily acetylated under conditions that leave the 12β-hydroxy compound 14, which is strongly H bonded, untouched.[12] Indeed,



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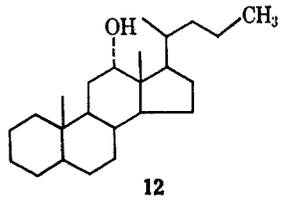

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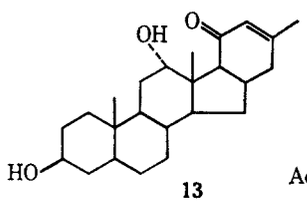

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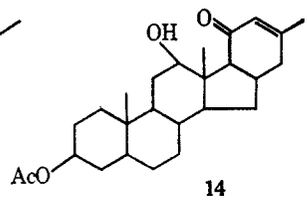

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the infrared spectra of methyl 12α-hydroxycholanate in carbon tetrachloride indicate intramolecular hydrogen bonding (Table II), whereas spectra of 5β-pregnan-12α-ol-20-one and 5β-pregnan-12α-ol indicate only intermolecular hydrogen bonding. As both of the latter are acetylated in higher yield than methyl 12α-hydroxycholanate, it might be inferred that, if comparable molecular associations take place in benzene–pyridine–acetic anhydride solution, the intramolecular H bonding of methyl 12α-hydroxycholanate is responsible for its low reactivity. This interpretation

(5) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 222.

(6) L. F. Fieser, S. Rajogopalan, E. Wilson, and M. Tishler, *J. Amer. Chem. Soc.*, **73**, 4133 (1951).

(7) A. Lardon, *Helv. Chim. Acta*, **30**, 597 (1947).

(8) The argument that the difference in acetylation results could be due to the difference in experimental conditions is somewhat muted by the observation that methyl cholate is converted into its 3,7-diacetate by acetic acid and acetyl chloride: H. Wieland and W. Kapitel, *Z. Physiol. Chem.*, **212**, 269 (1932).

(9) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., New York, N. Y., 1959, p 321.

(10) A. R. Butler and V. Gold, *J. Chem. Soc.*, 4362 (1961).

(11) Our short side chain is one atom shorter than Lardon's [$-C(=O)CH_3$ *vs.* $-C(=O)OCH_3$], and inspection of models shows that both are too short to reach the 12α-hydroxyl.

(12) M. E. Wall, F. I. Carroll, and G. S. Abernethy, Jr., *J. Org. Chem.*, **29**, 604 (1964).

TABLE II

INFRARED EVIDENCE FOR HYDROGEN BONDING

Compd no.	Name	4% solution	8% solution
8	Methyl 12α-hydroxycholanate	2.70, 2.8–2.9 (sh)[a]	2.70, 2.8–2.9 (sh)[a]
6	Methyl 7α-hydroxycholanate	2.70	2.70, 2.9 (sh)
7	5β-Pregnan-12α-ol	2.70	2.70, 2.90
11	5β-Pregnan-12α-ol-20-one	2.70	2.70, 2.85
12	12α-Cholanol	2.70	2.70, 2.8–2.9 (sh)

[a] $\lambda_{max}^{CCl_4}$ in μ; in 0.2-mm (4%) and 0.1-mm (8%) cells.

is faulty, however, for both 12α-cholanol (**12**) and methyl 7α-hydroxycholanate (**6**) also exhibit low reactivity, but neither hydrogen bonds intramolecularly. Consequently, although the side chain surely deactivates the 12α-hydroxyl, the manner in which this is accomplished is unknown, and other factors may also be important in the diacetylation of methyl cholate.

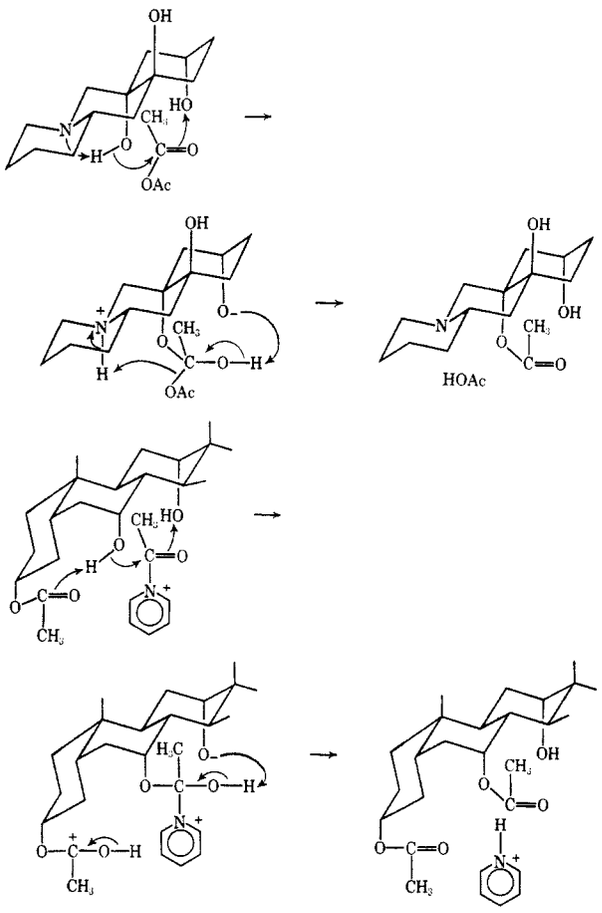
A 3α-acetoxy group, for example, although shown not to migrate to the 7 position, might conceivably influence the course of the reaction by directing acetylation toward the 7-hydroxyl. In that event, a compound lacking a functional group at carbon 3 might react differently toward acetic anhydride than does methyl cholate. For the purpose of testing this possibility, methyl 7α,12α-dihydroxycholanate (**9**), prepared by reduction of methyl 7α,12α-dihydroxy-3-oxocholanate,[13] was acetylated under conditions that convert methyl cholate into the 3,7-diacetate. The structure of product **15** was indicated by its oxidation to **16**, which along with methyl 3α,7α-diacetoxy-12-oxocholanate exhibits a positive Cotton effect (Scheme I).[14] Confirmation of the 12-oxo structure was obtained by Wolff–Kishner reduction of **16** to 7α-hydroxycholanic acid, which was converted with diazomethane into methyl 7α-hydroxycholanate (**6**). Selective acetylation of 7,12-diol **9** at the 7-hydroxyl proves that the presence or absence of a 3-acetoxy group does not materially influence the course of the reaction.

It is of some interest to note, however, that the yield obtained in this reaction, 56%, is a slightly less than the 66–70% yield of 3,7-diacetate obtained from methyl 3α-acetoxy-7α,12α-dihydrocholanate (**17**) under the same conditions. A similar enhancement of reactivity of the 7-hydroxyl by a 3α-acetoxy group is observed by comparing yields of 7-acetate produced from methyl 7α-hydroxycholanate (**6**), 3–7%, and methyl 3α-acetoxy-7α-hydroxycholanate (**18**), 19%. A much larger effect, however, is obtained in the enhancement of 7-hydroxyl reactivity by the 12α-hydroxyl group: compare **6** (3–7%) with **9** (56%) and **18** (19%) with **17** (66–70%). The significance of compound **17** is that it surely is the intermediate in the diacetylation of methyl cholate. The full explanation of the methyl cholate anomaly, then, must take into account not only 12-hydroxyl deactivation by the side chain, but also 7-hydroxyl enhancement by both the 3-acetoxy group and the 12-hydroxyl group. Bifunctional intramolecular catalysis of a possibly similar nature has been observed by Kupchan, *et al.*, in the acetylation of several perhydrobenzoquinolizine derivatives.[15,16]

(13) F. Nakada, *Steroids*, **2**, 45 (1963).

(14) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p 44.

(15) S. M. Kupchan, J. H. Block, and A. C. Isenberg, *J. Amer. Chem. Soc.*, **89**, 1189 (1967). One of the mechanisms proposed by these authors, the general acid–general base mechanism, is readily adaptable to methyl cholate 3-acetate. The adaptation is not wholly adequate, however, for neither does it embrace the role of the side chain, nor does it explain why an analogous process does not occur in which the side-chain ester carbonyl and the 7-hydroxyl combine to enhance reactivity of the 12-hydroxyl.



(16) The results in Table I for methyl 7α- and 12α-hydroxycholanate are not precise enough either to confirm or conflict with our preliminary finding that (under slightly different conditions) the 12-hydroxy compound reacts at a faster rate than its 7-hydroxy isomer (R. T. Blickenstaff and A. Sattar, Second International Congress on Hormonal Steroids, Milan, 1966, Abstract No. 391). It is interesting to note that a comparable difference in rates for the trimethylsilylation of these two isomers was observed recently [T. Briggs and S. R. Lipsky, *Biochim. Biophys. Acta*, **97**, 579 (1965)].

Experimental Section[17]

Methyl 7α-hydroxycholanate (**6**) was prepared by the action of diazomethane on 7α-hydroxycholanic acid, obtained by Wolff-Kishner reduction of methyl 7α-acetoxy-3,12-dioxocholanate, mp (**6**) 78.5–80.0° (lit.[18] mp 78–79°).

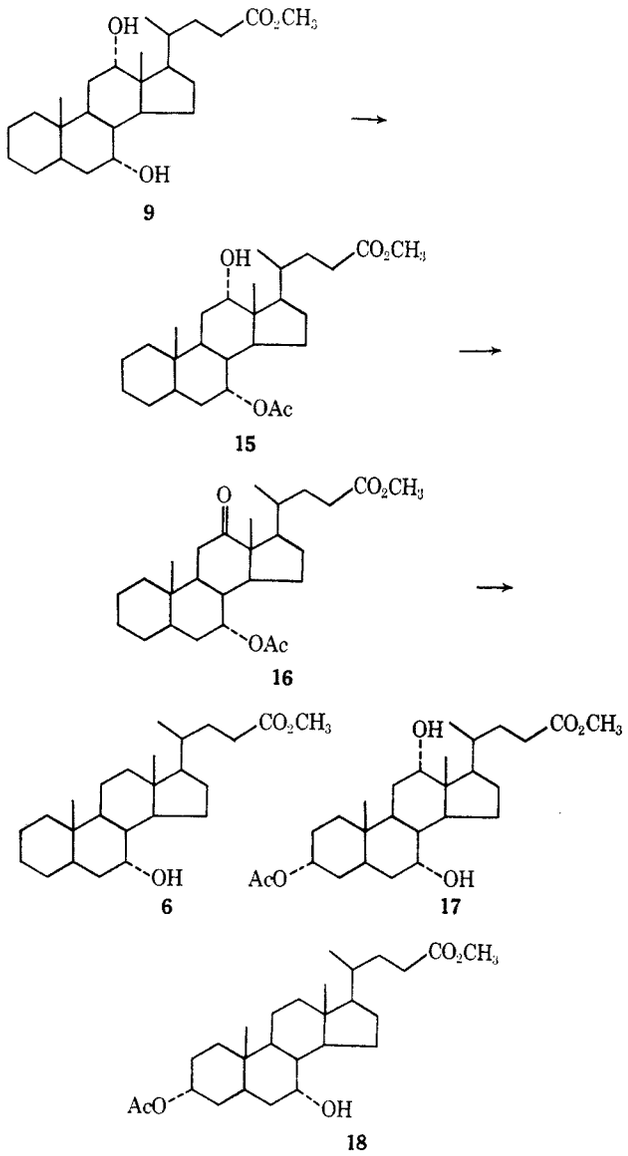
The **acetate** crystallized out of MeOH–H_2O, mp 95–96.2°.

Anal. Calcd for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.06; H, 10.43.

(17) Melting points were taken on a Unimelt apparatus and are uncorrected. Infrared spectra were determined in CCl_4 solution or as mineral oil mulls with an Infracord. Optical rotatory dispersion spectra were determined on a Rudolph prototype at Eli Lilly and Co. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

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

SCHEME I



5β-Pregnan-12α-ol (7) was prepared by Wolff–Kishner reduction of 2 g of 5β-pregnan-12α-ol-3,20-dione. The crude product (1.8 g) was recrystallized in MeOH–H_2O to give 1.3 g of 7: mp 100–101°; λ_{max}^{Nujol} 2.81 (OH), 9.33, 9.49, 9.70 μ.

Anal. Calcd for $C_{21}H_{36}O$: C, 82.83; H, 11.92. Found: C, 83.14; H, 11.87.

The **acetate** crystallized from MeOH–H_2O, mp 56.0–56.8°.

Anal. Calcd for $C_{23}H_{38}O_2$: C, 79.71; H, 11.05. Found: C, 80.01; H, 11.44.

Methyl 12α-hydroxycholanate (8) was prepared by hydrogenation of the Δ^3 analog, obtained from dehydrotosylation of methyl deoxycholate 3-monotosylate, mp (8) 117–118° (lit.[19] mp 120–121°).

Methyl 7α,12α-Dihydroxycholanate (9).—Methyl cholate (1) was oxidized with aluminum isopropoxide and acetone to methyl 7α,12α-dihydroxy-3-oxocholanate,[20] which was then reduced by the Wolff–Kishner method to 7α,12α-dihydroxycholanic acid. The latter was converted with refluxing methanolic HCl into methyl 7α,12α-dihydroxycholanate (9), mp 153–154° (lit.[18] mp 155–156°).

5β-Pregnan-12α-ol-20-one (11).—3α,12α-Diacetoxy-5β-pregnan-20-one (2.00 g) was hydrolyzed by heating it in a mixture containing 1.6 g of KOH, 8 ml of MeOH, and 8 ml of H_2O near reflux temperature for 15 min. The cooled mixture was diluted with 40 ml of H_2O and filtered; the precipitate was washed with H_2O and vacuum dried. The crude diol was further dried by axeotropic distillation of benzene; then without additional purification, it was tosylated with 2.0 g of *p*-toluenesulfonyl chloride in 14 ml of pyridine. After 3 hr at room temperature, the solution was poured on crushed ice, causing a gum to separate. Trituration of the gum in dilute HCl formed a solid, which was filtered, washed with H_2O and dried, 2.81 g. Crystallization from methanol gave **3α-tosyloxy-5β-pregnan-12α-ol-20-one**, mp 145–146°.

Anal. Calcd for $C_{28}H_{40}O_5S$: C, 68.82; H, 8.25; S, 6.56. Found: C, 68.77; H, 8.24; S, 6.28.

First and second crops (2.30 g) were combined for dehydrotosylation in 18 ml of collidine. The solution was refluxed 3 hr, cooled, diluted with ice, and acidified with H_2SO_4. Slowly a brown solid formed, which was filtered, washed with H_2O, and dried, 1.45 g. Two crystallizations from aqueous acetone gave the analytical sample of **5β-pregna-3-en-12α-ol-20-one**: mp 184–149°; λ_{max} 2.80 (OH) and 5.91 μ (C=O).

Anal. Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.50; H, 10.20.

Hydrogenation of the olefin in absolute ethanol with 5% Pd/C at 50 psi for 30 min gave **5β-pregnan-12α-ol-20-one** (11), after two crystallizations from aqueous acetone: mp 132–133°; λ_{max} 2.79 (OH) and 5.90 μ (C=O).

Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.75. Found C, 79.02; H, 10.51.

The **acetate** crystallized from MeOH–H_2O, mp 84–86°.

Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06. Found: C, 76.52; H, 10.27.

12α-Cholanol (12).—Reduction of methyl 12α-hydroxycholanate (8) with lithium aluminum hydride gave 12α,24-cholanediol, different preparations melting variously at 113–119, 119–124, 126–128, and 129–131°, but with similar ir spectra (lit.[21] mp 113–115°); λ_{max}^{Nujol} 2.91, 9.45, 9.68, 9.82 μ. Selective mesylation with methanesulfonyl chloride in pyridine gave 12α-hydroxycholan-24-yl mesylate: mp 89–95° (lit.[22] mp 97.4–98.6°); λ_{max}^{Nujol} 2.73, 8.55, 10.60, 10.90 μ. Reduction of the monomesylate with lithium aluminum hydride, under the conditions used for the reduction of 3α-hydroxycholan-24-yl tosylate to 3α-cholanol,[23] and chromatography on alumina gave 12α-cholanol (7), mp 102–103° (lit.[24] mp 100.9–103.3°).

The **acetate** crystallized out of MeOH–H_2O, mp 63.5–64.0°.

Anal. Calcd for $C_{26}H_{44}O_2$: C, 80.35; H, 11.41. Found: C, 80.54; H, 11.46.

Acetylation of Methyl 7α,12α-Dihydroxycholanate (9).—A warm solution of 1.00 g of 9 in 5.6 ml of benzene and 0.64 ml of pyridine was cooled to room temperature, and acetic anhydride (0.64 ml) was added. After 24 hr the solution was poured into 35 ml of H_2O, the flask was rinsed with 10 ml of ether, and the combined organic layer was washed with three 15-ml portions of water and evaporated to dryness: 1.06 g of crude product, mp 108–132°. An identical product was obtained with 1.00 g of 9, 11.5 ml of benzene, 0.9 ml of pyridine, and 0.9 ml of acetic anhydride. Crystallization of 2.12 g of the crude product from benzene–petroleum ether (bp 30–60°) gave a mixture (1.20 g, mp 124–142°) which was chromatographed on alumina. Benzene–ether 33:1 eluted 0.47 g of methyl 7α-acetoxy-12α-hydroxycholanate (15), and ether–methanol 1:1 eluted unreacted 9. Recrystallization of the first fraction from benzene–petroleum ether gave pure 15: mp 138–139°; λ_{max}^{Nujol} 2.80 (OH), 5.79, and 5.85 μ (C=O).

Anal. Calcd for $C_{27}H_{44}O_5$: C, 72.28; H, 9.88. Found: C, 72.04; H, 9.91.

It is likely that the mother liquor from the original crystallization contained additional product, for a small-scale acetylation (150 mg) of 9 in which the total crude steroid mixture was chromatographed as described above provided 93 mg (56%) of 15, 40 mg (27%) of 9, and no other products (thin layer chromatography), except that in one run a trace of fast moving component was observed, possibly diacetate.

Methyl 7α-Acetoxy-12-oxocholanate (16).—A solution of 252 mg of $Na_2Cr_2O_7 \cdot 2H_2O$ in 5.34 ml of glacial acetic acid was added to a solution of 377 mg of 15 in 5.34 ml of acetic acid.[25] After 20 min at room temperature, the solution was diluted with water to turbidity and refrigerated overnight. Filtering, washing with H_2O, and drying the solid product gave 352 mg of crude

(19) J. Barnett and T. Reichstein, *Helv. Chim. Acta*, **21**, 926 (1938).
(20) S. Kuwada and S. Morimoto, *Bull. Chem. Soc. Jap.*, **17**, 147 (1942).
(21) G. V. Rao and C. C. Price, *J. Org. Chem.*, **27**, 205 (1962).
(22) F. C. Chang, *J. Pharm. Sci.*, **53**, 1014 (1964).
(23) R. T. Blickenstaff and F. C. Chang, *J. Amer. Chem. Soc.*, **80**, 2726 (1958).
(24) R. T. Blickenstaff and F. C. Chang, *ibid.*, **81**, 2835 (1959).
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material that was satisfactory for the subsequent steps. Two crystallizations from acetone–H_2O gave the analytical sample of **16**: mp 172–173°; λ_{max}^{Nujol} 5.78 and 5.90 μ (C=O).

Anal. Calcd for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.81; H, 9.62.

Methyl 7α-acetoxy-12-oxocholanate was hydrolyzed by heating a mixture of 99 mg of **16**, 0.1 g of KOH, 0.3 ml of H_2O, 3 ml of acetone, and 5 ml of MeOH at reflux for 5 hr. The crude product was crystallized twice from methanol–water to give an analytical sample of **7α-acetoxy-12-oxocholanic acid**: mp 154–155°; λ_{max}^{Nujol} 2.8–2.9 (OH of carboxyl), 5.75 (ester and acid C=O), 5.87 (ketone C=O), 8.04, 9.80 μ.

Anal. Calcd for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32. Found: C, 71.95; H, 9.52.

Methyl 7α-acetoxy-12-oxocholanate gave a positive Cotton effect curve, $[\alpha]_{314\ m\mu}$ 790 (1.85%, dioxane). Methyl 3α,7α-diacetoxy-12-oxocholanate, mp 177–178.5° (lit.[6] mp 179–181°), prepared by dichromate oxidation of methyl cholate 3,7-diacetate, also gave a positive Cotton effect curve, $[\alpha]_{306\ m\mu}$ 785 (1.68%, dioxane).

Reduction of Methyl 7α-Acetoxy-12-oxocholanate.—A mixture of 304 mg of **16**, 3.34 ml of diethylene glycol, and 1.61 ml of 100% hydrazine hydrate was refluxed 1.5 hr, then heated to 240° with the condenser removed. Potassium hydroxide (0.6 g) was added and, after more H_2O had boiled off, the condenser was replaced and the mixture refluxed 4.5 hr. It was then cooled, diluted with H_2O, and acidified with HCl to pH 2. The resulting white precipitate was filtered, washed with H_2O, and dried over P_2O_5, yield 287 mg. The crude hydroxy acid was esterified by treatment of its methanolic solution with ethereal CH_2N_2. The crude product, an oil, was chromatographed twice on alumina and the fractions eluted by ether and by methanol were crystallized twice from acetone–H_2O to give methyl 7α-hydroxycholanate (**6**), mp 74–76.5°, ir same as that derived from methyl 7α-acetoxy-3,12-dioxocholanate.

Methyl cholate 3-acetate (**17**) is the sample described in ref 4; methyl lithocholate (**5**) was prepared from commercial lithocholic acid and methanolic HCl.

Methyl 3α-Acetoxy-7α-hydroxycholanate (**18**).—Chenodeoxycholic acid was converted into the methyl ester with diazomethane, but the product failed to crystallize even after chromatography. As it showed only one spot on tlc, it was acetylated as the oil by the method for preparation of methyl cholate 3-acetate.[26] The crude product crystallized with difficulty out of acetone–petroleum ether (bp 30–60°) to give fine needles of **18**: mp 57–58°; λ_{max}^{Nujol} 2.77, 5.72, 8.1, 8.60, 8.80, 9.28, 9.75, 10.20 μ.

(26) R. Grand and T. Reichstein, *Helv. Chim. Acta*, **28**, 344 (1945).

Anal. Calcd for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.31; H, 10.00.

Acetylation Procedure.—These conditions are similar to those under which methyl cholate is converted into the 3,7-diacetate. A solution of the steroid (0.37 mmol), acetic anhydride (0.10 ml), and dry (KOH) pyridine (0.10 ml) in 0.84 ml of benzene was kept at room temperature (25°) for 24 hr. Ether (3.2 ml) was used to transfer the reaction mixture to a separatory funnel containing 2.5 ml of water. The ethereal layer was washed with three portions (2.5 ml) of water and evaporated *in vacuo* to dryness. The residue was chromatographed on 30 times its weight of alumina in an 8-mm column, eluted by appropriate combinations of petroleum ether, benzene, ether, and ethanol, separations being followed by tlc. Plates were developed in 3–7% methanol in benzene, then sprayed with 50% H_2SO_4 and heated. In general, compounds lacking free hydroxyl groups (acetates of monohydroxy steroids) were eluted from the column with petroleum ether (bp 30–60°)–benzene mixtures, monohydroxy steroids with benzene–ether mixtures, and dihydroxy steroids with ether–ethanol mixtures. (In a few instances of incomplete separation of ROAc and ROH, the mixture was further separated on thin layer silica gel plates.) Appropriate cuts from the column were combined into acetate and unreacted steroid fractions, weighed, and identified by ir. The yields, based on weights of the acetate fractions, are given in Table I; 84–94% of the starting steroid was accounted for.

Registry No.—**1**, 1448-36-8; **6** (acetate derivative), 19684-60-7; **7**, 6618-40-2; **7** (acetate derivative), 19684-62-9; **11**, 19684-63-0; **11** (acetate derivative), 19684-64-1; **12** (acetate derivative), 19684-29-8; **15**, 19684-66-3; **16**, 19684-67-4; **18**, 19684-68-5; 3α-tosyloxy-5β-pregnan-12α-ol-20-one, 19684-40-3; 5β-pregna-3-en-12α-ol-20-one, 19684-69-6; 12α-hydroxycholan-24-yl mesylate, 1253-86-7; 7α-acetoxy-12-oxocholanic acid, 19684-30-1.

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