Intramolecular Catalysis. VI. Selectivity in 7α,12α-Dihydroxy Steroids and Enhancement of 12α-Hydroxyl Reactivity by Substituents at Carbon 3[1]

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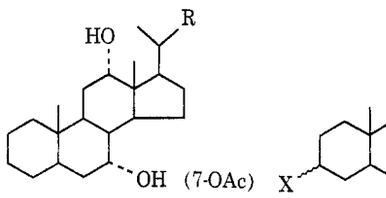
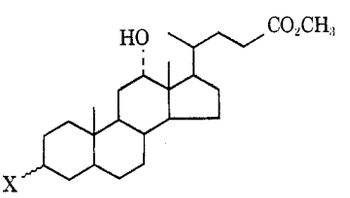
A series of 7α,12α-dihydroxy steroids (**1a, 1b, 1e, 1f, 1g, 1h**) was synthesized and compared regarding their reactivity with acetic anhydride and pyridine. All were acetylated selectively at the 7-hydroxyl and in comparable yields, indicating that the type of terminus of the side chain is immaterial with respect to preferential acetylation of the 7-hydroxyl. A series of 3-substituted 12α-hydroxy steroids was synthesized and similarly compared. Several 3 substituents enhance 12α-hydroxyl reactivity, notably oxo, chloro, and tosyloxy.

The preferential acetylation of methyl 3α-acetoxy-7α,12α-dihydroxy-5β-cholanate to the 3,7-diacetate in spite of the inherently greater reactivity of the 12-hydroxyl has been partially explained in terms of deactivation of the 12-hydroxyl by the side chain and activation of the 7-hydroxyl by both the 3α-acetoxy group and the 12-hydroxyl group.[2] A comparable explanation (except for reference to the 3-OAc group) would apply to the selective acetylation of methyl 7α,12α-dihydroxy-5β-cholanate (**1a**).[2] Without detailed knowledge of the mechanisms of these effects, it seemed possible that with other side chains the selectivity observed with **1a** might disappear or be reversed.



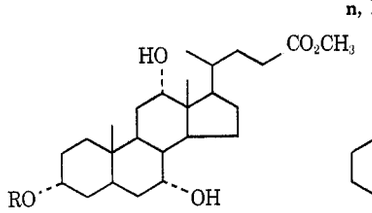
R

1a, $CH_2CH_2CO_2CH_3$ (**2a**)
b, $CH_2CH_2C(CH_3)_2OH$ (**2b**)
c, $CH_2CH_2CH_2OH$
d, $CH_2CH_2CH_2O_3SCH_3$
e, $CH_2CH_2CH_2Cl$ (**2e**)
f, $CH_2CH_2CH_2N(C_2H_5)_2$ (**2f**)
g, $CH_2CH_2CH_3$ (**2g**)
h, $CH_2CH_2CO_2H$ (**2h**)



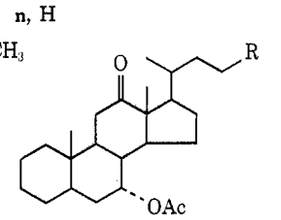
X

5a, O
b, $(CH_3)_2NN$
c, α-$(CH_3)_2N$
d, α-HO
e, α-AcO
f, α-BzO
g, α-CH_3OCO_2
h, α-N_3CO_2
i, α-TsO
j, β-N_3
k, β-NH_2
l, β-$(CH_3)_2N$
m, β-Cl
n, H



R

3a. H
b. CH_3SO_2



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4a. $C(CH_3)_2OH$
b. CH_2Cl
c. CH_3

We have synthesized a series of 7α,12α-dihydroxy steroids which differ in the structure of the side chain, and examined their acetylation behavior in order to determine (a) if the 7α-hydroxyl is preferentially acetylated, and (b) if the yield of 7-acetate is influenced appreciably by the side chain.

24,24-Dimethyl-5β-cholane-7α,12α,24-triol (**1b**, Table I) was synthesized by a Grignard reaction on methyl 7α,12α-dihydroxy-5β-cholanate (**1a**).[2] Reduction of **1a** with lithium aluminum hydride[3] gave 5β-cholane-7α,12α,24-triol (**1c**), which was also prepared in a more direct fashion from methyl cholate (**3a**) by selective mesylation (methanesulfonyl chloride and triethylamine in tetrahydrofuran at 0°) and, without isolating the intermediate 3-monomesylate (**3b**), reduction with lithium aluminum hydride. The triol **1c** was selectively mesylated to give 24-mesyloxy-5β-cholane-7α,12α-diol (**1d**), the intermediate for the synthesis of three more compounds in the series. The 24-mesylate **1d** reacted with pyridinium chloride in pyridine to give 24-chloro-5β-cholane-7α,12α-diol (**1e**), and with diethylamine to give 24-diethylamino-5β-cholane-7α,12α-diol (**1f**). Reduction of the mesylate **1d** with lithium aluminum hydride gave 5β-cholane-7α,12α-diol (**1g**).

As some of these compounds were only slightly soluble in the benzene medium used previously for acetylation comparisons,[2] the acetylations were carried out in pyridine (24 hr at 25°). The yields, based on weight of product isolated by column chromatography, are given in Table II. Three of the monoacetates were shown to be 7-acetates by oxidation to the corresponding 12-ketones **4**, which exhibited positive Cotton effect curves (the acid **2h** was converted to the methyl ester **2a**, identical with that previously described[2]).

The compounds in this series were chosen to include a range of electron-withdrawing groups [CO_2CH_3, Cl, and $N(C_2H_5)_2$] and electron-releasing groups (CH_3 and CO_2^-). The significant finding is that all compounds in the series acetylate selectively at the 7-hydroxyl to a nearly equal extent. Thus, deactivation of 12α-hydroxyl reactivity by the side chain is most likely a steric phenomenon, the exact nature of which is unlikely to depend on any particular kind of association between the terminal group and the hydroxyl. Deactivation has been discussed in terms of shielding,[4] which was not defined. Shielding by these types of

(1) Intramolecular Catalysis. (a) III: R. T. Blickenstaff, K. Atkinson, D. Breaux, E. Foster, Y. Kim, and G. C. Wolf, *J. Org. Chem.*, **36**, 1271 (1971). (b) IV: A. Sattar and R. T. Blickenstaff, *Steroids*, **17**, 357 (1971). (c) V: R. T. Blickenstaff and K. Sophasan, *Tetrahedron*, **28**, 1945 (1972). (d) Unpublished results from this laboratory.

(2) R. T. Blickenstaff and B. Orwig, *J. Org. Chem.*, **34**, 1377 (1969).

(3) R. T. Blickenstaff and F. C. Chang, *J. Amer. Chem. Soc.*, **80**, 2726 (1958).

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

TABLE I

12α-HYDROXY AND 7α,12α-DIHYDROXY STEROIDS[f]

No.	Compd	Preparation	Yield, %	Mp, °C	Ir, μ
1b	24,24-Dimethyl-5β-cholane-7α,12α,24-triol	Grignard reaction with CH_3I and 1a	66	153–154[a]	2.90–3.00
2b	7-Acetate of 1b			158–160[b]	2.78, 5.82
1d	24-Mesyloxy-5β-cholane-7α,12α-diol	CH_3SO_3Cl, 1c and $(C_2H_5)_3N$ in THF	54	169–170[b]	2.95–3.05, 7.42, 8.52
1e	24-Chloro-5β-cholane-7α,12α-diol	1d and pyridinium chloride in pyridine	48	173–175[b]	2.9–3.0
2e	7-Acetate of 1e			131–133[b]	2.77, 5.82
1f	24-Diethylamino-5β-cholane-7α,12α-diol	1d in refluxing $(C_2H_5)_2NH$ for 24 hr	81	149.5–150[b]	2.9–3.0
1g	5β-Cholane-7α,12α-diol	Reduction of 1d with $LiAlH_4$ in THF	78	204–205[b]	2.88–2.98
2g	7-Acetate of 1g			134–135[b]	2.77, 5.85
1h	7α,12α-Dihydroxy-5β-cholanic acid	Hydrolysis of 1a		206–207[b]	(lit.[g] 206)
4a	7α-Acetoxy-24,24-dimethyl-24-hydroxy-5β-cholan-12-one	Oxidation of 2b with $Na_2Cr_2O_7$ in AcOH	93	156–157[b]	2.95, 5.78, 5.88
4b	7α-Acetoxy-24-chloro-5β-cholan-12-one	Oxidation of 2e with $Na_2Cr_2O_7$ in AcOH	54	200–201[b]	5.78, 5.89
4c	7α-Acetoxy-5β-cholan-12-one	Oxidation of 2d with $Na_2Cr_2O_7$ in AcOH	65	183–184[b]	5.76, 5.89
5a	Methyl 12α-hydroxy-3-oxo-5β-cholanate	Oppenauer oxidation of methyl deoxycholate		138–140[b]	(lit.[h] 140–142)
5b	Methyl 3-(*N*,*N*-dimethylhydrazino)-12α-hydroxy-5β-cholanate	5a and $(CH_3)_2NNH_2$ in EtOH and pyridine	69.3	164–164.5[c]	3.08, 5.82, 6.18
5c	Methyl 3α-dimethylamino-12α-hydroxy-5β-cholanate	5a in refluxing DMF and HCO_2H	84.4	138–141[d]	2.9–3.05, 5.8
5f	Methyl3α-benzoyloxy-12α-hydroxy-5β-cholanate	Benzoyl chloride in pyridine		102–102.5	(lit.[i] 90–95)
5g	Methyl 3α-carbomethoxyoxy-12α-hydroxy-5β-cholanate	Methyl chloroformate in benzene and pyridine	58	180–182[b]	
5i	Methyl 3α-tosyloxy-12α-hydroxy-5β-cholanate	Tosyl chloride in pyridine		147	(lit.[i] 149–150)
5j	Methyl 3β-azido-12α-hydroxy-5β-cholanate	5j and NaN_3 in DMSO	67.4	124[b]	2.8, 4.77, 5.8
5k	Methyl 3β-amino-12α-hydroxy-5β-cholanate	Reduction of 5k with H_2 and Raney Ni	52	150–152[e]	3.17, 6.32
5m	Methyl 3β-chloro-12α-hydroxy-5β-cholanate	5j and pyridinium chloride	66	135	(lit.[i] 129–130)

[a] Analytical samples were recrystallized from toluene–hexane. [b] From methanol–H_2O. [c] From acetone. [d] From acetone–H_2O. [e] From benzene–petroleum ether. [f] Satisfactory analytical data (±0.3% for C, H, N, S, Cl) were reported for all new compounds listed in the table. [g] S. Kuwada and S. Morimoto, *Bull. Chem. Soc. Jap.*, **17**, 147 (1942). [h] A. S. Jones, *et al.*, *J. Chem. Soc.*, 2164 (1949); C. Djerassi, *Bull. Soc. Chim. Fr.*, 741 (1957); V. Burckhardt, *Helv. Chim. Acta*, **25**, 821 (1942). [i] B. F. MacKenzie, *J. Biol. Chem.*, **162**, 555 (1946). [j] F. C. Chang, *et al.*, *J. Amer. Chem. Soc.*, **79**, 2164 (1957).

TABLE II

ACETYLATION OF HYDROXY STEROIDS WITH ACETIC ANHYDRIDE AND PYRIDINE

Compd no.	Name	Yield of 7-acetate,[a] %
1a	Methyl 7α,12α-dihydroxy-5β-cholanate	66–73
1b	24,24-Dimethyl-5β-cholane-7α,12α,24-triol	68
1e	24-Chloro-5β-cholane-7α,12α-diol	64–69
1f	24-Diethylamino-5β-cholane-7α,12α-diol	61[b]
1g	5β-Cholane-7α,12α-diol	66–73
1h	7α,12-Dihydroxy-5β-cholanic acid	60–63

[a] Steroid (0.50 mmol) and Ac_2O (1.44 mmol) in pyridine (2.0 ml total volume), room temperature, 24 hr, yield determined by column chromatography. [b] Yield determined by glc (see Experimental Section).

side chains that are branched at C-20 recently has been ascribed to the steric effect of the C-21 methyl group,[1d] a finding consistent with the results in this communication.

Previous work has shown that the 12α-hydroxyl, though deactivated by the bile acid side chain, can be enhanced in reactivity by a 3α substituent. Thus, methyl deoxycholate 3-acetate (**5e**) gave a higher yield on acetylation of the 12-hydroxyl than methyl 12α-hydroxy-5β-cholanate (**5n**).[2] Similarly, 3α-acetoxy and 3α-tosyloxy groups were shown to enhance the yield of 12α-hydroxyl acetylation in a 5β-pregnan-20-one series.[1a] In order to determine what other substituents at C-3 might influence the 12α-hydroxyl, the series **5a–n** has now been synthesized and acetylated.

Methyl 12α-hydroxy-3-oxo-5β-cholanate (**5a**) was treated with 1,1-dimethylhydrazine to give the corresponding hydrazone **5b**, and with formic acid in DMF (Lekart reaction[5]) to give methyl 3α-dimethylamino-12α-hydroxy-5β-cholanate (**5c**). The 3α configuration for **5c** was shown by its comparison with the 3β epimer **5l**, to be described later. The acetate (**5e**), benzoate (**5f**), and tosylate (**5i**) were prepared by standard methods, as was the carbomethoxyoxy derivative (**5g**). The action of phosgene on methyl deoxycholate (**5d**) gave a chloroformate intermediate that reacted with sodium azide to give methyl 3α-azidoformoxy-12α-hydroxy-5β-cholanate (**5h**). The action of sodium azide on the tosylate (**5i**) gave methyl 3β-azido-12α-hydroxy-5β-cholanate (**5j**), reduction of which gave the corresponding amine **5k**. Reductive alkylation of **5k** with formaldehyde and hydrogen gave methyl 3β-dimethylamino-12α-hydroxy-5β-cholanate (**5l**). It did not crystallize, but was shown to differ from the isomer **5c** by ir and melting point compar-

(5) R. R. Savers, *J. Amer. Chem. Soc.*, **80**, 4721 (1958).

isons of their respective hydrochlorides (see Experimental Section). Methyl 3β-chloro-12α-hydroxy-5β-cholanate (**5m**) was prepared from the tosylate (**5i**) and pyridinium chloride.[6]

The compounds were acetylated as described previously,[2] except that in many cases it was more convenient to analyze the reaction mixture by thin layer chromatography (tlc) or by gas chromatography (glpc), rather than by miniature column chromatography (see Experimental Section for details). The results, shown in Table III, indicate that tlc and glpc gave

TABLE III

ACETYLATION OF 3-SUBSTITUTED METHYL 12α-HYDROXYCHOLANATES WITH ACETIC ANHYDRIDE AND PYRIDINE IN BENZENE

Compd no.	Name	Yield of acetate formed in 24 hr, % Column[a]	tlc[a]	glc[a]
5a	Methyl 12α-hydroxy-3-oxo-5β-cholanate	19		
5b	Methyl 3-dimethylhydrazino-12α-hydroxy-5β-cholanate		11	11–12
5c	Methyl 3α-dimethylamino-12α-hydroxy-5β-cholanate			15–16
5e	Methyl 3α-acetoxy-12α-hydroxy-5β-cholanate	11–13	16	15
5f	Methyl 3α-benzoyloxy-12α-hydroxy-5β-cholanate		16–17	
5g	Methyl 3α-carbomethoxyoxy-12α-hydroxy-5β-cholanate		16–20	
5h	Methyl 3α-azidoformoxy-12α-hydroxy-5β-cholanate		11–13	
5i	Methyl 12α-hydroxy-3α-tosyloxy-5β-cholanate		26–29	
5j	Methyl 3β-azido-12α-hydroxy-5β-cholanate		13	
5l	Methyl 3β-dimethylamino-12α-hydroxy-5β-cholanate			13
5m	Methyl 3β-chloro-12α-hydroxy-5β-cholanate	16		
5n	Methyl 12α-hydroxy-5β-cholanate	5–8	11	10

[a] Values labeled "Column" represent yield of product isolated by column chromatography.[2] Values labeled tlc and glc were obtained by thin layer chromatography and gas–liquid chromatography, respectively, as described in the Experimental Section. All compounds except **5a**, **5c**, **5l**, and **5n** were run in duplicate or triplicate.

comparable values, while column chromatography gave slightly lower values. The yields for the dimethylhydrazino (**5b**) and α-azidoformoxy (**5h**) derivatives, while numerically greater than that of the unsubstituted compound **5n**, are not significantly different. The α-dimethylamino (**5c**) and β-dimethylamino (**5l**), as well as the β-azido (**5j**) derivatives, are borderline, while the α-acetoxy group (**5e**) is confirmed as weakly enhancing. The strongest groups that enhance 12-hydroxyl acetylation are α-benzoyloxy (**5f**), α-carbomethoxyoxy (**5g**), β-chloro (**5m**), oxo (**5a**), and α-tosyloxy (**5e**). We are currently examining the effects of some of these groups on the rate of acetylation of the 7α-hydroxyl group.

(6) F. C. Chang, *et al.*, *J. Amer. Chem. Soc.*, **79**, 2164 (1957).

Experimental Section[7]

5β-Cholane-7α,12α,24-triol (**1c**).—This compound was prepared by two different routes. In the first, methyl 7α,12α-dihydroxy-5β-cholanate (**1a**, 12.198 g, 0.030 mol) was dissolved in 300 ml of THF (dried over molecular sieves). This solution was then slowly added to $LiAlH_4$ (4.554 g, 0.120 mol) suspended in 400 ml of THF. Then the final solution was refluxed for 16 hr and hydrolyzed with 5 *N* NaOH. When the excess $LiAlH_4$ was destroyed, the mixture was acidified with concentrated HCl. The solution was filtered into 2500 ml of water, whereupon the product readily precipitated. This solid was collected and recrystallized from methanol–water to give 10.039 g (89%) of the expected triol, mp 197–198°.

Although the above procedure worked quite well to give a good yield of reduced product, the starting ester could be prepared much less readily. In our hands the removal of the 3α-hydroxy group from methyl cholate was achieved in only a 30% yield (*via* Oppenauer oxidation and Wolff–Kishner reduction) and required an appreciable amount of time. In an effort to circumvent these difficulties an alternate approach to **2a** was sought. To this end, methyl cholate (13.640 g, 0.030 mol) was dissolved in 100 ml of dry THF and to this was added triethylamine (3.137 g, 0.031 mol). This homogeneous solution was cooled to 0° and subsequently methanesulfonyl chloride (3.551 g, 0.031 mol) in 50 ml of THF was added dropwise over a 45-min period. The mixture was left to warm to room temperature for 1 hr while a second solution containing $LiAlH_4$ (11.386 g, 0.300 mol) in 150 ml of THF at 0° was prepared. The first solution was then filtered directly into the second over 1 hr. The final mixture was allowed to stir overnight at 45–50°. The excess hydride was hydrolyzed with water and the mixture was acidified with HCl. It was then poured into 1500 ml of water and extracted with two 500-ml portions of $CHCl_3$. Combining the organic extracts, drying, and evaporating the solvent left a solid residue, which was recrystallized from methanol–water to give 7.809 g (69%) of white needles, mp 194–196°, ir 2.98 μ.

Anal. Calcd for $C_{24}H_{42}O_3$: C, 76.14; H, 11.18. Found: C, 75.99; H, 11.23.

Acetylation Procedure.—The acetylations of the 7,12-diol series (**1**) were carried out using 2.0 ml of a pyridine solution which was 0.25 *M* in steroid and 0.72 *M* in acetic anhydride. This solution was kept at 25° for 24 hr and then transferred to a separatory funnel with *ca.* 10 ml of ether. Subsequently it was washed three times with 10-ml portions of water [in the case of 7α,12α-dihydroxycholanic acid (**1h**), the organic solution was first washed with enough 5 *N* HCl to ensure that the free acid was present and not the carboxylate anion; the reaction mixture of **1f** was analogously washed with 5 *N* NaOH] and the organic layer was separated, dried briefly over Na_2SO_4, and filtered. The solvent was allowed to evaporate and the residue was chromatographed on 20 g of 30–60 mesh Florisil using 0.5–3% methanol in benzene as the eluent. The progress of the separations was followed by tlc (silica gel G developed in 3–10% methanol in benzene and subsequently sprayed with 50% H_2SO_4 and heated). In most instances separation was quite good and appropriate fractions (20 ml) were combined, the solvent was removed, and the weight of residue was recorded; both product and starting material were isolated. The acetates were identified (in the cases where they were unknown) by synthesizing them on a larger scale, recording their ir spectra, and obtaining analyses. A few per cent of a second product (possibly 7,12-diacetate) was usually observed; total recovery was 95% or better.

In the acetylation of the amine **1f**, all attempts to separate the product acetate from the starting material *via* column chromatography were unsuccessful. Consequently, a glpc analysis of the reaction mixture was carried out using a 5-ft column packed with OV 17 on Chromosorb G and at a column temperature of 273°. Three components were noted with retention times of 14.7, 16.5, and 18.2 min in a ratio of 2:61:37. The retention time of the last peak was exactly the same as that of the starting material (**1f**); the second peak was ascribed to the 7α-acetate; the first was probably due to either the 12α-acetate or the 7α,12α-diacetate.

(7) Compounds not listed in Table I are described in this section. Melting points were taken on a Unimelt apparatus and are uncorrected; the ir spectra were recorded on a Perkin-Elmer Infracord as Nujol mulls. The ORD and CD spectra were determined on a Cary Model 60 spectropolarimeter at Eli Lilly and Co., Indianapolis, Ind. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

The same problem of separation arose in the acetylation of **1h**; in this case, the crude residue was dissolved in 30 ml of methanol and 1 ml of concentrated HCl and refluxed for 2 hr to esterify the acid side chain. [We have found 5β-cholane-7α,12α-diol 7-acetate (**2g**) to be completely stable to these conditions.] The solvent was then removed and the methyl esters were separated on Florisil.

Methyl 3α-Azidoformoxy-12α-hydroxy-5β-cholanate (5h).—Methyl deoxycholate in cold toluene was treated with phosgene for 3 hr to give methyl 3α-chloroformoxy-12α-hydroxy-5β-cholanate. The solvent was evaporated under a stream of hot air and the residue was recrystallized from acetone–petroleum ether (bp 30–60°), mp 135°. Methyl 3α-chloroformoxy-12α-hydroxy-5β-cholanate (900 mg) and 1.8 g of sodium azide were dissolved in DMSO and heated at 75° for 3 hr. After standing at room temperature overnight, the mixture was diluted with water and extracted with chloroform to give 990 mg of crude material. This material was chromatographed on 22 g of Florisil; the product (751 mg) was eluted by 4:1 benzene–ether. Recrystallization from acetone–water gave 238 mg, mp 164–165° (analytical sample), and 400 mg, mp 161–162°, ir 4.58, 4.70 (N_3), 5.77, 5.84 μ (C=O).

Anal. Calcd for $C_{26}H_{41}N_3O_5$: C, 65.66; H, 8.69; N, 8.83. Found: C, 65.67; H, 8.64; N, 8.70.

Methyl 3β-Dimethylamino-12α-hydroxy-5β-cholanate (5l).—Methyl 3β-amino-12α-hydroxy-5β-cholanate (**5k, 1g**) was dissolved in 45 ml of methanol and 25 ml of 37% CH_2O, 205 mg of 5% Pd/C was added, and the mixture was hydrogenated under a hydrogen pressure of 36 psi for 3 days. The mixture was then filtered and the solvent was removed under reduced pressure. The residue was taken up in chloroform, the chloroform solution was washed with water and dried over sodium sulfate and the solvent was evaporated to give 946 mg of crude product. This material was dissolved in acetone and cooled. The acetone solution was then saturated with HCl followed by the addition of ether to give 570 mg of solid material, mp 254–256°. Approximately 70 mg of this material was treated with aqueous methanol–sodium carbonate to give the free amine, which we were unable to crystallize. The amine was purified by means of thin layer chromatography and isolated as an oil.

Anal. Calcd for $C_{27}H_{47}NO_3$: C, 74.78; H, 10.92; N, 3.23. Found: C, 74.72; H, 11.89; N, 3.15.

The infrared spectrum of methyl 3β-dimethylamino-12α-hydroxy-5β-cholanate hydrochloride showed absorptions at 3.78 and 4.07 (tertiary $^+$NH) and 5.82 μ (C=O) [lit.[8] 3.88 and 4.1 μ (tertiary $^+$NH)]. The α epimer showed absorptions at 3.74 and 3.97 (tertiary $^+$NH) and 5.76 μ (C=O) [lit.[11] 3.7 and 4.02 μ (tertiary $^+$NH)]. The hydrochloride salts of methyl 3β-dimethylamino-12α-hydroxy-5β-cholanate and its 3α epimer were prepared by treating a solution of the free amines in acetone with HCl (g) and precipitating the salts by addition of ether. The hydrochloride of the α derivative had a melting point of 252–253° and the β-epimer, mp 253–255°, mmp 234–238°.

Acetylation and Yield Determination By Thin Layer Chromatography and Gas Chromatography.—The steroid (0.37 mmol) was dissolved in 0.1 ml of pyridine, some benzene was added, and 0.1 ml of acetic anhydride was added, followed by enough benzene to make the total volume 1 ml. The reactions were carried out at room temperature (25 ± 1°) over a period of 24 hr and quenched with methanol to stop the reaction. The solvent was allowed to evaporate and 15–35 mg of the steroidal material was streaked on a silica gel G thin layer plate and developed in a suitable solvent system. Various solvent mixtures of benzene–ether and benzene–methanol were used. Thin layer chromatography plates were sprayed at one end with sulfuric acid to locate the bands. Once the bands were located, the two fractions were recovered separately and weighed.

From reaction mixtures that had evaporated to dryness, approximately 4 mg of the residue was redissolved in 1 ml or more of acetone or chloroform for chromatography in a Micro Tek 220 equipped with a flame ionization detector and a Disc integrator. Two to three separate injections of 1 μl each were averaged. Samples to be analyzed were chromatographed on either a 6-ft 1% OV-17 on Chrom G column (methyl 3α-acetoxy-12α-hydroxy-5β-cholanate, methyl 3-dimethylhydrazino-12α-hydroxy-5β-cholanate, methyl 3α- and 3β-dimethylamino-12α-hydroxy-5β-cholanate) or a 4-ft 3% polysulfone on Chrom Q column (methyl 12α-hydroxy-5β-cholanate). Column temperature ranged from 275 to 290° and a carrier gas (N_2) flow rate of 55 ml/min was used.

Registry No.—**1a,** 3701-54-0; **1b,** 38379-63-4; **1c,** 32624-95-6; **1d,** 38431-60-6; **1e,** 38431-61-7; **1f,** 38431-62-8; **1g,** 17041-50-8; **2b,** 38431-63-9; **2d,** 38379-65-6; **2e,** 38379-66-7; **2g,** 38379-67-8; **4a,** 38379-68-9; **4b,** 38379-69-0; **4c,** 38379-70-3; **5a,** 10538-58-6; **5b,** 38379-72-5; **5c,** 38379-73-6; **5c** HCl, 38379-74-7; **5g,** 38359-35-2; **5h,** 38359-36-3; **5j,** 38359-37-4; **5k,** 38359-38-5; **5l,** 38359-39-6; **5l** HCl, 38359-40-9; methyl 3α-chloroformoxy-12α-hydroxy-5β-cholanate, 38359-41-0.

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