Intramolecular Catalysis. VIII. Effects on the Acetylation of the 7α-Hydroxyl Group of Steroids. A ^{1}H Nuclear Magnetic Resonance Rate Method[1]

James F. Baker and R. T. Blickenstaff*

Veterans Administration Hospital and Biochemistry Department, Indiana University School of Medicine, Indianapolis, Indiana 46202

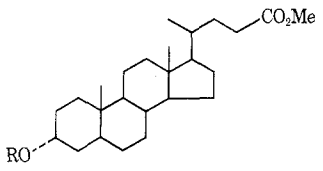
Received January 31, 1975

Analogs of methyl 7α-hydroxycholanoate were synthesized, and acetylation rates were determined by a new ^{1}H NMR method. Substituents at C-3 include α-acetoxy, α-tosyloxy, α-mesyloxy, α-succinoyloxy, α-p-nitrobenzoyloxy, α-dimethylamino, β-chloro, oxo, ethylenedioxy, and ethylenedithio. Substituents at C-12 include α-hydroxy and α-methoxy. The 3 esters were prepared by selective esterifications, the 3α-dimethylamino derivative by the Leuckart reaction on the corresponding 3-oxo compound, and the 12α-methoxy derivative by the action of methyl fluorosulfonate on methyl cholate 3,7-diacetate. Acetylations were carried out in NMR sample tubes, and rates were determined by measuring the relative intensities of angular methyl peaks, which shifted slightly on acetylation of the particular steroid. Most of the groups at C-3 afforded slight catalysis of 7α-hydroxyl acetylation. The 12α-hydroxyl was most effective, and the absence of catalysis by a 12α-methoxyl group points toward protonation in the rate-determining step.

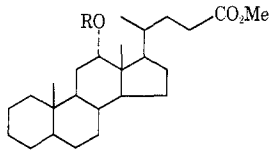
The 7α-hydroxyl group of methyl cholate 3-acetate (**13a**) was shown to be more reactive toward acetic anhydride than that of methyl 7α-hydroxy-5β-cholan-24-oate (**3a**) by comparing their 24-hr yields.[2] This was explained in terms of enhancement of 7α-hydroxyl reactivity by both the 3α-acetoxy group and the 12α-hydroxyl group, and these effects were shown to be intramolecular.[3] Reactivity of the 7α-hydroxyl was found not to be influenced by the type of side chain attached at C-17.[4] 3-Oxo, 3-chloro, and 3-tosyloxy groups were shown to enhance 12α-hydroxyl reactivity,[4] but the influence of these groups on the 7α-hydroxyl has not been studied. Methods for the determination of acetylation rates of these hydroxy steroids have been developed utilizing GLC,[3] uv,[3] ^{14}C,[5] and optical rotation.[6] In the present work a rate method employing ^{1}H nuclear magnetic resonance is applied to the study of the effects of substituents at C-3 and at C-12 on 7α-hydroxyl reactivity.

^{1}H nuclear magnetic resonance has been employed in the kinetic study of the esterification of methanol with acetic anhydride; measurements were based on the intensity of the singlet arising from the methoxyl group of the methyl acetate produced.[7] In the present method, we employed singlets arising from the angular methyl groups, whose chemical shifts are known to be influenced profoundly by substituents on the steroid ring system.[8] In particular, the 3α-acetoxy group exerts a 0.025-ppm downfield shift on the C-19 methyl resonance of 5β steroids in deuteriochloroform, while the 3α-hydroxyl exerts only a 0.008-ppm downfield shift.[8] In theory it should be possible to observe the progress of acetylation of a 3α-hydroxyl group by noting the gradual disappearance of the C-19 methyl resonance and the concomitant appearance of a new resonance about 0.02 ppm further downfield in such solutions. In practice, it was found possible to follow acetylations of the 3α-, 7α-, and 12α-hydroxyls by following shifts of either the C-18 or C-19 methyl resonances on samples dissolved in pyridine. Instead of a downfield shift, *upfield* shifts of 0.02–0.04 ppm of both methyl resonances were observed, the reversal most likely being related to complexing between steroid and solvent molecules.[9] While this is not a large separation, it is easily adequate in this case because of the sharpness of the angular methyl singlet resonances. Acetylations were carried out in the NMR sample tubes, and quantitation of the two methyl resonance peaks for either C-18 or C-19 methyl during acetylation permitted calculation of the second-order rate constants.

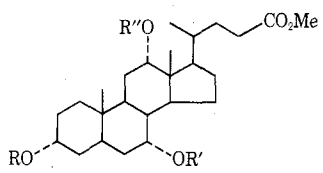
Most of the preparations of compounds for this study



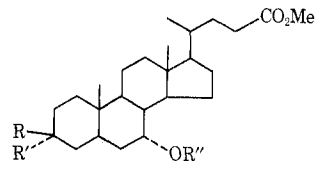
1a, R = H
b, R = Ac



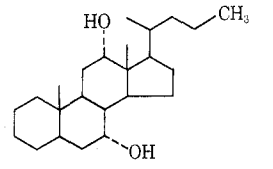
2a, R = H
b, R = Ac



	R	R′	R″
13a	Ac	H	H
13b	Ac	Ac	H
13c	Ac	Ac	Ac
13d	$O_2NC_6H_4CO_2$	H	H
14a	Ac	Ac	CH_3
14b	H	H	CH_3
14c	Ac	H	CH_3
15	O=	H	H
16	SCH_2CH_2S	H	H
17a	Ac	Ac	O=
17b	Ac	H	O=



	R	R′	R″
3a	H	H	H
3b	H	H	Ac
4a	H	AcO	H
4b	H	AcO	Ac
5a	O		H
6a	OCH_2CH_2O		H
6b	OCH_2CH_2O		Ac
7a	H	CH_3SO_3	H
7b	H	CH_3SO_3	Ac
8a	H	$C_7H_7SO_3$	H
8b	H	$C_7H_7SO_3$	Ac
9a	H	CH_2CO_2 CH_2CO_2H	H
9b	H	CH_2CO_2 CH_2CO_2H	Ac
10a	Cl	H	H
10b	Cl	H	Ac
11a	Me_2N	H	H
11b	Me_2N	H	CHO
12a	H	Me_2N	H
12b	H	Me_2N	Ac



18

were straightforward and are listed in Table I. Methyl 3α-dimethylamino-7α-hydroxy-5β-cholan-24-oate (**12a**) was prepared by the Leuckart reaction on the corresponding 3 ketone (**5a**),[22] and was separated from other minor products by chromatography on basic alumina followed by preparative TLC on silica gel. One minor component with a higher R_f (than **12a**) is tentatively presumed to be the 3 epimer (**11a**), as it exhibits a six-proton singlet at 2.27 ppm (dimethylamino), while **12a** exhibits the same at 2.38 ppm. This is completely consistent with the methyl dimethylamino-12α-hydroxy-5β-cholan-24-oates,[4] in which the faster moving 3β epimer has a 2.21- and the slower moving 3α epimer a 2.29-ppm resonance. The methyl cholate 3-*p*-nitro-

Table I
Synthesis of the 7α-Hydroxy Steroids[a]

Compd	Yield, %	Mp, °C (lit. value)	Ir absorption, selected bands, cm^{-1}	^1H NMR spectral assignments, δ
1a Methyl 3α-hydroxy-5β-cholan-24-oate	81	130–132[b] (lit.[10] 125–128)	3610 (OH), 1721 (C=O), 1106, 1010, 725	3.69 (s, 3 H, ester Me), 0.95 (s, 3 H, 19-Me), 0.64 (s, 3 H, 18-Me)
1b Methyl 3α-acetoxy-5β-cholan-24-oate[c]		135–136[b] (lit.[11] 133–135)	1736 (C=O), 1252 (acetate C–O), 1172, 1022	4.74 (broad m, 1 H, 3β-H), 3.69 (s 3 H, ester Me), 2.02 (s, 3 H, acetate Me), 0.94 (s, 3 H, 19-Me), 0.65 (s, 3 H, 18-Me)
2a Methyl 12α-hydroxy-5β-cholan-24-oate[d]		120.7–122.7[e] (lit.[12] 119–120)	3559 (OH), 1715 (C=O), 1205, 1174	4.15 (broad, 1 H, 12β-H), 3.62 (s, 3 H, ester Me), 0.92 (s, 3 H, 19-Me), 0.71 (s, 3 H, 18-Me)
2b Methyl 12α-acetoxy-5β-cholan-24-oate[f]		95–96[b] (lit.[10] 95–97)	1754 (C=O), 1247 (acetate C–O), 1168, 1031	5.25 (broad, 1 H, 12β-H), 3.65 (s, 3 H, ester Me), 2.14 (s, 3 H, acetate Me), 0.89 (s, 3 H, 19-Me), 0.66 (s, 3 H, 18-Me)
3a Methyl 7α-hydroxy-5β-cholan-24-oate[d]		80–81[b] (lit.[2] 75–79)	3584 (OH), 1724 (C=O) 1106	3.86 (broad, 1 H, 7β-H), 3.68 (s, 3 H, ester Me), 0.91 (s, 3 H, 19-Me), 0.65 (s, 3 H, 18-Me)
3b Methyl 7α-acetoxy-5β-cholan-24-oate		96–97[b] (lit.[2] 95–96)	1745 (C=O), 1242 (acetate C–O), 1157, 1017	4.86 (broad, 1 H, 7β-H), 3.67 (s, 3 H, ester Me), 2.03 (s, 3 H, acetate Me), 0.89 (s, 3 H, 19-Me), 0.64 (s, 3 H, 18-Me)
4a Methyl 3α-acetoxy-7α-hydroxy-5β-cholan-24-oate[g,h]	39	59–61[i] (lit.[13] 54–62)	3536 (OH), 1727 and 1715 (C=O), 1242 (acetate C–O), 1153, 971	4.56 (broad, 1 H, 3β-H), 3.86 (broad, 1 H, 7β-H), 3.68 (s, 3 H, ester Me), 2.00 (s, 3 H, acetate Me), 0.91 (s, 3 H, 19-Me), 0.66 (s, 3 H, 18-Me)
4b Methyl 3α,7α-diacetoxy-5β-cholan-24-oate[g]		131–132[j] (lit.[14] 128–130)	1730 (C=O), 1245 (acetate C–O), 1064, 1021, 969, 940	4.93 (broad, 1 H, 7β-H), 4.56 (broad, 1 H, 3β-H), 3.70 (s, 3 H, ester Me), 2.06 (s, 3 H, acetate Me), 2.03 (s, 3 H, acetate Me), 0.93 (s, 3 H, 19-Me), 0.67 (s, 3 H, 18-Me)
5a Methyl 7α-hydroxy-3-oxo-5β-cholan-24-oate[k]	41	122–124[b] (lit.[15] 128–129)	3521 (OH), 1739 (ester C=O), 1700 (keto	3.92 (broad, 1 H, 7β-H), 3.68 (s, 3 H, ester Me),

Table I
(Continued)

Compd	Yield, %	Mp, °C (lit. value)	Ir absorption, selected bands, cm^{-1}	^1H NMR spectral assignments, δ
			C=O), 1250, 1100	1.02 (s, 3 H, 19-Me), 0.71 (s, 3 H, 18-Me)
6a Methyl 3,3-ethylenedioxy-7α-hydroxy-5β-cholan-24-oate	42	114–116[b]	3636 (OH), 1736 (C=O), 1212, 1160, 1096	4.02 (broad s, 1 H, 7β-H), 3.88 (s, 4 H, ketal), 3.65 (s, 3 H, ester Me), 0.99 (s, 3 H, 19-Me), 0.66 (s, 3 H, 18-Me)
6b Methyl 7α-acetoxy 3,3-ethylenedioxy-5β-cholan-24-oate[c]		84–85[b]	1733 (C=O), 1238 (acetate C–O), 1220, 1099	5.05 (broad s, 1 H, 7β-H), 3.87 (s, 4 H, ketal), 3.64 (s, 3 H, ester Me), 2.00 (s, 3 H, acetate Me), 0.95 (s, 3 H, 19-Me), 0.60 (s, 3 H, 18-Me)
7a Methyl 7α-hydroxy-3α-methanesulfonoxy-5β-cholan-24-oate	23	133–134[f]	3545 (OH), 1721 (C=O), 1175 (SO_3), 920	4.51 (broad, 1 H, 3β-H), 3.84 (broad, 1 H, 7β-H), 3.69 (s, 3 H, ester Me), 3.01 (s, mesylate Me), 0.93 (s, 3 H, 19-Me), 0.67 (s, 3 H, 18-Me)
7b Methyl 7α-acetoxy-3α-methanesulfonoxy-5β-cholan-24-oate[c]		130–131[f]	1724 (C=O), 1238 (acetate C–O), 1223, 1167 (SO_3), 938	4.92 (broad, 1 H, 7β-H), 4.52 (broad, 1 H, 3β-H), 3.70 (s, 3 H, ester Me), 3.04 (s, 3 H, mesylate Me), 2.08 (s, 3 H, acetate Me), 0.96 (s, 3 H, 19-Me), 0.70 (s, 3 H, 18-Me)
8a Methyl 7α-hydroxy-3α-*p*-toluenesulfonoxy-5β-cholan-24-oate	76	129–131[e] (lit.[3] 128.5–129°)	3584 (OH), 1727 (C=O), 1170 (SO_3), 923, 868, 677	7.82 (d, 2 H, aromatic), 7.32 (d, 2 H, aromatic), 4.33 (broad, 1 H, 3β-H), 3.83 (broad, 1 H, 7β-H), 3.67 (s, 3 H, ester Me), 2.45 (s, 3 H, tosylate Me), 0.87 (s, 3 H, 19-Me), 0.64 (s, 3 H, 18-Me)
8b Methyl 7α-acetoxy-3α-*p*-toluenesulfonoxy-5β-cholan-24-oate		166–167[f]	1724 (C=O), 1227 (acetate C–O), 1174 (SO_3), 930	7.82 (d, 2 H, tosylate aromatic), 7.33 (d, 2 H, tosylate aromatic), 4.82 (broad, 1 H, 7β-H), 4.33 (broad, 1 H, 3β-H), 3.66 (s, 3 H, ester Me), 2.45 (s, 3 H, tosylate Me), 2.00 (s, 3 H,

Table I
(Continued)

Compd	Yield, %	Mp, °C (lit. value)	Ir absorption, selected bands, cm^{-1}	^1H NMR spectral assignments, δ
				acetate Me), 0.87 (s, 3 H, 19-Me), 0.63 (s, 3 H, 18-Me)
10a Methyl 3β-chloro-7α-hydroxy-5β-cholan-24-oate[m]	83	140–141[j]	3636 (OH), 1724 (C=O), 1105, 706	4.56 (broad, 1 H, 3α-H), 3.87 (broad, 1 H, 7β-H), 3.67 (s, 3 H, ester Me), 0.97 (s, 19-Me), 0.67 (s, 3 H, 18-Me)
10b Methyl 7α-acetoxy-3β-chloro-5β-cholan-24-oate[c]		150–152[j]	1721 (C=O), 1239 (acetate C–O), 1010, 700	4.90 (broad, 1 H, 7β-H), 4.56 (broad, 1 H, 3α-H), 3.66 (s, 3 H, ester Me), 2.02 (s, 3 H, acetate Me), 0.97 (s, 3 H, 19-Me), 0.60 (s, 3 H, 18-Me)
13a Methyl 3α-acetoxy-7α,12α-dihydroxy-5β-cholan-24-oate[g]		153–155[l] (lit.[16] 149–150)	1720 (C=O), 1640, 1240 (acetate C–O), 1160, 1010, 670	4.56 (broad, 1 H, 3β-H), 3.96 (broad, 1 H, 12β-H), 3.86 (broad, 1 H, 7β-H), 3.68 (s, 3 H, ester Me), 2.00 (s, 3 H, acetate Me), 0.90 (s, 3 H, 19-Me), 0.69 (s, 3 H, 18-Me)
13b Methyl 3α,7α-diacetoxy-12α-hydroxy-5β-cholan-24-oate[c]	67	188–190[b] (lit.[17] 187–188)		4.90 (broad, 1 H, 7β-H), 4.56 (broad, 1 H, 3β-H), 4.00 (broad, 1 H, 12β-H), 3.67 (s, 3 H, ester Me), 2.06 (s, 3 H, acetate Me), 2.02 (s, 3 H, acetate Me), 0.92 (s, 3 H, 19-Me), 0.68 (s, 3 H, 18-Me)
13c Methyl 3α,7α,12α-triacetoxy-5β-cholan-24-oate[c]		108–109[b] (lit.[18] 90–91)		5.07 (broad, 1 H, 12β-H), 4.90 (broad, 1 H, 7β-H), 4.56 (broad, 1 H, 3β-H), 3.65 (s, 3 H, ester Me), 2.09 (s, 3 H, acetate Me), 2.05 (s, 3 H, acetate Me), 2.00 (s, 3 H, acetate Me), 0.87 (s, 3 H, 19-Me), 0.69 (s, 3 H, 18-Me)
15 7α,12α-Dihydroxy-3-oxo-5β-cholan-24-oate[k]	11	178–179[b] (lit.[19] 171–173)	3540 (OH), 1740 (ester C=O), 1700 (keto C=O), 1190, 1082, 1040, 982	4.04 (broad m, 2 H, 7β-H and 12β-H), 3.71 (s, 3 H, ester Me), 1.04 (s, 3 H, 19-Me), 0.76 (s, 3 H, 18-Me)

Table I
(Continued)

Compd	Yield, %	Mp, °C (lit. value)	Ir absorption, selected bands, cm^{-1}	^1H NMR spectral assignments, δ
16 Methyl 7α,12α-dihydroxy-3,3-ethylenedithio-5β-cholan-24-oate	75	102–113[n]	3410 (OH), 1730 (C=O), 1192, 1166, 1066, 1030	4.02 (broad s, 1 H, 12β-H), 3.84 (broad s, 1 H, 7β-H), 3.70 (s, 3 H, ester Me), 3.28 (s, 4 H, thioketal), 0.94 (s, 3 H, 19-Me), 0.70 (s, 3 H, 18-Me)
17a Methyl 3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate[o]	87	183–184[l] (lit.[20] 179–181)	1752 (ester C=O), 1722 (keto C=O), 1252 (acetate C–O), 1070, 1031	5.04 (s, 1 H, 7β-H), 4.60 (broad m, 1 H, 3β-H), 3.68 (s, 3 H, ester Me), 2.04 (s, 6 H, acetate Me's), 1.03 (s, 6 H, C-18 and C-19 Me's), 0.82 (d, 3 H, 21-Me)
17b Methyl 3α-Acetoxy-7α-hydroxy-12-oxo-5β-cholan-24-oate[p]	42	194–195[b] (lit.[21] 194–195)	3436 (OH), 1727 and 1692 (C=O), 1258 (acetate C–O), 1085, 1032, 978	

[a] Satisfactory analytical data (±0.3% for C, H, S, Cl) were reported for **6a, 6b, 7b, 10a, 10b,** and **16; 7a** was identified by its spectra and by analysis of its acetate **7b.** [b] Recrystallized from methanol–water. [c] Prepared with acetic anhydride and pyridine. [d] Prepared by hydrogenation of the Δ^3 olefin. [e] Recrystallized from methanol. [f] Prepared with acetic acid, acetic anhydride, and *p*-toluenesulfonic acid. [g] Prepared with acetic anhydride in benzene. [h] Separated from the diacetate by chromatography on alumina. [i] Recrystallized from acetone–petroleum ether. [j] Recrystallized from acetone–water. [k] Prepared with aluminum isopropoxide and acetone. [l] Recrystallized from ether. [m] Prepared with pyridinium chloride and **8a.** [n] Did not crystallize from solution; obtained as an amorphous powder. [o] Prepared by oxidation of **13b** with sodium dichromate in acetic acid. [p] Prepared by hydrolysis of **17b,** then methanolic HCl, then acetic anhydride, benzene, and THF.

benzoate (**13d**)[3] and 5β-cholane-7α,12α-diol (**18**)[4] are the samples described earlier. After several unsuccessful attempts to alkylate the hydroxyl of methyl cholate 3,7-diacetate (**13b**),[23] methyl 3α,7α-diacetoxy-12α-methoxy-5β-cholan-24-oate (**14a**) was prepared from **13b** and methyl fluorosulfonate.[24] Alkaline hydrolysis gave the dihydroxy acid, which was not purified, but was converted to the methyl ester (**14b**). Selective acetylations of the 3α-hydroxyl were carried out by treating the steroid with acetic anhydride in benzene (**4a, 13a**) or THF (**14c**). In the case of **4a,** some of the diacetate was also produced.

Comparisons of acetylation rates of the 3α-, 7α-, and 12α-hydroxyls with each other by this method, and with other methods previously reported.[3,5] are given in Table II. Again the 12α-hydroxyl reacts about 1.4 times as fast as the 7α-hydroxyl, and the 3α-hydroxyl is many times as fast. These rates are faster than those obtained by the other two methods, owing in part to the higher temperature in the sample well of the NMR instrument (35° vs. 25°).

The effects of substituents on the acetylation rate of the 7α-hydroxyl are indicated by the rate constants listed in Table III. It can be seen that the 3α-tosyloxy (**8a**), 3α-mesyloxy (**7a**), 3β-chloro (**10a**), 3α-acetoxy (**4a**), 3-oxo (**5a**), and 3α-succinoyloxy (**9a**) groups are all mildly enhancing, and the 3α-dimethylamino (**12a**) somewhat more so (2.4 times H). By far the largest effects are observed for those compounds containing a 12α-hydroxyl group in addition to the 3 substituent. Methyl cholate 3-acetate (**13a**) exhibits a rate constant 9.3 times that of methyl 7α-hydroxy-5β-cholan-24-oate (**3a**), and 5.7 times that of methyl chenodeoxycholate 3-acetate (**4a**), illustrating the added effect of the 12α-hydroxyl group. The combination of 3-ethylenedithio and 12α-hydroxy is the most effective, giving a rate constant 14.6 times that of **3a.**

Table II
Acetylation Rates[a]

Compd	^1H NMR,[b] $k_2 \times 10^6$	GLC,[c] $k_2 \times 10^6$	^{14}C,[d] $k_2 \times 10^6$
1a Methyl 3α-hydroxy-5β-cholan-24-oate	82.1 (44.8)[b]	55.6 (69)	37.8 (95)
2a Methyl 12α-hydroxy-5β-cholan-24-oate	2.47 (1.35)	1.12 (1.38)	0.58 (1.5)
3a Methyl 7α-hydroxy-5β-cholan-24-oate	1.83 (1.00)	0.81 (1.00)	0.39 (1.0)

[a] Rate constants are expressed in M^{-1} sec^{-1}; figures in parentheses are relative rates. [b] Obtained at 35°, the temperature of the sample well. [c] Reference 3. [d] Reference 5.

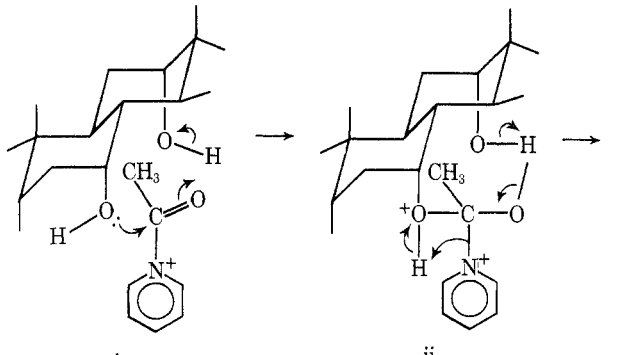
The mechanism of rate enhancement by the 3 substituents is not known, but a clue to the role of the 12α-hydroxyl is obtained by comparing methyl 3α,7α-dihydroxy-12α-methoxy-5β-cholan-24-oate 3-acetate (**14c**) with methyl cholate 3-acetate (**13a**). The hydroxyl and methoxyl groups have similar steric and inductive effects, so this large difference in rate constant (factor of 15) would appear to be related to the ability of the hydroxyl to contribute a proton. A plausible mechanism would employ protonation of

Table III
Acetylation Rates of 7α-Hydroxy Steroids

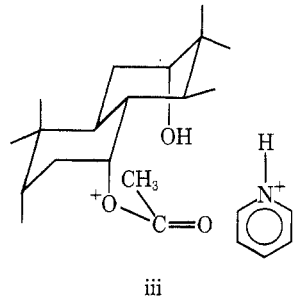
	Compd	$k_2 \times 10^6$, M^{-1} sec^{-1}
14c	Methyl 3α-acetoxy-7α-hydroxy-12α-methoxy-5β-cholan-24-oate	1.03 ● 0.11
6a	Methyl 3,3-ethylenedioxy-7α-hydroxy--5β-cholan-24-oate	1.49 ±0.17
3a	Methyl 7α-hydroxy-5β-cholan-24-oate	1.83 ± 0.03
8a	Methyl 7α-hydroxy-3α-*p*-toluenesulfonoxy-5β-cholan-24-oate	2.37 ± 0.21
7a	Methyl 7α-hydroxy-3α-methanesulfonoxy-5β-cholan-24-oate	2.63 ± 0.05
10a	Methyl 3β-chloro-7α-hydroxy-5β-cholan-24-oate	2.71 ± 0.13
4a	Methyl 3α-acetoxy-7α-hydroxy-5β-cholan-24-oate	2.90 ± 0.35
5a	Methyl 7α-hydroxy-3-oxo-5β-cholan-24-oate	3.23 ± 0.05
9a	Methyl 7α-hydroxy-3α-succinoyloxy-5β-cholan-24-oate	3.32 ± 0.20
12a	Methyl 3α-dimethylamino-7α-hydroxy-5β-cholan-24-oate	4.47 ± 0.58
13a	Methyl 3α-acetoxy-7α-12α-dihydroxy-5β-cholan-24-oate	17.00 ± 1.2
15	Methyl 7α,12α-dihydroxy-3-oxo-5β-cholan-24-oate	17.2 ± 1.9
18	5β-Cholane-7α,12α-diol	25.6 ± 1.1
13d	Methyl 7α,12α-dihydroxy-3-*p*-nitrobenzoyloxy-5β-cholan-24-oate	26.2[a] ± 3.6
16	Methyl 7α,12α-dihydroxy-3,3-ethylenedithio-5β-cholan-24-oate	26.7 ± 1.1

[a] A rate constant of 31.3 × 10^{-6} M^{-1} sec^{-1} was obtained by the uv method.[3]

the acetyl pyridinium ion in production of the tetrahedral intermediate ii, which then collapses to product (iii) and pyridinium ion. With respect to substituents at C-3, there



i ii



iii

is no obvious single mechanism by which all of the diverse groups used here [α-RSO_3, β-Cl, α-AcO, O=, α-HO_2C-$C_2H_4CO_2$, α-Me_2N, α-*p*-$O_2NC_6H_4CO_2$, and -$(CH_2S)_2$] can enhance acetylation of the 7α-hydroxyl; neither field effects nor inductive effects correlate with rates. Association between certain 3 substituents and the 7-hydroxyl is implied by the observation that upon acetylation appropriate resonances (acetate Me, mesylate Me, ketal and dithioketal methylenes) move downfield by 0.1–0.2 ppm. In the case of methyl 7α,12α-dihydroxy-3,3-ethylenedithio-5β-cholan-24-oate (**16**) this shift, rather than an angular methyl shift, was used to calculate the rate constant. These shifts are solvent dependent in the sense that they are substantially smaller (0.03) in deuteriochloroform.

The 7α,12α-dihydroxy steroids underwent a second upfield shift of the C-19 angular methyl representing acetylation of the 12-hydroxyl. These rates were calculated (Table IV) by assuming that the acetic anhydride concentration had been lowered by an amount equivalent to the steroid concentration (to accommodate that used in acetylation of the 7-hydroxyl). This method was validated by the finding of similar rate constants for the 12α-hydroxyls of methyl cholate 3-acetate (**13a**) and methyl cholate 3,7-diacetate (**13b**). The sequence differes in this series, suggesting that substituents at C-3 influence the 7- and 12-hydroxyls by different mechanisms.

Table IV
Acetylation Rates of 12α-Hydroxy Steroids

	Compd	$k_2 \times 10^6$ M^{-1} sec^{-1}
18	5β-Cholane-7α,12α-diol	1.27 ± 0.14
13a	Methyl 3α-acetoxy-7α,12α-dihydroxy-5β-cholan-24-oate	1.83 ± 0.12
13b	Methyl 3α,7α-diacetoxy-12α-hydroxy-5β-cholan-24-oate	1.99 ± 0.23
13d	Methyl 7α,12α-dihydroxy-3-*p*-nitrobenzoyloxy-5β-cholan-24-oate	2.74 ± 0.11
16	Methyl 7α,12α-dihydroxy-3,3-ethylenedithio-5β-cholan-24-oate	3.31 ± 0.29
15	Methyl 7α,12α-dihydroxy-3-oxo-5β-cholan-24-oate	3.70 ± 0.39

Experimental Section[25]

Methyl 7α-Hydroxy-3α-succinoyloxy-5β-cholan-24-oate (9a). A solution of 2.00 g (4.93 mmol) of methyl chenodeoxycholate (**4a**) in 25 ml of benzene was dried by distilling 15 ml of the benzene. Succinic anhydride (2.00 g, 19.7 mmol) and chloroform (25 ml) were added and the resulting suspension was stirred at room temperature for 5 days. Washing the suspension with three 100-ml portions of H_2O, drying (Na_2SO_4), and evaporating produced a pale yellow syrup which was chromatographed from benzene solution on 70 g of Florisil. Elution with 7% MeOH in ether gave recovered **4a**, and elution with MeOH gave 1.34 g of a glass, mp 139–150°. Analysis implied contamination with succinic acid, which was removed by dissolving the glass in 25 ml of H_2O, acidifying, extracting with benzene (4 × 25 ml), drying (Na_2SO_4), and evaporating the benzene. Dissolving the residue in dilute NaOH, acidifying, adding MeOH to partially dissolve the resulting precipitate, and gradual cooling gave 758 mg of **9a**: mp 79–90°; ir 3546 (OH), 1721 (C=O), 1155, 1074, 1000, 973 cm^{-1}; NMR δ 4.60 (broad, 1 H, 3β-H), 3.88 (broad, 1 H, 7β-H), 3.69 (s, 3 H, ester Me), 2.62 (s, 4 H, succinate CH_2's), 0.92 (s, 3 H, 19-Me), 0.67 (s, 3 H, 18-Me).

Anal. Calcd for $C_{29}H_{46}O_7$: C, 68.74; H, 9.15. Found: C, 68.75; H, 9.23.

Methyl 3α-Dimethylamino-7α-hydroxy-5β-cholan-24-oate (12a). A solution of 2.06 g (5.0 mmol) of methyl 7α-hydroxy-3-oxo-5β-cholanoate (**5a**) and 1.0 ml of 97% HCO_2H in 2 ml of DMF was heated at reflux for 5.5 hr. Acidification with 20 ml of 1 *N* HCl,

dilution with 50 ml of H_2O, and neutralization with aqueous $NaHCO_3$, drying (Na_2SO_4), evaporating, and chromatographing the residue on basic Al_2O_3 (Brockman activity I) gave three oily fractions: A, eluted by Et_2O–C_6H_6 (1:9), 4% overall yield, presumed to be a 7-formate **11b** [NMR δ 8.11 (s, 1 H, formate H), 5.03 (s, 1 H, 7β-H), 2.21 (s, 6 H, amine Me's)]; B, eluted by Et_2O–C_6H_6 (1:4), 8% yield, presumed to be the 3β epimer **11a** [NMR s 2.27 (s, 6 H, amine Me's)]; C, eluted by Et_2O–C_6H_6 (4:1) and further purified by preparative TLC, 16% yield, **12a:** ir 3150–3400 (OH), 1730 (C=O), 1160, 980, 750 cm^{-1}; NMR δ 3.88 (broad, 1 H, 7β-H), 3.70 (s, 3 H, ester Me), 2.38 (s, 6 H, amine Me's), 0.94 (s, 3 H, 19-Me), 0.69 (s, 3 H, 18-Me). It was characterized as the hydrochloride, an amorphous solid from trituration in ether–petroleum ether, mp 262–265°.

Anal. Calcd for $C_{27}H_{48}O_3NCl$: C, 68.98; H, 10.38; N, 2.98; Cl, 7.54. Found: C, 68.71; H, 10.10; N, 3.01; Cl, 7.80.

The acetate (**12b**), prepared as usual and purified by chromatography on basic Al_2O_3, eluted by Et_2O–C_6H_6 (1:5) was an oil: ir 1272 (C=O), 1240 (acetate C=O), 1158, 1010, 670 cm^{-1}; NMR δ 4.91 (broad, 1 H, 7β-H), 3.68 (s, 3 H, ester Me), 2.30 (s, 6 H, amine Me's), 2.06 (s, 3 H, acetate Me), 0.93 (s, 3 H, 19-Me), 0.66 (s, 3 H, 18-Me).

Anal. Calcd for $C_{29}H_{49}O_4N$: C, 73.22; H, 10.38; N, 2.95. Found: C, 73.47; H, 10.21; N, 3.12.

Methyl 3α,7α-Diacetoxy-12α-methoxy-5β-cholan-24-oate (14a). A solution of 10.0 g (19.8 mmol) of methyl cholate 3,7-diacetate (**13b**) in 70 ml of benzene was dried by distilling 20 ml of the benzene. Methyl fluorosulfonate (25 ml) was added slowly to the benzene solution at room temperature. After 12 hr H_2O was added and the mixture was carefully neutralized with $NaHCO_3$. Extraction into ether, washing, and drying gave 5.3 g of a yellow oil whose benzene solution was chromatographed on Al_2O_3 (150 g). Following a forerun with 10% Et_2O in C_6H_6, 25–30% Et_2O in C_6H_6 eluted the product, which was rechromatographed twice to give 1.8 g (18%) of an oil: ir 1721 and 1704 (C=O), 1235 (acetate C–O), 1094 (ether C–O), 1020, 680 cm^{-1}; NMR δ 4.90 (broad, 1 H, 7β-H), 4.57 (broad, 1 H, 3β-H), 3.67 (s, 3 H, ester Me), 3.33 (s, 3 H, ether Me), 2.05 (s, 6 H, acetate Me's), 0.92 (s, 3 H, 19-Me), 0.70 (s, 3 H, 18-Me).

Anal. Calcd for $C_{30}H_{48}O_7$: C, 69.20; H, 9.29. Found: C, 69.42; H, 9.33.

Methyl 3α,7α-Dihydroxy-12α-methoxy-5β-cholan-24-oate (14b). Hydrolysis of **14a** with KOH in 90% MeOH at reflux for 20 hr, acidification, and extraction into $HCCl_3$ gave the acid, an oil, which was esterified in methanolic HCl to give **14b,** prisms out of MeOH–H_2O, 76%: mp 189–190°; ir 3247 (OH), 1757 (C=O), 1115, 1094 (ether C–O), 998 cm^{-1}; NMR δ 3.87 (broad, 1 H, 7β-H), 3.70 (s, 3 H, ester Me), 3.32 (s, 3 H, ether Me), 0.92 (s, 3 H, 19-Me), 0.70 (s, 3 H, 18-Me).

Anal. Calcd for $C_{26}H_{44}O_5$: C, 71.52; H, 10.16. Found: C, 71.32; H, 10.30.

Methyl 3α-Acetoxy-7α-hydroxy-12α-methoxy-5β-cholan-24-oate (14c). A solution of 1.23 g (2.82 mmol) of **14b** in 15 ml of anhydrous THF containing 5 ml of Ac_2O was kept at 60–65° for 10 hr. Pyridine (1 ml) and H_2O (50 ml) were added to the solution at room temperature. Extraction into ether, washing (dilute $NaHCO_3$ and H_2O), and drying gave 1.51 g of an oil. Chromatography on 50 g of Al_2O_3 and elution with Et_2O–C_6H_6 (1:3) to remove forerun, followed by elution with Et_2O, gave 620 mg (46%) of **14c,** an oil: ir 3540 (OH), 1720 and 1702 (C=O), 1240 (acetate C–O), 1090 (ether C–O), 1068, 1020, 908, 730 cm^{-1}; NMR δ 4.57 (broad, 1 H, 3β-H), 3.85 (broad, 1 H, 7β-H), 3.69 (s, 3 H, ester Me), 3.32 (s, 3 H, ether Me), 2.04 (s, 3 H, acetate Me), 0.93 (s, 3 H, 19-Me), 0.70 (s, 3 H, 18-Me).

Anal. Calcd for $C_{28}H_{46}O_6$: C, 70.26; H, 9.69. Found: C, 70.42; H, 9.57.

Kinetic Runs. The steroid (0.370 mmol) was weighed into a 1-ml volumetric tube and dissolved in approximately 0.6 ml of pyridine (previously dried over molecular sieve). Acetic anhydride was weighed into a 3-ml volumetric tube and made up to the mark with anhydrous pyridine. At zero time, 0.20 ml of the Ac_2O solution was pipetted into the steroid solution, which was then immediately brought to the mark with anhydrous pyridine and mixed. The solution was transferred to a NMR sample tube, sealed with a cap and parafilm, and placed in a bath at 35 ± 1°. Periodically the tube was placed in the spectrometer and the spectrum from 5 to 0 ppm was traced. The time of the measurement was taken to be that time when the pen traced the particular methyl resonance of interest.

Peak heights of either the C-18 (usually) or C-19 methyl resonances for the alcohol and the acetate in each mixture were measured in millimeters from the spectrum base line. These peak heights were normalized by use of the peak height of an invariant resonance as an internal standard, usually the side-chain methyl resonance. Rate constants were calculated from these peak heights and starting concentrations using a program written in FORTRAN IV language for the CDC 6600 system and the standard rate expression

$$k = \frac{1}{t(b - a)} \ln \frac{a(b - x)}{b(a - x)}$$

where a = initial molar concentration of steroid, b = initial molar concentration of Ac_2O, and x = moles reacted at time t.

The computer calculated the second-order rate constant from a least-squares plot of log $(b - x)/(a - x)$ vs. t; data from a typical run are given in Table V.

Table V
Acetylation of Methyl 12α-Hydroxy-5β-cholan-24-oate[a]

Time, hr	ROH peak ht (corr)	ROAc peak ht (corr)	ROH, M	ROAc (x), M	Log $(b-x)/(a-x)$	% Reaction
8.4	107	16	0.320	0.049	1.156	13.2
21.9	85	36	0.260	0.109	1.305	29.6
33.6	72	41	0.235	0.134	1.380	36.4
45.5	75	61	0.203	0.166	1.491	45.1
58.2	67	71	0.179	0.190	1.587	51.4
68.8	62	82	0.159	0.210	1.684	56.9
103.0	46	93	0.122	0.247	1.909	67.1
130.6	41	96	0.109	0.260	1.999	70.4
166.5	38	106	0.097	0.272	2.106	73.8

[a] Initial molar concentrations: steroid, 0.369; Ac_2O, 1.067.

For the diols (**13a, 13d, 15, 16,** and **18**) there are two competing reactions occurring simultaneously, but preliminary work with methyl cholate 3-acetate (**13a**) indicated that the difference in rate between 7α-hydroxyl acetylation and 12α-hydroxyl acetylation was great enough that the two reactions could, as a good approximation, be considered sequential. Consequently, in calculating the rate constants for the 12-hydroxyl of these diols, it was assumed that the starting concentration of acetic anhydride was equal to the actual initial concentration minus the initial concentration of steroid. As a check of the value thus calculated in the case of methyl cholate 3-acetate (**13a**), runs were also carried out using the 3,7-diacetate (**13b**).

Each compound was run two to six times, the k_2's reported representing the mean for each compound.

Acknowledgment. Computations were performed at the Indiana University School of Medicine Research Computation Center and were supported in part by PHS Grant FR00162.

Registry No.—**1a,** 1249-75-8; **1b,** 3253-69-8; **2a,** 1249-70-3; **2b,** 1919-68-2; **3a,** 28050-19-3; **3b,** 19684-60-7; **4a,** 19684-68-5; **4b,** 2616-71-9; **5a,** 14773-00-3; **6a,** 54852-40-3; **6b,** 54852-41-4; **7a,** 54852-42-5; **7b,** 54852-43-6; **8a,** 28192-93-0; **8b,** 54852-44-7; **9a,** 54852-45-8; **10a,** 54852-46-9; **10b,** 54852-47-0; **11a,** 54852-48-1; **11b,** 54852-49-2; **12a,** 54852-50-5; **12a** HCl, 54852-51-6; **12b,** 54852-52-7; **13a,** 7443-91-6; **13b,** 3749-87-9; **13c,** 6818-44-6; **13d,** 28192-79-2; **14a,** 54852-53-8; **14b,** 54852-54-9; **14c,** 54852-55-0; **15,** 14772-99-7; **16,** 54852-56-1; **17a,** 28535-81-1; **17b,** 54852-57-2; **18,** 3701-54-0; succinic anhydride, 108-30-5.

References and Notes

(1) Part VII: *Steroids,* **23,** 895 (1974). Part VIII taken from the Ph.D. Thesis of James F. Baker, Indiana University.
(2) R. T. Blickenstaff and B. Orwig, *J. Org. Chem.,* **34,** 1377 (1969).
(3) R. T. Blickenstaff, K. Atkinson, D. Breaux, E. Foster, Y. Kim, and G. C. Wolf, *J. Org. Chem.,* **36,** 1271 (1971).
(4) G. C. Wolf, E. L. Foster, and R. T. Blickenstaff, *J. Org. Chem.,* **38,** 1276 (1973).
(5) A. Sattar and R. T. Blickenstaff, *Steroids,* **17,** 357 (1971).
(6) R. T. Blickenstaff and K. Sophasan, *Tetrahedron,* **28,** 1945 (1972).
(7) H. Schmid, H. Sofer, and B. Mayerböck, *Monatsh. Chem.,* **99,** 463 (1968).

(8) N. S. Bhacca and D. H. Williams, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Holden-Day, San Francisco, Calif., 1964, p 13.
(9) P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari, and E. Wenkert, *J. Am. Chem. Soc.*, **90**, 5480 (1968).
(10) F. C. Chang, R. T. Blickenstaff, A. Feldstein, J. R. Gray, G. S. McCaleb, and D. H. Sprunt, *J. Am. Chem. Soc.*, **79**, 2164 (1957).
(11) E. Seebeck and T. Reichstein, *Helv. Chim. Acta*, **26**, 536 (1943).
(12) J. Barnett and T. Reichstein, *Helv. Chim. Acta*, **21**, 926 (1938).
(13) E. Hauser, E. Baumgartner, and K. Meyer, *Helv. Chim. Acta*, **43**, 1595 (1960).
(14) Y. Sato and N. Ikekawa, *J. Org. Chem.*, **24**, 1367 (1959).
(15) H. B. Kagan and J. Jaques, *Bull. Soc. Chim. Fr*, 699 (1957).
(16) P. A. Plattner and H. Hauser, *Helv. Chim. Acta*, **27**, 748 (1944).
(17) L. F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **72**, 5530 (1950).
(18) W. S. Knowles, J. Fried, and R. C. Elderfield, *J. Org. Chem.*, **7**, 383 (1942).
(19) A. S. Jones, M. Webb, and F. Smith, *J. Chem. Soc.*, 2164 (1949).
(20) L. F. Fieser, S. Rajagopalan, E. Wilson, and M. Tishler, *J. Am. Chem. Soc.*, **73**, 4133 (1951).
(21) E. Berner, A. Lardon, and T. Reichstein, *Helv. Chim. Acta*, **30**, 1542 (1947).
(22) 3α-Amines were obtained by the Leuckart reaction also in the 5β-pregnane series: J. J. Panouse et al., *Bull. Soc. Chim. Fr.*, 1753 (1963).
(23) Attempted alkylation with $(CH_3)_2SO_4$ or CH_3I and strong bases gave no detectable reaction. Treatment with diazomethane and a Lewis acid provided, surprisingly, in each case, a high yield of the oxidation product, methyl 3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate (**17a**).
(24) M. G. Ahmed, R. W. Alder, G. H. James, M. L. Sinnott, and M. C. Whiting, *Chem. Commun.*, 1533 (1968).
(25) Melting points were determined on a Thomas-Hoover melting point apparatus and are corrected. Infrared spectra were recorded from smears or Nujol mulls on a Perkin-Elmer 247 spectrophotometer. Nuclear magnetic resonance spectra were obtained in deuteriochloroform on a Varian T-60 spectrophotometer. Elemental microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Application of the Wittig Reaction to the Synthesis of Steroidal Side Chains. Possibility of 3β-Phenoxy Formation as a Secondary Reaction

Jean Pierre Schmit, Marcel Piraux,* and Jean François Pilette

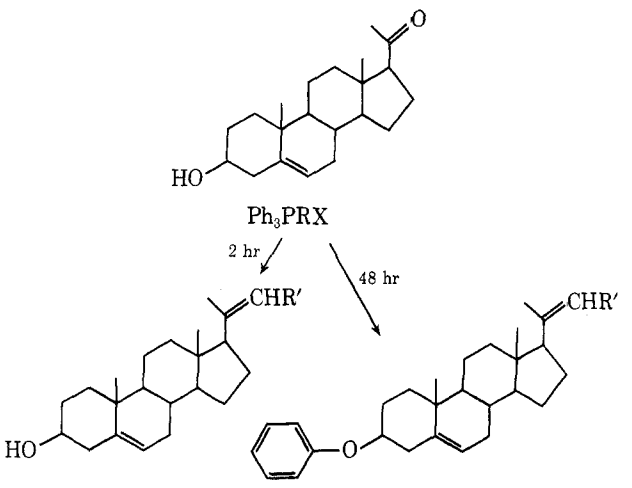
Department of Pharmacognosy and Natural Products Chemistry, Catholic University of Louvain, 1348 Louvain-la-Neuve, Belgium

Received September 18, 1974

The Wittig reaction of various alkylidenephosphoranes with Δ^5-pregnen-3β-ol-20-one has been studied. The formations of 3β-phenoxy derivatives in a secondary reaction is demonstrated.

With the available methods for the synthesis of side-chain steroids from C-20 and C-21 compounds, one obtains stereoisemeric 20-hydroxy compounds as an intermediate.[1] The latter, on being dehydrated at C-20 and then catalytically hydrogenated, yield a mixture of the two possible isomers. The dehydration of a 20-hydroxy intermediate in principle could give five different olefins: two $\Delta^{17(20)}$-dehydro compounds, the corresponding $\Delta^{20(21)}$ isomer, and two $\Delta^{20(22)}$ isomers.[2,3,4] The isomerism difficulty was partially resolved by Sondheimer and Mechoulam[5], who described the synthesis of $\Delta^{5,20(21)}$-cholestadien-3β-ol acetate by use of the Wittig reaction on 21-nor-20-ketocholesteryl acetate followed by hydrogenation. Subsequently, the Wittig reaction was applied, principally with triphenylphosphine methylene reagent,[6] to various keto steroids. In order to develop a simple route to various unambiguous isomeric side chain steroids, we investigated the Wittig reaction with Δ^5-pregnen-3β-ol-20-one (pregnenolone).

In the first study, isopentylidenephosphorane as the Wittig reagent in the presence of sodium *tert*-amylate[7] as base in benzene solution was found to give the $\Delta^{5,20(22)}$-cholestadien-3β-ol. Thin layer chromatography on Kieselgel with benzene as eluent revealed two products. The faster moving material, with a 0.9 R_f, was present in very low yield after the usual Wittig reaction time. The slower moving component, with a 0.6 R_f, isolated in 80% yield, was found to be the desired $\Delta^{5,20(22)}$-cholestadien-3β-ol. The fast-moving compound, which was initially present in negligible quantities, became the predominant compound with a longer reaction time. The ir spectrum of this secondary product shows the presence of bands at 1598, 1584, 1241, 760, and 694 cm^{-1}. NMR spectroscopy revealed two multiplets appearing respectively at 6.92 and 7.28 ppm. In comparison with the cholesterol the 3β proton was shifted 0.5 ppm to higher field and the 19 methyl peak was shifted 0.02 ppm to lower field. Protons H-6 and H-22 exhibited no observable shift change. Finally, mass spectroscopy gives a molecular ion at 76 units above the expected mass and a base peak 93 units lower than the molecular ion. All of these data support the replacement of the 3β-hydroxyl group by a 3β-phenoxy group in the minor product.



We have also carried out this reaction in the presence of cholesterol and triphenylphosphonium salt and obtained, in either benzene or toluene as solvent, 3β-phenoxycholesterol in high yield. As expected, no substitution occured when the phosphonium salt was absent.

The normal acetylated 20(22) condensation product was then selectively hydrogenated[8] in dioxane, in the presence of acetic acid and platinum oxide as catalyst, to give (80% yield) a product which was identical with the natural product in its spectroscopic properties, melting point, and specific rotation, $[\alpha]^{20}D$ −39.5° ($CHCl_3$). No other hydrogenated product was isolated. This selectivity results from two principal reasons. First, the Wittig reaction on pregnenolone gives a single condensation product (TLC proof and precise melting point, 124.5 ± 0.5°), which was found to be the 20(22) *E* isomer as evidenced by its 18-methyl NMR