INTRAMOLECULAR CATALYSIS. VII. THE NATURE OF SIDE CHAIN SHIELDING OF THE 12α-HYDROXYL GROUP OF STEROIDS[1]

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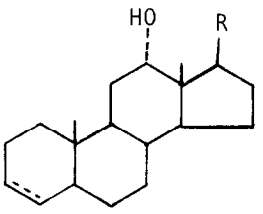
ABSTRACT

A series of 12α-hydroxy steroids with varying side chains was prepared, and their 24-hour acetylation yields were compared. 12α-Hydroxy-5β-pregnan-20-one (1b) was prepared from 3α,12α-diacetoxy-5β-pregnan-20-one (2) and also by side chain degradation of 12α-acetoxy-5β-cholanoic acid (5d). 21-Benzyl-5β-pregnan-12α-ol (1g) was synthesized by hydrogenation of the 21-benzylidine derivative of ketone 1b. 23-Phenyl-5β-norcholan-12α-ol (1k) was obtained by the Grignard reaction of 2-phenylethylmagnesium bromide and ketone 1b, dehydration, hydrogenation and hydride reduction; a similar sequence produced 20-methyl-5β-pregnan-12α-ol (1m). The acetylation results (Table II) imply that branching at C-20 may be more significant for 12α-hydroxyl reactivity than side chain length or type. An additional compound with an unbranched side chain, 21-nor-5β-cholan-12α-ol (14), was synthesized by a Grignard reaction on the 21-bromo intermediate 11b. Acetylation rates determined by glc indicate (Table III) that compounds with unbranched side chains have 12α-hydroxyl groups about ten times as reactive as their analogs with 20-methyl groups.

The 12α-hydroxy steroids in Table I have been shown previously to acetylate under standard conditions in differing yields depending on the nature of the side chain.[2,3] Excluding the tert.-alcohol 1f, it would seem that the larger the side chain, the less reactive the 12α-hydroxyl toward acetic anhydride and pyridine. Compound 1f is anamolous, however, in having a relatively short side chain and an unreactive 12α-hydroxyl group.[4] In considering the influence of the side chain on 12α-hydroxyl reactivity, another fact to be accounted for is the observation that a series of bile acid derivatives with 7α- and 12α-hydroxyl groups

TABLE I

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

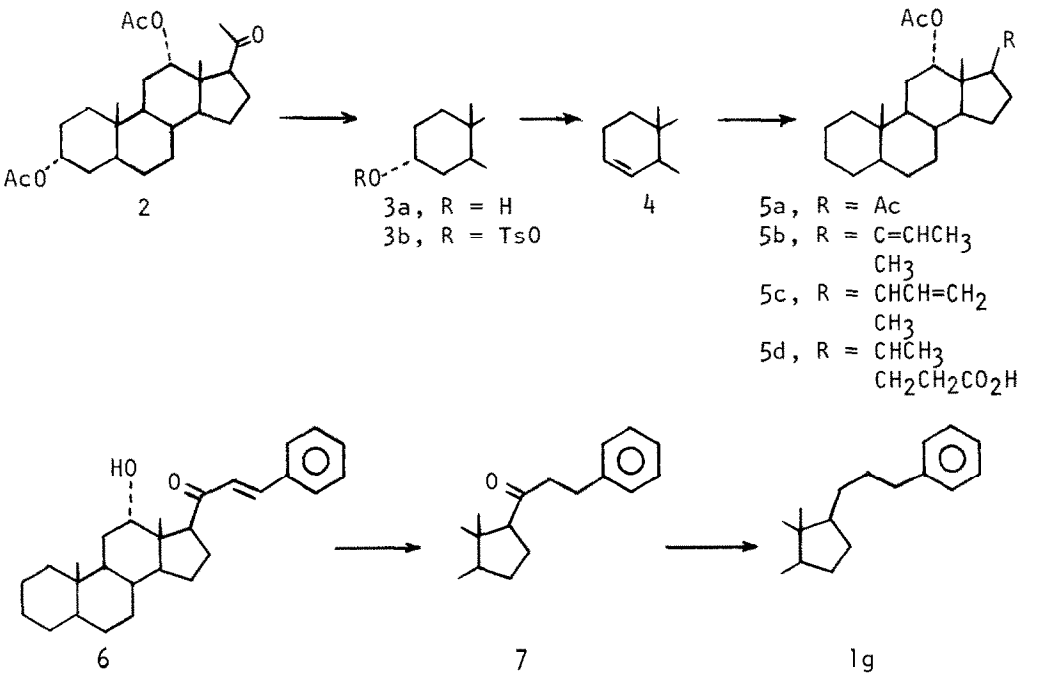


Compd. No.	R	Yield of acetate, %
1a	CH_2CH_3	45-50
1b	$C(=O)CH_3$	18-21
1c	$CH(CH_3)CH_2CH_2C(CH_3)_2OH$	10-12
1d	$CH(CH_3)CH_2CH_2CH_3$	5-10
1e	$CH(CH_3)CH_2CH_2CO_2CH_3$	5-8
1f	$C(CH_3)_2OH$ (Δ^3)	<1

[a]Steroid (0.37 mmol), Ac_2O (0.10 ml), pyridine (0.10 ml), and benzene (0.84 ml), room temperature, 24 hr.

acetylate preferentially at position 7 irrespective of the functional group terminating the side chain.[5] These two observations lead us to explore further the relationship between side chain structure and 12α-hydroxyl reactivity.

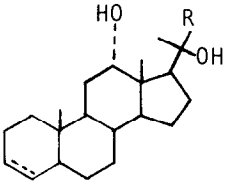
The compounds in Table II were synthesized and their 24-hour acetylation yields were compared. Compound 1a was prepared by Wolff-Kishner reduction of 12α-acetoxy-5β-pregn-3-en-20-one (4), followed by hydrogenation of the 3-double bond; compounds 1b-f were prepared as described earlier.[2,3] The acetate 5a of 12α-hydroxy-5β-pregnan-20-one (1b), a key intermediate to many of the remaining compounds, was prepared also by two other routes. In one, 3α,12α-diacetoxy-5β-pregnan-20-one (2) was selectively hydrolyzed to the monoacetate 3a; tosylation of mono-



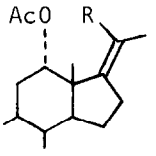
acetate 3a, dehydrotosylation of tosylate 3b and hydrogenation of the olefin 4 gave the acetoxy ketone 5a. Alternatively, the acetate 5d of 12α-hydroxy-5β-cholanoic acid (1i) was oxidized with lead tetraacetate[6] to 24-nor-5β-chol-22-en-12α-yl acetate (5c). The structure of the product was confirmed by the 910 cm^{-1} band for terminal methylene. The double bond was isomerized to 20-22 by the action of lithium and ethylene diamine.[7] Ozonolysis[8] of the olefin 5b (possibly a mixture of cis and trans isomers) gave hydroxy ketone 1b, which was acetylated to the ester 5a.

The condensation of benzaldehyde and hydroxy ketone 1b gave 21-benzylidene-12α-hydroxy-5β-pregnan-20-one (6), which on hydrogenation and Wolff-Kishner reduction gave 21-benzyl-5β-pregnan-12α-ol (1g). The acid 1h was obtained by hydrolysis of the corresponding methyl ester, an intermediate in the synthesis of the ester 1e;[2] hydrolysis of ester 1e similarly gave the acid 1i. The ketal 1j was prepared in

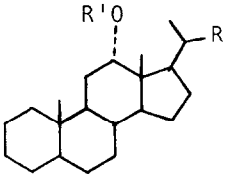
the customary fashion. The action of 2-phenylethylmagnesium bromide on ketone 1b produced diol 8, which was dehydrated in refluxing acetic acid-acetic anhydride; hydrogenation of the olefin 9a gave compound 10a, which could not be hydrolyzed in refluxing methanolic sodium hydroxide. It was reduced to the alcohol 1k with sodium bis-(2-methoxyethoxy)aluminum hydride in refluxing benzene. The configuration at C-20 in compounds 10a and 10b is assumed to be the natural one, based on the findings of Bergmann et al. in a similar series of synthetic steps.[9] Compounds 8, 9a, 10a and 1k, all oils, were purified by chromatography and characterized by ir spectra. 20-Methyl-5β-pregn-3-en-12α,20-diol (1f)[3] was similarly dehydrated and the olefin 9b hydrogenated to isopropyl compound 10b; reduction of the latter with lithium aluminum hydride gave crystalline 20-methyl-5β-pregnan-12α-ol (1m).



8, R = $C_2H_4C_6H_5$
1f R = CH_3 (3)



9a, R = $C_2H_4C_6H_5$
9b, R = CH_3 (3)



10a, R = $C_2H_4C_6H_5$; R' = Ac
1k, R = $C_2H_4C_6H_5$; R' = H
10b, R = CH_3; R' = Ac
1m, R = CH_3; R' = H

The 12 -hydroxy steroids 1a, 1b, 1d-k, and 1m, were acetylated with acetic anhydride and pyridine under previously reported conditions;[2] 24-hour yields were determined either by gas chromatography or by thin layer chromatography (see Experimental), and are reported in Table II. The results reveal a hitherto unrecognized correlation between side chain structure and 12α-hydroxyl reactivity. While many of the compounds with large side chains (compounds 1e, 1d, 1i, 1j, 1k) have relatively unreactive 12α-hydroxyl groups, the clearest distinction between reactive and unreactive analogs is based on branching at C-20.

TABLE II

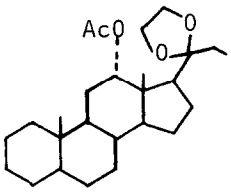
ACETYLATION OF HYDROXY STEROIDS WITH ACETIC ANHYDRIDE AND PYRIDINE[a]

Compd. No.	R	No. runs	Yield of acetate, %
1a	CH_2CH_3	4	55[b]
1g	$CH_2CH_2CH_2C_6H_5$	4	53.5[b]
1b	$C(=O)CH_3$	2	19.5[c]
1h	$CH(CH_3)CH_2CH_2CO_2H$ (Δ^3)	2	18[c]
1i	$CH(CH_3)CH_2CH_2CO_2H$	2	13[c]
1e	$CH(CH_3)CH_2CH_2CO_2CH_3$	4	10.8[b]
1j	$C(CH_3)(OCH_2CH_2O)$	2	8.5[c]
1d	$CH(CH_3)CH_2CH_2CH_3$	4	7.8[b]
1k	$CH(CH_3)CH_2CH_2C_6H_5$	4	6.2[b]
1m	$CH(CH_3)_2$	4	4.7[b]
1f	$C(CH_3)_2OH$ (Δ^3)	2	1.5[c]

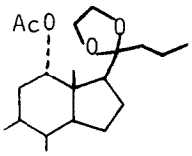
[a]Steroid (0.37 mmol), Ac_2O (0.10 ml), pyridine (0.10 ml), in benzene to make 1.00 ml, 28±1°, 24 hr. [b]By glc. [c]By tlc.

Unbranched side chains, whether short (as in compound 1a) or long (compound 1g), give rise to very reactive 12α-hydroxyl groups, while the least reactive compound in the series, tert.-alcohol 1f, is the most highly branched at C-20 (counting the tert.-hydroxyl group).[10]

It seemed desirable at this point to investigate specifically the effect of branching at C-20 on 12α-hydroxyl group reactivity. Consequently, rate studies were carried out on the three pairs of compounds in Table III; each pair contains a 21-methyl compound and its 21-nor analog. The acetate of ketal 1j was brominated in methylene chloride in the presence of calcium oxide[11] to give the corresponding 21-bromo



11a, X = H
11b, X = Br



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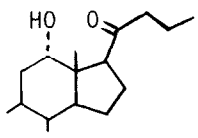

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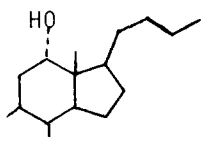

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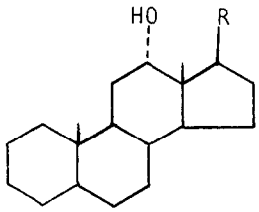
derivative 11b. Its reaction with ethylmagnesium bromide gave ketal 12, which was not purified but was hydrolyzed to the hydroxy ketone 13. Wolff-Kishner reduction gave 21-nor-5β-cholan-12α-ol (14), which crystallized as a methanol solvate.

The rates of acetylation of these three pairs of compounds were determined by glc,[3] and the resulting rate constants were calculated on a 6600 computer.[12] As clearly indicated in Table III, the 21-nor compounds acetylate faster than their 21-methyl counterparts, roughly an order of magnitude faster. These results may be interpreted in

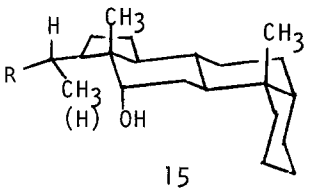
TABLE III

RATES OF ACETYLATION OF 12α-HYDROXY STEROIDS WITH BRANCHED VERSUS UNBRANCHED SIDE CHAINS[a]



	R	$k_2 \times 10^6$ $M^{-1}sec^{-1}$		R	$k_2 \times 10^7$ $M^{-1}sec^{-1}$
1a	CH_2CH_3	8.38	1m	$C(CH_3)_2$	6.07
1g	$CH_2CH_2CH_2C_6H_5$	11.51	1k	$CH(CH_3)CH_2CH_2C_6H_5$	7.27
14	$CH_2CH_2CH_2CH_3$	10.35	1d	$CH(CH_3)CH_2CH_2CH_3$	12.34

[a]Steroid (0.37 mmol), Ac_2O (0.10 mmol), pyridine (0.10 mmol), in benzene made to 1.00 ml, 28±1°.



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terms of the conformation implied in structure 15. Examination of models indicates that any R as large or larger than methyl will tend to assume an orientation away from the C-18 angular methyl group as shown. This conformation requires the C-21 methyl group to point toward the 12α-hydroxyl. With the A ring inhibiting approach of reagent from one direction and the C-21 similarly inhibiting from the other direction, the 12α-hydroxyl in these compounds is unreactive. Replacing the C-21 methyl group with hydrogen relieves a portion of this steric inhibition and results in a significantly more reactive 12α-hydroxyl group. Side chain shielding in these compounds may be defined as steric inhibition by the C-21 methyl group. The C-21 methyl group has been implicated previously in influencing the stereochemistry of reactions of C-12 substituents. Reduction of 12-oximinocholanes (5α and 5β) with sodium and ethanol gave 12α-aminocholanes exclusively, rather than the equatorial 12β-amines.[13] Reduction of 12-ketocholanoic acid or of 5β-cholan-12-one with lithium and ammonia or sodium and 1-propanol gave the corresponding 12α-hydroxy derivatives, but an analogous spirostane derivative where the E ring causes the C-21 methyl to be oriented farther away from the 12-substituent, gave rise to a β-hydroxy product.[14] X-ray diffraction studies have not been carried out on 12α-hydroxy steroids with flexible side chains, but those on steroids lacking a 12α-hydroxyl show conformations roughly similar to that indicated by structure 15.[15] Thus, the present work

for the first time presents kinetic evidence that is consistant with product stereochemistry and with X-ray evidence on crystals.

EXPERIMENTAL

GENERAL: Melting points were taken on a Unimelt apparatus and are uncorrected. Infrared spectra were taken on smears or mineral oil mulls on a Perkin-Elmer 247 spectrophotometer. NMR spectra were obtained in deuterochloroform on a Varian T-60 spectrometer, with tetramethylsilane as internal standard. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

5β-Pregnan-12α-ol (1a). A mixture of 12α-acetoxy-5β-pregn-3-en-20-one (4, 1.0 g), NaOH (3.0 g), 85% hydrazine hydrate (9 ml) and diethylene glycol (30 ml) was refluxed 2 hr, the condenser was removed until the temp reached 200° C, and refluxing was continued for 2 hr. The mixture was cooled, acidified to pH 2 with HCl, diluted with 100 ml of H_2O and extracted twice with 150 ml of ether. The solution was dried and evaporated, leaving a residue which was dissolved in CCl_4 and chromatographed on 30 g of Al_2O_3. After several cuts of CCl_4 and CCl_4-C_6H_6 mixtures, benzene eluted 410 mg of product; recrystallization in MeOH-H_2O gave the analytical sample of 5β-pregn-3-en-12α-ol, m.p. 61-62°; nmr δ 5.56 (broad q, 3-H,4-H), 3.80 (broad t, 12β-H), 0.96 (s, 19-CH_3), 0.62 (s, 18-CH_3); ir 3546 (OH), 1645 (weak, C=C) cm^{-1}.

Anal. Calcd for $C_{21}H_{34}O$: C, 80.70; H, 10.60. Found: C, 80.40; H, 10.90.

Hydrogenation of the olefin (1 g) in MeOH (50 ml) over PtO_2 (100 mg) at 50 psi overnight gave 5β-pregnan-12α-ol, recrystallized from MeOH-H_2O, m.p. 99.5-100.5° (lit[2] 100-101°); nmr δ 3.79 (broad t, 12β-H), 0.93 (s, 19-CH_3), 0.60 (s, 18-CH_3); ir 3450 (OH) cm^{-1}.

12α-Hydroxy-5β-pregnan-20-one (1b). The acetate 5b (12 g) in 250 ml of CH_2Cl_2 was ozonized at -70°, the solution was evaporated to dryness at 35-40° and the residue, in acetone, was titrated to a permanent brown color with Jones reagent (7 ml of 26% CrO_3 in 23% H_2SO_4). Dilution with H_2O, extraction into C_6H_6 and washing gave 10 g of a mixture of 1b and 5a. Alkaline hydroxysis (25 g of NaOH, 125 ml of MeOH, 125 ml of H_2O, reflux 30 min) and chromatography on 300 g of Al_2O_3 gave 5.5 g of 1b, eluted by C_6H_6 and C_6H_6-Et_2O 2:3, identical to that obtained via 3 and 4.[2]

21-Benzyl-5β-pregnan-12α-ol (1g). Wolff-Kishner reduction of 1.5 g of 7 under the conditions described for 1a gave about 2 g of crude 1g, which was chromatographed on 80 g of Al_2O_3. Benzene eluted 350 mg of material lacking aromatic bands in the ir, then C_6H_6-Et_2O eluted 600 mg of 1g, ir 3311 (OH), 1592 (arom. ring) cm^{-1}. It was analyzed as the acetate, nmr δ 7.22 (s, phenyl) 5H, 5.00 (broad t, 12β-H) 1H, 2.05 (s, acetate CH_3) 3H, 0.90 (s, 19-CH_3) 3H, 0.60 (s, 18-CH_3) 3H.

Anal. Calcd for $C_{30}H_{44}O_2$: C, 82.51; H, 10.15. Found: C, 82.27; H, 10.32

12α-Hydroxy-5β-pregnan-20-one ethyleneketal (1j). The ketal was prepared in the usual way from 1b, ethylene glycol and p-TsOH in refluxing C_6H_6, utilizing a Dean-Stark trap containing molecular sieve 4A. Recrystallization of the crude solid in MeOH-H_2O gave 1j, m.p. 124-125°; ir 3571 (OH) cm^{-1}.

Anal. Calcd for $C_{23}H_{38}O_3$: C, 76.28; H, 9.66. Found: C, 76.60; H, 10.08.

23-Phenyl-24-nor-5β-cholan-12α-ol (1k). The acetate 10a (1.2 g) was cleaved by reduction with 5 ml of sodium bis-(2-methoxyethoxy)aluminum hydride in 25 ml of C_6H_6 at reflux 16 hr. Careful acidification of the reaction mixture, extraction into Et_2O and washing gave 770 mg of an oil which was chromatographed on 25 g of Al_2O_3. After the column was developed with petroleum ether-C_6H_6 1:1, C_6H_6 eluted 320 mg of pure 1k, a single compound by tlc and glc, but which refused to crystallize; ir 3248 (OH), 1592 (arom. ring) cm^{-1}.

Anal. Calcd for $C_{29}H_{44}O$: C, 85.23; H, 10.8 . Found: C, 85.37; H, 10.83.

20-Methyl-5β-pregnan-12α-ol (1m). The acetate 10b (600 mg) was reduced with 200 mg of $LiAlH_4$ in 25 ml of tetrahydrofuran at reflux for 6 hr. The usual isolation gave 500 mg of crude product which was recrystallized from MeOH-H_2O: 1m, m.p. 122-124°; ir 3559 (OH) cm^{-1}. Two more recrystallizations from MeOH-H_2O gave the analytical sample, m.p. 140-141°.

Anal. Calcd for $C_{22}H_{38}O$: C, 82.95; H, 12.02. Found: C, 83.03; H, 11.86.

12α-Acetoxy-3α-hydroxy-5β-pregnan-20-one (3a). 3α,12α-Diacetoxy-5β-pregnan-20-one (10 g) was selectively hydrolyzed by standing at room temp 16 hr in 200 ml of MeOH containing 4 ml of 11.5 M HCl. Slow addition of 200 ml of H_2O pptd the product (8.5 g), which was recrystallized in MeOH-H_2O to give the analytical sample, m.p. 208-209°; ir 3946 (OH), 1703 (acetate C=O), 1690 (keto C=O), 1265 (acetate C-O) cm^{-1}.

Anal. Calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.69. Found: C, 73.57; H, 9.70.

12α-Acetoxy-3α-tosyloxy-5β-pregnan-20-one (3b). Tosylation of 8.5 g of 3a with 8.5 g of p-toluenesulfonyl chloride in 30 ml of dry pyridine was carried out at 4° for 16 hr. Dilution with H_2O, extraction into C_6H_6 and washing gave 13.0 g of yellow crude 3b, which was decolorized with C and recrystallized from MeOH to give the analytical sample, m.p. 158-159°; nmr δ 7.78 (d, phenyl) 2H, 7.30 (d, phenyl) 2H, 5.06 (broad s, 12β-H) 1H, 4.34 (broad m, 1H), 2.36 (s, tosylate CH_3) 3H, 2.06 (s) 3H, 1.93 (s) 3H, 0.78 (s, 19-CH_3) 3H, 0.57 (s, 18-CH_3) 3H;

ir 1718 (acetate C=O), 1695 (keto C=O), 1590 (arom. ring), 1240 (acetate C-O), 1165 (SO_3) cm^{-1}.

Anal. Calcd for $C_{30}H_{42}O_6S$: C, 67.89; H, 7.98; S, 6.04. Found: C, 67.31; H, 7.70; S, 6.32.

12α-Acetoxy-5β-pregn-3-en-20-one (4). Refluxing a solution of 6 g of 3b in 20 ml of 2,4,6-collidine for 3.5 hr, pouring the solution into 400 ml of ice-cold 4 M HCl, extracting 3 times with C_6H_6, washing and drying gave 4.5 g of crystalline product. Recrystallization in MeOH-H_2O gave the analytical sample, m.p. 102-103° (one preparation m.p. 110-111.5°); nmr δ 5.6 (broad m, 3-H,4-H) 2H, 5.18 (broad t, 12β-H) 1H, 2.12 (d) 3H, 2.02 (s) 3H, 0.94 (s, 19-CH_3) 3H, 0.69 (s, 18-CH_3) 3 H; ir 1720 (acetate C=O), 1695 (keto C=O), 1602 (weak, C=C), 1240 (acetate C-O) cm^{-1}.

Anal. Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.93; H, 9.58.

12α-Acetoxy-5β-pregnan-20-one (5a). Crude 4 (8.0 g.) was hydrogenated in MeOH (200 ml.) over PtO_2 (450 mg.) at 50 psi overnight. The product was chromatographed from CCl_4 on Al_2O_3 (200 g.); fractions eluted by CCl_4-C_6H_6 mixtures and by C_6H_6 were combined and recrystallized in MeOH-H_2O to give the product, m 82-84° (lit[2] 84-86°); 1723 (acetate C=O), 1697 (keto C=O), 1240 (acetate C-O) cm^{-1}.

24-Nor-5β-chol-22-en-12α-yl acetate (5c). A solution of 25 g of 12α-acetoxy-5β-cholanoic acid (5d)[19] in 600 ml of benzene was distilled in a N_2 atmosphere until 100 ml had collected. Cupric acetate (500 mg), anh pyridine (1.0 ml), and vacuum-dried $Pb(OAc)_4$ (45 g) were added and the mixture refluxed 4 hr under N_2. The suspension was washed twice with 200 ml of ethylene glycol, 3 times with 3 M HCl, and 6 times with 5% NaOH. Acidification of the alkaline solution pptd 10 g of recovered 5d. The C_6H_6 solution was dried and evaporated to give 12 g of olefin, which was chromatographed from petroleum ether on Al_2O_3. Elution with petroleum ether-CCl_4 1:1 gave pure 5c in 43% conversion, m.p. 103-104°; nmr δ 5.7 (broad m), 5.12 (broad t), 4.9 (d), 2.13 (s, acetate CH_3), 1.02 (s, 21-CH_3), 0.93 (s, 19-CH_3), 0.80 (s, 18-CH_3); ir 1734 (C=O), 1627 (C=C), 908 (=CH_2) cm^{-1}.

Anal. Calcd for $C_{24}H_{40}O_2$: C, 80.59; H, 10.82. Found: C, 80.89; H, 10.88.

24-Nor-5β-chol-20(22)-en-12α-yl acetate (5b). Ethylenediamine (250 ml, previously dried over Na and distilled) was heated to 80-85° under N_2 and lithium ribbon (12 g) was added in pieces. After the solution was refluxed 20 min, a hot solution of 12 g of 5c in a minimum volume of ethylenediamine was added. After refluxing 12 min, the solution was cooled and diluted with ice water. Extraction into C_6H_6 and washing gave 10.5 g of crude 20(22)-olefin, an oil which gave a single spot by tlc; ir 3460 (OH), 3020 (olefinic CH) cm^{-1}.

It was converted to the acetate 5b in refluxing Ac_2O containing p-TsOH. Chromatography of the crude product on Al_2O_3 and elution with CCl_4-petroleum ether 1:1 gave a colorless oil which darkened on standing at room temp; nmr δ 5.5 (broad m), 5.0 (broad s), 2.05 (s, acetate CH_3), 0.93 (s, 19-, 21- and 23-CH_3), 0.60 (s, 18-CH_3); ir 1730 (acetate C=O), 1644 (C=C), 1245 (acetate C-O) cm^{-1}.

21-Benzyl-12α-hydroxy-5β-pregnan-20-one (7). A solution of 1b (2.5 g) and benzaldehyde (2.5 g) in 0.2 N ethanolic KOH (60 ml) stood at room temp in the dark for 12 hr. Neutralization with 2 N HCl, extraction into ether and washing gave 3.5 g of crude product, which was chromatographed on 100 g of Al_2O_3. The column was developed with C_6H_6, then ether-C_6H_6 1:4 eluted 2.4 g of the benzylidine derivative 12α-hydroxy-23-phenyl-21,24-bisnor-5β-chol-22-en-20-one (6), m 173-174°; nmr δ 7.9-6.8 (m, 22-H, 23-H, phenyl) 7H, 3.95 (broad s, 12β-H) 1H, 3.5 (m, 17α-H) 1H, 0.92 (s, 19-CH_3) 3H, 0.63 (s, 18-CH_3) 3H; ir 3597 (OH), 3448 (OH), 1672 (C=O), 1597 (C=C) cm^{-1}.

Anal. Calcd for $C_{28}H_{38}O_2$: C, 82.70; H, 9.42. Found: C, 82.62; H, 9.38.

Hydrogenation of the benzylidine derivative (2.4 g.) over PtO_2 (200 mg.) in MeOH (200 ml.) at 50 psi gave 7, m 49-51°; nmr δ 7.30 (s, phenyl), 3.92 (broad s, 12β-H), 2.85 (s), 0.95 (s, 19-CH_3), 0.60 (d, 18-CH_3); ir 3450 (OH), 1678 (C=O) cm^{-1}.

20-(2-Phenylethyl)-5β-pregnan-12α-yl acetate (10a). A solution of 1 g of 1b in 20 ml of anh C_6H_6 was added slowly to 2-phenylethylmagnesium bromide (prepared from 2 ml of 2-phenylethyl bromide and 0.4 g of Mg) in 20 ml of Et_2O. After the ether was distilled out, the solution was refluxed 12 hr. The usual workup gave 1.5 g of crude product which was dissolved in C_6H_6 and chromatographed on 50 g of Al_2O_3. The column was developed with benzene, benzene-ether mixtures, ether, and finally 1% MeOH in benzene. The first fraction, 150 mg., exhibited OH (3440) and aromatic ring (1582 cm^{-1}) bands in the ir, and is, presumably, the C-20 epimer produced in smaller amount. The second fraction, 350 mg. of 8 contaminated by a minor amount of 1b, an oil; nmr δ 7.26 (s, phenyl), 4.05 (broad s), 3-2.6 (m), 0.90 (s, CH_3), 0.85 (s, CH_3), 0.62 (s, 18-CH_3); ir 3400 (OH), 1680 (C=O), 1600 cm^{-1} (arom. ring). The third fraction, 250 mg., lacked aromatic bands in the ir and was not identified.

The second fraction was dehydrated by 4 hours of refluxing in AcOH 5 ml.) and Ac_2O (2.5 ml.). Partitioning the product in H_2O-C_6H_6, washing and drying the C_6H_6 layer, and evaporating gave 0.3 g. of 9a contaminated by 1b), and oil; nmr δ 7.26 (s, phenyl) 5H, 4.93 (broad s, 12β-H) 1H, 2.00 (s, acetate CH_3) 3H, 0.90 (s, 19-CH_3, 21-CH_3) 6H, 0.63 (s, 18-CH_3); ir 1730 (acetate C=O), 1600 (arom. ring), 1240 cm^{-1} (acetate C-O).

Hydrogenation of 300 mg. of 9a in 20 ml. of MeOH over PtO_2 (50 mg.) at 50 psi for 12 hours, and chromatography on 20 g. of Al_2O_3 gave 155 mg. of 10a (eluted by CCl_4 and CCl_4-C_6H_6 mixtures up to 1:1) and 100 mg. of the acetate of 1b (eluted by Et_2O-C_6H_6 1:4). The product 10a was an oil;

nmr δ 7.2 (s, phenyl) 5H, 5.1 (s, 12β-H) 1H, 2.0 (s, acetate CH_3) 3H, 0.89 (s, 19-CH_3, 21-CH_3) 6H, 0.70 (s, 18-CH_3) 3H; 1730 (C=O), 1605 (arom. ring), 1247 cm^{-1} (acetate C-O).

Anal. Calcd for $C_{31}H_{46}O_2$: C, 82.61; H, 10.29. Found: C, 82.86; H, 10.21.

20-Methyl-5β-pregnan-12α-yl acetate (10b). A solution of 800 mg of 20-methyl-5β-pregn-3-en-12α,20-diol (1f)[3] in 10 ml of AcOH and 5 ml of Ac_2O was refluxed 2.5 hr. The solution was dissolved in C_6H_6 and washed to give 830 mg of crude 9b [ir 1754 (C=O), 1660 (C=C), 1247 (C-O) cm^{-1}], which was not purified, but was hydrogenated in MeOH (50 ml.) over PtO_2 (100 mg.) at 50 psi overnight to give 10b, an oil; ir 1720 (C=O), 1238 (C-O) cm^{-1}.

12α-Acetoxy-5β-pregnan-20-one ethyleneketal (11a). The ketal was prepared in the usual way from 1b-acetate, ethylene glycol, p-TsOH and refluxing benzene; it recrystallized from EtOH-H_2O to give long, white crystals mp 113-115°. Recrystallization from MeOH-H_2O gave the analytical sample mp 115.5-117°; nmr δ 5.12 (t, 12β-H) 1H, 3.90 (m, ketal methylenes) 4H, 2.08 (s, acetate CH_3) 3H, 1.22 (s, 21-CH_3) 3H, 0.90 (s, 19-CH_3) 3H, 0.82 (s, 18-CH_3) 3H; ir 1742 (C=O), 1250 (C-O) cm^{-1}.

Anal. Calcd for $C_{25}H_{40}O_4$: C, 74.12; H, 9.96. Found: C, 74.27; H, 9.89.

12α-Acetoxy-21-bromo-5β-pregnan-20-one ethyleneketal (11b). A solution of 1.5 ml of Br_2 in 4 ml of CH_2Cl_2 was added slowly to a stirred suspension containing 3.5 g of 11a and 1.5 g of CaO in 30 ml of CH_2Cl_2 at -10°. The mixture was stirred for an additional 30 min, then poured into 50 ml of 10% aq $NaHCO_3$; the organic layer was washed twice with 50 ml of 5% aq $Na_2S_2O_3$ and with H_2O, dried and evaporated in vacuo to give 4 g of crude 11b. It was chromatographed on 120 g of Al_2O_3; after development with petroleum ether-C_6H_6 mixtures up to 1:1, the column yielded two bromine-containing products: A, 1.6 g, eluted by petroleum ether-C_6H_6 3:7, C_6H_6, and C_6H_6-Et_2O 4:1; B, 1.4 g, eluted by C_6H_6-Et_2O 3:2 and Et_2O. The latter was not characterized, but the former was recrystallized from EtOH-H_2O to give 11b, m 129-130°; nmr δ 5.20 (m, 12β-H) 1H, 4.7-3.7 (m, ketal methylenes) 4H, 2.10 (s, acetate CH_3) 3H, 0.93 (s) 3H, 0.87 (s, 19-CH_3) 2H, 0.66 (d, 18-CH_3) 3H.

Anal. Calcd for $C_{25}H_{39}O_4Br$: C, 61.01; H, 7.94; Br, 16.23. Found: C, 62.00; H, 8.13; Br, 16.52.

21-Nor-5β-cholan-12α-ol (14). A solution of 1.2 g of 11b in 100 ml of C_6H_6 was added slowly to a solution of ethylmagnesium bromide (prepared from 5.43 g of EtBr and 1.22 g of Mg) in 75 ml of Et_2O. The mixture was heated without a condenser until the temp reached 80°, then refluxed for 12 hr. The cooled reaction mixture was worked up as usual giving 1.0 g of crude 12. It was hydrolyzed by refluxing for 24 hr in 100 ml of acetone containing 1 ml of 10 M HCl. The usual

workup gave 700 mg of an oil with ir bands for OH (3370 cm^{-1}) and C=O (1695 cm^{-1}), indicating that hydrolysis of both ester and ketal groups had occurred. A Wolff-Kishner reduction similar to that described for 1a gave 470 mg of solid 14. Recrystallization from MeOH-H_2O gave the analytical sample, m.p. 91-92°; ir 3289 (OH) cm^{-1}.

Anal. Calcd for $C_{23}H_{40}O \cdot 1/3CH_3OH$: C, 81.67; H, 12.13. Found: C, 81.89; H, 11.74.

Acetylation yield and rate determinations. The 24-hr yields (Table II) were determined by tlc and glc technics dexcribed previously,[5] except that reactions were run in a dry box at 28±1°. Rates were determined by the glc method described previously;[3] reactions were followed to at least 70% completion (3-22 days). A computor program using Fortran IV CDC version 3.0 for use with a Control Data 6600 computor[12] was used to calculate rates based on the standard expression

$$k_2 = \frac{1}{t(b-a)} \ln \frac{a(b-x)}{b(a-x)}$$

where a = starting concentration of steroid, b = starting concentration of acetic anhydride, and x = concentration of each having reacted at time t. The value of a was taken as 0.370 M based on the sample of steroid weighed, and b was assumed to be 1.065 M based on the volume of Ac_2O pipetted. At appropriate intervals duplicate aliquots were injected into the gas chromatograph; peak area ratios were everaged to calculate mmols of ROH and ROAc. A typical run for 1a is as follows:

Hr	Peak Area Ratio ROH/ROAc	mmol ROH	mmol ROAc	$\ln\frac{(b-x)}{(a-x)}$	Completion, %
2	160/25, 196/28	0.322	0.048	1.145	13.0
3	180/31, 174/28	0.317	0.053	1.155	14.3
4	180/35, 166/34	0.308	0.062	1.175	16.6
5	310/65, 250/61	0.302	0.068	1.190	18.5
6	220/53, 190/49	0.296	0.074	1.203	20.0
7	165/49, 210/57	0.288	0.082	1.222	22.1
8	173/59, 160/53	0.277	0.093	1.250	25.2
12	214/127, 237/142	0.232	0.138	1.380	37.4
18	190/149, 172/134	0.208	0.162	1.464	43.9
24	66/70, 94/105	0.177	0.193	1.588	52.1
36	72/132, 79/145	0.131	0.239	1.838	64.7
48	60/151, 55/143	0.104	0.266	2.033	71.9
72	43/156, 30/141	0.072	0.298	2.354	80.4

A least squares plot of $\ln(b-x)/(a-x)$ vs time permitted the calculation $k_2 = 7.32 \times 10^{-6}$ $M^{-1}sec^{-1}$; three other determinations gave 9.51, 8.39 and 8.31 $\times 10^{-6}$. The mean, 8.38×10^{-6} is reported in Table III; 4 determinations were made for all compounds except 1k, for which only 2 determinations were made.

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REFERENCES

1. Taken from the Ph.D. thesis of Kenneth Atkinson, Indiana University.

2. Blickenstaff, R. T., and Orwig, B., J. ORG. CHEM. 34, 1377 (1969).

3. Blickenstaff, R. T., Atkinson, K., Breaux, D., Foster, E., Kim, Y., and Wolf, G. C., J. ORG. CHEM. 36, 1271 (1971).

4. It differs from the other compounds by having also a double bond in the A ring, but as will be shown later, this is not considered the cause of unreactivity of the hydroxyl group.

5. Wolf, G. C., Foster, E. L., and Blickenstaff, R. T., J. ORG. CHEM. 38, 1276 (1973).

6. Narula, A. S., and Sukh, D., TETRAHEDRON 27, 1119 (1971).

7. Barton, D. H. R., McCapra, F., May, P. J., and Thudium, F., J. CHEM. SOC., 1297 (1960).

8. Narula, A. S., and Sukh, D., TETRAHEDRON LETTERS, 1733 (1969); see also ref. 6.

9. Bergmann, E. D., Rabinovitz, M., and Levinson, Z. H., J. AM. CHEM. SOC. 81, 1239 (1959).

10. The 3-double bond of 1f is not likely to be responsible for its low reactivity. Introduction of a 3-double bond into 1i increased acetylation yield (see 1h).

11. Amos, S. S., and Ziegler, P., CAN. J. CHEM. 37, 345 (1959).

12. Program written by James Baker, these laboratories, to be published.

13. Alauddin, M., and Martin-Smith, J., J. ORG. CHEM. 28, 886 (1963); Ahmed, S., Alauddin, M., Caddy, B., Martin-Smith, M., Sidwell, W. T. L., and Watson, T. R., AUST. J. CHEM. 24, 521 (1971).

14. Huffman, J. W., Alabran, D. M., Bethea, T. W., and Ruggles, A. C., J. ORG. CHEM. 29, 2963 (1964).

15. Gopalakrishna, E. M., Cooper, A., and Nordon, D. A., ACTA CRYST. B25, 143 and 1601 (1969); Komeichi, Y., Osawa, Y., Duax, W. L., and Cooper, A., STEROIDS 15, 619 (1970).