analytical sample: mp 156.5–157 °C; TLC ($CHCl_3$–MeOH, 95:5) R_f 0.38; IR, NMR, and mass spectra were as expected. Anal. ($C_{29}H_{42}ClNO$) C, H, N.

N-[(20S)-3β-Hydroxy-5-pregnen-20-yl]-p-chlorophenylacetamide (3e) (0.25 g, 18%), mp 175–177 °C, was isolated when the aforementioned column was eluted with chloroform–methanol (98:2). An analytical sample was obtained after recrystallization from acetone: mp 178.5–180.5 °C; TLC ($CHCl_3$) R_f 0.27; IR, NMR, and mass spectra were as expected. Anal. ($C_{29}H_{40}ClNO_2$) C, H, N.

3β-Hydroxy-22,23-dinor-5-cholenic Acid N-Benzylamide (4). 3β-Acetoxy-22,23-dinor-5-cholenic acid (1.00 g, 2.60 mmol) was suspended in 20 mL of anhydrous ether, and 0.7 mL of distilled thionyl chloride was added along with a few drops of 10% pyridine–benzene solution. The mixture was stirred at room temperature for 40 min. An additional 0.3 mL of thionyl chloride was added and the mixture was allowed to stand at room temperature for 2 h. The excess thionyl chloride was removed under reduced pressure by several coevaporations with dry benzene. The crude acid chloride was then dissolved in 25 mL of dry benzene, benzylamine (0.60 g, 6.18 mmol) was added with ice bath cooling, and the mixture was allowed to stand at room temperature for 16 h. Evaporation of the solvent and recrystallization of the crude amide from methanol gave 700 mg (57%) of the desired amido ester. A portion of the amido ester was hydrolyzed with 2% methanolic KOH to give desired amide **4**: mp 234–235 °C (acetone); TLC ($CHCl_3$–MeOH, 95:5) R_f 0.79; IR, NMR, and mass spectra were as expected. Anal. ($C_{29}H_{41}NO_2$) C, H, N.

Enzyme-Inhibition Studies. Materials and Methods. Mature bovine adrenals were purchased frozen from Pel-Freez Biologicals, Inc., Rogers, AR. [26-^{14}C]Cholesterol (specific activity 46.0 mCi/mmol) and [7-^{3}H]cholesterol (specific activity 10.9 Ci/mmol) were obtained from New England Nuclear Corp. The incubation vials were the same as the scintillation vials listed below. A Precision Scientific (catalog no. 68351-C) heated vacuum desiccator was used to volatilize isocaproic acid. All scintillation counting was done using a Packard Model 2425 Tri-Carb liquid scintillation spectrometer. The samples were counted in 22-mm glass vials with polyethylene-lined caps using 10 mL of Budget Solve (all from Research Products International Corp., Elk Grove Village, IL).

All other materials and methods were as described previously.[5]

Acknowledgment. The authors are grateful to John Ip for his assistance during the biological evaluation and to Richard Dvorak for obtaining mass spectra.

Inhibition of Cholesterol Side-Chain Cleavage. 4.[1] Synthesis of A or B Ring Modified Azacholesterols

Matthias C. Lu,* Norma G. Delaney,

Department of Medicinal Chemistry, College of Pharmacy, University of Illinois at the Medical Center, Chicago, Illinois 60612

and Raymond E. Counsell

Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan 48109. *Received March 27, 1981*

A number of A or B ring modified 20- and 22-azacholesterol analogues (**1** and **2**, respectively) were synthesized in an attempt to ascertain the structural requirements for inhibition of the cholesterol side-chain cleavage reaction in bovine adrenocortical mitochondrial acetone powder preparations. The inhibition studies of these analogues revealed that (1) the 3-methyl ethers were as active as the parent compounds and that (2) reduction of the Δ^5 double bond greatly lessened the inhibitory activity. The studies demonstrated a crucial role of the Δ^5 double bond for inhibitory activity, while a free hydroxyl group at C-3 is not essential for this action. Furthermore, as in the parent compounds, 22-azacholesterol analogues were more potent than their 20-azacholesterol counterparts.

The enzymatic cleavage of the cholesterol side chain between C-20 and C-22 to produce pregnenolone is a key step in the biosynthesis of adrenal steroid hormones. This reaction is catalyzed by a specific cytochrome P-450 of adrenocortical mitochondria,[2] and (22*R*)-22-hydroxycholesterol and (20*R*,22*R*)-20,22-dihydroxycholesterol appear to be obligatory intermediates in this reaction.[3]

Inhibitors of cholesterol side-chain cleavage (CSCC) are of interest not only as potential therapeutic agents in diseases associated with hyperfunctioning adrenal glands[4] but also as biochemical tools in the elucidation of the mechanism of the enzymatic reaction.[4b,5] Our previous studies with azacholesterols[6] revealed that the 22-azacholesterol epimer (**1a**) having the same configuration as cholesterol at C-20 was clearly the most active inhibitor. 20-Azacholesterol (**2a**) was somewhat less inhibitory, but the β configuration at C-17 was not essential for its inhibitory action. Furthermore, our recent studies[7] and the studies of Burstein et al.[8] have indicated that other structural modifications of the side chain can lead to potent inhibitors of CSCC. In view of the importance of the steroid nucleus in the substrate specificity of the CSCC enzyme,[9] the preparation of a number of A or B ring modified analogues of both 22- and 20-azacholesterol (**1** and **2**, respectively) was undertaken to further ascertain the structural requirements for the CSCC system in adrenal preparations.

(1) Taken in part from the Ph.D. Dissertation of N.G.D., University of Illinois at the Medical Center, Chicago, IL, 1980. For the previous paper in this series, see ref 7.
(2) L. D. Wilson and B. W. Harding, *Biochemistry*, **9**, 1621 (1970).
(3) S. Burstein and M. Gut, *Steroids*, **28**, 115 (1976).
(4) (a) T. E. Temple and G. W. Liddle, *Annu. Rev. Pharmacol.*, **10**, 199 (1970); (b) P. E. Graves, V. I. Uzgiris, and H. A. Salhanick, *Steroids*, **35**, 543 (1980).
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(6) M. C. Lu, P. Afiatpour, C. B. Sullivan, and R. E. Counsell, *J. Med. Chem.*, **15**, 1284 (1972).
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(8) S. Burstein, Y. Letourneux, H. L. Kimball, and M. Gut, *Steroids*, **27**, 361 (1976).
(9) (a) M. Morisaki, C. Duque, N. Ikekawa, and M. Shikita, *J. Steroid Biochem.*, **13**, 545 (1980); (b) E. C. Trout, Jr., and W. Arnett, *Proc. Soc. Exp. Biol. Med.*, **136**, 469 (1971).

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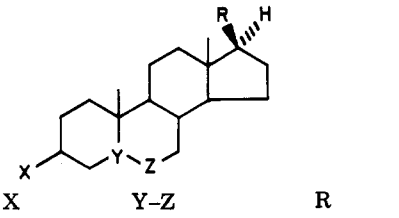
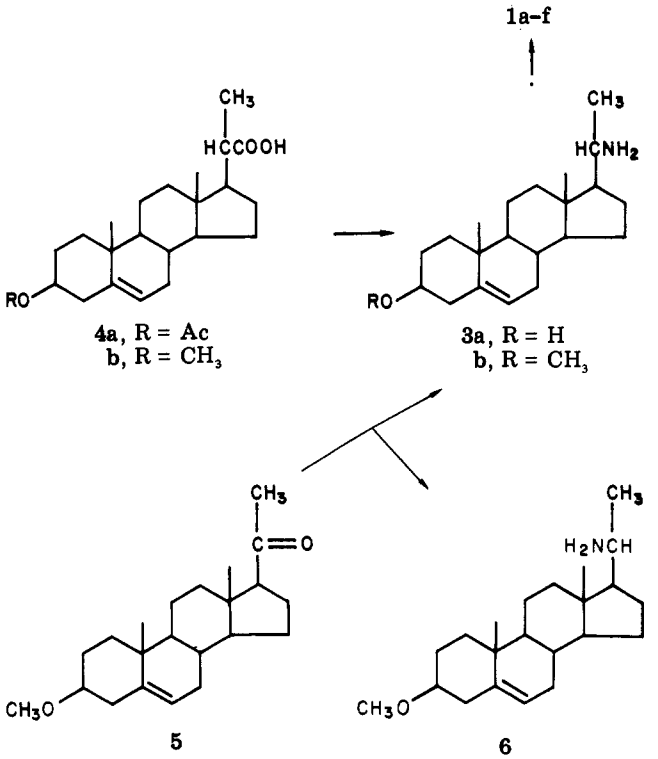


	X	Y–Z	R
1a	β-OH	C=CH	
b	β-OH	(5α)CHCH$_2$	
c	β-OCH$_3$	C=CH	
d	β-OCH$_3$	C=CH	
e	β-OCH$_3$	(5α)CHCH$_2$	
f	=O	(5α)CHCH$_2$	
2a	β-OH	C=CH	
b	β-OH	(5α)CHCH$_2$	
c	β-OCH$_3$	C=CH	
d	β-OCH$_3$	(5α)CHCH$_2$	
e	α-OCH$_3$	(5α)CHCH$_2$	

Scheme I. Synthesis of 22-Azacholesterol Analogues



Preparation of 22-Azacholesterol Analogues (1). All of the desired A or B ring modified 22-azacholesterol analogues (1) were readily prepared from the known 20*S* primary amine **3a** or its corresponding 3β-methyl ether **3b**. Amine **3a** was prepared from acid **4a** by the procedure of Julian et al.[10] involving a Curtius rearrangement. The desired starting amine **3b** was prepared via two synthetic routes as depicted in Scheme I.

Conversion of pregnenolone 3-methyl ether **5** to its oxime, followed by reduction with sodium in 1-propanol, gave a 1:1 mixture of the epimeric amines **3b** and **6** in 81% yield. The epimers were separated by column chromatography on silica gel, yielding 45% each of the less polar (20*R*)-amine **6** and the more polar (20*S*)-amine **3b**, with 10% overlap. The stereochemistry of the amines was initially assigned according to the general rule[11] that for 20-aminopregnanes the 20*R* epimer is less polar on TLC than the 20*S* epimer. Furthermore, it has been reported that in the NMR analysis of 20-aminopregnanes the 21-methyl resonance for the 20*S* epimer is downfield relative to that for the 20*R* epimer by 0.07–0.11 ppm.[11] In keeping with this analysis, the 21-methyl resonance for (20*S*)-amine **3b** was 0.10-ppm downfield relative to (20*R*)-amine **6**. Finally, the structural assignment was unequivocally verified when (20*S*)-amine **3b** was prepared in 89% yield via a stereospecific Curtius rearrangement from 3β-methoxy-22,23-dinor-5-cholenic acid (**4b**) by a direct adaptation of Julian's procedure.[10]

Reductive alkylation (method A) of (20*S*)-amine **3b** with isovaleraldehyde and sodium borohydride ($NaBH_4$) afforded a mixture of secondary amines. Column chromatographic separation gave **1c** and **1d** in 81 and 11% yield, respectively. The formation of minor product **1d** in this reaction was anticipated, since the isovaleraldehyde obtained from Aldrich Chemical Co. contained β-methylbutyraldehyde as a contaminant which could not be separated by distillation.

22-Azacholesterol (**1a**) and its methyl ether **1c** were then subjected to catalytic hydrogenation (method B) over platinum catalyst, yielding the (5α)-5,6-dihydro analogues **1b** and **1e** in 76 and 62% yield, respectively. It has been well established that catalytic hydrogenation of 3β-substitutued Δ^5-steroids yields the 5α-isomer almost exclusively.[12]

Oppenauer oxidation of **1b** with aluminum *tert*-butoxide and cyclohexanone in refluxing toluene gave **1f** in 55% yield after chromatographic purification.

Preparation of 20-Azacholesterol Analogues (2). 20-Azacholesterol **2a** was prepared from **7a** (Scheme II) by an adaptation of the literature procedure.[13] Compound **2c** was readily prepared (method C) from 3β-methoxy-5-androsten-17-one[14] (**7b**) and 4-methylpentylamine via lithium aluminum hydride reduction of the imine formed, followed by reductive N-methylation of intermediate **8b** with formaldehyde and $NaBH_4$. In a similar fashion, **2e** was prepared from 3α-methoxy-5α-androstan-17-one.[15]

Catalytic hydrogenation of **2a** and **2c** over platinum as described before (method B) yielded the desired (5α)-

(10) P. L. Julian, E. W. Meyer, and H. C. Printy, *J. Am. Chem. Soc.*, **70**, 887 (1948).
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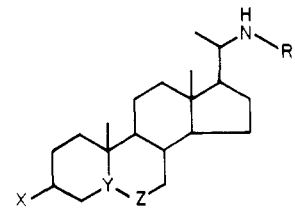
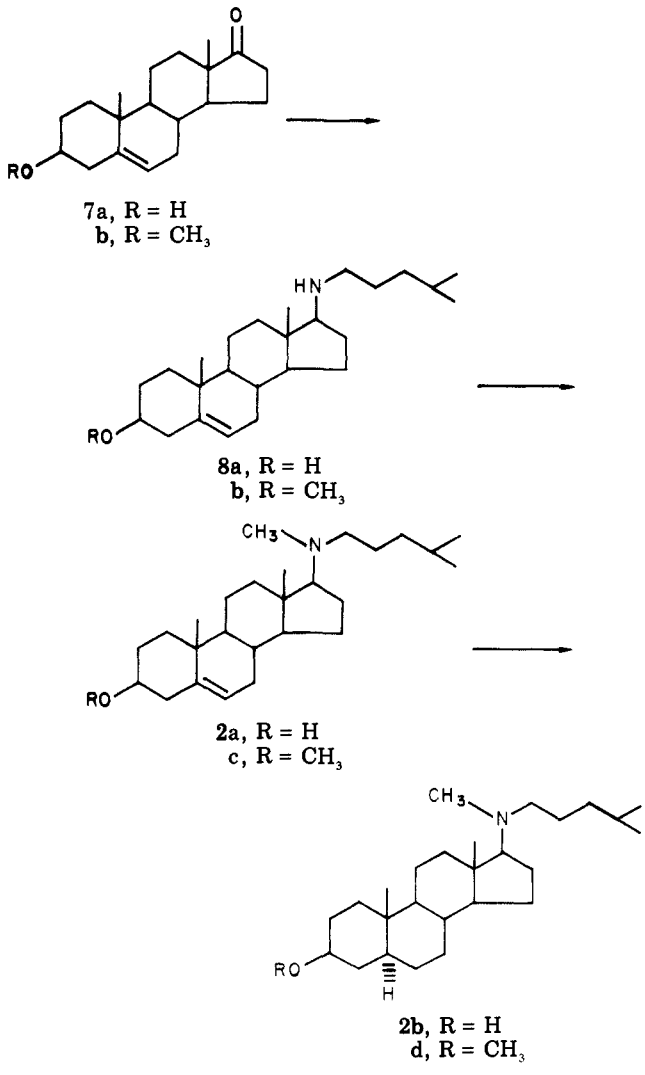
Table I. Cholesterol Side-Chain Cleavage Inhibitory Activity of Some A or B Ring Modified 22-Azacholesterol Analogues



no.	Substitution X	Substitution Y–Z	Substitution R	% inhibn of side-chain cleavage at various inhib concns[a] 100 μM	10 μM	1 μM
1a	(β)-OH	C=CH	3-Me-Bu	93 ± 8	75 ± 11	10 ± 9
1b	(β)-OH	(5α)CHCH$_2$	3-Me-Bu	28 ± 8		
1c	(β)-OCH$_3$	C=CH	3-Me-Bu	99 ± 5	86 ± 6	14 ± 8
1d	(β)-OCH$_3$	C=CH	2-Me-Bu	100 ± 6	89 ± 7	12 ± 6
1e	(β)-OCH$_3$	(5α)CHCH$_2$	3-Me-Bu	21 ± 4		
1f	=O	(5α)CHCH$_2$	3-Me-Bu	3 ± 8		

[a] Error limits are standard deviations.

Scheme II. Synthesis of 20-Azacholesterol Analogues



7a, R = H
b, R = CH$_3$

8a, R = H
b, R = CH$_3$

2a, R = H
c, R = CH$_3$

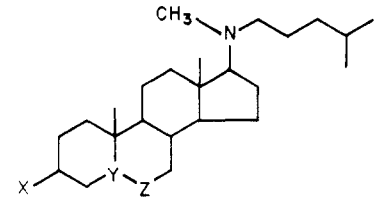
2b, R = H
d, R = CH$_3$

5,6-dihydro analogues **2b** and **2d** in 92 and 32% yield, respectively.

Results and Discussion

Inhibition Studies. Assays for CSCC activity were performed as described previously.[6,7] The inhibitory activity of the test compounds was determined at a final concentration of 100 μM or less, and each percent inhibition represents the results of at least six data points and two separate experiments. The error limits are the standard deviations from the mean.

Table II. Cholesterol Side-Chain Cleavage Inhibitory Activity of Some A or B Ring Modified 20-Azacholesterol Analogues



no.	substitution X	substitution Y–Z	% inhibn of side-chain cleavage at various inhib concns[a] 100 μM	10 μM
2a	(β)-OH	C=CH	78 ± 10	29 ± 6
2b	(β)-OH	(5α)CHCH$_2$	26 ± 7	
2c	(β)-OCH$_3$	C=CH	86 ± 14	30 ± 11
2d	(β)-OCH$_3$	(5α)CHCH$_2$	5 ± 14	
2e	(α)-OCH$_3$	(5α)CHCH$_2$	3 ± 8	

[a] Error limits are standard deviations.

Tables I and II show the inhibitory activities of the 22- and 20-azacholesterol analogues tested, respectively. The inhibitory activities of the parent compounds **1a** and **2a** were in good agreement with our previous studies.[6]

In the A or B ring modified 22-azacholesterol series (Table I), the following were observed: (1) replacement of the 3β-hydroxyl group by 3β-methoxyl (**1c** vs. **1a**) had essentially no effect on the inhibitory activity, (2) reduction of the Δ^5 double bond of either 22-azacholesterol or its methyl ether drastically decreased the inhibitory activity (**1a** vs. **1b**; **1c** vs. **1e**), (3) the 3-oxo,(5α)-5,6-dihydro analogue, **1f**, was inactive, and (4) replacement of the 3-methylbutyl side chain with a 2-methylbutyl side chain (**1c** vs. **1d**) had no effect on the inhibitory activity.

In the A or B ring modified 20-azacholesterol series (Table II) the same trends were observed: (1) the 3-methyl ether was as active as the parent compound (**2c** vs. **2a**), (2) reduction of the Δ^5 double bond greatly lessened the inhibitory activity (**2a** vs. **2b**; **2c** vs. **2d**), and (3) compound **2e**, in which the Δ^5 double bond was reduced and the 3β-hydroxyl group was replaced by a 3α-methoxyl group, was devoid of activity.

The results of the inhibition studies of the A or B ring modified 20- and 22-azacholesterol analogues indicate that

Table III. A or B Ring Modified Azasterols

no.	method (yield, %)	crystn solvent	mp, °C	formula	anal.
1a	A (80)	acetone	129–130	$C_{26}H_{45}NO$	known[a]
1b	B (76)	acetone	140–141	$C_{26}H_{47}NO$	C, H, N
1c	A (81)	acetone	82–83	$C_{27}H_{47}NO$	C, H, N
1d	A (11)	acetone	86–86.5	$C_{27}H_{47}NO$	C, H, N
1e	B (62)	acetone–H_2O	67–67.5	$C_{27}H_{49}NO$	C, H, N
1f	b (55)	EtOH–H_2O	127.5–128.5	$C_{26}H_{45}NO$	C, H, N
2a	C (48)	acetone–H_2O	125–125.5	$C_{26}H_{45}NO$	known[a]
2b	B (92)	acetone	138–138.5	$C_{26}H_{47}NO$	C, H
2c	C (50)	acetone	59–61.5	$C_{27}H_{47}NO$	C, H
2d	B (32)	MeOH–acetone	38–40	$C_{27}H_{49}NO$	C, H
2e	C (52)	acetone–H_2O	68–69	$C_{27}H_{49}NO$	C, H

[a] See ref 13. [b] See Experimental Section.

a Δ^5 double bond is an absolute requirement for inhibitory activity toward the adrenal CSCC enzyme system. A free 3-hydroxyl group is not required, since the methyl ethers are equal in potency to their free hydroxyl counterparts. This is not surprising, since a free hydroxyl group is not strictly required for substrate activity[16] while the Δ^5 double bond appears to provide substrate specificity in the CSCC reaction by adrenal preparations.[9b,17]

Though the results of this investigation do not allow any conclusions to be drawn about the stereochemical requirements at C-3 for inhibition, since modification at C-3 as in the 3α-methoxy derivative **2e** or the 3-oxo derivative **1f** was accompanied by reduction of the Δ^5 double bond, it is highly probable that these requirements will also parallel those for substrate activity. Future studies using 22-azacholesterol analogues which retain the Δ^5 double bond and have modified stereochemistry at C-3 should prove informative in this regard.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 337 spectrophotometer. ^1H NMR spectra were obtained in $CDCl_3$ on a Varian T-60A spectrometer equipped with a Nicolet TT-7 Fourier transform accessory. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as the internal standard. Mass spectra were obtained at 70 eV using a Hitachi Perkin-Elmer RMU-D6 single-focusing mass spectrometer. TLCs were run on 2.5 × 10 cm strips coated with silica gel and a fluorescent indicator (Eastman chromagram sheet no. 13181). The following solvent systems were employed: A, $CHCl_3$–MeOH–concentrated NH_4OH (191.5:7:1.5); B, $CHCl_3$–MeOH–concentrated NH_4OH (94:5:1); C, $CHCl_3$–MeOH (95:5); D, benzene–ethyl acetate (1:1). Column chromatographic separations utilized J. T. Baker silica gel, 60–200 mesh. Height to diameter ratios were normally 25–35:1 with 40–50 g of silica gel per gram of mixture. Analyses of the 22-azacholesterol analogues were performed by Micro-Tech Laboratories, Skokie, IL, while analyses of the 20-azacholesterol analogues were done by Spang Microanalytical Lab, Ann Arbor, MI, and all agreed within ±0.4% of the theoretical values.

Steroidal precursors were obtained from Steraloids, Inc., Pawling, NY.

Reduction of 3β-Methoxy-5-pregnen-20-one Oxime and Separation of the Epimeric Amines (3b and 6). To a solution of 3β-methoxy-5-pregnen-20-one oxime[18] (10.88 g, 31.5 mmol) in 650 mL of 1-propanol was added 24 g of metallic sodium portionwise over a period of 1.25 h. After 4 h of reflux, most of the solvent was evaporated. The residue was diluted with 500 mL of water and the 1-propanol layer was separated. The 1-propanol layer was diluted with 100 mL of water and extracted with 250 mL of ether. The combined aqueous layers were again extracted with ether (2 × 150 mL), and finally, the combined organic extracts were washed with water (2 × 100 mL) and evaporated to dryness. The residue was redissolved in ether, dried (anhydrous $MgSO_4$), and evaporated to give 12.37 g of crude solid, mp 93–102 °C (lit.[19] mp 93–97 °C). Analysis by TLC (solvent C) indicated the presence of two amines together with faster moving impurities.

A 10-g portion of the amines was separated by chromatography on silica gel. Elution with chloroform–methanol–concentrated ammonium hydroxide (191.5:7:1.5) gave the **(20*R*)-3β-methoxy-5-pregnen-20-amine** (**6**; 3.28 g, 40%), mp 120–121.5 °C. An analytical sample was obtained after recrystallization from hexane: mp 123–123.5 °C; TLC R_f (solvent A) 0.52, R_f (solvent B) 0.87; ^1H NMR δ 0.75 (s, C-18 CH_3), 1.01 (s, C-19 CH_3), 1.01 (d, J = 6.2 Hz, C-21 CH_3), 1.37 (NH_2), 3.35 (s, OCH_3), 5.40 (br, =C*H*); mass spectrum, m/e (relative intensity) 331 (2, M^+), 44 (100, α-cleavage). Anal. ($C_{22}H_{37}NO$) C, H, N.

Further elution of the same column gave overlapping fractions of the two epimers (0.58 g, 9%). The **(20*S*)-3β-methoxy-5-pregnen-20-amine** (**3b**; 3.33 g, 38%) was finally eluted with chloroform–methanol–concentrated ammonium hydroxide (94:5:1). An analytical sample was obtained after recrystallization from hexane: mp 101–101.5 °C; TLC R_f (solvent A) 0.28, R_f (solvent B) 0.59; ^1H NMR δ 0.68 (s, C-18 CH_3), 1.00 (s, C-19 CH_3), 1.11 (d, J = 6.3 Hz, C-21 CH_3), 1.32 (NH_2) 3.35 (s, OCH_3), 5.38 (br, =C*H*); mass spectrum identical with **6**. Anal. ($C_{22}H_{37}NO$) C, H, N.

Stereospecific Synthesis of (20*S*)-3β-Methoxy-5-pregnen-20-amine (3b). This procedure was adapted from the one reported by Julian et al.[10] for the preparation of amine **3a** from acid **4a**. Thus, a solution of acid **4b**[20] (0.72 g, 2.00 mmol) in 50 mL of anhydrous ether containing a few drops of a 10% solution of pyridine in benzene was treated with 3 mL of thionyl chloride at room temperature for 3.5 h. After workup, the acid chloride was dissolved in 15 mL of acetone and the solution cooled to 10 °C. A solution of 0.26 g of sodium azide in 1.4 mL of water was then added portionwise so as not to increase the reaction temperature. After the addition was completed, the reaction mixture was stirred for 15 min, 15 mL of ice-cold water was added, and the off-white crystalline precipitate was filtered. The still moist crystals were added immediately to a solution of 2 mL of water in 15 mL of glacial acetic acid and heated gently on a steam bath. After approximately 5 min, nitrogen was evolved and the solid dissolved. The temperature of the solution was maintained at 60–65 °C for 1 h, and then the still hot solution was steam distilled until 75 mL of distillate was collected. The hot residue was made strongly basic with NaOH pellets and allowed to cool, and the product was extracted into dichloromethane (4 × 25 mL). The combined organic extracts were washed with water, dried (anhydrous Na_2SO_4), and evaporated to give **3b** as an off-white solid (0.59 g, 89%), mp 98–99 °C. The NMR, IR, and mass spectra and TLC (solvents A and B) of this sample were identical with those obtained for **3b** prepared by reduction of the oxime.

(16) (a) P. R. Raggatt and M. W. Whitehouse, *Biochem. J.*, 101, 819 (1966); (b) F. Gasparini, A. Wolfson, R. Hochberg, and S. Lieberman, *J. Biol. Chem.*, **254**, 6650 (1979).
(17) L. Aringer, P. Eneroth, and L. Nordström, *J. Steroid Biochem.*, 11, 1271 (1979).
(18) O. R. Rodig, P. Brown, and P. Zaffaroni, *J. Org. Chem.*, **26**, 2431 (1961).
(19) G. V. Rao and C. C. Price, *J. Org. Chem.*, **27**, 205 (1962).
(20) B. Riegel, E. W. Meyer, and J. Beiswanger, *J. Am. Chem. Soc.*, **65**, 325 (1943).

Reaction of (20*S*)-3β-Methoxy-5-pregnen-20-amine (3b) with Isovaleraldehyde and $NaBH_4$. General Method A. A solution of isovaleraldehyde (2.68 g, 31.12 mmol) in 50 mL of methanol was added dropwise over a period of 20 min to a solution of amine **3b** (2.06 g, 6.22 mmol) in 200 mL of methanol. After 21 h of reflux, the solution was cooled to 5–10 °C. Sodium borohydride (9.5 g, 250 mmol) was added portionwise over a period of 1 h, and the reaction mixture was allowed to stir at 5–10 °C for 2.25 h. Acetone (10 mL) was added dropwise to decompose the excess borohydride, and the reaction mixture was poured onto 200 g of crushed ice. After extraction into chloroform and workup, 3.36 g of a colorless oil was obtained. TLC (solvent C) showed the major product to be a secondary amine (R_f 0.40; nitroprusside–acetaldehyde,[21] green) with a small amount of another secondary amine (R_f 0.51). Some starting amine **3b** and other nonpolar impurities were also evident.

Purification of the mixture by chromatography on silica gel with chloroform–methanol–concentrated ammonium hydroxide (191.5:7:1.5) as eluent provided 1.03 g of a mixture of secondary amines, followed by 1.33 g (53%) of **(20*S*)-*N*-(3-methylbutyl)-3β-methoxy-5-pregnen-20-amine (1c)**, mp 81–81.5 °C. An anlytical sample was obtained after recrystallization from acetone (colorless needles): mp 82–83 °C; TLC R_f (solvent D) 0.26; ^1H NMR δ 0.90 (d, J = 5.8 Hz, C-26 and C-27 CH_3), other assignments similar to **3b**; mass spectrum, m/e (relative intensity) 401 (<1, M^+), 114 (100, α-cleavage). Anal. ($C_{27}H_{47}NO$) C, H, N.

The mixture of secondary amines obtained from the first column (1.03 g) was rechromatographed on a column of silica gel packed in benzene–ethyl acetate (7:3). Elution with benzene–ethyl acetate (1:1) yielded 280 mg (11%) of **(20*S*)-*N*-(2-methylbutyl)-3β-methoxy-5-pregnen-20-amine (1d)**, mp 85–85.5 °C. An analytical sample was obtained after recrystallization from acetone (colorless needles): mp 86–86.5 °C; TLC R_f (solvent D) 0.40; ^1H NMR as expected; mass spectrum identical with **1c**. Anal. ($C_{27}H_{47}NO$) C, H, N.

Further elution with the same solvent provided the remainder of **1c** (710 mg, 28%). The combined yield of **1c** after both columns was 81%.

(20*S*)-20-[(3-Methylbutyl)amino]-5α-pregnan-3β-ol (1b). General Method B. A solution of **1a** (0.91 g, 2.35 mmol) in 35 mL of 95% ethanol to which had been added 0.6 mL of concentrated HCl was hydrogenated over 125 mg of platinum oxide at atmospheric pressure and room temperature. After hydrogen uptake ceased (approximately 1.5 h), the catalyst was removed by filtration through Celite and the solvent was evaporated. The acidic residue was dissolved in 70 mL of 50% aqueous methanol and adjusted to pH 10–11 with K_2CO_3. Most of the methanol was evaporated, and the remaining aqueous suspension was diluted with 25 mL of water and extracted with chloroform (100 mL). The chloroform layer was washed with water, dried (anhydrous Na_2SO_4), and evaporated to give **1b** (0.70 g, 76%) as a colorless residue, which crystallized upon addition of acetone to a white solid, mp 138–140 °C. NMR showed the absence of the alkene proton. Recrystallization from acetone afforded the analytical sample (colorless needles): mp 140–141 °C; TLC R_f (solvent C) 0.34; ^1H NMR δ 0.77 (s, C-19 CH_3), other assignments as expected; IR and mass spectra as expected. Anal. ($C_{26}H_{47}NO$) C, H, N.

(20*S*)-20-[(3-Methylbutyl)amino]-5α-pregnan-3-one (1f). To a solution of **1b** (0.38 g, 0.98 mmol) in 10 mL of dry toluene (freshly distilled from sodium) was added cyclohexanone (2 mL) and aluminum *tert*-butoxide (244 mg, 1.01 mmol). The reaction mixture was heated at reflux for 8 h, cooled, and diluted with 50 mL of benzene. The benzene solution was washed with 10% aqueous sodium potassium tartrate (4 × 20 mL) and water (2 × 25 mL), then dried (anhydrous Na_2SO_4) and evaporated to give 1.28 g of a yellow oil. Purification by chromatography on silica gel with chloroform–methanol (93:7) as eluent gave **1f** (0.21 g, 55%) as a white crystalline solid, mp 121–123 °C. Two recrystallizations from aqueous ethanol gave the analytical sample: mp 127.5–128.5 °C; TLC R_f (solvent C) 0.35; IR ($CHCl_3$) 1715 (C=O) cm^{-1}; ^1H NMR δ 0.98 (s, C-19 CH_3) other assignments as expected; mass spectrum as expected. Anal. ($C_{26}H_{45}NO$) C, H, N.

***N*-Methyl-*N*-(4-methylpentyl)-3β-methoxy-5-androsten-17β-amine (2c). General Method C.** A solution of 3β-methoxy-5-androsten-17-one[14] (**7b**; 3.00 g, 10 mmol), 4-methylpentylamine (1.50 g, 15 mmol), and *p*-toluenesulfonic acid (100 mg) in 50 mL of benzene was heated at reflux overnight. The water formed during the reaction was removed with the aid of a Dean–Stark trap. The reaction mixture was cooled to room temperature, washed with water (50 mL), and dried (K_2CO_3). Evaporation of the solvent gave an oily residue which was taken up in dioxane (60 mL) and then reduced by heating under reflux with lithium aluminum hydride (1.90 g) for 20 h. The excess hydride was cautiously decomposed by successive addition of 75% aqueous dioxane (20 mL), 25% NaOH (5 mL), and water (5 mL). The reaction mixture was then filtered while hot, the solids were washed with hot 2-propanol, and the filtrate was evaporated to dryness. The residue was dissolved in 2-propanol–HCl solution and diluted with ether. The HCl salt was collected by filtration. Liberation of the free base and recrystallization of crude amine **7b** from acetone–methanol gave pure **N-(4-methylpentyl)-3β-methoxy-5-androsten-17β-amine (8b**; 2.16 g, 57%): mp 49–51 °C; IR, NMR, and mass spectra were as expected. Anal. ($C_{26}H_{45}NO$) C, H.

A solution of amine **8b** (0.37 g, 0.95 mmol) and 0.6 mL of 37% aqueous formaldehyde in 10 mL of methanol was stirred for 2 h at room temperature and then cooled to 5–10 °C. Sodium borohydride (900 mg, 24 mmol) was added portionwise over a 40-min period, and stirring was continued at 5–10 °C for 1 h and then at room temperature for 2 h. After workup, 0.44 g of crude **2c** was obtained. Recrystallization from acetone gave 190 mg (50%) of **2c** as colorless crystals: mp 59–61.5 °C; TLC R_f (solvent C) 0.57 (did not stain with nitroprusside–acetaldehyde); ^1H NMR δ 0.82 (s, C-18 CH_3), 0.86 (d, J = 5.5, C-26 and C-27 CH_3), 1.00 (s, C-19 CH_3), 2.22 (s, CH_3N), 3.35 (s, CH_3O), 5.39 (br, =CH); mass spectrum, m/e (relative intensity) 401 (18, M^+), 330 (63, α-cleavage), 154 (100, α-cleavage). Anal. ($C_{27}H_{47}NO$) C, H.

Enzyme-Inhibition Studies. Materials and Methods. Mature bovine adrenals were purchased frozen from Pel-Freez Biologicals, Inc., Rogers, AR. [26-^{14}C]Cholesterol (specific activity 46.0 mCi/mmol) and [7-^3H]cholesterol (specific activity 10.9 Ci/mmol) were obtained from New England Nuclear Corp. The incubation vials were the same as the scintillation vials listed below. A Precision Scientific (catalog no. 68351-C) heated vacuum desiccator was used to volatilize isocaproic acid. All scintillation counting was done using a Packard Model 2425 Tri-Carb liquid scintillation spectrometer. The samples were counted in 22-mm glass vials with polyethylene-lined caps using 10 mL of Budget Solve (all from Research Products International Corp., Elk Grove Village, IL).

All other materials and methods were as described previously.[6]

Acknowledgment. The authors are grateful to John Ip for his assistance during the biological evaluation and to Richard Dvorak for obtaining mass spectra. This research was supported in part by USPHS Grant HE-11274 (University of Michigan Medical School).

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