matrix least squares. In the final refinement, anisotropic thermal parameters were used for the heavier atoms, and isotropic temperature factors were used for the hydrogen atoms and the four carbon and oxygen atoms of the 2-propanol molecule. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indexes are $R = 0.086$ and $wR = 0.095$ for the 1271 observed reflections. The final difference map has no peaks greater than ± 0.3 eÅ^{-3}, except for one peak of 0.6 eÅ^{-3} found about 1 Å from two carbon atoms of the 2-propanol molecule.

The absolute configuration was determined by carrying out two refinements, one using the correct value of the imaginary part of the anomalous dispersion correction for chlorine ($\Delta f''$) and the other with the sign of $\Delta f''$ reversed (equivalent to refining the antipode). The weighted discrepancy indexes at the end of the two refinements were 0.0955 and 0.1002 for $\Delta f''$ and $-\Delta f''$, respectively. Thus, according to the test described by Hamilton,[30] the absolute configuration is established at better than the 0.995 confidence level.

Crystal Structure of Molindone Hydrochloride. A sample (Endo Laboratories) was recrystallized from 1:1 methanol–acetonitrile and air dried. The crystal data are summarized in Table III. The intensity data were measured as above. The size of the crystal used for data collection was approximately 0.5 × 0.10 × 0.08 mm; the data were corrected for absorption. Of the 2202 independent reflections for $\theta < 57$, 1811 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved and refined as above. The final discrepancy indexes are $R = 0.042$ and $wR = 0.049$ for the 1811 observed reflections. The final difference map has no peaks greater than ± 0.2 eÅ^{-3}.

Pharmacology. Discrete Avoidance Response in Rats. Male Charles River CD rats were trained and tested in experimental chambers equipped with a response lever, a grid floor for delivery of electric shock (unconditioned stimulus; UCS), and a speaker for presentation of auditory stimuli (conditioned stimulus; CS). Behavioral trials were presented at 2-min intervals during each 1-h session. Each trial consisted of a 15-s CS, continuing for an additional 15 s accompanied by the UCS (1.0 mA, 350 V.A.C. scrambled). The rats could terminate a trial at any time by pressing the response lever. A response during the CS period was considered an avoidance response, while a reponse occurring during the UCS period was an escape response.

Trained rats which maintained a reliable control base line of avoidance behavior of zero to three avoidance failures per session were used to test experimental compounds. One control and one experimental session were alternated during each week. The compounds were adminstered 60 min before the start of each session to a minimum of three rats at each dose level over a range of doses. Rats received vehicle alone during control sessions.

For each dose group, the proportion of trials in which the rats failed to exhibit an avoidance or an escape response was determined, using the ten trial session segment in which the largest such effect occurred. The dose required to produce a 50% block of avoidance responding (ABD_{50}) was computed by regression analysis ($y = a + b \log x$), and 95% confidence limits were computed as described by Tedeschi et al.[31] The lowest dose required to produce a 20% block of escape responding was estimated graphically from a dose–effect curve.

Acknowledgment. We thank members of our Physical Chemistry Department for obtaining the spectral and microanalytical data, Elliot Chiang for checking several synthetic procedures, and Mary Zolcinski for technical assistance with the discrete avoidance test.

Supplementary Material Available: Tables I–VIII list final atomic parameters, final anisotropic thermal parameters, bond lengths, and bond angles for (–)-1·HCl and molindone hydrochloride. Table IX lists atomic coordinates for dexclamol conformer B (7 pages). Ordering information is given on any current masthead page.

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Inhibition of Cholesterol Side-Chain Cleavage. 3.[1] 22-Azacholesterol Analogues Bearing Aryl-Substituted Side Chains

Norma G. Delaney and Matthias C. Lu*

Department of Medicinal Chemistry, College of Pharmacy, University of Illinois at the Medical Center, Chicago, Illinois 60612. *Received February 27, 1981*

The potent inhibitory activity of 22-azacholesterol analogue **2a**, in which the (3-methylbutyl)amino side chain had been replaced by the (phenylethyl)amino side chain, on the conversion of cholesterol to pregnenolone prompted the synthesis and enzymatic studies of two series of 22-azacholesterol analogues bearing (arylalkyl)amino and (arylalkyl)amido side chains. The potent inhibitory activity of both the amines (**2**) and the amides (**3**) indicated that a basic nitrogen was not a requirement for inhibitory activity. However, the amide analogue (**4**) in which the positions of the carbonyl and the nitrogen were interchanged was a much poorer inhibitor. The inhibitory activities in the phenylacetamido series were decreased by electron-withdrawing groups on the aromatic ring, while an electron-donating group effected a small increase.

Inhibitors of cholesterol side-chain cleavage (CSCC) are of interest as potential biochemical tools in the elucidation of the mechanism of the CSCC reaction, as well as potential therapeutic agents for the modulation of the excessive production of adrenocortical hormones caused by various disease states.[2] One such inhibitor, aminoglutethimide (α-ethyl-α-*p*-aminophenylglutarimide), has been used in the treatment of such conditions,[3] but its unwanted side effects[4] make it desirable to find more specific in-

(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 5.

(2) (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).

(3) (a) R. Cash, A. J. Brough, M. P. Cohen, and P. S. Satch, *J. Clin. Endocrinol.*, **27**, 1239 (1967); (b) L. M. Fishman, G. W. Liddle, D. P. Island, N. Fleischer, and O. Kuchel, *ibid.*, **27**, 481 (1967); (c) R. I. Misbin, J. Canary, and D. Willard, *J. Clin. Pharmacol.*, **16**, 645 (1976); (d) R. J. Santen, A. Lipton, and J. Kendall, *J. Am. Med. Assoc.*, **230**, 1661 (1974).

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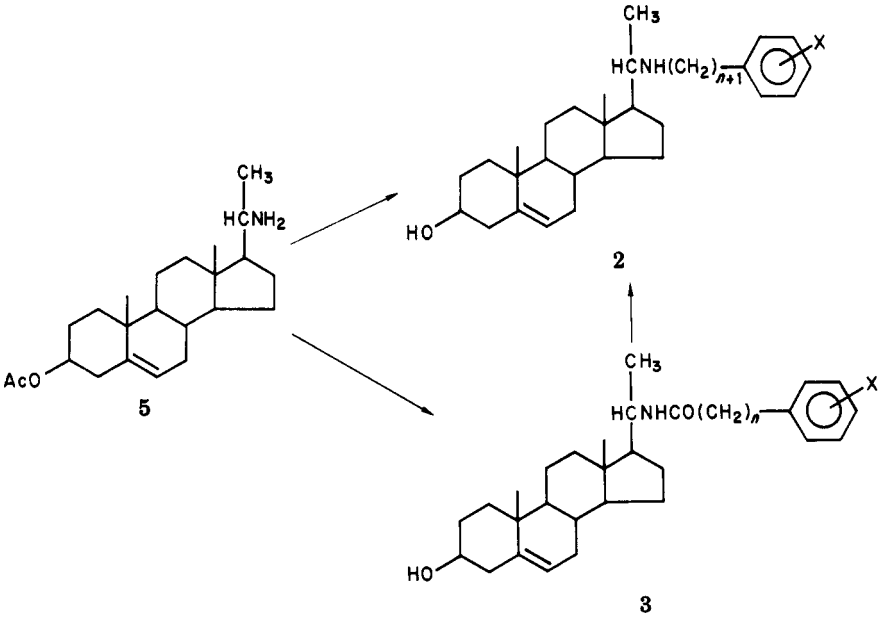
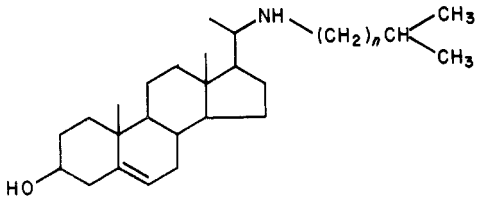
0022-2623/81/1824-1034$01.25/0

Scheme I

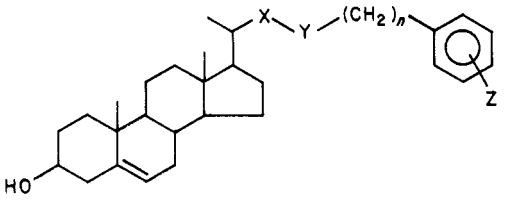


hibitors. Our previously study[5] with a number of side-chain-modified analogues of 22-azacholesterol (**1a**), a po-



1a, $n = 2$
b, $n = 3$
c, $n = 1$

tent competitive inhibitor, revealed that increasing (**1b**) or shortening (**1c**) the side chain length by one methylene has little effect on inhibitory activity. Introduction of a phenyl ring in place of the C-25, C-26, and C-27 fragments of 22-azacholesterol, as in the (phenylethyl)amino analogue **2a**, produced a compound which was just as active as 22-



2a, X-Y = $NHCH_2$; $n = 1$; Z = H
b, X-Y = $NHCH_2$; $n = 2$; Z = H
c, X-Y = $NHCH_2$; $n = 0$; Z = H
d, X-Y = $NHCH_2$; $n = 1$; Z = p-OCH_3
e, X-Y = $NHCH_2$; $n = 1$; Z = p-Cl
3a, X-Y = NHCO; $n = 1$; Z = H
b, X-Y = NHCO; $n = 2$; Z = H
c, X-Y = NHCO; $n = 0$; Z = H
d, X-Y = NHCO; $n = 1$; Z = p-OCH_3
e, X-Y = NHCO; $n = 1$; Z = p-Cl
f, X-Y = NHCO; $n = 1$; Z = p-NO_2
g, X-Y = NHCO; $n = 1$; Z = m-I
4, X-Y = CONH; $n = 1$; Z = H

azacholesterol, suggesting that larger side chains can be accommodated. This paper represents a continuation of our structure–activity relationship studies in this direction.

Two series of side-chain (arylalkyl)amino (**2**) and (arylalkyl)amido (**3**) analogues of 22-azacholesterol with electron-donating or electron-withdrawing substituents on the aromatic ring were synthesized. These substituents might enhance the binding of the inhibitor to the enzyme as well as alter the electronic character of the side chain. By replacement of the amino nitrogen with an amide nitrogen, the importance of the basic character of the side-chain nitrogen might be determined, as well as the effect of introducing added bulk and rigidity around C-22 and C-23. The unsubstituted phenyl analogues with one additional (**2b**, **3b**) and one fewer (**2c**, **3c**) side-chain methylene were prepared to examine the effect of the side-chain length in each series. Amide analogue **4**, in which the position of the carbonyl and the nitrogen had been interchanged, was also prepared to explore the importance of the relative orientation of this function for inhibitory activity.

Chemistry. Two synthetic routes were considered for the preparation of the target compounds **2** and **3** depicted in Scheme I. The starting material for both routes was (20*S*)-3β-acetoxy-5-pregnen-20-amine (**5**), prepared from 3β-acetoxy-22,23-dinorcholenic acid by the procedure of Julian et al.[6] involving a Curtius rearrangement. The first route involved the reaction of **5** with appropriately substituted phenylalkanoic acid derivatives to form amides **3** with subsequent reduction to give target amines **2**. Alternatively, target compounds **2** could be prepared directly from **5** via reductive alkylation with an appropriate aldehyde and sodium borohydride ($NaBH_4$).

Initial attempts to prepare amides **3d–g** by the reaction of **5** with substituted phenylacetyl chlorides in benzene with triethylamine or other bases to scavenge the hydrogen chloride formed produced impractical yields because dehydrohalogenation and subsequent polymerization of the phenylacetyl chlorides made isolation and purification of the desired amides difficult. It was felt that a two-phase reaction, whereby the troublesome base would have only limited access to the organic phase where the acylation was occurring, would circumvent the polymerization problem.

(5) M. C. Lu, P. Afiatpour, C. B. Sullivan, and R. E. Counsell, *J. Med. Chem.*, **15**, 1284 (1972).

(6) P. L. Julian, E. W. Meyer, and H. C. Printy, *J. Am. Chem. Soc.*, **70**, 887 (1948).

Thus, the Schotten–Baumann method, modified for this purpose, gave amides **3** in 72 to 89% yield from **5** and the appropriate acyl chloride (method A). Alternatively, amides **3** could be obtained from **5** via DCC coupling. However, isolation of the desired product from an acylurea side-product and dicyclohexylurea required repeated column chromatography.

Attempts to effect reduction of **3** with lithium aluminum hydride in dioxane at 100 °C were unsuccessful. Therefore, alternate routes to the desired amines **2** were explored.

Direct N-alkylation of amines using sodium borohydride and carboxylic acids has been reported.[7,8] While these methods have been used most successfully in the preparation of tertiary amines, secondary amines have been obtained in variable yield. It was felt that the somewhat sterically hindered steroidal primary amine **5** would be a good candidate for monoalkylation.

Following the method of Marchini,[8] $NaBH_4$ was initially reacted with 3 equiv of the carboxylic acid in benzene solution to form the $NaBH_4$–carboxylic acid complex. The reaction was completed by addition of the amine **5** and subsequent heating (method B). Analysis by TLC showed a complex mixture of products but no unreacted primary amine **5**. Treating the TLC plate with nitroprusside–acetaldehyde spray reagent[9] verified that one of the major products was a secondary amine. Variation of the number of equivalents of the complex or the reaction time failed to improve the proportion of secondary amine formed. The crude acetates were hydrolyzed by boiling in methanolic base and then separated by column chromatography.

Two major products were formed—the desired secondary amine **2** and the amide **3**. There was no evidence of N,N-dialkylation. In 1978, Gribble et al.[10] reported that alkylation of aliphatic amines by Marchini's method leads to amide and amine–borane formation. Thus, amides can be expected as byproducts in this method. All of the carboxylic acids tried gave approximately the same product distribution as judged by TLC. Differences in the isolated yields of products were likely due to the variability in the chromatographic separations. Attempts to prepare a *p*-nitrophenylethylamino derivative by this method failed. Only unidentified colored products were isolated.

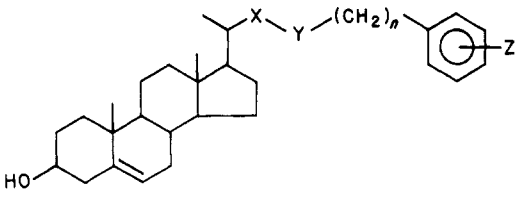
Compound **2c** was prepared from **5** via reductive alkylation with benzaldehyde and $NaBH_4$. Other amines **2**, however, were not prepared by this method, since the required substituted phenylacetaldehydes are not commercially available nor readily prepared.

The reverse amide **4** was obtained in good yield by treatment of the 3β-acetoxy-22,23-dinorcholenic acid chloride with benzylamine.

Results and Discussion

Inhibition Studies. Assays for CSCC activity were performed according to the methods of Doering[11] and Lu et al.,[5] with the one major deviation that frozen bovine adrenals were used for the preparation of the adrenocortical mitochondrial acetone powder. [7-^{3}H,26-^{14}C]Cholesterol was employed as substrate at a concentration of 3.3 μM. The cleavage product, ^{14}C-labeled isocaproic acid, was removed by heating in a vacuum oven. The enzyme activity was then calculated from the changes in the isotope ratios at 2 h vs. zero time. The inhibitory activity of the test compounds was determined at a final concentration of 100 μM or less. In all determinations, the inhibitors were dissolved in 10 μL of DMF and added to the incubation mixture prior to the addition of substrate. Three controls containing only 10 μL of DMF and no inhibitor were run in each experiment. Also, three flasks containing 3 μM 22-azacholesterol (K_i = 2.2 μM) were included to validate the experiment. 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 I. Cholesterol Side-Chain Cleavage Inhibitory Activity of Some 22-Azacholesterol Analogues Bearing Aryl-Substituted Side Chains



no.	X-Y	n	Z	% inhibn of side-chain cleavage at various inhib concns[a] 100 μM	10 μM	1 μM	0.1 μM
2a	$NHCH_2$	1	H		93 ± 5	24 ± 4	
2b	$NHCH_2$	2	H		78 ± 8	24 ± 7	
2c	$NHCH_2$	0	H		88 ± 10	27 ± 4	
2d	$NHCH_2$	1	*p*-OCH_3		78 ± 15	40 ± 9	−6 ± 12
2e	$NHCH_2$	1	*p*-Cl		86 ± 9	22 ± 3	
3a	NHCO	1	H		74 ± 8	44 ± 7	28 ± 10
3b	NHCO	2	H		80 ± 6	32 ± 13	19 ± 20
3c	NHCO	0	H	84 ± 7	84 ± 5	53 ± 10	9 ± 6
3d	NHCO	1	*p*-OCH_3		86 ± 9	49 ± 6	38 ± 20
3e	NHCO	1	*p*-Cl		63 ± 7	30 ± 6	25 ± 19
3f	NHCO	1	*p*-NO_2	39 ± 8	49 ± 13	22 ± 6	
3g	NHCO	1	*m*-I		73 ± 10	18 ± 12	
4	CONH	1	H	45 ± 1	31 ± 11		

[a] Error limits are standard deviations.

Table I shows the inhibitory activities of the 22-azacholesterol analogues tested. The percent inhibitions for 22-azacholesterol (**1a**) and the (phenylethyl)amino analogue **2a** were in good agreement with results previously reported.[5] These data clearly indicate that several modifications can be made without observing significant departure from the inhibitory activity of the lead compound, the (phenylethyl)amino analogue **2a**. These included (1) addition or deletion of one methylene in the (phenylethyl)amino side chain (**2b** and **2c**), (2) replacement of the (phenylethyl)amino side chain by a phenylacetamido side chain (**3a**), or (3) addition or deletion of one methylene in the phenylacetamido side chain (**3b** and **3c**).

The structural requirements of the side chain for binding as an inhibitor as revealed by this study thus appear to be less stringent than those for substrate activity. While cholesterol analogues with increased side-chain length are poor substrates for the enzyme,[12] the 22-azacholesterol analogues with aryl-substituted side chains are as effective as inhibitors as 22-azacholesterol itself.

Substitution of an amide functional group for the secondary amine did not decrease the inhibitory activity (**2a** vs. **3a**, **2b** vs. **3b**, and **2c** vs. **3c**). Thus, a basic nitrogen

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(11) C. H. Doering, *Methods Enzymol.*, **15**, 591–595 (1969).
(12) M. Morisaki, C. Duque, N. Ikekawa, and M. Shikita, *J. Steroid Biochem.*, **13**, 545 (1980).

Table II. 22-Azacholesterol Analogues Bearing Aryl-Substituted Side Chains

no.	method (yield, %)	crystn solvent	mp, °C	formula	anal.
2a	B (20)	acetone	121-123	$C_{29}H_{43}NO$	known[b]
2b	B (39)	95% EtOH	130-130.5	$C_{30}H_{45}NO$	C, H, N
2c	a	acetone	159.5-160	$C_{28}H_{41}NO$	C, H, N
2d	B (15)	acetone	135-135.5	$C_{30}H_{45}NO_2$	C, H, N
2e	B (53)	acetone	156.5-157	$C_{29}H_{42}ClNO$	C, H, N
3a	A (89)	MeOH	206-206.5	$C_{29}H_{41}NO_2$	C, H, N
3b	B (24)	acetone	216.5-219	$C_{30}H_{43}NO_2$	C, H, N
3c	A (85)	MeOH	201.5-203	$C_{28}H_{39}NO_2$	C, H, N
3d	A (72)	acetone-H_2O	121-125	$C_{30}H_{43}NO_3$	C, H, N
3e	B (18)	acetone	178.5-180.5	$C_{29}H_{40}ClNO_2$	C, H, N
3f	A (83)	95% EtOH	216.5-218	$C_{29}H_{40}N_2O_4$	C, H, N
3g	A (89)	EtOH-H_2O	181-183	$C_{29}H_{40}NO_2I$	C, H, N
4	a	MeOH	233-234	$C_{29}H_{41}NO_2$	C, H, N

[a] See Experimental Section. [b] See ref 5.

is not a prerequisite for inhibition. However, the amide analogue in which the positions of the carbonyl and the nitrogen were interchanged was a much poorer inhibitor (4 vs. 2a). Thus, the relative orientation of this group is important for inhibitory activity.

The inhibitory activities in the phenylacetamido series were decreased by electron-withdrawing groups on the aromatic ring (3e or 3f vs. 3a), while an electron-donating substituent (3d) effected a small increase. The basis for these substituent effects is not apparent at this time. Significant substituent effects could not be observed within the smaller (phenylethyl)amino series.

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). 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 were performed by Micro-Tech Laboratories, Skokie, IL, and agreed within ±0.4% of the theoretical values.

All of the substituted phenylalkanoic acids needed were commercially available, except *m*-iodophenylacetic acid which was prepared from *m*-iodotoluene by the literature method.[13]

(20*S*)-20-(Benzylamino)-5-pregnen-3β-ol (2c). A solution of amine 5[6] (1.00 g, 2.78 mmol) and benzaldehyde (1.48 g, 13.9 mmol) in 125 mL of absolute methanol was heated at reflux for 2 h and then cooled to 5–10 °C. Sodium borohydride (4.25 g) was added portionwise over a 20-min period, and the reaction mixture was stirred for an additional 2 h at 5–10 °C. Acetone (5 mL) was added dropwise to decompose the excess hydride, and then the reaction mixture was poured onto 150 g of crushed ice. The aqueous suspension was extracted with chloroform (3 × 100 mL), and the chloroform layer was dried (anhydrous Na_2SO_4) and evaporated to give 2.65 g of a pale tan oil. The oil was warmed at 65 °C in vacuo (0.05 mm) to remove benzyl alcohol. The resultant white residue was then boiled for 2 h in 40 mL of 2% methanolic KOH to hydrolyze the ester. The methanol was evaporated, and the residue was suspended in water, adjusted to pH 8–9 with 5 N HCl, and extracted into chloroform (4 × 50 mL). The chloroform layer was dried (anhydrous $MgSO_4$) and evaporated to give 1.09 g of an off-white residue which resisted crystallization. The solid was chromatographed on silica gel with chloroform–hexane (7:3) as initial eluent. Elution with chloroform–methanol (95:5) yielded 2c (0.87 g, 77%) as a white crystalline solid, mp 156–159 °C. Recrystallization from acetone gave the analytical sample: mp 159.5–160 °C; TLC ($CHCl_3$–MeOH, 98:2) R_f 0.35; ^1H NMR δ 0.66 (s, C-18 CH_3), 0.99 (s, C-19 CH_3), 1.15 (d, J = 6.1 Hz, C-21 CH_3), 1.72 (NH, OH), 3.74 (q, J_{AB} = 13 Hz, ArCH_2N), 5.34 (br, =CH), 7.28 (s, ArH); mass spectrum, m/e (relative intensity) 407 (<1, M^+), 134 (100, α-cleavage), 91 (26, $C_7H_7^+$). Anal. ($C_{28}H_{41}NO$) C, H, N.

***N*-[(20*S*)-3β-Hydroxy-5-pregnen-20-yl]-*p*-nitrophenylacetamide (3f) via the Schotten–Baumann Procedure. General Method A.** A solution of amine 5 (1.00 g, 2.78 mmol) in 15 mL of benzene was stirred vigorously with a solution of NaOH (123 mg, 3.06 mmol) in 5 mL of water. To this was added a solution of *p*-nitrophenylacetyl chloride (611 mg, 3.06 mmol) in 5 mL of benzene in small portions over a period of 5 min. An additional 10 mL of benzene was used to rinse any precipitate from the sides of the flask. After stirring at room temperature for 15 min, the reaction mixture was diluted with 100 mL of chloroform, extracted with successive aliquots of 2% aqueous Na_2CO_3 (3 × 15 mL), 10% aqueous HCl (2 × 20 mL), and water (2 × 20 mL), then dried (anhydrous Na_2SO_4), and evaporated to give 1.44 g of the solid amido ester: mp 253–255.5 °C; TLC ($CHCl_3$) R_f 0.42. A portion of the solid (750 mg, 1.44 mmol) was boiled in 62 mL of 2% methanolic KOH for 25 min to hydrolyze the ester. Most of the methanol was evaporated, 100 mL of water was added, and the aqueous slurry was adjusted to approximately pH 7 with 5 N HCl. The product was extracted into chloroform (3 × 50 mL) and the combined chloroform extracts were washed with water, dried (anhydrous Na_2SO_4), and evaporated to give the required amide (0.57 g, 83%). Recrystallization from methanol gave the analytical sample 3f: mp 216.5–218 °C; TLC ($CHCl_3$–MeOH, 98:2) R_f 0.40; IR (KBr) 1643 cm^{-1} (NHC=O); ^1H NMR δ 0.70 (s, C-18 CH_3), 0.99 (s, C-19 CH_3), 1.14 (d, J = 6.5 Hz, C-21 CH_3), 3.59 (s, ArCH_2CO), 5.19 (NHCO), 5.32 (br, =CH), 7.82 (center of an AA′XX′ system ranging from 7.36 to 8.28, para-disubstituted benzene); mass spectrum, m/e (relative intensity) 480 (32, M^+), 44 (100, $CH_3CH{=}NH_2^+$). Anal. ($C_{29}H_{40}N_2O_4$) C, H, N.

(20*S*)-20-[[2-(*p*-Chlorophenyl)ethyl]amino]-5-pregnen-3β-ol (2e) via Reaction of 5 with a $NaBH_4$–Carboxylic Acid Complex. General Method B. A vigorously stirred slurry of *p*-chlorophenylacetic acid (3.92 g, 22.94 mmol) in 10 mL of dry benzene (freshly distilled from sodium) was cooled to 10–15 °C, and $NaBH_4$ (263 mg, 6.96 mmol) was added in small portions. The mixture was stirred for 1.5 h at 10–15 °C, followed by the addition in one portion of a solution of amine 5 (1.00 g, 2.78 mmol) in 20 mL of dry benzene. The solution was heated at reflux for 17 h, then cooled, and diluted with 200 mL of ether. The ether solution was washed with 2 N NaOH solution (5 × 50 mL), followed by water (4 × 50 mL) until the extracts were neutral. The ether solution was dried (anhydrous Na_2SO_4) and evaporated to give 1.45 g of an off-white solid. The ester was hydrolyzed in 2% methanolic KOH as described under general method A. After workup, 1.36 g of a yellow residue was chromatographed on silica gel with $CHCl_3$–hexane (7:3) as initial eluent. Elution with $CHCl_3$–MeOH (95:5) yielded 2e (0.67 g, 53%) as a white crystalline solid, mp 156–156.5 °C. Recrystallization from acetone gave an

(13) J. F. J. Dippy and R. H. Lewis, *J. Chem. Soc.*, 644 (1936).

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.

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0022-2623/81/1824-1038$01.25/0