was carried out in a manner very similar to that used by Hurd and Dowbenko[6] to convert 2,2-dimethylcoumaran to 2,2-dimethyl-3-coumaranone. Thus a mixture of 8.1 g (0.05 mol) of 2,2,6-trimethylcoumaran (4), 8.9 g (0.05 mol) of N-bromosuccinimide, 0.05 g of benzoyl peroxide, and 150 ml of dry carbon tetrachloride was refluxed for 2 hr and processed in the manner of Hurd and Dowbenko to give 7.6 g (63%) of 3-bromo-2,2,6-trimethylcoumaran as a colorless liquid: bp 80–81° (1.5 mm); nmr spectrum (CCl_4) δ 1.3 (s, 3, C_2 CH_3), 1.5 (s, 3, C_2 CH_3), 2.2 (s, 3, C_6 CH_3), 4.9 (s, 1, C_3 H), and 6.3–7.2 (m, 3, aromatic).

The entire amount of 3-bromo-2,2,6-trimethylcoumaran was dissolved in a mixture of 12 ml of glacial acetic acid and 7.5 g of freshly fused potassium acetate. The mixture was heated at 120° for 10 min, allowed to stand at room temperature overnight, and then heated on a steam bath for 3 hr. The crude product, 3-acetoxy-2,2,6-trimethylcoumaran, was isolated in the same way that Hurd and Dowbenko isolated 3-acetoxy-2,2-dimethylcourmaran except that the product was not distilled. Instead, the crude acetoxycoumaran was dissolved in a solution of 50 ml of methanol and 5.0 g of potassium hydroxide, and the solution was refluxed for 1 hr. It was then diluted with 100 ml of water, saturated with sodium chloride, and extracted with ether. After drying the ethereal extract, solvent was removed and the crude product, 3-hydroxy-2,2,6-trimethylcoumaran (3.1 g), solidified spontaneously. After recrystallization from hexane, the hydroxycoumaran appeared as colorless crystals, mp 66–66.5°; its ir spectrum (NaCl) showed the expected hydroxyl stretching vibration at 3370 cm^{-1} and a band at 1280 cm^{-1} assignable to the ether stretching vibration.

The 3-hydroxy-2,2,6-trimethylcoumaran (2.5 g) in 20 ml of pyridine was added to a slurry of chromium trioxide (4.5 g) in 45 ml of pyridine at 20°. The reaction mixture was allowed to stand overnight at 20–25° and was then diluted with 200 ml of water. The mixture was extracted with ether. The ethereal extract was washed with dilute aqueous hydrochloric acid, then water, and dried over calcium sulfate. Evaporation of the ether gave 2.1 g of 2,2,6-trimethyl-3-coumaranone as colorless needles, mp 51.5–52.5° from hexane (lit.[7] 52°), whose ir spectrum showed a strong carbonyl stretching vibration at 1725 cm^{-1}. The coumaranone formed a semicarbazone derivative, mp 244–246° from ethanol (lit.[7] 250°).

Registry No.—6, 25594-08-5; 2-isobutenyl-5-methylphenol, 25594-09-6; 3-bromo-2,2,6-trimethylcoumaran, 25594-10-9; 3-hydroxy-2,2,6-trimethylcoumaran, 25594-11-0; 2,2,5,7-tetramethylcoumaran, 25594-12-1; 2,2-dimethyl-5-(1,1,3,3-tetramethylbutyl)coumaran, 25594-13-2; 2,3-dihydro-2,2-dimethylnaphtho[1,2-*b*]-furan, 25594-14-3.

Acknowledgment.—The authors wish to acknowledge the contribution of Messrs. R. Capwell and L. Petrakis who helped to establish the constitution of some of the products, of D. L. Bash who willingly carried out a number of the preparative reactions, and of Dr. Wm. I. Gilbert for his interest and encouragement in this work.

Synthesis and Characterization of C_3 and C_{17} Steroidal Amines

ROBERT GLASER[1a] AND EDMOND J. GABBAY[1b]

School of Chemistry, Rutgers, the State University, New Brunswick, New Jersey 08903, and Department of Chemistry, University of Florida, Gainesville, Florida 32601

Received March 16, 1970

The synthesis and characterization of 5α-androstane C_3 and C_{17} amines are reported. Primary, tertiary, and quaternary mono- and diammonium salts of 5α-androstane have been synthesized. The salts are found to interact selectively with nucleic acids.[3]

For the past three years, considerable work has been devoted in our laboratory to the elucidation of the interaction specificity of nucleic acid systems with mono- and polyammonium salts.[2] It is well known that these compounds interact very strongly with polynucleotides. This paper reports the synthesis and characterization of steroidal amines, of 5α-androstane, *i.e.*, primary, tertiary, and quaternary ammonium salts as well as various epimers (3α, 3β, 17α, and 17β). The interaction specificity of these salts with various nucleic acids has been studied by proton magnetic resonance, ultraviolet, circular dichroism, viscometry, and T_m of helix–coil transitions. While this is reported elsewhere,[3] the results indicate that the steroidal amines selectively stabilize the DNA helical structure, while causing the ribose-containing acids to unravel and denature. The temperature-dependent pmr experiments show that single-stranded random coils interact with the steroidal amines *via* electrostatic and hydrogen- and hydrophobic-type bonding. The capacity to form H bonding in the random coils is shown to be greater than that of the helix.[3]

Results and Discussion

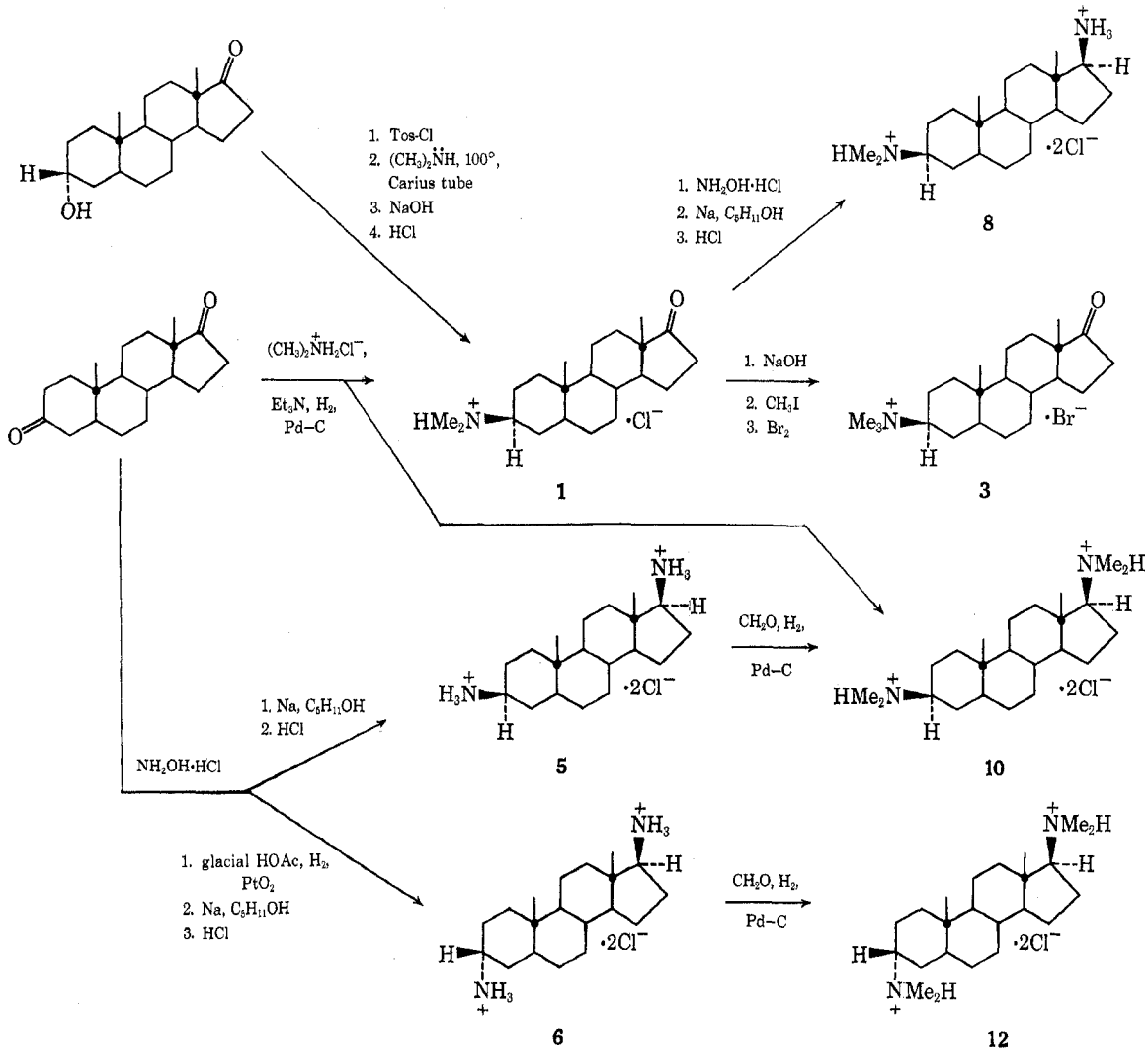
The synthetic scheme for the preparation of the steroidal amines is straightforward and is outlined in Schemes I and II. The 3-amino-17-oxo-(5α)-androstane derivatives, **1** and **2**, were synthesized by an SN2 reaction of dimethylamine with the appropriate 3-tosylate 17-oxo-(5α)-androstane precursor. For example, the 3β-tosylate 17-oxo intermediate was allowed to react with dimethylamine in a sealed tube to give the 3α-dimethylamino-17-oxo-(5α)-androstane product as the tosylate salt. Conversion to the free base and acidification with HCl afforded the salt **2**. In a similar manner, the 3β epimer was obtained. The stereochem-

(1) (a) NDEA Predoctoral Fellow, 1966–1969. (b) To whom correspondence should be addressed: Department of Chemistry, University of Florida, Gainesville, Fla. 32601.

(2) (a) E. J. Gabbay, *Biochem.*, **5**, 3036 (1966); (b) E. J. Gabbay, *Biopolymers*, **5**, 727 (1967); (c) R. Glaser and E. J. Gabbay, *ibid.*, **6**, 243, (1968); (d) E. J. Gabbay and R. R. Shimshak, *ibid.*, **6**, 255 (1968); (e) E. J. Gabbay and R. Kleinman, *J. Amer. Chem. Soc.*, **89**, 7123 (1967); (f) E. J. Gabbay, R. Kleinman, and R. Shimshak, *Biopolymers*, **6**, 993 (1968); (g) E. J. Gabbay, R. Kleinman, and R. R. Shimshak, *J. Amer. Chem. Soc.*, **90**, 1927 (1968); (h) E. J. Gabbay, *ibid.*, 5257 (1968); (i) E. J. Gabbay and J. Mitschele, *Biochem. Biophys. Res. Commun.*, **34**, 53 (1969); (j) E. J. Gabbay, *J. Amer. Chem. Soc.*, **90**, 6574 (1968); (k) *ibid.*, **91**, 5136 (1969); (l) E. J. Gabbay and M. Malin, submitted for publication in *J. Biol. Chem.*; (m) E. J. Gabbay, B. L. Gaffney, and L. A. Wilson, *Biochem. Biophys. Res. Commun.*, **35**, 854 (1969); (n) E. J. Gabby, B. L. Gaffney, R. Glaser, and D. Z. Denney, *Chem. Commun.*, 1507 (1969).

(3) E. J. Gabbay and R. Glaser, *J. Biol. Chem.*, in press.

SCHEME I



istry at the 3 position on the steroid nucleus was consistent with pmr spectra.[4]

The identical 3β-dimethylamino-17-oxo-(5α)-androstane salt (**1**) was prepared in greater yield by selective reductive amination of the 3-keto group in 3,17-dioxo-(5α)-androstane by condensation under hydrogen atmosphere with dimethylamine hydrochloride in the presence of triethylamine and palladized charcoal. A by-product of this reaction afforded the 3β,17β-bis(dimethylamino)-(5α)-androstane salt (**10**) in approximately 1% yield. The bisdimethylamino derivative **10** was synthesized by another route, and this will be described later.

The 3-(tertiary amino)-17-oxo derivatives, **1** and **2**, were converted to the corresponding 3-(quaternary amino)-17-oxo compounds, **3** and **4**, by conversion to the free base and alkylation with methyl iodide. However, the resulting iodide salt always turned from light yellow to black on exposure to light, even after repeated purification. It was therefore decided to convert the iodide salt into the bromide salt by addition of excess bromine. The bromide salt of the 3-(quaternary amino)-17-oxo compounds, **3** and **4**, were found to be stable upon exposure to light.

The 3β,17β-bis(dimethylamino)-(5α)-androstane salt (**10**), previously mentioned, was prepared in larger yields by the following route. The 3,17-dioxo-(5α)-androstane starting material was converted to the bisoxime and then stereoselectively reduced with sodium in *n*-amyl alcohol according to the method of Dodgson and Haworth[5] to give the bis(primary amino) salt **5**. It was shown by Shoppee, *et al.*,[6] that the 3-oxime in 5α steroids is stereoselectively reduced with sodium in alcohol to give the 3β-amine, whereas the epimeric 3α-amine is stereoselectively obtained by platinum oxide

(4) The following pmr empirical formula which relates the width at half height ($\Delta\nu_{1/2}$) of a complex multiplet in a rigid system was utilized to determine whether or not the pmr assignment for the steroidal N-methyne protons was consistent with the proposed structure. Using this relationship, the α-methyne proton would have a relatively broader width at half height ($\Delta\nu_{1/2}$ = 9–21 cps) compared with that for the β-methine proton ($\Delta\nu_{1/2}$ = 2–14 cps). The pmr spectra of the 3β-dimethylamino derivative, **1**, showed a complex multiplet at 6.90 ± 0.33 τ ($\Delta\nu_{1/2}$ = 25 cps), and the 3α-dimethylamino derivative, **2**, showed a complex multiplet at 6.70 ± 0.15 τ ($\Delta\nu_{1/2}$ = 8 cps). Thus, the pmr assignments for the N-methyne protons were found to be consistent with the proposed structure. See (a) J. N. Shoolery and M. J. Rogers, *J. Amer. Chem. Soc.*, **80**, 5121 (1958); (b) R. U. Lemieux,

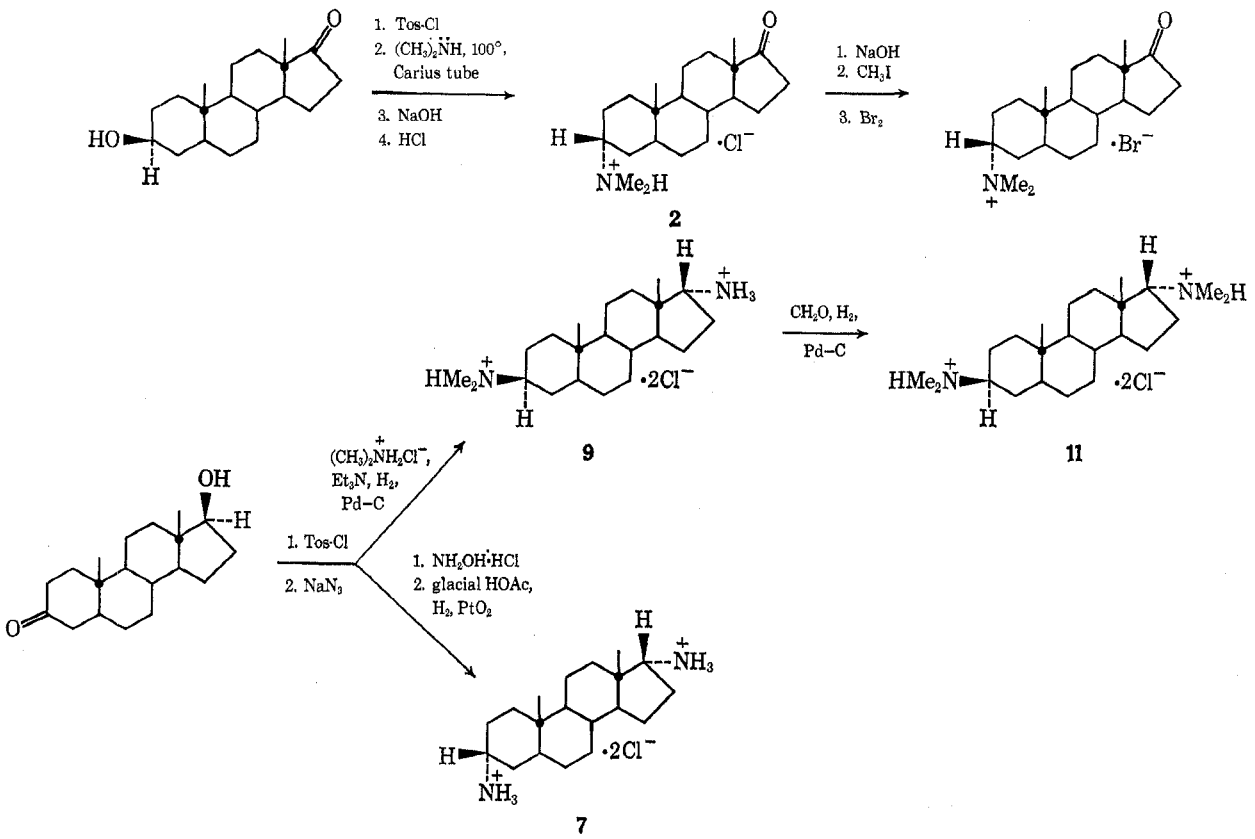
$$\Delta\nu_{1/2} = J_{AX} + J_{BX}$$

R. K. Kulling, H. J. Bernstein, and W. G. Schneider, *ibid.*, **80**, 6098 (1958); C. A. Grob and R. A. Whol, *Helv. Chim. Acta*, **48**, 1610 (1965).

(5) D. P. Dodgson and R. D. Haworth, *J. Chem. Soc.*, 67 (1952).

(6) C. W. Shoppee, D. E. Evans, H. C. Richards, and G. H. R. Summers, *ibid.*, 1649 (1956).

SCHEME II



catalyzed hydrogenation in glacial acetic acid.[7] In addition, Shoppee, *et al.*,[6] found sterically hindered C-17 oximes were stereoselectively reduced to give the amine arising from approach of the reducing agent to the least hindered side of the steroid.[8] The 3β,17β-bis(primary amino) salt **5** was converted to the corresponding bis(tertiary amino) salt **10** by reductive condensation with formaldehyde. This product was found to be identical in all respects (melting point and infrared and nmr spectra) with the material produced in low yield by reductive amination of 3,17-dioxo-(5α)-androstane with dimethylamine hydrochloride, as discussed earlier.

The 3-oxime of the 3,17-bisoxime intermediate was selectively reduced to the 3α-amino-17-hydroxyamino intermediate by platinum oxide catalyzed hydrogenation in glacial acetic acid, and the infrared spectrum was consistent with the proposed structure. The 3α,17β-bis(primary amino) derivative **6** was then obtained by sodium reduction in *n*-amyl alcohol followed by acidification with hydrochloric acid. The nmr spectrum in deuterium oxide (DSS standard) showed a complex multiplet at τ 6.44 ± 0.16 ($\Delta\nu_{1/2}$ = 8 cps) and 6.94 ± 0.28 ($\Delta\nu_{1/2}$ = 16 cps) corresponding to the 3β-$\overset{+}{\text{N}}$-methyne and 17α-$\overset{+}{\text{N}}$-methyne protons, respectively. The corresponding 3α,17β-bis(tertiary amino) compound **12** was then obtained by reductive condensation of the 3α,17β-bis(primary amino) compound **6** with formaldehyde.

(7) Reduction of the 3-oxime with lithium aluminum hydride afforded an epimeric mixture of amines.

(8) Shoppee, *et al.*,[6] found this to occur when the oxime was reduced by sodium in alcohol, platinum oxide catalyzed hydrogenation in glacial acetic acid, or lithium aluminum hydride.

3-Oxo-17β-hydroxy-(5α)-androstane was converted to the tosylate and then reacted with sodium azide to give the 3-oxo-17α-azido intermediate. The 3-keto function underwent reductive amination with dimethylamine hydrochloride under hydrogen atmosphere while the 17α-azide function was simultaneously reduced to yield the 3β-dimethylamino-17α-amino-(5α)-androstane salt (**9**).

The 3-oxo-17α-azide intermediate was also utilized to form the 3α,17α-bis(primary amino) salt **7**. This was accomplished by forming the 3-oxime, and then platinum oxide catalyzed hydrogenation in glacial acetic acid to reduce the 3-oxime and 17α-azido functions.

The 3β-dimethylamino-17β-amino-(5α)-androstane salt (**8**) was obtained by conversion of the tertiary amino keto salt **1** to the oxime, followed by reduction of the oxime by sodium in *n*-amyl alcohol.

All derivatives of the steroidal amines I and II were characterized by infrared, pmr, ORD, and elemental analysis, and the results were found to be consistent with the proposed structures.

Experimental Section

Melting points were determined on Mel-Temp apparatus and are uncorrected. Unless otherwise stated, pmr spectra of the neutral compounds were taken in $CDCl_3$ with TMS acting as internal standard and in D_2O with DSS as internal standard for the polar ammonium salts. The spectra were recorded on a Varian A-60 spectrometer. The infrared spectra were run in KBr pellets unless specified, and the group frequencies listed are unlabeled for stretch frequencies, and labeled b for bending frequencies. The ORD and CD spectra of the steroidal amines were recorded on a Cary 60 spectropolarimeter. Analyses were performed by George Robertson, Florham Park, N. J.

3β-Dimethylamino-17-oxo-(5α)-androstane Hydrochloride Hemihydrate (1).—To 2.00 g (6.9 mmol) of 3α-hydroxy-17-

oxo-(5α)-androstane, 6.0 g (32 mmol) of *p*-toluenesulfonyl chloride and 40 cc of dry pyridine were added. The reaction mixture was heated for 4 days at 48°. Upon cooling of the reaction mixture and addition of 25 cc of water, a solid separated which was washed successively with water, 1 *N* hydrochloric acid, and water. After the solid was dissolved in 1 cc of chloroform, excess *n*-hexane was added, and a solid separated which was recrystallized from acetone–water to give 0.51 g (16.5% yield) of the intermediate compound, 3α-*p*-toluenesulfonyl-17-oxo-(5α)-androstane as needles, mp 137.5–138.5° dec. The infrared spectrum showed absorptions at 2910 (C–H), 1735 (five-membered cyclic C=O), 1345 and 1180 (S=O), and 897 cm^{-1} (S–O–C). The nmr spectrum showed a two-proton doublet at τ 2.28 (J = 8 cps, aromatic 3′ protons), a two-proton doublet at τ 2.75 (J = 8 cps, aromatic 2′ protons), a one-proton complex multiplet at τ 5.29 ± 0.10 ($\Delta\nu_{1/2}$ = 7 cps, 3β-methyne proton), a three-proton singlet at τ 7.58 (aromatic methyl protons), a 22-proton complex multiplet at τ 8.50 ± 0.98 (methylene and methyne protons), a three-proton singlet at τ 9.17 (C-19 methyl protons), and a three-proton singlet at τ 9.25 (C-18 methyl protons).

The intermediate tosylate compound (0.460 g) was allowed to react with 5 cc of dimethylamine. The reaction mixture was heated in a sealed tube at 100° for 48 hr. Upon evaporation of the excess dimethylamine, a solid separated. After the solid was dissolved in 1 cc of chloroform, excess *n*-hexane was added and a solid separated. The solid was dissolved in hot water and excess aqueous sodium hydroxide was added. The solid which separated was extracted with chloroform. After evaporation of the chloroform layer, the free amine was obtained as an oil. After addition of hydrochloric acid, a solid separated which was recrystallized from ethanol–ether to give 0.040 g (10.7% yield) of 3β-dimethylamino-17-oxo-(5α)-androstane hydrochloride hemihydrate (**1**), mp 271–273° dec. The ORD of the sample (2.5×10^{-3} *M* in water) showed a positive Cotton effect, with a molecular rotation for the first extremum of $[\Phi]_{306}$ +9200, λ_0 290 mμ, and $[\Phi]_{271}$ −8280 for the second extremum. The infrared spectrum showed absorptions at 2920 (C–H), 2570 and 2440 (tertiary $\overset{+}{N}$–H), 1740 (five-membered cyclic C=O), 1640 (N–H, b), and 1475 cm^{-1} (C–H, b). The nmr spectrum showed a one-proton complex multiplet at τ 6.90 ± 0.33 ($\Delta\nu_{1/2}$ = 25 cps, 3α-methyne proton), a six-proton singlet at τ 7.19 (N-methyl protons), a 22-proton complex multiplet at τ 8.54 ± 0.95 (methylene and methyne protons), a three-proton singlet at τ 9.12 (C-19 methyl protons), and a three-proton singlet at τ 9.17 (C-18 methyl protons).

Anal. Calcd for $C_{21}H_{36}NOCl \cdot {}^1/_2H_2O$: C, 69.48; H, 10.28. Found: C, 69.45; H, 10.40.

Compound **1** was prepared in greater yield by another route. To 5.00 g (17.3 mmol) of 3,17-dioxo-(5α)-androstane, 100 cc of ethanol, 2 cc of triethylamine, 10 g of dimethylamine hydrochloride, and 1.0 g of 10% palladized charcoal were added. The reductive condensation reaction was carried out in a Parr shaker under a hydrogen atmosphere for a period of 25 hr at 36-psi pressure. After filtration of the catalyst and evaporation of the solvent, a solid separated which was dissolved in a blend of 50:50 chloroform–1 *M* hydrochloric acid. The aqueous layer was saved for subsequent work-up. Upon separation of the organic layer and evaporation of the solvent, a solid separated which was recrystallized out of chloroform–hexane to give 4.90 g of solid, mp 260–262° dec. Further recrystallization of 3.40 g of solid from water gave 1.14 g (19.0% yield) of 3β-dimethylamino-17-oxo-(5α)-androstane hydrochloride hemihydrate (**8**), mp 271–274° dec. The melting point and infrared and nmr spectra were found to be identical with those of the material prepared by the method described above.

3α-Dimethylamino-17-oxo-(5α)-androstane Hydrochloride Sesquihydrate (2).—To 2.00 g (6.9 mmol) of 3β-hydroxy-17-oxo-(5α)-androstane, 6.0 g (32 mmol) of *p*-toluenesulfonyl chloride and 40 cc of dry pyridine were added. The reaction mixture was heated for 4 days at 48°. Upon cooling of the reaction mixture and addition of 25 cc of water, an oil separated. Purification of the oil by trituration yielded a solid which was washed successively with water, 1 *N* hydrochloric acid, and water and then recrystallized from acetone–water to give 2.04 g (66.0% yield) of the intermediate compound, 3β-*p*-toluenesulfonyl-17-oxo-(5α)-androstane as needles, mp 151–152° (lit.[9] 160–161°). The infrared spectrum showed absorptions at 2860 (C–H), 1725 (five-membered cyclic C=O), 1340 and 1170 (S=O), and 925 cm^{-1} (S–O–C). The nmr spectrum showed a two-proton crude doublet at τ 2.19 (J = 8 cps, aromatic 3′ protons), a two-proton crude doublet at τ 2.66 (J = 8 cps, aromatic 2′ protons), a one-proton complex multiplet at τ 5.69 ± 0.28 (3α-methyne proton), a three-proton singlet at τ 7.55 (aromatic methyl protons), a 22-proton complex multiplet at τ 8.45 ± 1.18 (methylene and methyne protons), a three-proton singlet at τ 9.18 (C-19 methyl protons), and a three-proton singlet at τ 9.20 (C-18 methyl protons).

The tosylate (1.66 g) was allowed to react with 10 cc of dimethylamine. The reaction mixture was heated in a sealed tube at 100° for 48 hr. Upon evaporation of the excess dimethylamine, a solid separated. The solid was dissolved in hot water and excess aqueous sodium hydroxide was added; the solid which separated was extracted with chloroform. Upon evaporation of the chloroform layer, the free amine was obtained as an oil. After addition of hydrochloric acid, a solid separated which was recrystallized from ethanol–ether to give 0.57 g (40.3% yield) of 3α-dimethylamino-17-oxo-(5α)-androstane hydrochloride sesquihydrate (**2**), mp 202–205° dec. The ORD of the sample (2.5×10^{-3} *M* in water) showed a positive Cotton effect with a molecular rotation for the first extremum of $(\Phi)_{306}$ +8200, λ_0 290 mμ, and $[\Phi]_{270}$ −7960 for the second extremum. The infrared spectrum showed absorptions at 2930 (C–H), 2700 and 2480 (tertiary $\overset{+}{N}$–H), 1735 (five-membered cyclic C=O), 1635 (N–H, b), and 1460 cm^{-1} (C–H, b). The nmr spectrum showed a one-proton complex multiplet at τ 6.70 ± 0.15 ($\Delta\nu_{1/2}$ = 8 cps, 3β-methyne proton), a six-proton at τ 7.14 (N-methyl protons), a 22-proton complex multiplet at τ 8.42 ± 0.93 (methylene and methyne protons), and a six-proton singlet at τ 9.13 (C-18, C-19 methyl protons).

Anal. Calcd for $C_{21}H_{36}NOCl \cdot 1{}^1/_2H_2O$: C, 66.20; H, 10.32. Found: C, 66.05; H, 10.50.

3β-Trimethylammonium-17-oxo-(5α)-androstane Bromide Hemihydrate (3).—To 0.150 g (0.41 mmol) of **1**, excess aqueous sodium hydroxide was added, and the solid which separated was extracted with chloroform. Upon evaporation of the chloroform layer, the free amine was obtained as a solid. To the free amine, 1 cc of ethanol and 2.5 cc of methyl iodide were added. The reaction mixture was heated in a sealed tube for 18 hr at 100°. Upon evaporation of the solvent, a black solid separated. After the solid was recrystallized from ethanol–ether, a yellow solid separated which became black upon exposure to light. The solid was dissolved in ethanol and 0.3 cc of bromine was added, which caused the solution to change color from black to orange. After addition of excess ether, a solid separated which was recrystallized from ethanol–ether to give 0.041 g (23.8% yield) of 3β-trimethylammonium-17-oxo-(5α)-androstane bromide hemihydrate (**3**), mp 287° dec. The ORD of the sample (2.5×10^{-3} *M* in water) showed a positive Cotton effect with a molecular rotation for the first extremum of $[\Phi]_{306}$ +8040, λ_0 290 mμ, and $[\Phi]_{271}$ −6920 for the second extremum. The infrared spectrum showed absorptions at 2920 (C–H), 1740 (five-membered cyclic C=O), and 1470 cm^{-1} (C–H, b). The nmr spectrum showed a one-proton complex multiplet at τ 6.47 ± 0.16 (3α-methyne proton), a nine-proton singlet at τ 6.90 (N-methyl protons), a 22-proton complex multiplet at τ 8.43 ± 1.04 (methylene and methyne protons), a three-proton singlet at τ 9.10 (C-19 methyl protons), and a three-proton singlet at τ 9.13 (C-18 methyl protons).

Anal. Calcd for $C_{22}H_{38}NOBr \cdot {}^1/_2H_2O$: C, 62.69; H, 9.33. Found: C, 62.79; H, 9.38.

3α-Trimethylammonium-17-oxo-(5α)-androstane Bromide Hemihydrate (4).—Using the method described for **3**, 0.150 g (0.39 mmol) of **2** was allowed to react to give 0.031 g (18.9% yield) of 3α-trimethylammonium-17-oxo-(5α)-androstane bromide hemihydrate (**4**), mp 277° dec. The ORD of the sample (2.5×10^{-3} *M* in water) showed a positive Cotton effect with a molecular rotation for the first extremum of $[\Phi]_{306}$ +9760, λ_0 291 mμ, and $[\Phi]_{271}$ −7960 for the second extremum. The infrared spectrum showed absorptions at 2920 (C–H) and 1735 (five-membered cyclic C=O), and 1475 cm^{-1} (C–H, b). The nmr spectrum showed a one-proton complex multiplet at τ 6.72 ± 0.10 (3β-methyne proton), a nine-proton singlet at τ 6.97 (N-methyl protons), a 22-proton complex multiplet at τ 8.51 ± 0.89 (methylene and methyne protons), a three-proton singlet at τ 9.12 (C-19 methyl protons), and a three-proton singlet at τ 9.16 (C-18 methyl protons).

(9) J. Iriarte, G. Rosenkranz, and R. Sondheimer, *J. Org. Chem.*, **20**, 542 (1955).

Anal. Calcd for $C_{22}H_{38}NOBr \cdot {}^1/_2H_2O$: C, 62.69; H, 9.33. Found: C, 62.55; H, 9.41.

3β,17β-Diamino-(5α)-androstane Dihydrochloride Hemihydrate (5).—To 3,17-dioxo-(5α)-androstane, 8.3 g (120 mmol) of hydroxylamine hydrochloride, 8.3 cc of pyridine, and 100 cc of ethanol were added. The reaction mixture was refluxed for 1 hr, during which time a white solid separated. After evaporation of the solvent and washing with water, the solid was recrystallized out of ethanol to give 3.36 g (73.4% yield) of 3,17-bis(hydroxyamino)-(5α)-androstane, mp 267–270° (lit. 260–261° [10] and 271–273° [5]). The infrared spectrum showed absorptions at 3270 (O–H), 1670 (C=N), and 940 cm^{-1} (N–OH).

The bisoxime (3.96 g) was added to 450 cc of *n*-amyl alcohol and reduced with 25 g of sodium according to the method of Dodgson and Haworth.[5] After acidification of the solution with hydrochloric acid and evaporation of the solvent, a solid separated. The solid was dissolved in hot water and excess sodium hydroxide was added; the solid which separated was extracted with chloroform. After evaporation of the chloroform layer, the free amine was obtained as an oil. Upon addition of hydrochloric acid, a solid separated which was recrystallized from ethanol–acetone to give 4.04 g (88.0% yield) of 3β,17β-diamino-(5α)-androstane dihydrochloride hemihydrate (5), mp >370° dec. The ORD of the sample (2.5×10^{-3} *M* in water) gave a very slight positive plain curve in the region of 200–300 mμ. The infrared spectrum showed absorptions at 3440 (primary $\overset{+}{N}$–H), 2910 (C–H), 1985 ($\overset{+}{N}H_3$, b), 1590 (N–H, b), 1490 (N–H, b), and 1445 cm^{-1} (C–H, b). The nmr spectrum showed a two-proton complex multiplet at τ 6.92 ± 0.33 (N-methyne protons), a 22-proton complex multiplet at τ 8.62 ± 1.07 (methylene and methyne protons), a three-proton singlet at τ 9.18 (C-19 methyl protons), and a three-proton singlet at τ 9.20 (C-18 methyl protons).

Anal. Calcd for $C_{19}H_{36}N_2Cl_2 \cdot {}^1/_2H_2O$: C, 61.28; H, 10.02. Found: C, 61.34; H, 10.09.

3α,17β-Diamino-(5α)-androstane Dihydrochloride Dihydrate (6).—The intermediate bisoxime (3.2 g) described in the preparation of 12 was added to 120 cc of glacial acetic acid, and 0.60 g of platinum oxide was added. The sterically less hindered 3-oxime was stereoselectively reduced to the 3α-amine under a hydrogen atmosphere in a Parr shaker for 15 hr at 36 psi according to the method of Shoppee, *et al.*[6] After filtration of the catalyst and evaporation of the solvent, an oil separated. Upon addition of hot water, followed by filtration of the water-insoluble residue, excess sodium hydroxide was added, and the solid which separated was extracted with chloroform and then ether. Upon evaporation of the combined organic layers, 2.4 g (78.5% yield) of the solid crude 3α-amino-17-hydroxyamino-(5α)-androstane was obtained and used without further purification. The infrared spectrum showed absorptions at 3310 (N–H and O–H), 2910 (C–H), 1660 (C=N), 1580 (N–H, b), 1440 (C–H, b), and 940 cm^{-1} (N–OH).

The above intermediate (2.4 g) was added to 350 cc of *n*-amyl alcohol. The oxime was reduced to the β-amine with 21 g of sodium in boiling *n*-amyl alcohol according to the method of Dodgson and Haworth.[5] After acidification with hydrochloric acid and evaporation of the solvent, a solid separated. The solid was dissolved in hot water and excess sodium hydroxide was added; the solid which separated was extracted with chloroform. After evaporation of the chloroform layer, the free amine was obtained as an oil. Upon addition of hydrochloric acid, a solid separated which was recrystallized from ethanol–acetone to give 1.6 g (51.0% yield) of 3α,17β-diamino-(5α)-androstane dihydrochloride dihydrate (6), mp >370° dec. The ORD of the sample (2.5×10^{-3} *M* in water) gave a very slight positive plain curve in the region of 200–300 mμ. The infrared spectrum showed absorptions at 3460 (primary $\overset{+}{N}$–H), 2940 (C–H), 2000 ($\overset{+}{N}H_3$, b), 1615 (N–H, b), 1505 (N–H, b), and 1450 cm^{-1} (C–H, b). The nmr spectrum showed a one-proton complex multiplet at τ 6.44 ± 0.16 ($\Delta\nu_{1/2}$ = 8 cps, 3β-methyne proton), a one-proton complex multiplet at τ 6.94 ± 0.28 ($\Delta\nu_{1/2}$ = 16 cps, 17α-methyne proton), a 22-proton complex multiplet at τ 8.52 ± 0.88 (methylene and methyne protons), a three-proton singlet at τ 9.18 (C-19 methyl protons), and a three-proton singlet at τ 9.22 (C-18 methyl protons).

Anal. Calcd for $C_{19}H_{36}N_2Cl_2 \cdot 2H_2O$: C, 57.13; H, 10.09. Found: C, 57.35; H, 10.14.

3α,17α-Diamino-(5α)-androstane Dihydrochloride Monohydrate (7).—To 2.0 g (6.9 mmol) of 3-oxo-17β-hydroxy-(5α)-androstane, 6.0 g (32 mmol) of *p*-toluenesulfonyl chloride and 40 cc of dry pyridine were added. The reaction mixture was heated for 4 days at 48°, according to the method of Elks and Shoppee.[11] Upon cooling of the reaction mixture and addition of 15 cc of water, a solid separated which was washed successively with water, 1 *N* hydrochloric acid, and water and then recrystallized from acetone to give 2.8 g (90.9% yield) of the intermediate compound, 3-oxo-17β-*p*-toluenesulfonyl-(5α)-androstane as needles, mp 180–181°. The infrared spectrum showed absorptions at 2910 and 2860 (C–H), 1700 (six-membered cyclic C=O), 1340 and 1165 (S=O), and 960 cm^{-1} (S–O–C). The nmr spectrum showed a two-proton crude doublet at τ 2.24 (J = 8 cps, aromatic 3′ protons), a two-proton crude doublet at τ 2.70 (J = 8 cps, aromatic 2′ protons), a one-proton triplet at τ 5.75 (J = 8 cps, 17α-methyne proton), a three-proton singlet at τ 7.56 (aromatic methyl protons), a 22-proton complex multiplet at τ 8.53 ± 1.09 (methylene and methyne protons), a three-proton singlet at τ 9.01 (C-19 methyl protons), and a three-proton singlet at τ 9.19 (C-18 methyl protons).

The intermediate tosylate (1.8 g) was allowed to react with 2.1 g (32 mmol) of sodium azide in 25 cc of absolute ethanol and 2 cc of water. The reaction mixture was heated in a sealed tube for 18 hr at 130°. Upon cooling of the reaction mixture, 25 cc of water and 100 cc of chloroform were added. After evaporation of the chloroform layer, an oil was obtained. Upon addition of *n*-hexane to the oil, a solid separated which was shown to be unreacted starting material by comparison of its infrared spectrum with that of the starting material. Evaporation of the hexane filtrate gave an oil of 1.2 g (95.5% yield) of the crude intermediate compound, 3-oxo-17α-azido-(5α)-androstane, which was used without further purification. The infrared spectrum (thin film on sodium chloride) showed absorptions at 2930 and 2870 (C–H), 2100 (N=N), 1710 (six-membered cyclic C=O), and 1440 cm^{-1} (C–H, b).

The crude intermediate compound, 3-oxo-17α-azido-(5α)-androstane (1.25 g), was allowed to react with 1.6 g (22.8 mmol) of hydroxylamine hydrochloride in 25 cc of ethanol and 2 cc of pyridine. The reaction mixture was heated under reflux for 4 hr. Upon evaporation of the solvent, a solid separated. After washing of the solid with 20 cc of water, 1.24 g (94.1% yield) of the crude intermediate compound, 3-hydroxyamino-17α-azido-(5α)-androstane, was obtained and was utilized without further purification. The infrared spectrum showed absorptions at 3280 (O–H), 2920 (C–H), 2110 (N=N), 1650 (C=N), 1440 (C–H, b), and 960 cm^{-1} (N–OH).

The crude intermediate compound, 3-hydroxyamino-17α-azido-(5α)-androstane (1.24 g), was added to 50 cc of glacial acetic acid, and 0.25 g of platinum oxide was added. The reduction of the 3-oxime to the 3α-amine and the corresponding reduction of the azide to the amine were carried out under a hydrogen atmosphere in a Parr shaker for 20 hr at 36 psi. After filtration of the catalyst and evaporation of the solvent, an oil separated which was dissolved in a blend of 50:50 chloroform–1 *N* hydrochloric acid. Upon separation of the aqueous layer and addition of excess sodium hydroxide, a solid separated which was extracted with chloroform. After evaporation of the chloroform layer, the free amine was obtained as an oil. Upon addition of hydrochloric acid, a solid separated which was recrystallized from ethanol–acetone to give 0.07 g (5.0% yield) of 3α,17α-diamino-(5α)-androstane dihydrochloride monohydrate (7), mp 370° dec. The ORD of the sample (2.5×10^{-3} *M* in water) gave a very slight positive plain curve in the region of 200–300 mμ. The infrared spectrum showed absorptions at 3460 (primary $\overset{+}{N}$–H), 2920 (C–H), 2040 ($\overset{+}{N}H_3$, b), 1600 (N–H, b), 1510 (N–H, b), and 1440 cm^{-1} (C–H, b). The nmr spectrum showed a two-proton complex multiplet at τ 6.66 ± 0.28 (N-methyne protons), a 22-proton complex multiplet at τ 8.42 ± 1.05 (methylene and methyne protons), and a six-proton singlet at τ 9.11 (C-18, C-19 methyl protons).

Anal. Calcd for $C_{19}H_{36}N_2Cl_2 \cdot H_2O$: C, 59.82; H, 10.04. Found: C, 59.67; H, 10.27.

(10) (a) L. Ruzicka, P. Meister, and V. Prelog, *Helv. Chim. Acta*, **30**, 867 (1947); (b) P. Crabbe, M. J. Durazo, R. M. Salama, and P. G. Holton, *Bull. Soc. Chim. Belg.*, **71**, 203 (1962).

(11) J. Elks and C. W. Shoppee, *J. Chem. Soc.*, 241 (1953).

3β-N,N-Dimethylamino-17β-amino-(5α)-androstane Dihydrochloride Monohydrate (8).—To 1.5 g (4.1 mmol) of **1**, 1.5 g (22 mmol) of hydroxylamine hydrochloride, 2 cc of pyridine, and 60 cc of ethanol were added. The reaction mixture was refluxed for 1 hr, during which time a white solid separated. After evaporation of the solvent and washing with water, the solid was dried to give 1.2 g (78% yield) of the crude intermediate compound, 3β-dimethylamino-17-hydroxyamino-(5α)-androstane hydrochloride, which was used without further purification. The infrared spectrum (Nujol mull) showed absorptions at 3280 (O–H), 2620 (tertiary $\overset{+}{N}$–H, b), 1670 (C=N), and 930 cm^{-1} (N–OH).

The crude intermediate compound (1.12 g) was added to 200 cc of *n*-amyl alcohol. The oxime was reduced with 12 g of sodium in boiling *n*-amyl alcohol according to the method of Dodgson and Haworth.[5] After acidification with hydrochloric acid and evaporation of the solvent, a solid separated. The solid was dissolved in hot water and excess sodium hydroxide was added; the solid which separated was extracted with chloroform. Upon addition of hydrochloric acid, a solid separated which was recrystallized from ethanol–acetone to give 0.62 g (50.1% yield) of 3β-N,N-dimethylamino-17β-amino-(5α)-androstane dihydrochloride monohydrate (**8**), mp 360° dec. The ORD of the sample (2.5×10^{-3} *M* in water) gave a very slight positive plain curve in the region of 200–300 mμ. The infrared spectrum showed absorptions at 3400 (primary $\overset{+}{N}$–H), 2870 (C–H), 2690 (tertiary $\overset{+}{N}$–H), 2070 ($\overset{+}{N}H_3$, b), 1610 (N–H, b), 1515 (N–H, b), and 1460 cm^{-1} (C–H, b). The nmr spectrum showed a two-proton complex multiplet at τ 6.87 ± 0.37 (N-methyne protons), a six-proton singlet at τ 7.18 (N-methyl protons), a 22-proton complex multiplet at τ 8.51 ± 0.76 (methylene and methyne protons), a three-proton singlet at τ 9.17 (C-19 methyl protons), and a three-proton singlet at τ 9.20 (C-18 methyl protons).

Anal. Calcd for $C_{21}H_{40}N_2Cl_2 \cdot H_2O$: C, 61.59; H, 10.34. Found: C, 61.64; H, 10.43.

3β-N,N-Dimethylamino-17α-amino-(5α)-androstane Dihydrochloride Monohydrate (9).—The crude intermediate compound (1.30 g), 3-oxo-17α-azido-(5α)-androstane, described in the preparation of **7** was allowed to react with 3.35 g (41 mmol) of dimethylamine hydrochloride, 1 cc of triethylamine, 1.0 g of 10% palladized charcoal, and 125 cc of ethanol. The reductive condensation and azide reduction was carried out in a Parr shaker under hydrogen atmosphere for 42 hr at 36 psi. After filtration of the catalyst and evaporation of the solvent, a solid separated. The solid was dissolved in hot water and excess sodium hydroxide was added; the solid which separated was extracted with ether. After evaporation of the ether layer, the free amine was obtained as an oil. Upon addition of hydrochloric acid, a solid separated which was recrystallized from ethanol–acetone to give 0.20 g (11.9% yield) of 3β-N,N-dimethylamino-17α-amino-(5α)-androstane dihydrochloride monohydrate (**9**), mp 316° dec. The ORD of the sample (2.5×10^{-3} *M* in water) gave a very slight positive plain curve in the region from 200–300 mμ. The infrared spectrum showed absorptions at 3480 (primary $\overset{+}{N}$–H), 2940 (C–H), 2680 (tertiary $\overset{+}{N}$–H, b), 2110 ($\overset{+}{N}H_3$, b), 1640 (N–H, b), 1510 (N–H, b), and 1440 cm^{-1} (C–H, b). The nmr spectrum showed a two-proton complex multiplet at τ 6.81 ± 0.40 (N-methyne protons), a six-proton singlet at τ 7.18 (N-methyl protons), a 22-proton complex multiplet at τ 8.43 ± 0.94 (methylene and methyne protons), and a six-proton singlet at τ 9.17 (C-18, C-29 methyl protons).

Anal. Calcd for $C_{21}H_{40}N_2Cl_2 \cdot H_2O$: C, 61.59; H, 10.34. Found: C, 61.47; H, 10.16.

3β,17β-Bis(dimethylamino)-(5α)-androstane Dihydrochloride (10).—To 3.86 g (10.4 mmol) of **5**, 50 cc of water was added, and the pH was adjusted to 7.0 with aqueous sodium hydroxide. The methylation was accomplished by reductive condensation with 71 g (850 mmol) of 37% formaldehyde solution and hydrogen in the presence of 1.0 g of 10% palladized charcoal in a total volume of 150 cc. The reaction was carried out in a Parr shaker for 40 hr at 36 psi. After filtration of the catalyst and evaporation of the solvent, a solid separated. The solid was dissolved in hot water and excess sodium hydroxide was added; the solid which separated was extracted with ether. After evaporation of the ether layer, the free amine was obtained as an oil. Upon addition of hydrochloric acid, a solid separated which was recrystallized from ethanol–acetone to give 2.54 g (58.5% yield) of 3β,17β-bis(dimethylamino)-(5α)-androstane dihydrochloride (**10**), mp >370° dec (lit.[4] >360° dec). The ORD of the sample (2.5×10^{-3} *M* in water) gave a very slight positive plain curve in the region from 200–300 mμ. The infrared spectrum showed absorptions at 2930 (C–H), 2560 and 2460 (tertiary $\overset{+}{N}$–H 1470 (N–H, b), and 1440 cm^{-1} (C–H, b). The nmr spectrum showed a two-proton complex multiplet at τ 6.93 ± 0.33 (N-methyne protons), a six-proton singlet at τ 7.19 (17 N-methyl protons), a 22-proton complex multiplet at τ 8.40 ± 0.92 (methylene and methyne protons), a three-proton singlet at τ 9.10 (C-19 methyl protons), and a three-proton singlet at τ 9.19 (C-18 methyl protons).

Anal. Calcd for $C_{23}H_{44}N_2Cl_2$: C, 65.86; H, 10.57. Found: C, 65.89; H, 10.63.

Compound **10** was also prepared by another route. To 5.00 g (17.3 mmol) of 3,17-dioxo-(5α)-androstane, 100 cc of ethanol, 2 cc of triethylamine, and 1.0 g of palladized charcoal were added. The reductive condensation reaction was carried out in a Parr shaker under hydrogen atmosphere for 25 hr at 36 psi. After filtration of the catalyst and evaporation of the solvent, a solid separated which was dissolved in a blend of 50:50 chloroform–1 hydrochloric acid. The organic layer was saved for subsequent work-up, and yielded **1**. Upon separation of the aqueous layer and addition of excess sodium hydroxide, the basic solution was extracted with ether. After evaporation of the ether layer, the free amine was obtained as an oil. The oil was dissolved in acetone and excess hydrochloric acid was added. A solid separated which was recrystallized from ethanol–acetone to give 0.10 g (1.4% yield) of 3β,17β-bis(dimethylamino)-(5α)-androstane dihydrochloride (**10**), mp >370° dec. The melting point and infrared and nmr spectra were found to be identical with those of the material prepared by the method described above.

3β,17α-Bis(dimethylamino)-(5α)-androstane Dihydrochloride (11).—Using the method described for **10**, 0.125 g (0.30 mmol) of **9** was allowed to react to give 0.047 g (42.3% yield) of 3β,17α-bis(dimethylamino)-(5α)-androstane dichloride (**11**), mp 313° dec. The ORD of the sample (2.5×10^{-3} *M* in water) gave a very slight positive plain curve in the region of 200–300 mμ. The infrared spectrum showed absorptions at 2940 (C–H), 2580 and 2470 (tertiary $\overset{+}{N}$–H, b), 1710 (N–H, b), 1640 (N–H, b), and 1470 cm^{-1} (C–H, b). The nmr spectrum showed a two-proton complex multiplet at τ 6.77 ± 0.33 (N-methyne protons), a 12-proton singlet at τ 7.12 (N-methyl protons), a 22-proton complex multiplet at τ 8.57 ± 1.02 (methylene and methyne protons), a three-proton singlet at τ 9.11 (C-19 methyl protons), and a three-proton singlet at τ 9.16 (C-18 methyl protons).

Anal. Calcd for $C_{23}H_{44}N_2Cl_2$: C, 65.86; H, 10.57. Found: C, 65.96; H, 10.62.

3α,17β-Bis(dimethylamino)-(5α)-androstane Dihydrochloride Monohydrate (12).—Using the method described for **10**, 1.40 g (3.5 mmol) of **6** was allowed to react to give 0.56 g (36.6% yield) of 3α,17β-bis(dimethylamino)-(5α)-androstane dihydrochloride monohydrate (**12**), mp 287° dec. The ORD of the sample (2.5×10^{-3} *M* in water) gave a very slight positive plain curve in the region of 200–300 mμ. The infrared spectrum showed absorptions at 2930 (C–H), 2650 and 2480 (tertiary $\overset{+}{N}$–H), 1630 (N–H, b), and 1450 cm^{-1} (C–H, b). The nmr spectrum showed a two-proton complex multiplet at τ 6.90 ± 0.37 (N-methyne protons), a 12-proton singlet at τ 7.07 (N-methyl protons), a 22-proton complex multiplet at τ 8.42 ± 0.95 (methylene and methyne protons), a three-proton singlet at τ 9.08 (C-19 methyl protons), and a three-proton singlet at τ 9.12 (C-18 methyl protons).

Anal. Calcd for $C_{23}H_{44}N_2Cl_2 \cdot H_2O$: C, 63.14; H, 10.60. Found: C, 63.07; H, 10.79.

Registry No.—**1**, 25383-09-9; **2**, 25383-11-3; **3**, 25383-12-4; **4**, 25383-13-5; **5**, 14968-31-1; **6**, 25383-15-7; **7**, 25383-16-8; **8**, 25383-18-0; **9**, 25383-19-1; **10**, 14967-50-1; **11**, 25383-21-5; **12**, 25383-22-6; 3α-*p*-toluenesulfonyl-17-oxo-5α-androstane, 25383-10-2; 3-oxo-17β-*p*-toluenesulfonyl-5α-androstane, 25383-17-9.

Acknowledgment.—This work was supported by Grants GM13597, GM15308, and GM15309 from the U. S. Public Health Service.