method of Shore, *et al.*,[5] employing a later modification[6] where one-tenth the usual amount of *o*-phthalaldehyde was used. Duplicate 1-ml aliquots of plasma were analyzed; fluorescence of the final solutions was measured on a Turner fluorometer[7] equipped with 7-60 primary and 2-A secondary filters. The lower limit of sensitivity was 0.005 μg of histamine/ml of initial sample, and the precision of reading samples was ±0.0005 μg. Recoveries of added histamine from plasma averaged 92% (range 90–96%). Average deviations of single determinations from their means did not exceed 4%. Serotonin did not contribute in this assay.

Experimental Section

Physical and analytical data are listed in Tables II and III.

A. N,N′-(1-Benzyl-4-phenyl-4-piperidylmethyl)terephthalamide Dihydrochloride (XIV).—To a chilled mixture of 2.0 g (7.1 mmoles) of 4-phenyl-4-aminomethyl-1-benzylpiperidine (Aldrich Chemical Co.) in 15 ml of anhydrous methylene chloride was added (dropwise) a mixture of 0.73 g (3.6 mmoles) of terephthaloyl dichloride in 10 ml of anhydrous CH_2Cl_2. The mixture was stirred at room temperature 1 hr, then allowed to stand at room temperature overnight. The white solid was collected by filtration and recrystallized from absolute methanol, giving 1.83 g (67%) of white crystals, mp 283–290°. A sample was converted to the free amine (partitioned between $CHCl_3$ and 10% NaOH) and recrystallized from 2-methoxyethanol.

B. N,N′-(4-Phenyl-4-piperidylmethyl)terephthalamide (VIII).—To 500 mg of 100% Pd catalyst were added 20 ml of glacial acetic acid and 1.3 g (1.7 mmoles) of XIV. The mixture was hydrogenated at 70° and atmospheric pressure, taking up the theoretical amount of hydrogen in 1 hr. The mixture was diluted with 25 ml of water, the catalyst was removed by filtration, and the filtrate was evaporated *in vacuo* to dryness. The gummy material was taken up in water and alkalized (pH 10) with 10% NaOH to precipitate a white solid. The solid was collected by filtration and washed thoroughly with water to leave 0.74 g. Recrystallization from 2-methoxyethanol afforded 0.56 g (65%) of white crystals.

C. 1-Carbomethoxy-4-cyano-4-phenylpiperidine (XIX).—To a mixture of 5.0 g (22.5 mmoles) of 4-phenyl-4-cyanopiperidine hydrochloride, 100 ml of water, 2.2 g (54 mmoles) of NaOH, and 50 ml of $CHCl_3$ was slowly added 2.0 ml (27 mmoles) of methyl chloroformate. The mixture was vigorously stirred at room temperature for 2 hr, chilled, and acidified to pH 2 with 6 *N* HCl. The mixture was stirred (chilled) for 1 hr, and the chloroform layer was removed and washed with water. The $CHCl_3$ extract was dried ($MgSO_4$) and evaporated *in vacuo* to dryness to give an orange syrup which crystallized upon standing to yield 5.0 g (91%) of white crystals. An analytical sample was obtained from cyclohexane.

D. 1-Methyl-4-aminomethyl-4-phenylpiperidine Dipicrate (XXII).—To a cold suspension of 7.7 g (0.2 mole) of $LiAlH_4$ in 175 ml of anhydrous tetrahydrofuran was slowly added 5.0 g (22.3 mmoles) of XIX. The mixture was refluxed 15 hr. Excess $LiAlH_4$ was decomposed by the careful addition of absolute ethanol. The reaction mixture was then treated with water, stirred briefly, and evaporated *in vacuo* to near dryness. The pasty material was extracted with 1-butanol, which was dried ($MgSO_4$) and evaporated *in vacuo* to yield 3.64 g (80%) of orange syrup. A sample was converted to the picrate and recrystallized from 2-methoxyethanol.

1-Methyl-4-hydroxymethyl-4-phenylpiperidine Terephthalate (XXIX).—To a chilled mixture of 1.02 g (5 mmoles) of 1-methyl-4-phenyl-4-hydroxymethylpiperidine[3] in 10 ml of anhydrous CH_2Cl_2 was added a suspension of 0.505 g (2.5 mmoles) of terephthaloyl chloride in 10 ml of of anhydrous CH_2Cl_2. The mixture was stirred at room temperature (25°) for 18 hr, and the white crystals were collected by filtration. Trituration with acetone gave 1.25 g of crude material. Recrystallization from 2-propanol–absolute methanol yielded 0.292 g (11%), mp 276–279°.

Anal. Calcd for $C_{34}H_{40}N_2O_4 \cdot 2HCl \cdot H_2O$: C, 64.6; H, 7.03; N, 4.43. Found: C, 64.6; H, 7.09; N, 4.50.

(5) P. A. Shore, A. Burkhalter, and V. H. Cohn, Jr., *J. Pharmacol. Exptl. Therap.*, **127**, 182 (1959).

(6) D. von Redlich and D. Glick, *Anal. Biochem.*, **10**, 459 (1965).

(7) Model 110 was equipped with a high-sensitivity conversion kit, No. 110-865, and a microcuvette adaptor, No. 110-66.

16-Aza Steroids

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The preparation of several 16-azaandrostenes is described, utilizing 3β-hydroxy-16,17-secoandrost-5-ene-16,17-dioic acid (I) as starting material. The latter compound was transformed, *via* its diester IIa, half-ester IIb, acid chloride IIc, and isocyanate III, into 3β-hydroxy-16-azaandrost-5-en-17-one (V). In a similar manner 16-azaestrone was prepared from the methyl ether of marrianolic acid. The results of some preliminary biological tests are reported.

The introduction of a nitrogen atom into the steroid nucleus has led to compounds exhibiting diverse biological activity.[1] Although most of the ring D aza steroids previously described[2] are analogs of D-homosteroids, the preparation of various 17-aza steroids has been reported[3] in which the nitrogen is incorporated in a five-membered D ring. Studies on the corresponding 16-aza series were initiated by Bachmann and Ramirez, who described[4] the synthesis of *dl*-16-azadeoxyisoequilenin and *dl*-16-azadeoxyequilenin. A recent report on the preparation of the 16-aza derivatives of estrone[5] prompts us to describe our related studies in this field.

In this connection, a diester of 3β-hydroxy-16,17-secoandrost-5-ene-16,17-dioic acid (I) appeared attractive as a starting material for the 16-azaandrostenes. Due to the steric hindrance of the tertiary carboxyl group, the diacid I has usually been converted to the dimethyl ester[6] with diazomethane. We have found that I was more conveniently esterified with the diethyl

(1) M. Martin-Smith and M. F. Sugrue, *J. Pharm. Pharmacol.*, **16**, 569 (1964).

(2) L. Tokes in "Steroid Reactions," C. Djerassi, Ed., Holden Day, Inc., San Francisco, Calif., 1963, pp 502–531.

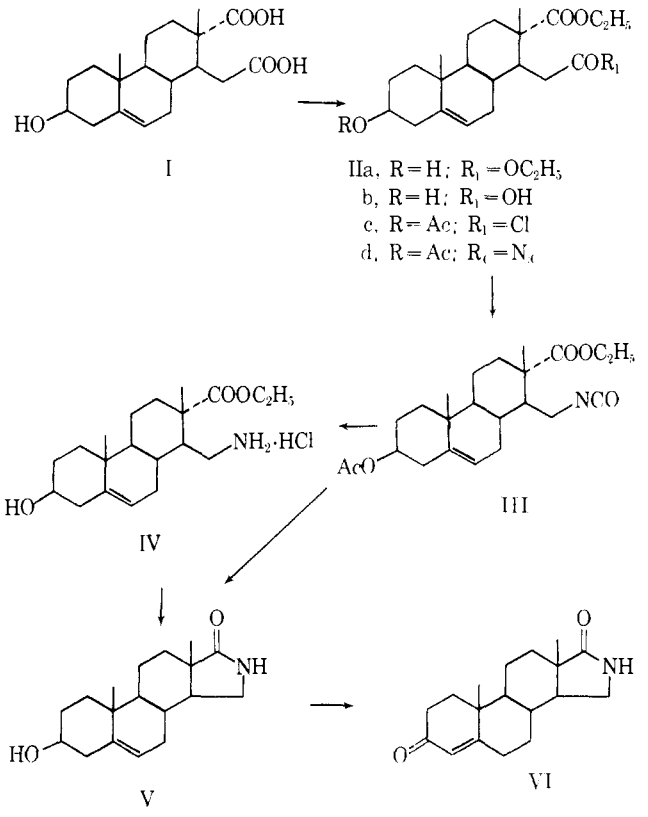
(3) (a) S. Rakhit and M. Gut, *J. Org. Chem.*, **29**, 859 (1964); (b) S. Rakhit and M. Gut, *Steroids*, **4**, 291 (1964).

(4) W. E. Bachmann and F. Ramirez, *J. Am. Chem. Soc.*, **72**, 2527 (1950).

(5) J. S. Baran, U. S. Patent 3,257,412 (June 21, 1966).

(6) (a) J. Heer and K. Miescher, *Helv. Chim. Acta*, **30**, 786 (1947); (b) E. B. Hershberg, E. Schwenk, and E. Stahl, *Arch. Biochem.*, **19**, 300 (1948); (c) C. von Seeman and G. A. Grant, *J. Am. Chem. Soc.*, **72**, 4073 (1950).

CHART I



acetal of dimethylformamide[7] to give the diethyl ester IIa[8] in 78% yield (see Chart I).

Mild hydrolysis of the diester IIa gave the half-ester IIb which was converted, *via* its acetate, to the corresponding acid chloride IIc. Reaction of the latter compound with sodium azide gave the azide IId which, without isolation, was transformed on heating to the isocyanate III. Attempts to prepare the amine IV by direct acid hydrolysis of the isocyanate III were unsuccessful. However, treatment of III with *t*-butyl alcohol gave the *t*-butylurethan which cleaved readily in the presence of acid[9] to give the amine hydrochloride IV. The latter compound underwent smooth cyclization in the presence of dilute base to give 3β-hydroxy-16-azaandrost-5-en-17-one (V).

In a more convenient procedure, the isocyanate III was converted directly and in high yield to the lactam V on treatment with dilute base. Oxidation of V by the Oppenauer method gave the expected 3-keto lactam VI.

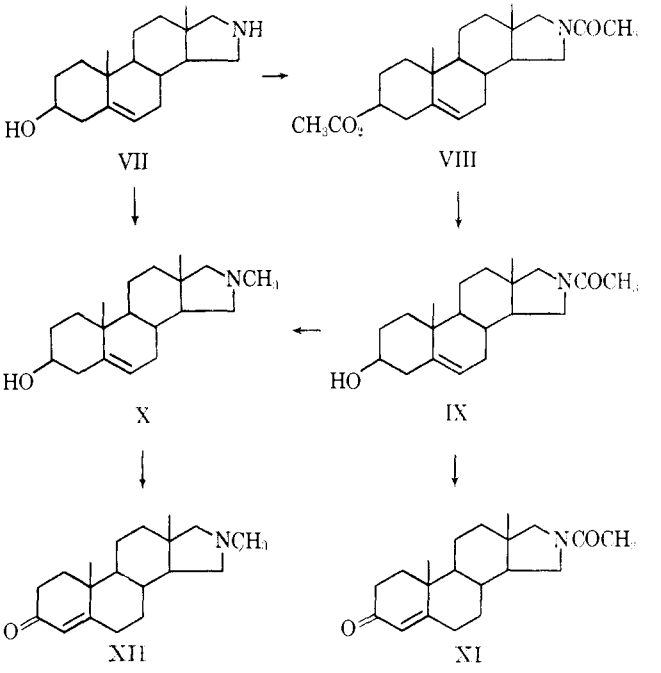
Vigorous reduction of the lactam V with lithium aluminum hydride in dioxane gave the amine VII. Treatment of the latter compound with acetic anhydride in pyridine gave the N-acetyl acetate VIII, which was hydrolyzed to the corresponding alcohol IX. Reaction of the amine VII with formaldehyde and formic acid gave the N-methyl derivative X. Oppenauer oxidation of IX and X gave the corresponding ketones XI and XII, respectively (see Chart II).

(7) (a) H. Vorbrüggen, *Angew. Chem. Intern. Ed. Engl.*, **2**, 211 (1963); (b) H. Brechbühler, H. Büchi, E. Hatz, J. Schreiber, and E. Eschenmoser, *ibid.*, **2**, 212 (1963); *Helv. Chim. Acta*, **48**, 1746 (1965).

(8) First prepared from the diacid and diazoethane by S. Kuwada and K. Nakamura, *J. Pharm. Soc. Japan*, **58**, 841 (1938); *Chem. Abstr.*, **33**, 2530 (1939).

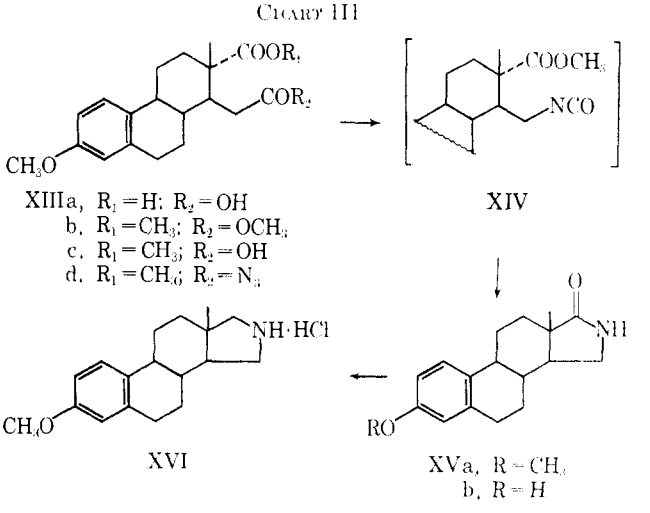
(9) H. C. Beyerman and J. S. Bontekoe, *Proc. Chem. Soc.*, 249 (1961).

CHART II



Our studies on the preparation of 16-azaestrone utilized as starting material the methyl ether of marrianolic acid (XIIIa)[10] (see Chart III). The dimethyl

CHART III



ester XIIIb[10] of the latter compound was hydrolyzed with potassium carbonate solution to give the known half-ester XIIIc[11] which was converted, *via* the acid chloride, to the corresponding azide XIIId. The crude azide rearranged on heating in benzene solution to afford the isocyanate XIV, which on treatment with dilute potassium hydroxide was converted directly to the methyl ether of 16-azaestrone (XVa).[5] The latter compound was demethylated readily with pyridine hydrochloride to give 16-azaestrone (XVb). Reduction of XVa with lithium aluminum hydride in dioxane solution gave the corresponding amine, which was isolated as the hydrochloride (XVI). Baran has previously reported[5] the isolation of this amine as the hydrobromide salt.

(10) (a) M. Levitz, *J. Am. Chem. Soc.*, **75**, 5352 (1953); (b) J. C. Sheehan, R. E. Coderre, and P. A. Cruickshank, *ibid.*, **75**, 6231 (1953).

(11) First prepared by J. Heer and K. Miescher, *Helv. Chim. Acta*, **29**, 895 (1946).

Biological Results.—None of the 16-azaandrostenes described herein exhibited any significant antigonadotropic activity when assayed orally for 10 days in immature male rats at 1 mg/rat/day. The compounds were evaluated for progestational activity in the McPhail assay and were found inactive.[12] The compounds in this series did not exhibit any significant uterotropic activity when assayed orally for 3 days in the immature female rat at 1 mg/rat/day.

When assayed in ovariectomized rats,[13] both 16-azaestrone (XVb) and its methyl ether XVa exhibit very weak estrogenic activity, having less than 10^{-4} of the activity of estradiol benzoate. Both compounds exhibit antiestrogenic activity[14] and cause a 42 and 38% inhibition, respectively, of the uterine weight increase due to estradiol benzoate.

Experimental Section[15]

Diethyl Ester of 3β-Hydroxy-16,17-secoandrost-5-ene-16,17-dioic Acid (IIa).—To a suspension of 10.0 g (0.028 mole) of 3β-hydroxy-16,17-secoandrost-5-ene-16,17-dioic acid (I) (mp 246–249° dec)[16] and 100 ml of benzene was added 15 g (0.10 mole) of the diethyl acetal of dimethylformamide and the resulting stirred mixture was heated under reflux for 3 hr (the mixture became clear after about 40 min). The cooled reaction mixture was filtered through a bed of Celite (made up in ether) and the filtrate was diluted with an equal volume of ether. The organic solution was washed twice with 2 *N* HCl, twice with 2 *N* NaOH, and once with water. The dried (Na_2SO_4) solvent was removed under reduced pressure and the resulting semicrystalline oil was crystallized from ether–hexane to give 8.84 g (78%) of IIa as an off-white solid, mp 93–95.5° (vac), lit.[8] mp 103.5–104.5°.

Monoethyl Ester of 3β-Hydroxy-16,17-secoandrost-5-ene-16,17-dioic Acid (IIb).—A solution of 34.0 g (0.61 mole) of KOH in 340 ml of water was added to a solution of 34.0 g (0.087 mole) of IIa in 680 ml of methanol and the resulting mixture was heated under reflux for 1 hr. The reaction mixture was cooled immediately and most of the solvent was removed under reduced pressure, care being taken that the bath temperature did not rise above 45°. The residue was diluted with about 1 l. of water and was then extracted with ether–methylene chloride (3:1). The aqueous layer was cooled in ice water and acidified to congo red with about 500 ml of 6 *N* HCl. The resulting precipitate was filtered and washed with water. The wet compound was crystallized from acetone–water (1:1) to give 24.7 g (76%) of IIb, mp 176.5–178° (vac), lit.[8] mp 176–177.5°.

Acetate of 1,2,3,4,4a,4b,5,6,7,8,10,10a-Dodecahydro-7-hydroxy-1-chloroformylmethyl-2,4b-dimethyl-2-phenanthrenecarboxylic Acid Ethyl Ester (IIc).—To a solution of 185 g (0.51 mole) of the dry half-ester IIb in 1280 ml of anhydrous pyridine was added 785 ml of freshly distilled acetic anhydride and the reaction mixture was allowed to stand at room temperature for 48 hr. The reaction mixture was poured into 7 l. of water and was extracted three times with ether–CH_2Cl_2 (3:1). The organic layers were washed three times with 2 *N* HCl and once with water. The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to give 206 g of crude acetate, which was used directly for the next step.

Oxalyl chloride (300 ml) was added rapidly to a stirred solution of the crude acetate (206 g) in 2 l. of benzene, and the temperature of the reaction mixture was then maintained at 66–69° for 1.5 hr by heating in an oil bath. An additional 50 ml of oxalyl chloride was then added, and heating and stirring were continued for an additional 30 min. The reaction mixture was cooled and the solvents were removed under reduced pressure, care being taken that the bath temperature did not rise above 45°. The residue was diluted with benzene and the solution was evaporated to dryness under reduced pressure in order to remove the last traces of oxalyl chloride. The crude product was slurried with 300 ml of hexane (no attempt should be made to heat or "crystallize" at this point) and the resulting precipitate was filtered and washed with hexane to give 197 g (91%) of the crude acid chloride IIc, mp 114–122°, which is sufficiently pure for the next step. A small portion was crystallized from hexane to give the analytical sample, mp 130.5–132° (vac), $[\alpha]^{26}D$ −74.5° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 5.54 and 5.86 μ.

Anal. Calcd for $C_{23}H_{33}ClO_5$: C, 65.00; H, 7.83; Cl, 8.34. Found: C, 65.27; H, 7.95; Cl, 8.09.

Acetate of 1,2,3,4,4a,4b,5,6,7,8,10,10a-Dodecahydro-7-hydroxy-1-isocyanatomethyl-2,4b-dimethyl-2-phenanthrenecarboxylic Acid Ethyl Ester (III).—To a vigorously stirred and cooled (5–10°) solution of 197 g (0.463 mole) of IIc in 4 l. of acetone was added a solution of 154 g (2.37 moles) of NaN_3 in 620 ml of water over a 20-min period. The reaction was stirred in the cold for an additional 20 min and was then diluted with 3.5 l. of water. The mixture was extracted four times with benzene and the benzene layers were washed twice with water and once with brine. The combined benzene layers were dried (Na_2SO_4) and about 250 ml of benzene was evaporated under reduced pressure in order to remove the last traces of water.

The remaining benzene solution (of crude azide IId) was heated under reflux with stirring for 1.5 hr, whereupon the evolution of gas had ceased. The reaction mixture was cooled and the solvent was removed under reduced pressure to give a yellow oil which crystallized upon addition of 200 ml of hexane. The material was filtered and crystallized from ether–hexane (1:10) to give 131 g (70%) of isocyanate (III), mp 122–127° (vac). Crystallization from ether–hexane gave the analytical sample, mp 127–128° (vac), $[\alpha]^{27}D$ −54.6° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 4.39 and 5.80 μ.

Anal. Calcd for $C_{23}H_{33}NO_5$: C, 68.46; H, 8.24; N, 3.47. Found: C, 68.19; H, 8.03; N, 3.62.

The corresponding methyl ester had mp 161.5–163.5°, $[\alpha]^{28}D$ −55.2° (*c* 1.0, $CHCl_3$), λ_{max}^{KBr} 4.41 and 5.81 μ.

Anal. Calcd for $C_{22}H_{31}NO_5$: C, 67.84; H, 8.02; N, 3.60. Found: C, 68.11; H, 8.30; N, 3.66.

Hydrochloride of 1,2,3,4,4a,4b,5,6,7,8,10,10a-Dodecahydro-7-hydroxy-1-aminomethyl-2,4b-dimethyl-2-phenanthrenecarboxylic Acid Ethyl Ester (IV).—A solution of 22.0 g (54.5 mmoles) of the isocyanate III in 220 ml of *t*-butyl alcohol was heated under reflux for 22 hr and then was cooled and evaporated to dryness under reduced pressure. The residue was dissolved in 150 ml of 4 *N* ethanolic HCl, and the solution was heated under reflux for 10 min. The cooled reaction mixture was evaporated to dryness and the residue crystallized from ethanol–ethyl acetate to give 7.68 g (38%) of the hydrochloride of IV, mp 239–240.5° dec (vac). Crystallization from ethanol–ethyl acetate gave the analytical sample, mp 239.5–240.5° dec (vac), λ_{max}^{KBr} 5.85 μ.

Anal. Calcd for $C_{20}H_{34}ClNO_3$: C, 64.58; H, 9.21; Cl, 9.53; N, 3.77. Found: C, 64.53; H, 9.46; Cl, 9.70; N, 3.91.

3β-Hydroxy-16-azaandrost-5-en-17-one (V). A. From the Isocyanate III.—A solution of 100 g (1.79 moles) of KOH in 100 ml of water was added to a suspension of 100 g (0.248 mole) of the isocyanate III (mp 122–127° (vac)) in 940 ml of methanol. The reaction mixture became warm and clear and was allowed to stand at ambient temperature overnight. It was evaporated under reduced pressure to about one-quarter of the original volume and was diluted with 2 l. of water. The resulting precipitate was filtered, washed with water, and crystallized directly from ethanol (charcoal), whereupon 50.8 g (71%) of lactam V, mp 272–274° (vac), came out of the boiling solution. An additional 9.4 g of product (mp 266–272° (vac)) was obtained upon concentration of the mother liquors. Crystallization from chloroform–acetonitrile gave the analytical sample, mp 272–274° (vac), $[\alpha]^{25}D$ −73.9° (*c* 1.0, $CHCl_3$), λ_{max}^{KBr} 2.98 and 5.95 μ.

Anal. Calcd for $C_{18}H_{27}NO_2$: C, 74.70; H, 9.40; N, 4.84. Found: C, 75.02; H, 9.23; N, 4.89.

B. From the Amine Hydrochloride IV.—To a solution of 100 mg (0.27 mmole) of the hydrochloride IV in 1.0 ml of methanol was added a solution of 150 mg (2.7 mmoles) of KOH in 0.15 ml of water. The mixture was heated on the steam bath for 10 min and was then evaporated to dryness. The residue was

(12) Assayed at a dosage of 1 mg/day sc in the rabbit.

(13) Assayed at a dosage of 1 mg/day *po* in the rat.

(14) Administered orally to rats at a dosage of 20,000:1 by weight against estradiol benzoate, the latter being administered subcutaneously.

(15) Melting points were determined in capillary tubes and are corrected. The infrared spectra were recorded on a Beckman Instrument Model IR-9 and the ultraviolet spectra on a Cary recording spectrophotometer Model 14M. Celite is a diatomaceous silica product (Johns-Manville Company). Merck-Darmstadt silica gel (0.2–0.5 mm) was used for column chromatography. Florisil (60–100 mesh) is a synthetic magnesium silicate (Floridin Co., Hancock, W. Va.)

(16) Prepared by the method of W. J. Adams, D. K. Patel, V. Petrow, and I. A. Stuart-Webb, *J. Chem. Soc.*, 297 (1956).

diluted with water and the resulting precipitate was filtered, washed with water, and dried to give 69 mg (89%) of lactam V, mp 272.5–274° (vac). Crystallization from ethanol–acetonitrile gave 48 mg of product, mp 273–274° (vac), identical with the sample prepared by method A.

16-Azaandrost-4-ene-3,17-dione (VI).—A solution of 5.0 g (17.3 mmoles) of the lactam V in 200 ml of dioxane, 170 ml of toluene, and 50 ml of cyclohexanone was heated to boiling and 30 ml of the solvent mixture was removed by distillation. To this hot solution was added rapidly 5.0 g of aluminum isopropoxide in 50 ml of toluene, and distillation was continued with simultaneous addition of fresh toluene. After 30 min an additional 1.50 g of aluminum isopropoxide was added and distillation was continued for 2 hr, a total of 300 ml of distillate being collected.

The solution was heated under reflux for an additional 4 hr and then allowed to stand at room temperature overnight. The reaction mixture was acidified with 400 ml of 10% H_2SO_4 solution and was extracted with benzene. The organic layers were washed three times with 10% K_2CO_3 solution, once with brine and dried (Na_2SO_4). The solution was evaporated to dryness at aspirator vacuum, the residual cyclohexanone being removed under high vacuum. The residue was crystallized from ethyl acetate to give 2.95 g (59.5%) of VI, mp 238.5–240° (vac). Crystallization from ethyl acetate gave the analytical sample: mp 239.5–241° (vac); $[\alpha]^{25}D$ +126° (*c* 1.0, $CHCl_3$); $\lambda_{max}^{C_2H_5OH}$ 239 mμ (ε 16,900); $\lambda_{max}^{CHCl_3}$ 2.90, 5.88, and 6.00 μ.

Anal. Calcd for $C_{19}H_{25}NO_2$: C, 75.22; H, 8.77; N, 4.87. Found: C, 75.32; H, 8.71; N, 4.88.

3β-Hydroxy-16-azaandrost-5-ene (VII).—To a solution of 25.0 g (0.61 mole) of $LiAlH_4$ in 1 l. of boiling dioxane was added rapidly a suspension of 25.0 g (0.0865 mole) of the lactam V in 1 l. of dioxane. The resulting mixture was heated under reflux for 20 hr in a nitrogen atmosphere. To the cooled (ice bath) reaction mixture was added slowly 150 ml of water and the reaction mixture was heated under reflux for an additional 30 min. The hot mixture was filtered through a bed of Celite, the latter being well washed with hot dioxane and hot chloroform. The filtrate was evaporated to dryness and the residue was crystallized from methanol–ethyl acetate to yield 18.7 g of VII, mp 234–236.5° (vac). An additional 1.6 g of product (mp 234–236.5° (vac)) was recovered from the mother liquors (total yield, 85.5%). Crystallization from methanol–ethyl acetate gave the analytical sample, mp 235.5–237.5° (vac), $[\alpha]^{25}D$ −80.0° (*c* 1.01, $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 2.78 μ.

Anal. Calcd for $C_{18}H_{29}NO$: C, 78.49; H, 10.61; N, 5.09. Found: C, 78.78; H, 10.58; N, 4.88.

3β-Hydroxy-N-acetyl-16-azaandrost-5-ene Acetate (VIII).—To a solution of 3.0 g of VII in 60 ml of hot pyridine was added 12 ml of freshly distilled acetic anhydride and the resulting solution was heated under reflux for 2 hr. The cooled reaction mixture was poured into 400 ml of cold water, and the precipitate was extracted with ether–CH_2Cl_2 (3:1). The organic layers were washed twice with 1 *N* HCl, once with 5% $NaHCO_3$ solution, and once with water. The combined organic layers were dried (Na_2SO_4) and evaporated to dryness. The residue was crystallized from acetone–hexane to give 3.43 g of crude solid. A portion (1.0 g) was recrystallized from acetone–hexane to give 0.928 g of VIII, mp 175–176.5° (vac). One further recrystallization from acetone–hexane gave the analytical sample, mp 175–176.5° (vac), $[\alpha]^{25}D$ −118° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 5.79 and 6.13 μ.

Anal. Calcd for $C_{22}H_{33}NO_3$: C, 73.50; H, 9.25; N, 3.90. Found: C, 73.38; H, 9.35; N, 3.93.

3β-Hydroxy-N-acetyl-16-azaandrost-5-ene (IX).—A solution of 4.9 g (13.6 mmoles) of crude VIII in 130 ml of methanol and 13 ml of 10% NaOH was heated under reflux for 5 min and was then allowed to stand at room temperature for 30 min. The reaction mixture was evaporated to about 20 ml and the residue was diluted with 400 ml of water. The resulting precipitate was filtered, washed with water and dried to give 3.98 g (92%) of crude IX, mp 189–192° (vac). Crystallization from ethyl acetate gave the analytical sample, mp 192–194° (vac), $[\alpha]^{25}D$ −112.4° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 2.77 and 6.15 μ.

Anal. Calcd for $C_{20}H_{31}NO_2$: C, 75.67; H, 9.84; N, 4.41. Found: C, 75.33; H, 9.98; N, 4.38.

3β-Hydroxy-N-methyl-16-azaandrost-5-ene (X).—A solution of 18.9 g (68.7 mmoles) of VII in 195 ml of 98% aqueous formic acid and 145 ml of 36% aqueous formaldehyde was heated under reflux for 2.5 hr (nitrogen atmosphere). An additional 145 ml of HCHO solution was then added and the reaction mixture was again heated under reflux for 2.5 hr. The resulting mixture was evaporated to near dryness under reduced pressure. The residue was taken up in 800 ml of 10% methanolic KOH and the solution was heated under reflux for 20 min. The solvents were then removed under reduced pressure and the residue was diluted with 600 ml of water. The mixture was extracted three times with ether–CH_2Cl_2 (3:1), and the organic layers were washed once with water. The combined organic layers were dried (Na_2SO_4) and evaporated to dryness. The product was crystallized from methanol–acetonitrile to give 12.56 g (63%) of X, mp 166–168° (vac). Crystallization from acetonitrile gave the analytical sample, mp 168–169.5° (vac), $[\alpha]^{25}D$ −108.8° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{5\%Cr}$ 3.19 μ.

Anal. Calcd for $C_{19}H_{31}NO$: C, 78.84; H, 10.80; N, 4.84. Found: C, 78.78; H, 10.69; N, 4.84.

N-Acetyl-16-azaandrost-4-en-3-one (XI).—A solution of 4.31 g (13.6 mmoles) of IX in 350 ml of toluene and 43 ml of cyclohexanone was heated to boiling and 30 ml of solvents was removed by distillation. To this hot solution was added rapidly 4.3 g of aluminum isopropoxide in 43 ml of toluene and distillation was continued. After 1 hr an additional 1.25 g of aluminum isopropoxide was added and distillation was continued for 30 min, a total of 85 ml of distillate being collected. The reaction mixture was filtered from the insoluble aluminum salts, and the filtrate was steam distilled. The residue was extracted with ether–CH_2Cl_2 (3:1), and the organic layers were washed with water, dried (Na_2SO_4), and evaporated. The resulting oil was dissolved in benzene and filtered through a short column of Florisil. Evaporation of the eluates gave an oil which crystallized from CH_2Cl_2–ether–hexane to yield 1.95 g (46%) of XI, mp 131–133° (vac). Further crystallization from the same solvent system gave the analytical sample, mp 133–135° (vac), $[\alpha]^{25}D$ +16.8° (*c* 1.1, $CHCl_3$), $\lambda_{max}^{C_2H_5OH}$ 240 mμ (ε 17,150), $\lambda_{max}^{CHCl_3}$ 6.00 and 6.15 μ.

Anal. Calcd for $C_{21}H_{29}NO_2$: C, 76.15; H, 9.27; N, 4.41. Found: C, 76.29; H, 9.51; N, 4.38.

N-Methyl-16-azaandrost-4-en-3-one (XII).—A solution of 8.0 g (27.6 mmoles) of X in 640 ml of toluene and 80 ml of cyclohexanone was heated to boiling and 50 ml of the solvent was removed by distillation. To this hot solution was added rapidly 8.0 g of aluminum isopropoxide in 80 ml of toluene and distillation was continued with simultaneous addition of fresh toluene. After 1 hr an additional 2.40 g of aluminum isopropoxide was added and distillation was continued for 30 min, a total of 250 ml of distillate being collected. The reaction mixture was cooled and filtered, and the filtrate was steam distilled. The residue was taken up in CH_2Cl_2, washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to give the crude amine as a solid. The latter material was dissolved in 300 ml of ethyl acetate and was treated with anhydrous HCl, to yield 7.00 g of crude hydrochloride, mp 289–290.5° (vac). This salt was dissolved in water and was treated with 300 ml of 1 *N* KOH solution. The liberated base was isolated with ether–CH_2Cl_2 (3:1). After evaporation to dryness, the residue was dissolved in benzene and filtered through a short column of Florisil to give 5.2 g of crude product. Crystallization from aqueous methanol followed by crystallization from ether–hexane gave 3.0 g of pure XII, mp 112.5–114.5° (vac), $[\alpha]^{25}D$ +60.4° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{C_2H_5OH}$ 240 mμ (ε 17,700), $\lambda_{max}^{CHCl_3}$ 6.01 μ.

Anal. Calcd for $C_{20}H_{29}NO$: C, 79.59; H, 10.17; N, 4.87. Found: C, 79.49; H, 10.20; N, 4.73.

2-Methoxycarbonyl-7-methoxy-2-methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-acetic Acid (Monomethyl Ester of Marrianolic Acid Methyl Ether) (XIIIc).—Estrone methyl ether was oxidized with KOI to give marrianolic acid methyl ether (XIIIa).[10] The latter compound was converted readily with CH_2N_2 to the corresponding dimethyl ester (XIIIb), mp 73–75°.[10]

A solution of 20.0 g (0.145 mole) of K_2CO_3 in 100 ml of water was added to a solution of 20.0 g (0.0555 mole) of the diester XIIIb in 400 ml of methanol. The reaction mixture was heated under reflux for 5.5 hr and was then allowed to stand at room temperature overnight. Most of the solvent was evaporated, and the residue was diluted with 500 ml of water. The aqueous solution was washed with ether and the combined ether washes were dried (Na_2SO_4) and concentrated to give 2.3 g of crude solid. Crystallization from methanol gave 1.73 g (8.5%) of recovered starting material (XIIIb), mp 73.5–76°. The aqueous layer was then acidified to congo red with 3 *N* HCl and the resulting mixture was extracted three times with ether. The

ether layers were washed with water, dried (Na_2SO_4), and evaporated to give 18.1 g of colorless oil which was chromatographed on 270 g of silica gel (0.2–0.5 mm). Elution with ethyl acetate–benzene (1:4) and finally with pure ethyl acetate gave 16.1 g of colorless oil. Crystallization from ether–hexane gave 11.56 g of half-ester (XIIIc), mp 98–101.5°. An additional 2.51 g of product, mp 93–97.5°, was recovered from the mother liquors (total yield 73%). Crystallization from ether–hexane gave the analytical sample: mp 100.5–102.5°; $[\alpha]^{25}D$ +70.4° (*c* 1.0, C_2H_5OH); $\lambda_{max}^{C_2H_5OH}$ 219 mμ (ε 8470), 278 (2060), 287 (1950); $\lambda_{max}^{CHCl_3}$ 2.77 and 5.81 μ. This compound was described previously as an oil, $[\alpha]^{20}D$ +61 ± 4° (*c* 0.19, alcohol).[11]

Anal. Calcd for $C_{20}H_{26}O_5$: C, 69.34; H, 7.57. Found: C, 69.64; H, 7.69.

16-Azaestrone 3-Methyl Ether (XVa).—To a solution of 12.5 g (0.0362 mole) of XIIIc in 63 ml of benzene was added 12.5 ml of oxalyl chloride, and the resulting mixture was heated at 65° for 50 min. An additional 6.0 ml of oxalyl chloride was then added and heating was continued for 30 min. The reaction mixture was evaporated to dryness and the residue was crystallized from ether–hexane to give 11.68 g of crude acid chloride, mp 88.5–92°, $\lambda_{max}^{CHCl_3}$ 5.54 and 5.80 μ. This compound has been described previously as an oil.[11]

A solution of 9.0 g (0.138 mole) of NaN_3 in 35 ml of water was added over a 10-min period to a cold (0–5°) solution of 11.38 g of crude acid chloride in 228 ml of acetone. The mixture was allowed to stir in the ice bath for an additional 15 min and was then diluted with 1 l. of water. The mixture was extracted with ether and the ether extracts were washed with water, dried (Na_2SO_4), and evaporated to give the crude azide XIIId as an oil, $\lambda_{max}^{CHCl_3}$ 4.67 (sharp) and 5.81 μ. A solution of this azide in 400 ml of benzene was heated under reflux for 1 hr at which time the evolution of gas had ceased. The benzene was then removed under reduced pressure to give the crude isocyanate XIV as an oil, $\lambda_{max}^{CHCl_3}$ 4.40 (broad) and 5.81 μ.

A solution of 11.3 g (0.2 mole) of KOH in 11.5 ml of water was added to a solution of the crude isocyanate in 115 ml of methanol. The reaction mixture was heated under reflux for 1.5 hr and was then diluted with 500 ml of water. The resulting precipitate was filtered, washed with water, and dried to give 7.53 g (73% yield from the half-ester XIIIc) of the lactam XVa, mp 210.5–212.5° (vac). Crystallization from CH_2Cl_2–ether gave the analytical sample: mp 211–212° (vac); $[\alpha]^{25}D$ +78.4° (*c* 1.0, C_2H_5OH); $\lambda_{max}^{C_2H_5OH}$ 220 mμ (ε 8600), 279 (1980), 287 (1780); $\lambda_{max}^{CHCl_3}$ 2.92 and 5.92 μ; lit.[5] mp 202–205°, $[\alpha]D$ +70.5° ($CHCl_3$).

Anal. Calcd for $C_{18}H_{23}NO_3$: C, 75.75; H, 8.12; N, 4.91. Found: C, 75.56; H, 8.15; N, 5.08.

16-Azaestrone (XVb).—A mixture of 4.0 g (0.014 mole) of XVa and 80 g (0.79 mole) of pyridine hydrochloride was heated with stirring at 210° for 40 min in an atmosphere of nitrogen. The reaction mixture was then cooled and diluted with 600 ml of 2 *N* HCl. The resulting precipitate was filtered, washed with water, and dried to give 3.40 g of crude product, mp 362–364° (vac). Crystallization from chloroform–ethanol gave 1.88 g of XVb, mp 360–362.5° (vac). A further 0.89 g of XVb (mp 362–364°) was recovered from the mother liquors (total yield, 2.77 g, 73%). Crystallization from $CHCl_3$–ethanol gave the analytical sample: mp 362.5–364.5° (vac); $[\alpha]^{25}D$ +82.5° (*c* 0.1, C_2H_5OH); $\lambda_{max}^{C_2H_5OH}$ 220 mμ (ε 7500), 280 (2090), 288 (1810); λ_{max}^{KBr} 2.93, 5.95, and 6.01 μ.

Anal. Calcd for $C_{17}H_{21}NO_2$: C, 75.24; H, 7.80; N, 5.16. Found: C, 75.31; H, 7.78; N, 5.17.

16-Azaestra-1,3,5(10)-trien-3-ol 3-Methyl Ether Hydrochloride (XVI).—A solution of 1.38 g (4.84 mmoles) of XVa in 46 ml of dry dioxane was added rapidly to a boiling solution of 1.38 g (36.4 mmoles) of $LiAlH_4$ in 46 ml of dry dioxane and the resulting mixture was heated under reflux for 20 hr in a nitrogen atmosphere. Water (7.4 ml) was added slowly to the ice-cold reaction mixture which was then heated under reflux for 30 min. The hot mixture was filtered through a bed of Celite, the latter being washed with hot dioxane. The filtrate was evaporated to dryness and the resulting colorless oil was dissolved in benzene and passed through a short column of Florisil. Evaporation of the eluates gave an oil which was dissolved in CH_2Cl_2, washed three times with 2 *N* HCl, dried (Na_2SO_4), and evaporated. The residue was crystallized from CH_2Cl_2–ether to give 1.16 g (78%) of XVI, mp 298.5–301° (vac). Crystallization from ethanol gave the analytical sample: mp 298–301° (vac); $[\alpha]^{25}D$ +76° (*c* 0.5, ethanol); $\lambda_{max}^{C_2H_5OH}$ 219 mμ (ε 8050), 279 (2000), 287 (1810).

Anal. Calcd for $C_{18}H_{26}ClNO$: C, 70.22; H, 8.51; Cl, 11.52; N, 4.55. Found: C, 70.25; H, 8.28; Cl, 11.55; N, 4.53.

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Enzyme Inhibitors. XV. A New Irreversible Inhibitor of Adenosine Deaminase[1]

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Previous studies have shown that 9-(*p*-bromoacetamidobenzyl)adenine (XVI) and 9-(*m*-bromoacetamidobenzyl)adenine (XVII) are both good reversible inhibitors of adenosine deaminase but that XVI causes an irreversible inactivation of this enzyme at a much higher rate than does XVII. In a continuation of studies on the effect of isomers on the inhibition of adenosine deaminase, a variety of 9-(*ortho*-substituted benzyl)-6-substituted purines have been synthesized. These compounds were weaker reversible inhibitors of adenosine deaminase than was 9-benzyladenine. However, 9-(*o*-bromoacetamidobenzyl)adenine, even though it was a weaker reversible inhibitor of this enzyme than XVI or XVII, was found to be a good irreversible inhibitor of adenosine deaminase. Evidence is presented that this irreversible inactivation of adenosine deaminase proceeds through an initial reversible enzyme–inhibitor complex and not by a random bimolecular reaction between the enzyme and the inhibitor.

Recent studies have shown that 9-(*p*-bromoacetamidobenzyl)adenine and 9-(*m*-bromoacetamidobenzyl)adenine are both good reversible inhibitors of adenosine deaminase obtained from calf intestinal mucosa.[2–4] However, when these compounds were evaluated as irreversible inhibitors of this enzyme, it was found that the *para* isomer was a good irreversible inhibitor,[3] whereas the *meta* isomer was a poor irreversible inhibitor of adenosine deaminase.[4] Since the chemical reactivity of the *meta* isomer is greater than that of the *para* isomer when 4-(*p*-nitrobenzyl)pyridine is used as the nucleophilic reagent,[4] it would appear that the differences in the rates at which these two compounds irreversibly inactivate adenosine deaminase is due to the difference in the environment on the enzyme in which the alkylating group of the inhibitor

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