Synthesis and Evaluation of Antimicrobial Properties of Amidinoazaandrostanes and Guanidinoazaandrostanes

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Abstract □ The intermediate, 17α-methyl-4-aza-5α-androstan-17β-ol (III) required for the synthesis of 4-amidino-17α-methyl-4-aza-5α-androstan-17β-ol (VII) and 4-(β-guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol (VI) was obtained through a reaction of 17α-methyl-3,5-*seco*-4-norandrostan-17β-ol-5-on-3-oic acid with ammonium hydroxide followed by two reductions (platinum dioxide and lithium aluminum hydride). Condensation of III with chloroacetonitrile under anhydrous condition, followed by reduction of the nitrile with lithium aluminum hydride, gave 4-(β-aminoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol (V). The reaction of V with 2-methyl-2-thiopseudourea sulfate and of III-hydrochloride with cyanamide provided the title compounds, VI-sulfate and VII-hydrochloride, respectively. A serial dilution assay showed the title compounds to have antimicrobial activity against *Saccharomyces cerevisiae*. This activity is especially significant since this is the greatest activity thus far obtained in a 4-azaandrostane with a hydroxyl group at the 17β-position.

Keyphrases □ 4-Amidino-17α-methyl-4-aza-5α-androstan-17β-ol and 4-(β-guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol —synthesis, antimicrobial activity □ 4-Azasteroids—synthesis, antimicrobial activity of amidino- and guanidinoazaandrostanes □ Antimicrobial activity—4-amidino-17α-methyl-4-aza-5α-androstan-17β-ol and 4-(β-guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol

Doorenbos and coworkers (1–5) synthesized 4-azasteroids which have a wide variety of pharmaceutical properties such as coronary dilatory, hypocholesterolemic, hypercholesterolemic, hypotensive, and antimicrobial. The active azasteroids inhibited the growth of various Gram-positive bacteria, yeasts, and molds (3, 6). 4-Methyl-4-aza-5α-cholestane has quite a high antimicrobial activity, *i.e.*, approximately 1 μg/ml is bactericidal (1) against Gram-positive bacteria, yeast, and molds.

Previous studies indicated that 4-aza-5α-androstanes possessing a free 17β-hydroxyl (2) are devoid of antimicrobial activity except at very high concentrations. Explanation for the lack of activity in this molecule may be microbial degradation enhanced by hydroxyl substitution. It is commonly known that testosterone (17β-hydroxyl) is inactive orally but that deactivation is blocked by a 17α-methyl group. The objectives of this investigation were to test this hypothesis by synthesizing additional azasteroids through the introduction of the "antimicrobially active moiety," amidine or guanidine functions to azasteroids, namely a 4-azaandrostane, and to evaluate the resulting antimicrobial activity (7–9). Guanidine or amidine derivatives of azasteroids have not been prepared previously, however.

DISCUSSION

Synthesis—17α-Methyltestosterone was used as starting material for the synthesis of 4-amidino-17α-methyl-4-aza-5α-androstan-17β-ol (VII) and 4-(β-guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol (VI) (Scheme I). Catalytic hydrogenation of the double bond of 17α-methyl-4-aza-5-androsten-17β-ol-3-one (I) (10) was accomplished with platinum dioxide in acetic acid (11). The course of the reaction was monitored by the disappearance of UV absorption at 233 nm, and the reduction product, 17α-methyl-4-aza-5α-androstan-17β-ol-3-one (II), gave a disappearance of a weak shoulder at 840 cm^{-1} which is characteristic of the carbon–carbon double bond in this lactam. 17α-Methyl-4-aza-5α-androstan-17β-ol (III), obtained from lithium aluminum hydride reduction of II, was condensed with chloroacetonitrile under anhydrous acetone to obtain 4-(β-cyanoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol (IV). The condensation failed to occur when such solvents as anhydrous benzene, dioxane, and dimethyl sulfoxide were used. The nitrile function of IV was reduced by refluxing lithium aluminum hydride in tetrahydrofuran, and 4-(β-aminoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol (V) was obtained.

For the preparation of VI, V was reacted with 2-methyl-2-thiopseudourea salt (12). The reaction of cyanamide with III gave VII. The 2-methyl-2-thiopseudourea method was unsuccessful in the preparation of VI. Only the sulfate salt of the starting amine was isolated (13).

Biological[1]—An antimicrobial screen, using a reported method (4), was employed to determine the approximate antimicrobial potency of VII and VI. As a standard, 4-methyl-4-aza-5α-cholestane (2), a 4-azasteroid of known activity, was tested by the same procedure. The biological data are presented in Table I.

The first tubes of each series contained 100 μg of sample; succeeding tubes contained 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 μg. The level of activity of the compounds was determined by choosing the last tube in which there was no noticeable growth of the organism. No statistical analyses were employed. To ensure that the cells were viable under test conditions, tubes containing steroid were used as blanks. The assay was duplicated. The following media were utilized: tryptic soy broth for bacteria and mycophil broth for molds and yeasts.

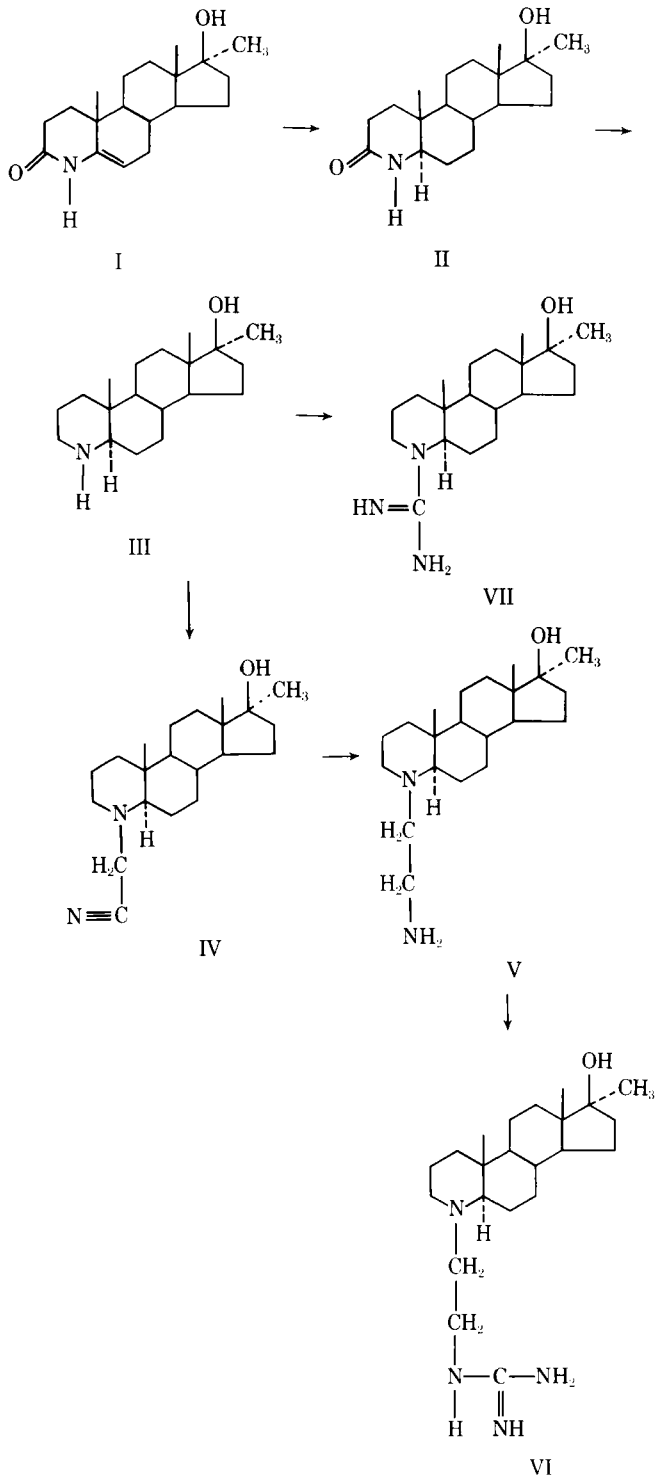
The activity of VI against *Saccharomyces cerevisiae* is especially significant since this is the greatest degree of activity thus far obtained in 4-azaandrostanes with a free hydroxyl group at the 17β-position. Previous studies indicated that 4-aza-5α-androstanes possessing a free 17β-hydroxyl (2) are detrimental to the antimicrobial activity.

EXPERIMENTAL[2]

Compound I—A solution of 20.00 g (0.0062 mole) of 17α-methyl-3,5-*seco*-4-norandrostan-17β-ol-5-on-3-oic acid in 200 ml of concentrated aqueous ammonium hydroxide was heated in a pressure vessel under a nitrogen atmosphere at 180° for 7 hr. The mixture was cooled, and the white solid was separated by filtration to obtain 17.47 g (92%). Crystallization from acetone gave 16.83 g (89%) of white needles, mp 256–258° [lit. (10) mp 256–258°]; IR (KBr): 3090 and 3210 (N—H), 1673 (lactam C=O), and

[1] The cell cultures used were obtained from the University of Mississippi, Department of Biology. For *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*, the strains are identified by the numbers ATCC 10231, K-257, and ATCC 4157, respectively. *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Bacillus cereus* have been part of University stock cultures for many years.

[2] Melting points were taken in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus unless otherwise indicated, and the elemental analyses were obtained from Galbraith Laboratories, Knoxville, Tenn. A Perkin-Elmer 202 UV-visible spectrophotometer was used to record the UV spectra. The R_f values were determined by applying a methanolic solution of the steroid at a rate of 50–100 μg of the steroid as a spot on a silica gel TLC plate (Eastman chromatogram sheet) and developing in a Brinkmann jar in the following solvent systems: Solvent A, benzene–methanol–ethyl acetate (85:10:5); and Solvent B, chloroform–methanol–ammonia (85:14:1). The steroids were detected by iodine vapor.



Scheme I

840 and 1630 (C=C) cm^{-1}; λ_{max}: 233 nm; R_f 0.16 and 0.71 in Solvents A and B, respectively.

Compound II—To a solution of 150 ml acetic acid were added 4.00 g (0.013 mole) of I and 0.35 g of platinum dioxide. The reaction mixture was hydrogenated at 46 psi and 55° for 3 days. After removing the catalyst, evaporating the solvent *in vacuo*, and recrystallizing the residue from acetone, 3.65 g (82%) of white needles was obtained, mp 245–247°; IR (KBr): 1673 (lactam C=O) cm^{-1} and absence of a shoulder at 840 cm^{-1}; R_f 0.11 and 0.67 in Solvents A and B, respectively.

Anal.—Calc. for $C_{19}H_{32}NO_2$: C, 74.71; H, 10.23; N, 4.59. Found: C, 74.92; H, 10.23; N, 4.55.

Compound III—To a solution of 2.00 g (0.006 mole) of II in 250 ml of dry tetrahydrofuran was added, in small portions, 0.97 g (0.026 mole) of lithium aluminum hydride. The mixture was refluxed with stirring for 24 hr. Excess hydride was destroyed with tetrahydrofuran saturated with water. The mixture was filtered and the filtrate was evaporated *in vacuo* to yield 1.35 g of a yellow oily residue. Crystallization from acetone gave 1.21 g (75%) of white needles, mp 200–202°; IR (KBr): disappearance of lactam C=O at 1673 cm^{-1}; R_f 0.73 in Solvent B.

Anal.—Calc. for $C_{19}H_{33}NO$: C, 78.29; H, 11.41; N, 4.81. Found: C, 78.02; H, 11.22; N, 4.86.

Compound IV—To a stirred solution of 5.00 g (0.016 mole) of III and 2.43 g (0.032 mole) of chloroacetonitrile in 750 ml of anhydrous acetone was added 8.00 g of anhydrous sodium carbonate. The mixture was refluxed with vigorous stirring for 17 hr and filtered when hot. The filtrate was evaporated *in vacuo* to obtain a light-yellow oily residue. Crystallization from ethanol gave 3.29 g (64%) of a white solid, mp 183–185°; IR (KBr): 2260 (C≡N) cm^{-1}; R_f 0.72 in Solvent B.

Anal.—Calc. for $C_{21}H_{34}N_2O$: C, 76.31; H, 10.37; N, 8.51. Found: C, 76.27; H, 10.26; N, 8.64.

Compound V-Dihydrochloride—To a stirred solution of 1.00 g (0.003 mole) of IV in 150 ml of dry tetrahydrofuran was added, in small portions, 0.57 g (0.015 mole) of lithium aluminum hydride. The mixture was refluxed with stirring for 17 hr. After decomposing excess lithium aluminum hydride with methanol and then water, the mixture was filtered and the filtrate was evaporated *in vacuo* to obtain a yellow oily residue. All attempts to obtain crystals were unsuccessful; IR (neat): 3090 and 3210 (N—H) cm^{-1} and disappearance of the nitrile peak at 2260 cm^{-1}. The yellow oily residue, V, was dissolved in 100 ml of anhydrous ether and was treated with hydrogen chloride until a gummy solid formed. After chilling, the solid was separated by filtration and the filtrate was evaporated *in vacuo* to obtain a light-yellow solid. Crystallization from a mixture of ethanol and ether gave 0.92 g (77%) of white solid, mp 281–284°; IR (KBr): absence of nitrile peak at 2260 cm^{-1}; R_f 0.74 in Solvent B.

Anal.—Calc. for $C_{21}H_{40}Cl_2N_2O$: C, 61.90; H, 9.90; N, 6.88. Found: C, 61.59; H, 9.79; N, 6.78.

Compound VI-Sulfate—One gram (0.0028 mole) of V and 0.45 g (0.0058 mole) of 2-methyl-2-thiopseudourea sulfate were dissolved in 50 ml of 50% aqueous ethanol and heated under reflux with vigorous stirring for 1 hr (13). After chilling, the reaction mixture was filtered to obtain a gray solid. Crystallization from 50% aqueous acetone gave 2.02 g (82%) of a cream-colored solid, mp >300°; IR (KBr): 3100 and 3300 (guanidine absorption) and 1620 and 1660 (C=N) cm^{-1}; R_f 0.30 in Solvent B.

Anal.—Calc. for $C_{44}H_{82}N_8O_6S$: C, 62.08; H, 9.71; N, 13.13. Found: C, 61.89; H, 9.79; N, 13.08.

Compound VII-Hydrochloride—III-Hydrochloride (III-HCl) was made in the usual manner. Equal molar amounts of III-HCl (0.8 g, 0.0024 mole) and cyanamide (0.15 g, 0.0024 mole) were added to 20 ml of water and refluxed with vigorous stirring for 3 days. The reaction mixture was evaporated *in vacuo* to obtain a yellow oily residue. Crystallization from a mixture of ethanol and ether gave 0.62 g (70%) of white needles, mp 274–275°; IR (KBr): 3100 and 3300 (N—H) and 1620 and 1660 (C=N) cm^{-1}; R_f 0.47 in Solvent B.

Anal.—Calc. for $C_{20}H_{36}ClN_3O$: C, 64.93; H, 9.81; Cl, 9.58; N, 11.36. Found: C, 65.11; H, 9.91; Cl, 9.51; N, 11.21.

Table I—Minimum Inhibitory Concentrations of Steroids (Expressed in Micrograms per Milliliter)

Microorganisms	VI	VII	ND-502[a]
Bacillus cereus	—[b]	—[b]	3.12
Escherichia coli	—[b]	—[b]	—[b]
Staphylococcus aureus	—[b]	—[b]	6.25
Saccharomyces cerevisiae	6.53	25	3.12
Aspergillus niger	—[b]	—[b]	3.12
Candida albicans	—[b]	—[b]	6.25

[a] 4-Methyl-4-aza-5α-cholestane. [b] No inhibition.

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Oscillopolarography of Mercaptopurine and Its Synthesis Intermediates Using a Commercial Oscilloscope and an Adapter Circuit

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Abstract □ Details are given of an adapter circuit to be used with a storage oscilloscope fitted with a camera back for the recording of oscillopolarograms with the dropping mercury electrode. The satisfactory operation of the unit was shown by studies with zinc, cadmium, cupric, and plumbous ions in various supporting electrolytes. The unit was also used for an oscillopolarographic study of 6-purinethiol (mercaptopurine) and its synthesis intermediates: purine-6(1*H*)-one (hypoxanthine), 4,5-diamino-6-hydroxypyrimidine (4,5-diaminohypoxanthine), 4,5-diamino-6-hydroxy-2-thiopyrimidine (4,5-diamino-2-thiouracil), and 4-amino-6-hydroxy-2-thiopyrimidine (4-amino-2-thiouracil).

Keyphrases □ Oscillopolarography—mercaptopurine and synthesis intermediates, using commercial oscilloscope and adapter circuit □ Mercaptopurine and synthesis intermediates—oscillopolarographic study

The first experiments in oscillopolarography (1, 2) used alternating current to investigate the electrode processes in polarography and followed the changes in electrode potential on the oscilloscope. Since the commercial introduction[1] of the polaroscope, papers have shown that the method is applicable to qualitative and quantitative analyses of inorganic and organic compounds, to the determination of decomposition kinetics, and to following fast electrode processes. Many substances that are inactive in dc polarography give characteristic ac oscillopolarograms. The technique supplements classical polarography but has its own established theory and instrumentation (3–6).

This study was an investigation of 6-purinethiol (mercaptopurine) (I), an antitumor agent, and four synthesis intermediates which might be present in mercaptopurine: purine-6(1*H*)-one (hypoxanthine) (II), 4,5-diamino-6-hydroxypyrimidine (4,5-diaminohypoxanthine) (III), 4,5-diamino-6-hydroxy-2-thiopyrimidine (4,5-diamino-2-thiouracil) (IV), and 4-amino-6-hydroxy-2-thiopyrimidine (4-amino-2-thiouracil) (V) (7).

EXPERIMENTAL

Equipment—The following were used: adapter circuit to give the desired function, $dE/dt = f(E)$ as illustrated in Fig. 1 (any oscillator capable of a 10-v peak-to-peak output can be used in the circuit); type 546 B storage oscilloscope with type 2B67 time base and 3A3 dual-trace differential amplifier, fitted with an oscilloscope camera[2] having a film pack holder[3]; dropping mercury electrode[4], 3-sec drop time; and refrigerated, heated water bath and circulator[5].

Technique—The oscillopolarogram was recorded by photographing the oscilloscope face just before detachment of the drop. To test the proper functioning of the equipment, oscillopolarograms were obtained for zinc, plumbous, cadmium, and cupric ions in various supporting electrolytes including 1 *N* solutions of hydrochloric acid, sulfuric acid, lithium chloride, sodium hydroxide, lithium hydroxide, and potassium nitrate. Best results were obtained in 1 *N* lithium chloride. Polarograms for the four ions are shown in Fig. 2. The *Q* values for the ions are shown in Table I; calibration curves for quantitative determination are shown in Fig. 3.

A study was then carried out on Compounds I–V, using as the supporting electrolyte solutions of 1 *N* H_2SO_4, NaOH, LiCl, KNO_3, and LiOH and also potassium chloride solutions buffered

[1] Zavody Prumyslove Automatizace, Prague, Czechoslovakia, 1950.

[2] Tektronix, Inc., Portland, Ore.
[3] Polaroid.
[4] Catalog No. 3-2942, F. H. Sargent Co., Springfield, N.J.
[5] Forma Jr., Forma Scientific, Inc., Marietta, Ohio.