Synthesis and Antimicrobial Properties of 17β-Isopentyloxy-4-aza-5α-androstane and the 4-Methyl Derivative

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Abstract □ Synthesis of 17β-isopentyloxy-4-aza-5α-androstane was accomplished through a set of reactions involving oxidative opening of an A-ring α,β-unsaturated ketone, ring closure, and two reductions. The Williamson synthesis of ethers was employed to introduce the 17β-isopentyloxy group; ketal formation of the 3-oxo group protected the α,β-unsaturated ketone during this reaction. A serial dilution assay showed the title compound and the 4-methyl derivative to have antimicrobial activity against Gram-positive bacteria, yeasts, and molds.

Keyphrases □ 17β-Isopentyloxy-4-aza-5α-androstane and 4-methyl derivative—synthesis, antimicrobial activity □ 4-Aza-steroids—synthesis, antimicrobial activity of 17β-isopentyloxy-4-aza-5α-androstane and 4-methyl derivative □ Antimicrobial activity—17β-isopentyloxy-4-aza-5α-androstane and 4-methyl derivative □ Structure–activity relationships—4-aza-steroids (17β-isopentyloxy-4-aza-5α-androstane) and antimicrobial activity

Doorenbos and his coworkers (1–4) synthesized 4-aza-steroids which have a wide variety of pharmacological properties such as coronary dilatory, hypocholesterolemic, hypercholesterolemic, hypotensive, and antimicrobial. In certain of these compounds (*e.g.*, 4-methyl-4-aza-5α-cholestane), antimicrobial activity was quite high, *i.e.*, approximately 1 mcg./ml. bactericidal (1) against Gram-positive bacteria, yeasts, and molds.

Previous studies indicated that 4-aza-5α-androstanes possessing a free 17β-hydroxyl (2) and 4-aza-5α-pregnanes possessing a free 20-hydroxy (3, 4) are devoid of antimicrobial activity except at very high concentrations. This showed a necessity for the lack of a hydrophilic group in this region of the molecule. Further explanation for the lack of activity in these molecules 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. This hypothesis was applied to 4-aza-5α-androstan-17β-ol by the synthesis and testing of 17α-methyl-4-aza-5α-androstan-17β-ol, but this compound was also inactive in antimicrobial testing (1).

Alkylation of the 20-hydroxy group in the pregnane series (5) produced a highly active agent, 4-aza-22α-oxa-5α-cholestane. To evaluate structure–activity relationships along these lines, the synthesis and antimicrobial properties of 17β-isopentyloxy-4-aza-5α-androstane and the 4-methyl derivative are reported here.

DISCUSSION

Synthesis—Testosterone was utilized as starting material for the synthesis of 17β-isopentyloxy-4-aza-5α-androstane (VIII) and the 4-methyl derivative IX (Scheme I). The 3-ethylenedioxy derivative II was prepared by the usual method (6, 7). The reaction was very sensitive to traces of water and acid, but the addition of anhydrous potassium carbonate and a few drops of pyridine immediately before workup improved the yield. The ketal II was formed to prevent alkylation of the α,β-unsaturated ketone in the conversion of II to III.

The ether linkage was formed *via* a base-catalyzed condensation of the alcohol II with isopentyl *p*-toluenesulfonate. Yields from this reaction were about 30% until large excesses of newly purchased and carefully protected (anhydrous storing) potassium *tert*-butoxide and addition of dimethyl sulfoxide boosted yields to a respectable range.

Hydrolysis of the ketal-ether III in an acetic acid–water medium, with a small amount of *p*-toluenesulfonic acid as a strong acid catalyst, produced the isopentyl ether of testosterone, IV. It was necessary to conduct the hydrolysis under a nitrogen atmosphere since air oxidation caused formation of dark-colored oils which were not characterized.

Ozonolysis of IV yielded 17β-isopentyloxy-3,5-*seco*-4-norandrostan-5-on-3-oic acid (V). Ethers are known to react with ozone, although at a slower rate than olefins and unsaturated ketones, to produce carboxylic acids, esters, and aldehydes. TLC analyses of reactions run with varied amounts of ozone showed that 0.4 mole excess of ozone per mole of steroid gave the desired product V containing a little starting material. This material was suitable for the subsequent ring closure.

The keto-acid-ether V, when heated under nitrogen in concentrated ammonium hydroxide in a pressure vessel, gave 17β-isopentyloxy-4-aza-5-androsten-3-one (VI). This compound resisted attempts at recrystallization and persisted in forming gels. Elemental analyses were not obtained but the IR, NMR, and UV spectra were consistent with the assigned structure. In addition, the next step gave a crystalline product whose structure was confirmed by all spectral data and accurate elemental analyses.

The enamine-lactam-ether VI was converted to the lactam-ether VII by low pressure catalytic hydrogenation, giving the 5α-product only (8).

The final products, 17β-isopentyloxy-4-aza-5α-androstane (VIII) and 17β-isopentyloxy-4-methyl-4-aza-5α-androstane (IX), were produced from VII by lithium aluminum hydride reduction and the Eschweiler–Clarke *N*-methylation, respectively.

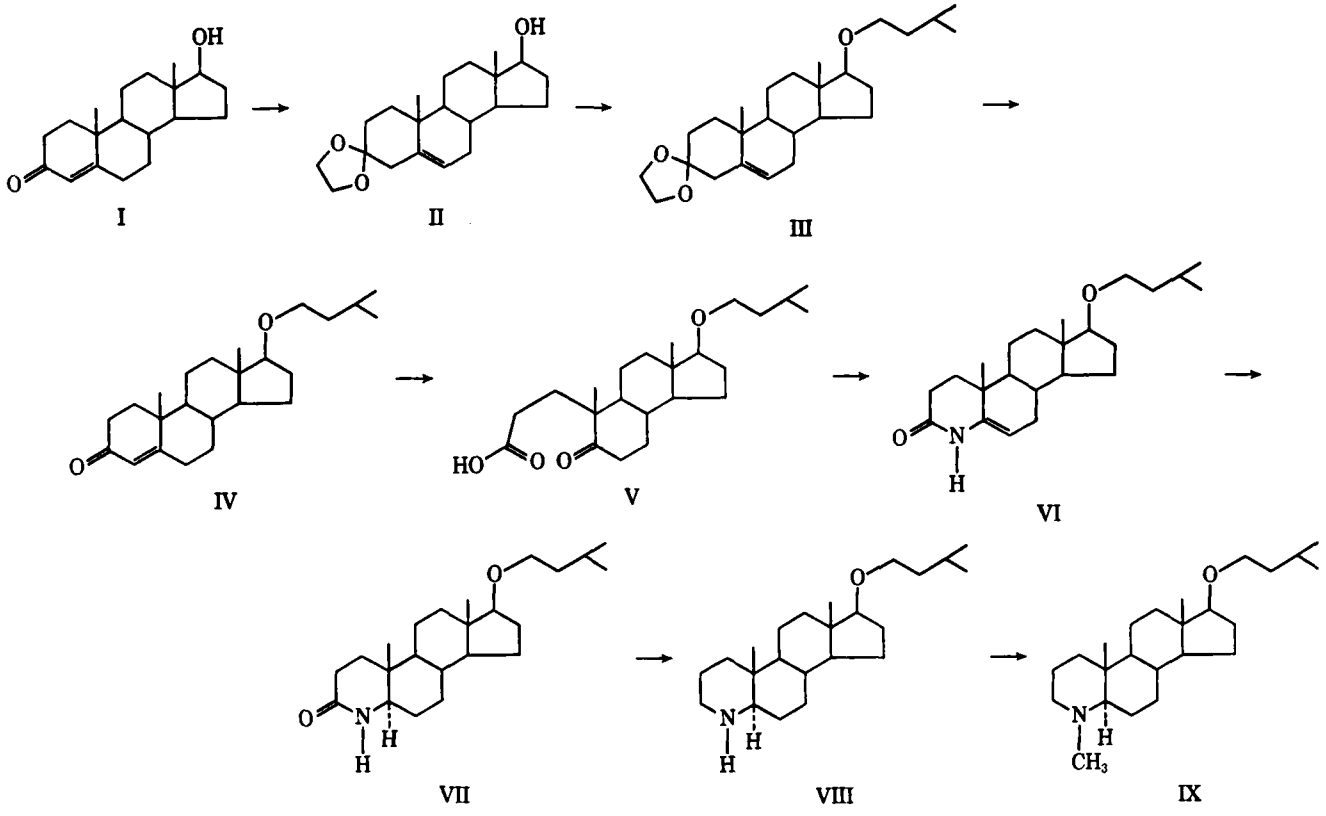
Biological[1]—A twofold serial dilution assay was used to determine the approximate antimicrobial potency of 17β-isopentyloxy-4-

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

Microorganisms	Steroids		
	VIII	IX	4-Aza-steroid[a]
Saccharomyces cerevisiae	12.5	12.5	12.5
Candida albicans	12.5	50.0	50.0
Aspergillus niger	3.12	3.12	50.0
Staphylococcus aureus	12.5	50.0	12.5
Bacillus cereus	12.5	12.5	25.0
Escherichia coli	—[b]	—	—

[a] 4-Methyl-4-aza-5α-cholestane (ND-502). [b] No inhibition.

[1] The cell cultures used were obtained from the Department of Biology, University of Mississippi. 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* cultures have been part of University stock cultures for many years and are typed periodically by students using microscopic and biochemical methods.



Scheme I

aza-5α-androstane (VIII) and the *N*-methyl derivative IX. For comparison, 4-methyl-4-aza-5α-cholestane[2], a 4-aza-steroid of known activity, was tested by the same procedure.

The first tube of each series contained 100 mcg. of sample; succeeding tubes contained 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 mcg. 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 no steroid were used as blanks. The assay was duplicated.

The biological data are presented in Table I. The growth medium for the bacteria was tryptic soy broth; for the fungi, it was mycophil broth.

EXPERIMENTAL[3]

3-Ethylenedioxy-5-androsten-17β-ol (II)—A solution of 25 g. (0.086 mole) of testosterone, 55 ml. (61.35 g., 1.011 mole) of ethylene glycol, 1.2 g. (0.007 mole) of *p*-toluenesulfonic acid, and 1.5 l. of benzene was refluxed 5 days in a 3-l. boiling flask fitted with a Dean-Stark trap, condenser, and drying tube. Water was removed from the trap each day. After adding 10–15 g. of anhydrous potassium carbonate and stirring until room temperature was reached, the solution was divided in half. Each half was washed with three 500-ml. portions of 4% sodium carbonate and dried over anhydrous potassium carbonate. A few drops of pyridine were added and the solvent was evaporated to dryness *in vacuo*. The slightly yellow solid residue was recrystallized from acetone and a few drops of pyridine to yield 22.87 g. (79%) of product, m.p. 180–182° [lit. (9) m.p. 182–183°]; IR: 3450 (OH), 1130, 1095, 1055, and 1025 (C—O stretching) cm.$^{-1}$ and absence of carbonyl absorption.

3-Ethylenedioxy-17β-isopentyloxy-5-androstene (III)—To a solution of 13.98 g. (0.042 mole) of II and 500 ml. of dry tetrahydrofuran, in a carefully dried 1-l. three-necked boiling flask fitted with dropping funnel, condenser, and drying tubes, was added 8.0 g. (0.071 mole) of potassium *tert*-butoxide with refluxing under nitrogen over 5 hr. To this solution was added dropwise 10.2 g. (0.042 mole) of isopentyl *p*-toluenesulfonate and 8.5 ml. of dimethyl sulfoxide in 100 ml. of tetrahydrofuran. Refluxing was continued for an additional 19 hr. Addition of potassium *tert*-butoxide and isopentyl *p*-toluenesulfonate was repeated as follows: (*a*) 8.0 g. (0.071 mole) potassium *tert*-butoxide with refluxing for 5 hr. and 6.0 g. (0.028 mole) isopentyl *p*-toluenesulfonate in 100 ml. tetrahydrofuran with refluxing 19 hr., and (*b*) 8.0 g. (0.071 mole) of potassium *tert*-butoxide with refluxing for 17 hr. After cooling and removing the solvent *in vacuo*, the residue was digested in refluxing benzene; the benzene mixture was washed with six portions of 500 ml. of water, dried over anhydrous potassium carbonate, and evaporated to dryness. Recrystallization of the solid residue from methanol and a few drops of pyridine gave shiny leaflets, 12.00 g. (70%), m.p. 130–131°; IR: 1135, 1105, 1065, and 1025 (C—O stretching) cm.$^{-1}$ and absence of hydroxyl absorption.

Anal.—Calc. for $C_{26}H_{42}O_3$: C, 77.56; H, 10.51. Found: C, 77.31; H, 10.37.

17β-Isopentyloxy-4-androsten-3-one (IV)—A solution of 8.31 g. (0.021 mole) of III, 0.13 g. (0.68 mmole) of *p*-toluenesulfonic acid, 2 ml. (0.111 mole) of water, and 200 ml. of acetic acid in a 250-ml. boiling flask was stirred under nitrogen for 1 hr. at ambient temperature. After mixing the reaction solution with 500 ml. of ether, it was washed with three 1-l. portions of water, three 340-ml. portions of cold 4% sodium carbonate solution, and 1 l. of water. Then it was dried over anhydrous potassium carbonate, filtered, and evaporated *in vacuo* to give 7.01 g. of a viscous oil which solidified.

Purification Method A—An analytical sample was obtained by passing 1.63 g. of this substance through 25 g. of grade III neutral alumina in a 2-cm. column with *n*-hexane. Evaporation of the solvent and several recrystallizations from methanol gave 157 mg. of

[2] ND-502.

[3] All melting points were determined on a Thomas-Hoover capillary melting-point apparatus, and the elemental analyses were obtained from Galbraith Laboratories, Knoxville, Tenn. The maximum wavelength of absorption in the UV spectra was determined on a Perkin-Elmer model 202, and the absorbance was determined on a Beckman model DU spectrophotometer. The IR spectra were recorded on a Perkin-Elmer model 137B and, unless indicated otherwise, all spectra were determined in KBr. Specific rotations were determined on a Perkin-Elmer model 141 automatic polarimeter as 2% solutions in chloroform.

Appreciation is extended to Mrs. R. L. Settine of the Chemistry Department, University of Mississippi, for the NMR spectra, which were recorded with a Varian model A-60-A spectrometer. The NMR spectra sample solutions were 40 mg. of sample/0.5 ml. of chloroform-*d*, with tetramethylsilane as the internal reference.

white solid, m.p. 65–66°; IR 1680, 1620 (3-carbonyl and 4-double bond), 1135, 1114, and 1095 (C—O stretching) cm.$^{-1}$.

Anal.—Calc. for $C_{24}H_{38}O_2$: C, 80.39; H, 10.68. Found: C, 80.22; H, 10.77

Purification Method B—Sublimation of 7.0 g. of IV through a distance of 2.6 cm. at 180° (wax bath temperature) and 0.250 mm. gave 6.61 g. (90%) of a white solid, m.p. 63–64° and IR identical to that obtained by Method A. Method B was routinely used for purification of this compound.

17β-Isopentyloxy-3,5-*seco*-4-norandrosten-5-on-3-oic Acid (V)—To a solution of 6.61 g. (0.018 mole) of IV, 50 ml. of ethyl acetate, and 150 ml. of glacial acetic acid was added 0.025 mole of ozone at ice bath temperature. After addition of 10 ml. of 30% hydrogen peroxide and 20 ml. of water and stirring for 16 hr., the solution was mixed with 600 ml. of ether. This solution was washed with four 500-ml. portions of water and extracted with two 300-ml. portions of 8% sodium hydroxide, and the basic solutions were made acidic to litmus by addition of 47% (v/v) phosphoric acid with cooling. After extracting the oily precipitate of each fraction with three 250-ml. portions of ether, washing with four 500-ml. portions of water, drying over anhydrous magnesium sulfate, and filtering, the filtrate was evaporated *in vacuo* to give a viscous oil which was not further purified. The IR spectrum (liquid film) gave major bands at 3500–3000 (carboxyl OH), 1725, 1700 (5-ketone and carboxyl carbonyl), 1130, 1085, and 1040 (C—O stretching) cm.$^{-1}$.

17β-Isopentyloxy-4-aza-5-androsten-3-one (VI)—A solution of the viscous oil V dissolved in 200 ml. of concentrated ammonium hydroxide and cooled in a dry ice–acetone bath was placed in an autoclave, evacuated at the water pump, sealed, and heated to 170° for 22 hr. After cooling, a creamy-white solid was collected, washed with water, and dried *in vacuo* at 70° for 6–8 hr., giving 3.36 g. of VI (51% based on the quantity of IV used), m.p. 226–227° dec.; IR: 3190, 3050 (NH), 1690, 1675 (C=C and lactam carbonyl), 1135, 1100, and 1090 (C—O stretching) cm.$^{-1}$; $UV_{max}^{95\%\,EtOH}$: 235 nm. (ε 13,600); NMR (CCl_4): δ 7.77 (broad, NH), 4.75 (broad, vinyl), 3.30 (t, CH and CH_2 adjacent to the ether linkage), 1.04 (s, C-19 methyl), 0.88 (d, isopropyl group in the side chain), and 0.72 (s, C-18 methyl).

17β-Isopentyloxy-4-aza-5α-androstan-3-one (VII)—A mixture of 3.36 g. (9.3 mmoles) of VI, 0.5 g. of platinum oxide, and 100 ml. of glacial acetic acid was hydrogenated at 46 p.s.i. and 60° for 36 hr. The UV spectrum indicated the presence of a small amount of non-reduced compound, so hydrogenation was continued at 74° and 46 p.s.i. for 15 hr. (after adding an additional 0.2 g. of platinum oxide). After removing the catalyst, evaporating the solvent *in vacuo*, and recrystallizing the residue from acetonitrile, 2.30 g. (68%) of white needles was obtained, m.p. 241–242°; IR: 3125, 3025 (NH), 1675, 1620 (lactam carbonyl), 1135, 1115, 1110, and 1100 (C—O stretching) cm.$^{-1}$; NMR (CCl_4): δ 7.96 (broad, NH), 3.36 (t, CH and CH_2 adjacent to the ether linkage), 0.90 (d, isopropyl group in the side chain), 0.90 (s, C-19 methyl), and 0.72 (s, C-18 methyl).

Anal.—Calc. for $C_{23}H_{39}NO_2$: C, 76.40; H, 10.87; N, 3.87. Found: C, 76.49; H, 11.01; N, 3.78.

17β-Isopentyloxy-4-aza-5α-androstane (VIII)—A slurry of 5.0 g. (0.132 mole) of lithium aluminum hydride in 200 ml. of ether was added dropwise with magnetic stirring to a solution of 2.30 g. (6.4 mmoles) of VII and 600 ml. of dry ether in a 1-l. three-necked flask fitted with a dropping funnel and condenser with drying tube. Refluxing was continued for 41 hr. After decomposing excess lithium aluminum hydride with methanol and then water, adding 350 ml. of ether and 200 ml. of water, separating the salts and water from the ether phase, and washing with 500 ml. of water, 500 ml. of 9% sodium hydroxide, and two 500-ml. portions of water, the solvent was evaporated *in vacuo* to yield 2.26 g. of a yellow oily solid. Crystallization from acetonitrile gave 1.34 g. (62%) of prisms, m.p. 68–70°; $[\alpha]_D^{23}$ + 25.1° ($CHCl_3$); IR: 3220 (NH), 1140, 1120, 1100, and 1095 (C—O stretching) cm.$^{-1}$ and absence of carbonyl absorption; NMR ($CDCl_3$): δ 3.60 (t, CH and CH_2 adjacent to the ether linkage), 0.92 (s, C-19 methyl), 0.86 (d, isopropyl group in the side chain), 0.76 (s, C-18 methyl), and a proton under the methylene absorption which exchanged with D_2O.

Anal.—Calc. for $C_{23}H_{41}NO$: C, 79.48; H, 11.89; N, 4.03. Found: C, 79.26; H, 11.94; N, 4.23.

17β-Isopentyloxy-4-methyl-4-aza-5α-androstane (IX)—A solution of 0.51 g. (1.47 mmoles) of VIII, 5 ml. (0.098 mole) of 90% formic acid, and 5.0 ml. (0.063 mole) of formalin was heated for 6.5 hr. at 90° and 12 hr. at 55°. After cooling, concentrated ammonium hydroxide was added dropwise until the solution turned red litmus blue. The mixture was cooled in an ice bath and filtered, and the precipitate was washed with water and dried at 70° *in vacuo* to give 0.51 g. of white solid. Recrystallization from acetonitrile yielded 0.47 g. (87%) of colorless needles, m.p. 94–95°; $[\alpha]_D^{23}$ + 6.3° ($CHCl_3$); IR: 2925, 2825, 2750, 2700 [Bohlmann bands (10)], 1140, 1120, 1100, and 1070 (C—O stretching) cm.$^{-1}$ and absence of NH stretching; NMR ($CDCl_3$): δ 3.48 (t, CH and CH_2 adjacent to the ether linkage), 2.22 (s, *N*-methyl), 0.98 (s, C-19 methyl), 0.92 (d, isopropyl group of the side chain), and 0.77 (s, C-18 methyl).

Anal.—Calc. for $C_{24}H_{43}NO$: C, 79.72; H, 11.99; N, 3.87. Found: C, 79.54; H, 11.93; N, 3.69.

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