Steroids XXII

4-Dialkylaminoethyl-4-aza-cholestanes and Androstanes

By NORMAN J. DOORENBOS* and ROBERT E. HAVRANEK†

The synthesis of 4-dimethylaminoethyl-17α-methyl-4-aza-5-androsten-17β-ol-3-one (II) and 4-diethylaminoethyl-17α-methyl-4-aza-5-androsten-17β-ol-3-one from 17α-methyl-3,5-seco-4-norandrostan-17β-ol-5-on-3-oic acid is described. Conditions for preparing the 5-hydroxy intermediates are reported. These lactams were converted into quaternary salts. II was reduced with lithium aluminum hydride and subsequently with hydrogen and platinum to obtain 4-dimethylaminoethyl-17α-methyl-4-aza-5α-androstan-17β-ol. Similar syntheses, beginning with 3,5-seco-4-norcholestan-5-on-3-oic acid, are described. Some of these derivatives possess antimicrobial properties.

PREVIOUS reports have described 4-azasteroids with antimicrobial (2–5), hypotensive (6), anti-inflammatory (1, 6), and hypocholesterolemic (6, 7) activities. This paper describes the synthesis of some 4-dialkylaminoethyl-4-aza-steroids. The procedures used are similar to those reported earlier (8–10), but the products were much more difficult to purify.

17α - Methyl - 3,5 - seco - 4 - norandrostan-17β-ol-5-on-3-oic acid (I) was condensed with *N,N*-dimethylaminoethylamine and *N,N*-diethylaminoethylamine at reflux temperatures under a nitrogen atmosphere to yield 4-dimethylaminoethyl - 17α - methyl - 4 - aza - 5 - androsten - 17β-ol-3-one (II) and 4-diethylaminoethyl-17α-methyl-4-aza-5-androsten-17β-ol-3-one (IV), respectively. If milder conditions were used in the preparation and purification of II, the intermediate, 4-dimethylaminoethyl-17α-methyl-4-aza-androstane-5ξ,17β-diol-3-one (VII), could be isolated. Each of these lactams was converted into a quaternary salt to aid in characterization and to provide derivatives with possible antimicrobial activity. VII was conveniently dehydrated by heating above its melting point (8, 11).

The enamine lactams (II and IV) absorb at 234 mμ which is characteristic of such substances (9, 10). The 5-hydroxy derivative absorbs at a lower wavelength. It is interesting to note that the quaternary salts of the enamine lactams absorb at about 220 mμ. The quaternary nitrogen has caused a hypsochromic shift.

II was reduced with lithium aluminum hydride to 4-dimethylaminoethyl-17α-methyl-4-aza-5-androsten-17β-ol (IX) and subsequently with hydrogen and platinum in ethanol solution to 4-dimethylaminoethyl - 17α - methyl - 4 - aza-5α-androstan-17β-ol (X).

4-Dimethylaminoethyl-4-aza-5-cholesten-3-one (XII) was prepared by the condensation of 3,5-seco-4-norcholestan-5-on-3-oic acid (XI) and *N,N*-dimethylaminoethylamine. XII was obtained as an oil and resisted all attempts at crystallization. It was characterized by physical data and by preparation of the picrate and methiodide salts. A 5-hydroxy derivative (XIV) of XII was obtained under milder reaction conditions.

Microbiological screening, using procedures previously reported (1), revealed that VII, VIII, XIV, and XV possess bacteriostatic activity at concentrations ranging between 1 and 10 mcg./ml. In marked contrast, the enamine lactams (II, IV, and XII) and their quaternary salts (III, V, VI, and XIII) showed little activity. A detailed report of this study, which is still in progress, will be made at a later date. (Scheme I.)

EXPERIMENTAL[1]

4 - Dimethylaminoethyl - 17α - methyl - 4 - aza-5-androsten-17β-ol-3-one (II).—Twenty grams (0.06 *M*) of 17α-methyl-3,5-seco-4-norandrostan-17β-ol-5-on-3-oic acid (10, 12) and 100 ml. of *N,N*-dimethylaminoethylamine were mixed and refluxed 26 hr. under a nitrogen atmosphere. Excess amine was distilled, and 200 ml. of dry ether was added. White crystals (8.9 Gm.) separated upon refrigeration. Recrystallization from ethyl acetate yielded 8.0 Gm. of white needles, m.p. 138.5–140°.

The mother liquors were concentrated and chromatographed on neutral alumina, activity grade I,

Received January 18, 1965, from the School of Pharmacy, University of Maryland, Baltimore.
Accepted for publication May 18, 1965.
This investigation was supported by research grant CA-04132 from the National Cancer Institute and gifts of steroid intermediates from the Cancer Chemotherapy National Service Center, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

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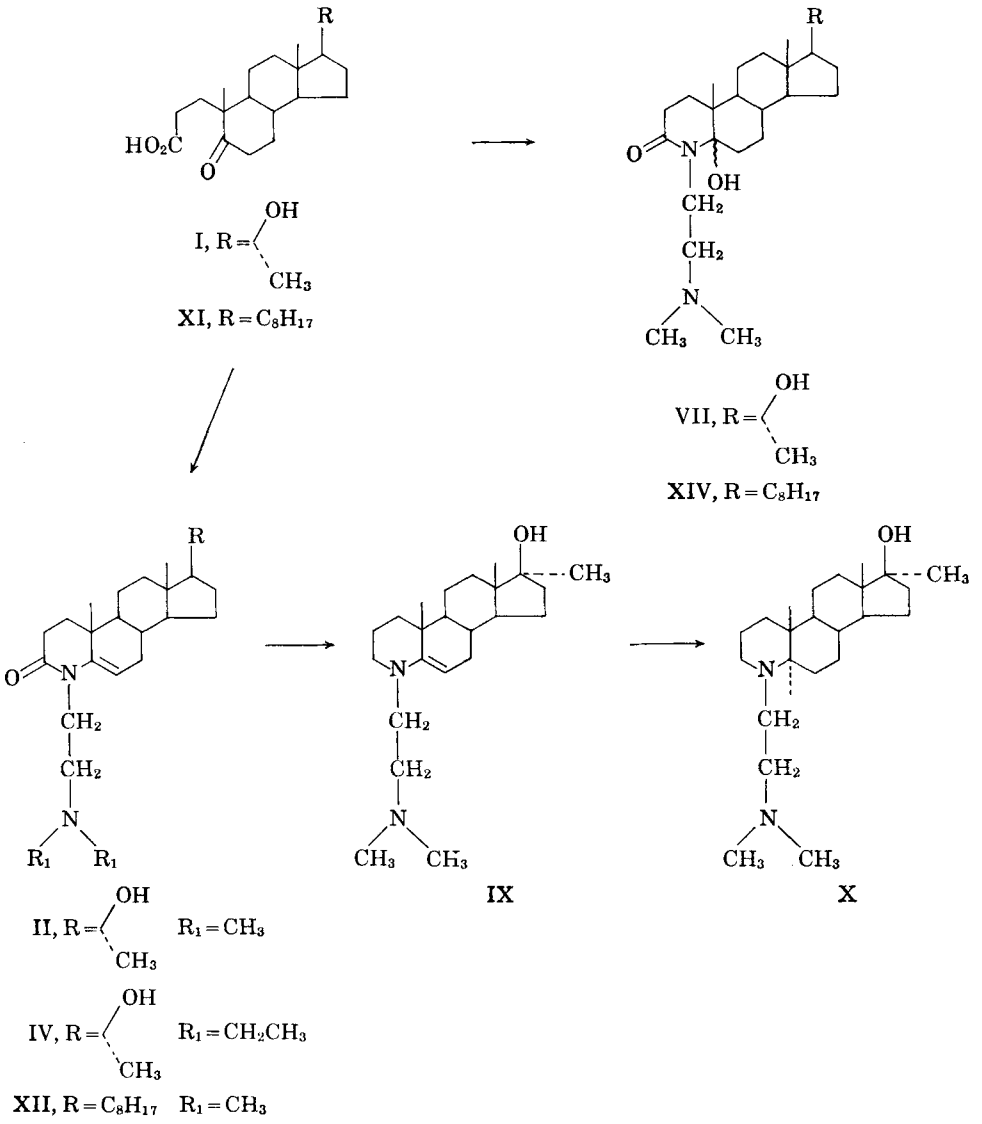
[1] Melting points are uncorrected and were determined on a Thomas-Hoover melting point apparatus. Optical rotations were determined on 1% solutions in $CHCl_3$ at 25°. Ultraviolet spectra were obtained with a Perkin-Elmer Spectracord using 95% ethanol solutions. Infrared spectra were obtained with a Perkin-Elmer Infracord using KBr pellets. Analyses were obtained from Drs. Weiler and Strauss, Oxford, England, and Schwarzkopf Microanalytical Laboratories.



Scheme I

to obtain an additional 7.0 Gm. of pure product, m.p. 138.5–140°. The product was eluted with a 1:1 mixture of ethyl acetate and benzene. Total yield, 15.0 Gm. (72%); $[\alpha]_D$ −200°; $\lambda_{max.}$ 235 mμ (log ε 4.10), and 6.14 μ with an inflection at 6.94 μ.

Anal.—Calcd. for $C_{23}H_{38}N_2O_2$: C, 73.75; H, 10.23; N, 7.48. Found: C, 73.78; H, 10.10; N, 7.30.

4 - Dimethylaminoethyl - 17α - methyl - 4 - aza-5-androsten-17β-ol-3-one Methiodide (III).—Three grams of II was dissolved in a mixture of 30 ml. of dry ether and 2 ml. of absolute ethanol and treated with 4 Gm. of methyl iodide. Two glass beads were placed in the 150-ml. flask used for this reaction, and the flask was sealed with an aluminum foil covered cork. Rapid swirling of the flask induced crystallization of white granular crystals. After 6 hr., the reaction mixture was refrigerated and then filtered to obtain 3.9 Gm. of III, m.p. 217–219°. This product was recrystallized from ethanol–dry ether to obtain 3.6 Gm. (86%); m.p. 222–223°; $[\alpha]_D$ −185°; $\lambda_{max.}$ 220 mμ (log ε 4.37) and 6.14 μ with an inflection at 6.93 μ.

Anal.—Calcd. for $C_{24}H_{41}IN_2O_2$: C, 55.81; H, 8.00; I, 24.57. Found: C, 55.74; H, 8.28; I, 24.81.

4 - Diethylaminoethyl - 17α - methyl - 4 - aza-5-androsten-17β-ol-3-one (IV).—Five grams of I was treated with *N,N*-diethylaminoethylamine in a manner similar to that used in the preparation of II. The product was obtained as an oil; $\lambda_{max.}$ 234 mμ and 6.14 μ with a slight inflection at 6.04 μ. All attempts to crystallize this product were unsuccessful, even though spectra were almost identical to those of II. The hydrochloride and hydrobromide salts were prepared, but they were very hygroscopic.

4 - Diethylaminoethyl - 17α - methyl - 4 - aza-5-androsten-17β-ol-3-one Methiodide (V).—Four grams of the oily residue (IV) was treated with methyl iodide in the manner used to prepare III. Four grams of crude salt upon refrigeration was recrystallized from ethanol–dry ether to yield 2.8

Gm. (51%) of white granular crystals, m.p. 218–220°; $[\alpha]_D$ −109°; $\lambda_{max.}$ 219 mμ (log ε 4.37) and 6.14 μ with an inflection at 6.03 μ.

Anal.—Calcd. for $C_{26}H_{45}IN_2O_2$: C, 57.34; H, 8.33; I, 23.30; N, 5.14. Found: C, 57.07; H, 8.06; I, 23.30; N, 5.07.

4 - Diethylaminoethyl - 17α - methyl - 4 - aza-5-androsten-17β-ol-3-one Ethiodide (VI).—The oily residue of IV (6.2 Gm.) was treated with ethyl iodide in a manner similar to the preparation of III. The yield of crude product was 2.7 Gm. Recrystallization from ethanol–ether yielded 2.0 Gm. (24%) of VI as off-color white cubic crystals; m.p. 222.5–225°; $[\alpha]_D$ −106°; $\lambda_{max.}$ 219 mμ (log ε 4.37) and 6.13 μ with an inflection at 6.03 μ.

Anal.—Calcd. for $C_{27}H_{47}IN_2O_2$: C, 58.05; H, 8.48; I, 22.72; N, 5.02. Found: C, 57.76; H, 7.84; I, 23.02; N, 4.84.

4 - Dimethylaminoethyl - 17α - methyl - 4-azaandrostan-5ξ,17β-diol-3-one (VII).—In the preparation of II, it was not unusual to obtain an excellent yield of 4-dimethylaminoethyl-17α-methyl-4-azaandrostane-5ξ, 17β-diol-3-one (VII) and little, if any, of the desired product. VII was never isolated as a solid, but its presence could be demonstrated by the absence of C=C absorption in the infrared, the hypsochromic shift in the absorption max. in the ultraviolet spectrum (from 235 to 219 mμ), and by analysis of its methiodide.

It was found that VII dehydrated rapidly when heated to temperatures of 250–300°. We adopted the procedure of heating VII at 300° for 4 min. in a nitrogen atmosphere. We have evidence that the dehydration was complete in a few seconds. The added time was just insurance. II was obtained in excellent yield following dehydration.

4 - Dimethylaminoethyl - 17α - methyl - 4-azaandrostan-5ξ,17β-diol-3-one Methiodide (VIII).—The methiodide was prepared from VII by the same procedure used for the preparation of III. Two grams of VII yielded 2.45 Gm. (87%) of VIII as white granular crystals, after crystallization from ethanol–ether; m.p. 222–223°; $[\alpha]_D$ −126°; $\lambda_{max.}$ 219 mμ (log ε 4.38) and 6.14 μ.

Anal.—Calcd. for $C_{24}H_{43}IN_2O_3$: C, 53.93; H, 8.11; I, 23.74. Found: C, 53.87; H, 7.59; I, 23.74.

4 - Dimethylaminoethyl - 17α - methyl - 4 - aza-5-androsten-17β-ol (IX).—Ten grams of II was dissolved in 400 ml. of dry ether and added dropwise to a stirred solution containing 3 *M* excess of lithium aluminum hydride dissolved in 200 ml. of dry ether. After the addition was complete, the mixture was refluxed with stirring for 12 hr. Excess hydride was carefully decomposed with water and the mixture filtered using a sintered-glass funnel. After washing the inorganic salts with ether, the ether solutions were combined, dried over sodium sulfate, and the ether evaporated. The residue was crystallized from ethyl acetate to yield 8.9 Gm. (92%) of IX as white crystals, m.p. 151–152°; $[\alpha]_D$ −175°; $\lambda_{max.}$ 6.10 μ (weak, C=C).

Anal.—Calcd. for $C_{23}H_{40}N_2O$: C, 76.61; H, 11.18; N, 7.77. Found: C, 76.61; H, 10.98; N, 7.70.

4 - Dimethylaminoethyl - 17α - methyl - 4 - aza-5α-androstan-17β-ol (X).—A solution of 4.0 Gm. of IX in 300 ml. of absolute ethanol was treated with hydrogen in the presence of 500 mg. of platinum catalyst at 100° and 500 p.s.i. for 10 hr. An oil was obtained after filtering the catalyst and distilling the solvent. The oil was dissolved in hot ethyl acetate–ethanol (3:1) and cooled to obtain 3.0 Gm. of pink platelets, m.p. 175–176°. Since this product gave an incorrect elemental analysis, it was chromatographed on neutral alumina, activity grade I. The product was eluted with a 1:1 mixture of ethyl acetate and benzene. Evaporation of the solvent and recrystallization from ethyl acetate–ethanol (3:1) yielded white platelets, m.p 175–176° (2.8 Gm., 70%); $[\alpha]_D$ −25°.

Anal.—Calcd. for $C_{23}H_{42}N_2O$: C, 76.18; H, 11.68; N, 7.73. Found: C, 75.86; H, 11.28; N, 8.13.

4 - Dimethylaminoethyl - 4 - aza - 5 - cholesten-3-one (XII).—Six grams of 3,5-seco-4-norcholestan-5-on-3-oic acid (XII) (8) and 100 ml. of *N,N*-dimethylaminoethylamine were mixed and refluxed 24 hr. under a nitrogen atmosphere. Excess amine was distilled, and 200 ml. of dry ether was added. Since crystallization could not be induced, the ether solution was washed with water until neutral to litmus. The ether was evaporated, and attempts were made to crystallize the resulting oil from various solvents, both before and after chromatographing the product on neutral alumina. The product did not crystallize. The hydrochloride and hydrobromide salts were prepared. These salts were very hygroscopic and failed to crystallize.

Spectra of the oil obtained in this reaction indicated that it was the desired product: $\lambda_{max.}$ 235 mμ and 6.14μ with an inflection at 6.04 μ.

The picrate of XII was prepared in ether solution and subsequently recrystallized from methanol to obtain yellow crystals, m.p. 192–194°.

Anal.—Calcd. for $C_{36}H_{55}N_5O_8$: C, 63.04; H, 8.08; N, 10.21. Found: C, 62.85; H, 8.31; N, 10.86.

4 - Dimethylaminoethyl - 4 - aza - 5 - cholesten-3-one Methiodide (XIII).—The methiodide was prepared from 3.6 Gm. of XII using the same procedure used for III. Recrystallization of the product from methanol yielded 3.5 Gm. (68%) of XIII as white cottonlike crystals, m.p. 264–266°; $[\alpha]_D$ −120°; $\lambda_{max.}$ 219 mμ (log ε 4.38) and 6.13 μ with an inflection at 6.03 μ.

Anal.—Calcd. for $C_{31}H_{55}IN_2O$: C, 62.19; H, 9.26; N, 4.68. Found: C, 61.78; H, 9.21; N, 4.67.

4 - Dimethylaminoethyl - 4 - azacholestan - 5ξ-ol-3-one (XIV).—In the preparation of XII, it was often observed that the product formed in excellent yield was 4-dimethylaminoethyl-4-azacholestan-5ξ-ol-3-one (XIV) and not the desired XII. XIV was the product obtained when the reflux time was short, *e.g.*, 1 hr.

XIV was always obtained as an oil which resisted all attempts at crystallization. Its structure was confirmed by the lack of C=C absorption in the infrared, the low wavelength absorption in the ultraviolet (219 mμ rather than 235 mμ), and by analysis of its quaternary salt. It was established that XIV was rapidly dehydrated by heating at 300°. The product formed in excellent yield was XII.

4 - Dimethylaminoethyl - 4 - azacholestan - 5ξ-ol-3-one Methiodide (XV).—The methiodide was prepared from 5.5 Gm. of XIV following the same

procedure used for III. Recrystallization from 95% ethanol yielded 5.0 Gm. (66%) of XV as white cottonlike crystals, m.p. 258–259°; $[\alpha]_D$ −81°; $\lambda_{max.}$ 6.13 μ with no inflection at 6.03 μ.

Anal.—Calcd. for $C_{31}H_{37}IN_2O_2$: C, 60.57; H, 9.35; I, 20.65; N, 4.56. Found: C, 60.79; H, 9.09; I, 20.62; N, 4.42.

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Catharanthus lanceus VII

Isolation of Tetrahydroalstonine, Lochnerinine, and Periformyline

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A continuing study of *Catharanthus lanceus* leaf alkaloids for antineoplastic compounds has led to the isolation of tetrahydroalstonine, lochnerinine, and a new alkaloid, periformyline. Details concerning the isolation of these alkaloids and the structure elucidation of periformyline, the first example of an $N_{(b)}$-substituted formyl indole alkaloid to be found in nature, are presented.

As part of a continuing search for new and active antineoplastic agents from the various alkaloid fractions of *Catharanthus lanceus*, nine crystalline compounds have been isolated from both the leaf and root alkaloid fractions. One of these is the antineoplastic alkaloid leurosine (1–3).

Of the three alkaloids reported herein, tetrahydroalstonine and lochnerinine represent alkaloids previously reported in *C. roseus* (4, 5) while the third, periformyline, represents a new alkaloid of novel structure.

The infrared absorption spectrum (Fig. 1) and ultraviolet absorption spectrum of isolated tetrahydroalstonine and a reference sample were identical. Their R_f values and chromogenic reactions to the ceric ammonium sulfate (CAS) detecting reagent (6, 7) in three different solvent systems were also identical (8).

The identity of lochnerinine was shown by comparison of our data with that found in the literature and is presented in Table I (5, 9). The infrared absorption spectrum (Fig. 2) seemed identical with that in the literature and the mass spectrum of lochnerinine furnished by Bose (10) was in good agreement with that of our

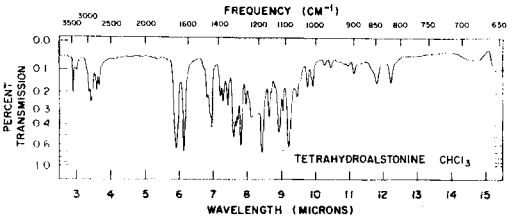

Fig. 1.—Infrared absorption spectrum of tetrahydroalstonine.

Table I.—Identification of Lochnerinine

	Lit. (5, 9)	Observed Data
M.p., °C.	168–169°	164–166°
Specific rotation (chloroform)	−424° (16°C.)	−442° (26°C.)
U.V. max.	247 mμ (log ε 4.19)	247 mμ (log ε 4.22)
(in ethanol)	326 mμ (log ε 4.33)	326 mμ (log ε 4.41)
Mass spectrum mol. wt.	382	382
Base peak	138	138

Received April 1, 1965, from the Departments of Pharmacognosy and Pharmaceutical Chemistry, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pa.
Accepted for publication June 2, 1965.
Presented to the Scientific Section, A.PH.A., Detroit meeting, March 1965.
This investigation was supported by research grants CA 08228-01 and HE-06162 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md., and from Eli Lilly and Co., Indianapolis, Ind.
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