Steriods XXIV

Synthesis of Some Pyrimidino- and Imidazolino-Steroids

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3,5-Seco-4-norcholestan-5-on-3-oic acid reacts with 1,3-diaminopropane and ethylenediamine at reflux temperatures to yield 1′,4′,5′,6′-tetrahydropyrimidino-[*a*-4,3]-4-aza-5-cholestene (III) and 2′,3′-dihydroimidazolino[*a*-4,3]-4-aza-5-cholestene (V). 1′,4′,5′,6′-Tetrahydropyrimidino[*a*-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (IV) and 2′,3′-dihydroimidazolino[*a*-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (VI) were prepared in a similar manner. The C=N bond in each of these azastroides was reduced smoothly with lithium aluminum hydride. III possesses hypocholesterolemic, anti-inflammatory, hypotensive, diuretic, antibacterial, and antifungal activities. The other derivatives are less active.

ALTHOUGH a number of heterocyclic steroids have been described in recent years, the preparation of 4-azasteroids with a heterocyclic ring fused at positions 3 and 4 has not been reported. This paper describes the first synthesis of some pyrimidino- and imidazolino-4-azasteroids.

3,5-Seco-4-norcholestan-5-on-3-oic acid (I) and 17α-methyl-3,5-seco-4-norandrostan-17β-ol-5-on-3-oic acid (II) were prepared by the ozonolysis of 4-cholesten-3-one and 17α-methyltestosterone, respectively, using a modification of the procedure of Bolt (1).

The reaction of I and II with 1,3-diaminopropane at elevated temperatures yielded 1′,4′,5′,6′-tetrahydropyrimidino[*a*-4,3]-4-aza-5-cholestene (III) and 1′,4′,5′,6′-tetrahydropyrimidino-[*a*-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (IV). These structures were assigned on the basis of analyses and spectra. (Scheme I.) Elemental analyses were in agreement with the assigned structures indicating the absence of oxygen. The intense absorption peaks at 6.15 μ were therefore assigned to C=N stretching (2). The weak absorption peaks at 6.03 μ were assigned to C=C stretching. These pyrimidinosteroids exhibited no absorption that could be assigned NH or NH_2.

Structures of this type have been prepared by similar reactions. Imidazoles may be obtained by heating monoacylethylenediamines (3). Reppe synthesized a pyrimidinoimidazole by heating *N*-γ-aminopropylpyrrolidone (4). (Scheme II.)

The reaction of I and II with ethylenediamine under reflux (116°) resulted in the formation of 2′,3′-dihydroimidazolino[*a*-4,3]-4-aza-5-cholestene (V) and 2′,3′-dihydroimidazolino[*a*-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (VI), respectively. The structures of these azasteroids were established by analysis and spectra, as with III and IV.

The C=N bond in III, IV, V, and VI was reduced by treatment with lithium aluminum hydride to yield hexahydropyrimidino[*a*-4,3]-4-aza-5-cholestene (VII), hexahydropyrimidino[*a*-4,3]-17α-methyl-4-aza-5-androstan-17β-ol (VIII), tetrahydroimidazolino[*a*-4,3]-4-aza-5-cholestene (IX) and tetrahydroimidazolino[*a*-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (X), respectively. The reduction of the C=N double bond was established by the loss of absorption at 6.15 μ (C=N) and the appearance of absorption at 3.00 μ (NH). The C=C was not affected as indicated by absorption at 6.03 μ. The configuration at position 3 has not been established. It is possible that the products consist of mixtures of 3α and 3β isomers. Attempts to isolate such isomers have not been successful.

BIOLOGICAL DATA[1]

Compound III, administered subcutaneously in sesame oil, propylene glycol, or ethanolic saline solution, exhibited hypocholesterolemic, anti-inflammatory, and diuretic activities. In an endocrine screen in adrenalectomized rats, 2 mg./Kg./day for 7 days lowered plasma cholesterol levels from the control values of 98 mg. to 58 mg./100 ml. of plasma. The incorporation of labeled mevalonic acid into cholesterol by rat liver homogenates was inhibited 86% at 1×10^{-5} moles/L. and 96% by 1×10^{-4} moles/L. of III. The subcutaneous injection of 20 mg./Kg./day for 7 days in adrenalectomized adult male rats gave the anti-

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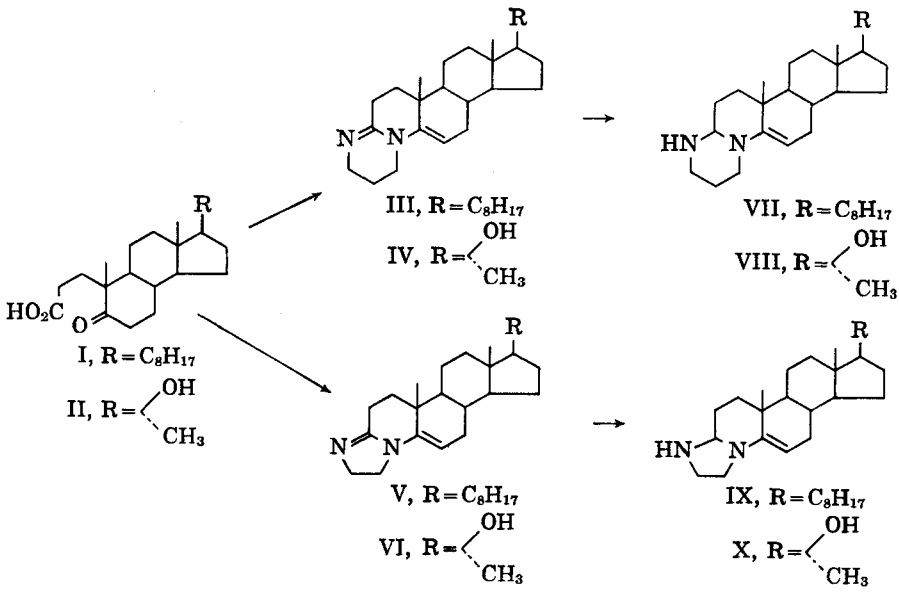
[1] The biological data, with the exception of the antimicrobial data, were furnished by Smith Kline and French Laboratories. The antimicrobial data were obtained in our laboratory by a procedure described by Smith, R. F., Shay, D. E., and Doorenbos, N. J., *J. Bacteriol.*, **85**, 1295(1963). Detailed results of antimicrobial studies being carried out on these pyrimidino- and imidazolinosteroids will be published separately.



Scheme I

Scheme II

inflammatory activity of 5 mg./Kg./day of hydrocortisone in a filter paper granuloma assay.[2] In a followup study, it was demonstrated that III had no effect upon corticogenesis in an *in vitro* assay using quartered adrenal glands. The adrenal glands of rats treated with III increase in weight and the possible resulting increased *in vivo* biosynthesis of anti-inflammatory steroids may account for the activity of III. Sodium elimination increased an average of about fivefold in the endocrine screen. This was accompanied by a significant increase in urine volume.

Compound III possessed hypotensive activity when administered subcutaneously in sesame oil solution to Sprague-Dawley rats. The effect on blood pressure was much greater after administration for several days and was much greater in DCA-hypertensive than in normotensive rats. Blood pressure, for example, was lowered from an average of 240 to 170 mm. Hg by the subcutaneous administration of 60 mg./Kg. on day 1 and 20 mg./Kg. on days 2–5.

IV, in contrast to III, strongly inhibited sodium elimination. Sodium elimination in the endocrine screen was lowered to one-fifth of the normal value when IV was administered subcutaneously at 20 mg./Kg./day. IV showed no other significant activities in the endocrine screen. It possessed no androgenic, anabolic, antiandrogenic, or antianabolic activity.

V inhibited cholesterol biosynthesis. VII possessed anti-inflammatory activity but had marginal hypocholesterolemic and no hypotensive activities.

[2] Filter paper pellets weighing approximately 6.3 mg./pellet were implanted subcutaneously after being soaked in 0.02 ml. of 10% formalin solution.

Each of the steroids screened was inactive when administered by oral intubation.

III, V, VII, and IX were found capable of inhibiting the growth *in vitro*, of many Gram-positive bacteria and fungi at concentrations ranging between 1 and 50 mcg./ml.

EXPERIMENTAL[3]

3,5-Seco-4-norcholestan-5-on-3-oic Acid (I).—4-Cholestan-3-one (25 Gm., 0.065 mole) was dissolved in a mixture of 300 ml. of glacial acetic acid and 100 ml. of ethyl acetate and treated with a 30% excess of ozone (7) at 0°. Water (100 ml.) and 30% hydrogen peroxide (15 ml.) were added. The mixture was left in an icebox for 3 days. The crystals which had formed were collected by filtration and recrystallized from hot *n*-hexane to obtain 17.5 Gm. (70%) of white needles, m.p. 151–153°. [Reported m.p. 154–155° (1) and 154–154.5° (5).]

17α-Methyl-3,5-seco-4-norandrostan-17β-ol-5-on-3-oic Acid (II).—17α-Methyltestosterone (15.1 Gm., 0.05 mole) was dissolved in 150 ml. of glacial acetic acid and treated with a 30% excess of ozone[4] at 0°. The mixture was treated with 200 ml. of water and 10 ml. of 30% hydrogen peroxide and left standing in an icebox for 3 days. The product was filtered and recrystallized from acetone to obtain 12.2 Gm. (75%) of II as white needles, m.p. 196–197°. [Reported m.p. 196–197° (1).]

1′,4′,5′,6′-Tetrahydropyrimidino[a-4,3]-4-aza-5-cholestene (III).—A mixture of 2.02 Gm. (0.005 mole) of 3,5-seco-4-norcholestan-5-on-3-oic acid (I) and 10 Gm. (0.14 mole) of 1,3-diaminopropane was refluxed in an atmosphere of nitrogen for 1 hr.

[3] The melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Analyses were obtained from Drs. Weiler and Strauss, Oxford, England, and Schwarzkopf Microanalytical Laboratory. Ultraviolet spectra were obtained on a Perkin-Elmer Spectracord spectrophotometer. Infrared spectra were obtained on a Perkin-Elmer Infracord spectrophotometer.

[4] The ozone used in this investigation was furnished by a Welsbach T-23 Ozone Generator.

Excess 1,3-diaminopropane was distilled and the temperature raised to 210–220° for 10 min. The oily product was crystallized from ether–acetonitrile to obtain 1.74 Gm. (82%) of 1′,4′,5′,6′-tetrahydropyrimidino[*a*-4,3]-4-aza-5-cholestene (III) as a pale yellow solid, m.p. 116–120°. An analytical sample was obtained as a white crystalline solid by two recrystallizations from acetonitrile, m.p. 124–125°. $[\alpha]_D^{25}$ −46.5° (c 0.45, chloroform); $\lambda_{max.}^{EtHO}$ 252 mμ (log ε 4.07); $\lambda_{max.}^{CHCl_3}$ 6.03 (moderate peak, C=C) and 6.15 μ (C=N).

Anal.—Calcd. for $C_{29}H_{48}N_2$: C, 82.01; H, 11.39; N, 6.60. Found: C, 81.64; H, 11.34; N, 6.96.

1′,4′,5′,6′-Tetrahydropyrimidino[a-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (IV).—A mixture of 1.50 Gm. (0.0047 mole) of 17α-methyl-3,5-seco-4-norandrostan-17β-ol-5-on-3-oic acid (II) and 10 Gm. (0.14 mole) of 1,3-diaminopropane was refluxed 3 hr. in a nitrogen atmosphere. The mixture was diluted with 40 ml. of water. The white needles which separated on cooling were filtered and recrystallized from 80% ethanol to yield 1.12 Gm. (71%) of 1′,4′,5′,6′-tetrahydropyrimidino-[*a*-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (IV) as white needles, m.p. 240–241° dec. $[\alpha]_D^{25}$ −112° (c 0.5, chloroform); $\lambda_{max.}^{EtOH}$ 250 mμ (log ε 4.13); $\lambda_{max.}^{CHCl_3}$ 2.75 (OH), 6.03 (C=C), and 6.15 μ (C=N).

Anal.—Calcd. for $C_{22}H_{32}N_2O$: C, 77.14; H, 10.01; N, 8.18. Found: C, 77.33; H, 10.21; N, 7.86.

2′,3′-Dihydroimidazolino[a-4,3]-4-aza-5-cholestene (V).—A solution of 2.02 Gm. (0.005 mole) of 3,5-seco-4-norcholestan-5-on-3-oic acid (I) in 10 ml. of ethylenediamine was refluxed in a nitrogen atmosphere. After 16 hr., excess ethylenediamine was distilled and the residue crystallized from acetonitrile to give 1.31 Gm. (64%) of 2′,3′-dihydroimidazolino[*a*-4,3]-4-aza-5-cholestene (V) as pale yellow crystals, m.p. 117–118°. $[\alpha]_D^{25}$ −3.88° (c 1.0, chloroform); $\lambda_{max.}^{EtOH}$ 256 mμ (log ε 4.15); $\lambda_{max.}^{CHCl_3}$ 6.03 (C=C) and 6.15 μ (C=N).

Anal.—Calcd. for $C_{28}H_{46}N_2$: C, 81.89; H, 11.29; N, 6.82. Found: C, 81.33; H, 11.68, N, 6.84.

2′,3′-Dihydroimidazolino[a-4,3]-4-aza-17α-methyl-5-androsten-17β-ol (VI).—This compound was prepared in a manner similar to the synthesis of 2′,3′-dihydroimidazolino[*a*-4,3]-4-aza-5-cholestene (V), except that the reaction mixture was refluxed 7 hr. From 3.22 Gm. (0.01 mole) of 17α-methyl-3,5-seco-4-norandrostan-17β-ol-5-on-3-oic acid (II) and 20 ml. of ethylenediamine, 2.85 Gm. (86%) of 2′,3′-dihydroimidazolino[*a*-4,3]-4-aza-17α-methyl-5-androsten-17β-ol (VI) was obtained upon recrystallization from 70% methanol, m.p. 229–230° dec. $[\alpha]_D^{32}$ −30.5° (c 1.0, chloroform); $\lambda_{max.}^{EtOH}$ 258 mμ (log ε 4.17); $\lambda_{max.}^{CHCl_3}$ 2.75 (OH), 6.03 (C=C), and 6.15 μ (C=N).

Anal.—Calcd. for $C_{21}H_{32}N_2O$: C, 76.78; H, 9.82; N, 8.53. Found: C, 76.36; H, 9.62; N, 8.25.

Hexahydropyrimidino[a-4,3]-4-aza-5-cholestene (VII).—III (4.25 Gm., 0.01 mole) was added to a refluxing solution of 4 Gm. of lithium aluminum hydride in 800 ml. of anhydrous ether by means of a Soxhlet extractor. The addition was complete in 1 hr. The mixture was refluxed 6 hr. The excess hydride was destroyed with wet ether and water. The precipitate was filtered and washed with ether. The ether was evaporated after the solution had been dried over sodium sulfate. The white solid residue was crystallized from acetone–methanol to yield 3.68 Gm. (86%) of hexahydropyrimidino[*a*-4,3]-4-aza-5-cholestene (VII), m.p. 146–147°. $[\alpha]_D^{25}$ −104.63° (c 0.5, chloroform); $\lambda_{max.}^{CHCl_3}$ 3.00 (NH) and 6.03 μ (weak, C=C).

Anal.—Calcd. for $C_{28}H_{50}N_2$: C, 81.62; H, 11.81; N, 6.57. Found: C, 81.14; H, 11.61; N, 7.20.

Hexahydropyrimidino[a-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (VIII).—This compound was prepared in a manner similar to the synthesis of VII. From 343 mg. (0.001 mole) of IV and 500 mg. of lithium aluminum hydride, 270 mg. (80%) of hexahydropyrimidino[*a*-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (VIII) was obtained. The analytical sample was recrystallized from aqueous acetone to give m.p. 192–193°. $[\alpha]_D^{25}$ −151.2° (c 1.0, chloroform); $\lambda_{max.}^{CHCl_3}$ 2.75 (OH), 3.00 (NH), and 6.03 μ (weak, C=C).

Anal.—Calcd. for $C_{22}H_{36}N_2O$: C, 76.69; H, 10.53; N, 8.13. Found: C, 76.40; H, 10.42, N, 8.22.

Tetrahydroimidazolino[a-4,3]-4-aza-5-cholestene (IX).—V (1.03 Gm., 0.0025 mole) was added to a solution of 1 Gm. of lithium aluminum hydride in 200 ml. of anhydrous ether by means of a Soxhlet extractor. After the addition was completed, the mixture was refluxed 2.5 hr. Excess hydride was destroyed with water, and the inorganic salts were filtered and washed with ether. The ether solution was evaporated and the residue crystallized from methanol to yield 0.62 Gm. (60%) of tetrahydroimidazolino[*a*-4,3]-4-aza-5-cholestene (IX), m.p. 94–96°. $[\alpha]_D^{32}$ −80.8° (c 0.25, chloroform); $\lambda_{max.}^{CHCl_3}$ 3.00 (NH) and 6.03 μ (weak, C=C).

Anal.—Calcd. for $C_{28}H_{48}N_2$: C, 81.49; H, 11.72; N, 6.79. Found: C, 81.23; H, 11.57; N, 7.07.

Tetrahydroimidazolino[a-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (X).—Following a procedure similar to that for IX, 500 mg. (0.0016 mole) of IV and 500 mg. of lithium aluminum hydride gave 410 mg. (82%) of tetrahydroimidazolino[*a*-4,3]-17α-methyl-4-aza-5-androsten-17β-ol (X). Recrystallization from ether–petroleum ether (b.p. 30–60°) gave an analytical sample, m.p. 165–167°. $[\alpha]_D^{32}$ −44.0° (c 0.5, chloroform); $\lambda_{max.}^{CHCl_3}$ 2.75 (OH), 3.00 (NH), and 6.03 μ (weak, C=C).

Anal.—Calcd. for $C_{21}H_{34}N_2O$: C, 76.31; H, 10.37; N, 8.48. Found: C, 75.77; H, 10.14; N, 8.96.

REFERENCES

(1) Bolt, C. C., *Rec. Trav. Chim.*, **70**, 940(1951).
(2) Brown, D. J., and Evans, R. F., *J. Chem. Soc.*, **1962**, 527.
(3) Hofmann, K., "The Chemistry of Heterocyclic Compounds. Part I. Imidazole and Its Derivatives," Interscience Publishers, Inc., New York, N. Y., 1953, p. 214.
(4) Reppe, W., *et al.*, *Ann.*, **596**, 211(1955).
(5) Turner, R. B., *J. Am. Chem. Soc.*, **72**, 579(1950).