Structural biochemistry. IV. 3β-Hydroxy-17β-(L-prolyl)amino-androst-5-ene[1–3]

G. R. PETTIT,[4] R. L. SMITH, A. K. DAS GUPTA,[5] AND J. L. OCCOLOWITZ[6]

Department of Chemistry, University of Maine, Orono, Maine, and Department of Chemistry, Arizona State University, Tempe, Arizona

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The synthesis of the title compound III was studied in detail and the following combination of methods was found reliable and convenient. The oxime derivative I*b* of ketone I*a* was reduced with sodium–ethanol to 3β-hydroxy-17β-amino-androst-5-ene. The configurational assignment for amine II*a* was supported by the results of a comparison with the 17α-epimer and by a proton magnetic resonance study of both isomers. Selective reaction between amine II*a* and carbobenzoxy-L-proline was achieved with Woodward's reagent K. Of several procedures explored for removing the carbobenzoxy protecting group from amide II*c*, palladium-catalyzed hydrogenolysis proved quite satisfactory. Hydrogenolysis of carbamate II*b* to yield prolyl amide III was realized without affecting the Δ^5 olefin system. A mass spectral study of amine III and the corresponding 5α-derivative IV confirmed the latter observation. A brief review of procedures for the synthesis of steroidal amines was also presented.

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To provide synthetic steroidal peptides of possible use in advancing knowledge of both steroid transport and alteration of hormone properties, we initially developed methods for the synthesis of proline- and arginine-containing steroidal peptides derived from 3β-hydroxy-17β-amino-5α-androstane (1–3). The present investigation was undertaken to develop procedures satisfactory for the synthesis of peptides derived from steroids bearing both alcohol and olefin groups. More specifically, 3β-hydroxy-Δ^5-type steroids, which are immediate precursors of the 3-oxo-Δ^4-system, an important characteristic of many biologically active steroids, was of initial interest. Accordingly, the synthesis of proline derivative III was undertaken for the reason just outlined and for comparison with previously described (3) amide IV.

Although the synthesis of an important intermediate, 17β-amine II*a*, had been achieved by sodium–ethanol reduction (4*a*) of oxime I*b* or by reductive amination (4*b*) of ketone I*a*, the need for relatively large quantities with a firmly established stereochemistry prompted a re-study of routes to amine II*a*. Among a variety of methods[7]

[1]For part III in this series, see ref. 1. This paper also can be considered as part XXXVI in the series entitled Steroids and Related Natural Products.

[2]A preliminary report of the present study was presented (April 9, 1964) at the 3rd International Symposium on the Chemistry of Natural Products, Kyoto, Japan (see also ref. 2). The present contribution is also based on part of the doctoral dissertation submitted by R. L. Smith to the Graduate School, University of Maine, Orono, Maine, August 1965.

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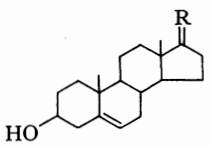
[4]Present address: Department of Chemistry, Arizona State University, Tempe, Arizona.

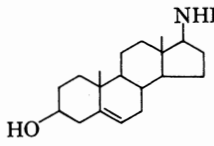
[5]Present address: Research Division, East India Pharmaceutical Works, Calcutta, India.

[6]Present address: Defence Standards Laboratories, Maribyrnong, Victoria, Australia.

[7]Nucleophilic displacement of steroid alkyl halides or *p*-toluenesulfonate esters was among the earliest methods employed, and the technique has recently been extended to azide nucleophiles (5*a*). Catalytic reduction of steroidal oximes with palladium (5*b*), platinum (5*c*), or nickel (5*d*) has been used for obtaining axial amines. Lithium aluminium hydride reduction (5*e*) or lithium–ammonia–alcohol (5*f*) reduction of steroidal oximes generally yields axial–equatorial mixtures. Reductive amination of steroidal ketones by application of the Leuckart reaction (5*g*) gives predominantly equatorial amines. Recently, Schmitt and his collaborators have pursued a careful investigation of reductive amination involving both imine and enamine intermediates generated *in situ* from ketones and primary or secondary amines. Here catalytic (5*h*), hydride (5*i*), diborane (5*i*), formic acid (5*j*), and chemical (5*k*) reduction with aluminium and mercury(II) chloride in the presence of protic solvents has been explored. The catalytic approach generally gives a mixture of epimeric amines, whereas the hydride and chemical reduction methods give the thermodynamically favored equatorial amines. Steroidal amines have also been obtained recently by photochemical transformation of azide precursors (5*l*). The Beckman (5*m*), Curtius (5*n*), and Schmidt (5*o*) reactions have been the most dependable for the stereospecific generation of steroidal amines.



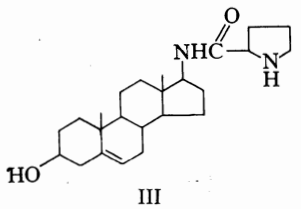
Ia R = O
Ib R = NOH



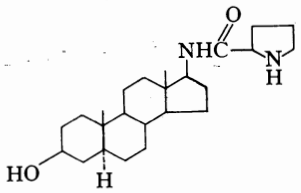
IIa R = H
IIb R = $COCH_2NHCO_2CH_2C_6H_5$

IIc R = CO–N(CO$_2$CH$_2$C$_6$H$_5$)

IId R = $COCHCH_2$–(indole)
NH
CO_2
$CH_2C_6H_5$



III



IV

for preparing steroidal amines, the classical sodium–alcohol reduction of oximes seemed well suited to the requirements set for amine II*a*. A cursory examination of both the catalytic (platinum) and lithium aluminium hydride reduction of oxime I*b* as compared to the sodium–ethanol (3) technique reinforced this view. Eventually the sodium–ethanol route was studied in detail and amine II*a* was obtained routinely in approximately an 85% yield. Thin-layer chromatograms of amine II*a* with various solvent systems failed to reveal the presence of the axial epimer. Since conversion of ketone I*a* into oxime I*b* is virtually quantitative, the overall transformation to equatorial amine II*a* was quite practical. The 17β-configuration was confirmed by comparing amine II*a* with a sample[8] of 3β-hydroxy-17α-amino-androst-5-ene obtained by lithium aluminium hydride reduction of the corresponding 17α-azide. Comparison of both epimers by infrared spectra and thin-layer chromatographic behavior (chloroform–methanol or chloroform as eluent) gave nearly identical results. However, a mixture melting point determination was depressed and a comparison of the proton magnetic resonance (p.m.r.) responses was particularly rewarding. Proton magnetic resonance spectra of the 17β- and 17α-amino epimers exhibited C-18 angular methyl group responses as sharp singlets at 0.65 and 0.69 δ, respectively. Further, the axial 17α-proton of equatorial amine II*a* appeared as a broad signal at 2.57 δ, whereas the equatorial 17β-proton of the axial amine appeared further downfield as a well-defined doublet centered at 2.83 δ.[9]

With the stereochemistry of amine II*a* established beyond doubt, the next step involved peptide bond formation. The reaction between carbobenzoxy glycine, carbobenzoxy-L-proline, or carbobenzoxy-L-tryptophan and amine II*a* in the presence of the phenylisoxazolium reagent (1, 8) and triethylamine was evaluated. The reactions involving glycine and tryptophan were included for comparison purposes. Although the glycine (II*b*) and proline (II*c*) derivatives were readily obtained, depending upon the reaction conditions, tryptophan amide II*d* was accompanied by three to seven other substances.

To preserve both nuclear unsaturation and the 3β-hydroxy substituent, removal of the carbobenzoxy group from carbamate II*c* was attempted by several mild techniques. The procedure of initial choice was

[8]We are grateful to Dr. C. H. Robinson for providing this specimen (see ref. 6).

[9]Axial protons attached to rigid five or six membered ring systems are known to display p.m.r. responses at higher fields than their equatorial counterparts. For reviews pertinent to this subject, see ref. 7.

catalytic hydrogenolysis, but the concomitant reduction of the 5-ene appeared to be a serious potential disadvantage. Hydrogenolysis of carbamate II*c* with palladium black as catalyst was complete in 2 h, and after purification, amine III was obtained in a 60% yield. Interestingly, no evidence for the presence of the 5α-steroid IV was detected. Although both amines III and IV have nearly the same melting point and a mixture melting point is inconclusive, comparison p.m.r. spectra provided convincing evidence for the structure assigned amine III. Preservation of nuclear unsaturation was apparent by a complex signal at 5.12 δ in the p.m.r. spectrum of amine III attributable to the C-6 vinyl proton. Conclusive evidence for the preservation of the olefin system was provided by a mass spectral study. Olefin III provided a molecular ion at *m/e* 386 (Fig. 1) unaccompanied by an M + 2 fragment corresponding to saturated steroid IV (Fig. 2). The ions at *m/e* 288 and 290, respectively, for amines III and IV also reinforced this conclusion.

Reductive cleavage of the carbobenzoxy group with triethylsilane – palladium(II) chloride (9) also gave prolylamine III in a practical yield (70%). After catalytic hydrogenolysis with palladium or by the palladium(II) chloride procedure, amine III was obtained as the hydrochloride derivative. Apparently, enough hydrogen chloride was absorbed by the palladium catalyst to yield an ammonium salt. As with other steroidal peptides we have so far examined, amine III was readily protonated (for example, during chromatographic purification) and converted into solvates.

Solvolysis of carbamate II*c* with warm anhydrous trifluoroacetic acid (10) or cleavage of the carbobenzoxy group with anhydrous hydrogen bromide (11) at dry ice temperature proved less satisfactory than the preceding two methods. After purification, amine III was obtained in less than 25% conversion.

The ketone → oxime → amine route to 17β-amine II*a* followed by peptide bond formation with Woodward's reagent K and catalytic hydrogenolysis of the protecting group, as illustrated for the synthesis of steroidal amine III, seems a generally useful approach to steroids of this type.

EXPERIMENTAL

Brief descriptions of solvents, reagents, and column chromatographic absorbents appear in two preceding contributions (1, 3). Solvent extracts of aqueous solutions were dried over magnesium sulfate. All analytical samples were colorless, and R_f values were determined (1) on silica gel G plates. Melting points were recorded on a Kofler melting point apparatus, and mixture melting points were determined in open Kimble glass capillary tubes, using a silicon oil bath. The infrared (in potassium bromide) and p.m.r. (in deuteriochloroform solution on a Varian model A-60 spectrometer with tetramethylsilane as an internal standard) spectra were recorded by R. A. Hill and P. A. Whitehouse, University of Maine. Mass spectra were obtained with a CEC-21-103C mass spectrometer equipped with a direct-inlet system. The instrument was run with the electron multiplier and a fine exit slit. The ionizing energy of the mass spectrometer was maintained at 70 eV and the ionizing current at 50 μA. Optical rotation values were determined (in chloroform solution at 20° unless otherwise noted) in the laboratories of Dr. C. Janssen, Beerse, Belgium, and were provided by Dr. P. Demoen. Elemental analytical data were provided by Dr. A. Bernhardt, Max-Planck Institut, Mülheim, Germany.

3β-Hydroxy-17-oximino-androst-5-ene

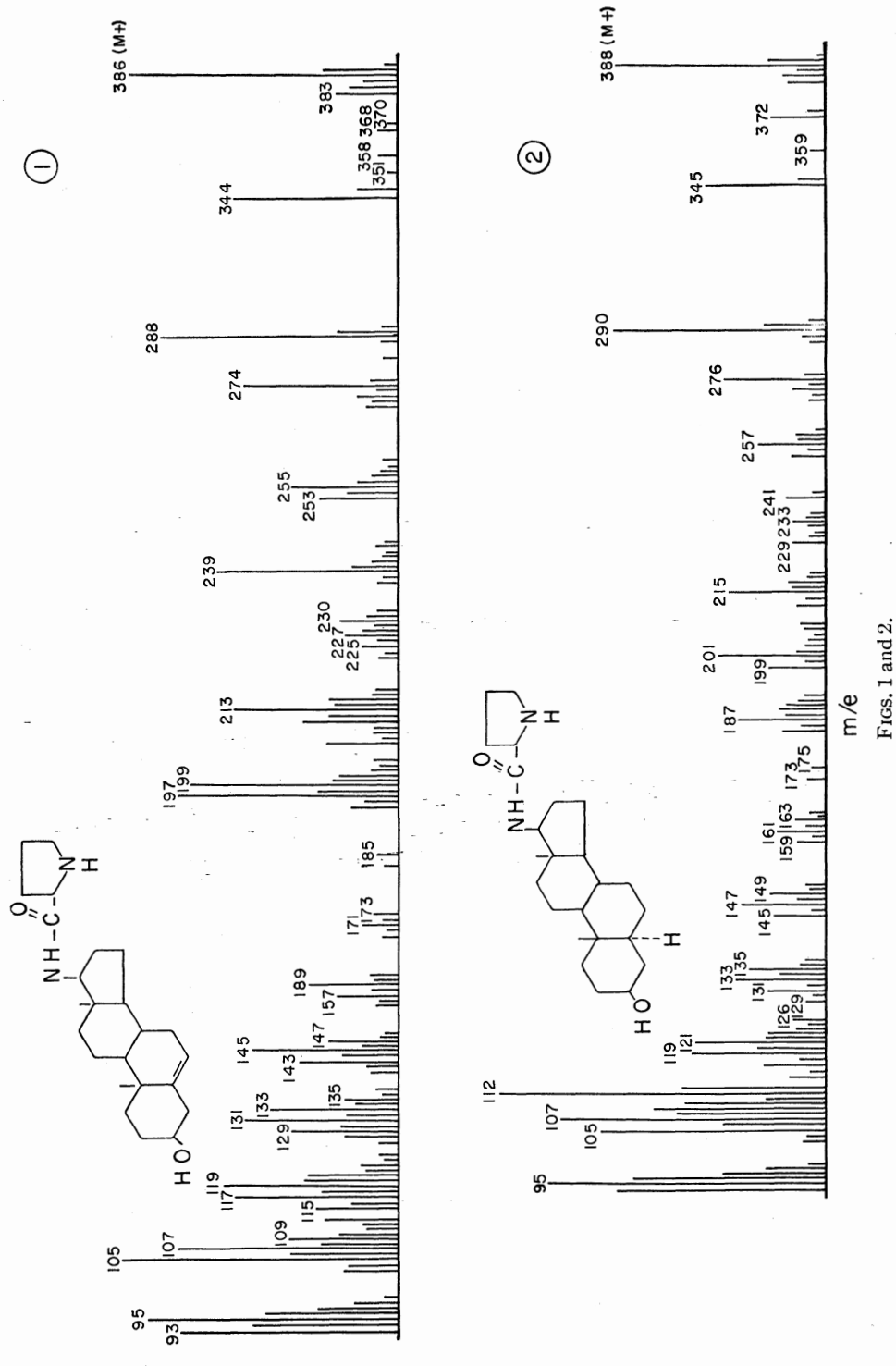
The following two experiments leading to amine II*a* represent improved modifications of prior methods. Each experiment was repeated a number of times, and the procedures described below were found to be convenient and reliable.

A warm solution of hydroxylamine hydrochloride (0.9 g) in ethanol (10 ml of 95%) – water (1 ml) was added to a warm solution of 3β-hydroxy-17-oxo-androst-5-ene (I*a*, 1.0 g) in pyridine (10 g). The resulting solution was heated at reflux for 5 h, cooled to room temperature, and diluted with water (100 ml). The product which separated was collected and washed successively with water, 5% aqueous hydrochloric acid, and water to yield (quantitative) 1.0 g of oxime I*b* melting at 193–197° (sintering from 160°). Four recrystallizations from chloroform–methanol gave a pure sample as fine needles: R_f 0.45 in chloroform–methanol (19:1); m.p. 203° (sintering from 160°); ν_{max} 3 400 (broad), 2 920, 1 674, 1 468 – 1 432 (broad), 1 372, 1 360, 1 066, 1 038 – 1 020 (broad), 950, and 930 cm^{-1}. Melting points of 191–193° and 204–206° have been reported (12) for oxime I*b*.

Anal. Calcd. for $C_{19}H_{29}NO_2$: C, 75.20; H, 9.65; N, 4.61. Found: C, 75.08; H, 9.80; N, 4.56.

*3β-Hydroxy-17β-amino-androst-5-ene (II*a*)*

Sodium (60.0 g, freshly cut) was added (during 1 h) in small increments to a refluxing solution of oxime I*b* (10.0 g) in absolute ethanol (500 ml). After the sodium was added, stirring and heating at reflux



FIGS. 1 and 2.

were continued for 1.75 h. The hot mixture was diluted with warm (60°)[10] water (5 l) and allowed to stand at room temperature for 16 h. The solid which separated was collected, washed with water (3 × 100 ml), and dried. The light, tan-colored solid weighed 8.4 g (85%) and melted at 164° with sintering from 158°. Two recrystallizations from aqueous ethanol (with Norite A) and one recrystallization from benzene–tetrahydrofuran yielded a pure specimen as microcrystals: R_f 0.75 in chloroform–methanol (9:1)[11] and 0.36 in chloroform; m.p. 170–172° with sintering from 152° (another sample that was recrystallized from methanol – ethyl acetate melted at 165–168°); $[\alpha]_D$ −65.6° (*c*, 1.83) and −55.6° (*c*, 0.27 in methanol); ν_{max} 3 400 – 3 020 (broad), 2 920, and 1 734 cm^{-1}; p.m.r. responses at 0.65 (singlet, three protons), 1.00 (singlet, three protons), and 2.57 (multiplet, one proton) δ. Melting points ranging from 158–159° to 166–168° and optical rotations of $[\alpha]_D^{20}$ −54°, $[\alpha]_D^{25}$ −67.8°, and $[\alpha]_D$ −80° have been reported (4, 14) for amine II*a*.

Anal. Calcd. for $C_{19}H_{31}NO$: C, 78.84; H, 10.79; N, 4.84. Found: C, 78.72, 78.42; H, 10.75, 10.80; N, 4.88, 4.99.

The 17β-configuration assigned to amine II*a* was confirmed by comparison with an authentic sample[8] of 3β-hydroxy-17α-amino-androst-5-ene, which exhibited the following physical constants: R_f 0.75 in chloroform[11]–methanol (9:1) and 0.36 in chloroform;[11] m.p. 192–194° (sintering from 168°); $[\alpha]_D$ −100°; p.m.r. responses at 0.69 (singlet, three protons), 1.00 (singlet, three protons), 2.78–2.88 (doublet, one proton), 3.33 (broad, one proton), and 5.13–5.19 (doublet, one proton) δ. The melting point and p.m.r. data were recorded with the equipment employed in the preceding experiment for the 17β-isomer II*a*. A mixture melting point determination employing both epimers gave a value of 165–170°; however, an infrared spectral comparison showed only minor differences.

*3β-Hydroxy-17β-(N$^{\alpha}$-carbobenzoxy-glycyl)amino-androst-5-ene (II*b*)*

Glycine derivative II*b* was prepared essentially as described in the following experiment leading to proline derivative II*c*. The active intermediate prepared from carbobenzoxy glycine (0.88 g), *N*-ethyl-5-phenylisoxazolium-3′-sulfonate (1.0 g, Woodward's reagent K), and triethylamine (0.4 g) in acetonitrile (40 ml) was treated with amine II*a* (1.2 g). In this experiment, the amino acid dissolved within 90 min at ice-bath temperature, and condensation with amine II*a* was begun at that point. After 24 h at room temperature, the mixture of solution and solid phase was dissolved in chloroform and concentrated (*in vacuo*) to dryness. A solution of the residue in chloroform was washed as described in experiment II*c*; after removal (*in vacuo*) of solvent, the solid was recrystallized from chloroform – ethyl acetate to yield 1.5 g melting at 199–200°. Recrystallization from the same solvent yielded crystal rosettes melting at 201–202°. The analytical sample displayed $[\alpha]_D$ −66.7° (*c*, 0.87), and a solvent composed of 19:1 chloroform–methanol was used for thin-layer chromatography.

Anal. Calcd. for $C_{29}H_{40}N_2O_4$: C, 72.47; H, 8.39; N, 5.83; O, 13.32. Found: C, 72.30; H, 8.41; N, 6.06; O, 13.42.

*3β-Hydroxy-17β-(N$^{\alpha}$-carbobenzoxy-L-prolyl)amino-androst-5-ene (II*c*)*

In a typical experiment, Woodward's reagent K (5.1 g) and triethylamine (2.0 g) were added to a cold (ice bath), rapidly stirred solution of carbobenzoxy-L-proline (4.6 g) in dry acetonitrile (250 ml). While the reactants slowly dissolved, stirring, with cooling, was continued. After 2 h, solution was complete and amine II*a* (5.8 g) was added. Before solvent was removed *in vacuo*, the solution was stirred and maintained at room temperature for 22 h. The tan viscous residue was extracted with chloroform (150 ml), and the resulting solution was washed successively with water, 5% aqueous hydrochloric acid, water, 5% aqueous sodium bicarbonate, and water. Removal of solvent *in vacuo* gave a buff-colored foam. A solution of the residue in 2:1 ethyl acetate – chloroform was chromatographed on neutral alumina. Elution with the same solvent (400 ml) gave a pure (as evidenced by thin-layer chromatography) crystalline fraction, which was recrystallized from ethyl acetate – chloroform (4:1) to provide 4.8 g (46%) melting at 194–198°. Concentration of the mother liquors gave two additional crystal crops: 0.8 g (8%), m.p. 194–198°, and 1.7 g (16%) melting at 194–198°. Three recrystallizations from chloroform–hexane gave an analytical specimen as sparkling plates: R_f 0.68 in 9:1 chloroform–methanol; m.p. 194–198.5° with a phase change at 114–115°; $[\alpha]_D$ −113.9° (*c*, 1.83); ν_{max} 3 400 – 3 280 (doublet), 2 929, 1 710 – 1 655 (broad), 1 550, 1 420 (shoulders at 1 470 and 1 450), 1 356, and 1 124 cm^{-1}; p.m.r. responses at 0.57 (singlet, three protons), 0.99 (singlet, three protons), 4.20 (broad, one proton), 4.94 (singlet, two protons), 5.04–5.18 (complex, one proton), and 7.04 (singlet, five protons) δ.

Anal. Calcd. for $C_{32}H_{44}N_2O_4$: C, 73.81; H, 8.52; N, 5.38. Found: C, 73.66; H, 8.72; N, 5.22.

In another experiment, a pure sample that was recrystallized from ethyl acetate – hexane melted at 194–195° and exhibited $[\alpha]_D$ −104.2° (*c*, 3.2).

Anal. Calcd. for $C_{32}H_{44}N_2O_4$: O, 12.29. Found: C, 73.86; H, 8.73; N, 5.27; O, 12.41.

*3β-Hydroxy-17β-(N$^{\alpha}$-carbobenzoxy-L-tryptophyl)-amino-androst-5-ene (II*d*)*

The preceding experiment (see II*c*) was repeated with amine II*a* (1.2 g) and the active intermediate from carbobenzoxy-L-tryptophan (1.4 g), Woodward's reagent K (1.0 g), and triethylamine (0.4 g) in nitromethane (15 ml) – acetonitrile (40 ml). As

[10]Dilution with cold water yields a finely divided suspension which is difficult to filter.

[11]Chloroform used as eluent was first saturated with concentrated ammonium hydroxide. Unless this precaution was observed, the amine appeared as two overlapping spots on the thin-layer chromatogram. The impurity was attributed to protonation of the amine. In earlier experiments, a solvent composed of 130:15:1 chloroform – methanol – ammonium hydroxide did not completely retard salt formation. A similar ammonium hydroxide technique has been described by Lábler and Černý (13).

evidenced by thin-layer chromatography (99:1 chloroform–methanol as the mobile phase), the product II*d* was accompanied by minor amounts of two other substances. In other experiments, as many as seven other components comprised the product. Thus, a solution of the mixture (2.8 g) in benzene was chromatographed on neutral alumina (100 g). A series of fractions eluted by 1:1 benzene–chloroform were combined (1.4 g total) and recrystallized from methanol–chloroform. The resulting solid melted at 130–133° (sintering from 128°) and showed $[\alpha]_D$ −39.3° (*c*, 0.64).

Anal. Calcd. for $C_{38}H_{47}N_3O_4$: C, 74.84; H, 7.77; N, 6.89; O, 10.50. Found: C, 74.64; H, 7.86; N, 6.96; O, 10.62.

3β-Hydroxy-17β-(L-prolyl)amino-androst-5-ene (III)

(a) By Catalytic Hydrogenolysis

During 2 h at room temperature, hydrogen was passed into a rapidly stirred solution of carbamate II*c* (1.0 g) in methanol (50 ml) containing suspended palladium black (0.5 g, Englehard Industries, Inc.). Exit gases were passed into a barium hydroxide trap and, as reduction progressed, a solid (presumably the hydrochloride salt from hydrogen chloride contained on the catalyst surface) separated from the reaction mixture. For this reason, the mixture was diluted with additional methanol (50 ml). The solution was filtered through a Celite layer and evaporated (*in vacuo*) to a solid, which was predominantly the hydrochloride derivative of amine III. The residue was partitioned between 5% aqueous sodium bicarbonate (25 ml) and chloroform (3 × 50 ml), and the combined chloroform extracts were concentrated *in vacuo* to a beige solid: yield 0.46 g (60%), m.p. 216–218° with resolidification at 223° and final melting at 235°. Four recrystallizations from chloroform–hexane (1:1) gave a sample as platelets with an optical rotation value of −97.1° (*c*, 0.72). Results of elemental analyses and a Beilstein test indicated that the specimen just described was a chloroform solvate. When the analytical sample was prepared with only methanol as solvent, shiny platelets melting at 225–228° were obtained which represented pure amine III: R_f 0.26 in chloroform–methanol (9:1); $[\alpha]_D$ −118.8° (*c*, 1.28); ν_{max} 2 410 – 2 320 (doublet), 2 930, 1 644, 1 525, and 1 070 cm^{-1}; p.m.r. responses at 0.69 (singlet, three protons), 0.98 (singlet, three protons), and 5.12 (complex, one proton) δ.

Anal. Calcd. for $C_{24}H_{38}N_2O_2$: C, 74.58; H, 9.91; N, 7.25; O, 8.28; mol. wt. 386. Found: C, 74.17; H, 10.26; N, 7.40; O, 8.28; mol. wt. (by mass spectrometry) 386.

A mixture melting point determination with amine III and the 5α-derivative (3) IV (calcd. mol. wt. 388, found by mass spectrometry 388) was inconclusive. Although both amines III and IV showed identical mobility (4:1 chloroform–methanol as eluent) on a thin-layer chromatography plate, their reaction to hot sulfuric acid was different. After the plate was sprayed with sulfuric acid and heated for approximately 15–20 min, olefin III gave a deep-yellow color whereas the 5α-steroid IV turned a greenish color.

A solution of amine III (0.17 g) in methanol (5 ml) was diluted with ethereal hydrogen chloride (20 ml). The solid (0.15 g) which separated was collected and recrystallized (3 times) from 1:1 methanol – diethyl ether. A pure sample of *3β-hydroxy-17β-(L-prolyl)amino-androst-5-ene hydrochloride* was obtained as fine needles melting at 263°: R_f 0.24 in chloroform–methanol (4:1); ν_{max} 3 350 – 3 200 (doublet), 2 900 (shoulders at 2 750 and 2 590), 1 670, 1 555, and 1 045 cm^{-1}.

Anal. Calcd. for $C_{24}H_{39}ClN_2O_2$: C, 68.16; H, 9.28; Cl, 8.38. Found: C, 68.34; H, 9.43; Cl, 8.21.

(b) By Reaction with Triethylsilane – Palladium(II) Chloride

Palladium(II) chloride (0.1 g, Amend Chemical and Drug Co., New York) was added to a warm mixture composed of carbamate II*c* (1.0 g), triethylsilane (0.5 g, Pierce Chemical Co., Rockford, Illinois), triethylamine (0.2 ml), and dry xylene (20 ml). The mixture was heated at reflux for 4 h, with stirring, cooled to room temperaure, and diluted with methanol (20 ml); then the liquid phase was passed through a Celite layer. The yellow filtrate was diluted with chloroform (200 ml) and washed with water, 5% aqueous sodium bicarbonate, and water. Removal of solvent *in vacuo* gave a yellow oil which slowly solidified. A solution of the solid in 1:1 benzene–chloroform was chromatographed on neutral alumina. Fractions eluted with the same solvent (250 ml) and chloroform (50 ml) contained three impurities (as evidenced by thin-layer chromatography with 9:1 chloroform–methanol as the mobile phase). Continued elution with 9:1 chloroform–methanol yielded 0.54 g (70%) of amine III. The hydrochloride derivative was identical[12] with the specimen prepared by method *a*.

(c) By Solvolysis with Trifluoroacetic Acid

Carbamate II*c* (1.0 g) was added to warm (40°) anhydrous trifluoroacetic acid (37 g, purified by distillation from phosphorus pentoxide, b.p. 70–72°). The deep-purple solution was stirred and maintained at 40° for 5 h. Solvent was removed *in vacuo*, and the purple, oily residue was dissolved in chloroform and washed with 5% aqueous sodium bicarbonate and water. After evaporation (*in vacuo*) of solvent, the yellow solid was dissolved in 1:1 benzene–chloroform and chromatographed on neutral alumina (20 g). Chromatographic purification of amine III was achieved as described above in method *b*. By this means a 0.17 g yield (22%) of amine III was realized. The product was characterized[12] as the hydrochloride derivative.

(d) By Reaction with Anhydrous Hydrogen Bromide

Approximately 10 ml of hydrogen bromide (purified by passage through a trap containing concentrated sulfuric acid) was condensed in a reaction vessel containing carbamate II*c* (1.0 g), and cooled (solid carbon dioxide – chloroform) to approximately

[12]The identical composition of both samples was confirmed by infrared spectral comparison, thin-layer chromatographic comparison (R_f 0.24 in 4:1 chloroform–methanol), and mixture melting point determination.

−77°. After 0.5 h, excess hydrogen bromide was removed *in vacuo* and the pale-yellow residue was partitioned between 5% aqueous sodium bicarbonate and chloroform. The chloroform extract was washed with water and concentrated to a colorless foam, which was chromatographed (see method *b*) on neutral alumina (30 g). As with method *c*, amine III was accompanied by two other substances. Recovered amine III weighed 0.1 g (13%) and was identified[12] after conversion into the hydrochloride derivative.

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