tered off and washed well with chloroform and the combined washings and filtrates were evaporated to dryness with the rotary evaporator. The brown solid residue was extracted with 100 ml. of boiling benzene, the benzene evaporated and the residue recrystallized from methanol to give 0.8 g. (50%) of pale yellow needles of 21-methoxy-4-formyltropone of m.p. 183–184°. The reported m.p. was 182–183°.

**2-Methoxy-4-carboxytropone (XI).**—To a cold basic suspension of fresh silver oxide, prepared by adding 6.8 g. (0.04 mole) of silver nitrate to a solution of 2.8 g. (0.07 mole) of sodium hydroxide dissolved in 100 ml. of water, was added 3.3 g. (0.02 mole) of 2-methoxy-4-formyltropone over 15 min. with stirring. The mixture was allowed to stand at ice-bath temperature for an additional half-hour then warmed to room temperature over one-half hour. The silver was filtered off and washed with water and the solution acidified with cold 10% hydrochloric acid. The precipitated acid was filtered off and recrystallized from 25% methanol in water to give 2.5 g. (70%) of pale yellow needles of 2-methoxy-4-carboxytropone, m.p. 253–254°. The reported m.p.[1] was 258°.

**2-Amino-4-carboxytropone (XII).**—2-Methoxy-4-carboxytropone (1.7 g.) and liquid ammonia (20 ml.) were sealed in a glass tube and the tube was stored in a steel bomb for 3 days at room temperature. (On some of the runs the tube exploded inside of the bomb. This could be largely prevented by charging the bomb with about 150 p.s.i. of $N_2$ after introducing the frozen (Dry Ice) tube and later cooling the entire apparatus to Dry Ice temperature before opening the bomb.) The contents of the tube were then transferred to a beaker and the ammonia was allowed to evaporate (caution: splattering). The solid residue was taken up in 25 ml. of water and acidified with 10% hydrochloric acid. The precipitated acid was filtered off and weighed 1.45 g. (93%). Three recrystallizations from aqueous ethanol gave the analytical sample as small orange needles, m.p. 280° d. (sealed tube).

*Anal.* Calcd. for $C_8H_7O_3N$: C, 58.18; H, 4.28. Found: C, 57.93; H, 4.41.

**2-Acetamino-4-carboxytropone (XIII).**—Treatment of 2-amino-4-carboxytropone with excess acetic anhydride in pyridine on the steam-bath for 45 min. followed by acidification with 10% hydrochloric acid in ice gave a yellow-brown solid. Two recrystallizations from ethanol with decolorizing charcoal gave a pale yellow powder of m.p. 230° dec. (sealed tube).

*Anal.* Calcd. for $C_{10}H_9O_4N$: C, 57.97; H, 4.38. Found: C, 57.50; H, 4.50.

**Acid Chloride of 2-Acetamino-4-carboxytropone.**—To a stirred suspension of 0.25 g. of the dry potassium salt of 2-acetamino-4-carboxytropone in 10 ml. of benzene was added 0.1 ml. of oxalyl chloride in 5 ml. of benzene. Stirring was continued for 3 hr. at room temperature during which time a gas was evolved and the solution became yellow. The mixture was filtered and evaporated to dryness on the rotary evaporator to give the crude acid chloride as a yellow solid. Treatment with *p*-bromoaniline in benzene gave the *p*-bromoanilide derivative which melted, after two recrystallizations from benzene, at 225–227°.

*Anal.* Calcd. for $C_{16}H_{13}O_3N_2Br$: C, 53.20; H, 3.63; Found: C, 53.52; H, 3.89.

**2-Amino-6-styryltropone (VI).**—A mixture of 1.5 g. of 2-methoxy-6-styryltropone (Va) and 30 ml. of liquid ammonia was allowed to stand in a sealed tube at room temperature for 45 hr. After evaporation of the ammonia the product was recrystallized from 100 ml. of benzene to give 0.85 g. of orange plates, m.p. 135–136°. An additional 0.35 g. was obtained by treating the filtrate with decolorizing charcoal and evaporating to 20 ml. The total yield was 1.2 g. (86%). Three more crystallizations from benzene (with charcoal treatment) gave the analytical sample as sparkling golden plates, m.p. 137–138°.

*Anal.* Calcd. for $C_{15}H_{13}NO$: C, 80.69; H, 5.87. Found: C, 80.24; H, 5.80.

**The acetyl derivative VIa** was prepared with acetic anhydride and pyridine, and melted, after three crystallizations from heptane containing 5% of benzene, at 179.5–181°.

*Anal.* Calcd. for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70. Found: C, 76.96; H, 5.98.

**2-Hydrazino-6-styryltropone (VII).**—To a suspension of 0.238 g. of 2-methoxy-6-styryltropone in 1 ml. of methanol and 1 ml. of dioxane was added 0.1 g. of 95% hydrazine. The mixture was warmed on the steam-bath for 5 min. and cooled; the crystals were collected (0.21 g., 88%) and after three crystallizations from methanol gave brilliant red crystals, m.p. 162.5–163.5°.

*Anal.* Calcd. for $C_{15}H_{14}N_2O$: C, 75.60; H, 5.92. Found: C, 75.91; H, 5.79.

**4-(β-Carboxyvinyl)-tropolone (XIV).**—A mixture of 0.75 g. of 4-formyltropolone, 0.52 g. of malonic acid, 1 ml. of piperidine and 20 ml. of pyridine was heated on the steam-bath for 12 hr. The resulting solution was poured over ice and acidified with 10% hydrochloric acid and the acidic solution was continuously extracted with ether for 24 hr. Evaporation of the dried ether solution and recrystallization of the solid residue from ethanol gave yellow needles of 4-(β-carboxyvinyl)-tropolone, m.p. 226.5–227.5° dec. (sealed tube). The compound gave an immediate green-brown color with ethanolic ferric chloride solution.

*Anal.* Calcd. for $C_{10}H_8O_4$: C, 62.60; H, 4.20. Found: C, 62.84; H, 4.37.

ROCHESTER 20, N. Y.

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[CONTRIBUTION NO. 111 FROM THE INSTITUTO DE QUÍMICA DE LA UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO]

# The Beckmann Rearrangement of the Acetoxime of $\Delta^{5,16}$-Pregnadien-3β-ol-20-one Acetate with Boron Trifluoride

BY J. ROMO AND A. ROMO DE VIVAR

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Beckmann rearrangement of $\Delta^{5,16}$-pregnadien-3β-acetoxy-20-acetoximino (Ib) with boron trifluoride etherate in benzene afforded dehydro epiandrosterone acetate (IIb), whereas in acetic anhydride the rearrangement followed a different course and two products were isolated: 17β-methyl-18-nor-$\Delta^{5,13(14)}$-isopregnadien-3β,16α-diol-20-one diacetate (IIIa) and 16-acetyl-17-acetylamino-$\Delta^{5,16}$-androstadien-3β-ol acetate (VIIa); several derivatives are described.

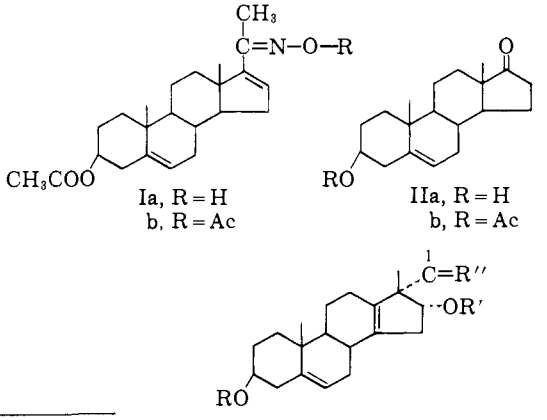
The Beckmann rearrangement of $\Delta^{5,16}$-pregnadien-3β-ol-20-one 3-acetate 20-oxime (Ia) has been studied by G. Rosenkranz, *et al.*[1] Using *p*-acetamidobenzenesulfonyl chloride as catalyst, the reaction afforded dehydroepiandrosterone (IIa) and working under appropriate conditions the intermediate amide VI could be isolated. In view of the current interest in this degradation of $\Delta^{16}$-20-ketones to androstane derivatives we considered the use of boron trifluoride as catalyst in this reaction. Recently Hauser and Hoffenberg[2] have used boron trifluoride in the Beckmann rearrange-

(1) G. Rosenkranz, O. Mancera, F. Sondheimer and C. Djerassi, *J. Org. Chem.*, **21**, 520 (1956).

(2) Ch. R. Hauser and D. S. Hoffenberg, THIS JOURNAL, **77**, 4885 (1955).

ment of some acetoximes and obtained in fairly good yield the corresponding amides. Therefore the 20-acetoxime of $\Delta^{5,16}$-pregnadien-3$\beta$-ol-20-one-3-acetate (Ib) was prepared and subjected to the action of boron trifluoride etherate in benzene solution and thus there was obtained dehydroepiandrosterone acetate (IIb); but when the rearrangement of the acetoxime Ib or the oxime Ia was carried in acetic anhydride it undertook a different course and two products A and B, were isolated by chromatographic separation. Product A analyzed for $C_{25}H_{34}O_5$, showed m.p. 216–218°, $[\alpha]^{20}D$ −14° ($CHCl_3$). The infrared spectrum showed a band at 5.80 $\mu$ (shoulder at 5.88 $\mu$). It formed an oxime and perbenzoic acid treatment afforded a diepoxide, while potassium bicarbonate saponification furnished the free derivative (m.p. 189–191°); product A was identified as 17$\beta$-methyl-18-nor-$\Delta^{5,13}$-isopregnadien-3$\beta$,16$\alpha$-diol-20-one 3,16-diacetate (IIIa), which had been obtained by Heusler and Wettstein[3] by rearrangement of 16$\alpha$,17$\alpha$-oxido-$\Delta^5$-pregnen-3$\beta$-ol-20-one acetate with *p*-toluenesulfonic acid in acetic anhydride. We repeated this reaction and found the substance to be identical with product A by mixed melting point and infrared comparison.[4]

Several derivatives of IIIa were prepared: lithium aluminum hydride reduction afforded the 3,16,-20-triol IIId; the keto group in IIIa was eliminated by hydrogenolysis of the cycloethylene mercaptol IIIf whereupon the diacetate IIIg was obtained; potassium bicarbonate saponification of IIIg yielded IIIh which on Oppenauer oxidation afforded the $\Delta^4$-3-ketone V.

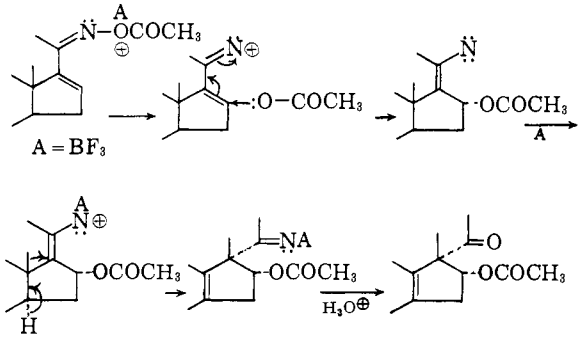


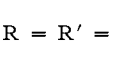
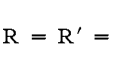
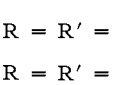
Ia, R = H
b, R = Ac

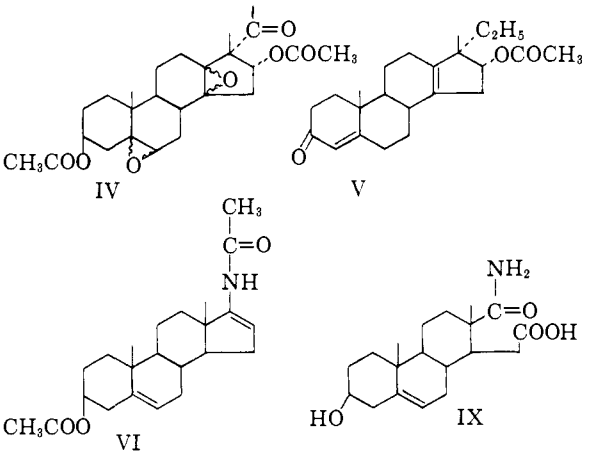
IIa, R = H
b, R = Ac

(3) K. Heusler and Wettstein, *Chem. Ber.*, **87**, 1301 (1954).

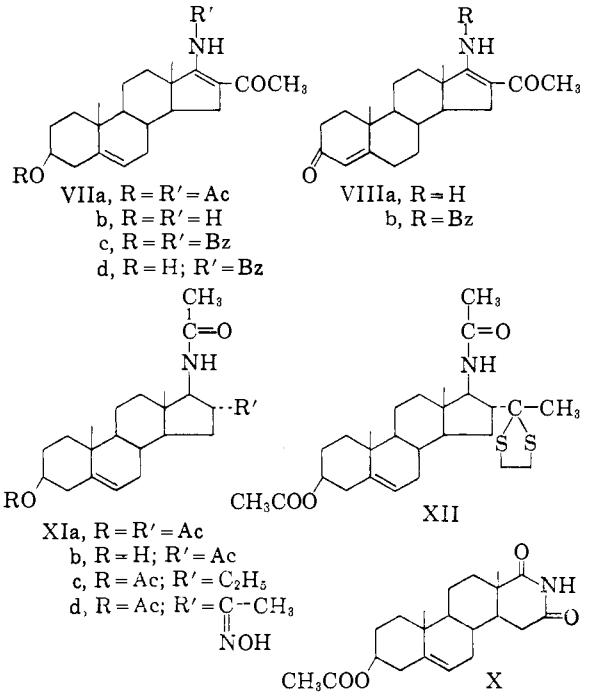
(4) The following mechanism for the formation of product A has been suggested to us by Dr. Gilbert Stork, Columbia University, to whom we are indebted.



IIIa, R = R′ = Ac; R″ = O
b, R = R′ = Ac; R″ = N·OH
c, R = R′ = H; R″ = O
d, R = R′ = H; R″ = 
e, R = R′ = Ac; R″ = 
f, R = R′ = Ac; R″ = 
g, R = R′ = Ac; R″ = $H_2$
h, R = H; R′ = Ac; R″ = $H_2$



IV V VI IX



VIIa, R = R′ = Ac
b, R = R′ = H
c, R = R′ = Bz
d, R = H; R′ = Bz

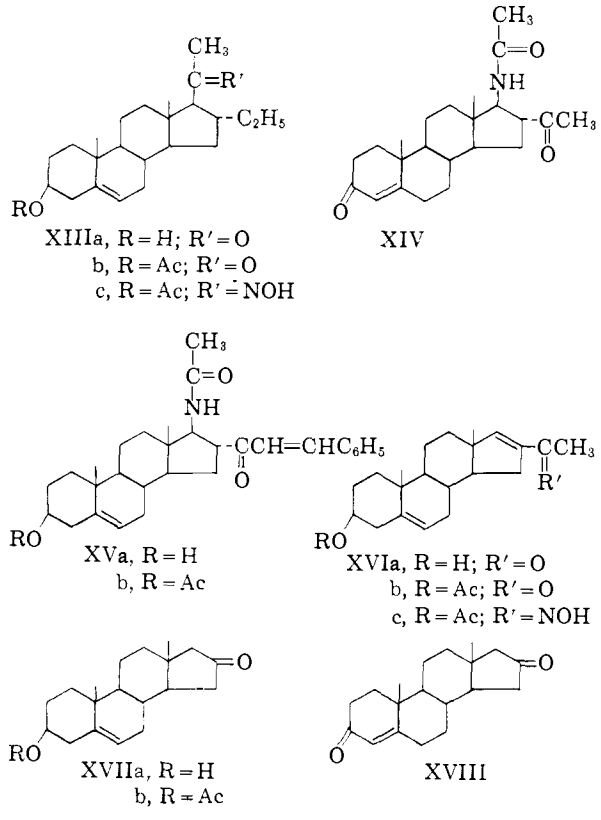
VIIIa, R = H
b, R = Bz

XIa, R = R′ = Ac
b, R = H; R′ = Ac
c, R = Ac; R′ = $C_2H_5$
d, R = Ac; R′ = 

XII X

Product B contained nitrogen, analyzed for $C_{25}H_{35}O_4N$ and showed m.p. 173–175°. Potassium hydroxide hydrolysis yielded the free compound ($C_{21}H_{31}O_2N$, m.p. 245–246°) which on reacetylation regenerated product B; the free compound gives a blue color with methanolic ferric chloride and a bluish-green precipitate with copper acetate in methanol solution upon addition of a few drops of ammonium hydroxide; the ultraviolet spectrum gave a maximum at 314 m$\mu$ (log $\epsilon$ 4.22)

and the infrared curve showed bands at 2.80, 2.90, 3.08, 6.20, 6.35 and 6.70 $\mu$. The formation of a copper chelate and the ultraviolet and infrared spectra strongly indicated the presence of a $\beta$-amino-$\alpha,\beta$-unsaturated ketone grouping. Combes and Combes,[5a] Ueno and Martell[5b] and Holtzclaw, *et al.*,[6] have described the formation of copper chelates of $\beta$-amino-$\alpha,\beta$-unsaturated ketones, while Cromwell, *et al.*,[7] have found that the grouping $-NH-C{=}C-CO-$ shows a maximum in the region of 306–308 m$\mu$, which is very close to the maximum observed in our derivative. The infrared spectra of these amino ketones have been reported by the aforementioned authors (see ref. 5b, 6 and 7), and particularly the bands at 6.20 (hydrogen bonded carbonyl), 6.35 and 6.70 $\mu$ (C=C stretching in the hydrogen bonded system) found in our free compound closely correspond to those reported for the $\beta$-amino-$\alpha,\beta$-unsaturated ketones (see ref. 6).




Further evidence of the presence of the $\beta$-amino-$\alpha,\beta$-unsaturated ketone grouping and its position arises from the following experiments.

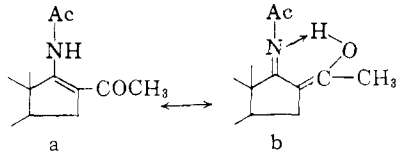
Alkaline hydrogen peroxide oxidation of the free compound furnished an amido acid ($C_{19}H_{29}O_4N$, m.p. 254–256°) which upon acetylation afforded a neutral acetate ($C_{21}H_{29}O_4N$, m.p. 260–262°). These derivatives proved to be identical with the 3$\beta$ - hydroxy - 16,17 - seco - $\Delta^5$ - androsten - 16,17-dioic acid-17-amide (IX) and 3$\beta$-acetoxy-16,17-seco-$\Delta^5$-androsten-16,17-imide (X) prepared by Regan and Hayes.[8] The formation of the acid IX would imply an oxidative splitting of a $\Delta^{16}$-double bond with elimination of a 16-acetyl group; therefore a 16-acetyl-17-amino-$\Delta^{16}$-androstene moiety comes into consideration. Another fact which strongly supports this structure is the introduction of an acetyl group into the amide VI by treatment with acetic anhydride in the presence of boron trifluoride etherate which produces the same product B, thus showing that the acetyl group is attached to the 16-position; then, formula VIIa must represent the structure of product B, and VIIb that of the hydrolysis of B. This structure was definitely proved in the following manner.

Selective hydrogenation of the 16-double bond of VIIa with Adams catalyst afforded the dihydro derivative XIa, which formed an oxime (XId) and a benzal derivative XVa; the 16-acetyl side chain of XIa was transformed into the ethyl group XIc by hydrogenolysis of the cycloethylene mercaptol XII.

Product XIc proved to be identical with 16$\alpha$-ethyl - 17 - acetylamino - $\Delta^5$ - androsten - 3$\beta$-ol-acetate which was synthesized by addition of ethylmagnesium iodide to $\Delta^{5,16}$-pregnadien-3$\beta$-ol-20-one acetate; Marker and Crooks[9] used this method to prepare several 16-alkyl-pregnenolones in which the 16-alkyl group is known to have the $\alpha$-configuration.[10]

The 16$\alpha$-ethyl-$\Delta^5$-pregnen-3$\beta$-ol-20-one (XIIIa) was converted into the acetate XIIIb and its oxime XIIIc; the latter by Beckmann rearrangement with tosyl chloride in pyridine furnished the acetylamino derivative XIc identical with that obtained by hydrogenolysis of the mercaptol XII.

It is pertinent to note that the hydrogenation of VIIa afforded the dihydro derivative XIa with the 16-acetyl group alpha oriented and it is well known that in the hydrogenation of a $\Delta^{16}$-double bond the hydrogen enters from the rear producing in this way beta oriented groups at positions 16 and 17. It is possible that under the hydrogenation conditions (in presence of acetic acid) equilibration takes place affording the more stable alpha oriented side chain[11] or that equilibration takes place when the cycloethylene mercaptol XII is formed, due to the acidic character of the zinc chloride used as catalyst. It is also of interest to point out that $\beta$-amino-$\alpha,\beta$-unsaturated ketones (a) are in equilibrium with structure (b)[6] and if the double bond of the latter imine is hydrogenated, then the 16-side




(5) (a) A. Combes and C. Combes, *Bull. soc. chim. France*, [3] **7**, 778 (1892); (b) K. Ueno and A. E. Martell, *J. Phys. Chem.*, **59**, 998 (1955).

(6) H. F. Holtzclaw, Jr., J. Collman and R. M. Alire, THIS JOURNAL, **80**, 1100 (1958).

(7) N. H. Cromwell, F. A. Miller, A. R. Johnson, R. L. Frank and D. J. Wallace, *ibid.*, **71**, 2337 (1949).

(8) B. M. Regan and F. N. Hayes, *ibid.*, **78**, 639 (1956). We are indebted to Dr. Regan for furnishing us with samples of these substances.

(9) R. E. Marker and H. M. Crooks, Jr., *Ibid.* **64**, 1280 (1942).

(10) J. Romo, J. Lepe and M. Romero, *Bol. Inst. de Quím. U.N.A.M.*, No. 2, Vol. IV, 125 (1952).

(11) J. Fajkos and F. Sorm, *Chem. Listy*, **51**, 579 (1959).

chain rearranges to the more stable $\alpha$-configuration.

Several derivatives of the amino-ketone VIIb were prepared: The dibenzoate VIIc was obtained by treatment of VIIb with benzoyl chloride in pyridine, while Schotten-Baumann benzoylation afforded the monobenzoate VIId; Oppenauer oxidation of VIId yielded the diketone VIIIb; alkaline hydrolysis of VIIIb furnished the amino-diketone VIIIa.

Potassium hydroxide treatment of the dihydro-aminoketone XIa only removed the 3-acetyl group yielding XIb, which on Oppenauer oxidation afforded the diketone XIV.

The dihydro aminoketone XIa lost the elements of acetamide on treatment with *p*-toluenesulfonic acid in acetic anhydride solution, yielding 16-acetyl-$\Delta^{5,16}$-androstadien-3$\beta$-ol acetate (XVIb) which on alkaline hydrolysis furnished the free derivative XVIa; these two compounds have been described by Fajkos and Sorm,[11] and the physical constants of our products agree well with those reported (see Experimental part).

The Beckmann rearrangement of 16-acetyl-$\Delta^{5,16}$-androstadien-3$\beta$-ol acetate-oxime (XVIc) furnished $\Delta^5$-androstene-3$\beta$-ol-16-one acetate (XVIIb) which on saponification yielded the free compound (XVIIa). These substances have been described by Huffman et al.[12] and by Fajkos and Sorm,[13] and our physical constants are in good agreement with those reported by the aforementioned authors.

**Acknowledgment.**—We wish to thank Dr. G. Rosenkranz of Syntex, S. A. for a generous gift of steroids.

## Experimental[14]

**Acetoxime of $\Delta^{5,16}$-Pregnadiene-3$\beta$-ol-20-one 3-Acetate** (Ib).—A solution of 10 g. of the oxime Ia in 30 ml. of anhydrous pyridine and 30 ml. of acetic anhydride was heated on the steam-bath for one hour and then poured into 300 ml. of cold water; the precipitate was collected, washed thoroughly with water and crystallized from chloroform–methanol, thus affording prismatic needles (9.35 g.), m.p. 195–196°; the analytical sample showed m.p. 195–197°, $[\alpha]^{20}D$ −41°.

*Anal.* Calcd. for $C_{25}H_{35}O_4N$: C, 72.25; H, 8.98; H, 3.37. Found: C, 72.18; H, 8.49; N, 3.47.

**$\Delta^5$-Androsten-3$\beta$-ol-17-one Acetate** (IIb).—To a solution of 2 g. of the acetoxime Ib in benzene, 30 ml., was added 3 ml. of boron trifluoride etherate and the mixture was left for 3 hours at room temperature, diluted then with cold water, washed with 5% sodium carbonate solution and water, dried over anhydrous sodium sulfate and evaporated to a small volume. The ketone IIb crystallized upon addition of hexane (410 mg.), m.p. 164–166°; one crystallization from acetone–hexane raised the m.p. to 168–170°, $[\alpha]^{20}D$ −2°; it gave no depression in mixed m.p. with an authentic specimen and the infrared spectra were identical.

**Beckmann Rearrangement of $\Delta^{5,16}$-Pregnadien-3$\beta$-ol-20-one 3-Acetate-20-oxime[1] (Ia) with Boron Trifluoride Etherate in acetic anhydride.**—A suspension of 50 g. of the oxime Ia in 300 ml. of acetic anhydride was treated with 100 ml. of boron trifluoride etherate in the course of 20 minutes at a temperature below 15° with mechanical stirring; during the addition all of the oxime went into solution. The dark solution was kept for 1 hour between 10–15° and then at room temperature overnight; the mixture was poured into 1300 ml. of ice-water, the oily precipitate was extracted with ether, the ether extract was washed with water, with 5% sodium carbonate solution and again with water, dried over sodium sulfate and evaporated to dryness. The oily residue (46 g.) was dissolved in benzene–hexane 1:1 and chromatographed on 1000 g. of alumina (washed with ethyl acetate) the first fractions eluted with benzene–hexane 1:1 crystallized on evaporation to yield 4.3 g. of product A (IIIa), m.p. 208–212°; the analytical sample showed m.p. 216–218° (prisms from acetone–methanol), $[\alpha]^{20}D$ −14°, $\lambda_{max}$ 5.8 $\mu$ (shoulder at 5.88 $\mu$) (the m.p. of the substance showed no depression in a mixture with a sample obtained by the method of Heusler and Wettstein,[3] and the infrared spectra were identical; this authors report m.p. 213–214.5°, $[\alpha]^{26}D$ −10 ± 4° ($CHCl_3$)).

*Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27; O, 19.30. Found: C, 72.53; H, 8.23; O, 19.15.

The last crystalline fractions eluted with benzene–hexane 1:1, with benzene–hexane 2:1, 3:1, 4:1 and with benzene were combined and recrystallized from acetone–methanol, thus furnishing fluffy needles of product $\beta$ (VIIIb) (39.5 g.), m.p. 168–172°; the analytical sample showed m.p. 173–175° (from acetone–methanol), $[\alpha]^{20}D$ −205°; $\lambda_{max}$ 310 m$\mu$, log $\epsilon$, 4.10; $\lambda_{max}$ 5.85, 6.7, 6.40 $\mu$.

*Anal.* Calcd. for $C_{25}H_{35}O_4N$: C, 72.60; H, 8.53; N, 3.39. Found: C, 72.66; H, 8.58; N, 3.35.

When the reaction was carried out with the acetoxime Ib the same results were obtained.

**17$\beta$-Methyl-18-nor-$\Delta^{5,13}$-isopregnadiene-3$\beta$,16$\alpha$-diol-20-one 20-oxime-3,16-diacetate** (IIIb) was prepared by reaction with hydroxylamine hydrochloride in pyridine and methanol. It showed m.p. 226–228.5° dec., $[\alpha]^{20}D$ −111° (Heusler and Wettstein[3] report m.p. 230–232°).

*Anal.* Calcd. for $C_{25}H_{35}O_5N$: C, 69.90; H, 8.21; N, 3.26; O, 18.62. Found: C, 70.47; H, 8.23; N, 3.53; O, 18.21.

**5,6,13,14-Di-oxido-17$\beta$-methyl-18-nor-isopregnan-3$\beta$,16$\alpha$-diol-20-one 3,16-Diacetate.**—A solution of 1 g. of the diacetate IIIa in 10 ml. of chloroform was mixed with a solution of 800 mg. of monoperphthalic acid in 20 ml. of ether and the mixture was kept overnight at 5° and then for 4 hours at room temperature, washed with 5% sodium carbonate solution and water, dried and evaporated to dryness. The residue crystallized upon the addition of methanol in the form of small plates (490 mg.), m.p. 235–236°; recrystallization from acetone–ether raised the m.p. to 241–242°, $[\alpha]^{20}D$ −64°, $\lambda_{max}$ 5.80 $\mu$ (shoulder at 5.87 $\mu$).

*Anal.* Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.67; O, 25.09. Found: C, 67.59; H, 7.70; O, 25.10.

**17$\beta$-Methyl-18-nor-$\Delta^{5,13}$-isopregnadien-3$\beta$,16$\alpha$-diol-20-one** (IIIc).—A solution of 600 mg. of the diacetate IIIa in 30 ml. of methanol was mixed with 600 mg. of potassium bicarbonate in 10 ml. of water and the mixture was refluxed for 1 hour, diluted with water and extracted with chloroform; the chloroform extract was washed with water and evaporated to dryness in vacuum. Crystallization of the residue from acetone–hexane yielded small prisms (360 mg.), m.p. 176–181°; further recrystallizations from acetone–ether raised the m.p. to 189–191°, $[\alpha]^{20}D$ −123°; $\lambda_{max}$ 2.8, 2.95, 5.9 $\mu$ (reported[3] m.p. 189–191°, $[\alpha]^{20}D$ −131 ± 4° ($CHCl_3$)).

*Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 75.84; H, 9.12.

**17$\beta$-Methyl-18-nor-$\Delta^{5,13}$-isopregnadien-3$\beta$,16$\alpha$,20-triol** (IIId).—One gram of the diacetate IIIa was dissolved in 50 ml. of anhydrous tetrahydrofuran and slowly added to a suspension of 800 mg. of lithium aluminum hydride in 150 ml. of ether and the mixture was refluxed for 1 hour; the excess of hydride was decomposed with a few drops of ethyl acetate, then poured into water and acidified with 10% sulfuric acid; the precipitate was extracted with ethyl acetate, washed with water, concentrated to small volume and crystallized by the addition of ether, thus yielding 640 mg., m.p. 183–185°; the analytical sample was obtained as large needles by crystallization from benzene–hexane, m.p. 202–204°, $[\alpha]$ −207°, $\lambda_{max}$ 2.9–3.0 $\mu$.

*Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.98; H, 9.76.

(12) M. N. Huffman, M. H. Lott and A. Tillotson, *J. Biol. Chem.*, **218**, 567 (1956).

(13) J. Fajkos and F. Sorm, *Chem. Listy*, **50**, 282 (1956).

(14) Melting points are uncorrected. Rotations were determined at 20° in chloroform, unless noted otherwise. The ultraviolet absorption spectra were determined in 95% ethanol solution in a Beckman DK2 spectrophotometer. The infrared spectra were determined in chloroform solution on a Perkin–Elmer double-beam spectrophotometer (unless stated otherwise). The microanalyses were performed by Dr. Franz Pascher, Bonn, Germany.

**The triacetate (IIIe)** (acetic anhydride and pyridine at room temperature overnight) showed m.p. 121–122° (small prisms from hexane), $[\alpha]^{20}D$ −149.5°, $\lambda_{max}$ 5.85 μ.

*Anal.* Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35; O, 20.94. Found: C, 70.83; H, 8.36; O, 21.17.

**Cycloethylene Mercaptol (IIIf).**—A solution of 1 g. of the diacetate IIIa and 1 g. of ethanedithiol in 50 ml. of acetic acid was treated with 1 ml. of a saturated solution of hydrogen bromide in glacial acetic acid and the mixture was kept for 3 hours at room temperature, poured into 300 ml. of cold water and rapidly extracted with ether; the organic layer was washed with 5% sodium hydroxide solution and water, evaporated to dryness and the oily residue crystallized from methanol, thus giving 510 mg. of mercaptol in the form of needles, m.p. 137–140°; the analytical sample showed m.p. 149–151° (from acetone–methanol), $[\alpha]^{20}D$ −108°.

*Anal.* Calcd. for $C_{27}H_{38}O_4S_2$: C, 66.10; H, 7.81; S, 13.04; O, 13.04. Found: C, 65.87; H, 8.07; S, 12.78; O, 13.19.

**17β-Methyl-17α-ethyl-18-nor-$\Delta^{5,13}$-androstadien-3β,16α-diol 3,16-Diacetate (IIIg).**—The mercaptol IIIf (600 mg.) was hydrogenolyzed in 125 ml. of ethanol with 5 g. of Raney nickel[15] by refluxing for 3 hours, the nickel was removed by filtration and the solution evaporated to dryness; crystallization from acetone–methanol afforded small plates (380 mg.), m.p. 118–120°; further recrystallizations from acetone–methanol raised the m.p. to 123–124°, $[\alpha]^{20}D$ −176°, $\lambda_{max}$ 5.85 μ.

*Anal.* Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06; O, 15.98. Found: C, 75.26; H, 9.00; O, 16.19.

**17β-Methyl-17α-ethyl-18-nor-$\Delta^{4,13}$-androstadien-16α-ol-3-one acetate (V).**—The diacetate IIIg (1 g.) in methanol (40 ml.) was saponified with sodium hydroxide (1 g.) in water (10 ml.) by refluxing for 1 hour, the solution was diluted with water and extracted with ether, the ethereal extract was washed with water and evaporated to dryness. The oily residue was dissolved in 40 ml. of toluene and 10 ml. of cyclohexanone, 10 ml. was distilled to eliminate moisture; a solution of 800 mg. of aluminum isopropylate in 10 ml. of toluene then was added and the mixture refluxed for 1 hour, diluted with benzene and washed with 5% hydrochloric acid and water and the organic solvents were removed by steam distillation. The oily residue was extracted with ether, dried over sodium sulfate, evaporated to dryness (890 mg. of residue), dissolved in hexane and chromatographed on washed alumina. The crystalline fractions eluted with hexane were combined and recrystallized from acetone–hexane, m.p. 144–146° (240 mg.). The analytical sample showed m.p. 147–148° (prismatic needles from acetone–hexane), $[\alpha]^{20}D$ −53.6°; $\lambda_{max}$ 238–240 mμ, log ε, 4.17; $\lambda_{max}$ 5.85, 6.05 μ.

*Anal.* Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05; O, 13.46. Found: C, 77.41; H, 9.09; O, 13.73.

**16-Acetyl-17-amino-$\Delta^{5,16}$-androstadien-3β-ol (VIIb).**—A mixture of 4.7 g. of the diacetate VIIa and 60 ml. of methanol was treated with a solution of 4 g. of potassium hydroxide in 10 ml. of water and refluxed for 1 hour; the solution was concentrated to half its volume, diluted with water and the precipitate collected; crystallization from methanol–acetone furnished 2.46 g., m.p. 242–245°, and further recrystallizations from methanol–acetone raised the m.p. to 245–246°, $[\alpha]^{20}D$ −136°; $\lambda_{max}$ 314 mμ, log ε, 4.22; $\lambda_{max}$ 2.80, 2.90, 3.08, 6.20, 6.35 and 6.70 μ.

A methanolic solution of the product gives a blue color with aqueous ferric chloride and gives a bluish-green precipitate with a copper acetate–ammonia solution.

*Anal.* Calcd. for $C_{21}H_{31}O_2N$: C, 76.55; H, 9.48; N, 4.25; O, 9.71. Found: C, 76.39; H, 9.50; N, 4.39; O, 10.05.

Reacetylation with acetic anhydride and pyridine regenerates the diacetate VIIa.

**3β-Hydroxy-16,17-seco-$\Delta^5$-androsten-16,17-dioic Acid 17-Amide (IX).**—A solution of the amino-ketone VIIb (3.38 g.) and potassium hydroxide (4 g.) in methanol (120 ml.) and water (10 ml.) was mixed with 10 ml. of 35% hydrogen peroxide and the solution was refluxed 20 minutes; 10 ml. more of hydrogen peroxide was added and the reflux prolonged for 20 minutes more; the solution was concentrated to a small volume, diluted with water, filtered and acidified with 10% hydrochloric acid solution. The precipitate was collected, washed with water and crystallized from chloroform–methanol, furnishing needles (840 mg.), m.p. 235–239°. The analytical sample showed m.p. 254–256° dec., $[\alpha]^{20}D$ −68° (dioxane); $\lambda_{max}$ (Nujol) 2.95, 5.87, 6.1 μ. (This product showed no depression in mixed m.p. with an authentic sample; Regan[8] reported m.p. 255–257° dec.)

*Anal.* Calcd. for $C_{19}H_{29}O_4$: C, 68.03; H, 8.71; N, 4.18; O, 19.08. Found: C, 68.26; H, 8.79; N, 4.57; O, 19.19.

**16,17-Seco-$\Delta^5$-androsten-3β-ol-16,17-imide Acetate (X).**—A mixture of 300 mg. of the acid IX, 2 ml. of acetic anhydride and 2 ml. of pyridine were heated on the steam-bath for 1 hour, poured into water and the precipitate collected; crystallization from methanol afforded needles (210 mg.), m.p. 260–262°, $[\alpha]^{20}D$ −138°; $\lambda_{max}$ 3.0 (NH), 5.82, 5.9 μ. The mixed m.p. with an authentic specimen[8] showed no depression and the infrared spectra were identical).

*Anal.* Calcd. for $C_{21}H_{29}O_4N$: C, 70.17; H, 8.13; O, 17.80; N, 3.90. Found: C, 70.59; H, 8.19; O, 17.65; N, 3.72.

**Treatment of 17-Acetylamino-$\Delta^{5,16}$-androstadien-3β-ol Acetate (VI) with Acetic Anhydride and Boron Trifluoride Etherate.**—A solution of the acetylamino derivative VI (2.4 g.) in acetic anhydride (20 ml.) was treated with 4 ml. of boron trifluoride etherate and kept for 1 hour at a temperature, below 15°, poured into 200 ml. of cold water and worked up as in the isolation of product **B**. Crystallization from acetone–hexane yielded fluffy needles (1.915 g.), m.p. 170–172°. This product showed no depression in mixed m.p. with the acetyl aminoketone VIIa and the infrared spectra were superimposable.

**16α-Acetyl-17-acetylamino-$\Delta^5$-androsten-3β-ol Acetate (XIa).**—The acetylaminoketone VIIa (1 g.) in 80 ml. of ethyl acetate and 20 ml. of acetic acid was hydrogenated with 100 mg. of Adams catalyst until 1 mole of hydrogen was absorbed (3 hours); the catalyst was removed by filtration and the solution washed with dilute sodium carbonate and water, evaporated to a small volume and crystallized by the addition of ether (prisms (835 mg.), m.p. 226–230°); further crystallizations from methanol–ether raised the m.p. 239–241°, $[\alpha]^{20}D$ −77°; $\lambda_{max}$ 2.95, 5.83, 6.0 μ.

*Anal.* Calcd. for $C_{25}H_{37}O_4N$: C, 72.25; H, 8.98; O, 15.40; N, 3.37. Found: C, 72.40; H, 8.50; O, 15.34; N, 3.25.

**The oxime XId** showed m.p. 298–299° (from chloroform–methanol), $[\alpha]^{20}D$ −81°.

*Anal.* Calcd. for $C_{25}H_{38}O_4N_2$: C, 69.73; H, 8.90; O, 14.86; N, 6.51. Found: C, 69.95; H, 8.86; O, 14.91; N, 6.43.

**16α-Acetyl-17-acetylamino-$\Delta^5$-androsten-3β-ol (XIb).**—The diacetate XIa (600 mg.) was saponified with 30 ml. of methanol and 400 mg. of potassium hydroxide by refluxing for 1 hour. The mixture was diluted with water, extracted with chloroform and the extract washed with water and concentrated. Upon the addition of ether there crystallized the product as plates (450 mg.), m.p. 256–258°. The analytical sample showed m.p. 265–267° (from methanol–ether), $[\alpha]^{20}D$ −91° (dioxane).

*Anal.* Calcd. for $C_{23}H_{35}O_3N$: C, 73.95; H, 9.45; O, 12.85; N, 3.75. Found: C, 74.11; H, 9.42; O, 13.08; N, 3.78.

**16α-Acetyl-17-acetylamino-$\Delta^4$-androsten-3-one (XIV).**—The monoacetate XIb (880 mg.) was dissolved in 50 ml. of toluene and 10 ml. of cyclohexanone, 10 ml. of solvent was distilled to remove moisture and a solution of 400 mg. of aluminum isopropylate in 10 ml. of toluene was added. The mixture was refluxed for 1 hour, then washed with dilute hydrochloric acid and the volatile components were removed by steam distillation. The semi-solid residue was extracted with chloroform, washed with water and evaporated to dryness; crystallization from acetone–ether afforded shiny plates (580 mg.), m.p. 205–207°. The analytical sample showed m.p. 210–212° (from acetone–ether), $[\alpha]^{20}D$ +58°; $\lambda_{max}$ 239–240 mμ, log ε, 4.09; $\lambda_{max}$ 2.95, 5.9 and 6.05 μ.

*Anal.* Calcd. for $C_{23}H_{33}O_3N$: C, 74.36; H, 8.95; O, 12.92; N, 3.77. Found: C, 74.00; H, 8.87; O, 13.45; N, 3.81.

**Benzal Derivative of 16α-Acetyl-17-acetylamino-$\Delta^5$-an-**

(15) Prepared following the method described by R. Mozingo, D. E. Wolfrom, S. A. Harris and K. Folkers, THIS JOURNAL, **65**, 1013 (1943).

**drosten-3$\beta$-ol (XVa).**—Benzaldehyde (0.1 ml.) was added to a solution of the diacetate XIa (200 mg.) and potassium hydroxide (300 mg.) in methanol (10 ml.). The mixture was kept at room temperature overnight, whereupon long needles crystallized which were collected and washed thoroughly with methanol, m.p. 313–315° (210 mg.). The analytical sample showed m.p. 314–316° (from acetic acid–methanol).

*Anal.* Calcd. for $C_{30}H_{39}O_3N$: C, 78.05; H, 8.52; N, 3.03. Found: C, 77.78; H, 8.48; N, 2.83.

**The acetate** showed m.p. 304–306° (small needles from pyridine); $\lambda_{max}$ 290–292 m$\mu$, log $\epsilon$ 4.25.

*Anal.* Calcd. for $C_{32}H_{41}O_4N$: C, 76.31; H, 8.21; N, 2.78. Found: C, 76.63; H, 8.21; N, 2.82.

**Cycloethylene Mercaptol of 16$\alpha$-Acetyl-17-acetylamino-$\Delta^5$-androsten-3$\beta$-ol Acetate (XII).**—The diacetate XIa (1.5 g.) and ethanedithiol (3 ml.) were dissolved in anhydrous dioxane (20 ml ) and freshlv fused and finely powdered zinc chloride (15 g.) and anhydrous sodium sulfate (10 g.) were added. The semi-solid mixture was kept at room temperature overnight, poured into water and extracted with chloroform; the organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. Crystallization of the residue from ethyl acetate–hexane afforded needles (1.62 g.), m.p. 191–193°; further crystallizations raised the m.p. to 194–195° (from acetone–ether), $[\alpha]^{20}D$ −78°.

*Anal.* Calcd. for $C_{27}H_{41}O_3S_2N$: C, 65.96: H, 8.41; O, 9.77; S, 13.01; N, 2.85. Found: C, 65.71; H, 8.24; O, 9.46; S, 12.80; N, 2.73.

**16$\alpha$-Ethyl-17-acetylamino-$\Delta^5$-androsten-3$\beta$-ol Acetate (XIc). By Hydrogenolysis of the Cycloethylene-mercaptol XII.**—To a solution of the mercaptol XII (1.5 g.) in 200 ml. of ethanol there was added 15 g. of Raney nickel and the suspension was refluxed for 3 hours, filtered and the solvent concentrated to small volume. There separated shiny plates, m.p. 183–185°, which upon further crystallizations from acetone–hexane showed m.p. 195–196°, $[\alpha]^{20}D$ −120.3°; $\lambda_{max}$ 2.95, 5.83, 6.03 $\mu$.

*Anal.* Calcd. for $C_{25}H_{39}O_3N$: C, 74.77; H, 9.76; O, 11.95; N, 3.49. Found: C, 74.52; H, 9.71; O, 12.21; N, 3.46.

**16$\alpha$-Ethyl-$\Delta^5$-pregnen-3$\beta$-ol-20-one Acetate (XIIIb).**—From a solution of 3 g. of $\Delta^{5,16}$-pregnadien-3$\beta$-ol-20-one acetate in 100 ml. of benzene there was distilled 20 ml. to remove moisture and a solution of ethylmagnesium iodide (prepared from 12 g. of ethyl iodide) in 25 ml. of ether was added and the mixture refluxed for 3 hours, poured into cold water and acidified with diluted hydrochloric acid. The organic layer was diluted with 100 ml. of ether and washed with water, dried over anhydrous sodium sulfate and evaporated to dryness (weight 3.05 g.). The ketonic and non-ketonic fractions were separated with Girard T reagent. The ketonic fractions weighed 2.2 g. and were acetylated by heating for 1 hour on the steam-bath with 6 ml. of acetic anhydride and 6 ml. of pyridine, poured into water and the semi-solid precipitate was extracted with ether; the ether extract was washed with dilute hydrochloric acid, sodium carbonate solution and water and then evaporated to dryness. Crystallization from methanol furnished shiny plates, m.p. 120–121° (1.88 g.). The analytical sample showed m.p. 123–125°, $[\alpha]^{20}D$ −78° $\lambda_{max}$ 5.85 $\mu$.

*Anal.* Calcd. for $C_{25}H_{38}O_3$: C, 77.67; H, 9.91; O, 12.42. Found: C, 77.32; H, 9.90; O, 12.81.

**The oxime** (prepared by the usual method: hydroxylamine hydrochloride and pyridine) showed m.p. 156–158°, $[\alpha]^{20}D$ −79°.

*Anal.* Calcd. for $C_{25}H_{39}O_3N$: C, 74.77; H, 9.79; O, 11.95; N, 3.49. Found: C, 75.16; H, 9.78; O, 11.71; N, 3.31.

**16$\alpha$-Ethyl-17-acetylamino-$\Delta^5$-androsten-3$\beta$-ol Acetate (XIc). By Beckmann Rearrangement of the Oxime XIIIc.**—Tosyl chloride (2 g.) was added to a solution of the oxime XIIIc (2 g.) in anhydrous pyridine (40 ml.), the mixture was kept at room temperature for 3 hours, poured into 200 ml. of 10% sulfuric acid solution and extracted with ether, washed with water and concentrated to a small volume, thus giving plates (1.36 g.), m.p. 194–196°; further crystallization from acetone raised the m.p. to 196–197°, $[\alpha]$ −120.8° (the mixed m.p. with the product obtained by hydrogenolysis of the mercaptol XII showed no depression and the infrared spectra were identical).

**16-Acetyl-17-benzoylamino-$\Delta^{5,16}$-androstadien-3$\beta$-ol 3-benzoate (VIIc)** (prepared with benzoyl chloride and pyridine, 1 hour on the steam-bath) showed m.p. 279–281° (needles from acetone), $[\alpha]^{20}D$ −44°.

*Anal.* Calcd. for $C_{35}H_{39}O_4N$: C, 78.18; H, 7.31; O, 11.90; N, 2.61. Found: C, 78.75; H, 7.43; O, 11.31; N, 2.51.

**16-Acetyl-17-benzoylamino-$\Delta^{5,16}$-androstadien-3$\beta$-ol (VIId).**—The aminoketone VIb (1 g.) was suspended in a solution of potassium hydroxide (4 g.) in water (40 ml.), benzoyl chloride (2 g.) was added, the flask was stoppered and shaken for 20 minutes. It was then diluted with water and the precipitate collected, washed thoroughly with water and crystallized from acetone–ether, thus furnishing prisms (810 mg.), m.p. 186–192°; further crystallizations from acetone–ether raised the m.p. to 218–220°, $[\alpha]^{20}D$ −51°.

*Anal.* Calcd. for $C_{28}H_{35}O_3N$: C, 77.56; H, 8.14; O, 11.07; N, 3.23. Found: C, 77.38; H, 8.27; O, 10.69; N, 3.19.

**16-Acetyl-17-benzoylamino-$\Delta^{4,16}$-androstadien-3-one (VIIIb).**—The monobenzoate VIId (750 mg.) was dissolved in toluene (50 ml.) and cyclohexanone (10 ml.), a few ml. of the solution was distilled to remove moisture and then a solution of aluminum isopropylate (500 mg.) in toluene (5 ml.) was added. The mixture was refluxed for 1 hour and then the product was isolated as in previous cases. Crystallization from chloroform–ether furnished fluffy needles (520 mg.), m.p. 220–222°. The analytical sample showed m.p. 221–222° (from chloroform–ether), $[\alpha]^{20}D$ +94°; $\lambda_{max}$ 242, 326–330 m$\mu$, log $\epsilon$, 4.47, 4.10; $\lambda_{max}$ 2.95 (weak), 6.03, 6.30, 6.40 $\mu$.

*Anal.* Calcd. for $C_{28}H_{33}O_3N$: C, 77.92; H, 7.71; O, 11.12; N, 3.25. Found· C, 77.87; H, 7.89; O, 11.33; N, 2.69.

**16-Acetyl-17-amino-$\Delta^{4,16}$-androstadien-3-one (VIIIa).**—The benzoate VIIIb (400 mg.) was dissolved in a solution of potassium hydroxide (400 mg.) in methanol (30 ml.) and refluxed for 1 hour, diluted with water and the precipitate extracted with chloroform; the extract was washed with water, evaporated to dryness and the residue crystallized from acetone–ether, yielding prisms (280 mg.), m.p. 123–125°; the analytical sample showed m.p. 123–125°, resolidified and remelts at 161–162° (from acetone–ether). The methanolic solution gives a blue color with ferric chloride; $[\alpha]^{20}D$ −8°; $\lambda_{max}$ 238–240 m$\mu$, log $\epsilon$, 4.22; $\lambda_{max}$ 2.92, 3.08, 6.05, 6.20, 6.35 $\mu$.

*Anal.* Calcd. for $C_{21}H_{29}O_2N$: C, 77.02; H, 8.93; O, 9.77; N, 4.28. Found: C, 76.69; H, 8.77; O, 9.67; N 4.29.

**16-Acetyl-$\Delta^{5,16}$-androstadien-3$\beta$-ol Acetate (XVIb).**—The aminoketone XIa (300 mg.) and *p*-toluenesulfonic acid (40 mg.) were dissolved in acetic anhydride (8 ml.) (1 drop of pyridine was added) the solution was refluxed for 1.5 hours and then poured into cold water. When the acetic anhydride had hydrolyzed, the precipitate was extracted with ether, the organic layer washed with dilute sodium carbonate solution and water. Upon concentration of the extract there crystallized 70 mg. of unreacted product. The mother liquor was evaporated to dryness and the residue crystallized from methanol, thus yielding needles (105 mg.), m.p. 191–195°; further crystallization from acetone–methanol raised the m.p. 201–203°, $[\alpha]^{20}D$ −132°; $\lambda_{max}$ 240–242 m$\mu$, log $\epsilon$, 4.07; $\lambda_{max}$ 5.73, 6.05, 6.3 (weak) $\mu$ [reported[11] m.p. 200–201°, $[\alpha]^{20}D$ −130° ($CHCl_3$); $\lambda_{max}$ 240 m$\mu$; $\epsilon$ 9780.]

*Anal.* Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05; O, 13.46. Found: C, 77.40; H, 8.79; O, 13.82.

**The oxime XVIc** showed m.p. 212–215° dec. (from chloroform–methanol), $[\alpha]^{20}D$ −128°.

*Anal.* Calcd. for $C_{23}H_{33}O_3N$: C, 74.36; H, 8.95; O, 12.92; N, 3.77. Found: C, 73.98; H, 8.99; O, 13.27; N, 4.12.

**16-Acetyl-$\Delta^{5,16}$-androstadien-3$\beta$-ol (XVIa).**—The acetate XVIb (1 g.) in methanol (30 ml.) was mixed with a solution of potassium bicarbonate (1 g.) in water (10 ml.) and refluxed for 1 hour. The solution was concentrated to a small volume, diluted with water and the precipitate was collected. Crystallization from acetone–ether afforded

prisms (540 mg.), m.p. 170–172°; the analytical sample showed m.p. 177–179° (from acetone–ether), $[\alpha]^{20}D$ −115°; $\lambda_{max}$ 240–242 mμ, log ε, 4.16; $\lambda_{max}$ 2.95, 6.05, 6.30 μ (reported[11] m.p. 181–182°, $[\alpha]^{20}D$ −132°).

*Anal.* Calcd. for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62; O, 10.18. Found: C, 79.98; H, 9.53; O, 10.11.

**Δ⁵-Androsten-3β-ol-16-one Acetate (XVIIb).**—The solution of the oxime XVIc (1.3 g.) and tosyl chloride (1.3 g.) in anhydrous pyridine (30 ml.) was treated as in the previous case. Crystallization from pentane furnished needles (510 mg.), m.p. 129–130.5°, and 220 mg. more from the mother liquors, m.p. 121–123°. The analytical sample showed m.p. 129.5–130.5°, $[\alpha]^{20}D$ −222°, $\lambda_{max}$ 5.82 μ. (Huffman, *et al.*,[12] reported m.p. 127.5–128°; Fajkos and Sorm,[13] m.p. 134–135°, $[\alpha]^{20}D$ −238° ($CHCl_3$).)

*Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15; O, 14.53. Found: C, 76.69; H, 9.13; O, 14.28.

**The free alcohol XVIIa** showed m.p. 163–165°, $[\alpha]^{20}D$ −235°; $\lambda_{max}$ 3, 5.78 μ (reported by Huffman, *et al.*,[12] m.p. 163.5–165°, $[\alpha]^{20}D$ −242° in $CHCl_3$; Fajkos and Sorm,[13] m.p. 168–169°, $[\alpha]^{20}D$ −255° in $CHCl_3$).

*Anal.* Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79; O, 11.10. Found: C, 79.42; H, 9.82; O, 11.29.

**Δ⁴-Androstene-3,16-dione (XVIII).**—Oppenauer oxidation of the free alcohol XVIIa (750 mg.) furnished thick prisms (410 mg.), m.p. 152–153° (from acetone–hexane), $[\alpha]^{20}D$ −90°, $\lambda_{max}$ 240 mμ, log ε, 4.22; $\lambda_{max}$ 5.78, 6.05 μ (reported[13] m.p. 152–153°, $[\alpha]^{20}D$ −90.5° in $CHCl_3$).

*Anal.* Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15; O, 11.17. Found: C, 79.48; H, 9.13; O, 11.45.

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[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE]

# Cellulose Ion Exchange and Rotatory Dispersion Studies with the Bacitracin Polypeptides

BY WM. KONIGSBERG AND L. C. CRAIG

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Bacitracin has been shown to undergo a change at a *p*H of 4 or less which has been traced to an epimerization of the terminal isoleucine residue. The resulting stereoisomers showing different antibiotic activity have been separated by cellulose ion exchange chromatography. Anomalous rotatory dispersion behavior has been found which has been shown to be connected with the thiazoline complex. Data have been obtained which suggest an interaction of the thiazoline grouping with a ring peptide linkage.

In spite of the careful work done in a number of laboratories[1-3] on the fractionation of the mixture of polypeptides present in commerical bacitracin, a recent paper[4] has indicated that a very subtle type of heterogeneity still has persisted even in bacitracin A. The heterogeneity was correlated with small shifts in optical activity and was of particular importance to the antibiotic potency.

The sequential formula I proposed independently in two laboratories[5,6] contains a thiazoline ring structure which is unique in naturally occurring peptides. Recently, however, the possibility of such a ring being present[7,8] as one of the energy rich states in certain natural products of considerable biological interest has been raised. No relationship of these substances to bacitracin, other than the possible common occurrence of the thiazoline ring, has been shown. Irrespective of this possibility, a substance with formula I would be expected to undergo transformation easily, as indeed has been found to be the case with bacitracin A. It now appears very likely that a type of tautomeric or resonating system of linkages exists in bacitracin A which has not thus far been clearly elucidated. Chemical procedures such as those involving direct hydrolysis with acid may act by a preliminary rearrangement or splitting as is now known to happen with the thiazoline ring and thus fail to reveal the more subtle aspects of the structure or structures. For example, there is reason to believe[6,9] that the phenylalanine residue is in some way connected to the isoleucine-cysteine residues which form the thiazoline ring system. Yet a rigid structural formula does not permit a covalent bond except by having two nitrogens linked to the carbon which emerges on hydrolysis as the carbonyl of the phenylalanyl residue. Although not accepted ordinarily in peptide chemistry as a valid linkage, unusual configurations such as those found in the ergot peptides[10,11] apparently can stabilize the linkage to the extent that it becomes a significant member of the various resonance and tautomeric forms of the amide linkage.

Often the interpretation of complex and labile structures is greatly assisted by spectroscopic methods. Indeed ultraviolet absorption measurements have been very helpful[9] in supporting the thesis of the thiazoline ring in bacitracin A. Although infrared studies have been made in this Laboratory, clear interpretation has been hindered by the multiplicity of groups present which could contribute to specific bands.

Recently the introduction of the Rudolph spectrophotometric polarimeter has considerably facilitated the use of rotatory dispersion in the study of natural products. Its application to the bacitracin polypeptides together with new absorption

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