to give **5**: 49.5 g (75%), bp 143.5° (1 mm). *Anal.* Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.41; H, 7.76.

Carolic Acid (1).—A solution of 50 g of ethyl ε-benzyloxy-β-ketocaproate in 20 ml of absolute alcohol, 1 ml of carbon tetrachloride, and 100 ml of dry ether was added to 4.7 g of magnesium. After all of the magnesium had dissolved the solvents were removed by distillation under reduced pressure. The residue was dissolved in 60 ml of dry ether and to this solution was added 30 g of acetyllactyl chloride[16] diluted with 30 ml of dry ether. The reaction mixture was set aside overnight and then treated cautiously with 100 ml of 5% sulfuric acid under cooling with an ice–salt mixture. The ether layer was separated and the water layer was washed with 50 ml of ether. The combined ether solution was washed once with dilute sulfuric acid and then with water, dried, and concentrated. The residue was dissolved in 200 ml of 4 *N* sodium hydroxide and set aside for 24 hr. Addition of an excess of 5% sulfuric acid under cooling separated 26 g of an oily substance (**7**) which was extracted with ether. The product could not be purified by either distillation or crystallization and was used directly for the following reaction.

The crude product (**7**, 2.9 g, 0.01 mole) was reduced at atmospheric pressure with 1 g of 10% palladium–charcoal catalyst in 100 ml of methanol containing 0.5 ml of hydrochloric acid at room temperature (about 240 ml of hydrogen was absorbed). After absorption had ceased the solvent was removed and the residue was recrystallized from ethanol to give 1.6 g of **1** as needles, mp 113°. *Anal.* Calcd for $C_9H_{10}O_4$: C, 59.33; H, 5.53. Found: C, 59.32; H, 5.58.

Reduced Product of Carolic Acid Anhydride (19).—Crude **7** (2.9 g, 0.01 mole) was reduced with 1 g of 10% of palladium–charcoal catalyst at atmospheric pressure in 100 ml of methanol at room temperature (about 720 ml of hydrogen was absorbed). After absorption had ceased the solvent was removed and the residue was heated under reduced pressure. The solid product was recrystallized from ether to give 0.8 g of white crystals, mp 54°. *Anal.* Calcd for $C_9H_{12}O_3$: C, 64.27; H, 7.19. Found: C, 64.01 H, 7.45.

α-Acetyl-γ-phenyltetronic acid (13) was prepared in a similar manner from ethyl acetoacetate and acetylmandelyl chloride in 65% yield and was recrystallized from petroleum ether (bp 104.5°). It melted at 104.5°. The infrared spectrum (KBr) of this compound exhibited sharp bands at 1600, 1660, and 1745 cm^{-1}. *Anal.* Calcd for $C_{12}H_{12}O_4$: C, 66.05; H, 4.62. Found: C, 66.09; H, 4.62.

α-Acetyl-γ-methyltetronic acid (14) was prepared similarly from ethyl acetoacetate and α-acetoxypropionyl chloride in 60% yield and was recrystallized from petroleum ether. This compound melted at 55° and exhibited the sharp infrared bands (KBr) at 1625, 1665, and 1755 cm^{-1}. *Anal.* Calcd for $C_7H_8O_4$: C, 53.84; H, 5.16. Found: C, 53.94; H, 5.37.

α-Ethyl-γ-methyltetronic Acid (17).—A solution of 1.56 g (0.01 mole) of **14** in 100 ml of methanol was reduced with 1 g of 10% palladium–charcoal catalyst at atmospheric pressure and room temperature (about 480 ml of hydrogen was absorbed). After absorption had ceased the solvent was removed to give 1.3 g of **17** which was recrystallized from petroleum ether to give white needles, mp 81.5°. The infrared spectrum (KBr) of this compound exhibited sharp bands at 1655 and 1725 cm^{-1}. *Anal.* Calcd for $C_7H_{10}O_3$: C, 59.14; H, 7.09. Found: C, 58.92; H, 7.13.

Reaction of γ-Benzyloxybutyric Acid with Thionyl Chloride.—To a mixture of **2** (19.4 g, 0.1 mole) and 30 ml of petroleum ether was added 23.8 g (0.2 mole) of thionyl chloride at room temperature. After an evolution of gas had ceased, solvent and excess thionyl chloride were distilled under reduced pressure and then the residue was distilled under reduced pressure to yield 8.0 g of benzyl chloride, bp 41° (3.0 mm), and 6.0 g of γ-butyrolactone, bp 56° (3.0 mm). The former was characterized by its isothiourea hydrochloride, mp 148°, and the latter was characterized by its infrared sharp bands at 1770 cm^{-1} (liquid film). *Anal.* Calcd for C_7H_7Cl: C, 66.42; H, 5.57. Found: C, 66.30; H, 5.50. Calcd for $C_4H_6O_2$: C, 55.80; H, 7.03. Found: C, 55.91; H, 6.95.

Registry No.—**1**, 485-40-5; **2**, 10385-30-5; **4**, 10385-31-6; **5**, 10378-08-2; γ-butyrolactone, 96-48-0; benzyl chloride, 100-44-7; isothiourea hydrochloride, 538-28-3; **13**, 10385-32-7; **14**, 10385-33-8; **17**, 10385-34-9; **19**, 10385-35-0.

(16) R. Anschütz and W. Bertram, *Ber.*, **36**, 468 (1903).

Synthesis of Epimeric 3-Ureido-Δ⁴-Androsten-17-ones[1]

HEINZ KAUFMANN[2] AND DAVID K. FUKUSHIMA

Institute for Steroid Research, Montefiore Hospital and Medical Center, New York, New York 10467

Received January 10, 1967

3β-Ureido-Δ⁴-androsten-17-one was prepared from 3-oximino-Δ⁴-androsten-17-one ethylene ketal by zinc reduction to 3β-amino-Δ⁴-androsten-17-one followed by carbamylation of the amine with silicon tetraisocyanate or nitrourea. 3α-Ureido-Δ⁴-androsten-17-one was formed stereoselectively from 3-hydroxy-Δ⁴-androsten-17-one ethylene ketal and urea. The corresponding 17β-hydroxy derivatives were prepared. The orientation of the ureido group was proved by chemical and physical methods.

Recently a nitrogen-containing steroid was isolated following administration of 11β-hydroxy-Δ⁴-androstene-3,17-dione to man.[3] The compound, ureasterone (I), was characterized as 3α-ureido-11β-hydroxy-Δ⁴-androsten-17-one. It was subsequently demonstrated that ureasterone could be readily synthesized[4] stereoselectively from both 3α,11β- and 3β,11β-dihydroxy-Δ⁴-androsten-17-one and urea in aqueous acetic acid solution at 50°. Since the α orientation of the ureido group in ureasterone (I) was assigned principally from nmr studies, a stereospecific synthesis of 3β-ureido-Δ⁴-androsten-17-one (IIa) was undertaken to compare it with the product formed from the 3-hydroxy Δ⁴-steroids and urea. This paper reports the synthesis and reactions of the epimeric 3-ureido Δ⁴-steroids.

In 1956, Joska and Šorm[5] described the reduction of testosterone oxime with zinc dust in ethanolic acetic acid to 3-amino-Δ⁴-androsten-17β-ol (IIIa), but they did not assign the orientation of the 3-amino group. In the present study this reduction was repeated and the product was shown to be the 3β-amino epimer from its nmr spectrum. This stereoselective method was therefore employed for the synthesis of 3β-amino-Δ⁴-androsten-17-one (VIa) which was carbamylated to yield the desired 3β-ureido-Δ⁴-androsten-17-one (IIa). In order to retain the 17-ketone in the synthesis, this group was protected by ketalization in the starting material, Δ⁴-androstene-3,17-dione 17-ethylene ketal

(1) This work was supported by a research grant from the American Cancer Society and by a grant (CA 07304) from the National Cancer Institute, National Institutes of Health.

(2) Visiting Scientist, 1965–1966.

(3) S. Noguchi and D. K. Fukushima, *J. Biol. Chem.*, **241**, 761 (1966).

(4) D. K. Fukushima, S. Noguchi, H. L. Bradlow, B. Zumoff, K. Kozuma, L. Hellman, and T. F. Gallagher, *ibid.*, **241**, 5336 (1966).

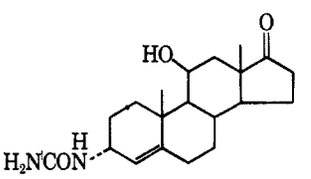
(5) J. Joska and F. Šorm, *Collection Czech. Chem. Commun.*, **21**, 754 (1956).

(IV). Conversion of IV to 3-oximino-Δ^4-androsten-17-one 17-ethylene ketal (V) yielded a 1:1 mixture of two compounds, presumably the *syn* and *anti* oximes. In trial runs of the zinc-reduction step the same results were obtained whether the 1:1 mixture or samples containing predominantly one of the isomeric compounds were used. Therefore the isomeric mixture of ketal V was reduced with zinc dust in ethanolic acetic acid solution to give the desired 3β-amino-Δ^4-androsten-17-one (VIa). The protective ethylene ketal group was also removed under the reduction conditions employed. The amino compound VIa was isolated from the crude reduction mixture by precipitation as the ether-insoluble acetate salt VIb. The free amino compound was readily recovered by ether extraction of an alkaline aqueous solution of the salt. The free amine VI was an oil and apparently was not very stable, turning dark on standing. Although the acetate salt VIb was stable it could not be readily purified and the acetamido derivative VIc was therefore prepared for characterization of the 3β-amino steroid (VIa). Nmr studies confirmed the formation of the 3β-amino group in the zinc reduction of the oxime. The vinylic proton at C-4 appeared as a narrow multiplet centered at δ 5.27 in VIa and 5.23 in VIc. Additional evidence for the β orientation is reported below for the ureido derivatives.

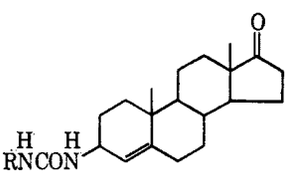
It was important to use mild conditions for the formation of the ureido group from the unsaturated amine in order to avoid elimination reactions leading to dienes. An appropriate reaction between amines and silicon tetraisocyanate was recently reported by Neville and MacGee.[6] The method was successfully applied to the synthesis of 3β-ureido-Δ^4-androsten-17-one (IIa) with some modification. The yield was about 30% with substantial formation of a less polar by-product in contrast to the simpler amine which yielded the corresponding ureide in almost quantitative yields. The by-product did not stain with *p*-dimethylaminobenzaldehyde (absence of $CONH_2$),[7] but gave a pink spot with 77% H_2SO_4 (typical for 3-substituted Δ^4-steroids) on thin layer chromatography. Further characterization of this by-product will be reported at a later time.

In the synthesis of saturated steroid 3-ureides the carbamylation of the steroid amine was achieved with nitrourea in boiling ethanol–water (1:1).[8,9] Reaction of the acetate salt of 3β-amino-Δ^4-androsten-17β-one (VIb) with this reagent gave a 50% yield of the ureide IIa. In this case no by-product was formed, but as the conditions were more vigorous than in the silicon tetraisocyanate method, some cleavage of IIa took place leading to $\Delta^{3,5}$-diene and free urea. This method however proved to be more advantageous than the silicon tetraisocyanate method since the acetate salt VIb could be used as starting material instead of the free amine VIa.

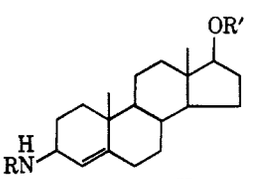
The unsaturated 3β-ureide (IIa) as well as the N-acetyl derivative (IIb) exhibited a narrow multiplet centered at δ 5.25 and 5.28, respectively, for the vinylic proton at C-4 with the proton at C-3 in the axial α orientation. Further evidence for the orientation of the 3β-ureido group was obtained by catalytic hydrogenation of 3β-ureido-Δ^4-androsten-17-one (IIa) in acetic acid with Adams catalyst. A mixture of epimeric 3β-ureidoandrostan-17β-ols was obtained with the 5β epimer being predominant. The stereoselective synthesis of the saturated 3β-ureido compounds which were used for comparison purposes was reported previously.[9] Sodium borohydride reduction of 3β-ureido-Δ^4-androsten-17-one (IIa) afforded 3β-ureido-Δ^4-androsten-17β-ol (IIIc). The ureide IIIc was identical with that prepared by the carbamylation of the unsaturated amine (IIIa). This series of reactions confirms the formation of the 3β-amino epimer upon reduction of testosterone oxime with zinc dust in ethanol–acetic acid.[5]



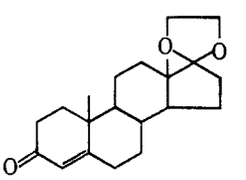
I



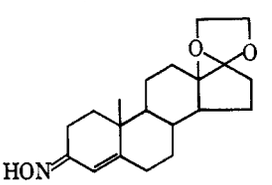
IIa, R=H
b, R=$COCH_3$



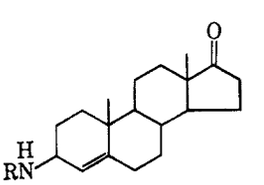
IIIa, R=R'=H
b, R=R'=$COCH_3$
c, R=$CONH_2$; R'=H



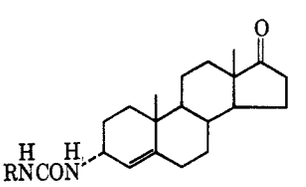
IV



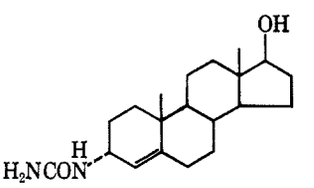
V



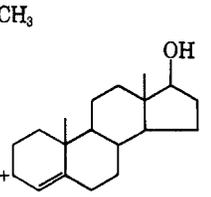
VIa, R=H
b, R=$H_2^+ \cdot CH_3COO^-$
c, R=$COCH_3$



VIIa, R=H
b, R=$COCH_3$



VIII



IX

The epimeric 3α-ureido-Δ^4-17-keto steroid (VIIa) was prepared by the method employed in the synthesis of ureasterone. Δ^4-Androstene-3,17-dione 17-ethylene ketal (IV) was reduced with sodium borohydride to a mixture of 3α- and 3β-hydroxy Δ^4-steroids. The crude reduction mixture was treated with aqueous acetic acid and urea at 50°. During the acid treatment the

(6) R. G. Neville and J. J. MacGee, *Can. J. Chem.*, **41**, 2123 (1963).
(7) G. B. Marini-Bettolo and G. Trabacchi, *Biochem. Biophys. Acta*, **21**, 258 (1956).
(8) T. L. Davis and K. Blanchard, *J. Am. Chem. Soc.*, **51**, 1790 (1929).
(9) A. Yagi, J. Liang, and D. K. Fukushima, *J. Org. Chem.*, **32**, 713 (1967).

protective ethylene ketal group was removed and 3α-ureido-Δ⁴-androsten-17-one (VIIa) was obtained directly. Nmr studies of the 3α-ureido steroid and its N-acetyl derivative showed the vinylic proton at C-4 as an ill-defined doublet centered at δ 5.38 (J = 4 cps) for VIIa and 5.44 (J = 5 cps) for VIIb, indicative of the presence of the 3β-equatorial proton. Further evidence for the 3α orientation of the ureido group in VII was achieved by catalytic hydrogenation of VIIa with palladium on charcoal in ethanol and with Adams catalyst. With the former catalyst the reduction products were the C-5 epimers of 3α-ureidoandrostan-17-one, whereas with Adams catalyst the corresponding 17β-hydroxy derivatives were obtained. The C-5 epimers could not be readily separated but the mixtures were identified by comparison of the infrared spectra of authentic samples of the saturated 3α-ureido steroids prepared by another route.[9]

3α-Ureido-Δ⁴-androsten-17-one (VIIa) could more readily be prepared from testosterone. Sodium borohydride reduction to Δ⁴-androstene-3α- and -3β,17β-diols and reaction with urea in aqueous acetic acid afforded 3α-ureido-Δ⁴-androsten-17β-ol (VIII). The stability of the allylic ureido group to oxidation was demonstrated by the conversion of VIII to the 17-keto steroid VIIa with chromic acid in pyridine or in acetic acid.

Although it has been shown that 3-hydroxy Δ⁴-steroids give rise to a 1:1 equilibrium mixture of C-3 epimeric alcohols in aqueous acid catalyzed reaction[10] and similarly to a mixture of methoxy epimers in methanol,[11] the reaction with urea is stereoselective for the 3α epimer. The mixtures of the alcohols and ethers arise from the reversible reaction with the intermediate carbonium ion IX. The ureido steroids are formed by the axial (3α) attack by urea on the carbonium ion at C-3 but the products are stable and do not re-form the carbonium ion intermediate under the conditions studied. The product of reaction with urea is therefore under kinetic control.

The results of the present study confirms the assignment of the structure, 3α-ureido-11β-hydroxy-Δ⁴-androsten-17-one (I), to ureasterone.

Experimental Section[12]

3-Oximino-Δ⁴-androsten-17-one 17-Ethylene Ketal (V).—A solution of 5 g of hydroxylamine hydrochloride and 12 g of sodium acetate in 50 ml of water was added to a solution of 5.11 g of Δ⁴-androstene-3,17-dione 17-ethylene ketal (IV, mp 145–146°) in 500 ml of methanol. The mixture was refluxed for 2 hr, diluted with water, and extracted with ether. The extract was washed with 5% sodium bicarbonate solution and then with water until neutral. After drying over magnesium sulfate, filtering, and evaporating the solvent, 5.0 g of crude oxime was obtained. This material showed two spots, R_f 0.18 and 0.32, when chromatographed on thin layer of silica gel GF with ethyl acetate–cyclohexane (1:1). Further purification of each isomer by thin layer chromatography again yielded a mixture of the same two compounds. The mixture of V crystallized from ethanol, mp 220–223°. It was found that this mixture, presumably the *syn* and *anti* oximes, could be used without further purification for the next step.

Anal. Calcd for $C_{21}H_{31}NO_3$: C, 73.01; H, 9.05; N, 4.06. Found: C, 73.03; H, 9.04; N, 4.02.

3β-Amino-Δ⁴-androsten-17-one (VIa) and Acetate Salt VIb.—To a hot solution of 7.6 g of oxime V in 350 ml of ethanol and 700 ml of glacial acetic acid was added 140 g of zinc dust with vigorous stirring.[5] The mixture was refluxed for 2 hr. The cooled reaction mixture was filtered and the precipitate was washed thoroughly with ethanol. The filtrate was evaporated almost to dryness, neutralized with 10% sodium hydroxide solution, and extracted with ether. The extract was washed with water, dried over magnesium sulfate, and filtered, and the solvent was evaporated. The residue was dissolved in 75 ml of ether and about 20 drops of glacial acetic acid was added to give 3.4 g of 3β-amino-Δ⁴-androsten-17-one acetate salt (VIb). The compound did not melt but decomposed slowly above 140°: $[\alpha]^{28}D$ + 98.3° (ethanol); R_f 0.5 on tlc with upper layer of the mixture of *n*-butyl alcohol–acetic acid–water (4:1:5); ν_{max}^{KBr} 2220, 1738, 1640, 1550 (br), 1050, 1014, 917, 815, and 652 cm⁻¹.

The **acetate salt VIb** (2.0 g) was shaken with 10% sodium hydroxide solution and ether in a separatory funnel. The ether layer was washed with water until neutral and dried over magnesium sulfate, and the solvent was evaporated to give 1.57 g of yellow oil which could not be crystallized. 3β-Amino-Δ⁴-androsten-17-one (VIa) turned dark brown on standing and therefore should be used for further reaction as soon as possible: ν_{max}^{KBr} 3455, 3350, 3275, 1745, 1587, 1053, 1009, and 670 cm⁻¹; δ 0.90 (s, 18-CH_3), 1.08 (s, 19-CH_3), 3.33 (m, 3α-H), and 5.27 (nm, C-4 vinylic H).

3β-Acetamido-Δ⁴-androsten-17-one (VIc).—The acetate salt VIb (200 mg) was converted to the free amino compound as described above and acetylated with pyridine and acetic anhydride to give 160 mg of crude acetamido VIc. Several recrystallizations from benzene yielded 45 mg of pure 3β-acetamido-Δ⁴-androsten-17-one (VIc): mp 189–192°; $[\alpha]^{23}D$ +68.2°; ν_{max}^{KB} 3290, 3078, 1743, 1645, 1558, 1117, 1058, 1005, and 604 cm⁻¹; δ 0.90 (s, 18-CH_3), 1.07 (s, 19-CH_3), 1.97 (s, $NCOCH_3$), 4.42 (m, 3α-H), and 5.18 (nm, C-4 vinylic H).

Anal. Calcd for $C_{21}H_{31}NO_2$: C, 76.55; H, 9.49. Found: C, 76.44; H, 9.70.

3β-Ureido-Δ⁴-androsten-17-one (IIa). A. With Silicon Tetraisocyanate.—A solution of 1.5 g of the free amine VIa in 30 ml of anhydrous benzene was added to 0.75 ml of silicon tetraisocyanate and 50 ml of anhydrous benzene with stirring. After 2 hr ethyl acetate–methanol (9:1) and water were added and the mixture was filtered through a layer of Celite. The filtrate was washed with water and dried over sodium sulfate, and the solvent was evaporated to give 2.2 g of yellow foam. This material consisted of the desired ureido steroid IIa and an unknown by-product in a ratio of 2:1 according to tlc in ethyl acetate–methanol (9:1), R_f 0.27 and 0.47, respectively. The unsaturated ureido steroid IIa stains bluish pink with 77% sulfuric acid, a bright yellow with *p*-dimethylaminobenzaldehyde, and purple with Zimmermann reagent. The mixture was separated in two portions on a column (1.4 m high and 2.5 cm i.d.) filled with 400 g of silica gel; the solvent system was ethyl acetate–methanol (9:1). Fractions of 30 ml were collected and the dropping rate was adjusted to 45 ml/hr. From the two runs 390 mg of the by-product was obtained in fractions 30–35 and 865 mg of ureido steroid IIa was obtained in fractions 50–65. The by-product was recrystallized from methanol yielding 267 mg of pure crystals, mp 206–208°; it did not stain with *p*-dimethylaminobenzaldehyde but gave a pink color with 77% sulfuric acid. The ureido steroid IIa was recrystallized from methanol–ether to give 550 mg of 3β-ureido-Δ⁴-androsten-17-one: mp 123–127°; $[\alpha]^{28}D$ +80°; ν_{max}^{KBr} 3470 (br), 3360 (br), 3210 (br), 1740, 1680–1615 (br), 1600, 1545 (br), 1123, 1056, and 1012 cm⁻¹; δ 0.88 (s, 18-CH_3), 1.05 (s, 19-CH_3), 4.16 (m, 3α-H), and 5.25 (nm, C-4 vinylic-H).

Anal. Calcd for $C_{20}H_{30}N_2O_2 \cdot 0.5H_2O$: C, 70.76; H, 9.21; N, 8.25. Found: C, 70.76; H, 9.15; N, 8.21.

B. With Nitrourea.—A solution of 100 mg of nitrourea in 15 ml of water was added to a solution of 250 mg of acetate salt of the Δ⁴-3β-amine in 15 ml of ethanol. The mixture was refluxed for 75 min, cooled, and extracted with ethyl acetate. The extract was washed with 5% sodium carbonate solution and water and dried over sodium sulfate, and the solvent was evaporated to give 239 mg of crude product. Chromatography on 25

(10) D. K. Fukushima, S. Dobriner, and H. L. Bradlow, *Biochemistry*, **5**, 178 (1966).

(11) W. R. Nes and U. H. Kim, *Steroids*, **1**, 594 (1963).

(12) All melting points were determined on a micro hot stage and were corrected. Optical rotations were determined in chloroform unless otherwise stated. Nmr spectra were determined in deuteriochloroform on a Varian A-60 spectrometer with tetramethylsilane as internal reference. The chemical shifts are given in δ = ppm; s = singlet, t = triplet, m = multiplet, brm = broad multiplet, and nm = narrow multiplet. Infrared spectra were determined on a Beckman IR-9 spectrophotometer; s = shoulder, br = broad.

g of silica gel and elution with ethyl acetate–ethanol (95:5) afforded 115 mg of 3β-ureido-Δ^4-androsten-17-one (IIa).

3β-(N-acetylureido)-Δ^4-androsten-17-one (IIb).—3β-Ureido-Δ^4-androsten-17-one (IIa, 75 mg) was acetylated with 2 ml of pyridine and 2 ml of acetic anhydride at 38° for 3 days. Two recrystallizations from methanol yielded 55 mg of 3β-(N-acetylureido)-Δ^4-androsten-17-one (IIb): mp 258–260°; $[\alpha]^{28}D$ +67.1°; ν_{max}^{KBr} 3280, 3240, 3115, 1742, 1700 (doublet), 1550, 1250, 1060, and 1015 cm^{-1}; δ 0.88 (s, 18-CH$_3$), 1.07 (s, 19-CH$_3$), 2.12 (s, NCOCH$_3$), 4.30 (m, 3α-H), and 5.25 (nm, C-4 vinylic H).

Anal. Calcd for $C_{22}H_{32}N_2O_3$: C, 70.93; H, 8.66; N, 7.52. Found: C, 71.12; H, 8.74; N, 7.61.

3β-Ureido-Δ^4-androsten-17β-ol (IIc). A.—A solution of testosterone oxime (12 g) in 500 ml of ethanol and 1 l. of acetic acid was reduced[5] with 200 g of zinc dust in the same manner as the 3-oximino 17-ethylene ketal (V). The acetate salt of 3β-amino-Δ^4-androsten-17β-ol was obtained: 5.4 g, mp 188–189° (lit.[5] mp 186–190°). This salt (660 mg) was shaken with 5% sodium hydroxide solution and ether. The ether solution was washed with water and dried, and the solvent was evaporated. 3β-Amino-Δ^4-androsten-17β-ol (IIIa) gave the following signals in the nmr spectrum: δ 0.78 (s, 18-CH$_3$), 1.07 (s, 19-CH$_3$), 3.66 (t, J = 9 cps, 17α-H), and 5.23 (brs, C-4 vinylic H). The amine was acetylated with acetic anhydride and pyridine. Recrystallization from methanol afforded 465 mg of 3β-acetamido-Δ^4-androsten-17β-ol acetate (IIIb): mp 236–237°; $[\alpha]^{23}D$ −1.8°; ν_{max}^{KBr} 3300, 3070, 1735, 1640, 1550, and 1245 cm^{-1}; δ 0.82 (s, 18-CH$_3$), 1.03 (s, 19-CH$_3$), 1.97 (s, OCOCH$_3$), 2.04 (s, NCOCH$_3$), 4.55 (m, 3α-H, 17α-H), and 5.18 (nm, C-4 vinylic H); R_f 0.19, ethyl acetate–cyclohexane (7:3).

Anal. Calcd for $C_{23}H_{35}NO_3$: C, 73.95; H, 9.44; N, 3.74. Found: C, 74.09; H, 9.26; N, 3.66.

A solution of 3.5 g of the 3β-amine acetate salt and 1.3 g of nitrourea in 1 l. of ethanol and 280 ml of water was refluxed for 100 min. The reaction was worked up as described for the ureido steroid IIa. Chromatography on silica gel and elution with ethyl acetate–ethanol (9:1) afforded 1.32 g of 3β-ureido-Δ^4-androsten-17β-ol (IIIc). Recrystallization from methanol–ethyl acetate yielded the ureido alcohol (IIIc): mp 233–242 dec; $[\alpha]^{25}D$ −10° (ethanol); ν_{max}^{KBr} 3492, 3360, 3316, 1670, 1605, and 1545 cm^{-1}.

Anal. Calcd for $C_{20}H_{32}N_2O_2$: C, 72.25; H, 9.70; N, 8.43. Found: C, 72.70; H, 9.83; N, 8.27.

B.—A solution of 270 mg of 3β-ureido-Δ^4-androsten-17-one (IIa) and 75 mg of sodium borohydride in 75 ml of 80% ethanol was stored at room temperature for 1 hr. The mixture was extracted with chloroform, washed with water, and dried over sodium sulfate. Removal of the solvent yielded 249 mg of yellow product which on chromatography on silica gel yielded 189 mg of 3β-ureido-Δ^4-androsten-17β-ol (IIIc). Recrystallization from methanol–ethyl acetate yielded IIIc, mp 241–243° dec. The infrared spectrum was identical with that obtained above.

Catalytic Reduction of 3β-Ureido-Δ^4-androsten-17β-ol.—A suspension of 90 mg of 3β-ureido-Δ^4-androsten-17β-ol (IIIc) and 20 mg of Adams catalyst in 25 ml of acetic acid was shaken with hydrogen at atmospheric pressure and room temperature for 2 days. After chromatography on silica gel, 67 mg of product was obtained. Recrystallization from ethanol yielded 20 mg of 3β-ureido-5α-androstan-17β-ol uncontaminated with the 5β epimer as judged by infrared spectrometery. The mother liquor (47 mg) was predominantly 3β-ureido-5β-androstan-17β-ol as judged from its infrared spectrum and from the C-19 methyl proton signals in the nmr spectrum.[9]

3α-Ureido-Δ^4-androsten-17-one (VIIa).—A solution of 4.55 g of Δ^4-androsten-3,17-dione 17-ethylene ketal (IV) and 1.35 g of sodium borohydride in 130 ml of methanol was stirred at room temperature for 3 hr. Examination by thin layer chromatography of the product demonstrated almost complete reduction of the Δ^4-3-keto group and predominant formation of Δ^4-3β-hydroxy epimer. Without further purification the reduction products, 3α- and 3β-hydroxy-Δ^4-androsten-17-one 17-ethylene ketals were dissolved in 200 ml of dioxane and added to a solution of 200 g of urea in 2 l. of water and 400 ml of acetic acid. The mixture was stirred at 50° for 2 hr. The mixture was cooled and extracted with ethyl acetate which was washed with 10% sodium hydroxide solution and water and dried, and the solvent was evaporated to give 5.2 g of residue. Recrystallization from methanol afforded 1.80 g of 3α-ureido-Δ^4-androsten-17-one (VIIa), mp 205–211°. Chromatography of the mother liquor on silica gel and elution with ethyl acetate–ethanol (9:1) afforded 1.41 g of VIIa. Crystallization from methanol yielded an additional 0.77 g of pure VIIa. The analytical sample of 3α-ureido-Δ^4-androsten-17-one melted at 213–215°: $[\alpha]^{25}D$ +238°; ν_{max}^{KBr} 3450, 3378, 1728, 1690, 1655, 1600, 1585, 1515, 1055, 1014, and 853 cm^{-1}; δ 0.90 (s, 18-CH$_3$), 1.00 (s, 19-CH$_3$), 4.12 (m, 3β-H), and 5.38 (ill-defined doublet, J = 4 cps, C-4 vinylic H).

Anal. Calcd for $C_{20}H_{30}N_2O_2$: C, 72.69; H, 9.15; N, 8.48. Found: C, 72.48; H, 9.28; N, 8.26.

3α-(N-Acetylureido)-Δ^4-androsten-17-one (VIIb).—3α-Ureido-Δ^4-androsten-17-one (VIIa, 500 mg) was acetylated with 20 ml of pyridine and 20 ml of acetic anhydride at 37° for 5 days. The product (709 mg) contained some starting material, the desired N-acetyl derivative VIIb, and two minor impurities as judged by thin layer chromatography. Separation was achieved by using a column [550 g of silica gel; column 4 × 75 cm; solvent system ethyl acetate–cyclohexane (7:3); dropping rate 40 drops/min and 30-ml fraction]. Fractions 88–138 contained 539 mg of the N-acetyl derivative VIIb. Crystallization from methanol gave 376 mg of 3α-(N-acetylureido)-Δ^4-androsten-17-one (VIIb): mp 208–211°; $[\alpha]^{25}D$ +228°; ν_{max}^{KBr} 3280, 3238, 3118, 1740, 1693, 1542, 1232, 1007, 850, 710, 690, 670, and 595 cm^{-1}; δ 0.90 (s, 18-CH$_3$), 1.05 (s, 19-CH$_3$), 2.13 (s, NCOCH$_3$), 4.30 (nm, 3β-H), and 5.44 (ill-defined doublet, J = 5 cps, C-4 vinylic H).

Anal. Calcd for $C_{22}H_{32}N_2O_3$: C, 70.93; H, 8.66; N, 7.52. Found: C, 70.97; H, 8.63; N, 7.49.

3α-Ureido-Δ^4-androsten-17β-ol (VIII).—A solution of 500 mg of testosterone and 200 mg of sodium borohydride in 16 ml of methanol was kept at room temperature for 1 hr. The reduction product, predominantly the 3β epimer of Δ^4-androstene-3α- and -3β,17β-diol, was dissolved in 20 ml of dioxane and added to a solution of 20 g of urea in 200 ml of water and 40 ml of acetic acid. The mixture was kept at 50° for 24 hr, and then extracted with ethyl acetate. The organic layer was washed with sodium hydroxide solution and water and dried, and the solvent was evaporated. Crystallization of the product from ethyl acetate–methanol yielded 430 mg of 3α-ureido-Δ^4-androsten-17β-ol (VIII), mp 219–227°. The analytical sample melted at 224–234°: $[\alpha]^{24}D$ +158° (ethanol); ν_{max}^{KBr} 3492, 3400, 3340, 1668, 1638 (s), 1590 (s), and 1545 cm^{-1}.

Anal. Calcd for $C_{20}H_{32}N_2O_2$: C, 72.25; H, 9.70; N, 8.43. Found: C, 72.47; H, 9.62; N, 8.30.

A solution of 60 mg of chromic acid in 6 ml of pyridine was added to a solution of 30 mg of 3α-ureido-Δ^4-androsten-17β-ol in 1 ml of pyridine. The mixture was stored at room temperature for 40 hr. Recrystallization of the oxidation product from ethyl acetate–methanol gave 16 mg of 3α-ureido-Δ^4-androsten-17-one (VIIa), mp 207–213°. The infrared spectrum was identical with that of the 17-ketone VIIa prepared above.

Oxidation of VIII could also be carried out with 2% chromic acid in 90% acetic acid; 30 mg of VIII afforded 20 mg of the 17-ketone VIIa.

Catalytic Reduction of 3α-Ureido-Δ^4-androsten-17-one (VIIa). A. With Adams Catalyst.—A suspension of 50 mg of 3α-ureido-Δ^4-androsten-17β-one (VIIa) and 18 mg of Adams catalyst in 25 ml of acetic acid was shaken with hydrogen at atmospheric pressure and room temperature for 24 hr. The reduction product was triturated with hot acetone and methanol to give 12 mg of 3α-ureido-5β-androstan-17β-ol, mp 237–246° (lit. mp 225–243°). The infrared spectrum was identical with that of the authentic sample. The mother liquor was triturated with ethyl acetate–methanol to give 10 mg of a mixture of the C-5 epimers of 3α-ureidoandrostan-17β-ol. Acetylation of this mother liquor and thin layer chromatography in ethyl acetate afforded 10 mg of 3α-(N-acetylureido)-5β-androstan-17β-ol acetate[9] as judged by infrared spectrometry.

B. With Palladium.—A suspension of 300 mg of 3α-ureido-Δ^4-androsten-17-one (VIIa) and 100 mg of palladium on charcoal in 75 ml of ethanol was shaken with hydrogen at atmospheric pressure and room temperature for 22 hr. The reduction product was chromatographed on 25 g of silica gel containing 10 ml of ethanol. Elution with 5% ethanol in methylene chloride afforded 266 mg of a mixture of 3α-ureido-5β-androstan-17-one and its 5α epimer as judged by infrared spectrometry.[9] Elution with 10% ethanol in methylene chloride yielded 18 mg of 3α-ureido-5β-androstan-17β-ol which was only slightly contaminated with the 5α epimer.[9]

Registry No.—IIa, 10561-79-2; IIb, 10498-61-0; IIIa, 10498-62-1; IIIb, 10588-73-5; IIIc, 10498-63-2; *syn* V, 10498-64-3; *anti* V, 10498-65-4; VIa, 10498-

66-5; VIb, 10498-67-6; VIc, 10498-68-7; VIIa, 10498-69-8; VIIb, 10498-70-1; VIII, 10498-71-2.

Acknowledgment.—We wish to acknowledge the interest and support of Dr. T. F. Gallagher throughout this investigation. We are grateful to Mrs. Beatrice S. Gallagher for the determination and interpretation of the infrared spectra, to Mrs. Julia Liang for the nmr spectra, and to Millie Smulowitz for invaluable technical assistance.

Stereochemistry and Kinetic Isotope Effects in the Formolytic Rearrangement, Substitution, and Elimination Reactions of Androsterone *p*-Toluenesulfonate[1]

JUDITH RAMSEYER AND H. HIRSCHMANN

Department of Medicine, Western Reserve University and the University Hospitals, Cleveland, Ohio

Received December 8, 1966

The formolysis of 3α-*p*-toluenesulfonoxy-5α-androstan-17-one gave mainly 5α-androst-2-en-17-one and lesser amounts of the Δ^3 isomer and of the formates of 3β-, 2β-, 3α-, and (in traces) 2α-hydroxy-5α-androstan-17-one. The four formates are listed in the declining order of their yields after correction is made for the slow addition of formic acid to the olefin which gave mainly the 3α and 2β, some of the 2α, and traces of the 3β isomers. The rearrangement of the 3α-tosylate to the 2β-formate proceeds in part (about 20%) *via* the 2α-tosylate which was isolated after an incomplete reaction. The formolysis of the latter was 0.35 times as fast as that of androsterone tosylate and gave, beside olefins, the 2β-formate. The 2β-tritiated androsterone tosylate lost less tritium on formolysis (6%) than the 2α-tritiated analog (23%). This indicates that the elimination reaction of the (axial) 3α-tosylate was predominantly *cis*. Its rearrangement to the 2β-formate, however, proceeds mainly by the migration of the 2β-hydrogen. This unusual steric result, retention of configuration at C-2, is attributed to the shielding effect of the departing tosylate ion, since such a mechanism would also accommodate the lesser pathway to the 2β-formate *via* the 2α-tosylate. Kinetic isotope effects were calculated for the formation of the main reaction products. When the starting tosylate was axially tritiated at C-2, the 3β-formate showed an isotope effect, k_H/k_T, that was significantly lower than that for the disappearance of the tosylate or those for the formation of the 2β- and 3α-formates and of the mixture of olefins. The implications of these results are compared with previously suggested schemes for the solvolysis of *cis*-4-*t*-butylcyclohexyl tosylate. In the additions of formic and of trifluoroacetic acid to the Δ^2-olefin, the entry of the nucleophilic agent was predominantly from the α side and thereby differed from the course of other ionic addition reactions of 5α-steroidal Δ^2-olefins.

After the initial work of Stoll[2] in 1932, solvolytic reactions of 3-tosyloxy steroids were investigated many times. In the absence of the Δ^5 double bond and of vicinal substituents, the course of the substitution reaction was found to be uniform and resulted in a product of inverted configuration at C-3. This was accompanied by varying amounts of olefin. This course was observed, for example, with equatorial tosylates in acetic acid containing acetate ion,[3] and with both axial and equatorial tosylates in pyridine,[4] piperidine,[4] and alcohols.[5,6] The methanolysis of the equatorial 5α-cholestan-3β-ol tosylate is of particular interest because Pappas, *et al.*,[7] found no acceleration of the rate upon the addition of sodium methoxide and concluded that a reaction *via* a carbonium ion had yielded only the methyl ether of inverted configuration. The conversion of androsterone sulfate to 2α- and 2β-hydroxy steroids on acid hydrolysis[8] suggested the existence of reaction paths from a C-3 cation which had not been observed when such a carbonium ion was generated from the tosylate. In searching for rearrangement products of the tosylate it seemed best to use a solvent of greater ionizing power and lower nucleophilic characteristics than methanol. The present report describes the solvolysis of androsterone *p*-toluenesulfonate[9] in formic acid.

To minimize possible effects of toluenesulfonic acid or its anion,[10] we conducted the formolysis at great dilution (0.0005 *M*). It proceeded at a good rate at room temperature and gave after alkaline hydrolysis of the formates, the products shown in Table I, runs 2 and 3.[11] As in the hydrolysis of androsterone sulfate (Table I, run 1) all four isomers hydroxylated at C-2 and C-3 (**4**, **5**, **6**, and **8**) were obtained but in significantly different yields, because the 2β-hydroxy compound was now the main alcoholic product and the yields of the 2α and 3α isomers were relatively diminished. Only part of the formate fraction was obtained from the tosylate by a direct route, because the olefin undergoes a slow addition reaction. A solution of the androstenone recovered from the formolysis (which was about a 9:1 mixture of the Δ^2 and Δ^3 isomers) and of an equimolar amount of *p*-toluenesulfonic acid in formic acid gave, after a reaction time of 163 hr and after alkaline hydrolysis of the formates, the four alcohols in yields shown in run 4 of Table I. The reaction appears to be irreversible because the 2β-formate which presumably is the least stable of the four isomers failed to give

(1) Supported by U. S. Public Health Service Grants CA 01679 and AM 09105 and a Research Career Program Award K6-AM-14367. Some of the results have been given in a preliminary report: J. Ramseyer and H. Hirschmann, *Federation Proc.*, **24**, 534 (1965).

(2) W. Stoll, *Z. Physiol. Chem.*, **207**, 147 (1932).

(3) P. A. Plattner and A. Fürst, *Helv. Chim. Acta*, **26**, 2266 (1943).

(4) L. C. King and M. J. Bigelow, *J. Am. Chem. Soc.*, **74**, 3338 (1952).

(5) H. R. Nace, *ibid.*, **74**, 5937 (1952); J. R. Lewis and C. W. Shoppee, *J. Chem. Soc.*, 1375 (1955).

(6) R. Gardi, R. Vitali, and A. Ercoli, *Gazz. Chim. Ital.*, **92**, 632 (1962).

(7) N. Pappas, J. A. Meschino, A. A. Fournier, and H. R. Nace, *J. Am. Chem. Soc.*, **78**, 1907 (1956).

(8) J. Ramseyer, J. S. Williams, and H. Hirschmann, *Steroids*, **9**, 347 (1967).

(9) D. A. Swann and J. H. Turnbull, *Tetrahedron*, **20**, 1265 (1964).

(10) A. Streitwieser, Jr., T. D. Walsh, and J. R. Wolfe, Jr., *J. Am. Chem. Soc.*, **87**, 3682 (1965); A. Streitwieser, Jr., and T. D. Walsh, *ibid.*, **87**, 3686 (1965).

(11) In run 3, which was conducted under standard conditions, a very small amount of benzene and of acetone was used to give an instantaneous solution of the tosylate in formic acid which dissolves crystals of the tosylate only on prolonged shaking. The very similar yields of run 2, which was made without these cosolvents, serve to show that the results were not decisively influenced by the use of acetone as might be argued from the observations of H. Weiner and R. A. Sneen, *ibid.*, **87**, 287 (1965).