Laboratories and Dr. T. E. Eble of these Laboratories for the penicillic acid, to Mr. George C. Prescott for preparation of the methyl penicillate, and to Mr. Lambertus Scholten and Dr. George Pish for determination of the ultraviolet absorption spectra.

Experimental

Penicillic Acid.—Recrystallization from hot water with subsequent drying in moist air gave the monohydrate, m. p. 62–63° (lit.,[1] 64–65°). The monohydrate was found to lose water on standing in a desiccator over calcium chloride at room temperature. The melting point of the anhydrous acid was found to be 85–86° (lit.,[1] 87°).

Methyl Penicillate.—This compound was prepared by the potassium carbonate–methyl iodide method according to Raphael,[6] and was recrystallized from dilute methanol; m. p. 35–36° (lit.,[5] 35°).

Potassium Penicillate.—An aqueous solution of penicillic acid was titrated to *p*H 8 with dilute potassium hydroxide and lyophilized. The resulting solid was dissolved in 90% acetone and four volumes of anhydrous acetone were added. The salt crystallized rapidly in the form of very slender needles; m. p. 195–196°. The analytical sample was dried *in vacuo* over phosphorus pentoxide at 58°.

Anal.[10] Calcd. for $C_8H_9O_4K \cdot 1.5H_2O$: C, 40.84; H, 5.14. Found (average of four determinations): C, 40.87; H, 5.10.

Attempts to prepare the anhydrous salt were abandoned when it was found that drying for 18 hours over phosphorus pentoxide at 100° and 40–50 μ pressure removed only about one-half of the water of crystallization.

Infrared Absorption Spectra.—A Perkin–Elmer (model 12B) recording infrared spectrophotometer with a sodium chloride prism was used. Unless otherwise indicated, the spectra given in Fig. 1 were obtained on Nujol mulls which were prepared by the usual method.[3] An 0.05-mm. sodium chloride cell was used for the chloroform solutions and an 0.05-mm. calcium fluoride cell was used for the deuterium oxide solution of potassium penicillate.

Ultraviolet Absorption Spectra.—A Cary (model 11) recording ultraviolet spectrophotometer was used. A 1-cm. cell was used, except in the case of chloroform solutions, in which an 0.05-mm. cell was used.

(10) Analyses by Clark Microanalytical Laboratory, Urbana, Illinois, and by our Microanalytical Laboratory under the supervision of Mr. William A. Struck.

Summary

1. Infrared absorption spectra indicate that the equilibrium between the cyclic and open chain forms of penicillic acid exists in aqueous solutions but not in the solid state.

2. Methyl penicillate has the open chain structure (III).

3. Ultraviolet absorption spectra are of doubtful value in determining whether compounds of this type exist in the cyclic or open chain form.

KALAMAZOO, MICHIGAN RECEIVED MARCH 31, 1950

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroids. VI.[1] The Wohl–Ziegler Bromination of Steroidal 1,4-Dien-3-ones. Partial Synthesis of Δ^6-Dehydroestrone and Equilenin

BY ST. KAUFMANN, J. PATAKI, G. ROSENKRANZ, J. ROMO AND CARL DJERASSI

The reaction of N-bromosuccinimide (Wohl–Ziegler bromination) with cross-conjugated doubly-unsaturated ketones does not appear to have been studied[2] until very recently. In 1949, Martens[3] reported the isolation of 6-bromo-$\Delta^{1,4}$-cholestadien-3-one (IIc) from the Wohl–Ziegler bromination of $\Delta^{1,4}$-cholestadien-3-one (Ic), but subsequent dehydrobromination led to an oil, which formed an unstable and analytically impure semicarbazone.[3a] An independent investigation of this reaction with certain androstane derivatives yielded well-crystallized derivatives, which served as starting materials for the first partial synthesis of Δ^6-dehydroestrone (Δ^6-isoequilin) (IV) and equilenin (VI) from non-aromatic steroids.

$\Delta^{1,4}$-Androstadiene-3,17-dione (Ia)[4,5] readily reacted with N-bromosuccinimide in carbon tetrachloride solution in the presence of benzoyl peroxide to afford in 89% yield the corresponding 6-bromo derivative IIa, which was dehydrobrominated on short boiling with γ-collidine in 62% yield to $\Delta^{1,4,6}$-androstatriene-3,17-dione (IIIa); similar reactions with $\Delta^{1,4}$-androstadien-17-ol-3-one 17-acetate[5] led to $\Delta^{1,4,6}$-androstatrien-17-ol-3-one 17-acetate (IIIb). These triply unsaturated steroid ketones proved to be identical with material prepared by another method[6] and exhibited a characteristic ultraviolet absorption spectrum (Fig. 1) with maxima at 222, 256 and 298 mμ.

When the trienone IIIa in mineral oil solution[7] was passed through a glass tube, packed with helices and heated to 600°, 40% of an alkali-soluble phenol, m. p. 261–263°, $[\alpha]^{20}D$ −127° (dioxane), was obtained which exhibited an ultraviolet absorption spectrum (Fig. 1) typical of a phenol with an additional conjugated double bond. The substance possessed approximately one-third the biological activity of estrone (rats) and formed an acetate (m. p. 140.5°) and a benzoate (m. p.

(1) Paper V, Rosenkranz, Pataki, Kaufmann, Berlin and Djerassi, THIS JOURNAL, **72**, 4081 (1950).

(2) Djerassi, *Chem. Revs.*, **43**, 271 (1948).

(3) Martens, *Ann.*, **563**, 131 (1949).

(3a) See however Romo, Djerassi and Rosenkranz, *J. Org. Chem.*, **15**, 896 (1950).

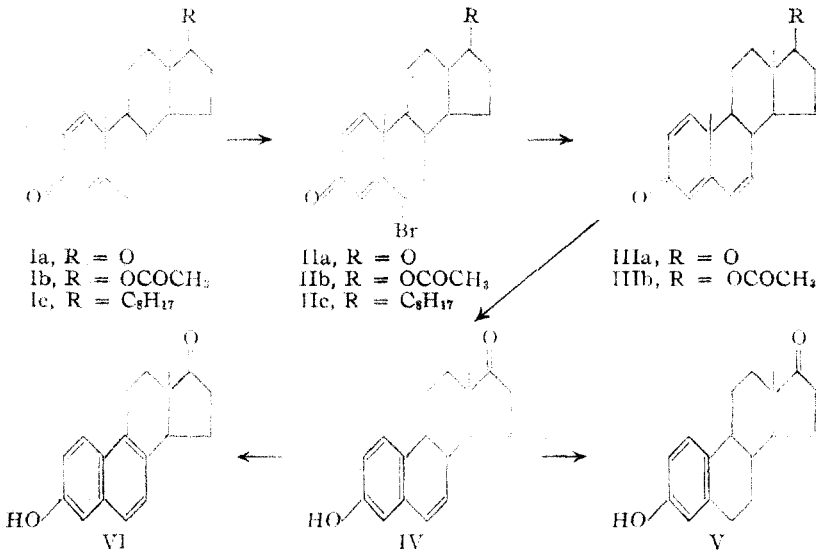
(4) Djerassi and Scholz, *J. Org. Chem.*, **13**, 697 (1948).

(5) Inhoffen, Zuehlsdorff and Huang-Minlon, *Ber.*, **73**, 451 (1940).

(6) Djerassi, Rosenkranz, Romo, Kaufmann and Pataki, THIS JOURNAL, **72**, 4534 (1950).

(7) This superior modification of the conventional tetralin vapor phase aromatization of steroidal, 1,4-dien-3-ones (I) (Inhoffen in Fiat Report No. 996, London, 1947, H. M. Stationery Office; Djerassi and Scholz, THIS JOURNAL, **71**, 3962 (1949)) is due to Hershberg, Rubin and Schwenk, *J. Org. Chem.*, **15**, 292 (1950).

202.5°). Furthermore, catalytic hydrogenation with palladium-on-charcoal afforded estrone (V) in 90% yield, characterized by direct comparison with the natural hormone and its acetate. These reactions clearly established the structure of the aromatization product as Δ^6-dehydroestrone (Δ^6-isoequilin) (IV). Pearlman and Wintersteiner[8] have prepared a Δ^6-isoequilin through an interesting series of transformations from equilin, which agreed in all its properties with our product with the exception of the rotation for which a value of $[\alpha]D$ +150° (dioxane) was reported. In the meanwhile,[6] other Δ^6-dehydrophenols of the steroid series have been prepared, which were also characterized by a strongly negative rotation, so that it appeared most likely that the rotation reported earlier[8] was due to an error. Correspondence with Dr. O. Wintersteiner of the Squibb Institute for Medical Research proved this to be the case and a redetermination of the rotation as well as direct comparison of the two phenols and their benzoates confirmed their identity.[9]



Finally, Δ^1-dehydroestrone (IV) as the acetate was smoothly dehydrogenated to equilenin (VI) on heating in acetic acid with selenium dioxide for ten to fifteen minutes. This further strengthens[9] the belief[10] for the steric identity of estrone and equilenin at carbon atoms 13 and 14 and also constitutes the first partial synthesis of equilenin from a non-aromatic steroid. Aside from total synthesis,[11] this hormone has so far only been obtained from mare's urine and by dehydrogenation of its dihydroderivative equilin.[12] If one considers the ready dehydrogenation of Δ^6-dehydroestrone (IV) to equilenin (VI), it is remarkable that this reaction does not occur to any detectable extent in the vapor phase aromatization of the trienone IIIa, which proceeds at 600° as compared to the dehydrogenation which can be accomplished at *ca.* 110°. This clearly emphasizes that the elimination of the angular methyl group of steroidal 1,4-dien-3-ones (I and III) does not constitute a dehydrogenation process but the elimination of methylene radicals in a little understood manner.

Experimental[13,14]

6-Bromo-$\Delta^{1,4}$-androstadiene-3,17-dione (IIa).—A solution of 42 g. of $\Delta^{1,4}$-androstadiene-3,17-dione (Ia)[4] in 400 cc. of carbon tetrachloride was refluxed with 27 g. of N-bromosuccinimide and 2 g. of benzoyl peroxide for seventy-five minutes. After filtration of succinimide, the filtrate was cooled in ice until crystallization was complete; the resulting 6-bromo derivative (48 g., 89% yield, m. p. 178–180° (dec.)) was satisfactory for the next step. Only fair analytical results were obtained, even after three recrystallizations from ether–hexane; m. p. 189–192° (Kofler, dec.), $[\alpha]^{20}D$ + 118°, 115.6° (dioxane), u. v. maximum at 250 mμ (log *E* 4.34).

Anal. Calcd. for $C_{19}H_{23}O_2Br$: C, 62.82; H, 6.38. Found: C, 63.66; H, 6.47.

$\Delta^{1,4,6}$-Androstatriene-3,17-dione (IIIa).—The above 6-bromo derivative IIa (62 g.) was dehydrobrominated by refluxing with 200 cc. of collidine for fifteen minutes and then poured into dilute sulfuric acid. The product was extracted with ethyl acetate, washed free of collidine with dilute acid, dried and concentrated to *ca.* 50 cc.; after chilling in ice, 27 g. of crystals with m. p. 161–163° was obtained. An additional 3 g. with the same m. p. was isolated from the mother liquors, raising the total yield to 62%. The analytical sample crystallized as very large, hexagonal plates from ethyl acetate; m. p. 164.5–166° (Kofler), $[\alpha]^{20}D$ +82.6°, +72.5° (dioxane), u. v. maxima

(8) Pearlman and Wintersteiner, *J. Biol. Chem.*, **130**, 35 (1939); **132**, 605 (1940). Recently, Hershberg, *et al.*, ref. 7 suggested that the "isoequilin" of Inhoffen [*Naturwissenschaften*, **25**, 125 (1937)] may be Δ^6-isoequilin arising from aromatization of impure $\Delta^{1,4,6}$-androstatriene-3,17-dione, which in turn was supposed to have been obtained from impure 2,4,6-tribromoandrostane-3,17-dione. Not only do the physical constants of Inhoffen's products preclude such a possibility, but it has in fact been indicated (ref. 4) that this tribromo derivative is most likely the 2,4,16-tribromo isomer.

(9) Rosenkranz, Djerassi, Kaufmann, Pataki and Romo, *Nature*, **165**, 814 (1950); Pearlman and Wintersteiner, *ibid.*, p. 815.

(10) Shoppee, *ibid.*, **161**, 207 (1948); Klyne, *ibid.*, **161**, 434 (1948); Stork and Singh, *ibid.*, **165**, 816 (1950).

(11) Bachmann, Cole and Wilds, THIS JOURNAL, **62**, 824 (1940); Johnson, Petersen and Gutsche, *ibid.*, **69**, 2942 (1947).

(12) Dirscherl and Hanusch, *Z. physiol. Chem.*, **236**, 131 (1935). The dehydrogenation of estrone (V) (Butenandt, Wolff and Karlson, *Ber.*, **74**, **1308 (1941)**) is accompanied by inversion and yields *d*-isoequilenin [*cf.* Bachmann and Dreiding, THIS JOURNAL, **72**, 1323 (1950)].

(13) Melting points, marked Kofler, were determined on the Kofler block and are corrected; all others were carried out in capillaries and are uncorrected unless noted otherwise. Rotations were determined on *ca.* 60–100 mg. of substance in chloroform solution (unless indicated otherwise) in a 2-dcm. tube of 10-cc. capacity. When two values are given, the first one always refers to chloroform solution. Ultraviolet absorption spectra were measured in 95% ethanol solution with a Beckman Quartz Photoelectric Spectrophotometer.

(14) The microanalyses were carried out in our Microanalytical Department under the direction of Srta. Amparo Barba. The Srtas. Paquita Revaque and Ann Rochman were responsible for all spectra and rotations.

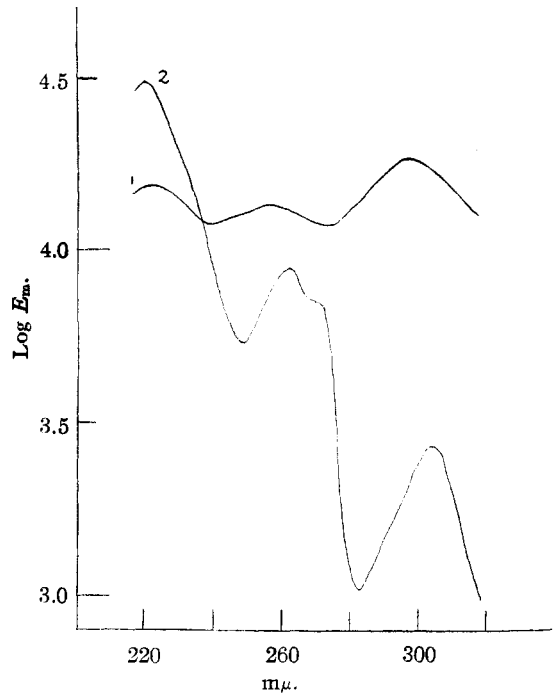


Fig. 1.—Ultraviolet absorption spectra (in 95% ethanol solution): curve 1, $\Delta^{1,4,6}$-androstatriene-3,17-dione (IIIa); curve 2, Δ^6-dehydroestrone (IV).

(Fig. 1) at 222 mμ (log E 4.19), 256 mμ (log E 4.13) and 298 mμ (log E 4.27).

Anal. Calcd. for $C_{19}H_{22}O_2$: C, 80.81; H, 7.85. Found: C, 80.95; H, 7.95.

6-Bromo-$\Delta^{1,4}$-androstadien-17-ol-3-one 17-Acetate (IIb).—The Wohl–Ziegler bromination was carried out as above for one-hundred minutes, with 2.15 g. of dienolone acetate (Ib),[5] 1.18 g. of N-bromosuccinimide and 75 mg. of peroxide. Filtration, evaporation to dryness and trituration with ether afforded 1.45 g. (55%) of colorless needles, m. p. 142–144° (slight decomposition, Kofler), which remained unchanged after recrystallization from hexane–acetone; u. v. maximum at 248 mμ (log E 4.32).

Anal. Calcd. for $C_{21}H_{27}O_3Br$: C, 61.91; H, 6.68. Found: C, 62.50; H, 6.93.

$\Delta^{1,4,6}$-Androstatrien-17-ol-3-one 17-Acetate (IIIb).—The dehydrobromination of the 6-bromo-17-acetate (IIb) (1.3 g.) was carried out in the usual manner by refluxing for twenty-five minutes with 10 cc. of collidine (0.6 g., 94% collidine hydrobromide isolated) and yielded 0.7 g. (67%) of the desired trienolone acetate with m. p. 151–153° (Kofler), $[\alpha]^{20}D$ −9°, which exhibited the identical absorption spectrum (maxima at 222, 256 and 298 mμ) as the specimen prepared by the alternate and preferred method[6]; a mixed melting point determination further confirmed the identity of the two products.

Δ^6-Dehydroestrone (IV).—The aromatization was carried out essentially by the general method of Inhoffen as modified by Hershberg, Rubin and Schwenk[7]: A solution of 5.5 g. of $\Delta^{1,4,6}$-androstatriene-3,17-dione (IIIa) in 550 cc. of mineral oil was passed at a rate of 2 cc. per second through a glass tube filled with helices and heated to 600°. After cooling overnight in the ice-box, the light tan crystals were filtered and washed free of mineral oil with hexane; yield 2.1 g. (40%), m. p. 255–262°. Recrystallization from methanol afforded stout prisms, m. p. 261–263° (Kofler), $[\alpha]^{20}D$ −127° (dioxane), u. v. maxima (Fig. 1) at 220 mμ (log E 4.49), 262 mμ (log E 3.95) and 304 mμ (log E 3.44). A mixed m. p. determination, kindly carried out by Dr. O. Wintersteiner, showed no depression on admixture with his sample.[8] The substance possessed one-third the estrogenic potency of estrone in rats.

Anal. Calcd. for $C_{18}H_{20}O_2$: C, 80.56; H, 7.51. Found: C, 80.69; H, 7.30.

Two grams of Δ^6-dehydroestrone was heated for two hours on the steam-bath with 20 cc. of pyridine and 5 cc. of acetic anhydride, poured into water, the product collected and recrystallized from methanol; yield 2.05 g., m. p. 136–139°. The analytical sample of **Δ^6-dehydroestrone acetate** was obtained from methanol with m. p. 140–140.5° (cor.), $[\alpha]^{20}D$ −113.7° (dioxane); reported,[8] m. p. 140–141°.

Anal. Calcd. for $C_{20}H_{22}O_3$: C, 77.39; H, 7.14. Found: C, 77.71; H, 7.11.

Benzoylation of the phenol by the Schotten–Baumann procedure, followed by two recrystallizations from ethanol–chloroform, yielded **Δ^6-dehydroestrone benzoate** as colorless needles with m. p. 200.5–202.5° (Kofler); no depression in m. p. was observed on admixture with a sample (m. p. 202°) of Pearlman and Wintersteiner,[8] which however had given poor analytical results.

Anal. Calcd. for $C_{25}H_{24}O_3$: C, 80.62; H, 6.50. Found: C, 80.79; H, 6.43.

Lithium Aluminum Hydride Reduction of Δ^6-Dehydroestrone Acetate.—To a mixture of 0.5 g. of lithium aluminum hydride in 100 cc. of ether was added slowly a solution of 1.5 g. of Δ^6-dehydroestrone acetate in 100 cc. of ether. After refluxing for thirty minutes, water and dilute acid was added, the layers were separated and the ethereal solution was dried and evaporated. Recrystallization from methanol gave 1.1 g. (84%) of **Δ^6-dehydroestradiol** with m. p. 225–227° (Kofler), $[\alpha]^{20}D$ −179° (dioxane), which exhibited the same absorption spectrum and gave no depression in m. p. on admixture with an authentic specimen prepared from testosterone.[6]

Hydrogenation of Δ^6-Dehydroestrone to Estrone (V).—The theoretical amount of hydrogen was consumed in twenty-five minutes when a solution of 0.5 g. of Δ^6-dehydroestrone (IV) in 80 cc. of ethyl acetate was shaken with 100 mg. of 10% palladium-on-charcoal (American Platinum Works) in an atmosphere of hydrogen. Filtration, evaporation and recrystallization from methanol yielded 0.45 g. (90%) of estrone (V), m. p. 256–258° (cor.), $[\alpha]^{20}D$ +162° (dioxane); the ultraviolet absorption spectrum was identical with that of the natural hormone and a mixture of the two specimens showed m. p. 257–259°.

Acetylation of a sample of the phenol in the usual manner and recrystallization from hexane–ether led to estrone acetate, m. p. 125–127° (cor.) (m. p. 126–127° on admixture with authentic estrone acetate), $[\alpha]^{20}D$ +148° (dioxane).

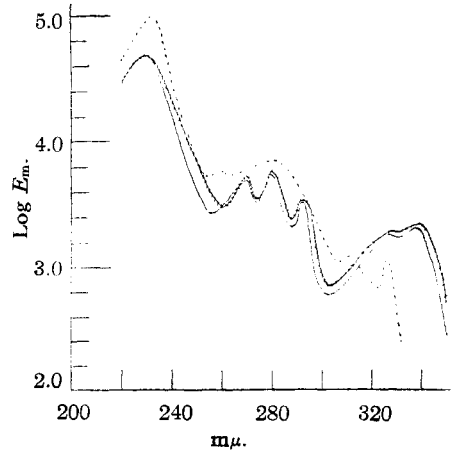


Fig. 2.—Ultraviolet absorption spectra: ——, natural equilenin (VI); -|-|-|-|-|-, semi-synthetic equilenin; -------, equilenin acetate.

Dehydrogenation of Δ^6-Dehydroestrone to Equilenin (VI).—A solution of 2.5 g. of Δ^6-dehydroestrone acetate in 70 cc. of glacial acetic acid was refluxed with 0.43 g. of freshly sublimed selenium dioxide for ten to fifteen minutes in a current of nitrogen. After filtration of selenium and pouring into water, there was obtained 2.4 g. of crude **equilenin acetate** with a reddish color, which was removed on filtration in hexane–benzene (5:1) solution through a short column of alumina. Recrystallization from methanol gave long prisms, with a slight pink tinge, m. p. 156–157° (cor.), $[\alpha]^{20}D$ +72°; no depression was observed on admixture with the acetate (m. p. 157–158°) prepared from the natural hormone. The ultraviolet absorption spectrum is shown in Fig. 2.

Anal. Calcd. for $C_{20}H_{20}O_2$: C, 77.90; H, 6.54. Found: C, 78.16; H, 6.23.

Saponification of the acetate by boiling with 5% methanolic potassium hydroxide solution for twenty-five minutes, followed by sublimation *in vacuo* and recrystallization from dilute ethanol, yielded **equilenin** (VI), m. p. 256–258° (red melt), $[\alpha]^{20}D$ +86.4° (dioxane); the m. p. was undepressed on mixing with a sample of the natural hormone (kindly supplied by Ayerst, McKenna and Harrison, Ltd.). The characteristic spectrum is given in Fig. 2 with maxima at 230, 270, 280, 292, 328 and 340 mμ and was nearly indistinguishable from that of the natural hormone.[15]

(15) Our log E values, for both the natural and semi-synthetic sample, were about 0.2 unit lower than those reported earlier (ref. 11) and the last maximum was observed at 340 mμ (in agreement with Dirscherl and Hanusch, *Z. physiol. Chem.*, **233**, 13 (1935)) rather than at 345 mμ (ref. 11). Our values were obtained on a Beckman Spectrophotometer which had been calibrated both in regard to wave length (benzene) and extinction (potassium chromate in alkali) by the procedure of Hogness, *et al.*, *J. Phys. Chem.*, **41**, 379 (1937).

Anal. Calcd. for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81. Found: C, 80.89; H, 6.75.

Summary

The Wohl–Ziegler bromination of $\Delta^{1,4}$-androstadiene-3,17-dione (and of the corresponding 17-acetoxy derivative) proceeds smoothly and after dehydrobromination of the intermediate 6-bromo compound IIa leads to $\Delta^{1,4,6}$-androstatriene-3,17-dione (IIIa). Aromatization in mineral oil solution of IIIa at 600° results in the formation of Δ^6-dehydroestrone (Δ^6-isoequilin) (IV) in 40% yield, whose structure was proved by hydrogenation to estrone (V) and by dehydrogenation to equilenin (VI). This constitutes the first partial synthesis of equilenin from a steroid with an angular methyl group at C-10.

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MEXICO CITY, D. F.

RECEIVED FEBRUARY 9, 1950

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroids. VII.[1] Contribution to the Bromination of Δ^4-3-Ketosteroids and a New Partial Synthesis of the Natural Estrogens

BY CARL DJERASSI, G. ROSENKRANZ, J. ROMO, ST. KAUFMANN AND J. PATAKI

$\Delta^{1,4}$-Dien-3-ones (XI) of the steroid series represent the key intermediates in the partial synthesis of the female sex hormones, estrone and estradiol from non-aromatic steroids possessing an angular methyl group at C-10, since thermal treatment results in aromatization of ring A with formation of the desired phenol (XIV).[2,3] This process is now used commercially and the required dienones have so far only been prepared by dibromination of saturated 3-keto*allo*steroids followed by dehydrobromination of the resulting 2,4-dibromo derivatives. The alternate approach involving as a starting material a Δ^4-3-ketosteroid (I), such as testosterone, appeared attractive and was studied in very considerable detail by Inhoffen[2] and by Butenandt.[4] Their study was limited to a model Δ^4-3-ketosteroid, Δ^4-cholesten-3-one (Ic) and was concerned primarily with the behavior of this ketone toward bromine. These reactions, which at times proved to be very involved, led these workers[2,4] to the conclusion that mono- and poly-bromination (up to *eight* moles of bromine) of Δ^4-3-ketosteroids (I) results in substitution at C-4 and in ring B and that, therefore, these ketones are useless as starting materials for the partial synthesis of the estrogens. Since a reinvestigation of this problem in this laboratory has provided a novel partial synthesis of all of the major natural estrogens and has thus thrown open to question the structure assignments of a considerable number of compounds,[2,4] a detailed discussion of the bromination of Δ^4-3-ketosteroids (I) is necessary.

At the outset it should be noted that most of the work done on the bromination of Δ^4-3-ketosteroids (I) was carried out in acetic acid, alone or diluted with another solvent, and the present discussion is limited to this type of solvent. It is stated[2,4,5] that *monobromination* of Δ^4-cholesten-3-one (Ic) in ether–acetic acid leads to the 6-bromo derivative (IIc). This substance (m. p. 132°, u. v. maximum at 248 mμ) is known and has been prepared by a number of methods[5,6] but *it has never been isolated* in the monobromination of Δ^4-cholesten-3-one (Ic) in acetic acid, because a difficultly separable mixture is formed,[7] and there-

(1) Paper VI, Kaufmann, Pataki, Rosenkranz, Romo and Djerassi, THIS JOURNAL, **72**, 4531 (1950).

(2) *Cf.* Inhoffen, *Angew. Chem.*, **53**, 473 (1940); *ibid.*, **59**, 207 (1947).

(3) (a) Hershberg, Rubin and Schwenk, *J. Org. Chem.*, **15**, 292 (1950); (b) Wilds and Djerassi, THIS JOURNAL, **68**, 2125 (1946); Djerassi and Scholz, *ibid.*, **71**, 3962 (1949).

(4) Butenandt, Schramm and Kudsus, *Ann.*, **531**, 176 (1937).

(5) Inhoffen, *Ber.*, **69**, 2141 (1936).

(6) Ruzicka, Bosshard, Fischer and Wirz, *Helv. Chim. Acta*, **19**, 1147 (1936); Dane, Wang and Schulte, *Z. physiol. Chem.*, **245**, 80 (1936); Reich and Lardon, *Helv. Chim. Acta*, **29**, 671 (1946); Martens, *Ann.*, **563**, 131 (1949).

(7) Unpublished observation; *cf.* also ref. 5, p. 2145.