characteristic of Δ^7-sterols (Fig. 2), and as expected[14] the chromophore was somewhat less intense than that of Δ^7-cholestenol. The infrared spectrum was identical to that of a sample of Δ^7-spinastenol prepared from α-spinasterol, and was also very similar to that of Δ^7-cholestenol.[19]

(19) D. R. Johnson, D. R. Idler, V. W. Meloche and C. A. Baumann, THIS JOURNAL, **75**, 52 (1953).

Acknowledgment.—We are indebted to Dr. O. Wintersteiner of the Squibb Company for a generous sample of α-spinasteryl acetate and to Dr. E. S. Wallis, Princeton University, for samples of α_3-sitosteryl acetate and benzoate.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY OF THE SOUTHWESTERN MEDICAL SCHOOL]

16-Substituted Steroids. VIII. 1,3,5(10)-Estratrien-3-ol-16-one

BY MAX N. HUFFMAN[1] AND MARY HARRIET LOTT[1]

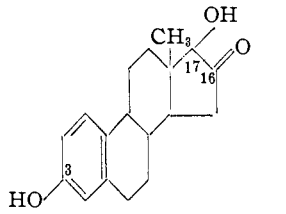
RECEIVED MARCH 19, 1953

The mild Clemmensen reduction of 1,3,5(10)-estratrien-3,17β-diol-16-one produces a mixture of estrone and 1,3,5(10)-estratrien-3-ol-16-one. The latter was characterized as the semicarbazone, acetate, benzoate, palmitate, methyl ether and benzyl ether. The shifting of the carbonyl from C_{17} to C_{16} on the naturally-occurring estratrien nucleus resulted in a powerful rotatory effect in the negative direction. The mechanism by which 1,3,5(10)-estratrien-3-ol-16-one is formed in the Clemmensen reduction of 1,3,5(10)-estratrien-3,17β-diol-16-one is discussed.

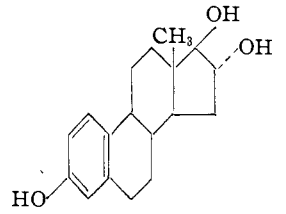
In 1943, 1,3,5(10)-estratrien-3,17β-diol-16-one[2,3] (I) was submitted to reduction with amalgamated zinc in aqueous ethanolic hydrochloric acid in the hope of obtaining one of the four possible 16,17-epimers of estriol (II). There was obtained instead a material which was soluble in 0.5 *N* sodium hydroxide, and which gave both a monobenzoate and a semicarbazone whose analyses agreed unexpectedly with those of a parent $C_{18}H_{22}O_2$ compound.

The mild Clemmensen reduction of 1,3,5(10)-estratrien-3,17β-diol-16-one was repeated several times, always with the finding that a ketonic material of varying melting point was obtained whose analysis was correct for estrone (IV). One such preparation, under assay, contained 50% estrone; another such preparation, after further reduction of carbonyl to carbinol, furnished pure estradiol-3,17β (VII).

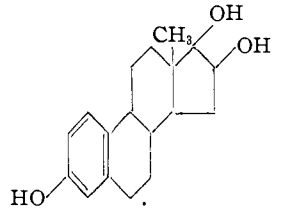
It finally became clear that our reduction of 1,3,5(10)-estratrien-3,17β-diol-16-one[4,5] produced a varying mixture of estrone and an isomer of estrone. We were eventually successful in obtaining pure the new $C_{18}H_{22}O_2$ steroid, which showed an optical rotation of $[\alpha]^{25}D$ $-87°$ (95% ethanol). That this new compound possesses the unaltered, naturally-occurring estratrien nucleus was established by hydrogenolysis[4] of its 3-benzoxy-16-diethyl thioketal to desoxoestrone benzoate (followed by saponification to desoxoestrone) as shown by mixed melting point comparison using authentic desoxoestrone benzoate (and using authentic desoxoestrone). Inasmuch as the new 1,3,5(10)-estratrien-3-ol-monoketone was formed from the reduction of a parent molecule which bears oxygen functions at C_{16} and C_{17}, the only logical structural formulation for it is



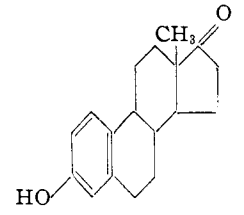
I



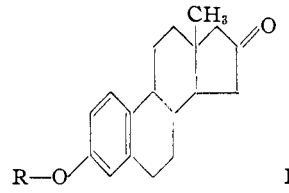
II



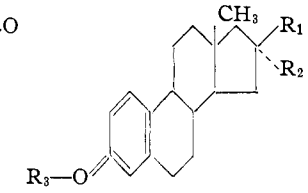
III



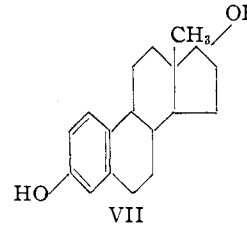
IV



Va (R = (H)
Vb (R = C—CH_3)
Vc (R = C—C_6H_5)
Vd (R = C—$(CH_2)_{14}$—CH_3)
Ve (R = CH_3)
Vf (R = CH_2—C_6H_5)



VIa (R_1 + R_2 = N—NH—CO—NH_2; R_3 = H)
VIb (R_1 = R_2 = S-C_2H_5; R_3 = C—C_6H_5)



VII

that of a 1,3,5(10)-estratrien-3-ol-16-one (Va), for it cannot bear the carbonyl at C_{17}.

The optical rotation value for 1,3,5(10)-estratrien-3-ol-16-one ($-87°$) is interestingly some 250° more negative than that of naturally-occurring estrone

(1) Oklahoma Medical Research Foundation, Oklahoma City' Oklahoma.

(2) M. N. Huffman, THIS JOURNAL, **64**, 2235 (1942).

(3) M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **172**, 325 (1948).

(4) M. N. Huffman and M. H. Lott, THIS JOURNAL, **71**, 719 (1949).

(5) In this manuscript we are using the presently accepted terminology for the stereo configuration at C_{17} of the steroid nucleus. Structural determinations in the field of steroidal 16,17-ketols and 16,17-glycols previously presented by us[4] were based upon the general belief then held that the C_{17}-carbinol of testosterone and estradiol possessed the α-configuration.

(+168°).[6] It is to be remembered that the introduction of a ketone group at C_{16} in estradiol confers a large negative rotation to the molecule; thus, estradiol (VII) has an optical rotation value of +80°,[6] whereas 1,3,5(10)-estratrien-3,17β-diol-16-one (I) has a value of −102°.[3] All 16-keto-17β-hydroxysteroids so far prepared by us in the androgen series have likewise shown a similar negative rotation effect over that of the parent 17β-hydroxysteroid. This we regard as further convincing evidence that the new isomeric estrone is correctly formulated.

1,3,5(10)-Estratrien-3-ol-16-one was further characterized as the 3-acetate (Vb), 3-benzoate (Vc), 3-palmitate (Vd), 3-methyl ether (Ve) and 3-benzyl ether (Vf).

The actual mechanism by which 1,3,5(10)-estratrien-3-ol-16-one is formed in this Clemmensen reduction is not known. We do not believe that either estriol (II) or 16-epiestriol (isoestriol-A)[7,4] (III) can be intermediates, for the former triol is known to dehydrate preferentially to the 17-ketone,[8,9] and the latter triol is not appreciably altered by a 5-hour subjection to the rigors of this reduction. It is, of course, possible that the 17-hydroxyl group of 1,3,5(10)-estratrien-3,17β-diol-16-one (I) is replaced by a chlorine group which is in turn reductively removed by the zinc.[10] The fact that a 17-ketone[11] is also formed in the Clemmensen reduction of a 16-keto-17β-hydroxysteroid indicates, in our opinion, that some kind of keto–carbinol tautomerization is taking place.

We believe our communication[12] in regard to the preparation of 16-keto-17-desoxy-steroids by the reduction of 16-keto-17-hydroxysteroids is the first example in the literature wherein a true Clemmensen reduction had been found to remove the carbinol from α-ketol while leaving the ketone group intact. W. T. Smith, Jr., has independently confirmed the finding that a Clemmensen reduction of an α-ketol may furnish the ketone with the hydroxyl reductively removed.[13] Thus, he showed that butyroin yields 50–60% octanone-4 upon Clemmensen reduction. He furthermore showed that 4,5-octanediol could not be considered as an intermediate in the reaction.

In 1948, Wilds and Johnson[14] prepared by total synthesis a structural isomer of estrone bearing the carbonyl group at position 16 of the steroid nucleus. This compound, however, was a stereoisomer and did not possess the naturally-occurring estratrien nucleus.

(6) E. A. Doisy, "Sex and Internal Secretions," 2nd Ed., The Williams and Wilkins Company, Baltimore, Md., 1939, p. 846.

(7) M. N. Huffman and H. H. Darby, THIS JOURNAL, **66**, 150 (1944).

(8) A. Butenandt and F. Hildebrandt, *Z. physiol. Chem.*, **199**, 243 (1931).

(9) G. F. Marrian and G. A. D. Haslewood, *Biochem. J.*, **26**, 25 (1932).

(10) R. Stoermer in Houben, "Die Methoden der organischen Chemie," 3rd Ed., Georg Thieme, Leipzig, 1925, p. 228.

(11) After the Clemmensen reduction of androstane-3β,17β-diol-16-one it has proved easy to isolate both androstane-3β-ol-17-one and androstane-3β-ol-16-one. This research will be described in paper IX of this series.

(12) M. N. Huffman and M. H. Lott, THIS JOURNAL, **73**, 878 (1951).

(13) W. T. Smith, Jr., *ibid.*, **73**, 1883 (1951).

(14) A. L. Wilds and T. L. Johnson, *ibid.*, **70**, 1166 (1948).

The biological activity of 1,3,5(10)-estratrien-3-ol-16-one is being investigated.

Acknowledgment.—We wish to thank Dr. O. Wintersteiner for the gift of desoxoestrone and desoxoestrone benzoate used for mixed melting point comparison. Our thanks are due to Dr. E. W. D. Huffman for the microanalyses reported in this publication. To the U. S. Standard Products Company and to G. D. Searle and Company the authors are particularly indebted for financial assistance rendered during the course of this research.

Experimental[15]

Mild Clemmensen Reduction of 1,3,5(10)-Estratrien-3,17β-diol-16-one.—In 45 ml. of 95% ethanol containing 3 ml. of 6 *N* hydrochloric acid was dissolved 603 mg. of 1,3,5(10)-estratrien-3,17β-diol-16-one[3] (m.p. 232–234.5°) (I). To this solution was added 15 g. of amalgamated zinc (20-mesh), and the resulting mixture refluxed on the steam-bath for five hours, with the addition of 3-ml. portions of 6 *N* hydrochloric acid at 15-minute intervals. At the end of the 5-hour reflux period the mixture was cooled, diluted with water, and extracted with ethyl ether. After having been washed with 3% sodium bicarbonate and with water, the ethereal phase was evaporated to yield a white crystalline residue. Semicarbazone formation was effected without further purification.

Semicarbazone of 1,3,5(10)-Estratrien-3-ol-16-one.—The crude steroid (of the preceding paragraph) was treated with a solution of 0.60 g. of semicarbazide hydrochloride and 0.84 g. of crystalline sodium acetate in 36 ml. of ethanol plus 4 ml. of water. The mixture was refluxed on the steam-bath for one hour and then diluted with 25 ml. of water. After two days in the ice-box, the white crystalline semicarbazone was filtered and washed well with water. A recrystallization from methanol yielded 320 mg. decomposing at 244.5–247°. Of this material, 100 mg. was recrystallized successively from 95% ethanol, chloroform–absolute ethanol and absolute ethanol (twice) to give 46 mg. of felt-like semicarbazone melting at 246.5–248° dec. (VIa).

Anal. Calcd. for $C_{19}H_{25}O_2N_3$: C, 69.70; H, 7.70; N, 12.84. Found: C, 68.92, 69.03; H, 7.66, 7.70; N, 12.68, 12.75.

This semicarbazone was once obtained with a melting point of 254–257° dec., and from it there was obtained after hydrolysis a practically quantitative yield of 1,3,5(10)-estratrien-3-ol-16-one. It is not known whether this higher melting material represents a crystalline modification of the product melting at 246.5–248° dec.

Hydrolysis of 1,3,5(10)-Estratrien-3-ol-16-one Semicarbazone.—Semicarbazone from the foregoing experiment (220 mg., dec. 244.5–247°) (VIa) was dissolved in 75 ml. of ethanol and 25 ml. of 2 *N* hydrochloric acid, and the mixture refluxed on the steam-bath for two hours. Then to the hot solution 5 ml. of pyruvic acid in 20 ml. of water was mixed in and the refluxing continued for an additional 5 minutes. After a day at room temperature, the solution was diluted with 275 ml. of water and extracted with 400 ml. of ethyl ether. The separated ethereal phase was washed with dilute hydrochloric acid, with 5% sodium carbonate and with water. After evaporation of the ether, the white crystalline residue was recrystallized twice from aqueous methanol (once with the aid of charcoal) to give 135 mg. melting at 242.5–243.5° dec. Two recrystallizations from absolute methanol gave 36 mg. of fine feathery needles which melted at 243.5–245.5° with decomposition; $[\alpha]^{25}D$ −87° (*c*, 0.43 in 95% ethanol) (Va).

Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 80.04, 79.93; H, 8.22, 8.15.

Benzoylation of 1,3,5(10)-Estratrien-3-ol-16-one.—In 30 ml. of warm 0.5 *N* sodium hydroxide were dissolved 37 mg. of pure 1,3,5(10)-estratrien-3-ol-16-one (Va). The cooled solution was then treated with 0.50 ml. of benzoyl chloride, and shaken vigorously for ten minutes. After having stood at room temperature for 24 hours, the benzoate was filtered,

(15) Melting points are uncorrected. Microanalyses and optical rotations are by Dr. E. W. D. Huffman, Denver, Col.

washed copiously with water, and dried at room temperature. Two recrystallizations from acetone–ethanol yielded 44 mg. of fine needles (Vc) melting sharply at 223.5–224.5°, with a slight decomposition.

Anal. Calcd. for $C_{25}H_{26}O_3$: C, 80.18; H, 7.00. Found: C, 80.09, 80.21; H, 6.99, 6.92.

Acetylation of 1,3,5(10)-Estratrien-3-ol-16-one.—Pure 1,3,5(10)-estratrien-3-ol-16-one (48 mg.) (Va) was acetylated in 1 ml. of dry pyridine with 1 ml. of acetic anhydride. The reaction mixture was left at room temperature for 24 hours and the acetate then precipitated with 50 ml. of ice-water. After several hours at room temperature, it was filtered, washed copiously with water, and dried in the warm closet. The acetate was recrystallized twice from aqueous methanol (treatment with charcoal) to give 49 mg. of material melting at 132–133° (Vb). A final crystallization from 80% methanol yielded 38 mg. of tiny leaves with unchanged melting point.

Anal. Calcd. for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.72, 76.87; H, 7.72, 7.78.

1,3,5(10)-Estratrien-3-ol-16-one Benzyl Ether.—Pure 1,3,5(10)-estratrien-3-ol-16-one (Va) (50 mg.) was covered with 113 mg. of anhydrous potassium carbonate, 0.10 ml. of benzyl chloride added, and the latter rinsed down with 2.5 ml. of 95% ethanol. The mixture was refluxed on the steam-bath for 1.5 hours, and then 0.6 ml. of water added. The mixture was chilled overnight and the benzyl ether collected on the filter, washed with cold 75% ethanol, washed with water, and then dried in the warm closet. The steroid was recrystallized three times from 95% ethanol in order to sharpen the melting point of the silky needles to 156–156.5° (35 mg.) (Vf).

Anal. Calcd. for $C_{25}H_{28}O_2$: C, 83.30; H, 7.83. Found: C, 83.18, 83.25; H, 7.79, 7.85.

1,3,5(10)-Estratrien-3-ol-16-one Methyl Ether.—To 50 mg. of pure 1,3,5(10)-estratrien-3-ol-16-one (Va) was added a solution of 4.0 g. of 85% potassium hydroxide in 9 ml. of water. The mixture was agitated, cooled, and then treated with 1.0 ml. of methanol. It was then heated on the steam-bath under reflux until solution was effected, after which 1 ml. of dimethyl sulfate was added. After the reaction had subsided, an additional 1 ml. of dimethyl sulfate was employed. The mixture was refluxed for 15 minutes and then diluted with 5 ml. of water. After a day at room temperature the crude methyl ether was removed by filtration, washed copiously with water, and then recrystallized from aqueous acetone. Two recrystallizations from aqueous methanol (charcoal) gave colorless plates melting sharply at 124–124.5° (30 mg.) (Ve).

Anal. Calcd. for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 80.28, 80.35; H, 8.41, 8.49.

1,3,5(10)-Estratrien-3-ol-16-one Palmitate.—In 1.0 ml. of dry pyridine was dissolved 53 mg. of pure 1,3,5(10)-estratrien-3-ol-16-one (Va), and the solution treated with 0.50 ml. of palmitoyl chloride. A thick, white precipitate formed at once with the evolution of heat. The mixture was diluted further with 1.5 ml. of dry pyridine and agitated continuously for 15 minutes. Then 0.25 ml. of water was added, the mixture agitated for an additional five minutes, and finally diluted with 40–50 ml. of cold water. After 24 hours at room temperature the palmitate was filtered, washed well with water, and dried *in vacuo*. Several recrystallizations from 95% ethanol afforded 64 mg. of crystalline 1,3,5(10)-estratrien-3-ol-16-one palmitate (Vd) as waxy plates melting at 110.5–111.5°.

Anal. Calcd. for $C_{34}H_{52}O_3$: C, 80.26; H, 10.30. Found: C, 80.17, 80.12; H, 10.20, 10.32.

The Transformation of 1,3,5(10)-Estratrien-3-ol-16-one Benzoate to Desoxoestrone.—To 179 mg. of 1,3,5(10)-estratrien-3-ol-16-one benzoate (m.p. 223–224.5°) (Vc) were added 500 mg. of anhydrous sodium sulfate and 250 mg. of freshly fused zinc chloride. The mixture was at once covered with 10 ml. of ethyl mercaptan, the flask stoppered, and the mixture thoroughly agitated. The benzoate soon dissolved with the development of a pink color. After three days in the ice-chest the mixture was evaporated under vacuum at 30° in order to remove excess mercaptan. The residue was taken up alternately in 1 *N* sodium hydroxide and in ethyl ether, the two phases thoroughly shaken, and the separated ethereal phase washed twice with water containing traces of pyridine. To the ether were added 1 ml. of pyridine, 25 ml. of absolute ethanol and 30 ml. of benzene, and the resulting solution was evaporated cautiously to an oily residue which, after a thorough drying *in vacuo* over sulfuric acid, furnished the thioketal as a straw-colored oil (VIb).

To this oil were added 3 g. of modified Raney nickel catalyst and 30 ml. of absolute ethanol. The mixture was refluxed for seven hours on the steam-bath, cooled and filtered free of catalyst. To the filtrate was added 30 ml. of 2 *N* potassium hydroxide, and the alkaline solution refluxed for 45 minutes. The resulting solution was distilled down to low volume, acidified with dilute hydrochloric acid, and taken up in ether. The ether was washed with 3% sodium carbonate and with water. The ethereal solution was dried and evaporated, yielding an oil which soon crystallized. To remove ketones the crystalline material was treated with 30 ml. of *n*-propyl alcohol, 0.24 g. of carboxymethoxylamine hemihydrochloride, 0.37 g. of potassium acetate and 10 ml. of water under reflux for 1.75 hours, after which the reaction mixture was taken up in ethyl ether, and the latter washed with water, with 3% sodium carbonate and with water. Evaporation of the ethereal phase furnished a nonketonic fraction, which after three crystallizations from aqueous ethanol gave 77 mg. of long, white needles melting at 133–134°. After admixture with authentic desoxoestrone[16] there was no depression of this melting point. Before analysis the sample was dried for 3 hours *in vacuo* over phosphorus pentoxide at 100°.

Anal. Calcd. for $C_{18}H_{24}O$: C, 84.32; H, 9.44. Found: C, 84.35, 84.36; H, 9.37, 9.40.

Desoxoestrone Benzoate.—In 2 ml. of pyridine was dissolved 39 mg. of the desoxoestrone (m.p. 133–134°) from the preceding experiment, and the solution treated with 0.5 ml. of benzoyl chloride. After 24 hours at room temperature the reaction was stopped by the addition of water. After two days at 5° the reaction was taken up in ethyl ether, and the ether washed with water, with 3% sodium bicarbonate and again with water. The ether was evaporated to dryness, and the residue recrystallized from aqueous methanol and from methanol–acetone. There was obtained 44 mg. of tiny, felt-like needles of desoxoestrone benzoate melting at 169–169.5°. This product did not depress the melting point of authentic desoxoestrone benzoate.[16]

Anal. Calcd. for $C_{25}H_{28}O_2$: C, 83.29; H, 7.83. Found: C, 83.04, 82.96; H, 7.91, 7.86.

Attempts to Dehydrate 16-Epiestriol (Isoestriol-A). (a) Using Ethanolic Hydrochloric Acid.—16-Epiestriol[17] (190 mg.) (III) was dissolved in 20 ml. of 95% ethanol, and 10 ml. of 6 *N* hydrochloric acid added. After having been refluxed for 3 hours on the steam-bath, the solution was diluted with three volumes of water. After a day in the ice-box the crystallized steroid was filtered off and recrystallized from aqueous ethanol. The recovery of unchanged triol was 160 mg.

(b) **Using Aqueous Ethanolic Hydrochloric Acid and Amalgamated Zinc.**—A solution of 121 mg. of 16-epiestriol[17] (III) in 9 ml. of 95% ethanol was refluxed for 5 hours, 0.6-ml. portions of 6 *N* hydrochloric acid being added at 15-minute intervals (another 2 ml. of ethanol added at the end of 3 hours of refluxing). The reaction mixture was worked up as described for the first experiment in Experimental. The steroid residue was crystallized once from aqueous ethanol and once from 95% ethanol to give 75 mg. of unchanged triol.

OKLAHOMA CITY, OKLAHOMA

(16) Kindly furnished us by Dr. O. Wintersteiner.

(17) We agree the trivial name 16-epiestriol is preferable to isoestriol-A, as suggested by Fieser and Fieser in "Natural Products Related to Phenanthrene," 3rd Ed., Reinhold Publishing Corporation, New York, N. Y., 1949, p. 318.