tion with hydrochloric acid gave an immediate precipitate of 6-phenyl-5-chlorouracil melting at 260–261° to a clear oil. The compound contained chlorine, and was dried for analysis at 100–110°.

*Anal.* Calcd. for $C_{10}H_7O_2N_2Cl$: N, 12.58. Found: N, 12.32.

## Summary

1. 5,5-Dichloro-6-hydroxy-6-phenylhydrouracil (II) is formed in good yield by the combined action of superoxol and hydrochloric acid on 6-phenyluracil.

2. This hydrouracil compound is characterized by its behavior when digested with concentrated hydrochloric acid. This treatment leads to complete destruction of the pyrimidine ring with formation of benzoic acid and ammonium chloride.

3. A reaction mechanism has been proposed to explain this unexpected decomposition of (II) by action of hydrochloric acid.

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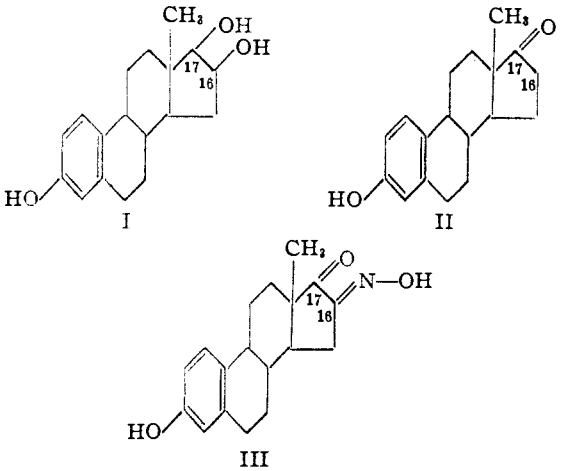
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

# 16-Substituted Steroids. I. Isoestriol-A

BY MAX N. HUFFMAN[1] AND HUGH H. DARBY

In 1930 Marrian[2] in England and Doisy[3] in America succeeded in isolating from human pregnancy urine the estrogen which is now known as estriol or theelol. Browne,[4] working in Collip's laboratory, subsequently obtained this hormone in pure form from human placenta. Since the isolation of estrone in 1929 by Doisy and coworkers,[5] some seven or eight different estrogens have been obtained from natural sources. Estriol (I) is, however, the only naturally-occurring estrogen in which position number 16 on the steroid nucleus is occupied by a functional group.

In our search for an abnormal estrogen metabolite that may be concerned in the etiology of cancer of the genital organs, we have prepared several steroids[6] of the estrogen series in which position number 16 on the steroid nucleus is functionally substituted. In this paper is reported in detail the preparation of an epimer of theelol. It is to be noted that there exists the theoretical possibility for four stereoisomeric estriols, considering only the spatial arrangements of the carbinols at positions 16 and 17 on the estrane skeleton. On the reduction of estrone (II) (which is ketonic at position 17) to the secondary alcohol stage, two isomeric estradiols are possible. Both these estradiols are known.[7] Similarly, the reduction of 16-ketoestrone (an $\alpha$-diketone) should give rise to four isomeric estriols. Previously only one estriol was known—the naturally occurring theelol. The isomer prepared by us has been designated isoestriol-A in view of the fact that the geometric arrangement of the two carbinols at positions 16 and 17 has not as yet been determined. It is our goal to prepare the remaining two isomers in order that all four compounds of the series $\Delta$1,3,5-estratrientriol-3,16,17 be on hand for experimentation on tumor production.




Isoestriol-A was prepared from estrone by a five step synthesis involving benzoylation of estrone, formation of the 16-oximino derivative, saponification of the ester, reductive hydrolysis of the oximino group, and, finally, hydrogenation of the ketol (or ketols).

Litvan and Robinson[8] in 1938 first prepared the 16-oximino derivative of a steroid when they submitted estrone methyl ether to reaction with isoamyl nitrite in a medium of *t*-butyl alcohol and potassium *t*-butoxide. We followed their procedure with modifications but were forced to employ an ester of estrone in order that the free phenol might be easily recovered. Estrone benzoate was chosen in the hope that with it, under the conditions of our reaction, saponification would occur much less readily than nitrosation. Such was very likely the situation, for, after saponification 16-oximinoestrone (III) was obtained in 80% yield.

(1) National Research Fellow in the Medical Sciences, 1941–1942.

(2) Marrian, *Biochem. J.*, **24**, 435 (1930).

(3) Doisy, Thayer, Levin and Curtis, *Proc. Soc. Exp. Biol. Med.*, **28**, 88 (1930).

(4) Browne, cited by Collip, *Proc. Calif. Acad. Med.*, **1**, 38 (1931).

(5) Doisy, Veler and Thayer, *Am. J. Physiol.*, **90**, 329 (1929).

(6) Huffman, THIS JOURNAL, **64**, 2235 (1942).

(7) Schwenk and Hildebrandt, *Naturwissenschaften*, **21**, 177 (1933); Whitman, Wintersteiner and Schwenk, *J. Biol. Chem.*, **118**, 792 (1937).

(8) Litvan and Robinson, *J. Chem. Soc.*, 1997 (1938).

Recently Stodola, Kendall and McKenzie[9] found that the mild reduction of 16-oximinodehydroisoandrosterone using zinc and aqueous acetic acid yielded only a small amount (less than 5%) of the expected amine, the bulk of the compound having been changed to a mixture of isomeric α-ketols. Our experience with the zinc–acetic acid reduction of 16-oximinoestrone was similar, as we rocovered a nitrogen-free material in over 90% yield. The exact composition of this material is not known, but certainly the preponderating component is an α-ketol. On further reduction of the α-ketolic material with hydrogen and Adams catalyst a triol (m.p. 267–269°)[10] was obtained which on admixture with authentic theelol (m.p. 271°) showed a depression of some 10°. Furthermore, the new triol gave a triacetate melting at 152° and a monomethyl ether melting at 141–142°, as contrasted against the analogous derivatives[11] of theelol which melt at 127 and 159°, respectively. The values[11] listed in the literature for the optical rotation of estriol vary from +57 to +66°, while that of isoestriol-A was found to be +88°. Isoestriol-A is also less soluble in ethanol than naturally-occurring estriol.

## Experimental

**Benzoylation of Estrone.**[11]—Estrone (II) was benzoylated by the Schotten–Baumann method. For each gram of estrone, 570 cc. of 0.5 *N* potassium hydroxide and 10 cc. of benzoyl chloride were employed.

**16-Oximinoestrone (16-Isonitrosoestrone) (III).**—Potassium metal (1.0 g.) was dissolved in 40 cc. of *t*-butyl alcohol and 766 mg. of powdered estrone benzoate (m. p. 215–216°) added. The mixture was stirred for five hours to dissolve the ester and then 0.8 cc. of freshly-prepared isoamyl nitrite pipetted into the solution, which at once turned red in color. After the red solution had been stirred for four hours, another 0.8-cc. portion of isoamyl nitrite was added, and the stirring continued for an additional eight hours. The whole procedure was conducted at room temperature under an atmosphere of dry nitrogen.

The reaction mixture was transferred to a separatory funnel containing a solution of 10 g. of glycine in 150 cc. of water, and the whole shaken with ethyl ether until solution of all components was effected. After separation, the ether was extracted once with water and then with 0.5 *N* potassium hydroxide. In order to ensure complete saponification, the yellow potassium hydroxide layer was allowed to remain at room temperature for four hours. It was then extracted twice with ether, acidified with concentrated hydrochloric acid, and placed at 0°. A bulky white precipitate soon formed. After standing overnight, the precipitate filtered easily, and it was submitted to copious washing with water. On drying in the desiccator over calcium chloride, 495 mg. (81% yield)[12] of α-oximino ketone melting at 210–211° was obtained. On recrystallization from aqueous methanol, the 16-oximinoestrone crystallized in small tufts of fine, practically colorless needles which melted at 214–215° with decomposition.

*Anal.* Calcd. for $C_{18}H_{21}O_3N$: N, 4.68. Found: 4.59.

16-Oximinoestrone is a typical α-oximino ketone[13] in that its solution is colorless in ether but yellow in alkali.

**Reduction of 16-Oximinoestrone with Zinc and Acetic Acid.**—16-Oximinoestrone (397 mg.) was dissolved in 12.0 cc. of glacial acetic acid and 0.5 cc. of water. Zinc dust (1.0 g.) was added and the mixture swirled in a water-bath at 40–45°. The α-oximino ketone soon dissolved to give a yellow solution with a greenish fluorescence. Water[14] (11.5 cc.) was then added and the solution heated under reflux in an oil-bath held at a temperature of 120–125°. At first the color of the solution deepened to an intense green but this gradually disappeared, and at the end of 1.5 hours only a slight color remained. The hot solution was then decanted from the zinc into a separatory funnel containing an ice-cold aqueous solution of 9.0 g. of sodium hydroxide. A further rinsing of the zinc was performed with 5.0 cc. of hot acetic acid and the latter combined with the previous decantation. The mixture was shaken out with ethyl ether, the ether separated and washed successively with 1.0 *N* hydrochloric acid, aqueous bicarbonate, and water. Evaporation of the ether yielded a crystalline, almost colorless residue which on recrystallization from aqueous methanol with the use of charcoal gave 320 mg. The principal constituent in this product is an α-ketol which will be described in a subsequent publication.

**Isoestriol-A.**—The 320 mg. of α-ketolic product from the zinc–acetic acid reduction of 16-oximinoestrone was dissolved in 150 cc. of 0.5 *N* sodium hydroxide and 600 mg. of Adams catalyst added. After being shaken for twenty-four hours under hydrogen, the solution was filtered from the platinum and the latter washed with 0.5 *N* sodium hydroxide. The alkaline filtrate was acidified and extracted with ethyl ether. After being washed with aqueous bicarbonate and then with water, the ether was distilled off. In order to remove any possible unreduced ketone, the ether residue was treated with carboxymethoxylamine by a procedure previously published.[15] The non-ketonic fraction was recrystallized twice from acetone to yield 110 mg. of white microscopic crystals melting at 267–269°. This melting point remained the same after treatment with charcoal and successive recrystallizations from acetone, methanol and ethanol. A mixed melting point taken with authentic theelol[16] showed a depression of about 10 degrees. $[\alpha]^{29.5}D$ +88° (0.227% solution in 95% ethanol).

*Anal.* Calcd. for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 74.88; H, 8.40.

**Isoestriol-A Methyl Ether.**—Isoestriol-A (35 mg.) was dissolved in 20 cc. of 0.5 *N* sodium hydroxide and 0.5 cc. of redistilled dimethyl sulfate added. After being shaken for one and one-half hours the reaction mixture was left at room temperature overnight. The white precipitate was then filtered, washed several times with 0.5 *N* sodium hydroxide, and finally with water. After being dried in the desiccator the product was dissolved in methanol, treated with charcoal, and recrystallized from aqueous methanol at 0°. The yield was 12 mg. of white microscopic crystals melting at 141–142°. For analysis the ether was dried to constant weight *in vacuo* over phosphorus pentoxide at 80°.

*Anal.* Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.23; H, 8.72.

**Isoestriol-A Triacetate.**—To 42 mg. of Isoestriol-A, 3 cc. of acetic anhydride and 3 cc. of pyridine were added. The solution was heated for three hours on the steam-bath, cooled and diluted with 50 cc. of cold water. Well-formed

(9) Stodola, Kendall and McKenzie, *J. Org. Chem.*, **6**, 841 (1941).

(10) All melting points in this paper are uncorrected.

(11) Doisy, in "Sex and Internal Secretions," Williams and Wilkins Co., Baltimore, p. 866 (1939).

(12) This reaction has been carried out several times, and the yield is usually around 80%. Once, however, we obtained a 96% yield of pure product.

(13) Meyer and Jacobson, "Lehrbuch der Organischen Chemie," Veit & Co., Leipzig, Sec. Ed., I, Part 2, p. 829 (1913).

(14) After conducting several zinc–acetic acid reductions on 16-oximinoestrone by the procedure of Stodola, Kendall and McKenzie, we decided that it was advantageous to use a more dilute solution of acid and to reflux longer if necessary, since, by this alteration, less fluorescing by-product seemed to be formed.

(15) Huffman, MacCorquodale, Thayer, Doisy, Smith and Smith, *J. Biol. Chem.*, **134**, 594 (1940).

(16) Kindly supplied us by Dr. Louis Levin, College of Physicians and Surgeons, Columbia University.

crystals came out at once; the mixture was left at 0° overnight, filtered, and the crystals washed with water and dried in the desiccator over calcium chloride and potassium hydroxide. On recrystallization from methanol at 0°, long slender needles were slowly deposited; yield, 24 mg. melting at 152°.

*Anal.* Calcd. for $C_{24}H_{30}O_6$: C, 69.54; H, 7.30. Found: C, 69.13; H, 7.51.

**Solubility of Isoestriol-A.**—No direct solubility measurements were made; however, in attempting to prepare a solution for polarimetry it was found impossible to dissolve 23.66 mg. of the triol in 3.00 ml. of 95% ethanol at room temperature. The solubility of theelol in 95% ethanol has been found to be 1.20 g. (10°) and 1.65 g. (30°) in 100 g. of solvent.[17] It was further observed that isoestriol-A dissolves in 0.5 *N* sodium hydroxide much more readily than does theelol.

**Ultraviolet Absorption Spectrum.**—Ultraviolet absorption spectra of isoestriol-A and of theelol were obtained by use of the Hilger medium spectrograph. The two curves were identical, each with the other, and with that of theelol previously published by Callow.[18] This finding was anticipated, since isomers of this nature are not expected to show differences in ultraviolet absorption spectra.

(17) Doisy, Huffman, Thayer and Doisy, *J. Biol. Chem.*, **138**, 283 (1941).

(18) Callow, *Biochem. J.*, **30**, 906 (1936).

**Acknowledgment.**—The authors wish to thank Miss Georgiana Becker for technical assistance and to express their appreciation to Mr. William Saschek for the micro-analyses reported in this paper. The authors are very grateful to Mr. Floyd H. Eggert, U. S. Standard Products Company, for the generous gift of crude estrone. To Professor Hans T. Clarke, who furnished the facilities for this research and who was a constant counselor throughout, they are deeply indebted.

## Summary

1. 16-Oximinoestrone has been prepared from estrone benzoate.

2. Using 16-oximinoestrone as an intermediate, an estriol isomeric with theelol has been synthesized. Like theelol, the new estriol is a Δ1,3,5-estratrientriol-3,16,17.

WOODWORTH, WISCONSIN RECEIVED OCTOBER 22, 1943

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF DUKE UNIVERSITY]

# The Use of Certain Phenyl Esters in the Reformatsky Reaction[1]

BY MELVIN S. BLOOM AND CHARLES R. HAUSER

The Reformatsky reaction using ethyl esters instead of aldehydes or ketones as the carbonyl component has not generally been very satisfactory.[2] Since phenyl esters have a more reactive carbonyl group than the corresponding ethyl esters (judging from their relative rates of alkaline hydrolysis)[3] it seemed possible that phenyl esters might serve satisfactorily as the carbonyl component in the Reformatsky reaction. This has been realized in certain cases but not in others.

It has been found that, although ethyl benzoate fails, phenyl benzoate undergoes satisfactorily the Reformatsky reaction with ethyl α-bromoisobutyrate to form ethyl benzoyldimethylacetate.

$$C_6H_5CO_2C_6H_5 + BrC(CH_3)_2CO_2C_2H_5 \xrightarrow{Zn} C_6H_5COC(CH_3)_2CO_2C_2H_5 + C_6H_5OZnBr$$

The yield (52%) of the β-keto ester obtained by this method is essentially the same as that obtained by the benzoylation of ethyl isobutyrate in the presence of the triphenylmethide ion.[4]

Phenyl esters are satisfactory as the carbonyl component in the Reformatsky reaction apparently only when neither the phenyl ester nor the bromoester has α-hydrogens. With 4-phenylphenyl acetate and ethyl α-bromoisobutyrate, ethyl α,α-dimethylacetoacetate was obtained in only low yield (11%), while with phenyl benzoate and ethyl α-bromoacetate or with 4-phenylphenyl propionate and ethyl α-bromoacetate, only very low yields of the respective β-keto esters appeared to be formed. Since most of the zinc was used up in these experiments, the low yields in these cases appears to be due to the predominance of side or subsequent reactions, which, among others, may involve the self-condensation of the phenyl ester (when it has an α-hydrogen) and the enolization of the β-keto ester formed from bromoesters having α-hydrogens. Both of these reactions could probably be effected by the organozinc halide which is presumably the intermediate in the Reformatsky reaction.[2]

(1) Paper XXI on "Condensations"; paper XX, THIS JOURNAL, **65**, 2051 (1943).

(2) See especially Shriner, "Organic Reactions," Roger Adams, Editor-in-Chief, John Wiley and Sons, New York, N. Y., 1942, Chapter I.

(3) See Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1940, p. 211.

(4) Hauser and Renfrow, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, New York, N. Y., 1943, p. 268.

## Experimental

**Ethyl Benzoyldimethylacetate.**—In a 500-cc. three-necked flask fitted with a mechanical stirrer, a separatory funnel, and a reflux condenser protected with a calcium chloride tube was placed 16.4 g. (0.25 mole) of zinc foil which had been sandpapered and cut into small strips. A mixture of 48.8 g. (0.25 mole) of ethyl α-bromoisobutyrate (b. p. 49–51° (10 mm.)), 49.6 g. (0.25 mole) of phenyl benzoate (m. p. 68–68.5°), 100 cc. of dry benzene, and 150 cc. of dry toluene was placed in the separatory funnel. About 50 cc. of this solution was added to the zinc and the flask was heated to 150° in an oil-bath until the reaction started. The mixture was then stirred and the rest of the solution introduced at such a rate that gentle refluxing occurred, about thirty minutes being required. Refluxing and stirring were continued for an additional three hours. The flask was cooled in an ice-bath and its contents poured into ice-cold 10% sulfuric acid with vigorous stirring. The acid layer was separated and the benzene–toluene layer was washed with water. The combined wash water and