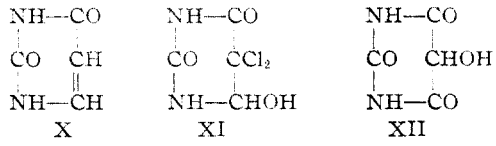
Formation of 5-Chloro-5-bromoxyhydroorotic Acid (VIII).—This acid is formed in excellent yield by the action of bromine water at ordinary temperature on the oxide of 6-methyl-6-oxy-5-chloro-1,5-bicyclouracil (II). The pyrimidine is moderately soluble in hot water and crystallizes in the form of prismatic crystals melting at 192–193°. It was dried for analysis at 100–110°. *Anal.* Calcd. for $C_5H_4O_5N_2BrCl$: N, 9.63. Found: N, 9.63.

The Conversion of 5,5-Dihalogenated-6-hydroxyhydroorotic Acids to Dialuric Acid.—The two new hydroorotic acid derivatives, namely: 5,5-dichloroxyhydroorotic acid (VII) and 5-chloro-5-bromoxyhydroorotic acid (VIII) bear the same structural relationship to orotic acid as 5,5-dichloroxyhydrouracil (XI) does to the uracil molecule (X).

The author now finds that these two hydropyrimidines (VII) and (VIII) cannot be distinguished from 5,5-dichlorhydrouracil (XI) by application of the standard



X XI XII

Wheeler and Johnson color reaction for uracil.[10] By application of their well-known technique the 6-carboxyl groups in the two pyrimidines (VII) and (VIII) are removed by the action of barium hydroxide yielding at once dialuric acid (XII), and its characteristic purple-colored barium salt.

These are the first pyrimidines of the halogenated 6-hydroxyhydrouracil type, so far discovered, which have responded to this characteristic color reaction reported by Wheeler and Johnson as a specific color test for uracil and cytosine.[10]

Summary

1. The oxide of 6-methyl-6-oxy-5-chloro-1,5-bicylouracil is converted smoothly by reduction to a true uracil derivative.

2. The chemical action of three different oxidizing reagents has thus far been studied namely: superoxol and hydrochloric acid, bromine water and nitric acid.

3. The methyl group of this 1,5-bicyclopyrimidine compound is oxidized to carboxyl by the action of all three reagents, with regeneration of the normal pyrimidine structure, yielding characteristic derivatives of hydroorotic acid.

BETHANY, CONNECTICUT RECEIVED JULY 20, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

The Acid Hydrolysis of a 6-Aryl-5,5-dichloroxyhydrouracil[1]

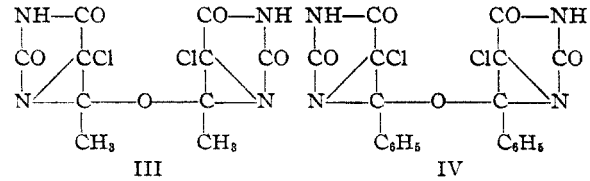
BY TREAT B. JOHNSON[2]

In the light of the results obtained during the previous experimentation on acid hydrolysis of 5,5-dichloro-5-hydroxy-6-methylhydrouracil[3] (I), it became of increased interest to the author to undertake a similar study with the simplest aromatic representative of this heterocyclic pyrimidine series, namely, 5,5-dichloro-6-hydroxy-6-phenylhydrouracil (II). Very little is known about the chemistry of such aromatic pyrimidine compounds as represented by (II).

I II

Judging from the experience of the author in other fields of pyrimidine research, it might be predicted that the study of such aryl derivatives as (II) would disclose further evidence in support of reaction mechanisms that have previously been postulated[3] regarding positions 1-, 5- and 6- in this type of hydrouracil compound. The pyrimidine (II) is easily prepared, without substitution of chlorine in the 6-phenyl group, by the action of superoxol and concentrated hydrochloric acid on 6-phenyluracil.[4] It is much less soluble in water than the corresponding 6-methylpyrimidine compound (I).

It has been shown by the author[3] that the 6-methylpyrimidine (I) undergoes a characteristic change when digested with strong hydrochloric acid yielding the first representative of a new type of pyrimidine compound to which has been assigned the constitution of the oxide of 6-methyl-6-oxy-5-chloro-1,5-bicyclouracil (III). In fact, the 1,5-bicyclouracil structure expressed in this formula (III) is favored by the oxide structure functioning in position 6- of the pyrimidine molecule.



III IV

The author now finds that 5,5-dichloro-6-hydroxy-6-phenylhydrouracil (II) is much less stable, under the influence of strong hydrochloric acid, than its corresponding 6-methyl-analog (I). No evidence has thus far been obtained by the author of the formation of a condensed 1,5-bicyclouracil compound (IV) by digestion of (II) with this mineral acid.

(1) Researches on Pyrimidines, CLXXXII.

(2) Experimental work conducted in the Bethwood Research Laboratory, Bethany, Connecticut.

(3) Johnson, THIS JOURNAL, **65**, 1220 (1943).

(4) Fischer and Roeder, *Ber.*, **34**, 3763 (1901); Wheeler and Merriam, *Am. Chem. J.*, **29**, 490 (1903); Johnson and Hemingway, THIS JOURNAL, **37**, 280 (1915).

TABLE I

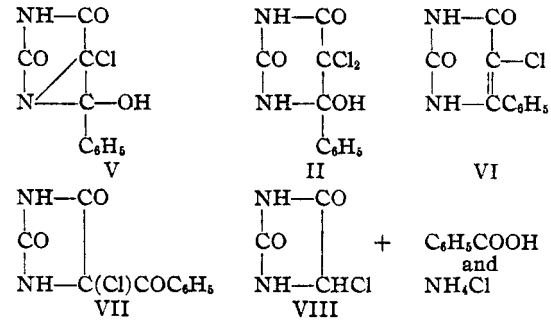
ACID HYDROLYSIS OF 5,5-DICHLORO-6-HYDROXY-6-PHENYLHYDROURACIL (II)

Reactant, g.	Hydrochloric acid, cc.	Digestion period, hr.	Reaction products	Analyses, $C_{10}H_8O_3N_2Cl_2$ Calcd.	Found
The hydropyrimidine (II), 1	100 of concd. acid	2 at 100°	Reactant recovered = 0.75 g., NH_4Cl identified	C, 43.66	43.9
				H, 2.91	3.17
				N, 10.18	10.09
1	100	6 at 100°	Reactant completely dextroyed. Benzoic acid and NH_4Cl identified		
1	100	4 at 100°	Recovered 0.4 g. benzoic acid and 4 mg. of reactant m. p. 209–210°. 5-Chloro-6-phenyluracil identified m. p. 260–261°. Also NH_4Cl	N, 10.18	9.95
1	100	4 at 100°	Recovered 0.5 g. of benzoic acid, and 3.5 mg. of reactant. NH_4Cl identified		

While aryl derivatives of uracil-like 6-phenyluracil and 5-chloro-6-phenyluracil, for example, can be heated with strong hydrochloric acid without degradation of the pyrimidine cycle, the hydrouracil derivative (II) suffers complete destruction of the pyrimidine ring by such treatment with formation of benzoic acid and ammonium chloride. The only pyrimidine degradation product identified after the hydrolysis was a few milligrams of 5-chloro-6-phenyluracil (VI). The results of four hydrolysis experiments obtained after two, four and six hours of digestion with strong hydrochloric acid are recorded in Table I of the experimental part of this paper. The author obtained no evidence of the formation of cinnamic acid or any derivative of this acid as a degradation product of (II).

The most surprising result of this hydrolytic change by the action of hydrochloric acid is the quantitative conversion of (II) to benzoic acid. To explain this molecular transformation of the pyrimidine (II), the author has presupposed the possibility of formation of different intermediate compounds, but he concludes at present, that the formation of a 1,5-bicyclouracil derivative (V) is probably the first intermediate product of hydrolytic change. According to this interpretation of reaction we are dealing with an hypothetical **ethylene-imide** cycle, which would be predicted to be unstable due to the influence of a free hydroxyl group in position 6- of (V) and (II), and to revert in acid solution to the hypothetical hydantoin derivative (VII) following scission of the **ethylene-imide** cycle in the 1,6-position of (V). In the corresponding hydrolysis study of the pyrimidine (I) the **ethylene-imide** ring structure is stabilized by the absence of hydroxyl due to the formation of the oxide or ether structure functioning in position 6- of (III).

This intermediate, hydantoin derivative (VII) would be predicted from our present knowledge of the chemistry of such heterocyclic compounds to undergo degradation easily, by hydrolysis with strong hydrochloric acid, leading to complete destruction of the hydantoin ring and production of benzoic acid and ammonium chloride. The hydantoin expressed by formula (VIII) has not, thus far, been isolated as a product of reaction. The study of the behavior on acid hydrolysis of aryl and alkyl substituted pyrimidine compounds of types (I) and (II) will be continued by the author.



Experimental Part

Formation of 5,5-Dichloro-6-hydroxy-6-phenylhydrouracil from 6-Phenyluracil.—Two grams of finely pulverized 6-phenyluracil[4] was stirred into a cold mixture of 30 cc. of superoxol and concentrated hydrochloric acid, respectively. The pyrimidine did not dissolve at any stage of the operation, and was allowed to stand for fully twenty-four hours when the colorless reaction product was filtered off and purified by recrystallization from hot water. It separated on cooling in the form of flat prisms melting at 209–210° with violent effervescence. The compound contained chlorine, and was dried at 100–110° before analysis.

Anal. Calcd. for $C_{10}H_8O_3N_2Cl_2$: N, 10.18. Calcd. for $C_{10}H_7O_2N_2Cl$: N, 12.58. Found: N, 9.75, 9.86.

In a second experiment 3.5 g. of 6-phenyluracil was triturated with a mixture of 50 cc. of superoxol and 50 cc. of concentrated hydrochloric acid for twenty-four hours. We obtained 4.0 g. of a colorless product melting at 195–196° with decomposition. After recrystallization from hot water this product melted at 209–210° with violent effervescence.

Reduction of 5,5-Dichloro-6-hydroxy-6-phenylhydrouracil (II). Formation of 5-Chloro-6-phenyluracil (VI).—One gram of the hydrouracil derivative mixed with 10 cc. of hydriodic acid (sp. gr. 1.5) and 0.5 g. of red phosphorus was heated to 100°, and the mixture diluted with 20 cc. of water. This was then digested for fifteen minutes at the boiling point and finally cooled. Following filtration, the phosphorus residues were triturated with dilute sodium hydroxide and filtered giving a clear alkaline solution. Acidifica-

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.

BETHANY, CONNECTICUT RECEIVED AUGUST 6, 1943

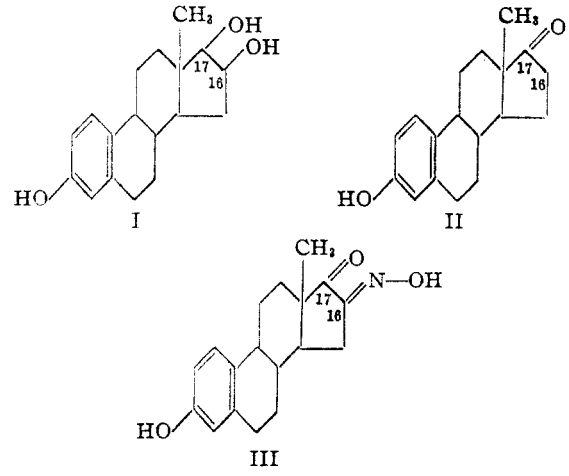
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

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 α-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 Δ1,3,5-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).