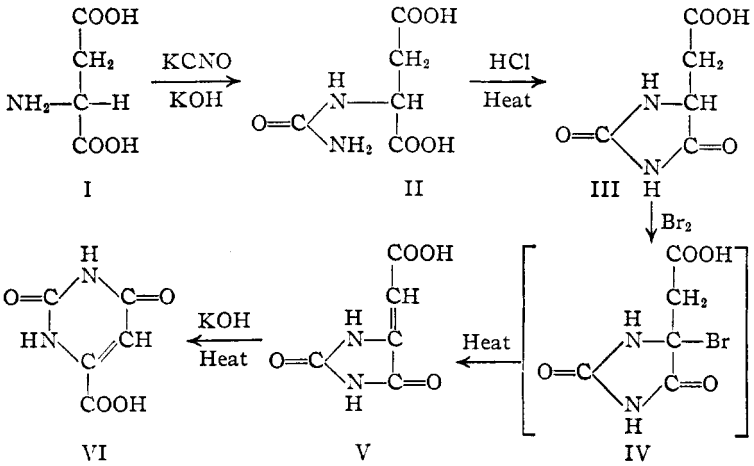
[CONTRIBUTION FROM THE KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

Synthesis of Orotic Acid from Aspartic Acid

BY JOSEPH F. NYC AND HERSCHEL K. MITCHELL

Since the isolation of orotic acid (4 carboxyuracil) from milk by Biscaro and Belloni,[1] the compound has been synthesized in a number of different laboratories by several different methods.[2-6]

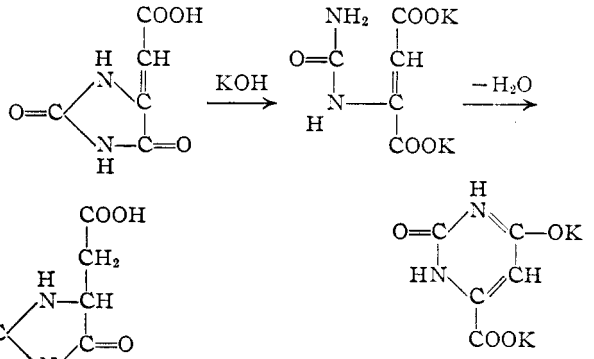
Recently it has been shown that orotic acid can be obtained in a good yield from 5-(carboxy-methylidine)-hydantoin by treatment with alkali.[7] Consequently the total synthesis of orotic acid from aspartic acid has been investigated. The series of reactions involved in this synthesis may be represented by the following equations



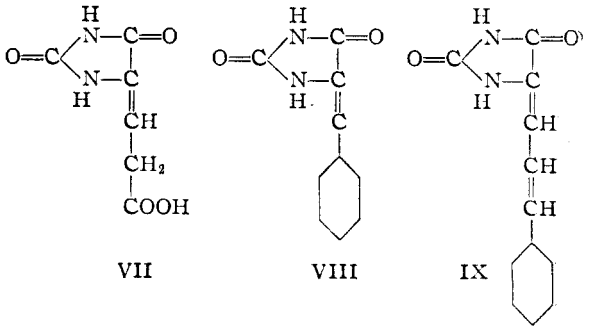
Compound III was obtained by Gabriel[8] directly from I without isolation of compound II. In the present work it has been found advantageous to isolate the intermediate II, since it is less soluble than III and can be more easily separated from by-products of the first reaction.

Gabriel[8] also reported the isolation of compound IV, which was then heated dry to obtain V. This procedure has been revised for the present purposes, since it has been found that V is obtained directly from the reaction mixture of III and bromine in acetic acid by crystallization from hot water.

In a previous publication[7] the mechanism for conversion of V [5-(carboxy-methylidine)-hydantoin] to VI (orotic acid) was not considered. A plausible mechanism for this reaction involves the opening of the hydantoin ring to form an unsaturated hydantoic acid followed by closure to formthe pyrimidine derivative in accordance with the reactions



Although the first reaction would be expected to take place as a result of alkaline treatment, the ring closure giving the pyrimidine would not ordinarily be expected until acidification of the solution containing the hydantoic acid. It has been demonstrated, however, by following changes of absorption spectra in alkaline solutions that the salt of orotic acid is produced during the alkaline treatment. In order to test the possibility that the orotic acid is formed by a rearrangement other than that suggested by the above reactions, 5-(carboxyethylidene)-hydantoin (VII), benzalhydantoin (VIII) and cinnamalhydantoin IX were prepared and subjected to alkaline treatment.



The course of reaction was followed by determinations of the absorption spectra of the reaction mixtures. In all three cases, in contrast to the similar treatment of 5-(carboxy-methylidine)-hydantoin, the absorption bands nearly disappeared, approaching those that would be expected for hydantoic acids rather than those of substituted pyrimidines.

(1) G. Biscaro and E. Belloni, *Ann. Soc. Chim. Milano*, XI, fasch I and II (1905); *Chem. Zentr.*, **76**, II, 63, 64 (1905).

(2) H. L. Wheeler, *Am. Chem. J.*, **38**, 358 (1905).

(3) R. Behrend and K. Struve, *Ann.*, **378**, 163 (1910).

(4) M. Bachstez, *Ber.*, **63A**, 1000 (1930).

(5) T. B. Johnson and E. F. Schroeder, THIS JOURNAL, **53**, 1989 (1931).

(6) G. E. Hilbert, *ibid.*, **54**, 2076 (1932).

(7) H. K. Mitchell and J. F. Nyc, THIS JOURNAL, **69**, 674 (1947).

(8) S. Gabriel, *Ann.*, **348**, 50 (1906).

Experimental

Ureidosuccinic Acid (II).—This compound has been described a number of times in the literature. A mixture of 13 g. of aspartic acid, 8 g. of potassium cyanate, and 100 ml. of 1 *N* potassium hydroxide was allowed to stand at room temperature for sixteen hours. The solution was acidified with concentrated hydrochloric acid and after two hours of standing the hydantoic acid was filtered off and recrystallized from water. Yield was 10.5 g. (68%), m. p. 178–180°.

Anal. Calculated for $C_5H_8N_2O_5$: C, 34.10; H, 4.56; N, 15.91. Found: C, 34.29; H, 4.79; N, 15.79.

5-(Acetic Acid)-hydantoin (III).—Ureidosuccinic acid (10.5 g.) was dissolved in 35 ml. of 20% hydrochloric acid and the mixture evaporated nearly to dryness on a hot-plate. The residue was recrystallized from water. Yield was 7.9 g. (84%), m. p. 214–216°.

5-(Carboxy-methylidine)-hydantoin (V).—A mixture of 1.28 g. of bromine, 4 g. of 5-(acetic acid)-hydantoin and 16 ml. of glacial acetic acid was heated with shaking at 100° for one and one-half hours in a sealed tube. The precipitated product was filtered off and suspended in 50 ml. of boiling water for twenty minutes. Water was added to the boiling suspension until all of the solid had dissolved. On cooling 5-(carboxy-methylidine)-hydantoin crystallized. Yield was 2.92 g. (73%); m. p. above 400°. Absorption spectrum Fig. 2B (see also reference 7).

Anal. Calcd. for $C_5H_4N_2O_4$: C, 38.48; H, 2.56; N, 17.94. Found: C, 38.50; H, 2.84; N, 18.22.

Orotic Acid (VI).—In a previous publication[7] it was reported that 5-(carboxy-methylidine)-hydantoin can be converted to orotic acid by treatment with alkali at 100°. It has been found that a much better yield is obtained by treatment at a lower temperature. Six hundred mg. of 5-(carboxy-methylidene)-hydantoin was dissolved in 20 ml. of 1 *M* potassium hydroxide in a constant temperature bath at 64°. The course of the reaction was followed by sampling at fifteen minute intervals and determining the absorption spectrum on each sample diluted in 0.1 *M* potassium hydroxide. Absorption curves are given in Fig. 1 for 0 (A), 15 (B), 60 (C) and 120 (D) minutes. Curves A and D correspond to spectra in alkaline solution of beginning and final products. Following the two-hour reaction period the solution was acidified with concentrated hydrochloric acid. The precipitated orotic acid was collected and recrystallized from water; yield of anhydrous compound, 557 mg. (93%); decomposition temperature by immersion of sample in a bath at 320°, 343–345°. The absorption spectrum of the product corresponded to that previously described.[7]

Anal. Calcd.: N, 17.94. Found: N, 18.19.

5-(Carboxy-ethylidene)-hydantoin.—This compound was prepared from glutamic acid by the same method described above for the preparation of 5-(carboxy-methylidene)-hydantoin.

Anal. Calcd. for the intermediate 5-(propionic acid)-hydantoin, $C_6H_8N_2O_4$: C, 41.8; H, 4.69; N, 16.28. Found: C, 42.08; H, 4.79; N, 16.36; m. p. 171–173°. Calcd. for 5-(carboxy-ethylidene)-hydantoin, $C_6H_6N_2O_4$: C, 42.33; H, 3.54; N, 16.45. Found: C, 42.03; H, 3.39; N, 16.20; m. p. 220–222°. Absorption spectrum, Figure 2A. Both of these compounds have been prepared by Dakin.[9]

Benzal-hydantoin.—This compound was prepared according to the procedure of Wheeler and Hoffman[10]; m. p. 220–221, absorption spectrum, Fig. 2C. On treatment with 1 mole of potassium hydroxide at 100° for two hours, the characteristic absorption bands of the compound practically disappeared and a pyrimidine derivative could not be isolated.

Cinnamal-hydantoin.—The same procedure utilized above for preparation of benzal-hydantoin was utilized for synthesis of cinnamal-hydantoin. The product is a yellow compound crystallizing from 95% alcohol, m. p. (dec.) 270–275°. Absorption spectrum Fig. 2D.

Anal. Calcd. for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.01; H, 4.85; N, 12.84. The

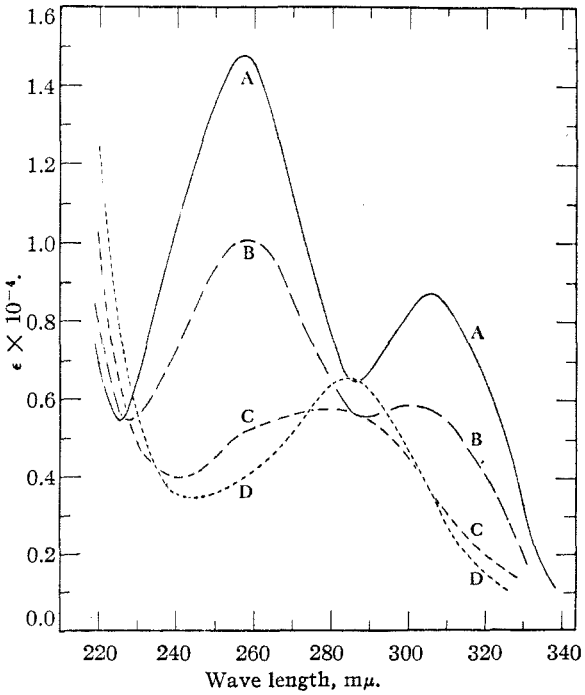


Fig. 1.—Changes in absorption spectrum during the conversion of 5-(carboxy-methylidene)-hydantoin to orotic acid in alkaline solution; A, 0; B, 15; C, 60; D, 120 minutes.

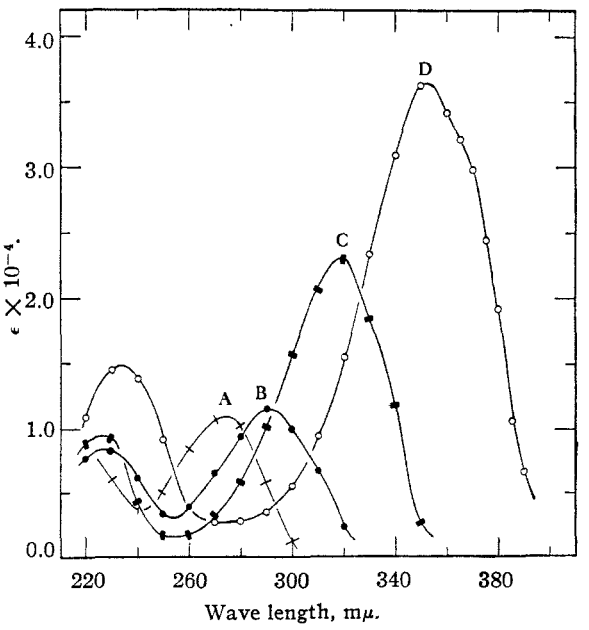


Fig. 2.—Absorption spectra of hydantoins in water: A, 5-(carboxyethylidene)-hydantoin; B, 5-(carboxy-methylidene)-hydantoin; C, benzalhydantoin; D, cinnamalhydantoin.

(9) H. D. Dakin, *Biochem. J.*, **13**, 402 (1919).

(10) H. L. Wheeler and C. Hoffman, *Am. Chem. J.*, **45**, 368 (1911).

compound reacted with alkali in a fashion similar to 5-(carboxy-ethylidene)-hydantoin and benzal-hydantoin and no evidence was obtained for conversion to a pyrimidine. This compound has been previously prepared by Johnson and Wrenshall.[11]

Discussion

The over-all yield of orotic acid obtained from aspartic acid was 39%. This yield is considerably better than by methods previously described in the literature. In addition, all of the reactions are technically simple and the yields are easily reproduced.

It appears most probable that the conversion of 5-(carboxy-methylidene)-hydantoin to orotic acid goes through an intermediate hydantoic acid which recyclizes rapidly in the presence of alkali to give a pyrimidine ring. Although the hydantoic acid has not been isolated the alternative mechanism involving direct ring enlargement is made improbable by the fact that three related hydantoins are not converted to pyrimidines on alkaline treatment.

(11) T. B. Johnson and R. Wrenshall, THIS JOURNAL, **37**, 2138 (1915).

It is of interest to note both the increase in $\epsilon_{max.}$ and wave length of maxima with increased conjugation in the four hydantoins described (Fig. 2).

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Summary

1. The total synthesis of orotic acid from aspartic acid has been described. The over-all yield obtained was 39%.

2. The rearrangement of 5-(carboxymethylidene)-hydantoin to orotic acid has been discussed.

3. Absorption spectra are given for 5-(carboxy-ethylidene)-hydantoin, 5-(carboxy-methylidene)-hydantoin, benzal-hydantoin and cinnamalhydantoin.

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Synthesis of Cephalin[1]

BY W. GORDON ROSE

A supply of cephalin uncontaminated by other phospholipids is desired in this Laboratory as a substrate for certain enzyme investigations as well as for contemplating coupling experiments with purothionin.[1a] Since pure cephalin has never been obtained from natural sources, synthesis seemed to be the more promising alternative to obtain this material. Grün and Limpächer[2] described a synthesis of cephalin by heating distearin with phosphoric anhydride and adding ethanolamine carbonate to this reaction mixture. The product that was obtained sintered at 80°, melted with the formation of a meniscus at 177° and decomposed at 185°. Kabashima and Susuki[3a] and Kabashima[3b] also described a synthesis of cephalin. Their procedure consisted of heating together bromoethylamine picrate and the mono-silver salt of dipalmitoglycerophosphoric acid. They obtained a yield of 0.5 g. of dipalmitocephalin that melted at 77° from 5 g. of the above silver salt. In spite of the poor yield, the Kabashima procedure appeared to be the more attractive because it seemed less likely that the groups attached to the glycerol would change places, and because of the probability that the unprotected amino group of ethanolamine also participated in the reaction with the phosphatidic acid. After several disappointing experiments on the acylation of glycerophosphoric acid with palmitoyl chloride by the Schotten–Baumann procedure, it appeared that dipalmitoglycerophosphoric acid was more readily accessible by the phosphorylation of dipalmitin.

α,γ-Dipalmitin was first prepared by the direct acylation of glycerol with palmitoyl chloride and dry pyridine. The reaction mixture was allowed to stand several days before adding ether and washing out the pyridine with dilute acid. The product so obtained melted at 68–69°. Averill, Roche and King[4a] reported 69.5°, and Jackson, Daubert, King and Longnecker[4b] reported 72.5°, obtained by Fischer's α-iodohydrin procedure. α,γ-Dipalmitin which we subsequently prepared through the trityl chloride procedure[5] melted at 73.5°. Grün[6] reported 72.6° for the melting point of α,γ-dipalmitin prepared from dihydroxyacetone. Dipalmitin prepared in this Laboratory through the iodohydrin procedure melted at 72.0–72.5° and hence was somewhat less pure

(1) Enzyme Research Laboratory Contribution No. 104.

(1a) Balls, Hale and Harris, *Cereal Chem.*, **19**, 279–288 (1942).

(2) Grün and Limpächer, *Ber.*, **60**, 151 (1927).

(3) (a) Kabashima and Susuki, *Proc. Imp. Acad. (Japan)*, **8**, 492–495 (1931); (b) Kabashima, *Ber.*, **71**, 76–80; 1071–1073 (1938).

(4) (a) Averill, Roche and King, THIS JOURNAL, **51**, 870 (1929); (b) Jackson, Daubert, King and Longenecker, *ibid.*, **66**, 289 (1944).

(5) Verkade, van der Lee and Meerburg, *Rec. Trav. Chim.*, **54**, 716–724 (1935).

(6) Schönfeld Hefter, "Fette und Fettprodukte," Vol. I, "Chemie und Gewinning der Fette," Julius Springer, Vienna, 1936, p. 251.