the incorporation of isotopic nitrogen in positions 1 and 3 is described. The formation of uric acid from the reaction of isotopic urea with uramil or with 2,6-dioxy-4,5-diaminopyrimidine has been studied.

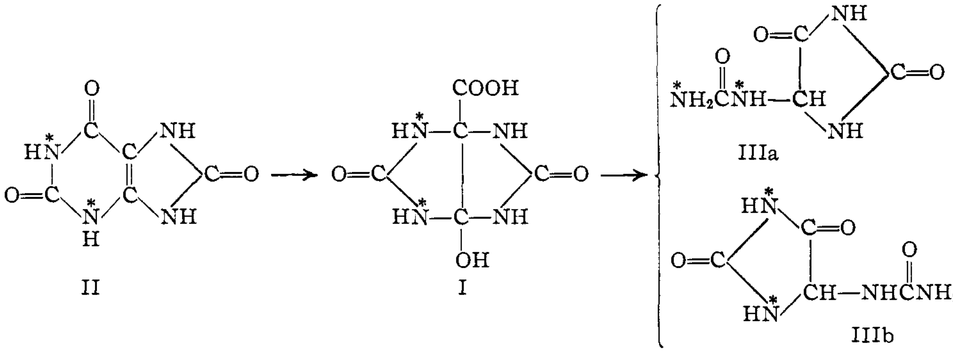
NEW YORK, NEW YORK RECEIVED MAY 19, 1947

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

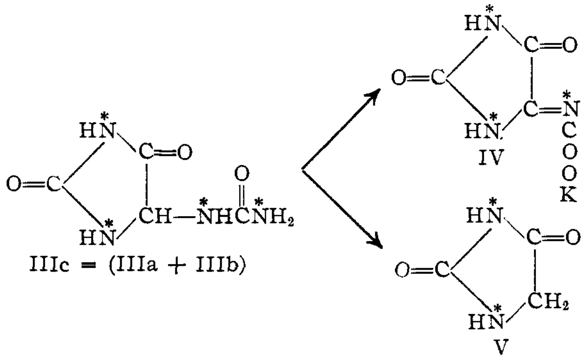
The Mechanism of the Oxidation of Uric Acid Studied with Isotopic Nitrogen as a Tracer[1]

BY LIEBE F. CAVALIERI AND GEORGE BOSWORTH BROWN

The oxidation of uric acid with potassium permanganate in alkaline solution results in the formation of allantoin,[2,3,4] while oxidation with nitric acid or chlorine produces alloxan.[5,6] In the alkaline permanganate oxidation it has been suggested[3] that the reaction proceeds *via* the symmetrical intermediate I. In support of this hypothesis, Fischer and Ach[7] have shown that both 1- and 7-methyluric acids yield upon oxidation 3-methylallantoin and that both 3- and 9-methyluric acids yield 1-methylallantoin. Schuler and Reindel[8] isolated the silver salt of an intermediate compound to which they assigned the symmetrical structure (I) originally postulated by Behrend. Klemperer[9] has also obtained what is believed to be this compound by the action of uricase on uric acid.



We have studied the oxidation of uric acid (II) containing an excess of isotopic nitrogen in positions 1 and 3. II was oxidized to allantoin with alkaline permanganate. If the symmetrical intermediate I is formed, the N^{15} will be present in only one pair of the nitrogen atoms of the molecule. The cleavage of either ring of this molecule will result in the formation of allantoin. The result in one case will be the introduction of the isotopic nitrogen into the urea moiety of the allantoin (IIIa) and in the other case into the ring of the allantoin (IIIb).[10] The allantoin isolated will contain the same number of molecules of each of IIIa and IIIb. Statistically, therefore, the allantoin will have the isotopic nitrogen distributed uniformly among all four nitrogens of the molecule.



A sample of the allantoin obtained (IIIc) was oxidized to potassium oxonate (IV) with potassium permanganate, while a second portion was converted by reduction with hydriodic acid to hydantoin (V). Isotope analyses showed that the nitrogen of the potassium oxonate and that of the hydantoin contained the same atom per cent. excess of N^{15} as the allantoin. The isotopic nitro-

---

[1] The authors gratefully acknowledge the use of funds from The Office of Naval Research and The Barker Welfare Foundation.

[2] Biltz and Schauder, *J. prakt. Chem.*, [2] **106**, 114 (1923).

[3] Behrend, *Ann.*, **333**, 146 (1904).

[4] Hartman, Moffett and Dickey, "Organic Syntheses," Coll. Vol. II, 21 (1943).

[5] Liebig and Wöhler, *Ann.*, **26**, 256 (1838).

[6] Biltz and Hehn, *ibid.*, **413**, 60 (1916).

[7] Fischer and Ach, *Ber.*, **32**, 2723 (1899).

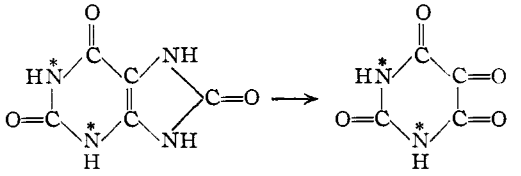
[8] Schuler and Reindel, *Z. physiol. Chem.*, **208**, 248 (1932).

[9] Klemperer, *J. Biol. Chem.*, **160**, 111 (1945).

[10] In the work of Fischer and Ach[7] only the substituted allantoins with the methyl group on the hydantoin ring were isolated. This may have been due to failure to isolate an accompanying allantoin with the methyl substituent on the urea moiety, or it may be that no such allantoin is formed because of a preferential cleavage of the unsubstituted ring of a methylated I.

gen originally present in the 1 and 3 positions of the uric acid was thus proven to have become distributed equally between the imidazolone and the urea moieties of the allantoin and the isotope in the urea moiety was shown to be equally distributed between its two nitrogens. This evidence furnishes direct proof of the formation of a symmetrical intermediate in this oxidation of uric acid to allantoin.

When the uric acid, labelled with isotopic nitrogen in the 1 and 3 positions, was oxidized either with nitric acid or with chlorine, the atom per cent. excess of N^{15} in the alloxan (VI) formed was twice that of the uric acid from which it was derived.



II VI

This demonstrates that the nitrogen atoms of the alloxan were derived solely from the 1 and 3 nitrogens of the uric acid. Thus the oxidation in acid media is a direct cleavage of the imidazolone ring with the formation of the alloxan from the pyrimidine ring alone.

Experimental

Uric Acid.—Two samples of uric acid containing N^{15} in the 1 and 3 positions, synthesized according to the directions given in another communication,[11] were shown to contain, respectively, 0.43 and 0.34 atom per cent. excess N^{15}.[12]

Allantoin.—Uric acid (1.20 g., 0.43 atom per cent. excess N^{15}) yielded 866 mg. of allantoin when oxidized according to the procedure of Hartman, Moffett and Dickey.[4] Recrystallization from water yielded 647 mg., (57%); 0.41 atom per cent. excess N^{15}.

Anal. Calcd. for $C_4H_6O_3N_4$: N, 35.4. Found: N, 35.3.[13]

Potassium Oxonate.—Allantoin was oxidized to potassium oxonate according to Biltz and Giesler.[14] Allantoin (100 mg.) yielded 85 mg. of potassium oxonate. Recrystallization from water gave 50 mg., (40%); 0.41 atom per cent. excess N^{15}.

Anal. Calcd. for $C_4H_2O_4N_3K$: N, 21.5. Found: N, 21.8.

Hydantoin.—The procedure of Baeyer[15] for the reduction of allantoin to hydantoin was simplified as follows. Allantoin (200 mg.) was refluxed with 2 cc. of hydriodic acid, containing a trace of hypophosphite, for eight minutes. The mixture was then evaporated to dryness with a stream of air. Three cc. of acetone was added to the dry residue and the solution was placed in an ice-box for one-half hour. The hydantoin (70 mg.) was collected by filtration and washed with fresh acetone and then recrystallized from ethyl alcohol. Yield was 57 mg. (45%); 0.43 atom per cent. excess N^{15}.

Anal. Calcd. for $C_3H_4O_2N_2$: N, 28.0. Found: N, 28.6.

Alloxan (a) Nitric Acid Oxidation.—The procedure for the oxidation of uric acid was similar to that of Nightingale[16] for the oxidation of alloxantin to alloxan. Uric acid (400 mg., 0.34 atom per cent. excess N^{15}) yielded 50 mg. of alloxan, 0.67 atom per cent. excess N^{15}.

Anal. Calcd. for $C_4H_2O_4N_2 \cdot 4H_2O$: N, 13.1. Found: N, 13.2.

(b) **Chlorine Oxidation.**—Uric acid (400 mg., 0.34 per cent. excess N^{15}) was treated with 107 mg. of potassium chlorate, 0.67 cc. of concentrated hydrochloric acid and 1.1 cc. of water.[17] After all the solid had gone into solution, the mixture was evaporated with a stream of air to about one-half the volume. After standing in the ice-box overnight, 100 mg. of alloxan crystallized, 0.69 atom per cent. excess N^{15}.

Anal. Calcd. for $C_4H_2O_4N_2 \cdot 4H_2O$: N, 13.1. Found: N, 13.5.

Summary

By the use of isotopic nitrogen as a tracer, it has been demonstrated that the oxidation of uric acid to allantoin with alkaline potassium permanganate proceeds through a symmetrical intermediate. Oxidation with either nitric acid or chlorine results in a direct cleavage of the imidazolone ring to produce alloxan.

NEW YORK, N. Y. RECEIVED JULY 31, 1947

---

[11] Cavalieri, Blair and Brown, THIS JOURNAL, **70**, 1241 (1948).

[12] We are indebted to Mr. Stephen Friedland of this Laboratory for the isotope analyses.

[13] We are indebted to Miss Alice Angelos for the nitrogen determinations.

[14] Biltz and Giesler, *Ber.*, **46**, 3413 (1913).

[15] Bayer, *Ann.*, **130**, 158 (1864).

[16] Nightingale, "Organic Syntheses," **23**, 5 (1946).

[17] Nightingale, *ibid.*, **23**, 6 (1946).