and chlorohydroxy compounds. This scheme is self-consistent and is in harmony with accepted theories of the Walden inversion and double bond addition reactions.

According to this scheme, epoxidation of the unsaturated acids with organic per-acids, or oxidation with alkaline potassium permanganate, takes place by *cis*-addition, whereas reaction with hypochlorous acid proceeds by *trans*-addition. Furthermore, it is postulated that opening (either in alkaline or acidic media) and re-forming of the oxirane ring, as well as replacement of a hydroxyl group of 9,10-dihydroxystearic acid by halogen, are each accompanied by an inversion. An inversion is also postulated when halogen is replaced by hydroxyl in the 9,10(10,9)-halohydroxystearic acids, and an explanation is offered for the formation of high- or low-melting 9,10-dihydroxystearic acid when 9,10(10,9)-iodohydroxystearic acids (from oleic acid) are treated with dilute or concentrated alkali, respectively.

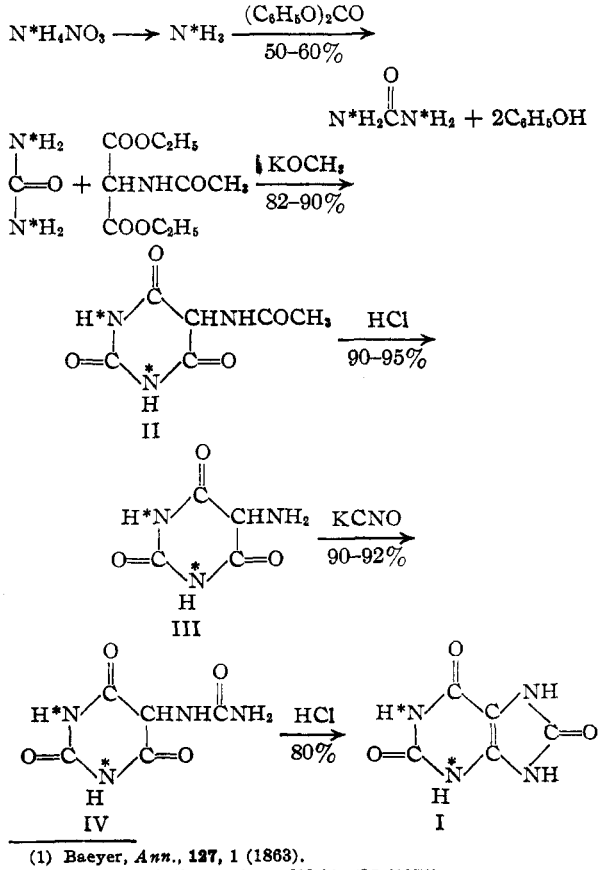
PHILADELPHIA 18, PA. RECEIVED JULY 10, 1947

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

The Synthesis of Uric Acid Containing Isotopic Nitrogen

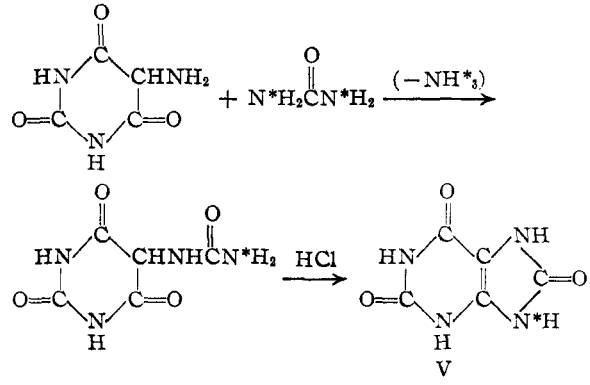
BY LIEBE F. CAVALIERI, VIRGINIA E. BLAIR AND GEORGE BOSWORTH BROWN

The existing procedures[1,2,3,4,5,6] for the synthesis of uric acid involve many steps and the over-all yields are low, based upon the urea used. For the introduction of isotopic nitrogen a modified synthesis has been developed in order to insure optimum utilization of the isotope. This synthesis introduces N^{15} in the pyrimidine ring (positions 1 and 3) (I) as shown in the following equations:

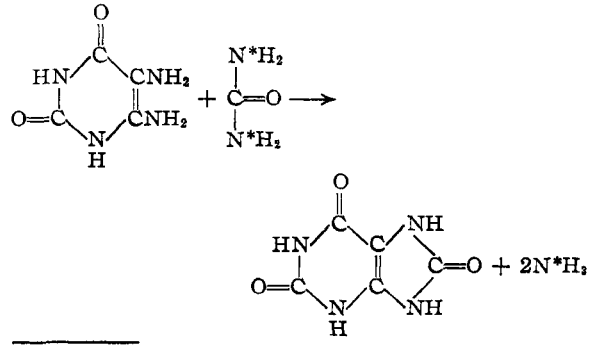


The over-all yield in this synthesis was 55%, based upon the urea used. The yields afforded by the older procedures can be estimated to be about 27%.

A second synthesis of uric acid (V) incorporating N^{15} presumably in position 9 was carried out according to the method of Grimaux.[2]



It will be noted that one amino group of the urea molecule was eliminated in the initial reaction. Cyclization with hydrochloric acid produced uric acid (V) containing about 1 atom of isotopic nitrogen. Since in the analogous reaction of 2,6-dioxy-4,5-diaminopyrimidine with urea[7] both of the amino groups of the urea were eliminated and no isotopic nitrogen was introduced into the uric



(1) Baeyer, *Ann.*, **127**, 1 (1863).
(2) Grimaux, *Bull. soc. chim.*, [2] **31**, 535 (1879).
(3) Fischer, *Ber.*, **30**, 559 (1897).
(4) Horbaczewski, *Monatsh.*, **8**, 201 (1887).
(5) Behrend and Roosen, *Ann.*, **251**, 235 (1889).
(6) Behrend, *ibid.*, **441**, 215 (1925).
(7) Levene and Senior, *J. Biol. Chem.*, **25**, 618 (1916).

acid it may be presumed that in the uric acid (V) formed from urea and uramil the isotopic nitrogen was introduced chiefly in position 9.

Experimental

Urea.—The preparation of urea from diphenyl carbonate and ammonia was reported by Hentschel.[8] Bloch and Schoenheimer[9] introduced the use of copper powder as the catalyst. The preparation is described here since the technique used was more reproducible, in our hands, than that of Bloch and Schoenheimer.

A three-necked flask was equipped with a reflux condenser, dropping funnel and an inlet tube for nitrogen. A potassium hydroxide drying tower was placed on top of the condenser and a wide-mouth tube from this was led into a glass bomb tube, 15 cm. above the bottom of the bomb. An outlet tube from the bomb tube was passed through an acid trap containing a known quantity of standard acid. A flask of liquid nitrogen was placed around the bomb tube to condense and freeze the isotopic ammonia.

A solution of 4.088 g. (0.0511 mole) of $N^{15}H_4NO_3$ (32.0 atom % excess N^{15}) in 25 cc. of water was introduced into the three-necked flask. The bomb tube was charged with an intimate mixture of 4.95 g. (0.0231 mole) of diphenyl carbonate and 130 mg. of copper powder. Before the ammonia was liberated from the ammonium nitrate, the apparatus was swept with nitrogen for ten minutes. The bomb tube was then immersed in the liquid nitrogen. While continuing the slow stream of nitrogen, an excess of sodium hydroxide was slowly introduced from the funnel and the generating flask was heated to boiling. Care must be exercised not to allow the ammonia to be liberated too rapidly since the inlet tube in the bomb may become clogged with crystalline ammonia. The generating flask was heated for three hours and about 5% of uncondensed ammonia was collected in the acid trap. The bomb tube was transferred to a solid carbon dioxide-bath and sealed. It was then heated in a water-bath at 90–100° for four hours. The contents of the tube were dissolved in a minimal amount (about 50-cc.) of warm water and the copper powder was removed by filtration. The filtrate was extracted with six 50-cc. portions of chloroform. The aqueous layer was decolorized and evaporated to dryness on a water-bath. The yield of crude urea was 0.977 g. Recrystallization from acetone gave 0.830 g. of urea (57%, based upon the ammonia condensed); m. p. 133.5–135° (stage).

Acetyluramil (Potassium Salt).—A three-necked flask was equipped with a Hershberg stirrer, reflux condenser and glass stopper. The apparatus was dried and potassium methylate was prepared by adding absolute methanol (dried over Drierite) to 0.9 g. (0.23 mole) of clean potassium under benzene. The solvents were removed under reduced pressure and to the dry residue was added 1.180 g. (0.0197 mole) of urea in 4 cc. of absolute methanol. Ethyl acetamidomalonate[10] (4.3 g., 0.02 mole) was dissolved in 8 cc. of methanol and added in one portion to the urea solution. The mixture was refluxed and stirred for four hours. The white precipitate of potassium acetyluramil was collected by filtration; yield 3.74 g. (85%). An analytical sample of acetyluramil was prepared as follows: potassium acetyluramil (500 mg.) was dissolved in 15 cc. of concentrated hydrochloric acid at room temperature. The solution was added to 100 cc. of ethanol and cooled overnight. The precipitate which formed weighed 400 mg.

Anal. Calcd. for $C_6H_7O_4N_3$: N, 22.70. Found: N, 22.50.[11]

Uramil.—The potassium acetyluramil (3.61 g., 0.0161 mole) was hydrolyzed to uramil in 30 cc. of concentrated hydrochloric acid. The mixture was heated to boiling for about five minutes at which time all the solid had gone into solution. The uramil was precipitated by diluting with 70 cc. of water; yield 2.16 g. (94%). An analytical sample was prepared by dissolving 100 mg. of uramil in dilute sodium hydroxide and reprecipitating with hydrochloric acid; recovery 72 mg.

Anal. Calcd. for $C_4H_5O_3N_3$: C, 33.57; H, 3.52. Found: C, 33.01; H, 3.60.

Pseudouric Acid (Potassium Salt).—Potassium pseudourate was prepared by a modification of the procedure of Baeyer.[1] Uramil (2.16 g., 0.0151 mole) was added in one portion to a boiling solution of 55 g. of potassium cyanate in 260 cc. of water. The uramil dissolved producing a clear pink solution and potassium pseudourate precipitated almost immediately. The solution was refluxed for one-half hour while stirring. Upon cooling, 3.14 g. (92%) was obtained.

Uric Acid.—Potassium pseudourate was converted to uric acid according to the procedure of Fischer.[3] Yields of 72 to 80% were obtained, after two recrystallizations.

Anal. Calcd. for $C_5H_4O_3N_4$: C, 35.71; H, 2.39; N, 33.33. Found: C, 35.81; H, 2.50; N, 33.25.

The isotopic preparation contained 34.05% total nitrogen (calcd. 33.52% with allowance for the N^{15} present), and 16.1 atom % excess N^{15} (expected 16.0%).[12]

Absorption Spectra of Uric Acid.—The ultraviolet absorption spectrum of synthetic samples prepared by either method (determined upon a solution of 0.013 mg. per cc. at *p*H 7.0; 0.1 *M* phosphate buffer) showed maxima of ϵ 12,700 at 292 mμ and of ϵ 10,100 at 235 mμ. A commercial sample of uric acid,[13] purified in the same manner as the synthetic sample, exhibited slightly lower maxima (ϵ = 11,500 and 8,820) at the same wave lengths. The data on the commercial sample agree with the values previously reported.[14,15]

Uric Acid from Urea and Uramil (Isotopic Nitrogen in the 9 Position).—Urea (550 mg., 0.0092 mole, 0.97 atom % excess N^{15}) was fused with 500 mg. (0.0035 mole) of uramil[16] at 150–170° for forty-five minutes. The cooled melt was extracted with 20 cc. of boiling water and the insoluble material was removed by filtration. The filtrate was decolorized and evaporated to about 6 cc. Upon cooling, 355 mg. (50%) of ammonium pseudourate precipitated. This was dissolved in aqueous sodium hydroxide and pseudouric acid was precipitated by acidification with hydrochloric acid; yield, 200 mg. (35%). This pseudouric acid contained 0.28 atom % excess N^{15}. This was converted to uric acid (calcd. N, 33.34; found 33.18) which also contained 0.28 atom % excess N^{15}. This is slightly more than the theoretical of 0.24 atom % N^{15} excess for the introduction of one nitrogen atom from the urea used.

Uric Acid from 2,6-Dioxy-4,5-diaminopyrimidine.—Urea (700 mg., 0.0117 mole, 0.97 atom % excess N^{15}) was fused with 468 mg. (0.0011 mole) of 2,6-dioxy-4,5-diaminopyrimidine sulfate[9] for one hour at 150–170°. The cooled melt was washed with 10 cc. of water and filtered. The residue was dissolved in dilute sodium hydroxide, decolorized with charcoal and precipitated by the addition of acetic acid. It was dissolved again in alkali and precipitated by the addition of hydrochloric acid. The product was then recrystallized from water and the 163 mg. of uric acid obtained was shown to contain no excess isotopic nitrogen.

Summary

An improved synthesis of uric acid, suitable for

(8) Hentschel, *Ber.*, **17**, 1284 (1884).

(9) Bloch and Schoenheimer, *J. Biol. Chem.*, **138**, 176 (1941).

(10) Snyder and Smith, THIS JOURNAL, **66**, 350 (1944).

(11) We are indebted to Dr. Elek of the Rockefeller Institute for Medical Research for the analyses.

(12) We are indebted to the M. W. Kellogg Co. for the mass spectrometer determinations of isotopic nitrogen.

(13) Eastman Kodak Co.

(14) Fromherz and Hartmann, *Ber.*, **69**, 2420 (1936).

(15) Stimson and Reuter, THIS JOURNAL, **65**, 153 (1943).

(16) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, New York, 1943, p. 617.

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

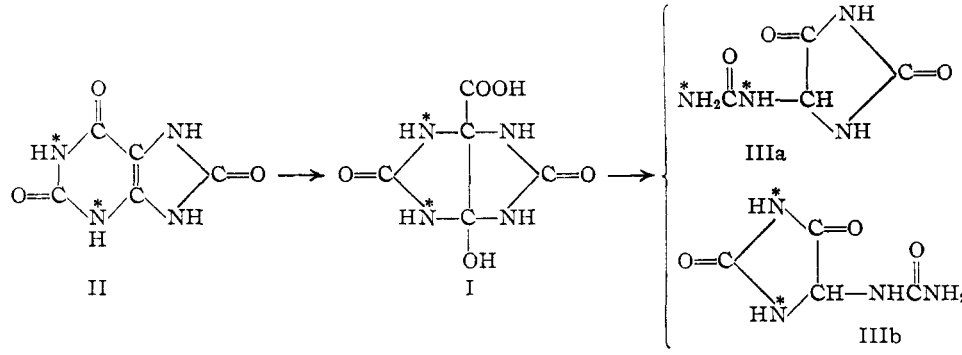
[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

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

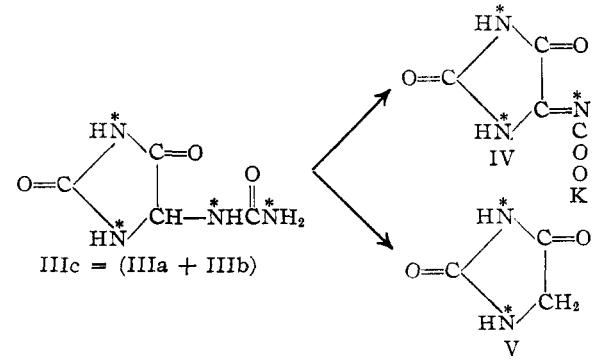
BY LIEBE F. CAVALIERI AND GEORGE BOSWORTH BROWN

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.