Anal.[3] Calcd. for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 69.12; H, 7.74.

When a mixture of 0.2 mole each of ethyl acetate and acetophenone was added to 0.4 mole of lithium amide in liquid ammonia and the reaction completed in refluxing ether as described above, there was obtained a 47% yield of the β-hydroxy ester.

The β-hydroxy ester was identified by dehydration with phosphorus oxychloride in benzene solution to form ethyl β-methylcinnamate, b.p. 146–149° at 17 mm. (reported 146–148° at 16.5 mm.),[4] and by saponification of the latter ester to give β-methylcinnamic acid, m.p. 97–98° (reported 97–98°).[4]

Ethyl Acetate with Cyclohexanone.—This condensation was carried out essentially as described above with acetophenone employing 0.2 mole each of ethyl acetate and cyclohexanone, and 0.42 mole of lithium amide. There was obtained, after a small forerun of cyclohexanone, 25.4 g. (69%) of ethyl 1-hydroxycyclohexyl acetate (IIB), b.p. 124–126° at 18 mm.

Anal.[3] Calcd. for $C_{10}H_{18}O_3$: C, 64.49; H, 9.74. Found: C, 64.54; H, 9.80.

The β-hydroxy ester was identified by saponification to form cyclohexanolacetic acid, m.p. 63–64° (reported 62–64°).[5]

(4) S. Lindenbaum, *Ber.*, **50**, 1270 (1917).
(5) O. Wallach, *Ann.*, **347**, 328 (1906).

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On the Mechanism of the Oxidation of Uric Acid by Alkaline Peroxide[1a]

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The action of alkaline peroxide on uric acid (I) has been studied by several authors,[2a,b] who assigned the following structures to the degradation products, oxonic acid (II) and allantoxaidin (III). it became clear that this scheme could not account for the results.[3] While carbon number 6 appeared in the first molecule of carbon dioxide liberated, carbons 2, 4 and 8 did not act as precursors for the second molecule of carbon dioxide, which therefore must originate from carbon number 5. Because of this and in view of the ease with which oxonic acid can be oxidized to cyanuric acid (IX)[4] and allantoxaidin split to formic acid (VI) and biuret (VII),[2b] Brandenberger[3] proposed structure IV and V for oxonic acid and allantoxaidin, respectively. The present paper reports experiments confirming this hypothesis by a stepwise degradation of uric acid labeled in position 4 with C^{14} to biuret and formic acid and by chemical and spectroscopic studies of allantoxaidin and analogs.

The oxonic acid was prepared by a modification of the method of Moore and Thomas[2b] as the potassium salt. This was then transformed into the silver salt which is decarboxylated readily to allantoxaidin in dilute hydrochloric acid. This method permitted an easy separation of the inorganic acid and salt from the reaction products, which would be difficult otherwise. The CO_2 evolved in the preceding steps was collected separately as barium carbonate. The allantoxaidin was hydrolyzed to biuret and formic acid with concentrated ammonia. The results of the degradation experiments on uric acids labeled in positions 4 and 5, respectively, are given in Table I. It is seen that the carboxyl group of oxonic acid is derived from carbon 5 of the uric acid, and that carbon 4 of the uric acid is found *entirely* in the formic acid obtained from the allantoxaidin. The latter observation cannot be explained on the basis of the old reaction mechanism. The results of the above experiments can be explained readily, however, if oxonic acid and allantoxaidin are assigned the triazine structures IV and V.

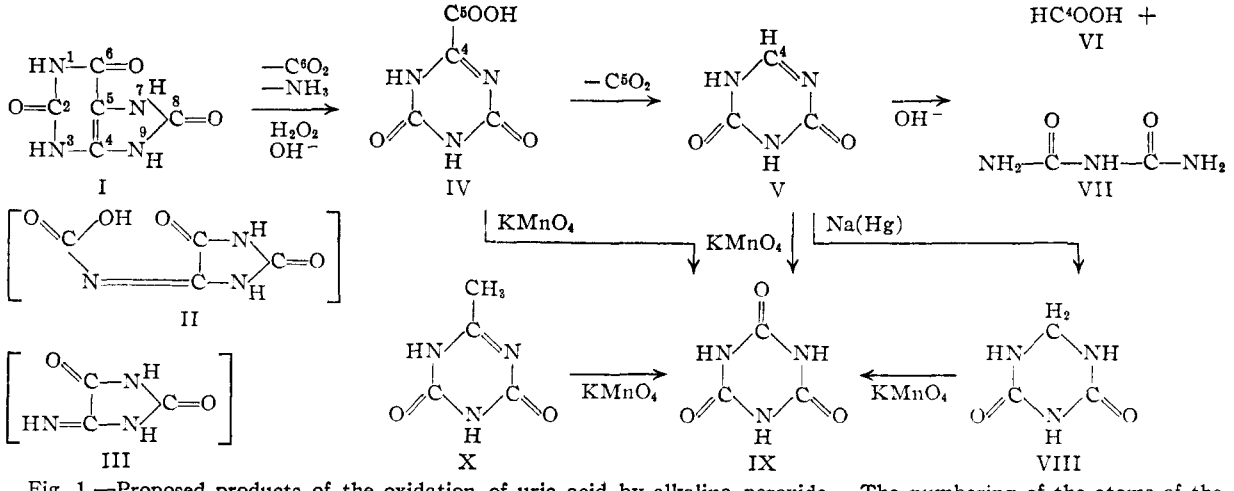

Fig. 1.—Proposed products of the oxidation of uric acid by alkaline peroxide. The numbering of the atoms of the degradation products refers to the numbering of the corresponding atoms in the uric acid molecule.

However, when the reaction was followed with uric acid labeled in positions 2, 4, 6 and 8 with C^{14}, Sodium amalgam reduces allantoxaidin to a dihydro derivative of the empirical formula $C_3H_5O_2N_3$ (VIII). Acid permanganate easily oxidizes this compound to cyanuric acid, a fact which strongly indicates a *s*-triazine structure.

(1) (a) Supported by grants from the Damon Runyon Memorial Fund for Cancer Research, Inc., and the National Cancer Institute, National Institutes of Health, United States Public Health Service. (b) Predoctoral Fellow of the National Science Foundation (1953–1954). The material of this report was submitted in partial fulfillment of the requirements for the degree of Master of Science, Massachusetts Institute of Technology.

(2) (a) C. S. Venable, THIS JOURNAL, **40**, 1099 (1918); (b) F. J. Moore and R. M. Thomas, *ibid.*, **40**, 1120 (1918).

(3) H. Brandenberger, *Helv. Chim. Acta*, **37**, 641 (1954).
(4) H. Biltz and R. Robl, *Ber.*, **54B**, 2441 (1921).

6-Methyl-2,4-dioxytetrahydro-*s*-triazine (X) was prepared according to Ostrogovich[5] and its absorption spectrum in the ultraviolet compared to that of allantoxaidin at different *p*H values. A great similarity of the absorption curves of the two compounds was noted. The wave lengths of the maximal absorption and the extinction coefficients are given in the Experimental part. Parabanic acid and hydantoin, whose structures would be similar to the old structure for allantoxaidin, do not have an ultraviolet absorption of the above-mentioned type, but instead exhibit only end absorption above 215 mμ.

The 6-methyltriazine also shows many chemical similarities to allantoxaidin such as ease of oxidation to cyanuric acid, hydrolysis in concentrated ammonia to acetic acid and biuret and reduction by sodium amalgam with uptake of two hydrogens.[6]

TABLE I

OXIDATION OF C^{14}-LABELED URIC ACIDS

Compound	Specific activity, c.p.m./μM. Uric acid-4-C^{14}	Uric acid-5-C^{14}
Uric acid	84	22.5
First CO_2	0.2	0.4
Second CO_2	0.1	18.5
Allantoxaidin	89	0.0
Formic acid	87	..
Biuret	0.1	..

Experimental

4-C^{14}-Uric Acid.—4-C^{14}-Hypoxanthine was synthesized by Dr. Walter Brooks by the method of Shaw and Woolley.[7,8] This was converted to 4-C^{14}-uric acid by the action of xanthine oxidase.

5-C^{14}-Uric Acid.—5-C^{14}-Hypoxanthine was prepared from 2-C^{14}-glycine in an *in vitro* pigeon liver system, synthesizing inosinic acid.[9] The hypoxanthine, obtained by hydrolysis of the inosinic acid, was converted to uric acid with xanthine oxidase. Uric acid was isolated by a standard procedure after addition of carrier.[10]

Potassium Oxonate and First CO_2 Fraction.—Five hundred milligrams of uric acid was dissolved in a solution of 1.7 g. of KOH in 15 ml. of water and 7.5 ml. of 30% hydrogen peroxide. After standing for 24 hours, a small amount of manganese dioxide was added to destroy the excess peroxide, and the MnO_2 then removed by centrifugation. Four ml. of glacial acetic acid was added to bring the solution to about *p*H 5, and the carbon dioxide evolved was collected in saturated $Ba(OH)_2$ solution. The reaction mixture was cooled in ice while the potassium oxonate crystallized out; yield 360 mg., 63%.

Anal. Calcd. for $C_4H_2O_4N_3K$: K, 20.04. Found: K, 20.66.[11]

Allantoxaidin and Second CO_2 Fraction.—The potassium oxonate was dissolved in the minimum amount of water and 3 ml. of 10% silver nitrate solution added. The precipitate of silver oxonate was centrifuged off and washed with water. This was suspended in 3 ml. of water and 2 ml. of concentrated hydrochloric acid was added. The solution was warmed to 60° to hasten the decarboxylation reaction and the carbon dioxide evolved was collected as $BaCO_3$. When carbon dioxide evolution ceased, the silver chloride was centrifuged off, the supernatant taken to dryness *in vacuo* and washed with water to remove the HCl. The product was recrystallized from methanol; yield 109 mg., 52%. This product was dried at 100° over P_2O_5 for 24 hours for analysis.

Anal. Calcd. for $C_3H_3N_3O_2$: C, 31.87; H, 2.67; N, 37.17. Found: C, 32.03; H, 2.69; N, 37.36.[11]

Hydrolysis of Allantoxaidin to Formic Acid and Biuret.—Seventy milligrams of allantoxaidin were heated with 5 ml. of concentrated ammonium hydroxide on the steam-bath until the volume was reduced to 2 ml. Then 3 ml. more of ammonium hydroxide was added and the volume taken down again to 2 ml. One ml. of 0.5 *M* lead nitrate solution and 10 ml. of ethanol were added. The lead formate precipitate was centrifuged off and washed with ethanol. These washings were added to the supernatant solution. The lead formate was treated with 2 ml. of 10% $HgSO_4$ and 1 ml. of concentrated sulfuric acid to oxidize the formic acid to carbon dioxide, which was collected as barium carbonate.

Oxidation of Allantoxaidin and 6-Methyl-2,4-dioxytetrahydrotriazine to Cyanuric Acid.—The acid permanganate method of Biltz[4] was used to oxidize both compounds. Oxidation of the 6-methyltriazine to cyanuric acid also could be accomplished by boiling with 10% nitric acid or by heating with bromine water.

Reduction of Allantoxaidin with Sodium Amalgam.—One hundred milligrams of allantoxaidin was dissolved in 10 ml. of water and heated to 100° on a steam-bath. Ten grams of 2% sodium amalgam was added slowly with stirring while the solution was maintained slightly acid at all times with HCl. When hydrogen evolution stopped, the solution was cooled in ice and the product precipitated. After recrystallization from hot water the product sublimed at 260° (uncorrected) and melted with decomposition at 287° when sealed in a capillary. It exhibited only end absorption in the ultraviolet region; yield 40 mg., 39%.

Anal. Calcd. for $C_3H_5N_3O_2$: C, 31.32; H, 4.38; N, 36.53. Found: C, 31.90; H, 4.38; N, 36.68.[11]

ULTRAVIOLET ABSORPTION OF ALLANTOXAIDIN AND 6-METHYL-2,4-DIOXYTETRAHYDRO-*s*-TRIAZINE

Compound	Maximum, mμ, at *p*H 2.2	Molecular extinction coeff. × 10^{-3} at *p*H 2.2	Maximum, mμ, at *p*H 11.6	Molecular extinction coeff. × 10^{-3} at *p*H 11.6
Allantoxaidin	235	5.75	252.5	7.75
6-Methyl-2,4-dioxytetrahydro-*s*-triazine	232.5	1.85	250	2.15

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(5) A. Ostrogovich, *Ann.*, **288**, 318 (1895).

(6) A. Ostrogovich and A. Ostrogovich, *Gazz. chim. ital.*, **66**, 48 (1936).

(7) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **181**, 89 (1949).

(8) E. Shaw, *ibid.*, **185**, 439 (1950).

(9) M. P. Shulman, J. C. Sonne and J. M. Buchanan, *ibid.*, **196**, 499 (1952).

(10) J. C. Sonne, J. M. Buchanan and Adelaide M. Delluva, *ibid.*, **173**, 69 (1948).

(11) All microanalyses were carried out by the Microchemical Laboratory, M.I.T., Dr. S. M. Nagy.

The Synthesis of 9,10-Dimethyl-1,2-benzanthracene-9,10-C^{14} [1]

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The potent carcinogen 9,10-dimethyl-1,2-benzanthracene has been labeled with C^{14} in the 9,10-positions as indicated below. By means of the Grignard reaction, *o*-bromotoluene and $C^{14}O_2$ gave carboxy-labeled *o*-toluic acid. Oxidation with alkaline potassium permanganate followed by sublimation[2] resulted in phthalic anhydride in 67% yield from $BaC^{14}O_3$. After condensing the anhydride with naphthalene by conducting the Friedel–Crafts reaction in ethylene chloride (a solvent recom-

(1) This work was supported by a Cancer Control Grant of the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

(2) W. Werth, *Ber.*, **7**, 1057 (1874).