

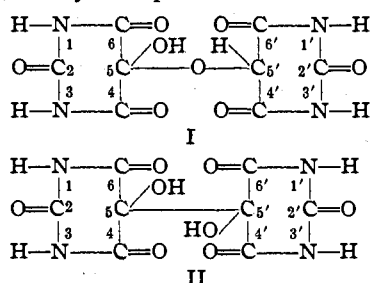
4. The diketones were only 80–90% enolized by the reagent; the relative positions of the two acetyl groups had little effect on the degree of enolization. MINNEAPOLIS, MINN. RECEIVED FEBRUARY 23, 1937

[CONTRIBUTION FROM KENT CHEMISTRY LABORATORY, UNIVERSITY OF CHICAGO]

## Studies in the Alloxantine Series<sup>1</sup>

BY DOROTHY NIGHTINGALE<sup>2</sup>

The formation and many of the reactions of alloxantine<sup>3</sup> can be explained by either the hemiacetal structure (I) of Piloty and of Slimmer and Stieglitz, or by the pinacol structure (II).



On the basis of the hemiacetal structure, it should be possible to prepare two isomeric methyl alloxantines and two unsymmetrical dimethyl alloxantines, namely, 1-methylalloxantine and 1'-methylalloxantine, and 1,3-dimethylalloxantine and 1',3'-dimethylalloxantine. The first step, therefore, was to try to prepare the two possible monomethyl alloxantines, and then to determine by means of their hydrolytic products whether or not they maintained their identity. According to the pinacol formula they should be identical.

The 1-methylalloxantine can be prepared from methylalloxan and dialuric acid, and would be expected to yield these compounds on hydrolysis. Similarly, 1'-methylalloxantine should be formed by the union of alloxan and methylalodialuric acid, and on hydrolysis should yield these same products. If the pinacol structure is correct, then either compound could yield a mixture of dialuric acid, methylalodialuric acid, alloxan and methylalloxan. Tartar<sup>4</sup> concluded that the unsymmetrical di-

methylalloxantines maintained their identity because the dimethyl murexides which he obtained from them gave dimethylalloxan and uramil, alloxan and dimethyluramil, respectively, when hydrolyzed with dilute hydrochloric acid. No further evidence was presented for their structures.

Alloxantine can be hydrolyzed with the following reagents and the hydrolytic products separated nearly quantitatively: (a) hydrochloric acid and the subsequent treatment of the solution with hydroxylamine hydrochloride, (b) hydroxylamine hydrochloride, (c) primary amines, (d) potassium acetate solution. When the mono or dimethyl alloxantines are used, however, this is no longer true. Solubilities are changed and yields lowered so that the hydrolytic products furnish little definite quantitative evidence as to probable structure.

Method (d) is useful for the isolation of only one hydrolytic product, namely, dialuric acid. According to Koech<sup>5</sup> freshly prepared potassium acetate solution hydrolyzes alloxantine to give alloxan and a quantitative yield of the slightly soluble potassium dialurate. The potassium salt of methylalodialuric acid is very soluble in water, making this reaction of use only in detecting dialuric acid. A large excess of potassium acetate seems to be necessary for a quantitative yield of the potassium dialurate. With the theoretical amount, only a small quantity of potassium dialurate is precipitated. The reaction was applied to the methyl alloxantines with interesting results.

One rather unexpected result was observed, namely, the formation of potassium dialurate from 1'-methylalloxantine prepared from either 1-methylalodialuric acid or its derivative 1-methyluramil. This result can be explained on the assumption that there is oxidation-reduction of methylalodialuric acid by alloxan after hydrolysis.

According to Biilmann and Bentzon<sup>6</sup> alloxan-

(1) Abstract of a portion of a dissertation presented by the author in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Chicago in 1928. The investigation was carried out under the supervision of the late Professor Julius Stieglitz.

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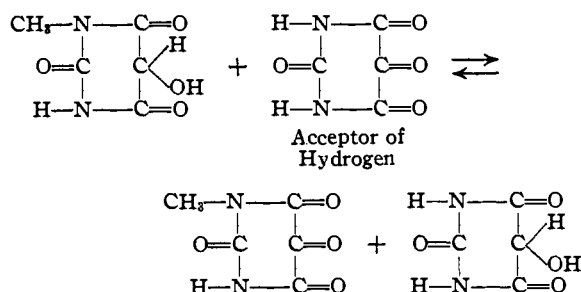
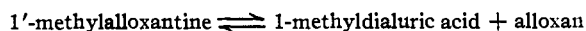
(3) For a bibliography and brief discussion of the possible structures of alloxantine, see Davidson and Epstein, *J. Org. Chem.*, **1**, 305 (1936).

(4) H. V. Tartar, doctoral dissertation, University of Chicago, 1920.

(5) Koech, *Ann.*, **315**, 254 (1901).

(6) Biilmann and Bentzon, *Ber.*, **51**, 522 (1918).

tine is 78% dissociated in saturated aqueous solution into dialuric acid and alloxan. The extent and rate of dissociation of 1'-methylalloxantine should be somewhat the same as for alloxantine, since the methyl radical of the ureide group should not affect the group of atoms which are involved in the hydrolysis. In an aqueous solution of 1'-methylalloxantine there would be the equilibrium



When potassium acetate is added, it reacts to form the slightly soluble potassium dialurate, more dialuric acid is formed to satisfy the equilibrium, and thus the 1'-methylalloxantine is destroyed completely. This is indicated by the fact that the potassium dialurate is precipitated slowly, completion of the reaction requiring several hours, whereas potassium dialurate is precipitated in a few minutes in excellent yield when either alloxantine or 1-methylalloxantine is hydrolyzed under the same conditions. This interpretation is in accordance with the hemiacetal formula for the alloxantines.

The acetyl and benzoyl derivatives of alloxantine prepared by Friedrich<sup>7</sup> from the corresponding acylalloyuric acid and alloxan are stable compounds which melt without decomposition and are soluble in alcohol as well as in water. These facts suggested the possibility of preparing isomeric acyl methyl alloxantines which might have different melting points. The benzoyl derivatives were chosen because they do not hydrolyze as readily in aqueous solution as do the acetyl derivatives.

Benzoyldialuric acid and methylalloxan or dimethylalloxan reacted readily to form the corresponding benzoyl methyl or dimethyl alloxantines, but there was no evidence of any reaction between benzoylmethylalloyuric acid and the alloxans.

(7) Friedrich, *Ann.*, **344**, 7 (1906).

## Experimental

**1-Methylalloxantine.**—The compound can be prepared by either of the following methods: (1) the reaction between dialuric acid and methylalloxan, first proposed by Andreasch,<sup>8</sup> and later more carefully developed by Biltz and Heyn,<sup>9</sup> yielding about 30%; and (2) the reaction of uramil and methylalloxan in dilute hydrochloric acid solution, an application of the method recently described by Davidson and Epstein<sup>3</sup> but first developed by Tartar<sup>4</sup> for the unsymmetrical dimethyl alloxantines. The yield is nearly quantitative; hence this method, described below, was used more frequently.

Uramil (2.8 g.) and an excess of methylalloxan (4 g.) were added to 25 cc. of water containing 2 cc. of concentrated hydrochloric acid. The mixture was kept at a temperature of 50° for three hours, with the addition of a little water from time to time to keep the volume constant. The mixture was cooled and the colorless crystals collected on a filter, washed with water, alcohol and ether, and dried in a vacuum desiccator; yield 5 g.

*Anal.* Calcd. for  $\text{C}_9\text{H}_8\text{N}_4\text{O}_8 \cdot 3\text{H}_2\text{O}$ : N, 15.82. Found: (Kjeldahl) N, 15.72.

**1'-Methylalloxantine.**—Freshly prepared methyluramil (2.5 g.) and alloxan monohydrate (2.5 g.) were added to 20 cc. of water containing 2 cc. of concentrated hydrochloric acid. The mixture was kept at a temperature of about 60° for two and one-half hours, following the procedure given above; yield 4 g.

*Anal.* Calcd. for  $\text{C}_9\text{H}_8\text{N}_4\text{O}_8 \cdot 3\text{H}_2\text{O}$ : N, 15.82. Found: (Kjeldahl) N, 16.00.

Both compounds when recrystallized melt with decomposition at 226°, and soon turn red when exposed to air, especially if at all moist. The dried samples were kept in well-stoppered bottles in a desiccator. In order to avoid as far as possible oxidation-reduction during solution, the methylalloxantines for the hydrolysis experiments were not recrystallized. The compounds were recrystallized for analysis.

**Hydrolysis of 1-Methylalloxantine.**—The 1-methylalloxantine (3 g.) was dissolved in hot boiled water (75 cc.) and to this solution was added a solution of potassium acetate prepared as follows: 1.2 g. of potassium hydroxide was dissolved in 10 cc. of water and glacial acetic acid added drop by drop until the solution was just acid to litmus. Crystals of potassium dialurate began to separate rapidly as soon as the potassium acetate solution was added to the solution of 1-methylalloxantine. The solution was kept hot but not boiling, to prevent the precipitation of the difficultly soluble methylalloxantine. The precipitation was complete in about fifteen minutes. The crystals of potassium dialurate were collected on a filter, washed with alcohol and ether and dried in a vacuum desiccator; yield 1.17 g., theoretical 1.54 g.

*Anal.* Calcd. for  $\text{C}_9\text{H}_8\text{N}_4\text{O}_8\text{K}$ : N, 15.38. Found: (Kjeldahl) N, 15.37.

**Hydrolysis of 1'-Methylalloxantine.**—The 1'-methylalloxantine (3 g.) was hydrolyzed under the same conditions as 1-methylalloxantine. After standing for about

(8) Andreasch, *Monatsh.*, **3**, 431 (1882).

(9) Biltz and Heyn, *Ber.*, **52**, 1310 (1919).

fifteen minutes, crystals of potassium dialurate began to form slowly. The precipitation required several hours for completion. As has already been pointed out, this relatively long interval is of theoretical significance. The yield of potassium dialurate was 1.35 g. The yield of salt possible if all of the 1'-methylalloxantine is converted into dialuric acid and methylalloxan is 1.54 g.

*Anal.* Calcd. for  $C_4H_2N_2O_4K$ : N, 15.38. Found: (Kjeldahl) N, 15.23.

The experiment was repeated several times, using 1'-methylalloxantine prepared by the Biltz-Andreasch directions as well as by Tartar's method, with the same results.

In support of the assumption that the formation of potassium dialurate from 1'-methylalloxantine is the result of intermolecular oxidation-reduction after hydrolysis, the following experiment was carried out. Methylalloyuric acid (1.7 g.) was dissolved in hot water (60 cc.). To this solution was added the 10 cc. of potassium acetate solution prepared as described above. Alloxan monohydrate (1.6 g.) dissolved in water (15 cc.) was added at once. The solution was kept hot to prevent the precipitation of the methylalloyuric acid. After a time, crystals began to separate and after several hours precipitation was complete. The crystals of potassium dialurate were collected on a filter and dried; yield 1.2 g., theoretical 1.8 g.

*Anal.* Calcd. for  $C_4H_2N_2O_4K$ : N, 15.38. Found: (Kjeldahl) N, 15.29.

**Hydrolysis of the Unsymmetrical Dimethylalloxantines.**—The unsymmetrical dimethylalloxantines were prepared in the same manner as the monomethyl alloxantines and were hydrolyzed with potassium acetate as described above. From 3 g. of 1,3-dimethylalloxantine an almost quantitative yield of 1.18 g. of potassium dialurate was obtained in a short time. A 2-g. sample of 1',3'-dimethylalloxantine gave 0.65 g. of potassium dialurate, a 66% yield if all of the alloxan is reduced completely by dimethylalloyuric acid to dialuric acid. This latter reaction proceeded slowly, as with 1'-methylalloxantine.

**Benzoyl-1-methylalloxantine.**—Friedrich's<sup>7</sup> directions were used to prepare benzoyldialuric acid. To a solution of the acid (1.5 g.) in 90 cc. of hot water, methylalloxan (1.5 g.) was added. Crystals separated when the solution was cooled and the beaker scratched. The benzoylmethyl alloxantine was collected on a filter, washed with water and finally with ether, and recrystallized from hot absolute alcohol. The yield was 0.72 g. of purified product which melted with decomposition at about 233°.

*Anal.* Calcd. for  $C_{16}H_{12}N_4O_9$ : N, 13.86. Found: (Kjeldahl) N, 13.93.

**Benzoyl-1,3-dimethylalloxantine** was prepared in the same manner from benzoyldialuric acid (1.5 g.) dissolved in 100 cc. of hot water and dimethylalloxan (1.7 g.). The yield was 1.3 g. The compound decomposed at 237°.

*Anal.* Calcd. for  $C_{17}H_{14}N_4O_9$ : N, 13.40. Found: (Kjeldahl) N, 13.41.

**Benzoylmethylalloyuric acid**, a compound not described in the literature, was prepared as follows: 1-methyl-

dialuric acid (5 g.) was added to 25 g. of benzoyl chloride and the mixture heated on an oil-bath for half an hour. A reaction began at about 120° with the evolution of hydrogen chloride. The methylalloyuric acid went into solution at about 150°. Crystals separated when the mixture was cooled and the sides of the flask scratched. After standing for several hours, the crystals were collected on a Büchner funnel and as much of the benzoyl chloride as possible removed by suction. The compound was washed with ether until the odor of the acid chloride was no longer noticeable; yield 6 g., melting point 185–187°. Repeated recrystallization of the compound from hot water did not change its melting point.

*Anal.* Calcd. for  $C_{12}H_{10}N_2O_4$ : N, 10.68. Found: (Kjeldahl) N, 10.60.

The potassium salt of benzoylmethylalloyuric acid was prepared from 2.5 g. of the acid dissolved in 70 cc. of 95% alcohol and a solution of 3.2 g. of potassium acetate in 30 cc. of alcohol. Colorless crystals separated rapidly when the two solutions were mixed. After standing some time, the crystals were collected on a filter, washed with alcohol and ether, and dried in a vacuum desiccator.

*Anal.* Calcd. for  $C_{12}H_9N_2O_5K \cdot 1H_2O$ : K, 12.30; N, 8.80. Found: K, 12.38; N, 8.83.

For the preparation of benzoyl-1'-methylalloxantine benzoylmethylalloyuric acid (1.5 g.) was dissolved in 125 cc. of hot water and to this solution was added alloxan (0.5 g.). After several hours the crystals which separated (1.3 g.) were collected and dried. Their melting point was 185–187° and the melting point of a mixture of these crystals and benzoylmethylalloyuric acid was also 185–187°.

## Summary

1. The experimental evidence indicates that methylalloxantine may exist in the form of two isomers, as represented by the hemiacetal formula. In aqueous solution 1-methylalloxantine would dissociate into dialuric acid and methylalloxan, and when potassium acetate solution is added, potassium dialurate is precipitated rapidly. Similarly, 1'-methylalloxantine would yield methylalloyuric acid and alloxan. The alloxan appears to be reduced slowly by the methylalloyuric acid to dialuric acid, which is precipitated slowly as the difficultly soluble potassium dialurate. Similar results were obtained with 1,3-dimethylalloxantine and 1',3'-dimethylalloxantine.

2. Methods are given for the preparation of the following compounds not described in the literature: benzoyl-1-methylalloyuric acid, benzoyl-1-methylalloxantine, benzoyl-1,3-dimethylalloxantine.

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