

Papers of the American Museum of Natural History. The qualitative grouping of the sites is expressed by letters, A—Aztec, M—Mazapan, T—Teotihuacan, E—early or "archaic" cultures.

¹ Vaillant, George C., "Excavations at Zacatenco," *Anthropological Papers of the American Museum of Natural History*, 32, part 1, New York (1930).

² Vaillant, George C., "Excavations at Ticoman," *Anthropological Papers of the American Museum of Natural History*, 32, part 2, New York (1931).

³ A comprehensive study of this site is about to be published by the Department of Historic Monuments of the Mexican Government. It will give a fuller periodization than the above.

ISOVIOLURIC ACID (ALLOXAN-6-OXIME)*

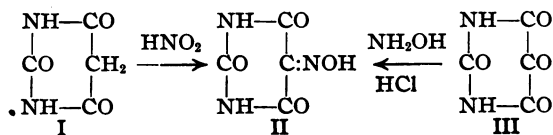
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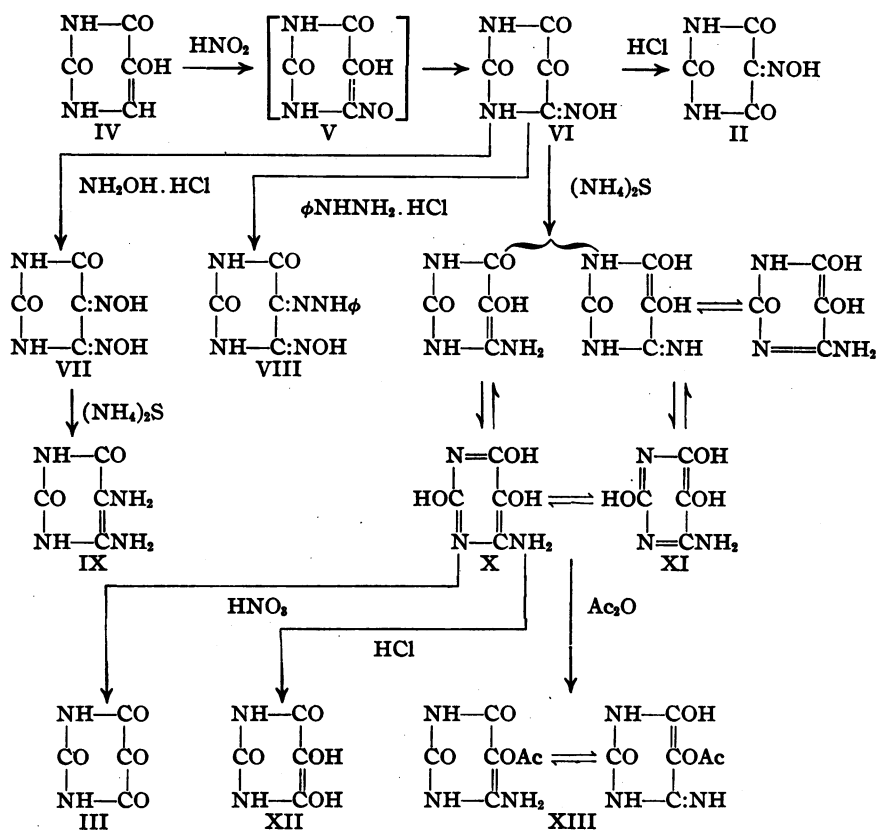
Introduction

Since its discovery by Baeyer¹ violuric acid (II) has been of perennial interest to chemists, largely on account of the beautifully colored salts which it forms. Baeyer prepared this substance by the action of nitrous acid on barbituric acid (I), while Ceresole² showed that it could be produced by the action of hydroxylamine hydrochloride on alloxan (III).

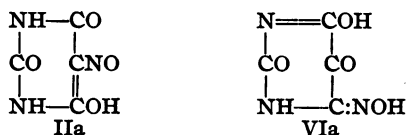


We have found that the action of nitrous acid on isobarbituric acid (IV) leads to the formation of an isomer of violuric acid which we term isovioluric acid (VI). Since the phenolic character of isobarbituric acid has been established by its behavior toward acetic anhydride, hydroxylamine, hypobromous acid,³ diazomethane⁴ and potassium ferricyanide,⁵ it was anticipated that 5-hydroxy-6-nitrosouracil (V) would be obtained. The behavior of the product, however, indicated it to be the tautomeric form (VI) (alloxan-6-oxime).

* Contribution from the Chemical Laboratories of Columbia University, with the cooperation of the Research Department of the American Manufacturers of Toilet Articles. The authors gratefully acknowledge financial aid received from the Warren fund of the American Academy of Arts and Sciences.



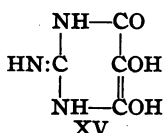
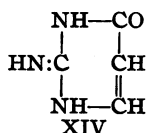
Properties of Isovioluric Acid.—Although isovioluric acid is lemon-yellow in color as compared with the pale amber of violuric acid, the salts of isovioluric acid are generally orange to red as compared with the much deeper red to blue colors of the violurates. These color relationships may be accounted for by considering the structural formulas involved. In VI we find two adjacent carbonyl groups, while in II the two corresponding carbonyl groups are separated by a third carbon atom. These formulas account for the difference in the depth of color of the two acids. In the case of the salts, on the other hand, we may consider the violurates to be derived from the nitrosophenol⁶ form (IIa) of violuric acid, which is colored on account of the NO group present, while in the isoviolurates we may assume that salt formation involves merely the enolization of an NH—CO group to N:COH (VIa).



On account of the reactive 5-carbonyl group which it has in common with alloxan, isoviolic acid oxidizes potassium iodide in acid solution, and undergoes condensations with phenylhydrazine and hydroxylamine. The latter reactions yield alloxan-6-oxime-5-phenylhydrazone (VIII) and alloxan-5,6-dioxime (VII), respectively. The dioxime may be reduced to 5,6-diaminouracil (IX),⁷ thus allowing a new method of entering the purine group. Like alloxan, isoviolic acid is sensitive to alkali. Thus, with dilute sodium hydroxide it gives an orange-red salt which soon decolorizes, however, probably on account of a rearrangement similar to that of alloxan to alloxanic acid.⁸

Since isoviolic acid represents an unusual oxime of alloxan, it is not surprising to find that dilute hydrochloric acid rearranges it to violuric acid. The simplest mechanism which suggests itself for this reaction involves two steps: (1) the hydrolysis of isoviolic acid to alloxan and hydroxylamine, and (2) the recondensation of these to form violuric acid.

Isouramil.—Isoviolic acid is readily reduced by means of dilute ammonium sulfide to $\text{C}_4\text{H}_5\text{O}_3\text{N}_3$. Owing to the benzenoid system of oscillating double bonds present in the completely enolized uracil ring, this substance may be represented by two desmotropic formulas; namely, 5-hydroxy-6-aminouracil (X) and 5,6-dihydroxycytosine (= dialuric acid-4-imine) (XI). Because of its close relationship to uramil we propose the name isouramil for this substance. The properties of isouramil are readily accounted for by means of formula XI, which relates it to dialuric acid. In fact, isouramil is converted into dialuric acid (XII) by means of dilute hydrochloric acid, and, like dialuric acid, it is easily oxidized to alloxan (in this case, with the simultaneous hydrolysis of the amino group). Accordingly, it is a strong reducing agent toward silver nitrate or Fehling's solution. Furthermore, like dialuric acid, it gives a blue color with ferric chloride and ammonia, as well as a purple precipitate with barium hydroxide. The purple precipitate from isouramil is, however, decolorized by excess of the reagent. This behavior distinguishes isouramil from dialuric acid, but is in agreement with the experience of Wheeler and Johnson,⁹ who found that isocytosine (XIV), when treated with bromine water and barium hydroxide, gave a blue color which was also discharged by excess of the barium hydroxide. In this case, dialuric acid-2-imine (XV) may be assumed to be an intermediate product in the formation of the colored precipitate.¹⁰ Finally, isouramil is converted into a monoacetyl derivative (XIII) by means of acetic anhydride.



Experimental

Preparation of Isovioluric Acid (VI).—Six and three-tenths g. (0.05 mol.) of isobarbituric acid¹¹ was dissolved in 40 cc. of 10% sodium hydroxide (0.10 mol.) together with 3.7 g. of sodium nitrite (0.055 mol.). This solution was then stirred into a mixture of 18 g. (0.3 mol.) of glacial acetic acid and 50 g. of ice. Stirring was continued for half an hour, whereupon the light yellow precipitate was filtered by suction and washed with cold water. Air drying yielded 8.2 g. (85% of theory) of analytically pure isovioluric acid dihydrate.

Anal. Calcd. for $\text{C}_4\text{H}_5\text{O}_4\text{N}_3 \cdot 2\text{H}_2\text{O}$: N, 21.8; H_2O , 18.7%. Found: N, 22.2; H_2O (110°), 18.0%.

Dehydration at 110° gave bright yellow anhydrous isovioluric acid, which melted at about 250° (dec.).

Anal. Calcd. for $\text{C}_4\text{H}_3\text{O}_4\text{N}_3$: C, 30.6; H, 1.9; N, 26.8%. Found: C, 30.5; H, 2.3; N, 26.7%.

Isovioluric acid is insoluble in water and alcohol in the cold, but is readily soluble hot. It dissolves in sodium hydroxide or ammonia to give orange solutions which soon become decolorized. With barium hydroxide, it gives a brick-red precipitate. In the presence of sodium acetate, it gives red colors with Ni^{++} , Co^{++} , Ag^+ , reddish brown with Fe^{++} and green with Cu^{++} .

Rearrangement of Isovioluric Acid (VI) to Violuric Acid (II).—One and ninety-three-hundredths g. (0.01 mol.) of isovioluric acid dihydrate was suspended in a solution of 2 cc. (0.02 mol.) of concentrated hydrochloric acid in 25 cc. of water. The mixture was heated under reflux in a boiling water bath for four hours. The isovioluric acid quickly went into solution and was replaced by a faint yellow precipitate (alloxan-5,6-dioxime) which gradually redissolved as the heating progressed. On allowing the reaction mixture to stand in the cold, large, clear, pale yellow crystals of violuric acid slowly deposited. Yield, 0.68 g. (43% of theory). By using the mother liquor for the rearrangement of a second batch of isovioluric acid, a yield of 1.10 g. (70% of theory) was obtained. The product was identified by means of its colored salts particularly the deep blue ferrous salt, and by its decomposition at 245–50°.

Alloxan-5,6-dioxime.—One gram of anhydrous isovioluric acid was dissolved in 35 cc. of absolute alcohol and treated with one equivalent of powdered hydroxylamine hydrochloride. The mixture was heated under

reflux for one hour. A precipitate began to separate within a few minutes after heating was begun. The product was filtered, washed with absolute alcohol and dried at 80°. Yield, 1.07 g. (88% of theory) of a faint yellow powder, m. 242° (dec.).

Anal. Calcd. for $C_4H_4O_4N_4 \cdot H_2O$: C, 25.3; H, 3.1; N, 29.5%. Found: C, 25.1; H, 3.1; N, 30.1%.

Alloxan-5,6-dioxime is much less soluble than isoviolic acid, being insoluble even in hot water, alcohol or glacial acetic acid. It does not lose its molecule of water at 110°. It dissolves in alkali and ammonia to give orange-brown solutions, which, unlike those of violuric acid, are stable. In the presence of sodium acetate, it gives brown precipitates with Ni^{++} and Co^{++} , an orange precipitate with Ag^+ and a greenish brown color with Cu^{++} .

Reduction of Alloxan-5,6-dioxime (VII).—One gram of alloxan-5,6-dioxime was suspended in 25 cc. of water and to this was added 10 cc. of 20% ammonium sulfide solution. The orange-colored mixture was gently heated until the reaction occurred and all went into solution. It was then boiled to remove the excess of ammonium sulfide, and finally filtered to separate the sulfur formed. The addition of dilute sulfuric acid to the filtrate gave 0.45 g. of 5,6-diaminouracil sulfate dihydrate,¹² which was recrystallized from hot water and analyzed.

Anal. Calcd. for $(C_4H_6O_2N_4)_2 \cdot H_2SO_4 \cdot 2H_2O$: C, 23.0; H, 4.3%. Found: C, 23.3; H, 5.1%.

Alloxan-6-oxime-5-phenylhydrazone (VIII).—The preparation of this product was similar to that of the dioxime given above. An 80% yield of brilliant yellow platelets, m. 268° (dec.) was obtained.

Anal. Calcd. for $C_{10}H_9O_4N_5$: C, 48.6; H, 3.6; N, 28.4%. Found: C, 48.2; H, 4.6; N, 28.8%.

Reduction of Isoviolic Acid. Isouramil (XI).—To 7.8 g. of isoviolic acid dihydrate suspended in 100 cc. of water was added 50 cc. of 20% ammonium sulfide. The reaction mixture was gently shaken. The reaction occurred readily with slight evolution of heat. The reaction mixture was then largely evaporated under diminished pressure to remove ammonia and hydrogen sulfide. The residue consisted of an aqueous suspension of isouramil and sulfur, which was acidified with acetic acid, and filtered. The solid was washed with water, alcohol and ether, and then extracted with carbon disulfide for one hour in the apparatus of Clarke and Kirner¹³ to remove the sulfur. This treatment left 5.2 g. (91% of theory) of a flesh-colored powder which turned deep pink on standing.

Anal. Calcd. for $C_4H_5O_3N_3$: C, 33.6; H, 3.5; N, 29.4%. Found: C, 32.7; H, 3.8; N, 29.3%.

Isouramil does not melt below 290°. It is insoluble in hot water, alcohol or acetic acid. It dissolves in sodium hydroxide, ammonia and dilute hydrochloric acid. With barium hydroxide it gives a purple precipitate which turns white with excess of the reagent. An ammoniacal solution of isouramil gives a lilac precipitate with barium chloride and a deep blue color with ferric chloride. It reduces neutral or ammoniacal silver nitrate as well as Fehling's solution in the cold.

Hydrolysis of Isouramil to Dialuric Acid (XII).—A solution of one-half g. of isouramil in 10 cc. of dilute hydrochloric acid (1:1) was boiled for a few minutes and then cooled. A deposit of crystals weighing 0.25 g. was obtained, which melted at 214° (dec.) and gave a beautiful purple precipitate with barium hydroxide (which was not discharged by excess of the reagent). These properties characterized the product as dialuric acid.

Oxidation of Isouramil to Alloxan (III).—To 1.43 g. of isouramil was gradually added a mixture of 1.1 cc. of concentrated nitric acid and 1.1 cc. of water. The reaction proceeded with the vigorous evolution of gas. The mixture was finally warmed gently to complete the reaction and 5 cc. of glacial acetic acid added. On standing, 1.00 g. of large transparent crystals separated. These gave a white precipitate with barium hydroxide, and violuric acid with hydroxylamine by hydrochloride. These properties identified the product as alloxan hydrate.¹⁴

Acetylation of Isouramil.—One-half g. of isouramil was suspended in 10 cc. of acetic anhydride and the mixture boiled for half an hour. Complete solution did not occur during this time, but on adding boiling water, all the solid went into solution. On cooling, there was obtained 0.25 g. of a white powder, which darkened but did not melt below 275°. The product gave no color with ferric chloride and ammonia and dissolved in barium hydroxide to a colorless solution, indicating that the 5-hydroxyl group of isouramil had been masked. This was confirmed by the following analysis:

Anal. Calcd. for $C_6H_7O_4N_3$: C, 38.9; H, 3.8; N, 22.7%. Found: C, 39.0; H, 4.0; N, 23.1%.

When boiled in dilute hydrochloric acid for a few minutes the product was hydrolyzed to dialuric acid (barium hydroxide test).

Summary

1. The action of nitrous acid on isobarbituric acid yields isovioluric acid (alloxan-6-oxime), which is rearranged to violuric acid (alloxan-5-oxime) by means of acid, but otherwise behaves much like alloxan.
2. Reduction of isovioluric acid yields isouramil (5,6-dihydroxycytosine), which resembles dialuric acid in its chemical properties, and is converted into the latter by means of dilute acid.

- ¹ Baeyer, *Ann. Chem.*, **127**, 200 (1863); **130**, 140; **131**, 292 (1864).
² Ceresole, *Ber. chem. Ges.*, **16**, 1133 (1883).
³ Behrend and Roosen, *Ann. Chem.*, **251**, 235 (1889).
⁴ Biltz and Paetzold, *Ann. Chem.*, **433**, 64 (1923).
⁵ Davidson and Baudisch, *J. Biol. Chem.*, **64**, 619 (1925).
⁶ Hantzsch and Isherwood, *Ber. chem. Ges.*, **42**, 986 (1909); Hantzsch and Issaias, *Ibid.*, 1000.
⁷ Traube, *Ber. chem. Ges.*, **33**, 1371 (1900); *Ann. Chem.*, **432**, 266 (1923).
⁸ Biltz, Heyn and Bergius, *Ann. Chem.*, **413**, 68 (1916).
⁹ Wheeler and Johnson, *J. Biol. Chem.*, **3**, 183 (1907).
¹⁰ Gregory, *J. prak. Chem.*, [1], **32**, 278 (1844), showed that dialuric acid gave a white precipitate with barium ion, but that it was readily oxidized by air to alloxantine, which gave a purple precipitate. The role of air in the formation of a purple barium salt from dialuric acid was also appreciated by Liebig and Wöhler, *Ann. Chem.*, **26**, 279 (1838). For a modern interpretation of the color of barium alloxantinate, see Hantzsch, *Ber. chem. Ges.*, **54**, 1267 (1921) and Retinger, *J. Am. Chem. Soc.*, **39**, 1059 (1917).
¹¹ Davidson and Baudisch, loc. cit.
¹² Traube, loc. cit. Levene and Senior, *J. Biol. Chem.*, **25**, 607 (1916).
¹³ Clarke and Kirner, *Organic Syntheses*, **2**, 49 (1922).
¹⁴ Biltz, Heyn and Bergius, loc. cit. Ceresole, loc. cit.

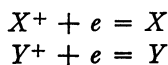
CONCENTRATION OF THE H^2 ISOTOPE OF HYDROGEN BY THE FRACTIONAL ELECTROLYSIS OF WATER*

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If the reactions



are both possible cathodic reactions, the one having the lower cathodic potential will be favored to the practical exclusion of the other during an electrolysis under equilibrium conditions. Though the normal electrode potentials of the isotopes of all elements except hydrogen must be so nearly the same that no appreciable separation can be expected from any small differences, this may not be true in the case of the hydrogen isotopes because of the very large mass ratio.

In addition to a possible small difference in the normal electrode potentials of these two isotopes, the mechanism of the formation of hydrogen gas at the cathode involves the diffusion of the two species of ions through the cathode film and the subsequent union of the discharged ions to produce hydrogen molecules, in both of which processes (differences in "polari-