

actants. The condensation system diethyl ketone-resorcinol was studied, leading to the direct synthesis of a crystalline, non-polymeric phenolic

coumarane, the structure of which was proved.

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[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY AND SOILS, UNITED STATES DEPARTMENT OF AGRICULTURE]

## Isoguanine from the Croton Bean

BY JOSEPH R. SPIES

Naturally occurring isoguanine was first isolated by Cherbuliez and Bernhard from the croton bean (*Croton tiglium* L.). It occurs as the aglycone fragment of the glycoside 2-oxy-6-aminopurine-*d*-ribose.<sup>1</sup>

We confirmed the observation of E. Fischer<sup>2</sup> and Cherbuliez and Bernhard<sup>1</sup> that isoguanine, unlike guanine,<sup>3</sup> is apparently not convertible to xanthine by reaction with nitrous acid. Xanthine was obtained from natural isoguanine in good yield, however, by the action of hydrochloric acid<sup>4</sup> and this reaction affords an unusual example of the deamination of a compound resistant to the action of nitrous acid.

The conversion of naturally occurring isoguanine to xanthine is confirmation for its accepted structure, 2-oxy-6-aminopurine.

Isoguanine has been found to crystallize from water in large rosetts, containing one and one-half molecules of water of crystallization. The optical properties of the substance were kindly determined by George L. Keenan of the Food and Drug Administration.

### Experimental

**Isolation of Isoguanine.**—The crotonoside was extracted from ground, unshelled croton beans (*Croton tiglium* L.) with methanol and isolated by the method of Cherbuliez and Bernhard.<sup>1</sup> The purified riboside was hydrolyzed by heating on the water-bath with 100 parts of 0.1 *N* sulfuric acid for four hours. The crude sulfate which separated on cooling was purified by recrystallizations from 5% sulfuric acid after charcoal decolorization. The amorphous base was obtained by dissolving 5 g. of the sulfate ( $C_6H_5ON_5$ )<sub>2</sub>H<sub>2</sub>SO<sub>4</sub>·H<sub>2</sub>O in 75 ml. of 1 *N* sodium hydroxide. To the solution was added 10 ml. of glacial acetic acid and 100 ml. of water. The precipitated base was filtered off, washed with water, and dried in vacuum over phosphorus pentoxide; yield 3.4 g.

(1) E. Cherbuliez and K. Bernhard, *Helv. Chim. Acta*, **15**, 464 and 978 (1932); cf. Spies and Drake, *THIS JOURNAL*, **57**, 774 (1935).

(2) E. Fischer, *Ber.*, **30**, 2247 (1897).

(3) Strecker, *Ann.*, **118**, 167 (1861); E. Fischer, *ibid.*, **215**, 309 (1882).

(4) E. Fischer, *Ber.*, **43**, 805 (1910), used hydrochloric acid to deaminate guanine.

**Crystallization of Isoguanine Hydrate.**—One gram of pure isoguanine was dissolved in 37 ml. of 0.33 *N* sodium hydroxide. The alkaline solution was filtered into 2 liters of distilled water, heated to 65°. Twenty milliliters of glacial acetic acid was added to the hot solution, which was allowed to cool slowly to room temperature. After six hours, the clear solution was placed in the refrigerator at 5°. The solution was still clear after twenty-four hours but on the third day crystallization had commenced. After twelve days, the crystals were filtered off and washed with a little water. The crystals attained a length up to 4 mm.; yield 0.61 g. The solubility of isoguanine (calculated on basis of the anhydride) is approximately 0.21 mg./ml. at 5° in water containing about 1% acetic acid.

*Anal.*<sup>5</sup> Calcd. for  $C_6H_5ON_5 \cdot 1.5H_2O$ : C, 33.71; H, 4.53; N, 39.32; H<sub>2</sub>O, 15.17. Found: C, 34.5, 34.4; H, 4.31, 4.37; N, 39.5, 39.5; H<sub>2</sub>O (dried in vacuum at 110°), 14.71, 14.62. Calcd. for  $C_6H_5ON_5$ : C, 39.74; H, 3.34; N, 46.36. Found: C, 39.5, 39.4; H, 3.31, 3.30; N, 46.2, 46.2.

Microscopical examination of the crystals ( $C_6H_5ON_5 \cdot 1.5H_2O$ ) in ordinary light showed them to consist largely of colorless rods, usually terminated by a plane at right angles, also narrower needle-like forms. The refractive indices are as follows:  $n_\alpha$  1.516;  $n_\beta$  indt.;  $n_\gamma > 1.734$  (immersion method). An intermediate index value  $n$  1.734 (methylene iodide) occasionally is shown on very slender rods when the long dimension is oriented perpendicular to the vibration plane of the lower nicol (crosswise).  $n_\alpha$  is the most commonly occurring index and is always shown on the rods when their long dimension lies parallel to the vibration plane of the lower nicol (lengthwise). The maximum index value is always shown crosswise on the rods. The double refraction is extremely strong ( $n_\gamma - n_\alpha = > 0.218$ ). With crossed nicols, the extinction is straight and the sign of the elongation is negative. Anomalous interference colors, consisting of peculiar and characteristic blues and purples, are quite significant for the substance with cross nicols. The rods invariably extinguish sharply. The compound apparently crystallizes in the orthorhombic system.

**Xanthine from 2-Oxy-6-aminopurine.**—Two grams of anhydrous isoguanine was refluxed with 200 ml. of 25% hydrochloric acid (starting concentration) for forty-seven hours. The hydrochloric acid was evaporated on the steam-bath and the residue was washed with 50 ml. of

(5) The author is indebted to Thomas H. Harris, Jr., for the microanalytical determinations reported in this paper.

water. The water-insoluble residue was boiled up with 40 ml. of 0.05 *N* hydrochloric acid and filtered hot. The residue was washed with a little hot water on the filter. The insoluble portion was dissolved in 50 ml. of warm dilute ammonium hydroxide and the cooled solution filtered. The ammoniacal solution was evaporated to dryness and the residue dissolved in 3 l. of boiling water. The filtered solution was cooled overnight at 5°. The white crystalline precipitate was separated and dried in a vacuum over phosphorus pentoxide; yield 1.06 g. *Anal.* Calcd. for  $C_5H_4O_2N_4$ : N, 36.8. Found: N, 36.6.

The xanthine was identified by its conversion to bromocaffeine (m. p. 208–209°) and ethoxycaffeine (m. p. 140°) by Fischer's methods.<sup>6</sup> Mixed melting points with equal

quantities of bromocaffeine and ethoxycaffeine prepared from caffeine<sup>7</sup> showed no depression.

### Summary

1. The structure of the aglycone from the crotonoside is confirmed as 2-oxy-6-aminopurine by its transformation to xanthine.

2. Isoguanine (2-oxy-6-aminopurine) has been obtained in crystalline form and its optical crystallographic properties are recorded.

3. An unusual example of the use of constant boiling hydrochloric acid for deamination of a compound, resistant to the action of nitrous acid, is cited.

(6) E. Fischer, "Untersuchungen in der Puringruppe," 1882–1906, pp. 462–463.

(7) Fischer, *ibid.*, pp. 93–95.

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## Some Salts of 2-Oxy-6,8-diaminopurine

By JOSEPH R. SPIES AND THOMAS H. HARRIS, JR.

The third of the three isomeric diamino-oxy-purines was first prepared by Cherbuliez and Bernhard from isoguanine,<sup>1</sup> by reduction of the colored diazonium coupling compound. These authors did not describe characterizing salts of the new purine, presumably due to an insufficient amount of the substance.<sup>2</sup>

This paper describes a method for the preparation of 2-oxy-6,8-diaminopurine, some of its properties and several salts. Table I shows the formulas of the crystalline salts which have been prepared by usual methods. All of these salts exhibit birefringence when viewed with a polarizing microscope. The sulfate of 2-oxy-6,8-diaminopurine, like that of isoguanine, retains one molecule of water of crystallization even on heating in vacuum at 139°. Unlike isoguanine, 2-oxy-6,8-diaminopurine forms salts with both acetic acid and carbon dioxide.

### Experimental

#### Preparation of 2-Oxy-6,8-diaminopurine Sulfate.—

Twelve grams of finely pulverized 2,4-dichloroaniline (1.5 mol) was diazotized in 58 ml. of concentrated hydrochloric acid by the slow addition of 5.4 g. of sodium nitrite in 7.5 ml. of water. The suspension was kept at 0–5° and

stirred mechanically. The diazonium salt practically all dissolved when crushed ice was added to the acid suspension.

To 10 g. of 2-oxy-6-aminopurine sulfate, dissolved in 400 ml. of 1.2 *N* sodium hydroxide (0–10°), stirred mechanically, was added one-half the cold diazonium solution. Eighteen and one-half milliliters of 19 *N* sodium hydroxide was then added and the remaining diazonium solution was dropped in as before. The cold red solution was stirred for fifteen minutes. Carbon dioxide was passed in until the solution was neutral to phenolphthalein. The red precipitate was filtered off and washed with cold water. This precipitate was dissolved in 600 ml. of 1.5% sodium hydroxide and heated and stirred during the gradual addition of sodium hydrosulfite (approx. 180 g.). The solution, which should be kept alkaline, finally turns from red to yellow. The voluminous precipitate which formed when the cooled solution was saturated with carbon dioxide was filtered off and washed with water. (Acetic acid may be used in place of carbon dioxide.) The slightly yellow precipitate was dissolved in 1300 ml. of boiling 5% sulfuric acid, decolorized with carbon, filtered and allowed to cool to 5°. The sulfate was recrystallized from 1 liter of 5% sulfuric acid. The fine hair-like crystals were washed with water and dried at 110° in a vacuum: yields 2–4 g. Two further recrystallizations from 5% sulfuric acid produced no change in nitrogen content of the sulfate.

**The Base.**—Purified 2-oxy-6,8-diaminopurine sulfate was dissolved in warm dilute sodium hydroxide and precipitated from the cooled solution with carbon dioxide. The carbonate was dissolved in boiling water (about 1 g./l.) and the hot solution was filtered through a Seitz sterilizing pad to remove a little colloidal carbon. The clear solution was boiled gently for some time and allowed to cool in a flask closed with an Ascarite tube.

(1) Cherbuliez and Bernhard, *Helv. Chim. Acta*, **15**, 471 (1932); Spies, *THIS JOURNAL*, **61**, 350 (1939).

(2) K. Bernhard, "Recherches sur la Graine de Croton (*Croton tiglium* L.)," Thèse (Genève), 1932, described the sulfate and hydrochloride. The water content of the hydrates does not agree with that reported by us, however.