Reduction of Alloxan in Aqueous Solution. By Hydrogen Sulfide* I.

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Study of the reduction of alloxan in aqueous solution with hydrogen sulfide reveals that, in deciding the products formed (at 25° or the boiling temperature), temperature is not a factor (except in so far as decomposition may be accelerated by rise in temperature). It is possible, by appropriate choice of the proportion of hydrogen sulfide introduced, to produce at will alloxantin dihydrate or dialuric acid monohydrate, regardless of the temperature or the initial alloxan concentration.

N CONNECTION with a study of the causation of experimental diabetes in animals by administration of aqueous alloxan solution, we have examined the effect of various reductants on alloxan in aqueous solution, and have encountered behavior not in precise concordance with predictions based on the information at present available in the literature. We have therefore reinvestigated the conditions under which alloxantin and dialuric acid are formed when hydrogen sulfide is the reductant.

EARLY OBSERVATIONS

In 1838, Wöhler and Liebig (1) found that, on passing some hydrogen sulfide into a concentrated aqueous solution of alloxan at room temperature, sulfur was precipitated and colorless crystals also separated (Experiment 1). This mixed precipitate was filtered off, treated with boiling water (in which the colorless crystals dissolved), and the sulfur was filtered off. On cooling the filtrate, colorless crystals of a material they called "alloxantin" were obtained. Elementary analysis of this material indicated the formula C₈H₁₀N₄O₁₀, or, more precisely (2-4), $C_8H_6N_4O_8\cdot 2H_2O.$

On the other hand, they observed that, on passing excess hydrogen sulfide into a boiling aqueous solution of this alloxantin, "eine neue Veränderung" occurred (Experiment 2). A further quantity of sulfur was precipitated, possibly implying that hydrogen sulfide has a more potent reducing action in the boiling reaction mixture than at room temperature. The organic product, which they named "dialuric acid," could not be isolated free (by them), but they prepared its ammonium salt; this had an elementary analysis indicating the formula C₈H₁₄N₆O₈ (modern, $C_4H_7N_3O_4$). From this they deduced that dialuric acid has the formula C₈H₈N₄O₈ (modern, C₄H₄N₂O₄), and suggested that alloxantin and dialuric acid might be compounds of alloxan with hydrogen. The same ammonium salt could be isolated by treating a boiling alloxan solution with excess hydrogen sulfide and then with ammonium carbonate (Experiment 3). [A few years later, Gregory (5) succeeded in isolating dialuric acid monohydrate by the action of dilute hydrochloric acid on the ammonium salt.]

Wöhler and Liebig (1) decided that alloxantin represents an intermediate stage of reduction, between alloxan and dialuric acid, for the following reason (Experiment 4). They passed hydrogen sulfide into a *boiling* aqueous solution of alloxan until reduction was judged to be complete; the solution was sufficiently dilute that, on cooling, organic product did not crystallize out. Addition of some alloxan solution to the filtrate now caused crystallization of some alloxantin. [They were puzzled, however, as to how to reconcile the above results with their observation that, if an alloxan solution was treated with some hydrogen sulfide in the cold and the mixed precipitate was filtered off, addition of some alloxan solution to the filtrate gave a second crop of alloxantin, suggesting that the mother liquor had contained dialuric acid. This observation was confirmed by their pupil, Schlieper (6).]

Subsequent authors misquoted Wöhler and Liebig's Experiment 4 and implied that 1 mole of alloxan was added to 1 mole of dialuric acid to yield 1 mole of alloxantin. Thus, with no additional experimental proof, Strecker (7) depicted alloxantin as a 1:1 compound of alloxan with dialuric acid, and Baeyer (8), also without further proof, utilized this formulation. One of Baeyer's equations, modernized, is as follows:

 $C_4H_2N_2O_4 \cdot H_2O + C_4H_4N_2O_4 \cdot H_2O =$ Dialuric acid Alloxan monohydrate monohydrate

> $C_8H_6N_4O_8\cdot 2H_2O$ (1) Alloxantin dihydrate

This interpretation of Wöhler and Liebig's experimental observations does not appear to have been verified (9) until 1913 (and, even then, not very precisely).

PRESENT WORK

It appeared that one of the simplest ways of testing these hypotheses would be to repeat Wöhler and Liebig's experiments, first determining how much sulfur is liberated on passing excess hydrogen sulfide into cold, and into boiling, aqueous solutions of given weights of alloxan monohydrate. According to Baeyer's theory, the equations (modernized) for these reactions should be:

$$2C_{4}H_{2}N_{2}O_{4} \cdot H_{2}O + H_{2}S = C_{8}H_{6}N_{4}O_{8} \cdot 2H_{2}O + S \quad (2)$$

$$C_{4}H_{2}N_{2}O_{4} \cdot H_{2}O + H_{2}S = C_{4}H_{4}N_{2}O_{4} \cdot H_{2}O + S \quad (3)$$

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We soon learned that, in at least one respect, the behavior observed at room temperature depended on the initial concentration of alloxan monohydrate; if this was at or below a certain critical value (xGm. per liter), the precipitate finally deposited consisted solely of sulfur; above this concentration, an organic compound also crystallized out.

It was found that, in partial agreement with Wöhler and Liebig's observations (1), treatment of aqueous alloxan solution with excess hydrogen sulfide, first at room temperature and then at the boiling temperature of the mixture, proceeds in accordance with Eq. 3. Surprisingly, however, in the cold (25°) also, treatment of a solution of less than the "critical" concentration with excess hydrogen sulfide causes the liberation of the amount of sulfur demanded by Eq. 3 (instead of Eq. 2); there was no immediate evidence for a halting of the reaction at the "half-way" stage represented by Eq. 2. [However, the observation that, in the zone of alloxan concentrations above a certain figure, z (but below the "critical" concentration x), some colorless crystals formed and then redissolved, during the treatment with hydrogen sulfide, suggested that the reaction might have proceeded via that pathway.]

After filtering off the sulfur produced in the reaction at 25°, a good yield of colorless, crystalline organic product (X) was readily isolated by cooling the filtrate in ice, provided that the initial alloxan concentration had been at or only slightly below x. We now proceeded to determine the effect of excess hydrogen sulfide on cold and boiling solutions of this supposed "alloxantin dihydrate." According to Baeyer's theory, there should be no reaction at room temperature, but, in the boiling solution, reaction should occur as in Eq. 4.

$$C_{8}H_{6}N_{4}O_{8}\cdot 2H_{2}O + H_{2}S = 2C_{4}H_{4}N_{2}O_{4}\cdot H_{2}O + S$$
 (4)

It was found that if X was dissolved in cold deaerated water, and excess hydrogen sulfide was passed in, no sulfur was liberated; this is not at variance with expectations. But when the same treatment was performed at the boiling temperature of the solution, there was also no liberation of sulfur. Hence, compound X was dialuric acid monohydrate and, under the above conditions, Eq. 4 represents, as far as the over-all effect and final result is concerned, a possibly nonexistent reaction. To summarize, the only reaction for which we found positive evidence (at this stage in the investigation) is that shown in Eq. 3; in other words, treatment of alloxan solution (of concentration less than x) with excess hydrogen sulfide yields *dialuric acid* as the sole final organic product, no matter whether the reaction be performed at 25° or at the boiling temperature.

Tentatively, it therefore seemed that, under the above conditions, alloxantin may be regarded as nonexistent—a conclusion in partial conformity with Piloty's statement (10), confirmed by later workers (11-16), that an aqueous solution of alloxantin behaves as a solution of alloxan plus dialuric acid. Indeed, Hill and Michaelis (17) had come to the conclusion that "the intermediary form, known as alloxantin in the crystalline state, does not exist to any appreciable extent" in solution.

Consequently, it seemed necessary to repeat Wöhler and Liebig's Experiment 4 on the formation of alloxantin. On adding a deaerated aqueous solution of 1 molar proportion of alloxan monohydrate to a deaerated aqueous solution of 1 molar proportion of dialuric acid (prepared as above, from an alloxan solution of the "critical" concentration), an excellent yield of crystalline alloxantin dihydrate was deposited (Eq. 1), thus reconfirming Wöhler and Liebig's result and Baeyer's formulation.

Being now possessed of authentic alloxantin dihydrate, we proceeded to study the effect of an excess of hydrogen sulfide on cold and hot aqueous solutions of given weights of the compound. Treatment of a saturated or undersaturated, deaerated, aqueous solution at room temperature proceeded according to Eq. 4, with no deposition of organic product. (When a cold, aqueous suspension was so treated, all the alloxantin dihydrate dissolved and no organic crystals separated if the proportion of alloxantin dihydrate to water was less than that corresponding to x. Hence z represents the alloxan monohydrate concentration giving a saturated solution of alloxantin composition.) Reaction near the boiling temperature also followed Eq. 4. Because dialuric acid is more soluble than alloxantin in both cold and hot water, the result is independent of the initial alloxantin concentration.

On the other hand, as regards the reduction of alloxan solutions, the "critical" concentration x is obviously determined by the weight of alloxan monohydrate which will give a saturated solution of dialuric acid in the presence of excess hydrogen sulfide (and crystal nuclei of dialuric acid monohydrate). In the absence of these nuclei, there appears in respect to dialuric acid a supersolubility zone, the lower limit of which is defined by x. Although it affects the solubilities, in the reaction mixture, of the various compounds involved, the temperature at which the reaction is performed does not influence the results (except in so far as decomposition may occur near the boiling temperature). Instead, the real controlling factor, hitherto apparently unrecognized, is the proportion of hydrogen sulfide introduced. It was found that, for alloxan solutions below the "critical" concentration, addition at 25° of exactly 1 mole of hydrogen sulfide per 2 moles of alloxan proceeded according to Eq. 2. Alloxantin dihydrate crystallized out if the resulting aqueous solution was supersaturated therewith.

Exactly the same kind of result was obtained with alloxan solutions above the "critical" concentration. That is to say, using excess hydrogen sulfide, all the alloxantin was reduced to dialuric acid in accordance with Eq. 4 (over-all, Eq. 3). The "critical" concentration has no significance in this respect.

To summarize, alloxantin dihydrate cannot be prepared by the action of excess hydrogen sulfide on aqueous alloxan solutions under any conditions studied, despite the numerous reports to this effect in the literature. It is thus evident that, in the preparation of alloxantin dihydrate (1, 5, 18-21), either the use of *excess* hydrogen sulfide is to be avoided (because of the resulting complete transformation to dialuric acid), or sufficient concentrated alloxan solution should be subsequently added to permit recovery as crystalline alloxantin dihydrate. [The latter method was employed by Schlieper (6).]

In the same connection, although dialuric acid monohydrate has been prepared many times in the past, it is doubtful if it has often been *isolated* hitherto [cf., Baeyer (8)] in pure form. In preparing dialuric acid monohydrate, heating is not only unnecessary but may be deleterious; furthermore, operation at room temperature is simpler.

DISCUSSION

It should be noted that, in describing their preparation (Experiment 1) of alloxantin dihydrate from alloxan monohydrate, Wöhler and Liebig (1) did not stipulate the use of excess hydrogen sulfide, but some subsequent authors (5, 6, 19, 21) have definitely advocated the use of excess of this reductant and state that alloxantin dihydrate then crystallized out. Just because the latter is less soluble (in water at room temperature) than is either alloxan monohydrate or dialuric acid monohydrate, it was assumed that the organic precipitate formed on treatment with excess hydrogen sulfide was alloxantin dihydrate. As we have shown, the latter would only be obtained if less than an excess of hydrogen sulfide were deliberately or fortuitously introduced. But alloxantin dihydrate could readily result as an artifact through the subsequent action of atmospheric oxygen on the dialuric acid monohydrate during filtration, drying, or recrystallization.] The same reasoning probably also applies to such reductions as that of ninhydrin to hydrindantin (22), where the use of excess hydrogen sulfide has been advocated. Furthermore, despite the claims in the literature, we see no reason for believing that the action of the various other reductants recommended for use instead of hydrogen sulfide differs from that outlined above, if the reducing power of these reductants is comparable to that of hydrogen sulfide. Such reductants include zinc and hydrochloric acid (1, 23), sodium amalgam (8, 9), hydriodic acid (14, 24), tin and hydrochloric acid (8, 13), and stannous chloride (1, 2, 9, 13, 23-29). It should be noted that, in 1930, Biilmann and Mygind (30) proved that use of stannous chloride for the preparation of dimethylalloxantin by Biltz's method (31) actually yields methyldialuric acid.

EXPERIMENTAL

Deaerated Water.—Distilled water was deaerated by adding a boiling stone, boiling under reflux for at least five minutes, and then cooling in ice to room temperature, under an atmosphere of oxygen-free nitrogen.

Moist Hydrogen Sulfide.—Hydrogen sulfide was passed through a reversed, empty 500-ml. Drechsel gas-washing bottle and then through 250 ml. of deaerated water in a similar bottle.

Moist Oxygen-Free Nitrogen.—Nitrogen was passed through: (a) a 500-ml. Drechsel bottle containing a solution (prepared fresh each day) of 25 Gm. of sodium hydroxide plus 5 Gm. of pyrogallol in 250 ml. of deaerated water; (b) a reversed, empty 500-ml. bottle; and (c) a 500-ml. bottle containing 250 ml. of deaerated water. The resulting gas will be referred to as "nitrogen."

Alloxan Monohydrate.—Alloxan monohydrate was dried to constant weight over soda lime and phosphorus pentoxide in the vacuum desiccator at room temperature. It was colorless, and readily and completely soluble in five volumes of cold water. It assayed 99–100% alloxan monohydrate by Tipson and Cretcher's method (32).

Action of Excess Hydrogen Sulfide on Alloxan Monohydrate.—(a) At 25°.—Distilled water (1 L.) was placed in a tared, 1,500-ml. Erlenmeyer flask and deaerated; 16.01 Gm. (0.1 mole) of alloxan monohydrate was added, and the mixture weighed and then swirled gently until the alloxan had dissolved. Hydrogen sulfide was immediately passed in, with resulting liberation of colloidal sulfur followed by deposition of colorless crystals. When ca. 7 Gm. of hydrogen sulfide had been absorbed, the sulfur readily settled out. The precipitate was now filtered off by suction, while hydrogen sulfide was passed in. [The filtering apparatus (preflushed with hydrogen sulfide) was a simplified macro version of that used in microanalysis (33); a tared Gooch crucible having a coarse, fritted-glass septum was employed.] The crucible plus precipitate was then immediately transferred to a vacuum desiccator (preflushed with hydrogen sulfide) and dried overnight over soda lime and phosphorus pentoxide; wt. of precipitate, 6.8 Gm. The precipitate was suspended in 250 ml. of water, the suspension boiled under reflux during fifteen minutes and filtered hot, and the precipitate redried; wt., 3.2 Gm. (unchanged on further washing and drying).

The solubility of alloxantin dihydrate at room temperature has been variously reported as 8.182 Gm. per liter of water acidulated with hydrochloric acid (4), 2.8 Gm. per liter of aqueous solution (12), 2.886 Gm. per liter of aqueous solution saturated with carbon dioxide (14), and ca. 2.674 Gm. per liter of solution in 0.05 N aqueous hydrochloric acid (16). The solubility of dialuric acid monohydrate at room temperature is said to be ca. 4.053 Gm. per liter of solution in 0.05 N aqueous hydrochloric acid (16). From our experiment reported above, it is seen that the organic product is soluble to the extent of ca. 12.6 Gm. per liter of water at 25° when the solution is saturated with hydrogen sulfide.

Consequently, in order to obtain a mother liquor containing all the organic product, the experiment was repeated, except that 1,300 ml. of water was used. On passing in hydrogen sulfide, the colorless crystals, which started to form soon after liberation of sulfur commenced, slowly redissolved and, after a time, only sulfur was visible. The weight of this precipitate (dry) was 3.2 Gm. (unchanged on treatment with boiling water as described above). (The main *mother liquor* was now heated and kept at the boil for sixty minutes, under reflux, while hydrogen sulfide was admitted through a tube passing down the middle tube of the condenser. No more sulfur was liberated.)

To permit ready isolation of the organic product formed below the "critical" concentration, the experiment was now repeated in a separatory funnel. except that, after excess hydrogen sulfide had been passed in, 100 ml. of carbon disulfide was added. The opalescence in the aqueous layer disappeared, the carbon disulfide acquired a yellow color, and a trace of insoluble, white material separated at the liquid-liquid interface. The carbon disulfide layer was separated, and evaporated to dryness; wt. of sultur, 3.2 Gm. The aqueous layer was now cooled to $+2^{\circ}$ in ice, while hydrogen sulfide was passed in; colorless crystals were deposited. The flask was stoppered and kept overnight in the refrigerator, the mixture was filtered as described above, and the crystals (washed with carbon disulfide and deliberately left reeking with carbon disulfide and hydrogen sulfide) dried in the vacuum desiccator (Desiguard) over soda lime and phosphorus pentoxide under high vacuum (with trap cooled in Dry Icechloroform); wt. (dry), 9.5 Gm. From its infrared absorption spectrum (34), this material proved to be dialuric acid monohydrate.

For isolation of the organic product crystallizing out at room temperature when the strength of the original alloxan solution was above the "critical" concentration, 32.02 Gm. of alloxan monohydrate in 1,273 ml. of water was treated with excess hydrogen sulfide at 25°, yielding 25.5 Gm. of initial precipitate (dry); extraction of the precipitate with cold carbon disulfide gave 6.4 Gm. of sulfur and 19.1 Gm. of colorless crystals insoluble in carbon disulfide. [From its infrared absorption spectrum (34), the latter proved to be dialuric acid monohydrate.] The aqueous filtrate gave no more free sulfur on retreatment with hydrogen sulfide. It was then cooled in ice to 2°, saturated with hydrogen sulfide, and kept overnight in the refrigerator, yielding a further 7.1 Gm. (dry) of colorless crystals of dialuric acid monohvdrate.

(b) At the Boiling Temperature.-The second experiment described above was repeated except that, after the distilled water (1,300 ml.) had been boiled under reflux (Liebig condenser with large bulbs), it was only allowed to cool slightly. Then 16.01 Gm. of alloxan monohydrate was added; some decomposition apparently took place since a gas was evolved. Hydrogen sulfide was immediately passed in (through a tube passing through the center tube of the condenser), giving colloidal sulfur; this gas stream was continued while the suspension was reheated and then boiled for seventy minutes. The sulfur was now present as a yellow suspension which settled readily; it was filtered (hot) while hydrogen sulfide was passed in; wt. (dry) = 0.93 Gm. (unchanged by further washing and drying).

Now, Wöhler and Liebig (35) have demonstrated that, on boiling an aqueous solution of alloxan, carbon dioxide is liberated and a mixture of alloxantin and parabanic acid results. Since this reaction had apparently occurred to some extent in the above experiment, the reaction was repeated with the following modification. After the water had been deaerated, it was cooled in ice to room temperature while hydrogen sulfide was passed in to saturation. The alloxan monohydrate was then added, giving an immediate precipitate of sulfur. The gas stream was continued, while the suspension was reheated and then boiled for five minutes. Since all the free sulfur was now present as a yellow suspension which settled readily, and since no more was liberated, it was filtered from the hot suspension as described above; wt. (dry) = 3.2 Gm. (unchanged by extraction with boiling water and drying). Dialuric acid monohydrate was isolated from the cooled filtrate in the usual manner.

Direct Preparation of Alloxantin (Only) from Alloxan at Room Temperature.—(a) Below the "Critical" Concentration.—Deaerated water (515 ml.) was saturated with hydrogen sulfide (1.7 Gm.) at room temperature. To this solution was added a freshly prepared solution of 16.01 Gm. of alloxan monohydrate in 785 ml. of deaerated water, and the flask rapidly stoppered. Sulfur was immediately liberated. Carbon disulfide (100 ml.) was added

and the flask quickly stoppered. After standing overnight at room temperature, colorless crystals had separated, the odor of hydrogen sulfide had disappeared, and the aqueous layer was no longer opalescent. The mixture was filtered with slight suction (under nitrogen atmosphere) and the crystals washed with carbon disulfide and dried; wt. (dry), 12.2 Gm. (The carbon disulfide layer was separated, and evaporated to dryness; wt. of sulfur, 1.6 Gm.)

(b) Above the "Critical" Concentration.—Deaerated water (1,030 ml.) was saturated with hydrogen sulfide (3.4 Gm.), as above, and to the solution was added a freshly prepared solution of 32.02 Gm. of alloxan monohydrate in 270 ml. of deaerated water. After treatment as above, there was obtained 28.2 Gm. (dry) of colorless crystals (and 3.2 Gm. of sulfur).

Action of Excess Hydrogen Sulfide on Alloxantin Dihydrate.—(a) At 25°.—Dry, finely powdered alloxantin dihydrate (3.22 Gm.; 0.01 mole) was suspended in 1,110 ml. of deaerated water at 25°, and moist hydrogen sulfide was passed in until there was no further gain in weight. After stoppering the flask and keeping it overnight at room temperature, the undissolved material was filtered off and dried; wt., 0.32 Gm. (unchanged on extracting with boiling water and drying).

The experiment was repeated, using a magnetically stirred suspension of 16.1 Gm. of alloxantin dihydrate in 1,300 ml. of deaerated water. After saturation with hydrogen sulfide, the mixture was kept at room temperature for three days, with occasional stirring, and then filtered; wt. of sulfur, 1.60 Gm. The aqueous filtrate was cooled in ice, while passing in hydrogen sulfide, stoppered, and kept overnight in the refrigerator. The colorless needlelike crystals were filtered off under hydrogen sulfide, and dried under high vacuum; wt. (dry), 9.0 Gm. Examination of its infrared absorption spectrum (34) showed this material to be dialuric acid monohydrate.

The following method is more convenient. A suspension of 16.1 Gm. of alloxantin dihydrate in 1,300 ml. of water was prepared in a separatory funnel. After saturating the suspension with hydrogen sulfide, the free sulfur was extracted with two 100-ml. portions of carbon disulfide, and dialuric acid monohydrate, crystallized from the aqueous layer by cooling as above, was filtered off, under slight hydrogen sulfide pressure, and dried under high vacuum.

(b) At the Boiling Temperature.-Distilled water (600 ml.) was deaerated by boiling under reflux, and allowed to cool somewhat while hydrogen sulfide was passed in through the condenser. Dry alloxantin dihydrate (32.22 Gm.) was quickly added and the mixture reheated to boiling while hydrogen sulfide was passed in. After passing the gas for sixty minutes, reaction was judged to be complete; the mixture was then filtered hot by means of a filtering apparatus preflushed with hydrogen sulfide. The precipitate (sulfur) was dried in the vacuum desiccator; wt., 2.01 Gm. (unchanged by extraction with 250 ml. of boiling water under reflux and drying). (The reaction had therefore not proceeded to completion, possibly because of partial decomposition of alloxantin in the boiling solution.) The initial mother liquor was cooled in ice, while passing in hydrogen sulfide, stoppered, and kept overnight in the refrigerator. The colorless crystals of dialuric acid monohydrate were filtered off under hydrogen sulfide, and dried under high vacuum; wt., 21.50 Gm.

(c) At 89°.-Because some decomposition apparently occurred under the above conditions, the reaction was repeated with the following modifications. After the water (600 ml.) had been deaerated, it was cooled in ice to room temperature while hydrogen sulfide was passed in to saturation. Finely powdered alloxantin dihydrate (32.22 Gm.) was then added, and hydrogen sulfide was passed in while the suspension was very slowly heated under reflux until all the alloxantin had dissolved, giving a clear, colorless solution (temp. 89°) with sulfur floating on the surface. The mixture was cooled to about 46° and carbon disulfide (100 ml.) was added in portions; the sulfur all dissolved and colorless crystals were deposited in the upper, aqueous layer. The mixture was now cooled in ice, while continuing the gas stream, filtered under hydrogen sulfide, and the crystals of dialuric acid monohydrate washed with carbon disulfide and dried under high vacuum; wt., 27.2 Gm. (The carbon disulfide layer was separated from the aqueous mother liquor, which was then washed with four 25-ml. portions of carbon di-The carbon disulfide solution and washings sulfide. were combined and evaporated to dryness, yielding





drochloric acid. 2, solution 1 treated with excess hydrogen sulfide at 25° (less the absorbances of hydrogen sulfide and colloidal sulfur). 3, 0.0002 M alloxantin dihydrate in 0.03 N hydrochloric acid treated as for solution 2.

sulfur; wt., 3.2 Gm.) This is a satisfactory method for preparing dialuric acid monohydrate from alloxantin dihydrate.

Ultraviolet Absorption Spectra of Alloxan and Dialuric Acid in Aqueous Solution.-In 1905, Hartley (36) discovered that the ultraviolet absorption spectrum of a freshly prepared aqueous solution of dialuric acid exhibits a peak at 270 m μ , whereas this peak is absent from the spectrum of aqueous alloxan solution. Freshly prepared aqueous alloxantin solution also showed a peak at 270 $m\mu$. [These peaks disappeared if the solutions were kept; such stale solutions were studied by other workers (26, 37), who reported no peak.]

For confirmation of the results obtained on treatment of dilute aqueous solutions of alloxan and alloxantin with excess hydrogen sulfide, the ultraviolet absorption spectra were therefore studied. These spectra were obtained with a Cary recording spectrophotometer; the maximum error in the wave-length calibration of the instrument was ± 5 Å.

As shown in Fig. 1, the height of the peak at 270 $m\mu$ observed on treating 0.0002 M alloxan monohydrate (in 0.03 N hydrochloric acid) with excess hydrogen sulfide was half that for 0.0002 M alloxantin dihydrate (in 0.03 N hydrochloric acid) similarly treated. This agrees with Eq. 3 and 4, respectively; it contradicts the work of Labes and Freisburger (38). Calculated as dialuric acid monohydrate, $\epsilon = 3,725 \pm 50$. [Patterson, et al. (29) found $\epsilon = 2,900$ in 10% sulfuric acid and $\epsilon = 3,200$ in 1% hydrochloric acid containing cysteine.] The curve for the untreated alloxan solution (Fig. 1) agrees with that in the literature (37).

SUMMARY

1. Treatment of aqueous alloxan solutions with excess hydrogen sulfide at room temperature yields dialuric acid (monohydrate), not alloxantin (dihydrate).

2. Because alloxan gradually decomposes in aqueous solution at the boiling temperature, the reduction of alloxan to dialuric acid is more satisfactorily performed at room temperature.

Alloxantin dihydrate is readily prepared 3. by addition of 2 molar proportions of alloxan to 1 molar proportion of hydrogen sulfide (in aqueous solution).

4. Treatment of aqueous solutions or suspensions of alloxantin dihydrate with excess hydrogen sulfide yields dialuric acid (monohydrate). Some decomposition occurs at the boiling temperature, but at 89° or 25° the yield is excellent.

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The Determination of Analgetic Potency by a Quantal Method^{*,†}

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A modification of the Hardy-Wolff-Goodell radiant-heat method into a quantal procedure is described. Patterned after ED50 bioassays, this modification determines the fifty per cent analysic dose (AD_{50}) of an analysic when groups of white rats are exposed forty minutes after treatment with the analgetic to a selected stimulus (320 millicalories/sec./cm.²) for the standard period of three seconds. Analgesic potency is measured by comparing the AD_{60} of an analgetic with the AD_{60} of morphine. The method is rapid and convenient and appears to be well suited for the screening and evaluation of analgetics.

THE Hardy-Wolff-Goodell (1) radiant-heat method has won wide acceptance as a reliable procedure for analgesimetric studies. However, it has generally been used to obtain data based on graded responses. Involving, as it does, the tedious step-wise determination of pain thresholds with varying successive intensities of stimulus both before and after treatment of the test animal with the analgetic, the procedure for obtaining this type of response is slow and time-consuming. Moreover, the data so obtained are difficult to evaluate because the exact relationship between rise in pain threshold and analgetic potency is subject to various interpretations.

The modification of the Hardy-Wolff method from a graded response procedure to a quantal one patterned after an ED₅₀ quantal assay seems to offer a means of avoiding these drawbacks. This modification would involve the treating of successive groups of test animals with graded doses of an analgetic, exposing them, after a certain time interval, to a certain selected intensity of radiant-heat stimulus for the standard period of three seconds, and determining the percentage of each group that has attained analgesia as shown by failure to react to the stimulus. From the data so obtained a dose-per cent effect curve can be constructed and the dose of the analgetic that would cause analgesia in 50 per cent of animals determined. The 50 per cent analgesic dose (AD₅₀) of the analgetic can then be compared with the AD₅₀ of a standard analgetic like morphine, and estimation of its potency can be made in terms of morphine. One advantage of such a procedure is that it effects a considerable saving of time and labor by not determining the pretreatment thresholds, as these are known to be normally distributed. Secondly, the actual determination of the after-treatment

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