XXXI. NOTE ON SOME EXPERIMENTS WITH GLYOXALINE DERIVATIVES.

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This note deals (1) with the behaviour of glyoxaline-formaldehyde in the organism of the dog, (2) with the condensation of glyoxaline-formaldehyde and malonic acid, (3) with the action of sulphuric acid on α -hydroxy- β -glyoxaline-propionic acid.

I. Furfuraldehyde, when injected hypodermically into the dog, was shown by Jaffé and Cohn [1887] to be changed principally into pyromucic acid and the condensation production of this acid with glycine, namely pyromycuric acid, but to the extent of a few per cent.; there is also produced furfurylacryluric acid, which is formed from glycine and furfurylacrylic acid. This latter observation induced us to administer glyoxaline-formaldehyde to a dog, in the hope that it might similarly be converted into urocanic acid, identified by Hunter [1912] as glyoxalineacrylic acid, which in two dogs has been observed as an abnormal, yet constant product of metabolism.

Accordingly 2 g. of glyoxaline-formaldehyde, for which we are indebted to the kindness of Dr F. L. Pyman [1916], was given hypodermically in 40 cc. of water to a small terrier bitch. The 280 cc. of urine, collected in the first 24 hours, was evaporated in vacuo and the residue was extracted with methyl alcohol, which removed the whole of the substances giving Pauly's reaction. After evaporation of the methyl alcohol, the residue was dissolved in 200 cc. of 5 % sulphuric acid and precipitated with 90 g. of phosphotungstic acid (a slight excess); the precipitate, filtered off next day, dissolved almost completely in 280 cc. of boiling water, and on cooling a large part remained in solution. From the phosphotungstate insoluble in cold water no base could be isolated, but the solution yielded glyoxalinecarboxylic acid, as follows. The bases, after recovery by means of barium hydroxide and

evaporation to dryness, were almost completely soluble in methyl alcohol, which left on evaporation a residue consisting largely of urea, but also giving a strong Pauly reaction. Most of the urea was removed by adding dilute nitric acid and the filtrate was neutralised and evaporated. The residue, dissolved in a little hot dilute alcohol, deposited a small quantity of a glyoxaline derivative which on recrystallisation from a mixture of methyl alcohol and acetone formed thin needles, closely resembling glyoxaline-carboxylic acid. A crystal of the acid obtained from the dog grew in a solution of the synthetic acid. The acid from the dog's urine melted at 272–273°, another specimen, recrystallised from water, at 279°, the synthetic acid at 275–276°, the mixture of the two at 276°. (The melting point depends greatly on the rate of heating; in the above cases the bath was previously heated to 260°.) The acid from the dog's urine on melting yielded a distillate which crystallised on cooling and then had the melting point 86–87°. (Glyoxaline melts at 88°.)

Micro-Kjeldahl. 0.0040 g. gave 4.95 cc. N/70 NH_3 . N, 24.75 %. Calculated for $\text{C}_4\text{H}_4\text{O}_2\text{N}_2$:N, 25.0 %. This formation of glyoxalinecarboxylic acid from its aldehyde is therefore analogous to the production of pyromucic acid, observed by Jaffé and Cohn, but the yield was very small and no trace of urocanic acid could be detected, perhaps because the glyoxaline ring is broken down too readily by oxidation.

II. It was thought that urocanic acid might be formed *in vitro* from the aldehyde and malonic acid, with subsequent removal of carbon dioxide.

The substance II (*glyoxaline-methylidene-malonic acid*) is readily produced but so far it has been impossible to transform it into urocanic acid (III).

Knoevenagel [1904] showed that a variety of aldehydes readily condense with malonic acid and allied substances, when the reaction is catalysed by the addition of ammonia or a primary or secondary amine. The base is supposed to unite with the aldehyde giving a product which reacts readily

with the malonic acid. In the present case it is interesting to note that the basic glyoxaline-formaldehyde readily condenses with malonic acid without addition of a catalyst, on simply warming the two substances in aqueous solution on the water-bath. The aldehyde (1 g.), malonic acid (1.2 g.) and water (25 cc.) were heated together. A practically clear solution resulted from which almost at once the condensation product (1.6 g.) began to separate as a finely crystalline meal. The substance is sparingly soluble in water or acetic acid, and very slightly soluble in alcohol or ether. It crystallises from water in prisms, which melt at 214° with decomposition and evolution of much gas. It gives a finely crystalline sparingly soluble *nitrate*, thus resembling urocanic acid. The substance is strongly acidic and may be fairly accurately titrated with baryta and phenolphthalein, the acid neutralising two equivalents of alkali. Permanganate is readily decolorised in both alkaline and acid solution.

Micro-Kjeldahl. 0.0128 g. gave 9.65 cc. N/70 NH_3 . N, 15.1 %. Calculated for $\text{C}_7\text{H}_6\text{O}_4\text{N}_2$: N, 15.4 %.

All efforts to remove one molecule of carbon dioxide from the substance, so as to yield urocanic acid, were unsuccessful. The substance was heated directly with and without glycerol to 200° , with water in a sealed tube to 150° , with acids and with alkalies. Carbon dioxide was in many cases readily removed, but urocanic acid could not be isolated from the products of the reaction. With water at 150° the reaction product was very soluble in water and could not be crystallised.

III. With a view to obtaining glyoxaline-acetaldehyde in the same way that acetaldehyde is formed from lactic acid, α -hydroxy- β -glyoxaline-propionic acid was heated with sulphuric acid, and a molecular proportion of carbon monoxide was indeed eliminated. The primary reaction is no doubt expressed as follows:

but then the aldehyde undergoes the further loss of a molecule of water and two molecules are condensed, the whole reaction being represented as follows:

The constitution of the base $\text{C}_{10}\text{H}_8\text{N}_4$, containing two glyoxaline rings, has

not yet been established, but it is evidently closely analogous to the hydrocarbon $C_{16}H_{12}$ obtained by Breuer and Zincke [1878] by heating phenylacetaldehyde and allied compounds with sulphuric acid, diluted with an equal volume of water. The hydroxy-glyoxaline-propionic acid was prepared from histidine by Fränkel's method [1903] and the substance containing a molecule of water of crystallisation was heated with three times its weight of sulphuric acid. With acid more dilute than 75 % no carbon monoxide is evolved until the acid has been concentrated. With 85 % acid evolution is slow at 150° , but complete at $170-180^{\circ}$. With 90 % acid carbon monoxide begins to appear at 125° and is liberated completely at $150-160^{\circ}$. With acid of a still higher concentration charring is apt to occur. The best concentration is therefore 85-90 % of H_2SO_4 and the yield of carbon monoxide approaches closely to the theoretical.

0.312 g. of hydrated α -hydroxy- β -glyoxaline-propionic acid (= 1/50 gram-molecule) was heated with 1 cc. of 85 % sulphuric acid in a test tube in a metal bath. Previous experiments had shown that no trace of carbon dioxide is formed in the reaction, so the air in the test tube was swept out by means of a current of carbon dioxide and subsequently the monoxide formed was collected in the same way in a Schiff nitrometer. The gas evolved measured 48.0 cc. at 20° and 760 mm., of this 0.2 cc. was absorbed by alkaline pyrogallol and 46.6 cc. by ammoniacal cuprous chloride, leaving a residue of 1.2 cc. The oxygen and the residue, probably nitrogen, were doubtless introduced as air from the Kipp apparatus.

46.6 cc. CO (moist) at 20° and 760 mm. = 0.052 g. or 93 % of the theoretical.

At the end of the experiment the sulphuric acid solution was pale brown and strongly fluorescent; the fluorescence is due to a substance hardly soluble in hot water, but readily in alcohol, which could not be obtained pure. The sulphuric acid may after dilution be neutralised with barium carbonate and after filtering hot, the base $C_{10}H_8N_4$ crystallises from the filtrate, or it may be obtained more simply by the addition of concentrated ammonium hydroxide, which at once precipitates it; yield 55-68 % of the theoretical. The deficiency is due to the formation of the fluorescent substance and of a substance readily soluble in water but not in alcohol, which can be obtained from the final mother liquor after quantitative removal of the sulphuric acid by barium hydroxide. This second substance is probably glyoxalinecarboxylic acid; it crystallised from water in thin needles, closely resembling the synthetic acid, and melted at the same temperature (284°).

Properties of the base C₁₀H₈N₄.

The base is readily soluble in cold alcohol, but only slightly in hot water (about 1 %), from which it is best recrystallised by addition of charcoal. In the crude state it forms small characteristic lozenge- or diamond-shaped plates, with rounded corners, but after recrystallisation irregular leaflets are obtained, which may be a centimetre long but still tenaciously retain a little colouring matter; by sublimation in the vacuum of a Gaede pump it is, however, obtained quite white. The substance crystallised from water or dilute alcohol has the composition C₁₀H₈N₄, H₂O. The water of crystallisation is given off in a vacuum over phosphorus pentoxide at 100°, but under atmospheric pressure only at a considerably higher temperature. The anhydrous substance is very hygroscopic, and the hydrated substance was therefore analysed, after drying in a steam-bath until constant.

0.1408 g.; 0.0635 g. H₂O and 0.03093 g. CO₂,
 0.00965 g. (Micro-Kjeldahl) gave 13.4 cc. N/70 NH₃,
 0.0592 heated to 150° in air-bath until constant lost 0.0053 g.
 0.1041 heated over P₂O₅ at 10 mm. and 100° lost 0.0090 g.

The molecular weight was determined by the micro-method due to one of us [Barger, 1904].

0.0508 g. in 0.81 g. ethyl alcohol was equivalent to 0.325 g. β-naphthol, whence M.W. = 193.

	C	H	N	H ₂ O	M.W.
Found	59.9	5.0	27.8	8.7, 8.9	193
Calc. for C ₁₀ H ₈ N ₄ , H ₂ O	59.4	5.0	27.7	8.9	202

Heated to 150° the hydrated substance sinters without melting; plunged suddenly into a bath at 160° it melts, but gradually solidifies again to the anhydrous substance, which melts at 235–238°. In a Gaede pump vacuum it sublimes with metal bath at 260°. It has not been possible to crystallise the anhydrous substance from non-aqueous solvents although the original crystalline form is preserved when the substance is dehydrated at 100° in a vacuum; exposed to the air, the anhydrous substance rapidly takes up rather more than a molecule of water.

The substance C₁₀H₈N₄ is a base, readily soluble in hydrochloric or acetic acid. The *nitrate* is, however, very slightly soluble and crystallises well from water; the *picrate* is hardly soluble in boiling water. The base has practically no effect on the blood pressure or at most, exerts a very slight pressor action.

Action of other reagents on α -hydroxy- β -glyoxaline-propionic acid.

By heating the above acid with baryta to 205° , Fränkel [1903] obtained a substance, very slightly soluble in water and melting at 216° , in quantity insufficient for analysis. We repeated this experiment on a much smaller scale but could not isolate Fränkel's substance nor the base $C_{10}H_8N_4$ and therefore cannot draw any conclusion as to their possible identity.

With syrupy phosphoric acid at 200° no reaction occurs. At 220 – 240° about one molecule of carbon monoxide is given off and the solution becomes intensely fluorescent; after removal of phosphoric acid by baryta and evaporation, the residue was found to consist, for the smaller part, of the fluorescent substance, slightly soluble in cold water, readily in alcohol, and for the larger part of a substance readily soluble in water but insoluble in alcohol. The second substance on heating with 85 % sulphuric acid to 180° is transformed into the crystalline base $C_{10}H_8N_4$.

Concentrated hydrochloric acid at 235° acts in much the same way as phosphoric acid, yielding a fluorescent mixture of substances.

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