amylase is a single enzyme which has for its specific function the degradation of starch to maltose.

Conclusions.

1. *Salivary amylase in sterilized saliva without preservative is relatively stable for one year.* This relative stability may vary from practically no change to that of over 50% of its former amyloclastic activity, the variation depending probably on slight differences in the composition of the saliva.

2. The causes which lower the stability of salivary amylase in saliva are not solely the degrading action of bacteria, mould spores, yeast plants and special preservatives. The inherent chemical weakness of the enzyme molecule, rather, must be taken into account, which weakness may be increased by the presence of temperatures from 18 to 30°, diffused light and compounds in the saliva.

3. *Salivary amylase in saliva is relatively stable for a year when preserved with toluene, thymol and chloroform.* Toluene has the least destructive action on the enzyme and thymol and chloroform follow in order.

4. Saliva may be kept for 2.5 years under the ordinary laboratory conditions without preservative and may still show a form of amyloclastic activity.

NEW ORLEANS, LOUISIANA.

[CONTRIBUTION FROM THE OTHO S. A. SPRAGUE MEMORIAL INSTITUTE AND THE DEPARTMENT OF PATHOLOGY OF THE UNIVERSITY OF CHICAGO.]

STUDIES ON PROTEINOGENOUS AMINES.

I. THE SYNTHESIS OF β-IMIDAZOLYLETHYLAMINE (HISTAMINE).

BY KARL K. KOESSLER AND MILTON TH. HANKE.

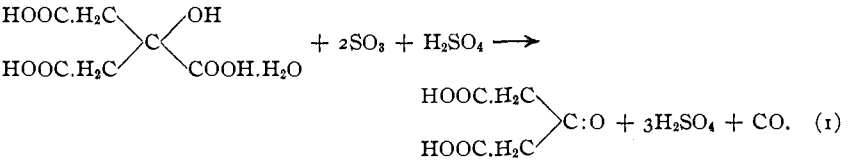
Received August 12, 1918.

The synthesis of β-imidazolylethylamine (β-aminoethylglyoxaline) reported herewith is based on the synthesis of this substance reported by F. L. Pyman.[1] Diaminoacetone dihydrochloride, obtained from citric acid, is heated with sodium sulfocyanide. The thioglyoxaline thus formed, according to Gabriel's[2] general method, is oxidized with nitric acid and through action of nitrous acid formed the hydroxymethylglyoxaline is obtained; over the chloro-compound the nitril is prepared which on reduction yields the amine. While we have followed in the main Pyman's procedure, several additions and improvements have been made which warrant a detailed report of some of the steps involved in the synthesis of this substance which, on account of its remarkable physiological properties, is of great interest to the biochemist.

[1] F. L. Pyman, *J. Chem. Soc.*, **99**, 668 (1911).

[2] Gabriel, *Ber.*, **26**, 2204 (1893); *Ibid.*, **27**, 1037 (1894).

The Preparation of Acetonedicarbonic Acid.[1]



The method employed by us differs from that of Pechmann in the following details: Finely powdered commercial citric acid containing one molecule of water of crystallization was used instead of the heat-dehydrated substance. To insure the entire absence of water in the reaction mixture, fuming sulfuric acid containing 18.3% of sulfur trioxide was used in the proportion of two pounds of sulfuric to one pound of citric acid. The fuming acid was not diluted with concd. sulfuric acid. The reaction mixture was not agitated. Instead of heating on the boiling water bath, a bath kept at 65–70° was used, the reaction mixture being allowed to lie quietly in the bath. Sulfur dioxide was never evolved at this temperature. The reaction was over about 5 minutes after the cabon monoxide flame went out, and this point was easily recognized by the fact that the solution no longer effervesced. The total time of heating was 55 minutes.

The flask and contents were now cooled to a temperature near 0° and 500 cc. of water added in small portions, the mixture being thoroughly cooled after each addition. After about 200 cc. of water had been added, crystals began to form, and they increased in quantity with each further addition. The resulting semi-solid pasty mass was filtered through art canvas on an 8-inch Büchner funnel and pressed down with a pestle until it was no longer sticky. The product so obtained was over 80% acetone dicarbonic acid. The average yield for 10 experiments was 330 g. of 80% acetone dicarbonic acid from 450 g. of citric acid, which is 86% of the theory. Pechmann obtained 225 g. of equally pure product from 450 g. of citric acid, which is 60% of the theory.

The Preparation of Di-isonitrosoacetone.[2]

$$CO.(CH_2 - COOH)_2 + 2HNO_2{}^{3} \longrightarrow CO[C(:NOH)COOH]_2 + 2H_2O \longrightarrow CO.(CH:NOH)_2. \quad (2)$$

The method employed by us was a very close duplicate of that used by

[1] Pechmann, *Ann.*, **261**, 155 (1891).

[2] Pechmann, *Ber.*, **19**, 2465 (1886).

[3] The nitrous acid is formed partially by the interaction of sodium nitrite with the acetonedicarbonic acid, but largely by interaction with the sulfuric acid which is present as an impurity in the acetonedicarbonic acid. At first it is the sulfuric acid that furnishes the necessary hydrogen ions. Until it has been completely converted into sodium sulfate, the mixture has a pale yellow color, and carbon dioxide is freely evolved. After the free sulfuric acid has been entirely neutralized, the solution changes color from yellow to bluish brown and the evolution of carbon dioxide almost ceases al-

Pechmann,[1] the only significant difference being in the amount of sodium nitrate used. Operating on 100 g. of crude acetone dicarbonic acid, we found that 60 g. of 93% sodium nitrate was sufficient to complete the reaction. If more nitrate was used the yield was not improved, if less, the product was impure.

The Preparation of Diaminoacetone Chlorostannite.[2]

$$CO(CH{:}NOH)_2 + 5SnCl_2 + 10HCl \longrightarrow [CO(CH_2 \cdot NH_3)_2]SnCl_4 + 2H_2O + 4SnCl_4. \quad (3)$$

Although the principle of the preparation as carried out by us was identical with that described by Kalischer,[3] we found it difficult to obtain uniform results by following the details given in the literature. The large excess of hydrochloric acid present early in the course of the reaction produced a marked decomposition of the di-isonitrosoacetone which gave rise to a brown-colored solution and a decreased yield of the desired substance. The temperature employed by Kalischer was so low that 48 hours were required to complete the reaction. We deemed it best, therefore, to give the process we employed in detail.

400 g. of crystalline stannous chloride was mixed with 110 cc. of 37% hydrochloric acid in a two-liter round-bottomed flask. The mixture became very cold as most of the stannous chloride dissolved. Finely powdered di-isonitrosoacetone was slowly added to this mixture in small portions so that about 15 g. had been added in the course of an hour. The reaction was strongly exothermic. Since the reaction proceeded most rapidly and efficiently at room temperature, it was necessary to cool the mixture somewhat after each addition.[4]

After about 15 g. of di-isonitrosoacetone had been added, the mixture was treated with 110 cc. of 37% hydrochloric acid, which dissolved most of the remaining stannous chloride. The remaining di-isonitrosoacetone obtained from 100 g. of acetonedicarbonic acid, 15–20 g., was then slowly added as described above in the course of another hour.

The final mixture so obtained was of a semisolid consistency, due to the presence of diaminoacetone chlorostannite, which began to precipitate

though the nitrous acid formed is still absorbed. This would seem to indicate that the sodium salt of di-isonitrosoacetonedicarbonic acid does not lose carbon dioxide readily. To complete the reaction it is necessary to add a mineral acid to decompse the above sodium salt. Enough of the mineral acid will have been added when the color of the mixture changes again from bluish brown to yellow.

[1] *Ber.*, **19**, 2465 (1886).

[2] Kalischer, *Ibid.*, **28**, 1519 (1895).

[3] *Ibid.*, **28**, 1519 (1895).

[4] If the mixture became distinctly warm to the touch, decomposition of the di isonitrosoacetone occurred and poor yields were obtained. If the mixture was too thoroughly cooled, equally poor yields were obtained, probably due to incomplete reduction.

early in the course of the reaction. To insure a complete precipitation of the chlorostannite, 50 cc. of 37% hydrochloric acid was added and the resulting pale brown mixture placed in an ice box overnight. It was then filtered through art canvas on a 6-inch Büchner funnel. The crystals were washed first with conc. hydrochloric acid and then with 95% alcohol to free them from stannous chloride, and adhering mother liquor. Dried at 100° for 5 hours, the perfectly pure, white crystals weighed from 80–87 grams. Since the equivalent of 33 g. of di-isonitrosoacetone was reduced, the above yield is about 83% of the theory.

The Preparation of Diaminoacetone Hydrochloride from the Tin Double Salt.

$$[CO(CH_2 — NH_3)_2]SnCl_4 + H_2S \longrightarrow CO(CH_2 — NH_2,HCl)_2 + SnS\downarrow + 2HCl. \quad (4)$$

Kalischer found it hard to free the solution from tin by using hydrogen sulfide. This was due to the fact that he added hydrochloric acid to the fairly concentrated aqueous *suspension* of the salt. As can be seen from the above equation, hydrochloric acid is liberated in the course of the reaction, and if its concentration exceeded that necessary for dissolving stannous sulfide, precipitation would cease. We found it possible to obtain a product entirely free from tin by *dissolving* 140.4 g. of the tin double salt—0.4 mole—in 3000 cc. of water and saturating the solution with hydrogen sulfide under pressure. The colorless solution obtained after filtering from the precipitated stannous sulfide was freed from water and hydrochloric acid by distillation *in vacuo* at 60–80°. A colorless to pale yellow entirely crystalline solid was obtained, weighing 65–67 g., which is slightly more than the theory—64.4 g.—the excess weight being hydrochloric acid. This product was used directly for the next preparation, which was carried out in the same flask.

The Preparation of 2-Thiol- (4 or 5) -aminomethylglyoxaline Hydrochloride.[1]

$$O:C(CH_2 — NH_2.HCl)_2 + NaSCN \longrightarrow \begin{matrix} HC — NH \\ \| \quad \diagdown \\ C — N \quad C — SH \\ | \\ CH_2 — NH_2.HCl \end{matrix} + NaCl + H_2O \quad (5)$$

In an attempt to isolate the *free base* of the above substance, Pyman was forced to resort to a rather laborious series of recrystallizations with consequent loss of material. We found that the easily obtainable hydrochloride which was also obtained but not used by Pyman, could be used in place of the free base for the next preparation. Sodium thiocyanate

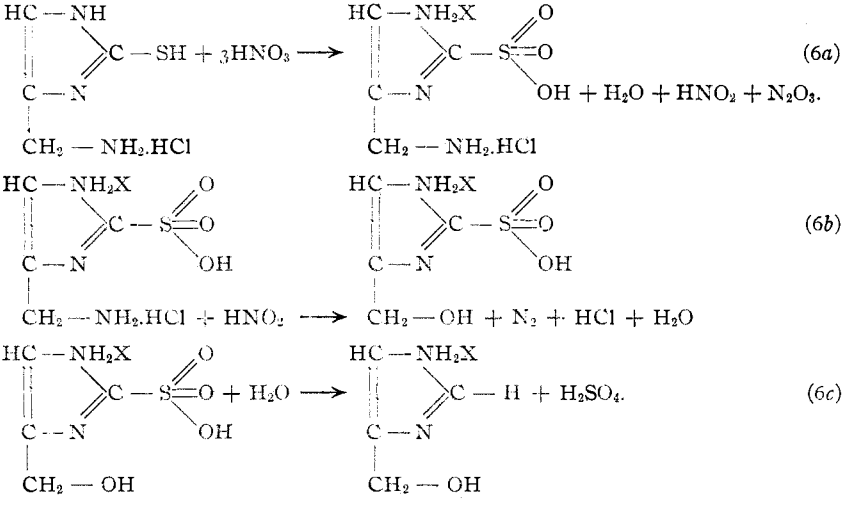
[1] This and the subsequent reactions were first carred out by Pyman and are reported in the *J. Chem. Soc.*, 99, 668 (1911).

proved to be superior to the potassium salt for reasons that will be given below. The method used was briefly as follows:

The diaminoacetone hydrochloride obtained above, representing 64.4 g. of dry salt, was dissolved in 60 cc. of water and heated on the boiling water bath. 35 g. of dry, chemically pure sodium thiocyanate was added to the solution, one-half immediately, and the rest in the course of 20 minutes. After another 60 minutes of heating the reaction mixture was cooled in an ice bath for two hours and the precipitated 2-thiol-(4 or 5)-aminomethylglyoxaline *hydrochloride* filtered on a Büchner funnel. The crystals were washed free from adhering mother liquor with 50% alcohol in which they are not very soluble. Dried at 100° for two hours the white to pale yellow crystals[1] weighed from 53–56 g., which is 82% of the theory.

The filtrate from the above crystals was freed from water, alcohol, etc., by distillation *in vacuo* at 50°. The resulting solid mass was repeatedly extracted with 95% alcohol to free it from thiocyanic acid, sodium thiocyanate and coloring matter. The crystals (C)[2] which were largely sodium chloride, but which contained some 2-thiol-(4 or 5)-aminomethylglyoxaline hydrochloride, were collected on a filter, dried at 100° and reserved for the next preparation.

The Preparation of (4 or 5)-Hydroxymethylglyoxaline Picrate.



It was found advisable in carrying out the above oxidation to use more water and less nitric acid than specified by Pyman, and to use the *hydrochloride* of 2-thiol-(4 or 5)-aminomethylglyoxaline in place of the free

[1] These crystals were free from sodium chloride. When potassium thiocyanate is used, the product weighs about 5 g. more due to the admixture of potassium chloride.

[2] This is a mark for future reference.

base. By boiling the solution obtained, after the oxidation had been completed, for one hour instead of for 10 minutes, complete hydrolysis of the sulfonic acid derivative was insured. The picrate so obtained was quite pure, and recrystallization was unnecessary.

A concentrated aqueous solution of 16.55 g. of 2-thiol-(4 or 5)-aminomethylglyoxaline *hydrochloride*—0.1 mole—was slowly added to a gently boiling solution of 25 cc. of 70% nitric acid in 400 cc. of water contained in a two-liter flask. The resulting pale yellow solution was then boiled for one hour, no attempt being made to prevent concentration. The final volume was about 20 cc. The well-cooled solution was neutralized with dry sodium carbonate, instead of sodium hydroxide, and poured into a hot solution of 30 g. of 90% picric acid in 700 cc. of water. The mixture was cooled in an ice bath for two hours and the precipitated picrate filtered off on a Büchner funnel and washed with 300 cc. of water. Dried for 24 hours in air the pure picrate melted with decomposition at 207° and weighed 29.5 g., which is 90% of the theory.

It was found possible to conduct an entirely similar oxidation on the solid (C) (see above), by adding the dry solid from two experiments to a single oxidation mixture of 25 cc. of 70% nitric acid and 400 cc. of water. From 20 to 28 g. of pure picrate was always obtained.[1,2]

The Preparation of (4 or 5)-Hydroxymethylglyoxaline Hydrochloride.

Picrates are usually freed from picric acid by treating them with hydrochloric acid, filtering from the precipitated picric acid and extracting the resulting solution with ether. When large quantities of picrates have to be liberated, this process is always accompanied by considerable loss of time and material, and is made disagreeable by the large volumes of ether and acid that it is necessary to employ. By using benzene at its boiling point, we have found it possible to liberate large quantities of picrate without difficulty and without loss of material. This process ought to be applicable to the liberation of any picrate when *the resulting hydrochloride is insoluble in hot benzene.*

100 cc. of 37% hydrochloric acid, 250 cc. of water and 500 cc. of benzene were placed in a two-liter flask and immersed in a water bath kept at 80°. 100 g. of 4-hydroxymethylglyoxaline picrate was rapidly added to this mixture. The contents of the flask were then thoroughly and repeatedly mixed until the solid picrate had passed into solution. This required only a few minutes. The benzene layer, which was nearly sat-

[1] This raises the effective yield of 2-thiol-(4 or 5)-aminomethylglyoxaline hydrochloride to 90% of the theory.

[2] It would be entirely impractical to conduct this oxidation on a similar mixture containing potassium instead of sodium chloride because of the insolubility of potassium picrate. This is perhaps the best reason for using sodium instead of potassium thiocyanate for the preparation of 2-thiol-(4 or 5)-aminomethylglyoxaline hydrochloride.

urated with picric acid, was removed as completely as possible by decantation.[1] The aqueous acid solution was then extracted 5 times in the same flask with hot benzene to remove the picric acid as completely as possible. The last traces of picric acid were removed by heating the pale yellow solution with a few grams of Kahlbaum's highest grade animal charcoal and filtering the cooled solution through a hard, folded, water-soaked filter paper. The residual benzene was left on the filter along with the charcoal and coloring matter. The clear, and usually colorless, solution was freed from water and hydrochloric acid by distillation *in vacuo* at 60–80°. An entirely crystalline pale yellow solid, weighing slightly more than the theory, was left in the flask. The further purification of this solid was accomplished rather simply as follows:

The crystalline mass was loosened from the flask with a steel wire and transferred to a mortar with 100 cc. of acetone. The lumps were rapidly pulverized under acetone, the mixture transferred to a Büchner funnel, and the crystals washed with acetone until they were white. Dried *in vacuo* for 24 hours, the pure white crystals had a melting interval of from 107 to 109° and weighed from 39.8 to 40 g., which is 97% of the theory.

The above rather efficient method for purifying the crude product is applicable only when the pure picrate is liberated. When this is not the case, recrystallization from absolute alcohol has to be resorted to as given by Pyman.

The (4 or 5)-chloromethylglyoxaline hydrochloride and (4 or 5)-cyanomethylglyoxaline were prepared, with no significant modifications, according to the directions given by Pyman. The yields were slightly better than those reported by that author.

The Preparation of Imidazolylethylamine Dichloride (Histamine Dichloride).

$$C_3H_3N_2.CH_2.CN + 4Na + 4HOC_2H_5 \longrightarrow C_3H_3N_2.CH_2.CH_2.NH_2 + 4NaOC_2H_5. \quad (7)$$

The method described by Pyman gave a very poor yield of the substance in the form of picrate. By a radical change in the method we have found it possible to obtain a good yield of very pure histamine in its most desirable form, the hydrochloride.

Five g. of cyanomethylglyoxaline, m. p. 138–40°, was treated with 50 cc. of absolute alcohol in a 500 cc. round-bottomed flask. The flask was suspended from a Liebig condenser by means of a cork stopper, and the alcohol heated to boiling by means of a small flame which impinged against

[1] By distilling the benzene solution of picric acid from a water bath, pure benzene is recovered. The residue of picric acid benzene left in the flask can be converted into picric acid by heating for some hours with water on the boiling water bath. By cooling the solution and adding conc. hydrochloric acid to it, the picric acid can be recovered to the extent of 95%.

an asbestos gauze. A one-half inch air space between the gauze and the bottom of the flask prevented superheating and charring of the sodium ethylate which was precipitated in the course of the reaction. Eight g. of metallic sodium, about two equivalents, was rapidly added in small pieces through the condenser, the time of addition being 5 minutes. After boiling for 45 minutes, 20 cc. of absolute alcohol was added through the condenser to facilitate the solution of the sodium.

After the sodium had dissolved, the mixture was allowed to cool somewhat. 50 cc. of water was then added, and the resulting clear, yellow solution was freed from alcohol, ammonia and some water by distillation *in vacuo* at 50°, until the residual solution had a volume of about 25 cc. The strongly alkaline aqueous solution was then transferred to a graduated separatory funnel with enough water to give a final volume of about 50 cc. This solution was then extracted 5 times with amyl alcohol, using 50 cc. for each extraction.[1] The first 3 extracts were pale yellow in color and contained practically all the histamine.[2,3] The remaining extracts were colorless. The fifth extract was free from histamine.

The strongly alkaline aqueous layer was conserved and used for the preparation of glyoxaline acetic acid (see below).

The combined amyl alcohol extracts were dried over anhydrous sodium carbonate, filtered from the sodium carbonate, using a Büchner funnel, and the clear, pale yellow filtrate extracted 5 times in a separatory funnel with 0.5 *N* hydrochloric acid. 100 cc. of acid was used for each of the first 3 extracts, and 50 cc. each for the remaining two extracts.

The combined aqueous acid extracts were pink in color. Heating for one hour on the water bath changed the color to orange-red. A small quantity of Kahlbaum's highest grade animal charcoal was added to the hot solution. The charcoal absorbed the color entirely. The colorless solution obtained by filtration was freed from water and hydrochloric acid by distillation *in vacuo* at 60°.[4] The pale yellow, slightly gummy, crystalline solid, which still contained a small quantity of sodium chloride, weighed from 7.2 to 7.8 g. It was treated with one cc. of 20% hydrochloric acid, 10 cc. of absolute alcohol, and digested on the boiling water bath. The large lumps were pulverized with a flattened glass rod. The

[1] The two layers must be very completely separated, which by ordinary gravity settling takes fully 20 minutes for each extraction. By using a centrifuge, an even better separation can be effected in a few minutes.

[2] Amyl alcohol extracts histamine quantitatively from a strongly alkaline aqueous solution. The sodium salt of glyoxaline acetic acid remains just as quantitatively in the alkaline aqueous layer.

[3] The presence of histamine was determined by means of Pauly's reaction.

[4] A small quantity of sodium chloride usually separated when the final volume of the solution was about 15 cc. This was removed by filtration after adding 30 cc. of absolute alcohol to the mixture.

coloring matter and gum were entirely soluble in the alcohol, the crystalline solid only slightly soluble. After the resulting mixture had stood for 15 hours in an ice box, it was filtered, the crystals being washed freely with absolute alcohol.

Dried at 100° for two hours, and then finally for 48 hours in a vacuum the pure white crystalline solid had the following properties:

(1) It melted to a clear liquid at 244–246° (corr.).

(2) It was entirely free from ammonium chloride.

(3) It had an intense rose-red imidazole reaction with *p*-diazobenzene sulfonate.

(4) It left a residue on ignition at dull red heat of 3.92%, *i. e.*, 0.5000 g. ignited in a platinum crucible left a residue of 0.0196 g., which proved to be sodium chloride.

(5) 0.1 g. of the solid gave 0.30 g. dipicrate in the form of heavy, flat, truncated pyramids, m. p. 238–240°, from 60 cc. of water. This is the recorded melting point of histamine dipicrate.

(6) A nitrogen determination by the Kjeldahl method indicated that the solid was 94.8% pure histamine dichloride, *i. e.*, 0.6012 g. of solid took 9.28 cc. *N* HCl to neutralize the generated ammonia, as compared to 9.79 cc. demanded for 100% histamine dichloride.

(7) A Van Slyke amino nitrogen determination gave the following results: 0.0619 g. of substance gave 8.15 and 8.20 cc. of nitrogen gas at 24° and 749 mm. If the material was really only 94.8% pure, the actual weight of pure histamine dichloride used was 0.0587 g.

% amino nitrogen. Calc., 7.61%. Found, 7.64 and 7.68%.

(8) A chlorine determination gave the following results: 0.1913 g. of solid took 20.93 cc. of 0.1 *N* $AgNO_3$. If the solid is considered to be 94.8% histamine dichloride and 3.92% sodium chloride, 20.89 cc. 0.1 *N* $AgNO_3$ should have been used.

(9) A large guinea pig died inside of 4 minutes from asphyxia due to bronchial spasm when it was given an intracardial injection of 1 mg. of the substance. A *post mortem* examination showed the lungs to be widely and permanently distended.

(10) An intradermic injection of 0.25 mg. into a 160 lb. man produced the following series of symptoms: There was a marked visceral vasoconstriction within the first 30 seconds, the pulse rising from 80 to 130, with increased facial color. This was followed in the next 30 seconds by a vasodilation accompanied by a rapid drop in the pulse rate, considerable sweating and facial pallor.

A large welt surrounded by a red halo formed around the point of injection. This welt had subsided materially within the first hour but did not disappear entirely until 6 hours later.

A marked spasm of the pylorus gave rise to a feeling of nausea and an expulsion of the perfectly digested stomach contents some 5 hours after the injection.

A very painful headache developed within the first hour and had not entirely subsided 8 hours after the injection.

This series of ten tests proves conclusively that the substance really was histamine dichloride. 4.2 to 4.8 g. of pure histamine dichloride were obtained from 5 grams of cyanomethylglyoxaline, which is from 50–56% of the theory.

A very small second crop of histamine dichloride weighing from 0.15 to 0.20 g., m. p. 210–230°, was obtained from the filtrate from the above solid, which distilled to an oil weighing 2.6 g., by recrystallizing from 3

cc. of absolute alcohol. The purity of this second crop was determined approximately by conversion into the dipicrate. Thus 7.3 g., obtained from a number of experiments, when treated with 21 g. of 90% picric acid dissolved in 2000 cc. of water, gave 18 g. of pure histamine dipicrate, m. p. 241°. The 2000 cc. of solution could contain 1.40 g. of picrate.[1] The maximum total weight would then be 19.4 g. Since 22.3 g. are demanded by theory, the material must have been about 85% pure.

The Separation of Methyl Glyoxaline.

The filtrate from the above two crops of histamine dichloride was freed from alcohol by distillation *in vacuo* at 60°.. The combined oil from the reduction of 65 g. of cyanide was dissolved in water, treated with 15 g. of sodium hydroxide and again subjected to distillation *in vacuo*. The semi-solid mass was then extracted repeatedly with ether, about 2500 cc. of ether being used in all. The pale yellow ethereal extract was dried over sodium hydroxide and the ether removed by distillation. 14 g. of pale yellow oil was left in the flask, which was proved to be (4 or 5)-methylglyoxaline by converting it into the picrate. There was thus obtained 36 g. of picrate, m. p. 159–160°. The recorded melting point of methyl glyoxaline picrate is 159–160°.

The Separation of Glyoxaline Acetic Acid.

The strongly alkaline aqueous solution obtained from the reduction of 65 g. of cyanomethylglyoxaline, freed from imidazolylethylamine and methylglyoxaline by extraction with amyl alcohol, was treated with 37% hydrochloric acid until the resulting solution was strongly acid. The solution was then freed from sodium chloride by concentrating *in vacuo*, adding alcohol and filtering. The filtrate from the sodium chloride was freed from water, alcohol and hydrochloric acid by distillation *in vacuo* at 70°. The residue left in the flask was then extracted with a small quantity of hot methyl alcohol, which dissolved the glyoxaline acetic acid hydrochloride readily, but left most of the sodium chloride behind. The brown solid left after the methyl alcohol had been removed by distillation was dissolved in water and the solution decolorized with animal charcoal. The resulting colorless solution was freed from water by distillation *in vacuo* at 60°. The dry, white solid left in the flask weighed 14.35 g. It was triturated in the flask with 20 cc. of hot absolute alcohol, set aside in an ice box to crystallize for 15 hours, and filtered. There was thus obtained 11.1 g. of white needles, m. p. 220–222° (corr.), which were nearly pure glyoxaline acetic acid hydrochloride. One recrystallization from 5 cc. of water and 25 cc. of absolute alcohol gave 7.0 g. of very pure substance, m. p. 226–228° (corr).

[1] Unpublished data.

THE APPROXIMATE AVERAGE QUANTITIES OF THE PRODUCTS THAT CAN BE OBTAINED FROM TEN POUNDS OF CITRIC ACID.

	Obtained, grams.
Citric acid	4530
Acetone dicarbonic acid	3300
Di-isonitroso acetone	1089
Diamino acetone chlorostannite	2640
Diaminoacetone hydrochloride	1200
2-Thiol-4-aminomethylglyoxaline hydrochloride (pure)	950
4-Hydroxymethylglyoxaline picrate	..
(1) From the above 950 g. 2-thiol compound	1670
(2) From the solid (C) (see experimental part)	185
4-Hydroxymethylglyoxaline hydrochloride	740
4-Chloromethylglyoxaline hydrochloride	700
4-Cyanomethylglyoxaline	200
Imidazolylethylamine dihydrochloride	165

CHICAGO, ILLINOIS.

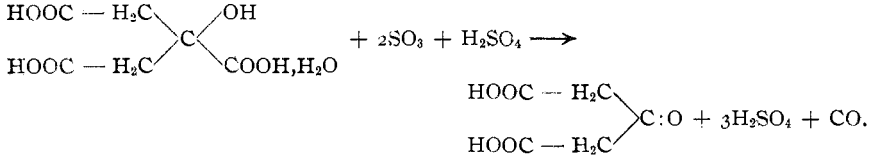
[CONTRIBUTION FROM THE OTHO S. A. SPRAGUE MEMORIAL INSTITUTE AND THE DEPARTMENT OF PATHOLOGY OF THE UNIVERSITY OF CHICAGO.]

THE ELECTRONIC CONSTITUTIONS OF ACETOACETIC AND CITRIC ACIDS AND SOME OF THEIR DERIVATIVES.

BY MILTON TH. HANKE AND KARL K. KOESSLER.

Received August 12, 1918.

The first step in the synthesis of imidazolyl ethyl amine[1] is the preparation of acetone dicarbonic acid by treatment of citric acid with fuming sulfuric acid.



It will be noticed from the above equation that it is the central carboxyl group that is removed as carbon monoxide. This selective action toward the central carboxyl group is hard to explain unless the electrical constitution of citric acid is considered. The following formula shows how much of the electrical constitution of citric acid can be directly foretold by inspection and by a knowledge of its simplest reactions.[2]

It will be noted that the unknown electrical charges are limited entirely to the linkages between the carbon atoms. In attempting to solve the problem of the direction of electrical field in these bonds, the easiest point of attack is the union between the carboxyl groups and the carbon atoms to which they are attached.

[1] See preceding article.

[2] The heavy dashes represent electrical fields of undetermined polarity.