3. The compounds giving Pauly's reaction are probably not histidine, since Weidel's reaction, as modified by Fischer, or Knoop's reaction with bromine were both negative.

4. Pauly's reaction is not a specific reaction for histidine, unless other compounds, such as tyrosine, *p*-oxyphenylethylamine, β-iminazolylethylamine, adrenaline, etc., are removed; but a general reaction for a class of compounds yet to be determined.

DETROIT, MICH.

[CONTRIBUTION FROM THE PHARMACOLOGICAL LABORATORY OF THE UNIVERSITY OF MINNESOTA.]

TRI-AMMONIUM CITRATE.

BY ROBERT A. HALL.

Received November 20, 1914.

The ammonium salts of citric acid were seemingly first investigated by Heldt[1] in 1843, although in Berzelius' Lehrbuch der Chemie IV the statement is made that these compounds are very difficult to prepare and that no analysis of them had been made. Heldt's method of preparation was to neutralize a solution of citric acid with ammonia and by evaporation crystallize out the salts. His efforts to obtain the monoammonium citrate were unsuccessful, as his thick syrupy liquid always gave star-shaped groups of crystals containing varying amounts of ammonia. With the diammonium citrate he was more successful, as he obtained this salt in two separate, distinct crystalline forms. From a strong, concentrated solution after standing some hours, there separated out a mass of interlaced rhomboidal prisms; but upon slow evaporation in the cold winter air, the salt separated at the bottom of the vessel as a ring of massed oblique rhomboidal prisms with rectangular sides and semi-circular back-sides. In their composition both salts were identical and afforded a very interesting example of dimorphic organic compounds. Both crystalline forms rapidly absorbed moisture from the air and were soluble in boiling alcohol from which, upon cooling, they separated as oily drops. Their taste is agreeably acid with a cooling, bitter after effect. They were dried by pressing between filter paper and an elementary analysis made of their hydrogen and carbon content; the nitrogen was determined by precipitation as platinum ammonium hydrochloride. Both crystalline forms were found to have the composition represented by the formula $(NH_4)_2C_6H_6O_7$, that is, both were the diammonium citrate. Both salts were stable on heating to 100°.

Heldt's efforts at preparing the triammonium citrate were unsuccessful. He attempted to prepare this salt by evaporating a citric acid solution saturated with ammonia, but obtained the diammonium citrate. A

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[1] Heldt, *Lieb. Ann.*, 47, 167 (——).

boiling alcoholic solution of citric acid, neutralized with ammonia, became cloudy upon cooling and deposited the salt as oily drops which did not crystallize even upon long standing.

Heusser,[1] in 1853, was evidently unacquainted with Heldt's earlier work on the ammonium citrates, for he says, "Concerning these salts (Citrates of Ammonium) I find nothing in the literature other than the observation in Berzelius's Lehrbuch der Chemie IV that they can be prepared unmixed only with great difficulty and no analyses thereof have been made." He attempted the preparation of the ammonium citrates but, in so far as the preparation of the triammonium citrate was concerned, he was no more successful than Heldt had been. He obtained the diammonium citrate and a compound that was probably a mixture of the di- and triammonium salts. His methods were essentially the same as those of Heldt.

F. Sestini,[2] in 1879, prepared a *neutral ammonium citrate* in crystalline form by neutralizing a solution of citric acid with ammonia and evaporating this solution over quick-lime in an atmosphere of ammonia. The crystals thus obtained were dried between filter paper and analyzed, the ammonia content and water of crystallization only being determined. The salt had approximately the composition represented by the formula $(NH_4)_3C_6H_5O_7 + H_2O$, that is, the salt was triammonium citrate with one molecule of water of crystallization. The crystals were monoclinic and were very unstable, going over into the diammonium citrate upon being dried at 105–110°.

Van Itallie,[3] in 1908, for the purpose of investigating the failure of calcium to produce a precipitate when added to an ammoniacal solution containing oxalate and citrate, repeated the preparation of triammonium citrate by Sestini's method and with the same results. He says, "The compound is not easy to purify for analysis, as is easily understood, because it is so hygroscopic and so easily dissociated." In his analysis he determined the citric acid and ammonia content of his salt, as well as the water of crystallization. He, too, obtained the triammonium citrate with one molecule of water. From the data obtained in his physical-chemical measurements, using Schreinemaker's "Reste-Methode" of plotting his curves, he demonstrated that the triammonium citrates containing one or more molecules of water must necessarily be unstable.

The writer first prepared the salt, normal triammonium citrate, in 1911, shortly after the completion of his investigations which were published in the papers, "The Physical Properties of Aqueous Solutions Containing Ammonia and Citric Acid"[4] and the "Preparation of Neutral

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[1] *Pogg. Ann.*, 88, 121 (1853).

[2] *Gazz. chim. ital.*, **9**, 298 (1879).

[3] *Z. anorg. Chem.*, **60**, 358 (1908).

[4] THIS JOURNAL, **33**, 711 (1911).

Ammonium Citrate Solutions by the Conductivity Method."[1] The salt thus obtained reacted alkaline to rosolic acid, although the analyses showed it to have the composition of normal triammonium citrate. As this was contradictory to all the literature then existing on the subject, it was deemed advisable to make further investigation. In the spring of 1912, further samples of the salt were made, analyzed, and certain physical and chemical properties were observed.[2] The method of preparation and general properties of the salt were reported, for record, at the meeting of the Academy of Science of St. Louis, April 21, 1913.[3] An informal oral announcement of the preparation and properties of the salt was also made at the following May meeting of the St. Louis Section of the American Chemical Society. Publication was deferred, as it was desired to continue the investigation of the salt along the following lines: (1) As to its physical-chemical properties—it was deemed necessary to determine experimentally the concentration of the NH_3 and citric acid ions in both a dilute solution and in a solution of the concentration used in the fertilizer analysis; also, to ascertain by determinations of the ionization constants the proper indicator to be used in testing the neutrality of the so-called "neutral" ammonium citrate solutions. (2) The character and effect of the growth that so frequently occurs in so-called neutral solutions of ammonium citrate. (3) The pharmacological properties of the salt.

In the summer of 1913 the investigation of the physical chemical properties had scarcely been begun in the Kent Chemical Laboratory, University of Chicago, when Dr. Hildebrand's paper, "Some Applications of the Hydrogen Electrode in Analysis, Research, and Teaching"[4] appeared. Hildebrand had anticipated a part of the investigation planned and had announced his intention of investigating and determining the proper indicator for concentrated solutions of neutral ammonium citrate, so this part of the research was abandoned. The recent appearance of his paper on "The Preparation of 'Neutral' Ammonium Citrate"[5] enabled me to employ his results in the examination of the salt under investigation and to complete that part of it. Although the investigation of the growth formation in the solution and the pharmacological properties of the salt is still incomplete, yet the importance of the salt, to the fertilizer chemist in particular, is thought to be sufficient justification for publishing the results so far obtained.

Hantzsch,[6] in 1902, found, in his investigations of the behavior of weak

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[1] *J. Ind. Eng. Chem.*, **3**, 559 (1911).

[2] I am indebted to Messrs. John T. Ragsdale, Jr., and H. W. Ramsey, my students, of St. Louis, for their assistance in making these preliminary analyses.

[3] *Transactions of the Academy of Science of St. Louis*, **22**, No. 6, 40 (1913).

[4] THIS JOURNAL, **35**, 847 (1913).

[5] *J. Ind. Eng. Chem.*, **6**, 577 (1914).

[6] *Ber.*, **35**, 226, 2724 (1902); **40**, 3798 (1907).

acids and pseudo-acids towards ammonia, that, by passing perfectly dry ammonia gas into solutions of weak organic acids and pseudo-acids in perfectly anhydrous indifferent solvents as ether or benzol, the ammonium salts of these weak acids and pseudo-acids and tautomeric forms of chloroamids, nitro derivatives, etc., hitherto regarded as unstable because of the dissociating effect of water, could be prepared and obtained pure in quantity. If proper care were taken to keep every trace of water from the compound until it had been freed from the mother liquor and perfectly dried, then these bodies were unaffected by moisture and could be freely exposed to the air. This suggested to me that triammonium citrate could be prepared by passing dry ammonia gas into a solution of anhydrous citric acid in an inactive anhydrous solvent, as ether or alcohol. This could be done by dissolving the anhydrous citric acid (made anhydrous by carefully heating the acid, *in vacuo*, to its melting temperature and carefully maintaining this temperature until the water of crystallization was completely removed) in an anhydrous indifferent solvent and then passing through the solution in excess a stream of perfectly dry ammonia gas. Since the diammonium citrate is soluble in boiling alcohol,[1] absolute alcohol probably could preferably be used as the solvent and precipitation would not occur until the formation of the triammonium citrate began. Moreover, only the pure triammonium salt would be obtained if the solution were kept at the boiling point until the acid had been completely converted into the normal salt. The yield would be quantitative. The normal salt could be freed from its mother liquor by decantation and by repeated washings with the anhydrous solvent finally removing the last trace of the solvent by suction on the filter pump. The salt, being thus perfectly dried, should be stable; it could then be exposed to the moisture of the air without undergoing decomposition, etc. The solvent and washings could be freed from ammonia and recovered in the usual way, thus reducing the loss incurred in the preparation to that of the excess of ammonia gas used (and even this loss could be prevented, if desired, by recovery of the ammonia). Results obtained fully justified these conclusions.

Experimental.

Preparation of the Normal Triammonium Citrate Salt.—1. Citric acid was freed from its water of crystallization by heating it to 150°, the melting point of the acid. The temperature was not allowed to go much above this point as the acid is decomposed by a higher heat. The removal of the water of crystallization was facilitated by the use of the vacuum pump. A weighed amount of this water-free acid was placed in a round bottom flask whose mouth was sufficiently large to permit the use of a rubber stopper through which three tubes passed. One tube was

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[1] Heldt, *Loc. cit.*

for the admission of the ammonia gas; this tube extended nearly to the bottom of the flask and was slightly bent outward to avoid coming in contact with the extended flanges of the stirrer when in motion. Through the center of the stopper passed the stirrer which was propelled by a motor of a size sufficient to preclude the probability of the stirrer stopping during the experiment. The third opening was for the entrance of the lower end of the reflux condenser tube, which served the double purpose of an exit tube for the excess of ammonia gas and also for the condensation and return into the flask of the alcohol used as solvent, thus preventing the loss of alcohol. The upper end of the condenser was connected with a tightly fitting tube which conducted the excess ammonia out of doors. The ammonia gas was obtained from a tank of liquid ammonia, or was prepared by allowing strong ammonia water to drop into a flask containing solid sodium hydroxide and ammonium chloride. In either case, for the purpose of completely drying the ammonia, the gas was first passed through a reflux condenser and then through several upright towers filled with sodium hydroxide sticks. Absolute alcohol, distilled over sodium, sufficient to dissolve completely the citric acid and also to cover completely the stirrer when in motion, was added and brought to the boiling temperature by heating on an electric bath. Ammonia gas was then slowly bubbled into the boiling solution which, throughout the course of the experiment, was vigorously stirred by the electrically propelled stirrer. For some few minutes, dependent upon the concentration of the acid solution and upon the rate of admission of the gas, no precipitation occurred. This was as expected and was probably due to the formation of the mono and diammonium citrates which are soluble in boiling alcohol.[1] As soon as the formation of the triammonium citrate began, precipitation occurred and continued until all the acid had been converted into triammonium citrate. When the precipitation was completed the heat was discontinued, but the ammonia gas was allowed to pass through the solution until the flask had become entirely cool. This served to prevent air bearing water vapor from entering the flask as the flask cooled. The supernatant liquid was decanted from the salt which was washed several times by decantation with absolute alcohol, until the washings were free from ammonia, the salt then being pressed dry on the Büchner funnel, using the thin rubber sheet cover with the suction pump as suggested by Cortner.[2] The salt was further pressed out on a clay plate, dried for an hour on a *dry* steam bath, and then placed in a desiccator over sulfuric acid for 24 hrs. The yield of triammonium citrate was quantitative. The alcohol used as solvent and washings was freed from ammonia and recovered in the usual

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[1] Heldt, *Loc. cit.*

[2] THIS JOURNAL, **36**, 1967 (1914).

way, thus making the only loss in the preparation that of the excess of ammonia used.

2. The method of preparation was varied by substituting for anhydrous citric acid purest citric acid, containing one molecule of water of crystallization. Otherwise the preparation was carried out as given above. A salt as pure and as stable as that obtained from the anhydrous acid was obtained. The yield, however, was only 85%, as much of the salt adhered to the sides of the flask.

3. Again the method of preparation was varied by omission of the use of the (electrically propelled) stirrer. The flask was shaken by hand from time to time. There was observed a tendency of the precipitate to clog and stick to the walls of the flask. The flask was tightly corked up and allowed to stand for a week. It was found that the salt obtained was the normal triammonium citrate. The yield was not over 75%.

4. Another variation in the method of preparation was introduced, the substitution of ordinary 95% alcohol for the absolute alcohol used above, the other conditions remaining as given in the first method of preparation. From 60 g. of anhydrous citric acid about 30 g. of the normal salt was obtained. As the mixture of the di- and triammonium citrates adhered closely to the walls of the flask, it was easy to separate the pure salt.[1]

5. Dry ammonia gas was passed into a cold anhydrous alcoholic solution of citric acid; precipitation occurred at once. The precipitate was very viscid and adhered to the walls of the flask and to the tube admitting the ammonia when the flask was shaken. A small amount of the precipitate was removed and, after being freed from the mother liquor, was found to be acid to litmus. The salt thus separated out was either the mono- or diammonium citrate—probably a mixture of the two. It was very deliquescent and unstable.[2] The passage of the ammonia was continued for several hours. The contents of the flask became warm from the heat of the reaction. A small sample was then removed from the flask and found to react acid. The flask was tightly corked and set aside for a week. It was then found that the salt had an alkaline reaction to rosolic acid. The salt was washed and dried as given above and was found to be triammonium citrate. The yield was not good.

Other solvents of citric acid, as ether, acetone, etc., were substituted for alcohol but were found to be unsatisfactory in that the insolubility of the mono- and diammonium citrates in these solvents led to their pre-

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[1] While it has not been put to the experimental test yet, it is very likely that the citric acid with its water of crystallization can be used in this method also.

[2] Investigation of the mono- and diammonium citrates is being continued in this laboratory.

cipitation along with the normal salt and thus gave mixtures instead of the pure salt.

Properties of Triammonium Citrate.—Triammonium citrate, prepared from a solution of the anhydrous citric acid in absolute alcohol or from a solution of the pure citric acid containing water of crystallization, is a beautiful white, crystalline salt. It is stable in so far that it can be exposed to the air for more than 24 hrs. without appreciable change, can be heated for an hour or more on a dry steam bath, or kept indefinitely in a desiccator over sulfuric acid. Upon prolonged heating on the steam bath or prolonged exposure to the air the salt loses ammonia and doubtless passes over into the diammonium citrate.[1] It is exceedingly soluble in water and can not be recrystallized from its water solution by evaporation. When a saturated water solution, cooled in a freezing mixture of ice and salt, is poured into absolute alcohol similarly cooled, a mass of long, fine crystals is obtained. The crystals were unstable and were not analyzed.[1] They are probably the triammonium citrate with one or more molecules of water of crystallization.[2] (It is hoped to take advantage of the winter cold of Minnesota to prepare and to isolate this salt in a pure form by carrying out the preparation altogether in the out door cold.) If a saturated water solution of triammonium citrate at room temperature is added to alcohol, oily drops are obtained but no crystals.[1] The water solution of triammonium citrate is alkaline to rosolic acid, thus fulfilling the prediction of Hildebrand.[3] On exposure to the air a water solution of the salt loses ammonia and reacts acid.

Since the diammonium citrate is soluble in boiling alcohol, it is evident that the triammonium citrate salt that has become acid by loss of ammonia can be purified either by the removal of the diammonium citrate by extraction with boiling absolute alcohol or by passing through the finely powdered salts suspended in hot alcohol an excess of dry ammonia gas and working it up as in the original preparation.

Analyses.

The carbon, hydrogen and nitrogen content was determined by elementary analyses of a sample of the salt that had been dried in the steam oven for an hour and then dried further for some days over sulfuric acid in a desiccator.

I. 0.2501 g. of the salt gave: 0.1583 g. of H_2O and 0.2712 g. CO_2; calc. for $(NH_4)_3C_6H_5O_7$: H = 7.05, C = 29.61; found: 7.04 and 29.58.

II. 0.2115 g. of subst. gave 0.3660 g. of N; calc. for $(NH_4)_3C_6H_5O_7$: 17.28%; found: 17.30%.

III. Assuming that the salt was pure triammonium citrate an 0.1 *N* solution was made.

50 cc. of this solution = 0.4050 g. of the salt, gave on distillation with excess standard alkali 0.08513 g. of NH_3. Calc. for $(NH_4)_3C_6H_5O_7$: 21.01%; found: 21.02.

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[1] Heldt, *Loc. cit.*

[2] Van Itallie, *Ibid.*

[3] Hildebrand, *Ibid.*

The excess of alkali in the distillation flask was determined and the citric acid content of the salt thus found. Calc. citric acid for $(NH_4)_3C_6H_5O_7$: 78.98%; found: 78.96.

For analysis and trial by a chemist in actual comparison with solutions used in the daily determination of phosphoric acid content of fertilizers, a sample of the salt analyzed above was sent to Dr. Paul Rudnick, Chicago. His report is given below:

"We made up a solution of ammonium citrate from the triammonium citrate which you sent us. The quantity received was a little more than sufficient for 500 cc. in all. Its reaction to azo-litmin, by the method described in my paper[1] 'On the Preparation of Neutral Ammonium Citrate,' was neutral. Analysis by a determination of both the NH_3 and citric acid content of the solution showed that the salt which you sent me must, therefore, have been 99.92% pure triammonium citrate. The specific gravity of this solution was 1.0898 at 20°. Accordingly, we added a sufficient amount of the salt to make the specific gravity 1.09 at 20°. The ratio between ammonia and citric acid was then that required by the Patten and Marti standard.[2]

"We then prepared a solution of neutral ammonium citrate from pure citric acid and ammonia and analyzed four different samples of fertilizers with each of the two solutions. The results obtained were as follows:

INSOLUBLE PHOSPHORIC ACID.

	Solution triammonium citrate. Per cent.	Official neutral ammonium citrate solution. Per cent.
10 and 5	0.74	0.71
Acid phosphate	0.10	0.10
Complete fertilizer containing acid phosphate only	2.29	2.27
Complete fertilizer containing acid phosphate and bone	4.55	4.58

"The concordance between these results is all that could be expected."

Dr. Rudnick's results corroborate fully those obtained in this and in other laboratories,[3] the data of which it is unnecessary to give. The importance of this salt to the fertilizer chemist is manifest. His literature of recent years has been replete with easy, simple, and exact methods of preparing the "neutral ammonium citrate" solutions used in the determination of the insoluble phosphoric acid content of commercial fertilizers. While these valuable and interesting physical-chemical and analytical methods have in the main contributed somewhat to rendering more easy the preparation of this difficult solution, yet in no one method, or combination of these methods, has the solution of the problem been found. No two chemists have been able to prepare "neutral ammonium citrate solutions" that were in exact agreement one with the other as to their neutrality or their ammonia and citric acid content. It is hoped that the successful preparation of this salt, triammonium citrate, in a stable, workable form marks the beginning of the end of his troubles. It would

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[1] Rudnick, *J. Ind. Eng. Chem.*, 5, 12, 998 (1913).

[2] Patten and Marti, *Ibid.*, 5, 567 (1913).

[3] Grateful appreciation is hereby expressed to Dr. Rudnick and the others who have so kindly contributed to the success of this investigation.

seem so in view of the very evident fact that it is but necessary to weigh out, or add to water a sufficient amount of the salt to give the required specific gravity, and the solution is ready. The ease with which the solution can be prepared from the pure salt would preclude the necessity of making up large quantities of the solution so that deterioration by standing would be avoided. Uniformity in the results obtained in this very important analyses would thus be predicated upon uniformity of content of the ammonium citrate content of the solution. The difference in the hydrogen ion concentration of the normal and neutral solutions of ammonium citrate is so very slight[1] that the difference in the analytical results are negligible; and the ease and certainty of preparation of an accurate solution from the pure normal triammonium citrate salt would seem to predetermine the question in favor of the use of the triammonium citrate. Since, however, the neutral solution is prescribed legally as the solution to be used in the fertilizer analysis and since any change in the legal status would be dependent upon the report of chemists who have tried out the salt, this preliminary publication is made, although, as has been noted, the investigation of the triammonium citrate as contemplated and desired is still incomplete.

Summary.

Triammonium citrate can be obtained readily and easily as a stable compound by the passage of anhydrous ammonia gas into citric acid dissolved in an anhydrous solvent. The best solvent for this purpose is absolute alcohol heated to its boiling point. The yield of triammonium citrate is quantitative. Ninety-five per cent. alcohol may be used, but the yield is not quantitative.

The salt is a stable, crystalline substance. Analyses show it to have the composition represented by the formula $(NH_4)_3C_6H_5O_7$. It reacts alkaline to rosolic acid. It is exceedingly soluble in water and can not be recrystallized from its water solution. Precipitated, in the cold, from an aqueous solution by addition of alcohol an unstable crystalline form of the salt is obtained.

From practical tests in laboratories where daily analyses of fertilizers are made it has been shown that the salt lends itself readily to the making of solutions of 1.09 sp. gr. at 20°; that this solution of normal ammonium citrate gives, in parallel phosphoric acid determinations, results practically identical with the most carefully prepared "neutral" ammonium citrate solutions and can, therefore, well be substituted for the so-called neutral ammonium citrate solution.

MINNEAPOLIS, MINN.

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[1] Hildebrand, *J. Ind. Eng. Chem.*, **6**, 578 (July, 1914).