Summary

1. This paper describes an application of Crismer's method for measuring quantitatively the amount of water in methyl alcohol.

2. The method is based upon the determination of solution temperatures of the three component systems methyl alcohol-water-ligroin, and methyl alcohol-water-hexane; the exact amount of water present in a given case is determined, once for all, by specific-gravity measurements.

3. The wide range of solution temperatures observed with the two solvents used makes the method accurate and sensitive.

4. The use of pure hexane as solvent is recommended, since by its use a set of permanent data can be obtained.

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[CONTRIBUTION FROM THE DIVISION OF BIOCHEMISTRY AND PHARMACOLOGY, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL]

OBSERVATIONS UPON THE SYNTHESIS OF PYRROLE- AND PYRROLIDONE-CARBOXYLIC ACIDS, AND UPON THE SYNTHESIS OF ALPHA-THIONIC ACID OF PYRROLE[1]

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Introduction

One of the problems which continually confront biochemists is the lack of a satisfactory supply of the constituents of the protein molecule. With the exception of glutamic acid[2] which is used in Japan as a condiment the production of the amino acids is extremely limited. In certain instances the known methods of preparation are such as to yield products of doubtful purity. This is particularly true of the amino acids which contain the pyrrole ring. While our attempts to synthesize proline have been unsatisfactory some additional information regarding the behavior of certain of the pyrrole compounds has been gained.

The two methods for the synthesis of proline which are best known are those of Sörensen[3] and of Fischer and Zemplén.[4] The former carried out his synthesis by permitting the diethyl ester of sodium phthalimidomalonic acid to react with trimethylene-bromide with the formation of α-bromopropyl-phthalimidomalonic ester. When this substance is boiled with potassium hydroxide, proline, glycine and a number of other products

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[1] This investigation has been aided by a grant from the Research Board of the University of California.

[2] Ikeda, Eighth Intern. Cong. Appl. Chem., **18**, 147 (1912). Ikeda and Suzuki, U. S. pat. 1,015,891, 1,035,591 (1912). Schmidt and Foster, *Proc. Soc. Exptl. Biol. Med.*, **18**, 205 (1921).

[3] Sörensen, *Compt. rend. trav. lab. Carlsberg*, **6**, 137 (1905).

[4] Fischer and Zemplén, *Ber.*, **42**, 1022, 2989 (1909).

result. This synthesis is expensive due to the cost of the initial products. The Fischer and Zemplén synthesis involves the breaking of the piperidine ring by oxidation with potassium permanganate, which yields benzoyl-*d*-aminovalerianic acid. The latter substance is brominated and then boiled with alkali. In both syntheses proline is contaminated with secondary products of the reaction which are very difficult to remove.

Experiments with Pyrrolidone-α-Carboxylic Acid

Pyrrolidone-α-carboxylic acid affords a possible source for the synthesis of proline. This product is readily formed from glutamic acid by heating at 150–160°,[5] and in turn it is easily hydrolyzed to glutamic acid by boiling with concd. hydrochloric acid.[6] Fischer and Boehner[7] saw in pyrrolidone-α-carboxylic acid a possible source for proline and their work consisted in an attempt to reduce the ethyl ester of this substance by means of sodium and alcohol. The yield, however, was extremely poor. The following reagents were used by us in our attempts to reduce pyrrolidone-α-carboxylic acid: zinc amalgam, tin and hydrochloric acid, sodium and ethyl alcohol, sodium amalgam and water, aluminum and sodium hydroxide, aluminum amalgam, and phosphorus and hydriodic acid in sealed tubes. Catalytic reduction was tried with platinum as a catalyst[8] using water, glacial acetic acid and alcohol as solvents and hydrogen under pressure of one atmosphere. The catalytic reductions were attempted at both room temperatures and 100°. In no instance was there a measurable reduction.

Attempts were also made to induce the carbonyl group of the pyrrolidone compound to react with phenylhydrazine and hydroxylamine but without success. Negative results were also obtained in the attempts to reduce the ethyl ester of the pyrrolidone compound with platinum as a catalyst.

Experiments with the α-Carboxylic Acid of Pyrrole

A series of experiments was next carried out in an effort to reduce the amide of the α-carboxylic acid of pyrrole. Fischer[9] attempted this reduction, using phosphonium iodide and hydriodic acid but found he could obtain only pyrroline-α-carboxylic acid. In our experiments the amide was prepared by the method of Schwanert.[10] Catalytic reduction was attempted with hydrogen in the presence of platinum and palladium. Various strengths of alcohol with and without the addition of different amounts of hydrochloric acid were employed as solvents. No reduction

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[5] Haitinger, *Monatsh.*, **3**, 228 (1882).

[6] Foreman, *Biochem. J.*, **8**, 481 (1914).

[7] Fischer and Boehner, *Z. physiol. Chem.*, **65**, 118 (1910).

[8] Prepared both by the methods of Adams and Kaufmann, THIS JOURNAL, **45**, 3029 (1923), and Willstätter and Hatt, *Ber.*, **45**, 1472 (1912).

[9] Fischer and Van Slyke, *Ber.*, **44**, 3166 (1911). Fischer and Gerlach, *Ber.*, **45**, 2453 (1912).

[10] Schwanert, *Ann. Chem. Pharm.*, **116**, 270 (1860).

was obtained when pyromucic acid was added to the flask, indicating that the catalyst had been poisoned. A control experiment with pyromucic acid alone showed that this substance readily absorbed hydrogen.

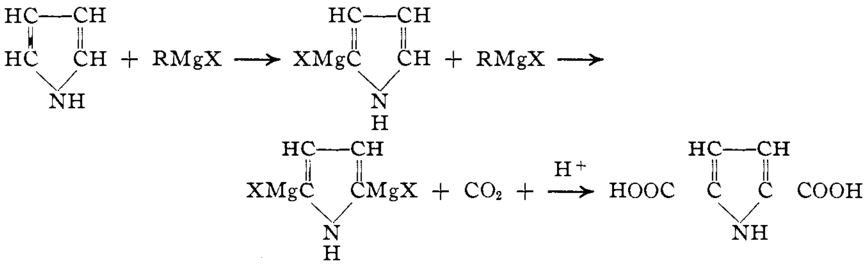
Synthesis of the α-Carboxylic Acid of Pyrrole

Another source for the α-carboxylic acid of pyrrole was found in the method developed by Oddo,[11] and the later improvement of Gilman and Pickens.[12] They obtained the acid by passing carbon dioxide through an ether solution of pyrrylmagnesium iodide and decomposing the resulting compound with sulfuric acid. Oddo reports a yield of 25–30%. Since a better yield is desirable we found it advantageous to modify the conditions of preparation.

Methylmagnesium iodide was prepared according to the optimum conditions which have been developed for the Grignard reagent by Gilman.[13] Our reagent was found to be 0.62 *N*. From a large number of runs we found that the following gave the best yield. A solution of 10 cc. of pyrrole in 100 cc. of absolute ether was slowly added during rapid stirring to 338 cc. of the Grignard reagent. The mixture was cooled with ice, and carbon dioxide was slowly passed through the flask for an hour. An excess of normal sodium hydroxide solution was added, the mixture heated to boiling and the resulting magnesium hydroxide removed by filtration. The filtrate was treated with charcoal, concentrated to a small volume, cooled and sufficient hydrochloric acid was added to make the reaction just acid to litmus. A large proportion of the desired product was separated at this point. The supernatant liquid was further acidified and the last traces of the product were extracted with ether. The yield was 6.46 g., or 40%.

These studies to obtain a maximum yield throw some light upon the mechanism of the reaction. The optimum molecular proportion for the synthesis of pyrrylmagnesium iodide is one of pyrrole to 1.4 of methylmagnesium iodide. If an amount of the latter substance in excess of this ratio is employed, it is used up in the formation of acetic acid.

It might be expected that, instead of the reaction proceeding up to the extent of about 40% of the available pyrrole and the excess of any available methylmagnesium iodide being converted into acetic acid, the reaction might proceed as follows.



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[11] Oddo, *Gazz. chim. ital.*, **39**, I, 649 (1909); **44**, I, 482 (1914); *Ber.*, **43**, 1012 (1910).

[12] Gilman and Pickens, This Journal, **47**, 245 (1925).

[13] Gilman, *ibid.*, **45**, 154 (1923).

The products of this reaction should be α,α'-dicarboxylic acid of pyrrole as well as α-carboxylic acid of pyrrole and acetic acid. The recent work of Dufford[14] and his co-workers on the luminescence of Grignard compounds when exposed to the action of a stream of oxygen might throw some light upon the position of the —MgI group in pyrrylmagnesium iodide. When a stream of oxygen was allowed to play on the surface of a solution of pyrrylmagnesium iodide, no luminescence was observed. In view of Dufford's experiments these results may, indicate that the —MgI remains on the nitrogen until carbon dioxide has been blown into the solution. In the light of the more recent experiments of Evans and Diepenhorst[15] however, this can but little more than confirm the conclusions of the previous observation in which it was noted that α,α'-dicarboxylic acid of pyrrole is not obtained even when a large excess of methylmagnesium iodide is used in the preparation of pyrrylmagnesium iodide.

Further Attempts to Reduce the α-Carboxylic Acid of Pyrrole

Using the α-carboxylic acid of pyrrole which was synthesized by the Grignard method, numerous attempts at catalytic reduction were made. The same solvents as those previously mentioned were used. In contrast to the earlier attempts the catalyst was never poisoned as was shown by its ability to reduce xylene. In only one case was hydrogen absorbed, in a 50% solution of alcohol containing 2 cc. of concd. hydrochloric acid, and 1.7 g. of the α-carboxylic acid of pyrrole. More than the calculated amount necessary for complete reduction was absorbed, using the platinum oxide catalyst. The resulting solution did not blacken in air. It was thoroughly extracted with ether and only a trace of the original was found in the ether extract. After careful neutralization with sodium hydroxide the solution was evaporated in a vacuum to dryness. The dried residue was thoroughly extracted with absolute alcohol. The brown alcoholic solution, when evaporated to dryness, left a dark residue which resembled proline before it crystallizes. Sörensen[16] has called attention to the use of phosphotungstic acid as a qualitative test for proline. The alcoholic residue was dissolved in water and treated with phosphotungstic acid. A voluminous white precipitate resulted. Attempts were made to identify this residue but failed on account of the small amount of material available. The evidence, however, did not indicate that the substance was either proline phosphotungstate whose nitrogen content was found to be 1.1%, or pyrrolidine tungstate which when prepared under identical conditions gave a nitrogen value of 1.3%. More studies upon this compound are in progress.

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[14] Dufford, Nightingale and Calvert, THIS JOURNAL, **47**, 95 (1925). Evans and Dufford, *ibid.*, **45**, 278 (1923). Dufford, Calvert and Nightingale, *ibid.*, **45**, 2058 (1923).

[15] Evans and Diepenhorst, THIS JOURNAL, **48**, 715 (1926).

[16] Sörensen, *Compt. rend. trav. lab. Carlsberg*, **6**, 137 (1905). McCay and Schmidt, *J. Gen. Physiol.*, **9**, 333 (1926).

E. Fischer[17] has previously described the phenylhydantoin as the best derivative to characterize proline. Following his directions the proline derivative was readily prepared from a solution of pure proline while the unknown residue yielded brown, feather-shaped crystals of indefinite melting point.

Incidental observations showed that the oxidation of the α-carboxylic acid of pyrrole to form melanin-like compounds is dependent upon the hydrogen-ion concentration of the solution. Alkaline solutions can be boiled for some time without the least darkening, while solutions acidified with hydrochloric acid when exposed to the air at room temperatures turn black in several hours. No darkening was observed in acidified solutions when kept in an atmosphere of hydrogen, even after a period of six months.

The Reaction between Methylmagnesium Iodide and Pyrrolidine

Experiments were also carried out to determine whether methylmagnesium iodide reacts with pyrrolidine in the same manner as it does with pyrrole. Accordingly 5 g. of pyrrolidine was treated with the calculated amount of methylmagnesium iodide. The evolution of methane showed that it reacted in the manner customary to secondary amines. After carbon dioxide was blown through the reagent in the usual manner, it was acidified with sulfuric acid and extracted with ether. The ether extract contained a considerable amount of pyrrolidine. The acid was removed from the ether-extracted solution before it was evaporated to dryness in a vacuum. A slight, black residue resulted which was found to contain no proline.

These experiments indicate that in the saturated pyrrolidine ring the —MgX portion of the Grignard reagent does not migrate to the alpha position under the experimental conditions employed here. Since it remains attached to the nitrogen it is removed by the subsequent treatment with carbon dioxide and a strong acid.

Synthesis of the α-Dithionic Acid of Pyrrole

Since pyrrole itself is readily oxidized by the oxygen of the air we might expect any compound containing both it and thio groups to be very readily subject to oxidation. No such compounds are known and it is possible that if prepared they would possess considerable interest.

Houben[18] was the first to employ the Grignard reagent for the synthesis of dithionic acids. He treated RMgX compounds with carbon disulfide in place of carbon dioxide and obtained the resulting substances which he called carbithionic acids. In more recent times a number of these acids have been produced by treating aldehydes with ammonium polysulfide.[19] A search of the literature reveals no such acid in which a pyrrole nucleus or a nitrogen atom is involved in a nucleus that holds a dithionic acid group.

[17] Fischer, *Z. physiol. Chem.*, **88,** 189 (1913).

[18] Houben, *Ber.*, **39,** 3219 (1906).

[19] Bruni and Levi, *Atti. accad. Lincei*, **32,** 5 (1923).

The synthesis of α-dithionic acid of pyrrole was carried out as follows. The methylmagnesium iodide was prepared using 26.7 g. of magnesium and 156 g. of methyl iodide in 500 cc. of absolute ether. To this solution was added 67 g. of pyrrole in 500 cc. of absolute ether. Sixty g. of carbon disulfide was then added while the pyrrylmagnesium iodide was well stirred.

After the addition of the carbon disulfide an excess of sodium hydroxide was added, the solution was heated to boiling and extracted four times with fresh ether. The black precipitate of magnesium hydroxide was then filtered off with suction. A considerable amount of the product was recovered from the precipitate by boiling up several times with water, filtering and adding to the main portion of the solution. The solution of the sodium salt was concentrated to a small volume and acidified with sulfuric acid. The reddish oil that was thus set free was again extracted with ether in which it was readily soluble. The ether solution was dried over sodium sulfate. A portion was evaporated to dryness in a vacuum desiccator. This left a dark brown oil which later turned to a (partly crystalline) pasty mass. This solid was almost completely soluble in alkali and ether. It was only slightly soluble in acid and practically completely soluble in chloroform, ethyl alcohol and carbon disulfide.

Five-cc. portions of the ether solution were titrated in water with phenolphthalein as an indicator. Because of the color of the thionic acid the end-point was rather difficult to distinguish. The titration with sodium hydroxide solution showed an approximate yield of 40%. A portion of the ether solution of the acid was added to a large excess of water. This was divided into three portions and served for the preparation of the iron, mercury and lead salts. These were precipitated upon the addition of ferrous chloride and the acetates of mercury and lead, respectively. The iron salt formed a deep green precipitate which turned black in the air. The rate of blackening was much increased by exposure to sunlight. The mercury salt formed a light yellow, colloidal solution which was precipitated as a yellow salt upon boiling. The lead salt was a deep red and precipitated readily.

Attempts were made to find a solvent for recrystallizing the lead salt, but it seemed practically insoluble in all the ordinary solvents. This may prove a method for the quantitative determination of lead. It was only slightly soluble in boiling acetone. The lead salt was dried for three hours at 110° in the electric oven. Nitrogen was determined in the salt by the Kjeldahl method and the sulfur by a modification of the Carius method. The latter had to be modified, due to the fact that a lead salt was being analyzed. The lead salt was heated in a sealed tube with nitric acid for the usual length of time. At the conclusion, half the sulfur was present as lead sulfate. This was dissolved in ammonium acetate and precipitated with barium nitrate. The method of precipitation with barium nitrate is known to give high results and hence is not usually employed. In both cases the sulfur analyses were too high and the checks were poor.

Anal. Calcd.: N, 5.7; S, 26.1. Found: N, 5.5, 5.5; S, 27.7, 28.7.

The α-dithionic acid of pyrrole was easily oxidized to a black powder when exposed to the air. Its solution was also readily blackened by light. The compound reacted with benzoyl chloride to give a precipitate of black shining crystals.

Summary

1. Attempts to synthesize proline by reduction of pyrrolidone-α-carboxylic acid and the α-carboxylic acid of pyrrole are described, all of which yielded negative results.
2. Considerable information concerning the synthesis of pyrrole-α-carboxylic acid with the aid of the Grignard reagent has been accumulated.

The luminescence of unsaturated compounds was used to throw additional light on the structure of pyrrylmagnesium iodide.

3. The reaction between pyrrolidine and methylmagnesium iodide has been studied. It has been shown that the —MgX group does not migrate to the alpha position under the experimental conditions studied.

4. The synthesis of the α-dithionic acid of pyrrole is described.

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[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY, CORNELL UNIVERSITY]

PYROGALLOLSULFONEPHTHALEIN, SULFONEGALLEIN, 2,3,4-TRIHYDROXY-BENZOYL-BENZENE-ORTHO-SULFONIC ACID (THE INTERMEDIATE ACID), AND SOME OF THEIR DERIVATIVES

BY W. R. ORNDORFF AND NATHANIEL FUCHS[1]

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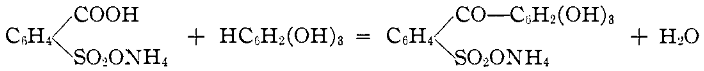
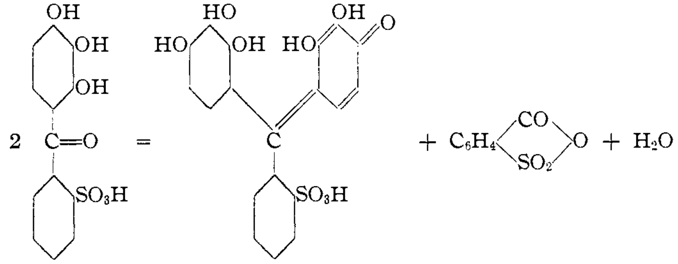
The ammonium salt of 2,3,4-trihydroxy-benzoyl-benzene-*o*-sulfonic acid was prepared by heating the acid ammonium salt of *o*-sulfobenzoic acid with pyrogallol.

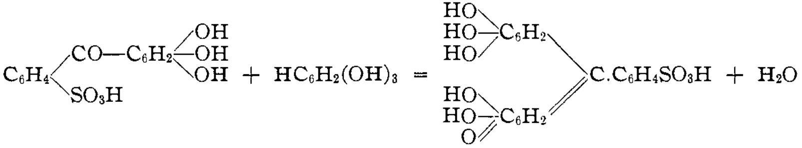


$$C_6H_4\left\langle\begin{array}{l}\mathrm{COOH}\\ \mathrm{SO_2ONH_4}\end{array}\right. + HC_6H_2(OH)_3 = C_6H_4\left\langle\begin{array}{l}\mathrm{CO—C_6H_2(OH)_3}\\ \mathrm{SO_2ONH_4}\end{array}\right. + H_2O$$

The free acid was obtained by passing dry hydrogen chloride into the ammonium salt suspended in absolute ethanol. When heated alone at 140°, it forms pyrogallolsulfonephthalein, *o*-sulfobenzoic anhydride and water.



Pyrogallolsulfonephthalein also results when the trihydroxy acid is heated with pyrogallol.



$$C_6H_4\left\langle\begin{array}{l}\mathrm{CO—C_6H_2}\left\langle\begin{array}{l}\mathrm{OH}\\ \mathrm{OH}\\ \mathrm{OH}\end{array}\right.\\ \mathrm{SO_3H}\end{array}\right. + HC_6H_2(OH)_3 = \left.\begin{array}{l}\left.\begin{array}{l}\mathrm{HO}\\ \mathrm{HO}\\ \mathrm{HO}\end{array}\right\rangle C_6H_2\\ \left.\begin{array}{l}\mathrm{HO}\\ \mathrm{HO}\\ \mathrm{O}\end{array}\right\rangle C_6H_2\end{array}\right\rangle C.C_6H_4SO_3H + H_2O$$

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[1] This article is based on a thesis submitted to the Faculty of the Graduate School of Cornell University by Nathaniel Fuchs, Grasselli Fellow in Chemistry, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. An abstract of this paper was presented at the seventieth meeting of the American Chemical Society at Los Angeles, in 1925.