A NEW SYNTHESIS OF GLUTAMIC ACID*

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Glutamic acid has been prepared previously by the racemization (1) of the natural dextro form and by three synthetic methods. The first synthesis, that by Wolff (2) in 1890, is principally of theoretical interest as it involves the preparation of six intermediate substances starting with levulinic acid and the yield of final product is not given. In 1925 Knoop and Oesterlin (3) obtained 1.1 gm. (23 per cent yield) of glutamic acid from \( \alpha \)-ketoglutaric acid and alcoholic ammonia with hydrogen and palladium black as reducing agents. In the same year Keimatsu and Sugasawa (4) reported a synthesis with acrolein as starting material. None of these methods appears to be as satisfactory as the method described herewith.

The following reactions are involved in the present synthesis of glutamic acid.

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\begin{align*}
\text{COOC}_2\text{H}_5 & \quad \text{COOC}_2\text{H}_5 \\
| & | \\
\text{CH}_5 & \quad \text{CNOH} \\
\text{COOC}_2\text{H}_5 & \quad \text{COOC}_2\text{H}_5 \\
\text{I} & \quad \text{II} \\
& \quad \text{III} \\
\text{COOC}_2\text{H}_5 & \quad \text{COOC}_2\text{H}_5 \\
\text{CHNHCOC}_2\text{H}_5 & \quad \text{C}((\text{CH}_2\text{CH}_2\text{COOC}_2\text{H}_5)\text{NHCOC}_2\text{H}_5) \\
\text{COOC}_2\text{H}_5 & \quad \text{COOC}_2\text{H}_5 \\
\text{IV} & \quad \text{V}
\end{align*}
\]

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\begin{align*}
\text{Method 1} & \\
\text{COOC}_2\text{H}_5 & \xrightarrow{\text{CHNH}_2 \cdot \text{HCl}} \text{COOC}_2\text{H}_5 \\
\text{III} & \xrightarrow{\text{ClCH}_2\text{CH}_2\text{COOC}_2\text{H}_5 \quad (\text{C}_2\text{H}_5\text{ONa})} \text{C}((\text{CH}_2\text{CH}_2\text{COOC}_2\text{H}_5)\text{NH}_2 + \\
\text{V-A} & \\
\text{COOC}_2\text{H}_5 & \xrightarrow{3\text{H}_2\text{O} \quad (\text{HCl})} \text{CHNH}_2 \cdot \text{HCl} + \text{CO}_2 + 3\text{C}_2\text{H}_5\text{OH} + \\
\text{COOC}_2\text{H}_5 & \xrightarrow{\text{Ag}_2\text{O}} \text{CHNH}_2 \\
\text{VI} & \xrightarrow{\text{CH}_2\text{CH}_2\text{COOH}} \\
\text{Method 2} & \\
\text{Isonitrosomalonic ester (II) was prepared from malonic ester and nitrous acid by the method of Cerchez (5).} \\
\text{The preparation of aminomalonic ester (III) presents some}
unusual difficulties whether the reduction of isonitrosomalonic ester is carried out in acid, alkaline, or neutral medium. In acid solution an amino derivative is formed, while the ester may be hydrolyzed by alkaline reduction. If neutral solutions are used, an active metal or amalgam catalyst is required.

Since Johnson and Nicolet (6) obtained a 47 per cent yield of aminomalonic ester hydrochloride from the reduction of 10 gm. of isonitrosomalonic ester by hydrogen sulfide in alkaline solution, it seemed desirable to test this method with larger quantities of the isonitroso ester. 71.6 gm. (0.38 mol) of isonitrosomalonic ester and 22.1 gm. (0.38 mol plus 1 gm. excess) of potassium hydroxide were mixed with 400 cc. of water. Aliquot portions of this mixture were withdrawn for analysis of amino nitrogen by the Van Slyke gasometric method. It was shown that in 12 hours, the time used by Johnson and Nicolet, the reduction was 97 per cent complete. As no data on the reaction of nitrous acid with aminomalonic ester could be found, the deaminization chamber was shaken for an arbitrarily chosen time of 10 minutes.

Calculated on the basis of the isonitrosomalonic ester taken, the final yield of aminomalonic ester hydrochloride was only 14.2 per cent of the theoretical, while it was 73.4 per cent based on the amino substances present in the combined ether fractions. These findings confirm the statement of Johnson and Nicolet that with more than 10 to 20 gm. of isonitrosomalonic ester the percentage yield is decreased. That this is due to the hydrolysis of the ester to aminomalonic acid was verified by isolating from the mother liquor 30.4 gm. of lead aminomalonate, which was converted to 10.2 gm. of crystalline aminomalonic acid (37 per cent of the theoretical amount). The method of Baeyer (7) was used for isolating these substances.

Johnson and Nicolet state that aminomalonic ester hydrochloride, prepared in this manner, is practically pure, while Cerchez (8) found that it was hygroscopic and difficult to purify. The product prepared in the present investigation was impure and contained nitrogen other than that in the amino form. It is interesting to note that the reaction of aminomalonic ester with nitrous acid is not complete in 5 minutes. Thus the behavior of this aminodicarboxylic acid resembles that of the aminomonocarboxylic acids (9) in which the amino group is present in other than the α position.
Calculated for C\textsubscript{7}H\textsubscript{10}O\textsubscript{4}NCl. N 6.62. Found N (Kjeldahl) 6.37, 6.25, 6.25; (Van Slyke) 4.44 (5 min.), 4.84 (10 min.), 5.14 (30 min.), 5.10 (60 min.).
Calculated. Cl 16.77. Found Cl (Volhard) 16.69, 16.72.

Catalytic methods for the reduction of isonitrosomalonic ester are generally unsatisfactory. After treating 5 gm. of isonitrosomalonic ester for 2 days with hydrogen and platinum black, Putochin (10) obtained a 60 per cent yield of aminomalonic ester hydrochloride. It was shown by Locquin and Cerchez (11) that hydrogen and nickel in an alcoholic medium did not reduce the ester in 8 hours at room temperature. In the present work an attempted reduction with hydrogen and platinum black gave negative results. Here the catalyst was prepared by the method of Adams, Voorhees, and Shriner (12) and its activity tested with maleic acid as described by Adams and Voorhees (13). The absorption of hydrogen was negligible in 3 hours, with a 25 gm. sample of isonitrosomalonic ester dissolved in 95 per cent ethyl alcohol, even though the catalyst was reactivated during this time and activator ions (ferrous and zinc) were added. The results were similar with ethyl acetate as solvent.

Locquin and Cerchez (11) were not able to effect reduction with magnesium amalgam but these authors, as well as Putochin (10) and Piloty and Neresheimer (14), found that aluminum amalgam is a satisfactory reducing agent. In the present investigations the reduction with aluminum amalgam went smoothly when the conditions established by Cerchez were followed. However, it is not easy to agree with Cerchez that aluminum is an effective agent only when in the form of platelets 20 to 25 mm. long, 6 to 8 mm. wide, and exactly 0.3 mm. thick. Since aluminum foil was used successfully in the present work, it is our opinion that the reducing power of aluminum amalgam depends on the purity of the metal, while the dimensions of the pieces should influence only the rate of the reduction.

The intermediate benzoylaminomalonic ester (IV) is a stable crystalline compound easily prepared in almost theoretical yield. The benzoyl radical not only fully protects the amino group from reaction with the halogen acid ester but it is easily removed from the resulting reaction product (V) by acid hydrolysis. When free aminomalonic ester was used by Keimatsu and Kato (15) for the synthesis of aspartic acid, a 33 per cent yield of iminodiacetic acid,
Fig. 1. Photomicrograph of \( d \)-glutamic acid. \( \times 69.75 \)

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Figs. 2 to 5. Photomicrographs of \( dL \)-glutamic acid. Figs. 2, 3, and 4 \( \times 69.75 \); Fig. 5 \( \times 16.5 \).

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difficult to separate from the amino acid, was formed. If the amino group is protected by the phthalimido radical, as used by Sørensen and Andersen (16) for the synthesis of aminoadipic acid and by Dunn and Smart (17) for aspartic acid, hydrolytic cleavage of the group is so difficult that decomposition products are formed in addition to the desired amino acid.

As described in the experimental part, 8 gm. (51.8 per cent of the theoretical amount) of dl-glutamic acid (VII) of high purity were prepared. Photomicrographs of this product and of d-glutamic acid, isolated from ajinomoto, are shown in Figs. 1 to 5. On account of their low solubility in cold water both forms usually crystallize in balls or in needles, as shown by Keenan (18). However, when crystallization proceeds slowly, they form characteristic four-sided plates, needles, and pyramids as shown in Figs. 1 to 5 and as described by Fischer, Kropp, and Stahlschmidt (1).

Experiments for the preparation of glutamic acid through the reaction of sodium phthalimidomalonic ester with ethylene chlorohydrin were unsuccessful. Here, as found previously (17) for similar halogen compounds, the desired addition product could not be isolated although the liberation of chloride ions was complete, as shown by Volhard analyses. Similarly, all attempts to prepare pure sodium derivatives of either aminomalonic ester or benzoylaminomalonic ester were unsatisfactory. Such salts are easily formed from sodium and these esters in absolute ethyl alcohol or anhydrous toluene, but on exposure to air they are converted to impure, colored solids which react slowly and incompletely with β-chloropropionic ester.

EXPERIMENTAL

Isonitrosomalonic Ester (II)—In making numerous preparations of isonitrosomalonic ester a high yield of crude product was easily obtained but the maximum yield of vacuum-distilled material was about 70 per cent of the theoretical. It was found necessary to distil the ester at pressures lower than 12 mm. to avoid decomposition. The most satisfactory yields of ester resulted when the sodium nitrite solution was allowed to drop slowly, over a period of about 3 days, into the malonic ester-acetic acid mixture contained in a long narrow tube. When the time of addition was extended to 9 days, the yield was not improved.
The vacuum-distilled product was a colorless oil, b.p. 150–152° at 4.8 mm. Cerchez found 172° at 12 mm.

Aminomalonic Ester (III)—Isonitrosomalonic ester, in 100 gm. portions, was reduced with aluminum amalgam by the method of Cerchez. The yield of vacuum-distilled product, b.p. 85–88° at 4 to 5 mm., was about 50 per cent. On account of the instability of this almost colorless oil Cerchez converted it to the hydrochloride or the oxalate, but according to our experience the pure ester undergoes no apparent decomposition when preserved for many days in a glass-stoppered bottle.

Analysis
Calculated for C_{12}H_{13}O_{4}N. N 8.00. Found (Kjeldahl). N 7.73, 7.73.

The reaction of aminomalonic ester with nitrous acid (Van Slyke method) was found to be about 60 per cent complete in 5 minutes and approximately 88 per cent in 4 hours.

The aluminum foil was amalgamated and the activity of the amalgam tested by methods devised by Dr. H. A. Spoehr and Dr. W. G. Young at the Division of Plant Biology, Carnegie Institution of Washington, Stanford University, California. Aluminum to be amalgamated is washed with warm, dilute sodium hydroxide solution until a vigorous evolution of gas occurs. It is then washed with several portions of distilled water and once with 95 per cent ethyl alcohol. A 1 per cent solution of mercuric chloride in 95 per cent ethyl alcohol is poured on the aluminum and left in contact with it for 20 seconds. After the bichloride solution is decanted and the amalgam washed once with 95 per cent ethyl alcohol, the amalgam is ready for immediate use.

The activity of the aluminum is determined as follows: 2 gm. of aluminum pieces, amalgamated as described, are placed in a beaker and covered with 50 cc. of distilled water. If the sample is active, a reaction begins at once, giving considerable heat and a strong evolution of hydrogen, and continues steadily until at the end of not more than 24 hours it is complete. At this time all of the metal will have disappeared to give a fine precipitate of aluminum hydroxide. Baker's aluminum foil, No. 11,530, was found to be more active than other samples of aluminum sheet or wire tested in this work or in the many experiments of Spoehr and Young.
Benzoylaminomalonic Ester (IV)—Aminomalonic ester was benzyolated in the usual manner. From 30 gm. of vacuum-distilled ester, a 47 gm. (98 per cent) yield of white, crystalline product, m.p. 62–63°, was obtained.

Analysis
Calculated for C₁₄H₁₆O₆N₂. N 5.02. Found (Kjeldahl). N 5.04, 4.94, 4.96.

Propane-α-Benzoylamino-α,α,γ-Tricarboxylic Acid Triethyl Ester (V)—Purified sodium, 4.03 gm. (0.174 mol), was dissolved in 500 cc. of absolute ethyl alcohol and then 47 gm. (0.168 mol) of benzoylaminomalonic ester and 30.4 gm. (0.158 mol) of vacuum-distilled ethyl-β-bromopropionate (prepared by the method of Kendall and McKenzie (19)) were added to the cold alcoholic solution. The mixture, which became warm at once and deposited sodium bromide, was refluxed for 1½ hours on a water bath. According to Volhard analyses for halogen ions, approximately 45 minutes are required for the completion of the reaction. The alcohol was distilled and 100 cc. of water added to dissolve the sodium bromide. The lower oily layer was separated from the aqueous portion and the volatile material removed by distillation at 100° at 4 mm. The ester could not be distilled at 4 mm. without decomposition. The yield was 57.5 gm. (90 per cent).

Analysis

Propane-α-Amino-α,α,γ-Tricarboxylic Acid Triethyl Ester (V-A)—β-Chloropropionic acid was prepared by the method of Jacobs and Heidelberger (20) and the acid esterified by the method used by Conrad (21) for chloroacetic ester. The triethyl ester derivative (V-A) was prepared essentially by the procedure described above for the triethyl ester (V). The yield of residual oil, dried for 2 hours at 110°, was 30 gm. (66 per cent), 28.7 gm. (0.164 mol) of vacuum-distilled aminomalonic ester being used. The product, which partly crystallized on standing, contained some yellow oil and was probably a mixture of the desired substance with some ethane-α-carboxylic acid ethyl ester-β-iminomalonic ester (V-B) and aminomalonic ester. However no evidence could
be obtained that the latter was present since an attempt to isolate glycine, as its benzoyl derivative, from the final acid hydrolysate was unsuccessful.

*Analysis*

Calculated for $\text{C}_{12}\text{H}_{14}\text{O}_4\text{N}$. N 5.09. Found (Kjeldahl). N 5.95, 5.88.

**Glutamic Acid (VII). Method 1**—The intermediate benzoyl-amino ester (V) and an excess of concentrated hydrochloric acid were refluxed in an all-glass apparatus for 16 hours, after which the elimination of carbon dioxide was complete, as shown by testing the evolved gas with barium hydroxide solution. After the solution was cooled and the precipitated benzoic acid filtered, the filtrate was heated to remove volatile material. The chloride ion content of the residual liquid was measured by a Volhard analysis of an aliquot portion and the chloride ions removed from the liquid with silver oxide. The filtrate from the silver chloride precipitate was evaporated to 100 cc., 200 cc. of ethyl alcohol were added, and the white, flocculent precipitate of $dl$-glutamic acid separated after cooling in an ice bath. The yield from 48.9 gm. of the intermediate ester was 8.01 gm., first crop, and 1.83 gm., second crop, or a total of 9.84 gm. (51.8 per cent). The melting point of the product, crystallized once from distilled water and dried at 110°, was 193.5–194.5° (corrected). When $dl$-glutamic acid is recrystallized many times, it melts at 198° (2) or 199° (1).

It is believed that the yield can be increased appreciably when larger quantities are used, as it was found by amino nitrogen analyses that about 1 gm. of glutamic acid was retained by the benzoic acid and silver chloride residues. Also about 10 per cent of the final glutamic acid solution was lost accidentally.

*Analysis*


**Method 2**—By a hydrolysis procedure identical with that described above 32 gm. of the intermediate substance (V-A) were refluxed with 50 cc. of concentrated hydrochloric acid for 14$\frac{1}{2}$ hours. After further treatment the hydrolysate yielded a total of 5.9 gm. (36 per cent) of glutamic acid. This product was crystallized once from water and alcohol and dried at 110° for 15 hours.
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Analysis
Calculated for C₅H₈O₄N. N 9.52. Found N (Kjeldahl) 9.42, 9.45; (Van Slyke) 9.56, 9.57.

The authors are indebted to Dr. W. G. Young of this department for valuable suggestions and for the loan of his catalytic hydrogenation apparatus.

SUMMARY
1. A new synthesis of glutamic acid has been described in which a 51.8 per cent yield of this amino acid has been obtained. Photomicrographs are given for the active and racemic modifications of glutamic acid, and their characteristic crystalline forms shown.
2. It has been found that the benzoyl radical effectively protects the amino group of aminomalonic ester when the latter is used for the synthesis of an aminodicarboxylic acid.
3. Hydrogen sulfide in alkaline solution proved to be an unsatisfactory agent for the reduction of isonitrosomalonic ester because the reduced product undergoes hydrolysis with the formation of aminomalonic acid.
4. Aminomalonic ester has been prepared in satisfactory yield from isonitrosomalonic ester and amalgamated active aluminum. Procedures have been devised for testing the activity of aluminum and for the preparation of aluminum amalgam. It is believed that the reducing activity of aluminum depends upon its state of purity rather than on any purely physical factor as claimed by Cerchez.

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