No one of the methods proposed for the synthesis of aspartic acid is entirely satisfactory as a practical procedure. The synthesis, described in a recent paper (1) from this laboratory, has been utilized in preparing 50 to 60 gm. lots of recrystallized aspartic acid but even here the beginning reagents are expensive, the over-all yield is only about 30 per cent of the theoretical, and the process is time-consuming because of the number of steps involved.

In searching for a convenient and inexpensive method of synthesis it was apparent that reactions with malic, succinic, maleic, or fumaric acid were the most promising. It was expected that ammonia could be made to react satisfactorily with chlorosuccinic or bromosuccinic acid, prepared from any one of the four dibasic acids, or directly with maleic or fumaric acid to give aspartic acid in good yield. Various methods for the preparation of the intermediate bromosuccinic (2–6) and chlorosuccinic acids (7–9) have been reported, whereas Fischer and Raske (10) were able to prepare 2.2 gm. (16 per cent of theory) of crude aspartic acid from bromosuccinic acid and aqueous ammonia. In our experiments with malic and fumaric acids the yields of chlorosuccinic and bromosuccinic acids were poor and only the insoluble ammonium salts resulted when these acids were treated with liquid ammonia or with a saturated solution of the dry gas in alcohol, ether, or pyridine. The formation of ammonium bromosuccinate in ether is in accord with the observations of McMaster and Magill (11).

The direct addition of ammonia to maleic and fumaric acids has been investigated by Engel (12) and Stadnikoff (13). When the amination was conducted at 140–150° in aqueous or alcoholic solution, Engel obtained a 30 to 35 per cent yield of aspartic
acid, although the quantity of amino acid synthesized and the analytical evidence of its purity were not reported. Stadnikoff isolated 3 gm. of diethyl aspartate, 2 gm. of diethyl iminosuccinate, and a small amount of an unidentified third substance by the fractional distillation in vacuo of the esterified products resulting from the reaction of 20 gm. of fumaric acid and an excess of ammonia. Numerous experiments were performed in the present investigation in attempting to confirm Engel's work but the results were negative.

In view of the exhaustive investigations of Morsch (14), who has studied the reaction of ammonia with a number of unsaturated esters, we were encouraged to try the addition of ammonia to fumaric ester. Subsequently, it was found that this reaction had been investigated by Koerner and Menozzi (15) who obtained diethyl aspartate in addition to a small amount of a crystalline substance which they believe to be aspartimide, COCH₂CH₂NH₂CO.\[\text{NH}\]

In a later paper by Fischer and Koenigs (16) it was shown that this substance is more probably the isomeric diketopiperazine diacetamide (the diamide of the diketopiperazine of aspartic acid),

\[
\text{NH}_2\text{COCH}_2\text{CHCONH}\quad \text{NHCOCHCH}_2\text{CONH}_2
\]

Koerner and Menozzi isolated aspartic acid from the alkaline hydrolysate of the crystalline product, but no yields were stated.

In our experiments it was found that alcoholic ammonia reacts with diethyl fumarate, in 24 hours at 100° and 6 atmospheres pressure, to give principally the crystalline diketopiperazine diacetamide. The latter is readily hydrolyzed by sodium hydroxide to aspartic acid which is conveniently isolated as the copper salt. A 78 gm. yield (59 per cent of theory) of analytically pure aspartic acid was prepared by the procedures described in the experimental part.

**EXPERIMENTAL**

*Diethyl Fumarate*—The method of Corson, Adams, and Scott (17) was used in preparing 419 gm. of diethyl fumarate from 348 gm. of Eastman's Practical fumaric acid.
Diketopiperazine Diacetamide—Glass, ginger ale bottles (Canada Dry) were used for the pressure reactions described below. Bottles of this type resisted a pressure of 7.3 atmospheres when tested at 100° with 25 per cent aqueous ammonia, whereas the pressure of the experimental solution was only about 6 atmospheres at 100°. Bottles to be used for pressure reactions should be well wrapped with cheese-cloth to minimize the danger from flying glass in case of an explosion. An iron pipe, similar to that described by Dakin (19), was used in one pressure experiment but it proved to be unsatisfactory partly because of its weight and unwieldiness but mainly because the chemical reactions appeared to take an entirely different course than in glass, perhaps due to some catalytic effect of the iron.

82 gm. (4.8 mols) of dry ammonia gas and 172 gm. (1.0 mol) of diethyl fumarate, b.p. 213-215° uncorrected, were dissolved in 1100 cc. of 99.85 per cent ethyl alcohol. This solution was divided equally among five ginger ale bottles each fitted with a firmly wired rubber stopper and a yard of cheese-cloth wrapping. The bottles were immersed in a water bath which was heated to boiling and maintained at this temperature for 24 hours. The solid crust, which formed on the sides of the bottle, was separated from the liquid material and the latter was distilled to dryness in vacuo. The combined solids were practically free from oil indicating that the diethyl fumarate and the intermediate diethyl aspartate had been converted almost completely to diketopiperazine diacetamide. The latter crystallizes from water as needles which do not melt sharply but decompose when heated above 250°.

C₆H₁₂N₄O₄. Calculated, N 24.6; found (Kjeldahl), 24.3, 24.5

Copper Aspartate—The crude diketopiperazine diacetamide was refluxed for 6 hours in an oil bath with 750 cc. of 6 N sodium hydroxide. The resulting solution was neutralized to methyl red with 6 N hydrochloric acid and filtered. After several unsuccessful attempts to crystallize aspartic acid directly from the filtrate the latter was treated with a hot solution of 220 gm. (10 per cent excess) of copper acetate monohydrate in 1450 cc. of distilled

1 The pressure-temperature-concentration relations of aqueous ammonia are given by Mollor (18).
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water. Crystals of the light blue copper aspartate began to form within an hour. As stated by Hofmeister (20) these crystals appear as tyrosine-like clusters when viewed under the microscope. After standing overnight in the ice box, the voluminous precipitate was filtered and a sample recrystallized from water. When dried to constant weight in vacuo over sulfuric acid, the purified salt appeared to be the trihydrate, a finding in agreement with the observations of Cook and Woolf (21).

\[ \text{C}_4\text{H}_5\text{O}_4\text{NCu-3H}_2\text{O}. \text{ Calculated}, \text{ N } 5.63; \text{ found (Van Slyke), } 5.60, 5.60 \]

Aspartic Acid—The crude copper aspartate was suspended in 2 liters of 1 N acetic acid in a 4 liter flask and saturated with hydrogen sulfide. By maintaining a slight pressure of the hydrogen sulfide and intermittently shaking the flask, the decomposition of the copper salt was completed in about 2 hours. The resulting suspension was boiled gently for about 30 minutes, the coagulated cupric sulfide filtered, and the filtrate evaporated until crystals began to separate. After adding 3 liters of 95 per cent ethyl alcohol and standing overnight in the ice box the resulting crystals of aspartic acid were filtered and dried for 24 hours at 55°.

The mother liquor was distilled under reduced pressure until solid began to separate. Then about 1 liter of distilled water was added, the solution distilled to dryness in vacuo, and the residue converted to aspartic acid through the copper salt as previously described.

The yield of analytically pure aspartic acid was 64 gm., first crop, 14 gm., second crop, or a total of 78 gm. (59 per cent of theory). The weight of crude amino acid was 129 gm. (96 per cent of theory) and of once recrystallized material 101 gm. (76 per cent of theory). It is probable that the yield of analytically pure material can be increased since some decomposition occurred during the vacuum distillations.

\[ \text{C}_4\text{H}_4\text{O}_4\text{N}. \text{ Calculated}, \text{ N } 10.53; \text{ found (Van Slyke), first crop, } 10.46, 10.46, 10.68, 10.73; \text{ second crop, } 10.54, 10.62, 10.69 \]

SUMMARY

It has been shown that analytically pure aspartic acid may be conveniently and inexpensively synthesized from diethyl fumarate.
and alcoholic ammonia through the intermediate diketopiperazine diacetamide and copper aspartate.

**BIBLIOGRAPHY**

THE SYNTHESIS OF ASPARTIC ACID
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