The Optical Resolution of DL-Aspartic Acid, DL-Glutamic Acid, DL-Asparagine and DL-Glutamine by Preferential Crystallization*

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(Received December 28, 1964)

Several studies of optical resolutions of organic compounds by mechanical sorting[1,2] and by preferential crystallization[3-11] have been reported.

DL-Asparagine was first resolved by Piutti[2] using Pasteur's mechanical sorting method.[1] Ostromisslensky[3] also resolved DL-asparagine by seeding its supersaturated aqueous solution

* Contribution No. 46 of the Institute of Molecular Evolution, University of Miami.

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with crystals of glycine, which has no asymmetric carbon. Although the optical activity of the resolved asparagine was not recorded, one seeding experiment resulted in the crystallization of the L-form, while the other induced crystallization of the D-from. The investigator could not predict which from of asparagine would crystallize in any given experiment. L-Histidine hydrochloride and some D-histidine hydrochloride were isolated by the successive crystallizations of the mixture of DL-histidine hydrochloride and L-histidine hydrochloride. DL-Threonine was resolved by seeding with L-threonine.[8] DL-Aspartic acid was partially resolved by preferential crystallization with optically active glutamic acid.[10] DL-Aspartic acid was totally resolved from its supersaturated copper complex solution by seeding it with an L- or D-aspartic acid copper complex.[11] DL-Glutamic acid was resolved completely from its supersaturated aqueous solution by seeding.[9] This type of simple optical resolution by the preferential crystallization procedure is the most advantageous method providing we can obtain an optically pure isomer. However, in the resolution of the DL-amino acids, most of the methods used are impractical or result in a partial resolution, with the exception of DL-glutamic acid[9] and the copper complex of DL-aspartic acid.[11]

In this report, the total optical resolution of DL-α-amino acid from its supersaturated solution by an inoculation procedure will be described. The method will be applied to the optical resolution of DL-aspartic acid, DL-glutamic acid, DL-asparagine, and DL-glutamine.

One important condition in the optical resolution by the crystallization procedure is that the attraction between L- and D-isomers be weak; preferably, the compound should be a racemic mixture (conglomerate). However, most α-amino acids form racemic compound crystals upon crystallization from an aqueous solution.[12,13] For this reason the seeding procedure did not give any optically active aspartic acid when a pure aqueous DL-aspartic acid solution was seeded by an optically active isomer.[11] The ammonium formate which was added to the aqueous aspartic acid solution may act as an agent (I) to stabilize the supersaturated solution, (II) to slow down the crystallization rate, and (III) to weaken the attraction between the D- and L-molecules, thus forming a racemic compound.

Formate ($HCOO^-$) and ammonium (NH_4^+) ions might not be essential for this type of resolution of amino acid. An aqueous solution of glycine was also used for this purpose. However, the results of the resolution using glycine were not as good as those obtained using ammonium formate.

Experimental

The Resolution of DL-Aspartic Acid.—DL-Aspartic acid (14.0 g.) was dissolved in a mixture of ammonium formate (25.0 g.) and 30 ml. of water at 80°C in a water bath. The hot solution was then filtered and cooled slowly to 43°C. Into this supersaturated solution 0.50 g. of finely-pulverized L-aspartic acid ($[\alpha]_D^{25}$ +24.6°, 6 N HCl) was seeded and mixed. The mixing is important, and the procedure should be standardized in order to obtain the best results. This is also true in the resolution of other amino acids. The seeded solution was allowed to stand undisturbed at room temperature (23°C) for 15 min. The crystals which were precipitated were filtered and washed with cold water. 1.57 g. of L-aspartic acid (including seed) was obtained (1st L-asp). When this was recrystallized from 39 ml. of water, 1.28 g. of purified L-aspartic acid crystals were obtained; $[\alpha]_D^{25}$ +24.6°, (*c* 2.13, 6 N HCl).

Found: C, 36.09; H, 5.34; N, 10.57. Calcd. for $C_4H_7O_4N$: C, 36.09; H, 5.30; N, 10.52%.

Into the filtrate, 0.50 g. of pulverized D-aspartic acid ($[\alpha]_D^{25}$ −24.3°, 6 N HCl) was added. The mixture was then kept undisturbed at room temperature (23°C) for 45 min. 1.69 g. of crystals (including seed) was thus obtained (1st D-asp). When this was recrystallized from water, 1.39 g. of purified D-aspartic acid was obtained; $[\alpha]_D^{25}$ −23.6° (*c* 2.03, 6 N HCl). (Found: C, 36.11; H, 5.30; N, 10.24%.) In the mother liquor, 2.50 g. of DL-aspartic acid was dissolved at 80°C. The solution was then filtered and cooled to 43°C at room temperature. Into the supersaturated solution, 0.50 g. of L-aspartic acid was seeded. After 15 min., 1.79 g. of 2nd L-asp was obtained. By seeding the filtrate with D-aspartic acid, 1.70 g. of 2nd D-asp was obtained after the solution had stood for 45 min. The same procedures were used to obtain 3rd L-asp, 1.79 g. and 3rd D-asp, 1.78 g. after dissolving 2.50 g. of DL-aspartic acid in the mother liquor. The nitro-

TABLE I. THE RESOLUTION OF DL-ASPARTIC ACID

	Yield, g.*1	$[\alpha]_D^{25}$*2	Optical purity, %*3
1st L	1.57	+24.6	99
1st D	1.69	−23.6	95
2nd L	1.79	+24.4	98
2nd D	1.70	−23.7	96
3rd L	1.79	+24.8	100
3rd D	1.78	−23.0	93

*1 DL-Aspartic acid, 14.0 g., was resolved by seeding of 0.50 g. of L- and D-aspartic acids.
*2 Specific rotations were measured in 6 N hydrochloric acid.
*3 Specific rotation of pure L-aspartic acid: $[\alpha]_D^{25}$ +24.8°, 6 N hydrochloric acid.

12) T. Ogawa, *J. Chem. Soc. Japan, Ind. Chem. Sec.* (*Kōgyō Kagaku Zassi*), **52**, 69, 71, 102, 103, 104 (1949).
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gen analyses of 2nd L-, 2nd D-, 3rd L-, and 3rd D-aspartic acids agreed with the theoretical values. The specific rotations are listed in Table I.

The Resolution of DL-Glutamic Acid.—DL-Glutamic acid monohydrate (5.0 g.) was dissolved in a mixture of 20 ml. of water and 5.0 g. of ammonium formate at 80°C. The solution was then filtered, and the filtrate was cooled slowly. 0.25 g. of L-glutamic acid ($[\alpha]_D^{25}$ +31.5°, 6 N HCl) was seeded in the supersaturated solution at 43°C. The L-glutamic acid which was crystallyized was filtered after it had stood 20 min. at room temperature (23°C). L-Glutamic acid (0.97 g.) (1st L-glu) was obtained. This was recrystallized from 12 ml. of water. $[\alpha]_D^{25}$ +28.5° (*c* 2.05, 6 N HCl).

Found: C, 40.96; H, 6.18; N, 9.62. Calcd. for $C_5H_9O_4N$: C, 40.81; H, 6.17; N, 9.52%.

To the filtrate, 0.25 g. of D-glutamic acid ($[\alpha]_D^{25}$ −30.1°, 6 N HCl) was seeded. After it had stood 20 min. at room temperature (23°C), 1.04 g. of crystals were obtained (1st D-glu). The D-glutamic acid was recrystallized from water. $[\alpha]_D^{25}$ −27.5° (*c* 2.10, 6 N HCl). (Found: C, 40.83; H, 6.21; N, 9.65%.) 1.50 g. of DL-glutamic acid monohydrate was dissolved in the mother liquor, and the resolution procedures were repeated to isolate 2nd L- (0.87 g.), 2nd D- (0.90 g.), 3rd L- (1.13 g.), and 3rd D- (1.03 g.) glutamic acids. The specific rotations of these isolated glutamic acids are listed in Table II. The results of the nitrogen analyses

Table II. The resolution of DL-glutamic acid

	Yield, g.*1	$[\alpha]_D^{25}$*2	Optical purity, %*3
1st L	0.97	+28.5	91
1st D	1.04	−27.5	88
2nd L	0.87	+30.8	99
2nd D	0.90	−26.3	84
3rd L	1.13	+27.2	87
3rd D	1.03	−27.5	88

*1 DL-Glutamic acid, 5.0 g., was resolved by seeding of 0.25 g. of L- and D-glutamic acids.
*2 Specific rotations were measured in 6 N hydrochloric acid.
*3 Specific rotation of pure L-glutamic acid: $[\alpha]_D^{25}$ +31.2°, 6 N hydrochloric acid.

of 2nd L-, 2nd D-, 3rd L- and 3rd D-glutamic acids agreed with the theoretical values.

The Resolution of DL-Asparagine.—DL-Asparagine monohydrate (10.0 g.) was dissolved at 60°C in 40 ml. of water containing 10.0 g. of ammonium formate. After the solution had been filtered and cooled slowly to about 40°C, it was seeded with 0.50 g. of L-asparagine monohydrate ($[\alpha]_D^{25}$ +30.6°, 3.4 N HCl). The solution was then kept undisturbed at room temperature for 30 min. The crystals which were precipitated were filtered and washed with cold water and ethanol. A total of 2.43 g. of L-asparagine monohydrate (1st L-asp NH_2) was obtained. When this was recrystallized from 12 ml. of water and 24 ml. of ethanol, 1.98 g. of purified L-asparagine was obtained; $[\alpha]_D^{25}$ +28.6° (*c* 3.10, 3.6 N HCl).

Found: C, 31.92; H, 6.44; N, 18.52. Calcd. for $C_4H_{10}O_4N_2$: C, 32.00; H, 6.11; N, 18.66%.

0.50 g. of D-asparagine ($[\alpha]_D^{25}$ −30.3°, 3.4 N HCl) was seeded in the filtrate. After 30 min. of undisturbed standing at room temperature, the crystals which were precipitated were isolated (1st D-asp NH_2). Yield, 3.05 g. This was recrystallized from water and alcohol; 2.54 g., $[\alpha]_D^{26}$ −29.7° (*c* 2.93, 3.6 N HCl). (Found: C, 32.32; H, 6.79; N, 18.76%.) In the mother liquor 4.50 g. of DL-asparagine monohydrate was then dissolved. The resolution procedures were repeated in a manner similar to that described above. Second L- (2.68 g.) and 2nd D- (2.48 g.) asparagines were thus obtained. The specific rotations are listed in Table III. The elemental analyses of 2nd L- and D-asparagines agreed with the theoretical values.

Table III. The resolution of DL-asparagine

	Yield, g.*1	$[\alpha]_D^{25}$*2	Optical purity, %*3
1st L	2.43	+28.6	93
1st D	3.05	−29.7	97
2nd L	2.68	+30.0	98
2nd D	2.48	−28.9	94

*1 DL-Asparagine monohydrate, 10.0 g., was resolved by seeding of 0.50 g. of L- and D-asparagine monohydrate.
*2 Specific rotations were measured in 3.6 N hydrochloric acid.
*3 Specific rotation of pure L-asparagine: $[\alpha]_D^{25}$ +30.6°, 3.6 N hydrochloric acid.

The Resolution of DL-Glutamine.—DL-Glutamine was resolved under the same conditions as were used with DL-asparagine (DL-glutamine 10.0 g.; ammonium formate, 10.0 g., and 40 ml. of water). 0.5 g. of L-glutamine ($[\alpha]_D^{25}$ +32.8°, 1 N HCl) was seeded in a supersaturated glutamine solution. After 20 min., 2.05 g. of L-glutamine (1st L-glu NH_2) was obtained. This was recrystallized from 16 ml. of water and 30 ml. of ethanol; 1.65 g., $[\alpha]_D^{25}$ +32.8° (*c* 2.22, 1 N HCl).

Found: C, 41.06; H, 7.00; N, 18.67. Calcd. for $C_5H_{10}O_3N_2$: C, 41.09; H, 6.90; N, 19.19%.

From the filtrate, 2.32 g. of D-glutamine (1st D-glu NH_2) was obtained by the seeding of D-glutamine

Table IV. The resolution of DL-glutamine

	Yield, g.*1	$[\alpha]_D^{25}$*2	Optical purity, %*3
1st L	2.05	+32.8	100
1st D	2.32	−31.4	96
2nd L	2.45	+32.5	99
2nd D	2.38	−31.1	95
3rd L	2.04	+32.9	100
3rd D	2.15	−30.5	93

*1 DL-Glutamine, 10.0 g., was resolved by seeding of 0.5 g. of each L-glutamine and D-glutamine.
*2 Specific rotations were measured in 1 N hydrochloric acid.
*3 Specific rotation of pure L-glutamine: $[\alpha]_D^{25}$ +32.8°, 1 N hydrochloric acid.

after 30 min.'s standing. This was recrystallized from water and alcohol; 1.86 g., $[\alpha]_D^{25}$ −31.4° (*c* 2.31, 1 N HCl). (Found: C, 41.48; H, 6.99; N, 19.21%.)

To the mother liquor 4.40 g. of DL-glutamine was then added and the resolution procedures repeated. Second L- (2.45 g.), 2nd D- (2.38 g.), 3 rd L- (2.04 g.) and 3rd D-glutamines (2.15 g.) were obtained. The results are shown in Table IV. The results of the nitrogen analysis of 2nd L-, 2nd D-, 3rd L- and 3rd D-glutamines agreed with the theoretical values.

This work was supported by Grant NsG-173-62 and NsG-689 from the National Aeronautics and Space Administration. Thanks are extended to Dr. Sidney W. Fox for his encouragement and to Mr. Arthur I. Holden for his measurement of optical rotation.

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