[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

Resolution of Glutamic Acid with 1-Hydroxy-2-aminobutane[1,2]

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Unsubstituted DL-glutamic acid has been resolved by use of an optical isomer of the readily available 1-hydroxy-2-aminobutane. The amine used was obtained by resolution with either tartaric acid or glutamic acid. Attempts to extend this method to aspartic acid resulted in partial resolution only. Formyl-DL-phenylalanine, however, was resolved to give the pure isomers readily. Mutual solubilizing effects are described for diastereomers of the hydroxybutaneammonium hydrogen glutamate.

Increased interest in improved resolutions of amino acids stems from growing knowledge of the effects of the L- and D-forms in nutrition,[4,5] of inhibition of bacterial growth,[6,7] of the occurrence of D-residues in antibiotics[6,8] and in other biological manifestations. Particular interest in the enantiomorphs of glutamic acid arises from the technological value of the sodium salt of the L-form,[9] from the disputed suggestion that D-glutamic acid characterizes tumor protein,[10,11] and from the need in nutritional experiments for amino acid enantiomorphs of non-biological origin.

Nearly all of the numerous recorded resolutions of amino acids involve in part acylation and deacylation of amino acids. This is true even for the Greenstein methods, which represent an outstanding advance in the provision of ample quantities of optically active forms.[12,13] Scattered reports of resolution of unsubstituted amino acids are recorded.[14-19] No such report has, however, been found in the corresponding literature search for resolutions of glutamic acid. Glutamic acid has been resolved through the benzoyl derivative as the strychnine salt[20]; through the *p*-nitrobenzoyl derivative also as the strychnine salt[21]; by cyclization to pyroglutamic acid, resolution of the quinine salts, and decyclization[22]; by enzymic resolution through the chloroacetyl derivative with hog kidney enzyme,[12] and through the carbobenzoxy derivative similarly treated[13]; through the carbobenzoxyglutamic acid and anilide by hydrogenolysis[23] and by hydrolysis[24]; and D-glutamic acid has been prepared by enzymic destruction[25] and microbial destruction of the L-isomer.[26-30]

In a study of modern techniques and reagents for resolution of amino acids, the active forms of 1-hydroxy-2-aminobutane ("butanolamine") were found to be uniquely useful for resolving glutamic acid. The amine is inexpensive, conveniently resolvable into both forms with tartaric acid or glutamic acid and easily purifiable by distillation. When the (−)-amine, for example, is combined with DL-glutamic acid in aqueous ethanol, 50–80% of the expected amount of D-glutamate crystallizes first; after filtration 20–35% of nearly pure isomeric L-glutamate and subsequent other less distinct crops separate. Each active acid and the amine are easily recovered.

The pattern of crystallization of the isomeric salts is somewhat unusual. Each was found to increase the solubility of the other.[31] Attempted selective seeding facilitated crystallization but did not change the order of separation. The suggested mutual solubilizing effect was confirmed by determinations of solubility alone and with diastereomer. Although this phenomenon can explain delayed separation of L-glutamate until most of the D-glutamate precipitates, it does not explain the precipitation of the virtually pure first major crop of D-glutamate. The proportion of water present is believed also to influence this peculiar behavior.

Attempts to resolve aspartic acid by use of the same conditions were mainly unsuccessful; L-aspartic acid of 70% optical purity was recovered.

In attempts to resolve acylamino acids with butanolamine, successful conditions were found with formyl-DL-phenylalanine. In this resolution, configurationally alternate precipitation was again encountered.

Experimental

Resolution of 1-Hydroxy-2-aminobutane; (−)-1-Hydroxybutane-2-ammonium Hydrogen L-Tartrate.—(+)-

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(2) From the Ph.D. theses of Ralph B. Fearing (1951) and Frederick H. Radke (1952).

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Tartaric acid (930 g., 6.2 moles) was dissolved in 1500 ml. of water and treated with, under vigorous stirring and cooling, 553 g. (6.2 moles) of 1-hydroxy-2-aminobutane which had been purified by vacuum distillation. After the dark red solution was cooled overnight in the refrigerator, 458 g. of silky needles of (−)-1-hydroxybutane-2-ammonium hydrogen L-tartrate monohydrate, m.p. 101.5°, had precipitated. The red brown color was removed by washing with 500 ml. of absolute ethanol. The crude solid was dissolved in 220 ml. of hot water and 250 ml. of 95% ethanol was added; yield 378 g., m.p. 102.5–103° uncor., $[\alpha]^{23}D$ +10.5° ± <0.1° (H_2O, *c* 5).

Anal. Calcd. for $C_8H_{17}O_7N \cdot H_2O$: N, 5.4; neut. equiv., 257; H_2O, 7.0. Found: N, 5.3; neut. equiv., 259; H_2O, 7.0.

The filtrate was used to recrystallize another crop obtained by evaporation of the orginal filtrate. Another 212 g. was thus obtained, total 590 g., 74%. In other runs higher yields were obtained. The original unredistilled Commercial Solvents amine also could be used.

(−)-1-Hydroxy-2-aminobutane.—The (−)-1-hydroxybutane-2-ammonium hydrogen L-tartrate monohydrate (590 g., 2.3 moles) almost all dissolved in 1500 ml. of water. It was then treated with successive small portions of calcium hydroxide, with stirring, until the *p*H was constant at 10.6 (*ca.* 230 g. of calcium hydroxide was required). The calcium tartrate was removed by filtration and washed with 100 ml. of water. (−)-1-Hydroxy-2-aminobutane was separated by fractional distillation; 138 g., 68%, b.p. 80° (13 mm.), or 54° (1.5 mm.), d^{26} 0.9390; $[\alpha]^{26}D$ −9.92° ± <0.1°.

Stoll, Peyer and Hofmann recorded +9.8° for the (+)-1-hydroxy-2-aminobutane.[32] Approximately 2% of the aqueous fraction of the distillate was 1-hydroxy-2-aminobutane, as determined by titration.

In another run, 340 g. (1.34 moles) of the tartrate was dissolved in 850 ml. of 70% methanol and calcium hydroxide (132 g., 2.36 moles) was added in portions until the *p*H rose to 10.5. After 4 hr. of stirring, the suspension was filtered, and the calcium tartrate was washed with 500 ml. of water. The combined filtrate and washings was fractionated through a 50-cm. column to yield 107 g. (83%). The rotation of −9.1° and density of 0.9443 indicated the presence of about 8% water. The 92% amine was satisfactory for the resolution of glutamic acid.

Resolution of Glutamic Acid.—In a typical run DL-glutamic acid[33] (73.5 g., 0.50 mole) and a 92% aqueous solution of (−)-1-hydroxy-2-aminobutane (49.5 g., 0.50 mole) were stirred vigorously, and 20 ml. of water was added in small portions. The reaction was noticeably exothermic. The sirup was heated with stirring on a water-bath at 60° until nearly all of the DL-glutamic acid dissolved. The sirup was filtered through No. 1 filter paper in a jacketed büchner funnel heated with CCl_4 vapors. It was necessary to use 40 ml. of hot water to wash the sirup through the filter paper. Enough ethanol was added to the solution to bring the volume to one l. After 20 hr. in the refrigerator 2.0 g. was collected as a first crop, m.p. 139–142°; $[\alpha]^{22}D$ −1.1° ± < 0.1° (H_2O, *c* 5).

The second crop was collected one day later, yielding 40 g., 68%, of (−)-1-hydroxybutane-2-ammonium hydrogen D-glutamate, m.p. 146–147°; $[\alpha]^{22}D$ −3.3° ± <0.1° (H_2O, *c* 5).

Anal. Calcd. for $C_9H_{20}O_5N_2$: N, 11.85. Found: N, 11.9.

The third crop was very small, while the fourth, fifth and sixth crops were (−)-1-hydroxybutane-2-ammonium hydrogen L-glutamate, yield 21 g., 36%. Later crops were optically impure.

(−)-1-Hydroxybutane-2-ammonium hydrogen D-glutamate and (−)-1-hydroxybutane-2-ammonium hydrogen L-glutamate were prepared from their optically pure components, and the constants checked with the salts obtained from the resolution. For the L-glutamate, m.p. 115–117° was found; $[\alpha]^{22}D$ −9.7° ± 0.2° (H_2O, *c* 5).

Anal. Calcd. for $C_9H_{20}O_5N_2$: N, 11.85. Found: N, 11.8.

In other runs, the yield of (−)-aminium D-glutamate was 64, 79, 70, 72 and 51%. The corresponding yields of L-glutamate in the first three cases were 32, 20 and 23%; the others were not worked up.

In two instances, the original precipitate of D-glutamate did not separate within 48 hr., but did so promptly on s eding. In these two instances, the proportions of water in the alcoholic solvent were 7 and 8%. Difficulties in precipitation were not encountered at 6% water or less. A few attempts to interfere with the normal succession of precipitation by seeding with the diastereomer were unsuccessful; the pattern of a major separation of the D-glutamate followed by a minor separation of L-glutamate was quite rigid. In those few instances in which the products failed to exhibit the requisite m.p. or rotation it proved to be easy to purify the material to the desired constants by washing with successive small portions of absolute ethanol, because of the solubility behavior which is noted in a later section.

Recovery of optically impure glutamic acid and of 1-hydroxy-2-aminobutane could be effected from the mother liquors of the D-glutamate. Concentration, adjustment of *p*H to 3.2, dilution with ethanol, precipitation and filtration gave the glutamic acid. In some cases relatively pure hydrochloride of the amine could be obtained by concentration of the mother liquors, in others the *p*H was raised to *ca.* 10.6 with alkali, and the amine recovered by distillation.

D-Glutamic Acid.—(−)-1-Hydroxybutane-2-ammonium hydrogen D-glutamate (25.0 g., 0.106 mole) was dissolved in 100 ml. of water and concd. hydrochloric acid was added to bring the *p*H to 3.16 ± 0.04. Absolute ethanol (200 ml.) was added to the suspension and it was filtered within ten min. The D-glutamic acid was washed with 200 ml. of absolute ethanol and the washings and mother liquor were allowed to stand at room temperature for the precipitation of further crops. A total of 15.0 g., 96%, was collected; $[\alpha]^{33}D$ −31.0° ± 0.0° (6 *N* HCl, *c* 5).

L-Glutamic Acid.—A 1.0-g. sample of (−)-1-hydroxybutane-2-ammonium hydrogen L-glutamate yielded 0.60 g., 97%, of L-glutamic acid; $[\alpha]^{23}D$ +31.0° ± 0.1° (6 *N* HCl, *c* 2).

Resolution of 1-Hydroxy-2-aminobutane with L-Glutamic Acid.—L-Glutamic acid (General Mills, recrystallized from water; 294 g., 2.00 moles) and redistilled 1-hydroxy-2-aminobutane (178 g., 2.00 moles) were dissolved in 180 ml. of water on a water-bath at 70°. The sirupy solution was filtered to remove a small amount of undissolved L-glutamic acid. Enough absolute ethanol was added to the filtrate to bring the volume to 2 l., and the liquid was cooled in the refrigerator. Seeding with (−)-1-hydroxybutane-2-ammonium hydrogen L-glutamate crystallized the crop of (+)-1-hydroxybutane-2-ammonium hydrogen L-glutamate. The precipitate was washed with absolute ethanol. The yield was 150 g., 64%, m.p. 146–147°; $[\alpha]^{22}D$ +3.2° ± <0.1° (H_2O, *c* 5).

Anal. Calcd. for $C_9H_{20}O_5N_2$: N, 11.85. Found: N, 11.9.

A recovery of 63% of (−)-aminium L-glutamate was obtained from the mother liquor. In another run by this procedure the proportions were changed to one mole of salt, 100 ml. of water and 1900 ml. of absolute ethanol. Two recrystallizations gave an 81% yield of the optically pure (+)-aminium L-glutamate. In another run a proportion of one mole of salt and 150 ml. of water to 1850 ml. of absolute ethanol yielded 70% of the optically pure salt.

(+)-1-Hydroxy-2-aminobutane.—(+)-1-Hydroxybutane-2-ammonium hydrogen L-glutamate (50 g., 0.21 mole) was dissolved in 150 ml. of water and the solution was brought to a *p*H of 3.2, the isoelectric point of glutamic acid, by the dropwise addition of concd. hydrochloric acid. To complete the precipitation of L-glutamic acid, 200 ml. of absolute ethanol was added. A total of 30.1 g., 97%, of L-glutamic acid was collected. The aqueous ethanolic solution of (+)-1-hydroxy-2-aminobutane hydrochloride was concentrated under reduced pressure. Calcium hydroxide and ammonium hydroxide were used to raise the *p*H to 10.6. Three volumes of absolute ethanol were added and a small amount of solid was separated by filtration. After evaporation under reduced pressure the final yield was 11.4 g., 61%. The specific rotation compared favorably to +9.8° reported in the literature[32]; $[\alpha]^{25}D$ +9.7° ±0.0°. In another run, cold alcoholic sodium hydroxide was used to raise the *p*H to 10.6 in the decomposition of the (+)-aminium L-glutamate and the sodium glutamate was fil-

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tered from an alcoholic solution from which the (+)-1-hydroxy-2-aminobutane was recovered.

Reaction of 1-Hydroxy-2-aminobutane with D-Glutamic Acid.—This experiment was carried out to check the results of the reaction of the amine with L-glutamic acid and to prepare the diastereomer, (∓)-1-hydroxybutane-2-ammonium hydrogen D-glutamate.

D-Glutamic acid (14.7 g., 0.10 mole) and commercial solvents 1-hydroxy-2-aminobutane (8.9 g., 0.10 mole) were dissolved in 15 ml. of water on a water-bath at 70°. The warm sirup was filtered on a büchner funnel with the aid of 15 ml. of warm water and 220 ml. of absolute ethanol was added to the filtrate. A flocculent white precipitate of 0.6 g. of D-glutamic acid formed immediately. The second crop of crystals was 11.6 g. of 88% optically pure (−)-aminium D-glutamate. Recrystallization by solution in 30 ml. of water and the addition of 470 ml. of absolute ethanol yielded 9.2 g., 78%, m.p. 146–147°; $[\alpha]^{25}D$ −3.2° ±0.1° (H_2O, *c* 5).

The mother liquor from the first crop was cooled with dry ice and 1.0 g. of 69% optically pure (−)-aminium D-glutamate precipitated. Three partial evaporations and the addition of absolute ethanol failed to cause further precipitation. Finally 2-propanol was added to the sirup and a gum formed immediately. The 2-propanol was decanted and the gum was dried over calcium chloride in a vacuum desiccator at 0.5 mm. yielding 2.8 g., 24%, of crude (+)-1-hydroxybutane-2-ammonium hydrogen D-glutamate. The deliquescent white compound was then dried for 90 min. over phosphorus pentoxide at oil-pump pressure at the temperature of refluxing chloroform. The m.p. of the dried compound was 98–104°; $[\alpha]^{29}D$ +9.5 ± 1.0° (H_2O, *c* 3).

Partial Resolution of Aspartic Acid.—National Aniline DL-aspartic acid (6.65 g., 0.05 mole) and 96% (−)-1-hydroxy-2-aminobutane (4.46 g., 0.05 mole) were stirred on a water-bath at 60° with 2 ml. of water until a sirup formed. The addition of 100 ml. of absolute ethanol and storage in the refrigerator failed to produce crystallization, so the solution was concentrated at room temperature. When the volume was reduced by half, 2.4 g. of salt, m.p. 128–132°, was collected. Two grams of the aspartate was dissolved in 5 ml. of water and enough concd. hydrochloric acid was added to bring the *p*H to 3.0, the isoelectric point of aspartic acid. Enough ethanol was added to bring the volume to 40 ml.; yield 1.2 g.; $[\alpha]^{22}D$ +10.0 ±0.4° (6 *N* HCl, *c* 2.69).

The value reported in the literature for L-aspartic acid in 6 *N* HCl is +24.6°[34]; thus this preparation was of 70% optical purity.

Recrystallization of DL-Amino Acids from Aqueous (−)-1-Hydroxy-2-aminobutane.—Experiments were performed on DL-leucine, DL-phenylalanine and DL-valine to determine whether resolution might result from simple recrystallization from the optically active solvent. The amino acids were essentially insoluble in the anhydrous amine. For the aqueous amine only the attempt with valine is described. A mixture of 21 ml. of the amine and 11 ml. of water was heated to dissolve 5.7 g. of DL-valine. After overnight cooling, the precipitate was treated with alcohol containing 16% acetic acid to leave 3 g. of valine. This material was optically inactive as were similar products from the two other amino acids.

The selectivity of reaction with unsubstituted dicarboxylic acid may be used for separations as illustrated in the next section.

Separation of Leucine and Glutamic Acid by 1-Hydroxy-2-aminobutane.—L-Glutamic acid (14.7 g., 0.100 mole), DL-leucine (13.1 g., 0.100 mole) and 1-hydroxy-2-aminobutane (9.4 g., 0.105 mole) were warmed with 50 ml. of water at 60° for 30 min. The suspension was filtered to return 12.9 g. (98.5%) of DL-leucine ($[\alpha]D$ 0.0°). The filtrate was adjusted to *p*H 3.2 with 12 *N* hydrochloric acid and was then treated with 300 ml. of absolute ethanol. There was recovered 12.6 g. (86%) of L-glutamic acid; $[\alpha]^{25}D$ +31.0° ± 0.1° (6 *N* HCl, *c* 4).

Effect of Diastereomer on Solubility of Hydroxybutane-ammonium Glutamate.—An indication of the relative solubility of the diastereomers was obtained by recording the weights of added and of undissolved salt in 83 vol. % ethanol, following successive additions and alternate warming to 70° and cooling. The solubilities, 1.2 g. per 100 ml. and 1.1 g. per 100 ml., respectively, for the (−)-aminium D-glutamate and the (−)-aminium L-glutamate indicated that there was no significant difference in the solubilities of the diastereomers in 83% ethanol. In an experiment typically illustrative of the mutual solubilizing effect 16 g. of (−)-1-hydroxybutane-2-ammonium hydrogen D-glutamate brought 13 g. of (−)-1-hydroxybutane-2-ammonium hydrogen L-glutamate into solution in 100 ml. of 83% ethanol. Whereas there was little difference in the solubility of the diastereomers alone with a change in temperature, the diastereomers together showed a marked change in solubility with thermal change.

Formyl-DL-phenylalanine.—DL-Phenylalanine was formylated in 80% yield by the method of du Vigneaud, Dorfmann and Loring[35] applied to cystine. The product m. 168°; Fischer and Schoeller[36] reported 169–170°.

Resolution of Formylphenylalanine.—Formylphenylalanine (165 g., 0.84 mole) was dissolved with 75 g. (0.84 mole) of (−)-1-hydroxy-2-aminobutane in 600 ml. of 1-butanol and decolorized with Norit. To this was added 600 ml. each of benzene and of Skelly D. One crop of crystals was removed after 6 hr., another after overnight standing. Both crops were virtually pure (−)-1-hydroxybutane-2-ammonium formyl-D-phenylalaninate, m.p. 128–129°; 58 g. (50%). Recrystallization from 200 ml. of 1-butanol gave 40 g. of rosettes, m.p. 129–130°; $[\alpha]^{29}D$ −52.5° ± 0.2° (*c* 4.4 in 95% ethanol).

Anal. Calcd. for $C_{14}H_{22}O_4N_2$: N, 9.93. Found: N, 9.97.

To the filtrate was added another 1000 ml. of Skelly D, which deposited a small amount of oil, followed by 24 g. of (−)-1-hydroxybutane-2-ammonium formyl-L-phenylalaninate. Recrystallization from 70 ml. of 1-butanol and 70 ml. of benzene gave 20 g., m.p. 106–107°; $[\alpha]^{29}D$ +42.7° ± 0.6° (*c* 1.6 in 95% ethanol).

Anal. Calcd. for $C_{14}H_{22}O_4N_2$: N, 9.93. Found: N, 9.96.

Solubility determinations indicated closely similar values for the two diastereomers in the solvent system employed.

Recovery of D- and of L-Phenylalanine.—The formylphenylalanines were recovered from the salts by solution in four parts of water and addition of about an equal part of 5–6 *N* hydrochloric acid. After being washed with water, the formylphenylalanine precipitates were hydrolyzed to yield the phenylalanine isomers by the method of Fischer and Schoeller.[36] Rotations were $[\alpha]^{29}D$ +35.1° ± 1.0° and −35.0° ± 1.0° for 1% solutions in water.

Further crops of formylphenylalanine salt could be recovered from the mother liquors of the treatment of the formylphenylalanine with amine. Attempts to perfect the method were not continued inasmuch as it was found that unsubstituted phenylalanine could be resolved by use of methylcinchoninium hydroxide.[37]

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AMES, IOWA

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