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RESOLUTION OF GLUTAMIC ACID WITH 2-AMINOBUTAN-1-OL

by

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Approved:

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I. INTRODUCTION

The resolution of glutamic acid is of considerable interest for many reasons. D-Glutamic acid, although available commercially in small quantities, in usually prepared as needed in most laboratories. L-Glutamic acid is commercially available in large quantities, but it is a by-product of the manufacture of wheat starch (1) and the processing of sugar beets (2), and hence its supply is limited by the quantity of these products manufactured. Since L-glutamic acid is utilized, as the monosodium salt, in large quantities as a condiment, it is possible that its demand may create an incentive for the preparation of synthetic glutamic acid. Should such a synthesis prove feasible, a method of resolution which is readily adaptable to large scale production must be developed to yield the commercially desirable L-glutamic acid. D-Glutamic acid is relatively tasteless and without nutritional value.

At present the resolution of DL-glutamic acid is employed to obtain D-glutamic acid only, since the supply of

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(1) M. J. Blish, "Wheat Gluten", in Anson and Edsall, eds., "Advances in Protein Chemistry", Academic Press, New York, 1945, vol. 2, p. 356.

(2) C. L. Royal, United States Patent 2,373,342 (1945). Abstracted in *C.A.*, *39*, 4510 (1945).

L-glutamic acid is meeting the demand, and since DL-glutamic acid is much less accessible than L-glutamic acid.

Interest in D-glutamic acid was aroused to a high level during the last decade and a half by the work of Kögl and coworkers. D-Glutamic acid, according to Kögl (1), is present in malignant tissue in a quantity proportional to the malignancy of the tumor tissue. This abnormal presence of D-glutamic acid in tumor tissues is an uncomplicated explanation for the formation of such tissue. However, most workers in the field do not accept Kögl's theory, and do not accept his experimental results as valid (2). Kögl's work did stimulate interest in the "unnatural" D-glutamic acid in the field of cancer research, and it is certain that much more work would be done if D-glutamic acid were more conveniently accessible for inclusion in experiments such as those dealing with animal feeding and tissue metabolism.

No reports were found in the literature presenting an actual resolution of underivatized DL-glutamic acid. Either the α-carboxyl or the amino group was blocked, thus disrupting the zwitterion to make possible the necessary diastereomeric salts or derivatives of DL-glutamic acid.

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(1) F. Kögl and H. Erxleben, *Z. physiol. Chem.*, *258*, 57 (1939).

(2) J. A. Miller, *Cancer Research*, *10*, 65 (1950).

This required the removal of the blocking group by hydrolysis or hydrogenolysis after the separation of the diastereomers had been achieved. This over-all procedure involved time-consuming steps which were costly in material and which it was desirable to eliminate. A simple, rapid method of resolution for DL-glutamic acid is much needed, and it is the purpose of the experiments which follow to determine whether or not the method presented fulfills these requirements.

It was reported by Fearing (1) that (-)2-aminobutan-1-ol could be used to resolve DL-glutamic acid. This small scale run yielded 42% of the D-glutamic acid, recovered from a diastereomeric salt, and 23% of the L-glutamic acid, recovered from the mother liquor. It was stated that (-)2-aminobutan-1-ol-D-glutamate formed a crystalline precipitate, while its diastereomer, (-)2-aminobutan-1-ol-L-glutamate, formed an oil. It was considered worthwhile to investigate this method, since it involves the resolution of underivatized DL-glutamic acid, and rapidly gives a good yield of D-glutamic acid.

The literature was searched for methods of resolution of amino acids which do not require derivatization of the amino or the carboxyl group of the amino acid; for some

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(1) R. B. Fearing, Organic Bases and Other Agents for Amino Acid Resolution. Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library. 1951. p. 84

experimental results which show the need for both antipodes of amino acids, especially of glutamic acid; for methods of resolution of DL-glutamic acid and methods of resolution by L-glutamic acid; and for methods of resolution of racemic 2-aminobutan-1-ol. A review of methods of resolution of amino acids in general is found in the thesis of Fearing (1).

The initial purpose of the investigation described in this thesis was to determine the reproducibility of the resolution of DL-glutamic acid with (-)2-aminobutan-1-ol. After the successful completion of this study, the determination of the solubility characteristics of the diastereomers, (-)2-aminobutan-1-ol-D-glutamate and (-)2-aminobutan-1-ol-L-glutamate, was undertaken. Since (+)tartaric acid, the commercially available enantiomorph, yields (-)2-aminobutan-1-ol, a pathway to (+)2-aminobutan-1-ol was sought in order to facilitate a resolution of DL-glutamic acid which would yield predominantly L-glutamic acid. Finally the possible application of 2-aminobutan-1-ol to the resolution of other amino acids was investigated.

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(1) R. B. Fearing, Organic Bases and Other Agents for Amino Acid Resolution. Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library. 1951. p. 5.

II. REVIEW OF LITERATURE

A. Studies Requiring D-Amino Acids

1. Natural sources

Only in the last two decades has adequate experimental evidence been presented for the presence of D-amino acids in nature. Previously the conditions under which the occurrence of D-amino acids in natural products was established often left doubt as to the possibility of racemization, which would produce a misleading specific rotation (1). However, the isolation of DL-alanine from the nonprotein fraction of the mushroom Bolelus edulis is accepted as well authenticated and racemization during isolation was unlikely (1,2). The discovery of D-amino acids in antibiotics such as penicillin, gramicidin, tyrocidine, and gramicidin S, in the alkaloids of ergot, and in the capsular substance of several bacteria has recently focussed attention on the general distribution of D-amino acids in nature.

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(1) A. Neuberger, "Stereochemistry of Amino Acids", in Anson and Edsall, eds., "Advances in Protein Chemistry", Academic Press, New York, 1948, vol. 4, p. 363.

(2) E. Winterstein, C. Reuter, and R. Korolew, Landw. Versuchs-Stat., 79-80, 541 (1913). Original not seen. Abstracted in J. Chem. Soc. Abstracts, 104, 433 (1913).

Early claims of the isolation of D-amino acids from natural sources were reviewed by Neuberger and were discounted for technical reasons (1). Recently the hydrolysis of the capsules of *Bacillus anthracis* yielded D-glutamic acid (2,3) since the capsule was composed of a high molecular weight polypeptide of D-glutamic acid (4). The capsular substance of *Bacillus mesentericus* was found to be a high molecular weight polypeptide of D-glutamic acid as determined by the isolation of only D-glutamic acid from the capsular substance which had been purified by dialysis (5). Bruckner and Oskolás (5) hypothesized that the parallelism between the virulence and the capsule-forming capacity of the anthrax bacillus can be explained on the basis that the basic material of the capsule strongly protects the capsule against the effects of L-polypeptide proteases of higher organisms.

Jenkins and Ciereszko (6) found that whereas the

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(1) A. Neuberger, "Stereochemistry of Amino Acids", in Anson and Edsall, eds., "Advances in Protein Chemistry", Academic Press, New York, 1948, vol. 4, p. 363.

(2) V. Bruckner and G. Ivanovics, *Z. physiol. Chem.*, *247*, 281 (1937).

(3) G. Bruckner, G. Ivanovics, and M. K. Oskolás, *Magyar Chem. Folyoirat*, *45*, 131 (1939). Original not seen. Abstracted in *C.A.*, *34*, 3766 (1940).

(4) W. E. Hanby and H. N. Rydon, *Biochem. J.*, *40*, 297 (1946).

(5) G. Bruckner and M. K. Oskolás, *Acta Univ. Szegediensis, Acta Chem. et Phys.*, [N.S.] *1*, 144 (1943). Original not seen. Abstracted in *C.A.*, *41*, 7423 (1947).

(6) L. T. Jenkins and L. S. Ciereszko, *J. Biol. Chem.*, *191*, 305 (1951).

extracellular polypeptide of *Bacillus subtilis* was composed of only D-glutamic acid, the cellular glutamic acid was L-glutamic acid. Chargaff (1) found only L-glutamic acid and L-aspartic acid in *Corynebacterium diphtheriae* and *Phytomonas tumefaciens*, an etiological agent of plant tumors. Relatively large quantities of D-amino acids were found in the acid hydrolyzates of the cells of *Lactobacillus arabinosus* and *Bacillus brevis*, a smaller quantity in the cells of *Torulopsis utilis*, and none in the cells of *Penicillium chrysogenum* (2,3). Unhydrolyzed cells of *Bacillus brevis*, added to the diet of rats, led to the excretion of D-amino acids, mostly D-aspartic acid, in the urine (3).

2. Tumor protein

The controversial question of D-amino acid content in tumor protein was reviewed through 1949 by Miller (4). After carefully considering the evidence in the literature for and against Kögl's hypothesis that D-amino acids appear in tumor protein, Miller arrived at the conclusion that Kögl has yet to unequivocally prove his hypothesis. Since 1949 much has been published concerning this hypothesis, and some of this

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(1) E. Chargaff, *J. Biol. Chem.*, 130, 29 (1939).
(2) M. S. Dunn, M. N. Camien, S. Shankman, and H. Block, *J. Biol. Chem.*, 168, 43 (1947).
(3) C. M. Stevens, P. E. Halpern, and R. P. Gigger, *J. Biol. Chem.*, 190, 705 (1951).
(4) J. A. Miller, *Cancer Research*, 10, 65 (1950).

literature will be presented because of its bearing on D-glutamic acid.

Kögl fed rats having chemically induced tumors water containing D_2O, and after 17 days of feeding found an equal quantity of the deuterium in both the D- and the L-glutamic acids that were isolated from tumor hydrolyzate (1). Rats with chemically induced tumors were fed deuterio-DL-glutamic acid and after 5 and 15 days of feeding, analysis of the tumor protein revealed that the deuterium content of the L-glutamic acid was practically nil, but that of the D-glutamic acid was definitely above experimental error (2).

Subcutaneous injections of deuterio-DL-glutamate in rats having benzopyrene-induced tumors indicated on analysis of the tumor protein that the deuterium content of the D-glutamic acid was much higher than that of the L-glutamic acid. Injection of deuterio-D-glutamic acid resulted in a high content in the tumor protein, whereas injection of deuterio-L-glutamic acid produced no deuterio-D-glutamic acid. Therefore, Kögl (3) concluded that D-glutamic acid was directly used in the protein synthesis, and that there was no enzyme causing a Walden inversion of L-glutamic acid to D-glutamic acid during the protein synthesis.

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(1) F. Kögl, A. J. Klein, H. Erxleben, and G. J. van Veersen, Rec. trav. chim., 69, 822 (1950).
(2) Ibid., p. 834.
(3) Ibid., p. 841.

Not unrelated to Kögl's conclusion is Mayer's statement which points out that foods containing albuminoids also contain small amounts of D-amino acids. The harmful effects of these acids are neutralized by natural elimination or by deamination in persons with normal hepatic and renal functions. However, in persons having hepatic or renal malfunctions or in those having a precancerous focus, D-glutamic acid accumulates at certain points and changes the stereochemical structure of certain proteins which act in tissue formation (1).

Glutamic acid was estimated in the plasma of 80 patients suffering from malignant growths (2). The mean concentration was elevated markedly above the normal level. In patients with benign growths, the level of glutamic acid in the plasma did not differ significantly from the normal.

In an analysis of normal and tumor tissues of 6 patients, Mondolfo and Camboni (3) noted that in the hydrolyzates of neoplastic tissue there is a greater quantity of some amino acids than in healthy tissue of the same origin. The most consistent differences were found in the contents of glutamic

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(1) C. Mayer, *Paris Méd.*, *41*, 155 (1951). Original not seen. Abstracted in *C.A.*, *45*, 9683 (1951).

(2) W. J. McGanity and E. W. McHenry, *Can. Med. Assoc. J.*, *65*, 219 (1951). Original not seen. Abstracted in *C.A.*, *46*, 2668 (1952).

(3) V. Mondolfo and V. Camboni, *Boll. soc. ital. biol. sper.*, *26*, 131 (1950). Original not seen. Abstracted in *C.A.*, *44*, 9551 (1950).

acid, threonine, and tyrosine, which were always higher in neoplastic tissue. The amount of D-glutamic acid was higher in normal tissue than in neoplastic tissue in quantities which were sometimes higher and at other times lower than those of L-glutamic acid.

In contrast to the concept of an abnormal abundance of D-amino acids at tumor sites, D-tryptophan and D-lysine, when used in a diet to replace the "natural" L-tryptophan and L-lysine, strongly retarded the growth of spontaneous mammary tumors of a strain of mice. Penicillin and aureomycin were fed to suppress intestinal bacterial growth which might have been the source of enough L-tryptophan and L-lysine to give inconsistent results (1). However, Kögl did not claim that all D-amino acids were present in tumor tissue in abnormal quantities.

3. Bacterial products

Bacillus brevis which generates the polypeptide antibiotics, tyrocidine and gramicidin, that contain D-amino acids is incapable of the utilization of D-valine, D-alanine, and D-glutamic acid unless glucose is present (2). Fifty per cent

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(1) A. E. Gessler, K. S. McCarty, and M. C. Parkinson, Exptl. Med. Surg., 8, 168 (1950).

(2) A. S. Konikova and N. N. Dobbert, Biokhimiya, 13, 115 (1948). Original not seen. Abstracted in C.A., 42, 7832 (1948).

of the amino acid content of *Bacillus brevis* protein is of the D-configuration, and practically the same proportion of D-amino acids is found in the protein regardless of whether the bacteria are grown on glucose and L-glutamic acid or on glucose and D-glutamic acid (1).

In addition to gramicidin and tyrocidine, gramicidin S and polymyxin are recently discovered cyclic polypeptide antibiotics which contain D-amino acids. The interesting question is raised as to whether the D-amino acids are necessary for the cyclic structure and for the antibiotic activity of these polypeptides, or whether L-amino acids can be substituted for the D-amino acids without changing the basic structure or losing antibiotic activity. Gramicidin S, the lowest in molecular weight of the four, is considered to be a cyclopentapeptide, or possibly a cyclodecapeptide. The structure or gramicidin S is considered to be (-α-(L-valyl)-L-ornithyl-L-leucyl-D-phenylalanyl-L-prolyl-) occurring in a closed peptide chain once or twice (2).

Harris and Work (3,4) have synthesized peptides related to gramicidin S in an effort to determine whether or not

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(1) A. S. Konikova and N. N. Dobbert, *Biokhimiya*, *13*, 115 (1948). Original not seen. Abstracted in *C.A.*, *42*, 7832 (1948).

(2) R. Consden, A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, *41*, 596 (1947).

(3) J. I. Harris and T. S. Work, *Biochem. J.*, *46*, 196 (1950).

(4) *Ibid.*, p. 582.

D-phenylalanine is necessary for antibiotic activity. The antibacterial activity of L-leucyl-D-phenylalanyl-L-proline methyl ester was equal to that of L-leucyl-L-phenylalanyl-L-proline ethyl ester. Likewise α-(L-valyl)-L-ornithyl-L-leucyl-D-phenylalanyl-L-proline methyl ester had the same antibacterial activity as α-(L-valyl)-DL-ornithyl-L-leucyl-L-phenylalanyl-L-proline ethyl ester. A tenfold increase in inhibition was achieved over the former pentapeptide by tosylating the free amino group of valine. The resultant tosylated pentapeptide methyl ester and a tosylated pentapeptide amide were approximately 0.2% as effective as gramicidin S. This work and similar experiments led Harris and Work to conclude that the presence of D-amino acid residue alone did not give rise to the antibacterial activity in the cyclic polypeptides. Their tosylated pentapeptide amide and ester presented only the γ-amino group of ornithine as a free reactive group, just as does gramicidin S. The tosylated pentapeptide amide is about as close to the structure of gramicidin as is possible without cyclization. Therefore, Harris and Work postulated that the biological activity of gramicidin S is intimately related to its cyclic structure. The key question of whether D-phenylalanine is essential to the cyclic structure awaits its answer in the actual synthesis of gramicidin S itself. An effective ring closure method for a pentapeptide awaits discovery.

4. Inhibition

In some cases the atypical isomers of amino acids exhibit interference with the normal metabolism of amino acids in general. Both D-valine and D-leucine inhibited the growth of *Lactobacillus arabinosus* and *Escherichia coli* strains when added to a medium containing the usual full complement of amino acids (1,2,3). According to Mario (4) subcutaneous or oral administration of valine and norleucine in 5-15 g. quantities was effective in controlling infection. The configuration of the valine and norleucine was not given. The desirability of determining which isomer is responsible for this effect is apparent.

D-Glutamic acid, D-lysine, and D-arginine had an antibacterial effect on *Staphylococcus aureus* (5,6). DL-Tryptophan is not as effective in meeting the growth requirements of *Lactobacillus arabinosus* as half as much L-tryptophan (7).

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(1) S. W. Fox, M. Fling, and G. N. Bollenback, *J. Biol. Chem.*, **155**, 465 (1944).
(2) M. Fling and S. W. Fox, *J. Biol. Chem.*, **160**, 329 (1945).
(3) Y. Kobayashi, M. Fling, and S. W. Fox, *J. Biol. Chem.*, **174**, 391 (1948).
(4) O. Mario, *Minerva med.*, **38**, I, 578 (1947). Original not seen. Abstracted in *C.A.*, **41**, 6974 (1947).
(5) E. Jeney, *Hung. Acta Physiol.*, **1**, 142 (1948).
(6) E. Jeney, *Orvosok Lapja*, **4**, 1010 (1948). Original not seen. Abstracted in *C.A.*, **43**, 9146 (1949).
(7) J. M. Prescott, B. S. Schweigert, C. M. Lyman, and K. A. Kuiken, *J. Biol. Chem.*, **178**, 727 (1949).

The atypical forms of alanine (1) and serine (2) are inhibitory toward the growth of *Escherichia coli*. The ciliated protozoan *Tetrahymena geleii* is inhibited by D-leucine, D-valine, and D-phenylalanine (3). Jeney (4) postulated that the two amino acids of penicillin liberated during the course of its action were responsible for its antibiotic effect. The D-amino acid residue of penicillin is essential to its antibacterial activity since the L-analog gives no such effect (5).

In a rather unusual phenomenon, exhibited by *Lactobacillus mannitopoeus*, glycine and DL-serine were capable of inhibiting the interconversion of L- and D-alanine; in the presence of either inhibitor, both enantiomorphs are required by the bacteria (6). Camien and Dunn hypothesized that the greater reduction of D-amino acid activity than of L-amino acid activity, as illustrated by lactic acid bacteria, is a general phenomenon (7).

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(1) Y. Kobayashi, M. Fling, and S. W. Fox, *J. Biol. Chem.*, *174*, 391 (1948).

(2) B. D. Davis and W. K. Maas, *J. Am. Chem. Soc.*, *71*, 1865 (1949).

(3) G. W. Kidder and V. C. Dewey, *Proc. Natl. Acad. Sci.*, *33*, 347 (1947).

(4) E. Jeney, *Hung. Acta Physiol.*, *1*, 142 (1948).

(5) V. duVigneaud, F. H. Carpenter, R. W. Holley, A. H. Livermore, and J. R. Rachele, *Science*, *104*, 431 (1946).

(6) M. N. Camien and M. S. Dunn, *J. Biol. Chem.*, *185*, 553 (1950).

(7) M. N. Camien and M. S. Dunn, *J. Biol. Chem.*, *184*, 283 (1950).

Evidence for the inhibitory effect of *D*-amino acids in animals is less clear than that for bacteria, but a preponderance of evidence indicates that competitive inhibition does take place. Wretlind (1) noted that at a level of 20% in a purified diet 10 *DL*-amino acids gave a greater growth response in rats than a level of 30 or 40%. A mixture of *L*-amino acids duplicating the amino acid composition of β-lactoglobulin supported the same growth in mice as did whole protein, whereas the feeding of certain *DL*-amino acids in the mixture impaired the growth response (2). In rats *DL*-leucine, -tryptophan, and -aspartic acid prevented growth in comparison with the *L*-isomers (3). *L*-serine did not present the clinical and pathological alterations given by *DL*-serine (4). *DL*-threonine and *DL*-phenylalanine in amounts equivalent to those present in 2% hydrolyzed casein aggravated a niacin-tryptophan deficiency in rats (5). In liver tissue slices from 60 day old rats *D*-methionine inhibited the uptake of *L*-methionine (6).

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(1) K. A. J. Wretlind, *Acta Physiol. Scand.*, *15*, 304 (1948). Original not seen. Abstracted in *C.A.*, *42*, 8285 (1948).

(2) E. Brand and D. K. Bosshardt, *Abstracts of Papers, 114th Am. Chem. Soc. Meeting*, p. 38C (1948).

(3) C. E. Graham, S. W. Hier, H. K. Waitkoff, S. M. Saper, W. G. Bibler, and E. I. Pentz, *J. Biol. Chem.*, *185*, 97 (1950).

(4) C. Artom, W. H. Fishman, and R. P. Morehead, *Proc. Soc. Exptl. Biol. Med.*, *60*, 284 (1945).

(5) L. V. Hankes, L. M. Henderson, W. L. Brickson, and C. A. Elvehjem, *J. Biol. Chem.*, *174*, 873 (1948).

(6) L. V. Simpson and H. Tarver, *Arch. Biochem.*, *25*, 384 (1950).

That the retardation of growth is probably due to the inhibition of proteases is supported by enzymic inhibition experiments. D-leucine inhibits the hydrolytic behavior of peptidases from several types of muscle (1). D-Histidine, -phenylalanine, -alanine and -isoleucine competitively inhibited the action of carboxypeptidase isolated from beef pancreas (2).

5. Metabolism

In experimental and developmental uses of mixtures of amino acids in nutrition, the non-utilization of the "unnatural" D-forms of certain of the essential amino acids is a troublesome problem in a manner very closely related to the inhibition phenomena previously mentioned. Tryptophan is utilized in both the D- and L-forms by rats, but only the L-form is used by man; hence, assays of protein preparations with rats may not give a clear prediction of their value for human beings with regard to tryptophan (3).

Rats utilized DL-amino acid mixtures in casein and casein hydrolyzates at a slow rate compared to casein hydrolyzates

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(1) E. Abderhalden and R. Abderhalden, Fermentforschung, 16, 445 (1942).

(2) E. Elkins-Kaufman and H. Neurath, J. Biol. Chem., 175, 893 (1948).

(3) A. A. Albanese, "Protein and Amino Acid Requirements of Mammals", Academic Press, New York, 1950, p. 68.

containing the same L-amino acids (1). At the levels fed, no symptoms of toxicity were produced, but a slight depression of growth may have occurred.

The problem of feeding certain DL-amino acids in diets and media, the DL-form being the only available pure form, may be solved by replacing them with the corresponding α-keto acid and a nitrogen source. The α-keto analogs of valine and isoleucine, as the sole sources of these amino acids, promote excellent growth in rats (2). They may undergo asymmetric amination *in vivo* to supply the corresponding L-amino acids.

The mammalian circulatory system is capable of removing D-amino acids. Boulanger isolated a D-amino acid dehydrogenase from defatted hog kidney powder which acted rapidly on D-glutamic acid but did not act on L-glutamic acid (3). The presence and exact functions of D-peptidases and D-amino acid oxidases and dehydrogenases in animal systems has not been adequately explained (4).

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(1) G. B. Ramasarma, L. M. Henderson, and C. A. Elvehjem, *J. Nutrition*, **38**, 177 (1949).

(2) W. C. Rose, L. C. Smith, M. Womack, and M. Shane, *J. Biol. Chem.*, **181**, 307 (1949).

(3) P. Boulanger, *Compt. rend. soc. biol.*, **137**, 521 (1943).

(4) E. Waldschmidt-Leitz, *Ergebn. Enzymforsch.*, **9**, 193 (1943). Original not seen. Abstracted in *Chem. Zentr.*, **114**, II, 1719 (1943).

Homogenized kidney from rats deficient in vitamin B_6 exhibited only one third as much D-amino acid oxidase activity as did normal rat kidney homogenates (1). A supplement of D-amino acids to vitamin B_6 deficient rats greatly depressed dietary nitrogen utilization. Simultaneous administration of vitamin B_6 completely overcame this effect.

The physiological functions of glutamic acid are of prime importance. Weil-Malherbe (2), considering the significance of glutamic acid for the metabolism of nervous tissue, stated that the three enzymic reactions involving glutamic acid (deamination, transamination, and amidation) might perhaps be integrated into a system for the deionization and removal of intracellular ammonia. Glutamic acid is the only amino acid oxidized in the brain to any appreciable extent by a specific enzyme. Transamination of glutamic acid serves as a buffer system which takes up the sudden influx of NH_3 and insures its gradual disposal during restitution. Glutamic acid is known to maintain the respiration of nervous tissue, and may also be necessary for maintaining its function. It may also exert, through its special activity, its influence on the permeability of the nervous cell membrane. If D-glutamic acid were to appear at the sites of such reactions, it

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(1) K. L. Armstrong, G. Feldott, and H. A. Lardy, Proc. Soc. Exptl. Biol. Med., 73, 159 (1950).
(2) H. Weil-Malherbe, Physiol. Revs., 30, 549 (1950).

might affect their efficiency. Such a possibility has yet to be experimentally clarified.

The previously mentioned experimental work emphasizes the definite need for investigations utilizing D-amino acids as well as L- and DL-amino acids. Such investigations can be expedited by an ample supply of D-amino acids, made available by simple resolutions.

B. Resolutions of Unsubstituted Amino Acids

1. DL-Lysine

Kearley and Ingersoll (1) found that the crystallization of the diastereomeric lysine (+)-dibenzoyl hydrogen tartrates from 2-propanol gave physically distinct salts of D-lysine and L-lysine. The D-lysine salt precipitated first as a hard crust of hexagonal plates with an 82% yield, and within an hour after its removal, a 76% yield of the L-lysine salt precipitated as fine fragile needles. A third smaller crop was rich in the D-salt. If the two diastereomers crystallized together, the crop was dissolved in the aqueous 2-propanol for reprecipitation. One recrystallization was required for the D-salt and two recrystallizations were required for the L-salt. Decomposition

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(1) F. J. Kearley and A. W. Ingersoll, J. Am. Chem. Soc., 73, 5783 (1951).

with hydrochloric acid permitted recovery of the resolving agent and the active lysine hydrochlorides. N-Acetyl-3,5-dibromo-L-tyrosine and a 17% water solution of DL-lysine gave a crystalline precipitate of the L-lysine salt while the D-lysine salt remained in the liquors. The yield of the L-lysine salt was 84%, and the yield of L-lysine hydrochloride was 67%.

DL-Lysine was resolved with L-glutamic acid by Emmick (1). An 86% yield of crude L-lysine-L-glutamate-H_2O precipitated from an 84% methanol solution upon seeding. Purification through a bed of Dowex 50, elution with NH_4OH, and the addition of the calculated amount of HCl gave L-lysine hydrochloride upon the addition of ethanol. An 82% yield of 95% optical purity and 98.5% chemical purity was obtained.

2. DL-Glutamic acid

High quality D-glutamic acid was prepared from the DL-compound by the specific enzymic decarboxylation of L-glutamic acid using a decarboxylase prepared from *Escherichia coli* cells (2). The enzymic reaction required 4 hours, and the recovery of D-glutamic acid required dilution, centrifugation, evaporation to dryness under reduced

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(1) R. D. Emmick, United States Patent 2,556,907, (1951). Abstracted in *C.A.*, 46, 525 (1952).

(2) M. N. Camien, L. E. McClure, and M. S. Dunn, *Arch. Biochem.*, 28, 220 (1950).

pressure, solution, filtration and saturation with HCl to yield D-glutamic acid hydrochloride. The over-all purified yield of D-glutamic acid was 58% after the pH was brought to 3.2 with ethanolamine and after recrystallization.

Osteux and Boulanger (1) used sedimented Clostridium welchii with DL-glutamic acid and got a 67% yield of D-glutamic acid by a procedure similar to that of Camien, McClure, and Dunn.

So specific are amino acid oxidases and decarboxylases that they can be used to determine the contamination of one optical isomer by the other to less than 1 part in 1000 (2). Enzymic resolution methods gave optical isomers which contained less than 0.1% of the enantiomorph. Of 15 commercial samples checked, only three showed a purity equivalent to those prepared by enzymic resolution, and 12 contained 0.6-16.3% of the enantiomorph.

The first observations that some molds specifically utilize L-glutamic acid were made in the latter part of the last century. It was observed that Penicillium glaucum utilized the L-glutamic acid; consequently, the D-glutamic

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(1) R. Osteux and P. Boulanger, Bull. soc. chim. biol., 32, 1059 (1950).
(2) A. Meister, L. Levintow, R. B. Kingsley, and J. P. Greenstein, J. Biol. Chem., 192, 535 (1951).

acid was recovered from the medium (1,2). Pringsheim (3) found that *Aspergillus niger* preferentially utilized L-glutamic acid in its media. Ehrlich (4) used a yeast strain in the presence of glucose to utilize the L-glutamic acid and leave the D-form in the medium.

C. Resolutions with Unsubstituted Amino Acids

A synthetic racemate of cis-2-(4-carboxylbutyl)-3,4-ureylenetetrahydrothiophene (biotin) was resolved with L-arginine (5). The D-salt was precipitated by diluting the water solution with 4 volumes of 2-propanol and cooling. Decomposition with mineral acid gave biotin. The other diastereomer could be obtained by the concentration of the filtrate.

While d-oxymethylene camphor and d-camphorsulfonic acid were found adequate in the resolution of small quantities of α,β-diphenyl-β-hydroxyethylamine, they were

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(1) E. Schulze and E. Bosshard, *Z. physiol. Chem.*, 10, 134 (1886).

(2) A. Menozzi and G. Appiani, *Gazz. chim. ital.*, 24 I, 370 (1894). Original not seen. Abstracted in *J. Chem. Soc. Abstracts*, 66, 498 (1894).

(3) H. Pringsheim, *Z. physiol. Chem.*, 65, 96 (1910).

(4) F. Ehrlich, *Biochem. Z.*, 63, 379 (1914).

(5) K. Folkers and D. E. Wolf, United States Patent 2,441,141 (1948). Abstracted in *C.A.*, 42, 6381 (1948).

inadequate for large scale preparation (1). L-Glutamic acid was successfully used as a resolving agent. The pure levorotatory isomer was obtained from the less soluble glutamate in 60% yield, whereas the other enantiomorph was obtained from the mother liquor in 39% yield.

The previously mentioned resolution of underivatized lysine with L-glutamic acid also fits into the category of resolutions with unsubstituted amino acids (2).

Although pyroglutamic acid is not truly an amino acid, its close relationship to glutamic acid merits mention of its use as a resolving agent. Dearborn and Stekol (3) formed pyroglutamic acid from L-glutamic acid, and with it resolved dl-phenylethylamine, dl-2-aminooctane and dl-2-aminoheptane. One diastereomer precipitated, while the other was not recovered, but was decomposed in the concentrated mother liquor with NaOH to yield the optically active amine.

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(1) J. Weijlard, K. Pfister, E. F. Swanezy, C. A. Robinson, and M. Tishler, *J. Am. Chem. Soc.*, 73, 1216 (1951).; M. Tishler, Private communication, 1952.

(2) R. D. Emmick, United States Patent, 2,556,907 (1951). Abstracted in *C.A.*, 46, 525 (1952).

(3) R. J. Dearborn and J. A. Stekol, United States Patent 2,528, 267 (1950). Abstracted in *C.A.*, 45, 2984 (1951).

D. Resolutions with Enzymes and Derivatized DL-Glutamic Acid

1. Papain

When needed for experimental work in these laboratories, D-glutamic acid had been resolved by a procedure based on the specific formation of carbobenzoxy-L-glutamic acid anilide by papain, which was activated by cysteine (1). Optimum conditions for the pH and the buffer concentration were determined in these laboratories, and were applied to the procedure (2). The yield of D-glutamic acid was 50%, while the L-glutamic acid was not recovered, since it is commercially available. Fig. 1 presents the series of reactions in the procedure, which is time consuming. The incubation alone required 72 hours.

2. Purified hog kidney enzyme

Purified hog kidney enzyme specifically hydrolyzes N-acylated-L-amino acids, thus presenting a method of resolving N-acylated-DL-amino acids. This procedure has been applied to DL-alanine, -methionine, -valine, -threonine, -isoleucine, -serine, -leucine, -aspartic, and -glutamic

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(1) J. S. Fruton, G. W. Irving, and M. Bergmann, J. Biol. Chem., 133, 703 (1940).
(2) S. W. Fox and Y. Kobayashi, J. Am. Chem. Soc., 73, 353 (1951).

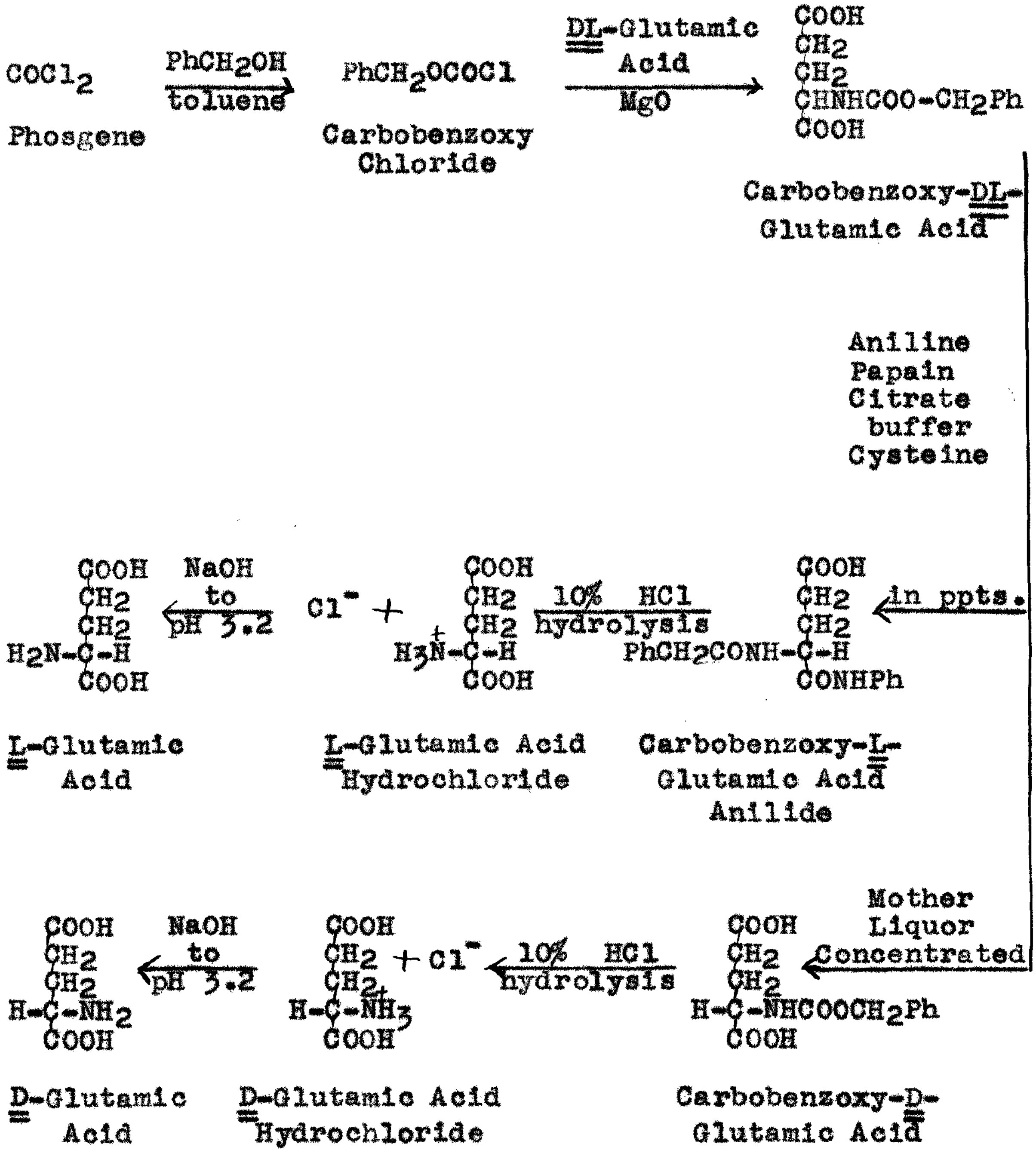

Fig. 1 Resolution of DL-Glutamic Acid Using the Formation of Carbobenzoxy-L-Glutamic Acid Anilide

acids (1), and hence has been applied to a greater variety of amino acids than any other single method. Large scale preparations can be conveniently carried out; for example, 8-10 kilograms of fresh hog kidneys were treated to yield enough of the enzyme in 5-8 days to hydrolyze 100 moles of N-acylated methionine. Chloroacetylation is advantageous since the chloroacetylated amino acids hydrolyze at a much faster rate than do the acetylated. The chloroacetylated amino acid was subjected to the purified hog kidney enzyme, with the progress of the reaction being followed by the ninhydrin-CO_2 procedure. When the analysis indicated that L-glutamic acid liberation was completed, these steps were carried out successively: acidification, decolorization, concentration, and precipitation with alcohol. Recrystallization from water and alcohol resulted in a 40-55% yield of the L-amino acid.

The D-amino acid was isolated by evaporating the mother liquor to dryness *in vacuo*, extraction with ethyl acetate, evaporation to dryness, solution in acetone, evaporation of acetone, hydrolysis with HCl, and adjustment of the pH to 3.2 with LiOH. The yield was from 30-60%. The advantages attributed to this method of resolution are simplicity,

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(1) V. E. Price, J. B. Gilbert, and J. P. Greenstein, *J. Biol. Chem.*, *179*, 1169 (1949).

rapidity of operation, and the possibility of working with unlimited quantities of the material at low cost.

Carbobenzoxy-DL-glutamic acid has been substituted for chloroacetyl-DL-glutamic acid to yield a more readily isolated compound and a purer D-glutamic acid, which was made possible by the recovery of carbobenzoxy-D-glutamic acid through catalytic hydrogenation (1). These advantages are weighed against the disadvantages of higher cost and a much slower enzymatic reaction. The yields were 83% L-glutamic acid and 77% D-glutamic acid. Glutamic acid was the only amino acid for which this deviation would work.

E. Resolution of DL-Glutamic Acid by Fractionation

1. Strychnine salt

Emil Fischer (2) resolved benzoyl-DL-glutamic acid for the first chemical resolution of DL-glutamic acid. The strychnine salt of benzoyl-D-glutamic acid precipitated, while the salt of benzoyl-L-glutamic acid was not recovered, but was decomposed by NaOH to yield benzoyl-L-glutamic acid from the mother liquor. A 15% yield of D-glutamic acid was achieved upon HCl hydrolysis of the benzoyl-D-glutamic

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(1) L. Levintow, J. P. Greenstein, and R. B. Kingsley, *Arch. Biochem. Biophys.*, **31**, 77 (1951).

(2) E. Fischer, *Ber.*, **32**, 2451 (1899).

acid. This procedure, which included benzoylation, salt formation and fractionation, strychnine recovery, and hydrolysis, offers few advantages. The low yield and the undesirability of strychnine are strong disadvantages. Winter adapted this resolution to p-nitrobenzoyl-DL-glutamic acid (1). Repeated crystallization of the strychnine salt yielded p-nitrobenzoyl-D-glutamic acid, which was hydrogenated to p-aminobenzoyl-D-glutamic acid.

2. Quinine salt of pyroglutamic acid

Glutamic acid, when heated up to the melting point, loses a molecule of water to form pyroglutamic acid. Sugasawa (2) dehydrated DL-glutamic acid to form DL-pyroglutamic acid, which was reacted with quinine to form the quinine salt. The quinine salt of D-pyroglutamic acid, the diastereomer which precipitated, was decomposed and separated by successive treatment with alkali, acid, and absolute alcohol. Fifteen per cent hydrochloric acid formed D-glutamic acid from the D-pyroglutamic acid. The L-glutamic acid was isolated by a similar treatment of the mother liquor.

A modification by Hillmann and Elies (3) simplified this

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(1) H. C. Winter, *J. Am. Chem. Soc.*, **62**, 3266 (1940).

(2) S. Sugasawa, *J. Pharm. Soc. Japan.*, **537**, 934 (1926). Original not seen. Abstracted in *C.A.*, **21**, 2664 (1927).

(3) G. Hillmann and A. Elies, *Z. physiol. Chem.*, **283**, 31 (1948).

procedure considerably. Picric acid formed a quinine picrate after decomposing the quinine salt of D-pyroglutamic acid. The insoluble quinine picrate was removed, leaving the D-pyroglutamic acid in solution. Hydrochloric acid hydrolysis yielded D-glutamic acid hydrochloride as before. The yields were raised to 65% D-glutamic acid hydrochloride from 44%, and to 37% L-glutamic acid hydrochloride from 30%.

3. Supersaturation and precipitation

This ingenious preparation of radioactive optically active glutamic acid is not, in a strict sense, a resolution. It might be called a resolution from the standpoint of the radioactive glutamic acid, since it began as optically inactive and finished as optically active isomers. However, this was achieved at the expense of non-radioactive, optically active glutamic acid. The synthetic radioactive DL-glutamic acid was dissolved with a quantity of non-radioactive D-glutamic acid hydrochloride equal to the yield of radioactive D-glutamic acid hydrochloride possible from the DL-compound (1). Upon cooling, radioactive D-glutamic acid hydrochloride separated from the supersaturated solution. After recrystallization and treatment with NH_4OH, radioactive D-glutamic acid was obtained. The radioactive L-glutamic acid

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(1) F. Kögl, J. Halberstadt, and T. J. Barendregt, Rec. trav. chim., 68, 387 (1949).

was prepared by the same procedure. In each case a 102.5% yield of the optically active, radioactive hydrochloride was reported.

4. Paper chromatography

Paper chromatography, with 1-methyl-(β-phenylisopropyl)-amine as a solvent, showed a difference in R_f values of 0.02 for D- and L-glutamic acid (1). The difference was considered to be in part due to the asymmetric character of the cellulose. While not a working resolution, this separation is a step toward an ideal type of resolution--the separation of optical isomers by the retention of one of the isomers on a chromatographic column.

F. Resolution of Some Amino Alcohols

1. (-)2-Aminobutan-1-ol

The reaction of equimolar amounts of (+)tartaric acid and racemic 2-aminobutan-1-ol in water resulted in the precipitation of (-)2-aminobutan-1-ol (+)hydrogen tartrate (2). The addition of calcium hydroxide to this salt formed insoluble calcium tartrate and left (-)2-aminobutan-1-ol in a

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(1) M. Kotake, T. Sakan, N. Nakamura, and S. Senoh, J. Am. Chem. Soc., 73, 2973 (1951).

(2) R. B. Fearing, Organic Bases and Other Agents for Amino Acid Resolution, Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library, 1951. p. 37.

water solution, from which it was separated by fractional distillation.

2. (+)2-Aminobutan-1-ol

(+)2(Benzylamine)butan-1-ol was prepared from racemic 2-benzylaminobutan-1-ol by resolution with mandelic acid (1). (+)2-Aminobutan-1-ol oxalate was formed from the (+)2-(benzylamino)butan-1-ol by the action of oxalic acid, H_2, and PdO_2. Alcoholic KOH precipitated potassium oxalate from an alcohol solution, leaving (+)2-aminobutan-1-ol which was purified by fractional distillation. The specific rotation was +9.8°.

3. Related compounds

Similar compounds, some 1-phenyl-2-methyl-aralkylamino-1-propanols (2), and threo-p-nitrophenyl-2-amino-1,3-propanediol (3) were resolved with (+)tartaric acid. The (+)-tartaric acid salt was decomposed with alkali.

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(1) A. Stoll, J. Peyer, and A. Hofmann, Helv. Chim. Acta, 26, 929 (1943).

(2) I. G. Farbenind. A. G., British Patent 318,488 (1928). Original not seen. Abstracted in C.A., 24, 2240 (1930).

(3) H. Ikeda and H. Ikeda, J. Sci. Research. Inst. (Tokyo). 45, 8 (1951). Original not seen. Abstracted in C.A., 45, 9000 (1951).

III. EXPERIMENTAL PROCEDURES AND RESULTS*

A. Resolution of 2-Aminobutan-1-ol

1. (-)2-Aminobutan-1-ol (+)hydrogen tartrate

A procedure which is essentially that of Fearing (1) was used to prepare this optically active salt. Mallinckrodt (+)tartaric acid (930 g., 6.2 moles) was dissolved in 1500 ml. of water, and to this was added Commercial Solvents 2-aminobutan-1-ol (553 g., 6.2 moles) with vigorous stirring and cooling. For this reaction the 2-aminobutan-1-ol was distilled under reduced pressure in order to remove a yellow impurity. After the dark red solution was cooled overnight in the refrigerator, the first crop of long silky crystals was collected and washed with 500 ml. of absolute ethanol, which removed a red-brown color. The initial crude yield was 458 g., 57%, of (-)2-aminobutan-1-ol (+)hydrogen tartrate monohydrate, m.p. 101½°. Succeeding crops of the long silky crystals were collected by further cooling the mother liquor and later by concentrating the mother liquor and adding absolute ethanol. All crops were recrystallized by

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* All melting points are uncorrected

(1) R. B. Fearing, Organic Bases and Other Agents for Amino Acid Resolution. Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library. 1951. p. 37.

dissolving them in a minimum of hot water, followed by the addition of absolute ethanol. The addition of ethanol formed a precipitate of long needles, whereas water alone formed a hard salt cake which was difficult to remove from the crystallizing dish. Some of the crude product (464 g.) was dissolved in 220 ml. of hot water and 250 ml. of 95% ethanol were added. After overnight standing, the recrystallization yielded 373 g., 80% of theory, m.p. $102\frac{1}{2}$-103°.

$[\alpha]_D^{23} = +10.5^\circ \pm 0.02^\circ$ (Water, C,5, l=2)

The specific rotation was the same as that reported by Fearing (1). A tabulation of the recrystallized crops is presented in Table 1. A total of 799.1 g. of the 2-aminobutan-1-ol (+)hydrogen tartrate was collected. A specific rotation of +10.5° was found for 489 g., which is a yield of 62% of pure (-)2-aminobutan-1-ol (+)hydrogen tartrate monohydrate. Crops having specific rotations of +10.5°, +10.6°, or +10.7° were found suitable for the preparation of (-)2-aminobutan-1-ol which was used for the resolution of DL-glutamic acid. Thus a yield of 534 g., 68%, was available for the preparation of (-)2-aminobutan-1-ol.

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(1) R. B. Fearing, Organic Bases and Other Agents for Amino Acid Resolution. Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library. 1951. p. 37.

Table 1

Crops of (-)2-Aminobutan-1-ol (+)Hydrogen Tartrate

Crop	Yield (g.)	M.P. (°C.)	$[\alpha]_D$ *
1	373	102½-103	10.5°
2	96.5	102½-103	10.5
3	28.0	103-104	10.6
4	65.0	130-135	23.0
5	59.0	143-144	23.3
6	5.4	98-99	14.0
7	34.0	98-100	18.4
8	11.0	88-99	10.5
9	7.0	98-100	19.6
10	4.0	98-99	10.6
11	13.0	145-146	23.8
12	8.2	102-103	10.5
13	12.0	102-103	10.7
14	13.0	91-92	14.3
15	31.0	97-98	17.3
16	39.0	115-130	22.4

* Water, C,5, *l*=2. Temperature 23-24°.
All specific rotations were positive.

2. (-)2-Aminobutan-1-ol

(-)2-Aminobutan-1-ol (+)hydrogen tartrate monohydrate (340 g., 1.34 moles) was dissolved in 850 ml. of 70% methanol, and calcium hydroxide (132 g., 2.36 moles) was added in portions until the pH became 10.5. After 4 hours of stirring, the suspension was filtered, and the calcium tartrate was washed with 500 ml. of H_2O. The combined filtrate and mother liquor were fractionated with a 50 cm. column. The fraction boiling between 42° and 0.4 mm. and 55° and 0.2 mm. was collected. The yield was 106.7 g.

$$[\alpha]_D^{22} = -9.14^\circ \pm 0.03^\circ \quad (\underline{l}=2)$$

The density, 0.9443, indicated that the liquid was 92% 2-aminobutan-1-ol and 8% water. This calculation was based on the assumption of a linear relationship between the density and the concentration of 2-aminobutan-1-ol and water. Fearing (1) reported a density of 0.9390 and a specific rotation of -9.92° for the pure compound. The yield of (-)2-aminobutan-1-ol was 83% of the theoretical. A concentration of 1.9% of (-)2-aminobutan-1-ol was found in the water fraction of the distillate; therefore, the 92% solution was not purified further to prevent additional loss of the product.

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(1) R. B. Fearing, Organic Bases and Other Agents for Amino Acid Resolution. Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library. 1951. p. 39.

This solution was found satisfactory for the resolution of DL-glutamic acid.

B. Resolution of DL-Glutamic Acid with (-)2-Aminobutan-1-ol

1. DL-Glutamic acid

L-Glutamic acid was racemized according to a modification of the procedure of Arnow and Opsahl (1). General Mills L-glutamic acid (500 g., 3.4 moles) was heated at 190-195° for 4½ hours in a liter round bottom flask warmed with a heating mantle. Immediately after the heating was stopped, 770 ml. of 20% HCl (4.2 moles) were added. A condenser was attached and the black solution was refluxed for 4 hours. The hot solution was then decolorized with 12 g. of Norit A and filtered through a steam-heated funnel. After overnight standing the filtrate yielded a large crop of crystals which were washed with 500 ml. of 12N HCl, 500 ml. of absolute ethanol, and 100 ml. of ether. The yield of DL-glutamic acid hydrochloride was 425 g., 68% of theory, m.p. 198°. The mother liquor was brought to a pH of 3.2 with concentrated NaOH solution to recover any DL-glutamic acid which did not precipitate as the hydrochloride. The 425 g. of DL-glutamic acid hydrochloride were dissolved in 600 ml. of hot water, and

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(1) L. E. Arnow and J. C. Opsahl, J. Biol. Chem., 134, 649 (1940).

4.5N NaOH was added until the pH reached 3.2. The crystals were filtered after overnight standing and were washed with 500 ml. each of cold water, ethanol, and ether. The yield was 320 g., 95% of theoretical. Recrystallization from water resulted in 270 g. as a first crop. After all crops of DL-glutamic acid were collected, the yield was 350 g., 70% of the starting material, m.p. 194°.

$$[\alpha]_D^{23} = 0.0^\circ \pm 0.04^\circ \quad (6N\ HCl,\ c, 5,\ l=2)$$

In a second run this procedure was modified to eliminate the isolation of the hydrochloride. After the refluxing with 20% HCl was completed, 20 g. Darco animal charcoal were added. The mixture was filtered through a heated funnel. To the filtrate was added 50% NaOH until the pH reached 3.0. The final volume was 1500 ml. After overnight standing filtration resulted in a crop of brown crystals which were immediately recrystallized from 2000 ml. of water with the addition of 5 g. of Darco. A yield of 330 g., 66% of the starting material, was achieved from this simplified procedure.

$$[\alpha]_D^{23} = 0.0^\circ \pm 0.01^\circ \quad (6N\ HCl,\ c, 5,\ l=2)$$

2. (-)2-Aminobutan-1-ol-D-glutamate and (-)2-aminobutan-1-ol-L-glutamate

Several runs were made by this procedure. The results from two such runs are presented in Table 2. A typical run follows:

DL-Glutamic acid (73.5 g., 0.5 mole) and 92% (-)2-aminobutan-1-ol (48.5 g., 0.5 mole) were stirred vigorously, and 20 ml. of water were added in small portions. The reaction was noticeably exothermic. The syrup was heated with stirring on a 60° water bath until all of the DL-glutamic acid had dissolved except a trace. The syrup 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 syrup through the filter paper. The filter paper gained 0.9 g. during the filtration, indicating that 0.9 g. of DL-glutamic acid did not react. Enough absolute ethanol was added to the filtrate to bring the volume to one liter. Some of the ethanol was used to wash the filter paper and funnel. After twenty hours in the refrigerator, 2.0 g. were collected as a first crop, m.p. 139-142°.

$$[\alpha]_D^{22} = -1.1^\circ \pm 0.04^\circ \quad \text{(Water, C,5, } l\text{=2)}$$

This first crop was 84% optically pure based on values found for (-)2-aminobutan-1-ol-D-glutamate and (-)2-aminobutan-1-ol-L-glutamate. The second crop was collected one day later yielding 40 g., 68% of theory, m.p. 146-147°.

$$[\alpha]_D^{22} = -3.3^\circ \pm 0.04^\circ \quad \text{(Water, C,5, } l\text{=2)}$$

The third crop was very small, while the fourth, fifth, and sixth crops were (-)2-aminobutan-1-ol-L-glutamate. Later crops were optically impure. All crops were washed with absolute ethanol and were dried in a vacuum desiccator.

3. D-Glutamic acid and L-glutamic acid

D-Glutamic acid was prepared from (-)2-aminobutan-1-ol-D-glutamate by the dropwise addition of 12N HCl to a solution made by dissolving the salt in a relatively small volume of water. When a pH of 3.2 was reached, two or more volumes of absolute ethanol were added to make the D-glutamic acid nearly insoluble while the (-)2-aminobutan-1-ol hydrochloride remained in the ethanol solution. A typical run follows:

Twenty-five grams of (-)2-aminobutan-1-ol-D-glutamate were dissolved in 100 ml. of H_2O and 12N HCl was added to bring the pH to 3.16 ± 0.04. A relatively large proportion of the HCl was added in the pH range between 3.8 and 3.6 although the pH of the water solution of (-)2-aminobutan-1-ol-D-glutamate was 7.0. Absolute ethanol (200 ml.) was added to the suspension and it was filtered within ten minutes. The D-glutamic acid was washed with 200 ml. of absolute ethanol, and the washings and the mother liquor were allowed to stand at room temperature for the precipitation of further crops. A total of 15.0 g., 96% of theory, were collected.

$$[\alpha]^{33}_{D} = -31.0^\circ \pm 0.1^\circ \quad (6N\ HCl,\ C,2\ l=2)$$

A 1.0 g. sample of (-)2-aminobutan-1-ol-L-glutamate yielded 0.6 g. of L-glutamic acid, 97% of theory.

$$[\alpha]^{23}_{D} = +31.0^\circ \pm 0.1^\circ \quad (6N\ HCl,\ C,2,\ l=2)$$

Further data concerning the recovery of glutamic acid is reported in a later section. This specific rotation, +31.0°, was the same as that of a known sample in 6N HCl. The literature value was +31.2° (1), but it was taken with a 4 decimeter polarimeter tube. The constants reported were for the diastereomeric salts and the D-glutamic or L-glutamic acid without recrystallization. Recrystallization was not necessary in this procedure because the (-)2-aminobutan-1-ol hydrochloride was very soluble in the alcohol solution. Table 2 presents the results of the two runs. Yields of 68% and 64% of theory were obtained for (-)2-aminobutan-1-ol-D-glutamate, and 26% and 32% of theory for (-)2-aminobutan-1-ol-L-glutamate. Some observations made during these runs follow:

Seeding was tried with both diastereomeric salts on several occasions but did not affect the order of precipitation. For instance, after a crop of (-)2-aminobutan-1-ol-L-glutamate was separated, seeding was attempted with (-)2-aminobutan-1-ol-D-glutamate. However, the next crop was (-)2-aminobutan-1-ol-L-glutamate.

2-Propanol was tried in place of ethanol as a precipitating agent. It caused the simultaneous precipitation of both diastereomers.

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(1) C. L. A. Schmidt, "The Chemistry of Amino Acids and Proteins", Charles C. Thomas, Springfield, Illinois, 1944, p. 1175.

Table 2

Resolution of DL-Glutamic Acid with (-)2-Aminobutan-1-ol

Crop	M.P. °C.	Yield g.	% of Theoretical[a] Yield of Diastereomer	$[\alpha]_D$ of (-)2-[b] Aminobutan-1-ol Glutamate	$[\alpha]_D$ of[c] Glutamic Acid
Run 1					
1	139-142	2.0		-1.1°	
2	146-147	40.1	68	-3.3	-31.0°
3	107-110	0.1			
4	112-113	10.0	17	-10.1	+31.0
5	112-115	7.0	12	-9.7	+31.0
6	115-116	4.0	7	-9.2	+28.6
7	129-132	0.5		-5.4	
8	128-132	27.7		-7.2	+11.7
9	110-115	2.7		-8.6	
Run 2					
1	145-146	38.0	64	-3.3	-30.5
2	100-105	16.0	27	-9.4	+30.2
3		3.0	5	-9.7	+28.1
4		3.0		-5.2	-2.17

a L-Glutamic acid with a specific rotation of +28.6° Was purified to a specific rotation of +31.0° in an 87% yield by one recrystallization, and hence is included in the yield.

b (Water, C,5, l=2). The temperature range was from 22°-24°.

c (6N HCl, C,1.5-4, l=2). The temperature range was from 22°-24°.

The time elapsed before the first crop of crystals was removed did not seem to affect the quality of the first large precipitate. It was necessary to remove this large crop before the next would form.

(-)2-Aminobutan-1-ol-D-glutamate was mildly hygroscopic, whereas (-)2-aminobutan-1-ol-L-glutamate was strongly hygroscopic and was actually deliquescent when the atmosphere was high in humidity.

C. Solubility Experiments with (-)2-Aminobutan-1-ol-D-Glutamate and (-)2-Aminobutan-1-ol-L-Glutamate

1. (-)2-Aminobutan-1-ol-D-glutamate

D-Glutamic acid (3.7 g., 0.025 mole) and 92% (-)2-aminobutan-1-ol (2.40 g., 0.025 mole) formed a syrup upon the addition of 14 ml. of H_2O. Heating to 60° in a water bath resulted in a nearly complete solution. Filtration through a heated funnel removed 25 mg. of unreacted D-glutamic acid, m.p. 205-206°. Heating at 60° on a water bath removed all of the excess water feasible; then absolute ethanol was added to the syrup. A white precipitate formed immediately. The yield was 5.0 g., 83% of theory, m.p. 147-148°.

$$[\alpha]_D^{25} = -3.3^\circ \pm 0.0^\circ \quad (\text{Water, C,5, } l=2)$$

Fearing (1) reported the specific rotation as -3.2°, and the melting point as 146-147°.

A small scale reaction was carried out to clarify the role of water in the salt formation. A 0.025 molar run by the previously mentioned procedure resulted in a 68% yield, m.p. 147-149°. The reaction took place with only the 0.002 g. of H_2O present in the 2-aminobutan-1-ol. This plus the fact that heat is given off from stirring of 2-aminobutan-1-ol with glutamic acid indicated that water was not necessary for salt formation, but the reaction required water for completion. The salt formation for the resolution of DL-glutamic acid left a small amount of the DL-glutamic acid on the filter paper, and this residue indicated that an excess of (-) 2-aminobutan-1-ol was not present.

2. (-)2-Aminobutan-1-ol-L-glutamate

L-Glutamic acid (14.7 g., 0.1 mole) and 92% (-)2-aminobutan-1-ol (9.7 g., 0.1 mole) were stirred with 22 ml. of water in a 60° water bath until solution was nearly complete. Upon filtration 0.025 g. of L-glutamic acid, m.p. 198-200°. were removed from the syrup. After the evaporation of some of the water, 240 ml. of absolute ethanol were added, and

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(1) R. B. Fearing, Organic Bases and Other Agents for Amino Acid Resolution. Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library. 1951. p. 85.

the solution was placed in the refrigerator. No immediate precipitate was noticeable at this point as it was for (-)2-aminobutan-1-ol-D-glutamate. A milky-white oil which formed did not crystallize so the ethanol-water solvent was distilled in vacuo as much as feasible, and 2-propanol was added to the syrup. The white oil, which formed immediately, changed to a sticky solid. After two days a very difficult filtration yielded 20 g., 85% of theory, of (-)2-aminobutan-1-ol-L-glutamate, m.p. 115-117°.

Anal. Calc'd. for $C_9H_{20}O_5N_2$: N, 11.85

Found: N, 11.82 (micro Kjeldahl)

$[\alpha]^{22}_{D} = -9.7^\circ \pm 0.02^\circ$ (Water, C,5, l=2)

Fearing (1) reported that (-)2-aminobutan-1-ol-L-glutamate formed an oil which did not crystallize. While this salt crystallized only with difficulty, it did form a clearly defined solid compound. However, there was a great difference in the ease of precipitation between the diastereomers, (-)2-aminobutan-1-ol-D-glutamate and (-)2-aminobutan-1-ol-L-glutamate. Emmick (2) reported a monohydrate for L-lysine-L-glutamate. Such a possibility is ruled out for (-)2-aminobutan-1-ol-L-glutamate because of the nitrogen analysis and

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(1) R. B. Fearing, Organic Bases and Other Agents for Amino Acid Resolution. Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library. 1951. p.84.

(2) R. D. Emmick, United States Patent 2,556,907 (1951). Abstracted in C.A., 46, 525 (1952).

the nearly quantitative yields of L-glutamic acid separated from the salt. The sample for elemental analysis was dried over P_2O_5 at the temperature of refluxing chloroform at 0.35 mm. The sample for optical analysis was dried over $CaCl_2$ at room temperature at 20 mm.

3. The approximate solubility of the diastereomers separately and together

An indication of the relative solubility of the diastereomers was achieved by adding the solid to a solution of 83% ethanol by volume. Two ml. of absolute ethanol and 0.40 ml. of water were pipetted into each of two 100 x 13 mm. test tubes equipped with rubber stoppers. The tubes were immersed in a mineral oil bath to the level of the ethanol solution. The solid was added in small portions with stirring until a small visible quantity remained undissolved. Then the bath was brought up to 70° and allowed to cool. When no more solid would dissolve, the loss in weight of the sample bottle was used to determine the approximate solubility. The volume of solvent in the tube did not visibly change. For (-)2-aminobutan-1-ol-D-glutamate, 29.4 mg. dissolved in 2.4 ml. of the 83% ethanol by volume. For (-)2-aminobutan-1-ol-L-glutamate, 25.5 mg. dissolved in 2.4 ml. of the ethanol solution. These solubilities, 1.2 g. per 100 ml. and 1.1 g. per 100 ml., respectively, indicated that there was no significant difference in the solubilities of the diastereomers at this concentration.

An indication of the solubility behavior of one diastereomer in the presence of the other was obtained by starting with a weighed amount of one diastereomer and adding the other until solution took place. Then enough of the first diastereomer was added until a small quantity of the solid phase was present. In one typical experiment approximately 0.30 g. of (-)2-aminobutan-1-ol-L-glutamate were suspended in 2.4 ml. of 83% ethanol by volume, and 0.3864 g. of (-)2-aminobutan-1-ol-D-glutamate were added to effect solution. Then a small quantity of the former salt was added. The temperature was raised from 25 to 75° after each addition. The weight of the (-)2-aminobutan-1-ol-L-glutamate was 0.3108 g. Thus 16 g. of (-) 2-aminobutan-1-ol-D-glutamate were required to bring 13 g. of (-)2-aminobutan-1-ol-L-glutamate into solution in 100 ml. of 83% ethanol by volume.

The reverse procedure of solubilizing (-)2-aminobutan-1-ol-L-glutamate with (-)2-aminobutan-1-ol-D-glutamate was also carried out. A relatively large quantity of the D-glutamate was required in order to have some of the solid phase present. In this case 0.3897 g. of the L-glutamate were required to bring 0.7630 g. of the D-glutamate into solution. 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 a change in temperature. The differential appeared to be greater in the case of the L-glutamate solubilizing the D-glutamate. On the basis of 100 ml. of 83% ethanol solution by volume, 14 g. of the L-glutamate were brought into solution by 31 g. of the D-glutamate.

4. Variation of solubility with ethanol concentration

The solubilities of the diastereomers were studied over a range of ethanol concentrations in an effort to determine the effect of the ratio of ethanol to water on solubility. (-)2-Aminobutan-1-ol-D-glutamate (0.7000 g.) and (-)2-aminobutan-1-ol-L-glutamate (0.7000 g.) were each placed in a 20 ml. rubber-stoppered test tube with 10 ml. of 97% ethanol by volume. The suspension was stirred vigorously and then was heated from 20° to 40° in a mineral oil bath. After the solid had settled, 1.00 ml. of the solution was pipetted into a tared 10 ml. Erlenmeyer flask. Then 0.50 ml. of water was added to the test tube, forming 9.5 ml. of a 91% ethanol solution by volume. After stirring, warming, and cooling, 1.0 ml. was removed for a sample and 0.5 ml. of distilled water were added to the test tube, forming 9.0 ml. of an

86.5% ethanol solution. A continuation of this procedure for lower concentrations yielded the samples for Table 3. All of the samples were taken at 24°. The samples were weighed and dried to constant weight at a temperature of 70-80°. Ethanol was added after each weighing to facilitate drying. From the weight of the dry residue, the weight percentage of (-)2-aminobutan-1-ol-D-glutamate or (-)2-aminobutan-1-ol-L-glutamate was calculated. Melting point determinations proved that decomposition had not taken place. Table 3 presents the results of this experiment.

D. Resolution of 2-Aminobutan-1-ol with L-Glutamic Acid

1. (+)2-Aminobutan-1-ol-L-glutamate

General Mills L-glutamic acid (294 g., 2.00 moles) and Commercial Solvents vacuum distilled racemic 2-aminobutan-1-ol (178 g., 2.00 moles) were dissolved with 180 ml. of water in a 70° water bath. The syrupy solution was filtered through a heated funnel to remove a small amount of undissolved L-glutamic acid. Enough absolute ethanol was added to bring the volume to two liters, and the liquid was cooled in the refrigerator. Seeding with (-)2-aminobutan-1-ol-L-glutamate crystallized the first crop of (+)2-aminobutan-1-ol-L-glutamate. The crystals were washed with absolute ethanol. The yield was 150 g., 64% of theory, m.p. 146-147°.

Table 3

Solubilities of (-)2-Aminobutan-1-ol-D-Glutamate and (-)2-Aminobutan-1-ol-L-Glutamate in Different Concentrations of Ethanol

% Ethanol by Volume	% Dry (-)2-Aminobutan-1-ol-D-Glutamate by Weight	% Dry (-)2-Aminobutan-1-ol-L-Glutamate by Weight
97	0.15	0.41
91	3.0	2.2
87	6.3	3.7
81	7.5	6.0
76	7.3	5.8
71	6.8	4.9
67	6.4	5.1
61	6.0	4.5
56		4.4
51		4.4

Anal. Calc'd. for $C_9H_{20}O_5N_2$: N, 11.85

Found: N, 11.89 (micro Kjeldahl)

$[\alpha]_D^{22} = +3.2° \pm 0.04°$ (Water, C,5, $\underline{l}$=2)

These values are in close agreement with those found for the enantiomorph, (-)2-aminobutan-1-ol-D-glutamate, which had a rotation of -3.3° and an m.p. of 146-147°. A mixed melting point with (-)2-aminobutan-1-ol-D-glutamate was depressed.

Further crops were collected by allowing the mother liquor and the ethanol washings from the previous crop to cool in the refrigerator. After no more precipitation occurred the mother liquor was concentrated *in vacuo* with a water pump and a 60° water bath. Absolute ethanol was added and a further crop was collected. This procedure was repeated until the last two crops were precipitated from a solvent of Skelly A, Skelly B, and absolute ether. The remainder of the salt, 15%, did not crystallize from any solvent tried. The final yield of (+)2-aminobutan-1-ol-L-glutamate was 150 g., 64% of theory, and that of (-)2-aminobutan-1-ol-L-glutamate of 96% or more optical purity was 63% of theory. Table 4 lists the crops and their rotations.

L-Glutamic acid (147 g., 1.00 mole) and 2-aminobutan-1-ol (89.0 g., 1.00 mole) were mixed with 80 ml. of water and the volume was diluted to two liters with absolute ethanol. A

Table 4

Resolution of Racemic 2-Aminobutan-1-ol with L-Glutamic Acid

Crop	Yield (g.)	% of Theoretical Yield of Diastereomer	$[\alpha]_D$ *
1	150.	64	+3.2°
2	3.6		-3.8
3	6.1		+2.5
4	55.0		-0.1
5	25.0	11	-9.2
6	20.0	9	-9.2
7	17.2		-4.4
8	75.0	32	-9.7
9	3.3		+2.8
10	2.3		+0.7
11	4.6		0.0
12	8.9	4	-9.6
13	16.5	7	-9.6
14	16.0		-8.7

* (Water, C,5, l=2)
Temperature range, 22°-25°

yield of 116.1 g. of (+)2-aminobutan-1-ol-L-glutamate of 85% optical purity was recovered. Recrystallization from 60 ml. of water and 1940 ml. of absolute ethanol resulted in 101.2 g. of a 96% optical purity. Recrystallization from 100 ml. of water and 1900 ml. of absolute ethanol yielded 95.0 g., 81%, of optically pure (+)2-aminobutan-1-ol-L-glutamate. In another molar run 150 ml. of water and 1850 ml. of absolute ethanol gave 83.0 g., 70% of theory, of optically pure L-glutamate.

2. (+)2-Aminobutan-1-ol

(+)2-Aminobutan-1-ol-L-glutamate (50g., 0.21 mole) was dissolved in 150 ml. of water and the solution was brought to a pH of 3.2, the isoelectric point of glutamic acid, by the dropwise addition of 12N HCl. To aid in the completeness of the precipitation of L-glutamic acid, 200 ml. of absolute ethanol were added. A total of 30.1 g. of L-glutamic acid was collected, which is 97% of theory. The water-ethanol solution of (+)2-aminobutan-1-ol was concentrated by evaporation *in vacuo*. Calcium hydroxide was used in conjunction with ammonium hydroxide to raise the pH to 10.6. Three volumes of absolute ethanol were added and a small amount of solid was separated by filtration. After evaporation *in vacuo* the final yield was 11.4 g., 61% of the theoretical.

The specific rotation compared favorably to +9.8° reported in the literature (1).

$$[\alpha]_D^{25} = +9.7^\circ \pm 0.02^\circ \quad (\underline{l}=2)$$

In addition to the above method for decomposing the (+)2-aminobutan-1-ol hydrochloride, two others were successfully used to give good yields of (+)2-aminobutan-1-ol. In one the glutamic acid was removed at its isoelectric point in an ethanol suspension and the cold mother liquor, which contained the (+)2-aminobutan-1-ol hydrochloride, was brought to a pH of 10.7 with cold alcoholic NaOH. Evaporation and filtration yielded an alcoholic solution of (+)2-aminobutan-1-ol. In the second method, cold alcoholic NaOH was added to the (+)2-aminobutan-1-ol-L-glutamate suspended in ethanol until the pH became 10.8. The insoluble sodium glutamate was removed by filtration, and the (+)2-aminobutan-1-ol was recovered from the alcohol.

E. Reaction of 2-Aminobutan-1-ol with D-Glutamic Acid

This experiment was carried out to check the results of the reaction of 2-aminobutan-1-ol with L-glutamic acid and to prepare the fourth diastereomer, (+)2-aminobutan-1-ol-D-glutamate.

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(1) A. Stoll, J. Peyer, and A. Hofman, Helv. Chim. Acta, 26, 929 (1943).

D-Glutamic acid (14.7 g., 0.10 mole) and Commercial Solvents 2-aminobutan-1-ol (8.9 g., 0.10 mole) were dissolved with 15 ml. of water on a 70° water bath. The syrup was filtered with the aid of 15 ml. of H_2O, and 220 ml. of absolute ethanol were added to the filtrate. A flocculent white precipitate formed immediately. This white precipitate was 0.6 g. of D-glutamic acid, which was removed by filtration. The second crop of crystals was 11.6 g. of 88% optically pure (-)2-aminobutan-1-ol-D-glutamate. Recrystallization from 30 ml. of water and 470 ml. of absolute ethanol yielded 9.2 g., 78% of theory, m.p. 146-147°.

$$[\alpha]_D^{25} = -3.2^\circ \pm 0.02^\circ \quad (\text{Water, C, 5, } \underline{l}=2)$$

The mother liquor from the first crop was cooled with dry ice, and 1.0 gram of 69% optically pure (-)2-aminobutan-1-ol-D-glutamate precipitated. Three evaporations and the addition of absolute ethanol failed to cause further precipitation. Finally 2-propanol was added to the syrup, and a gum formed immediately. The 2-propanol was decanted from the gum, and the gum was dried over $CaCl_2$ in a vacuum desiccator at 0.5 mm. yielding 2.8 g., 24% of theory, of the crude material. The deliquescent white compound was then dried for 90 minutes over P_2O_5 at a pressure of 0.1 mm. and the temperature of refluxing chloroform, m.p. 98-104°.

$[\alpha]_D^{29} = +9.5^\circ \pm 1.0^\circ$ (Water, C, 3.03, l=2)

These results checked with those found with the resolution of 2-aminobutan-1-ol-L-glutamate. The (+)2-aminobutan-1-ol-D-glutamate was more difficult to isolate than was (-)2-aminobutan-1-ol-L-glutamate, but this could probably be alleviated by a larger scale preparation.

F. Attempted Resolution of DL-Aspartic Acid with (-)2-Aminobutan-1-ol

An attempt was made to apply the method successfully used with glutamic acid to aspartic acid, the other dicarboxylic amino acid.

National Aniline DL-aspartic acid (6.65 g., 0.05 mole) and 96% (-)2-aminobutan-1-ol (4.46 g., 0.05 mole) were stirred on a 60° water bath with 2 ml. of H_2O until a syrup formed. This reaction appeared to be more exothermic than the reaction of DL-glutamic acid with (-)2-aminobutan-1-ol. The addition of 100 ml. of absolute ethanol and storage in the refrigerator failed to produce crystallization so the solution was concentrated *in vacuo* at room temperature. When the volume was reduced by half, 2.4 g. of (-)2-aminobutan-1-ol aspartate were collected on a Büchner funnel, m.p. 128-132°. Two grams of the aspartate were dissolved in 5 ml. of water and enough 12N HCl was added to bring the pH to 3.0, the isoelectric point for aspartic acid. Enough ethanol was added to bring the volume to 40 ml. The yield of aspartic acid was 1.2 g., the theoretical amount.

$$[\alpha]_D^{22} = +10.0^\circ \pm 0.4^\circ \quad (6N\ HCl,\ c, 2.69,\ l=2)$$

The value reported in the literature for L-aspartic acid in 6N HCl is +24.6°(1); thus this preparation was of 70% optical purity. The rotation is of the opposite direction from that of glutamic acid prepared under the same conditions.

Several other attempts to resolve the DL-aspartic acid did not meet with success. However, the above results indicate that the separation might be accomplished if the proper solvent were found.

G. Attempted Resolution of DL-Phenylalanine with (-)2-Aminobutan-1-ol

DL-Phenylalanine did not form a salt with (-)2-aminobutan-1-ol. It was recovered unchanged from the reaction mixture.

H. Attempted Resolution of DL-Leucine with (-)2-Aminobutan-1-ol

DL-Leucine also does not possess enough acid strength to form a salt with (-)2-aminobutan-1-ol. This difference

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(1) C. L. A. Schmidt, "The Chemistry of Amino Acids and Proteins", Charles C. Thomas, Springfield, Illinois, 1944, p. 1175.

in salt forming ability of leucine and glutamic acid was illustrated by the following experiment:

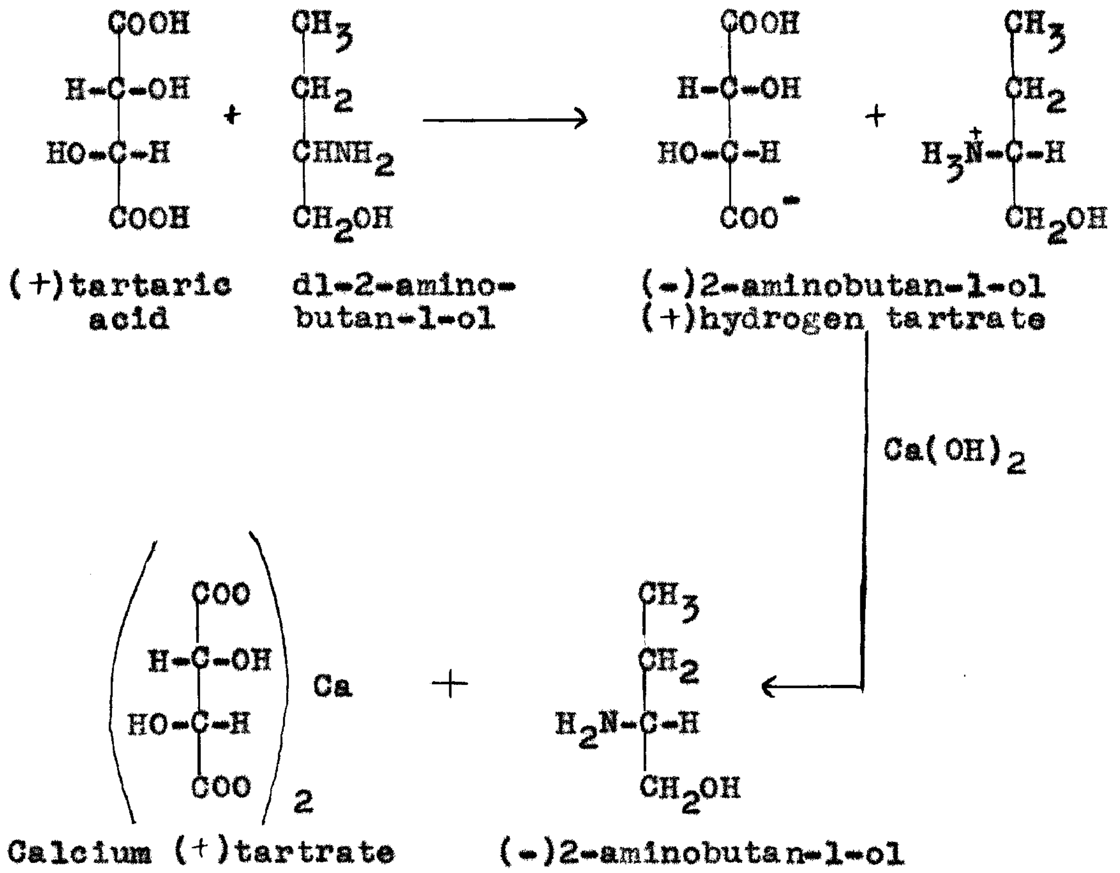
General Mills L-glutamic acid (14.7 g., 0.100 mole), Dow DL-leucine (13.1 g., 0.100 mole), and Commercial Solvents 2-aminobutan-1-ol (9.4 g., 0.105 mole) were warmed with 50 ml. of water on a 60° water bath for 30 minutes. The suspension was filtered and 12.9 g., 98.5% of the starting material, of DL-leucine was recovered. The leucine showed no optical rotation indicating that no contaminating L-glutamic acid was present. The filtrate was brought to a pH of 3.2 with 12N HCl and 300 ml. of absolute ethanol were added. A yield of 12.6 g. of L-glutamic acid, 86% of the starting material was recovered.

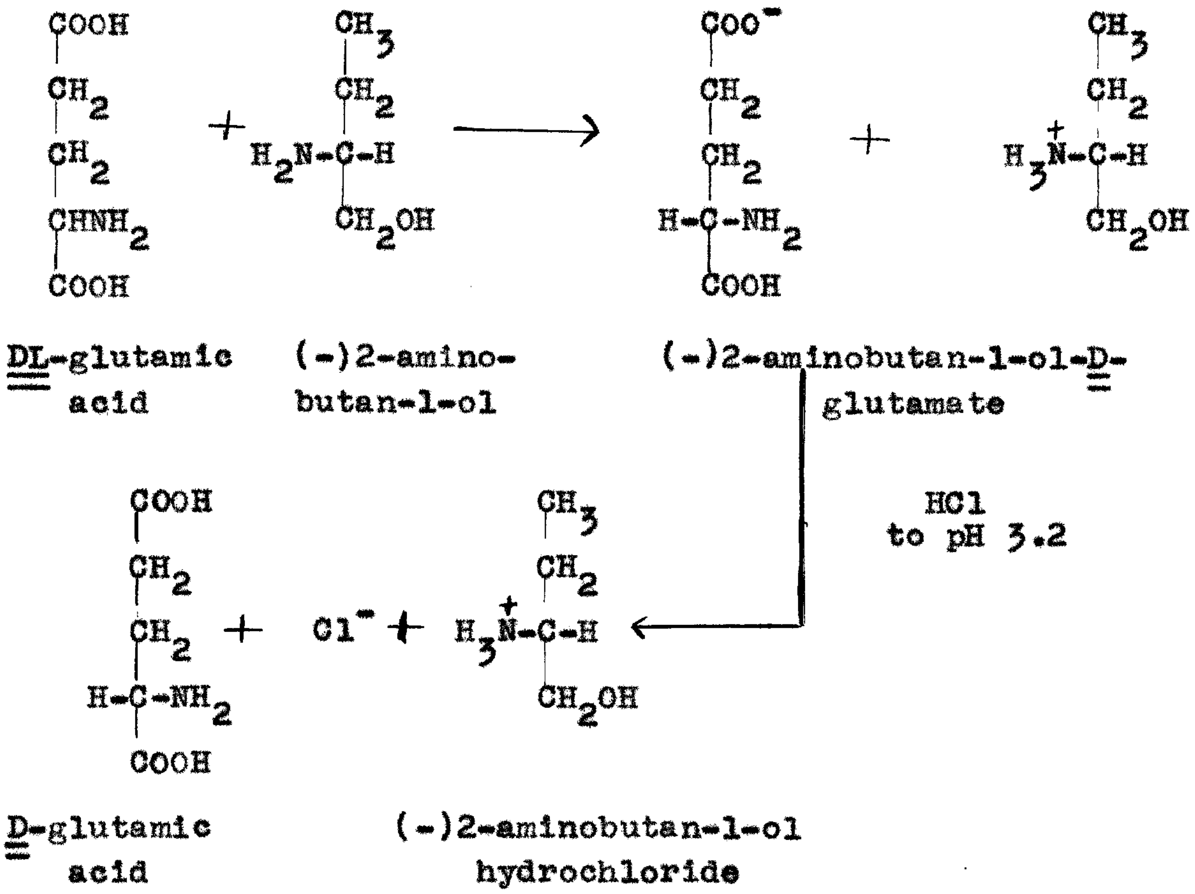
$$[\alpha]_D^{25} = +31.0^\circ \pm 0.1^\circ \quad (6N\ HCl,\ C,4,\ l=2)$$

No leucine contaminated the L-glutamic acid recovered from this reaction. Such a purification procedure could be used to purify glutamic or aspartic acid from less acid contaminants.

IV. DISCUSSION AND CONCLUSIONS

The resolution of DL-glutamic acid with (-)2-aminobutan-1-ol has been proved to be reproducible and to give a good yield of D-glutamic acid. The following equations present the essential steps in the preparation of D-glutamic acid, beginning with the resolution of 2-aminobutan-1-ol and following with the resolution of DL-glutamic acid with (-)2-aminobutan-1-ol.





This resolution is very economical in comparison with other methods for the resolution of DL-glutamic acid since (+)tartaric acid, racemic 2-aminobutan-1-ol and calcium hydroxide are all available commercially and since few steps are involved. The two resolving agents, (+)tartaric acid and (-)2-aminobutan-1-ol, can be recovered for recycling. D-glutamic acid can be prepared within three days, allowing overnight cooling in the refrigerator for the crystallizations. If (-)2-aminobutan-1-ol were kept on the reagent shelf, the preparation of D-glutamic acid could be carried out within 24 hours to give a good yield in comparison with other methods. These statements would also apply to L-glutamic acid if it were to be resolved with (+)2-aminobutan-1-ol.

At no time during this resolution were any of the optically active compounds exposed to any reagent which would be likely to cause racemization. Calcium hydroxide is not soluble enough to give a high hydroxide ion concentration, which could cause a serious problem in this respect.

In other resolution procedures recrystallization is necessary for an optically pure compound, but in this procedure the first large crop of the diastereomer is optically pure, and the glutamic acid precipitated by adjusting the pH to the isoelectric point is optically pure.

Two of the difficulties encountered during the resolution were caused by the sensitivity of the diastereomeric salts to heat and water vapor. The long exposure to heat above 80° resulted in the decomposition of some of the (-)2-aminobutan-1-ol-D-glutamate or (-)2-aminobutan-1-ol-L-glutamate, which was a handicap in the drying of the salts. (-)2-Aminobutan-1-ol-D-glutamate is hygroscopic, and (-)2-aminobutan-1-ol-L-glutamate is deliquescent.

The salt formation of glutamic acid with 2-aminobutan-1-ol offers the possibility of separating glutamic acid from impurities which will not form a salt with 2-aminobutan-1-ol nor dissolve in the small volume of water used as a solvent. Hence, the following filtration would remove the impurities. Those contaminants which would dissolve in the syrup probably would not precipitate at the isoelectric point of glutamic acid.

Under present practices this resolution of glutamic acid would be advantageous in the preparation of D-glutamic acid for experimental use, or for the preparation of L-glutamic acid free from contamination of other amino acids for exacting work such as microbiological assay, where even very small quantities of some contaminants, such as occur in the commercial product from natural sources, are very undesirable. Resolved synthetic DL-glutamic acid would avoid such contamination.

A new resolution of 2-aminobutan-1-ol was presented. L-glutamic acid formed the salt, (+)2-aminobutan-1-ol-L-glutamate, which crystallized as a large crop, and from which L-glutamic acid can be recovered almost quantitatively. While the over-all yield of (+)2-aminobutan-1-ol is not high, the unprecipitated salt can be decomposed for the recovery of the starting materials. If a commercial synthesis and resolution were developed for L-glutamic acid, some of the resultant L-glutamic acid could be used to resolve 2-aminobutan-1-ol to replace recyclizing losses of the latter, while little or no L-glutamic acid is lost.

D-Glutamic acid and 2-aminobutan-1-ol led to the same type of differential precipitation of diastereomers as did L-glutamic acid with 2-aminobutan-1-ol. In these two reactions all four of the optical isomers of glutamic acid and 2-aminobutan-1-ol were encountered. The two antipodes of greatest interest are (-)2-aminobutan-1-ol-D-glutamate and (+)2-aminobutan-1-ol-L-glutamate, since they are the ones which made up the initial large crop of crystals when (-)2-aminobutan-1-ol and L-glutamic acid were used as resolving agents. When (-)2-aminobutan-1-ol was used as a resolving agent for DL-glutamic acid, the (-)2-aminobutan-1-ol-D-glutamate formed the large crop. If (+)2-aminobutan-1-ol resolved DL-glutamic acid, (+)2-aminobutan-1-ol-L-glutamate would make up the large crop.

The solubility experiments with (-)2-aminobutan-1-ol-D-glutamate and (-)2-aminobutan-1-ol-L-glutamate did not provide a good explanation for the initial large precipitation of (-)2-aminobutan-1-ol-D-glutamate in the resolution of glutamic acid with (-)2-aminobutan-1-ol. The solubility tests with the individual diastereomers indicated no significant difference in the quantity dissolved in aqueous ethanol. The diastereomers together in a solution indicated that (-)2-aminobutan-1-ol-L-glutamate should have crystallized first, since the L-glutamate had a greater solubilizing effect on the D-glutamate than that of the D-glutamate on the L-glutamate. Since 2-aminobutan-1-ol has two polar groups and glutamic acid has three polar groups, the relationship of the configuration to the interionic bonding probably is the governing factor in the order of precipitation.

Heating (-)2-aminobutan-1-ol over P_2O_5 in a drying pistol did not cause a significant loss in weight, indicating that a hydrate which would lose water to P_2O_5 did not form. The nitrogen analysis also proved that a hydrate which would not lose water to P_2O_5 did not form. While (-)2-aminobutan-1-ol-D-glutamate crystallized readily when formed from its optically active components, (-)2-aminobutan-1-ol-L-glutamate crystallized only with great difficulty. The L-glutamate was much more hygroscopic than the D-glutamate. (+)2-Aminobutan-1-ol-D-glutamate, the enantiomorph of (-)2-aminobutan-1-ol-L-glutamate, was more hygroscopic than (-)2-aminobutan-1-ol-D-glutamate. These facts point to a difference in the affinity for water. It is conceivable that the (-)2-aminobutan-1-ol-L-glutamate binds a greater share of the water than (-)2-aminobutan-1-ol-D-glutamate, hence forcing the D-glutamate out of solution.

These solubility experiments emphasize the necessity of not placing reliance upon the solubility behavior of the diastereomers alone in solvents. The resolution of glutamic acid with (-)2-aminobutan-1-ol would not have been discovered on this basis, since the solubilities of the diastereomers were approximately the same in the ethanol solutions.

The application of the resolution with 2-aminobutan-1-ol to other amino acids appears to be limited by the acid strength of these acids. Even the necessity for a blocking group should not exclude 2-aminobutan-1-ol as a resolving agent because of its abundant supply and its simple resolution.

Aspartic acid, which formed a salt with 2-aminobutan-1-ol, did not follow the crystallization behavior of the glutamic acid salt. An optically impure L-aspartic acid was obtained under the conditions for which D-glutamic acid was obtained. The fact that DL-glutamic acid is a racemic mixture and DL-aspartic acid is a racemic compound may have significance in this connection (1).

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(1) H. B. Dalton and C. L. A. Schmidt, J. Biol. Chem., 103, 549 (1933).

V. SUMMARY

1. The literature concerning the occurrence of D-amino acids in nature, the resolution of underivatized amino acids, the resolution of glutamic acid, and the resolution of 2-aminobutan-1-ol has been reviewed.

2. The resolution of 2-aminobutan-1-ol with (+)tartaric acid was studied.

3. The resolution of DL-glutamic acid with (-)2-aminobutan-1-ol has been shown to be simple and reproducible.

4. Solubility experiments showed that the diastereomers (-)2-aminobutan-1-ol-D-glutamate and (-)2-aminobutan-1-ol-L-glutamate were about equally soluble in aqueous ethanol individually. The two diastereomers together were much more soluble than would be expected from their individual solubilities. The solubilizing effect of (-)2-aminobutan-1-ol-L-glutamate on the (-)2-aminobutan-1-ol-D-glutamate was greater than the solubilizing effect of (-)2-aminobutan-1-ol-D-glutamate on the (-)2-aminobutan-1-ol-L-glutamate.

5. The resolution of 2-aminobutan-1-ol with L-glutamic acid offered a convenient preparation for (+)2-aminobutan-1-ol, which in turn could be used to resolve DL-glutamic acid

to give a good yield of L-glutamic acid.

6. The resolution of 2-aminobutan-1-ol with D-glutamic acid followed the pattern of the resolution with L-glutamic acid.

7. The four optical isomers of 2-aminobutan-1-ol-glutamate were isolated during these resolutions; two of the isomers were characterized.

VI. ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. Sidney W. Fox for his encouragement and guidance during the course of this investigation.