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RESOLUTION OF LYSINE

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This invention relates to the resolution of amino acids and more particularly to a novel process for the resolution of lysine and a new compound thereby obtained.

Naturally occurring lysine is optically active, its aqueous solutions rotating the plane of polarized light to the right and this form is commonly designated as l(+)-lysine. In this form, lysine is an essential component of animal diets whereas the enantiomorphous d(-)-lysine has no known nutritional value.

Synthetic lysine as produced, for example, by the process of Eck and Marvel, Organic Syntheses Collective, vol. II, page 374, or by the process described in application Serial No. 62,246, filed November 26, 1948, now Patent No. 2,498,300, is optically inactive and consists of equal parts of the biologically active l(+)-isomer and the biologically inactive d(-)-isomer. Thus the synthetic product can be doubled in nutritional value by converting its d(-)-lysine content into the l(+)-form while retaining the quantity of the latter initially present. This can be accomplished by separating the isomers (resolution) followed by racemization of the d(-)-lysine fraction to partially convert d(-)-lysine into l(+)-lysine; and repeating this procedure until the lysine is converted entirely to the desired l(+)-form.

The most commonly used method of resolving dl-mixtures involves combination with an optically active compound known as the resolving agent, followed by fractional crystallization of the resulting mixture of compounds (diastereoisomers.) For a practical resolution, it is necessary to find a combination of resolving agent and solvent which will give good crystallization behavior, together with a pronounced difference in solubility between the diastereoisomers. Berg (J. Biol. Chem. 115, 9-15 (1936)) obtained l(+)-lysine by combining dl-lysine with d-camphoric acid and crystallizing the product from 50% aqueous methanol. This method has been found to yield at best only about 2/3 of the available l(+)-lysine and to be subject to rather wide variations in yield and purity of product. In addition, d-camphoric acid is relatively expensive and is of uncertain availability in this country, since it is prepared from imported natural (optically active) camphor. Camphoric acid prepared from synthetic camphor is the optically inactive dl-camphoric acid, which cannot be used as a resolving agent.

An object of this invention is to provide a new and improved process for the resolution of lysine. Another object is to provide a new resolving agent

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for lysine which is economical to use, is readily available and produces high yields of the desired isomer of lysine. A further object is to provide a new and useful process by which a mixture of l(+)-lysine and d(-)-lysine may be converted to l(+)-lysine. Still another object of the invention is to provide a new and useful compound having valuable properties as a food supplement. These and other objects will be apparent from the ensuing description of the invention.

The above objects are attained in accordance with this invention which comprises reacting an optically active glutamic acid or its ammonium salt with a mixture of l(+)-lysine and d(-)-lysine and fractionally crystallizing the product from a methanol-water mixture.

The reaction between the lysine and the glutamic acid is readily carried out by merely mixing the two in solution. As reaction solvent, I prefer to use water or the solvent in which the fractional crystallization is to be conducted. Preferably, I mix aqueous solutions of dl-lysine and l(+)-glutamic acid or its ammonium salt, add methanol to the mixture and permit crystallization to occur, seeding if necessary. The glutamic acid salt of l(+)-lysine crystallizes, leaving d(-)-lysine glutamate in the mother liquor. When the ammonium glutamate is used, I prefer to heat to remove ammonia before adding the methanol.

The invention is more specifically illustrated by the following examples:

Example 1

A water solution (50 cc.) containing 0.124 mole of free dl-lysine was mixed with l(+)-glutamic acid (18.23 g., 0.124 mole) and diluted with water to a total weight of 118 g. The mixture was warmed to dissolve the glutamic acid, then cooled to room temperature and mixed with 272 cc. of methanol. The solution was seeded with l(+)-lysine l(+)-glutamate monohydrate and stirred forty hours at room temperature. The solid which precipitated during the stirring was filtered off, washed with 100 cc. of methanol and dried. Yield 16.67 g. (86% of theoretical l(+)-lysine l(+)-glutamate monohydrate). The specific rotation of the product in water at 12% concentration and room temperature was +3.6°. Kjeldahl analysis of material prepared in the above manner showed 13.6% N; calc. for lysine glutamate monohydrate: 13.5% N.

The product remaining after the removal of analytical samples (12.96 g.) was heated in an oven at 80-85° C. for 39 hours. The loss in weight

(0.75 g.) was equivalent to one mole of water per mole of lysine glutamate. The dehydrated product was dissolved in water to make 468 g. of solution and was passed through a 200 cc. bed of "Dowex 50" cation-exchange resin in ammonium form, which was then washed with water. Ammonium glutamate was recovered from the effluent. The resin bed was extracted with 10% aqueous ammonia and the effluent freed of ammonia by evaporation. The resulting lysine solution was shown by titration to be equivalent to the lysine glutamate fed to the resin bed. It was treated with the calculated quantity of HCl for conversion to lysine monohydrochloride and the latter recovered by partial evaporation, followed by the addition of ethyl alcohol to precipitate the product. The yield of l(+)-lysine monohydrochloride was 95%; its specific rotation (+9.0°) indicated 95% optical purity and its chloride ion content (19.1%) corresponded to 98.5% chemical purity.

Example 2

An aqueous solution of ammonium l(+)-glutamate (1299 g. of solution, 0.103 mole ammonium glutamate) was mixed with an aqueous solution of free dl-lysine (44.9 g. solution, 0.103 mole lysine). The solution was clarified by adding charcoal and filtering and was concentrated on a steam bath under reduced pressure to a total weight of 98 g. (30.2 g. lysine glutamate, 67.8 g. water). Methanol (226 cc.) was added, the mixture seeded with l(+)-lysine l(+)-glutamate monohydrate and stirred for 46 hours. The precipitate was filtered off, washed with three 50 cc. portions of methanol and air-dried. The yield was 13.89 g. (87% of the theoretical l(+)-lysine l(+)-glutamate monohydrate). The product had a specific rotation of +3.7°, corresponding to 97% optical purity.

Example 3

The following table shows the effects on yield and optical purity of l(+)-lysine l(+)-glutamate monohydrate when the composition of the crystallization mixture is varied.

Molar Ratio l(+)-Glu- tamic Acid dl-Lysine	Solvent: cc. per g. Lysine Glutamate		Gross Yield, Per Cent of Theoretical l(+)-Lysine Glutamate Monohydrate	Optical Purity of Product, Per Cent
	Water	Methanol		
1:1	2.25	7.5	87	97
1:1	2.25	9.0	128	78
0.6:1	3.75	12.5	65	97
1:1	2.25	8.0	87	97
1:1	2.25	8.5	113	81
1:1	2.25	7.0	83	98
1:1	2.25	8.25	89	97
1:1	2.25	6.0	62	98
1:1	1.00	1.78	59	98
1:1	0.5	0.5	66	90
1:1	11.3	101	58	96
1:1	11.2	214	51	96

While the foregoing examples describe only the application of glutamic acid in the resolution of racemic or dl-lysine, the same method may obviously be applied to partially resolved compositions, i. e., those in which the ratio of l(+)-lysine to d(-)-lysine is other than 1:1. Also, it will be noted that the optical purity of the l(+)-lysine l(+)-glutamate produced is in general somewhat less than 100%. If it is desired to obtain a product of higher purity, this may be accomplished by recrystallizing the initial product, e. g., from a solvent similar in composition to that originally used. If desired, d(-)-

lysine glutamate may be recovered from the mother liquor. The d(-)-lysine, either free or as glutamate, may be racemized to the dl form. Preferably, the glutamate is treated to recover free d(-)-lysine, which then may be racemized, by a conventional method, e. g., by heating with an acid.

Broadly stated, the process of this invention comprises reacting free lysine containing substantial proportions of both stereoisomers with an optically active glutamic acid or its ammonium salt and separating the resulting diastereoisomers of lysine glutamate by fractional crystallization. Preferably, I employ the l(+)-glutamic acid or its ammonium salt and fractionally crystallize the l(+)-lysine salt from a mixture of methanol and water, as hereinafter described. This method results in a high yield of l(+)-lysine l(+)-glutamate of high optical purity, together with reasonably rapid crystallization, well-formed crystals and free-flowing mother liquor.

The proportions of lysine:glutamic acid:water:methanol for satisfactory results are interdependent and may be varied within wide limits. The theoretical proportion of glutamic acid to lysine is a 1:1 molar ratio, and excellent results are obtainable with this ratio. However, good results can be obtained and some economy of materials effected with a lower proportion of glutamic acid, e. g., that equivalent only to the l(+)-lysine present. Proportions outside these limits can obviously be used, but show no advantage and are in general uneconomical.

The solvent employed for fractional crystallization is a mixture of methanol and water in the proportions given below. Among a number of solvents tested (including ethanol water mixture), this was the only one giving satisfactory results. The composition of the solvent (methanol-water ratio) must be within the range of about 1:1 to about 20:1 vol. methanol per vol. of water. With lower proportions of methanol, the solubility of the product is too high for satisfactory operation. This results in a viscous mother liquor which is difficult to filter and tends to give a low yield. With higher proportions of methanol, the total solubility of lysine glutamate is strongly depressed, so that the total volume of the charge in proportion to the quantity of lysine which can be satisfactory resolved becomes excessive. Generally, best results are obtained with a methanol to water ratio of about 1.5:1 to about 5:1, by volume.

The quantity of lysine glutamate treated by a given quantity of solvent should be adjusted in relation to the solvent composition to give a maximum yield of l(+)-lysine l(+)-glutamate of maximum optical purity. As an example, excellent results are obtained by crystallizing a composition consisting of equimolar quantities of dl-lysine and l(+)-glutamic acid from a solvent containing 2.25 cc. of water and 8.25 cc. of methanol per gram of lysine glutamate. The ratio of total solvent to weight of the lysine glutamate will vary, depending on the methanol:water ratio in the solvent, because of corresponding variations in solubility; but in any event the proportion of solvent must be sufficiently low to form a supersaturated solution, in order that crystallization can occur.

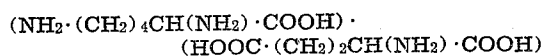
The temperature at which the crystallization is carried out is not an important factor in respect to yields, but will influence the concentration and rate. Thus, a higher temperature increases the total solubility of both diastereoisomers and also

increases the rate of approach to equilibrium. A lower temperature has the opposite effects. It is preferred to operate at about room temperature, e. g., at about 15 to 30° C., since excellent results are thus obtained with minimum operating and equipment costs.

l(+)-Lysine l(+)-glutamate ordinarily crystallizes from aqueous methanol with one mole of water (i. e., as a monohydrate). The water of hydration can be removed by prolonged heating in a dry atmosphere or under vacuum, but is recovered on exposure to moist air. The hydrated salt melts at 180-182° C. (sealed tube).

Its specific rotation $[\alpha]_D$ at room temperature and 12% concentration is +4.0°. The specific rotation of dl-lysine l(+)-glutamate is approximately -2.2°.

It is to be understood that the term "lysine glutamate" as used herein refers to the glutamic acid salt of lysine containing one mole of glutamic acid and one mole of lysine. Its formula in the anhydrous state may be written:



Lysine and glutamic acid may be recovered from lysine glutamate by any of several methods, e. g., by acidification of a concentrated aqueous solution with HCl to yield lysine hydrochloride in solution and precipitate glutamic acid. A particularly convenient and effective method comprises passing a solution of lysine glutamate through a bed of a cation-exchange material, preferably in its ammonium form. When this is done, ammonium glutamate is recovered from the effluent and may be converted to glutamic acid by known methods or heated with free dl-lysine in water solution to expel ammonia and prepare for a subsequent resolution. The lysine which is adsorbed by the ion-exchange material may then be eluted with an acid, e. g., hydrochloric acid, or with a base, e. g., ammonium hydroxide. Elution with ammonium hydroxide is especially desirable, since evaporation of the effluent leaves free lysine which may be recovered as such or converted to any desired salt, e. g., the monohydrochloride.

In place of the glutamic acid, I may use optically active ammonium glutamate, which readily reacts with lysine, setting free ammonia. When using ammonium glutamate, I prefer to heat the resulting lysine glutamate solution to drive off the ammonia, after which I may add the methanol and permit crystal formation.

In a preferred cyclic process, dl-lysine aqueous solution is mixed with an aqueous solution of ammonium l(+)-glutamate, the mixture is heated, e. g., to around 100° C., to substantially completely drive off ammonia, and to concentrate the solution by evaporation, if necessary. Methanol in suitable proportion (as hereinabove indicated) is then added and, if necessary, a few crystals of l(+)-lysine l(+)-glutamate are added to induce crystallization. The resulting crystals of l(+)-lysine l(+)-glutamate are recovered by filtration and dissolved in water. The resulting aqueous solution of l(+)-lysine l(+)-glutamate is passed through a bed of a cation-exchange resin which has previously been treated with ammonium hydroxide solution. The effluent from the cation-exchange resin bed is an aqueous solution of ammonium glutamate, which is recycled to react with dl-lysine, as above. The cation-exchange resin then is treated with ammonium hydroxide, regenerating the cation-ex-

change resin and resulting in an aqueous solution of l(+)-lysine as effluent.

Any conventional cation-exchange material may be utilized in the above cyclic process. I prefer to use a synthetic resin cation-exchange material, for example: sulfonated condensation products of formaldehyde and phenolic compounds; sulfonated copolymers of divinyl benzene such as sulfonated styrene/divinyl benzene copolymers; divinyl benzene copolymers containing carboxyl groups such as divinyl benzene/methacrylic acid copolymers; sulfonated coals; and the like. Generally, I prefer to use the sulfonated divinyl benzene/styrene copolymers.

While I prefer to use l(+)-glutamic acid or its ammonium salt as resolving agent, the opposite optically active form of this acid and salt (d(-)-glutamic acid and ammonium d(-)-glutamate) may be employed, if desired. In such case, using the above described crystallization solvent, d(-)-lysine d(-)-glutamate is separated as the crystalline product of the fractional crystallization, leaving l(+)-lysine glutamate in the mother liquor. The lysine salts of d(-)-glutamic acid may be converted to free lysine by the methods employed for the lysine salts of l(+)-glutamic acid.

I claim:

1. The process which comprises reacting an optically active compound selected from the group consisting of glutamic acid and ammonium glutamate with a mixture consisting of an aqueous solution of dl-lysine and fractionally crystallizing the product from a solvent consisting of a mixture of water and methanol in the proportions of about 1 to about 20 volumes of methanol to 1 volume of water to recover optically active lysine glutamate.

2. The process which comprises reacting a compound selected from the group consisting of l(+)-glutamic acid and ammonium l(+)-glutamate with an aqueous solution containing l(+)-lysine and d(-)-lysine and fractionally crystallizing the resulting lysine glutamate from a solvent consisting of a mixture of water and methanol in the proportions of about 1 to about 20 volumes of methanol to 1 volume of water so as to separate crystals of l(+)-lysine l(+)-glutamate.

3. The process which comprises reacting an optically active compound selected from the group consisting of glutamic acid and ammonium glutamate with a mixture of l(+)-lysine and d(-)-lysine and fractionally crystallizing the resulting lysine glutamate from a solvent consisting of a mixture of water and methanol in the proportions of about 1 to about 20 volumes of methanol to 1 volume of water.

4. The process which comprises reacting a compound selected from the group consisting of l(+)-glutamic acid and ammonium l(+)-glutamate with an aqueous solution of a mixture of l(+)-lysine and d(-)-lysine and fractionally crystallizing the resulting l(+)-lysine glutamate from a solvent consisting of a mixture of water and methanol in the proportions of about 1.5 to about 5 volumes of methanol to 1 volume of water.

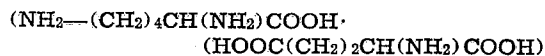
5. The process which comprises mixing an aqueous solution of an optically active ammonium glutamate with a mixture of l(+)-lysine and d(-)-lysine, heating the resulting solution to drive off ammonia, then mixing methanol with

the solution in the proportion equivalent to about 1.5 to about 5 volumes of methanol to 1 volume of the water present and separating crystals of l(+)-lysine glutamate from the solution.

6. The process which comprises mixing an aqueous solution of ammonium l(+)-glutamate with a mixture of l(+)-lysine and d(-)-lysine, heating the resulting solution to drive off ammonia, then mixing methanol with the solution in the proportion equivalent to about 1.7 to about 4.5 volumes of methanol to one volume of the water present, separating crystals of l(+)-lysine glutamate from the solution, flowing an aqueous solution of said crystals through a bed of a cation-exchange resin which previously has been treated with ammonium hydroxide, then treating said resin with ammonium hydroxide and recovering an effluent an aqueous solution of l(+)-lysine.

7. An optically active lysine glutamate.

8. The l(+)-lysine salt of l(+)-glutamic acid, which in its anhydrous form has the formula:



9. l(+)-Lysine l(+)-glutamate monohydrate.
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