Patented Feb. 19, 1952

2,586,154

UNITED STATES PATENT OFFICE

2,586,154

RACEMIZATION OF AMINO ACID

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No Drawing. Application June 29, 1950, Serial No. 171,245

8 Claims. (Cl. 260—534)

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This invention relates to the racemization of amino acids and more particularly it relates to a novel process for the racemization of lysine.

Lysine is one of the amino acids which occur in nature as constituents of proteins. The lysine which occurs naturally 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 enantiomorphic d(—)-lysine has no known nutritional value.

Lysine which has been synthesized from optically inactive materials, for example, by the process of Eck and Marvel, Organic Syntheses Collective vol. II, page 374, or by the process described in U. S. P. 2,498,300 is optically inactive and consists of equal parts of the biologically active l(+)-isomer and the biologically inactive d(—)-isomer. Thus, its nutritional value can be doubled by converting its d(—)-lysine content into the l(+)-form while retaining the quantity of the latter initially present.

It is possible to convert synthetic lysine completely to the biologically active form by first separating the isomers (resolution) and then converting the inactive d(—)-lysine back to the dl-mixture (racemization). By a repetition of these operations, the lysine is eventually converted entirely into the l(+)-form. As a practical matter it is unnecessary and usually impossible to effect a complete separation of the l(+)-lysine and the d(—)-isomer. The requirements for an economically feasible process are that a substantial portion of the l(+)-lysine be isolated in pure form in the resolution step and that no serious loss of total lysine occur in either step.

Resolution of dl-lysine is usually effected by combining the material to be resolved with an optically active compound known as the resolving agent and fractionally crystallizing the product. This may be accomplished by known methods. For example, in a method disclosed by C. P. Berg in the Journal of Biological Chemistry, vol. 115, pages 9–15, (1936), d-camphoric acid is used as a resolving agent to obtain l(+)-lysine. This author also shows the use of l-camphoric acid to obtain d(—)-lysine.

The racemization of lysine, or conversion of the optically active material to the racemic mixture, has previously been accomplished by heating the isomer with hydrochloric acid. This method is disclosed in Ber. 35, 3778, (1902). Although good recoveries of lysine are obtainable by this method, the mixture of hydrochloric acid

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and lysine is highly corrosive to metals, particularly at the temperatures necessary to effect racemization at a practical rate. Thus, expensive corrosion resistant equipment is necessary if this method is to be used. Furthermore, the use of hydrochloric acid requires equipment capable of withstanding elevated pressures. Attempts to use this method by reducing the proportion of hydrochloric acid to that stoichiometrically equivalent to the lysine present have failed to obviate this serious disadvantage. Another method for the racemization of lysine which is disclosed in U. S. P. 2,071,327 consists in heating the amino acid with acetyl chloride in the presence of acetic acid. Obviously, this method is even less desirable than the one described above.

It is an object of this invention to provide a simple, practical and economical process for the racemization of lysine. A further object is to provide a new and improved process by which a mixture of l(+) and d(—)-isomers of lysine may easily be recovered from the reaction medium.

Still another object is to provide a rapid process for the racemization of lysine which may be operated at atmospheric pressure. These and other objects will be apparent from the following description of the invention.

The above objects are attained in accordance with this invention by heating optically active lysine in combination with phosphoric acid.

In one mode of operating the process of this invention phosphoric acid is added to an aqueous solution of optically active lysine or lysine hydrochloride and the resulting solution is boiled to remove water until the desired temperature of operation is reached. A reflux condenser is then attached and heating is continued until racemization is substantially complete. The racemized lysine may be recovered by diluting the racemization mixture and passing the resulting solution into contact with a cation-exchange material which adsorbs the lysine. Lysine may then be displaced from the cation-exchange material by elution with a base, for example, ammonia, and free lysine obtained by evaporation to remove the ammonia. An acid, for example, hydrochloric acid, may be utilized if desired to remove the lysine from the cation-exchange material in which case the lysine may be recovered as the hydrochloride.

Racemization in accordance with this invention may be carried out by heating either free lysine or lysine monohydrochloride in combination with phosphoric acid. When the racemization is carried out using the hydrochloride a somewhat

longer time is required but better recovery of lysine is obtained and higher yields of optically active lysine are obtained in the subsequent resolution step.

The time required for complete racemization will vary with the temperature as well as the ratio of optical isomers present in the lysine treated. For example, complete racemization of l(+)-lysine has been obtained by heating with phosphoric acid during 1 hour at about 170° to 175° C. Lysine containing about 70% of the d(−)-isomer was completely racemized by heating with phosphoric acid during 1 hour at 155° to 165° C. Racemization of l(+)-lysine monohydrochloride required 4 hours at 160° to 162° C. and about 8 hours at 150° to 153° C. In general the racemization should be operated within the range 110° to 210° C. A temperature of about 160° C. is usually satisfactory. At lower temperatures the racemization is slower while at higher temperatures, although the rate of racemization is increased, side reactions and decomposition of lysine may occur with resulting low recoveries of lysine.

Although racemization may be carried to completion in accordance with this invention it is unnecessary and may sometimes be undesirable in practical operation to racemize completely. Since mixtures containing varying proportions of l(+)-lysine and d(−)-lysine may be resolved without difficulty it is entirely satisfactory to stop the racemization at any desired point. However, it is of course desirable to increase substantially the proportion of the desired isomer in the mixture.

Although lysine may be racemized by heating in combination with phosphoric acid in the absence of water it is preferable to have present at least 2% by weight of water based upon the weight of the mixture in order to minimize dehydration of the lysine. The racemization may also be accomplished in the presence of large amounts of water, for example, 50% by weight, but such large amounts of water will involve a longer time for racemization because of the lower temperature of operation or pressure equipment in order to obtain the preferred temperature of operation. In general it is desirable to adjust the amount of water utilized in accordance with the temperature at which the operation is to be carried out at atmospheric pressure.

The proportion of phosphoric acid to lysine should be at least equimolar in order to obtain good results. An excess of phosphoric acid is satisfactory. For example, a ratio of 2 moles of phosphoric acid to 1 mole of lysine has been used with excellent results. A greater excess may be utilized if desired but in most cases no advantage is obtained thereby.

When the racemization has been carried to the desired point the lysine may be separated from the phosphoric acid and the latter is suitable for reuse in the racemization process. Various methods may be utilized for the separation of lysine from the phosphoric acid. For example, a compound capable of forming an insoluble phosphate may be added to the aqueous mixture to precipitate the phosphate which is then filtered off leaving lysine in solution. Calcium or barium hydroxides are examples of materials suitable for this purpose.

A preferred method of isolating the lysine following racemization is to pass the diluted racemization mixture into contact with a cation-exchange material in the acid form which adsorbs the lysine and allows the phosphoric acid to pass through. In this mode of operation the phosphoric acid is recovered in condition for reutilization without further treatment. The lysine adsorbed on the cation-exchange material may be recovered by eluting the material with a material capable of liberating lysine therefrom and recovering lysine from the elutriate. For example, an acid or a base may be utilized. A preferred material for eluting the cation-exchange material to displace lysine is ammonia since the lysine may then be readily recovered in free form by boiling to expel ammonia followed by evaporation. If desired, the cation-exchange material containing the lysine may be eluted with an acid, for example, hydrochloric acid, in which case the lysine may be isolated as the hydrochloride.

Any of the well-known cation-exchange materials may be utilized. As commonly used the term cation-exchange materials refers to a class of insoluble polymeric materials which contain acidic groups capable of combining with a variety of cations to form insoluble salts. A typical example of a cation-exchange material is described in U. S. P. 2,366,007. Other examples include polyphenolsulfonic acids, sulfonated hydrocarbon polymers and polycarboxylic acids. Sulfonated coals and natural and synthetic zeolites having cation-exchange properties are also suitable in the practice of this invention. The selection of a particular cation-exchange material will depend upon such considerations as capacity, exchange rate, mechanical ruggedness and chemical stability as well as cost. Any of the commercially available cation-exchange materials are suitable. For example, satisfactory results may be obtained with cation-exchange materials sold under the names "Dowex-50," "Amberlite IR-100," "Amberlite IR-105," "Amberlite IR-120," "Ionac C-200," "Duolite C-3," "Amberlite IRC-50" and "Permutit Q."

A preferred class of cation-exchange materials are those in which the acidic groups are sulfonic acid groups as exemplified by the sulfonated hydrocarbon polymers. These cation-exchange materials are particularly valuable in the practice of this invention since they possess excellent chemical and physical stability under the conditions utilized, are readily available, and have high capacity and exchange rate.

Various forms of the cation-exchange material may be utilized but the acid form or ammonium form are most suitable and the acid form is usually preferred since this permits recovery of the phosphoric acid in free form thus avoiding the necessity of converting the ammonium or other form to the free acid for reuse in the racemization step.

When the acid form of a cation-exchange material is used the cation-exchange material must be a strong acid type, for example, a material in which the acidic group are sulfonic acid groups. Thus the cation-exchange material when used in the acid form should be sufficiently strong to liberate the phosphoric acid.

The following examples illustrate the invention.

Example 1

A solution of 12.80 g. (0.07 mole) of l(+)-lysine monohydrochloride and 10 ml. 85% phosphoric acid in 30 ml. water was boiled until the temperature reached 185° C. and was then heated under reflux at 185 to 192° C. for 1 hour. The solution, after dilution with water, showed zero rotation indicating complete racemization. The solution

was diluted to 1 liter and the lysine adsorbed on a column of cation-exchange resin. The resin was eluted with HCl and the HCl elutriate evaporated to dryness. The residue was dissolved in hot 95% ethyl alcohol and acetone was then added to precipitate the lysine dihydrochloride. The recovered lysine dihydrochloride weighed 14.74 g., 96 percent of the theoretical. It contained 31.6% Cl^- (theory 32.4) and a water solution showed zero rotation.

Example 2

To a water solution containing 0.07 mole free l(+)-lysine was added 10 ml. 85% phosphoric acid. The mixture was heated until the temperature reached 170° C. and was then heated under reflux at 170–175° C. for 1 hour. The mixture, after dilution with water, showed zero rotation. The lysine dihydrochloride, recovered as described in Example 1, weighed 14.44 g., 94 percent of the theoretical.

Example 3

To a water solution of 0.14 mole free lysine containing about 70% d(−)-isomer, was added 20 ml. 85% phosphoric acid. The mixture was boiled until the temperature reached 155° C. and was then heated under reflux at 155–165° C. for 1 hour. The mixture, after dilution with water, showed zero rotation. The lysine dihydrochloride recovered as described in Example 1, weighed 30.64 g., 99.9 percent of the theoretical.

Example 4

To 68 g. of l(+)-lysine monohydrochloride was added 53.5 ml. 85% phosphoric acid and about 20 ml. water. The mixtures was boiled until the temperature reached 160° C. A reflux condenser was then attached and the mixture heated at 160 to 162° C. for 4 hours. Samples (5 ml.) were taken at various intervals, diluted to 25 ml., and the rotation observed. The results are summarized in the table below:

Time of Heating at 160 to 162° C. (Hours)	Observed Rotation (Degrees)
0	4.57
1	2.01
1.5	1.21
2	0.83
2.5	0.55
3	0.31
3.5	0.14
4	0

Example 5

The same procedure as in Example 4 was used. The racemization was run at 150 to 153° C. The results are summarized in the table below.

Time of Heating at 150–153° C.	Observed Rotation (Degrees)
0	4.74
1	3.39
2	2.56
3	2.01
4	1.31
5	0.90
6	0.59
7	0.38
8	0.24

Example 6

A water solution of 1.055 mole free lysine containing about 70% d(−)-isomer was mixed with 143 ml. 85% phosphoric acid and the solution boiled until the temperature reached 160° C. The solution was then heated under reflux at 160° C. for 1 hour, diluted with water, and boiled 15 minutes. It was found to be completely racemized. Free lysine was recovered by adsorbing the lysine on a cation-exchange resin and eluting it with ammonia; the recovery was 92.5 percent. Resolution of the lysine with d-camphoric acid in aqueous methanol gave a 40 percent yield of l(+)-lysine d-camphorate, 94% optically pure.

The methanol was stripped from the mother liquor which was then treated with 114 ml. 85% phosphoric acid. The theoretical quantity of d-camphoric acid was recovered by filtration and extraction with ether. The racemization and reconversion to free lysine was repeated with an 89 percent recovery of lysine. This was resolved with d-camphoric acid and gave a 43 percent yield of 95% optically pure l(+)-lysine d-camphorate.

The resolution mother liquor was again converted to lysine phosphate, racemized and reconverted to free lysine. The recovery of lysine was 87 percent.

Example 7

A series of runs similar to Example 6 was made in which lysine monohydrochloride was treated with phosphoric acid and racemized at 160° C. The results are shown in the table below.

Run No.	Racemization time (Hrs.)	Per Cent Recovery of Free Lysine	l(+)-lysine d-Camphorate	
			Per Cent Yield	Per Cent Optical Purity
1[1]	3	97.5	58	93
2	2¾	98	49	95

[1] In the first run 97.5% optically pure l(+)-lysine monohydrochloride was used as the starting material.

The process of this invention is superior to the prior art process utilizing hydrochloric acid in the surprising and unexpected speed with which racemization is accomplished. It has been found that racemization of lysine with hydrochloric acid at 185° C. requires about 12 hours as compared with only about 1 hour for the process of this invention at a temperature of 170° to 175° C. Thus the racemization with phosphoric acid may be carried out at a much lower temperature and still require only a fraction of the time required by the prior art method. In addition racemization with phosphoric acid may be carried out at atmospheric pressure.

Although the process of this invention is useful in racemizing optically active lysine from any source, it is of particular utility as a part of the resolution-racemization cycle involved in converting dl-lysine or other mixture of l(+) and d(−)-lysine to a pure optical isomer. This procedure which may vary in detail consists essentially in resolving lysine by known methods, for example, the method described by Berg, separating the desired isomer, heating the remaining undesired isomer in combination with phosphoric acid to effect racemization, recovering a mixture of l(+) and d(−)-lysine and returning the latter to the resolution step.

When the lysine to be racemized in accordance with this invention is in the form of its compound with a resolving agent such as optically active camphoric acid, the latter may be recovered from solution upon the addition of phosphoric acid followed by filtration and extraction with ether and racemization is then carried out

by heating the remaining solution of lysine in combination with phosphoric acid as described above.

Throughout this specification and in the appended claims the term lysine, unless otherwise indicated, is intended to include within its scope both free lysine and lysine hydrochloride or other salt of lysine.

I claim:

1. Process for the racemization of lysine which comprises heating optically active lysine in combination with phosphoric acid.

2. Process for the racemization of lysine which comprises heating optically active lysine in combination with phosphoric acid in the presence of water.

3. The process of claim 2 wherein the temperature is maintained at 110° to 210° C.

4. The process of claim 2 wherein the concentration of phosphoric acid is at least equimolar.

5. Process which comprises resolving a mixture of l(+) and d(−)-lysine, separating a desired optically active isomer, heating the remaining undesired optically active isomer in combination with phosphoric acid and recovering a mixture of l(+) and d(−)-lysine.

6. Process which comprises resolving a mixture of l(+) and d(−)-lysine into l(+)-lysine and d(−)-lysine, separating l(+)-lysine, heating the remaining d(−)-lysine in combination with phosphoric acid in the presence of water and recovering a mixture of l(+) and d(−)-lysine.

7. Process which comprises heating optically active lysine in combination with phosphoric acid in the presence of water, passing an aqueous solution of the racemized lysine obtained thereby into contact with a cation-exchange material, recovering phosphoric acid, eluting said cation-exchange material with a material capable of displacing lysine from said cation-exchange material and recovering lysine from the elutriate.

8. Process which comprises heating optically active lysine in combination with phosphoric acid in the presence of water, passing an aqueous solution of the racemized lysine obtained thereby into contact with a cation-exchange material, recovering phosphoric acid, eluting said cation-exchange material with ammonia and recovering lysine from the elutriate.

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