The present invention relates to a novel process for the preparation of optically active amino acids and is more particularly concerned with the resolution of alkyl esters of DL-tryptophane into an alkyl D-tryptophane and alkyl L-tryptophane, the D-camphorsulfonates of alkyl DL-tryptophane, and a process for the preparation of D- and L-tryptophane.

It is well known that α-amino acids are fundamental in the field of nutrition and physiological chemistry. Of the thirty or twenty isolated α-amino acids, a small number have been shown to be indispensable for the maintenance of animal life. Man requires eight such indispensable amino acids, among them tryptophane, while rats require an additional two. It has been further established that the L-optical isomers are the only ones found in proteins and produced by nature. Since the D-amino acids are either not at all or only partially used by the animal, it is desirable to separate the D-amino acids from mixtures of synthetically obtained DL-acids. Inasmuch as L-tryptophane is considered an indispensable amino acid, the present improved process for the production thereof is of considerable importance.

The process of the present invention involves the formation of the D-camphorsulfonates of an intermediate in the synthesis of tryptophane, namely, an alkyl DL-tryptophane (David I. Weisblat and Douglas A. Lyttle, U. S. Patent 2,557,041, issued June 12, 1951), wherein the alkyl group contains from one to four carbon atoms, inclusive. An alkyl L-tryptophane-D-camphorsulfonate is practically insoluble in ethyl acetate, while the corresponding diastereoisomer is soluble. Treating the separated salts with a base results in formation of the optically active alkyl esters of tryptophane which are converted to D- and L-tryptophane by hydrolysis. It is an object of the present invention to provide a new and simplified process for the preparation of pure L-tryptophane and D-tryptophane. Another object of the invention is the provision of a process for the separation of optical isomers of alkyl tryptophanes in substantially pure form. Another object of the invention is to provide alkyl D-tryptophanes-D-camphorsulfonates and alkyl D-tryptophanes-D-camphorsulfonates, two sets of diastereoisomers which possess entirely different solubility characteristics in ethyl acetate, and a process for the production of these alkyl tryptophanes-D-camphorsulfonates. Other objects of the present invention will be apparent to those skilled in the art to which this invention pertains.

Due to the fact that L-tryptophane is valuable in cases of undernourishment (Rose, Fed. Proc. 8, 546 (1949)), especially in those cases where patients are efeebled to the extent of being unable to utilize protein containing food properly, and in the prevention and treatment of pellagra (The Merck Index, Merck & Co., Inc., Rahway, N. J., page 981, 1952), any different procedures have been used for the separation of L-tryptophane from mixtures of synthetically produced tryptophane. The most common practice of separation is to convert DL-tryptophane into an N-acyl derivative thereof and then use the N-acyl-DL-tryptophane to form a salt with an optically active base, such as brucine, quinine, or d-phenylethylamine. (Vigneaud and Sealock, J. Biol. Chem. 96, 511 (1932)), separating the thus-obtained diastereoisomers by taking advantage of their different solubility characteristics, and by hydrolyzing the separated diastereoisomers to obtain D- and L-tryptophane. Neuberg and Mandl (U. S. Patent 2,511,867, issued June 20, 1950) employed a biochemical method of hydrolysis for acyl-DL-tryptophanes, wherein an amidase hydrolyzes the N-acyl-L-tryptophane to L-tryptophane while the D-isomer remains intact.

One distinct advantage of the present invention consists in the fact that the resolution is accomplished by means of a readily formed and easily decomposed salt rather than through a derivative such as an amid. Moreover, the starting material of the present invention, an alkyl ester of DL-tryptophane, is an intermediate for the preparation of either DL-tryptophane or of the separated diastereoisomers. Another advantage of the present invention is the fact that the resolving agent, D-camphorsulfonic acid, is of relative low cost compared to some of the natural alkaloids which have been used previously, and can easily be recovered in the process. The resolution of the present process does not include any fractional crystallization, but is carried out by simply removing the insoluble diastereoisomer from the soluble diastereoisomer through filtration. Usage of the tryptophane ester instead of the N-acyltryptophane also facilitates the final hydrolysis, since the ester can be hydrolyzed at room temperature, while the hydrolysis of an amid, as used in the prior art, requires more vigorous conditions, such as refluxing the amid with an aqueous acid for a period of several hours, which favors the formation of a larger amount of side-products. A further advantage of the present invention is the fact that the optically active esters of tryptophane have importance as starting materials for the synthesis of peptides.

The starting materials of the present invention are the lower-alkyl esters of DL-tryptophane containing from one to four carbon atoms, inclusive. The preparation of these esters is described in detail by David I. Weisblat and Douglas A. Lyttle, U. S. Patent 2,557,041, issued June 12, 1951. Representative starting esters include: methyl DL-tryptophane, ethyl DL-tryptophane, propyl and isopropyl DL-tryptophane, and butyltryptophane.

In carrying out the process of the present invention, an alkyl DL-tryptophane, wherein the alkyl group contains from one to four carbon atoms, inclusive, with ethyl DL-tryptophane being preferred, is dissolved in ethyl acetate and a solution of D-camphorsulfonic acid in ethyl acetate is added. Equal molar weights of alkyl DL-tryptophane and D-camphorsulfonic acid are used, which in case of ethyl tryptophane amounts to equal weights in grams. The ethyl acetate solutions of alkyl DL-tryptophane and D-camphorsulfonic acid are preferably concentrated solutions, near the saturation point. D-camphorsulfonic acid is preferably dissolved in warm ethyl acetate. Crystallization of the alkyl L-tryptophane D-camphorsulfonate may be induced either by seeding or scratching the walls of the vessel, and the thus-precipitated material is separated by filtration and washed with ethyl acetate. Neither concentration nor temperatures are critical, but chilling the solution of alkyl DL-tryptophane after the addition of the D-camphorsulfonic acid assures better precipitation of alkyl L-tryptophane D-camphorsulfonate. The alkyl D-tryptophane D-camphorsulfonate is obtained from its ethyl acetate solution (filtrate) by concentration of the solution at reduced pressure.

It is preferable, but not necessary, to purify the separated alkyl D- and alkyl L-camphorsulfonate, which may
be done by recrystallization from mixtures of organic solvents, such as ethyl acetate or other like solvents, or by dissolving the camphorsulfonates in water and extracting the water layer with a water-insoluble organic solvent, such as diethyl ether, chloroform, benzene or other like solvents. The alkyl D- and the alkyl L-tryptophanes are obtained from their D-camphorsulfonate salts by adding an equivalent or slight excess of a base to the aqueous solutions of their salts. A one Normal ammonium hydroxide solution is preferred, but solutions of alkali-metal hydroxides, such as sodium or potassium hydroxide, in tenth Normal solution or barium hydroxide in tenth Normal solution can also be used; excess of strong bases will produce hydrolysis of the ester. Since the alkyl esters of tryptophane are water-insoluble, they are removed from the solution by an organic solvent which is insoluble in water, e.g., benzene, chloroform, diethyl ether, methylene chloride, or other like solvent. In a preferred embodiment of the invention, an aqueous solution of the D-camphorsulfonate of the selected optically active alkyl tryptophane isomer is shaken in a separatory funnel with an aqueous solution of ammonium hydroxide and diethyl ether. The water layer is discarded and the ether layer is concentrated to dryness to yield the desired optically active alkyl tryptophane isomer. The thus-obtained crude alkyl tryptophanes are converted to the respective D- and L-tryptophane by hydrolysis with a base. For this purpose the selected alkyl tryptophane, dissolved in a solvent such as methyl alcohol, ethyl alcohol, propyl alcohol, acetone or other like solvent, with ethyl alcohol being preferred, is contacted with a solution of a base, such as sodium hydroxide, potassium hydroxide, or barium hydroxide. In a preferred embodiment, an alcoholic solution of the selected alkyl tryptophane is mixed with a concentrated aqueous solution of sodium hydroxide containing from ten to thirty percent by weight in sodium hydroxide and is allowed to stand for a period of eight to 24 hours at about room temperature, that is, between about fifteen and thirty degrees centigrade. Higher temperatures or lower temperatures are also operative, but present no advantages. The thus-obtained alkali-metal salts of tryptophane yield tryptophane by treatment with dilute mineral acids or organic acids, such as dilute hydrochloric acid, dilute sulfuric acid, acetic acid, formic acid or other like acids, with acetic acid preferred. The mixture is usually brought to a pH between 4.5 to 6.5, to achieve complete precipitation of the tryptophane. At higher pH ranges, some of the tryptophane remains in solution as an alkali-metal salt, while at lower pH ranges some of the tryptophane remains in solution as the quaternary amine salt of tryptophane. The thus-precipitated D- or L-tryptophane is separated from the aqueous solution by filtration and is, after washing with water and solvents such as alcohol and ether, sufficiently pure for oral administration.

The following examples are illustrative of the process of the present invention, but are not to be construed as limiting.

Example 1A.—Ethyl L-tryptophane D-camphorsulfonate

Forty-two grams of ethyl DL-tryptophane was dissolved in 200 milliliters of hot ethyl acetate. Thirty milliliters of ethyl acetate was used to complete the transfer of the D-camphorsulfonic solution. The solution was stirred and crystallization of the sparingly soluble ethyl L-tryptophane D-camphorsulfonate was started by seeding the solution. The mixture was chilled after the crystallization appeared to be complete. The white ethyl L-tryptophane D-camphorsulfonate was filtered with suction and washed with 120 milliliters of cold ethyl acetate, combining the wash liquid with the mother liquor. The crystalline material was recrystallized from a twenty percent solution of ethyl alcohol in ethyl acetate and dried in vacuo. The yield was 31.5 grams (75 percent) of ethyl L-tryptophane D-camphorsulfonate of melting point 179 to 180 degrees centigrade (uncorrected).

1B.—Ethyl D-tryptophane D-camphorsulfonate

The combined mother liquor and wash liquid of Example 1A was concentrated to dryness at reduced pressure and the thus-obtained, solid ethyl D-tryptophane D-camphorsulfonate was recrystallized from acetone.

IC.—Ethyl L-tryptophane

Thirty-one and one-half grams of ethyl L-tryptophane D-camphorsulfonic acid, dissolved in 500 milliliters of water in a one-liter separatory funnel, was vigorously shaken with 200 milliliters of ether and 100 milliliters of one Normal ammonium hydroxide until all solid disappeared. The layers were separated, aqueous sodium phosphate extracted twice with 100-milliliter portions of ether, and the resulting ether extracts were combined and dried over anhydrous magnesium sulfate. The solution was filtered and concentrated at reduced pressure. The last traces of ether were removed at fifty degrees centigrade in vacuo and ethyl L-tryptophane was obtained, which was used for the preparation of L-tryptophane.

ID.—Ethyl D-tryptophane

The ethyl D-tryptophane D-camphorsulfonate as obtained in Example 1B was dissolved in 300 milliliters of water and extracted with ether to remove ether-soluble impurities. The water solution was made alkaline with ammonium hydroxide and extracted with ether. Removal of the ether from the ether extracts in vacuo yielded ethyl D-tryptophane which may alternatively be used for the preparation of D-tryptophane.

1E.—L-tryptophane

Ethyl L-tryptophane, as obtained in Example 1C, was dissolved in 45 milliliters of ethyl alcohol and thereto was added eighteen grams of sodium hydroxide. The solution was allowed to stand overnight at room temperature. The pH was adjusted to 5.9 with glacial acetic acid and crystalline material was separated. After the mixture had stood in the ice-box overnight, the L-tryptophane was filtered and washed with water, alcohol and ether. The dried product was ground (equal to sixty percent theoretical, based on ethyl DL-tryptophane) and had a melting point of 268 degrees centigrade.

1F.—D-tryptophane

Crude ethyl D-tryptophane was dissolved in sixty milliliters of 95 percent ethyl alcohol and thereto was added 24.4 grams of 20 percent sodium hydroxide solution. After standing overnight, the D-tryptophane was recovered from the mixture by acidification with acetic acid as in Example 1E. Twelve grams of D-tryptophane, equal to a yield of 65 percent theoretical, based on ethyl DL-tryptophane, were obtained. The melting point of the D-tryptophane was 274 degrees centigrade.

Example 2A.—Methyl L-tryptophane D-camphorsulfonate

Substitution of methyl DL-tryptophane for ethyl DL-tryptophane in Example 1A yielded methyl L-tryptophane D-camphorsulfonate which was precipitated and recovered by filtration.

2B.—Methyl D-tryptophane D-camphorsulfonate

By concentrating the filtrate and washings of Example 2A, methyl D-tryptophane D-camphorsulfonate was obtained.
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2C.—Methyl L-tryptophane and methyl D-tryptophane

By treating the D-camphorsulfonates of methyl D- and methyl L-tryptophane, obtained in Examples 2A and 2B, with ammonia as shown in Examples 1C and 1D, the methyl D- and methyl L-tryptophane were obtained.

2D.—L-tryptophane and D-tryptophane

L-tryptophane and D-tryptophane were prepared by treating methyl L-tryptophane, and methyl D-tryptophane, respectively, in methanol solutions with potassium hydroxide and acidifying the solution of the potassium salts of L- and D-tryptophane with one-twentieth Normal hydrochloric acid solution to a pH between five and six to precipitate the L-tryptophane and D-tryptophane.

In a manner similar to Examples 1A, 1B, 2A, and 2B, propyl D- and propyl L-tryptophane D-camphorsulfonates are prepared from propyl DL-tryptophane; isopropyl D- and isopropyl L-tryptophane D-camphorsulfonates are prepared from isopropyl DL-tryptophane; 2-ethylbutyl D- and Normal butyl L-tryptophane D-camphorsulfonates are prepared from Normal butyl L-tryptophane D-camphorsulfonate and evaporating the filtrate to obtain alkyl D-tryptophane D-camphorsulfonate. From these camphor-D-sulfonates the following optically active esters are obtained in the manner described in Examples 1C, 1D and 2C; propyl L- and propyl D-tryptophane; isopropyl L- and isopropyl D-tryptophane and butyl L- and butyl D-tryptophane, which by hydrolysis with a base and treatment with an acid yield L-tryptophane and D-tryptophane, respectively.

It is to be understood that the invention is not to be limited to the exact details of operation or exact compounds shown and described as obvious modifications and equivalents will be apparent to one skilled in the art, and the invention is therefore to be limited only by the scope of the appended claims.

We claim:

1. In a process for the production of optical isomers of tryptophane the steps which comprise: treating alkyl DL-tryptophane wherein the alkyl group contains from one to four carbon atoms, inclusive, dissolved in ethyl acetate, with a solution of D-camphorsulfonic acid in ethyl acetate to obtain a precipitate of alkyl L-tryptophane D-camphorsulfonate and a solution of alkyl D-tryptophane D-camphorsulfonate in ethyl acetate, and filtering the thus-obtained mixture to obtain the precipitated alkyl L-tryptophane D-camphorsulfonate.

2. In a process for the production of optical isomers of tryptophane the steps which comprise: treating alkyl DL-tryptophane wherein the alkyl group contains from one to four carbon atoms, inclusive, dissolved in ethyl acetate, with a solution of D-camphorsulfonic acid in ethyl acetate to obtain a precipitate of alkyl L-tryptophane D-camphorsulfonate and a solution of alkyl D-tryptophane D-camphorsulfonate in ethyl acetate, filtering the thus-obtained mixture to obtain the precipitated alkyl L-tryptophane D-camphorsulfonate and evaporating the filtrate to obtain alkyl D-tryptophane D-camphorsulfonate.

3. The process of claim 2 wherein the alkyl DL-tryptophane is ethyl DL-tryptophane.

4. In a process for the production of optical isomers of tryptophane the steps which comprise: treating alkyl DL-tryptophane wherein the alkyl group contains from one to four carbon atoms, inclusive, dissolved in ethyl acetate, with a solution of D-camphorsulfonic acid in ethyl acetate to obtain a precipitate of alkyl L-tryptophane D-camphorsulfonate and a solution of alkyl D-tryptophane D-camphorsulfonate in ethyl acetate, filtering the thus-obtained mixture to obtain the precipitated alkyl L-tryptophane D-camphorsulfonate, evaporating the filtrate to obtain alkyl D-tryptophane D-camphorsulfonate, and treating the thus-separated isomeric alkyl tryptophane D-camphorsulfonates with a base to yield the corresponding alkyl D- and alkyl L-tryptophanes.

5. The process of claim 4 wherein the starting alkyl DL-tryptophane is ethyl DL-tryptophane.

6. The process of claim 4 wherein the base used to decompose the separated, optically active alkyl tryptophane D-camphorsulfonates is aqueous ammonium hydroxide.

7. A process for the production of optical isomers of tryptophane which comprises: treating alkyl DL-tryptophane wherein the alkyl group contains from one to four carbon atoms, inclusive, dissolved in ethyl acetate, with a solution of D-camphorsulfonic acid in ethyl acetate to obtain a precipitate of alkyl L-tryptophane D-camphorsulfonate and a solution of alkyl D-tryptophane D-camphorsulfonate in ethyl acetate, filtering the thus-obtained mixture to obtain the precipitated alkyl L-tryptophane D-camphorsulfonate, evaporating the filtrate to obtain alkyl D-tryptophane D-camphorsulfonate, treating the separated isomeric alkyl tryptophane D-camphorsulfonates with a base to yield the corresponding alkyl D- and L-tryptophanes, hydrolyzing the thus-obtained alkyl tryptophanes with a base to obtain the separated isomeric salts of tryptophane and treating these salts with an acid to obtain D- and L-tryptophane.

8. The process of claim 7 wherein the starting alkyl DL-tryptophane is ethyl DL-tryptophane.

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