RESOLUTION OF RACEMIC PHENYLALANINE

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(Received for publication, April 14, 1950)

Phenylalanine has previously been resolved by fractional crystallization of the brucine salt of formyl-DL-phenylalanine (1) or of the cinchonine salt of benzoyl-DL-phenylalanine (2). Enzymatic methods have also been used for the resolution. According to the method of Gilbert, Price, and Greenstein (3), racemic phenylalanine is resolved by subjecting its N-chloroacetylated derivative to asymmetric hydrolysis by a carboxypeptidase present in beef pancreas. A preparation of the enantiomorphs of DL-phenylalanine has also been reported by making use of the difference in rates of synthesis by papain of the anilides of acetyl DL-phenylalanylglycine (4).

In this paper, a simple method is described, which is based upon the asymmetric hydrolysis of the isopropyl ester of DL-phenylalanine by an enzyme preparation derived from pancreas. Complete digestion of the ester yields free L-phenylalanine and the isopropyl ester of D-phenylalanine. The latter is easily separated by virtue of its solubility in ether and alcohol. The ethyl ester of DL-phenylalanine is hydrolyzed in the same manner. However, the isopropyl ester is not hydrolyzed appreciably by water at room temperature and is therefore more suitable for the purpose. Resolution of amino acids by asymmetric hydrolysis of their esters has previously been used to obtain the isomers of leucine (5), methionine (6, 7), and tryptophan (8).

EXPERIMENTAL

Isopropyl Ester of DL-Phenylalanine—100 gm. of DL-phenylalanine are suspended in 1400 ml. of an anhydrous solution of isopropyl alcohol containing 10 per cent of anhydrous hydrochloric acid. The mixture is refluxed for 2 hours. A clear solution is obtained, which is evaporated to dryness on a steam bath in vacuo. To the hydrochloride of the isopropyl ester thus obtained are added 300 ml. of distilled water and 500 ml. of ether. While stirring, the mixture is cooled to −10°. A 25 per cent solution of ammonium hydroxide is added until the reaction of the mixture is faintly alkaline to phenolphthalein. The ether layer is separated and

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the water solution is extracted with two portions of 250 ml. of ether. The combined ether extracts, containing the isopropyl ester, are dried over anhydrous sodium sulfate. The sodium sulfate is removed and washed with 100 ml. of anhydrous ether. The ether extracts are distilled in vacuo, and the fraction distilling between 128-131° at 5 mm. contains the isopropyl ester of DL-phenylalanine; yield 110 to 116 gm. (88 to 93 per cent of theory).

C₁₅H₂₁O₂N. Calculated, C 69.5, H 8.3, N 6.8; found, C 69.7, H 8.3, N 6.9

**Enzyme Preparation**—The enzyme used in this investigation was prepared from raw pancreas desiccated and defatted at 37°. The pancreas powder is gently shaken in distilled water for half an hour, filtered, and the filtrate used for the resolution procedure. The rate of hydrolysis of the L ester is shown in Fig. 1. It is evident that the D ester is completely resistant for at least 50 hours.

**Preparation of L-Phenylalanine**—100 gm. of the isopropyl ester of DL-phenylalanine are gently shaken for 24 hours at room temperature with 400 ml. of a filtered 2.5 per cent extract of the pancreas powder. At the end of this time, there is a heavy precipitate of L-phenylalanine. While stirring, 10 ml. of 25 per cent ammonium hydroxide and 400 ml. of ether are added to the digestion mixture. The stirring is continued for an hour, when the mixture is filtered and the precipitate is washed with three 25 ml. portions of absolute alcohol.

1 Viobin Corporation, Monticello, Illinois.
The precipitate of L-phenylalanine is further extracted by being stirred for an hour at gentle boiling temperature with a mixture of 300 ml. of ether and 100 ml. of absolute alcohol. After filtration, the precipitate is allowed to stand overnight at room temperature under 200 ml. of ether. The ether, which has evaporated, is then restored, and the mixture is stirred for an hour and filtered. After being washed twice with 50 ml. of ether, the precipitate is allowed to dry in the air.

A second crop of L-phenylalanine is obtained from the digestion mixture in the following way. The ether layer is separated and the water solution is extracted with two 100 ml. portions of ether, concentrated in vacuo to 150 to 200 ml., treated with one-third of its volume of 95 per cent alcohol, and chilled overnight. The precipitate is washed with two 25 ml. portions of 30 per cent alcohol and two like portions of absolute alcohol. The combined precipitates of impure L-phenylalanine should weigh 37 to 40 gm.

All of the ether extracts, containing the isopropyl ester of D-phenylalanine, are combined, dried over anhydrous sodium sulfate, and saved for the preparation of D-phenylalanine.

The impure L-phenylalanine is recrystallized from 11 times its weight of hot distilled water after treatment with norit. 0.33 volume of 95 per cent alcohol is added and the whole is chilled overnight. The crystals are washed with 30 per cent alcohol and absolute alcohol as above. The L-phenylalanine thus obtained contains 99.0 to 99.5 per cent of the L isomer and weighs 24 to 30 gm. To obtain an analytically pure sample, the L-phenylalanine is again recrystallized and washed as outlined above. The product thus obtained should weigh 19 to 23 gm. (49 to 58 per cent of theory). Analysis of a typical preparation yielded the following result.

\[
\text{C}_{9} \text{H}_{11} \text{O}_{2} \text{N}. \text{ Calculated, N 8.48; found, N 8.44}
\]

\[
\left[\alpha\right]_{D}^{22} = -35.0 \text{ to } -35.2 \ (c = 2, \text{ in distilled water})
\]

The values reported in the literature are -35.1 (1), -34.8 (3), -35.3 (9).

Preparation of D-Phenylalanine—The combined ether extracts from the preparation of L-phenylalanine are filtered, the sodium sulfate is washed with 100 ml. of anhydrous ether, and the ether is evaporated. The resulting oil is shaken for 24 hours with 100 ml. of a 5 per cent aqueous extract of the pancreas powder in order to remove any contamination with the L ester. After the addition of 5 ml. of a 25 per cent solution of ammonium hydroxide, the mixture is extracted with three 75 ml. portions of ether. The combined ether extracts are dried over anhydrous sodium sulfate and concentrated on a steam bath with the aid of a water pump. The remaining oil (weighing 38 to 42 gm.) is refluxed for 12 hours with
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7 volumes of 20 per cent hydrochloric acid. The solution is evaporated to dryness in vacuo and for a second time after adding 300 ml. of distilled water. The hydrochloride of D-phenylalanine thus obtained is dissolved in 250 ml. of water, decolorized with 0.5 gm. of norit, and the clear solution neutralized with 25 per cent ammonium hydroxide and treated with 90 ml. of 95 per cent alcohol. The crystals which separate overnight are washed with alcohol as above and should weigh 20 to 23 gm.

The impure D-phenylalanine is recrystallized by the same technique as the L enantiomorph. The analytically pure D-phenylalanine thus obtained weighs 12 to 15 gm. (30 to 38 per cent of theory). Analysis of a typical preparation yielded the following results.

\[
\text{C}_{6}\text{H}_{13}\text{O}_{2}\text{N}. \quad \text{Calculated}, \text{N} 8.48; \text{found}, \text{N} 8.55
\]

\[
[\alpha]_{D}^{\text{N}} = +35.0 \text{ to } +35.2 \ (c = 2, \text{ in distilled water})
\]

The values reported in the literature are +35.08 (2), +34.8 (3), +34.56 (10).

SUMMARY

A simple and effective method is described for the preparation of both isomers of phenylalanine by the asymmetric hydrolysis of the isopropyl ester of DL-phenylalanine.

The author wishes to thank Dr. William C. Rose for his helpful suggestions and for generously making available the facilities of his laboratory.

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