PREPARATION OF D- AND L-METHIONINE FROM DL-METHIONINE BY ENZYMATIC RESOLUTION*

BY CHARLES A. DEKKER AND JOSEPH S. FRUTON

(From the Department of Physiological Chemistry, Yale University, New Haven)

(Received for publication, December 19, 1947)

It has been shown previously (1, 2) that the synthesis of CO-NH linkages by papain as well as by other proteinases is characterized by extreme stereoselective specificity. Thus, upon the addition of cysteine-activated papain to a mixture of carbobenzoxy-DL-glutamic acid and aniline, there is formed carbobenzoxy-L-glutamic acid anilide, which separates in crystalline form. The carbobenzoxy-D-glutamic acid which remains in solution may then be hydrogenated to yield D-glutamic acid (2).

As noted elsewhere (3), it appears desirable to examine the feasibility of this enzymatic resolution method for the preparation of the optically active isomers of several amino acids (methionine, threonine, valine, and isoleucine) whose DL forms are now readily accessible by synthesis. In the present communication there is described the preparation, by the enzymatic resolution of DL-methionine, of D- and of L-methionine. A method for the resolution of DL-methionine has been described previously by Windus and Marvel (4), who used the classical procedure of Emil Fischer. Duschinsky and Jeannerat (5) prepared L-methionine from the racemate by the selective oxidation of the D isomer with D-amino acid oxidase.

When carbobenzoxy-DL-methionine is incubated with aniline in the presence of cysteine-activated papain, carbobenzoxy-L-methionine anilide crystallizes with a yield of 95 per cent. From the filtrate, there may be isolated carbobenzoxy-D-methionine which, on catalytic hydrogenation, is converted to D-methionine of satisfactory purity.

It was intended to obtain L-methionine by the hydrolysis of carbobenzoxy-L-methionine anilide with hydrochloric acid. The main product of the cleavage was found to be L-methionine, however, but S-benzyl-l-homocysteine. The formation of this compound from carbobenzoxy-L-methionine anilide may be explained by assuming the formation of benzyl chloride in the course of the acid hydrolysis of the carbobenzoxy group, followed by the conversion of the thioether to a sulfonium ion. The preferential elimination of the methyl group during the decomposition of the

* This study was aided by grants from the American Cancer Society (on recommendation of the Committee on Growth of the National Research Council) and from the Rockefeller Foundation.

471
sulfonium ion would then lead to the formation of S-benzyl-L-homocysteine.

\[
\begin{align*}
\text{CH}_3 & \quad + \text{S--CH}_2\text{C}_6\text{H}_5 & \quad \text{S--CH}_2\text{C}_6\text{H}_5 \\
\text{S} & \quad \text{CH}_2 & \quad \text{CH}_3 \\
\text{CH}_2 & \quad \text{CH}_2 & \quad \text{CH}_2 \\
\text{C}_6\text{H}_5\text{CH}_2\text{OCO--NHCH} & \quad \text{COOH} & \quad \text{COOH}
\end{align*}
\]

Support for this interpretation comes from the observation that, if DL-methionine is refluxed with benzyl chloride and hydrochloric acid, S-benzyl-L-homocysteine is formed.

The application of the enzymatic resolution method thus offers an additional method for the preparation of S-benzyl-L-homocysteine. This compound was first obtained by du Vigneaud and Patterson (6) from DL-methionine by the conversion of the latter substance to DL-homocysteine, which was then reduced with sodium in liquid ammonia. Treatment with benzyl chloride gave S-benzyl-DL-homocysteine which was resolved by fractional crystallization of the brucine salts of the N-formyl derivatives. Du Vigneaud and Patterson prepared D- and L-homocysteine from the optically active S-benzyl derivatives by treatment with sodium in liquid ammonia.

The difficulty encountered in the attempted preparation of L-methionine from carbobenzyloxy-DL-methionine may be avoided if, in place of the carbobenzyloxy derivative, the benzoyl compound is employed in the enzymatic resolution. In the presence of papain, benzoyl-L-methionine anilide is formed in 97 per cent yield and pure L-methionine may be prepared by acid hydrolysis of this derivative. The isolation of pure benzoyl-D-methionine from the filtrate of the anilide proved to be difficult, in contrast to the situation found in the case of the corresponding carbobenzyloxy compound. Hydrolysis of the impure benzoyl-D-methionine gave partially racemic preparations of the amino acid.

The ready availability of the optically active isomers of methionine makes possible their use in the synthesis of peptides of L- and of D-methionine. The preparation of such peptides will be the subject of a subsequent communication.

**EXPERIMENTAL**

*Carbobenzyloxy-DL-methionine*—To a solution of 30 gm. (0.2 mole) of L-methionine in 100 cc. of 2 n NaOH, there were added 37.5 gm. (0.22 mole)
of carbobenzoxy chloride and 120 cc. of 2 N NaOH in five portions over a period of 30 minutes. The reaction mixture was kept at 0° and stirred continuously during this time. After further stirring for 1 hour at room temperature, the solution was acidified to Congo red with concentrated hydrochloric acid, causing the precipitation of crystalline carbobenzoxy-DL-methionine. The crystals were collected and dried over phosphorus pentoxide in vacuo. Yield, 50 gm. (88 per cent); m.p., 110-112°. After recrystallization from 40 per cent ethanol, the substance melted at 112°.

C_{13}H_{20}O_2NS. Calculated. C 55.1, H 6.1, N 4.9
283.2 Found. " 55.2, " 6.0, " 4.9

Enzymatic Synthesis of Carbobenzoxy-L-methionine Anilide—14.5 gm. (0.05 mole) of carbobenzoxy-DL-methionine were dissolved in 50 cc. of N NaOH and added to 9.5 cc. (0.11 mole) of aniline. A solution of 0.6 gm. of cysteine hydrochloride in 20 cc. of water was added, followed by 40 cc. of 0.2 M citrate buffer (pH 5.0). A solution obtained by the extraction of 3 gm. of crude papain (dried papaya latex) with 40 cc. of water was then introduced and the reaction mixture was diluted to 250 cc. with water. Separation of the anilide began immediately, crystallization being induced by vigorous shaking of the flask. The reaction mixture was placed in a constant temperature bath at 38° for 4 days. The anilide which separated was removed daily and the filtrate was replaced in the bath after adjusting the pH to 5 with a few drops of concentrated hydrochloric acid. The combined precipitates weighed 8.7 gm. (95 per cent). The compound was recrystallized from 40 per cent ethanol; m.p., 162.5°.

C_{19}H_{22}O_2NS. Calculated. C 63.6, H 6.2, N 7.8
388.3 Found. " 63.5, " 6.2, " 8.1
[\alpha]_b^0 = -14.8° (1.45% in glacial acetic acid)

Carbobenzoxy-D-methionine—The filtrate remaining from the enzymatic synthesis of carbobenzoxy-L-methionine anilide was heated to boiling to coagulate the proteins and was decolorized with a few gm. of Darco. The clear filtrate was acidified to Congo red with concentrated hydrochloric acid. The syrup which separated crystallized after being kept in the ice-box for 24 hours. The crystals were dissolved in ether and the ethereal solution was extracted with an aqueous solution of potassium bicarbonate. Upon acidification of the aqueous layer, there separated crystalline carbobenzoxy-D-methionine which was collected and washed with cold water. Yield, 7 gm. (85 per cent). After recrystallization from 50 per cent ethanol, the substance melted at 69-70°.

C_{13}H_{17}O_2NS. Calculated. C 55.1, H 6.1, N 4.9
283.2 Found. " 54.9, " 6.1, " 4.8
\textbf{\textit{d-Methionine}}—2.85 gm. (0.01 mole) of carbobenzoxy-\textit{d}-methionine were dissolved in 20 cc. of methanol containing a few drops of glacial acetic acid and hydrogenated with palladium black as the catalyst. The hydrogenation required 11 hours and fresh catalyst was added at the end of 5 hours. 50 cc. of hot water were added to dissolve the free amino acid, and the catalyst was removed by filtration and washed on the filter with more hot water. The filtrate and washings were concentrated to dryness \textit{in vacuo} and the residue was extracted with ether to remove any unchanged carbobenzoxy-\textit{d}-methionine. The ether-insoluble material was recrystallized from 75 per cent ethanol. The crystalline \textit{d}-methionine was collected, washed with small amounts of absolute alcohol and ether, and dried over phosphorus pentoxide. Yield, 1.0 gm. (67 per cent).

\begin{align*}
\text{C}_5\text{H}_{11}\text{O}_2\text{NS} & \quad \text{Calculated. C} 40.2, \text{H} 7.5, \text{N} 9.4 \\
149.2 & \quad \text{Found. " 40.1, " 7.4, " 9.1 \\
[\alpha]_D^\circ & = -21.5^\circ \text{ (1.2\% in 0.2 N hydrochloric acid)}
\end{align*}

Windus and Marvel (4) report $[\alpha]_D^{25} = -21.18^\circ$ (0.8 per cent in 0.2 N hydrochloric acid).

\textbf{\textit{S-Benzyl-L-homocysteine}}—2.5 gm. (0.0067 mole) of carbobenzoxy-\textit{l}-methionine anilide were refluxed with 20 cc. of concentrated hydrochloric acid for 10 hours. The hydrolysate was extracted with ether and the aqueous layer was concentrated under reduced pressure. The concentrate was neutralized with saturated lithium hydroxide solution, yielding a heavy white precipitate which was collected and washed with small amounts of water, alcohol, and ether. The product was recrystallized from boiling water. Yield, 0.5 gm. (30 per cent); m.p., 243–244\textdegree{} (with decomposition). Du Vigneaud and Patterson (6) report 247–252\textdegree{} for \textit{S}-benzyl-\textit{d}-homocysteine.

\begin{align*}
\text{C}_7\text{H}_{13}\text{O}_2\text{NS} & \quad \text{Calculated. C} 58.6, \text{H} 6.7, \text{N} 6.2 \\
225.2 & \quad \text{Found. " 58.6, " 6.7, " 6.1 \\
[\alpha]_D^\circ & = +27.2^\circ \text{ (1\% in N hydrochloric acid)}
\end{align*}

Du Vigneaud and Patterson (6) found for \textit{S}-benzyl-\textit{d}-homocysteine $[\alpha]_D^{25} = -25^\circ$ (1 per cent in N hydrochloric acid).

\textbf{\textit{S-Benzyl-DL-homocysteine}}—1.5 gm. (0.01 mole of \textit{DL}-methionine) were dissolved in 20 cc. of concentrated hydrochloric acid, 1.4 cc. (0.012 mole) of benzyl chloride were added, and the mixture was refluxed for 11 hours. The reaction mixture, after being cooled, was extracted with ether, and the aqueous layer was concentrated to a syrup under reduced pressure. The residue was taken up in 30 cc. of water and neutralized with saturated lithium hydroxide solution. The resulting precipitate was collected and washed successively with cold water, ethanol, and ether. Yield, 1.0 gm. (45 per cent). After recrystallization from boiling water, the substance

**Benzoyl-DL-methionine**—This compound was prepared according to the directions of Steiger (7) for the benzoylation of amino acids. 32 gm. of the benzoyl derivative were obtained from 20 gm. of DL-methionine, representing a yield of 95 per cent. The crude material melted at 145°-150°. After recrystallization from 33 per cent ethanol, the compound melted sharply at 151°. This value agrees with that reported by Hill and Robson (8).

**Enzymatic Synthesis of Benzoyl-L-methionine Anilide**—19.45 gm. (0.077 mole) of benzoyl-DL-methionine were dissolved in 77 cc. of N NaOH and 13.9 gm. (0.154 mole) of aniline were added. A solution of 0.2 M citrate buffer (pH 5.0). The enzyme solution, prepared by extracting 4.6 gm. of papain with 62 cc. of water, was introduced, and the mixture was agitated and placed in a constant temperature bath at 38°. Another 190 cc. of buffer and more water were added gradually over a period of 2 hours, making the total volume 750 cc. After 18 hours, the benzoyl-L-methionine anilide which had separated was collected and the filtrate was replaced in the bath. The first crop weighed 11.3 gm. (90 per cent). Another 0.9 gm. was collected after the next 24 hours, giving a total yield of 12.2 gm. (97 per cent). The compound was purified by recrystallization first from ethyl acetate and then from ethanol-water; m.p., 159°.

**Acid Hydrolysis of Benzoyl-L-methionine Anilide**—2.2 gm. (0.0067 mole) of benzoyl-L-methionine anilide were suspended in 150 cc. of 6 N hydrochloric acid and refluxed on a sand-bath for 12 hours. After cooling the mixture, the benzoic acid which had crystallized was removed by filtration. The filtrate was concentrated under reduced pressure to 50 cc. and then was extracted with two 35 cc. portions of ether. The aqueous layer was concentrated and the residue was taken up in 15 cc. of absolute alcohol. The addition of 5 cc. of pyridine caused the crystallization of L-methionine. The crystals were collected and washed successively with absolute alcohol and ether. Yield, 0.5 gm. (50 per cent). The rotation of the unrecrystallized material was \([\alpha]_D^{24} = +20.7°\) (1 per cent in 0.2 N hydrochloric acid). After recrystallization from 75 per cent ethanol, the optical activity was unchanged. The substance melted at 281° (corrected) with previous darkening and shrinkage. Mueller (9) reports a melting point of 283° for L-methionine.

\[
\text{C}_{14}\text{H}_{11}\text{O}_{2}\text{N}_{3}\text{S. Calculated. C 40.2, H 7.5, N 9.4}
\]

\[
149.2 \text{ Found. } ^{\circ} \text{ C 40.0, } ^{\circ} \text{ H 7.3, } ^{\circ} \text{ N 9.1}
\]
Acid Hydrolysis of Benzoyl-d-methionine—The filtrate from the enzymatic synthesis of benzoyl-l-methionine anilide was heated to boiling to coagulate the proteins, was decolorized with Darco and filtered, and the filtrate was concentrated in vacuo to 185 cc. After acidification to Congo red, the benzoyl-d-methionine separated as an oil which crystallized readily upon stirring and scratching. The crude crystals were collected, washed with a small amount of cold water, and dried over phosphorus pentoxide and sodium hydroxide. Yield, 9.4 gm. (96 per cent). Partial purification was effected by the extraction of an ethyl acetate solution of this material with aqueous potassium bicarbonate, and acidification of the bicarbonate solution. The crystalline material which resulted was washed and dried as before. Further purification by recrystallization was unsuccessful because the solubility of benzoyl-d-methionine proved to be greater than that of benzoyl-nL-methionine. Therefore, 2.55 gm. (0.01 mole) of the partially purified benzoyl-n-methionine were suspended in 250 cc. of 10 per cent hydrochloric acid and refluxed on a sand-bath for 12 hours. The benzoic acid which had separated was removed by filtration, and the filtrate was concentrated under reduced pressure to a volume of 125 cc. After extraction with two 60 cc. portions of ether, the solution was further concentrated in vacuo and the residue was taken up in 18 cc. of absolute alcohol. The alcoholic solution was clarified by filtration and the methionine was precipitated by the addition of 4 cc. of pyridine. Yield, 1.0 gm. (67 per cent). The optical rotation of the product was [α]_b^25 = -16.7° (1.7 per cent in 0.2 N hydrochloric acid). Recrystallization of 0.9 gm. of the above material from 100 cc. of 75 per cent ethanol yielded 0.6 gm. of methionine with a rotation of [α]_b^25 = -15.7° (0.7 per cent in 0.2 N hydrochloric acid), thus indicating that the material was being enriched with respect to D-methionine rather than D-methionine.

An additional fraction (0.16 gm.) was recovered from the mother liquor, and this had a rotation of [α]_b^25 = -20.7° (0.8 per cent in 0.2 N hydrochloric acid).

C₈H₁₀O₂NS. Calculated. C 40.2, H 7.5, N 9.4
149.2 Found. " 40.4, " 7.5, " 9.2

Although reasonably pure d-methionine was obtained, the procedure was less satisfactory than that involving the hydrogenation of carbobenzoxy-d-methionine.

SUMMARY

DL-Methionine has been resolved by the action of papain on a mixture of carbobenzoxy-DL-methionine and aniline. The anilide of the L isomer separates in crystalline form, and from the filtrate there may be isolated carbon-
benzoxymethylmethionine which upon catalytic hydrogenation is converted to D-methionine. Acid hydrolysis of carbobenzoxymethylmethionine anilide yields S-benzyl-L-homocysteine. L-Methionine may be prepared by the enzymatic synthesis of benzoyl-L-methionine anilide from benzoyl-DL-methionine and aniline, followed by acid hydrolysis of the anilide.

BIBLIOGRAPHY

PREPARATION OF d- AND l-METHIONINE FROM dl-METHIONINE
BY ENZYMATIC RESOLUTION
Charles A. Dekker and Joseph S. Fruton