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The Use of N-Benzylsulfonyl-α-amino Acids in Enzymatic Syntheses of the L-Phenylhydrazides, and for Enzymatic Resolutions[1,2]

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The enzymatic synthesis (using papain) of a number of N-benzylsulfonyl-L-α-amino acid phenylhydrazides has been accomplished. The stereospecific behavior of papain in this reaction has been demonstrated. The N-benzylsulfonyl-L-α-amino acid phenylhydrazides reacted with ferric chloride to give a high yield of the N-benzylsulfonyl-L-α-amino acids. The enzymatic resolution of D,L-methionine and L-methionyl-D,L-methionine is reported.

In a previous paper,[3] it was shown that benzylsulfonyl chloride is a very useful reagent for peptide syntheses. The preparation of N-benzylsulfonyl derivatives of an extensive list of amino acids was reported. It was also shown that the benzylsulfonyl group is cleaved by sodium in liquid ammonia, Raney nickel, hydriodic acid and hydrobromic acid. Both the synthesis and the cleavage of the derivatives were accomplished without racemization of optically active amino acids. The usefulness of this reagent was demonstrated by using it in the preparation of the dipeptides, L-leucyl-L-leucine and L-methionyl-D,L-methionine.

Since Bergmann and co-workers[4] demonstrated that proteolytic enzymes may, under suitable conditions, cause the synthesis of peptide bonds as well as their hydrolyses, there has been an interest in the use of proteolytic enzymes in the resolution of amino acids and the enzymatic synthesis of peptides. At the present stage of its development, this method cannot be compared in usefulness for peptide syntheses with other chemical methods. One of the principal difficulties lies in the removal of amide or anilide groups without the accompanying destruction of the peptide.

However, acylated-α-amino acid phenylhydrazides, which may be prepared by enzymatic syntheses, are oxidized with mild oxidizing agents to give acylated-α-amino acids. Waldschmidt-Leitz and Kühn[5] oxidized glycylglycine phenylhydrazide with copper acetate and obtained glycylglycine. We have shown[6] that phenylhydrazide groups may be quantitatively removed from N-carboallyloxy-α-amino acid phenylhydrazides by oxidation with ferric chloride.

When N-carbobenzoxy-α-amino acids[7] or N-carboallyloxy-α-amino acids[8] are used in an enzymatic synthesis with papain, both the D- and L-isomers react. On the other hand, when an acetylated-α-amino acid[7] is used, the reaction proceeds with almost complete specificity for the L-antipode.

It was of interest to see if N-benzylsulfonyl-α-amino acids could be used in enzymatic syntheses, and if the reaction was stereospecific enough for enzymatic resolutions of amino acids and dipeptides.

Discussion of Results

The enzymatic synthesis of the phenylhydrazides of six N-benzylsulfonyl-L-α-amino acids and three N-benzylsulfonyl dipeptides has been accomplished. The results are listed in Table I. The enzymatic reactions of the N-benzylsulfonyl-α-amino acids with phenylhydrazine in the presence of papain were stereospecific. The specific rotations and melting points of the phenylhydrazides of N-benzylsulfonyl-L-α-amino acids. prepared from D,L-α-amino acids through enzymatic syntheses, were the same as those of the corresponding phenylhydrazides prepared from L-α-amino acids. The phenylhydrazides of three N-benzylsulfonyl-α-amino acids were chemically synthesized in order to compare their melting points and specific rotations with those of the corresponding phenylhydrazides from enzymatic syntheses.

However, the yield of this enzymatic reaction depends upon the amino acids which are used. For example, N-benzylsulfonyl-D,L-alanine, N-benzylsulfonyl-D,L-leucine and N-benzylsulfonyl-D,L-methionine reacted with phenylhydrazine at a *p*H 4.7 in the presence of papain to give the L-phenylhydrazides with yields of 10, 46 and 74%, respectively.

The reactions of N-benzylsulfonyl-α-amino acids with phenylhydrazine in the presence of papain are slower than the corresponding reactions of N-carbobenzoxy or N-carboallyloxy derivatives of the amino acids. This may be due in part to the low solubility of the N-benzylsulfonyl-α-amino acids at a *p*H 4.7. In order to increase the reaction rates, relatively high concentrations of papain were used

Fox and co-workers[9] investigated the papain-catalyzed reaction of some glycine-containing benzoyl dipeptides with aniline. When glycine was adjacent to the benzoyl group and alanine, valine, leucine or glycine was terminal, a transamidation reaction was observed. When the glycine was C-terminal and alanine, valine or leucine was interior, a direct coupling occurred leading to the synthesis of the benzoyl dipeptide anilide. However, in the study being reported here, N-benzylsulfonyl derivatives of glycylglycine, L-leucyl-L-leucine and L-methionyl-D,L-methionine reacted with phenylhydrazine in the presence of papain to yield the N-benzylsul-

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(2) Abstracted in part from a thesis presented to the Graduate Faculty of the State College of Washington by Chi-Hsieh Peng in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1956.

(3) H. B. Milne and C. H. Peng, THIS JOURNAL, **79**, 639 (1957).

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(6) H. B. Milne, J. E. Halver, D. S. Ho and M. S. Mason, THIS JOURNAL, **79**, 637 (1957).

(7) E. L. Bennett and C. Niemann, *ibid.*, **72**, 1798 (1950).

(8) H. B. Milne and C. M. Stevens, *ibid.*, **72**, 1742 (1950).

(9) G. Tollin, M. Winitz and S. W. Fox, *Federation Proc.*, **15**, 371 (1956).

TABLE I

ENZYMATIC SYNTHESES OF L-PHENYLHYDRAZIDES OF N-BENZYLSULFONYLAMINO ACIDS

N-Benzylsulfonyl derivative of	Wt., g.	Phenylhydrazine hydrochloride, g.	Papain, g.	M.p., °C.	Yield,[a] %	Formula of L-phenylhydrazides	Analyses, nitrogen, % Calcd.	Found
Glycine	2.29	1.44	1.0	198	83	$C_{15}H_{17}N_3O_3S$	13.16	13.29
D,L-Alanine	1.22	1.00	1.0	174–176	5	$C_{16}H_{19}N_3O_3S$[b]	12.61	12.50
L-Leucine	1.43	1.00	1.0	183–184	58	$C_{19}H_{25}N_3O_3S$[c]	11.19	11.09
D,L-Leucine	4.28	2.17	2.5	183–184	23	$C_{19}H_{25}N_3O_3S$[d]	11.19	11.00
D-Leucine[e]	1.00	0.70	1.0		None			
L-Methionine	1.00	1.00	1.0	163–164	76	$C_{18}H_{23}N_3O_3S_2$[f]	10.68	10.48
D,L-Methionine	1.00	1.00	1.0	163–164	37	$C_{18}H_{23}N_3O_3S_2$[g]	10.68	10.83
D-Methionine[h]	1.00	1.00	1.0		None			
L-Phenylalanine	1.60	0.72	0.8	124–126	61	$C_{22}H_{23}N_3O_3S$[i]	10.26	9.98
L-Lysine	2.27	0.72	1.0	172–173	80	$C_{26}H_{32}N_4O_3S_2$[j]	10.29	10.11
Glycylglycine	2.86	1.44	1.2	199–200	84	$C_{17}H_{20}N_4O_4S$	14.89	14.92
L-Leucyl-L-leucine	1.00	1.00	1.0	232–234	70	$C_{25}H_{36}N_4O_4S$[k]	11.47	11.25
L-Methionyl-D,L-methionine	2.50	1.70	1.0	175–176	40	$C_{23}H_{32}N_4O_4S_3$[l]	10.68	10.63
L-Methionyl-D-methionine[m]	0.10	0.10	0.1		None[n]			

[a] Yields are based on the weights of recrystallized product obtained from total substrate used. [b] $[\alpha]^{26}D$ $-37.3 \pm 1.0°$ (c 1.00, butanone). [c] $[\alpha]^{26}D$ $-30.1 \pm 0.8°$ (c 1.00, butanone). The phenylhydrazide was collected by a series of successive incubations. [d] $[\alpha]^{26}D$ $-30.4 \pm 0.9°$ (c 1.00, butanone). [e] No phenylhydrazide was obtained after 20 days of incubation. [f] $[\alpha]^{26}D$ $-16.6 \pm 0.8°$ (c 1.50, butanone). [g] $[\alpha]^{26}D$ $-16.4 \pm 0.8°$ (c 1.50 butanone). [h] No phenylhydrazide was obtained after 15 days of incubation. [i] $[\alpha]^{26}D$ $-25.2 \pm 0.9°$ (c 0.50, acetone). [j] $[\alpha]^{26}D$ $-15.2 \pm 1.0°$ (c 0.462, acetone). [k] $[\alpha]^{26}D$ $-48.5 \pm 1.0°$ (c 1.00, butanone). [l] $[\alpha]^{25}D$ $-22.3 \pm 0.9°$ (c 1.00, butanone). The phenylhydrazide was collected after 2 hours of incubation. [m] This substrate was prepared by the resolution of N-benzylsulfonyl-L-methionyl-D,L-methionine. [n] No phenylhydrazide was obtained after 300 hours of incubation.

fonyl dipeptide phenylhydrazides. There was no evidence of the transamidation.

The usefulness of N-benzylsulfonyl-α-amino acids in enzymatic resolutions was demonstrated by resolving N-benzylsulfonyl-D,L-methionine. The N-benzylsulfonyl-L-methionine phenylhydrazide, formed in the enzymatic reaction, was oxidized with ferric chloride to yield N-benzylsulfonyl-L-methionine. N-Benzylsulfonyl-D-methionine was recovered from the filtrate of the enzymatic reaction. Both pure L- and D-methionine were obtained by reacting the N-benzylsulfonyl derivatives with sodium in liquid ammonia. D,L-Methionine has previously been resolved by the reaction of N-acyl-D,L-methionine with aniline in the presence of papain.[10,11] However, as the acyl and anilide groups were removed by hydrolyses, the method would not be satisfactory for the resolution of a dipeptide.

The enzymatic reaction of N-benzylsulfonyl-L-methionyl-D,L-methionine with phenylhydrazine has now been studied. N-Benzylsulfonyl-L-methionyl-D,L-methionine reacted with phenylhydrazine in the presence of papain to give an 84% yield of N-benzylsulfonyl-L-methionyl-L-methionine phenylhydrazide. The phenylhydrazide group was removed by oxidation with ferric chloride, and the N-benzylsulfonyl group was removed by sodium in liquid ammonia to yield L-methionyl-L-methionine. The N-benzylsulfonyl-L-methionyl-D-methionine, from the filtrate of the enzymatic reaction, was converted to L-methionyl-D-methionine with sodium in liquid ammonia.

N-Benzylsulfonyl-L-methionyl-L-methionine, obtained by the oxidation of its phenylhydrazide, gave a specific rotation, $[\alpha]^{26}D$ $-25.2°$ (c 1.00, N sodium hydroxide), and N-benzylsulfonyl-L-methionyl-D-methionine from the filtrate gave a specific rotation, $[\alpha]^{26}D$ $-51.3°$ (c 1.00, N sodium hydroxide). The average of these two specific rotations is approximately equal to that of the starting material, N-benzylsulfonyl-L-methionyl-D,L-methionine $[\alpha]^{26}D$ $-38.2°$ (c 1.00, N sodium hydroxide).[3] L-Methionyl-L-methionine gave a specific rotation $[\alpha]^{25}D$ $+26.1°$ (c 2.00, water) and L-methionyl-D-methionine from the reduction of N-benzylsulfonyl-L-methionyl-D-methionine gave a specific rotation $[\alpha]^{25}D$ $+75.8°$ (c 1.00, water). The average of these two specific rotations is approximately equal to that of L-methionyl-D,L-methionine, $[\alpha]^{25}D$ $+51.6°$ (c 1.00, water).[3]

As the specific rotation of the L-methionyl-L-methionine agrees with the reported[12] value, it may be concluded from the above that the reaction of N-benzylsulfonyl-L-methionyl-D,L-methionine with phenylhydrazine in the presence of papain is stereospecific, and that N-benzylsulfonyl-L-methionyl-D-methionine and L-methionyl-D-methionine are optically pure.

Experimental[13]

Papain.—Commercial papain (Nutritional Biochemicals Corporation) was purified by the procedure of Grassmann[14] and Bergmann and Fraenkel-Conrat[4] as modified by Bennett and Niemann.[7] After three or four successive treatments with hydrogen sulfide, followed by precipitation with methanol, the precipitate was lyophilized, and a white powder was obtained.

Enzymatic Syntheses of Phenylhydrazides of N-Benzylsulfonyl-L-α-amino Acids.—These phenylhydrazides were prepared according to the method of Bergmann and Fraenkel-Conrat.[4] Some of the results are listed in Table I. The N-benzylsulfonyl-α-amino acid was dissolved in 4–5 equivalents of N sodium hydroxide solution and the resulting

(10) C. A. Dekker and J. S. Fruton, *J. Biol. Chem.*, **173**, 471 (1948).

(11) D. G. Doherty and E. A. Popenoe, *ibid.*, **189**, 447 (1951).

(12) C. A. Dekker, S. P. Taylor and J. S. Fruton, *ibid.*, **180**, 155 (1949).

(13) All N-benzylsulfonyl derivatives of α-amino acids and dipeptides in this research are prepared according to our procedures.[3]

(14) W. Grassmann, *Biochem. Z.*, **279**, 131 (1935).

solution was treated successively with 1–2 equivalents of phenylhydrazine hydrochloride in 30 ml. of water, 1.5–2.0 equivalents of L-cysteine hydrochloride[15] in 30 ml. of water and 50 ml. of 1 *M* acetic acid–sodium acetate buffer (*p*H 4.7). If an insoluble phenylhydrazine salt precipitated the mixture was heated to 40° and diluted with water until it became homogeneous. The total volume of the solution was about 150–200 ml. After adjusting the *p*H to 4.7 with 3 *N* hydrochloric acid, papain was added, and nitrogen was bubbled through the solution for a few minutes. The flask was stoppered, and the solution was then incubated in a water-bath at 40°; after several days, the product was collected by filtration. After incubating the filtrate for several days, a second crop of crystals was obtained. The crude product was recrystallized from acetone–water.

N-Benzylsulfonyl-L-leucine Phenylhydrazide.—N-Benzylsulfonyl-L-leucine (2.85 g.) was dissolved in 200 ml. of anhydrous ether and cooled in an ice-bath. Pulverized phosphorus pentachloride (2.5 g.) was added, and the reaction was allowed to proceed with cooling and swirling for one hour. This mixture was filtered rapidly through a sintered glass filter. When the ether was removed *in vacuo*, the product crystallized. The crystals were washed with dry petroleum ether, dried *in vacuo*, and redissolved in 100 ml. of anhydrous ether. This ethereal solution was added dropwise to a mixture of 10 ml. of *N* sodium hydroxide solution, 4 ml. of phenylhydrazine and 50 ml. of water, which was cooled in an ice-bath and vigorously stirred mechanically. At the end of the addition of the ethereal solution, the mixture was stirred for an additional one-half hour. The slightly yellow precipitate was washed with 30 ml. of ether and decolorized with charcoal during recrystallization from acetone–water. The crystalline product, 3.52 g. (94% yield), melted at 183–184°, $[\alpha]^{26}D$ −30.0 ± 0.8° (*c* 1.00, butanone).

Anal. Calcd. for $C_{19}H_{25}N_3O_3S$: C, 60.77; H, 6.71; N, 11.19. Found: C, 60.37; H, 6.60; N, 11.10.

N-Benzylsulfonyl-D-leucine Phenylhydrazide.—N-Benzylsulfonyl-D-leucine (1.00 g.) was used to prepare its phenylhydrazide in the usual manner. The crystalline product, 1.25 g. (95% yield), melted at 183–184°, $[\alpha]^{26}D$ +29.9 ± 0.9° (*c* 1.00, butanone).

Anal. Calcd. for $C_{19}H_{25}N_3O_3S$: C, 60.77; H, 6.71; N, 11.19. Found: C, 60.05; H, 6.85; N, 11.15.

N-Benzylsulfonyl-D,L-methionine Phenylhydrazide.—N-Benzylsulfonyl-D,L-methionine (1.52 g.) was dissolved in a mixture of 20 ml. of dry dioxane and 50 ml. of anhydrous ether and cooled in an ice-bath. Pulverized phosphorus pentachloride (1.04 g.) was added, and the reaction was allowed to proceed in the usual manner. The phenylhydrazide was recrystallized from acetone–water; 1.12 g. (57% yield), m.p. 172–173°.

Anal. Calcd. for $C_{18}H_{23}N_3O_3S_2$: C, 54.93; H, 5.89; N, 10.68. Found: C, 54.72; H, 5.78; N, 10.87.

Conversion of N-Benzylsulfonyl-L-leucine Phenylhydrazide to N-Benzylsulfonyl-L-leucine.—N-Benzylsulfonyl-L-leucine phenylhydrazide (0.5 g.), obtained from the enzymatic reaction of N-benzylsulfonyl-D,L-leucine with phenylhydrazine, was dissolved in 50 ml. of acetone, and the solution was warmed to 35–40°. To this solution, 4.0 g. of ferric chloride ($FeCl_3 \cdot 6H_2O$) in 8 ml. of water was added dropwise. The reaction mixture was stirred mechanically during the addition of the reagent. After the addition of the reagent, the mixture was refluxed for two hours. The yellow-green solution was diluted with 20 ml. of water, cooled to room temperature, and then made alkaline to litmus paper with 3 *N* sodium hydroxide solution. The black residue was removed by centrifugation, and the solution was evaporated *in vacuo* until no odor of acetone remained. The solution was acidified (congo red) with concentrated hydrochloric acid. White crystals were obtained and recrystallized from ethanol–water; 0.33 g. (87% yield), m.p. 133°, $[\alpha]^{26}D$ −23.1 ± 1.0° (*c* 1.00, *N* sodium hydroxide). A mixed melting point determination with an authentic sample of N-benzylsulfonyl-L-leucine showed no depression.

Anal. Calcd. for $C_{13}H_{19}NO_4S$: N, 4.91; neut. equiv., 285.35. Found: N, 5.00; neut. equiv., 286.

(15) One gram of L-cysteine hydrochloride per 100 ml. of the reaction mixture was used.

Resolution of D,L-Methionine. (a) **N-Benzylsulfonyl-L-methionine Phenylhydrazide.**—N-Benzylsulfonyl-D,L-methionine (6.27 g.) was dissolved in a solution consisting of 80 ml. of *N* sodium hydroxide solution and 50 ml. of water, and the resulting solution was treated successively with 2.98 g. of phenylhydrazine hydrochloride in 20–30 ml. of water, 3 g. of L-cysteine hydrochloride in 20–30 ml. of water, 100 ml. of 1 *M* acetic acid–sodium acetate buffer (*p*H 4.7), and 3 g. of papain. This enzymatic reaction then was allowed to proceed in the usual manner. The phenylhydrazide was collected in successive fractions and recrystallized from acetone–water; 3.49 g. (86% based on the weight of L-substrate), m.p. 163–164°, $[\alpha]^{26}D$ −16.5 ± 0.9° (*c* 1.50, butanone).

Anal. Calcd. for $C_{18}H_{23}N_3O_3S_2$: N, 10.68. Found: 10.48.

(b) **Conversion of N-Benzylsulfonyl-L-methionine Phenylhydrazide to N-Benzylsulfonyl-L-methionine.**—The phenylhydrazide (1.97 g.) from the above enzymatic reaction was dissolved in 100 ml. of acetone, and the solution was warmed to 35–40°. To this solution, 15 g. of ferric chloride ($FeCl_3 \cdot 6H_2O$) in 30 ml. of water was added dropwise. The time required for the addition of ferric chloride solution was one hour. The reaction mixture was stirred mechanically during the addition of the reagent and then heated under reflux for three hours. The resulting slightly yellow solution was cooled to room temperature and made alkaline to litmus paper with 50% sodium hydroxide solution. The black residue was removed by centrifugation, and the solution was evaporated *in vacuo* until no odor of acetone remained. The solution was acidified (congo red) with concentrated hydrochloric acid, saturated with sodium chloride, and extracted with three 50-ml. portions of ether. The ethereal solution was washed with 20 ml. of water. The solution was dried over anhydrous magnesium sulfate and evaporated to dryness *in vacuo*. The product was recrystallized from anhydrous ether–hexane; 1.26 g. (83% yield), m.p. 90–91°, $[\alpha]^{26}D$ −13.8 ± 0.6° (*c* 1.00, *N* sodium hydroxide). A mixed melting point determination with an authentic sample of N-benzylsulfonyl-L-methionine showed no depression.

Anal. Calcd. for $C_{12}H_{17}NO_4S_2$: N, 4.62; neut. equiv., 303.39. Found: N, 4.60; neut. equiv., 304.

(c) **L-Methionine.**—N-Benzylsulfonyl-L-methionine (1.00 g.) was dissolved in 200 ml. of liquid ammonia. To this mixture was added, with mechanical stirring during one-half hour, 0.21 g. of metallic sodium in 15 approximately equal portions. A blue color persisted for one minute after the last addition of sodium. The mixture was treated with 0.2 ml. of methyl iodide, stirred mechanically for a few minutes, and then allowed to evaporate spontaneously. The residue was dried *in vacuo* and dissolved in 10 ml. of water. The solution was filtered, and the filtrate was neutralized with 3 *N* hydrochloric acid to *p*H 5.8. L-Methionine was collected after the addition of 20 ml. of ethanol and recrystallized from water–ethanol; 0.38 g. (77% yield), m.p. 280–283° (with decomposition), $[\alpha]^{25}D$ −8.0 ± 0.6° (*c* 0.80, water). The reported[16] value is $[\alpha]^{25}D$ −8.11 ± 0.5° (*c* 0.80, water).

Anal. Calcd. for $C_5H_{11}NO_2S$: C, 40.25; H, 7.43; N, 9.39. Found: C, 40.66; H, 7.49; N, 9.10.

(d) **N-Benzylsulfonyl-D-methionine.**—The filtrate from the enzymatic preparation of N-benzylsulfonyl-L-methionine phenylhydrazide was acidified (congo red) with concentrated hydrochloric acid, saturated with sodium chloride and extracted with three 50-ml. portions of ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, decolorized with charcoal and evaporated *in vacuo*. The residue was recrystallized twice from anhydrous ether–hexane. The white crystals, 1.88 g. (60% based on the weight of D-substrate), melted at 90–91°, $[\alpha]^{26}D$ +13.5 ± 1.1° (*c* 1.00, *N* sodium hydroxide). A mixed melting point determination with an authentic sample of N-benzylsulfonyl-D-methionine showed no depression.

Anal. Calcd. for $C_{12}H_{17}NO_4S_2$: N, 4.62; neut. equiv., 303.39. Found: N, 4.85; neut. equiv., 306.

(e) **D-Methionine.**—N-Benzylsulfonyl-D-methionine (0.4 g.) was allowed to react with 0.13 g. of metallic sodium in 100 ml. of liquid ammonia in the usual manner. D-Meth-

(16) W. Windus and C. S. Marvel, THIS JOURNAL, **53**, 3490 (1931).

ionine (0.14 g., 71% yield) was obtained after recrystallization from water–ethanol; m.p. 281–285° (with decomposition), $[\alpha]^{25}D$ +8.1 ± 0.5° (*c* 0.80, water). The reported[16] value is $[\alpha]^{25}D$ +8.12 ± 0.5° (*c* 0.80, water).

Anal. Calcd. for $C_5H_{11}NO_2S$: C, 40.25; H, 7.43; N, 9.39. Found: C, 40.30; H, 7.58; N, 9.30.

Resolution of L-Methionyl-D,L-methionine. (a) **N-Benzylsulfonyl-L-methionyl-L-methionine Phenylhydrazide.**—N-Benzylsulfonyl-L-methionyl-D,L-methionine (3.0 g.) was dissolved in 40 ml. of *N* sodium hydroxide solution. To this solution was added successively 2 g. of phenylhydrazine hydrochloride in 30 ml. of water, 2 g. of L-cysteine hydrochloride in 30 ml. of water, 50 ml. of 1 *M* acetic acid–sodium acetate buffer (*p*H 4.7) and 1.5 g. of papain. This enzymatic reaction was allowed to proceed in the usual manner. After the reaction mixture had been incubated at 40° for two hours, N-benzylsulfonyl-L-methionyl-L-methionine phenylhydrazide was collected by filtration and recrystallized from acetone–water; 1.51 g. (84% based on the weight of L-substrate), m.p. 175°, $[\alpha]^{26}D$ −22.1 ± 0.7° (*c* 1.00, butanone). After another 411 hours of incubation, no appreciable amount of phenylhydrazide was obtained.

Anal. Calcd. for $C_{23}H_{32}N_4O_4S_3$: N, 10.68. Found: N, 10.63.

(b) **Conversion of N-Benzylsulfonyl-L-methionyl-L-methionine Phenylhydrazide to N-Benzylsulfonyl-L-methionyl-L-methionine.**—The phenylhydrazide (1.2 g.) from the above enzymatic reaction was oxidized with 8 g. of ferric chloride ($FeCl_3 \cdot 6H_2O$) in the usual manner as for N-benzylsulfonyl-L-methionine phenylhydrazide. Slightly yellow crystals were obtained after acidification with concentrated hydrochloric acid and dissolved in 50 ml. of ethyl acetate. This solution was extracted with three 30-ml. portions of *N* sodium bicarbonate solution. The aqueous solution was evaporated *in vacuo* until no odor of ethyl acetate remained and acidified (congo red) with concentrated hydrochloric acid. After refrigerating the mixture overnight, white crystals were obtained which were recrystallized from ethanol–water; 0.74 g. (75% yield), m.p. 107–108°, $[\alpha]^{26}D$ −25.2 ± 0.9° (*c* 1.00, *N* sodium hydroxide).

Anal. Calcd. for $C_{17}H_{26}N_2O_5S_3$: C, 46.98; H, 6.03; N, 6.45; neut. equiv., 434.58. Found: C, 47.00; H, 6.17; N, 6.40; neut. equiv., 436.

(c) **L-Methionyl-L-methionine.**—N-Benzylsulfonyl-L-methionyl-L-methionine (0.40 g.) was dissolved in 200 ml. of liquid ammonia. To this mixture was added, with mechanical stirring during one-half hour, 0.08 g. of metallic sodium in 15 approximately equal portions. A blue color persisted for one minute after the last addition of sodium. The mixture was treated with 0.15 ml. of methyl iodide, stirred for a few minutes more, and then allowed to evaporate spontaneously. The residue was dried *in vacuo* and dissolved in 10 ml. of water. The solution was filtered; the filtrate was neutralized with hydrobromic acid (20%) to *p*H 6–7 and then evaporated to dryness under reduced pressure. The residue was extracted into 40 ml. of hot 95% ethanol. The insoluble residue was discarded. The alcoholic solution was evaporated to 10 ml. *in vacuo* and diluted with 30 ml. of ether. The white crystals were recrystallized from 80% ethanol; 0.21 g. (81% yield), m.p. 224–226° (with decomposition), $[\alpha]^{25}D$ +26.1 ± 0.7° (*c* 2.00, water). The reported[12] value is $[\alpha]^{25}D$ +26.5° (*c* 2, water).

Anal. Calcd. for $C_{10}H_{20}N_2O_3S_2$: C, 42.83; H, 7.19; N, 9.99. Found: C, 42.36; H, 7.29; N, 9.75.

(d) **N-Benzylsulfonyl-L-methionyl-D-methionine.**—The filtrate from the enzymatic preparation of N-benzylsulfonyl-L-methionyl-L-methionine phenylhydrazide was acidified (congo red) with concentrated hydrochloric acid and extracted with three 50-ml. portions of ethyl acetate. The ethyl acetate solution was extracted with three 20-ml. portions of *N* sodium bicarbonate solution. The resulting aqueous solution was evaporated *in vacuo* until no odor of ethyl acetate remained and acidified (congo red) with concentrated hydrochloric acid. White crystals were obtained after refrigerating the mixture for a few hours. The product was recrystallized from ethanol–water; 0.54 g. (36% based on the weight of D-substrate), m.p. 136–137°, $[\alpha]^{26}D$ −51.3 ± 0.7° (*c* 1.00, *N* sodium hydroxide).

Anal. Calcd. for $C_{17}H_{26}N_2O_5S_3$: C, 46.98; H, 6.03; N, 6.45; neut. equiv., 434.58. Found: C, 46.80; H, 5.83; N, 6.70; neut. equiv., 431.

(e) **L-Methionyl-D-methionine.**—N-Benzylsulfonyl-L-methionyl-D-methionine (0.35 g.) in 200 ml. of liquid ammonia was treated with 0.07 g. of metallic sodium in the usual manner. L-Methionyl-D-methionine was recrystallized from 80% ethanol; 0.20 g. (89% yield), m.p. 236–238° (with decomposition), $[\alpha]^{25}D$ +75.8 ± 1.2° (*c* 1.00, water).

Anal. Calcd. for $C_{10}H_{20}N_2O_3S_2$: N, 9.99. Found: N, 10.26.

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[CONTRIBUTION FROM THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Preparation and Properties of the Isomeric Forms of α-Amino- and α,ε-Diaminopimelic Acid

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Resolution of α-aminopimelic acid, as its N-acetyl derivative, has been effected *via* the asymmetric action of hog renal acylase I at *p*H 7.0. The yield of the optically pure L- and D-isomers, which showed $[\alpha]D$ +21.5° and $[\alpha]D$ −21.0° (1% in 5 *N* HCl) values, was 77 and 48%, respectively. In addition, a modified procedure, whereby the three isomeric forms of α,ε-diaminopimelic acid may be secured, is described. Such modification consists in a fractional separation of the carbobenzoxy derivatives of the *meso*- and DL-forms from the inactive synthetic epimeric mixture. Conversion of the DL-form to the corresponding amino acid amide proceeded through hydrogenolysis of carbobenzoxy-DL-α,ε-diaminopimelic acid amide. Resolution of the racemic amino acid amide with a purified hog kidney amidase preparation subsequently resulted in the optically pure L- and D-isomers, which showed $[\alpha]D$ values of +45.0° and −45.5° (1% in 1 *N* HCl), respectively, in agreement with previous results. Infrared spectra for each of the stereoisomeric forms of α-amino- and α,ε-diaminopimelic acid, as well as the apparent dissociation constants of the latter, are presented.

The natural occurrence of α-aminopimelic acid as a component of green plants has been reported recently by Virtanen and Berg.[1] This compound, isolated in milligram quantities, was characterized *via* chromatographic, melting point and titration data. Paucity of the natural material unfortunately permitted neither determination of its optical rotation nor configuration. It is with such identification and characterization of the L- and D-antipodes of α-aminopimelic acid with which the present communication is, in part, concerned. In addition, a modification and improvement of the procedure for the preparation of the three isomeric forms of α,ε-diaminopimelic acid, previously de-

(1) A. I. Virtanen and A. M. Berg, *Acta Chem. Scand.*, **8**, 1725, 1085 (1954).