TABLE II

ENZYMATIC SYNTHESIS OF PHENYLHYDRAZIDES

Substrate	Yield,[a] %	M.p., °C.	$[\alpha]^{22}D$	Formula	Nitrogen, % Calcd.	Found
N-Carboallyloxy-D,L-phenylalanine[b]	37	163–164	−26.5°[c]	$C_{19}H_{25}O_3N_3$	12.4	12.42
N-Carboallyloxy-D,L-alanine[b]	28.8	115–116	−60.3[d]	$C_{13}H_{17}O_3N_3$	16.0	15.60
O,N-Dicarboallyloxy-L-tyrosine	94	162–163	− 8.5[e]	$C_{23}H_{25}O_6N_3$	9.57	9.83
N-Carbopropyloxy-L-tyrosine	75	185–186	− 3.4[e]	$C_{12}H_{23}O_4N_3$	11.75	11.52

[a] Yield based on total substrate. [b] Because the enzyme is not always optically specific for carboallyloxy-L-amino acids,[5] the derivatives of phenylalanine and alanine may not be optically pure. [c] (*c* 1, chloroform). [d] (*c* 3, chloroform). [e] (*c* 1, 95% ethanol).

twice to remove any iron salts or hydrochloric acid present and then decolorized by boiling 15 minutes with charcoal. Upon filtering and evaporating the solvent a light yellow oil remained. The final product was dried at 2 mm. over phosphorus pentoxide for 24 hr.; yield 1.58 g. (85%), $[\alpha]^{22}D$ −14.6° (*c* 3.7, chloroform); for authentic sample carboallyloxy-L-leucine $[\alpha]^{22}D$ −14.5° (*c* 3.7, chloroform).[5]

Anal. Calcd.: neut. equiv., 215. Found: neut. equiv., 217.

Oxidation of N,N-Dicarboallyloxy-L-lysine Phenylhydrazide.—Dicarboallyloxy-L-lysine phenylhydrazide (6.22 g.) was oxidized in the same manner as carboallyloxy-L-leucine phenylhydrazide and the dicarboallyloxy-L-lysine recovered as a dark oil. The oil was taken up in benzene and boiled with charcoal for 15 minutes. The mixture was filtered and the solvent evaporated yielding a light yellow oil. The product was dried under 2 mm. over phosphorus pentoxide; yield 4.16 g. (86%), $[\alpha]^{22}D$ −8.2° (*c* 12, 11% concd. HCl and 89% methyl Cellosolve); authentic sample N,N-dicarboallyloxy-L-lysine, $[\alpha]^{22}D$ −8.4° (*c* 12, 11% concd. HCl and 89% methyl Cellosolve).[5,3]

Anal. Calcd.: neut. equiv., 314. Found: neut. equiv., 317.

Oxidation of O,N-Dicarboallyloxy-L-tyrosine Phenylhydrazide.—O,N-Dicarboallyloxy-L-tyrosine phenylhydrazide (0.44 g.) was oxidized in the same manner described above. The recovered light oil was dissolved in 95% ethyl alcohol and boiled with charcoal for 15 minutes. Upon filtration and evaporation of the solvent, crystals formed. The crystals were dissolved in hot 50% ethyl alcohol and the solution put in a refrigerator for 2 days; yield 0.32 g., m.p. 105° uncor., $[\alpha]^{22}D$ +28.9° (*c* 3, chloroform). A sample of O,N-dicarboallyloxy-L-tyrosine prepared directly from L-tyrosine had m.p. 104–105°, $[\alpha]^{22}D$ +29.2° (*c* 3, chloroform). A mixed melting point was 104°.

Anal. Calcd. for $C_{17}H_{19}O_7N$: C, 58.45; H, 5.48; N, 4.01; neut. equiv., 349. Found: C, 58.48; H, 5.46; N, 4.09; neut. equiv., 347.

Carboallyloxy-L-leucyl-L-leucine Phenylhydrazide.—Carboallyloxy-L-leucine phenylhydrazide (6.8 g.) was dissolved in 100 ml. of methylal and 650 mg. of platinum oxide was added. The mixture was shaken in a Parr hydrogenation apparatus under a pressure of 30 lb. of hydrogen for 2 hr. The catalyst was removed by filtration. The solvent was distilled under reduced pressure leaving a dark oil. The oil was dissolved in 100 ml. of dry ether and dry hydrogen chloride gas passed through the solution, yielding 4.35 g. of L-leucine phenylhydrazide hydrochloride which was used in the enzymatic reaction without further purification.

Carboallyloxy-L-leucine (5.00 g.), 4.35 g. of L-leucine phenylhydrazide hydrochloride and 2.0 g. of cysteine hydrochloride was dissolved in 300 ml. of 3 *M* acetate buffer. The *p*H of the solution was 4.7. Three grams of papain was added and the solution incubated at 40°. After 55 hr., the crystalline product was collected and recrystallized from alcohol to yield 4.49 g. (63.5% based on L-leucine phenylhydrazide hydrochloride) of carboallyloxy-L-leucyl-L-leucine phenylhydrazide, m.p. 185–186°, $[\alpha]^{22}D$ −91.0° (*c* 0.86, chloroform).

Anal. Calcd. for $C_{22}H_{34}O_4N_4$: C, 63.13; H, 8.13; N, 13.39. Found: C, 62.93; H, 8.08; N, 13.25.

Carboallyloxy-L-leucyl-L-leucine.—Carboallyloxy-L-leucyl-L-leucine phenylhydrazide (2.04 g.) was dissolved in 30 ml. of 95% ethanol and then warmed to 35°. To this solution 15 g. of ferric chloride hexahydrate dissolved in 25 ml. of water was slowly added. The mixture was stirred until nitrogen gas was no longer liberated. The solution was made basic to litmus with 1 *N* sodium hydroxide and the ferric hydroxide precipitate removed by centrifugation. The supernatant solution was acidified with concentrated hydrochloric acid and extracted with five 25-ml. portions of ether. Ligroin was added to the ether solution until it became turbid. The solution was then cooled and the resulting crystals collected; yield 0.69 g. (43%), m.p. 113–114°, $[\alpha]^{22}D$ −25.1° (*c* 2, chloroform).

Anal. Calcd. for $C_{16}H_{28}O_5N_2$: C, 58.5; H, 8.53; N, 8.53; neut. equiv., 328.4. Found: C, 58.74; H, 8.59; N, 8.40; neut. equiv., 329.5.

L-Leucyl-L-leucine.—Carboallyloxy-L-leucyl-L-leucine (0.44 g.) was hydrogenated with platinum oxide in ethanol, containing concentrated hydrogen chloride, in the usual manner. The peptide was recrystallized from ethanol and dried *in vacuo* over phosphorus pentoxide; 0.138 g. (42%), m.p. 259–261°, $[\alpha]^{22}D$ −13.5° (*c* 1.2, 1 *N* sodium hydroxide); Fischer[12] reported m.p. 270°, $[\alpha]^{22}D$ −13.4° (*c* 8.1, 1 *N* sodium hydroxide).

Anal. Calcd. for $C_{12}H_{24}O_3N_2$: N, 11.5. Found: N, 11.2.

PULLMAN, WASHINGTON

(12) E. Fischer, *Ber.*, **39**, 2893 (1906).

[CONTRIBUTION FROM THE FULMER CHEMICAL LABORATORY, THE STATE COLLEGE OF WASHINGTON]

The Use of Benzylsulfonyl Chloride in Peptide Syntheses[1,2]

BY H. BAYARD MILNE AND CHI-HSIEH PENG

RECEIVED JULY 23, 1956

The use of benzylsulfonyl chloride as a reagent in peptide syntheses has been investigated. N-Benzylsulfonyl derivatives of 25 amino acids have been prepared and characterized. The N-benzylsulfonyl group is easily cleaved from amino acid derivatives by sodium in liquid ammonia or Raney nickel. It is slowly cleaved by hydriodic acid and by hydrobromic acid. Both the preparation and the cleavage of the N-benzylsulfonyl derivatives of optically active amino acids are accomplished without racemization. The application of this reagent to peptide syntheses through the preparation of L-leucyl-L-leucine and L-methionyl-D,L-methionine is reported.

(1) Presented in part before the Pacific Slope Biochemical Conference, Seattle, Washington, June 12, 1956.

(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.

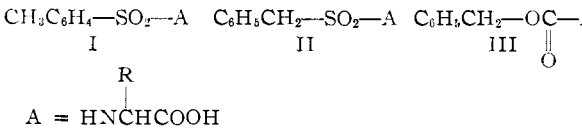
Among the many reagents used for peptide syntheses,[3] carbobenzoxy chloride[4] and *p*-toluenesulfonyl chloride[5,6] are particularly important.

Carbobenzoxy chloride has been most widely used, but it has the disadvantage of instability and difficulty of crystallizing certain derivatives.[7] In the case of certain N-carbobenzoxyamino acids—*e.g.*, N-carbobenzoxy-L-leucine—the conversion of the acid to acid chloride is not entirely satisfactory.[3]

In attempting to surmount these difficulties, recent modifications of the method have been introduced. These include the use of the carboallyloxy[8] group, the carbo-*p*-bromobenzoxy[9] group and the carbo-*p*-nitrobenzoxy[7,10] group.

p-Toluenesulfonyl chloride is commercially available and stable, but the *p*-toluenesulfonyl derivatives of several amino acids have been reported as oils,[11] and the yields are low.

In attempt to find a new reagent with the advantages of carbobenzoxy chloride and *p*-toluenesulfonyl chloride, but without the disadvantages, benzylsulfonyl chloride now has been studied as a reagent in peptide syntheses. It seemed possible that it might prove to be useful because the structure of N-benzylsulfonylamino acids (II) are related somewhat to both the corresponding N-carbobenzoxyamino acids (III) and N-*p*-toluenesulfonylamino acids (I).



I II III

In compounds of the types I and III, either the *p*-toluenesulfonyl group of I or the carbobenzoxy group of III can be removed by reduction with sodium in liquid ammonia. Kraus and White[12] reported that sodium benzenesulfonate reacts with sodium in liquid ammonia to yield benzene. Suter and Milne[13] have shown that sodium benzylsulfonate is cleaved by sodium in liquid ammonia. It follows that II might be cleaved by sodium in liquid ammonia as easily as I and III.

N-Benzylsulfonyl derivatives of 25 amino acids have been prepared, including N-benzylsulfonylglycine and N-benzylsulfonyl-D,L-alanine which were prepared by Johnson and co-workers.[14,15] These are excellent crystalline solids and include N-benzylsulfonyl derivatives of L-hydroxyproline, D,L- and L-isoleucine, L-leucine and L-proline, which yield N-carbobenzoxy derivatives that are oils.[7]

The yields of the N-benzylsulfonylamino acids in Table I are approximately the same as the reported yields for the corresponding N-*p*-toluenesulfonylamino acids, but they are lower than the yields reported for the N-carbobenzoxyamino acids. Because this list of crystalline N-benzylsulfonyl derivatives in Table I is more complete than any corresponding list of other amino acid derivatives, these results should be very useful for the identification and characterization of amino acids.

The N-benzylsulfonyl group is cleaved readily from amino acid derivatives by sodium in liquid ammonia. L-Tyrosine and L-leucine, recovered from the cleavage of their N-benzylsulfonyl derivatives by this method, did not show any loss of optical activity. When this procedure was used with the N-benzylsulfonyl derivative of L-leucyl-L-leucine, the dipeptide was recovered in good yield without evidence of racemization. When sodium was added slowly to N-benzylsulfonyl-D,L-methionine in a relatively large amount of liquid ammonia, a 78% yield of D,L-methionine was recovered.

Raney nickel has also been found to be a very effective reagent for cleaving the N-benzylsulfonyl group from amino acid derivatives. No racemization was observed nor was the peptide linkage in N-benzylsulfonyl-L-leucyl-L-leucine broken under these conditions. It was necessary to carry out the cleavage at room temperature because unidentified products were formed when N-benzylsulfonylamino acids were refluxed with Raney nickel.

When N-benzylsulfonylglycine was cleaved with hydriodic acid in glacial acetic acid, hydrogen sulfide was liberated. This same phenomenon was observed by Fischer[5] when benzylsulfonamide was reduced with hydriodic acid and phosphonium iodide.

Our results indicate that the ease of cleavage of the benzylsulfonyl group is almost the same as that of the *p*-toluenesulfonyl group. It was cleaved readily by sodium in liquid ammonia and Raney nickel, slowly by hydriodic acid or hydrobromic acid, and not at all by hydrogenation with Adams platinum oxide.

In order to compare this method with other methods of preparing dipeptides, and also to test the usefulness of benzylsulfonyl chloride in the preparation of a dipeptide containing a carbon–sulfur bond, L-leucyl-L-leucine and a diastereomeric mixture of L-methionyl-L-methionine and L-methionyl-D-methionine were prepared. In the synthesis of these two dipeptides, all intermediate N-benzylsulfonyl derivatives except N-benzylsulfonyl-L-methionyl chloride were obtained readily in crystalline form. Over-all yields of 40–43 and 33–40% of the theoretical amount of L-leucyl-L-leucine and L-methionyl-D,L-methionine, respectively (on the basis of the amino acid used), were obtained.

Experimental[16]

Preparation of N-Benzylsulfonyl Derivatives of Amino Acids.[17] By Procedure A.—The chosen amino acid

(3) J. S. Fruton, "Advances in Protein Chemistry," Vol. V, eds. M. L. Anson, J. T. Edsall and K. Bailey, Academic Press, Inc., New York, N. Y., 1949, p. 1.

(4) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(5) E. Fischer, *ibid.*, **48**, 93 (1915).

(6) R. Schönheimer, *Z. physiol. Chem.*, **154**, 203 (1926).

(7) F. H. Carpenter and D. T. Gish, THIS JOURNAL, **74**, 3818 (1952).

(8) C. M. Stevens and R. Watanabe, *ibid.*, **72**, 725 (1950).

(9) D. M. Channing, P. B. Turner and G. T. Young, *Nature*, **167**, 487 (1951).

(10) D. T. Gish and F. H. Carpenter, THIS JOURNAL, **75**, 950 (1953).

(11) E. W. McChesney and W. K. Swann, *ibid.*, **59**, 1116 (1937).

(12) C. A. Kraus and G. F. White, *ibid.*, **45**, 768 (1923).

(13) C. M. Suter and H. B. Milne, *ibid.*, **65**, 582 (1943).

(14) T. B. Johnson and J. A. Ambler, *ibid.*, **36**, 372 (1914).

(15) T. B. Johnson and G. C. Bailey, *ibid.*, **38**, 2135 (1916).

(16) All melting points were determined in a micro hot stage (except as otherwise noted) and are corrected. All samples having a melting point higher than 100° were dried for analysis *in vacuo* over phosphorus pentoxide at 80° and the others at 60°. Microanalyses were performed by the Galbraith Microanalytical Laboratories, Knoxville Tennessee, or the Weiler and Strauss Microanalytical Laboratory, Oxford, England. Benzylsulfonyl chloride (m.p. 92–93°) was furnished by the Heyden Chemical Corporation.

(17) In both procedures, D,L-alanine, L-alanine, D-alanine, β-alanine

(usually 0.01–0.03 mole) was dissolved in 1.2 equivalents of *N* sodium hydroxide solution, and the solution was cooled in an ice-bath. To the mixture, 1.2 equivalents of pulverized benzylsulfonyl chloride and 1.4 equivalents of *N* sodium hydroxide solution were added in five approximately equal portions. The reaction mixture was cooled and shaken vigorously for 15 to 20 minutes between successive additions (except in the case of L-proline, when 30 minutes was allowed). Finally the mixture was shaken for an additional hour at room temperature and made basic to litmus paper with *N* sodium hydroxide solution. Unreacted material was separated by filtration. The filtrate was washed in a separatory funnel with two 20-ml. portions of ether, and dissolved ether was removed from the aqueous layer under reduced pressure. The solution was acidified (congo red) slowly with concentrated hydrochloric acid. The derivative which separated was recrystallized from an appropriate solvent as indicated in Table I.

Procedure B.—A solution consisting of 0.03 equivalent of the amino acid, 30 ml. of *N* sodium hydroxide solution and 30 ml. of dioxane was prepared. Fifty milliliters of *N* sodium hydroxide solution and 0.045 equivalent of benzylsulfonyl chloride in 50 ml. of dioxane were added simultaneously in ten approximately equal portions. Between the additions of reagents, the mixture was shaken vigorously for 15 to 20 minutes (mechanically) in an ice-bath; then the mixture was shaken for one to two additional hours at room temperature. After the solution had been made basic to litmus paper, it was filtered, and the filtrate was washed with two 40-ml. portions of ether. The aqueous solution was acidified (congo red) with concentrated hydrochloric acid, saturated with sodium chloride and extracted with three 50-ml. portions of ethyl acetate. The ethyl acetate solution was washed with 30 ml. of water, and 100 ml. of petroleum ether (b.p. 30–60°) was added. The derivative was then extracted with three 50-ml. portions of *N* sodium bicarbonate solution, and the aqueous solution was evaporated *in vacuo* until the odor of ethyl acetate no longer persisted. This aqueous solution was acidified (congo red) slowly with concentrated hydrochloric acid and stirred vigorously. The derivative which separated was recrystallized from an appropriate solvent as indicated in Table I.

Cleavage of N-Benzylsulfonyl Groups from Amino Acid Derivatives. (a) With Sodium in Liquid Ammonia.—The general procedure of Sifferd and du Vigneaud[18] was used, and the results with several amino acid derivatives are listed in Table II. A solution of N-benzylsulfonylamino acid in liquid ammonia was treated slowly with small pieces of metallic sodium under mechanical stirring until a blue color persisted for two or three minutes. The ammonia was allowed to evaporate spontaneously. The residue was dried *in vacuo* and then dissolved in 20 to 30 ml. of water. The *p*H of the solution was adjusted to the isoelectric point of the amino acid with 3 *N* hydrochloric acid. The crude amino acid was collected, after the addition of ethanol or saturation with sodium chloride, and it was recrystallized from water–ethanol or water.

(b) With Raney Nickel.—The Raney nickel was prepared according to the procedure of Covert and Adkins.[19] A solution containing the N-benzylsulfonylamino acid, 15 to 20 ml. of *N* sodium hydroxide solution, 80 ml. of 95% ethanol and 20 ml. of freshly prepared Raney nickel was stirred mechanically for 20 hours at room temperature under a nitrogen atmosphere. The Raney nickel was removed by centrifugation and washed with 10 to 20 ml. of *N* sodium hydroxide solution. The washings were combined with the alcoholic solution. The solution was evaporated at reduced pressure to remove the ethanol. The resulting solution was treated with 0.5 g. of ammonium chloride and 2 ml. of concentrated ammonia water and then saturated with hydrogen sulfide. The resulting dark mixture was allowed to stand for several hours, and then it was acidified (congo red) with concentrated hydrochloric acid. The dark precipitate was removed by centrifugation, and the colorless solution was concentrated at reduced pressure to a volume of about 20 ml. The *p*H of this solution was adjusted to the isoelectric point of the amino acid with 3 *N* sodium hydroxide, and the product was crystallized, after the addition of 30 to 40 ml. of ethanol or saturation with sodium chloride. The amino acid was recrystallized from water–ethanol or water. Details are shown in Table III.

(c) With 50% Hydriodic Acid.—N-Benzylsulfonylglycine (2.29 g.) in 50 ml. of glacial acetic acid and 40 g. of 50% hydriodic acid were refluxed for eight hours.[20] The solvent and unreacted hydriodic acid were partially removed by distillation; water was added simultaneously to keep the volume of liquid unchanged. The resulting solution was cooled to room temperature and filtered to remove unreacted starting material. Sodium hydroxide solution (50%) was added to neutralize the acidic solution until the *p*H was 6.06. The resulting solution gave a positive ninhydrin test and was used to prepare hippuric acid. The yield of hippuric acid was 1.12 g. (63% based on the weight of N-benzylsulfonylglycine), m.p. 187–188°. A mixed melting point determination with an authentic sample of hippuric acid showed no depression.

(d) With 48% Hydrobromic Acid.—The N-benzylsulfonylamino acid, 48% hydrobromic acid and phenol were heated under reflux for the period indicated in Table IV. The mixture then was cooled rapidly and filtered to remove unreacted starting material. The filtrate was washed into a separatory funnel with two 30-ml. portions of carbon tetrachloride, and then the mixture was neutralized carefully with 50% sodium hydroxide solution to the isoelectric point of the amino acid. The amino acid was collected, after addition of ethanol, by filtration and recrystallized from water–ethanol.

N-Benzylsulfonyl-L-leucyl Chloride.[21]—This acid chloride was prepared according to the procedure which Carpenter and Gish[7] used to prepare carbo-*p*-nitrobenzoxy-L-leucyl chloride. N-Benzylsulfonyl-L-leucine (5.70 g.) was dissolved in 200 ml. of anhydrous ether. Pulverized phosphorus pentachloride (4.55 g.) was added, and the reaction was allowed to proceed with swirling in an ice-bath for one hour. Then the mixture was brought quickly to room temperature and filtered rapidly through a sintered glass filter. Ether was removed *in vacuo*. The crystals were washed with dry petroleum ether and then recrystallized from anhydrous ether–petroleum ether (b.p. 30–60°); 5.8 g. (96% yield), m.p. 67°, $[\alpha]^{25}D$ −20.7 ± 0.6° (*c* 1.00, butanone).

Anal. Calcd. for $C_{13}H_{18}ClNO_3S$: C, 51.39; H, 5.97; N, 4.61. Found: C, 51.10; H, 6.03; N, 4.63.

N-Benzylsulfonyl-L-leucyl-L-leucine Ethyl Ester.—A mixture of 11.74 g. of L-leucine ethyl ester hydrochloride, prepared by the method described by Foreman,[22] 300 ml. of anhydrous ether and 11 ml. of triethylamine was cooled in an ice-bath and stirred mechanically. To this mixture an ethereal solution of freshly prepared N-benzylsulfonyl-L-leucyl chloride (15.32 g. in 100 ml. of anhydrous ether) was added dropwise. When the addition of the ethereal solution was completed, the mixture was stirred for five hours at room temperature. The mixture was filtered quickly. The residue was washed with 50 ml. of water and then dissolved in 50 ml. of ether. This ethereal solution was combined with the filtrate from the reaction mixture. The resulting solution was washed successively with two 40-ml. portions of *N* hydrochloric acid, 40 ml. of water, two 40-ml. portions of *N* sodium bicarbonate solution and two 40-ml. portions of water. After the solution had been dried over

and L-histidine derivatives separated after the addition of sodium chloride (2.5 g. per 10 ml. of solution); derivatives of D,L-α-aminobutyric acid, L-arginine, L-hydroxyproline, L-lysine, D,L-methionine, L-methionine, D-methionine, D,L-norvaline, D,L-ornithine, D,L-phenylalanine, L-phenylalanine and D,L-valine usually separated as oils and solidified after standing in the cold. (If they did not solidify in the cold, they were extracted with ether or ethyl acetate and solidified after evaporation of the solvent *in vacuo*.) The derivatives of D,L-aspartic acid, D,L-glutamic acid, D,L-serine and D,L-threonine were so soluble in water that they did not separate from aqueous solution after acidification. These derivatives were prepared by procedure B. After the addition of sodium chloride, the derivatives were extracted with three 50-ml. portions of ethyl acetate in the case of D,L-aspartic acid and D,L-glutamic acid, or with three 50-ml. portions of ether in the case of D,L-serine and D,L-threonine. The resulting solutions were dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield the crystalline products.

(18) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(19) L. W. Covert and H. Adkins, THIS JOURNAL, **54**, 4116 (1932).

(20) The presence of hydrogen sulfide in the vapor was demonstrated by the darkening of wetted lead acetate paper.

(21) This acid chloride decomposes gradually over a period of several weeks at room temperature, but is stable in the refrigerator.

(22) F. W. Foreman, *Biochem. J.*, **13**, 378 (1919).

TABLE I

N-BENZYLSULFONYL DERIVATIVES OF AMINO ACIDS

N-Benzylsulfonyl derivative of	M.p., °C.	Yield,[a] %, for Proc. A	Yield,[a] %, for Proc. B	Solvent for recrystn.	Formula	Analyses: Neut. equiv. Calcd.	Neut. equiv. Found	Nitrogen, % Calcd.	Nitrogen, % Found
D,L-Alanine[b]	164–165	41	40	Hot water	$C_{10}H_{13}NO_4S$	243.27	243	5.76	5.81
L-Alanine[c]	127–128		29	Hot water	$C_{10}H_{13}NO_4S$	243.27	243	5.76	5.58
D-Alanine[d]	128		36	Hot water	$C_{10}H_{13}NO_4S$	243.27	244	5.76	5.76
β-Alanine	149–150	38		Ethanol–water	$C_{10}H_{13}NO_4S$	243.27	244	5.76	5.75
D,L-α-Amino-butyric acid	116–117	48		Ethanol–water	$C_{11}H_{15}NO_4S$	257.30	260	5.44	5.60
L-Arginine[e]	84–86	27		Acetone–ether	$C_{20}H_{26}N_4O_6S_2$	482.56	479	11.61	11.50
D,L-Aspartic acid	106–107	26		Ethyl acetate–chloroform	$C_{11}H_{13}NO_6S$	143.64	145	4.88	4.74
L-Cystine[f]	172–173	61		Water–acetone	$C_{20}H_{24}N_2O_8S_4$	274.32	270	5.11	5.00
D,L-Ethionine	95		65	Ether–petroleum ether	$C_{13}H_{19}NO_4S_2$	317.41	316	4.41	4.45
D,L-Glutamic acid	131–132		40	Ethyl acetate–chloroform	$C_{12}H_{15}NO_6S$	150.65	151	4.65	4.65
Glycine[g]	151–152	44		Hot water	$C_9H_{11}NO_4S$	229.25	230	6.11	5.95
Glycylglycine	192–193	70		Ethanol–water	$C_{11}H_{14}N_2O_5S$	286.30	285	9.79	9.82
L-Histidine[h]	182–185	38		Water–acetone	$C_{13}H_{15}N_3O_4S$	309.33	304	13.59	13.56
L-Hydroxyproline[i]	143–144	32		Ether–hexane	$C_{12}H_{15}NO_5S$	285.31	287	4.91	4.98
D,L-Isoleucine[j]	113	30		Ethanol–water	$C_{13}H_{19}NO_4S$	285.35	285	4.91	4.84
L-Isoleucine[k]	146–147	39		Ethanol–water	$C_{13}H_{19}NO_4S$	285.35	285	4.91	4.80
D,L-Leucine	113–114	52	58	Ethanol–water	$C_{13}H_{19}NO_4S$	285.35	284	4.91	4.82
L-Leucine[l]	133–134	63	72	Ethanol–water	$C_{13}H_{19}NO_4S$	285.35	284	4.91	4.83
D-Leucine[m]	133–134	41	55	Ethanol–water	$C_{13}H_{19}NO_4S$	285.35	285	4.91	4.81
L-Lysine[n]	144–145	27	50	Ethanol–water	$C_{20}H_{26}N_2O_6S_2$	454.55	458	6.16	6.24
D,L-Methionine	110–111	42	47	Ethanol–water	$C_{12}H_{17}NO_4S_2$	303.39	302	4.62	4.58
L-Methionine[o]	90–91	58	70	Ether–hexane	$C_{12}H_{17}NO_4S_2$	303.39	304	4.62	4.48
D-Methionine[p]	90–91		49	Ether–hexane	$C_{12}H_{17}NO_4S_2$	303.39	307	4.62	4.70
D,L-Norleucine	103–104	40		Ethanol–water	$C_{13}H_{19}NO_4S$	285.35	285	4.91	4.95
D,L-Norvaline[q]	84–85	41		Ethanol–water	$C_{12}H_{17}NO_4S$	271.33	272	5.16	5.00
D,L-Ornithine	54–55	48		Ethanol–water	$C_{19}H_{24}N_2O_6S_2$	440.52	442	6.36	6.40
D,L-Phenylalanine	151–152	47		Ethanol–water	$C_{16}H_{17}NO_4S$	319.37	320	4.39	4.35
L-Phenylalanine[r]	156–157	28	58	Ethanol–water	$C_{16}H_{17}NO_4S$	319.37	319	4.39	4.49
L-Proline[s]	107–108	22		Ether	$C_{12}H_{15}NO_4S$	269.31	271	5.20	5.20
D,L-Serine	123–124		47	Ethyl acetate–petroleum ether	$C_{10}H_{13}NO_5S$	259.27	259	5.40	5.32
D,L-Threonine	143–144		51	Ethyl acetate–petroleum ether	$C_{11}H_{15}NO_5S$	273.30	273	5.13	5.11
D,L-Tryptophan[t]	95–96	65	52	Ethanol–water	$C_{18}H_{20}N_2O_5S$	376.42	380	7.44	7.50
D,L-Tyrosine	195–196	43		Acetone–water	$C_{23}H_{23}NO_7S_2$	489.54	486	2.86	2.90
L-Tyrosine[u]	191–192	40		Acetone–water	$C_{23}H_{23}NO_7S_2$	489.54	484	2.86	2.74
D,L-Valine[v]	123–124	53	58	Ethanol–water	$C_{12}H_{17}NO_4S$	271.33	272	5.16	5.06

[a] Yields are based on the weights of the products after one recrystallization. [b] This derivative was reported by Johnson and Bailey,[15] m.p. 164–165°. [c] $[\alpha]^{26}D$ −38.5 ± 1.0° (*c* 1.00, *N* sodium hydroxide). [d] $[\alpha]^{26}D$ +38.7 ± 1.0° (*c* 1.00, *N* sodium hydroxide). [e] $[\alpha]^{26}D$ −8.7 ± 1.0° (*c* 1.00, *N* sodium hydroxide). The derivative precipitated from acetone–ether as an oil; when it was dried under reduced pressure, it was converted to a glass-like solid. [f] $[\alpha]^{26}D$ +72.2 ± 1.2° (*c* 1.00, *N* sodium hydroxide). [g] This derivative was reported by Johnson and Ambler,[14] m.p. 149–150°, and also by Saunders, Stacey and Wilding,[23] m.p. 152°. [h] $[\alpha]^{25}D$ −22.2 ± 0.9° (*c* 1.00, *N* sodium hydroxide). [i] $[\alpha]^{24}D$ −40.6 ± 0.9° (*c* 1.00, *N* sodium hydroxide). [j] The derivative was recrystallized from ethanol–water as a hydrate, m.p. 74–75°, which loses water of hydration at about 90°. The presence of water of hydration was indicated by the fact that when the sample was dried to constant weight at 100° it decreased 7.30% in weight; the calculated loss for 1.25 moles of water of hydration is 7.31%. [k] $[\alpha]^{24}D$ −39.9 ± 0.9° (*c* 1.00, *N* sodium hydroxide). [l] $[\alpha]^{26}D$ −23.1 ± 0.9° (*c* 1.00, *N* sodium hydroxide). [m] $[\alpha]^{26}D$ +23.7 ± 0.6° (*c* 1.00, *N* sodium hydroxide). [n] $[\alpha]^{26}D$ −9.9 ± 0.6° (*c* 1.00, *N* sodium hydroxide). [o] $[\alpha]^{26}D$ −13.5 ± 0.5° (*c*, 1.00, *N* sodium hydroxide). [p] $[\alpha]^{26}D$ +13.6 ± 1.0° (*c* 1.00, *N* sodium hydroxide). [q] The derivative was recrystallized from ethanol–water in the cold as a needle-like crystalline hydrate, m.p. 50–51°. The presence of water of hydration was indicated by the fact that when the sample was dried to constant weight at 100° it decreased 6.58% in weight; the calculated loss for 1.0 mole of water of hydration is 6.23%. [r] $[\alpha]^{25}D$ −12.6 ± 0.3° (*c* 1.50, *N* sodium hydroxide). [s] $[\alpha]^{25}D$ −72.3 ± 0.8° (*c* 1.00, *N* sodium hydroxide). The derivative was dissolved in a small amount of ether, and crystallization was induced by slow evaporation of the solvent. [t] The derivative was a monohydrate. *Anal.* Calcd.: C, 57.43; H, 5.36; H_2O, 4.79. Found: C, 57.40; H, 5.32; H_2O, 4.82. [u] $[\alpha]^{24}D$ −9.2 ± 0.8° (*c* 1.00, *N* sodium hydroxide). [v] The derivative was recrystallized from ethanol–water as a hydrate, m.p. 65.5–66.5°, and the water of hydration was removed *in vacuo* over phosphorus pentoxide. The presence of water of hydration was indicated by the fact that when the sample of the derivative was dried to constant weight at 100° it decreased 7.40% in weight; the calculated loss for 1.20 moles of water of hydration is 7.38%.

anhydrous sodium sulfate, the ether was removed *in vacuo*. The product separated as crystals and was recrystallized from ether–petroleum ether (b.p. 30–60°); 16.41 g. (76% yield), m.p. 122–123°, $[\alpha]^{27}D$ −48.7 ± 1.0° (*c* 3.33, absolute ethanol).

(23) B. C. Saunders, G. J. Stacey and I. G. E. Wilding, *Biochem. J.*, **36**, 368 (1942).

TABLE II

CLEAVAGE OF N-BENZYLSULFONYLAMINO ACIDS WITH SODIUM IN LIQUID AMMONIA

N-Benzylsulfonyl derivative of	Wt., g.	Sodium, g.	Liquid ammonia, ml.	Yield,[a] %	Formula of the product	Analyses, % Calcd.	Found
Glycine	1.00	0.35	200	73	$C_2H_5NO_2$[b]		
Glycylglycine	1.00	.30	200	79	$C_4H_8N_2O_3$[c]		
L-Leucine	2.85	.60	200	73	$C_6H_{13}NO_2$[d]	C, 54.94	55.03
						H, 10.00	10.17
						N, 10.68	10.70
D,L-Methionine	1.52	.29	500	78	$C_5H_{11}NO_2S$	C, 40.25	40.39
						H, 7.43	7.46
						N, 9.39	9.49
D,L-Phenylalanine	1.00	.32	200	78	$C_9H_{11}NO_2$	C, 65.43	65.82
						H, 6.71	6.71
						N, 8.48	8.31
L-Tyrosine	0.80	1.00	100	71	$C_9H_{11}NO_3$[e]	C, 59.66	59.43
						H, 6.12	5.95
						N, 7.73	7.61

[a] Yields are based on the weights of the products after one recrystallization. [b] Glycine was characterized by converting it to hippuric acid, m.p. 186–187°. A mixed melting point determination with an authentic sample of hippuric acid showed no depression. [c] Glycylglycine was characterized by converting it to N-benzoylglycylglycine, m.p. 207–208°. (Fischer[24] reported m.p. 208°.) [d] $[\alpha]^{25}D$ −10.4 ± 0.4° (*c* 2.043, water). The reported[25] value is $[\alpha]^{25}D$ −10.58° (*c* 2.043, water. [e] $[\alpha]^{18}D$ −13.3 ± 0.7° (*c* 0.906, 3 *N* sodium hydroxide). The reported[26] value is $[\alpha]^{18}D$ −13.2° (*c* 0.906, 3 *N* sodium hydroxide).

TABLE III

CLEAVAGE OF N-BENZYLSULFONYLAMINO ACIDS WITH RANEY NICKEL

N-Benzylsulfonyl derivative of	Wt., g.	Yield,[a] %	Formula of the product	Analyses, % Calcd.	Found
Glycine	3.45	73	$C_2H_5NO_2$[b]		
L-Leucine	2.85	72	$C_6H_{13}NO_2$[c]	C, 54.94	55.06
				H, 10.00	9.94
				N, 10.68	10.70
L-Phenylalanine	2.00	65	$C_9H_{11}NO_2$[d]	C, 65.43	65.33
				H, 6.71	6.64
				N, 8.48	8.30
D,L-Threonine	2.00	77	$C_4H_9NO_3$[e]		
L-Tyrosine	1.50	87	$C_9H_{11}NO_3$[f]	C, 59.66	59.66
				H, 6.12	6.12
				N, 7.73	7.85
D,L-Tyrosine	2.45	84	$C_9H_{11}NO_3$	C, 59.66	59.69
				H, 6.12	6.15
				N, 7.73	7.70

[a] Yields are based on the weights of the products after one recrystallization. [b] Glycine was characterized by converting it to hippuric acid, m.p. 187–188°. A mixed melting point determination with an authentic sample of hippuric acid showed no depression. [c] $[\alpha]^{25}D$ −10.3 ± 0.6° (*c* 2.043, water). The reported[25] value is $[\alpha]^{25}D$ −10.58° (*c* 2.043, water). [d] $[\alpha]^{20}D$ −35.9 ± 1.3° (*c* 1.936, water). The reported[27] value is $[\alpha]^{20}D$ −35.14° (*c* 1.936, water). [e] D,L-Threonine was characterized by converting it to N-benzoyl-D,L-threonine, m.p. 143°. (West and Carter[28] reported m.p. 143–144°.) A mixed melting point determination with an authentic sample of N-benzoyl-D,L-threonine showed no depression. [f] $[\alpha]^{18}D$ −13.2 ± 0.8° (*c* 0.906, 3 *N* sodium hydroxide). The reported[26] value is $[\alpha]^{18}D$ −13.2° (*c* 0.906, 3 *N* sodium hydroxide).

Anal. Calcd. for $C_{21}H_{34}N_2O_5S$: C, 59.13; H, 8.03; N, 6.57. Found: C, 59.38; H, 7.99; N, 6.60.

N-Benzylsulfonyl-L-leucyl-L-leucine.—N-Benzylsulfonyl-L-leucyl-L-leucine ethyl ester (6.73 g.) was suspended in 300 ml. of 0.5% potassium hydroxide solution, and this mixture was heated under reflux for 15 to 20 minutes until it became homogeneous. The solution was cooled rapidly to room temperature and extracted with 40 ml. of ether. Dissolved ether was evaporated at reduced pressure. A milky solution resulted following acidification of the aqueous solution (congo red) with concentrated hydrochloric acid and, after a short time, white silky crystals separated. They were recrystallized from acetone–water; 6.01 g. (96% yield), m.p. 140–141°, $[\alpha]^{24}D$ −52.9 ± 0.8° (*c* 1.00, *N* sodium hydroxide).

Anal. Calcd. for $C_{19}H_{30}N_2O_5S$: C, 57.26; H, 7.59; N, 7.03; neut. equiv., 398.51. Found: C, 57.29; H, 7.67; N, 6.98; neut. equiv., 394.

L-Leucyl-L-leucine Hydrate. (a) Using Sodium in Liquid Ammonia.—A solution of 1.00 g. of N-benzylsulfonyl-L-leucyl-L-leucine in 200 ml. of liquid ammonia was treated with 0.19 g. of sodium in the usual manner. The yield was 0.55 g. (85% based on the hydrate). For analysis the peptide was recrystallized from 95% ethanol, from which it separated as a hydrate.[30] On the hot stage, the crystals, which had been dried in air, were observed to undergo a change in crystalline form at about 155°. The new crystals sublimed from between the cover slips without melting. When the crystals were put in a sealed capillary tube and heated in an oil-bath, they melted at 253–256° cor. with

TABLE IV

CLEAVAGE OF N-BENZYLSULFONYLAMINO ACIDS WITH 48% HYDROBROMIC ACID

N-Benzylsulfonyl derivative of	Wt., g.	Phenol, g.	Time of reflux, hr.	48% HBr, ml.	Product	Yield,[a] %
Glycine	2.29	1.0	0.67	25	Glycine[b]	47[c]
L-Leucine	2.85	2.0	8	40	L-Leucine[d]	35

[a] Yields are based on the weights of the products after one recrystallization. [b] Glycine was not isolated, but was characterized by converting it to hippuric acid, m.p. 187°. A mixed melting point determination with an authentic sample of hippuric acid showed no depression. [c] The yield is based on hippuric acid. [d] L-Leucine was characterized by converting it to 3,5-dinitrobenzoyl-L-leucine, m.p. 185–186°. (Saunders[29] reported m.p. 186–187°.) A mixed melting point determination with an authentic sample of 3,5-dinitrobenzoyl-L-leucine showed no depression.

(24) E. Fischer, *Ber.*, **38**, 605 (1905).

(25) M. P. Stoddard and M. S. Dunn, *J. Biol. Chem.*, **142**, 329 (1942).

(26) O. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930).

(27) E. Fischer and W. Schoeller, *Ann.*, **357**, 1 (1907).

(28) H. D. West and H. E. Carter, *J. Biol. Chem.*, **119**, 109 (1937).

(29) B. C. Saunders, *Biochem. J.*, **28**, 580 (1934).

(30) The same phenomenon was noted by Fischer[31] in his original preparation of L-leucyl-L-leucine and also by Carpenter and Gish[7] in their preparation of L-leucyl-L-leucine using carbo-*p*-nitrobenzoxy chloride.

(31) E. Fischer, *Ber.*, **39**, 2893 (1906).

decomposition,[32] $[\alpha]^{22}D$ −13.2 ± 0.9° (*c* 0.95, *N* sodium hydroxide). The sample for analysis and the determination of specific rotation was dried in air.

Anal. Calcd. for $C_{12}H_{24}N_2O_3 \cdot 0.75H_2O$: C, 55.90; H, 9.97; N, 10.87; H_2O, 5.24. Found: C, 55.99; H, 10.03; N, 10.53; H_2O, 5.39.

(b) **Using Raney Nickel.**—One g. of N-benzylsulfonyl-L-leucyl-L-leucine was reduced with 20 ml. of Raney nickel in the usual manner. The sample for analysis and the determination of specific rotation was dried in air. The yield was 0.55 g. (85% based on the hydrate), m.p. 253–256° with decomposition, measured in a capillary tube and corrected),[32] $[\alpha]^{22}D$ −13.1 ± 1.0° (*c* 0.95, *N* sodium hydroxide).

Anal. Calcd. for $C_{12}H_{24}N_2O_3 \cdot 0.75H_2O$: C, 55.90; H, 9.97; N, 10.87; H_2O, 5.24. Found: C, 55.94; H, 10.10; N, 10.80, H_2O, 5.36.

N-Benzylsulfonyl-L-methionyl Chloride.—N-Benzylsulfonyl-L-methionine (0.78 g.) was used to prepare N-benzylsulfonyl-L-methionyl chloride in the same manner as that used for the preparation of N-benzylsulfonyl-L-leucyl chloride. The sirupy product was precipitated from ether–petroleum ether (b.p. 30–60°), 0.81 g. (98% yield), and characterized by converting it to N-benzylsulfonyl-L-methioninamide.

N-Benzylsulfonyl-L-methioninamide.—The product from the above reaction was dissolved in 30 ml. of anhydrous ether, and the solution was dropped into 50 ml. of cold, concentrated ammonia water with vigorous stirring. The mixture was stirred for two hours at room temperature. The ethereal layer was separated, and after evaporation at reduced pressure to remove ether, no appreciable quantity of the product was obtained. The aqueous layer was evaporated on the steam-bath to near dryness and then cooled to room temperature. The residue was washed with 30 ml. of water and recrystallized from ethanol–water. The yield was 0.58 g. (75% based on the weight of N-benzylsulfonyl-L-methionine), m.p. 141–142°, $[\alpha]^{25}D$ +14.4 ± 1.2° (*c* 1.00, butanone).

Anal. Calcd. for $C_{12}H_{18}N_2O_3S_2$: C, 47.66; H, 6.00; N, 9.26. Found: C, 47.74; H, 6.20; N, 9.20.

N-Benzylsulfonyl-L-methionyl-D,L-methionine Ethyl Ester.—D,L-Methionine ethyl ester hydrochloride[33] (2.12 g.), prepared by the method described by Foreman,[22] was dissolved in 10 ml. of anhydrous chloroform, and 40 ml. of anhydrous ether and 3 ml. of triethylamine were added. The mixture was cooled in an ice-bath and stirred mechanically. To this mixture, 50 ml. of an ethereal solution of N-benzylsulfonyl-L-methionyl chloride, which had just been prepared from 2.0 g. of N-benzylsulfonyl-L-methionine in the usual manner, was added dropwise. The precipitate, triethylamine hydrochloride, was extracted with 30 ml. of water, and the ethereal solution was washed successively with two 30-ml. portions of *N* hydrochloric acid, 30 ml. of water, two 30-ml. portions of *N* sodium bicarbonate solution and two 30-ml. portions of water. After the solution had been dried over anhydrous sodium sulfate, the ether was removed *in vacuo*. The product separated in a solid state and was recrystallized from anhydrous acetone–petroleum ether (b.p. 30–60°); 2.35 g. (77% yield), m.p. 99–100°, $[\alpha]^{25}D$ +9.7 ± 0.5° (*c* 1.00, butanone).

Anal. Calcd. for $C_{19}H_{30}N_2O_5S_3$: C, 49.32; H, 6.54; N, 6.06. Found: C, 49.46; H, 6.69; N, 5.92.

N-Benzylsulfonyl-L-methionyl-D,L-methionine.—N-Benzylsulfonyl-L-methionyl-D,L-methionine ethyl ester (3.5 g.) was dissolved in 100 ml. of methanol, and 30 ml. of *N* potassium hydroxide solution was added. After the solution had been allowed to stand for ten hours at room temperature, the methanol was removed at reduced pressure. The solution was diluted with 20 ml. of water and washed with two 20-ml. portions of ether. The dissolved ether was removed *in vacuo*, and the solution was acidified (congo red) with concentrated hydrochloric acid. An oily precipitate formed; this solidified after standing in the refrigerator for two days. This white precipitate, m.p. 104–106°, amounted to 3.13 g. (95% yield). It was recrystallized from ether–hexane in the cold. It melted at 108–109°, $[\alpha]^{26}D$ −38.2 ± 1.2° (*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, 47.08; H, 6.08; N, 6.43; neut. equiv., 430.

Diastereomeric Mixture[34] of L-Methionyl-L-methionine and L-Methionyl-D-methionine.—N-Benzylsulfonyl-L-methionyl-D,L-methionine (1.50 g.) was dissolved in 400 ml. of liquid ammonia. To this mixture was added, with mechanical stirring during one hour, 0.21 g. of metallic sodium in 30 approximately equal portions. A blue color persisted for one minute after the last addition of sodium. The mixture was treated with 0.3 ml. of methyl iodide and then allowed to evaporate spontaneously. The residue was dried *in vacuo* and dissolved in 10–15 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 with 40 ml. of hot 95% ethanol. The insoluble substance was discarded. The alcoholic solution was evaporated to 10 ml. *in vacuo* and diluted with 40 ml. of ether. White crystals were obtained and recrystallized from ethanol–ether; 0.76 g. (79% yield), m.p. 230–233° (with decomposition), $\alpha^{25}D$ +51.6 ± 0.9° (*c* 1.00, water).

Anal. Calcd. for $C_{10}H_{20}N_2O_3S_2$: C, 42.83; H, 7.19; N, 9.99. Found: C, 42.84; H, 6.92; N, 9.75.

PULLMAN, WASHINGTON

(32) Fischer[31] reported a melting point of 270° cor. for L-leucyl-L-leucine hydrate and a calculated specific rotation for the anhydrous compound of $[\alpha]^{20}D$ −13.36° (*c* 8.83, *N* sodium hydroxide). Carpenter and Gish[7] reported a melting point of 252–254° uncor., measured in a sealed capillary tube, for L-leucyl-L-leucine hydrate and a calculated specific rotation for the anhydrous compound of $[\alpha]^{22}D$ −13.7° (*c* 0.95, *N* sodium hydroxide).

(33) This hydrochloride was recrystallized from chloroform–anhydrous ether, m.p. 71–74°. *Anal.* Calcd. for $C_7H_{16}ClNO_2S$: Cl, 16.59; N, 6.55. Found: Cl, 16.35; N, 6.40.

(34) The diastereomeric mixture was resolved into L-methionyl-L-methionine and L-methionyl-D-methionine using an enzymatic reaction which will be reported in detail at a later date. The resulting L-methionyl-L-methionine had a specific rotation $[\alpha]^{25}D$ +26.1° (*c* 2.00, water), and the L-methionyl-D-methionine had a specific rotation $[\alpha]^{25}D$ +75.8° (*c*, 1.00, water). The average of these two (+50.80°) was essentially the same as the diastereomeric mixture (+51.6°). From this it may be concluded that the diastereomeric mixture contains approximately equal amounts of the two dipeptides.