fied after standing for several days at room temperature. The solid when recrystallized from an absolute ethanol-Skellysolve C mixture weighed 9.0 g. (43%) and melted at 105–107°.

Anal. Calcd. for $C_{10}H_{11}NO_2S$: N, 6.69; S, 15.32. Found N, 6.63; S, 15.21.

2-(2'-Hydroxyethyl)-3,4-dihydro-1,4,2-benzothiazine Hydrochloride (VII).—This compound was prepared in two ways: (a) A suspension of 6.5 g. (0.17 mole) of lithium aluminum hydride in 600 ml. of dry ether was stirred in a flask containing a condenser and drying tube while 25 g. (0.1 mole) of solid VIII was added in portions. After the addition the suspension was stirred and refluxed for 3 hours, the excess reductant and the complex salts decomposed with 20 ml. of ethanol, 20 ml. of water and 2 ml. of glacial acetic acid and the suspension filtered. After the filtrate was washed twice with water it was dried over anhydrous magnesium sulfate. The addition of an ethereal solution of hydrogen chloride precipitated an oil that solidified. The product (12 g., 52%) which was light-pink after it was recrystallized from an absolute ethanol-ether mixture melted at 145–148°.

Anal. Calcd. for $C_{10}H_{14}ClNOS$: N, 6.04; S, 13.83. Found: N, 6.02; S, 13.88.

(b) A slurry of 4 g. (0.02 mole) of VI in 200 ml. of dry ether was added to a suspension of 1 g. (0.026 mole) of lithium aluminum hydride and the reduction conducted the same as in method (a) (above). The precipitated pink hydrochloride salt (1.25 g., 28%) after it was recrystallized from an absolute ethanol-ether mixture showed no melting-point depression when mixed with a sample prepared by a similar reduction of VIII. These two samples also were shown to be identical by their ultraviolet and infrared absorption spectra.

3,4-Dihydro-1,4,2-benzothiazine[18,19,24,25] was prepared from 3,4-dihydro-3-oxo-1,4,2-benzothiazine[17] in nearly quantitative yield by reduction with lithium aluminum hydride in ether; m.p. 36–37° (lit.[18] m.p. 35°).

Anal. Calcd. for C_8H_9NS: N, 9.26. Found: N, 9.19.

The 4-phenylthiocarbamyl derivative melted at 128–129° (lit.[18,24] m.p. 129°).

Anal. Calcd. for $C_{15}H_{14}N_2S_2$: N, 9.78. Found: N, 9.82.

Acknowledgment.—The authors wish to thank Mr. M. E. Auerbach and Mr. K. D. Fleischer and staffs for the analytical data and corrected melting points, Dr. F. C. Nachod and staff for absorption spectra, and Mrs. Grace W. Schraver for her technical assistance.

(24) O. Hromatka, M. Vacutny, J. Angle and K. Wiltschke, *Monatsh.*, **88**, 822 (1957).

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RENSSELAER, N. Y.

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

Studies on Diastereoisomeric α-Amino Acids and Corresponding α-Hydroxy Acids. X. The Preparation of β-Hydroxy-β-methylaspartic Acid

BY LEO BENOITON, MILTON WINITZ, ROBERTA F. COLMAN, SANFORD M. BIRNBAUM AND JESSE P. GREENSTEIN

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β-Hydroxy-β-methylaspartic acid was prepared in 60% yield by allowing a mixture of pyruvic acid and copper glycinate in *N* NaOH to stand at 5° for 12–18 hours. The compound gives a bright yellow initial color with ninhydrin on paper chromatograms. Proof of the structure of the isolated compound was obtained by means of elemental analyses, reduction to β-methylaspartic acid, reaction with ninhydrin which yielded 2 moles of carbon dioxide per mole of compound, and reaction with periodate to yield ammonia. The synthetic amino acid was composed of nearly equal amounts of the two theoretically possible diastereomers as shown by the ready separation of the latter on columns of Dowex 1-acetate eluted with acetic acid.

Earlier studies on the properties of N-pyruvoylglycine revealed that the characteristic absorption in the ultraviolet of aqueous solutions of this compound, as well as its ability to form a crystalline dinitrophenylhydrazone, were irreversibly lost when the compound was exposed to *p*H levels higher than 10.[1,2] A reinvestigation of this phenomenon with the aid of paper chromatography has since revealed that when a solution of pyruvoylglycine in *N* NaOH was allowed to stand at room temperature glycine began to appear after 1 hour and gradually increased to a maximum after 6 days. At this time alanine made its appearance while glycine decreased, the ratio of glycine to alanine reaching an apparently constant value of approximately 2:1 by the 18th day of standing as determined by the intensity of the ninhydrin spots. At the same time that glycine appeared, a hitherto unrecognized ninhydrin spot also made its appearance, which was initially bright yellow in color, gradually darkening through grayish-brown to the usual amino acid purple after 24 hours. The R_f values of this new compound in several solvents were very close to those of γ-hydroxyglutamic acid under the same conditions.[3,4]

Substantially the same results were obtained with mixtures of glycine and pyruvic acid. Thus, when an equimolar mixture of glycine and pyruvic acid was allowed to stand in *N* NaOH solution at room temperature, alanine and the unknown compound began to appear within a few hours. With further standing, the concentration of alanine increased, that of glycine decreased, whilst that of the unknown compound remained apparently constant.[5]

(1) M. Errera and J. P. Greenstein, *Arch. Biochem. Biophys.*, **14**, 477 (1947); **15**, 445 (1947).

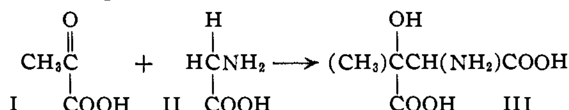
(2) S-C. J. Fu, V. E. Price and J. P. Greenstein, *ibid.*, **31**, 83 (1951).

(3) L. Benoiton and L. P. Bouthillier, *Can. J. Chem.*, **33**, 1473 (1955).

(4) L. Benoiton, M. Winitz, S. M. Birnbaum and J. P. Greenstein, THIS JOURNAL, **79**, 6192 (1957).

(5) The formation of alanine under these conditions clarifies the earlier observation on the isolation of this amino acid from the acid hydrolysate of previously alkalinized pyruvoylglycine.[1] It is evident that pyruvoylglycine is readily hydrolyzed on standing in alkali even at room temperature, the alanine formed by secondary reaction increasing with time. Hydrolysis of pyruvoylglycine, of previously alkalinized pyruvoylglycine, or of equimolar amounts of glycine and pyruvic acid in refluxing *N* NaOH for 2 hours, leads to still greater amounts of alanine. On the other hand, pyruvoylglycine exposed to alkali for only a relatively brief time and then acid-hydrolyzed, leads

Although it is possible to conceive of the condensation of glycine with the enol form of pyruvic acid as leading to γ-hydroxyglutamic acid,[2] the fact that the unknown compound yields an immediate yellow color with ninhydrin whereas γ-hydroxyglutamic acid yields an immediate purple color under the same conditions, indicated that, despite the identical mobilities on paper in different solvents, the two compounds were not identical. As an alternative to γ-hydroxyglutamic acid, the structurally isomeric β-hydroxy-β-methylaspartic acid (III) arising from an aldol-type condensation between the α-carbon atoms of pyruvic acid (I) and glycine (II) was considered. In order to determine whether this possibility was justifiable, it was necessary to prepare the unknown compound in quantity and to investigate its properties.



Inasmuch as the feasibility of preparing α-amino-β-hydroxy acids in good yield by the condensation of an aldehyde with the copper complex of glycine has recently been shown by Akabori and his associates,[6] an investigation was made of the reaction of pyruvic acid with copper glycinate. Under certain experimental conditions the principal product was indeed the hitherto unknown β-hydroxy-β-methylaspartic acid (III), and this compound was found to possess the same R_f values and color reactions with ninhydrin noted for the compound on the paper chromatograms of the alkaline reaction mixture of glycine and pyruvic acid.

Results and Discussion

Synthesis of β-Hydroxy-β-methylaspartic Acid.—Copper glycinate was first allowed to react with pyruvic acid under the conditions employed by Sato, Okawa and Akabori to prepare threonine from acetaldehyde and copper glycinate,[6] namely, warming the reactants at 50° for 1 hour in the presence of sodium carbonate. The final reaction mixture was found by paper chromatography to contain glycine, alanine, a periodate-resistant amino acid with an R_f in the range of the dicarboxylic amino acids, all in about the same proportion, and the desired β-hydroxy-β-methylaspartic acid in much larger amount. An increase in the duration or temperature of the reaction caused a decrease in the amounts of glycine, alanine, and β-hydroxy-β-methylaspartic acid, and a relative increase in the amount of the periodate-resistant amino acid. When, however, the reaction was conducted at 5°, only β-hydroxy-β-methylaspartic acid and glycine appeared on the paper chromatograms in significant quantity.[7]

to the formation only of glycine. The non-enzymatic transamination of pyruvic acid to alanine, under conditions somewhat different from those described, has been studied extensively by Herbst; *cf.* R. M. Herbst, THIS JOURNAL, **58**, 2238 (1936).

(6) M. Soto, K. Okawa and S. Akabori, *Bull. Chem. Soc., Japan*, **30**, 937 (1957).

(7) Prior to paper chromatography, the aliquot of reaction mixture was desalted by shaking in the presence of a few grams of Dowex 50 (H^+)(20–50 mesh). The amino acids were then displaced from the resin by shaking the latter in 5 *N* NH_4OH, and the solution subsequently evaporated.

The β-hydroxy-β-methylaspartic acid was isolated from the basic reaction mixture by column chromatography. The copper was first removed as the sulfide, and the solution then run through a column of Dowex 1-acetate. The sodium ions and the glycine were washed through with water, and the retained β-hydroxy-β-methylaspartic acid displaced from the resin with acetic acid until the ninhydrin reaction was negative. After evaporation of the solvent the residue was pure III. The method was found to be simple, rapid and adaptable to reasonably large quantities, a 4.5 × 40 cm. column being sufficient to purify about 17 g. of amino acid. The yield is about 60–70% of the theoretical. The amino acid crystallizes from water–ethanol as the dihydrate which readily loses the water of hydration when dried at 100° over phosphorus pentoxide *in vacuo*. It forms, like aspartic acid, a very insoluble copper salt, as well as crystalline N-benzoyl and N-carbobenzoxy derivatives.

Properties of β-Hydroxy-β-methylaspartic Acid.—When chromatographed on paper, β-hydroxy-β-methylaspartic acid has R_f values nearly identical with those of its structural isomer, γ-hydroxyglutamic acid, namely, 0.26 in *sec*-butyl alcohol–formic acid–water (4:1:1), 0.13 in phenol saturated with 10% sodium citrate, and 0.23 in 1-butanol–acetic acid–water (15:3:7). However, it is distinguished by its unique color reactions with ninhydrin. Thus, when a solution of β-hydroxy-β-methylaspartic acid is spotted on filter paper and the paper sprayed with a solution of ninhydrin in acetone and subsequently heated, a bright yellow spot appears. The yellow color gradually changes to gray-brown after 4 to 8 hours, and finally to purple after 18 to 24 hours. If, however, the paper is sprayed with a basic ninhydrin solution[8] and heated the yellow spot appears, but almost immediately turns to purple.

As a derivative of aspartic acid, β-hydroxy-β-methylaspartic acid would be expected to yield on reaction with ninhydrin 2 moles of carbon dioxide per mole of compound. This was indeed found to be true. The structural isomer, γ-hydroxyglutamic acid as a derivative of glutamic acid would, under the same conditions, be expected to yield only 1 mole of carbon dioxide per mole of compound, and this also was found to be the case.[3,4] Again, as an α-amino-β-hydroxy acid, β-hydroxy-β-methylaspartic acid would be expected to yield ammonia nearly quantitatively on reaction with periodate, and this was found to be true, in contrast with the complete resistance of γ-hydroxyglutamic acid to the periodate reagent.

Conversion of β-Hydroxy-β-methylaspartic Acid to β-Methylaspartic Acid.—A further proof of the structure of the new amino acid was afforded by its reduction to β-methylaspartic acid through the agency of refluxing concentrated hydriodic acid in the presence of red phosphorus. After removal of unchanged β-hydroxy-β-methylaspartic acid as the insoluble copper salt, the filtrate was con-

(8) R. Kay, D. Harris and C. Entenman, *Arch. Biochem. Biophys.*, **63**, 14 (1956), describe a solution (0.5 g. of ninhydrin, 0.5 ml. of *N* NaOH, 75 ml. of 95% ethanol and 25 ml. of water) which is very suitable for this purpose.

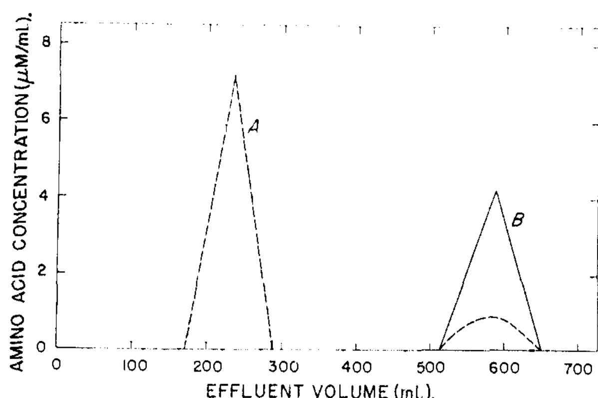


Figure 1.—Separation of the racemic diastereoisomers of 40 mg. of β-hydroxy-β-methylaspartic acid on Dowex 1-acetate eluted with 0.5 *N* acetic acid. Broken line represents re-chromatogram on one resin column of original peak A, solid line represents re-chromatogram on another resin column of original peak B.

centrated and β-methylaspartic acid isolated and crystallized. Analysis of this product, and comparison of its properties with an authentic sample of β-methylaspartic acid prepared by the method of Dakin,[9,10] resulted in its unequivocal identification.

Separation of the Diastereomeric Racemates.—β-Hydroxy-β-methylaspartic acid (III) possesses two asymmetric carbon atoms and theoretically should exist in two racemic steric modifications. To investigate this possibility, the compound was chromatographed on an ion-exchange resin in much the same manner previously shown to be successful for the separation of the two racemic diastereomers of γ-hydroxyglutamic acid.[4] Thus, 40 mg. of synthetic β-hydroxy-β-methylaspartic acid in 25 ml. of water was passed over a 2.5 × 33 cm. column of Dowex 1-acetate (100–200 mesh). A volume of 750 ml. of 0.5 *N* acetic acid was employed as the eluent at a rate of about 75 ml. per hour, fractions of 10 ml. volume being collected. Aliquots of each fraction were chromatographed and the concentration of amino acid determined by the intensity of the color with ninhydrin. Two distinct concentration maxima, 370 ml. of eluent fluid apart, were apparent. Each peak in the concentration–elution volume was fairly sharp, the earlier peak (A) being somewhat higher than the later peak B. The fractions comprising each peak were combined and chromatographed separately. Each column was eluted with 750 ml. of 0.5 *N* acetic acid as before, and the same fractionation procedure was employed. Peak B on re-chromatographing gave a single symmetrical peak at the same elution volume as before. Peak A on re-chromatographing yielded evidence of 2 peaks, one quite large and symmetrical at the same elution volume as that obtained with the parent fractions, and the other very small and coincident with that of peak B. The re-chromatogram evidently separated a small amount of the B form which originally emerged with the A form in the first chromatogram. Background flow was practically nil for both Dowex 1 columns, and the volume distance between peaks was 360 ml. of eluting fluid. Figure 1 illustrates the results obtained.

(9) H. D. Dakin, *J. Biol.*, **141**, 945 (1941).

(10) L. Benoiton, S. M. Birnbaum, M. Winitz and J. P. Greenstein, *Arch. Biochem. Biophys.*, in press (1959).

Preliminary experiments on chromatographing each peak on Whatman No. 1 paper previously buffered with phthalate at *p*H 2.8 (the probable isoelectric point of the amino acid) suggested that a clear-cut separation of the diastereomers could be achieved by two-dimensional chromatography using phenol saturated with citrate and dihydrogen phosphate in one direction and lutidine–ethanol–water (55:20:25) in the other direction. According to all the evidence acquired, the amounts of the two diastereomers comprising the synthetic amino acid were nearly equal. The compound was subjected to the possible action of a wide variety of snake venom L-amino acid oxidases, hog kidney D-amino acid oxidase and bacterial decarboxylases, and found to be entirely resistant.

Experimental

β-Hydroxy-β-methylaspartic Acid.—An aqueous solution containing 5.3 g. (0.06 mole) of pyruvic acid neutralized with sodium bicarbonate was added to a suspension of 11.5 g. (0.05 mole) of copper glycinate in 200 ml. of *N* sodium hydroxide. The suspension was shaken for a few minutes, and then placed in the refrigerator (at 5°) overnight. The next day, the clear blue solution was filtered to remove the small amount of cuprous oxide that had separated out, the filtrate was diluted to a more convenient volume, and hydrogen sulfide was bubbled through the stirred solution. The copper sulfide was filtered off with the aid of charcoal, the solution concentrated under vacuum to remove as much hydrogen sulfide as possible, treated with charcoal again, and the solution (200 ml.) finally placed on a 4.5 × 40 cm. column of Dowex 1-acetate previously washed with water. The sodium ions and any residual glycine were washed through with water (1.5–2 liters). The column was then inverted over a suction flask and the resin drawn into the flask with suction. Dilute acetic acid was added to the resin and the slurry was stirred until no more bubbles of hydrogen sulfide were liberated. The resin slurry was then poured back into the column and *N* acetic acid was allowed to flow through the column until no more ninhydrin-positive material was present in the eluate. The eluate (3–4 liters) was evaporated to dryness with a rotary evaporator, the solid residue washed with ethanol and finally acetone, the final traces of acetone removed with a jet of air, and the product as the dihydrate recrystallized from water–ethanol; yield 12.0 g. or 60%. The compound is a mixture of two racemic diastereomers (Fig. 1). Treatment with ninhydrin or with alkaline periodate yielded close to 2 moles of CO_2 and 1 mole of NH_3, respectively.

Anal. Calcd. for $C_5H_9NO_5 \cdot 2H_2O$: C, 30.2; H, 6.6; N, 7.0. Found: C, 30.3; H, 6.7; N, 7.0.

The amino acid loses its water of hydration when dried under vacuum at 100° over phosphorus pentoxide.

Anal. Calcd. for $C_5H_9NO_5$: C, 36.8; H, 5.6; N, 8.6. Found: C, 37.0; H, 5.5; N, 8.5.

The copper salt was prepared by adding copper carbonate to a boiling aqueous solution of the amino acid, filtering rapidly, and allowing the filtrate to cool.

Anal. Calcd. for $(C_5H_6NO_5)_2 \cdot Cu_3 \cdot 2H_2O$: Cu, 34.9; C, 22.0; H, 2.6; N, 5.1. Found: Cu, 34.9; C, 22.1; H, 4.1; N, 4.9.

Benzoyl-β-hydroxy-β-methylaspartic Acid.—Three grams of amino acid was treated with benzoyl chloride under the usual Schotten–Baumann conditions. Upon subsequent acidification of the solution a fine precipitate came out of solution. The product was collected by filtration, had a sharp m.p. of 121° and was evidently benzoic acid. The filtrate was therefore extracted with ethyl acetate, the extract dried over sodium sulfate, and concentrated under vacuum. The residue was crystallized from ethyl acetate,

m.p. 163–164°, yield 1.5 g. Whether this derivative, and the one following, is still a mixture of racemic diastereomers or only a single racemic form due to fractionation, cannot be stated at this time.

Anal. Calcd. for $C_{12}H_{13}NO_6$: C, 53.9; H, 4.9; N, 5.2. Found: C, 53.9; H, 5.0; N, 5.2.

Carbobenzoxy-β-hydroxy-β-methylaspartic acid was prepared by carbobenzoxylation of the amino acid in the presence of excess sodium bicarbonate. The derivative, extracted from the acidified solution with ethyl acetate, was crystallized from chloroform, m.p. 154–156°. The compound is soluble in water, just as is its structural isomer, carbobenzoxy-γ-hydroxyglutamic acid.[4]

Anal. Calcd. for $C_{13}H_{15}NO_7$: C, 52.5; H, 5.1; N, 4.7. Found: C, 52.4; H, 5.1; N, 4.7.

Reduction of β-Hydroxy-β-methylaspartic Acid to β-Methylaspartic Acid with Hydriodic Acid.—Five grams of amino acid was refluxed 24 hr. in 250 ml. of 57% hydriodic acid containing 2 g. of red phosphorus. Paper chromatography of an aliquot, desalted by absorbing the amino acids onto Dowex 50 followed by elution with ammonium hydroxide, showed the reduction to be far from complete (less than 50%). The red phosphorus in suspension was filtered off, the hydriodic acid removed by distillation under vacuum, and the residue resubmitted to the same treatment as before. This was then repeated a third time after which the amount of hydroxyamino acid remaining was roughly estimated at about 20–30%. The mixture was filtered, repeatedly evaporated to dryness under vacuum, treated with silver carbonate to completely rid the solution of iodide, the solution filtered, treated with H_2S, filtered again, and the solution finally concentrated under vacuum to completely remove the H_2S. The residue was dissolved in 250 ml. of water and the solution boiled for 10 minutes in the presence of excess copper carbonate. The insoluble copper salt of the unchanged starting material thereby crystallized, while the much more soluble copper salt of the product remained in solution. The mixture was cooled several hours, filtered, and the filtrate freed of copper by H_2S treatment. The final solution was evaporated to an oil, the oil dissolved in a few ml. of water, the solution treated with charcoal and the amino acid crystallized by the addition of ethanol; yield 0.8 g. The product, recrystallized from water–ethanol, was chromatographically identical with a sample of authentic β-methylaspartic acid obtained by synthesis,[9,10] namely, R_f 0.44 in *sec*-butyl alcohol–formic acid–water (4:1:1) and 0.21 in phenol saturated with 10% sodium citrate.

Anal. Calcd. for $C_5H_9NO_4$: C, 40.8; H, 6.2; N, 9.5. Found: C, 40.9; H, 6.3; N, 9.3.

BETHESDA, MD.

[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS, AND THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Preparation and Disulfide Interchange Reactions of Unsymmetrical Open-chain Derivatives of Cystine

BY LEONIDAS ZERVAS, LEO BENOITON, ELLINOR WEISS, MILTON WINITZ AND JESSE P. GREENSTEIN

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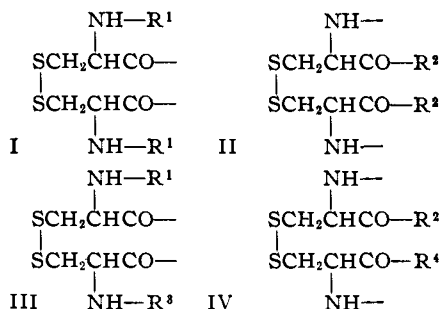
Preparation of mono-(carbobenzoxyglycyl)-L-cystine was achieved by treatment of an excess of L-cystine in aqueous alkali with carbobenzoxyglycyl chloride. Decarbobenzoxylation of the compound by the action of hydrogen bromide in glacial acetic acid permitted the subsequent isolation of pure, crystalline monoglycyl-L-cystine. The bis-methyl, bis-benzyl, monomethyl and monobenzyl ester derivatives of monocarbobenzoxy-L-cystine were prepared by the usual esterification procedures. Conversion of the first-mentioned derivative to N^{α}-carbobenzoxy-$N^{\alpha'}$-trityl-L-cystine bis-methyl ester was effected by the action of trityl chloride in chloroform containing triethylamine. Treatment of such product with hydrazine did not lead to the expected α-monohydrazide but rather induced a rapid disulfide interchange with the formation of the symmetrical bis-trityl-L-cystine bis-methyl ester and bis-carbobenzoxy-L-cystine bis-hydrazide as the only isolable products. Comparable disulfide interchange of this same compound as well as of monocarbobenzoxy- and monoglycyl-L-cystine was catalyzed by alkali in both aqueous and methanolic solution. The rate and extent of such interchange in basic solution was shown to increase with an increase in *p*H.

Since substitution at both amino or both carboxyl functions of the symmetrical cystine molecule can involve either the same or different substituents, derivatives of this amino acid may be classified as symmetrical (I and II), unsymmetrical (III and IV) or combinations thereof. An im-



pressive number of symmetrical open-chain peptides of cystine are presently accessible *via* a variety of synthetic routes,[1] whilst the synthesis of oxytocin, vasopressin and analogs thereof[2] has at the same time provided several unsymmetrical peptides of cystine wherein the disulfide linkage is implicated in a cyclic structure. In contrast, the development of methods which would permit the preparation of unsymmetrical open-chain derivatives of the type represented by structures (III) and (IV) has hitherto received only scant attention. Thus, an attempt by Fischer and Gerngross,[3] in 1909, to prepare monoglycyl- and monoleucyl-L-cystine through aminolysis of their respective mono-α-haloacyl-L-cystine precursors culminated in the isolation, in each instance, of a material which was presumed to be the desired product. Such presumption was nonetheless tinged with an

(1) *Cf.* J. S. Fruton, *Adv. in Protein Chem.*, **5**, 1 (1949); W. Grassmann and E. Wünsch, *Fortschr. Chem. org. Naturstoffe*, **13**, 445 (1956); M. Goodman and G. W. Kenner, *Adv. in Protein Chem.*, **12**, 465 (1957); J. P. Greenstein and M. Winitz "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., Vol. II, in press.

(2) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis, THIS JOURNAL, **76**, 3115 (1954); V. du Vigneaud, D. T. Gish and P. G. Katsoyannis, *ibid.*, **76**, 4751 (1954); P. G. Katsoyannis, *ibid.*, **79**, 109 (1957); C. Ressler and V. du Vigneaud, *ibid.*, **79**, 4511 (1957); V. du Vigneaud, M. F. Bartlett and A. Jöhl, *ibid.*, **79**, 5572 (1957).

(3) E. Fischer and O. Gerngross, *Ber.*, **42**, 1485 (1909).