dry refluxing benzene was added over a period of 1.5 hr. with stirring 70 g. (0.5 mole) of O-ethyl hydrogen methylphosphonothioate.[17] The mixture was refluxed for an additional period of 75 min. and then allowed to stand at room temperature overnight. Stirring and refluxing was resumed again for an additional 4 hr., after which 25 cc. of water was added to the stirred mixture in order to dissolve the white crystalline material which had formed upon cooling. The upper benzene layer was separated, dried with anhydrous magnesium sulfate and Drierite and filtered, and the benzene evaporated from the filtrate under reduced pressure. Distillation of the brownish oily residue resulted in some decomposition of the distillation charge while 17.5 g. of forerun was collected between 40 and 97° at 0.2–0.3 mm. pressure; a white crystalline solid, apparently diethylaniline hydrochloride, deposited in the still-head. The distillation was interrupted, the cooled liquid residue dissolved in 80 cc. of diethyl ether and the solution washed once with 50 cc. of water. After drying with Drierite, the ether was evaporated under reduced pressure and the orange-brown, liquid residue was distilled to yield 57.0 g. (50%) of O-ethyl O-(2-ethylthioethyl) methylphosphonothioate, b.p. 108–110° (0.100 mm.), $n^{25}D$ 1.5123. The brown liquid distillation residue (5 g.) was discarded.

Anal. Calcd. for $C_7H_{17}O_2PS_2$: C, 36.82; H, 7.51; S, 28.08. Found: C, 36.4; H, 7.6; S, 28.15.

Acknowledgment.—The authors wish to express their appreciation to Mr. Harold Finegold for the determination of the infrared and nuclear magnetic resonance spectra and their interpretation and to personnel of the Analytical Research Branch, Chemical Research Division, for performing all analyses reported in this paper.

ARMY CHEMICAL CENTER, MD.

(17) A paper describing the preparation of this compound and of analogs and homologs is in preparation.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Use of N-Formylamino Acids in Peptide Synthesis

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Several representative peptides and peptide derivatives have been synthesized using an N-formyl blocking group and the carbodiimide coupling method. Racemization was not observed, and optimum conditions for acidic hydrolysis of the N-formyl function were determined. Similar peptide syntheses employing the mixed anhydride procedure led to extensive racemization. It has been shown that an N-formyl peptide ester can be extended on either the amino or carboxyl end by selective hydrolysis.

The N-formyl group has long been known to undergo acid hydrolysis[2] and alcoholysis[3] under conditions which generally do not rupture a peptide linkage. Although the N-formyl group has been proposed previously[4,5] it has not gained wide acceptance as an amino protective function. Two major disadvantages of the use of an N-formylamino acid in peptide synthesis are tendency toward racemization, characteristic of many acylamino acids under treatment which may lead to azlactone formation, and instability toward reagents conventionally employed to "activate" the carbonyl function for coupling.[6] With the advent of the carbodiimide method of peptide bond formation,[7] which operates under exceptionally mild conditions, it becomes feasible to employ sensitive and labile blocking groups, including the N-formyl function.

In this communication, using N-formylamino acids, a comparison has been made between two of the recent methods of amide bond formation, namely, the mixed carbonic anhydride[8,9] and the carbodiimide procedures. Also, conditions for the removal of the N-formyl group have been studied carefully.

An N-formyl group can be introduced readily without racemization of the parent amino acids by formylation in the presence of acetic anhydride, using a modification of the procedure of du Vigneaud.[10] For the solvolysis studies of N-formyl derivatives, simple DL-formyl peptides including formyl-DL-valine anilide, formyl-DL-phenylalanine amide, racemic formylvalylphenylalanine and its methyl ester were prepared from the corresponding formyl-DL-amino acids *via* the mixed carbonic anhydride procedure. The solvolytic conditions employed are considerably milder than those reported previously, but the actual time required for complete deformylation varies with different amino acids. In general, an N-formyl group can be removed smoothly by treatment of the formyl peptide with a slight excess of 0.5 *N* hydrochloric acid in methanol (or in water–dioxane if the peptide free acid is used) at room temperature for a period of 48 hours or at reflux temperature for one hour. The yields are high, ranging from 80–95%.

In order to test the tendency toward racemization of the mixed carbonic anhydride procedure as applied to N-formylamino acids, we chose to prepare acetylphenylalanylglycine anilide for comparison with the optically pure product obtained previously.[11] The mixed carbonic anhydride derived from formyl-L-phenylalanine and ethyl chloroformate was treated with glycine anilide. The product N-formylphenylalanylglycine anilide had a

(1) Aided by a Contract from the Office of Naval Research, Washington, D. C.

(2) E. Fischer and O. Warburg, *Ber.*, **38**, 3997 (1905).

(3) T. Curtius, *ibid.*, **16**, 753 (1883).

(4) A. Hillmann and G. Hillmann, *Z. Naturforsch.*, **6B**, 340 (1951).

(5) S. G. Waley, *Chemistry & Industry*, 107 (1953).

(6) For example, F. R. King, J. W. Clark-Lewis, D. D. A. Kidd and G. R. Smith, *J. Chem. Soc.*, 1039 (1954), reported very low yields using an azide method of coupling; and unpublished results in this Laboratory indicate substantial decomposition of many N-formylamino acids by interaction with such reagents as thionyl chloride and phosphorus pentachloride.

(7) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).

(8) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).

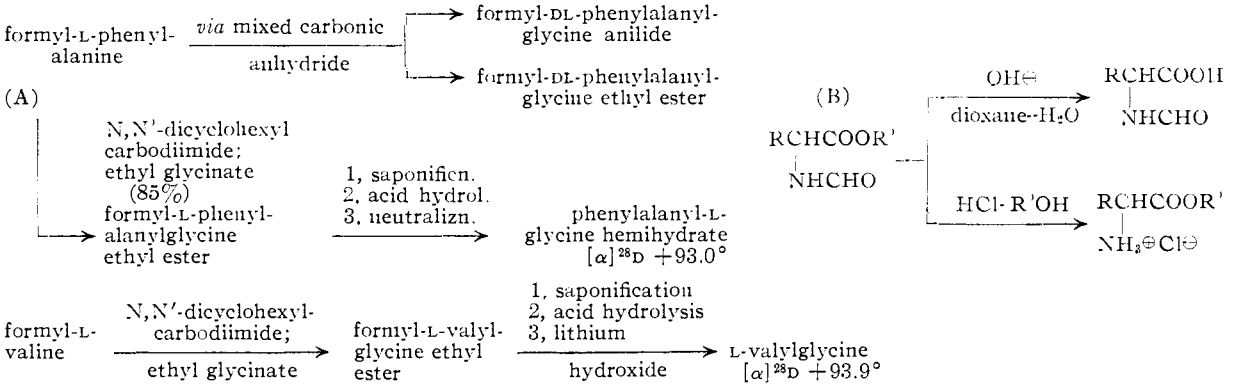
(9) J. R. Vaughan, Jr., THIS JOURNAL, **73**, 3547 (1951).

(10) V. du Vigneaud, R. Dorfmann and H. S. Loring, *J. Biol. Chem.*, **98**, 577 (1932).

(11) J. C. Sheehan, D. W. Chapman and R. W. Roth, THIS JOURNAL, **74**, 3822 (1952).

very low rotation. Hydrolysis followed by acetylation afforded completely racemized acetylphenylalanylglycine anilide in good yield. In a similar manner, racemic formylphenylalanylglycine ethyl ester was obtained from formyl-L-phenylalanine.

The condensation of optical active formylamino acids by the mixed carbonic anhydride procedure



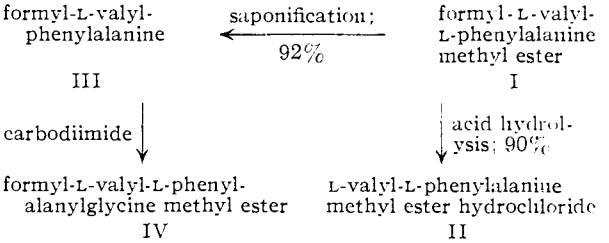
was repeated using modified conditions, which were claimed to have minimized racemization.[12] By using a variety of solvent systems, lowering the reaction temperature and reducing the reaction time allowed for the formation of the mixed anhydride, the optical purity of the formyl peptides was not improved and the yield decreased markedly.

It is generally believed that the racemization of an N-acylamino acid during peptide synthesis involves azlactonization[13,14] and the extent of racemization depends greatly on the "activation" of the carboxyl function for peptide formation as well as the nature of the acyl function attached to the amino moiety. The use of a mixed anhydride derived from an N-formylamino acid and another acid stronger than carbonic, such as a carboxylic acid, would be expected to favor azlactonization. However, Waley,[5] without experimental details, reported the synthesis of two "optically pure" dipeptides using N-formylamino acids and a mixed carboxylic anhydride procedure.[15]

Formyl-L-phenylalanine when condensed with glycine ethyl ester in the presence of N,N'-dicyclohexylcarbodiimide led to an optically active formylphenylalanylglycine ethyl ester in good yield. Saponification of the ester group and then acid deformylation yielded phenylalanylglycine hydrochloride from which the dipeptide was generated as a hemihydrate. The hemihydrate of L-phenylalanylglycine has a rotation higher than the equivalent rotation of the monohydrate reported.[11] By a similar procedure, unracemized valyl-L-glycine was prepared of identical rotation to that prepared by a different route.[16] The maintenance of optical integrity, using the carbodiimide procedure with these N-formylamino acids, is demonstrated. However, the failure to observe racemization in these representative carbodiimide couplings probably should be attributed to the neutral and exceptionally mild reaction conditions which prevail, and is not necessarily an indication that peptide synthesis employing carbodiimides is mechanistically or functionally incapable of producing racemization (A).

Although the formyl group is solvolyzed readily in dilute acid, it is surprisingly resistant toward basic hydrolysis. Saponification of esters of formylamino acids in aqueous dioxane gave the corresponding free acids in yields consistently better than 85%. The difference in stability of the formyl group and the ester group in acid and base thus makes it possible to apply the formyl method in a stepwise synthesis of polypeptides (B).

In order to demonstrate that an N-formyl peptide ester can be extended on either the carboxyl or the amino end by selective hydrolysis of the ester group or the formyl group, an optically active tripeptide formyl-L-valyl-L-phenylalanlyglycine ethyl ester was prepared. Formyl-L-valine was condensed with L-phenylalanine methyl ester by the carbodiimide procedure to give an excellent yield of formyl-L-valyl-L-phenylalanine methyl ester (I). Acid hydrolysis of I in dilute methanolic hydrochloric acid revealed the amino function and L-valyl-L-phenylalanine methyl ester (II) could be obtained in 90% yield; basic hydrolysis of I in aqueous dioxane regenerated the carbonyl function and formyl-L-valyl-L-phenylalanine (III) could be obtained in 92% yield. The condensation of III and glycine ethyl ester by N,N'-dicyclohexylcarbodiimide afforded the optically active tripeptide, formyl-L-valyl-L-phenylalanylglycine ethyl ester (IV).



The formyl group offers several important advantages as a potential peptide protective function. (1) The formyl group can be introduced easily in high yield without racemization of the parent amino acid. (2) Formyl amino acids, when used

(12) J. R. Vaughan, Jr., THIS JOURNAL, **74**, 6137 (1952); J. R. Vaughan, Jr., and J. A. Eichler, *ibid.*, **75**, 5556 (1953).

(13) V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **99**, 143 (1932).

(14) T. Wieland, *Angew. Chem.*, **63**, 7 (1951).

(15) T. Wieland and R. Sehring, *Ann.*, **569**, 122 (1950).

(16) E. Fischer and H. Scheibler, *ibid.*, **363**, 136 (1908).

in conjunction with the carbodiimide procedure, did not racemize in the representative cases investigated. (3) The formyl group can be removed selectively by acid hydrolysis and the peptide chain can be extended on the amino end. (4) The ester group can be removed selectively by saponification and the peptide chain can be extended on the carboxyl end.

Experimental[17]

Preparation of N-Formylamino Acids.—DL-Formylamino acids can be prepared in 78–90% yields following essentially the procedure of du Vigneaud for the formylation of DL-cystine.[10] Acetic anhydride (83 ml.) was added dropwise to a mixture of approximately 0.10 mole of the amino acid in 250 ml. of 88% formic acid at a rate to maintain the temperature of the reaction mixture between 50–60°. After the addition was complete, the mixture was stirred at room temperature for 1 hour at the end of which time 80 ml. of ice-water was introduced, and the mixture was concentrated at reduced pressure. The crystalline residue could be easily recrystallized from water or aqueous ethanol.

For the preparation of optically active formylamino acids, 98% formic acid was used (0.10 mole of the amino acid in 210 ml. of 98% formic acid) and acetic anhydride (70 ml.) was added at a temperature between 5–15°. The recrystallized yields ranged from 85–90%.[18]

Formyl-DL-valine Anilide.—To a solution of 1.45 g. (0.01 mole) of formyl-DL-valine and 2.38 ml. (0.01 mole) of tributylamine in 10 ml. of chloroform was added 1.09 g. (0.01 mole) of ethyl chloroformate. The mixture was cooled in an ice-bath for 15 minutes and 0.93 g. (0.01 mole) of aniline was introduced. A colorless precipitate separated immediately with vigorous evolution of carbon dioxide. After stirring at 0–5° for 15 minutes, the precipitate was collected, washed with hot water and methanol, and recrystallized from aqueous acetic acid; 1.35 g. (62%), m.p. 183–184.5°.

Anal. Calcd. for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.17; H, 7.51; N, 12.50.

DL-Valine Anilide Hydrochloride.—A solution of 1.32 g. (6.0 millimoles) of DL-formylvaline anilide in 6.0 ml. of 5% methanolic hydrochloric acid (5.5 ml. of 38% aqueous hydrochloric acid in 60 ml. of absolute methanol) was stored at room temperature for a period of 2 days. Removal of solvent at reduced pressure gave a colorless residue which was washed thoroughly with anhydrous ether (weight 1.28 g., 93%, m.p. 135–144°). Crystalline hydrochloride was obtained by gradual addition of absolute ether to a methanolic solution of the crude product; 0.71 g., m.p. 165–166°.

Formyl-DL-phenylalanine Amide.—A solution of 2.90 g. (0.015 mole) of formyl-DL-phenylalanine and 3.58 ml. (0.015 mole) of tributylamine in 15 ml. of benzene, cooled in an ice-water mixture, was treated with 1.63 g. (0.015 mole) of ethyl chloroformate. The mixture was stirred for 15 minutes and gaseous ammonia was introduced until no further precipitation was observed. The precipitate was collected by filtration and washed thoroughly with cold water; 1.50 g. (52%), m.p. 152–153.5°. An analytical sample was prepared by two recrystallizations from water, m.p. 153–154°.

Anal. Calcd. for $C_{10}H_{12}N_2O_2$: C, 62.43; H, 6.30; N, 14.58. Found: C, 62.41; H, 6.31; N, 14.62.

DL-Phenylalanine Amide Hydrochloride.—A solution of 0.35 g. (1.69 millimoles) of formyl-DL-phenylalanine amide in 10 ml. of 5% methanolic hydrochloric acid was stored at room temperature for 2 days. Removal of solvent and excess hydrochloric acid under reduced pressure gave a water-soluble residue which was recrystallized from methanol–chloroform to give 0.28 g. (83%) of small prisms, m.p. 234–235° dec. Two recrystallizations from the same solvent mixture afforded an analytical sample, m.p. 237.8–238.8° dec.

Anal. Calcd. for $C_{19}H_{13}NOCl$: N, 13.9. Found: N, 13.4.

A sample of the hydrochloride in an aqueous solution was treated with a slight excess of diethylamine. Concentration at reduced pressure gave a colorless residue of DL-phenylalanine amide which, after an aqueous digestion was recrystallized from chloroform, m.p. 137–139° (reported[19] m.p. 137–141).

Formylvalylphenylalanine Methyl Ester.—A solution of 2.18 g. (0.015 mole) of formyl-DL-valine and 2.08 ml. (0.015 mole) of triethylamine in 7 ml. of dioxane was treated with 1.63 g. (0.015 mole) of ethyl chloroformate at −5°. The mixture was maintained at −5° for 15 min. and 2.68 g. (0.015 mole) of DL-phenylalanine methyl ester was introduced. After 15 min. of stirring at 0°, and then 15 min. at room temperature, the mixture was concentrated under reduced pressure. When the oily residue was triturated with 15 ml. of water it coagulated slowly to a fluffy precipitate. The mixture was stored at 0–5° and a colorless precipitate of m.p. 125–165° was collected 24 hours later; 2.66 g. (58%).

Three recrystallizations from methanol–water yielded a crystalline product of m.p. 165–166°, weight 1.80 g. (35%). The compound was recrystallized from benzene with another recrystallization from methanol–water, and analyzed; m.p. 166.4–166.8°.

Anal. Calcd. for $C_{16}H_{22}O_4$: C, 62.72; H, 7.24; N, 9.15. Found: C, 62.71; H, 7.45; N, 9.27.

Formylvalylphenylalanine.—A solution of 2.18 g. (0.015 mole) of formyl-DL-valine in 7 ml. of dioxane and 2.08 ml. (0.015 mole) of triethylamine was treated with 1.63 g. (0.015 mole) of ethyl chloroformate. The mixture was stirred at 0–5° for 15 min. With vigorous stirring, a second solution of 2.48 g. (0.015 mole) of DL-phenylalanine in 3.75 ml. of 4 *N* sodium hydroxide was introduced. After 25 min. of stirring at 0°, the mixture was acidified with 5 ml. of 3 *N* hydrochloric acid and the crystalline product soon separated. The mixture was stored overnight at 0–5°, and the prismatic precipitate (1.4 g., 32%, m.p. 209–211°) was collected by filtration. Two recrystallizations from water gave an analytical sample, m.p. 218–219°.

Anal. Calcd. for $C_{15}H_{20}N_2O_4$: C, 61.62; H, 6.90; N, 9.59. Found: C, 61.69; H, 7.18; N, 9.42.

Valylphenylalanine Methyl Ester Hydrochloride. A. From Formylvalylphenylalanine Methyl Ester.—A solution of 0.32 g. (1.0 millimole) of formylvalylphenylalanine methyl ester in 1 ml. of methanol was treated with 1.1 ml. of 1 *N* methanolic hydrochloric acid (2 ml. of concentrated hydrochloric acid diluted to 24 ml. with absolute methanol). After storage at room temperature for 2 days, water-soluble needles separated from the solution. The mixture was evaporated at reduced pressure and the crystalline residue was washed thoroughly with methanol–ether (1:3). The crude yield amounted to 0.308 g. (95%), m.p. 228–230. One recrystallization from absolute methanol afforded an analytical sample 0.28 g., 85%, m.p. 232–233°.

Anal. Calcd. for $C_{15}N_{23}N_2O_3Cl$: N, 8.90. Found: N, 8.81.

B. From Formylvalylphenylalanine.—According to the procedure given above, 0.19 g. (0.60 millimole) of formylvalylphenylalanine was treated with 4.5 ml. of 5% methanolic hydrochloric acid at room temperature for a period of 25 hours. Crystalline hydrochloride was obtained in 94% yield, 0.17 g., m.p. 232–233°. A mixed melting point with the hydrochloride from procedure A gave no depression.

Anal. Calcd. for $C_{15}H_{23}N_2O_3Cl$: C, 57.22; H, 7.36; N, 8.90. Found: C, 57.02; H, 7.56; N, 9.13.

Formyl-DL-phenylalanylglycine Anilide.—A solution of 1.84 g. (0.01 mole) of formyl-L-phenylalanine and 1.39 ml. (0.01 mole) of triethylamine in 10 ml. of purified methylene chloride, cooled to −5°, was treated with 1.09 g. (0.01 mole) of freshly distilled ethyl chloroformate. The mixture was maintained at −5° for 15 min. and a precooled solution of 1.87 g. (0.01 mole) of glycine anilide hydrochloride and 1.39 ml. (0.01 mole) of triethylamine in 40 ml. of purified methylene chloride was introduced. A colorless precipitate separated immediately. After 20 min. of stirring at 0° and then for 30 min. at room temperature, the precipitate was collected and triturated with small portions of 5% hydrochloric acid, 5% sodium bicarbonate and water, weight

(17) All melting points are uncorrected (capillary tube). We are indebted to Dr. S. M. Nagy and his associates for the microanalyses.

(18) Physical constants of the N-formylamino acids agreed in general with literature values; E. Fischer and W. Schoeller, *Ann.*, **357**, 1 (1907); M. A. Nyman and R. M. Herbst, *J. Org. Chem.*, **15**, 108 (1950).

(19) E. Koenigs and B. Mylo, *Ber.*, **41**, 4439 (1908).

2.23 g. (70%), m.p. 209–210°. One recrystallization from ethanol–water gave 1.8 g. of an optically inactive material, m.p. 213.5–214.5°. A sample was recrystallized from the same solvent mixture for analysis.

Anal. Calcd. for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91. Found: C, 66.54; H, 5.89; N, 12.69.

DL-Phenylalanylglycine Anilide Hydrochloride.—DL-Formylphenylalanylglycine anilide (0.488 g., 1.5 millimoles) was dissolved in a mixture of 3 ml. of methanol and 1 ml. of water by gentle warming. On cooling, 1.8 ml. of 1 *N* methanolic hydrochloric acid was added. The solution was stored at room temperature for 24 hours, during which time traces of water-insoluble crystals separated. The mixture was redissolved by warming and another ml. of methanol–hydrochloric acid was added. After 10 hours of storage at room temperature, the solvent was evaporated under reduced pressure and the residue was washed thoroughly with methanol–ether (1:2). The crude product weighed 0.36 g., m.p. 211–214°.

A crystalline hydrochloride was obtained by crystallization from methanol–ether, weight 0.35 g. (70%), m.p. 214–215° dec. Recrystallization from the same solvents gave an analytical sample, m.p. 217–219°.

Anal. Calcd. for $C_{17}H_{20}N_3O_2Cl$: C, 61.15; H, 6.04; N, 12.60. Found: C, 60.80; H, 6.30; N, 12.47.

Acetyl-DL-phenylalanylglycine Anilide.—To a solution of DL-phenylalanylglycine anilide hydrochloride (0.20 g., 0.67 millimole) in 7 ml. of water at 0–5° was added 1.4 ml. of 5% sodium bicarbonate (0.67 millimole) and then 1.0 ml. of acetic anhydride. As the acetic anhydride dissolved, colorless precipitate soon separated. The mixture was stirred at 0–5° for 5 min. and a second 1-ml. portion of acetic anhydride was added. When all of the acetic anhydride had dissolved, 3 ml. of glacial acetic acid was introduced and the precipitate was dissolved by gentle warming on a steam-bath. The product was allowed to crystallize from the cooled solution. Recrystallization from methanol–water (1:1) yielded 0.19 g. (94%) of colorless needles, m.p. 195.5–196°. A sample was recrystallized once from acetic acid–water and once from methanol–water for analyses; m.p. 195.5–196°.

Anal. Calcd. for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 67.24; H, 6.11; N, 12.37.

Formyl-DL-phenylalanylglycine Ethyl Ester.—To a solution of 2.90 g. (0.015 mole) of formyl-L-phenylalanine and 2.08 ml. (0.015 mole) of triethylamine in 8 ml. of purified methylene chloride at 8° was added 1.63 g. (0.015 mole) of ethyl chloroformate in 3 ml. of methylene chloride. After 15 min. at −8°, a precooled solution of 2.09 g. (0.015 mole) of glycine ethyl ester hydrochloride and 2.08 ml. (0.015 mole) of triethylamine in 40 ml. of methylene chloride was introduced. Stirring was continued at −5° for 20 min. and at room temperature for 30 min. The methylene chloride layer was extracted twice with small portions of 5% hydrochloric acid, 5% sodium bicarbonate and water, dried and concentrated at reduced pressure. Washing of the semi-crystalline residue with anhydrous ether yielded 2.31 g. of a colorless residue, m.p. 88–89.5°. Recrystallization from carbon tetrachloride gave 2.29 g. (50%) of small prisms, m.p. 93–95°. A sample was recrystallized from carbon tetrachloride; m.p. 98.5–99°, $[\alpha]$ 0°.

Anal. Calcd. for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found: C, 59.91; H, 6.50; N, 10.12.

Formyl-L-phenylalanylglycine Ethyl Ester.—A solution of 1.83 g. (0.01 mole) of formyl-L-phenylalanine and 1.03 g. (0.01 mole) of glycine ethyl ester in a mixture of 20 ml. of dioxane and 20 ml. of methylene chloride was cooled in an ice-bath. With vigorous stirring a second solution of 2.06 g. (0.01 mole) of N,N′-dicyclohexylcarbodiimide in 5 ml. of methylene chloride was introduced. The solution became cloudy at the end of 8 min. and a large amount of precipitate separated within 10 min. Stirring was continued at ice-bath temperature for 3.5 hours. Removal of the urea by filtration followed by concentration of the filtrate under reduced pressure gave a colorless oil which was taken up in 150 ml. of methylene chloride. The methylene chloride solution, after thorough washing with 5% hydrochloric acid, 5% sodium bicarbonate, and water, was dried and evaporated to a thick paste under diminished pressure. On addition of carbon tetrachloride, a colorless solid separated which was recrystallized from methylene chloride–carbon tetrachloride yielding 2.25 g. (85%) of elongated needles, m.p. 130–131°. The product was recrystallized from water for analyses; m.p. 131–132°, $[\alpha]^{26}D$ +4.4° (42.8 mg. in 1.9 ml. of absolute ethanol).

Anal. Calcd. for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.14; H, 6.67; N, 10.07.

Formyl-L-phenylalanylglycine.—A solution of 1.0 g. (3.6 millimoles) of formyl-L-phenylalanylglycine ethyl ester in 9.3 ml. of dioxane was treated with 3.6 ml. of 1.02 *N* sodium hydroxide. After storage at room temperature for 50 min., an additional 0.3 ml. of sodium hydroxide was introduced; 15 min. later, the solution was acidified with an equivalent amount of *N* hydrochloric acid. Removal of solvents under reduced pressure gave a crystalline residue which was recrystallized from water, yielding 0.80 g. (89%) of colorless needles, m.p. 203–204°. A sample was recrystallized from water for analyses; m.p. 203–204°, $[\alpha]^{28}D$ +15.1° (28.3 mg. in 1.3 ml. of 0.5 *N* sodium carbonate).

Anal. Calcd. for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.20. Found: C, 57.63; H, 5.66; N, 11.02.

L-Phenylalanylglycine Hemihydrate.—L-Formylphenylalanylglycine (0.67 g., 2.68 millimoles) was dissolved in a mixture of 6 ml. of water and 7 ml. of ethanol by gentle warming. As the temperature of the mixture was cooled to 45°, 3.0 ml. of 1.0 *N* hydrochloric acid was introduced, and the solution was allowed to cool to room temperature gradually. After storage overnight at room temperature, the crystalline product which separated was redissolved by warming and the solution was maintained at 45° for 40 min. Removal of solvents under reduced pressure gave a semi-solid which was taken up in 5 ml. of water. The water-insoluble starting material (72 mg.) was recovered by filtration. To the filtrate, triethylamine was added dropwise to a *p*H of 6. Concentration of this solution under reduced pressure gave a thick paste, which crystallized as needles on addition of 15 ml. of acetone; 300 mg. (65%), m.p. 257–258°. Recrystallization from water yielded a hemihydrate; m.p. 258.5–259°, $[\alpha]^{28}D$ +93.0° (26.1 mg. in 1.3 ml. of water) or $[\alpha]^{28}D$ +49.3° (22.4 mg. in 1.3 ml. of glacial acetic acid).

Anal. Calcd. for $C_{11}H_{14}N_2O_3 \cdot {}^1/_2H_2O$: C, 57.12; H, 6.54; N, 12.11. Found: C, 57.28; H, 6.86; N, 12.15.

Formyl-L-valylglycine Ethyl Ester.—A solution of 0.90 g. (6.0 millimoles) of formyl-L-valine and 0.61 g. (6.0 millimoles) of glycine ethyl ester in 20 ml. of methylene chloride and 20 ml. of dioxane was treated with 1.40 g. (6.8 millimoles) of N,N′-dicyclohexylcarbodiimide in 10 ml. of methylene chloride. After 10 hours at room temperature, the precipitated urea was removed by filtration and the filtrate was concentrated at reduced pressure. A solution of the residue in methylene chloride was washed thoroughly with 5% hydrochloric acid, 5% sodium bicarbonate and water. After extraction of the washings with additional methylene chloride, the combined organic layers were dried and concentrated at reduced pressure. The residue was taken up in a small amount of hot water and an additional quantity of the insoluble urea was removed by filtration. From the filtrate, which had been concentrated to a volume of 5 ml. and stored for 10 hours at 0–5°, 0.70 g. of colorless needles (m.p. 156–157°) was collected. A second crop of 0.30 g. (m.p. 156–156.5°) could be obtained by concentration of the mother liquor. The combined yield, 1.0 g. (72%), was recrystallized from ethyl acetate.

Anal. Calcd. for $C_{10}H_{18}N_2O_4$: C, 52.16; H, 7.88; N, 12.17. Found: C, 52.30; H, 8.16; N, 12.24.

Formyl-L-valylglycine.—A solution of 0.60 g. (2.6 millimoles) of formyl-L-valylglycine ethyl ester in 9 ml. of dioxane and 3.0 ml. of 1.0 *N* sodium hydroxide was stored at room temperature for 1 hour. The mixture was neutralized with an equivalent amount of *N* hydrochloric acid and evaporated under diminished pressure. The crystalline residue was recrystallized from water yielding 0.47 g. (90%) of fine needles of m.p. 207–208°, $[\alpha]^{28}D$ −50.9° (23.6 mg. in 1.8 ml. of 70% ethanol).

Anal. Calcd. for $C_8H_{14}N_2O_4$: C, 47.51; H, 6.98; N, 13.86. Found: C, 47.67; H, 7.13; N, 13.92.

L-Valylglycine.—A mixture of 0.387 g. (1.9 millimoles) of L-formylvalylglycine in 2.67 ml. of 1.0 *N* hydrochloric acid, 2 ml. of water and 1 ml. of methanol, was brought into solution by gentle warming. The solution was allowed to cool to room temperature slowly and was stored at this temperature for 48 hours. During this period, the mixture

was warmed occasionally to dissolve some of the starting material that had precipitated. After neutralization with an equivalent of lithium hydroxide, the solution was lyophilized to a colorless powder, which crystallized from methanol–ethyl acetate; 0.275 g. (83%), m.p. 270–272°. A sample was recrystallized twice from the same solvents for analyses; m.p. 271–272°, $[\alpha]^{23}D$ +93.9° (20.0 mg. in 1.3 ml. of water), reported[16] $[\alpha]^{21}D$ +93.3° (10% solution in water).

Anal. Calcd. for $C_7H_{14}N_2O_3$: C, 48.26; H, 8.10; N, 16.09. Found: C, 48.30; H, 8.09; N, 15.82.

Formyl-L-valyl-L-phenylalanine Methyl Ester.—A solution of 0.87 g. (6.0 millimoles) of formyl-L-valine, 1.07 g. (6.0 millimoles) of phenylalanine methyl ester and 1.30 g. (6.6 millimoles) of N,N′-dicyclohexylcarbodiimide in 40 ml. of purified methylene chloride was stirred overnight at room temperature. After removal of the precipitated urea by filtration, the filtrate, diluted with 50 ml. of methylene chloride, was washed with successive small portions of 5% hydrochloric acid, 5% sodium bicarbonate and water. Concentration of the dried methylene chloride layer at reduced pressure yielded 1.40 g. (80%) of a crystalline residue, which was recrystallized from water to give 1.13 g. of small needles, m.p. 148–149.5°, $[\alpha]^{28}D$ −43.2° (18.5 mg. in 1.2 ml. of methanol).

Anal. Calcd. for $C_{16}H_{22}O_4$: C, 62.72; H, 7.24; N, 9.13. Found: C, 62.49; H, 7.38; N, 9.23.

L-Valyl-L-phenylalanine Methyl Ester Hydrochloride.—A mixture of 0.80 ml. of 5% methanolic hydrochloric acid and 0.5 ml. of methanol containing 0.20 g. (0.66 millimole) of formyl-L-valylphenylalanine methyl ester was stored at room temperature for 48 hours. During this period the mixture was shaken occasionally; complete solution resulted at the end of 10 hours. After addition of an equal volume of anhydrous ether, the crystalline hydrochloride separated in fine, long needles; 0.185 g. (90%), m.p. 192–195°. One recrystallization from methanol–ether yielded 0.165 g. of an analytical sample, m.p. 196–196.5°, $[\alpha]^{28}D$ +26.6° (33.6 mg. in 1.2 ml. of water).

Anal. Calcd. for $C_{15}H_{23}N_2O_3Cl$: C, 57.23; H, 7.36; N, 8.90. Found: C, 57.45; H, 7.53; N, 8.99.

Formyl-L-valyl-L-phenylalanine.—A mixture of 0.42 g. (1.34 millimoles) of formyl-L-valyl-L-phenylalanine methyl ester, 3.2 ml. of dioxane and 1.44 ml. of 1.0 *N* sodium hydroxide was stored at room temperature for 1 hour. At the end of this period, an equivalent amount of *N* hydrochloric acid was added and the solution was lyophilized to a colorless powder. Crystallization of the powder from water yielded 0.37 g. (92%) of needles, m.p. 203–204°, $[\alpha]^{28}D$ −31.4° (9.9 mg. in 1.2 ml. of methanol).

Anal. Calcd. for $C_{15}H_{20}N_2O_4$: C, 61.62; H, 6.90; N, 9.59. Found: C, 61.82; H, 7.09; N, 9.52.

Formyl-L-valyl-L-phenylalanylglycine Ethyl Ester.—A suspension of the salt derived from the addition of 0.086 g. (0.83 millimole) of glycine ethyl ester in 30 ml. of methylene chloride to a solution of 0.242 g. (0.83 millimole) of formyl-L-valylphenylalanine in 15 ml. of dioxane was treated with 0.190 g. (0.92 millimole) of N,N′-dicyclohexylcarbodiimide in 5 ml. of methylene chloride. The mixture was stirred overnight at room temperature. As the salt dissolved slowly, crystalline urea separated. After removal of the urea by filtration and washing of the urea with more methylene chloride, the combined methylene chloride solution was extracted with small portions of 5% hydrochloric acid, 5% sodium bicarbonate and water. The residue, obtained from evaporation of the methylene chloride solution, was dissolved in hot water plus a small quantity of methanol. An additional amount of the insoluble urea could be removed by filtration. When most of the methanol was evaporated, the hot filtrate was allowed to cool gradually and the tripeptide separated as flocculated solid. Recrystallization of the solid in the same manner afforded 0.15 g. (40%) of fine needles, m.p. 187–188°, $[\alpha]^{28}D$ −12.5° (9.5 mg. in 1.1 ml. of glacial acetic acid).

Anal. Calcd. for $C_{19}H_{27}N_3O_5$: C, 60.46; H, 7.21; N, 11.14. Found: C, 60.65; H, 7.45; N, 10.91.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

A New Synthesis of Cysteinyl Peptides[1]

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Using a new protective system for the sulfhydryl and amino groups of cysteine, cysteinyl peptides and peptide derivatives have been prepared in good yield in crystalline form without detectable racemization. Cysteine was converted to a thiazolidine derivative by reaction with acetone; the nitrogen may be blocked by formylation. After formation of the new peptide bond the formyl group was removed by acid-catalyzed solvolysis and the thiazolidine ring was disrupted by mild acid hydrolysis or by mercuric chloride treatment. The new method permits the synthesis of cysteinyl peptides through the carboxyl function (L-cysteinylglycine) or through the amino group (γ-L-glutamyl-L-cysteine) or both.

In recent years, there has been a growing interest in the synthesis of cysteinyl peptides in connection with the studies of many peptides and proteins of biological importance, of which cysteine is an essential constituent. Due to the sensitivity of the cysteine molecule toward oxidation and elimination, it usually is necessary to protect β-sulfhydryl function in addition to the amino group or the carboxyl group during synthesis. Customarily this is accomplished by transformation of the β-sulfhydryl group to a S-benzyl thioether[3] or oxidation to the cysteinyl derivative[4,5] with the amino group blocked usually by the N-carbobenzoxy procedure. After the condensation is complete, both the carbobenzoxy group and the S-benzyl group or the disulfide linkage can be cleaved readily by reductive means.[5,6]

In this communication, a new[1] protective system for the synthesis of cysteinyl peptides which does not require the conventional reductive method, is reported. The synthesis consists of a thiazolidine

(1) The essential features of this method were reported in 1952 at the 122nd Meeting of the American Chemical Society and summarized in the Meeting Abstracts (J. C. Sheehan and W. A. Armstrong, Abs. No. 23, p. 15M) in a paper entitled, "A New Synthesis of Peptides Derived from Cysteine and Penicillamine." Since the preparation of this manuscript for publication, a paper has appeared (F. E. King, J. W. Clark-Lewis and R. Wade, *J. Chem. Soc.*, 880 (1957)) describing substantially our same 1952 scheme as, "A New Route to Cysteinyl-peptides," although our aforementioned meeting abstract was acknowledged. Compounds II, III and VI were reported in the Sheehan and Armstrong 1952 reference and also in the King, *et al.*, 1957 publication.

(2) James Flack Norris Memorial Fellow, 1953–1954. Aided in part by a contract from the Office of Naval Research.

(3) J. L. Wood and V. du Vigneaud, *J. Biol. Chem.*, **130**, 109 (1939).

(4) N. W. Pirie, *Biochem. J.*, **25**, 614 (1931).

(5) C. R. Harington and T. H. Mead, *ibid.*, **29**, 1602 (1935).

(6) (a) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935); (b) H. S. Loring and V. du Vigneaud, *ibid.*, **111**, 385 (1935).