

Synthesis of Tryptophan Peptides *

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Synthetic methods are described for the synthesis of the *p*-toluenesulfonates of glycyl-L-tryptophanamide, L-tryptophylglycyl-L-tryptophanamide, and glycyl-L-tryptophylglycyl-L-tryptophanamide. During the course of this work several carbobenzoxy and trityl derivatives of tryptophan peptides were prepared, and the applicability of various coupling methods was examined. Attention is drawn to the possible formation of β -oxyindolylalanine derivatives upon decarbobenzoylation of protected tryptophan peptides by HBr-acetic acid.

During the course of recent studies on the polymerization of dipeptide amides by cathepsin C (Würz *et al.*, 1962), it was found that glycyl-DL-tryptophanamide acetate is an excellent substrate for this enzyme. Near pH 7.5, no measurable hydrolysis of the carboxyl-terminal amide bond occurred, the extent of liberation of NH_4^+ approached that expected for complete reaction of the L-isomer, and the polymeric product (obtained in about 70% yield) corresponded to an octapeptide amide. In order to study the mechanism of this reaction further and to compare this cathepsin C-catalyzed polymerization with that of other dipeptide amides (e.g., glycyl-L-phenylalaninamide, glycyl-L-tyrosinamide, L-alanyl-L-phenylalaninamide), analytically pure glycyl-L-tryptophanamide was needed. Although the corresponding carbobenzoxydipeptide amide was readily obtained by Würz *et al.* (1962) with the mixed anhydride method (isobutylchlorocarbonate), unexpected difficulty was encountered in preparing, by catalytic hydrogenolysis, an analytically pure sample of the acetate or hydrochloride of glycyl-L-tryptophanamide. In the present communication, a method is given for the synthesis of glycyl-L-tryptophanamide by hydrogenolysis of the carbobenzoxydipeptide amide (prepared in better yield by the carbodiimide method) with palla-

dium oxide, and isolation of the dipeptide amide as the *p*-toluenesulfonate. In contrast to the pink products previously obtained, this salt was free of color, probably because acid was not added until after the hydrogenolysis and because the insolubility of the resulting salt tended to reduce the side-reactions leading to the formation of colored by-products. Preliminary enzymic experiments with the pure dipeptide amide *p*-toluenesulfonate have shown that it is completely deamidated by cathepsin C at pH 5, and gives rise to a polymeric peptide at pH 7.5; the results of the study of the action of cathepsin C on glycyl-L-tryptophanamide and related compounds will form the subject of a future communication.

Previous work on the polymerization of glycyl-L-tyrosinamide by cathepsin C (Würz *et al.*, 1962; Fruton and Knappenberger, 1962) had led to the hypothesis that the tetrapeptide amide glycyl-L-tyrosylglycyl-L-tyrosinamide is an intermediate in the polymerization reaction only when it is formed and held by the enzyme. In connection with proposed studies on the mechanism of cathepsin C action, the synthesis of the tetrapeptide amide glycyl-L-tryptophylglycyl-L-tryptophanamide was therefore undertaken.

Several routes for the synthesis of the tryptophan-containing tetrapeptide amide were examined, and observations were made on the behavior of tryptophan peptides that may be more generally useful. It was found that tritylglycyl-L-tryptophan methyl ester can readily be prepared by the carbodiimide method, and that this compound can be detritylated in good yield

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by means of *p*-toluenesulfonic acid to yield the dipeptide ester *p*-toluenesulfonate. This product, upon coupling with carbobenzoxy-L-tryptophan by the mixed-anhydride method (ethylchlorocarbonate), gave the expected carbobenzoxytripeptide ester. Similarly, carbobenzoxy-L-tryptophan and glycyl-L-tryptophanamide *p*-toluenesulfonate gave the expected carbobenzoxytripeptide amide in good yield. Some difficulty was encountered, however, in the isolation of an analytically pure uncolored product after catalytic hydrogenolysis of the carbobenzoxytripeptide amide. Although the chromatographic behavior and absorption spectrum of the *p*-toluenesulfonate of the tripeptide amide did not reveal the presence of impurities, traces of pink color could not be removed in repeated trials, and the nitrogen analysis gave a slightly low value.

The above tripeptide amide *p*-toluenesulfonate was coupled with carbobenzoxyglycine by the mixed-anhydride method (ethylchlorocarbonate) to yield the expected carbobenzoxytetrapeptide amide in crystalline form, melting at 196–198° after recrystallization. Catalytic hydrogenolysis of this product in the manner found suitable for carbobenzoxyglycyl-L-tryptophanamide gave the tetrapeptide amide *p*-toluenesulfonate. Attempts to prepare the carbobenzoxytetrapeptide amide by the coupling of carbobenzoxyglycyl-L-tryptophan (as its benzylammonium salt) with glycyl-L-tryptophanamide *p*-toluenesulfonate by the carbodiimide method gave only amorphous products with much lower melting points. It should be noted that the analytical data for C, H, and N were in accord with the theory, and that in optical activity the product was similar to that prepared from carbobenzoxyglycine and the tripeptide amide (where no racemization is expected). In general, it may be suggested that the carbodiimide method, when applied to the synthesis of tryptophan peptides, can sometimes lead to unfavorable results, and the mixed-anhydride method appears to be preferable.

Evidence was also obtained for the suitability of the coupling method developed by Woodward *et al.* (1961) in the synthesis of tryptophan peptides. For example, the reaction of carbobenzoxy-L-tryptophan and glycine benzyl ester, after activation with *N*-ethyl-5-phenylisoxazolium-3'-sulfonate, gave an excellent yield of the expected product. This method was applied to the coupling of tritylglycyl-L-tryptophan (as its diethylammonium salt) with glycyl-L-tryptophanamide *p*-toluenesulfonate, and the crude product was detritylated by means of *p*-toluenesulfonic acid. The resulting tetrapeptide amide *p*-toluenesulfonate exhibited the same chromatographic and spectroscopic properties as the material obtained from the coupling of carbobenzoxyglycine and L-tryptophylglycyl-L-tryptophanamide.

Recent studies on the synthesis of peptide hormones having adrenocorticotrophic and melanophore-stimulating activity have led to several

reports on the synthesis of tryptophan peptides, since the active peptides all contain one L-tryptophan residue. In particular, some synthetic routes have involved the use of the HBr-acetic acid method (Ben-Ishai and Berger, 1952; Ben-Ishai, 1954) for the decarbobenzoylation of protected tryptophan-containing peptides (Hofmann and Lande, 1961; Schwyzler and Kappeler, 1961). During our attempts to prepare analytically pure samples of glycyl-L-tryptophanamide from the carbobenzoxy compound, it was found that treatment with HBr-acetic acid led to rapid coloration of the reaction mixture (see also Boissonas *et al.*, 1958). The product isolated from the reaction mixture had an absorption spectrum which differed from that of the authentic glycyl-L-tryptophanamide in having a minimum near 253 m μ , instead of 245 m μ , and a much lower molecular extinction coefficient near 280 m μ than expected. Paper chromatography of an aqueous solution of the product revealed the presence of at least two major components, one of which appeared to be identical with glycyl-L-tryptophanamide as judged by the ninhydrin and Ehrlich reactions. The other chromatographic component did not appear to be L-tryptophan, glycyl-L-tryptophan, or glycine, and gave a greenish-yellow reaction with Ehrlich's reagent. Upon paper electrophoresis at pH 6.5, only one ninhydrin-positive component was detected; its mobility corresponded to that of an analytically pure sample of glycyl-L-tryptophanamide toluenesulfonate. These observations suggest that the treatment with HBr-acetic acid had led to partial conversion of the peptide amide to a β -oxyindolylalanine derivative; this amino acid gives a yellow color with Ehrlich's reagent, and its absorption spectrum in water exhibits a maximum near 250 m μ ($\epsilon_{\text{mol}} = 7250$), a shoulder near 282 m μ ($\epsilon_{\text{mol}} = \text{ca. } 1500$), and a minimum near 227 m μ ($\epsilon_{\text{mol}} = 3250$) (Cornforth *et al.*, 1951). In this connection, attention should be drawn to the studies of Lawson *et al.* (1960) on the formation of oxindole compounds upon the bromination of indoles. It may be suggested, therefore, that when tryptophan derivatives are decarbobenzoylated by the HBr-acetic acid method, the partial oxidation to β -oxindolylalanine derivatives is a possibility. With peptides of complex structure, paper chromatography may not lead to the separation of the impurity from the desired product, but a complete absorption spectrum may be expected to reveal the presence of the oxindole compound, and permit an estimate of the amount present.

In relation to the problem of the treatment of carbobenzoxy peptides with HBr-acetic acid it may be added that carbobenzoxyglycyldehydrophenylalaninamide can be converted in this manner to the hydrobromide of glycyldehydrophenylalaninamide, without appreciable saturation of the double bond, as shown by the similarity of its absorption spectrum to that of acetyldehydrophenylalanine (Fruton *et al.*, 1947).

EXPERIMENTAL¹

Glycyl-L-tryptophanamide *p*-Toluenesulfonate.—Carbobenzoxylglycyl-L-tryptophanamide was prepared by coupling carbobenzoxylglycine (6 g) with L-tryptophan methyl ester hydrochloride (7.5 g) in methylene chloride (100 ml) in the presence of triethylamine (3.03 g) and dicyclohexylcarbodiimide (6 g). The resulting sirupy ester (10 g, 83%) was converted to the amide in the usual manner (Würz *et al.*, 1962). Yield of crystalline product, 8.9 g (92%); after recrystallization from methanol-ether, the substance melted at 161–163° (Würz *et al.*, 1962, reported m.p. 169–170°; Smith, 1948, reported softening at 117–121° and m.p. 145°).

The carbobenzoxydipeptide amide (2.1 g) was subjected to catalytic hydrogenolysis in absolute ethanol (100 ml) in the presence of 0.5 g of palladium oxide. The evolution of CO₂ ceased after 3 hours; the reaction mixture was filtered, and to the chilled filtrate was added a solution of 0.95 g *p*-toluenesulfonic acid monohydrate in 200 ml of ether. Complete precipitation was effected by the further addition of 100 ml of petroleum ether. The crystalline product was collected, and recrystallized from 80% ethanol-ether. Yield, 1.9 g (83%); m.p. 160–161° (decomp.). $[\alpha]_D^{25} = 17.8^\circ$ (c, 2 in water). An aqueous solution of the product gave an absorption spectrum with a maximum at 280 m μ ($\epsilon_{\text{mol}} = 5480$), and a minimum at 245 m μ ($\epsilon_{\text{mol}} = 2220$). *p*-Toluenesulfonic acid makes a negligible contribution to the absorption at 280 m μ . At 245 m μ , its molecular extinction coefficient is 150.

Anal. Calcd. for C₂₀H₂₄N₄O₃S (432.5): C, 55.5; H, 5.6; N, 12.9. Found: C, 55.5; H, 5.8; N, 12.7.

Tritylglycyl-L-tryptophan Methyl Ester.—A mixture of 3.17 g of tritylglycine (Zervas and Theodoropoulos, 1956), L-tryptophan methyl ester hydrochloride (2.5 g), triethylamine (1.0 g), and dicyclohexylcarbodiimide (2 g) in methylene chloride (50 ml) was stirred at room temperature for 6 hours. The filtrate was washed rapidly twice with 10% aqueous Na₂CO₃ and water, dried over exsiccated Na₂SO₄, and concentrated *in vacuo* at 35°. The crystalline residue (3 g, 60%) was recrystallized from acetone. M.p. 187°.

Anal. Calcd. for C₃₃H₃₁N₃O₃ (517.6): C, 76.5; H, 6.0; N, 8.1. Found: C, 76.3; H, 6.0; N, 8.1.

Tritylglycyl-L-tryptophan Diethylammonium Salt.—To a suspension of 1.1 g of tritylglycyl-L-tryptophan methyl ester in 20 ml of acetone was added 1 ml of 2 N NaOH. The mixture was heated on the steam bath for 2–3 minutes, left at room temperature for 1 hour, and concentrated to dryness. Water (100 ml) was added, insoluble

material (0.4 g of unreacted ester) was filtered off, and the filtrate was acidified with acetic acid. The resulting precipitate was dried and dissolved in 5 ml of acetone, and diethylamine (0.2 ml) was added to the solution. The product crystallized upon chilling; yield 0.4 g (53%). After recrystallization from acetone, it melted at 173°.

Anal. Calcd. for C₃₈H₄₀N₄O₃ (576.7): C, 75.0; H, 7.0; N, 9.7. Found: C, 74.8; H, 6.8; N, 9.8.

Glycyl-L-tryptophan Methyl Ester *p*-Toluenesulfonate.—Tritylglycyl-L-tryptophan methyl ester (5.17 g) was suspended in acetone (15 ml), and *p*-toluenesulfonic acid monohydrate (1.95 g) was added. The resulting solution was heated under reflux for 5 minutes on the steam bath and chilled, and ether was added. The product was recrystallized from isopropanol-ether. Yield 3.5 g (77%); m.p. 176–178°.

Anal. Calcd. for C₂₁H₂₃N₃O₃S (447.4): C, 56.3; H, 5.5; N, 9.4. Found: C, 56.5; H, 5.4; N, 9.4.

Carbobenzoxylglycyl-L-tryptophan Benzylammonium Salt.—The sirupy carbobenzoxylglycyl-L-tryptophan methyl ester (3.8 g) was saponified in 20 ml of ethanol with 5 ml of 2 N NaOH. After 1 hour at room temperature, the solvent was removed *in vacuo* at 40°, 50 ml of water was added, and the aqueous solution was extracted with ether and acidified to Congo Red with hydrochloric acid. The resulting oil could not be crystallized. It was extracted with ethyl acetate, and the organic layer was washed with water and dried over exsiccated Na₂SO₄. Upon the addition of benzylamine (2 ml) and 50 ml of ether, the salt crystallized. It was recrystallized from ethanol-ether. Yield, 2.5 g (55%); m.p. 174°.

Anal. Calcd. for C₂₃H₃₀N₄O₃ (502.6): C, 66.9; H, 6.0; N, 11.1. Found: C, 66.8; H, 5.9; N, 11.1.

Carbobenzoxyl-L-tryptophylglycyl-L-tryptophan Methyl Ester.—Carbobenzoxyl-L-tryptophan (1.65 g; prepared according to Smith, 1948) and triethylamine (0.5 g) were dissolved in freshly purified dioxane (15 ml), the solution was chilled, and ethylchlorocarbonate (0.54 g) was added. After about 4 minutes, glycyl-L-tryptophan methyl ester *p*-toluenesulfonate (2.2 g) and triethylamine (0.5 g) were added. After 10 minutes, the mixture was diluted with 300 ml of water. The resulting precipitate was collected and washed thoroughly with 10% aqueous sodium bicarbonate and water. It was recrystallized from dimethylformamide-ether. Yield 1.9 g (69%); m.p. 180–182°.

Anal. Calcd. for C₃₃H₃₅N₅O₆ (597.6): C, 66.3; H, 5.8; N, 11.6. Found: C, 66.1; H, 5.5; N, 11.1.

A product having the same melting point was obtained by coupling in the presence of dicyclohexylcarbodiimide. However, the yield of recrystallized product was unsatisfactory. Prolonged exposure of the reaction mixture to dioxane, as in this coupling method, leads to the formation of deeply colored products.

¹ All melting points are uncorrected. We are indebted to Dr. Stephen M. Nagy (Department of Chemistry, Massachusetts Institute of Technology) for the microanalyses reported in this paper.

Carbobenzoxy-L-tryptophylglycyl-L-tryptophanamide.—To 10 ml of freshly distilled tetrahydrofuran were added carbobenzoxy-L-tryptophan (1.65 g), ethylchlorocarbonate (0.5 g), and triethylamine (1.0 g). After 5 minutes, a solution of glycyl-L-tryptophanamide *p*-toluenesulfonate (2.1 g) and triethylamine (1.0 g) in 90% tetrahydrofuran was added with vigorous shaking. After the reaction mixture had been kept at room temperature for 10 minutes, 300 ml of water was added, and the resulting precipitate was collected and washed thoroughly with 10% sodium bicarbonate and water. Yield, 2.6 g (80%); m.p. 209–210°. After recrystallization from acetonitrile-ether, it melted at 212–214°.

Anal. Calcd. for $C_{32}H_{32}N_6O_5$ (580.6): C, 66.2; H, 5.5; N, 14.4. Found: C, 66.1; H, 5.6; N, 14.3.

L-Tryptophylglycyl-L-tryptophanamide *p*-Toluenesulfonate.—The carbobenzoxytripeptide amide (1.4 g) was suspended in 50 ml of absolute ethanol and subjected to hydrogenolysis in the presence of 0.5 g of palladium oxide. After 3 hours, CO_2 evolution had ceased, and the peptide amide was isolated as the *p*-toluenesulfonate by the addition to the filtrate of 0.48 g *p*-toluenesulfonic acid monohydrate in ether. In repeated trials, although the ethanol solution was colorless, the addition of acid (even in the cold) gave a pink precipitate. The color could not be removed by recrystallization from isopropanol-ether. Upon paper chromatography (Whatman No. 1) in *n*-butanol-pyridine-acetic acid-water (15:10:3:12), the preparation gave a single ninhydrin-positive spot of R_F 0.77. In 80% ethanol, the substance showed an absorption maximum at 280 $m\mu$ ($\epsilon_{mol} = 11,600$), and a minimum at 244 $m\mu$ ($\epsilon_{mol} = 3420$). The data from elementary analysis accorded best with those calculated for the hemihydrate (*cf.* Hofmann and Lande, 1961).

Anal. Calcd. for $C_{31}H_{34}N_6O_6S$ (618.6): C, 60.1; H, 5.5; N, 13.6. Found: C, 58.7; H, 5.5; N, 12.9.

Carbobenzoxyglycyl-L-tryptophylglycyl-L-tryptophanamide.—The mixed anhydride formed in tetrahydrofuran (10 ml) from carbobenzoxyglycine (0.5 g) and ethylchlorocarbonate (0.27 g) in the presence of triethylamine (0.25 g) was mixed with a solution of L-tryptophylglycyl-L-tryptophanamide *p*-toluenesulfonate (1.5 g) and triethylamine (0.5 g) in 90% tetrahydrofuran. After 10 minutes, 300 ml of water was added, and the resulting precipitate was filtered, washed thoroughly with 10% sodium bicarbonate and water, and dried. The amorphous colored material was dissolved in 30 ml of isopropanol, heated under reflux for 3–4 minutes in the presence of 0.5 g of Norite, and filtered. By the addition of 20 ml of dry ether, a slightly colored amorphous material (0.15 g) was obtained. The filtrate was evaporated to dryness, and, upon treatment of the resulting oil with 90% ethanol, a crystalline product (0.5 g; 34%) melting at 190–192° was obtained. $[\alpha]_D^{25} = -13.2^\circ$ (c,

1 in dimethylformamide). Further recrystallization from ethanol raised the m.p. to 196–198°.

Anal. Calcd. for $C_{34}H_{35}N_7O_6$ (637.7): C, 64.0; H, 5.5; N, 15.4. Found: C, 64.1; H, 5.5; N, 15.0.

The preparation of the carbobenzoxy tetrapeptide amide was also attempted by the coupling of carbobenzoxyglycyl-L-tryptophan benzylammonium salt (1.25 g) with glycyl-L-tryptophanamide *p*-toluenesulfonate in 5 ml of dimethylformamide in the presence of 0.5 g dicyclohexylcarbodiimide. The reaction mixture was left at room temperature overnight and filtered, and 500 ml of water was added to yield a precipitate that was washed thoroughly with 10% sodium bicarbonate and water. Yield 1.1 g (73%). No crystalline product could be obtained from this amorphous material. After precipitation from ethyl acetate-ether, it softened at 115° and melted at about 140°. $[\alpha]_D^{25} = -14.0^\circ$ (c, 1 in dimethylformamide).

Anal. Calcd. for $C_{34}H_{35}N_7O_6$ (637.7): C, 64.0; H, 5.5; N, 15.4. Found: C, 63.7; H, 5.8; N, 15.2.

Glycyl-L-tryptophylglycyl-L-tryptophanamide *p*-Toluenesulfonate.—The crystalline carbobenzoxy tetrapeptide amide (400 mg) that melted at 192–193° was subjected to catalytic hydrogenolysis with palladium oxide in 90% ethanol. Addition of *p*-toluenesulfonic acid monohydrate (150 mg) and ether gave 200 mg (50%) of a product that was precipitated with isopropanol-ether. Paper chromatography (Whatman No. 1) with *n*-butanol-pyridine-acetic acid-water (15:10:3:12) gave a single spot with an R_F of 0.69 (ninhydrin and Ehrlich).

Anal. Calcd. for $C_{33}H_{37}N_7O_6S$ (675.7): C, 58.6; H, 5.5; N, 14.5. Found: C, 58.2; H, 5.4; N, 14.5.

An attempt was made to prepare this compound by the coupling of tritylglycyl-L-tryptophan diethylammonium salt (1.44 g) and glycyl-L-tryptophanamide (1.08 g) in the presence of *N*-ethyl-5-phenylisoxazolium-3'-sulfonate (0.63 g) and triethylamine (0.35 ml), with dimethylformamide (10 ml) as the solvent. After 18 hours, 400 ml of water was added, and the product (1.5 g) was collected. It was dissolved in ethanol, a few drops of diethylamine were added, and the desired product was precipitated by the addition of water containing NaCl. This product (0.6 g) was detritylated by treatment with 0.18 g of *p*-toluenesulfonic acid monohydrate in 5 ml of absolute ethanol. The mixture was heated under reflux for 3 minutes, ether was added, and the resulting product was reprecipitated from ethanol-ether. Yield, 0.43 g. This product showed the same chromatographic behavior as the one prepared from the carbobenzoxy compound.

The two samples of the tetrapeptide amide tetrasulfonate gave absorption spectra that were identical with respect to the positions of the maxima and minima; the principal maximum was at 280 $m\mu$ ($\epsilon_{mol} = 12,360$), and the minimum

at 245 $m\mu$ ($\epsilon_{mol} = 3540$).

Carbobenzoxy - L - tryptophylglycine Benzyl Ester.—Carbobenzoxy-L-tryptophan (4 g; 0.02 mole) and glycine benzyl ester *p*-toluenesulfonate (7 g; 0.02 mole; prepared according to Zervas *et al.*, 1957) were coupled by the mixed-anhydride procedure (ethylchlorocarbonate) in 20 ml of tetrahydrofuran. Yield 6.9 g (75%). After recrystallization from ethyl acetate-petroleum ether, the substance melted at 117–119°.

Anal. Calcd. for $C_{23}H_{27}N_3O_5$ (485.5): C, 69.3; H, 5.6; N, 8.7. Found: C, 69.4; H, 5.8, N, 8.7.

The synthesis of the same compound by coupling 0.005 mole quantities of the reactants in the presence of *N*-ethyl-5-phenylisoxazolium-3'-sulfonate with acetonitrile as the solvent gave a yield of 1.9 g (89%) of crude product, m.p. 115–117°. After recrystallization as before, 1.6 g (m.p. 117–119°) was obtained.

Treatment of Carbobenzoxyglycyl - L-tryptophanamide with HBr in Acetic Acid.—The carbobenzoxy dipeptide amide (200 mg) was suspended in 5 ml of a freshly-prepared solution of 2 *N* HBr in glacial acetic acid. After 2 hours at room temperature, the substance had dissolved, ether was added, and the resulting product was collected. Yield, 160 mg (93%). An aqueous solution of the product gave an absorption spectrum with a maximum at 281 $m\mu$ ($\epsilon_{mol} = 4200$) and a minimum at 253 $m\mu$ ($\epsilon_{mol} = 2600$), assuming a molecular weight of 341. Upon paper chromatography (Whatman No. 1) in *n*-butanol-pyridine-acetic acid-water (15:10:3:12), three ninhydrin-positive components (R_F 0.57, 0.44, 0.29 [faint]) were observed; the reference substances used were glycyl-L-tryptophanamide *p*-toluenesulfonate (R_F 0.57), L-tryptophan (R_F 0.56), glycyl-L-tryptophan (R_F 0.47), and glycine (R_F 0.25). With *n*-butanol-formic acid-water (15:3:1), two ninhydrin-positive spots (R_F 0.19, 0.07) were observed; the faster-moving component corresponded to the authentic dipeptide amide, and also gave a red spot (turning dark green) with Ehrlich's reagent (2% solution of *p*-dimethylaminobenzaldehyde in 5% hydrochloric acid). The slower-moving compound gave a greenish-yellow spot with Ehrlich's reagent. In the latter chromatographic system, glycyl-L-tryptophan had an R_F value of 0.38. Upon paper electrophoresis (Whatman No. 3) at pH 6.5 (pyridine-acetic acid-water, 20:1:200) and 300 volts for 3 hours, only one ninhydrin-positive component was observed. Its movement toward the cathode (5.0 cm) corresponded to that of an authentic sample of glycyl-L-tryptophanamide.

After chromatography with *n*-butanol-formic acid-water and elution of the slower-moving component with water, the resulting solution showed an absorption spectrum with a maximum at 256 $m\mu$; the ratio of absorbance at 256 $m\mu$ to that at 282 $m\mu$ (where a lesser peak was noted) was 4.4. It should be noted in this connection that prolonged exposure of the hydrobromide to the

acidic chromatographic solvent led to a marked change in the absorption spectrum of the more rapid component with an R_F similar to that of authentic glycyl-L-tryptophanamide; maximal absorption was observed at 256 $m\mu$ and 280 $m\mu$ (ratio of absorbance, 2.6).

Upon decarboxylation of carbobenzoxyglycyl-L-tryptophanamide with HBr-acetic acid in the presence of 20% diethylphosphite (Boissonas *et al.*, 1958), the product obtained upon precipitation with ether gave an absorption spectrum (in water) having a maximum at 280 $m\mu$ and a minimum at 251 (ratio, 1.8). This ratio may be compared to that for the product of HBr treatment in the absence of diethylphosphite ($A_{281}/A_{253} = 1.6$), and for the authentic glycyl - L - tryptophanamide *p*-toluenesulfonate ($A_{280}/A_{245} = 2.7$).

Glycyldehydrophenylalaninamide Hydrobromide.—Carbobenzoxyglycyldehydrophenylalaninamide (200 mg; Doherty *et al.*, 1943) was dissolved in 3 ml of glacial acetic acid containing 0.05 g HBr, and the solution was heated under reflux for 1 minute. After 30 minutes at room temperature, ether (about 80 ml) was added, and the resulting precipitate was dissolved in water. The aqueous solution was filtered and evaporated to dryness, and the crystalline residue was washed with absolute ethanol. Yield, 150 mg. An aqueous solution (pH 4.5) of the product showed an absorption spectrum with a maximum at 278 $m\mu$ ($\epsilon_{mol} = 15,170$).

Anal. Calcd. for $C_{11}H_{13}N_3O_2Br$ (300.1): C, 44.0; H, 4.7; N, 14.0. Found: C, 43.6; H, 4.7; N, 13.7.

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