The N-acetyl compound was prepared with acetic anhydride at 25°. It was crystallized from benzene–petroleum ether as hard granules, m.p. 99°.

Anal. Calcd. for $C_{11}H_{19}NO_2$: C, 66.97; H, 9.71; N, 7.10. Found: C, 66.56; H, 9.56; N, 7.14.

N-Methyl-10-hydroxydecahydroquinoline (XII).—To a mixture of 5 ml. of 98% formic acid and 5 ml. of 40% formalin was added 0.2 g. of XI and the solution heated for two hours on a steam cone. The solvents were removed *in vacuo*, the residue made strongly alkaline and extracted with 2 25-ml. portions of ether. Concentration of the dried extract gave 170 mg. of oil which was converted to the hydrochloride with ethereal HCl and separated as cubes, m.p. 187–193°.

The picrate was obtained from an aqueous solution of the hydrochloride; it separated as tufts of fine yellow needles, liquefied at 153–154° and cleared at 157°.

Anal. Calcd. for $C_{16}H_{22}N_4O_8$: C, 48.24; H, 5.57; N, 14.07. Found: C, 48.04; H, 5.74; N, 13.74.

The methiodide was prepared in benzene. It was crystallized from methanol–ether as colorless granules, m.p. 236–237° with slight decomposition.

Anal. Calcd. for $C_{11}H_{22}INO$: C, 42.45; H, 7.13; N, 4.50. Found: C, 41.77; H, 6.86; N, 4.33.

2-Keto-1-azabicyclo[5.3.0]decane (XIV).—To a solution of 0.34 g. of ketolactam IX in 10 ml. of methanol were added 50 mg. of PtO_2, 3 drops of acetic acid and 1 drop of water. After four hours of hydrogenation the only uptake observed was that corresponding to reduction of the catalyst. After addition of 1 ml. of concd. hydrochloric acid hydrogenation was rapid and ceased after 1 hour with the absorption of one molar equivalent. The solution was filtered and concentrated to a colorless crystalline residue. The compound was recrystallized by slow evaporation from methylene chloride; it separated as clusters of needles which began to sublime at 95° and melted at 103–105°.

Anal. Calcd. for $C_9H_{16}NOCl$: C, 56.99; H, 8.50; N, 7.39; Cl, 18.69. Found: C, 57.27; H, 8.69; N, 7.37; Cl, 18.61.

The free lactam was obtained by saturating an alkaline solution of the hydrochloride with sodium sulfate and extracting with ether. The compound is a colorless oil which crystallized below 0°. It did not form a picrate in ether.

1-Azabicyclo[5.3.0]decane (XIII). A. By Reduction of the Ketolactam (IX).—To a solution of 0.8 g. of ketolactam IX in 10 ml. of dioxane was added 1 g. of powdered lithium aluminum hydride, and the mixture refluxed for 48 hours. Excess hydride was decomposed with ethyl acetate and water and the suspension was concentrated to dryness *in vacuo*. The residue was extracted with 3 25-ml. portions of hot petroleum ether (65–67°) and the combined extracts concentrated to a colorless oil (0.36 g.) with strong amine odor. The oil was dissolved in 10 ml. of ether and converted to the picrate. The crystalline precipitate was washed with cold water and recrystallized twice from methanol by slow evaporation. It separated as clusters of large needles, m.p. 215–216°, undepressed on admixture with the picrate of XIII (m.p. 214–215°) prepared by Clemmensen reduction of 1-ketoquinolizidine.[20] The infrared spectra were identical.

Anal. Calcd. for $C_{15}H_{20}N_4O_7$: C, 48.91; H, 5.47; N, 15.21. Found: C, 49.09; H, 5.18; N, 14.94.

B. By Reduction of Lactam (XIV).—To a suspension of 50 mg. of lactam hydrochloride XIV in 10 ml. of dioxane was added 0.2 g. of powdered lithium aluminum hydride and the mixture refluxed for 24 hours. After addition of water and concentration to dryness, the residue was extracted with 2 × 20 ml. of ether and the base precipitated as the picrate. The yellow precipitate of long plates was recrystallized twice from methanol; it separated as large needles, m.p. 214–215°. A mixed m.p. with authentic picrate[20] gave no depression.

Acknowledgment.—The authors thank Dr. William C. Alford and his associates, all of this Institute, for carrying out the microanalyses, Mr. Wm. M. Jones for measuring the infrared spectra, and Mrs. Anne Wright for the ultraviolet spectra.

(20) *Cf.* ref. 11. This sample was kindly supplied by Dr. Nelson J. Leonard.

BETHESDA 14, MARYLAND

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY AND THE NEW YORK STATE PSYCHIATRIC INSTITUTE]

Thioesters of Glutamic Acid[1]

BY HOWARD SACHS AND HEINRICH WAELSCH

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The syntheses of S-α- and γ-glutamylglutathione *via* the corresponding thiophenyl esters of carbobenzyloxyglutamic acid are reported. The protecting group was removed by treatment with a mixture of glacial acetic acid, hydrobromic acid and phenol. The homogeneity and structures of the compounds were confirmed in a number of ways. When the corresponding carbobenzyloxythioglutamic acids were used as the starting material, mixtures of α- and γ-isomers were obtained indicating rearrangement during the synthetic procedure.

Recently, a study of the non-enzymatic and enzymatic hydrolysis and transfer reactions of S-acetyl GSH[2] has been reported.[3] The possibility that thioesters of amino acids may be intermediates in the synthesis of amide bonds has been discussed. It was hoped that some information on the role of thioesters of amino acids in biosynthetic processes might be obtained by the use of model substrates and in particular of thioesters of glutamic acid.

In this paper the syntheses of S-α- and S-γ-glutamyl GSH (I, II) are presented.[4]

In the syntheses of glutamyl thioesters the α- or γ-derivative has to be prepared with the exclusion of the other isomer. Also, despite the confirmed homogeneity of the starting material it is necessary to prove unequivocally the structure of the final product in order to exclude rearrangement during the synthetic procedure. This stringent requirement for the glutamyl compounds is a consequence

(1) This work was supported in part by grants from the National Institute of Neurological Disease and Blindness (Grant B-226-C) of the National Institute of Health, United States Public Health Service and by a contract between the Office of Naval Research and the Psychiatric Institute.

(2) The following abbreviations and symbols are used (*cf.* ref. 5a): GSH, glutathione; Z, carbobenzyloxy, $C_6H_5CH_2OCO$; Bz, $C_6H_5CH_2$; Et, C_2H_5; Glu-, $NHCH(CH_2CH_2COOH)CO$, $C_5H_7O_3N$; when the γ-carboxyl group of glutamic acid is substituted, the substituent in the γ-position is indicated below the line: Glu; otherwise, a free γ-COOH group is implied. Configuration follows compound in parentheses and refers to the glutamic acid moiety since only thiophenol or natural GSH served as the mercaptan, *e.g.*, N-carbobenzyloxy-L-glutamic acid α-thiophenyl ester, Z.Glu.S.C_6H_5(L); N-carbobenzyloxy S-γ-L-glutamylglutathione, Z.Glu.OH(L); S-α-L-glutamylglutathione, H.Glu.SG(L).

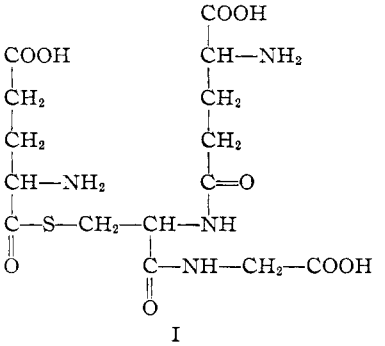
(3) H. Strecker, P. Mela and H. Waelsch, *J. Biol. Chem.*, **212**, 223 (1955).

(4) A preliminary report has been given, *cf.* H. Sachs, *Federation Proc.*, **13**, 951 (1954); H. Sachs, H. J. Strecker and H. Waelsch, *Science*, **120**, 791 (1954).

of the presence of two carboxyl groups whose very favorable steric relationship make possible the interconversion of isomers.[5]

Synthesis of S-α- and S-γ-glutamyl GSH was attempted by way of the corresponding carbobenzyloxy-mono-thioglutamates prepared from the mixed anhydride of ethylcarbonic acid and the γ-ethyl or α-benzyl ester of Z. glutamic acid according to the procedures of Sheehan[6] and Cronyn[7] for other acylaminothio acids. Because of the stability of thio acids in alkali, removal of the ester protecting the other carboxyl group of glutamic acid was readily accomplished. However, it was observed that the final products always consisted of mixtures of the α- and γ-isomers when pure α-benzyl or γ-ethyl esters of Z. glutamic acid were used as starting material.[8]

These results are reminiscent of similar rearrangements previously reported for other glutamyl derivatives[5] and suggests the interesting possibility



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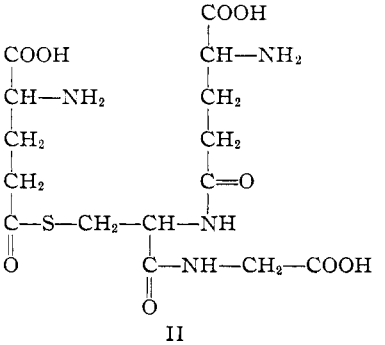
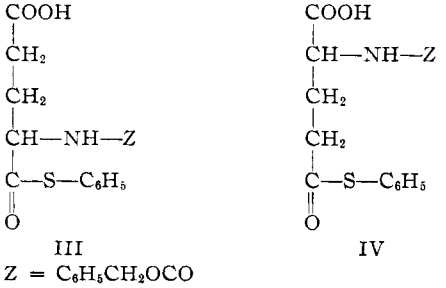


II



III

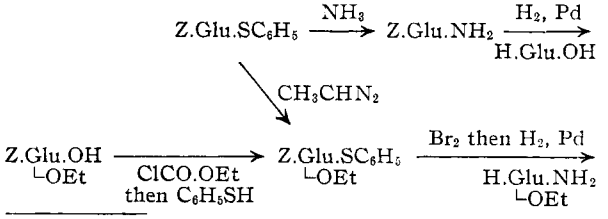
IV

Z = $C_6H_5CH_2OCO$

that conversion of γ- to α-glutamyl bonds may occur under biological conditions.[9]

In their extensive studies of thioesters and related compounds, Wieland and his group have shown that an acyl group may readily be transferred from thiophenol to other mercaptans such as GSH,[10] and it appeared probable that the rearrangement experienced with the thio acids might be avoided by the use of thiophenyl esters of glutamic acid, with subsequent transfer of the Z. glutamyl moiety to GSH. The pure α- and γ-thiophenyl esters of Z. glutamic acid (III, IV) were prepared by heating Z. L-glutamic acid anhydride with thiophenol under two different experimental conditions which favored the formation of one isomer. The milder conditions employed for the preparation of the α-thiophenyl-Z. glutamate gave the thioester of L-glutamic acid whereas the more rigorous conditions used for the synthesis of the γ-isomer resulted in racemization of the glutamic acid moiety. The carbobenzyloxy group of the thioester of Z. glutamic acid was removed without cleavage of the thioester linkage by treatment with a mixture of glacial acetic acid, phenol or thiophenol and hydrobromic acid.[11] Phenol was added as a trapping agent for any bromine which may have been formed.[12] This procedure may be of general usefulness in the preparation of aminoacyl thioesters.[13]

The homogeneity of each derivative was confirmed in a number of ways. For example, Z-L-glutamic acid α-thiophenyl ester was characterized in the following manner: (a) the thio-ester was treated with concentrated ammonia and the Z. group removed by catalytic hydrogenolysis. The presence of isoglutamine was shown by analysis of the solution and by chromatography; (b) treatment with diazoethane gave crystalline Z.α-thiophenyl-γ-ethyl L-glutamate, identical with an authentic compound prepared from N-Z-γ-ethyl L-glutamate and thiophenol; (c) oxidative cleavage (bromine) of the thioester group of this latter compound, removal of the Z. group and subsequent analysis gave values consistent with γ-ethyl glutamate. This series of reactions is



(5) (a) H. Sachs and E. Brand, THIS JOURNAL, **76**, 1815 (1954); (b) Clayton and Kenner, *Chem. and Ind.*, 1205 (1953); (c) P. J. Fodor, A. Miller, A. Neidle and H. Waelsch, *J. Biol. Chem.*, **203**, 991 (1953); (d) A. R. Battersby and J. C. Robinson, *J. Chem. Soc.*, 259 (1955).

(6) J. C. Sheehan and D. A. Johnson, THIS JOURNAL, **74**, 4726 (1952).

(7) M. W. Cronyn and J. Jiu, *ibid.*, **74**, 4726 (1952).

(8) Synthesis and properties of the thioglutamic acids will be reported in the near future.

(9) H. Waelsch, *Advances in Enzymology*, **13**, 313 (1952).

(10) For a recent review *cf.* T. Wieland, *Angew. Chem.*, **66**, 507 (1954).

(11) The removal of the carbobenzyloxy group from amino acid esters and peptides with hydrobromic acid in glacial acetic acid has been reported from several laboratories; *cf.* G. V. Anderson, J. Blodinger and A. D. Welcher, THIS JOURNAL, **74**, 5309 (1952); D. Ben-Ishai, *J. Org. Chem.*, **19**, 62 (1954); N. F. Albertson and F. C. McKay, THIS JOURNAL, **75**, 5323 (1953).

(12) D. I. Weisblat, B. J. Magerlein, A. R. Hanze, D. R. Myers and S. T. Rolfson, *ibid.*, **75**, 3625 (1953).

(13) Simultaneous with our preliminary account of this work,[4] Schwyzer (*Helv. Chim. Acta*, **37**, 647 (1954)) reported the preparation of several amino acid and peptide thioester hydrobromides by treating the corresponding carbobenzyloxy derivatives with hydrobromic acid in glacial acetic acid under carefully controlled anhydrous conditions, which are not imperative when phenol is added to the reaction mixture as described here.

Of some interest are the low values of amino nitrogen obtained when aqueous solutions of the α-thiophenyl ester of glutamic acid were subjected to the nitrous acid procedure[14] in the presence of acetic acid. When the determination was carried out in a solution of mineral acid or in acetic acid with subsequent addition of dilute mineral acid most of the nitrogen was recovered. The low values in the former case are probably partly a result of decomposition to ninhydrin negative material (pyrrolidonecarboxylic acid or diketopiperazine[15]), and partly to formation of a stable diazo ester. The facile conversion to a diazo compound would be expected in view of the ease of activation (loss of a proton and stabilization of the negative charge) of the α-carbon of other acyl thioesters (*i.e.*, acetyl CoA).

Experimental[16]

Methods.—For analysis, all compounds were dried to constant weight at 100° *in vacuo* over P_2O_5. Carboxyl and amino nitrogen and carbon determinations were performed according to Van Slyke, *et al.*[17,18] All m.p.'s are corrected.

α-Thiophenyl Z-L-Glutamate.—Z-L-Glutamic acid anhydride[19] (8 g., 0.03 mole) was dissolved in 80 ml. of thiophenol and 10 ml. of pyridine and the mixture was permitted to stand at room temperature for 4 to 6 hours. The solution was distilled *in vacuo* to a sirup; 50 ml. of ethyl acetate was added and removed *in vacuo*. This procedure was repeated 3 times in order to remove residual thiophenol. The final sirup was taken up in 50–75 ml. of ethyl acetate–ether (1:1), washed with dilute hydrochloric acid and then water and dried over sodium sulfate. Petroleum ether was added until the solution became turbid and the ester began to crystallize. The material was recrystallized from ethyl acetate–petroleum ether with a yield of 3.5 g. An additional 2.0 g. was obtained by adding more petroleum ether to the first mother liquor. The total yield of pure ester was 5–6 g. (55–65%), soft needles, m.p. 113–114°; $[\alpha]^{26}D$ −44.8° (1.7% in glacial acetic acid). *Anal.* Calcd. for $C_{19}H_{19}O_5NS$ (373): C, 61.2; N, 3.8. Found: C, 61.0; N, 3.8.

Characterization of Z.Glu.S.C_6H_5: Isoglutamine.—Concentrated ammonia (2 ml.) was added to 0.056 g. (0.15 mmole) of Z.Glu.S.C_6H_5 and after 5–10 minutes at room temperature 5 ml. of water was added to the mixture. The solution was concentrated *in vacuo*, washed with ether, acidified with 1 *N* hydrochloric acid and extracted with ethyl acetate. The ethyl acetate solution was washed with water and taken to dryness *in vacuo*. The residue was dissolved in 2 ml. of 80% acetic acid and hydrogenolyzed with palladium on charcoal. When the hydrogenolysis was completed the solution was brought to 10 ml. with water and aliquots subjected to chromatography and analysis for free ammonia, labile amide ammonia and carboxyl, amino and total nitrogen. Calculated for isoglutamine: $C_5H_{10}O_3N_2$ (146): free NH_3, 0.0; labile amide NH_3, 0.0; carboxyl N, 0.0; ratio total N/amino N, 2.0/1.0. Found: free NH_3, 0.0; labile amide NH_3 (10% TCA, 70°, 70 minutes) 0.0; carboxyl N (*p*H 2.5), 0.0; ratio total N/amino N, 2.1/1.0 (yield 88%). Chromatography (phenol–water) gave a single spot corresponding to isoglutamine (or glutamine). Paper electrophoresis at *p*H 4.5 (0.005 *N* acetate buffer) was incapable of separating the two amides. With the same buffer, however, isoglutamine moved far ahead of glutamine toward the cathode if the sample to be chromatographed was dissolved in 10–30% acetic acid. Under these conditions a sample of the above solution gave a single spot indistinguishable from authentic isoglutamine and easily separable from the γ-isomer. Glutamic acid which was inseparable from glutamine in this system could easily be identified on a chromatogram developed with phenol–water.

α-Thiophenyl-γ-ethyl Z-L-Glutamate.—Z.Glu.S.C_6H_5(L) (0.059 g., 0.16 mmole) was treated with ethereal diazoethane. After removal of the solvent *in vacuo* the residue was taken up in glacial acetic acid; upon the addition of water 0.06 g. (93%) of crystalline material precipitated, m.p. 98–99°. *Anal.* Calcd. for $C_{21}H_{23}O_5NS$ (401): N, 3.5. Found: N, 3.5. This derivative did not depress the m.p. of the authentic γ-ethyl-α-thiophenyl Z-L-glutamate. The authentic derivative was prepared essentially according to the procedure of Wieland, *et al.*[20] The mixed ethylcarbonic acid anhydride of γ-ethyl Z-L-glutamate (1.6 mmoles) was treated with thiophenol (5.0 mmoles) for 90 minutes at 60–70°. The product was recrystallized from acetic acid–water and ether–petroleum ether. The yield of pure compound was 90%, m.p. 98–99°; $[\alpha]^{26}D$ −41.4° (2.0% in glacial acetic acid). *Anal.* Calcd. for $C_{21}H_{23}O_5NS$ (401): C, 62.9; N, 3.5. Found: C, 62.5; N, 3.5.

The other possible isomer, α-ethyl-γ-thiophenyl Z-L-glutamate, prepared from α-ethyl Z-L-glutamate as described above was not obtained in crystalline form.

γ-Ethyl Glutamate.—Z.Glu.S.C_6H_5 was esterified with ethereal diazoethane as described above. The solvent was removed *in vacuo* and the sirup was taken up in 70% acetic acid. Bromine water was added until the yellow color persisted, and the material was then hydrogenated in the usual manner. Analysis of the solution indicated an equivalent amount of carboxyl, amino and total nitrogen, a result which would be expected for γ-ethyl glutamate (although it should be noted that the recovery in this case was poor (50–60%)).

Optical Purity.—As seen above the Z.Glu.S.C_6H_5 was optically active in glacial acetic acid and the γ-ethyl derivative was identical with the authentic L-isomer. A sample of Z.Glu.S.C_6H_5 was hydrolyzed in 6 *N* hydrochloric acid and the specific rotation of the free glutamic acid was determined. Found: $[\alpha]^{26}D$ +30.0° (1.1% in 6 *N* hydrochloric acid).

γ-Thiophenyl Z-DL-Glutamate.—Z.L-glutamic acid anhydride (5.0 g., 0.019 mole) was dissolved in 20 ml. of dioxane and the solution warmed to 100°. Thiophenol (20 ml.) and tri-*n*-butylamine (4.8 ml., 0.02 mole) were added and the temperature was maintained at 100° for 30 minutes. The product was isolated in exactly the same manner as described above for the α-isomer. It was recrystallized from ethyl acetate–petroleum ether or methanol–water. The yield of pure compound was 30–50%, m.p. 134–135°. *Anal.* Calcd. for $C_{19}H_{19}O_5NS$(373): C, 61.2; N, 3.8. Found: C, 61.2; N, 3.7.

Although the α-derivative was shown to be optically pure the γ-compound was completely racemized. It gave zero rotation in glacial acetic acid or ethanol and hydrolysis in 6 *N* hydrochloric acid yielded DL-glutamic acid. Under milder conditions or in the absence of base, racemization might be avoided. However, no attempts were made along these lines since the DL-compound was sufficient for the present purposes.

The homogeneity and structure of this compound was demonstrated by treatment with ammonia, removal of the Z.group and analysis of the solution as described above. Z-γ-DL-Glutamylthiophenyl ester, 0.069 g.; final volume 10 ml.; calcd. for glutamine $C_5H_{10}O_3N_2$(146): ratio total N: carboxyl N: amino N, 2.0:1.0:1.8.[21] Found: total N: carboxyl N:amino N, 2.1:1.0:1.8. The solution contained no free ammonia but the presence of labile ammonia was indicated by the gradual color development with Nessler reagent, a behavior characteristic for glutamine. Phenol-water chromatograms and paper electrophoresis (acetate buffer, *p*H 4.5, sample in acetic acid solution) gave single spots corresponding to glutamine.

(14) D. D. Van Slyke, *J. Biol. Chem.*, **9**, 185 (1911); **12**, 275 (1912); **16**, 121 (1913).

(15) H. Sachs and H. Waelsch, unpublished results.

(16) We are indebted to T. Zelmenis for the analytical results.

(17) D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen and P. Hamilton, *J. Biol. Chem.*, **141**, 627 (1941).

(18) D. D. Van Slyke, R. Steele and J. Plazin, *ibid.*, **192**, 769 (1951). This rapid wet combustion method for total carbon has greatly facilitated the work with the compounds described in this report, many of which are very hygroscopic. By the use of this method a variety of determinations (total C, total N, carboxyl and amino N) can be carried out on the same solution.

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

(20) T. Wieland, W. Schafer and E. Bokelmann, *Ann.*, **573**, 99 (1951).

(21) Based on the observation that glutamine under these conditions yields approximately 1.8 equivalents of amino nitrogen; *cf.* A. C. Chibnall and R. G. Westall, *Biochem. J.*, **26**, 122 (1932); H. Sachs and E. Brand, THIS JOURNAL, **76**, 3601 (1954).

S-α-Z-L-Glutamyl GSH.—GSH (1.4 g., 4.5 mmoles), was dissolved in 60 ml. of solution containing lithium carbonate (2.2 g., 30 mmoles) and adjusted to *p*H 7–8 with 2.68 ml. of 5.6 *N* hydrobromic acid; this solution was added slowly (1–3 minutes) with stirring to 5.0 g. (13.4 mmoles) of Z.Glu.S.C_6H_5(L) in 20 ml. of purified dioxane. The homogeneous reaction mixture gradually became turbid as thiophenol precipitated. After 10 minutes at room temperature the reaction was completed (as indicated by the absence of a positive nitroprusside test[22] with sodium carbonate as the base and the presence of material giving a delayed nitroprusside reaction with ammonia; the solution was then acidified with 2.68 ml. of 5.6 *N* hydrobromic acid, and extracted with ethyl acetate and then ether. Unreacted Z.Glu.S.C_6H_5(L) may be recovered from the organic phase or this solution may be used directly for coupling reactions with more GSH or other mercaptans. The aqueous phase was taken down *in vacuo* to a small volume and ethanol and ether added. The precipitated material was washed repeatedly with ethanol and then recrystallized from water–isopropyl alcohol–ether. The yield of pure material was 1.7–2.1 g. (70–80%); m.p. 179–180° dec., $[\alpha]^{26}D$ −7.3° (3.2% in 0.01 *N* hydrobromic acid). *Anal.* Calcd. for $C_{23}H_{30}O_{11}N_4S$(570.6): C, 48.4; N, 9.7; carboxyl N, 2.4; amino N, 2.4.[23] Found: C, 48.0; N, 9.8; carboxyl N, 2.4 (*p*H 1.0); amino N, 4.7 (3 min.) 6.2 (30 min.).[24]

A sample of this compound was treated with ammonia as described above for the Z.glutamyl thiophenyl esters. After removal of the Z. group, analysis of aliquots of the solution showed no free ammonia, no labile amide nitrogen, no carboxyl nitrogen (*p*H 2.5) and amide nitrogen (hydrolysis in 6 *N* hydrochloric acid) and amino nitrogen values equal to $^1/_2$ of the total nitrogen. In confirmation of the analytical results chromatography (phenol–water) and paper ionophoresis showed the presence of a single component corresponding to isoglutamine.

S-γ-Z-Glutamyl GSH Hydrobromide.—γ-Thiophenyl ester of Z-DL-glutamate 1.0 g. (2.7 mmoles) in 5 ml. of dioxane was treated with 0.28 g. (0.9 mmole) of GSH and 1.5 g. (15 mmoles) of potassium bicarbonate in 15 ml. of water. After the reaction was completed (15–20 minutes), 2.85 ml. of 5.6 *N* hydrobromic acid (16 mmoles) was added and the mixture extracted with several portions of ether. The aqueous phase was taken to dryness *in vacuo* and the residue extracted with four 30-ml. portions of glacial acetic acid. The solution was concentrated *in vacuo* to a volume of 5–10 ml., 25 ml. of acetone was added and the insoluble material centrifuged off and discarded. Addition of several volumes of ether gave an oil which crystallized on trituration with ether. The material was recrystallized from glacial acetic acid–ether, or purified by extracting with hot aqueous acetone, concentrating the solution *in vacuo* and precipitating the product with ether. The yield was 0.45 g. (77%), m.p. 167–169° dec. (with sintering at 110°).

Anal. Calcd. for $C_{23}H_{30}O_{11}N_4S$·HBr (651.5): N, 8.6; amino N, 2.15; carboxyl N, 2.15; bromide 12.4. Found: N, 8.6, amino N, 4.0 (3 min.), 5.0 (30 min.)[24]; carboxyl N, 2.0 (*p*H 4.7), bromide, 12.7.

α-Thiophenyl L-Glutamate Hydrobromide.—Z.Glu.S.C_6H_5 (0.25 g., 0.67 mmole) was dissolved in 2 ml. of a mixture of thiophenol and glacial acetic acid (1:1) and 1 ml. of hydrobromic acid in glacial acetic acid (35 g./100 ml.) was added. After carbon dioxide evolution was completed (1–2 hr.) most of the solvent was removed *in vacuo* and the product permitted to crystallize in the ice-box (crystallization may be induced by adding ether). The yield of pure material was 0.19 g. (90%); m.p. 171–174°; $[\alpha]^{25}D$ +92.8° (1.3% in 0.1 *N* hydrochloric acid). *Anal.* Calcd. for $C_{11}H_{13}O_3NS$·HBr (320.2): N, 4.4; amino N, 4.4; carboxyl N, 0.0. Found: N, 4.4; amino N, 4.1; carboxyl N, 0.18.[25]

The amino nitrogen analysis was performed by dissolving the sample in dilute hydrochloric acid; solution in water led to low values (20–30% of theory). However, if in separate runs the nitrogen evolved after 3 minutes was ejected and 2 cc. of 0.5 *N* hydrochloric acid added to the Van Slyke chamber, after 2.5 hours 60% of the missing nitrogen was recovered.

S-α-Glutamyl-GSH Dihydrobromide.—Z.Glu.SG(L) (0.8 g., 1.4 mmoles) was suspended in 5 ml. of a mixture of glacial acetic acid and phenol (0.8 g. of phenol in 5 ml. of glacial acetic acid); 1.5–2.0 ml. of hydrobromic acid in glacial acetic acid (35 g./100 ml.) was added to yield a homogeneous solution; (in the event that the starting material is impure and contains salt or unreacted GSH, a separation is effected at this stage since most of the salt and GSH·HBr will not dissolve under these conditions). An additional 2–3 ml. of hydrobromic–acetic acid was added and the mixture permitted to stand at room temperature. Carbon dioxide evolution occurred immediately and after 15–20 minutes the product began to crystallize out of solution. When the reaction was complete (2–5 hours), the mixture was cooled in ice and the product centrifuged and washed repeatedly with acetone and ether. The material was dissolved in a minimum amount of 0.05 *N* hydrobromic acid, treated with charcoal and acetone and ether were added. The resulting oil was then triturated with acetone to give 0.5 g. (60%) of crystalline material, $[\alpha]^{23}D$ +8.5° (0.95% in 0.1 *N* hydrochloric acid). *Anal.* Calcd. for $C_{16}H_{24}O_9N_4S$·2HBr (598.0): C, 30.2; N, 9.4; amino N, 4.7; carboxyl N, 2.4. Found: C, 30.0; N, 9.5; amino N, 6.9[24] (3 min.); carboxyl N, 1.9 (*p*H 4.7).

S-γ-Glutamyl-GSH Dihydrobromide.—This compound was prepared as described above for the α-isomer. After carbon dioxide evolution was completed the solution was concentrated *in vacuo* and crystallization occurred. Yield of pure material was 60–80%. *Anal.* Calcd. for $C_{16}H_{24}O_9N_4S$·2HBr·H_2O (616): N, 9.1; amino N, 4.6; carboxyl N, 4.6; bromide, 26.0. Found: N, 9.1; amino N, 5.8[24]; carboxyl N, 2.4[26]; bromide, 26.0.

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(22) F. Lynen, *Ann.*, **574**, 33 (1951).

(23) Calculated for one amino nitrogen.

(24) The high values are due to the GSH moiety; *cf.* ref. 21.

(25) This small amount of carboxyl nitrogen probably arises from some decomposition to glutamic acid.

(26) The low carboxyl nitrogen values are due to rapid decomposition to pyrrolidonecarboxylic acid.