dry tetrahydrofuran was allowed to reflux 4 hr. Upon evaporation of the solvent and crystallization of the residue from ethanol there was obtained 9.4 g. (45%) 1-phenyldihydrouracil, m.p. and mixed m.p. 184–186°.

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Synthesis of Mixed α- and γ-Glutamylhomocysteinylglycines[1]

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Received July 1, 1958

The participation of glutathione in cell growth and division and in various enzyme catalyzed reactions suggests that an investigation of glutathione homologs and analogs for inhibitory properties might be of interest and potential therapeutic value. A number of such compounds have been investigated by Kermack and Matheson[4] and were found to be inhibitors of the glyoxalase reaction. In these compounds alteration of glutathione was limited to *S*-alkylation and replacement of the cysteinyl residue with glycyl, alanyl, and cysteyl residues. Since the thiol group of glutathione is functionally important, it appeared to be of interest to synthesize a thiol-containing homolog and in this paper the synthesis of γ-glutamylhomocysteinylglycine accompanied by the α-tripeptide is reported.

The synthesis[5] was effected by condensation of *N*-*p*-tosyl-γ-L-glutamyl azide with *S*-benzyl-DL-homocysteinylglycine ethyl ester followed by saponification and then simultaneous debenzylation and detosylation with sodium in liquid ammonia. The required *N*-*p*-tosyl-γ-L-glutamyl azide was prepared from *N*-*p*-tosyl-γ-L-glutamyl hydrazide,[6] the latter being prepared from L-glutamic acid by a known sequence of reactions[7] and *S*-benzylhomocysteinylglycine ethyl ester was prepared by decarbobenzoxylation of *N*-carbobenzoxymethionylglycine ethyl ester with ethanolic hydrogen chloride[8] or with acetic acid–hydrogen bromide.[5]

In view of the fact that the synthetic route gave rise to a mixture[9] of α- and γ-tripeptides, rearrangement of a γ-glutamyl derivative to an α-glutamyl derivative must have occurred during the course of the synthesis. As a rule, rearrangements[10–12] of γ-glutamyl and β-aspartyl peptides to the corresponding α-peptides occur under conditions which permit nucleophilic attack by the peptide nitrogen atom on the carbonyl carbon atom of the α-carboxyl function, the α-carboxyl being suitably substituted, and expulsion of the substituent group. Usually these requirements are met by an alkaline reaction medium and an esterified α-carboxyl group. In the present case, the α-carboxyl group is not substituted and it is difficult to visualize rearrangement occurring *via* peptide nitrogen attack with expulsion of an oxide or hydroxide ion. Since the *N*-*p*-tosyl-γ-L-glutamyl hydrazide is free[6,13] of α-hydrazide, rearrangement to an α-derivative must have occurred during azide formation or subsequent reaction of azide with the amine moiety.[14] A similar rearrangement has been definitely shown to occur[15] when *N*-carbobenzyloxy-γ-L-glutamyl hydrazide is used *via* the azide, for γ-peptide synthesis.

The tripeptide mixture has no inhibitory effect on the growth of Sarcoma 180 in the mouse.[16] The γ-peptide[4] is an inhibitor of the glyoxalase reaction.[4]

(1) This work was supported, in part, by a grant from The American Cancer Society.

(2) Abstracted in part from the doctoral thesis, June 1957, of Frank Draus.

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(4) W. O. Kermack and N. A. Matheson, Proc. Biochem. Soc., *Biochem. J.*, **57**, XXII (1954).

(5) During the course of this work a synthesis of γ-glutamylhomocysteinylglycine *via* condensation of the γ-azide of *N*-carbobenzoxyglutamic acid with *S*-benzyl-homocysteinylglycine ethyl ester was reported by E. C. Herrick and C. W. Todd, U.S. Patents **2,723,972** and **2,723,973**; *Chem. Abstr.*, **50**, 4214, 4215 (1956). Paper chromatography of the product yielded only one spot. Other evidence for the absence of α-peptide in the product is not presented.

(6) J. Rudinger, *Coll. Czechoslov. Chem. Communs.*, **19**, 365 (1954).

(7) C. R. Harrington and R. C. Morridge, *J. Chem. Soc.*, 706 (1940).

(8) O. Gawron and F. Draus, *J. Org. Chem.*, **23**, 1040 (1958).

(9) As shown by α-carboxyl CO_2 determination. Chromatography on paper yielded only one spot.

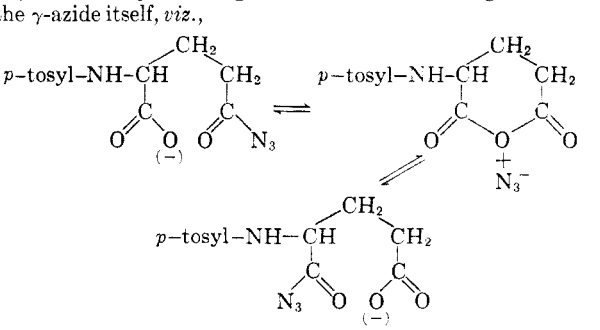
(10) E. Sondheimer and R. Holley, *J. Am. Chem. Soc.*, **76**, 2467 (1954).

(11) A. R. Battersby and J. C. Robinson, *J. Chem. Soc.*, 259 (1955).

(12) D. W. Clayton, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc.*, 371 (1956).

(13) A potentiometric titration gave a theoretical titration curve for a compound with one carboxyl group of pK 3.65 and no indication of a carboxyl group with a higher pK value. The pK_a value of 3.65 is typical for the α-carboxyl group of *N*-acyl-γ-substituted glutamic acid derivatives (Ref. 10).

(14) A likely rearrangement involves rearrangement of the γ-azide itself, *viz.*,



This is an elaboration of the suggestion of Sachs and Brand (Ref. 15). The anhydride, of course, would yield a mixture of α- and γ-peptides.

(15) H. Sachs and E. Brand, *J. Am. Chem. Soc.*, **76**, 1815 (1954).

(16) Courtesy of Drs. C. C. Stock and R. Barclay, Sloan-Kettering Institute for Cancer Research.

EXPERIMENTAL

*N-carbobenzoxy-*DL*-methionylglycine ethyl ester.* To a solution of 5.66 grams (0.02 mole) of *N*-carbobenzoxy-DL-methionine[17] and 2.04 grams (0.02 mole) of triethylamine in 50 ml. of 1:1 chloroform, toluene, cooled to 0°, 2.41 grams (0.02 mole) of isovaleryl chloride was added. After 2 hr. standing at 0°, a cold solution of 2.79 grams (0.02 mole) of ethyl glycinate hydrochloride and 2.04 grams (0.02 mole) of triethylamine in 50 ml. of chloroform was added to the reaction mixture. The reaction mixture was allowed to stand in the cold overnight and then washed with water, 3% sodium bicarbonate and again with water. It was then dried with sodium sulfate and after removal of the drying agent by filtering, petroleum ether was added to turbidity. On cooling, 4.42 grams (60%) of *N*-carbobenzoxy-DL-methionylglycine ethyl ester, m.p. 75–77°, lit.[18] 72–74°, was obtained.

*N-p-tosyl-*γ*-*L*-glutamyl hydrazide.* A solution of 1 g. (0.0036 mole) of *N*-*p*-tosyl-2-pyrrolidone-5-carboxylic acid[6] and 1 g. of 85% hydrazine hydrate in 15 ml. of absolute alcohol was refluxed for 1 hr. After cooling to 0°, the hydrazinium salt, m.p. 135–136°, of *N*-*p*-tosyl-γ-L-glutamyl hydrazide separated out as a heavy oil which on being scratched crystallized.

Anal. Calcd. for $C_{12}H_{21}O_5N_5S$: N, 20.08. Found: N, 20.03.

The free hydrazide was obtained by dissolving the above hydrazinium salt in 5 ml. of water and acidifying to *p*H 3 with 4*N* sulfuric acid. Upon acidification the hydrazide crystallizes from solution and on recrystallization from ethanol-water melts at 203–205°, lit.[6] 207°.

*N-p-Tosyl-*γ*-L-glutamyl azide.* A solution of the azide in chloroform was prepared for immediate use. To a suspension of 6.3 grams (0.02 mole) of *N*-*p*-tosyl-γ-L-glutamyl hydrazide in 100 ml. of cold water, 10 ml. of concentrated hydrochloric acid and 100 ml. of chloroform were added. After cooling the reaction mixture to 0° in an ice salt bath, a cold solution of 2.28 grams (0.03 mole) of sodium nitrite in 20 ml. of water was added with stirring. Stirring was continued for 6 min. after which time the chloroform layer was separated. The aqueous layer was shaken with 20 ml. of cold chloroform and the combined chloroform extracts were washed with 20 ml. of cold water and after drying over anhydrous sodium sulfate the chloroform solution of azide was ready for use.

*N-p-Tosyl-*γ*-*L*-glutamyl-S-benzyl-*DL*-homocysteinylglycine ethyl ester.* To a cold chloroform solution of the azide from 0.02 mole of *N*-*p*-tosyl-γ-L-glutamyl hydrazide, a cold solution of 9.4 grams (0.02 mole) of *S*-benzyl-DL-homocysteinylglycine ethyl ester hydrobromide,[5] 6.4 ml. of *N*,*N*-diethylaniline and 2.78 ml. of triethylamine in 100 ml. of chloroform was added with stirring over a period of 30 min. The reaction mixture was then stirred in the cold for 3 hr. and allowed to stand overnight at room temperature. The resulting yellow solution was washed with 40 ml. of *N* hydrochloric acid, then with four 25-ml. portions of 0.5*N* hydrochloric acid and finally with three 25-ml. portions of distilled water. After drying over anhydrous magnesium sulfate, solvent was removed at reduced pressure to give 7.0 grams (59%) of the desired ester as a noncrystallizable oil.

Anal. Calcd. for $C_{27}H_{35}N_3O_8S_2$: Neut. equiv., 593; N, 7.09. Found: Neut. equiv., 589; N, 7.01.

*N-p-Tosyl-*γ*-*L*-glutamyl-S-benzyl-*DL*-homocysteinylglycine.* Seven g. of the corresponding ester was dissolved in 75 ml. of dioxane and to this solution 37 ml. of *N* sodium hydroxide was added. Saponification was allowed to proceed for 75 min. at room temperature and at the end of this period an equivalent amount of *N* hydrochloric acid was added. The reaction mixture was then concentrated *in vacuo* and the residue was extracted several times with ethyl acetate. The combined extracts were dried with anhydrous sodium sulfate and after removal of ethyl acetate *in vacuo*, 6.3 g. of *N-p-tosyl-*γ-L-glutamyl-*S*-benzyl-DL-homocysteinylglycine was obtained as an oil.

Anal. Calcd. for $C_{25}H_{31}N_3O_8S_2$: Neut. equiv., 282.5; C, 53.20; H, 5.50; N, 7.45. Found: Neut. equiv., 280.0; C, 52.96; H, 5.89; N, 7.50.

γ-L-*Glutamyl-*DL*-homocysteinylglycine.* A solution of 6.3 grams (0.011 mole) of *N*-*p*-tosyl-γ-L-glutamyl-*S*-benzyl-DL-homocysteinylglycine in 800 ml. of dry liquid ammonia was treated with 1.7 grams of sodium, the sodium being added portionwise and with vigorous stirring. After addition of the sodium, stirring was continued for 30 min. and then 7.0 g. of ammonium chloride was added with continued stirring. The solvent was then allowed to evaporate at room temperature and residual traces were removed with a water aspirator. The residue was dissolved in 35 ml. of water and after adjustment of *p*H to 7.0, the solution was extracted several times with ether. The aqueous solution was then cooled to 0° and following adjustment of *p*H to 1.5 with 5*N* sulfuric acid, mercuric sulfate reagent[19] was added dropwise until precipitation of tripeptide was complete. The mercury salt after washing with 20 ml. of cold, 0.5*N* sulfuric acid and with three 25-ml. portions of cold water, was decomposed by treating it in aqueous suspension with hydrogen sulfide for 8 hr. at room temperature. After removal of mercuric sulfide by filtration, inorganic sulfate was removed by treatment with barium hydroxide and excess barium was removed with 0.1*N* sulfuric acid. After filtering, the solution was lyophilized to yield 1.4 g. of amorphous tripeptide.

Anal. Calcd. for $C_{11}H_{18}N_3O_2S$: N, 13.11; amino N, 4.29; α-carboxyl CO_2, 4.29. Found: N, 13.50; amino N, 4.18; α-carboxyl CO_2, 2.09.

The amino acid composition of the tripeptide was confirmed by two dimensional chromatography[20] of an acid hydrolyzate and by amino acid analysis. Chromatography indicated the presence of glycine, homocysteine, and glutamic acid, the amino acid ratio, glycine[21]:homocysteine[22,23]:glutamic acid,[24] was found to be 1.00:1.06:1.14.

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(17) C. A. Dekker and J. S. Fruton, *J. Biol. Chem.*, **173**, 471 (1948).

(18) C. A. Dekker, S. Taylor, and J. S. Fruton, *J. Biol. Chem.*, **180**, 155 (1949).

(19) V. du Vigneaud and D. L. Miller, *Biochem. Preps.*, **2**, **88**, (1952).

(20) Utilizing 85% phenol in an ammonia atmosphere for the first dimension and 80% propanol-water containing 0.1% *N*-ethylmaleimide for the second dimension.

(21) A. R. Patton, *J. Biol. Chem.*, **108**, 267 (1935).

(22) A. E. Mirsky and M. L. Anson, *J. Gen. Physiol.*, **18**, 302 (1935).

(23) O. Gawron and J. Keil, unpublished work.

(24) P. P. Cohen, *Biochem. J.*, 551 (1939).