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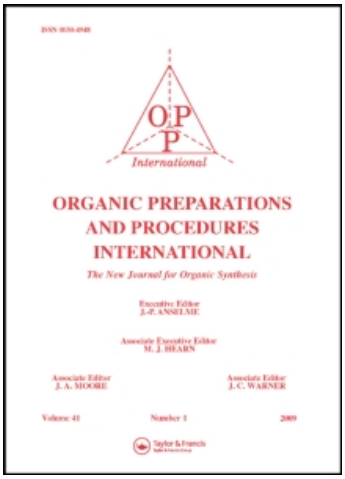

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S-CARBOXYMETHYL DERIVATIVES OF L-CYSTEINYLGLYCINE AND GLYCYL-L-CYSTEINE

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S-CARBOXYMETHYL DERIVATIVES OF L-CYSTEINYLGLYCINE AND GLYCYL-L-CYSTEINE†

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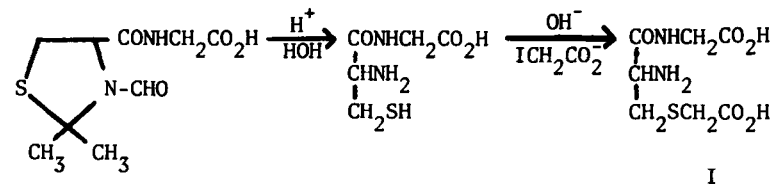
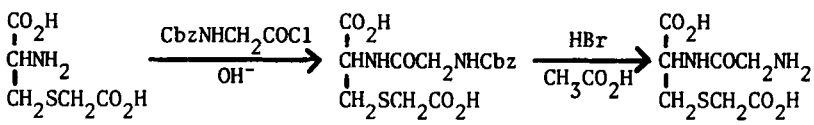
Cysteinylglycine is a biologically important compound since it is formed during the γ-glutamyl cycle[1] and has been identified as occurring naturally in wheat germ, human serum, murine lymphoblasts and E. coli.[2] It has been measured by treatment with iodoacetic acid followed by ion-exchange chromatography of the resultant S-carboxymethyl derivative (I).[2]



I



II

The pure carboxymethyl derivative has not been described. Pure samples of this and the isomeric N-glycyl-S-carboxymethylcysteine (II) were required for the identification of cystinylglycine as a component of blood plasma.[3] This paper reports the synthesis and physical properties of these substances.

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EXPERIMENTAL[4]

S-Carboxymethyl-L-cysteinylglycine (I).- L-N-(3-formyl-2,2-dimethylthiazolidine-4-carboxyl)-glycine (3.20 g, 0.013 mole) was prepared and hydrolyzed as described by Sheehan and Yang.[5] The solution was kept in an argon atmosphere, cooled, neutralized by the addition of 1 N NaOH and concentrated in vacuo to a pasty white crystalline mass. This material was dissolved in 50 ml of water (argon atmosphere), transferred to a beaker, and carboxymethylated by the addition of 2.66 g (0.014 mole) of iodoacetic acid followed by addition of 21 ml of 1 N NaOH to adjust the pH of the stirred solution to 6.0. Additional alkali (7 ml) was gradually added to maintain the pH at 6.0-6.2. After 30 min. the pH decreased very slowly; 6 ml more of 1 N NaOH was added slowly to increase the pH to 8.0, at which time no further change in pH occurred.

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The solution was then adjusted to pH 2.0 by the addition of 6 N HCl, passed through a 2 x 20 cm column of Amberlite CG-120(H^+) (100-200 mesh), the resin was washed with 400 ml of water and the product was eluted with 3 N NH_4OH. The solvent was removed in vacuo. The resulting light tan syrup was dissolved in 20 ml of warm water, treated with Norit and filtered, and the resulting colorless solution was passed through a 2 x 20 cm column of Biorad AG-1X8 (acetate form, 100-200 mesh). The resin was washed with water and the I was eluted with 3 N acetic acid. The eluate was concentrated in vacuo to a syrup, which crystallized after standing overnight in a vacuum desiccator over solid NaOH. The compound was recrystallized from 5 ml of water to yield 1.68 g (55%) of product, mp. 173-175° (dec.). For analysis, 1.5 g was recrystallized from 4 ml of water to yield 1.0 g of mp. 175-176° (dec.); $\alpha]_D^{26}$ + 53.7° (1% in water). (100% optical purity of the peptides was not established).

NMR (CF_3CO_2H): δ 3.42 (2H, t, CH_2S), 3.65 (2H, s, CH_2CO_2), 4.35 [2H, d, CH_2(gly)], 4.68 (H, broad s, CH), 7.77 (3H, broad s, NH_3^+), 8.12 (H, t, CONH).

Anal. Calcd for $C_7H_{12}O_5N_2S$: C, 35.56; H, 5.12; N, 11.86; S, 13.57

Found: C, 35.48; H, 5.20; N, 11.85; S, 13.44

N-Glycyl-S-carboxymethyl-L-cysteine (II).- A solution of carbobenzoxyglycyl chloride[6] prepared from 10.5 g (0.05 mole) of carbobenzoxyglycine in 80 ml of anhydrous ether was added in 4 equal portions along with 4 equal portions of 1 N NaOH (45 ml) to a well shaken solution of 7.2 g (0.04 mole) of carboxymethyl-L-cysteine in 90 ml of 1 N NaOH (temp. $< 5^{\circ}C$).[7] After the addition was completed the reaction mixture was extracted twice with 50 ml portions of ether; the ethereal extracts were discarded. The residual aqueous phase was acidified to pH 1.8 (conc. HCl) and extracted twice with 50 ml portions of ethyl acetate. The extracted aqueous layer was left in the refrigerator overnight and the unreacted carboxymethylcysteine that crystallized was collected [2.6 g, mp. 183-200° (dec.)]. The ethyl acetate extracts were washed with 0.005 N HCl, then with water, dried over anhyd. Na_2SO_4 and the ethyl acetate was removed *in vacuo*. The residual oil (7.5 g) could not be induced to crystallize.

This material was cooled in an ice bath and 25 ml of 22% HBr in glacial acetic acid was added. The mixture was allowed to warm to room temperature and shaken. After 2 hrs., 10 ml of HBr/HOAc was added and shaking was continued for 4 hrs., at which time the oil had dissolved completely. This solution was poured into 500 ml of ether, the sticky cake that formed was broken up and the ether was decanted and discarded. This procedure was repeated four times and the solid was collected on a filter and dried *in vacuo* over solid NaOH. The resulting sticky paste was dissolved in 25 ml of water and the solution was adjusted to pH 2.2 by the addition of 3 N NaOH, treated with Norit and filtered. The clear colorless solution was then purified by chromatography on Amberlite CG-120(H^+) and Biorad AG-1X8 (acetate form) in the manner described for the purification of I. The acidic eluate from the Biorad AG-1X8 column was concentrated to dryness *in vacuo*

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and dried overnight in a vacuum desiccator over solid NaOH. The resulting semi-crystalline mass (1.2 g) was dissolved in 5 ml of hot water; the solution was cooled in the refrigerator overnight and the resulting crystalline carboxymethylcysteine was collected, washed and dried to yield 0.40 g, mp. 194-195° (dec.). The combined mother liquor and washings were concentrated to dryness in vacuo, the residue was dissolved in 5 ml of hot water, 10 ml of ethanol was added and the clear solution was cooled, left in the refrigerator overnight and the product was collected and dried to afford 0.47 g (5%), mp. 189-190° (dec.), $\alpha]_D^{30}$ − 22.5° (1% in water).

NMR (CF_3CO_2H): δ 3.35 (2H, d, CH_2S), 3.52 (2H, s, CH_2CO_2), 4.32 [2H, d, CH_2(gly)], 5.12 (H, m, CH), 7.48 (3H, broad s, NH_3^+), 8.07 (H, d, CONH).

Anal. Calcd for $C_7H_{12}O_5N_2S$: C, 35.56; H, 5.12; N, 11.86; S, 13.57

Found: C, 35.42; H, 5.30; N, 11.76; S, 13.64

Chromatographic properties.- Ion-exchange chromatography was carried out with a Spinco Model 120 amino acid analyzer using a 0.9 x 150 cm column of Biorad Aminex Q-150 S resin. Elution was effected with pH 3.15, 0.30 N Li citrate buffer and an initial column temperature of 30° which was increased to 60° after 9 hrs; flow rates of 30 ml/hr of buffer and 15 ml/hr of ninhydrin were used. II is eluted at 302 ml, 4 ml before glycine, the color yield with ninhydrin is 1.12 that of glycine and the 570/440 nm absorbance ratio is 8.35. I is eluted at 338 ml, 32 ml after glycine, the color yield is 1.07 that of glycine, and the 570/440 nm ratio is 5.38.

Thin-layer chromatography was done on Polygram® Cel 300 (Brinkmann) with solvent systems A, 1-butanol:acetic acid:H_2O - 4:1:2, and B, pyridine: acetone:3 N NH_4OH - 50:30:25. For II, R_f values in A, 0.40, B, 0.11; color with ninhydrin (collidine), yellow. For I, R_f in A, 0.44, B, 0.13; color with ninhydrin, olive-gray.

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