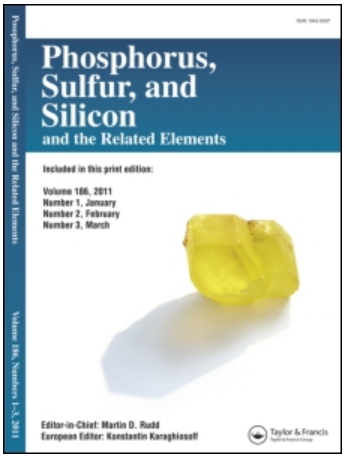

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STABILITY OF SALTS OF N-CARBOXY-, N-THIOCARBOXY-, AND N-DITHIOCARBOXYGLYCINE[1]

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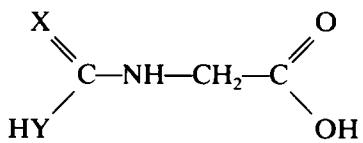
Salts of N-carboxy-, N-thiocarboxy-, and N-dithiocarboxyglycine (**1–3**) have been prepared and their stability in aqueous solution investigated by NMR spectroscopy. Salts of N-carboxyglycine (**1**) undergo partial hydrolysis in aqueous solution, giving the same equilibrium mixtures as those formed in the reaction of glycine with carbonate. Salts of N-dithiocarboxyglycine (**2**) are stable in aqueous solution, even at 100°C. The disodium salt **2d**, which has often been employed as a reaction intermediate but never adequately characterized, has been synthesized by several independent methods. Salts of N-thiocarboxyglycine (**3**) are stable in aqueous solution at 25°C but not at 100°C.

Key words: Glycine, amino acids, carbon dioxide, carbon disulfide, carbonyl sulfide, carbamates, carbonates, ^{1}H NMR, ^{13}C NMR, hydrolysis, stability.

INTRODUCTION

Interactions of proteins with carbon dioxide and carbon disulfide are important from a physiological viewpoint.[2,3] Because these interactions are reversible, they are potentially useful for the chemical modification of food proteins, such as soy protein, where the nutritional value and digestibility of the protein must not be impaired.

In this paper, the stability of the products of such interactions is examined with the amino acid glycine as a model. In the presence of strong bases, glycine reacts with carbon dioxide, carbon disulfide, and carbonyl sulfide to form salts of N-(carboxymethyl)carbamic acid (N-carboxyglycine, **1**) and its thio analogues **2** and **3**. Several alkali (Na, K) and alkaline earth (Ca, Ba) salts of each class are known,[4–8] but only **2** is known to form an ammonium salt.[9,10] Efforts to prepare some of the missing members of each class are reported and the stability of the salts in aqueous solution is investigated with ^{1}H or ^{13}C NMR as the probe.[11]



1, X = Y = O
2, X = Y = S
3, X = O, Y = S

RESULTS AND DISCUSSION

Salts of N-Carboxyglycine (1)

The disodium salt **1d**, prepared according to Farthing,[4] was found to be stable to air but unstable to water. The salt, a white, free-flowing powder, was unaffected by drying for one hour at 110°C in a forced draft oven,[12] but its NMR spectrum, taken 15 minutes after solution in D_2O, showed a pair of singlets at δ_H 3.62 and 3.38 ppm whose relative intensity gradually changed over a 5 hour period from 2.6:1 to 1:1, after which there was no further change. In a separate experiment, the pH of a 0.5*M* solution of **1d** in water was found to be 9.79 immediately after mixing and remained constant for over two weeks. The δ_H 3.38 signal was pH-sensitive with respect to line position and intensity, shifting upfield upon the addition of NaOD or Na_2CO_3 and downfield upon the addition of CO_2, whereas the δ_H 3.62 signal did not shift (Table I). In each case equilibrium was established rapidly.

This behavior is consistent with partial hydrolysis of **1d** to glycine and CO_2 (Eq 1), where the δ_H 3.62 signal represents CH_2 in the carbamate **1** and the pH-sensitive signal represents CH_2 in glycine. Hydrolysis is at a maximum in ambient water and is repressed by CO_2 or OH^-.

$$^-O_2CNHCH_2CO_2^- + H_2O \rightleftharpoons NH_2CH_2CO_2^- + CO_2 + OH^- \qquad (1)$$

This work complements previous studies on the interaction of glycine with carbon dioxide and alkali in aqueous solution in which the equilibrium of Eq 1 was approached from right to left.[13-20] The extent of carbamate formation at equilibrium, whether measured by titration,[13-17] ^1H NMR[2,17,18] or ^{13}C NMR,[18,19] is at a maximum at pH 9–10 and falls off rapidly at pH values outside this region. The velocity constant for the decomposition of carbamate is 0.0027 min^{-1} at pH 10 and 18°C.[15] The reactive species are carbon diodide and glycine anion. Below pH 9, most of the glycine is in zwitterion form;[21] above pH 10, most of the carbon dioxide is in carbonate/bicarbonate form.[13-15]

Stadie and O'Brien[14] were able to determine the velocity of carbamate formation in solutions containing glycine and sodium carbonate by following the change in pH, but clearly this should be possible only when one reagent or the other is in excess. Indeed, we found that the pH of an aqueous solution of glycine and sodium

TABLE I

^1H NMR Chemical Shifts of **1d** in D_2O at Equilibrium

Additive	Carbamate δCH_2	Glycine δCH_2	Percent Carbamate
None	3.62	3.38	49
CO_2	3.62	3.52	64
Na_2CO_3	3.61 (3.69)[a]	3.27 (3.26)[a]	77
NaOD	3.62	3.16	91[b]

[a]Lemieux and Barton.[2]

[b]Stable for 5 hr but falling to 62, 42, 20 and 0% after 1, 4, 10 and 26 days, respectively.

carbonate rises when sodium carbonate is in excess, falls when glycine is in excess, and remains constant when the reagents are equimolar.

Reaction of glycine with ammonium carbonate under the conditions employed with sodium carbonate gave only recovered glycine, but when the reaction was allowed to continue overnight a white, crystalline product was obtained which had the characteristics expected for the diammonium salt **1a**. The product dissolved readily in water giving a pH of 8.5, and gassed strongly when acidified. Its NMR spectrum in D_2O showed a pair of singlets at δ_H 3.62 (CH_2, **1a**) and 3.55 (CH_2, glycine) in a ratio of 1:2; the latter shifted upfield with gaseous ammonia and downfield with CO_2, whereas the former did not shift. The product, unfortunately, was unstable, reverting to glycine after 30 minutes at 110°C or 20 hours at room temperature. Based on the final weight of glycine, it was calculated that the **1a** was 42% degraded at the time it was isolated. Efforts to improve this procedure by changing the ratio of carbon dioxide to ammonia or by modifying the workup were only partially successful.

An experiment in which carbon dioxide was passed into a solution of glycine and tetramethylammonium hydroxide in methanol produced an exothermic reaction but the products were glycine (84.8%, NMR [D_2O] δ_H 3.58), which separated during the reaction, and tetramethylammonium bicarbonate (NMR [D_2O] δ_H 3.18; lit.[22a] 3.15). The affinity of tetramethylammonium hydroxide for carbon dioxide was sufficient to displace the glycine from its tetramethylammonium salt.

Salts of N-Dithiocarboxyglycine (2)

Though salts of N-dithiocarboxyglycine have been reported in some 40 papers since the first one was prepared by Körner[23] in 1908, only the ammonium, barium and calcium salts (**2a–c**) have been adequately characterized. All three are crystalline compounds that redden when exposed to air and revert to glycine and carbon disulfide upon acidification.[5,6,10] The only NMR data given for these compounds was that of Castillo *et al.*[24] who reported δ_H 4.03 for the barium salt **2b**.

The NMR spectra of the three salts **2a–c** in D_2O showed only a sharp singlet for the CH_2 protons at δ_H 4.13, unaffected by the addition of carbon disulfide or base. All but the barium salt **2b** were also sufficiently soluble in methanol-d_4 to show a signal at 4.13. The D_2O spectra of **2b** and **2c** were unaffected by brief (5 minutes) immersion of the NMR tubes in boiling water. The ^{13}C NMR spectrum of **2c** in D_2O, recorded at 25°C over an elapsed time of 15 hours, showed no evidence of hydrolysis.

The disodium salt **2d**, though never isolated in the pure state, has often been prepared as an intermediate for further reactions.[3,25–29] Some ancillary characteristics have been reported.[13,30–33] Bode *et al.*[6] found that the reaction of glycine with carbon disulfide in aqueous methanol, which yielded the calcium salt **2c** without difficulty, produced substantial quantities of red by-products from which the sodium salt **2d** could not be separated when the base was sodium hydroxide. In our hands, reaction in aqueous or anhydrous methanol produced some **2d** (identified by NMR), but the conversion was poor and the product had an exceedingly offensive odor. Xanthate formation could account for the poor conversion and the intense yellow color but not for the accompanying stench.

Much better results were obtained when the alcohol was omitted (method A). Reaction was slow owing to the insolubility of the carbon disulfide, but the NMR spectrum of the reaction mixture showed only the signals for glycine and **2d** and the progress of the reaction could be easily followed by measuring their relative intensity. On a 0.2 molar scale the reaction was complete in 30 hours. Zahradnik[34] found the reaction to be pseudomonomolecular, and determined the rate of formation of **2d** to be 0.0180 minute^{-1} in pH 9.3 buffer at 40°C. The **2d** prepared in this manner was a yellow oil which was soluble in water and methanol, insoluble in other organic solvents, and analyzed as a trihydrate. Its NMR spectrum in D_2O consisted of a sharp singlet at δ_H 4.13, unaffected by time, by brief boiling (5 minutes), or by the addition of carbon dioxide or NaOD. It was soluble in DSS-free D_2O but precipitated DSS from D_2O solution. When acidified with hydrochloric acid, it reverted to glycine and carbon disulfide. Like Zahradnik,[35] we found no evidence for the formation of rhodanine (lit.[22b] NMR [dmso-d_6] δ_H 4.23), a reported product of cyclization of **2d** in sulfuric acid.[28]

The NMR spectrum of **2d** in methanol-d_4 showed only a singlet at δ_H 4.13, but when the salt was dissolved in methanol and taken to dryness the NMR spectrum in either D_2O or methanol-d_4 showed an extra singlet at 3.37 (CH_3), corresponding to a **2d** monomethanol solvate. The extra singlet only vanished when the solvate was redissolved in a little water and taken to dryness.

The disodium salt **2d** was also prepared by five other methods. The reaction of glycine with sodium trithiocarbonate (Eq 2, method B), incomplete even after three weeks at 25°C, gave a mixture of **2d** (71.4%) and glycine (26.2%) that retained glycine tenaciously despite its insolubliity in methanol. Passing carbon dioxide into a solution of the mixture in methanol removed some of the glycine in the form of its N-carboxy salt **1d** (which is also insoluble in methanol), but to ensure complete removal of the glycine it was necessary to supply extra base, preferably in the form of sodium carbonate. The **2d** remaining after this treatment was identical to that of method A.

$$NH_2CH_2CO_2H + Na_2CS_3 \rightarrow NaS_2CNHCH_2CO_2Na + H_2S \qquad (2)$$

The reaction of glycine with sodium methyl xanthate (Eq 3, method C) gave an 85.1% yield of **2d** after 3 days in methanol at room temperature. The product, a deep yellow oil with a disagreeable stench, was contaminated with xanthate (9.0%). In water, the conversion of glycine to **2d** under the same conditions was only 45.4%.

$$NH_2CH_2CO_2H + 2\ CH_3OCS_2Na \rightarrow NaS_2CNHCH_2CO_2Na + 2\ CH_3OH + CS_2 \qquad (3)$$

The reaction of **1d** with carbon disulfide (Eq 4, method D), inspired by a method that we have used to prepare the N-dithiocarboxy derivative of ethylenediamine,[36] gave a 76.6% conversion of **1d** to **2d** after four days at 25°C. The product, after removal of the unreacted **1d**, contained glycine, which presumably arose from **1d** by hydrolysis in the mildly alkaline medium. Removal of the glycine by the procedure described under method B gave **2d**, identical to the product of method A.

$$\mathbf{1d} + CS_2 \rightarrow \mathbf{2d} + CO_2 \qquad (4)$$

Finally, **2d** was prepared from **2a** by displacement of ammonia with sodium hydroxide (method E), and from **2c** by precipitation of calcium as the carbonate (method F). Because of the purity of the **2a** and **2c** and the mildness of the reaction conditions, the **2d** prepared by these methods was colorless.[37]

The crystalline diammonium salt **2a** was also prepared from **2c** by method F. The product was identical (NMR) to that prepared by Budevsky's method.[10] Both reddened within a day and were brownish purple by the end of a month, but their ^{1}H NMR spectra remained unchanged.

Salts of N-Thiocarboxyglycine (3)

Little is known of this class of compounds. Müller-Litz[8] prepared the calcium and barium salts **3b** and **3c**, both colorless crystalline solids, by the reaction of glycine with carbonyl sulfide at 0°C and characterized them by infrared. The kinetics of the reaction have been investigated.[38] The NMR spectra and stability characteristics of the products were unknown.

The NMR spectrum of the calcium salt **3c** in D_2O showed only a singlet for the CH_2 protons at δ_H 3.69, unaffected by the addition of base. The ^{13}C NMR spectrum of **3c**, like that of **2c**, showed no evidence of dissociation over a 15 hour period at 25°C.

When carbonyl sulfide was passed into a solution of glycine in aqueous ammonium hydroxide or sodium hydroxide at 25°C (method A), a new signal appeared in the NMR spectrum at δ_H 3.67, unaffected by the addition of carbon dioxide or NaOD. The conversion of glycine to **3a** or **3d** was 12.2% after six hours (NH_4OH) or 21.2% after three hours (NaOH). Efforts to drive the reaction to completion, however, were unsuccessful, owing we believe to depletion of the base. Carbonyl sulfide is rapidly degraded by sodium hydroxide to sulfide and carbonate.[39]

Similar results were obtained with method D. When carbonyl sulfide was passed into a solution of **1d** in water at 25°C, the conversion of **1d** to **3d** was 20.6% after four hours and no higher after 48 hours. The NMR signal for **3d** in either D_2O or methanol-d_4 was a singlet at δ_H 3.67. The product reverted to glycine upon acidification with hydrochloric acid.

The disodium salt **3d** was finally prepared in pure state by the reaction of **3c** with sodium carbonate (method F). The product, a colorless glass, showed a single NMR line at δ_H 3.67. Unlike **2d**, the **3d** was 56.6% hydrolyzed to glycine and carbonyl sulfide after 5 minutes at 100°C.

EXPERIMENTAL

General Methods.[40] Melting points were corrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. ^{1}H NMR spectra were taken on a Varian EM-360L with internal lock, and ^{13}C NMR spectra on a Varian VXR-200 at 50.3 MHz; chemical shifts are reported relative to DSS (δ_H and δ_C) or TMS (δ_H). Disodium N-carboxyglycinate (**1d**) and calcium N-thiocarboxyglycinate (**3c**) were prepared by the procedures of Farthing[4] and Müller-Litz,[8] respectively. Diammonium, barium, and calcium N-dithiocarboxyglycinate (**2a–c**) were prepared by the procedures of Budevsky *et al.*,[10] Musil and Irgolic,[7] and Bode *et al.*,[6] respectively. Sodium methyl xanthate, NMR (D_2O) δ_H 4.01, was prepared by the reaction of carbon disulfide with sodium hydroxide in methanol. All other reagents were used as obtained.

Disodium N-Carboxyglycinate (**1d**).[4] This salt, a free-flowing air-stable powder, was dried in a forced-draft oven for 1 hr at 110°C without change. NMR (D_2O): See Table 1. A saturated solution in methanol-d_4 or dmso-d_6 produced no detectable NMR signal even at high amplification.

Diammonium N-Carboxyglycinate (**1a**). Ammonium carbonate (13.69 g, 0.12 mol) was added to a solution of glycine (7.50 g, 0.1 mol) and concentrated ammonium hydroxide (13.1 mL, 0.2 mol) in water (25 mL), and stirred overnight in a stoppered flask. Next day, the solution was treated with ethanol (75 mL), whereupon it separated into two layers. Methanol (75 mL) was added to the lower layer, depositing suddenly a mass of snow-white solids which were collected on a filter, rinsed with methanol and vacuum dried, giving 9.95 g of **1a** as a white, crystalline solid, mp 227°C dec., with an ammoniacal odor; NMR (D_2O): δ_H 3.62 (CH_2, **1a**); see text.

Diammonium N-Dithiocarboxyglycinate (**2a**).[10] The **2c** (9.73 g, 0.04 mol) was added to a solution of ammonium carbonate (4.56 g, 0.04 mol), conc. ammonium hydroxide (1 mL) and water (20 mL), stirred for 2 hr, and filtered. The filtrate and washings were treated with a tenfold excess of ethanol, set aside overnight in a refrigerator and filtered, giving 5.10 g (62.8%) of **2a** as long, lustrous needles which rapidly reddened on storage; NMR (D_2O or methanol-d_4): δ_H 4.13.

Barium N-Dithiocarboxyglycinate (**2b**).[7] NMR (D_2O, sat'd): δ_H 4.13 (s, CH_2); weak signal, even at high amplification, and no signal in methanol-d_4.

Calcium N-Dithiocarboxyglycinate (**2c**).[6] The product, recrystallized from water (10 mL/g) and precipitated with ethanol (5 mL/g), exhibited no tendency to redden on storage. NMR (D_2O or methanol-d_4): δ_H 4.13 (s, CH_2); NMR (D_2O) δ_C 52.3 (s, CH_2), 177.7 (s, C=O), and 212.6 (s, C=S).

Disodium N-Dithiocarboxyglycinate (**2d**). *A. From glycine and carbon disulfide.* Glycine (15.01 g, 0.2 mol) was added to a solution of sodium hydroxide (16.00 g, 0.4 mol) in water (50 mL) and stirred by means of a heavy oval magnetic stirring bar until the glycine dissolved. The solution was then purged with argon, treated with carbon disulfide (30 mL, 0.5 mol), and stirred until the glycine was all consumed (30 hr). The progress of the reaction was readily followed by measuring the intensity of the new signal at 248 Hz relative to the glycine signal at about 191 Hz in the ^1H NMR spectrum of the crude reaction mixture. After 48 hr, the mixture was stripped of water and excess carbon disulfide under reduced pressure, taken up in methanol (100 mL), filtered, and stripped of methanol under reduced pressure. The product, a pale yellow oil, was taken up water (10 mL), stripped, redissolved in water (10 mL), stripped again, and finally dried in a vacuum desiccator in the presence of potassium hydroxide pellets giving 48.67 g (97.7%) of **2d** as a pale yellow oil, n_D^{20} 1.6131, soluble in water and methanol and insoluble in other organic solvents; NMR (D_2O or methanol-d_4): δ_H 4.13 (s, CH_2).

Anal. Calcd. for $C_3H_3NNa_2O_2S_2.3H_2O$: C, 14.46; H, 3.64; N, 5.62; Na, 18.45. Found: C, 14.47; H, 3.84; N, 5.64; Na, 18.69.

B. From glycine and sodium trithiocarbonate. A solution of glycine (7.51 g, 0.1 mol) in 40% aqueous sodium trithiocarbonate (38.54 g, 0.1 mol) was stirred under argon for one week, during which the color changed from bright red to pale yellow but the pH (9–9.5) was unchanged. The conversion of glycine to **2d**, by ^1H NMR, was 39.7%. Another 0.1 mol of sodium trithiocarbonate was added and stirring continued for two more weeks. The solution, again pale yellow, was filtered, stripped of water under reduced pressure and worked up as for method A, giving 15.12 g of yellow oil containing **2d** (71.4%) and unreacted glycine (26.2%). To remove the latter, the yellow oil was redissolved in methanol (100 mL), treated with powdered anhydrous sodium carbonate (5.0 g), gassed with carbon dioxide for 15 min, filtered and worked up as before giving 13.61 g (54.6%) of **2d** as a yellow oil; NMR (D_2O): δ_H 4.13 (s, CH_2).

C. From glycine and sodium methyl xanthate. Glycine (0.75 g, 0.01 mol) was added to a solution of sodium methyl xanthate (2.60 g, 0.02 mol) in methanol (10 mL), stirred for 3 days, filtered, stripped of methanol under reduced pressure and worked up as for Method A giving 2.12 g (85.1%) of **2d** as a deep yellow oil (STENCH) contaminated with xanthate (9.0%); NMR (D_2O): δ_H 4.13 (s, CH_2) and 4.01 (s, CH_3).

D. From **1d**. A solution of **1d** (6.52 g, 0.04 mol) in water (50 mL) was treated with carbon disulfide (6.0 mL, 0.1 mol) and stirred under argon overnight. No carbon disulfide remained, but the conversion (NMR) was only 35.8%. More carbon disulfide (12.0 mL) was added and stirring continued under argon for three more days, raising the conversion to 76.6%. The solution was stripped of water and

excess carbon disulfide under reduced pressure, taken up in methanol (100 mL), shaken for 1 hr and filtered, giving 1.24 g, (19.0%) of recovered **1d**. The filtrate, worked up as for method B, yielded 5.60 g (56.2%) of **2d** as a yellow oil; NMR (D_2O): δ_H 4.13 (s, CH_2).

E. From **2a**. The **2a** (6.10 g, 0.03 mol) was added to a solution of sodium hydroxide (2.40 g, 0.06 mol) in water (10 mL), stirred under argon for 1 hr, and stripped of water under reduced pressure, giving 6.58 g (88.0%) of **2d** as a colorless oil, n_D^{20} 1.6043; NMR (D_2O): δ_H 4.13 (s, CH_2).

F. From **2c**. The **2c** (9.73 g, 0.04 mol) was added to a solution of sodium carbonate (4.24 g, 0.04 mol) in water (20 mL), stirred for 2 hr, filtered, and stripped of water under reduced pressure. The residue was taken up in methanol (50 mL) and worked up as for method A, giving 9.09 g (91.2%) of **2d** as a colorless oil, n_D^{20} 1.6126; NMR (D_2O): δ_H 4.13 (s, CH_2).

Calcium N-Thiocarboxyglycinate (**3c**).[8] The product, recrystallized from water (15 mL/g) containing a little calcium hydroxide, exhibited no tendency to redden on storage. NMR (D_2O): δ_H 3.69 (s, CH_2); δ_C 46.1 (s, CH_2), 179.3 (s, C=O), and 184.2 (s, C=S). A saturated solution in methanol-d_4 produced no detectable NMR signal, even at high amplification.

Disodium N-Thiocarboxyglycinate (**3d**). The **3c** (9.09 g, 0.04 mol) was added to a solution of sodium carbonate (4.24 g, 0.04 mol) in water (20 mL), stirred for 2 hr, filtered, and stripped of water under reduced pressure. The residue was taken up in methanol (50 mL) and worked up as for method A, giving 5.77 g (61.9%) of **3d** as a colorless glass, soluble in water and methanol and insoluble in other organic solvents; NMR (D_2O or methanol-d_4): δ_H 3.67 (s, CH_2).

Anal. Calcd. for $C_3H_3NNa_2O_3S.2H_2O$: C, 16.75; H, 3.28; N, 6.51; Na, 21.37. Found: C, 16.66; H, 3.40; N, 6.40; Na, 21.33.

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40. Names of companies or commercial products are given solely for the purpose of providing specific information. Their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.