# Syntheses of Hydroxyalkyl and Mercaptoalkyl Derivatives of Sulfur-containing Amino Acids

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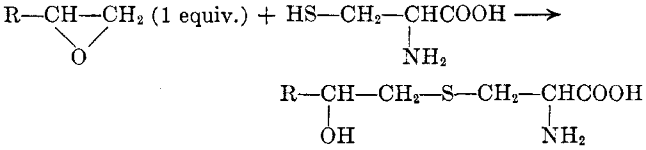
Some S-, N-, and S,N-hydroxyalkyl derivatives of cysteine, homocysteine, cystine, and methionine were prepared by the reaction of alkylene oxides or alkylene carbonates with the amino acids under basic conditions. S-Mercaptoalkyl derivatives of cysteine were obtained by a similar reaction with alkylene sulfides.

S-, N-, or S,N-hydroxyalkyl and mercaptoalkyl derivatives of sulfur-containing amino acids such as, cysteine, cystine, homocysteine, and methionine were synthesized. These derivatives are potential antimetabolites.[1-5]

In previous work[6] we prepared the S-, and S,N-(2-hydroxypropyl) derivatives of cysteine by reaction of the amino acid with propylene oxide. S-(2-Hydroxyethyl)cysteine was similarly prepared, but was not isolated in a pure condition.[7] The kinetics of the addition of ethylene oxide to cysteine was also investigated[8]; the rate was found to increase with increase in the pH of the reaction mixture. Cysteine or methionine treated with ethylene oxide[9] gave products which could not be crystallized, while N-acetyl methionine gave on similar treatment a product which was shown indirectly to be S-(2-hydroxyethyl)-N-acetylmethionine; *i.e.*, hydroxyalkylation of the sulfur atom occurred to yield the corresponding sulfonium derivative. N,N′-bis(2-hydroxyethyl)cystine was obtained on heating a basic aqueous solution of cystine with ethylene oxide.[10] The same reaction carried out in the absence of base and for a longer time led to the formation of a liquid morpholone derivative, as in the case of glycine.[11]

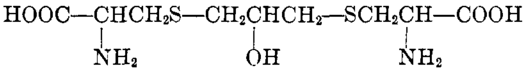
The hydroxyalkylations and mercaptoalkylations were realized under basic conditions according to the following scheme.[6]



$$\mathrm{R{-}\underset{O}{\underbrace{CH{-}CH_2}}\ (1\ equiv.) + HS{-}CH_2{-}\underset{NH_2}{C}HCOOH \longrightarrow R{-}\underset{OH}{C}H{-}CH_2{-}S{-}CH_2{-}\underset{NH_2}{C}HCOOH}$$

Using two or more equivalents of alkylene oxide, and longer reaction times, the amino group was also hydroxyalkylated. The formation of both these derivatives can be easily followed by paper chromatography. The S,N-dihydroxyalkyl derivatives have higher $R_f$ values than the S-hydroxyalkyl derivatives, which in turn have higher values than the starting amino acids.

With epichlorhydrin, 3,3′-(2-hydroxypropane-1,3-dithio)dialanine (I) was obtained.



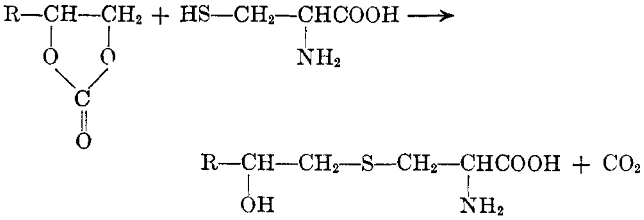
$$\mathrm{HOOC{-}\underset{NH_2}{C}HCH_2S{-}CH_2\underset{OH}{C}HCH_2{-}SCH_2\underset{NH_2}{C}H{-}COOH} \qquad \mathrm{(I)}$$

The opening of the epoxide ring in the reaction of the alkylene oxides with the amino acids under basic conditions is most probably that required by theory, in that the primary carbon atom is attached to the nucleophile.[12]

Attempts were made to use a large excess of the alkylene oxide in reaction with the amino acids, to obtain dihydroxyalkylation on the nitrogen. These derivatives could then on replacement of the hydroxyl groups by chlorine yield nitrogen mustards based on sulfur containing amino acids. However, except for the case of styrene oxide with methionine, we were unable to isolate dihydroxyalkylation products with other alkylene oxides and amino acids.

In the preparation of the hydroxyalkyl derivatives we encountered some experimental difficulties. Many of the derivatives were soluble in alcohol and water and it was difficult to separate completely the required derivatives from triethylamine hydrochloride or sodium chloride formed from cysteine hydrochloride and from neutralization of the reaction mixture, so that low yields were obtained. In some cases it was found that it is better to neutralize the reaction mixture with hydroiodic acid, since the sodium iodide formed is very soluble in water and organic solvents. Also it was found effective to use a weakly acidic resin (Amberlite IRC-50) to remove excess alkali.

Hydroxyalkylation was also realized by reaction of the amino acids under basic conditions with cyclic carbonates as follows:



$$\mathrm{R{-}\underset{\underset{O}{\overset{\|}{\underset{\diagdown\ \diagup}{O\quad C\quad O}}}}{CH{-}CH_2}} + \mathrm{HS{-}CH_2{-}\underset{NH_2}{C}HCOOH} \longrightarrow \mathrm{R{-}\underset{OH}{C}H{-}CH_2{-}S{-}CH_2{-}\underset{NH_2}{C}HCOOH + CO_2}$$

However, longer reaction times and more drastic conditions were needed as compared to those required in the reaction with alkylene oxides, and hydroxyalkylation of the nitrogen did not occur. In liquid ammonia no reaction occurred with the alkylene carbonates, in contrast to that with alkylene oxides, probably due to the low reaction temperature.

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TABLE I

PREPARATION OF S-, N-, AND S,N-HYDROXYALKYL DERIVATIVES OF SULFUR-CONTAINING AMINO ACIDS

| Alkylene Oxide | Equiv. | Amino acid[a] | Base[b] | Substitution on S or N | Reaction time, hr. | Temp., °C. | Yield, % | Crystallization solvent | $R_f$ | M.p., °C. | Formula | Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found | N (Van Slyke) Calcd. | N (Van Slyke) Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ethylene | 1.1 | Cysteine | TEA | S | 1<br>3 | −10<br>25 | 59 | Water–acetone | 0.65 | 210 | $C_5H_{11}NO_3S$ | 36.4 | 36.2 | 6.7 | 6.4 | 8.5 | 8.5 | 8.5 | 8.7 |
| Ethylene | 3.0 | Cysteine | TEA | S, N[c] | 1 week | 25 | 15 | Absolute ethanol | .90 | 203 | $C_7H_{15}NO_4S$ | 40.2 | 40.0 | 7.2 | 7.3 | 6.7 | 6.9 | . . . | . . . |
| Butylene | 3.0 | Cysteine | TEA | S, N[c] | 48 | 50 | 15 | Water–acetone | .98 | 227 | $C_{11}H_{23}NO_4S$ | 50.0 | 49.4 | 8.7 | 8.7 | 5.3 | 5.3 | 0.0 | 0.0 |
| Butylene | 2.2 | Methionine[d] | NaOH | N | 40 | 50 | 50 | Water–ethanol | .98 | 240 | $C_9H_{19}NO_4S$ | 48.9 | 48.7 | 8.6 | 8.6 | 6.3 | 5.9 | 0.0 | 0.0 |
| Propylene | 2.2 | Methionine[d] | NaOH | N | 48 | 25 | 44 | Water–ethanol | .98 | 236 | $C_8H_{17}NO_3S$ | 46.4 | 46.3 | 8.2 | 8.3 | 6.8 | 6.6 | . . . | . . . |
| Propylene | 2.2 | S-Benzylcysteine | NaOH | N | 48 | 25 | 50 | Water | 1.0 | 214 | $C_{13}H_{19}NO_3S$ | 58.0 | 57.5 | 7.0 | 6.7 | 5.2 | 5.1 | . . . | . . . |
| Propylene | 4.0 | Cystine | NaOH | N[e] | 36 | 25 | 21 | Water | 0.95 | 247 | $C_{12}H_{24}N_2O_6S_2$ | 40.4 | 39.8 | 6.8 | 6.9 | 7.9 | 8.1 | 0.0 | 0.0 |
| Styrene | 1.1 | Cysteine[d] | NaOH | S | 36 | 50 | 40 | Water–acetone | .85 | 197 | $C_{11}H_{15}NO_3S$ | 54.7 | 54.3 | 6.2 | 5.9 | 5.8 | 5.7 | 5.8 | 5.8 |

[a] L-Cysteine, L-cystine, and DL-methionine were used. [b] In all cases 1 equiv. of amino acid was used and 1–1.1 equiv. of base. [c] The reaction mixture contained also the mono-substituted derivative. It was evaporated to dryness and extracted with boiling absolute ethanol, from which the derivative precipitated on cooling. [d] The reaction mixture was acidified with hydroiodic acid, evaporated to dryness, and extracted with absolute ethanol to remove sodium iodide formed. [e] Acidification of the reaction mixture resulted in the precipitation of the product, unreacted cystine, and a substance having $R_f$ 0.63 (probably mono-substituted derivative). The required product was extracted with boiling water and recrystallized twice.

Exclusive preparation of N-hydroxyalkyl derivatives of cysteine was accomplished by N-hydroxyalkylation of S-benzylcysteine, and then splitting off the benzyl protecting group by reaction with metallic sodium in liquid ammonia. These derivatives can also be obtained by reducing the doubly N-substituted cystine derivative.

Sulfoxide derivatives of the S-hydroxyalkyl derivatives were easily obtained by reaction with hydrogen peroxide in acetic acid. They have lower melting points and $R_f$ values than the starting materials.

Propiolactone reacted with cysteine under basic conditions to give β-carboxyethylcysteine.[13–15] This is similar to the reaction of propiolactone with amines where N-alkyl-β-alanines are formed.[16]

## Experimental

Melting points were determined in a Fisher–Johns apparatus and the ascending method of paper partition chromatography was used (80% phenol).

Only typical examples are described, the rest summarized in Table I.

**S-(2-Hydroxy-*n*-butyl)-L-cysteine.**—L-Cysteine hydrochloride (3.1 g., 0.02 mole) was dissolved, under nitrogen, in water (25 ml.) and ethanol (15 ml.) and triethylamine (2 g., 0.02 mole) were added, followed by butylene oxide (1.6 g., 0.022 mole). The reaction mixture was shaken and left for 30 hr. at room temperature. It gave a negative test for free cysteine with nitroprusside, and was evaporated to complete dryness *in vacuo* from a water bath at 50°. The residue was extracted by boiling absolute ethanol to remove triethylamine hydrochloride, and the insoluble substance filtered and washed with absolute ethanol; yield 1.7 g. (44%). Recrystallized from water–acetone, it decomposed at 214° and had $R_f$ 0.82.

*Anal.* Calcd. for $C_7H_{15}NO_3S$: C, 43.5; H, 7.8; N, 7.3; N (Van Slyke), 7.3. Found: C, 44.1; H, 8.1; N, 7.4; N (Van Slyke), 7.5.

**N-(2-Hydroxy-2-phenylethyl)-DL-methionine and N-Di(2-hydroxy-2-phenylethyl)-DL-methionine.**—DL-Methionine (7.45 g., 0.05 mole) was dissolved in 3 *N* sodium hydroxide solution (16.7 ml., 0.05 mole) and ethanol (60 ml.) and styrene oxide (12 g., 0.1 mole) were added. The reaction mixture was flushed with nitrogen, heated with shaking for 24 hr. at 50°, left for 1 week at room temperature, and then acidified with hydrochloric acid (1 *N*) to pH 6. The precipitate was filtered and washed with water. It contained the monosubstituted derivative slightly contaminated with the disubstituted derivative. The latter is much more soluble in ethanol, and was separated by extraction with ethanol; yield 8 g. (59%). Recrystallized from hot water, it decomposed at 212°, and had $R_f$ 0.96. It showed a negative reaction on boiling with ninhydrin solution.

*Anal.* Calcd. for $C_{13}H_{19}NO_3S$: C, 57.9; H, 7.0; N, 5.2. Found: C, 57.7; H, 6.9; N, 4.9.

In the original filtrate a yellow oil separated which solidified. It was triturated with a small volume of ethanol and filtered, yield 0.6 g. (3%), m.p. with decomposition 138°, raised to 138°–140° on recrystallization from hot ethanol. It is slightly soluble in hot water and contrary to the monosubstituted derivative it gave no reaction with ninhydrin on paper chromatograms.

*Anal.* Calcd. for $C_{21}H_{27}NO_4S$: C, 64.8; H, 6.9; N, 3.6. Found: C, 64.5; H, 6.7; N, 3.6.

**S-(2-Hydroxy-*n*-propyl)-DL-homocysteine.**—DL-Homocystine (1.08 g., 0.004 mole) was dissolved in liquid ammonia (150 ml.). Sodium metal was added slowly with mechanical stirring until the solution became permanently blue. Powdered ammonium chloride was added cautiously until the solution was just decolorized. Propylene oxide (0.58 g., 0.01 mole) was added and the solution was left until all the ammonia had evaporated. The residual ammonia was evaporated at a water pump, and the

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residue dissolved in water (50 ml.), and passed through a column packed with 3 equivalents of a weak cation exchange resin (crosslinked polymethacrylic acid, Amberlite IRC 50), to remove excess alkali. The eluent (pH 4–5) was evaporated to dryness *in vacuo*. The residue was taken up in a small volume of water, decolorized with Norite, and the product precipitated by acetone. It was recrystallized from water–acetone, yield 0.5 g. (33%), m.p. with decomposition 233°, $R_f$ 0.78.

*Anal.* Calcd. for $C_7H_{15}NO_3S$: C, 43.5; H, 7.8; N, 7.3; N (Van Slyke), 7.3. Found: C, 43.4; H, 8.0; N. 7.4; N (Van Slyke), 7.4.

**3,3'-(2-Hydroxypropane-1,3-dithio)di-L-alanine.**—L-Cystine (3.6 g., 0.015 mole) was dissolved in liquid ammonia (150 ml.). Sodium was added as before, followed by epichlorhydrin (0.92 g., 0.01 mole) and the ammonia was left to evaporate overnight. Residual ammonia was evaporated *in vacuo*, and the residue was taken up in water (100 ml.) and passed through a column packed with Amberlite IRC 50 to remove excess alkali, and then through a column packed with Amberlite IR 120 (nuclear sulfonic acid type cation exchange resin). The sodium ions and the product were held on the column while the chloride ions were removed as hydrochloric acid. The column was washed with water until the effluent gave a negative reaction with silver nitrate. The column was eluted with ammonia (5%), and the eluate evaporated *in vacuo* yielding 1.9 g. (43%) of the crude product. Recrystallized from water–acetone it decomposed at 228° and had $R_f$ 0.35. It is insoluble in ethanol.

*Anal.* Calcd. for $C_9H_{18}N_2O_5S_2$: C, 36.2; H, 6.0; N, 9.4. Found: C, 35.6; H, 6.3; N, 9.1.

**S-(2-Hydroxyethyl)-L-cysteine.**—L-Cysteine hydrochloride (3.1 g., 0.02 mole) was dissolved in water (15 ml.), ethanol (15 ml.) and triethylamine (2 g., 0.02 mole) added, followed by ethylene carbonate (5.3 g., 0.06 mole). The reaction mixture was flushed with nitrogen, and shaken in a closed vessel for 100 hr. at 50°. The reaction mixture gave a negative test for free cysteine with nitroprusside, and was evaporated to complete dryness *in vacuo*. The residue was extracted several times with boiling absolute ethanol to remove triethylamine hydrochloride, filtered, and washed with ethanol, yield 1.8 g. (56%). It was dissolved in a small volume of water and recrystallized by the addition of a large volume of acetone; m.p. with decomposition 210°, $R_f$ 0.65.

*Anal.* Calcd. for $C_5H_{11}NO_3S$: C, 36.4; H, 6.7; N, 8.5. Found: C, 36.3; H, 6.7; N, 8.7.

**S-(2-Hydroxy-*n*-propyl)-L-cysteine Sulfoxide.**—S-(2-Hydroxy-*n*-propyl)-L-cysteine (0.5 g., 0.0028 mole) was dissolved in hot acetic acid (8 ml.), and cooled to 10°. Hydrogen peroxide 30% solution (0.5 ml.) was added, the reaction mixture was held at 10° for 1 hr., and left overnight at room temperature. It was evaporated *in vacuo* at 50°, and acetone added to the residue which solidified. The product was filtered and washed with acetone, yield 0.5 g. (92%). Recrystallized from water–acetone, it decomposed at 150° and had $R_f$ 0.65 as compared to $R_f$ 0.77 for the starting material.[6]

*Anal.* Calcd. for $C_6H_{13}NO_4S$: C, 36.9; H, 6.7; N, 7.2; N (Van Slyke) 7.2. Found: C, 37.4; H, 6.8; N, 7.3; N (Van Slyke), 7.3.

**N-(2-Hydroxy-*n*-propyl)-L-cysteine.**—S-Benzyl-N-(2-hydroxy-*n*-propyl)-L-cysteine (1.9 g., 0.007 mole) was dissolved in liquid ammonia (150 ml.). Sodium metal was added as usual and the ammonia was left overnight to evaporate. The residue was dissolved in deaerated water, acidified with hydroiodic acid, and the product precipitated with acetone; yield 0.7 g. (55%); m.p. 205° (on second recrystallization from water–acetone), $R_f$ 0.95. This $R_f$ is that of the cystine derivative formed on oxidation by air.

*Anal.* Calcd. for $C_6H_{13}NO_3S$: N, 7.8; N (Van Slyke), 0.0. Found: N, 7.6; N (Van Slyke), 0.0.

**S-(2-Carboxyethyl)-L-cysteine.**—L-Cysteine hydrochloride (3.1 g., 0.02 mole) was dissolved in 50% ethanol (20 ml.) and sodium hydroxide 4 *N* solution (10 ml., 0.04 mole). Propiolactone (2.2 g., 0.03 mole) was added, the reaction mixture flushed with nitrogen and kept for 5 days at room temperature. The reaction mixture was acidified to pH 6, and the precipitated derivative was filtered off. The filtrate was evaporated *in vacuo*, and both the residue and precipitate were crystallized from water–ethanol; yield 2.8 g. (73%), m.p. and m.m.p. 212°,[13] on recrystallization from 50% ethanol.

*Anal.* Calcd. for $C_6H_{11}NO_4S$: C, 37.3; H, 5.7; N, 7.2; N (Van Slyke), 7.2. Found: C, 37.0; H, 5.8; N, 6.9; N (Van Slyke), 7.2.

**S-(2-Mercaptoethyl)-L-cysteine.**—L-Cysteine hydrochloride (3.1 g., 0.02 mole) was dissolved in 50% ethanol (20 ml.) and sodium hydroxide 3 *N* solution (6.6 ml., 0.02 mole). Ethylene sulfide (1.8 g., 0.03 mole) was added at 0° under nitrogen and a white precipitate formed immediately. The reaction mixture was left overnight at room temperature and filtered. The precipitate, m.p. around 180°, gave no reaction with ninhydrin and was insoluble in water, ethanol, acetone, dimethylformamide, and tetrahydrofuran, and is probably polyethylene sulfide. The filtrate was acidified to pH 6, with hydroiodic acid, and the precipitate was filtered and recrystallized from water–ethanol; yield 0.25 g. (8%), m.p. with decomposition 233°, $R_f$ 0.64. It gave a positive reaction with nitroprusside for sulfhydryl groups. Similar yields were obtained on using triethylamine.

*Anal.* Calcd. for $C_5H_{11}NO_2S_2$: C, 33.1; H, 6.1; N, 7.7. Found: C, 33.0; H, 6.3; N, 7.6.

**S-(2-Mercapto-*n*-propyl)-L-cysteine.**—L-Cysteine hydrochloride (3.1 g., 0.02 mole) was dissolved in 50% ethanol (20 ml.), the solution neutralized to pH 7 with concentrated sodium bicarbonate solution, cooled to 0°, and propylene sulfide (1.5 g., 0.02 mole) added. The reaction mixture was flushed with nitrogen kept in the cold for 1 hr. and then for 2 days at room temperature. Propylene sulfide, which is weaker as a monomer than its lower homologue, did not polymerize appreciably under these conditions. A precipitate formed on standing, which contained free sulfhydryl groups. The solution was acidified to pH 6 and filtered. The filtrate was evaporated *in vacuo*, and the residue was recrystallized from hot deaerated water–acetone. Total yield, including product that initially precipitated was 3 g. (80%), m.p. with decomposition 211°, $R_f$ 0.78. It gave a positive reaction for sulfhydryl groups.

*Anal.* Calcd. for $C_6H_{13}NO_2S_2$: C, 36.9; H, 6.7; N, 7.2; N (Van Slyke) 7.2. Found: C, 37.5; H, 6.6; N, 6.9; N (Van Slyke), 7.1.