the ratio 1:2 with the pKa 9.3, and nitrite ion was conclusively identified as present.

The same phenomena were observed for the alkaline degradation of β-aminooxypropionic acid (Table III).

It can be concluded that carbonate or hydroxylamine is not an end product of the mild alkaline degradation of aminooxyalkanoic acid (no pKa's 6.0–6.5 are observable) and that the most probable sequence is the hydrolysis to hydroxylamine and some carboxylic acid, with a subsequent fast degradation of hydroxylamine to ammonia and nitrite ion.

Figure 5 plots the ammonia and nitrite produced as a function of time in 0.01 *M* sodium hydroxide at 60° as based on the above discussion. Apparent first-order plots for the production of ammonia and nitrite are given in Fig. 6 for aminooxyacetic acid with an apparent half-life of 110 hours under these conditions.

It is interesting to note that although β-aminooxypropionic acid is quite stable at 60° below pH 2 and between pH 7–10, degradation other than alkaline also occurs with an apparent maximum at pH values of *ca.* 3–4. This implies a significant acid catalyzed hydrolysis of the neutral molecule where

$$H_2NO{-}CH_2CH_2COOH + H_3O^+ \rightarrow H_3\overset{+}{N}OH + HOCH_2CH_2COOH \quad \text{(Eq. 3)}$$

the hydroxylamine was identified by the appearance of a pKa 6.0 since hydroxylamine does not readily degrade at this pH. It may be inferred that at a pH less than 2 the protonated compound, $^+H_3NO{-}CH_2CH_2COOH$, is the major species and resists hydrogen ion attack. At a pH greater than 7, the hydrogen ion and hydroxyl ion concentrations are insufficient for catalytic action.

Biological Activity and the Chemistry of Aminooxyalkanoic Acids.—The increased biological activity of aminooxyacetic acid over all other compounds studied (4, 7) can be assigned to its unusual chemical characteristics which include the absence of any apparent basicity of the aminooxy group in aqueous solution, perhaps due to a tendency to exist in the form of a five-membered ring. This in turn may be responsible for its ability to cross cell walls.

The ability of these α-aminooxyalkanoic acids to release hydroxylamine *in vitro* is of interest in light of the known action of hydroxylamine as an inhibitor of transaminase. This suggests that these α-aminooxyalkanoic acids may uniquely carry and release hydroxylamine at the physiological site, wheras hydroxylamine itself is too readily metabolized or not readily transportable. Of course, the possibility that the intact acids are the primary agent is not refuted.

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Sulfide Derivatives of Cysteine II

Some Sulfonamide Derivatives of Cysteine and Methionine

By MATTHEW VERDERAME

Seven new sulfide derivatives of cysteine have been prepared. In addition sulfonamides of cysteine, L-3-mercapto-N-sulfanilylalanine, and methionine, DL-N-sulfanilylmethionine, among others, have also been prepared. Except for L-3-[(3′-hydroxy-4′-carboxyphenyl)-carbamylmethylthio]-alanine (compound 2), which was slightly effective against a *Klebsiella pneumoniae* infection in Swiss mice, all other compounds tested for various activities showed a lack of physiological effectiveness.

A PREVIOUS paper (1) was concerned with the synthesis and physiological testing of several new amide derivatives of L-3-carboxymethylthioalanine as possible antiviral agents. This paper is, in part, involved with the synthesis and testing of seven additional sulfide derivatives of cysteine. The second half of this publication essentially deals with the preparation and biological study of L-3-mercapto-N-sulfanilylalanine, DL-N-sulfanilylmethionine, and other related compounds.

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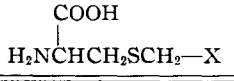
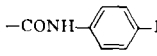
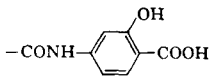
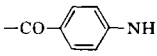
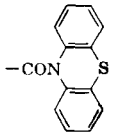
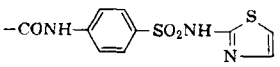
DISCUSSION

The sulfide derivatives of cysteine, L-3-(substituted)-methylthioalanines (Table I), were made by

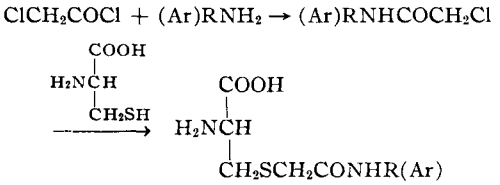
TABLE I.—L-3-(SUBSTITUTED)-METHYLTHIOALANINES



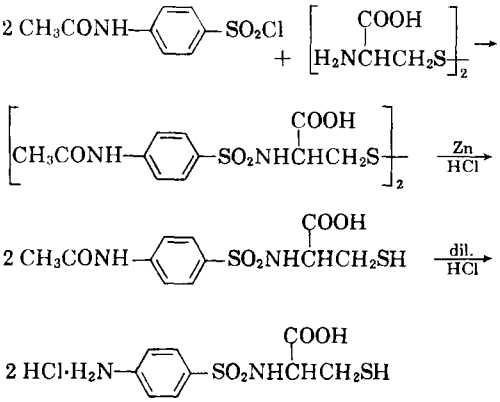
No.	X	Formula	Yield, %	M.P.,[a] °C. (decompn.)	Ref.[b]	Nitrogen, % Calcd.	Nitrogen, % Found.[c]	Sulfur, % Calcd.	Sulfur, % Found
1		$C_{13}H_{17}N_3O_4S$	38.8	208	(4)	13.50	13.32	10.30	9.88
2		$C_{12}H_{14}N_2O_6S$	18.0	203	(5)	8.92	8.90	10.20	10.18
3		$C_{11}H_{14}N_2O_3S$	60.2	130	...	11.02	10.92	12.61	12.65
4		$C_8H_{14}N_2O_3S$	34.4	183	(6)	12.84	13.01	14.69	15.02
5		$C_{17}H_{16}N_2O_3S_2$	61.9	165	(7)	7.77	7.42	17.79	17.60
6		$C_{11}H_{16}N_4O_5S_3$	10.3	164	(8)	13.45	12.99	23.10	23.32
7		$C_{14}H_{15}NO_2S$	56.5	197	...	5.36	5.19	12.27	11.51

[a] All melting points are uncorrected. [b] These references pertain to the synthesis of the corresponding N-chloroacetyl precursors made by treating those substituted amines grouped under the X column with chloroacetyl chloride. [c] The analytical data were determined at the Sterling-Winthrop Research Institute.

the alkylation of cysteine according to procedures previously described (1-3).



The new sulfonamides of cysteine and methionine (Table II) were made in the usual manner starting with N-acetylsulfanilyl chloride (ASC) to acylate the amino groups of these amino acids, followed by the acid hydrolysis of the acetamido grouping. In the case of cysteine, the acylation was first performed on cystine and the resulting product then reduced to free the mercapto group, according to the following reactions



EXPERIMENTAL

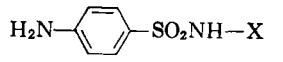
Most of the starting materials were obtained from Eastman. Others were purchased from Matheson (*p*-aminosalicylic acid and DL-methionine) and Merck (sulfathiazole).

L-3-(Substituted)-methylthioalanines (Table I).—Compounds 1 and 6 were recrystallized from water. Compound 4 was recrystallized from water and alcohol. Number 3 required two recrystallizations from water and a third from equal parts absolute ethanol and methanol, followed by the addition of ether. Number 7 was purified from acetic acid plus water. For compound 5, equal parts of alcohol and water was used initially, and then water and acetic acid. Compound 2 required alternate treatment with dilute hydrochloric acid and sodium bicarbonate solutions. The base, added to the filtered, acid solution of the crude material, yielded the amphoteric compound, which redissolved by the addition of more bicarbonate solution. Acidification of the filtered, basic solution produced a pure compound.

L - N,N' - Bis - (*p* - acetamidobenzenesulfonyl)-cystine.—A mixture consisting of 39.2 Gm. (0.168 mole) of ACS, 20.0 Gm. (0.084 mole) of L(−)cystine, 13.6 Gm. (0.336 mole) of sodium hydroxide dissolved in 500 ml. of water, and 300 ml. of ether was vigorously agitated for 45 minutes at room temperature. The layers were separated and the aqueous portion chilled and acidified with concentrated hydrochloric acid to pH 1. The product was reduced at once without further purification. For analysis, a portion was recrystallized once from isopropyl alcohol and twice from alcohol; m.p. 216° (decompn.).

Anal.—Calcd. for $C_{22}H_{26}N_4O_{10}S_4$: N, 8.83; S, 20.20. Found: N, 8.82; S, 19.6.

TABLE II.—SOME SULFONAMIDE DERIVATIVES OF CYSTEINE AND METHIONINE



No.	X	Formula	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
8	—CH(COOH)CH$_2$SH	$C_9H_{12}N_2O_4S$	10.14	10.29	23.21	23.40
9	—CH(COOH)CH$_2$SCH$_2$CH$_3$	$C_{11}H_{16}N_2O_4S_2$	9.20	9.08	21.07	21.5
10	—C$_6$H$_4$—SO$_2$NHCH(COOH)CH$_2$SC$_2$H$_5$	$C_{17}H_{21}N_3O_6S_3$	9.14	8.75	20.93	20.03
11	—CH(COOH)CH$_2$CH$_2$SCH$_3$	$C_{11}H_{16}N_2O_4S_2$	...	[a]	21.07	22.38

[a] Carbon and hydrogen were also determined. *Anal.*—Calcd.: C, 43.40; H, 5.30. Found: C, 43.77; H, 5.17.

L - 3 - Mercapto - N - acetylsulfanilylalanine.—The crude L-N,N′-Bis-(*p*-acetamidobenzenesulfonyl)-cystine was treated with 9.0 Gm. (0.138 mole) of zinc dust, 90 ml. (0.168 mole) of a 10% solution of hydrochloric acid, 110 ml. methyl alcohol, and 75 ml. of water. The mixture was then heated at about 55° for 1.5 hours, with shaking. To effect a solution of the product, the mixture was heated to near boiling, filtered, and chilled. The white product thus obtained was essentially pure and amounted to 14.6 Gm., or 27.4%, based on the quantity of ASC used initially. A sample, recrystallized twice from alcohol, melted at 214–217°.

Anal.—Calcd. for $C_{11}H_{14}N_2O_5S_2$: N, 8.80; S, 20.14. Found: N, 8.85; S, 19.9.

L-3-Mercapto-N-sulfanilylalanine (Compound 8).—The previously isolated 14.6 Gm. (0.046 mole) of L-3-mercapto-N-acetylsulfanilylalanine was refluxed for 30 minutes with 125 ml. of 6 *N* hydrochloric acid solution. The mixture was filtered while hot, cooled, and neutralized with a solution of 20% sodium hydroxide. The 8.0 Gm. of product, obtained by two recrystallizations from hot water, corresponded to a 63% yield; m.p. 170–173°. For analytical data, see Table II.

L - 3 - Ethylthio - N - (N′ - acetylsulfanilyl)-alanine.—By the procedure previously described, a mixture of 13.4 Gm. (0.090 mole) of L-3-ethylthioalanine (2), 22.2 Gm. (0.095 mole) of ASC, 7.6 Gm. (0.190 mole) of sodium hydroxide in 150 ml. of water, and 150 ml. of ether, stirred for 2 hours at room temperature, yielded 17.4 Gm. of product, which was recrystallized twice from alcohol and water. Yield, 55.9%; m.p. 190–193°.

Anal.—Calcd. for $C_{13}H_{18}N_2O_5S_2$: N, 8.09; S, 18.51. Found: N, 8.02; S, 18.69.

L-3-Ethylthio-N-sulfanilylalanine (Compound 9).—From the hydrolysis of 17.4 Gm. (0.051 mole) of L-3-ethylthio-N-(N′-acetylsulfanilyl)-alanine with 125 ml. of 6 *N* hydrochloric acid solution for 30 minutes was obtained 11.1 Gm. of product, following two recrystallizations from alcohol and water. Yield, 70.6%; m.p. 182–184°.

L - 3 - Ethylthio - N - [(N′ - sulfanilyl) - sulfanilyl]-alanine (Compound 10).—As previously described, the crude product obtained from a mixture of 7.7 Gm. (0.025 mole) of L-3-ethylthio-N-sulfanilylalanine, 6.5 Gm. (0.028 mole) of ASC, 2.2 Gm. (0.056 mole) of sodium hydroxide in 80 ml. of water, and 80 ml. of ether, was hydrolyzed directly with 60 ml. of 6 *N* hydrochloric acid solution for one-half hour. The product thus obtained was recrystallized three times from alcohol and water, yielding 1.5 Gm. or 12%, based on L-3-ethylthio-N-sulfanilylalanine; m.p. 127° (decompn.).

DL-N-(N′-Acetylsulfanilyl)-methionine.—A mixture of 13.9 Gm. (0.093 mole) of DL-methionine, 21.6 Gm. (0.093 mole) of ASC, 6.8 Gm. (0.186 mole) of sodium hydroxide dissolved in 110 ml. of water, and 110 ml. of ether, agitated for 1 hour, yielded an oil, upon acidification of the aqueous phase. The oil solidified in a desiccator over calcium chloride after 3 days. Partial purification was effected by triturating the solid in a mortar three times with 50-ml. portions of ether, the mixture being filtered each time. The undissolved substance was then recrystallized once from ethyl acetate and again from water. The 12.0 Gm. of product thus obtained corresponded to a 37.6% yield; m.p. 163–164°.

Anal.—Calcd. for $C_{13}H_{18}N_2O_5S_2$: C, 45.07; H, 5.24; N, 8.09; S, 18.51. Found: C, 44.98; H, 4.97; N, 7.92; S, 18.67.

DL-N-Sulfanilylmethionine (Compound 11).—Hydrolysis of 12.0 Gm. (0.035 mole) of DL-N-(N′-acetylsulfanilyl)-methionine with 95 ml. of 6 *N* hydrochloric acid solution in the usual way produced 4.2 Gm. of product (39.8%), after two recrystallizations from ethyl acetate; m.p. –144146°.

BIOLOGICAL RESULTS[1]

Compounds 2, 5, 7, 8, and 10 were ineffective intraperitoneally or subcutaneously against a *M. pyogenes* infection in Swiss mice. Also, compounds 8, 9, 10, and 11 were inactive *in vivo* against a staphylococcus infection orally and subcutaneously. Against a *Trichomonas gallinae* infection in hamsters, compound 7 was without effect.

[1] The author wishes to express his thanks to A. J. Arrowsmith, S. D. Sobell, D. A. Berberian, and R. G. Slighter, among others, of the Sterling-Winthrop Research Institute for performing the biological studies on these compounds.

As possible antimetabolites for calcium pantothenate, phenylalanine, cysteine, and folic acid, compounds 3, 4, 5, 6, and 11 were ineffective. Given intraperitoneally to Swiss mice having a *D. pneumoniae I* infection, compounds 2, 3, 4, and 6 were of no value. Compound 1 was ineffective as a possible spermatocidal agent. Also ineffective against a *Klebsiella pneumoniae* infection in Swiss mice given by various routes were compounds 1, 3, 4, 5, and 6. Compound 2, however, did show slight effectiveness.

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Effect of Phentolamine and Tolazoline on Some Metabolic Processes of Heart Homogenates

BY HARRY C. FROEDE and HAROLD C. HEIM

The addition of phentolamine enhances oxygen uptake of rat heart homogenates respiring in the presence of added succinate, but evokes an inhibitory effect with pyruvate as the substrate. These effects are not observed when phentolamine is replaced by tolazoline. Oxidation of α-ketoglutarate is not affected by either drug nor is the activity of a purified malic dehydrogenase. The addition of oxaloacetate to homogenates inhibits oxygen uptake and the inhibitory effect is not abolished by the prior addition of phentolamine. The total α-keto acid content of homogenates respiring in the presence of succinate was found to be somewhat higher than in those homogenates to which both succinate and phentolamine were added at the beginning of the experiments.

TOLAZOLINE and phentolamine are both 2-substituted imidazolines which evoke adrenergic blocking effects. It has been reported that the most pronounced side effects elicited by these drugs are related to a direct stimulation of the myocardium and include cardiac arrhythmias, anginal pain, tachycardia, and fibrillation (1–4).

A survey of the literature reveals that no studies have been undertaken to determine whether phentolamine or tolazoline affect metabolic processes of the heart, *in vitro*. Therefore, because of the widely held belief that drugs produce effects on tissues, at least in part, through an alteration of intracellular reactions, an investigation of the effects of these two drugs on oxidative processes of heart homogenates was conducted.

EXPERIMENTAL

Conventional manometric techniques were used to determine the effect of phentolamine and tolazoline on oxygen uptake in the presence of different added substrates including succinate, α-ketoglutarate, and pyruvate. Hearts from adult Sprague-Dawley rats of both sexes were used. The animals were stunned and decapitated, following which the hearts were immediately removed, cut open, and washed in cold 0.1*M* phosphate buffer. The ventricles were then dissected free, blotted dry on filter paper, weighed, and placed in a glass homogenizer immersed in ice and containing sufficient buffer so that 1.0 ml. of homogenate contained 50 mg., wet weight, of ventricle. The homogenates were centrifuged for 10 minutes at 480 × g in a Servall RC-2 centrifuge with an SS-34 rotor and at a temperature of 3°. The sediment was discarded and the supernatant was used as the homogenate. Ten animals were used in each experiment and each determination of oxygen uptake was performed in duplicate. No more than 10 minutes elapsed between the time the animals were killed and the preparation of the homogenates.

The concentrations of reagents added to the flasks are included in *Results*. The temperature of the bath was 37° and the gas phase was air. After a 10-minute equilibration period, the side arm contents were tipped into the vessels, the manometers closed, and readings taken at 15-minute intervals for a total of 90 minutes.

Keto-acid determinations were performed according to the method of Friedemann (5). The contents of the main compartments of two Warburg vessels were pooled and 2.0 ml. of the ensuing mixture was pipetted into 8.0 ml. of cold, 10% trichloroacetic acid solution. After centrifugation at 3000 × g for 15 minutes, 3.0 ml. of the clear supernatant

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