substituted for a nitro group is confirmed by the very low K_0' values of benzoate in the anti-3-NB and anti-4-NB systems, and of benzoate, *m*-phthalate, trimesate and N-3-nitro-5-carboxyphenylsuccinamate in the anti-3,5-NB system.

Effect of Substitution of Iodine for a Nitro Group in the Homologous Position.—In the anti-3-NB and anti-4-NB systems, the presence of an iodine atom in the nitro-specific position contributes markedly to the free energy of interaction with antibody. In the anti-3-NB system, the ΔF° value for N-3-iodophenylsuccinamate is 3.5 kcal./mole more negative than that of N-phenylsuccinamate; and in the anti-4-NB system, the value for N-4-iodophenylsuccinamate is 2.4 kcal./mole more negative than that of the unsubstituted compound. These energies represent 60 to 65% of the values associated with substitution of a nitro group in each of the corresponding homologous positions. The effectiveness of an iodo group in each case is consistent with the hypothesis that the interaction of the nitro group with antibody is largely the result of non-polar forces. Despite the fact that an iodo group does not ordinarily form hydrogen bonds, it still contributes a large fraction of the energy of interaction of the homologous nitro group with each antibody. The remaining difference can probably be ascribed to a difference in complementariness rather than to the nature of the force of attraction although hydrogen bonding cannot be ruled out completely. (The fact that a large change in ΔF° can be associated with a small change in the size of the substituent group is substantiated by the comparison of chloro and iodo substitution, discussed below.)

The possibility of ion–dipole interaction with a positive charge in the molecule similarly appears improbable. A positive charge, if present, would interact strongly with carboxylate; actually, substitution of carboxylate for nitro results in each case in a large decrease in K_0', despite the fact that carboxylate and nitro are almost identical in size and configuration.

Substitution of a chloro group for a hydrogen atom in the nitro-specific position in the anti-3-NB or anti-4-NB systems similarly results in improved combination with antibody. In the latter system, the chloro is nearly as effective as iodo; in the former, the chloro group is considerably less effective (the ratio of K_0' values is about 18/1). The higher K_0' values of the iodo compounds, as compared with chloro derivatives, may be attributed to the closer similarity in size of iodine to the homologous nitro group.

The effect of substitution of a halogen for nitro in the anti-3,5-NB system cannot readily be discussed because disubstituted halogen derivatives were not tested and because of the uncertainty in the K_0' value of N-3-nitrophenylsuccinamate in the first run (Table III).

Other Observations.—The anti-3-NB antibody was found to fit closely about the 3-nitrophenylsuccinamate ion, as shown by the fact that placing a second nitro group on the opposite side (in the 5-position) increased the free energy of combination by about 1.3 kcal./mole. If a carboxylate group is placed in the 5-position instead of the nitro group, there is a much greater increase (3.6 kcal./mole) which may be due either to the hydration of the carboxylate, which may increase its effective size; or to the presence of a negative charge in or near the combining site of the antibody, which would repel the carboxylate-substituted hapten.

BUFFALO, NEW YORK

[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION[1]]

Cysteine Thioethers from Chloroethylenes[2]

BY L. L. MCKINNEY, A. C. ELDRIDGE AND J. C. COWAN

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Both *cis*-dichloroethylene and vinylidene chloride reacted with disodium cysteinate in liquid ammonia to give the same dithioether I in good yield, but *trans*-dichloroethylene failed to react. Trichloroethylene reacted to give only a monothioether II, while tetrachloroethylene gave both a mono- (III) and a dithioether (IV). The hyperconjugation and conjugative effect of a vinyl group attached to a sulfur atom is evidenced by a bathochromic shift in the ultraviolet which is accentuated by the presence of chlorine atoms on the vinyl group. The trichloroethylene derivative II is unique in giving a typical mustard gas color test and in exhibiting a high order of toxicity which is not necessarily a function of chlorine beta to sulfur.

Introduction

The discovery that treatment of disodium L-cysteinate with trichloroethylene in liquid ammonia produced S-(dichlorovinyl)-L-cysteine which on oral administration to calves produced an aplastic anemia syndrome identical in all respects to that observed from trichloroethylene-extracted soybean oil meal[3] prompted a further study of the thioethers formed from chloroethylenes. This paper describes the products obtained by treating disodium cysteinate in liquid ammonia with *cis*-dichloroethylene, vinylidene chloride or tetrachloroethylene, along with further observations of the reaction with trichloroethylene and comparison of the properties of the products.

(1) One of the Divisions of the Agricultural Research Service, U. S. Department of Agriculture.

(2) Presented before the Division of Agricultural and Food Chemistry at the Symposium on Deleterious Compounds in Foods and Feeds, 134th Meeting, American Chemical Society, Chicago, Illinois, September 7–12, 1958.

(3) (a) L. L. McKinney, F. B. Weakley, A. C. Eldridge, R. E. Campbell, J. C. Cowan, J. C. Picken, Jr., and H. E. Biester, THIS JOURNAL, **79**, 3932 (1957); (b) L. L. McKinney, J. C. Picken, Jr., F. B. Weakley, A. C. Eldridge, R. E. Campbell, J. C. Cowan and H. E. Biester, *ibid.*, **81**, 909 (1959).

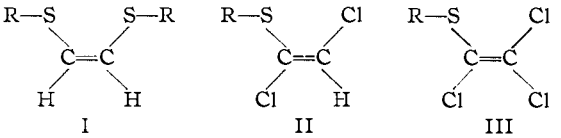
Truce, *et al.*,[4] reported studies on the reaction of sodium *p*-toluenethiolate with *cis*-dichloroethylene in ethanol at elevated temperatures to produce *cis*-1,2-bis-(*p*-tolylmercapto)-ethene. *trans*-Dichloroethylene failed to react with the thiolate under these conditions. Treatment of vinylidene chloride with the thiolate produced the same compound as did *cis*-dichloroethylene.[5]

Cusa and McCombie[6] refluxed an alcoholic solution of sodium thiophenoxide with trichloroethylene and obtained phenyl-1,2-dichlorovinyl sulfide. Further treatment with thiophenoxide failed to displace additional chlorine. Tetrachloroethylene was found to react with two molecular portions of sodium thiophenoxide to give the symmetrical compound, PhS–CCl=CCl–SPh, which did not react further with the thiophenoxide.

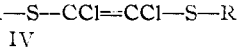
Backer, *et al.*,[7] showed that 1,2-dichloro-1-*t*-butylthioethene was obtained when an alcoholic solution of equimolar portions of sodium *t*-butylthiolate and trichloroethylene were refluxed.

Discussion

Both *cis*-dichloroethylene and vinylidene chloride reacted with disodium cysteinate in liquid ammonia to give the same compound in good yield; however, *trans*-dichloroethylene failed to react. The infrared curves of these reaction products were identical. These results parallel those obtained by treating sodium arylthiolate with these vinyl halides in ethanol at elevated temperatures.[4–6] Hence the same reaction mechanism is suggested, and structure I, *cis*-1,2-di-(1,1-aminocarboxy-2-thioethyl)-ethylene, has been tentatively assigned to the compound.



I II III



IV

R = —CH_2—CH(NH_2)—COOH

Attempts to isolate an intermediate in which only one chlorine atom in *cis*-dichloroethylene or vinylidene chloride was replaced by the cysteine radical were unsuccessful. Chromatographic analysis indicated that a small amount of equimolecular reaction product was present in the *cis*-dichloroethylene reaction mixture. These observations, coupled with the failure of *trans*-dichloroethylene to react, indicate that the reaction is facilitated where *trans*-dehydrohalogenation is possible in accordance with the elimination–addition mechanism proposed by Truce, *et al.*[4]

As previously reported,[3] trichloroethylene reacts nearly quantitatively with disodium cysteinate in liquid ammonia within 2 hr., or the time required to evaporate the ammonia, to give S-(dichlorovinyl)-L-cysteine. Since it has been shown that trichloroethylene reacts with sodium thiolates to produce 1,2-dichlorovinyl compounds,[6,7] the cysteine derivative might also be expected to have this structure. Attempts to treat two or more molecular portions of cysteine with trichloroethylene resulted in only slight replacement of two or three chlorine atoms even though the reaction mixture stood for 8 days. The slow rate of the reaction is attributed to the *trans* chlorine structure shown in II, which precludes *trans*-dehydrohalogenation. A *cis* chlorine configuration would allow *trans*-dehydrohalogenation, and the reaction would be expected to proceed. In view of these and other considerations, *i.e.*, infrared data, chemical reactivity, toxicity and comparison with the analog from tetrachloroethylene, structure II, S-(*trans*-dichlorovinyl)-L-cysteine, has been tentatively assigned to the reaction product of trichloroethylene and disodium L-cysteinate.

Excess tetrachloroethylene reacted with disodium L-cysteinate to give a mixture of S-(1,2,2-trichlorovinyl)-L-cysteine (III) and 1,2-di-(1,1-aminocarboxy-2-thioethyl)-1,2-dichloroethylene (IV) from which compound III was isolated in 15% yield based on the cysteine. Although the reaction was slower than when trichloroethylene was used to produce compound II, a strict comparison is precluded because acetone added to the liquid ammonia to prevent freezing of tetrachloroethylene could affect the reaction rate. Compound IV was obtained in good yield by allowing two molecular portions of disodium L-cysteinate and one of tetrachloroethylene to stand in liquid ammonia containing 10% acetone for 5 days. A small amount of III was present in this reaction mixture. Both reaction mixtures contained a small amount of ninhydrin positive material with low R_f indicating a tendency for more than two chlorine atoms to be replaced by cysteine. Structure IV is assigned on the basis of elementary analysis and also because Cusa and McCombie[6] found that the symmetrical compound was obtained by refluxing thiophenol and tetrachloroethylene in the presence of sodium ethoxide.

On treatment at 80° for 1 hr. with 2 *N* sodium hydroxide, all four compounds gave a strong fuchsin test for aldehyde and, with the exception of I, all liberated chloride; the nitroprusside test for sulfhydryl groups and the phenylhydrazine–potassium ferricyanide test for formaldehyde were negative. Compound II alkylated 4-(*p*-nitrobenzyl)-pyridine (4-NBP)[8] to give an intense blue color typical of mustard gases whereas compound III gave a rose color which did not turn blue on treatment with alkali; IV failed to give any color.

According to Truce and Boudakian[5] infrared spectra of polar-substituted olefins exhibit C–H in-plane bending bands for the *cis* configuration at 7.73–7.78 μ and for the *trans* at 8.44–8.55 μ whereas the C–H out-of-plane bending vibrations of *trans* occur at 10.62–11.25 μ, the exact location depending upon the nature of the polar groups. The intense absorption of cysteine at 7–8 μ obscures evaluation of the infrared spectra in this range. The strong absorption at 8.30 μ and the medium band at 11.20

(4) W. E. Truce, M. M. Boudakian, R. F. Heine and R. J. McManimie, THIS JOURNAL, **78**, 2743 (1956).

(5) W. E. Truce and M. M. Boudakian, *ibid.*, **78**, 2748 (1956).

(6) N. Cusa and H. McCombie, *J. Chem. Soc.*, 767 (1937).

(7) H. J. Backer, J. Strating and J. F. A. Hazenberg, *Rec. trav. chim.*, **72**, 813 (1953).

(8) (a) T. A. Geissman, H. Hochman and R. T. Fukuto, THIS JOURNAL, **74**, 3313 (1952); (b) J. Epstein, R. W. Rosenthal and R. J. Ess, *Anal. Chem.*, **27**, 1435 (1955).

μ for I might argue for a *trans* configuration and lend some doubt to the assigned structure. Absorption bands are noted in the range for both *cis* and *trans* configurations for II and III as expected. However, absorption in the range for *trans* only is noted for IV.

The intense absorption at 250–280 mμ appears to be characteristic of ethylene groups adjacent to a sulfur atom. The molecular extinction coefficients of I and IV are 8,800 as compared to 3,200 for II[3] and 4,700 for III, indicating the role played by the sulfur and chlorine atoms in the hyperconjugation effect.[9]

Several attempts to measure the optical activity of S-(*trans*-dichlorovinyl)-L-cysteine (II) gave a value of zero even though measurements were made on a 4.5% solution. This result was somewhat disturbing because the other three compounds were optically active indicating that racemization of disodium L-cysteinate did not occur in liquid ammonia. Furthermore, S-(dichlorovinyl)-L-glutathione prepared in this Laboratory was found to be optically active.[3b] It is suspected that the crystalline S-(dichlorovinyl)-L-cysteine contained an impurity, possibly cystine, which cancelled out its low order of optical activity. Nevertheless, failure to demonstrate optical activity demands assumption of the L-form.

S-(Dichlorovinyl)-L-cysteine (II) was unique not only in its property to give a mustard gas reaction with 4-NBP but also in its high order of toxicity. Neither I nor IV exhibited toxicity toward the mold (*Mucor ramannianus*), the yeast (*Saccharomyces pastorianus*), algae, soybeans or aquarium fish (*Lebistes reticulatus*). Both II and III were toxic to each of the above. However, II was 20 times as toxic to the yeast as III, 10 times as toxic to the mold, 2 times as toxic to algae, 3 times as toxic to soybeans and 2.5 times as toxic to the fish. The lower toxicity of III compared to II and the apparent non-toxicity of IV suggest that tetrachloroethylene-extracted soybean oil meal might be less toxic than is trichloroethylene-extracted soybean oil meal.

When pancreatic digests of toxic soybean protein, obtained from known toxic trichloroethylene-extracted soybean oil meal, were spray-dried, substantial amounts of the toxicity as determined by calf-assay were lost.[3b,10] This toxicity loss can be explained by assuming that pancreatin contains a thionase similar to that reported by Binkley[11] and that this thionase liberates the low boiling compound, 1-thiol-1,2-dichloroethylene (ClCH=CClSH), which is lost on spray-drying the pancreatic digest. Paper chromatography of alcoholic extracts of the pancreatic digests with 70:30 1-propanol–water yielded a spot at R_f 0.90 which contained sulfur, gave a positive 4-NBP test and was ninhydrin and nitroprusside negative. These extracts also inhibited the growth of *M. pastorianus*. Pancreatic digests of protein from hexane-extracted soybean meal failed to give this spot and the extract was not toxic to the test mold. These tests indicate the presence of a low-molecular-weight toxic compound containing sulfur and the dichlorovinyl group but not an amino or sulfhydryl group. Whether it is a hydrolysis product of S-(dichlorovinyl)-L-cysteine was not established.

S-(Dichlorovinyl)-L-cysteine (II) treated with the plant thionase reported by Gmelin, *et al.*,[12] failed to give either the nitroprusside test for a sulfhydryl compound or the 2,4-dinitrophenylhydrazine test for pyruvic acid.

Experimental

Analyses. (a).—Ascending paper chromatograms were developed with different solvent systems as shown in Table I. Spots were detected by spraying chromatograms with ninhydrin (for α-amino groups), iodoplatinate reagent (for sulfur), nitroprusside reagent (for sulfhydryl groups) and 4-(*p*-nitrobenzyl)-pyridine (4-NBP)[8] (for alkylation resulting from active chloride). After spraying with 0.4% alcoholic 4-NBP, the chromatogram was heated at 110–120° over a hotplate for 5 minutes or until a rose color appeared; then it was sprayed with 0.2 *N* NaOH to give the characteristic blue color. A densitometer was used to estimate the relative proportions of ninhydrin color in mixtures. The R_f values obtained for pertinent compounds are shown in Table I.

TABLE I

R_f VALUES FOR DIFFERENT SOLVENT MIXTURES[a]

	$R_f \times 100$		
Compound	70:30 1-PrOH–H_2O	70:30 C_6H_5OH–H_2O	80:20 C_6H_5OH–H_2O
L-Cysteine[b]	55	83	87
L-Cystine	16	16	10
I	25	45	30
II	69	77	80
III	80	81	77
IV	36	42	30

[a] Ratios of solvent mixtures are on volume basis. Whatman No. 1 paper was used. [b] In the presence of N-ethylmaleimide.

(b).—Chloride analysis of mother liquors and reaction mixtures were by gravimetric measurement of AgCl. The cysteine-silver complex becomes soluble at 60° and was separated from AgCl by filtering the hot acidified mixtures. Purification of AgCl was effected by twice dissolving with ammonium hydroxide, precipitating with dilute nitric acid and filtering while hot.

(c).—Infrared measurements were made on potassium bromide discs. L-Cysteine gave absorption bands in microns at 3.40(S); 3.95(W); 4.85(M); 6.25(S); 7.00(S); 7.15(S); 7.40(S); 7.70(S); 7.85(W); 8.35(M); 8.75(M); 9.05(W); 9.40(S); 10.00(W); 10.05(S); 11.55(S); 12.45(M); 12.40(M); 13.00(W); 13.30(M); 14.50(S).

(d).—Ultraviolet absorption measurements were made on aqueous solutions of compounds II and III, and at *p*H 2.0 with HCl for compounds I and IV.

Liquid Ammonia Reaction Procedure.—Sodium metal was added to dry liquid ammonia (250–300 ml.) contained in a reaction flask, protected from atmospheric moisture and equipped with a stirrer. Portions of cysteine were added until the blue color disappeared, indicating the formation of the disodium salt of cysteine. The process was repeated until the desired amount of cysteine (0.1 mole) was added.

The chloroethylene was then added, either dropwise from a buret or diluted with liquid ammonia and decanted slowly. Tetrachloroethylene (m.p. −22.4°) was dissolved in acetone equal to 10% of the volume of liquid ammonia to depress its freezing point and cooled on solid carbon dioxide prior to adding to the reaction flask.

After evaporating the ammonia, the residue was dissolved in 50–100 ml. of water and the residual ammonia removed by evacuation. The *p*H at this stage was 10–12 and

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(10) L. L. McKinney, F. B. Weakley, R. E. Campbell, A. C. Eldridge, J. C. Cowan, J. C. Picken, Jr., and N. R. Jacobson, *J. Am. Oil Chemists' Soc.*, **34**, 461 (1957).

(11) F. Binkley, *J. Biol. Chem.*, **186**, 287 (1950).

(12) R. Gmelin, G. Hasenmaier and G. Strauss, *Z. Naturforsch.*, Pt. b, **12**, 687 (1957). Dr. Gmelin kindly furnished the enzyme.

was reduced to 5.0 by the addition of acetic acid. Where good yields of thioethers were obtained, copious precipitation occurred and more water had to be added for stirring. After standing overnight in a refrigerator, the precipitate was dissolved in hot water, decolorized with carbon and cooled to yield crystals. The more water-soluble compounds, II and III, required an equal volume of ethanol to ensure complete precipitation. During manipulation of the reaction products some unreacted cysteine was often oxidized to cystine which was found on chromatographic analysis of the crude product. This cystine was solubilized by adding a small amount of potassium cyanide during recrystallization.

cis-**Dichloroethylene Reaction.**—*cis*-1,2-Di-(1,1-aminocarboxy-2-thioethyl)-ethylene (I) was prepared by treating disodium L-cysteinate (0.0580 mole) with *cis*-dichloroethylene, $n^{15}D$ 1.4520 (0.0264 mole). At the end of 48 hr. the reaction appeared to be complete as judged by the nitroprusside test. The crude product (5.58 g., 80% yield) was crystallized from hot water to give 4.4 g. (63% yield) of hexagonal platelets similar to those of cystine; dec. at 250 ± 10°; *soly.* H_2O: $0.15^{100°}$ and $0.03^{5°}$; $[\alpha]^{26}D$ −5.9 (*c* 0.678, *N* HCl); *neutral equiv.* in 10% formaldehyde, 133 (calcd. 133). *Infrared* absorption bands in microns for I: 6.10(S); 6.25(VS); 6.70(VS); 7.05(VS); 7.40(S); 7.65(M); 7.90(W); 8.30(S); 8.85(S); 9.10(W); 9.50(M); 10.35(S); 11.20(S); 11.80(VS); 12.20(S); 12.90(S); 14.30(W). *Ultraviolet* absorption *p*H 2 with HCl: λ_{max} 248 mμ, ε 8,800. *Paper chromatography:* R_f values are shown in Table I, positive ninhydrin, ultraviolet and iodoplatinate tests. *Anal.* Calcd. for $C_8H_{14}O_4N_2S_2$: C, 36.09; H, 5.25; N, 10.51; S, 24.04. Found: C, 36.41; H, 5.24; N, 10.38; S, 24.24. Treatment for 1 hr. with 2 *N* NaOH at 80° gave a strong fuchsin test for aldehyde and a negative nitroprusside test. Chloride analysis on the mother liquor showed presence of 0.048 mole of AgCl from 0.0264 mole of the dichloroethylene or 91% of the total.

When equimoles (0.0271 mole) of disodium L-cysteinate and *cis*-dichloroethylene were allowed to react for 3 hr., I was isolated in 40% yield. Paper chromatography gave ninhydrin positive spots for I, cysteine and a trace of cystine. The experiment was repeated in which the reaction mixture was held at −50° with solid carbon dioxide to yield only 10% of I. Chromatographic examination of this reaction mixture with 70:30 1-propanol–H_2O showed a weak ninhydrin spot at R_f 0.50 which was 4-NBP positive and ultraviolet absorbing. Comparison of these properties with those of II and III indicated that 1-S-(2-chlorovinyl)-cysteine (R–S–CH=CHCl) was present.

trans-**Dichloroethylene Reaction.**—*trans*-Dichloroethylene was distilled through a 12-inch packed column to give a fraction boiling at 48–49° with $n^{15}D$ 1.4485. This *trans*-dichloroethylene (0.0259 mole) was allowed to stand with disodium L-cysteinate (0.0569 mole) in liquid ammonia for 8 days. There was no precipitation upon neutralizing the aqueous solution of ammonia-free residue with acetic acid to *p*H 5.0. Examination by paper chromatography gave ninhydrin spots for cysteine, a trace of cystine and a small spot for II. Chloride analysis showed the presence of 0.00428 mole, or 4.1% of the total chlorine was liberated from the dichloroethylene. The slight reaction is attributed to contamination of *trans*-dichloroethylene by the more stable *cis* isomer.

Vinylidene Chloride Reaction.—*cis*-1,2-Di-(1,1-aminocarboxy-2-thioethyl)-ethylene (I) was also obtained by treating disodium L-cysteinate (0.0567 mole) with vinylidene chloride (0.0258 mole) in liquid ammonia for 48 hr. The crude precipitate (5.2 g., 76% yield) was dissolved in 9 l. of water at 90°, decolorized with carbon and set in a refrigerator for 2 days to yield 4.06 g. of crystals (60% overall yield). *Anal.* Calcd. for $C_8H_{14}O_4N_2S_2$: C, 36.05; H, 5.25; N, 10.50; S, 24.02. Found: C, 36.13; H, 5.21; N, 10.32; S, 24.25. The crystal form, solubility, paper chromatograms, infrared and ultraviolet absorptions were identical with those of the product obtained from *cis*-dichloroethylene reaction. Chloride analysis on the mother liquor showed the presence of 0.0381 mole of AgCl or 74% of the total chlorine from the vinylidene chloride.

In an attempt to obtain the equimolar reaction product, disodium cysteinate (0.03 mole) was treated with vinylidene chloride (0.06 mole) in liquid ammonia for 26 hr. After removal of ammonia, the aqueous alkaline solution of the residue was neutralized with acetic acid as described and no precipitation occurred. After standing in a refrigerator overnight a precipitate (1.0 g.) was removed which on crystallization from hot water yielded 0.85 g. (10% on the cysteine) of I. Paper chromatograms of the reaction mixture gave a spot for cysteine and none that could be attributed to the equimolecular reaction product. The chromatograms also indicated that the actual recovery of I from the reaction mixture was poor.

Trichloroethylene Reaction.—The properties of S-(*trans*-dichlorovinyl)-cysteine (II) have been reported.[3] A total of six preparations have been made in which equimoles of cysteine and trichloroethylene, and 10% excess cysteine were used. In two of these reactions ammonia was allowed to evaporate slowly over a 12-hour period. In the other four reactions ammonia was evaporated with the aid of a stream of air on the reaction flask within 3 hours after adding the trichloroethylene. Chloride analysis on each mother liquor showed the presence of 0.9 to 1.0 equivalent based on trichloroethylene. Chromatographic analyses did not show a ninhydrin spot indicative of more than one cysteine moiety reacting with trichloroethylene. The yield of crystalline product was 70–80% and chromatograms showed appreciable amounts remaining in the mother liquor and filtrates. *Optical activity,* $[\alpha]^{26}D$ 0 (*c* 4.554, *N* HCl). *Infrared* absorption bands in microns for II: 6.30(S); 6.60(S), 7.20(S); 7.45(M); 8.40(M); 8.80(S); 9.55(W); 10.20(M); 10.35(M); 10.90(S); 11.10(W); 11.45(W); 11.80(M); 12.30(S); 12.65(W); 13.00(M); 14.00(M); 14.80(W); 15.10(M). Treatment of II for 0.5 hr. with 2 *N* NaOH at 80° gave a strong test for chloride; a paper chromatogram developed with 70:30 1-propanol–H_2O showed 75% of the ninhydrin positive material at R_f 0.69 (compound II) and 25% at R_f 0.23 which gave a positive fuchsin test.

Attempts to isolate a product analogous to I or IV by treatment of 2 molecular portions of disodium cysteinate with 1 of trichloroethylene in liquid ammonia were unsuccessful although chromatographic evidence was obtained that such a product was formed. Disodium cysteinate (0.0515 mole, 5.9 g.) in liquid ammonia was allowed to stand for 24 hr. with trichloroethylene (0.0223 mole, 2.0 ml.). No precipitate was obtained on neutralizing the aqueous reaction mixture with acetic acid. The solution was evaporated under vacuum to 25 ml. and left in a refrigerator overnight to yield 1.32 g. of microscopic crystals. Ninhydrin spots on a paper chromatogram developed with 70:30 1-propanol–H_2O were measured with a densitometer: 60% at R_f 0.69; 30% at R_f 0.36; and 10% at R_f 0.06; all three spots were ultraviolet absorbing and gave positive iodoplatinate tests. Only the R_f 0.69 spot gave a positive 4-NBP test. Chromatographic analysis of the mother liquor gave the same distribution of these spots and additional spots for cysteine and cystine. Fractionation by alcoholic precipitation of barium salts yielded fractions containing about 80% of the R_f 0.35 and 0.06 spots, but chromatographically pure fractions were not attained.

Treatment of equimolecular portions of sodium S-(dichlorovinyl)-L-cysteinate and disodium cysteinate in liquid ammonia for 7 days gave substantially the same results.

Tetrachloroethylene Reaction.—S-(1,2,2-Trichlorovinyl)-L-cysteine (III) was prepared by treating disodium cysteinate (0.0294 mole, 3.562 g.) with excess tetrachloroethylene (0.049 mole, 5 ml.) in liquid ammonia for 67 hours. The crude precipitate (5.2 g.) was chromatographed and found to consist of about 33% of III, 41% of IV along with 8% of cystine, 10% of cysteine and 8% of an unknown with low R_f. The crude precipitate (5.0 g.) was dissolved in 50 ml. of water adjusted to *p*H 11 with sodium hydroxide and barium chloride was added. Upon addition of an equal volume of ethanol a precipitate formed. The filtrate was adjusted to *p*H 4.8 with acetic acid from which crystals (1.1 g., 15% yield on cysteine) were obtained. These crystals were chromatographically pure for compound III; m.p. 158–159° dec.; $[\alpha]^{26}D$ +3.5 (*c* 2.718, *N* HCl); *soly.* 50% EtOH: $5.0^{70°}$; $0.1^{4°}$. *Paper Chromatography:* positive ninhydrin, ultraviolet and iodoplatinate tests; a pink spot appeared on spraying with 4-NBP which did not turn blue on treatment with alkali. Treatment with 2 *N* NaOH at 80° for 1 hr. gave strong fuchsin and chloride tests and negative nitroprusside test. *Anal.* Calcd. for $C_5H_8O_2NSCl_3$: C, 24.15; H, 2.39; N, 5.59; S, 12.79; Cl, 42.4. Found: C, 24.31; H, 2.59; N, 5.60; S, 13.0; Cl, 42.3. *Infrared* absorption bands in microns: 6.25(S);

6.60(S); 7.00(W); 7.15(S); 7.40(M); 7.70(M); 7.80(M); 8.10(W); 8.35(W); 8.75(M); 9.50(W); 10.20(M); 10.30-(M); 10.75(W); 11.30(S); 11.75(S); 12.15(W); 12.75(W); 13.20(S); 14.05(W); 15.05(M). *Ultraviolet* absorption in water: λ_{max} 264 mμ, ε 4,700.

1,2-Di-(1,1-aminocarboxy-2-thioethyl)-1,2-dichloroethylene (IV) was prepared by treating excess disodium L-cysteinate (0.431 mole, 5.22 g.) with tetrachloroethylene (0.0196 mole, 2.0 ml.) in liquid ammonia for 5 days. The crude precipitate (6.17 g.) was amorphous and chromatographic analysis showed that it consisted of 64% of IV, 3% of III, 23% of cystine and 9% of cysteine. The precipitate (4.0 g.) was dissolved in 2.5 l. of water at 80° to which 0.1 g. of potassium cyanide was added and allowed to cool slowly in a Dewar container to yield 1.4 g. (33% on the tetrachloroethylene) of needle-like crystals. These crystals were chromatographically pure; m.p. 189–192° dec.; $[\alpha]^{26}D$ +31.7 (*c* 0.76, *N* HCl); *soly.* H_2O: 0.25[80°]; 0.03[4°]. *Paper chromatography:* positive ninhydrin, ultraviolet and iodoplatinate tests; negative 4-NBP test. Treatment with 2 *N* NaOH at 80° for 1 hr. gave strong fuchsin and chloride tests and negative nitroprusside test. *Anal.* Calcd. for $C_8H_{12}O_2N_2S_2Cl_2$: C, 28.69; H, 3.58; N, 8.35; S, 19.13; Cl, 21.15. Found: C, 29.03; H, 3.77; N, 8.28; S, 19.18; Cl, 21.05. *Infrared* absorption bands in microns: 6.15(S); 6.25(S); 6.65(S); 6.95(S); 7.10(S); 7.40(W); 7.60(W); 7.85(W); 8.35(M); 8.70(S); 9.40(M); 10.10(M); 10.20-(W); 11.20(W); 11.40(S); 11.75(S); 12.40(M); 12.60(W); 13.05(W); 13.50(S); 14.20(M). *Ultraviolet* absorption *p*H 2 with HCl; λ_{max} 281 mμ, ε 8,800. Chloride analysis on the mother liquor showed the presence of 0.02365 mole or 1.21 equivalents based on tetrachloroethylene.

Toxicity. (a).—Fungicidal activity was determined by the disc assay method using *Saccharomyces pastorianus*, Hansen (NRRL Y-139) and *Mucor ramannianus* Moel., (NRRL 1839) as described by Pridham, *et al.*[13] Toxicity of the cysteine thioethers as determined by growth inhibition of two fungi is shown in Table II.

TABLE II

INHIBITION OF FUNGI GROWTH BY CYSTEINE THIOETHERS[a]

μg. on 12.5 mm. paper disc[c]	Diameter of inhibition zone, mm. *S. pastorianus* II[b]	*S. pastorianus* III	*M. ramannianus* II	*M. ramannianus* III
1.0	0	0	0	0
2.5	18	0	15	0
5.0	19	0	18	0
10	21	0	22	0
20	22	13	24	0
30	24	15	26	..
50	..	18	..	18

[a] Compounds I and IV failed to cause growth inhibition in amounts up to 50 μg. [b] II also inhibited the growth of *Aspergillus niger* and *Sarcina lutea* but failed to inhibit *Bacillus subtilis* (NRRL B-765), *Trichoderma viridae* (NRRL 1700), *Ceratostomella ulmi* (NRRL 2356), and *Corynebacterium flaccumfaciens* (NRRL B-729) at the 30 μg. level. [c] Applied in 0.10 ml. of neutral aqueous solution.

(b).—Algaecidal activity was determined by serial dilution of the thioethers in water. The *p*H of each solution was adjusted to 6.5 by addition of dilute sodium hydroxide. To each flask containing 100 ml. of solution, or aqueous control, was added 4 mg. of a commercial tropical fish food as nutrient. The flasks were then inoculated with 5 ml. of water which was green with algae from spontaneous growth, and they were allowed to stand for 10 days at room temperature (25 ± 3°) in sunlight. Optical densities were read at 540 mμ against water containing nutrient and inoculant. The results are shown in Table III.

(13) T. G. Pridham, L. A. Lindenfelser, O. L. Shotwell, F. H. Stodola, R. G. Benedict, C. Foley, R. W. Jackson, W. J. Zaumeyer, W. H. Preston, Jr., and J. W. Mitchell, *Phytopathology*, **46**, 568 (1956).

TABLE III

INHIBITION OF ALGAE GROWTH BY CYSTEINE THIOETHERS[a]

Concn., p.p.m.	Optical densities at 540 mμ II	III
0	0.463	0.463
10	.141	...
25	.123	...
50	.016	...
100	.016	0.208
200	.001	.016
300	...	.001
400	...	.004
500	...	.004

[a] Concentrations of 500 p.p.m. of compounds I and IV gave optical densities identical with the control containing only water and nutrients.

(c).—Toxicity to soybeans was determined by soaking the beans overnight in neutral solutions of the thioethers whereupon the beans gained 1.5 times their weight; the beans were then drained and germinated at room temperature (25 ± 3°) between layers of moist cotton for a total of 48 hr., including soaking time. By then the control beans, soaked in water, exhibited sprouts about 1 inch long; beans soaked in more than 100 p.p.m. of II or more than 300 p.p.m. of III failed to sprout in this length of time. At the end of 96 hr., the control beans were green with healthy sprouts about 2 inches long while the poisoned beans were chlorotic and exhibited curled sprouts 1/4 to 1/2 inch long. Beans soaked in solutions containing 500 p.p.m. of I or IV did not differ from the controls.

(d).—The toxicity of thioethers containing chlorine, *i.e.*, compounds II, III and IV, was tested on guppies (*Lebistes reticulatus*, Peters). Two fish were placed in 100 ml. of neutralized thioether solution contained in a 250-ml. beaker, and the volume periodically adjusted to compensate for evaporation. The fish were fed a commercial tropical fish food and in cases where solutions became cloudy or littered with debris and other growth, the fish were temporarily removed and the solutions filtered; *p*H adjustments were then made with either HCl or NaOH as required to maintain neutrality. The results are shown in Table IV where the days to death is the average for two or more fish. Fish that died in solutions containing 100 p.p.m. or less of II exhibited hemorrhagic spots.

TABLE IV

TOXICITY OF THIOETHERS[a] TO GUPPIES (*Lebistis reticulatus*, Peters)

Concn., p.p.m.	Days to death RSCCl=CHCl[b] (II)	RSCCl=CCl$_2$[b] (III)
25	43	..
50	38	..
100	24	..
200	8	..
300	3.5	17
400	3.0	10.5
500	..	7

[a] No deaths occurred in 5 weeks in 400 and 500 p.p.m. of 1,2-di-(1,1-aminocarboxy-2-thioethyl)-1,2-dichloroethylene (IV). [b] R = $-CH_2-CH(NH_2)-COOH$.

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PEORIA, ILLINOIS