hydrofuran (20 ml.) was stirred and cooled while ethyl vinyl ether (3.67 g., 0.051 mole) was added over 10 minutes. The temperature rose spontaneously to 35°, where it was checked by slight external cooling. Fifteen minutes after addition was complete the solvent was removed under reduced pressure and the product was distilled through a 25-cm. Vigreux column; b.p. 74–75° (3.5 mm.), $n^{25}D$ 1.4978. Much tar formation occurred in the still-pot during distillation. The yield of faintly pink **2-chloro-2-ethoxyethanesulfinyl chloride** was 4.57 g. (48%). The product was stored at Dry Ice temperature. The infrared spectrum shows bands at 3.40, 3.45, 3.50, 6.82, 6.98, 7.26, 7.33, 7.50, 7.91, 8.26, 8.60–9.10, 9.65, 10.08, 10.90, 11.25, 12.15 and 13.44 $\mu$. The broad unresolved band at 8.60–9.10 $\mu$ probably consists both of (C—O—C) and (S=O). The (S=O) band was found at 8.55–8.75 $\mu$ in two aliphatic sulfinyl chlorides in this Laboratory.

*Anal.* Calcd. for $C_4H_8Cl_2O_2S$: C, 25.14; H, 4.22; Cl, 37.11. Found: C, 25.81; H, 4.40; Cl, 37.21.

**Reaction of Sulfinyl Chloride 8 with Salt 1.**—A solution of sulfinyl chloride **8** (0.52 g., 0.0027 mole) in DME (5 ml.) was added over 10 minutes to a suspension of salt **1** (1.00 g., 0.0054 mole) in DME (12 ml.). After 30 minutes the mixture was filtered, and the filtrate was evaporated to dryness *in vacuo*. The resulting sirup was stirred with methylene chloride (100 ml.). The hygroscopic orange solid which deposited was collected by filtration under nitrogen. The solid weighed 0.91 g. (104% crude yield) and the infrared spectrum showed it to consist of a mixture of *cis-cis*-**5** and *trans-trans*-**5**.[1a] The methylene chloride extract was chromatographed on a 1 × 10-cm. column of acid-washed alumina. There was thus obtained 0.08 g. (7%) of 2,3-dicyano-5-ethoxy-5,6-dihydro-1,4-dithiin, which was identified by the infrared spectrum.

**Reaction of Disodium *trans-trans*-5 with Sulfinyl Chloride 8.**—A solution of sulfinyl chloride **8** (0.58 g., 0.0030 mole) in DME (4 ml.) was added over 10 minutes to a solution of disodium *trans-trans*-**5** (1.00 g., 0.0032 mole) in DME (12 ml.). The temperature was maintained at 0° by an ice-bath. After 10 minutes the mixture was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was extracted twice with boiling ether and once with cold methylene chloride. The insoluble solid remaining weighed 0.41 g. (62%), and the infrared spectrum showed it to be slightly impure dithiin **2**. The combined ether and methylene chloride extracts were concentrated and chromatographed on a 1 × 10-cm. column of acid-washed alumina. There was thus obtained 0.02 g. (15%) of 2,3-dicyano-5-ethoxy-5,6-dihydro-1,4-dithiin which was identified spectrally.

**Reaction of Disodium *trans-trans*-5 with Sulfinyl Chloride 8 in the Presence of Methyl Vinyl Ether.**—A solution of sulfinyl chloride **8** (0.59 g., 0.0031 mole) in DME (5 ml.) was added at 0° to a solution of disodium *trans-trans*-**5** (1.00 g., 0.0031 mole) and methyl vinyl ether (1.50 g., 0.026 mole) in DME (10 ml.). After 5 minutes the mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was chromatographed on a 1 × 20-cm. column of acid-washed alumina. Elution with 50:50 methylene chloride–ether gave 0.75 g. (62%) of crude product whose infrared spectrum showed it to be methoxy adduct **9** contaminated with a small amount of ethoxy adduct **6**. The product was submitted to a second chromatographic purification from which 0.53 g. (44%) of essentially pure methoxy adduct **9** was isolated.

**Pyrolysis of Trithiole Oxide 4.**—A Pyrex tube (0.5″ i.d.) was packed to a depth of 1.5″ with small quartz cylinders and brought to a temperature of 250°. The internal pressure was adjusted to about 10 mm. while a slow stream of nitrogen was passed through the apparatus. Over 15 minutes a freshly prepared sample of pure trithiole oxide **4** (0.20 g., 0.00106 mole) was fed into the top of the tube. A liquid nitrogen trap was attached directly to the outlet to quench volatile products. Under these conditions little material reached the trap, and most of the product condensed at the base of the pyrolysis tube just below the furnace. The solid was collected (0.1 g.) and the infrared spectrum showed it to be essentially pure dithiin **2** contaminated with traces of **4**.

A similar experiment was run in which ethyl vinyl ether (~ 2 g.) was condensed on the walls of the liquid nitrogen trap before the pyrolysis began. The pyrolysis was carried out at 200°, and the addition of **4** (0.15 g., 0.00080 mole) required 5 minutes. The trap was allowed to warm to 25°. The "infrared" spectrum of the small amount of solid formed in the trap showed it to be essentially pure dithiin **2**. The ultraviolet spectrum of the product was indicative of dithiin **2**, but a sizable shoulder at 342 m$\mu$ was observed which is characteristic of the ethoxy adduct **6**. The product was not further investigated.

**Reaction of Salt 1 with Bis-(trifluoromethyl)-1,2-dithiete (10).**—A suspension of salt **1** (1.98 g., 0.0106 mole) in DME (20 ml.) was stirred and cooled at 0° in an ice-bath. Bis-(trifluoromethyl)-1,2-dithiete (**10**)[6] (2.41 g., 0.0106 mole) was added dropwise over 5 minutes. A deep red color developed as soon as the addition was begun. The mixture was allowed to stir for 3 hours at 0° and then 3 hours at 25°. The solvent was evaporated in a stream of dry nitrogen and the residue was extracted with hot benzene. From the benzene extract was isolated 0.6 g. of tricyano-1,4-dithiino[c]-isothiazole (**14**),[1a] m.p. 173.4–174.5°. The infrared spectrum of the product was identical with that of an authentic sample of **14**.[1a]

**Reaction of Trithiole Oxide 4 with Bis-(Trifluoromethyl)-1,2-dithiete (10).**—Over 3 minutes trithiole oxide **4** (0.100 g., 0.00053 mole) was added in small portions to a freshly prepared solution of diethiete **10**[5] (0.120 g., 0.00053 mole) in DME (3 ml.). After 30 minutes a small precipitate of sulfur had deposited. The solution was evaporated *in vacuo* after 2 hours to a light yellow residue which was extracted with two 25-ml. portions of boiling petroleum ether. The insoluble portion weighed 0.05 g. (87%), m.p. 194–199° dec., and the infrared spectrum showed it to be almost pure dithiin **2**. From the petroleum ether extract was obtained 0.065 g. of tan needles, m.p. 50°, whose infrared spectrum indicated it was an isomer of 2,3,4a,6,7,8a-hexakis-(trifluoromethyl)-4a,8a-dihydro-1,4-dithiino[2,3-b]-1,4-dithiin, a known product of self condensation of **10**.[5]

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[CONTRIBUTION FROM THE VENABLE CHEMICAL LABORATORY, THE UNIVERSITY OF NORTH CAROLINA, CHAPEL HILL, N. C.]

# Chemistry of Aliphatic Disulfides. IV. Studies on the Synthesis of Open-chain Unsymmetrical Cystine Derivatives[1,2]

BY RICHARD G. HISKEY AND WILLIAM P. TUCKER[3,4]

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The sulfenyl thiocyanate method for the preparation of unsymmetrical disulfides has been applied to several unsymmetrical cystine derivatives. Unsymmetrical disulfides can also be obtained directly from the appropriate S-(2-tetrahydropropyranyl) thioether by treatment with thiocyanogen followed by a second mercaptan.

The importance of combined cystine in the stabilization of secondary and tertiary protein structure is generally recognized although little understood.[5] For example, fission of the disulfide

(1) Supported in part by research grant A-3416 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, United States Public Health Service.

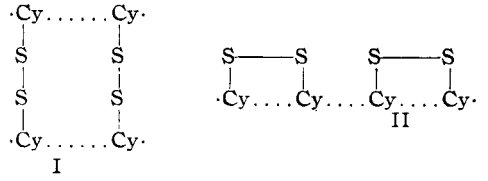
(2) Part III of this series, R. G. Hiskey and F. I. Carroll, *J. Am. Chem. Soc.*, **83**, 4647 (1961).

(3) R. J. Reynolds Tobacco Co. Fellow, 1959.

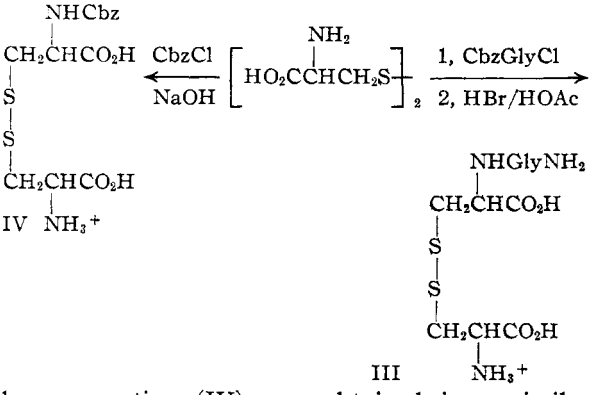
(4) Abstracted in part from a dissertation submitted by W. P. Tucker to the University of North Carolina in partial fulfillment of the requirements for the Ph.D. degree, August, 1962.

(5) P. D. Boyer in "The Enzymes," P. D. Boyer, H. Lardy and K.

bond in homones or enzymes containing cystine frequently results in loss of biological activity presumably due to destruction of the unique conformation necessary for biological action. Although the primary structure of several proteins is known, the size of these molecules, *e.g.*, ribonuclease or insulin, makes a systematic study of their chemical reactions difficult. Thus, if molecules of type I or II or combinations thereof could be prepared, a number of interesting "model" compounds would be available whose properties could be studied and compared with the larger natural polypeptides.

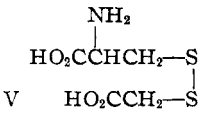


The presence of two amino and two carboxyl groups in cystine permits the existence of several types of peptides. Zervas, *et al.*,[6] have pointed out that although synthetic methods are available for the preparation of symmetrical open-chain[7] and some[8] unsymmetrical cyclic cystine peptides, a general method for the synthesis of all types of cystine derivatives and in particular of unsymmetrical open-chain peptides, *e.g.*, I, is not available. The first unsymmetrical open-chain cystine peptide to be prepared, monoglycyl-L-cystine (III), was obtained only recently[6] by treatment of a large excess of cystine with carbobenzoxyglycyl chloride followed by decarbobenzoxylation. Monocarbobenzoxycystine (IV) was obtained in a similar manner. Although the preparation of III, IV and the other derivatives reported is of great value, the method employed is admittedly limited to the simpler cystine derivatives.

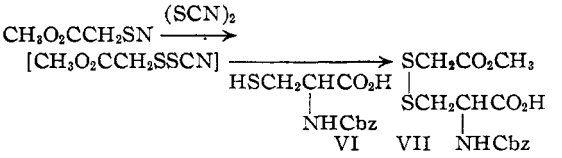


A major obstacle to the preparation of unsymmetrical cystine derivatives is disulfide interchange. Earlier reports[9,10] established that the process occurs in strongly acidic solution and is catalyzed by sulfenium ions. The base-catalyzed interchange which probably proceeds by a different mechanism was studied by Zervas, *et al.* The rate of decomposition of IV and the extent of interchange was found to increase with an increase in *p*H. At *p*H 7.5 considerable interchange occurred while at *p*H 6.5 no interchange was detected. Thus, if molecules such as I and II are to be constructed, several factors must be considered: (a) The formation of the disulfide bond in the last stages of the synthesis is essential. (b) The method used to form the disulfide bonds should involve conditions which do not promote disulfide interchange. (c) The reactivity of the thiol group demands suitable protective groups which can be removed without affecting other reactive functional groups, including disulfide bonds, in the molecule. (d) Several protective groups which could be selectively removed from various sulfur atoms should be available.

Of the various procedures available for the formation of unsymmetrical disulfides, the sulfenyl thiocyanate method[11] appeared most promising. Although L-1-amino-3,4-dithiapentane-1,5-dicarboxylic acid (V) had previously been prepared[12] by the action of thiocyanogen on L-cysteine, the conditions employed were not suited for large scale preparations. Further, the low solubility of cysteine in organic solvents together with the



possibility of interaction between the free amino group and thiocyanogen dictated that N-protected cysteine derivatives be employed. Since Zervas, *et al.*,[6] had demonstrated that the carbobenzoxy group could be removed from monocarbobenzoxyglycylcystine to afford III, N-carbobenzoxycysteine (VI) was initially studied. To determine the reactivity of VI toward a sulfenyl thiocyanate the preparation of 1-carboxy-1-(carbobenzoxyamino)-5-carbomethoxy-3,4-dithiapentane (VII) was attempted. The reaction proceeded smoothly to afford a 56% yield of VII. In order to determine whether a sulfenyl thiocyanate could be generated



from VI the synthesis of IV was considered. Treatment of VI with thiocyanogen followed by addition of cysteine hydrochloride to the intermediate sulfenyl thiocyanate provided a 57% yield of IV. The specific rotation of IV, $[\alpha]^{27}D$ $-111°$, was in reasonable agreement with the values of $[\alpha]^{23}D$ $-117°$ and $-120°$ previously reported.[6,13]

Myrbäck, Academic Press, Inc., New York, N. Y., Vol. I, part II, 1959.

(6) L. Zervas, L. Benoiton, E. Weiss, M. Winitz and J. P. Greenstein, *J. Am. Chem. Soc.*, **81**, 1729 (1959).

(7) The nomenclature used in ref. 6 has been adopted in this work.

(8) Although oxytcin, vasopressin and a number of derivatives (see ref. 6 for leading references) were prepared by air oxidation of unblocked cysteine residues, the work of D. Jarvis, H. N. Rydon and J. A. Schofield, *J. Chem. Soc.*, 1752 (1961), and earlier papers indicate complex mixtures may result when this method is employed.

(9) A. P. Ryle and F. Sanger, *Biochem. J.*, **60**, 535 (1955).

(10) R. E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **80**, 1666 1958).

(11) R. G. Hiskey, F. I. Carroll, R. M. Babb, J. O. Bledsoe, R. T. Puckett and B. W. Roberts, *J. Org. Chem.*, **26**, 1152 (1961).

(12) H. Lamfrom and S. O. Nielsen, *Compt. rend. Lab. Carlsberg, Ser. Chim.*, **30**, 360 (1958).

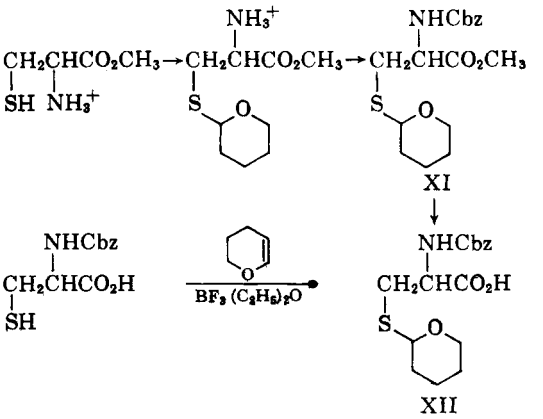
(13) R. Marshall, M. Winitz, S. M. Birnbaum and J. P. Greenstein, *J. Am. Chem. Soc.*, **79**, 4538 (1957).

Thus it appeared that open-chain unsymmetrical cystine derivatives could be obtained *via* sulfenyl thiocyanates provided the amino group of the initial cysteine derivative was protected.

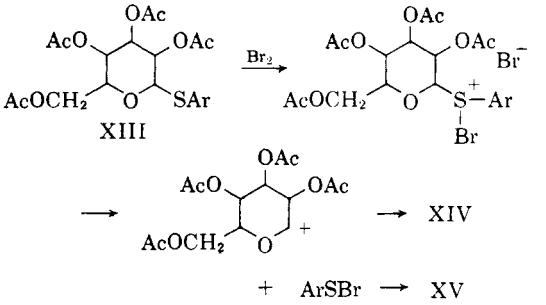
Since a limited number of cysteine derivatives are accessible, various thiol protective groups were considered.[14] The blocking groups investigated included the S-benzylthiomethyl group,[15] the S-*p*-nitrobenzyl group,[16] the S-tetrahydropyranyl group[17] and the S-triphenylmethyl group.[18,19] The S-benzylthiomethyl group affords crystalline derivatives of cysteine which are easily manipulated. The group is reported to be removed from N-carbobenzoxy-S-benzylthiomethylcysteine (VIII) with aqueous mercuric chloride and from S-benzylthiomethylcysteine with warm N hydrochloric acid. However, when either N-carbobenzoxyglycyl-S-benzylthiomethyl-L-cysteine (IX) or N-carbobenzoxy-S-benzylthiomethylcysteinylglycine (X) was treated with excess mercuric chloride in a mixture of either aqueous ethanol or acetone and aqueous hydrochloric acid, no thiol could be detected. Peptides IX and X were also inert to silver nitrate.

Although the classical[20] S-benzyl group could not be employed, since sodium in liquid ammonia also removes the N-carbobenzoxy group, the S-*p*-nitrobenzyl group was studied. This blocking group has been successfully removed from cysteine by catalytic hydrogenation.[16] Preliminary experiments were, however, disappointing. When N-carbobenzoxyglycyl-S-*p*-nitrobenzylcysteine was reduced, no pure thiol could be isolated although the requireda mount of hydrogen was slowly consumed.[21]

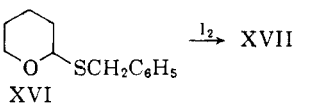
The S-tetrahydropyranyl group was effectively employed by Holland and Cohen[17] who found the hemithioacetal could be cleaved from cysteine derivatives with either dilute acid or silver nitrate. A key intermediate employed by these workers, N - carbobenzoxy - S - (2 - tetrahydropyranyl)-L-cysteine (XII), was prepared *via* the protected ester XI. A modified procedure which has proved considerably more convenient and is applicable to a number of S - (2 - tetrahydropyranyl) thioethers involves the treatment of VI with dihydropyran in the presence of boron fluoride. The utility of the S-tetrahydropyranyl group is limited by two features. The derivatives are usually obtained as sirups and if the group is removed with a heavy metal, the isolation of the desired thiol is difficult.[22]




Therefore, an alternate method for the removal of the S-tetrahydropyranyl group was desired. A possible approach was suggested by the earlier work of Bonner[23,24] who found that bromination of alkyl and aryl tetra-O-acetyl-β-D-thioglucopyranosides (XIII) produced tetra-O-acetyl-α-D-glucopyranosyl bromide (XIV) and the dialkyl or diaryl disulfide (XV).[25] The conversion presumably involved the formation of an intermediate sulfonium ion which could decompose to the sulfenyl bromide by ejection of a stabilized carbonium ion. Addition of bromide ion to the carbonium ion and decomposition of the arylsulfenyl bromide




would then afford the observed products. In a similar experiment benzyl 2-tetrahydropyranyl sulfide (XVI) was treated with one equivalent of iodine in N,N-dimethylformamide at room temperature. Dibenzyl disulfide (XVII) was produced in 68% yield.




From the similarity between the halogens and thiocyanogen it was anticipated that treatment of XVI with thiocyanogen would afford the corresponding sulfenyl thiocyanate XVII (R = $C_6H_5CH_2$). Addition of a second thiol should yield the unsymmetrical disulfide XVIII and presumably 2-thiocyanotetrahydropyran. Treatment of XVI with thiocyanogen followed by addition of mercaptosuccinic acid produced 3-carboxy-6-phenyl-4,5-dithiahexanoic acid (XVIIIa, R =

(14) The thiol protective groups commonly employed have recently been summarized by J. P. Greenstein and M. Winitz in "Chemistry of Amino Acids," John Wiley and Sons, Inc., New York, N. Y., Vol. 2, 1961.

(15) P. J. E. Pimlott and G. T. Young, *Proc. Chem. Soc.*, 257 (1958).

(16) C. Berse, R. Boucher and L. Piche, *J. Org. Chem.*, **22**, 805 (1957).

(17) G. F. Holland and L. A. Cohen, *J. Am. Chem. Soc.*, **80**, 3765 (1958).

(18) L. Velluz, G. Amiard, J. Bartos, B. Goffinet and R. Heymes, *Bull. soc. chim. France*, 1464 (1956).

(19) A complete account of our work with the S-triphenylmethyl group is given in the following paper.

(20) V. du Vigneaud and G. L. Miller, *J. Biol. Chem.*, **116**, 469 (1949).

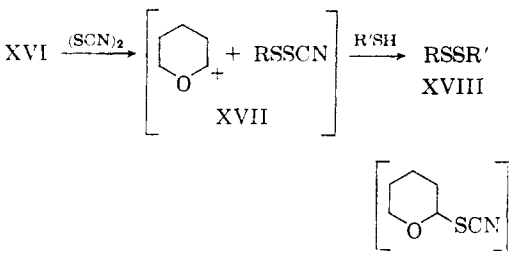
(21) Hydrogenation experiments were carried out by Mr. J. P. Dickerson.

(22) The disadvantages of preparing mercaptans *via* heavy metal mercaptides have been discussed by R. E. Benesch and R. Benesch, *Biochim. Biophys. Acta*, **28**, 658 (1957).

(23) W. A. Bonner, *J. Am. Chem. Soc.*, **70**, 770, 3491 (1948).

(24) W. A. Bonner and A. Robinson, *ibid.*, **72**, 356 (1950).

(25) Recently this reaction has been extended to phenyl 2-tetrahydropyranyl sulfide and phenyl 1-ethoxyethyl sulfide; W. A. Bonner, P. J. Werth and M. Roth, *J. Org. Chem.*, **27**, 1575 (1962).




$C_6H_5CH_2$, R′ = $CH(CO_2H)CH_2CO_2H$) in 58% yield. The same compound was produced in 25% yield from S-(2-tetrahydropyranyl)-succinic acid and benzyl mercaptan. Similar treatment of XVI with thiocyanogen and cysteine hydrochloride gave XVIIIb (R = $C_6H_5CH_2$, R′ = $CH_2CH(NH_3^+)CO_2H$) in 59% yield. No attempt was made in any experiment to isolate the product derived from the tetrahydropyranyl portion of the molecule.

Since the S-(2-tetrahydropyranyl) derivatives of cysteine are usually obtained as sirups because of the introduction of a second asymmetric site, the use of a hemithioacetal which would not contain a new asymmetric center was tested. Phenylthiomethyl methyl ether (XIXa, R = $C_6H_5$), prepared from sodium thiophenoxide and chloromethyl ether, was treated with thiocyanogen and β-thionaphthol. The desired unsymmetrical disulfide, phenyl β-naphthyl disulfide (XVIIIc, R = $C_6H_5$, R′ = $C_{10}H_7$) was obtained in 69% yield. In a similar manner benzylthiomethyl methyl

$$RSCH_2OCH_3 \xrightarrow[2,\ R'SH]{1,\ (SCN)_2} XVIII$$

XIX

ether (XIXb, R = $C_6H_5CH_2$) afforded 40% of XVIIIa when treated with thiocyanogen and mercaptosuccinic acid. The benzylthiomethyl derivatives of benzyl mercaptan and mercaptosuccinic acid were, however, unaffected by thiocyanogen.

These data indicate that the sulfenyl thiocyanate method can be applied to the synthesis of unsymmetrical cystine derivatives. Of considerably more interest, however, is the fact that unsymmetrical disulfides can be obtained directly from suitably blocked thiols thus eliminating the necessity of a second unblocking step. The application of this general method to cystine derivatives and the uses of other blocking groups is discussed in the accompanying paper.[26]

## Experimental[27]

**N-Carbobenzoxycysteine (VI)** was obtained in 75% yield from N,N-dicarbobenzoxycystine by the method of Foye and Verderame,[28] m.p. 77.5–79°, reported[28] m.p. 78.5–80°.

**1-Carboxy-1-(carbobenzoxyamino)-3-carbomethoxy-3,4-dithiapentane (VII).**—Following the procedure previously described[11] 2.12 g. (0.020 mole) of methyl thioglycolate in 50 ml. of ether was added dropwise with stirring to a cold solution of thiocyanogen prepared from 10.0 g. (0.03 mole) of lead thiocyanate and 4.0 g. (0.025 mole) of bromine in 100 ml. of ether. The addition required 1.25 hours and was followed by the addition of 5.12 g. (0.02 mole) of VI in 40 ml. of ether. During the addition, 20 minutes, the temperature was maintained at 0°. After stirring 0.5 hour at 0° and 0.5 hour at room temperature the ether solution was washed six times with water, dried, and evaporated to yield a semi-solid. Recrystallization from a chloroform–*n*-hexane mixture afforded 3.84 g. (56%) of white crystals melting at 75–77°.

*Anal.* Calcd. for $C_{14}H_{17}NO_6S_2$: C, 46.78; H, 4.77; S, 17.84. Found: C, 46.83; H, 4.92; S, 17.62.

**Monocarbobenzoxycystine (IV).**—To a solution of 0.02 mole of thiocyanogen in 150 ml. of cold ether was added 5.11 g. (0.02 mole) of VI in 125 ml. of ether. The solution was stirred during the addition which required 1.5 hours. A solution containing 3.52 g. (0.02 mole) of cysteine hydrochloride monohydrate in 80 ml. of freshly distilled N,N-dimethylformamide was then added in 0.5 hour. An additional 100 ml. of N,N-dimethylformamide was added and the resulting solution stirred 0.5 hour at 0 and 1 hour at room temperature. Removal of the solvents afforded a pink oil which was treated essentially as described by Zervas, *et al.*[6]

The oil was dissolved in the required amount of aqueous diethylamine (5 g. per 100 ml.) and treated immediately with excess acetic acid. The crystalline solid was filtered, washed, and dried *in vacuo*. The disulfide, 4.02 g. (57%), appeared as a white powder, $[\alpha]^{27}D$ − 111° (*c* 1 in 5 *N* HCl), reported[6] $[\alpha]^{25}D$ − 126° (*c* 1 in 5 *N* HCl). The preparation was identical in all respects (infrared spectrum and $R_f$ values on Whatman #1 paper in butanol:water:acetic acid solvent and on silica gel (T.L.C.) using a chloroform–methanol solvent) to the material obtained *via* N-carbobenzoxy-S-(2-tetrahydropyranyl)-cysteine,[26] N-carbobenzoxy-S-tritylcysteine[26] and cystine.[6,26]

**S-Benzylthiomethylcysteine** was obtained in 71% yield by the method of Pimlott and Young.[15] The compounded decomposed at 193–194°, reported[15] m.p. 193° dec.

**N-Carbobenzoxy-S-benzylthiomethylcysteine.**—A cold solution of 15.44 g. (0.06 mole) of S-benzylthiomethylcysteine in 60 ml. of 1 *N* sodium hydroxide was treated simultaneously with 13.2 g. (0.077 mole) of carbobenzoxy chloride and 60 ml. of 1 *N* sodium hydroxide. After 1 hour at room temperature the solution was diluted with water, washed twice with ether, and acidified to congo red. The precipitated oil was extracted with ether and the extracts were washed, dried and evaporated. The resulting oil crystallized after 3 days and was recrystallized from an ethyl acetate–hexane mixture to give 19.5 g. (80%) of white solid, m.p. 68–70°. The material was previously reported as a sirup.[15]

***p*-Nitrophenyl N-Carbobenzoxy-S-benzylthiomethylcysteinate.**—Treatment of 9.15 g. (0.023 mole) of N-carbobenzoxy-S-benzylthiomethylcysteine with 3.92 g. (0.028 mole) of *p*-nitrophenol and 4.83 g. (0.023 mole) of N,N-dicyclohexylcarbodiimide in 75 ml. of tetrahydrofuran[29] afforded 5.42 g. (46%) of the *p*-nitrophenyl ester, m.p. 105.5–106° from ethanol.

*Anal.* Calcd. for $C_{25}H_{24}N_2O_8S_2$: C, 58.58; H, 4.72; S, 12.51. Found: C, 58.66; H, 4.77; S, 12.51.

**Methyl S-Benzylthiomethylcysteinate.**—A 12.87 g. (0.05 mole) sample of S-benzylthiomethylcysteine was added in portions to a solution of 6.56 g. (0.06 mole) of thionyl chloride in 30 ml. of methanol at −5°. The mixture was allowed to warm to room temperature and held at 40° for 3 hours. The solvent was removed and the solid residue washed with ether and recrystallized from a methanol–ether mixture to yield 12.65 g. (88%) of the methyl ester hydrochloride, m.p. 152.5–154°, reported[15] m.p. 145–146°.

**Methyl N-Carbobenzoxyglycyl-S-benzylthiomethylcysteinate.**—An ethyl acetate solution of N-carbobenzoxyglycyl azide prepared in the usual manner from 6.7 g. (0.03 mole) of N-carbobenzoxyglycyl hydrazide[30] was added to a cold solution of methyl S-benzylthiomethylcysteinate, prepared from 9.24 g. (0.03 mole) of the hydrochloride in 150 ml.

(26) R. G. Hiskey and W. P. Tucker, *J. Am. Chem. Soc.*, **84**, 4794 (1962).

(27) The amino acids used in this work are of the L-configuration and were obtained from the Mann Research Laboratories. Optical rotations were performed with a Rudolf polarimeter, model 80, equipped with a model 200 photoelectric attachment. Elemental analysis by Micro-Tech Laboratories, Skokie, Ill. Melting points were taken in capillary tubes and are uncorrected.

(28) W. O. Foye and M. Verderame, *J. Am. Pharm. Assoc.*, **46**, 273 (1957).

(29) M. Bodansky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

(30) E. F. Erlanger and E. Brand, *ibid.*, **73**, 3508 (1951).

of ethyl acetate. The solution was kept at 0° for 48 hours, washed with 5% sodium bicarbonate, water and dried. Removal of the solvent afforded 11.44 g. (88%) of an oil whose infrared spectrum exhibited the expected absorption peaks for the protected dipeptide ester.

**N-Carbobenzoxyglycyl-S-benzylthiomethylcysteine (IX).**—A 2.00 g. (0.0043 mole) sample of the above oily dipeptide ester was dissolved in 50 ml. of methanol and allowed to stand at room temperature for 1.5 hours with 8.6 ml. of 1 *N* sodium hydroxide solution. The solution was treated with Norite, filtered, and diluted with 250 ml. of water. The mixture was acidified to congo red with 6 *N* hydrochloric acid and the precipitated solid was recrystallized from an acetone–ether mixture to yield 1.69 g. (89%) of protected dipeptide, m.p. 127–128°.

*Anal.* Calcd. for $C_{21}H_{24}N_2O_5S_2$: C, 56.23; H, 5.39; S, 14.28. Found: C, 56.34; H, 5.44; S, 13.88.

**Treatment of N-Carbobenzoxyglycyl-S-benzylthiomethylcysteine (IX) with Silver Nitrate.**—To 0.49 g. (0.001 mole) of the protected cysteine derivative in 20 ml. of acetone was added 25 ml. of 1 *N* silver nitrate solution. Precipitation began immediately. The mixture was allowed to stand overnight at room temperature, filtered and the solid washed with water. The solid was suspended in 30 ml. of water and treated with hydrogen sulfide for 40 minutes. The solution was acidified and the excess hydrogen sulfide removed with nitrogen. The mixture was extracted several times with warm ethyl acetate and the silver sulfide filtered and washed again with warm ethyl acetate. Evaporation of the filtrate and combined washings afforded only starting material.

A similar experiment in which mercuric chloride was substituted for silver nitrate was conducted at 75° for 0.5 hour and yielded only starting material. No evidence of thiol could be detected with sodium nitroprusside.

**Treatment of N-Carbobenzoxyglycyl-S-benzylthiomethylcysteine (IX) with Mercuric Chloride.**—To 0.49 g. (0.001 mole) of IX in 25 ml. of acetone and 25 ml. of aqueous 1 *N* hydrochloric acid was added 2.20 g. (0.008 mole) of mercuric chloride. The solution was heated at 75° for 30 minutes. Workup in the manner described afforded only starting material. No thiol could be detected. Similar treatment of X gave identical results.

**S-*p*-Nitrobenzylcysteine** was prepared in 41% yield by the procedure previously described[16]; m.p. 172.5–174° from water, reported[16] m.p. 262–263° for the monohydrate.

*Anal.* Calcd. for $C_{10}H_{12}N_2O_4S$: C, 47.87; H, 4.72; N, 10.94; S, 12.51. Found: C, 46.67; H, 4.76; N, 10.52; S, 11.98.

**Ethyl S-*p*-Nitrobenzylcysteinate Hydrochloride.**—Esterification of 10.4 g. (0.04 mole) of S-*p*-nitrobenzylcysteinate with 6 ml. of thionyl chloride in 50 ml. of ethanol afforded 12.4 g. (97%) of ester hydrochloride, m.p. 161–163°, reported[16] 172–173°.

**Ethyl Carbobenzoxyglycyl-S-*p*-nitrobenzylcysteinate.**—To 6.45 g. (0.02 mole) of the ethyl ester hydrochloride suspended in 35 ml. of chloroform was added 5.5 ml. (0.04 mole) of triethylamine and 6.6 g. (0.04 mole) of *p*-nitrophenyl carbobenzoxyglycinate in 50 ml. of chloroform. The solution was refluxed for 48 hours and evaporated. The residue was washed with 0.5 *N* ammonium hydroxide, water, 1.0 *N* hydrochloric acid and water. Recrystallization from dilute ethanol afforded 7.8 g. (82%) of dipeptide ester, m.p. 101–102°.

*Anal.* Calcd. for $C_{22}H_{25}N_3O_7S$: C, 55.57; H, 5.30; N, 8.84; S, 6.74. Found: C, 55.64; H, 5.54; N, 8.69; S, 7.11.

**Attempted Hydrogenolysis of Ethyl Carbobenzoxyglycyl-S-*p*-nitrobenzylcysteinate.**—Several hydrogenation experiments were performed using 10% palladium-on-charcoal catalyst in the following solvents: absolute ethanol, ethanol–acetic acid, dioxane–*N* hydrochloric acid and dioxane–concentrated hydrochloric acid. In each case hydrogen was absorbed, but no thiol could be isolated. In the latter solvent system a sodium nitroprusside test in the presence of sodium cyanide on the reduction product gave the characteristic color test.

**Methyl S-(2-tetrahydropyranyl)-cysteine hydrochloride (XI)** was prepared according to the procedure of Holland and Cohen.[17] The material was obtained as a sirup.

**N-Carbobenzoxy-S-(2-tetrahydropyranyl)-cysteine (XII).**—To a solution of 5.12 g. (0.02 mole) of N-carbobenzoxy-L-cysteine and 1.68 g. (0.02 mole) of freshly distilled dihydropyran in 60 ml. of cold ether was added 2.84 g. (0.02 mole) of boron fluoride–diethyl ether complex. The solution was allowed to stand at 0° for 0.5 hour and at room temperature for 1 hour, after which it was washed three times with water, dried and concentrated to a clear sirup. The infrared spectrum of this material was identical to the spectrum of the sample obtained by alkaline hydrolysis of XI.[17]

The sirup was dissolved in 250 ml. of ether and treated with 2.14 g. (0.02 mole) of benzylamine in 50 ml. of ether. The resulting benzylamine salt, 8.02 g. (90%), melted at 105–107°.

*Anal.* Calcd. for $C_{23}H_{30}N_2O_5S$: C, 62.00; H, 6.78; N, 6.28; S, 7.25. Found: C, 61.37; H, 6.60; N, 6.12; S, 7.70.

**Benzyl 2-tetrahydropyranyl sulfide (XVI)** was prepared by the procedure of Parham and Anderson.[31] The fraction boiling at 120–124° (1 mm.) (75%) was collected; $n^{25}D$ 1.5580, reported[32] b.p. 86–87° (2 mm.).

**S-(2-Tetrahydropyranyl)-mercaptosuccinic Acid.**—To a solution containing 7.51 g. (0.05 mole) of mercaptosuccinic acid and 3.36 g. (0.04 mole) of dihydropyran in 200 ml. of ether was added 5.0 ml. (0.04 mole) of boron fluoride–diethyl ether complex. The solution was allowed to stand at room temperature for 2 hours, washed 4 times with water, and dried. The solid residue obtained by evaporation of the solvent was crystallized from benzene containing a minimum amount of acetone. Removal of the acetone and slow cooling afforded 6.50 g. (70%) of solid, m.p. 127–130°. Two recrystallizations from benzene–acetone raised the melting point to 144–145°.

*Anal.* Calcd. for $C_9H_{14}O_5S$: C, 46.20; H, 6.04; S, 14.30. Found: C, 46.49; H, 6.06; S, 13.74.

**Phenylthiomethyl methyl ether (XIXa)** was prepared in 17% yield by the method of Marvel and Porter.[33] The fraction boiling at 64–68° at 4 mm. was collected, $n^{16}D$ 1.5637, reported[34] b.p. 128° at 18 mm.

**Benzylthiomethyl methyl ether (XIXb)** was prepared by the method of Fehnel and Carmack[35] in 93% yield, b.p. 72–74° at 0.8 mm., $n^{22}D$ 1.5496, reported[35] b.p. 128° at 18 mm.

**3-Carboxy-6-phenyl-4,5-dithiahexanoic Acid (XVIIIa).**

**A. From Benzyl 2-Tetrahydropyranyl Sulfide.**—To a cold stirred solution of 0.025 mole of thiocyanogen in 150 ml. of ether was added 5.20 g. (0.025 mole) of XVI in 100 ml. of ether. The addition required 1 hour. The yellow solution was treated with 3.75 g. (0.025 mole) of solid mercaptosuccinic acid, added in several portions over a 1.75-hr. period. The ether solution was washed free of thiocyanate ion, dried and evaporated to yield 4.13 g. of solid. Recrystallization from an ethyl acetate–benzene mixture (1:4) afforded 4.00 g. (58%) of XVIIIa, m.p. 147–148°, reported[11] m.p. 147–148°.

**B. From S-(2-Tetrahydropyranyl)-mercaptosuccinic Acid.**—A solution of 0.0125 mole of thiocyanogen in 150 ml. of ether was treated with 2.93 g. (0.0125 mole) of S-(2-tetrahydropyranyl)-mercaptosuccinic acid in 100 ml of an ether–dioxane mixture (4:1). The reaction was conducted in the usual manner and afforded 2.19 g. (64.5%) of very crude disulfide, m.p. ~ 128°. Recrystallization from an ethyl acetate–benzene solvent gave 0.86 g. (25%) of XVIIIa, m.p. 144–146°.

**2-Amino-6-phenyl-4,5-dithiahexanoic Acid Hydrochloride (XVIIIb) from Benzyl 2-Tetrahydropyranyl Sulfide.**—A cold solution of XVI (5.20 g., 0.025 mole) in 80 ml. of N,N-dimethylformamide was added to 0.025 mole of thiocyanogen in 150 ml. of cold ether. The yellow mixture was treated with 3.94 g. (0.025 mole) of cysteine hydrochloride in 50 ml. of N,N-dimethylformamide, stirred for 5 hours and poured into 500 ml. of cold ether. The resulting solid was filtered, washed with ether and dried. Recrystalli-

(31) W. E. Parham and E. L. Anderson, *J. Am. Chem. Soc.*, **70**, 4187 (1948).

(32) F. Kipnis and J. Ornfeld, *ibid.*, **73**, 822 (1951).

(33) C. S. Marvel and P. K. Porter, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 377.

(34) J. de Lattre, *Bull. soc. chim. Belg.*, **26**, 323 (1912).

(35) E. A. Fehnel and M. Carmack, *J. Am. Chem. Soc.*, **71** 84 (1949).

zation from an ethanol–ether mixture afforded 4.15 g. (59%) of white powder XVIIIb, m.p. 175–180° dec., reported[36] m.p. 175–180° dec. A mixture melting point with cysteine hydrochloride (m.p. 175–178°) was depressed.

**Phenyl β-Naphthyl Disulfide (XVIIIc) from Phenylthiomethyl Methyl Ether.**—To 0.05 mole of thiocyanogen in 250 ml. of cold ether was added 7.7 g. (0.05 mole) of XIXa. After 0.5 hour, 8.1 g. (0.05 mole) of β-thionaphthalene was added in portions. Workup in the usual manner and crystallization of the residue from ethanol afforded 8.50 g. (63.5%) of XVIIIc, m.p. 74–74.5°; reported[37,38] m.p. 76°, 67.5°. The mixture melting point with β-thionaphthalene was depressed.

**3-Carboxy-6-phenyl-4,5-dithiahexanoic Acid (XVIIIa) from Benzylthiomethyl Methyl Ether.**—A solution of 4.20 g. (0.025 mole) of XIXb in 100 ml. of acetone was added to 0.025 mole of thiocyanogen in 150 ml. of ether at 0. The yellow solution was then treated with 3.75 g. (0.025 mole) of mercaptosuccinic acid. Evaporation of the solvent afforded a brown solid which was dried over potassium hydroxide, washed with *n*-hexane and crystallized from water to yield 2.70 g. (40%) of XVIIIa, m.p. 148–149°. A mixture melting point with an authentic sample of disulfide was not depressed.

(36) H. Bretschneider and W. Klötzer, *Monatsh. Chem.*, **81**, 589 (1950).

(37) H. Lecher and M. Wittwer, *Ber.*, **55B**, 1474 (1922).

(38) H. Emde, German Patent 804,572 (1951); *C. A.*, **46**, 529 (1952).

[CONTRIBUTION FROM THE VENABLE CHEMICAL LABORATORY, THE UNIVERSITY OF NORTH CAROLINA, CHAPEL HILL, N. C.]

# Chemistry of Aliphatic Disulfides. V. Preparation of Some Open-chain Unsymmetrical Cystine Derivatives from Thioethers of Cysteine[1,2]

BY RICHARD G. HISKEY AND WILLIAM P. TUCKER[3]

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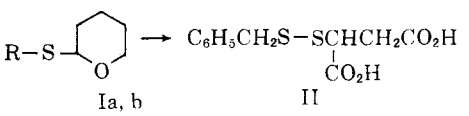
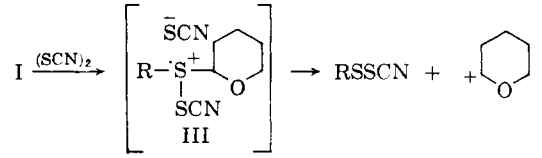
Several unsymmetrical disulfides, including two unsymmetrical open-chain cystine derivatives, have been prepared in reasonable yield from the corresponding S-triphenylmethyl or S-(2-tetrahydropyranyl)-thioethers using thiocyanogen-zinc chloride. The S-benzhydryl thioethers can likewise be removed with this agent.

In the preceding report[2] it was established that the sulfenyl thiocyanate method[4] can be applied to the synthesis of unsymmetrical open-chain cystine derivatives. Of additional interest was the observation that unsymmetrical disulfides could be obtained directly from S-(2-tetrahydropyranyl) thioethers and related hemithioacetals. For example, a 58% yield of 3-carboxy-6-phenyl-4,5-dithiahexanoic acid (II) resulted from treatment of benzyl 2-tetrahydropyranyl sulfide (Ia, R = $C_6H_5CH_2$) with thiocyanogen and mercaptosuccinic acid. A somewhat lower yield of II (25%) resulted from a similar reaction involving S-(2-



Ia, b II

tetrahydropyranyl)-succinic acid (Ib, R = $CH(CO_2H)CH_2CO_2H$), thiocyanogen and benzyl mercaptan. The facile cleavage of the hemithioacetals by electrophiles is presumably due to the fact that a stabilized carbonium ion can be ejected from the intermediate sulfonium salt III. To



III

test this view several other thioethers capable of reacting in a similar manner were considered.

From the viewpoint of peptide synthesis the S-triphenylmethyl group has several desirable features. S-Tritylcysteine derivatives are usually easily handled solids (in contrast to the S-tetrahydropyranyl analogs) and have been successfully employed for the synthesis of peptides.[5,6] In these preparations the trityl group was used to block the nitrogen and sulfur functions of cysteine; the former group could be selectively removed with warm acetic acid. Detritylation of both functions could be accomplished with dry hydrogen chloride in chloroform. The expectation that trityl sulfides would cleave with thiocyanogen was strengthened by the reported[7] iodine-catalyzed conversion of phenyltrityl sulfide to triphenylcarbinol and diphenyl disulfide.

Preparation of the desired S-trityl thioethers was readily accomplished in good yield by treatment of the appropriate mercaptan with triphenylcarbinol and boron fluoride. When benzyl triphenyl methyl sulfide (IVa, R = $C_6H_5CH_2$) was added to a thiocyanogen solution followed by mercaptosuccinic acid, II was obtained in 20–28% yield with a com-

IVa, b

parable amount of trityl thiocyanate. A similar reaction involving S-tritylmercaptosuccinic acid (IVb, R = $CH(CO_2H)CH_2CO_2H$), thiocyanogen and benzyl mercaptan produced II in 43% crude yield. Dibenzyl sulfide did not react with thiocyanogen.

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(2) Part IV of this series, R. G. Hiskey and W. P Tucker, *J. Am. Chem. Soc.*, **84**, 4789 (1962).

(3) Abstracted in part from a dissertation submitted by W. P. Tucker to the University of North Carolina in partial fulfillment of the requirements for the Ph.D. degree, August, 1962.

(4) R. G. Hiskey, F. I. Carroll, R. M. Babb, J. O. Bledsoe, R. T. Puckett and B. W. Roberts, *J. Org. Chem.*, **26**, 1152 (1961).

(5) G. Amiard, R. Heymes and L. Velluz, *Bull. soc. chim. France*, 698 (1956).

(6) L. Velluz, G. Amiard, J. Bartos, B. Goffinet and R. Heymes, *ibid.*, 1464 (1956).

(7) D. S. Tarbell and D. P. Harnish, *J. Am. Chem. Soc.*, **74**, 1862 (1952).