Chemistry of Aliphatic Disulfides. XII. Synthesis of Unsymmetrical Open-Chain Cystine Derivatives[1-3]

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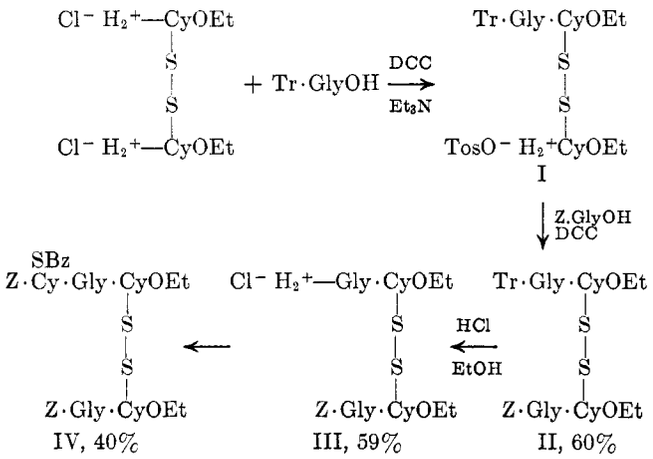
The sulfenylthiocyanate method of disulfide synthesis has been extended to several unsymmetrical cystine derivatives. The selective removal of the S-trityl group from ethyl N-carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycinate with a sulfenylthiocyanate has also been demonstrated.

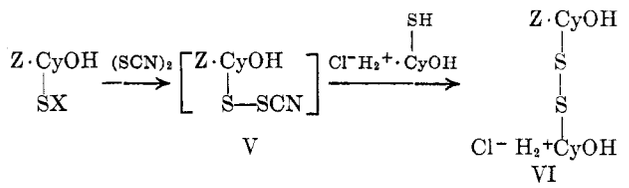
The development of methods suitable for the preparation of unsymmetrical open-chain cystine derivatives presents an unsolved synthetic problem. Although several reports on this subject have appeared,[6-8] virtually no compounds of this type are known. The earlier synthetic approaches have involved the formation of a suitably protected cystine derivative which could be coupled with another amino acid residue. This procedure requires that the disulfide bridge be introduced at an early stage in the projected synthesis. For example, Rydon and Serrao[8] condensed a large excess of L-cystine diethyl ester with N-tritylglycine. The crude, partially protected dipeptide derivative (I), obtained in unspecified yield, was condensed with N-carbobenzoxyglycine to provide II. Treatment of II with hydrogen chloride selectively removed the N-trityl group and afforded III which was coupled with N-carbobenzoxy-S-benzyl-L-cysteine to yield IV.



While this approach is useful for small molecules containing unsymmetrically substituted cystine, the construction of large molecules by this approach would be difficult at best. Furthermore, with peptides containing two or more cystine residues, disulfide interchange is likely to occur during the various deblocking or coupling steps. This possibility leads to some uncertainty as to the precise location of the disulfide bridges.

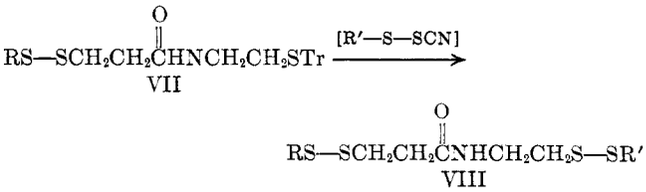
The alternate method for the preparation of unsymmetrical cystine derivatives involves formation of the disulfide bridge in the latter stages of the synthesis. This scheme requires the use of sulfur protective groups that will withstand the conditions used for the coupling of various residues and the removal of amino and carboxy protective groups; however, the sulfur protective group must be easily removed at a later stage when disulfide bond formation is required. If a molecule containing several cystine residues is desired, S-protective groups of differing lability are necessary. Furthermore, if stepwise formation of several disulfide bonds is anticipated, an oxidation method which would provide the direct conversion of a thio ether to a disulfide, without interaction with neighboring sulfur-sulfur bonds, would be desirable.

In an earlier report, the general applicability of the sulfenylthiocyanate method of disulfide synthesis to cystine derivatives was suggested.[9] In these preliminary experiments N-carbobenzoxy-L-cysteine and several derivatives [S-trityl, S-benzhydryl, and S-(2-tetrahydropyranyl)] were treated with thiocyanogen or a thiocyanogen–zinc chloride reagent. Addition



X = —H, $C(C_6H_5)_3$, $CH(C_6H_5)_2$

of cystine hydrochloride to the sulfenylthiocyanate (V), provided monocarbobenzoxy-L-cystine (VI). Subsequently, it was demonstrated[1] that the added zinc chloride was unnecessary when S-trityl derivatives were employed; a molecule such as VII could be converted to the bis unsymmetrical disulfide (VIII) using only thiocyanogen. The present report describes the



application of the sulfenylthiocyanate method to the synthesis of several other unsymmetrical cystine derivatives and considers the selective oxidation of various

(1) Part XI of this series: R. G. Hiskey and D. N. Harpp, *J. Am. Chem. Soc.*, **87**, 3965 (1965).

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(3) The following abbreviations have been adopted throughout the text: Z = $C_6H_5CH_2OCO$; Tr = $(C_6H_5)_3C$; Bzh = $(C_6H_5)_2CH$; Bz = $C_6H_5CH_2$; BhOC = $(C_6H_5)_2CHOCO$; DCC = N,N,-dicyclohexylcarbodiimide; DMF = N,N-dimethylformamide.

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(5) Abstracted in part from the dissertation of E. L. Smithwick, Jr., submitted to the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the Ph.D. degree, June 1966.

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

(7) H. Zahn and H. G. Otten, *Ann. Chem.*, **653**, 139 (1962).

(8) H. N. Rydon and F. O. dos S. P. Serrao, *J. Chem. Soc.*, 3638 (1964).

(9) R. G. Hiskey and W. P. Tucker, *J. Am. Chem. Soc.*, **84**, 4789, 4794 (1962).

sulfur atoms within the same molecule using thiocyanogen.

In order to determine the optimum conditions necessary for the preparation of unsymmetrical cystine derivatives, a model system was initially studied. N-Carbobenzoxy-S-(carbomethoxymethylthio)-L-cysteine (IX) was prepared by the sulfenylthiocyanate method and the effect of the reaction conditions on product yield were determined (Table I). The effect of several added catalysts on the yield of disulfide was also studied. Boron trifluoride has been found to increase the amount of symmetrical disulfide formed in the reaction while zinc chloride and thiocyanogen appear to enhance disulfide decomposition. An indication of the latter possibility was indicated by a control experiment in which thiocyanogen and zinc chloride were allowed to stand for 1 hr with diethyl N,N'-biscarbobenzoxy-L-cystinyldiglycinate; only 41% of

TABLE I

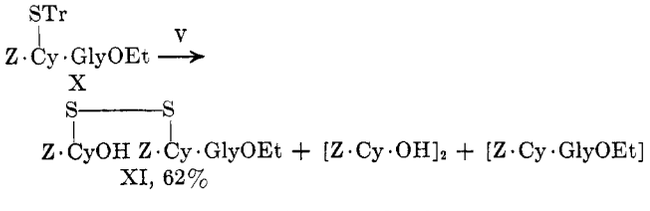
PREPARATION OF S-(2-CARBOMETHOXYMETHYLTHIO)-N-CARBOBENZOXY-L-CYSTINE

$$\text{RSH} \xrightarrow[\text{2. R'SH or R'STr}]{\text{1. (SCN)}_2} \text{RSSR'}$$

RSH	Addn time of RSH, min	R'SH or R'STr	Catalyst	Yield,[e] %
a	40	*b*	$Hg(OAc)_2$	64[f]
b	40	*a*	$Hg(OAc)_2$	51[f]
a	40	*b*	$Hg(OAc)_2$[d]	..
a	40	*b*	NaOAc	64[g]
b	90	*a*	NaOAc	78[c]
a	90	*c*	NaOAc	56[g]
a	90	*c*	...	60[g]

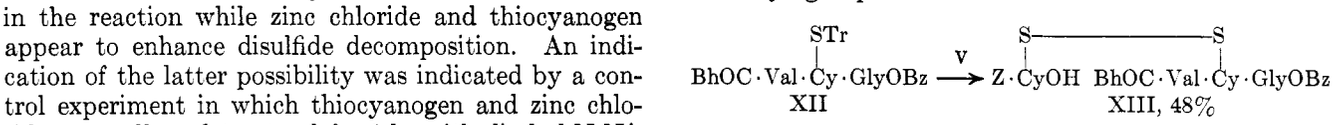
[a] Methyl thioglycolate. [b] N-Carbobenzoxyl-L-cysteine. [c] N-Carbobenzoxy-S-trityl-L-cysteine. [d] Mercury(II) acetate added before addition of the first mercaptan. [e] Lit.[9] mp 75–77°. [f] Product isolated by crystallization, mp 73–75°. [g] Product isolated by silicic acid chromatography, mp 77–79°.

the disulfide could be recovered. Addition of sodium acetate to the sulfenylthiocyanate was previously found[1] to give higher yields of VIII; however, the yield of IX seemed to depend to a greater extent on the time and order of addition of the initial thiol. Thus, the synthesis of ethyl N,N-biscarbobenzoxy-L-cystinylmonoglycinate (XI) was attempted using thiocyanogen alone. Addition of ethyl N-carbobenzoxy-S-trityl-L-cysteinylglycinate (X) to V provided a crude product which was a mixture of the three possible disulfides and trityl isothiocyanate. The isothiocyanate could be removed by extraction; however, attempts to further purify the mixture of disulfides by fractional crystallization failed. Pure XI was obtained in 62%



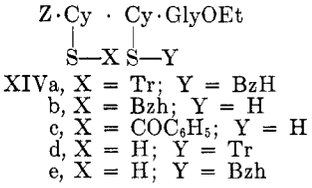
yield by elution from a silicic acid column; an alternate procedure on a larger scale which provided XI in 50% yield involved crystallization of XI from aqueous pyridine. Disulfide XI was coupled to several amino acid and dipeptide esters (see the Experimental Section). In these reactions 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide[10] was the coupling agent of choice. The use of N,N-dicyclohexylcarbodiimide invariably provided a product contaminated with what appeared to be the N-acylurea derivative.

The conditions developed for the synthesis of XI were applied to the preparation of XIII. Treatment of benzyl N-benzhydryloxycarbonyl-L-valyl-S-trityl-L-cysteinylglycinate (XII) with V, followed by silicic acid chromatography afforded XIII in 48% yield. These results indicate that the N-benzhydryloxycarbonyl group[11] is stable to the reaction conditions



required for sulfenylthiocyanate formation and subsequent cleavage of the S-trityl group.

Since the sulfenylthiocyanate method appeared applicable to the preparation of unsymmetrical cystine derivatives, the selectivity of the thio ether cleavage was evaluated. A convenient group of model compounds, the ethyl N-carbobenzoxy-L-cysteinyl-L-cysteinylglycinate derivatives (XIV), was available from previous investigations.[12] The purpose of these studies[3] was to determine the relative reactivity toward thiocyanogen (or a sulfenylthiocyanate) of the thiol, S-trityl, and S-benzhydryl groups. Treatment of three of the four thiols (XIVb, c, e) with 0.5 equiv



XIVa, X = Tr; Y = BzH
b, X = Bzh; Y = H
c, X = COC_6H_5; Y = H
d, X = H; Y = Tr
e, X = H; Y = Bzh

of thiocyanogen provided the corresponding symmetrical cystine derivatives (XV, XVI, XVII) in good yield.

XV, X = COC_6H_5
XVI, X = Bzh
XVII, X = Bzh
XVIII, X = Tr

These results indicate that the S-benzoyl (as expected) and the S-benzhydryl groups are considerably less reactive toward thiocyanogen than the thiol group. However, when XIVd was treated with thiocyanogen, a mixture of disulfides was always obtained. One component of the mixture was established as XVIII by comparison of the tlc pattern of the mixture with authentic XVIII (prepared by air oxidation of XIVd). The other product(s) was (were) not identified but presumably result from simultaneous oxidation of the sulfur atom protected by the S-trityl group.

Additional information concerning the relative reactivity of the S-trityl and S-benzhydryl groups toward thiocyanogen was obtained by preparation of unsymmetrical disulfide XIX. When XIX was prepared *via* XIVb and the S-trityl derivative (XIVa) in the

(10) J. C. Sheehan, P. A. Cruickshank, and G. L. Boshart, *J. Org. Chem.*, **26**, 2527 (1961).

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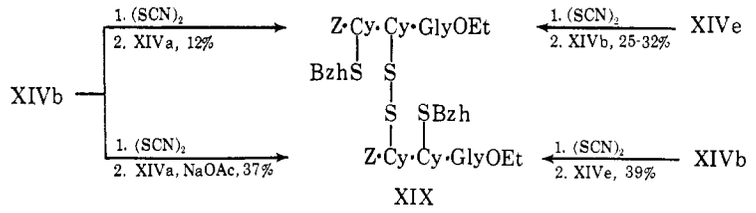

XIX

absence of added sodium acetate, a 12% yield of the unsymmetrical cystine derivative was obtained; use of sodium acetate in this system increased the yield of XIX to 37%. Approximately the same yield of XIX resulted when the two thiols (XIVb, e) were employed. Reversal of the order of addition of the thiols gave essentially the same results. These experiments demonstrate that the sulfenylthiocyanate method provides a reasonable synthetic route to simple unsymmetrical cystine derivatives; application of this procedure to larger peptides and a description of the conditions for oxidation of S-benzhydryl thio ethers will be reported separately.

Experimental Section[13]

Preparation of S-(2-Carbomethoxymethylthio)-N-carbobenzoxy-L-cysteine (IX). General Procedure.—To a cold, stirred solution of 0.0026 mole of thiocyanogen in 35 ml of methylene chloride was added during either 40 or 90 min, a solution of 0.0026 mole of the mercaptan. Five minutes after addition was complete, 0.0026 mole of the catalyst was added. The second mercaptan or S-trityl thio ether (0.0026 mole) was added during 40 min; stirring was continued in the cold for 1 hr and at room temperature for 1–2 hr. The reaction mixture was filtered and the filtrate was diluted with methylene chloride. The organic layer was extracted with aqueous, saturated sodium chloride until the aqueous extract gave a negative ferric chloride test. The methylene chloride solution was dried, treated with activated carbon, and filtered and the filtrate was concentrated *in vacuo*. The residue was applied to a 3 × 50 cm column of 200 mesh silicic acid and eluted with benzene–dioxane (9:2). Fractions were collected at 42-min intervals and analyzed by thin layer chromatography. Those fractions containing the desired product were combined and concentrated *in vacuo*. The residue was dissolved in chloroform, treated with activated carbon, filtered, and crystallized by addition of *n*-hexane to the filtrate. The yields of IX are given in Table I.

Action of Thiocyanogen and Zinc Chloride on Diethyl N,N'-Biscarbobenzoxy-L-cystinyldiglycinate.—A solution of 0.203 g (0.3 mmole) of diethyl N,N'-biscarbobenzoxy-L-cystinyldiglycinate[14] in 150 ml of ethyl acetate was treated with a solution of thiocyanogen prepared from 0.124 g (0.38 mmole) of lead thiocyanate and 0.048 g (0.3 mmole) of bromine in 13 ml of ethyl acetate; the solution also contained 0.04 g (0.29 mmole) of zinc chloride. The reaction mixture was stirred for 1 hr at 2° and evaporated *in vacuo*. The residue was washed with ether (two 25-ml portions). Recovered disulfide (0.083 g 40.8%) melted at 167–168°; a mixture melting point with authentic starting material was not depressed.

Ethyl N-carbobenzoxy-S-trityl-L-cysteinylglycinate (X) was prepared according to the procedure of Zervas and Photaki[15] in 93% yield, mp 114–115° (lit.[15] mp 112–113°).

Preparation of Ethyl N,N'-Biscarbobenzoxy-L-cystinylmonoglycinate (XI).—To a cold, stirred solution of 0.0026 mole of thiocyanogen in 35 ml of methylene chloride was added 0.663 g (0.0026 mole) of N-carbobenzoxy-L-cysteine in 25 ml of methylene chloride over a period of 1.5 hr. The suspension was stirred at 0° for 0.25 hr after addition was complete and treated with a solution of 1.51 g (0.0026 mole) of ethyl N-carbobenzoxy-S-trityl-L-cysteinylglycinate (X) in 25 ml of methylene chloride. Stirring was continued at 0° for 1 hr and at room temperature for 2 hr. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate and the solution was extracted with saturated aqueous sodium chloride until the aqueous extract gave a negative ferric chloride test for thiocyanate ion. The ethyl acetate solution was dried, treated with activated carbon, filtered, and evaporated to yield 2.10 g of white solid which was thoroughly triturated with cold benzene. After standing at about 10° for 1 hr the gel-like residue was filtered, washed with hexane, and dried *in vacuo* to afford 1.30 g of material shown by tlc to consist of three components. The mixture was purified by gradient elution chromatography (5% dioxane in methylene chloride to 15% dioxane in methylene chloride) on a silicic acid column (3 × 68 cm). Fractions were collected automatically at regular intervals and their content was determined by tlc. Fractions containing the desired product were combined and evaporated *in vacuo*. The residue was dissolved in ethyl acetate, treated with activated carbon, filtered, and crystallized by the addition of *n*-hexane to yield 0.96 g (62%) of XI: mp 147–149°, $[\alpha]^{27}D$ −159.3 (*c* 1.1, DMF).

Anal. Calcd for $C_{26}H_{31}N_3O_9S_2$: C, 52.60; H, 5.26; N, 10.80; S, 7.08. Found: C, 52.50; H, 5.23; N, 10.74; S, 7.07.

In an alternate approach the reaction was carried out on a 0.02-mole scale. The crude residue remaining after evaporation of the ethyl acetate was placed in a Soxhlet cup and extracted with *n*-hexane. The hexane-insoluble residue was dissolved in 20 ml of pyridine and diluted with 100 ml of water. The precipitated crude diester (4.1 g) was filtered; the aqueous pyridine solution was neutralized with concentrated hydrochloric acid and extracted with ethyl acetate. The organic extracts were evaporated *in vacuo* and the residue was crystallized from an ethyl acetate–ether–ethanol mixture to afford 5.9 g (50%) of XI: mp 148–150°, $[\alpha]^{27}D$ −162.9 (*c* 1.1, DMF).

Preparation of N-Carbobenzoxy-S-(N'-carbobenzoxy-L-cysteinylglycine ethyl ester)-L-cysteinyl-L-serine Methyl Ester.—To a stirred solution of 1.19 g (0.002 mole) of XI and 0.320 g (0.00205 mole) of L-serine methyl ester hydrochloride in 10 ml of methylene chloride was added 0.285 ml (0.00205 mole) of triethylamine. The reaction mixture was cooled to 0° and 0.040 g (0.0021 mole) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide was added. Stirring was continued for 1 hr at 0° and for 18 hr at room temperature. The reaction mixture was diluted with 200 ml of ethyl acetate and the resulting solution was extracted successively with 2 *N* sulfuric acid, 0.5% sodium bicarbonate, and water. The ethyl acetate extract was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was dissolved in methanol and precipitated with water to afford 1.0 g of crude product. Recrystallization from ethyl acetate–hexane provided 0.85 g (61%) of the desired disulfide: mp 162–164°, $[\alpha]^{27}D$ −117.7° (*c* 0.52, DMF).

Anal. Calcd for $C_{30}H_{38}N_4O_{11}S_2$: C, 51.86; H, 5.52; N, 8.08; S, 9.25. Found: C, 51.75; H, 5.51; N, 7.92; S, 9.39.

An attempt to prepare the substance using DCC as the coupling agent afforded a 55% yield of crude material, mp 172–176°, shown by tlc to consist of two components.

Preparation of N-Carbobenzoxyglycyl-L-serine Methyl Ester.—To a stirred suspension of 4.18 g (0.02 mole) of N-carbobenzoxyglycine and 3.19 g (0.0205 mole) of L-serine methyl ester hydrochloride in 25 ml of methylene chloride was added 2.83 ml (0.0205 mole) of triethylamine. After 0.25 hr the reaction mixture was cooled to −10° and 4.0 g (0.021 mole) of 1-ethyl-3-dimethylaminopropyl)carbodiimide hydrochloride was added. Stirring was continued for several hours during which time the reaction mixture was diluted to 200 ml with ethyl acetate and extracted successively with 2 *N* sulfuric acid, 5% sodium bicarbonate, and water. The ethyl acetate solution was dried, concen-

(13) Melting points are uncorrected and were taken using a Kofler hot stage. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill., and Crobaugh Laboratories, Charleston, W. Va. Optical rotations were measured with a Rudolph Model 200 polarimeter equipped with a Model 80 photoelectric attachment and a Perkin-Elmer polarimeter Model 141 (glass cell). Amino acids were obtained from Mann Research Laboratories, New York, N. Y. Thin layer chromatograms were used as criteria of homogeneity and were carried out on microscope slides using silica gel G and GF_{254}. Chromatograms were developed with iodine vapor or ninhydrin.

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(15) L. Zervas and I. Photaki, *J. Am. Chem. Soc.*, **84**, 3887 (1962).

trated *in vacuo* to about 30 ml, and diluted with *n*-hexane. The product appeared as 4.28 g (69%) of white solid: mp 94–95°, $[\alpha]^{27}D$ 25.30° (*c* 1.02, $CHCl_3$) (lit.[16] mp 91–93°).

Anal. Calcd for $C_{14}H_{18}N_2O_6$: C, 54.35; H, 5.85; N, 9.05. Found: C, 54.11; H, 6.00; N, 8.94.

Preparation of Glycyl-L-serine Methyl Ester Hydrochloride.—To a suspension of 1.5 g of 10% palladium on carbon in 50 ml of methanol was added 3.10 g (0.01 mole) of N-carbobenzoxyglycyl-L-serine methyl ester and 0.85 ml of concentrated hydrochloric acid. The flask was thoroughly flushed with nitrogen and hydrogen was bubbled into the solution at atmospheric pressure for 1.5 hr. (Tlc of aliquots withdrawn at regular intervals showed that no starting material remained after 1 hr.) Removal of the solvent provided a clear oil which was dried *in vacuo*, triturated with ether, and dried to afford 2.0 g (94%) of the dipeptide. The substance exhibited a single spot when chromatographed on Whatman No. 1 paper using *n*-butyl alcohol–acetic acid–water (4:1:5).

Anal. Calcd for $C_6H_{13}ClN_2O_4$: C, 33.85; H, 6.17; Cl, 16.69; N, 13.15. Found: C, 33.96; H, 6.65; Cl, 16.95; N, 12.80.

Preparation of N-Carbobenzoxy-S-(N′-carbobenzoxy-L-cysteinylglycine ethyl ester)-L-cysteinylglycyl-L-serine Methyl Ester.—To a stirred solution of 1.19 g (0.002 mole) of XX and 0.45 g (0.0021 mole) of glycyl-L-serine methyl ester hydrochloride in 10 ml of DMF and 10 ml of methylene chloride was added 0.292 ml (0.0021 mole) of triethylamine. The solution was cooled to −10° and 0.40 g (0.0021 mole) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide was added. Stirring was continued for 1 hr at 0° and for 12 hr at room temperature. The reaction mixture was diluted with saturated sodium chloride solution and the methylene chloride layer was separated and evaporated *in vacuo*. The crude product was filtered, dissolved in methanol, and precipitated with 2 *N* sulfuric acid. The precipitate (shown by tlc to consist of two components) was dissolved in 5% methanol in methylene chloride and filtered through a 2.5 × 12 cm Florisil column. The eluate was collected and concentrated to a yellow residue which was dissolved in methanol, treated with activated carbon, and filtered, and the filtrate was evaporated *in vacuo* to dryness. The product was dissolved in boiling ethyl acetate and precipitated with ether to afford 0.81 g (50%) of the disulfide: mp 133–135°, $[\alpha]^{27}D$ −124.3° (*c* 0.74, DMF).

Anal. Calcd for $C_{33}H_{41}N_5O_{12}S_2$: C, 51.90; H, 5.41; N, 9.20; S, 8.40. Found: C, 51.91; H, 5.68; N, 9.07; S, 8.36.

When the preparation was attempted using DCC, a solid which melted at 115–118°, was obtained. The material was shown by tlc to consist of two components.

Preparation of N-Carbobenzoxy-L-glutamic Acid α-Ethyl Ester.—The N,N-dicyclohexylamine salt of the title compound was prepared in 48% yield according to the procedure described by Weygand and Hunger,[17] mp 155–157°, unchanged by one recrystallization from water (lit.[17] mp 160–161°). The salt was converted to the title compound with Dowex 50W X-8 (H^+); no attempt was made to crystallize the free acid.

Preparation of L-Glutamic Acid α-Ethyl Ester γ-*t*-Butyl Ester Oxalate.—N-Carbobenzoxy-L-glutamic acid α-ethyl ester was converted into the *t*-butyl ester using the procedure of Schwyzer and Kappeler.[18] Hydrogenation of the diester (0.1 mole) was conducted in ethanol with 10% palladium-on-charcoal catalyst. Removal of the catalyst and evaporation (*in vacuo*) afforded a yellow oil. An ether solution of the oil was treated with an ethereal solution of 6.3 g (0.05 mole) of oxalic acid dihydrate. The resulting salt was recrystallized from water and dried to give 11.4 g (71%) of product: mp 131–133°, $[\alpha]^{25}D$ 18.1° (*c* 0.73, ethanol).

Anal. Calcd for $C_{13}H_{23}NO_8$: C, 48.58; H, 7.21; N, 4.36. Found: C, 48.45; H, 7.35; N, 4.17.

Preparation of L-Glutamic Acid α-Ethyl Ester γ-*t*-Butyl Ester.—To a suspension of 0.643 g (0.002 mole) of L-glutamic acid α-ethyl ester γ-*t*-butyl ester oxalate salt in 10 ml of cold methylene chloride was added 8.90 ml of 0.45 *N* dry ammonia in methylene chloride. The suspension was stoppered, stirred at 0° for 0.5 hr, and filtered. The insoluble material was dried *in vacuo* to afford 0.248 g (100%) of ammonium oxalate. The methylene chloride was evaporated *in vacuo* and the residue was dried over sodium hydroxide for 0.5 hr *in vacuo* to afford 0.460 g (99% conversion) of the free amine, shown by tlc to consist of a single, ninhydrin-positive spot. The amine was coupled immediately as described below.

Preparation of N-Carbobenzoxy-S-(N′-carbobenzoxy-L-cysteinylglycine ethyl ester)-L-cysteinyl-L-glutamic Acid α-Ethyl Ester γ-*t*-Butyl Ester.—To a cold, stirred solution of 1.19 g (0.002 mole) of XI and 0.460 g (0.0021 mole) of L-glutamic acid α-ethyl ester γ-*t*-butyl ester in 10 ml of DMF and 10 ml of methylene chloride was added 0.400 g (0.0021 mole) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. Stirring was continued for 1 hr at ice temperature and for 18 hr at room temperature. The reaction mixture was diluted with 200 ml of water and allowed to stand for several hours, and the aqueous DMF solution was decanted. The methylene chloride solution was diluted to 200 ml with ethyl acetate and extracted successively with cold 2 *N* sulfuric acid, 0.5% sodium bicarbonate, and water. The ethyl acetate solution was dried, concentrated to about 50 ml, and treated with 100 ml of *n*-hexane. Collection of the precipitate afforded 1.21 g (75%) of the disulfide: mp 105–107°, $[\alpha]^{27}D$ −108.0° (*c* 1.0, DMF).

Anal. Calcd for $C_{37}H_{56}N_4O_{12}S_2$: C, 55.00; H, 6.24; N, 6.92; S, 7.95. Found: C, 55.21; H, 6.16; N, 6.92; S, 8.08.

When the preparation was attempted using DCC as the coupling agent, a mixture, mp 104–107°, shown by tlc to consist of two components, resulted.

Preparation of N-Benzhydryloxycarbonyl-L-valine Dicyclohexylammonium Salt.—A mixture of 4.75 g (0.04 mole) of L-valine, 15.21 g (0.06 mole) of benzhydrylazidoformate, 5.04 g (0.06 mole) of sodium bicarbonate, 40 ml of 1 *N* sodium hydroxide, 60 ml of water, and 200 ml of dioxane was stirred at room temperature for 18 hr. The reaction mixture was filtered and added to 800 ml of cold water containing 0.20 equiv of sulfuric acid. The mixture was extracted several times with 200-ml portions of ethyl acetate and the extracts were combined and extracted with six 200-ml portions of water. The dried extract was concentrated to 150 ml, and treated with 7.2 g (0.04 mole) of N,N-dicyclohexylamine. Dilution with 150 ml of ether and addition of *n*-hexane to the cloud point afforded 13.8 g of solid. Recrystallization from ethyl acetate–hexane afforded 12.4 g (61%) of product: mp 157–158°, $[\alpha]^{26}D$ 7.7° (*c* 0.415, $CHCl_3$) [lit.[11] mp 156–157°, $[\alpha]^{24}D$ 6.01° (*c* 0.396, $CHCl_3$)].

Preparation of Benzyl S-Trityl-L-cysteinylglycinate Hydrochloride.—To a cold, stirred solution of 5.17 g (0.01 mole) of N-(*o*-nitrosulfenyl)-S-trityl-L-cysteine[19] and 4.05 g (0.012 mole) of benzyl glycinate *p*-toluenesulfonate was added 1.67 ml (0.012 mole) of triethylamine. After 5 min, 2.20 g (0.012 mole) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride was added. Stirring was continued for 12 hr at room temperature. The solvent was evaporated *in vacuo* and the resulting oil was partitioned in water and ether. The ether layer was extracted with cold 2 *N* sulfuric acid, sodium bicarbonate solution, and saturated aqueous sodium chloride. The ether layer was dried and evaporated to yield 6.5 g of solid (shown by tlc to consist of two components). The mixture was dissolved in benzene and applied to a 3.5 × 18 cm column of acid-washed Florisil. Elution with benzene provided 5.5 g (83%) of a powder which was homogeneous by tlc. A cold solution of 2.64 g (0.004 mole) of the protected dipeptide derivative in 100 ml of dry ether was treated with 4.0 ml of 3.2 *N* hydrogen chloride in ether. The mixture was allowed to stand at −10° for 2 hr. The solid was collected and recrystallized from ethyl acetate–hexane to provide 1.9 g (87%) of white powder: $[\alpha]^{27}D$ 30.1° (*c* 1.03, $CHCl_3$).

Anal. Calcd for $C_{31}H_{31}ClN_2O_3$: C, 68.10; H, 5.72; Cl, 6.48; N, 5.14; S, 5.86. Found: C, 68.01; H, 5.96; Cl, 6.68; N, 5.26; S, 6.08.

Preparation of N-Benzhydryloxycarbonyl-L-valyl-S-trityl-L-cysteinylglycine Benzyl Ester (XII).—To a cold, stirred suspension of 5.08 g (0.01 mole) of N-benzhydryloxycarbonyl-L-valine N,N-dicyclohexylamine salt and 5.75 g (0.0105 mole) of S-trityl-L-cysteinylglycine benzyl ester hydrochloride was added 2.0 g (0.0105 mole) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. Stirring was continued for 1 hr at −10° and for 12 hr at room temperature. The solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate and extracted with cold 2 *N* sulfuric acid, water and aqueous, saturated sodium chloride. The ethyl acetate solution was dried and concentrated to about 100 ml. Addition of *n*-hexane afforded

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6.0 g (73%) of the desired product: mp 191.5–192.5°, $[\alpha]^{27}D$ −12.5° (*c* 1.1, $CHCl_3$).

Anal. Calcd for $C_{50}H_{49}N_3O_6S$: C, 73.25; H, 6.02; N, 5.13; S, 3.91. Found: C, 72.94; H, 6.06; N, 4.96; S, 3.63.

Preparation of N-Benzhydryloxycarbonyl-L-valyl-S-(N′-carbobenzoxy-L-cysteine)-L-cysteinylglycine Benzyl Ester.—A solution of 1.27 g (0.005 mole) of N-carbobenzoxy-L-cysteine in 100 ml of methylene chloride was added during 2.5 hr to a cold, stirred solution of 0.0053 mole of thiocyanogen in 250 ml of methylene chloride. One-fourth hour after addition was complete, 4.10 g (0.005 mole) of XII was added. Stirring was continued for 8 hr as the reaction mixture gradually warmed to room temperature. The reaction mixture was filtered and the filtrate was extracted with water until the aqueous extracts gave a negative ferric chloride test. The methylene chloride solution was concentrated to 200 ml dried, and evaporated to a white residue which was thoroughly triturated with boiling ether. After standing overnight at 0°, the insoluble portion was removed by filtration, washed with cold ether, and dried *in vacuo* to give 3.75 g of material shown by tlc to consist of three components. Adsorption on silicic acid and elution with methylene chloride and dioxane (5 to 15% dioxane in methylene chloride) gave 2.0 g (48%) of the disulfide: mp 131–133°, $[\alpha]^{27}D$ −118.2° (*c* 1.1, DMF).

Anal. Calcd for $C_{42}H_{46}N_4O_{10}S_2$: C, 60.70; H, 5.58; N, 6.75; S, 7.72. Found: C, 60.78; H, 5.62; N, 7.04; S, 7.52.

Preparation of Diethyl N,N′-Bis(carbobenzoxy-S-benzoyl-L-cysteinyl)-L-cystinyldiglycinate (XV).—A solution of 0.99 g (1.25 mmoles) of XIVc in 30 ml of methylene chloride was treated with 0.69 mmole of thiocyanogen in 10 ml of methylene chloride and 0.2 g (0.66 mmole) of boron trifluoride–diethyl etherate in 3 ml of methylene chloride. The reaction mixture was stirred at 0° for 1 hr, evaporated *in vacuo*, and extracted with warm petroleum ether (bp 30–60°). The petroleum ether extract provided 0.29 g (79.2%) of trityl isothiocyanate, mp 136–138° (from methanol).

The petroleum ether insoluble portion of the residue was washed with cold water, dried, and recrystallized from dioxane to provide 0.61 g (89.2%) of a white powder, mp 206–209°. After three recrystallizations from dioxane, 0.19 g of white powder was obtained; the material frothed at 209–210° and melted up to 223°, $[\alpha]^{25}D$ −107.9° (*c* 1.00, DMF).

Anal. Calcd for $C_{50}H_{56}N_6O_{14}S_4$: C, 54.93; H, 5.16; N, 7.19; S, 11.73. Found: C, 54.85; H, 5.21; N, 7.86; S, 11.55.

Preparation of Diethyl N,N′-Bis(N-carbobenzoxy-S-benzhydryl-L-cysteinyl)-L-cystinyldiglycinate (XVI).—A solution containing 1.22 g (2.0 mmole) of XIVb in 200 ml of ethyl acetate was added to a solution of 1.0 mmole of thiocyanogen in 50 ml of ethyl acetate. The mixture was stirred at 0° for 1.5 hr, concentrated *in vacuo*, and diluted with ether. The resulting solid was washed with ether and precipitated from 30 ml of ethyl acetate. The disulfide appeared as 1.13 g (92.8%) of white powder: mp 182–184°, $[\alpha]^{24}D$ −80.5 (*c* 1.11, DMF).

Anal. Calcd for $C_{62}H_{68}N_6O_{12}S_4$: C, 61.16; H, 5.63; N, 6.90; S, 10.54. Found: C, 61.55; H, 5.81; N, 6.81; S, 10.98.

When the oxidation of the thiol was conducted in ethanol using 0.1 *N* iodine–potassium iodide solution, the yield of XVI was 70%, mp 182–184°, $[\alpha]^{24}D$ −80.4 (*c* 1.05, DMF). The thiol was converted to the disulfide in 93.2% yield by air oxidation of an ethanolic solution containing triethylamine; however, the oxidation required 2 days and was not adaptable to large scale.

Preparation of Diethyl N,N′-Bis(carbobenzoxy-L-cystinyl)bis(S-benzhydryl-L-cysteinylglycinate) (XVII).—A solution of 0.26 mmole of thiocyanogen in 12 ml of ethyl acetate was decanted from the precipitated lead bromide into a stirred solution containing 0.305 g (0.5 mmole) of XIVe in 30.5 Xl of ethyl acetate at 0°. Stirring was continued for 2 hr at 0°. The reaction mixture was evaporated *in vacuo*, diluted with ether, and filtered. The resulting white powder was recrystallized twice from ethyl acetate to provide 0.165 g (55%) of disulfide, mp 196–198°. One additional recrystallization from methylene chloride–hexane raised the melting point to 197–198.5°, $[\alpha]^{24}D$ −95.5° (*c* 1.02, DMF).

Anal. Calcd for $C_{62}H_{68}N_6O_{12}S_4$: C, 61.16; H, 5.63; N, 6.90; S, 10.54. Found: C, 61.08; H, 5.55; N, 7.06; S, 10.44.

When the oxidation of XIVe was conducted in ethanol using 0.1 *N* iodine–potassium iodide, the disulfide was obtained in 92.1% yield: mp 196–198°, $[\alpha]^{24}D$ −95.6 (*c* 1.22, DMF). A mixture melting point with the disulfide prepared using thiocyanogen was not depressed.

Preparation of Diethyl N,N′-Bis(carbobenzoxy-L-cystinyl)bis(S-trityl-L-cysteinylglycinate) (XVIII).—A solution containing 0.30 g (0.044 mmole) of XIVd and 2 drops of triethylamine in 10 ml of ethanol was cooled in an ice bath and treated with air for 6 days. The ethanol and triethylamine were replenished during the oxidation. The solvent was removed *in vacuo* and a chloroform solution of the residue was filtered through 6 g of silicic acid. The chloroform was removed *in vacuo* and the residue was recrystallized several times from an ethyl acetate–hexane solvent. The disulfide was obtained as 0.08 g (26.6%) of white powder: mp 132–134°, $[\alpha]^{25}D$ −63.1° (*c* 0.45, DMF).

Anal. Calcd for $C_{74}H_{76}N_6O_8S_4$: C, 64.89; H, 5.59; N, 6.14; S, 9.36. Found: C, 64.67; H, 5.71; N, 6.13; S, 9.34.

When the thiol was allowed to react with thiocyanogen in ethyl acetate solution a two-component mixture was obtained which could not be purified.

Preparation of XIX. A. From Ethyl N-Carbobenzoxy-S-benzhydryl-L-cysteinyl-L-cysteinylglycinate (XIVb) and Ethyl N-Carbobenzoxy-S-trityl-L-cysteinyl-S-benhydryl-L-cysteinylglycinate (XIVa).—To a cold solution of 0.573 mmole of thiocyanogen in 5 ml of methylene chloride and 2 ml of ethyl acetate was added a solution of 0.305 g (0.5 mmole) of XIVb in 5 ml of methylene chloride. The addition required 20 min. After stirring for 25 min, a solution containing 0.426 g (0.5 mmole) of XIVa in 5 ml of methylene chloride was added and the mixture was stirred for 4 hr at 5°. The solution was allowed to warm to room temperature, filtered, and washed with water. The dried organic layer was evaporated *in vacuo* and the residue was extracted with hot petroleum ether (bp 30–60°, three 20-ml portions). Evaporation of the petroleum ether extract provided 0.9 g (40%) of tritylisothiocyanate.

The residue was warmed in 10 ml of 50% aqueous methanol and filtered and the insoluble portion was dissolved in ethyl acetate. The solution was filtered and concentrated *in vacuo* and the precipitated solid (0.31 g) was dissolved in chloroform–ethyl acetate (1:1) and filtered through Florisil. Evaporation of the eluate provided a powder which was recrystallized from a methylene chloride–ethyl acetate mixture to yield 0.074 g (12%) of XIX: mp 186–189°, $[\alpha]^{24}D$ −85.6 (*c* 0.98, DMF). A mixture melting point with a sample of the disulfide obtained *via* the thiols was 186–189°.

B. From XIVb and XIVa Using Sodium Acetate Catalyst.—To a solution containing 1.5 mmoles of thiocyanogen in 15 ml of methylene chloride and 6 ml of ethyl acetate was added 0.915 g (1.5 mmoles) of XIVb in 15 ml of methylene chloride. The addition required 24 min. The cold solution was stirred for 5 min and treated with 1.28 g (1.5 mmoles) of XIVa in 15 ml of methylene chloride and 0.123 g (1.5 mmoles) of sodium acetate. The reaction mixture was stirred for 4 hr at 0–20°. The usual work-up gave a powder which was recrystallized twice from a methylene chloride–ethyl acetate mixture to yield 0.67 g (36.6%) of XIX: mp 186–188°, $[\alpha]^{24}D$ −86.8° (*c* 1.12, DMF). A mixture melting point with the sample obtained in A was 186–188°.

Preparation of XIX. A. From XIVe and XIVb.—To a cold, stirred solution of 0.625 mmole of thiocyanogen in 13 ml of ethyl acetate was added a solution containing 0.305 g (0.5 mmole) of XIVe in 15 ml of ethyl acetate. The dropwise addition required 10 min. After stirring for 30 min the sulfenylthiocyanate solution was treated with 0.305 g of XIVb in 15 ml of ethyl acetate. The second thiol was added in one portion. The reaction mixture was stirred for 3 hr at 7–24° and evaporated *in vacuo* and the residue was washed with 10 ml of cold methanol. The resulting white powder was recrystallized four times from ethyl acetate to provide 0.15 g (24.6%) of XIX: mp 188–190° (187–189° from a methylene chloride–petroleum ether mixture), $[\alpha]^{24}D$ −87.9° (*c* 0.79, DMF).

Anal. Calcd for $C_{62}H_{68}N_6O_{12}S_4$: C, 61.16; H, 5.63; N, 6.90; S, 10.54. Found: C, 61.03; H, 5.48; N, 7.07; S, 10.49.

When both additions of the thiols were conducted at −70° and the resulting product was purified by filtration of a chloroform–ethyl acetate (9:1) solution of the residue through a silicic acid column (15 g), a 31.6% yield of XIX was obtained: mp 189–191.5°, mmp 187–190°.

B. From XIVb and XIVe.—A cold, stirred solution of 1.13 mmoles of thiocyanogen in 20 ml of methylene chloride and 5 ml of ethyl acetate was treated with 0.61 g (1.0 mmole) of XIVb in 25 ml of methylene chloride. The dropwise addition required 40 min. The reaction mixture was stirred 30 min and treated with a solution containing 0.61 g (1.0 mmole) of XIVe in 25 ml

of methylene chloride. The addition required 15 min. The reaction mixture was stirred for 15 min and evaporated *in vacuo* and the residue was washed with ether. The crude product was dissolved in a chloroform–ethyl acetate mixture (9:1) and filtered through silicic acid (20 g). The solvent was evaporated and the residue was suspended in ethyl acetate, warmed, and filtered. The insoluble portion was dissolved in a minimum amount of methylene chloride, diluted with ethyl acetate and concentrated. The precipitated powder was dried to yield 0.473 g (38.9%) of white powder: mp 188–191°, $[\alpha]^{24}D$ −86.6° (*c* 0.93, DMF). A mixture melting point with the sample obtained in A was 188–190°.

The Pyrolysis of Unsymmetrical Dialkyl Sulfoxides. Rates of Alkene Formation and Composition of the Gaseous Products

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The relative yields of alkenes produced in the thermal decomposition of some unsymmetrical dialkyl sulfoxides were determined. The olefinic products produced are those predicted on the basis of the currently accepted mechanism involving the loss of a sulfenate moiety and any one of the available β hydrogens. Secondary alkyl groups are cleaved to form alkenes more readily than primary alkyl groups when both are embodied in the same molecule. Cleavage of a *sec*-butyl group results in the formation of 1-butene and a mixture of the 2-butenes. Sulfoxides having both a primary and a secondary alkyl group decompose at a substantially greater rate than sulfoxides in which both alkyl groups are primary.

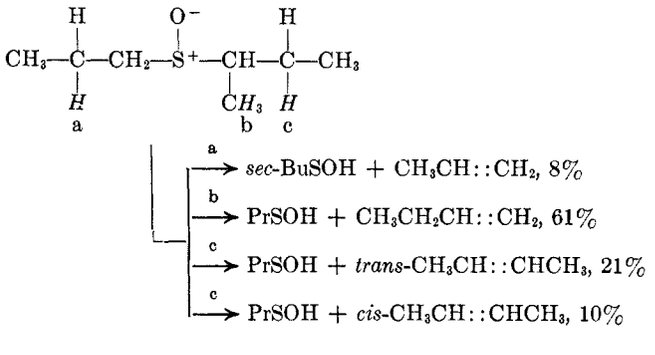
The thermal instability of certain dialkyl sulfoxides was noted as early as 1875.[1] More recently, several studies[2–6] have dealt with the general features of the sulfoxide pyrolysis reaction including the nature of the products formed. The sulfoxides in these studies were either symmetrical[3,4,6] or had only one alkyl group which could be cleaved to form alkenes.[2,3,5] In one study[7] unsymmetrical sulfoxides were considered, but the composition of the olefinic products was not reported.

The present work was undertaken to gain insight on the relative yields of alkenes produced in the pyrolysis of unsymmetrical dialkyl sulfoxides of such structure that both alkyl groups could afford alkenes. Five sulfoxides of the type RSOR′ were prepared of such structure that the alkenes produced would identify the alkyl groups from which they were formed by pyrolytic cleavage.

The sulfoxides were prepared by hydrogen peroxide oxidation of appropriate dialkyl sulfoxides of high purity. The sulfoxides studied were all either racemic mixtures or mixtures of diastereoisomers depending on whether the asymmetry was inherent in the unsymmetrical sulfoxide or whether additional asymmetry was present in a *sec*-butyl group. The neat sulfoxides were pyrolyzed in a controlled-temperature reactor and the quantity of gas was measured by water displacement. The composition of the gas was determined by gas–liquid partition chromatography. Satisfactory first-order rates were observed, although there was some acceleration of the rate after 2 half-lives.

A. *n*-Propyl *sec*-Butyl Sulfoxide.—A thorough discussion of the pyrolysis of *n*-propyl *sec*-butyl sulfoxide will serve to illustrate the methodology of the study. Chart I shows what products might be expected on the basis of the Ei mechanisms proposed by Kingsbury and Cram.[2] Since one alkyl group is primary (*n*-propyl) and the other is secondary (*sec*-butyl), the ratio of propene to the isomeric butenes indicates the relative ease of cleavage of a primary carbon–sulfur bond compared to a secondary carbon–sulfur bond. Since the *sec*-butyl group embodies two types of β hydrogens, b and c, the ratio of 1-butene to the isomeric 2-butenes reveals whether or not electrical effects operating on the acidity of the β hydrogens are significant as have been shown to be the case in the base-catalyzed cleavage of tetraalkylammonium ions[8,9] and trialkylsulfonium ions.[10,11] Our results, given in Table I, show that, in the pyrolysis of dialkyl sulfoxides, an inductive effect is not of extreme importance within the *sec*-butyl group since the yield of 1-butene is only twice the yield of the 2-butenes. Statistically, on the basis of the numbers of b and c hydrogens, the yield ratio of 1-butene to the 2-butenes would be 3:2. It is concluded, therefore, that the attack of a strongly basic oxygen atom on an acidic β hydrogen is not of overriding importance in the rate-determining step. In this respect the reaction most closely resembles the pyrolysis of amine oxides.[12] A comparison with some

CHART I



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