Chemistry of Aliphatic Disulfides. XV. Bisdisulfide Peptide Derivatives[1-3]

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Several unsymmetrical bisdisulfides containing other functional groups have been prepared. The chemical modification of various groups has been studied; esterification, acid-catalyzed ester hydrolysis, and amide formation could be accomplished in the presence of the sulfur–sulfur bonds.

In a previous paper, the use of unsymmetrical bisdisulfides as models for the synthesis of biscystinyl peptides was described.[6] The present report provides an account of our efforts to obtain unsymmetrical bisdisulfides containing various functional groups and to determine reaction conditions for the introduction and removal of functional groups in the presence of disulfide bonds.

Unsymmetrical bisdisulfides have been prepared by the reaction of sulfenyl thiocyanates with S-trityl[6] and S-benzhydryl[7,8] thioethers; however, the former sulfur-protecting group has been demonstrated to be more labile toward a sulfenyl thiocyanate.[8] Thus the desired bisdisulfides, N-(7-carboxy-3,4-dithiahexyl)-5-phenyl-4,5-dithiapentanoic acid amide (II), and N-carboxy-3-({2-[3-(phenyldithio)propionamido]ethyl}-dithio)-N-benzyl-L-alanine ester (III) were prepared from N-(2-tritylthioethyl)-5-phenyl-4,5-dithiapentanoic acid amide (I) (Scheme I). The acid, II, was also prepared

SCHEME I

$$HSCH_2CH_2CO_2H \xrightarrow{(SCN)_2} [NCSSCH_2CH_2CO_2H] \longrightarrow C_6H_5SSCH_2CH_2CONHCH_2CH_2STr$$

I, 32–48%

$$TrSCH_2CH_2CO_2H \xrightarrow[2.\ I]{1.\ (SCN)_2}$$

IV

$$C_6H_5SSCH_2CH_2CONHCH_2CH_2SSCH_2CH_2CO_2H$$

II, 47%

$$HSCH_2CHCO_2H \xrightarrow[2.\ I]{1.\ (SCN)_2}$$

NHZ

$$C_6H_5SSCH_2CH_2CONHCH_2CH_2SSCH_2CHCO_2H$$

NHZ

III, 33–42%

from I and 5,5,5-triphenyl-4-thiapentanoic acid (IV). The other bisdisulfides studied (Table I) were obtained either from I, *via* a thiocyanogen reaction, or from II and III, by coupling with the appropriate amine.

TABLE I

BISDISULFIDE DERIVATIVES, $C_6H_5SSCH_2CH_2CONHCH_2CH_2SSR$

Compd	Terminal group, R	Yield, %	Prep method
V	$-CH_2CH_2CONHCH_2CH_2OH$	88	*b*
VI	$-CH_2CH_2CONHCH_2CH_2OCCH_3$ (O)	46	*c*
VII	$-CH_2CH_2CONHCH_2CH_2STr$	62	*b*
VIII	$-CH_2CHCO_2CH_3$ (HNZ)	67	*d*
IX	$-CH_2CH_2CONHCHCO_2CH_2C_6H_5$ ($CH_2CH_2CH_2CH_2NHZ$)	36	*b*
XII	$-CH_2CHCONHCH_2CO_2CH_2CH_3$ (HNBhOC)	53	*a*
XIV	$-CH_2CHCONHCHCONHCHCONHCH_2CO_2tBu$ (HNZ; CH_2; $CH_2C_6H_5$; SiBM)	81	*b*
XV	$-CH_2CHCONHCHCO_2CH_2CH_3$ (HNZ; $CH_2CH_2SCH_3$)	70	

[a] Thiocyanogen reaction with II. [b] Coupling reaction with IV or V and DCC or WSC. [c] Acetylation of V. [d] Fischer esterification of V.

The instability of simple unsymmetrical open-chain cystine derivatives toward various acidic and basic reagents has been previously noted.[9] In general, hydroxide and alkoxide ions, hydrazine, and aqueous solutions of amines appear to induce disulfide interchange. Similar results have been obtained with hydrogen bromide in a variety of solvents and with neat trifluoroacetic acid. However, in more complex molecules the presence of side chains and various functional groups might influence the stability of the disulfide bond toward these reagents. Thus the introduction and removal of various groups in the bisdisulfides were studied. The stability of the disulfide bonds in II and III toward various amines, in the presence of carboxyl-activating reagents, was demonstrated by the formation of several amide derivatives of II and III (Table I). When II was allowed to react with ethanolamine in the presence of WSC, N-{7-[3-(phenyldithio)propionamido]-4,5-dithiaheptanamido}-2-hydroxyethylamine (V) was produced in 88% yield. Acetylation of V, using acetic anhydride at 80°, provided N-{7-[3-(phenyldithio)propionamido]-4,5-dithiaheptanamido}-2-acetoxyethylamine (VI); however, when V was treated with *p*-toluenesulfonyl chloride in pyridine complete decomposition of the starting material occurred. Several attempts to prepare the sulfur analog of V were unsuccessful. When N-{7-[3-(phenyldithio)propionamido]-4,5-dithiaheptanamido}-4,4,4-triphenyl-3-thiabutylamine (VII), obtained by coupling II and 2-tritylthioethylamine, was treated with silver nitrate in pyridine and methanol,[10] extensive decomposition of the starting material resulted; a similar experiment involving I also led to de-

(1) Part XIV of this series: R. G. Hiskey and M. A. Harpold, *J. Org. Chem.*, **32**, 3191 (1967).

(2) Supported by Research Grant RG-7966 from the National Institute of General Medical Sciences of the National Institutes of Health, U. S. Public Health Service.

(3) The following abbreviations have been incorporated into the text: Tr, trityl; Z, carbobenzoxy; For, formyl; BhOC, benzhydryloxycarbonyl; iBM, isobutoxymethyl; WSC, 1-ethyl-3-(N,N-dimethylaminopropyl)carbodiimide hydrochloride; DMF, N,N-dimethylformamide; DCC, N,N,-dicyclohexylcarbodiimide; DEA^+, N,N-diethylammonium.

(4) Abstracted in part from a dissertation submitted by Michael A. Harpold to the University of North Carolina in partial fulfillment of the requirements for the Ph.D. degree, June 1967.

(5) National Science Foundation Cooperative Fellow, 1964–1966.

(6) R. G. Hiskey and D. N. Harpp, *J. Am. Chem. Soc.*, **87**, 3965 (1965).

(7) R. G. Hiskey and M. A. Harpold, *Tetrahedron*, **23**, 3923 (1967).

(8) R. G. Hiskey, T. Mizoguchi, and E. L. Smithwick, Jr., *J. Org. Chem.*, **32**, 97 (1967).

(9) See R. G. Hiskey and E. L. Smithwick, Jr., *J. Am. Chem. Soc.*, **89**, 437 (1967), for pertinent references.

(10) L. Zervas and I. Photaki, *ibid.*, **84**, 3887 (1962).

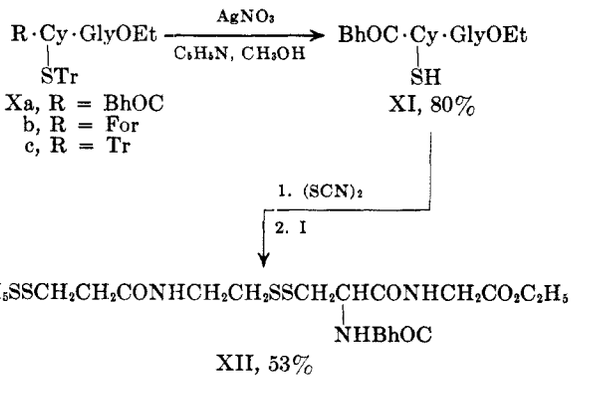
composition and none of the desired thiol could be detected.

Esterification of III using methanol and sulfuric acid proceeded normally and provided N-carboxy-3-({2-[3-(phenyldithio)propionamido]ethyl}dithio)-N-benzyl-L-alanine methyl ester (VIII) in 67% yield. The ester, VIII, had previously been prepared[6] from I and methyl N-carbobenzoxy-L-cysteinate. Attempts to introduce a *t*-butyl ester into II using the isobutylene–sulfuric acid method were unsuccessful and extensive decomposition of II resulted. As anticipated, VIII could not be converted into III using 0.05 *N* sodium hydroxide solution; III was also destroyed when treated with 1 equiv of N,N-dicyclohexylamine or excess triethylamine.

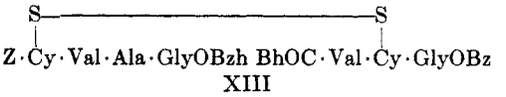
Although previous experiments[9] indicated that the N-carbobenzoxy group could not be removed from unsymmetrical open-chain cystine derivatives by the usual reagents without disulfide interchange or catalyst poisoning, larger molecules in which the N-carbobenzoxy group was separated from the disulfide bond might exhibit a different behavior. Accordingly N^{ϵ}-carboxy-N^{α}-{7-[3-(phenyldithio)propionamido]-4,5-dithiaheptanamido}-N-benzyl-L-lysine benzyl ester (IX) was prepared by the WSC coupling of benzyl N^{ϵ}-carbobenzoxy-L-lysinate with II. The use of 5% palladium on barium sulfate in a variety of solvents led either to decomposition or recovered starting material. Likewise the action of hydrogen chloride and *p*-toluenesulfonic acid afforded only decomposition products.

In order to evaluate other amino protective groups, N^{ϵ}-carboxy-N^{α}-{7-[3-(phenyldithio)propionamido- 4,5-dithioheptanamido-N-benzyl-L-lysine benzyl ester (IX) and N-[N-carboxy-3({2-[3-(phenyldithio)propionamido]ethyl}dithio)-N-benzhydryl-L-alanyl]-glycine ethyl ester (XII) were prepared. The N^{ϵ}-carbobenzoxy-L-lysine derivative, IX, was obtained in 36% yield by the WSC-catalyzed coupling of benzyl N^{ϵ}-carbobenzoxy-L-lysinate with II; XII was prepared by the thiocyanogen reaction between I and ethyl N-benzhydryloxycarbonyl-L-cysteinylglycinate (XI). The mercaptan, XI, was obtained by detritylation of ethyl N-benzhydryloxycarbonyl-S-trityl-L-cysteinylglycinate (Xa). The N-formyl and N-trityl derivatives of S-trityl-L-cysteinylglycine ethyl ester (Xb, c) were also prepared; however, when either Xb or Xc were subjected to the action of thiocyanogen in ethyl acetate, a ninhydrin-positive product resulted, suggesting that both N-protective groups were cleaved (Scheme II).

SCHEME II



In earlier studies[9] the N-benzhydryloxycarbonyl group was cleanly removed from XIII by the action of boron trifluoride etherate in acetic acid. However,



XIII

when XII was subjected to the cleavage conditions, the desired amine was contaminated with other products of similar tlc mobility and the amine could not be obtained in a pure form. Thus the N-benzhydryloxycarbonyl group may not be as generally compatible with unsymmetrical open-chain cystine derivatives as initially believed.

The general question of the stability of disulfides containing thioether residues was of interest in view of the lack of success in the preparation of disulfides containing thiol side chains. Thus, N-{3-phenyl-N-[3-(1-thia-5-oxa-4-methylhexyl)-N-{N-carboxy-3-[(2-[3-(phenyldithio)propionamido]ethyl)dithio]-N-benzyl-L-alanyl}-L-alanyl]-L-alanyl}glycine *t*-butyl ester (XIV) and N-[N-carboxy-3-({2-[3-(phenyldithio)propionamido]-ethyl}dithio)-N-benzyl-L-alanyl]-DL-methionine ethyl ester (XV) were prepared. Both thioethers were stable in acetic acid or DMF solvent and gave no indication of thioether disulfide interactions.

Experimental Section[11]

N-(7-Carboxy-3,4-dithiahexyl)-5-phenyl-4,5-dithiapentanoic amide (II) was prepared in yields of 32–48% by the procedure of Hiskey and Harpp,[6] mp 100.5–102° (lit.[6] mp 98.5–100.5°).

The title compound could also be prepared by a similar procedure which employed both trityl thioethers; a solution of thiocyanogen was generated in 25 ml of ethyl acetate from 2.0 g (0.062 mole) of lead thiocyanate and 0.67 g (0.041 mole) of bromine. To this stirred mixture at ice temperature was added a slurry of 1.34 g (0.04 mole) of IV in 50 ml of ethyl acetate. The addition required 15 min and the reaction was monitored by tlc (system A). After 1.6 hr the clear supernatant contained no starting thioether and 2.08 g (0.04 mole) of I was added in one portion along with 5 ml of additional ethyl acetate. The reaction mixture was allowed to warm to room temperature and filtered; the filtrate was washed with water and saturated sodium chloride solution before drying.

The dried solution was concentrated *in vacuo* and the solid residue was dissolved in chloroform, treated with charcoal, and filtered; the filtrate was concentrated to 25 ml before cooling to 0°. The first crop of product appeared as 0.51 g, mp 97–99°, homogeneous on tlc with mobility equal to authentic material. The mother liquor was concentrated and chromatographed on silica gel to recover a second crop of identical material, 0.22 g. The combined product (0.73 g, 47.4%) was used to obtain a mixture melting point with authentic material which was not depressed.

N-Carboxy-3-({2-[3-(phenyldithio)propionamido]ethyl}dithio)-N-benzyl-L-alanyl ester (III) was prepared in yields of 33–42% by the method of Hiskey and Harpp,[6] mp 93–95° (lit.[6] mp 93–93.5°).

N-{7-[3-(Phenyldithio)propionamido]-4,5-dithiaheptanamido}-2-hydroxyethylamine (V) was prepared at −10° from 1.0 g (2.6 mmoles) of II, 0.16 g (2.6 mmoles) of ethanolamine, and 0.52 g (2.7

(11) Melting points are uncorrected and were obtained in capillary tubes or with a Kofler apparatus when the desired range exceeded 200°. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter, using a glass cell. Thin layer chromatograms were conducted on microscope slides or on 5 × 20 and 20 × 20 cm Pyrex plates using uniform coatings of silica gel GF_{254}. The chromatograms were developed with iodine vapor and/or viewed under ultraviolet light. Solvent systems for tlc were benzene–dioxane–acetic acid (90:25:4, system A), *n*-butyl alcohol–acetic acid–water (4:1:5, system B), chloroform–ethyl acetate (1:1, system C), chloroform–benzene (1:1, system D), and chloroform–methanol (9:1, system E). Commercial reagents were of the highest quality available and were purified as necessary.

mmoles) of WSC in 40 ml of ethyl acetate. The reaction mixture was allowed to warm to room temperature while stirring for 12 hr and the ethyl acetate solution was washed with water, 2 *N* sulfuric acid, water, and saturated sodium chloride solution before drying. The solvent was removed *in vacuo* to yield a white solid residue which was recrystallized from ethyl acetate; tlc (system A) indicated a trace of impurity. The crude product was dissolved in chloroform and chromatographed on a 1.5 × 40 cm column of Florisil (40 g, 100 mesh). The major component was eluted from the column with chloroform–ethyl acetate (1:1) and was recrystallized from ethyl acetate–*n*-hexane. The product appeared as 0.80 g (88%) of white solid, mp 107–108°; homogeneous on tlc (system A); ν_{max}^{KBr} 3340, 3290, 3060, 2930, 1640, 1540, 1420, 1260, 1055, 735, 680 cm^{-1}.

Anal. Calcd for $C_{15}H_{24}N_2O_3S_4$: C, 45.69; H, 5.75; N, 6.66; S, 30.49. Found: C, 45.73; H, 5.83; N, 6.73; S, 30.31.

N{7-[3-(Phenyldithio)propionamido]-4,5-dithiaheptanamido}-2-acetoxyethylamine (VI) was prepared by heating a mixture of 0.42 g (1 mmole) of V in 5 ml of acetic anhydride at 80° for 15 min while stirring under a calcium chloride drying tube. At the end of the heating period the clear solution was allowed to cool to room temperature while stirring overnight. The reaction mixture was poured into 100 ml of cold water and the white precipitate was recovered by filtration, washed with water, and dried *in vacuo*. The crude product was dissolved in chloroform and applied to a 1.5 × 40 cm column of silicic acid (40 g, 100–200 mesh). The desired product was eluted from the column with 60–70% ethyl acetate in chloroform and recrystallized from ethyl acetate–*n*-hexane. The product appeared as 0.21 g (46%) of white crystals: mp 98–98.5°; homogeneous on tlc (system A); ν_{max}^{KBr} 3380, 1740, 1640, 1550, 1255, 1050, 950, 740, 690 cm^{-1}.

Anal. Calcd for $C_{18}H_{26}N_2O_4S_4$: C, 46.73; H, 5.67; N, 6.06; S, 27.72. Found: C, 46.98; H, 5.92; N, 6.19; S, 28.01.

2-Tritylthioethylamine hydrochloride was prepared *via* 2-tritylthioethylammonium chloride. The hydrochloride was prepared by the procedure used for 2-benzhydrylthioethylammonium chloride.[7] The crude product was precipitated from the reaction mixture with ethyl ether, charcoaled, and recrystallized from ethyl acetate. The product was obtained in 86% yield: mp 125–128°, with softening at 120°; homogeneous on tlc (system B); ninhydrin positive and nitroprusside negative. An additional recrystallization from ethyl acetate with a trace of ether raised the melting point to 128–130°; ν_{max}^{KBr} 3550, 3200–2700, 2080, 1600, 1520, 1480, 1440, 1030, 745, 700 cm^{-1}.

Anal. Calcd for $C_{21}H_{22}ClNS$: C, 70.86; H, 6.23; N, 3.94; S, 9.01. Found: C, 70.73; H, 6.50; N, 4.07; S, 8.68.

Conversion to the free amine was accomplished in 82% yield by partitioning the hydrochloride between ethyl acetate and aqueous sodium hydroxide. The organic solution was separated, washed with water and saturated sodium chloride solution, and dried. The limited solubility of the product required that the dried solution be heated prior to filtration to remove the drying agent. The filtrate was concentrated *in vacuo* and the solid residue was recrystallized from ethyl acetate. The product appeared as white crystals, mp 94–96° (lit.[6] mp 94–96.5°). The product was homogeneous on tlc (system B) and a mixture melting point with authentic material was not depressed.

N-{7-[3-(Phenyldithio)propionamido]-4,5-dithiaheptanamido}-4,4,4-triphenyl-3-thiabutylamine VII was prepared by stirring a mixture of 1.0 g (2.6 mmoles) of II, 0.83 g (2.6 mmoles) of 2-tritylthioethylamine, and 0.52 g (2.7 mmoles) of WSC in 100 ml of acetone and 1 ml of DMF at −10°. The cooling bath was removed and the reaction mixture was allowed to warm to room temperature while stirring for 12 hr. The solvent was removed *in vacuo* and the oily residue was dissolved in ethyl acetate and washed with water, 2 *N* sulfuric acid, water, and saturated sodium chloride solution. After drying, the solution was concentrated *in vacuo* to produce a yellow oil which was treated with charcoal and crystallized from ethyl acetate. The product appeared as 1.1 g (62%) of white crystals: mp 104–106°; homogeneous on tlc (systems A and B); ν_{max}^{KBr} 3280, 3060, 2910, 1645, 1560, 1440, 1260, 740, 695 cm^{-1}. An additional recrystallization gave material melting at 103–104°.

Anal. Calcd for $C_{35}H_{38}N_2O_2S_5$: C, 61.91; H, 5.64; N, 4.13; S, 23.61. Found: C, 61.98; H, 5.69; N, 4.47; S, 23.40.

Attempted Detritylation of I.—When I was treated with silver nitrate and pyridine in an attempt to produce the analogous mercaptan *via* the silver mercaptide procedure, a tacky yellow precipitate was obtained. Further experimentation revealed that this material was impure (tlc, system A) and treatment with hydrogen sulfide produced several nitroprusside-positive components.

Attempted Detritylation of VII.—As in the application of the silver mercaptide procedure to I, the desired mercaptan could not be produced in this manner. The presumed silver mercaptide of the bisdisulfide was treated with hydrogen sulfide in a methylene chloride–acetone slurry and the resultant solution contained at least three very polar components, as evidenced by tlc mobility (system B) and an unsuccessful attempt to resolve the mixture *via* column chromatography.

Reaction of V with *p*-Toluenesulfonyl Chloride.—A solution of 0.42 g (1 mmole) of V in 1.5 ml of pyridine was treated with 0.47 g (2.5 mmoles) of *p*-toluenesulfonyl chloride and the resultant yellow solution was heated at 80° for 15 min with occasional swirling. The orange reaction mixture was poured into 100 ml of ice water and extracted into 100 ml of ethyl acetate. This solution was washed with water and saturated sodium chloride solution and dried. The solution displayed four spots on tlc (system D); the mixture could not be resolved by column chromatography.

Reaction of III with Amines. A. With 1 Equiv of Dicyclohexylamine.—A solution of 1.05 g (2 mmoles) of III in 60 ml of ethyl ether and 20 ml of ethyl acetate was treated with a solution of 0.39 ml (2 mmoles) of dicyclohexylamine in 10 ml of ether. The solution was stirred at room temperature and after 1 hr a white precipitate appeared. Stirring was continued overnight and the precipitate was collected by filtration, washed with ether and *n*-hexane, and dried *in vacuo*. The mother liquor was examined by tlc (system A) and was discarded due to the degree of heterogeneity. The solid from the reaction was also observed to contain at least three components and this mixture could not be resolved beyond a recovery of 60 mg (6%) of starting material, mp 92–94°. A mixture melting point was not depressed.

B. With an Excess of Triethylamine.—A slurry of 0.53 g (1 mmole) of III in 10 ml of xylene was stirred at room temperature and treated with 0.5 ml (3.7 mmoles) of triethylamine. The solid dissolved and reappeared as a yellow oil after 3–4 min. The oil was dissolved in ethyl acetate, washed with water, and dried. The oily residue after removal of the solvent *in vacuo* was observed to contain at least five components when examined by tlc (system A).

N-Carboxy-3-({2-[3-(phenyldithio)propionamido]ethyl}dithio)-N-benzyl-L-alanine methyl ester (VIII) was prepared by the esterification of III by the Fisher method. A solution of 0.53 g (1 mmole) of III in 15 ml of anhydrous methanol was treated with 5 drops of concentrated sulfuric acid and stirred at room temperature for 20 hr, then at reflux for 10 min. The solvent was removed *in vacuo* and the residual oil was dissolved in ethyl acetate, washed with 25 ml of 3% sodium bicarbonate solution, water, and saturated sodium chloride solution, and dried. The dried solution was concentrated *in vacuo* and the residue was dissolved in benzene and applied to a 1.5 × 40 cm column of Florisil (40 g, 100 mesh). The elution was monitored by tlc (system A) and the desired material was removed from the column with benzene–ethyl acetate (1:1). The solvent was removed *in vacuo* to provide an oil which crystallized upon drying *in vacuo*. The crude solid was recrystallized from ethyl acetate–*n*-hexane to yield 362 mg (67%) of product, mp 74.5–75° (lit.[6] mp 74.5–75.5°). A mixture melting point with authentic material was not depressed.

Attempted Saponification of VIII.—A solution of 135 mg (0.25 mmole) of VIII in 10 ml of dioxane was treated with 5 ml of 0.05 *N* sodium hydroxide solution and the resulting solution was stirred at room temperature for 2.5 hr. The reaction mixture was cooled, diluted with 100 ml of ethyl acetate, and the organic layer was washed with 0.2 *N* hydrochloric acid, water, and saturated sodium chloride solution. After drying, the solvent was removed *in vacuo* and the residual oil was dissolved in chloroform and examined by tlc (system A). The solution contained at least four components; an attempt to resolve the mixture by column chromatography failed.

N^{ϵ}-Carboxy-N^{α}-{7-[3-(phenyldithio)propionamido]-4,5-dithiaheptanamido}-N^{ϵ}-benzyl-L-lysine benzyl ester (IX) was prepared at −10° by adding 0.94 g (2.5 mmoles) of II and 0.50 g (2.6 mmoles) of WSC to a solution containing 1.0 g (2.5 mmoles) of benzyl N^{ϵ}-carbobenzoxy-L-lysinate hydrochloride[12] and 0.35 ml (2.5 mmoles) of triethylamine in 100 ml of ethyl acetate and 4 ml of DMF. The reaction mixture was allowed to warm to

(12) Purchased from Cyclo Chemical Corp., Los Angeles, Calif.

room temperature while stirring for 10 hr and the solution was washed with water, 2 *N* sulfuric acid, water, and saturated sodium chloride solution before drying. The solvent was removed *in vacuo* and the oily residue was crystallized from ethyl acetate–*n*-hexane. The product appeared as 0.65 g (36%) of white solid: mp 80–82°; homogeneous on tlc (system A); ν_{max}^{KBr} 3300, 3050, 2930, 2860, 1750, 1680, 1645, 1530, 1265, 1180, 1055, 740, 690 cm^{-1}. An additional recrystallization from ethyl acetate–*n*-hexane gave product with mp 81.5–82.5°; $[\alpha]^{24}D$ −26.1° (*c* 1.0, chloroform).

Anal. Calcd for $C_{35}H_{43}N_3O_6S_4$: C, 57.59; H, 5.94; N, 5.76; S, 17.57. Found: C, 57.70; H, 6.08; N, 5.57; S, 17.43.

N,S-Ditrityl-L-cysteine diethylammonium salt (XVIc) was prepared in 60% yield, mp 192–194° (lit.[13] mp 192–193°).

Ethyl N,S-ditrityl-L-cysteinylglycinate (Xc) was prepared by the method of Amiard, *et al.*, and used as a chromatographically pure foam (tlc, system A).

N-Formyl-S-trityl-L-cysteine diethylammonium salt (XVIb) was prepared in 86% yield by the method of Zervas and Photaki,[10] mp 162–165° (lit.[10] mp 165°).

Ethyl N-formyl-S-trityl-L-cysteinylglycinate (Xb) was prepared in 66% yield by the method of Zervas and Photaki,[10] mp 77–79° (lit.[10] mp 78–79°).

N-Benzhydryloxycarbonyl-S-trityl-L-cysteine diethylammonium salt (XVIa) was prepared in 73% yield by the method of Smithwick,[14] mp 165–166° (lit.[14] mp 167.5–169°).

Ethyl N-benzhydryloxycarbonyl-S-trityl-L-cysteinylglycinate (Xa) was prepared by stirring a mixture of 6.47 g (0.01 mole) of XVIa and 1.40 g (0.01 mole) of ethyl glycinate hydrochloride in 50 ml of ethyl acetate at ice temperature for 5 min. To this solution was added 2.1 g (0.011 mole) of WSC and the stirring was continued at 0–5° for 3 hr and at room temperature for 6 hr. The reaction mixture was washed with 100-ml portions of water, 1 *N* sulfuric acid, water, and saturated sodium chloride solution. After drying, the solvent was removed *in vacuo* to yield a white foam which was dissolved in chloroform and applied to a 2.5 × 80 cm column of silica gel (150 g, 0.05–0.2 mm). The elution was monitored by tlc (system A) and the major product was removed from the column with 10% ethyl acetate in chloroform. After removing the solvent *in vacuo*, the white foam weighed 4.8 g (73%). A portion was dissolved in ethyl acetate, precipitated as a viscous oil with *n*-hexane, and triturated with *n*-hexane until it became solid. Careful recrystallization from ether–*n*-hexane gave white needles: mp 137–138° with softening at 134°; ν_{max}^{KBr} 3310, 3055, 3030, 2925, 1730, 1675, 1498, 1210, 1030, 745, 700 cm^{-1}; $[\alpha]^{27}D$ 0.92° (*c* 0.54, chloroform).

Anal. Calcd for $C_{40}H_{38}N_2O_5S$: C, 72.92; H, 5.81; N, 4.25; S, 4.87. Found: C, 73.19; H, 5.96; N, 4.27; S, 4.83.

Ethyl N-benzhydryloxycarbonyl-L-cysteinylglycinate (XI) was prepared by adding a solution of 0.43 g (2.5 mmoles) of silver nitrate in 1 ml of water, 10 ml of acetone, 20 ml of ethanol, and 0.20 g (2.5 mmoles) of Xa in 15 ml of acetone and 15 ml of ethanol. The addition was conducted with rapid stirring and after 5 min the reaction mixture was placed in the dark and allowed to stand at room temperature for 2 hr with occasional swirling. The precipitate was obtained by filtration and washed with ethanol and ether before drying *in vacuo*. The silver mercaptide was slurried in 100 ml of ethyl acetate while hydrogen sulfide was passed through the mixture for 15 min. The silver sulfide was separated by filtration and the filtrate was concentrated *in vacuo* to a gelatinous solid residue. This material was recrystallized from ethyl acetate–*n*-hexane after treatment wih charcoal. The product appeared as 0.52 g: mp 153–159°; nitroprusside positive and homogeneous on tlc (system, A); ν_{max}^{KBr} 3300, 3050, 2970, 2920, 1730, 1690, 1630, 1530, 1260, 1225, 1031, 740, 690 cm^{-1}. The mother liquor was reprocessed to obtain a second crop of product, mp 150–155°, and identical on tlc with the first crop. The combined yield was 0.83 g (80%) of chromatographically pure and nitroprusside-positive product. An analytical sample was prepared by an additional recrystallization from ethyl acetate–*n*-hexane; mp 157–159°; $[\alpha]^{27}D$ −2.58° (*c* 0.56, chloroform).

Anal. Calcd for $C_{21}H_{24}N_2O_5S$: C, 60.56; H, 5.81; N, 6.73; S, 7.70. Found: C, 60.95; H, 5.89; N, 6.77; S, 7.79.

(13) G. Amiard, R. Heymes, and L. Velluz, *Bull. Soc. Chim. France*, 698 (1956).

(14) E. L. Smithwick, Jr., Ph.D. Thesis, University of North Carolina, 1966.

N-[N-Carboxy-3-({2-[3-(phenyldithio)propionamido]ethyl}-dithio)-N-benzhydryl-L-alanyl]glycine ethyl ester (XII) was prepared by the usual thiocyanogen procedure in which the thiocyanogen was generated in 5 ml of ethyl acetate from 0.5 g (1.7 mmoles) of lead thiocyanate and 0.16 g (1 mmole) of bromine. This mixture was stirred at ice temperature and a solution of 416 mg (1 mmole) of XI in 10 ml of methylene chloride was added over a 15-min period. After stirring for 5 min, a solution of 526 mg (1 mmole) of I in 20 ml of methylene chloride was added. This addition required 10 min and the reaction mixture was stirred at 0–5° for 2.5 hr, and at room temperature for 2 hr. The reaction mixture was filtered and the filtrate was washed with cold 5% sodium bicarbonate solution until a negative thiocyanate test was observed with ferric chloride solution. Following washing with water and saturated sodium chloride solution, the solution was dried and the solvent was removed *in vacuo*. The crude product was dissolved in chloroform and applied to a 1.5 × 80 cm column of silica gel (100 g, 0.05–0.2 mm). The elution was monitored by tlc (system C) and the major product was removed with 25% ethyl acetate in chloroform. The solvent was evaporated *in vacuo* and the solid residue was recrystallized from ethyl acetate–*n*-hexane. The product appeared as 364 mg (53%) of white powder: mp 68–70°; homogeneous on tlc (systems A and C); ν_{max}^{KBr} 3400, 3080, 2940, 1750, 1720, 1680, 1650, 1530, 1255, 1180, 1030, 738, 690 cm^{-1}. An additional recrystallization raised the melting point to 69–70°; $[\alpha]^{24}D$ −6.6° (*c* 0.5, $CHCl_3$).

Anal. Calcd for $C_{32}H_{37}N_3O_6S_4$: C, 55.87; H, 5.42; N, 6.11; S, 18.64. Found: C, 55.19, 56.06; H, 5.36, 5.39; N, 5.96; S, 18.83.

Diethyl N,N'-dibenzhydryloxycarbonyl-L-cystinyldiglycinate was isolated from an unsuccessful attempt to prepare XII by the thiocyanogen method. The modified procedure utilized addition periods which were much longer than in the successful procedure. The purification procedure involved column chromatography and the major product was eluted from the column with 10% ethyl acetate in chloroform. The solvent was removed *in vacuo* to yield a white powder, mp 164–167°. This solid was recrystallized from ethyl acetate–*n*-hexane to produce a white powder (0.21 g, 67%) which melted at 166–168°; homogeneous on tlc (system A); ν_{max}^{KBr} 3320, 3055, 2980, 2955, 2930, 1740, 1690, 1660, 1530, 1250, 1040, 765, 745, 695 cm^{-1}; $[\alpha]^{24}D$ −15.8° (*c* 0.5, chloroform).

Anal. Calcd for $C_{42}H_{46}N_4O_{10}S_2$: C, 60.70; H, 5.58; N, 6.86; S, 7.73. Found: C, 60.60; H, 5.71; N, 6.55; S, 7.79.

Attempted Preparation of N-[3-({2-[3-(Phenyldithio)propionamido]ethyl}dithio)-L-alanyl]glycine Ethyl Ester.—When solutions of XII in glacial acetic acid were treated with boron trifluoride etherate to affect removal of the benzhydryloxycarbonyl nitrogen protecting group in the manner described by Hiskey and Smithwick,[9] tlc (system A) indicated that the ninhydrin-positive mixture contained at least four components. Various modifications in the purification procedure failed to resolve this mixture.

When the reaction mixture was lyophilized rather than neutralized as in the previous experiment, a pink residue resulted which could ultimately be resolved to one ninhydrin-positive component (tlc, system B) plus two other smaller components, but the major component could not be isolated and purified.

N-{3-Phenyl-N-[3-(1-thia-5-oxa-4-methylhexyl)-N-{N-carboxy-3-[(2-[3-(phenyldithio)propionamido]ethyl)dithio]-N-benzyl-L-alanyl}-L-alanyl]-L-alanyl}glycine *t*-butyl ester (XIV) was prepared at ice temperature by adding 264 mg (0.5 mmole) of III and 210 mg (0.55 mmole) of WSC in 5 ml of methylene chloride to a solution of 257 mg (0.55 mmole) of *t*-butyl S-isobutoxymethyl-L-cysteinyl-L-phenylalanylglycinate[15] in 5 ml of methylene chloride. The reaction mixture was allowed to warm to room temperature while stirring overnight. The clear solution was diluted with 50 ml of methylene chloride, transferred to a separatory funnel, and washed with 50-ml portions of water, 2 *N* sulfuric acid, water, and saturated sodium chloride solution. After drying, the solvent was evaporated *in vacuo* to produce a white semisolid which was recrystallized from ethyl acetate–*n*-hexane. The product was obtained in two crops as a white powder: 396 mg (81%); mp 115–120°; homogeneous on tlc (system A); ν_{max}^{KBr} 3360, 3050, 2990, 1750, 1700, 1640, 1535, 1250, 1150, 1070, 745, 695 cm^{-1}. An additional recrystallization from ethyl acetate–*n*-hexane raised the melting point to 120–121°, $[\alpha]^{25}D$ −15.0° (*c* 0.5, chloroform).

(15) This material was prepared by Mr. J. T. Sparrow.

Anal. Calcd for $C_{45}H_{61}N_5O_9S_6$: C, 55.36; H, 6.30; N, 7.17; S, 16.42. Found: C, 55.89; H, 6.44; N, 7.15; S, 16.62.

N-[N-Carboxy-3-({2-[3-(phenyldithio)propionamido]ethyl}dithio)-N-benzyl-L-alanyl]-DL-methionine ethyl ester (XV) was prepared at −10° by adding 0.40 g (2.1 mmoles) of WSC to a solution of 1.05 g (2 mmoles) of III and 0.35 g (2 mmoles) of DCC in 40 ml of ethyl acetate. The mixture was stirred at ice temperature for 1 hr, then for 4 hr while warming to room temperature. The solution was transferred to a separatory funnel and washed with 200-ml portions of water, 2 *N* sulfuric acid, water, 5% sodium bicarbonate solution, water, and saturated sodium chloride solution. After drying, the solvent was removed *in vacuo* and the residual oil solidified upon drying *in vacuo*. The crude product was recrystallized from ethyl acetate–*n*-hexane to obtain 0.95 g (70%) of white buttons: mp 85–87°; homogeneous on tlc (system A); ν_{max}^{KBr} 3280, 3050, 2960, 2910, 1735, 1690, 1660, 1270, 1225, 1040, 740, 695 cm^{-1}. Two recrystallizations raised the melting point to 94–95°; $[\alpha]^{24}D$ 4.5° (*c* 1.0, chloroform).

Anal. Calcd for $C_{29}H_{39}N_3O_6S_5$: C, 50.78; H, 5.73; N, 6.13; S, 23.37. Found: C, 51.05; H, 5.91; N, 6.02; S, 23.23.

Ethyl DL-methionate (XVII) was prepared by the thionyl chloride esterification method of Brenner and Huber.[16] Fractional distillation gave product with bp 110–113° (1.7–2.0 mm); $n^{31}D$ 1.4763 (17% of theory based on DL-methionine) (lit.[17] bp 112° (2.5 mm); $n^{20}D$ 1.4819).

Registry No.—V, 15297-41-3; VI, 15297-42-4; 2-tritylthioethylamine hydrochloride, 15297-43-5; VII, 15296-99-8; VIII, 15297-01-5; IX, 15297-32-2; Xa, 15297-02-6; XI, 15297-03-7; XII, 15297-04-8; diethyl N,N-dibenzhydryloxycarbonyl-L-cystinyldiglycinate, 15297-05-9; XIV, 15297-00-4; XV, 15297-06-0.

Acknowledgment.—We are indebted to Dr. Kurt L. Loening of the Chemical Abstracts Service for assistance in the nomenclature of the bisdisulfide derivatives.

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Chemistry of Aliphatic Disulfides. XVI. Studies on the Alkoxide Cleavage of 1,6-Diphenyl-3,4-dithia-1,6-hexanedione[1,2]

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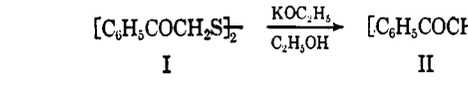
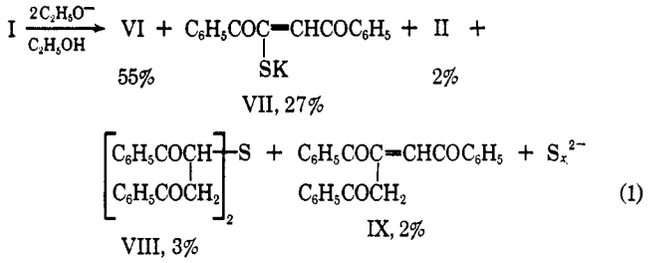
Treatment of 1,6-diphenyl-3,4-dithia-1,6-hexanedione (I) with 2 equiv of potassium ethoxide provides dipotassio-1,4-diphenyl-1,4-butanedione 2,3-dimercaptide (VI) and potassio-1,4-diphenyl-2-butene-1,4-dione 2-mercaptide (VII) as the major products. A mechanism for the formation of VII is proposed to involve VI as the precursor

Groth[4] reported the unique conversion of 1,6-diphenyl-3,4-dithia-1,6-hexanedione (diphenacyl disulfide, I) into 1,4-diphenyl-1,4-butanedione (II) with 1



equiv of potassium ethoxide. Subsequent studies[5,6] confirmed these observations and clarified the structures of other products formed by the action of alkoxides on I. The results of these experiments are summarized in Chart I. It is pertinent to note that the nature of the products resulting from the action of ethoxide ion on I vary with the concentration of base used; at low base concentration 1,5-diphenyl-3-thiapentane-1,5-dione (III) resulted while at higher base concentrations the dipotassio mercaptide, VI, was formed. Since VI was previously proposed as a possible precursor of II, the formation and reactions of this substance were studied in more detail.

Cleavage of I with 2 Equiv of Potassium Ethoxide.—Treatment of 1,6-diphenyl-3,4-dithia-1,6-hexanedione (I) with 2 equiv of potassium ethoxide in ethanol gave dipotassio-1,4-diphenyl-1,4-butanedione-2,3



dimercaptide (VI) in 55.2% yield (eq 1). In addition small amounts of 1,4-diphenyl-1,4-butanedione (II), 3,5-dibenzoyl-1,7-diphenyl-4-thia-1,7-heptanedione (VIII), and 3-benzoyl-1,5-diphenyl-2-pentene 1,5-dione (IX) were isolated. When the aqueous extract of the reaction mixture was adjusted to pH 9 with carbon dioxide, a red-brown, nitroprusside-positive solid was obtained in 26.8% yield. The water-soluble salt was identified as potassio-1,4-diphenyl-2-butene-1,4-dione 2-mercaptide (VII) on the basis of the following evidence. The nmr spectrum of VII in D_2O showed an aromatic multiplet centered at τ 1.27 (3.85 H), a singlet at 2.00 (1.00 H), and an aromatic multiplet centered at 2.30 (6.30 H). The infrared spectrum of the salt showed a broad peak at 1635–1625, a medium peak at 1560, and a broad peak at 1500–1425 cm^{-1}. The infrared spectrum of VII along with its alkylation and acylation products contain a band in the 1560–1550-cm^{-1} region which is assigned as C=C absorption. Although this band falls considerably lower than the usual C=C stretching frequency, there is evidence that sulfur-linked C=C ab-

(1) This investigation was supported by Public Health Service Fellowship 1-F3-GM-31,871-01 and by Research Grant RG 7966 from the National Institutes of General Medical Sciences.
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