Preparation and Mass Spectral Properties of Cystine and Lanthionine Derivatives. A Novel Synthesis of L-Lanthionine by Selective Desulfurization[1]

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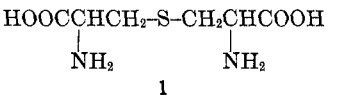
Received April 28, 1970

A useful conversion of certain cystine derivatives to the corresponding L-lanthionine compounds is described. *N,N'*-Dicarbobenzoxy-L-cystine diethyl ester (**9**) and *N,N'*-bis(trifluoroacetyl)-L-cystine dimethyl ester (**7**) were selectively desulfurized in high yield by tris(diethylamino)phosphine to the corresponding L-lanthionine derivatives. The trifluoroacetyl derivative of L-lanthionine was hydrolyzed to optically pure L-(+)-lanthionine (**1**). However, the peptide ethyl *N,N'*-dicarbobenzoxy-*O*-methyl-L-cystinylglycinate (**11**) under the same conditions rearranged to the symmetrical diethyl *N,N'*-dicarbobenzoxy-L-cystinyldiglycinate. The mass spectral fragmentation of cystine, lanthionine, and cysteamine derivatives is also discussed.

Because of the recognized importance of cystine in biological systems, this amino acid has received wide attention.[3] The sulfide analog, lanthionine (**1**), was first isolated as an artifact in wool hydrolysates[4,5] as a mixture of stereoisomers in 1941 and synthesized by du



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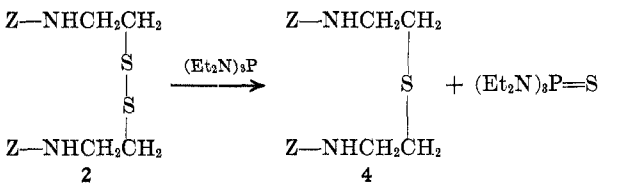
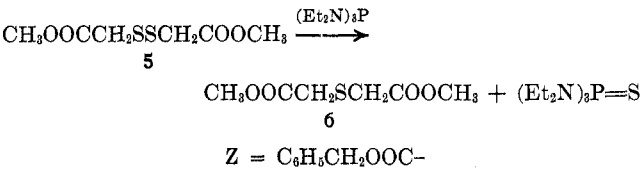
Vigneaud and Brown in the same year.[6] The first report of naturally occurring lanthionine was made in 1966 by Sloane and Untch[7] who isolated both L- and *meso*-lanthionine from the free amino acid pool of chick embryo. Subsequently, L-lanthionine has been found in the antibiotic, Nisin,[8] in the deprotonized haemolymph of various insects,[9] most notably the silkworm and Japanese Oak Moth, and in plant pollen.[10] The absence of the major sulfur-containing amino acids (cystine, cysteine, and methionine) in these sources is interesting. While several synthetic schemes for *meso*- and DL-lanthionine have been reported,[11] the only stereospecific synthesis of L-lanthionine involves the condensation of L-cysteine with methyl L-β-chloroalanate followed by strong alkaline hydrolysis. Low yields, coupled with problems of racemization,[12] render this approach unattractive for the synthesis of larger lanthionine peptides.

Results and Discussion

It appeared to us that selective removal of a sulfur atom from appropriate cystine derivatives would afford a convenient synthetic route to optically pure lanthionine and its derivatives. We have recently found[1,13] that, in simple disulfide systems (*e.g.*, dibenzyl or diamyl disulfide), aminophosphines can effect such a selective desulfurization. Since carboxylic acids are known to react with aminophosphines,[1,14] it was necessary to use cystine derivatives protected as the methyl or ethyl esters for this study. Preliminary work showed that the amide function would not interfere in the desulfurization as *N,N'*-dicarbobenzoxycysteamine (**2**) was desulfurized in 70% yield in refluxing benzene. Similarly, it was demonstrated that the ester function would not interfere since bis(carbomethoxymethyl) disulfide (**5**) was quantitatively desulfurized to the correspond-





ing sulfide **6** in less than 2 min at room temperature.

One cystine derivative chosen for study was *N,N'*-bis(trifluoroacetyl)-L-cystine dimethyl ester (**7**). The trifluoroacetyl (TFA) group was chosen as it can be removed under mild alkaline conditions (0.1 *N* NaOH). In addition, the enhanced volatility provided by the TFA group[15] would allow for a mass spectral study of the cystine and lanthionine derivatives. Disulfide **7** was prepared in 96% yield by the reaction of L-cystine methyl ester hydrochloride with trifluoroacetic anhydride in trifluoroacetic acid.

The desulfurization of disulfide **7** by aminophosphine **3** afforded the corresponding lanthionine derivative **8** in 96% yield, $[\alpha]^{25}D$ −21.6°. Structure proof of **8** obtains from its elemental analysis and mass spectrum (*vide infra*). Mild alkaline hydrolysis of **8** gave a 64% yield of L-(+)-lanthionine (**1**). The infrared spectrum of **1** was identical with that reported[7] for L-(+)-lanthionine and different from both meso and racemic lanthio-

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(1) Organic Sulfur Chemistry. Part IV. For part III, see D. N. Harpp and J. G. Gleason, *J. Org. Chem.*, **35**, 3259 (1970).

(2) Holder of an NRCC Studentship, 1968–1969.

(3) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Wiley, New York, N. Y., 1961, pp 1879–1924.

(4) M. J. Horn, D. B. Jones, and S. J. Ringel, *J. Biol. Chem.*, **138**, 141 (1941).

(5) W. R. Cuthbertson and H. Phillips, *Biochem. J.*, **39**, 7 (1945).

(6) G. B. Brown and V. du Vigneaud, *J. Biol. Chem.*, **140**, 767 (1941); V. du Vigneaud and G. B. Brown, *ibid.*, **138**, 151 (1941).

(7) N. H. Sloane and K. G. Untch, *Biochemistry*, **5**, 2658 (1966).

(8) (a) E. Gross and J. L. Morell, *FEBS Lett.*, 61 (1968); (b) E. Gross and J. L. Morell, *J. Amer. Chem. Soc.*, **92**, 2920 (1970).

(9) D. R. Rao, A. H. Ennor, and B. Thorpe, *Biochemistry*, **6**, 1208 (1967).

(10) V. Rossetti, *Ann. Chim. (Rome)*, **56**, 935 (1966); *Chem. Abstr.*, **66**, 397 (1967).

(11) Reference 3, p 2675.

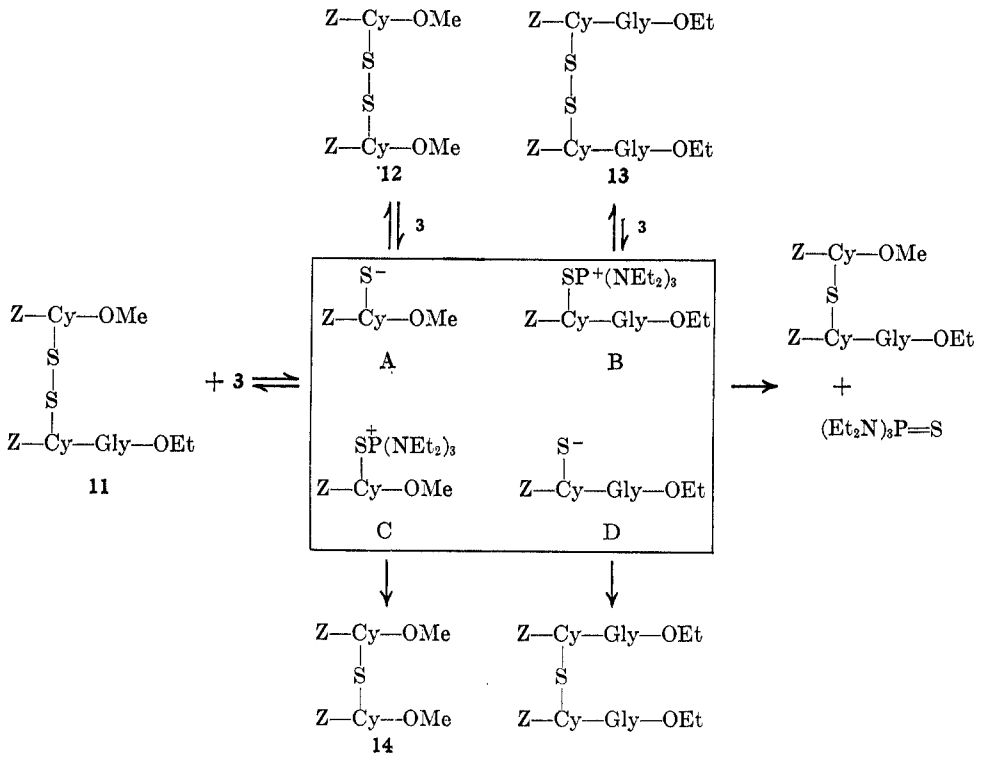
(12) Lanthionine undergoes complete racemization in 3–4 hr in 2.4 *N* NaOH solution; this reaction is much faster than previously reported.[7]

(13) D. N. Harpp, J. G. Gleason, and J. P. Snyder, *J. Amer. Chem. Soc.*, **90**, 4181 (1968).

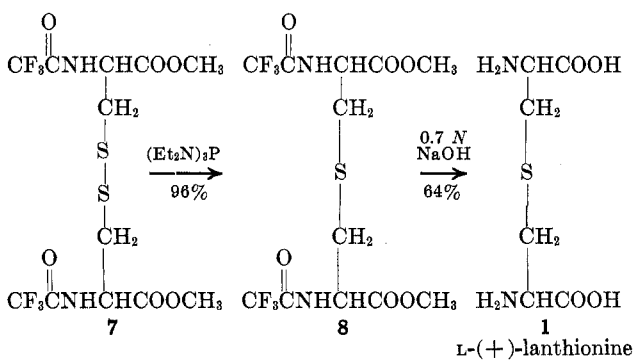
(14) R. Burgada, *Ann. Chim. (Rome)*, 347 (1963).

(15) F. Weygand, A. Prox, E. C. Jorgensen, R. Axen, and P. Kirchner, *Z. Naturforsch. B*, **18**, 93 (1963).

SCHEME I



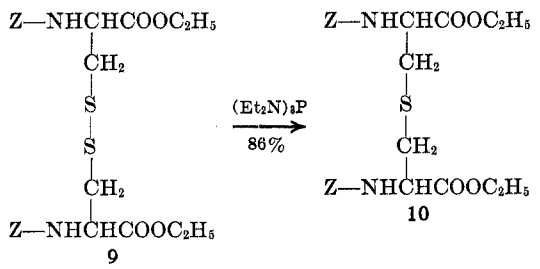
nine. The optical rotation of **1** in acid, $[\alpha]^{25}_{578}$ +4.0°, compares favorably with that previously reported[9]



L-(+)-lanthionine

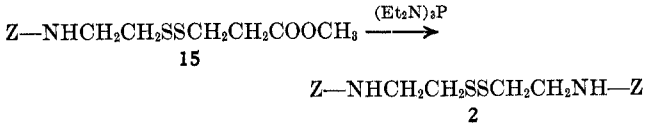
($[\alpha]^{25}_{578}$ +2.36°, +5.00°). Measurement of the optical rotation in base (2.4 *N* NaOH) gave a value of +9.4° (lit.[7] $[\alpha]_D$ +8.4°). On the basis of the above data, we conclude that this material is of high optical purity.

The versatility of the carbobenzoxy group makes lanthionine derivative **10** a useful starting material for peptide synthesis. This compound was prepared in 86% yield by desulfurization of *N,N'*-dicarbobenzoxy-L-cystine diethyl ester (**9**).



We felt it of considerable interest to examine the desulfurization of some unsymmetrical disulfides since most naturally occurring cystine peptides are of this type. An attempt was made to desulfurize the unsymmetrical peptide **11**; however, only the symmetrical disulfide **13** could be isolated. This observation would suggest that a phosphine-catalyzed equilibration[16] of disulfides **11**, **12**, and **13** (Scheme I) takes place. Presumably the extreme insolubility of **13** removes it from the reaction as rapidly as it is formed, while the formation of sulfide **14** proceeds *via* the remaining ion fragments A and C. As a result, the major products of the reaction are disulfide **13**, sulfide **14**, and phosphine sulfide.

A similar result was obtained for the unsymmetrical disulfide **15**. As in the case of peptide **11**, the symmetrical disulfide **2** was isolated in 88% yield. Since in this case no tris(diethylamino)phosphine sulfide was observed, equilibration of these disulfides must occur much faster than does desulfurization.



Mass Spectral Properties.—As might be expected, many of the spectral properties of the cystine and lanthionine derivatives are very similar. However, the fragmentation reactions which occur under electron impact in the mass spectrometer should be quite different.[17] To determine the effect of the sulfide and disulfide groups on the fragmentation of cystine and lanthionine derivatives, a detailed examination of the mass spectra of the TFA derivatives **7** and **8**, the carbo-

(16) The equilibration of disulfides **11**, **12**, and **13** may also occur *via* a mercaptide exchange reaction; see, for example, G. Dalman, J. McDermed, and G. Gorin, *J. Org. Chem.*, **29**, 1480 (1964).

(17) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967, pp 276–296.

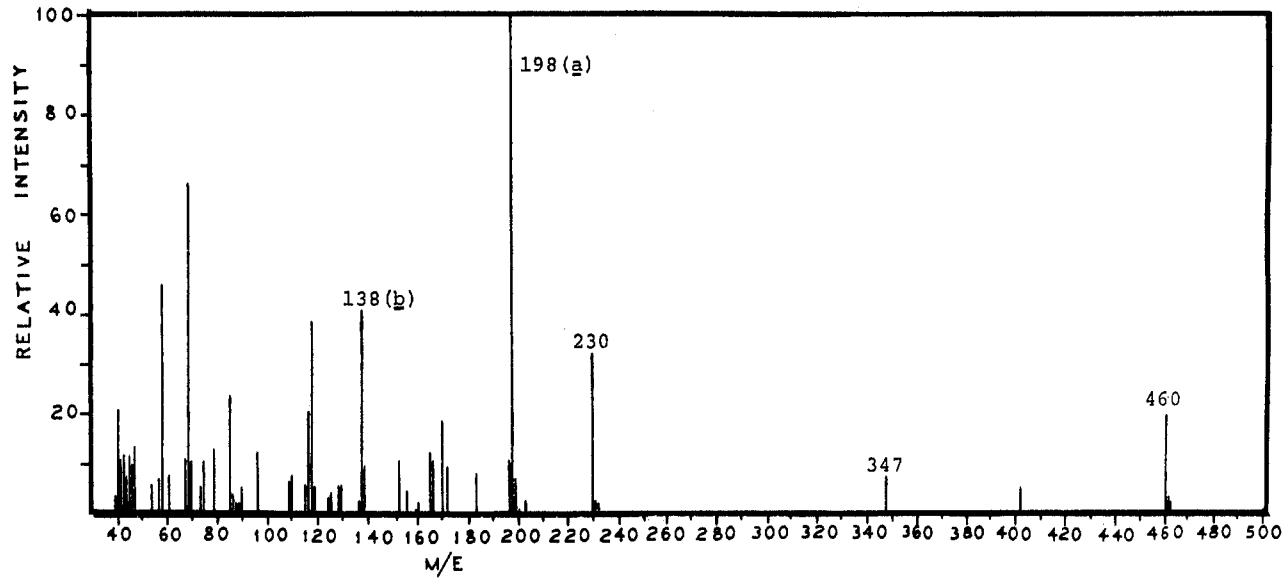


Figure 1.—*N,N'*-Bis(trifluoroacetyl)-L-cystine dimethyl ester (**7**).

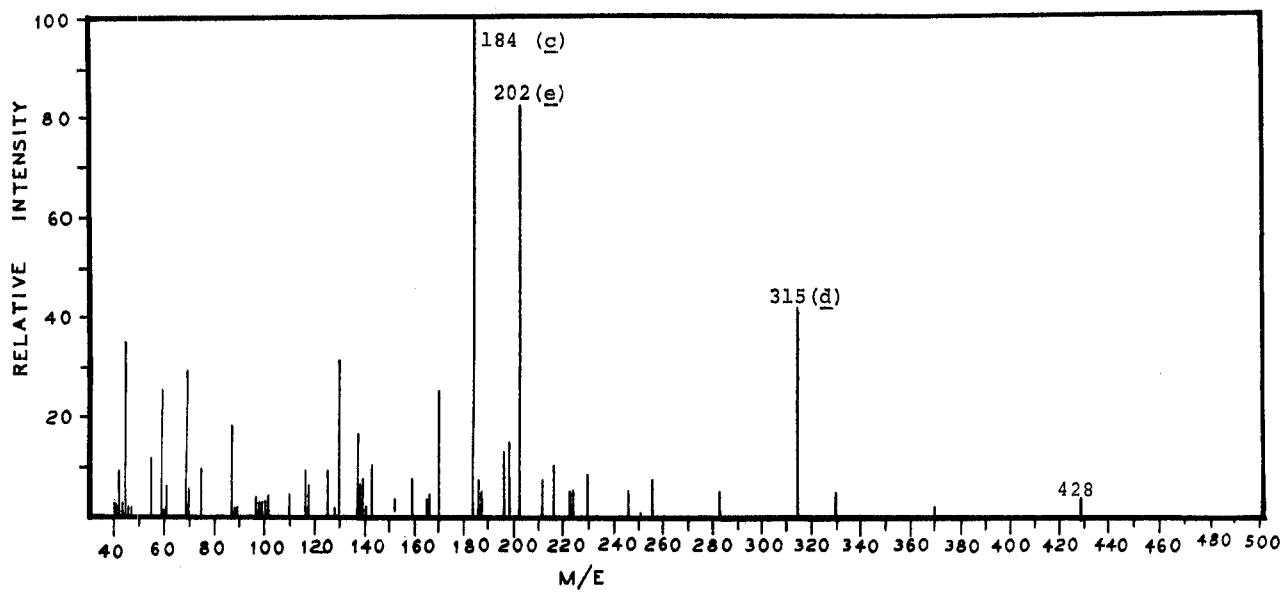
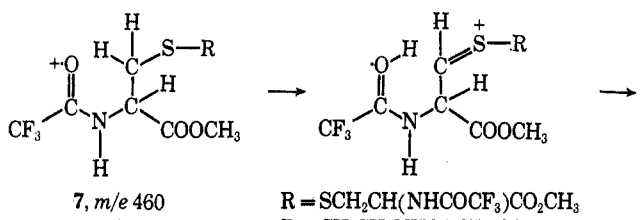


Figure 2.—*N,N'*-Bis(trifluoroacetyl)-L-lanthionine dimethyl ester (**8**).

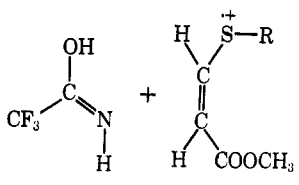
benzoxy derivatives **9** and **10**, and cysteamine derivatives **2**, **4**, and **15** was undertaken. The mass spectrum of the trifluoroacetylcystine dimethyl ester **7** (Figure 1) showed an intense molecular ion at *m/e* 460 (20%) with the base peak at *m/e* 198 arising from cleavage α to the disulfide (Scheme II). This ion a may be formulated as either an open chain ion a_1 or as an oxazoline ion a_2.[18]

The formation of ion a appears unique in that it is not observed in other acetyl and trifluoroacetyl amino acid esters.[19] The mass spectrum of *N,N'*-bis(trifluoroacetyl)-L-lanthionine dimethyl ester (**8**) (Figure 2) is radically different from the spectrum of the cystine derivative **7**. Here the major fragmentation (Scheme III) occurs β to the sulfide to form ion c, a process which is common to most acyl amino acid esters.[19a] Of more interest in the spectrum of **8** is the



7, *m/e* 460
8, *m/e* 428

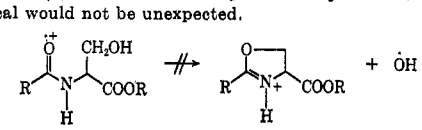
R = $SCH_2CH(NHCOCF_3)CO_2CH_3$
R = $CH_2CH(NHCOCF_3)CO_2CH_3$



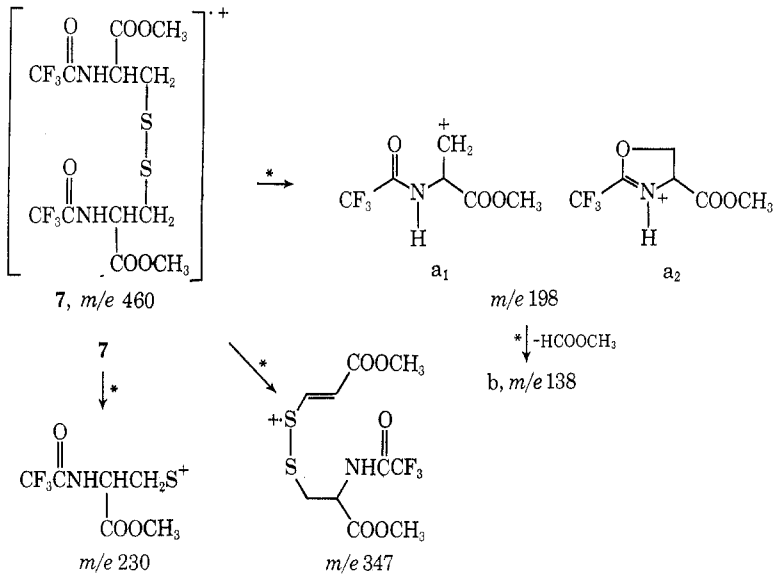
m/e 347
m/e 315

(18) It would appear from preliminary deuterium-labeling studies that both ions a_1 and a_2 are formed since *N,N'*-dideuterio-**7** (from **7** by D_2O exchange) shows the loss of both methyl formate and methyl formate-d_1 in the fragmentation process 198 → 138.

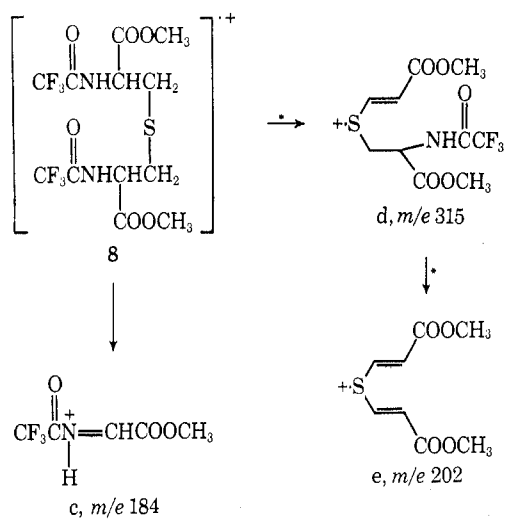
(19) (a) K. Heyns and H. F. Grützmacher, *Justus Liebigs Ann. Chem.*, **698**, 24 (1966). (b) This includes acetylserine ethyl ester where the loss of an OH radical would not be unexpected.



SCHEME II



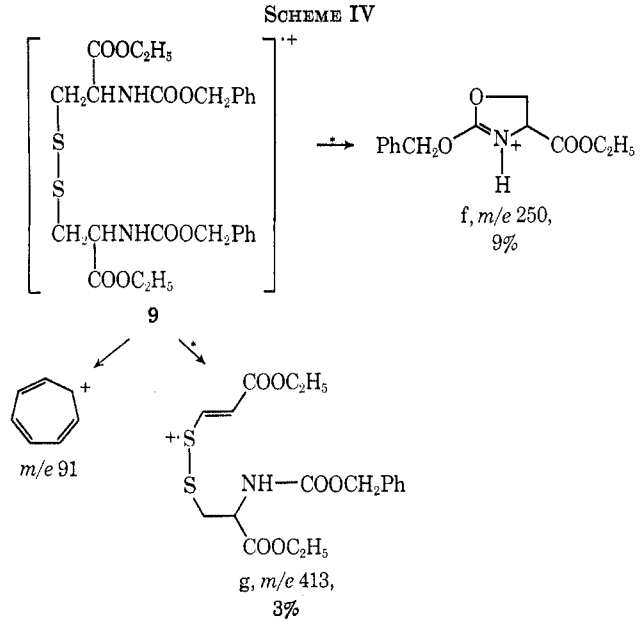
SCHEME III



loss of the elements of trifluoroacetamide to form ion d of *m/e* 315 which is 40% of the base peak. This ion subsequently loses another trifluoroacetamide molecule to form an intense ion e at *m/e* 202. The formation of d presumably results from hydrogen migration and cleavage of the C–N bond. This fragmentation is analogous to a McLafferty rearrangement;[20] however, unlike the McLafferty rearrangement, the charge is retained on the olefin fragment. It should be noted that no normal McLafferty rearrangement occurs as evidenced by the lack of an ion at *m/e* 113, $(CF_3CONH_2)\cdot^+$. This unusual fragmentation is observed only in special circumstances, as, for example, in the fragmentation of *N*-acetyl-β-phenylalanine esters to form styrene esters.[19a,20b]

While the formation of the oxazoline ion is the major pathway for the trifluoroacetyl disulfide **7** with this "reversed"[21] McLafferty rearrangement occurring to a small degree, exactly the opposite behavior is observed for the sulfide. This dichotomy of behavior must be due to an inherent difference between sulfide and disulfide groups. To further explore this mass spectral behavior, the spectra of several analogous *N*-carbobenzoxy derivatives were examined. The mass spectrum of *N*,*N*′-dicarbobenzoxy-L-cystine diethyl ester (**9**) (Figure 3) exhibited both oxazoline formation (ion f, *m/e* 250, 9%) and reversed[21] McLafferty rearrangement (ion g, *m/e* 413, 3%) (Scheme IV). As was the case for disulfide **7**, oxazoline formation predominates, here in a ratio of 3:1.

SCHEME IV



In contrast, relatively little oxazoline formation is observed in the mass spectrum (Figure 4) of the lanthionine derivative **10**; the reversed McLafferty rearrangement is the major fragmentation process (h, *m/e* 381, and i, *m/e* 230). This parallels the observations in the TFA derivatives.

The mass spectra of several structurally analogous cysteamine derivatives were studied to further explore this sulfide–disulfide dichotomy. The mass spectrum

(20) (a) Reference 17, p 155; (b) F. W. McLafferty, "Interpretation of Mass Spectra," W. A. Benjamin, New York, N. Y., 1966, pp 123–131. (c) While we have not yet been successful in verifying β-hydrogen transfer, there appears to be no other logical pathway.

(21) The term "reversed" is used here to emphasize that the charge resides on the olefin fragment in contrast to the normal McLafferty rearrangement.

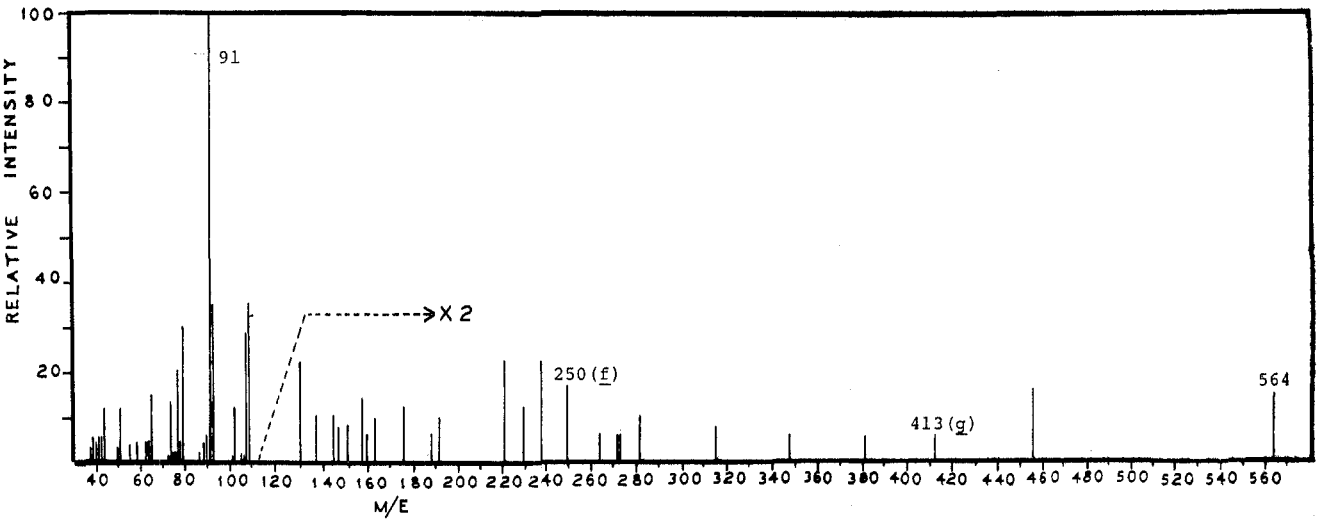


Figure 3.—*N*,*N*′-Dicarbobenzoxy-L-cystine diethyl ester (**9**).

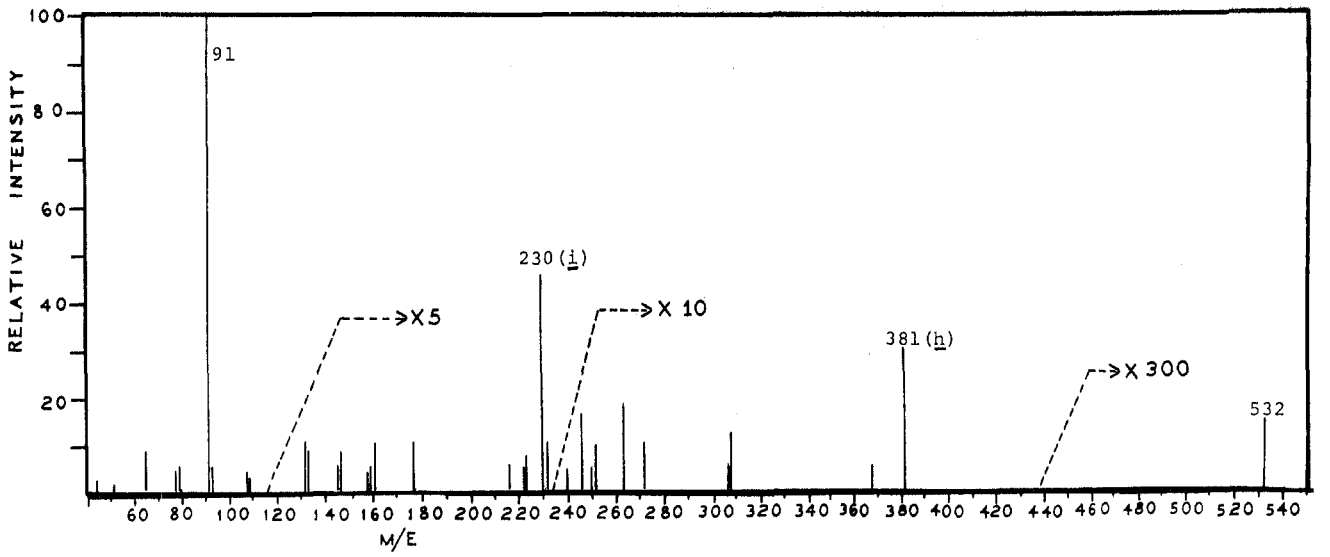
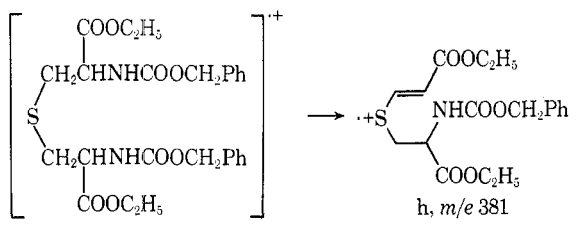
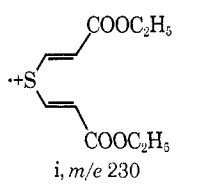


Figure 4.—*N*,*N*′-Dicarbobenzoxy-L-lanthionine diethyl ester (**10**).



10

h, *m/e* 381

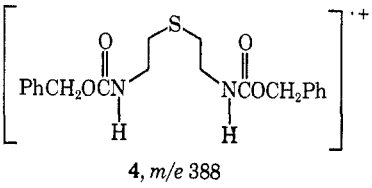


i, *m/e* 230

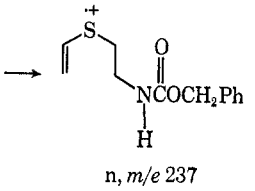
of *N*,*N*′-dicarbobenzoxycysteamine (**2**) (Figure 5) had a strong ion at *m/e* 178 (8%) corresponding to the oxazoline ion (Scheme V) and a smaller ion at *m/e* 269 (1%) corresponding to a reversed McLafferty rearrangement.[21] The predominance of oxazoline formation again parallels the observation in the cystine series. Other ions observed in this spectrum and their origins are shown in Scheme V. Note that the oxazoline ion may arise from several pathways, although only path a (**2** → j → k) and path b (**2** → k) appear to be of major importance.

The mass spectrum of the unsymmetrical disulfide, *N*-carbobenzoxy-2-aminoethyl 2′-carbomethoxyethyl disulfide (**15**) (Figure 6), possessed a strong peak at *m/e* 178. This peak may be ascribed to *either* oxazoline ion l or ion m resulting from reversed McLafferty rearrangement. A high resolution spectrum of *m/e* 178 showed clearly the presence of two ions; the major ion at *m/e* 178.0879 (85%) was the oxazoline ion l (calcd for $C_{10}H_{12}NO_2$: 178.0868), while the minor ion (15%), *m/e* 178.0128 (calcd for $C_6H_{10}O_2S_2$: 178.0122), corresponded to the fragment m resulting from the reversed McLafferty rearrangement (Scheme VI).

In contrast to the behavior of **2** and **15**, the sulfide derivative of **2**, *N*,*N*′-dicarbobenzoxy-2,2′-diaminodiethyl sulfide (**4**) (Figure 7), showed very little oxazoline



4, *m/e* 388



n, *m/e* 237

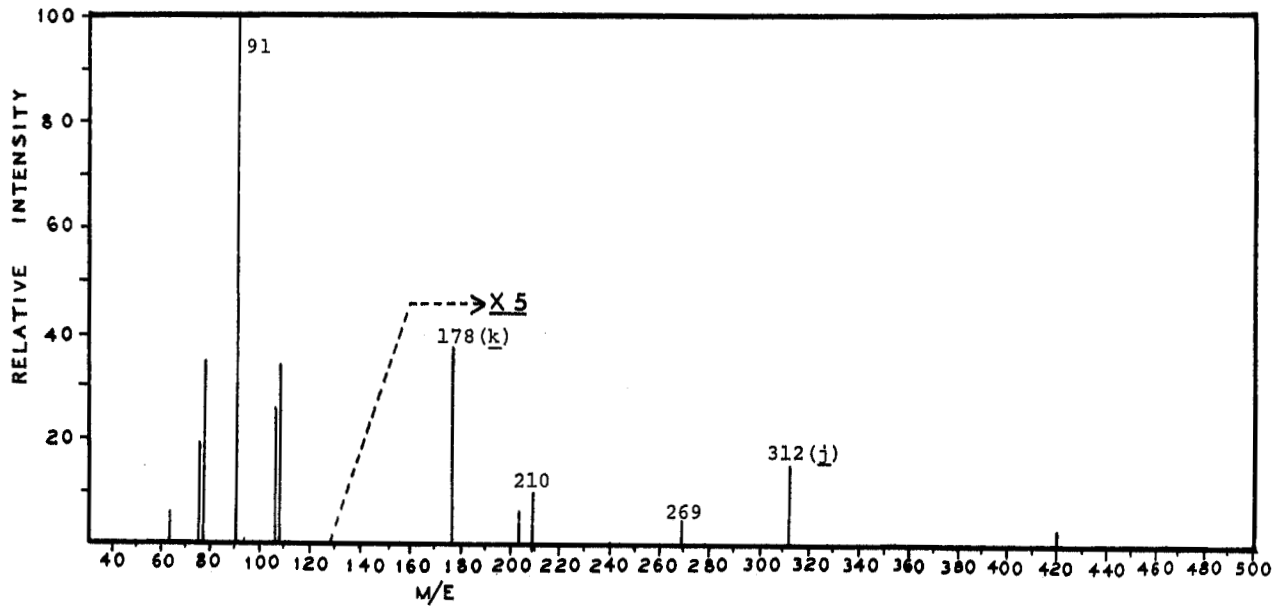
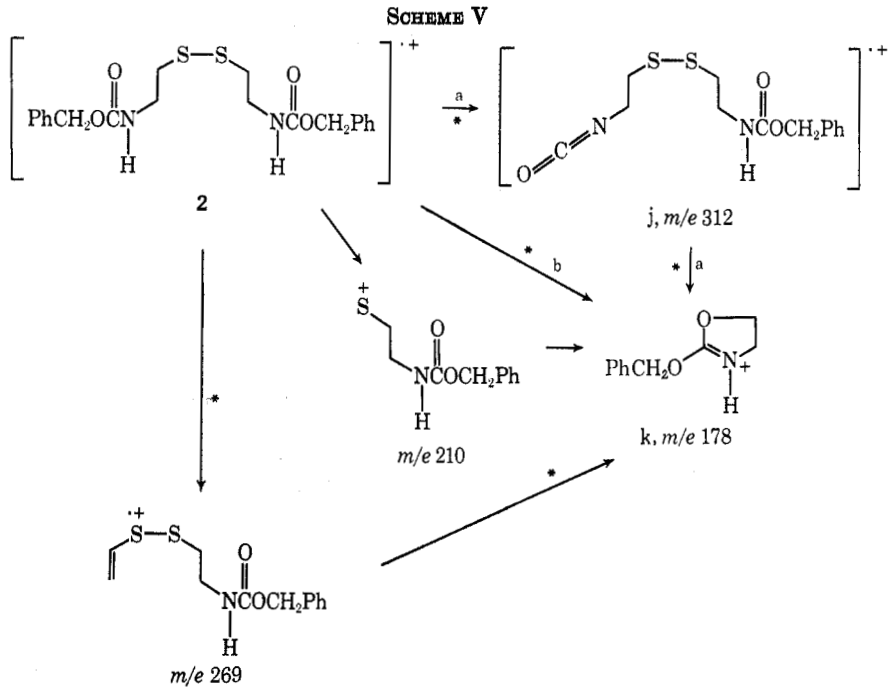
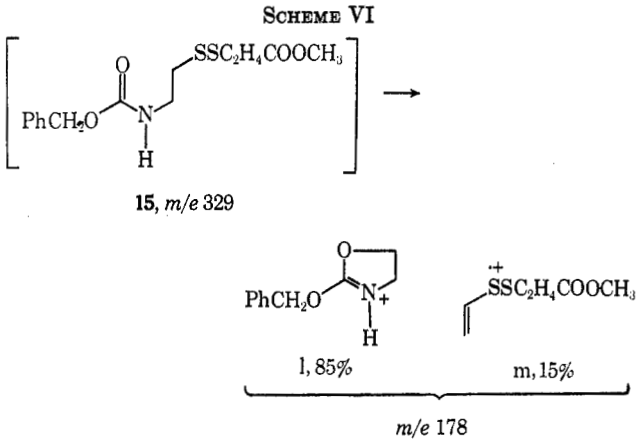


Figure 5.—*N,N'*-Dicarbobenzoxycysteamine (2).

SCHEME V



SCHEME VI



formation, but again showed only the formation of the vinyl sulfide ion n at *m/e* 237 (5%).

The major difference in the spectra of the sulfides as compared with the corresponding disulfides lies in the relative amounts of the oxazoline to reversed McLafferty processes (Table I). The ratio is high for the di-

TABLE I
ION ABUNDANCES

Compd	Parent ion, %	Oxazoline ion (X), %	Vinyl sulfide (disulfide) ion (Y), %	X/Y
		Disulfides		
7	19	100	9	11
9	7	9	3	3
2	0.4	8	2	4
15	3	4	0.7	6
		Sulfides		
8	4	15	42	0.35
10	0.005	0.5	3	0.2
4		0.5	5	0.1

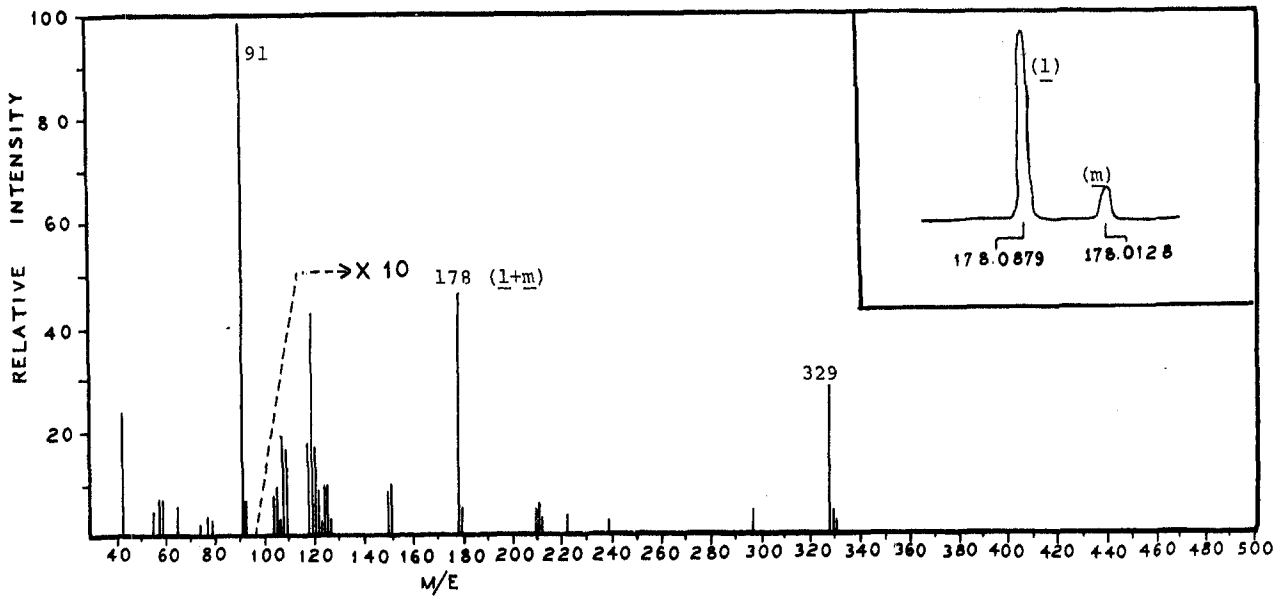
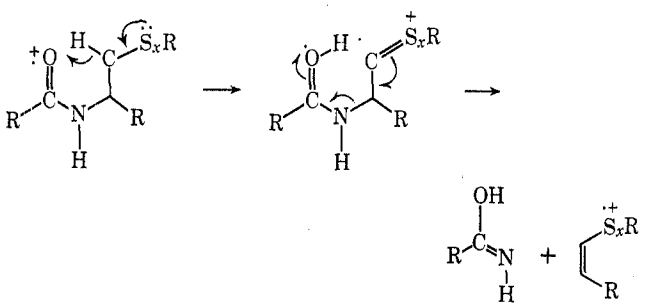


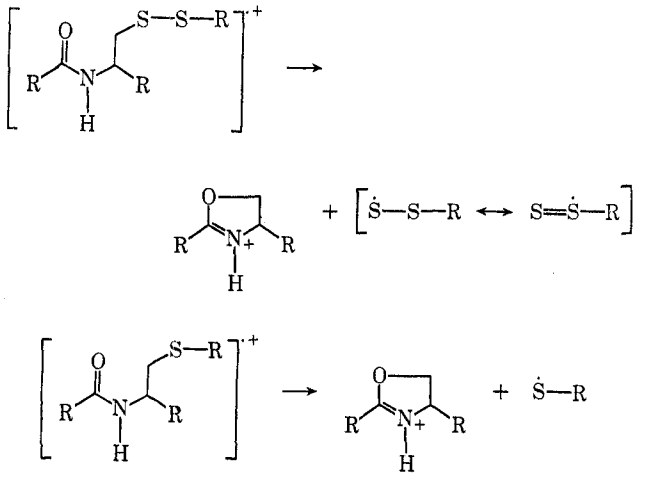
Figure 6.—*N*-Carbobenzoxy-2-aminoethyl 2′-carbomethoxyethyl disulfide (15).

sulfides and low for the sulfides. This difference between disulfides and sulfides could be the result of two additive effects. The electron-donor ability of sulfur would assist in the transfer of a hydrogen to the carbonyl oxygen during vinyl sulfide (disulfide) formation. Sulfides, better electron donors than disulfides,[22] would



$x = 1, 2$

be more likely to undergo this reversed McLafferty rearrangement. In contrast, the increased stability of the sulfthiyl radical (RSS·) over the thiyl radical (RS·) (which has been attributed to both inductive and resonance effects)[23] would result in the preferred formation of the oxazoline ion from disulfides rather than from sulfides.



Experimental Section

Melting points were determined on a Gallenkamp block and are corrected. Mass spectra were obtained on an AEI-MS-902 mass spectrometer at 70 eV using a direct-insertion probe. Nmr spectra were recorded on a Varian Associates A-60 spectrometer. Optical rotations were measured on a Carl Zeiss photoelectric precision polarimeter.

***N,N′*-Dicarbobenzoxy-2,2′-diaminodiethyl Sulfide (4).**—To a suspension of 0.210 g (0.5 mmol) of *N,N′*-dicarbobenzoxycysteamine[24] (2) in 2 ml of dry benzene was added 0.20 g (0.8 mmol) of tris(diethylamino)phosphine. After the mixture was refluxed for 4 hr, the reaction was diluted with 25 ml of hexane. On standing, colorless crystals were obtained, 0.131 g (68%), mp 99–100°, which after crystallization from ethanol afforded an analytical sample: mp 99–100°; ir (KBr) 3150 (NH) and 1680 cm^{-1} (OCONH).

Anal. Calcd for $C_{20}H_{24}N_2O_4S$: C, 61.83; H, 6.22; N, 7.21; S, 8.25. Found: C, 61.81; H, 6.25; N, 7.04; S, 8.39.

Bis(carbomethoxymethyl) Sulfide (6).—To a solution of 4.20 g (17.5 mmol) of bis(carbomethoxymethyl) disulfide (5) in 10 ml of dry benzene was added slowly 6.0 g (24.3 mmol) of tris(diethylamino)phosphine. When the exothermic reaction was complete (about 2 min), the solvent was removed *in vacuo* and the residue distilled to afford 2.96 g (84%) of the sulfide 6: bp 82–84° (0.1 mm); nmr (CCl_4) τ 6.24 (singlet, 3 H), 6.62 (singlet, 2 H); ir (film) 1730 cm^{-1} (–COO–). Upon oxidation with hydrogen peroxide, the sulfide 6 yielded a crystalline sulfone, mp 111–112° (lit.[25] mp 114–116°).

***N,N′*-Bis(trifluoroacetyl)-L-cystine Dimethyl Ester (7).**—A suspension of 4.50 g of cystine dimethyl ester hydrochloride in 15 ml of trifluoroacetic acid was cooled to −5°; 10 ml of trifluoroacetic anhydride was added dropwise. The resulting solution was stirred for 1 hr at −5° and then 1 hr at room temperature. The reaction mixture was poured over 200 ml of ice–H_2O; the mixture was stirred for 10 min and filtered; the crystalline product was washed well with water and then dried *in vacuo* to yield 6.2 g (95%) of white crystals: mp 152–154°; $[\alpha]^{25}D$ −183° (*c* 2.5, MeOH) (lit.[15] mp 152–153°; $[\alpha]^{25}D$ −194°).

***N,N′*-Bis(trifluoroacetyl)-L-lanthionine Dimethyl Ester (8).**—To a suspension of 2.30 g (5.0 mmol) of disulfide in 25 ml of dry benzene was added slowly 1.40 g (5.5 mmol) of tris(diethylamino)phosphine. The resulting mixture was stirred under N_2 for 10 min. The suspended amide slowly dissolved and then reprecipitated as a gel. After addition of 50 ml of hexane, the re-

(22) M. Good, A. Major, J. Nog-Chaudhuri, and S. McGlynn, *J. Amer. Chem. Soc.*, **83**, 4329 (1961).

(23) E. Muller and J. B. Hyne, *ibid.*, **91**, 1907 (1969).

(24) We acknowledge the generous gift of this compound from Professor Richard G. Hiskey.

(25) H. J. Backer and W. Stevens, *Recl. Trav. Chim. Pays-Bas*, **59**, 444 (1940).

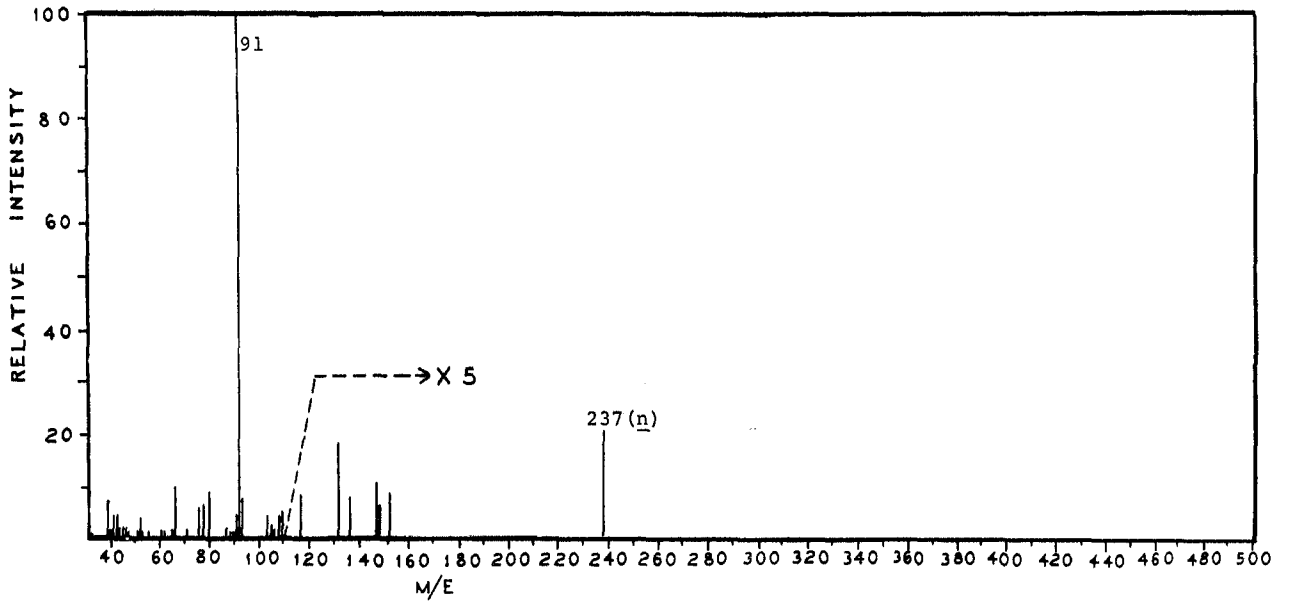


Figure 7.—*N,N'*-Dicarbobenzoxy-2,2'-diaminodiethyl sulfide (4).

sulting suspension was filtered and the white crystals were washed well with hexane to yield 2.07 g (96%) of white crystals, mp 103–109°.

After three recrystallizations from aqueous methanol, an analytical sample was obtained: mp 117–118°; $[\alpha]^{25}D$ −32.4° (*c* 0.4 MeOH); ir (KBr) 3300 (NH), 1760 (-COO-), and 1705 cm^{-1} (CONH).

Anal. Calcd for $C_{12}H_{14}N_2O_6SF_6$: C, 33.57; H, 3.30; N, 6.54; S, 7.49; F, 26.63. Found: C, 33.92; H, 2.91; N, 6.59; S, 7.61; F, 27.05.

L-(+)-**Lanthionine** (1).—A solution of 1.290 g (3.0 mmol) of the bis(trifluoroacetyl)lanthionine dimethyl ester **8** in 15 ml of dioxane was cooled to 0° in an ice bath; 27 ml of 1.0 *N* NaOH was added slowly. After 0.5 hr at 5°, the mixture was acidified with 12 ml of 2 *N* HCl. After adjusting the pH to 6.0, the solvent was removed under vacuum. To the residue was added 15 ml of H_2O and the crystalline L-(+)-lanthionine was collected by filtration and dried *in vacuo*, yield 0.398 g (64%) of white crystals: mp 295–296° dec; $[\alpha]^{25}D$ +9.4° (*c* 1.4, 2.4 *N* NaOH) (lit.[7] mp 295° dec, $[\alpha]^{25}D$ +8.4°); $[\alpha]^{25}_{578}$ +4.0° (*c* 1.0, 1 *N* HCl) (lit.[9] $[\alpha]^{25}_{578}$ +2.36°, +5.00°). The infrared spectrum of this material was identical with that reported[7] for L-(+)-lanthionine.

N,N'-**Dicarbobenzoxy-L-lanthionine Diethyl Ester** (10).—To a suspension of 2.261 g (4.0 mmol) of *N,N'*-dicarbobenzoxy-L-cystine diethyl ester[24] (9) in 10 ml of dry benzene was added slowly 1.20 g (4.8 mmol) of tris(diethylamino)phosphine. An exothermic reaction occurred and the peptide dissolved. After the mixture was stirred for 1 hr, the solvent was removed under vacuum and the residue chromatographed over silica gel. The phosphine sulfide (1.09 g, 99%) was eluted with 9:1 hexane–ethyl acetate, followed by a small amount of impurities (0.05 g). Elution with 1:1 hexane–ethyl acetate afforded a colorless oil which on standing crystallized to give 1.83 g (86%) of white crystals, mp 63–67°, which after three recrystallizations from cyclohexane afforded an analytical sample: mp 67–68°; $[\alpha]^{25}D$ −15.9° (*c* 1.1, MeOH); ir (KBr) 3320 (NH), 1750 (-COO-), and 1690 cm^{-1} (-OCONH). The infrared spectrum of the analytical sample was identical with that of the crude (mp 63–67°) crystals obtained from the column.

Anal. Calcd for $C_{26}H_{32}N_2O_8S$: C, 58.62; H, 6.06; N, 5.26; S, 6.02. Found: C, 58.68; H, 6.20; N, 5.37; S, 6.22.

Reaction of Ethyl *N,N'*-**Dicarbobenzoxy-***O*-**methyl-L-cystinylglycinate** (11) **with Tris(diethylamino)phosphine.**—A suspension of 131 mg (0.22 mmol) of **11**[24] and 100 mg (0.4 mmol) of phosphine **3** in 100 ml of anhydrous ether was stirred for 2 hr during which time the texture of the suspension changed. Filtration afforded a white crystalline material, 60 mg (82% based on complete conversion of **11** to **13**), mp 165–170°, which was identical (melting point, ir, and nmr) with that of the authentic disulfide **13**. The tlc (chloroform) of the filtrate of **13** showed the presence of tris(diethylamino)phosphine sulfide and sulfide **14**, both identified by comparison with authentic samples.

N-**Carbobenzoxy-2-aminoethyl 2'-Carbomethoxyethyl Disulfide** (15).—A solution of 0.50 g (1.57 mmol) of 3-[2-(*N*-carbobenzoxy)aminoethyl]dithiopropanoic acid and 1.0 ml of phosphorus trichloride in 10 ml of chloroform was stirred at room temperature for 1 hr. The excess phosphorus trichloride and chloroform were removed under vacuum and the residue diluted with 10 ml of methanol. After the mixture was stirred for 10 min, the solvent was removed under vacuum and the residue chromatographed over silica gel. Elution with chloroform afforded an oil which resisted all attempts at crystallization. Removal of all traces of solvent *in vacuo* afforded a colorless oil, 0.405 g (70%), which was homogeneous on tlc (silica gel, $CHCl_3$): ir (film) 3180 (NH) and 1720 cm^{-1} (broad, -OCO- and OCONH); nmr ($CDCl_3$) τ 2.58 (singlet, 5 H, aromatic), 4.6 (broad, 1 H, NH), 4.80 (singlet, 2 H, benzylic), 6.21 (singlet, 3 H, $-OCH_3$), 6.40 (quartet, 2 H, $-CH_2N$), 7.1 (multiplet, 6 H); mass spectrum, parent ion at *m/e* 329.0757 (calcd for $C_{14}H_{19}NO_4S_2$: 329.0755).

Anal. Calcd for $C_{14}H_{19}NO_4S_2$: C, 51.05; H, 5.81; N, 4.25; S, 19.43. Found: C, 50.81; H, 5.59; N, 4.36; S, 19.22.

Reaction of Disulfide 15 with Tris(diethylamino)phosphine.—To a solution of 0.33 g (1.0 mmol) of **15** in 3 ml of dry benzene was added 0.30 g (1.2 mmol) of tris(diethylamino)phosphine. A white precipitate which formed immediately on addition of the phosphine was obtained by filtration as colorless crystals, 0.184 g (88% based on complete conversion of **15** to **2**): mp 124–124.5°; mmp 124–125°, identical (ir, nmr) with that of the authentic disulfide **2**.

Registry No.—**1**, 922-55-4; **2**, 26542-61-0; **4**, 26630-73-9; **6**, 16002-29-2; **7**, 26527-24-2; **8**, 26527-25-3; **9**, 26527-26-4; **10**, 26527-27-5; **13**, 2790-85-4; **15**, 26599-16-6.