8.04 (O-C_6H_4), 9.60, and 9.80 μ (O-CH_3); nmr ($CDCl_3$) δ 2.73 (s, 6 H, =S(CH_3)$_2$, 3.82 (s, 3 H, -OCH_3), 6.85 (d, 2 H, aromatic), and 8.03 ppm (d, 2 H, aromatic).

Anal. Calcd for $C_{10}H_{13}NO_2S$: C, 56.85; H, 6.20; N, 6.63; S, 15.18. Found: C, 56.54; H, 5.92; N, 6.70; S, 14.86.

N-(*p*-Methoxybenzoyl)-1-propanesulfenamide (6).—*n*-Propyl sulfide (5.91 g, 0.05 mol) was added to 10 g (0.05 mol) of methyl N-chloro-*p*-methoxybenzimidate during 10 min. There was only very slight exotherm of reaction (2° rise) and no gas evolved under these conditions. The resultant reaction mixture was slowly heated to 135° during 3.5 hr and maintained at 135–137° temperature for 2.0 hr. During this time the theoretical quantity (1120 ml) of methyl chloride (identified by mass spectrometry) had been collected. The reaction product was filtered to give a tacky, pale yellow solid which was washed with cyclohexane and dried to yield 11.30 g (100%). This product was extracted with five 400-ml portions of boiling petroleum ether (63–75°). The yellow insoluble amorphous residue was discarded and the extracts were allowed to stand for 2 days. The crystallized product was collected by filtration and dried to yield 3.45 g (31%), mp 86.5–89°. An analytical sample was prepared by recrystallization of 0.5 g from 200 ml of petroleum ether (63–75°): yield 0.43 g; mp 92–93.5°; ir (KBr) 3.05 (N-H), 3.52 (O-CH_3), 6.08 (amide C=O), 6.24, 6.36, and 6.66 μ (aromatic C=C); nmr ($CDCl_3$) δ 0.99 (t, 2 H, -SCH_2CH_2-), 1.64 (sextet, 2 H, -$CH_2CH_2CH_3$), 2.79 (t, 3 H, -CH_2CH_3), 3.82 (s, 3 H, CH_3-OC_6H_4-), 6.89 (m, 2 H, aromatic), 7.45 (s, 1 H, -NH-), and 7.82 ppm (m, 2 H, aromatic).

Anal. Calcd for $C_{11}H_{15}NO_2S$: C, 58.64; H, 6.71; N, 6.22. Found: C, 58.62; H, 6.74; N, 6.42.

Registry No.—**1** *syn*, 23632-48-6; **1** *anti*, 23632-47-5; **2** *syn*, 24978-55-0; **2** *anti*, 25024-02-6; **3**, 19397-91-2; **3** (HCl), 24978-57-2; **4**, 25024-03-7; **5**, 24978-58-3; **6**, 24978-59-4; **7**, 23847-33-8.

Nucleophilic Scission of Disulfides by Selenolate. Synthesis and Some Properties of Acyclic Thiolselenenates[1,2]

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Received January 27, 1970

Various types of compounds possessing a sulfur–sulfur bond, *e.g.* diaryl disulfides (ArSSAr), dialkyl disulfides (RSSR), sulfenyl thiocyanates (RSSCN), sulfenyl sulfites ($RSSO_3^-$), and sulfenyl thiosulfates ($RSS_2O_3^-$), are susceptible to nucleophilic attack.[3] The ionic scission of sulfur–sulfur bonds by nucleophilic agents has been repeatedly investigated and, on the basis of kinetic studies, appears to involve an S_N2 mechanism.[4]

This paper describes the scission of disulfides by selenolate. As an *a priori* consideration, this type of reaction would be expected to proceed readily. On the one hand the valence shell electrons of selenium are highly polarizable,[5] resulting in highly nucleophilic species;[6,7] on the other hand sulfur in a disulfide bridge is capable of using the empty 3d orbitals in the transition state, facilitating nucleophilic scission of S–S bonds.[4] Added interest in this type of reaction stemmed from a possible general application of this method for the synthesis of thiolselenenates, particularly those which are aliphatic and acyclic in nature; it appears that the first compound of this type, *viz.* 1-amino-3-selena-4-thiatetradecane, was prepared only recently.[8]

In this study 2-naphthylsulfenylthiocyanate (**2**),[9] a relatively stable compound among the labile sulfenylthiocyanates, was allowed to react with the selenolate of N-carbobenzoxy-L-selenocysteine diphenylmethyl ester (**1**);[10] the thiolselenenate (**3**) was isolated in moderate yield following chromatographic separation from the diselenide (**4**) and disulfide (**5**) (Scheme I). In independent experiments it was noticed that **2** is rather labile in an alkaline medium giving rise to the disulfide **5**; since it was felt that the moderate yield of **3** may have been associated with the lability of the substrate **2**, experiments were repeated using increasing amounts of **2** (up to 3 mol equiv). The fact that neither the yield of **3** nor the ratio of **3** to **4** were changed indicated that the moderate yield of product could be due to an intrinsic instability of **3**, a possibility in line with earlier observations with thiolselenenates.[8,11]

In order to eliminate definitively possible interference by a base-labile substrate, **2** was replaced with the more stable sulfenyl sulfite (Bunte salt); pure unsymmetrical disulfides have been prepared in weakly alkaline reaction media using sulfenyl sulfites.[12] However, when the anion **1** treated with sodium S-benzylthiosulfate (**6**)[13] the desired thiolselenenate (**7**) was isolated only in somewhat higher yield (Scheme II). This again pointed to an instability of the thiolselenenate.

Further semiquantitative studies with **3** and **7** involving solvent variation *per se* showed that disproportionation takes place, the rate depending in first approximation on the polarity of the solvent. Essentially, instantaneous disproportionation of **3** and **7** occurs in basic media and a rapid disproportionation also takes place in acidic media as illustrated by the attempt to decarbobenzoxylate **3** which resulted in L-selenocystine. From these and other findings[8,11] it appears that aliphatic acyclic thiolselenenates are exceedingly reactive molecules, while cyclic thiolselenenates are generally more stable,[14–16] although excep-

(1) This work was supported by U. S. Publich Health Service Grants AM-10080, AM-13567 of the National Institute of Arthritis and Metabolic Diseases and by the U. S. Atomic Energy Commission.

(2) The following abbreviations have been adopted: Z = $C_6H_5CH_2OCO$, DMF = N,N-dimethylformamide, AcOH = acetic acid, EtOH = ethanol, MeOH = methanol, Et_2O = diethyl ether, EtOAc = ethyl acetate.

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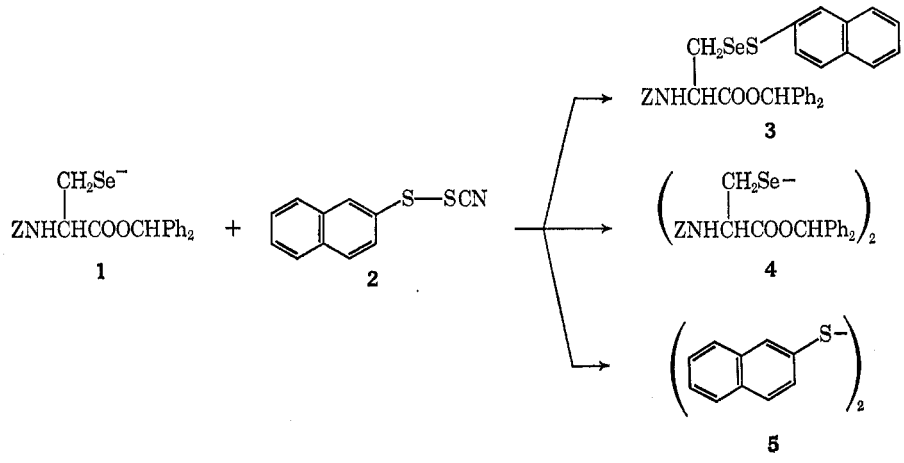
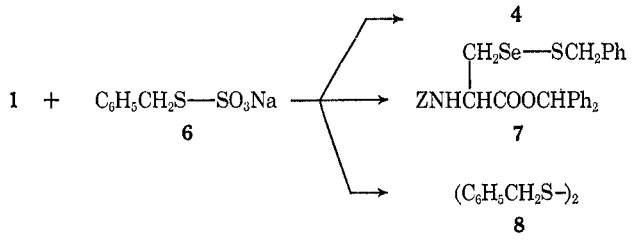
SCHEME I



SCHEME II



tions are encountered.[17] If thiolselenenates occur in living organisms, their high reactivity would dictate that their existence would be transient. Hence, one would expect to find thiolselenenates to be involved in catalytic processes rather than as a permanent component of protein structures.

Experimental Section[18]

N-Carbobenzoxy-Se-(naphthyl-2-thio)-L-selenocysteine Diphenylmethyl Ester (3).—Sodium (0.107 g) was dissolved in EtOH (5 ml); the resulting solution was degassed and all subsequent steps were performed under hydrogen atmosphere. To the solution, saturated with hydrogen selenide gas, N-carbobenzoxy-O-tosyl-L-serine diphenylmethyl ester[19] (1.86 g) dissolved in 5 ml of degassed DMF was added. The reaction mixture was stirred for an additional 0.5 hr and NaOH (0.17 g) dissolved in 1 ml of degassed water was then added, immediately followed by 2-naphthylsulfenyl thiocyanate (2)[9] (1.8 g) in degassed DMF (5 ml). The reaction was exothermic and the mixture turned dark and solid separated. Stirring was continued for 3 hr, and the reaction mixture was subsequently diluted with EtOAc (80 ml) and shaken thoroughly with water (30 ml); at this stage 0.4 g of 2-naphthyl disulfide, mp 138–139° (lit.[9] mp 139–140°), which was insoluble in both the aqueous and organic phases, was isolated by filtration. From the filtrate the aqueous layer was separated and the organic layer was washed with three 25-ml portions of water, dried, and evaporated to dryness under vacuum. The residual semisolid was dissolved in a minimum volume of boiling EtOAc; on standing a second batch of 2-naphthyl disulfide separated as crystalline yellow solid to yield 0.5 g, mp 138.5–139.5°. The filtrate, after separation of this solid, was then evaporated under vacuum and the viscous mass was tested on silica gel G tlc in the solvent system EtOAc:C_6H_6 (1:9, v/v). Two spots, Zahn's reagent[20] positive (the one spot with the higher R_f value was later found to correspond to **3**, see below; the R_f value of the other spot was identical with that of authentic **4**)[10] and one Zahn's reagent negative spot (a pronounced white spot with an R_f value corresponding to authentic **5**) were detected. Compound **3** was eluted from a silica gel G column with C_6H_6 containing 1.5% EtOAc and crystallized from EtOAc–MeOH in clusters of fine needles: yield 0.5 g (24%); mp 85.5–86.5°; $[\alpha]^{30.5}D$ −75.0° (2% in DMF).

Anal. Calcd for $C_{34}H_{29}NO_4SSe$: C, 65.2; H, 4.66; N, 2.24. Found: C, 65.2; H, 4.66; N, 2.22.

N-Carbobenzoxy-Se-(benzylthio)-L-selenocysteine Diphenylmethyl Ester (7).—Sodium hydrogen selenide was prepared from sodium (0.107 g) dissolved in absolute EtOH (5 ml). Under hydrogen atmosphere N-carbobenzoxy-O-tosyl-L-serine diphenylmethyl ester (1.86 g) in degassed DMF (5 ml) was added and stirring was continued for 0.5 hr. Base (0.17 g of NaOH dissolved in 1 ml of degassed water) was added, followed by sodium S-benzylthiosulfate (6)[13] (2.26 g) in 3 ml of degassed water. The reaction mixture, after stirring under hydrogen for 3 hr, was diluted with EtOAc (80 ml) and washed with four 25-ml portions of water. The organic layer was separated and dried, and the solvent was removed under reduced pressure. The resulting oil was chromatographed on a silica gel G column and three fractions were collected. The first fraction eluted with C_6H_6 containing 1% EtOAc, yielded upon evaporation a solid which after crystallization from 95% EtOH was identified as benzyl disulfide (**8**), 0.5 g, mp 69–70° (lit.[21] mp 69–70°). The second fraction eluted with C_6H_6 containing 2% EtOAc, also gave a crystalline solid upon evaporation of solvent. Recrystallization from an EtOAc–EtOH mixture gave **7** in 36% yield (0.7 g), mp 74–75°, $[\alpha]^{30.5}D$ −57.0° (2% in DMF).

Anal. Calcd for $C_{31}H_{29}NO_4SSe$: C, 63.0; H, 4.95; N, 2.37. Found: C, 63.0; H, 5.08; N, 2.32.

The third fraction eluted with C_6H_6 containing 3% EtOAc, was identified as bis(diphenylmethyl)bis(N-carbobenzoxy) L-selenocystinate (**4**) after crystallization from an EtOAc–EtOH mixture; yield, 0.85 g, mp 101–102° (lit.[10] mp 101–102°); in addition the ir spectra taken in KBr were superimposable.

L-Selenocystine.—The thiolselenenate **3** (0.25 g) was dissolved in dry AcOH (1 ml) and 4 *N* HBr in AcOH (1 ml) was added while stirring. After 15 min the reaction mixture was diluted with anhydrous Et_2O (25 ml) and chilled, and the solid precipitate was filtered and washed with a small volume of cold anhydrous Et_2O. The solid was suspended with 2 ml of water and the insoluble material was collected by filtration (this material was identified by melting point and ir as 2-naphthyl disulfide). The pH of the filtrate was adjusted to 5 whereupon a yellow solid separated which was collected by filtration, washed with a small volume of water, and dried *in vacuo* over P_2O_5 to yield 0.05 g (75%). On the basis of melting point, superimposable ir, and specific optical rotation the material was identified as L-selenocystine.[10]

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(18) All melting points were determined with a Thomas-Hoover capillary melting point apparatus and are corrected. The infrared spectra were recorded on a Perkin-Elmer 457 infrared spectrophotometer in pressed disks of KBr at a concentration of 0.3%. The optical rotations were determined with a Carl Zeiss photoelectric precision polarimeter (0.005°). The elementary analyses were carried out by Galbraith Laboratories, Knoxville, Tenn.

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Registry No.—3, 24978-13-0; 7, 24978-14-1.

Acknowledgment.—The authors are thankful to Mrs. Ingrid Mintz for technical assistance.

Solvent Modification in Merrifield Solid-Phase Peptide Synthesis

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Received February 11, 1970

Occasionally during the use of Merrifield solid-phase peptide synthesis from seemingly simple syntheses, steps occur where part of the peptide chain stops growing.[2-5] We encountered such a step at glutamine during the synthesis of the peptide H_2N-Ser-Arg-Phe-Gly-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Ser-Pro-Phe-Gly-Lys-COOH.[6] Although the use of trifluoroacetic acid[2] and double couplings[4] in two different solvents improved our synthesis somewhat, the use of a mixed solvent system of methylene chloride and dimethylformamide (DMF) gave the best results for our peptide. We interpret this to mean that our step was caused by a tertiary structure peculiar to this peptide, and we suggest that this solvent system may be generally useful for problems of this type.

Table I shows the results of several experiments using a variety of deblocking agents, coupling reagents, and reaction times. Each experiment was run in triplicate. Figure 1 is a graphic interpretation of Table I. Only the first five residues, HOOC-Lys-Gly-Phe-Pro-Ser-NH_2, could be completely coupled using methylene chloride as the solvent for dicyclocarbodiimide (DCCI), even when trifluoroacetic acid in methylene chloride was used for deblocking. Only 70% of the sixth amino acid, glutamine, could be added as an active ester in DMF within 6 hr. However, if 1.5 *M* of urea was added to DMF, glutamine could be added to an extent of 90% after 6 hr and the reaction was complete after 24 hr. If DMF (1/3 by volume) was added to the DCCI–methylene chloride couplings and allowed to react 6 hr, glycine (7th), glutamic acid (8th), and alanine (9th) could be coupled completely. If only DCCI–methylene chloride was used, just 50% of the chain continued to grow. Knowing this, amino acids 6 through 13 were coupled using DMF while it was not necessary for the coupling of the remaining three amino acids.

It should be mentioned that Merrifield,[2] while showing the usefulness of trifluoroacetic acid, actually used DCCI with DMF and methylene chloride as solvents in adding histidine while making bradykinin, since histidine was partly dissolved in DMF for the coupling. It appears that while deblocking with trifluoroacetic acid can overcome many of the chain-termination problems in peptide synthesis, the combination of deblocking with trifluoroacetic acid and coupling with DCCI in DMF and methylene chloride might prove more satisfactory.

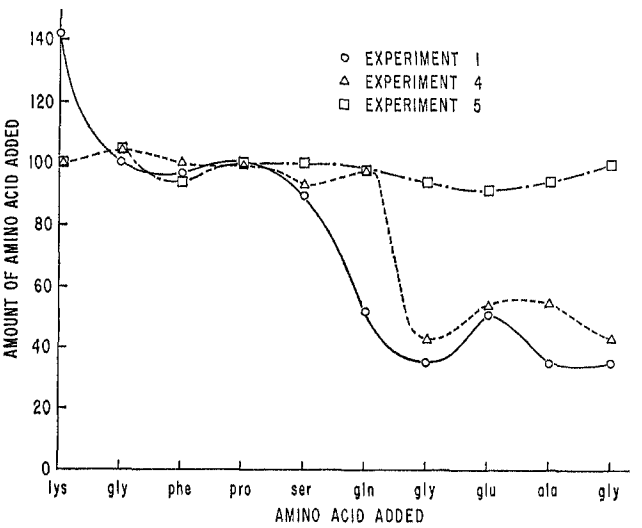

Figure 1.—Graphical interpretation of experiments 1, 4, and 5.

Experimental Section

Dry chloromethylated copolystyrene–2% divinylbenzene (20 g) (Biorad Beads S-X-2, 200–400 mesh, capacity 1.1 milliequiv/g) was mixed with 20 mm of both triethylamine and *e*,N-carbobenzoxy-*a*,N-*t*-butoxylysine in 80 ml of ethanol. The mixture was refluxed for 46 hr. The resin was washed in ethanol, methylene chloride, water and methanol and then dried. The resin contained 0.2 mmol of blocked lysine per gram of resin. The following cycle of deprotection, neutralization, and coupling was carried out on 1 g of resin with a total solution volume of 10 ml for each residue added: (1) three washes with the deprotecting solvent–acetic acid, propionic acid, or methylene chloride; (2) 30 min of reacting with the deprotecting agent—acetic acid and 1 *M* HCl, propionic acid and 0.8 *M* HCl, both with 1% by volume mercaptoethanol, or 50% trifluoroacetic acid in 50% methylene chloride with 5% by volume mercaptoethanol;[7] (3) three washes with the deprotecting solvent—acetic acid, propionic acid, or methylene chloride; (4) two washes with ethanol; (5) three washes with chloroform; (6) neutralization for 10 min with a mixture of 12.5% by volume of triethylamine and 87% by volume of chloroform; (7) three washed with chloroform; (8) three washes with methyl chloride if DCCI coupling or three washes with DMF if active ester coupling; (9) the coupling step depended upon the experiment and the amino acid being added as shown in Table I. It consists of one of the following procedures: (A) addition of 5 ml of methylene chloride containing 2.2 mmol of blocked amino acid and equilibration for 10 min, (B) addition of 5 ml of a solution of DMF and methylene chloride (60:40) containing 2.2 mmol of blocked amino acid with 10 min of equilibration time, or (C) addition of 10 ml of DMF containing 1.5 *M* urea with 4 mmol of the active ester of glutamine; (10) addition of 3 ml of DCCI (66 gm DCCI/400 ml of methylene chloride) followed by 2 ml of methylene chloride. This step is not performed for active ester additions. Coupling times are given in Table I.

Periodically, 8 mg of deblocked peptide resin was dried and hydrolyzed with 1 ml of concentrated HCl and 1 ml of propionic acid for 2 hr at 130° in a sealed tube.[8] From preliminary results,

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(2) R. B. Merrifield, *Recent Progr. Horm. Res.*, **23**, 460 (1967).
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(4) Private communication from Dr. Robert Colescott.
(5) Unpublished results of Professor A. B. Robinson while making portions of cytochrome c.
(6) This peptide was made for Dr. E. Eylar at The Salk Institute for Biological Studies, La Jolla, Calif.
(7) Mercaptoethanol is unstable in trifluoroacetic acid and another reducing agent is more advisable. Unpublished observations of J. Sharp and F. Westall.
(8) Unpublished procedure of J. Scotchler and R. Losier.