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Studies on Peptides. LXXXIII.[1,2] Behavior of S-Substituted Cysteine Sulfoxides under Deprotecting Conditions in Peptide Synthesis

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Sulfoxides of Cys(S-*p*-methoxybenzyl) and Cys(S-benzyl) were prepared by oxidation with sodium perborate. Hydrogen fluoride and methanesulfonic acid converted the former sulfoxide to S-*p*-methoxyphenylcysteine or S-*p*-hydroxyphenylcysteine in the presence of anisole or phenol, respectively, while the latter sulfoxide resisted the actions of these deprotecting reagents. Thiophenol appears to be useful as a powerful reducing reagent for protected cysteine sulfoxides.

Keywords——S-*p*-methoxybenzylcysteine sulfoxide; S-benzylcysteine sulfoxide; hydrogen fluoride treatment of cysteine sulfoxide; methane sulfonic acid treatment of cysteine sulfoxide; sodium in liquid ammonia treatment of cysteine sulfoxide; S-*p*-methoxyphenylcysteine; S-*p*-hydroxyphenylcysteine; reduction of cysteine sulfoxide with thiophenol

Sulfoxides of S-substituted cysteine derivatives have hitherto been little considered in peptide synthesis. In 1977, Live *et al.*[4] briefly reported the reduction of the sulfoxide of the Cys(S-methylbenzyl) residue by treatment with acetone in hydrogen bromide–acetic acid, during the course of the solid phase synthesis of oxytocin. As in the case of methionine sulfoxide, the possibility of partial oxidation of S-substituted cysteine residues to the corresponding sulfoxide during peptide synthesis cannot be excluded. The chemical properties of the sulfoxide of Cys(MBzl), as well as that of Cys(Bzl), especially under acidolytic deprotecting conditions, were therefore examined.

Oxidation of Z(OMe)-Cys(MBzl)-OH[5] with sodium perborate gave the corresponding sulfoxide [Ia, abbreviated as Z(OMe)-Cys(MBzl)(O)-OH] quantitatively. The product appeared to be a mixture of two stereoisomers as regards the configuration of the sulfoxide moiety, as predicted on the basis of a similar oxidation of N^{α}-protected methionine derivatives.[6] The sulfoxides of Z-Cys(MBzl)-OH[7] and Boc-Cys(MBzl)-OH[8] were similarly prepared for characterization.

TFA treatment[9] of the sulfoxide (Ia) afforded H-Cys(MBzl)(O)-OH as a crystalline compound. When the sulfoxide (Ia) was exposed to hydrogen fluoride[10] in the presence

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2) Cysteine and its derivatives are of the L-configuration. The following abbreviations are used: Z=-benzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, MBzl=*p*-methoxybenzyl, TFA=trifluoroacetic acid.
3) Location: *Sakyo-ku, Kyoto, 606, Japan.*
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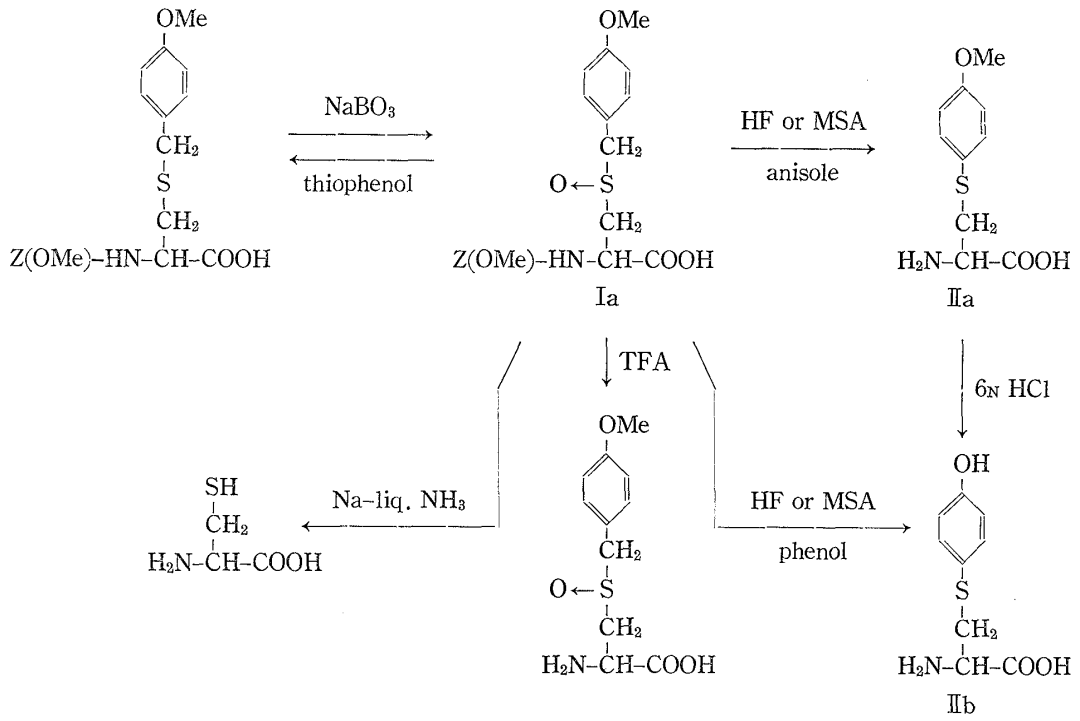

Fig. 1. Chemical Behavior of Z(OMe)-Cys(MBzl)(O)-OH

of anisole under deprotecting conditions, the recovery of cysteine was unsatisfactory, and a compound less soluble in water was obtained as a major component. Nuclear magnetic resonance (NMR) spectral examination of this product revealed the presence of a singlet peak due to the three protons of the methoxy group; however, two proton signals due to the benzyl moiety were missing. This product was therefore assigned as S-*p*-methoxyphenylcysteine (IIa).[11] The same compound was isolated when the sulfoxide (Ia) was treated with methanesulfonic acid (MSA) in the presence of anisole.[12] When phenol was used as a cation scavenger, these two deprotecting reagents, hydrogen fluoride and MSA, afforded S-*p*-hydroxyphenylcysteine (IIb) as a main product (Fig. 1).

Formation of S-*p*-methoxyphenylcysteine (IIa) and S-*p*-hydroxyphenylcysteine from the sulfoxide (Ia) may be explained as the result of a substitution reaction initiated by protonation of the oxygen atom of the sulfoxide followed by electrophilic attack of anisole or phenol at the *p*-position and subsequent hydrolysis, as pointed out by Blackwood *et al.*[13] and Goethals and Radziyzky[14] in similar S-substitution reactions of the sulfoxides of tetracycline thio compounds and dimethylsulfoxide, respectively.

The compounds, IIa and IIb, emerged from a short column of an amino acid analyzer at retention times of 38 and 26 min, respectively. Under usual 6 N HCl hydrolytic conditions for peptides and proteins,[15] the 38-minute compound (IIa) was completely converted to the 26-minute compound (IIb) by hydrolysis of the *p*-methoxy group. The above results indicate that if the 26-minute compound (IIb) is detected in a 6 N HCl hydrolysate of peptides deprotected with hydrogen fluoride or MSA in the presence of anisole, the parent protected peptides will be contaminated with partially oxidized Cys(MBzl) derivatives. The presence of the sulfoxide of Cys(MBzl) in protected peptides can also be estimated from the presence of cysteic

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acid in 6 N HCl hydrolysates. When 6 N HCl hydrolysis is performed in an evacuated tube as usual, the hydrolysate contains cysteine and cystine, but no cysteic acid. When the sulfoxide (Ia) was subjected to 6 N HCl hydrolysis (without anisole or phenol), cysteic acid was detected in 13% yield, besides cysteine and cystine. Hydrolysis of Ia with 3 N *p*-toluenesulfonic acid[16] also afforded cysteic acid (5.8%).

It should be noted here that if protected peptides are hydrolyzed with 6 N HCl in the presence of phenol in order to obtain a better recovery of tyrosine,[17] the presence of the 26-minute compound (IIb), if any, is not a direct indication of the presence of the Cys(MBzl)-(O) residue in the parent protected peptides. Treatment of cystine (not cysteine) with 6 N HCl in the presence of phenol also afforded the 26-minute compound in 5.8% yield. Partial oxidation of cysteine to cystine is usually observable during the acid hydrolysis of protected cysteine peptides, and thus the possibility cannot be excluded that the 26-minute compound was formed by the direct reaction of phenol and cystine partially formed during the hydrolysis.

Z(OMe)-Cys(Bzl)-OH[18] was similarly oxidized by sodium perborate to the corresponding sulfoxide [Ib, abbreviated as Z(OMe)-Cys(Bzl)(O)-OH]. The sulfoxides of Z-Cys(Bzl)-OH[19] and Boc-Cys(Bzl)-OH[20] were also prepared for characterization. Unlike the sulfoxide (Ia), the sulfoxide (Ib) resisted the action of hydrogen fluoride or MSA, giving the N^{α}-deprotected sulfoxide, H-Cys(Bzl)(O)-OH, as a major product, even in the presence of a reasonable amount of anisole or phenol. Different behavior of the sulfoxide (Ib) from that of Ia may be due to subtle differences in stability between benzyl cations with or without the electron-donating substituent at the *p*-position. Hydrolysis of the sulfoxide (Ib) with 6 N HCl gave a low recovery of cystine approximately 21%, besides cysteic acid, 0.5%. The appearance of a large amount of ammonia indicated that most of the sulfoxide (Ib) was decomposed under hydrolytic conditions.

These findings indicate that the sulfoxide of Cys(MBzl) or Cys(Bzl), if any, in synthetic protected peptides, should be reduced before deprotection with hydrogen fluoride or MSA, otherwise satisfactory recovery of cysteine cannot be expected. Reduction with sodium in liquid ammonia[21] regenerated cysteine (determined as cystine) from the sulfoxide (Ia or Ib) in approximately 60 to 70% yield, though some formation of an unidentified and less water-soluble product was noted. A number of reducing reagents for methionine sulfoxide are

Table I. Reduction of Protected Cysteine and Methionine Sulfoxides with Thiol Compounds

Reagents	Reduction of (%)									
	Z(OMe)-Cys-(Bzl)(O)-OH			Z(OMe)-Cys-(MBzl)(O)-OH			Z(OMe)-Met(O)-OH		H-Met-(O)-OH	
	8 hr	24 hr	36 hr	8 hr	24 hr	36 hr	8 hr	24 hr	4 hr	8 hr
Mercaptoethanol	8.1	19.5	34.6	15.7	25.9	42.0	22.0	31.5	72.2	98.7
Thioglycolic acid	7.5	16.4	19.9	11.8	12.6	31.5	31.4	60.2	100	
Dithiothreitol	5.6	16.9	23.4	13.3	20.2	39.6	20.4	35.0	94.0	100
Ethanedithiol	33.2	52.5	56.3	36.7	55.7	67.1	25.0	31.0	45.6	46.7
Thiophenol	38.6	82.0	98.1	32.0	85.8	100	67.0	92.0	37.9	41.5

Reduction was performed at 70°.

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known.[22] We felt that the reduction of protected cysteine sulfoxides in an organic solvent was somewhat difficult compared to the reduction of methionine sulfoxide in aqueous solution. When Z(OMe)-Cys(Bzl)(O)-OH and Z(OMe)-Cys(MBzl)(O)-OH in dimethylformamide was incubated with various reducing reagents, such as thioglycolic acid, dithiothreitol, mercaptoethanol or ethanedithiol, at 70° for 36 hr, reduction proceeded, at best, to the extent of 35% to 67%. We found, however, that when thiophenol was used as a reducing reagent, nearly quantitative reduction proceeded under the conditions mentioned above (Table I). By increasing the amount of thiophenol (20 equiv.) and increasing the incubation temperature to 85°, preparative reduction could be performed within 6 hr.

Experimental evidence obtained here on the behavior of S-substituted cysteine sulfoxide derivatives under acidolytical deprotecting conditions may be useful in the synthesis of peptides containing cysteine, especially those with a large number of disulfide bridges.

Experimental

Thin-layer chromatography (TLC) was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8:3:1), Rf_2 $CHCl_3$–MeOH–AcOH (9:1:0.5), Rf_3 MeCN–H_2O (3:1), Rf_4 *n*-BuOH–AcOH–H_2O (4:1:5).

Z(OMe)-Cys(MBzl)(O)-OH (Ia)——A mixture of Z(OMe)-Cys(MBzl)-OH (3.95 g, 9.8 mmol) in AcOEt (40 ml) and $NaBO_3 \cdot 4H_2O$ (1.65 g, 1.1 equiv.) in H_2O (20 ml) was stirred at room temperature overnight, then acidified with citric acid. The resulting powder was collected by filtration and washed with H_2O–NaCl. The organic phase of the filtrate was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and evaporated down. The residual solid was combined with the powder obtained above and recrystallized from tetrahydrofuran (THF) and ether; yield 3.43 g (84%), mp 148—151°, $[\alpha]_D^{22}$ −72.8° (c=1.1, dimethylformamide (DMF)). Rf_1 0.34, Rf_2 0.59. *Anal.* Calcd. for $C_{20}H_{23}NO_7S$: C, 56.99; H, 5.50; N, 3.32. Found: C, 56.89; H, 5.53; N, 3.33.

Boc-Cys(MBzl)(O)-OH——This compound was similarly prepared; yield 73%. mp 176—177°, $[\alpha]_D^{22}$ −71.6° (c=1.4, DMF). Rf_2 0.43. *Anal.* Calcd. for $C_{16}H_{23}NO_6S$: C, 53.76; H, 6.49; N, 3.92. Found: C, 53.93; H, 6.58; N, 3.67.

Z-Cys(MBzl)(O)-OH——This compound was similarly prepared; yield 73%. mp 133—135°, $[\alpha]_D^{22}$ −54.1° (c=1.0, DMF). Rf_2 0.38. *Anal.* Calcd. for $C_{19}H_{21}NO_6S \cdot H_2O$: C, 55.73; H, 5.66; N, 3.42. Found: C, 55.67; H, 5.45; N, 3.33.

H-Cys(MBzl)(O)-OH——Z(OMe)-Cys(MBzl)(O)-OH (3.03 g, 7.2 mmol) was treated with TFA (6 ml) in the presence of anisole (1.6 ml) in an ice-bath for 60 min and dry ether was added. The resulting powder was dissolved in 5% NH_4OH and the solution was adjusted to pH 6 with 1 N HCl to yield a white powder, which was reprecipitated by adjusting the pH as mentioned above, yield 1.48 g (80%), mp 176—177°, $[\alpha]_D^{22}$ +6.7° (c=0.7, 50% AcOH). Rf_3 0.47. ^1H NMR (CF_3COOD): δ 3.72 (2H, d, β-CH_2), 3.97 (3H, s, OMe), 4.47 (2H, s, benzyl CH_2), 5.02 (1H, t, α-CH), 7.14 (2H, d, aromatic H), 7.44 (2H, d, aromatic H). *Anal.* Calcd. for $C_{11}H_{15}NO_4S$: C, 51.34; H, 5.88; N, 5.44. Found: C, 51.55; H, 5.88; N, 5.33.

S-*p*-Methoxyphenylcysteine (IIa)——(a) Treatment of H-Cys(MBzl)(O)-OH with HF-anisole: H-Cys(MBzl)(O)-OH (105 mg) was treated with HF (approximately 4 ml) in the presence of anisole (1 ml, 25 equiv.) in an ice-bath for 45 min. The excess HF was removed by evaporation and the residue was washed with ether. The resulting powder was collected by filtration, dissolved in H_2O (4 ml) and the pH of the solution was adjusted to 6 with dil. NH_4OH to form a white powder, which was collected by filtration and washed with H_2O; yield 47 mg (54%), mp 189—190°, $[\alpha]_D^{22}$ +11.8° (c=0.5, 50% AcOH). Rf_3 0.59. ^1H NMR (CF_3COOD): δ 3.62 (2H, d, β-CH_2), 3.96 (3H, s, OMe), 4.48 (1H, t, α-CH), 7.06 (2H, d, aromatic H), 7.51 (2H, d, aromatic H). *Anal.* Calcd. for $C_{10}H_{13}NO_3S$: C, 52.84; H, 5.77; N, 6.16; S, 14.11. Found: C, 53.12; H, 5.90; N, 5.93; S, 13.83.

(b) Treatment of Z(OMe)-Cys(MBzl)(O)-OH with MSA-anisole: Z(OMe)-Cys(MBzl)(O)-OH (3.43 g) was treated with MSA (14 ml) in the presence of anisole (3.5 ml, 4 equiv.) at room temperature for 60 min and dry ether was added. The oily residue was washed with ether, dissolved in water and the solution was neutralized with Et_3N to yield a white powder, which was collected by filtration and washed with the upper

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phase of *n*-BuOH–AcOH–H_2O (4: 1: 5); yield 0.77 g (42%), mp 189—190°, $[\alpha]_D^{22}$ +10.6° (c=0.4, 50% AcOH). Rf_3 0.59. Its ^1H NMR spectra were identical with those of the compound obtained in (a). Retention time on the short column of an amino acid analyzer was 38 min.

S-*p*-Hydroxyphenylcysteine (IIb)——(a) Treatment of Z(OMe)–Cys(MBzl)(O)–OH with MSA-phenol: Z(OMe)–Cys(MBzl)(O)–OH (420 mg) was treated with MSA (0.8 ml) in the presence of phenol (370 mg, 4 equiv.) in an ice-bath for 15 min and at room temperature for 45 min, then dry ether was added. The resulting oily precipitate was dissolved in H_2O (2 ml) and the solution was neutralized with 5% NH_4OH to form a gummy precipitate, which was obtained as a solid on trituration with EtOH (Rf_3 0.44 main, 0.51 faint). This crude material was purified by partition chromatography[23] on Sephadex G-15 with the solvent system *n*-BuOH–AcOH–H_2O (4: 1: 5). Fractions containing the substance of Rf_3 0.44 were collected, the solvent was evaporated off and the residue was recrystallized from EtOH; yield 100 mg (47%), mp 199—201°, $[\alpha]_D^{22}$ +7.8° (c=0.4, 50% AcOH). Rf_3 0.44. Rf_4 0.51. ^1H NMR (CF_3COOD): δ 3.59 (2H, d, β-CH_2), 4.47 (1H, t, α-CH), 7.00 (2H, d, aromatic H), 7.52 (2H, d, aromatic H). *Anal.* Calcd. for $C_9H_{11}NO_3S$: C, 50.69; H, 5.20; N, 6.57. Found: C, 50.54; H, 5.13; N, 6.29.

(b) Treatment of Z(OMe)–Cys(MBzl)(O)–OH with HF-phenol: Z(OMe)–Cys(MBzl)(O)–OH (420 mg) was treated with HF in the presence of phenol (370 mg, 4 equiv.) as described for II-a and the product was isolated in essentially the manner described above; yield 89 mg (42%). Its ^1H NMR spectra were identical with those of the compound obtained in (a). Retention time on the short column of an amino acid analyzer was 26 min.

Hydrolysis of S-*p*-Methoxyphenylcysteine with 6 N HCl——S-*p*-Methoxyphenylcysteine (IIa) (258 mg) was heated with 6 N HCl at 110° for 24 hr in a sealed tube. After removal of the solvent by evaporation, the residue was neutralized with 5% NH_4OH and the resulting powder was recrystallized from 50% MeOH and then EtOH; yield 70 mg (29%), mp 196—200°. Identity of the compound with S-*p*-hydroxyphenylcysteine was confirmed by comparison of their ^1H NMR spectra and retention times on an amino acid analyzer.

Hydrolysis of H-Cys(MBzl)(O)-OH with 6 N HCl or 3 N *p*-Toluenesulfonic Acid——H–Cys(MBzl)(O)–OH (1.12 mg) was hydrolyzed with 6 N HCl (1 ml) at 110° for 20 hr and the hydrolysate was examined on an amino acid analyzer: cysteic acid 12.9% and cysteine plus cystine 82.1% recovery. When 3 N *p*-toluenesulfonic acid was used: cysteic acid 5.8%, cysteine plus cystine 94.2% recovery.

Hydrolysis of Z(OMe)-Cys(MBzl)(O)-OH with 6 N HCl-Phenol——Z(OMe)–Cys(MBzl)(O)–OH (1.4 mg) was hydrolyzed with 6 N HCl (1 ml) in the presence of phenol (1.2 mg, 4 equiv.) at 110° for 24 hr. When the hydrolysate was examined on an amino acid analyzer, the following peaks were detected, though they were not quantified on the short column, 26 min (S-*p*-hydroxyphenylcysteine); on the long column, 28 min (cysteic acid), 90 min (cysteine), 114 min (cystine).

Hydrolysis of Cystine with 6 N HCl-Phenol——Cystine (8 mg) was heated with 6 N HCl (2 ml) in the presence of phenol (15 mg, 4 equiv.) as mentioned above and the hydrolysate was examined on an amino acid analyzer. On the short column, 26 min (5.8%); on the long column, 114 min (cystine 92.4%).

Treatment of Z(OMe)-Cys(MBzl)(O)-OH with Na in Liquid NH_3——Z(OMe)–Cys(MBzl)(O)–OH (99 mg, 0.24 mmol) was treated with Na (22 mg, 4 equiv.) in liquid NH_3 until the blue color persisted for 10 sec. After addition of NH_4Cl (10 mg), NH_3 was evaporated off and the residue was examined on an amino acid analyzer. On the long column, 28 min (unidentified small peak, cysteic acid ?), 114 min (cystine 68%).

Z(OMe)-Cys(Bzl)(O)-OH (Ib)——A mixture of Z(OMe)–Cys(Bzl)–OH (17.39 g, 46 mmol) in AcOEt (170 ml) and $NaBO_3 \cdot 4H_2O$ (7.84 g, 1.1 equiv.) in H_2O (85 ml) was stirred at room temperature overnight. After acidification with 5% citric acid, the product was extracted with AcOEt. The extract was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then condensed. The residue was triturated with ether and recrystallized from MeOH and ether; yield 13.01 g (71%), mp 161—163°, $[\alpha]_D^{22}$ −19.8° (c=0.8, DMF). Rf_2 0.42. *Anal.* Calcd. for $C_{19}H_{21}NO_6S$: C, 58.30; H, 5.41; N, 3.58. Found: C, 58.06; H, 5.35; N, 3.60.

Boc-Cys(Bzl)(O)-OH——This compound was similarly prepared; yield 70%, mp 176—177°, $[\alpha]_D^{22}$ −40.8° (c=1.1, DMF). Rf_2 0.37. *Anal.* Calcd. for $C_{15}H_{21}NO_5S$: C, 55.03; H, 6.47; N, 4.28. Found: C, 55.22; H, 6.56; N, 4.18.

Z-Cys(Bzl)(O)-OH——This compound was similarly prepared; yield 76%, mp 161—163°, $[\alpha]_D^{22}$ −46.7° (c=0.8, DMF). Rf_2 0.47. *Anal.* Calcd. for $C_{18}H_{19}NO_5S$: C, 59.82; H, 5.30; N, 3.88. Found: C, 60.03; H, 5.51; N, 4.02.

H-Cys(Bzl)(O)-OH——Z(OMe)–Cys(Bzl)(O)–OH (9.07 g) was treated with TFA (20 ml) in the presence of anisole (5 ml) in an ice-bath for 60 min and dry ether was added. The resulting powder was suspended in H_2O (20 ml) and the pH of the solution was adjusted to 6 with 5% NH_4OH. The crystalline product was collected by filtration and precipitated from 5% NH_4OH with 1 N HCl; yield 3.43 g (65%), mp 170—171°, $[\alpha]_D^{22}$ −24.7° (c=0.8, 50% AcOH). Rf_3 0.57. *Anal.* Calcd. for $C_{10}H_{13}NO_3S$: C, 52.84; H, 5.77; N, 6.16. Found: C, 53.02; H, 5.76; N, 6.17.

Treatment of H-Cys(Bzl)(O)-OH with HF——H–Cys(Bzl)(O)–OH (102 mg, 0.45 mmol) was treated with HF (approximately 5 ml) in the presence of anisole (1.2 ml, 25 equiv.) in an ice-bath for 45 min. After

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evaporation of HF, the residue was washed with ether, dissolved in H_2O and the pH of the solution was adjusted to 6 with 5% NH_4OH to yield a white precipitate, which was collected by filtration; yield 77 mg (recovery 75%), mp 170—172°, Rf_3 0.57. Retention time (127 min) on the long column was identical with that of an authentic sample of H–Cys(Bzl)(O)–OH. When the filtrate was examined on an amino acid analyzer, an unidentified peak (119 min on the long column) was detected.

Treatment of H-Cys(Bzl)(O)-OH with MSA——H–Cys(Bzl)(O)–OH (239 mg) was treated with MSA (5.7 ml) in the presence of anisole (1.1 ml, 10 equiv.) in an ice-bath for 15 min and at room temperature for 60 min, then dry ether was added. The residue was dissolved in H_2O and the pH of the solution was adjusted to 6 with 5% NH_4OH. The *Rf* value (Rf_3 0.57) of the resulting powder (194 mg, 81%) was identical with that of the starting material.

Hydrolysis of Z(OMe)-Cys(Bzl)(O)-OH with 6 N HCl——When the hydrolysate (24 hr hydrolysis) was examined on an amino acid analyzer, cysteic acid 0.5%, cystine 21.1% and a large amount of ammonia were detected.

Treatment of H-Cys(Bzl)(O)-OH with Na in Liquid NH_3——H–Cys(Bzl)(O)–OH (313 mg) was treated with Na (*ca.* 300 mg) in liquid NH_3 (approximately 30 ml) until the blue color persisted for 5 sec and it was then discharged with NH_4Cl (20 mg). After evaporation of NH_3, the residue was dissolved in H_2O and the solution was examined on an amino acid analyzer; cystine (114 min, 56.5%) and cysteine (90 min, 4%). Some water-insoluble material was isolated.

Reduction of Protected Cysteine Sulfoxides with Various Thiol Compounds——The sulfoxide (Ia or Ib) (100 mg each) in DMF (1 ml) was incubated at 70° in the presence of various reducing reagents (10 equiv.). At intervals, an aliquot was examined by TLC with the solvent system $CHCl_3$–MeOH–AcOH (9 : 1 : 0.5) and the amount of reduced material (ninhydrin stain) was determined with a Shimadzu dual-wavelength thin-layer chromatogram scanner. The results are listed in Table I. Reduction of Z(OMe)–Met(O)–OH in DMF and H–Met(O)–OH in H_2O were also carried out for comparison.

Reduction of Z(OMe)-Cys(MBzl)(O)-OH with Thiophenol——Z(OMe)–Cys(MBzl)(O)–OH (2.08 g, 4.8 mmol) in DMF (10 ml) was heated with thiophenol (9.8 ml, 20 equiv.) at 85° for 6 hr; the starting material disappeared and a new spot of Rf_1 0.62 was detected on TLC. The solvent was evaporated off and the residue was dissolved in 5% NH_4OH. The aqueous phase, after washing with ether, was acidified with 5% citric acid and the resulting precipitate was extracted with AcOEt. The extract was washed with H_2O–NaCl, dried over Na_2SO_4 and then condensed. Treatment of the residue with *n*-hexane afforded a powder, which was recrystallized from AcOEt and *n*-hexane to give a product identical with an authentic sample of Z(OMe)–Cys(MBzl)–OH; yield 1.86 g (97%), mp 78—80°, $[\alpha]_D^{22}$ −36.9° (*c*=1.0, MeOH). Rf_1 0.62, Rf_2 0.72. *Anal.* Calcd. for $C_{20}H_{23}NO_6S$: C, 59.24; H, 5.72; N, 3.45. Found: C, 59.15; H, 5.62; N, 3.41.

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