caused by aniline, a type II compound. These observations suggest that 11-OH-$\Delta^8$-THC and hexobarbital bind to the same cytochrome P-450. The major difference of our system from that of Kupfer *et al.* appears to be the use of higher concentrations of 11-OH-$\Delta^8$-THC in the present study. Addition of lower concentrations ($<25$ μM) of 11-OH-$\Delta^8$-THC to untreated rabbit and rat microsomes did not produce any significant spectral change, although it produced a type I spectral change with PB-treated microsomes containing higher levels of P-450.

It is well known that various substrates of the microsomal monooxygenase system bind to cytochrome P-450 and produce spectral changes.[18] The present results thus afford further support for the participation of rabbit and rat liver microsomal cytochrome P-450 in the further metabolism of 11-OH-$\Delta^8$-THC. However, the present observations also show that the affinity of 11-OH-$\Delta^8$-THC for cytochrome P-450 ($K_s$ μM=402.1 for rabbits and 76.6 for rats) is considerably lower than that of $\Delta^8$-THC (88.7 for rabbit and 10.1 for rats).

18) J.B. Schenkmann, H. Remmer, and R.W. Estabrook, *Mol. Pharmacol.*, 3, 113 (1967).

[Chem. Pharm. Bull. 28(6)1942—1945(1980)]

# Studies on Peptides. XCVI.[1,2] Behavior of S-Acetamidomethylcysteine Sulfoxide under Deprotecting Conditions in Peptide Synthesis

HARUAKI YAJIMA, KENICHI AKAJI, SUSUMU FUNAKOSHI, NOBUTAKA FUJII, and HIROSHI IRIE

*Faculty of Pharmaceutical Sciences, Kyoto University*[3]

(Received January 25, 1980)

The sulfoxide of Boc–Cys(S–acetamidomethyl)–OH was prepared by oxidation with sodium perborate. Mercuric acetate and iodine failed to cleave the S-protecting group from the sulfoxide. Hydrogen fluoride and methanesulfonic acid partially converted the sulfoxide to S-*p*-methoxyphenylcysteine in the presence of anisole. A reducing reagent, thiophenol, converted the sulfoxide to acetamidomethyl phenyl sulfide and $N^{\alpha}$-Boc–S–(phenylthio)cysteine.

**Keywords**——S-acetamidomethylcysteine sulfoxide; mercuric acetate treatment; iodine treatment; hydrogen fluoride treatment; methanesulfonic acid treatment; thiophenol treatment

Recently, we examined the chemical properties of the sulfoxide of Cys(MBzl), as well as that of Cys(Bzl).[4] In this paper, we report on the behavior of the sulfoxide of Cys(Acm), another important cysteine derivative in peptide chemistry, especially under various deprotecting conditions.

1) Part XCV: M. Takeyama, K. Koyama, K. Inoue, T. Kawano, H. Adachi, T. Tobe, and H. Yajima, *Chem. Pharm. Bull.*, **28**, 1873 (1980).
2) Cysteine and its derivatives are of the L-configuration. The following abbreviations are used: Boc=*tert*-butoxycarbonyl, MBzl=*p*-methoxybenzyl, Bzl=benzyl, Acm=acetamidomethyl, TFA=trifluoroacetic acid, MSA=methanesulfonic acid.
3) Location: *Sakyo-ku, Kyoto, 606, Japan.*
4) H. Yajima, S. Funakoshi, N. Fujii, K. Akaji, and H. Irie, *Chem. Pharm. Bull.*, **27**, 2151 (1979).

The Acm group was first introduced by Veber *et al.*[5] as a useful thiol protecting group for cysteine, because of its characteristic chemical properties. This group can be removed selectively by treatment with iodine,[6] as well as mercuric (II) acetate, though the S-MBzl group was later found removable by treatment with mercuric acetate in TFA.[7] This group survives under various deprotecting condition used for the S-Bzl and S-MBzl groups, such as treatment with Na in liq. $NH_3$[8] or HF.[9,10] It resists even MSA. As mentioned previously,[4] the possibility of partial oxidation of S-substituted cysteine residues to the corresponding sulfoxide during peptide synthesis cannot be excluded. We therefore decided to examine the chemical properties of the sulfoxide of this useful cysteine derivative.

Oxidation of Boc–Cys(Acm)–OH[5] with sodium perborate gave the corresponding sulfoxide [I, abbreviated as Boc–Cys(Acm)(O)–OH] quantitatively. However, the isolation yield decreased to less than 50%, because of its large solubility in water. 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 $N^{\alpha}$-protected methionine derivatives.[11] TFA treatment of the sulfoxide (I) afforded H–Cys(Acm)(O)–OH as a crystalline compound.

The behaviors of the sulfoxide (I) under conditions used for deprotection of the parent derivative were first examined. When H–Cys(Acm)(O)–OH was treated with mercuric (II) acetate according to Veber *et al.*[5] or with iodine according to Kamber,[6] no change was observed on TLC and no cysteine or cystine was detected using an amino acid analyzer. The results indicate that if Cys(Acm) residues were oxidized to the corresponding sulfoxide during peptide synthesis, this S-protecting group would no longer be removable by the usual treatments.

Next, we examined the behavior of the sulfoxide (I) with HF and MSA,[12] which are known deprotecting reagents for the S–MBzl and S–Bzl groups and other protecting groups. When Boc–Cys(Acm)(O)–OH was treated with HF in the presence of anisole in an ice-bath for 1 hour, besides the $N^{\alpha}$-deprotected sulfoxide (approximately 10%), two products were detected on TLC. Though we failed to isolate the minor product, the major product was characterized as S–*p*-methoxyphenylcysteine, identical with the product derived from H–Cys(MBzl)(O)–OH under the same conditions.[4] The results indicate that if peptides contaminated with the sulfoxide were exposed to HF, most of the sulfoxide would be transformed to other compounds, such as S–*p*-methoxyphenylcysteine, depending upon the scavengers employed, though a part of the sulfoxide and the parent residue, Cys(Acm), would remain unchanged.

When Boc–Cys(Acm)(O)–OH was next similarly treated with MSA in the presence of anisole, three products were detected on TLC. Though we failed to characterize the minor component, the other two products were identified, using an amino acid analyzer, as cystine and S–*p*-methoxyphenylcysteine. The results indicate that if peptides contaminated with the sulfoxide were exposed to MSA, in spite of the inertness of the parent derivative to MSA, cystine would be regenerated from the sulfoxide to a certain degree (around 48%) and the remainder would be transformed to other compounds, such as S–*p*-methoxyphenylcysteine, as in the case of HF treatment, depending on the scavengers employed.

---

5) D.F. Veber, J.D. Milkowski, R.G. Denkewalter, and R. Hirschmann, *Tetrahedron Lett.*, **1968**, 3057; D.F. Veber, J.D. Milkowski, S.L. Varga, R.G. Denkewalter, and R. Hirschmann, *J. Am. Chem. Soc.*, **94**, 5456 (1972).

6) B. Kamber, *Helv. Chim. Acta*, **54**, 927 (1971).

7) M. Fujino and O. Nishimura, *J.C.S. Chem. Commun.*, **1976**, 998; O. Nishimura, C. Kitada, and M. Fujino, *Chem. Pharm. Bull.*, **26**, 1576 (1978).

8) R.H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

9) S. Akabori, S. Sakakibara, Y. Shimonishi, and Y. Nobuhara, *Bull. Chem. Soc. Jpn.*, **37**, 433 (1964).

10) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jpn.*, **40**, 2164 (1967).

11) N. Fujii, T. Sasaki, S. Funakoshi, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, **26**, 650 (1978).

12) H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.*, **23**, 1164 (1975).

Next, H–Cys(Acm)(O)–OH was treated with Na in liq. $NH_3$. Besides the parent sulfoxide, cysteic acid and a number of unidentified peaks, cystine was detected in only 10% yield using an amino acid analyzer.

From these results, it can be concluded that satisfactory recovery of cysteine from Cys(Acm)(O) is difficult to achieve by available deprotecting procedures. Previously, we found that thiophenol, among various sulfur compounds tested, is a powerful reducing reagent for the sulfoxides of Cys(MBzl) and Cys(Bzl).[4] Boc–Cys(Acm)(O)–OH was therefore treated with thiophenol at 80° for 12 hours; the starting material disappeared and two products were detected by staining with $Ce(SO_4)_2$ on TLC. However, none of these compounds was the expected reduced compound, Boc–Cys(Acm)–OH. These two compounds were then isolated by column chromatography on silica and identified on the basis of spectral data as acetamidomethyl phenyl sulfide and $N^{\alpha}$-Boc–S–(phenylthio)cysteine (Fig). The parent compound, Boc–Cys(Acm)–OH, remained unchanged under the above reducing conditions. Cys(Acm)(O) was found to be susceptible to thiophenolate ion and behaved quite differently from the sulfoxides of Cys(MBzl) and Cys(Bzl).

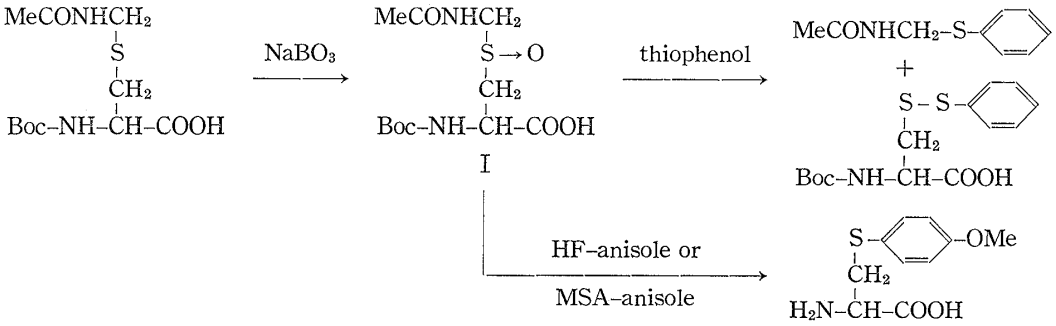


Fig. Chemical Behavior of Boc–Cys(Acm)(O)–OH

Thus, we conclude that if Cys(Acm) residues are partially oxidized to the corresponding sulfoxide during peptide synthesis, no appropriate procedures exist at present for the satisfactory recovery of cysteine. The presence or absence of Cys(Acm)(O) in synthetic peptides can be determined by hydrolysis with 6 N HCl in the presence of phenol. A peak with a retention time of 26 minutes on the short column of an amino acid analyzer is due to the product derived from the sulfoxide, since a hydrolysate of the parent compound, Cys(Acm), did not give such a peak. This compound was assigned as S–*p*-hydroxyphenylcysteine, identical with the reported product[4] derived from Cys(MBzl)(O) under identical conditions or from S–*p*-methoxyphenylcysteine by treatment with 6 N HCl.

## Experimental

Thin-layer chromatography was performed on silica gel (Kieselgel G, Merck) and *Rf* values refer to the following solvent systems: $Rf_1$ $CHCl_3$–MeOH–AcOH (9: 1: 0.5), $Rf_2$ *n*-BuOH–AcOH–AcOEt–$H_2O$ (1: 1: 1: 1), $Rf_3$ *n*-BuOH–AcOH–$H_2O$ (10: 2: 3), $Rf_4$ *n*-BuOH–AcOH–pyridine–$H_2O$ (4: 1: 1: 2), $Rf_5$ $CHCl_3$–MeOH (10: 0.5).

**Boc-Cys(Acm)(O)-OH**——$NaBO_3 \cdot 4H_2O$ (2.32 g, 1.1 equiv.) in $H_2O$ (10 ml) was added to a solution of Boc–Cys(Acm)–OH (4.0 g, 13.7 mmol) in AcOEt (30 ml) and the mixture was stirred at room temperature overnight; starting material disappeared completely as determined by TLC. The aqueous phase was separated from the AcOEt layer, washed with AcOEt and then acidified with citric acid. No precipitate was obtained, and attempts to extract the sulfoxide with organic solvents were unsuccessful. Storage of the acidified solution in a refrigerator for 2 days afforded a crystalline mass, which was recrystallized twice from MeOH and EtOH; isolation yield 1.79 g (42%), mp 159.5—161°, $[\alpha]_D^{18}$ −46.3° ($c$=1.1, MeOH), $Rf_1$ 0.20. *Anal.* Calcd for $C_{11}H_{20}N_2O_6S$: C, 42.84; H, 6.54; N, 9.09. Found: C, 42.58; H, 6.69; N, 8.91.

**H-Cys(Acm)(O)-OH**——Boc–Cys(Acm)(O)–OH (400 mg, 1.3 mmol) was treated with TFA (1.1 ml) in the presence of anisole (0.3 ml) in an ice-bath for 60 min, then dry ether was added. The resulting powder was collected by filtration and dissolved in $H_2O$ (3 ml). After neutralization with $Et_3N$, the solution was concentrated and the residue was recrystallized twice from EtOH; yield 167 mg (62%), mp 168° dec., $[\alpha]_D^{18}$

$-9.4°$ ($c=0.9$, $H_2O$), $Rf_2$ 0.34, $Rf_3$ 0.24, $Rf_4$ 0.29. The retention time on the long column of an amino acid analyzer was 49 min. *Anal.* Calcd for $C_6H_{12}N_2O_4S \cdot 1/4H_2O$: C, 33.87; H, 5.92; N, 13.17. Found: C, 34.01; H, 5.92; N, 13.20.

**Treatment of H-Cys(Acm)(O)-OH with Mercuric Acetate**——According to Veber *et al.*,[5] H–Cys(Acm)(O)–OH (100 mg, 0.48 mmol) was dissolved in $H_2O$ (10 ml) and the pH of the solution was adjusted to 4.0 with AcOH. Mercuric(II) acetate (153 mg, 0.48 mmol) was added and the mixture, after readjusting the pH to 4.0, was stirred at room temperature for 60 min. No change was observed on TLC ($Rf_3$ 0.24). When the solution was examined using an amino acid analyzer, no cysteine or cystine was detected.

**Treatment of H-Cys(Acm)(O)-OH with Iodine**——According to Kamber,[6] a solution of $I_2$ (76 mg, 2.5 equiv.) in MeOH (2.4 ml) was added dropwise to a stirred solution of H–Cys(Acm)(O)–OH (50 mg, 0.24 mmol) in $H_2O$–MeOH (1: 1, 4 ml) at 20° over a period of 30 min. Stirring was continued for 45 min. No change was observed on TLC ($Rf_2$ 0.34, $Rf_4$ 0.29). When the solution was examined using an amino acid analyzer, no cysteine or cystine was detected.

**Treatment of Boc-Cys(Acm)(O)-OH with HF**——Boc–Cys(Acm)(O)–OH (500 mg, 1.62 mmol) was treated with HF (*ca.* 4 ml) in the presence of anisole (0.35 ml, 2 equiv.) at $-5°$ for 60 min. After removal of HF by evaporation, the residue was washed with ether and dissolved in $H_2O$ (5 ml). The pH of the solution was adjusted to 7 with 5% $NH_4OH$. When examined with a Shimadzu dual wavelength TLC scanner, three components were detected; a compound with $Rf_2$ 0.63 (72.5%, isolated as described below), a minor compound with $Rf_2$ 0.52 (8.4%, not identified) and an additional minor compound with $Rf_2$ 0.23 (10%, presumably H–Cys(Acm)(O)–OH). The solution was stored in a refrigerator overnight. The resulting powder was collected by filtration, washed with the upper phase of *n*-BuOH–AcOH–$H_2O$ (4: 1: 5) and recrystallized from 40% AcOH to afford a compound with $Rf_2$ 0.63; isolation yield 100 mg (27%); mp 190—192°, $[\alpha]_D^{18}$ +10.1° ($c=0.6$, 50% AcOH), $Rf_2$ 0.63. $^1$H-NMR ($CF_3COOD$): $\delta$ 3.60 (2H, d, $\beta$-$CH_2$), 3.96 (3H, s, OMe), 4.45 (1H, t, $\alpha$-CH), 7.03 (2H, d, aromatic H), 7.55 (2H, d, aromatic H). The spectral data are identical with those of S-*p*-methoxyphenylcysteine (lit.[4] mp 189—190°, $[\alpha]_D^{22}$ +11.8° in 50% AcOH). The retention time on the short column of an amino acid analyzer was 38 min. *Anal.* Calcd for $C_{10}H_{13}NO_3S$: C, 52.84; H, 5.77; N, 6.16. Found: C, 52.58; H, 5.80; N, 5.90.

**Treatment of Boc-Cys(Acm)(O)-OH with MSA**——Boc–Cys(Acm)(O)–OH (800 mg, 2.60 mmol) was treated with MSA (2.3 ml) in the presence of anisole (0.56 ml, 2 equiv.) at 20° for 80 min, then dry ether was added and the resulting powder was dissolved in $H_2O$ (7 ml). When the solution was examined by TLC and with an amino acid analyzer, three components were detected. The compound with $Rf_2$ 0.63 (retention time on the short column, 38 min) was identified as S-*p*-methoxyphenylcysteine; a minor compound with $Rf_2$ 0.52 was not identified and a compound with $Rf_2$ 0.36 (47.6%, retention time on the long column, 116 min) was identified as cystine.

**Treatment of H-Cys(Acm)(O)-OH with Na in Liq. $NH_3$**——H–Cys(Acm)(O)–OH (40 mg, 0.19 mmol) was treated with Na (18 mg, 4 equiv.) in liq. $NH_3$ (approximately 5 ml) 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 with an amino acid analyzer. Several peaks were detected on the long column with the following retention times; 30 min (cysteic acid, 19%), 49 min (H–Cys(Acm)(O)–OH, minor), 56 min (H–Cys(Acm)–OH, 21%), 62 min (unknown, minor), 77 min (unknown, minor), 99 min (cysteine, minor), 106 min (unknown, minor), 116 min (cystine, 10%).

**Hydrolysis of Boc-Cys(Acm)(O)-OH with 6 N HCl**——Boc–Cys(Acm)(O)–OH (0.198 mg) was hydrolyzed with 6 N HCl (1 ml) in a sealed tube at 110° for 24 hr and the hydrolysate was examined with an amino acid analyzer. A peak with a retention time of 116 min (cystine) was detected on the long column.

**Hydrolysis of Boc-Cys(Acm)(O)-OH with 6 N HCl in the Presence of Phenol**——Boc–Cys(Acm)(O)–OH (0.248 mg) was hydrolyzed with 6 N HCl (1 ml) in the presence of phenol (0.3 mg, 4 equiv.) at 110° for 24 hr. When the hydrolysate was examined with an amino acid analyzer, the following peaks were detected: on the short column, 26 min (assigned as S-*p*-hydroxyphenylcysteine,[4] 41%), and on the long column, 116 min (cystine, 16%).

**Treatment of Boc-Cys(Acm)(O)-OH with Thiophenol**——Boc–Cys(Acm)(O)–OH (200 mg, 0.65 mmol) in DMF was treated with thiophenol (1.33 ml, 20 equiv.) at 80° for 12 hr. When examined by TLC, the starting material had completely disappeared and two new spots ($Rf_5$ 0.32, $Rf_5$ 0.48) were detected by Ce-$(SO_4)_2$ staining. The solvent was evaporated off and the residue was extracted with ether. The organic phase was washed with $H_2O$, dried over $Na_2SO_4$ and concentrated. The residue was dissolved in a small amount of $CHCl_3$ and purified by column chromatography on silica gel (Kieselgel 60, Merck, 1.8×19 cm), eluting with $CHCl_3$ (100 ml), then $CHCl_3$–MeOH (20: 0.5 v/v, 50 ml) and finally $CHCl_3$–MeOH (10: 0.5, 150 ml); yield of an oily compound with $Rf_5$ 0.48, 35 mg (30%), MS *m/e*: 181 ($M^+$ $C_9H_{11}NOS$). $^1$H-NMR ($CDCl_3$): $\delta$ 1.90 (3H, s, Me), 4.62 (2H, d, $CH_2$), 7.16—7.45 (5H, aromatic H). Yield of an oily compound with $Rf_5$ 0.32, 44 mg (21%), $[\alpha]_D^{18}$ $-14.8°$ ($c=1.6$, $CHCl_3$). MS *m/e*: 329 ($M^+$ $C_{14}H_{19}NO_4S_2$). $^1$H-NMR ($CDCl_3$): $\delta$ 1.43 (9H, s, Boc), 3.18 (2H, d, $\beta$-$CH_2$), 4.56 (1H, m, $\alpha$-CH), 7.19—7.69 (5H, m, aromatic H).

**Acknowledgement** This investigation was supported in part by a grant (477928) from the Ministry of Education, Science and Culture, Japan.