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Studies on Peptides. LXXIV.[1,2] Convenient Procedure for the Preparation of Methionine Sulphoxide Derivatives

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Sodium perborate can be used for oxidation of N^{α}-protected Met derivatives to the corresponding (*R*,*S*)-sulphoxide. Thus a new derivative, Z(OMe)-Met-(*R*,*S*)-sulphoxide was easily prepared in good yield. Sodium metaperiodate was also employed for this purpose.

Keywords——methionine sulphoxide; methionine sulphone; sodium perborate oxidation; sodium metaperiodate oxidation; hydrogen peroxide oxidation

As described in a series of papers,[1,4-6] we have been evaluating the usefulness of methanesulphonic acid (MSA) as a deprotecting reagent at the final step of peptide synthesis and found that for the synthesis of peptides containing methionine its sulphur atom has to be protected as the sulphoxide, otherwise, to which the methyl group of anisole, a cation scavenger, is transformed.[6] Preliminarily, a model peptide, H-Tyr-Gly-Gly-Gly-Lys-Met-Gly-OH named endorphin,[7] was synthesized after deprotection of Z(OMe)-Tyr-Gly-Gly-Gly-Lys(Z)-Met(O)-Gly-OH with MSA followed by reduction of the sulphoxide moiety to the parent amino acid residue with dithiothreitol.[1] Thus in the MSA procedure, it became necessary to secure an enough quantity of Met(O) derivatives as the starting material of peptide synthesis.

The use of Met(O) in peptide synthesis was first introduced by Iselin[8] in 1961. Oxidation of Z-Met-OH with hydrogen peroxide gives Z-Met-(*R*,*S*)-sulphoxide and, in some instances, the corresponding sulphone also, depending on the conditions employed. Z- and Boc-Met-(*S*)-sulphoxide[9] were only obtained starting with the pre-separated isomer, methionine-(*S*)-sulphoxide. Bordignon *et al.*[10] reported that oxidation by tetrachloroauric (III) acid proceeds stereospecifically to give Met-(*S*)-sulphoxide and we applied this reagent for the synthesis of endorphin stated above.

We now examined more safer and easier procedures for the preparation of this amino acid derivatives. Three N^{α}-protected derivatives, Z-, Boc-, and Z(OMe)-Met-OH, in ethyl acetate were exposed to mild oxidants available, including hydrogen peroxide,[8,9] such as sodium metaperiodate, sodium perborate, chloramine T (CT), tribromocresol (TBC) and N-chlorosuccinimide (NCS). Latter three reagents are those examined by Patchornik *et*

1) Part LXXIII: H. Irie, N. Fujii, H. Ogawa, H. Yajima, M. Fujino, and S. Shinagawa, *Chem. Pharm. Bull.* (Tokyo), **25**, 2929 (1977).
2) The following abbreviations were used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Met(O)=methionine sulphoxide. Met used here is of the (*S*)-configuration.
3) Location: *Sakyo-ku, Kyoto, 606 Japan.*
4) H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.* (Tokyo), **23**, 1164 (1975).
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al.[11–13] for oxidative cleavage of the tryptophan residue in proteins. Thin-layer chromatography of the reaction mixtures exhibited two close spots with different intensities depending upon reagents employed and the high *Rf* value corresponded to that of Met-sulphone prepared by the known procedure.[14]

These results suggest that conditions for the specific oxidation of the sulphur atom of Met to the corresponding sulphoxide, free from contamination with the sulphone, seems difficult to establish. However by recrystallization of the products from methanol and ethyl acetate, we could isolate each Met sulphoxide derivative, except the product of CT oxidation, as a chromatographically homogeneous substance (the low *Rf* substance) respectively. Physical constants of these products were listed in Table I, II and III in the experimental section. When rotation values of the Boc- and Z-derivatives were compared with those of references,[8,9] it can be realized that products obtained are a mixture of two diasteroisomers of (*S*)-(*S*) and (*S*)-(*R*) configuration, though they look as if a single component on thin layer chromatography resectively. The ratios of the diastereoisomers seem to differ each other, depending upon reagents employed for oxidation as previously predicted by Lavine[15] during the oxidation of Met by potassium iodate or iodine.

It should be mentioned that the hydrogen peroxide oxidation gave, besides the sulphoxide, a small amount of the sulphone also and even the sulphoxide is a heterogenous mixture of the two diastereoisomers. Among reagents tested, the CT oxidation gave the high *Rf* substances (corresponding sulphone) predominantly, which were poorly solidified. Oxidation by TBC and NCS completed within 30 minutes at room temperature giving the corresponding sulphoxides (the low *Rf* substances) as the main products respectively, but these oxidations suffer some disadvantages. The former reagent has to be prepared before use and in the latter, a certain amount of deprotection of the Boc and Z(OMe) groups was observed, because of the acidity of the reaction mixture.

When sodium metaperiodate was employed for oxidation, the starting material disappeared within 7 hours at room temperature and, in the case of sodium perborate, overnight reaction was required. In both cases, the corresponding sulphoxides (the low *Rf* substances) were the main products and, especially in the latter, the formation of the sulphones were negligible. From experiences so far examined, we wish to conclude that sodium perborate, rather sodium metaperiodate, seems to be a very convenient reagent to convert the N^{α}-protected Met derivatives to the corresponding sulphoxides, which are a mixture of the diastereoisomers, but not far from unity, as seen from the rotation figures of Boc- and Z-Met(O)-OH prepared by this reagent. Confirmatively, Z(OMe)-Met(O)-OH prepared by the sodium perborate was deprotected by trifiuoroacetic acid in the presence of anisole and the resulting sulphoxide was submitted to the amino acid analyser. In the long column of the analyser, nearly equal amounts of the (*S*)-(*S*) and (*S*)-(*R*) diastereoisomers were detected. Z(OMe)-Met(O)-OH, as an example of a new procedure for the preparation of Met(O)-derivatives by sodium metaperiodate or perborate, was given in the experimental section.

Experimental

Thin-layer chromatography was performed on silica gel (Kieselgel G. Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–AcOH (9: 1: 0.5 v/v) and Rf_2 $CHCl_3$–MeOH–H_2O (8: 3: 1 v/v).

Preliminary Oxidation Test——Three N^{α}-protected derivatives (0.01 mol each), Z–Met–OH, Boc–Met–OH and Z(OMe)–Met–OH, in AcOEt (10 ml) were oxidized at room temperature (22°) by the following reagents: a) 30% H_2O_2 (1.36 ml, 1.2 equiv.) in the presence of pyridinium chloride (1.16 g, 1 equiv.) in H_2O (5 ml)

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overnight. b) $NaIO_4$ (2.35 g, 1.1 equiv.) in H_2O (10 ml) for 7 hr. c) $NaBO_3$ (1.84 g, 1.1 equiv.) in H_2O (10 ml) overnight. d) TBC (0.42 g, 1.1 equiv.) in H_2O (5 ml) for 30 min. e) NCS (0.16 g, 1.1 equiv.) in H_2O (5 ml) for 30 min. f) CT (3.12 g, 1.1 equiv.) in H_2O (15 ml) for 3 hr. When each starting material disappeared on thin-layer chromatography, the solution was acidified with 5% citric acid. The organic phase was separated, washed with H_2O–NaCl, dried over Na_2SO_4 and then condensed *in vacuo*. The residue was triturated with AcOEt and recrystallized from MeOH and AcOEt.

(I) Z–Met(O)–OH: Physical constants of the products (Rf_2 0.60) were listed in Table 1. Z–Met(O_2)–OH (Rf_2 0.63) formed during the oxidation was removed by recrystallization.

Table I. Z-Met(O)-OH prepared by Various Oxidants

Reagent	mp °C	$[\alpha]_D^{22}$ in AcOH	Yield
H_2O_2[a)]	98—101	+1.9	63
$NaIO_4$	100—104	+2.5	61
$NaBO_3$	91—93	+3.9	84
TBC	92—94	+0.5	55
NCS	89—92	+5.5	59
CT	Not purified		

a) lit.[9] Z-Met-(*R*,*S*)-sulphoxide: mp 97—100°, $[\alpha]_D$+3.2° in AcOH.
Z-Met-(*S*)-sulphoxide: mp 112—114°, $[\alpha]_D$+52.8° in AcOH.
Z-Met-(*R*)-sulphoxide: mp 117—119°, $[\alpha]_D$−43.9° in AcOH.

(II) Boc–Met(O)–OH: The title compound is partly soluble in H_2O and this property caused the decrease of isolation yield. Physical constants of the products (Rf_1 0.40) were listed in Table II. The sulphone (Rf_1 0.48) detected during the oxidation was removed by recrystallization.

Table II. Boc-Met(O)-OH prepared by Various Oxidants

Reagent	mp °C	$[\alpha]_D^{22}$ in DMF	yield
H_2O_2[a)]	116—118	−13.9	44
$NaIO_4$	113—115	−11.8	69
$NaBO_3$	116—119	−12.6	49
TBC	101—104	−10.6	41
NCS	Not purified		
CT	Not purified		

a) lit.[9] Boc-Met-(*R*,*S*)-sulphoxide: mp 123—125°, $[\alpha]_D$−9.8° in DMF.
Boc-Met-(*S*)-sulphoxide: mp 142—145°, $[\alpha]_D$+41.6° in DMF.

(III) Z(OMe)–Met(O)–OH: Physical constants of the products (Rf_1 0.39) were listed in Table III. The sulphone (Rf_1 0.42) detected during the oxidation was removed by recrystallization.

Table III. Z(OMe)-Met(O)-OH prepared by Various Oxidants

Reagent	mp °C	$[\alpha]_D^{22}$ in AcOH	yield
H_2O_2	97—100	+6.4	81
$NaIO_4$	98—100	+2.4	84
$NaBO_3$	97—100	+8.4	79
TBC	98—101	+3.3	61
NCS	97—101	+1.9	63
CT	77—79	−9.5	52

Z(OMe)–Met(O)–OH——a) Oxidation by $NaIO_4$: A mixture of Z(OMe)–Met–OH (15.67 g) in AcOEt (100 ml) and $NaIO_4$ (13.9 g, 1.3 equiv.) in H_2O (80 ml) was stirred efficiently at room temperature for 7 hr until the starting material disappeared on thin-layer chromatography. Two spots, Rf_1 0.39 and 0.42 (faint spot), were detected. After acidification with citric acid, the organic phase was separated, washed with H_2O–NaCl, dried over Na_2SO_4 and then condensed *in vacuo*. The residue was triturated with AcOEt and recrystallized twice from MeOH and AcOEt; yield 11.82 g (72%), mp 97—99°, $[\alpha]_D^{22}$ +2.9° (c=2.1, AcOH).

Rf_1 0.39, Rf_2 0.61. *Anal.* Calcd. for $C_{14}H_{19}NO_6S$: C, 51.05; H, 5.81; N, 4.25. Found: C, 51.09; H, 5.75; N, 4.25.

b) Oxidation by $NaBO_3$: Z(OMe)-Met-OH (15.67 g) in AcOEt (100 ml) was similarly oxidized by $NaBO_3$ (9.23 g, 1.2 equiv.) in H_2O (50 ml) at room temperature overnight. On thin-layer chromatography, two spots, Rf_1 0.39 (main spot) and 0.42 (very faint spot), were detected. The product was isolated as stated above and recrystallized from MeOH and AcOEt; yield 12.99 g (79%), mp 98—101°, $[\alpha]_D^{22}$ +8.4° (c=1.9, AcOH). Rf_1 0.39. *Anal.* Found: C, 51.30; H, 6.05; N, 4.16.

The product (0.50 g) was treated with trifluoroacetic acid (2 ml) in the presence of anisole (0.5 ml) in an ice-bath for 60 min and dry ether was added. The precipitated powder was dissolved in a small amount of H_2O and the solution was neutralized with Et_3N. After evaporation of the solvent, EtOH was added to the residue to afford the powder, which was recrystallized from H_2O and EtOH; yield of H-Met(O)-OH 0.21 g (81%), mp 238° (dec.), Rf 0.29 in *n*-BuOH-AcOH-AcOEt-H_2O (1:1:1:1), $[\alpha]_D^{22}$ +35.3° (c=0.9, in 1 N HCl). (lit.[8] H-Met-(*R*,*S*)-sulphoxide, H_2O_2 oxidation product, $[\alpha]_D$ +33.6° in 1 N HCl). Ratios of the (*S*)-(*S*) and (*S*)-(*R*) isomers determined in the long column of the amino acid analyser (Hitachi KLA-5) were 1 (retention time 55 min): 1.074 (retention time 57 min). The standard sample of H-Met(O)-OH [(*S*)-(*S*)], was prepared through the corresponding picrate.[16] mp 248—251°, $[\alpha]_D^{25}$ +123.3° (c=2.0, 1 N HCl). (lit.[8] $[\alpha]_D$ +127.2° in 1 N HCl, lit.[16] $[\alpha]_D$ +131° in 1 N HCl).

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N-[(N-Nitrosobenzylamino)methyl]benzamide as a Direct Benzylating Agent

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A new application of N-[(N-nitrosobenzylamino)methyl]benzamide as a direct benzylating agent has been realized by benzylation of several protic materials. Ferric chloride functions as a catalyst in the benzylation of ethanol, acetic acid, phenol, and thiols.

Keywords——N-[(N-nitrosobenzylamino)methyl]benzamide; benzylating agent; decomposition of N-nitrosoamine; ferric chloride; phenyldiazomethane

Recently the convenient diazoalkane generation from N-[(N-nitrosoalkylamino)methyl]-amides has been reported[2] from this laboratory. In the present paper we wish to report on several new applications of N-[(N-nitrosoalkylamino)methyl]amides as a direct alkylating agent through diazoalkane generation *in situ*.

In alkali solution, N-[(N-nitrosoalkylamino)methyl]benzamides have been known to be susceptible to the decomposition into diazoalkanes.[2]

$$2C_6H_5CONHCH_2\underset{\underset{NO}{|}}{N}CH_2R \xrightarrow{^-OH} 2RCHN_2 + (C_6H_5CONH)_2CH_2 + CH_2O + H_2O$$

1) Location: *2-2-1 Oshika, Shizuoka 422, Japan.*
2) M. Sekiya, Y. Ohashi, Y. Terao, and K. Ito, *Chem. Pharm. Bull.* (Tokyo), **24**, 369 (1976).