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L-METHIONINE OXIDATION : NOVEL AND UNANTICIPATED TRANSFORMATIONS WITH 4-^{t} BUTYL IODOXYBENZENE

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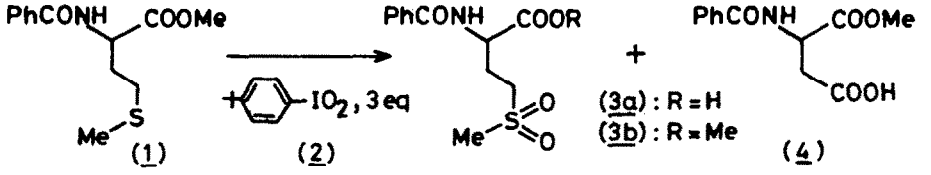
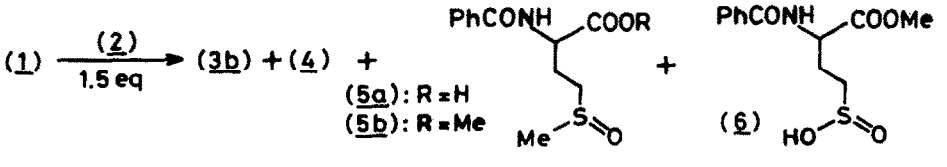
ABSTRACT : 4-^{t}Butyl iodoxybenzene transforms Bz-Met-OMe to products arising from, S oxidation and C-H insertion followed by degradation. Z-Met (sulfoxide)-OMe is very effective in bringing about ester hydrolysis via intramolecular attack. The S oxidation to sulfoxides and then to sulfones can be monitored and controlled, proceeds with chiral retention, affects neither the peptide bond nor the protecting groups and has been further illustrated with, Z-Gly-Met-OMe, Z-Met-OMe and Z-S(benzyl)-Cys-OMe.

The selective transformation of the methionine side chain is an important operation in protein synthesis and rupture, a recent example being in the humulin synthesis, enabling the separation of the β -galactosidase fragment from insulin chains[1]. This paper reports of a study of the reaction of N-benzoylmethionine methyl ester (Bz-Met-OMe, 1)[2] and related compounds with 4-^{t}butyl iodoxybenzene (2)[3] revealing, inter alia, unexpected, novel and useful facets pertaining to (1) as well as (2).

The reaction of (1) with 3 eq of (2) in refluxing PhCl for 3 h, gave Bz-Met (sulfone)-OH(3a, 48%), Bz-Met(sulfone)-OMe(3b, 10%) and Bz-Asp(β -OH)-OMe(4, 14%) :

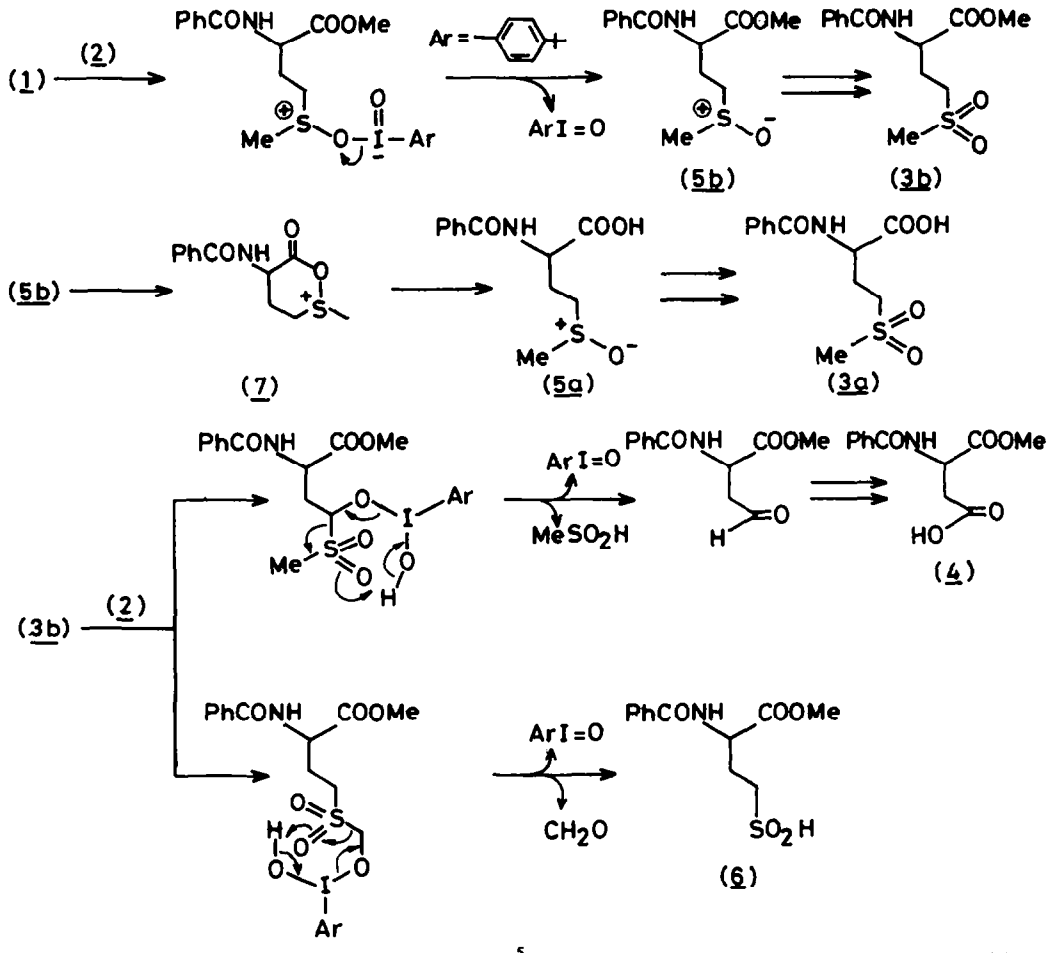


The formation of (3a) and (4) was quite unexpected and a detailed examination of the oxidation of (1) with 1.5 eq of (2) yielded, (3b) (29%), (4) (9%), (5a) (12%), (5b) (36%) and (6) (12%) :



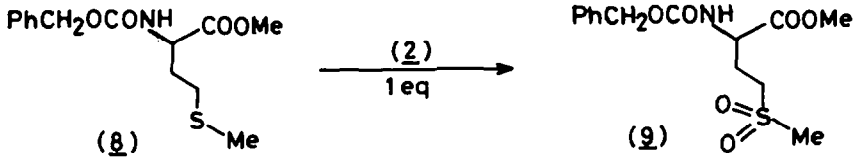
The genesis of acids (3a) and (5a) can be rationalized on the basis of intramolecular cyclization of the initially formed sulfoxide (5b) leading to (7) followed by hydrolysis to (5a)[4] and by further oxidation to (3a). The logical extension of this, namely, the possible use of methionine S-oxides in peptide rupture, is under study. The formation of the remaining products further reinforce the notion of 4-^{t}butyl iodoxybenzene as an ozone equivalent[3]. Whilst pathways leading to (5b) and (3b) are unexceptional, those to (4), and (6) likely involve C-H insertion of (2) and fragmentation (SCHEME 1). The pathways envisaged in these transformations are similar to those involved in the reaction of dialkylsulfides with ozone leading

SCHEME I

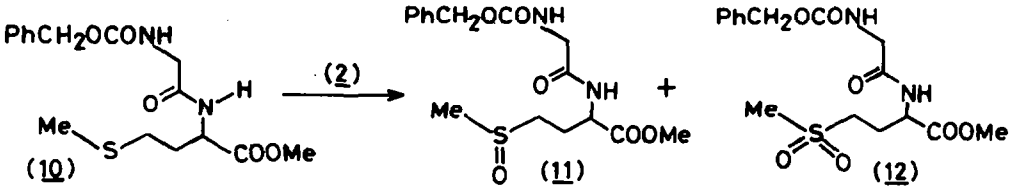


to oxidation and, fragmentation without selectivity[5]. In the present case also, the isolation of (4) and (6) in the same range of yields, suggests lack of selectivity in the (2) C-H insertion. The (1)→(4) degradation opens up the possibility of the use of methionine or related compounds as placid precursors of aspartic acid in protein synthesis[6], provided higher selectivity and yields could be obtained.

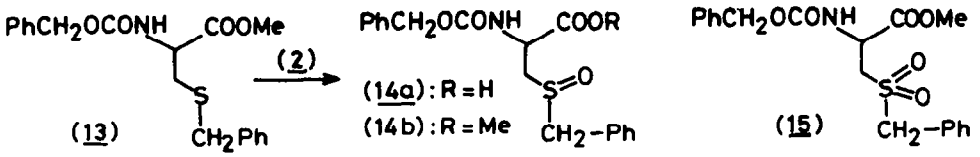
Using 1 eq. of the reagent (2) and by careful monitoring of the reaction by tlc, Z-Met(sulfone)-OMe(9) was obtained in 57% yields from Z-Met-OMe(8), thus making the sulfone transformation experimentally viable.



No perceptible loss of chirality was noted in the side chain oxidation with (2). Neither was the peptide bond affected as demonstrated with the transformation of Z-Gly-Met-OMe(10) to Z-Gly-Met (sulfoxide)-OMe (11, 42%) and Z-Gly-Met(sulfone)-OMe(12, 26%) :



S-Protected cysteine presented a profile similar to that of methionine towards (2). Z-S(benzyl)-Cys-OMe(13) on treatment with 1.5 eq. of (2) gave Z-S(benzyl)-Cys(sulfoxide)-OMe(14b, 30%), Z-S(benzyl)-Cys(sulfoxide)-OH(14a, 7%) and Z-S(benzyl)Cys(sulfone-OMe(15, 18%) :



We feel that 4-^{t}butyl iodoxybenzene has potential for use in the side chain modification of peptides.

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EXPERIMENTAL[7]

I. The reaction of 4-^{t}butyl iodoxybenzene (2) with N-benzoyl methionine methyl ester(Bz-Met-OMe,1): Isolation of Bz-Met(sulfone)-OH(3a), Bz-Met(sulfone)-OMe(3b) and Bz-Asp-(β-OH)-OMe(4).

A stirred solution of (1)[8] (0.518 g, 1.94 mmol) in chlorobenzene(13 ml) was admixed, in lots, with (2) (1.70 g, 5.82 mmol, 3 eq), refluxed for 3 h when a clear solution was obtained, cooled solvents evaporated in vacuo, the residue triturated with satd. $NaHCO_3$ (≈ 50 ml) for 3 h, extracted with EtOAc (3x30 ml), dried, evaporated and the residue chromatographed on silica gel. Elution with EtOAc:PhH::60:40 gave, 0.056 g(10%) of (3b), mp 118-120°C(EtOAc). (Found:C,51.68; H,5.39;N,4.37; Calc. for $C_{13}H_{17}NO_5S$:C,52.17; H,5.68; N,4.68%); IR: ν_{max} (KBr) cm^{-1} 3300, 1730, 1630, 1560, 1290, 1120; ^{1}H-NMR: δ ($CDCl_3$)2.9(s,3H), 3.0-3.41(m,4H), 3.75(s,3H), 4.9(q,1H), 7.1-7.9(m,5H); m/z:299(M^+).

The bicarbonate extract was cooled, made acidic to pH~ 3(2N H_2SO_4), saturated with NaCl, extracted with EtOAc(3x30 ml), dried and evaporated. The residue (0.510g) totally free from non-acidic compounds (tlc), consisted of (3a) (mp 229°C[9], 48%) and (4) (mp 126°C, 14%). This analysis was made on the basis of separation of the corresponding methyl esters-prepared in quantitative yields with diazomethane-by preparative tlc, using PhH:EtOAc::7:3 as the developer. Compounds (3b)(vide supra) and Bz-Asp (β-OMe)-OMe thus obtained were found identical to that of authentic samples. Bz-Asp(β-OMe)-OMe : mp 88-89°C (benzene-hexane); IR: ν_{max} (KBr) cm^{-1} 3300, 1730(br), 1640, 1530; ^{1}H-NMR : δ($CDCl_3$) 3.1(dd,2H), 3.7(s,3H), 3.8(s,3H), 5.1(m,1H), 7.4-8.0(m,6H); $[\alpha]_D^{25}$ = 70(c,0.46,$CHCl_3$).

II. The reaction of (1) with restricted amount of reagent (2) : Isolation of, (3a), (3b), (4), Bz-Met(sulfoxide)-OH(5a), Bz-Met(sulfoxide)-OMe(5b) and BzNHCH($CH_2CH_2SO_2H$)COOMe(6) :

The reaction of (1) (0.801 g, 3 mmol) with (2) (1.314 g, 4.5 mmol) was carried out and processed as described in Experiment I. The neutral residue on chromatography over silica gel and elution with PhH:EtOAc::3:7 gave 0.255 g(29%) of (3b) followed by 0.106 g (12%) of (6); mp. 132°C(benzene-hexane); IR: ν_{max} (KBr) cm^{-1} 3300, 1735, 1625, 1570, 1030; ^{1}H-NMR : δ($CDCl_3$) 2.8-3.5(m,4H), 3.8(s,3H), 4.9(q,1H), 7.2-8.12(m,5H); m/z : 285(M^+), 269(M^+-16).

Further elution with PhH:EtOAc:: 1:4 gave 0.304 g(36%) of (5b); colorless glassy mass; (Found:C, 55.25; H,6.13; N,4.56; Calc. for $C_{13}H_{17}NO_4S$: C,55.12; H,6.00; N,4.94%); IR : ν_{max} (neat) 3300, 1730, 1635, 1565, 1030; ^{1}H-NMR: δ ($CDCl_3$) 2.56(s,3H), 2.63-3.10(m,4H), 3.73(s,3H), 4.85(q,1H), 7.3-8.0(m,5H); m/z:283(M^+), 267(M^+-16).

The acidic residue, arising from processing of the bicarbonate extract as described in Experiment I, was found to be free from neutral products (tlc) and consisted of (4) (9%) and (5a) (12%). This analysis was made on the basis of separation of the corresponding methyl esters by column chromatography using PhH:EtOAc(3:2 and then 1:1) as eluent. Bz-Asp -di OMe) and (5b) thus obtained were found to be identical with authentic samples.

III. The reaction of N-benzyloxycarbonyl methionine methyl ester(8) with (2) : Isolation of Z-Met(sulfone)-OMe(9).

The reaction of (8)[10] (0.24 g, 0.808 mmol) and (2) (0.284 g, 0.972 mmol) was carried out as described in Experiment I. The oxidation was monitored by tlc and terminated after 2.5 h. Work-up as described in Experiment I yielded only neutral residue, which on chromatography over silica gel and elution with PhH:EtOAc::1:1 gave 0.150 g (57%) of (9) mp.89°C(benzene) (Found:C, 50.84; H,5.86; N, 4.01; Calc. for $C_{14}H_{19}NO_6S$: C,51.06; H,5.77; N,4.25%); IR : ν_{max} (KBr) cm^{-1} 3310, 1725, 1680, 1520, 1310, 1120; ^{1}H-NMR : δ($CDCl_3$) 2.38 (m,2H), 2.88(s,3H), 2.93-3.38(m,2H), 3.76(s,3H), 4.53(m,1H), 5.09(s,2H), 5.78(d,1H), 7.31(s,5H); m/z: 329(M^+).

IV. The reaction of N-benzyloxycarbonyl glycyl methionine methyl ester (Z-Gly-Met-OMe, 10) with(2): Isolation of Z-Gly-Met(sulfoxide)-OMe(11) and Z-Gly-Met(sulfone)-OMe(12).

To a stirred solution of (10)[11] (1.416 g, 4 mmol) in chlorobenzene (30 ml) was added (2) (1.46 g, 5 mmol) at rt. The mixture was held at 80-90°C for 1.5 h, cooled solvents evaporated in vacuo and the residue chromatographed over silica gel. Elution with EtOAc:MeOH::98:2 gave 0.400 g(26%) of (12); thick syrupy liquid; (Found: C,49.69; H,5.42; N,7.24; Calcd. for $C_{16}H_{22}N_2O_7S$: C, 49.74; H,5.69; N,7.25%; IR: ν_{max}(neat) cm^{-1} 3340, 1735, 1680, 1540, 1300, 1140, ^{1}H-NMR : δ ($CDCl_3$) 2.83(s,3H), 2.92-3.44(m,4H), 3.7(s,3H), 3.77-4.72(m,3H), 5.05(s,2H), 5.94(br,2H), 7.26(s,5H); m/z : 386(M^+).

Further elution with EtOAc:MeOH::1:1 gave 0.620 g (42%) of (11); thick syrupy liquid; (Found: C,51.80; H,5.62; N,7.31; Calc. for $C_{16}H_{22}N_2O_6S$: C,51.89; H,5.94; N,7.56%); IR : ν_{max}(neat) cm^{-1} 3330, 1740, 1670, 1540, 1045; ^{1}H-NMR : δ ($CDCl_3$) 2.51(s,3H), 2.58-3.28(m,4H), 3.7(s,3H), 3.88(m,2H), 4.78(m,1H), 5.08(s,2H), 5.98(br,2H), 7.28(s,5H); m/z : 370(M^+).

V. The reaction of N-benzyloxycarbonyl(S-benzyl) cysteine methyl ester Z-(S-benzyl)-Cys-OMe(13) with (2) : Isolation of Z-(S-benzyl)-Cys-(sulfoxide)-OH(14a), Z-(S-benzyl)-Cys-(sulfoxide)-OMe(14b) and Z-(S-benzyl)-Cys-(sulfone)-OMe(15).

A stirred solution of (13)[12] (0.359 g, 1 mmol) in chlorobenzene (15 ml) admixed with (2) (0.438 g, 1.5 mmol), refluxed for 1.5 h, cooled, solvents evaporated, the residue triturated with aquoues $NaHCO_3$ for 3.5 h, extracted with EtOAc(3x20 ml), dried, evaporated and the residue chromatographed over silica gel. Elution with benzene gave 0.068 g(18%) of (15); mp 175°C (benzene-hexane); (Found: C,58.22; H,5.39; N,3.22; Calc. for $C_{19}H_{21}NO_5S$: C,58.31; H,5.37; N,3.58%); IR: ν_{max}(KBr) cm^{-1} 3315, 1730, 1685, 1520, 1300, 1130, ^{1}H-NMR : δ($CDCl_3$) 3.5(d,2H), 3.71(s,3H), 4.2(s,2H), 4.72(m,1H), 5.08(s,2H), 5.9(br,1H), 7.3(s,10H); m/z : 391 (M^+).

Further elution with PhH:EtOAc::7:3 gave 0.111 g(30%) of (14b); mp. 119-121°C (benzene); (Found: C,60.28; H,5.77; N, 3.43; Calc. for $C_{19}H_{21}NO_5S$: C,60.80; H,5.60; N,3.73%); IR : ν_{max}(KBr) cm^{-1} 3310, 1730, 1685, 1520, 1030; ^{1}H-NMR : δ ($CDCl_3$) 3.06(d,2H), 3.61(s,3H), 3.91(s,2H), 4.57(br,1H), 5.01(s,2H), 6.11(Br,1H), 7.2(s,10H); m/z : 375(M^+).

The acidic residue, arising from processing of the bicarbonate extract as described in Experiment I, was found to be free of neutral products(tlc) and consisted of (14a) (7%). This analysis was made on the basis of diazomethane esterification and isolation of (14b) by chromatography over silica gel and elution with PhH:EtOAc::7:3.

REFERENCES AND NOTES

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2. All the amino acids used in this study have L configuration.
3. S. Ranganathan, D. Ranganathan and S.K. Singh, Tetrahedron Lett., 4955 (1985).
4. The water required for the (7)→(3a) change must be from reagent (2), which, we feel, retains moisture inspite of good drying. However, under conditions of the (1)→(3a) transformation, Bz-Phe-OMe is recovered unchanged, thus showing that the moisture alone cannot lead to the ester hydrolysis; further, esters of compounds related to those studied here are not affected in pH3 phosphate buffer in aq. MeCN, at rt.
5. P.S. Bailey and A.Y. Khashab J. Org. Chem., 43, 675(1978).
6. Problems are encountered in carrying on an early aspartic acid residue in peptide synthesis. These could perhaps be obviated if a suitable placid precursor could be identified.
7. MPs are not corrected. IR spectra were recorded on a PE-377 instrument as neat or KBr discs. NMR spectra were obtained ~5% solutions in $CDCl_3$ on FT-R 600 instrument. The chemical shifts are recorded in ppm with TMS at 0.00 as internal standard. Mass spectra were obtained on a Jeol instrument. Silica gel (Acme) was used for TLC and column chromatography (100-200 mesh). Reactions were monitored wherever possible by TLC. The organic extracts were invariably dried over anhydrous $MgSO_4$ and solvents evaporated in vacuo. Chromatography columns were prepared as slurry in hexane. Preliminary elutions were done with hexane and then with benzene to remove 4-^{t}butyl iodobenzene and, in the case of N-benzyloxycarbonyl protected amino acids, small and varying amounts of benzyl carbamate.
8. Bz-Met-OMe(1) was prepared by benzoylation of Met-OMe.HCl in aqueous bicarbonate, mp 75°C.
9. The reaction of Bz-Met-OMe(1) with Ru^{VIII}, at rt, gave a 65% yield of Bz-Met(sulfone)-OH(3a) and none of (4). Therefore (3a) could be obtained pure, was characterized, converted to (3b) and compared with authentic sample (S. Ranganathan, D. Ranganathan, D. Bhattacharyya and S. Shanthy, unpublished).
10. C.A. Dekker, S.P. Taylor and J.S. Fruton, J. Biol. Chem., 180, 155(1949).
11. Z-Gly-Met-OMe : HOBt(10 mmol) followed by DCC(10 mmol) was added to stirred Z-Gly(10 mmol) in dry CH_2Cl_2(20 ml). Met-OMe(10 mmol) in dry CH_2Cl_2(10 ml) was then added, left stirred overnight at rt, filtered, washed with CH_2Cl_2(2 x 15 ml), evaporated, the residue taken up in CH_2Cl_2, washed with 2 N HCl (2 x 15 ml), satd. bicarbonate (20 ml), satd NaCl, dried, evaporated, and the residue chromatographed on silica gel. Elution with EtOAc:benzene(1:1) gave 2.6 g(74%) of (10), viscous liquid; (Found:C,54.01; H,6.70; N,7.76; Calc. for $C_{16}H_{22}N_2O_5S$: C,54.23; H,6.21; N,7.90%); IR: ν_{max}(neat) cm^{-1} 3340, 1740, 1680, 1540; ^{1}H-NMR: δ ($CDCl_3$) 2.06(s,3H), 2.46(m,4H), 3.7(s,3H), 3.8-4.2(m,2H), 4.7(m,1H), 5.1(s,2H), 5.67(br,2H), 7.3(s,5H); m/z:354(M^+).
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