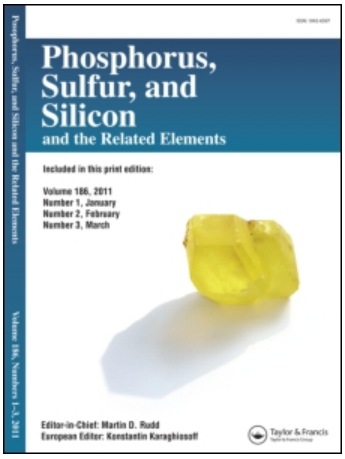

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SYNTHESIS AND PROPERTIES OF N-(DIISOPROPYLOXYPHOSPHORYL)- CYSTEINE AND ITS DERIVATIVES

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SYNTHESIS AND PROPERTIES OF N-(DIISOPROPYLOXYPHOSPHORYL)-CYSTEINE AND ITS DERIVATIVES

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N-(diisopropyloxyphosphoryl)cysteine and N,N-bis-(diisopropyloxyphosphoryl)cystine were synthesized, and their interconversion was studied. There was no N→S phosphoryl group migration. In contrast, the N-(diisopropyloxyphosphoryl)serine and threonine compounds exhibited N to O phosphoryl group migration.

Key words: Diisopropyl phosphite; phosphorylated amino acid; N-phosphorylated cysteine; N,N-bis-(diisopropyloxyphosphoryl)cystine.

INTRODUCTION

In our laboratory we have previously synthesized a series of phosphorylated amino acids and low molecular weight peptides using various dialkyl phosphites as the phosphorylating reagent.[1–3] Hijiya[4] and Baanwarth[8] have also reported the similar methods. Many phosphorylated peptides display the important biological activity,[6] and some of them contain one or several cysteine or cystine residues. We therefore undertook the phosphorylation of cysteine in order to understand the chemical, physical and biological properties of phosphorylated cysteine derivatives.

RESULTS AND DISCUSSIONS

Initially the cysteine **1** was phosphorylated with diisopropyl phosphite in a solution of triethylamine, carbon tetrachloride, methanol and water.[1] It was found that the expected product N-(diisopropyloxyphosphoryl)cysteine **3** was not isolated if more than three equivalents of triethylamine were used or if the reaction temperature was kept above 0°C. Instead, a crystalline material **2** was obtained (in 70% yield), it was only soluble in methanol and dimethyl sulfoxide (DMSO) and recrystallized from MeOH and Et_2O. Fast atom bombardment mass spectroscopy (FAB-MS) of this substance demonstrated a peak at m/z 569 [MH^+] and a peak at m/z 401 was observed resulting from successive loss of four propylene molecules from 569 [MH^+].

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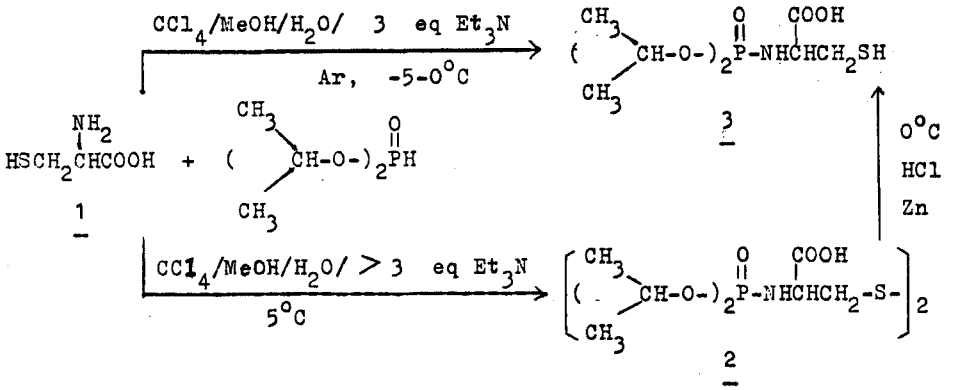
This observation was consistent with the report that the N-diisopropyloxyphosphoryl amino acids easily lose the propylene molecular fragment upon fast atom bombardment.[7-9] These results suggested the presence of two N-diisopropyloxyphosphoryl groups in this compound. The ^1H NMR spectrum of the compound displayed a triplet at 5.20 ppm for the proton of amino group and a broad peak of one proton at 3.84 ppm for carboxylic acid group, as indicated by D_2O exchange. The abnormal upfield shift of the carboxylic group might be due to the highly shielding effects of the phosphoryl ester group. Since the X-ray analysis shows the very short distance between the acidic proton and phosphoryl oxygen. The detail X-ray data will be reported in another paper. No signal was observed for a free thiol group in the compound, nor did it give a positive color test with sodium nitroferricyanide [$Na_2Fe(CN)_5NO$]. These results proved that the compound is the N,N-bis-(diisopropyloxyphosphoryl)cystine **2** (Scheme I).



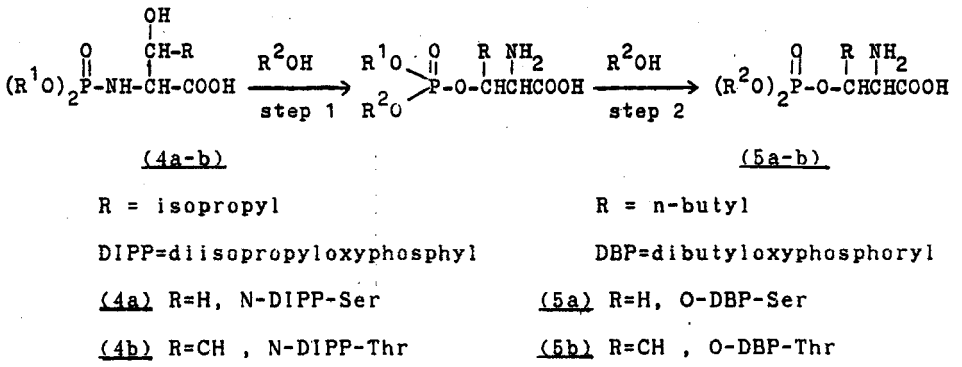
SCHEME I. The reaction of cysteine with diisopropyl phosphite.

Oxidation of the thiol group was prevented by carrying out the reaction with three equivalents of triethylamine, one equivalent of amino acid and diisopropyl phosphite in 2 ml water at a temperature below 0°C under the protection of Ar. The obtained oily product **3** gives a peak in the FAB-MS at m/z 286 [MH^+], with a fragmentation peak at 202 (MH^+-2 × 42), it indicates that the molecule lose two propylene molecules from one diisopropyloxyphosphoryl group. Its ^{13}C NMR and ^{31}P NMR spectra were similar to those of compound **2**, but its ^1H NMR spectrum displayed a triplet at 1.62 ppm for the thiol group. The proton resonances for the thiol, amino and carboxyl groups were all exchanged with D_2O. The IR spectrum of **3** showed the absorption at 2550 cm^{-1} for the thiol group. Also the compound gave a positive color test with the $Na_2Fe(CN)_5NO$. Furthermore, the compound **2** was reduced with Zn powder in 2 N HCl at 0°C to give the same oily substance. These phenomena confirm that the oily product is N-(diisopropyloxyphosphoryl)cysteine **3** (Scheme I).

It was previously reported that N-(diisopropyloxyphosphoryl)serine and N-(diisopropyloxyphosphoryl)threonine were so labile that they took place esters exchange on the phosphorus atom, as well as the migration of the dialkyloxy phosphoryl group from amino to hydroxy group in the alcoholic solution (Scheme II).[10]

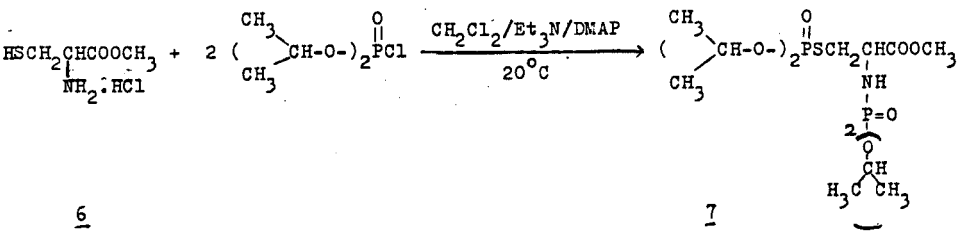
It is thought that the thiol group of **3** might have the similar behavior. Treatment of **3** with n-butanol and glacial acetic acid (10%) at 40°C for 24 hr under argon

resulted in no change by ^{31}P NMR, MS or TLC. This result means that there is no N to S migration, contrary to the N-phosphorylated serine and threonine **4a–b** (Scheme II) where N to O migration took place.



SCHEME II. The phosphoric ester exchange and the N→O migration in N-(diisopropyloxyphosphoryl)serine and threonine.

In the cysteine only the amino but not the thiol group can be phosphorylated easily in the mild basic condition, even if two equivalents of diisopropyl phosphite were used. However, the disopropyl chlorophosphate could be used for the phosphorylation of cysteine methyl ester. The product **7** was obtained in CH_2Cl_2 at 40°C after 48 hr. Compound **7** have two ^{31}P NMR chemical shifts at 24.03 ppm and 4.48 ppm for the P—S and P—N bonds respectively. In particularly, if catalytic amount of N-dimethylaminopyridine (DMAP) was added, the reaction time could be shortened to 12 hr at 20°C and the yield was up to 72%.



SCHEME III. The synthesis of N,S-bis-(diisopropyloxyphosphoryl)cysteine.

In conclusion, under very mild basic condition the cysteine could be phosphorylated on the amino group with the persistence of a free thiol group. But with a stronger basic condition the thiol group was oxidized to a disulfide linkage, which could be reduced back by zinc in acidic media. The methyl ester of cysteine could be phosphorylated both on the amino and thiol groups by the phosphochloridate reagents.

The N-phosphorylated cysteine has an inert phosphoryl group which would not give any chemical reaction under a mild acidic condition, whereas in the corresponding N-phosphoryl serine or threonine compounds, the phosphoryl group migration and ester exchange occurred. This substantial difference between the serine and cysteine might be the intrinsic character to divert their obligation in the enzyme's chemistry.[11–14]

EXPERIMENTAL

^1H NMR, and ^{13}C NMR spectra were taken on a JEOL FX-100 spectrometer with TMS as the internal standard in $CDCl_3$. The spectrum of N,N-bis-(diisopropyloxyphosphoryl)cystine **2** was recorded in DMSO as a solvent. The ^{31}P NMR spectra were recorded on the same instrument with 85% H_3PO_4 as an external reference. Positive ion FAB-MS were obtained on a KYKY ZhP-5 double focusing mass spectrometer (scientific Instrument Factory, Beijing, China) equipped with a standard KYKY fast argon atom gun. Elemental analyses were performed by the Analytical Laboratory, Institute of Chemistry, Academia Sinica, Beijing, China. IR spectra were recorded on a Perkin Elmer 180 spectrometer. Melting points are uncorrected.

Synthesis of N,N-bis-(diisopropyloxyphosphoryl)cystine, **2**. A mixture of diisopropyl phosphite (1.7 ml, 0.01 mol) and 5 ml CCl_4 was added dropwise to a suspension of L-cysteine (1.21 g, 0.01 mol) in 10 ml Et_3N, 10 ml H_2O and 5 ml MeOH cooled to 5°C. The mixture was stirred at 5°C for 10 hr. Then 10 ml H_2O was added and the mixture was extracted with ether (30 ml × 3). The water layer was adjusted to pH 3 by adding 2 N HCl, and then extracted with a mixed solvent of t-BuOH and EtOAc (1:1.5). The combined extraction solvents were dried with $MgSO_4$ and evaporated under reduced pressure at room temperature. A crystalline product (2 g, yield 70%) was obtained which was recrystallized with MeOH and ether, mp: 166–168°C. ^{31}P NMR: 5.45 ppm, ^1H NMR: 1.22 (d, 24H, J = 7Hz, $(CH_3)_2C$—), 2.9–3.27 (m, 6H, =N—CH— and CH_2—S—), 3.84 (bs, 2H, —COOH, disappears with D_2O), 4.3–4.7 (m, 4H, =CH—O—), 5.22 (t, 2H, J = 11 Hz, =NH, disappears with D_2O), ^{13}C NMR: 23.59 (d, J = 3Hz, $(CH_3)_2C$—), 36.98 (s, CH_2—S—), 53.89 (s, =N—CH=), 69.69 (d, J = 5.86 Hz, =CH—O—), 173.0 (d, J = 3 Hz, —COOH). FAB-MS: m/z 569 (MH^+, 14.7), 401 (MH^+ − 4 × 42, 9.7), 286 (33.8), 284 (20.9), 254 (18), 252 (12), 202 (17), 200 (13.4), 124 (98), 98 (100), Anal. calcd. for $C_{18}H_{38}O_{10}N_2P_2S_2$: C, 38.03; H, 6.69; N, 5.09; S, 11.26; Found: C, 38.37; H, 6.71; N, 5.04; S, 11.12: IR (KBr): 3300 (=NH), 1720 (C=O), 1200 (P=O), 990 (P—O) cm^{-1}.

Reduction of N,N-bis-(diisopropyloxyphosphoryl)cystine, **2**. Zinc powder (2.6 g, 0.04 mol) and 80 ml of 2 N HCl were added to a solution of 5 ml MeOH and **2** (1.14 g, 0.002 mol) under 0°C, stirred the suspension solution for 1 hr at 0°C. The mixture was filtered and the filtrate was extracted with ether (50 ml × 3). The ether was washed with saturated NaCl solution, dried with $MgSO_4$ and distilled under reduced pressure. A 1.0 g of oily product was obtained in 87% yield. ^{31}P NMR: 5.45 ppm; ^1H NMR: 1.37–1.40 (d, 12 H, J = 6 Hz, $(CH_3)_2C$—), 1.62 (t, 1H, J = 8 Hz, —SH, disappears with D_2O), 2.95 (dd, 2H, JcH_2-sH = 8 Hz, JcH-cH_2-s = 4 Hz, CH_2—S—, when added D_2O, the doublet of doublets has transformed into a doublet and J = 4 Hz, this means that the influence of thiol group has been eliminated), 4.0–4.12 (m, 1H, —N—CH—), 4.5 (t, 1H, J = 12 Hz, =NH, disappears with D_2O), 4.6–4.82 (m, 2H, =CH—O—), 10.7 (s, 1H, —COOH, disappears with D_2O); ^{13}C NMR: 23.55 (d, J = 5 Hz, $(CH_3)_2C$—), 28.75 (s, CH_2—S—), 55.42 (s, =N—CH—), 72.04 (d, J = 6 Hz, =CH—O—), 172.6 (d, J = 9 Hz, —COOH); FAB-MS: m/z 286 (MH^+, 9.1), 244 (6.8), 202 (57), 156 (100), 124 (96), 98 (71), Anal. calcd. for $C_9H_{20}NSPO_5$: C, 37.89; H, 7.02; N, 4.91; S, 11.23; Found: C, 37.72; H, 6.93; N, 5.07; S, 10.98: IR (KBr); 3280 (=NH), 2550 (—SH), 1720 (C=O), 1180 (P=O), 1000 (P—O) cm^{-1}.

Synthesis of N-(diisopropyloxyphosphoryl)cysteine **3**. The method is similar to N,N-bis-(diisopropyloxyphosphoryl)cystine **2**, but the reaction temperature must be kept under 0°C, and only three equivalent of Et_3N and 2 ml water was used.

Synthesis of N,S-bis-(diisopropyloxyphosphoryl)cysteine **7**. The cysteine methyl ester hydrochloride **6** (0.85 g, 5 mmol) is added to the solution of anhydrous CH_2Cl_2 (15 ml), Et_3N (0.7 ml) and DMAP (60 mg) in 5 min, then diisopropyl chlorophosphate (2 g, 10 mmol) and Et_3N (2.1 ml) are dropped under Ar protection at 0°C in 2 hr, stirred the solution at 20°C for 12 hr. Adding 1.2 N HCl 10 ml to the solution, and extracted it with EtOAc (10 ml × 3). Combining the EtOAc solution and washed with 1.2 N HCl, 1 mol Na_2CO_3 and saturated NaCl respectively, and dried with $MgSO_4$ and then concentrated. The crude product 2.71 g is obtained which is chromatographed on the silica gel 60 H column eluted with ether and chloroform. The pure N,S-bis-(diisopropyloxyphosphoryl)cysteine methyl ester 1.82 g in 72% yield was obtained. ^{31}P NMR: 4.48 ppm, 24.04 ppm; ^1H NMR: 1.2–1.5 (m, 24H, $(CH_3)_2C$—), 3.25 (dd, 2H, J = 6 Hz, —CH_2—S), 3.71 (t, 1H, J = 10 Hz, =NH, disappears with D_2O), 3.77 (s, 3H, —OCH_3), 4.03–4.50 (m, 1H, =N—CH), 4.43–4.93 (m, 4H, =CH—O—); ^{13}C NMR: 23.53 (t, J = 4.4 Hz, $(CH_3)_2C$—), 34.82 (t, J = 6 and 4 Hz, CH_2—S—. The ^{13}C data of the $(CH_3)_2C$— and CH_2—S— group in **7** both should be a doublet of doublets, but two of the central lines may be too closed to be resolved on the computer, so that we only saw a triplet), 52.2 (s, —OCH_3), 54.68 (d, J = 4.4 Hz, =N—CH—), 72.1 (q, Jc-o-p-n = 6 Hz, Jc-o-p-s = 7.3 Hz, =CH—O—), 171.37 (d, J = 6 Hz, —COOH); FABMS: m/z 464 (MH^+, 95), 422 (27), 380 (24), 338 (44), 296 (100), Anal. calcd.

for $C_{16}H_{35}NSP_2O_8$: C, 41.47; H, 7.56; N 3.02; S, 6.91; Found: C, 41.14; H, 7.49; N, 2.86; S, 7.02: IR (KBr): 3220 (=NH), 1745 (C=O), 1250 (P=O), 1000 (P—O) cm^{-1}.

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