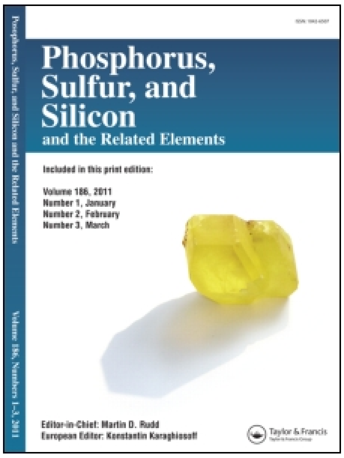

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STUDIES ON DIASTEREOMERIC N-PHOSPHONOAMINO ACID ESTERS CONTAINING A PHOSPHORUS-CARBON BOND

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STUDIES ON DIASTEREOMERIC N-PHOSPHONOAMINO ACID ESTERS CONTAINING A PHOSPHORUS-CARBON BOND

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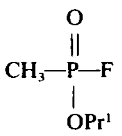
Four N-phosphonoamino acid methyl esters have been synthesized. The structures were supported by ^{31}P, ^1H, NMR, IR and FAB-MS data. Two stereoisomers of each compound appeared in the ^{31}P NMR spectra. N-(O-isopropyl methyl-phosphonyl)L-serine methyl ester was more reactive in acid than N-(O,O-diisopropyl) phosphoryl-L-serine ester, which underwent rapid N→O migration of the phosphorus group.

Key words: Phosphonoamino acid; phosphorus-carbon bond; NMR; N→O migration.

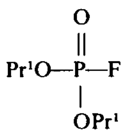
INTRODUCTION

Acetylcholinesterase (AChE, EC3.1.1.7) can be inhibited by many organophosphorus compounds, especially those containing a phosphorus-carbon bond.[1] Isopropyl methylphosphonofluoridate (Sarin), for example, is a powerful inhibitor of AChE; its inhibition rate of the enzyme activity is ten times that of diisopropyl fluorophosphate (DFP).[2,3] Another characteristic of these compounds is that their stereoisomers have great differences in chemical properties and biological activity. It had been shown that two stereoisomers of Sarin inhibited the activity of AChE with a difference of 4000 to 1.[4] How to explain the mechanism of phosphorus acting on the enzymes or proteins is a complex problem. Recently, our studies on the phosphorylation of amino acids and their derivatives provided some important results.[5] For example, N-phospho-amino acids could undergo some bio-mimic reactions, such as: ester exchange at phosphorus, N→O migration of the phosphorus group, self-activation to peptide formation, and esterification of amino acids.[6] In this paper, the syntheses of N-phosphoryl-L-amino acid methyl esters containing a phosphorus-carbon bond and their comparison to the N-(O,O-diisopropyl)phosphoryl analogues is reported.



$$CH_3-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OPr^i}{|}}{P}}-F$$

(Sarin)



$$Pr^iO-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OPr^i}{|}}{P}}-F$$

(DFP)

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RESULTS AND DISCUSSION

Two main methods of phosphorylation of amino acids and their derivatives were used, namely the reaction of dialkyl chlorophosphate with amino acid esters in anhydrous solvent[7] and the reaction of dialkyl phosphite in water.[8] Here isopropyl

TABLE I

NMR chemical shifts of N-phosphonoamino acid methyl esters

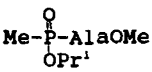
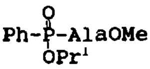
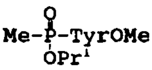
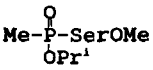
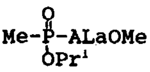
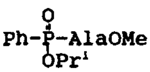
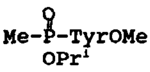
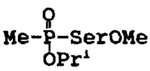
Entry Formula	Structure	^{31}P NMR δ ppm	^{1}H NMR ($CDCl_3$) δ ppm	
1 $C_8H_{18}NO_4P$	 $\mathrm{Me{-}\underset{OPr^{i}}{\overset{O}{P}}{-}AlaOMe}$	30.03 30.44 (CH_2Cl_2)	1.17-1.21(d) 3H 1.40-1.43(d) 3H 3.66(s) 3H 4.50-4.70(w) 1H	1.31-1.34(d) 6H 2.80-2.90(w) 1H 3.79-3.87(m) 1H
2 $C_{13}H_{20}NO_4P$	 $\mathrm{Ph{-}\underset{OPr^{i}}{\overset{O}{P}}{-}AlaOMe}$	18.79 19.22 (EtOAc)	1.16-1.39(m) 9H 3.65(s) 3H 4.69-4.77(m) 1H	2.20-2.50(w) 1H 3.82-4.05(m) 1H 7.42-7.78(m) 5H
3 $C_{14}H_{22}NO_5P$	 $\mathrm{Me{-}\underset{OPr^{i}}{\overset{O}{P}}{-}TyrOMe}$	32.46 33.12 ($CHCl_3$)	1.27-1.86(m) 9H 3.12-3.62(m) 2H 3.99-4.19(m) 2H 9.0-9.5(w) 1H	2.93(s) 1H 3.85(s) 3H 6.91-7.11(m) 4H
4 $C_8H_{18}NO_5P$	 $\mathrm{Me{-}\underset{OPr^{i}}{\overset{O}{P}}{-}SerOMe}$	32.60 32.21 ($CHCl_3$)	1.23-1.38(m) 6H 2.76(s) 1H 3.76(s) 3H 3.82-3.99(m) 2H	1.43-1.55(m) 3H 3.0-3.2(w) 1H 4.05-4.20(m) 1H 4.60-4.75(m) 1H
5 $C_8H_{18}NO_5P$	 $\mathrm{Me{-}\underset{OPr^{i}}{\overset{O}{P}}{-}OSerOMe}$	20.55 21.03 (Ether)	1.23-1.44(d) 6H 3.85-4.25(m) 2H 4.80-5.10(m) 1H 3.76(s) 3H	1.63-1.74(m) 3H 4.50-4.62(m) 1H 6.20-6.60(w) 2H

TABLE II

IR and positive ion FAB-MS data of N-phosphono-amino acid esters

Entry	Compounds	M.W	IR (cm^{-1})	M^+	Fragments m/e M^+-42	M^+-60	M^+-102
1	 $\mathrm{Me{-}\underset{OPr^{i}}{\overset{O}{P}}{-}ALaOMe}$	223	1790	224(100)	182(100)	164(50)	122(100)
2	 $\mathrm{Ph{-}\underset{OPr^{i}}{\overset{O}{P}}{-}AlaOMe}$	285	1770	286(100)	243(100)	226(10)	184(100)
3	 $\mathrm{Me{-}\underset{OPr^{i}}{\overset{O}{P}}{-}TyrOMe}$	315	1740	316(20)	273(60)	256(5)	213(100)
4	 $\mathrm{Me{-}\underset{OPr^{i}}{\overset{O}{P}}{-}SerOMe}$	239	1750	240(100)	198(60)	180(10)	138(20)

methylphosphonochloridate and isopropyl phenylphosphonochloridate were used as phosphorylation reagents to synthesize N-phosphono-amino acid esters (Tables I and II). All reactions were completed within one hour in yields of 42–65%. L-tyrosine and L-serine which contain a hydroxy group in the side-chain were phosphorylated only at the α-amino group by chlorophosphates.

Similar to the N-(O,O-diisopropyl)phosphoryl(DIPP)-amino acid esters, the methyl (or phenyl) phosphonyl-L-alanine and L-tyrosine methyl esters 1–3 were stable from room temperature to 40°C. Unexpectedly methylphosphono-L-serine methyl ester was very reactive, and produced two new products shown in Figure 1(B) in 0.1 N HCl solution at room temperature. The IR and ^{31}P, ^{1}H NMR data indicated that they were diastereomeric compound 5 (δ 20.55, δ 21.03 ppm) and O-isopropyl methylphosphonic acid (δ 27.24 ppm). This proved that compound 4 was more

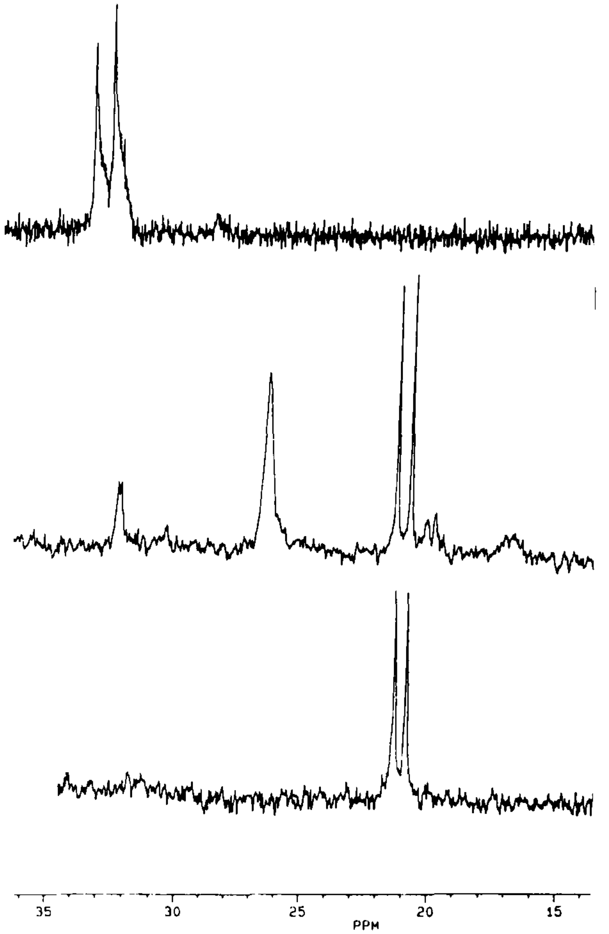
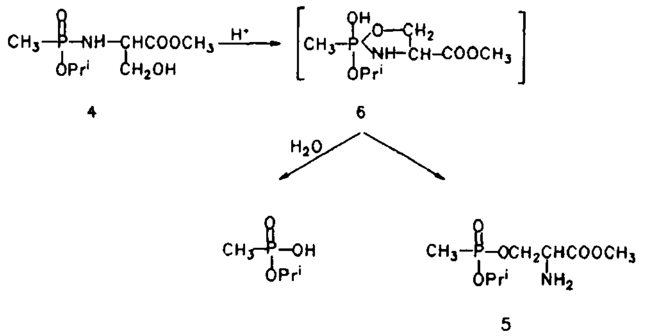
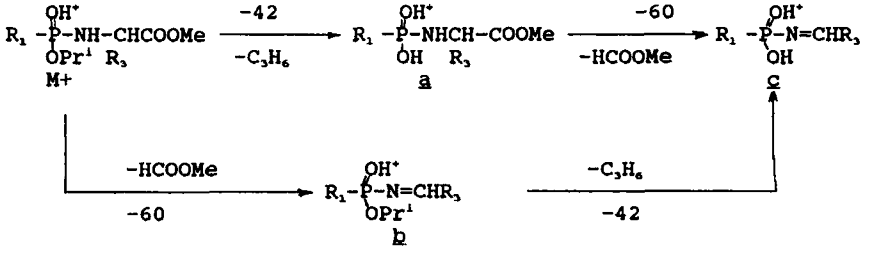


FIGURE 1 ^{31}P NMR spectra showing N→O migration of N-(O-isopropyl methylphosphono-L-serine methyl ester 4, (A) represents diastereomeric compound 4 (δ 32.60, 32.21 ppm). (B) 4 is treated with 0.1 N hydrochloric acid at room temperature and produces new ^{31}P-NMR signals, which represent diastereomeric compound 5 (δ 21.56, 22.10 ppm) and O-isopropyl methylphosphonic acid (δ 27.24 ppm), respectively; (C) corresponds to the final compound 5 (δ 20.55, 21.03 ppm).



SCHEME 1 The mechanism of N→O migration of the phosphorus group in N-(O-isopropyl methylphosphonyl)-L-serine methyl ester.



SCHEME 2 The fragment ions of N-phosphono-amino acid methyl esters.

labile and underwent dephosphorylation and N→O migration. The analogue of DIPP-L-serine methyl ester could only be hydrolyzed by refluxing in 5–7% HCl for 6 hours.[9] The mechanism may involve a five-membered heterocyclic intermediate 6 formed by nucleophilic attack of the OH group on serine with the phosphorus, followed by ring opening to the ester of the final product, which is shown in Scheme 1.

Two ^{31}P NMR signals corresponding to two diastereoisomers of each compound 1–5 are shown in Table I. The difference in the chemical shifts (δ) ranged from 0.4–0.9 ppm. Previous experiments had indicated that the diastereoisomers of phosphoramides could be separated using fractional crystallization when the value of δ was about 0.68 ppm.[10] So this concept might be used to separate the stereoisomers of the above compounds 1–5.

The FAB-MS for the N-phosphono-amino acid methyl esters is shown in Table II. It not only gave all molecular ion peaks but also three main fragments derived from successive loss of one isopropylene and formyl methyl ester (a–c) illustrated in Scheme 2.

CONCLUSIONS

Diastereoisomers of N-phosphono-amino acid methyl esters containing a phosphorus-carbon bond can be easily synthesized through a reaction of amino acid methyl esters with phosphonochloridates and followed by ^{31}P NMR, ^{1}H NMR and

FAB-MS. Each pair of stereoisomers of the compounds 1–5 gave two ^{31}P NMR signals. The N-(O-isopropyl methylphosphonyl) L-serine methyl ester was more labile than DIPP-homologue, which could easily underwent N → O migration of the phosphorus group in acidic media. Further studies on the separation of stereoisomers of the compounds will be continued.

EXPERIMENTAL

^{31}P-NMR and ^{1}H-NMR spectra were recorded on a Brucker ACP-200 spectrometer, chemical shifts of ^{1}H-NMR are referenced to internal tetramethylsilane (TMS), ^{31}P-NMR spectra are referenced to external 85% H_3PO_4. Positive-ion FAB-MS data were obtained on a KYKY Zhp-5 double-focusing mass spectrometer from Scientific Instrument Factory, Beijing, China, equipped with a KYKY fast-atom gun. IR spectra were measured on a Shimadzu IR-408 spectrometer.

N-(O-isopropyl methylphosphonyl)-L-alanine methyl ester 1. To a solution of L-alanine methyl ester hydrochloride (5 mmol 0.7 g) in 20 ml dry chloroform and 4 ml N-methyl morpholine cooled to 0°C was added a mixture of isopropyl methylphosphonochloridate (5 mmol 0.79 g) and 10 ml dry $CHCl_3$. The mixture was stirred for 40 minutes and then washed with water (10 ml × 1), 0.1 N HCl (10 ml × 2), saturated $NaHCO_3$ (10 ml × 1) and water (10 ml × 1). The $CHCl_3$ phase was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was extracted with petroleum ether (30–60°C), yield 0.7 g (63%) oily product 1.

N-(O-isopropyl)phenylphosphono-L-alanine methyl ester 2. Similarly, a reaction of L-alanine methyl ester hydrochloride (5 mmol 0.7 g) with equal mole isopropyl phenylphosphonochloridate (1.1 g) gave a white solid (0.8 g, 58%) 2.

N-(O-isopropyl methylphosphonyl)-L-tyrosine methyl ester 3. Tyrosine methyl ester hydrochloride (5 mmol 1.16 g) was dissolved in 30 ml CH_2Cl_2, 6 ml N-methyl morpholine added and the mixture solution cooled to 0°C. Isopropyl methylphosphonochloridate (5 mmol 0.79 g) was added and stirred for a hour. The solution was washed with water (10 ml × 1), 0.1 N citric acid (10 ml × 2), water (10 ml × 1) and dried over anhydrous $MgSO_4$. After removal of the solvent in vacuo, an oily yellow residue (1.23 g) was washed with petroleum ether (30–60°C), then dried in vacuo, yielding a foam-like solid at lower temperature (1.0 g, 64%) 3.

N-(O-isopropyl methylphosphonyl-L-serine methyl ester 4. A mixture of L-serine methyl ester hydrochloride (10 mmol 1.56 g), 20 ml dry chloroform and 6 ml N-methyl morpholine was cooled to 0°C and added to a solution of isopropyl methylphosphonochloridate (10 mmol 1.57 g) in 10 ml chloroform. The mixture was stirred for 40 minutes, solvent removed in vacuo, the residue dissolved in 50 ml dry ether and filtered. The filtrate was concentrated and then washed with petroleum ether (30–60°C) to give an oily product (1.0 g, 42%) 4.

O-(O-isopropyl methylphosphonyl)-L-serine methyl ester 5. N-(O-isopropyl methylphosphonyl)-L-serine methyl ester (0.5 g) was added 0.1 N HCl and stirred for 30 minutes at room temperature. The product was extracted with ether, dried over $MgSO_4$ and concentrated in vacuo, to give an oily product (0.2 g, 44%) 5.

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