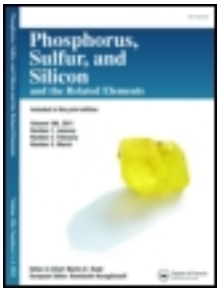

Phosphorus, Sulfur, and Silicon and the Related Elements

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SYNTHESIS AND ANTICHOLINESTERASE ACTIVITY OF SOME OXAZAPHOSPHOLINE AND BENZO-1,3,2-DIOXAPHOSPHOLENE, DIAZAPHOSPHOLINE 2-ONES CONTAINING 2-AMINO ACID SUBSTITUTION

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SYNTHESIS AND ANTICHOLINESTERASE ACTIVITY OF SOME BENZO-1,3,2-DIOXAPHOSPHOLENE, OXAZAPHOSPHOLINE AND DIAZAPHOSPHOLINE 2-ONES CONTAINING 2-AMINO ACID SUBSTITUTION

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Synthesis of the titled compounds has been described. ^{1}H NMR, IR band frequencies and MS fragmentation and rearrangement peaks were analyzed and discussed in detail. Studying the inhibitory effect of these compounds on acetylcholinesterase (AChE) showed that dioxaphospholenes were stronger inhibitors than oxazaphospholines and diazaphospholines. This was explained by increasing the number of nitrogen atoms around the phosphorus atom in the later two series, which reduces the electrophilic character of the phosphorus atom by the overlapping between the $d\pi - p\pi$ orbitals of the phosphorus and the neighboring nitrogen atoms, and hence reducing the electrophilic attack of the phosphorus atom on a nucleophilic center at the esteratic site of the enzyme. Steric factor of the amino acid moiety showed stronger effect than the electronic factor on the inhibition activity. the observed order was glycine > glutamic > methaionine > phenylalanine > alanine.

Keywords: Dioxaphospholene; oxazaphospholine; diazaphospholine; acetylcolinesterase inhibitors

INTRODUCTION

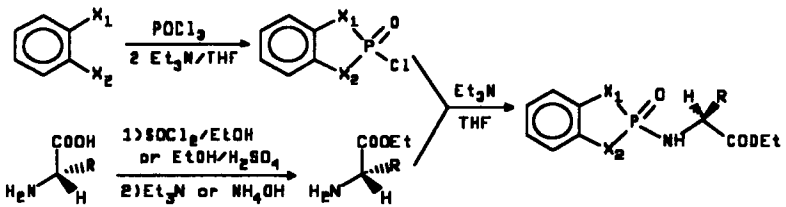
Much information is available on the synthesis and chemical properties of 1, 3, 2-ring systems containing phosphorus and two atoms of oxygen and/or nitrogen. Stereospecific synthesis of 1, 3, 2-oxazaphospholidin-2-ones were prepared from chiral ephedrine; sodium alkoxides opened the ring by P-N rather than P-O bond cleavage.[1] Synthesis and conforma-

tional analysis of 2-anilino-1, 3, 2-oxaphospholanes showed that the ring adopts either an envelop or a twist-envelope conformation with the anilino moiety being equatorial.[2] In contrast with a 2-dimethylamino substituent, a 2-anilino group was found to prefer the axial orientation in 4-methyl-1, 3, 2-dioxaphosphorinan ring system.[3] The oxidation of 2-methoxy or limethylamine-2-S(Se)-4-methyl-1, 3, 2-dioxaphosphorinanes with hydrogen peroxide to 2-oxo derivatives proceeded with net retention of configuration at the phosphorus atom.[4] Treatment by NaH and CS_2 followed by alkylation converted the P-N bond to P-S bond with retention of configuration at the phosphorus atom in 2-phenylamino-1, 3, 2-dioxaphosphorinanes and 1, 3, 2-oxazaphospholidines.[5] However, less information is available on the biological effects of these ring systems. It was found that 4-H-1, 3, 2-oxazaphosphoridines derived from α-amino acids possess fairly high insecticidal activity.[6,7] Small changes in the substituents of phosphorus atom changed markedly the insecticidal activity of 1, 3, 2-benzoxazaphosphorine 2-ones or 2-thions[8] and 1, 3, 2-oxazaphospholidines.[6] 4-Substituted phenoxy dinaphtho [2, -d:1', 2'-f][1, 3, 2-] dioxaphosphepin 4-oxides[9] or sulfides[10] were screened for their anticholinesterase activity. Therefore, in the effort of studying the adverse effects of some organophosphorus compound,[11–14] three series of benzo-1, 3, 2-dioxaphospholene, oxazaphospholine and diazaphospholine 2-ones containing 2-amino acid substitution were synthesized and tested for their anticholinesterase activities.

RESULTS AND DISCUSSIONS

The reactions of phosphoryl chloride with catecole, *o*-aminophenol or *o*-phenylenediamine in the presence of triethylamine[1,15] yield benzo-1, 3, 2-dioxaphospholene, oxazaphospholine or diazaphospholine 2-chloro 2-ones respectively. The reactions were diluted in THF to minimize the polymerization side reaction. The products reacted with α-amino acid esters in equimolar amount of Et_3N to give the titled compounds **1–15**. The reaction seemed to be affected by the α-alkyl substituents of α-amino acids. Electron withdrawing groups reduce the nucleophilicity of the amino nitrogen and thus the reaction requires more time and gives relatively lower yield. The reactions were followed by TLC chromatography. α-Amino acid ethyl esters were prepared from L-(S)-α-amino acids and

ethanol in acidic medium of concentrated H_2SO_4[16] or $SOCl_2$/EtOH[17,18] to generate HCl gas. The resulted α-amino acid ester sulfates or chlorides were then neutralized with concentrated ammonia or Et_3N. Amino acids with α-electron withdrawing substituents as in glutamic acid gave higher yield by $SOCl_2$ procedure. The synthesis of these compounds was outlined in the following scheme.



X_1 = O, X_2 = O, R = H (Gly) **1**
X_1 = O, X_2 = O, R = CH_3 (Ala) **2**
X_1 = O, X_2 = O, R = $PhCH_2$ (Phe) **3**
X_1 = O, X_2 = O, R = EtOOC-CH_2-CH_2 (Glu) **4**
X_1 = O, X_2 = O, R = CH_3-S-CH_2-CH_2 (Met) **5**
X_1 = O, X_2 = N, R = H (Gly) **6**
X_1 = O, X_2 = N, R = CH_3 (Ala) **7**
X_1 = O, X_2 = N, R = $PhCH_2$ (Phe) **8**
X_1 = O, X_2 = N, R = EtOOC-CH_2-CH_2 (Glu) **9**
X_1 = O, X_2 = N, R = CH_3-S-CH_2-CH_2 (Met) **10**
X_1 = N, X_2 = N, R = H (Gly) **11**
X_1 = N, X_2 = N, R = CH_3 (Ala) **12**
X_1 = N, X_2 = N, R = CH_3 (Ala) **13**
X_1 = N, X_2 = N, R = EtOOC-CH_2-CH_2 (Glu) **14**
X_1 = N, X_2 = N, R = CH_3-S-CH_2-CH_2 (Met) **15**

The characteristic IR bands of compounds **1–15** (Table I) showed two skeletal aromatic C=C stretching bands at 1430–1480 and 1590–1620 cm^{-1}. The carbonyl ethyl ester band appeared at 1720–1730 cm^{-1}. The shorter frequency of this band along with its lower intensity than expected suggests its involvement in a hydrogen bond with the nearby amino proton in a five member ring as presented in Figure 1. The N-H peak is broad and fall in the region 3300–3350 cm^{-1} which confirms the hydrogen bond formation.[19] The P=O str. band appeared in the range 1175–1220 cm^{-1} (the higher limits for compounds with glutamic or methaionine moiety). This peak is usually overlapped with the broad C-O str. band.

^1H NMR of these compounds (Table II) showed that the four aromatic protons are similarly affected by the two ortho substituents to give singlet at 7.2–7.5 ppm. Protons of both aliphatic and aromatic amino groups gave broad multiplets because of the electrical quadrupole moment of the nitrogen nucleus and the splitting by the nearby phosphorus and hydrogen atoms. These protons were deshielded by the effect of the neighboring

P=O group to give signals at 6.60–6.95 ppm. The methylene protons α to the amino group in glycinate moiety are chemically equivalent and appeared at ~ 3.7 ppm as multiplet or doublets of doublets because of the splitting by the phosphorus and NH proton with coupling constants 14.1 and 6.0 Hz respectively (Figure 1). The methyne proton α to the amino group in other compounds was split further by other neighboring protons to give multiplets. The two methylene protons in the phenylalanine moiety are diastereotropic and hence they are not equivalent. They appear either as triplet or as two overlapping doublets of doublets at ~ 3.1 ppm with coupling constants J_{gem} 10.0 Hz and J_{vic} 7.2 Hz (Figure 1). The same methylene protons in other compounds split further by other protons to give multiplets.

TABLE I Infrared absorbance band frequencies (cm^{-1}) of compounds **1–15**

Compound	*N-H*	*C(O)=O*	*Aromatic C=C str.*	*P-O*	*P-N or P-O*
1	3300	1730	1600, 1430	1175	1000, 1040
2	3300	1730	1600, 1440	1190	1000, 1040
3	3350	1720	1610, 1455	1180	960, 1040
4	3300	1730	1460	1220	1000, 1920
5	3350	1730	1620, 1460	1210	1020, 1000
6	3350	1730	1590, 1480	1200	1000, 900
7	3350	1720	1600, 1475	1200	1000, 900
8	3350	1720	1600, 1480	1210	1000, 900
9	3300	1730	1480	1220	1040, 900
10	3350	1730	1605, 1480	1200	1000, 900
11	3350	1730	1605, 1480	1205	1000, 900
12	3350	1730	1605, 1480	1200	1000, 900
13	3300	1730	1605, 1480	1200	1000, 900
14	3350	1730	1480	1210	1040, 900
15	3350	1730	1605, 1480	1210	1000, 900

TABLE II ^{1}H NMR data of compounds **1–15**

Compound	*Aromatic H's*	*NH,s*	*OCH_2-CH_3*	*Relative to Aliphatic NH*			
				α	β	*γCH_2 or Ph*	δ *SMe*
1	7.50s(4H)	6.90m(H)	4.13q(2H), 1.25t(3H)	3.71m(2H)			
2	7.32s(4H)	6.95m(H)	4.10q(2H), 1.30t(3H)	3.73m(H)	1.40d(3H)		
3	7.26s(4H)	6.82m(H)	4.05q(2H), 1.27t(3H)	3. 52m(H)	3.10dd(2H)[1]	7.18m(5H)	
4	7.29s(4H)	6.81m(H)	4.12q(4H), 1.28t(6H)	3.53m(H)	2.25m(2H)	2.36t(2H)	
5	7.32s(4H)	6.83m(H)	4.06q(2H), 1.25t(3H)	3.60m(H)	1.90m(2H)	2.60t(2H)	2.05s(3H)
6	7.40s(4H)	6.90m(2H)	4.15q(2H), 1.26t(3H)	3.75m(2H)			
7	7.25s(4H)	6.75m(2H)	4.10q(2H), 1.28t(3H)	3.70m(H)	1.39d(3H)		
8	7.28s(4H)	6.73m(2H)	4.10q(2H), 1.25t(3H)	3.71m(H)	3.05t(2H)	7.16m(5H)	
9	7.28s(4H)	6.78m(2H)	4.20q(4H), 1.29t(6H)	3.70m(H)	2.26m(2H)	2.33t(2H)	
10	7.28s(4H)	6.67m(2H)	4.18q(2H), 1.27t(3H)	3.65m(H)	1.80m(2H)	2.63t(2H)	2.09s(3H)
11	7.30s(4H)	6.85m(3H)	4.13q(2H), 1.28t(3H)	3.70dd(2H)[2]			
12	7.25s(4H)	6.76m(3H)	4.10q(2H), 1.26t(3H)	3.63m(H)	1.41d(3H)		
13	7.26s(4H)	6.70m(3H)	4.15q(2H), 1.28t(3H)	3.72m(H)	3.11t(2H)	7.15m(5H)	
14	7.20s(4H)	6.60m(3H)	4.20q(4H), 1.28t(6H)	3.80m(H)	2.29m(2H)	2.34(2H)	
15	7.30s(4H)	6.75m(3H)	4.10q(2H), 1.30t(3H)	3.70m(H)	1.80m(2H)	2.60t(2H)	2.08s(3H)

1. Two overlapping doublets of doublets, J_{gem} 10.0Hz, J_{vic} 7.2Hz.
2. $J_{HC\text{-}NP}$ 14.1Hz, $J_{HC\text{-}NH}$ 6.0Hz.

Mass spectra of these compounds showed that the molecular ion peak may vary from a weak peak as in compound **1** (m/z 257, 0.35%) to the base peak as in compound **15** (m/z 355, 100%). The base peak of compound **1** (m/z 155) results from M-(NH-CH_2-COOEt) fragment. This compound gave also a fragmentation and a rearrangement peaks at m/z 99 and 109 respectively as depicted in Figure 2. Compound **15** gave fragmentation peak for M-(CH-COOEt) and rearrangement peak for $(H\text{-}COOEt)^+$ at m/z 269 and 74 respectively as shown in Figure 2.

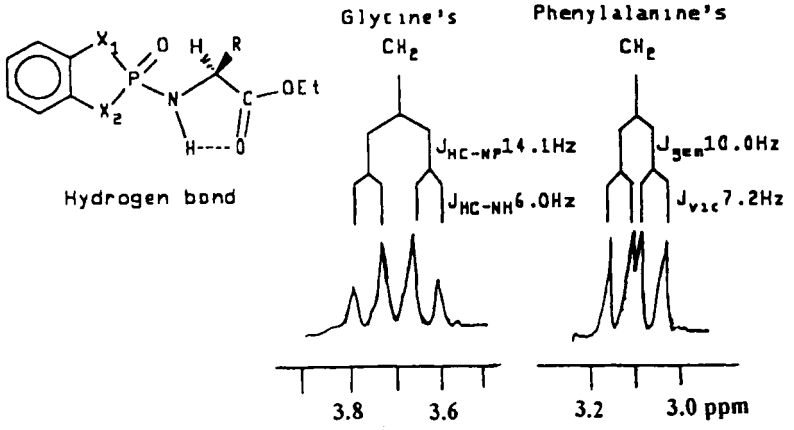


FIGURE 1 Hydrogen bonding and coupling constants of compounds **1–15**

The inhibition percentages of serum acetylcholinesterase enzyme (AChE) by compounds **1–15** at concentration 1 ppm ranged from 7 to 76 % as presented in Table III. It was found that a series of *O*-ethyl *O*-2-isopropoxycarbonylphenyl *N*-alkykphosphoramidates and amidothioates were generally poor inhibitors to serum AChE. The inhibition percentage ranged from 0 to 45 % at inhibitor concentration > 30 ppm.[20] Dioxaphospholene derivatives (**1–5**) were stronger inhibitors than both oxazaphospholine (**6–10**) and diazaphospholine (**11–15**) derivatives. This could be explained by reducing the electrophilic character of the phosphorus atom in the later two series by the overlapping between the dπ-pπ orbitals of the phosphorus and the neighboring nitrogen atoms, and hence reducing the electrophilic attack of the phosphorus atom on a nucleophilic center at the esteratic site of the enzyme as was reported previously.[13]

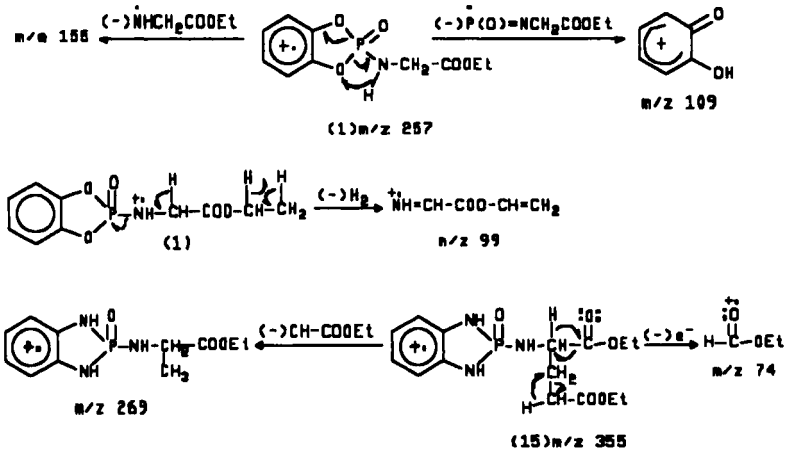


FIGURE 2 Fragmentations and rearrangements of compounds **1** and **15** by mass spectrometry

Therefore, increasing the number of nitrogen atoms bonded to the phosphoryl group decreases the inhibition process. This also explained the lower activity of phosphoramidates compared to their phosphate ester analogous towards nucleophilic attack.[21,22] The inhibition effect decreased generally according to the following order of amino acid moieties: glycine > glutamic > methaionine > phenylalanine > alanine. This order shows that branching of α-amino acids at α carbon decreases markedly the inhibition effect in the three series (glycine derivatives were much more effective than their analogues). The electronic factor may also play rule but at smaller extend since the inhibition activities of α-branched amino acid moieties increases with the presence of electron withdrawing groups.e. glutamic > methaionine > phenylalanine > alanine.

EXPERIMENTAL

Solvents and reagents were reagent grade. Catecole was purified by crystallization from benzene then sublimed under vacuum. *o*-Aminophenol was crystallized from ethanol. *o*-Phenylenediamine was crystallized from aqueous 1 % sodium hyposulphite, washed with ice-water and dried in a vacuum desiccator. Phosphoryl chloride was freshly purified by fractional

distillation.[23] The reactions were monitored till completion by thin layer chromatography (TLC). α-Amino acids used were of L-(S)-configuration. ^{1}H NMR spectra were executed on a Varian EM 390 spectrometer by using $CDCl_3$/TFAA as a solvent and TMS as an internal standard. GC-MS analyses were performed in electron impact mode on a Finnigan Mat GCQ spectrometer equipped with Rtx-5MS column with 30 m long, 0.25 mm I.D. and 0.25 μm film thickness (df). The oven temperature program was: initial value 40°C for 3 min then increased by 15°C/min till 250°C (5 min). Injection and detection temperatures were 150 and 250°C respectively. IR spectra were recorded on a Nicolet 460 FT-IR spectrometer.

Esterification of α-amino acids

For esterification of α-amino acids, two procedures[16–18] were modified as follows:

1) To a suspension of 0.06 mol amino acid in 40 mL absolute ethanol was added slowly 8 mL concentrated sulfuric acid. The mixture was refluxed for 2 hours during which all materials were dissolved. The solution was cooled to 0°C and neutralized with concentrated aqueous ammonia solution. After filtration and removal of the solvent, the residue was extracted with CH_2Cl_2 or THF then dried and evaporated to give pure α-amino acid ester (yield 60–70%).

2) Thionyl chloride was added dropwise to a suspension of amino acid in ethanol at 0°C in a molar ratio 1.1: 1:8 respectively. The reaction mixture was left overnight during which it gave a clear solution. The solution was evaporated to get rid of excess HCl then dissolved in CH_2Cl_2 or THF and neutralized either with the addition of equimolar amount of triethylamine while stirring at room temperature or with concentrated NH_4OH at 0°C. The solution was filtered and worked up as described previously. This procedure gave better yield with glutamic acid (80%).

Synthesis of benzo-1, 3, 2-dioxaphospholene, oxazaphospholine and diazaphospholine 2-one derivatives 1–15

To an ice-cooled stirred solution of 4.6 g (0.03 mol) phosphoryl chloride in 50 mL THF was added dropwise a solution of 0.03 mol of catecole, *o*-ami-

nophenol or o-phenylenediamine and 6.06 g (0.06 mol) triethylamine in THF. After stirring for 3 hours, a mixture of 0.03 mol of a suitable α-amino acid ester, 0.03 mol triethylamine and THF was added slowly while cooling. The reaction mixture was stirred overnight then filtered and evaporated. the crude product was subjected to silica gel column chromatography and eluted with hexane/CH_2Cl_2 solvent system. Yields were 50–60% while purities as checked by GC-MS were > 97%.

TABLE III The inhibition percentages of acetylcholinesterase by compounds **1–15**

Compound	*% AChE inhibition (±SD)*	*Compound*	*% AChE inhibition (± SD)*	*Compound*	*% AChE inhibition (± SD)*
1	75.90 (±1.86)	**6**	67.90 (±1.05)	**11**	37.82 (±0.87)
2	7.24 (±0.21)	**7**	8.61 (±0.22)	**12**	11.00 (±0.27)
3	14.86 (±0.26)	**8**	7.20 (±0.17)	**13**	12.75 (±0.25)
4	58.80 (±1.14)	**9**	27.70 (±0.67)	**14**	27.29 (±0.48)
5	27.03 (±0.57)	**10**	18.50 (±0.58)	**15**	24.05 (±0.45)

Anticholinesterase activity

Blood samples were freshly drawn from 3- to 6-month old male Swiss white mice and centrifuged at 5000 rpm to collect the serum as enzyme source. The clear serum was then frozen until used in the enzyme assay within two days. The assay was measured by the method of Ellman et al (1961).[24] The inhibition percentage was determined in triplicate at concentration 1 ppm of each pesticide (dissolved in ethanol) in the assay solution.

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