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Synthesis and Quantitative Structure–Activity Relationship of a New Series of Chiral 4-Alkoxycarbonyl-2-(alkylamino)-1,3,2-oxa or thiazaphospholidine-2-ones

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ABSTRACT: *Twenty-one of the chiral 4-alkoxycarbonyl-2-(α-alkyl-α-ethoxycarbonyl methylamino)-1,3-2-thia or oxazaphospholidine-2-ones have been synthesized by cyclization of* L*-serine or* L*-cysteine ethyl or n-octyl ester with phosphoryl chloride followed by reaction with a suitable* L*-amino acid ethyl ester. Proton NMR, IR, and mass spectra of these compounds have been discussed in detail. These compounds inhibited up to 68.52% of acetylcholinesterase (AChE) at the 1 ppm concentration level. Regression analysis showed that AChE inhibition was determined by both the steric and electronic effects of the alkyl groups of the amino acid. The enzyme inhibition correlated directly with the steric bulk of the alkyl groups, indicating a steric requirement for maximizing inhibitor–enzyme interaction and an inverse relationship with the electron-donating ability of the alkyl groups. This supports the concept of a nucleophilic attack mechanism of a hydroxyl group of a serine amino acid in the enzyme active center on the partially positive phosphorus atom of the oxazaphospholidines and thiazaphospholidines, with correlation coefficients of 0.999 and 0.838, respectively. Results also indicated that the steric requirement was more important than the electronic factor in affecting the inhibition process, which explained the high activity of compounds containing the isoleucine moiety. The high AChE inhibition activity of these compounds and the expected nontoxic products of their in vivo hydrolysis make them eligible for pesticidal application.* © 1999 John Wiley & Sons, Inc. Heteroatom Chem 10: 475–480, 1999

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INTRODUCTION

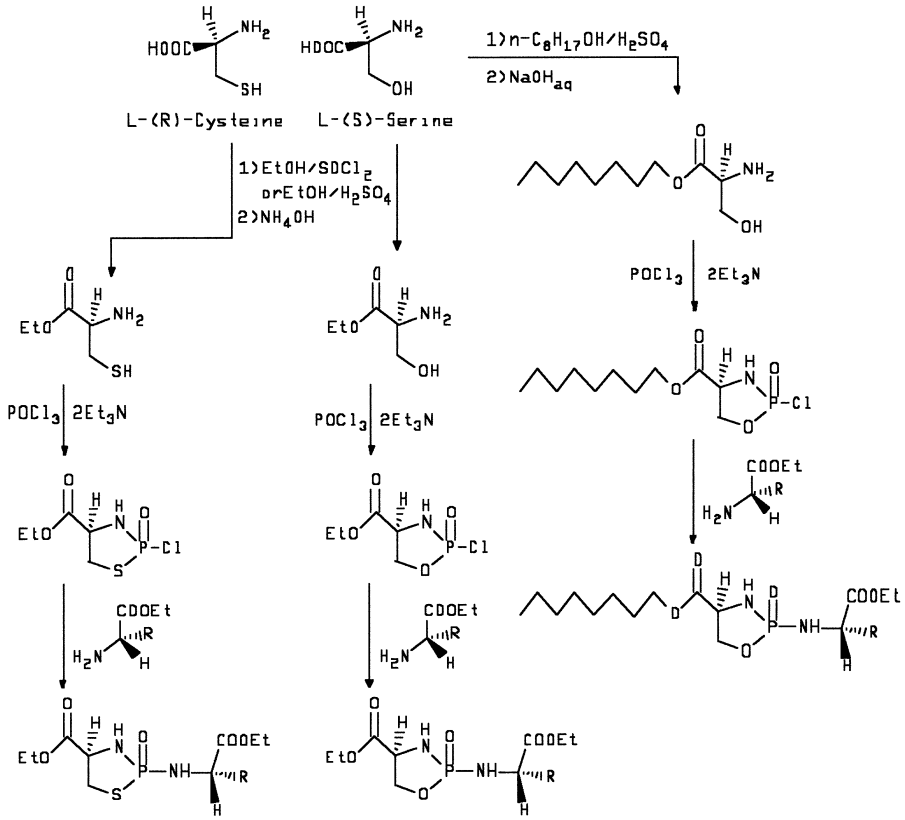
It has been found that 4-*H*-1,3,2-oxazaphosphoridines derived from α-amino acids possess fairly high insecticidal activity [1,2]. 4-*H*-1,3,2-Benzoxaphosphorins also showed some insecticidal activity [3]. We have found that 1,3,2-benzoxazaphospholine-2-ones and related ring systems containing a 2-amino acid ester substituent are active anticholinesterase agents [4]. Synthesis and structural assignments of 1,3,2-oxazaphospholidines derived from (−)-ephedrine have been reported [5]. Incorporating amino acid esters in some drugs lowered their toxicity and enhanced their cellular uptake [6], which can be attributed to producing nontoxic products upon in vivo hydrolysis of these drugs. Coupling this information with our effort to study the adverse effects of pesticides [7,8] and to correlate their chemical structures with their biological activities [9,10], a new series of the titled compounds were synthesized, characterized, and tested as acetylcholinesterase (AChE) inhibitors. These compounds are expected, upon hy-

drolysis, to give nontoxic materials, mainly amino acids and to possess anticholinesterase activity. A quantitative structure–activity relationship was also developed.

RESULTS AND DISCUSSION

Esterification of L-(*R*)-cysteine or L-(*S*)-serine, followed by reaction with phosphoryl chloride and 2 moles of triethylamine, yielded cyclic phosphoramidothiolic chlorides. A polycondensation side reaction was minimized by dilution with benzene. Addition of the appropriate L-α-amino acid ethyl ester gave 4-alkoxycarbonyl-2-(α-alkyl-α-ethoxycarbonyl methylamino)-1,3,2-thia or oxazaphospholidine-2-ones, (**1–8**) and (**9–21**), respectively, as presented in Scheme 1.

Infrared characteristic bands of compounds (**1–21**), as listed in Table 1, have $\nu_{N\text{-}H}$ at 3479–3344 cm^{-1}, $\nu_{C=O}$ at ~1740 cm^{-1}, $\nu_{P\text{-}N}$ at ~1020 cm^{-1}, and $\nu_{P=O}$ interfered with $\nu_{C\text{-}O}$ stretching bands at 1239–1203 cm^{-1}. The methylene protons of the cyclic ring are diastereotopic, and hence they are chemically nonequivalent. In their proton NMR spectra, they split each other and are split further by a neighboring methyne proton and the phosphorus atom to appear as a multiplet at ~3.2 ppm. The methyne proton is also split by each of the methylene protons, by NH, and by the phosphorus atom to give a multiplet at ~3.7 ppm. The ethyl group appears as a quartet and triplet at ~4.1 and 1.3 ppm, respectively; OCH_2 protons of the octyl group (compounds **16–21**) give a triplet at ~4.0 ppm and the other octyl proton peaks fall in the region of 0.8–1.5 ppm. Regarding the amino acid portion of the acyclic moiety, the methylene or methyne protons α to the NH group appear



R = H (Gly) **1**
= Me (Ala) **2**
= *i*-Pr (Val) **3**
= *s*-Bu (Ile) **4**
= 5-imidazolyl-CH_2 (His) **5**
= $PhCH_2$ (Phe) **6**
= $EtOOCCH_2CH_2$ (Glu) **7**
= $MeSCH_2CH_2$ (Met) **8**

R = H (Gly) **9**
= Me (Ala) **10**
= *i*-Pr (Val) **11**
= *s*-Bu (Ile) **12**
= 5-imidazolyl-CH_2 (His) **13**
= $EtOOCCH_2CH_2$ (Glu) **14**
= $MeSCH_2CH_2$ (Met) **15**

R = H (Gly) **16**
= *i*-Pr (Val) **17**
= *s*-Bu (Ile) **18**
= 5-imidazolyl-CH_2 (His) **19**
= $EtOOCCH_2CH_2$ (Glu) **20**
= $MeSCH_2CH_2$ (Met) **21**

SCHEME 1

TABLE 1 Infrared Band Frequencies (cm^{-1}) of Compounds **1–21**

Compound	*N–H*	*C=Ostr.*	*P=O C–O str.*	*P–N*
1	3456	1734	1212	1017
2	3446	1734	1222	1016
3	3446	1739	1217	–
4	3383	1734	1225	1023
5	3469	1733	1222	1019
6	3363	1732	1207	1023
7	3344	1733	1203	1026
8	3357	1740	1222	1029
9	3476	1739	1208	1016
10	3446	1739	1217	1013
11	3436	1743	1215	1015
12	3446	1744	1209	1016
13	3429	1739	1215	1013
14	3436	1739	1222	1014
15	3479	1747	1225	1023
16	3355	1746	1233	1025
17	3350	1747	1232	1025
18	3370	1747	1227	1023
19	3364	1745	1224	1022
20	3380	1734	1232	1024
21	3375	1748	1239	1024

as a multiplet at ~3.7 ppm. Protons of each of the two methylene groups in glutamic or methionine are diastereotopic. The methylene group β to the NH group (next to the chiral center) gives a multiplet at ~1.85 ppm, whereas the γ methylene group appears as a triplet at ~2.3 ppm for glutamic and ~2.6 ppm for methionine. Detailed assignments are given in Table 2.

Studying the rearrangement and fragmentation patterns of these compounds by mass spectrometry assists not only an identifying their structures but also in analyzing and identifying related organophosphorus compounds or pesticides [11]. Assignment of the important peaks in the mass spectra of compounds **2** and **21** are presented in Scheme 2. The molecular ion peak of these high molecular weight compounds is either weak (**2**) or absent (**21**). Compound **2** gave a fragmentation peak at *m/e* 209, and it gave the base peak at *m/e* 225 upon rearrangement with hydrogen atom transfer. Compound **21** undergoes successive fragmentation and rearrangement steps. Successive loss of CH_2CH_2SMe and OH fragments gave ions at *m/e* 363 and 346, respectively. The latter (97.9%) is stabilized by distribution of the positive charge on the central nitrogen, α-CH, NH, and oxygen atom. This ion could lose a methyl group to give an *m/e* 331 ion (30.1%) or lose a C_2H_2 molecule through the McLafferty rearrangement to give an ion at *m/e* 318 (64.7%), which is also stabilized by a positive charge distribution as depicted in Scheme 2. The *m/e* 318 ion, upon rupturing of the $C_8H_{13}OOCCH=CH_2$ group, gave the base peak ion with *m/e* 138 (100%).

The inhibition percentages of acetylcholinesterase by the synthesized 1,3,2-thia or oxazaphospholidine-2-one compounds (**1–21**) at a concentration level of 1 ppm are listed in Table 3. The inhibition varied from 5 to 68%. Multiple regression analysis has been employed extensively to correlate chemical structure with bioactivity [12–14]. Taft's steric (Es) and polar (σ^*) parameters have been used successfully to study the stereo-electronic effects of alkyl groups on chemical reactivity in aliphatic systems [15]. The 1,3,2-thiazaphospholidines **1–4** differ only in their alkyl substituents on the α-carbon to the amino group. The same is true for the 1,3,2-oxazaphospholidines **9–12**. Correlating AChE inhibition percentage (% AChE inh.) with the polar and steric parameters of the alkyl groups, and their squares in both series by stepwise regression analysis yielded equations 1 and 2, respectively. The descriptive values used in this study for H, Me, *i*-Pr and *s*-Bu substituents were (0, −0.49, −0.68, and −0.70) for the polar parameter and (0, −1.24, −1.71, and −2.37) for the steric parameter, respectively.

$$\log\ \%\ \text{AChE inh.} = 1.494 + 0.195\ (\text{Es})^2 - 2.017\ (\sigma^*)^2 \quad (1)$$

$$n = 4,\ R = 0.838,\ s = 0.233$$

$$\log\ \%\ \text{AChE inh.} = 1.403 + 0.375\ (\text{Es})^2 - 3.411\ (\sigma^*)^2 \quad (2)$$

$$n = 4,\ R = 0.999,\ s = 0.022$$

where n = number of compounds, R = correlation coefficient, and s = standard error with respect to the equation at 95% confidence interval.

Inspection of equations 1 and 2 revealed a few points. First, AChE inhibition in the two series was determined by both the steric and electronic effects of the alkyl groups of the amino acids. Second, the enzyme inhibition increased by increasing the steric bulk of the alkyl groups, indicating a steric requirement for maximizing inhibitor–enzyme interaction. Third, the enzyme inhibition was inversely related to the electron-donating ability of the alkyl groups, which supports the concept of a nucleophilic attack mechanism of a hydroxyl group of a serine amino acid in the enzyme active center on the partially positive phosphorus atom [9,16, 17]. This hydroxyl group normally attacks the carbonyl carbon of acetylcholine, the natural enzyme substrate, while a carboxylate ion in the enzyme coordinates to the tertiary amine in the substrate [17]. However, with organophosphorus compounds, the hydroxyl group

TABLE 2 Proton NMR Data for Compounds **1–21**

		Protons of Cyclic Ring			Substituents on N			
Compound	*OCH_2CH_3 $OCH_2C_7H_{15}$*	*CH*	*CH_2*	*NH*	*α CHorCH$_2$*	*β CH_2orCH$_3$*	*γ or δ CH_2, CH_3, Ph or 5-imidazolyl*	*SMe*
1	4.16(4H, q), 1.4(6H, t)	3.68(H, m)[a]	3.19(2H, m)	4.7	3.68(2H, m)[a]			
2	4.18(4H, q), 1.3(6H, t)	3.68(H, m)[a]	3.10(2H, m)	4.7	3.68(H, m)[a]	1.36(3H, d)		
3	4.10(4H, q), 1.35(6H, t)[a]	3.60(H, m)[b]	3.10(2H, m)	4.7	3.60(H, m)[b]	1.35(H, m)[a]	1.35(6H, d)[a]	
4	4.15(4H, q), 1.30(6H, t)[a]	3.60(H, m)[b]	3.10(2H, m)	4.7	3.60(H, m)[b]	1.30(H, m)[a]	1.0, 1.30(8H, m)[a]	
5	4.05(4H, q), 1.34(6H, t)	3.55(H, m)[a]	3.16(2H, m)[b]	–	3.55(H, m)[a]	3.16(2H, m)[b]	7.3(2H, m)	
6	4.10(4H, q), 1.20(6H, t)	3.66(H, m)[a]	3.10(2H, m)[b]	–	3.66(H, m)[a]	3.10(2H, m)[b]	7.20(5H, m)	
7	4.25(6H, q), 1.32(9H, m)	3.75(H, m)[a]	3.10(2H, m)	–	3.75(H, m)[a]	1.90(2H, m)	2.35(2H, t)	
8	4.20(4H, q), 1.28(6H, t)	3.62(H, m)[a]	3.14(2H, m)	–	3.62(H, m)[a]	1.82(2H, m)	2.60(2H, t)	2.10(3H, s)
9	4.12(4H, q), 1.40(6H, t)	3.80(H, m)[a]	3.12(2H, m)	–	3.80(2H, m)[a]			
10	4.15(4H, q), 1.39(6H, t)	3.70(H, m)[a]	3.12(2H, m)	4.7	3.70(H, m)[a]	1.48(3H, d)		
11	4.10(4H, q), 1.40(6H, t)[a]	3.68(H, m)[b]	3.10(2H, m)	4.7	3.68(H, m)[b]	1.40(H, m)[a]	1.40(6H, d)[a]	
12	4.06(4H, q), 1.40(6H, t)[a]	3.65(H, m)[b]	3.10(2H, m)	–	3.65(H, m)[b]	1.40(H, m)[a]	1.0, 1.40(8H, m)[a]	
13	4.10(4H, q), 1.40(6H, t)	3.67(H, m)[a]	3.12(2H, m)[b]	–	3.67(H, m)[a]	3.12(2H, m)[b]	7.50(2H, m)	
14	4.15(6H, q), 1.40(9H, m)	3.70(H, m)[a]	3.20(2H, m)	–	3.70(H, m)[a]	1.88(2H, m)	2.35(2H, t)	
15	4.10(4H, q), 1.40(6H, t)	3.70(H, m)[a]	3.20(2H, m)	–	3.70(H, m)[a]	1.90(2H, m)	2.70(2H, t)	2.12(3H, s)
16	4.03(2H, q + 2H, t), 0.8–1.5(18H, m)	3.60(H, m)[a]	3.34(2H, m)	–	3.60(2H, m)[a]			
17	4.00(2H, q + 2H, t), 0.8–1.5(18H, m)[a]	3.63(H, m)[b]	3.35(2H, m)	–	3.63(H, m)[b]	0.8–1.5(H, m)[a]	0.8–1.5(6H, d)[a]	
18	4.00(2H, q + 2H, t), 0.8–1.5(18H, m)[a]	3.60(H, m)[a]	3.35(2H, m)	–	3.60(H, m)[b]	0.8–1.5(H, m)[a]	0.8–1.5(8H, m)[a]	
19	4.02(2H, q + 2H, t), 0.8–1.5(18H, m)	3.60(H, m)[a]	3.35(2H, m)[b]	–	3.60(H, m)[a]	3.35(2H, m)[b]	7.20(2H, m)	
20	4.00(4H, q + 2H, t), 0.8–1.5(21H, m)	3.70(H, m)[a]	3.35(2H, m)	–	3.70(H, m)[a]	1.91(2H, m)	2.35(2H, t)	
21	4.00(2H, q + 2H, t), 0.8–1.5(15H, m)	3.62(H, m)[a]	3.38(2H, m)	–	3.62(H, m)[a]	1.80(2H, m)	2.50(2H, t)	2.12(3H, s)

[a,b]Signals of the same sign are overlapped.

is phosphorylated, and the carboxylate residue in the enzyme coordinates with an electrophilic carbon of either carboxylate ester in the inhibitor and thus deactivates the enzyme toward acetylcholine, as presented in the Scheme 3 mechanism.

The similarity between acetylcholine and the synthesized phospholidines in interacting with AChE explains their inhibition activity even at low concentration (1 ppm). In addition, the anticipation of giving nontoxic products upon in vivo hydrolysis, for example, amino acids and alcohols, makes these compounds eligible for pesticidal applications. Equations 1 and 2 also explain the high activity of compounds containing the isoleucine moiety in both series (**4, 12**), since incorporating a bulky group has two opposite effects on enzyme inhibition: enhancing the steric interaction with the enzyme and destabilizing the nucleophilic attack. However, the square of the *s*-butyl steric descriptor (5.617) is much higher than the square of the polar descriptor (0.49), which compensates the large polar weighting factor compared with that of the steric factor, causing the steric factor to be more important in affecting the inhibition process in both series. In contrast, in the 1,3,2-oxazaphospholidine series with an octyl group (**16–21**), compound **18** containing the isoleucine moiety showed the lowest inhibition activity within the series, which can be attributed to the bulky octyl group that causes this series to be more sensitive toward the steric effect.

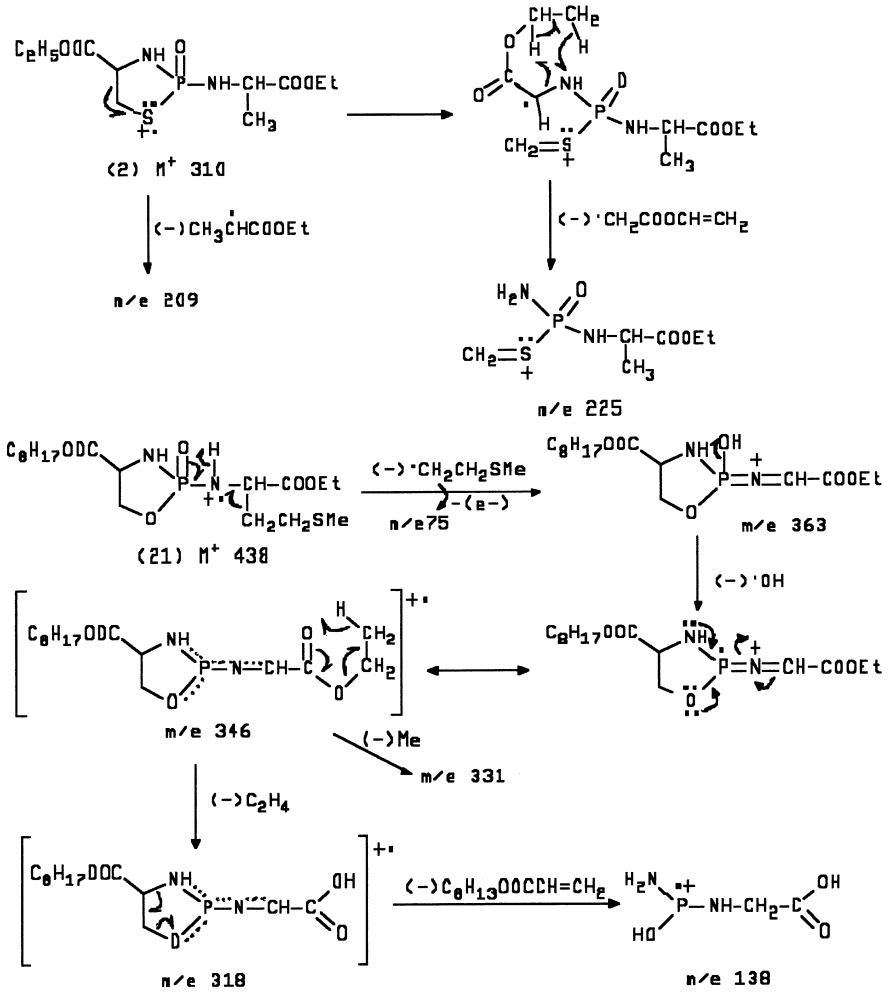
EXPERIMENTAL

Instruments and Reagents

α-Amino acids used were ACS grade and of the L-configuration. Chemicals were purified and dried before use according to standard procedures. Reactions were followed until completion by thin-layer chromatography. Infrared spectra were recorded on a Nicolet 460 FT-IR spectrometer. Proton NMR spectra were executed on a Varian EM 390 spectrometer by using $CDCl_3$/TFAA as a solvent. GC–MS analyses were performed in the electron impact mode on a Finnigan Mat GCQ spectrometer equipped with a Rtx-5MS column that is 30 m long, 0.25 mm i.d., and has a 0.25 μm film thickness (df). The oven temperature program was as follows: initial value at 40°C for 3 minutes, then increased by 15°C/min to 250°C (5 minutes). Injection and detection temperatures were 150 and 250°C, respectively.

Preparation of Ethyl α-Amino Acid Esters

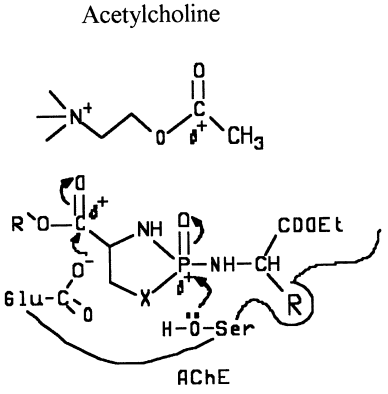
The esterification was performed according to the reported procedures [4,6].



SCHEME 2

TABLE 3 The Observed AChE Inhibition Percentages by Compounds **1–21**

Compound	*% AChE Inh* (± *SD*)	*Compound*	*% AChE Inh* (± *SD*)
1	25.25 (±0.41)	**12**	68.52 (±0.49)
2	31.62 (±0.43)	**13**	5.13 (±0.10)
3	10.80 (±0.24)	**14**	62.73 (±0.58)
4	39.64 (±0.52)	**15**	43.02 (±0.39)
5	3.68 (±0.07)	**16**	28.54 (±0.32)
6	29.71 (±0.35)	**17**	25.39 (±0.26)
7	15.27 (±0.19)	**18**	9.88 (±0.12)
8	38.95 (±0.56)	**19**	12.89 (±0.16)
9	24.82 (±0.38)	**20**	38.91 (±0.29)
10	15.07 (±0.24)	**21**	39.67 (±0.27)
11	8.17 (±0.14)		



SCHEME 3

Preparation of n-Octyl Serine and Cysteine Esters

L-(*S*)-Serine or L-(*R*)-cysteine (60 mmol), 8 mL of concentrated sulfuric acid, and 85 g of 1-octanol were refluxed for 2 hours. After cooling, the two fractions of the reaction mixture (ether and water) were separated. The aqueous layer was washed twice with ether to get rid of excess octanol. The aqueous layer was then neutralized with aqueous NaOH. The free amino acid ester was extracted twice with ether. The ether layer was washed with water, dried over anhydrous $MgSO_4$, and evaporated.

Synthesis of 2-(α-Alkyl, α-Ethoxycarbonyl Methylamino)-4-Alkoxycarbonyl 1,3,2-oxa or thiazaphospholidines

To a stirred and cooled (0°C) solution of 3.47 g (22.6 mmol) of phosphoryl chloride in 30 mL of benzene, a mixture of 22.6 mmol of L-(*S*)-serine or L-(*R*)-cysteine ester and 4.47 g (45.2 mmol) of Et_3N in 20 mL of benzene was added dropwise. The reaction mixture was stirred for 4 hours, then cooled again, and a mixture of 22.6 mmol of the appropriate L-amino acid ester, 2.24 g (22.6 mmol) of Et_3N, and 20 mL of benzene was added. The reaction mixture was stirred overnight and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with 5% HCl, and then with water. The organic layer was dried over anhydrous $MgSO_4$, evaporated, and subjected to silica gel column chromatography with a C_6H_6/CH_2Cl_2 solvent system.

Determination of Anticholinesterase Activity

Blood samples were freshly drawn from 3- to 6-month-old Swiss white mice and centrifuged at 5000 rpm to collect the serum as the enzyme source. The clear serum was then frozen until used within two days in the enzyme assay. The assay was measured by the method of Ellman et al. [18]. The inhibition percentage was determined in triplicate at a concentration of 1 ppm of each pesticide in the assay solution. The pesticide was added as a solution in ethanol.

Statistical Analysis

A computerized multiple regression analysis was performed by stepwise introduction of a new parameter with minimization of the sum of squared deviations. The analysis was stopped when the correlation was no longer improved significantly, as evidenced by the Student's *t*-test. The parameters used in the regression analysis were obtained from the literature [12,15].

REFERENCES

[1] Hirashima A.; Eto, M. Agric Biol Chem 1983, 47, 2831.
[2] Tawata, S.; Kuwano, E.; Eto, M. Agric Biol Chem 1980, 44, 1489.
[3] Yoshikawa, H.; Fughigami, K.; Shono, T. J Pesticide Sci 1986, 11, 631.
[4] Ali, H. M. Phosphorus, Sulfur and Silicon, in press.
[5] Cooper, D. B.; Hall, C. R.; Harrison, J. M.; Inch, T. D. J Chem Soc Perkin Trans 1977, 1, 1969.
[6] Chen, R.-Y.; Wang, H.-L.; Zhou, J. Heteroat Chem 1994, 5, 497.
[7] Habiba, R. A.; Ali, H. M.; Ismail, S. M. M. J Agric Food Chem 1992, 40, 1852.
[8] Ismail, S. M. M.; Ali, H. M.; Habiba, R. A. J Agric Food Chem 1993, 41, 610.
[9] Ali, H. M.; Mostafa, A. A. Environ Toxicol Chem 1999, 18, 167.
[10] Ali, H. M.; Mostafa, A. A.; El-Zohry, M. F. Heteroatom Chem in press.
[11] Wilkins, J. P. G.; Pestic Sci 1990, 29, 163.
[12] Roy, N. K.; Nidiry, E. S. J.; Vasu, K.; Bedi, S.; Lalljee, B.; Singh, B. J Agric Food Chem 1996, 44, 3971.
[13] Fukuto, T. R.; Metcalf, R. L.; Jones, R. L.; Myers, R. O. J Agric Food Chem 1969, 17, 923.
[14] Chen, J.; Wang; L.; Lu, G.; Zhao, T. Bull Environ Contam Toxicol 1997, 58, 372.
[15] Taft, R. W., Jr. Steric Effects in Organic Chemistry Newman, M. S., Ed.; Wiley & Sons: New York, 1962.
[16] Quistad, G. B.; Fukuto, T. R.; Metcalf, R. L. J Agric Food Chem 1970, 18, 189.
[17] Hassall, K. A. The Biochemistry And Uses of Pesticides. 2nd ed.; Macmillan Press Ltd: Hong Kong, 1990, 92–96.
[18] Ellman, G. L.; Courtney, K. D.; Andres, V.; Jr.; Featherstone, R. M. Biochem Pharmacol 1961, 7, 88.