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Synthesis, fungitoxicity and quantitative structure activity relationship of *O*-aryl *O*-2-chloroethyl *O*-ethyl phosphates

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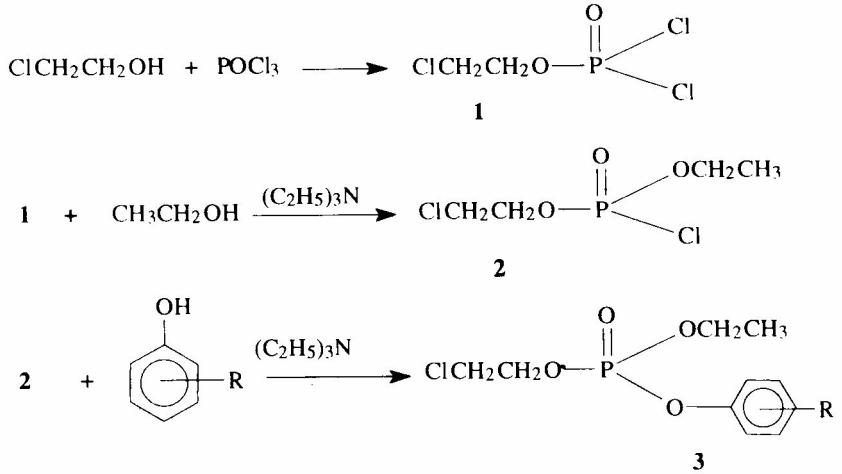
Nineteen new *O*-aryl *O*-2-chloroethyl *O*-ethyl phosphates **3** have been synthesised and tested for their fungicidal activity *in vitro* against *Rhizoctonia bataticola* and *Alternaria alternata*. *O*-2-Chloroethyl *O*-ethyl *O*-(pentachlorophenyl) phosphate **3s** shows highest activity against both the tested fungi and possesses better activity compared to the standard ediphenphos. Quantitative structure activity relationship studies provide excellent correlation between fungicidal activities and physico-chemical parameters like hydrophobicity ($\Sigma\pi$), electronic effect ($\Sigma\sigma$) and few position specific STERIMOL parameters like $\Sigma L_{(o)}$, $\Sigma L_{(m)}$, $\Sigma B1_{(o)}$ and $\Sigma B4_{(m)}$ of phenyl ring substituents

A few organophosphates have been commercially used as fungicides. The fungicidal activities of some of the series of phosphates have been reported earlier from our laboratory[1,2]. In continuation of this work of finding new, safe and potent fungicide molecules and to see the effect of replacing the smaller methyl group of *O*-aryl *O*-2-chloroethyl *O*-methyl phosphates[2] by ethyl group, the present study on the synthesis, fungitoxicity and quantitative structure activity relationship of *O*-aryl *O*-2-chloroethyl *O*-ethyl phosphates **3** has been carried out, which is reported in this paper.

The phosphates **3** were synthesised by the condensation of different substituted phenols with *O*-ethyl *O*-2-chloroethyl phosphorochloridate **2** (Scheme I), which in turn was synthesised by condensing *O*-2-chloroethylphosphoryl dichloride[2] **1** with ethanol. The fungicidal activity of the phosphates **3a-s** was studied against *Rhizoctonia bataticola* and *Alternaria alternata*. The phosphates **3**, their physical data and fungicidal activities (ED_{50}) are given in Table I. It is clear from the results that the introduction of Cl, NO_2 and $C(CH_3)_3$ groups in benzene ring, in general, enhanced the activity of the phosphates while the presence of OCH_3 and CH_3 groups in the benzene ring results in a decrease in the fungitoxicity of phosphates against both the tested fungi. In dimethyl substituted



Scheme I

Table I — Physical data and fungitoxicity of *O*-aryl *O*-2-chloroethyl O-ethylphosphates **3a-s**

Compd	R	Yield (%)	ED_{50} values (μg mL^{-1}) against	
			R. bataticola	*A. alternaria*
3a	H	59	133.70	122.77
3b	2-Cl	56	188.80	070.85
3c	4-Cl	58	080.57	102.03
3d	2-OCH_3	49	642.33	560.11
3e	4-OCH_3	48	188.51	226.67
3f	2-CH_3	49	143.35	186.07
3g	3-CH_3	50	097.80	111.76
3h	4-CH_3	52	164.91	064.68
3i	4-$C(CH_3)_3$	55	050.77	041.04
3j	2-NO_2	48	066.54	109.10
3k	4-NO_2	51	156.98	093.94
3l	3,4-$(CH_3)_2$	48	198.63	137.36
3m	2,3-$(CH_3)_2$	45	143.65	076.99
3n	2,4-$(CH_3)_2$	51	079.37	078.58
3o	3,5-$(CH_3)_2$	50	190.86	182.75
3p	2,4-$(CH_3)_2$	59	050.65	026.69
3q	2,4,6-Cl_3	61	029.31	031.06
3r	2,4,5-Cl_3	64	028.86	019.65
3s	Cl_5	65	011.23	012.52
Ediphenphos	—	—	040.00	050.00

All compounds from **3a-j** and **3l-r** were liquids purified by column chromatography whereas compounds **3k** and **3s** were solids with m.ps 115-16 and 67-68° respectively.

componds, except the 2,4-$(CH_3)_2$ analog **3n**, other analogs have lower activity compared to unsubstituted phenol analog **3a**. The activity increases with increase in the number of chloro substituents in the phenyl ring which follows the order: mono < di < tri < penta. The most active compound in this series is the pentachloro analog **3s** against both the fungi with ED_{50} values of 11.23 and 12.52 ppm against *R. bataticola* and *A. alternata*, respectively. A few other analogs too expressed better activity compared to the reference standard ediphenphos. These were **3q** (29.31 ppm) and **3r** (28.86 ppm) against *R. bataticola* and **3p** (26.69 ppm), **3q** (31.06 ppm) and **3r** (19.65 ppm) against *A. alternata*.

Quantitative Structure Activity Relationships

The modulation of fungicidal activity in a congeneric series can be attributed to the electronic, hydrophobic and steric properties of the benzene ring substituents. This effect can be studied in a quantitative way by multiple regression analysis using substituent constants for the above properties. In the present study, $-\log ED_{50}(M)$ is taken as dependent variable and substituent constant parameters (Table II) as independent variable. The ED_{50} values given in Table I were converted to molar concentrations and their negative logrithms are given in Table III.

The Hansch π values were used as a measure of relative hydrophobicity of substituents, Hammetts electronic parameter σ_p and σ_m as measure of relative electronic influence of substituents, and Verloop STERIMOL parameters L, B1 and B4, which represent length, minimum and maximum width of the groups, respectively.

The results obtained by the multiple regression analysis are expressed below together with their statistical values, n is the number of compounds used in regression; r is the correlation coefficient; s is the standard deviation and F is the ratio of correlation and expresses significance index with respect to the equations. The figures in parentheses below the regression coefficients are the standard error of respective constants. The correlation matrix of the parameters appearing in the regression equations (1) to (8) is given in Table IV.

R. bataticola

When π alone, the linear term of hydrophobicity, was taken, Eqn (1) was obtained. It shows that hydrophobicity alone contributes about 64% towards the fungicidal activity of phosphates.

$$-\text{Log } ED_{50}(M) = 3.216 + 0.327\ \Sigma\pi \quad (\pm 0.062)$$

$$s=0.249,\ n=18,\ r=0.799,\ F=28.17(F_{1,16}=8.53) \qquad ...(1)$$

Table II — Substituent parameters used for correlation analysis

R	π	σ	L	B1	B4
H	0.00	0.00	2.06	1.00	1.00
Cl	0.71	0.23 (0.37)	3.52	1.80	1.80
NO_2	-0.28	0.78	3.44	1.70	2.44
OCH_3	-0.02	-0.27	3.98	1.35	2.87
CH_3	0.56	-0.17 (-0.07)	3.00	1.52	2.40
$C(CH_3)_3$	1.68	-0.20	4.11	2.59	2.97

σ values given in the parentheses are for *meta*-substutuents while others are for *ortho*- and *para*- substituents.

Table III - Observed and calculated -log ED_{50}(M) values of *O*-aryl *O*-2-chloroethyl *O*-ethyl phosphates **3a-s**

Compd	R	-Log ED_{50}(M) against			
		R. bataticola		*A. alternata*	
		Obsd	Calcd[a]	Obsd	Calcd[b]
3a	H	3.30	3.24	3.33	3.30
3b	2-Cl	3.20	3.53	3.63	3.59
3c	4-Cl	3.57	3.53	3.47	3.65
3d	2-OCH_3	2.66	3.23	2.72	2.72
3e	4-OCH_3	3.19	3.13	3.11	3.18
3f	2-CH_3	3.29	3.33	3.18	3.39
3g	3-CH_3	3.45	3.26	3.40	3.32
3h	4-CH_3	3.23	3.33	3.63	3.42
3i	4-$C(CH_3)_3$	3.80	3.72	3.89	3.91
3j	2-NO_2	3.67	3.45	3.45	3.45
3k	4-NO_2	3.29	3.45	3.52	3.54
3l	3,4-$(CH_3)_2$	3.17	3.35	3.33	3.44
3m	2,3-$(CH_3)_2$	3.31	3.35	3.58	3.40
3n	2,4-$(CH_3)_2$	3.57	3.43	3.57	3.51
3o	3,5-$(CH_3)_2$	3.19	3.19	3.20	3.33
3p	2,4-Cl_2	3.82	3.81	4.10	3.94
3q	2,4,6-Cl_3	4.10	4.10	4.07	4.22
3r	2,4,5-Cl_3	4.11	4.06	4.27	4.11
3s	Cl_5	4.59	4.60	4.54	4.58

Values calculated from [a]Eqn (3), [b]Eqn (8)

A combination of π and σ parameters improves the correlation (r=0.923) and gives Eqn (2).

$$-\text{Log } ED_{50}(\text{M}) = 3.224 + 0.241\Sigma\pi + 0.398\Sigma\sigma \quad (\pm 0.045) \quad (\pm 0.086)$$
$$s=0.164,\ n=18\ r=0.923,\ F=42.96(F_{2,15}=6.36) \qquad ...(2)$$

$$-\text{Log } ED_{50}(\text{M}) = 3.460 + 0.280\Sigma\pi + 0.376\Sigma\sigma - 0.110\Sigma B4_{(m)} \quad (\pm 0.044) \quad (\pm 0.077) \quad (\pm 0.050)$$
$$s=0.147,\ n=18,\ r=0.943,\ F=37.76(F_{3,14}=5.56) \qquad ...(3)$$

Eqn (2) attributes 89% variability in fungicidal activity to hydrophobic and electronic parameters of the benzene ring substituents. Further inclusion of $B4_{(m)}$ parameter in Eqn (2) gives Eqn (3) with better correlation (r=0.943). The parameters π, σ and $B4_{(m)}$ contribute about 89% towards fungicidal activity of phosphates **3a-s**. Although the significance index (F) value for Eqn (3) is less than that for Eqn (2), it is found that the contribution made by $B4_{(m)}$ is significant (Cald $F_{1,14}$=4.92 and Table $F_{1,14}$ =4.60) at 95% level.

Introduction of any other parameter to Eqn (3) did not improve the correlation significantly. Therefore, Eqn (3) is taken as the best fitted equation for expressing the fungicidal activity of *O*-aryl *O*-2-chloroethyl *O*-ethyl phosphates. This equation was significant at 99% level and explained 89% variation in fungicidal activity exhibited by the members of phosphate series. The –log ED_{50}(M) values calculated from Eqn (3) are comparable to the observed values (Table III) except for **3d**, which was excluded from the regression analysis. The positive sign associated with π and σ terms in Eqn (3) indicates that the fungicidal activity of the compounds **3a-s** increases with the increase in the values of these parameters while the negative sign associated with $B4_{(m)}$ indicates that with the decrease in the value of $B4_{(m)}$, the fungitoxicity of phosphates increases.

A. alternata

Eqns (4) to (8) were obtained for the fungicidal activity of *O*-aryl *O*-2-chloroethyl *O*-ethyl phosphates against *A. alternata*.

$$-\text{Log } ED_{50}(\text{M}) = 3.229 + 0.365\Sigma\pi \quad (\pm 0.063)$$
$$s=0.261,\ n=19,\ r=0.817,\ F=34.03(F_{1,17}=8.40) \qquad ...(4)$$

Eqn (4) was obtained when π parameter alone was used in the regression analysis and expresses that hydrophobicity alone contributes about 67% towards fungicidal activity of phosphates.

$$-\text{Log } ED_{50}(\text{M}) = 3.249 + 0.278\Sigma\pi + 0.373\Sigma\sigma \quad (\pm 0.053) \quad (\pm 0.103)$$
$$s=0.199,\ n=19,\ r=0.904,\ F=35.70(F_{2,16}=6.23) \qquad ...(5)$$

$$-\text{Log } ED_{50}(\text{M}) = 3.867 + 0.355\Sigma\pi + 0.406\Sigma\sigma - 0.152\Sigma L_{(m)} \quad (\pm 0.058) \quad (\pm 0.093) \quad (\pm 0.067)$$
$$s=0.177,\ n=19,\ r=0.930,\ F=31.86(F_{3,15}=5.42) \qquad ...(6)$$

Table IV- Correlation matrix of the parameters used in the regression equations

Parameters	$\Sigma\pi$	$\Sigma\sigma$	$\Sigma L_{(o)}$	$\Sigma L_{(m)}$	$\Sigma B1_{(o)}$	$\Sigma B4_{(m)}$
$\Sigma\pi$	1.000					
$\Sigma\sigma$	0.443	1.000				
$\Sigma L_{(o)}$	0.536	0.596	1.000			
$\Sigma L_{(m)}$	0.647	0.340	0.251	1.000		
$\Sigma B1_{(o)}$	0.638	0.676	0.955	0.307	1.000	
$\Sigma B4_{(m)}$	0.403	0.076	- 0.024	0.872	0.020	1.000

Introduction of σ term in Eqn (4) gives Eqn (5) with better correlation (r=0.904). According to Eqn (5), a combination of π and σ terms explains about 82% variability in fungicidal activity of phosphates. Further addition of $L_{(m)}$ term to Eqn (5) gives Eqn (6) which expresses the combined effect of π, σ and $L_{(m)}$ parameters on fungitoxicity and attributes 86% variability in fungicidal activity to these parameters. Subsequent addition of $L_{(o)}$ and $B1_{(o)}$ terms gives Eqns (7) and (8), respectively. Eqn (8) expresses the effect of π, σ, $L_{(o)}$, $L_{(m)}$ and $B1_{(o)}$ parameters on fungicidal activity of phosphates against *A. alternata*, and accounts for 92% variability in fungitoxicity.

$$-\text{Log}ED_{50}(\text{M}) = 4.543 + 0.413\Sigma\pi + 0.517\Sigma\sigma - 0.188\Sigma L_{(m)} - 0.118\Sigma L_{(o)}$$
$$(\pm 0.057)\quad (\pm 0.095)\quad (\pm 0.061)\quad (\pm 0.052)$$
$$s=0.157,\ n=19,\ r=0.949,\ F=31.82(F_{4,14}=5.08) \qquad \ldots(7)$$

$$-\text{Log}ED_{50}(\text{M}) = 4.268 + 0.354\Sigma\pi + 0.431\Sigma\sigma - 0.159\Sigma L_{(m)}$$
$$(\pm 0.062)\quad (\pm 0.100)\quad (\pm 0.059)$$
$$-0.334\Sigma L_{(o)} + 0.531\Sigma B1_{(o)}$$
$$(\pm 0.128)\quad (\pm 0.291)$$
$$s=0.145,\ n=19,\ r=0.960,\ F=30.35(F_{5,13}=4.86) \qquad \ldots(8)$$

Further inclusion of any other parameter to Eqn (8) did not improve the correlation. So, Eqn (8) was chosen as the best fit for expressing the fungicidal activity of *O*-aryl *O*-2-chloroethyl *O*-ethyl phosphates **3** against *A. alternata* The values of significance index (F) for Eqn (6) to (8) were less than that for Eqn (5).But it was found that the contribution by $L_{(o)}$ and $L_{(m)}$ was significant at 95% level (Calcd $F_{1,15}$=5.24, Table $F_{1,15}$=4.54; Calcd $F_{1,14}$=5.12, Table $F_{1,14}$=4.60). Although the contribution by $B1_{(o)}$ is nonsignificant (Calcd $F_{1,13}$=3.33), it was included in the regression equation as its addition increases the value of correlation coefficient r from r=0.949 for Eqn (7) to r=0.960 for Eqn (8). All the equations from (4) to (8) were significant at 99% level. The positive sign associated with B, F and $B1_{(o)}$ terms indicates that the fungicidal activity of phosphates against *A. alternata* increases with the increase in the values of π, σ and $B1_{(o)}$ parameters. While the negative sign associated with $L_{(o)}$ and $L_{(m)}$ terms indicates that the fungicidal activity of the phosphates will increase with the decrease in the value of these two parameters. The -log ED_{50}(M) values calculated from Eqn (8) are comparable to the observed values (Table III).

The results of QSAR studies indicate that the fungicidal activity of *O*-aryl *O*-2-chloroethyl *O*-ethyl phosphates **3a-s** against *R. bataticola* and *A. alternata* is mainly attributed to the hydrophobic and electronic parameters of the benzene ring substituents. Fungitoxicity increases with increased hydrophobicity and electron withdrawing nature of the benzene ring substituents. These two parameters account for 82 to 85% variability in fungicidal activity. Besides this, a few steric parameters expressed as STERIMOL parameters $L_{(o)}$, $L_{(m)}$, $B1_{(o)}$ and $B4_{(m)}$ also contribute towards fungicidal activity.

Experimental Section

General. Melting points are uncorrected. IR spectra were recorded in CCl_4 on a Nicolet 400 spectrometer, and ^{1}H NMR spectra on a Varian EM-360L 60 MHz spectrometer in CCl_4 (chemical shifts in δ, ppm) using TMS as internal standard.

***O*–2–Chloroethylphosphoryl dichloride 1.** It was prepared by the method of Singh *et al.*[2] (b.p. 100° at 20mm).

***O*-Aryl *O*–2-chloroethyl *O*-ethyl phosphate 3: General procedure.** Compound 1 (0.1 mole) was reacted with C_2H_5OH (0.12 mole) at 5-10°C in benzene (200 mL) while stirring. After the addition of C_2H_5OH was over, triethyl amine (0.1 mole dissolved in 10 mL benzene) was added to the reaction mixture and the stirring continued for another 2 hr. Thereafter, phenol or substituted phenol (0.1 mole) dissolved in benzene (100 mL) was added dropwise to the reaction mixture at 0-5°C while stirring, followed by the addition of triethyl amine (0.1 mole). Stirring was continued for another 2 hr at room temperature. The precipitated amine hydrochloride was filtered off and solvent was distilled off on a water-bath to get the crude product. It was recrystallized from appopriate solvent, if solid or by column chromatography on silica column, if liquid. The purity of the compounds was checked by TLC on

silica gel coated plates using benzene-acetone (80:20) as developing solvent and iodine vapours as visualising agent. **3a** (R=H) — IR[3] (cm^{-1}): 1285 (P=O), 1190 (P-O-C, aromatic), 1212 (P-O-C, aliphatic); ^1H NMR[4]: 7.4 (m, 5H, phenyl), 4.3 (m, 4H, $-OCH_2CH_2Cl$, $-OCH_2-CH_3$), 3.75 (t, J=6Hz, CH_2Cl), 1.4 (t, J=6Hz, 3H, $-CH_3$).

Fungitoxicity assay. Fungicidal testing of phosphates **3** was carried out against *R. bataticola* (Taub.) Butler and *A. alternata* (Fr.) Keissler following the poisoned food technique using standard potato-dextrose agar as described by Nene and Thapliyal[5]. Ediphenphos was used as the reference standard for comparison. The ED_{50} values (Table I) were calculated on a log probit scale from the per cent inhibition of growth data for 5 concentrations. The maximum concentration used was 1 mg mL^{-1}.

QSAR study. For QSAR anlaysis, the ED_{50} values were converted to -log ED_{50}(M) values. The structure activity correlation was done by the multiple regression analysis technique[6] using -log ED_{50}(M) values as the dependent variable and physico-chemical parameters for hydrophobic, electronic and steric properties of each compound of the series as independent variable. The values for various physico-chemical parameters were taken from literature[7,8]. The value of π and σ for multisubstituted phenols were obtained by adding the values of all the ring substituents corresponding to their position. While the values for steric parameters were used as the sum of values for each *ortho-*, *meta-* and *para*-position.

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