JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Organophosphorus Derivatives Containing Piperazine Dithiosemicarbazones as Chemotherapeutants against Fungal Pathogens of Sugarcane

RUCHI CHANDRA, O. P. PANDEY, AND S. K. SENGUPTA*

Chemistry Department, DDU Gorakhpur University, Gorakhpur 273009, India

Five novel organophosphorus derivatives have been synthesized by the reactions of *O*,*O*-diethylchlorophosphate with piperazine dithiosemicarbazones. The derivatives have been characterized on the basis of analyses and spectral (IR, ^{1}H NMR) data. Fungicidal activities of these derivatives against *Colletotrichum falcatum*, *Fusarium oxysporum*, and *Curvularia pallescence* have been evaluated. The screening results have been correlated with the structural features of the tested compounds. Organophosphorus derivatives containing 1,4-bis(4-chlorobenzaldehyde)piperazine dithiosemicarbazone and 1,4-bis(4-methoxybenzaldehyde)piperazine dithiosemicarbazone proved to be more active than some prevalent commercial synthetic fungicides.

KEYWORDS: Organophosphorus derivatives; synthesis; IR; NMR; fungal pathogens; sugarcane

INTRODUCTION

Sugarcane, a major source of sugar and important cash crop, is extensively grown worldwide. Sugarcane is known to be affected by fungal, viral, bacterial, and phytoplasma pathogens that are responsible for considerable economic loses (*1*, *2*). Among fungi, *Colletotrichum falcatum* (red rot) and *Fusarium oxysporum* (associated with wilt syndrome) are the most important pathogens that may be transmitted primarily by planting infected setts. *Curvularia pallescence* and *Curvularia lunata* (causing leaf spots) are the important foliar pathogens of sugarcane, sometimes causing enormous losses to the sugar industry. A number of synthetic organic compounds, namely,, dithiocarbamates, carbamates, and hydrazides, are now known to be useful in the control of various fungal diseases of plants (*2*). As an alarming level of loss is being caused to sugarcane by fungal attacks, it has become important to search for new fungicides to minimize disease incidence.

A few recent studies (*3*−*6*) from our laboratory have shown that on the basis of suitable logic, organic molecules incorporating phosphorus may be designed such that they may be less dangerous in use without losing their value as effective pesticides. One of the useful properties of phosphorus compounds is their relatively low stability and rapid metabolic breakdown in plants, in animal organisms, in soil, and in other components of the environment with the formation of products that are safe for human beings and domestic animals. Another important feature of these compounds is the high selectivity of their action. The discovery of the mechanism of action (*7*) of organophosphorus compounds make it possible to develop the fundamental principles of the directed synthesis of new substances and to establish the cause of their selective action on an organism. Studies on organophosphorus derivatives could constitute a new and promising field of application in the national economy. The present study was therefore undertaken to evaluate the antifungal efficacy of some newly synthesized organophosphorus compounds against various important fungal pathogens of sugarcane.

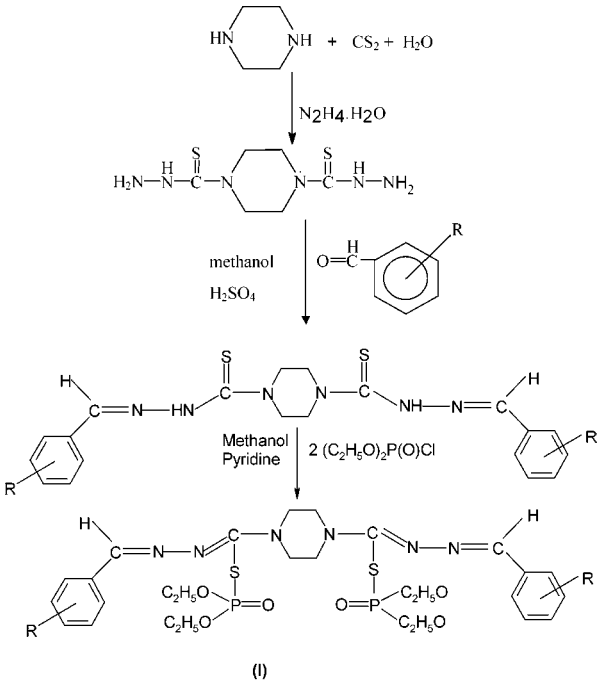

Figure 1. Synthetic route of organophosphorus derivatives containing substituted piperazine dithiosemicarbazones. R = H ($BPPTH_2$), 2-Cl ($BOCPTH_2$), 4-Cl ($BCPTH_2$), 4-OCH_3 ($BMPTH_2$) , or 4-NO_2 ($BNPTH_2$).

* Author to whom correspondence should be addressed (e-mail Sengupta2002@yahoo.co.in).

10.1021/jf040134m CCC: $30.25
Published on Web 02/18/2005

Table 1. Reactions of *O,O*-Diethylchlorophosphate with Piperazine Dithiosemicarbazone

reactants taken (g)					found (calcd) %			
$(C_2H_5O)_2POCl$	substituted piperazine dithiosemicarbazone[a]	molar ratio	reflux time (h)	product color, yield (%)	C	H	N	S
1.8	$BCPTH_2$ (2.3)	2:1	10	$[\{(C_2H_5O)_2PO\}_2$(BCPT)] yellowish brown, 59	44.00 (44.74)	5.12 (5.09)	11.02 (11.18)	8.51 (8.53)
1.8	$BPPTH_2$ (2.0)	2:1	10	$[\{(C_2H_5O)_2PO\}_2$(BPPTH)] yellowish brown, 61	49.20 (49.26)	5.80 (5.90)	12.23 (12.30)	9.30 (9.39)
1.8	$BMPTH_2$ (2.3)	2:1	10	$[\{(C_2H_5O)_2PO\}_2$ (BMPT)] yellow, 52	48.30 (48.38)	5.81 (5.94)	11.0 (11.28)	8.52 (8.60)
1.8	$BNPTH_2$ (2.5)	2:1	16	$[\{(C_2H_5O)_2PO\}_2$ (BNPT)] yellow, 55	43.41 (43.51)	4.92 (4.95)	14.44 (14.50)	8.18 (8.29)
1.8	$BOCPTH_2$ (2.3)	2:1	16	$[\{(C_2H_5O)_2O\}_2$(BOCPT)] yellow, 60	44.60 (44.74)	5.10 (5.09)	11.04 (11.18)	8.44 (8.53)

[a] $BCPTH_2$, 1,4-bis(4-chlorobenzaldehyde)piperazine dithiosemicarbazone; $BPPTH_2$, 1,4-bis(benzaldehyde)piperazine dithiosemicarbazone; $BMPTH_2$, 1,4-bis(4-methoxybenzaldehyde)piperazine dithiosemicarbazone; $BNPTH_2$, 1,4-bis(4-nitrobenzaldehyde)piperazine dithiosemicarbazone; $BOCPTH_2$, 1,4-bis(2-chlorobenzaldehyde)piperazine dithiosemicarbazone.

MATERIALS AND METHODS

The reactions of *O,O*-diethylchlorophosphate were carried out under inert atmosphere and anhydrous conditions. Special precautions were taken to exclude moisture from the apparatus and chemicals as the starting materials (*O,O*-diethylchlorophosphate) and reactions were susceptible to hydrolysis. Glass apparatus with interchangeable joints were used throughout the work. All of the organic solvents used were of analytical reagent grade. The solvents were purified and dried using the method described in the literature (*8*). *O,O*-Diethylchlorophosphate was prepared (*9*) by adding a solution of pyridine, absolute ethanol, and benzene dropwise to an ice-cooled solution of phosphorus oxychloride in benzene at temperature below 20 °C. After 3 h of stirring, the pyridine hydrochloride was filtered off. The product, after distillation, was obtained as a colorless liquid (bp 60−63 °C/2.5 mmHg). All reactions were carried out in the hood. A hood is a specially constructed workplace that has, at the least, a powered vent to suck noxious fumes outside. The details of analysis and physical measurements were the same as reported earlier (*6*).

For antifungal activity, all compounds were tested against all test fungi by the food poison technique (*10*) at three concentrations (10, 100, and 1000 ppm). For this, the desired amount of chemical was dissolved in 0.5 cm^3 of solvent and mixed with the culture medium on the basis of the volume of medium in each Petri plate (80 mm diameter). Oatmeal agar medium was used for all test fungi. In controls, the same amount of medium containing the requisite amount of solvent was poured in place of test chemicals. A mycelial disk (5 mm diameter) obtained from the periphery of 2-week-old cultures was taken and transferred to the center of each Petri plate. Plates were incubated for 7 days at 28 ± 2 °C. Each treatment was repeated three times, and the inhibition was recorded relative to percent mycelial inhibition calculated using the formula

$$[(dc - dt)/dc] \times 100$$

where d*c* is the average diameter of the mycelial colony of the control and d*t* is the average diameter of the mycelial colony of the treatment.

Reactions of *O,O*-Diethylchlorophosphate with Piperazine Dithiosemicarbazones. *O,O*-Diethylchlorophosphate was added to a solution of the appropriate piperazine dithiosemicarbazone in 2:1 molar ratio, respectively, in methanol (~40 cm^3) in the presence of pyridine (5 cm^3), and the reaction mixture was refluxed for 10−18 h. The solid product was separated out and filtered and crystallized from acetone.

RESULTS AND DISCUSSION

Reactions of *O,O*-diethylchlorophosphate with thiosemicarbazones derived by the condensation of piperazine dithiosemicarbazide with benzaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzaldehyde, 4-nitrobenzaldehyde, or 4-methoxybenzaldehyde have been carried out in methanol in the presence of pyridine, and a variety of organophosphorus derivatives have been isolated according to **Figure 1**. The methods used for the preparation and isolation of these compounds gave materials of good purity as supported by their analyses and TLC. The elemental analyses and physical properties of the organophosphorus derivatives are given in (**Table 1**). The organophosphorus derivatives are found to be soluble in chloroform, dimethylformamide, tetrahydrofuran, and dimethyl sulfoxide. All of these compounds are yellow or brown in color. The compounds melt in the temperature range of 145−205 °C.

Table 2. ^1H NMR Data (δ Scale) of Organophosphorus Derivatives Containing Piperazine Dithiosemicarbazones

compound	$C_4H_4N_2$	phenyl group	C_2H_5	CH_3
$[\{(C_2H_5O)_2PO\}_2$ (BPPT)]	3.45 (s)	7.30 (s)	2.50 (t), 4.15 (q)	
$[\{(C_2H_5O)_2PO\}_2$ (BOCPT)]	3.48 (s)	7.60–7.75 (m)	2.65 (t), 4.00 (q)	
$[\{(C_2H_5O)_2PO\}_2$ (BCPT)]	3.47 (s)	7.62–7.72 (m)	2.55 (t), 4.25 (q)	
$[\{(C_2H_5O)_2PO\}_2$ (BMPT)]	3.45 (s)	7.72–7.85 (m)	2.60 (t), 4.20 (q)	3.40 (s)
$[\{(C_2H_5O)_2PO\}_2$ (BNPT)]	3.46 (s)	7.70–7.82 (m)	2.58 (t), 4.25 (q)	

Infrared Spectra. These thiosemicarbazones can exist either as a thione or the thiol tautomeric forms or as an equilibrium mixture of both forms, because they have a thioamide, −NH−C(=S) function. The IR spectra in the solid state do not show any ν(S−H) band but exhibit a medium ν(N−H) band at ~3150 cm^{-1}, indicating that in the solid state they remain mainly in the thione form. However, in solution they readily convert to the thiol tautomeric form with the concomitant formation of phosphorus complex by the protonated mercapto form of the ligands. This is indicated by the the absence of a −NH band in the organophosphorus derivatives. The IR spectra of the organophosphorus derivatives also show a new medium-intensity band at ~700 cm^{-1} owing to conversion (*11*) of C=S to C−S^-. The formation of a bond between the thiol sulfur and phosphorus is supported (*6*) by the appearance of band at ~620 cm^{-1}, assigned to ν(P−S−C).

The spectra of dithiosemicarbazones show bands at ~1570−1560 cm^{-1} due to (*11*) ν(C=N), which remain almost at the same position in the corresponding organophosphorus derivatives, suggesting noninvolvement of the azomethine nitrogen atom in bond formation with phosphorus. However, the loss of N(2)H's from the two dithiosemicarbazone moieties, by thione−thiol tautomerism, produces an additional carbon−nitrogen double bond N(2)=C(S), which is indicated by the appearance of a band at ~1590 cm^{-1} in the organophosphorus derivatives. In addition, all organophosphorus derivatives show bands at ~1015−1030 and ~1280−1295 cm^{-1} assignable (*6*) to ν(P−O−C) (alkyl) and ν(P=O) vibrations, respectively.

Table 3. Fungitoxic Screening Data of Organophosphorus Derivatives Containing Piperazine Dithiosemicarbazones

	% mycelial inhibition								
	Colletotrichum falcatum			*Fusarium oxysporum*			*Curvularia pallescence*		
compound	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm
$BCPTH_2$	24.8	35.4	68.2	28.7	41.6	64.8	38.5	49.2	68.4
$[\{(C_2H_5O)_2PO\}_2$ (BCPT)]	42.5	72.8	100.0	37.8	76.2	100.0	66.6	88.2	100.0
$BPPTH_2$	14.1	28.9	53.6	14.3	25.6	48.8	20.6	32.1	57.4
$[\{(C_2H_5O)_2PO\}_2$ (BPPT)]	25.8	40.2	73.4	24.8	35.6	66.5	32.8	49.5	75.0
$BMPTH_2$	22.1	37.6	65.9	35.3	40.2	66.8	28.6	45.8	67.2
$[\{(C_2H_5O)_2PO\}_2$ (BMPT)]	36.4	58.6	100.0	53.2	70.6	100.0	42.2	80.5	100.0
$BNPTH_2$	11.6	24.3	46.6	16.2	23.9	44.8	22.7	31.1	59.6
$[\{(C_2H_5O)_2PO\}_2$ (BNPT)]	25.8	40.8	65.2	27.5	40.2	62.6	35.6	58.2	89.2
$BOCPTH_2$	21.2	30.8	60.3	25.5	42.0	69.2	27.4	47.3	66.2
$[\{(C_2H_5O)_2PO\}_2$ (BOCPT)]	55.2	70.6	85.6	56.8	82.4	100.0	47.7	86.8	88.2
mean	27.9	44.0	71.9	32.0	47.8	72.3	36.3	56.9	77.1
SD	13.26	17.26	18.17	14.13	20.88	20.64	13.62	21.19	16.02

Table 4. Efficacy of Organophosphorus Derivatives Compared with Synthetic Fungicides against Sugarcane Pathogens

		MIC (ppm) against		
common name of fungicide/chemical	trade name	*C. falcatum*	*F. oxysporum*	*C. pallescence*
carbendazim	Bavistin	4000	3000	4000
copper oxychloride	Blitox 50	4000	3000	2000
mancozeb	Dithane M-45	4000	3000	4000
thiophanate methyl	Topsin M	4000	4000	4000
$[\{(C_2H_5O)_2PO\}_2$(BCPT)]		1000	1000	1000
$[\{(C_2H_5O)_2PO\}_2$(BMPT)]		1000	1000	1000

Proton Magnetic Resonance Spectra. The proton magnetic resonance spectra of the derivatives have been recorded in DMSO-d_6 **(Table 2)** The line intensities were determined by planimetric integration. A comparison of the spectra of the ligands with the complexes leads to the following conclusions:

(a) The signal of N(2)H is seen at δ ~12.60−12.95 in the dithiosemicarbazones. In the organophosphorus derivatives neither this signal nor a thiol SH signal is visible.

(b) The chemical shift at ~3.35 ppm may be due to piperazine ring protons, which shifted slightly downfield in the organophosphorus derivatives.

(c) The signal due to aromatic ring protons appears in the region δ 7.30−7.85.

(d) The signals due to ethoxy groups appear at δ ~2.5−2.65 (triplet due to CH_3 group) and at δ ~4.0−4.25 (quartet due to CH_2 group) in the spectra of all organophosphorus derivatives.

Antifungal Activity. The organophosphorus derivatives containing piperazine dithiosemicarbazones show **(Table 3)** promising results in inhibiting the mycelial growth of all the test fungi. There is significant alteration in the antifungal activity with the change in the nature of organic group attached to *O*,*O*-diethylphosphate moiety. For any particular species of fungus, organophosphorus derivatives containing dithiosemicarbazones are found to be more effective as compared to other reported derivatives. In this series, the best activities were recorded with *O*,*O*-diethylphosphate derivatives containing 1,4-bis(4-chlorobenzaldehyde)piperazine dithiosemicarbazone and 1,4-bis(4-methoxybenzaldehyde)piperazine dithiosemicarbazone. Two compounds show inhibition of 100% for all of the test fungi at 1000 ppm concentration.

The minimum inhibitory concentrations (MIC) of the most active *O*,*O*-diethylphosphate derivatives were determined. Three concentrations, 1000, 2000, and 3000 ppm, of each test compound with respect to the culture medium were prepared. The fungistatic/fungicidal nature of the active chemicals was determined in three replicates using the test fungi following the procedure of Garbour and Houston (*12*). This was done by observing if revival of growth of the inhibition mycelial disks occurred following transfer to a chemical-free medium.

The two dithiosemicarbazone derivatives, namely, *O*,*O*-diethylphosphate derivatives containing 1,4-bis(4-chlorobenzaldehyde)piperazine dithiosemicarbazone and 1,4-bis(4-methoxybenzaldehyde)piperazine dithiosemicarbazone, showed superiority over the commercial fungicides Bavistin, Blitox-50, Topsin-M, and Dithiane M-45 during the present study.

These compounds are 2−4 times more active than the tested commercial fungicides **(Table 4)**, which are being used in sugarcane fungal disease management.

ACKNOWLEDGMENT

We are thankful to Dr. G. P. Rao, Sugarcane Research Station, Kunraghat, Gorakhpur, for helpful discussion and suggestions.

LITERATURE CITED

(1) Agnihotri, V. P. *Diseases of Sugarcane and Sugarbeet*; Oxford and IBH: New Delhi, India, 1990; pp 4−38.

(2) Sengupta, S. K.; Pandey, O. P.; Rao, G. P. Potentialities of some newly synthesized organophosphorus derivatives as fungicides against sugarcane pathogens. In *Sugarcane Pathology, Vol. I: Fungal Diseases*; Rao, G. P., Bergamin Filho, A., Magarey, R. C., Autrey, L. J. C., Eds.; Science Publishers: Enfield, NH, 2000; pp 279−300.

(3) Jaiswal, A. K.; Rao, G. P.; Pandey, O. P.; Sengupta, S. K. Efficacy of organophosphorus derivatives against fungal pathogens of sugarcane. *J. Agric. Food Chem.* **1998**, *46*, 1609−1613.

(4) Sengupta, S. K.; Pandey, O. P.; Rao, G. P.; Shahi, S. P.; Jaiswal, A. K. Fungitoxicity of newly synthesized organophosphorus derivatives containing alkyl xanthates. *Sugarcane* **1998**, *5*, 17−20.

(5) Sengupta, S. K.; Pandey, O. P.; Rao, G. P.; Vishen, P. Efficacy of organophosphorus derivatives containing chalcones/chalcone semicarbazones against fungal pathogens of sugarcane. *Metal Based Drugs* **2002**, *8*, 293−302.

(6) Sengupta, S. K.; Pandey, O. P.; Rao, G. P.; Dwivedi, A.; Vishen, P. Efficacy of organophosphorus derivatives containing substituted chalcone thiosemicarbazones and dithiocarbazates against fungal pathogens of sugarcane. *Phosphorus, Sulphur Silicon Relat. Elem.* **2003**, *178*, 839−849.
(7) Fest, C.; Schmidt, K. J. *The Chemistry of Organophosphorus Pesticides*; Springer-Verlag: New York, 1982.
(8) Vogel, A. I. *A Text Book of Practical Organic Chemistry Including Qualitative Organic Analysis*, 3rd ed.; Longmans: London, U.K., 1956.
(9) Chaturvedi, K. Organic derivatives of phosphorus as pesticides. Ph.D. Thesis, University of Gorakhpur (India), 1993.
(10) Grover, R. K.; J. D. Moore, Toximetric studies of fungicides against brown rot organism *Sclerotinia fruticola* and *S. laxa*. *Phytopathology* **1962**, *52*, 876−880.
(11) Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*; Wiley-Interscience: New York, 1997.
(12) Garbour, R. H.; Houston, B. R. An inhibitor of *Verticillium albo-atrum* in cotton seed. *Phytopathology* **1959**, *49*, 449−450.

Received for review March 15, 2004. Revised manuscript received December 2, 2004. Accepted December 3, 2004.

JF040134M