Most of chlorbromuron remained in the soil in the 0–2.5-cm depth (Ragab et al., 1979), and probably little or no residues reached potato roots.

ACKNOWLEDGMENT

We thank Dow Chemical U.S.A., Michigan, and Ciba-Geigy, Montreal, for their courtesy in supplying analytical standards and J. C. Dumphy and F. A. Stewart of the Fredericton Research Station for their assistance in conducting field work.

Registry No. Sinox PE, 88-85-7; maloran, 13360-45-7.

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Received for review October 26, 1981. Revised manuscript received June 7, 1982. Accepted November 19, 1982. Contribution No. 1742, Kentville, Nova Scotia, Canada.

A Novel Class of Fungicides: (2-Pyridylthio)methyl Benzoate *N*-Oxides

The reaction of chloromethyl benzoates with sodium pyrithione afforded (2-pyridylthio)methyl benzoate *N*-oxides having in vitro fungicidal activity on a variety of agriculturally important fungi (e.g., *Alternaria solani* and *Phytophthora infestans*). The title compounds have demonstrated a spectrum of activity similar to that of zinc pyrithione, suggesting that release of the fungicidal 2-mercaptopyridine 1-oxide moiety may be significant. The synthesis of thiomethyl benzoate analogues and their bioevaluation are described.

The role of fungicides in seed storage and in the prevention and cure of systemic plant fungal infections is a significant area of interest to the agricultural scientist and farmer alike. The antifungal and antibacterial properties of 2-mercaptopyridine 1-oxide and its salts have long been known (Ladd, 1954; Albert et al., 1956; Leonard et al., 1956; Sijpesteijn et al., 1958). It was of interest to synthesize derivatives of 2-mercaptopyridine 1-oxide which had the capacity to hydrolytically release the active moiety after application. Such a protecting group was found to be the methyl benzoate system.

EXPERIMENTAL SECTION

Chemistry. The general synthetic scheme is shown in Figure 1. The acid chlorides (Ia–d) were commercially available (Aldrich Chemical Co.) and were used without further purification. Infrared spectra (IR) were recorded with a Perkin-Elmer Model 727B instrument. All ^{1}H NMR spectra were obtained with a Hitachi Perkin-Elmer Model R-24B 60-MHz high-resolution spectrometer. NMR samples were prepared in $CDCl_3$ or Me_2SO-d_6 containing 1% Me_4Si; chemical shifts are reported in ppm (δ) relative to $\delta(Me_4Si) = 0$. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected.

The syntheses of IIa–d were accomplished with moderate yields (20–70%) by using anhydrous zinc chloride and paraformaldehyde at 100 °C (Henry, 1901; Descude', 1901) and purified by distillation. The syntheses of the (2-pyridylthio)methyl benzoate *N*-oxides (IVa-d) were carried out in low yields (8–41%) by using sodium pyrithione (III, sodium omadine, 90%) in a simple displacement reaction. The low yields are apparently a consequence of hydrolysis of the product during the reaction. The procedure for the synthesis of IVa serves as an example.

(2-Pyridylthio)methyl Benzoate N-Oxide (IVa). Chloromethyl benzoate (IIa) (distilled: bp 75–77 °C/2.0 mm; 4.8 g, 28.1 mmol) in 10 mL of 2-propanol was added dropwise to a rapidly stirring solution of sodium pyrithione (III, 6.71 g, 45.0 mmol) in 25 mL of 2-propanol under anhydrous conditions and at ambient temperature. After 1 h, the mixture was filtered to remove precipitated sodium chloride and the filtrate concentrated at reduced pressure to yield a white solid. Recrystallization from toluene afforded 1.5 g of IVa (21% yield): mp 124 °C dec; IR (CO) ν 1750 cm^{-1}, ν (NO) 840 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 5.85 (2 H, s), 7.50 (8 H, m), 8.20 (1 H, d, 6 Hz). Anal. Calcd for $C_{13}H_{11}NO_3S$: C, 59.78; H, 4.25; N, 5.36. Found: C, 59.95; H, 4.36; N, 5.36.

The procedures for the synthesis of IVb–d were identical with that for IVa.

(2-Pyridylthio)methyl 2-Chlorobenzoate N-Oxide (IVb). The data are as follows: 41% yield; mp 123 °C; IR ν (CO) 1740 cm^{-1}, ν (NO) 840 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.90 (2 H, s), 8.50 (8 H, m). Anal. Calcd for $C_{13}H_{10}ClNO_3S$: C, 52.79; H, 3.38; N, 4.74. Found: C, 52.56; H, 3.28; N, 4.75.

(2-Pyridylthio)methyl 2-Methylbenzoate N-Oxide (IVc). The following data were found: 29% yield; mp 123 °C dec; IR ν (CO) 1700 cm^{-1}, ν (NO) 840 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.55 (3 H, s), 5.70 (2 H, s), 7.50 (8 H, m), 8:20 (1 H, d, 6 Hz).

(2-Pyridylthio)methyl 4-Methoxybenzoate N-Oxide (IVd). The data were as follows: 8% yield; mp 140 °C dec;

0021-8561/83/1431-0451$01.50/0

Table I. In Vitro Fungicidal Activities of IVa-d Compared with Those of Zinc Pyrithione and Captan

fungus	concn, ppm[a]	% control IVa	IVb	IVc	IVd	zinc pyrithione	captan
Alternaria solani	500	100	75	80	70	100	63
	20	50				29	
Phytophthora infestans	500	100	100	85	100	100	50
	20	100	50		72	100	
Pythium sp.	500	100	60		100	83	0
	20	100	0		0	0	
Sclerotium rolfsii	500	65	75	35	40	100	0

[a] Concentrations given are those of the test solutions described under Experimental Section.

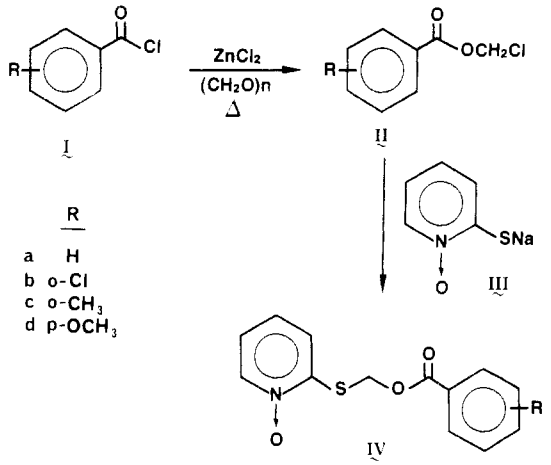

Figure 1. General synthetic scheme.

IR ν (CO) 1750 cm^{-1}, ν (NO) 837 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.85 (3 H, s), 5.75 (2 H, s), 7.50 (8 H, m).

(2-Pyridylsulfonyl)methyl Benzoate N-Oxide (V). (2-Pyridylthio)methyl benzoate *N*-oxide (IVa, 6.71 g, 22.0 mmol) and sodium tungstate (50 mg) were dissolved in 20 mL of acetic acid and heated to 40 °C. Hydrogen peroxide (30%, 5 mL, 50 mmol) was added slowly and the mixture heated to 80 °C for 1 h. The reaction mixture was then poured into 200 mL of cold water and extracted with 100 mL of chloroform, and the chloroform extract was washed with 100 mL of water. After drying with magnesium sulfate, filtration and evaporation, the chloroform extract yielded 2.84 g of V as a gum (38% yield): mp 80–82 °C dec; IV ν (CO) 1750 cm^{-1}, ν (NO) 850 cm^{-1}, ν (SO_2) 1160, 1345 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.00 (2 H, s), 7.20–8.4 (9 H, m).

(2-Pyridylthio)methyl 2-Methylbenzoate (VI). (2-Pyridylthio)methyl 2-methylbenzoate *N*-oxide (IVc, 0.36 g, 1.3 mmol) and phosphorus trichloride (0.20 g, 1.45 mmol) were dissolved in 25 mL of chloroform. After being stirred at ambient temperature for 15 min, the mixture was evaporated under reduced pressure to afford 0.34 g of VI (100% yield): mp 97 °C dec; IR ν (CO) 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.50 (3 H, s), 6.05 (2 H, s), 7.10–8.0 (8 H, m).

Bioevaluation. The general procedure for compounds IVa–d is as follows. The compound (20 mg) was dissolved in 20 mL of acetone, giving a 1000-ppm solution. Aliquots of this solution were diluted with sterile distilled water to provide 500 and 20 ppm of test solutions. Sterilized (autoclaved) 12.7-mm diameter filter paper disks were dipped in the test soution and air-dried (four disks per concentration tested). The treated disks were then placed on mycological ager (Difco Laboratories, Inc., 1953) in standard Petri dishes (150 × 20 mm). Agar plugs (7-mm diameter) removed from culture plates of healthy mycelial growth were placed mycelial side down on the center of the disks and incubated at 29 °C for 2–3 days. Control disks were treated with acetone alone.

Table II. In Vitro Fungicidal Activities of IVa, IVc, and Related Analogues at 500 ppm

compound	% control *A. solani*	*P. infestans*	*S. rolfsii*
IVa, R = H	100	100	65
IVc, R = CH_3	80	85	35
V	5	5	0
VI	40	0	0

Scoring was done by measuring the radius of the mycelial growth from the disk outer edge to the growth outer edge. Percent control was obtained by using eq 1,

$$\text{percent control} = \frac{\text{AR(control)} - \text{AR(treated)}}{\text{AR(control)}} \times 100 \quad (1)$$

where AR is the average radius. Four fungi were used in this study: *Alternaria solani, Phytophthora infestans, Pythium* sp., and *Sclerotium rolfsii.*

RESULTS AND DISCUSSION

The in vitro fungicidal activities IVa–d, zinc pyrithione, and the known fungicide, captan, are shown in Table I. In general, the in vitro activities of the title compounds were found to be superior to those of captan, which was included for reasons of comparison. In addition, these compounds exhibited in vivo activity against rice blast (*Piricularia oryzae*) (Davis, 1981).

The overall pattern of activity for the (2-pyridylthio)-methyl benzoate *N*-oxides is similar to that for zinc pyrithione. This similarity suggests that the fungicidal properties of compounds IVa–d could be attributed to a release of the active moiety, 2-mercaptopyridine 1-oxide. This supposition is further substantiated by the observed loss of fungicidal activity (Table II) when the sulfur atom is oxidized to a sulfone [(2-pyridylsulfonyl)methyl benzoate *N*-oxide, V] or when the pyridyl nitrogen is deoxygenated [(2-pyridylthio)methyl 2-methylbenzoate, VI]. Curiously, when the entire pyridyl *N*-oxide group is replaced by phenyl, as in the case of (phenylthio)methyl benzoate, fungicidal activity is lost and postemergence herbicidal activity typical of thiophenols is gained (Bell, 1981). These limited structure–activity relationships thus provide additional evidence in support of a release mechanism involving hydrolysis of either the benzoate ester moiety, or more likely, the hemithioacetal linkage.

ACKNOWLEDGMENT

We thank John R. Kasinskas for the in vitro fungicidal screening.

Registry No. Ia, 98-88-4; Ib, 609-65-4; Ic, 933-88-0; Id, 100-07-2; IIa, 5335-05-7; IIb, 36893-95-5; IIc, 59874-33-8; IId, 75447-66-4; III, 3811-73-2; IVa, 84099-36-5; IVb, 84099-37-6; IVc, 84099-38-7;

IVd, 84099-39-8; V, 84099-40-1; VI, 84099-41-2.

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Received for review April 23, 1982. Revised manuscript received November 1, 1982. Accepted November 19, 1982.

Determination of Emodin in Feeds

Emodin from feeds was extracted into aqueous acetonitrile and partitioned into chloroform. After concentration of the chloroform extract, emodin was detected by thin-layer chromatography and quantitatively determined by high-performance liquid chromatography. Recoveries from various materials averaged from 80 to 90%.

The fungal pigment emodin (1,3,8-trihydroxy-6-methylanthraquinone) is widely distributed, occurring in the genera *Aspergillus* (Wells et al., 1975), *Cladosporium*, *Chaetomium*, *Penicillium*, and *Penicillopsis* (Shibata et al., 1964). Wells et al. (1975) and Kinosita and Shikata (1965) have reported on its toxicity. In these studies, it appeared that emodin acted synergistically with other fungal metabolites. Because of its toxicity and wide distribution, a simple method for its determination in agricultural commodities would be useful. Rai et al. (1975) developed a HPLC separation for emodin and other anthraquinones, while Danilovic and Naumovic-Stevanovic (1965) described a thin-layer method for some anthraquinones. These studies, however, did not attempt the analysis of an anthraquinone metabolite from a complex mixture. A procedure utilizing a liquid–liquid partition cleanup followed by thin-layer or liquid chromatography is described.

EXPERIMENTAL SECTION

Reagents and Equipment. Emodin was purchased from the Aldrich Chemical Co. (Milwaukee, WI); solvents were commercially supplied reagent or pesticide grades. Silica gel plates were prepared from silica gel G purchased from Sigma (St. Louis, MO). Sufficient water was added to the silica gel so that bubbles were not trapped in the slurry following shaking (about 30 g of silica gel to 70 mL of water). Layers 0.25 mm thick were spread on glass plates, allowed to air-dry, and then activated for about 2 h at 120 °C. Plates were kept in a cabinet over desiccant until used.

The liquid chromatograph was a Waters Model 6000 pump with the U6K injector (Waters Associates, Milford, MA) and a Beckman Model 153 ultraviolet absorbance detector. Columns used were 25 cm × 4.6 mm i.d. with 10-μm silica gel packing (Beckman Instruments, Fullerton, CA) and 10-μm C_{18} reversed-phase packing (Alltech Associates, Deerfield, IL).

Extraction. Fifty grams of the feed or ground grain was extracted with 250 mL of 80:20 acetonitrile–water either by blending for 5 min or by mechanical shaking for 30 min. The mixture was filtered through fluted filter paper and a measured amount of filtrate taken (e.g., 150 mL). The filtrate was partitioned twice with 100-mL portions of hexane, discarding the hexane layers. Seventy-five milliliters of water was added to 150 mL of filtrate, and the aqueous acetonitrile extract was partitioned with 2 × 50 mL portions of chloroform. The lower chloroform layers were combined and taken to dryness on a rotary evaporator. The residue was transferred to a graduated tube with 9:1 chloroform–methanol and brought to a desired volume, e.g., 0.5 or 1.0 mL. An aliquot was then taken for analysis by thin-layer or liquid chromatography. (**Caution**: good ventilation is required when using chloroform and acetonitrile.)

Chromatography. Aliquots of the extract were spotted on silica gel plates, previously described, along with standards of emodin dissolved in chloroform. Many solvent systems may be employed. The following systems were used in this work (with R_f values in parentheses): 50:50:2:1 hexane–chloroform–acetic acid–methanol (0.40), 88:12:1 chloroform–acetone–water and 100:1:1 chloroform–methanol–acetic acid (0.50). Emodin is visible to the naked eye in quantities as low as 0.2–0.5 μg as a yellowish orange spot. When exposed to ammonia vapor, the spot becomes pink and more intense. Emodin exhibited a yellowish orange fluorescence under short- and long-wave ultraviolet light. Detection is sometimes complicated by oily material in the extract, which alters the R_f value and may overload the plate capacity, but 1 or 2 ppm of emodin may usually be detected by thin-layer chromatography.

High-performance liquid chromatography (HPLC) may also be utilized in the determination of emodin, particularly for quantification. While Rai et al. (1975) used a pellicular silica with a cyclohexane–ethyl acetate gradient, microparticulate silica HPLC packings were used in this study. Solvents used were 80:20:1 methanol–water–acetic acid for reversed-phase separations, while 95:5:1 isooctane–2–propanol–acetic acid was used with silica gel columns. At the low part per million levels, monitoring at 254 or 280 nm with the sensitivity set at 0.08 absorbance unit full scale was satisfactory. On the reversed-phase system, 0.5 μg of emodin gave about 30% full-scale deflection with a retention of about 9 min, when a monitoring wavelength of 280 nm at 0.08 absorbance unit full scale was used. Detection limits by HPLC are estimated at

0021-8561/83/1431-0453$01.50/0