O,O-Dialkyl O-[p-(N-Alkylcarbamoyl)phenyl] Phosphorothionates: A Promising New Series of Toxicants for the Control of Imported Fire Ants

William E. McHenry,* Rose Mary Stiffin, Thomas H. Fisher, and Earl G. Alley

A series of O,O-dialkyl O-[p-(N-alkylcarbamoyl)phenyl] phosphorothionates were synthesized where, for the O,O-dimethyl derivatives, R = isopropyl, isobutyl, isoamyl, sec-butyl, n-amyl, and n-octyl and, for the O,O-diethyl derivatives, R = isopropyl, isobutyl, and sec-butyl. The toxicities of the phosphorothionates to the imported fire ant were determined for 1.0, 0.5, 0.1, and 0.05% solutions of toxicants in soybean oil in a prescreening laboratory test designed to measure delayed toxicity. Eight of the nine compounds tested exhibited delayed toxicity at one or more of the concentrations tested. Two of the compounds were screened against whole laboratory colonies of imported fire ants, and both compounds exhibited control at one or more of the concentrations tested.

The imported fire ants, *Solenopsis richteri* and *Solenopsis invicta*, continue to pose a serious infestation problem for the southeastern United States. Since the mid-1960s Mirex, a chlorinated hydrocarbon, was the recommended toxicant for control of this pest (Lofgren et al., 1964), but because of the discovery of Mirex residues in nontarget organisms (Ludke et al., 1971; Mehendal et al., 1972), the toxicity of Mirex to certain estuarine animals (Kaiser, 1974), and problems associated with its unusual stability in the environment (Alley, 1973), the Environmental Protection Agency canceled the registration of Mirex on June 30, 1978. Currently, tetrahydro-5,5-dimethyl-2(1*H*)-pyrimidinone, [3-[4-(trifluoromethyl)-phenyl]-1-[2-(4-(trifluoromethyl)phenyl]ethenyl]-2-propenylidine]hydrazone (Amdro), is the only registered bait toxicant that is recommended for the control of the imported fire ant (Williams et al. 1980). There is considerable interest in the development of alternative imported fire ant toxicants.

In order for a toxicant to be effective at controlling the imported fire ant, the toxicant must kill the queen. This is difficult because the queen is located far down the colony's food chain. Stringer et al. (1964) have described three requirements toxicants must meet in order to cause queen mortality. The requirements are (1) the toxicant should not be repellant to the ants when combined with a food attractant, (2) the toxicant must be transferable between ants, and (3) the toxicant must kill with delayed toxicity, preferably over as wide a range of concentrations as possible. U.S. Department of Agriculture scientists have developed a laboratory procedure for screening potential imported fire ant toxicants for delayed toxicity, and they have evaluated over 5000 chemicals as possible alternative imported fire ant toxicants with very few nonhalogenated toxicants exhibiting delayed toxicity (Lofgren et al., 1967).

We report here the synthesis of a series of O,O-dialkyl O-[p-(N-alkylcarbamoyl)phenyl] phosphorothionates and their evaluation as potential imported fire ant toxicants.

EXPERIMENTAL SECTION

Instrumentation and Equipment. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Infrared spectra (IR) were determined on a Perkin-Elmer Model 283B spectrophotometer. The IR spectra for solid samples were determined by incorporating the sample into a pellet of potassium bromide. Nuclear magnetic resonance (NMR) spectra were determined on a Varian Associates A-60 nuclear magnetic resonance spectrometer. Tetramethylsilane was used as the internal standard, and chloroform-*d* or acetone-d_6 was used as the solvent. Mass spectra data (MS, GLC–MS) were obtained by using the Hewlett-Packard Model 5930 mass spectrometer. The preparation of the phosphorothionates was conducted in a vented Labconco Carcinogen Box.

Materials. Phenyl *p*-hydroxybenzoate was obtained from Trans World Chemicals. All amines, sodium hydride and diethyl and dimethyl phosphorochloridothionate were obtained from Aldrich Chemical Co. Acetone was distilled over phosphorus pentoxide prior to use.

General Procedure for Preparation of N-Alkyl-p-hydroxybenzamides. A modification of the method reported by Spacht (1967) was utilized for the synthesis of the hydroxybenzamides. This method involves refluxing a solution composed of phenyl *p*-hydroxybenzoate (0.0234 mol) and 15 mL of a primary amine overnight. Much of the excess amine was removed from the reaction mixture via distillation under reduced pressure. The oily material that remained was then dissolved in 100 mL of chloroform and washed first with two 50-mL aliquots of 6 N HCl to remove any residual amine and then with two 50-mL aliquots of water to remove the phenol. After the solution was dried, with anhydrous sodium sulfate, the volume of solution was reduced to approximately 40 mL. In some cases crystals grew directly from this solution; in other cases ethyl ether was added to induce crystallization. The amides were then recrystallized from either chloroform or ethanol. The following are physical characteristics and yields of the isolated products. *N-Isopropyl-p-hydroxybenzamide* (**2a**): mp 160–161 °C (chloroform); 85% yield; ^{1}H NMR (acetone-d_6) δ 1.2 (d, 6 H), 4.2 (m, 1 H), 6.9 (d, 2 H), 7.8 (m, 3 H); mass spectrum *m/e* 179 (M^+). *N-Isobutyl-p-hydroxybenzamide* (**2b**): mp 114–115 °C (chloroform); 70% yield; ^{1}H NMR (acetone-d_6) δ 0.9 (d, 6 H), 1.8 (m, 1 H), 3.2 (t, 2 H), 6.8 (d, 2 H), 7.75 (m, 3 H); mass spectrum *m/e* 193 (M^+). *N-Isoamyl-p-hydroxybenzamide* (**2c**): mp 152–153 °C (chloroform); ^{1}H NMR (acetone-d_6) δ 0.9 (d, 6 H), 1.5 (m, 3 H), 3.4 (m, 2 H), 6.85 (d, 2 H), 7.75 (m, 3 H); mass spectrum *m/e* 207 (M^+). *N-sec-Butyl-p-hydroxybenzamide* (**2d**): mp 157–158 °C (chloroform); 70% yield; ^{1}H NMR (acetone-d_6) δ 0.9 (t, 3 H), 1.2 (d, 3 H), 1.6 (m, 2 H), 4.1 (m, 1 H), 6.9 (d, 2 H), 7.8 (m, 3 H); mass spectrum *m/e* 193 (M^+). *N-n-Amyl-p-hydroxybenzamide* (**2e**): mp 108–110 °C (ethanol); 84% yield; ^{1}H NMR (acetone-d_6) δ 0.93 (5 H), 1.4 (m, 4 H), 3.51 (m, 2 H), 6.8 (d, 2 H), 7.8 (m, 3 H); mass spectrum *m/e* 207 (M^+).

Department of Chemistry, Mississippi State University, Mississippi State, Mississippi 39762 (W.E.M., R.M.S., and T.H.F.), and Mississippi State Chemical Laboratory Mississippi State, Mississippi 39762 (E.G.A.).

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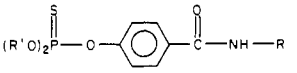
Table I. Fire Ant Percent Mortality Data for Several Concentrations of Phosphorothionates (3) in Soybean Oil



3	R'	R	1% day 1	1% day 14	0.5% day 1	0.5% day 14	0.1% day 1	0.1% day 14	0.05% day 1	0.05% day 14	toxicity[a] class
a	Me	*i*-Pr	98	100	93	100	22	98	2	93	III_b
b	Me	*i*-Bu	50	100	0	100	0	100	0	72	III_b
c	Me	*i*-Am	2	100	0	98	3	53	3	18	III_a
d	Me	*sec*-Bu	93	100	78	100	7	100	0	77	III_b
e	Me	*n*-Am	2	98	2	97	2	87	2	62	III_b
f	Me	*n*-Oct	0	8	0	12	0	8	0	0	I_a
g	Et	*i*-Pr	65	100	13	100	0	100	0	0	III_b
h	Et	*i*-Bu	0	100	0	100	0	92	0	42	IV
i	Et	*sec*-Bu	7	77	0	97	0	68	0	77	III_a

[a] Refer to Lofgren's imported fire ant toxicant classification system (Banks et al., 1977).

N-n-Octyl-p-hydroxybenzamide (**2f**): mp 62 °C (ethanol); 70% yield; ^1H NMR (acetone-d_6) δ 0.9 (m, 3 H), 1.35 (br s, 12 H), 3.2 (m, 2 H), 6.85 (d, 2 H), 7.8 (m, 3 H); mass spectrum *m/e* 249 (M^+).

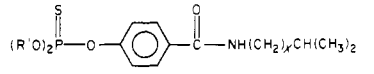
Phosphorothionate Synthesis. The *O,O*-dimethyl (and *O,O*-diethyl) *O*-[*p*-(*N*-alkylcarbomoyl)phenyl] phosphorothionates were synthesized by a modification of previously described method (Lorentz et al., 1971; Welch and Waters, 1978). A suspension of sodium hydride (0.006 mol, 0.144 g) in 3 mL of acetone was treated with *N*-alkyl-*p*-hydroxybenzamide (0.003 mol) dissolved in 3 mL of acetone. The resulting mixture was stirred for 15–30 min in an ice bath. Dimethyl (diethyl) phosphorochloridothionate (0.003 mol) was added dropwise to the mixture. The ice bath was removed and the mixture was stirred at room temperature for 60–90 min. The reaction mixture was then transferred to a 10-mL concentrator tube, and the solvent was removed by blowing a stream of nitrogen over the sample while heating at 35 °C. The reaction mixture was dissolved in ethyl ether and extracted with three 25-mL portions of water followed by extraction with 25-mL portions of 2% aqueous sodium carbonate.

After being dried over sodium sulfate, the solution was filtered, and the ether was removed by heating (35 °C) and blowing a stream of dry nitrogen over it. The tube containing the product was then connected to a vacuum pump, and unreacted dialkyl phosphorochloridothionate and any remaining ether were removed by heating (50 °C) under reduced pressure (180 mtorr). The product was then dissolved in chloroform-*d* with tetramethylsilane as an internal standard, and the NMR spectrum was run. The structure of each phosphorothionate **3** reported here was consistent with the IR and NMR spectra obtained for it. In each case the NMR spectrum indicated above 90% purity and the phosphorothionates were used without further purification.

Toxicity Test. The primary laboratory screening procedures used to evaluate the bait toxicants were described by Williams et al. (1980). The tests were designed to screen new toxicants for delayed toxicity. Each toxicant was tested at 1.0, 0.5, 0.1, and 0.05% concentrations in soybean oil (sbo). The results of these studied are shown in Table I.

Representative compounds were subjected to a secondary screening test that evaluates the toxicity of the compounds to whole colonies of ants. This test, described by William et al. (1980), involved dissolving the compound to be tested in soybean oil and feeding the bait to the ants either directly or after impregnation on a granular carrier (extruded corn pellets; Quaker Oats Co., Chicago, IL). The ants had access to the bait for 96 h. The bait was then removed and the colony fed honey–water (1:1) and Banks' diet. General observations on the status of the colony and mortality were recorded weekly. The test were continued until the queen, brood, and 90% or more the workers were dead or until the colony recovered and returned to normal. The latter recovery condition was considered to be in effect after the queen resumed egg laying and all stages of brood were present. The results of these studies are shown in Table II.

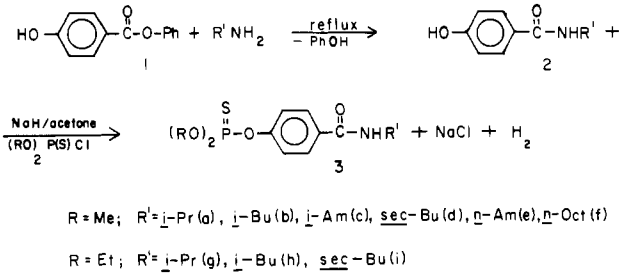
Table II. Mortality Data for Phosphorothionates



3	R'	X	π^a	rel toxicities[b]	delayed toxicities[c]
a	Me	0	1.53	98	0.1-0.05
b	Me	1	1.99	50	0.5-0.1
c	Me	2	2.54	2	1.0-0.5
g	Et	0	1.53	65	0.5-0.1
h	Et	1	1.99	0	1.0-0.1

[a] Calculated values for the *N*-alkyl groups by assuming additivity of the following groups: CH_3, 0.56; CH_2, 0.45; CH, 0.41; C, 0.30. [b] The additivity method of Leo et al. (1975) was used. [b] Relative toxicities are based on mortality data for day 1 at 1.0% toxicant in soybean oil. [c] Delayed toxicities are given in concentrations at which this phenomenon occurs.

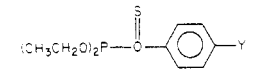
Scheme I



RESULTS AND DISCUSSION

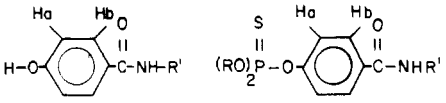
An outline of the synthetic pathway followed for the synthesis of the nine phosphorothionates used in this study is given in Scheme I. The *N*-alkyl-*p*-hydroxybenzamides (**2**) were synthesized via aminolysis of phenyl *p*-hydroxybenzoate (Spacht, 1967). Structural assignments were based on NMR, IR, and mass spectral data and yields ranged from 32 to 82%. The *N*-alkyl-*p*-hydroxybenzamides were converted to *O,O*-dialkyl *O*-aryl phosphoro-

Table III. A Comparison of the Effects Ester and Amide Substituents Have on the Toxicities of Phosphorothionates to the Imported Fire Ant



Y	1% day 1	1% day 14	0.1% day 1	0.1% day 14	reference
4-CO_2-*i*-Pr	98	100	18	75	Fisher et al. (1980)
4-CONH-*i*-Pr	65	100	0	100	this paper
4-CO_2-*i*-Bu	100	100	2	42	Fisher et al. (1980)
4-CONH-*i*-Bu	0	100	0	92	this paper

thionates (3) by treating the phenols with sodium hydride (NaH) in anhydrous acetone to prepare the phenoxide ions and then adding the phosphorochloridothionate to this solution. Since the phenoxide ion is a much better nucleophile than phenol, it was not necessary to heat or add a base catalyst to the mixture. This procedure was followed in order to minimize the thiono–thiolo rearrangement (Eto, 1974). Structures of the phosphorothionates were assigned from their NMR spectra. The hydrogens ortho to the phenolic hydroxy group experienced between a 0.2- and 0.3-ppm downfield shift when the hydroxy group was phosphorylated.



H_b: 7.7–7.8 ppm H_a: 6.8–6.9 ppm

H_b: 7.77–7.8 ppm H_a: 7.1–7.15 ppm

The most critical of the three criteria for an effective imported fire ant toxicant is delayed toxicity (Stringer et al., 1964). Delayed toxicity is defined as less than 15% mortality on day 1 and greater than 90% mortality on day 14. Very little is known about the physiochemical nature of delayed toxicity. Fisher et al. (1980) evaluated a series of *O,O*-diethyl *O*-(carboalkyloxyphenyl) phosphorothionates (4) as potential imported fire ant toxicants and observed that the compounds killed too fast at high concentrations (1.0% toxicant in soybean oil) and exhibited too little toxicity at low concentrations (0.01% toxicant in soybean oil); phosphorothionates 4 (R = Me or *i*-Pr) showed delayed kill at several concentrations between 1.0% and 0.1% toxicant in soybean oil. At the 0.1% toxicant in soybean oil concentration, their toxicity was very dependent on the lipophilicity of the carboalkoxy group with the least lipophilic compounds (shorter chain alkoxy groups) exhibiting the highest mortality. From the results of the QSAR study on *O,O*-diethyl *O*-(carboalkoxyphenyl) phosphororthionates, it appeared that phosphorothionates that have substituents attached to the aryl group with similiar electronic properties but with less lipophilic character than esters may show increased delayed toxicity (Fisher et al., 1980).

Hammett σ and Hansch π (Hansch et al., 1973) values were used to compare electronic and lipophilic properties of our phosphorothionates. Nine *O,O*-dialkyl *O*-[*p*-(*N*-alkylcarbamoyl)phenyl] phosphorothionates were synthesized that had enough structural variation to test our hypothesis on delayed toxicity. The amide group was chosen because (1) amides are not as susceptible to hydrolysis as esters [this hydrolysis is a detoxification reaction that may become important at low concentration (O'Brien, 1960)], (2) amides are not as lipid soluble as the corresponding esters [for example, Hansch π values for the carbomethoxy and the *N*-methylcarbamoyl groups are −0.01 and −1.64, respectively (Hansch et al., 1973); this indicates that the carbomethoxy group is 46.7 times more lipid soluble than the *N*-methylcarbamoyl], and (3) amides and esters have similar electronic properties [the Hammett σ values for the carbomethoxy and the *N*-methylcarbamoyl groups are 0.45 and 0.36, respectively (Hansch et al., 1973)].

The mortality data for the nine phosphorothionates made in this study are given in Table I. The mortality data is given only for day 1 and day 14 for each toxicant at four concentrations in soybean oil. More attention was given to the isoalkyl groups than the *n*-alkyl groups because Fisher et al. (1980) observed that the isopropyl derivative exhibited better delayed toxicity than long straight chain alkyl groups in the carboalkoxyphenyl phosphorothionates. Five of the nine phosphorothionates synthesized for this study had *N*-isoalkyl groups. The data for the "*N*-isoalkyl" series are shown in Table II. Within these homologous series of (*N*-isoalkylcarbamoyl)phenyl phosphorothionates, the toxicity was inversely proportional to lipophilicity at 1% toxicant in soybean oil at day 1, with the least lipophilic derivatives exhibiting the greatest toxicity to the imported fire ant. The concentration at which delayed toxicity was first observed in the series was directly proportional to lipophilicity. The most lipophilic toxicant in both series exhibited delayed toxicity at the highest concentration tested. For **3c**, the delayed toxicity occurred over a 2-fold dosage range, whereas for **3h**, the delayed toxicity occurred over a 10-fold dosage range. The isomeric

Table IV. Mortality in Laboratory Colonies of Red Imported Fire Ants Treated with Indicated Concentration of Toxicants 3e and 3g in Soybean Oil

compound	% concn of 3 in soybean oil	mortality, %, in each colony at indicated no. of weeks posttreatment[a] 1	2	3	4	5	6	7	8
3e	0.5	3	5	8	10CN				
	0.5	7	8	40	40CR				
	1.0	60	85	87	87	87	87	87	87CR
	1.0	80D							
	1.0	90	94	96	97	97	97	97	97CR
	1.0	65	85	92	93D				
3g	0.1	40	45	46	55	55	60	61	62CR
	0.1	40	65	66	68	80	81	81	81CR
	0.5	65	90	92	98D				
	0.5	50	65	65	68	68	69	69	69CR
	1.0	40	60	75	95	96	96	96	96D
	1.0	75D							
Amdro	2.5	20			68D				

[a] D = colony dead; CR = colony appears to be recovering; CN = colony normal.

sec-butyl (**3d**) and isobutyl derivatives (**3b**) exhibited similar toxic behavior to the imported fire ants in the prescreening test.

The *O,O*-dialkyl *O*-[*p*-(*N*-alkylcarbamoyl)phenyl] phosphorothionates exhibited uncommon delayed toxicity for organophosphorous compounds. Of the approximately 800 organophosphorous compounds that have been evaluated as possible imported fire ant toxicants, only 10 have exhibited delayed toxicity. All 10 are halogenated phosphorothionates (Banks et al., 1977). A comparison of the ester phosphorothionate derivatives (**4**) to the amide derivative (**3**) (Table III) indicates that the toxicities of the 0.1% solutions of the amides were only slightly more toxic than those of corresponding ester derivatives to the imported fire ant, but substitution of the amide group for a ester group greatly enhanced the delayed killing property of the phosphorothionates.

Phosphorothionates **3b** and **3e** exhibited delayed toxicity over almost a 10-fold concentration range while **3h** (R′ = Et; R = *i*-Bu) turned out to be one of the few known *class iv* (Banks et al., 1977) imported fire ant toxicants. Phosphorothionates **3e** and **3g** were chosen to undergo evaluation in the secondary screening tests against whole colonies of imported fire ants. This test should reveal any inherent difficulties in using these compounds as imported fire ant toxicants, such as lack of bait acceptance or poor distribution of the bait throughout the mound. In these tests the chemicals were offered to the ants in soybean oil impregnated on an extruded corn pellet carrier (5 g/colony) for 4 days, and a special laboratory diet was fed for the rest of the test. General observations on the status of the colony and mortality were recorded weekly. The test was continued until the queen, brood, and 90% or more of the workers were dead or until the colony recovered and returned tonormal (Williams et al., 1980). The results of this test are given in Table IV. Both baits were able to control the imported fire ant colonies at the 1% toxicant in soybean oil concentration. Toxicant **3g** baits were, as expected, considerably more toxic than those of **3e**. The former compound caused considerable ant mortality even at the 0.5% and 0.1% toxicant in soybean oil levels. The baits were acceptable to the imported fire ant, and no problems surfaced that would indicate any difficulty in the bait being distributed throughout the colony.

The data presented in this report provides evidence that *O,O*-dialkyl *O*-[*p*-(*N*-alkylcarbamoyl)phenyl phosphorothionates are a very promising new series of imported fire ant toxicants that may provide a viable alternative chemical method for controlling the imported fire ant.

ACKNOWLEDGMENT

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Degradation of the Toxicant AC 217,300 in Amdro Imported Fire Ant Bait under Field Conditions

Robert K. Vander Meer,* David F. Williams, and Clifford S. Lofgren

The rate of decomposition of AC 217,300 [tetrahydro-5,5-dimethyl-2(1*H*)-pyrimidinone [3-[4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethenyl]-2-propenylidene]hydrazone], the active component of Amdro fire ant bait, was determined under ambient summer climatic conditions. Samples analyzed by GLC showed rapid decomposition during daylight hours but no decomposition during evening hours. No thermal decomposition was detected in the absence of light; therefore, the decomposition was attributed to photolysis. Concurrent bait toxicity bioassays corroborated the results obtained from the chemical studies. Our results indicate that the time of application may influence the efficacy of Amdro fire ant bait.

The red imported fire ant (RIFA), *Solenopsis invicta*, Buren, infests large areas of nine Southeastern and Southern states and has been the subject of extensive control efforts since 1957 (Lofgren et al., 1975). From 1962 to 1978 mirex bait was the method of choice for control of RIFA; however, in 1978 the Environmental Protection Agency canceled the registration of mirex because it was a possible carcinogen. The main reason for the paucity

Insects Affecting Man and Animals Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, Florida 32604.