These data support a structure in which oxidation of the ring at the 2 position is followed by ring scission to yield an aldehyde. Formation of E-8 may be depicted by the reaction sequence which follows:

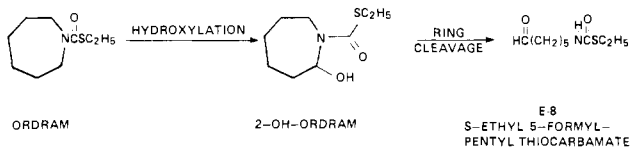

Figure 13 shows the proposed metabolism of [^{14}C]Ordram by the rat based on the identification of metabolites present in 0–48 h urine. The major route of metabolism appears to involve sulfoxidation and conjugation with glutathione to ultimately give rise to the mercapturic acid derivative.

This mercapturic acid accounted for 35.4% of the urinary ^{14}C in the present study as compared to only 2% in the study reported by Hubbell and Casida (1977). This discrepancy may be due to the different routes of administration (oral vs. ip) and/or doses (72 vs. 187 mg/kg) employed for the two studies.

No metabolites which cochromatographed with the Ordram-glutathione or Ordram-cysteine derivatives were detected in these studies. Inclusion of Ordram sulfoxide in this pathway is based on studies by Casida et al. (1975a, b) and Lay et al. (1975). The sulfoxide can also presumably undergo hydrolysis to yield HMI.

Another significant degradative pathway involves ring hydroxylation to yield the 2-, 3-, and 4-hydroxy derivatives, excreted predominantly as the glucuronide conjugates. No sulfate conjugates were detected.

Two basic metabolites which cochromatographed with hydrolysis products from 3- and 4-hydroxy-Ordram were identified as 3- and 4-hydroxy-HMI. As indicated above, sulfoxidation is presumed to occur prior to liberation of the hydroxyimine moiety. Another minor metabolite which appears to be a cyclic carbamate may be formed by internal rearrangement of 3-hydroxy-Ordram or its sulfoxide.

The data show that Ordram is readily degraded by the rat to more polar products which are excreted primarily in the urine. Only 0.1% of the urinary ^{14}C was identified as unchanged Ordram after an oral dose of 72 mg/kg. Major metabolic pathways include sulfoxidation, conjugation with glutathione, ring hydroxylation, and liberation of the unmodified and hydroxylated cyclic imine moiety.

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Insecticidal Properties of Phosphonamidothioate Esters and Derivatives

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A series of *S*-alkyl methyl- or ethylphosphonamidothioate esters analogous to methamidophos (*O,S*-dimethyl phosphoramidothioate) and acephate (*O,S*-dimethyl *N*-acetylphosphoramidothioate) was synthesized and evaluated for toxicological properties. Although *S*-methyl methyl- and ethylphosphonamidothioate were more effective against the housefly than methamidophos, the acylated derivatives showed variable toxicity but all were substantially less effective than acephate. The acylated derivatives, however, showed improved mouse toxicity. *S*-Methyl methyl- and ethylphosphonamidodithioate were less active against the housefly than methamidophos.

Acephate or *O,S*-dimethyl *N*-acetylphosphoramidothioate is one of the most interesting new insecticides discovered during the past decade (Magee, 1974). Compared to many organophosphorus insecticides, acephate has the virtue of being relatively simple in structure and of low mammalian toxicity (rat oral LD_{50} = 900 mg/kg). On the other hand, methamidophos (*O,S*-dimethyl phosphoramidothioate), a compound which differs from acephate only in the replacement of the acetyl moiety by a hydrogen atom, is relatively toxic to mammals (rat oral LD_{50} = 20 mg/kg) although of about equal insecticidal activity. Recent studies have indicated that acephate and related esters are converted in vivo to methamidophos in insects and it is methamidophos which is responsible for intoxication (Kao and Fukuto, 1977; Khasawinah et al., 1978). In mammals, relatively little methamidophos is formed, thus accounting for the safety of acephate.

In an earlier study (Quistad et al., 1970), we described the outstanding insecticidal activity of a few phosphon-

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amidothioate esters related to methamidophos. For example, *S*-ethyl ethylphosphonamidothioate with a housefly LD_{50} of 0.64 μg/g was about twofold more toxic to the housefly than methamidophos. Unfortunately, this compound was also about twofold more toxic to the white mouse. Because of the high degree of selectivity achieved by converting methamidophos to acephate, it was of interest to examine the effect of acylation on the mammalian toxicity and insecticidal activity of *S*-alkyl alkylphosphonamidothioate esters. This report is concerned with the synthesis and evaluation of the toxicological properties of a series of acylated *S*-alkyl alkylphosphonamidothioates and some related esters.

MATERIALS AND METHODS

Synthesis. *O*-Alkyl alkylphosphonamidothioates were prepared by the reaction between the appropriate *O*-alkyl alkylphosphonochloridothioate (Hoffman et al., 1958) and ammonia (Quistad et al., 1970). The corresponding *N*-methyl or *N,N*-dimethyl analogues were prepared by reacting the chloridothioate with methyl- or dimethylamine. The *O*-alkyl alkylphosphonamidothioates were converted to the corresponding *S*-alkyl alkylphosphonamidothioates (**1–7**) by removing the *O*-alkyl moiety and realkylating with methyl *p*-toluenesulfonate or triethyloxonium fluoroborate according to Magee (1974). In some cases (**8–10**) the *S*-alkyl alkylphosphonamidothioates were obtained by isomerization of the thionates by reaction with iodomethane (Quistad et al., 1970).

The *O*-alkyl *N*-acyl-*P*-alkylphosphonamidothioates were prepared through the *N*-trimethylsilyl intermediate according to the following example. To a stirred mixture of 10 g of *O*-methyl ethylphosphonamidothioate, 8.7 g of triethylamine, and 50 mL of dry benzene was added 9.4 g of trimethylchlorosilane at room temperature and under a nitrogen atmosphere. The mixture was heated at reflux for 1 h and filtered, and the intermediate *O*-methyl *N*-trimethylsilyl-*P*-ethylphosphonamidothioate was distilled, bp 65–75 °C (0.2 mm), in about 50% purity. This intermediate was then heated at reflux with an equivalent amount of acetyl chloride for 1 h, the mixture was filtered and distilled to give impure *O*-methyl *N*-acetyl-*P*-ethylphosphonamidothioate, bp 110–112 °C (0.4 mm), n^{25}_D 1.5192. After washing an ether solution of the impure material several times with water, redistillation at 95–96 °C (0.15 mm) gave 7.6 g of final product: n^{25}_D 1.5179; NMR (CCl_4, δ, Me_4Si) 7.45 (bd, 1 H, NH, J_{P-H} = 10 Hz), 3.69 (d, 3 H, OCH_3, J_{P-H} = 15 Hz), 2.37 (bm, 2 H, PCH_2), 2.13 (d, 3 H, $COCH_3$, J = 2 Hz), 1.18 (t, 3 H, CH_2CH_3, J_{P-H} = 24 Hz). Other *O*-alkyl *N*-acyl-*P*-alkylphosphonamidothioates were prepared similarly except the intermediate trimethylsilyl derivatives were not isolated. *O*-Methyl *N*-hexanoyl-*P*-ethylphosphonamidothioate was purified by column chromatography, using SilicAR (Mallinckrodt) and chloroform–hexane (9:1) as solvent. All other acylated *O*-methyl phosphonamidothioates were purified by distillation.

S-Alkyl *N*-acyl-*P*-alkylphosponamidothioates (**11–20**) were prepared in the same manner as the *S*-alkyl alkylphosphonamidothioates by O-dealkylation and S-alkylation of the *O*-methyl *N*-acyl-*P*-alkylphosphonamidothioates using sodium benzenethiolate as the dealkylating agent and methyl *p*-toluenesulfonate or triethyloxonium fluoroborate as the alkylating agent.

O-Methyl *N*-(2-tetrahydropyranyl)-*P*-methylphosphonamidothioate was prepared as follows. To a mixture of 10 g of *O*-methyl methylphosphonamidothioate and 6.4 g of 2,3-dihydropyran in 50 mL of benzene was added a few drops of hydrogen chloride saturated ether. After allowing the mixture to stand overnight the product was distilled: bp 108–10 °C (0.03 mm); mp 49–50 °C; NMR ($CDCl_3$, δ, Me_4Si) 4.81–3.26 (m, 4 H, P-N$HCHOCH_2$), 3.62 (d, 3 H, OCH_3, J_{P-H} = 13 Hz), 1.85 (d, 3 H, P-CH_3, J_{P-H} = 16.1 Hz), 1.56 (m, 6 H, pyranyl-CH_2). The *O*-methyl *N*-(2-tetrahydropyranyl)-*P*-methylphosphonamidothioate was converted to the corresponding *S*-methyl analogue (**21**) by reaction with iodomethane as previously described (Quistad et al., 1970).

The preparation of the *S*-methyl alkylphosphonamidodithioates (**22–25**) was carried out by using methyl- or ethylthionophosphine sulfide (Newallis et al., 1962) as the starting material. *S*-Methyl methyl- or ethylphosphonodithioic acid was prepared by reacting methanethiol with the appropriate thionophosphine sulfide (Schrader, 1963), which was then converted to the respective *S*-methyl methyl- or ethylphosphonochloridodithioate by the action of sulfuryl chloride (Chupp and Newallis, 1962). Treatment of the chloridodithioate with ammonia or amine in the usual manner gave the *S*-methyl methyl- or ethylphosphonamidodithioates **22–25**.

The structures of all intermediates and final compounds listed in Table I and Table II were verified by NMR using a Varian T-60 spectrometer and, in many cases, by mass spectral analysis using a Finnigan Model 1015 mass spectrometer, interfaced with a digital computer controlled data acquisition and reduction system (System-150). Elemental analyses were by C. F. Geiger, Ontario, Calif.

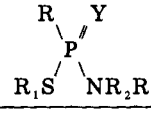
Toxicological Evaluation. Housefly toxicity was determined on the susceptible NAIDM strain, *Musca domestica*, by topical application according to previously described methods (March and Metcalf, 1949). Synergized toxicity was determined by co-treating houseflies with a constant dosage of 500 μg/g of piperonyl butoxide. Mouse toxicity was determined according to Hollingworth et al. (1967). Three- to 6-month-old Swiss white mice (about 25 g/mouse) were treated orally using water or propylene glycol as the carrying agent. The highest dosage tested was 100 mg/kg.

RESULTS AND DISCUSSION

Data for the toxicity of the various *S*-alkyl alkylphosphonamidothioates and derivatives are presented in Table II. The data show that highest toxicity to houseflies is associated with the *S*-methyl or *S*-ethyl *P*-alkylphosphonamidothioates containing small *P*-alkyl moieties (**1**, **4**, **5**, and **6**). Increasing the chain length of the *P*-alkyl moiety resulted in abrupt reduction in housefly toxicity with the *P*-butyl analogue **10**. Branching in the *P*-alkyl moiety drastically reduced housefly toxicity and the *P*-isopropyl analogue **9** was devoid of insecticidal activity. As in the case of the *S*-alkyl *O*-alkyl phosphoramidothioates, substitution on the nitrogen atom greatly reduced insecticidal activity of the *S*-methyl *P*-methylphosphonamidothioates (compare **1**, **2**, and **3**) and the *N,N*-dimethyl analogue **3** was insecticidally inactive. On the other hand, the *N,N*-dimethyl analogue of the *S*-ethyl *P*-ethylphosphonamidothioate (**7**) with a housefly LD_{50} of 60 μg/g was moderately active. It was, however, about 100-fold less effective than the unsubstituted compound **6**.

The outstanding housefly toxicity of the simple *S*-alkyl alkylphosphonamidothioates prompted us to examine the toxicological properties of their acylated derivatives. Considerable difficulty was initially experienced in the synthesis of these compounds (**11–20**), particularly with the acylation reaction. Methods commonly used for the acylation of *O,O*-dialkyl phosphoramidothioates (Magee, 1974) were unsuccessful with the phosphonamidothioates. Acylation eventually was achieved by proceeding through

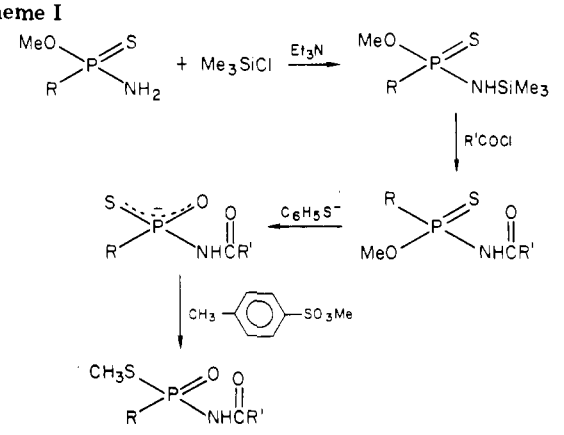
Table I. Physical Properties of *S*-Alkyl Phosphonamidothioates and Related Derivatives



no.	R	R_1	R_2	R_3	Y	mp, °C	bp, °C/mm	$n^{25}D$	analysis theory	analysis found
1	Me	Me	H	H	O	76-78			C 19.20	C 19.09
									H 6.44	H 6.83
2	Me	Me	H	Me	O	40-42			C 25.90	C 26.42
									H 7.19	H 7.27
3	Me	Me	Me	Me	O	46-48			C 31.36	C 31.81
									H 7.90	H 7.65
4	Me	Et	H	H	O	44-45			C 25.90	C 26.19
									H 7.19	H 7.31
5	Et	Me	H	H	O	65-67[a]				
6	Et	Et	H	H	O	46-48[a]				
7	Et	Et	Me	Me	O		76/0.3[a]	1.4959		
8	*n*-Pr	Me	H	H	O	65-67			C 31.37	C 32.47
									H 7.89	H 8.68
9	*i*-Pr	Me	H	H	O	60-62			C 31.37	C 32.26
									H 7.89	H 7.45
10	*n*-Bu	Me	H	H	O	94-96			C 35.91	C 36.29
									H 8.44	H 8.02
11	Et	Me	H	$C(O)CH_3$	O	59.5-61			C 33.14	C 33.39
									H 6.68	H 6.86
12	Et	Et	H	$C(O)CH_3$	O	48-50			C 36.92	C 37.09
									H 7.23	H 7.51
13	Et	Me	H	$C(O)C_2H_5$	O			1.5104[b]	C 36.92	C 36.31
									H 7.23	H 7.55
14	Et	Et	H	$C(O)C_2H_5$	O	70-71.5			C 40.18	C 40.07
									H 7.71	H 7.84
15	Et	Et	H	C(O)-*n*-C_3H_7	O	43-44			C 43.04	C 42.73
									H 8.13	H 8.74
16	Et	Me	H	C(O)-*i*-C_3H_7	O	86-86.5			C 40.18	C 42.38
									H 7.71	H 8.10
17	Et	Et	H	C(O)-*i*-C_3H_7	O	77-78			C 43.04	C 43.81
									H 8.13	H 8.58
18	Et	Me	H	C(O)-*n*-C_4H_9	O	42-44			C 43.04	C 43.74
									H 8.13	H 9.29
19	Et	Et	H	C(O)-*n*-C_5H_{11}	O			1.4942	C 47.79	C 47.41
									H 8.82	H 8.51
20	Et	Me	H	$C(O)CH{=}CMe_2$	O	92-93			C 43.43	C 44.41
									H 7.29	H 7.83
21	Me	Me	H	$\overline{CHO(CH_2)_2CH_2}$	O	98-100			C 40.19	C 39.79
									H 7.65	H 7.97
22	Me	Me	H	H	S		91/0.025	1.6250	C 17.01	C 17.44
									H 5.71	H 5.88
23	Et	Me	H	H	S		90-91/0.05	1.6086	C 23.21	C 23.67
									H 6.49	H 6.49
24	Et	Me	H	Me	S		96/0.05	1.5846	C 28.39	C 28.95
									H 7.15	H 6.88
25	Et	Me	Me	Me	S		54.5/0.025	1.5636	C 32.76	C 32.89
									H 7.70	H 7.58

[a] Quistad et al. (1970). [b] Purified by column chromatography using silica gel and chloroform-methanol as solvent.

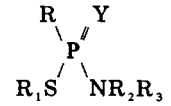
Scheme I



the *N*-trimethylsilylphosphonamidothioate intermediate according to Scheme I. O-Demethylation and realkylation with either methyl *p*-toluenesulfonate or triethyloxonium fluoroborate gave the desired products.

In general, acylation of the *S*-methyl or *S*-ethyl methyl- and ethylphosphonamidothioates resulted in compounds **11–20** which were relatively ineffective against houseflies compared to the parent phosphonamidothioates. Whereas acylation of methamidophos with the acetyl or propionyl moieties gave products, e.g., acephate, which were equal to methamidophos in insecticidal activity, acylation of the phosphonamidothioates gave products of widely varying activity, i.e., from 4.5 to >500 μg/g. Acylation of *S*-methyl ethylphosphonamidothioate gave products of higher housefly toxicity than the corresponding *S*-ethyl derivatives (compare **11** with **12**, **13** with **14**, and **16** with **17**) even though the parent *S*-ethyl ethylphosphonamidothioate was slightly more active than the *S*-methyl analogue. *S*-Methyl *N*-propionyl-*P*-ethylphosphonamidothioate (**13**) with a housefly LD_{50} of 4.5 μg/g showed the highest activity of

Table II. Toxicological Properties of Phosphonamidothioates and Derivatives



no.	R	R_1	R_2	R_3	Y	housefly LD_{50}, μg/g alone	housefly LD_{50}, μg/g +PB[a]	mouse LD_{50}, mg/kg
1	Me	Me	H	H	O	1.0	0.63	10
2	Me	Me	H	Me	O	205	120	
3	Me	Me	Me	Me	O	>500		>100
4	Me	Et	H	H	O	0.65		
5	Et	Me	H	H	O	0.81[b]	0.47	16
6	Et	Et	H	H	O	0.64[b]		16[b]
7	Et	Et	Me	Me	O	60[b]		
8	*n*-Pr	Me	H	H	O	4.5	3.0	30
9	*i*-Pr	Me	H	H	O	>500		>100
10	*n*-Bu	Me	H	H	O	52		35
11	Et	Me	H	$C(O)CH_3$	O	52	19.7	>100
12	Et	Et	H	$C(O)CH_3$	O	153	33	>100
13	Et	Me	H	$C(O)C_2H_5$	O	4.5		>100
14	Et	Et	H	$C(O)C_2H_5$	O	150	3.8	>100
15	Et	Et	H	C(O)-*n*-C_3H_7	O	92	15.5	>100
16	Et	Me	H	C(O)-*i*-C_3H_7	O	114	73	>100
17	Et	Et	H	C(O)-*i*-C_3H_7	O	>500	99	
18	Et	Me	H	C(O)-*n*-C_4H_9	O	87	18	>100
19	Et	Et	H	C(O)-*n*-C_5H_{11}	O	350	52	
20	Et	Me	H	$C(O)CH{=}CMe_2$	O	>500	>500	>100
21	Me	Me	H	$CHO(CH_2)_2CH_2$	O	45	11	<10
22	Me	Me	H	H	S	15.8	8.5	
23	Et	Me	H	H	S	11.5		55
24	Et	Me	H	Me	S	250		>100
25	Et	Me	Me	Me	S	>500		>100

[a] Piperonyl butoxide was applied at a constant dose of 500 μg/g. [b] From Quistad et al. (1970).

the acylated derivatives but it was more than fivefold less active than the parent compound.

In some cases toxicity of the acylated derivatives to houseflies was increased substantially by co-treatment with piperonyl butoxide, e.g., **14** was synergized about 40-fold from 150 to 3.8 mg/kg. This suggests that variability in toxicity of the acylated derivatives may be attributable in part to variability in their susceptibility to oxidative detoxication.

Although the acylated derivatives were poorer insecticides than the unacylated compounds, without exception acylation resulted in significant improvement in mammalian toxicity. None of the acylated derivatives was toxic to mice at 100 mg/kg, which was the highest dosage tested. In comparison, the mouse LD_{50} of **5** and **6**, the parent phosphonamidothioate, was 16 mg/kg. In contrast to the acylated phosphonamidothioates, the single *N*-pyranyl derivative **21** was more toxic to mice than the parent phosphonamidothioate **1**. This was not surprising owing to the greater lipophilicity of **21** (Kao and Fukuto, 1977) and the likelihood of its reversion to the parent phosphonamidothioate in the acidic environment of the mouse's stomach. Since improvement in mammalian safety and insecticidal activity was not achieved, other *N*-pyranyl derivatives were not examined.

S-Methyl phosphonamidodithioate analogues were synthesized and examined for toxicological properties (**22**–**25**). While **22** and **23** were moderately toxic to houseflies, they were less effective than the corresponding phosphonamidothioates (**1** and **5**). Substitution on the nitrogen atom also resulted in drastically reduced housefly toxicity.

One of the acylated derivatives (**13**) was examined for systemic activity in the cotton plant (Wustner and Fukuto, 1973). Laboratory tests revealed that **13** gave 6 weeks control of the cotton aphid (*Aphis gossypii*) but was ineffective against the mite (*Tetranychus cinnabarinus*), perforator (*Bucculatrix thurberiella*), and salt marsh caterpillar (*Estigmene acrea*). Under the same conditions acephate effectively controlled mites, aphids, and perforators for a period of 4–7 weeks. Overall, the acylated phosphonamidothioates were insecticidally disappointing.

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