Altering Translocation Characteristics of Methamidophos by N-Acylation with Dicarboxylic Half-Acid Esters

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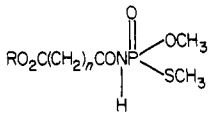
The phloem mobility of methamidophos is increased when the organophosphorus insecticide is acylated with half-acid esters of short-chain dicarboxylic acids. Several derivatives were synthesized and bioassayed. Toxicity of each derivative to cabbage looper larvae [*Tricoplusia ni* (Hübner)] was measured and phloem transport in soybeans (*Glycine max* L.) assessed. Ethanedioic acid, 1-phosphoramidothionic *O,S*-dimethyl ester 2-monoethyl ester, and propanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 3-monoethyl ester, were as toxic to insects as methamidophos. Toxicity to rats, however, was significantly reduced by N-acylation. N-Acylated analogues with four or more carbons in the dioic moiety were less phloem mobile. The feeding toxicity of hexanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 6-monoethyl ester, was equal to that of methamidophos, but systemic activity was lost.

Systemic properties not currently found in commercial insecticides are necessary for a chemical to be effective against many cryptic forest insect pests. To increase translocation characteristics in plants as a possible method of controlling these pests, we have been studying ways to chemically alter commercially available insecticides. One possible approach is to acylate pesticides with dicarboxylic acids (Crisp, 1972).

To continue this work, we synthesized a number of monoesterified dicarboxylic acid derivatives of methamidophos (I and Table I):



I

R = ethyl or methyl
n = 1–7

This report discusses results of bioassays of the insecticidal activities of the derivatives of methamidophos by methods that also assayed their systemic properties.

MATERIALS AND METHODS

Stauffer R-29534 (ethanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 2-monoethyl ester) was furnished by the Stauffer Chemical Co., Richmond, CA. Methamidophos and experimental compounds OR-20014 (buteneoic acid, 2,3(*EZ*),3-phosphoramidothionic acid *O,S*-dimethyl ester 1-monoethyl ester) and OR-14115 (*N*-formylphosphoramidothionic acid, *O,S*-dimethyl ester) were furnished by Chevron Chemical Co., Richmond, CA. Methamidophos was purified to 95% or better by solvent extraction and recrystallization from methylene chloride. Microchemical analyses were by the Microchemical Laboratory, Chemistry Department, University of California, Berkeley, CA. Tests on rat toxicity were done by Unilab Corp. of Oakland, CA. Monoester dicarboxylic acid chlorides were either commercial products or were synthesized by metatheses with oxalyl chloride of half-hydrolyzed dicarboxylic acid diesters.

The method of synthesis used was direct acylation of methamidophos with an excess of the appropriate half-ester dicarboxylic acid chloride in methylene chloride at room temperature for several days. At elevated temperatures many byproducts were formed. The use of acid acceptors or acylation catalysts in the reaction was deleterious, however, because the reaction is acid catalyzed. Two examples, considered typical acylation procedures, are the following.

Compound 3: Malonylmethamidophos. Methamidophos (2.5 g, 0.018 mol) of 95% or better purity was stirred for 2 days at room temperature with 4.5 g of ethylmalonyl chloride in 25 mL of methylene chloride and a small amount of anhydrous magnesium sulfate. The reaction mixture was hydrolyzed in excess water by stirring at room temperature for 2 h. The organic phase was separated and washed with water, dilute sodium bicarbonate, and water again. The solution was dried and evaporated to an oil that was chromatographed on a silica gel column (30 × 2.4 cm; E. Merck). Elutions made with hexane and methylene chloride removed less polar byproducts. The product was eluted with 25% acetone in methylene chloride. Evaporation of solvents gave 1.9 g (41%) of propanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 3-monoethyl ester (**3**). Anal. Calcd for $C_7H_{14}NO_5PS$: C, 32.9; H, 5.4. Found: C, 33.3; H, 5.43. Analyses by IR and NMR were compatible with the chemical structure of the product. Rat oral toxicity was approximately 125 mg/kg.

Compound 5: Glutarylmethamidophos. Methamidophos (5.3 g, 0.038 mol) was stirred for 4 days at room temperature with 8.9 g (0.054 mol) of methyl 4-(chloroformyl)butyrate (Aldrich Chemical Co.) in 50 mL of methylene chloride containing a small amount of anhydrous magnesium sulfate. At the end of the first day, an additional 1.2 g of acid chloride were added. The reaction mixture was hydrolyzed, extracted, washed, and chromatographed. The yield of pentanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 5-monomethyl ester (**5**), as a colorless oil was 4.5 g (42%). Anal. Calcd for $C_8H_{16}NO_5PS$: C, 38.4; H, 5.7. Found: C, 38.6, H, 6.5. Analyses by IR and NMR were compatible with the chemical structure of the product.

Bioassay Procedures. Bioassay methods to assay symplastic systemic insecticidal activity of the compounds will be described in a later publication (Crisp et al., 1981). The bioassay was done with cabbage looper [*Tricoplusia ni* (Hübner)] larvae and soybean plants (*Glycine max* L.) (Tables II and III). Data from the bioassay were analyzed

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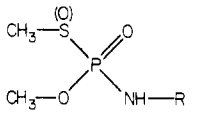
Table I. Compound Number, Abbreviated Name, CAS Name, and Molecular Structure



compd no.	abbreviated name	CAS name	-R
1	methamidophos	phosphoramidothioic acid, *O,S*-dimethyl ester	-H
2	oxalyl	ethanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 2-monoethyl ester	$-C(O)C(O)OCH_2CH_3$
3	malonyl	propanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 3-monoethyl ester	$-C(O)CH_2C(O)OCH_2CH_3$
4	succinyl	butanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 4-monomethyl ester	$-C(O)(CH_2)_2C(O)OCH_3$
5	glutaryl	pentanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 5-monomethyl ester	$-C(O)(CH_2)_3C(O)OCH_3$
6	adipyl	hexanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 6-monoethyl ester	$-C(O)(CH_2)_4C(O)OCH_2CH_3$
7	azelayl	nonanedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 9-monomethyl ester	$-C(O)(CH_2)_7C(O)OCH_3$
8	maleyl	butenedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 2,3(*E*),4-monoethyl ester	$-C(O)CH{=}CHC(O)OCH_2CH_3$ (*E*)
9	fumaryl	butenedioic acid, 1-phosphoramidothionic acid *O,S*-dimethyl ester 2,3(*Z*),4-monoethyl ester	$-C(O)CH{=}CHC(O)OCH_2CH_3$ (*Z*)
10	formyl	*N*-formylphosphoramidothionic acid, *O,S*-dimethyl ester	-C(O)H
11	des-*S*-malonyl	propanedioic acid, 1-phosphoramidothionic acid *O,O*-dimethyl ester 3-monoethyl ester	

Table II. Toxicity of *N*-Acylmethamidophos Derivatives to Cabbage Looper Larvae[a]

compd no.	*N*-acyl moiety	no. of carbons in acyl moiety	*N*	NC	slope ± SE	LD_{50}	LC_{50}	CL	TR
			(A) Topical Test Carbon *N*-Acyl Analogues						
1	methamidophos	0	398	30	2.66 ± 0.26	12		10-13	1
10	formyl	1	100	22	*c*	>200		*b*	>17
2	oxalyl	2	1037	20	1.66 ± 0.13	55		41-83	5
3	malonyl	3	845	60	1.74 ± 0.26	96		78-125	8
8	*cis*-maleyl	4	50	10	1.74 ± 0.91	226		*b*	19
9	*trans*-fumaryl	4	49	9	1.67 ± 0.63	72		*b*	6
4	succinyl	4	1030	88	1.90 ± 0.14	156		109-288	13
5	glutaryl	5	399	30	1.28 ± 0.24	204		124-563	17
6	adipyl	6	148	30	1.26 ± 0.37	157		84-1198	13
7	azelayl	9	50	10	*b*	>200		*b*	>17
			(B) Feeding Test Carbon *N*-Acyl Analogues						
1	methamidophos	0	458	70	0.83 ± 0.09		21	11-34	1.0
10	formyl	1	771	120	2.50 ± 0.30		65	59-89	3.2
2	oxalyl	2	584	64	1.89 ± 0.16		12	3-29	0.6
3	malonyl	3	1009	75	1.96 ± 0.13		30	15-56	1.5
8	*cis*-maleyl	4	574	85	1.98 ± 0.18		132	*b*	6.0
9	*trans*-fumaryl	4	*d*						
4	succinyl	4	546	75	2.16 ± 0.18		8	6-11	0.4
5	glutaryl	5	809	219	2.02 ± 0.18		236	145-352	11.5
6	adipyl	6	656	119	4.29 ± 0.41		31	27-34	1.5
7	azelayl	9	575	121	6.18 ± 0.54		18	15-23	0.9

[a] *N* = number of insects in treatment; NC = number of insects in control; slope = slope of the regression line; SE = standard error of the slope of the regression line; LD_{50} = concentration required to kill 50% of the insects in the topical tests; CL = confidence limits; TR = toxicity ratio = LD_{50} of derivative – LD_{50} of methamidophos. Feeding and translocation tests are LC_{50} values in micrograms per gram fresh weight of tissue. [b] No confidence limits computed because $g \geq 0.50$ at the 90% level. [c] No significant regression was obtained. [d] Compound was not tested in this bioassay.

statistically (Russell et al., 1977). Regression lines were compared by likelihood ratio tests (Savin et al., 1977; Robertson et al., 1980).

RESULTS AND DISCUSSION

Compounds **2–11** (Table I) represent systematic studies of substitutions at two sites on methamidophos (**1**), the parent toxicant, and at several sites on the *N*-acyl moiety: (a) N-acylation (**2–10**), (b) N-acylation with increasing methylene groups in the *N*-acyldioic moiety (**2–7** and **10**), (c) cis and trans double bond in the 2,3 position of the *N*-succinyl moiety (**8** and **9**), and (d) replacement of the methylthio group with a methoxy and the phosphoryl oxygen with sulfur to yield the thion (**11**) analogue of the malonyl derivative. These substitutions tested the possibility that β-oxidation and the malonate semialdehyde pathway for fatty acid oxidation may be involved in generating a toxicant from some *N*-acyl dioic methamidophos analogues.

The N-acylated derivatives and methamidophos were tested for insecticidal activity with mixed-sex cabbage looper larvae. The multiple screening system included

Table III. Effects of Two Sites of Application on the Systemic Toxicity of Methamidophos and Nine *N*-Acylmethamidophos Analogues[a]

compd no.	*N*-acyl moiety	no. of carbons in acyl moiety	*N*	NC	slope ± SE	LC_{50}	CL	TR	PTT
		(A) Cotyledon Treatments with *N*-Acyl Analogues							
1	methamidophos	0	700	172	2.00 ± 0.17	76	54-106	1.0	27
10	formyl	1	180	60	*e*	>1000	*b*	>13	*d*
2	oxalyl	2	1200	193	0.88 ± 0.88	83	65-105	1.1	14
3	malonyl	3	473	75	0.70 ± 0.08	434	135-3393	5.7	7
8	*cis*-maleyl	4	304	72	1.02 ± 0.15	671	429-1290	9	*d*
9	*trans*-fumaryl	4	*c*						
4	succinyl	4	606	87	1.26 ± 0.14	316	87-1138	4	3
5	glutaryl	5	635	98	0.99 ± 0.16	>1000	*b*	>13	*d*
6	adipyl	6	625	93	0.54 ± 0.37	>1000	*b*	>13	0
7	azelayl	9	601	84	0.48 ± 0.13	>1000	*b*	>13	*d*
		(B) Primary Leaf Treatments with *N*-Acyl Analogues							
1	methamidophos	0	536	95	1.12 ± 0.16	203	95-341	1.0	10
10	formyl	1	594	96	0.37 ± 0.16	>1000	*b*	>5.0	*d*
2	oxalyl	2	717	109	1.14 ± 0.14	235	72-728	1.2	5
3	malonyl	3	850	95	1.39 ± 0.12	77	47-115	0.4	39
8	*cis*-maleyl	4	254	36	0.08 ± 0.11	>1000	*b*	>5	*d*
9	*trans*-fumaryl	4	*c*						
4	succinyl	4	464	69	4.11 ± 0.47	264	126-582	1.3	3
5	glutaryl	5	360	49	1.75 ± 0.37	874	*b*	>4.3	*d*
6	adipyl	6	408	64	2.10 ± 0.29	646	499-838	3.2	5
7	azelayl	9	455	77	2.31 ± 0.25	411	129-932	2.0	5

[a] *N* = number of insects in treatment; NC = number of insects in control; slope = slope of regression line; SE = standard error of the slope of the regression line; CL = 95% confidence limits; LC_{50} = concentration required to kill 50% of the larvae in the topical tests; TR = toxicity ratio = LD_{50} of derivative – LD_{50} of methamidophos; PTT = percent toxicant translocated = (feeding LC_{50} – translocated LC_{50}) × 100. Feeding and translocation tests are LC_{50} values. [b] No confidence limits computed because $g \geq 0.50$ at the 90% level. [c] Compound was not tested in this bioassay. [d] Absence of LC_{50} or confidence limits voids determination of PTT. [e] No significant regression was obtained.

assessment of the topical toxicity of the compounds expressed as LD_{50} (micrograms per gram of insect) and assessment of the feeding toxicity (a) as determined by treating mature primary leaves and feeding them directly to larvae where the LC_{50} is expressed as micrograms per milliliter of tissue, (b) after translocation from treated cotyledon leaves (source) to terminal growth (sink) and expressed as LC_{50} (micrograms per milliliter of tissue), and (c) after transport from dosed primary leaves (source) and expressed as the LC_{50} (micrograms per milliliter of tissue).

For reasons of convenience and by convention, we refer to the LC_{50} estimate as the apparent toxicity. The toxicity ratio for two compounds A and B is defined as the ratio of their LC_{50} estimates if any of the following conditions hold: (a) the response lines are parallel but not equal, (b) the response lines are neither parallel nor equal, but both compounds have estimable nonoverlapping confidence limits for their LC_{50} values, or (c) the lines are not parallel or equal, but one component has an estimable confidence interval for LC_{50} and the other is virtually ineffective (resulting in an inequality for the toxicity ratio.)

Topical toxicity tests of N-acylated methamidophos derivatives with odd and even numbers of carbons in the *N*-acyl moiety were compared with methamidophos (1) (Table II). Formyl (10) and azelayl (7) were nontoxic. Oxalyl (2) and malonyl (3) were not different from each other but were both less toxic than methamidophos (1). A trend of increasing LD_{50} values with increasing numbers of methylene carbons in the dioic moiety is suggested in the central cluster consisting of oxalyl (2), malonyl (3), succinyl (4), and glutaryl (5). However, we cannot state emphatically that they are different from each other, because the confidence limits overlap. Glutaryl (5) was not different from adipyl (6). No difference was noted for odd- and even-numbered methylene groups. The lack of a difference between odd and even series and the suggestion that increasing lipophilicity increases the LD_{50} value are interpreted to mean that these N-acylated analogues are being metabolized by an amidase rather than by β-oxidation.

The likelihood ratio test showed that the regression lines for the following pairs were equal: 2 with 9, 4 with 8, and 6 with 8. The hypothesis of parallelism was accepted for the following pairs: 1 with 9, 2 with 8, 4 with 6, 4 with 9, 6 with 8, and 8 with 9.

A hypothetical pathway for the biodegradation of succinyl (4) to methamidophos (1) with eight possible intermediates is shown (Figure 1). We tested four of the possible intermediates—fumaryl (9), maleyl (8), oxalyl (2), and formyl (10)—as their esters. If we assume that deesterification and conjugation with CoA occurs, then the fumaryl (9) analogue is an intermediate in the β-oxidation pathway. The topical LD_{50} values indicate that analogues tested in this pathway have some level of toxicity, except *N*-formyl (10). The results of the bioassays of *N*-*cis*-maleyl (8) and *N*-*trans*-fumaryl (9) were of particular value, because both are potential substrates in the pathway. The LD_{50} values indicate that both the cis and trans analogues have toxicity; however, confidence limits for the respective LD_{50} values could not be established.

Overall, the reduced toxicity of compounds 4–7, compared with that of methamidophos (1), was expected. The toxicity probably should decrease as methylene groups are added between the two 1-oxoethylenes because (a) as the molecular size of the *N*-acyl moiety increases, the concentration of methamidophos decreases, (b) lipophilicity increases as methylene groups are added and this may result in slower rates of deesterification of the simple methyl and ethyl esters, and (c) esterified N-acylated derivatives that remain esterified in vivo cannot be metabolized to methamidophos. Although insect toxicities were reduced for some of these N-acylated analogues, these data augment structure–activity studies of other phosphoramidothioates (Eto, 1974; Eto et al., 1977; Gaughan,

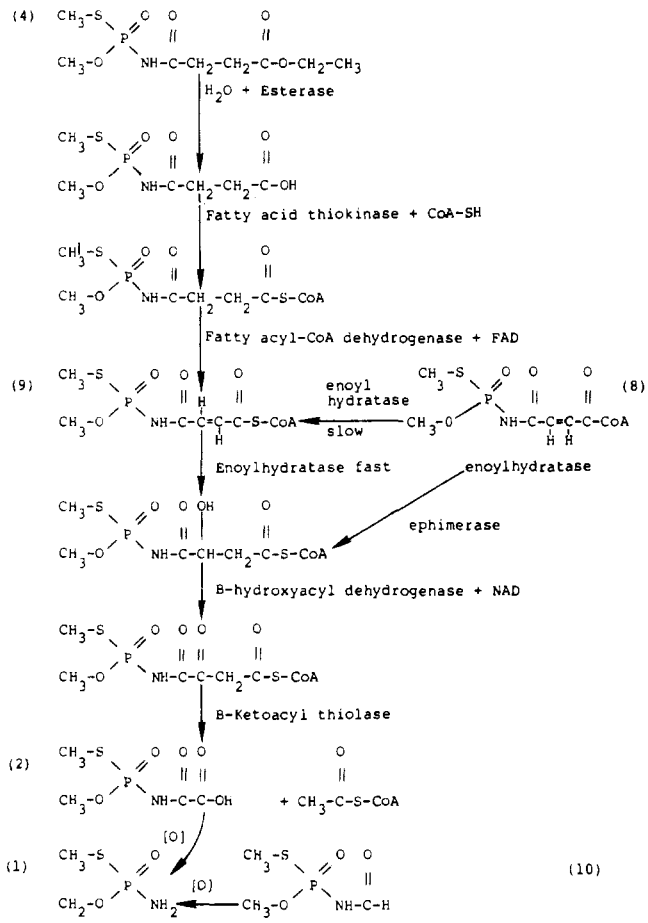


Figure 1. Hypothetical pathway for degradation of succinyl (**4**), with cis and trans intermediates (**8**; **9**), and end product oxalyl (**2**) and an alternate pathway via formyl (**10**), all resulting in the final generation of methamidophos (**1**).

1974; Hajjar and Hodgson, 1980; Hall and Inch, 1977; Hussain et al., 1974; Kao and Fukuto, 1977; Magee, 1973; Lorenz, 1974; Suksayretrup and Plapp, 1977; Wustner et al., 1978).

Feeding toxicities (Table IIB) were measured in the event that the topical toxicities were minimized by slow cuticular penetration and to establish an LC_{50} value for use in the numerator of the percent toxicant translocated (PTT) (Crisp et al., 1981) equation. In the feeding tests, the cabbage looper larvae and the toxicants had a 96-h period to interact with living plant tissue. The test materials in the treated leaf did not translocate, and the insects were forced to eat the chemical residues. With TR as a guide (Table IIB), the two dioic N-acylated derivatives, succinyl (**4**) and oxalyl (**2**), were possibly more toxic after interacting with plant tissue, although the maleyl (**8**) was less toxic, suggesting a slow conversion to fumaryl (**9**), which may be further metabolized to oxalyl (**2**) (Figure 1). The likelihood ratio test rejected the hypothesis for parallelism or equality when comparing methamidophos (**1**) with any of the N-acylated compounds with even carbon numbers in the *N*-acyl moiety. The hypothesis that the regression line for oxalyl (**2**) was parallel with that for succinyl (**4**) and maleyl (**8**) was accepted ($p > 0.05$). The LC_{50} values for adipyl (**6**) and methamidophos (**1**) are similar. The slope of the regression line was 4.29 for adipyl (**6**) and 0.83 for methamidophos (**1**). Their log dose–response profiles, therefore, are different.

Feeding tests with odd-numbered carbon N-acylated derivatives showed that malonyl (**3**) and azelayl (**7**) had overlapping confidence limits with methamidophos (**1**). The hypotheses of parallelism and equality for response curves with methamidophos (**1**) and malonyl (**3**) were both rejected. The slope of the response curve for methamidophos (**1**) is <1.0, and it was not parallel with any of the N-acylated derivatives in the feeding test. Curves for formyl (**10**) were found to be parallel with those for malonyl (**3**) and glutaryl (**5**).

Systemic toxicity was assayed with two bioassays designed to measure the symplastic activity. Cotyledons or primary leaves of soybean plants were treated with methamidophos (**1**) or N-acylated analogues, and the terminal growth was bioassayed with cabbage looper larvae. Toxicants translocated from cotyledons or primary leaves to the terminal growth use the symplastic transport pathway. The bioassays, therefore, yield two useful types of information: a positive response indicates that a solute was translocated from source to sink; a cholinergic response indicates that a toxicant was translocated.

For each test compound, an LC_{50} value was established by treating many plants with different concentrations and replicating the treatments several times. The LC_{50} value is the concentration of solute required at the photosynthate source which, after transport, will yield 50% mortality in the sink tissue. The mortality data do not reflect the chemical nature of the toxicant; rather, they provide information on the concentration of all the toxicants translocated from source to sink.

The systemic activity after cotyledon treatment with even-numbered carbon N-acylated methamidophos analogues is compared with methamidophos for LC_{50} values, toxicity ratios, and percent total toxicant translocated (Table IIIA). The compounds have been arranged in each of the four tests so as to reflect decreasing carbon numbers in the *N*-acyl moiety from the top of the table downward. None of the N-acylated derivatives were more toxic than methamidophos (**1**). The toxicity ratio of oxalyl (**2**) was similar to that of methamidophos (**1**), but the slopes of the two response curves were different. Long-chain N-acylated dioic esters (**6**) are not translocated or do not produce toxic metabolites that translocate.

In the tests of the odd-numbered carbon N-acylated compounds (Table IIIA), azelayl (**7**) and glutaryl (**5**) had regression line slopes < 1.0 and LC_{50} values > 1000 μg/mL of tissue. These compounds are considered to be inactive. The response of formyl (**10**) in the various bioassays was capricious. Only in the feeding test was activity observed (Table IIB). Contact toxicity (Table IIA) and translocation (Table III) were negligible. Formyl (**10**) may not be able to penetrate cuticular tissue in plants and insects. Plant amidase and oxidation may be rapidly and efficiently converting formyl (**10**) to *O*,*S*-dimethyl phosphate, CO_2, and NH_3. *O*,*S*-Dimethyl phosphate is phloem mobile, but, because it has no cholinergic activity, it would not be detected in the sink tissue by the systemic bioassays. Feeding insects are affected, however, indicating that the N–C bond is broken by an insect gut amidase or that the intact formyl (**10**) is cholinergic, when introduced by feeding.

Results for azelayl (**7**) showed unusual selectivity. In the cotyledon tests (Table IIIA) and in the topical tests (Table IIA), no activity was observed. Plant cotyledon tissue and insect larval cuticular tissue, therefore, apparently do not contain enzymes capable of degrading a long-chain dicarboxylic acid. In the feeding tests (Table IIB), the toxicity of azelayl (**7**) equaled that of methamidophos (**1**). These data suggest that insect gut can rapidly degrade the substrate to toxic intermediates.

Cotyledon tests with dioic moieties containing odd-numbered carbon showed that malonyl (**3**) and methamidophos (**1**) were systemic, where 7 and 27% translo-

cation was estimated. At the LD_{50} the toxicity ratio of malonyl (3) was 5.7. The hypotheses for parallelism and equality of response for the regression lines of the pair (1 with 3) were uniformly rejected.

Translocation of even-numbered carbon N-acylated analogues from treated primary leaves (source) to terminal trifoliate leaves (sink) is another test of systemic activity that is specific for phloem transport. This type of test is a better evaluation of systemic characteristics for field studies than the cotyledon test. Maleyl (8) was immobile (Table IIIB), suggesting that mature primary leaves do not convert it to *N*-[L-(+)-3-hydroxysuccinyl]methamidophos, which is needed as a substrate for β-oxidation. LC_{50} values for methamidophos (1), oxalyl (2), and succinyl (4) are similar. Because succinyl (4) was a substrate, the hydrating enzyme enoyl-CoA-hydratase is not the reason for lack of activity with maleyl (8). Rather, the interpretation suggests that these mature leaves do not contain 3-hydroxyacyl-CoA ephimerase for the conversion of *N*-[L-(−)-3-hydroxysuccinyl]methamidophos to the active *N*-[L-(+)-3-hydrooxysuccinyl]methamidophos.

None of the N-acylated analogues in the even-carbon series had PTT estimates exceeding 5%, although an estimated 10% of methamidophos (1) was translocated.

The systemic activity in primary leaf treatments of odd-numbered carbon *N*-acyl derivatives are compared with that of methamidophos (1) in Table IIIB. Data obtained for the azelayl derivative (7) and methamidophos (1) can be interpreted as similar to that for the malonyl derivative (3). Only malonyl (3) required a lower concentration of solute than methamidophos (1) to produce an LC_{50} in the sink tissue. The hypotheses of parallelism and equality for the adipyl (6) and azelayl (7) pair were accepted ($p > 0.05$); therefore, primary leaf treatments may be processing these two long-chain *N*-acyl dioic analogues in a similar manner. The response curves for 5 and 4 were parallel ($p > 0.05$), as well as for oxalyl (2) with malonyl (3). Primary leaf treatments, therefore, produced response curves that were parallel in most tests.

In general, data from the primary leaf bioassay indicate that the phloem mobile activity of malonyl (3) has been improved by N-acylation of methamidophos with short-chain dicarboxylic half-acid esters. The overall performance of methamidophos (1), oxalyl (2), and malonyl (3), when quantified on the basis of LC_{50} values, is essentially equivalent. Further evaluation of other simple dicarboxylic acids as substrates for N-acylation reactions would seem to be a fruitful area for improving systemic activity.

The major function of the *N*-acyl dioic moiety on methamidophos is to stimulate phloem loading and facilitate long-distance transport. If β-oxidation pathways' enzymes and amidase hydrolyze the N-acylated insecticide before phloem loading, systemic activity will not have been increased. The ideal systemic molecule for long-distance transport would contain an ionizable carboxylic acid that can be removed only in the alkaline environment of the sieve-tube sap. In this way, the acid moiety on the proinsecticide can facilitate phloem loading but not interfere with insect feeding toxicity at the sink (Crisp, 1972; Crisp and Look, 1979).

In this study, a systematic synthesis of a few methamidophos analogues was done to make specific substrates for β-oxidation. Enzymes associated with β-oxidation are most likely compartmentalized in the glyoxysomes and oxalyl (2) must be N-deacylated in this subcellular particulate. The data presented do not eliminate the possibility that amidase-type enzymes could hydrolyze the *N*-acyl moiety and the differences in LD_{50} and LC_{50} result from structural and electrophilic variations on the *N*-acyl moiety that relate to substrate specificity for this enzyme.

The lower molecular weight *N*-acyl dioic esters of methamidophos have favorable mammalian toxicities. Oxalyl (2), for example, has a rat acute oral LD_{50} of 501 mg/kg and malonyl (3), 125 mg/kg. If selectivity and safety are desired characteristics in a systemic insecticide, the translocation results for oxalyl (2) and malonyl (3) suggest that these two novel *N*-acyl dioic esters have favorable systemic and mammalian toxicities to justify further systemic research and development.

The thion analogue of compound 3 is compound 11. Replacement of the methylthio group of malonyl (3) with a methoxy group yields a nontoxic molecule and, in the presence of insect or plant tissue, the activity is not improved.

The phosphoramide configuration of methamidophos is unique for phloem transport characteristics because it can mimic a weak acid. Crisp and Look (1979) found that 4-chlorophenoxyacetic acid could mimic 4-chlorophenoxyacetamide. Ethophone and glyphosate are phloem mobile because of their acid groups. The $=P(O)—NH_2$ group of phosphoramidothioate insecticides, therefore, may mimic the $=P(O)—OH$ group in the phosphate plant growth regulators and herbicides. This characteristic may explain why most of the other organophosphorus insecticides are not phloem mobile to the same extent as the phosphoramidates, which have phloem-loading characteristics. The amine moiety can provide the proton for phloem loading, and it may be the only phosphorus substitute in the organophosphorus insecticides that can do this.

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