Chemistry and Action of *N*-Phenylmaleamic Acids and Their Progenitors as Selective Herbicide Antidotes

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Several N-substituted maleimides and related compounds were prepared and tested for their effects on plant growth. *N*-Alkylmaleimides were inactive but *N*-(4-chlorophenyl)maleimide (CPMI) protected sorghum from injury caused by alachlor without reducing its herbicidal activity. The highest protection was obtained on simultaneous application of CPMI and alachlor immediately after sowing. CPMI possessed both high botanical and chemical specificity, protecting only sorghum among the six crops evaluated and only against alachlor; it was ineffective as an antidote for EPTC or chlorsulfuron. Structural features favorable for high antidotal activity are the unsubstituted maleimide ring for *N*-(4-chlorophenyl)maleimides and a 4-chloro, 4-fluoro, or 4-methyl substituent on the phenyl ring for the almost equally active *N*-phenylmaleimides, -isomaleimides, and -maleamic acids. CPMI and *N*-(4-chlorophenyl)isomaleimide undergo rapid hydrolysis to *N*-(4-chlorophenyl)maleamic acid (CPMA). *N*-Phenylmaleimides and -isomaleimides thus appear to be proantidotes and *N*-phenylmaleamic acids are the actual antidotes. CPMI and CPMA, as the earlier antidotes R-25788 and flurazole, increase the glutathione level in sorghum roots.

Plant thiols including glutathione (GSH) are involved in herbicide detoxification and the action of herbicide antidotes (Corbett et al., 1984; Fedtke, 1982; Hatzios and Penner, 1982). The antidote, *N*,*N*-diallyl-2,2-dichloroacetamide (R-25788), enhances sulfate metabolism and elevates the GSH content in several plant systems (Adams et al., 1983; Ezra and Gressel, 1982; Lay et al., 1975; Rennenberg et al., 1982). It also increases the GSH-dependent metabolism in corn of 2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide (alachlor) (Mozer et al., 1983) and of *S*-ethyl dipropylthiocarbamate (EPTC) via its sulfoxide (Lay and Casida, 1976). The protective action of R-25788 extends to several other herbicides detoxified on reaction with GSH in plants (Hatzios, 1983; Pallos and Casida, 1978). Some of the herbicides undergoing facile reaction with GSH may owe their herbicidal activity to analogous derivatization of critical enzyme, receptor, or membrane thiols. Thus, chemicals which elevate or deplete GSH and other thiols in plants may serve as antidotes or herbicides, respectively.

Maleimides like *N*-ethylmaleimide (NEM) and *N*-(4-chlorophenyl)maleimide (CPMI) are known thiol reagents (Adams et al., 1983; Augustin et al., 1978) and related *N*-aryl cyclic imides possess herbicidal (Ohta et al., 1976) or fungicidal (Fujinami et al., 1972) activity. We therefore synthesized a series of *N*-phenylmaleimides and related compounds (isomaleimides and maleamic acids) (Figure 1) and evaluated their phytotoxicity, antidotal activity, reactivity as thiol reagents, and influence on thiol levels in plants. Contrary to expectations, the maleimides and isomaleimides are antidotes rather than herbicides, e.g., for alachlor in sorghum (Kirino et al., 1985), and appear to be proantidotes acting after conversion to the corresponding maleamic acids.

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MATERIALS AND METHODS

Chemicals. The compounds were either obtained commercially or synthesized according to known methods (Cotter et al., 1961; Hargreaves et al., 1970; Roderick, 1957; Sauers, 1969). Maleamic acids (MAs) were prepared by the reaction of maleic anhydride with the repective amines in toluene. Maleimides (MIs) were synthesized by dehydration of the MAs with acetic anhydride and sodium acetate or by direct heating. Isomaleimides (IMs) were obtained by dehydration of the MAs with dicyclohexylcarbodiimide in toluene. The structure of the compounds was confirmed by their 90-MHz ^{1}H NMR spectra. Compounds not reported previously are *N*-(3-acetylphenyl)-maleimide, mp 103–105 °C, and *N*-(substituted phenyl)-isomaleimides: 4-F, mp 75–76 °C; 4-Et, mp 58–60 °C; 4-*n*-Bu, mp 45–46 °C; 4-CN, mp 120–122 °C; 3-Cl, n^{22}_D 1.5946; 3-COMe, n^{22}_D 1.5733; 2,4-Cl_2, mp 151–153 °C; 2,4-$(OMe)_2$, mp 122–124 °C dec; 2,6-Me_2, n^{22}_D 1.5815; 3,4-Cl_2, mp 123–124 °C. All melting points are uncorrected.

[^{14}C]*N*-(4-Chlorophenyl)maleamic acid ([^{14}C]CPMA) (3 mCi/mmol) was prepared from [U-^{14}C]4-chloroaniline and purified by thin-layer chromatography (TLC). [^{14}C]-Alachlor labeled at the methoxy group was used at a specific activity of 17 mCi/mmol.

Biological Tests. Each MI, IM, MA, or related compound was formulated as a 10% emulsifiable concentrate (EC) containing 30% ATLOX-1045 (polyoxyethylene sorbitol laurate type emulsifier) and 60% *N*,*N*-dimethylformamide (w/w). Commercial preparations were used for the herbicides alachlor (45% EC, Monsanto, St. Louis, MO), EPTC (98.5% technical grade, Stauffer, Richmond, CA), and chlorsulfuron (2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-benzenesulfonamide) (94.4% technical grade, Du Pont, Wilmington, DE, dissolved in acetone at 1 mg/mL) and the herbicide antidotes flurazole (benzyl 2-chloro-4-(trifluoromethyl)-5-thiazolecarboxylate (48% flowable, Monsanto) and R-25788 (analytical grade, Stauffer).

The test plants were sorghum [*Sorghum bicolor* (L.) Moench, c.v., G-499-GBR], corn (*Zea mays* L., c.v., XL-72B), rice (*Oryza sativa* L., c.v., M-101), wheat (*Triticum aestivum* L., c.v., Yecora rojo), cotton (*Gossypium hirsutum* L., c.v., SJ-2), soybean [*Glycine max* (L.) Merr., c.v.,

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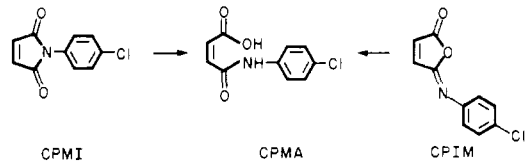

Figure 1. Three types of herbicide antidotes, *N*-(4-chlorophenyl)maleimide (CPMI), *N*-(4-chlorophenyl)isomaleimide (CPIM), and *N*-(4-chlorophenyl)maleamic acid (CPMA).

Amsoy-71], barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.], Johnson grass [*Sorghum halepense* (L.) Pers.], and green foxtail [*Setaria viridis* (L.) Beauv.]. Styrofoam pots (180-mL volume, 7-cm diameter, with holes in the bottom) were filled with 200 g of fresh water-washed sand (16 mesh) and placed within similar pots without holes (double pot technique). Seeds of the test plants were placed on top of the sand and covered with an additional 50 g of sand to give approximatley 1-cm sowing depth. Each test chemical or formulation as above was diluted with water and administered either at 50 mL per pot immediatley after sowing or, in application timing tests, at 50 mL per pot immediately after sowing and 25 mL at twice the concentration for later treatments. The plants were maintained at 28 °C under 12-h photoperiod with a mixture of fluorescent and "GROLUX" lamps (F40 plant light, General Electric). Plant injury was rated visually from 0 (no or slight injury) to 3 (severe injury) relative to the untreated control 8 days after sowing. Shoot fresh weight and height were determined 14 days after sowing for application timing tests and 10 days for antidotal effect and spectrum tests except 13 days with rice. Data are presented as mean values of the percentages of the appropriate untreated controls with two experiments each with three replicates.

[^{14}C]CPMA Uptake and Translocation. Five seeds of sorghum were sown in a glass beaker (20 mL) filled with washed sand. CPMA solution (6 mL) at 10 ppm containing ca. 0.1 μCi of [^{14}C]CPMA was applied immediately after sowing. Four and ten days after application, plants were removed from the sand, washed, dried, and autoradiographed.

Effect of CPMA and Flurazole on Alachlor Uptake, Distribution, and Metabolism. Sorghum (50 seeds) was planted in sand as described above (double pot technique) and treated with 50 mL of 1 ppm alachlor (analytical grade) solution containing 1.3 μCi of [^{14}C]alachlor with or without CPMA (100 ppm) or flurazole (10 ppm). Four and seven days after planting, the seedlings were harvested and thoroughly washed to remove sand particles. Four seedings from each of three replicates were separated into roots, seeds, and shoots and their radioactivity content was determined by combustion with a sample oxidizer (Packard, Donner's Grove, IL) and liquid scintillation counting (LSC). Two seedlings without seeds were dried overnight and autoradiographed to localize the [^{14}C]alachlor and its degradation products. The roots and shoots of the rest of the seedlings were weighed and ground in ethanol (20 mL) with a mortar and pestle. The homogenate was centrifuged at 15 000*g* for 15 min at 4 °C and the supernatant collected. The plant residue was reextracted twice with ethanol by using centrifugation and then dried overnight for combustion and LSC. All supernatants were combined and brought to a final volume of 100 mL, and aliquots (2 × 1 mL) were taken for LSC. The remaining ethanol extract was evaporated below 40 °C under reduced pressure and redissolved in 1 mL of methanol–chloroform (2:1 v/v). An aliquot (100 μL) was applied to a precoated TLC plate (silica gel 60 F-254, E. Merck, West Germany) and chromatographed with unlabeled and [^{14}C]alachlor by using two separate solvent systems: (A) benzene–methanol (19:1 v/v) and (B) *n*-butanol–acetic acid–water (6:3:2 v/v/v).

Effect of Antidotes and Alachlor on GSH Level in Sorghum. Sorghum was planted in sand as described above and treated immediately after sowing with alachlor at 1 ppm with or without antidotes. Fresh roots (0.5–1.0 g) harvested 4 and 7 days after sowing were homogenized in 8 volumes of trichloroacetic acid solution (5% w/v) and the precipitated proteins were removed by centrifugation (17 000*g*, 30 min at 4 °C). An aliquot (1 mL) of the supernatant was added to 1.5 mL of 0.2 M phosphate buffer, pH 7.4, and the pH was adjusted to 7.5 with 0.3 N NaOH (approximately 1 mL). After addition of 5,5′-dithiobis(2-nitrobenzoic acid) reagent (0.75 μmol in 0.5 mL of 0.1 M phosphate buffer, pH 7.4), the absorbance was measured at 412 nm (Adams et al., 1983). The same procedure was employed with barnyardgrass. The time course for the effect of alachlor and CPMA on the GSH level was examined with 4-day-old sorghum seedlings. Alachlor (1 ppm) and CPMA (100 ppm) were applied as a sand drench. The plants were harvested 4, 8, 24, 48, and 72 h after treatment, and their root GSH content was determined.

GSH Reactivity in Vitro. CPMI, CPMA, NEM, and alachlor (1 μM) were mixed (vortex) with GSH (0.01 or 0.1 μM) in 0.2 M phosphate buffer, pH 7.4, at 5 °C. The candidate thiol reagents were added in *N*,*N*-dimethylformamide (1% v/v). After 15 min at 5 °C the unreacted GSH was determined as described above.

Hydrolysis of Maleimides and Isomaleimides. The 4-chlorophenyl derivatives in the MI, IM, and MA series were added in methanol to 0.2 M phosphate buffer (pH 7.4 and 6.8) to give final concentrations of 0.05 μM test compound and 10% methanol. The UV spectra were recorded hourly until no further change was observed.

RESULTS

Effect of Maleimides and Alachlor Alone or in Combination on Sorghum and Barnyardgrass. CPMI does not injure sorghum or barnyardgrass and selectively protects sorghum from alachlor injury without reducing its herbicidal activity against barnyardgrass (Table I). Other *N*-(4-chlorophenyl)imides and -amides are also active as herbicides or antidotes. The 3,4,5,6-tetrahydrophthalimide, 3,4-dimethylmaleimide, and acetamide were phytotoxic and none had useful antidotal activity. In contrast, the succinimide was not phytotoxic and showed selective antidotal activity, although it was not as effective as CPMI. The crotonamide, phthalimide, 3-methylmaleimide, and *N*-alkylmaleimides including NEM were of much less interest as phytotoxicants or antidotes. The reference antidote flurazole slightly retarded sorghum growth at 100 ppm with complete protection against alachlor injury but, in contrast to CPMI, it also slightly protected barnyardgrass from the herbicidal action (Table I).

Effect of Phenyl Substituents on Activity of *N*-Phenylmaleimides, *N*-Phenylisomaleimides, *N*-Phenylmaleamic Acids and Related Compounds as Antidotes for Alachlor Injury in Sorghum. The three superior antidotes in each of the MI, IM, and MA series have the 4-Cl, 4-Me, or 4-F substituent (Table II). This similarity of response for the MI, IM, and MA types is repeated with each phenyl substituent examined, although overall the potency trend is MI > MA > IM. In general, substitution in the 4-position provides greater activity than in the 2- or 3-position and disubstitution reduces activity.

When compared on a quantitative basis, the superior antidotes (i.e., 4-Cl, 4-Me, and 4-F) are of similar activity

Table I. Effect of Preemergence Application of Maleimides and Related Compounds at 100 ppm and Alachlor at 0 or 1 ppm on Growth of Sorghum and Barnyardgrass

compd	plant injury rating[a] for indicated alachlor level, ppm: sorghum 0	sorghum 1	barnyardgrass 0	barnyardgrass 1
controls				
none	0	3	0	3
flurazole	0	0	0	2
N-(4-chlorophenyl) derivatives				
maleimide (CPMI)	0	0	0	3
3-methylmaleimide	1	3	1	3
3,4-dimethylmaleimide	2	3	3	3
tetrahydrophthalimide	3	3	3	3
phthalimide	1	2	1	3
succinimide	0	1	0	3
acetamide	1	3	2	3
crotonamide	1	2	1	3
N-alkylmaleimides[b]	0	3	0	3

[a] Ratings: graded response from 0 = no or slight injury to 3 = severe injury determined 8 days after sowing. [b] *N*-Alkylmaleimides $\overline{RNC(O)CR_1{=}CR_2C(O)}$ gave ratings for both sorghum and barnyardgrass of 0 for no alachlor and 3 for 1 ppm alachlor. The analogues examined were R, R_1, R_2: Me, H, H; Et, H, H; Et, Me, H; Et, Me, Me; *n*-Pr, H, H; *i*-Pr, H, H; *i*-Bu, H, H. As the only exceptions, the second, third, and seventh compounds in this sequence gave a rating of 1 for no alachlor with sorghum.

Table II. Effect of Phenyl Substituents on the Activity of *N*-Phenylmaleimides (MI), *N*-Phenylisomaleimides (IM), and *N*-Phenylmaleamic Acids (MA) at 100 ppm as Antidotes for Alachlor Injury at 1 ppm in Sorghum

phenyl substituent	plant injury rating[a] compd type MI	IM	MA	av
	Superior			
4-Cl	0	0	0	0.0
4-Me	0	0	0	0.0
4-F	0	1	0	0.3
	Good			
2-Me	0	1	1	0.7
4-OMe	0	1	1	0.7
H	1	1	1	1.0
4-NO_2	1	1	1	1.0
2,4-Cl_2	1	1	1	1.0
	Fair			
3-Cl	2	1	1	1.3
3,4-Cl_2	1	2	1	1.3
2,6-Me_2	1	2	1	1.3
3-COMe	1	2	2	1.7
4-Et	2	1	2	1.7
4-OEt	2	1	2	1.7
4-COMe	1	2	2	1.7
	Poor			
4-*n*-Bu	2	2	2	2.0
4-CN	2	2	2	2.0
2,4-$(OMe)_2$	2	3	2	2.3
2-Cl	2	3	2	2.3

[a] Test conditions and ratings as in Table I. The rating was consistently 3.0 without an antidote.

in the MI, IM, and MA series (Table III). The antidotal effectiveness was considerably less at 10 than at 50 ppm in contrast to flurazole and R-25788. However, flurazole even at 10 ppm inhibited sorghum growth. The trans isomer of 4-chlorophenylmaleamic acid (fumaramic acid) and the saturated derivative (succinamic acid) possessed much lower antidotal activity than the corresponding MA (Table III).

Table III. Effect of Preemergence Application of *N*-Phenylmaleimides, *N*-Phenylisomaleimides, *N*-Phenylmaleamic Acids and Related Compounds as Antidotes for Alachlor Injury in Sorghum

candidate antidote	shoot fresh weight, % of control[a]: antidote alone, 50 ppm	alachlor, 3 ppm, with antidote at indicated ppm: 10	50
none (control)	100	20	20
maleimides			
4-Cl	99	55	82
4-Me	84	38	75
4-F	94	42	72
isomaleimides			
4-Cl	99	50	77
4-Me	99	48	61
4-F	82	43	71
maleamic acids			
4-Cl	91	49	77
4-Me	98[b]	43	60
4-F	106	50[b]	81
fumaramic acid			
4-Cl	100	38	41[b]
succinamic acid			
4-Cl	110[b]	37	50
R-25788	85 (98)[c]	65	79
flurazole	73 (75)[c]	68	74

[a] Untreated control: 72 mg/plant 10 days after sowing. The same relationships are evident throughout with plant height as the criterion. [b] SE values of 13–18%. In all other cases SE values of up to 12%. [c] Antidote alone at 10 ppm.

Table IV. Effect of Application Time on Action of Three Antidotes for Alachlor Injury in Sorghum

antidote compd (ppm)	antidote treatment day	shoot fresh weight, % of control[a]: antidote alone	antidote with alachlor treatment, 2 ppm, on indicated day: 0	2	4
none (control)		100	53	29	58
CPMI (100)	0	96	85	61[b]	70[b]
	2	97		53	70
	4	92			80[b]
flurazole (10)	0	86	81	77	77
	2	91[b]		82	83
	4	90			84
R-25788 (10)	0	99	93	40	62
	2	100		47	74
	4	101			61

[a] Untreatment control: 80 mg/plant 14 days after sowing. [b] SE values of 14–16%. In all other cases SE values of up to 12%.

Relationship between Application Time and Antidotal Effect of CPMI, Flurazole, and R-25788 in Sorghum. Alachlor under the conditions used is more phytotoxic when applied at 2 than at 0 days after sowing (Table IV) possibly due to greater root exposure to the herbicide. At 7 days after sowing alachlor caused no crop injury. Simultaneous treatment of CPMI at 100 ppm with alachlor at 2 ppm immediately after sowing showed the highest protection, as compared to delayed application of any combination of the two compounds. Flurazole at 10 ppm gave good and consistent protection at all application

Table V. Spectrum of Antidotal Action with Various Crops and Herbicides on Preemergence Applications

herbicide and antidote (ppm)	shoot fresh weight, % of control[a]					
	sorghum	corn	rice	wheat	cotton	soybean
antidote alone						
CPMI (100)	97[b]	86[b]	89	108	95	58
flurazole (10)	86[b]	103	90	92	96	97
R-25788 (10)	94[b]	97	93	95	99	100
alachlor (2)						
none	41[b]	107	0	45	76	91
CPMI (100)	80	76	0	67[b]	63	52
flurazole (10)	78	99	14	48	77	91
R-25788 (10)	73	92[b]	0	75	72	82
EPTC (10)						
none	0	101	0	0	0	46
CPMI (100)	0	81	0	0	0	38
flurazole (10)	0	97	0	0	10	52
R-25788 (10)	0	102	0	0	0	44
chlorsulfuron (20 ppb)						
none	102[c]	81[d]	61	94	65	41
CPMI (100)	87[c]	66[d]	77	108[b]	67	17
flurazole (10)	100[b]	84[b,c]	86	100	57	37
R-25788 (10)	94	91[c]	79	106[b]	56	35

[a] Untreated controls (mg/plant): sorghum 65, corn 457, rice 35, wheat 114, cotton 435, and soybean 456. [b] SE values of 13–19%. In all other cases SE values of up to 12%. [c] Slight inhibition of root growth. [d] Severe inhibition of root growth.

times. The antidotal effect of R-25788 at 10 ppm was similar to that of CPMI at 100 ppm. All antidotes at any application time showed little or no phytotoxicity.

Spectrum of Antidotal Action with Various Crops and Herbicides. The antidotal effectiveness was compared for CPMI, flurazole, and R-25788 on six major crops against alachlor, EPTC, and chlorosulfuron injury (Table V). The antidotes alone were not phytotoxic except for CPMI with soybean. Alachlor at 2 ppm severely damaged sorghum, rice, and wheat with lesser injury to cotton. Protection against alachlor injury was provided by all three antidotes in sorghum and also by CPMI and R-25788 in wheat. EPTC at 10 ppm caused severe injury to all crops but corn and the antidotes did not alleviate its phytotoxicity in sensitive crops. Chlorsulfuron at 20 ppb showed strong phytotoxicity in soybean, cotton, and rice and good tolerance in wheat. This herbicide did not greatly decrease the fresh weight of sorghum and corn shoots but almost completely inhibited their root elongation. R-25788 showed slight protection of corn from chlorsulfuron injury in which the root inhibition was considerably lowered. No one of the antidotes at 10 or 100 ppm under the present conditions was phytotoxic to barnyardgrass, Johnson grass, or green foxtail, whereas alachlor at 2 ppm completely killed these weeds. CPMI and R-25788 did not reduce the herbicidal activity of alachlor but flurazole completely protected Johnson grass from this herbicide.

Hydrolytic Stability of *N*-(4-Chlorophenyl)maleimide and *N*-(4-Chlorophenyl)isomaleimide. CPMI and the corresponding isomaleimide (CPIM) were rapidly hydrolyzed to CPMA in pH 7.4, 0.2 M phosphate buffer at 25 °C (Figure 2) with half-life times of ~2 h. Similar results were obtained at pH 6.8. CPMA was stable under these conditions and no hydrolysis was observed over a period of 15 days.

Uptake and Distribution of [^{14}C]CPMA and [^{14}C]-Alachlor and Metabolism of [^{14}C]Alachlor in Sorghum. Autoradiography revealed that on uptake in sorghum [^{14}C]CPMA was accumulated mainly in the roots and lower shoots of both 4- and 10-day-old plants, with slight translocation to the shoot and some accumulation in the coleptile and first node.

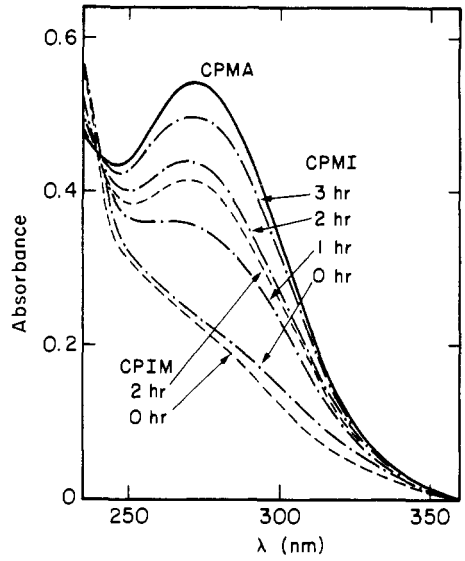

Figure 2. Ultraviolet spectra illustrating hydrolytic conversion of a representative maleimide and isomaleimide to the corresponding maleamic acid.

Table VI. Effect of Antidotes on Uptake and Distribution of Radioactivity from [^{14}C]Alachor in Sorghum Plants

antidote	^{14}C, %			
	uptake[a]	distributn, % of total		
		root	seed	shoot
4 Days after Treatment				
none	100 ± 20[b]	47 ± 10	41 ± 8	12 ± 5
CPMA	218 ± 38	51 ± 7	37 ± 7	12 ± 9
flurazole	209 ± 15	49 ± 8	39 ± 3	12 ± 6
7 Days after Treatment				
none	100 ± 11[b]	73 ± 8	15 ± 5	12 ± 2
CPMA	96 ± 12	80 ± 7	12 ± 4	8 ± 4
flurazole	145 ± 9	76 ± 1	17 ± 4	7 ± 2

[a] 6110 dpm/plant at 4 days and 26 340 dpm/plant at 7 days. [b] SE values.

[^{14}C]Alachlor applied preemergence was readily absorbed by sorghum seedlings. Immediately following emergence (4 days after sowing), the ^{14}C was mainly found in the seeds and roots and CPMA and flurazole significantly increased the uptake without altering the distribution pattern (Table VI). Seven days after treatment, the total amount absorbed was increased by 2–4-fold and the only antidote-related differences were in increased uptake with flurazole and reduced shoot levels with the antidotes. Autoradiography revealed that at 7 days the ^{14}C was distributed throughout the plant.

[^{14}C]Alachlor metabolism was unaffected by the antidotes based on analyses of the plant material 7 days after treatment involving ethanol extraction and TLC. The extraction recovered 75–80% of the total ^{14}C in both roots and shoots with no differences between alachlor alone and alachlor with CPMA treatments. No parent compound was found in the root or shoot extracts and all metabolites were at R_f 0.00 vs. alachlor at R_f 0.68 in solvent system A. TLC system B revealed 4 major root metabolites at R_f 0.34, 0.45, 0.48, and 0.63 (alachlor R_f was 0.95) and two minor metabolites at R_f 0.4 and 0.6. No qualitative nor quantitative differences were found between the alachlor alone and alachlor with CPMA treatments. Shoot extracts had three major products (R_f 0.33, 0.50, and 0.61 in solvent system B) and an additional major spot in the solvent front which contained large amounts of extracted chlorophyll. The lack of antidote-related differences also extended to the shoot extracts.

Table VII. Effect of Preemergence Application of Various Antidotes on Glutathione (GSH) Content in Sorghum Roots

antidote	alachlor, ppm	GSH, μmol/g fresh wt		ratio rel to control	
		4 days	7 days	4 days	7 days
none	0	0.87 ± 0.01[a]	0.47 ± 0.04	1.00	1.00
	1	1.00 ± 0.06	0.55 ± 0.04	1.15	1.17
CPMI	0	1.38 ± 0.05	0.80 ± 0.10	1.59	1.70
(100 ppm)	1	1.46 ± 0.05	0.78 ± 0.03	1.68	1.66
CPMA	0	1.42 ± 0.05	0.77 ± 0.04	1.63	1.64
(100 ppm)	1	1.49 ± 0.08	0.78 ± 0.04	1.71	1.66
flurazole	0	1.69 ± 0.02	1.18 ± 0.13	1.94	2.51
(10 ppm)	1	1.73 ± 0.02	1.09 ± 0.07	1.99	2.32
R-25788	0	1.59 ± 0.01	0.95 ± 0.03	1.83	2.02
(10 ppm)	1	1.59 ± 0.01	1.05 ± 0.06	1.83	2.23

[a] SE values.

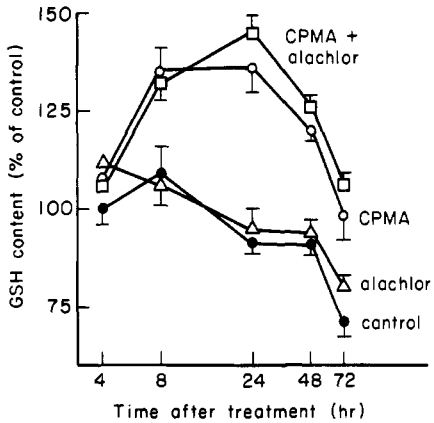

Figure 3. Effect of *N*-(4-chlorophenyl)maleamic acid (CPMA) and alachlor alone and in combination on the glutathione (GSH) content of sorghum roots. GSH content is relative to the control at 4 h.

Effects of Antidotes on GSH Content in Sorghum Roots. Alachlor gave a 15% and the antidotes (CPMI, CPMA, flurazole and R-25788) a 60–130% increase in the GSH content (determined as free thiol) in sorghum roots 4 and 7 days after sowing and treatment (Table VII). The ratio for elevation of GSH content was maintained or increased between days 4 and 7 even though the absolute content of GSH declined. Simultaneous treatment with the antidotes and alachlor increased the GSH content in the same pattern obtained with the antidotes alone. Barnyardgrass roots contained lower levels of GSH (0.51 ± 0.02 and 0.28 ± 0.05 μmol/g of fresh weight at 4 and 7 days after sowing, respectively) but CPMA did not induce any increase in this level. A more detailed analysis of the antidote-induced changes in GSH content in 4-day-old sorghum plants is shown in Figure 3. The elevated GSH content was evident at 8–48 h after application of CPMA with or without alachlor.

The maleimides NEM and CPMI reacted rapidly with GSH whereas CPMA and alachlor did not. Under the test conditions, the unreacted GSH was 100% in the control, 24 ± 4% for NEM, 36 ± 5% for CPMI, and 93 ± 6% for each of CPMA and alachlor, with similar results at a 10:1 and 100:1 ratio of candidate thiol reagent:GSH.

DISCUSSION

Alachlor and some other chloroacetanilides provide selective control of grass and certain broad-leafed weeds in soybean and corn. Their selective action is extended to sorghum with suitable antidotes or safeners, including flurazole (Brinker et al., 1982) and appropriate acetonitrile derivatives (e.g., cyometrinil and CGA-92194) (Rufener et al., 1982). Alachlor is detoxified in part by GSH conjugation which is facilitated by antidote action (Fedtke, 1981; Hatzios and Penner, 1982; Leavitt and Penner, 1979; Mozer et al., 1983; Stephenson and Ezra, 1982; Stephenson et al., 1983). Compounds that alter GSH levels may therefore influence the phytotoxicity of alachlor or be herbicidal themselves.

Maleimides were selected for emphasis because of their reactivity with thiols, e.g., NEM and CPMI in the present studies. The *N*-alkylmaleimides were neither phytotoxic nor alachlor antidotes, suggesting they may be too unstable. *N*-Phenylmaleimides, however, exhibit selective antidotal activity, protecting sorghum but not barnyardgrass from alachlor. Substitution of the maleimide ring with one or two methyl groups or a tetramethylene moiety results in compounds that are herbicides (see also Ohta et al., 1976) rather than antidotes. The present study establishes that the unsubstituted maleimide ring is favorable but not essential for antidotal activity, indicating the importance of an interaction between the unsaturated bond and a site critical for antidotal effect. *N*-Phenylmaleimides, -isomaleimides, and -maleamic acids have similar substituent effects at the benzene ring on antidotal activity with a clear advantage for chloro, fluoro, or methyl substituents at the 4-position. Disubstitution at any position of the benzene ring reduces the activity. The *N*-phenylmaleimides and -isomaleimides are sufficiently unstable that they undoubtedly hydrolyze to the corresponding maleamic acids before exerting their antidotal action. These results along with the low activity of the trans isomer of CPMA indicate the importance for optimal potency of suitable steric character around both the benzene ring and the unsaturated bond.

The antidotal actions of flurazole and R-25788 are more general (Hatzios, 1983, 1984; Schafer et al., 1980; Stephenson and Ezra, 1982) than that of maleimide CPMI relative to both the herbicide and the plant. CPMI acts only with alachlor in sorghum and less so in wheat. This may be an advantage for CPMI since the action of flurazole extends to protecting the weed Johnson grass from alachlor (Schafer et al., 1981). The activity of CPMI is dependent on proper timing, e.g., simultaneously with the herbicide immediately after sowing or potentially as a seed dressing, whereas timing is less critical with the more potent and stable flurazole.

The mechanism of antidotal action appears to involve conversion of the maleimides and isomaleimides to the maleamic acids, e.g., CPMA. The antidote does not act by inhibiting alachlor uptake or distribution or modifying the metabolic products. Thus, [^{14}C]CPMA and [^{14}C]-alachlor are readily absorbed by sorghum plants but radiocarbon from [^{14}C]alachlor is more extensively translocated. The present study does not evaluate if the rate or site of alachlor metabolism in the plant is altered. All of the antidotes examined here increased the sorghum GSH content and CPMA did not increase the GSH level of barnyardgrass. The initial effect of CPMI could conceivably be due to its reaction with thiols (Augustin et al., 1978) but this is not consistent with either the elevated GSH level in vivo or the similar action of CPMA which does not react with thiols. The *N*-(substituted phenyl)-maleamic acids therefore become a new class of herbicide antidotes useful as probes in examining species specificity and the factors controlling GSH synthesis and functions in plants.

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Registry No. 4-ClPMI, 1631-29-4; 4-MePMI, 1631-28-3; 4-

FPMI, 6633-22-3; 2-MePMI, 4067-01-0; 4-OMePMI, 1081-17-0; PMI, 941-69-5; 4-NO_2PMI, 4338-06-1; 2,4-Cl_2PMI, 26396-57-6; 3-ClPMI, 1204-35-9; 3,4-Cl_2PMI, 19844-27-0; 2,6-Me_2PMI, 1206-49-1; 3-AcPMI, 95695-43-5; 4-EtPMI, 76620-00-3; 4-OEtPMI, 19077-60-2; 4-AcPMI, 1082-85-5; 4-*n*-BuPMI, 65833-02-5; 4-CNPMI, 31489-18-6; 2,4-$(OMe)_2$PMI, 67154-42-1; 2-ClPMI, 1203-24-3; 4-ClPIM, 19990-27-3; 4-MePIM, 19990-25-1; 4-FPIM, 95695-44-6; 2-MePIM, 95048-06-9; 4-OMePIM, 19990-24-0; PIM, 19990-26-2; 4-NO_2PIM, 59333-51-6; 2,4-Cl_2PIM, 95695-45-7; 3-ClPIM, 71783-37-4; 3,4-Cl_2PIM, 72291-57-7; 2,6-Me_2PIM, 64363-00-4; 3-AcPIM, 95695-46-8; 4-EtPIM, 63408-20-8; 4-OEtPIM, 94934-03-9; 4-AcPIM, 19990-28-4; 4-*n*-BuPIM, 95695-49-1; 4-CNPIM, 64868-92-4; 2,4-$(OMe)_2$PIM, 95695-50-4; 2-ClPIM, 71782-74-6; 4-ClPMA, 7242-16-2; 4-MePMA, 24870-11-9; 4-FPMA, 780-05-2; 2-MePMA, 53616-19-6; 4-OMePMA, 24870-10-8; PMA, 555-59-9; 4-NO_2PMA, 36342-10-6; 2,4-Cl_2PMA, 95695-47-9; 3-ClPMA, 18196-80-0; 3,4-Cl_2PMA, 21395-61-9; 2,6-Me_2PMA, 31460-31-8; 3-AcPMA, 95695-48-0; 4-EtPMA, 55750-37-3; 4-OEtPMA, 51992-13-3; 4-AcPMA, 24870-12-0; 4-*n*-BuPMA, 6953-82-8; 4-CNPMA, 31460-28-3; 2,4-$(OMe)_2$PMA, 95695-51-5; 2-ClPMA, 53616-16-3; R-25788, 37764-25-3; EPTC, 759-94-4; 3-methylmaleimide, 1072-87-3; 3,4-dimethylmaleimide, 17825-86-4; tetrahydrophthalimide, 85-40-5; phthalimide, 85-41-6; succinimide, 123-56-8; acetamide, 60-35-5; crotonamide, 23350-58-5; 1-methylmaleimide, 930-88-1; 1-ethylmaleimide, 128-53-0; 1-ethyl-3-methylmaleimide, 31217-72-8; 1-ethyl-3,4-dimethylmaleimide, 34316-52-4; 1-propylmaleimide, 21746-40-7; 1-isopropylmaleimide, 1073-93-4; 1-isobutylmaleimide, 4120-68-7; furaramic acid, 2987-87-3; succinamic acid, 638-32-4; alachlor, 15972-60-8; chlorsulfuron, 64902-72-3; flurazole, 72850-64-7; glutathione, 70-18-8.

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