Pretilachlor [2-Chloro-*N*-(2,6-diethylphenyl-*N*-(2-propoxyethyl)acetamide]- and Butachlor [*N*-(Butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide]-Induced Accumulation of Phytoalexin in Rice (*Oryza sativa*) Plants

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The chloroacetamide herbicides pretilachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(2-propoxyethyl)-acetamide] and butachlor [*N*-(butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide] trigger accumulation of the phytoalexins momilactone A and sakuranetin in rice leaves. This ability was found to be specific to pretilachlor and butachlor. The accumulation of these phytoalexins was related to the herbicide concentration and period of exposure and was followed by the appearance of necrotic lesions on the rice leaves.

Keywords: *Phytoalexin; rice plant; accumulation; pretilachlor; butachlor*

INTRODUCTION

The accumulation of phytoalexins in response to microbial invasion is considered a defense mechanism in higher plants (Bailey, 1982). The compounds known as rice plant phytoalexins include the diterpenoid phytoalexins momilactones A and B (Cartwright et al., 1977); oryzalexins A–E (Akatsuka et al., 1983, 1985; Kono et al., 1984, 1985; Sekido et al., 1986; Kato et al., 1993) and S (Kodama et al., 1992a; Tamogami et al., 1993), and the flavonoid phytoalexin sakuranetin (Kodama et al., 1992b). Their production in rice plants is triggered by fungal invasion or by abiotic treatments with heavy metals (Kodama et al., 1988a) and ultraviolet irradiation (Kodama et al., 1988b). Phytoalexin accumulation is also brought about by, or associated with, cell death or stress caused by chemicals. For example, acifluorfen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid], a diphenyl ether herbicide, increases phytoalexin contents in the leaves of various crops, e.g. soybean (*Glycine max*) (Kömives and Casida, 1983). This means that phytotoxic compounds, such as herbicides, have the potential to trigger the accumulation of phytoalexins. As our interest is in chemicals that trigger phytoalexin accumulation in the rice plant, we screened various types of herbicides for their elicitation of momilactone A and sakuranetin (Figure 1). Momilactone A, a typical diterpenoid phytoalexin, and sakuranetin, an important new type of phytoalexin with a flavonoid skeleton, are accumulated in much higher amounts than other phytoalexins in rice plants.

MATERIALS AND METHODS

Apparatus. Phytoalexin contents were analyzed in a Shimadzu QP-1000 gas chromatograph–mass spectrometer. A bonded phase fused silica capillary column (250 m × 0.2 mm, 0.33 μm film thickness, Hewlett-Packard) was inserted directly into the ion source of the machine.

Plant Material. Rice plants (*Oryza sativa* L. Nipponbare) were cultivated in a greenhouse. At the sixth leaf stage, the fifth leaves were detached and assayed for elicitor activity.

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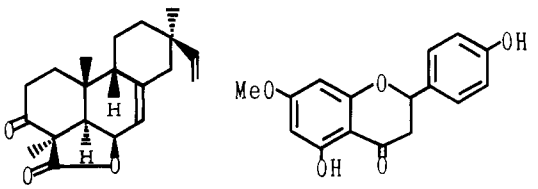

momilactone A sakuranetin

Figure 1. Typical phytoalexins found in rice plants.

Chemicals. The phytoalexins momilactone A and sakuranetin were isolated from ultraviolet-irradiated rice leaves (Kodama et al., 1988b, 1992b). The herbicides tested for elicitor activity were all gifts from Dr. T. Takematsu (Utsunomiya University, Japan).

Procedure. Elicitor activity was measured as the accumulation of phytoalexins as reported elsewhere (Kodama et al., 1988a).

1. Elicitation. Droplets of the test herbicide solution (1 mM, 25 μL) were applied to press-injured spots on rice leaves. The treated leaves were kept under high humidity. After an appropriate incubation period, the leaf spots were treated with methanol to obtain the phytoalexins. The crude extract containing the phytoalexins was purified with Bond Elute (C_{18} octadecyl, Varian) prior to analysis.

2. Analysis. Phytoalexins obtained from the rice leaves were quantified by gas chromatography–mass spectrometry–selected ion monitoring (GC–MS–SIM). Results were compared with values obtained for authentic compounds.

RESULTS AND DISCUSSION

We examined a variety of common herbicides (Table 1) for elicitor activity and found that pretilachlor and butachlor (Figure 2) greatly increased the content of momilactone A and sakuranetin in rice leaves. Other herbicides tested did not. We therefore made a more detailed examination of the elicitor activity of chloroacetamide-type herbicides.

Time courses of dose–responses for elicitor activity were examined for pretilachlor, butachlor, and alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide]. Seventy-two hours after treatment with pretilachlor and butachlor, the accumulation of momilactone A was greatly increased, evidence that verified

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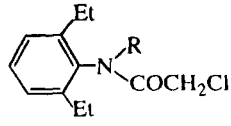
Table 1. Herbicides Screened for Elicitor Activity

mode of action	herbicide name
photosynthesis inhibition	simetryn [*N,N'*-diethyl-6-(methythio)-1,3,5-triazine-2,4-diamine], atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine], bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide]
glutamate synthesis inhibition	bialaphos [(2-amino-4-methylphosphinobutyryl)alanylalanine sodium salt]
cell division inhibition	trifluralin [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine], swep [methyl (3,4-dichlorophenyl)carbamate)], terbutol [2,6-bis(1,1-dimethylethyl)-4-methylphenyl methylcabamate), dymron [1-(α,α-dimethylbenzyl)-3-(*p*-tolyl)urea]
protein synthesis inhibition	pretilachlor, butachlor, alachlor, perfluidone [1,1,1-trifluoro-*N*-[2-methyl-4-(phenylsulfonyl)phenyl]methanesulfonamide], thiobencarb[[*S*-(4-chlorophenyl)methyl]diethylcarbamothioate]
chlorophyll synthesis inhibition	pyrazolate [4-(2,4-dichlorobenzoyl)-1,3-dimethyl-1*H*-pyrazol-5-yl *p*-toluenesulfonate], oxadiazon [3-[2,4-dichloro-5-(1-methylethoxy)phenyl]-5-(1,1-dimethylethyl)-1,3,4-oxadiazol-2(3*H*)-one]
bleaching	chlomethoxynil (2,4-dichlorophenyl 3-methoxy-4-nitrophenyl ether)
auxin	naproanilide [2-(2-naphthyloxy)propionanilide]
auxin inhibition	credazine [3-(*o*-tolyloxy)pyridazine], maleic hydrazide (1,2-dihydro-3,6-pyridazinedione), flurecol-butyl (butyl 9-hydroxyfluorene-9-carboxylate)



R	Name
$CH_2CH_2OCH_2CH_2CH_3$	pretilachlor
$CH_2OCH_2CH_2CH_2CH_3$	butachlor
CH_2OCH_3	alachlor

Figure 2. Structures of the herbicides tested.

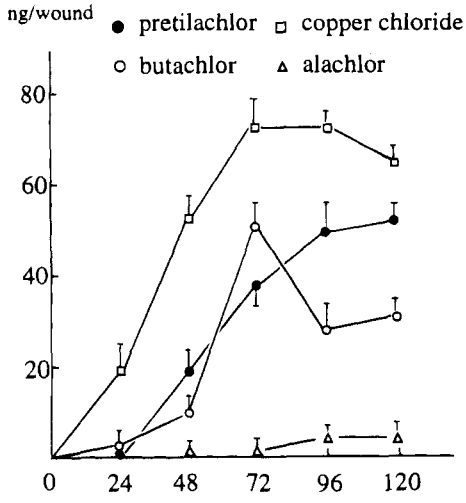


Figure 3. Momilactone A contents in rice leaves at various times after treatment with 1 mM elicitor. Each point is the mean of two determinations. Bars indicate SE.

accumulation was dependent on the exposure period (Figures 3 and 4). In contrast, alachlor showed negligible activity, in spite of its chemical structure similarity to pretilachlor and butachlor. The potent elicitor copper dichloride (Kodama et al., 1988a), however, had activity similar to that of pretilachlor and butachlor for sakuranetin but greater than that for momilactone A. The optimum dose of pretilachlor for the accumulation of both momilactone A and sakuranetin appears to be 2.5 mM (Figures 5 and 6). Butachlor showed similar accumulation activity; its optimum dose was about 1 mM for sakuranetin and about 2.5 mM for momilactone A. Alachlor showed negligible activity at all doses. The wound margins (areas surrounding the wound) treated with pretilachlor and butachlor remained dark green, showing there was no severe damage, whereas wound margins treated with alachlor were pale yellow, indica-

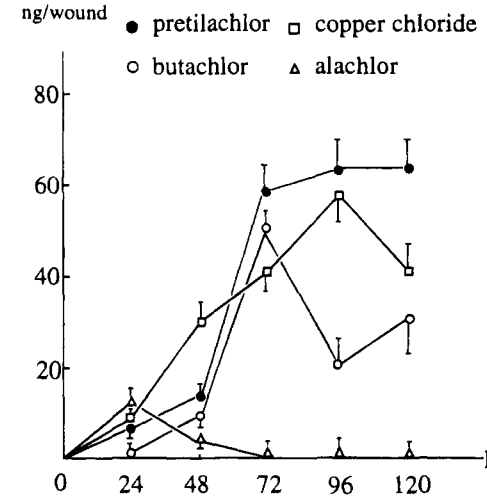


Figure 4. Sakuranetin contents in rice leaves at various times after treatment with 1 mM elicitor. Each point is the mean of two determinations. Bars indicate SE.

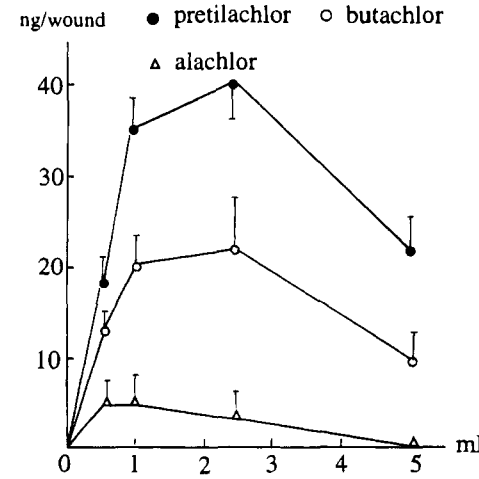


Figure 5. Momilactone A contents in rice leaves at various doses of elicitors 96 h after treatment. Each point is the mean of two determinations. Bars indicate SE.

tive of severe tissue damage. Cell death or cell stress caused by heavy metals (Kodama et al., 1988a) and by ultraviolet irradiation (Kodama et al., 1988b) may trigger the accumulation of phytoalexins. The accumulation caused by pretilachlor and butachlor, however, is not the result of nonspecific herbicidal cell death or stress because it appears to be specific for these two herbicides, whereas alachlor was inactive. Sakuranetin production has been suggested to occur by *de novo*

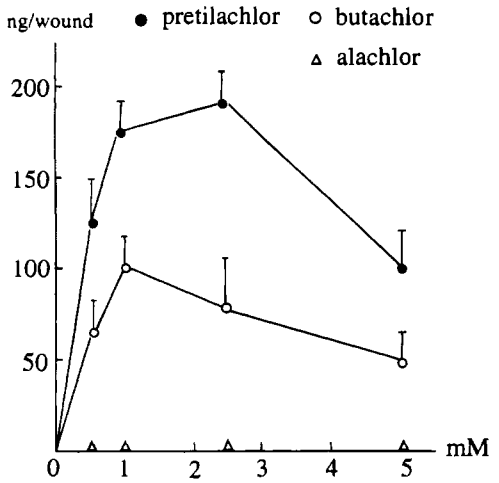


Figure 6. Sakuranetin contents in rice leaves at various doses of elicitors 96 h after treatment. Each point is the mean of two determinations. Bars indicate SE.

synthesis from phenylalanine (Kodama et al., 1992b). Results of a study of herbicide action and phenolic metabolism in plants indicate that certain herbicides have phenylalanine ammonialyase (PAL)-inducing activity (Kömives and Casida, 1982). The action of pretilachlor and butachlor may reflect increased PAL activity leading to the accumulation of sakuranetin. A clear understanding of the elicitor activity mechanism is needed to determine the mode of action of chloroacetamide-type herbicides.

The accumulation of phytoalexins in rice plants triggered by herbicides is interesting in another respect. We have suggested that in rice plant the phytoalexins momilactones A and B and the oryzalexins A–D and S may be synthesized from a common precursor, geranyl geranyl pyrophosphate (Tamogami et al., 1993). Geranyl geranyl pyrophosphate is a known precursor of kaurene in the pathway to the well-known plant growth hormone gibberellic acid. If production of these phytoalexins affects the biosynthesis of gibberellic acid, then elicitors (chemicals that trigger accumulation of phytoalexins) themselves may function as plant growth regulators. In rice plants, elicitor activity therefore may be required for plant protection and growth regulation.

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