Flower Induction in Asparagus Seedlings by Anilide and Benzamide Derivatives

Takane Hara, Naoko Wada, and Hajime Iwamura*

Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

Two series of anilides (N-phenylalkanamides) and N-alkylbenzamides induced flowers in 1-month-old seedlings of *Asparagus officinalis* L. The active compounds in both series all had a p-halo- or m,p-dihalobenzene and an alkane moiety of three to five carbons. The most potent compounds caused about 90% of the plants to flower when the seeds were germinated in their presence and planted in vermiculite. The flowering rate was as high as that caused by the s-triazine and carbamate compounds we measured earlier, but the inhibition of germination and emergence from vermiculite was in general less. N-Cyclohexyl-p-chlorobenzamide was more useful for selection of males of this perennial plant, which are commercially desirable, than the previous triazine and carbamate compounds.

Asparagus is a dioecious plant, and males are preferred for commercial production because of their greater vigor, yield, and longevity. It is impossible to distinguish the sexes until flowering, which normally starts in the second or third year. Based on the finding that the s-triazine herbicide atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and the urea herbicide DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] induce flowering in 1-month-old seedlings of this perennial plant (Abe and Kameya, 1986), we have tested s-triazine and carbamate anticytokinins (Shimizu et al., 1989, 1990), finding that they induce flowering more effectively and are less toxic than the original herbicides (Abe et al., 1987; Yanosaka et al., 1989). This has made it possible to select males in the seedling stage and to grow fields of males only. In this study, we developed a new series of flower-inducing compounds that have anilide and benzamide structures and found that they are more effective for this use than the previously evaluated s-triazine and carbamate compounds.

MATERIALS AND METHODS

Chemicals. Anilides 1–26 were prepared by reaction of an aniline with an excess (1.5×) of an appropriate acid chloride in anhydrous toluene at room temperature with stirring overnight. The reaction mixture was washed with water, aqueous NaHCO₃, 1 N HCl, and water, and the toluene layer was dried over anhydrous Na₂SO₄ and evaporated at reduced pressure. The residue was purified by recrystallization from ethanol or ethanol–H₂O or by chromatography on a silica gel (Wakogel C-200) column with n-hexane, n-hexane–acetone, n-hexane–EtOAc, or n-hexane–CHCl₃ as the solvent. The preparation of carbamates 27–30 was reported previously (Shimizu et al., 1990). Benzamides 31–37 and the related compounds 38 and 39 were similarly prepared from an appropriate benzoyl chloride and an alkyl- or cycloalkylamine. Structures were identified by ¹H NMR spectra recorded on a JEOL PMX-60 spectrometer in CDCl₃ or Me₂SO-d₆ with tetramethylsilane as the internal reference and by elemental analysis for C, H, and N within the error of ±0.3%.

Flower Induction. The method was essentially the same as that reported previously (Yanosaka et al., 1989). The chemicals were dissolved in dimethyl sulfoxide, and the solution was diluted with distilled water to an appropriate concentration so that the final concentration of the organic solvent did not exceed 0.5% (v/v). Forty seeds of *Asparagus officinalis* L. cv. Mary Washington 500W (a widely used cultivar) were placed in a Petri dish (90 mm in diameter, 15 mm high) that contained three layers of filter paper (No. 2, Toyo Rosi Kaisha) and 20 mL of the test solution. The seeds were incubated at 25 °C for 12 days with a

12-h period of light from fluorescent lamps (National FL 40SS.D/37 and FL 200SD, 80 W m⁻¹). The germination percentages were noted at the end of the incubation period. The germinated seeds were washed in running water for 10 min, planted in vermiculite, and grown for 13 days at 25 °C under the same light conditions as above. The flowering rate (percent) was expressed as (no. of plants with flowers) (no. of plants that emerged from vermiculite) × 100. The maximum range of the experimental error was ±10%. Not all of the germinated seeds that had been transplanted emerged from the vermiculite, so the emergence percentage was estimated as (no. of plants that emerged from vermiculite)/(no. of seedlings planted) × 100. The range of experimental error was within ±8% during germination and emergence. Means are given in Tables I and II.

RESULTS

The compounds listed in Tables I and II were assayed with *A. officinalis* L. cv. Mary Washington 500W, a representative cultivar in worldwide use. Of the anilides 1–26, the most active compounds were 18, 19, and 11, which gave flowering rates of 80–90%. Of the p-chloroanilides (1–3) with a n-alkanoyl in the acyl moiety, n-propyl 1 and n-butyl 2 gave higher flowering rates than n-amyl 3. Provided that length was appropriate, the compounds with suitable bulkiness because of one or two methyl branches or cyclization in the acid moiety (11, 15, and 17–19) were generally active. The results suggested that there is preferable steric condition for activity. Movement of the p-chloro substituent to the meta position gave 4, which had a weak effect, and movement to the ortho position gave 5, with no effect. Unsubstituted 9 had little activity. The replacement of the chlorine atom at para of 2 by fluorine or bromine atoms gave the less potent compounds 7 and 8. The 3,4-dichloro compounds 6 and 18 were more potent than the corresponding p-chloro derivatives 2 and 17. Compound 22 with electronically neutral 3,4-dimethyl substituent was far more active than the corresponding 21 with strong electron-withdrawing 3,4-difluoro group. The results indicated that the benzene substituents had position-specific steric and hydrophobic effects rather than electronic ones. In Table I, some representative potent carbamate compounds, 27–30, reported earlier (Yanosaka et al., 1989) are shown for comparison. Compounds 2, 6, and 18 of the present series correspond structurally to 27, 29, and 30, respectively, and they also were potent.

Benzamides are compounds in which the aminocarbonyl group is in the reverse orientation to that in anilides. The assay results are shown in Table II. Of o-, m-, and p-

Table I. Effects of Anilides and Related Compounds on Germination, Emergence, and Flowering of A. officinalis L. Cv. Mary Washington 500W

compd no.	structure X	structure R	germination, % 100 µM	200 µM	400 µM	emergence, % 100 µM	200 µM	400 µM	flowering, % 100 µM	200 µM	400 µM
					Anilides						
1	p-Cl	n-Pr	85	85	58	100	100	95	30	55	63
2	p-Cl	n-Bu	88	93	85	95	95	95	53	63	29
3	p-Cl	n-Am	98	88	85	100	100	100	15	15	15
4	m-Cl	n-Bu	93	95	90	100	95	90	15	11	28
5	o-Cl	n-Bu	95	95	83	100	95	100	0	0	0
6	3,4-Cl₂	n-Bu	95	95	68	100	95	85	25	74	65
7	p-F	n-Bu	95	88	80	100	95	95	10	21	11
8	p-Br	n-Bu	88	90	85	100	100	95	20	35	11
9	H	n-Bu	85	88	85	95	100	100	0	5	15
10	p-Cl	allyl	88	90	58	95	100	90	32	10	15
11	3,4-Cl₂	1-Me-n-Bu	93	88	90	95	95	95	37	80	58
12	3,4-Cl₂	1-Me-Am	90	95	88	95	95	95	42	42	47
13	p-Cl	2-Me-n-Bu	98	90	80	95	100	80	32	60	56
14	3,4-Cl₂	1-Et-Pr	95	88	88	90	95	90	22	37	22
15	3,4-Cl₂	t-Bu	90	93	95	95	100	90	63	70	78
16	H	neopentyl	90	85	75	100	100	100	10	25	20
17	p-Cl	neopentyl	93	88	80	100	100	100	65	58	75
18	3,4-Cl₂	neopentyl	95	98	83	90	95	90	78	90	67
19	p-Cl	cyclopropyl	88	80	40	80	70	93	50	86	57
20	p-Cl	cyclohexyl	95	93	88	100	100	95	35	30	26
21	3,4-F	cyclohexyl	95	90	85	95	75	75	21	0	0
22	3,4-Me₂	cyclohexyl	95	98	88	90	60	65	61	50	39
23	p-Cl	2-(cyclopentyl)-Et	88	90	80	95	95	96	0	5	5
24	p-Cl	benzyl	98	95	93	100	100	95	35	40	30
25	p-Cl	Ph	90	90	90	100	100	100	50	55	20
26	p-Cl	p-Cl-Ph	95	93	83	100	100	100	35	55	60
					Carbamates[a]						
27	p-Cl	O-n-Pr	95	75	70	75	75	55	67	95	64
28	p-CF₃	O-n-Pr	98	98	88	80	80	70	75	81	93
29	3,4-Cl₂	O-n-Pr	83	73	57	86	86	75	54	84	67
30	3,4-Cl₂	O-t-Bu	80	78	75	100	95	85	45	63	77
	control[b]			90			100			0	

[a] Cited from a previous report (Yanosaka et al., 1989). [b] With no addition to the medium except for 0.5% (v/v) dimethyl sulfoxide.

Table II. Effects of Benzamides and Related Compounds on Germination, Emergence, and Flowering of A. officinalis L. Cv. Mary Washington 500W

compd no.	structure X	structure R	germination, % 100 µM	200 µM	400 µM	emergence, % 100 µM	200 µM	400 µM	flowering, % 100 µM	200 µM	400 µM
31	p-Cl	n-Bu	88	83	58	95	95	95	26	47	42
32	p-Cl	neopentyl	100	85	83	100	90	100	50	50	65
33	p-F	cyclohexyl	90	93	85	100	95	95	60	74	68
34	p-Cl	cyclohexyl	98	90	93	100	100	100	80	75	70
35	m-Cl	cyclohexyl	95	93	93	84	89	81	59	78	93
36	o-Cl	cyclohexyl	98	95	93	72	71	49	18	48	67
37	3,4-Cl₂	cyclohexyl	90	83	83	72	55	67	40	71	41

compd no.	structure X	structure R	germination, % 100 µM	200 µM	400 µM	emergence, % 100 µM	200 µM	400 µM	flowering, % 100 µM	200 µM	400 µM
38	Et	cyclohexyl	65	95	90	77	71	61	0	15	0
39	n-Pr	cyclohexyl	90	95	98	67	89	41	4	12	7
	control[a]			90			100			0	

[a] See footnote b of Table I.

chlorobenzamides 34–36, the potency of the ortho compound 34 was lowest, but not as low as that of the corresponding o-anilide 5. N-n-Butyl 31 was less potent than the corresponding N-cyclohexyl 34. The relationship was the reverse of that seen between anilides 2 and 20. However, the compounds with a neopentyl of intermediate bulk, anilide 17 and benzamide 32, had about the same potency. Of all the compounds, compound 34 gave the highest flowering rate, 80%, at 100 µM, the lowest concentration tested, and high percentages of germination and emergence. The nonaromatic compounds 38 and 39 were only weakly active.

DISCUSSION

The anilide series of compounds have structures in which the oxygen atom of the carbamates reported before (Yanosaka et al., 1989) is replaced by a methylene. Benzamides are compounds in which the amide group is inserted in reverse to its orientation in anilides. The structure–activity profiles of these new series of compounds seemed to correspond roughly to those observed for the carbamate compounds, with differences in details reflecting a somewhat different mode of interaction with the receptive site. That all three series of compounds have flower-inducing activity in asparagus seedlings suggests that the function responsible for it is usually amide –CONH–. The mechanism of action of the new series of compounds is being explored, as is that of the carbamate compounds (Yanosaka et al., 1991). So far, flower induction occurs only in *A. officinalis* at the germination stage (Yanosaka et al., 1990).

Carbamate and amide functions are chemically similar, but they may be different in their toxicity, stability against metabolic attack, and permeation into plant tissues, causing differences in efficacy. The inhibition by the present series of compounds of germination and emergence from vermiculite were generally less than that of the previously described carbamates. In practical use, a low concentration should give a flowering rate, but germination and emergence percentages are also important, since yields after selection of the preferred males are the products of these three factors. This product for compound **34** at 100 μM was 78%, that for carbamate **27** was 53% at 200 μM, and that for compound **28** was 57% at 400 μM. The toxicity of compound **34** toward germination and emergence was about 20% less than that of the carbamates.

Supplementary Material Available: Table of analytical data concerned with the compounds prepared (1 page). Ordering information is given on any current masthead page.

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