Structural Requirements of Phenoxyalkanoic Acids and Related Compounds for Promotion of Flowering in *Sagittaria pygmaea* Miq.

Hitoshi Kuramochi,[†] Takumi Yoshimura,[‡] Takeshige Miyazawa,[§] Makoto Konnai,[†]
Tetsuo Takematsu,[†] and Koichi Yoneyama*,[†]

Weed Science Center, Utsunomiya University, 350 Mine-machi, Utsunomiya 321, Japan, K·I Chemical
Research Institute Company Ltd., Shioshinden Fukude-cho, Iwata-gun, Shizuoka, 437-12, Japan, and
Kumiai Chemical Industry Company Ltd., 1-4-26, Ikenohata, Taitoh-ku, Tokyo 110, Japan

The flowering of a perennial paddy weed, *Sagittaria pygmaea (S. pygmaea)* Miq., was promoted by 2,6-diisopropylphenoxyacetic acid, as well as by gibberellin A_3 (GA_3). Therefore, structural requirements of phenoxyalkanoic acids and related compounds for promotion of flowering in the weed were investigated. Among the synthesized phenoxyalkanoic acids, only the compounds having two bulky alkyl substituents on the 2- and 6-positions of the phenyl ring promoted flowering. In particular, 2,6-diisopropyl- and 2,6-di-*tert*-butylphenoxyacetic acids were highly effective promoters of flowering. 2,6-Diisopropylphenylacetic and -thioacetic acids were also active, but the corresponding phenylaminoacetic acid and a benzoic acid analogue were inactive on the promotion of flowering of the weed. Although the active compounds including 2,6-diisopropylphenoxyacetic acid may be classified as anti-auxins, typical anti-auxins including 2,6-dichlorophenoxyacetic acid did not promote flowering. These results demonstrate that the structural requirements of these compounds for promotion of flowering of *S. pygmaea* are somewhat similar but not identical to those for anti-auxins.

Keywords: *Flowering; phenoxyalkanoic acid; Sagittaria pygmaea Miq.; structural requirements*

INTRODUCTION

The flowering of *Sagittaria pygmaea* Miq., one of the troublesome perennial paddy weeds in Japan, is not under photoperiodic control, and the weed begins to produce flowers and tubers 50−60 days after planting irrespective of the planting date (Kusanagi, 1978, 1984). Gibberellic acid (GA_3) has been reported to promote flowering in *S. pygmaea* (Takasawa et al., 1981) and in two cultivars of Chinese arrowhead (*S. trifolia*) (Tanimoto, 1989), suggesting that endogenous GAs play an important role in floral induction in *Sagittaria* species. Our previous study with *S. pygmaea* supported this possibility, since GA_3 promoted flowering and furthermore two types of GA biosynthetic inhibitors, uniconazole [(*E*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol] and prohexadione (4-ethoxycarbonyl-3-hydroxy-5-oxo-3-cyclohexenecarboxylic acid), inhibited flowering (Kuramochi et al., 1996). We also found that AC-94377 [1-(4-chloro-1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)cyclohexanecarboxamide], which shows GA-like effects in various bioassays (Shanks, 1976), was a strong promoter of flowering. The flowering of *S. pygmaea*, however, was promoted not only by GA_3 and AC-94377 but also by 2,6-diisopropylphenoxyacetic acid. The promotive effect of AC-94377 can be attributable to its GA-like activity, while 2,6-diisopropylphenoxyacetic acid is inactive in bioassays typical for GAs. These synthetic compounds AC-94377 and the phenoxyacetic acid also promoted flowering of arrowhead (*S. trifolia* L.) and Chinese arrowhead (unpublished results). The lack of GA-like activity of the phenoxyacetic acid led us to consider that the compound may show GA-like activity only against *Sagittaria* species or that it may affect GA biosynthesis or metabolism of these plants.

A large number of substituted phenoxyalkanoic acids have been used as plant growth regulators and herbicides. They are potent auxins or anti-auxins, depending on their chemical structures (Åberg, 1961). In these phenoxy compounds, substituents on the phenyl ring and on the α-carbon atom have pronounced effects on their activities. For example, the introduction of a chlorine atom to the 6-position of 2,4-D (2,4-dichlorophenoxyacetic acid), a typical auxin, affords an anti-auxin (Åberg, 1961). In addition, an α,α-dimethyl derivative of 2,4-D, 2-(2,4-dichlorophenoxy)isobutyric acid, is also an anti-auxin (Wain and Wightman, 1957). However, none of these phenoxyalkanoic acids have been reported to exhibit GA-like effects. Therefore, 2,6-diisopropylphenoxyacetic acid is quite a unique compound in its promotive effect on the flowering of *Sagittaria* species. In this paper, structural requirements of phenoxyalkanoic acids and related compounds for promotion of flowering in *S. pygmaea* are described and are discussed in relation to those for auxin and anti-auxin activities.

MATERIALS AND METHODS

Plant Materials and Assay Methods. Plant materials and assay methods were essentially the same as in our previous report (Kuramochi et al., 1996). Tubers of *Sagittaria pygmaea* Miq. were collected locally from paddy fields in winter and were stored in moist vermiculite at 4 °C until use. All of the test compounds were examined for their effects on the flowering of *S. pygmaea* using ceramic pots and plastic containers in a greenhouse. Ceramic pots (15 cm i.d.) were filled with paddy soil and excessively watered to create paddy conditions. Four tubers of *S. pygmaea* in uniform size (0.07−0.08 g) were germinated in an incubator at 30 °C in the dark for 24 h and then transplanted to each pot to a depth of 2 cm. Aqueous solutions of the chemicals at the dosages of 0, 0.125,

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* Author to whom correspondence should be addressed (fax, +81-28-636-7662; e-mail, yoneyama@cc.utsunomiya-u.ac.jp).

[†] Utsunomiya University.

[‡] K·I Chemical Research Institute Co., Ltd.

[§] Kumiai Chemical Industry Co., Ltd.

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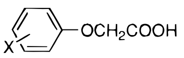
Table 1. Promotive Activity of Ring-Substituted Phenoxyacetic Acids on Flowering of *S. pygmaea*[a]



		flowering promotion	
chemicals	X	ED_{50} (kg/ha)	flowering plants (%) at 1 kg/ha
1	2,6-diisopropyl	0.25	91.7
2	2,6-di-*tert*-butyl	1.00	63.3
3	2,6-di-*sec*-butyl	4.00	13.3
4	2,6-dimethyl	>8.00	13.3
5	2,6-diethyl	>8.00	13.3
6	2,6-dimethoxy	>8.00	8.3
7	2,6-dichloro	>8.00	15.0
8	2-isopropyl-6-methoxy	4.00	20.0
9	2-isopropyl-6-propyl	1.00	61.7
10	2-isopropyl-6-ethyl	1.00	51.7
11	2-isopropyl-6-methyl	>8.00	13.3
12	2-isopropyl-6-bromo	>8.00	13.3
13	2-isopropyl	>8.00	13.3
14	2,6-diisopropyl-4-methyl	>8.00	8.3
15	2,6-diisopropyl-4-methoxy	>8.00	6.7
16	2,6-diisopropyl-4-chloro	>8.00	15.0
17	2,6-diisopropyl-4-amino	>8.00	13.3
AC-94377		0.125	86.7
GA_3		0.125	96.7
control			13.3

[a] Activities are expressed as ED_{50} values (kg/ha) and as the percent of the number of flowering plants in the plots treated with chemicals at 1 kg/ha.

0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 kg/ha were added to the irrigation water on the following day, and the pots were placed in a greenhouse maintained at 25−30 °C under natural daylight conditions. The level of flooding water was maintained at 3 cm throughout the experiments. The number of flowering plants was counted 45 days after treatment, and the promotive activities were expressed as ED_{50} values. The ED_{50} values indicate dosages (kg/ha) at which 50% of the treated plants produce flowers. In the experiment using plastic containers (20 cm × 30 cm × 15 cm), 10 tubers were planted to each container. The application rate of the chemicals was 1.0 kg/ha. The results were averaged and expressed as percent of the number of flowering plants in the treated plots. Under these experimental conditions, 13.3% (1.3 ± 0.7 plants/10 plants) of the untreated plants produced flowers. All of the experiments were repeated two times with three replications.

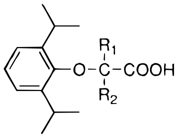
Chemicals. Phenoxyalkanoic acids and related compounds were synthesized according to the methods described in the literature (Synerholm and Zimmerman, 1945; Fawcett et al., 1953, 1955).

RESULTS

As reported previously, 2,6-diisopropylphenoxyacetic acid promoted not only flowering but shoot growth of *S. pygmaea* (Kuramochi et al., 1996). A similar tendency was also observed for the other compounds examined in this study, and the promotive activities on flowering were well-correlated to those on shoot elongation. Therefore, in general, the stronger promoters of flowering exhibited stronger promotion of shoot growth (data not shown). Some of the test compounds including 2,6-diisopropylphenoxyacetic acid slightly retarded the growth of the weed at dosages higher than 4.0 kg/ha. In particular, 2,6-diisopropylbenzoic acid showed a rather strong growth inhibition and inhibited shoot growth at dosages higher than 0.5 kg/ha.

Aromatic Substituents. Among the 2,6-disubstituted phenoxyacetic acids listed in Table 1, only the compounds having two bulky alkyl groups promoted flowering of *S. pygmaea*. The highest activity was observed in 2,6-diisopropylphenoxyacetic acid (**1**), while 2,6-dimethyl (**4**) and 2,6-diethyl (**5**) derivatives were not active even at the highest dosage tested (8 kg/ha). In the plots treated with 2,6-diisopropyl- and 2,6-di-*tert*-butylphenoxyacetic acids at 1 kg/ha, 91.7 and 63.3% of the plants produced inflorescence, respectively. By contrast, compounds having substituents other than alkyl groups, 2,6-dimethoxy (**6**) and 2,6-dichloro (**7**) derivatives, were inactive in the assay. 2-Isopropyl-6-substituted phenoxyacetic acids (**8**−**10**) showed moderate to strong promotion of flowering except for the 6-methyl (**11**) and 6-bromo (**12**) derivatives. All of the trisubstituted derivatives (**14**−**17**) were inactive in the assay. In particular, 2,6-diisopropyl-4-methyl (**14**) and 4-methoxy (**15**) derivatives showed a slight inhibition of flowering at 1 kg/ha.

Table 2. Promotive Activity of α-Substituted Phenoxyacetic Acids on Flowering of *S. pygmaea*[a]



			flowering promotion	
chemicals	R_1	R_2	ED_{50} (kg/ha)	flowering plants (%) at 1 kg/ha
18	methyl	H	0.50	78.3
19	ethyl	H	2.00	36.7
20	propyl	H	4.00	18.3
21	methyl	methyl	2.00	15.0

[a] Activities are expressed as ED_{50} values (kg/ha) and as the percent of the number of flowering plants in the plots treated with chemicals at 1 kg/ha.

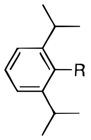
Substituent on α-Carbon Atom. Although the replacement of an α-hydrogen with an alkyl group affords optical isomers, synthesized compounds were assayed as racemic mixtures. Compounds having α-substituents listed in Table 2 were rather strong promoters of flowering. In general, the promotive effects of the α-substituted derivatives decreased with an increase in the α-side chain length.

Other Types of Compounds Having a 2,6-Diisopropylphenyl Moiety. The promotive activities of other types of compounds having a 2,6-diisopropylphenyl moiety are listed in Table 3. Among the compounds which lack a carboxyl group, 2-(2,6-diisopropylphenoxy)ethanol (**22**) showed the highest activity. In compounds **24**−**26**, the oxygen atom of the phenoxy moiety was replaced with a sulfur atom (**24**), methylene (**25**), or amino group (**26**). Although 2,6-diisopropylphenylthioacetic acid (**24**) and the phenylpropionic acid (**25**) were strong promoters of flowering, 2,6-diisopropylphenylaminoacetic acid (**26**) did not promote flowering even at the highest dosage. In addition, 2,6-diisopropylphenylacetic acid (**27**) was active, whereas 2,6-diisopropylbenzoic acid (**28**) was inactive in the assay.

DISCUSSION

Rather strict structural requirements are observed in the phenoxyalkanoic acids and related compounds for promotion of flowering of *S. pygmaea*. In particular, aromatic substituents were found to be major determinants of the activity. Only the compounds having bulky alkyl groups on both the 2- and 6-positions of the phenyl ring are active promoters of the flowering (Table 1). In these aromatic substituents, branched alkyl groups are

Table 3. Promotive Activity of Compounds Having a 2,6-Diisopropylphenyl Moiety on Flowering of *S. pygmaea*[a]



chemicals	R	flowering promotion: ED_{50} values (kg/ha)	flowering promotion: flowering plants (%) at 1 kg/ha
22	OCH_2CH_2OH	0.50	80.0
23	OCH_2CN	8.00	13.3
24	SCH_2COOH	2.00	45.0
25	CH_2CH_2COOH	0.50	83.3
26	$NHCH_2COOH$	>8.00	15.0
27	CH_2COOH	1.00	65.0
28	COOH	>8.00	11.7

[a] Activities are expressed as ED_{50} values (kg/ha) and as the percent of the number of flowering plants in the plots treated with chemicals at 1 kg/ha.

preferable for high activity. For example, the 2,6-diisopropyl derivative (**1**) is far more active than the 2-isopropyl-6-propyl derivative (**9**). Slightly decreased activity of 2,6-di-*tert*-butylphenoxyacetic acid (**2**) as compared to 2,6-diisopropylphenoxyacetic acid (**1**) may indicate that the *tert*-butyl group is too bulky to fit the receptor site. One of the aromatic substituents may be an alkoxy group, since the 2-isopropyl-6-methoxy derivative (**8**) was more active than the 2-isopropyl-6-methyl derivative (**11**). The lack of activity in mono- (**6**) and dihalogenated (**7**) derivatives can be interpreted to mean that the introduction of electron-withdrawing groups eliminates possible interactions between the molecule and the receptor site. Furthermore, the introduction of a *para* substituent to the active 2,6-diisopropylphenoxyacetic acid resulted in a complete loss of activity. These results clearly indicate that the substituents on the *ortho* positions interact with a rather unconstrained region of the receptor site, while there exist strict structural requirements for the *para* position of the phenyl ring.

According to the reports on structure−activity relationships of phenoxyalkanoic acids and related compounds for auxin and anti-auxin activities (Thompson et al., 1946; Wain and Wightman, 1957; Åberg, 1961), 2,6-disubstituted compounds, e.g. 2,6-dichlorophenoxyacetic acid (**7**), are classified as anti-auxins (Wain and Wightman, 1957; Åberg, 1961). In fact, 2,6-diisopropylphenoxyacetic acid (**1**) showed an antagonistic effect against 2,4-D (data not shown). However, the promotive effect of 2,6-diisopropylphenoxyacetic acid on the flowering of *S. pygmaea* may not be due to its anti-auxin activity, since 2,6-dichlorophenoxyacetic acid (**7**), a known anti-auxin (Wain and Wightman, 1957; Åberg, 1961), and all of the 2,4,6-trisubstituted derivatives, possibly anti-auxins, were inactive in the promotion of flowering. Furthermore, 2,3,5-triiodobenzoic acid (2,3,5-TIBA), a typical anti-auxin, did not promote flowering in the plant (data not shown).

The effects of the α-alkyl group on the promotive activity are somewhat similar to those reported for auxin or anti-auxin activity of phenoxyalkanoic acids (Smith et al., 1952; Wain and Wightman, 1957; Åberg, 1961; Fredga and Olsson, 1969). Although the data in Table 3 were obtained with racemic mixtures, effects of optical isomerism may be small in these 2,6-diisopropylphenoxyalkanoic acids in which rather bulky groups occupy both of the *ortho* positions (Åberg, 1961). However, one of the enantiomers of 2-(2,6-diisopropylphenoxy)propionic acid (**18**) may be more active than 2,6-diisopropylphenoxyacetic acid (**1**), since the inactive antipode may antagonize against the active one in the racemate (Fredga and Olsson, 1969).

Flowering promotive effectiveness of other types of compounds having a 2,6-diisopropylphenyl moiety demonstrates that the moiety itself is important for the activity. In addition, at least one bridging atom or group between the 2,6-diisopropylphenyl moiety and a carboxyl group is needed for activity. In the case of auxins, the introduction of an oxygen bridging group into 2,4-dichlorophenylacetic acid increases auxin activity, whereas the activities of the corresponding sulfur and amino derivatives are rather low (Thompson et al., 1946; Fawcett et al., 1955). Similar effects of the bridging group on activity are observed for the promotion of flowering, but the amino derivative (**26**) was almost inactive in this case. Although 2-(2,6-diisopropylphenoxy)ethanol (**22**) and 2,6-diisopropylphenoxyacetonitrile (**23**) which lack a carboxyl group exhibited strong and weak promotive activities, respectively, they might not be active in themselves. In soil or in the treated plants, some biochemical or chemical conversion giving rise to the active phenoxyacetic acid is likely to occur under the experimental conditions (Wain and Smith, 1976).

To date, numbers of attempts have been made to explain structural requirements for auxin or anti-auxin activity (e.g. Porter and Thimann, 1965; Kaethner, 1977; Farrimond et al., 1978; Katekar, 1979). Structural requirements of phenoxyalkanoic acids for promotion of flowering in *S. pygmaea* seem to be similar to those for anti-auxin activity, whereas typical anti-auxins including 2,6-dichlorophenoxyacetic acid (**7**) did not promote flowering. Furthermore, though GA_3 and AC-94377 promoted flowering of *S. pygmaea*, the most active 2,6-diisopropylphenoxyacetic acid is not active in bioassays for GAs. Therefore, any bioassay methods for auxins, anti-auxins, and GAs are not applicable for assessing the promotive activity of 2,6-disubstituted phenoxyacetic acids and related compounds on the flowering of *S. pygmaea*. Further study is needed to clarify structure−activity correlation and mechanisms involved in the promotion of flowering of *S. pygmaea*.

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