Preparation and Plant Growth-Regulatory Activity of N'-Chloro-Substituted Phenyl- and Benzyl-N-furfuryloxamides

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N'-Chlorophenyl- and N'-chlorobenzyl-N-furfuryloxamides (6b—e and 7b—d) were synthesized through the reaction of chloro-substituted anilines (12b—e) or chloro-substituted benzylamines (13b—d) with N-furfuryloxamic acid sodium salt (10), which was prepared *via* condensation of methyl oxalate (8) with furfurylamine, followed by hydrolysis of the resulting amide-ester with sodium hydroxide. The activity of the prepared compounds (6 and 7) as plant growth regulators was examined by a germination assay using rape and leek seeds.

N'-(3-Chlorophenyl)-N-furfuryloxamide (6c) showed a little effectiveness as an inhibitor, whereas the parent compound, N'-phenyl-N-furfuryloxamide (6a) inhibited the root growth of rape seedlings. The 2-chlorophenyl derivative (6b) inhibited the root growth of rape seedlings as potently as 6a, but sufficiently promoted the root growth of leek seedlings. Further, the 4-chlorophenyl derivative (6d) promoted the root growth of both species. On the other hand, in the series of chlorinated benzyl derivatives (7b—d), N'-(3-chlorobenzyl)-N-furfuryloxamide (7c) showed a markedly superior inhibitory effect in both species, but 2- and 4-chlorobenzyl derivatives (7b and 7d) inhibited the root growth of only rape seedlings.

Key words　　furan derivative; plant growth regulator; plant growth activity; rooting; halogenated oxamide; amidation

Plant growth regulators have a wide range of bio-physiological actions, modulating germination, rooting, elongation, flowering, fruiting, fruit abscission, and defoliation.[1] They are widely used in very small quantities in commercial agriculture for stimulation of rooting, control of fruit setting and development, promotion of ripening and delaying senescence.[2]

Many commercial plant growth regulators are synthetic organic compounds, which frequently have a haloaromatic group as a partial structure. For example, 4-chloro-phenoxyacetic acid (1; 4-CPA) is used to enhance fruiting of tomato and eggplant. Thus, tomato ripening was accelerated by treatment with 0.2% 4-CPA (1).[3] N-[4-Chloro-2-(hydroxyphenylmethyl)phenyl]-4-pyridine-carboxamide (2; inabenfide) has a selective antilodging effect in rice with no phytotoxic effects.[4] It has been reported that (E)-1-(4-chlorophenyl)-4,4-dimethyl-2-[(1,2,4-triazol)-1-yl]-1-pentan-3-ol (3; paclobutrazole) significantly reduced growth of the root zone of young greenhouse-grown tissue-culture-propagated Gala apple trees,[5] and (E)-1-(4-chlorophenyl)-4,4-dimethyl-2-[(1,2,4-triazol)-1-yl]-1-penten-3-ol (4; uniconazole) effectively promoted flowering of Japanese pear (*Pyrus serotina*) cultivar Kousui.[6] It has also been suggested that 4 can be used as antitranspirant.[7] 5-Chloroindoleacetic acid (5) strongly inhibits the growth of wheat roots,[8] and stimulates pea pericarp elongation.[9]

We previously showed[10] that N'-phenyl- and N'-benzyl-N-furfuryloxamides (6a) inhibited root growth in seedlings of rape, *Brassica campestris* L. (Dicotyledoneae). As a continuation of that work, we were interested in the biological activity of chloro-substituted derivatives of 6a and its related compound (7a). We report here the chemical synthesis of several chloro-substituted oxamide derivatives (6b—e and 7b—d) and testing of their plant growth-regulatory activity by means of germination assay using rape and leek seeds.

Preparation of N'-(Chloro-Substituted Phenyl)- and

N'-(Chloro-Substituted Benzyl)-N-furfuryloxamides (6b—e and 7b—d)　　We synthesized a series of test compounds (6b—e and 7b—d), as well as 6a and 7a.[10] First, dimethyl oxalate (8) was hydrolyzed with equimolar water at 60 °C for 1 h in the presence of potassium acetate to form the potassium salt of oxalic acid monomethyl ester, which was converted into methyl N-furfuryloxamate (9) (Chart 2). Subsequently, hydrolysis of 9 in sodium hydroxide solution at room temperature afforded the sodium salt (10) of N-furfuryloxamic acid.[11]

Next, as shown in Chart 2, the activation–amidation methodology was applied to the reaction of N-furfuryl-oxamic acid sodium salt (10) with chloro-substituted anilines (12b—e) or chloro-substituted benzylamines (13b—d) to afford N'-substituted N-furfuryloxamides (6

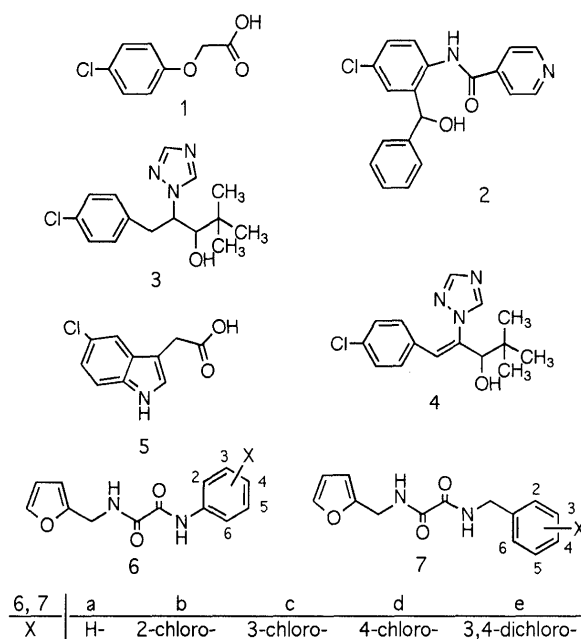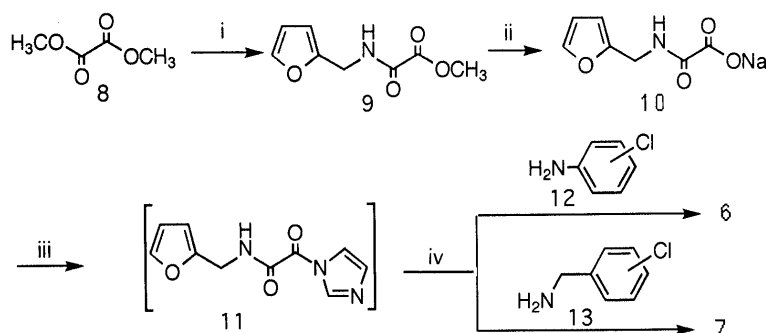| 6, 7	a	b	c	d	e
X	H-	2-chloro-	3-chloro-	4-chloro-	3,4-dichloro-

Chart 1

i, hydrolysis using KOAc to give a half ester, activation using 1,1'-oxalyldiimidazole(ODI), and then furfurylamine. ii, NaOH. iii, activation using ODI. iv, amidation

Chart 2

Table 1. Root Growth-Modulating Activities of N'-Substituted N-Furfuryloxamides (6 and 7)

Compd.	N'-Substituent	Dicotyledoneae: Rape; *Brassica campestris* L. Root growth (mm)[a]					Monocotyledoneae: Leek; *Allium tuberosum* ROTTLER Root growth (mm)[a]				
		Concentration (M)					Concentration (M)				
		1.0×10^{-3}	5.0×10^{-4}	1.0×10^{-4}	5.0×10^{-5}	Control	1.0×10^{-3}	5.0×10^{-4}	1.0×10^{-4}	5.0×10^{-5}	Control
6a	Phenyl	23±12.1**	18±13.6**	26± 8.3**	25±12.0**	54±23.2	2±3.4	3±3.8	2±1.8**	3±3.2	4±3.8
6b	2-Chlorophenyl	24± 5.7**	33±12.0**	28±10.5**	33±11.6**	61±29.4	10±6.4**	10±6.9**	8±7.2	10±7.4**	6±5.2
6c	3-Chlorophenyl	38±13.9**	49±25.2	43±19.0*	43±20.0*	54±27.0	7±4.2	6±4.5	5±3.6	5±3.0*	6±3.5
6d	4-Chlorophenyl	60±31.3	72±29.2**	67±27.8**	68±24.6**	51±20.3	12±9.0**	12±6.5**	9±5.4**	8±5.0	6±3.5
6e	3,4-Dichlorophenyl	32±23.7**	34±31.1**	44±31.6*	44±32.2*	60±25.3	4±4.0	3±3.9	2±2.9*	4±3.9	5±4.7
7a	Benzyl	28±30.1	27±26.2*	20±33.4*	44±48.9	32±37.8	1±1.4	1±1.3	1±1.2	1±1.4	2±2.3
7b	2-Chlorobenzyl	22±15.5**	35±20.5**	30±18.8**	40±18.5**	60±21.3	1±3.2*	4±3.5	2±3.0	4±3.4	3±4.0
7c	3-Chlorobenzyl	7±12.8**	22±18.4**	36±13.3**	47±10.2**	67±16.2	1±1.3**	1±1.6**	1±3.2**	3±3.2	4±3.8
7d	4-Chlorobenzyl	36±11.1**	41±16.6**	49±14.9**	37±14.9**	65±17.7	3±3.8	4±3.7	3±3.5	3±3.6	4±4.1

a) The values represent mean ± S.D. of 40 seeds after seven days (*A. tuberosum*: ten days). Significant differences from the corresponding control level are indicated, * and ** showing $p < 0.05$ and $p < 0.01$, respectively. Quantity of light: $127 \, \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Temperature 25 °C. Relative humidity of 60%. Experimental size: 20 seeds/group, 2 groups.

and 7) in yields of 46% to 82%. All nine of the prepared N-furfuryloxamide derivatives (6a—e and 7a—d) were examined for plant growth-modulating activity.

Root Growth-Modulating Activity Plant growth-modulating activity was assayed according to Inamori *et al.*[12] using seeds of rape, *Brassica campestris* L. (Brassicaceae), as a dicotyledon and leek, *Allium tuberosum* ROTTLER (Lilliaceae), as a monocotyledon. The root length (in millimeters) of the seedlings was measured and averaged. The results are summarized in Table 1.

Our initial expectation was that the chlorine-substituted test compounds (6b—e and 7b—d) would be more potent inhibitors of root growth than the unsubstituted compounds (6a and 7a). However, the biological activity of 6b—e and 7b—d in seedlings varied depending upon the position of substitution of chlorine. For example, the 3-chlorophenyl derivative (6c) showed little or no effectiveness as an inhibitor, whereas the parent compound, N'-phenyl-N-furfuryloxamide (6a) inhibited the root growth of rape seedlings. The 2-chlorophenyl derivative (6b) inhibited the root growth of rape seedlings as potently as 6a, but markedly promoted the root growth of leek seedlings. Further, the 4-chlorophenyl derivative (6d) promoted the root growth of both species. Thus, 6d may be a promising lead compound for the development of rooting accelerators. Interestingly, the 3,4-dichlorophenyl compound (6e) tended to be a weaker inhibitor of the root

growth of rape seedlings in comparison with the parent compound 6a.

All of the chlorinated benzyl derivatives (7b, c) inhibited the root growth of rape seedlings. N'-(3-Chlorobenzyl)-N-furfuryloxamide (7c) showed an especially potent inhibitory effect at the highest concentration examined, 1×10^{-3} M.

In summary, chlorination did not always increase the root growth-inhibitory activity of N'-phenyl-N-furfuryloxamide (6a) in the germination assay using rape and leek seeds. Indeed, N'-(4-chlorophenyl)-N-furfuryloxamide (6d) promoted the root growth of both rape and leek seedlings.

Experimental

Methyl oxalate, oxalyl chloride, imidazole, dimethyl sulfoxide (DMSO), furfurylamine, chloro-substituted anilines (12b, c) and chloro-substituted benzylamines (13b—d) were purchased from commercial sources and used as received. N'-Phenyl-N-furfuryloxamide (6a) and N'-benzyl-N-furfuryloxamide (7a) were prepared according to the reported procedures.[10] Melting points were taken on a Yanagimoto melting point apparatus. All melting points are uncorrected. IR spectra were measured on a Hitachi model 270-30 IR spectrophotometer. NMR spectra were measured on a Bruker DPX-400 spectrometer (400 MHz) using tetramethylsilane as an internal reference, and chemical shifts were recorded as δ-values.

N'-(2-Chlorophenyl)-N-furfuryloxamide (6b) 6b was prepared according to the reported procedure.[10] A solution of oxalyl chloride (1.28 g, 10 mmol) in acetonitrile (10 ml) was added dropwise to an ice-cold, stirred

solution of imidazole (2.7 g, 40 mmol) in acetonitrile (150 ml). The mixture was stirred at room temperature for 5 min, then a suspension of N-furfuryloxamic acid sodium salt (10) (1.8 g, 10 mmol) and methanesulfonic acid (1 g, 10 mmol) in acetonitrile (10 ml) was added rapidly in a single portion. The mixture was stirred at room temperature for 20 min, and then a solution of 2-chloroaniline (12b) (1.3 g, 10 mmol) in acetonitrile (10 ml) was added dropwise at room temperature. The resultant mixture was stirred for 3 h at 40 °C. The solvent was removed *in vacuo*, and the residue was poured onto ice and extracted with ethyl acetate. Washing of the ethyl acetate extract with 5% hydrochloric acid and water, followed by drying and evaporation of the solvent left 2.3 g (82%) of the crude product (6b). Recrystallization from toluene gave 6b of mp 115—117 °C. IR (KBr) cm^{-1}: 1683 (CO). ^{1}H-NMR (DMSO-d_6) δ: 4.3 (d, 2H, $J = 6.1$ Hz, -CH$_2$-), 6.2—7.5 (m × 3, 1H × 3, furan-4H, -3H and -5H), 7.4—7.5 (m, 5H, phenyl-3H, -4H, -5H and -6H), 9.4 (t, 1H, -CH$_2$-NH-), 10.8 (s, 1H, -CO-NH-). *Anal.* Calcd for C$_{13}$H$_{11}$ClN$_2$O$_3$: C, 56.03; H, 3.98; N, 10.05. Found: C, 56.31; H, 3.90; N, 10.09.

N'-(3-Chlorophenyl)-*N*-furfuryloxamide (6c) 6c was prepared as above through the reaction of 10 with 3-chloroaniline (12c) in 68% yield. Recrystallization from ethyl acetate gave 6c, mp 153—155 °C. IR (KBr) cm^{-1}: 1662 (CO). ^{1}H-NMR (DMSO-d_6) δ: 4.3—4.4 (d, 2H, $J = 5.7$ Hz, -CH$_2$-), 6.2—7.9 (m × 3, 1H × 3, furan-4H, -3H and -5H), 7.2—7.5 (m, 4H, phenyl-2H, -4H, -5H and -6H), 9.4 (t, 1H, -CH$_2$-NH-), 10.1 (s, 1H, -CO-NH-). *Anal.* Calcd for C$_{13}$H$_{11}$ClN$_2$O$_3$: C, 56.03; H, 3.98; N, 10.05. Found: C, 56.23; H, 3.68; N, 9.81.

N'-(4-Chlorophenyl)-*N*-furfuryloxamide (6d) 6d was prepared as above through the reaction of 10 with 4-chloroaniline (12d) in 46% yield. Recrystallization from methanol gave 6d, mp 192—193 °C. IR (KBr) cm^{-1}: 1665 (CO). ^{1}H-NMR (DMSO-d_6) δ: 4.3 (d, 2H, $J = 6.1$ Hz, -CH$_2$-), 6.2—7.5 (m × 3, 1H × 3, furan-4H, -3H and -5H), 7.3—7.4 (two d, each 2H, phenyl-2H, -6H, and phenyl-3H, -5H), 9.4 (t, 1H, -CH$_2$-NH-), 10.8(s, 1H, -CO-NH-). *Anal.* Calcd for C$_{13}$H$_{11}$ClN$_2$O$_3$: C, 56.03; H, 3.98; N, 10.05. Found: C, 56.09; H, 3.65; N, 9.87.

N'-(3,4-Dichlorophenyl)-*N*-furfuryloxamide (6e) 6e was prepared as above through the reaction of 10 with 3,4-dichloroaniline (12e) in 52% yield. Recrystallization from ethyl acetate gave 6e, mp 192—193 °C. IR (KBr) cm^{-1}: 1668 (CO). ^{1}H-NMR (DMSO-d_6) δ: 4.3—4.4 (d, 2H, $J = 5.9$ Hz, -CH$_2$-), 6.2—8.1 (m × 3, 1H × 3, furan-4H, -3H and -5H), 7.5—7.8 (m, 3H, phenyl-2H, -5H and -6H), 9.4 (t, 1H, -CH$_2$-NH-), 11.0 (s, 1H, -CO-NH-). *Anal.* Calcd for C$_{13}$H$_{10}$Cl$_2$N$_2$O$_3$: C, 49.86; H, 3.22; N, 8.95. Found: C, 49.67; H, 3.29; N, 8.98.

N'-(2-Chlorobenzyl)-*N*-furfuryloxamide (7b) 7b was prepared as above through the reaction of 10 with 2-chlorobenzylamine (13b) in 81% yield. Recrystallization from ethyl acetate gave 7b, mp 135—136 °C. IR (KBr) cm^{-1}: 1656 (CO). ^{1}H-NMR (DMSO-d_6) δ: 4.3 and 4.4 (each d, each 2H, $J = 6.3$, 6.2 Hz, each -CH$_2$-), 6.2—7.5 (three m, each 1H, furan-3H, -4H and -5H), 7.2—7.3 (m, 4H, phenyl-3H, -4H, -5H and -6H), 9.2 and 9.3 (each t, each 1H, each -NH-). *Anal.* Calcd for C$_{14}$H$_{13}$ClN$_2$O$_3$: C, 57.45; H, 4.48; N, 9.57. Found: C, 57.55; H, 4.47; N, 9.58.

N'-(3-Chlorobenzyl)-*N*-furfuryloxamide (7c) 7c was prepared as above through the reaction of 10 with 3-chlorobenzylamine (13c) in 73% yield. Recrystallization from toluene gave 7c, mp 135—136 °C. IR (KBr) cm^{-1}: 1656 (CO). ^{1}H-NMR (DMSO-d_6) δ: 4.3 (two d, each 2H, each $J = 6.3$ Hz, each -CH$_2$-), 6.2—7.5 (three m, each 1H, furan-3H, -4H and -5H), 7.2—7.4 (m, 4H, phenyl-2H, -4H, -5H and -6H), 9.2 and 9.4 (each t, each 1H, each -NH-). *Anal.* Calcd for C$_{14}$H$_{13}$ClN$_2$O$_3$: C, 57.45; H, 4.48; N, 9.57. Found: C, 57.46; H, 4.42; N, 9.51.

N'-(4-Chlorobenzyl)-*N*-furfuryloxamide (7d) 7d was prepared as above through the reaction of 10 with 4-chlorobenzylamine (13d) in 50% yield. Recrystallization from toluene gave 7d, mp 135—136 °C. IR (KBr) cm^{-1}: 1653 (CO). ^{1}H-NMR (DMSO-d_6) δ: 4.3 (two d, each 2H,

$J = 6.6$ Hz, each -CH$_2$-), 6.2—7.5 (three m, each 1H, furan-3H, -4H and -5H), 7.2 and 7.3 (each d, each 2H, $J = 8$ Hz, phenyl-2H, -6H and phenyl-3H, -5H), 9.2 and 9.4 (each t, each 1H, each -NH-). *Anal.* Calcd for C$_{14}$H$_{13}$ClN$_2$O$_3$: C, 57.45; H, 4.48; N, 9.57. Found: C, 57.29; H, 4.36; N, 9.52.

Plant Growth-Inhibitory Activity Test This test was carried out according to the method reported by Inamori *et al.*[12] DMSO solution (1.0 ml) containing an oxamide derivative (6a—e and 7a—d) or DMSO alone (1.0 ml) as a control was diluted in 100 ml of sterilized agar (0.8%, Nacalai Tesque, Inc.) to give concentrations of 5×10^{-5} M, 1.0×10^{-4} M, 5×10^{-4} M and 1.0×10^{-3} M. Agar containing a test chemical or DMSO as a control was poured into a sterilized culture jar of 500 ml. Then 20 seeds of each plant species, sterilized with 70% ethanol and 1% NaClO, were put on the agar and left for seven days (*A. tuberosum*; ten days) at 25 °C under a relative humidity of 60% and a light intensity of 127 μmol·m^{-2}·s^{-1}. The plant growth-inhibitory activity was expressed as the root length of the seedlings in millimeters. The results are summarized in Table 1.

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