Preparation and Root Growth-Modulatory Activity of N-Substituted 2-Acetylamino-2-ethoxycarbonyl-3-(2-furyl)propanamides

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N-Substituted 2-acetylamino-2-ethoxycarbonyl-3-(2-furyl)propanamides (8) were synthesized through the reaction of amines (13) with 2-acetylamino-2-ethoxycarbonyl-3-(2-furyl)propanoic acid (3b), which was prepared via condensation of 2-(bromomethyl)furan (10b) with diethyl acetamidomalonate, followed by partial hydrolysis of the resultant diethyl ester (3a) in the presence of barium hydroxide. However, bulky amines such as tert-butylamine or 2-trifluoromethylaminine did not afford the corresponding diamides (8).

The biological activity of the prepared diamides (8) as root growth modifiers was examined by germination assay using rape and leek seeds. N-(5-Bromo-2-thiazolyl)- and N-(4-chloro-2-benzothiazolyl)-2-acetylamino-2-ethoxycarbonyl-3-(2-furyl)propanamides (8h, i) both potently inhibited the root growth of rape seedlings, but were less effective in the case of leek seeds. The herbicide 2,4-dichlorophenoxyacetic acid completely inhibited root growth in both cases.

Key words: furan derivative; plant growth regulator; herbicidal activity; rooting; propanamide; amidation

Herbicides and plant growth regulators are widely used in crop production to improve productivity.1) Repeated use of the same herbicide or herbicides with the same mechanism of action, however, can lead to the appearance of weed species with tolerance to the applied agrochemicals, such as sulfonylurea-resistant paddy weeds or triazine-resistant weeds.2) Therefore, the continued development of novel herbicides and plant growth regulators is essential to supplement the current limited treatment options for resistant weeds.3)

We are interested in furan-containing structures, since it has been reported that 1-3-(2-furyl)alanine (1)5) used as a 500 mg/l spray on tomatoes completely prevented infection by Phytophthora infestans,3) and 1-3-(3-furyl)alanine (2) has fungicidal activity.5) These compounds (1, 2) were prepared from 2-(2-furylmethyl)- and 2-(3-furylmethyl)-2-acetylamidopropanedioic acid diethyl esters (3a, 4), respectively.

In addition, 3-(2-furyl)propionic acid (5) was proven to be a weak growth accelerator by Tamari.5) On the basis of this report, Kato et al. examined the phytophylgrowth-inhibitory activity of 3-(2-furyl)propionates (6) and 3-(2-furyl)propionamides (7). However, they did not show a remarkable herbicidal activity.5)

In this study, we examined the possibility that 2-acetylamino-2-ethoxycarbonyl-3-(2-furyl)propanamides (8)3) having one ester group and two amide groups at the same carbon, might have plant growth-regulatory activity. We report herein the preparation of the diamides (8), and their testing in germination assays using rape and leek seeds.

Preparation of N-substituted 2-acetylamino-2-ethoxycarbonyl-3-(2-furyl)propanamides (8) The synthetic route reported here was designed to prepare a series of test diamides (8) from the half acid (3b) obtained via the diester (3a),8) as shown in Chart 2. Compound 3a has been synthesized from the reaction of diethyl acetamidomalonate with 2-(chloromethyl)furan (10a), itself obtained from the reaction of 2-furylmethanol (9) with thionyl chloride in the presence of pyridine.9) However, 10a is extremely unstable and decomposes during purification by distillation to give hydrogen chloride, which catalyzes polymerization of the furan ring with explosive violence.10) To avoid this difficulty, we used 3-(bromomethyl)furan (10b)11) as an equivalent of 10a. An important consideration is that the procedure for the preparation of 10b does not require a base such as pyridine as a scavenger of hydrogen chloride.11)

First, 2-furylmethanol (9) in THF was allowed to react with phosphorus tribromide at 0 °C for 1.5 h to yield the corresponding bromo compound (10b), which could be extracted from the reaction mixture with ether, and used without further purification. Then, 10b was directly treated with diethyl acetamidomalonate at 70 °C in the presence of sodium ethoxide as a base to afford the corresponding diethyl ester (3a) in 81% yield. The success of this reaction using the bromo compound (10b) in place of the chloro compound (10a) allowed us to develop a convenient method of preparation of the title compounds (8).

Attempted partial hydrolysis of the diethyl ester (3a) according to the reported procedure, using 10% sodium hydroxide for 1 h at room temperature, gave a mixture of...
the half acid (3b) and full hydrolysate, the dicarboxylic acid (3c). These products are difficult to separate from each other by chromatography or recrystallization. However, we could obtain the half acid (3b) in 87% yield when the partial hydrolysis of the diethyl ester (3a) was carried out with barium hydroxide instead of sodium hydroxide. This may be because the diethyl ester (3a) is hydrolyzed to afford the barium salt of the half acid (3b), which is sparingly soluble and precipitates from the solution.

Next, the carboxyl group of the half acid (3b) was activated with 1,1'-oxalylidimidazole (ODI) to form an imidazolide intermediate (11), which reacted with amines (13) to afford the corresponding diamides (8) in 51—71% yield. Since the average yield of 8 is about 60%, it seems that the carbonyl group of the imidazolide intermediate (11) is not readily amidated, presumably because of steric crowding by the nearby quaternary carbon atom and imidazole ring. Application of the activation–amidation methodology for the reaction of the half acid (3b) with tert-butylamine or 2-(trifluoromethyl)aniline, having a bulky alkyl group around the amino group, did not afford the desired diamides (8), but provided 2-acetylamino-3-(2-furyl)propanoic acid ethyl ester (12) instead. In addition, decarboxylation of the half acid (3b) occurred to form 12 in 66% yield accompanied with the formation of tarry matter when a mixture of 3b and ODI was heated without any amine (13) at 60 °C for 1.5 h. The mechanism of the formation of 12 is unclear, but we speculate that, as the reaction to afford the diamides (8) does not proceed readily, prolonged heating of the imidazolide intermediate (11) results in C1–C2 (quaternary carbon) bond and C1–N1 (imidazole) bond cleavages accompanied with the transfer of a proton from an unidentified source to the C2 carbon to form 12.

All nine of the prepared diamides (8a—i), as well as the diethyl ester (3a) and the half acid (3b), were tested for plant growth-modulating activity.

**Root Growth-Modulating Activity** This activity was assayed according to the reported procedure using seeds of rape, *Brassica campestris* L. (Brassicaceae), as a dicotyledon and leek, *Allium tuberosum* Rottler (Liliaceae), as a mono-}

The half acid (3b) showed 66% inhibition for rape and 47% inhibition for leek at the concentration of 1.0 × 10^{-3} M, whereas the diethyl ester (3a) showed little activity. Among the N-substituted amides (8), neither the N-butyl derivative (8a) nor the N-phenyl derivative (8b) was inhibitory. However, the N-(4-chlorophenyl) derivative (8c) showed 49% inhibition for rape, and 29% inhibition for leek. Interestingly, we found that the N-(2-furylmethyl) derivative (8d) and N-(2-thiazolyl) derivative (8e) inhibited root growth of rape, but promoted that of leek. We therefore examined the effect of substituent groups on the heterocycles. The (5-nitro-2-thiazolyl) derivative (8g) showed 94% inhibition activity for rape, and 54% inhibition for leek. Thus, the nitro group on
the thiazole ring markedly enhanced the inhibitory activity. Similarly, the bromo derivative (8h) and the chloro derivative (8i) showed complete inhibition of rape root growth and substantial inhibition of leek root growth. The positive control (2,4-D; 14) completely inhibited root growth of both species.

In conclusion, the condensation reaction of (2-bromomethyl)furan (10b) with diethyl acetylamonolate, with subseuent partial hydrolysis of the adduct (3a) with barium hydroxide, has been shown to be a convenient general method for the preparation of the half acid (3b), and application of traditional activation–amidation methodology to 3b smoothly afforded N-substituted 2-acetylamino-2-ethoxy carbonyl-3-(2-furyl)propanamides (8). Evaluation of 8 for plant growth-modulating activity revealed that the N-(5-bromo-2-thiazolyl) and N-(4-chloro-2-benzothiazolyl) derivatives (8h, i) completely inhibited root growth of rape seedlings and partially inhibited that of leek seedlings. These findings suggest that the compounds (8h, i) show selective phytotoxicity towards germination of dicotyledons. A halogen group such as bromine or chlorine on the heterocycles seems to play an important role in the appearance of the selective toxicity.

Experimental

Diethyl acetylamonolate, oxalyl chloride, imidazole, dimethyl sulfoxide (DMSO) and amines (13a—i) were purchased from commercial sources and used as received. 2-(Bromomethyl)furan (10b) was prepared by the reported procedure.1

Table 2. Concentration Dependence of Plant Growth-Modulating Activities of Compounds 8h, 8i and 14

<table>
<thead>
<tr>
<th>Compd.</th>
<th>N′-Subs. groups</th>
<th>Dicotyledoneae</th>
<th>Monocotyledoneae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rape; <em>Brassica campestris</em> L.</td>
<td>Leek; <em>Allium tuberosum</em> R. T. L.</td>
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<tr>
<td></td>
<td></td>
<td>Growth (mm)</td>
<td>Inhibition (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>1.0×10⁻³ (m)</td>
</tr>
<tr>
<td>8h</td>
<td>2-(5-Bromothiazolyl)</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>8i</td>
<td>4-Chloro-2-benzothiazolyl</td>
<td>100</td>
<td>91</td>
</tr>
<tr>
<td>14</td>
<td>2,4-D (c)</td>
<td>100</td>
<td>99</td>
</tr>
</tbody>
</table>

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.

- 2-Acetiminono-2-(2-furylmethyl)propionic Acid Diethyl Ester (3a)
  - Diethyl acetylamonolate (8.2 g, 38 mmol) was dissolved in a sodium ethoxide solution [prepared from 0.85 g of sodium (0.037 g-atm) and 100 ml of absolute ethanol]. To this stirred solution, the ether solution (5.9 g, 20 mmol) was added to 50 ml of water. The mixture was stirred for 24 h. The precipitated product was collected by filtration. The precipitate was dried over anhydrous Na2SO4. The organic solvent was evaporated in vacuo, followed by drying under Na2SO4 and extraction of the solvent to afford 4.7 g (87%) of the crude product (3b). Recrystallization from

- 2-Acetiminono-2-ethoxycarbonyl-3-(2-furyl)propanoic Acid (3b)
  - A mixture of an ethanol solution (5 ml) of 2-acetiminono-2-(2-furylmethyl) propionic acid diethyl ester (3a) (5.9 g, 20 mmol) was added to 50 ml of 4% Ba(OH)2 solution. The reaction mixture was stirred at room temperature for 24 h. The precipitated product was collected by filtration. The precipitate was suspended in water (50 ml). The aqueous mixture was acidified with 5% HCl and extracted with ethyl acetate. The organic solution was washed with brine, followed by drying under Na2SO4 and evaporation of the solvent to afford 2.4 g (72%) of the crude product (3b). Recrystallization from

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Decarboxylation of the Half Acid (3b) Leading to 2-Acetamidino-3-(2-furyl)propanoic Acid Ethyl Ester (12) A solution of oxalyl chloride (1.1 g, 8.6 mmol) in acetonitrile (5 ml) was added dropwise to an ice-cold, stirred solution of imidazole (2.4 g, 35 mmol) in acetonitrile (50 ml). The mixture was stirred at room temperature for 15 min, then 2-acetamidino-3-(2-furyl)propanamide (8) General Procedure

A solution of oxalyl chloride (1.1 g, 8.6 mmol) in acetonitrile (50 ml). The mixture was stirred at room temperature for 15 min, then 2-acetamidino-3-(2-furyl)propanamide (8) was prepared as above through the reaction of the half acid (3b) (2.1 g, 8 mmol) with 2-aminothiazole (13a) (0.9 g, 8.8 mmol) in 68% yield, mp 228—231 °C (EtOH/toluene). IR (KBr) cm⁻¹: 3256 (N-H), 1746 (ester CO), 1698 (amide CO), 1652 (amide CO). 'H-NMR (DMSO-d₆) δ: 1.16 (3H, t, J = 7 Hz, -CH₂CH₃), 1.96 (3H, s, -COCH₃), 3.58–3.73 (two 1H, each d, J = 15 Hz, furan-3H, 4H and 5H), 6.2—6.6 (three m, each 1H, furan-3H, 4H, 5H), 7.41—7.58 (1H, t, J = 3 Hz, thiazole-5H), 7.32 (1H, d, J = 3 Hz, thiazole-4H), 7.54 (1H, s, NH), 7.18 (1H, s, NH). Anal. Calcd for C₁₆H₂₄N₂O₅: C, 58.12; H, 6.72; N, 11.52. Found: C, 58.26; H, 6.87; N, 11.65.

N-(5-Methyl-2-thiazolyl)-2-acetamidino-2-ethoxycarbonyl-3-(2-furyl)propanamide (8f) 8f was prepared as above through the reaction of the half acid (3b) (2.1 g, 8 mmol) with 2-amino-5-methylthiazole (13b) (1.3 g, 8.8 mmol) in 66% yield. mp 176—179 °C (EtOH/toluene). IR (KBr) cm⁻¹: 3304 (N-H), 1746 (ester CO), 1700 (amido CO), 1644 (amido CO). 'H-NMR (DMSO-d₆) δ: 1.16 (3H, t, J = 7 Hz, -CH₂CH₃), 1.96 (3H, s, -COCH₃), 3.58—3.73 (two 1H, each d, J = 15 Hz, furan-3H, 4H and 5H), 6.2—6.6 (three m, each 1H, furan-3H, 4H, 5H), 7.41—7.58 (1H, t, J = 3 Hz, thiazole-5H), 7.32 (1H, d, J = 3 Hz, thiazole-4H), 7.54 (1H, s, NH), 7.18 (1H, s, NH). Anal. Calcd for C₁₆H₂₄N₂O₅S: C, 51.28; H, 4.84; N, 11.96. Found: C, 51.20; H, 5.05; N, 11.83.

N-(5-Nitro-2-thiazolyl)-2-acetamidino-2-ethoxycarbonyl-3-(2-furyl)propanamide (8g) 8g was prepared as above through the reaction of the half acid (3b) (2.1 g, 8 mmol) with 2-amino-5-nitrothiazole (12g) (1.3 g, 8.8 mmol) in 62% yield. mp 182—185 °C (ether/AcOEt). IR (KBr) cm⁻¹: 3402 (N-H), 1750 (ester CO), 1700 (amido CO), 1640 (amide CO). 'H-NMR (DMSO-d₆) δ: 1.15 (3H, t, J = 7 Hz, -CH₂CH₃), 1.97 (3H, s, -COCH₃), 3.58—3.73 (two 1H, each d, J = 15 Hz, furan-3H, 4H and 5H), 6.2—6.6 (three m, each 1H, furan-3H, 4H, 5H), 7.41—7.58 (1H, t, J = 3 Hz, thiazole-5H), 7.32 (1H, d, J = 3 Hz, thiazole-4H), 7.85 (1H, s, NH), 13.33 (1H, s, NH). Anal. Calcd for C₁₅H₁₇N₃O₅S: C, 45.45; H, 4.40; N, 14.14. Found: C, 45.65; H, 4.39; N, 14.38.

N-(5-Bromo-2-thiazolyl)-2-acetamidino-2-ethoxycarbonyl-3-(2-furyl)propanamide (8h) 8h was prepared as above through the reaction of the half acid (3b) (2.1 g, 8 mmol) with 2-amino-5-bromothiazole (13h) (2.1 g, 8.8 mmol) in 51% yield. mp 198—200 °C (ether/AcOEt). IR (KBr) cm⁻¹: 3402 (N-H), 1750 (ester CO), 1700 (amido CO), 1640 (amide CO). 'H-NMR (DMSO-d₆) δ: 1.14 (3H, t, J = 7 Hz, -CH₂CH₃), 1.95 (3H, s, -COCH₃), 3.57—3.73 (two 1H, each d, J = 15 Hz, furan-3H, 4H and 5H), 6.2—6.6 (three m, each 1H, furan-3H, 4H, 5H), 6.35 (1H, m, furan-3H), 7.53 (1H, m, furan-5H), 8.17 (1H, s, thiazole-4H), 8.65 (1H, s, NH), 13.33 (1H, s, NH). Anal. Calcd for C₁₅H₁₇N₃O₅SBr: C, 45.45; H, 4.40; N, 14.14. Found: C, 45.65; H, 4.39; N, 14.38.

N-(4-Chloro-2-benzothiazolyl)-2-acetamidino-2-ethoxycarbonyl-3-(2-furyl)propanamide (8i) 8i was prepared as above through the reaction of the half acid (3b) (2.1 g, 8 mmol) with 2-amino-4-chlorobenzothiazole (13j) (1.63 g, 8.8 mmol) in 63% yield. mp 154—157 °C (ether/AcOEt). IR (KBr) cm⁻¹: 3420 (N-H), 1780 (ester CO), 1720 (amido CO), 1660 (amide CO). 'H-NMR (DMSO-d₆) δ: 1.16 (3H, t, J = 7 Hz, -CH₂CH₃), 1.99 (3H, s, -COCH₃), 3.60—3.82 (two 1H, each d, J = 15 Hz, furan-3H, 4H and 5H), 6.2—6.6 (three m, each 1H, furan-3H, 4H, 5H), 7.55—7.60 (1H, s, benzothiazole-5H), 7.33 (1H, t, J = 8 Hz, benzothiazole-4H), 8.05 (1H, s, NH), 12.85 (1H, s, NH). Anal. Calcd for C₁₆H₁₇ClN₃O₅SBr: C, 52.35; H, 4.13; N, 9.64. Found: C, 52.32; H, 4.12; N, 9.52.

Plant Growth-modulating Activity Test

This test was carried out according to the method reported by Inamori et al. A DMSO solution (1.0 ml) containing an amide derivative (8a—8i) 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 $5 \times 10^{-3}$, $1.0 \times 10^{-4}$, $5 \times 10^{-5}$, and $1.0 \times 10^{-3}$ m. Agar containing a test chemical or DMSO as a control was poured into a sterilized 500 ml culture jar. 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 $\text{mole} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. The plant growth-inhibitory activity was expressed as the root length of the seedlings in millimeters. The results are summarized in Tables 1 and 2.

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References and Notes