Plant Growth Activities of Aspyran, Asperentin, and its Analogues Produced by the Fungus *Aspergillus* sp.

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Aspyran (**1**), a novel compound, and the known isocoumarin asperentin (**2**), also known as cladosporin, together with its analogues **3**–**6** were isolated from *Aspergillus* sp. and their structures established by spectroscopic methods including 2D NMR spectroscopy. The effects of **1**–**6** on plant growth were examined by bioassays using lettuce and rice seedlings. Compounds **1** and **3** promoted the root growth of the seedlings, while **2** and **5** were inhibitory. Compounds **4** and **6** did not show any effect on the growth of lettuce and rice seedlings, respectively.

Key words: Aspyran, Asperentin, *Aspergillus*

Introduction

Fungi have been proven to be a valuable source for the discovery of novel natural products, many of them leading to agrochemical (Kim *et al*., 1995; Barrios-Gonzalez *et al*., 2003; Avalos *et al*., 2007) and biomedical (Pearce, 1997; Lien, 1990; Molnar *et al*., 2010) developments. So far, we have isolated and characterized chloroisosulochrin (Shimada *et al*., 2001), myxostiol (Kimura *et al*., 2002), 4-hydroxykigelin (Shimada *et al*., 2004), brevicompanine C (Kimura *et al*., 2005), citrinolactone A (Kuramata *et al*., 2007), and rubralactone (Kimura *et al*., 2007). In the course of our screening search for new plant growth regulators, using bioassays with lettuce and rice seedlings suitable for developing new herbicides, we found plant growth regulators in the culture filtrate of the fungus *Aspergillus* sp. Our investigation of the metabolites of this fungus has now led to the isolation of a new metabolite designated aspyran (**1**) and five known isocoumarin analogues, **2**–**6**, from the culture filtrate. We report here the isolation, structural determination, and effects on plant growth of **1**–**6**.

Material and Methods

General experimental procedures

Melting points were determined using a Yanagimoto (Kyoto, Japan) micromelting point apparatus and are uncorrected. Optical rotation data were determined with a Horiba (Kyoto, Japan) SEPA-200 polarimeter. The UV spectra were recorded on a Shimazu (Kyoto, Japan) UV-2000 spectrophotometer and the IR spectra on a JASCO (Tokyo, Japan) FT IR-7000 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded with a JEOL (Tokyo, Japan) JNM-ECD 500 NMR spectrometer at 500 and 125 MHz, respectively. Chemical shifts are expressed in δ values with solvents as internal standards. High-resolution electron impact mass spectrometry (HREIMS) data were obtained with a JEOL JMS-SX 102 mass spectrometer. Silica gel (Wako Pure Chemical Industries, Ltd., Osaka, Japan; 75–150 μm) was used for column chromatography. Precoated silica gel plates (Merck, Darmstadt, Germany; Kieselgel 60 F254, 0.2 mm) were used for preparative thin-layer chromatography (TLC).

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Fungal material and fermentation

Aspergillus sp. was collected from the soil in the city of Tottori in April 2004, and identified by light microscopy and by its production of previously characterized bioactive metabolites (Scott *et al.*, 1971; Grove, 1972; Springer *et al.*, 1981). A voucher specimen (No. F653) is deposited at the Laboratory of Bioorganic Chemistry, Department of Biological and Environmental Chemistry, Faculty of Agriculture, Tottori University, Tottori, Japan. Eighty 500-ml Erlenmeyer flasks, each containing 250 ml of malt extract medium supplemented with 3% polypeptone, were individually inoculated with an 1-cm^2 agar plug taken from a stock culture of the fungus maintained at 20 °C on potato dextrose agar. The fungus was statically grown at 24 °C for 21 d.

Extraction and isolation

The culture filtrate (20 l) was adjusted to pH 2.0 with 2 M HCl and extracted twice with EtOAc. The combined extracts were partitioned twice with a saturated $NaHCO_3$ solution. The EtOAc-soluble, neutral phases were combined and concentrated *in vacuo*. The $NaHCO_3$ phases were combined and adjusted to pH 2.0 with 2 M HCl. The acidic solution was extracted twice with EtOAc, and the EtOAc-soluble, acidic phases were combined and concentrated *in vacuo*. The resulting acidic residue (2.0 g) was first fractionated by column chromatography on silica gel (benzene/EtOAc). The fraction (0.13 g) obtained by eluting with 30% EtOAc was purified twice by preparative TLC (*n*-hexane/EtOAc, 7:3, v/v) to yield 11 mg of aspyran (**1**) as colourless crystals. The resulting neutral residue (5.0 g) was first fractionated by column chromatography on silica gel (*n*-hexane/EtOAc). The fraction (0.72 g) obtained by eluting with 30% EtOAc was purified twice by preparative TLC (benzene/EtOAc, 7:3, v/v) to yield 12 mg of asperentin (**2**) as colourless crystals and 22 mg of asperentin-6-*O*-methyl ether (**3**) as colourless crystals. The other fraction (1.0 g) obtained by eluting with 70% EtOAc was fractionated for a second time by column chromatography on silica gel (*n*-hexane/acetone). The fraction (0.22 g) obtained by eluting with acetone was further purified by preparative TLC ($CHCl_3$/MeOH, 97:3, v/v) to yield 26 mg of 5'-hydroxyasperentin (**4**) as white powder and 22 mg of 4'-hydroxyasperentin (**5**) as white powder. The other first-column fraction (1.4 g) obtained by eluting with EtOAc was purified twice by preparative TLC ($CHCl_3$/MeOH, 97:3, v/v) to yield 8 mg of asperentin-8-*O*-methyl ether (**6**) as white powder.

Aspyran (**1**): M.p. 58–61 °C. – $[\alpha]_D^{20}$ –40.0° (*c* 0.1, EtOH). – UV/vis (EtOH): λ_{max} (lg ε) = 217 (4.10), 256 (3.56), 299 nm (3.20). – IR (KBr): ν = 3270 (OH), 2900–2300 (COOH), 1650 (C=O), 1624 (C=C), 1454, 1379, 1249, 1174, 847 cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 1.13 (d, *J* = 6.6 Hz, 3H, 6'-CH_3), 1.37 (m, 2H, 5'-H), 1.42–1.73 (m, 4H, 4',3'-H), 2.82 (dd, *J* = 13.7, 4.8 Hz, 1H, 7-H), 3.06 (dd, *J* = 13.7, 7.8 Hz, 1H, 7-H), 3.99 (m, 1H, 2'-H), 4.21 (m, 1H, 6'-H), 6.16 (d, *J* = 2.3 Hz, 1H, 5-H), 6.28 (d, *J* = 2.3 Hz, 1H, 3-H), 11.32 (br. s, 1H, O*H*). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 17.5 (q, *C*H_3), 17.6 (t, C-4'), 29.7 (t, C-5'), 30.5 (t, C-3'), 40.5 (t, C-7), 69.8 (d, C-6'), 72.1 (d, C-2'), 102.2 (d, C-3), 107.2 (s, C-1), 111.2 (d, C-5), 142.1 (s, C-6), 160.5 (s, C-4), 164.2 (s, C-2), 172.5 (s, *C*OOH).

Asperentin (**2**): M.p. 184–186 °C. – $[\alpha]_D^{20}$ –70.0° (*c* 0.1, EtOH). – UV/vis (EtOH): λ_{max} (lg ε) = 217 (4.18), 269 (4.07), 305 nm (3.88). – IR (KBr): ν = 3408 (OH), 1657 (lactone), 1595, 1479, 1386, 1265, 1207, 1170, 1125, 1108 cm^{-1}. – ^1H NMR (500 MHz, methanol-d_4): δ = 1.08 (d, *J* = 6.4 Hz, 3H, 6'-CH_3), 1.21 (m, 2H, 5'-H), 1.58–1.63 (m, 5H, 9, 3', 4'-H), 2.02 (m, 1H, 9-H), 2.81 (m, 2H, 4-H), 3.81 (m, 1H, 6'-H), 4.05 (m, 1H, 2'-H), 4.56 (m, 1H, 3-H), 6.10 (d, *J* = 2.3 Hz, 1H, 7-H), 6.12 (d, *J* = 2.3 Hz, 1H, 5-H). – ^{13}C NMR (125 MHz, methanol-d_4): δ = 19.3 (t, C-4'), 20.1 (q, *C*H_3), 31.5 (t, C-5'), 32.8 (t, C-3'), 34.5 (t, C-4), 39.3 (t, C-9), 68.4 (d, C-6'), 68.5 (d, C-2'), 77.9 (d, C-3), 101.6 (s, C-8a), 102.3 (d, C-7), 108.0 (d, C-5), 143.6 (s, C-4a), 165.5 (s, C-8), 166.4 (s, C-6), 171.4 (s, C-1). – HREIMS: *m/z* = 292.1315 [M^+]; calcd. for $C_{16}H_{20}O_5$, 292.1314.

Asperentin-6-O-methyl ether (**3**): M.p. 93–98 °C. – $[\alpha]_D^{20}$ +70.0° (*c* 0.1, EtOH). – UV/vis (EtOH): λ_{max} (lg ε) = 219 (4.21), 267 (4.17), 302 nm (3.80). – IR (KBr): ν = 2940, 1659 (lactone), 1630, 1580, 1437, 1379, 1201, 1156 cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 1.21 (d, *J* = 6.4 Hz, 3H, 6'-CH_3), 1.34 (m, 2H, 3'-H), 1.66–1.73 (m, 4H, 4', 5'-H), 1.83–1.96 (m, 2H, 9-H), 2.88 (d, *J* = 6.9 Hz, 2H, 4-H), 3.82 (s, 3H, OCH_3), 3.95 (m, 1H, 6'-H), 4.08 (m, 1H, 2'-H), 4.72 (m, 1H, 3-H), 6.24 (d, *J* = 2.3 Hz, 1H, 5-H), 6.31 (d, *J* = 2.3 Hz, 1H, 7-H), 11.22 (s, 1H, O*H*). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.3 (t, C-4'), 19.0 (q, *C*H_3), 30.9 (t, C-3', 5'), 33.8 (t, C-4), 39.5 (t, C-9), 55.5 (q, O*C*H_3), 66.2 (d, C-2'), 67.5 (d,

C-6'), 76.4 (d, C-3), 99.4 (d, C-7), 101.8 (s, C-8a), 106.1 (d, C-5), 141.1 (s, C-4a), 164.5 (s, C-8), 165.7 (s, C-6), 169.7 (s, C-1). – HREIMS: *m/z* = 306.1466 [M$^+$]; calcd. for $C_{17}H_{22}O_5$, 306.1467.

5'-Hydroxyasperentin (**4**): M.p. 220–223 °C. – $[\alpha]_D^{20}$ –36.1° (*c* 0.08, EtOH). – UV/vis (EtOH): λ_{max} (lg ε) = 218 (4.21), 269 (4.17), 303 nm (3.88). – IR (KBr): ν = 3294 (OH), 3034, 1659 (lactone), 1591, 1493, 1373, 1265, 1241, 1191 cm^{-1}. – ^{1}H NMR (500 MHz, methanol-d_4): δ = 1.10 (d, *J* = 6.4 Hz, 3H, 6'-C*H*$_3$), 1.20–1.25 (m, 2H, 4'-H), 1.59–1.85 (m, 2H, 3'-H), 1.68–1.85 (m, 2H, 9-H), 2.73–2.83 (m, 2H, 4-H), 3.61 (m, 1H, 5'-H), 3.84–3.92 (m, 2H, 2',6'-H), 4.56 (m, 1H, 3-H), 6.10 (d, *J* = 2.3 Hz, 1H, 5-H), 6.11 (d, *J* = 2.3 Hz, 1H, 7-H). – ^{13}C NMR (125 MHz, methanol-d_4): δ = 13.4 (q, *C*H$_3$), 27.4 (t, C-3'), 30.3 (t, C-4), 34.6 (t, C-4), 40.6 (t, C-9), 66.7 (d, C-3), 69.2 (t, C-5'), 72.6 (d, C-2'), 78.1 (d, C-6'), 101.8 (s, C-8a), 102.5 (d, C-5), 108.2 (d, C-7), 143.8 (s, C-4a), 165.9 (s, C-8), 166.6 (s, C-6), 171.7 (s, C-1). – HREIMS: *m/z* = 308.1251 [M$^+$]; calcd. for $C_{16}H_{20}O_6$, 308.1260.

4'-Hydroxyasperentin (**5**): M.p. 197–199 °C. – $[\alpha]_D^{20}$ –36.1° (*c* 0.08, EtOH). – UV/vis (EtOH): λ_{max} (lg ε) = 220 (4.22), 269 (4.20), 303 nm (3.92). – IR (KBr): ν = 3398 (OH), 2938, 1657 (lactone), 1634, 1383, 1241, 1170 cm^{-1}. – ^{1}H NMR (500 MHz, methanol-d_4): δ = 1.17 (d, *J* = 6.4 Hz, 3H, 6'-C*H*$_3$), 1.30 (m, 1H, 5'-H), 1.63 (m, 1H, 3'-H), 1.75–1.84 (m, 2H, 9, 3'-H), 1.92 (m, 1H, 5'-H), 2.29 (m, 1H, 9-H), 2.92 (m, 2H, 4-H), 3.77 (m, 1H, 6'-H), 3.94 (m, 1H, 4'-H), 4.38 (m, 1H, 2'-H), 4.62 (m, 1H, 3-H), 6.20 (d, *J* = 2.3 Hz, 1H, 5-H), 6.21 (d, *J* = 2.3 Hz, 1H, 7-H). – ^{13}C NMR (125 MHz, methanol-d_4): δ = 21.9 (q, *C*H$_3$), 34.0 (t, C-4), 37.1 (t, C-9), 39.0 (t, C-3'), 43.4 (t, C-5'), 64.5 (t, C-4'), 66.2 (d, C-6'), 69.8 (d, C-2'), 77.8 (d, C-3), 101.4 (s, C-8a), 102.0 (d, C-5), 107.8 (d, C-7), 143.3 (s, C-4a), 165.5 (s, C-8), 166.2 (s, C-6), 171.2 (s, C-1). – HREIMS: *m/z* = 308.1261 [M$^+$]; calcd. for $C_{16}H_{20}O_6$, 308.1260.

Asperentin-8-O-methyl ether (**6**): M.p. 230–235 °C. – $[\alpha]_D^{20}$ +20.0° (*c* 0.1, EtOH). – UV/vis (EtOH): λ_{max} (lg ε) = 227 (4.27), 265 (4.21), 298 nm (4.01). – IR (KBr): ν = 3300 (OH), 2930, 1676 (lactone), 1607 (C=C), 1439, 1348, 1253, 1089 cm^{-1}. – ^{1}H NMR (500 MHz, pyridine-d_5): δ = 1.10 (d, *J* = 6.4 Hz, 3H, 6'-C*H*$_3$), 1.20–1.25 (m, 4H, 4', 5'-H), 1.59–1.85 (m, 2H, 3'-H), 1.68–1.85 (m, 2H, 9-H), 2.73–2.83 (m, 2H, 4-H), 3.80 (s, 3H, OC*H*$_3$), 3.84–3.92 (m, 2H, 2', 6'-H), 4.56 (m, 1H, 3-H), 6.10 (d, *J* = 2.3 Hz, 1H, 5-H), 6.11 (d, *J* = 2.3 Hz, 1H, 7-H). – ^{13}C NMR (125 MHz, pyridine-d_5): δ = 19.0 (t, C-4'), 20.0 (q, *C*H$_3$), 31.3 (t, C-3'), 32.1 (t, C-5'), 36.0 (t, C-4), 39.5 (t, C-9), 56.2 (q, O*C*H$_3$), 67.1 (d, C-2'), 67.3 (d, C-6'), 74.8 (d, C-3), 100.0 (d, C-7), 106.7 (s, C-8a), 107.7 (d, C-5), 145.1 (s, C-4a), 162.5 (s, C-8), 164.4 (s, C-6), 170.0 (s, C-1). – HREIMS: *m/z* = 306.1470 [M$^+$]; calcd. for $C_{17}H_{22}O_5$, 306.1467.

*Acetylation of **1***

Compound **1** (2 mg) was acetylated with acetic anhydride (0.2 ml) and pyridine (0.4 ml) for 24 h at room temperature. Purification by preparative TLC (*n*-hexane/EtOAc, 7:3, v/v) gave a diacetyl derivative of **1** (1.3 mg).

Diacetylaspyran: HREIMS: *m/z* = 291.1232 [M$^+$ – (COOH + CH$_2$)]; calcd. for $C_{16}H_{19}O_5$, 291.1232.

Bioassay for the growth of lettuce seedlings

Lettuce seeds (*Lactuca sativa* cv. Kingcisco) were purchased from Takii Nursery and sown in a Petri dish (150 mm x 25 mm) lined with filter paper containing deionized H_2O. After 1 d under continuous light [100 μE/(m^{2}s)] from fluorescent lamps (FLR40S D/M; Toshiba, Tokyo, Japan) at 24 °C, twelve seedlings were selected for uniformity (radicles of 2 mm) and transferred into a mini-Petri dish (35 mm x 15 mm) lined with filter paper containing 1 ml of deionized H_2O and a defined amount of the test compound. The Petri dishes were kept for 4 d under the same conditions as the seedlings. The lengths of the hypocotyl and root were measured, and their mean values were compared with those of a untreated control (Kusano *et al.*, 1998). All experiments were performed in triplicate.

Bioassay for the growth of rice seedlings

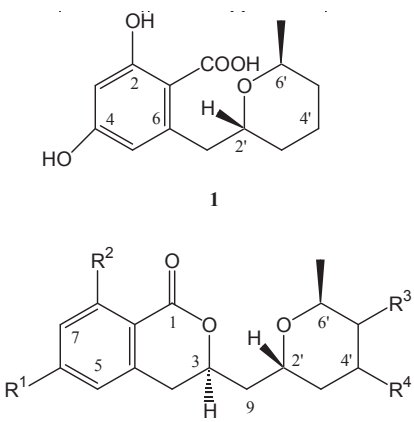
Rice seeds (*Oryza sativa* L.) were sterilized with 75% (v/v) ethanol for 30 s, immersed in sodium hypochlorite solution (antiformin) for 2 h, rinsed under running water for 3 h, and transferred to a Petri dish (150 mm x 25 mm) containing deionized water. After 3 d under continuous light (see above) at 30 °C, seven seedlings were selected for uniformity (radicles of 2 mm) and transferred into a test tube (35 mm x 15 mm) containing 1 ml of deionized water and a defined amount of the test compound. The test tube was sealed with polyethylene film and incubated for 7 d under continuous light (as above) at 30 °C. The lengths

of the stem and primary root were measured and their mean length values compared with those of a untreated control (Kusano *et al.*, 1998). All experiments were performed in triplicate.

Results and Discussion

The EtOAc-soluble, neutral fraction (5.0 g) from the filtrate of *Aspergillus* sp. was purified by silica gel column chromatography and preparative TLC to afford compounds **2**–**6** which were identified as asperentin (**2**), asperentin-6-*O*-methyl ether (**3**), 5'-hydroxyasperentin (**4**), 4'-hydroxyasperentin (**5**), and asperentin-8-*O*-methyl ether (**6**), respectively, by comparing their physicochemical properties with those reported (Scott *et al.*, 1971; Grove, 1972; Springer *et al.*, 1981; Jacyno *et al.*, 1993) (Fig. 1). The EtOAc-soluble, acidic fraction (2.0 g) from the filtrate of *Aspergillus* sp. was purified by silica gel column chromatography and preparative TLC to afford **1** (Fig. 1).

Compound **1** was obtained as colourless crystals. The molecular formula of **1** was established as $C_{14}H_{18}O_5$ by the ^{13}C and ^{1}H NMR spectra and the HREI mass spectrum of a diacetyl derivative. The ^{13}C and ^{1}H NMR spectra and ^{1}H-^{13}C pulsed field gradient-heteronuclear multiple quantum coherence spectroscopy (^{1}H-^{13}C PFG-HMQC) experiments indicated the presence of one methyl, four methylene, two *O*-substituted aliphatic methine, two aromatic methine, two *O*-substituted aromatic quaternary, two aromatic quaternary, and one carbonyl carbon atoms or groups, respectively. The IR bands from 2900 cm^{-1} to 2300 cm^{-1} and 1650 cm^{-1}, and a signal at δ_C 172.5 ppm in the ^{13}C NMR spectrum indicated the presence of a carboxy group. A signal at δ_H 11.32 ppm, being a D_2O-exchangeable signal, in the ^{1}H NMR spectrum indicated the presence of a hydrogen-bond hydroxy group. An IR band at 3270 cm^{-1}, two signals at δ_C 160.5 ppm and 164.2 ppm in the ^{13}C NMR spectrum, and the remaining atoms from the molecular formula indicated the presence of another hydroxy group. The ^{1}H NMR spectrum indicated the presence of aromatic signals at δ_H 6.16 ppm and 6.28 ppm with *meta*-coupling (J = 2.3 Hz). These two aromatic signals in the ^{1}H NMR spectrum and six sp^2 carbon atoms in the ^{13}C NMR spectrum indicated the presence of a tetra-substituted benzene ring. Two signals at δ_H 2.82 ppm and 3.06 ppm in the ^{1}H NMR spectrum and a signal at δ_C 40.5 ppm in the ^{13}C NMR spectrum indicated the presence of a benzylic methylene group. A comparison of the ^{13}C and ^{1}H NMR spectra of **1** with those of **2** indicated the presence of a methyl-substituted oxane ring (Jacyno *et al.*, 1993). In the ^{1}H-^{13}C pulsed field gradient heteronuclear multiple-bond correlation (^{1}H-^{13}C PFG-HMBC) spectrum (Fig. 2), the methyl proton at δ_H 1.13 ppm was correlated with the methylene and oxygenated methine carbon atoms at δ_C 29.7 ppm and 69.8 ppm. The benzylic methylene proton at δ_H 3.06 ppm was correlated with the oxygenated methine, aromatic methine, and aromatic quaternary carbon atoms at δ_C 72.1 ppm, 111.2 ppm, and 142.1 ppm, respectively. The aromatic methine proton at δ_H 6.16 ppm was correlated with the benzylic



	R^1	R^2	R^3	R^4
2	OH	OH	H	H
3	OMe	OH	H	H
4	OH	OH	OH	H
5	OH	OH	H	OH
6	OH	OMe	H	H

Fig. 1. Chemical structures of asypran (**1**), asperentin (**2**), asperentin-6-*O*-methyl ether (**3**), 5'-hydroxyasperentin (**4**), 4'-hydroxyasperentin (**5**), and asperentin-8-*O*-methyl ether (**6**).

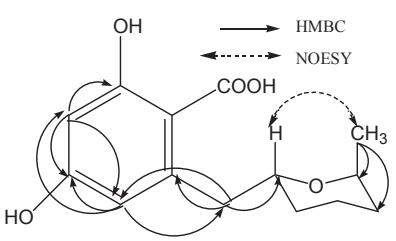


Fig. 2. HMBC (→) and NOESY correlations (⟷) of **1**.

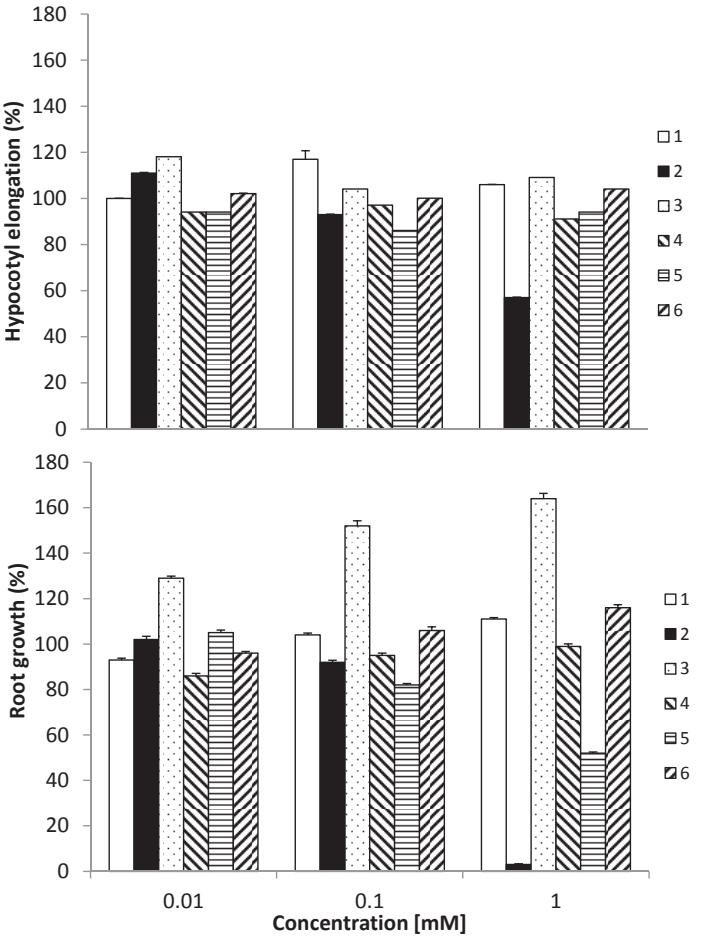


Fig. 3. Effects of compounds **1**–**6** on the growth of lettuce seedlings, in % of the control. Each value is presented as the mean ± SE (n = 3).

methylene, another aromatic methine, and *O*-substituted aromatic quaternary carbon atoms at δ_C 40.5 ppm, 102.2 ppm, and 160.5 ppm, respectively. Another aromatic methine proton at δ_H 6.28 ppm was correlated with the aromatic methine and two *O*-substituted aromatic quaternary carbon atoms at δ_C 111.2 ppm, 160.5 ppm, and 164.2 ppm, respectively. A detailed analysis of the long-range correlation led to a planar structure of **1**. The relative stereochemistry of **1** was deduced from the ^{1}H NMR coupling constants and nuclear Overhauser effect spectroscopy (NOESY) correlations (Fig. 2). The stereochemistry of 2'-H was assigned to be β with axial orientation in the view of two coupling constants of 7-H (J = 4.8 and 7.8 Hz). The stereochemistry of the methyl group was assigned to be β with axial orientation, since NOEs were observed between the methyl group and 2'-H. These results indicated that the relative configurations at C-2' and C-6' in **1** were *R** and *S**, respectively. The relative stereochemistry of C-2' and C-6' in **1** was the same as in **2** (Springer *et al.*, 1981). From these results, **1** was established to be (2'*R**,6'*S**)-2,4-dihydroxy-6-(6-methyl-tetrahydro-2*H*-pyran-2-yl-methyl)benzoic acid; it was named aspyran.

Compound **2** is known to inhibit the growth of etiolated wheat coleoptiles and spore germination of *Penicillium* and *Aspergillus* spp. (Springer *et al.*, 1981; Jacyno *et al.*, 1993), but there is no information about the effect of **2**–**6** on the growth of lettuce and rice seedlings. With lettuce seedlings (Fig. 3), **2** strongly inhibited root growth (4% of

control) at 1 mM, and **5** showed weak inhibitory activity on root growth at the same concentration. In contrast, **3** particularly increased root growth in proportion to its concentration from 0.01 to 1 mM. Compounds **1** and **6** weakly enhanced root elongation in the concentration range of 0.01 to 1 mM, while compound **4** did not show any effect in this concentration range. With rice seedlings (Fig. 4), **2** decreased root growth in proportion to its concentration from 0.01 to 1 mM, while **5** was weakly inhibitory (61% of the control at 1 mM). In contrast, **3** enhanced root elongation to 151% and 144% at the concentrations of 0.1 and 1 mM, respectively. Compounds **1** and **4** enhanced root growth to 132% and 117%, respectively, at a concentration of 1 mM. Compound **6** did not show any effect in the concentration range of 0.01 to 1 mM.

From the results of **1**, the carboxy group and the hydroxy group in the molecule of **1** might play an important role in the effect on root growth (Yoshikawa *et al*., 1979; Vazquez *et al*., 1983). The effect of the hydroxy substitution in the oxane ring on the activity was evaluated by comparing the results among **2**, **4**, and **5**. Hydroxylation at the 4'-position in the oxane ring was unfavourable to the inhibitory activity, and 5'-OH did not affect root growth. Then, the effect of the introduction of a methoxy group in the isocoumarin moiety on root growth was evaluated by comparing the results among **2**, **3**, and **6**. The introduction of a methoxy group in the 6-position of the isocoumarin moiety enhanced the root growth-promoting effect, but a methoxy group in the 8-position did not affect the root growth activity.

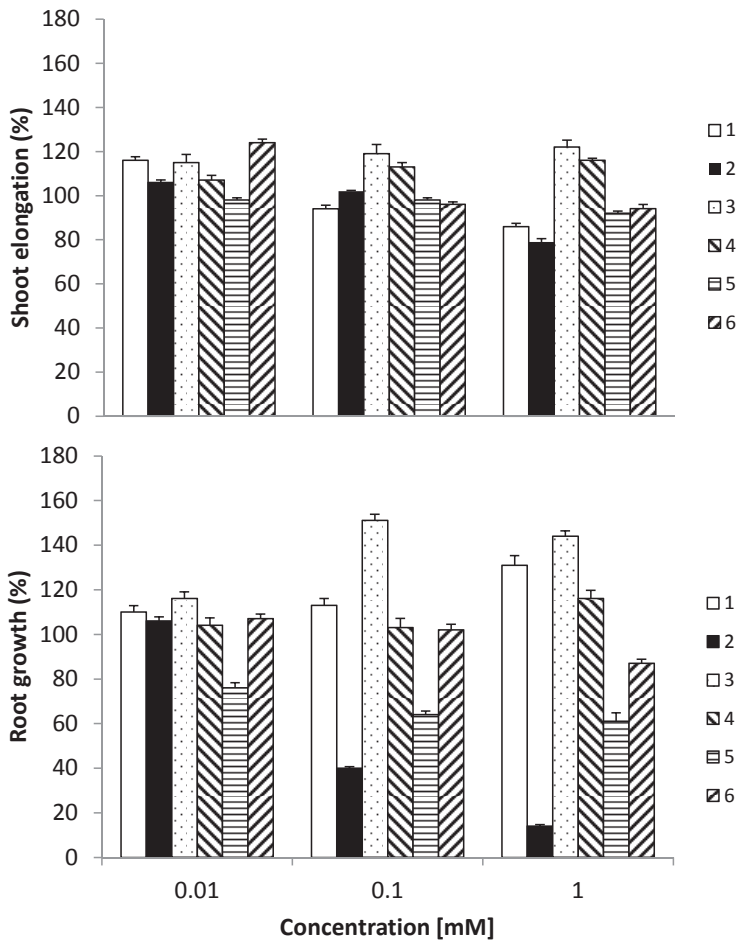


Fig. 4. Effects of compounds **1**–**6** on the growth of rice seedlings, in % of the control. Each value is presented as the mean ± SE (n = 3).

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