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Phenolic Constituents of *Acorus gramineus*

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The purification of a MeOH extract from the rhizome of *Acorus gramineus* (Araceae) using column chromatography furnished two new stereoisomers of phenylpropanoid, acoraminol A (**1**) and acoraimol B (**2**). It also furnished 17 known phenolic compounds, β-asarone (**3**), asaraldehyde (**4**), isoacoramone (**5**), propioveratrone (**6**), (1'*R*,2'*S*)-1',2'-dihydroxyasarone (**7**), (1'*S*,2'*S*)-1',2'-dihydroxyasarone (**8**), 3',4'-dimethoxycinnamyl alcohol (**9**), 3',4',5'-trimethoxycinnamyl alcohol (**10**), kaempferol 3-methyl ether (**11**), 2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (**12**), hydroxytyrosol (**13**), tyrosol (**14**), (2*S*,5*S*)-diveratryl-(3*R*,4*S*)-dimethyltetrahydrofuran (**15**), (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol (**16**), 7*S*,8*S*-*threo*-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-*O*-4'-neolignan (**17**), 7*S*,8*R*-*erythro*-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-*O*-4'-neolignan (**18**), and dihydroyashsbushiketol (**19**). The structures of the new compounds were elucidated by analysis of spectroscopic data including 1D and 2D NMR data. The absolute configurations of **1** and **2** were determined using the convenient Mosher ester procedure. Compounds **5-19** were isolated for the first time from this plant source. The isolated compounds were tested for cytotoxicity against four human tumor cell lines *in vitro* using a Sulforhodamine B (SRB) bioassay.

Key words: *Acorus gramineus*, Araceae, Acoraminol A, Acoraminol B, Cytotoxicity

INTRODUCTION

Acorus gramineus (Araceae), which is distributed throughout Korea, Japan, and China, has been used as a Korean traditional medicine for learning and memory improvement, sedation and analgesia (Liao et al., 1998). Moreover, this herb has long been used for the treatment of stomach ache (Tang and Eisenbrand, 1992) and swelling as well as for the extermination of insects (Wang et al., 1998). Several pharmacologically active phenolics, such as β-asarone, α-asarone and phenylpropenes have been reported from this rhizome (Greca et al., 1989). Previous pharmacological studies on *A. gramineus* found that its extracts showed neuroprotective (Chun et al., 2008) and antibacterial activities (Lee et al., 2004).

In our continuing study on bioactive natural products from Korean traditional medicinal plants, we investigated the rhizomes of *A. gramineus*. In this study, we isolated two new stereoisomers of phenylpropanoid, acoraminol A (**1**) and acoraminol B (**2**), along with seventeen known phenolic compounds (**3**-**19**) including four lignan derivatives (**15**-**18**) from its MeOH extract. Their structures were elucidated by spectroscopic data including 1D and 2D NMR and comparisons with reported data. The isolated compounds were tested for cytotoxicity against four human tumor cells *in vitro* using a Sulforhodamine B (SRB) bioassay. This paper describes the isolation and structural elucidation of **1** and **2**, and the cytotoxic activity of all the compounds (**1**-**19**).

MATERIALS AND METHODS

General experimental procedure

Melting points were determined on a Gallenkamp

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melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 Polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded with a Schimadzu UV-1601 UV-Visible spectrophotometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer. EIMS and FABMS data were obtained on a JEOL JMS700 mass spectrometer, and HR-ESIMS data were obtained on an Agilent 1100LC/MSD trap SL LC/MS. Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector and Apollo Silica 5 μ column (250 × 10 mm) or Econosil RP-18 10 μ column (250 × 10 mm). Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh) was used for column chromatography. TLC was performed using Merck precoated Silica gel F_{254} plates and RP-18 F_{254s} plates. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Low-pressure liquid chromatography was performed over Merck LiChroprep Lobar-A Si 60 (240 × 10 mm) or LiChroprep Lobar-A RP-18 (240 × 10 mm) columns with a FMI QSY-0 pump (ISCO).

Plant materials

The rhizome parts of *A. gramineus* were collected on Jeju Island, Korea in March 2009, and identified by one of us (K.R.Lee). A voucher specimen (SKKU-NPL-0910) of the plant was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation

The rhizome parts of *A. gramineus* (15 kg) were extracted at room temperature with 80% MeOH to give a MeOH extract (825 g). The extract was dissolved in water (2 L) and then successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, yielding 166, 14, 5, and 47 g of residues, respectively. The hexane-soluble fraction (62 g) was separated over a silica gel column with *n*-hexane-EtOAc (11:1) to yield eight fractions (H1-H8). Fraction H3 (24 g) was separated over an RP-C_{18} silica gel column with 80% MeOH and was purified by a silica gel prep. HPLC using *n*-hexane-EtOAc (10:1) to yield compounds **3** (2 g) and **4** (19 mg). Fraction H5 (1 g) was subjected to Sephadex LH-20 column chromatography (CH_2Cl_2-MeOH = 1:1) and was purified by an RP-C_{18} prep. HPLC (60% MeOH) to give compound **6** (5 mg). Fraction H6 (606 mg) was subjected to Sephadex LH-20 column chromatography (CH_2Cl_2-MeOH = 1:1) and was purified by using a silica gel prep. HPLC using *n*-hexane-EtOAc (2:1) to yield compounds **5** (4 mg) and **15** (6 mg). The $CHCl_3$ fraction (14 g) was separated over an RP-C_{18} silica gel column with 50% MeOH to give seven fractions (C1-C7). Fraction C1 (800 mg) was subjected to a silica gel column chromatography ($CHCl_3$-MeOH = 40:1) and was purified by an RP-C_{18} prep. HPLC (55% MeOH) to obtain compounds **7** (8 mg) and **8** (63 mg). Fraction C2 (497 mg) was subjected to a silica Lobar A-column ($CHCl_3$-MeOH = 40:1) to give four subfractions (C21-C24). Subfraction C21 (157 mg) was subjected to Sephadex LH-20 column chromatography (CH_2Cl_2-MeOH = 1:1) and was purified by an RP-C_{18} prep. HPLC (65% MeOH) to yield compounds **1** (6 mg), **2** (3 mg), **9** (9 mg) and **10** (3 mg). Subfraction C24 (84 mg) was purified by an RP-C_{18} prep. HPLC (55% MeOH) to yield compound **17** (40 mg). Fraction C3 (544 mg) was subjected to a silica gel column ($CHCl_3$-EtOAc-MeOH = 10:1:1) and was purified by an RP-C_{18} prep. HPLC (55% MeOH) to give compound **16** (5 mg). The EtOAc fraction (5 g) was separated over an RP-C_{18} silica gel column using a solvent system of 50% MeOH to give eight fractions (E1-E8). Fraction E2 (800 mg) was subjected to a silica gel column ($CHCl_3$-MeOH = 20:1) and was purified by an RP-C_{18} prep. HPLC (30% MeOH) to afford compound **13** (9 mg). Fraction E3 (807 mg) was subjected to a silica gel column ($CHCl_3$-MeOH = 20:1) to give five subfractions (E31-E35). Subfraction E32 (53 mg) was subjected to Sephadex LH-20 column chromatography (MeOH 80%) and was purified by a silica gel prep. HPLC using $CHCl_3$-MeOH (35:1) to yield compound **14** (5 mg). Subfraction E33 (53 mg) was subjected to Sephadex LH-20 column chromatography (MeOH 80%) and was purified by a silica gel prep. HPLC using $CHCl_3$-MeOH (25:1) to give compounds **18** (5 mg) and **19** (5 mg). Subfraction E33 (53 mg) was subjected to Sephadex LH-20 column chromatography (MeOH 80%) and was purified by an RP-C_{18} prep. HPLC (45% MeOH) to afford compound **12** (5 mg). Fraction E7 (90 mg) was subjected to a silica Lobar A-column ($CHCl_3$-MeOH = 25:1) and was purified by a silica gel prep. HPLC using a solvent system of $CHCl_3$-MeOH (40:1) to yield compound **11** (9 mg).

Acoraminol A (1)

White oil; $[\alpha]_D^{25}$ −12.3° (*c* 0.30, $CHCl_3$); IR ν_{max} cm^{-1}: 3410, 2937, 1608, 1512, 1460, 1206; UV (MeOH) λ_{max} nm: 209, 231, 291; HR-ESI-MS *m/z*: 279.1202 [M + Na]$^+$ (calcd for $C_{13}H_{20}NaO_5$, 279.1208); ^{1}H- ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz): see Table I.

Acoraminol B (2)

Yellow oil; $[\alpha]_D^{25}$ −14.3° (*c* 0.15, $CHCl_3$); IR ν_{max} cm^{-1}: 3419, 2971, 1618, 1512; UV (MeOH) λ_{max} nm: 207, 230,

290; HR-ESI-MS *m/z*: 279.1203 $[M + Na]^+$ (calcd for $C_{13}H_{20}NaO_5$, 279.1208); ^{1}H- ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz): see Table I.

β-Asarone (3)
Yellow oil; $[\alpha]_D^{25}$ −14.0° (*c* 1.85, $CHCl_3$); IR ν_{max} cm^{-1}: 2937, 2834, 1608, 1512, 1463, 1209; FAB-MS *m/z*: 208 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.83 (3H, dd, *J* = 7.0, 1.8 Hz, H-3'), 3.74 (3H, s, -OCH_3), 3.79 (3H, s, -OCH_3), 3.83 (3H, s, -OCH_3), 5.76 (1H, dq, *J* = 10.6, 6.8 Hz, H-2'), 6.47 (1H, dq, *J* = 10.6, 1.8 Hz, H-1'), 6.53 (1H, s, H-3), 6.84 (1H, s, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 14.9 (C-3'), 56.3 (-OCH_3), 56.5 (-OCH_3), 56.7 (-OCH_3), 97.9 (C-3), 114.5 (C-6), 118.4 (C-5), 124.5 (C-2'), 125.3 (C-1'), 142.7 (C-1), 148.9 (C-2), 151.8 (C-4).

Asaraldehyde (4)
Colorless oil; $[\alpha]_D^{25}$ +39.3° (*c* 0.95, $CHCl_3$); IR ν_{max} cm^{-1}: 2942, 2833, 1664, 1607, 1514, 1213; FAB-MS *m/z*: 197 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.88 (3H, s, -OCH_3), 3.90 (3H, s, -OCH_3), 3.95 (3H, s, -OCH_3), 6.48 (1H, s, H-6), 7.30 (1H, s, H-3), 10.29 (1H, s, H-1'); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 56.4 (-OCH_3×2), 56.5 (-OCH_3), 96.3 (C-6), 109.3 (C-3), 117.6 (C-2), 143.8 (C-4), 156.0 (C-1), 158.9 (C-5), 188.2 (C-1').

Isoacoramone (5)
Colorless oil; $[\alpha]_D^{25}$ −17.0° (*c* 0.20, $CHCl_3$); IR ν_{max} cm^{-1}: 2945, 2832, 1453; ESI-MS *m/z*: 225 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.16 (3H, t, *J* = 7 Hz, H-3'), 2.99 (2H, q, *J* = 7 Hz, H-2'), 3.88 (3H, s, -OCH_3), 3.91 (3H, s, -OCH_3), 3.95 (3H, s, -OCH_3), 6.50 (1H, s, H-3), 7.43 (1H, s, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 8.8 (C-3'), 37.3 (C-2'), 56.3 (-OCH_3), 56.4 (-OCH_3), 56.5 (-OCH_3), 96.8 (C-3), 113.0 (C-6), 119.5 (C-1), 143.3 (C-5), 153.8 (C-4), 155.3 (C-2), 200.9 (C-1').

Propioveratrone (6)
Colorless gum; $[\alpha]_D^{25}$ −10.7° (*c* 0.25, $CHCl_3$); IR ν_{max} cm^{-1}: 3386, 2940, 1595, 1418; FAB-MS *m/z*: 195 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.22 (3H, t, *J* = 7.0 Hz, H-3'), 2.96 (2H, q, *J* = 7.2 Hz, H-2'), 3.94 (6H, s, -OCH_3×2), 6.88 (1H, d, *J* = 8.5 Hz, H-5), 7.54 (1H, s, H-2), 7.58 (1H, d, *J* = 8.0 Hz, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 8.7 (C-3'), 31.5 (C-2'), 56.2 (-OCH_3), 56.3 (-OCH_3), 110.2 (C-5), 110.5 (C-2), 122.7 (C-6), 130.4 (C-1), 149.3 (C-3), 153.3 (C-4), 199.7 (C-1').

(1'*R*,2'*S*)-1',2'-Dihydroxyasarone (7)
Colorless gum; $[\alpha]_D^{25}$ +31.0° (*c* 0.40, $CHCl_3$); IR ν_{max} cm^{-1}: 3379 , 2943, 2834, 1207; FAB-MS *m/z*: 242 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.32 (3H, d, *J* = 6.5 Hz, H-3'), 3.83 (3H, s, -OCH_3), 3.86 (3H, s, -OCH_3), 3.90 (3H, s, -OCH_3), 4.08 (1H, m, H-2'), 4.88 (1H, br s, H-1'), 6.53 (1H, s, H-3), 6.98 (1H, s, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 17.8 (C-3'), 56.4 (-OCH_3×2), 56.8 (-OCH_3), 70.6 (C-2'), 73.8 (C-1'), 97.6 (C-3), 112.2 (C-6), 120.2 (C-1), 143.5 (C-5), 149.3 (C-4), 151.1 (C-2).

(1'*S*,2'*S*)-1',2'-Dihydroxyasarone (8)
Yellow oil; $[\alpha]_D^{25}$ +78.7° (*c* 1.10, $CHCl_3$); IR ν_{max} cm^{-1}: 3389, 2939, 1513, 1207; FAB-MS *m/z*: 242 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.07 (3H, d, *J* = 6.5 Hz, H-3'), 3.83 (3H, s, -OCH_3), 3.86 (3H, s, -OCH_3), 3.90 (3H, s, -OCH_3), 3.98 (1H, m, H-2'), 4.56 (1H, br s, H-1'), 6.52 (1H, s, H-3), 6.86 (1H, s, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 18.9 (C-3'), 56.4 (-OCH_3×2), 56.8 (-OCH_3), 71.5 (C-2'), 75.6 (C-1'), 97.7 (C-3), 112.4 (C-6), 120.7 (C-1), 143.4 (C-5), 149.4 (C-4), 151.3 (C-2).

3',4'-Dimethoxycinnamyl alcohol (9)
Colorless oil; $[\alpha]_D^{25}$ +18.6° (*c* 0.45, $CHCl_3$); IR ν_{max} cm^{-1}: 3361, 2944, 2833; FAB-MS *m/z*: 195 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.89 (3H, s, -OCH_3), 3.90 (3H, s, -OCH_3), 4.32 (2H, br s, H-3'), 6.26 (1H, dt, *J* = 15.7, 5.8 Hz, H-2'), 6.56 (1H, br d, *J* = 15.7 Hz, H-1'), 6.83 (1H, d, *J* = 8.0 Hz, H-6), 6.92 (1H, d, *J* = 8.0 Hz, H-5), 6.95 (1H, s, H-2); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 56.1 (-OCH_3), 56.2 (-OCH_3), 64.1 (C-3'), 109.2 (C-5), 111.4 (C-2), 119.9 (C-6), 126.8 (C-2'), 130.0 (C-1), 131.4 (C-1'), 149.2 (C-3), 149.3 (C-4).

3',4',5'-Trimethoxycinnamyl alcohol (10)
Colorless oil; $[\alpha]_D^{25}$ +10.3° (*c* 0.15, $CHCl_3$); IR ν_{max} cm^{-1}: 3385, 1604; ESI-MS *m/z*: 225 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.85 (3H, s, -OCH_3), 3.86 (3H, s, -OCH_3), 3.87 (3H, s, -OCH_3), 4.33 (2H, dd, *J* = 5.6, 1.4 Hz, H-3'), 6.29 (1H, dt, *J* = 15.8, 5.6 Hz, H-2'), 6.52 (1H, br d, *J* = 15.8 Hz, H-1'), 6.63 (2H, s, H-2, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 56.3 (-OCH_3×2), 61.1 (-OCH_3), 63.9 (C-3'), 103.8 (C-2, C-6), 128.3 (C-2'), 131.4 (C-1'), 132.6 (C-1), 138.2 (C-4), 153.6 (C-3, C-5).

Kaempferol 3-methyl ether (11)
Yellow powder; m.p. 270-275°C; $[\alpha]_D^{25}$ +14.0° (*c* 0.45, CH_3OH); IR ν_{max} cm^{-1}: 3378, 1606; UV (MeOH) λ_{max} nm: 203, 267, 280, 349; ESI-MS *m/z*: 301 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 3.78 (3H, s, -OCH_3), 6.19 (1H, d, *J* = 2.0 Hz, H-6), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.91 (2H, d, *J* = 9.0 Hz, H-3', H-5'), 7.96 (2H, d, *J* = 9.0 Hz, H-2', H-6'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 59.4 (-OCH_3), 93.6 (C-8), 98.6 (C-6), 104.7 (C-10), 115.4 (C-3', C-5'), 121.4 (C-1'), 130.2 (C-2', C-6'), 138.3 (C-3), 156.9 (C-2), 157.3 (C-9), 160.5 (C-4'), 161.9 (C-5), 164.8 (C-7), 178.8 (C-4).

2-[4-(3-Hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (12)
Colorless gum; $[\alpha]_D^{25}$ +18.4° (*c* 0.25, MeOH); IR ν_{max} cm^{-1}: 3382, 1602; ESI-MS *m/z*: 257 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 1.79-1.84 (2H, m, H-2'), 2.63 (2H, t, *J* = 7.7 Hz, H-1'), 3.55 (2H, t, *J* = 6.6 Hz, H-3'), 3.74 (4H, d, *J* = 5.1 Hz, H-1", H-3"), 3.84 (3H, s, -OCH_3), 4.15 (1H, q, *J* = 5.1 Hz, H-2"), 6.73 (1H, dd, *J* = 8.1, 2.2 Hz, H-6), 6.85 (1H, d, *J* = 2.2 Hz, H-2), 6.99 (1H, d, *J* = 8.1 Hz, H-5); ^{13}C-NMR (CD_3OD, 125 MHz): δ 31.5 (C-1'), 34.4 (C-2'), 55.3 (-OCH_3), 60.9 (C-1", C-3"), 61.0 (C-3'), 82.2 (C-2"), 112.9 (C-2), 118.3 (C-5), 120.7 (C-6), 137.1 (C-1), 145.7 (C-4), 152.0 (C-3).

Hydroxytyrosol (13)
Brown oil; $[\alpha]_D^{25}$ +30.6° (*c* 0.02, MeOH); IR ν_{max} cm^{-1}: 3375, 2946, 1606, 1447; FAB-MS *m/z*: 154 $[M]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 2.66 (2H, t, *J* = 7.3 Hz, H-1'), 3.67 (2H, t, *J* = 7.3 Hz, H-2'), 6.52 (1H, dd, *J* = 8.0, 2.1 Hz, H-6), 6.59 (1H, d, *J* = 2.1 Hz, H-2), 6.67 (1H, d, *J* = 8.0 Hz, H-5); ^{13}C-NMR (CD_3OD, 125 MHz): δ 38.5 (C-1'), 63.4 (C-2'), 115.1 (C-2), 115.9 (C-5), 120.0 (C-6), 130.6 (C-1), 143.4 (C-4), 145.0 (C-3).

Tyrosol (14)
Brown oil; $[\alpha]_D^{25}$ +32.6° (*c* 0.37, MeOH); IR ν_{max} cm^{-1}: 3359, 2940, 1514, 1444; FAB-MS *m/z*: 138 $[M]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 2.71 (2H, t, *J* = 7.0 Hz, H-1'), 3.67 (2H, t, *J* = 7.3 Hz, H-2'), 6.70 (2H, dd, *J* = 8.0, 2.1 Hz, H-3, H-5), 7.02 (2H, dd, *J* = 8.0, 2.1 Hz, H-2, H-6); ^{13}C-NMR (CD_3OD, 125 MHz): δ 38.2 (C-1'), 63.4 (C-2'), 114.9 (C-3, C-5), 129.7 (C-2, C-6), 130.7 (C-1), 155.6 (C-4).

(2*S*,5*S*)-Diveratryl-(3*R*,4*S*)-dimethyltetrahydrofuran (15)
Colorless oil; $[\alpha]_D^{25}$ −16.6° (*c* 0.30, $CHCl_3$); IR ν_{max} cm^{-1}: 3381, 2968, 1600; UV (MeOH) λ_{max} nm: 206, 276, 393; ESI-MS *m/z*: 373 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.60 (1H, d, *J* = 7.0 Hz, H-9'), 1.01 (1H, d, *J* = 6.3 Hz, H-9), 2.45 (2H, m, H-8, H-8'), 3.87 (3H, s, -OCH_3), 3.88 (3H, s, -OCH_3), 3.89 (3H, s, -OCH_3), 3.90 (3H, s, -OCH_3), 4.66 (1H, d, *J* = 9.1 Hz, H-7), 5.46 (1H, d, *J* = 5.4 Hz, H-7'), 6.82-6.87 (4H, m, H-5, H-5', H-6, H-6'), 6.92 (1H, d, *J* = 1.3 Hz, H-2'), 6.96 (1H, d, *J* = 1.8 Hz, H-2); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 9.7 (C-9'), 12.1 (C-9), 43.7 (C-8), 47.8 (C-8'), 56.1 (-OCH_3×2), 56.2 (-OCH_3×2), 85.0 (C-7'), 85.9 (C-7), 109.4 (C-2), 109.7 (C-2'), 111.1 (C-5'), 111.2 (C-5), 118.3 (C-6'), 118.7 (C-6), 133.5 (C-1'), 135.9 (C-1), 148.0 (C-4), 148.7 (C-3), 149.0 (C-4'), 149.4 (C-3').

(7*S*,8*R*)-Dihydrodehydrodiconiferyl alcohol (16)
Colorless oil; $[\alpha]_D^{25}$ +14.2° (*c* 0.50, MeOH); IR ν_{max} cm^{-1}: 3371, 2939, 1607, 1517, 1460; UV (MeOH) λ_{max} nm: 212, 282; FAB-MS *m/z*: 360 $[M]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 1.80 (2H, m, H-8'), 2.62 (2H, t, *J* = 7.2 Hz, H-9'), 3.45 (1H, m, H-8), 3.46 (1H, dd, *J* = 11.2, 6.4 Hz, H-9), 3.56 (2H, t, *J* = 6.4 Hz, H-9'), 3.74 (1H, dd, *J* = 11.2, 5.6 Hz, H-9), 3.79 (3H, s, -OCH_3), 3.82 (3H, s, -OCH_3), 5.47 (1H, d, *J* = 6.2 Hz, H-7), 6.72 (1H, d, *J* = 8.0 Hz, H-5), 6.78 (2H, s, H-2', H-6'), 6.83 (1H, dd, *J* = 8.0, 1.6 Hz, H-6), 6.95 (1H, d, *J* = 1.6 Hz, H-2); ^{13}C-NMR (CD_3OD, 125 MHz): δ 31.7 (C-7'), 34.6 (C-8'), 54.2 (C-8), 55.2 (-OCH_3), 55.6 (-OCH_3), 61.1 (C-9'), 63.8 (C-9), 87.8 (C-7), 109.4 (C-2), 113.0 (C-2'), 115.0 (C-5), 116.8 (C-6'), 118.6 (C-6), 128.6 (C-5'), 133.6 (C-1), 135.7 (C-1'), 144.0 (C-3'), 146.3 (C-4), 146.4 (C-4'), 147.9 (C-3).

7*S*,8*S*-*threo*-4,7,9,9'-Tetrahydroxy-3,3'-dimethoxy-8-*O*-4'-neolignan (17)
Colorless gum; $[\alpha]_D^{25}$ +10.2° (*c* 0.25, MeOH); IR ν_{max} cm^{-1}: 3386, 1604; UV (MeOH) λ_{max} nm: 207, 228, 280; ESI-MS *m/z*: 401 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 1.80 (1H, m, H-8'), 2.62 (2H, t, *J* = 7.0 Hz, H-7'), 3.55 (2H, t, *J* = 6.5 Hz, H-9'), 3.79 (3H, s, -OCH_3), 3.80 (3H, s, -OCH_3), 4.19 (1H, m, H-8), 4.87 (1H, d, *J* = 6.0 Hz, H-7), 6.68-7.02 (6H, m, H-2, H-5, H-6, H-2', H-5', H-6'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 31.5 (C-7'), 34.4 (C-8'), 55.2 (-OCH_3), 55.4 (-OCH_3), 60.7 (C-9), 61.0 (C-9'), 73.0 (C-7), 86.6 (C-8), 110.6 (C-2), 112.8 (C-2'), 114.7 (C-5), 118.5 (C-5'), 119.6 (C-6), 120.9 (C-6'), 132.6 (C-1), 137.1 (C-1'), 145.8 (C-4), 146.0 (C-4'), 147.7 (C-3), 150.5 (C-3').

7*S*,8*R*-*erythro*-4,7,9,9'-Tetrahydroxy-3,3'-dimethoxy-8-*O*-4'-neolignan (18)
Colorless gum; $[\alpha]_D^{25}$ +16.6° (*c* 0.25, MeOH); IR ν_{max} cm^{-1}: 3375, 1513; UV (MeOH) λ_{max} nm: 209, 227, 280; ESI-MS *m/z*: 401 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 1.80 (1H, m, H-8'), 2.60 (2H, t, *J* = 7.0 Hz, H-7'), 3.54 (2H, t, *J* = 6.5 Hz, H-9'), 3.79, (3H, s, -OCH_3), 3.80 (3H, s, -OCH_3), 4.28 (1H, m, H-8), 4.83 (1H, d, *J* = 4.0 Hz, H-7), 6.65-7.02 (6H, m, H-2, H-5, H-6, H-2', H-5', H-6'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 31.5 (C-7'), 34.3 (C-8'), 55.2 (-OCH_3), 55.3 (-OCH_3), 61.0 (C-9), 62.3 (C-9'), 73.0 (C-7), 85.5 (C-8), 110.7 (C-2), 112.9 (C-2'), 114.5 (C-5), 118.5 (C-5'), 119.8 (C-6), 120.7 (C-6'), 133.0 (C-1), 136.9 (C-1'), 145.8 (C-4), 146.1 (C-4'), 147.5 (C-3), 150.7 (C-3').

Dihydroyashsbushiketol (19)
Colorless needle; $[\alpha]_D^{25}$ −215.3° (*c* 0.10, MeOH); IR ν_{max} cm^{-1}: 3394, 2923, 1604, 1424; FAB-MS *m/z*: 283 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.65-1.81 (2H, m,

H-8'), 2.55 (4H, t, J = 7.0 Hz, H-7, H-7'), 2.74 (2H, m, H-8), 2.90 (2H, m, H-10), 4.03 (1H, q, J = 7.0 Hz, H-9'), 7.19 (10H, br s, H-1, H-2, H-3, H-4, H-5, H-1', H-2', H-3', H-4', H-5'); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 29.8 (C-7'), 32.0 (C-7), 38.3 (C-8'), 45.3 (C-8), 49.5 (C-10), 67.1 (C-9'), 126.1 (C-4), 126.5 (C-4'), 128.5 (C-2, C-6), 128.7 (C-2', C-6', C-3', C-5'), 128.8 (C-3, C-5), 140.9 (C-1'), 142.0 (C-1), 211.3 (C-9).

Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of 1 and 2

Compound **1** (1.5 mg) in deuterated pyridine (0.75 mL) was transferred to a clean NMR tube. (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride (10 μL) was added to the NMR tube immediately under a N_2 gas stream. The NMR tube was then shaken carefully to thoroughly mix the sample and the MTPA chloride. The NMR reaction tube was left at room temperature overnight. The reaction was then completed to afford the (*R*)-MTPA ester derivative (**1r**) of **1**. Treatment of **1** (1.5 mg) with (*R*)-MTPA-Cl (10 μL) as described above yielded the corresponding (*S*)-MTPA ester **1s**. Similarly, treatment of **2** with (*S*)- and (*R*)-MTPA-Cl afforded the respective Mosher esters **2r** and **2s**. The ^{1}H-NMR spectra of **1r**, **1s**, **2r**, and **2s** were measured directly in the NMR reaction tubes.

1s. ^{1}H-NMR (pyridine-d_5, 500 MHz): δ 1.168 (3H, d, J = 6.5 Hz, H-3'), 3.261 (3H, s, -OCH_3), 3.688 (3H, s, -OCH_3), 3.704 (3H, s, -OCH_3), 3.767 (3H, s, -OCH_3), 4.207 (1H, dq, J = 8.0, 6.5 Hz, H-2'), 5.681 (1H, d, J = 8.0 Hz, H-1'), 6.717 (1H, s, H-3), 7.175 (1H, s, H-6).

1r. ^{1}H-NMR (pyridine-d_5, 500 MHz): δ 1.218 (3H, d, J = 6.5 Hz, H-3'), 3.249 (3H, s, -OCH_3), 3.688 (3H, s, -OCH_3), 3.704 (3H, s, -OCH_3), 3.752 (3H, s, -OCH_3), 4.202 (1H, dq, J = 8.0, 6.5 Hz, H-2'), 5.364 (1H, d, J = 8.0 Hz, H-1'), 6.717 (1H, s, H-3), 7.175 (1H, s, H-6).

2s. ^{1}H-NMR (pyridine-d_5, 500 MHz): δ 1.271 (3H, d, J = 6.5 Hz, H-3'), 3.334 (3H, s, -OCH_3), 3.691 (3H, s, -OCH_3), 3.735 (3H, s, -OCH_3), 3.769 (3H, s, -OCH_3), 4.365 (1H, dq, J = 6.5, 4.5 Hz, H-2'), 5.753 (1H, d, J = 4.5 Hz, H-1'), 6.704 (1H, s, H-3), 7.176 (1H, s, H-6).

2r. ^{1}H-NMR (pyridine-d_5, 500 MHz): δ 1.324 (3H, d, J = 6.5 Hz, H-3'), 3.320 (3H, s, -OCH_3), 3.676 (3H, s, -OCH_3), 3.720 (3H, s, -OCH_3), 3.762 (3H, s, -OCH_3), 4.345 (1H, dq, J = 6.5, 4.5 Hz, H-2'), 5.697 (1H, d, J = 4.5 Hz, H-1'), 6.700 (1H, s, H-3), 7.141 (1H, s, H-6).

Test for cytotoxicity *in vitro*

Sulforhodamine B bioassays (SRB) were used as cytotoxicity screening methods (Skehan et al., 1990). Cytotoxicity assays for each compound were done *in vitro* at the Korea Research Institute of Chemical Technology against four cultured human tumor cell lines: A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxrubicin was used as a positive control. The cytotoxicity of doxorubicin against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines were IC_{50} 0.02, 0.01, 0.01, and 0.04 μM, respectively.

RESULTS AND DISCUSSION

Compound **1** was obtained as white oil. The HR-ESI-MS ($[M + Na]^+$ at m/z 279.1202, calcd for 279.1208) and ^{1}H- and ^{13}C-NMR spectral data of **1** gave the molecular formula of $C_{13}H_{20}O_5$. The IR spectrum of **1** indicated the presence of an OH (3410 cm^{-1}) and an aromatic system (1608 cm^{-1}). The ^{1}H-NMR spectrum of **1** (Table I) showed two aromatic protons at δ 6.54 (1H, s) and 6.83 (1H, s), one methyl proton at δ 1.01 (3H, d, J = 6.5 Hz) and four methoxy protons at δ 3.24 (3H, s, alcoholic OMe), 3.82 (3H, s), 3.85 (3H, s), and 3.91 (3H, s). The ^{13}C-NMR spectrum of **1** indicated 13 carbon resonances, which were classified by DEPT and HMQC experiments as six aromatic carbons at δ 97.6, 111.0, 118.5, 143.8, 149.4, and 152.7, a methyl at δ 17.9, two oxygenated methines at δ 71.7 and 81.8, and four methoxy carbons at δ 56.3, 56.7, 56.8, and 56.9. Further analysis showed that the data of **1** were very similar to those of the known compound, 1-(2,4,5-

Table I. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectral data of compounds **1-2** in $CDCl_3$ (δ in ppm)

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		118.5		117.9
2		152.7		152.3
3	6.54 (s)	97.6	6.54 (s)	97.6
4		149.4		149.3
5		143.8		143.7
6	6.83 (s)	111.0	6.92 (s)	111.4
1'	4.42 (d, 8.0)	81.8	4.60 (d, 4.5)	80.9
2'	3.82 (dq, 8.0, 6.5)	71.7	3.98 (dq, 6.5, 4.5)	71.7
3'	1.01 (d, 6.5)	17.9	1.09 (d, 6.5)	18.1
2-OCH_3	3.91 (s)	56.9[a]	3.90 (s)	57.4[b]
4-OCH_3	3.85 (s)	56.8[a]	3.86 (s)	56.8[b]
5-OCH_3	3.82 (s)	56.7[a]	3.81 (s)	56.5[b]
1'-OCH_3	3.24 (s)	56.3	3.30 (s)	56.3

Assignments were based on 2D NMR methods, including HMQC and HMBC. Well-resolved couplings are expressed with coupling patterns and coupling constants (in Hz) given in parentheses.

*NMR assignments with the same superscript may be interchanged.

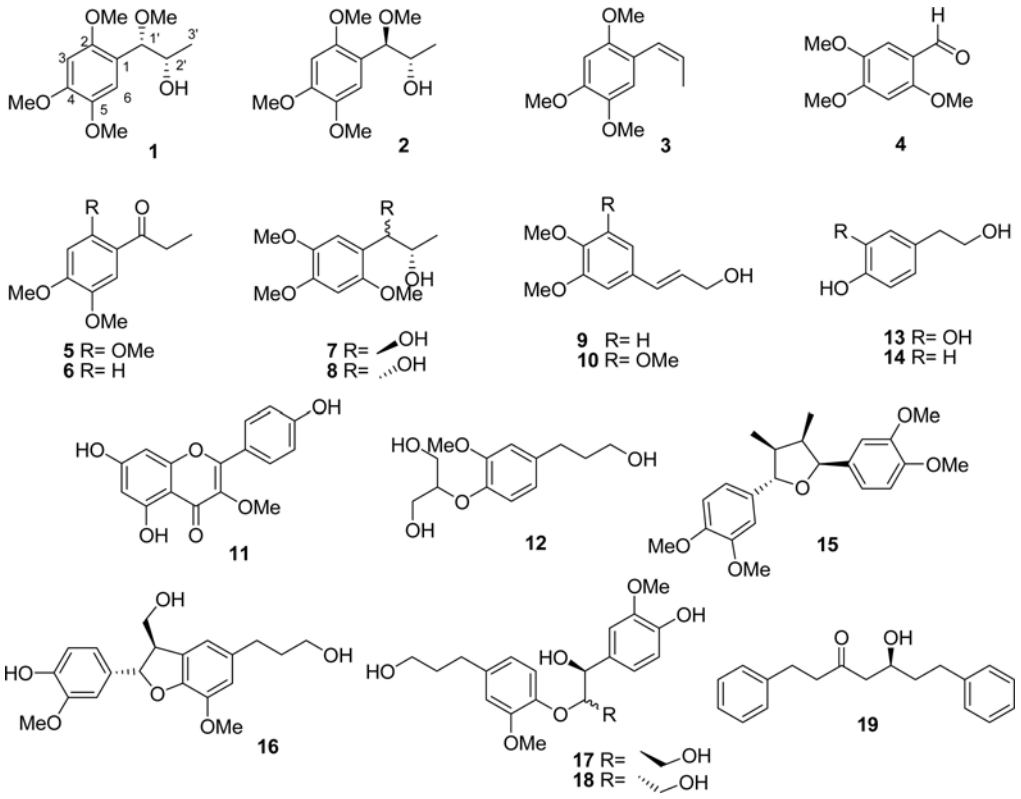

Fig. 1. The structures of compounds isolated from *A. gramineus*

trimethoxyphenyl)-1-methoxypropan-2-ol, whose absolute configuration has not been confirmed (Nawamaki and Kuroyanagi, 1996). The structure of **1** was unambiguously confirmed by the HMBC experiment, which showed correlations of H-1' (δ 4.42)/C-1 (δ 118.5), C-2 (δ 152.7), and C-6 (δ 111.0), H-3' (δ 1.01)/C-2' (δ 71.7) and C-1' (δ 81.8), and H-6 (δ 6.83)/C-1' (δ 81.8). The absolute configuration of **1** was established on the basis of the convenient Mosher ester procedure (Su et al., 2002). Treatment of **1** with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA)-Cl gave the (*S*)- and (*R*)-MTPA esters **1s** and **1r**, respectively. Diagnostic ^{1}H-NMR chemical shift differences between the MTPA esters of **1** [δ_S - δ_R] (Fig. 2) revealed the absolute configuration at C-2' to be *S*. The coupling constant (J = 8.0 Hz) for H-1' and H-2' suggested that these protons have a *cis*-orientation and, thus the absolute configuration at C-1' was confirmed to be *S* (Herrera Braga et al., 1984). On the basis of the above evidence, the structure of **1** was established as shown in Fig. 1 and named acoraminol A.

Compound **2**, obtained as yellow oil, had a molecular formula of $C_{13}H_{20}O_5$, as obtained from the HR-ESI-MS ([M + Na]$^+$ at m/z 279.1203, calcd for 279.1208) and ^{1}H- and ^{13}C-NMR spectral data of **2**. The IR spectrum of **2** indicated the presence of an OH (3419 cm^{-1}) and an aromatic system (1618 cm^{-1}). Inspection of the ^{1}H- and ^{13}C-NMR data of **2** (Table I) revealed that these data were very similar to those of **1**, except for the chemical shifts from C-1' to C-3' [δ 4.60 (1H, d, J = 4.5 Hz, H-1'), 3.98 (1H, dq, J = 6.5, 4.5 Hz, H-2'), and 1.09 (3H, d, J = 6.5 Hz, H-3'); δ 80.9 (C-1'), 71.7

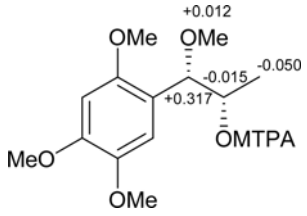

Fig. 2. Values of δ_S - δ_R (data obtained in pyridine-d_5) of the MTPA esters of **1**

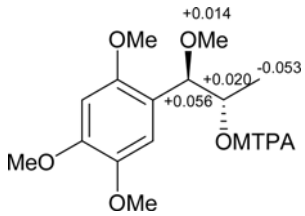

Fig. 3. Values of δ_S - δ_R (data obtained in pyridine-d_5) of the MTPA esters of **2**

(C-2'), and 18.1 (C-3') in **2**; δ 4.42 (1H, d, J = 8.0 Hz, H-1'), 3.82 (1H, dq, J = 8.0, 6.5 Hz, H-2'), and 1.01 (3H, d, J = 6.5 Hz, H-3'); δ 81.8 (C-1'), 71.7 (C-2'), and 17.9 (C-3') in **1**]. Analysis of the 2D NMR data of **2** (HMQC, HMBC, and NOESY) led to unambiguous ^{1}H- and ^{13}C-NMR assignments (Table I), and confirmed **2** to be the stereoisomer of **1**. As described for **1**, the absolute configuration of **2** was determined using the convenient Mosher ester procedure (Su et al., 2002), which proved the *S*-configuration for C-2' (Fig. 3). In addition, the coupling constant (J = 4.5 Hz) for H-1' and H-2' indicated that these protons have a *trans*-orientation and, thus the absolute configuration at C-1' was determined to be *R* (Herrera Braga et al., 1984). Based on the above considerations, the structure of **2** was established as shown in Fig. 1 and named acoraminol B.

Compounds **3**-**19** were identified by comparing their ^{1}H-, ^{13}C-NMR, and MS spectra with the literature to be β-asarone (**3**) (Patra and Mitra, 1981), asaraldehyde (**4**) (Nawamaki and Kuroyanagi, 1996), isoacoramone (**5**) (Santos and Chaves, 1999), propioveratrone (**6**) (Joshi et al., 2005), (1'*R*,2'*S*)-1',2'-dihydroxyasarone (**7**) (Freire et al., 2005), (1'*S*,2'*S*)-1',2'-dihydroxyasarone (**8**) (Freire et al., 2005), 3',4'-dimethoxycinnamyl alcohol (**9**) (Feliciano et al., 1986), 3',4',5'-trimethoxycinnamyl alcohol (**10**) (Feliciano et al., 1986), kaempferol 3-methyl ether (**11**) (Valesi, 1972), 2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (**12**) (Kouno et al., 1992), hydroxytyrosol (**13**) (Capasso et al., 1992), tyrosol (**14**) (Capasso et al., 1992), (2*S*,5*S*)-diveratryl-(3*R*,4*S*)-dimethyltetrahydrofuran (**15**) (Prasad et al., 1995), (7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol (**16**) (Kuang et al., 2009), 7*S*,8*S*-*threo*-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-*O*-4'-neolignan (**17**) (Matsuda and Kikuchi, 1996), 7*S*,8*R*-*erythro*-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-*O*-4'-neolignan (**18**) (Matsuda and Kikuchi, 1996), and dihydroyashsbushiketol (**19**) (Asakawa, 1970). Compounds **5**-**19** were isolated for the first time from this plant source.

The isolated compounds were tested for cytotoxicity against four human tumor cells *in vitro* using a SRB bioassay. Compound **11** exhibited good cytotoxic activity against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines (IC_{50}: 11.37, 5.74, 7.19 and 9.06 μM, respectively). Compound **15** showed moderate cytotoxic activity against A549, SK-OV-3, SK-MEL-2, and HCT15 cells (IC_{50}: 14.05, 19.27, 32.14 and 12.86 μM, respectively). The other compounds showed little cytotoxicity (IC_{50} > 30 μM).

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