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Isolation and Structural Identification of Minor Constituents from *Sasa borealis*

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Abstract – Compounds of (+)-5,5'-dimethoxylariciresinol (**1**) and (3*S*,5*R*,6*S*,7*E*)-5,6-epoxy-3-hydroxy-7-megastigmen-9-one (**2**) were isolated from an EtOAc extract of the whole plant of *Sasa borealis* (Hack.) Makino (Gramineae) for the first time in the present investigation. The structures of compounds were identified by analysis of spectral data including 1D- and 2D-NMR spectra as well as by comparison of their data with the published values. These compounds have never been isolated previously from the family Gramineae.
Keywords – *Sasa borealis*, Gramineae, (+)-5,5'-dimethoxylariciresinol, (3*S*,5*R*,6*S*,7*E*)-5,6-epoxy-3-hydroxy-7-megastigmen-9-one.

Introduction

Sasa borealis (Hack.) Makino is a perennial plant belonging to the family Gramineae which is composed of 550 genera with 10,000 species (Ohmoto *et al.*, 1970; Koh and Jeon, 2003). Previous phytochemical studies on the *Sasa* species are including triterpenes, flavonoids, and flavonolignans (Yoon *et al.*, 2000; Nakajima *et al.*, 2003). However, there have not been many studies on *S. borealis*. In the present study, compounds including a known lignan (**1**) and a megastigmen (**2**) were isolated from the whole plant of *S. borealis* for the first time.

Materials and Methods

Plant material – The whole plant of *S. borealis* was purchased from the Kyungdong Oriental Herbal market, Korea, in February 2003.

General experimental procedure – Optical rotations were measured with a P-1010 polarimeter (Jasco, Japan) at 25°C. UV and IR spectra were recorded on a U-3000 spectrophotometer (Hitachi, Japan) and a FTS 135 FT-IR spectrometer (Bio-Rad, CA), respectively. 1D and 2D NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA). TMS was used as an internal standard. EIMS was obtained on a JMS 700 Mstation HRMS spectrometer (JEOL, Japan). LRESIMS were recorded on VG Biotech platform mass spectrometer (VG Biotech, U.K.). TLC analysis was performed on Kieselgel 60 F_{254} (Merck, Germany) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich) followed by charring at 110°C for 5-10 min. Silica gel (230-400 mesh, Merck, Germany), RP-18 (YMCGEL ODS-A, 12 nm, S-150 μm), and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. All solvents used for chromatographic separations were distilled before use.

Extraction and isolation – The dried and ground whole plants (4 kg) of *S. borealis* were extracted three times with MeOH (1 L×3) overnight at room temperature. The solvent was evaporated *in vacuo* to give a concentrated MeOH extract, which was then diluted with distilled water to afford an aqueous MeOH solution (1 L). The aqueous solution was defatted with *n*-hexane (2 L× 6) and subsequently partitioned with EtOAc (2 L×4) and *n*-BuOH (1 L×5). Each of these fractions was concentrated *in vacuo* to dryness to provide the residues of *n*-hexane (23 g), EtOAc (17.7 g) and *n*-BuOH (22.7 g). The EtOAc soluble-extract (17.7 g) was chromatographed over silica gel as stationary phase using a CH_2Cl_2-MeOH gradient from 1 : 0 to 0 : 1 v/v as mobile phase to give eleven fractions (I-XI). Fractions VI and VII were combined and separated over a further silica gel column chromatography (3×30 cm) and eluted with *n*-hexane-acetone gradient from 50 : 1 to 0 : 1 v/v to give 18 subfractions. Subfraction VI-10 (80 mg) was separated over a Sephadex LH-20 column chromatography (2×80 cm) and eluted with pure 100% MeOH. Fraction VI-10-4 out of VI-10 subfractions was purified by reverse phase column chromatography (3 ×30 cm) with MeOH-H_2O gradient from 1 : 1 to 1 : 0 v/v

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Table 1. ^{1}H NMR data of compounds **1** and **2**

Position	**Compound 1** (δ_H)	Position	**2** (δ_H)
2	6.64 (2H, s)	2a[b]	1.56 (1H, ddd, *J* = 13.2, 3.6, 1.6)
6	6.64 (2H, s)	2b[b]	1.27 (1H, m)
7	4.79 (1H, d, J^a = 6.0)	3.77 (1H, m)	3
8	2.32 (1H, m)	4α[b]	2.27 (1H, ddd, *J* = 14.4, 5.2, 1.6)
9	3.86 (2H, m)	4β[b]	1.66 (1H, dd, *J* = 14.4, 8.4)
2'	6.52 (2H, s)	7	7.13 (1H, d, *J* = 15.8)
6'	6.52 (2H, s)	8	6.13 (1H, d, *J* = 15.8)
7'	2.95 (1H, dd, *J* = 13.2, 4.8)	10	2.26 (3H, s, C<u>H</u>$_3$-10)
	2.53 (1H, dd, *J* = 13.6, 11.2)	11	1.20 (3H, s, C<u>H</u>$_3$-11)
8'	2.70 (1H, m)	12	0.92 (3H, s, C<u>H</u>$_3$-12)
9'	3.97 (1H, dd, *J* = 8.4, 6.4)	13	1.15 (3H, s, C<u>H</u>$_3$-13)
	3.69 (1H, dd, *J* = 8.4, 6.8)		
3,3',5,5'-OCH$_3$	3.80 (12H, s)		

[a] Coupling constant in Hz.

[b] α and β represent equatorial and axial protons, respectively. They were assigned stereo-chemically.

Table 2. ^{13}C NMR data of compounds **1**and **2**

Position	**1** (δ_H)		**2** (δ_H)
1	131.3 (s)	1	35.6 (s)
2	102.4 (d)	2	47.6 (t)
3	146.9 (s)	3	63.6 (s)
4	133.8 (s)	4	41.5 (t)
5	146.9 (s)	5	67.8 (s)
6	102.4 (d)	6	70.0 (s)
7	82.9 (d)	7	143.8 (d)
8	52.6 (d)	8	133.6 (d)
9	60.9 (t)	9	197.3 (s)
1'	131.3 (s)	10	27.4 (q)
2'	105.1 (d)	11	25.3 (q)
3'	146.9 (s)	12	30.6 (q)
4'	133.8 (s)	13	20.2 (q)
5'	146.9 (s)		
6'	105.1 (d)		
7'	33.8 (t)		
8'	42.4 (d)		
9'	72.8 (t)		
3,3',5,5'-OCH$_3$	56.3(q)		

and yielded compound **1** (2.5 mg, R_f = 0.3 at 1 : 1 ratio of MeOH:H_2O). Fraction V (652 mg) was chromatographed over a reverse phase column (3×30 cm) with MeOH-H_2O gradient from 3 : 2 to 7 : 3 v/v to give eight subfractions. Subfraction V-1 was further purified by a reverse phase column chromatography (3×30 cm) with MeOH-H_2O gradient from 1 : 1 to 1 : 0 v/v and yielded compound **2** (2.1 mg, R_f = 0.4 at 1 : 1 ratio of *n*-hexane:EtOAc).

(+)-5,5'-Dimethoxylariciresinol (1) – Colorless crystals, $[\alpha]_D^{25}$: + 20.0° (*c* 0.12, MeOH); IR (film) ν_{max} (cm^{-1}): 3406, 1612, 1518; UV λ_{max} MeOH (log ε): 272 nm (3.81); LREIMS *m/z* (% rel. int.) 420 ([M]$^+$, 100), 210 (23), 194 (14), 167 (89); ^{1}H-NMR (acetone-d_6, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) data in Table 1 and 2, respectively.

(3*S*,5*R*,6*S*,7*E*)-5,6-epoxy-3-hydroxy-7-megastigmen-9-one (2) – Amorphous solid, $[\alpha]_D^{25}$: −30.8° (*c* 0.1, MeOH); IR (film) ν_{max} (cm^{-1}): 3432, 2927, 1677, 1378, 1257, 1179, 1032, 989, 671; UV λ_{max} MeOH (log ε): 224 nm (3.23); LREIMS *m/z* (% rel. int.) 223 ([M-H]$^+$, 7), 207 (9), 167 (19), 149 (57); ^{1}H-NMR (acetone-d_6, 400 MHz) and ^{13}C-NMR (acetone-d_6, 100 MHz) data in Table 1 and 2, respectively.

Results and Discussion

Repeated column chromatography of the EtOAc-soluble fraction of *S. borealis* led to the isolation of (+)-5,5'-dimethoxylariciresinol (**1**) (Achenbach *et al.*, 1988; Ida *et al.*, 1994), and (3*S*,5*R*,6*S*,7*E*)-5,6-epoxy-3-hydroxy-7-megastigmen-9-one (Macias *et al.*, 1999; Duan *et al.*, 2002; D'Abrosca *et al.*, 2004) (**2**). To determine the structures of compounds **1** and **2**, the combined analyses with a series of 1D and 2D NMR, infrared, and mass spectra were accomplished. In addition, all physical and

spectroscopic data obtained in this present study were compared with those published before.

Compound **1** showed a $[M]^+$ ion peak at *m/z* 420 in the EIMS spectrum, which together with ^{13}C NMR data was consistent with the molecular formula of $C_{22}H_{28}O_8$. In the ^1H-NMR spectrum, the protons corresponding to four methoxy groups showed down field shifted peaks at δ_H 3.80 (12H, s, $OC\underline{H}_3$-3, $OC\underline{H}_3$-5, $OC\underline{H}_3$-3', and $OC\underline{H}_3$-5'). Four aromatic protons at δ_H 6.52 (2H, s, H-2' and H-6') and δ_H 6.64 (2H, s, H-2 and H-6) came out symmetrically. These results indicate two phenol rings defined by IR peaks (see Extraction and Isolation) have two-methoxy groups attached at *meta*-position each other and these aromatic rings are linked symmetrically. The optical rotation of (+)- and (±)- forms were reported to be +14.4° (Kinzo *et al.*, 1991) and +5° (Achenbach et al., 1988), respectively. Compound **1** was measured as +20°, therefore, it was identified as (+)-5,5'-dimethoxylariciresinol.

Compound **2** was identified as $C_{13}H_{20}O_3$ from EIMS [*m/z* 224, M^+] and ^{13}C NMR data. The ^1H NMR spectrum of **2** displayed signals corresponding to two coupled olefinic protons at δ_H 6.13 and 7.13 (each 1H, d, J = 15.8 Hz, H-7 and H-8), an oxygenated methine proton at δ_H 3.77 (1H, m, H-3), two sets of cyclic methylene protons at δ_H 1.66 and 2.27 (each 1H, H-4α, H-4β), and at δ_H 1.27 and 1.56 (each 1H, H-2α, H-2β), and four methyl groups at δ_H 0.93, 1.15, 1.20, and 2.26 (each 3H, s, $C\underline{H}_3$-10, $C\underline{H}_3$-11, $C\underline{H}_3$-12, and $C\underline{H}_3$-13). The large coupling constant between H-7 and H-8 (J = 15.8 Hz) indicates the *E* geometry for the double bond. The ^{13}C NMR spectrum showed 13 carbon signals including a conjugated ketone (C-9) at δ_C 197.3, one double bond between C-7 and C-8 detected at δ_C 133.6 and 143.8, four methyl groups (C-10, C-11, C-12, and C-13) at δ_C 20.2, 25.3, 27.4, and 30.6, two methylene groups (C-2 and C-4) at δ_C 41.5 and 47.6, one oxygenated methine (C-3) at δ_C 63.6, and three quaternary carbons (C-1, C-5, and C-6) at δ_C 35.6, 67.8, and 70.0, respectively. Especially, two methyl groups of H-11 and H-12 on C-1 and the cyclic methylene protons of H-2α, 2β and H-4α, 4β were assigned (see Table 1) stereo-chemically using ^1H-^1H NOESY, COSY and ^1H-^{13}C HMBC data and the structure obtained by energy minimization and conformational search with SYBYL program (Tripos). H-11 exists as an axial conformation shown Fig. 1 which verified by NOE correlation with H-8 while H-12 showed NOE correlation with H-3 not H-8. The H-2β proton was defined by stronger NOE peak intensity to H_3-11 than to H_3-12, while H-2α showed no difference in NOE intensity with those methyl protons.

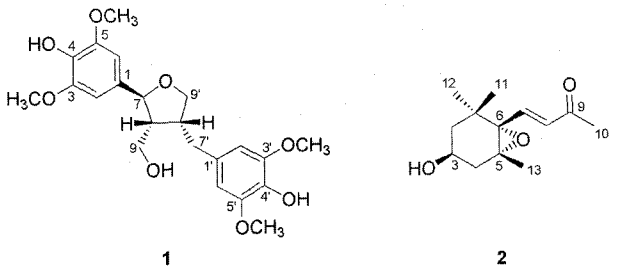

Fig. 1. Structures of compounds **1** and **2**.

The H-4β proton could be assigned by NOE peak with H-8 because the NOE correlation between H-4α and H-8 was not detected. The long range coupling with small value (1.6 Hz) between H-2α and H-4α was able due to their planar W-shape conformation. The spectral data were comparable to those of 3-hydroxy-5,6-epoxy-7-megastigmen-9-one (D'Abrosca *et al.*, 2004).

Accordingly, the structures of compounds **1** and **2** were identified as (+)-5,5'-dimethoxylariciresinol and (3*S*,5*R*,6*S*,7*E*)-5,6-epoxy-3-hydroxy-7-megastigmen-9-one, respectively. This is the first report on the isolation of compounds **1** and **2** from the whole plant of *S. borealis*.

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Reference

D'Abrosca, B., Dellagreca, M., Fiorentino, A., Monaco, P., Oriano, P., and Temussi, F., Structure elucidation and phytotoxicity of C_{13} *nor*-isoprenoids from *Cestrum parqui*. *Phytochemistry*, **65**, 497-505 (2004).

Achenbach, H., Stocker M., and Constenala, M. A., Flavonoids and other constituents of *Bauhinia manca*. *Phytochemistry*, **27**, 1835-1841 (1988).

Duan, H., Takishi, Y., Momota, H., Ohmoto, Y., and Taki, T., Immunosuppressive constituents from *Saussurea medusa*. *Phytochemistry*, **59**, 85-90 (2002).

Ida, Y., Satoh, Y., Ohtsuka, M., Nagasao, M., and Shoji, J., Phenolic constituents of *Phellodendron amurense* bark. *Phytochemistry*, **35**, 209-215 (1994).

Kinzo, J., Higuchi, H., Fukui, K., and Nohara, T., Lignoids from Albizziae Dortes. II. A Biodegradation pathway of syringaresinol. *Chem. Pharm. Bull.*, **39**, 2952-2955 (1991).

Koh, K. S. and Jeon, E. S., Ferns, Fern-Allies and seed-bearing plants of Korea. Iljinsa, Seoul (2003).

Macias, F. A., Oliva, R., M., Varela, R, M., Torres, A., and Molinillo, J. M. G., Alleochemicals from sunflower leaves cv. Peredovick. *Phytochemistry*, **52**, 613-621 (1999).

Nakajima, Y., Young, S.Y., and Kunugi, A., Six new flavonolignans from *Sasa veitchii* Rehder. *Tetrahedron*, **59**, 8011-8015 (2003).

Ohmoto, T. and Ikuse, M., Triterpenoids of the Gramineae. *Phytochemistry*, **9**, 2137-2148 (1970).

Yoon, K.D., Kim, C.-Y., and Huh, H., The flavone glycosides of *Sasa borealis*. *Kor. J. Pharmacogn.*, **31**, 224-227 (2000).

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