Three Terpenes and One Phenolic Compound from *Sasa borealis*

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Four compounds of previously known structures, friedeline (1), 3-hydroxyglutinol (2), *p*-hydroxybenzealdehyde (3), and squalene (4) were obtained from the *n*-hexane- and EtOAc-soluble fractions of the dried whole plants of *Sasa borealis*. The structures of 1-4 were identified by the interpretation of their spectroscopic data including 1D and 2D NMR as well as by comparison with previously reported values. This is the first report on the isolation of compounds 1-4 from *S. borealis*.

Key words: *friedeline, Gramineae, 3-hydroxyglutinol, p-hydroxybenzealdehyde,* Sasa borealis, *squalene*

The family Gramineae is composed of 550 genera with 10,000 species and has been known to contain lower terpenes, indolyalkylamines, alkaloids, flavonoids, and some glycosides [Frahn & Illman, 1973; Koh & Jeon, 2003; Ohmoto *et al.*, 1970; Ohmoto *et al.*, 1974; Wassel *et al.*, 1987; Yoon *et al.*, 2000]. *Sasa* species (Gramineae) are mostly small, fast spreading species, often with relatively large, broad, ribbed leaves and arching culms [Koh & Jeon, 2003]. Major components of the genus *Sasa* including triterpenoids, flavonoids, and flavonolignans [Nakajima *et al.*, 2003; Yoon *et al.*, 2000] have generated intense interest due to their pharmacological effectiveness to retard spontaneous mammary tumorigenesis and to suppress apoptosis induced by oxidative stress (Akao *et al.*, 2004; Ren *et al.*, 2004]. *Sasa borealis* (Hack.) Makino has been used in traditional medicines for the treatment of burns, hemoptysis, and uremia in Asia [Namba & Ki, 1982]. Previous phytochemical investigations have reported that this plant contains several secondary metabolites such as flavonoids, flavone glucosides, triterpenoids, flavonolignans, and lignans [Jeong *et al.*, 2006; Jeong *et al.*, 2005; Yoon *et al.*, 2000]. In contrast, both chemical and biological studies on this plant remain minimal.

Here, a study of chemical constituents of *S. borealis* was undertaken as a part of our research program to search for bioactive compounds from native medicinal plants in Korea. Chromatographic separations of the *n*-hexane- and EtOAc-soluble fractions of the dried whole plants of *S. borealis* led to the isolation of four compounds of previously known structures, friedeline (**1**), 3-hydroxyglutinol (**2**), *p*-hydroxybenzealdehyde (**3**), and squalene (**4**) (Fig. 1). All the isolates (**1-4**) were identified by spectroscopic methods including 1D and 2D NMR techniques as well as by comparison with literature data. To the best of our knowledge, compounds (**1-4**) were obtained from *S. borealis* for the first time. We report herein the isolation and identification of these compounds.

Materials and Methods

Plant materials. *Sasa borealis* (Hack.) Makino purchased from Kyungdong Oriental Herbal Market, Korea, in February 2003, and was identified by Emeritus Professor, Chang Soo Yook at Kyung Hee University, Korea. A voucher specimen (No. EA226) was deposited at Natural Product Chemistry Laboratory, College of Pharmacy, Ewha Womans University, Korea.

General experimental procedures. Optical rotations were measured with a P-1010 polarimeter (Jasco, Japan) at 25°C. UV and IR spectra were recorded on a U-3000

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Abbreviations: EIMS, electron impact mass spectrometry; HMBC, heteronuclear multiple bond correlation; HRMS, high resolution mass spectrometry; LRESIMS, low resolution electrospray ionization mass spectrometry; TLC, thin-layer chromatography; TMS, tetramethylsilane

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) with TMS as 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, UK). 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 (YMC · GEL ODS-A, 12 nm, S-150 ìm), 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 whole plants (4 kg) were extracted with MeOH (20 L × 3) overnight at room temperature. The combined MeOH extracts was evaporated *in vacuo* to give a dark residue (96 g). The MeOH extract was suspended in water, and then partitioned successively with *n*-hexane, EtOAc, and *n*-BuOH to afford the residues of *n*-hexane (23 g), EtOAc (17.7 g), and *n*-BuOH (22.7 g), respectively. The *n*-hexane-soluble extract (23 g) was mixed with Celite (38 g) and subjected to silica gel column chromatography (230-400 mesh, 7 × 30 cm) using *n*-hexane-EtOAc gradient (from 19 : 1 to 0 : 1 v/v) as an elution system to provide thirteen fractions (FI-FXIII). The EtOAc soluble-extract (17.7 g) was chromatographed over silica gel column (230-400 mesh, 6.5 × 30 cm) using a CH_2Cl_2-MeOH gradient (from 1 : 0 to 0 : 1 v/v) as mobile phase to give eleven fractions (F01-F11). Compounds **1** (163.6 mg, 0.00409%) and **2** (45 mg, 0.00113%) were obtained from FIII (1.2 g) and FVI (1.1 g), respectively, by recrystallization in MeOH. F03 (40 mg) was further fractionated over a sephadex LH-20 column chromatography (2 × 80 cm) and eluted with $CHCl_3$-MeOH (1 : 2), to yield compound **3** (8.9 mg, 0.00022 %, R_f = 0.7; *n*-hexane-EtOAc = 1 : 1). Compound **4** (67.8 mg, 0.00170 %, R_f = 0.8; *n*-hexane-CH_2Cl_2 = 1 : 1) was purified from a fraction (FI and FII, 2.5 g) through a silica gel column chromatography (3.5 × 30 cm) using *n*-hexane-CH_2Cl_2 (9 : 1) as solvent system.

Friedeline (**1**): Colorless needles; $[\alpha]_D^{25}$ −75.8° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 338 nm (2.13); IR (film) ν_{max} 2932, 2862, 1714, 1454, 1389, 1223, 1180, 1040 cm^{-1}; EIMS *m/z* (% rel. int.) 426 $[M]^+$ (15), 411 (10), 341 (13), 302 (27), 273 (100). ^1H NMR (400 MHz, $CDCl_3$) d 2.39 (1H, ddd, *J* = 14.0, 5.3, 2.0 Hz, H-2a), 2.30 (1H, m, H-2b), 2.25 (1H, m, H-4), 1.97 (1H, m, H-1a), 1.75 (1H, m, H-6), 1.68 (1H, dd, *J* = 12.0, 5.2 Hz, H-1b),

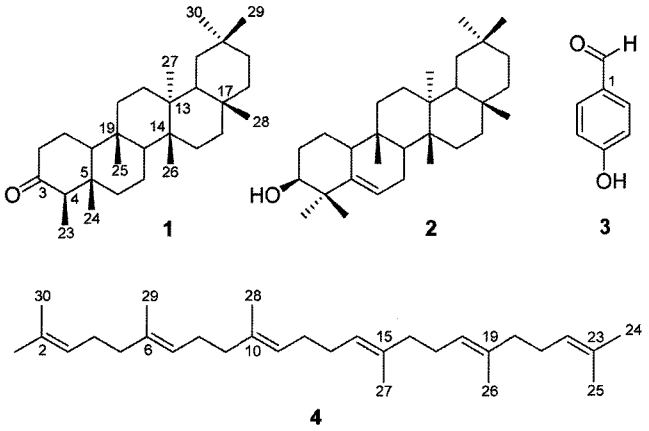

Fig. 1. Structures of compounds 1-4 from *S. borealis*.

1.22 (1H, m, H-19), 1.18 (3H, s, C$\underline{H}_3$-28), 1.05 (3H, s, C$\underline{H}_3$-27), 1.01 (3H, s, C$\underline{H}_3$-26), 1.00 (3H, s, C$\underline{H}_3$-30), 0.95 (3H, s, C$\underline{H}_3$-29), 0.89 (3H, d, *J* = 6.8Hz, C$\underline{H}_3$-23), 0.87 (3H, s, C$\underline{H}_3$-25), 0.73 (3H, s, C$\underline{H}_3$-24); ^{13}C NMR (100 MHz, $CDCl_3$) d 213.0 (C-3), 59.7 (C-10), 58.4 (C-4), 53.3 (C-8), 43.0 (C-18), 42.4 (C-5), 41.8 (C-2), 41.5 (C-6), 39.9 (C-13), 39.5 (C-22), 38.5 (C-14), 37.7 (C-9), 36.3 (C-16), 35.9 (C-11), 35.6 (C-19), 35.3 (C-29), 33.0 (C-21), 32.7 (C-15), 32.4 (C-28), 32.1 (C-30), 30.8 (C-12), 30.2 (C-17), 28.4 (C-20), 22.5 (C-1), 20.5 (C-26), 18.9 (C-27), 18.5 (C-7), 18.2 (C-25), 14.9 (C-24), 7.1 (C-23).

3-Hydroxyglutinol (**2**): Colorless needles; $[\alpha]_D^{25}$ +46.7° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 365 nm (2.51); IR (film) ν_{max} 3561, 1448, 1373 cm^{-1}; EIMS *m/z* (% rel. int.) 426 $[M]^+$ (10), 274 (100), 259 (75), 205 (30), 134 (20), 95 (23). ^1H NMR (400 MHz, $CDCl_3$) d 5.63 (1H, br d, *J* = 6.0 Hz, H-6), 3.46 (1H, br s, H-3), 1.16 (3H, s, C$\underline{H}_3$-28), 1.14 (3H, s, C$\underline{H}_3$-24), 1.09 (3H, s, C$\underline{H}_3$-26), 1.04 (3H, s, C$\underline{H}_3$-23), 1.01 (3H, s, C$\underline{H}_3$-27), 0.99 (3H, s, C$\underline{H}_3$-30), 0.95 (3H, s, C$\underline{H}_3$-29), 0.85 (3H, s, C$\underline{H}_3$-25); ^{13}C NMR (100 MHz, $CDCl_3$) d 141.8 (C-5), 122.2 (C-6), 76.5 (C-3), 49.9 (C-10), 47.6 (C-8), 43.3 (C-18), 41.0 (C-4), 39.5 (C-14), 39.2 (C-22), 38.0 (C-13), 36.2 (C-16), 35.3 (C-19), 35.1 (C-9), 34.8 (C-15), 34.7 (C-29), 33.3 (C-21), 32.6 (C-30), 32.3 (C-11), 32.3 (C-28), 30.6 (C-12), 30.3 (C-17), 29.2 (C-23), 28.5 (C-20), 28.0 (C-2), 25.7 (C-24), 23.9 (C-7), 19.8 (C-26), 18.6 (C-27), 18.4 (C-1), 16.4 (C-25).

p-Hydroxybenzaldehyde (**3**): Pale yellow solids; UV (MeOH) λ_{max} (log ε) 238 nm (3.21); IR (film) ν_{max} 3207, 1675, 1607, 1458 cm^{-1}; EIMS *m/z* (%) 122 $[M]^+$ (42), 121 (53), 83 (68), 71 (85).

^1H NMR (400 MHz, acetone-d_6) δ 9.85 (1H, s, C$\underline{H}$O), 7.80 (2H, dd, *J* = 8.8, 2.6 Hz, H-2 and H-6), 7.01 (2H, dd, *J* = 8.4, 1.8 Hz, H-3 and H-5);

^{13}C NMR (100 MHz, acetone-d_6) δ 191.1 (C$\underline{H}$O), 164.1

(C-4), 132.9 (C-2 and C-6), 130.5 (s, C-1), 116.8 (C-3 and C-5).

Squalene (**4**): Oil: UV (MeOH) λ_{max} (log ε) 344 nm (2.06); IR (film) ν_{max} 2927, 1707, 1448, 1379 cm^{-1}; EIMS *m/z* (% rel. int.) 410 $[M]^+$ (5), 341 (8), 137 (17), 81 (40), 69 (100). ^1H NMR (400 MHz, $CDCl_3$) δ 5.12 (6H, m, H-3, H-7, H-11, H-14, H-18, and H-22), 2.03 (20H, m, H-4, H-5, H-8, H-9, H-12, H-13, H-16, H-17, H-20, and H-21), 1.68 (6H, s, CH_3-25 and CH_3-30), 1.60 (18H, s, CH_3-1, CH_3-24, CH_3-26, CH_3-27, CH_3-28, and CH_3-29); ^{13}C NMR (100 MHz, $CDCl_3$) d 135.2 (C-6 and C-19), 135.0 (C-10 and C-15), 131.3 (C-2 and C-23), 124.6 (C-3 and C-22), 124.5 (C-7 and C-18), 124.4 (C-11 and C-14), 40.0 (C-5 and C-20), 40.0 (C-9 and C-16), 28.6 (C-12 and C-13), 27.1 (C-4 and C-21), 27.0 (C-8 and C-17), 26.0 (C-1 and C-24), 18.0 (C-25 and C-30), 16.3 (C-26 and C-29), 16.3 (C-27 and C-28).

Results and Discussion

Four known compounds, friedeline (**1**), 3-hydroxyglutinol (**2**), *p*-hydroxybenzealdehyde (**3**), and squalene (**4**), were isolated from the *n*-hexane- and EtOAc-soluble fractions of the dried whole plants of *S. borealis* using repeated chromatographic fractionation procedure. The structural identifications of the isolates (**1-4**) were carried out by spectroscopic analysis including 1D and 2D NMR techniques as well as by comparison of spectral and physical data with those reported in literature (Fig. 1).

Compound **1** was obtained as colorless needles and had a molecular formula of $C_{30}H_{50}O$, which was deduced from its EIMS (*m/z* 426, $[M]^+$) and confirmed by ^{13}C-NMR and DEPT analysis. The 1D NMR data (^1H- and ^{13}C-NMR) of **1** and those of published analogous compounds supported that compound **1** was a friedelane-type of triterpene [Akihisa *et al.*, 1992; Chang *et al.*, 1999; Leong & Harrison, 1999; Lin *et al.*, 1989]. In particular, the ^1H-NMR spectrum of **1** indicated the presence of eight methyl groups (δ_H 1.18, 1.05, 1.01, 1.00, 0.95, 0.89, 0.87, and 0.73) and ^{13}C-NMR spectrum showed the 30 carbons including a conjugated ketone (C-3) at δ_C 213.0. The positions of quaternary carbons and methyl groups were assigned by HMBC correlations and comparison with NMR data reported previously [Akihisa *et al.*, 1992; Chang *et al.*, 1999]. Therefore, compound **1** was identified as friedeline. Compound **2** was obtained as colorless needles. The EIMS showed $[M]^+$ at *m/z* 426, consistent with the molecular formula $C_{30}H_{50}O$. The eight methyl signals at δ_H 1.16, 1.14, 1.09, 1.04, 1.01, 0.99, 0.95, and 0.85 were also observed from its ^1H-NMR spectrum. Compound **2** showed similar ^1H- and ^{13}C-NMR spectral data to those of **1** except for the presence of a double bond signals at δ_H 5.63 (1H, br d, J = 6.0 Hz, H-6), δ_C 122.2 (C-6), and 141.8 (C-5). Compound **2** had a hydroxyl group at C-3 instead of the carbonyl group in **1**. In addition, the methyl group was attached at C-4 in **2**. These spectral data revealed that **2** was identical to 3-hydroxyglutinol. Its structure was established based on comparison of ^1H- and ^{13}C-NMR spectral data with those of reference values [Akihisa *et al.*, 1992; Leong & Harrison, 1999; Lin *et al.*, 1989]. The EIMS spectrum of compound **3** showed $[M]^+$ ion peak at *m/z* 122, consistent with the molecular formula $C_7H_6O_2$. The ^1H-NMR spectrum of **3** displayed characteristic signals for an aldehyde proton at δ_H 9.85 (1H, s, CHO) and a pair of double of doublets (AB pattern) at δ_H 7.80 (2H, dd, J = 8.8, 2.6 Hz, H-2 and H-6), 7.01 (2H, dd, J = 8.4, 1.8 Hz, H-3 and H-5) and indicated the existence of aromatic system composed of *ortho*- and *meta*-coupled protons. The ^{13}C-NMR spectrum showed a carbonyl group signal of aldehyde moiety at d 191.1 and two symmetrical aromatic carbon signals at d 132.9 (C-2 and C-6) and 116.8 (C-3 and C-5). Hence, the structure of **3** was identified as *p*-hydroxybenzealdehyde and further verification of this structure was conducted by comparison with previously reported NMR data [Jang *et al.*, 2004]. Compound **4** was obtained as red oil, and its molecular formula was deduced as $C_{30}H_{50}$ on the basis of EIMS (*m/z* 410, $[M]^+$) and ^{13}C-NMR analysis. The ^1H-NMR spectrum of **4** indicated the presence of eight methyl groups [δ 1.60 (18H, s, CH_3-1, CH_3-24, CH_3-26, CH_3-27, CH_3-28, and CH_3-29) and δ 1.68 (6H, s, CH_3-25 and CH_3-30)], ten methylenes [δ 2.03 (20H, m, H-4, H-5, H-8, H-9, H-12, H-13, H-16, H-17, H-20, and H-21)], and six vinylic proton signals [δ 5.12 (6H, m, H-3, H-7, H-11, H-14, H-18, and H-22)]. These spectral data revealed that compound **4** was identified as squalene, which was established by comparison of its physical and spectral data with those of literature values [He *et al.*, 2002; Jautelat *et al.*, 1970]. To the best of our knowledge, compounds **1-4** were never reported from *S. borealis* previously.

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References

Akao Y, Seki N, Nakagawa Y, Yi H, Matsumoto K, Ito Y, Ito K, Funaoka M, Maruyama W, Naoi M, and Nozawa Y (2004) A highly bioactive lignophenol derivative from

bamboo lignin exhibits a potent activity to suppress apoptosis induced by oxidative stress in human neuroblastoma SH-SY5Y cells. *Bioorg Med Chem* **12**, 4791-4801.

Akihisa T, Yamamoto K, Tamura T, Kimura Y, Iida T, Nambara T, and Chang FC (1992) Triterpenoid ketones from *Lingnania chungii* McClure: arborinone, friedelin and glutinone. *Chem Pharm Bull* **40**, 789-791.

Chang CW, Wu TS, Hsieh YS, Kuo SC, and Chao PDL (1999) Terpenoids of *Syzygium formosanum*. *J Nat Prod* **62**, 327-328.

Frahn JL and Illman RJ (1973) Separation of some indolylalkylamines from their metho cation derivatives using neutral polystyrene resin. Application to the alkaloids of *Phalaris tuberosa* (gramineae). *J Chromatogr* **87**, 187-191.

He HP, Cai Y, Sun M, and Corke H (2002) Extraction and purification of squalene from *Amaranthus Grain*. *J Agric Food Chem* **50**, 368-372.

Jang DS, Han AR, Park G, Jhon GJ, and Seo EK (2004) Flavonoids and aromatic compounds from the rhizomes of *Zingiber zerumbet*. *Arch Pharm Res* **27**, 386-389.

Jautelat M, Grutzner JB, and Roberts JD (1970) Natural-abundance carbon-13 nuclear magnetic resonance spectra of terpenes and carotenes. *Proc Natl Acad Sci USA* **65**, 288-292.

Jeong YH, Lee J, Kwon Y, and Seo EK (2006) Reverse-phase HPLC method for identification of diastereomeric constituents from *Sasa borealis*. *Yakhak Hoechi* **50**, 21-25.

Jeong YH, Nam JW, Lee NY, Seo EK, and Kwon Y (2005) Isolation and structural identification of minor constituents from *Sasa borealis*. *Nat Prod Sci* **11**, 170-173.

Koh K and Jeon ES (2003) Ferns, Fern-Allies and seed-bearing plants of Korea. pp. 736-740, Iljinsa, Seoul.

Leong YW and Harrison LJ (1999) (20R,23E)-eupha-8,23-diene-3b,25-diol from *Tripetalum cymosum*. *Phytochem* **50**, 849-857.

Lin YL, Kuo YH, and Chen YL (1989) Two new clerodane-type diterpenoids, clerodinins A and B, from *Clerodendron brachyanthum* Schauer. *Chem Pharm Bull* **37**, 2191-2193.

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

Namba T and Ki HB (1982) Pharmacognogical studies on the crude drug "Zhu'-ye" and Nambusaceous plants (VII). On the botanical origins of the folk medicine "Kuma-zasa" on the Japanese markets and the comparative anatomical studies of the leaves of the Genus *Sasa*. *Shoyakugaku Zasshi* **36**, 43-54.

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

Ohmoto T, Uzawa S, and Tanaka R (1974) Triterpenoids and the related compounds from gramineae plants. *Shoyakugaku Zasshi* **28**, 1-6.

Ren M, Reilly RT, and Sacchi N (2004) *Sasa* health exerts a protective effect on Her2/NeuN mammary tumorigenesis. *Anticancer Res.* **24**, 2879-2884.

Wassel G, El-Menshawi B, Saeed A, Mahran G, and Reisch J (1987) New sources of pyrrolizidine alkaloids: genus *Cordia* (Ehretiaceae) and *Schismus* (Gramineae). *Sci Pharm* **55**, 163-166.

Yoon KD, Kim CY, and Huh H (2000) The flavone glycosides of *Sasa borealis*. *Saengyak Hakhoechi* **31**, 224-227.