Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

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Published online: 15 Apr 2015.

Bioscience, Biotechnology, and Biochemistry, 2015
Vol. 79, No. 9, 1402–1405

Note

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Received February 12, 2015; accepted March 10, 2015
http://dx.doi.org/10.1080/09168451.2015.1031079

A new aliphatic alcohol, (2*R*,6*R*)-oct-7-ene-2,6-diol (1), and seven other known compounds (2-8) were isolated from *Acorus gramineus* rhizomes. The structure of 1 was elucidated by a combination of extensive spectroscopic analyses, including 2D NMR, HR-MS, and the modified Mosher's method. Compounds 3-8 displayed consistent antiproliferative activities against the cell lines tested with IC_{50} values ranging from 7 to 48 μm.

Key words: *Acorus gramineus*; Araceae; aliphatic alcohol; cytotoxicity

Acorus gramineus (Araceae) is an aquatic plant that possesses a faculty of water purification. It is distributed throughout Korea, Japan, and China. *A. gramineus* rhizomes have been used in Korean traditional medicine since ancient times. It has been used for sedation as well as treatment of various conditions, including cranial nerve disease, stomach ache, tinnitus, and edema.[1,2)] In an earlier pharmacological study, phenolics such as β-asarone, α-asarone, phenylpropenes, and methyl chavicol have been reported from this rhizome, which were shown to have antibacterial, antifungal, and anti-inflammatory effects.[3–6)] Recently, our phytochemical investigations on *A. gramineus* revealed the presence of various bioactive constituents with antitumor and cytotoxic activities.[7–9)] Our continued interest in discovering cytotoxic constituents from this plant led us to investigate the cytotoxic metabolites of *A. gramineus* rhizomes. A bioassay-guided fractionation and chemical investigation of its MeOH extract resulted in the isolation and identification of a new aliphatic alcohol, (2*R*,6*R*)-oct-7-ene-2,6-diol (**1**), which represents only the second natural example of octanediol along with seven known compounds (**2**–**8**) (Fig. 1). In this paper, we report the isolation and structure elucidation of the isolates and their cytotoxic activity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines.

The rhizomes of *A. gramineus* (15 kg) were extracted with 80% aqueous MeOH at room temperature and filtered. The filtrate was evaporated under vacuum to obtain a MeOH extract (825 g), which was suspended in distilled H_2O (2 L) and successively solvent-partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, yielding 166, 14, 5, and 47 g of residues, respectively. Each fraction was evaluated for cytotoxicity against A549, SK-OV-3, and SK-MEL-2 cells using sulforhodamine B (SRB) assay. The *n*-hexane-soluble and EtOAc-soluble fractions showed significant cytotoxic activity against all three cancer cell lines that were tested. The *n*-hexane-soluble fraction (62 g) was separated over an silica gel column chromatography with *n*-hexane-EtOAc (11:1) to yield eight fractions (H1-H8). Fraction H1 (2 g) was extracted with petroleum ether yielding P1 (542 mg), which was separated over an RP-C_{18} silica gel column chromatography using a solvent system of 95% MeOH to provide four fractions (P11–P14). Fraction P12 (80 mg) was applied to a silica gel column chromatography with $CHCl_3$-MeOH (40:1) to obtain two subfractions (P121–P122). Fraction P121 (44 mg) was purified by semi-preparative reverse-phase HPLC (250 mm × 10 mm i.d., 10 μm, Econosil RP-18 column, flow rate; 2 mL/min, 93% MeOH), and further purified by semi-preparative normal-phase HPLC (250 mm × 10 mm i.d., 5 μm, Apollo Silica column) with a solvent system of *n*-hexane-EtOAc (30:1, flow rate; 2 mL/min) to yield compound **4** (24 mg). Fraction H6 (606 mg) was applied to Sephadex LH-20 column chromatography using a solvent system of CH_2Cl_2-MeOH (1:1) to give two subfractions (H61–H62). Fraction H61 (547 mg) was subjected to an RP lobar column using a solvent system of MeOH-H_2O (2:3), and further purified by semi-preparative normal-phase HPLC with a solvent system of *n*-hexane-EtOAc (1.5:1, flow rate; 2 mL/min) to obtain compounds **5** (6 mg) and **6** (3 mg). Another active fraction, EtOAc-soluble fraction (5 g) was separated over an RP-C_{18} silica gel column chromatography using a

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Abbreviations: DMAP, 4-(dimethylamino)pyridine; SRB, sulforhodamine B.

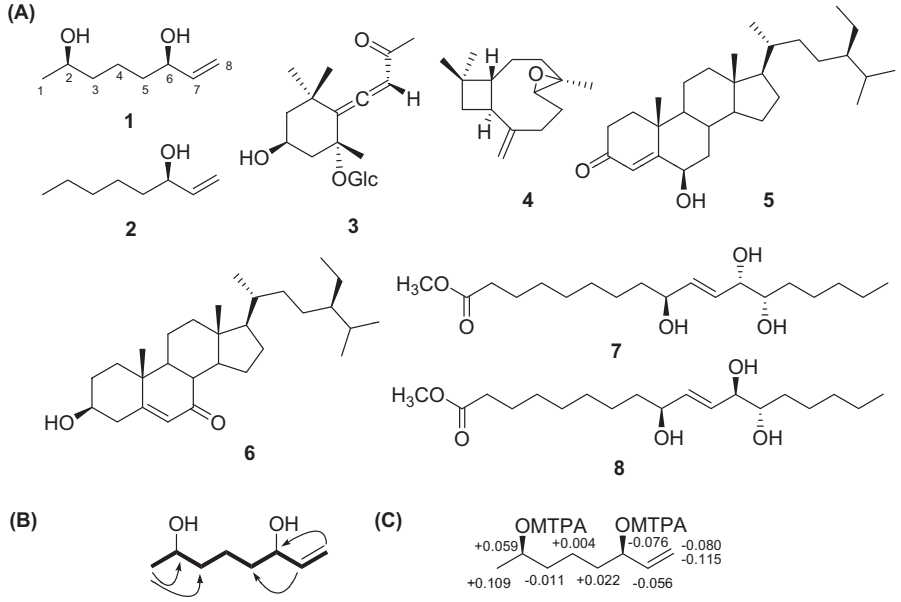

Fig. 1. Structures of compounds **1-8** (A), ^{1}H-^{1}H COSY correlations (bond), and key HMBC correlations (H→C) of **1** (B), and Δδ values ($\delta_S - \delta_R$) in ppm of the two MTPA diesters derived from **1** (C).

solvent system of MeOH-H_2O (1:1) to provide eight fractions (E1–E8). Fraction E4 (310 mg) was applied to a silica gel column chromatography with $CHCl_3$-MeOH (20:1) to obtain two subfractions (E41–E42). Fraction E41 (76 mg) was subjected to Sephadex LH-20 column chromatography using a solvent system of MeOH-H_2O (4:1), and further purified by semi-preparative normal-phase HPLC with a solvent system of $CHCl_3$-MeOH (30:1, flow rate; 2 mL/min) to yield compounds **1** (2 mg) and **2** (2 mg). Fraction E8 (110 mg) was subjected to a silica gel column chromatography with $CHCl_3$-MeOH (20:1) to obtain fraction E81 (19 mg), which was purified by semi-preparative reverse-phase HPLC (80% MeOH), and further purified by semi-preparative normal-phase HPLC with a solvent system of $CHCl_3$-MeOH (30:1, flow rate; 2 mL/min) to give compounds **7** (4 mg) and **8** (3 mg). The BuOH-soluble fraction (47 g) was also investigated and separated over a silica gel column chromatography using a solvent system of $CHCl_3$-MeOH (5:1) to obtain four fractions (B1–B4). Fraction B2 (3.8 g) was applied to RP-C_{18} silica gel column chromatography using a solvent system of MeOH-H_2O (1:3) to yield five subfractions (B21–B25). Fraction B24 (430 mg) was further purified by semi-preparative normal-phase HPLC with a solvent system of $CHCl_3$-MeOH (9:1, flow rate; 2 mL/min) to afford compound **3** (14 mg).

Compound **1** was isolated as a colorless gum with a negative optical rotation ($[\alpha]_D^{25}$ −26.2 (*c* 0.30, MeOH), and the molecular formula $C_8H_{16}O_2$ was deduced from the molecular ion peak $[M + Na]^+$ at *m/z* 167.1050 (calcd. for $C_8H_{16}O_2Na$, 167.1048) in the positive-ion HR-ESIMS. The IR absorptions of **1** implied the presence of OH functional group (3383 cm^{-1}) in **1**. The ^{1}H NMR data (Table 1) of **1** showed typical signals for mono-substituted double bond protons at δ_H 5.89 (1H, ddd, *J* = 17.5, 10.5, 6.0 Hz), 5.23 (1H, d, *J* = 17.5 Hz), and 5.12 (1H, d, *J* = 10.5 Hz), two oxygenated methines at δ_H 4.12 (1H, m), and 3.83 (1H, m), and one methyl proton at δ_H 1.21 (3H, d, *J* = 6.0 Hz). The ^{13}C

Table 1. ^{1}H and ^{13}C NMR data of compound **1** in $CDCl_3$[a]

	1	
Position	δ_H	δ_C
1	1.21 d (6.0)	23.7 q
2	3.83 m	68.2 d
3	1.48 m	39.2 t
4	1.46 m	21.6 t
5	1.58 m	37.0 t
6	4.12 m	73.3 d
7	5.89 ddd (17.5, 10.5, 6.0)	141.3 d
8	5.23 d (17.5); 5.12 d (10.5)	114.9 t

[a] ^{1}H and ^{13}C NMR data were recorded at 500 and 125 MHz, respectively. Coupling constants (in Hz) are given in parentheses.

NMR data (Table 1) displayed a total of 8 carbon signals composed of one double bond at δ_C 141.3 and 114.9, two oxygenated methine carbons at δ_C 73.3 and 68.2, three methylene carbons at δ_C 39.2, 37.0, and 21.6, and one methyl carbon at δ_C 23.7, which were also verified by the analysis of DEPT data. Detailed inspection of the ^{1}H and ^{13}C NMR data revealed that the ^{1}H and ^{13}C NMR spectra of **1** were quite similar to those of (3*R*)-oct-1-en-3-ol (**2**),[10] which was also isolated from this plant, with apparent differences being the signals for an oxygenated methine at δ_H 3.83 and δ_C 68.2 in **1**. The position of replacement was confirmed to be C-2 by the ^{1}H-^{1}H COSY correlations of H-1/H-2/H-3 and HMBC correlations of H-1/C-2 and H-1/C-3 (Fig. 1). The full NMR assignments of **1** were performed by analyzing the ^{1}H-^{1}H COSY, DEPT, HMQC, and HMBC spectroscopic data (Table 1). The absolute configurations of C-2 and C-6 of **1** were established on the basis of the modified Mosher's method[11] Treatment of **1** with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*S*)-MTPA-Cl] and 4-(dimethylamino)pyridine (DMAP) in pyridine gave the (*R*)-MTPA diester **1r**. Similar treatment of **1** with (*R*)-(−)-MTPA-Cl afforded the (*S*)-MTPA diester **1s**. Analysis of the ^{1}H NMR chemical shift differences ($\Delta\delta_{S-R}$)

of the two MTPA diesters allowed for the assignment of the absolute configurations of C-2 and C-6 as 2*R* and 6*R* (Fig. 1). Thus, the structure of **1** was established as (2*R*,6*R*)-oct-7-ene-2,6-diol.[12)]

To the best of our knowledge, aliphatic alcohols have been generally isolated as aliphatic alcohol glycosides.[13–15)] Their aglycones have been rarely isolated up to this point. Compound **1** represents only the second natural example of octanediol as one of the aglycones; the first octanediol reported, 7-octene-1,6-diol, was isolated from *Aeschynanthus bracteatus*.[16)] The known compounds were identified as (3*R*)-oct-1-en-3-ol (**2**),[10)] citroside B (**3**),[17)] (−)-caryophyllene oxide (**4**),[18)] 6β-hydroxystigmast-4-en-3-one (**5**),[19)] 3β-hydroxystigmast-5-en-7-one (**6**),[19)] methyl (9*S*,12*S*,13*S*)-9,12,13-trihydroxy-10*E*-octadecenoate (**7**),[20)] and methyl (9*S*,12*R*,13*S*)-9,12,13-trihydroxy-10*E*-octadecenoate (**8**).[21)] All the compounds, except for compound **4**, were reported for the first time from this plant.

The SRB assay was used to evaluate the antiproliferative activities of the isolated compounds **1-8**,[22)] which were tested against four human tumor cell lines, including A549 (non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). The IC_{50} values of each compound were determined and are summarized in Table 2. The data demonstrated that compounds **3-8** displayed consistent antiproliferative activities against all of the cell lines tested, with IC_{50} values ranging from 7 to 48 μm. No cytotoxic effects were observed for aliphatic alcohols (**1-2**), including novel compound **1** (IC_{50} >50 μm). Among the isolates **1-8**, (−)-caryophyllene oxide (**4**) displayed the most potent cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines (IC_{50}: 27, 14, 7, and 17 μm, respectively).

In conclusion, we isolated and identified a new aliphatic alcohol, (2*R*,6*R*)-oct-7-ene-2,6-diol (**1**), together with seven known compounds (**2-8**) from *A. gramineus* rhizomes, and evaluated their antiproliferative activities against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines. (−)-Caryophyllene oxide (**4**) showed the most potent cytotoxicity against all of the tested cell lines. Novel compound **1** represents only the second natural example of octanediol. This is the first time that other known compounds, except for compound **4**, were reported to be isolated from this plant.

Table 2. Cytotoxic activities of compounds **1**–**8**.

	IC_{50} (μm)[a]			
Compounds	A549	SK-OV-3	SK-MEL-2	HCT-15
1	>50	>50	>50	>50
2	>50	>50	>50	>50
3	17	27	31	45
4	27	14	7	17
5	>50	48	38	>50
6	45	33	26	48
7	39	>50	40	>50
8	46	>50	38	>50
Etoposide[b]	2	2	1	2

[a]IC_{50} values of compounds against each cancer cell line. IC_{50} value was defined as concentration (μm) causing 50% inhibition of cell growth in vitro.
[b]Etoposide was used as a positive control.

Supplemental material

The supplemental material for this paper is available at http://10.1080/09168451.2015.1031079.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology [2013R1A1A2A10005315].

References

[1] Liao JF, Huang SY, Jan YM, Yu LL, Chen CF. Central inhibitory effects of water extract of Acori graminei rhizoma in mice. J. Ethnopharmacol. 1998;61:185–193.
[2] Tang W, Eisenbrand G. Chinese drugs of plant origin. New York, NY: Springer; 1992. p. 45–46.
[3] Greca MD, Monaco P, Previtera L, Aliotta G, Pinto G, Pollio A. Allelochemical activity of phenylpropanes from *Acorus gramineus*. Phytochemistry. 1989;28:2319–2321.
[4] Lee JY, Lee JY, Yun BS, Hwang BK. Antifungal activity of beta-asarone from rhizomes of *Acorus gramineus*. J. Agric. Food. Chem. 2004;52:776–780.
[5] Perrett S, Whitfield PJ. Anthelmintic and pesticidal activity of *Acorus gramineus* (Araceae) is associated with phenylpropanoid asarones. Phytother. Res. 1995;9:405–409.
[6] McGaw LJ, Jager AK, van Staden J. Isolation of β-asarone, an antibacterial and anthelmintic compound, from *Acorus calamus* from South Africa. S. Afr. J. Bot. 2002;68:31–35.
[7] Kim KH, Kim HK, Choi SU, Moon E, Kim SY, Lee KR. Bioactive lignans from the rhizomes of *Acorus gramineus*. J. Nat. Prod. 2011;74:2187–2192.
[8] Kim KH, Moon E, Kim HK, Oh JY, Kim SY, Choi SU, Lee KR. Phenolic constituents from the rhizomes of *Acorus gramineus* and their biological evaluation on antitumor and anti-inflammatory activities. Bioorg. Med. Chem. Lett. 2012;22:6155–6159.
[9] Kim KH, Moon E, Kang KS, Kim SY, Choi SU, Lee KR. Alkaloids from *Acorus gramineus* rhizomes and their biological activity. J. Braz. Chem. Soc. 2015;26:3–8.
[10] Mosandl A, Heusinger G, Gessner M. Analytical and sensory differentiation of 1-octen-3-ol enantiomers. J. Agric. Food. Chem. 1986;34:119–122.
[11] Kim KH, Choi SU, Kim YC, Lee KR. Tirucallane triterpenoids from *Cornus walteri*. J. Nat. Prod. 2011;74:54–59.
[12] (2*R*,6*R*)-Oct-7-ene-2,6-diol (1): colorless gum; $[\alpha]^{25}_{D}$-26.2 (*c* 0.30, MeOH); IR (KBr) ν_{max} 3357, 2945, 2832, 1451, 1116, 1032, 674 cm^{-1}; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; ESIMS (positive-ion mode) *m/z*: 167 $[M + Na]^{+}$. HR-ESIMS (positive-ion mode) *m/z*: 167.1050 $[M + Na]^{+}$ (calcd for $C_8H_{16}O_2Na$, 167.1048).
[13] Kanchanapoom T, Kasai R, Picheansoonthon C, Yamasaki K. Megastigmane, aliphatic alcohol and benzoxazinoid glycosides from *Acanthus ebracteatus*. Phytochemistry. 2001;58:811–817.
[14] Uemura Y, Sugimoto S, Matsunami K, Otsuka H, Takeda Y, Kawahata M, Yamaguchi K. Microtropins A–I: 6′-O-(2″S,3″R)-2″-ethyl-2″,3″-dihydroxybutyrates of aliphatic alcohol β-D-glucopyranosides from the branches of *Microtropis japonica*. Phytochemistry. 2013;87:140–147.
[15] Nakamura S, Li X, Matsuda H, Yoshikawa M. Bioactive constituents from Chinese natural medicines. XXVIII. Chemical structures of acyclic alcohol glycosides from the roots of *Rhodiola crenulata*. Chem. Pharm. Bull. 2008;56:536–540.

[16] Li SM, Yang XW, Shen YH, Feng L, Wang YH, Zeng HW, Liu XH, Tian JM, Shi YN, Long CL, Zhang WD. Chemical constituents of *Aeschynanthus bracteatus* and their weak anti-inflammatory activities. Phytochemistry. 2008;69: 2200–2204.

[17] Zhang Z, Zhang W, Ji YP, Zhao Y, Wang CG, Hu JF. Gynostemosides A-E, megastigmane glycosides from *Gynostemma pentaphyllum*. Phytochemistry. 2010;71:693–700.

[18] Krebs HC, Rakotoarimanga JV, Habermehl GG. Isolation of spatulenol and (−)-caryophyllene oxide from *Vernonia mollissima* don and ^{1}H and ^{13}C reassignment by two-dimensional NMR spectroscopy. Magn. Reson. Chem. 1990;28:124–128.

[19] Della Greca M, Monaco P, Previtera L. Stigmasterols from *Typha latifolia*. J. Nat. Prod. 1990;53:1430–1435.

[20] Miura A, Kuwahara S. A concise synthesis of pinellic acid using a cross-metathesis approach. Tetrahedron. 2009;65:3364–3368.

[21] Thomas CP, Boeglin WE, Garcia-Diaz Y, O rcia-Di VB, Brash AR. Steric analysis of epoxyalcohol and trihydroxy derivatives of 9-hydroperoxy-linoleic acid from hematin and enzymatic synthesis. Chem. Phys. Lipids. 2013;167–168:21–32.

[22] Song JH, Choi HJ, Song HH, Hong EH, Lee BR, Oh SR, Choi K, Yeo SG, Lee YP, Cho S, Ko HJ. Antiviral activity of ginsenosides against coxsackievirus B3, enterovirus 71, and human rhinovirus 3. J. Ginseng Res. 2014;38:173–179.