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**Note**

A new aliphatic alcohol, (2R,6R)-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 IC₅₀ 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, (2R,6R)-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 H₂O (2 L) and successively solvent-partitioned with n-hexane, CHCl₃, EtOAc, and n-ButOH, 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-C₁₈ 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 CHCl₃-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 CH₂Cl₂-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-H₂O (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-C₁₈ silica gel column chromatography using a

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

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solvent system of CHCl3-MeOH (5:1) to provide eight fractions (E1–E8). Fraction E4 (310 mg) was applied to a silica gel column chromatography using a solvent system of CHCl3-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 Sephadex LH-20 column chromatography using a solvent system of MeOH-H2O (1:1) to provide eight fractions (E4, E5, E6, E7, E8). Fraction E4 (310 mg) was applied to semi-preparative normal-phase HPLC with a solvent system of MeOH-H2O (20:1) to obtain two subfractions (E41, E42). Fraction E42 (76 mg) was subjected to Sephadex LH-20 column chromatography with a solvent system of MeOH-H2O (1:3) to yield compound 4 (4 mg) and 5 (2 mg). Fraction E8 (110 mg) was subjected to a silica gel column chromatography with CHCl3-MeOH (9:1, flow rate; 2 mL/min) to afford compound 8 (14 mg).

Compound 1 was isolated as a colorless gum with a negative optical rotation ($\alpha_{	ext{D}}^{25} = -26.2$ (c 0.30, MeOH), and the molecular formula C8H16O2 was deduced from the molecular ion peak [M + Na]+ at m/z 167.1050 (calcd. for C8H14O2Na, 167.1048) in the positive-ion HR-ESIMS. The IR absorptions of 1 revealed the presence of OH functional group (3383 cm$^{-1}$). The NMR data (Table 1) displayed a total of 8 carbon signals composed of one double bond at $\delta_{C}$ 141.3 and 114.9, two oxygenated methine carbons at $\delta_{C}$ 73.3 and 68.2, three methylene carbons at $\delta_{C}$ 39.2, 37.0, and 21.6, and one methyl carbon at $\delta_{C}$ 23.7, which were also verified by the analysis of DEPT data. Detailed inspection of the 1H and 13C NMR data of compound 1 in CDCl3$^*$.

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_{	ext{H}}$</th>
<th>$\delta_{C}$</th>
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<td>1</td>
<td>1.21 d (6.0)</td>
<td>23.7 q</td>
</tr>
<tr>
<td>2</td>
<td>3.83 m</td>
<td>68.2 d</td>
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<tr>
<td>3</td>
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<td>4</td>
<td>1.46 m</td>
<td>21.6 t</td>
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<tr>
<td>5</td>
<td>1.58 m</td>
<td>37.0 t</td>
</tr>
<tr>
<td>6</td>
<td>4.12 m</td>
<td>73.3 d</td>
</tr>
<tr>
<td>7</td>
<td>5.89 ddd (17.5, 10.5, 6.0)</td>
<td>141.3 d</td>
</tr>
<tr>
<td>8</td>
<td>5.23 d (17.5); 5.12 d (10.5)</td>
<td>114.9 t</td>
</tr>
</tbody>
</table>

$^*$1H and 13C NMR data were recorded at 500 and 125 MHz, respectively. Coupling constants (in Hz) are given in parentheses.
of the two MTPA diesters allowed for the assignment of the absolute configurations of C-2 and C-6 as 2R and 6R (Fig. 1). Thus, the structure of 1 was established as (2R,6R)-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 (3R)-oct-1-en-3-ol (2),\(^{(17)}\) 3β-citrostadiene B (3),\(^{(18)}\) (±)-caryophyllene oxide (4),\(^{(18)}\) 6β-hydroxy-stigmast-4-en-3-one (5),\(^{(19)}\) 3β-hydroxy-stigmast-5-en-7-one (6),\(^{(19)}\) methyl (9S,12S,13S)-9,12,13-trihydroxy-10E-octadecenoate (7),\(^{(20)}\) and methyl (9S,12R,13S)-9,12,13-trihydroxy-10E-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 IC₅₀ 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 IC₅₀ values ranging from 7 to 48 μM. No cytotoxic effects were observed for aliphatic alcohols (1-2), including novel compound 1 (IC₅₀ >50 μM). Among the isolates 1-8, (±)-caryophyllene oxide (4) displayed the most potent cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC₅₀: 27, 14, 7, and 17 μM, respectively).

In conclusion, we isolated and identified a new aliphatic alcohol, (2R,6R)-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.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>A549 (μM)</th>
<th>SK-OV-3 (μM)</th>
<th>SK-MEL-2 (μM)</th>
<th>HCT-15 (μM)</th>
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<td>&gt;50</td>
<td>38</td>
<td>&gt;50</td>
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<tr>
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<td>2</td>
</tr>
</tbody>
</table>

*IC₅₀ values of compounds against each cancer cell line. IC₅₀ value was defined as concentration (μM) causing 50% inhibition of cell growth in vitro.

### Supplemental material

The supplemental material for this paper is available at [http://10.1080/09168451.2015.1031079](http://10.1080/09168451.2015.1031079).

### Disclosure statement

No potential conflict of interest was reported by the authors.

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### References

12. (2R,6R)-Oct-7-ene-2,6-diol (1): colorless gum; [α]D — 26.2 (c 0.30, MeOH); IR (KBr) νmax 3357, 2945, 2832, 1451, 1116, 1032, 744 cm⁻¹; 1H (500 MHz) and 13C (125 MHz) NMR data, see Table 1; ESI-MS (positive-ion mode) m/z: 167 [M + Na⁺].
13. HR-ESI-MS (positive-ion mode) m/z: 167.1050 [M + Na⁺] (caled for C₉H₁₆O₂Na, 167.1048).


