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Short communication

# Chemical constituents of the bulbs of *Haemanthus multiflorus*

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ABSTRACT

Six new (**1**–**6**) and four known (**7**–**10**) phenolic glycosides were isolated from the bulbs of *Haemanthus multiflorus* (Amaryllidaceae). The structures of the new compounds were determined on the basis of spectroscopic analysis and of chromatographic analysis of the hydrolyzed products. Moreover, compound **1** showed moderate cytotoxicity against HL-60 human promyelocytic leukemia cells.

## 1. Introduction

Plants of the family Amaryllidaceae contain a number of alkaloids with different basic chemical structures and significant biological activities (Zhong, 2007). Previously, we examined the chemical constituents of several Amaryllidaceae plants such as *Habranthus brachyandrus* and *Lycoris albiflora*, resulting in the isolation of alkaloids, flavan derivatives, a hydroxybutyric acid glucoside, neolignans, and acetophenone derivatives (Jitsuno et al., 2009, 2011). Moreover, some alkaloids including lycorine, haemultine, haemanthidine, and chlidanthine have been isolated from the bulbs of *Haemanthus multiflorus* Martyn (Abdallah et al., 1989; Fales and Wildman, 1961), a plant of the Amaryllidaceae family that is found mainly in the tropical regions of Africa (Tsukamoto, 1989) and is cultivated worldwide for ornamental purposes. As part of our ongoing phytochemical study of Amaryllidaceae plants, we investigated the chemical constituents of the bulbs of *H. multiflorus*, focusing on phenolic glycosides. As a result, six new (**1**–**6**) and four known (**7**–**10**) phenolic glycosides were isolated, and the structures of the glycosides were determined on the basis of spectroscopic analysis and of chromatographic analysis of the hydrolyzed products. The isolated compounds were evaluated for their cytotoxic activity against HL-60 human promyelocytic leukemia cells.

## 2. Experimental

### 2.1. General experimental procedures

The instruments and experimental conditions were the same as those described in a previous paper (Yokosuka et al., 2016). However, in addition to silica gel and octadecylsilanized (ODS) silica gel, NH-silica gel (Fuji-Silysia Chemical, Aichi, Japan) was used for column chromatography (CC).

The bulbs of *H. multiflorus* Martyn were obtained from Sakata Seed Corporation (Kanagawa, Japan) in June 2014. A voucher specimen has been deposited in the specimen room of Tokyo University of Pharmacy and Life Sciences (voucher number: KS-2014-001).

### 2.2. Extraction and isolation

The bulbs of *H. multiflorus* (7.7 kg) were extracted with MeOH, and the MeOH extract (490 g) was passed through a porous-polymer polystyrene resin (Diaion HP-20™) column and successively eluted with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc. The 50% MeOH fraction (10 g) was subjected to silica gel CC, eluted with gradient mixtures of $CHCl_3$–MeOH–$H_2O$ (90:10:1; 40:10:1; 10:10:1) and finally with MeOH alone, to give 16 fractions (A–P). Fraction D was purified by preparative HPLC using MeCN–$H_2O$ (1:4) as the mobile phase to give **10** (1.7 mg). Fraction H was purified by NH-silica gel CC, eluted with EtOAc–MeOH (9:1; 4:1; 3:1; 2:1), and by preparative HPLC using MeCN–$H_2O$ (1:2) as the mobile phase to give **9** (0.9 mg). Fraction J was subjected to ODS silica gel CC eluted with MeCN-$H_2O$ (1:4) to give 10 subfractions (Ja–Jj). Fraction Jb was further separated by silica gel CC eluted with EtOAc-MeOH-$H_2O$ (90:10:1; 60:10:1; 40:10:1) yielding 13 fractions (Jba–Jbm). Fraction Jbg was purified by ODS silica gel CC, eluted with MeOH–$H_2O$ (1:3), and by preparative HPLC using MeCN–$H_2O$ (1:4) and MeOH–$H_2O$ (1:1) as the mobile phases to give **6** (1.7 mg). Fraction Jc was purified by ODS silica gel CC, eluted with MeOH–$H_2O$ (1:2), and by preparative HPLC using MeCN–$H_2O$ (1:4) as the mobile phase to give **5** (1.6 mg), **7** (10.4 mg), and **8** (6.6 mg). Fraction Jf was purified by silica gel CC, eluted with EtOAc–MeOH–$H_2O$ (90:10:1), and by preparative HPLC using MeCN–$H_2O$ (1:4) as the mobile phase to give **1** (8.0 mg) and **2** (3.6 mg). Fraction N was

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**Table 1**
$^{1}H$ and $^{13}C$ NMR chemical shift assignments for **1–6.**[a]

| **1** | | | | **2** | | | | **3** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $^{1}H$ | $^{13}C$ | | | $^{1}H$ | $^{13}C$ | | | $^{1}H$ | $^{13}C$ |
| | 1 | | 107.5 | | 1 | | 107.9 | | 1 | | 106.7 |
| | 2 | | 164.7 | | 2 | | 164.8 | | 2 | | 164.3 |
| | 3 | | 107.9 | | 3 | | 107.8 | | 3 | | 106.9 |
| | 4 | | 162.8 | | 4 | | 163.0 | | 4 | | 162.6 |
| | 5 | 6.31, s | 90.9 | | 5 | 6.34, s | 91.1 | | 5 | 6.77, s | 90.8 |
| | 6 | | 162.6 | | 6 | | 162.9 | | 6 | | 161.8 |
| | 7 | | 205.1 | | 7 | | 205.2 | | 7 | | 203.9 |
| | 8 | 2.60, s | 33.3 | | 8 | 2.60, s | 33.3 | | 8 | 2.53, s | 33.0 |
| | MeO-6 | 3.91, s | 56.2 | | MeO-6 | 3.92, s | 56.4 | | MeO-6 | 3.88, s | 55.9 |
| | Me-3 | 2.03, s | 7.6 | | Me-3 | 2.03, s | 7.7 | | Me-3 | 2.34, s | 8.0 |
| Glc | 1′ | 5.01, d (7.4)[b] | 101.4 | Glc | 1′ | 4.99, d (7.4) | 101.7 | Glc-I | 1′ | 5.64, d (7.3) | 102.0 |
| | 2′ | 3.48 | 74.7 | | 2′ | 3.44 | 74.8 | | 2′ | 4.30 | 75.2 |
| | 3′ | 3.47 | 78.0 | | 3′ | 3.43 | 78.0 | | 3′ | 4.32 | 78.4 |
| | 4′ | 3.35 | 71.3 | | 4′ | 3.33 | 71.5 | | 4′ | 4.21 | 71.3 |
| | 5′ | 3.63 | 77.0 | | 5′ | 3.65 | 77.2 | | 5′ | 4.39 | 77.7 |
| | 6′ | 4.00, dd (12.1, 1.4) | 67.9 | | 6′ | 4.04, dd (10.9, 1.5) | 68.8 | | 6′ | 4.91, br d (10.3) | 70.6 |
| | | 3.56, dd (12.1, 1.2) | | | | 3.58, dd (10.9, 6.7) | | | | 4.36 | |
| Rha | 1″ | 4.67, d (1.5) | 102.0 | Api | 1″ | 4.94, d (2.8) | 110.8 | Glc-II | 1″ | 5.01, d (7.8) | 105.6 |
| | 2″ | 3.70 | 72.0 | | 2″ | 3.86, d (2.8) | 77.9 | | 2″ | 4.00, dd (8.3, 7.8) | 74.6 |
| | 3″ | 3.60 | 72.3 | | 3″ | | 80.4 | | 3″ | 4.19 | 78.5 |
| | 4″ | 3.33 | 73.9 | | 4″ | 3.71, d (10.2) | 74.9 | | 4″ | 4.22 | 71.6 |
| | 5″ | 3.60 | 69.8 | | | 3.92, d (10.2) | | | 5″ | 3.88 | 78.4 |
| | 6″ | 1.18, d (6.3) | 17.8 | | 5″ab | 3.52, s | 65.2 | | 6″ | 4.92, dd (11.8, 2.0) | 62.6 |

| **4** | | | | **5** | | | | **6** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $^{1}H$ | $^{13}C$ | | | $^{1}H$ | $^{13}C$ | | | $^{1}H$ | $^{13}C$ |
| | 1 | | 107.9 | | 1 | | 107.9 | | 1 | | 160.8 |
| | 2 | | 167.6 | | 2 | | 167.6 | | 2 | 6.29, d (2.2) | 96.7 |
| | 3 | 6.29, d (2.3) | 97.8 | | 3 | 6.25, br s | 97.8 | | 3 | | 162.9 |
| | 4 | | 165.3 | | 4 | | 165.4 | | 4 | 6.16, dd (2.2) | 95.6 |
| | 5 | 6.23, d (2.3) | 93.0 | | 5 | 6.25, br s | 93.0 | | 5 | | 162.9 |
| | 6 | | 164.6 | | 6 | | 164.7 | | 6 | 6.29, d (2.2) | 96.7 |
| | 7 | | 204.9 | | 7 | | 205.0 | | MeO-3 | 3.74, s | 55.9 |
| | 8 | 2.58, s | 33.2 | | 8 | 2.59, s | 33.2 | | MeO-5 | 3.74, s | 55.9 |
| | MeO-6 | 3.89, s | 56.4 | | MeO-6 | 3.91, s | 56.6 | | | | |
| Glc-I | 1′ | 4.98, d (7.3) | 101.2 | Glc | 1′ | 4.98, d (7.3) | 101.2 | Glc | 1′ | 4.86, d (7.5) | 102.2 |
| | 2′ | 3.46 | 74.7 | | 2′ | 3.42 | 74.7 | | 2′ | 3.41 | 74.9 |
| | 3′ | 3.47 | 77.8 | | 3′ | 3.44 | 77.7 | | 3′ | 3.43 | 77.8 |
| | 4′ | 3.38 | 71.4 | | 4′ | 3.38 | 71.4 | | 4′ | 3.37 | 71.5 |
| | 5′ | 3.75 | 77.3 | | 5′ | 3.69 | 77.3 | | 5′ | 3.63 | 77.2 |
| | 6′ | 3.80, dd (11.4, 6.5) | 70.3 | | 6′ | 4.12, dd (11.2, 1.6) | 69.7 | | 6′ | 4.10, dd (11.4, 2.1) | 69.5 |
| | | 4.14, dd (11.4, 1.7) | | | | 3.76, dd (11.2, 6.2) | | | | 3.77, dd (11.4, 5.3) | |
| Glc-II | 1″ | 4.32, d (7.8) | 105.0 | Ara | 1″ | 4.28, d (6.7) | 105.1 | Ara | 1″ | 4.27 d (6.8) | 104.9 |
| | 2″ | 3.22 | 75.1 | | 2″ | 3.57, dd (8.8, 6.7) | 72.4 | | 2″ | 3.56, dd (8.8, 6.8) | 72.4 |
| | 3″ | 3.22 | 78.0 | | 3″ | 3.50, dd (8.8, 3.4) | 74.2 | | 3″ | 3.49, dd (8.8, 3.4) | 74.2 |
| | 4″ | 3.30 | 71.5 | | 4″ | 3.77 | 69.5 | | 4″ | 3.77 | 69.5 |
| | 5″ | 3.22 | 78.0 | | 5″ | 3.83, dd (12.4, 3.2) | 66.7 | | 5″ | 3.82, dd (12.4, 3.2) | 66.9 |
| | 6″ | 3.65, dd (12.0, 5.9) | 62.7 | | | 3.48, dd (12.4, 1.9) | | | | 3.45, dd (12.4, 1.8) | |
| | | 3.86, dd (12.0, 2.0) | | | | | | | | | |

[a] Compound **1**, **2**, and **4–6** were measured in $CD_3OD$, and **3** in $C_5D_5N$.
[b] Values in parentheses are coupling constants in Hz.

chromatographed on ODS silica gel eluted with MeOH–$H_2O$ (2:5) and on silica gel eluted with EtOAc–MeOH–$H_2O$ (40:10:1) to yield **3** (16.7 mg) and **4** (16.6 mg).

### 2.3. 2,4-Dihydroxy-6-methoxy-3-methylacetophenone 4-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (1)

Amorphous powder; $[\alpha]_D^{25}$ −104.7 (*c* 0.10, MeOH); UV (MeOH) $\lambda_{max}$ nm (log ε): 285.5 (4.31), 209.0 (4.35); IR $\nu_{max}$ (film) $cm^{-1}$: 3389 (OH), 2926 (CH), 1619 (C=O); $^{1}H$ NMR (500 MHz, $CD_3OD$) and $^{13}C$ NMR (125 MHz, $CD_3OD$) spectroscopic data, see Table 1; HR-ESI-TOF-MS *m/z*: 527.1729 $[M+Na]^+$ (calculated for $C_{22}H_{32}NaO_{13}$, 527.1741).

### 2.4. Acid hydrolysis of 1

A solution of **1** (2.0 mg) in 1 M HCl (dioxane–$H_2O$, 1:1, 2 mL) was heated at 95 °C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-96SB (Organo, Tokyo, Japan) column. The reaction mixture was diluted with $H_2O$ (10 mL) and extracted with EtOAc (10 mL × 3). The EtOAc-soluble phase was chromatographed on silica gel and eluted with hexane–$Me_2CO$ (4:1) to give 2,4-dihydroxy-6-methoxyacetophenone (**1a**, 0.6 mg). The $H_2O$-soluble phase was dissolved in $H_2O$ (1 mL), passed through a Sep-Pak $C_{18}$ cartridge (Waters, Milford, MA, USA), and analyzed by HPLC under the following conditions: column, Capcell Pak $NH_2$ UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido); solvent,