# A New Megastigmane Glucoside from the Aerial Parts of *Erythronium japonicum*

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**Abstract** – The purification of the MeOH extract from the aerial parts of *Erythronium japonicum* using column chromatography furnished a new megastigmane glucoside, erthrojaponiside (**1**), together with six known megastigmane derivatives (**2 - 7**). The structure of the new compound (**1**) was determined through 1D and 2D NMR spectral data analysis and chemical means. The isolated compounds (**1 - 7**) were tested for cytotoxicity against four human tumor cells *in vitro* using a Sulforhodamin B (SRB) bioassay.

**Keywords** – *Erythronium japonicum*, Liliaceae, Megastigmane, Cytotoxicity.

## Introduction

*Erythronium japonicum* (Liliaceae) is a plant that is widely distributed throughout Japan, China, and Korea. This indigenous herb is an edible wild vegetable that is traditionally used as a folk medicine for the treatment of stomach and digestive disorders (Lee *et al.*, 1994). Previous phytochemical investigations on this plant reported the isolation of sterol, steroidal saponin, fatty acid and flavonoid (Isono, 1976; Yasuta *et al.*, 1995; Moon and Kim, 1992; Lee *et al.*, 1994). Some biological studies, *e.g.* anticancer (Shin *et al.*, 2004), antioxidant and cytotoxicity activities (Heo *et al.*, 2007) of the MeOH extract of this source have also been reported. As parts of our continuing search for biologically active compounds from Korean medicinal plants, we have investigated the constituents from the aerial parts of *E. japonicum*. Column chromatographic separation of the MeOH extract led to isolation of a new megastigmane glucoside, erthrojaponiside (**1**), together with six known megastigmane derivatives (**2 - 7**). The structure of **1** was elucidated by spectroscopic methods, including 1D and 2D NMR. The isolated compounds (**1 - 7**) were tested for cytotoxicity against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15 cells) *in vitro* using a SRB bioassay.

## Experimental

**General** – Optical rotations were measured on a Jasco

P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. FAB and HRFAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra, including $^1$H-$^1$H COSY, HMQC, HMBC and NOESY experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz ($^1$H) and 125 MHz ($^{13}$C) with chemical shifts given in ppm ($\delta$). Preparative HPLC was conducted using a Gilson 306 pump with Shodex refractive index detector and Apollo Silica 5 column ($250 \times 22$ mm i.d.). Silica gel 60 (Merck, 70 - 230 mesh and 230 - 400 mesh) was used for column chromatography. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). TLC was performed using Merck precoated silica gel $F_{254}$ plates. Spots were detected on TLC under UV light or by heating after spraying with 10% $H_2SO_4$ in $C_2H_5OH$ (v/v).

**Plant materials** – The half dried aerial parts of *E. japonicum* (3 kg) were collected at Yangyang-gun in Gangwon-Do province in May 2008 and identified by one of the authors (K.R.Lee). A voucher specimen (SKKU-2009-04) of the plant was deposited at the School of Pharmacy at Sungkyunkwan University, Suwon, Korea.

**Extraction and isolation** – The aerial parts of *E. japonicum* (3 kg) were extracted with 80% MeOH at room temperature and filtered. The filtrate was evaporated under reduced pressure to give a MeOH extract (570 g), which was suspended in water (800 mL) and then successively partitioned with *n*-hexane, CHCl₃, EtOAc and *n*-BuOH, yielding 32, 8, 5, and 60 g, respectively. The n-BuOH soluble fraction (60 g) was chromatographed on a diaion HP-20 column, eluting with a gradient solvent

system consisting of 100% water and 100% MeOH, yielded two subfractions (A-B). Fraction B (17 g) was separated over a silica gel column (230 - 400 mesh, 360 g) with a solvent system of CHCl₃/MeOH/water (9 : 4 : 0.2) as the eluent to give six fractions (B1 - B6). Fraction B4 (3 g) was subjected to Sephadex LH-20 column chromatography eluted with 90% MeOH as to give three subfractions (B41 – B43). Subfraction B42 (1 g) was subjected to column chromatography (CC) over a silica gel (230 - 400 mesh, 20 g) eluted with a solvent system of CHCl₃/MeOH (5 : 1) to give four sub-fractions (B421 – B424). Subfraction B421 was purified with a RP-C₁₈ prep HPLC (35% MeOH) to yield **2** (4 mg, $R_t$ = 16 min) and **3** (4 mg, $R_t$ = 19 min). Subfraction B422 was purified with a RP-C₁₈ prep HPLC (35% MeOH) to yield **4** (19 mg, $R_t$ = 15 min) and **6** (17 mg, $R_t$ = 17 min). Subfraction B423 was purified with a RP-C₁₈ prep HPLC (35% MeOH) to yield **1** (4 mg, $R_t$ = 14 min) and **7** (6 mg, $R_t$ = 16 min). Compound **5** (10 mg, $R_t$ = 13 min) was obtained from subfraction B424 by RP-HPLC using 30% MeOH.

**Erthrojaponiside (1)** – Colorless gum. $[\alpha]_D^{25}$ : −20.3 (c 0.12, MeOH); IR (KBr) $\nu_{max}$ cm⁻¹: 3382, 2951, 1655, 1452, 1261, 1032, 799; UV (MeOH) $\lambda_{max}$ (log ) 217 (4.0), 275 (3.7) nm; FABMS m/z 403 [M + H]⁺; HRFABMS m/z 403.1968 [M + H]⁺; (calcd for C₁₉H₃₁O₉, 403.1968); ¹H-, ¹³C-NMR : see Table 1.

**Euodionoside A (2)** – Colorless gum, $[\alpha]_D^{25}$ : −40.5 (c 0.25, MeOH); ESI-MS m/z: 409.18 [M + Na]⁺; ¹H-NMR (CD₃OD, 500 MHz): δ 7.08 (1H, d, J = 16.0 Hz, H-7), 6.19 (1H, d, J = 16.0 Hz, H-8), 4.31 (1H, d, J = 8.0 Hz, H-1'), 3.98 (1H, m, H-3), 2.37 (1H, dd, J = 15.0, 7.0 Hz, H-4b), 2.29 (3H, s, CH₃-10), 1.91 (1H, dd, J = 15.0, 10.0 Hz, H-4a), 1.53 (2H, m, H-2), 1.25 (3H, s, CH₃-11), 1.17 (3H, s, CH₃-13), 0.97 (3H, s, CH₃-12); ¹³C-NMR (CD₃OD, 125 MHz): 198.9 (C-9), 142.5 (C-7), 133.3 (C-8), 101.8 (C-1'), 76.9 (C-3'), 76.8 (C-5'), 73.9 (C-2'), 70.9 (C-3), 70.8 (C-6), 70.6 (C-4'), 65.7 (C-5), 61.7 (C-6'), 39.7 (C-2), 37.2 (C-4), 34.7 (C-1), 26.3 (C-12), 26.1 (C-10), 23.7 (C-11), 20.2 (C-13).

**Icariside B₂ (3)** – Colorless gum, $[\alpha]_D^{25}$ : −102.1 (c 0.97, MeOH); FAB-MS m/z: 385.18 [M − H]⁻; ¹H-NMR (CD₃OD, 500 MHz): δ 7.16 (1H, d, J = 16.0 Hz, H-7), 6.19 (1H, d, J = 16.0 Hz, H-8), 4.34 (1H, d, J = 8.0 Hz, H-1'), 3.91 (1H, m, H-3), 2.40 (1H, m, H-4b), 2.29 (3H, s, CH₃-10), 1.81 (1H, m, H-4a), 1.74 (1H, m, H-2b), 1.41 (1H, m, H-2a), 1.21 (3H, s, CH₃-13), 1.19 (3H, s, CH₃-12), 0.96 (3H, s, CH₃-13); ¹³C-NMR (CD₃OD, 125 MHz): δ 199.1 (C-9), 144.1 (C-7), 132.6 (C-8), 101.8 (C-1'), 76.9 (C-3'), 76.8 (C-5'), 73.9 (C-2'), 71.6 (C-3), 70.5 (C-4'), 70.0 (C-6), 67.2 (C-5), 61.5 (C-6'), 44.0 (C-2),

**Table 1.** ¹H and ¹³C NMR data of **1** in CD₃OD. (δ in ppm, 500 MHz for ¹H and 125 MHz for ¹³C)ᵃ

| Position | 1 | |
|---|---|---|
| | $\delta_H$ | $\delta_C$ |
| 1 | | 41.4 |
| 2 | 3.69 d (11.0) | 71.3 |
| 3 | 3.83 dd (11.0, 4.5) | 77.9 |
| 4 | 4.24 d (4.5) | 69.9 |
| 5 | | 130.1 |
| 6 | | 139.4 |
| 7 | 7.24 d (16.0) | 142.8 |
| 8 | 6.14 d (16.0) | 133.9 |
| 9 | | 199.6 |
| 10 | 2.32 s | 26.1 |
| 11 | 1.05 s | 20.0 |
| 12 | 1.14 s | 25.3 |
| 13 | 1.86 s | 18.6 |
| 1' | 4.46 d (7.5) | 101.7 |
| 2' | 3.20-3.40 m | 73.7 |
| 3' | 3.20-3.40 m | 76.9 |
| 4' | 3.20-3.40 m | 70.3 |
| 5' | 3.20-3.40 m | 76.5 |
| 6' | 3.66 m, 3.85 m | 61.4 |

ᵃJ values are in parentheses and reported in Hz; the assignments were based on ¹H-¹H COSY, HMQC, and HMBC experiments.

37.0 (C-4), 34.8 (C-1), 28.3 (C-12), 26.3 (C-11), 24.3 (C-10), 19.0 (C-13).

**3β-Hydroxy-5α,6α-epoxy-β-ionone-2α-O-D-glucopyranoside (4)** – Colorless gum, $[\alpha]_D^{25}$ : −145.0 (c 0.14, MeOH); ESI-MS m/z: 425.17 [M + Na]⁺; ¹H-NMR (CD₃OD, 500 MHz): δ 7.13 (1H, d, J = 16.0 Hz, H-7), 6.18 (1H, d, J = 16.0 Hz, H-8), 4.32 (1H, d, J = 7.5 Hz, H-1'), 3.66 (1H, m, H-3), 3.17 (1H, d, J = 10.0 Hz, H-2), 2.43 (3H, dd, J = 15.0, 5.0 Hz, H-4b), 2.29 (3H, s, CH₃-10), 1.82 (1H, dd, J = 15.0, 10.0 Hz, H-4a), 1.32 (3H, s, CH₃-12), 1.16 (3H, s, CH₃-13), 1.00 (3H, s, CH₃-11); ¹³C-NMR (CD₃OD, 125 MHz): δ 199.1 (C-9), 143.6 (C-7), 132.5 (C-8), 105.4 (C-1'), 90.7 (C-2), 76.9 (C-3'), 76.8 (C-5'), 74.2 (C-2'), 70.1 (C-4'), 70.0 (C-6), 66.6 (C-5), 65.2 (C-3), 61.3 (C-6'), 40.5 (C-1), 38.4 (C-4), 26.3 (C-12), 25.4 (C-10), 18.4 (C-13), 17.4 (C-11).

**(2R,3R,5R,6S,9R)-3-Hydroxy-5,6-epoxy-β-ionol-2-O--D-glucopyranoside (5)** – Colorless gum, $[\alpha]_D^{25}$ : −82.5 (c 0.325, MeOH); ESI-MS m/z: 427.19 [M + Na]⁺; ¹H-NMR (CD₃OD, 500 MHz): δ 5.89 (1H, d, J = 16.0 Hz, H-7), 5.67 (1H, dd, J = 16.0, 6.0 Hz, H-8), 4.30 (1H, d, J = 8.0 Hz, H-1'), 4.27 (1H, m, H-9), 3.66 (1H, m, H-3), 3.15 (1H, d, J = 10.0 Hz, H-2), 2.39 (3H, dd, J = 15.0, 5.0 Hz,

H-4b), 1.75 (1H, dd, $J = 15.0, 10.0$ Hz, H-4a), 1.27 (3H, s, CH$_3$-12), 1.22 (3H, d, $J = 6.5$ Hz, CH$_3$-10), 1.16 (3H, s, CH$_3$-13), 1.00 (3H, s, CH$_3$-11); $^{13}$C-NMR (CD$_3$OD, 125 MHz): δ 138.0 (C-8), 124.6 (C-7), 105.3 (C-1'), 91.2 (C-2), 77.0 (C-3'), 76.9 (C-5'), 74.2 (C-2'), 70.2 C-4'), 70.1 (C-6), 67.4 (C-9), 65.8 (C-5), 65.4 (C-3), 61.3 (C-6'), 40.5 (C-1), 38.4 (C-4), 25.6 (C-12), 22.6 (C-10), 18.5 (C-13), 17.2 (C-11).

**(6$R$,9$R$)-3-Oxo-$\alpha$-ionol-9-$O$-$\beta$-D-glucopyranoside (6)** – Colorless gum, $[\alpha]_D^{25}$ : −116.0 ($c$ 0.79, MeOH); ESI-MS $m/z$: 409.18 [M + Na]$^+$; $^1$H-NMR (CD$_3$OD, 500 MHz): δ 5.86 (2H, m, H-7, 8), 4.42 (1H, m, H-9), 4.34 (1H, d, $J = 8.0$ Hz, H-1'), 2.51 (H, d, $J = 17.0$ Hz, H-2b), 2.14 (1H, d, $J = 17.0$ Hz, H-2a), 1.91 (3H, s, CH$_3$-13), 1.29 (3H, d, $J = 6.5$ Hz, CH$_3$-10), 1.03 (3H, s, CH$_3$-11), 1.02 (3H, s, CH$_3$-12); $^{13}$C-NMR (CD$_3$OD, 125 MHz): δ 200.0 (C-3), 166.1 (C-5), 134.1 (C-8), 130.4 (C-7), 126.0 (C-4), 101.6 (C-1'), 78.8 (C-6), 76.9 (C-3'), 76.8 (C-5'), 76.1 (C-9), 74.0 (C-2'), 70.5 (C-4'), 61.7 (C-6'), 49.5 (C-2), 41.2 (C-1), 23.52 (C-12), 22.3 (C-11), 20.0 (C-10), 18.3 (C-13).

**(6$R$,9$S$)-Megastigman-4-en-3-one-9,13-diol-9-$O$-glucopyranoside (7)** – Colorless gum, $[\alpha]_D^{25}$ : +27.1 ($c$ 0.70, MeOH); FAB-MS $m/z$: 387.19 [M − H]$^-$; $^1$H-NMR (CD$_3$OD, 500 MHz): δ 6.04 (1H, s, H-4), 4.36 (1H, d, $J = 16.0$ Hz, H-13b), 4.33 (1H, d, $J = 7.5$ Hz, H-1'), 4.19 (1H, d, $J = 16.0$ Hz, H-13a), 3.89 (1H, m, H-9), 2.56 (1H, d, $J = 18.0$ Hz, H-2b), 2.01 (1H, d, $J = 18.0$ Hz, H-2a), 1.94 (1H, m, H-6), 1.62 (2H, m, H$_2$-8), 1.51 (2H, m, H$_2$-7), 1.18 (3H, d, $J = 6.5$ Hz, CH$_3$-10), 1.10 (3H, s, CH$_3$-12), 1.01 (3H, s, CH$_3$-11); $^{13}$C-NMR (CD$_3$OD, 125 MHz): δ 200.0 (C-3), 166.1 (C-5), 134.1 (C-8), 130.4 (C-7), 126.0 (C-4), 101.6 (C-1'), 78.8 (C-6), 76.9 (C-3'), 76.8 (C-5'), 76.1 (C-9), 74.0 (C-2'), 70.5 (C-4'), 61.7 (C-6'), 49.5 (C-2), 41.2 (C-1), 23.52 (C-12), 22.3 (C-11), 20.0 (C-10), 18.3 (C-13).

**Cytotoxicity assay** – A sulforhodamine B bioassay (SRB) was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines (Skehan et al., 1990). The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin (Sigma Chemical Co., ≥ 98%) was used as a positive control. The cytotoxicities of doxorubicin against the A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines were IC$_{50}$ 0.0017, 0.0117, 0.0011 and 0.296 μM, respectively.

**Enzymatic hydrolysis of 1** – Compound **1** (3.0 mg) in 1 ml of H$_2$O and 4 mg of $\beta$-glucosidase (Emulsin) were stirred at 37 °C for 2 days, and then extracted with CHCl$_3$

three times, and the CHCl$_3$ extract was evaporated in *vacuo*. The CHCl$_3$ extract (2.5 mg) was purified using RP Silica HPLC 50% MeOH to afford an aglycone **1a** as a colorless gum $\{[\alpha]_D^{25}$: −37.0 ($c$ 0.1, MeOH); $^1$H-NMR (Pyridine-$d_5$, 500 MHz)$\}$ and glucose.

**1a**: Colorless gum; $[\alpha]_D^{25}$: −37.0 ($c$ 0.1, MeOH); $^1$H-NMR (Pyridine-$d_5$, 500 MHz): δ 7.36 (1H, d, $J = 16.5$ Hz, H-7), 6.30 (1H, d, $J = 16.5$ Hz, H-8), 4.40 (1H, d, $J = 4.0$ Hz, H-4), 4.17 (1H, d, $J = 10.5$ Hz, H-2), 4.08 (1H, dd, $J = 10.5, 4.0$ Hz, H-3), 2.29 (3H, s, H-10), 2.01 (3H, s, H-13), 1.27 (3H, s, H-12), 1.25 (3H, s, H-11); HR-FAB-MS (positive mode) m/z = 241.1441 [M + H]$^+$ (calcd for C$_{13}$H$_{21}$O$_4$, 241.1440).

**Preparation of the ($R$)- and ($S$)-MTPA ester derivatives of 1a** – **1a** (1.0 mg) in pyridine (400 μL) was stirred with 4-(dimethylamino)pyridine (2 mg) and ($R$)-(+)-$\alpha$-methoxy-$\alpha$-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl, 10 μL) at room temperature for 12 h. The reaction mixture was then passed through a silica gel Waters Sep-Pak Vac 6cc and eluted with CHCl$_3$-MeOH (20 : 1) to give ($S$)-Mosher ester (**1b**). In the same way, **1a** (1.0 mg) yielded ($R$)-MTPA ester (**1c**).

**1b**. $^1$H-NMR (Pyridine-$d_5$, 500 MHz): δ 1.785 (3H, s, H-13), 2.291 (3H, s, H-10), 4.100 (1H, d, $J = 11.5$ Hz, H-2), 4.485 (1H, dd, $J = 11.5, 4.5$ Hz, H-3), 6.118 (1H, d, $J = 4.5$ Hz, H-4), 6.228 (1H, d, $J = 16.0$ Hz, H-8).

**1c**. $^1$H-NMR (Pyridine-$d_5$, 500 MHz): δ 1.592 (3H, s, H-13), 2.229 (3H, s, H-10), 4.224 (1H, d, $J = 11.5$ Hz, H-2), 4.588 (1H, dd, $J = 11.5, 4.5$ Hz, H-3), 6.101 (1H, d, $J = 4.5$ Hz, H-4), 6.062 (1H, d, $J = 16.0$ Hz, H-8).

## Results and Discussion

Compounds **2 - 7** structures were determined by comparing $^1$H-, $^{13}$C-NMR, and MS spectral data with those in the literatures to be euodionoside A (**2**) (Yamamoto et al., 2008), icariside B$_2$ (**3**) (Otsuka et al., 1995), 3$\beta$-hydroxy-5$\alpha$,6$\alpha$-epoxy-$\beta$-ionone-2$\alpha$-$O$-D-gluco-pyranoside (**4**) (Habib et al., 2006), (2$R$,3$R$,5$R$,6$S$,9$R$)-3-hydroxy-5,6-epoxy-$\beta$-ionol-2-$O$-$\beta$-D-glucopyranoside (**5**) (Feng et al., 2007), (6$R$,9$R$)-3-oxo-$\alpha$-ionol-9-$O$-$\beta$-D-glucopyranoside (**6**) (Yamano et al., 2005), and (6$R$,9$S$)-megastigman-4-en-3-one-9,13-diol-9-$O$-glucopyranoside (**7**) (Sueyoshi et al., 1997). Compounds **2 - 7** were isolated from this source for the first time.

Compound **1** was obtained as a colorless gum. The molecular formula was determined to be C$_{19}$H$_{30}$O$_9$ from the molecular ion peak [M + H]$^+$ at $m/z$ 403.1968 (calcd for C$_{19}$H$_{31}$O$_9$, 403.1968) in the positive-ion HR-FAB-MS. The IR spectrum of **1** indicated the presence of hydroxy
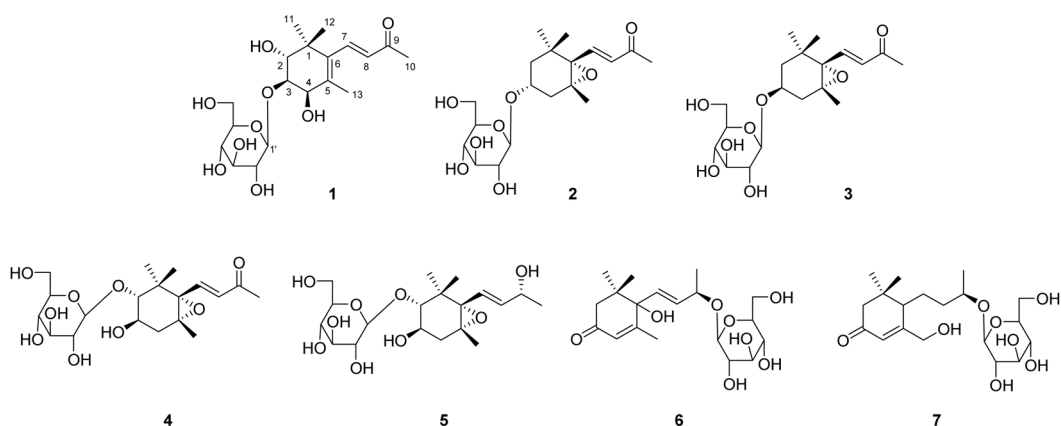
**Fig. 1.** The structures of **1 - 7** isolated from *E. japonicum*.

(3382 cm⁻¹) and ketone groups (1655 cm⁻¹). The ¹H-NMR spectrum of **1** displayed signals for four methyl groups at $\delta_H$ 2.32 (3H, s), 1.86 (3H, s), 1.14 (3H, s), and 1.05 (3H, s), three oxymethine proton signals at $\delta_H$ 4.24 (d, $J$ = 4.5 Hz), 3.83 (dd, $J$ = 11.0, 4.5 Hz), and 3.69 (d, $J$ = 11.0 Hz) and two olefinic proton signals at $\delta_H$ 7.24 (d, $J$ = 16.0 Hz), and 6.14 (d, $J$ = 16.0 Hz). In the ¹³C-NMR spectrum, 13 carbon signals appeared, including four methyl carbons at $\delta_C$ 26.1, 25.3, 20.0, and 18.6, three oxygenated methine carbons at $\delta_C$ 77.9, 71.3, and 69.9, four olefinic carbons $\delta_C$ 142.8, 139.4, 133.9, and 130.1, one quaternary carbon at $\delta_C$ 41.4, and one carbonyl carbon at $\delta$ 199.6. These spectral data implied that **1** was to be a megastigmane derivative (Xie *et al.*, 2005; Shinichi *et al.*, 1990). Additionally, the signals for D-glucopyranose appeared at $\delta_H$ 4.46 (1H, d, $J$ = 7.5 Hz), 3.85 (1H, m), 3.66 (1H, m), and 3.20-3.40 (3H, m) in the ¹H-NMR spectrum and $\delta_C$ 101.7, 76.9, 76.5, 73.7, 70.3, and 61.4 in the ¹³C-NMR spectrum. The coupling constant ($J$ = 7.5 Hz) of the anomeric proton signal at $\delta_H$ 4.46 of D-glucose indicated to be the $\beta$-form (Perkins *et al.*, 1977). The NMR spectral data of **1** were similar to those of komaroveside A isolated from *Cardamine komarovii* (Lee *et al.,* 2011), except for a additional oxygenated methine signal [$\delta_H$ 3.69 (d, $J$ = 11.0 Hz); $\delta_C$ 71.3]. The position of OH group was to be placed at C-2, based on the comparison of ¹H- and ¹³C-NMR chemical shifts of **1** with those of komaroveside A. This was confirmed by the HMBC experiment which showed correlations between the oxygenated methine signal ($\delta_H$ 3.69, H-2) and C-1, C-4, C-11 and C-12. The position of D-glucose was established by an HMBC experiment, in which a long-range correlation was observed between H-1' ($\delta$ 4.46) of D-glucose and C-3 ($\delta$ 77.9) of the aglycone (Fig. 2). The relative configuration of **1** was deduced on the basis of
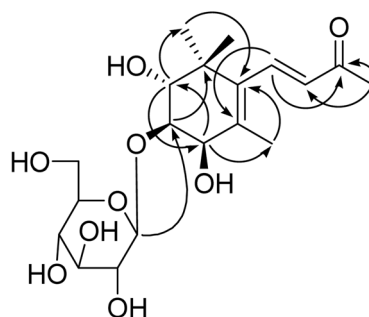


**Fig. 2.** Key HMBC ( $\rightarrow$ ) correlations of **1**.
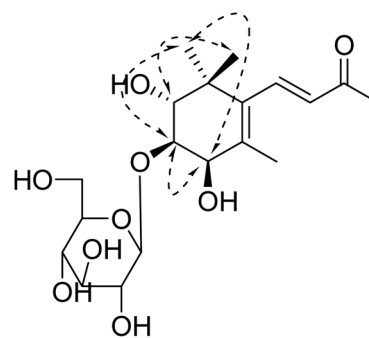


**Fig. 3.** Key NOESY ( ◄┈┈┈► ) correlations of **1**.

the $J$ values in the ¹H-NMR spectrum and the NOESY experiment (Fig. 3). The coupling constants of H-3 (dd, $J_{2,3}$ = 11.0 Hz and $J_{3,4}$ = 4.5 Hz), H-2 (d, $J_{2,3}$ = 11.0 Hz) and H-4 (d, $J_{3,4}$ = 4.5 Hz) in the ¹H-NMR spectrum were almost same as those of H-3/H-4 and H-3/H-2 in indaquassin B (Koike and Ohmoto, 1993). Moreover, NOESY correlations between H-3 ($\delta$ 3.83) and H-4 ($\delta$ 4.24), and no correlations between H-2 and H-3 and H-4 were observed (Fig. 3). These data suggested that the hydroxyl groups at C-3 and C-4 are to be *cis*- and at C-3 and C-2 *trans*-configurations, respectively. Enzymatic

**1b** : R = (*S*)-MTPA
**1c** : R = (*R*)-MTPA
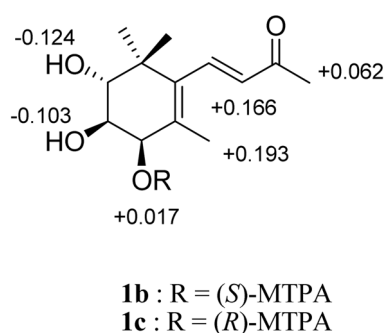
**Fig. 4.** Values of $\delta_S$ - $\delta_R$ of the MTPA esters of **1a**.

hydrolysis of **1** with $\beta$-glucosidase (emulsin) yielded 2,3,4-trihydroxy-5,7-megastigmadien-9-one (**1a**) and glucose; **1a** was identified by the $^1$H-NMR and HR-FAB-MS data and the sugar by co-TLC (EtOAc : MeOH : $H_2O = 9 : 3 : 1$, $R_f$ value : 0.2) with a D-glucose standard (Aldrich Co., USA) and its specific rotation value $\{[\alpha]_D^{25}$ : + 52° (*c* 0.03, $H_2O$)} (Gan *et. al.,* 2008). The absolute configuration at C-4 in **1a** was determined by a modified Mosher's method (Lee *et al.,* 2011). The treatment of **1a** with (*R*)-, and (*S*)-MTPA-Cl gave the (*S*)-, and (*R*)-MTPA esters **1b** and **1c**, respectively. The $^1$H-NMR data of two MTPA esters indicated that the absolute configuration at C-4 was to be *R* form (Fig. 4). Thus, the structure of **1** was determined to be 2$\alpha$,3$\beta$,4*R*-trihydroxy-5,7-megastigmadien-9-one-3-*O*-$\beta$-D-glucopyranoside, and named erthrojaponiside.

The cytotoxicities of compounds **1 - 7** against the A549 (a non small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT15 (colon adenocarcinoma) human cancer cell lines were evaluated using the SRB assay. All the compounds showed little cytotoxicity against any tested cell line ($IC_{50} > 30$ mM).

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