

## Reinvestigation of the Absolute Stereochemistry of Megastigmane Glucoside, Icariside B<sub>5</sub>

Katsuyoshi MATSUNAMI,<sup>a</sup> Hideaki OTSUKA,<sup>\*a</sup> Yoshio TAKEDA,<sup>b</sup> and Toshio MIYASE<sup>c</sup>

<sup>a</sup> Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University; 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8553, Japan; <sup>b</sup> Faculty of Pharmacy, Yasuda Women's University; 6–13–1 Yasuhigashi, Asaminami-ku, Hiroshima 731–0153, Japan; and <sup>c</sup> Faculty of Pharmaceutical Sciences, University of Shizuoka; 52–1 Yata, Suruga-ku, Shizuoka 422–8526, Japan. Received June 8, 2010; accepted July 1, 2010; published online July 27, 2010

**Icariside B<sub>5</sub> is one of the widely distributed megastigmane glucosides among plant sources. The absolute structure of icariside B<sub>5</sub> was reinvestigated by chemical conversion from the related compound and the application of the modified Mosher's method. As a result, the structure of icariside B<sub>5</sub> was revised to be (6*S*,9*S*)-6,9-dihydroxymegastigman-4-en-3-one 9-*O*-β-*D*-glucopyranoside.**

**Key words** icariside B<sub>5</sub>; dihydrovomifoliol-*O*-β-*D*-glucopyranoside; blumenol B; megastigmane glucoside

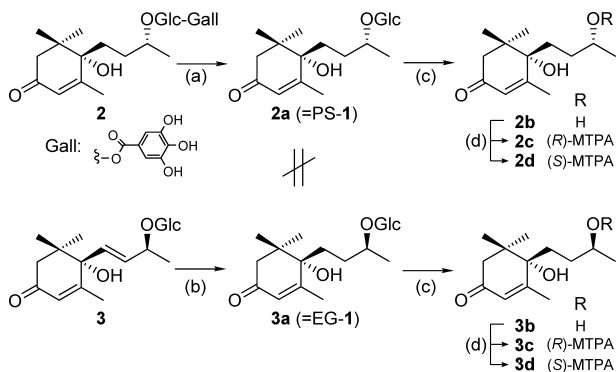
Blumenol B was isolated from the leaves of *Podocarpus blumei* in 1972.<sup>1)</sup> The absolute stereochemistry of blumenol B was then determined as (6*S*,9*R*) by chemical conversion of related compounds.<sup>2)</sup> In 1988, two groups independently reported the isolation of β-*D*-glucopyranoside of blumenol B, named differently as dihydrovomifoliol-*O*-β-*D*-glucopyranoside (PS-1) from *Pinus sylvestris* and icariside B<sub>5</sub> (EG-1) from *Epimedium grandiflorum* var. *thunbergianum*, respectively.<sup>3,4)</sup> (The former is designated as PS-1 and the latter EG-1 in this study for readers' convenience.) The absolute structures of the aglycones were determined to be the same as (6*S*,9*R*)-6,9-dihydroxymegastigman-4-en-3-one by comparisons of the spectral data of aglycones (PS-1a and EG-1a) obtained by enzymatic hydrolysis of glucosides (PS-1 and EG-1) with those reported for blumenol B.<sup>3,4)</sup> Thus compounds PS-1 and EG-1 were concluded to be the same compound until now, although the NMR spectra were measured in different solvents, e.g., methanol-*d*<sub>4</sub> and pyridine-*d*<sub>5</sub>, respectively.<sup>3,4)</sup> The modified Mosher's method is widely used for the determination of the absolute stereochemistry of chiral secondary alcohols.<sup>5)</sup> In this study, the absolute configurations of these compounds (PS-1 and EG-1) were reinvestigated using this reliable method.

First, a closely related compound, macarangioside A (2),<sup>6)</sup> was hydrolyzed with mild alkaline hydrolysis (Chart 1) to afford degalloyl-2 (2a), of which the NMR spectra (in methanol-*d*<sub>4</sub>) and specific optical rotation value {[α]<sub>D</sub><sup>24</sup> –2.2°

(*c*=0.12, MeOH)} were essentially identical to those reported for PS-1 {Ref.: [α]<sub>D</sub><sup>20</sup> –4.4° (*c*=0.80, MeOH)}.<sup>3)</sup> Thus 2a was elucidated to be identical to PS-1. Second, the <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of 2a were remeasured in pyridine-*d*<sub>5</sub> according to the literature for EG-1.<sup>4)</sup> However, the chemical shift values in pyridine-*d*<sub>5</sub> of 2a (Tables 1, 2) did not coincide with those reported for EG-1.<sup>4)</sup> These results clearly indicated that PS-1 and EG-1 were not the same compounds. The detailed inspection of <sup>13</sup>C chemical shift values measured in pyridine-*d*<sub>5</sub> between 2a (=PS-1) and the reported values for EG-1<sup>4)</sup> suggested that EG-1 must be the C-9 epimer of PS-1. According to the literature,<sup>3,4)</sup> the absolute structures of both PS-1 and EG-1 were determined in a similar manner, e.g., by comparison of the spectral data for aglycones with those reported for blumenol B without an adequate chiroptical method. Therefore we next performed detailed analysis to determine the absolute structures by chemical conversion and the modified Mosher's method for PS-1 and EG-1.

Enzymatic hydrolysis of 2a (=PS-1) afforded an aglycone (2b). The (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters were prepared using the conventional procedure (2c and 2d from 2b, see Experimental) (Chart 1). The distribution pattern of Δδ<sub>S-R</sub> values for 2c and 2d clearly demonstrated that 2b possessed the 9*R* configuration (Fig. 1a). In addition, the configuration of glucose was determined to be the *D*-series in HPLC analysis following acid hydrolysis of 2 and derivatization of the liberated glucose. The application of the β-*D*-glucosylation-induced shift-trend rule also supported the above result (Table 1).<sup>7)</sup> The absolute stereochemistry of C-6 was also confirmed based on the circular dichroism (CD) spectra. The CD spectral data for 2a and 2b were essentially identical to those of blumenol B, of which the absolute stereochemistry was determined by chemical and spectroscopic analyses.<sup>2)</sup> Therefore the structure of 2a (=PS-1, dihydrovomifoliol-*O*-β-*D*-glucopyranoside) was confirmed unambiguously to be (6*S*,9*R*)-6,9-dihydroxymegastigman-4-en-3-one 9-*O*-β-*D*-glucopyranoside, e.g., 9-*O*-β-*D*-glucopyranoside of blumenol B (Fig. 2).

Next, compound 3 was prepared from the closely related compound corchoionoside C (3), originally isolated by Yoshikawa *et al.*,<sup>8)</sup> which was also isolated from *Euodia meliaefolia* in our previous study.<sup>9)</sup> Partial hydrogenation of 3 afforded 3a (Chart 1). The physicochemical data, e.g., NMR



a) 0.1 M NaOMe in MeOH, rt, 1 h, b) PtO<sub>2</sub>/H<sub>2</sub>, 0 °C, 4 h, c) β-*D*-glucosidase, 37 °C, 12 h, d) EDC, DMAP, (*R*) and (*S*)-MTPAs in CH<sub>2</sub>Cl<sub>2</sub>, 35 °C, 12 h.

Chart 1

Table 1.  $^{13}\text{C}$ -NMR Spectral Data for Dihydrovomifoliol-*O*- $\beta$ -D-glucopyranoside (**2a**) and Icariside B<sub>5</sub> (**3a**)

C	<b>2a</b> (=PS-1) (C <sub>5</sub> D <sub>5</sub> N)	<b>2a</b> (=PS-1) (CD <sub>3</sub> OD)	<b>2b</b> (CD <sub>3</sub> OD)	<b>3a</b> (=EG-1) (C <sub>5</sub> D <sub>5</sub> N) <sup>a)</sup>	<b>3a</b> (=EG-1) (CD <sub>3</sub> OD)	<b>3b</b> (CD <sub>3</sub> OD)
1	42.3	42.9	43.0	42.3	43.2	43.0
2	50.8	51.2	51.2	50.6	51.2	51.2
3	197.6	201.1	200.9	198.0	201.1	200.9
4	126.4	126.8	126.7	126.1	126.8	126.7
5	168.5	171.7	171.7	168.9	171.8	171.8
6	78.1	79.4	79.2	78.1	79.5	79.3
7	34.8	34.9	35.3	33.7	34.8	35.8
8	33.3	33.6	35.4 (-1.8) <sup>b)</sup>	32.2	33.1	35.4 (-2.3) <sup>c)</sup>
9	75.0	76.3	69.0 (+7.3)	76.7	77.9	69.4 (+8.5)
10	20.3	20.1	23.5 (-3.4)	22.0	22.2	23.7 (-1.5)
11	24.8	24.7	24.7	24.4	24.5	24.6
12	24.2	24.2	24.1	24.0	24.1	24.1
13	21.6	21.7	21.8	21.6	22.0	21.8
1'	102.5	102.4		104.1	104.4	
2'	75.3	75.2		75.0	75.4	
3'	78.7	78.3		78.2	78.4	
4'	72.1	71.9		71.4	71.8	
5'	78.4	77.9		78.0	78.4	
6'	63.2	63.0		62.6	62.9	

a) Added one drop of D<sub>2</sub>O. b)  $\delta_{2a-2b}$ . c)  $\delta_{3a-3b}$ .

Table 2.  $^1\text{H}$ -NMR Spectral Data for Dihydrovomifoliol-*O*- $\beta$ -D-glucopyranoside (**2a**) and Icariside B<sub>5</sub> (**3a**) (600 MHz)

C	<b>2a</b> (C <sub>5</sub> D <sub>5</sub> N) (=PS-1)	<b>2a</b> (CD <sub>3</sub> OD) (=PS-1)	<b>2b</b> (CD <sub>3</sub> OD)	<b>3a</b> (C <sub>5</sub> D <sub>5</sub> N) <sup>a)</sup> (=EG-1)	<b>3a</b> (CD <sub>3</sub> OD) (=EG-1)	<b>3b</b> (CD <sub>3</sub> OD)
2	2.38 (1H, d, 18)	2.15 (1H, dd, 18, 1)	2.16 (1H, dd, 18, 1)	2.34 (1H, dd, 18, 1)	2.14 (1H, dd, 18, 1)	2.16 (1H, dd, 18, 1)
	2.77 (1H, d, 18)	2.60 (1H, d, 18)	2.58 (1H, d, 18)	2.82 (1H, d, 18)	2.65 (1H, d, 18)	2.59 (1H, d, 18)
4	6.06 (1H, br s)	5.83 (1H, dq, 1, 1)	5.83 (1H, dq, 1, 1)	5.96 (1H, br s)	5.82 (1H, br s)	5.83 (1H, dq, 1, 1)
7	2.09 (1H, m)	1.81 (1H, m)	1.77 (1H, ddd, 14, 12, 5)	2.26 (1H, ddd, 13, 13, 4)	1.84 (1H, ddd, 14, 13, 4)	1.79 (1H, ddd, 14, 12, 4)
	2.43 (1H, m)	2.03 (1H, m)	1.95 (1H, ddd, 14, 12, 4)	2.38 (1H, ddd, 13, 13, 5)	2.06 (1H, ddd, 14, 13, 5)	1.98 (1H, ddd, 14, 12, 5)
8	1.71 (1H, m)	1.49 (1H, m)	1.43 (1H, dddd, 13, 12, 5, 5)	1.75 (1H, dddd, 13, 13, 6, 5)	1.51 (1H, dddd, 13, 13, 7, 5)	1.40 (1H, dddd, 13, 12, 8, 5)
	2.18 (1H, m)	1.77 (1H, m)	1.65 (1H, dddd, 13, 12, 7, 4)	2.10 (1H, m)	1.78 (1H, dddd, 13, 13, 4, 4)	1.68 (1H, dddd, 13, 12, 5, 4)
9	4.06 (1H, dq, 6, 6)	3.81 (1H, m)	3.66 (1H, dqd, 7, 6, 5)	4.07 (1H, qdd, 6, 6, 6)	3.81 (1H, m)	3.65 (1H, dqd, 8, 6, 5)
10	1.27 (3H, d, 6)	1.17 (3H, d, 6)	1.15 (3H, d, 6)	1.34 (3H, d, 6)	1.24 (3H, d, 6)	1.16 (3H, d, 6)
11	1.24 (3H, s)	1.02 (3H, s)	1.02 (3H, s)	1.17 (3H, s)	1.02 (3H, s)	1.02 (3H, s)
12	1.20 (3H, s)	1.10 (3H, s)	1.10 (3H, s)	1.19 (3H, s)	1.09 (3H, s)	1.09 (3H, s)
13	2.13 (3H, br s)	2.04 (3H, d, 1)	2.04 (3H, d, 1)	2.11 (3H, d, 1)	2.04 (3H, d, 1)	2.04 (3H, d, 1)
1'	4.89 (1H, d, 8)	4.31 (1H, d, 8)		4.87 (1H, d, 8)	4.32 (1H, d, 8)	
2'	3.96 (1H, m)	3.13 (1H, dd, 9, 8)		3.93 (1H, dd, 9, 8)	3.14 (1H, dd, 9, 8)	
3'	4.23 (1H, m)	3.34 (1H, dd, 9, 9)		4.15 (1H, dd, 9, 9)	3.33 (1H, m)	
4'	4.19 (1H, m)	3.27 (1H, dd, 9, 9)		4.11 (1H, dd, 9, 9)	3.27 (1H, dd, 9, 9)	
5'	3.94 (1H, m)	3.25 (1H, m)		3.86 (1H, ddd, 9, 6, 2)	3.25 (1H, m)	
6'	4.33 (1H, m)	3.65 (1H, dd, 12, 5)		4.28 (1H, dd, 12, 6)	3.65 (1H, dd, 12, 6)	
	4.53 (1H, br d, 11)	3.85 (1H, dd, 12, 2)		4.46 (1H, dd, 12, 2)	3.84 (1H, dd, 12, 2)	

In parentheses, multiplicities and coupling constants (*J* in Hz). m: Multiplet or overlapped. Chemical shifts were determined by  $^1\text{H}$ - $^1\text{H}$  COSY and HMQC. a) Added one drop of D<sub>2</sub>O.

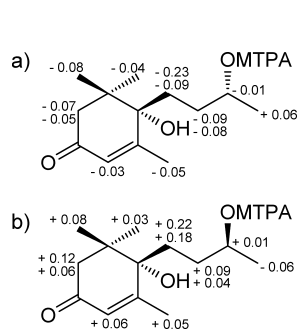


Fig. 1. Modified Mosher's Analysis

a) For **2b** and b) for **3b**.  $\Delta\delta$  values ( $\delta_S - \delta_R$ ) were shown in ppm.

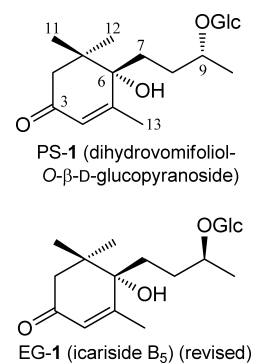


Fig. 2. Dihydrovomifoliol-*O*- $\beta$ -D-glucopyranoside (PS-1) and Revised Structure of Icariside B<sub>5</sub> (EG-1)

spectra (in pyridine-*d*<sub>5</sub>) (Tables 1, 2) and specific optical rotation value  $\{[\alpha]_D^{24} - 10.5^\circ (c=0.13, \text{MeOH})\}$  of **3a** were essentially identical to those reported for EG-1  $\{Ref.: [\alpha]_D^{25} - 12.9^\circ (c=0.62, \text{MeOH})\}$ ,<sup>4)</sup> which indicated that **3a** was identical to EG-1. The stereochemistry of **3** was determined by chemical conversion of roseoside, the 9-epimer of **3**, e.g., NaBH<sub>4</sub> reduction of the 9-ketonic functional group following the PCC oxidation of 9-OH of roseoside.<sup>8)</sup> Thus EG-1 (= **3a**) must have the same stereochemistry as **3**. We also performed the modified Mosher's analysis to confirm the above result. The (*R*)- or (*S*)-MTPA esters were prepared similarly as described above [**3c** and **3d** from the aglycone (**3b**), see Experimental]. The distribution patterns of  $\Delta\delta_{S-R}$  values for **3c** and **3d** clearly demonstrated that **3b** possessed the 9*S* configuration (Fig. 1b). The application of the  $\beta$ -D-glucosylation-induced shift-trend rule<sup>7)</sup> also supported the above result (Table 1 and Experimental). The CD spectral data for **3a** and **3b** were also essentially the same as those for blumenol B. Therefore the structure of icariside B<sub>5</sub> (EG-1) (= **3a**) was revised to be (6*S*,9*S*)-6,9-dihydroxymegastigman-4-en-3-one 9-*O*- $\beta$ -D-glucopyranoside, e.g., 9-*O*- $\beta$ -D-glucoside of 9-*epi*-blumenol B (Fig. 2). Finally, the NMR spectral data (CD<sub>3</sub>OD) of icariside B<sub>5</sub> (EG-1) isolated from *E. thunbergianum* var. *grandiflorum*<sup>4)</sup> were found to be identical to those of **3a**.

The specific optical rotation value of blumenol B (**2b**) prepared in this study was  $[\alpha]_D^{27} + 6.2^\circ (c=0.06, \text{CH}_3\text{OH})$ . Thus icariside B<sub>5</sub> (EG-1) was misidentified as a glucoside of blumenol B, probably due to the relatively small optical rotation value of the aglycone  $[Ref.: [\alpha]_D^{25} + 19.7^\circ (c=0.23, \text{CH}_3\text{OH})]$ .<sup>4)</sup> However, 9-*epi*-blumenol B (**3b**) prepared in this study also showed small optical rotation,  $[\alpha]_D^{27} + 16.6^\circ (c=0.05, \text{CH}_3\text{OH})$ , suggesting that it was difficult to distinguish these epimers by optical rotation values alone at that time. It is noteworthy that there is a slight but clear difference between blumenol B (**2b**) and 9-*epi*-blumenol B (**3b**) for H<sub>2</sub>-8 in the <sup>1</sup>H-NMR spectra, i.e., chemical shifts and coupling patterns of H<sub>2</sub>-8 geminal protons were inverted [ $\delta_{\text{H}} 1.43$  (1H, dddd, *J*=13, 12, 5, 5 Hz, H-8a for **2b**) and  $\delta_{\text{H}} 1.65$  (1H, dddd, *J*=13, 12, 7, 4 Hz, H-8b for **2b**),  $\delta_{\text{H}} 1.40$  (1H, dddd, *J*=13, 12, 8, 5 Hz, H-8a for **3b**) and  $\delta_{\text{H}} 1.68$  (1H, dddd, *J*=13, 12, 5, 4 Hz, H-8b for **3b**)] (Table 2). The detailed comparison of chemical shifts and coupling patterns of H<sub>2</sub>-8 provides important criteria to distinguish them from each other.

Recently, we have reported the empirical rule for the determination of the absolute stereochemistry of C-9 of the related megastigmane glucosides, simply by comparing the <sup>13</sup>C chemical shift values (in methanol-*d*<sub>4</sub>) of C-9 and C-10 and the anomeric carbon of the attached glucose, i.e., <sup>13</sup>C signals at ca.  $\delta_{\text{C}} 76$  (C-9), 20 (C-10), and 102 (C-1') indicate the 9*R* configuration, and ca.  $\delta_{\text{C}} 78$  (C-9), 22 (C-10), and 104 (C-1') indicate 9*S* for related compounds. The <sup>13</sup>C-NMR spectral data measured in methanol-*d*<sub>4</sub> for **2a** (=PS-1) and **3a** (=EG-1) also coincided with the above rule. This result further supports the usefulness of our empirical rule.<sup>10)</sup>

We reported the isolation of "icariside B<sub>5</sub>" from the leaves of *Macaranga tanarius* as a known compound in a previous paper, because PS-1 and EG-1 were considered to be the same compound at that time.<sup>11)</sup> We should correct that here by stating that we isolated dihydrovomifoliol-*O*- $\beta$ -D-glucopy-

ranoside (PS-1), not icariside B<sub>5</sub> (EG-1), from *M. tanarius*.

## Experimental

**General Experimental Procedures** HPLC was performed on octadecylsilylanized (ODS) silica gel (Inertsil ODS-3; GL Science, Tokyo, Japan;  $\Phi=6$  mm, *L*=250 mm), and the eluate was monitored with UV and refractive index detectors. Optical rotations were measured on a JASCO P-1030 polarimeter. IR spectra were recorded on a Horiba FT-710 Fourier transform infrared spectrophotometer and UV spectra on a JASCO V-520 UV/Vis spectrophotometer. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were obtained on JEOL ECA-600 spectrometers at 600 MHz for <sup>1</sup>H and 150 MHz for <sup>13</sup>C, with tetramethylsilane as an internal standard. Positive-ion high-resolution (HR)-electrospray ionization (ESI)-time-of-flight (TOF)-MS was recorded on an Applied Biosystem QSTAR XL spectrometer. CD spectra were obtained on a JASCO J-2600 spectropolarimeter.

**Mild Alkaline Hydrolysis of Macarangioside A (2)** A mixture of **2** (3.0 mg) and 0.1 M NaOMe in MeOH (1.0 ml) was allowed to stand at room temperature for 1 h under a N<sub>2</sub> atmosphere. Liberation of degalloylated compound **2a** was trailed by TLC analysis (CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O, 15:6:1, *R*<sub>f</sub> values, **2**: 0.32 and **2a**: 0.57). The reaction mixture was neutralized with Amberlite IR-120B (Organo) and **2a** (1.2 mg) was purified by preparative TLC [silica gel (0.25 mm thickness, applied for 9 cm and developed with CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O, 15:6:1 for 9 cm)]. The spectral data including chiroptical spectra were identical to those of PS-1. **2a** (=PS-1): Amorphous powder;  $[\alpha]_D^{24} - 2.2^\circ (c=0.12, \text{MeOH})$ ; IR  $\nu_{\text{max}}$  (film) cm<sup>-1</sup>: 3392, 2967, 2925, 1650, 1373, 1076, 1034; UV  $\lambda_{\text{max}}$  (MeOH) nm (log  $\epsilon$ ): 238 (3.77); <sup>13</sup>C-NMR (CD<sub>3</sub>OD)  $\delta_{\text{C}}$ : 201.1 (C-3), 171.7 (C-5), 126.8 (C-4), 102.4 (C-1'), 79.4 (C-6), 78.3 (C-3'), 78.0 (C-5'), 76.3 (C-9), 75.2 (C-2'), 71.9 (C-4'), 63.0 (C-6'), 51.2 (C-2), 42.9 (C-1), 34.9 (C-7), 33.6 (C-8), 24.7 (C-11), 24.2 (C-12), 21.7 (C-13), 20.1 (C-10); CD  $\Delta\epsilon$  (nm): +1.16 (328), -5.57 (253), +9.76 (221) (*c*=3.09×10<sup>-5</sup> M, MeOH); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 411.1980 [M+Na]<sup>+</sup> (Calcd for C<sub>19</sub>H<sub>32</sub>O<sub>8</sub>Na: 411.1989).

**Enzymatic Hydrolysis of 2a** Compound **2a** (1.2 mg) were dissolved in 1 ml of 100 mM acetate buffer (pH 5.0) and then hydrolyzed with  $\beta$ -D-glucosidase (Oriental Yeast Co., Ltd., Japan, 10 mg) at 37 °C for 12 h by reciprocal shaking. The reaction mixture was extracted twice with an equal amount of EtOAc. The aglycone (**2b**) (0.6 mg) was purified by preparative TLC from the EtOAc layer (CHCl<sub>3</sub>:MeOH, 10:1, *R*<sub>f</sub> 0.61). (6*S*,9*R*)-6,9-Dihydroxymegastigman-4-en-3-one (blumenol B) (**2b**): amorphous powder;  $[\alpha]_D^{27} + 6.2^\circ (c=0.06, \text{CH}_3\text{OH})$ ; IR  $\nu_{\text{max}}$  (film) cm<sup>-1</sup>: 3401, 2965, 2927, 1650, 1373, 1127; UV  $\lambda_{\text{max}}$  (MeOH) nm (log  $\epsilon$ ): 240 (3.99); <sup>13</sup>C- and <sup>1</sup>H-NMR (CD<sub>3</sub>OD): Tables 1 and 2; CD  $\Delta\epsilon$  (nm): +2.94 (326), -12.0 (251), +19.0 (219) (*c*=2.65×10<sup>-5</sup> M, MeOH); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 249.1466 [M+Na]<sup>+</sup> (Calcd for C<sub>13</sub>H<sub>22</sub>O<sub>3</sub>Na: 249.1461).

**Determination of Sugar Configuration** The previously described method<sup>12)</sup> was used with slight modifications. Glucosides (**2** and **3**, 0.2 and 0.3 mg, respectively) were hydrolyzed with 0.2 ml of 1 M HCl at 90 °C for 2 h and the reaction mixtures were passed through the packed MB-3 ion-exchange resin (0.5×5 cm) after washing with EtOAc (0.2 ml). After drying *in vacuo*, the residues were dissolved in anhydrous pyridine (0.1 ml) and reacted with L-cysteine methyl ester (0.5 mg) at 60 °C for 1 h. Then *o*-tolylisothiocyanate (1.4 mg in 70  $\mu$ l pyridine) was added to the mixtures and further incubated at 60 °C for 1 h. The reaction mixtures were directly analyzed using ODS HPLC [Cosmosil 5C<sub>18</sub>-ARII (Nacalai Tesque, Kyoto, Japan), 4.6×250 mm, 25 °C, 25% CH<sub>3</sub>CN-50 mM H<sub>3</sub>PO<sub>4</sub>, 0.8 ml/min, UV detection at 250 nm]. The peaks (18.1 min) were identical to the derivative of authentic D-glucose.

**Catalytic Hydrogenation of 3** Compound **3** (8.7 mg) was dissolved in MeOH (1.0 ml), then 2 mg of Adams' catalyst (PtO<sub>2</sub>) was added and stirred at 0 °C for 4 h under a H<sub>2</sub> atmosphere. Hydrogenation of **3** was monitored by ESI-MS analysis because of the similar *R*<sub>f</sub> values for reactant and product on TLC analysis. The reaction mixture was purified using HPLC to afford **3a** (1.3 mg) [Inertsil ODS-3 (GL Science), 6×250 mm, 25 °C, 15% CH<sub>3</sub>CN aq., 1.6 ml/min, 27.9 min]. (6*S*,9*S*)-6,9-Dihydroxymegastigman-4-en-3-one 9-*O*- $\beta$ -D-glucopyranoside, (=icariside B<sub>5</sub>) (**3a**) (=EG-1): amorphous powder;  $[\alpha]_D^{24} - 10.5^\circ (c=0.13, \text{MeOH})$ ; IR  $\nu_{\text{max}}$  (film) cm<sup>-1</sup>: 3388, 2964, 2928, 1650, 1373, 1076, 1028; UV  $\lambda_{\text{max}}$  (MeOH) nm (log  $\epsilon$ ): 244 (3.97); <sup>13</sup>C- and <sup>1</sup>H-NMR (C<sub>2</sub>D<sub>5</sub>N and CD<sub>3</sub>OD): Tables 1 and 2; CD  $\Delta\epsilon$  (nm): +1.92 (325), -7.01 (251), +11.6 (219) (*c*=2.82×10<sup>-5</sup> M, MeOH); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 411.1988 [M+Na]<sup>+</sup> (Calcd for C<sub>19</sub>H<sub>32</sub>O<sub>8</sub>Na: 411.1989).

**Enzymatic Hydrolysis of 3a (=EG-1)** The aglycone (**3b**) (0.5 mg) was prepared using a similar procedure from **3a** (=EG-1) (1.3 mg) as described

above. (TLC; CHCl<sub>3</sub>:MeOH, 10:1, *R<sub>f</sub>* 0.59). (6*S*,9*S*)-6,9-Dihydroxymegastigman-4-en-3-one (9-*epi*-blumenol B) (**3b**): amorphous powder;  $[\alpha]_D^{27} +16.6^\circ$  ( $c=0.05$ , CH<sub>3</sub>OH); IR  $\nu_{\max}$  (film) cm<sup>-1</sup>: 3401, 2965, 2926, 1650, 1373, 1129; UV  $\lambda_{\max}$  (MeOH) nm (log  $\epsilon$ ): 240 (4.02); <sup>13</sup>C- and <sup>1</sup>H-NMR (CD<sub>3</sub>OD): Tables 1 and 2; CD  $\Delta\epsilon$  (nm): +2.91 (326), -14.4 (251), +20.0 (219) ( $c=2.21 \times 10^{-5}$  M, MeOH); HR-ESI-TOF-MS (positive-ion mode)  $m/z$ : 249.1464 [M+Na]<sup>+</sup> (Calcd for C<sub>13</sub>H<sub>22</sub>O<sub>3</sub>Na: 249.1461).

**Preparation of (R)- and (S)-MTPA Esters from 2b and 3b** A solution of **2b** (0.3 mg) in 1 ml of dehydrated CH<sub>2</sub>Cl<sub>2</sub> was reacted with (*R*)-MTPA (19.1 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (19.2 mg) and 4-*N,N'*-dimethylaminopyridine (DMAP) (10.2 mg), and stored at 35 °C for 12 h. After the addition of 1 ml each of H<sub>2</sub>O and CHCl<sub>3</sub>, the solution was washed successively with 1 M HCl (1 ml), NaHCO<sub>3</sub>-saturated H<sub>2</sub>O (1 ml), and saturated brine (1 ml). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and then evaporated under reduced pressure. The residue was purified using preparative TLC [silica gel (0.25 mm thickness, applied for 9 cm and developed with CHCl<sub>3</sub>-(CH<sub>3</sub>)<sub>2</sub>CO (20:1) for 9 cm, *R<sub>f</sub>* 0.50, and eluted with CHCl<sub>3</sub>-MeOH (2:1)] to furnish the (*R*)-MTPA ester **2c** (0.3 mg, 51%). Through a similar procedure, the (*S*)-MTPA ester (**2d**) (*R<sub>f</sub>* 0.49, 0.3 mg, 51%) was prepared from **2b** (0.3 mg) using (*S*)-MTPA (17.2 mg), EDC (16.3 mg), and DMAP (8.1 mg). The (*R*)-MTPA ester **3c** (*R<sub>f</sub>* 0.44, 0.3 mg, 61%) was also prepared from **3b** (0.25 mg) using (*R*)-MTPA (17.4 mg), EDC (16.5 mg), and DMAP (8.9 mg). The (*S*)-MTPA ester **3d** (*R<sub>f</sub>* 0.45, 0.2 mg, 41%) was also prepared from **3b** (0.25 mg) using (*S*)-MTPA (16.3 mg), EDC (21.2 mg), and DMAP (11.0 mg). (6*S*,9*R*)-6,9-Dihydroxymegastigman-4-en-3-one 9-(*R*)-MTPA ester (**2c**): amorphous powder; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$ : 7.54—7.50 (2H, m, aromatic protons), 7.43—7.38 (3H, m, aromatic protons), 5.86 (1H, s, H-4), 5.08 (1H, m, H-9), 3.51 (3H, s, OMe), 2.43 (1H, d, *J*=18 Hz, H-2a), 2.24 (1H, d, *J*=18 Hz, H-2b), 1.98 (3H, s, H<sub>3</sub>-13), 1.92 (1H, m, H-8a), 1.81 (1H, m, H-7a), 1.73 (1H, m, H-8b), 1.61 (1H, m, H-7b), 1.30 (3H, d, *J*=6 Hz, H<sub>3</sub>-10), 1.05 (3H, s, H<sub>3</sub>-12), 1.03 (3H, s, H<sub>3</sub>-11); HR-ESI-TOF-MS (positive-ion mode)  $m/z$ : 465.1849 [M+Na]<sup>+</sup> (Calcd for C<sub>23</sub>H<sub>29</sub>O<sub>3</sub>F<sub>3</sub>Na: 465.1859). (6*S*,9*R*)-6,9-Dihydroxymegastigman-4-en-3-one 9-(*S*)-MTPA ester (**2d**): amorphous powder; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$ : 7.54—7.50 (2H, m, aromatic protons), 7.41—7.37 (3H, m, aromatic protons), 5.83 (1H, s, H-4), 5.07 (1H, m, H-9), 3.57 (3H, s, OMe), 2.36 (1H, d, *J*=18 Hz, H-2a), 2.19 (1H, d, *J*=18 Hz, H-2b), 1.93 (3H, s, H<sub>3</sub>-13), 1.84 (1H, m, H-8a), 1.64 (1H, m, H-8b), 1.58 (1H, m, H-7a), 1.52 (1H, m, H-7b), 1.36 (3H, d, *J*=6 Hz, H<sub>3</sub>-10), 1.01 (3H, s, H<sub>3</sub>-12), 0.95 (3H, s, H<sub>3</sub>-11); HR-ESI-TOF-MS (positive-ion mode)  $m/z$ : 465.1862 [M+Na]<sup>+</sup> (Calcd for C<sub>23</sub>H<sub>29</sub>O<sub>3</sub>F<sub>3</sub>Na: 465.1859). (6*S*,9*S*)-6,9-Dihydroxymegastigman-4-en-3-one 9-(*R*)-MTPA ester (**3c**): amorphous powder; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$ : 7.54—7.50 (2H, m, aromatic protons), 7.42—7.37 (3H, m, aromatic protons), 5.80 (1H, s, H-4), 5.14 (1H, m, H-9), 3.56 (3H, s, OMe), 2.27 (1H, d, *J*=18 Hz, H-2a), 2.16 (1H, d, *J*=18 Hz, H-2b), 1.94 (3H, s, H<sub>3</sub>-13), 1.82 (1H, m, H-8a), 1.60 (1H,

m, H-7a), 1.56 (1H, m, H-7b), 1.55 (1H, m, H-8b), 1.36 (3H, d, *J*=6 Hz, H<sub>3</sub>-10), 1.01 (3H, s, H<sub>3</sub>-12), 0.96 (3H, s, H<sub>3</sub>-11); HR-ESI-TOF-MS (positive-ion mode)  $m/z$ : 465.1869 [M+Na]<sup>+</sup> (Calcd for C<sub>23</sub>H<sub>29</sub>O<sub>3</sub>F<sub>3</sub>Na: 465.1859). (6*S*,9*S*)-6,9-Dihydroxymegastigman-4-en-3-one 9-(*S*)-MTPA ester (**3d**): amorphous powder; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$ : 7.53—7.50 (2H, m, aromatic protons), 7.42—7.38 (3H, m, aromatic protons), 5.86 (1H, s, H-4), 5.15 (1H, m, H-9), 3.50 (3H, s, OMe), 2.39 (1H, d, *J*=18 Hz, H-2a), 2.22 (1H, d, *J*=18 Hz, H-2b), 1.99 (3H, s, H<sub>3</sub>-13), 1.86 (1H, m, H-8a), 1.78 (2H, m, H<sub>2</sub>-7), 1.64 (1H, m, H-8b), 1.30 (3H, d, *J*=6 Hz, H<sub>3</sub>-10), 1.044 (3H, s, H<sub>3</sub>-12), 1.037 (3H, s, H<sub>3</sub>-11); HR-ESI-TOF-MS (positive-ion mode)  $m/z$ : 465.1866 [M+Na]<sup>+</sup> (Calcd for C<sub>23</sub>H<sub>29</sub>O<sub>3</sub>F<sub>3</sub>Na: 465.1859).

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