Reinvestigation of the Absolute Stereochemistry of Megastigmane Glucoside, Icariside B_5

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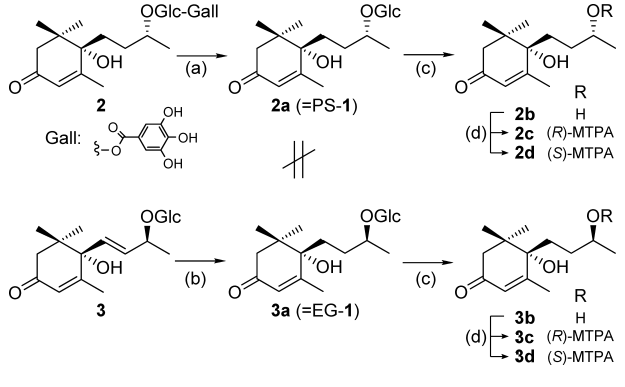
Icariside B_5 is one of the widely distributed megastigmane glucosides among plant sources. The absolute structure of icariside B_5 was reinvestigated by chemical conversion from the related compound and the application of the modified Mosher's method. As a result, the structure of icariside B_5 was revised to be (6*S*,9*S*)-6,9-dihydroxymegastigman-4-en-3-one 9-*O*-*β*-D-glucopyranoside.

Key words icariside B_5; dihydrovomifoliol-*O*-*β*-D-glucopyranoside; blumenol B; megastigmane glucoside

Blumenol B was isolated from the leaves of *Podocarpus blumei* in 1972.[1] The absolute stereochemistry of blumenol B was then determined as (6*S*,9*R*) by chemical conversion of related compounds.[2] 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_5 (EG-**1**) from *Epimedium grandiflorum* var. *thunbergianum*, respectively.[3,4] (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.[3,4] 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_4 and pyridine-d_5, respectively.[3,4] The modified Mosher's method is widely used for the determination of the absolute stereochemistry of chiral secondary alcohols.[5] 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**),[6] was hydrolyzed with mild alkaline hydrolysis (Chart 1) to afford degalloyl-**2** (**2a**), of which the NMR spectra (in methanol-d_4) and specific optical rotation value {$[\alpha]_D^{24}$ −2.2° (c=0.12, MeOH)} were essentially identical to those reported for PS-**1** {*Ref.*: $[\alpha]_D^{20}$ −4.4° (c=0.80, MeOH)}.[3] Thus **2a** was elucidated to be identical to PS-**1**. Second, the ^{1}H- and ^{13}C-NMR spectra of **2a** were remeasured in pyridine-d_5 according to the literature for EG-**1**.[4] However, the chemical shift values in pyridine-d_5 of **2a** (Tables 1, 2) did not coincide with those reported for EG-**1**.[4] These results clearly indicated that PS-**1** and EG-**1** were not the same compounds. The detailed inspection of ^{13}C chemical shift values measured in pyridine-d_5 between **2a** (=PS-**1**) and the reported values for EG-**1**[4] suggested that EG-**1** must be the C-9 epimer of PS-**1**. According to the literature,[3,4] 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**.



a) 0.1 M NaOMe in MeOH, rt, 1 h, b) PtO_2/H_2, 0 °C, 4 h, c) *β*-D-glucosidase, 37 °C, 12 h, d) EDC, DMAP, (*R*) and (*S*)-MTPAs in CH_2Cl_2, 35 °C, 12 h.

Chart 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 $\Delta\delta_{S-R}$ 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).[7] 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.[2] 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 EG-**1** was prepared from the closely related compound corchoionoside C (**3**), originally isolated by Yoshikawa *et al.*,[8] which was also isolated from *Euodia meliaefolia* in our previous study.[9] Partial hydrogenation of **3** afforded **3a** (Chart 1). The physicochemical data, *e.g.*, NMR

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Table 1. ^{13}C-NMR Spectral Data for Dihydrovomifoliol-*O*-*β*-D-glucopyranoside (**2a**) and Icariside B_5 (**3a**)

C	**2a** (=PS-**1**) (C_5D_5N)	**2a** (=PS-**1**) (CD_3OD)	**2b** (CD_3OD)	**3a** (=EG-**1**) (C_5D_5N)[a]	**3a** (=EG-**1**) (CD_3OD)	**3b** (CD_3OD)
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)[b]	32.2	33.1	35.4 (−2.3)[c]
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_2O. *b*) δ_{2a-2b}. *c*) δ_{3a-3b}.

Table 2. ^{1}H-NMR Spectral Data for Dihydrovomifoliol-*O*-*β*-D-glucopyranoside (**2a**) and Icariside B_5 (**3a**) (600 MHz)

C	**2a** (C_5D_5N) (=PS-**1**)	**2a** (CD_3OD) (=PS-**1**)	**2b** (CD_3OD)	**3a** (C_5D_5N)[a] (=EG-**1**)	**3a** (CD_3OD) (=EG-**1**)	**3b** (CD_3OD)
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}H–^{1}H COSY and HMQC. *a*) Added one drop of D_2O.

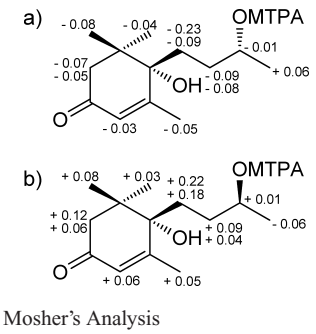

Fig. 1. Modified Mosher's Analysis

a) For **2b** and b) for **3b**. $\Delta\delta$ values (δ_S–δ_R) were shown in ppm.

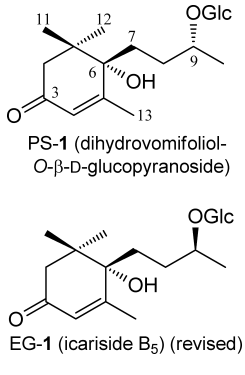

Fig. 2. Dihydrovomifoliol-*O*-*β*-D-glucopyranoside (PS-**1**) and Revised Structure of Icariside B_5 (EG-**1**)

spectra (in pyridine-d_5) (Tables 1, 2) and specific optical rotation value {$[\alpha]_D^{24}$ −10.5° (c=0.13, MeOH)} of **3a** were essentially identical to those reported for EG-**1** {*Ref.*: $[\alpha]_D^{25}$ −12.9° (c=0.62, MeOH)},[4] 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.*, $NaBH_4$ reduction of the 9-ketonic functional group following the PCC oxidation of 9-OH of roseoside.[8] 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 β-D-glucosylation-induced shift-trend rule[7] 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_5 (EG-**1**) (=**3a**) was revised to be (6*S*,9*S*)-6,9-dihydroxymegastigman-4-en-3-one 9-*O*-β-D-glucopyranoside, *e.g.*, 9-*O*-β-D-glucoside of 9-*epi*-blumenol B (Fig. 2). Finally, the NMR spectral data (CD_3OD) of icariside B_5 (EG-**1**) isolated from *E. thunbergianum* var. *grandiflorum*[4] 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° (c=0.06, CH_3OH). Thus icariside B_5 (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° (c=0.23, CH_3OH)].[4] However, 9-*epi*-blumenol B (**3b**) prepared in this study also showed small optical rotation, $[\alpha]_D^{27}$ +16.6° (c=0.05, CH_3OH), 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_2-8 in the ^{1}H-NMR spectra, *i.e.*, chemical shifts and coupling patterns of H_2-8 geminal protons were inverted [δ_H 1.43 (1H, dddd, J=13, 12, 5, 5 Hz, H-8a for **2b**) and δ_H 1.65 (1H, dddd, J=13, 12, 7, 4 Hz, H-8b for **2b**), δ_H 1.40 (1H, dddd, J=13, 12, 8, 5 Hz, H-8a for **3b**) and δ_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_2-8 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 ^{13}C chemical shift values (in methanol-d_4) of C-9 and C-10 and the anomeric carbon of the attached glucose, *i.e.*, ^{13}C signals at *ca.* δ_C 76 (C-9), 20 (C-10), and 102 (C-1′) indicate the 9*R* configuration, and *ca.* δ_C 78 (C-9), 22 (C-10), and 104 (C-1′) indicate 9*S* for related compounds. The ^{13}C-NMR spectral data measured in methanol-d_4 for **2a** (=PS-**1**) and **3a** (=EG-**1**) also coincided with the above rule. This result further supports the usefulness of our empirical rule.[10]

We reported the isolation of "icariside B_5" 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.[11] We should correct that here by stating that we isolated dihydrovomifoliol-*O*-β-D-glucopyranoside (PS-**1**), not icariside B_5 (EG-**1**), from *M. tanarius*.

Experimental

General Experimental Procedures HPLC was performed on octadesylsilanized (ODS) silica gel (Inertsil ODS-3; GL Science, Tokyo, Japan; Φ=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. ^{1}H- and ^{13}C-NMR spectra were obtained on JEOL ECA-600 spectrometers at 600 MHz for ^{1}H and 150 MHz for ^{13}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-720 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_2 atmosphere. Liberation of degalloylated compound **2a** was trailed by TLC analysis ($CHCl_3$: MeOH : H_2O, 15 : 6 : 1, *Rf* 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 $CHCl_3$: MeOH : H_2O, 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° (c=0.12, MeOH); IR ν_{max} (film) cm^{-1}: 3392, 2967, 2925, 1650, 1373, 1076, 1034; UV λ_{max} (MeOH) nm (log ε): 238 (3.77); ^{13}C-NMR (CD_3OD) δ_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 $\Delta\varepsilon$ (nm): +1.16 (328), −5.57 (253), +9.76 (221) (c=3.09×10^{-5} M, MeOH); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 411.1980 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_8Na$: 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 β-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 ($CHCl_3$: MeOH, 10 : 1, *Rf* 0.61). (6*S*,9*R*)-6,9-Dihydroxymegastigman-4-en-3-one (blumenol B) (**2b**): amorphous powder; $[\alpha]_D^{27}$ +6.2° (c=0.06, CH_3OH); IR ν_{max} (film) cm^{-1}: 3401, 2965, 2927, 1650, 1373, 1127; UV λ_{max} (MeOH) nm (log ε): 240 (3.99); ^{13}C- and ^{1}H-NMR (CD_3OD): Tables 1 and 2; CD $\Delta\varepsilon$ (nm): +2.94 (326), −12.0 (251), +19.0 (219) (c=2.65×10^{-5} M, MeOH); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 249.1466 $[M+Na]^+$ (Calcd for $C_{13}H_{22}O_3Na$: 249.1461).

Determination of Sugar Configuration The previously described method[12] 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*-tolylisothiothiocyanate (1.4 mg in 70 μl 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_{18}-ARII (Nacalai Tesque, Kyoto, Japan), 4.6×250 mm, 25 °C, 25% CH_3CN–50 mM H_3PO_4, 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_2) was added and stirred at 0 °C for 4 h under a H_2 atmosphere. Hydrogenation of **3** was monitored by ESI-MS analysis because of the similar *Rf* 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_3CN aq., 1.6 ml/min, 27.9 min]. (6*S*,9*S*)-6,9-Dihydroxymegastigman-4-en-3-one 9-*O*-β-D-glucopyranoside, (=icariside B_5) (**3a**) (=EG-**1**): amorphous powder; $[\alpha]_D^{24}$ −10.5° (c=0.13, MeOH); IR ν_{max} (film) cm^{-1}: 3388, 2964, 2928, 1650, 1373, 1076, 1028; UV λ_{max} (MeOH) nm (log ε): 244 (3.97); ^{13}C- and ^{1}H-NMR (C_5D_5N and CD_3OD): Tables 1 and 2; CD $\Delta\varepsilon$ (nm): +1.92 (325), −7.01 (251), +11.6 (219) (c=2.82×10^{-5} M, MeOH); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 411.1988 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_8Na$: 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; $CHCl_3$: MeOH, 10 : 1, *Rf* 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° (*c*=0.05, CH_3OH); IR ν_{max} (film) cm^{-1}: 3401, 2965, 2926, 1650, 1373, 1129; UV λ_{max} (MeOH) nm (log ε): 240 (4.02); ^{13}C- and ^1H-NMR (CD_3OD): Tables 1 and 2; CD $\Delta\varepsilon$ (nm): +2.91 (326), −14.4 (251), +20.0 (219) (*c*=2.21×10^{-5} M, MeOH); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 249.1464 $[M+Na]^+$ (Calcd for $C_{13}H_{22}O_3Na$: 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_2Cl_2 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_2O and $CHCl_3$, the solution was washed successively with 1 M HCl (1 ml), $NaHCO_3$–saturated H_2O (1 ml), and saturated brine (1 ml). The organic layer was dried over Na_2SO_4 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 $CHCl_3$–$(CH_3)_2CO$ (20 : 1) for 9 cm, *Rf* 0.50, and eluted with $CHCl_3$–MeOH (2 : 1)] to furnish the (*R*)-MTPA ester **2c** (0.3 mg, 51%). Through a similar procedure, the (*S*)-MTPA ester (**2d**) (*Rf* 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** (*Rf* 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** (*Rf* 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; ^1H-NMR ($CDCl_3$, 600 MHz) δ: 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_3-13), 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_3-10), 1.05 (3H, s, H_3-12), 1.03 (3H, s, H_3-11); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 465.1849 $[M+Na]^+$ (Calcd for $C_{23}H_{29}O_5F_3Na$: 465.1859). (6*S*,9*R*)-6,9-Dihydroxymegastigman-4-en-3-one 9-(*S*)-MTPA ester (**2d**): amorphous powder; ^1H-NMR ($CDCl_3$, 600 MHz) δ: 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_3-13), 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_3-10), 1.01 (3H, s, H_3-12), 0.95 (3H, s, H_3-11); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 465.1862 $[M+Na]^+$ (Calcd for $C_{23}H_{29}O_5F_3Na$: 465.1859). (6*S*,9*S*)-6,9-Dihydroxymegastigman-4-en-3-one 9-(*R*)-MTPA ester (**3c**): amorphous powder; ^1H-NMR ($CDCl_3$, 600 MHz) δ: 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_3-13), 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_3-10), 1.01 (3H, s, H_3-12), 0.96 (3H, s, H_3-11); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 465.1869 $[M+Na]^+$ (Calcd for $C_{23}H_{29}O_5F_3Na$: 465.1859). (6*S*,9*S*)-6,9-Dihydroxymegastigman-4-en-3-one 9-(*S*)-MTPA ester (**3d**): amorphous powder; ^1H-NMR ($CDCl_3$, 600 MHz) δ: 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_3-13), 1.86 (1H, m, H-8a), 1.78 (2H, m, H_2-7), 1.64 (1H, m, H-8b), 1.30 (3H, d, *J*=6 Hz, H_3-10), 1.044 (3H, s, H_3-12), 1.037 (3H, s, H_3-11); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 465.1866 $[M+Na]^+$ (Calcd for $C_{23}H_{29}O_5F_3Na$: 465.1859).

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