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Stereochemistry of megastigmane glucosides from *Glochidion zeylanicum* and *Alangium premnifolium*

Hideaki Otsuka[a,*], Eiji Hirata[b], Takakazu Shinzato[c], Yoshio Takeda[d]

[a]*Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima 734-8551, Japan*
[b]*Faculty of Agriculture, University of the Ryukyus, Okinawa 903-0129, Japan*
[c]*Subtropical Field Science Center, University of the Ryukyus, Okinawa 905-1427, Japan*
[d]*Faculty of Integrated Arts and Sciences, The University of Tokushima, Tokushima 770-8502, Japan*

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Abstract

From *Glochidion zeylanicum*, two megastigmane glucosides, 3- and 9-*O*-β-D-glucopyranosides of (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol (**1** and **2**, respectively), were isolated. Their structures were different from those of kiwiionoside (**3**) and actinidioionoside (**4**), isolated from *Actinidia chinensis* and *Actinidia polygama*, respectively, in the stereochemistry at the 9-positions. Alangionosides E (**5**) and O (**6**), isolated from the leaves of *Alangium premnifolium*, are also megastigmane glucosides, and the latter is closely related to **1** and actinidioionoside (**4**). However, the absolute configurations of the 9-position remained to be determined. They were analyzed to be *R* by means of a modified Mosher's method. Alangionoside E (**5**) is identical with corchoionoside A in all aspects. The name of corchoionoside A must be retained thereafter.

Keywords: *Glochidion zeylanicum*; Euphorbiaceae; Megastigmane glucoside; Alangionosides E and O; Stereochemistry

1. Introduction

In a previous paper (Otsuka et al., 2000), the isolation of butenolide glucosides, glochidionolactones A–F, from the leaves of *Glochidion zeylanicum* (Gaertn) A. Juss (Euphorbiaceae) were reported. Further investigation of the same plant afforded two new megastigmane glucosides (**1**,**2**). Their structures were elucidated on the basis of spectroscopic evidence and the spectroscopic analyses of the (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters (**1b** = **2b**, **1c** = **2c**) of their common aglycones (**1a** = **2a**). It was found that they are the diastereomers of kiwiionoside (**3**) (Murai et al., 1992) and actinidioionoside (**4**) (Murai and Tagawa, 1989) respectively, with alternate configuration at the 9-positions. The absolute stereostructure of similar compounds, alangionosides E (**5**) (Otsuka et al., 1995) and O (**6**) (6′-*O*-β-D-xylopyranoside of **3**) (Kijima et al., 1996), isolated from the leaves of *Alangium premnifolium* Ohwi (Alangiaceae) have remained to be determined. The absolute configurations of the 9-position of **5** and **6** were also analyzed by the same method. The NMR spectroscopic features of the epimeric pairs of megastigmane glucosides are discussed.

2. Results and discussion

From the leaves of *G. zeylanicum*, megastigmane glucosides **1** and **2**, were isolated. Their structures were elucidated by means of spectroscopic analyses, including a modified Mosher's method (Ohtani et al., 1991).

Compound **1**, $[\alpha]_D$ −38.0°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{34}O_9$ by negative-ion high-resolution (HR) FAB mass spectrometry. The ^1H and ^{13}C NMR (Table 1) spectra showed the presence of six signals assignable to β-glucopyranoses, the remaining 13 signals comprising three singlet and one doublet methyls, two methylenes, two methines with hydroxyl substituents, and three quaternary carbons, two of which bear a hydroxyl substitutent and one disubstituted *trans* double bond, which must form a megastigmane skeleton (Naves, 1964). The functionalities and their relative

* Corresponding author. Tel.: +81-82-257-5292; fax: +81-82-257-5294.
E-mail address: hotsuka@hiroshima-u.ac.jp (H. Otsuka).

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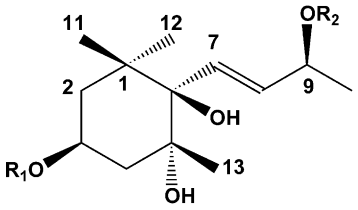
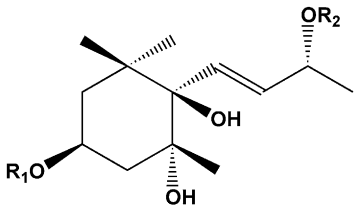
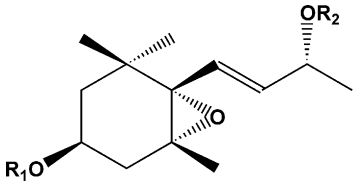


	R_1	R_2
1	Glc	H
1a (=2a)	H	H
1b (=2b)	(*R*)-MTPA	(*R*)-MTPA
1c (=2c)	(*S*)-MTPA	(*S*)-MTPA
2	H	Glc



	R_1	R_2
3	Glc	H
3a (=4a, 6a)	H	H
4	H	Glc
6	Glc(6)Xyl	H
6b	(*R*)-MTPA	(*R*)-MTPA
6c	(*S*)-MPTA	(*S*)-MTPA



	R_1	R_2
5	Glc	H
5a	H	H
5b	(*R*)-MTPA	(*R*)-MTPA
5c	(*S*)-MPTA	(*S*)-MTPA

arrangements on the six-membered ring, available from the results of one- and two-dimensional NMR spectroscopy, were the same as those of kiwiinoside (**3**) (Murai et al., 1992) and actinidioionoside (**4**) (Murai and Tagawa, 1989), which were isolated as pentaacetates from *Actinidia* spp. The absolute structure of kiwiinoside pentaacetate was determined to be 3*S*, 5*R*, 6*R*, and 9*R* by means of an X-ray crystallographic method in which β-glucopyranose was used as a probe, supposing that it is in D-series. The absolute configuration of the 3-position of **1** could be determined to be *S* by application of β-D-glucopyranosylation-induced shift-trends (see Table 1, **1** and **1a** or **6a**; Kasai et al., 1977), and other chiral centers on the ring were simultaneously determined to be 5*R* and 6*R*. However, the chirality of the 9-position must be determined independently. Therefore, **1** was enzymatically hydrolyzed, and then the aglycone **1a**, liberated was esterified with (*R*)- and (*S*)-MTPAs. Analysis of **1b** and **1c** by the ^{1}H NMR showed that the 9-position had the *S* configuration, opposite to that of kiwiionol (**3a**; Fig. 1), the aglycone of kiwiionoside, and the same as that of a non-glycosylated megastigmane, isolated from the leaves of *Euscaphis japonica* (Takeda et al., 2000). Therefore, the structure of **1** was elucidated to be (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol 3-*O*-β-D-glucopyranoside.

Table 1
^{13}C NMR data for (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol 3-*O*-β-D-glucopyranoside and 9-*O*-β-D-glucopyranoside (**1** and **2**, respectively), their common aglycone (**1a**), and alangionoside O (**6**) and its aglycone (**6a**) (100 MHz, CD_3OD)

C	1	2	6[a]	1a (=2a)	6a
1	40.9	40.8	40.8	40.8	40.7
2	44.6 (−2.0)[b]	46.6	44.5	46.6	46.5
3	73.4 (+8.1)	65.3	73.3	65.4	65.3
4	42.5 (−3.2)	45.7	42.7	45.8	45.7
5	77.8	78.5	77.8	77.8	77.8
6	79.2	79.3	79.1	79.0	79.0
7	131.0	135.9	131.1	131.2	131.3
8	136.2	133.1 (−3.1)[c]	136.2	136.2	136.2
9	69.2	75.5 (+5.9)	69.6	69.6	69.6
10	24.0	22.4 (−1.6)	24.3	24.1	24.2
11	27.6	28.1	27.6	27.6	27.5
12	26.3	26.5	26.4	26.2	26.1
13	27.2	27.2	27.3	27.1	27.1
1′	102.3	100.8	102.2		
2′	75.2	75.1	75.1		
3′	78.2	78.2	78.0		
4′	71.8	71.8	71.5		
5′	77.9	77.8	77.0		
6′	62.8	62.8	69.6		

[a] Data from Kijima et al. (1996), and those for the xylopyranose moiety were omitted.
[b] $\Delta\delta_{\mathbf{1}-\mathbf{1a}}$.
[c] $\Delta\delta_{\mathbf{2}-\mathbf{2a}}$.

Compound **2**, $[\alpha]_D$ −56.3°, was isolated as an amorphous powder, and spectroscopic data indicated that **2** had the same elemental composition as that of **1** and was the positional isomer regarding the glucopyranose moiety, like actinidioionoside (**4**), which has a sugar moiety on the hydroxyl group at the 9-position. Thus, the absolute configuration of the 9-position was expected to be *S* on the basis of β-D-glucopyranosylation-induced shift-trends (Kasai et al., 1977), and the absolute configurations of the ring carbons were assumed to be 3*S*, 5*R*, and 6*R* due to the co-occurrence of **1** in the same plant. This assumption was confirmed by means of the modified Mosher's method, as shown in Fig. 1 (see figures in parentheses). Retrogressive application of β-D-glucopyranosylation induced shift trends revealed that the glucose is in D-series. Thus, the structure of **2** was elucidated to be (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol 9-*O*-β-D-glucopyranoside. The absolute structure of the aglycone **4a** of actinidioionoside was expected to be the same as that of kiwiionol (**3a**), as both were isolated from the same genus. However, except for the 9-position, there is some uncertainty regarding the stereochemistry of the ring, since the stereochemistry of the aglycone was not fully identical with that of kiwiionol.

The absolute configurations of the 9-positions of alangionosides E (**5**) (Otsuka et al., 1995) and O (**6**) (Kijima et al., 1996), isolated from *A. premnifolium*, have remained

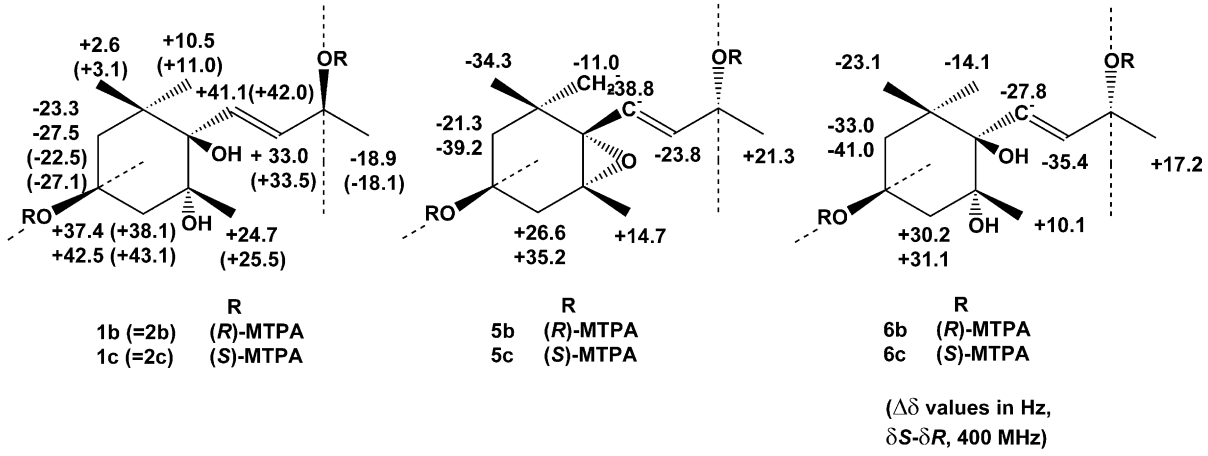


Fig. 1. Results with the modified Mosher's method for **1** and **2**, and alangionosides E and O (**5** and **6**). Figures are $\Delta\delta$ values in Hz (δS–δR).

to be determined. Thus, from an aglycone **5a**, previously obtained through the hydrolysis of **5**, (*R*)- and (*S*)-MTPA esters were prepared. Based on ^{1}H NMR spectral analysis, the absolute configuration of the 9-position was established to be *R*, which was the same as that of corchoionol A (see Fig. 1) (Yoshikawa et al., 1997, 1998). This evidence regretfully indicates that compound (**5**) has two different trivial names, as corchoionoside A isolated from *Corchorus olitorius,* whose stereochemistry had fully been determined, and as alangionoside E (**5**), whose stereochemical structure was elucidated in the present experiment. Therefore, although alangionoside E (**5**) first appeared in this journal, the name of corchoionoside A must be retained thereafter, since its structure was fully elucidated previously.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Union Giken PM-101 digital polarimeter. The FT–IR spectra were recorded on a Horiba FT-710 spectrophotometer. ^{1}H and ^{13}C NMR spectra were taken on a Jeol α-400 spectrometer (400 and 100 MHz, respectively) with TMS as the internal standard. HR-FAB–MS were carried out on a Jeol SX-102 mass spectrometer using PEG-400 as calibration matrix. Silica gel column chromatography (CC) and reversed-phase [octadecyl silica (ODS) gel] open CC (RPCC) were performed on silica gel 60 (Merck, 70–230 mesh) and Cosmosil 75C_{18}-OPN (Nacalai Tesque Co., Ltd., Kyoto) [$\Phi = 50$ mm, $L = 25$ cm, linear gradient: MeOH–H_2O (1:9, 1 l)→(1:1, 1 l)], fractions of 10 g being collected. The droplet countercurrent chromatograph (DCCC) (Tokyo Rikakikai, Tokyo) was equipped with 500 glass columns ($\Phi = 2$ mm, $L = 40$ cm), and the lower and upper layers of the solvent mixture of $CHCl_3$–MeOH–H_2O–*n*-PrOH (9:12:8:2) were used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to the order of elution of the mobile phase. (*R*)-(+)- and (*S*)-(−)-MTPAs were purchased from Nacalai Tesque Co., Ltd.

3.2. Plant materials

The leaves of *G. zeylanicum* were collected in Kunigami-son, Kunigami-gun, Okinawa, Japan, in August, 1990. The plant was identified by Anki Takushi of the Okinawa Prefectural Station of Forestry, whom the authors acknowledge, and a voucher specimen was deposited in the Herbarium of the Programs for Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (90-GZ-Okinawa-0822).

3.3. Extraction and isolation

The air-dried leaves of *G. zeylanicum* (4.72 kg) were extracted with MeOH three times. The MeOH extract was concentrated to 1.5 l and then 75 ml of H_2O was added to make a 95% aqueous solution. This solution was washed with 1.5 l of *n*-hexane and the methanolic layer was concentrated to a viscous gum. The gummy residue was suspended in 1.5 l of H_2O, and then extracted with 1.5 l each of EtOAc and *n*-BuOH, successively, to give 75.5 g and 108 g of EtOAc and *n*-BuOH soluble fractions, respectively. The *n*-BuOH soluble fraction (107 g) was subjected to highly porous synthetic resin cc (Diaion HP-20, Mitsubishi Chemical Co. Ltd., $\Phi = 80$ mm, $L = 55$ cm) using H_2O–MeOH (4:1, 6 l), (2:3, 6 l), (3:2, 6 l) and (1:4, 6 l), and MeOH (6 l), 2 l fractions were being collected. The residue (28.7 g in fractions 4–6) of the 20% MeOH eluent was subjected to silica gel (530 g) cc, with elution with $CHCl_3$ (1.5 l) and $CHCl_3$–MeOH [(99:1, 3 l), (49:1, 6 l), (24:1, 6 l), (47:3, 6 l), (23:2, 6 l), (9:1, 6 l), (7:1, 6 l), (17:3, 4.5 l), (4:1, 3 l) and (7:3, 3 l)], 500 ml fractions being collected. Combined fractions

65–78 (2.18 g) were separated by RPCC. The residue (534 mg) of fractions 36–60 was subjected to DCCC and the residue (77 mg) from fractions 12–18 was finally purified by preparative HPLC [ODS (Inertsil, $\Phi = 6$ mm, $L = 25$ cm, GL Science, Tokyo, Japan), MeOH–H_2O (1:4), 1.6 ml/min, detection: refractive index] to give 10 mg of **2** and 19 mg of **1** at 5.8 and 8.0 min, respectively.

3.3.1. (3S,5R,6R,7E,9S)-Megastigman-7-ene-3,5,6,9-tetrol 3-O-β-D-glucopyranoside

An amorphous powder; $[\alpha]_D^{24}$ −38.0° ($c = 1.00$, MeOH); IR ν_{max} (KBr) cm^{-1}: 3433, 2929, 2879, 1637, 1457, 1371, 1076; ^{1}H-NMR (CD_3OD) δ: 0.88 (3H, *s*, H_3-12), 1.12 (3H, *s*, H_3-11), 1.22 (3H, *s*, H_3-13), 1.27 (3H, *d*, $J = 6$ Hz, H_3-10), 1.59 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.73 (1H, *t*, $J = 12$ H, H-2ax), 1.77 (1H, *dd*, $J = 13$, 12 Hz, H-4ax), 1.95 (1H, *ddd*, $J = 13$, 4, 2 Hz, H-4eq), 3.15 (1H, *t*, $J = 8$ Hz, H-2′), 3.68 (1H, *dd*, $J = 2$, 5 Hz, H-6′a), 3.86 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 4.19 (1H, *tt*, $J = 12$, 4 Hz, H-4), 4.34 (1H, *quint.d*, $J = 6$, 1 Hz, H-9), 4.41 (1H, *d*, $J = 8$ Hz, H-1′), 5.79 (1H, *dd*, $J = 16$, 6 Hz, H-8), 6.07 (1H, *dd*, $J = 16$, 1 Hz, H-7); ^{13}C NMR (CD_3OD): Table 1; HR-FAB–MS (negative-ion mode) *m/z*: 405.2151 $[M-H]^-$(calc. for $C_{19}H_{33}O_9$: 405.2125).

3.3.2. (3S,5R,6R,7E,9S)-Megastigman-7-ene-3,5,6,9-tetrol 9-O-β-D-glucopyranoside

An amorphous powder, $[\alpha]_D^{24}$ −56.3° ($c = 0.65$, MeOH); ^{1}H NMR (CD_3OD) δ: 0.91 (3H, s, H_3-12), 1.11 (3H, *s*, H_3-11), 1.24 (3H, *s*, H_3-13), 1.32 (3H, *d*, $J = 6$ Hz, H_3-10), 1.46 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.66 (1H, *t*, $J = 12$ H, H-2ax), 1.73 (1H, *dd*, $J = 13$, 12 Hz, H-4ax), 1.78 (1H, *ddd*, $J = 13$, 4, 2 Hz, H-4eq), 3.67 (1H, *dd*, $J = 12$, 6 Hz, H-6′a), 3.85 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 4.06 (1H, *ddt*, $J = 13$, 12, 4 Hz, H-4), 4.41 (1H, *d*, $J = 8$ Hz, H-1′), 4.54 (1H, *quint.*, $J = 6$ Hz, H-9), 5.66 (1H, *dd*, $J = 16$, 6 Hz, H-8), 6.18 (1H, *d*, $J = 16$ Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB–MS (negative-ion mode) *m/z*: 405.2140 $[M-H]^-$ (calc. for $C_{19}H_{33}O_9$: 405.2125).

3.3.3. Enzymatic hydrolysis of 1 to 1a

Compound **1** (14 mg) was hydrolyzed with hesperidinase (20 mg) in 2 ml of H_2O at 37 °C for 24 h. The reaction mixture was concentrated, and then subjected to silica gel column (20 g, $\Phi = 15$ mm, $L = 20$ cm) chromatography with C_6H_6 (40 ml), C_6H_6–$CHCl_3$ (1:1, 40 ml), $CHCl_3$ (100 ml), and $CHCl_3$–MeOH (19:1, 100 ml, 9:1 100 ml, 17:3, 100 ml and 7:3, 300 ml), 10 ml fractions being collected. The aglycone (**1a**) and D-glucose were recovered from fractions 40–46 (5.3 mg, 63%) and 51–59 (3.8 mg, 62%), respectively. Aglycone (**1a**): Amorphous powder; $[\alpha]_D^{22}$ −21.1° ($c = 0.38$, MeOH); ^{1}H NMR (CD_3OD) δ: 0.88 (3H, *s*, H_3-12), 1.10 (3H, *s*, H_3-13), 1.22 (3H, *s*, H_3-10), 1.27 (3H, *d*, $J = 6$ Hz, H_3-10), 1.45 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.65 (1H, *t*, $J = 12$ Hz, H-2ax), 1.73 (1H, *dd*, $J = 11$, 2 Hz, H-4ax), 1.78 (1H, *ddd*, $J = 13$, 5, 2 Hz, H-4eq), 4.06 (1H, *m*, H-3), 4.34 (1H, *quint.d*, $J = 6$, 1 Hz, H-9), 5.79 (1H, *dd*, $J = 16$, 6 Hz, H-8), 6.07 (1H, *dd*, $J = 16$, 1 Hz, H-7); ^{13}C NMR (CD_3OD): see Table 1; HR-FAB–MS (negative-ion mode) *m/z*: 243.1593 $[M-H]^-$ (calc. for $C_{13}H_{23}O_4$: 243.1596). D-Glucose, $[\alpha]_D^{21}$ +43.4° ($c = 0.25$, H_2O, 24 h after being dissolved in the solvent).

3.3.4. Preparation of the (R)- and (S)-MTPA esters 1b and 1c from 1a

A solution of **1a** (2.6 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (36 mg) in the presence of 4-dicyclohexylcarbodiimide (DCC) (29 mg) and 4-dimethylaminopyridine (DMAP) (18 mg), the mixture being occasionally stirred at 25 °C for 30 min. After the addition of 1 ml each of H_2O and CH_2Cl_2, the solution was washed with 5% HCl (1 ml), $NaHCO_3$ saturated H_2O (1 ml), and brine (1 ml), successively. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness, and developed with $CHCl_3$–$(CH_3)_2CO$ (19:1) and eluted with $CHCl_3$–MeOH (9:1)] to furnish the ester, **1b** (3.3 mg, 46%). Through a similar procedure, **1c** (4.5 mg, 63%) was prepared from **1a** (2.6 mg) by use of (*S*)-MTPA (40 mg), DCC (27 mg), and DMAP (14 mg).

3.3.5. (3S,5R,6R,7E,9S)-Megastigman-7-ene-3,5,6,9-tetrol 3,9-di-(R)-MTPA ester (1b)

Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.80 (3H, *s*, H_3-12), 1.09 (3H, *s*, H_3-13), 1.25 (3H, *s*, H_3-11), 1.46 (3H, *d*, $J = 6$ Hz, H_3-10), 1.68 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.80 (1H, *t*, $J = 12$ Hz, H-2ax), 1.86 (1H, *ddd*, $J = 13$, 5, 2 Hz, H-4eq), 1.88 (1H, *dd*, $J = 13$, 11 Hz, H-4ax), 3.56 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.57 (3H, *q*, $J = 1$ Hz, –OCH_3), 5.47 (1H, *m*, H-3), 5.67 (1H, *quint.*, $J = 6$ Hz, H-10), 5.71 (1H, *dd*, $J = 16$, 6 Hz, H-8), 6.18 (1H, *d*, $J = 16$ Hz, H-7), 7.35–7.41 (6H, *m*, aromatic protons), 7.51–7.55 (4H, *m*, aromatic protons); HR-FAB–MS (negative-ion mode) *m/z*: 645.2271 $[M-CH_3O]^-$ (calc. for $C_{32}H_{35}O_7F_6$: 645.2287).

3.3.6. (3S,5R,6R,7E,9R)-Megastigman-7-ene-3,5,6,9-tetrol 3,9-di-(S)-MTPA ester (1c)

Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.82 (3H, *s*, H_3-12), 1.15 (3H, *s*, H_3-13), 1.27 (3H, *s*, H_3-11), 1.41 (3H, *d*, $J = 6$ Hz, H_3-10), 1.62 (1H, *ddd*, $J = 12$, 5, 2 Hz, , H-2eq), 1.74 (1H, *t*, $J = 12$ Hz, H-2ax), 1.95 (1H, *ddd*, $J = 13$, 5, 2 Hz, H-4eq), 2.02 (1H, *dd*, $J = 13$, 11 Hz, H-4ax), 3.53 (3H, *q*, $J = 1$ Hz, -OCH_3), 3.55 (3H, *q*, $J = 1$ Hz, –OCH_3), 5.48 (1H, *tt*, $J = 12$, 5 Hz, H-3), 5.66 (1H, *quint.d*, $J = 6$, 1 Hz, H-9), 5.79 (1H, *dd*, $J = 16$, 6 Hz, H-8), 6.28 (1H, *dd*, $J = 16$, 1 Hz, H-7), 7.34–7.42 (6H, *m*, aromatic protons), 7.51–7.56 (4H, *m*, aromatic protons); HR-FAB–MS (negative-ion mode) *m/z*: 645.2286 $[M-CH_3O]^-$ (calc. for $C_{32}H_{35}O_7F_6$: 645.2287).

*3.3.7. Enzymatic hydrolysis of **2** to **2a**, and preparation of the (R)- and (S)-MTPA esters **2b** and **2c***

Compound **2** (9.1 mg) was hydrolyzed with hesperidinase (10 mg) in 2 ml of H_2O at 37 °C for 12 h. The reaction mixture was concentrated and subjected to silica gel cc with the same solvent system as used for purification of **1a** to give 3.9 mg (69%) of an aglycone, **2a**. Using a similar procedure to that used for the preparation of **1b** and **1c** from **1a, 2b** (2.9 mg, 58%) and **2c** (3.1 mg, 62%) were prepared from **2a** (1.8 mg each) by use of the respective amounts of (*R*)- and (*S*)-MTPA (26 and 24 mg, respectively), DCC (19 and 20 mg, respectively), and DMAP (9 and 11 mg, respectively). The esters, **2b** and **2c**, gave essentially the same ^{1}H NMR spectra as those of **1b** and **1c**, respectively. The $\Delta\delta S$–δR values are included in Fig. 1 (see figures in parentheses).

*3.3.8. Preparation of the (R)- and (S)-MTPA esters **5b** and **5c***

Using a similar procedure to that used for the preparation of **1b** and **1c** from **1a, 5b** (12.0 mg, 81%) and **5c** (14.0 mg, 95%) were prepared from **5a** (5.1 mg each) by use of the respective amounts of (*R*)- and (*S*)-MTPA (49 and 48 mg), DCC (35 and 34 mg), and DMAP (18 and 19 mg). The developing solvent for preparative TLC was C_6H_6–$(CH_3)_2CO$ (9:1).

*3.3.9. (3S,5R,6S,7E,9R)-Megastigman-7-en-5,6-epoxy-3,9-diol 3,9-di-(R)-MTPA ester (**5b**)*

Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.95 (3H, *s*, H_3-11), 1.03 (3H, *s*, H_3-12), 1.10 (3H, *s*, H_3-13), 1.36 (3H, *d*, $J=7$ Hz, H_3-10), 1.39 (1H, *dd*, $J=14$, 9 Hz, H-2 pseudo-ax), 1.74 (1H, *ddd*, $J=13$, 3, 1 Hz, H-2 pseudo-eq), 1.77 (1H, *dd*, $J=15$, 7 Hz, H-4 pseudo-ax), 2.39 (1H, *ddd*, $J=15$, 4, 1 Hz, H-4 pseudo-eq), 3.53 (3H, *s*, $-OCH_3$), 3.55 (3H, *s*, $-OCH_3$), 5.12 (1H, *m*, H-3), 5.59 (1H, *quint.d*, $J=6$, 1 Hz, H-9), 5.71 (1H, *dd*, $J=16$, 6 Hz, H-8), 5.98 (1H, *dd*, $J=16$, 1 Hz, H-7), 7.35–7.43 (6H, *m*, aromatic protons), 7.49–7.55 (4H, *m*, aromatic protons); HR-FAB–MS (positive-ion mode) *m*/*z*: 681.2286 $[M+Na]^+$ (+NaI) (calc. for $C_{33}H_{36}O_7F_6Na$: 681.2263).

*3.3.10. (3S,5R,6S,7E,9R)-Megastigman-7-en-5,6-epoxy-3,9-diol 3,9-di-(S)-MTPA ester (**5c**)*

Amorphous powder, ^{1}H NMR ($CDCl_3$) δ: 0.93 (3H, *s*, H_3-11), 0.908 (3H, *s*, H_3-12), 1.14 (3H, *s*, H_3-11), 1.30 (1H, *dd*, $J=13$, 9 Hz, H-2 pseudo-ax), 1.41 (3H, *d*, $J=6$ Hz, H_3-10), 1.68 (1H, *ddd*, $J=13$, 3, 1 Hz, H-2 pseudo-eq), 1.85 (1H, *dd*, $J=15$, 7 Hz, H-4 pseudo-ax), 2.46 (1H, *ddd*, $J=15$, 6, 1 Hz, H-4 pseudo-eq), 3.53 (3H, *q*, $J=1$ Hz, $-OCH_3$), 3.55 (3H, *q*, $J=1$ Hz, $-OCH_3$), 5.16 (1H, *m*, H-3), 5.60 (1H, *quint.*, $J=6$ Hz, H-9), 5.66 (1H, *dd*, $J=16$, 6 Hz, H-8), 5.89 (1H, *d*, $J=16$ Hz, H-7), 7.34–7.44 (6H, *m*, aromatic protons), 7.49–7.54 (4H, *m*, aromatic protons); HR-FAB–MS (positive-ion mode) *m*/*z*: 681.2280 $[M+Na]^+$ (+NaI) (calcd. for $C_{33}H_{36}O_7F_6Na$: 681.2263).

*3.3.11. Preparation of (R)- and (S)-MTPA esters **6b** and **6c** from **6a***

Using a similar procedure to that used for the preparation of **1b** and **1c** from **1a**, **6b** (10.6 mg, 77%) and **6c** (10.7 mg, 77%) were prepared from **6a** (5.0 mg each) by use of the respective amount of (*R*)- and (*S*)-MTPA (58 mg and 57 mg), DCC (40 mg and 39 mg), and DMAP (19 mg each).

*3.3.12. (3S,5R,6R,7E,9R)-Megastigman-7-ene-3,5,6,9-tetrol 3,9-di-(R)-MTPA ester (**6b**)*

Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.86 (3H, *s*, H_3-12), 1.09 (3H, *s*, H_3-13), 1.28 (3H, *s*, H_3-11), 1.42 (3H, *d*, $J=7$ Hz, H_3-10), 1.70 (1H, *ddd*, $J=12$, 4, 2 Hz, H-2eq), 1.81 (1H, *t*, $J=12$ Hz, H-2ax), 1.87 (1H, *ddd*, $J=13$, 5, 2 Hz, H-4eq), 1.91 (1H, *dd*, $J=13$, 11 Hz, H-4ax), 3.53 (3H, q, $J=1$ Hz, $-OCH_3$), 3.55 (3H, q, $J=1$ Hz, $-OCH_3$), 5.49 (1H, *dddd*, $J=13$, 11, 5, 4 Hz, H-3), 5.65 (1H, *quint.*, $J=7$ Hz, H-9), 5.77 (1H, *dd*, $J=16$, 7 Hz, H-8), 6.30 (1H, *dd*, $J=16$, 1 Hz, H-7), 7.34–7.41 (6H, *m*, aromatic protons), 7.50–7.55 (4H, *m*, aromatic protons); HR-FAB–MS (negative-ion mode) *m*/*z*: 645.2271 $[M-CH_3O]^-$ (calc. for $C_{32}H_{35}O_7F_6$: 645.2287).

*3.3.13. (3S,5R,6R,7E,9R)-Megastigman-7-ene-3,5,6,9-tetrol 3,9-di-(S)-MTPA ester (**6c**)*

Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.80 (3H, *s*, H_3-12), 1.11 (3H, *s*, H_3-13), 1.24 (3H, *s*, H_3-11), 1.46 (3H, *d*, $J=7$ Hz, H_3-10), 1.61 (1H, *ddd*, $J=12$, 4, 2 Hz, H-2eq), 1.72 (1H, *t*, $J=12$ Hz, H-2ax), 1.94 (1H, *ddd*, $J=13$, 5, 2 Hz, H-4eq), 1.99 (1H, *dd*, $J=13$, 11 Hz, H-4ax), 3.55 (3H, *q*, $J=1$ Hz, $-OCH_3$), 3.56 (3H, *q*, $J=1$ Hz, $-OCH_3$), 5.48 (1H, *dddd*, $J=13$, 11, 5, 4 Hz, H-3), 5.67 (1H, *quint.*, $J=7$ Hz, H-9), 5.68 (1H, *dd*, $J=16$, 7 Hz, H-8), 6.22 (1H, *d*, $J=16$ Hz, H-7), 7.34–7.41 (6H, *m*, aromatic protons), 7.50–7.55 (4H, *m*, aromatic protons); HR-FAB–MS (negative-ion mode) *m*/*z*: 645.2299 $[M-CH_3O]^-$ (calc. for $C_{32}H_{35}O_7F_6$: 645.2287).

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