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Euodionosides A–G: Megastigmane glucosides from leaves of *Euodia meliaefolia*

Miwako Yamamoto [a], Takeyuki Akita [a], Yuka Koyama [a], Etsuko Sueyoshi [a], Katsuyoshi Matsunami [a], Hideaki Otsuka [a,*], Takakazu Shinzato [b], Atsushi Takashima [c], Mitsunori Aramoto [d], Yoshio Takeda [e]

[a] *Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan*
[b] *Subtropical Field Science Center, Faculty of Agriculture, University of the Ryukyus, 1 Sembaru, Nishihara-cho, Nakagami-gun, Okinawa 903-0213, Japan*
[c] *Yona Field, Subtropical Field Science Center, Faculty of Agriculture, University of the Ryukyus, 685 Aza Yona, Kunigami-son, Kunigami-gun, Okinawa 905-1427, Japan*
[d] *Iriomote Station, Tropical Biosphere Research Center, University of the Ryukyus, 870 Aza Uehara, Taketomi-cho, Yaeyama-gun, Okinawa 907-1541, Japan*
[e] *Faculty of Integrated Arts and Sciences, The University of Tokushima, 1 Minamijosanjima-cho, Tokushima 770-8502, Japan*

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Abstract

From a 1-BuOH-soluble fraction of the MeOH extract of leaves of *Euodia meliaefolia*, collected in Okinawa, seven megastigmane glucosides, named euodionosides A–G, were isolated together with three known megastgmane glucosides, and two aliphatic and three phenolic compounds. Their structures were elucidated through a combination of spectroscopic analyses and application of the modified Mosher's method.

Keywords: *Euodia meliaefolia*; Rutaceae; Euodionoside; Megastigmane glucoside; Modified Mosher's method

1. Introduction

The fruits of *Evodia rutaecaepa* (Rutaceae) are a well known crude drug included in the Japanese Pharmacopoeia XV and contain characteristic indole alkaloids, i.e. rutaecarpine and evodiamine. However, in reliable plant name databases (IPNI), such as the Index Kewensis, Australian Plant Name Index and Gray Card Index, the genus name *Evodia* is not listed under the Rutaceae. Instead, many *Euodia* species appear in the databases as Rutaceous plants. Probably, some previous investigators made a mistake due to the resemblance of the letters u and v. Thereafter, even an isolated compound was erroneously named evodiamine.

A closely related species, *E. meliaefolia*, is a tall deciduous tree of about 15 m in height and found in southern Kyushu through Okinawa, Taiwan and China (Hatusima, 1975). Currently no chemical and medicinal investigations of this plant have been reported. In a continuing study on Okinawan plants, the chemical constituents of *E. meliaefolia*, collected in Okinawa, were investigated.

From a 1-BuOH-soluble fraction of the MeOH extract of leaves of *E. meliaefolia*, 10 megastigmane glucosides (**1**–**10**) were isolated together with two known aliphatic [(*Z*)-3-hexenyl β-D-glucopyranoside (**11**) (Mizutani et al., 1988) and 3,7-dimethylocte-1-en-3,6,7-triol 6-*O*-β-D-glucopyranoside (**12**) (Manns, 1995)] and three phenolic [(+)-catechin (**13**) (Nay et al., 2001), syringin (**14**) DellaGreca

* Corresponding author. Tel./fax: +81 82 257 5335.
E-mail address: hotsuka@hiroshima-u.ac.jp (H. Otsuka).

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et al., 1998) and 1-β-D-glucopyranosyloxy-3-methoxy-5-hydroxybenzene (**15**) (Sakar et al., 1993)] compounds. Of the ten megastigmane glucosides, three were known compounds, namely spinoside A (**8**) (Çaliş et al., 2002), staphylionoside D (**9**) (Yu et al., 2005), and corchoionoside C (**10**) (Yoshikawa et al., 1997). This paper deals with structural elucidation of the seven new megastigmane glucosides.

2. Results and discussion

Air-dried leaves of *E. meliaefolia* were extracted with MeOH three times and the concentrated MeOH extract was partitioned with solvents of increasing polarity. The *n*-BuOH soluble fraction was separated by means of various chromatographic procedures including column chromatography (CC) on a highly porous synthetic resin (Diaion HP-20), then normal silica gel and reversed-phase octadecyl silica gel (ODS) CC, droplet counter-current chromatography (DCCC), and high-performance liquid chromatography (HPLC) to afford 15 compounds (**1**–**15**). The details and yields are given under Section 4. The structures of the new megastigmane glucosides (**17**) were elucidated on the basis of spectroscopic evidence, that obtained with the modified Mosher's method (Ohtani et al., 1991), and those of known compounds were identified by comparison of spectroscopic data with those reported in the literature (Fig. 1). The ^{13}C NMR spectroscopic data of spinoside A (**8**) in CD_3OD are included under Section 4 for the readers' convenience.

Euodionoside A (**1**), $[\alpha]_D^{26}$ −40.5, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{30}O_9$ by HR-ESI-TOF-MS. The IR spectrum indicated the presence of a sugar moiety (3395 cm^{-1}) and a ketonic functional group (1673 cm^{-1}), and the UV spectrum a conjugated system (235 nm). The ^1H and ^{13}C MR spectra showed the presence of six signals assignable to β-glucopyranose, the remaining 13 carbon signals comprising four singlet methyls, two methylenes, one methine with a hydroxyl substituent, and four quaternary carbons, and a disubstituted trans double bond, which must form the megastigmane skeleton. Judging from the chemical shifts, one (δ_H 2.29) of the methyl groups must be adjacent to the carbonyl functional group, and one (δ_C 200.1) and two (δ_C 72.1 and 67.0) of the quaternary carbons must have a carbonyl group and oxygen substituents, respectively. When five degrees of unsaturation and a deficiency of one oxygen atom for three hydroxyl groups are considered, an epoxy ring must be required for one more cyclic system between C-5 and C-6 of megastigmane skeleton. In the H–H COSY spectrum, H_2-2 through H_2-4 showed a significant correlation, and the correlations between H-7 (δ_H 7.08) and H-10 (δ_H 2.29), and the carbonyl carbon (δ_C 200.1) in the HMBC spectrum established a structure as **1** (Fig. 1). The hydroxyl group at the C-3 (δ_C 72.1) position is the sole site to be glucosylated. To clarify the relative arrangement of the substituents, the phase-sensitive (PH)-NOESY spectrum was examined. Judging from the key correlations between H-3 (δ_H 3.98) and H_3-11ax (δ_H 1.25), H_3-11 and H-7 (δ_H 7.08), and H-4eq (δ_H 2.38) and H_3-13 (δ_H 1.17), H-3, the epoxy ring is in an *anti* orientation, in contrast to in the case of icariside B_2, which was isolated from *Epimedium grandiflorum* var. *thunbergianum* (Miyase et al., 1987). On enzymatic hydrolysis, an aglycone (**1a**) and D-glucose were obtained. From the β-D-glucosylation-induced shift-trend between the ^{13}C NMR spectra of **1** and **1a**, the absolute configuration of the 3-position was determined to be *R* (Table 1, a) (Kasai et al., 1977). This was further confirmed by the modified Mosher's method (Fig. 2) (Ohtani et al., 1991). Therefore, the structure of euodionoside A (**1**) was elucidated to be (3*R*,5*R*,6S,7*E*)-megastigman-7-en-5,6-epoxy-3-ol-9-one 3-*O*-β-D-glucopyranoside, as shown in Fig. 1.

Euodionosides B (**2**), $[\alpha]_D^{25}$ 24.8, and C (**3**), $[\alpha]_D^{26}$ −56.3, were isolated as amorphous powders and their elemental compositions were determined to be $C_{19}H_{32}O_8$ by HR-FAB-MS. The ^1H and ^{13}C NMR spectra indicated that euodionosides B (**2**) and C (**3**) were compounds analogous to euodionoside A (**1**), except for the presence of hydroxyl groups at their 9-positions, instead of the carbonyl functional group found in **1**. The HMBC spectra showed a correlation between the anomeric proton (δ_H 4.30) and C-3 (δ_C 72.2) in the case of **2**, but between the former and (δ_H 4.28) and C-9 (δ_C 74.8) in the case of **3**. The stereochemistry of the ring system of **2** was expected to be the same as that of **1**, since the ^{13}C NMR spectrum of **2** showed essentially the same chemical shifts as that of **1**. On enzymatic hydrolysis of **2**, an aglycone (**2a**) ($[\alpha]_D$ +8.4) and D-glucose were obtained. The stereochemistry of the 9-position of **2a** was found with the modified Mosher's method to be *S*, and that of the ring portion was also confirmed by the PH-NOESY spectrum to comprise the 3*R*, 5*R* and 6*S* configurations (Fig. 2). The stereochemistry of the 9-position of euodionoside C (**3**) can be deduced with the β-D-glucosylation-induced shift-trend rule (Kasai et al., 1977) to be *S*, which is the same as that of **2a**, and that of the ring system was also expected to be the same as that of **2**. However, the stereochemistry of the ring system must be determined independently. Although the optical rotation value for the aglycone (**3a**) ($[\alpha]_D$ +5.2) of **3** was close to that of **2a**, the stereochemistry of **2a** was similarly established with the modified Mosher's method (Fig. 2). Therefore, the structures of euodionosides B (**2**) and C (**3**) were elucidated to be (3*R*,5*R*,6*S*,7*E*,9*S*)-megastigman-7-en-5,6-epoxy-3,9-diol 3- and 9-*O*-β-D-glucopyranosides, respectively.

Euodionoside D (**4**), $[\alpha]_D$ −42.7, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{34}O_9$ by HR-ESI-TOF-MS. On analyses of the NMR spectroscopic data, **4** was also expected to be a derivative of a megastigmane glucoside with four hydroxyl groups at the C-3, 5, 6, and 9 positions. The HMBC correlation between the anomeric proton (δ_H 4.38) and C-9 (δ_C 75.7) established that the sugar unit

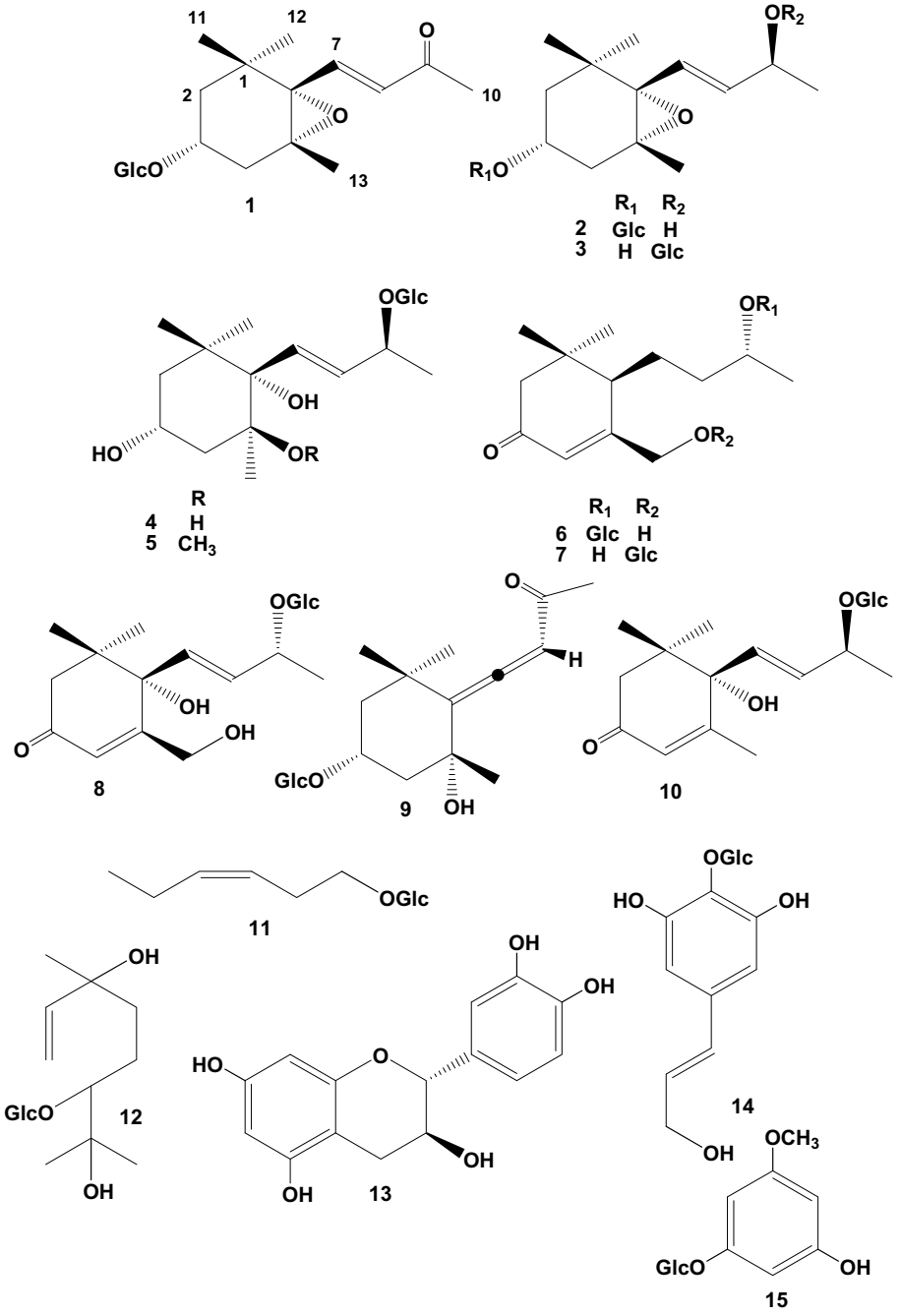

Fig. 1. Structures.

was linked to the hydroxyl group at the 9-position, and the coupling pattern of H-3 (δ_H 4.06, *dddd*, $J = 12, 12, 6, 6$ Hz) indicated that the hydroxyl group at the 3-position was in an equatorial orientation. From this evidence together with the results of comparison of the ^{13}C NMR spectroscopic data, the relative orientations of the substitutents of the ring system were found to be the same as those of (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol 9-*O*-β-D-glucopyranoside isolated from *Glochidion zeylanicum*. According to the β-D-glucosylation-induced shift-trend rule (Kasai et al., 1977) the absolute configuration of the 9-position was the same in the megastigmanes from *E. meliaefolium* and *G. zeylanicum*. However, although NMR data for the ring systems of these two compounds were essentially the same, the stereochemistries of the side chain and the ring system must be determined independently (Otsuka et al., 2003a). Thus, the modified Mosher's method was applied to the aglycone (**4a**) (Fig. 2), which was shown to have opposite absolute configurations to that from *G. zeylanicum*. Therefore, the structure of euodionoside D (**4**) was elucidated to be (3*R*,5*S*,6*S*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol 9-*O*-β-D-glucopyranoside, as shown in Fig. 1.

Table 1
^{13}C NMR spectroscopic data for euodionosides A–G (**1**–**7**) (CD_3OD, 100 MHz)

C	1	2	3	4	5	6	7
1	35.9	35.8	35.9	40.7	40.9	37.5	37.3
2	41.0 (−3.3)[a]	41.4	44.4	46.4	46.3	48.6	48.7
3	72.1 (+8.2)[a]	72.2	63.8	65.3	65.1	202.4	202.2
4	38.4 (−1.6)[a]	38.6	40.2	45.7	38.7	121.7	123.3
5	67.0	66.2	66.2	77.7	83.2	172.3	167.8
6	72.1	72.2	72.1	79.1	79.2	48.4	47.9
7	143.7	124.3	128.2	135.5	136.3	27.2	27.6
8	134.6	140.1	136.9	133.3	132.7	37.4 (−2.5)[b]	39.8
9	200.1	68.7	74.8	75.7	75.7	77.7 (+8.8)[b]	68.9
10	27.3	24.0	22.5	22.6	22.5	21.9 (−4.1)[b]	23.6
11	25.0	25.1	25.3	27.5	27.6	27.7	27.7
12	27.5	27.2	27.2	26.3	26.3	28.9	28.9
13	21.0	21.8	22.2	27.7	20.3	65.2	70.9
–O*C*H$_3$					48.7		
1′	103.0	102.9	101.4	100.7	100.5	104.0	103.5
2′	75.1	75.1	75.1	75.1	75.0	75.4	75.1
3′	78.1	78.1	78.4	78.4	78.4	78.3	78.3
4′	71.8	71.8	71.8	71.9	71.7	71.7	71.7
5′	78.0	77.9	78.1	78.0	78.1	77.9	78.2
6′	63.0	62.9	63.0	63.0	62.8	62.9	62.9

[a] $\Delta\delta_{\mathbf{1}-\mathbf{1a}}$.
[b] $\Delta\delta_{\mathbf{6}-\mathbf{7a}}$.

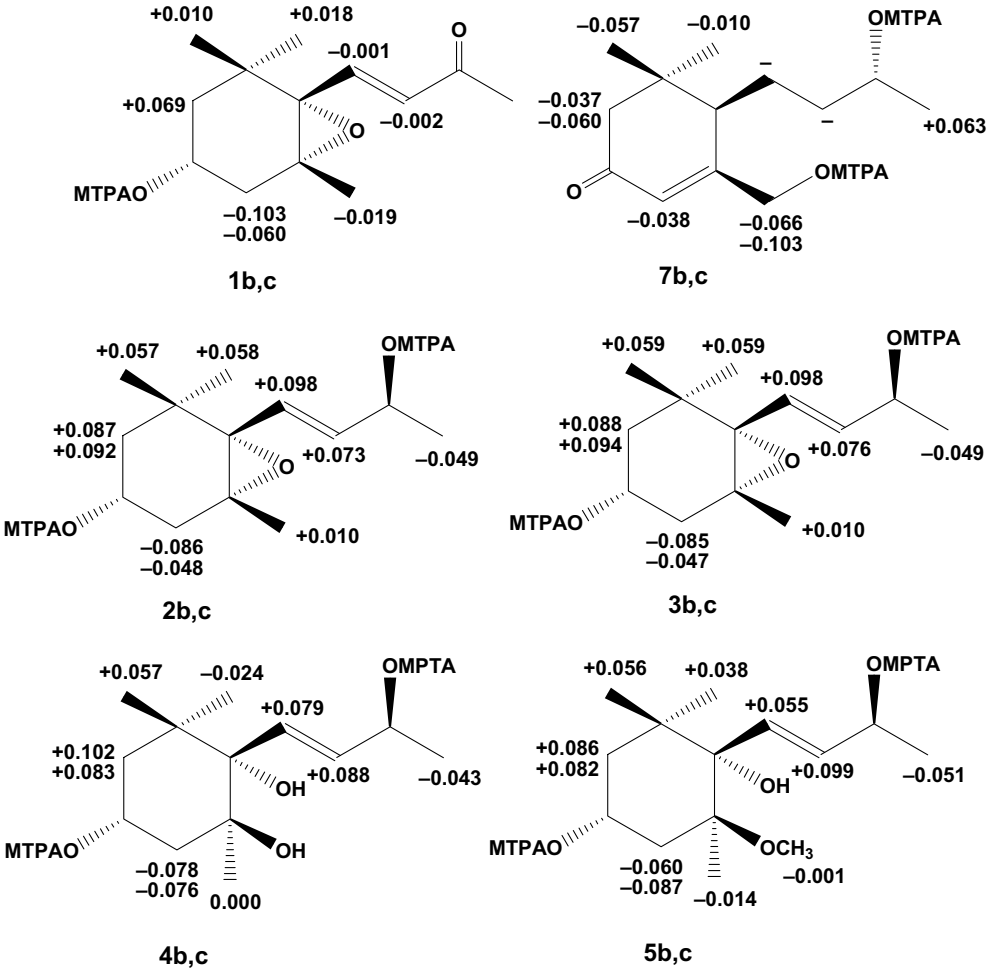

Fig. 2. Results with the modified Mosher's method ($\Delta_{\delta S-\delta R}$).

Euodionoside E (**5**), $[\alpha]_D$ −35.0, was isolated as an amorphous powder, and the one- and two-dimensional NMR spectroscopic data showed that it was a compound analogous to euodionoside D (**4**) with similar substituents on the six-membered ring. In the NMR spectra, a methoxyl signal was observed [δ_H 3.21 (3H, *s*) and δ_C 48.7 (*q*)], and the presence of an extra carbon atom was also supported by the HR-ESI-TOF-MS data ($C_{20}H_{36}O_9$). From the HMBC spectroscopic correlation between δ_H 3.21 and δ_C 83.2 (C-5), the position of the methoxyl group was assigned as C-5, and from the PH-NOESY spectrum, the relative orientations of the substituents were found to be the same as those of **4**. The absolute configurations of the ring system were also determined by the application of the modified Mosher's method to the aglycone (**5a**) (Fig. 2). Therefore, the structure of euodionoside E (**5**) was elucidated to be (3*R*,5*S*,6*S*,7*E*,9*S*)-megastigman-7-ene-5-methoxy-3,6,9-triol 9-*O*-β-D-glucopyranoside. A similar megastigmane derivative, which has a methoxyl group at the 6-position, was isolated as staphylionoside J from *Staphylea bumalda* (Yu et al., 2005).

Euodionosides F (**6**), $[\alpha]_D$ −0.7 and G (**7**), $[\alpha]_D$ +5.9, were isolated as amorphous powders and their elemental compositions were determined to be $C_{19}H_{32}O_8$ by HR-ESI-TOF-MS. The IR (1649 and 1651 cm^{-1}, respectively) and UV (238 and 237 nm, respectively) spectra showed the presence of a conjugated ketone, their NMR spectra being similar, with two singlet and one doublet methyls, and one primary and one secondary alcohol. Thus, one of the methyl groups was expected to be oxidized to a primary alcohol, whose proton signals (δ_H 4.16 and 4.32, and δ_H 4.37 and 4.53, respectively) showed correlation to C-5 resonances (δ_C 172.3 and δ_C 167.8) in the HMBC spectra. These facts were coincident with that C-13 was oxidized to a primary alcohol. The HMBC spectra also indicated that **6** and **7** were isomers of each other as to the positions of sugar linkages, the former had a sugar on the hydroxyl group at the 9-position and the latter on that at the 13-position, since correlation between anomeric protons and C-9 (δ_C 77.3) in 6, and C-13 (δ_C 70.9) in 7 was observed, respectively. The absolute configuration of the 9-position of **6** was determined to be *R* on comparison with a similar compound (Takeda et al., 1997) and that of the 6-position was also assigned as *R* according to the circular dichroism (CD) spectral data (Otsuka et al., 2003b). The absolute configuration of the 9-position was further supported by application of the β-D-glucosylation-induced shift-trend rule (Table 1, b) (Otsuka et al. (1995)), Therefore, the structure of euodionoside F (**6**) was elucidated to be (6*R*,9*R*)-megastigman-4-ene-9,13-diol 9-*O*-β-D-glucopyranoside. Since euodionoside G (**7**) was an isomeric form of **6**, the absolute configuration of the 9-position was expected to be the same as that of **6**. However, for the same reason as that for euodionoside D (**4**), the absolute configurations of the 6- and 9-positions were independently established by CD spectral analysis and the modified Mosher's method (Fig. 2), respectively. Although the exact $\Delta_{\delta S-\delta R}$ values for the H_2-7 and eight protons could not be calculated, due to overlapping of their signals, they obviously had minus signs, whereas that for the H_3-10 protons had a significant plus value (+0.063) (Fig. 2). As a result, the structure of euodionoside G (**7**) was elucidated to be (6*R*,9*R*)-megastigman-4-ene-9,13-diol 13-*O*-β-D-glucopyranoside. A compound closely related to **6** has been isolated from *G. zeylanicum* as glochidionionoside B, which has the 9*S* configuration (Otsuka et al., 2003).

3. Concluding remarks

Although fruits of closely related species, *E. rutaecarpa*, are used for stomachic antipyretic and diuretic in traditional Chinese medicine, there has been no report on the medicinal use of *E. meloaefolia*. Due to usage of leaves for this experiment, alkaloids are so far not isolated. Biological evaluation and chemical investigation of fruits of *E. meloaefolia* will be subject of a future study.

Megastigmane and its glycosides are currently an expanding class of compounds. Even with only 13 carbon atoms in the basic skeleton of megastigmane, several oxidation steps and glycosylation afforded many kinds of megastigmane derivatives and their glycosidic forms. In this experiment, seven new megastigmane glucosides were isolated from leaves of the title plant. Their stereostructures were established by the modified Mosher's method.

4. Experimental

4.1. General experimental procedures

Optical rotations and CD spectra were measured on JASCO P-1030 and JASCO J-720 polarimeters, respectively. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/Vis spectrophotometers, respectively. ^1H and ^{13}C NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane as an internal standard. Negative-ion HR-MS was performed with a JEOL SX-102 spectrometer in the FAB mode and positive-ion HR-MS with an Applied Biosystem QSTAR XL system ESI (Nano Spray)-TOF-MS.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC and reversed-phase [octadecyl silica gel (ODS)] open CC were performed on silica gel 60 (Merck, Darmstadt, Germany) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [$\Phi = 50$ mm, $L = 25$ cm, linear gradient: MeOH–H_2O (1:9, 1 L) → (1:1, 1 L), fractions of 10 g being collected], respectively. Droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns ($\Phi = 2$ mm, $L = 40$ cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–*n*-PrOH (9:12:8:2) were

used for the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; $\Phi = 6$ mm, $L = 25$ cm), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor.

Emulsin was purchased from Tokyo Chemical Industries Co. Ltd. (Tokyo, Japan), and crude hesperidinase was a gift from Tokyo Tanabe Pharmaceutical Co. Ltd. (Tokyo, Japan). (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acids (MTPA) were the products of Wako Pure Chemical Industry Co. Ltd. (Tokyo, Japan).

4.2. Plant material

Leaves of *E. meliaefolia* Benth. (Rutaceae) were collected in Okinawa, Japan, in August 2002, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (02-EM-Okinawa-0704).

4.3. Extraction and isolation

Dried leaves of *E. meliaefolia* (15.7 kg) were extracted three times with MeOH (45 l) at 25 °C for one week and then concentrated to 6 l *in vacuo*. The extract was washed with *n*-hexane (6 l, 531 g) and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in water (6 l) and then extracted with EtOAc (6 l) to give 165 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (6 l) to give a 1-BuOH-soluble fraction (409 g), and the remaining water-layer was concentrated to furnish 971 g of a water-soluble fraction.

A portion (154 g) of the 1-BuOH-soluble fraction was subjected to a Diaion HP-20 column ($\Phi = 50$ mm, $L = 50$ cm) using H_2O–MeOH (4:1, 8 l), (2:3, 8 l), (3:2, 8 l), and (1:4, 8 l), and MeOH (6 l), 1 l fractions were being collected. The residue (27.6 g in fractions 6–12) of the 20–40% MeOH eluent was subjected to silica gel (1.50 kg) CC, with elution with $CHCl_3$ (6 l) and $CHCl_3$–MeOH [(99:1, 6 l), (97:3, 6 l), (19:1, 6 l), (37:3, 6 l), (9:1, 6 l), (7:1, 6 l), (17:3, 6 l), (33:7, 6 l), (4:1, 6 l), (31:9, 6 l), (3:1, 6 l), and (7:3, 6 l)], 1 l fractions being collected. Combined fractions 31–39 (1.20 g) were separated by RPCC. The residue (383 mg) of fractions 62–78 was subjected to DCCC to give 25.4 mg of **11** in fractions 35–40, and the residue (44.5 mg) in fractions 44–52 was further purified by prep. HPLC with MeOH–H_2O (3:7) to give 7.6 mg of **13** at R_t 10 min. The residue (112 mg) in fraction 94–114 obtained on RPCC was subjected to DCCC to give 3.9 mg of **1** in fractions 63–72, and the residue (10.6 mg in fractions 21–27) was further purified by prep. HPLC with MeOH–H_2O (3:7) to give 3.9 mg of **4** at R_t 13 min. The residue (85 mg) in fractions 115–132 obtained on RPCC was also subjected to DCCC to give two fractions, 10.2 mg in fractions 90–105 and 37.3 mg in fractions 109–136. Prep. HPLC of the former fraction with CH_3CN–H_2O (3:17) gave 1.4 mg of **6** and 2.8 mg of **7** at R_ts 16 min and 18 min, respectively. The latter was found to be a pure compound, **14**.

The residue (1.50 g out of 3.03 g) in fractions 40–45 obtained on 15–17.5% MeOH eluate on silica gel CC was subjected to RPCC to give five fractions, 172 mg in fractions 37–47, 87.0 mg in fractions 62–65, 150 mg in fractions 81–89, 116 mg in fractions 98–104, and 101 mg in fractions 112–123. The first fraction was purified by DCCC to give 144 mg of **15** in fractions 21–29. The second fraction was purified by DCCC (40.1 mg in fractions 47–57) and then prep. HPLC with MeOH–H_2O (1:4) to afford 5.4 mg of **8** at R_t 30 min. The third fraction was subjected to prep. HPLC with MeOH–H_2O (1:3) to give two partially purified fractions at R_ts 34 min (36.0 mg) and 42 min (59.4 mg). The former was repeatedly purified by prep. HPLC with CN_3CN–H_2O (1:9) to give 15.3 mg of **9** and 5.2 mg of **10** at R_ts 56 min and 68 min, respectively. The latter gave 21.0 mg of **2** on prep. HPLC with CH_3CN–H_2O (13:87) at R_t 44 min. The residue of the fourth fraction was found to be a pure compound, **3**. The fifth fraction was subjected to prep. HPLC with MeOH–H_2O (19:31) to give 8.5 mg of **6** at R_t 18 min.

The residue (1.61 g) in fractions 46–51 obtained from the 17.5–20% MeOH eluate on silica gel CC was subjected to RPCC to give two fractions, 130 mg in fractions 40–46 and 20.0 mg in fractions 146–153. The former was further purified by DCCC to give 29.6 mg of **15** in fractions 53–58 and the latter was a pure compound, **5**.

4.4. Characterization data

4.4.1. Euodionoside A (*1*)

Amorphous powder; $[\alpha]_D^{26}$ −40.5 ($c = 0.25$, MeOH); IR ν_{max} (film) cm^{-1}: 3395, 2926, 1673, 1455, 1367, 1076; UV λ_{max} (MeOH) nm (log ε): 235 (3.75); ^1H NMR (CD_3OD, 400 MHz) δ: 7.08 (1H, *d*, $J = 16$ Hz, H-7), 6.20 (1H, *d*, $J = 16$ Hz, H-8), 4.31 (1H, *d*, $J = 8$ Hz, H-1′), 3.98 (1H, *dddd*, $J = 11$, 10, 7, 7 Hz, H-3), 3.87 (1H, *dd*, $J = 12$, 2 Hz, H-6′a), 3.65 (1H, *dd*, $J = 12$, 6 Hz, H-6′b), 3.42–3.25 (3H, *m*, H-3′,4′ and 5′), 3.12 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 2.38 (1H, *dd*, $J = 15$, 7 Hz, H-4pseudo-eq), 2.29 (3H, *s*, H_3-10), 1.98 (1H, *dd*, $J = 15$, 10 Hz, H-4pseudo-ax), 1.50 (1H, *dd*, $J = 11$, 7 Hz, H-2eq), 1.49 (1H, *dd*, $J = 11$, 11 Hz, H-2ax), 1.25 (3H, *s*, H_3-11), 1.17 (3H, *s*, H_3-13), 0.97 (3H, *s*, H_3-12); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 409.1856 $[M+Na]^+$ (calcd for $C_{19}H_{30}O_8Na$, 409.1832).

4.4.2. Euodionoside B (*2*)

Amorphous powder; $[\alpha]_D^{25}$ –24.8 ($c = 0.79$, MeOH); IR ν_{max} (film) cm^{-1}: 3367, 2965, 1653, 1453, 1379, 1075; ^1H NMR (CD_3OD, 400 MHz) δ: 5.84 (1H, *dd*, $J = 16$, 1 Hz, H-7), 5.67 (1H, *dd*, $J = 16$, 6 Hz, H-8), 4.30 (1H, *d*, $J = 8$ Hz, H-1′), 4.29 (1H, *qdd*, $J = 6$, 6, 1 Hz, H-9), 3.94 (1H, *dddd*, $J = 11$, 10, 7, 6 HZ, H-3), 3.86 (1H, *dd*, $J = 12$, 2 Hz, H-6b), 3.65 (1H, *dd*, $J = 12$, 6 Hz, H-6′b),

3.36–3.25 (3H, *m*, H-3′, 4′ and 5′), 3.12 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 2.32 (1H, *ddd*, *J* = 15, 7, 1 Hz, H-4pseudo-eq), 1.89 (1H, *dd*, *J* = 15, 10 Hz, H-4pseudo-ax), 1.49 (2H, *m*, H_2-2), 1.23 (3H, *d*, *J* = 6 Hz, H_3-10), 1.20 (3H, *s*, H_3-13), 1.16 (3H, *s*, H_3-11), 0.97 (3H, *s*, H_3-12); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-FAB-MS (negative-ion mode) *m*/*z*: 387.2028 $[M-H]^-$ (calcd for $C_{19}H_{31}O_8$, 387.2019).

4.4.3. Euodionoside C (3)

Amorphous powder; $[\alpha]_D^{24}$ −56.3 (*c* = 7.20, MeOH); IR ν_{max} (film) cm^{-1}: 3395, 2965, 1649, 1452, 1368, 1075; ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 6.01 (1H, *dd*, *J* = 16, 1 Hz, H-7), 5.57 (1H, *ddd*, *J* = 16, 7, 1 Hz, H-8), 4.52 (1H, *br dq*, *J* = 7, 6 Hz, H-9), 4.28 (1H, *d*, *J* = 8 Hz, H-1′), 3.86 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.81 (1H, *m*, H-3), 3.64 (1H, *dd*, *J* = 12, 6 Hz, H-6′b), 3.32–3.14 (4H, *m*, H-2′, 3′, 4′ and 5′), 2.21 (1H, *dd*, *J* = 15, 7 Hz, H-4pseudo-eq), 1.71 (1H, *dd*, *J* = 15, 10 Hz, H-4pseudo-ax), 1.46 (1H, *dd*, *J* = 12, 12 Hz, H-2ax), 1.28 (3H, *d*, *J* = 6 Hz, H_3-10), 1.27 (1H, *m*, H-2eq), 1.23 (3H, *s*, H_3-13), 1.17 (3H, *s*, H_3-11), 0.96 (3H, *s*, H_3-12); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-FAB-MS (negative-ion mode) *m*/*z*: 387.2021 $[M-H]^-$ (calcd for $C_{19}H_{31}O_8$, 387.2018).

4.4.4. Euodionoside D (4)

Amorphous powder; $[\alpha]_D^{24}$ −42.7 (*c* = 0.28, MeOH); IR ν_{max} (film) cm^{-1}: 3395, 2927, 1649, 1372, 1072, 1027; ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 6.16 (1H, *d*, *J* = 16 Hz, H-7), 5.63 (1H, *dd*, *J* = 16, 8 Hz, H-8), 4.52 (1H, dq, *J* = 8, 7 Hz, H-9), 4.38 (1H, *d*, *J* = 8 Hz, H-1′), 4.06 (1H, *dddd*, *J* = 12, 12, 6, 6 Hz, H-3), 3.87 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.65 (1H, *dd*, *J* = 12, 6 Hz, H-6′b), 3.35–3.18 (4H, *m*, H-2′, 3′, 4′ and 5′), 1.76 (2H, *m*, H_2-4), 1.65 (1H, *dd*, *J* = 12, 12 Hz, H-2ax), 1.45 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2eq), 1.31 (3H, *d*, *J* = 7 Hz, H_3-10), 1.173 (3H, *s*, H_3-12), 1.165 (3H, *s*, H_3-13), 0.85 (3H, *s*, H_3-11); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 429.2098 $[M+Na]^+$ (calcd for $C_{19}H_{34}O_9Na$, 429.2095).

4.4.5. Euodionoside E (5)

Amorphous powder; $[\alpha]_D^{24}$ −35.0 (*c* = 1.13, MeOH); IR ν_{max} (film) cm^{-1}: 3395, 2931, 1635, 1370, 1077, 1035; ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 6.13 (1H, *d*, *J* = 16 Hz, H-7), 5.59 (1H, *dd*, *J* = 16, 9 Hz, H-8), 4.49 (1H, *dq*, *J* = 9, 6 Hz, H-9), 4.37 (1H, *d*, *J* = 8 Hz, H-1′), 3.85 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.83 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 3.67 (1H, *dd*, *J* = 12, 6 Hz, H-6′b), 3.33–3.18 (4H, *m*, H-2′, 3′, 4′ and 5′), 3.21 (3H, *s*, –OCH_3), 2.10 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-4eq), 1.65 (1H, *dd*, *J* = 12, 12 Hz, H-2ax), 1.57 (1H, *dd*, *J* = 14, 12 Hz, H-4ax), 1.45 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2eq), 1.30 (3H, *d*, *J* = 6 Hz, H_3-10), 1.11 (3H, *s*, H_3-13), 1.10 (3H, *s*, H_3-12), 0.85 (3H, *s*, H_3-11); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 443.2244 $[M+Na]^+$ (calcd for $C_{20}H_{36}O_9Na$: 443.2251).

4.4.6. Euodionoside F (6)

Amorphous powder; $[\alpha]_D^{24}$ −0.70 (*c* = 0.55, MeOH); IR ν_{max} (film) cm^{-1}: 3396, 2930, 1649, 1076, 1037; UV λ_{max} (MeOH) nm (log*ε*): 238 (3.88); ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 6.60 (1H, *br s*, H-4), 4.33 (1H, *d*, *J* = 8 Hz, H-1′), 4.32 (1H, *dd*, *J* = 18, 1 Hz, H-13a), 4.16 (1H, *dd*, *J* = 18, 1 Hz, H-13b), 3.84 (1H, *m*, H-6′a), 3.82 (1H, *m*, H-9), 3.67 (1H, *dd*, *J* = 11, 6 Hz, H-6′b), 3.36–3.26 (3H, *m*, H-3′, 4′ and 5′), 3.15 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 2.58 (1H, *d*, *J* = 17 Hz, H-2a), 2.03 (1H, *d*, *J* = 17 Hz, H-2b), 1.92 (1H, m, H-6), 1.80-1.52 (4H, *m*, H_2-7 and 8), 1.24 (3H, *d*, *J* = 6 Hz, H_3-10), 1.11 (3H, *s*, H_3-12), 1.02 (3H, *s*, H_3-11); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; CD nm (Δ*ε*): 219 (+2.24), 278 (−0.11), 335 (+0.51) (*c* = 3.94 × 10^{-5} M, MeOH); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 411.1983 $[M+Na]^+$ (calcd for $C_{19}H_{32}O_8Na$, 411.1989).

4.4.7. Euodionoside G (7)

Amorphous powder; $[\alpha]_D^{27}$ +5.9 (*c* = 0.19, MeOH); IR ν_{max} (film) cm^{-1}: 3367, 2927, 1651, 1077, 1040; UV λ_{max} (MeOH) nm (log*ε*): 237 (3.57); ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 6.17 (1H, *br s*, H-4), 4.53 (2H, *dd*, *J* = 18, 2 Hz, H-13a), 4.37 (1H, *dd*, *J* = 18, 2 Hz, H-13b), 4.33 (1H, *d*, *J* = 8 Hz, H-1′), 3.88 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.68 (1H, *m*, H-9), 3.62 (1H, *dd*, *J* = 12, 6 Hz, H-6′b), 3.36–3.15 (4H, *m*, H-2′, 3′, 4′ and 5′), 2.54 (1H, *d*, *J* = 18 Hz, H-2a), 2.03 (1H, *t*, *J* = 6 Hz, H-6), 2.03 (1H, *d*, *J* = 18 Hz, H-2b), 1.80–1.52 (4H, *m*, H_2-7 and 8), 1.60 (3H, *d*, *J* = 6 Hz, H_3-10), 1.11 (3H, *s*, H_3-11), 1.03 (3H, *s*, H_3-12); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; CD nm (Δ*ε*): 235 (+1.78), 276 (−0.21), 337 (+0.29) (*c* = 3.22 × 10^{-5} M, MeOH); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 411.1995 $[M+Na]^+$ (calcd for $C_{19}H_{32}O_8Na$, 411.1989).

4.4.8. Enzymatic hydrolysis of euodionosides A (1)–E (5) and G (7)

Euodionoside A (**1**) (3.8 mg) in 2 ml of H_2O was hydrolyzed with emulsin (6.0 mg) and crude hesperidinase (6.0 mg) for 15 h at 37 °C. The reaction mixture was evaporated to dryness, and then the methanolic solution was absorbed on silica gel and subjected to silica gel CC (20 g, *Φ* = 18 mm, *L* = 18 cm) with $CHCl_3$ (100 ml) and $CHCl_3$–MeOH (19:1, 100 ml, 9:1, 100 ml, 17:3, 100 ml and 7:3, 300 ml), 12 ml fractions being collected. An aglycone (**1a**) (1.8 mg, 82%) and D-glucose (1.3 mg, 77%) were recovered in fractions 15–25 and 37–47, respectively. Aglycone (**1a**): An amorphous powder, $[\alpha]_D^{25}$ +27.9 (*c* = 0.12, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 7.08 (1H, *d*, *J* = 16 Hz, H-7), 6.20 (1H, *d*, *J* = 16 Hz, H-8), 3.85 (1H, *dddd*, *J* = 12, 10, 7, 4 Hz, H-3), 2.28 (3H, *s*, H_3-10), 2.24 (1H, *ddd*, *J* = 15, 7, 2 Hz, H-4pseudo-eq), 1.73 (1H, *dd*, *J* = 15, 10 Hz, H-4pseudo-ax), 1.48 (1H, *dd*, *J* = 12, 12 Hz, H-2ax), 1.33 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2eq), 1.24 (3H, *s*, H_3-11) 1.17 (3H, *s*, H_3-13), 0.96 (3H, *s*, H_3-12); ^{13}C NMR (CD_3OD, 100 MHz) *δ*: 143.8 (C-7), 134.6 (C-8), 63.9 (C-3), 44.3 (C-2), 40.0 (C-4), 36.0 (C-1), 27.6

(C-12), 27.3 (C-10), 25.2 (C-11), 21.4 (C-13), singlet carbons (C-5, 6 and 9) were not observed; HR-ESI-TOF-MS (positive-ion mode) *m/z*: 247.1307 $[M+Na]^+$ (calcd for $C_{13}H_{20}O_3Na$, 247.1304). D-Glucose: $[\alpha]_D^{25}$ +32.2° ($c = 0.087$, H_2O, 24 h after being dissolved in the solvent).

From euodionoside B (**2**) (12 mg), 6.8 mg (91%) of an aglycone (**2a**) and 4.9 mg (83%) of D-glucose were obtained: Aglycone (**2a**): an amorphous powder; $[\alpha]_D^{25}$ +8.4 ($c = 0.31$, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ: 5.85 (1H, *dd*, $J = 16$, 1 Hz, H-7), 5.67 (1H, *dd*, $J = 16$, 6 Hz, H-8), 4.30 (1H, *qd*, $J = 6$, 1 Hz, H-9), 3.81 (1H, *dddd*, $J = 12$, 10, 7, 4 Hz, H-3), 2.20 (1H, *ddd*, $J = 15$, 7, 2 Hz, H-4pseudo-ax), 1.71 (1H, *dd*, $J = 15$, 10 Hz, H-4pseuo-eq), 1.46 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.28 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.23 (3H, *d*, $J = 6$ Hz, H_3-10), 1.20 (3H, *s*, H_3-13), 1.15 (3H, *s*, H_3-11), 0.95 (3H, *s*, H_3-12); ^{13}C NMR (CD_3OD, 100 MHz) δ: 140.1 (C-8), 124.4 (C-7), 72.0 (C-6), 68.7 (C-9), 66.1 (C-5), 63.8 (C-3), 44.5 (C-2), 40.2 (C-4), 36.0 (C-1), 27.2 (C-12), 25.3 (C-11), 23.9 (C-10), 21.8 (C-13); HR-ESI-TOFMS (positive-ion mode) *m/z*: 249.1466 $[M+Na]^+$ (calcd for $C_{13}H_{22}O_5Na$, 249.1461). D-Glucose, $[\alpha]_D^{24}$ +41.3° ($c = 0.33$, H_2O, 24 h after being dissolved in the solvent).

From euodionoside B (**3**) (17 mg), 5.2 mg (49%) of an aglycone (**3a**) and 5.8 mg (69%) of D-glucose were obtained. Aglycone (**3a**): An amorphous powder; $[\alpha]_D^{24}$ +5.2 ($c = 0.41$, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ: 5.85 (1H, *dd*, $J = 16$, 1 Hz, H-7), 5.67 (1H, *dd*, $J = 16$, 6 Hz, H-8), 4.30 (1H, *qd*, $J = 6$, 1 Hz, H-9), 3.81 (1H, *dddd*, $J = 12$, 10, 7, 4 Hz, H-3), 2.20 (1H, *ddd*, $J = 15$, 7, 2 Hz, H-4pseudo-eq), 1.71 (1H, *dd*, $J = 15$, 10 Hz, H-4pseudo-ax), 1.46 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.28 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.23 (3H, *d*, $J = 6$ Hz, H_3-10), 1.20 (3H, *s*, H_3-13), 1.15 (3H, *s*, H_3-11), 0.95 (3H, *s*, H_3-12); ^{13}C NMR (CD_3OD, 100 MHz) δ: 140.1 (C-8), 124.4 (C-7), 72.0 (C-6), 68.7 (C-9), 66.1 (C-5), 63.8 (C-3), 44.5 (C-2), 40.3 (C-4), 36.0 (C-1), 27.2 (C-12), 25.3 (C-11), 23.9 (C-10), 21.8 (C-13); HR-ESI-TOFMS (positive-ion mode) *m/z*: 249.1466 $[M+Na]^+$ (calcd for $C_{13}H_{22}O_5Na$, 249.1464). D-Glucose, $[\alpha]_D^{27}$ +51.6 ($c = 0.38$, H_2O, 24 h after being dissolved in the solvent).

From euodionoside B (**4**) (3.8 mg), 1.8 mg (79%) of an aglycone (**4a**) and 1.1 mg (65%) of D-glucose were obtained. Aglycone (**4a**): An amorphous powder; $[\alpha]_D^{30}$ +18.6 ($c = 0.19$, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ: 6.06 (1H, *d*, $J = 16$ Hz, H-7), 5.63 (1H, *dd*, $J = 16$, 6 Hz, H-8), 4.34 (1H, *qd*, $J = 6$, 1 Hz, H-9), 4.05 (3H, *m*, H-3), 1.78 (1H, *ddd*, $J = 13$, 5, 2 Hz, H-4eq), 1.73 (1H, *dd*, $J = 13$, 12 Hz, H-4ax), 1.65 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.44 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.27 (3H, *d*, $J = 6$ Hz, H_3-10), 1.20 (3H, *s*, H_3-12), 1.14 (3H, *s*, H_3-13), 0.84 (3H, *s*, H_3-11); ^{13}C NMR (CD_3OD, 100 MHz) δ: 136.2 (C-7), 131.3 (C-8), 83.6 (C-6), 77.8 (C-5), 69.6 (C-9), 65.3 (C-3), 46.5 (C-2), 45.8 (C-4), 40.8 (C-1), 27.5 (C-11), 27.1 (C-13), 26.3 (C-12), 24.3 (C-10); HR-ESI-TOFMS (positive-ion mode) *m/z*: 267.1554 $[M+Na]^+$ (calcd for $C_{13}H_{24}O_4Na$, 247.1566). D-Glucose, $[\alpha]_D^{27}$ +25.0 ($c = 0.073$, H_2O, 24 h after being dissolved in the solvent).

From euodionoside B (**5**) (10.4 mg), 5.3 mg (83%) of an aglycone (**5a**) and 3.5 mg (78%) of D-glucose were obtained Aglycone (**5a**): An amorphous powder; $[\alpha]_D^{25}$ +25.5° ($c = 0.35$, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ: 5.95 (1H, *d*, $J = 16$ Hz, H-7), 5.65 (1H, *dd*, $J = 16$, 9 Hz, H-8), 4.21 (1H, *m*, H-9), 3.73 (1H, *m*, H-3), 3.09 (3H, *s*, -OMe), 1.99 (1H, *ddd*, $J = 14$, 4, 2 Hz, H-4eq), 1.55 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.45 (1H, *dd*, $J = 14$, 12 Hz, H-4ax), 1.35 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.15 (3H, *d*, $J = 6$ Hz, H_3-10), 1.02 (3H, *s*, H_3-12), 0.97 (3H, *s*, H_3-13), 0.74 (3H, *s*, H_3-11); ^{13}C NMR (CD_3OD, 100 MHz): δ 135.6 (C-7), 131.5 (C-8), 83.3 (C-5), 79.0 (C-6), 69.7 (C-9), 65.2 (C-3), 48.4 (–OCH_3), 46.5 (C-2), 40.9 (C-1), 38.8 (C-4), 27.7 (C-11), 26.4 (C-12), 24.2 (C-10), 19.7 (C-13); HR-ESI-TOFMS (positive-ion mode) *m/z*: 281.1729 $[M+Na]^+$ (calcd for $C_{14}H_{26}O_4Na$, 281.1723). D-Glucose, $[\alpha]_D^{25}$ +49.7 ($c = 0.23$, H_2O, 24 h after being dissolved in the solvent).

From euodionoside G (**7**) (2.5 mg), 1.2 mg (82%) of an aglycone (**7a**) and 1.0 mg (86%) of D-glucose were obtained. Aglycone (**7a**): An amorphous powder; $[\alpha]_D^{25}$ +61.7° ($c = 0.08$, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ: 6.06 (1H, *br s*, H-4), 4.30 (1H, *dd*, $J = 18$, 2 Hz, H-13), 4.15 (1H, *dd*, $J = 18$, 2 Hz, H-13), 3.66 (1H, *qd*, $J = 6$, 6 Hz, H-9), 2.54 (1H, *dd*, $J = 18$, 1 Hz, H-2), 2.03 (1H, *ddd*, $J = 18$, 1, 1 Hz, H-2), 1.94 (1H, *dd*, $J = 5$, 5 Hz, H-6), 1.821.49 (4H, *m*, H_2-7, 8), 1.16 (3H, *d*, $J = 6$ Hz, H_3-10), 1.11 (3H, *s*, H_3-11),1.02 (3H, *s*, H_3-12); ^{13}C NMR (CD_3OD, 100 MHz) δ: 172.3 (C-5), 121.7 (C-4), 68.9 (C-9), 65.1 (C-13), 48.7 (C-2), 48.4 (C-6), 39.9 (C-8), 37.4 (C-1), 28.9 (C-12), 28.0 (C-7), 27.6 (C-11), 23.6 (C-10); HR-ESI-TOF-MS (positive-ion mode) *m/z*: 249.1465 $[M+Na]^+$ (calcd for $C_{13}H_{22}O_2Na$, 249.1461). D-Glucose, $[\alpha]_D^{27}$ +20.4 ($c = 0$ .07, H_2O, 24 h after being dissolved in the solvent).

4.4.9. Preparation of (*R*)- and (*S*)-MPTA esters

A solution of **1a** (0.9 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (27 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardodiimide hydrochloride (EDC) (16 mg) and *N*,*N*-dimethyl-4-aminopyridine (4-DMAP) (11 mg), and then the mixture was occasionally stirred at 25 °C for 30 min. After the addition of 1 ml of CH_2Cl_2, the solution was washed with H_2O (1 ml), 5% HCl (1 ml), $NaHCO_3$–saturated H_2O, and then brine (1 ml), successively. The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), being applied for 18 cm, developed with $CHCl_3$–$(CH_3)_2CO$ (19:1) for 9 cm, and then eluted with $CHCl_3$–MeOH (9:1)] to furnish an ester, **1b** (1.2 mg, 68%). Through a similar procedure, **1c** (1.3 mg, 73%) was prepared from **1a** (0.9 mg) using (*S*)-MTPA (27 mg), EDC (17 mg), and 4-DMAP (12 mg).

(3*R*,5*R*,6*S*,7*E*)-Megastigman-7-en-5,6-epoxy-3-ol-9-one 3-*O*-(*R*)-MTPA ester (**1b**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.72–7.70 (2H, *m*, aromatic protons), 7.45–7.40 (3H, *m*, aromatic protons), 6.94 (1H, *d*, $J = 16$ Hz, H-7), 6.30 (1H, *d*, $J = 16$ Hz, H-8), 5.17 (1H, *m*, H-3), 3.53 (3H, *br s*, –OCH_3), 2.41 (1H, *ddd*, $J = 15$, 8, 2 Hz, H-4pseudo-eq), 2.28 (3H, *s*, H_3-10), 2.03 (1H, *dd*, $J = 15$, 10 Hz, H-4pseudo-ax), 1.70 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.41 (1H, *m*, H-2eq), 1.29 (3H, *s*, H_3-11), 1.20 (3H, *s*, H_3-13), 0.99 (3H, *s*, H_3-12); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 463.1700 $[M+Na]^+$ (calcd for $C_{26}H_{27}O_5F_3Na$, 463.1708).

(3*R*,5*R*,6*S*,7*E*)-Megastigman-7-en-5,6-epoxy-3-ol-9-one 3-*O*-(*S*)-MTPA ester (**1c**): An amorphous powder, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.70–7.69 (2H, *m*, aromatic protons), 7.54–7.51 (3H, *m*, aromatic protons), 6.94 (1H, *d*, $J = 16$ Hz, H-7), 6.30 (1H, *d*, $J = 16$ Hz, H-8), 5.17 (1H, *m*, H-3), 3.53 (3H, *q*, $J = 1$ Hz, -OCH_3), 2.35 (1H, *ddd*, $J = 15$, 8, 2 Hz, H-4pseudo-eq), 2.28 (3H, *s*, H_3-10), 1.92 (1H, *dd*, $J = 15$, 9 Hz, H-4pseudo-ax), 1.77 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.46 (1H, *m*, H-2eq), 1.30 (3H, *s*, H_3-11), 1.18 (3H, *s*, H_3-13),1.00 (3H, *s*, H_3-12); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 463.1705 $[M+Na]^+$ (calcd for $C_{26}H_{27}O_5F_3Na$, 463.1708).

From **2a** (4.3 mg and 2.5 mg), **2b** (5.0 mg, 40%) and **2c** (2.3 mg, 36%), respectively, were prepared.

(3*R*,5*R*,6*S*,7*E*,9*S*)-Megastigman-7-en-5,6-epoxy-3,9-diol 3,9-*O*-(*R*)-MTPA diester (**2b**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.517.49 (4H, *m*, aromatic protons), 7.417.36 (6H, *m*, aromatic protons), 5.98 (1H, *d*, $J = 15$ Hz, H-7), 5.74 (1H, *dd*, $J = 15$, 7 Hz, H-8), 5.64 (1H, *dq*, $J = 7$, 7 Hz, H-9), 5.12 (1H, *dddd*, $J = 12$, 10, 8, 4 Hz, H-3), 3.55 (3H, *br s*, –OCH_3), 3.52 (3H, *br s*, –OCH_3), 2.35 (1H, *ddd*, $J = 15$, 8, 2 Hz, H-4pseudo-eq), 1.98 (1H, *dd*, $J = 15$, 10 Hz, H-4pseudo-ax), 1.66 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.43 (3H, *d*, $J = 7$ Hz, H_3-10), 1.35 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.131 (3H, *s*, H_3-13), 1.126 (3H, *s*, H_3-11), 0.91 (3H, *s*, H_3-12); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 681.2250 $[M+Na]^+$ (calcd for $C_{33}H_{36}O_7F_6Na$, 681.2257).

(3*R*,5*R*,6*S*,7*E*,9*S*)-Megastigman-7-en-5,6-epoxy-3,9-diol 3,9-*O*-(*S*)-MTPA diester (**2c**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.527.49 (4H, *m*, aromatic protons), 7.417.35 (6H, *m*, aromatic protons), 5.98 (1H, *d*, $J = 15$ Hz, H-7), 5.74 (1H, *dd*, $J = 15$, 7 Hz, H-8), 5.61 (1H, *dq*, $J = 6$, 6 Hz, H-9), 5.14 (1H, *dddd*, $J = 12$, 10, 7, 4 Hz, H-3), 3.53 (3H, *br s*, –OCH_3), 3.52 (3H, *br s*, –OCH_3), 2.30 (1H, *ddd*, $J = 15$, 7, 1 Hz, H-4pseudo-eq), 1.89 (1H, *dd*, $J = 15$, 10 Hz, H-4pseudo-ax), 1.75 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.45 (1H, *ddd*, $J = 12$, 4, 1 Hz, H-2eq), 1.38 (3H, *d*, $J = 6$ Hz, H_3-10), 1.19 (3H, *s*, H_3-11), 1.14 (3H, *s*, H_3-13), 0.97 (3H, *s*, H_3-12); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 681.2242 $[M+Na]^+$ (calcd for $C_{33}H_{36}O_7F_6Na$, 681.2257).

From **3a** (2.9 and 2.3 mg), **3b** (5.5 mg, 65%) and **3c** (2.7 mg, 32%), respectively were prepared.

(3*R*,5*R*,6*S*,7*E*,9*S*)-Megastigman-7-en-5,6-epoxy-3,9-diol 3,9-*O*-(*R*)-MTPA diester (**3b**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.517.50 (4H, *m*, aromatic protons), 7.407.36 (6H, *m*, aromatic protons), 5.98 (1H, *d*, $J = 15$ Hz, H-7), 5.74 (1H, *dd*, $J = 15$, 7 Hz, H-8), 5.64 (1H, *dq*, $J = 7$, 7 Hz, H-9), 5.12 (1H, *dddd*, $J = 12$, 10, 8, 4 Hz, H-3), 3.55 (3H, *br s*, –OCH_3), 3.52 (3H, *br s*, –OCH_3), 2.35 (1H, *ddd*, $J = 15$, 8, 2 Hz, H-4eq), 1.98 (1H, *dd*, $J = 15$, 10 Hz, H-4ax), 1.66 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.43 (3H, *d*, $J = 7$ Hz, H_3-10), 1.35 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.13 (3H, *s*, H_3-11), 1.13 (3H, *s*, H_3-13), 0.91 (3H, *s*, H_3-12); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 681.2250 $[M+Na]^+$ (calcd for $C_{33}H_{36}O_7F_6Na$, 681.2257).

(3*R*,5*R*,6*S*,7*E*,9*S*)-Megastigman-7-en-5,6-epoxy-3,9-diol 3,9-*O*-(*S*)-MTPA diester (**3c**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.537.49 (4H, *m*, aromatic protons), 7.427.35 (6H, *m*, aromatic protons), 5.98 (1H, *d*, $J = 15$ Hz, H-7), 5.74 (1H, *dd*, $J = 15$, 7 Hz, H-8), 5.61 (1H, *dq*, $J = 7$, 7 Hz, H-9), 5.14 (1H, *dddd*, $J = 12$, 10, 8, 4 Hz, H-3), 3.54 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.52 (3H, *q*, $J = 1$ Hz, -OCH_3), 2.30 (1H, *ddd*, $J = 15$, 8, 2 Hz, H-4pseudo-eq), 1.89 (1H, *dd*, $J = 15$, 10 Hz, H-4pseudo-ax), 1.75 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.45 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.38 (3H, *d*, $J = 7$ Hz, H_3-10), 1.19 (3H, *s*, H_3-11), 1.14 (3H, *s*, H_3-13), 0.97 (3H, *s*, H_3-12); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 681.2268 $[M+Na]^+$ (calcd for $C_{33}H_{36}O_7F_6Na$, 681.2257).

From **4a** (0.9 mg each), **4b** (1.6 mg, 64%) and **4c** (1.2 mg, 48%) were prepared.

(3*R*,5*S*,6*S*,7*E*,9*S*)-Megastigman-7-ene-3,5,6,9-tetrol 3,9-*O*-(*R*)-MTPA diester (**4b**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.547.51 (4H, *m*, aromatic protons), 7.417.37 (6H, *m*, aromatic protons), 6.22 (1H, *d*, $J = 15$ Hz, H-7), 5.68 (1H, *dd*, $J = 16$, 7 Hz, H-8), 5.66 (1H, *q*, $J = 6$ Hz, H-9), 5.48 (1H, *m*, H-3), 3.56 (3H, *q*, $J = 1$ Hz, -OCH_3), 3.55 (3H, *q*, $J = 1$ Hz, -OCH_3), 1.99 (1H, *ddd*, $J = 13$, 5, 2 Hz, H-4eq), 1.94 (1H, *dd*, $J = 13$, 12 Hz, H-4ax), 1.72 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.62 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.52 (3H, *s*, H_3-13), 1.46 (3H, *d*, $J = 6$ Hz, H_3-10), 1.11 (3H, *s*, H_3-12), 0.80 (3H, *s*, H_3-11); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 699.2383 $[M+Na]^+$ (calcd for $C_{33}H_{38}O_8F_6Na$, 699.2363).

(3*R*,5*S*,6*S*,7*E*,9*S*)-Megastigman-7-ene-3,5,6,9-tetrol 3,9-*O*-(*S*)-MTPA diester (**4c**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.537.51 (4H, *m*, aromatic protons), 7.427.36 (6H, *m*, aromatic protons), 6.30 (1H, *d*, $J = 15$ Hz, H-7), 5.77 (1H, *dd*, $J = 16$, 7 Hz, H-8), 5.65 (1H, *q*, $J = 6$ Hz, H-9), 5.49 (1H, *m*, H-3) 3.56 (3H, *q*, $J = 1$ Hz, -OCH_3), 3.53 (3H, *q*, $J = 1$ Hz, –OCH_3), 1.92 (1H, *dd*, $J = 13$, 12 Hz, H-4ax), 1.86 (1H, *ddd*, $J = 13$, 5, 2 Hz, H-4eq), 1.82 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.70 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.52 (3H, *s*, H_3-13), 1.42 (3H, *d*, $J = 6$ Hz, H_3-10), 1.09 (3H, *s*, H_3-12, 0.86 (3H, *s*, H_3-11); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 699.2373 $[M+Na]^+$ (calcd for $C_{33}H_{38}O_8F_6Na$, 681.2257).

From **5a** (2.6 mg each), **5b** (3.4 mg, 49%) and **5c** (3.9 mg, 56%) were prepared.

(3*R*,5*S*,6*S*,7*E*,9*S*)-Megastigman-7-ene-5-methoxy-3,5,6,9-tetrol 3,9-*O*-(*R*)-MTPA diester (**5b**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.547.51 (4H, *m*, aromatic protons), 7.427.34 (6H, *m*, aromatic protons), 6.25 (1H, *d*, $J = 16$ Hz, H-7), 5.67 (1H, *qdd*, $J = 6$, 6, 6 Hz, H-9), 5.63 (1H, *dd*, $J = 16$, 6 Hz, H-8), 5.21 (1H, *dddd*, $J = 12$, 12, 4, 4 Hz, H-3), 3.56 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.55 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.26 (3H, *s*, –OCH_3 on C-5), 2.28 (1H, *ddd*, $J = 14$, 4, 2 Hz, H-4eq), 1.76 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.73 (1H, *dd*, $J = 14$, 12 Hz, H-4ax), 1.57 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.43 (3H, *d*, $J = 6$ Hz, H_3-10), 1.15 (3H, *s*, H_3-12), 1.00 (3H, *s*, H_3-13), 0.80 (3H, *s*, H_3-11); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 713.2516 $[M+Na]^+$ (calcd for $C_{34}H_{40}O_8F_6Na$, 713.2519).

(3*R*,5*S*,6*S*,7*E*,9*S*)-Megastigman-7-ene-5-methoxy-3,6,9-triol 3,9-*O*-(*S*)-MTPA diester (**5c**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.547.52 (4H, *m*, aromatic protons), 7.427.34 (6H, *m*, aromatic protons), 6.30 (1H, *d*, $J = 15$ Hz, H-7), 5.73 (1H, *dd*, $J = 15$, 7 Hz, H-8), 5.66 (1H, *qdd*, $J = 6$, 6, 6 Hz, H-9), 5.23 (1H, *dddd*, $J = 12$, 12, 4, 4 Hz, H-3), 3.55 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.53 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.26 (3H, *s*, –OCH_3 on C-5), 2.22 (1H, *ddd*, $J = 14$, 4, 2, Hz, H-4eq), 1.85 (1H, *dd*, $J = 12$, 12 Hz, H-2ax), 1.67 (1H, *dd*, $J = 14$, 12 Hz, H-4ax), 1.63 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.40 (3H, *d*, $J = 6$ Hz, H_3-10), 1.19 (3H, s, H_3-12), 0.98 (3H, s, H_3-13), 0.85 (3H, *s*, H_3-11); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 713.2523 $[M+Na]^+$ (calcd for $C_{34}H_{40}O_8F_6Na$, 713.2519).

From **7a** (0.7 mg and 0.5 mg), **7b** (1.5 mg, 74%) and **7c** (1.4 mg, 69%), respectively, were prepared.

(4*Z*,6*R*,9*R*)-Megastigman-4-ene-9,13-diol-3-one 9,13-*O*-(*R*)-MTPA diester (**7b**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.447.42 (4H, *m*, aromatic protons), 7.367.29 (6H, *m*, aromatic protons), 5.82 (1H, *br s*, H-4), 5.00 (1H, *m*, H-9), 4.81 (1H, *dd*, $J = 15$, 1 Hz, H-13), 4.67 (1H, *dd*, $J = 15$, 1 Hz, H-13), 3.47 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.41 (3H, *q*, $J = 1$ Hz, –OCH_3), 2.26 (1H, *d*, $J = 18$ Hz, H-2), 2.00 (1H, *d*, $J = 18$ Hz, H-2), 1.77 (1H, *dd*, $J = 5$, 5 Hz, H-6), 1.551.64 (4H, *m*, H_2-7 and 8), 1.20 (3H, *d*, $J = 6$ Hz, H_3-10), 0.92 (3H, *s*, H_3-11), 0.86 (3H, *s*, H_3-12); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 681.2270 $[M+Na]^+$ (calcd for $C_{33}H_{36}O_7F_6Na$, 681.2262).

(4*Z*,6*R*,9*R*)-Megastigman-4-ene-9,13-diol-3-one 9,13-*O*-(*S*)-MTPA diester (**7c**): An amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.457.42 (4H, *m*, aromatic protons), 7.367.33 (3H, *m*, aromatic protons), 7.30-7.29 (3H, m, aromatic protons), 5.78 (1H, *br s*, H-4), 5.00 (1H, *m*, H-9), 4.70 (1H, *dd*, $J = 15$, 1 Hz, H-13), 4.61 (1H, *dd*, $J = 15$, 1 Hz, H-13), 3.48 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.46 (3H, *q*, $J = 1$ Hz, –OCH_3), 2.20 (1H, *d*, $J = 18$ Hz, H-2), 1.96 (1H, *d*, $J = 18$ Hz, H-2), 1.70 (1H, *dd*, $J = 5$, 5 Hz, H-6), 1.501.62 (4H, *m*, H_2-7 and 8), 1.26 (3H, *d*, $J = 6$ Hz, H_3-10), 0.92 (3H, *s*, H_3-11), 0.85 (3H, *s*, H_3-12); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 681.2264 $[M+Na]^+$ (calcd for $C_{33}H_{36}O_7F_6Na$, 681.2262).

4.4.10. Known compounds isolated

Spinoside A (**8**): Amorphous powder; $[\alpha]_D^{22}$ +26.5 ($c = 0.21$, MeOH); ^{13}C NMR (CD_3OD, 100 MHz) δ: 201.3 (C-3), 169.1 (C-5), 133.8 (C-7 and 8), 123.2 (C-4), 101.3 (C-1′), 79.4 (C-6), 78.3 (C-3′), 78.1 (C-5′), 75.0 (C-2′), 74.7 (C-9), 71.8 (C-4′), 62.9 (C-6′), 61.4 (C-13), 50.8 (C-2), 42.8 (C-1), 24.3 (C-12), 23.4 (C-11), 22.3 (C-10); CD nm ($\Delta\varepsilon$) 238 (+12.0), 320 (−0.70) (Çaliş et al., 2002). Staphylionoside D (**9**): Amorphous powder; $[\alpha]_D^{25}$ −40.0 ($c = 0.25$, MeOH) (Yu et al., 2005). Corchoionoside C (**10**): Amorphous powder; $[\alpha]_D^{25}$ +61.5 ($c = 0.39$, MeOH) (Yoshikawa et al., 1997). (*Z*)-3-Hexenyl β-D-glucopyranoside (**11**): Amorphous powder; $[\alpha]_D^{25}$ −30.2 ($c = 2.39$, MeOH) (Mizutani et al., 1988). 3,7-Dimethyloct-1-ene-3,6,7-triol 6-*O*-β-D-glucopyranoside (**12**): Amorphous powder; $[\alpha]_D^{25}$ −3.8 ($c = 0.41$, MeOH) (Manns, 1995). (+)-Catechin (**13**): Amorphous powder; $[\alpha]_D^{30}$ +30.8 [$c = 0.49$, H_2O: $(CH_3)_2CO$ = 1:1] (Nay et al., 2001). Syringin (**14**): Amorphous powder; $[\alpha]_D^{25}$ −40.8 ($c = 0.37$, MeOH) (DellaGreca et al., 1998). 1-β-D-Glucopyranosyloxy-3-methoxy-5-hydroxybenzene (**15**): An off-white amorphous powder; $[\alpha]_D^{25}$ −59.7 ($c = 0.71$, MeOH) (Sakar et al., 1993).

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