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Crotonionosides A–G: Megastigmane glycosides from leaves of *Croton cascarilloides* Räuschel

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ABSTRACT

From the 1-BuOH-soluble fraction of a MeOH extract of leaves of *Croton cascarilloides*, collected in Okinawa, Japan, seven megastigmane glycosides, named crotonionosides A–G, were isolated together with three known megastigmane glucosides, dendranthemosides A and B, and citroside A. This structures were elucidated by a combination of spectroscopic analyses, HPLC analyses, and application of the modified Mosher's method.

1. Introduction

Croton cascarilloides Räuschel (Euphorbiaceae), an evergreen tree of about 0.5–2 m in height, is distributed in Japan (Kagoshima and Okinawa), Taiwan, Thailand and Vietnam. *C. oblongifolius* of the same genus in Thailand has been used as traditional medicine, e.g., as a tonic (leaves), teniacide (flowers), dysmenorrheal (fruit), and purgative (seeds), and for dyspepsia (bark) and dysentery (roots) (Ngamrojnavanich et al., 2003). Furthermore TPA (12-*O*-tetradecanoyl phorbol 13-acetate) from *C. tiglium*, has been used as a tumor promoter reagent (Evans and Taylor, 1983), and plaunotol from *C. sublyratus*, used as a cytoprotective antiulcer agent (Ogiso et al., 1978). In this study, we investigated the chemical constituents of *C. cascarilloides*, which is the only *Croton* species growing wild in Japan.

From the 1-BuOH-soluble fraction of a MeOH extract of *C. cascarilloides*, seven new megastigmane glycosides (**1**–**7**) were isolated together with three known megastigmane glucosides, dendranthemosides A and B (**8** and **9**) (Otsuka et al., 1992), and citroside A (**10**) (Umehara et al., 1988). This paper deals with structural elucidation of the new megastigmane glycosides.

2. Results and discussion

Air-dried leaves of *C. cascarilloides* were extracted with MeOH three times and the concentrated MeOH extract was partitioned with solvents of increasing polarity. The 1-BuOH-soluble fraction was separated by means of various chromatographic procedures including column chromatography (CC) on a highly-porous synthetic resin (Diaion HP-20), nomal and reversed-phase octadecyl silica gel (ODS) CC, droplet counter-current chromatography (DCCC), and high-performance liquid chromatography (HPLC), to afford ten compounds (**1**–**10**). The details and yields are given in Section 4. The structures of the new megastigmane glycosides (**1**–**7**) were elucidated on the basis of spectroscopic evidence and their absolute structures were confirmed by the modified Mosher's method (Ohtani et al., 1991), comparison of HPLC retention times with those of authentic compounds and the β-D-glucosylation-induced shift-trend rule (Kasai et al., 1977). Known compounds (**8**–**10**) were identified by comparison of the spectroscopic data with those reported in the literature.

Crotonionoside A (**1**), $[\alpha]_D^{26}$ −20.5, was isolated as an amorphous powder and its molecular formula was determined to be $C_{29}H_{42}O_{11}$ by HR-ESI-TOF–MS. The IR spectra exhibited absorptions for hydroxyl groups (3389 cm^{-1}), a conjugated ester carbonyl group (1701 cm^{-1}), and an aromatic ring (1596 cm^{-1}). The UV spectrum also indicated the presence of an aromatic ring (298 nm). In the ^1H NMR spectrum, signals for two doublet and two singlet methyls, three aromatic protons coupled in an ABX system, two pairs of *trans* olefinic protons, an anomeric proton (δ_H 4.34), and a methoxyl resonances were observed. The ^{13}C NMR spectrum displayed six signals assignable to a β-glucopyranoside, and six aromatic, two sp^2, one carbonyl and methoxyl carbons, the remaining 13 resonances comprising those of four methyls, two methylenes, three methines, a disubstituted *trans* double bond and two quaternary

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carbons. From the above evidence, the structure of crotonionoside A (**1**) was expected to be a megastigmane *O*-β-D-glucopyranoside with a feruloyl moiety. The absolute configuration of glucose was determined by HPLC analysis of the hydrolyzate of **1** using a chiral detector. The NMR spectroscopic data for the megastigmane skeleton was essentially the same as at for dendranthemoside A (**8**), a known megastigmane glucoside isolated from the title plant and originally from *Dendranthema shiwogiku* (Otsuka et al., 1992; Otsuka et al., 1993). The downfield shift of C-6′ was expected to be induced by esterification of the feruloyl moiety, and the linkage was confirmed by HMBC correlations between H-6′a and 6′b (δ_H 4.44 and 4.43) and C-9″ (δ_C 168.9). On mild alkaline hydrolysis of **1** with CH_3ONa, a glucoside (**1a**) and methyl ferulate were obtained. The NMR spectra of 1a were the same as those of **8**. However, turpinionoside A (**11**), which is an epimer of **8** at the 9-position, isolated from *Turpinia ternata* (Yu et al., 2002), also showed identical NMR spectra to those of **8**, and the optical rotation values of **8** and **11** were relatively too close to be distinguishable from each other [**8**: 44.2 (*c* 0.54, MeOH) and **11**: 34.8 (*c* 0.86, MeOH)]. The HPLC retention times of **8** and **11**, whose absolute structures have been unambiguously determined (Otsuka et al., 1993; Yu et al., 2002), were 16.0 min and 18.5 min, respectively, and the retention time of **1a** was found to be 16 min under the same conditions. Therefore, crotonionoside A (**1**) was elucidated to be (3*S*,5*R*,6*S*,7*E*,9*R*)-7-megastigmene-3,6,9-triol 3-*O*-β-D-(6′-*O*-feruloyl)glucopyranoside, as shown in Fig. 1.

Crotonionoside B (**2**), $[\alpha]_D^{26}$ −29.0, was isolated as an amorphous powder and its molecular formula was determined to be $C_{30}H_{44}O_{12}$ by HR-ESI-TOF–MS. The NMR spectra showed good similarity to those of **2**, except for appearance of a single aromatic signal for two protons in the ^{1}H NMR spectrum, instead of the ABX aromatic protons, observed of **1**. The ^{13}C NMR spectrum also indicated that the aromatic ring of the acyl moiety was substituted symmetrically and possessed two methoxyl groups. Thus, the feruloyl ester found in **1** must be replaced by a sinapoyl ester. The deacylated compound (**2a**) was confirmed to be identical with **8** on HPLC analysis. Therefore, crotonionoside B (**2**) was elucidated to be (3*S*,5*R*,6*S*,7*E*,9*R*)-7-megastigmene-3,6,9-triol 3-*O*-β-D-(6′-*O*-sinapoyl)glucopyranoside, as shown in Fig. 1.

Crotonionoside C (**3**), $[\alpha]_D^{24}$ 69.9, was isolated as an amorphous powder and its molecular formula was determined to be $C_{24}H_{42}O_{12}$. The IR spectrum indicated the presence of hydroxyl functions (3367 cm^{-1}) and an olefinic structure (1648 and 1457 cm^{-1}). The ^{13}C NMR spectrum showed the presence of five signals assignable to apiofuranose, while an anomeric proton (δ_H 5.39) was observed in the proton spectrum. The remaining 19 carbon resonances were similar to those of **8**, except for the signal for the 2′-position (δ_C 78.7) in the glucopyranose moiety. This downfield shift was indicative that the apiofuranose moiety was linked to the C-2′ hydroxyl group of the glucopyranose moiety, and the linkage was confirmed by the HMBC correlation cross peak between H-2′ (δ_H 3.32) and C-1″ (δ_C 110.5). Enzymatic hydrolysis of crotonionoside C (**3**) gave an aglycone (**3a**) and D series sugars. The absolute configuration of the aglycone was expected to be 3*S* according to the β-D-glucosylation-induced shift-trend rule (Kasai et al., 1977). The absolute configuration at the 9-position was determined by comparison of the HPLC retention times of aglycones (**8a** and **11a**) of **8** and **11**. The retention times of **8a** and **11a** were 16 min and 19 min, respectively, and a peak for the aglycone (**3a**) appeared at 16 min. Therefore, the aglycone (**3a**) of **3** was identical with **8a** and thus structure of **3** was elucidated to be (3*S*,5*R*,6*S*,7*E*,9*R*)-7-megastigmene-3,6,9-triol 3-*O*-β-D-(2′-*O*-β-D-apiofuranosyl)glucopyranoside (**3**), as shown in Fig. 1.

Crotonionosides D (**4**), $[\alpha]_D^{24}$ 38.5, and E (**5**), $[\alpha]_D^{22}$ −50.6, were isolated as amorphous powders and their molecular formulae were determined to be $C_{31}H_{46}O_{14}$ and $C_{35}H_{52}O_{16}$, respectively, by HR-ESI-TOF–MS. The NMR spectra of **4** and **5** indicated they were analogous compounds to crotonionoside C (**3**), and seven carbon signals for *p*-hydroxybenzoic acid were present in the ^{13}C NMR spectrum of **4** and **11** carbon resonances for sinapic acid in that of **5**. The linkages of the *p*-hydroxybenzoyl and sinapoyl moieties were confirmed by the HMBC correlation cross peaks between H-5″a and 5″b (**4**: δ_H 4.43 and 4.32) and C-7‴ (δ_C 168.1), and H-5″a and 5″b (5: δ_H 4.38 and 4.27) and C-9‴ (δ_C 169.0). On mild alkaline hydrolysis of **4** and **5** with CH_3ONa, methyl *p*-hydroxybezoate was obtained from **4**, and methyl sinapate from **5**, respectively, and common glucoside, crotonionoside C (**3**). Therefore, crotonionosides D and E (**4** and **5**) were elucidated to be (3*S*,5*R*,6*S*,7*E*,9*R*)-7-megastigmene-3,6,9-triol 3-*O*-β-D-[2′-*O*-β-D-(6″-*O*-*p*-hydroxybenzoyl)apiofuranosyl]glucopyranoside (**4**) and 3-*O*-β-D-[2′-*O*-β-D-(6″-*O*-sinapoyl)apiofuranosyl]glucopyranoside (**5**), respectively, as shown in Fig. 1.

Crotonionoside F (**6**), $[\alpha]_D^{23}$ −55.8, was isolated as an amorphous powder and its molecular formula was determined to be $C_{24}H_{42}O_{11}$ by HR-ESI-TOF–MS. The IR spectrum indicated the presence of hydroxyl groups (3366 cm^{-1}) and an olefinic structure (1647 and 1457 cm^{-1}). The ^{1}H and ^{13}C NMR spectra showed the presence of five signals assignable to apiofuranose, the remaining 19 ^{13}C NMR resonances being similar to those of alangionoside G (Otsuka et al., 1995a), except for C-2′ (δ_C 78.9). That the apiofuranose was attached to C-2′ position was confirmed by the HMBC correlation cross peak between H-2′ (δ_H 3.32) and C-1″ (δ_C: 110.6). Since, on enzymatic hydrolysis of **6**, the aglycone (6a) and D series sugars were obtained, the absolute configuration at the 3-position was expected to be *S* on the application of the β-D-glucosylation-induced shift-trend rule and that at the 9-position was determined to be *R*

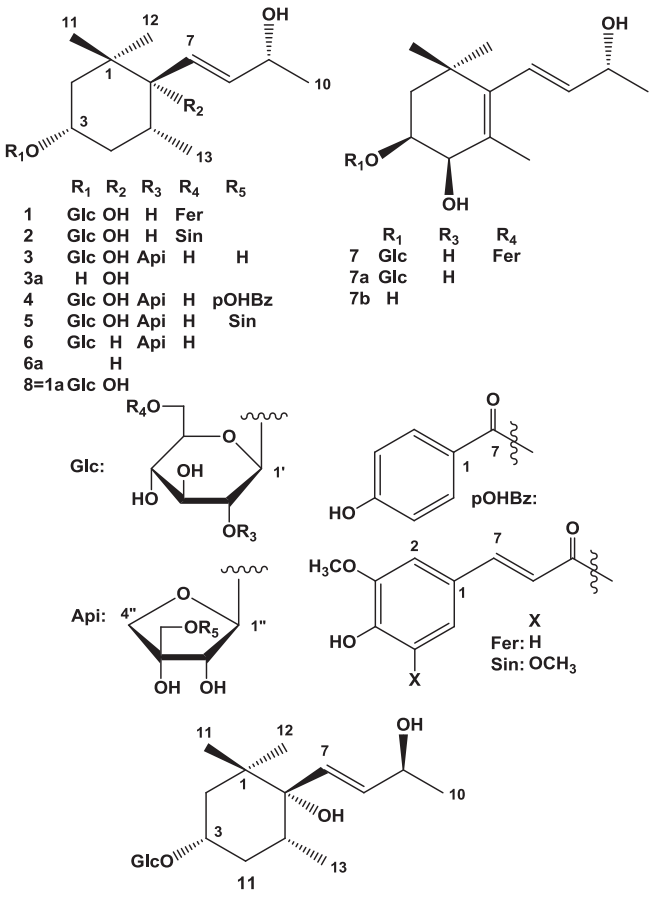

Fig. 1. Structures of isolated and reference compounds.

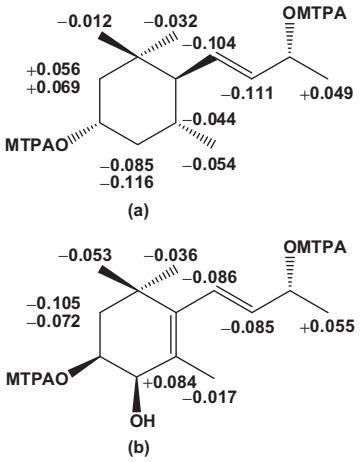

Fig. 2. Results with the modified Mosher's method for **6b** and **6c** (**a**), and **7c** and **7d** (**b**). $\Delta\delta$ Values are in ppm (δ_S–δ_R).

with the modified Mosher's method for 6a (Fig. 2). Therefore, 6 was elucidated to be (3*S*,5*R*,6*R*,7*E*,9*R*)-7-megastigmene-3,9-diol 3-*O*-β-D-(2′-*O*-β-D-apiofuranosyl)glucopyranoside (**6**), as shown in Fig. 1.

Crotonionoside G (**7**), $[\alpha]_D^{23}$ −63.6, was isolated as an amorphous powder and its molecular formula was determined to be $C_{29}H_{40}O_{11}$ by HR-ESI-TOF–MS. The IR spectrum indicated the presence of hydroxyl functions (3343 cm^{-1}), an ester linkage (1712 cm^{-1}), and an olefinic structure (1455 and 1630 cm^{-1}). The ^1H and ^{13}C NMR spectra showed the presence of signals assignable to ferulic acid, the remaining 19 carbon signals being similar to those of plucheoside B (Otsuka et al., 1995b: Uchiyama et al., 1989), except for C-6′ (δ_C: 64.9). The downfield shift at the 6′-position was expected to be induced by the linkage of the feruloyl moiet, and this attachment was confirmed by the HMBC correlation cross-peaks between H-6′a and 6′b (δ_H 4.49 and 4.48) and C-9″ (δ_C 169.0). Mild alkaline hydrolysis of crotonionoside G (**7**) gave a methyl ferulate and the corresponding deacylated glucoside (**7a**), whose NMR spectroscopic data were essentially the same as those of plucheoside B isolated from *Alangium premnifolium* (Otsuka et al., 1995b). Since the absolute configuration of plucheoside Bs, isolated from *A. premnifolium* and also from *Pluchea indica*, has not yet been determined, 7a was enzymatically hydrolyzed to give the aglycone (**7b**) and D-glucose. The absolute configuration of the aglycone (**7b**) was determined to be 3*R*, 4*R* and 9*R* by application of the modified Mosher's method (Fig. 2). Therefore, crotonionoside G (**7**) was elucidated to be (3*S*,4*R*,5*Z*,7*E*,9*R*)-5,7-megastigmadiene-3,4,9-triol 3-*O*-β-D-(6′-*O*-feruloyl)glucopyranoside (**7**).

3. Concluding remarks

Megastigmane derivatives have recently been isolated from many species of plants in many families, from dicotyledons as well as monocotyledons. However, although more than 600 *Croton* species are listed in the International Plant Name Index, there has been only one report of the isolation of a megastigmane from *Croton lechleri* (De Marino et al., 2008). This study implies that megastigmane derivatives are probably present in many *Croton* species.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a JASCO P-1030 polarimeter, where as IR spectra were recorded on a Horiba FT-710 Fourier transform infrared spectrophotometer and UV spectra on a JASCO V-520 UV/Vis spectrophotometer. ^1H- and ^{13}C NMR spectra were obtained on a JEOL JNM α-400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C, with tetramethylsilane (TMS) as an internal standard. Positive-ion HR-ESI-TOF–MS were recorded on an Applied Biosystem QSTAR XL spectrometer.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Chemical, Co., Ltd. (Tokyo, Japan). Silica gel CC was performed on silica gel 60 (Merck, Darmstadt, Germany) and reversed-phase (ODS) open CC (RPCC) on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ = 5 cm, L = 25 cm, linear gradient: MeOH–H_2O (1:9, 2 l) → (9:1, 2 l), 10 g/fraction]. The DCCC (Tokyo Rikakikai, Tokyo, Japan) was equipped with 300 glass columns (Φ = 2 mm, L = 40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–PrOH (9:12:8:2) were used as 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 ODS (Inertsil ODS-3; GL Science, Tokyo, Japan; Φ = 6 mm, L = 250 mm, flow rate; 1.6 ml/min), and the eluate was monitored with 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 *C. cascarilloides* Räuschel (Euphorbiaceae) were collected in Kunigami-son, Kunigami-gun, Okinawa, Japan, in July and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (04-CC-Okinawa-0628).

4.3. Extraction and isolation

Air-dried leaves of *C. cascarilloides* (6.53 kg) were extracted with MeOH (45 l) three times. The MeOH extract was concentrated to 6 l, washed with *n*-hexane (6 l, 59.1 g), methanolic solubles were concentrated to a viscous gum. The later was suspended in H_2O (6 l), and then partitioned with EtOAc (6 l) and 1-BuOH (6 l), successively, to give of EtOAc (100 g) and 1-BuOH-soluble fractions (126 g). The remaining aqueous solubles were concentrated to give a H_2O-soluble-fraction (263 g). The 1-BuOH-soluble fraction was subjected to Diaion HP-20 CC (Φ = 80 mm, L = 57 cm, 1 l/fraction), eluted with H_2O–MeOH (4:1, 6 l), (3:2, 6 l), (2:3, 6 l), and (1:4, 6 l) and MeOH (6 l). The residue (6.38 g) in fractions 10–14 was subjected to silica gel CC (Φ = 36 mm, L = 57 cm, 250 ml/fraction), and eluted with $CHCl_3$ (1.5 l), $CHCl_3$-MeOH (99:1, 1.5 l), (97:3, 1.5 l), (19:1, 1.5 l), (37:3, 1.5 l), (9:1, 1.5 l), (7:1, 1.5 l), (17:3, 1.5 l), (33:7, 1.5 l), (4:1, 1.5 l), (3:1, 1.5 l), and (7:3, 1.5 l), and MeOH (1.5 l). The residue (678 mg) in fractions 39–48 was subjected to RPCC, and the residue (73.7 mg) in fractions 88–91 was separated by DCCC to afford 8 (33.6 mg) in fractions 30–35 and 10 (17.3 mg) in fractions 43–48. The residue (778 mg) in fractions 49–58 on silica gel CC was subjected to RPCC and the residue (87.5 mg) in fractions 96–102) was separated by DCCC to give partially purified **3** (22.2 mg) in fractions 16–19. Crude **3** was purified by HPLC with 30% MeOH to give 3 (12.3 mg) from the peak at R_t 18 min.

The residue (6.90 g) in 15–18 fractions on Diaion HP-20 CC was subjected to silica gel CC (Φ = 36 mm, L = 57 cm, 250 ml/fraction), and eluted with $CHCl_3$ (1.5 l), $CHCl_3$–MeOH (99:1, 1.5 l), (97:3, 1.5 l), (19:1, 1.5 l), (37:3, 1.5 l), (9:1, 1.5 l), (7:1, 1.5 l), (17:3,

1.5 l), (33:7, 1.5 l), (4:1, 1.5 l), (3:1, 1.5 l), and (7:3, 1.5 l), and MeOH (1.5 l). The residue (619 mg) in fractions 34–41 was subjected to RPCC and the residue (20.6 mg) in fractions 86–92 was separated by DCCC to afford **9** (4.0 mg) in fractions 44–52. The residue (921 mg) in fractions 42–52 was subjected to RPCC and the residue (76.8 mg) in fractions 178–185 was separated by DCCC to give partially purified **6** (24.2 mg) in fractions 31–37. Crude **6** was purified by HPLC with 30% MeOH to afford **6** (10.2 mg) from the peak at R_t 32 min. The residue (29.9 mg) in fractions 208–211 on RPCC was separated by DCCC to give a residue (5.0 mg) in fractions 22–27, which was then purified by HPLC with 40% MeOH to yield **4** (2.6 mg) from the peak at R_t 33 min. The residue (54.4 mg) in fractions 221–226 on RPCC was separated by DCCC to give a residue (15.5 mg) in fractions 60–68, which was then purified by HPLC with 37.5% MeOH to give **5** (3.6 mg) from the peak at R_t 131 min.

The residue (11.7 g) in fractions 19–24 on Diaion HP-20 CC was subjected to silica gel CC (Φ = 50 mm, L = 57 cm, 500 ml/fraction), and eluted with $CHCl_3$ (3 l), $CHCl_3$–MeOH (99:1, 3 l), (97:3, 3 l), (19:1, 3 l), (37:3, 3 l), (9:1, 3 l), (7:1, 3 l), (17:3, 3 l), (33:7, 3 l), (4:1, 3 l), (3:1, 3 l), and (7:3, 3 l), and MeOH (3 l). The resulting residue (1.38 g) in fractions 33–43 was subjected to RPCC and the residue (99.6 mg in fractions 215–224) was separated by DCCC to give partially purified **2** (13.6 mg) in fractions 56–64. This was purified by HPLC with 37.5% MeOH to afford **2** (2.9 mg) from the peak at R_t 103 min. The residue (57.3 mg) in fractions 225–231 on RPCC was separated by DCCC to give a residue (12.3 mg) in fractions 53–62, followed by HPLC with 30% MeOH to afford **1** (5.4 mg) from the peak at R_t 21 min. The residue (55.2 mg) in fractions 240–243 on RPCC was separated by DCCC to give a residue (8.8 mg) in fractions 69–85, which was purified by HPLC with 2-PrOH–MeOH–H_2O (1:2:7) to afford **7** (1.7 mg) from the peak at R_t 51 min.

4.4. Characterization data

4.4.1. Crotonionoside A (**1**)

Amorphous powder; $[\alpha]_D^{26}$ −20.5 (*c* 0.39, MeOH); IR λ_{max} (film) cm^{-1}: 3389, 2967, 1701, 1631, 1596, 1456, 1370; UV ν_{max} (MeOH) nm (log ε): 324 (4.07), 298 (3.69), 234 (3.92), 215 (4.00); ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.64 (1H, *d*, *J* = 16 Hz, H-7″), 7.18 (1H, *d*, *J* = 2 Hz, H-2″), 7.07 (1H, *dd*, *J* = 8, 2 Hz, H-6″), 6.81 (1H, *d*, *J* = 8 Hz, H-5″), 6.38 (1H, *d*, *J* = 16 Hz, H-8″), 5.63 (1H, *dd*, *J* = 16, 6 Hz, H-8), 5.31 (1H, *dd*, *J* = 16, 1 Hz, H-7), 4.44 (1H, *dd*, *J* = 12, 6 Hz, H-6′a), 4.43 (1H, *dd*, *J* = 12, 3 Hz, H-6′b), 4.34 (1H, *d*, *J* = 8 Hz, H-1′), 4.22 (1H, *dqd*, *J* = 6, 6, 1 Hz, H-9), 3.89 (3H, s, 3″-OMe), 3.83 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 3.54 (1H, *ddd*, *J* = 9, 6, 3 Hz, H-5′), 3.37 (1H, *dd*, *J* = 9, 9 Hz, H-3′), 3.33 (1H, *m*, H-4′), 3.18 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 1.75 (2H, *overlapped*, H-4a and 5), 1.67 (1H, *dd*, *J* = 12, 12 Hz, H-2a), 1.52 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2b), 1.44 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-4b), 1.20 (3H, *d*, *J* = 6 Hz, H_3-10), 0.88 (3H, *s*, H_3-11), 0.86 (3H, *s*, H_3-12), 0.72 (3H, *d*, *J* = 6 Hz, H_3-13); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 589.2600 [MNa] (calcd for $C_{29}H_{42}O_{11}Na$, 589.2619).

4.4.2. Crotonionoside B (**2**)

Amorphous powder; $[\alpha]_D^{26}$ −29.0 (*c* 0.15, MeOH); IR λ_{max} (film) cm^{-1}: 3366, 2965, 2872, 1702, 1632, 1516, 1458, 1284; UV ν_{max} (MeOH) nm (log ε): 325 (3.98), 237 (3.97), 225 (3.95); ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.64 (1H, *d*, *J* = 16 Hz, H-7″), 6.91 (2H, *s*, H-2″ and 6″), 6.41 (1H, *d*, *J* = 16 Hz, H-8″), 5.62 (1H, *dd*, *J* = 16, 6 Hz, H-8), 5.28 (1H, *dd*, *J* = 16, 1 Hz, H-7), 4.45 (1H, *dd*, *J* = 12, 6 Hz, H-6′a), 4.43 (1H, *dd*, *J* = 12, 3 Hz, H-6′b), 4.34 (1H, *d*, *J* = 8 Hz, H-1′), 4.21 (1H, *dqd*, *J* = 6, 6, 1 Hz, H-9), 3.87 (6H, s, 3″ and 5″-OMe), 3.83 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 3.54 (1H,

Table 1
^{13}C NMR spectroscopic data for crotonionosides A–G (**1**–**7**) and the aglycones (**3a**, **6b** and **7b**) (100 MHz, CD_3OD).

C	1	2	3	3a	4	5	6	6a	7	7b
1	40.4	40.4	40.5	40.6	40.5	40.6	35.9	35.9	37.9	37.7
2	42.6	42.6	42.7	46.1	43.0	42.9	48.1	51.3	40.2	41.7
3	75.3	75.3	75.8	67.6	76.2	75.9	75.9	67.4	75.3	67.9
4	38.3	38.3	38.2	40.0	38.4	38.4	44.0	45.7	70.5	72.6
5	35.5	35.5	35.5	35.5	35.5	35.5	32.3	32.2	128.1	128.9
6	78.1	78.1	78.3	78.2	78.2	78.2	58.7	58.6	143.0	142.4
7	133.8	133.8	133.7	133.9	133.8	133.8	131.2	131.2	126.7	126.8
8	135.5	135.5	135.6	135.6	135.6	135.6	138.5	138.6	140.6	140.5
9	69.3	69.3	69.2	69.3	69.3	69.2	69.4	69.4	69.4	69.5
10	24.1	24.1	24.1	24.2	24.2	24.2	24.1	24.1	23.9	23.8
11	25.8	25.8	25.9	25.9	25.8	26.0	21.7	21.8	27.9	27.7
12	25.2	25.2	25.1	25.2	25.1	25.2	32.0	32.0	30.3	30.3
13	16.6	16.5	16.4	16.5	16.5	16.5	21.7	21.8	19.8	19.8
1′	103.0	103.0	101.5		101.7	101.5	101.6		103.3	
2′	75.1	75.1	78.7		79.0	79.0	78.9		75.6	
3′	78.2	78.1	78.1		78.9	78.6	78.1		77.9	
4′	72.4	72.4	71.5		72.0	72.0	71.9		72.1	
5′	76.1	76.1	77.7		77.7	77.7	77.7		76.7	
6′	64.9	64.9	62.9		62.9	62.9	62.9		64.9	
1″			110.5		110.6	110.3	110.6			
2″			78.6		78.7	78.6	78.7			
3″			80.8		79.2	79.2	80.7			
4″			75.4		75.6	75.7	75.4			
5″			66.3		68.4	68.7	66.3			
1‴ (1″)	127.7	126.6			122.2	126.7			128.0	
2‴ (2″)	111.8	107.1			133.2	107.2			111.9	
3‴ (3″)	150.8	149.5			116.3	149.6			150.8	
4‴ (4″)	149.4	139.6			163.8	139.9			149.5	
5‴ (5″)	116.6	149.5			116.3	149.6			116.6	
6‴ (6″)	124.3	107.1			133.2	107.2			124.2	
7‴ (7″)	146.9	147.1			168.1	147.5			147.1	
8‴ (8″)	115.5	115.9				115.9			115.4	
9‴ (9″)	169.0	168.9				169.0			169.0	
–OMe	56.6	56.9				56.6			56.6	

ddd, *J* = 9, 6, 3 Hz, H-5′), 3.37 (1H, *dd*, *J* = 9, 9 Hz, H-3′), 3.33 (1H, *m*, H-4′), 3.18 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 1.75 (2H, *overlapped*, H-4a and 5), 1.67 (1H, *dd*, *J* = 12, 12 Hz, H-2a), 1.51 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2b), 1.44 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-4b), 1.20 (3H, *d*, *J* = 6 Hz, H_3-10), 0.88 (3H, *s*, H_3-11), 0.86 (3H, *s*, H_3-12), 0.72 (3H, *d*, *J* = 6 Hz, H_3-13); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 619.2710 [MNa] (calcd for $C_{30}H_{44}O_{12}Na$, 619.2730).

4.4.3. Crotonionoside C (**3**)

Amorphous powder; $[\alpha]_D^{24}$ −69.9 (*c* 0.62, MeOH); IR λ_{max} (film) cm^{-1}: 3367, 2968, 1648, 1457, 1367; ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 5.73 (1H, *dd*, *J* = 15, 6 Hz, H-8), 5.55 (1H, *dd*, *J* = 15, 1 Hz, H-7), 5.39 (1H, *d*, *J* = 1 Hz, H-1″), 4.42 (1H, *d*, *J* = 7 Hz, H-1′), 4.29 (1H, *dqd*, *J* = 6, 6, 1 Hz, H-9), 4.08 (1H, *d*, *J* = 10 Hz, H-4″a), 3.94 (1H, *d*, *J* = 1 Hz, H-2″), 3.93 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 5.39 (1H, *d*, *J* = 1 Hz, H-1″), 3.85 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.72 (1H, *d*, *J* = 10 Hz, H-4″b), 3.65 (1H, *dd*, *J* = 12, 5 Hz, H-6′b), 3.64 (2H, *s*, H-5″), 3.47 (1H, *dd*, *J* = 9, 9 Hz, H-3′), 3.32 (1H, *m*, H-2′), 3.28 (1H, *dd*, *J* = 9, 9 Hz, H-4′), 3.24 (1H, *ddd*, *J* = 9, 5, 2 Hz, H-5′), 1.93 (1H, *dqd*, *J* = 12, 7, 4 Hz, H-5), 1.83 (1H, *dddd*, *J* = 12, 4, 4, 2 Hz, H-4 eq), 1.68 (1H, *dd*, *J* = 12, 12 Hz, H-2ax), 1.56 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2 eq), 1.49 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-4ax), 1.24 (3H, *d*, *J* = 6 Hz, H_3-10), 0.98 (3H, *s*, H_3-11), 0.91 (3H, *s*, H_3-12), 0.81 (3H, *d*, *J* = 7 Hz, H_3-13); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 545.2546 [MNa] (calcd for $C_{24}H_{42}O_{12}Na$, 545.2568).

4.4.4. Crotonionoside D (**4**)

Amorphous powder; $[\alpha]_D^{24}$ −38.5 (*c* 0.13, MeOH); IR λ_{max} (film) cm^{-1}: 3395, 2967, 2879, 1701, 1607, 1455, 1368; UV ν_{max} (MeOH) nm (log *ε*): 258 (4.04), 209 (4.04); ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 7.94 (2H, *d*, *J* = 9 Hz, H-2‴ and 6‴), 6.84 (2H, *d*, *J* = 9 Hz, H-3‴ and 5‴), 5.68 (1H, *dd*, *J* = 16, 6 Hz, H-8), 5.50 (1H, *dd*, *J* = 16, 1 Hz, H-7), 5.44 (1H, *d*, *J* = 1 Hz, H-1″), 4.43 (1H, *d*, *J* = 11 Hz, H-5″a), 4.42 (1H, *d*, *J* = 8 Hz, H-1′), 4.32 (1H, *d*, *J* = 11 Hz, H-5″b), 4.26 (1H, *dqd*, *J* = 6, 6, 1 Hz, H-9), 4.24 (1H, *d*, *J* = 10 Hz, H-4″a), 4.05 (1H, *d*, *J* = 10 Hz, H-2″), 3.85 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 3.85 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.84 (1H, *d*, *J* = 10 Hz, H-4″b), 3.65 (1H, *dd*, *J* = 12, 5 Hz, H-6′b), 3.49 (1H, *dd*, *J* = 9, 9 Hz, H-3′), 3.35 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 3.28 (1H, *m*, H-4′), 3.25 (1H, *m*, H-5′), 1.86 (1H, *dqd*, *J* = 12, 7, 4 Hz, H-5), 1.83 (1H, *m*, H-4a), 1.65 (1H, *dd*, *J* = 12, 12 Hz, H-2a), 1.48 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2b), 1.46 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-4b), 1.22 (3H, *d*, *J* = 6 Hz, H_3-10), 0.88 (3H, *s*, H_3-11), 0.78 (3H, *s*, H_3-12), 0.77 (3H, *d*, *J* = 7 Hz, H_3-13); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 665.2782 [MNa] (calcd for $C_{31}H_{46}O_{14}Na$, 665.2779).

4.4.5. Crotonionoside E (**5**)

Amorphous powder; $[\alpha]_D^{22}$ −50.6 (*c* 0.18, MeOH); IR λ_{max} (film) cm^{-1}: 3395, 2966, 2881, 1702, 1631, 1516, 1458, 1285; UV ν_{max} (MeOH) nm (log *ε*): 327 (4.08) 239 (4.05), 225 (4.03); ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 7.67 (1H, *d*, *J* = 16 Hz, H-7‴), 6.92 (2H, *s*, H-2‴ and 6‴), 6.43 (1H, *d*, *J* = 16 Hz, H-8‴), 5.68 (1H, *dd*, *J* = 15, 6 Hz, H-8), 5.51 (1H, *dd*, *J* = 15, 1 Hz, H-7), 5.44 (1H, *d*, *J* = 1 Hz, H-1″), 4.43 (1H, *d*, *J* = 8 Hz, H-1′), 4.38 (1H, *d*, *J* = 11 Hz, H-5″a), 4.27 (1H, *d*, *J* = 11 Hz, H-5″b), 4.26 (1H, *dqd*, *J* = 6, 6, 1 Hz, H-9), 4.21 (1H, *d*, *J* = 10 Hz, H-4″a), 3.97 (1H, *d*, *J* = 1 Hz, H-2″), 3.92 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 3.88 (6H, s, 3‴ and 5‴-OMe), 3.85 (1H, *overlapped*, H-6′a), 3.82 (1H, *d*, *J* = 10 Hz, H-4″b), 3.65 (1H, *dd*, *J* = 12, 5 Hz, H-6′b), 3.49 (1H, *dd*, *J* = 9, 9 Hz, H-3′), 3.35 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 3.28 (1H, *m*, H-4′), 3.23 (1H, *ddd*, *J* = 9, 5, 2 Hz, H-5′), 1.90 (1H, *dqd*, *J* = 12, 6, 4 Hz, H-5), 1.83 (1H, *m*, H-4a), 1.69 (1H, *dd*, *J* = 12, 12 Hz, H-2a), 1.54 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2b), 1.48 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-4b), 1.21 (3H, *d*, *J* = 6 Hz, H_3-10), 0.95 (3H, *s*, H_3-11), 0.87 (3H, *s*, H_3-12), 0.79 (3H, *d*, *J* = 6 Hz, H_3-13); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 751.3124 [MNa] (calcd for $C_{35}H_{52}O_{16}Na$, 751.3153).

4.4.6. Crotonionoside F (**6**)

Amorphous powder; $[\alpha]_D^{23}$ −55.8 (*c* 0.53, MeOH); IR λ_{max} (film) cm^{-1}: 3366, 2964, 1647, 1457, 1367; ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 5.45 (1H, *dd*, *J* = 15, 6 Hz, H-8), 5.30 (1H, *ddd*, *J* = 15, 10, 2 Hz, H-7), 5.38 (1H, *d*, *J* = 1 Hz, H-1″), 4.42 (1H, *d*, *J* = 8 Hz, H-1′), 4.22 (1H, *dqd*, *J* = 6, 6, 2 Hz, H-9), 4.05 (1H, *d*, *J* = 10 Hz, H-4″a), 3.94 (1H, *d*, *J* = 1 Hz, H-2″), 3.86 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 3.84 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.73 (1H, *d*, *J* = 10 Hz, H-4″b), 3.65 (1H, *dd*, *J* = 12, 5 Hz, H-6′b), 3.63 (1H, *d*, *J* = 11 Hz, H-5″a), 3.60 (1H, *d*, *J* = 11 Hz, H-5″b), 3.46 (1H, *dd*, *J* = 9, 9 Hz, H-3′), 3.32 (1H, *m*, H-2′), 3.26 (1H, *m*, H-4′), 3.23 (1H, *ddd*, *J* = 9, 5, 2 Hz, H-5′), 2.11 (1H, *dddd*, *J* = 12, 4, 4, 2 Hz, H-4a), 1.84 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2a), 1.51 (1H, *m*, H-5), 1.32 (1H, *dd*, *J* = 10, 10 Hz, H-6), 1.22 (3H, *d*, *J* = 6 Hz, H-10), 1.15 (1H, *dd*, *J* = 12, 12 Hz, H-2b), 1.01 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-4b), 0.91 (3H, *s*, H_3-12), 0.86 (3H, *s*, H_3-11), 0.83 (3H, *d*, *J* = 7 Hz, H_3-13); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 529.2635 [MNa] (calcd for $C_{24}H_{42}O_{11}Na$, 529.2619).

4.4.7. Crotonionoside G (**7**)

Amorphous powder; $[\alpha]_D^{23}$ −63.6 (*c* 0.11, MeOH); IR λ_{max} (film) cm^{-1}: 3343, 2926, 2856, 1712, 1630, 1599, 1515, 1455, 1261 ; UV ν_{max} (MeOH) nm (log *ε*): 323 (3.98), 214 (4.11); ^{1}H NMR (CD_3OD, 400 MHz) *δ*: 7.62 (1H, *d*, *J* = 16 Hz, H-7″), 7.16 (1H, *d*, *J* = 2 Hz, H-2″), 7.04 (1H, *dd*, *J* = 8, 2 Hz, H-6″), 6.81 (1H, *d*, *J* = 8 Hz, H-5″), 6.35 (1H, *d*, *J* = 16 Hz, H-8″), 5.97 (1H, *dd*, *J* = 16, 1 Hz, H-7), 5.46 (1H, *dd*, *J* = 16, 6 Hz, H-8), 4.50 (1H, *d*, *J* = 8 Hz, H-1′), 4.49 (1H, *dd*, *J* = 12, 6 Hz, H-6′a), 4.48 (1H, *dd*, *J* = 12, 3 Hz, H-6′b), 4.27 (1H, *dqd*, *J* = 6, 6, 1 Hz, H-9), 4.04 (1H, *br d*, *J* = 4 Hz, H-4), 3.91 (1H, *ddd*, *J* = 13, 4, 4 Hz, H-3), 3.89 (3H, *s*, 3″-OMe), 3.57 (1H, *ddd*, *J* = 9, 6, 3 Hz, H-5′), 3.41 (1H, *dd*, *J* = 9, 9 Hz, H-3′), 3.34 (1H, *m*, H-4′), 3.26 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 1.87 (1H, *dd*, *J* = 13, 13 Hz, H-2a), 1.82 (1H, *d*, *J* = 1 Hz, H_3-13), 1.58 (1H, *ddd*, *J* = 13, 4, 1 Hz, H-2b), 1.23 (3H, *d*, *J* = 6 Hz, H_3-10), 1.03 (3H, *s*, H_3-11), 0.99 (3H, *s*, H_3-12); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 587.2444 [MNa] (calcd for $C_{29}H_{40}O_{11}Na$, 587.2468).

4.4.8. Mild alkaline hydrolysis of crotonionosides A (**1**)–B (**2**), D (**4**)–E (**5**), and G (**7**)

To a solution of crotonionoside A (1) (5.9 mg) in MeOH (450 μl) was added 1 M CH_3ONa (50 μl), followed by standing at 20 °C for 4 h. The reaction mixture was neutralized with Amberlite IR-120B (H^+) and then evaporated to dryness. The residue was partitioned with $CHCl_3$ (2 ml)-H_2O (2 ml) to afford a megastigmane glycoside (**1a** = 8) (2.3 mg) from the H_2O-soluble fraction and methyl ferulate (**1b**) (1.0 mg) from the $CHCl_3$-soluble fraction.

In a similar manner to as for **1**, crotonionoside B (**2**) (2.2 mg) gave a megastismane glycoside (**2a** = 8) (1.0 mg) and methyl sinapate (**2b**) (0.4 mg).

Crotonionoside D (**4**) (2.6 mg) and E (**5**) (1.6 mg) gave **3** (1.7 mg) and a methyl *p*-hydroxybenzoate (**4b**) (0.1 mg), and **3** (0.9 mg) and methyl sinapate (**2b**) (0.4 mg), respectively.

Crotonionoside G (**7**) (1.7 mg) gave a megastismane glycoside (**7a**) (1.3 mg) and a methyl ferulate (**1b**) (0.2 mg).

Plucheoside B (**7a**); amorphous powder; $[\alpha]_D^{27}$ 45.6 (*c* 0.09, MeOH); NMR data were essentially the same as those reported (Otsuka et al., 1995b); HR-ESI TOF–MS (positive-ion mode) *m*/*z*: 411.2000 [MNa] (calcd for $C_{19}H_{32}O_8Na$, 411.1989).

*4.4.9. Enzymatic hydrolysis of crotonionoside C (**3**, 4a and **5a**), crotonionoside F (**6**) and plucheoside B (**7a**)*

Crotonionoside C (**3**) (3.4 mg) in H_2O (1 ml) was hydrolyzed with β-glucosidase (4.0 mg) and crude hesperidinase (7.5 mg) at 37 °C for 4 days. The reaction mixture was extracted with EtOAc (1 ml) to afford an aglycone (**3a**) (1.3 mg). The remaining H_2O layer was filtered and concentrated, and then analyzed by HPLC on an amino column [Asahipak NH2P-504E, CH_3CN–H_2O (4:1), 1 ml/min] with an optical rotation detector. The concentrated H_2O layer gave peaks for D-apiose and D-glucose at retention times 6.4 min and 20.0 min, respectively (positive optical rotation sign), and the peaks were identified by comparison with those of authentic D-apiose and D-glucose.

Aglycone (**3a**); amorphous powder; $[\alpha]_D^{26}$ −11.4 (*c* 0.07, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ: 5.72 (1H, *dd*, *J* = 16, 6 Hz, H-8), 5.55 (1H, *dd*, *J* = 16, 1 Hz, H-7), 4.29 (1H, *dqd*, *J* = 6, 6, 1 Hz, H-9), 3.80 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 1.93 (1H, *dqd*, *J* = 12, 6, 4 Hz, H-5), 1.67 (1H, *dddd*, J = 12, 4, 4, 2 Hz, H-4a), 1.66 (1H, *dd*, *J* = 12, 12 Hz, H-2a), 1.40 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2b), 1.39 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-4b), 1.24 (3H, *d*, *J* = 6 Hz, H_3-10), 0.98 (3H, *s*, H_3-11), 0.89 (3H, *s*, H_3-12), 0.81 (3H, *d*, *J* = 6 Hz, H_3-13); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 251.1620 [MNa] (calcd for $C_{13}H_{24}O_3Na$, 251.1617).

Using a similar procedure to that used for the hydrolysis of **3**–**3a, 4a** (1.7 mg) and **5a** (1.4 mg) were hydrolyzed to yield the common aglycone (**3a**) (0.4 mg and 0.3 mg) and sugars, which on analysis by HPLC gave peaks for D-apiose and D-glucose.

Crotonionoside F (**6**) (4.0 mg) was hydrolyzed to yield an aglycone (**6a**) (1.1 mg) and a sugar, which on analysis by HPLC gave a peak for D-glucose.

Aglycone (**6a**): amorphous powder; $[\alpha]_D^{26}$ −11.4 (*c* 0.07, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ: 5.45 (1H, *dd*, *J* = 15, 6 Hz, H-8), 5.30 (1H, *dd*, *J* = 15, 10, 2 Hz, H-7), 4.22 (1H, *dqd*, *J* = 6, 6, 2 Hz, H-9), 3.86 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 2.11 (1H, *m*, H-4a), 1.84 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2a), 1.51 (1H, *m*, H-5), 1.32 (1H, *dd*, *J* = 10, 10 Hz, H-6), 1.22 (3H, *d*, *J* = 6 Hz, H_3-10), 1.15 (1H, *dd*, *J* = 12, 12 Hz, H-2b), 1.01 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-4b), 0.91 (3H, *s*, H_3-12), 0.86 (3H, *s*, H_3-11), 0.83 (3H, *d*, *J* = 7 Hz, H_3-13); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 235.1670 [MNa] (calcd for $C_{13}H_{24}O_2Na$, 235.1673).

Compound **7a** (1.3 mg) was hydrolyzed to yield an aglycone (**7b**) (0.4 mg) and a sugar, which on analysis by HPLC gave a peak for D-glucose.

Aglycone (**7b**); amorphous powder; $[\alpha]_D^{25}$ −75.0 (*c* 0.02, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ: 6.02 (1H, *dd*, *J* = 16, 1 Hz, H-7), 5.50 (1H, *dd*, *J* = 16, 6 Hz, H-8), 4.30 (1H, *dqd*, *J* = 6, 6, 1 Hz, H-9), 3.82 (1H, *br d*, *J* = 4 Hz, H-4), 3.74 (1H, *ddd*, *J* = 13, 13, 4 Hz, H-3), 1.82 (3H, *s*, H_3-13), 1.78 (1H, *dd*, *J* = 13, 12 Hz, H-2a), 1.42 (1H, *ddd*, *J* = 12, 4, 1 Hz, H-2b), 1.27 (3H, *d*, *J* = 6 Hz, H_3-10), 1.06 (3H, *s*, H_3-11), 1.03 (3H, *s*, H_3-12); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 249.1456 [MNa] (calcd for $C_{13}H_{22}O_3Na$, 249.1461).

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

A solution of **6a** (0.5 mg) in 1 ml of dry CH_2Cl_2 was reacted with (*R*)-MTPA (23.4 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardodiimide hydrochloride (EDC) (16.0 mg) and *N*,*N*-dimethyl-4-aminopyridine (4-DMAP) (9.2 mg), were the mixture then being occasionally stirred at 25 °C for 30 min. After addition of CH_2Cl_2 (1 ml), the solution was successively washed with H_2O (1 ml), 5% HCl (1 ml), $NaHCO_3$-saturated H_2O (1 ml), and brine (1 ml). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure, with the residue purified by preparative TLC [silica gel (0.25 mm thickness), being applied for 18 cm, with development with $CHCl_3$-$(CH_3)_2CO$ (19:1) for 9 cm, and then eluted with $CHCl_3$–MeOH (9:1)] to furnish an ester, **6b** (0.9 mg). Through a similar procedure, **6c** (0.9 mg) was prepared from **6a** (0.9 mg) using (*S*)-MTPA (19.6 mg), EDC (15.4 mg), and 4-DMAP (10.7 mg).

(3*S*,5*R*,6*R*,7*E*,9*R*)-7-megastigmene-3,9-diol-3,9-*O*-(*R*)-MTPA diester (**6b**): amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.54–7.35 (10H, *m*, aromatic protons), 5.55 (1H, *qd*, *J* = 6, 4 Hz, H-9), 5.49 (1H, *d*, *J* = 4 Hz, H-8), 5.49 (1H, *d*, *J* = 8 Hz, H-7), 5.15 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 3.54 (3H, *br s*, -OMe), 3.53 (3H, *br s*, -OMe), 2.13 (1H, *dddd*, *J* = 12, 4, 4, 2 Hz, H-4a), 1.76 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2a), 1.62 (1H, *m*, H-5), 1.36 (1H, overlapped, H-6), 1.36 (1H, *d*, *J* = 6 Hz, H_3-10), 1.26 (1H, *dd*, *J* = 12, 12 Hz, H-2b), 1.14 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-4b), 0.90 (3H, *s*, H_3-12), 0.83 (3H, *s*, H_3-11), 0.83 (3H, *d*, *J* = 6 Hz, H_3-13); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 667.2481 [MNa] (calcd for $C_{33}H_{38}O_6F_6Na$, 667.2464).

(3*S*,5*R*,6*R*,7*E*,9*R*)-7-megastigmene-3,9-diol-3,9-*O*-(*S*)-MTPA diester (**6c**): amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.55–7.35 (10H, *m*, aromatic protons), 5.57 (1H, *qd*, *J* = 6, 4 Hz, H-9), 5.39 (1H, *d*, *J* = 8 Hz, H-7), 5.38 (1H, *d*, *J* = 4 Hz, H-8), 5.14 (1H, *dddd*, *J* = 12, 12, 4, 4 Hz, H-3), 3.56 (3H, *br s*, -OMe), 3.54 (3H, *br s*, -OMe), 2.05 (1H, *dddd*, *J* = 12, 4, 4, 2 Hz, H-4 eq), 1.82 (1H, *ddd*, *J* = 12, 4, 2 Hz, H-2a), 1.57 (1H, *m*, H-5), 1.41 (1H, *d*, *J* = 6 Hz, H_3-10), 1.32 (1H, *dd*, *J* = 12, 12 Hz, H-2b), 1.29 (1H, *dd*, *J* = 10, 8 Hz, H-6), 1.03 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-4ax), 0.87 (3H, *s*, H_3-12), 0.82 (3H, *s*, H_3-11), 0.77 (3H, *d*, *J* = 6 Hz, H_3-13); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 667.2443 [MNa] (calcd for $C_{33}H_{38}O_6F_6Na$, 667.2464).

*4.4.11. Preparation of (R)- and (S)-MPTA esters (**7c** and **7d**) from **7b***

Using a similar procedure to that used for the preparation of **6b** and **6c** from **6a**, 7c (5.5 mg) and **7d** (2.7 mg) were prepared from **7b** (0.2 and 0.2 mg, respectively) by use of the respective amounts of (*R*)- and (*S*)-MTPAs (31.9 mg and 29.0 mg), EDC (11.3 mg and 10.1 mg), and 4-DMAP (15.5 mg and 14.9 mg).

(3*S*,4*R*,5*Z*,7*E*,9*R*)-5,7-megastigmadiene-3,4,9-triol 3,9-*O*-(*R*)-MTPA diester (**7c**): amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.57–7.24 (10H, aromatic protons), 6.19 (1H, *dd*, *J* = 16, 1 Hz, H-7), 5.63 (1H, *dqd*, *J* = 6, 6, 1 Hz, H-9), 5.53 (1H, *dd*, *J* = 16, 6 Hz, H-8), 5.22 (1H, *ddd*, *J* = 13, 4, 4 Hz, H-3), 4.07 (1H, *br dd*, *J* = 4, 4 Hz, H-4), 3.58 (3H, *br s*, -OMe), 3.54 (3H, *br s*, -OMe), 2.05 (1H, *dd*, *J* = 13, 13 Hz, H-2a), 1.79 (3H, *d*, *J* = 1 Hz, H_3-13), 1.67 (1H, *ddd*, *J* = 13, 4, 1 Hz, H-2b), 1.41 (3H, *d*, *J* = 6 Hz, H_3-10), 1.10 (3H, *s*, H_3-12), 1.05 (3H, *s*, H_3-11); HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 681.2252 [MNa] (calcd for $C_{33}H_{36}O_7F_6Na$, 681.2257).

(3*S*,4*R*,5*Z*,7*E*,9*R*)-5,7-megastigmadiene-3,4,9-triol 3,9-*O*-(*S*)-MTPA diester (**7d**): amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.58–7.20 (10H, aromatic protons), 6.11 (1H, *dd*, *J* = 16, 1 Hz, H-7), 5.64 (1H, *dqd*, *J* = 6, 6, 1 Hz, H-9), 5.45 (1H, *dd*, *J* = 16, 6 Hz, H-8), 5.18 (1H, *ddd*, *J* = 13, 4, 4 Hz, H-3), 4.15 (1H, *br dd*, *J* = 4, 4 Hz, H-4), 3.56 (3H, *br s*, -OMe) 3.55 (3H, *br s*, -OMe), 1.94 (1H, *dd*, *J* = 13, 13 Hz, H-2a), 1.77 (3H, *d*, *J* = 1 Hz, H_3-13), 1.60 (1H, *ddd*, *J* = 13, 4, 1 Hz, H-2b), 1.46 (3H, *d*, *J* = 6 Hz, H_3-10), 1.07 (3H, *s*, H_3-12), 0.99 (3H, *s*, H_3-11); HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 681.2270 [MNa] (calcd for $C_{33}H_{36}O_7F_6Na$, 681.2257).

*4.4.12. Enzymatic hydrolysis of authentic dendranthemoside A (**8**) and turpinionoside A (**11**) for HPLC analyses*

Dendranthemoside A (**8**) (5.0 mg) and turpinionoside A (**11**) (5.0 mg), which were isolated and characterized in our laboratory, were hydrolyzed with β-glucosidase (5.5 mg each) in H_2O (2 ml each) at 37 °C for 24 h. The reaction mixtures were extracted with EtOAc (2 ml each), and then the organic layers were evaporated to dryness to afford aglycones (**8a**) (2.6 mg) and (**11a**) (2.4 mg), respectively.

Aglycone (**8a**): amorphous powder, $[\alpha]_D^{23}$ −13.5 (c 0.17, MeOH); the NMR data were essentially the same as the reported values (Otsuka et al., 1993); HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 251.1617 [MNa] (calcd for $C_{13}H_{24}O_3Na$, 251.1617).

Aglycone (**11a**): amorphous powder; $[\alpha]_D^{24}$ −14.6 (c 0.16, MeOH); NMR data were essentially the same as reported values (Yu et al., 2002); HR-ESI-TOF–MS (positive-ion mode) *m*/*z*: 251.1612 [MNa] (calcd for $C_{13}H_{24}O_3Na$, 251.1617).

4.4.13. HPLC analyses

With MeOH–H_2O (3:7, v/v), the retention times of **8** and **11** were 16 min and 18.5 min, respectively. Megastigmane glucoside (**1a** and **2a**), obtained by mild alkaline hydrolysis, was analyzed by HPLC with MeOH–H_2O (3:7, v/v) to give a peak at 16 min, which was the same as that of **8**.

With MeOH–H_2O (4:6, v/v), the retention times of **8a** and **11a** were 16 min and 19 min, respectively. Those of **3a**, 4b and **5b** were 16 min, which was the same as that of **8a**.

*4.4.14. Known compounds (**8**–**10**)*

Dendranthemoside A (**8**): amorphous powder; $[\alpha]_D^{30}$ −40.1 (*c* 0.56, MeOH) (Otsuka et al., 1992), Dendranthemoside B (**9**): amorphous powder; $[\alpha]_D^{24}$ −24.3 (*c* 0.27, MeOH) (Otsuka et al., 1992), Citroside A (**10**): amorphous powder; $[\alpha]_D^{25}$ −86.7 (*c* 1.15, MeOH) (Umehara et al., 1988).

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