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Bridelionosides A–F: Megastigmane glucosides from *Bridelia glauca* f. *balansae*

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Abstract

The chemical investigation of leaves of *Bridelia glauca* f. *balansae* afforded six megastigmane glucosides, named bridelionosides A–F, along with seven known megastigmane glucosides. Their structures were determined by a combination of spectroscopic analyses and by application of the modified Mosher's method.

Keywords: *Bridelia glauca* f. *balansae*; Euphorbiaceae; Megastigmane glucoside; Bridelionoside; Modified Mosher's method

1. Introduction

Bridelia glauca Bl. f. *balansae* (Tucht.) Hatusima (Euphorbiaceae) is an evergreen tree that grows to a height of about 10 m, and is distributed in Okinawa, Taiwan, southern China, Indochina and the Philippines (Hatusima, 1975). The isolation of balansenates I and II as long-chain esters, bridelone as an eburicane-like triterpenoid, bridelonine as a 'deimino-xanthine', and five adenine analogs from leaves of the title plant has been reported (Tsai et al., 2003). Our investigation of the leaves of the same plant species, collected in Okinawa, led to the isolation of six new megastigmane glucosides (**4**, **7**, **8** and **11**–**13**) (Fig. 1), to which bridelionosides A–F were assigned as trivial names, respectively, along with seven known megastigmane glucosides: 3-oxo-α-ionol glucoside (**1**) (Murakami et al., 1981), roseoside (**2**) (Otsuka et al., 1995), inamoside (**3**) (Aimi et al., 1990), megastigman-4-en-3-on-9,13-diol 9-*O*-β-glucopyranoside (**5**) (Peipp et al., 1997), alangionoside A (**6**) (Otsuka et al., 1994), ampelopsisionoside (**9**) (Inada et al., 1991), and actinidioionoside (**10**) (Murai and Tagawa, 1989). This paper deals with their structural elucidation.

2. Results and discussion

Air-dried leaves of *Bridelia glauca* f. *balansae* were extracted with MeOH three times with the concentrated MeOH extract partitioned with solvents of increasing polarity. The *n*-BuOH soluble fraction was separated by means of various chromatographic procedures including column chromatography (CC) on highly porous synthetic resin (Dioion HP-20), and then by normal silica gel and reversed-phase octadecyl silica gel (ODS) CC, droplet counter-current chromatography (DCCC), and high-performance liquid chromatography (HPLC) to afford 13 megastigmane glucosides (**1**–**13**). The details and yields are given in Section 4. The structures of the new compounds (**4**, **7**, **8** and **11**–**13**) were elucidated on the basis

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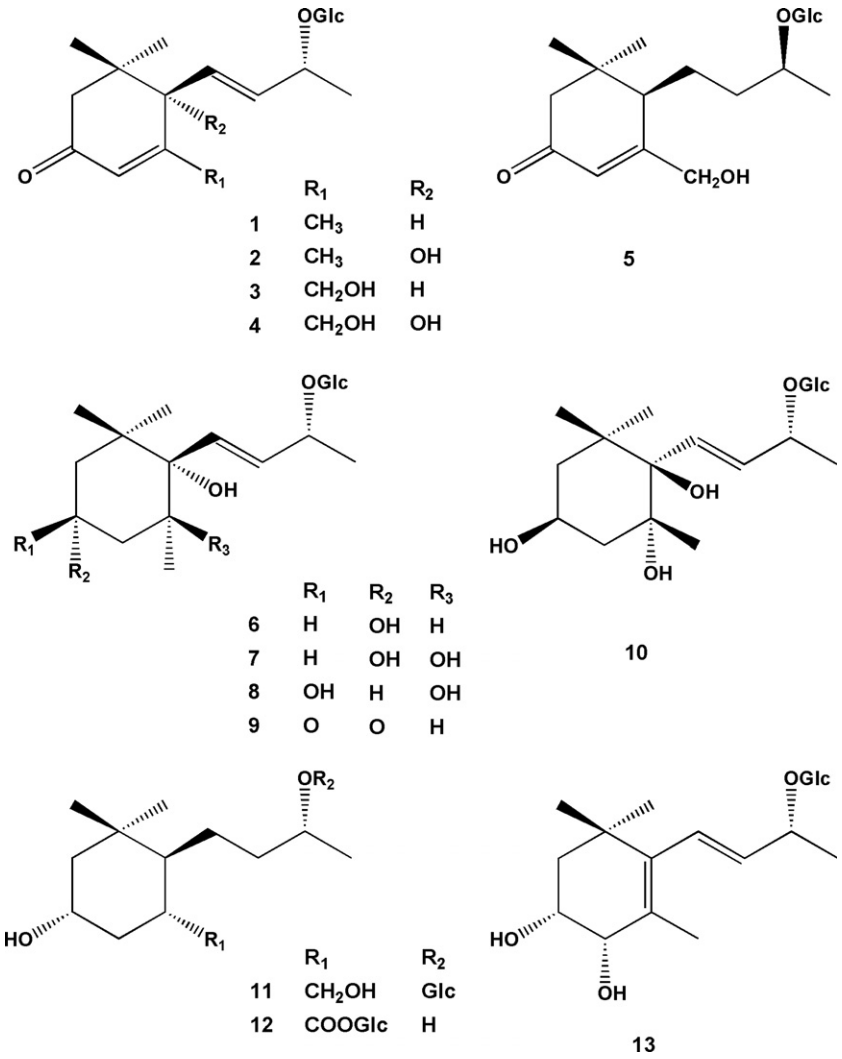

Fig. 1. Structures of compounds **1**–**13**.

of analysis of spectroscopic evidence, including a modified Mosher's method (Ohtani et al., 1991), and those of known compounds were identified by comparison of the spectroscopic data with those reported in the literature (Fig. 1). The absolute structure of megastigman-4-en-3-on-9,13-diol 9-*O*-β-glucopyranoside (**5**) remained to be determined and was elucidated in this experiment.

Bridelionoside A (**4**), $[\alpha]_D^{19} + 59.2°$, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{30}O_9$ by HR-FAB-MS. The ^1H and ^{13}C NMR spectra showed the presence of six signals assignable to β-glucopyranose, with the remaining 13 carbon signals comprising: two singlet and one doublet methyls; two methylenes one of which must possess an oxygen substituent (δ_C 61.3); one methine with a hydroxyl substituent; three quaternary carbons; and di- and trisubstituted double bonds, which must form a megastigmane skeleton (Naves, 1964). One of the quaternary carbons was expected to possess a ketonic oxygen functional group from the highly deshielded chemical shift (δ_C 201.2) in the ^{13}C NMR spectrum, and another was expected to possess a hydroxyl substituent. Therefore, the structure of **4** was assumed to be a megastigmane glucoside with two double bonds, as well as one ketonic and three hydroxyl functional groups. In the COSY spectrum, H-13a and 13b [δ_H 4.20 (*dd*, $J = 19$, 2 Hz) and 4.38 (*dd*, $J = 19$, 2 Hz)] showed cross-peaks with H-4 (δ_H 6.17), and in the HMBC spectrum, they showed cross-peaks with C-4, 5 and 6 (δ_C 123.1, 169.3 and 79.4, respectively). From the above evidence, **4** was expected to have a similar structure to spionoside A (**14**), isolated from *Capparis spinosa* (Çaliş et al., 2002). Although the chemical shift values in the ^{13}C NMR spectrum assigned to C-3, C-7, and C-9 in **4** were significantly different from those of spionoside A, because of the use of different solvents, they were similar to those reported for (6*S*,9*R*)-roseoside isolated from *Alangium premnifolium* (Otsuka et al., 1995), except that there was a carbinol instead of the methyl group at the 13-position. The downfield signal of C-9 (δ_C 77.4) in **4** indicated the 9*R* configuration compared with the upper field signal (δ_C 74.7) of (9*S*)-3-oxo-α-ionol (Pabst et al., 1992). The 6*S* configuration of **4** was determined from the extremes

at 233 nm ($\Delta\varepsilon$ +10.5) and 326 nm ($\Delta\varepsilon$ −0.81) in the circular dichroism (CD) spectrum, which was qualitatively the same as that ($\Delta\varepsilon_{241}$ +6.9 and $\Delta\varepsilon_{315}$ −1.8) of spionoside A. Thus, the structure of **4** was elucidated to be (6*S*,9*R*,4*Z*,7*E*)-megastigma-4,7-dien-3-one-6,9,13-triol 9-*O*-β-D-glucopyranoside, as an epimer of spionoside A at C-9 (Fig. 1).

Compound **5**, $[\alpha]_D^{20}$ + 27.1°, was isolated as an amorphous powder, and its ^{1}H and ^{13}C NMR spectra indicated the presence of a β-glucopyranose unit and a megastigmane skeleton. This suggested that **5** had a similar structure to **4**, except for the absence of a double bond between the 7- and 8-positions and a hydroxyl substituent at the 6-position. On comparison of the spectroscopic data with those in the literature, the structure of **5** was deduced to be that of megastigman-4-en-3-one-9,13-diol 9-*O*-β-D-glucopyranoside previously isolated from the roots of *Hordeum vulgare* (Peipp et al., 1997). However, its absolute structure remained to be determined. Since hydrolysis of **5** gave D-glucose, the stereochemistry at the 9-position was revealed to have a *S*-configuration on comparison of the ^{13}C NMR chemical shift of C-9 (δ_C 75.2) (De Marino et al., 2004) with that of blumenol C glucoside (δ_C 77.5), which is known to have the *R*-configuration (Takeda et al., 1997). The 6*R* configuration was confirmed from analysis of the CD spectrum which showed a positive extreme at 222 nm ($\Delta\varepsilon$ +2.11), in comparison with that ($\Delta\varepsilon_{218}$ +2.15) of blumenol C glucoside (Otsuka et al., 2003). Thus, the **5** was elucidated to be (6*R*,9*S*)-megastigman-4-en-3-one-9,13-diol 9-*O*-β-D-glucopyranoside (Fig. 1).

Compound **7**, $[\alpha]_D^{27}$ + 30.2°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{34}O_9$ by HR-FAB-MS. The ^{1}H and ^{13}C NMR spectra indicated the presence of a β-glucopyranose unit, a megastigmane skeleton possessing a *trans* double bond [δ_H 5.81 (*dd*, *J* = 16, 7 Hz) and 6.08 (*dd*, *J* = 16, 1 Hz)] between the 7- and 8-positions and four hydroxyl groups. Close inspection of the two-dimensional NMR spectrum established the structure as megastigman-7-ene-3,5,6,9-tetrol 9-*O*-β-glucopyranoside. From the coupling patterns of the adjacent well-resolved proton signals in the ^{1}H NMR spectrum, the H-3 signal resonating at δ_H 4.01 appeared as a triplet–triplet, and the coupling constant values of $J_{2ax\text{-}3}$ and $J_{3\text{-}4ax}$ were both calculated to be 12 Hz. Therefore, the hydroxyl group at the 3-position must have an equatorial orientation. On comparison of the chemical shift values in the ^{1}H and ^{13}C NMR spectra, the relative structure of the ring portion of **7** was assumed to be the same as that of actinidioionoside (**10**) isolated from *Actinidia polygama* (Murai and Tagawa, 1989); the absolute configuration at the 9-position was also expected to be *R* on the basis of the glucose-induced shift-trend (Kasai et al., 1977). After removal of the sugar by enzymatic hydrolysis, the aglycone **7a** was derivatized to the corresponding MTPA esters. The $\Delta\delta$ values of the MTPA esters are shown in Fig. 2. Thus, **7** was established to be (3*R*,5*S*,6*S*,7*E*,9*R*)-megastigman-7-ene-3,5,6,9-tetrol 9-*O*-β-D-glucopyranoside (Fig. 1).

Compound **8**, $[\alpha]_D^{15}$ − 2.1°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{34}O_9$ by HR-FAB-MS. The ^{1}H and ^{13}C NMR spectra indicated the presence of a β-glucopyranose unit and a megastigmane skeleton, and suggested that **8** was a compound analogous to **7**. Close inspection of the two-dimensional NMR spectrum allowed us to

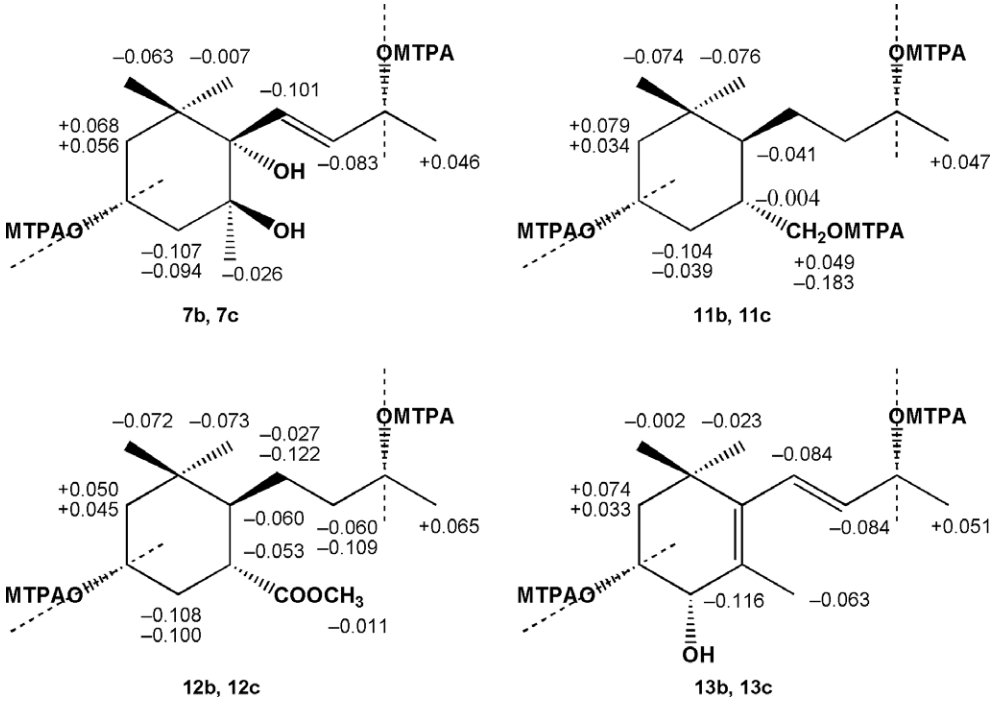

Fig. 2. Results with the modified Mosher's method ($\Delta\delta_S - \delta_R$).

establish its structure as megastigman-7-ene-3,5,6,9-tetrol 9-*O*-β-glucopyranoside. In the ^{1}H NMR spectrum, although the H-3 signal resonating at δ_H 4.07 appeared to be a multiplet, from the coupling patterns of adjacent well-resolved proton signals, the coupling constant values of $J_{2ax\text{-}3}$, $J_{2eq\text{-}3}$, $J_{3\text{-}4ax}$ and $J_{3\text{-}4eq}$ were calculated to be 4 Hz, 5 Hz, 3 Hz and 5 Hz, respectively. Therefore, the hydroxyl group at the 3-position must be in an axial orientation. The phase-sensitive (PH) NOESY spectrum indicated that the methyl group at the 5-position and the side chain at the 6-position were in an equatorial orientation (Fig. 3a). The hydroxyl substituent at the 3-position, together with another hydroxyl group at C-5 and an axial methyl group at the 1-position exhibiting a 1,3-diaxial relationship, may have caused perturbation of the ring system due to steric hindrance and thus caused the carbon signals to be broadened. From the above evidence, the relative structure of the ring portion of **8** was assumed to be the same as that of staphylionoside B isolated from *Staphylea bumalda* DC. (Yu et al., 2005), and the absolute configuration at the 9-position was expected to be *R* on the basis of the glucose-induced shift-trend (Kasai et al., 1977). After removal of the sugar by enzymatic hydrolysis, the aglycone **8a** was derivatized to the corresponding MTPA esters. The $\Delta\delta$ values of MTPA esters are shown in Fig. 3b. It is known that this is not an "irregular" arrangement, as opposite signs of the $\Delta\delta$ values for MTPA esters of 3α (axial)-cholesterol were also observed for corresponding protons of MTPA esters of 3β (equatorial)-cholesterol (Ohtani et al., 1991); that is, the absolute configurations at the 3-position of cholesterols could be established. Therefore, as to the axial hydroxyl group at the 3-position of **8**, the MTPA esters would show the opposite signs of $\Delta\delta$ values to those for the equatorial hydroxyl group (Fig. 3b). Thus, the structure of **8** was established to be (3*S*,5*S*,6*S*,7*E*,9*R*)-megastigman-7-ene-3,5,6,9-tetrol 9-*O*-β-D-glucopyranoside, as an epimer of **7** at C-3 (Fig. 1).

Compound **11**, $[\alpha]_D^{20}$ − 19.3°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{36}O_8$ by HR-FAB-MS. The ^{1}H and ^{13}C NMR spectra indicated the presence of a β-glucopyranose unit and a megastigmane skeleton including one oxymethylene (δ_C 66.0) and two oxymethines (δ_C 67.6 and 76.5). Close inspection on two-dimensional NMR established its structure to be megastigman-3,9,13-triol 9-*O*-β-glucopyranoside. The COSY correlations between the oxymethylene protons [δ_H 3.47 (*dd*, $J = 10$, 7 Hz) and 3.72 (*dd*, $J = 10$, 3 Hz)] at C-13 and the C-5 proton [δ_H 1.47 (m)] supported this structure. From the coupling patterns of the adjacent well-resolved proton signals in the ^{1}H NMR spectrum, the H-4 signal resonating at δ_H 1.06 appeared as a quartet, and the coupling constant values of $J_{3\text{-}4ax}$ and $J_{4ax\text{-}5}$ were both calculated to be 12 Hz. Therefore, the hydroxyl group at the 3-position and the hydroxymethyl group at the 5-position must be in an equatorial orientation. On comparison of the chemical shift values in the ^{1}H and ^{13}C NMR spectra, **11** was suggested to be a compound analogous to meliaionoside B isolated from *Melia toosendan* (Inada et al., 1991), and the absolute configuration at the 9-position was expected to be *R* on the basis of the glucose-induced shift-trend. After removal of the sugar by enzymatic hydrolysis, the aglycone **11a** was derivatized to the corresponding MTPA esters. The $\Delta\delta$ values of the MTPA esters are shown in Fig. 2. Thus, the structure of **11** was established to be (3*S*,5*R*,6*S*,9*R*)-megastigman-3,9,13-triol 9-*O*-β-D-glucopyranoside (Fig. 1).

Compound **12**, $[\alpha]_D^{20}$ − 15.2°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{34}O_9$ by HR-FAB-MS. The ^{1}H and ^{13}C NMR spectra indicated the presence of a β-glucopyranose

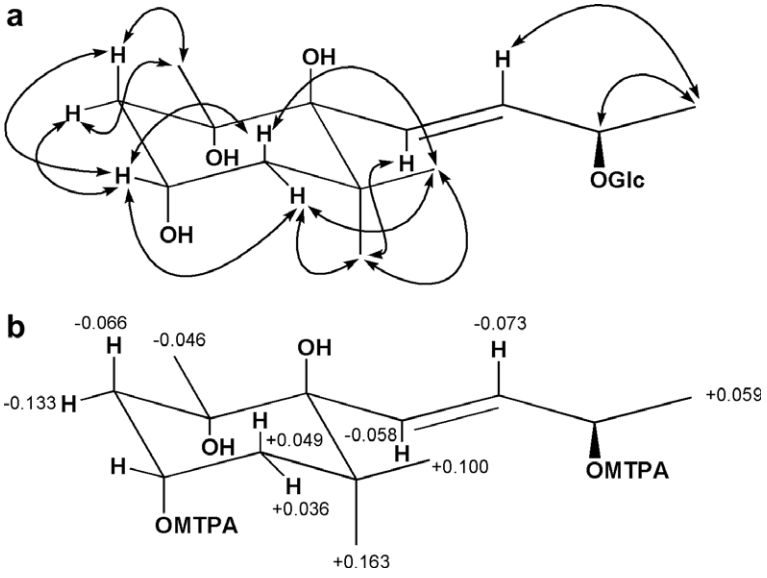

Fig. 3. Diagnostic PHNOESY correlations of (a) and results with the modified Mosher's method ($\Delta\delta_S - \delta_R$) (b) of **8**.

unit and a megastigmane skeleton, and suggested that **12** had a similar structure to **11**, except for possessing a carboxylic group instead of a hydroxymethyl group. Close inspection of the two-dimensional NMR established its structure to be β-glucopyranosyl 3,9-dihydroxy-megastigman-13-oate, because HMBC correlations from the C-5 proton [δ_H 2.46 (td, $J = 12$, 4 Hz)] and the anomeric proton [δ_H 5.47 (d, $J = 8$ Hz)] to the carbonyl carbon (δ_C 175.9) were observed. From coupling patterns of adjacent well-resolved proton signals in the ^{1}H NMR spectrum, the coupling constant values of $J_{2ax\text{-}3}$ and $J_{4ax\text{-}5}$ were both calculated to be 12 Hz. Therefore, the hydroxyl group at the 3-position and the carboxylic group at the 5-position must be in an equatorial orientation. To determine the absolute configuration at the 9-position, after removal of the sugar by mild alkaline hydrolysis, the methyl ester of aglycone **12a** was derivatized to the corresponding MTPA esters. The $\Delta\delta$ values of the MTPA esters are shown in Fig. 2. Thus, the structure of **12** was established to be β-D-glucopyranosyl 3,9-dihydroxy-(3*S*,5*R*,6*R*,9*R*)-megastigman-13-oate (Fig. 1).

Compound **13**, $[\alpha]_D^{20} + 60.0°$, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{32}O_8$ by HR-FAB-MS. The ^{1}H and ^{13}C NMR spectra showed the presence of six signals assignable to β-glucopyranose, with the remaining 13 carbon signals comprising two singlet and two doublet methyls, one methylene, three methines with hydroxyl substituents, and one quaternary carbon, and a disubstituted double bond and a tetrasubstituted double bond. Therefore, the structure of **13** was assumed to be a megastigmane glucoside with two double bonds and three hydroxyl functional groups. From the coupling patterns of adjacent well-resolved proton signals in the ^{1}H NMR spectrum, the coupling constant values of $J_{2ax\text{-}3}$ and $J_{3\text{–}4}$ were calculated to be 12 Hz and 4 Hz, respectively. Therefore, the hydroxyl groups at the 3- and 4-positions must be in equatorial and axial orientations, respectively. By comparison of the chemical shift values in the ^{1}H and ^{13}C NMR spectra, the relative structure of the ring portion of **13** was assumed to be the same as that of phlomisionoside isolated from *Philomis spinidens* (Takeda et al., 2002), and the absolute configuration at the 9-position was expected to be *R* on the basis of the glucose-induced shift-trend (Kasai et al., 1977). After removal of the sugar by enzymatic hydrolysis, the aglycone **13a** was derivatized to the corresponding MTPA esters. The $\Delta\delta$ values of MTPA esters are shown in Fig. 2. Thus, the structure of **13** was established to be (3*R*,4*S*,9*R*,5*Z*,7*E*)-megastigma-5,7-diene-3,4,9-triol 9-*O*-β-D-glucopyranoside (Fig. 1).

3. Concluding remarks

Although there are no reports on the medicinal uses of *B. glauca* f. *balansae*, a closely related species, *B. ovata* Decne., grown in Thailand, is used as an emetic, expectorant and purgative (Ponglux et al., 1987), whereas *B. stipularis* (L.) Bl. for venereal disease in Indonesia (Eisai, 1995). The biological evaluation of *B. glauca* f. *balansae* is thus also needed and thus will be subject of future study.

In this experiment, 13 megastigmane glucosides were isolated from leaves of the title plant. Even with only 13 carbon atoms in the basic skeleton of megastigmane, several oxidation stages and glycosylation afforded many kinds of megastigmane derivatives and their glycosidic forms. Megastigmane with carboxylic acid at the 13-position and its glucopyranosyl ester form was first isolated from nature in this study. The only similar compounds related to megastigmane with a carboxylic acid moiety are derivatives of abscisic acid, such as the glucosyl esters of phaseic and dihydrophaseic acids, isolated from *Lycopersicon esculentum* (Carrington et al., 1988); however, they have 15 carbon atoms in their skeletons. Thus, there are two more carbon atoms in the side chain of megastigmane.

4. Experimental

4.1. General

Optical rotations were measured on a JASCO P-1030 digital polarimeter. FT-IR spectra were recorded on a Horiba FT-710 spectrophotometer. CD spectra were obtained with a JASCO J-720 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL α-400 spectrometer (400 and 100 MHz, respectively) with TMS as internal standard. HR-FAB-MS were carried out on a JEOL SX-102 mass spectrometer using PEG-400 as the calibration matrix. Silica gel 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, Japan) [$\varnothing = 50$ mm, $L = 25$ cm, linear gradient: MeOH–H_2O (1:9, 1 l) → (1:1, 1 l)], respectively, fractions of 10 g being collected. The DCCC (Tokyo Rikakikai, Tokyo) was equipped with 500 glass columns ($\varnothing = 2$ 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 stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to the order of elution of the mobile phase. Preparative HPLC was performed using ODS (YMC-Pack, $\varnothing = 20$ mm, $L = 15$ cm, YMC, Kyoto, Japan) or ODS (Inertsil, $\varnothing = 6$ mm, $L = 25$ cm, GL Science, Tokyo, Japan) columns. (*R*)-(+)- and (*S*)-(−)-MTPAs were purchased from Nacalai Tesque Co., Ltd. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was from Kanto Chemical Co., Inc. (Tokyo, Japan). 4-Dimethylaminopyridine (DMAP) was from Wako Pure Chemical Industries, Ltd (Osaka, Japan).

4.2. Plant materials

Leaves of *B. glauca* f. *balansae* were collected in Taketomi-cho, Yaeyama-gun, Okinawa, Japan, in October

2001. A voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University (01-BGB-Okinawa-1024).

4.3. Extraction and isolation

Air-dried leaves at 50 degrees of *B. glauca* f. *balansae* (7.68 kg) were extracted with MeOH (15 l × 3) three times. The MeOH extract was concentrated *in vacuo* to 3 l and then H_2O (300 ml) was added to make a 90% aqueous solution. This solution was next washed with 3 l of *n*-hexane with the methanolic layer concentrated to a viscous gum. The gummy residue was suspended in H_2O (3 l), and then extracted with EtOAc (3 l) and *n*-BuOH (3 l), successively, to give 154 g of a *n*-BuOH soluble fraction. The *n*-BuOH soluble fraction was subjected to highly porous synthetic resin CC (Diaion HP-20, Mitsubishi Chemical Co., Ltd., $\varnothing = 70$ mm, $L = 50$ 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), 1 l fractions were being collected. The residue (19.7 g in fractions 5–7) of the 20–40% MeOH eluent was subjected to silica gel (500 g) CC, with elution with $CHCl_3$ (2 l) and $CHCl_3$–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)], 500 ml fractions being collected. Combined fractions 48–58 (2.43 g) were separated by RPCC. The residue (312 mg) of fractions 59–76 was subjected to DCCC to give 209 mg of **7** in fractions 19–29. The RPCC fractions 95–105 gave 63.2 mg of **10**.

The residue (15.8 g in fractions 8–11) of the 40% MeOH eluent was subjected to silica gel (440 g) CC, with elution with $CHCl_3$ (1.5 l) and $CHCl_3$–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)], 500 ml fractions being collected. Combined fractions 27–31 (1.31 g) were separated by RPCC. The residue (128 mg in fractions 87–91, 134 mg in fractions 102–112 and 72.8 mg in fractions 113–124) was subjected to DCCC to give **2** (72.4 mg) in fractions 73–85, **9** (80.1 mg) in fractions 86–99, and **5** (16.1 mg) in fractions 60–72. Combined fractions 36–43 (1.95 g) were separated by RPCC. The residue (332 mg) of fractions 52–62 was subjected to DCCC to give **4** (101 mg) in fractions 34–42. Combined RPCC fractions (95.1 mg in fractions 63–68, 269 mg in fractions 87–99 and 181 mg in fractions 100–113) were subjected to DCCC and HPLC to give **8** (12.7 mg) from the first, **3** (6.6 mg) from the second, **13** (6.0 mg) and **12** (10.9 mg) from the third residue. The combined RPCC fractions gave **11** (44.9 mg) in fractions 114–121. Combined fractions 44–53 (1.40 g) were separated by RPCC. The residue (105 mg) of fractions 94–104 was subjected to DCCC to give **6** (34.1 mg) in fractions 39–49.

The residue (26.1 g in fractions 17–22) of the 60–80% MeOH eluent was subjected to silica gel (550 g) CC, with elution with $CHCl_3$ (2 l) and $CHCl_3$–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)], 500 ml fractions being collected. Combined fractions 26–32 (2.74 g) were separated by RPCC. The residue (362 mg in fractions 119–133) was subjected to DCCC and HPLC to give **1** (6.0 mg).

4.4. Characterization data

4.4.1. Known compounds isolated

3-Oxo-α-ionol glucoside (**1**): Amorphous powder; $[\alpha]_D^{22} + 122^\circ$ ($c = 0.40$, MeOH). Roseoside (**2**): Amorphous powder; $[\alpha]_D^{20} + 100^\circ$ ($c = 0.92$, MeOH). Inamoside (**3**): Amorphous powder; $[\alpha]_D^{22} + 62.7^\circ$ ($c = 0.44$, MeOH). Alangionoside (**6**): Amorphous powder; $[\alpha]_D^{20} - 23.4^\circ$ ($c = 0.94$, MeOH). Ampelopsisionoside (**9**): Amorphous powder; $[\alpha]_D^{22} - 7.5^\circ$ ($c = 0.51$, MeOH). Actinidioionoside (**10**): Amorphous powder; $[\alpha]_D^{19} - 11.8^\circ$ ($c = 0.76$, MeOH).

4.4.2. Bridelionoside A (*4*)

Amorphous powder; $[\alpha]_D^{19} + 59.2^\circ$ ($c = 0.74$, MeOH); IR ν_{max} (film) cm^{-1}: 3367, 2933, 1653, 1519, 1074, 1036; UV λ_{max} (MeOH) nm ($\log\varepsilon$): 231 (4.03); ^1H NMR (CD_3OD) δ: 1.02 (3H, *s*, H_3-11), 1.05 (3H, *s*, H_3-12), 1.29 (3H, *d*, $J = 6$ Hz, H_3-10), 2.18 (1H, *d*, $J = 17$ Hz, H-2a), 2.49 (1H, *d*, $J = 17$ Hz, H-2b), 3.10 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 3.61 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 3.86 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 4.20 (1H, *dd*, $J = 19$, 2 Hz, H-13a), 4.33 (1H, *d*, $J = 8$ Hz, H-1′), 4.38 (1H, *dd*, $J = 19$, 2 Hz, H-13b), 4.41 (1H, *quint.d*, $J = 6$, 1 Hz), 5.87 (2H, *m*, H-7 and H-8), 6.17 (1H, *br. s*, H-4); for ^{13}C NMR (CD_3OD) spectrum, see Table 1; CD (MeOH) $\Delta\varepsilon$ (nm): +10.5 (233), −0.81 (326) ($c = 3.70 \times 10^{-5}$ M); HR-FAB-MS (negative-ion mode) *m*/*z*: 401.1805 $[M-H]^-$ (calcd for $C_{19}H_{29}O_9$: 401.1812).

4.4.3. (6R,9S)-Megastigman-4-en-3-one-9,13-diol 9-O-β-D-glucopyranoside (*5*)

Amorphous powder; $[\alpha]_D^{19} + 27.1^\circ$ ($c = 0.70$, MeOH); IR ν_{max} (film) cm^{-1}: 3366, 2928, 1656, 1077, 1035; UV λ_{max} (MeOH) nm ($\log\varepsilon$): 238 (3.82); ^1H NMR (CD_3OD) δ: 1.02 (3H, *s*, H_3-11), 1.11 (3H, *s*, H_3-12), 1.19 (3H, *d*, $J = 6$ Hz, H_3-10), 1.51 (2H, *m*, H_2-7), 1.65 (2H, *m*, H_2-8), 1.98 (1H, *m*, H-6), 2.01 (1H, *d*, $J = 18$ Hz, H-2a), 2.56 (1H, *d*, $J = 18$ Hz, H-2b), 3.15 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 3.67 (1H, *dd*, $J = 12$, 6 Hz, H-6′a), 3.88 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 3.89 (1H, *m*, H-9), 4.19 (1H, *dd*, $J = 16$, 2 Hz, H-13a), 4.34 (1H, *d*, $J = 8$ Hz, H-1′), 4.36 (1H, *dd*, $J = 16$, 2 Hz, H-13b), 6.05 (1H, *s*, H-4); for ^{13}C NMR (CD_3OD) spectrum, see Table 1; CD (MeOH) $\Delta\varepsilon$ (nm): +2.11 (222), −0.45 (272), +0.27 (329) ($c = 5.41 \times 10^{-5}$ M); HR-FAB-MS (negative-ion mode) *m*/*z*: 387.1998 $[M-H]^-$ (calcd for $C_{19}H_{35}O_8$: 387.2019).

4.4.4. Bridelionoside B (*7*)

Amorphous powder; $[\alpha]_D^{27} + 30.2^\circ$ ($c = 0.79$, MeOH); ^1H NMR (CD_3OD) δ: 0.83 (3H, *s*, H_3-12), 1.06 (3H, *s*, H_3-11), 1.18 (3H, *s*, H_3-13), 1.28 (3H, *d*, $J = 6$ Hz, H_3-10), 1.41

Table 1
^{13}C NMR spectroscopic data for compounds **4**, **5**, **7**, **8** and **11**–**13** (100 MHz, CD_3OD)

C	4	5	7	8	11	12	13
1	42.8	37.3	40.8	38.7	36.7	36.4	37.8
2	50.7	48.6	46.4	44.5	51.8	51.1	41.8
3	201.2	202.4	65.3	68.9	67.6	66.1	68.0
4	123.1	121.5	45.7	42.1	40.7	40.1	72.7
5	169.3	172.4	77.8	77.6	42.5	47.6	129.2
6	79.4	47.7	78.3	80.3	48.4	48.6	142.3
7	135.3	27.0	132.9	134.1	25.2	26.4	128.7
8	131.8	37.7	134.3	133.6	39.8	40.8	138.5
9	77.4	75.2	79.1	79.1	76.5	69.0	77.9
10	21.3	19.9	21.5	21.8	19.7	23.3	21.3
11	24.2	28.8	27.9	29.0	21.5	21.2	27.8
12	23.3	27.7	26.3	27.5	31.3	31.2	30.5
13	61.3	65.1	27.1	26.5	66.0	175.9	20.0
1′	102.9	102.1	102.6	102.9	102.6	95.8	102.5
2′	75.2	75.1	75.4	75.4	75.2	73.9	75.4
3′	78.0	78.1	77.9	78.0	78.2	78.1	78.2
4′	71.7	71.8	71.5	72.0	71.8	71.1	71.6
5′	78.1	77.9	78.3	78.3	77.8	78.8	78.0
6′	62.9	62.9	62.6	63.0	62.9	62.3	62.7

(1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.60 (1H, *t*, $J = 12$ Hz, H-2ax), 1.69 (1H, *t*, $J = 12$ Hz, H-4ax), 1.73 (1H, *ddd*, $J = 12$, 6, 2 Hz, H-4eq), 3.61 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 3.77 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 4.01 (1H, *tt*, $J = 12$, 6 Hz, H-3), 4.31 (1H, *d*, $J = 8$ Hz, H-1′), 4.39 (1H, *quint*, $J = 6$ Hz), 5.81 (1H, *dd*, $J = 16$, 7 Hz, H-8), 6.08 (1H, *dd*, $J = 16$, 1 Hz, H-7); for ^{13}C NMR (CD_3OD) δ spectrum, see Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 405.2152 $[M-H]^-$ (calcd for $C_{19}H_{33}O_9$: 405.2125).

*4.4.5. Bridelionoside C (**8**)*

Amorphous powder; $[\alpha]_D^{15} - 2.1°$ ($c = 0.48$, MeOH); ^1H NMR (CD_3OD) δ: 0.91 (3H, *s*, H_3-12), 1.15 (3H, *s*, H_3-11), 1.19 (3H, *s*, H_3-13), 1.33 (3H, *d*, $J = 7$ Hz, H_3-10), 1.55 (1H, *ddd*, $J = 14$, 5, 2 Hz, H-2eq), 1.71 (1H, *ddd*, $J = 14$, 5, 2 Hz, H-4eq), 1.78 (1H, *dd*, $J = 14$, 4 Hz, H-2ax), 2.03 (1H, *dd*, $J = 14$, 4 Hz, H-4ax), 3.59 (1H, *dd*, $J = 12$, 6 Hz, H-6′a), 3.85 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 4.07 (1H, *m*, H-3), 4.34 (1H, *d*, $J = 8$ Hz, H-1′), 4.40 (1H, *quint.d*, $J = 7$, 1 Hz, H-9), 5.83 (1H, *dd*, $J = 16$, 7 Hz, H-8), 6.17 (1H, *dd*, $J = 16$, 1 Hz, H-7); for ^{13}C NMR (CD_3OD) spectrum, see Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 405.2149 $[M-H]^-$ (calcd for $C_{19}H_{33}O_9$: 405.2125).

*4.4.6. Bridelionoside D (**11**)*

Amorphous powder; $[\alpha]_D^{20} - 19.3°$ ($c = 0.73$, MeOH); ^1H NMR (CD_3OD) δ: 0.79 (1H, *m*, H-6), 0.84 (3H, *s*, H_3-11), 0.96 (3H, *s*, H_3-12), 1.06 (1H, *q*, $J = 12$ Hz, H-4ax), 1.10 (1H, *t*, $J = 12$ Hz, H-2ax), 1.18 (3H, *d*, $J = 6$ Hz, H_3-10), 1.47 (1H, *m*, H-5), 1.60 (2H, *m*, H-7 and H-8), 1.65 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 2.10 (1H, *m*, H-4eq), 3.15 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 3.47 (1H, *dd*, $J = 11$, 7 Hz, H-13a), 3.67 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 3.71 (1H, *m*, H-3), 3.72 (1H, *dd*, $J = 11$, 3 Hz, H-13b), 3.85 (1H, *m*, H-9), 3.86 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 4.33 (1H, *d*, $J = 8$ Hz, H-1′); for ^{13}C NMR (CD_3OD) spectrum, see Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 391.2350 $[M-H]^-$ (calcd for $C_{19}H_{35}O_8$: 391.2332).

*4.4.7. Bridelionoside E (**12**)*

Amorphous powder; $[\alpha]_D^{20} - 15.2°$ ($c = 0.72$, MeOH); ^1H NMR (CD_3OD) δ: 0.86 (3H, *s*, H_3-12), 1.00 (3H, *s*, H_3-11), 1.06 (1H, *m*, H-7a), 1.10 (3H, *d*, $J = 6$ Hz, H_3-10), 1.20 (1H, *t*, $J = 12$ Hz, H-2ax), 1.29 (1H, *m*, H-6), 1.37 (1H, *m*, H-8a), 1.42 (1H, *q*, $J = 12$ Hz, H-4ax), 1.59 (1H, *m*, H-8b), 1.60 (1H, *m*, H-7b), 1.68 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 2.10 (1H, *m*, H-4eq), 2.46 (1H, *td*, $J = 12$, 4 Hz, H-5), 3.60 (1H, *sextet*, $J = 6$ Hz, H-9), 3.68 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 3.71 (1H, *m*, H-3), 3.84 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 5.47 (1H, *d*, $J = 8$ Hz, H-1′); for ^{13}C NMR (CD_3OD) spectrum, see Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 405.2120 $[M-H]^-$ (calcd for $C_{19}H_{33}O_9$: 405.2125).

*4.4.8. Bridelionoside F (**13**)*

Amorphous powder; $[\alpha]_D^{20} + 60°$ ($c = 0.40$, MeOH); ^1H NMR (CD_3OD) δ: 1.04 (3H, *s*, H_3-11), 1.06 (3H, *s*, H_3-12), 1.33 (3H, *d*, $J = 6$ Hz, H_3-10), 1.44 (1H, *ddd*, $J = 12$, 4, 1 Hz, H-2eq), 1.78 (1H, *t*, $J = 13$ Hz, H-2ax), 1.84 (3H, *d*, $J = 1$ Hz, H_3-13), 3.67 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 3.75 (1H, *dt*, $J = 13$, 4 Hz, H-3ax), 3.81 (1H, *dd*, $J = 12$, 3 Hz, H-6′b), 3.84 (1H, *br.d*, $J = 4$ Hz, H-4eq), 4.39 (1H, *d*, $J = 8$ Hz, H-1′), 4.43 (1H, *quint.d*, $J = 6$, 1 Hz, H-9), 5.92 (1H, *dd*, $J = 16$, 7 Hz, H-8), 6.08 (1H, *dd*, $J = 16$, 1 Hz, H-7); for ^{13}C NMR (CD_3OD) spectrum, see Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 387.2021 $[M-H]^-$ (calcd for $C_{19}H_{31}O_8$: 387.2019).

*4.4.9. Enzymatic hydrolysis of **5***

Compound **5** (11 mg) was hydrolyzed with hesperidinase (15 mg) in 2 ml of H_2O at 37 °C for 28 h. The reaction mixture was concentrated, and then subjected to silica gel (15 g, $\varnothing = 10$ mm, $L = 35$ cm) CC with $CHCl_3$ (100 ml) and $CHCl_3$–MeOH (19:1, 100 ml, 9:1, 100 ml, 17:3, 100 ml, 7:3, 300 ml), 12 ml fractions being collected. D-Glucose (4.1 mg) was recovered in fractions 32–36. D-Glucose, $[\alpha]_D^{20} + 47.1°$ ($c = 0.27$, H_2O, 24 h after being dissolved in the solvent).

*4.4.10. Enzymatic hydrolysis of 7 to **7a***

Bridelionoide B (**7**) (14 mg) was hydrolyzed with hesperidinase (20 mg) as above. The aglycone (bridelionol B) (5.6 mg) (**7a**) and 4.5 mg of D-glucose were recovered. Aglycone (**7a**): Amorphous powder; $[\alpha]_D^{20} + 24.1°$ ($c = 0.37$, MeOH); ^{1}H NMR (CD_3OD) δ: 0.87 (3H, *s*, H_3-12), 1.10 (3H, *s*, H_3-11), 1.22 (3H, *s*, H_3-13), 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), 1.74 (1H, *t*, $J = 12$ Hz, H-4ax), 1.78 (1H, *ddd*, $J = 12$, 6, 2 Hz, H-4eq), 4.05 (1H, *tt*, $J = 12$, 6 Hz, 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) δ: 24.2 (C-10), 26.2 (C-11), 27.1 (C-12), 27.6 (C-13), 40.8 (C-1), 45.8 (C-4), 46.6 (C-2), 65.4 (C-3), 69.6 (C-9), 77.8 (C-5), 79.0 (C-6), 131.2 (C-7), 136.2 (C-8); HR-FAB-MS (negative-ion mode) *m/z*: 243.1629 $[M-H]^-$ (calcd for $C_{13}H_{23}O_4$: 243.1596). D-Glucose, $[\alpha]_D^{20} + 36.7°$ ($c = 0.30$, H_2O, 24 h after being dissolved in the solvent).

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

A solution of **7a** (2.8 mg) in dry CH_2Cl_2 (1 ml) was reacted with (*R*)-MTPA (43 mg) in the presence of EDC (35 mg) and DMAP (23 mg), with the mixture being occasionally stirred at 25 °C for 30 min. After addition of 1 ml each of H_2O and CH_2Cl_2, the solution was successively washed with 5% HCl (1 ml), $NaHCO_3$ saturated H_2O (1 ml), and brine (1 ml). The organic layer was dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by preparative TLC silica gel 0.25 mm thickness developed with $CHCl_3$-$(CH_3)_2CO$ (9:1), and the product eluted with $CHCl_3$–MeOH (9:1) to furnish the ester, **7b** (4.5 mg, 50%). Through a similar procedure, **7c** (2.5 mg, 28%) was prepared from **7a** (2.8 mg) by use of (*S*)-MTPA (41 mg), EDC (35 mg), and DMAP (23 mg). Bridelionol B 3,9-di-(*R*)-MTPA ester (**7b**): Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.81 (3H, *s*, H_3-12), 1.15 (3H, *s*, H_3-11), 1.28 (3H, *s*, H_3-13), 1.41 (3H, *d*, $J = 6$ Hz, H_3-10), 1.62 (1H, *ddd*, $J = 12$, 6, 2 Hz, H-2eq), 1.74 (1H, *t*, $J = 12$ Hz, H-2ax), 1.95 (1H, *ddd*, $J = 13$, 6, 2 Hz, H-4eq), 2.01 (1H, *t*, $J = 13$ Hz, H-4ax), 3.53 (3H, *d*, $J = 1$ Hz, –OCH_3), 3.55 (3H, *d*, $J = 1$ Hz, –OCH_3), 5.48 (1H, *tt*, $J = 12$, 6 Hz, H-3), 5.66 (1H, *quint*, $J = 6$ Hz), 5.79 (1H, *dd*, $J = 16$, 7 Hz, H-8), 6.28 (1H, *dd*, $J = 16$, 1 Hz, H-7), 7.34–7.42 (6H, *m*, aromatic protons), 7.48–7.56 (4H, *m*, aromatic protons); HR-FAB-MS (negative-ion mode) *m/z*: 645.2288 $[M-CH_3O]^-$ (calcd for $C_{32}H_{35}O_7F_6$: 645.2287). Bridelionol B 3,9-di-(*S*)-MTPA ester (**7c**): Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.80 (3H, *s*, H_3-12), 1.09 (3H, *s*, H_3-11), 1.25 (3H, *s*, H_3-13), 1.46 (3H, *d*, $J = 6$ Hz, H_3-10), 1.68 (1H, *ddd*, $J = 12$, 6, 2 Hz, H-2eq), 1.80 (1H, *t*, $J = 12$ Hz, H-2ax), 1.86 (1H, *ddd*, $J = 13$, 6, 2 Hz, H-4eq), 1.91 (1H, *t*, $J = 13$ Hz, H-4ax), 3.55 (3H, *d*, $J = 1$ Hz, –OCH_3), 3.57 (3H, *d*, $J = 1$ Hz, –OCH_3), 5.47 (1H, *tt*, $J = 12$, 6 Hz, H-3), 5.67 (1H, *quint*, $J = 6$ Hz, H-9), 5.69 (1H, *dd*, $J = 16$, 6 Hz, H-8), 6.18 (1H, *d*, $J = 16$ Hz, H-7), 7.34–7.44 (6H, *m*, aromatic protons), 7.50–7.56 (4H, *m*, aromatic protons); HR-FAB-MS (negative-ion mode) *m/z*: 645.2260 $[M-CH_3O]^-$ (calcd for $C_{32}H_{35}O_7F_6$: 645.2287).

*4.4.12. Enzymatic hydrolysis of **8** to **8a***

Bridelionoside C (**8**) (13 mg) was hydrolyzed with hesperidinase (20 mg) in 2 ml of H_2O at 37 °C for 48 h. The reaction mixture was concentrated, and then workup in a similar manner to that for **7a** to give the aglycone (bridelionol C) (**8a**) (4.7 mg) and D-glucose (4.4 mg). Aglycone (**8a**): Amorphous powder; $[\alpha]_D^{25} - 39.4°$ ($c = 0.31$, MeOH); ^{1}H NMR (CD_3OD) δ: 0.90 (3H, *s*, H_3-12), 1.16 (3H, *s*, H_3-11), 1.18 (3H, *s*, H_3-13), 1.27 (3H, *d*, $J = 6$ Hz, H_3-10), 1.57 (1H, *ddd*, $J = 14$, 5, 2 Hz, H-2eq), 1.71 (1H, *ddd*, $J = 14$, 5, 2 Hz, H-4eq), 1.82 (1H, *dd*, $J = 14$, 4 Hz, H-2ax), 2.04 (1H, *dd*, $J = 14$, 4 Hz, H-4ax), 4.08 (1H, *m*, H-3), 4.34 (1H, *quint.d*, $J = 6$, 1 Hz), 5.77 (1H, *dd*, $J = 16$, 6 Hz, H-8), 6.15 (1H, *dd*, $J = 16$, 1 Hz, H-7); ^{13}C NMR (CD_3OD) δ: 24.1 (C-10), 26.1 (C-13), 27.7 (C-12), 29.0 (C-11), 38.8 (C-1), 42.1 (C-4), 44.4 (C-2), 69.0 (C-3), 69.7 (C-8), 77.6 (C-5), 80.3 (C-6), 132.0 (C-7), 135.5 (C-8); HR-FAB-MS (negative-ion mode) *m/z*: 243.1566 $[M-H]^-$ (calcd for $C_{13}H_{23}O_4$: 243.1596). D-Glucose, $[\alpha]_D^{26} + 34.8°$ ($c = 0.29$, H_2O, 24 h after being dissolved in the solvent).

*4.4.13. Preparation of (R)- and (S)-MTPA esters **8b** and **8c** from **8a***

Using a similar procedure to that used for the preparation of **7b** and **7c** from **7a**, **8b** (0.96 mg, 12%) and **8c** (2.0 mg, 26%) were prepared from **8a** (2.4 mg each) by use of the respective amounts of (*R*)- and (*S*)-MTPA (40 mg and 42 mg), EDC (30 mg and 33 mg), and DMAP (19 mg each). Bridelionol C 3,9-di-(*R*)-MTPA ester (**8b**): Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.76 (3H, *s*, H_3-12), 0.82 (3H, *s*, H_3-11), 1.10 (3H, *s*, H_3-13), 1.39 (3H, *d*, $J = 7$ Hz, H_3-10), 1.56 (1H, *ddd*, $J = 15$, 4, 1 Hz, H-2eq), 1.83 (1H, *ddd*, $J = 15$, 4, 2 Hz, H-4eq), 1.89 (1H, *dd*, $J = 15$, 4 Hz, H-2ax), 2.22 (1H, *dd*, $J = 15$, 4 Hz, H-4ax), 3.52 (3H, *s*, –OCH_3), 3.58 (3H, *s*, –OCH_3), 5.44 (1H, *quint.*, $J = 4$ Hz, H-3), 5.62 (1H, *quint.d*, $J = 7$, 1 Hz), 5.75 (1H, *dd*, $J = 16$, 7 Hz, H-8), 6.35 (1H, *d*, $J = 16$ Hz, H-7), 7.34–7.42 (6H, *m*, aromatic protons), 7.48–7.57 (4H, *m*, aromatic protons); HR-FAB-MS (negative-ion mode) *m/z*: 645.2299 $[M-CH_3O]^-$ (calcd for $C_{32}H_{35}O_7F_6$: 645.2287). Bridelionol C 3,9-di-(*S*)-MTPA

ester (**8c**): Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.86 (3H, *s*, H_3-12), 0.98 (3H, *s*, H_3-11), 1.05 (3H, *s*, H_3-13), 1.45 (3H, *d*, $J = 7$ Hz, H_3-10), 1.63 (1H, *ddd*, $J = 15$, 4, 2 Hz, H-2eq), 1.70 (1H, *ddd*, $J = 15$, 4, 2 Hz, H-4eq), 1.94 (1H, *dd*, $J = 15$, 4 Hz, H-2ax), 2.16 (1H, *dd*, $J = 15$, 4 Hz, H-4ax), 3.53 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.54 (3H, *q*, $J = 1$ Hz, –OCH_3), 5.43 (1H, *quint.*, $J = 4$ Hz, H-3), 5.64 (1H, *quint*, $J = 7$ Hz), 5.66 (1H, *dd*, $J = 15$, 7 Hz, H-8), 6.29 (1H, *d*, $J = 15$ Hz, H-7), 7.32–7.44 (6H, *m*, aromatic protons), 7.47–7.55 (4H, *m*, aromatic protons); HR-FAB-MS (negative-ion mode) *m*/*z*: 645.2302 $[M-CH_3O]^-$ (calcd for $C_{32}H_{35}O_7F_6$: 645.2287).

4.4.14. Enzymatic hydrolysis of *11* to *11a*

Bridelionoside D (**11**) (22.4 mg) was hydrolyzed with hesperidinase (20 mg) in 2 ml of H_2O at 37 °C for 29 h. The reaction mixture was concentrated, and processed in a similar manner to that for **7a** to give aglycone (bridelionol D) (**11a**) (5.8 mg) and D-glucose (2.6 mg). Aglycone (**11a**): Amorphous powder; $[\alpha]_D^{20} - 25.9°$ ($c = 0.39$, MeOH); ^{1}H NMR (CD_3OD) δ: 0.79 (1H, *ddd*, $J = 12$, 6, 2 Hz, H-6), 0.84 (3H, *s*, H_3-11), 0.97 (3H, *s*, H_3-12), 1.05 (1H, *ddd*, $J = 12$, 12, 12 Hz, H-4ax), 1.10 (1H, *t*, $J = 12$ Hz, H-2ax), 1.15 (3H, *d*, $J = 6$ Hz, H_3-10), 1.47 (1H, *m*, H-5), 1.57 (2H, *m*, H-7 and H-8), 1.65 (1H, *ddd*, $J = 12$, 4, 3 Hz, H-2eq), 2.12 (1H, *m*, H-4eq), 3.41 (1H, *dd*, $J = 11$, 8 Hz, H-13a), 3.71 (1H, *tq*, $J = 6$, 6 Hz H-9), 3.71 (1H, *dddd*, $J = 12$, 12, 4, 4 Hz, H-3), 3.72 (1H, *dd*, $J = 11$, 3 Hz, H-13b); ^{13}C NMR (CD_3OD) δ: 21.4 (C-11), 23.4 (C-10), 26.1 (C-7), 31.3 (C-12), 36.7 (C-1), 40.8 (C-4 and C-8), 42.7 (C-5), 48.5 (C-6), 51.8 (C-2), 66.1 (C-13), 67.5 (C-3), 69.1 (C-9); HR-FAB-MS (negative-ion mode) *m*/*z*: 229.1796 $[M-H]^-$ (calcd for $C_{13}H_{25}O_3$: 229.1804). D-Glucose, $[\alpha]_D^{27} + 32.7°$ ($c = 0.17$, H_2O, 24 h after being dissolved in the solvent).

4.4.15. Preparation of (*R*)- and (*S*)-MTPA esters *11b* and *11c* from *11a*

Using a similar procedure to that used for the preparation of **7b** and **7c** from **7a**, **11b** (0.8 mg, 7.6%) and **11c** (1.2 mg, 11%) were prepared from **11a** (2.9 mg each) by use of the respective amounts of (*R*)- and (*S*)-MTPA (38 mg and 45 mg), EDC (36 mg and 35 mg), and DMAP (40 mg and 41 mg). Bridelionol D 3,9,13-tri-(*R*)-MTPA ester (**11b**): Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.73 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-6), 0.82 (3H, *s*, H_3-11), 0.86 (3H, *s*, H_3-12), 1.13 (1H, *t*, $J = 12$ Hz, H-2ax), 1.18 (1H, *quint*, $J = 12$ Hz, H-4ax), 1.25 (3H, *d*, $J = 6$ Hz, H_3-10), 1.69 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 2.03 (1H, *m*, H-4eq), 3.49 (3H, *s*, –OCH_3), 3.50 (6H, *s*, –$OCH_3 \times 2$), 4.04 (1H, *dd*, $J = 11$, 6 Hz, H-13a), 4.51 (1H, *dd*, $J = 11$, 3 Hz, H-13b), 5.04 (1H, *m*, H-3), 5.06 (1H, *m*, H-9), 7.25–7.48 (9H, *m*, aromatic protons), 7.49–7.53 (6H, *m*, aromatic protons); HR-FAB-MS (positive-ion mode) *m*/*z*: 901.2999 $[M+Na]^+$ (+NaI) (calcd for $C_{43}H_{47}O_9F_9Na$: 901.2974). Bridelionol D 3,9,13-tri-(*S*)-MPTA ester (**11c**): Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.69 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-6), 0.75 (3H, *s*, H_3-11), 0.79 (3H, *s*, H_3-12), 1.07 (1H, *quint*, $J = 12$ Hz, H-4ax), 1.21 (1H, *t*, $J = 12$ Hz, H-2ax), 1.29 (3H, *d*, $J = 6$ Hz, H_3-10), 1.73 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.99 (1H, *m*, H-4eq), 3.47 (3H, *d*, $J = 1$ Hz, –OCH_3), 3.51 (3H, *d*, $J = 1$ Hz, –OCH_3), 3.55 (3H, *d*, $J = 1$ Hz, –OCH_3), 4.09 (1H, *dd*, $J = 11$, 5 Hz, H-13a), 4.32 (1H, *dd*, $J = 11$, 3 Hz, H-13b), 5.04 (1H, *m*, H-3), 5.07 (1H, *m*, H-9), 7.25–7.48 (9H, *m*, aromatic protons), 7.49–7.54 (6H, *m*, aromatic protons); HR-FAB-MS (positive-ion mode) *m*/*z*: 901.2974 $[M+Na]^+$ (NaI) (calcd for $C_{43}H_{47}O_9F_9Na$: 901.2974).

4.4.16. Mild alkaline hydrolysis of *12* to *12a*

Bridelionoside E (**12**) (10.0 mg) was treated with 1 ml of 0.1 M NaOH in MeOH at 25 °C for 2 h. The reaction mixture was diluted with H_2O (4 ml) and then extracted with $CHCl_3$ (2×4 ml, 2×2 ml). The combined organic layers were washed with brine (1 ml) and then neutralized with Amberlite IR-120B (H^+). After being dried (Na_2SO_4) the organic layer was concentrated, and subjected to silica gel (22 g, $\varnothing = 18$ mm, $L = 19$ cm) CC with $CHCl_3$ (100 ml) and $CHCl_3$–MeOH (19:1, 100 ml, 9:1, 100 ml, 17:3, 100 ml, 7:3, 300 ml), 12 ml fractions being collected. Bridelionoic acid methyl ester (**12a**) (6.1 mg) and D-glucose (2.9 mg) were recovered. Methyl ester (**12a**): Amorphous powder; $[\alpha]_D^{30} - 2.5°$ ($c = 0.41$, MeOH); ^{1}H NMR (CD_3OD) δ: 0.85 (3H, *s*, H_3-12), 0.99 (3H, *s*, H_3-11), 1.10 (3H, *d*, $J = 6$ Hz, H_3-10), 1.18 (1H, *t*, $J = 12$ Hz, H-2ax), 1.22 (1H, *ddd*, $J = 13$, 6, 3 Hz, H-6), 1.29 (1H, *m*, H-7a), 1.33 (2H, *m*, H-8), 1.39 (1H, *q*, $J = 12$ Hz, H-4ax), 1.61 (1H, *ddd*, $J = 13$, 5, 3 Hz, H-7b), 1.67 (1H, *ddd*, $J = 12$, 4, 3 Hz, H-2eq), 2.09 (1H, *ddd*, $J = 12$, 6, 3 Hz, H-4eq), 2.39 (1H, *td*, $J = 13$, 3 Hz, H-5), 3.57 (1H, *sextet*, $J = 6$ Hz, H-9), 3.67 (3H, *s*, –OCH_3), 3.70 (1H, *tt*, $J = 12$, 4 Hz, H-3); ^{13}C NMR (CD_3OD) δ: 21.1 (C-11), 23.4 (C-10), 26.9 (C-7), 31.2 (C-12), 36.5 (C-1), 40.3 (C-4), 41.3 (C-8), 47.4 (C-5), 48.8 (C-6), 51.3 (C-2), 52.2 (–OCH_3), 66.5 (C-3), 69.1 (C-9), 177.9 (C-13); D-Glucose, $[\alpha]_D^{20} + 26.0°$ ($c = 0.19$, H_2O, 24 h after being dissolved in the solvent).

4.4.17. Preparation of (*R*)- and (*S*)-MTPA esters *12b* and *12c* from *12a*

Using a similar procedure to that used for the preparation of **7b** and **7c** from **7a**, **12b** (3.0 mg, 38%) and **12c** (0.8 mg, 10%) were prepared from **12a** (3.0 mg each) by use of the respective amounts of (*R*)- and (*S*)-MTPA (40 mg and 49 mg), EDC (35 mg and 38 mg), and DMAP (21 mg and 24 mg). Methyl bridelionate 3,9-di-(*R*)-MTPA ester (**12b**): Amorphous powder; ^{1}H NMR ($CDCl_3$) δ: 0.85 (3H, *s*, H_3-12), 0.92 (3H, *s*, H_3-11), 1.01 (1H, *m*, H-7a), 1.23 (3H, *d*, $J = 6$ Hz, H3-10), 1.28 (2H, *m*, H-6 and H-8a), 1.34 (1H, *t*, $J = 12$ Hz, H-2ax), 1.56 (1H, *m*, H-8b), 1.57 (1H, *m*, H-7b), 1.66 (1H, *q*, $J = 12$ Hz, H-4ax), 1.73 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 2.22 (1H, *m*, H-4ax), 2.46 (1H, *td*, $J = 12$, 3 Hz, H-5), 3.51 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.52 (3H, *q*, $J = 1$ Hz, –OCH_3), 3.65 (3H, *s*, -$COOCH_3$), 4.97 (1H, *m*, H-9), 5.09 (1H, *tt*,

$J = 12$, 4 Hz, H-3), 7.35–7.42 (6H, *m*, aromatic protons), 7.47–7.54 (4H, *m*, aromatic protons); HR-FAB-MS (positive-ion mode) *m/z*: 713.2512 $[M+Na]^+$ (+NaI) (calcd for $C_{34}H_{40}O_8F_6Na$: 713.2525). Methyl bridelionate 3,9-di-(*S*)-MTPA ester (**12c**): Amorphous powder; ^1H NMR ($CDCl_3$) δ: 0.77 (3H, *s*, H_3-12), 0.85 (3H, *s*, H_3-11), 0.91 (1H, *m*, H-7a), 1.22 (2H, *m*, H-6 and H-8a), 1.30 (3H, *d*, H_3-10), 1.39 (1H, *t*, $J = 12$ Hz, H-2ax), 1.46 (1H, *m*, H-8b), 1.52 (1H, *m*, H-7b), 1.55 (1H, *q*, $J = 12$ Hz, H-3), 1.78 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 2.12 (1H, *m*, H-4eq), 2.40 (1H, *td*, 12, 3 Hz, H-5), 3.51 (3H, *q*, $J = 1$ Hz, $-OCH_3$), 3.52 (3H, *q*, $J = 1$ Hz, $-OCH_3$), 3.64 (3H, *s*, $-COOCH_3$), 4.98 (1H, *m*, H-9), 5.08 (1H, *tt*, $J = 12$, 4 Hz, H-3), 7.36–7.42 (6H, *m*, aromatic protons), 7.47–7.54 (4H, *m*, aromatic protons); HR-FAB-MS (positive-ion mode) *m/z*: 713.2498 $[M+Na]^+$ (+NaI) (calcd for $C_{34}H_{40}O_8F_6Na$: 713.2525).

4.4.18. Enzymatic hydrolysis of 13 to 13a

Bridelionoside F (**13**) (5.5 mg) was hydrolyzed with hesperidinase (10 mg) in 2 ml of H_2O at 37 °C for 29 h. The reaction mixture was treated as for **7a** to give aglycone (bridelionol F) (**13a**) (2.0 mg) and D-glucose (1.8 mg). Aglycone (**13a**): Amorphous powder; $[\alpha]_D^{20} + 113^\circ$ ($c = 0.13$, MeOH); ^1H NMR (CD_3OD) δ: 1.04 (3H, *s*, H_3-11), 1.06 (3H, *s*, H_3-12), 1.27 (3H, *d*, $J = 6$ Hz, H_3-10), 1.44 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.77 (1H, *t*, $J = 13$ Hz, H-2ax), 1.83 (3H, *d*, $J = 1$ Hz, H_3-13), 3.75 (1H, *dt*, $J = 13$, 4 Hz, H-3ax), 3.83 (1H, *br.d*, $J = 4$ Hz, H-4eq), 4.30 (1H, *quint.d*, $J = 6$, 1 Hz, H-9), 5.52 (1H, *dd*, $J = 16$, 6 Hz, H-8), 6.04 (1H, *dd*, $J = 16$, 1 Hz, H-7); ^{13}C NMR (CD_3OD) δ: 19.9 (C-13), 23.9 (C-10), 27.8 (C-11), 30.4 (C-12), 37.8 (C-1), 41.8 (C-2), 68.0 (C-3), 69.4 (C-9), 72.7 (C-4), 126.9 (C-7), 129.0 (C-5), 140.6 (C-8), 142.5 (C-6); HR-FAB-MS (negative-ion mode) *m/z*: 225.1501 $[M-H]^-$ (calcd for $C_{13}H_{21}O_3$: 225.1491). D-Glucose, $[\alpha]_D^{27} + 40.3^\circ$ ($c = 0.13$, H_2O, 24 h after being dissolved in the solvent).

4.4.19. Preparation of (R)- and (S)-MTPA esters 13b and 13c from 13a

Using a similar procedure to that used for the preparation of **7b** and **7c** from **7a**, **13b** (0.9 mg, 31%) and **11c** (0.8 mg, 27%) were prepared from **11a** (1.0 mg each) by use of the respective amounts of (*R*)- and (*S*)-MTPA (39 mg and 39 mg), EDC (21 mg and 22 mg), and DMAP (21 mg and 22 mg). Bridelionol F 3,9-di-(*R*)-MTPA ester (**13b**): Amorphous powder; ^1H NMR ($CDCl_3$) δ: 1.00 (3H, *s*, H_3-11), 1.12 (3H, *s*, H_3-12), 1.41 (3H, *d*, $J = 6$ Hz, H_3-10), 1.60 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.81 (3H, *d*, $J = 1$ Hz, H_3-13), 1.95 (1H, *t*, $J = 12$ Hz, H-2ax), 3.53 (3H, *d*, $J = 1$ Hz, $-OCH_3$), 3.56 (3H, *d*, $J = 1$ Hz, $-OCH_3$), 4.16 (1H, *m*, H-4), 5.20 (1H, *dt*, $J = 13$, 4 Hz, H-3), 5.54 (1H, *dd*, $J = 16$, 6 Hz, H-8), 5.63 (1H, *quint.d*, $J = 6$ Hz, H-9), 7.34–7.44 (6H, *m*, aromatic protons), 7.50–7.59 (4H, *m*, aromatic protons); HR-FAB-MS (positive-ion mode) *m/z*: 681.2301 $[M+Na]^+$ (+NaI) (calcd for $C_{33}H_{36}O_7F_6Na$: 681.2263). Bridelionol F 3,9-di-(*S*)-MTPA ester (**13c**): Amorphous powder; ^1H NMR ($CDCl_3$) δ: 1.00 (3H, *s*, H_3-11), 1.09 (3H, *s*, H_3-12), 1.46 (3H, *d*, $J = 6$ Hz, H_3-10), 1.63 (1H, *ddd*, $J = 12$, 4, 2 Hz, H-2eq), 1.75 (3H, *s*, H_3-13), 2.03 (1H, *t*, $J = 12$ Hz, H-2ax), 3.56 (3H, *d*, $J = 1$ Hz, $-OCH_3$), 3.58 (3H, *d*, $J = 1$ Hz, $-OCH_3$), 4.04 (1H, *m*, H-4), 5.21 (1H, *dt*, $J = 13$, 4 Hz, H-3), 5.46 (1H, *dd*, $J = 16$, 6 Hz, H-8), 5.63 (1H, *quint.d*, $J = 6$ Hz, H-9), 7.34–7.44 (6H, *m*, aromatic protons), 7.49–7.58 (4H, *m*, aromatic protons); HR-FAB-MS (positive-ion mode) *m/z*: 681.2306 $[M+Na]^+$ (+NaI) (calcd for $C_{33}H_{36}O_7F_6Na$: 681.2263).

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