Terpenic and Phenolic Glycosides from Leaves of *Breynia officinalis* Hemsl.

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From the leaves of *Breynia officinalis*, collected on Okinawa Island, six terpenic glucosides and six phenolic glycosides were isolated. Two of the terpenic glucosides were found to be known, and they were identified as turpinionoside B and betulalbuside A. The structures of the remaining terpenic glucosides were elucidated to be megastigmane glucosides, named breyniaionosides A—D, using spectroscopic analyses. The absolute structure of breyniaionoside D was determined using the modifed Mosher's method. The absolute structure of the known compound betulalbuside A was determined for the first time in this study. Six phenolic glycosides were found to be arbutin and its derivatives. Two known compounds were found to be robustaside A and eximine. New compounds were named isorobustaside A, and breyniosides A and B, and their structures were elucidated from spectroscopic evidence.

Key words *Breynia officinalis*; Euphorbiaceae; megastigmane glucoside; breyniaionoside; betulalbuside A; breynioside

Breynia officinalis (Euphorbiaceae) is a poisonous perennial shrub 1.5—4 m in height, and grows in Okinawa, Taiwan, and southern China. However, it is used as a remedy for healing wounds and edema, as an ointment, and for syphilis and intestinal hemorrhage due to overwork by oral administration.[1)] Continuing our survey of Okinawan resource plants, the constituents of *B. officinalis* were investigated.

From a *n*-BuOH-soluble fraction of the MeOH extract of the leaves of *B. officinalis*, 12 compounds were isolated. Five (**1**—**5**) were found to be megastigmane glucosides, one (**1**) of which was a known compound, identified as turpinionoside B.[2)] The other known compound, betulalbuside A (**6**) is 3,7-dimethylocta-1,6-dien-3,8-diol 8-*O*-β-D-glucopyranosiode, of which absolute configuration at the 3-position was determined for the first time. The known phenolic glucosides (**7**—**9**) were identified as arbutin,[3)] robustaside A,[4)] and eximine,[5,6)] respectively. New phenolic compounds **10**—**12** were also derivatives of arbutin, like compounds **8** and **9**. This paper deals with the structural elucidation of the new compounds.

Results and Discussion

Compounds **1**—**12** were isolated from the *n*-BuOH-soluble fraction of a MeOH extract of the leaves of *B. officinalis* by the combination of highly porous synthetic resin (Diaion HP-20), and normal- and reversed-phase column chromatography (RPCC). Droplet counter-current chromatography (DCCC) and ODS-HPLC were also performed for the purification of the compounds. The details and yields are given in Experimental.

Turpinionoside B (**1**) was isolated as an amorphous powder, and NMR spectra indicated the presence of a megastigmane skeleton and a glucopyranose unit. From spectroscopic data, including ^{1}H- and ^{13}C-NMR spectra, and $[\alpha]_D$ the structure of **1** was determined to be (3*S*,5*R*,6*S*,9*S*)-megastigman-7-ene-3,6,9-triol 9-*O*-β-D-glucopyranoside.[2)]

Breyniaionoside A (**2**), $[\alpha]_D$ −48.8°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{32}O_9$ using negative-ion high-resolution (HR) FAB-MS. The IR spectrum of **2** showed a strong absorption band at 3376 cm^{-1} suggestive of a glycosidic structure. The ^{1}H- and ^{13}C-NMR spectra showed signals attributable to a glucopyranosyl unit, and 13 carbon signals, which included two tertiary methyls at the geminal position, one secondary methyl, two methylenes, one methine, one quarternary carbon, one primary, secondary and tertiary carbinol, respectively, one disubstituted *trans* double bond, and a ketone functional group. The ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum showed series correlations from H-7 (δ_H 5.95), H-8 (δ_H 5.75), and H-9 (δ_H 4.47) to H$_2$-10 (δ_H 3.62). These data indicated that this aglycone is a derivative of megastigmane, of which the 10-methyl was oxidized to a primary carbinol. The secondary methyl on the ring had the equatorial orientation, since H-4 (δ_H 2.46) appeared as a triplet signal (J=13 Hz), and the side chain at the 6-position was in the equatorial orientation, as judged from the phase-sensitive nuclear Overhauser enhancement spectroscopy (PhNOESY) spectrum in which H-7 (δ_H 5.95) crossed both geminal methyls (δ_H 0.93, 0.97) at the 1-position. The ^{13}C-NMR spectrum and the sign of the Cotton effect in the CD spectrum of the ring part were essentially the same as those reported for ampelopsisionoside.[7)] The position of the glucosidic linkage was determined to be the hydroxyl group at the 9-postion, as judged from heteronuclear multiple bond correlation (HMBC) spectrum in which H-1′ (δ_H 4.36) crossed C-9 (δ_C 80.0) and H-9 (δ_H 4.47) C-1′ (δ_C 101.1). The mode of glucosidic linkage was deduced to be β from the coupling constant of the anomeric proton. Therefore the structure of

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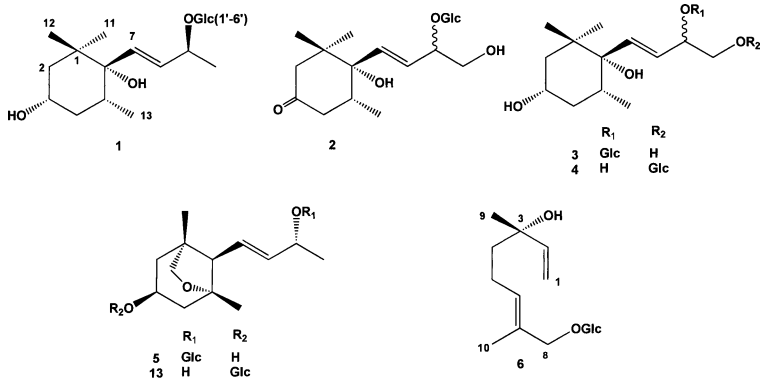

Chart 1

Table 1. ^{13}C-NMR Data for Breyniaionosides A—D (**2**—**5**) and Betulalbuside A (**6**) (CD_3OD, 100 MHz)

C	2	3	4	5	6
1	43.8	40.4	40.5	44.8	112.2
2	46.2	45.9	46.0	40.9	146.3
3	214.8	67.5	67.5	66.9	73.8
4	52.5	39.9	40.0	42.8	43.0
5	37.9	35.5	35.5	85.0	23.5
6	78.3	78.8	78.5	60.1	130.2
7	138.9	140.8	136.5	126.0	132.9
8	128.9	127.7	130.2	140.0	76.0
9	80.0	80.1	72.1	77.3	27.7
10	66.4	66.4	75.2	21.2	14.1
11	25.2	25.3	25.2	21.7	
12	25.1	25.9	25.9	78.8	
13	16.8	16.9	16.5	25.0	
1′	101.1	100.8	104.5	102.6	102.7
2′	75.0	75.0	75.2	75.3	75.1
3′	78.2	78.1	78.0	78.2	78.2
4′	71.7	71.7	71.8	71.6	71.8
5′	78.2	78.1	78.0	77.9	77.9
6′	62.8	62.8	62.9	62.8	62.9

breyniaionoside A (**2**) was elucidated to be (5*R*,6*S*,9ξ)-megastigman-7-ene-6,9,10-triol-3-one 9-*O*-β-D-glucopyranoside. The absolute configuration at the 9-position remains to be determined, since in the case of adjacent secondary and primary alcohols like 9,10-diol, the result of application of a β-D-glucopyranosylation-induced shift-trend[8] for determination of the absolute configuration of a secondary alcohol is known to be ambiguous,[9] and also the modified Mosher's method[10] probably leads to some ambiguity.

Breyniaionoside B (**3**), $[\alpha]_D$ −66.5°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{34}O_9$. The ^{1}H- and ^{13}C-NMR spectral data were similar to those of **2**, except for the absence of a ketone functional group and the presence of a secondary alcohol at δ_C 67.5 with δ_H 3.79 (tt, J=12, 4 Hz). From the coupling pattern of ring protons, the relative structure of the six-membered ring was judged to be the same as that of co-occurring turpinionoside B (**1**). Therefore the structure of breyniaionoside B (**3**) was elucidated to be (3*S**,5*R**,6*S**,9ξ)-megastigman-7-ene-3,6,9,10-tetrol 9-*O*-β-D-glucopyranoside. The absolute configuration of the 9-position and that on the ring remain to be determined for the same reason.

Breyniaionoside C (**4**), $[\alpha]_D$ −24.3°, was also isolated as an amorphous powder and its elemental composition was the same as that of **3**. The ^{1}H–^{1}H COSY spectrum showed that the spin-spin coupling systems of the ring moiety and the side chain were also the same as those of **3**. In the ^{13}C-NMR spectrum, the C-10 signal was shifted downfield (δ_C 66.4→75.2) and the C-9 one upfield (δ_C 80.1→72.1) compared with those of **3**. Thus **4** was assumed to be a positional isomer of **3** in terms of the sugar moiety. This assumption was confirmed by the HMBC spectrum, in which δ_H 3.65 (dd, J=10, 4 Hz) and 3.78 (dd, J=10, 7 Hz) (H_2-10) crossed δ_C 104.5 (C-1′) and δ_H 4.31 (d, J=8 Hz, H-1′) crossed δ_C 75.2. Therefore the structure of brenyaionoside C (**4**) was elucidated to be (3*S**,5*R**,6*S**,9ξ)-megastigman-7-ene-3,6,9,10-tetrol 10-*O*-β-D-glucopyranoside.

Breyniaionoside D (**5**), $[\alpha]_D$ −1.50°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{32}O_8$ based on negative-ion FAB-MS. The ^{1}H- and ^{13}C-NMR spectra showed signals attributable to a glucopyranosyl unit, and 13 carbon signals, which included two tertiary methyls, one secondary methyl, three methylenes, one of which bears an electronegative substitutent, two methines with an oxygen atom, two quarternary carbons, one of which bears an electronegative substitutent, and a *trans* double bond. The ^{1}H–^{1}H COSY spectrum showed two series of spin–spin couplings: from H-6 to H-10 and H_2-2 to H_2-4. Furthermore, H-2ax (δ_H 1.52) was coupled with H-12a (δ_H 3.47) due to long-range coupling *via* a w-letter interaction in 2 Hz. The HMBC spectrum show a cross peak between δ_H 3.72 (d, J=8 Hz, H-12b) and δ_C 85.0 (C-5). These results indicate the presence of one more ring system, which satisfies the degree of unsaturation, acquired by HR-FAB-MS. Thus the structure of **5** was assumed to have an epoxy ring between C-12 and C-5, like ascleposide C (**13**), isolated from *Asclepias fruticosa*.[11] Since the HMBC spectrum also showed a cross peak between H-1′ (δ_H 4.37) and C-9 (δ_C 77.3), breyniaionoside D (**5**) was a positional isomer of ascleposide C (**13**) in terms of the sugar moiety. On application of a β-D-glucopyranosylation-induced shift-trend[8] for determination of the absolute configuration, the 9-position was determined to have the *R* configuration, which is the same as in the case of **13**. Since there was no probe for elucidation of the absolute configuration of other carbons, breyniaionoside D (**5**) was enzymatically hydrolyzed and the modified Mosher's method[6] was applied for the aglycone (**5a**). As a result, the structure of the aglycone moiety was found to be the

same as that of **13**, including the absolute configuration. Therefore, the structure of breyniaionoside D (**5**) was elucidated to be (1*S*,3*S*,5*R*,6*R*,9*R*)-megastigman-7-ene-3,9-diol-5,12-epoxide 9-*O*-*β*-D-glucopyranoside.

Compound **6** was isolated as an amorphous powder and its elemental composition was $C_{16}H_{28}O_7$. The one- and two-dimensional NMR data showed that the planar structure of **6** was a known compound, betulalbuside A, isolated from *Betula alba*.[12] However, the absolute configuration of the 3-position was not fully determined. Thus commercially available (3*R*)-(−)-linalool was oxidized with SeO_2 to give (3*R*)-(−)-3,7-dimethylocta-1,6-dien-3,8-diol ($[\alpha]_D$ −8.60°).[12] Since the optical rotation value of the aglycone (**6a**) obtained on enzymatic hydrolysis showed $[\alpha]_D$ +7.59°, the absolute configuration of the 3-position was concluded to be *S*.

Isorobustaside A (**10**), $[\alpha]_D$ −51.4°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{21}H_{22}O_9$. The ^{1}H- and ^{13}C-NMR spectra showed the presence of a *para*-substituted benzene ring and a 6-acylated glucopyranose moiety as in robustaside A (**8**) and eximine (**9**). The acyl moiety comprises nine carbon atoms, which include a 1,4-disubstituted benzene ring, a cis double bond [δ_H 5.80 (d, J=12 Hz) and 6.89 (d, J=12 Hz)], and a carbonyl functional group. From these data, the structure of compound **10** was concluded to be a geometrical isomer of the acyl moiety of robustaside A isolated from *Grevillea robusta*, and it was named isorobustaside A according to the mother compound.

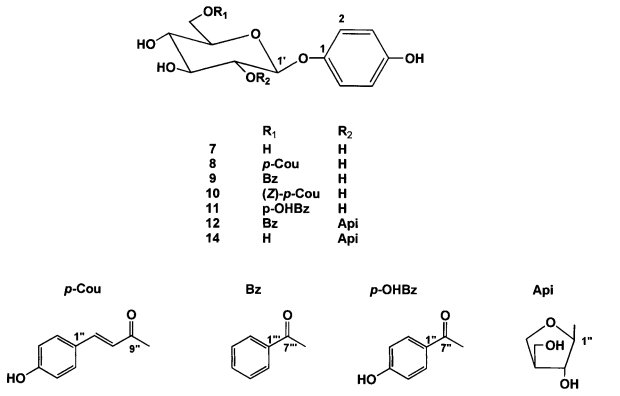

Chart 2

Table 2. ^{13}C-NMR Data for Isorobustaside A (**10**), Breyniosides A and B (**11**, **12**), and Seguinoside A (**14**) (CD_3OD, 100 MHz)

C	10	11	12	14[a]
1	154.2	152.3	152.2	152.4
2, 6	119.8	119.7	119.5	119.2
3, 5	116.3	116.7	116.7	116.7
4	152.4	153.9	153.9	153.8
1′	103.9	103.8	102.4	102.3
2′	75.0	75.0	78.6[b]	78.8
3′	78.0	78.1	78.7[b]	78.2
4′	71.9	72.1	72.2	71.5
5′	78.2	75.6	75.4	78.7
6′	64.4	65.1	65.5	62.8
1″	127.6	122.3	110.9	110.8
2″	133.7	133.0	78.2	77.9
3″	116.7	116.3	80.8	80.8
4″	160.1	163.6	75.5	75.5
5″	116.7	116.3	66.2	66.1
6″	133.7	133.0		
7″	145.4	168.0		
8″	115.9			
9″	168.1			
1‴			131.4	
2‴, 6‴			130.7	
3‴, 5‴			129.7	
4‴			134.4	
7‴			167.9	

a) Data from ref. 3. *b*) May be exchangeable.

Breynioside A (**11**), $[\alpha]_D$ −38.4°, was isolated as colorless needles (mp 244—246 °C) and its elemental composition was determined to be $C_{19}H_{20}O_9$. The ^{1}H- and ^{13}C-NMR data showed a close resemblance to those of eximine (**9**)[5,6] isolated from *Protea eximia*, except for the presence of 4-hydroxybenzoate instead of benzoate. Therefore the structure of breynioside A was elucidated to be 4″-hydroxyeximine.

Breynioside B (**12**), $[\alpha]_D$ −67.1°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{24}H_{28}O_{12}$. The ^{1}H- and ^{13}C-NMR data showed a close resemblance to eximine (**9**) with one more sugar moiety. From the characteristic carbon signals, the extra sugar moiety was concluded to be *β*-apiofuranose. When compared with reported data for seguinoside A (**14**) isolated from *Myrsine seguinii*,[3] the structure of breynioside B was concluded to be 6′-benzoylseguinoside A.

Experimental

General Experimental Procedures A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC was performed on silica gel 60 (E. Merck, Darmstadt, Germany) and reversed-phase [octadecyl silica gel (ODS)] open CC (RPCC) on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto) [Φ=50 mm, L=25 cm, linear gradient: MeOH–H_2O (1 : 9, 1 l)→(7 : 3, 1 l), fractions of 10 g were collected]. The DCCC (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ=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 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; GL Science, Tokyo, Japan; Φ=20 mm, L=250 mm), and the eluate was monitored with a UV detector at 254 nm and a reflective index monitor. *β*-D-Glucosidase (emulsin) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and crude hesperidinase was a gift from Tanabe Pharmaceutical Co. Ltd. (*R*)-(+)- and (*S*)-(−)-*α*-MTPAs were from Nacalai Tesque Co., Ltd. (Kyoto, Japan).

A melting point was determined with a Yanagimoto micro-melting point apparatus and is uncorrected. Optical rotations were measured on a Union Giken PM-101 digital polarimeter with a 1-cm cell or a JASCO DIP-360 polarimeter with a 10-cm cell under an accumulation of eight times. IR spectra were measured on a Horiba FT-710 Fourier transform infrared spectrophotometer and UV spectra on a JASCO V-520 UV/VIS spectrophotometer. CD spectrum was recorded on a JASCO J-720 spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM *α*-400 spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane (TMS) as an internal standard. Negative- and positive-ion HR-FAB-MS spectra were taken on a JEOL JMS SX-102 spectrometer.

Plant Material Leaves of *B. officinalis* HEMSL. (Euphorbiaceae) were collected in Kunigami-son, Kunigami-gun, Okinawa, Japan, in August 2000, and a voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University (00-BO-Okinawa-0828). A small amount of *B. officinalis* was also collected in Taketomi-cho, Yaeyama-gun, Okinawa, in November 2001 (01-BO-1104).

Extraction and Fractionation Air-dried leaves of *B. officinalis* (4.11 kg) were extracted three times with MeOH. The MeOH extract was concentrated to 3.0 l and then 150 ml of H_2O was added. This solution was washed with 3.0 l of *n*-hexane (159 g) and then the methanolic layer was concentrated to a viscous gum. The gummy residue was suspended in 3.0 l of H_2O, and then extracted successively with 3.0 l each of EtOAc and *n*-

BuOH to afford 111 g and 46.6 g of the EtOAc- and *n*-BuOH-soluble fractions, respectively. Evaporation of the H_2O layer gave 313 g of residue. The *n*-BuOH-soluble fraction was subjected to highly porous synthetic resin (Diaion HP-20) CC (Mitsubishi Chemical Co., Ltd.; Φ=80 mm, L=55 cm), using H_2O–MeOH (4 : 1, 6 l), (2 : 3, 6 l), (3 : 2, 6 l), and (1 : 4, 6 l), and MeOH (6 l), and 2-l fractions were collected. The residue (3.42 g) of the 20% MeOH eluate was subjected to silica gel (150 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (1 l), $CHCl_3$–MeOH (99 : 1, 1.5 l), (39 : 1, 1.5 l), (19 : 1, 1.5 l), (37 : 3, 1.5 l), (9 : 1, 3 l), (17 : 3, 3 l), (4 : 1, 3 l), (3 : 1, 3 l), and (7 : 3, 3 l)], and then $CHCl_3$–MeOH–H_2O (70 : 30 : 4, 3 l), and 250-ml fractions were collected]. The residue (0.92 g) of the 12.5—15% MeOH eluate was subjected to RPCC, and then the residue of fractions 28—35 (135 mg) was purified by DCCC to give 85.0 mg of **7** in fractions 21—26 as colorless needles. The residue (78 mg) of fractions 80—88 obtained on RPCC was purified by DCCC (31 mg in fractions 34—39), followed by HPLC with MeOH–H_2O (3 : 7) to give 18.1 mg of **2**. The residue (0.34 g) of 15—17.5% MeOH eluate obtained on silica gel CC was subjected to RPCC, and then the residue of fractions 70—79 (64 mg) was purified by DCCC (43 mg in fractions 16—21), followed by HPLC with MeOH–H_2O (3 : 17) to give 30.0 mg of **3** and 5.6 mg of **4**.

The residue (7.57 g) of the 40% MeOH eluate obtained on Diaion HP-20 CC was subjected to silica gel (450 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (2 l), $CHCl_3$–MeOH (99 : 1, 3 l), (39 : 1, 3 l), (19 : 1, 3 l), (37 : 3, 3 l), (9 : 1, 6 l), (17 : 3, 6 l), (4 : 1, 6 l), (3 : 1, 6 l), and (7 : 3, 6 l)], and then $CHCl_3$–MeOH–H_2O (70 : 30 : 4, 6 l), and 250-ml fractions were collected]. The residue (1.10 g) of the 7.5—10% MeOH eluate was subjected to RPCC, and then the residue of fractions 122—134 (124 mg) was purified by DCCC to give 27.3 mg of **6** in fractions 69—78. The residue (1.21 g) of the 10—12.5% MeOH eluate obtained on silica gel CC was subjected to RPCC, and then the residue of fractions 87—97 (152 mg) was purified by DCCC (38 mg in fractions 46—57), followed by HPLC with MeOH–H_2O (1 : 4) to afford 4.0 mg of **5**. The residue (226 mg) of fractions 105—120 obtained on RPCC was purified by DCCC (87 mg in fractions 44—55), followed by HPLC with MeOH–H_2O (7 : 13) to give 41.3 mg of **1**.

The residue (9.25 g) of the 60% MeOH eluate obtained on Diaion HP-20 CC was subjected to silica gel (450 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (2 l), $CHCl_3$–MeOH (99 : 1, 3 l), (39 : 1, 3 l), (19 : 1, 3 l), (37 : 3, 3 l), (9 : 1, 6 l), (17 : 3, 6 l), (4 : 1, 6 l), (3 : 1, 6 l), and (7 : 3, 6 l)], and then $CHCl_3$–MeOH–H_2O (70 : 30 : 4, 6 l), and 500-ml fractions were collected]. The residue (0.49 g) of the 10—12.5% MeOH eluate was subjected to RPCC, and then the residue (184 mg) of fractions 140—156 was purified by DCCC to give 95.0 mg of **9** in fractions 105—115. The residue (1.04 g) of the 12.5—15% MeOH eluate obtained on silica gel CC was subjected to RPCC, and then the residue (194 mg) of fractions 113—123 was purified by DCCC to give 127 mg of **11** in fractions 52—58. The residue (435 mg) of RPCC fractions 133—152 was subjected to DCCC to give 263 mg of a crystalline material in fractions 58—67. This was recrystallized from MeOH to give 11.4 mg of **8**. The residue of the mother liquor was purified by HPLC with MeOH–H_2O (2 : 3) to give 4.7 mg of **10**. The residue (0.77 g) of the 12.5—15% MeOH eluate obtained on silica gel CC was subjected to RPCC, and then the residue (142 mg) of fractions 154—166 was purified by DCCC to give 76 mg of **12** in fractions 70—82.

Breyniaionoside A (**2**): Amorphous powder, $[\alpha]_D^{27}$ −48.8° (*c*=1.21, MeOH). IR ν_{max} (film): 3376, 2931, 1639, 1076, 1037 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 0.93 (3H, s, H_3-12), 0.95 (3H, d, *J*=6 Hz, H_3-13), 0.97 (3H, s, H_3-11), 1.82 (1H, dd, *J*=13, 2 Hz, H-2eq), 2.12 (1H, dq, *J*=13, 2 Hz, H-4eq), 2.31 (1H, m, H-5), 2.46 (1H, t, *J*=13 Hz, H-4ax), 2.86 (1H, d, *J*=13 Hz, H-2ax), 3.20 (1H, ddd, *J*=9, 6, 2 Hz, H-5′), 3.26 (1H, dd, *J*=9, 7 H, H-2′), 3.26—3.32 (2H, overlapped, H-3′, 4′), 3.62 (2H, m, H_2-10), 3.65 (1H, dd, *J*=12, 6 Hz, H-6′a), 3.86 (1H, dd, *J*=12, 2 Hz, H-6′b), 4.36 (1H, d, *J*=7 Hz, H-1′), 4.47 (1H, m, H-9), 5.75 (1H, dd, *J*=16, 8 Hz, H-8), 5.95 (1H, d, *J*=16 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; CD: +153 (285) [θ] (nm) (*c*=0.011 M, MeOH); HR-FAB-MS (negative-ion mode) *m*/*z*: 403.1950 $[M-H]^-$ (Calcd for $C_{19}H_{31}O_9$: 403.1968).

Breyniaionoside B (**3**): Amorphous powder, $[\alpha]_D^{27}$ −66.5° (*c*=2.00, MeOH). IR ν_{max} (film): 3369, 2929, 1641, 1078, 1027 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 0.87 (3H, d, *J*=6 Hz, H_3-13), 0.88 (3H, s, H_3-12), 0.97 (3H, s, H_3-11), 1.40 (1H, q, *J*=12 Hz, H-4ax), 1.42 (1H, ddd, *J*=12, 4, 2 Hz, H-2eq), 2.31 (1H, m, H-5), 1.66 (1H, t, *J*=12 Hz, H-2ax), 1.69 (1H, m, H-4eq), 1.98 (1H, m, H-5), 3.18 (1H, ddd, *J*=9, 6, 2 Hz, H-5′), 3.26 (1H, dd, *J*=9, 7 Hz, H-2′), 3.29—3.31 (2H, overlapped, H-3′, 4′), 3.61 (2H, m, H_2-10), 3.65 (1H, dd, *J*=12, 6 Hz, H-6′a), 3.79 (1H, tt, *J*=12, 4 Hz, H-3), 3.85 (1H, dd, *J*=12, 2 Hz, H-6′b), 4.37 (1H, d, *J*=7 Hz, H-1′), 4.43 (1H, m, H-9), 5.63 (1H, dd, *J*=16, 8 Hz, H-8), 5.81 (1H, d, *J*=16 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) *m*/*z*: 405.2116 $[M-H]^-$ (Calcd for $C_{19}H_{33}O_9$: 405.2125).

Breyniaionoside C (**4**): Amorphous powder, $[\alpha]_D^{27}$ −24.3° (*c*=0.37, MeOH). IR ν_{max} (film): 3369, 2931, 1639, 1076, 1033 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 0.84 (3H, d, *J*=7 Hz, H_3-13), 0.87 (3H, s, H_3-12), 0.98 (3H, s, H_3-11), 1.39 (1H, q, *J*=12 Hz, H-4ax), 1.41 (1H, ddd, *J*=12, 4, 2 Hz, H-2eq), 1.66 (1H, t, *J*=12 Hz, H-2ax), 1.69 (1H, m, H-4eq), 1.95 (1H, m, H-5), 3.22 (1H, dd, *J*=9, 8 Hz, H-2′), 3.26—3.28 (3H, overlapped, H-4′, 5’), 3.37 (1H, t, *J*=9 Hz, H-3′), 3.63—3.68 (1H, overlapped, H-6′a), 3.65 (1H, dd, *J*=10, 4 Hz, H-10a), 3.78 (1H, dd, *J*=10, 7 Hz, H-10b), 3.78—3.83 (1H, overlapped, H-3), 3.87 (1H, dd, *J*=12, 2 Hz, H-6′b), 4.31 (1H, d, *J*=8 Hz, H-1′), 4.38 (1H, m, H-9), 5.71 (2H, s, H-7, 8); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) *m*/*z*: 405.2148 $[M-H]^-$ (Calcd for $C_{19}H_{33}O_9$: 405.2125).

Breyniaionoside D (**5**): Amorphous powder, $[\alpha]_D^{27}$ −1.50° (*c*=1.21, MeOH). IR ν_{max} (film): 3363, 2927, 1646, 1072, 1035 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 0.95 (3H, s, H_3-11), 1.15 (3H, s, H_3-13), 1.31 (3H, d, *J*=6 Hz, H_3-10), 1.48 (1H, t, *J*=13 Hz, H-4ax), 1.52 (1H, td, *J*=13, 2 Hz, H-2ax), 1.70 (1H, dd, *J*=13, 7 Hz, H-2eq), 1.82 (1H, dd, *J*=13, 7 Hz, H-4eq), 2.06 (1H, d, *J*=8 Hz, H-6), 3.19 (1H, dd, *J*=9, 8 Hz, H-2′), 3.23 (1H, ddd, *J*=9, 6, 2 Hz, H-5′), 3.34 (1H, t, *J*=9 Hz, H-4′), 3.36 (1H, t, *J*=9 Hz, H-3′), 3.47 (1H, dd, *J*=8, 2 Hz, H-12a), 3.67 (1H, dd, *J*=12, 6 Hz, H-6′a), 3.72 (1H, d, *J*=8 Hz, H-12b), 3.83 (1H, dd, *J*=12, 2 Hz, H-6′b), 4.37 (1H, d, *J*=8 Hz, H-1′), 4.00 (1H, tt, *J*=13, 7 Hz, H-3), 4.41 (1H, quintet, *J*=6 Hz, H-9), 5.78 (1H, dd, *J*=16, 8 Hz, H-7), 5.80 (1H, dd, *J*=16, 6 Hz, H-8); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) *m*/*z*: 387.1989 $[M-H]^-$ (Calcd for $C_{19}H_{31}O_8$: 387.2019).

Betulalbuside A (**6**): Amorphous powder, $[\alpha]_D^{23}$ −32.7° (*c*=1.89, MeOH). IR ν_{max} (film): 3431, 2925, 1650, 1076 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 1.26 (3H, s, H_3-9), 1.54 (2H, m, H_2-5), 1.67 (3H, br s, H_3-10), 2.09 (2H, m, H_2-4), 3.20 (1H, dd, *J*=9, 8 Hz, H-2′), 3.22 (1H, m, H-5′), 3.29 (1H, t, *J*=9 Hz, H-4′), 3.34 (1H, t, *J*=9 Hz, H-3′), 3.66 (1H, dd, *J*=12, 6 Hz, H-6′a), 3.85 (1H, dd, *J*=12, 2 Hz, H-6′b), 4.03 (1H, d, *J*=12 Hz, H-8a), 4.19 (1H, d, *J*=12 Hz, H-8b), 4.25 (1H, d, *J*=8 Hz, H-1′), 5.03 (1H, dd, *J*=11, 2 Hz, H-1a), 5.20 (1H, dd, *J*=18, 2 Hz, H-1b), 5.48 (1H, tq, *J*=7, 1 Hz, H-6), 5.91 (1H, dd, *J*=18, 11 Hz, H-2); ^{13}C-NMR (CD_3OD, 100 MHz): Table 1. HR-FAB-MS (negative-ion mode) *m*/*z*: 331.1765 $[M-H]^-$ (Calcd for $C_{16}H_{27}O_7$: 331.1757).

Isorobustaside A (**10**): Amorphous powder, $[\alpha]_D^{22}$ −51.4° (*c*=0.24, MeOH). IR ν_{max} (film): 3375, 1692, 1629, 1604, 1510, 1449, 1212, 1171, 1074, 832, 777 cm^{-1}. UV λ_{max} (MeOH): 225 (4.09), 300 (4.11), 311 (4.13) nm (log ε). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 3.41—3.44 (3H, m, H-2′, 3′, 4′), 3.59 (1H, m, H-5′), 4.31 (1H, dd, *J*=12, 6, H-6′a), 4.48 (1H, dd, *J*=12, 2 Hz, H-6′b), 5.80 (1H, d, *J*=12 Hz, H-8″), 6.64 (2H, d, *J*=9 Hz, H-3, 5), 6.72 (2H, d, *J*=9 Hz, H-3″, 5″), 6.89 (1H, d, *J*=12 Hz, H-7″), 6.91 (2H, d, *J*=9 Hz, H-2, 6), 7.60 (2H, d, *J*=9 Hz, H-2″, 6″), H-1′ was overlapped by a DHO signal. HR-FAB-MS (negative-ion mode) *m*/*z*: 417.1170 $[M-H]^-$ (Calcd for $C_{21}H_{21}O_9$: 417.1186).

Breynioside A (**11**): Colorless needles (MeOH), mp 244—246 °C, $[\alpha]_D^{22}$ −38.4° (*c*=1.12, MeOH). IR ν_{max} (film): 3369, 1697, 1607, 1509, 1280, 1211, 1047, 833, 769 cm^{-1}. UV λ_{max} (MeOH): 216 (3.94), 258 (4.04) nm (log ε). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 3.41—3.48 (3H, m, H-2′, 3′, 4′), 3.71 (1H, ddd, *J*=10, 7, 2 Hz, H-5′), 4.35 (1H, dd, *J*=12, 7, H-6′a), 4.47 (1H, d, *J*=8 Hz, H-1′), 4.72 (1H, dd, *J*=12, 2 Hz, H-6′b), 6.60 (2H, d, *J*=9 Hz, H-3, 5), 6.84 (2H, d, *J*=9 Hz, H-3″, 5″), 6.93 (2H, d, *J*=9 Hz, H-2, 6), 7.91 (2H, d, *J*=9 Hz, H-2″, 6″). ^{13}C-NMR (CD_3OD): Table 2. HR-FAB-MS (negative-ion mode) *m*/*z*: 391.1005 $[M-H]^-$ (Calcd for $C_{19}H_{19}O_9$: 391.1029).

Breynioside B (**12**): Amorphous powder, $[\alpha]_D^{23}$ −67.1° (*c*=0.89, MeOH). IR ν_{max} (film): 3421, 1708, 1603, 1509, 1290, 1227, 1076, 825, 773 cm^{-1}. UV λ_{max} (MeOH): 226 (4.03), 282 (3.33) nm (log ε). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 3.43 (1H, t, *J*=10 Hz, H-3′), 3.58 (1H, d, *J*=11 Hz, H-5″a), 3.61 (1H, d, *J*=11 Hz, H-5″b), 3.62 (2H, m, H-2′, 3′), 3.72 (1H, ddd, *J*=10, 8, 2 Hz, H-5′), 3.79 (1H, d, *J*=10 Hz, H-4″a), 3.98 (1H, d, *J*=2 Hz, H-2″), 4.10 (1H, d, *J*=10 Hz, H-4″b), 4.40 (1H, dd, *J*=12, 8 Hz, H-6′a), 4.71 (1H, dd, *J*=12, 2 Hz, H-6′b), 4.81 (1H, d, *J*=8 Hz, H-1′), 5.48 (1H, d, *J*=2 Hz, H-1″), 6.58 (2H, d, *J*=9 Hz, H-3, 5), 6.91 (2H, d, *J*=9 Hz, H-2, 6), 7.49 (2H, t, *J*=8 Hz, H-3‴, 5‴), 7.61 (1H, tt, *J*=8, 1 Hz, H-4‴), 8.02 (2H, dd, *J*=8, 1 Hz, H-2‴, 6‴). ^{13}C-NMR (CD_3OD): Table 2. HR-FAB-MS (negative-ion mode) *m*/*z*: 507.1524 $[M-H]^-$ (Calcd for $C_{24}H_{27}O_{12}$: 507.1503).

Enzymatic Hydrolysis of Breyniaionoside D (5) Breyniaionoside D (**5**, 3.8 mg) in 2 ml of H_2O was hydrolyzed with crude hesperidinase (5 mg)

at 37 °C for 24 h. The reaction mixture was partitioned with 2 ml of EtOAc. Evaporation of the organic layer resulted in 1.8 mg (81%) of aglycone (**5a**). Evaporation of the aqueous layer and trituration with MeOH gave glucose, which was identified on TLC. Aglycone (**5a**): Colorless syrup, ^{1}H-NMR (CD_3OD, 400 MHz) δ: 0.94 (3H, s, H_3-11), 1.14 (3H, s, H_3-13), 1.25 (3H, d, J=6 Hz, H_3-10), 1.49 (1H, dd, J=13, 10 Hz, H-4ax), 1.51 (1H, ddd, J=13, 10, 2 Hz, H-2ax), 1.69 (1H, dd, J=13, 10 Hz, H-2eq), 1.83 (1H, dd, J=13, 10 Hz, H-4eq), 2.03 (1H, m, H-6), 3.46 (1H, dd, J=8, 2 Hz, H-12a), 3.71 (1H, d, J=8 Hz, H-12b), 4.01 (1H, tt, J=13, 10 Hz, H-3), 4.28 (1H, quintet, J=6 Hz, H-9), 5.78 (1H, dd, J=16, 8 Hz, H-7), 5.80 (1H, dd, J=16, 6 Hz, H-8). ^{13}C-NMR (CD_3OD, 100 MHz) δ: 21.5 (C-11), 24.0 (C-10), 25.0 (C-13), 40.9 (C-2), 42.8 (C-4), 44.7 (C-1), 60.1 (C-6), 66.9 (C-3), 69.1 (C-9), 78.9 (C-12), 84.9 (C-5), 124.4 (C-7), 142.0 (C-8). HR-FAB-MS (negative-ion mode) m/z: 225.1499 $[M-H]^-$ (Calcd for $C_{13}H_{11}O_3$: 255.1491).

(*R*)- and (*S*)-MTPA Diesters of 5a A solution of **5a** (0.9 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (24 mg) in the presence of 1-ethyl-3-(3-dimethyalminopropyl)carbodiimide hydrochloride (12 mg) and *N*,*N*-dimethylaminopyridine (10 mg), and the mixture was occasionally stirred at 25 °C for 1 h. After the addition of 1 ml of CH_2Cl_2, 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 over Na_2SO_4 and then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25-mm thickness, Merck), applied for 18 cm, developed with $CHCl_3$–acetone (19 : 1) for 9 cm, and then eluted with $CHCl_3$–MeOH (9 : 1)] to furnish the 3,9-di-*O*-(*R*)-MTPA ester, **5b** (1.4 mg, 53%). Through a similar procedure, **5c** (1.3 mg, 50%) was prepared from **5a** (0.9 mg) by use of (*S*)-MTPA (26 mg), EDC (13 mg), and DMAP (9 mg).

3,9-Di-*O*-(*R*)-MTPA Ester (**5b**): Colorless syrup. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.91 (3H, s, H_3-11), 1.16 (3H, s, H_3-13), 1.37 (3H, d, J=6 Hz, H_3-10), 1.49 (1H, dd, J=13, 10 Hz, H-4ax), 1.60 (1H, ddd, J=13, 10, 2 Hz, H-2ax), 1.91 (1H, dd, J=13, 10 Hz, H-2eq), 1.98 (1H, dd, J=13, 10 Hz. H-4eq), 2.05 (1H, d, J=9 Hz. H-6), 3.52 (1H, dd, J=8, 2 Hz, H-12a), 3.52 (3H, q, J=1 Hz, –OCH_3), 3.54 (3H, q, J=1 Hz, –$COCH_3$), 3.85 (1H, d, J=8 Hz, H-12b), 5.39 (1H, tt, J=13, 10 Hz, H-3), 5.56 (1H, quintet, J=6 Hz, H-9), 5.70 (1H, dd, J=15, 6 Hz, H-8), 5.81 (1H, dd, J=15, 9 Hz, H-7), 7.32—7.43 (6H, m), 7.48—7.53 (4H, m) (aromatic protons). HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) m/z: 681.2258 $[M+Na]^+$ (NaI) (Calcd for $C_{33}H_{36}O_5F_3Na$: 681.2263).

3,9-Di-*O*-(*S*)-MTPA Ester (**5c**): Colorless syrup. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.87 (3H, s, H_3-11), 1.15 (3H, s, H_3-13), 1.42 (3H, d, J=6 Hz, H_3-10), 1.47 (1H, ddd, J=13, 10, 2 Hz, H-2ax), 1.56 (1H, dd, J=13, 10 Hz, H-4ax), 1.86 (1H, dd, J=13, 10 Hz, H-2eq), 2.01 (1H, dd, J=13, 10 Hz, H-4eq), 2.02 (1H, d, J=9 Hz. H-6), 3.50 (3H, br s, –OCH_3), 3.53 (1H, dd, J=8, 2 Hz, H-12a), 3.53 (3H, br s, –$COCH_3$), 3.83 (1H, d, J=8 Hz, H-12b), 5.41 (1H, tt, J=13, 10 Hz, H-3), 5.56 (1H, quintet, J=6 Hz, H-9), 5.61 (1H, dd, J=15, 6 Hz, H-8), 5.73 (1H, dd, J=15, 9 Hz, H-7), 7.34—7.40 (6H, m), 7.49—7.51 (4H, m) (aromatic protons). HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) m/z: 681.2258 $[M+Na]^+$ (NaI) (Calcd for $C_{33}H_{36}O_7F_6Na$: 681.2263).

$\Delta\delta S$–δR in Hz at 400 MHz: H-2ax, −52 Hz; H-2eq, −50 Hz; H-4ax, +29 Hz; H-4eq, +46 Hz; H-7, −33 Hz; H-8, −32 Hz; H_3-10, +21 Hz; H_3-11, −12 Hz; H-12a, −4 Hz; H_3-13, −3 Hz.

Enzymatic Hydrolysis of Betulalbuside A (6) Betulalbuside (**6**, 27.3 mg) in 2 ml of H_2O was hydrolyzed with emulsin (15 mg) at 37 °C for 24 h. The resultant hydrolyzate was separated by silica gel CC to give (*S*)-(+)-3,7-dimethylocta-1,6-dien-3,8-diol as an aglycone (**6a**) (11.4 mg, 82%) and 14.3 mg of D-glucose.

(*S*)-(+)-3,7-Dimethylocta-1,6-diene-3,8-diol (**6a**): Amorphous powder, $[\alpha]_D^{23}$ +7.59° (c=0.76, MeOH). IR ν_{max} (film): 3366, 2924, 1641, 1455, 1410, 1331, 999, 919 cm^{-1}. ^{1}H-NMR (CD_3OD, 400 MHz) δ: 1.25 (3H, s, H_3-9), 1.54 (2H, m, H_2-4), 1.63 (1H, br s, H-10), 2.07 (2H, m, H_2-5), 3.90 (2H, s, H_2-8), 5.03 (1H, dd, J=11, 2 Hz, H-1a), 5.19 (1H, dd, J=18, 2 Hz, H-1b), 5.38 (1H, tq, J=7, 1 Hz, H-6), 5.91 (1H, dd, J=18, 11 Hz, H-2). ^{13}C-NMR (CD_3OD, 100 MHz) δ: 13.7 (C-10), 23.4 (C-4), 27.7 (C-9), 43.1 (C-5), 69.0 (C-1), 73.8 (C-6), 112.1 (C-8), 126.9 (C-3), 135.9 (C-2), 146.3 (C-7). HR-FAB-MS (negative-ion mode) m/z: 169.1222 $[M-H]^-$ (Calcd for $C_{10}H_{17}O_2$: 169.1229).

D-Glucose: $[\alpha]_D^{22}$ +52.6° (c=0.95, H_2O, 24 h after being dissolved in the solvent).

Oxidation of (*R*)-(−)-Linalool (*R*)-(−)-Linalool (1.0 g) was oxidized with SeO_2 (0.87 g) in 40 ml of EtOH at 50 °C for 3 h.[13] The reaction mixture was diluted with H_2O and then extracted three times with EtOAc. The residue obtained on evaporation of the EtOAc layer was subjected to silica gel (100 g) CC with $CHCl_3$ and $CHCl_3$: MeOH (95 : 5). From the latter eluate, 228 mg of (*R*)-(−)-3,7-dimethylocta-1,6-diene-3,8-diol (**6b**) was obtained.

Amorphous powder, $[\alpha]_D^{22}$ −8.60° (c=0.76, MeOH). The ^{1}H- and ^{13}C-NMR spectra were essentially the same as those of the natural compound (**6a**).

Known Compounds Isolated Turpinionoside B (**1**): Amorphous powder, $[\alpha]_D^{22}$ −8.6° (c=0.76, MeOH).[2] Arbutin (**7**): Colorless needles (MeOH), mp 163—165 °C, $[\alpha]_D^{22}$ −55.8° (c=0.56, MeOH).[3] Robustaside A (**8**): Colorless needles (MeOH), mp 210—213 °C, $[\alpha]_D^{22}$ −67.9° (c=0.56, MeOH).[4] Eximine (**9**): Colorless needles (MeOH), mp 208—210 °C, $[\alpha]_D^{22}$ −38.6° (c=0.47, MeOH).[5,6]

Acknowledgements The authors are grateful for access to the superconducting NMR instrument in the Analytical Center of Molecular Medicine of the Graduate School of Biomedical Sciences, Hiroshima University. This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports, Culture and Technology of Japan (No. 13672216). Thanks are also due to the Okinawa Foundation for the financial support with an Okinawa Research Promotion Award (H.O.).

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