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ORIGINAL PAPER

Megastigmane glucosides and an unusual monoterpene from the leaves of *Cananga odorata* var. *odorata*, and absolute structures of megastigmane glucosides isolated from *C. odorata* var. *odorata* and *Breynia officinalis*

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Abstract From a 1-BuOH-soluble fraction of a MeOH extract of *Cananga odorata* var. *odorata*, collected at the Botanical Garden of Chiang Mai University, a new megastigmane glucoside, named canangaionoside, and an irregular monoterpene were isolated. A known compound, breyniaionoside A, which has been obtained from the leaves of *Breynia officinalis*, was also isolated, and its absolute structure was substantiated for the first time in this study. On this occasion, the absolute stereochemistries of structurally related megastigmane glucosides, breyniaionosides B and C, isolated from *B. officinalis* were examined.

Keywords *Cananga odorata* var. *odorata* · Annonaceae · Fragrant cananga · Megastigmane glucoside · *Breynia officinalis* · Euphorbiaceae · Canangaionoside

Introduction

Ylang-ylang is an essential oil, and has a euphoric and sedative effect on the nervous system. Therefore, it is globally used as an aroma therapy agent to remove uneasiness, tension, shock and panic, while the aphrodisiac qualities of the oil are useful for impotence and frigidity. The origin of this oil is the flowers of some *Cananga* species. *Cananga odorata* (Lam.) Hooker f. and Thomson var. *odorata* (Annonaceae) are called fragrant cananga or wild cananga, and *C. odorata* (Lam.) Hooker f. and Thomson var. *fruticosa* (Craib) Corner are species closely related to the former one and called shrubby cananga [1]. This paper deals with investigation of the constituents (**1**, **2**) of the leaves of *C. odorata* var. *odorata*, as well as the absolute stereochemistries of a known compound (**3**) [=breyniaionoside A (**4**)], isolated from the title plant, and structurally related megastigmane glucosides, breyniaionosides B (**5**) and C (**6**), isolated from *B. officinalis* were examined.

Results and discussion

Air-dried leaves of *C. odorata* var. *odorata* 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), and then normal silica gel and reversed-phase octadecyl silica gel (ODS) CC and high-performance liquid chromatography (HPLC) to afford four compounds (**1**–**3** and **7**) (Fig. 1). The details and yields are given under "Experimental." The structures of the new megastigmane glucoside, named canangaionoside (**1**), the unusual monoterpene (**2**), and the two known megastigmane glucosides, compound **3** [=breyniaionoside A (**4**)] [2] and citroside A (**7**), were examined [3]. The structure of the aglycone of

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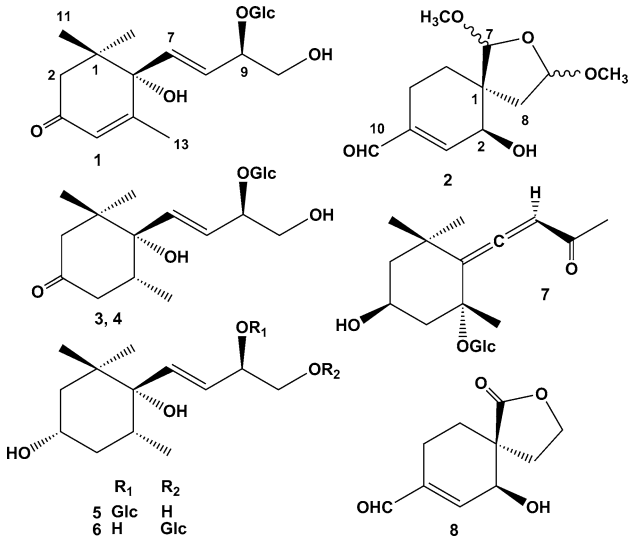


Fig. 1 Structures of compounds

breyniaionoside A (**4**), breyniaionol A, has been elucidated to be (5*R*,6*S*,7*E*)-megstigman-7-ene-6,9,10-triol-3-one. However, the absolute configuration at the 9-position remained to be clarified. In this study, the absolute structure of breyniaionoside A (**4**) was examined by the modified Mosher's method [4], and determination of the absolute structures of two other megastigmane glucosides, breyniaionosides B (**5**) and C (**6**), isolated from *Breynia officinalis* (Euphorbiaceae) was also performed.

Canangaionoside (**1**), $[\alpha]_D^{25}$ +52.0, was isolated as an amorphous powder, and its elemental composition was determined to be $C_{19}H_{30}O_9$ by HR-electrospray ionization (ESI)-MS. The IR spectrum exhibited absorptions for hydroxyl groups (3364 and 1075 cm^{-1}) and a conjugated ketone functional group (1651 cm^{-1}). In the ^{1}H-NMR spectrum, signals for three olefinic protons, two of which were coupled to each other with a coupling constant of 16 Hz, an anomeric proton, two singlet methyls and one doublet methyl ($J = 1$ Hz) were observed. The ^{13}C-NMR spectroscopic data showed the presence of six carbon signals assignable to β-glucopyranoside, and 13 carbon signal for four sp^2 carbons, three methyls, one methylene, one oxymethylene, one oxymethine, two quaternary carbons and one ketone. These carbons were expected to form a magastigmane skeleton with a *trans* double bond between the C-7 and C-8 positions. The ^{1}H-^{1}H COSY and HSQC spectra indicated that the oxymethine carbon must be adjacent to the *trans* double bond and that the oxymethine proton was further correlated to oxymethylene protons. Judging from the UV absorption at 232 nm, the carbonyl group must be conjugated with the remaining trisubstituted double bond. Two shielded methyl protons (δ_H 1.01 and 1.03) showed correlation cross peaks with C-2 (δ_C 50.7) and C-6 (δ_C 80.2), two olefinic protons (δ_H 5.79 and 6.10)

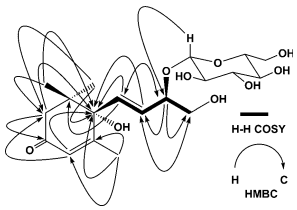


Fig. 2 ^{1}H-^{1}H COSY and diagnostic HMBC correlations of canangaionoside (**1**). *Dual arrowheads* denote HMBC correlations were observed both ways

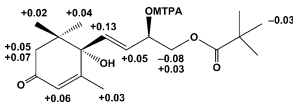


Fig. 3 Results of the modified Mosher's method of **1** ($\Delta\delta_{S-R}$)

with C-6, deshielded methyl protons (δ_H 1.93) with C-4 (δ_C 127.2), C-5 (δ_C 167.0), and C-6, and H-4 (δ_H 5.86) with C-2 in the HMBC spectrum (Fig. 2). The above evidence established the relative structure of the aglycone to be megastigma-4,7-diene-6,9,10-triol-3-one. The position of the sugar linkage was determined to be on the hydroxyl group at the 9-position by the HMBC spectrum, in which the anomeric proton (δ_H 4.29) showed a cross peak with C-9 (δ_C 79.5). The absolute configuration at the 6-position was determined to be *S*, judging from the positive and negative Cotton effects at 241 and 329 nm, respectively, in the CD spectrum [5]. The modified Mosher's method was applied to the aglycone (**1a**) of canangaionoside. Prior to α-methoxy-α-trifluoromethylphenyl acetic acid (MTPA) esterifications, the primary alcohol was protected as a pivaloyl ester. Figure 3 shows the results of the modified Mosher's method. The structure of canangaionoside (**1**) was elucidated to be (6*S*,9*R*,4*Z*,7*E*)-megastigma-4,7-diene-6,9,10-triol-3-one 9-*O*-β-D-glucopyranoside, as shown in Fig. 1. The aglycone of canangaionoside (**1**) was found to be an epimer as to the 9-position of cucumegastigmane I, which was isolated from *Cucumis sativus* [5].

Compound **2**, $[\alpha]_D^{24}$ +65.4, was isolated as an amorphous powder, and its elemental composition was established to be $C_{12}H_{18}O_5$ by HR-ESI-MS. The IR spectrum exhibited absorptions for hydroxyl groups (3381, 1097 and 1031 cm^{-1}) and a conjugated ketonic functional group (1685 cm^{-1}). The UV absorption at 232 nm also supported the presence of a conjugated enone system. In the ^{1}H-NMR spectrum, resonances for one aldehyde (δ_H 9.48), one olefinic (δ_H 6.74), two acetalic [δ_H 5.13 (dd) and 4.93 (s)], six aliphatic and one oxygenated methine (δ_H 4.00) protons were observed, along with two methoxyl signals. Other than two methoxyl signals, the ^{13}C-NMR spectrum exhibited ten signals for three methylene carbons, three oxygenated methine carbons, one trisubstituted double bond, one aldehyde carbon and one quaternary carbon. The double bond must be conjugated with the aldehyde, and from the elemental composition, compound **2** was presumed to possess a bicyclic system. Since a similar compound, canangone (**8**), was isolated from a closely related

Fig. 4 Diagnostic HMBC correlations for **2**

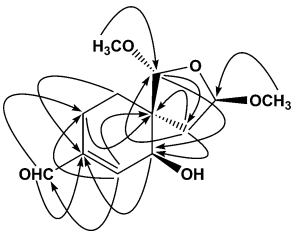


Fig. 5 Results of the modified Mosher's method of **4** ($\Delta\delta_{S\text{-}R}$)

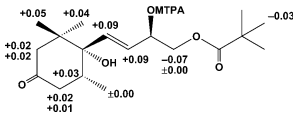
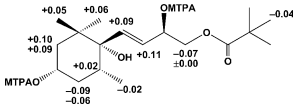


Fig. 6 Results of the modified Mosher's method of **5** ($\Delta\delta_{S\text{-}R}$)



plant, *C. odorata* forma *genuina* Hook. f. et Thomson [6], the structure of compound **2** was expected to be as shown in Fig. 1. The planar structure was supported by significant HMBC correlations, as shown in Fig. 4. Since, in the PS-NOESY spectrum, a significant correlation was observed between H-2 (δ_H 4.00) and H-8 (δ_H 1.99), the relative structure of the spiro junction was established to be as shown in Fig. 1. Although a mother compound has not yet been isolated, compound **2** is probably an artifact, formed through a dial. Thus, the geometry of methoxy groups and the absolute structure of **2** were not further examined.

Compound **3**, $[\alpha]_D^{25}$ −18.1, was isolated as an amorphous powder, and its elemental composition was determined to be $C_{19}H_{32}O_9$ by HR-ESI-MS. The NMR spectral data were essentially the same as those for breyniaionoside A, which was isolated from *Breynia officinalis* [2]. The CD spectrum of **3** was also superimposable on that of breyniaionoside A (**4**). However, the absolute configuration at the 9-position of breyniaionoside A (**4**) has not yet been determined. On enzymatic hydrolysis, **3** and **4** gave identical aglycones, breyniaionol A (**3a** = **4a**) and D-glucose. The *β*-D-glucopyranosylation-induced shift-trend rule suggested the absolute configuration at the 9-position to be *R* [7] ($\Delta\delta$ −3.2 ppm for C-8 and −1.4 ppm for C-10), which was further confirmed by the modified Mosher's method [4]. The primary alcohol of **4a** was protected as a pivalate, and (*R*)- and (*S*)-MTPA esters were prepared. Figure 5 shows the results of the modified Mosher's method for breyniaionol A (**4a**). One of the methylene protons at the 10-position showed an irregular value. Kusumi et al. [8] stated that this can be regarded as normal, since the corresponding protons of some compounds also exhibit irregular values and the pivaloyl methyls exhibited regular values. The structure of breyniaionoside A (**3** and **4**) was elucidated to be (5*R*,6*S*,7*E*,9*R*)-megastigman-7-ene-6,9,10-triol-3-one 9-*O*-*β*-D-glucopyranoside, as shown in Fig. 1.

From *B. officinalis*, structurally related megastigmane glucosides, breyniaionosides B (**5**) and C (**6**), have also been isolated (Fig. 1) [2]. However, their absolute structures remained to be determined. Thus, they were enzymatically hydrolyzed to give the common aglycone, breyniaionol B (=C) (**5a** and **6a**), and the modified Mosher's method was applied to breyniaionol B (**5a**) to establish the absolute configuration at the 9-position. As a result, as shown in Fig. 6, the absolute structures of breyniaionosides B (**5**) and C (**6**) were elucidated to be (3*S*,5*R*,6*S*,7*E*,9*R*)-megastigman-7-ene-3,6,9,10-tetrol 9- and 10-*O*-*β*-D-glucopyranosides, respectively, as shown in Fig. 1.

Experimental

General experimental procedure

Optical rotations and CD spectrum were measured on JASCO P-1030 and JASCO J-720 polarimeters, respectively. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/Vis spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM α-400 or ECA-600 spectrometer at 400 or 600 MHz and 100 or 150 MHz, respectively, with tetramethylsilane as an internal standard. Positive-ion HRESIMS was performed with an Applied Biosystem QSTAR XL system ESI (Nano Spray)-TOF-MS.

(*R*)- and (*S*)-MTPA, and *β*-glucosidase (emulsin) were the products of Wako Pure Chemical Industry Co., Ltd. (Tokyo, Japan).

Plant material

Leaves of *C. odorata* var. *odorata* (Annonaceae) were collected in March 2005 from cultivated species in the Botanical Garden, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand.

Extraction and isolation

Dried leaves of *C. odorata* var. *odorata* (1.17 kg) were extracted three times with MeOH (4.5 l) at 25°C for 1 week and then concentrated to 3 l in vacuo. The extract was washed with *n*-hexane (3 l, 20.1 g), and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in water (3 l) and then extracted with EtOAc (3 l) to give 14.0 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (3 l) to give a 1-BuOH-soluble fraction (27.4 g), and the remaining

water layer was concentrated to furnish 70.3 g of a water-soluble fraction.

The 1-BuOH-soluble fraction was subjected to Diaion HP-20 (purchased from Mitsubishi Kagaku, Tokyo, Japan) CC ($\Phi = 50$ mm, $L = 50$ cm), using H_2O-MeOH (4:1, 3 l), (2:3, 3 l), (3:2, 3 l) and (1:4, 3 l), and MeOH (3 l), with fractions of 500 ml being collected. The residue (2.08 g in fractions 8-10) obtained on Diaion HP-20 (20–40% MeOH eluate) was subjected to silica gel CC (125 g) with elution with a linear gradient from $CHCl_3$ (1 l) to $CHCl_3$-MeOH (1:1, 1 l), with fractions of 10 g being collected. The residue (78.6 mg) in fractions 46–50 was again separated by silica gel CC (50 g) with an isocratic solvent system of $CHCl_3$-MeOH (18:1), with fractions of 1 ml being collected. The residue (12.5 mg) in fractions 35–49 was purified by HPLC (MeOH-H_2O, 9:11) to give 1.4 mg of **2** from the peak at 12 min. The residue (336 mg) in fractions 79–89 was separated by ODS open CC to yield three subfractions, 143 mg in fractions 34–53, 34.0 mg in fractions 54–70 and 55.6 mg in fractions 71–90. The first subfraction was purified by HPLC (MeOH-H_2O, 1:3) to obtain 28.3 mg of **1** from the peak at 14 min. The second subfraction was purified by HPLC (MeOH-H_2O, 3:7) to obtain 3.0 mg of **3** from the peak at 15 min. The third subfraction was also purified by HPLC (MeOH-H_2O, 3:7) to give 21.7 mg of **7** from the peak at 23 min.

Canangaionoside (**1**)

Amorphous powder; $[\alpha]_D^{25}$ +52.0 (*c* 1.81, MeOH); IR ν_{max} (film) cm^{-1}: 3364, 2934, 1651, 1075, 1037; UV (MeOH) λ_{max} nm (log ε): 232 (4.16); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 6.10 (1H, dd, $J = 16$, 1 Hz, H-7), 5.86 (1H, q, $J = 1$ Hz, H-4), 5.79 (1H, dd, $J = 16$, 7 Hz, H-8), 4.45 (1H, m, H-9), 4.29 (1H, d, $J = 8$ Hz, H-1′), 3.83 (1H, dd, $J = 12$, 2 Hz, H-6′a), 3.63 (1H, dd, $J = 12$, 6 Hz, H-6′b), 3.63 (1H, dd, $J = 12$, 4 Hz, H-10a), 3.57 (1H, dd, $J = 12$, 6 Hz, H-10b), 3.26 (3H, m, H-2′, 3′ and 4′), 3.17 (1H, ddd, $J = 9$, 6, 2 Hz, H-5′), 2.60 (1H, d, $J = 17$ Hz, H-2a), 2.17 (1H, d, $J = 17$ Hz, H-2b), 1.93 (3H, d, $J = 1$ Hz, H_3-13), 1.03 (3H, s, H_3-11), 1.01 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD, 100 MHz): Table 1; CD (*c* 5.04 × 10^{-5} M, MeOH): $\Delta\varepsilon$ (nm): −0.71 (329), +17.3 (241); HR-ESI-MS (positive-ion mode) *m/z*: 425.1774 $[M+Na]^+$ ($C_{19}H_{30}O_9Na$ requires 425.1782).

Compound **2**

Amorphous powder; $[\alpha]_D^{24}$ +65.4 (*c* 0.11, MeOH); IR ν_{max} (film) cm^{-1}: 3381, 2836, 1685, 1178, 1097, 1031, 994; UV (MeOH) λ_{max} nm (log ε): 232 (4.13); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 9.48 (1H, s, H-10), 6.74 (1H, ddd, $J = 4$, 2, 2 Hz, H-3), 5.13 (1H, dd, $J = 6$, 5 Hz, H-9), 4.93 (1H, s, H-7), 4.00 (1H, br d, $J = 4$ Hz, H-2), 3.40 (3H, s, –OCH_3 on C-7), 3.36 (3H, s, –OCH_3 on C-9), 2.33 (1H, m, H-5a), 2.02 (1H, m, H-5b), 1.99 (1H, dd, $J = 14$, 6 Hz, H-8a), 1.86 (1H, ddd, $J = 14$, 9, 6 Hz, H-6a), 1.77 (1H, dd, $J = 14$, 5 Hz, H-8b), 1.77 (1H, m, H-6b); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 195.9 (C-10), 149.6 (C-3), 143.6 (C-4), 107.8 (C-7), 107.4 (C-9), 70.6 (C-2), 56.0 (CH_3O– at C-7), 55.4 (CH_3O– at C-9), 51.2 (C-1), 40.9 (C-8), 24.1 (C-5), 20.9 (C-6); HR-ESI-MS (positive-ion mode) *m/z*: 265.1047 $[M+Na]^+$ (Calcd for $C_{12}H_{18}O_5Na$: 265.1046).

Table 1 ^{13}C-NMR spectroscopic data for canangaionoside (**1**) (CD_3OD, 100 MHz)

C		C	
1	42.4	1′	101.5
2	50.7	2′	74.9
3	201.2	3′	78.2
4	127.2	4′	71.6
5	167.0	5′	78.1
6	80.2	6′	62.7
7	135.8		
8	128.9		
9	79.5		
10	66.0		
11	23.5		
12	24.7		
13	19.5		

Compound **3** [=breyniaionoside A (**4**)]

Amorphous powder; $[\alpha]_D^{25}$ −18.1 (*c* 0.20, MeOH); CD (*c* 4.01 × 10^{-5} M, MeOH): $\Delta\varepsilon$ (nm): +0.037 (294); HR-ESI-MS (positive-ion mode) *m/z*: 427.1941 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_9Na$: 427.1938).

Enzymatic hydrolysis of canangaionoside (**1**) to canangaionol (**1a**)

Canangaionoside (**1**) (10.5 mg) was hydrolyzed with emulsin (10 mg) in 400 µl of 100 mM acetate buffer (pH 5.0) at 37°C for 18 h. The reaction solvent was evaporated off, and the residue was purified by silica gel CC ($\Phi = 10$ mm, $L = 20$ cm) with elution with $CHCl_3$ (50 ml) and $CHCl_3$-MeOH [(19:1, 50 ml), (9:1, 50 ml), (17:3, 50 ml), and (7:3, 50 ml)], with fractions of 5 ml being collected. The aglycone (**1a**) (3.1 mg) was recovered in fractions 34–38 and glucose (4.4 mg) in fractions 59–64.

*Canangaionol (**1a**)*

Amorphous powder; $[\alpha]_D^{25}$ +211 (*c* 0.20; MeOH); ^{1}H-NMR (CD_3OD, 400 MHz): δ 5.90 (1H, dd, $J = 16$, 1 Hz, H-7), 5.84 (1H, m, H-4), 5.80 (1H, dd, $J = 16$, 5 Hz, H-8), 4.20

(1H, br ddd, $J = 6, 6, 6$ Hz, H-9), 3.51 (1H, dd, $J = 11, 5$ Hz, H-10a), 3.46 (1H, dd, $J = 11, 7$ Hz, H-10b), 2.52 (1H, d, $J = 17$ Hz, H-2a), 2.16 (1H, d, $J = 17$ Hz, H-2b), 1.92 (1H, s, H_3-13), 1.04 (3H, s, H_3-11), 1.01 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 201.2 (C-3), 167.4 (C-5), 132.6 (C-7), 132.5 (C-8), 127.2 (C-4), 80.1 (C-6), 73.7 (C-9), 67.3 (C-10), 50.8 (C-2), 42.4 (C-1), 24.5 (C-12), 23.4 (C-11), 19.6 (C-13); HR-ESI-MS (positive-ion mode) *m/z*: 263.1242 $[M+Na]^+$ (Cacld for $C_{13}H_{20}O_4Na$: 263.1253).

D-Glucose: $[\alpha]_D^{24}$ +50.4 (*c* 0.21, H_2O, 24 h after being dissolved in the solvent).

Preparation of 10-*O*-pivalate (**1b**) from canangaionol (**1a**)

To a solution of the aglycone (**1a**) (3.0 mg) in 100 µl of pyridine, 10 µl of pivaloyl chloride was added, and the reaction was performed for 3 h at 25°C. One milliliter of H_2O was added, followed by extraction with 2 ml of EtOAc three times. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by prep. TLC [$CHCl_3$-$(CH_3)_2CO$, 20:1] to give 1.2 mg of pivalate (**1b**).

*10-O-Pivaloyl canangaionol (**1b**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.96 (1H, dd, $J = 16$, 1 Hz, H-7), 5.90 (1H, m, H-4), 5.84 (1H, dd, $J = 16$, 5 Hz, H-8), 4.48 (1H, m, H-9), 4.20 (1H, dd, $J = 12$, 4 Hz, H-10a), 4.03 (1H, dd, $J = 12$, 7 Hz, H-10b), 2.46 (1H, d, $J = 17$ Hz, H-2a), 2.26 (1H, dd, $J = 17$, 1 Hz, H-2b), 1.89 (3H, d, $J = 1$ Hz, H_3-13), 1.22 (9H, s, CH_3– × 3), 1.08 (3H, s, H_3-11), 1.01 (3H, s, H_3-12); HR-ESI-MS (positive-ion mode) *m/z*: 347.1825 $[M+Na]^+$ (Calcd for $C_{18}H_{28}O_5Na$: 347.1834).

Preparation of (*R*)- and (*S*)-9-*O*-MTPA esters (**1c** and **1d**) from 10-*O*-pivaloyl canangaionol (**1b**)

A solution of **1b** (0.5 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (38 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (30 mg) and *N*,*N*-dimethyl-4-aminopyridine (DMAP) (20 mg), with the mixture being occasionally stirred at 25°C for 30 min. After the addition of 1 ml of CH_2Cl_2, the solution was washed with H_2O (1 ml), 5% HCl (1 ml), $NaHCO_3$-saturated H_2O and then brine (1 ml), successively. The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), being applied for 18 cm, developed with $CHCl_3$-$(CH_3)_2CO$ (19:1) for 9 cm, and then eluted with $CHCl_3$-MeOH (9:1)] to furnish an ester, **1c** (0.63 mg). Through a similar procedure, **1d** (0.72 mg) was prepared from **1b** (0.5 mg) using (*S*)-MTPA (50 mg), EDC (32 mg) and 4-DMAP (24 mg).

*10-O-Pivaloyl canangaionol 9-O-(R)-MTPA ester (**1c**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.54–7.51 (2H, aromatic protons), 7.41–7.37 (3H, m, aromatic protons), 5.87 (1H, m, H-4), 5.86 (1H, d, $J = 15$ Hz, H-7), 5.80 (2H, m, H-8 and 9), 4.40 (1H, dd, $J = 12$, 4 Hz, H-10a), 4.10 (1H, dd, $J = 12$, 7 Hz, H-10b), 3.57 (3H, q, $J = 1$ Hz, –OCH_3), 2.23 (1H, d, $J = 17$ Hz, H-2a), 2.18 (1H, d, $J = 17$ Hz, H-2b), 1.80 (3H, s, H_3-13), 1.17 (9H, s, CH_3– × 3), 1.04 (3H, s, H_3-11), 0.89 (3H, s, H_3-12); HR-ESI-MS (positive-ion mode) *m/z*: 547.2281 $[M+Na]^+$ (Calcd for $C_{28}H_{35}O_6F_3Na$: 547.2283).

*10-O-Pivaloyl canangaionol 9-O-(S)-MTPA ester (**1d**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.54–7.49 (2H, m, aromatic protons), 7.41–7.37 (3H, m, aromatic protons), 5.99 (1H, d, $J = 16$ Hz, H-7), 5.90 (1H, m, H-4), 5.85 (1H, dd, $J = 16$, 5 Hz, H-8), 5.74 (1H, m, H-9), 4.32 (1H, dd, $J = 12$, 3 Hz, H-10a), 4.13 (1H, dd, $J = 12$, 7 Hz, H-10b), 3.49 (3H, q, $J = 1$ Hz, –OCH_3), 2.30 (1H, d, $J = 17$ Hz, H-2a), 2.23 (1H, d, $J = 17$ Hz, H-2b), 1.83 (3H, s, H_3-13), 1.14 (9H, s, CH_3– × 3), 1.06 (3H, s, H_3-11), 0.95 (3H, s, H_3-12); HR-ESI-MS (positive-ion mode) *m/z*: 547.2271 $[M+Na]^+$ (Calcd for $C_{28}H_{35}O_6F_3Na$: 547.2283).

Enzymatic hydrolysis of compound **3** and breyniaionoside A (**4**) to its aglycone and breyniaionol A (**3a** and **4a**), respectively

Compound **3** (2.5 mg) was hydrolyzed in a similar manner as for **1**. The usual workup gave 0.27 mg of an aglycone (**3a**). Breyniaionoisde A (**4**) (8.5 mg) was also hydrolyzed enzymatically to give 4.9 mg of breyniaionol A (**4a**) and 3.2 mg of D-glucose.

*Aglycone (**3a**)*

Amorphous powder; $[\alpha]_D^{24}$ +5.2 (*c* 0.027, MeOH); ^{1}H-NMR (CD_3OD, 600 MHz) δ: 5.82 (1H, dd, $J = 16$, 5 Hz, H-8), 5.77 (1H, dd, $J = 16$, 1 Hz, H-7), 4.22 (1H, dddd, $J = 7, 5, 5, 1$ Hz, H-9), 3.54 (1H, dd, $J = 11$, 5 Hz, H-10a), 3.48 (1H, dd, $J = 11$, 7 Hz, H-10b), 2.86 (1H, d, $J = 14$ Hz, H-2a), 2.44 (1H, dd, $J = 14$, 13 Hz, H-4a), 2.28 (1H, dqd, $J = 13$, 7, 5 Hz, H-5), 2.12 (1H, ddd, $J = 14, 5, 2$ Hz, H-4b), 1.82 (1H, dd, $J = 14$, 2 Hz, H-2b),

0.96 (3H, s, H_3-12), 0.92 (3H, d, $J = 7$ Hz, H_3-13), 0.92 (3H, s, H_3-11); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 215.0 (C-3), 134.8 (C-7), 132.2 (C-8), 78.2 (C-6), 74.1 (C-9), 67.7 (C-10), 52.5 (C-2), 46.3 (C-4), 43.9 (C-1), 38.0 (C-5), 25.1 (C-11), 25.0 (C-12), 16.5 (C-13); HR-ESI-MS (positive-ion mode) *m/z*: 265.1413 $[M+Na]^+$ (Calcd for $C_{13}H_{22}O_4Na$: 265.1410).

*Breyniaionol A (**4a**)*

Amorphous powder; $[\alpha]_D^{24}$ +5.5 (*c* 0.49, MeOH); ^{1}H-NMR (CD3OD, 600 MHz) and ^{13}C-NMR (CD_3OD, 150 MHz): essentially the same as those of **3a**; HR-ESI-MS (positive-ion mode) *m/z*: 265.1415 $[M+Na]^+$ (Calcd for $C_{13}H_{22}O_4Na$: 265.1410).

Preparation of 10-*O*-pivalate (**4b**) from breyniaionol A (**4a**)

In a similar manner as for the preparation of **1b** from **1a**, **4b** was prepared from **4a** (4.9 mg) with 20 μl of pivaloyl chloride. The usual workup gave 2.6 mg of pivaloyl ester (**4b**).

*10-O-Pivaloyl breyniaionol A (**4b**)*

Amorphous powder; $[\alpha]_D^{24}$ −12.1 (*c* 0.07, MeOH); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 5.89 (1H, dd, $J = 16$, 1 Hz, H-7), 5.80 (1H, dd, $J = 16$, 5 Hz, H-8), 4.50 (1H, dddd, $J = 7$, 5, 4, 1 Hz, H-9), 4.21 (1H, dd, $J = 12$, 4 Hz, H-10a), 4.06 (1H, dd, $J = 12$, 7 Hz, H-10b), 2.83 (1H, d, $J = 14$ Hz, H-2a), 2.41 (1H, dd, $J = 14$, 13 Hz, H-4a), 2.29 (1H, dqd, $J = 13$, 7, 5 Hz, H-5), 2.22 (1H, ddd, $J = 14$, 5, 2 Hz, H-4b), 1.93 (1H, dd, $J = 14$, 2 Hz, H-2b), 1.24 (9H, s, CH_3 × 3), 0.96 (3H, s, H_3-12), 0.95 (3H, s, H_3-11), 0.91 (3H, d, $J = 7$ Hz, H_3-13); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 210.9 (C-3), 178.8 (–CO–), 135.3 (C-7), 129.4 (C-8), 77.4 (C-6), 70.7 (C-9), 68.4 (C-10), 51.5 (C-2), 42.5 (C-1), 45.1 (C-4), 39.0 [$(CH_3)_3C$)–], 36.5 (C-5), 27.3 (CH_3– × 3), 24.4 (C-11), 24.5 (C-12), 16.0 (C-13); HR-ESI-MS (positive-ion mode) *m/z*: 349.1991 $[M+Na]^+$ (Calcd for $C_{18}H_{30}O_5Na$: 349.1985).

Preparation of (*R*)- and (*S*)-9-*O*-MTPA esters (**4c** and **4d**) from 10-*O*-pivaloyl breyniaionol A (**4b**)

In a similar manner as for the preparation of **1c** and **1d** from **1b**, **4c** and **4d** were prepared from **4b** (0.75 mg each) with the respective amounts of the reagents, (*R*)- and (*S*)-MPTA (10.9 and 10.8 mg), EDC (16.5 and 16.2 mg) and DMAP (6.6 and 7.3 mg). The usual workup gave 0.83 mg (**4c**) and 0.72 mg (**4d**) of esters, respectively.

*10-O-Pivaloyl breyniaionol A 9-O-(R)-MTPA ester (**4c**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 7.55–7.53 (2H, m, aromatic protons), 7.44–7.37 (3H, m, aromatic protons), 5.89 (1H, dd, $J = 15$, 1 Hz, H-7), 5.81 (1H, br ddd, $J = 7$, 7, 3 Hz, H-9), 5.73 (1H, dd, $J = 16$, 7 Hz, H-8), 4.40 (1H, dd, $J = 12$, 3 Hz, H-10a), 4.15 (1H, dd, $J = 12$, 7 Hz, H-10b), 3.59 (3H, br s, CH_3O–), 2.78 (1H, d, $J = 14$ Hz, H-2a), 2.36 (1H, dd, $J = 13$, 13 Hz, H-4a), 2.24 (1H, m, H-5), 2.21 (1H, br dd, $J = 13$, 4 Hz, H-4b), 1.91 (1H, br d, $J = 14$ Hz, H-2b), 1.19 (9H, CH_3– × 3), 0.89 (3H, s, H_3-12), 0.86 (3H, s, H_3-11), 0.82 (3H, d, $J = 7$, H_3-13); HR-ESI-MS (positive-ion mode) *m/z*: 565.2380 $[M+Na]^+$ (Calcd for $C_{28}H_{37}O_7F_3Na$: 565.2383).

*10-O-Pivaloyl breyniaionol A 9-O-(S)-MTPA ester (**4d**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 7.55–7.53 (2H, m, aromatic protons), 7.44–7.38 (3H, m, aromatic protons), 5.98 (1H, dd, $J = 15$, 1 Hz, H-7), 5.82 (1H, dd, $J = 15$, 7 Hz, H-8), 5.77 (1H, br ddd, $J = 7$, 7, 3 Hz, H-9), 4.33 (1H, dd, $J = 12$, 3 Hz, H-10a), 4.15 (1H, dd, $J = 12$, 7 Hz, H-10b), 3.53 (3H, br s, CH_3O–), 2.80 (1H, d, $J = 14$ Hz, H-2a), 2.38 (1H, dd, $J = 13$, 13 Hz, H-4a), 2.27 (1H, m, H-5), 2.22 (1H, dd, $J = 13$, 4 Hz, H-4b), 1.93 (1H, dd, $J = 14$, 2 Hz, H-2b), 1.69 (9H, CH_3– × 3), 0.93 (1H, s, H_3-12), 0.91 (3H, s, H_3-11), 0.82 (3H, d, $J = 6$ Hz, H_3-13); HR-ESI-MS (positive-ion mode) *m/z*: 565.2386 $[M+Na]^+$ (Calcd for $C_{28}H_{37}O_7F_3Na$: 565.2383).

Enzymatic hydrolysis of breyniaionoside B (**5**) and breyniaionoside C (**6**) to their aglycones (**5a** = **6a**)

Breyniaionoside B (**5**) (10.2 mg) and breyniaionoside C (**6**) (4.0 mg) were hydrolyzed in a similar manner as for **1**. The usual workup gave 5.5 and 1.8 mg of the common aglycone, breyniaionol B (**5a** and **6a**, respectively), and 3.2 and 0.61 mg of D-glucose, respectively.

*Breyniaionol B (**5a**)*

Amorphous powder; $[\alpha]_D^{24}$ −9.9 (*c* 0.55, MeOH); ^{1}H-NMR (CD_3OD, 600 MHz) δ: 5.72–5.66 (2H, m, H-7 and 8), 4.18 (1H, dddd, $J = 7$, 5, 5, 1 Hz, H-9), 3.80 (1H, dddd, $J = 12$, 12, 4, 4 Hz, H-3), 3.51 (1H, dd, $J = 11$, 5 Hz, H-10a), 3.45 (1H, dd, $J = 11$, 7 Hz, H-10b), 1.95 (1H, dqd, $J = 12$, 7, 4 Hz, H-5), 1.68 (1H, dddd, $J = 12$, 4, 4, 2 Hz, H-4a), 1.65 (1H, dd, $J = 12$, 12 Hz, H-2a), 1.41 (1H, ddd, $J = 12$, 4, 2 Hz, H-2b), 1.39 (1H, ddd, $J = 12$, 12, 12 Hz, H-4b), 0.97 (3H, s, H_3-12), 0.86 (3H, s, H_3-11), 0.84 (3H, d, $J = 7$ Hz, H_3-13); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 136.5 (C-7), 131.0 (C-8), 78.4 (C-6), 74.3 (C-9), 67.8 (C-10), 67.5 (C-3), 46.0 (C-2), 40.5 (C-1), 40.0 (C-4), 35.5 (C-5), 25.9

(C-11), 25.2 (C-12), 6.5 (C-13); HR-ESI-MS (positive-ion mode) *m/z*: 267.1571 $[M+Na]^+$ (Calcd for $C_{13}H_{24}O_4Na$: 267.1566).

*Breyniaionol B (**6a**)*

Amorphous powder; $[\alpha]_D^{24}$ −12.2 (*c* 0.18, MeOH); ^{1}H- and ^{13}C-NMR spectra were indistinguishable from those of **5a**; HR-ESI-MS (positive-ion mode) *m/z*: 267.1570 $[M+Na]^+$ (Calcd for $C_{13}H_{24}O_4Na$: 267.1566).

Preparation of 10-*O*-pivalate (**5b**) from breyniaionol B (**5a**)

In a similar manner as for the preparation of **1b** from **1a**, **5b** was prepared from **5a** (3.2 mg) with 16 μl of pivaloyl chloride. The usual workup gave 1.62 mg of ester (**5b**).

*10-O-Pivaloyl breyniaionol B (**5b**)*

Amorphous powder; $[\alpha]_D^{23}$ −16.0 (*c* 0.16, MeOH); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 5.77 (1H, d, $J = 15$ Hz, H-7), 5.73 (1H, dd, $J = 15$, 5 Hz, H-8), 4.46 (1H, m, H-9), 4.18 (1H, dd, $J = 11$, 4 Hz, H-10a), 4.04 (1H, dd, $J = 11$, 7 Hz, H-10b), 3.87 (1H, dddd, $J = 12$, 12, 5, 5 Hz, H-3), 1.96 (1H, m, H-5), 1.80 (1H, ddd, $J = 12$, 12, 12 Hz, H-4a), 1.63 (1H, dd, $J = 12$, 12 Hz, H-2a), 1.51 (1H, br d, $J = 12$ Hz, H-2b), 1.36 (1H, br d, $J = 12$ Hz, H-4b), 1.23 (9H, CH_3 × 3), 0.98 (3H, s, H_3-11), 0.87 (3H, s, H_3-12), 0.83 (3H, d, $J = 7$ Hz, H_3-13); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 178.8 (CO), 136.5 (C-7), 128.5 (C-8), 77.2 (C-6), 70.8 (C-9), 68.5 (C-10), 66.7 (C-3), 45.3 (C-2), 39.5 (C-1), 39.3 (C-4), 39.0 [$(CH_3)_3C$–], 34.1 (C-5), 27.3 (CH_3– × 3), 25.1 (C-11), 24.6 (C-12), 15.9 (C-13); HR-ESI-MS (positive-ion mode) *m/z*: 351.2148 $[M+Na]^+$ (Calcd for $C_{18}H_{32}O_5Na$: 351.2141).

Preparation of (*R*)- and (*S*)-3,9-di-*O*-MTPA esters (**5c**, **5d**) from 10-*O*-pivaloyl breyniaionol B (**5b**)

In a similar manner as for the preparation of **1c** and **1d** from **1b**, **5c** and **5d** were prepared from **5b** (0.81 mg each) with the respective amounts of the reagents, (*R*)- and (*S*)-MPTA (22.2 and 21.0 mg), EDC (21.0 and 25.8 mg) and DMAP (23.4 and 16.4 mg). To complete the esterification, a prolonged reaction time (24 h) and an increased reaction temperature (40°C) were used. The usual workup gave 0.92 mg (**5c**) and 1.01 mg (**5d**) of esters, respectively.

*10-O-Pivaloyl breyniaionol B 3,9-di-O-(R)-MTPA ester (**5c**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 7.55–7.52 (4H, m, aromatic protons), 7.42–7.36 (6H, m, aromatic protons), 5.81 (1H, dd, $J = 16$, 1 Hz, H-7), 5.78 (1H, m, H-9), 5.64 (1H, dd, $J = 16$, 7 Hz, H-8), 5.20 (1H, dddd, $J = 12$, 12, 5, 5 Hz, H-3), 4.37 (1H, dd, $J = 12$, 3 Hz, H-10a), 4.14 (1H, dd, $J = 12$, 6 Hz, H-10b), 3.58 (3H, br s, CH_3O–), 3.55 (3H, br s, CH_3O–), 2.01 (1H, m, H-5), 1.89 (1H, m, H-4a), 1.76 (1H, dd, $J = 12$, 12 Hz, H-2a), 1.57 (1H, m, H-4b), 1.53 (1H, m, H-2b), 1.19 (9H, s, CH_3– × 3), 0.97 (3H, s, H_3-11), 0.82 (3H, s, H_3-12), 0.77 (3H, d, $J = 7$ Hz, H_3-13); HR-ESI-MS (positive-ion mode) *m/z*: 783.2941 $[M+Na]^+$ (Calcd for $C_{38}H_{46}O_9F_6Na$: 783.2938).

*10-O-Pivaloyl breyniaionol B 3,9-di-O-(S)-MTPA ester (**5d**)*

Amorphous powder; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 7.55–7.52 (4H, m, aromatic protons), 7.42–7.38 (6H, m, aromatic protons), 5.90 (1H, dd, $J = 15$, 1 Hz, H-7), 5.75 (1H, m, H-8), 5.74 (1H, m, H-9), 5.22 (1H, dddd, $J = 12$, 12, 5, 5 Hz, H-3), 4.30 (1H, dd, $J = 12$, 3 Hz, H-10a), 4.14 (1H, dd, $J = 12$, 6 Hz, H-10b), 3.56 (3H, br s, CH_3O–), 3.52 (3H, br s, CH_3O–), 2.03 (1H, m, H-5), 1.83 (1H, m, H-4a), 1.86 (1H, dd, $J = 12$, 12 Hz, H-2a), 1.62 (1H, br d, $J = 12$ Hz, H-2b), 1.48 (1H, ddd, $J = 12$, 12, 12 Hz, H-4b), 1.15 (9H, s, CH_3– × 3), 1.02 (3H, s, H_3-11), 0.88 (3H, s, H_3-12), 0.75 (3H, d, $J = 7$ Hz, H_3-13); HR-ESI-MS (positive-ion mode) *m/z*: 783.2932 $[M+Na]^+$ (Calcd for $C_{38}H_{46}O_9F_6Na$: 783.2938).

Sugar analysis

Glucose obtained from compound **3**, breyniaionosides A (**4**), B (**5**) and C (**6**) by enzymatic hydrolysis was analyzed with a chiral detector (JASCO OR-2090plus) on an amino column [Asahipak NH_2P-50 4E, CH_3CN-H_2O (4:1), 1 ml/min] to give peaks for D-glucose at 13.7 min. All peaks showed positive optical rotation signs. Peaks were identified by co-chromatography with authentic D-glucose.

Known compounds isolated

Citroside A (**7**): amorphous powder; $[\alpha]_D^{25}$ −102 (*c* 1.45, MeOH) [3].

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