

HETEROCYCLES, Vol. 80, No. 1, 2010, pp. 339 - 348. © The Japan Institute of Heterocyclic Chemistry
Received, 6th April, 2009, Accepted, 4th June, 2009, Published online, 8th June, 2009
DOI: 10.3987/COM-09-S(S)22

BUMALDOSIDES A, B AND C FROM THE LEAVES OF *STAPHYLEA*

BUMALDA

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Abstract – Two new aliphatic diglycosides and a phenolic glucoside (**4**, **5** and **7**) have been isolated from leaves of *Staphylea bumalda* DC., together with three known compounds, benzyl and phenethyl alcohol glycosides (**1** and **2**), and zingerone β -D-glucopyranoside (**6**). 2-Ethyl-3-methylmaleimide *N*-glucopyranoside (**3**) was first isolated as a free form. Their structures were determined on the basis of spectroscopic analysis.

INTRODUCTION

Staphylea bumalda (Staphyleaceae) can be found throughout eastern Asia, especially in China, Japan and Korea. It is a deciduous tree growing to about three to five meters high, and blooms in May to June. A decoction of its fruit is used as a cough remedy and its fresh roots are used for blood refreshment after delivery. The dried fruit is also used as a folk anti-diarrheal medicine.¹ In previous papers, the isolation of megastigmane glycosides¹ and olefinic acetogenin glucosides² was reported. Further extensive investigation of the 1-BuOH-soluble fraction of a MeOH extract of *S. bumalda* leaves afforded three new glycosides (**4**, **5** and **7**), together with four known glycosides (**1**, **2**, **3** and **6**). The structures of the new compounds were elucidated by spectroscopic analysis and by the chemical method. Those of known compounds were determined to be benzyl alcohol glucopyranoside (**1**),³ phenethyl alcohol β -D-glucopyranosyl(1'→6'')- β -D-*O*-glucopyranoside (**2**) and 2-ethyl-3-methyl-maleimide *N*- β -D-glucopyranoside (**3**) by comparison of reported spectroscopic data in the literature. Although compound **2** has been known as a synthetic glycoside,⁴ it was first isolated from tomato (*Lycopersicon esculentum*) as a natural product.⁵ Compound **3** was previously isolated as its acetate,^{6,7} and this is the first report of isolation of it as a natural form. Zingerone β -D-glucopyranoside (**6**) was isolated from *Pinus contorta* for the first time as a natural product,⁸ and was recently obtained as a biotransformation product.⁹

RESULTS AND DISCUSSION

Air-dried leaves of *S. bumalda* were extracted with MeOH and then the MeOH extract was concentrated. After the concentrate was washed with *n*-hexane, the MeOH extract was evaporated to a viscous gum and then suspended in H₂O. The suspension was extracted with EtOAc and 1-BuOH successively to give EtOAc- and 1-BuOH-soluble fractions, respectively. The 1-BuOH-soluble fraction was separated by various kinds of column chromatography (CC) on a highly porous synthetic polymer (Diaion HP-20), normal and reversed-phase silica gel, and droplet counter-current chromatography (DCCC), and preparative HPLC to give three new compounds (**4**, **5** and **7**). Compound **3** was isolated for the first time as a natural form.

2-Ethyl-3-methylmaleimide N - β -D-glucopyranoside (**3**), $[\alpha]_D -0.79$, was isolated as an amorphous powder, and based on mass spectral data, elemental composition of **3** was concluded to be C₁₃H₁₉O₇N. The ¹H- and ¹³C-NMR spectra exhibited the presence of β -glucopyranose moiety, two carbonyl carbons, one methyl on a double bond, one ethyl and one tetrasubstituted double bond. Close inspection of two-dimensional NMR spectra, the structure of compound **3** was concluded to be a β -glucopyranoside of maleinimide derivative as shown in Figure 1. Although the optical rotation value was relatively small, sugar analysis clearly demonstrated that the glucose was in D-series. Its aglycone was isolated from several sources as the aroma of fresh plants,^{10,11} wine,¹² tobacco,^{13,14} and tea.^{15,16} From the leaves of mangosteen⁶

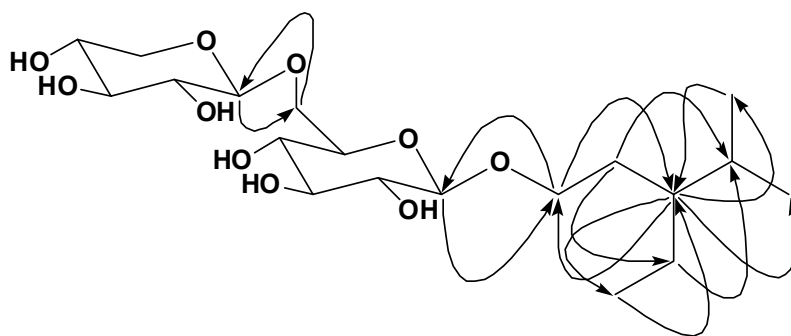
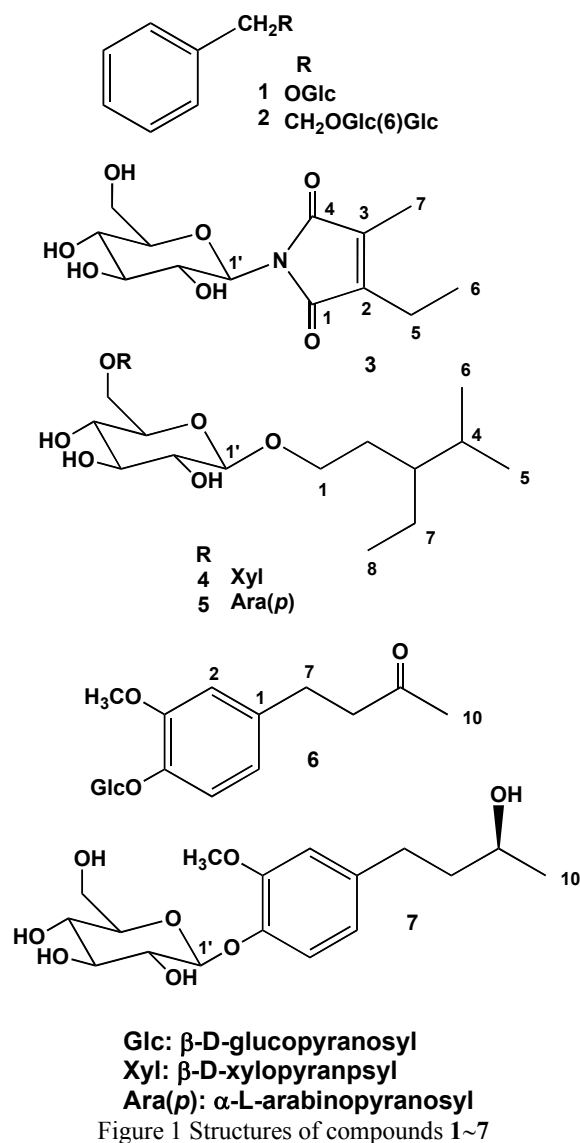


Figure 2 Diagnostic HMBC correlations of **4**

and Riesling wine,⁷ **3** was also isolated as a tetraacetyl derivative. This is the first report of isolation of **3**

Table 1. NMR spectroscopic data for bumaldosides A, B and C (**4**, **5** and **7**) (CD₃OD).

	⁴		⁵	⁷	
	C ^a	H ^b	C ^c	C ^c	H
1	70.2	3.54 ddd, 9, 9, 6 3.91 ddd, 9, 9, 6	70.3	139.0	
2	31.3	1.49 dddd, 15, 9, 7, 6 1.67 dddd, 15, 9, 6, 5	31.3	114.2	6.86 d, 2
3	43.6	1.20 m	43.6	146.1	
4	30.4	1.73 septet,d, 7, 4	30.4	150.8	
5	19.4	0.86 d, 7	19.4	118.5	7.07 d, 8
6	19.8	0.87 d, 7	19.8	121.9	6.74 dd, 8, 2
7	24.4	1.27 ddq, 15, 7, 7 1.36 dqd, 15, 7, 6	24.4	32.7	2.60 ddd, 14, 9, 7 2.65 ddd, 14, 10, 6
8	12.4	0.89 t, 7	12.4	42.1	1.68 dddd, 13, 9, 7, 6 1.72 dddd, 13, 9, 7, 6
9				67.9	3.72 quintet, 6
10				23.5	1.18 d, 6
-OCH ₃				56.8	3.85 s
1'	104.6	4.24 d, 8	104.5	103.1	4.84 d, 8
2'	75.2	3.17 dd, 9, 8	75.2	74.7	3.46 dd, 9, 8
3'	78.1	3.34 dd, 9, 9	78.1	77.9	3.47 dd, 9, 9
4'	71.6	3.32 dd, 9, 9	71.7	71.4	3.39 dd, 9, 9
5'	77.0	3.43 ddd, 9, 6, 2	76.9	78.2	3.38 m
6'	70.0	3.74 dd, 11, 6 4.04 dd, 11, 2	69.5	62.6	3.70 dd, 12, 6 3.86 dd, 12, 2
1''	105.6	4.32 d, 7	105.1		
2''	74.9	3.21 dd, 7, 9	72.4		
3''	77.7	3.31 dd, 10, 9	74.3		
4''	71.3	3.48 ddd, 10, 9, 5	69.4		
5''	67.0	3.19 dd, 11, 9 3.86 dd, 11, 5	66.7		

^a at 150 MHz, ^b at 600 MHz, ^c at 100 MHz.

as a free form. Therefore, the physical data for **3** are included in the Experimental section. Based on the structural resemblance, the aglycone of this compound is expected to be a photodegradation fragment of chlorophyll.¹⁷

Bumaldoside A (**4**), [α]_D −53.5, was isolated as an amorphous powder and its elemental composition was established to be C₁₉H₃₆O₁₀ by high resolution (HR) electrospray-ionization (ESI) mass spectrometry (MS). The IR spectrum exhibited strong absorption bands at 3370, 1076 and 1042 cm^{−1} for a hydroxyl group, and at 2958, 2931 and 2876 cm^{−1} for hydrocarbons. In the ¹H-NMR spectrum, one triplet methyl (δ_{H} 0.89) and two doublet methyls (δ_{H} 0.86 and 0.87), two anomeric protons [δ_{H} 4.24 (d, J = 8 Hz) and 4.32 (d, J = 7 Hz)] and three sets of oxymethylene protons (δ_{H} 3.54 and 3.91, 3.74 and 4.04, and 3.19

and 3.86) were observed, and the ^{13}C -NMR with DEPT spectra exhibited 11 signals assignable to primeverose [*O*- β -D-xylopyranosyl- (1 $\rightarrow$ 6) -*O*- β -D-glucopyranose],¹⁸ three methyls, three methylenes and two methine carbon signals. The connectivity of the proton signals was confirmed by the ^1H - ^1H COSY spectrum, in which protons were thoroughly traced from oxymethylene protons to all methyl groups. Thus the structure of **4** was established to be the primeveroside of 3-ethyl-4-methylpentanol, as shown in Figure 1 and the HMBC spectrum also supported the structure (Figure 2). Compound **4** was hydrolyzed and then the liberated sugars were identified as D-xylose and D-glucose.

Bumaldoside B (**5**), $[\alpha]_{\text{D}} -19.5$, was isolated as an amorphous powder and its elemental composition, analyzed by HR-ESI-MS, was the same as that of **4**. The ^1H - and ^{13}C -NMR spectra of the aglycone moiety were essentially superimposable on those of **4**. The ^{13}C -NMR spectrum also indicated the presence of 6-substituted glucopyranose and terminal arabinopyranose, and L-arabinose and D-glucose were identified as sugar components. Therefore, the structure of **5** was elucidated to be 3-ethyl-4-methylpentanol *O*- α -L-arabinopyranosyl (1 $\rightarrow$ 6)- β -D-glucopyranoside, as shown in Figure 1. The absolute configuration of the 3-position remains to be determined.²⁰

Bumaldoside C (**7**), $[\alpha]_{\text{D}} -41.8$, was isolated as an amorphous powder and its elemental composition was established to be $\text{C}_{17}\text{H}_{26}\text{O}_8$ by HR-ESI-MS. The IR spectrum indicated the presence of

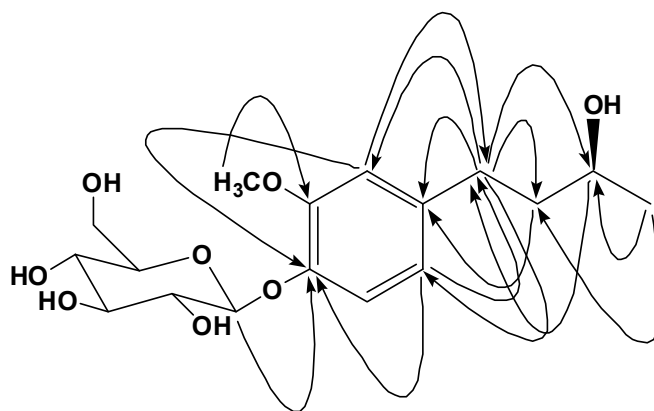


Figure 3 Diagnostic HMBC correlations of **7**

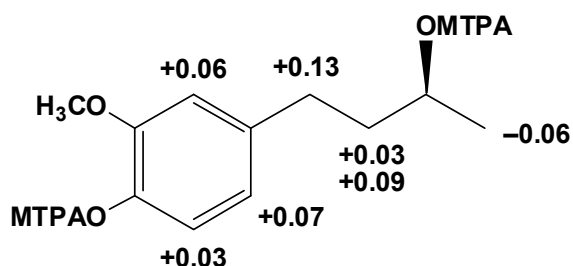


Figure 4 Results with the modified Mosher's method ($\Delta\delta_{\text{S}}-\delta_{\text{R}}$)

hydroxyl groups (3398 cm^{-1}) and an aromatic ring (1595 and 1511 cm^{-1}), and the UV absorption band at 275 nm also indicated the presence of the aromatic ring. In the ^1H -NMR spectrum, distinct signals of an anomeric proton ($\delta_{\text{H}} 4.84$), three aromatic protons ($\delta_{\text{H}} 6.74$, 6.86 and 7.07) coupled in an ABX system, and a doublet methyl ($\delta_{\text{H}} 1.18$) were observed. Based on the data obtained in the ^{13}C -NMR spectrum with DEPT experiment, six signals for a terminal glucose, six aromatic carbon signals, and one methyl, two methylene and one oxymethine signals were assigned. ^1H - ^1H COSY with HSQC spectrum revealed a sequence of proton signals from $\delta_{\text{H}} 2.60$ and 2.65 on C-7 to $\delta_{\text{H}} 1.68$ and 1.72 on C-8, and then $\delta_{\text{H}} 3.72$ on C-9, and finally to the methyl protons. The sugar moiety was placed at the 4'-phenolic hydroxyl group on the benzene ring based on the

correlation of the anomeric proton (δ_{H} 4.84) to the C-4' carbon atom (δ_{C} 150.8) in the HMBC spectrum (Figure 3). Other HMBC correlations also supported the structure of **7**, as shown in Figure 1. Glucose, obtained on acid hydrolysis of **7**, was determined to be in the D-series and the absolute configuration at the 9-position of the aglycone (**7a**) was determined by the modified Mosher's method²³ to be *S*. Levorotatory 9*R*-aglycone was isolated from *Taxus baccata*,²⁴ and 4-*O*-glucoside with 9*S*-aglycone, namely bumaldoside C (**7**), is known as a biotransformation product derived from zingerone with cultured cells of *Phytolacca americana*.⁹ From *Oxytropis myriophylla*, dextrorotatory 4-*O*-glucoside was claimed to be isolated without determination of the absolute configuration at the 9-position.²⁵ Thus, this is the first report of isolation of **7** with a fully detailed structure from a natural source.

EXPERIMENTAL

General experimental procedures

A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Chemical Co. Ltd (Tokyo, Japan). Silica gel column chromatography (CC) was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and reversed-phase [octadecyl silica gel (ODS)] open CC on Cosmosil 75C₁₈-OPN (Nacalai Tesque, Kyoto) [Φ = 50 mm, *L* = 25 cm, linear gradient: MeOH-H₂O (1 : 9, 1 L) $\rightarrow$ (7 : 3, 1 L), fractions of 10 g being collected]. Droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ = 2 mm, *L* = 40 cm), the lower and upper layers of a solvent mixture of CHCl₃-MeOH-H₂O-*n*-PrOH (9 : 12 : 8 : 2) being 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-3 (Inertsil; GL Science, Tokyo, Japan; Φ = 6 mm, *L* = 250 mm), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor. Crude hesperidinase was a generous gift from Tanabe Pharmaceutical Company Ltd. The (*R*)-(+)- and (*S*)-(-)- α -methoxy- α -trifluoromethylphenylacetic acids (MTPA) were purchased from Nacalai Tesque.

A melting point was determined with a Yanagimoto micromelting point apparatus and is uncorrected. Optical rotations were measured on a JASCO P-1030 digital polarimeter. IR spectra were measured on a Horiba FT-710 Fourier transform infrared spectrophotometer and UV spectra on a JASCO V-520 UV/Vis spectrophotometer. ¹H- and ¹³C-NMR spectra were taken on JEOL JNM α -400, λ -500 and ECA-600 spectrometers at 400, 500 or 600 MHz, and 100 or 150 MHz, respectively, with tetramethylsilane (TMS) as an internal standard. HR-ESI-MS (positive-ion mode) were measured with an Applied Biosystems QSTAR[®] XL NanoSpray[™] System. The absolute configuration of sugars was determined on a JASCO OR-2090*plus* optical rotation detector. (*R*)- and (*S*)- α -methoxy- α -trifluoromethylphenylacetic acids

(MTPA) were the products of Wako Pure Chemical Industry Co., Ltd. (Tokyo, Japan).

Plant material

Leaves of *Staphylea bumalda* DC. were collected in the suburbs of Hiroshima City, Japan, in June 2000, and a voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University (00-SB-Hiroshima-0618).

Extraction and isolation

The air-dried leaves of *S. bumalda* (5.71 kg) were extracted with MeOH (15 L $\times$ 3). Parts of the extraction and isolation procedures were described in the previous paper.¹

The 40% MeOH eluate (12.3 g) obtained on Diaion HP-20 column chromatography (CC) was subjected to silica gel (300 g) CC, with elution with CHCl₃ (2 L) and CHCl₃–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 21–29 (1.86 g) were separated by reversed-phase open CC. The residue (152 mg) in fractions 67–74 was subjected to DCCC to give a residue (18.3 mg) in fractions 68–76, which was then purified by HPLC (H₂O–MeOH, 3:1) to afford 8.7 mg of **3** from a peak at 13.1 min. The residues in fractions 75–85 (228 mg) and fractions 86–100 (174 mg) were subjected to DCCC to give 129 mg of **1** in fractions 62–76 and 10.4 mg of **6** in fractions 90–105, respectively. An aliquot (1.82 g) of combined fractions 30–36 (3.06 g) was separated by reversed-phase open CC. The residue (130 mg) in fractions 78–86 was subjected to DCCC, to give a residue (19.5 mg) in fractions 54–60, which was then purified by HPLC (H₂O–MeOH, 3:1) to yield 4.5 mg of **7** from a peak at 17.4 min. Combined fractions 41–51 (1.86 g) were separated by reversed-phase open CC. The residue (227 mg) in fractions 83–90 was subjected to DCCC to give a residue (39.6 mg) in fractions 25–27, which was then purified by HPLC (H₂O–MeOH, 3:1) to afford 14.4 mg of **2** from the peak at 21.3 min.

The 60% MeOH eluate (39.1 g) obtained on Diaion HP-20 column chromatography (CC) was subjected to silica gel (600 g) CC, with elution with CHCl₃ (2 L) and CHCl₃–MeOH [(99:1, 6 L), (49:1, 6 L), (19:1, 6 L), (37:3, 6 L), (23:2, 6 L), (9:1, 6 L), (7:1, 6 L), (17:3, 6 L), (4:1, 6 L), (3:1, 3 L) and (7:3, 6 L)], 500 mL fractions being collected. Combined fractions 69–81 (3.25 g) were separated by reversed-phase open CC. The residue (125 mg) in fractions 176–185 was subjected to DCCC to give a residue (79 mg) in fractions 67–88, which was then purified by HPLC (H₂O–MeOH, 11:9) to yield 3.3 mg of **5** and 6.2 mg of **4** from the peaks at 39.4 min and 43.0 min, respectively.

Bumaldoside A (4): Amorphous powder, $[\alpha]_D^{28}$ –53.5 (*c* 0.62, MeOH). IR $\nu_{\max}$ (film) cm^{–1}: 3370, 2958, 2931, 2876, 1633, 1076, 1042. ¹H and ¹³C-NMR: see Table 1. HR-ESI-MS (positive-ion mode) *m/z* 447.2211 [M + Na]⁺ (Calcd for C₁₉H₃₆O₁₀Na, 447.2200).

Bumaldoside B (5): Amorphous powder, $[\alpha]_D^{27} -19.5$ (c 0.42, MeOH). IR $\nu_{\max}$ (film) cm^{-1} : 3397, 2958, 2931, 2875, 1458, 1377, 1077, 1047, 1009. $^1\text{H-NMR}$ (MeOH, 400 MHz) δ : 4.32 (1H, d, $J = 7$ Hz, H-1"), 4.26 (1H, d, $J = 8$ Hz, H-1'), 4.09 (1H, dd, $J = 11, 2$ Hz, H-6'a), 3.91 (1H, ddd, $J = 9, 9, 6$ Hz, H-1a), 3.87 (1H, dd, $J = 12, 3$ Hz, H-5"a), 3.80 (1H, ddd, $J = 3, 3, 2$ Hz, H-4"), 3.74 (1H, dd, $J = 11, 5$ Hz, H-6'b), 3.58 (1H, ddd, $J = 9, 9, 6$ Hz, H-1b), 3.54 (1H, m, H-2"), 3.53 (1H, m, H-3"), 3.53 (1H, dd, $J = 12, 2$ Hz, H-5"b), 3.44 (1H, ddd, $J = 9, 5, 2$ Hz, H-5'), 3.36 (1H, dd, $J = 9, 9$ Hz, H-3'), 3.34 (1H, dd, $J = 9, 9$ Hz, H-4'), 3.18 (1H, dd, $J = 9, 8$ Hz, H-2'), 1.72 (1H, septet, $J = 7, 4$ Hz, H-4), 1.66 (1H, dddd, $J = 15, 9, 6, 5$ Hz, H-2a), 1.50 (1H, dddd, $J = 15, 9, 7, 6$ Hz, H-2b), 1.36 (1H, dqd, $J = 15, 7, 6$ Hz, H-7a), 1.27 (1H, ddq, $J = 14, 7, 7$ Hz, H-7b), 1.20 (1H, m, H-3), 0.89 (3H, t, $J = 7$ Hz, H₃-8), 0.87 (3H, d, $J = 7$ Hz, H₃-6), 0.85 (3H, d, $J = 7$ Hz, H₃-5). $^{13}\text{C-NMR}$: see Table 1. HR-ESI-MS (positive-ion mode) m/z 447.2208 $[\text{M} + \text{Na}]^+$ (Calcd for $\text{C}_{19}\text{H}_{36}\text{O}_{10}\text{Na}$, 447.2200).

Bumaldoside C (7): Amorphous powder, $[\alpha]_D^{30} -41.8$ (c 0.41, MeOH). IR $\nu_{\max}$ (film) cm^{-1} : 3398, 2965, 2927, 2878, 1595, 1511, 1266, 1222, 1073. UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 221 (3.91), 275 (3.43), 317 (2.94). $^1\text{H-NMR}$: see Table 1. $^{13}\text{C-NMR}$: see Table 1. HR-ESI-MS (positive-ion mode) m/z 381.1521 $[\text{M} + \text{Na}]^+$ (Calcd for $\text{C}_{17}\text{H}_{26}\text{O}_8\text{Na}$, 381.1519).

Known compounds isolated: Benzyl alcohol β -D-glucopyranoside (**1**), colorless needles, mp. 120-122 °C (MeOH), $[\alpha]_D^{26} -48.0$ (c 1.32, MeOH).³ Phenethyl alcohol β -D-glucopyranosyl(1'→6")- β -D-O-glucopyranoside (**2**), Amorphous powder, $[\alpha]_D^{26} -39.0$ (c 1.44, MeOH).^{4,5} 2-Ethyl-3-methylmaleimide N - β -D-glucopyranoside (**3**) Amorphous powder, $[\alpha]_D^{28} -0.79$ (c 0.75, MeOH). IR $\nu_{\max}$ (film) cm^{-1} : 3368, 2975, 2937, 2881, 1710, 1396, 1077. UV $\lambda_{\max}$ (CH₃OH) nm (log ϵ): 222 (4.10), 274 (3.26). $^1\text{H-NMR}$ (CD₃OD, 400 MHz) δ : 4.95 (1H, d, $J = 10$ Hz, H-1'), 4.31 (1H, dd, $J = 10, 9$ Hz, H-2'), 3.82 (1H, dd, $J = 12, 2$ Hz, H-6'a), 3.63 (1H, dd, $J = 12, 7$ Hz, H-6'b), 3.36 (3H, m, H-3', 4' and 5'), 2.44 (2H, q, $J = 8$ Hz, H₂-5), 1.93 (3H, s, H₃-7), 1.13 (3H, t, $J = 8$ Hz, H₃-6). $^{13}\text{C-NMR}$ (CD₃OD, 100 MHz) δ : 172.8 (C-4), 172.4 (C-1), 143.9 (C-2), 138.6 (C-3), 81.5 (C-1'), 80.8 (C-5'), 79.3 (C-3'), 71.6 (C-4'), 70.2 (C-2'), 62.9 (C-6'), 17.9 (C-5), 12.8 (C-6), 8.4 (C-7). HR-ESI-MS (positive-ion mode) m/z 324.1056 $[\text{M} + \text{Na}]^+$ (Calcd for $\text{C}_{13}\text{H}_{19}\text{O}_7\text{NNa}$, 324.1053). Zingerone β -D-glucopyranoside (**6**), $[\alpha]_D^{28} -24.3$ (c 1.04, MeOH).⁸

Acid hydrolysis

About 500 μg each of **3**, **4** and **5** was hydrolyzed with 1N HCl (0.1 mL) at 100 °C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 mL), and the water layers were analyzed with a chiral detector (JASCO OR-2090*plus*) on an amino column [Asahipak NH2P-50 4E, MeCN-H₂O (4:1), 1 mL/min]. Hydrolyzates of **3**, **4** and **5** gave peaks for D-glucose at 9.3 min, for D-xylose and D-glucose at 9.5 min and 13.7 min, and for L-arabinose and D-glucose at 9.3 min and 13.7 min,

respectively. All sugars showed a positive optical rotation sign. Peaks were identified by co-chromatography with authentic L-arabinose, D-xylose and D-glucose.

Enzymatic hydrolysis of bumaldoside C (7)

Bumaldoside C (**7**) (4.2 mg) in 2 mL of H₂O was hydrolyzed with crude hesperidinase (5.0 mg) for 12 h at 37 °C. The reaction mixture was evaporated to dryness, and then the methanolic solution was absorbed on silica gel and subjected to silica gel CC (10 g, Φ = 10 mm, L = 20 cm) with a linear gradient solvent system, from CHCl₃-MeOH (20 : 1, 100 mL) to CHCl₃-MeOH-H₂O (15 : 6 : 1, 100 mL), 5 g fractions being collected. An aglycone (**7a**) (1.9 mg, 82%) and D-glucose (1.4 mg, 67%) were recovered in fractions 10–12 and 41–43, respectively. Aglycone (**7a**): $[\alpha]_D^{27} +18.6$ (c 0.19, MeOH). ¹H NMR (CD₃OD, 600 MHz) δ : 6.77 (1H, d, J = 2 Hz, H-2), 6.69 (1H, d, J = 8 Hz, H-5), 6.62 (1H, dd, J = 8, 2 Hz, H-6), 3.72 (1H, dqd, J = 7, 6, 5 Hz, H-9), 3.83 (3H, s, CH₃O-), 2.64 (1H, ddd, J = 14, 10, 6 Hz, H-7a), 2.55 (1H, ddd, J = 14, 10, 7 Hz, H-7b), 1.71 (1H, dddd, J = 14, 10, 7, 6 Hz, H-8a), 1.66 (1H, dddd, J = 14, 10, 7, 5 Hz, H-8b), 1.17 (3H, d, J = 6 Hz, H₃-10). ¹³C NMR (CD₃OD, 150 MHz) δ : 148.9 (C-3), 145.6 (C-4), 135.4 (C-1), 121.8 (C-6), 116.2 (C-5), 113.3 (C-2), 68.0 (C-9), 56.5 (CH₃O-), 42.4 (C-8), 32.8 (C-7), 23.6 (C-10). HR-ESI-MS (positive-ion mode) m/z 219.0993 [$M + Na$]⁺ (Calcd for C₁₁H₁₆O₃Na, 219.0991). D-Glucose: $[\alpha]_D^{27} +29.8$ (c =0.14, H₂O).

Preparation of (*R*)- and (*S*)-MPTA esters (**7b** and **7c**) of **7a**

A solution of **7a** (0.8 mg) in 1 mL of dehydrated CH₂Cl₂ was reacted with (*R*)-MTPA (43.7 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (31 mg) and *N,N*-dimethyl-4-aminopyridine (4-DMAP) (17 mg), and then the mixture was occasionally stirred at 25 °C for 30 min and then 40 °C for 5 min. After the addition of 1 mL of CH₂Cl₂, the solution was washed with H₂O (1 mL), 4N HCl (1 mL), NaHCO₃-saturated H₂O, and then brine (1 mL), successively. The organic layer was dried over Na₂SO₄ 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 CHCl₃-(Me)₂CO (20:1) for 9 cm, and then eluted with CHCl₃-MeOH (9:1)] to furnish a diester, **7b** (1.1 mg), and a monoester (0.3 mg). Through a similar procedure, diester **7c** (0.53 mg) was prepared from **7a** (0.7 mg) using (*S*)-MTPA (39 mg), EDC (30 mg), and 4-DMAP (23 mg). A monoester (0.45 mg) was also obtained.

(*R*)-MTPA 4,9-*O*-diester (**7b**): ¹H NMR (CDCl₃, 500 MHz) δ : 7.72 (2H, m), 7.57–7.56 (2H, m), 7.49–7.44 (3H, m), 7.41–7.40 (3H, m) (aromatic protons of MTPA), 6.89 (1H, d, J = 8 Hz, H-5), 6.69 (1H, d, J = 2 Hz, H-2), 6.65 (1H, dd, J = 8, 2 Hz, H-6), 5.18 (1H, m, H-9), 3.79 (3H, s, CH₃O-), 3.72 (3H, br s, CH₃O-), 3.58 (3H, br s, CH₃O-), 2.50 (2H, m, H₂-7), 1.94 (1H, m, H-8a), 1.83 (1H, m, H-8b), 1.37 (3H, d, J = 6 Hz, H₃-10). HR-ESI-MS (positive-ion mode) m/z 651.1791 [$M + Na$]⁺ (Calcd for

C₃₁H₃₀O₇F₆Na, 651.1787).

(S)-MTPA 4,9-O-diester (7c): ¹H NMR (CDCl₃, 500 MHz) δ: 7.72 (2H, m), 7.56 (2H, m), 7.45–7.44 (3H, m), 7.42–7.40 (3H, m) (aromatic protons of MTPA), 6.92 (1H, d, *J* = 8 Hz, H-5), 6.75 (1H, d, *J* = 2 Hz, H-2), 6.72 (1H, dd, *J* = 8, 2 Hz, H-6), 5.18 (1H, br q, *J* = 6 Hz, H-9), 3.79 (3H, s, CH₃O-), 3.72 (3H, br s, CH₃O-), 3.56 (3H, br s, CH₃O-), 2.63 (2H, m, H₂-7), 2.03 (1H, m, H-8a), 1.86 (1H, m, H-8b), 1.31 (3H, d, *J* = 6 Hz, H₃-10). HR-ESI-MS (positive-ion mode) *m/z* 651.1793 [M + Na]⁺ (Calcd for C₁₃H₁₉O₇F₆Na, 651.1787).

ACKNOWLEDGEMENTS

The authors are grateful for access to the superconducting NMR instrument at the Analytical Center of Molecular Medicine of Graduate School of Biomedical Sciences, Hiroshima University, and an Applied Biosystem QSTAR XL system ESI-TOF-MS at the Analytical Center of Molecular Medicine and the Analysis Center of Life Science, respectively, of the Hiroshima University Faculty of Medicine.

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19. If the aglycone of **4** and **5** is supposed to be a fragment of the β -sitosterol side chain, it may have the *R* configuration. Cleavage of the cholesterol side chain through oxidative processes is conducted by P450scc,²⁰ and probably a similar mechanism operates to produce the aglycone of bumaldosides A (**4**) and B (**5**). Aglycone of bumaldosides A (**4**) and B (**5**) is known as a sex pheromone of queens of the slave-making ant, *Polyergus breviceps*.^{21,22} Synthetic work of both enantiomers revealed that only the *R* isomer is biologically active.
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