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# 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 glycosides[1] and olefinic acetogenin glucosides[2] 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 $H_2O$. 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.

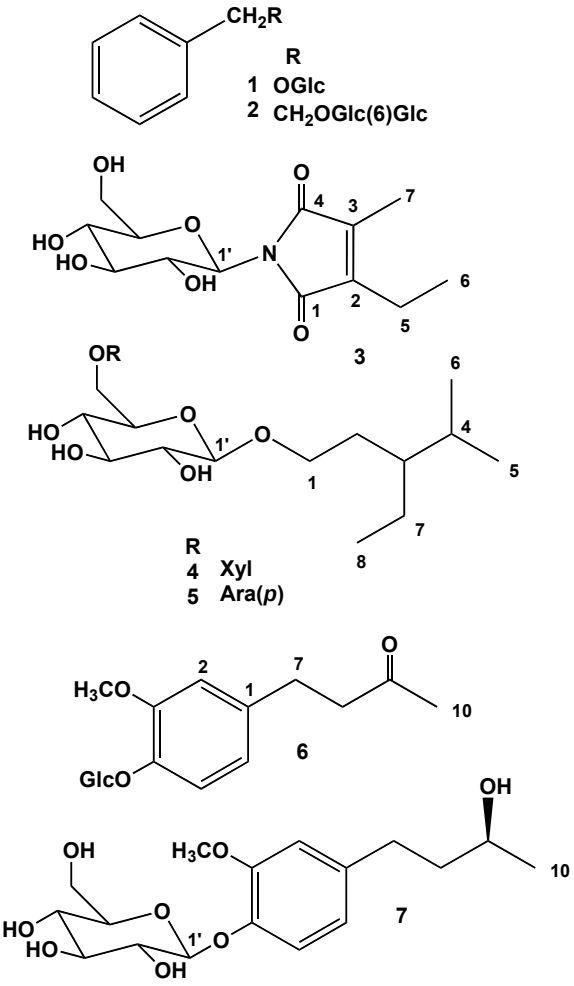


Figure 1 Structures of compounds **1**~**7**

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 $C_{13}H_{19}O_7N$. The $^1$H- and $^{13}$C-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 mangosteen[6] and Riesling wine,[7] **3** was also isolated as a tetraacetyl derivative. This is the first report of isolation of **3**

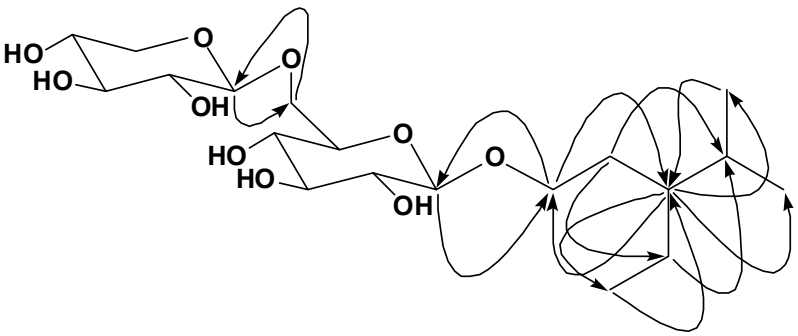

Figure 2 Diagnostic HMBC correlations of **4**

**Table 1.** NMR spectroscopic data for bumaldosides A, B and C (**4**, **5** and **7**) ($CD_3OD$).

| | **4** | | **5** | **7** | |
|---|---|---|---|---|---|
| | C[a] | H[b] | C[c] | C[c] | H |
| 1 | 70.2 | 3.54 ddd, 9, 9, 6<br>3.91 ddd, 9, 9, 6 | 70.3 | 139.0 | |
| 2 | 31.3 | 1.49 dddd, 15, 9, 7, 6<br>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<br>1.36 dqd, 15, 7, 6 | 24.4 | 32.7 | 2.60 ddd, 14, 9, 7<br>2.65 ddd, 14, 10, 6 |
| 8 | 12.4 | 0.89 t, 7 | 12.4 | 42.1 | 1.68 dddd, 13, 9, 7, 6<br>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<br>4.04 dd, 11, 2 | 69.5 | 62.6 | 3.70 dd, 12, 6<br>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<br>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**), $[\alpha]_D$ –53.5, was isolated as an amorphous powder and its elemental composition was established to be $C_{19}H_{36}O_{10}$ 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 $^1$H-NMR spectrum, one triplet methyl ($\delta_H$ 0.89) and two doublet methyls ($\delta_H$ 0.86 and 0.87), two anomeric protons [$\delta_H$ 4.24 (d, $J$ = 8 Hz) and4.32 (d, $J$ = 7 Hz)] and three sets of oxymethylene protons ($\delta_H$ 3.54 and 3.91, 3.74 and 4.04, and 3.19

and 3.86) were observe, and the $^{13}$C-NMR with DEPT spectra exhibited 11 signals assignable to primeverose [*O*-β-D-xylopyranosyl- (1→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 $^{1}$H-$^{1}$H 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**), $[\alpha]_D$ –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 $^{1}$H- 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-arabinopyrano-syl (1→6)-β-D-glucopyranoside, as shown in Figure 1. The absolute configuration of the 3-position remains to be determined.[20]

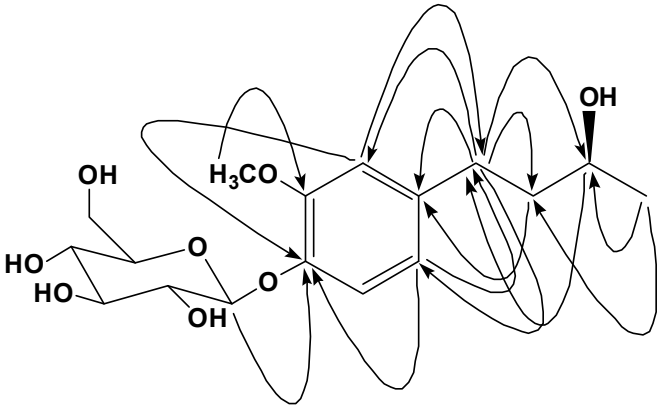


Figure 3 Diagnostic HMBC correlations of **7**

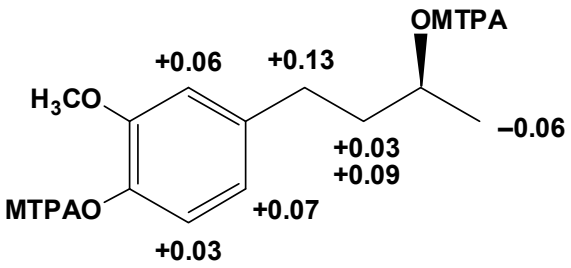


Figure 4 Results with the modified Mosher's method ($\Delta\delta_S$-$\delta_R$)

Bumaldoside C (**7**), $[\alpha]_D$ –41.8, was isolated as an amorphous powder and its elemental composition was established to be $C_{17}H_{26}O_8$ by HR-ESI-MS. The IR spectrum indicated the presence of 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 $^{1}$H-NMR spectrum, distinct signals of an anomeric proton ($\delta_H$ 4.84), three aromatic protons ($\delta_H$ 6.74, 6.86 and 7.07) coupled in an ABX system, and a doublet methyl ($\delta_H$ 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. $^{1}$H-$^{1}$H COSY with HSQC spectrum revealed a sequence of proton signals from $\delta_H$ 2.60 and 2.65 on C-7 to $\delta_H$ 1.68 and 1.72 on C-8, and then $\delta_H$ 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 ($\delta_H$ 4.84) to the C-4' carbon atom ($\delta_C$ 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 method[23] 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 75$C_{18}$-OPN (Nacalai Tesque, Kyoto) [$\Phi$ = 50 mm, $L$ = 25 cm, linear gradient: MeOH-$H_2O$ (1 : 9, 1 L) → (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 ($\Phi$ = 2 mm, $L$ = 40 cm), the lower and upper layers of a solvent mixture of $CHCl_3$-MeOH-$H_2O$-*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; $\Phi$ = 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-trifluoromethylpheylacetic 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. $^1$H- and $^{13}$C-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 × 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 $CHCl_3$ (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 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 ($H_2O$-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 ($H_2O$-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 ($H_2O$-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 $CHCl_3$ (2 L) and $CHCl_3$–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 ($H_2O$-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 $^{13}C$-NMR: see Table 1. HR-ESI-MS (positive-ion mode) *m*/*z* 447.2211 $[M + Na]^+$ (Calcd for $C_{19}H_{36}O_{10}Na$, 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. $^1$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,d, *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, $H_3$-8), 0.87 (3H, d, *J* = 7 Hz, $H_3$-6), 0.85 (3H, d, *J* = 7 Hz, $H_3$-5). $^{13}$C-NMR: see Table 1. HR-ESI-MS (positive-ion mode) *m*/*z* 447.2208 $[M + Na]^+$ (Calcd for $C_{19}H_{36}O_{10}Na$, 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). $^1$H-NMR: see Table 1. $^{13}$C-NMR: see Table 1. HR-ESI-MS (positive-ion mode) *m*/*z* 381.1521 $[M + Na]^+$ (Calcd for $C_{17}H_{26}O_8Na$, 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 $\lambda_{max}$ ($CH_3OH$) nm (log ε): 222 (4.10), 274 (3.26). $^1$H-NMR ($CD_3OD$, 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, $H_2$-5), 1.93 (3H, s, $H_3$-7), 1.13 (3H, t, *J* = 8 Hz, $H_3$-6). $^{13}$C-NMR ($CD_3OD$, 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 $[M + Na]^+$ (Calcd for $C_{13}H_{19}O_7NNa$, 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 ºC 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-$H_2O$ (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 $H_2O$ 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, $\Phi$ = 10 mm, $L$ = 20 cm) with a linear gradient solvent system, from $CHCl_3$-MeOH (20 : 1, 100 mL) to $CHCl_3$-MeOH-$H_2O$ (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). $^1$H NMR ($CD_3OD$, 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, C*H*$_3$O-), 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, $H_3$-10). $^{13}$C NMR ($CD_3OD$, 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 (*C*H$_3$O-), 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 $C_{11}H_{16}O_3Na$, 219.0991). D-Glucose: $[\alpha]_D^{27}$ +29.8 (*c*=0.14, $H_2O$).

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

A solution of **7a** (0.8 mg) in 1 mL of dehydrated $CH_2Cl_2$ was reacted with (*R*)-MTPA (43.7 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardodiimide 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 $CH_2Cl_2$, the solution was washed with $H_2O$ (1 mL), 4N 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 $CHCl_3$-$(Me)_2CO$ (20:1) for 9 cm, and then eluted with $CHCl_3$-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):** $^1$H NMR ($CDCl_3$, 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* = 2Hz, H-2), 6.65 (1H, dd, *J* = 8, 2 Hz, H-6), 5.18 (1H, m, H-9), 3.79 (3H, s, $CH_3O$-), 3.72 (3H, br s, $CH_3O$-), 3.58 (3H, br s, $CH_3O$-), 2.50 (2H, m, $H_2$-7), 1.94 (1H, m, H-8a), 1.83 (1H, m, H-8b), 1.37 (3H, d, *J* = 6 Hz, $H_3$-10). HR-ESI-MS (positive-ion mode) *m*/*z* 651.1791 $[M + Na]^+$ (Calcd for

$C_{31}H_{30}O_7F_6Na$, 651.1787).

**(*S*)-MTPA 4,9-*O*-diester (7c):** $^1$H NMR ($CDCl_3$, 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, $CH_3O$-), 3.72 (3H, br s, $CH_3O$-), 3.56 (3H, br s, $CH_3O$-), 2.63 (2H, m, $H_2$-7), 2.03 (1H, m, H-8a), 1.86 (1H, m, H-8b), 1.31 (3H, d, *J* = 6 Hz, $H_3$-10). HR-ESI-MS (positive-ion mode) *m*/*z* 651.1793 $[M + Na]^+$ (Calcd for $C_{13}H_{19}O_7F_6Na$, 651.1787).

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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 enatiomers revealed that only the *R* isomer is biologically active.

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