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# A New Stereoisomeric Acetogenic Glycoside from the Flower Buds of *Buddleja officinalis*

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*Buddleja officinalis* Maximowicz, which belongs to the Buddlejaceae, is a shrub tree and widely distributed in Asia, Africa, and America. It has been regarded as a traditional herbal medicine for the treatment of inflammation, conjunctivitis, headache, and clustered nebulae.[1,2,3] Previous phytochemical studies have led to the discovery of terpenoids, phenethyl glycosides, flavonoids and saponins.[2-5] The recent biological investigations on *B. officinalis* have been reported that the water extract showed the anti-inflammatory effect *via* negative regulation of NF-κB and ERK 1/2 signaling in BV-2 cells,[6] and down-regulation of intracellular ROS production and adhesion molecule expression in human umbilical vein endothelial cells,[7] and also exhibited the inhibitory effect on lipid accumulation during 3T3-L1 adipocyte differentiation.[8] Taken together, *B. officinalis* has been spotlighted by its various phytochemicals and bioactivities.

During our recent study on this plant, we investigated a new monoterpene and its analogues from the methylene chloride-soluble fraction.[9] In our continuing search for new constituents of *B. officinalis*, a new stereoisomeric acetogenic glycoside (**1**) along with four phenethyl glycosides (**2**-**5**), a benzyl glycoside (**6**), a phenylpropanoid glycoside (**7**), and two iridoid glycosides (**8** and **9**) were isolated from the *n*-butanol-soluble fraction. Eight known isolates were identified by the extensive analysis of spectroscopic data and comparison with the literature values, as $\rho$-hydroxy-phenethyl-*O*-$\beta$-D-glucopyranoside (**2**),[10] phenethyl-$\beta$-primeveroside (**3**),[11] phenethyl-*O*-$\beta$-D-gluco-pyranosyl-(1″→6′)-$\beta$-D-glucopyranoside (**4**),[12] phenethyl-*O*-$\beta$-D-glucopyranosyl-(1″→2′)-$\beta$-D-glucopyranoside (**5**),[13] benzyl-$\beta$-primeveroside (**6**),[11] syringin (**7**),[14] methylcatalpol (**8**),[15] and buddlejoside $A_4$ (**9**).[15] Herein, it deals with the isolation, structural characterization and hydrolysis of a new acetogenic glycoside.

Compound **1** was isolated as a white powder, and the molecular formula, $C_{18}H_{32}O_{11}$, was demonstrated by HRESIMS data coupled with 1D NMR spectra. The signals of $^1H$ and $^{13}C$ NMR showed two portions generated by sugars and an aglycone. First, in the sugar moiety, two sets of anomeric protons and carbons due to sophorose comprised of the 1→2 linked between two glucoses were observed at $\delta_H$ 4.59 (H-1″) and 4.42 (H-1′), and $\delta_C$ 105.1 (C-1″) and 103.2 (C-1′), respectively. The $\beta$ anomeric configuration of sophorose was demonstrated based on the large coupling constant of anomeric protons ($J_{1'2'}$, $J_{1''2''}$ = 7.7 Hz), and the presence of $\beta$-sophorose was further supported by the gas chromatography analysis of hydrolyzate of **1**, recognized HMBCs between H-1″ ($\delta_H$ 4.59) and C-2′ ($\delta_C$ 83.2), and the thorough

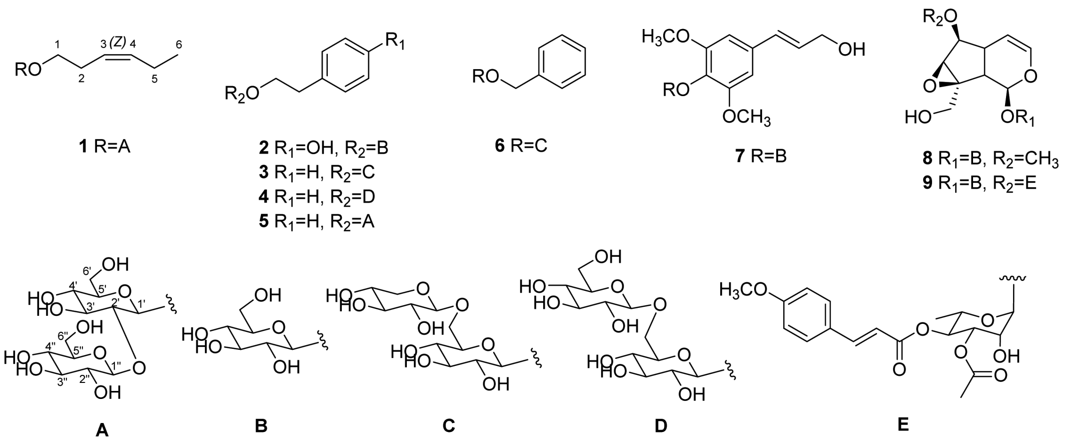

**Figure 1.** Isolated compounds from *B. officinalis* (**1-9**).

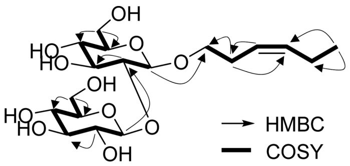


**Figure 2.** The selected HMBC and COSY correlations of **1**.

analysis of COSY spectrum (bold lines, Fig. 2). Second, the aglycone was comprised of a methyl, an oxygenated methylene, two methylene, and two methine carbons, which were determined by the full analysis of $^{13}$C NMR spectrum coupled with DEPT90 and 135 spectra. The discovery of splitting and coupling constant in $^{1}$H NMR spectrum allowed the aglycone of **1** was *n*-hexenol (Table 1). The geometry of the double bond was elucidated to be *cis* by the coupling constant of H-3 and H-4 ($J_{34}$ = 11.2 Hz), and the chemical shifts of C-3 and C-4 in $^{13}$C NMR were also identical with reported values [C-3: 126.1 (**1**), 126.0[16]; C-4: 134.6 (**1**), 134.6[16]]. Lastly, the connectivity of *β*-sophorose and (*Z*)-hexenol was determined by the observed HMBC cross peak between $\delta_H$ 4.42 and $\delta_C$ 70.5, indicating *β*-sophorose was attached at C-1. Taken together, compound **1** was determined as (*Z*)-3-hexenyl-*O*-*β*-D-glucopyranosyl-(1″→2′)-*β*-D-glucopyranoside. To the best of our knowledge, although the *trans* form of **1** was already investigated,[17] this is the first report of acetogenic glycoside in *B. officinalis*. Also, compounds **3**, **4**, and **6** were isolated from the genus of Buddlejaceae for the first time.

Among isolated compounds, some biological investigations on syringin (**7**) have been reported that it has an ability to raise the release of ACh from nerve terminals,[18] and inhibit the production of TNF-α and cytotoxic T cell proliferation.[19] Under consideration of these reports, further studies are needed to test the biological activities of the isolated compounds.

**Table 1.** NMR data of compound **1** ($CD_3OD$, 700 and 177 MHz)[a]

| Carbon No. | **1** | |
|---|---|---|
| | $\delta_H$ | $\delta_C$ |
| 1 | 3.88 (1H, dt, *J* = 9.1, 7.0 Hz)<br>3.57 (1H, dt, *J* = 9.1, 7.0 Hz) | 70.5 t[b] |
| 2 | 2.37 (2H, q, *J* = 7.0 Hz) | 28.9 d |
| 3 | 5.39 (1H, dtt, *J* = 11.2, 7.0, 1.4 Hz) | 126.1 d |
| 4 | 5.46 (1H, dtt, *J* = 11.2, 7.0, 1.4 Hz) | 134.6 d |
| 5 | 2.08 (2H, p, *J* = 7.0 Hz) | 21.7 t |
| 6 | 0.97 (3H, t, *J* = 7.0 Hz) | 14.8 s |
| Glc | | |
| 1′ | 4.42 (1H, d, *J* = 7.7 Hz) | 103.2 d |
| 2′ | 3.42 (1H, dd, *J* = 9.1, 7.7 Hz) | 83.2 d |
| 3′ | 3.37 (1H, m) | 77.8 d |
| 4′ | 3.32 (1H, m) | 71.4 d |
| 5′ | 3.55 (1H, m) | 77.9 d |
| 6′ | 3.86 (1H, dd, *J* = 11.9, 2.1 Hz)<br>3.66 (1H, dd, *J* = 11.9, 4.9 Hz) | 62.7 t |
| Glc | | |
| 1″ | 4.59 (1H, d, *J* = 7.7 Hz) | 105.1 d |
| 2″ | 3.22 (1H, dd, *J* = 9.1, 8.4 Hz) | 76.0 d |
| 3″ | 3.36 (1H, m) | 77.7 d |
| 4″ | 3.32 (1H, m) | 71.4 d |
| 5″ | 3.25 (1H, m) | 78.3 d |
| 6″ | 3.82 (1H, dd, *J* = 11.9, 2.8 Hz)<br>3.69 (1H, dd, *J* = 11.9, 6.3 Hz) | 62.7 t |

[a]Assignments aided by a combination of HMQC, HMBC, and COSY experiments. [b]Carbon multiplicity.

## Experimental Section

**General Experimental Procedures.** NMR spectra were recorded on a Bruker Avance III 700 MHz NMR spectrometer using $CD_3OD$ as solvent, and TMS was used as an internal standard. Chemical shifts are presented in ppm. Optical rotation was evaluated on JASCO P-2000. TLC analysis was performed on a precoated silica gel 60 $F_{254}$ (0.24 mm, Merck). Open column chromatography was performed using a silica gel (Kieselgel 60, 70-230 mesh, Merck), $RP_{18}$ (Part NO. 5982-5752, Agilent), MCI CHP20P gel (75-150 μM, Mitsubishi), and Sephadex LH-20 (GE Healthcare). Semi-preparative HPLC was performed on a Shimadzu Prominence UFLC with UV detector. HRTOF-MS and ESI-MS spectra were obtained using a Waters UPLC-QTOF micro, and a LCQ Fleet (Thermo Scientific), respectively. GC-MS spectra were acquired using an Agilent 6890/5973i.

**Plant Materials.** The flower buds of *B. officianlis* were purchased from a commercial market (Samhong medicinal herb market, Seoul, South Korea) in 2013. One of the authors (S.-Y. Park) performed botanical identification, and a voucher specimen has been deposited at the College of Pharmacy, Dankook University, South Korea.

**Extraction and Isolation.** The air-dried flower buds of *B. officinalis* (3 kg) were pulverized and then extracted with 100% methanol (24 L, three times) at room temperature. The methanolic filtrate was evaporated *in vacuo* to generate the methanolic extract (301 g), and the extract was partitioned with *n*-hexane, methylene chloride, ethyl acetate, *n*-butanol, and water, progressively. Among them, some of *n*-butanol extract (10 g) was loaded onto MCI gel to yield 4 subfractions (BOD1-BOD4) with a step gradient composed of methanol and water (40, 60, 80, 100% methanol). The subfraction BOD1 was further chromatographed on Sephadex LH-20 to give two portions (BOD1A-BOD1B), and BOD1A was then re-chromatographed on silica gel to generate 9 subfractions (BOD1A1-BOD1A9) with a step gradient solvent system composed of chloroform, methanol, and water (8:3:1 to 3:3:1). The subfractions BOD1A1, BOD1A6, and BOD1A7 was further purified by semi-preparative RP-HPLC (Ace, $C_{18}$, 21.2 × 250 mm, flow rate 7 mL/min) to furnish **1** (4.5 mg), **2** (3.0 mg), **3** (5.0 mg), **4** (4.0 mg), **5** (3.0 mg), **6** (3.5 mg), **7** (7.4 mg), **8** (12 mg), and **9** (2.0 mg).

**Compound 1:** White powder; $[\alpha]_D^{25}$ −17.0 (*c* 0.15, MeOH); HRESIMS *m/z* 423.1860 (calcd for $C_{18}H_{31}O_{11}$, 423.1866); $^{1}$H and $^{13}$C NMR in Table 1.

**Acid Hydrolysis of Compound 1 and Determination of Sugar Component.** Compound **1** (1.0 mg) was dissolved in 1.0 N HCl (1 mL), followed by heating at 120 °C in a water bath for 3 h. The solvent was evaporated *in vacuo*, and mixture was extracted with chloroform three times over. The hydrolyzate containing sugar portion in a vial dissolved in dry pyridine (100 μL), and then L-cystein methyl ester hydrochloride in dry pyridine (0.06 M, 100 μL) was added. After heating the mixture at 60 °C for 2 h, $NaBH_4$ (2.0 mg) was added into vial, and the reaction mixture was stirred for 1 h at room temperature. Trimethylsilylimidazole solution (100 μL) was added and the reaction mixture was then heated at 60 °C for 2 h. The reaction mixture was evaporated *in vacuo*, and the dried product was then partitioned with *n*-hexane and water. The *n*-hexane layer was analyzed by GC-MS: the standard sugar generated peak at $t_R$ 18.95 for D-glucose.

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**Supporting Information.** Spectroscopic data including 1D, 2D NMR, and HRTOF-MS are available as supporting information.

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