http://dx.doi.org/10.5012/bkcs.2014.35.7.2159

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

Chul Lee, Kwang-Woo Hwang,†,* and So-Young Park‡,*

Eco-friendly Material Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeonbuk 580-185, Korea
†Laboratory of Host Defense Modulation, College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea
**E-mail: khwang@cau.ac.kr*
‡Laboratory of Pharmacognosy, College of Pharmacy, Dankook University, Cheonan 330-714, Korea
**E-mail: soypark23@dankook.ac.kr*
Received January 17, 2014, Accepted March 10, 2014

Key Words : *Buddleja officinalis*, Acetogenic glycoside, Hydrolysis, NMR

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 *ρ*-hydroxy-phenethyl-*O*-*β*-D-glucopyranoside (**2**),[10] phenethyl-*β*-primeveroside (**3**),[11] phenethyl-*O*-*β*-D-gluco-pyranosyl-(1″→6′)-*β*-D-glucopyranoside (**4**),[12] phenethyl-*O*-*β*-D-glucopyranosyl-(1″→2′)-*β*-D-glucopyranoside (**5**),[13] benzyl-*β*-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 ^{1}H 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 δ_H 4.59 (H-1″) and 4.42 (H-1′), and δ_C 105.1 (C-1″) and 103.2 (C-1′), respectively. The β 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 *β*-sophorose was further supported by the gas chromatography analysis of hydrolyzate of **1**, recognized HMBCs between H-1″ (δ_H 4.59) and C-2′ (δ_C 83.2), and the thorough

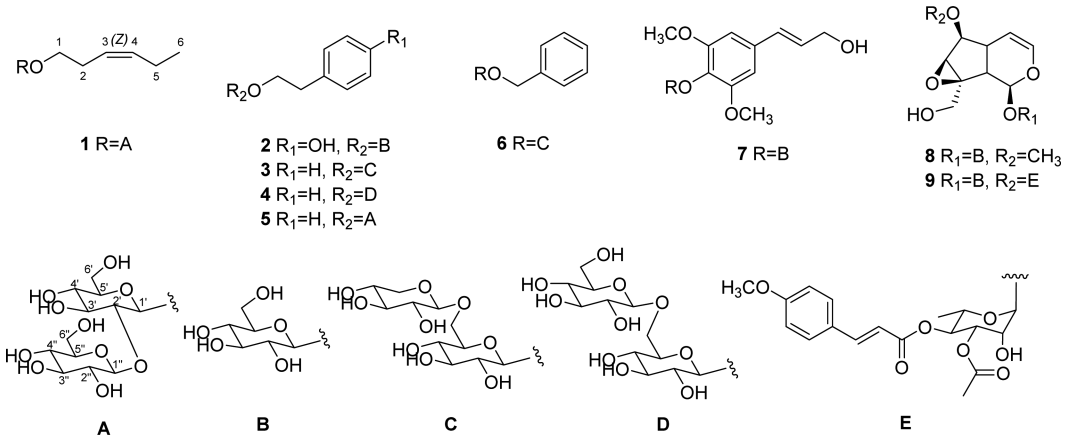


Figure 1. Isolated compounds from *B. officinalis* (**1**-**9**).

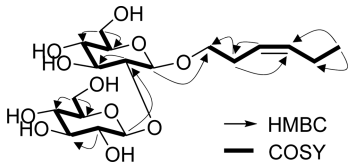


Figure 2. The selected HMBC and COSY correlations of **1**.

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 δ_H 4.42 and δ_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**	
	δ_H	δ_C
1	3.88 (1H, dt, *J* = 9.1, 7.0 Hz) 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) 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) 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.

Acknowledgments. We thank Prof. J. S. Oh, Dr. S. S. Hong, W. S. Jung at Gyeonggi Natural Products Research Institute for their help for NMR experiments, and Prof. D. Lee, and Dr. N. Kim at Korea University for providing MS experiment. This research was supported by the research fund from Institute of Bio-Science and Technology at Dankook University in 2011 and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0012877).

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

References

1. Ding, N.; Yahara, S.; Nohara, T. *Chem. Pharm. Bull.* **1992**, *40*, 780.
2. Tai, B. H.; Nhiem, N. X.; Quang, T. H.; Ngan, N. T. T.; Tung, N. H.; Kim, Y.; Lee, J.; Myung, C.; Cuong, N. M.; Kim, Y. H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3462.
3. Liao, Y. H.; Houghton, P. J.; Hoult, J. R. S. *J. Nat. Prod.* **1999**, *62*, 1241.
4. Guo, H.; Koike, K.; Li, W.; Satou, T.; Guo, D.; Nikaido, T. *J. Nat. Prod.* **2004**, *67*, 10.
5. Piao, M. S.; Kim, M. R.; Lee, D. G.; Park, Y.; Hahm, K. S.; Moon, Y. H.; Woo, E. R. *Arch. Pharm. Res.* **2003**, *26*, 453.
6. Oh, W. J.; Jung, U.; Eom, H. S.; Shin, H. J.; Park, H. R. *Molecules* **2013**, *18*, 9195.
7. Lee, Y. J.; Moon, M. K.; Hwang, S. M.; Yoon, J. J.; Lee, S. M.; Seo, K. S.; Kim, J. S.; Kang, D. G.; Lee, H. S. *Am. J. Chin. Med.* **2010**, *38*, 585.
8. Roh, C.; Park, M. K.; Shin, H. J.; Jung, U.; Kim, J. K. *Molecules* **2012**, *17*, 8687.
9. Lee, C.; Lee, S.; Park, S. Y. *Nat. Prod. Sci.* **2013**, *19*, 355.
10. Miyase, T. *Chem. Pharm. Bull.* **1987**, *35*, 1109.
11. Otsuka, H.; Takeda, Y.; Yammasaki, K. *Phytochemistry* **1990**, *29*, 3681.
12. Wang, L. B.; Wang, J. W.; Wang, C.; Sun, S. C.; Xu, B.; Wu, L. J. *Zhongguo Yaowu Huaxue Zazhi* **2012**, *22*, 220.
13. Yin, J. G.; Yuan, C. S.; Jia, Z. J. *Arch. Pharm. Res.* **2007**, *30*, 431.
14. Yang, E. J.; Kim, S. I.; Ku, H. Y.; Lee, D. S.; Lee, J. W.; Kim, Y. S.; Seong, Y. H. *Arch. Pharm. Res.* **2010**, *33*, 531.
15. Miyase, T.; Akahori, C.; Kohsaka, H.; Ueno, A. *Chem. Pharm. Bull.* **1991**, *39*, 2944.
16. Sueyoshi, E.; Yu, Q.; Matsunami, K.; Otsuka, H. *J. Nat. Med.* **2009**, *63*, 61.
17. Ma, Z.; Zhang, Y.; Song, S.; Xu, S. *Zhongguo Yaowu Huaxue Zazhi* **2008**, *18*, 300.
18. Liu, K. Y.; Wu, Y.-C.; Liu, I.-M.; Yu, W. C.; Cheng, J.-T. *Neuroscience Letters* **2008**, *434*, 195.
19. Cho, J. Y.; Nam, K. H.; Kim, A. R.; Park, J.; Yoo, E. S.; Baik, K. U.; Yu, Y. H.; Park, M. H. *J. Pharm. Pharmacol.* **2010**, *53*, 1287.