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BUDDLEJASAPONINS FROM THE FLOWERS OF *Buddleja officinalis*

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The genus *Buddleja* comprises around 100 species [1] native to tropical lands of America, Asia, and Africa. Most of them occur as bushes or small trees. The flowers, leaves, and root of various *Buddleja* species are used in folk medicine remedies in several parts of the world where they are indigenous. In traditional Chinese medicine, the flower buds of *B. officinalis*, called "Mi-meng-hua," are used for the treatment of conjunctival congestion and clustered nebulae [2]. It also is used to treat stroke, headache, and neurological disorders in traditional Korean medicine [3]. Besides its uses in folk medicine recipes, *B. officinalis* has also been cultured and its flowers utilized as food colorant in traditional festivals. In recent years, the genus *Buddleja* has been subjected to various investigations related to its chemistry [4]. Literature surveys indicated that several types of chemical compounds, including terpenoids, flavonoids, iridoids, and phenylethanoids, were isolated from the genus *Buddleja*. Continuing our study on the chemistry of *B. officinalis*, herein we report the isolation and structural elucidation of buddlejasaponin I, Ia, III, and α-amyrenone, which were first isolated from the flowers of *B. officinalis* along with mimengosides B, C, and E. Their structures were confirmed by NMR and ESI-MS spectral methods.

The fresh collection of flowers of *Buddleja officinalis* was dried, macerated, and repeatedly extracted with MeOH. The extract was then partitioned in various solvents and the chemical constituents separated by normal or reversed-phase flash chromatography. Based upon the results of TLC and further by NMR analysis, seven triterpenoids, buddlejasaponin I (**1**), buddlejasaponin III (**2**), buddlejasaponin Ia (**3**), mimengoside B (**4**) [5], mimengoside C (**5**) [6], mimengoside E (**6**) [6], and α-amirenone (**7**) [7] were isolated from the methanol extracts of the flowers. Of these, compounds **4**, **5**, and **6** were also first isolated and the structures identified by Guo and co-workers from the flowers of *B. officinalis* in 2004 [6], and compounds **1**, **2**, and **3** were isolated by Yamamoto from the aerial part of *B. japonica* in 1991 [8]. Until now, there are not many reports on both the isolation and biological activities of these compounds.

Compound **1** was obtained as a yellowish powder and its molecular formula, $C_{54}H_{88}O_{22}$, determined on the basis of ESI-MS at *m/z* 1111 $[M + Na]^+$, is in agreement with 54 carbon signals observed in the ^{13}C NMR spectra. The presence of four anomeric carbons at δ_C 105.13, 104.73, 103.45, and 102.83 in the ^{13}C NMR spectra suggested four sugar molecules in the sugar moiety of **1**. On the other hand, of the eight methyl groups, two (douplet, δ_C 16.89, 17.86) belonging to the sugar moiety and six (singlet, δ_C 12.56, 18.82, 20.21, 21.21, 24.07, 34.95) belonging to the aglycone moiety, which were observed in the ^1H NMR, ^{13}C NMR, and HMBC spectra, along with 24 other carbon aglycone signals (seven quaternary, seven methine, nine methylene carbons), appeared in the ^{13}C NMR and DEPT-135, which indicated that compound **1** was a triterpene glycoside with the oleane skeleton type. The presence of a long-range C-H correlation between C-3 (δ_C 84.21) and an anomeric proton (δ_H 4.48) in the HMBC spectra was evidence of the glycosidic linkage of fucopyranose to the C-3 position of the aglycone moiety. In addition, the 13,28-anhydro bridge in the aglycone moiety also point to the interaction of the proton H-28 (δ_H 3.63, 3.87) and the downfield carbon signal C-13 (δ_C 85.66) in the HMBC spectra. Finally, the existence of the 11,12-unsaturated bond was confirmed by two downfield signals in the ^1H and ^{13}C NMR [δ_H 5.38 (dd, J = 2.8, 10.4 Hz), 5.94 (d, J = 10.4 Hz) and δ_C 130.53, 134.22 ppm]. All of the above and comparison with the literature [8–10] showed that compound **1** was buddlejasaponin I {3β,16β,23α-trihydroxy-13,28-epoxyolean-11-en-3-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→3)]-[β-D-glucopyranosyl(1→2)]-β-D-fucopyranoside}.

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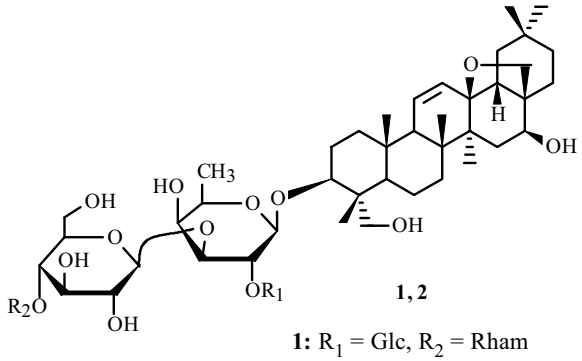

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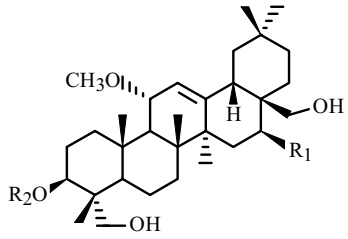

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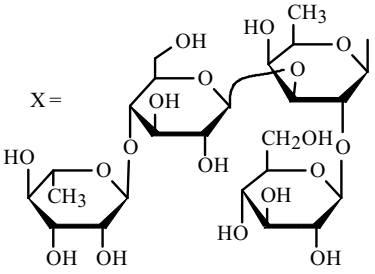

1: R_1 = Glc, R_2 = Rham
2: R_1 = H, R_2 = Xyl

3: R_1 = OH, R_2 = X
4: R_1 = H, R_2 = X

Compound **2** also was isolated as a yellowish powder, and its molecular weight was indicated by a peak in the ESI-MS spectrum at *m/z* 935 $[M + Na]^+$ corresponding to molecular formula $C_{47}H_{76}O_{17}$. In the ^{13}C NMR spectra, the slight differences of the aglycone carbon signals of **2** and **1** suggested the similarity of the aglycone moiety of these compounds. This was further confirmed by similar interactions observed in the HMBC spectra of **1** and **2**. The presence of three anomeric carbon signals in the ^{13}C NMR (δ_C 105.48, 105.38, 105.13 ppm) corresponding to three proton signals in the ^1H NMR (δ_H 4.59, 4.40, 4.36 ppm) suggested that there were three sugar molecules in **2**. In comparison with published reports [8, 11], compound **2** was identified as buddlejasaponin III {3β,16β,23-trihydroxy-13,28-epoxyolean-11-en-3-*O*-[β-D-xylopyranosyl(1→4)-β-D-glucopyranosyl(1→3)]-β-D-fucopyranoside}.

Like **1** and **2**, our observations of the ^1H and ^{13}C NMR of compound **3** showed that the structure of **3** also belongs to the oleane skeleton type. The suggested molecular formula of **3**, $C_{55}H_{92}O_{23}$, is based on the ESI-MS peak at *m/z* 1143 $[M + Na]^+$ and 55 carbon signals in the ^{13}C NMR and DEPT-135. The similarity of the ^{13}C NMR spectra at the sugar regions (δ_C 60–85, and 100–105 ppm) between **1** and **3** is evidences of the similarity of the sugar moiety in the structures of these compounds. Unlike **1**, the long-range correlations between proton H-11 (δ_H 3.92 ppm) and the two downfield carbon signals (δ_C 149.36, and 123.00 ppm) observed in the HMBC spectra indicated that the double bond is linked at C-12 and C-13. In addition, the existence of an interaction between proton H-11 and a carbon methoxy (δ_C 54.49 ppm) in the HMBC spectra also confirmed the methylation of the OH group at C-11. Finally, in compared with literature [10, 12], compound **3** was identified as buddlejasaponin Ia {3β,16β,23,28-tetrahydroxy-11-methoxyolean-12-en-3-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→3)]-[β-D-glucopyranosyl(1→2)]-β-D-fucopyranoside}.

Mimengoside B (**4**), $C_{55}H_{92}O_{22}$, white needle crystal, mp 259–260°C, $[\alpha]_D^{25}$ +2° (*c* 0.7, MeOH), ESI-MS *m/z* 1127 $[M + Na]^+$.

Mimengoside C (**5**), $C_{54}H_{88}O_{22}$, yellowish powder, $[\alpha]_D^{25}$ +85° (*c* 0.4 MeOH), ESI-MS *m/z* 1111 $[M + Na]^+$.

Mimengoside E (**6**), $C_{54}H_{88}O_{22}$, yellowish powder, $[\alpha]_D^{25}$ +70° (*c* 0.15 MeOH); ESI-MS *m/z* 1111 $[M + Na]^+$.

α-Amyrenone (**7**), $C_{30}H_{48}O$, white powder, ESI-MS *m/z* 425 $[M]^+$.

General Experimental Procedures. The nuclear magnetic resonance (^1H NMR, 400 MHz and ^{13}C NMR, 100 MHz) spectra were recorded on a Bruker DRX-NMR spectrometer (Germany) using Bruker′s standard pulse program. Chemical shifts were reported in ppm downfield from tetramethylsilane (TMS), with J in Hz. The electron spray ionization (ESI) mass spectra were recorded on an Agilent 1100 LC-MSD trap spectrometer. Silica gel (70–230, 230–400 mesh, Merck), and YMC RP-18 resins (30–50 μm, Fuji Silysia Chemicals Ltd.) were used as absorbents in the column chromatography. Thin layer chromatography (TLC) plates (Silica gel 60 F_{254} and RP-18 F_{254}, 0.25 μm, Merck) were purchased from Merck KGaA (Darmstadt, Germany). Spots were detected under UV radiation (254 and 365 nm) and by spraying the plates with 10% H_2SO_4 followed by heating with a heat gun.

Plant Material. The flowers of *Buddleja officinalis* were collected in Sapa town, Laocai Province, Vietnam in March 2007 and were identified by an experienced botanist at the Institute of Medicinal Materials, Ministry of Health, Hanoi, Vietnam. A voucher specimen (No. VN-814) was deposited at the Institute of Ecology and Biological Resources, VAST, Hanoi, Vietnam.

Extraction and Isolation. The dry flowers of *B. officinalis* Maxim. (2.0 kg) were extracted with methanol at room temperature three times. After removal of the solvent under reduced pressure, the crude extract (94.37 g) was dissolved in 1.0 L of H_2O to form a suspension that was successively partitioned with dichloromethane, ethyl acetate (EtOAc), and *n*-butanol to give dichloromethane (10.77 g), ethyl acetate (12.70 g), and *n*-butanol (27.29 g) extracts, respectively. The dichloromethane

extract was chromatographed on a silica gel column and eluted with a gradient of $CHCl_3$–MeOH (1:0–0:1, v/v) to afford seven fractions (D1a–g). Compound **7** (25 mg) was isolated from the D1d fraction by reverse phase (RP) column chromatography using an eluent of MeOH–Me_2CO (3:1, v/v). The *n*-butanol extract was then subjected to column chromatography using SiO_2 (70–230 mesh), eluting with Me_2CO–$CHCl_3$–H_2O (3:1:0.2, v/v/v) to give four fractions (B1a–d). Repeated silica gel column chromatography of fraction B1d with Me_2CO–EtOAc–H_2O (3:1:0.35, v/v/v) gave five subfractions (B2a–e). The B2a subfraction was further chromatographed using an YMC column and eluted by MeOH–H_2O (3:1, v/v) to yield compound **1** (40 mg) and compound **3** (34 mg). Next, the B2c fraction was subjected to an YMC column using an isocratic solvent of Me_2CO–H_2O (1.2:1, v/v). Combined with RP-TLC observation, compound **2** (15 mg) and compound **6** (28 mg) were isolated. Finally, compound **4** (17 mg) and compound **5** (33 mg) were also obtained from fraction B2d and B2e by an YMC column eluting with a mixture of MeOH–H_2O (4:1, v/v) and Me_2CO–H_2O (2.5:1, v/v), respectively.

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