

## Phenolic glycosides from the aerial parts of *Buddleja macrostachya* Benth.

Truong Thi Thu Hien<sup>1</sup>, Tran Hong Quang<sup>2</sup>, Nguyen Xuan Nhiem<sup>2</sup>, Bui Huu Tai<sup>2</sup>, Vu Phuong Phi<sup>3</sup>,  
Nguyen Thi Thu Hien<sup>4</sup>, Phan Van Kiem<sup>2\*</sup>

<sup>1</sup>Vietnam Military Medical University, 160 Phung Hung, Phuc La, Ha Dong, Hanoi

<sup>2</sup>Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST)

<sup>3</sup>Department of Pharmacy, 7B Military Hospital, Nguyen Ai Quoc, Tan Tien, Bien Hoa, Dong Nai

<sup>4</sup>Hanoi University of Mining and Geology, Tu Liem, Hanoi

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### Abstract

From the aerial parts of *Buddleja macrostachya* eight compounds, including: solidoside (1), echipurosides A (2), darendoside A (3), decaffeoylacteoside (4), 9-*O*- $\alpha$ -L-rhamnopyranosyl-4-hydroxy-cinnamic acid (5), 8-hydroxylinalool 3-*O*- $\beta$ -D-glucopyranoside (6), acteoside (7), and martynoside (8) were isolated. Their chemical structures were identified by NMR and MS spectroscopic analysis combined with a comparison to the reported values in the literature. This is the first time to report the isolation of echipurosides A, darendoside A, decaffeoylacteoside, 9-*O*- $\alpha$ -L-rhamnopyranosyl-4-hydroxy-cinnamic acid, and 8-hydroxylinalool 3-*O*- $\beta$ -D-glucopyranoside from the genus *Buddleja*.

**Keywords.** *Buddleja macrostachya*, Buddlejaceae, Phenolic glycoside.

### 1. INTRODUCTION

*Buddleja macrostachya* Benth. (Buddlejaceae family) is a deciduous shrub that grows up to 6 m in height and flowers annually during December. It is distributed in a wide area of the Asian countries, including China, India, Myanmar, Thailand, and Vietnam. In Vietnam, *B. macrostachya* is commonly distributed in Lao Cai province. In the Chinese traditional medicine, the *B. macrostachya* flowers have been used to treat hepatitis, conjunctivitis, and keratitis.<sup>[1]</sup> The leaves of *B. macrostachya* have been used for the treatment of bad sores and inflammation in the Indian traditional medicine.<sup>[2]</sup> Although many bioactive compounds, including iridoids<sup>[3,4]</sup> phenylethanoid glycosides<sup>[5]</sup>, flavonoids<sup>[6]</sup>, lignans<sup>[7]</sup>, and triterpenoid saponins<sup>[8]</sup> have been isolated from the *Buddleja* genus, the chemical composition of *B. macrostachya* has yet to be understood so far. In this study, we report the isolation and structural elucidation of eight compounds, including seven phenolic glycosides (1-5, 7 and 8) from the methanol extract of the aerial parts of *B. macrostachya* (Fig. 1).

### 2. MATERIAL AND METHODS

#### 2.1. Plant material

The aerial parts of *B. macrostachya* were collected in Sapa, Lao Cai province, Vietnam during September 2015. The scientific name was identified by Dr. Bui Van Thanh, Institute of Ecology and Biological Resources. A voucher specimen (NCCT-P02) was deposited at the Herbarium of the Institute of Marine Biochemistry, VAST.

#### 2.2. General experimental procedures

The NMR spectra were acquired on a Bruker AM500 FT-NMR spectrometer (500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C-NMR) with all chemical shifts are presented in ppm using tetrametylsilane (TMS) as an internal reference. ES-IMS spectra were taken on an Agilent 1100 spectrometer. Column chromatography (CC) was performed on silica gel 230-400 mesh or RP-18 resins (150  $\mu$ m, Fuji Silysia Chemical Ltd.). Compounds were visualized by spraying with aqueous 10 % H<sub>2</sub>SO<sub>4</sub> and heating for 5 minutes.

#### 2.3. Extraction and isolation

The dried and ground aerial parts of *B. macrostachya* (1.8 kg) were extracted with MeOH under sonication at room temperature. After being concentrated under reduced pressure, the MeOH

extract (BM, 130 g) was suspended in water and partitioned with *n*-hexane, CH<sub>2</sub>Cl<sub>2</sub>, and EtOAc to give the *n*-hexane, CH<sub>2</sub>Cl<sub>2</sub>, EtOAc, and water-soluble fractions. The EtOAc fraction (BME) was subjected to fractionation over reversed phase (RP) C<sub>18</sub> silica gel, eluted with MeOH-H<sub>2</sub>O (from 1:5 to 2:1, v/v) to give six fractions (BME1-BME6). Fraction BME6 was separated by RP C<sub>18</sub> column chromatography (CC), eluting with MeOH-H<sub>2</sub>O (1.5:1, v/v) to give **5** (5 mg). The aqueous fraction (BMW) was chromatographed over Diaion HP-20, eluting with a gradient step-wise of MeOH 25 %, 50 %, 75 %, and 100 % in H<sub>2</sub>O to provide four fractions (BMW1-BMW4). Fraction BMW2 was separated by silica gel CC, eluted with EtOAc-MeOH-H<sub>2</sub>O (10:1:0.01, v/v/v) to provide six subfractions (BMW2.1-BMW2.6). Subfraction BMW2.3 was isolated using RP C<sub>18</sub> CC and eluted with MeOH-H<sub>2</sub>O (1:3, v/v) to provide **1** (20 mg), and **6** (15 mg), and **7** (70 mg). Subfraction BMW2.4 was separated over a silica gel CC, eluting with CH<sub>2</sub>Cl<sub>2</sub>-MeOH-H<sub>2</sub>O (3.5:1:0.1, v/v/v) and further purified with RP C<sub>18</sub> CC using MeOH-H<sub>2</sub>O (1:3, v/v) as eluent to release **2** (11 mg), **3** (6 mg), and **4** (17 mg). Fraction BMW3 was subjected to a silica gel CC, eluted with CH<sub>2</sub>Cl<sub>2</sub>-MeOH-H<sub>2</sub>O (4:1:0.1, v/v/v) to provide seven subfractions (BMW3.1-BMW3.7). Subfraction BMW3.6 was further purified using RP C<sub>18</sub> CC, eluted with MeOH-H<sub>2</sub>O (1:1, v/v) to give **8** (25 mg).

**Salidroside (1):** white, amorphous powder; C<sub>14</sub>H<sub>20</sub>O<sub>7</sub>, M = 300; ESI-MS *m/z*: 323 [M+Na]<sup>+</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ<sub>H</sub>: 7.05 (d, *J* = 8.0 Hz, H-2 and H-6), 6.69 (d, *J* = 8.0 Hz, H-3 and H-5), 2.81 (t, *J* = 7.0 Hz, H<sub>2</sub>-7), 4.03 (q, *J* = 7.0 Hz, H-8a), 3.67 (q, *J* = 7.0 Hz, H-8b), 4.28 (d, *J* = 8.0 Hz, H-1'), 3.85 (br d, *J* = 12.0 Hz, H-6'a), 3.66 (dd, *J* = 5.0, 12.0 Hz, H-6'b); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz): see Table 1.

**Echipurosides A (2):** white, amorphous powder; C<sub>20</sub>H<sub>30</sub>O<sub>11</sub>, M = 446; ESI-MS *m/z*: 447 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ<sub>H</sub>: 7.09 (d, *J* = 8.0 Hz, H-2 and H-6), 6.73 (d, *J* = 8.5 Hz, H-3 and H-5), 2.86 (t, *J* = 7.5 Hz, H<sub>2</sub>-7), 4.00 (q, *J* = 7.5 Hz, H-8a), 3.85 (q, *J* = 7.5 Hz, H-8b), 4.30 (d, *J* = 8.0 Hz, H-1'), 4.00 (br d, *J* = 12.0 Hz, H-6'a), 3.63 (dd, *J* = 6.0, 12.0 Hz, H-6'b), 4.76 (s, H-1''), 1.28 (d, *J* = 6.5 Hz, H<sub>3</sub>-6''); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz): see Table 1.

**Darendosides A (3):** white, amorphous powder; C<sub>19</sub>H<sub>28</sub>O<sub>11</sub>, M = 432; ESI-MS *m/z*: 433 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ<sub>H</sub>: 7.09 (d, *J* = 8.5 Hz, H-2 and H-6), 6.72 (d, *J* = 8.5 Hz, H-3 and H-5), 2.84 (t, *J* = 7.5 Hz, H<sub>2</sub>-7), 4.05 (q, *J* = 7.5 Hz, H-8a), 3.69 (q, *J* = 7.5 Hz, H-8b), 4.37 (d, *J* = 7.5 Hz, H-1'),

3.49 (dd, *J* = 7.5, 9.0 Hz, H-2'), 3.87 (dd, *J* = 1.5, 12.0 Hz, H-6'a), 3.68 (dd, *J* = 5.5, 12.0 Hz, H-6'b), 5.39 (d, *J* = 1.0 Hz, H-1''), 4.01 (d, *J* = 10.0 Hz, H-4'a), 3.73 (d, *J* = 9.5 Hz, H-4'b), 3.62 (d, *J* = 2.5 Hz, H<sub>2</sub>-5''); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz): see Table 1.

**Decaffeoyllacteoside (4):** white, amorphous powder; C<sub>20</sub>H<sub>30</sub>O<sub>12</sub>, M = 462; ESI-MS *m/z*: 463 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ<sub>H</sub>: 6.71 (s, H-2), 6.70 (d, *J* = 8.5 Hz, H-5), 6.58 (d, *J* = 8.5 Hz, H-6), 2.80 (t, *J* = 7.5 Hz, H<sub>2</sub>-7), 4.03 (q, *J* = 7.5 Hz, H-8a), 3.71 (q, *J* = 7.5 Hz, H-8b), 4.32 (d, *J* = 8.0 Hz, H-1'), 5.17 (s, H-1''), 1.27 (d, *J* = 6.5 Hz, H<sub>3</sub>-6''); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz), see table 1.

**9-O-α-L-rhamnopyranosyl-4-hydroxycinnamic acid (5):** white, amorphous powder; C<sub>15</sub>H<sub>18</sub>O<sub>7</sub>, M = 310; ESI-MS *m/z*: 311 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ<sub>H</sub>: 7.05 (d, *J* = 8.5 Hz, H-2 and H-6), 6.83 (d, *J* = 8.5 Hz, H-3 and H-5), 7.69 (d, *J* = 16.0 Hz, H-7), 6.42 (d, *J* = 16.0 Hz, H-8), 5.07 (d, *J* = 1.5 Hz, H-1'), 1.31 (d, *J* = 6.0 Hz, H<sub>3</sub>-6''); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz): see Table 1.

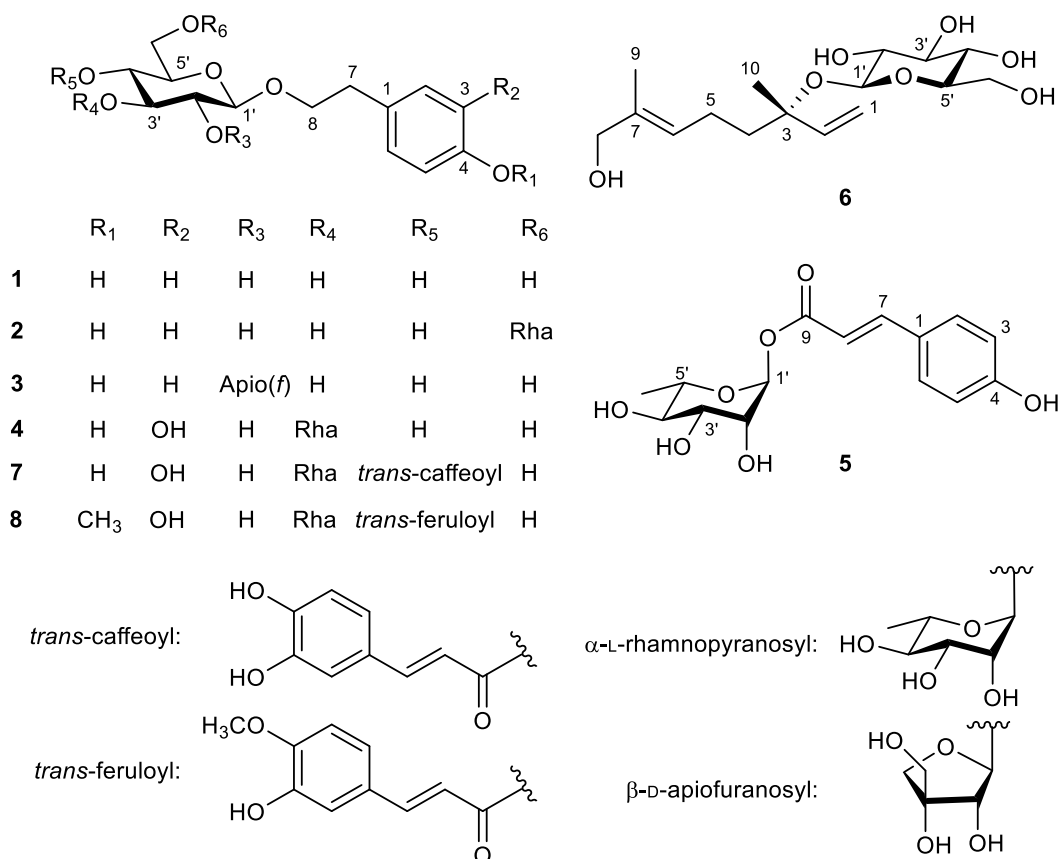
**8-Hydroxylinalool 3-O-β-D-glucopyranoside (6):** white, amorphous powder; C<sub>16</sub>H<sub>28</sub>O<sub>7</sub>, M = 332; ESI-MS *m/z*: 333 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ<sub>H</sub>: 5.23 (d, *J* = 18.0 Hz, H-1a), 5.20 (d, *J* = 11.0 Hz, H-1b), 6.12 (dd, *J* = 11.0, 18.0 Hz, H-2), 5.41 (t, *J* = 7.0 Hz, H-6), 3.92 (s, H<sub>2</sub>-8), 1.66 (s, H<sub>3</sub>-9), 1.36 (s, H<sub>3</sub>-10), 4.35 (d, *J* = 8.0 Hz, H-1'); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz), see table 1.

**Acteoside (7):** white, amorphous powder; C<sub>29</sub>H<sub>36</sub>O<sub>15</sub>, M = 624; ESI-MS *m/z*: 647 [M+Na]<sup>+</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) and <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz), see table 2.

**Martynoside (8):** white, amorphous powder; C<sub>31</sub>H<sub>40</sub>O<sub>15</sub>, M = 652; ESI-MS *m/z*: 653 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) and <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz), see table 2.

### 3. RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous, amorphous powder. Its molecular formula was established as C<sub>14</sub>H<sub>20</sub>O<sub>7</sub> by a sodium adduct ion [M+Na]<sup>+</sup> at *m/z* 323 in the ESIMS in combination with the <sup>13</sup>C NMR spectroscopic analysis. The <sup>1</sup>H NMR of **1** showed signals for two pairs of aromatic protons at δ<sub>H</sub> 7.05 (d, *J* = 8.0 Hz, H-2 and H-6) and 6.69 (d, *J* = 8.0 Hz, H-3 and H-5), suggesting the presence of a *para*-substituted benzene ring. The <sup>1</sup>H NMR spectrum further showed a signal for one anomeric proton δ<sub>H</sub> 4.28 (d, *J* = 8.0 Hz, H-1'), revealing that **1** has a monosaccharide unit in the structure. The analysis of the <sup>13</sup>C NMR and DEPT spectra led to identification of 14 carbon signals,

Figure 1: Chemical structures of compounds 1-8 from *B. macrostachya*Table 1:  $^{13}\text{C}$  NMR data ( $\text{CD}_3\text{OD}$ , 500 MHz) for compounds 1-6

C	1		2		3		4		5		6	
	$\delta_{\text{C}}^{\#1}$	$\delta_{\text{C}}$	$\delta_{\text{C}}$	$\delta_{\text{C}}^{\#2}$	$\delta_{\text{C}}$	$\delta_{\text{C}}^{\#3}$	$\delta_{\text{C}}$	$\delta_{\text{C}}^{\#4}$	$\delta_{\text{C}}$	$\delta_{\text{C}}^{\#5}$	$\delta_{\text{C}}$	
1	130.7	130.7	130.7	130.8	130.8	131.7	131.5	126.1	127.2	114.79	115.0	
2	130.9	130.9	130.9	130.9	130.9	117.3	117.1	130.8	131.2	144.50	144.5	
3	116.1	116.1	116.2	116.1	116.2	146.4	146.1	116.2	116.8	79.99	81.3	
4	156.8	156.7	156.7	156.8	156.7	144.9	144.6	160.8	161.3	41.97	41.2	
5	116.1	116.1	116.2	116.1	116.2	116.5	116.3	116.2	116.8	22.62	23.2	
6	130.9	130.9	130.9	130.9	130.9	121.5	121.3	130.8	131.2	124.88	127.0	
7	36.4	36.3	36.4	36.1	36.4	36.6	36.5	145.8	146.9	136.36	135.8	
8	72.1	72.1	72.2	72.0	72.0	72.3	72.1	114.5	115.2	68.08	68.9	
9								166.8	168.8	13.94	13.7	
10										23.58	23.5	
1'	104.4	104.3	104.4	103.3	103.3	104.4	104.2	91.0	93.2	99.79	99.2	
2'	75.1	75.0	75.1	78.4	78.7	75.8	75.6	72.8	74.6	75.38	75.1	
3'	77.9	77.8	78.0	78.3	78.6	84.6	84.5	71.9	70.4	78.85	78.2	
4'	71.7	71.6	71.7	71.7	71.7	70.3	70.2	74.3	75.1	71.83	71.7	
5'	78.1	78.0	76.8	77.8	77.9	78.0	77.8	70.1	69.3	78.11	77.6	
6'	62.8	62.7	68.1	62.7	62.7	62.8	62.6	18.8	18.2	62.96	62.8	
1''			102.2	110.5	110.5	102.9	102.7					
2''			72.2	78.0	77.8	72.5	72.3					
3''			72.4	80.7	80.7	72.4	72.2					
4''			74.0	75.4	75.3	74.1	74.0					
5''			69.8	66.2	66.2	70.2	70.0					
6''			18.0			17.9	17.9					

$^{\#1}\delta_{\text{C}}$  of salidoside in  $\text{CD}_3\text{OD}$  [9];  $^{\#2}\delta_{\text{C}}$  of darendoside A in  $\text{CD}_3\text{OD}$  [10];  $^{\#3}\delta_{\text{C}}$  of decaffeoylacteoside in  $\text{CD}_3\text{OD}$  [11];  $^{\#4}\delta_{\text{C}}$  of 9-O- $\alpha$ -L-rhamnopyranosyl-4-hydroxy-cinnamic acid in  $\text{CD}_3\text{OD}$  [12];  $^{\#5}\delta_{\text{C}}$  of 8-hydroxylinalool 3-O- $\beta$ -D-glucopyranoside in pyridine- $d_5$  [13].

including three methylenes [of which two were oxygenated:  $\delta_{\text{C}}$  72.1 (C-8) and 62.7 (C-6')], eight methines [including one anomeric carbon at  $\delta_{\text{C}}$  104.3 (C-1') and four aromatic carbons at  $\delta_{\text{C}}$  116.1 (C-2 and C-6) and 130.9 (C-3 and C-5)], and two non-protonated carbons at  $\delta_{\text{C}}$  130.7 (C-1) and 156.7 (C-4). This spectroscopic evidence suggested that **1** is a phenylethanoid glycoside derivative. Comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **1** with those of the reported phenylethanoid glycoside, salidroside revealed the identity<sup>[9]</sup>, suggesting that the structures of both compounds are identical. Thus, the structure of **1** was identified as shown in figure 1, named salidroside.

The molecular formula of **2** was identified as  $\text{C}_{20}\text{H}_{30}\text{O}_{11}$  by the presence of an ion  $[\text{M}+\text{H}]^+$  at  $m/z$  447 in the ESI-MS. Comparative analysis of the  $^{13}\text{C}$  NMR spectra of **2** with those of **1** revealed the close similarity, except for an additional presence of signals characteristic of a rhamnose unit [ $\delta_{\text{C}}$  102.2 (C-1''), 72.2 (C-2''), 72.4 (C-3''), 74.0 (C-4''), 69.8 (C-5''), and 18.0 (C-6'')] (table 1). The  $\alpha$ -oriented anomeric proton of the rhamnose was determined by its carbon chemical shift values of C-3 and C-5 positions.<sup>[14]</sup> The downfield carbon chemical shift of the oxymethylene carbon of the glucose at  $\delta_{\text{C}}$  68.1 (C-6') was indicative of the location of the *O*-glycosidic bond between two monosaccharides and therefore determining the  $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)-*O*- $\beta$ -glucopyranosyl sugar chain. Thus, the structure of compound **2** was determined as drawn in figure 1, named echipurosides A.<sup>[15]</sup>

The molecular formula of **3**,  $\text{C}_{19}\text{H}_{28}\text{O}_{11}$  was deduced by its ESI-MS:  $m/z$  433  $[\text{M}+\text{H}]^+$ . The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **3** were shown to be almost similar with those of **1**, except for and the additional presence of the carbon signals, including an anomeric carbon at  $\delta_{\text{C}}$  110.5 (C-1''), one oxymethine at  $\delta_{\text{C}}$  77.8 (C-2''), two oxymethylenes at  $\delta_{\text{C}}$  75.3 (C-4'') and 66.2 (C-5''), and one non-protonated carbon at  $\delta_{\text{C}}$  80.7 (C-3'') (table 1). These additional signals were found to be typical features of an apiose unit. Furthermore, the downfield shifted carbon signal of

C-2 of the glucose at 78.7 suggested that location of the apiose at C-2 of the glucose. From this analysis, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **3** were in a good agreement with those of the reported compound, darendoside A (Table 1).<sup>[10]</sup> The structure of **3** was further supported by analysis of the HSQC and HMBC spectra. In the HMBC spectrum, HMBC correlations from  $\delta_{\text{H}}$  5.39 (H-1'') to  $\delta_{\text{C}}$  78.7 (C-2') and from  $\delta_{\text{H}}$  4.37 (H-1') to  $\delta_{\text{C}}$  72.0 (C-8) allowed to identify the location of the  $\beta$ -D-apiofuranosyl-(1 $\rightarrow$ 2)-*O*- $\beta$ -D-glucopyranosyl at C-8 of the aglycone (figure 2). Therefore, compound **3** was identified as darendoside A.

The ESI-MS of **4** showed an ion peak  $[\text{M}+\text{H}]^+$  at  $m/z$  463 which was consistent with the molecular formula  $\text{C}_{20}\text{H}_{31}\text{O}_{12}$ . The  $^1\text{H}$  NMR spectrum of **4** exhibited signals characteristic of an ABX spin pattern at  $\delta_{\text{H}}$  6.71 (s, H-2), 6.70 (d,  $J = 8.5$  Hz, H-5), and 6.58 (d,  $J = 8.5$  Hz, H-6), indicating that **4** has a 1,3,4-trisubstituted aromatic ring (Table 1). The  $^1\text{H}$  NMR spectrum additionally displayed signals of two anomeric protons at  $\delta_{\text{H}}$  4.32 (d,  $J = 8.0$  Hz, H-1') and 5.17 (s, H-1''), suggesting that **4** possesses two sugar units in the molecule. The  $^{13}\text{C}$  and DEPT spectra contained 20 signals, of which eight carbons of a phenylethanoid aglycone and 12 carbons of a disaccharides could be assigned. The downfield chemical shift of an oxymethylene carbon of the aglycone at  $\delta_{\text{C}}$  72.1 (C-8) suggested that the sugar chain was attached to the aglycone at C-8. The signals for two sugars, including one glucose and one rhamnose were recognized by comparison with those reported in the literature.<sup>[11]</sup> The  $\beta$ -configurations for the anomeric proton of the glucose was deduced by the relatively large coupling constant ( $J = 8.0$  Hz), whereas the  $\alpha$ -oriented anomeric proton of the rhamnose was determined by its carbon chemical shift values of C-3 and C-5 positions.<sup>[14]</sup> In addition, the downfield shifted carbon signal at  $\delta_{\text{C}}$  84.5 suggested that the rhamnose was located at C-3 position of the glucose. This analysis was further supported by a comparison of the NMR data of **4** with those of the reported values

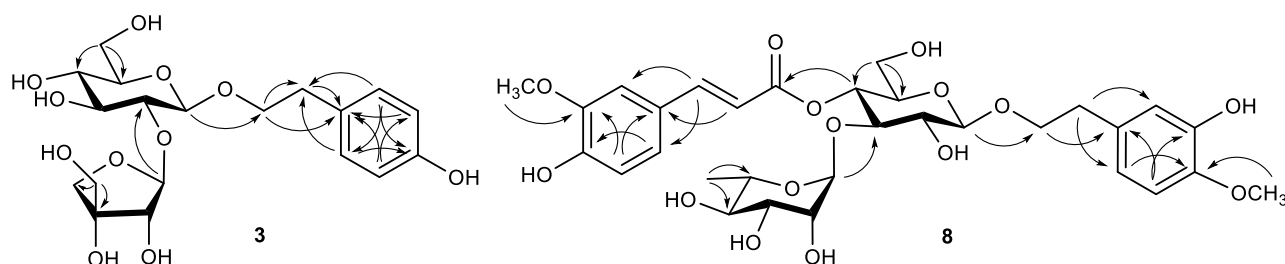


Figure 2: The selected HMBC correlations of compounds **3** and **8**

Table 2:  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for compounds **7** and **8**

C	$\delta_{\text{C}}^{\#1}$	<b>7</b>		$\delta_{\text{C}}^{\#2}$	<b>8</b>	
		$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ ( $J$ in Hz)		$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ ( $J$ in Hz)
1	131.74	131.4		132.90	132.8	
2	117.27	117.0	6.75, d (2.0)	117.08	117.0	6.72, br s
3	146.12	145.7		147.41	147.3	
4	144.65	144.2		147.57	147.5	
5	116.50	116.5	6.75, d (8.0)	112.83	112.8	6.80, d (8.5)
6	121.40	121.2	6.58, dd (2.0, 8.0)	121.16	121.1	6.67, dd (2.0, 8.5)
7	35.56	36.2	2.80, t (7.5)	36.59	36.5	2.80, t (7.5)
8	72.21	72.0	4.05, q (7.5)	72.15	72.0	4.05, q (7.5)
			3.72, q (7.5)			3.71, q (7.5)
1'	127.84	127.4		127.66	127.6	
2'	115.52	115.2	7.12, d (2.0)	111.75	111.8	7.17, s
3'	146.79	146.4		149.40	149.3	
4'	149.72	149.4		150.86	150.7	
5'	116.69	116.3	6.84, d (8.0)	116.51	115.1	6.80, d (8.0)
6'	123.21	123.2	6.97, dd (2.0, 8.0)	124.16	124.3	7.07, d (8.0)
7'	148.02	147.9	7.65, d (16.0)	147.91	147.8	7.66, d (16.0)
8'	114.93	114.5	6.33, d (16.0)	115.12	116.5	6.37, d (16.0)
9'	168.41	168.3		168.25	168.2	
1''	104.27	103.8	4.40, d (7.5)	104.25	104.2	4.36, d (8.0)
2''	76.24	75.8	3.45, dd (7.5, 8.5)	76.24	76.1	3.38, dd, (8.0, 9.0)
3''	81.69	81.5	3.86, t (9.5)	81.53	81.4	3.81, t, (9.0)
4''	70.81	70.1	4.98, t (9.5)	70.63	70.3	4.98, t (9.0)
5''	76.08	75.5	3.55, m	76.09	75.9	3.55, m
6''	62.49	62.0	3.68, br d (12.0)	62.41	62.3	3.62, br d (12.0)
			3.58, dd (5.5, 12.0)			3.52, dd (5.0, 12.0)
1'''	102.97	102.7	5.24, s	103.02	102.9	5.19, s
2'''	72.41	72.0	4.01, br d (3.5)	72.38	72.3	3.91, br d (3.5)
3'''	72.21	71.8	3.66, dd (3.5, 9.5)	72.08	72.0	3.58, dd (3.5, 9.0)
4'''	73.95	73.5	3.38, dd (9.0, 9.5)	73.79	73.7	3.29, t (9.0)
5'''	70.43	70.3	3.61, dq (6.5, 9.0)	70.44	70.6	3.58, dq (6.0, 9.0)
6'''	18.45	18.3	1.14, d (6.5)	18.47	18.4	1.09, d (6.0)
4-OCH <sub>3</sub>				56.48	56.5	3.91, s
3'-OCH <sub>3</sub>				56.45	56.4	3.91, s

<sup>a</sup>Recorded in CD<sub>3</sub>OD; <sup>b</sup>125 MHz; <sup>c</sup>500MHz; <sup>#1</sup>  $\delta_{\text{C}}$  of acteoside in CD<sub>3</sub>OD<sup>[16]</sup>; <sup>#2</sup>  $\delta_{\text{C}}$  of martynoside in CD<sub>3</sub>OD<sup>[17]</sup>

<sup>[11]</sup> Based on this evidence, the structure of **4** was elucidated as shown in Figure 1, named decaffeoylacteoside.

The molecular formula of **5** was established as C<sub>15</sub>H<sub>18</sub>O<sub>7</sub> by the presence of an ion [M+H]<sup>+</sup> at  $m/z$  311 in the ESI-MS. The  $^1\text{H}$  NMR of **5** contained signals for a *para*-substituted benzene ring [ $\delta_{\text{H}}$  7.05 (d,  $J$  = 8.5 Hz, H-2 and H-6) and 6.83 (d,  $J$  = 8.5 Hz, H-3 and H-5) and two olefinic protons at  $\delta_{\text{H}}$  7.69 (d,  $J$  = 16.0 Hz, H-7) and 6.42 (d,  $J$  = 16.0 Hz, H-8) (Table 1). The large coupling values ( $J$  = 16.0 Hz) of the two olefinic protons are the typical features of the *trans*-configured double bond. A resonance of an anomeric proton at  $\delta_{\text{H}}$  5.07 (d,  $J$  = 1.5 Hz, H-1') was also observed in the  $^1\text{H}$  NMR spectrum, suggesting that **5** possesses a monosaccharide unit in the structure. The  $^{13}\text{C}$  NMR spectra displayed 15 carbon

signals, including a carbonyl carbon at  $\delta_{\text{C}}$  168.8 (C-9), two olefinic methines at  $\delta_{\text{C}}$  146.9 (C-7) and 115.2 (C-8), two pair of aromatic methines and two aromatic non-protonated carbons, suggesting the structure of 4-hydroxy-cinnamic acid.<sup>[18]</sup> The six remaining carbon signals, including an anomeric carbon  $\delta_{\text{C}}$  93.2 (C-1') and a methyl at  $\delta_{\text{C}}$  18.2 (C-6') were assignable to a rhamnose unit. Based on the above observation, in combination with the good agreement when comparing the NMR data of **5** with those of the reported phenylpropanoid glycoside<sup>[12]</sup>, the structure of **5** was established as 9-*O*- $\alpha$ -L-rhamnopyranosyl-4-hydroxy-cinnamic acid.

The ESI-MS of **6** showed an ion [M+H]<sup>+</sup> at  $m/z$  333, corresponding with the molecular formula C<sub>16</sub>H<sub>29</sub>O<sub>7</sub>. The  $^1\text{H}$  NMR spectrum contained signals for two double bonds [ $\delta_{\text{H}}$  5.23 (d,  $J$  = 18.0 Hz, H-

1a), 5.20 (d,  $J = 11.0$  Hz, H-1b), 6.12 (dd,  $J = 11.0$ , 18.0 Hz, H-2), and 5.41 (t,  $J = 7.0$  Hz, H-6)], two methyls at  $\delta_{\text{H}}$  1.66 (s, H<sub>3</sub>-9) and 1.36 (s, H<sub>3</sub>-10), and a singlet of an oxymethylene group at  $\delta_{\text{H}}$  3.92 (s, H-8), and an anomeric proton at  $\delta_{\text{H}}$  4.35 (d,  $J = 8.0$  Hz, H-1') (table 1). The  $^{13}\text{C}$  NMR and DEPT spectra exhibited 16 carbon signals, including four olefinic carbons at  $\delta_{\text{C}}$  115.0 (C-1), 144.5 (C-2), 127.0 (C-6), and 135.8 (C-7), two aliphatic methylenes at  $\delta_{\text{C}}$  41.2 (C-4) and 23.2 (C-5), and two non-protonated carbons at  $\delta_{\text{C}}$  81.3 (C-3) and 135.8 (C-7), and six carbon signals which were assignable to a glucose unit (table 1). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **6** were found to be identical with those of 8-hydroxylinalool 3-*O*- $\beta$ -D-glucopyranoside<sup>[13]</sup>, revealing that the structures of both compounds are identical. Thus, the structure of **6** was determined as shown in Figure 1.

The molecular formula of **7**,  $\text{C}_{29}\text{H}_{36}\text{O}_{15}$  was identified by an ion  $[\text{M}+\text{Na}]^+$  at  $m/z$  647 in its ESI-MS. The  $^1\text{H}$  NMR spectrum contained signals characteristic of two aromatic rings possessing the ABX spin patterns [ $\delta_{\text{H}}$  6.75 (d,  $J = 2.0$  Hz, H-2), 6.75 (d,  $J = 8.0$  Hz, H-5), 6.58 (dd,  $J = 2.0$ , 8.0 Hz, H-6) and 7.12 (d,  $J = 1.5$  Hz, H-2'), 6.84 (d,  $J = 8.0$  Hz, H-5'), and 6.97 (d,  $J = 2.0$ , 8.0 Hz, H-6')], one trans-configured double bond [ $\delta_{\text{H}}$  7.65 (d,  $J = 16.0$  Hz, H-7') and 6.33 (d,  $J = 16.0$  Hz, H-8')], and two methylene groups of which one was oxygenated [ $\delta_{\text{H}}$  4.05 and 3.72 (H<sub>2</sub>-8)] (table 2). The  $^1\text{H}$  NMR spectrum additionally presented resonances for two anomeric protons at  $\delta_{\text{H}}$  4.40 (d,  $J = 7.5$  Hz, H-1'') and 5.24 (br s, H-1'''), implying the presence of two sugar units in the molecule. Analysis of the  $^{13}\text{C}$  NMR and HSQC spectra revealed 29 carbon signals, of which 22 carbons were assignable to the aglycone and 12 carbons belonged to the sugar moiety. The carbon signals for the aglycone, including three aromatic non-protonated carbons at  $\delta_{\text{C}}$  131.4 (C-1), 145.7 (C-3) and 144.2 (C-4), three aromatic methines at  $\delta_{\text{C}}$  117.0 (C-2), 116.5 (C-5), and 121.1 (C-6), along with two methylenes at  $\delta_{\text{C}}$  36.2 (C-7) and 72.0 (C-8) are characterized for a 2-(3,4-dihydroxy-phenyl)ethanol structural moiety (Table 2). In addition, the structure of a *trans*-caffeoyl moiety was observed through signals of a carbonyl carbon at  $\delta_{\text{C}}$  168.3 (C-9'), two olefinic carbons at  $\delta_{\text{C}}$  147.9 (C-7') and 114.5 (C-8'), and a 1,3,4-trisubstituted benzene ring. The 12 carbon signals of the sugar moiety were assigned to a glucose and a rhamnose by comparison with those reported in the literature<sup>[16]</sup>. In the HMBC spectrum, an HMBC correlation observed from  $\delta_{\text{H}}$  4.40 (H-1'') to  $\delta_{\text{C}}$  72.0 (C-8) indicated that the glucose is attached to the 2-

(3-hydroxy-4-methoxy-phenyl)ethanol moiety through an *O*-glycosidic linkage. An HMBC correlation observed from  $\delta_{\text{H}}$  5.24 (H-1''') to  $\delta_{\text{C}}$  81.5 (C-3'') suggested that the rhamnose was located at C-3 position of the glucose. The HMBC spectrum further showed an HMBC cross-peak of  $\delta_{\text{H}}$  3.29 (H-4'') with  $\delta_{\text{C}}$  168.3 (C-9'), suggesting that the caffeoyl group is located at C-4 position of the glucose. Based on the aforementioned spectroscopic analysis, compound **7** was identified as acteoside.

The molecular formula of **8** was established as  $\text{C}_{31}\text{H}_{40}\text{O}_{15}$  by the presence of an ion  $[\text{M}+\text{H}]^+$  at  $m/z$  653 in the ESI-MS. The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data of **8** were found to be nearly identical with those of **7**, except for the additional presence of signals for two methoxy groups in **8** [ $\delta_{\text{H}}$  3.91 (s)/ $\delta_{\text{C}}$  56.5 (4-OCH<sub>3</sub>) and  $\delta_{\text{H}}$  3.91 (s)/ $\delta_{\text{C}}$  56.4 (3'-OCH<sub>3</sub>)]. The location of the two methoxy groups at C-4 and C-3' was deduced by HMBC cross-peaks from  $\delta_{\text{H}}$  3.91 to  $\delta_{\text{C}}$  147.5 (C-4) and 149.40 (C-3') (figure 2). Thus, the structure of **8** was identified as illustrated in figure 1, named martynoside.

In conclusion, chemical investigation of the methanol extract of the aerial parts of *B. macrostachya* resulted in the isolation and identification of eight compounds. Of the isolates, echipurosides A, darendoside A, decaffeoylacteoside, 9-*O*- $\alpha$ -L-rhamnopyranosyl-4-hydroxy-cinnamic acid, and 8-hydroxylinalool 3-*O*- $\beta$ -D-glucopyranoside were isolated for the first time from the genus *Buddleja*.

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Corresponding author: **Phan Van Kiem**

Institute of Marine Biochemistry  
Vietnam Academy of Science and Technology  
18 Hoang Quoc Viet, Cau Giay, Hanoi, Viet Nam  
E-mail: [phankiem@vast.vn](mailto:phankiem@vast.vn); phankiem@yahoo.com