

## PHENOLIC GLYCOSIDES FROM *Antidesma ghaesembilla*

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### Abstract

Five phenolic glycosides, vanillyl alcohol 4-*O*- $\beta$ -D-glucopyranoside, 4-hydroxy-3,5-dimethoxybenzyl-*O*- $\beta$ -D-glucopyranoside, 3-hydroxy-4,5-dimethoxyphenyl-*O*- $\beta$ -D-glucopyranoside, 3,4,5-trimethoxyphenyl-*O*- $\beta$ -D-glucopyranoside, and sinapyl alcohol 4-*O*- $\beta$ -D-glucopyranoside (**1-5**), respectively were isolated from the methanol extract of the *Antidesma ghaesembilla* leaves. Their structures were elucidated by spectroscopic methods and in comparison with the published data. Compounds **1-3** were reported from Euphorbiaceae family for the first time, compounds **4** and **5** were reported from *Antidesma* genus for the first time.

**Keywords.** *Antidesma ghaesembilla*, Euphorbiaceae, phenolic.

### 1. INTRODUCTION

*Antidesma* is a genus of tropical plants belonging to Euphorbiaceae family and comprises about 100 species in the world and 29 species in Vietnam. The leaves of *Antidesma ghaesembilla* Gaertner have been used in traditional medicine to treat inflammation, infection, sore throat, and lung diseases [1]. In addition, a few phytochemical investigations of *A. ghaesembilla* has been studied [2]. Moreover, the leaves of this plant exhibited antioxidant and hypoglycemic activities [3]. Herein, we report the isolation and structure elucidation of five phenolic glycosides from the methanol extract of the leaves of *A. ghaesembilla*.

### 2. MATERIAL AND METHODS

#### 2.1. Plant Material

The leaves of *Antidesma ghaesembilla* Gaertner were collected in Dak Lak province, Vietnam, in March 2013 and identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature. A voucher specimen was deposited at Institute of Marine Biochemistry, VAST.

#### 2.2. General experimental procedures

All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for <sup>1</sup>H-NMR and 125 MHz for <sup>13</sup>C-NMR). NMR measurements, including <sup>1</sup>H-, <sup>13</sup>C-NMR, HSQC, and HMBC experiments, were carried out using 5-mm probe tubes at temperature of 22.2 °C. Melting points were recorded in Kofler micro-hostage apparatus. Optical rotations were determined on a Jasco DIP-1000 polarimeter. Column chromatography was performed using a silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (150  $\mu$ m, Fuji Silysia Chemical Ltd.), thin layer chromatography (TLC) using a pre-coated silica-gel 60 F<sub>254</sub> (0.25 mm, Merck) and RP-18 F<sub>254S</sub> plates (0.25 mm, Merck).

#### 2.3. Extraction and Isolation

The dried leaves of *A. ghaesembilla* (2.5 kg) were extracted in MeOH three times using sonicator to yield 115.0 g of a dark solid extract, which was then suspended in water and successively partitioned with dichloromethane and ethyl acetate (EtOAc) to give dichloromethane (AG1, 30.0 g), EtOAc (AG2, 20.0 g), and water layers (AG3, 65.0 g) after

removing solvent *in vacuo*. The water layer (AG3, 65.0 g) was chromatographed on a Diaion HP-20 column eluting with water to remove sugar component, then increase concentration of methanol in water (25, 50, 75, and 100 %) to yield four fractions, AG3A-AG3D. The AG3A fraction was chromatographed on a silica gel column eluting with EtOAc/MeOH/water (5/1/0.5, v/v/v) to give three fractions, AG3A1-AG3A3. The AG3A1 fraction was chromatographed on a RP-18 column eluting with MeOH/water (1/2, v/v) to yield compounds **1** (6.0 mg) and **3** (5.0 mg). The AG3A3 fraction was chromatographed on a RP-18 column eluting with MeOH/water (1/2, v/v) to yield compound **2** (5.0 mg). The AG3D fraction was chromatographed on a silica gel column eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH/water (5/1/0.1, v/v/v) to give three fractions, AG3D1-AG3D3. Finally, compounds **4** (7.0 mg) and **5** (5.0 mg) were obtained from the AG3D2 fraction using a RP-18 column, eluted with MeOH/water (1/2.5, v/v).

**Vanillyl alcohol 4-*O*- $\beta$ -D-glucopyranoside (1):** White amorphous powder, C<sub>14</sub>H<sub>20</sub>O<sub>8</sub>,  $[\alpha]_D^{25}$ : -47.0 (*c* = 0.1, MeOH), mp: 125-126°C, <sup>1</sup>H- and <sup>13</sup>C-NMR (CD<sub>3</sub>OD), see table 1.

**4-Hydroxy-3,5-dimethoxybenzyl-*O*- $\beta$ -D-glucopyranoside (2):** White amorphous powder, C<sub>15</sub>H<sub>22</sub>O<sub>9</sub>,  $[\alpha]_D^{25}$ : -50.0 (*c* = 0.1, MeOH), mp: 180-182°C, <sup>1</sup>H- and <sup>13</sup>C-NMR (CD<sub>3</sub>OD), see table 1.

**5-Hydroxy-3,4-dimethoxyphenyl-*O*- $\beta$ -D-glucopyranoside (3):** White amorphous powder, C<sub>14</sub>H<sub>20</sub>O<sub>9</sub>,  $[\alpha]_D^{25}$ : -25.0 (*c* = 0.1, MeOH), mp: 195-196°C, <sup>1</sup>H- and <sup>13</sup>C-NMR (CD<sub>3</sub>OD), see table 1.

**3,4,5-Trimethoxyphenyl-*O*- $\beta$ -D-glucopyranoside (4):** White amorphous powder, C<sub>15</sub>H<sub>22</sub>O<sub>9</sub>,  $[\alpha]_D^{25}$ : -30.0 (*c* = 0.1, MeOH), mp: 201-203 °C, <sup>1</sup>H- and <sup>13</sup>C-NMR (CD<sub>3</sub>OD), see table 2.

**Sinapyl alcohol 4-*O*- $\beta$ -D-glucopyranoside (5):** White amorphous powder, C<sub>17</sub>H<sub>24</sub>O<sub>9</sub>,  $[\alpha]_D^{25}$ : -18.0 (*c* = 0.1, MeOH), mp: 179-180 °C, <sup>1</sup>H- and <sup>13</sup>C-NMR (CD<sub>3</sub>OD), see table 2.

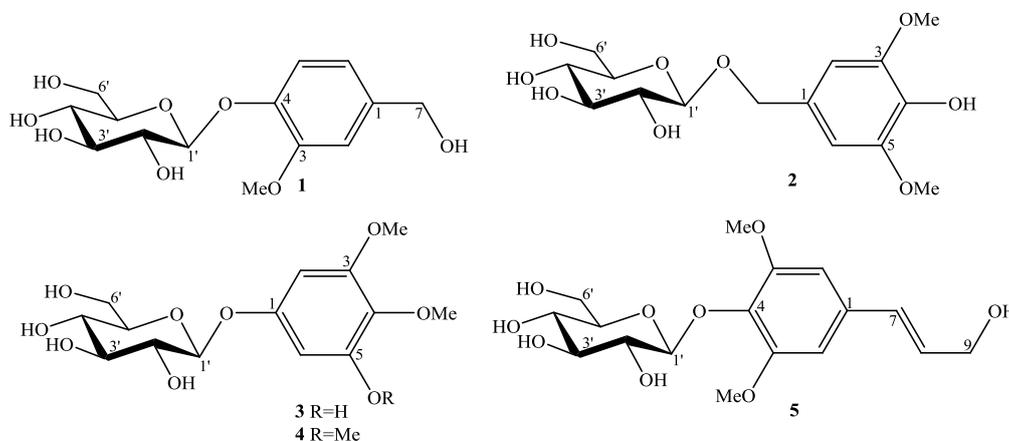


Figure 1: Chemical structures of compounds **1-5**

### 3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. The <sup>1</sup>H-NMR of compound **1** showed the following signals: three aromatic protons with ABX system at  $\delta_H$  6.90 (1H, dd, *J* = 2.0, 8.0 Hz), 7.04 (1H, d, *J* = 2.0 Hz), and 7.15 (d, *J* = 8.0 Hz) assigned to a phenolic aglycone; one anomeric proton at  $\delta_H$  4.89 (1H, d, *J* = 7.5 Hz) assigned to a sugar unit; one methoxy group at  $\delta_H$  3.89 (3H, s). The <sup>13</sup>C-NMR and DEPT spectra of **1** revealed signals of 14 carbons, including three non-protonated carbons at  $\delta_C$  137.76, 147.23, and 150.82; eight methine carbons at  $\delta_C$  71.35, 74.92, 77.84, 78.18, 102.98, 112.67, 117.99, and 120.72; two methylene carbons at  $\delta_C$  62.51 and 64.95; one

methoxy carbon at  $\delta_C$  56.69. The <sup>1</sup>H- and <sup>13</sup>C-NMR data (table 1) of **1** were similar to those of vanillyl alcohol 4-*O*- $\beta$ -D-glucopyranoside [4]. All the carbons were assigned to relevant protons by means of an HSQC experiment. The HMBC correlations between H-7 ( $\delta_H$  4.56) and C-1 ( $\delta_C$  137.76)/C-2 ( $\delta_C$  112.67)/C-6 ( $\delta_C$  120.72) suggested the position of hydroxyl group at C-7. The HMBC cross peak from H-2 ( $\delta_H$  7.04)/H-5 ( $\delta_H$  7.15) to C-3 ( $\delta_C$  150.82)/C-4 ( $\delta_C$  147.23); from methoxy group ( $\delta_H$  3.89) to C-3 ( $\delta_C$  150.82) confirmed the position of methoxy group at C-3. The large coupling constant of H-1' and H-2', *J* = 7.5 Hz and <sup>13</sup>C-NMR chemical shifts of sugar moiety ( $\delta_C$  102.98, 74.92, 77.84, 71.35, 78.18, and 62.51) suggested the presence of the  $\beta$ -D-

glucopyranosyl moiety in **1**. In addition, this sugar was attached to C-4 of aglycone by the observation of HMBC correlation between glc H-1' ( $\delta_{\text{H}}$  4.89) and C-4 ( $\delta_{\text{C}}$  147.23). Thus, the

structure of **1** was elucidated to be vanillyl alcohol 4-*O*- $\beta$ -D-glucopyranoside. As our knowledge, this compound was reported from Euphorbiaceae family for the first time.

Table 1: The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data for compounds **1-3** in  $\text{CD}_3\text{OD}$  and reference compounds

Pos.	<b>1</b>		<b>2</b>		<b>3</b>			
	$\delta_{\text{C}}^{\#}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., <i>J</i> , Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., <i>J</i> , Hz)	$\delta_{\text{C}}^{\$}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., <i>J</i> , Hz)
<b>Aglycone</b>								
1	137.7	137.76	-	129.48	-	155.8	155.83	-
2	112.6	112.67	7.04 (d, 2.0)	106.88	6.75 (s)	98.7	98.75	6.30 (d, 2.5)
3	150.8	150.82	-	149.15	-	151.8	151.85	-
4	147.2	147.23	-	136.30	-	133.2	133.26	-
5	117.9	117.99	7.15 (d, 8.0)	149.15	-	154.9	154.85	-
6	120.7	120.72	6.90 (dd, 2.0, 8.0)	106.88	6.75 (s)	94.8	94.92	6.36 (d, 2.5)
7	64.9	64.95	4.56 (s)	71.84	4.63 (d, 11.5) 4.83 (d, 11.5)			
8								
9								
3-OMe	56.6	56.69	3.89 (s)	56.78	3.87 (s)	56.4	56.38	3.82 (s)
4-OMe						61.1	61.11	3.74 (s)
5-OMe				56.78	3.87 (s)			
<b>OGlc</b>								
1'	102.9	102.98	4.89 (d, 7.5)	102.98	4.33 (d, 7.5)	102.9	102.93	4.80 (d, 7.5)
2'	74.9	74.92	3.51 (m)	75.13	3.26 (m)	74.9	74.91	3.44 (m)
3'	77.8	77.84	3.49 (m)	78.06	3.35 (m)	78.0	78.05	3.46 (m)
4'	71.3	71.35	3.43 (m)	71.79	3.31 (m)	71.5	71.51	3.38 (m)
5'	78.1	78.18	3.43 (m)	78.12	3.29 (m)	78.2	78.24	3.46 (m)
6'	62.5	62.51	3.71 (dd, 6.0, 12.0) 3.87 (dd, 2.0, 12.0)	62.88	3.71 (dd, 6.0, 12.0) 3.92 (dd, 2.0, 12.0)	62.6	62.61	3.71 (dd, 6.0, 12.0) 3.92 (dd, 2.0, 12.0)

$^{\#}\delta_{\text{C}}$  of vanillyl alcohol 4-*O*- $\beta$ -D-glucopyranoside in  $\text{CD}_3\text{OD}$  [4],

$^{\$}\delta_{\text{C}}$  of 5-hydroxy-3,4-dimethoxyphenyl-*O*- $\beta$ -D-glucopyranoside in  $\text{CD}_3\text{OD}$  [5].

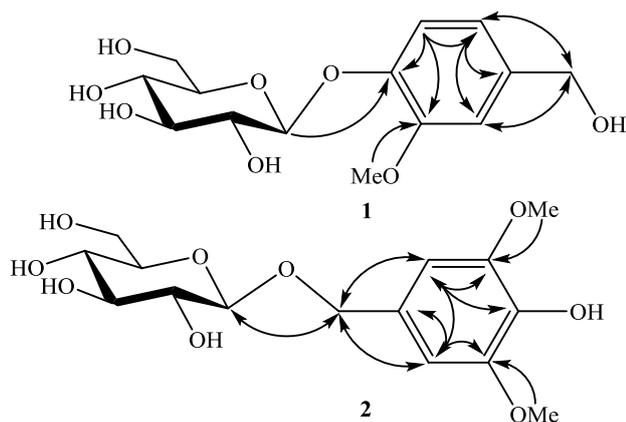


Figure 2: The key HMBC correlations of **1** and **2**

The  $^1\text{H}$ -NMR spectrum of **2** showed two aromatic protons  $\delta_{\text{H}}$  6.75 (2H, s), two methoxy groups at 3.87 (6H, s), and one anomeric proton at  $\delta_{\text{H}}$  4.33 (1H, d, *J* = 7.5 Hz). The  $^{13}\text{C}$ -NMR and DEPT spectra of **2** showed the signals of 15 carbons including four non-protonated at  $\delta_{\text{C}}$  129.48, 136.30, and 149.15  $\times$  2; seven methine at  $\delta_{\text{C}}$  71.79, 75.13, 78.06, 78.12, 102.98, and 106.88  $\times$  2; two methylene at  $\delta_{\text{C}}$  62.88, and 71.84; two methoxy carbons at  $\delta_{\text{C}}$  56.78  $\times$  2. Similar to **1**, the sugar in **2** was determined as  $\beta$ -D-glucopyranosyl. In addition, this sugar moiety at C-1 was confirmed by HMBC correlation from H-1' ( $\delta_{\text{H}}$  4.33) to C-7 ( $\delta_{\text{C}}$  71.84). The HMBC correlations between H-7 ( $\delta_{\text{H}}$  4.62 and 4.83) and C-1 ( $\delta_{\text{C}}$

129.48)/C-2/6 ( $\delta_C$  106.88); H-2/H-6 ( $\delta_H$  6.75) and C-3/C-5 ( $\delta_C$ 149.15)/C-4 ( $\delta_C$  136.30) indicated the positions of two methoxy groups at C-3/C-5 and hydroxyl group at C-4 of the aglycone. Consequently, the structure of **2** was determined as 4-hydroxy-3,5-dimethoxybenzyl-*O*- $\beta$ -D-glucopyranoside and the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of **2** were reported for the first time.

The  $^{13}\text{C}$ -NMR and DEPT spectra of **3** showed the presence of 15 carbons, including four non-protonated, seven methine, one methylene, and three methoxy carbons. Analytical  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of **3** indicated that its NMR data were identical to those of 5-hydroxy-3,4-dimethoxyphenyl-*O*- $\beta$ -D-glucopyranoside [5]. Moreover, the HMBC correlation between H-1' ( $\delta_H$  4.80) and C-1 ( $\delta_C$  155.83) confirmed that sugar moiety was located at

C-1 of aglycone. The HMBC correlations from methoxy groups ( $\delta_H$  3.74 and 3.82) to C-3 ( $\delta_C$ 154.85)/C-4 ( $\delta_C$ 133.26), respectively, suggested the positions of two methoxy groups at C-4 and C-5, and hydroxyl group at C-5 of aglycone. Based on the above evidence and in comparison NMR data of **3** to those compounds reported in the literature [5], the structure of **3** was defined as 5-hydroxy-3,4-dimethoxyphenyl-*O*- $\beta$ -D-glucopyranoside.

By similar way, the NMR data of **4** were almost similar to those of **3** except for an addition of methoxy group at C-3. Comparing NMR data of **4** to those of 3,4,5-trimethoxyphenyl-*O*- $\beta$ -D-glucopyranoside [7] were found in match. Thus, the structure of **4** was elucidated. Compound **4** was previously reported from Euphorbiaceae family [8]. But this is the first report from *Antidesma* genus.

Table 2: The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data for compounds **4-5** in  $\text{CD}_3\text{OD}$  and reference compounds

Pos.	<b>4</b>			<b>5</b>		
	$\delta_C^{\%}$	$\delta_C^a$	$\delta_H^a$ (mult., J, Hz)	$\delta_C^{\%}$	$\delta_C^a$	$\delta_H^a$ (mult., J, Hz)
<b>Aglycone</b>						
1	156.2	156.03	-	135.5	135.28	-
2	96.2	96.18	6.50 (s)	105.7	105.49	6.77 (s)
3	154.9	154.79	-	154.6	154.36	-
4	134.4	134.30	-	135.8	135.60	-
5	154.9	154.79	-	154.6	154.36	-
6	96.2	96.18	6.50 (s)	105.7	105.49	6.77 (s)
7				131.5	131.26	6.57 (d, 16.0)
8				130.2	130.05	6.34 (dt, 5.5, 16.0)
9				63.8	63.56	4.24 (d, 5.5)
3-OMe	56.7	56.55	3.83 (s)	57.4	57.04	3.88 (s)
4-OMe	61.4	61.22	3.72 (s)			
5-OMe	56.7	56.55	3.83 (s)	57.4	57.04	3.88 (s)
<b>OGlc</b>						
1'	103.3	103.20	4.84 (d, 7.5)	105.6	105.37	4.89 (d, 7.5)
2'	75.1	74.91	3.45 (m)	76.0	75.73	3.43 (m)
3'	78.2	78.08	3.47 (m)	78.0	77.83	3.50 (m)
4'	71.8	71.76	3.35 (m)	71.6	71.35	3.27 (m)
5'	78.6	78.41	3.45 (m)	78.5	78.35	3.43 (m)
6'	62.9	62.72	3.67 (dd, 6.0, 12.0) 3.93 (dd, 2.0, 12.0)	62.7	62.58	3.69 (dd, 6.0, 12.0) 3.81 (dd, 2.0, 12.0)

$^{\%}\delta_C$  of 3,4,5-trimethoxyphenyl-*O*- $\beta$ -D-glucopyranoside in  $\text{CD}_3\text{OD}$  [5],

$^{\%}\delta_C$  of sinapyl alcohol 4-*O*- $\beta$ -D-glucopyranoside in  $\text{CD}_3\text{OD}$  [6].

The  $^1\text{H}$ -NMR of compound **5** showed the signals of two aromatic protons at  $\delta_H$  6.77 (2H, s), two olefinic protons at 6.57 (1H, d,  $J = 16.0$  Hz) and 6.34 (1H, dt,  $J = 5.5$  and 16.0 Hz), two methoxy groups at 3.88 (6H, s); and one anomeric proton at  $\delta_H$  4.88 (1H,

d,  $J = 7.5$  Hz). The  $^{13}\text{C}$ -NMR and DEPT spectra of **5** showed signals of 17 carbons, including four non-protonated, nine methine, two methylene, and two methoxy carbons. The NMR data of **5** were identical to those of sinapyl alcohol 4-*O*- $\beta$ -D-glucopyranoside

[6]. The large coupling constant of H-7 and H-8,  $J = 16.0$  Hz indicated *E* configuration of double bond at C-7/C-8. Consequently, the structure of **5** was elucidated to be sinapyl alcohol 4-*O*- $\beta$ -D-glucopyranoside. This compound was isolated from *Phyllanthus urinaria* (Euphorbiaceae) [8].

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