

Article

## Two New Derivatives of 2, 5-Dihydroxyphenyl Acetic Acid from the Kernel of *Entada phaseoloides*

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**Abstract:** Two new aromatic compounds, butyl 2,5-dihydroxyphenyl acetate (**1**) and butyl 2-O- $\beta$ -D-glucopyranosyloxy-5-dihydroxyphenyl acetate (**2**), together with three known ones, methyl 2,5-dihydroxyphenyl acetate (**3**), ethyl 2,5-dihydroxyphenyl acetate (**4**) and 2-O- $\beta$ -D-glucopyranosyloxy-5-hydroxyphenyl acetic acid (**5**), were isolated from the EtOH extract of the kernel of *Entada phaseoloides*. The structures of the new compounds were elucidated by MS and NMR experiments. Compounds **1**, **3** and **4** displayed potent inhibitory activities against HIV-1 replication, with EC<sub>50</sub> values of 9.80  $\mu$ M, 11.70  $\mu$ M and 9.93  $\mu$ M, respectively.

**Keywords:** *Entada phaseoloides*; Leguminosae; 2,5-dihydroxyphenyl acetic acid; HIV-1

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### 1. Introduction

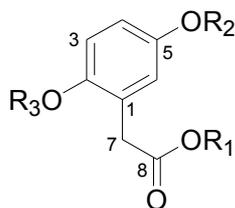
*Entada phaseoloides* (L.) Merr. is the sole species of *Entada* genus (Leguminosae), widely distributed in south China, esp. in Yunnan and Hainan provinces. The kernel of *E. phaseoloides* has been commonly used as an herbal medicine by the Dai nationality for the treatment of hemostasis and

detoxification [1]. Some investigations suggested that it had antidiabetic [2], anti-inflammatory [3], and molluscicidal activities [4]. In 1955, Barua first obtained a triterpene acid from its kernel [5]. After that, more compounds from this plant were reported, such as phenylacetic acid esters [6,7], triterpene saponins [8], phenolic acids [9,10], chalcone glycosides [11] and sulfur-containing amides [12,13]. As one of the components in Qi-wei Ke-Teng-Zi Wan [14], a famous formula of medicines used in the Dai nationality, the active constituents of the kernel of *E. phaseoloides* are still unknown. Herein we report the isolation, structure elucidation, and anti-HIV activity of five aromatic compounds from the kernel of *Entada phaseoloides*.

## 2. Results and Discussion

The EtOH extract from air-dried kernels (7 kg) of *E. phaseoloides* was extracted with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract was separated by repeated column chromatography to give five aromatic compounds (Figure 1) including two new ones (compounds **1**, **2**). The known compounds were readily identified as methyl 2,5-dihydroxyphenyl acetate (**3**) [1], ethyl 2,5-dihydroxyphenyl acetate (**4**) [6] and 2-O- $\beta$ -D-glucopyranosyloxy-5-hydroxyphenyl acetic acid (**5**) [6] by comparison of their spectroscopic data with published values.

**Figure 1.** Structures of compounds **1–5**.



- 1** R<sub>1</sub> = (CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub> R<sub>2</sub> = H R<sub>3</sub> = H  
**2** R<sub>1</sub> = (CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub> R<sub>2</sub> = H R<sub>3</sub> = glc  
**3** R<sub>1</sub> = CH<sub>3</sub> R<sub>2</sub> = H R<sub>3</sub> = H  
**4** R<sub>1</sub> = CH<sub>2</sub>CH<sub>3</sub> R<sub>2</sub> = H R<sub>3</sub> = H  
**5** R<sub>1</sub> = H R<sub>2</sub> = (CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub> R<sub>3</sub> = glc

Compound **1** was obtained as a white crystalline solid. The molecular formula was determined as C<sub>12</sub>H<sub>16</sub>O<sub>4</sub> (five degrees of unsaturation) on the basis of its TOF-ESI-MS at *m/z* 247.0963 [M+Na]<sup>+</sup>. The IR spectrum of **1** showed absorption bands at 3390 (OH), 1722 (C=O) cm<sup>-1</sup>. The <sup>1</sup>H-NMR data (Table 1) of **1** revealed similar structural features as those of **3**, except for the additional signals of the butyl group at  $\delta_{\text{H}}$  0.92 (3H, t, *J* = 7.5 Hz), 1.64 (2H, m), 1.37 (2H, m), 4.14 (2H, t, *J* = 6.5 Hz). This implied that the methyl in **3** was replaced by the butyl in compound **1**. HMBC correlation of H-9 with C-8 showed the butyl group was connected to the ester bond. Hence, **1** was elucidated as butyl 2,5-dihydroxyphenyl acetate.

Compound **2** was obtained as a white crystalline solid and displayed similar UV and IR profiles to those of **1**. The molecular formula was determined to be C<sub>18</sub>H<sub>26</sub>O<sub>9</sub> (six degrees of unsaturation) by analysis of its TOF-ESI-MS at *m/z* 409.1463 [M+Na]<sup>+</sup>. Compared with the NMR data with those of **1**, it revealed that **2** possessed similar units with those of **1** except that an additional glucose moiety [ $\delta_{\text{H}}$  4.69 (1H, d, *J* = 7.5 Hz), 3.41 (1, dd, *J* = 3.0, 6.0 Hz), 3.37 (1H, m), 3.39 (1H, m), 3.45 (1H, m),

3.87 (1H, dd,  $J = 1.5, 12.5$  Hz), 3.68 (1H, d,  $J = 12.5$  Hz)] was present in **2**. HMBC correlation from H-1' to C-2 demonstrated the glucose was connected to C-2. Upon acid hydrolysis, **2** afforded D-glucose, which was identified by co-TLC with authentic samples. The  $\beta$  configuration of the glucopyranose was confirmed by the large coupling constant ( $J = 7.5$  Hz) of its anomeric proton. Thus, the structure of **2** was established as butyl 2-O- $\beta$ -D-glucopyranosyloxy-5-dihydroxyphenyl acetate.

**Table 1.**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of compound **1** and **2** in MeOH- $d_4$  ( $\delta$  in ppm,  $J$  in Hz).

Position	1		2	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1	–	123.8	–	127.7
2	–	150.1	–	150.9
3	6.82 (d, 8.5)	119.1	7.06 (d, 9.0)	119.7
4	6.66 (dd, 8.5, 3.0)	116.2	6.65 (dd, 2.5, 9.0)	116.3
5	–	151.5	–	154.3
6	6.61 (d, 8.5)	117.1	6.64 (br s)	118.8
7	3.61 (s)	37.3	3.68 (s)	37.3
8	–	174.8	–	174.9
9	4.14 (t, 6.5)	66.1	4.08 (t, 6.5)	66.4
10	1.64 m	32.3	1.59 m	32.2
11	1.37 m	20.6	1.35 m	20.6
12	0.92 (t, 7.5)	14.5	0.91 (t, 7.5)	14.5
1'	–	–	4.69 (d, 7.5)	104.9
2'	–	–	3.41 (dd, 3.0, 6.0)	75.6
3'	–	–	3.30 m	78.6
4'	–	–	3.39 m	72.0
5'	–	–	3.45 m	78.5
6'	–	–	3.68 (d, 12.5)	63.2
			3.87 (dd, 1.5, 12.5)	

All isolated compounds were evaluated for anti-HIV activity against VSVG/HIV pseudotyped virus using zidovudine as the positive control. Compounds **1**, **3** and **4** exhibited inhibitory activities against HIV-1 replication with  $\text{EC}_{50}$  values of  $9.80 \mu\text{M}$ ,  $11.70 \mu\text{M}$  and  $9.93 \mu\text{M}$ , respectively, while the  $\text{EC}_{50}$  values of zidovudine was  $11.70 \text{ nM}$ . However, compounds **2** and **5** did not show inhibition at the concentration of  $10 \mu\text{M}$ .

### 3. Experimental

#### 3.1. General

NMR spectra were measured on a Bruker AM 500 NMR spectrometer as the internal reference and chemical shifts are expressed in ppm. TOF-ESI-MS spectra were measured on a Waters Synapt G2 mass spectrometer. EIMS data were recorded on a Zabspec E mass spectrometer. IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer. UV spectra were run on a Shimadzu UV-2550 UV/Vis spectrophotometer. TLC was performed on silica gel GF254 (10–40  $\mu\text{m}$ ; Qingdao Marine

Chemical, Inc., Qingdao, China). Column chromatography was performed on silica gel (100–200 or 200–300 mesh; Qingdao Marine Chemical, Inc.).

### 3.2. Plant Material

The kernal of *Entada phaseoloides* was collected from Xishuangbanna, Yunnan province, in January, 2010. The sample was identified by one of the authors C. Z. Peng, and a voucher specimen (No. 20100128) has been deposited in the herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

### 3.3. Extraction and Isolation

The air-dried kernels (7 kg) of *E. phaseoloides* were extracted with 95% and 50% EtOH (10 L) by reflux for 3 times. After removal of the solvent, the aqueous residue was partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract (23.3 g) was fractionated by silica gel column chromatography eluted with CH<sub>2</sub>Cl<sub>2</sub>/EtOAc (100:0–5:1) to give six fractions A–G. Fraction C (12.2 g) was subjected to silica gel column chromatography, eluted with petroleum ether/EtOAc (60:1–5:1) to give five fractions (C1–C5). Fraction C2 (4.5 g) was further separated over silica gel column chromatography and the material was eluted using petroleum ether/acetone (40:1–5:1) to afford five fractions (C21–C25). Fraction C22 (924 mg) was chromatographed on Sephadex LH-20 to yield **3** (20 mg). Fraction C23 (812 mg) was chromatographed on Sephadex LH-20 to yield **1** (20 mg) and **4** (8 mg). Fraction C24 (523 mg) was chromatographed on Sephadex LH-20 to yield **2** (15 mg) and **5** (25 mg).

### 3.4. Spectral Data

*Butyl 2, 5-dihydroxyphenyl acetate (1)* White crystals, mp 135–136 °C; UV (MeOH)  $\lambda_{\max}$  (log $\epsilon$ ) 204, 296 nm; IR (KBr)  $\nu_{\max}$  3390 (OH), 1722 (C=O), 1506, 1477, 953, cm<sup>-1</sup>; TOF-ESI-MS  $m/z$  247.0963 [M+Na]<sup>+</sup> (calcd 247.0948 for C<sub>12</sub>H<sub>16</sub>O<sub>4</sub>Na); EI-MS  $m/z$  (%) 224 [M]<sup>+</sup> (10), 150 (74), 122 (100), 94 (49), 56 (30), 41 (59); <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data see Table 1.

*Butyl 2-O- $\beta$ -D-glucopyranosyloxy-5-dihydroxyphenyl acetate (2)* White crystals, mp 135–136 °C; UV (MeOH)  $\lambda_{\max}$  (log $\epsilon$ ) 203, 224, 289 nm; IR (KBr)  $\nu_{\max}$  3380 (OH), 1717 (C=O), 1507, 1476, 990 cm<sup>-1</sup>; TOF-ESI-MS  $m/z$  409.1476 [M+Na]<sup>+</sup> (calcd 409.1463 for C<sub>18</sub>H<sub>26</sub>O<sub>9</sub>Na); EI-MS  $m/z$  (%) 224 [M-glc]<sup>+</sup> (64), 150 (100), 122 (42), 94 (8), 85 (12), 73 (14), 57 (20), 41 (21); <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data see Table 1.

### 3.5. Anti-HIV Activity Assay

Production of VSV-G/HIV pseudovirions: Human embryonic kidney 293T cells were transiently co-transfected with 3  $\mu$ g vesicular stomatitis virus glycoprotein (VSV-G) plasmid and 8  $\mu$ g Env-deficient HIV vector (pNL4-3-Luc-R<sup>-</sup>E<sup>-</sup>) in 100-mm plates by a standard Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> protocol. Sixteen h post-transfection, cells were washed by PBS, then added 10 mL fresh medium into each plate. Forty-eight h post-transfection, the supernatants, containing pseudotyped virions (VSVG/HIV), were collected and filtered through a 0.45  $\mu$ m filter. Virions were quantified by p24 concentrations

which were detected by ELISA (ZeptoMetrix, Buffalo, NY, USA; Cat.: 0801111) and diluted to 0.2 ng p24/mL which can be used directly or stored at  $-80\text{ }^{\circ}\text{C}$ .

Anti-HIV replication activity assay: One day prior to infection, 293T cells were seeded on 24-well plates with the density of  $6 \times 10^4$  cells per well. Compounds were incubated with cells 15 min ahead of infection. Forty eight h post-infection, infected cells were lysed in 50  $\mu\text{L}$  Cell Lysis Reagent (Promega, San Luis Obispo, CA, USA). Luciferase activity of cell lysate was measured by sirius luminometer (Berthold Detection System, Pforzheim, Germany) according to the manufacturer's instructions.

#### 4. Conclusions

Two new aromatic compounds, butyl 2,5-dihydroxyphenyl acetate (**1**) and butyl 2-O- $\beta$ -D-glucopyranosyloxy-5-dihydroxyphenyl acetate (**2**), together with three known ones, methyl 2,5-dihydroxyphenyl acetate (**3**), ethyl 2,5-dihydroxyphenyl acetate (**4**) and 2-O- $\beta$ -D-glucopyranosyloxy-5-hydroxyphenyl acetic acid (**5**), were isolated from the kernel of *Entada phaseoloides*. Compounds **1**, **3** and **4** displayed potent inhibitory activities against HIV-1 replication with  $\text{EC}_{50}$  values of 9.80  $\mu\text{M}$ , 11.70  $\mu\text{M}$  and 9.93  $\mu\text{M}$ , respectively.

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*Sample Availability:* Samples of the compounds **1–5** are available from the authors.

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