

Article

Two New Derivatives of 2, 5-Dihydroxyphenylacetic Acid from the Kernel of *Entada phaseoloides*

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Abstract: Two new aromatic compounds, butyl 2,5-dihydroxyphenylacetate (**1**) and butyl 2-O- β -D-glucopyranosyloxy-5-dihydroxyphenylacetate (**2**), together with three known ones, methyl 2,5-dihydroxyphenylacetate (**3**), ethyl 2,5-dihydroxyphenylacetate (**4**) and 2-O- β -D-glucopyranosyloxy-5-hydroxyphenylacetic 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 EC₅₀ values of 9.80 μ M, 11.70 μ M and 9.93 μ M, respectively.

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

1. Introduction

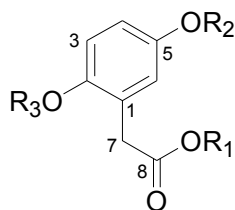
Entada phaseoloides (L.) Merr. is the sole species of *Entada* genus (Leguminosae), widely distributed in south China, esp. in Yunnan and Hainan provinces. The kernel of *E. phaseoloides* has been commonly used as a herbal medicine by the Dai nationality for the treatment of hemostasis and

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-dihydroxyphenylacetate (**3**) [1], ethyl 2,5-dihydroxyphenylacetate (**4**) [6] and 2-O- β -D-glucopyranosyloxy-5-hydroxyphenylacetic acid (**5**) [6] by comparison of their spectroscopic data with published values.

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



- 1** $R_1 = (CH_2)_3CH_3$ $R_2 = H$ $R_3 = H$
2 $R_1 = (CH_2)_3CH_3$ $R_2 = H$ $R_3 = \text{glc}$
3 $R_1 = CH_3$ $R_2 = H$ $R_3 = H$
4 $R_1 = CH_2CH_3$ $R_2 = H$ $R_3 = H$
5 $R_1 = H$ $R_2 = (CH_2)_3CH_3$ $R_3 = \text{glc}$

Compound **1** was obtained as a white crystalline solid. The molecular formula was determined as $C_{12}H_{16}O_4$ (five degrees of unsaturation) on the basis of Its TOF-ESI-MS at m/z 247.0963 $[M+Na]^+$. The IR spectrum of **1** showed absorption bands at 3390 (OH), 1722 (C=O) cm^{-1} . The ^1H -NMR data (Table 1) of **1** revealed similar structural features as those of **3**, except for the additional signals of the butyl group at 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 9-H with C-8 showed the butyl group was connected to the ester bond. Hence, **1** was elucidated as butyl 2,5-dihydroxyphenylacetate.

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 $C_{18}H_{26}O_9$ (six degrees of unsaturation) by analysis of its TOF-ESI-MS at m/z 409.1463 $[M+Na]^+$. 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 [δ_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 1'-H 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 β 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- β -D-glucopyranosyloxy-5-dihydroxyphenylacetate.

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

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{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 EC_{50} values of $9.80 \mu\text{M}$, $11.70 \mu\text{M}$ and $9.93 \mu\text{M}$, respectively, while the EC_{50} values of zidovudine was 11.70 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 μm ; 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 Professor Peng Chaozhong, 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 CH₂Cl₂/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-dihydroxyphenylacetate (1) White crystals, mp 135–136 °C; UV (MeOH) $\lambda_{\max}$ (log ϵ) 204, 296 nm; IR (KBr) $\nu_{\max}$ 3390 (OH), 1722 (C=O), 1506, 1477, 953, cm^{−1}; TOF-ESI-MS m/z 247.0963 [M+Na]⁺ (calcd 247.0948 for C₁₂H₁₆O₄Na); EI-MS m/z (%) 224 [M]⁺ (10), 150 (74), 122 (100), 94 (49), 56 (30), 41 (59); ¹H-NMR and ¹³C-NMR data see Table 1.

Butyl 2-O- β -D-glucopyranosyloxy-5-dihydroxyphenylacetate (2) White crystals, mp 135–136 °C; UV (MeOH) $\lambda_{\max}$ (log ϵ) 203, 224, 289 nm; IR (KBr) $\nu_{\max}$ 3380 (OH), 1717 (C=O), 1507, 1476, 990 cm^{−1}; TOF-ESI-MS m/z 409.1476 [M+Na]⁺ (calcd 409.1463 for C₁₈H₂₆O₉Na); EI-MS m/z (%) 224 [M-glc]⁺ (64), 150 (100), 122 (42), 94 (8), 85 (12), 73 (14), 57 (20), 41 (21); ¹H-NMR and ¹³C-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 μ g vesicular stomatitis virus glycoprotein (VSV-G) plasmid and 8 μ g Env-deficient HIV vector (pNL4-3-Luc-R[−]E[−]) in 100-mm plates by a standard Ca₃(PO₄)₂ 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 μ 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°C .

Anti-HIV replication activity assay: One day prior to infection, 293T cells were seeded on 24-well plates with the density of 6×10^4 cells per well. Compounds were incubated with cells 15 min ahead of infection. Forty eight h post-infection, infected cells were lysed in 50 μL 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-dihydroxyphenylacetate (**1**) and butyl 2-O- β -D-glucopyranosyloxy-5-dihydroxyphenylacetate (**2**), together with three known ones, methyl 2,5-dihydroxyphenylacetate (**3**), ethyl 2,5-dihydroxyphenylacetate (**4**) and 2-O- β -D-glucopyranosyloxy-5-hydroxyphenylacetic acid (**5**), were isolated from the kernel of *Entada phaseoloides*. Compounds **1**, **3** and **4** displayed potent inhibitory activities against HIV-1 replication with EC_{50} values of 9.80 μM , 11.70 μM and 9.93 μM , respectively.

Acknowledgments

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

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