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A new Antioxidative Resveratrol Trimer from the Roots and Stems of *Vitis quinquangularis*

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Abstract: Phytochemical investigation of *Vitis quinquangularis* has led to the isolation of a new resveratrol trimer, quinquangularol (**1**), along with three known stilbenoids **2**–**4**. The structure and relative configuration of **1** were established on the basis of spectroscopic evidence, especially HMBC and NOESY experiments. It showed potent antioxidant activity against DPPH (1,1-diphenyl-2-picrylhydrazyl) radical.

Keywords: *Vitis quinquangularis*; Vitaceae; resveratrol oligomer; quinquangularol; antioxidant; DPPH.

1. Introduction

Vitis quinquangularis Rehd is a species of *Vitis* in the flowering plant family Vitaceae. Native to China, *V. quinquangularis* is one of the most widely distributed East Asian wild species of *Vitis*. It grows in the mountain area and the bushes, and is distributed mainly in 17 provinces, south of Qinling Mountains and Mount Tai, such as Hubei, Hunan, Jiangxi, Guangxi, Guangdong, Yunnan and Guizhou [1]. *V. quinquangularis* is traditionally used as folk medicine for the treatment of physical injury [2].

Resveratrol is one of the most extensively studied plant polyphenol, since it was linked to the health benefits associated with red wine consumption (the so-called "French paradox") in the early 1990s [3-4]. Numerous pharmacological studies have demonstrated its great potential in preventing and treating an array of diseases, including cardiovascular disease, cancer, and neurodegenerative diseases [5].

In some particular families of plants (e.g., Vitaceae, the grapevine family), a number of phytoalexins were polymerized as resveratrol oligomers, via oxidative coupling of different number of units of resveratrol monomer [6]. Resveratrol oligomers have attracted considerable attention owing to their structural diversity and diverse biological activities, and they also play a vital role in plant chemical defense [7].

Our previous phytochemical investigations on Vitaceae plants have led to discovery of novel bioactive resveratrol oligomers [8–11]. Our continuing research on chemical constituents of the roots

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and stems of *V. quinquangularis* has now resulted in the isolation of a new resveratrol trimer, quinquangularol (**1**) together with three known stilbenoids; amurensin A (**2**) [12], vitisin A (**3**) [13], and resveratrol (**4**) [14]. **1** showed potent antioxidant activity in a DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals assay.

2. Materials and Methods

2.1. General experimental procedures

^{1}H-NMR spectra were recorded at 500 MHz, and ^{13}C-NMR spectra were recorded at 125 MHz with tetramethylsilane (TMS) and solvent signals as internal references. HR-ESI-MS data were acquired with a FT-ICR mass spectrometer. IR spectra were measured on a FT-IR spectrometer as KBr pellets. Column chromatography (CC) was carried out on silica gel (200–300 mesh from Qingdao Marine Chemical Co. Ltd., Qingdao, China).

2.2. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, MO, USA). Solvents (analytical grade) for extraction and CC were purchased from Huadong Chemicals (Hangzhou, China).

2.3. Plant Material

The roots and stems of *V. quinquangularis* Rehd were collected in May 2011 in Lishui, Zhejiang Province, China. The material was identified by Dr. Yunpeng Zhao (College of Life Sciences, Zhejiang University, Hangzhou, China). A voucher specimen (No. P110532) was deposited at the Department of Biology, Zhejiang University, China.

2.4. Extraction and Isolation

The plant material (2.3 kg) was extracted three times with EtOH (3 × 20 L) at room temperature. The solvent was evaporated *in vacuo* to produce a concentrated EtOH extract (170 g), which was then diluted with H_2O (1 L) to give an aqueous solution (1 L). The aqueous solution was extracted with EtOAc three times (3 × 2.0 L). The combined EtOAc layers were condensed *in vacuo* to provide an EtOAc extract (76 g), which was then subjected to silica gel CC (1 kg, 5 cm diameter column) eluted with light petroleum-EtOAc mixtures (10:1 to 1:10) to yield ten fractions. Fraction 4 (1.5 g) was subjected to preparative HPLC (column YMC-C18, 250 × 20 mm i.d.; solvent MeOH-H_2O, 55%:45%; flow rate 8 mL/min; detection 280 nm) to afford three pure isolates **2** (*t*R = 32 min, 51 mg), **3** (*t*R = 45 min, 75 mg) and **1** (*t*R = 71 min, 38 mg). Fraction 6 (0.8 g) was separated by preparative HPLC under similar conditions except for the ratio of MeOH: H_2O (40%:60%) to give compounds **4** (*t*R = 30 min, 120 mg).

*2.5. Quinquangularol (**1**)*

Colorless amorphous powder; $[\alpha]^{20}_{D}$ +42 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.3), 282 (3.3), 313 (3.1) nm; IR (KBr) ν_{max} 3385, 1612, 1519, 1440, 1335, 1238, 1170, 1004, and 829 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HR-ESI-MS *m/z* 729.2325 [M - H] $^{-}$ (calcd for $C_{43}H_{37}O_{11}$, 729.2336).

2.6. Antioxidant Activity

Antioxidant activity of 1 was determined by the DPPH assay as previously described [9]. Briefly, the reaction mixture containing sample solution (20 μL) and DPPH (180 μL, 150 μM) in ethanol was placed in a 96-well microplate and incubated at 37 °C for 30 min. The absorbance was measured at 517 nm by a microplate reader. IC_{50} value represents the concentration of a compound to scavenge 50% of DPPH radicals. Resveratrol was used as positive control.

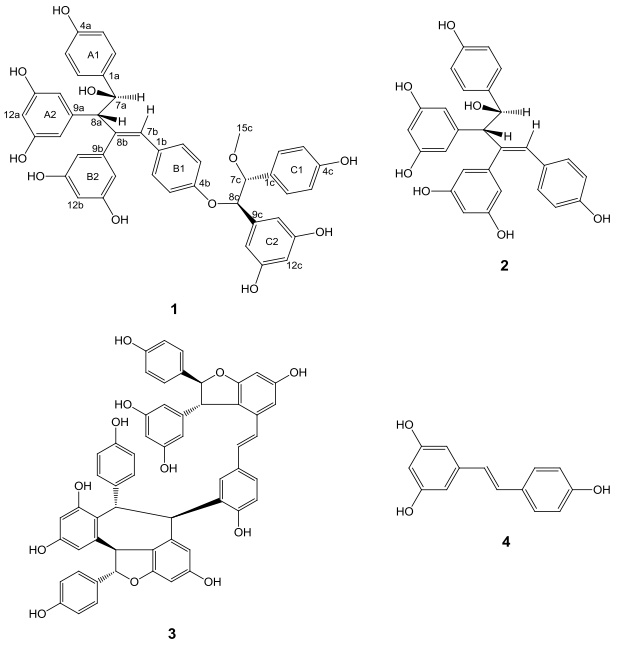

Figure 1. Chemical structures of stilbenoids from *V. quinquangularis* Rehd.

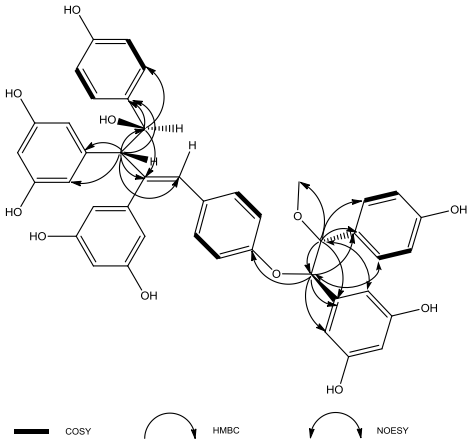

Figure 2. Key HMBC, ^{1}H–^{1}H COSY and NOESY correlations for quinquangularol (**1**).

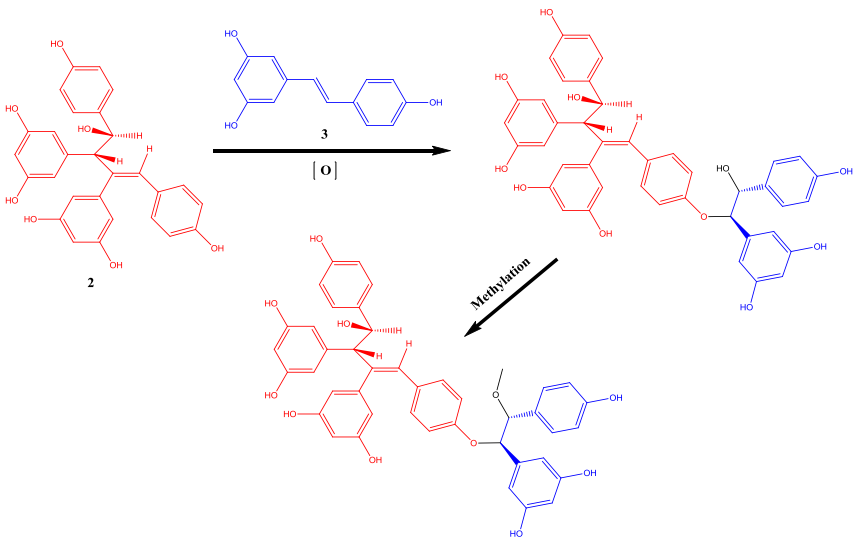

Figure 3. Postulated biogenetic pathway of quinquangularol (**1**).

3. Results and Discussion

3.1. Structure elucidation

The roots and stems of *V. quinquangularis* were extracted with ethanol at room temperature to yield a crude extract, which was then partitioned between ethyl acetate and water. The ethyl acetate extract was separated by silica gel column chromatography (CC), followed by preparative HPLC to afford compounds **1**–**4** (Figure 1).

Quinquangularol (**1**) was obtained as colorless amorphous powder, which was determined to have a molecular formula of $C_{43}H_{38}O_{11}$ from its HR-ESI-MS, which corresponded to a methylated resveratrol trimer, since the typical formula of resveratrol trimers is $C_{42}H_{32}O_9$. The ^{1}H-NMR and ^{1}H,^{1}H-COSY spectra of **1** showed the presence of three sets of *ortho*-coupled aromatic H-atoms assignable to three 4-hydroxyphenyl groups, three sets of 3,5-dihydroxyphenyl groups, an olefinic H-atom, two sets of mutually coupled methine H-atoms, and a hydroxymethyl group.

A comparison between the NMR data of **1** and amurensin A (**2**), previously isolated from *V. amurensis* [12], revealed that **1** contained **2** as partial structure (resveratrol units A and B). Compound **1** showed signals (Table 1, signals from 1c to 14c) corresponding to an additional resveratrol unit C, which was confirmed by HMBC data (Figure 2). The HMBCs H-8c/C-4b, and C-10c(14c) and H-7c/C-2c(6c), C-9c, and C-15c indicated that the resveratrol unit C(C-8c) formed an ether bond with the aromatic ring B1(C-4b), while the 4-hydroxyphenyl group (ring C1) and the hydroxymethyl group was attached at C-7c. Thus the structure of **1** was determined as shown in Figure 1. Its ^{1}H and ^{13}C-NMR data were assigned in Table 1.

In order to clarify the stereochemistry of **1**, a NOESY (Figure 2) experiment was carried out. The NOEs between H-8c and H-2c(6c), H-7c and H-10c(14c) illustrated that H-8c and H-7c should be *trans* oriented. A comparison of NMR data between molecules **1** and **2** [12] suggested that H-8a and H-7a has a *trans* orientation, which was identical with **2**. Thus the stereochemistry was as shown in Figure 1, and the metabolite was named as quinquangularol.

Based on previous investigation [7], we propose that **1** was probably generated by oxidative coupling between **2** and resveratrol, followed by a methylation step. A proposed biogenetic pathway was shown in Figure 3.

Table 1. ^{1}H and ^{13}C-NMR Data of quinquangularol (1) in MeOD [a].

Position	δc	δ_H(mult., *J* Hz)	Position	δc	δ_H(mult., *J* Hz)
1a	135.6		8b	142.4	
2a(6a)	129.5	6.98 (2H, *d*, 8.5)	9b	144.8	
3a(5a)	115.6	6.52 (2H, *d*, 8.5)	10b(14b)	109.0	5.95 (2H, *d*, 1.7)
4a	157.4		11b(13b)	159.4	
7a	75.9	4.97 (1H, *d*, 8.7)	12b	102.2	6.09 (1H, *d*, 1.6)
8a	64.7	3.62 (1H, *d*, 8.7)	1c	129.7	
9a	143.9		2c(6c)	130.2	6.89 (2H, *d*, 8.5)
10a(14a)	109.2	5.89 (2H, *d*, 1.8)	3c(5c)	115.8	6.57 (2H, *d*, 8.5)
11a(13a)	158.5		4c	158.3	
12a	101.5	5.87 [b]	7c	88.4	4.30 (1H, *d*, 7.0)
1b	131.6		8c	84.9	4.91 (1H, *d*, 7.0)
2b(6b)	131.2	6.80 (1H, *d*, 8.8)	9c	141.8	
3b(5b)	116.3	6.55 (1H, *d*, 8.8)	10c(14c)	107.3	5.99 (2H, *d*, 2.1)
4b	158.1		11c(13c)	159.2	
7b	127.9	6.63 (1H, *s*)	12c	102.9	5.97 (1H, *d*, 1.9)
			15c	57.2	3.18 (3H, *s*)

[a] ^{1}H-NMR spectra were measured at 500 MHz, and ^{13}C-NMR spectra were run at 125 MHz; [b] Overlapping (in the same column).

Compounds **2–4** were determined as amurensin A [12], vitisin A [13], and resveratrol [14], respectively, according to the spectroscopic data in the literature.

3.2. Antioxidant Activity

Antioxidant activity of **1** was determined by the DPPH assay as described [9]. Compound **1** showed potent scavenging activity against DPPH radical with IC_{50} = 48 μM, which was comparable with resveratrol (IC_{50} = 38 μM).

Acknowledgments

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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