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EXPERIMENTAL RESEARCH

Two new phenylpropanoids from the leaves of *Rauvolfia vomitoria*

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[Abstract] **Objective** Study on the non-alkaloid chemical constituents with novel structure and their biological activities from the *n*-butanol fraction of the leaves of *Rauvolfia vomitoria*. **Methods** The *n*-butanol fraction of *R. vomitoria* was separated by silica gel, ODS, and Sephadex LH-20 chromatography column, as well as semi-preparative HPLC to obtain five compounds. Their structures were identified by extensive spectroscopic analysis, including HRESIMS, 1D and 2D NMR, and ECD analysis. All the isolated compounds were evaluated their AChE inhibitory activities by Ellman's method with slight modification, their vasorelaxant activities against phenylephrine-induced contraction of rat mesenteric arteries, and their inhibitory activity of α-glucosidase employing pNPG as substrate. **Results** Two new phenylpropanoids (**1-2**) along with three known compounds, methyl *trans*-3,4,5-trimethoxycinnamate (**3**), methyl *cis*-3,4,5-trimethoxycinnamate (**4**), and 3,4,5-trimethoxybenzoic acid methyl ester (**5**), were isolated from the leaves of *R.vomitoria*. The five isolated compounds did not show significant tested activity. **Conclusion** Chemical investigation of the leaves of *R. vomitoria* led to the isolation and identification of two new phenylpropanoids (**1-2**), expanding the study of non-alkaloids in the genus of *Rauvolfia* and enriching the chemical diversity of this genus.

[Key words] *Rauvolfia vomitoria*; Phenylpropanoids; Vasorelaxant activity; Anti-AChE activity; α-glucosidase inhibitory activity

1 Introduction

Rauwolfia vomitoria Afzel (Apocynaceae) is native to tropical Africa and widely cultured in Yunnan, Guangxi, and Guangdong province, China[1]. This plant has been used to treat a variety of diseases such as hypertension, high fever, epilepsy, pain, and gastrointestinal diseases for a long time[1-3]. Previous phytochemistry investigation of *R. vomitoria* mainly focused on the chloroform fraction and led to isolation of 22 monoterpene indole alkaloids (MIAs)[2-7]. However, the chemical constituents of the *n*-butanol fraction of this plant have been rarely reported. To further explore the bioactive chemical components of this plant, a systematically chemical examination of the *n*-butanol fraction has been performed in our previous studies leading to the isolation of structurally diverse MIAs with anti-inflammatory activity, anti-acetylcholinesterase (anti-AChE)

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activity, and antitumor activity[8-11]. However, the non-MIAs composition of the *n*-butanol fraction in *R. vomitoria* has not been studied.

As a part of our continuing research for structurally unique and biologically active compounds especially non-MIAs from the *n*-butanol fraction of the leaves of *R. vomitoria*, two new phenylpropanoids **1**-**2**, and three known compounds methyl *trans*-3,4,5-trimethoxycinnamate (**3**)[12], methyl *cis*-3,4,5-trimethoxycinnamate (**4**)[12], and 3,4,5-trimethoxybenzoic acid methyl ester (**5**)[13] were obtained. The structures of two new compounds were elucidated by comprehensive spectroscopic methods, including HRESIMS, 1D and 2D NMR, and electronic circular dichroism (ECD) analysis. Interestingly, the phenylpropanoids were rarely reported from the genus of *Rauvolfia*, which expands the chemical diversity of this genus[14]. All isolated compounds were evaluated for their vasorelaxant activities against phenylephrine-induced contraction of rat mesenteric arteries, anti-AChE activities, and α-glucosidase inhibitory activities. Herein, the isolation, structure elucidation, as well as anti-AChE, vasorelaxant, and anti-α-glucosidase activities of all isolated compounds were reported.

2 Experimental section

2.1 General experimental procedures

UV spectra were carried out on an Agilent Cary 60 instrument. ECD spectra were obtained in methanol on a JASCO J-815 CD spectrometer. FT-IR spectra were obtained on a Bruker VERTEX70 instrument. Optical rotations were measured in MeOH on an InsMark FD polarimeter. HRESIMS spectra were recorded on a Waters I-Class Vion IMS QTOF spectrometer. NMR spectra were carried out on a Bruker Avance III HD-600 spectrometer. HPLC experiments were carried out on YMC Triart C_{18} Prep column (5 μm, 10 × 250 mm). Column chromatography (CC) was carried out with silica gel (100-200 mesh, 200-300 mesh), Sephadex LH-20 (40-70 μm), and ODS (20-45 μm).

2.2 Plant material

The leaves of *R. vomitoria* were gathered from Xishuangbanna, Yunnan Province, China in October 2017. The plant was identified by Dr. Tao Zhou (Xi'an Jiaotong University, Xi'an, China). A voucher specimen (No. 20171024) has been deposited at this institute.

2.3 Extraction and isolation

The dry leaves of *R. vomitoria* (18 kg) were extracted with 80% MeOH under reflux conditions. Then the chloroform extract and *n*-butanol extract at pH 9 were obtained via the method of pH gradient extraction as previously described[8]. The *n*-butanol fraction (158 g) was separated on a silica gel CC with the eluents of CH_2Cl_2/MeOH (a gradient from 12 : 1 to 1 : 1) to obtain eight fractions (Frs. A-H). Fr. C was performed on an ODS C_{18} with MeOH/H_2O gradient elution (from 30 : 70 to 70 : 30) to obtain Frs. C1-C3. Purification of Fr. C2 by silica gel CC and RP HPLC with MeCN/H_2O (25 : 75) to yield compounds **1** (4.8 mg, t_R 34.5 min) and **2** (5.1 mg, t_R 36.5 min). The subfraction Fr. C3 was separated further by silica gel CC and Sephadex LH-20 (MeOH) to give Fr. C3a. Compounds **5** (20.2 mg, t_R 29.3 min), **3** (19.8 mg, t_R 33.4 min), and **4** (17.1 mg, t_R 35.3 min) were obtained by RP HLPC eluting with MeOH/H_2O (60 : 40) from Fr. C3a.

(–)-(7*S*,8*R*)-4-hydroxy-3,3′-dimethoxy-8′,9′-dinor-8,4′-oxyneoligna-7,9-diol-7′-oate (**1**): colorless oil; $[\alpha]_D^{20}$ – 10 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.48), 217 (4.20), 264 (3.90), 287 (3.76), 301 (3.66) nm; CD (MeOH) 223 ($\Delta\varepsilon$ – 1.24), 238 ($\Delta\varepsilon$ – 0.72) nm; IR (KBr) ν_{max}:3 381, 2 939, 2 849, 1 703, 1 599, 1 509, 1 434, 1 269, 1 217, 1 027, 764 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 401.120 14 $[M + Na]^+$ (calcd. for $C_{19}H_{23}O_8Na^+$, 401.121 24).

(–)-(7*R*,8*R*)-4-hydroxy-3,3′-dimethoxy-8′,9′-dinor-8,4′-oxyneoligna-7,9-diol-7′-oate (**2**): colorless

Table 1 The ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of 1 and 2 in CD_3OD

Position	1		2	
	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C
1		134.0		133.9
2	7.04, d (1.4)	112.2	7.05, d (1.7)	111.8
3		148.8		149.0
4		147.3		147.4
5	6.70, d (7.8)	115.7	6.76, d (8.1)	116.0
6	6.85, dd (7.8, 1.4)	121.3	6.87, dd (8.1, 1.7)	120.8
7	4.82, d (5.3)	74.2	4.91, d (6.1)	74.0
8	4.58, ddd (5.3, 5.3, 5.3)	85.3	4.56, ddd (6.1, 5.8, 4.0)	86.0
9a	3.85, overlap	62.6	3.56, dd (12.0, 5.8)	62.2
9b			3.78, dd (12.0, 4.0)	
1′		124.3		124.4
2′	7.50, d (1.7)	114.2	7.59, d (2.0)	114.2
3′		151.2		151.1
4′		154.1		154.3
5′	7.03, d (8.3)	116.3	7.11, d (9.0)	116.3
6′	7.53, dd (8.3, 1.7)	124.6	7.59, dd (9.0, 2.0)	124.7
7′		168.5		168.5
3-OCH_3	3.80, s	56.5	3.83, s	56.5
3′-OCH_3	3.82, s	56.7	3.92, s	56.7
7′-OCH_3	3.85, s	52.6	3.88, s	52.6

oil; $[\alpha]_D^{20}$– 45 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.57), 218 (4.29), 264 (4.00), 287 (3.87), 300 (3.75) nm; CD (MeOH) 228 ($\Delta\varepsilon$ + 0.75), 236 ($\Delta\varepsilon$ – 0.72) nm; IR (KBr) ν_{max}:3 380, 2 945, 2 837, 1 703, 1 597, 1 509, 1 434, 1 269, 1 216, 1 023, 763 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 401.120 20 $[M + Na]^+$ (calcd. for $C_{19}H_{23}O_8Na^+$, 401.121 24).

2.4 AChE inhibitory activity evaluation

The AChE inhibitory activities of all isolated compounds (**1-5**) were evaluated by Ellman's method with slight modification as previously described[15]. All the determinations were performed in triplicate and at least in three independent runs. Galantamine was used as a positive control in this assay.

2.5 Vasorelaxant activity assay

The vasorelaxant activities of all isolated compounds (**1-5**) against phenylephrine-induced contraction of rat mesenteric arteries were measured as described previously[16]. All the determinations were performed in triplicate and at least in three independent runs. Phentolamine mesylate was used as a positive control in this assay.

2.6 α-Glucosidase inhibitory assay

The inhibitory activity of α-glucosidase was assessed through a slight modification of a previously reported method employing pNPG as substrate[17]. All experiments were performed in triplicate and acarbose was used as a positive control.

3 Results and discussion

Compound **1** was obtained as a colorless oil and possessed the molecular formula $C_{19}H_{22}O_8$ indicated by positive HRESIMS ion peaks at *m/z* 401.120 14 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_8Na^+$, 401.121 24). The IR spectrum of 1 exhibited characteristic absorption bands for hydroxy (3 381 cm^{-1}) and carbonyl (1 703 cm^{-1}) functionalities. The ^{1}H NMR spectrum of **1** (Table 1) exhibited the

presence of two 1,3,4-trisubstituted benzene ring systems [one ABX spin system at δ_H 7.04 (1H, d, J =1.4 Hz, H-2), 6.70 (1H, d, J = 7.8 Hz, H-5), and 6.85 (1H, dd, J = 7.8, 1.4 Hz, H-6); the other ABX spin system at δ_H 7.50 (1H, d, J = 1.7 Hz, H-2′), 7.03 (1H, d, J = 8.3 Hz, H-5′), 7.53 (1H, dd, J = 8.3, 1.7 Hz, H-6′)] and three methoxy groups [δ_H 3.85 (3H, s), 3.82 (3H, s), and 3.80 (3H, s)]. Besides the two 1,3,4-trisubstituted benzene ring signals (twelve carbons), the ^{13}C NMR, DEPT, and HSQC spectra of **1** showed seven extra carbon resonances ascribed to a carbonyl (δ_C 168.5), an oxygenated methylene group (δ_C 62.6), two oxygenated methine groups (δ_C 85.3 and 74.2), and three methoxy groups (δ_C 56.7, 56.5, and 52.6). The ^{1}H–^{1}H COSY cross-peaks of H-7/H-8/H-9 combined with the HMBC correlations from H-7 to C-1, C-2, and C-6, and from H-8 to C-4′ demonstrated the existence of a guaiacylglycerol group[18]. The HMBC correlations from 7′-OCH_3 to C-7′ and from H-2′/H-6′ to C-7′ supported the methoxycarbonyl group was attached at C-1′. The HMBC correlations from H-2′ to C-1′/C-3′/C-4′/C-6′ along with the NOESY correlation from H-2′ (δ_H 7.50, d, J = 1.7 Hz) to 3′-OCH_3 (δ_H 3.82, s) led to the establishment of methoxy group adjacent to C-3′. Meanwhile, the HMBC correlations from 3-OCH_3 (δ_H 3.80, s) to C-3 (δ_C 148.8) and the NOESY correlation from H-2 (δ_H 7.04, d, J = 1.4 Hz) to 3-OCH_3 (δ_H 3.80, s) suggested that this methoxyl group was connected to C-3. Hence, the planar structure of **1** was elucidated as shown in Fig. 1 and confirmed by the detailed 2D NMR analysis (Fig. 2).

The molecular formula of compound **2** was

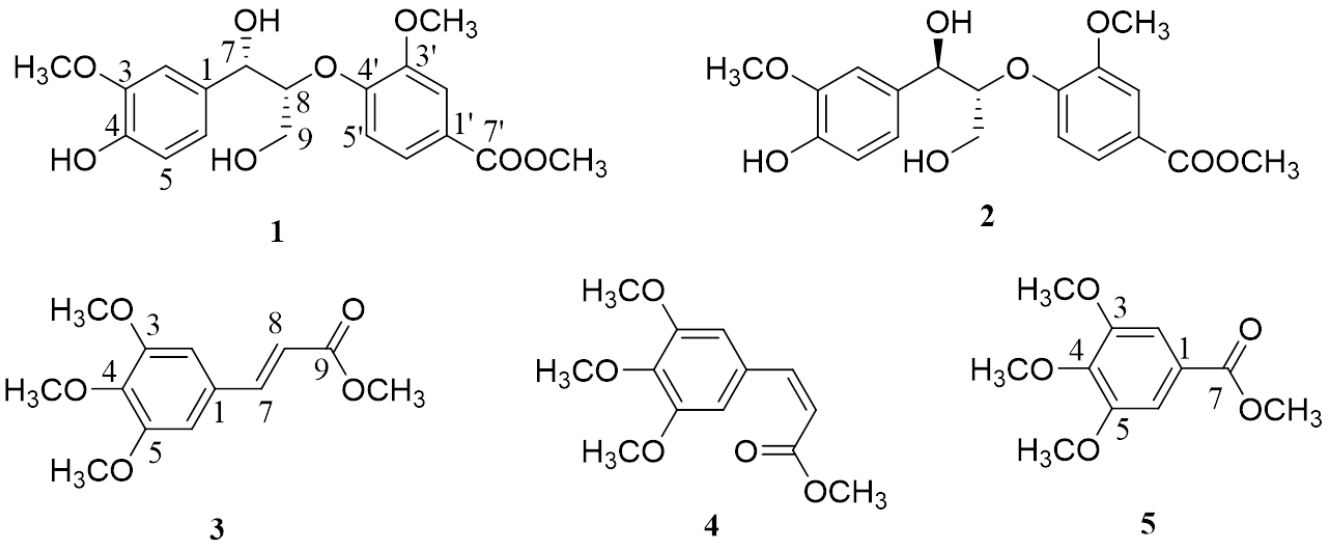

Fig. 1 Structures of the isolated compounds 1-5.

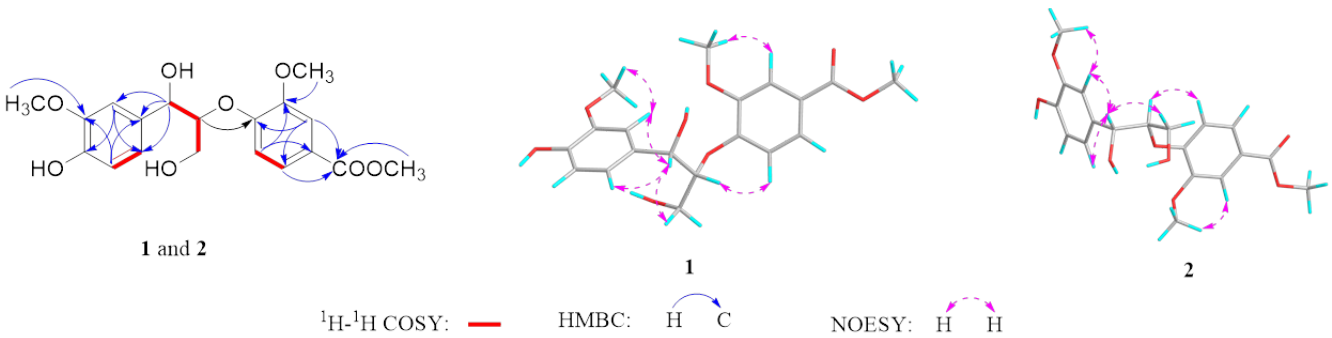

Fig. 2 ^{1}H–^{1}H COSY, key HMBC, and NOESY correlations of 1 and 2.

identified to be the same as that of **1** by positive HRESIMS ion peaks at *m/z* 401.120 20 $[M + Na]^+$ (calcd. for $C_{19}H_{22}O_8Na^+$, 401.121 24). The ^1H and ^{13}C NMR data of **2** were extremely similar to those of **1** with a very minor difference (Table 1) indicating compounds **1** and **2** shared the same planar structure (Fig. 1).

Previous studies had confirmed that the coupling constant value of $J_{7,8}$ and the $\Delta\delta_{C8-C7}$ value for the *erythro* guaiacylglycerol derivatives were smaller than those for *threo* isomer when the data were obtained in the same solvent[19-20]. Therefore, the *erythro* configuration of **1** and *threo* configuration of **2** was determined based on the smaller coupling constant of $J_{7,8}$=5.3 Hz and $\Delta\delta_{C8-C7}$ value (11.1 ppm) in **1** than those in **2** ($J_{7,8}$=6.1 Hz and $\Delta\delta_{C8-C7}$=12.0 ppm).

The absolute configurations of **1** and **2** were established based on their ECD spectra and optical rotation values. The negative ECD Cotton effects at 238 nm (Fig. 3) in compounds **1** and **2** indicated the 8*R* configuration for both **1** and **2**[19-24]. Therefore, the absolute configurations of compounds **1** and **2** can be defined as 7*S*,8*R* and 7*R*,8*R*, respectively. In addition, the negative optical rotation values of **1** ($[\alpha]_D^{20}$− 10) and **2** ($[\alpha]_D^{20}$− 45) were matched well with those of the reported similar compounds[18,20]. Therefore, compound **1** was determined to be (–)-(7*S*,8*R*)-4-hydroxy-3,3′-dimethoxy-8′,9′-dinor-8,4′-oxyneoligna-7,9-diol-7′-oate and compound **2** can be named as (–)-(7*R*,8*R*)-4-hydroxy-3,3′-dimethoxy-8′,9′-dinor-8,4′-oxyneoligna-7,9-diol-7′-oate.

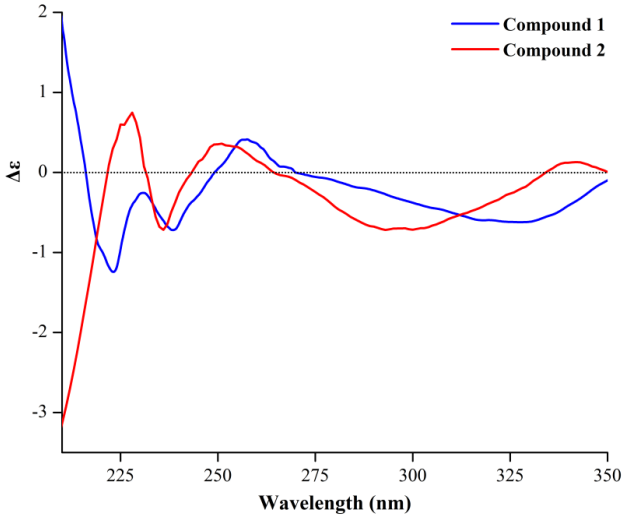

Fig. 3 ECD spectra of compounds 1 and 2.

Our previous study indicated that the *n*-butanol fraction of the extracts of *R. vomitoria* showed potential anti-AChE activity with an IC_{50} value of 141.97 ± 12.27 μg/mL leading to the isolation of some monoterpene indole alkaloids with anti-AChE activity[9], so all isolated compounds were evaluated for their anti-AChE activities. Unfortunately, none of them exhibited inhibition rates above 50% at the concentration of 200 μM, which may suggest that the anti-AChE activity of the extract is mainly caused by the alkaloids rather than non-alkaloids. All isolated compounds were also evaluated for their *α*-glucosidase inhibitory activity and vasorelaxant activity, since some neolignans have exhibited potent *α*-glucosidase inhibitory activity[25] and the extract of *R. vomitoria* was used to treat hypertension[5]. However, none of them showed obvious *α*-glucosidase inhibitory activity and vasorelaxant activity at a concentration of 400 and 200 μM, respectively.

4 Conclusion

In conclusion, chemical investigation of the leaves of *R. vomitoria* led to the isolation and identification of two new phenylpropanoids (**1-2**) and three known compounds (**3-5**). The structures of the isolates were determined based on spectroscopic interpretations, including HRESIMS, 1D and 2D NMR spectroscopic data, and ECD. Significantly, non-alkaloids were rarely reported in the genus of *Rauvolfia*, and our study expanded the chemical diversity of this genus. All isolated compounds were evaluated for their vasorelaxant activities and the inhibitory activities of AChE and *α*-glucosidase, unfortunately, none of them exhibited obvious bioactivities.

5 Conflicts of interest

These authors have no conflict of interest to declare.

6 Acknowledgments

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