

石岩枫中两个新的木脂素类化合物

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摘要: 从石岩枫 *Mallotus repandus* (willd) muell. -arg. 全草中分离得到两个新的木脂素类化合物, 根据理化性质和波谱鉴定其化学结构, 分别为: repandusin (**1**)、repanduthylin (**2**)。化合物 **1** 对 H₂O₂ 诱导的 PC12 细胞损伤有一定的保护作用, 化合物 **2** 对 H₂O₂ 诱导的 PC12 细胞损伤有微弱的保护作用。

关键词: 石岩枫; 木脂素类; H₂O₂ 诱导; PC12 细胞

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Two New Coumarinolignoids from *Mallotus repandus* (Willd) Muell. Arg.

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Abstract: Two new coumarinolignoids were isolated from the Rhizome of *Mallotus repandus* (willd) Muell. -Arg. Silica gel and Sephadex LH-20 column chromatographies were used to isolate the chemical constituents, and spectroscopic techniques were employed to elucidate their structures. Their structures were identified as repandusin (**1**) and repanduthylin (**2**). Compound **1** and **2** had medium and feeble protective effects to scathing PC12 cells which was induced by H₂O₂ respectively.

Key words: *Mallotus repandus*; coumarinolignoids; H₂O₂-induction; PC12 cells

Introduction

Mallotus repandus (Willd) Muell. Arg. (Euphorbiaceae) is a Chinese ethnic drug native to the south of Guangdong Province, Guizhou Province and Taiwan. It is widely used as a traditional medicine for rheumatic arthrositis, snake-bite, hepatitis and liver cirrhosis^[1]. Two diterpene lactones, mallorepine and bergenin have been isolated from this plant previously^[2-4]. In this study, we investigated the constituents of chloroform extract of *M. repandus*, and two new coumarinolignoids were isolated.

Materials and Methods

Instruments

IR spectra was obtained using a Vector 22 (KBr)

spectrometer. ¹H NMR (400 MHz), ¹³C NMR (100 MHz), and 2D-NMR spectra were recorded with an INOVA-400 spectrometer using TMS as internal standard and values were given in ppm. Column chromatography was performed on silica gel (Qingdao Haiyang Chemical Co. Ltd., China), Sephadex LH-20 (Sigma-Aldrich, America).

Plant materials

M. repandus was collected from Luodian county of Guizhou, China, in Oct 2007. It was identified by Prof. WEI Sheng-hua (Guiyang College of Traditional Chinese Medicine, Guiyang 550002, China).

Extraction and isolation

Air-dried powder of *M. repandus* 9.0 kg was extracted by 82% ethanol for three times and 60% ethanol for once. The extracts were combined and concentrated under vacuum. The residue obtained was suspended in water, extracted respectively with petroleum ether, chloroform and ethyl acetate to yield the petroleum ether extract, chloroform extract and ethyl acetate ex-

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tract. The chloroform layer (33 g) was concentrated and then subjected to silica gel column chromatography by eluting with petrol / EtOAc (80:1-2:1) and EtOAc / CH₃OH (20:1-1:1). Ten fractions were obtained, and fraction 8 (2 g) were purified over silica gel column and Sephadex LH-20 to obtain compound **1** (38 mg); Fractions 9 (4 g) were purified over silica gel column and recrystallization to afford compound **2** (49 mg).

Bioassay

PC12 cells were preactioned with certain concentration of samples for 24 hours, and the culture of the samples were discarded. Then join incubation media with H₂O₂ for some time to injury PC12 cells, and join H₂O₂ enzyme to terminate reaction and culture for 24 hours, test cells surviving and growing by MTT method. Compounds **1** and **2** were analyzed with three concentrations in a range between 2 μg/mL and 200 μg/mL, respectively by MTT method. Compound **1** showed medium protective effects to scathing PC12 cells which was induced by H₂O₂, and compound **2** showed feeble protective effects to that cells (Table 1).

Table 1 Protective effects of compound 1 and 2 against injury PC12 cells induced by H₂O₂

Sample	Ultimate concentration (μg/mL)	Restraining rate to normal PC12 cells (%)	Restraining rate to PC12 cells injured by H ₂ O ₂ (%)
Compound 1	200	7.11	34.18 ± 9.41
	20	0	43.40 ± 1.26
	2	0	9.880 ± 3.15
Compound 2	200	12.05	13.22 ± 9.18
	20	10.24	27.84 ± 9.51
	2	3.73	6.995 ± 10.8
α-Tocopherol	4.3	0	22.91 ± 2.45

Note: 1. α-Tocopherol is positive control; 2. the diffuence of compounds will influence the result.

Results and Discussion

Structural elucidation

Compound 1 was obtained as buff powder, found to be possess a molecular formula of C₂₁H₂₀O₉, as evidenced by positive mode HR-FAB-MS (*m/z* 417.1209, [M + H]⁺, calc 417.1186). The IR spectra of compound **1**

showed absorption bands for hydroxyl (3525 cm⁻¹), carbonyl group (1699 cm⁻¹). The ¹H NMR, ¹³C NMR and DEPT spectra displayed signals for 21 carbons, three methyl groups (δ_c 55.9, 56.2, 56.4), all of them were linked to oxygen; one methylene (δ_c 60.8); seven methines (δ_c 113.9, 144.6, 101.1, 116.4, 112.3, 77.6, 80.0); ten quaternary carbons (δ_c 160.9, 106.4, 133.1, 139.4, 112.0, 106.4, 149.4, 148.8, 127.6, 146.5); δ_c 160.9 were matched closely to the carbonyl group of IR spectra. In the ¹H NMR, the coupling constant of δ_H 6.45 and 7.75 was 9.6 Hz, which is representative coumarin that H-3 and H-4 have no substituent; H-5 is double apices, so H-6 has no substituent. In the ¹³C NMR, the chemical shift of δ_c 77.6 and 80.0 suggested their linking to an oxygen atom respectively, δ_c 160.9 confirm that compound **1** has a carbonyl group^[5].

Detailed analysis of the 2D-NMR data, including ¹H-¹H COSY, HMQC and HMBC, confirmed the above deduction. In the ¹H-¹H COSY, H-7' and H-8', H-3 and H-4, H-5 and H-6 were connected. In the HMBC spectrum, the C-2, C-5 and C-10 showed correlations to H-4, meanwhile, the C-7' and C-8' showed correlations to H-9'. The detailed ¹H NMR, ¹³C NMR and 2D-NMR data were shown in Table 2. The key ¹H-¹H COSY and HMBC spectra were expressed in Fig. 1.

Compound **2**, a white solid, had the molecular formula C₂₁H₂₄O₈ based on the positive-mode HR-FAB-MS (*m/z* 404.1477, [M + H]⁺, calc 405.1533). The IR spectrum of compound **2** showed absorption bands for hydroxyl (3490 cm⁻¹), 1469, 1090 cm⁻¹ and 750 cm⁻¹ indicated the presence of benzene ring, but there is no carbonyl group comparing to compound **1**. The ¹H NMR, ¹³C NMR and DEPT spectrum displayed signals for 21 carbons, three methyl groups (δ_c 49.7, 56.3, 56.4), all of them were linked to oxygen; three methylenes (δ_c 61.4, 62.9, 62.9); seven methines (δ_c 129.6, 100.7, 145.4, 105.6, 105.6, 77.4, 79.9); eight quaternary carbons δ_c 130.4, 130.0, 134.0, 108.6, 149.6, 138.2, 149.6); In the ¹H NMR, two methylenes in 6.82 and 6.94, which is representative coumarin that H-3 and H-4 changed to unsaturated bonds and had no substituent. In the ¹H-¹H COSY

spectrum, the link between H-7'/H-8', H-8'/H-9', H-3/H-4 and H-2/H-3 were observed clearly. In the HM-BC spectrum, the C-5, C-9 and C-10 showed correlations to H-4; the C-1' and C-2' showed correlations to H-7'. Meanwhile, C-7' and C-8' showed correlations to H-9'. The detailed data of compound **2** were shown in Table 3, and the key correlations of ¹H-¹H COSY and HMBC spectrum were indicated in Fig. 1^[6]. Repandusin (**1**) and repanduthylin (**2**) were separated from *M. Repandus* for the first time, and were related to coumarin. According to Naming Organic Compounds of

IUPAC, compound **1** relating to coumarinic anhydride and hence was named as repandusin and compound **2** was similar to lignan and hence was named as repanduthylin.

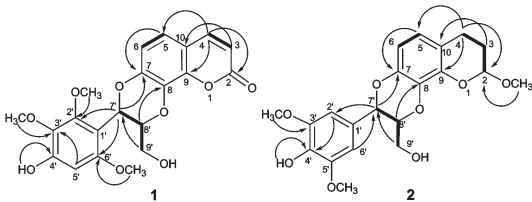


Fig. 1 Key HMBC (arrow, \curvearrowright) and ¹H-¹H COSY (bold line, —) correlations of compounds 1 and 2

Table 2 The ¹H NMR (400 MHz), ¹³C NMR (100 MHz) and 2D-NMR data of compound 1 (δ in ppm, J in Hz)

No.	δ _H	δ _C	HMBC	¹ H- ¹ H COSY
2		160.9		
3	6.45 (1H, d, 9.6)	113.9	C-2, C-10	H-4
4	7.75 (1H, d, 9.6)	144.6	C-2, C-5, C-8, C-9, C-10	H-3
5	6.74 (1H, d, 6.0)	101.1	C-3, C-4, C-10	H-6
6	7.28 (1H, d, 8.0)	116.4		H-5
7		106.4		
8		133.1		
9		139.5		
10		112.0		
1'		106.4		
2'		149.4		
3'		148.8		
4'		127.7		
5'	7.41 (1H, s)	112.3	C-1', C-3', C-4', C-10'	
6'		146.5		
7'	5.58 (1H, m, 8.0)	77.7	C-1', C-6'	H-8'
8'	4.50 (1H, m, 6.8)	80.0	C-7'	H-7'
9'	4.30 (1H, t), 3.93 (1H, t)	60.8	C-7', C-8'	
2'-CH ₃	3.59 (3H, s)	56.2	C-3'	
3'-CH ₃	3.69 (3H, s)	55.9	C-2'	
6'-CH ₃	3.76 (3H, s)	50.0		

Table 3 The ¹H NMR (400 MHz), ¹³C NMR (100 MHz) and 2D-NMR data of compound 2 (δ in ppm, J in Hz)

No.	δ _H	δ _C	HMBC	¹ H- ¹ H COSY
2	4.60 (1H, d, 4.8)	129.6	C-2	H-3
3	6.82 (2H, m, 11.6)	62.9	C-2, C-3	H-2, H-4
4	6.94 (2H, t, 9.6)	62.9	C-2, C-4, C-5, C-9, C-10	H-3
5	6.92 (1H, d, 6.4)	100.8	C-3, C-5, C-9, C-10	

6	7.09 (1H,d,1.6 Hz)	145.4	C-5, C-6, C-8, C-9	
7		130.4		
8		130.1		
9		134.0		
10		108.6		
2-CH ₃	3.62 (3H,s)	49.7		
3'-CH ₃	3.86 (3H,s)	56.4	C-3'	
5'-CH ₃	3.82 (3H,s)	56.4	C-5'	
1'		127.5		
2'	7.16 (1H,s)	105.6	C-1', C-3', C-4', C-2', C-7'	
3'		149.6		
4'		138.2		
5'		149.6		
6'	7.16 (1H,s)	105.6	C-1', C-3', C-4', C-6', C-7'	
7'	5.50 (1H,d,8.0)	77.4	C-1', C-2', C-6', C-8'	H-8'
8'	4.36 (1H,d,8.0)	79.9	C-7'	H-7', H-9'
9'	3.94 (1H,t), 3.91 (1H,t)	61.4	C-7', C-8'	H-8'

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