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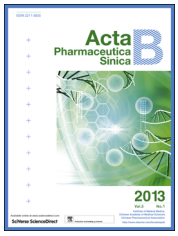

ORIGINAL ARTICLE

A new megastigmane from fresh roots of *Rehmannia glutinosa*

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KEY WORDS

Scrophulariaceae;
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Abstract A new megastigmane, rehmamegastigmane (**1**), together with eighteen known compounds lariciresinol (**2**), lariciresinol-4′-*O*-*β*-D-glucopyranoside (**3**), hierochin D (**4**), yemuoside YM1 (**5**), darendoside B (**6**), decaffeoylacteoside (**7**), jionoside B_1 (**8**), catalpol (**9**), ajugol (**10**), 6-*O*-vanilloylajugol (**11**), 6-*O*-*E*-feruloylajugol (**12**), rehmapicroside (**13**), rehmapicrogenin (**14**), 3-methoxy-2,6,6-trimethyl-cyclohexane-1-enecarboxylic acid (**15**), vanillic acid (**16**), hydroferulic acid (**17**), *threo*-1-(4-hydroxy-3-methoxyphenyl)-1,2,3-propanetriol (**18**), *p*-hydroxyphenylethyl alcohol (**19**) was isolated from the fresh roots of *Rehmannia glutinosa*. Compounds **2**–**6** and **16**–**18** were isolated from this plant for the first time.

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1. Introduction

The roots of *Rehmannia glutinosa* (Rehmanniae Radix) were originally recorded in *Shen Nong Ben Cao Jing* and classified as the upper class. The fresh roots can promote salivation, cool the blood, and stop bleeding[1]. The previous phytochemical studies on the *R. glutinosa* showed that iridoids, ionone glucosides, phenylethanoid glycosides, norcarotenoids, cerebrosides, as well as carbohydrates were the major components of this plant[2]. As a result of the continuous study on the chemical constituents of this plant, a new ionone together with eighteen known compounds was isolated from the 70% aqueous acetone extract of the plant, and their structures were determined to be rehmamegastigmane (**1**), lariciresinol (**2**)[3], lariciresinol-4′-*O*-*β*-D-glucopyranoside (**3**)[4], hierochin D (**4**)[5], yemuoside YM1 (**5**)[5], darendoside B (**6**)[6], decaffeoylacteoside (**7**)[7], jionoside B_1 (**8**)[8], catalpol (**9**)[9], ajugol (**10**)[8], 6-*O*-vanilloylajugol (**11**)[10], 6-*O*-*E*-feruloylajugol (**12**)[11], rehmapicroside (**13**)[12], rehmapicrogenin (**14**)[13], 3-methoxy-2,6,6-trimethylcyclohexane-1-enecarboxylic acid (**15**)[12], vanillic acid (**16**)[14], hydroferulic acid (**17**)[15], *threo*-1-(4-hydroxy-3-methoxyphenyl)-1,2,3-propanetriol (**18**)[16], *p*-hydroxyphenylethyl alcohol (**19**)[17]. This paper described their isolation and structural elucidation.

2. Results and discussion

Compound **1** was obtained as a colorless needles crystal (CH_3OH) with an optical rotation $[\alpha]_D^{25} = -25.4$ ($c = 0.19$, CH_3OH). Its molecular formula was determined to be $C_{15}H_{26}O_4$ on the basis of HR-ESI-MS at *m/z* 293.1723 $[M+Na]^+$. The IR spectrum of **1** showed absorption bands at 3425 and 1626 cm^{-1} which were ascribable to hydroxyl and double bond functions, respectively. The ^1H NMR spectrum (Table 1) showed that there were signals of four methyl groups [δ_H 1.84 (3H, s), 1.08 (3H, s), 1.03 (3H, s), and 0.87 (3H, s)], two *trans* olefinic proton groups [δ_H 6.33 (1H, d, $J = 16.1$ Hz), 6.22 (1H, d, $J = 16.1$ Hz)], an olefinic proton at [δ_H 5.63 (1H, t, $J = 13.4$, 6.7 Hz)], as well as one oxygenated methine proton at [δ_H 3.73 (1H, m)]. The ^{13}C NMR spectrum (Table 1), in combination with the HSQC and DEPT spectra exhibited 15 signals, including four methyls (δ_C 12.9, 17.9, 22.8 and 26.9), two oxygenated quaternary carbons (δ_C 75.6, 82.0), an oxygenated methine carbon (δ_C 74.5), an oxygenated methylene carbon (δ_C 59.4) and four olefinic carbons (δ_C 136.8, 135.2, 131.3, 130.4). The ^1H–^1H correlation spectroscopy (^1H–^1H COSY) experiment (Fig. 1) with **1** allowed sequential assignments of the homonuclear coupling correlations for $-CH_2-CH_2-CH(OH)-$ and $-CH=CH-C(CH_3)=CH-CH_2OH$, the latter of which were in a good agreement with those of the side chain, (2*E*,4*E*)-3-methylpenta dienoic ol[18–21]. The NMR spectra (Table 1) were very similar to the known compound of aeginetic acid[18,19] which was reported from *R. glutinosa*, except for the presence of an oxygenated methine (δ_C 74.5/δ_H 3.73) and an oxygenated methylene (δ_C 59.4/δ_H 4.23). This presumption was further corroborated by the HMBC long-range correlations (Fig. 2), correlations from H-11 (δ_H 4.23) to C-9 (δ_C 136.8) and C-10 (δ_C 130.4), from H-2 (δ_H 3.73) to C-1 (δ_C 44.7). Furthermore, the HMBC correlations from H-13 (δ_H 1.08) and H-14 (δ_H 0.87) to C-1 (δ_C 44.7), from H-15 (δ_H 1.03) to C-5 (δ_C 75.6) supported the presence of 2,5,6-trihydroxy-1,1,5-trimethylcyclohexyl fragment (A ring)[22]. In the NOE spectrum of 1 (Fig. 1), significant correlations of H-2 (δ_H 3.73) with H-14 (δ_H 0.87), and H-2 (δ_H 3.73) with H-15 (δ_H 1.03), indicated that these protons were located at *β*-orientated of the A ring. On the other hand, observation of the key NOE correlations of H-7 (δ_H 6.22) with H-13 (δ_H 1.08), revealed that these protons occupied the *α*-face of the A ring. It turned out that compound **1** was a derivative of aeginetic acid[18,19]. Compared to (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-*β*-D-glucopyranoside[23] and (1*S*,3*S*,5*R*,6*R*,9*R*)-3,9,12-trihydroxy-megastigmane-3-*O*-*β*-D-glucopyranoside[24], and based on further comparison of the NMR data with those in the literature, the absolute configuration of **1** at C-2 (δ_C 74.5), C-5 (δ_C 75.6) and C-6 (δ_C 82.0) were determined to be 2*S*,5*R*,6*R*. On the basis of the results described above, the structure of **1** was determined to be (7*E*,9*E*)-7-[(2*S*,5*R*,6*R*)-2,5,6-trihydroxy-1,1,5-trimethylcyclohexane]-9-methylpenta-7,9-dienoic-11-ol, named rehmamegastigmane.

The cytotoxic activities of **1** were evaluated on human tumor cell lines (MCF-7, MG63 and HepG2) *in vitro* using the MTT assay. Compound **1** showed a weak cytotoxic activity with an IC_{50} value of more than 100 μM. Since compound **1** are very rich in the roots of this plant, and compound **14** have the anticoagulant activity at a certain dosage[25], so further investigations of their bioactivities are necessary.

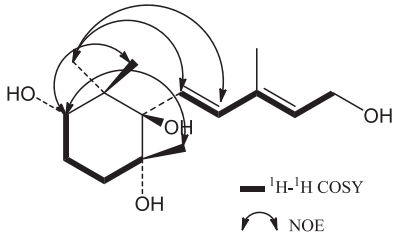

Figure 1 The ^1H–^1H COSY and key NOE correlations of **1**.

Table 1 ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **1** (in CD_3OD).

No.	δ_H	δ_C (DEPT)	No.	δ_H	δ_C (DEPT)
1		44.7 (C)	9		136.8 (C)
2	3.73 (1H, m)	74.5 (CH)	10	5.63 (1H, t, $J = 13.4$, 6.7 Hz)	130.4 (CH)
3	1.97, 1.52 (each 1H, m)	27.7 (CH_2)	11	4.23 (2H, d, $J = 6.7$ Hz)	59.4 (CH_2)
4	1.89, 1.50 (each 1H, m)	35.9 (CH_2)	12	1.84 (3H, s)	12.9 (CH_3)
5		75.6 (C)	13	1.08 (3H, s)	17.9 (CH_3)
6		82.0 (C)	14	0.87 (3H, s)	22.8 (CH_3)
7	6.22 (1H, d, $J = 16.1$ Hz)	131.3 (CH)	15	1.03 (3H, s)	26.9 (CH_3)
8	6.33 (1H, d, $J = 16.1$ Hz)	135.2 (CH)			

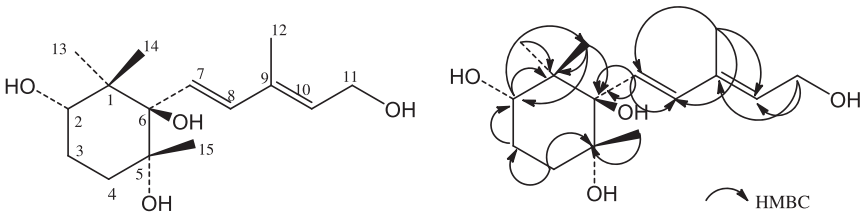

Figure 2 The chemical structure and key HMBC correlations of **1**.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded in acetone-d_6, D_2O or CD_3OD on a Bruker AVANCE III 500 spectrometer with TMS as internal standard. Optical rotation was measured with JASCO P-2000 digital polarimeter (Jasco Analytical Instruments, Easton, USA). IR spectra were recorded in KBr disks on a Shimadzu PC 8201 IR spectrometer. MS spectra were recorded on a APEX II mass spectrometer. HPLC was performed on a Waters Alliance 2695 separations module (Empower software) connected to a Waters 2998 photodiode array (PDA) detector (190–800 nm) equipped with a symmetry® C18 column (250 mm × 4.6 mm, I.D., 5 μm) (Waters, Ireland). Column chromatography was performed on silica gel (160–200 mesh, Qingdao Marine Chemical Co., Qingdao, China), Toyopearl HW-40, MCI gel CHP-20 and Diaion HP-20 (TOSOH Corp., Tokyo, Japan), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden). TLC was conducted on self-made silica gel G (Qingdao Marine Chemical Industry, Qingdao, China). EtOAc:EtOH:H_2O (10:2:1, *v/v*), CH_2Cl_2:MeOH (10:1, *v/v*), CH_2Cl_2:MeOH:H_2O (6:1:0.1, *v/v*) as the eluent. Spots were visualized by spraying with 10% H_2SO_4 in ethanol (*v/v*), followed by heating. The chemical reagents were supplied by Beijing Chemical Works (Beijing, China) and Tianjin 3 Reagent Plant (Tianjin, China).

3.2. Plant material

The fresh roots of *R. glutinosa* Libosch were collected in Wenxian, Henan Province, China, in July, 2011, and identified by Prof. Chengming Dong from Henan University of Traditional Chinese Medicine. A voucher specimen (No. 20110708A) has been deposited in Department of Natural Medicinal Chemistry, School of Pharmacy, Henan University of Traditional Chinese Medicine.

3.3. Extraction and isolation

The fresh roots (17.0 kg) of *R. glutinosa* were cut into small pieces and extracted with 70% aq. acetone three times at room temperature. The extracts were then concentrated in a vacuum evaporator to provide the extract (987 g). It was precipitated at the ethanol concentration of 60%, and the liquid supernatant was concentrated in a vacuum evaporator to yield gross extract (256 g), and then it was dissolved in suitable water, and subjected to Diaion HP-20 porous polymer resin and eluted with H_2O, 10%, 20%, 30%, 40% CH_3OH successively.

The fraction eluted with H_2O was subjected to Toyopearl HW-40 column chromatography eluting with CH_3OH /H_2O (from 10% to 100%) and then isolated and purified by Sephadex LH-20, Toyopearl HW-40, silica gel column chromatography, preparative HPLC and recrystallization to afford compounds **9** (56 mg), **16** (12 mg), **18** (17 mg), **19** (16 mg).

In the same way, compounds **10** (47 mg), **13** (16 mg), **14** (12 mg), **15** (18 mg) were successively obtained from the fraction eluted with 10% CH_3OH; compounds **1** (13 mg), **7** (16 mg), **17** (12 mg) were successively obtained from the fraction eluted with 20% CH_3OH; compounds **2** (12 mg), **3** (8 mg), **4** (17 mg), **5** (16 mg) were successively obtained from the fraction eluted with 30% CH_3OH; compounds **6** (21 mg), **8** (32 mg), **11** (19 mg), **12** (17 mg) were obtained from the fraction eluted with 40% CH_3OH.

3.4. Identification

Rehmamegastigmane (**1**): $C_{15}H_{26}O_4$, colorless needles crystal (CH_3OH). UV_{max} 276 nm, IR (KBr)ν_{max}: 3425, 3013, 2967, 2924, 1626, 1567, 1384, 1374 cm^{-1}; HR-ESI-MS: *m/z* 293.1723 $[M+Na]^+$; ^1H NMR (500 MHz, CD_3OD) spectral data and ^{13}C NMR (125 MHz, CD_3OD) spectral data, see Table 1.

3.5. Cytotoxicity assay

The cytotoxicity assay of compound **1** was evaluated against MCF-7, MG63 and HepG2 cells by MTT assay. All the cells were cultured in RPMI-1640 medium (Hyclone, Logan, UT), and supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and antibiotic (100 U/mL penicillin and 100 μg/mL streptomycin). Cells were plated in 96-well microassay culture plates (1×10^4 cells per well) and grown at 37 °C for 24 h in a 5% CO_2 incubator, and then treated with compound **1** at various concentrations (1, 10, 25, 50, 100 μM). After 24 h of treatment, 20 μL of 5 mg/mL MTT solution was added to each well and further incubated for 4 h. The cells in each well were then solubilized with DMSO (150 μL for each well). The optical density of each well was then measured on a microplate spectrophotometer at a wavelength of 490 nm. The IC_{50} values were determined by plotting the percentage viability *versus* concentration on a logarithmic graph and reading off the concentration at which 50% of cells remain viable relative to the control. Each experiment was repeated three times to get the mean values.

Acknowledgments

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