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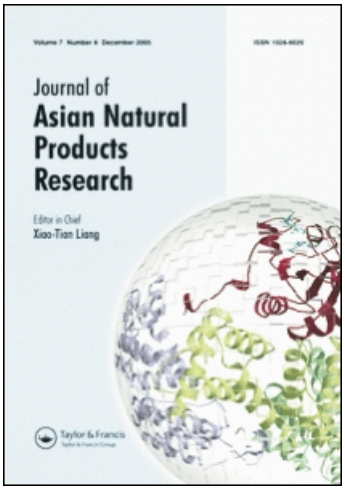

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
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To cite this Article Xiang, Lan , Liu, Xue-Hui , Fan, Guo-Qiang , Cui, Yu-Xin , Du, Li-Jun and Zheng, Jun-Hua(2005) 'Two new constituents from *Rheum sublanceolatum*', Journal of Asian Natural Products Research, 7: 4, 627 — 632
To link to this Article: DOI: 10.1080/10286020310001625076
URL: http://dx.doi.org/10.1080/10286020310001625076

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Journal of Asian Natural Products Research, Vol. 7, No. 4, August 2005, 627–632

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Two new constituents from *Rheum sublanceolatum*

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(Received 15 June 2003; revised 8 August 2003; in final form 10 September 2003)

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The chemical investigation of *Rheum sublanceolatum* has led to the isolation of two new constituents, the structures of which were identified on the basis of physical and spectroscopic analysis as 2-acetyl-3-methyl-6,8-dihydroxy-2,3,4,4-tetrahydronaphthalene-1-one-6-*O*-β-D-glucopyranoside (**1**) and 2,5-dimethyl-7-hydroxychromone-7-*O*-β-D-(6′-*O*-galloyl)-glucopyranoside (**2**).

Keywords: *Rheum sublanceolatum*; 2-Acetyl-3-methyl-6,8-dihydroxy-2,3,4,4-tetrahydronaphthalene-1-one-6-*O*-β-D-glucopyranoside; 2,5-Dimethyl-7-hydroxychromone-7-*O*-β-D-(6′-*O*-galloyl)-glucopyranoside

1. Introduction

Rhubarb used as a purgative drug in Chinese traditional medicine originates from three species, *Rheum palmatum*, *R. tanguticum* and *R. officinale*. There are about 60 species in the genus *Rheum*, of which 39 species and 2 variations are distributed in China [1]. An activity study showed that the crude drug of *R. sublanceolatum* C.Y. Cheng et Kao had the effect of scavenging superoxide radicals more significantly than thirty other species [2]. We previously reported the isolation and identification of some anthraquinone and non-anthraquinone derivatives from *R. sublanceolatum* [3,4]. Further investigation led to the isolation of two new constituents, compounds **1** and **2**. We report here the isolation and structure elucidation of these two new compounds.

2. Results and discussion

Compound **1**, a white amorphous powder (acetone), mp 202–203°C, showed dark yellow fluorescence under ultraviolet light (365 nm). Its IR spectrum revealed the absorptions of

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2005 Taylor & Francis Group Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020310001625076

free carbonyl, conjugated carbonyl and benzene ring (1717, 1630 and 1615, 1580 cm^{-1}). The negative HRFABMS exhibited a pseudo-molecular formula of $C_{19}H_{23}O_9$ and a fragment ion at *m/z* 233.0819 $[M - 1-162]^-$ indicated the presence of one sugar moiety, which was further determined to be glucose according to ^{13}CNMR data compared with those of glucose in literature [5]. The β configuration was confirmed from the coupling constant (7.5 Hz) of the anomeric proton. Two methyl proton signals at δ 2.23 (3H, s), 0.97 (3H, d, $J = 6.0$ Hz) and two signals at δ 6.42 (1H, d, $J = 2.0$ Hz), 6.47 (1H, br.s) could easily be observed from the ^{1}H NMR spectrum. The ^{1}H–^{1}H COSY spectrum revealed that the methyl proton at δ 0.97 correlated with the methine group at δ 2.53, which is connected with another methine group at δ 3.72 and one methene proton at δ 2.68. The above evidence indicates the presence of the fragment —CH—CH(CH_3)—CH_2—, which was further confirmed by DEPT, HMQC and HMBC spectra. The proton signals of the methene group also correlated with aromatic carbons at δ 107.9, 110.9 and 146.5 in the HMBC spectrum, indicating that the above fragment is connected to the benzene ring. Another terminal proton of this fragment, *i.e.* the methine at δ 3.72, is correlated with the carbons at δ 19.1, 31.7 and 35.7, as well as with carbonyl carbons at δ 206.1, 200.4 and the acetyl carbon at δ 30.5. This indicates a —CO—CH—CO—CH_3 fragment, which was further confirmed by the correlations of terminal methyl proton of this fragment with a carbonyl carbon at δ 206.1 and a tertiary carbon at δ 67.0. The aromatic carbon at δ 107.9 was assigned as C-5 as its proton is correlated with the secondary carbon at δ 35.7. It was deduced that the carbon at δ 110.9 is C-9 and another carbon at δ 163.9 is C-6 from the evidence that both of 5-H and 7-H are connected to the quaternary carbon at δ 110.9 and 163.9 through ^2J and ^3J correlations in the HMBC spectrum. The anomeric proton of glucose has a ^3J correlation with the carbon at δ 163.9, demonstrating that the glucose moiety is connected to C-6. The hydroxyl proton at δ 12.3 is correlated with C-7 (δ 101.2), C-8 (δ 164.3) and C-9 (δ 110.9), confirming that the hydroxyl group is at C-8. Compound **1** was therefore determined to be 2-acetyl-3-methyl-6,8-dihydroxy-2,3,4,4-tetrahydronaphthalene-1-one-6-*O*-β-D-glucopyranoside [figure 1(a)].

Corresponding to the keto-form of compound **1**, the NMR signals of the enol-form can also be seen with a ratio of 1:13.6 against the keto-form from analysis of the proton signal intensity. However, the enol-form signals were not so obvious and were sometimes ignored easily. Further study confirmed the enol-form definitely through analysis of a mixture of keto-enol tautomers with the ratio of 1.6:1. When the methanol solution containing predominantly the keto-form of compound **1** was kept at room temperature for a long time, the resultant precipitate became pink and the keto-form transformed into the enol-form significantly. When $NaOCD_3$ solution was added to compound **1**, it changed into the enol-form totally [figure 1(b)]. The assignment of two tautomers of compound **1** and its mixture are listed in tables 1–3, respectively.

Compound **2**, white needle crystals (ethyl acetate), mp 180–181°C, showed blue fluorescence under ultraviolet light (365 nm) and a dark blue color with $FeCl_3$ reagent. The peaks at 1692, 1645, 1614 and 1565 cm^{-1} in the IR spectrum suggest an ester carbonyl, conjugated carbonyl and benzene group. The negative HRFABMS showed the pseudo-molecular formula $C_{24}H_{23}O_{12}$. The ^{1}H NMR spectrum reveals two isolated methyl groups at δ 2.20, 2.63, an isolated aromatic proton at δ 6.01 and two meta-coupled aromatic protons at δ 6.99 and δ 6.92. All of these are typical signals of 2,5-dimethyl-7-hydroxychromone [6]. Two aromatic protons at δ 6.94 (2H, s) and three hydroxyl peaks at downfield regions are characteristic signals of gallic acid. The sugar moiety was glucose because its carbon signals were similar to those of

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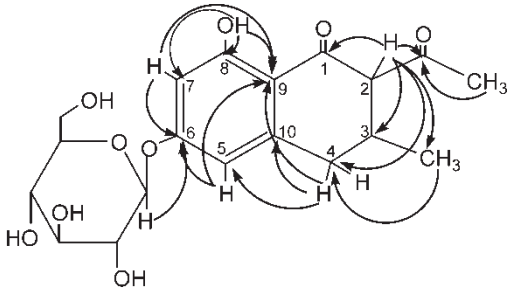
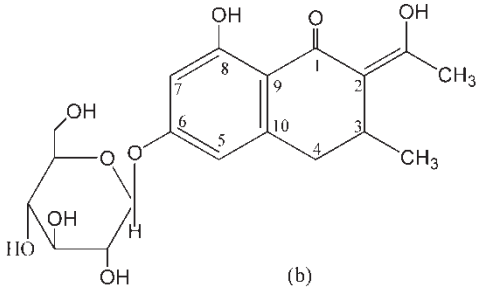


(a)



(b)

Figure 1. (a) Structure of the keto-form of **1** and important HMBC correlations. (b) Enol-form of **1**.

Table 1. NMR data of compound **1** (keto-form) in DMSO-d_6 (500 MHz for ^{1}H and 125 MHz for ^{13}C, *J* in Hz).

No.	^{13}C	^{1}H	*No.*	^{13}C	^{1}H
1	200.4		8	164.3	
2	67.0	3.72 (1H, d, *J*=11.0)	9	110.9	
2-CO-CH_3	206.1		10	146.5	
	30.5	2.23 (3H, s)			
3	31.7	2.53 (1H, m)	1′	99.5	5.0 (1H, d, *J*=7.5)
3-CH_3	19.1	0.97 (3H, d, *J*=6.0)	2′	73.0	3.22 (1H, t, *J*=7.5, 8.5)
4	35.7	2.89 (1H, dd, *J*=3.5, 16.5)	3′	77.1	3.39 (1H, t)
		2.68 (1H, dd, *J*=11.0, 16.0)			
5	107.9	6.47 (1H, br.s)	4′	69.5	3.15 (1H, t, *J*=9.5, 8.5)
6	163.9		5′	76.4	3.28 (1H, t, *J*=8.5, 9.5)
7	101.2	6.42 (1H, d, *J*=2.0)	6′	60.5	3.67 (1H, br.d)
8-OH		12.3 (1H, s)			3.46 (1H, dd, *J*=5.5, 12.0)

Table 2. NMR data of **1** (enol-from) in $NaOCD_3 + CD_3OD$ (500 MHz for ^{1}H and 125 MHz for ^{13}C; *J* in Hz).

No.	^{13}C	^{1}H	*No.*	^{13}C	^{1}H
1	194.1		8	165.1	
2	112.2		9	114.0	
2═C(OH)─CH_3	180.9		10	142.8	
	19.2	2.46 (3H, s)			
3	28.1	3.22 (1H, m)	1′	100.7	4.96 (1H, d, *J*=7.5)
3─CH_3	19.2	0.87 (3H, d, *J*=6.0)	2′	74.3	3.42–3.49 (m)
4	36.9	2.89 (1H, dd, *J*=5.5, 14.5)	3′	77.5	3.42–3.49 (m)
		2.44 (1H, br.d, *J*=14.5)			
5	106.8	6.28 (1H, br.s)	4′	70.8	3.42–3.49 (m)
6	160.9		5′	77.5	3.42–3.49 (m)
7	102.4	6.28 (1H, br.s)	6′	61.8	3.91 (1H, dd, *J*=1.5, 12.0)
					3.75 (1H, dd, *J*=5.0, 12.0)

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Table 3. NMR data of **1** (mixture of keto and enol-form with a ratio of 1.6:1) in CD_3OD (500 MHz for ^1H and 125 MHz for ^{13}C; *J* in Hz).

	Keto-form		*Enol-form*	
No.	^{13}C	^1H	^{13}C	^1H
1	201.0		192.8	
2	68.4	3.55 (1H, d, *J*=10.5)	111.2	
2═C(OH)─CH_3			179.6	
			19.0	2.14 (3H, s)
2-CO─CH_3	207.7			
	30.5	2.27 (3H, s)		
3	32.9	2.57 (1H, m)	29.8	3.02 (1H, m)
3-CH_3	19.2	1.04 (3H, d, *J*=6.5)	20.1	1.00 (3H, d, *J*=6.0)
4	36.9	2.96 (1H, dd, *J*=3.5, 16.5)	36.5	3.04 (1H, dd, *J*=3.5, 16.5)
		2.68 (1H, dd, *J*=11.0, 16.5)		2.65 (1H, dd, *J*=11.0, 16.5)
5	108.3	6.48 (1H, d, *J*=2.5)	110.0	6.45 (1H, d, *J*=2.5)
6	164.9		164.9	
7	102.3	6.43 (1H, d, *J*=2.5)	102.3	6.41 (1H, d, *J*=2.5)
8	165.7		165.7	
9	112.0		112.0	
10	147.3		146.0	
1′	100.6	4.99 (1H, d, *J*=7.5)	100.6	5.00 (1H, d, *J*=7.5)
2′	74.0	3.39–3.49 (m)	74.0	3.39–3.49 (m)
3′	77.7	3.39–3.49 (m)	77.7	3.39–3.49 (m)
4′	70.5	3.39–3.49 (m)	70.5	3.39–3.49 (m)
5′	77.2	3.39–3.49 (m)	77.2	3.39–3.49 (m)
6′	61.7	3.89 (1H, dd, *J*=2.0, 12.0)	61.7	3.89 (1H, dd, *J*=2.0, 12.0)
		3.69 (1H, dd, *J*=5.5, 12.0)		3.69 (1H, dd, *J*=5.5, 12.0)

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glucose in the literature [5]. Furthermore, the coupling constant (7.0 Hz) of the anomeric proton demonstrated that the glucose has a β configuration. The HMBC spectrum shows that the anomeric proton of glucose is correlated with a carbon at δ 159.6, suggesting it is connected to C-7. The gallic acid is connected to H-6′ of glucose as the H-6′ proton signals have moved downfield at δ 4.55 and 4.18. Compound **2** was therefore confirmed to be 2,5-dimethyl-7-hydroxychromone-7-*O*-β-D-(6′-*O*-galloyl)-glucopyranoside (figure 2).

3. Experimental

3.1 General Experimental Procedures

Melting points were determined on a XT_{4A} micromelting point apparatus and are uncorrected. UV spectra were measured on a TU-1800PC spectrophotometer. IR spectra

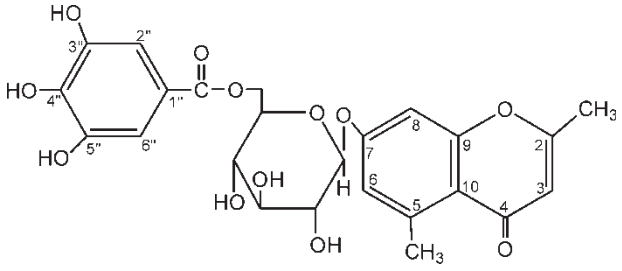

Figure 2. Structure of **2**.

were recorded on a Nicolet 5DX-FTIR spectrophotometer. The HRFABMS spectra were obtained on an APEXII mass spectrometer. The NMR experiments were performed with a Varian-INOVA-500 instrument, using TMS as internal standard. Column chromatography was carried out on silica gel (Qingdao Haiyang Chemical Group Co., Qingdao, China) or polyamide (Zhejiang Taizhou Siqin Chemical Plant, Zhejiang, China).

3.2 Plant Material

The radix and rhizoma of *Rheum sublancelatum* were collected from Qinghai Province of China in August 1999 and were identified by Professor Chen Hu-biao at the School of Pharmaceutical Sciences, Peking University. A voucher specimen (Q99143) has been deposited in the School of Pharmaceutical Sciences, Peking University.

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3.3 Extraction and Isolation

The dried powder (6 kg) of the radix and rhizoma of *R. sublanceolatum* was refluxed with 95% EtOH three times and the resultant extract was concentrated to a residue *in vacuo*. The residue was then partitioned between water and light petroleum, ethyl acetate, n-butanol, sequentially. The ethyl acetate extract (100 g) was subjected to a silica-gel column and eluted with chloroform and methanol with increasing polarity; 160 fractions were obtained. The recrystallization of Fr. 128–138 afforded compound **2** (15 mg). Fr. 63–74 were subjected to polyamide chromatography and eluted with chloroform mixed with methanol; the resultant Fr. 15–27 were further fractioned on a polyamide column eluted with water and ethanol, and the resultant Fr. 2–17 were then subjected to silica-gel column chromatography eluted with ethyl acetate–methanol–water (100:5:5) to afford Fr. 15–17. Compound **1** (12.8 mg) was obtained through repeated precipitation of Fr. 15–17 with acetone.

3.4 Characterization of New Compounds

2-Acetyl-3-methyl-6,8-dihydroxy-2,3,4,4-tetrahydronaphthalene-1-one-6-*O*-β-D-glucopyranoside (1). A white amorphous powder (acetone); UV λ_{max} (MeOH) (nm): 326,

Table 4. NMR data of **2** in DMSO-d_6 (500 MHz for ^{1}H and 125 MHz for ^{13}C; *J* in Hz).

No.	^{13}C	^{1}H	*No.*	^{13}C	^{1}H
2	164.4		1′	99.5	5.11 (1H, d, *J*=7.0)
2-CH_3	19.1	2.20 (3H, s)	2′	73.0	3.33 (m)
3	110.8	6.01 (1H, s)	3′	76.0	3.31 (m)
4	178.2		4′	69.7	3.29 (m)
5	141.3		5′	73.9	3.82 (m)
5-CH_3	22.2	2.63 (3H, s)	6′	63.3	4.55 (1H, br.d, *J*=10.5) 4.18 (1H, dd, *J*=6.0, 10.5)
6	116.6	6.79 (1H, br.s)	1″	119.2	
7	159.6		2″,6″	108.5	6.94 (2H, s)
8	101.2	6.92 (1H, br.s)	3″, 5″	145.4	
9	158.7		4″	138.4	
10	116.1		—O—CO—	165.6	

280, 220. IR ν_{max} (KBr) (cm^{-1}): 1717, 1630, 1615, 1580. FAB-MS (*m*/*z*): 397 $[M + 1]^+$, 234 $[M - 162]^+$, negative-HRFAB-MS (*m*/*z*): 395.1345 $[M - 1]^-$ (calcd. for $C_{19}H_{23}O_9$: 395.1347), 233.0819 $[M - 1-162]^-$ (calcd. for $C_{13}H_{13}O_4$: 233.0819). ^1H and ^{13}C NMR: see table 1.

2,5-Dimethyl-7-hydroxychromone-7-*O*-β-D-(6′-*O*-galloyl)-glucopyranoside (2). White needle crystals (ethyl acetate); UV λ_{max} (MeOH) (nm): 276, 249, 219. IR ν_{max} (KBr) (cm^{-1}): 1692, 1675, 1645, 1614, 1565. FAB-MS (*m*/*z*): 505 $[M + 1]^+$, negative-FAB-MS (*m*/*z*): 503.1186 $[M - 1]^-$ (calcd. for $C_{24}H_{23}O_{12}$: 503.1195). ^1H and ^{13}C NMR: see table 4.

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