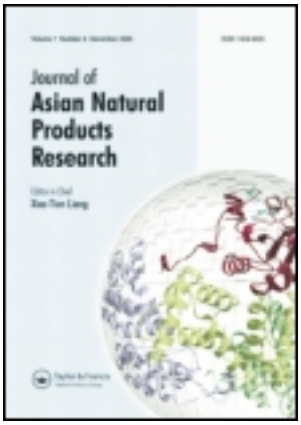

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
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Available online: 10 Oct 2011

To cite this article: Wen-Hao Chen, Juan Chen & Yan-Ping Shi (2011): Anthraquinones and stilbenes from the roots and rhizomes of Rhubarb, Journal of Asian Natural Products Research, 13:11, 1036-1041

To link to this article: http://dx.doi.org/10.1080/10286020.2011.613828

Journal of Asian Natural Products Research
Vol. 13, No. 11, October 2011, 1036–1041

Anthraquinones and stilbenes from the roots and rhizomes of Rhubarb

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(*Received 3 June 2011; final version received 9 August 2011*)

Two new anthraquinone glucosides [aloe-emodin 8-*O*-β-D-(6′-galloyl)glucopyranoside (**1**) and rhein 8-*O*-β-D-(6′-galloyl)glucopyranoside (**2**)], together with 16 known compounds (**3**–**18**), were isolated from the roots and rhizomes of *Rheum hotaoense* C.Y. Cheng et C.T. Kao. Their structures were elucidated on the basis of extensive investigation of 1D and 2D NMR, HR-ESI-MS, and chemical evidence. In addition, the free-radical-scavenging activity was tested using the 1,1-diphenyl-2-picrylhydrazyl assay.

Keywords: *Rheum hotaoense*; rhubarb; anthraquinones; stilbenes; DPPH assay

1. Introduction

Rhubarb is an important herbal medicine for the treatment of blood stagnation syndrome and is used as a purgative agent in Chinese, Japanese, and Korean traditional medicines. In the Chinese Pharmacopoeia, rhubarb consists of the roots and rhizomes of *Rheum officinale* Baill., *Rheum palmatum* L., and *Rheum tanguticum* Maxim. ex Balf., all of which belong to Sect. *Palmata* [1]. Aside from Sect. *Palmata*, species from Sect. *Rheum* are also used for rhubarb drugs in local areas of China. These unofficial species include *Rheum hotaoense* C.Y. Cheng et C.T. Kao, *Rheum franzenbachii* Munt., and *Rheum emodi* Wall. They exhibit much weaker purgative activities than official species due to the absence of sennosides and due to the relatively low content of anthraquinones [2]. Stilbenes, which are the major constituents of unofficial rhubarbs, were also different among the species, and exhibit potent antioxidant [3] and anti-allergic activities [4].

The chemical profiles of six rhubarb species (including official and unofficial) were analyzed by Ye *et al.* [5] using the HPLC/DAD/ESI-MS^n method, and it was found that their phenolic patterns showed significant difference, and the precise structures have to be determined further. In this study, the roots and rhizomes of *R. hotaoense* C.Y. Cheng et C.T. Kao were examined chemically leading to the isolation and structural elucidation of two new anthraquinone glucosides (**1** and **2**) together with 16 known compounds (**3**–**18**; Figure 1). Furthermore, the free-radical-scavenging activity on 1-diphenyl-2-picrylhydrazyl (DPPH) of the crude fractions and compounds **1**–**3** was also described.

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder, and its molecular

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ISSN 1028-6020 print/ISSN 1477-2213 online

http://dx.doi.org/10.1080/10286020.2011.613828
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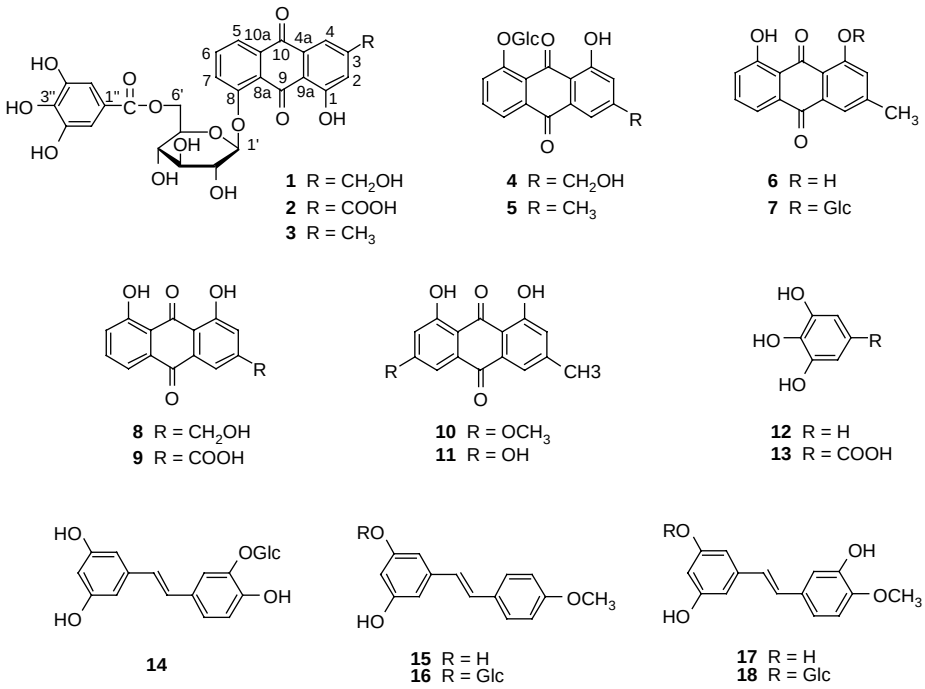

Figure 1. Chemical structures of compounds **1**–**18**.

formula of $C_{28}H_{24}O_{14}$ was determined from the negative-ion HR-ESI-MS (*m/z* 583.1084 $[M - H]^-$). The IR spectrum showed absorption bands ascribable to hydroxyl, free and chelated carbonyl, and aromatic functions at 3465, 1692, 1628, 1469, and 1081 cm^{-1}. The UV spectrum of **1** exhibited absorption maxima at 218, 256, 280, and 410 nm, indicating the presence of an anthraquinone system [3].

The ^1H NMR spectrum showed signals due to oxygenated methylene protons [δ_H 4.60 (2H, s, 3-CH_2OH)], an anomeric proton [δ_H 5.25 (1H, d, $J = 7.6$ Hz, Glc-1′-H)], a galloyl group [δ_H 6.98 (2H, s, galloyl-2″, 6″-H)], two aromatic protons [δ_H 7.25, 7.62 (each 1H, both s, 2-H, 4-H)], a 1,2,3-trisubstituted benzene ring [δ_H 7.69 (1H, d, $J = 7.6$ Hz, 7-H); 7.70 (1H, dd, $J = 7.2$, 7.6 Hz, 6-H); 7.82 (1H, d, $J = 7.2$ Hz, 5-H)], and a chelated hydroxyl proton [δ_H 12.86 (1H, br s, 1-OH)]. These data together with the ^{13}C NMR spectral data (Table 1) for **1** were similar to those of chrysophanol 8-*o*-β-D-(6′-galloyl)glucopyranoside (**3**), previously isolated from a Korean rhubarb *Rheum undulatum* L. [3]. The major differences between the two compounds were that the methyl group in **3** was replaced by the oxygenated methylene resonances (δ_H 4.60, δ_C 62.1) in **1**. This was further confirmed by the HMBC correlations from the oxygenated methylene at δ_H 4.60 to C-2 (δ_C 120.8), C-3 (δ_C 152.3), and C-4 (δ_C 116.1). The β-glucopyranosyl unit could be located at C-8 on the basis of the HMBC correlation observed between the anomeric proton signal at δ_H 5.25 and the carbon resonance at δ_C 158.0 (C-8). The negative ESI-MS showed significant fragment ion at *m/z* 431 $[M - C_7H_5O_4]^-$, ascribable to the loss of the galloyl unit. Furthermore, the linkage of the galloyl to the glucopyranosyl unit was established at C-6′ by the downfield shift of C-6′ (δ_C 63.4) and H-6′ (δ_H 4.28, 4.50), as well as the HMBC correlation between H-6′ and C-7″ (δ_C 165.7)

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** (δ values, in DMSO-d_6)[a].

	1		**2**	
No.	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
1		161.7 s		161.3 s
2	7.25 (s)	120.8 d[b]	7.74 (s)	124.0 d
3		152.3 s		140.5 s
4	7.62 (s)	116.1 d	8.12 (s)	118.5 d
5	7.82 (d, 7.2)	120.7 d[b]	7.84 (d, 7.2)	120.7 d
6	7.70 (dd, 7.2, 7.6)	135.8 d	7.74 (dd, 7.2, 8.4)	136.0 d
7	7.69 (d, 7.6)	122.3 d	7.68 (d, 8.4)	122.3 d
8		158.0 s		158.1 s
9		187.6 s		187.6 s
10		182.1 s		181.8 s
4a		132.3 s		132.6 s
8a		120.7 s		119.4 s
9a		115.5 s		118.9 s
10a		134.9 s		134.8 s
1-OH	12.86 (br s)		12.73 (br s)	
3-CH_2OH	4.60 (s)	62.1 t		
3-COOH				166.5 s
1′	5.25 (d, 7.6)	100.3 d	5.26 (d, 7.6)	100.3 d
2′	3.55 (m)	73.3 d	3.54 (m)	73.3 d
3′	3.42 (m)	76.5 d	3.42 (m)	76.5 d
4′	3.40 (m)	69.8 d	3.40 (m)	69.8 d
5′	3.85 (m)	74.1 d	3.85 (m)	74.1 d
6′	4.50 (m), 4.28 (m)	63.4 t	4.49 (m), 4.24 (m)	63.5 t
1″		119.4 s		119.4 s
2″, 6″	6.98 (s)	108.8 d	6.97 (s)	108.8 d
3″, 5″		145.6 s		145.7 s
4″		138.6 s		138.7 s
7″		165.7 s		165.8 s

[a] Assignments were based on DEPT135, HSQC, and HMBC experiments.
[b] Assignments (within the same column) may be interchangeable.

(Figure 2). Alkaline hydrolysis of **1** with 1.0% NaOMe/MeOH furnished **4**, which was identified by thin layer chromotography (TLC). Further detailed analysis of the ^{1}H, HSQC, and HMBC NMR spectroscopic data allowed unambiguous assignments for all of the ^{1}H and ^{13}C NMR signals of **1**. Thus, the structure of **1** was elucidated as aloe-emodin 8-*o*-β-D-(6′-galloyl)glucopyranoside.

Compound **2** was also obtained as a yellow amorphous powder, and its

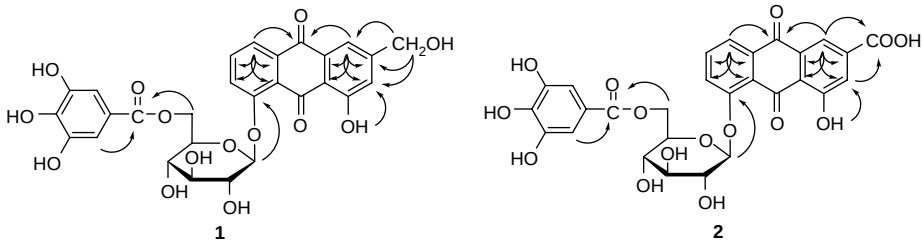

Figure 2. Key HMBC (H → C) correlations of **1** and **2**.

Table 2. DPPH radical scavenging activities of fractions and compounds **1**–**3**.

Sample	SC_{50} (μg/ml)	Sample	SC_{50} (μM)
EtOAc-soluble fraction	8.8	**1**	13.6
n-BuOH-soluble fraction	15.5	**2**	12.7
30% EtOH-eluted fraction	6.9	**3**	11.1
70% EtOH-eluted fraction	14.7	Quercetin	9.3

molecular formula of $C_{28}H_{22}O_{15}$ was determined from the negative-ion HR-ESI-MS (*m/z* 597.0874 $[M - H]^-$). The ^1H and ^{13}C NMR (Table 1) spectra closely resembled those of **1**, except for the presence of the signal for an additional carboxyl group (δ_C 166.5, s), and the absence of the oxygenated methylene resonances at C-3 as found in **1**, suggesting that the carboxyl group is linked to C-3 in **2**. This was further confirmed by the HMBC correlations from H-2 and H-4 to the carbonyl group (δ_C 166.5) (Figure 2). Thus, the structure of **2** was elucidated as rhein 8-*o*-β-D-(6′-galloyl)glucopyranoside.

The known compounds (**3**–**18**) were determined to be chrysophanol 8-*o*-β-D-(6′-galloyl)glucopyranoside (**3**) [3], aloe-emodin 8-*o*-β-D-glucopyranoside (**4**) [6], chrysophanol 8-*o*-β-D-glucopyranoside (**5**) [7,8], chrysophanol (**6**) [6,7], chrysophanol 1-*o*-β-D-glucopyranoside (**7**) [8], aloe-emodin (**8**) [6], rhein (**9**) [6], physcion (**10**) [7], emodin (**11**) [6,7], pyrogallic acid (**12**), gallic acid (**13**) [9], piceatannol 3′-*o*-β-D-glucopyranoside (**14**) [7], desoxyrhapontigenin (**15**) [7], desoxyrhaponticin (**16**) [7], rhapontigenin (**17**) [7], and rhaponticin (**18**) [7], respectively. Compounds **5** and **7** were isolated as an approximately 1:1 mixture of isomers, which was different from the ratio of 5:3 (or 3:5) reported in the literature [10].

The DPPH radical scavenging activity of the EtOAc- and *n*-BuOH-soluble, 30% and 70% EtOH-eluted fractions and compounds **1**–**3** were examined. The 30% EtOH-eluted fraction showed the most potent scavenging activity (SC_{50} 6.9 μg/ml) compared with all the other fractions. As shown in Table 2, compounds **1** and **2** were slightly less potent than the standard quercetin. In agreement with previous study, compound **3** showed potent radical scavenging activity. These results confirmed that the galloyl moiety enhanced the activity [3].

3. Experimental

3.1 General experimental procedures

UV and IR spectra were recorded on a Shimadzu UV-240 spectrophotometer and a Nicolet NEXUS-670 FT-IR spectrophotometer, respectively. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter with a 1 dm cell. NMR spectra were recorded on a Bruker AVANCE III-400 spectrometer with TMS as an internal reference (δ). ESI-MS and HR-ESI-MS were measured on a Waters® ACQUITY™ TQ Detector and a Bruker APEX-II mass spectrometer, respectively. D101 macroporous adsorptive resins (granularity: 0.30–1.25 mm) were supplied by the Cangzhou Baoen Chemical Industry Co., Ltd (Cangzhou, China). DPPH was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Silica gel (200–300 mesh) used for column chromatography (CC) and silica gel GF_{254} (10–40 μm) for TLC were both supplied by the Qingdao Marine Chemical Factory (Qingdao, China). TLC was detected at 254 nm and spots were visualized by spraying with 5% H_2SO_4 in C_2H_5OH (v/v) followed by heating.

3.2 Plant material

The roots and rhizomes of *R. hotaoense* C.Y. Cheng et C.T. Kao were purchased in

July 2009 from Huanghe Medicinal Material Market in Gansu, China, and were identified by adjunct Professor Huan-Yang Qi of Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, where a voucher specimen (ZY2010DH01) is deposited.

3.3 Extraction and isolation

The air-dried powders of roots and rhizomes of *R. hotaoense* C.Y. Cheng et C.T. Kao (4.5 kg) were extracted with 95% EtOH (3 × 2 h) at 60°C. The crude extract was concentrated *in vacuo* to afford a dark brown residue, which was suspended in H_2O and fractionated by successive partitioning with EtOAc and *n*-BuOH. The *n*-BuOH-soluble fraction was dissolved in H_2O and subjected to CC over D101 and eluted with H_2O and 30% and 70% EtOH, successively. The EtOAc-soluble fraction (220 g) was subjected to silica gel CC, using a step gradient-elution technique, employing mixtures of petroleum ether–acetone as solvent, to afford six fractions [Fre 1 (20:1, 10 g), Fre 2 (10:1, 15 g), Fre 3 (5:1, 36 g), Fre 4 (3:1, 45 g), Fre 5 (2:1, 32 g), and Fre 6 (1:1, 20 g)] according to TLC analysis. Fraction Fre 2 was chromatographed on silica gel with petroleum ether–EtOAc gradient system to give three subfractions (Fre 2.1–Fre 2.3). Further purification of subfraction Fre 2.1 (2.4 g) through repeated chromatography with petroleum ether–acetone (8:1) as eluant over silica gel yielded **6** (100 mg). Fre 2.2 (5.2 g) was subjected to silica gel CC with petroleum ether–EtOAc (5:1) as eluant followed by recrystallization to yield **11** (25 mg), **8** (100 mg), and **10** (10 mg), respectively. Fre 3, Fre 4, and Fre 5 were separated using the same procedure as Fre 2 to afford **15** (13 mg), **12** (22 mg), **17** (1 g), **9** (20 mg), and **13** (15 mg), respectively.

The 30% EtOH fraction (300 g) was subjected to silica gel CC with $CHCl_3$—MeOH as solvent, to afford five fractions [Fr1 (10:1, 18 g), Fr2 (5:1, 45 g), Fr3 (3:1, 36 g), Fr4 (2:1, 26 g), and Fr5 (1:1, 128 g)] and **18** (12.5 g). Fraction Fr2 was chromatographed on silica gel with a $CHCl_3$—MeOH gradient system to give three subfractions. The subfractions 2 (8.6 g) and 3 (15.5 g) were further separated by repeated chromatography over silica gel ($CHCl_3$—MeOH, 4:1) which afforded **14** (27 mg) and **4** (36 mg), respectively. Fraction Fr3 was subjected to silica gel CC with $CHCl_3$—MeOH (3:1) as eluant followed by recrystallization to yield **1** (25 mg). Fraction Fr4 was subjected to silica gel CC with $CHCl_3$—MeOH (2:1) as eluant followed by recrystallization to yield **2** (31 mg). Likewise, a similar isolation procedure adopted for the 70% EtOH fraction (180 g) afforded six compounds in the following order: **8** (10 mg), **17** (14 mg), **5** and **7** as a mixture (1:1, 2.7 g), **16** (1.2 g), and **3** (31 mg).

*3.3.1 Aloe-emodin 8-O-β-D-(6′-galloyl)glucopyranoside (**1**)*

A yellow amorphous powder. $[\alpha]_D^{20}$ + 23 (*c* 1.0, MeOH). UV (MeOH)/nm: λ_{max} (log ε) 218 (1.23), 256 (0.66), 280 (0.47), and 410 (0.18). IR (KBr) ν_{max}: 3465, 1692, 1628, 1469, 1276, and 1081 cm^{-1}. ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data, see Table 1. ESI-MS *m/z*: 583 $[M - H]^-$, 431 $[M - C_7H_5O_4$ (galloyl)$]^-$. HR-ESI-MS *m/z*: 583.1084 $[M - H]^-$ (calcd for $C_{28}H_{23}O_{14}$, 583.1094).

*3.3.2 Rhein 8-O-β-D-(6′-galloyl)glucopyranoside (**2**)*

A yellow amorphous powder. $[\alpha]_D^{20}$ + 10 (*c* 1.1, MeOH). UV (MeOH)/nm: λ_{max} (log ε) 220 (0.59), 258 (0.32), 280 (0.21), and 410 (0.10). IR (KBr) ν_{max}: 3471, 1700, 1631, 1359, 1270, and 1074 cm^{-1}. ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data, see Table 1. ESI-MS *m/z*: 597 $[M - H]^-$, 445 $[M - C_7H_5O_4$ (galloyl)$]^-$. HR-ESI-MS

m/z: 597.0874 $[M - H]^-$ (calcd for $C_{28}H_{21}O_{15}$, 597.0886).

3.4 Alkaline hydrolysis of 1

A solution of **1** (2 mg) in 1.0% NaOMe—MeOH (1.0 ml) was stirred at room temperature for 1 h. The reaction mixture was neutralized with CH_3COOH. Compound **4** was detected in the reaction mixtures by co-TLC comparison with the authentic sample ($CHCl_3$–MeOH–H_2O, 12:6:1; $R_f = 0.45$).

3.5 Scavenging activity of DPPH radicals

The DPPH assay was carried out according to the modified method of Chen et al. [11]: aliquots of 30 μl of an ethanol solution containing each sample were added to 3 ml of 100 μM ethanol solution of DPPH. Absorbance at 517 nm was determined after 30 min at room temperature, and the percent antioxidant activity (AA) was calculated using the following formula:

$$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100]/Abs_{control}\}.$$

Ethanol (3 ml) was used as a blank. 100 μM of DPPH ethanol solution (3 ml) was used as a negative control. The SC_{50} values denote the concentration of test sample required to scavenge 50% DPPH free radicals. Quercetin was used as a positive control.

Acknowledgements

This work was financially supported by the National Key Technology Research and Development Program of China (No. 2007BAI37B05), the Western High-Tech Project Action Plan of Chinese Academy of Sciences (No. KG-CX2-YW-510), and the National Natural Science Foundation of China (No. 21075127).

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