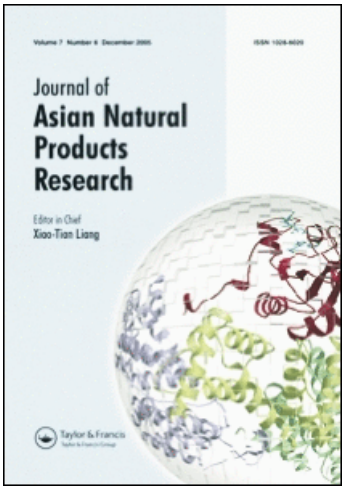

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Online publication date: 27 July 2010

To cite this Article Xu, Chuan-Lian , Zheng, Yi-Nan , Yang, Xiu-Wei , Li, Xiang-Gao , Li, Xiang and Chen, Quan-Cheng(2007) 'Raddeanalin, a new flavonoid glycoside from the leaves of *Salix raddeana* Laksh.', Journal of Asian Natural Products Research, 9: 5, 415 — 419
To link to this Article: DOI: 10.1080/10286020500034444
URL: http://dx.doi.org/10.1080/10286020500034444

Journal of Asian Natural Products Research, Vol. 9, No. 5, July–August 2007, 415–419

Raddeanalin, a new flavonoid glycoside from the leaves of *Salix raddeana* Laksh.

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(Received 23 August 2004; revised 22 October 2004; in final form 30 October 2004)

A new flavonoid glycoside, diosmetin 7-*O*-β-D-xylopyranosyl(1–6)-β-D-glucopyranoside, named raddeanalin (**1**), was isolated from the ethanolic extract of the leaves of *Salix raddeana* Laksh. together with three known compounds, kaempferol 3-*O*-glucoside (**2**), quercetin 3-*O*-glucoside (**3**) and quercetin 3-*O*-rutinoside (**4**). The structure of new compound was established by the spectral data and chemical properties.

Keywords: *Salix raddeana* Laksh.; *Salix*; Flavonoid glycoside; Raddeanalin

1. Introduction

Salix raddeana Laksh. belongs to Salicaceae family [1] and is distributed in the area of Changbai mountain of east northern China. Its leaves are used as a Chinese folk medicine for the treatment of antibacterium and diminishing inflammation, and are especially useful in curing stomatitis. As part of our continuing investigation on the flavonoid constituents of *Salix* species, we have examined the leaves of *Salix malsudana* Koidz and *Salix babylonica* L. [2], and we wish to report here the isolation and structural elucidation of a new flavonoid glycoside from the leaves of *S. raddeana* Laksh.

2. Results and discussion

The 20% ethanolic extract of the air-dried leaves of *Salix raddeana* Laksh. was partitioned with petroleum ether, chloroform and n-BuOH, successively. The n-BuOH soluble fraction was purified by polyamide column chromatography to afford a new compound, **1** (Scheme 1).

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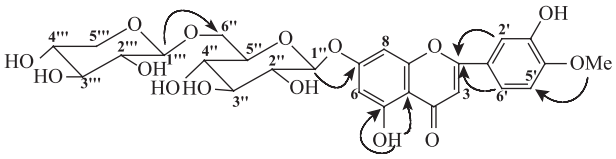
Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500034444



Scheme 1. Structure and the key HMBC correlations of **1**

Compound **1** was isolated as yellowish powder, mp 243–245°C. Its IR spectrum showed absorption bands at ν_{max} 3480–3520 (OH), 1624 (C═O), 2980 (C—H), 2865 (OMe), 1480–1010 (*O*-glycosyl) and 875 cm^{-1}. It was recognised as a flavonoid with 3- or 5-, or *ortho*-dihydroxyl groups from its colour reaction in the 2% $AlCl_3$/EtOH test. The UV absorption of **1** in MeOH (254 and 348 nm) and a bathchromic shift of 38 nm with $AlCl_3$/HCl suggested that it is a 5-hydroxyflavone compound [3,4]. In addition, it gave also characteristic diagnosis of glycoside from its positive reactions to the α-naphthol and the absorption bands at ν_{max} 1480–1010 (*O*-glycosyl) cm^{-1} in the IR spectrum. Therefore, compound **1** was a flavonoid glycoside.

Twenty-seven carbon signals in the ^{13}C NMR spectrum (table 1) exhibited the presence of a flavonoid nucleus and a saccharide chain contained two sugar units in **1**. The nine carbon signals of the aglycone in the DEPT spectrum were at δ 182.0, 164.2, 163.0, 161.2, 157.0, 105.5, 103.8, 99.6 and 94.8, suggesting that **1** was diosmetin [5,6]. According to signals in the HSQC and HMBC spectra of **1**, two singlets at δ 3.88 (3H, s)

Table 1. NMR spectral data of **1** (500 MHz, DMSO-d_6).

No.	*^{13}C, DEPT*	*^{1}H, HSQC*	*HMBC*
2	164.2 s		H-3, H-2′, H-6′
3	103.8 d	6.83, s	
4	182.0 s		H-3
4a	105.5 s		H-3, H-6, H-8, C_5—OH
5	161.2 s		H-6, H-8, C_5—OH
6	99.6 d	6.49, d, 2.0	H-8
7	163.0 s		H-1″, H-6, H-8
8	94.8 d	6.82, d, 2.0	H-6
8a	157.0 s		H-8
1′	122.9 s		H-3, H-2′, H-5′
2′	113.2 d	7.47, d, 2.0	H-6′
3′	146.8 s		H-2′, H-5′, $C_{3'}$—OH
4′	151.3 s		H-2′, H-5′, H-6′
5′	112.2 d	7.15, d, 8.5	$C_{4'}$—OMe
6′	118.9 d	7.60, d, 8.5	H-2′
1″	99.9 d	5.06, d, 7.5	H-2″
2″	73.1 d	3.30, m	$C_{2''}$—OH
3″	75.6 d	3.66, t, 6.5	$C_{3''}$—OH
4″	69.3 d	3.25, m	C4″—OH
5″	76.2 d	3.32, m	
6″	65.7 t	2.99, dd, 5.5, 11.0	H-1‴
		3.70, dd, 5.5, 11.0	
1‴	104.2 d	4.17, d, 7.5	H-2‴, H_a-5‴, H_b-5‴
2‴	73.4 d	3.02, dd, 3.5, 7.5	$C_{2'''}$—OH
3‴	76.5 d	3.09, ddd, 3.5, 8.8. 8.8	$C_{3'''}$—OH
4‴	69.5 d	3.29, m	$C_{4'''}$—OH
5‴	68.4 t	3.64, t, 5.5	H-5‴a
		3.95, dt, 3.5, 9.5	
C_5—OH		12.95, s	
C_4'—OMe	55.8 t	3.88, s	
C_3'—OH		9.45, s	

and 6.83 (1H, s) were assigned to methoxy groups at C-4′ and H-3; five aromatic protons at δ 6.49 (1H, d, $J = 2.0$ Hz), 6.82 (1H, d, $J = 2.0$ Hz), 7.15 (1H, d, $J = 8.5$ Hz), 7.47 (1H, d, $J = 2.0$ Hz), and 7.60 (1H, dd, $J = 2.0$, 8.5 Hz) were assigned to H-6, H-8, H-5′, H-2′ and H-6′; two singlets at δ 9.45 (1H, br s) and 12.95 (1H, br s) were assigned to C-3′ hydroxyl and a chelated hydroxyl (C—5—OH) proton, respectively, in the ^{1}H NMR spectrum of **1**. These data further suggested that aglycone of **1** is diosmetin [6].

In the ^{13}C NMR spectrum of **1**, the carbon signals at δ 104.2, 73.4, 76.5, 69.5, 68.4 and δ 99.9, 73.1, 75.6, 69.3, 76.2, 65.7 indicated the presence of one xylose and 6-substituted glucose. In the periodate oxidation of **1** with sodium metaperiodate [7], it showed that the sugar groups were present in pyranose form. β-xylose and β-glucose were detected after acid hydrolysis of **1** by comparison with authentic sugar samples. The ESI-TOF-MS of **1** gave a quasimolecular ion at *m/z* 593 $[M - 1]^+$, and fragment ions at *m/z* 462 [M–xylosyl]$^+$, 300 [M–xylosyl–glucosyl]$^+$ (aglycone). Its molecular formula $C_{27}H_{29}O_{15}$ was deduced by HR-ESI-TOF-MS at [*m/z* 593.1518 $[M - 1]^+$. The signals at δ 4.17 (1H, d, $J = 7.5$ Hz) and 5.06 (1H, d, $J = 7.5$ Hz) were assigned to H-1‴ and H-1″ of xylosyl and glucosyl moieties, respectively, which were coincident with ^{1}H NMR and HSQC spectra. The large J values indicated β-glycosidic linkages in all cases. In the HMBC spectrum of **1** (scheme 1, table 1), the signal of glucosyl anomeric proton at δ 5.06 was correlated with that of diosmetin C-7 at δ 163.0; the signal of xylosyl anomeric proton at δ 4.17 was correlated with that of the glucose C-6 at δ 68.5 (t), respectively, suggesting that the glucosyl group was connected with the diosmetin at C-7, and xylosyl group was connected with the glucosyl group at C-6.

On the basis of the above evidence, the structure of **1** was identified as a new flavonoid glycoside, diosmetin 7-*O*-β-D-xylopyranosyl (1 → 6)-β-D-glucopyranoside, named raddeanalin.

In addition, three known flavonoid glycosides, kaempferol 3-*O*-glucoside (**2**) [8,9], quercetin 3-*O*-glucoside (**3**) [9] and quercetin 3-*O*-rutinoside (**4**) were isolated. They were all identified by colour reactions, UV, IR, ^{1}H NMR and ^{13}C NMR spectral analysis, and by comparison with that of the authentic sample.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Bejing X-4 apparatus and are uncorrected. IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR Spectrometer with KBr pellets and UV spectra were acquired on Shimadzu UV-240 spectrometer. NMR spectra were performed on a Varian INOVA-500 spectrometer in DMSO-d_6 at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts are given in δ relative to TMS as an internal standard. HR-ESI-TOF-MS were performed on an MDS SCIEX API QSTAR mass spectrometer. ESI-MS were obtained on a Finnigan MAT LCQ (5 kV, metal capillary temperature at 200°C, atomisation gas as N_2).

3.2 Plant material

The leaves of *Salix raddeana* Lasksh. were collected at the area of Changbai Mountain, Jilin province, China in June 2000. A voucher specimen is deposited in the Herbarium of the

College of Traditional Chinese Medicinal Material, Jilin Agriculture University. The plant was identified by Mr Shaobo Fan.

3.3 Extraction and isolation

The air-dried leaves (4.8 kg) of *S. raddeana* Laksh. were extracted with 20% EtOH two times. The combined ethanolic extract was concentrated and successively extracted with petroleum ether (60–90°C), chloroform and n-BuOH. The n-BuOH-soluble fractions were evaporated to dryness *in vacuo* to yield a yellow-brown residue. Four spots were detected on polyamide-coated TLC using HOAc—BuOH—HCOOH—H_2O (5:3:1:1) as developing agent. The n-BuOH extracts were purified by polyamide column chromatography and gradient elution was accomplished with EtOH—H_2O (0, 10–70%). The 40% EtOH fraction was concentrated to yield compound **1** (80 mg). The 30% EtOH fraction was concentrated and purified by HPLC, using MeOH—H_2O (36:64) as mobile phase, Zorbax SB C_{18} as the chromatographic column, and 254 nm as monitor wavelength, to yield known compounds **2**, **3**, and **4**, respectively.

3.3.1 Diosmetin 7-O-β-D-xylopyranosyl (1–6)-β-D-glucopyranoside (1). Yellowish powder, mp 243–245°C; IR (KBr) ν_{max}: 3480–3520 (—OH), 1624 (C═O), 2950 (C—H), 2865 (OMe), 1480–1010 (*O*-glycosyl) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 2.99 (1H, dd, $J = 5.5$, 11.0 Hz, Ha-6″), 3.02 (1H, dd, $J = 3.5$, 7.5 Hz, H-2‴), 3.09 (1H, ddd, $J = 3.5$, 8.8. 8.8 Hz, H-3‴), 3.25 (1H, m, H-4″), 3.29 (1H, m, H-4‴), 3.30 (1H, m, H-2″), 3.32 (1H, m, H-5″), 3.64 (1H, t, $J = 5.5$ Hz, Ha-5‴), 3.66 (1H, t, $J = 6.5$ Hz, H-3″), 3.70 (1H, dd, $J = 5.5$, 11.0 Hz, Hb-6″), 3.88 (3H, s, $C_{4'}$—OMe), 3.95 (1H, dt, $J = 3.5$, 9.5 Hz, Hb-5‴), 4.17 (1H, d, $J = 7.5$ Hz, H-1‴), 4.88 (1H, d, $J = 4.5$ Hz, $C_{3'''}$—OH), 4.95 (1H, d, $J = 5.0$ Hz, $C_{4'''}$—OH), 4.97 (1H, d, $J = 4.5$ Hz, $C_{2'''}$—OH), 5.06 (1H, d, $J = 7.5$ Hz, H-1″), 5.18 (1H, d, $J = 5.0$ Hz, $C_{3''}$–OH), 5.22 (1H, d, $J = 4.5$ Hz, $C_{4''}$—OH), 5.44 (1H, d, $J = 4.5$ Hz, $C_{2''}$—OH), 6.49 (1H, d, $J = 2.0$ Hz, H-6), 6.82 (1H, d, $J = 2.0$ Hz, H-8), 6.83 (1H, s, H-3), 7.15 (1H, d, $J = 8.5$ Hz, H-5′), 7.47 (1H, d, $J = 2.0$ Hz, H-2′), 7.60 (1H, dd, $J = 2.0$, 8.5 Hz, H-6′), 9.45 (1H, s, $C_{3'}$—OH), 12.95 (1H, s, C_5—OH); ^{13}C NMR data is shown in table 1. ESI-TOF-MS: *m/z* 593 $[M - 1]^+$, 462 $[M-xylosyl]^+$, 300 $[M-xylosyl-glucosyl]^+$ (aglycone); HR-ESI-TOF-MS: *m/z* 593.1518 $[M - 1]^+$ (calcd for $C_{27}H_{29}O_{15}$, 593.1511).

3.3.2 Acid hydrolysis of 1. Compound 1 (30 mg) in 20 ml MeOH was refluxed with 10 ml of 9% HCl in a boiling water bath for 5–6 h. After the reaction solution was cooled, a yellow precipitate was obtained by filtration. The precipitate was chromatographed on Sephadex LH-20, eluting with $CHCl_3$/MeOH (5:1), to yield the aglycone (3.2 mg), which was identified as diosmetin by comparison with authentic sample. The aqueous hydrolysate after acid hydrolysis was neutralised with $BaSO_4$. The aqueous filtrate was concentrated, and then subjected to PC, with n-BuOH/AcOH/H_2O = 4:1:5 as developer and aniline hydrogen phthalate as detecting agent [10]. The result showed the presence of β-xylose ($R_f = 0.31$) and β-glucose ($R_f = 0.25$).

3.3.3 Periodate oxidation of 1. Compound 1 (10 mg) was dissolved in MeOH and treated with sodium metaperiodate for 24 h. The liberation of formic acid and periodates were

estimated by the Jones method, which also showed that both the sugars were present in pyranose form [7].

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