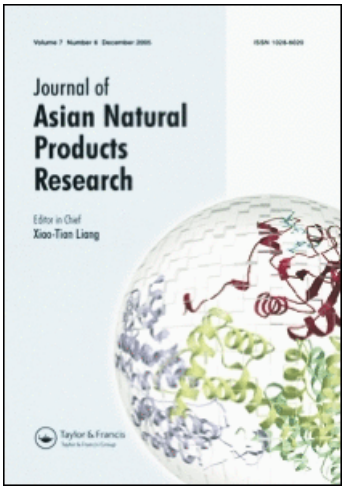

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ORIGINAL ARTICLE

Two new secoiridoid glycosides from *Verbena officinalis*

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Two new secoiridoid glycosides, verbenoside A (**1**) and verbenoside B (**2**), have been isolated from the ethanol extract of the aerial parts of *Verbena officinalis* L. Their structures were elucidated on the basis of spectroscopic evidences, especially 1D, 2D NMR, and MS experiments.

Keywords: *Verbena officinalis*; secoiridoid glycoside; verbenoside A; verbenoside B

1. Introduction

Verbena officinalis L. (Verbenaceae) is widely used in traditional Chinese medicine for the treatment of amenia, algomenorrhea, cameroon fever, laryngalgia, onco-carbuncle, edema, and pyretic stranguria [1]. In the course of our investigation on biologically active constituents from *V. officinalis*, we had isolated iridoid glycosides, triterpenes, phenethanol glycosides, and fatty acid esters from this herb [2,3]. Continuation of this work led to the isolation of two new secoiridoid glycosides, verbenoside A (**1**), and verbenoside B (**2**). Many activities of secoiridoid glycosides have been reported, such as antioxidant [4–6], radical scavenging [7,8], antihyperlipemia [9], and immunomodulatory effect [10]. This paper reports the isolation and structural elucidation of the two new compounds.

2. Results and discussion

The *n*-BuOH soluble fraction of the ethanol extract of the aerial parts of *V. officinalis* was subjected to column chromatography over silica gel, Sephadex LH-20, and finally semipreparative HPLC to yield two new compounds **1** and **2**.

Compound **1** was obtained as a white amorphous powder. The HR-ESI-MS spectrum showed a quasi-molecular ion peak at *m/z* 747.2105 $[M + Na]^+$, indicating the molecular formula $C_{33}H_{40}O_{18}$. ^1H and ^{13}C NMR spectral data (Table 1) and DEPT experiment disclosed the presence of 4 ester carbonyls, 3 sp^2 quaternary carbons, 13 sp^3 methines (11 of which were oxygen bearing), 6 sp^2 methines, 4 sp^3 methylenes (3 of which were oxygen bearing), 1 sp^2 methylene, and 2 methyl groups. The ^1H NMR spectral data of **1** exhibited the characteristic signals of secoiridoid glycoside at δ 5.46 (1H, d, $J = 1.4$ Hz, H-1), 7.61 (1H, d, $J = 2.4$ Hz, H-3), 5.57 (1H, m, H-8), 5.37 (1H, m, Ha-10), and 5.30 (1H, m, Hb-10). Comparison of the NMR spectral data of **1** (Table 1) with those of sweroside [11] showed that **1** had the moiety of sweroside; however, the

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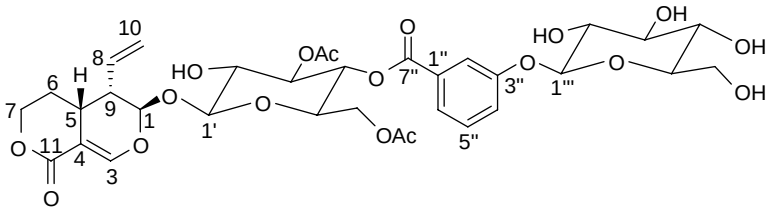
Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (CD_3OD).

	1		**2**	
Position	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	98.6	5.46 (d, $J = 1.4$)	98.8	5.32 (d, $J = 1.8$)
3	153.9	7.61 (d, $J = 2.4$)	153.5	7.56 (d, $J = 2.4$)
4	106.3		106.7	
5	28.6	3.14 (m)	28.8	2.89 (m)
6	26.0	1.82 (m), 1.75 (m)	25.9	1.74 (m), 1.64 (m)
7	69.9	4.47 (m), 4.38 (m)	70.1	4.45 (m), 4.33 (m)
8	133.2	5.57 (m)	132.9	5.48 (m)
9	44.0	2.76 (m)	43.6	2.69 (m)
10	121.2	5.37 (m), 5.30 (m)	121.4	5.25 (2H, m)
11	168.5		168.2	
1′	99.9	4.95 (d, $J = 7.9$)	98.1	4.94 (d, $J = 8.1$)
2′	72.9	3.55 (m)	74.9	4.74 (m)
3′	75.9	5.32 (m)	75.5	3.64 (m)
4′	71.4	5.25 (m)	71.5	3.56 (m)
5′	73.0	4.07 (m)	75.6	3.75 (m)
6′	63.6	4.31 (dd, $J = 12.3, 4.5$), 4.21 (dd, $J = 12.3, 2.8$)	65.1	4.72 (br d, $J = 12.0$), 4.58 (dd, $J = 12.0, 5.1$)
1″	114.7		114.8	
2″	125.1	7.49 (m)	153.0	
3″	147.8		147.5	
4″	124.4	7.45 (br d, $J = 8.1$)	123.9	7.43 (dd, $J = 8.1, 1.2$)
5″	120.4	6.87 (t, $J = 8.1$)	120.3	6.88 (t, $J = 8.1$)
6″	123.8	7.42 (br d, $J = 8.1$)	124.8	7.59 (dd, $J = 8.1, 1.2$)
7″	169.8		171.1	
1‴	103.4	4.90 (d, $J = 8.4$)	103.2	4.91 (d, $J = 8.7$)
2‴	75.0	3.53 (m)	75.0	3.54 (m)
3‴	77.9	3.47 (m)	77.9	3.48 (m)
4‴	71.4	3.41 (m)	71.4	3.41 (m)
5‴	78.5	3.42 (m)	78.5	3.43 (m)
6‴	62.6	3.89 (d, $J = 11.5$), 3.70 (dd, $J = 12.3, 4.5$)	62.6	3.89 (br d, $J = 12.0$), 3.70 (m)
2′-O*C*OCH$_3$			171.9	
2′-OCO*C*H$_3$			21.2	2.00 (3H, s)
3′-O*C*OCH$_3$	172.0			
3′-OCO*C*H$_3$	20.7	1.96 (3H, s)		
6′-O*C*OCH$_3$	172.3			
6′-OCO*C*H$_3$	20.8	2.00 (3H, s)		

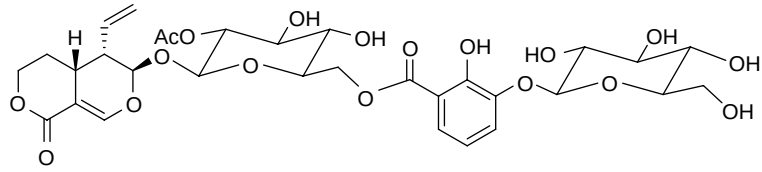
Note: Assignments were made on the basis of DEPT, HMBC, and HMQC experiments.

positions at 3′, 4′, 6′ of sweroside were acylated in **1** (Figure 1). The presence of two acetyl groups was suggested by the NMR spectral data [δ 1.96 (3H, s) and δ 20.7 and 172.0; δ 2.00 (3H, s) and δ 20.8 and 172.3], which was supported by HMBC and HMQC spectral data. The two acetyl groups were attached at C-3′ and C-6′ positions determined by the correlations of the carbonyl carbons of acetyl groups with H-3′ (δ 5.32, 1H, m) and H-6′ (δ 4.31, 1H, dd, $J = 12.3$, 4.5 Hz, H_a-6′; 4.21, 1H, dd, $J = 12.3$, 2.8 Hz, H_b-6′), respectively, in the HMBC spectrum (Figure 2).

Four signals at δ 7.49 (1H, m, H-2″), 7.45 (1H, br d, $J = 8.1$ Hz, H-4″), 6.87 (1H, t, $J = 8.1$ Hz, H-5″), and 7.42 (1H, br d, $J = 8.1$ Hz, H-6″) in the ^{1}H NMR spectrum and the corresponding carbon signals at δ 114.7 (C-1″), 125.1 (C-2″), 147.8 (C-3″), 124.4 (C-4″), 120.4 (C-5″), 123.8 (C-6″), and 169.8 (C-7″) (Table 1) implied the



1



2

Figure 1. Structures of compounds **1** and **2**.

presence of one 3-oxygen-substituted benzoyl group [12], which was determined by the HMBC and HMQC spectral data. In addition, the presence of one more β-D-glucopyranosyl moiety was deduced from the observation of anomeric signal at δ 4.90 (1H, d, $J = 8.4$ Hz, H-1‴) in the ^{1}H NMR spectrum and carbon signals at δ 103.4 (C-1‴), 75.0 (C-2‴), 77.9 (C-3‴), 71.4 (C-4‴), 78.5 (C-5‴), and 62.6 (C-6‴) in the ^{13}C NMR spectrum, which was confirmed by the detailed analysis of ^{1}H NMR, HMBC, and HMQC spectral data.

Cross peaks between H-1/C-1′ (δ 99.9), H-1′ (δ4.95, 1H, d, $J = 7.9$ Hz)/C-1 (δ98.6), H-4′ (δ 5.25, 1H, m)/C-7″, and H-1‴/C-3″ in the HMBC spectrum confirmed the connections of these substructures. Analysis of the fragments of EI-MS certified the correctness of the structural elucidation. From the above-mentioned evidence, the structure of **1** was elucidated to be 3′,6′-diacetyl-4′-{[3-(1-β-D-glucopyranosyloxy)]benzoyl}sweroside and named verbenoside A.

Compound **2** was isolated as a white amorphous powder, and its molecular formula was determined to be $C_{31}H_{38}O_{18}$ by HR-ESI-MS at *m/z* 721.1950 $[M + Na]^+$. The spectral data of compound **2** were almost identical with those of compound **1**, except for the absence of one acetyl group. Additionally, there was one more hydroxyl group at C-2″ in **2**, which was determined by the deshielded carbon signal of C-2″ (δ 153.0) in **2** (Table 1). Another obvious difference between **1** and **2** was due to the substituted positions of the acetyl group and the benzoyl moiety. According to the HMBC correlations between H-6′ (δ 4.72, 1H, br d, $J = 12.0$ Hz,

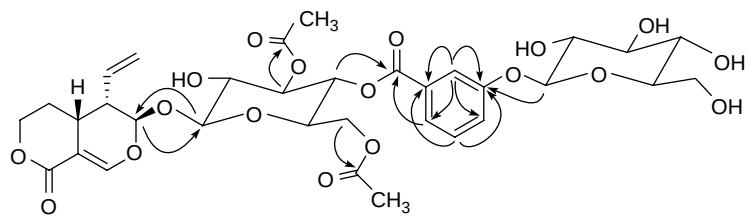


Figure 2. Key HMBC correlations of compound **1**.

H_a-6′; 4.58, 1H, dd, $J = 12.0, 5.1$ Hz, H_b-6′) and C-7″ at δ 171.1, H-2′ at δ 4.74 (1H, m) and the carbonyl at δ 171.9, the acetyl and the benzoyl moiety were assigned at C-2′ and C-6′ positions, respectively. Thus, the structure of **2** was determined to be 2′-acetyl-6′-{[2-hydroxy-3-(1-β-D-glucopyranosyloxy)]benzoyl}sweroside and named verbenoside B.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 MC using a 10 cm cell tube. The UV spectra were recorded on a Shimadzu UV-2201 spectrometer. The IR spectra were recorded on a Bruker IFS-55 spectrometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker-ARX-300 instrument and ^{13}C NMR (150 MHz) and 2D NMR spectra were recorded on a Bruker-AV-600 NMR instrument in CD_3OD. Chemical shifts were measured using residual MeOH-d_4 multiplet (δ_H 3.31 and δ_C 49.15) as an internal standard. HR-ESI-MS were recorded on a Bruker microTOF-Q spectrometer and EI-MS on GCMS-QP5050A. Silica gel (200–300 mesh for column chromatography and GF_{254} for TLC) was obtained from Qingdao Marine Chemical Company (Qingdao, China). Sephadex LH-20 was purchased from Amersham BioSciences (Uppsala, Sweden). Semipreparative HPLC was carried out using a Shimadzu system (LC-10AT pump, RID-10A detector, YMC-pack ODS-AM, AM-324: 300 × 10 mm).

3.2 Plant material

The aerial parts of *V. officinalis* L. was collected at Yichang (Hubei Province, China) in 2002 and the botanical identification was made by Prof. Changgong Zhang, Pharmacognosy Laboratory of Pharmacy College, Tongji Medical University. A voucher specimen (MBC 20020710) has been deposited at the Department of Pharmacognosy, Shenyang Pharmaceutical University, Shenyang, China.

3.3 Extraction and isolation

The aerial parts of *V. officinalis* (5 kg) were extracted with 95% EtOH (3 × 40 liters) at room temperature. The EtOH extract (350 g) was suspended in H_2O (5 liters) and partitioned with petroleum ether (3 × 5 liters), EtOAc (3 × 5 liters), and *n*-BuOH (3 × 5 liters), successively. The *n*-BuOH soluble extract (132 g) was subjected to silica gel column eluting with $CHCl_3$–MeOH (100:0 → 0:100) to yield 14 fractions (A–N). Fraction F (2.1 g, $CHCl_3$–MeOH 6:1) was subjected to silica gel column eluting with EtOAc–MeOH (100:0 → 0:100) to yield four fractions (FI–FIV). Fraction FIII (0.5 g, EtOAc–MeOH 8:1) was subjected to a Sephadex LH-20 column using MeOH to give two fractions (FIIIa and FIIIb). Fraction FIIIb (0.15 g) was purified by semipreparative HPLC (MeOH:H_2O 40:60, flow rate of 1.0 ml/min) to give **1** (5.2 mg) and **2** (9.6 mg).

3.3.1 Verbenoside A (*1*)

A white amorphous powder; $[\alpha]_D^{20} = -466.7$ ($c = 0.15$ in CH_3OH). UV (CH_3OH) λ_{max} nm (log ε): 313 (1.10), 243 (1.70), 211 (1.91). IR (KBr) ν_{max} cm^{-1}: 3420, 2968, 1617, 1400, 1251, 1055, 1015. ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD) spectral data: see Table 1; EI-MS *m/z* 300 (2), 247 (34), 197 (10), 179 (13), 151 (9), 137 (14), 127 (100); HR-ESI-MS *m/z* 747.2105 $[M + Na]^+$ (calcd for $C_{33}H_{40}O_{18}Na$, 747.2112).

3.3.2 Verbenoside B (*2*)

A white amorphous powder; $[\alpha]_D^{20} = -122.5$ ($c = 2.0$ in CH_3OH). UV (CH_3OH) λ_{max} nm (log ε): 310 (0.74), 243

(1.35), 214 (1.49). IR (KBr) ν_{max} cm^{-1}: 3418, 2924, 1617, 1401, 1249, 1067. ^1H NMR (300 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectral data: see Table 1; EI-MS *m/z* 523 (1), 503 (1), 429 (4), 195 (1), 179 (9), 127 (100); HR-ESI-MS *m/z* 721.1950 $[M + Na]^+$ (calcd for $C_{31}H_{38}O_{18}Na$, 721.1956).

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