Two New Sesquiterpene Glucosides from *Dennstaedtia scabra* (WALL.) MOORE

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Two new sesquiterpene glucosides onitioside A (1) and dennstoside B (2), were isolated from the 95% EtOH extract of *Dennstaedtia scabra* (WALL.) MOORE, together with seven known compounds, onitisin (3), pterosin A (4), pinocembrin (5), pinocembrin 7-rutinoside (6), kaempferol (7), nicotiflorin (8), and galangin (9). Their structures were determined by extensive spectroscopic analysis.

Key words *Dennstaedtia scabra*; sesquiterpene glucoside; onitioside A; dennstoside B

Dennstaedtia scabra (WALL.) MOORE, belonging to the Dennstaedtiaceae family, is widely distributed in south China. The whole plant is used as traditional Chinese medicine to treat cold, headache, rheumatism, and palsy.[1)] Previous phytochemical studies on this plant revealed the presence of pterosin-sesquiterpenes, illudane-type sesquiterpenes, and their glycosides.[2,3)] Many of these compounds exhibit various bioactivities such as antiprotozole[4)] and cytotoxic activities.[5,6)] As a part of our research work on bioactive metabolites from ferns of China,[7—9)] phytochemical investigation on the fronds of *D. scabra* was conducted and two new sesquiterpene glucosides (**1**, **2**) were isolated together with seven known compounds onitisin (**3**),[2)] pterosin A (**4**),[10)] pinocembrin (**5**),[11)] pinocembrin 7-rutinoside (**6**),[12)] kaempferol (**7**),[13)] kaempferol 3-rutinoside (**8**),[13)] and galangin (**9**).[14)] In the present paper, we report the isolation and structure elucidation of compounds **1** and **2**.

Results and Discussion

Compound **1** was obtained as colorless oil. Its molecular formula was determined as $C_{21}H_{30}O_9$ by HR-electrospray ionization (ESI)-MS (neg.) *m*/*z*: 425.1797 (Calcd for 425.1811). The IR spectrum showed the presence of hydroxyl (3416 cm^{-1}), carbonyl (1687 cm^{-1}), and an aromatic ring (1599, 1509, 1461 cm^{-1}) groups. The ^{13}C-NMR spectra of **1** revealed the presence of one glycopyranose moiety [δ 61.1 (t), 70.0 (d), 73.3 (d), 76.9 (d), 77.0 (d), 103.6 (d)]. The remaining carbons including an aromatic ring [δ 127.5 (s), 130.7 (s), 130.9 (s), 136.8 (s), 137.9 (s), 150.1 (s)], one ketone carbonyl δ 209.5 (s), three methyl [δ 12.7 (q), 12.9 (q), 21.8 (q)], four methylenes [δ 32.7 (t), 33.5 (t), 60.0 (t), 73.5 (t)], and one quaternary carbon δ 49.7 (s). Among them, two carbons [δ 60.0 (t), δ 3.40 (m); δ 73.5 (t), δ 3.29 (d), 3.93 (d)] were ascribed as oxygen-bearing atoms. Considering the types of compounds previously isolated from this plant,[2,10)] together with the characteristic NMR signals discussed above, compound **1** can be ascribed to be a pterosin-sesquiterpene glycoside.

The ^{1}H- and ^{13}C-NMR data of **1** were very similar to those of **3** except that **1** had a more glycopyranose moiety. The locations of the sugar units of **1** were confirmed by a heteronuclear multiple bond coherence (HMBC) experiment (Fig. 2), in which correlations of H-1′ (δ 4.10) with C-10a (δ 73.5), H-10a (δ 3.29, 3.93) with [C-1 (δ 209.5), C-2 (δ 49.7), C-3 (δ 33.5), C-10b (δ 21.8)] were observed. Acidic hydrolysis of **1** gave D-glucose moiety, which was determined to have a β configuration on the basis of the large coupling constants of the anomeric protons [δ 4.10 (d, J=7.6 Hz)]. Assignment of glucosidic protons system was achieved by analysis of ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC) experiments.

Acidic hydrolysis of **1** also afforded aglycone (**1a**), which was suggested to be compound **3** by comparing their NMR data (Table 1) and optical rotation values $[\alpha]_D^{15.3}$ −24.65 (c=0.14, MeOH).[2)] Thus the structure of compound **1** was elucidated as shown in Fig. 1 and named onitioside A.

Compound **2**, obtained as colorless oil, had a molecular formula of $C_{32}H_{40}O_{12}$ determined by HR-ESI-MS (neg.) *m*/*z*: 615.2469 (Calcd for 615.2442). Its IR absorptions indicated the presence of hydroxyl groups (3428 cm^{-1}), a ketone group (1723 cm^{-1}), and an aromatic ring group (1604, 1515, 1446 cm^{-1}). The ^{13}C-NMR spectra showed resonances characteristic of a sesquiterpene, a glycopyranose moiety [δ 62.3 (t), 76.4 (d), 72.2 (d), 73.7 (d), 74.4 (d), 96.8 (d)], a *p*-coumaroyl group [δ 167.0 (s), 114.8 (d), 146.3 (d), 126.7 (d), 2×131.1 (d), 2×116.7 (d), 160.8 (s)], and an acetyl group [δ 20.9 (q), 169.8 (s)]. The double bond of the *p*-coumaroyl group was suggested as *trans*-due to the coupling constant (J=15.5 Hz). The sesquiterpene possessed a ketone carbonyl δ 221.4, two olefinic carbons [δ 124.2, 141.7], an oxygenated secondary carbon δ 67.1, two oxygenated quaternary carbons [δ 72.3, 83.3], and two methylenes in upfield [δ 7.6, 8.3]. Along with the knowledge of previously isolated compounds from this plant,[2,3)] the basic skeleton of compound **2** was identified as an illudane-type sesquiterpene.

By carefully comparing the MS and NMR data of **2** with those of illudane-type sesquiterpene glycosides,[3)] compound **2** might be $C_9H_8O_3$ acid ester of dennstoside A. Assignment of glycosidic protons system was achieved by analysis of ^{1}H–^{1}H COSY and HSQC experiments. Connectivities of the sugar, acetyl group, and *p*-coumaroyl moiety were confirmed by the HMBC correlations of H-1′ (δ 4.76) with C-4 (δ 83.3), H-2′ (δ 4.79) with C-1‴ (δ 169.8) and H-4′ (δ 4.87) with C-1″ (δ 167.0), respectively. Comparing the NMR data

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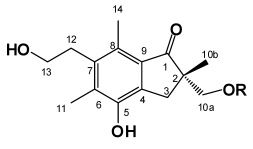
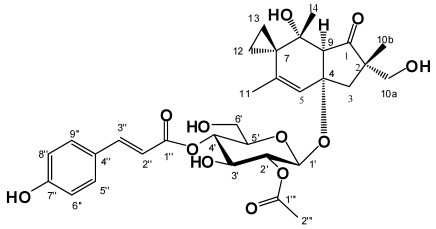
Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1**[a], **1a**[a] and **2**[b]. δ in ppm, J in Hz

No.	**1**		**2**		**1a**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		209.5 (s)		221.4 (s)		210.8 (s)
2		49.7 (s)		53.3 (s)		50.9 (s)
3	H_α: 3.18 (d, 17.2)	33.5 (t)	H_α: 2.74 (d, 14.4)	44.3 (t)	H_α: 3.09 (d, 17.6)	33.5 (t)
	H_β: 2.60 (d, 17.2)		H_β: 1.83 (d, 14.4)		H_β: 2.54 (d, 17.6)	
4		130.7 (s)		83.3 (s)		130.4 (s)
5		150.1 (s)	5.83 (br s)	124.2 (d)		150.0 (s)
6		130.9 (s)		141.7 (s)		131.7 (s)
7		136.8 (s)		30.8 (s)		136.6 (s)
8		127.5 (s)		72.3 (s)		127.2 (s)
9		137.9 (s)	2.67 (br s)	65.1 (d)		138.3 (s)
10a	3.93 (d, 9.2)	73.5 (t)	3.24 (d, 10.4)	67.1 (t)	3.50 (d, 10.4)	66.6 (t)
	3.29 (d, 9.2)		3.70 (d, 10.4)		3.28 (d, 10.4)	
10b	1.03 (s)	21.8 (q)	1.03 (s)	20.5 (q)	0.95 (s)	21.1 (q)
11	2.21 (s)	12.9 (q)	1.54 (br s)	19.7 (q)	2.21 (br s)	12.8 (q)
12	2.80 (t, 7.6)	32.7 (t)	0.54 (m)	8.2 (t)	2.80 (t, 8.0)	32.7 (t)
				1.15 (m)		
13	3.40 (t, 7.6)	60.0 (t)	0.82 (m)	7.6 (t)	3.39 (t, 8.0)	60.0 (t)
14	2.47 (s)	12.7 (q)	1.12 (s)	23.6 (q)	2.47 (s)	12.7 (q)
Glc						
1′	4.10 (d, 7.6)	103.6 (d)	4.76 (d, 8.0)	96.8 (d)		
2′	2.83 (m)	73.3 (d)	4.79 (t, 8.0)	74.4 (d)		
3′	3.05 (overlapped)	77.0 (d)	3.87 (t, 9.2)	73.7 (d)		
4′	2.97 (overlapped)	70.0 (d)	4.87 (t, 9.2)	72.2 (d)		
5′	2.98 (overlapped)	76.9 (d)	3.54 (overlapped)	76.4 (d)		
6′	3.61 (d, 11.6)	61.1 (t)	3.55 (overlapped)	62.3 (t)		
	3.38 (d, 11.6)					
p-Coumaroyl						
1″				167.0 (s)		
2″			6.33 (d, 15.6)	114.8 (d)		
3″			7.63 (d, 15.6)	146.3 (d)		
4″				126.7 (s)		
5″ 9″			7.55 (d, 8.8)	131.1 (d)		
6″ 8″			6.89 (d, 8.8)	116.7 (d)		
7″				160.8 (s)		
Acetyl						
1‴				169.8 (s)		
2‴			1.93 (s)	20.9 (q)		

a) Measured in DMSO-d_6. *b*) Determined in acetone-d_6.



1 R = Glc
3 R = H

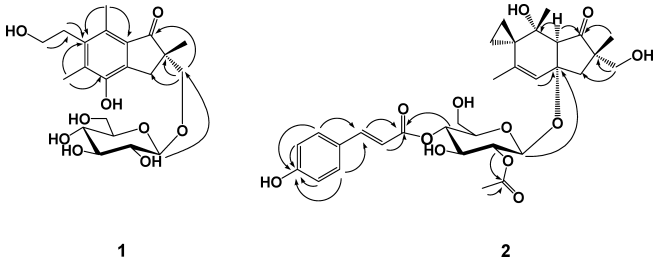


2

Fig. 1. Structures of Compounds **1**—**3**

with those of the known compounds,[15] the hexose should be glucose.

The relative stereochemistry of **2** was deduced from rotating frame Overhauser effect spectroscopy (ROESY) data. The cross peaks in the ROESY spectrum between H_β-3 (δ 1.83) and H-10b, H-3 (δ 1.83) and H-14, H-10b and H-14 showed that these hydrogens were in β-orientation, while the correlations between H_α-3 (δ 2.74) and H-1′, H-1′, and H-9 indicated that these hydrogens were α-oriented. Thus the stereochemistry of **2** was revealed the same as dennstoside



1 **2**

Fig. 2. Selected HMBC Correlations for Compounds **1** and **2**

A.[3] Therefore the structure of **2** was established as shown in Fig. 1, and named dennstoside B.

Experimental

General Experimental Procedures Optical rotation was measured on a Horiba SEPA-300 polarimeter. IR spectra were obtained by Tensor 27 FT-IR spectrometer with KBr pellets. The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AV-400 spectrometers in acetone-d_6 and DMSO-d_6 at room temperature (δ in ppm, J in Hz). FAB-MS was carried out on a VG Autospec-3000 spectrometer. HR-ESI-MS was recorded with an API QSTAR Pulsar i spectrometer. Silica gel (200—300 mesh), Silica gel H (Qingdao Marine Chemical Ltd., China), and LiChroprep RP-18 silica gel (40—63 μm, Merck, Dramstadt, Germany) were used for column chromatography. Fractions were monitored by TLC and spots visualized by heating silica gel plates immersed with 15% H_2SO_4 in ethanol. Solvents were distilled prior to use. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Shimadzu PRC-ODS (K) column. Sephadex LH-20 (Amersham Pharmacia biotech, Sweden).

Plant Material The aerial parts of *D. scabra* were collected from Jinpin, Yunnan Province, China in July 2007 and identified by professor Xiao Cheng at Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 200706A03) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The dried and powdered plant materials (1.1 kg) were extracted with 95% ethanol (8.0 l, each 2 d) three times. After evaporation of the solvent *in vacuo*, the concentrate was suspended into H_2O and partitioned successively with ethyl acetate. The ethyl acetate extract (30 g) was chromatographed on a silica gel column eluted with $CHCl_3$–MeOH (1 : 0 to 5 : 5) to give five fractions 1—5. Fraction 2 was further subjected to column chromatograph (CC) over silica gel (petroleum ether–acetone 9 : 1 to 6 : 4) to obtain four subfractions 2.1—2.4. Subfraction 2.2 was subjected to silica gel CC using petroleum ether–acetone (6 : 1) as eluent to yield **3** (20 mg) and **4** (826.5 mg). Subfraction 2.3 was subjected to Sephadex LH-20 ($CHCl_3$: MeOH = 1 : 1) to obtain **5** (50 mg), **7** (10 mg) and **9** (12 mg). Fraction 3 was eluted with $CHCl_3$–MeOH (1 : 1) over silica gel CC then further purified by HPLC (MeOH : H_2O = 38 : 62) and (MeOH : H_2O = 52 : 48) to yield **1** (132 mg) and **2** (14 mg). Fraction 4 was subjected to Sephadex LH-20 ($CHCl_3$: MeOH = 1 : 1) to obtain two subfractions 4.1—4.2. Fraction 4.1 was further purified by HPLC (MeOH : H_2O = 40 : 60) to obtain **8** (86 mg). Fraction 4.2 was first eluted with $CHCl_3$–MeOH (6 : 1) over silica gel then further purified by Sephadex LH-20 ($CHCl_3$: MeOH = 1 : 1) to yield **6** (2.5 g).

Onitioside A (**1**): Colorless oil. $[\alpha]_D^{19.3}$ −5.49 (c = 0.25, MeOH). UV λ_{max} (MeOH) nm (ε): 271.6 (5955), 231.6 (9926), 215.6 (9638), 198.6 (6847). IR (KBr) cm^{-1}: 3416, 2925, 1687, 1599, 1461, 1380, 1306, 1161, 1078, 1039. ^{1}H- and ^{13}C-NMR: see Table 1. FAB-MS (neg.) m/z: 425 $[M-H]^-$, 263 $[M-162-H]^-$. HR-ESI-MS (neg.) m/z: 425.1797 (Calcd for $C_{21}H_{29}O_9^-$, 425.1811).

Dennstoside B (**2**): Colorless oil. $[\alpha]_D^{18.3}$ −133.7 (c = 0.09, MeOH). UV (MeOH) λ_{max} (MeOH) nm (ε): 314.4 (16040), 212.6 (19248), 196.2 (11067). IR (KBr) cm^{-1}: 3428, 2932, 1723, 1631, 1604, 1515, 1446, 1376, 1248, 1165, 1046. ^{1}H- and ^{13}C-NMR: see Table 1. FAB-MS (neg.) m/z: 615 $[M-H]^-$. HR-ESI-MS (neg.) m/z: 615.2469 (Calcd for $C_{32}H_{39}O_{12}^-$, 615.2442).

Acidic Hydrolysis of Compound 1 Compound **1** (8 mg) was hydrolyzed with 2 M HCl–dioxane (1 : 1, 4 ml) under reflux for 6 h. The reaction mixture was extracted with $CHCl_3$ five times (4 ml×5) to obtain the aglycone. It was suggested to be compound **3** by comparing their NMR data and optical rotation values $[\alpha]_D^{15.3}$ −24.65 (c = 0.14, MeOH). The aqueous layer was neutralized with 2 M $NaHCO_3$ then dried to give a monosaccharide mixture. Then, a solution of the sugar mixture in pyridine (2 ml) was added to L-cysteine methyl ester hydrochloride (about 1.5 mg) and kept at 60 °C for 1 h. Next, trimethylsilylimidazole (about 1.5 ml) was added to the reaction mixture in ice water and kept at 60 °C for 30 min. The mixture was subjected to GC analysis, run on a Shimadzu GC-14C gas chromatograph equipped with a 30 m×0.32 mm i.d. 30QC2/AC-5 quartz capillary column and an H_2 flame ionization detector with the following conditions: column temperature, 180—280 °C; programmed increase, 3 °C/min; carrier gas, N_2 (1 ml/min); injector and detector temperature, 250 °C; injection volume, 4 μl; and split ratio, 1/50. The configuration of D-glucose for compound **1** was determined by comparison of the retention times of the corresponding derivatives with that of standard D-glucose, giving a peak at 19.066 min.

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