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Two New Iridoid Glycosides from the Root Barks of *Sambucus williamsii* Hance

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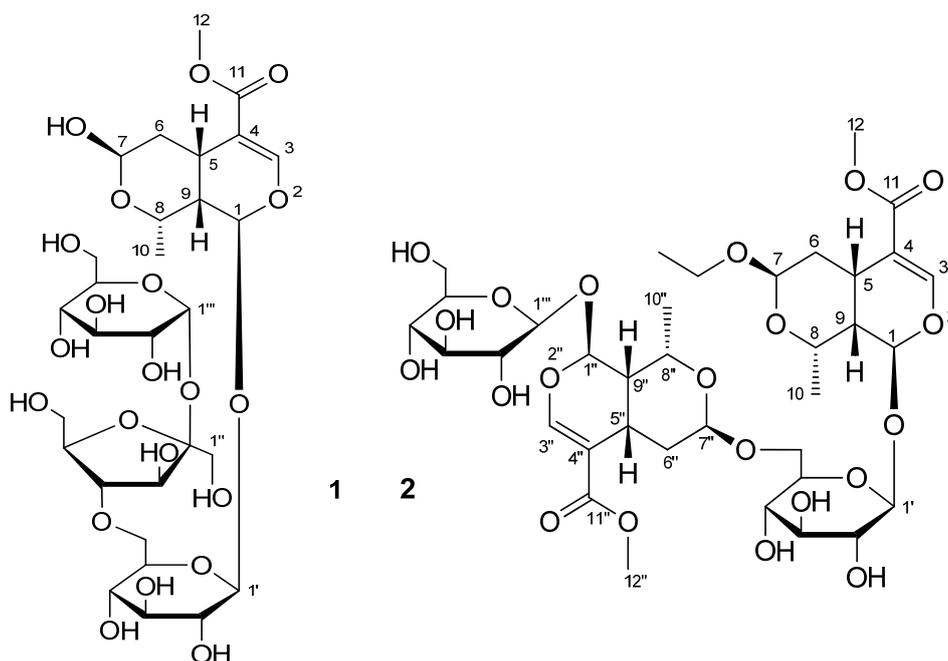
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Abstract: Two new iridoid glycosides, named williamsoside C (**1**) and williamsoside D (**2**) were isolated from the root barks of *Sambucus williamsii* Hance. Their structures were established on the basis of extensive spectroscopic analysis (1D, 2D NMR and HRESIMS) and chemical studies as α -D-glucopyranosyl (1 \rightarrow 2)- β -D-fructofuranosyl (4 \rightarrow 6)- β -morroneiside (**1**) and 7 β -O-ethyl morroneiside-(6'-O-7'')- β -morroneiside (**2**), respectively.

Keywords: *Sambucus williamsii* Hance; root barks; iridoid glycosides

1. Introduction

Sambucus williamsii Hance is a deciduous shrub or small tree widely distributed in China [1] and used for centuries for the treatment of inflammation [2] and bone fractures and joint diseases [3]. The chemical composition of *S. williamsii* has been extensively studied. Triterpenoids, flavonoids, lignans and the iridoids were reported [4,5]. In our present work, we investigated fraction of the root barks of *S. williamsii* obtained from a macroporous resin by elution with 50% ethanol. Our extraction and separation method can greatly enrich fractions in iridoid compounds so trace iridoids can be isolated. In this paper, we present the isolation and structural characterization of the two new iridoid morroneisides (in Figure 1) on the basis of the interpretation of their spectral data, including 1D, 2D NMR and HRESIMS data.

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

2. Results and Discussion

Compound **1** was obtained as a white amorphous powder and showed positive results for the Molisch reagent. Its molecular formula was determined as $C_{29}H_{46}O_{21}$ by the positive HRESIMS data. The UV spectrum of the compound displayed an absorption maximum at 239 nm, which is the characteristic of an iridoid skeleton, and intense IR bands at $3,450$ and $1,708\text{ cm}^{-1}$, which indicated the presence of hydroxyl and ester carbonyl functionalities, respectively.

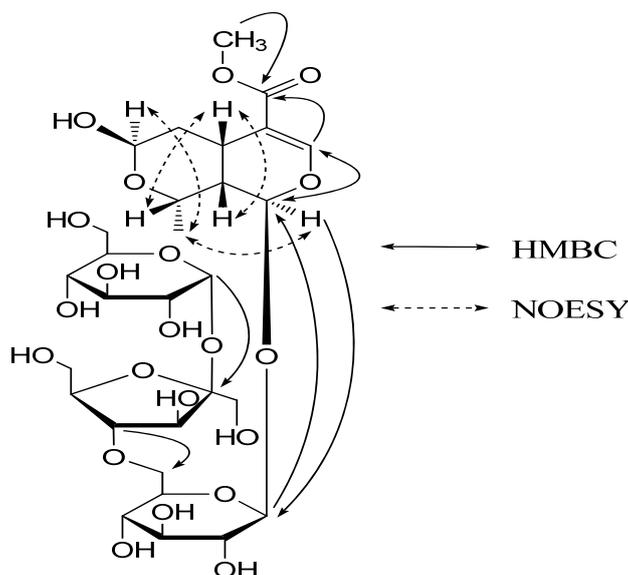
Detailed interpretation of 1D and 2D NMR data of **1** confirmed an iridoid structure, the presence of two glucopyranosyl and a fructofuranosyl moieties. The $^1\text{H-NMR}$ spectrum (Table 1) of **1** in CD_3OD showed signals diagnostic for an iridoid glycoside at δ_{H} 5.89 (1H, d, $J = 9.2$ Hz, H-1), 7.50 (1H, s, H-3), 4.78 (1H, d, $J = 7.6$ Hz, H-1') and 5.43 (1H, d, $J = 3.8$ Hz, H-1'''). In addition, the signals at δ_{H} 3.68 (3H, s) and 1.33 (3H, d, $J = 6.8$ Hz) were attributed to Me-12 and Me-10, respectively. The $^{13}\text{C-NMR}$ spectrum of **1** (Table 1) showed resonances for 29 C-atoms, including two quaternary carbons, six methines, one methylenes, and two methyls belonging to the aglycone moiety, two glucopyranosyl groups and a fructofuranosyl group.

Comparison of the NMR data of **1** and β -morrisonide [4] indicated the presence of an additional fructofuranosyl and an additional glucopyranosyl unit. In addition, the NMR data of C-6' of **1** were significantly deshielded by comparison with those of β -morrisonide. This indicated that **1** was a β -morrisonide derivative with a fructofuranosyl and a glucopyranosyl moieties located at C-6', which was verified by correlations from H_2 -6' to C-4'', from H-4'' to C-6', from H-1''' to C-2'' and from H-2'' to C-1''' in the HMBC spectrum of **1**. Acid hydrolysis of **1** afforded glucose and fructose, which were identified by TLC comparison with authentic samples. The glucose and fructose isolated from the hydrolysate gave optical rotations of $[\alpha]_{\text{D}}^{20} +35.3$ (c 0.05, MeOH) and $[\alpha]_{\text{D}}^{20} -90.2$ (c 0.025, MeOH), respectively, indicating that they were D-glucose and D-fructose, respectively [6–9]. The coupling constants of anomeric signals indicated that terminal glucosyl linkage is in α -configuration and inner

glucosyl linkage is in β -configuration. Comparison of ^{13}C -NMR chemical shifts with literature data [10,11] indicated D-fructose in **1** is in β -configuration.

The stereochemistry of **1** was established based on the NOESY experiment (Figure 2). The NOESY spectrum showed correlations of H-1/Me-10, Me-10/H-7, H-5/H-8 and H-5/H-9. These data indicated that compound **1** has the same stereochemistry at C-5, C-7, C-8 and C-9 as that of β -morrisonide and the hydroxy group bound to C-7 is in the β orientation. Thus, the structure of **1** was identified to be α -D-glucopyranosyl (1 \rightarrow 2)- β -D-fructofuranosyl (4 \rightarrow 6)- β -morrisonide, with the structure shown in Figure 1, and it was named williamsoside C.

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



Compound **2** was obtained as a white amorphous powder and showed positive results for the Molisch reagent. Its molecular formula was established as $\text{C}_{36}\text{H}_{54}\text{O}_{21}$ by the positive HRESIMS data. The IR absorption bands at $3,355$ and $1,692\text{ cm}^{-1}$, respectively, indicated the presence of hydroxyl groups and a carbonyl group in the molecule.

The ^1H - and ^{13}C -NMR spectra (Table 1) of **2** resemble those of β -morrisonide [4], but is more complex. Whereas the ^1H - and ^{13}C -NMR spectrum of **2** shows two sets of signals, this distribution of signals is consistent with a dimeric structure. The analysis of NMR spectra indicated the presence of two distinct iridoid units, which are hereafter referred to as units A and B. The ^1H -NMR spectrum showed signals at δ_{H} 7.50 (1H, s), 5.84 (1H, d, $J = 9.5$ Hz), 1.39 (1H, dt, $J = 3.7, 13.6$ Hz), 1.85 (1H, dd, $J = 4.7, 13.6$ Hz), 4.76 (1H, br.d, $J = 4.6$ Hz) and 1.29 (3H, d, $J = 6.9$ Hz) which were assigned to H-3, H-1, H-6, H-7 and Me-10 of unit A, based on analysis of the HMQC and HMBC spectra. Analysis of the ^{13}C -NMR spectra revealed signal values similar to those reported for 7β -O-ethyl morrisonide [12]. The remaining spectral data revealed a second iridoid unit due to part B of the new iridoid. Signals at δ_{H} 7.50 (1H, s), 5.87 (1H, d, $J = 9.7$ Hz), 1.49 (1H, dt, $J = 3.7, 13.6$ Hz), 1.97 (1H, dd, $J = 4.6, 13.7$ Hz), 4.94 (1H, d, $J = 3.2$ Hz) and 1.34 (3H, d, $J = 6.9$ Hz) in the ^1H -NMR spectrum were assigned to H-3'', H-1'', H-6'', H-7'' and Me-10'' of unit B. One significant difference observed for this part was the lack of an ethoxy group. The ^{13}C -NMR data of this moiety indicated signals identical to those of β -morrisonide [4], which was confirmed by 2D NMR data analysis (Table 1).

Table 1. ¹H- and ¹³C-NMR data for compounds **1** and **2** in CD₃OD (400 MHz for ¹H and 100 MHz for ¹³C).

No.	1		No.	2	
	δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)
1	95.7	5.89 (1H, d, 9.2)	1	96.0	5.84 (1H, d, 9.5)
3	154.5	7.50 (1H, s)	3	154.5	7.50 (1H, s)
4	111.7	-	4	111.7	-
5	28.0	3.06 (1H, dt, 4.4, 12.8)	5	27.8	3.06 (1H, m)
6	33.7	1.51 (1H, dt, 4.8, 13.6) 1.97 (1H, dd, 3.6, 13.6)	6	34.0	1.39 (1H, dt, 3.7, 13.6) 1.85 (1H, dd, 4.7, 13.6)
7	92.7	5.00 (1H, br.d, 3.2)	7	97.8	4.76 (1H, br.d, 4.6)
8	66.5	4.36 (1H, dq, 2.0, 6.8)	8	66.0	4.26 (1H, dq, 2.1, 6.9)
9	40.3	1.83 (1H, m)	9	40.4	1.79 (1H, m)
10	19.7	1.33 (3H, d, 6.8)	10	19.7	1.29 (3H, d, 6.9)
11	168.7	-	11	168.6	-
12	51.8	3.68 (3H, s)	12	51.8	3.68 (3H, s)
1'	100.1	4.78 (1H, d, 7.6)	-OCH ₂ CH ₃	63.6	3.41 (1H, o) 3.64 (1H, o)
2'	75.0	3.21 (1H, m)			
3'	78.0	3.36 (1H, m)	-OCH ₂ CH ₃	15.4	1.19 (3H, t, 7.1)
4'	71.6	3.26 (1H, m)	1'	100.4	4.78 (1H, d, 7.9)
5'	78.6	3.06 (1H, dt, 4.4, 12.8)	2'	75.4	3.20 (1H, m)
6'	69.4	3.84 (2H, o)	3'	78.0	3.37 (1H, m)
1''	63.8	3.62 (2H, s)	4'	71.8	3.34 (1H, m)
2''	105.6	-	5'	76.8	3.47 (1H, m)
3''	78.7	4.10 (1H, d, 8.4)	6'	68.0	3.62 (1H, o) 3.96 (1H, dd, 1.8, 12.0)
4''	76.3	3.99 (1H, t, 8.2)			
5''	82.1	3.90 (1H, o)	1''	95.7	5.87 (1H, d, 9.7)
6''	62.3	3.80 (1H, o) 3.72 (1H, o)	3''	154.5	7.50 (1H, s)
1'''	93.4	5.43 (1H, d, 3.8)	4''	111.8	-
2'''	73.3	3.41 (1H, m)	5''	28.0	3.06 (1H, m)
3'''	74.8	3.68 (1H, m)	6''	34.0	1.49 (1H, dt, 3.7, 13.6) 1.97 (1H, dd, 4.6, 13.7)
4'''	71.4	3.34 (1H, m)	7''	99.3	4.94 (1H, d, 3.2)
5'''	74.2	3.84 (1H, m)	8''	66.4	4.30 (1H, dq, 2.1, 7.0)
6'''	62.8	3.67 (1H, dd, 4.8, 11.8) 3.86 (1H, dd, 2.0, 11.8)	9''	40.3	1.79 (1H, m)
			10''	19.8	1.34 (3H, d, 6.9)
			11''	168.7	-
			12''	51.8	3.66 (3H, s)
			1'''	100.1	4.78 (1H, d, 7.9)
			2'''	75.0	3.20 (1H, m)
			3'''	78.0	3.37 (1H, m)
			4'''	71.6	3.28 (1H, m)
			5'''	78.5	3.28 (1H, m)
			6'''	62.8	3.63 (1H, o) 3.86 (1H, dd, 2.0, 11.6)

The absence of the ethoxyl of unit B, when compared to β -morrisonide, and observation of deshielding of C-6' (δ_C 68.0), when compared to the 7 β -O-ethyl morroniside (C-6', δ_C 62.8), was the first indication for the attachment of units A and B between C-6' and C-7''. The partial structures A and B were reasonably connected to each other by HMBC correlations. The correlations from H₂-6' with C-7'' and from H-7'' with C-6' in HMBC strongly indicated the connection between unit A and B through an ether linkage between C-6' and C-7''.

The stereo configuration of the substituent group at C-7 and C-7'' was determined to be the β -orientation on the basis of the obvious NOESY correlations between H-7/Me-10, Me-10/H-1, H-7''/Me-10'' and Me-10''/H-1''. On the basis of above data, the structure of **2** was identified to be 7 β -O-ethyl morroniside-(6'-O-7'')- β -morrisonide, with the structure shown in Figure 1, and it was named williamsoside D.

3. Experimental

3.1. General

Optical rotations were measured with a PE-241 digital polarimeter. UV spectra were recorded on a Shimadzu UV-1601 spectrometer. IR spectra were recorded on a Shimadzu FTIR-8400S spectrometer. NMR spectra were recorded on a Bruker DPX 400 NMR instrument (at 400 MHz for ¹H-NMR and 100 MHz for ¹³C-NMR). Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) used as internal standard, and coupling constants are given in Hz. HRESIMS were carried out on Waters Xevo QTOF mass spectrometer. Preparative HPLC (Waters, Delta 600-2487) was performed on a Hypersil-ODS II column (10 μ m, 20 \times 300 mm, Yilite, Dalian, China).

3.2. Plant Material

The root barks of *S. williansii* were collected in August 2008 from the Fangzheng district, Heilongjiang Province, China, and identified by the author Zhen-Yue Wang. A voucher specimen (20080079) has been deposited at Heilongjiang University of Chinese Medicine, Harbin, China.

3.3. Extraction and Isolation

The dried root barks (5.0 kg) were extracted with 95% EtOH (2 \times 10 L) for 2 h. The EtOH extracts was concentrated under reduced pressure and fractioned on an AB-8 macroporous resin column (8 \times 60 cm) with H₂O, 50% and 95% EtOH-H₂O to give three fractions (H₂O fraction, 50% EtOH-H₂O fraction, 95% EtOH-H₂O fraction). The 50% EtOH-H₂O fraction (52.0 g) was repeatedly column chromatographed on silica gel with a gradient of CHCl₃/MeOH (15:1 \rightarrow 1:1) solvents as eluents to afford 10 fractions: Fraction 1–10. Fraction 4 (20 g) continues silica gel chromatography elution with CHCl₃/MeOH (10:1 to 5:1) to afford a number of sub-fractions A1–A4. Compound **1** (45.5 mg) was obtained by prep. HPLC chromatography of the sub-fraction A2 (3 g) and elution with MeOH/H₂O (2:5). A3 (7 g) was separated on ODS-A column with MeOH/H₂O (1:4 to 1:0) as eluent, to produce five sub-fractions (B1–B5). The sub-fraction B3 (2 g) was purified by prep. HPLC with MeOH/H₂O (3:10) to afford **2** (35.1 mg).

Williamsoside C (1): White amorphous powder. $[\alpha]_D^{20} -16.5$ (c 0.075, MeOH). UV (MeOH) λ_{max} (log ϵ) nm: 239 (3.10). IR (KBr): $\nu = 3,450, 2,500, 1,708, 1,632 \text{ cm}^{-1}$. HRESIMS (positive): $m/z = 731.2619$ (calc. for $C_{29}H_{47}O_{21}$, 731.2610, $[M+H]^+$), 1H - and ^{13}C -NMR: see Table 1.

Williamsoside D (2): White amorphous powder. $[\alpha]_D^{20} -12.5$ (c 0.05, MeOH). UV (MeOH) λ_{max} (log ϵ) nm: 241 (3.05). IR (KBr): $\nu = 3,355, 2,450, 1,692, 1,637 \text{ cm}^{-1}$. HRESIMS (positive): $m/z = 823.3230$ (calc. for $C_{36}H_{55}O_{21}$, 823.3236, $[M+H]^+$), 1H and ^{13}C -NMR: see Table 1.

Acid Hydrolysis of **1** and **2**. To a solution of **1** and **2** (each, 15 mg) in MeOH (5 mL) was added 5% H_2SO_4 (5 mL) and the mixture was refluxed for 8 h. Each reaction mixture was then neutralized with saturated sodium carbonate and extracted with ethyl acetate (EtOAc, 2×10 mL) to give an aqueous fraction containing sugars and an EtOAc fraction containing the aglycone part. The aqueous phase was dried by using a N_2 stream. The residues were separately subjected to CC over silica gel with MeCN- H_2O (8:1) as the eluent to yield glucose and fructose from **1**, and glucose from **2**, respectively. The solvent systems $Me_2CO/H_2O/CHCl_3/MeOH$ (15:1:2:2) and $CHCl_3/MeOH/water$ (6:4:1) [6,7] were used for TLC identification of glucose and fructose.

4. Conclusions

As a part of our chemical investigation on *S. williamsii*, two new iridoid glycosides, α -D-glucopyranosyl (1 \rightarrow 2)- β -D-fructofuranosyl (4 \rightarrow 6)- β -morrnioniside (**1**) and 7 β -O-ethyl morroniside-(6'-O-7'')- β -morrnioniside (**2**) were isolated. Their structures were established on the basis of spectroscopic evidence. The discovery of compounds **1** and **2** represents a further addition to number and diversity of iridoid glycoside compounds.

Acknowledgments

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Sample Availability: Samples of williamsosides C and D are available from the authors.

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