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PHENYLPROPANOIDS AND FLAVONOID GLYCOSIDES FROM *LYSIONOTUS PAUCIFLORUS*

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Key Word Index—*Lysionotus pauciflorus*; Gesneriaceae; phenylpropanoid glycosides; flavone glycosides; nevadensin.

Abstract—A new phenylpropanoid glycoside, α-(3,4-dihydroxyphenyl)ethyl-(2′-*O*-α-L-rhamno-pyranosyl-3′-*O*-β-D-apiofuranosyl-4′-*O*-*E*-caffeoyl)-β-D-glucopyranoside, verbascoside, and two new flavone glucosides, nevadensin 7-*O*-β-D-glucoside and nevadensin 7-*O*-[α-L-rhamnosyl(1 → 6)]-β-D-glucoside, have been isolated from the aerial parts of *Lysionotus pauciflorus*. The structures have been elucidated on the basis of spectroscopic data and chemical correlation.

INTRODUCTION

Lysionotus pauciflorus is widely distributed in southern China and has been used in traditional Chinese medicine for the treatment of lymph node tuberculosis, cough with tachypnoea and rheumatic pains [1]. As a result of our studies on the glycosidic constituents we have already reported the isolation and structural determination of a new phenylpropanoid α-(3,4-dihydroxyphenyl)ethyl-[3′,6′-*O*-bis(β-D-apiofuranosyl)-4′-*O*-*E*-caffeoyl]-β-D-glucopyranoside (paucifloside) [2] and nevadensin 5-*O*-glucosides [3]. This paper deals with the isolation and structural elucidation of further phenylpropanoid and flavone glycoside components of this plant.

RESULTS AND DISCUSSION

The *n*-butanol-soluble fraction of the methanolic extract of the powdered aerial parts of *L. pauciflorus* was separated by repeated column chromatography using Sephadex LH-20 and polyamide with methanol and water as eluents. Compounds **1**, **2**, **3** and **4** were purified finally by preparative HPLC on RP-18 material.

Compounds **1** and **4** showed a strong fluorescence on the TLC plate (UV 365 nm), which could be intensified by natural product/PEG reagent. Acid hydrolysis with 2 N TFA at 100°C yielded apiose, rhamnose, and glucose in case of **1**, and rhamnose and glucose in case of **4**, respectively. The sugars were identified by TLC analysis. The positive-ion FAB-mass spectrum of **1** showed a quasi molecular ion peak at *m/z* 763 $[M+Li]^+$, corresponding to the molecular formula $C_{34}O_{19}H_{44}$, and a fragment ion at *m/z* 467 $[M+Li-(\text{pentose}-H_2O)-(\text{desoxy}-\text{hexose})]^+$ indicative of the cleavage of a pentose and a desoxy-hexose. The positive-ion FAB-mass spectrum of **4** showed a quasi molecular ion peak at *m/z* 631 $[M+Li]^+$, corresponding to the molecular formula $C_{29}O_{15}H_{36}$, and a fragment ion at *m/z* 485 $[M+Li-(\text{desoxy}-\text{hexose})]^+$, indicative of the cleavage of a desoxy-hexose.

The ^{1}H NMR spectra of **1** and **4** revealed the characteristic signals of β-(3,4-dihydroxyphenyl)ethoxy and *E*-caffeoyl units by comparsion with reported data [3–5]. Eight aromatic and olefinic protons were assigned for both compounds by integration of the signals. Two doublets of **1** at δ 6.25 and 7.59 with the intensity of one proton each and a coupling constant of 15.9 Hz indicated the presence of a double bond with the *E* configuration of the caffeic acid moiety. Two aromatic ABX systems were observed between δ 6.25–7.59. A doublet with a coupling constant $J = 8$ Hz at δ 6.69, a doublet with a coupling constant $J = 2$ Hz at δ 6.97 and a double doublet with coupling constants $J = 8$ and 2 Hz at δ 5.58 belonged to the first ABX system. A doublet at δ 7.07, a doublet at δ 6.80 and a double doublet at δ 6.98 with the same corresponding coupling constants as the first ABX system belonged to

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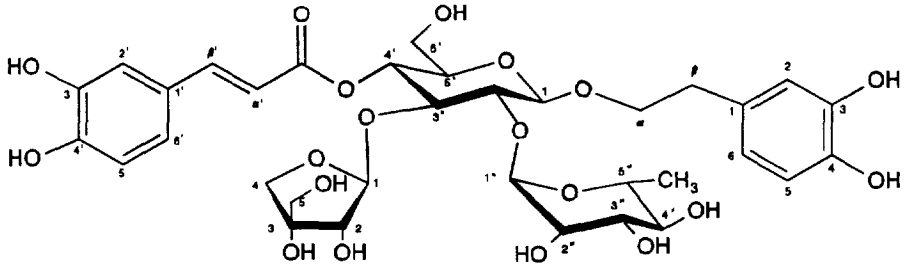
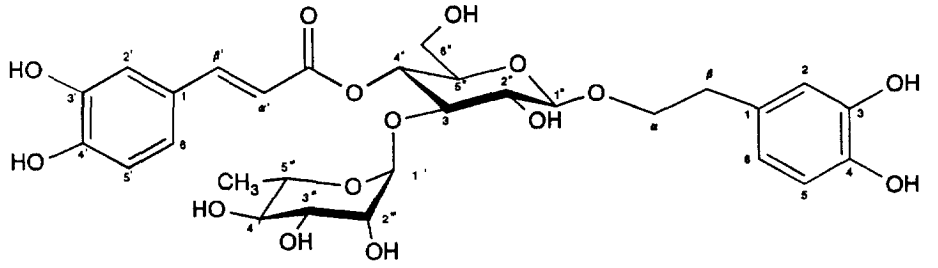
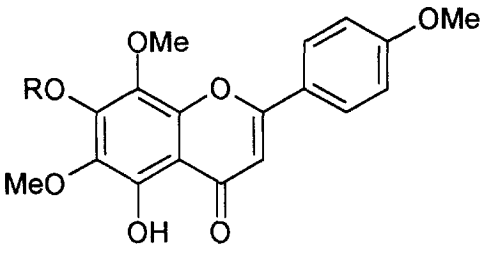
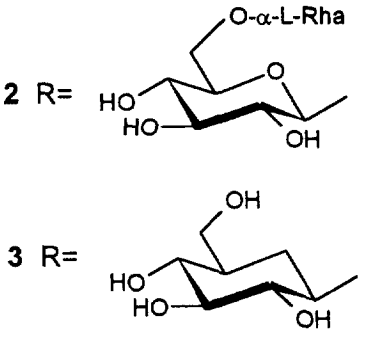


Lysionotoside **1**



Verbascoside **4**





5 R= H, Nevadensin

the second ABX system. The spin-couplings between olefinic protons, aromatic protons and α,β-protons of the aglycone were confirmed by the correlations in the ^{1}H-^{1}H COSY spectrum. The anomeric protons of **1** appeared as doublets at δ 4.38 (J = 7.7 Hz) for glucose, at δ 5.21 (J = 2.2 Hz) for apiose and at δ 5.28 (J = 1.3 Hz) for rhamnose. The signal of the methyl group as doublet at δ 1.12 indicated, that **1** contained a rhamnose. From biogenetic considerations, glucose and apiose should be D-sugars and rhamnose a L-sugar. According to the coupling constants, glucose and apiose possessed the β-D-configuration and rhamnose the α-L-configuration.

The ^{13}C NMR spectrum of **1** suggested also the presence of 3,4-dihydroxyphenethyl, caffeoyl, glucosyl, apiosyl and rhamnosyl moieties. Based on significant deshielding of H-4 of glucose (δ 4.93), the caffeoyl unit should be connected to C-4 of glucose [3–5]. The signal at δ 62.4 should be derived from C-6 of glucose. The significant upfield shift of this signal indicated, that the OH-group at C-6 of glucose was free. Three signals were found near 80 ppm. One of them at δ 80.4 ppm came from apiose, because it was determined as a quarternary C-atom based on a DEPT experiment. The other two signals at δ 80.0 and δ 80.2 gave the information, that both C-2 and

C-3 of glucose were attached to other sugars. The NOESY spectrum showed the following correlations: H-3 of glucose → H-1 of apiose; H-2 of glucose → H-1 of rhamnose. Both apiose and rhamnose are terminal sugars. The site of attachment of sugar residues to the aglycone, sugar sequence and conformations were also ascertained by ^{1}H-^{1}H-COSY, ^{13}C-^{1}H-COSY and DEPT.

On the basis of the spectral data, the structure of **1** was determined as α-(3,4-dihydroxyphenyl)ethyl-*O*-(2′-*O*-α-L-rhamno-pyranosyl-3′-*O*-β-D-apiofuranosyl-4′-*O*-*E*-caffeoyl)-β-D-glucopyranoside. This new compound was named lysionotoside.

Compound **4** contained a glucose and a rhamnose and appeared also as a phenylpropanoid glycoside. Comparsion of the ^{1}H NMR and ^{13}C NMR data of **4** with literature data [6], showed that **4** represented the known compound verbascoside.

Compounds **2** and **3** were obtained as yellow amorphous powders. In both cases, hydrolysis with 6% aqueous methanolic HCl yielded the flavone aglycone nevadensin (mp 197–198°C; $[M]^+$ at *m/z* 344 corresponding to $C_{18}H_{16}O_7$). Detailed analyses of its ^{1}H NMR, ^{13}C NMR, mass and UV spectra proved the identity of the aglycone as the recently identified nevadensin [3]. The sugar moieties of **2** and **3** were identified by TLC as glucose and rhamnose, and glucose, respectively. This was confirmed by ^{1}H NMR and ^{13}C NMR spectral data. For compound **2**, the coupling constant of the anomeric protons were ca 8 Hz and 4 Hz, which indicated a β-glucose and an α-rhamnose. For compound **3**, the coupling constant of the anomeric proton was ca 8 Hz, which indicated a β-glucose. The FAB-mass spectrum of **2** exhibited ion peaks at *m/z* 659 $[M+Li]^+$ and 345 $[aglycone+H]^+$, suggesting also that **2** contains a glucose and a rhamnose unit. The FAB-mass spectrum of **3** exhibited ion peaks at *m/z* 513 $[M+Li]^+$ and 345 $[aglycone+H]^+$, indicative that **3** contains only a glucose unit. The addition of shift reagents showed, that both **2** and **3** contain a free C-5 hydroxyl group and no C-7 or C-4′ hydroxyl groups.

From the ^{13}C NMR spectra of **2** and **3**, carbon signals attributed to the aglycone moiety were compared with the data reported for nevadensin and our own data [3] to confirm that the aglycone of **2** and **3** is nevadensin. For both compounds, an upfield shift of C-7 (ca 2 ppm), and a downfield shift of C-6 (5.3 ppm) and C-8 (4.7 ppm) also indicated that the sugar moiety was linked at C-7 [7]. The ^{1}H NMR spectra of **2** and **3**, measured in dimethylsulfoxide-d_6, both showed a proton signal for the C-5-hydroxyl group (δ 12.70, 12.50 (*br*) [8]). This confirmed a free hydroxyl group at position C-5 for both **2** and **3**. The ^{13}C NMR spectrum of **2** showed the C-6 signal of the glucose at δ 66.6. This indicated that the C-6 position of glucose was substituted, and suggested that the linkage between the glucose and rhamnose was 1 → 6. The attachment sites of the sugar residues to the aglycone, sugar sequence and conformations were also ascertained by ^{13}C NMR, DEPT and 2D NMR spectra, ^{1}H-^{1}H COSY and HMQC.

On the basis of the spectral data, the structures of **2** and **3** were determined as nevadensin 7-*O*-[α-L-rhamnopyranosyl(1 → 6)]-β-D-glucopyranoside and nevadensin 7-*O*-β-D-glcopyranooside, respectively. Both are reported for the first time as natural products.

EXPERIMENTAL

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Mps are uncorr. FAB-MS was measured on a MS 80 RFA instrument (Kratos) in glycerol–LiCl at 7 kV. TLC of sugars: precoated TLC plates (Merck silica gel 60 F_{254}) were used and developed with $CHCl_3$–MeOH–H_2O (13:10:2). The plates were sprayed with aniline hydrogenphthalate reagent and heated at 100° for 10 min. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 360 spectrometer and 2D NMR was performed with a JEOL GSX 400N and Bruker AM 400 instrument in MeOH-d_4 and DMSO-d_6 using TMS as int. standard.

Isolation of constituents

The plant material was collected in Guangxi, South China, and identified by Prof. Lou Zhicen, Beijing Medical College. A voucher specimen is deposited at the Institut für Pharmazeutische Biologie, München. Dried powdered aerial parts of *L. pauciflorus* (1 kg) were defatted in a Soxhlet with 2.5 l *n*-hexane for 24 h. The air-dried plant material was further extracted with 2.5 l MeOH for 24 h, yielding after evpn a syrupy brown residue (80 g). The MeOH extract was suspended in H_2O–satd *n*-BuOH. On evapn of the solvent, ca 30 g residue was obtained. The *n*-BuOH-soluble fr. of the MeOH extract was separated by repeated CC over Sephadex LH-20 (2 × 120 cm). 5 main fractions were obtained. Compounds **1** and **4** were isolated from fr. II and fr. III. Compounds **2** and **3** were obtained from fr. IV by repeated CC over polyamide (1.5 × 50 cm) with MeOH and MeOH–H_2O (4:1). Final purification was performed by semi-prep. reversed-phase HPLC (Merck LiChrosorb, RP-18, 250 × 10 mm; 7 μm) with MeCN–H_2O (1:4) and UV detection at 210 nm to give 40 mg **1**, 4.9 mg **3**, 31 mg **2** and 50 mg **4**.

Acid hydrolysis

Compounds **2** and **3** (2 mg each) were treated with 2 N HCl in a sealed tube at 100° for 1 h. The aglycone was extracted with Et_2O and subjected to HPLC to detect nevadensin while sugars were identified in the aq. layer by TLC. Compounds **1** and **4** were treated with 2 N TFA at 100° for 1 h. The soln was extracted with EtOAc and the aq. layer was used to identify sugars by TLC.

Table 1. ^{13}C NMR (100 MHz) and ^{1}H NMR (500 MHz) data of compounds **1** and **4** (in CD_3OD, TMS as int. standard; chemical shifts in δ values (ppm))

C/H	δ (ppm) [^{13}C NMR]		δ (ppm) [^{1}H NMR]		J (Hz)	
	1	**4**	**1**	**4**	**1**	**4**
Aglycone						
C-1	131.5	131.4	6.69 *d*	6.69 *d*	2.0	2.0
CH-2	115.2	116.3				
C-3	146.2	144.0				
C-4	144.7	145.5				
CH-5	116.4	117.1	6.68 *d*	6.67 *d*	8.08.0	
CH-6	121.3	121.2	6.56 *dd*	6.57 *dd*	2.0/8.0	2.0/8.0
CH_2-α	72.3	72.0	3.71 *m*/4.08 *m*	3.74–3.98		
CH_2-β	36.6	36.2	2.79 *dt*	2.79 *t*	2.7/7.0	7.0
Caffeoyl moiety						
C-1′	127.6	127.6				
CH-2′	114.2	115.1	7.07 *d*	7.06	2.0	2.0
C-3′	147.0	146.6				
C-4′	150.2	149.1				
CH-5′	114.6	116.3	6.80 *d*	6.78 *d*	8.0	8.0
CH-6′	123.4	123.1	6.97 *dd*	6.94 *dd*	2.0/8.0	2.0/8.0
CH-α′	117.1	115.3	6.25 *d*	6.28	15.9	15.9
CH-β′	148.0	147.8	7.59 *d*	7.57 *d*	15.9	15.9
Glucose						
CH-1″	104.2	104.0	4.38 *d*	4.37 *d*	7.7	7.9
CH-2″	80.0	75.6	3.66 *dd*	3.29–3.90 *m*	3.4/9.0	
CH-3″	80.4	81.5	3.85 *t*	3.74	9.0	
CH-4″	70.5	70.1	4.93	4.90	9.0	
CH-5″	76.4	75.7	3.51–3.62	3.29–3.90		
CH_2-6″	62.4	62.1	3.51–3.62	3.29–3.90		
Rhamnose						
CH-1‴	102.1	102.6	5.28 *d*	5.17 *d*	1.3	1.3
CH-2‴	72.6	72.2	3.60/3.65	3.29–3.90		
CH-3‴	72.5	72.1	3.29/3.90	3.29–3.90		
CH-4‴	75.9	73.7	3.30 *t*	3.29–3.90	10.2	
CH-5‴	76.4	70.2	3.60–3.95	3.29–3.90		
CH_3-6‴	18.7	18.2	1.12 *d*	1.09 *d*	6.1	6.0
Apiose						
CH-1⁗	111.4		5.21 *d*		2.2	
CH-2⁗	78.6		3.68 *d*		2.2	
C-3⁗	80.2					
CH_2-4⁗	74.8		3.60–3.65			
CH_2-5⁗	65.8		3.31			

Lysionotoside (**1**). White amorphous powder; $C_{34}O_{19}H_{44}$; UV: λ_{max}^{MeOH}nm: 221, 293, 334 nm; FAB-MS: *m/z* 763 $[M+Li]^+$, 467 $[M+Li-(apiose-H_2O)-rhamnose]^+$; ^{1}H and ^{13}C NMR data: see Table 1.

Nevadensin 7-*O*-[α-L-rhamnopyranosyl(1 → 6)]-β-D-glucopyranoside (**2**). $C_{30}O_{16}H_{36}$. UV: λ_{max}^{MeOH}nm: 283, 330: +$AlCl_3$ 350; +$AlCl_3$+HCl 302, 350; +NaOAc +H_3BO_3 283, 330; FAB-MS: *m/z* 659 $[M+Li]^+$, 345 [aglycone+H]$^+$; ^{1}H NMR (360 MHz, DMSO-d_6): δ 3.85 (3H, *s*, -OCH_3 4′), 3.80 (3H, *s*, -OCH_3 6), 3.93 (3H, *s*, -OCH_3 8), 6.93 (1H, *s*, H-3), 7.13 (2H, *d*, J = 9.0 Hz, H-3′,5′), 8.02 (2H, *d*, J = 9.0 Hz, H-2′,6′), 5.23 (1H, *d*, J = 7.4 Hz, glucose H-1), 4.41 (1H, *d*, J = 1.3 Hz rhamnose H-1), 12.70 (1H, *s*, OH-5); ^{13}C NMR: see Table 2.

Nevadensin 7-*O*-β-D-glucopyranoside (**2**). $C_{24}O_{12}H_{26}$. UV: λ_{max}^{MeOH}nm: 283, 330: +$AlCl_3$ 350; +$AlCl_3$+HCl 302, 350; +NaOAc+H_3BO_3 283, 330; FAB-MS: *m/z* 513 $[M+Li]^+$, 345 [aglycone+H]$^+$; ^{1}H NMR (360 MHz, DMSO-d_6): δ 3.85 (3H, *s*, -OCH_3 4′), 3.81 (3H, *s*, -OCH_3 6), 3.93 (3H, *s*, -OCH_3 8), 6.96 (1H, *s*, H-3), 7.14 (2H, *d*, J = 9.0 Hz, H-3′,5′), 8.03 (2H, *d*, J = 9.0 Hz, H2,′6′), 5.29 (1H, *d*, J = 7.4 Hz, glucose H-1), 12.50 (1H, *br*, OH-5); ^{13}C NMR: see Table 2.

Verbascoside (**4**). White amorphous powder; $C_{29}O_{15}H_{36}$; UV: λ_{max}^{MeOH}nm: 221, 293, 334 nm; FAB-

Table 2. ^{13}C NMR spectral data for compounds **2** and **3** (^{13}C NMR in CD_3OD, TMS as int. standard; chemical shifts in δ values (ppm))

C	Nevadensin [3]	2	3
C-2	163.1	162.4	162.5
C-3	103.0	103.4	103.3
C-4	182.3	182.5	182.5
C-5	145.4	148.4	148.4
C-6	131.6	136.0	135.9
C-7	150.9	148.7	148.9
C-8	128.0	132.7	132.7
C-9	148.4	145.1	145.1
C-10	103.1	106.6	106.4
C-1′	123.0	123.0	123.0
C-2′,6′	128.2	128.0	128.0
C-3′,5′	114.7	114.7	114.7
C-4′	162.4	163.4	163.4
C-OMe-6	61.2	61.5	61.5
C-OMe-8	60.2	60.1	60.1
C-OMe-4′	55.6	55.5	55.5
Glucose			
C-1″		102.8	102.6
C-2″		73.9	73.9
C-3″		76.8	76.8
C-4″		69.9	69.9
C-5″		75.7	77.5
C-6″		66.6	61.0
Rhamnose			
C-1‴		100.8	
C-2‴		70.3	
C-3‴		70.6	
C-4‴		71.8	
C-5‴		68.4	
C-6‴		17.8	

mass spectrum: *m/z* 737 $[M+Li]^+$, 485$[M+Li-$ Rhamnose$]^+$; ^1H and ^{13}C NMR data see Table 1.

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