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NEVADENSIN GLYCOSIDES FROM *LYSIONOTUS PAUCIFLORUS*

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Key Word Index—*Lysionotus pauciflorus*; Gesneriaceae; flavone glycosides; nevadensin; 5,4-dihydroxy-6,8,4′-trimethoxyflavone; nevadensin 5-glucoside and 5-glucosyl (1→6) glucoside.

Abstract—Two new flavone glucosides, nevadensin 5-*O*-β-D-glucoside and nevadensin 5-*O*-β-D-glucosyl(1→6)β-D-glucoside, have been isolated from the aerial parts of *Lysionotus pauciflorus*. The structures have been determined by means of UV, mass spectral and one- and two-dimensional ^{1}H and ^{13}C NMR techniques.

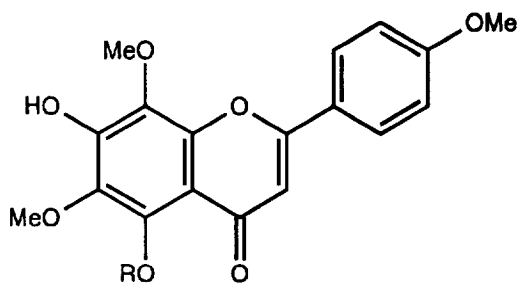
INTRODUCTION

Lysionotus pauciflorus Maxim. (Gesneriaceae) is widespread in south China. It has been used in traditional Chinese medicine for the treatment of lymph node tuberculosis, cough with tachypnoea and rheumatic pains [1]. In the course of our search for anti-inflammatory active compounds, we have investigated the constituents of a methanolic extract of the aerial parts of *L. pauciflorus*. Recently, we have reported the isolation and structural determination of the new phenylpropanoide paucifloside [2]. This paper deals with the isolation and structural elucidation of two new flavone glucosides, nevadensin 5-glucoside (**1**) and nevadensin 5-glucosyl(1→6)glucoside (**2**).

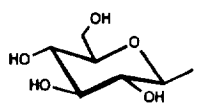
RESULTS AND DISCUSSION

The *n*-butanol-soluble fraction of the methanolic extract of the powdered aerial parts of *L. pauciflorus* was separated by repeated column chromatography over Sephadex LH-20 and polyamide with methanol and water as eluents. Compounds **1** and **2** were purified by preparative HPLC. Nevadensin (**3**) crystallized as yellow needles from a fraction of the chloroform extract subjected to LH-20 column chromatography.

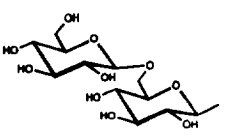
Compound **3** was identified by EI mass spectrometry, which gave molecular ion peak at *m/z* 344, corresponding to the molecular formula $C_{18}H_{16}O_7$, and fragment ion peaks at *m/z* 329, 197, 169 and 133. The ^{1}H NMR spectrum revealed the characteristic signals of a substituted flavone. A set of AB signals was due to the aromatic ring B. The integration indicated four protons in this AB system. From the ^{1}H NMR spectrum, it was deduced that the methoxyl group was linked at C_4 of the B ring. A singlet signal at 6.59 ppm suggested the existence of H-3. UV spectral analysis using the shift reagents $AlCl_3$, $AlCl_3$–HCl, sodium acetate and sodium acetate–H_3BO_3 indicated the presence of two free hydroxyl groups at C-5 and C-7 and two methoxyl groups at C-6 and C-8. No hydroxyl group was observed at C-3. The ^{13}C NMR spectrum of



1 R=



2 R=



3 R = H

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Table 1. ^{13}C NMR spectral data for compounds **1–3***

C	Nevadensin (**3**)	**1**	**2**
C-2	163.1	162.0	162.0
C-3	103.0	105.7	105.7
C-4	182.3	177.3	177.3
C-5	145.4	140.0	140.2
C-6	131.6	132.9	133.2
C-7	150.9	147.2	147.6
C-8	128.0	127.7	127.8
C-9	148.4	144.2	144.2
C-10	103.1	105.7	105.8
C-1′	123.0	123.1	123.1
C-2′, 6′	128.2	127.8	127.7
C-3′, 5′	114.7	114.7	114.6
C-4′	162.4	160.4	160.3
C-OMe-6	61.2	60.9	61.0
C-OMe-8	60.2	60.4	60.9
C-OMe-4′	55.6	55.4	55.5
Glucose-1			
C-1		105.4	105.4†
C-2		73.9	73.9†
C-3		76.5	76.8†
C-4		69.9	69.9†
C-5		77.5	76.5†
C-6		61.1	68.4
Glucose-2			
C-1			103.0†
C-2			73.5†
C-3			76.7†
C-4			69.3†
C-5			76.2†
C-6			61.2

*100 MHz in DMSO-d_6; TMS as int. standard; chemical shifts in δ values (ppm).

†Assignments might be interchanged.

3 supported the presence of these units (Table 1). Therefore, **3** was identified as nevadensin, 5,4-dihydroxy-6,8,4′-trimethoxyflavone, which has been isolated previously from *L. pauciflorus* [3].

Compounds **1** and **2** were obtained as yellow amorphous powders. In both cases, hydrolysis with 6% aqueous methanolic HCl yielded the flavone aglycone **3** (mp 197–198°; $[M]^+$ at *m/z* 344 corresponding to $C_{18}H_{16}O_7$). Detailed analyses of its ^1H NMR, ^{13}C NMR, mass and UV spectra proved the identity of the aglycone as nevadensin. The sugar moiety of **1** and **2** was identified as glucose by TLC. This was confirmed by ^{13}C and ^1H NMR spectral data. For **1** and **2**, the coupling constant of the anomeric protons was *ca* 8 Hz, which indicated a β-glucose. The FAB mass spectrum of **1** showed ion peaks at *m/z* 513 $[M + Li]^+$ and 345 $[aglycone + H]^+$. The FAB mass spectrum of **2** exhibited ion peaks at *m/z* 675 $[M + Li]^+$, 513 $[675 - 162\ (glucose)]^+$ and 345 $[aglycone + H]^+$, suggesting that **2** contains two glucose units. The ^1H NMR spectrum of peracetylated **2** exhibited signals for one acetyl group at 2.46 ppm, belonging to the aglycone, and seven acetyl groups in the range 1.80–2.12 ppm belonging to the sugar moiety, which confirmed the presence of two glucose units in **2**.

From the ^{13}C NMR spectra of **1** and **2**, carbon signals attributed to the aglycone moiety were compared with the data for nevadensin and published data [4] to confirm that the aglycone of **1** and **2** is nevadensin. For both compounds, an upfield shift of *ca* 5 ppm of the C-4 resonance was observed, which is typically associated with glycosylation of the C-5 hydroxyl. An upfield shift of C-5 (5.4 ppm) and a downfield shift of C-6 (1.5 ppm) also indicated that the sugar moiety was linked at C-5 [4]. The ^1H NMR spectra of **1** and **2**, measured in dimethyl sulphoxide-d_6, did not show a proton signal for the 5-hydroxyl group (it would appear at 12–13 ppm [5]). This suggested that there was no free hydroxyl group at position C-5, and also explained why the UV spectra of nevadensin and **1** were slightly different. This is in accordance with the conclusion that the sugar moiety is connected to C-5 of **1** and **2**.

The ^{13}C NMR spectrum of **2** showed the C-6 signals of both glucoses at 61.2 and 68.4 ppm, respectively. This suggested that the C-6 position of one glucose was substituted and that it was free in the other, and indicated that the linkage between the two glucoses is $1 \rightarrow 6$. The sites of attachment of sugar residues to the aglycone, sugar sequence and conformations were also ascertained by ^{13}C NMR, DEPT and 2D NMR spectra such as ^1H–^1H COSY and HMQC.

On the basis of the spectral data, the structures of **1** and **2** were determined as nevadensin 5-*O*-β-D-glucopyranoside and nevadensin 5-*O*-β-D-glucopyranosyl $(1 \rightarrow 6)$-β-D-glucopyranoside, respectively. Both are reported for the first time as natural products.

Nevadensin has been reported as a compound possessing anti-inflammatory activity in the carragenan-induced rat paw oedema model [6], as well as hypotensive [7] and tuberculostatic effects [3]. The *n*-hexane and chloroform extracts of *L. pauciflorus* exerted strong inhibitory activity *in vitro* in the cyclooxygenase (COX) inhibition assay. The IC_{50} values were determined as 7 and 30 $\mu g\ ml^{-1}$, respectively. However, paucifloside, nevadensin and its glucosides showed no or only weak activity in the assay. Therefore, the search for the COX inhibitory active principle of *L. pauciflorus* will be continued.

EXPERIMENTAL

The plant material was collected in China and identified by Prof. Lou Zhicen, Beijing Medical College. A voucher specimen is deposited at the Institut für Pharmazeutische Biologie, München.

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 hydrogen phthalate reagent and heated at 100° for 10 min. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM360 spectrometer and 2D NMR was performed by a JEOL GSX 400N instrument in MeOH-d_4 and DMSO-d_6 using TMS as int. standard.

Extraction and isolation. Dried powdered aerial parts of *L. pauciflorus* (200 g) were defatted in a Soxhlet with 1 l *n*-hexane for 12 hr. The air-dried plant material was extracted with 2 l MeOH for 24 hr, yielding after evapn a syrupy brown residue (20.5 g). The MeOH extract was suspended in H_2O, and extracted ×2 with H_2O-satd *n*-BuOH. On evapn of the solvent, 7.2 g residue was obtained. The *n*-BuOH-soluble fr. of the MeOH extract was sepd by repeated CC over Sephadex LH-20 and polyamide (ICN) with MeOH and MeOH–H_2O 4:1) as eluents, respectively. Final isolation was performed by semi-prep. reversed-phase HPLC (Merck LiChrosorb, RP-18, 250 × 10 mm; 7 μm) with AcCN–H_2O (1:4) and UV detection at 210 nm to give 12 mg **1** and 17 mg **2**.

Acid hydrolysis. Each glucoside (2 mg) was treated with 2 N HCl in a sealed tube at 100° for 1 hr. The aglycone was extracted with Et_2O and subjected to HPLC to detect nevadensin, while sugars were identified in the aq. layer by TLC.

Nevadensin 5-O-β-D-glucopyranoside (**1**). $C_{24}O_{12}H_{26}$. UV λ_{max}^{MeOH}: 216, 269, 332; +$AlCl_3$ 269, 310(sh), 334; +$AlCl_3$ + HCl 308, 334 nm; FAB-MS: 513 $[M + Li]^+$, 345 $[M - glucose - Li + H]^+$; ^1H NMR (CD_3OD, δ ppm): 3.89 (3H, *s*, -OMe 4′), 3.94 (3H, *s*, -OMe 6), 4.00 (3H, *s*, OMe 6), 6.7 (1H, *s*), 7.1 (2H, *d*, $J = 9$ Hz), 8.00 (2H, *d*, $J = 9$ Hz). ^{13}C NMR: see Table 1.

Nevadensin 5-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (**2**). $C_{30}O_{17}H_{36}$. UV λ_{max}^{MeOH}: 216, 269, 332; +$AlCl_3$ 269, 310(sh), 334; +$AlCl_3$ + HCl 308, 334 nm; FAB-MS: 675 $[M + Li]^+$, 513 [M-glucose + Li]$^+$, 345 $[M - 2 \times Glucose + H]^+$; ^1H NMR (CD_3OD, δ ppm): 3.89 (3H, *s*, -OMe 4′), 3.94 (3H, *s*, -OMe 6), 4.00 (3H, *s* -OMe 8), 4.33 (1H, *d*, $J = 8$ Hz), 4.06 (1H, *d*, $J = 7.5$ Hz) 6.64 (1H, *s*), 7.11 (2H, *d*, $J = 8.9$ Hz), 7.99 (2H, *d*, $J = 8.9$ Hz); ^{13}C NMR: see Table 1.

Nevadensin (**3**). $C_{18}O_7H_{16}$. mp. 198–199°; UV λ_{max}^{MeOH}: 283, 330; +$AlCl_3$ 210, 352; +$AlCl_3$ + HCl 308, 351; +NaOAc 283, 273; +NaOAc + H_3BO_3: 283, 321 nm; EI-MS: 344 $[M]^+$, 329, 277, 197, 169, 133; ^1H NMR ($CDCl_3$, δ ppm): 3.90 (3H, *s*, -OMe 4′), 4.02 (3H, *s*, -OMe 6), 4.04 (3H, *s*, -OMe 8), 6.59 (1H, *s*, H-3), 7.03 (2H, *d*, $J = 8.9$ Hz, H-3′, 5′), 7.89 (2H, *d*, $J = 8.9$ Hz, H-2′, 6′), 6.54 (1H, *s*, OH-7), 11.82 (1H, *br*, OH-5); ^{13}C NMR: see Table 1.

COX assay. The test was performed with COX from sheep seminal vesicle microsomes. COX activity was determined on a microtitre scale [8]. The incubation mixt. contained 1 μg enzyme prepn in 190 μl 0.1 M Tris buffer (pH 8.0), 1 nM reduced glutathione, 1 mM epinephrin hydrogen tartrate and 0.05 mM Na_2EDTA. The test substance (10 μl) dissolved in EtOH p.a. was added and preincubated for 5 min. The reaction was started by addition of 10 μl 4.5 μM 1-^{14}C-arachidonic acid (0.05 μCi) and incubated for 20 min at 37°. The reaction was stopped by adding 10 μl 10% HCO_2H. Arachidonic acid and its labelled metabolites were sepd by RP-HPLC, detected by radioactivity monitoring and quantified via peak areas. Activity was calculated as % inhibition of PGE_2 formation [9].

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