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PHENYLPROPANOID GLYCOSIDES FROM *NEWBOULDIA LAEVIS* ROOTS

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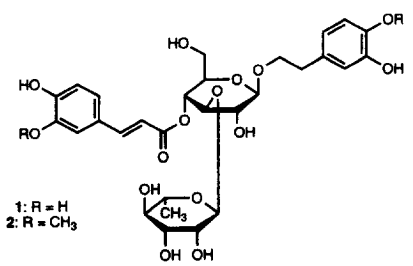
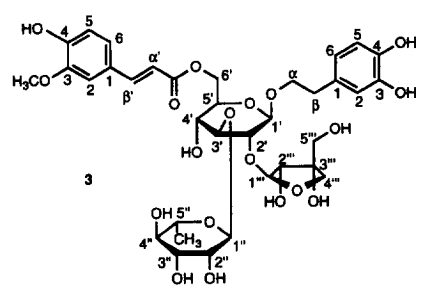
Key Word Index—*Newbouldia laevis*; Bignoniaceae; phenylpropanoid glycosides; newbouldioside.

Abstract—Three phenylpropanoid glycosides have been isolated from the methanol extract of *Newbouldia laevis* roots. One compound is new; its structure has been established by spectroscopic (UV, IR, FAB-MS, ^{1}H and ^{13}C NMR) and chemical methods as 2-(3,4-dihydroxyphenyl)ethyl-*O*-*β*-D-apiofuranosyl-(1→2)-*O*-*α*-L-rhamnopyranosyl-(1→3)-6-*O*-*E*-feruloyl-*β*-D-glucopyranoside. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Newbouldia laevis Seem. (Bignoniaceae) is a shrub or a small tree widely used in traditional medicine in western Africa [1]. Previous studies on the roots have led to the isolation of four alkaloids [2, 3], several naphthoquinones [3, 4] and a tetrasaccharide [5]. As the former work was mostly carried out on lipophilic extracts, the methanolic extract was investigated to complete the knowledge on this plant. From this extract, verbascoside (**1**), martynoside (**2**) and a new phenylpropanoid glycoside (**3**) have been isolated. Isolation and structure elucidation are given in this paper.





RESULTS AND DISCUSSION

Dried and powdered roots of *Newbouldia laevis* were extracted successively with dichloromethane and methanol. The methanolic extract was suspended in H_2O and extracted with EtOAc and then *n*-BuOH. The BuOH portion was fractionated on silica gel. The phenylpropanoid glycosides **1**–**3** were isolated by further separation by MPLC, gel filtration on Sephadex LH-20 and semi-preparative HPLC.

Compounds **1** and **2** were identified as verbascoside (**1**) and martynoside (**2**) by comparing their spectroscopical data (UV, IR, NMR and FAB mass spectra) with literature values [6, 7].

Compound **3** showed the same UV spectrum as **1** and **2** with maxima at 329 and 291 nm, which suggested that it was a phenylpropanoid glycoside. This was confirmed by the IR spectrum: bands for hydroxyl groups (3400 cm^{-1}, *br*) and aromatic rings (1590 and 1505 cm^{-1}) were observed. Furthermore, the bands of an *α*,*β*-unsaturated ester were found at 1685 cm^{-1} ($\nu_{C=O}$) and 1625 cm^{-1} ($\nu_{C=C}$) [6]. Acid hydrolysis with 0.1 N H_2SO_4 afforded apiose and rhamnose (identified by TLC) while acid hydrolysis with 2 N HCl yielded rhamnose, glucose (also identified by TLC) and ferulic acid. Apiose was obviously decomposed under these strong acid conditions. Ferulic acid was identified by HPLC (comparison with an authentic sample). FAB-MS (negative ion mode) showed a peak at *m/z* 769

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Table 1. ^{1}H NMR spectral data of **3** (200.06 MHz, CD_3OD). Chemical shifts δ in ppm relative to TMS; J in Hz

	Aglycone		*Glucose*		*Rhamnose*
2	6.67 ($d, J = 1.8$)	1′	4.41 ($d, J = 7.7$)	1″	5.01 ($d, J = 1.3$)
5	6.63 ($d, J = 8.0$)	2′	3.46 ($dd, J = 10.0, 7.7$)	2″	3.94–4.00*
6	6.54 ($dd, J = 8.0, 2.0$)	3′	3.62 ($dd, J = 10.7, 10.0$)	3″	3.69 ($dd, J = 9.5, 2.9$)
α	3.94 (m)	4′	3.35–3.50*	4″	3.35–3.45*
	3.71 (m)	5′	3.50–3.60*	5″	3.85–3.95*
β	2.77 ($t, J = 7.6$)	6′a	4.51 ($dd, J = 11.8, 2.0$)	6″	1.25 ($d, J = 6.1$)
		6′b	4.34 ($dd, J = 11.8, 5.7$)		
	Apiose		*Acyl moiety*		
1‴	5.20 ($d, J = 1.9$)	2	7.15 ($d, J = 1.7$)		
2‴	3.91 ($d, J = 1.9$)	5	6.79 ($d, J = 8.3$)		
4‴a	3.70 ($d, J = 9.7$)	6	7.01 ($dd, J = 8.3, 1.7$)		
4‴b	3.98 ($d, J = 9.7$)	α′	6.38 ($d, J = 15.9$)		
5‴	3.57 (s)	β′	7.61 ($d, J = 15.9$)		
		OMe	3.86 (s)		

* Signal pattern unclear due to overlapping.

$[M-H]^-$, confirming the molecular formula $C_{35}H_{46}O_{19}$. Further peaks were found at m/z 637 $[M-H-132]^-$, 623 $[M-H-146]^-$ and 593 $[M-H-176]^-$, indicating an apiosyl, a rhamnosyl and a feruloyl moiety attached to the core sugar.

The signals for the anomeric protons of the sugars in ^{1}H NMR (see Table 1) were found at δ 4.41 (d, $J = 7.7$ Hz; H—C(1′) of β-D-glucose), 5.01 ($d, J = 1.3$ Hz; H—C(1″) of α-L-rhamnose) and 5.20 ($d, J = 1.9$ Hz; H—C(1‴) of α or β-D-apiose). The β-configuration for apiose was confirmed by the shift of C-1‴ in the ^{13}C NMR (δ 111.1, see Table 2 [8]). The configuration of the hydroxyl groups at C-2‴ and C-3‴ of apiose was determined by the coupling of the C-5‴ protons (see Table 1). In the *erythro* form of apiose, the protons of C-5‴ are magnetically equivalent, while in the *threo* form, a coupling between these two protons, giving two doublets, can be observed [9]. As in the ^{1}H NMR spectrum only a singlet was observed at 3.57 ppm, the apiose in **3** was found to be in the *erythro* form and therefore the hydroxyl groups at C-2‴ and C-3‴ are *cis*-orientated.

Further signals in the ^{1}H NMR spectrum were attributed to 3,4-dihydroxy-phenylethyl alcohol (ABX-system at δ 6.54, dd, $J = 8.0$, 2.0 Hz, 1H; 6.63, $d, J = 8.0$ Hz, 1H and 6.67, d, $J = 1.8$ Hz, 1H) and to (*E*)-ferulic acid (ABX-system at δ 6.79, d, $J = 8.3$ Hz, 1H; 7.01, $dd, J = 8.3, 1.7$ Hz, 1H and 7.15, d, $J = 1.5$ Hz, 1H; δ 3.86, s, 3H, OCH_3). The *trans*-configuration of ferulic acid was shown by the shift and couplings of the α′- and β′-proton in the ^{1}H NMR (δ 6.38, d, $J = 15.9$ Hz, 1H and 7.61, d, $J = 15.9$ Hz, 1H).

The ^{13}C NMR data (see Table 2) indicated glucose to be the core sugar with the phenylethyl alcohol moiety linked to C-1′. A downfield shift ($\Delta\delta = +2.25$) of C-6′ of **3** in the ^{13}C NMR compared with **1** and **2**, together with a downfield shift of H-6′ in the ^{1}H NMR (δ 4.34, dd, $J = 11.9$, 5.7 Hz and δ 4.51, dd, $J = 11.8, 2.0$ Hz), indicated the site of acylation to be C-6′ of the core sugar glucose [7, 10]. A downfield shift of C-3′ and C-2′ in the ^{13}C NMR suggested that the other sugar moieties (rhamnose and apiose) were attached to C-3′ and C-2′ of glucose. Evidence for the position of rhamnose and apiose was found by NOE measurements (200 MHz, CD_3OD). Irradiation of the anomeric proton of rhamnose at 5.01 ppm showed an enhancement of H-3′ at 3.62 ppm. Irradiation of the anomeric proton of apiose at 5.20 ppm gave an enhancement of H-2′ at 3.46 ppm. The assignments of protons (see Table 1) were based on the results of the DQFCOSY experiment in CD_3OD and on the short-range heteronuclear correlation spectrum (HMQC). Further evidence for the attachment of the sugars was found in the long-range heteronuclear correlation spectrum (HMBC). Correlations were observed between H-2′ and C-1′ and C-1‴ as well as between H-3′ and C-4′, C-2′, C-1′ and C-1″. Thus, **3** was established as 2-(3,4-dihydroxyphenyl)ethyl-*O*-β-D-apiofuranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→3)-6-*O*-*E*-feruloyl-β-D-glucopyranoside and named newbouldioside.

Phenylpropanoid glycosides are widely distributed in the plant kingdom. Antioxidant, enzyme inhibiting and immunomodulatory effects have been reported for this class of compounds [11]. Some antibacterial and antiviral activities have also been mentioned [11]; in our antibacterial and antifungal tests, however, no growth inhibiting properties of **1**–**3** were observed.

The chemotaxonomy of phenylpropanoid glycosides is also of interest. The largest number has so far been found in Scrophulariaceae, Oleaceae and Plantaginaceae [11]. In the Bignoniaceae, phenylpropanoid glycosides have been found in *Campsis chinensis*, *Deplanchea speciosa* and several *Mussatia* species [11]. In certain families, phenylpropanoid and

Table 2. ^{13}C NMR spectral data for compounds **1–3** (50 MHz, CD_3OD)

	1	2	3		1	2	3
Aglycone				*Acyl moiety*			
1	131.4	132.9	131.4	1	127.6	127.5	127.6
2	116.5	112.8	116.3	2	114.7	111.7	111.5
3	144.7	147.5	144.6	3	149.8	149.5	149.3
4	146.1	147.4	146.1	4	146.8	151.2	150.6
5	117.1	117.1	117.1	5	116.5	116.6	116.5
6	121.2	121.1	121.2	6	123.2	124.4	124.3
α	72.3	72.1	72.3	α′	115.2	114.9	115.2
β	36.6	36.6	36.7	β′	148.0	147.9	147.1
OCH_3		56.5		C=O	168.3	168.3	169.0
				OCH_3		56.4	56.4
Glucose				*Apiose*			
1′	104.2	104.2	103.4	1‴			111.1
2′	76.2	76.2	79.7	2‴			78.4
3′	81.6	81.5	85.7	3‴			80.5
4′	70.5*	70.6*	70.5*	4‴			75.2
5′	76.0	76.0	75.2	5‴			65.7
6′	62.3	62.4	64.6				
Rhamnose							
1″	103.0	103.0	103.0				
2″	72.3	72.3	72.3				
3″	72.0	72.0	72.1				
4″	73.8	73.8	73.7				
5″	70.4*	70.4*	70.6*				
6″	18.5	18.4	17.9				

* Assignments within the same column may be interchangeable.

iridoid glycosides occur together. Such a coexistence has been proved for the Bignoniaceae [12]; however, no iridoid glycoside has been isolated from *Newbouldia laevis* yet.

EXPERIMENTAL

General. TLC: silica gel 60 F_{254} Al sheets (Merck). CC: silica gel (63–200 μm, Merck; 600 × 55 mm i.d.). MPLC: home-packed LiChroprep RP-18 column (15–25 μm; 460 × 36 mm i.d.). Semi-prep. HPLC: Shimadzu LC-10AD pump, Knauer LiChrosorb-RP-18 column (7 μm, 250 × 16 mm i.d.). UV: Varian DMS 100S UV visible spectrophotometer. Spectra were recorded in MeOH. Optical rotation: Perkin-Elmer 241 MC polarimeter. IR: Philips PU 9716 infrared spectrometer. ^1H and ^{13}C NMR: Varian VXR 200. HMBC and HMQC NMR spectra: Bruker AMX 2600. Spectra were measured in CD_3OD at 200.06 MHz and 600.13 MHz for proton and 50.30 MHz and 150.91 MHz for carbon, respectively. TMS was used as internal standard. FAB-MS: Finnigan MAT TSQ-700 triple-stage quadrupole instrument. The purity of the compounds was checked by HPLC using a Nova-Pak RP-18 column (5 μm, 150 × 3.9 mm i.d.).

Plant material. Roots of *Newbouldia laevis* were collected in 1994 in Seredou, province of Macenta, Guinea. A voucher specimen (No. 94084) is deposited at the Institut de Pharmacognosie et Phytochimie, Lausanne, Switzerland.

Extraction and isolation. The powdered roots (958 g) were extracted at room temperature successively with CH_2Cl_2 and MeOH to afford 6.8 g and 107.6 g of extract, respectively.

A portion of the MeOH-extract (99.0 g) was suspended in H_2O and extracted with EtOAc and BuOH, to yield 3.78 g and 12.53 g of extract, respectively. From the BuOH-extract, 12.34 g were subjected to CC on silica gel, using mixtures of $CHCl_3$–MeOH and MeOH–H_2O of increasing polarity, giving fractions 1–10.

Fr. 2 was subjected to gel filtration on Sephadex LH-20, using a mixture of $CHCl_3$–MeOH (1:1). Further purification by semi-preparative HPLC with MeOH–H_2O (45:55) yielded compound **2** (21.5 mg) and, after another purification step on Sephadex LH-20, compound **1** (13.5 mg). Compound **1** (142.2 mg) was also obtained from fr. 3 by MPLC on RP-18 with MeOH–H_2O (30:70) and further purification by semi-preparative HPLC (MeOH–H_2O 34:66) and from fr. 7 with two steps of gel filtration on Sephadex LH-20 (MeOH and MeOH–$CHCl_3$ 1:1, respectively). Fr. 7 yielded compound **3** (13.0 mg) after gel filtration on Sephadex LH-20 with MeOH, MeOH–$CHCl_3$ (1:1) and MeOH–H_2O (1:1), respectively. Compound **3** (8.7 mg) was finally obtained from fr. 8 by gel filtration on

Sephadex LH-20 with systems of MeOH–$CHCl_3$ (1:1) and MeOH–H_2O (1:1).

Newbouldioside. (**3**): Yellow amorphous powder. $[\alpha]_D$ −50.7° (MeOH; *c* 1.15). IR ν_{max}^{KBr} cm^{-1}: 3 400, 2 920, 1 685, 1 625, 1 590, 1 505, 810. UV λ_{max}^{MeOH} nm (log ε): 206 (4.38), 214 (4.28), 232 (4.10), 291 (4.05), 329 (4.16). FAB-MS *m/z* 769 $[M]^{[mom]}$, 637 [M–H − 132 (apiosyl)]$^-$, 623 [M–H − 146 (rhamnosyl)]$^-$, 593 [M–H − 176 (feruloyl)]$^-$. ^1H NMR and ^{13}C NMR see Tables 1 and 2.

Hydrolysis of **3** *with* 2 *N* HCl. 1.9 mg of **3** were refluxed in 10 ml 2 N HCl for 3 hr. The mixture was cooled and then extracted with BuOH. The organic layer was evaporated to dryness and a 0.1% solution was injected in HPLC (gradient: CH_3CN–H_2O (0.05% TFA) 5:95→65:35 in 30 min (1 ml/min)). Comparison with an authentic sample led to the detection of ferulic acid. The aqueous layer was neutralized with $NaHCO_3$, filtered and evaporated to dryness. The residue was dissolved in 0.5 ml pyridine. Comparison on TLC with authentic samples gave glucose and rhamnose.

Hydrolysis with 0.1 *N* H_2SO_4. 4.4 mg of **3** were refluxed in 10 ml 0.1 N H_2SO_4 for 1 hr. The mixture was cooled and 10 ml H_2O were added. The aqueous layer was extracted with BuOH, then neutralized with $NaHCO_3$, filtered and evaporated to dryness. The residue was treated in the same way as above. Comparison with authentic samples on TLC (solvent system MeOH–H_2O–EtOAc–HOAc 3:3:13:4) yielded rhamnose and apiose.

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