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Chevalierinoside B and C: Two new isoflavonoid glycosides from the stem bark of *Antidesma laciniatum* Muell. Arg (*syn. Antidesma chevalieri* Beille)

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ABSTRACT

Chevalierinosides B (**1**) and C (**2**), two new isoflavonoid glycosides, characterized as biochanin A 7-O-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside] and genistein 7-O-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside], together with the known isoflavonoids, chevalierinoside A (**3**) and genistein 7-O-β-D-glucopyranoside (**4**), kaempferol 3-O-β-D-glucopyranoside (**5**) and triterpenes, friedelin (**6**), betulinic acid (**7**), 30-oxobetulinic acid (**8**), 30-hydroxybetulinic acid (**9**), were isolated from the stem bark of *Antidesma laciniatum* Muell. Arg. (syn. *Antidesma chevalieri* Beille). Their structures were established by direct interpretation of their spectral data, mainly HR-TOFESIMS, 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (COSY, NOESY, TOCSY, HSQC and HMBC), and by comparison with the literature.

1. Introduction

Isoflavonoids represent a group of secondary metabolites widely distributed in higher plants. They often occurred in plants as glycosides in which one or more of the phenolic hydroxyl groups or aromatic carbons are combined with sugar residues (Mackova et al., 2006; Veitch, 2007; Botta et al., 2009; Yao-Kouassi et al., 2008; Farag et al., 2001; Wang et al., 2006; Tang et al., 2008). Isoflavonoids have attracted considerable interest because of a large variety of biological activities, such as antioxidant (Komiyama et al., 1989; Harper et al., 1999), antiplasmodial (Yenesew et al., 2003; Kraft et al., 2000), cytotoxic (Cottiglia et al., 2005; Nkengfack et al., 2001), anti-inflammatory (Laupattarakasem et al., 2004; Rahman et al., 2003) and antimicrobial (Sato et al., 2003; Redko et al., 2007). Recently, we reported the isolation of one new isoflavonoid glycoside named chevalierinoside A (Djouossi et al., 2014), from the stem bark of a small tree, *Antidesma laciniatum* Muell. Arg. (syn. *Antidesma chevalieri* Beille), used in Africa to prevent miscarriage and to treat intestinal complaints (Schmelzer, 2008). During phytochemical investigation of the methanol extract of the stem bark of this small tree (Phyllantaceae), we found some minor compounds that were difficult to separate due to the small quantity of the extract. As an extension of our previous work, we now report the isolation of two new isoflavonoid glycosides, chevalierinosides B (**1**) and C (**2**), together with the known chevalierinoside A (**3**), genistein 7-O-β-D-glucopyranoside (**4**), kaempferol 3-O-β-D-glucopyranoside (**5**), friedelin (**6**), betulinic acid (**7**), 30-oxobetulinic acid (**8**), 30-hydroxybetulinic acid (**9**), from the methanol extract of the stem bark of the same highest and oldest species, founded in the same Region during another fieldtrip collection.

2. Results and discussion

Compound **1** was obtained as a yellowish gum. Its molecular formula $C_{27}H_{30}O_{14}$ was determined on the basis of its HR-TOFESIMS spectrum exhibiting a pseudo-molecular ion peak at *m*/*z* 601.1537 $[M+Na]^+$ (calcd. 601.1533) and confirmed by ^{13}C

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NMR and DEPT analysis. This was in accord with an isoflavone having one hydroxyl, one methoxyl, and one pentosyl-hexosyl substitutions. The IR spectrum showed absorption bands for hydroxyl (3500–3200 cm^{-1}), carbonyl (1656 cm^{-1}) and aromatic (1614 and 1581 cm^{-1}) functionalities. The NMR spectral data for the aglycon moiety were in agreement with those of 5,7-dihydroxy-4′-methoxyisoflavone (biochanin A) (Santos et al., 1995). The ^{1}H NMR spectrum of **1** exhibited signals at δ 8.20 (H-2, *s*), 6.71 (H-8, *d*, *J* = 2.3 Hz), 6.52 (H-6, *d*, *J* = 2.3 Hz), 7.52 (H-2′/H-6′, *d*, *J* = 8.9 Hz) and 7.02 (H-3′/H-5′, *d*, *J* = 8.9 Hz) indicative of substitutions on carbons 5, 7 and 4′ of the isoflavone. The signal at δ 3.86 (*s*) correlated to the ^{13}C NMR signal at δ 159.7 in the HMBC spectrum (Table 1), confirming that the methoxyl group was located at C-4′ in biochanin A as observed with chevalierinoside A (**3**) (Djouossi et al., 2014) and lanceolarin (Rao et al., 1989) and not at C-5 as in eriosemaside C (Ma et al., 1999). After acid hydrolysis of **1**, only two sugar units, glucose and apiose were detected by TLC while the aglycon was also identified to biochanin A as previously described (Djouossi et al., 2014; Santos et al., 1995). The difference between chevalierinoside A (**3**) and **1** was the absence of the rhamnosyl moiety in **1**. This was supported by the ^{1}H NMR spectrum of **1** which displayed only two sugar anomeric protons at δ 5.16 (*d*, *J* = 7.5 Hz) and 5.48 (*d*, *J* = 1.7 Hz) giving correlations with two anomeric carbon at δ 98.8 and 109.5 respectively in the HSQC spectrum (Table 1). The chemical shift of the terminal oxymethylene carbon (C-6; δ 61.0) of the glucopyranosyl moiety also confirmed the absence of substitution at this position (Djouossi et al., 2014). Complete assignments of each sugar proton system were achieved by analysis of $^{1}H-^{1}H$ COSY, TOCSY and NOESY spectra while carbons were assigned from HSQC and HMBC spectra. Evaluation of the spin-spin couplings and chemical shifts allowed the identification of one terminal β-apiofuranosyl (Api) and one inner β-glucopyranosyl (Glc) units. The D-configuration for glucose and apiose was suggested by comparison of the NMR data of **1** with those of chevalierinoside A (**3**) (Djouossi et al., 2014), and, was confirmed by GC–MS after derivatization.

The HMBC spectrum correlations between H-1 (δ 5.16) of Glc and C-7 (δ 163.3) of the aglycon indicated that Glc was linked at C-7 of the aglycon. Furthermore, the HMBC correlation between H-1 (δ 5.48) of Api and C-2 (δ 77.3) of Glc established the connectivity between Api and Glc. This was supported in comparison between ^{13}C data of **1** with those of eriosemaside C (Ma et al., 1999) possessing the same glycosidic linkage and lanceolarin (Rao et al., 1989) possessing the apiose unit linked in position 6 of the glucose unit. Therefore, the oligosaccharide at C-7 of the aglycon was established as *O*-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside]. On the basis of the above evidences, the structure of compound **1** was determined as biochanin A 7-*O*-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside], a new isoflavonoid glycoside named chevalierinoside B.

Compound **2**, a yellowish gum, had a pseudo-molecular ion peak at *m*/*z* 587.1379 $[M+Na]^{+}$ (calcd. for $C_{26}H_{28}O_{14}Na$, 587.1377) in the HR-TOFESIMS, one methyl fewer than compound **1**. Comparison of their NMR spectroscopic data revealed that **2** differed only in ring B of the aglycon moiety. The shielding of C-4′ (δ 157.5) and H-3′/H-5′ (δ 6.88), and the deshielding of C-3′/C-5′ (δ 114.9) indicated the absence of methyl on the hydroxyl at C-4′ (Table 1). These NMR spectral data were in agreement with those of 5,7,4′-trihydroxyisoflavone (genistein) (Chaturvedula and Prakash, 2013). Acid hydrolysis of **2** also afforded apiose and glucose, detected by TLC, analysis of 1D- and 2D-NMR spectral data of **2** permitted the identification of one terminal β-apiofuranosyl (Api) and one inner β-glucopyranosyl

Table 1
^{1}H (600 MHz) DEPT and ^{13}C (150 MHz) NMR data of compounds **1** and **2** in CD_3OD, and some HMBC correlations of **1** and **2**.

No	**1**		**2**		HMBC (H→C)
	δ_C	δ_H (*mult*; *J*)	δ_C	δ_H (*mult*; *J*)	
2	154.1	8.20 (s)	153.9	8.17 (s)	3, 4, 9, 1′
3	123.1		123.7		
4	181.1		181.8		
5	162.0		162.2		
6	99.6	6.52 (d, 2.3)	99.5	6.52 (d, 2.2)	5, 7, 8, 10
7	163.3		163.2		
8	94.4	6.71 (d, 2.3)	94.4	6.71 (d, 2.2)	6, 7, 9, 10
9	157.9		157.9		
10	107.0		106.6		
1′	122.9		121.9		
2′	130.0	7.52 (d, 8.9)	130.0	7.42 (d, 8.8)	3, 4′
3′	113.5	7.02 (d, 8.9)	114.9	6.88 (d, 8.8)	1′, 4′, 5′
4′	159.7		157.5		
5′	113.5	7.02 (d, 8.9)	114.9	6.88 (d, 8.8)	1′, 4′
6′	130.0	7.52 (d, 8.9)	130.0	7.42 (d, 8.8)	3, 4′
4′-OMe	54.3	3.86 (s)		–	4′
Glc					
1	98.8	5.16 (d, 7.5)	98.8	5.16 (d, 7.5)	7, Glc-3
2	77.3	3.70 (dd, 8.8, 7.5)	77.3	3.69 (dd, 9.2, 7.5)	Glc-1, Api-1
3	77.0	3.65 (t, 8.8)	77.0	3.65 (t, 9.2)	Glc-2, Glc-4
4	69.8	3.43 (dd, 9.6, 8.8)	69.8	3.43 (t, 9.3)	Glc-5, Glc-6
5	76.9	3.52 (m)	76.9	3.53 (m)	
6	61.0	3.73 (dd, 12.3, 5.9)	61.0	3.73 (dd, 12.2, 5.8)	Glc-4
		3.92 (dd, 12.3, 2.2)		3.92 (dd, 12.2, 2.2)	Glc-4
Api					
1	109.5	5.48 (d, 1.7)	109.5	5.47 (d, 1.7)	Api-3, Api-4, Glc-2
2	76.7	3.97 (d, 1.7)	76.7	3.97 (d, 1.7)	Api-5
3	79.3		79.3		
4	74.0	3.83 (d, 9.6)	74.0	3.83 (d, 9.6)	Api-1, Api-2, Api-3, Api5
		4.04 (d, 9.6)		4.04 (d, 9.6)	
5	64.4	3.56 (s)	64.4	3.55 (s)	Api-2, Api-3, Api4

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(Glc) units, as in **1**. The D-configuration for glucose and apiose was determined by GC–MS after derivatization. The HMBC correlation between H-1 (δ 5.16) of Glc and C-7 (δ 163.2) of the aglycon and H-1 (δ 5.47) of Api and C-2 (δ 77.3) of Glc, confirmed the same diglycoside moity as in **1**. Thus, compound **2** was established as genistein 7-*O*-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside], named chevalierinoside C.

Structures of known compounds were determined as two isoflavonoids, one flavonoid and four triterpenes by means of *Co*-TLC, and by comparative analysis of their physical and spectral data with those reported in the literature for chevalierinoside A (**3**) (Djouossi et al., 2014), genistein 7-O-β-D-glucopyranoside (**4**) (Fedoreyev et al., 2008), kaempferol 3-O-β-D-glucopyranoside (**5**) (Shahat et al., 2005), friedelin (**6**) (Chandler and Hooper, 1979), betulinic acid (**7**) (Sholichin et al., 1980), 30-oxobetulinic acid (**8**) (Macias et al., 1998) and 30-hydroxybetulinic acid (**9**) (Mayer, 1996).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 spectrometer equipped with a cryoplatform (^{1}H at 600 MHz and ^{13}C at 150 MHz). 2D-NMR experiments were performed using standard Bruker microprograms (Xwin-NMR version 2.1 software). Chemical shifts (δ) are reported in parts per million (ppm) with the solvent signals as reference relative to TMS ($\delta = 0$) as internal standard, while the coupling constants (*J* values) are given in Hertz (Hz). The IR spectra were recorded with a Shimadzu FT-IR-8400S spectrophotometer. UV spectra were determined as methanol solution with a Cary 50 UV/VIS Spectrophotometer. HR-TOFESIMS experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK) with an electrospray source. The samples were introduced by direct infusion in a solution of MeOH at a rate of 5 μL min^{-1}. GC–MS experiments were carried out on an MD 800 instrument. Column chromatography (CC) was performed on silica gel 60 (70–230 mesh, Merck) and gel permeation on Sephadex LH-20 while TLC was carried out on silica gel GF_{254} pre-coated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C, or by visualizing with an UV lamp at 254 and 365 nm.

3.2. Plant material

The stem bark of *Antidesma laciniatum* Muell. Arg. (syn. *Antidesma chevalieri* Beille) was collected at Bansoa, Menoua Division, West Region of Cameroon, in January 2013. Authentication was done by Mr Victor Nana, a botanist of the Cameroon National Herbarium, Yaoundé, where the voucher specimen (No. 9667/SRF/Cam) is deposited.

3.3. Extraction and isolation

The dried and powdered plant material (7 kg) was extracted by percolation with methanol (3× 15 L, 72 h) at room temperature affording 120 g of crude extract after evaporation of the solvent under vacuum.

A portion (90 g) of this extract was subjected to silica gel column chromatography (CC) eluting with gradient mixtures of *n*-hexane-EtOAc (1:0, 9:1, 4:1, 7:3, 1:1, 3:7 and 0:1) followed by EtOAc-MeOH (19:1, 9:1, 17:3, 4:1, 1:1 and 0:1). 57 fractions, each 300 mL, were collected and combined on the basis of their TLC profiles to give five fractions noted F1 to F5. Fraction F1 was purified on silica gel CC eluted with *n*-hexane-EtOAc (19:1 and 9:1) and 32 fractions (25 mL each) were collected. Re-crystallization of fractions [12–24] gave friedelin (**6**) (34.5 mg). Fraction F2 was purified on silica gel CC eluted with *n*-hexane-EtOAc (4:1, 3:2 and 1:1) and 33 sub-fractions (25 mL each) were collected. Re-crystallization of fractions [5–11], [16–20] and [25–29] afford betulinic acid (**7**) (52 mg), 30-oxobetulinic acid (**8**) (12 mg) and 30-hydroxybetulinic acid (**9**) (10.5 mg), respectively. Fractions F3 and F4 were respectively passed through sephadex LH-20 CC eluted with methanol. 42 fractions of 10 mL each were collected from F3 and 37 others from F4. Fractions [23–40] from F3 (3 g) and [10–25] from F4 (2.5 g) were combined and passed through silica gel CC eluted with EtOAc-MeOH (1:0, 19:1 and 9:1). 46 fractions (50 mL each), were collected and combined on the basis of their TLC profiles. The fractions [1–13] (1.2 g), [14–21] (0.7 g) and [22–46] (1.3 g) were purified separately through sephadex LH-20, eluted with methanol, yielding genistein 7-O-β-D-glucopyranoside (**4**) (12 mg) and kaempferol 3-O-β-D-glucopyranoside (**5**) (6 mg) from [1–13], compounds **1** (15 mg) and **2** (17 mg) from [14–21], and chevalierinoside A (**3**) (29 mg) from [22–46]. Attempts of purification of fraction F5 failed.

3.4. New compounds information

Chevalierinoside B (**1**): yellowish gum; $[\alpha]_D^{23}$ −49.5 (*c* = 3.3, MeOH); IR (NaCl) ν_{max} (cm^{-1}): 3500–3200 (OH); 2925; 1656 (C–O), 1614, 1581 (aromatic); 1440; 1290; 1249; 1180; 1068; ^{1}H and ^{13}C NMR data, see Table 1; HR-TOFESIMS *m/z*: 601.1537 $[M+Na]^+$ (calcd. for $C_{27}H_{30}O_{14}Na$, 601.1533).

Chevalierinoside C (**2**): yellowish gum; $[\alpha]_D^{23}$ −36.0 (*c* = 0.50, MeOH); IR (NaCl) ν_{max} (cm^{-1}): 3500–3200 (OH); 2920; 1656 (C–O), 1612, 1575 (aromatic); 1251; 1180; 1072; ^{1}H and ^{13}C NMR data, see Table 1; HR-TOFESIMS *m/z*: 587.1379 $[M+Na]^+$ (calcd. for $C_{26}H_{28}O_{14}Na$, 587.1377).

3.5. Acid hydrolysis and GC–MS analysis of 1 and 2

Each of compound **1** (10 mg) and **2** (10 mg) was respectively dissolved in MeOH-2N HCl (1:4) (10 mL) and refluxed at 80 °C for 3 h. After removal of MeOH under reduced pressure, the aqueous layer was extracted with CH_2Cl_2 (3× 5 mL). The combined CH_2Cl_2 extracts were washed with H_2O and evaporated to dryness to afford biochanin A (1.5 mg) (Santos et al., 1995) from **1**, and genistein (2 mg) (Chaturvedula and Prakash, 2013) from **2**. Each aqueous layer was neutralized by dilute NaOH. The sugar components were analyzed by co-TLC with the mixture $CHCl_3$/MeOH/H_2O (70:30:2). After spraying, apiose gave a weak yellow spot at Rf 0.79, and glucose gave a blue spot at Rf 0.71.

Each previous aqueous layer was concentrated to dryness. The residue obtained was dissolved in pyridine (1 mL), then $(CH_3)_3SiNHSi(CH_3)_3$ (1 mL) was added. After 10 min at room temperature, the solution was blown to dryness with a stream of nitrogen. The residue was dissolved in diethyl ether then subjected to GC–MS analysis.

Trimethylsilyl ether derivatives were separated using an HP Ac-5 capillary column (0.25 m × 30 m). Nitrogen was used as the carrier gas. The initial column oven temperature was 180 °C, then increased at 5 °C min^{-1} to a final value of 240 °C. The sugars were determined by comparison of retention times (t_R) with standard sugars: t_R (min) Glc 6.86, Api 2.78.

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