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SHORT COMMUNICATION

CHEVALIERINOSIDE A: A NEW ISOFLAVONOID GLYCOSIDE FROM THE STEM BARK OF *ANTIDESMA CHEVALIERI* BEILLE (EUPHORBIACEAE)

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ABSTRACT. Chevalierinoside A (**1**), a new isoflavonoid glycoside determined as biochanin A 7-*O*-[α-L-rhamnopyranosyl-1→6-*β*-D-apiofuranosyl-1→2-*β*-D-glucopyranoside], together with the known friedelin (**2**), friedelan-3β-ol (**3**) and betulinic acid (**4**), were isolated from the stem bark of *Antidesma chevalieri* Beille. Their structures were established by direct interpretation of their spectral data, mainly TOF-HRESIMS, 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (COSY, ROESY, TOCSY, HSQC and HMBC), and by comparison with the literature.

KEY WORDS: *Antidesma chevalieri*, Euphorbiaceae, Isoflavonoid glycoside, Chevalierinoside A

INTRODUCTION

The genus *Antidesma* (Euphorbiaceae) contains about 170 species, forest shrubs and trees, distributed in the world [1, 2]. *Antidesma chevalieri* Beille (syn. *Antidesma laciniatum* Muell. Arg. var. laciniatum) is a tree of about 9 m high encountered in Africa, particularly in Cameroon, Democratic Republic of Congo and Equatorial Guinea [3], and occupies a prominent position in traditional African medicine [4]. Previous studies on the genus afforded terpenoids, steroids, flavonoids [2] and alkaloids [5], some of which possessed various biological activities [5-7]. Particularly, squalene, (*E*)-phyt-2-en-1-ol, sitosterol and amentoflavone are the only compounds previously isolated from the leaves of *Antidesma laciniatum* Muell. Arg. [2]. Continuing with the phytochemical investigation of plants of the genus *Antidesma*, we herein report the isolation and structure elucidation of a novel isoflavonoid glycoside, chevalierinoside A (**1**) from the methanol extract of the stem bark of *A. chevalieri* Beille.

EXPERIMENTAL

Melting points were recorded with a Reichert microscope and are uncorrected. Optical rotations were measured on a Belligham and Stanley ADP 220 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

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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. TOF-ESIMS and HR-TOF-ESI 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}. Column chromatography was run on Merck silica gel 60 (70-230 mesh) 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.

Plant material. The stem bark of *Antidesma chevalieri* Beille was collected at Bansoa, Menoua Division, West Region of Cameroon, in July 2011. Authentication was done by Mr Victor Nana, a botanist of the Cameroon National Herbarium, Yaoundé, where a voucher specimen (No. 9667/SRF/Cam) has been deposited.

Extraction and isolation. The dried and powdered plant material (3 kg) was extracted by percolation with methanol (3 x 10 L, 72 h) at room temperature affording 50 g of crude extract after evaporation of the solvent under vacuum. A portion (47 g) of this extract was subjected to silica gel column chromatography eluting with gradient mixtures of *n*-hexane-EtOAc (10:0, 9:1, 4:1, 7:3, 1:1, 3:7 and 0:10) followed by EtOAc-MeOH (19:1, 9:1, 17:3, 4:1, 1:1 and 0:10). 40 fractions of 300 mL each were collected and combined on the basis of their TLC profiles to give 5 major fractions: F1 (1-8, 12.9 g), F2 (9-16; 7.7 g), F3 (17-27; 2.3 g), F4 (28-35; 3.1 g) and F5 (36-40; 2.2 g). F1 and F2 were respectively purified on silica gel column chromatography eluted with *n*-hexane-EtOAc (19:1 and 9:1 for F1, 4:1, 3:2, and 1:1 for F2). 18 sub-fractions of 25 mL each were collected from F1. Re-crystallization of sub-fractions 7-8 and 10-11 gave friedelin (20 mg) and friedelan-3β-ol (18 mg) respectively. From F2, 23 sub-fractions of 25 mL each were collected affording betulinic acid (33 mg) after re-crystallization of sub-fractions 6-8. F4 was passed through sephadex LH-20 column eluted with methanol. 20 sub-fractions of 10 mL each were collected. Sub-fractions 12-18 (D) were combined and passed through silica gel column chromatography eluted with EtOAc-MeOH (19:1 and 9:1). Purification of D3 (combined fractions 10-14 from D) through sephadex LH-20 eluted with methanol yielded compound **1** (31 mg). Attempts of purifications of fractions F3 and F5 failed.

*Chevalierinoside A (**1**)*. Yellowish gum, $[\alpha]_D^{23}$ - 4° (*c* 0.53, MeOH). IR (NaCl) ν_{max} (cm^{-1}): 3500-3200 (OH); 1654 (C = O), 1608, 1583 (aromatic). ^{1}H- and ^{13}C-NMR data, see Table 1. TOF-ESIMS *m/z*: 1471.5 $[2M+Na]^+$, 747.2 $[M+Na]^+$. TOF-ESIMS-MS 747 *m/z*: 747.2 $[M+Na]^+$, 615.2 $[(M+Na) - 132]^+$, 469.1 $[(M+Na) – 132 - 146]^+$, 307.1 $[(M+Na) - 132 - 146 - 162]^+$, TOF-HRESIMS *m/z*: 747.2105 $[M+Na]^+$, calcd. for $C_{33}H_{40}O_{18}Na$: 747.2112.

Acid hydrolysis and GC-MS analysis. Compound **1** (10 mg) was dissolved in MeOH-2 N 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 x 5 mL). The combined CH_2Cl_2 extracts were washed with H_2O and evaporated to dryness to afford the aglycon (2 mg) identified as 5,7-dihydroxy-4'-methoxyisoflavone by comparison of its 1D-NMR data with those of literature [8]. The aqueous layer was neutralized by dilute NaOH. The sugar components were analyzed by co-TLC with the mixture $CHCl_3$/MeOH/H_2O (7:3:0.2). After spraying, apiose gave a weak yellow spot at Rf 0.78, rhamnose gave a green spot at R_f 0.75 and glucose gave a blue spot at Rf 0.70.

The 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.

GC-MS experiments were carried out on an MD 800 instrument. Trimethylsilyl ether derivatives were separated using an HP Ac-5 capillary column (0.25 x 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 6.87, Rha 4.32, Api 2.80.

RESULTS AND DISCUSSION

The high resolution time of flight electrospray ionisation mass spectrometry (HR-TOF-ESI-MS) of compound **1** (Figure 1) exhibited a pseudo-molecular ion peak at *m/z* 747.2105 $[M+Na]^+$ (calculated. 747.2112) consistent with a molecular formula $C_{33}H_{40}O_{18}$ and confirmed by ^{13}C NMR and DEPT analysis. This was in accord with an isoflavone having one hydroxyl, one methoxyl, and one pentosyl-deoxyhexosyl-hexosyl substitutions. The TOF-ESIMSMS of the $[M+Na]^+$ ion peak at *m/z* 747 gave significant fragment ion peaks observed at *m/z* 615 $[(M+Na)-132]^+$, 469.1 $[(M+Na)-132-146]^+$, and 307 $[(M+Na)-132-146-162]^+$ corresponding, respectively, to the successive loss of one pentosyl, one deoxyhexosyl and one hexosyl moieties from the pseudomolecular ion. The UV spectrum showed two maxima absorption at 300 and 260 nm, characteristic of isoflavone. The IR spectrum showed absorption bands for hydroxyl (3500-3200 cm^{-1}), carbonyl (1654 cm^{-1}) and aromatic (1608 and 1583 cm^{-1}) functionalities. The NMR spectral data for the aglycon moiety were in agreement with those of biochanin A [8]. In the ^1H NMR spectrum of **1**, signals at δ 7.93 (H-2, *s*), 6.56 (H-8, *d*, *J* = 2.2 Hz), 6.41 (H-6, *d*, *J* = 2.2 Hz), 7.39 (H-2'/H-6', *d*, *J* = 8.7 Hz) and 6.92 (H-3'/H-5', *d*, *J* = 8.7 Hz) indicated substitutions on carbons 5, 7 and 4' of the isoflavone. The signal at δ_H 3.78 (*s*) correlated to the ^{13}C NMR signal at δ_C 159.7 in the HMBC spectrum (see Table 1 and Figure 2) suggesting that the methoxyl group was located at C-4'. After acid hydrolysis of **1**, glucose, apiose and rhamnose were detected by TLC and the aglycon was identified (ESIMS, ^1H- and ^{13}C- NMR) as 5,7-dihydroxy-4'-methoxyisoflavone (biochanin A) [8]. The ^1H NMR spectrum of **1** displayed three sugar anomeric protons at δ 4.88 (*d*, *J* = 7.7 Hz), 4.69 (*brs*) and 5.33 (*d*, *J* = 1.6 Hz) giving correlations with three anomeric carbon atoms at δ 99.2, 100.4 and 109.5, respectively in the HSQC spectrum (Table 1) confirming that this compound contains three sugar units. Complete assignments of each sugar proton system were achieved by analysis of ^1H-^1H COSY, ROESY and TOCSY spectra (see the main correlations on Figure 3) while carbons were assigned from HSQC and HMBC spectra. Starting from the anomeric proton at δ_H 4.69 (*brs*), a terminal α-rhamnopyranosyl (Rha) with its methyl signal observed at δ_H 1.18 (*d*, *J* = 6.0 Hz) was identified after evaluation of the spin-spin couplings and carbon chemical shifts. Starting from the anomeric proton at δ 4.88 (*d*, *J* = 7.7 Hz), a seven spin system was identified corresponding to an inner β-glucopyranosyl (Glc) unit. The deshielding signals of carbons C-2'' (δ_C 77.6) and C-6'' (δ_C 66.5) suggest a disubstitution of this unit. From the last anomeric signal at δ 5.33 (*d*, *J* = 1.6 Hz), the structure of one terminal β-apiofuranosyl (Api) was elucidated. The analysis of COSY spectra showed correlations between proton H-1'''' and proton H-2'''' (δ 3.91, *d*, *J* = 1.6 Hz) and between protons of two isolated AB system, one at 3.80 and 3.90 (*d*, *J* = 9.8 Hz, H-4'''') and the second at 3.53 (*s*, 2H, H-5''''). In the HMBC spectra the proton H-1'''' was correlated with the methylene C-4'''' (δ_C 74.2) and a quaternary carbon C-3'''' (δ_C 79.1), the proton H-2'''' was also correlated with carbons C-3'''' and C-4'''' and carbons C-2'''', C-3'''' and C-4'''' were correlated with the methylene H-5'''' (δ_H 3.53). The rOe effect observed between H-2'''' and H-5'''' confirmed the structure of the apiofuronosyl moiety [8]. The sugar units were confirmed by thin layer chromatography after hydrolysis. The D-configuration for

glucose and apiose, and L-configuration for rhamnose were suggested by comparison of the NMR data of **1** with those of related compounds (including biochanin A 7-*O*-*α*-L-rhamnopyranosyl-1→6-*β*-D-glucopyranoside and biochanin A 7-*O*-*β*-D-apiofuranosyl-1→5-*β*-D-apiofuranosyl-1→6-*β*-D-glucopyranoside) [9-12], and were confirmed by GC-MS after derivatization.

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

No	δ^{13}C	δ ^{1}H (*mult* , *J*)	HMBC (H → C)
2	153.5	7.93 (*s*)	3, 4, 9, 1’
3	123.6		
4	181.0		
5	162.0		
6	100.1	6.41 (*d*, 2.2)	5, 7, 8, 10
7	162.9		
8	95.1	6.56 (*d*, 2.2)	6, 7, 9, 10
9	157.7		
10	107.1		
1’	122.8		
2’	130.1	7.39 (*d*, 8.7)	3, 4’
3’	114.0	6.92 (*d*, 8.7)	1’, 4’, 5’
4’	159.7		
5’	114.0	6.92 (*d*, 8.7)	1’, 4’, 3’
6’	130.1	7.39 (*d*, 8.7)	3, 4’
4’-OMe	55.3	3.78 (*s*)	4’
Glc			
1’’	99.2	4.88 (*d*, 7.7)	7, Glc-3’’, Glc-5’’
2’’	77.6	3.62 (*m*)	Glc-1’’, Api-1’’’’
3’’	77.0	3.54 (*m*)	Glc-1’’, Glc-5’’
4’’	70.0	3.35 (*t, 9.1*)	Glc-3’’, Glc-5’’, Glc-6’’
5’’	75.6	3.51 (*m*)	
6’’	66.5	4.00 (*d, 9.2*) 3.53 (*d, 9.2*)	Glc-4’’, Rha-1’’’ Glc-4’’, Rha-1’’’
Rha			
1’’’	100.4	4.69 (*brs*)	Glc-6’’, Rha-2’’’, Rha-3’’’, Rha-5’’’
2’’’	70.4	3.87 (*dm, 3.7*)	Rha-3’’’, Rha-4’’’
3’’’	71.3	3.73 (*dd, 9.5, 3.7*)	Rha-4’’’, Rha-5’’’
4’’’	72.9	3.30 (*t, 3.5*)	Rha-3’’’, Rha-5’’’, Rha-6’’’
5’’’	68.3	3.60 (*m*)	
6’’’	17.2	1.18 (*d*, 6.0)	Rha-4’’’, Rha-5’’’
Api			
1’’’’	109.5	5.33 (*d, 1.6*)	Glc-2’’, Api-3’’’’, Api-4’’’’
2’’’’	76.6	3.91 (*d, 1.6*)	Api-4’’’’
3’’’’	79.1		
4’’’’	74.2	3.80 (*d, 9.9*) 3.90 (*d, 9.9*)	Api-1’’’’, Api-2’’’’, Api-3’’’’, Api-5’’’’ Api-1’’’’, Api-2’’’’, Api-3’’’’, Api-5’’’’
5’’’’	65.0	3.53 (*s*)	

In the HMBC spectrum correlation observed between H-1’’ (δ 4.88) of Glc and C-7 (δ 162.9) of the aglycon indicated that Glc was linked at C-7 of the aglycon. Furthermore the HMBC correlations observed between H-1’’’’ (δ 5.33) of Api and C-2’’ (δ 77.6) of Glc and

between H-1''' (δ4.69) of Rha and C-6'' (δ66.5) of Glc established the connectivities between Api and Glc and between Glc and Rha (see Table 1 and Figure 2). These were confirmed by ROESY correlations (see Figure 3) observed between H-2'' (δ3.63) of Glc and H-1'''' (δ5.33) of Api, and between H-1''' (δ 4.69) of Rha and 2H-6'' (δ 4.00/3.53) of Glc. Hence, the oligosaccharide at C-7 of the aglycon was established as *O*-[α-L-rhamnopyranosyl-1→6-β-D-apiofuranosyl-1→2-β-D-glucopyranoside]. Thus, the structure of compound **1** was established as biochanin A 7-*O*-[α-L-rhamnopyranosyl-1→6-β-D-apiofuranosyl-1→2-β-D-glucopyranoside] a new isoflavonoid glycoside named chevalierinoside A.

Structures of compounds **2-4** (Figure 1) were determined by means of *Co* TLC, spectroscopic data, and by comparative analysis of their physical and spectral data with those reported in the literature for friedelin [13], friedelan-3β-ol [13] and betulinic acid [14], respectively.

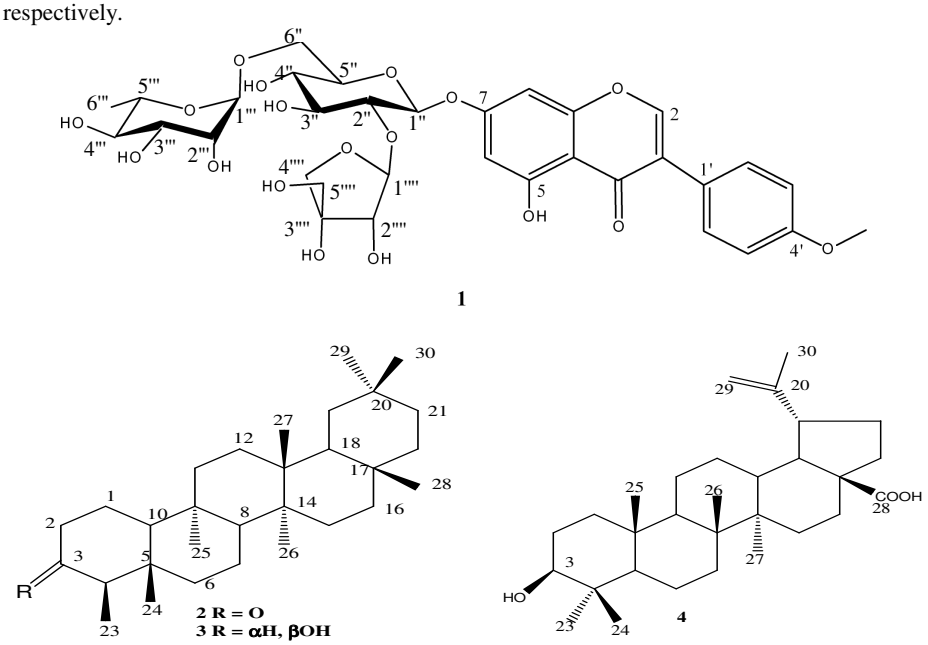

Figure 1. Structures of the isolated compounds.

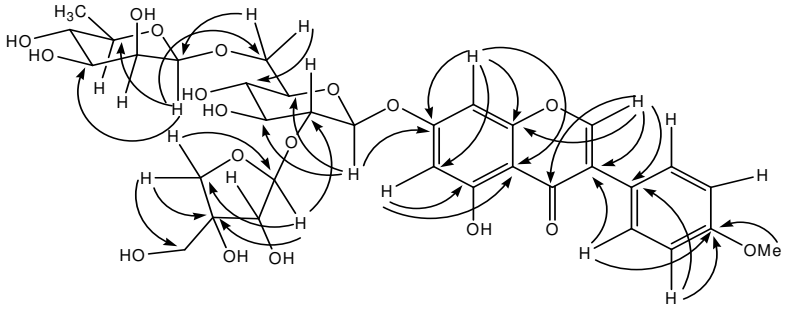

Figure 2. Important HMBC correlations of compound **1**.

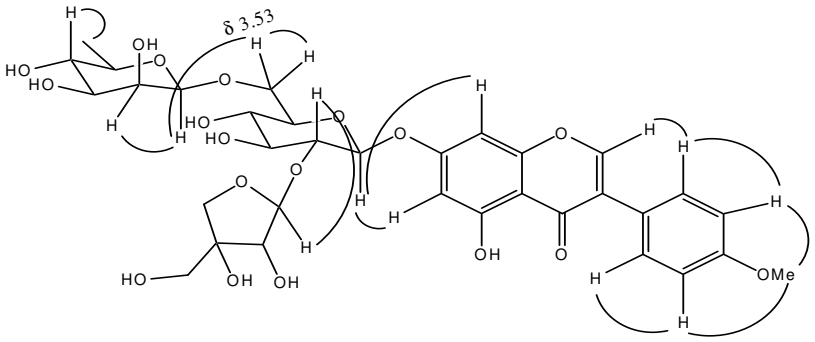

Figure 3. Key ROESY correlations of compound **1**.

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