Justicialosides A and B, two new flavone glycosides from the leaves of *Ruspolia hypocrateriformis* (Vahl) Milne-Redh. (Acanthaceae)

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ABSTRACT

Two new flavone glycosides, luteolin 7-*O*-*α*-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside (**1**) and chrysoeriol 7-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside (**2**), along with five known compounds, luteolin 7-*O*-*β*-D-apiofuranosyl-(1→2)-β-D-xylopyranoside (**3**), grandulosides A and B (**4** and **5**), luteolin 7-*O*-[*β*-D-glucopyranosyl-(1→2)-*α*-L-rhamnosyl-(1→6)]-*β*-D-glucopyranoside (**6**) and 10*H*-quindoline (**7**) were isolated from the leaves of *Ruspolia hypocrateriformis* (Acanthaceae). Their structures were elucidated by spectroscopic means, including 1D and 2D NMR, HRESIMS and by comparison with published data.

Keywords: Ruspolia hypocrateriformis; Acanthaceae; Medicinal plant; Glycosylated flavones; Luteolin; Justicialoside

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1. Introduction

Ruspolia hypocrateriformis (Vahl) Milne-Redh., synonym *Justicia hypocrateriformis* Vahl, is a small shrub with scattered growth measuring above 1 m in height and is native to Africa (Burch and Demmy, 1986). A decoction of the leaves of this plant is used as a remedy in Cameroon for the treatment of anaemia and fever associated with malaria as well as for the management of diarrhoea (Adjanohoun *et al*., 1996; Noumi, 2015). Previous studies reported the isolation of pyrrolidine alkaloids from the roots (Roessler *et al*., 1978; Neukomm *et al*., 1983); the aqueous extract of the leaves was shown to possess antidiarrhoeal and antioxidant properties (Agbor *et al*., 2014). As a part of our continuing study on the secondary metabolites of Cameroonian medicinal plants (Wansi *et al*., 2016; Guetchueng *et al*., 2017, 2018a, 2018b), we have undertaken the phytochemical investigation of *Ruspolia hypocrateriformis*. The present work describes the isolation and identification of two new flavone glycosides (**1** and **2**) from the leaves of this plant (Figure 1).

2. Results and discussion

A combination of reversed-phase solid-phase-extraction and reversed-phase preparative HPLC purification of the methanolic extract of the leaves of *R. hypocrateriformis* afforded seven compounds; two new flavone glycosides, luteolin 7-*O*-*α*-L-rhamnopynosyl-(1→2)-*β*-D-xylopyranoside (**1**) and chrysoeriol-7-*O*-*α*-L-rhamnopynosyl-(1→2)-*β*-D-xylopyranoside (**2**), named justicialosides A and B (Figure 1), respectively, and five known compounds, luteolin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-xylopyranoside (**3**) (Koffi *et al*., 2013), grandulosides A and B (**4** and **5**) (Tagoutsop *et al*., 2017), luteolin 7-*O*-[*β*-D-glucopyranosyl-(1→2)-*α*-L-rhamnosyl-(1→6)]-*β*-D-glucopyranoside (**6**) (Koffi *et al*., 2013) and 10*H*-quindoline (**7**) (Subbaraju *et al*., 2004). The structures of all compounds were elucidated by spectroscopic means, and by comparison with respective published data (for known compounds).

Both compounds **1** and **2** were isolated as yellow amorphous powders. The molecular formula $C_{26}H_{28}O_{14}$ of **1** was determined from its HR ESI-MS spectrum obtained in +ve ion mode, where the sodiated ion peak was observed at *m/z* 565.1559, calculated for $[C_{26}H_{28}O_{14}+H]^+$, 565.1552, consistent with thirteen DBE. The ^{1}H NMR (Table 1) spectrum of **1** displayed 17 signals, which could be attributed to a glycosylated molecule bearing two

sugar moieties. The aglycone part of the compound was identified as luteolin by the presence of an ABX system resonating at δ_H 7.25 (d, *J* = 8.3 Hz), 7.50 (dd, *J* = 2.2, 8.3 Hz) and 7.88 (d, *J* = 2.2 Hz), characteristic of a 3,4-dihydroxylated B ring; an AX system appearing as two doublet signals at δ_H 6.94 and 6.96 (*J* = 2.1 Hz), typical of a *meta*-dihydroxylated A ring, and one singlet at δ_H 6.91 assignable to the H-3 proton of the luteolin C ring. The sugar moieties were identified by the presence of two anomeric proton signals at δ_H 5.62 (d, *J* = 7.5 Hz) and 6.38 (d, *J* = 1.6 Hz) and a doublet methyl at δ_H 1.78 (*J* = 6.1 Hz) showing a cross peak correlation in the HSQC spectrum with the carbon signal resonating at δ_C 18.6 suggesting a rhamnose as one of the sugars. Complete assignment of the protons and carbons (Table 1) of the sugar units in **1** was achieved by analysis of its 2D NMR spectra including COSY, HSQC, HMBC and TOCSY spectra. The sugars were identified as *β*-D-xylopyranose and *α*-L-rhamnose with their anomeric protons resonating at δ_H 5.62 (d, *J* = 7.5 Hz) and 6.38 (d, *J* = 1.6 Hz), respectively, by comparison of their spectroscopic data with those reported in the literature (Beier and Mundy, 1980; Agrawal, 1992). In the HMBC spectrum (Figure 2), a correlation could be observed between the proton H-1″ (δ_H 5.62) of the xylose and the carbon C-7 (δ_C 163.2) of the aglycone, confirming its direct attachment to the luteolin skeleton. Other key correlations observed between the proton H-1‴ (δ_H 6.38) of the rhamnose and the carbon C-2″ of the xylose (δ_C 77.4) and between the proton H-2″ (δ_H 4.47) of the xylose and the carbon C-1‴ (δ_C 102.3) of the rhamnose confirmed attachment of the rhamnosyl unit on the carbon C-2″ of the xylose, making it a disaccharyl moiety linked to luteolin skeleton. The ^{13}C NMR data (Table 1) for all the 26 carbon atoms required for this luteolin glycoside could be fully obtained from HSQC and HMBC spectra. Thus, compound **1** was identified as luteolin 7-*O*-*α*-L-rhamnopynosyl-(1→2)-*β*-D-xylopyranoside, a new flavone glycoside, and was named justicialoside A.

The molecular formula $C_{27}H_{30}O_{14}$ of **2** was determined from its HR ESI-MS spectrum in +ve ion mode by the presence of the peak at *m/z* 579.1713 calculated for $[C_{27}H_{30}O_{14}+H]^+$, 579.1708, consistent with thirteen DBE. Its 1D and 2D NMR spectra, similar to those of **1,** also suggested a luteolin glycoside skeleton. The only difference was the presence of an additional signal in the ^{1}H NMR (Table 1) spectrum of **2** of a singlet attributable to a methoxyl group at δ_H 3.99 (3H). This was confirmed by the ^{1}H-^{13}C direct correlation observed in its HSQC spectrum between this signal and the carbon resonating at δ_C 55.4. In the HMBC spectrum, a correlation was observed between the signal of the methoxy proton

and a carbon resonating at δ_C 147.7 confirmed its position on the C-3′ carbon of the luteolin skeleton (Figure 2). Thus, all the ^{1}H and ^{13}C NMR data (Table 1) of **2** led to its identification as chrysoeriol 7-*O*-*α*-L-rhamnopynosyl-(1→2)-*β*-D-xylopyranoside, a new flavone glycoside, and was given the trivial name justicialoside B.

3. Materials and methods

3.1. General experimental procedures

Analytical HPLC was performed on a Dionex UPLC 3000 (Thermoscientific, UK) coupled with a photo-diode-array (PDA) detector (Thermoscientific). Extracts and fractions were analyzed on a Phenomenex C_{18} column (150 × 4.6 mm, 5 μm, Phenomenex, USA). An Agilent 1200 Infinity series preparative HPLC system coupled with a PDA detector (Agilent, UK) was used to isolate compounds; a Hichrom ACE C_{18} preparative column (150 × 21.2 mm, 5 μm) was used. The column temperature was set at 25°C. The chromatogram was monitored at variable UV–vis wavelengths (205, 320 and 366 nm). NMR spectroscopic analyses were performed in CD_3OD or Pyr-d_5 solution on a Bruker AMX600 NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C). HR ESI-MS analyses were performed on a Xevo G2-S ASAP or LTQ Orbitrap XL1 spectrophotometer or on an on an Agilent 6200 Series Accurate-Mass Time-of-Flight (TOF) LC/MS.

3.2. Plant material

The leaves of *Ruspoli*a *hypocrateriformis* were collected in Melen, Yaoundé, Centre Region, Cameroon, in June 2015, and identified at the Cameroon National Herbarium, where the voucher specimen 37822/SFR was deposited.

3.3. Extraction and isolation of compounds

The air-dried and powdered leaves (450.5 g) were successively extracted with *n*-hexane, DCM and MeOH to obtain 13.6, 10.2 and 41.8 g of dark green, brown green and dark brown extracts, respectively, using a Soxhlet extractor. A portion of the dried MeOH extract (2 g) was suspended in 10 mL of 10% MeOH-water and loaded on to a Strata C-18-E cartridge (Phenomenex, USA) (20 g), previously washed with MeOH (50 mL), followed by

equilibration with water (100 mL). The cartridge was eluted with MeOH-water mixture of decreasing polarity to obtain four fractions: 20, 50, 80 and 100% MeOH in water (200 mL each) (F1-F4, respectively). All fractions were concentrated to dryness using a combination of rotary evaporator and freeze-dryer and stored at 4°C until further use. Fraction F2 (428.6 mg) was purified by preparative HPLC using an ACE prep-column (150×21.2 mm, Hichrom Ltd, UK), flow rate 10 mL/min, mobile phase gradient of water (A) and methanol (B) both containing 0.1% TFA: 30-75% B, 0-20 min, monitored at wavelengths 320 and 366 nm to yield compounds **1** (3.3 mg), **3** (5.1 mg), **5** (0.9 mg), **6** (4.4 mg) and **7** (1.6 mg) having retention times 17.3, 15.5, 18.9, 10.2 and 17.8 min, respectively. F3 (361.8 mg) was subjected to preparative RP-HPLC as above using a different mobile phase gradient of water (A) and methanol (B) both containing 0.1% TFA: 45 - 50 % B, 0-10 min and 50 - 60 % B from 10 - 30 min monitored at wavelengths 205 nm to yield compounds **2** (2.6 mg), **4** (2.9 mg), and more of **3** (3.1 mg) and **5** (1.2 mg) having the retention 17.5, 19.2, 14.7 and 24.8 min, respectively.

*3.3.1. Justicialoside A (**1**)*

Yellow powder (3.3 mg); $[\alpha]_D^{25}$ = -38.5 (c 0.001, MeOH). HR ESI-MS *m/z* 565.1559, $[M+H]^+$ (calculated for $C_{26}H_{28}O_{14}H^+$, 565.1552). See Table 1 for ^{1}H NMR (600 MHz, Pyr-d_5) and ^{13}C NMR (150 MHz, Pyr-d_5) data.

3.3.2. Justicialoside B (**2**)

Yellow powder (2.6 mg); $[\alpha]_D^{25}$ = -29.3 (c 0.003, MeOH). HR ESI-MS *m/z* 579.1713 $[M+H]^+$ (calculated for $C_{27}H_{30}O_{14}H^+$, 579.1708). See Table 1 for ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data.

4. Conclusion

Two new luteolin glycosides (**1** and **2**), together with five known phytochemicals, were isolated from the leaves of *Ruspolia hypocrateriformis* (Vahl) Milne-Redh. (synonym *Justicia hypocrateriformis*).

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Appendix A. Supplementary data

NMR and MS spectra of compounds **1** and **2**.

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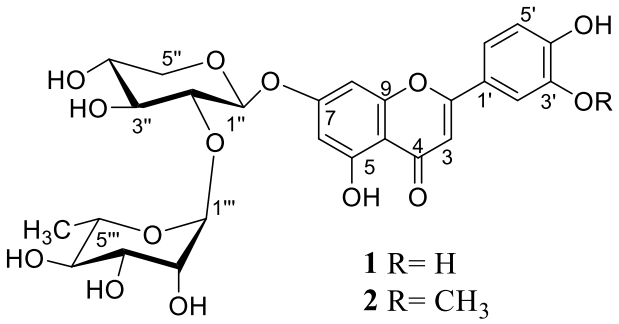

Figure 1. Structures of justicialosides A (**1**) and B (**2**)

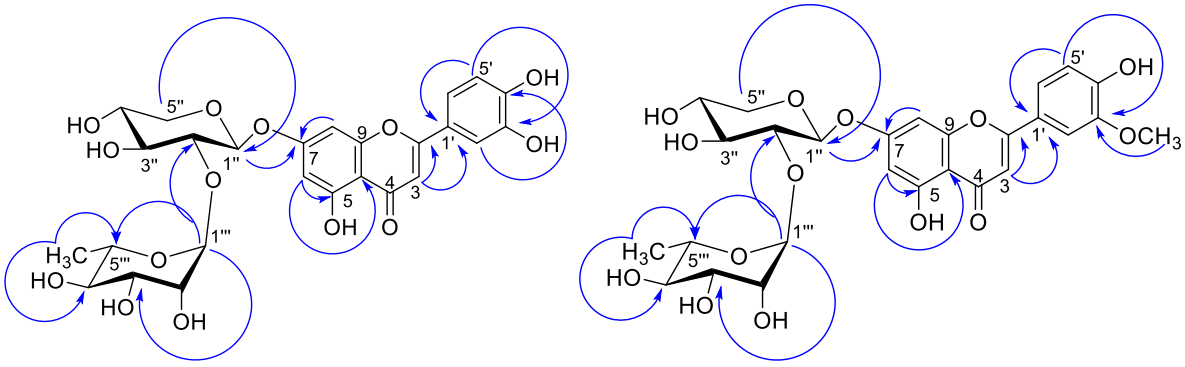

Figure 2. Key HMBC correlations compounds **1** and **2**

Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of compounds **1** and **2**

Position	**1**[a]		**2**[b]	
	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C
2	-	165.1	-	165.0
3	6.91 s	104.0	6.73 s	103.2
4	-	182.5	-	182.1
5	-	162.4	-	160.9
6	6.94 d (*2.1*)	99.9	6.46 d (*2.1*)	99.5
7	-	163.2	-	162.5
8	6.96 d (*2.1*)	95.0	6.79 d (*2.1*)	94.5
9	-	157.4	-	156.8
10	-	106.5	-	105.1
1′	-	122.3	-	121.5
2′	7.88 d (*2.2*)	114.1	7.55 d (*1.9*)	109.5
3′	-	147.6	-	147.7
4′	-	151.0	-	150.2
5′	7.25 d (*8.3*)	116.5	6.97 d (*8.4*)	115.3
6′	7.50 dd (*2.2*, *8.3*)	119.4	7.58 dd (*1.9*, *8.4*)	120.5
1″	5.62 d (*7.5*)	100.2	5.21 d (*7.3*)	99.0
2″	4.47 dd (*7.6*, *9.0*)	77.4	3.69 m	77.6
3″	4.28 m	78.6	3.61 m	70.7
4″	4.75 m	70.5	3.60 m	77.2
5″	3.80 m	67.0	3.49 m	65.6
	4.31 m		3.98 m	
1‴	6.38 d (*1.6*)	102.3	5.28 d (*1.5*)	101.2
2‴	4.80 m	72.1	3.96 *m*	70.7
3‴	4.54 dd (*3.3*, *9.3*)	72.4	3.61 m	69.7
4‴	4.32 m	74.0	3.41 t (*9.6*)	72.6
5‴	4.75 m	69.9	3.89 m	68.6
6‴	1.78 d (*6.1*)	18.6	1.32 d (*6.2*)	16.9
OCH_3	-	-	3.99 s	55.4

a- ran in Pyr-d_5; b- ran in CD_3OD