Lucidin Type Anthraquinone Glycosides from *Putoria calabrica*

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Two new lucidin type anthraquinone glycosides, putorinoside A (1) and putorinoside B (2) were isolated from *Putoria calabrica*, in addition to two known anthraquinone glycosides, lucidin 3-*O*-β-glucopyranoside (3) and lucidin 3-*O*-primeveroside (4). Based on spectroscopic data, putorinosides A and B were identified as 2-hydroxymethyl-1-methoxy-3,5,6-trihydroxyanthraquinone 3-*O*-β-glucopyranoside and 2-hydroxymethyl-1-methoxy-3,6-dihydroxyanthraquinone 3-*O*-β-glucopyranoside, respectively.

Key words anthraquinone glycoside; lucidin-type anthraquinone; putorinosides A and B; *Putoria calabrica*; Rubiaceae

The Rubiaceae family is well known for containing anthraquinones. Our previous research on a Rubiaceaous plant, *Putoria calabrica* (L. fil) DC resulted in the isolation of iridoid, flavonoid and lignan glycosides.[1,2] Further investigation of the methanolic extract of the plant furnished four anthraquinone glycosides of lucidin type, **1**—**4**. In this paper, we present the isolation and structure elucidation of the new compounds, **1** and **2**.

Putorinoside A (**1**) was obtained as an amorphous red powder. The positive and negative ion electrospray ionization (ESI) mass spectra of **1** displayed the pseudomolecular ions, $[M+Na]^+$ and $[M-H]^-$ at *m/z* 501 and 477, respectively. The molecular formula of $C_{22}H_{22}O_{12}$ was derived from high resolution (HR)-FAB-MS ($[M+H]^+$, *m/z* 479.1183, $\Delta=0.7$ mmu). An unsaturation number of 12, deduced from the MS and ^{13}C-NMR data (Table 1), suggested the presence of four rings. The UV spectrum of **1** showed absorption bands at λ_{max} 268, 304 (sh), 345 and 432 nm, indicative of an anthraquinone structure. **1** further displayed IR bands at ν_{max} 3400 (broad), 1668 and 1641 cm^{-1} for hydroxyl group(s) and unchelated and chelated carbonyl functions, respectively. The ^{13}C-NMR spectrum (DMSO-d_6, Table 1) contained signals for all 22 carbons. Sixteen signals were accounted for by the anthraquinone aglycone, among them four signals belonging to aromatic carbons bearing an oxygen atom (δ 150.0, 151.5, 160.4, 160.6), two signals belonging to a *p*-quinone moiety (δ 179.0, 187.9), and two belonging to oxygenated aliphatic carbons (δ 51.9, 62.6). The distortionless enhancement by polarization transfer (DEPT)-135 experiment indicated that the latter signals were due to an oxymethylene (δ 51.9 t) and a methoxyl (δ 62.6 q) group. Also present were signals belonging to the six carbons of a hexose unit within **1**. All these data revealed that **1** was an anthraquinone glycoside with two hydroxy, one methoxy and one oxymethylene substituents. The ^{1}H-NMR spectrum (Table 1, DMSO-d_6) contained, for the aglycone moiety, two doublets at δ 7.20 and 7.58 (each representing 1H, $J=8.0$ Hz), an aromatic proton singlet at δ 7.75 (1H), a complex multiplet of 2H at δ 4.60, a methoxy function (δ 3.82) and two D_2O-exchangeable protons (broad, OH) at δ 10.6 and δ 12.4. The sugar moiety demonstrated an anomeric proton signal at δ 5.10 as a doublet ($J=7.8$ Hz), four overlapping protons between δ 3.24—3.40 and an oxymethylene group at δ 3.53 (dd, $J=4.5$, 11.0 Hz) and 3.70 (dd, $J=2.0$, 11.0 Hz). From this data, associated with the ^{13}C-NMR data, it was obvious that the sugar unit was β-glucopyranose. The site of glycosylation and the substitution pattern of the anthraquinone aglycone could be deduced by two-dimensional (2D)-NMR experiments, particularly by gradient heteronuclear multiple bond connectivity (gHMBC). The methoxy protons exhibited a long range coupling with C-1 (δ 160.6 s) of ring C, confirming its location on this carbon atom. Likewise, the long distance correlations from H_2-15 (δ 4.60 m) to C-2 (δ 121.1 s) and C-3 (δ 160.4 s) served to position the hydroxymethyl function at C-2. Upon observation of a prominent HMBC cross peak between the anomeric proton (δ 5.10, H-1′) and C-3, the site of glycosylation was predicted to be at C-3. This interpretation was confirmed by further HMBC correlations between H_2-15/C-3 and H-4/C-3. Thus, the isolated aromatic proton signal at δ 7.75 (s) was ascribed to C-4. The $^2J_{CH}$ and $^3J_{CH}$ correlations obtained from H-4 to C-2, C-10, C-13 and C-14 unambigiously proved this assignment. The C-10 carbonyl carbon (δ 187.9) was deshielded by *ca.* 9 ppm compared to that of C-9 (δ 179.0), suggesting strong hydrogen bonding with a phenolic hydroxyl group.[3,4] Thus, C-5 (δ 150.0) was recognized to bear a *peri*-hydroxyl function to C-10 carbonyl. The chemical shift of one of the exchangeable protons (δ 12.4, br s) further corroborated this suggestion.[3,4] Since two aromatic signals at δ 7.20 (H-7) and δ 7.58 (H-8) coupled with the coupling constant of $J_{AB}=8.0$ Hz, they had to be *ortho* positioned, and thus, the second phenolic hydroxyl group (δ 10.6, br s, exchangeable) had to reside at C-6 (δ 151.5). This substitution was confirmed by ^{1}H–^{1}H correlation spectroscopy (COSY) couplings, and ^{1}H–^{13}C long range correlations ($^3J_{CH}$) between H-7/C-5, H-7/C-12 and H-8/C-6, H-8/C-9. Based upon these evidences, **1** was determined to be a new anthraquinone glycoside, 2-hydroxymethyl-1-methoxy-3,5,6-trihydroxyanthraquinone 3-*O*-β-glucopyranoside.

Putorinoside B (**2**) was obtained as an amorphous orange powder. The positive and negative ion ESI mass spectra of **2** exhibited the pseudomolecular ions, $[M+Na]^+$ and $[M-H]^-$ at *m/z* 485 and 461, respectively. The molecular formula, $C_{22}H_{22}O_{11}$ (*m/z* 463.1219, 16 amu less than that of **1**), was established by positive HR-FAB-MS and ^{13}C-NMR spectra (Table 1). Many of the carbon and proton resonances of **2** were almost identical to those of **1**, implying a close similarity in their structures. Significant differences between **1** and **2** in the ^{1}H- and ^{13}C-NMR spectra (Table 2) were restricted to

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Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Data of **1** and **2** (DMSO-d_6; δ in ppm, J in Hz).

C/H atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		160.6 s		160.4 s
2		121.1 s		120.4 s
3		160.4 s		160.5 s
4	7.75 s	108.9 d	7.69 s	109.1 d
5		150.0 s	7.41 br s	111.5 d
6		151.5 s		162.6 s
7	7.20 d (8.0)	120.8 d	7.21 br d (8.0)	121.9 d
8	7.58 d (8.0)	120.6 d	8.02 d (8.0)	129.7 d
9		179.0 s		179.6 s
10		187.9 s		182.3 s
11		115.7 s		134.0 s
12		125.2 s		125.3 s
13		135.5 s		135.8 s
14		132.2 s		131.7 s
15	4.60 m	51.9 t	4.64 d (11.0)	52.0 t
			4.56 d (11.0)	
1′	5.10 d (7.8)	101.1 d	5.06 d (7.8)	101.1 d
2′	3.37[a)]	73.3 d	3.36[a)]	73.3 d
3′	3.34[a)]	76.0 d	3.34[a)]	76.0 d
4′	3.24[a)]	69.4 d	3.25[a)]	69.4 d
5′	3.40[a)]	77.4 d	3.41[a)]	77.3 d
6′	3.53 dd (11.0, 4.5)	60.5 t	3.54 dd (5.0, 11.0)	60.5 t
	3.70 dd (11.0, 2.0)		3.71 br d (11.0)	
OCH_3	3.82 s	62.6 q	3.83 s	62.7 q
5-OH	12.4 br s			
6-OH	10.6 br s			

a) Multiplicity is unclear due to overlapping.

the ring A, where one of the phenolic hydroxy groups was replaced by an aromatic proton signal (δ_H 7.41 br s, δ_C 115.1 d). The upfield shift of the C-10 carbonyl function (δ 182.3) in conjunction with the IR data (1668 cm^{-1}, unchelated carbonyl function) suggested that the *peri*-hydroxy group (C-5) in **1** was absent in **2**. Three bond HMBC couplings between the pairs H-5/C-7, H-5/C-10; H-7/C-5, H-7/C-12; and H-8/C-6, H-8/C-9 further supported the presence of only one OH function in ring A, namely at C-6 (δ 162.6). Detailed inspection of the gHSQC, DQF-COSY, and gHMBC data (Fig. 1) substantiated the structure of **2** as shown. Compound **2** is 2-hydroxymethyl-1-methoxy-3,6-dihydroxyanthraquinone 3-*O*-β-glucopyranoside.

The spectroscopic data for the known glycosides, lucidin 3-*O*-β-glucopyranoside (**3**) and lucidin 3-*O*-primeveroside (**4**), showed good accordance to those of previously reported.[5)]

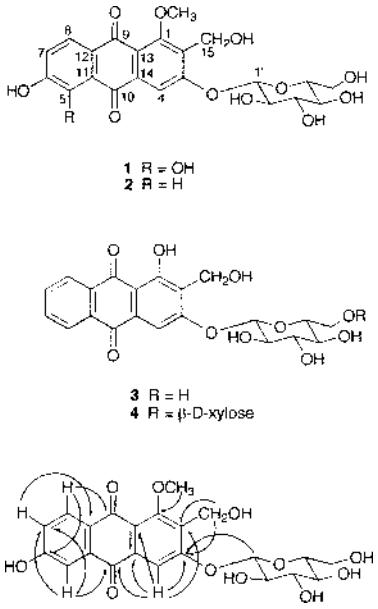

Fig. 1. Observed HMBC Correlations for Putorinoside B (**2**)

Experimental

General Experimental Procedures and Plant Material As reported in previous study.[2)]

Extraction and Isolation The air-dried and powdered plant material (90 g) was extracted with MeOH (500 ml×2) for 6 h at 45 °C and filtered. The filtrate was concentrated to dryness *in vacuo*. The resulting residue was dissolved in H_2O and partitioned with $CHCl_3$. The H_2O phase was lyophilized (15 g, yield 15%) and preadsorbed onto SiO_2 (25 g). This mixture was applied to a Si gel column (250 g) using the mixture of CH_2Cl_2–MeOH–H_2O (80 : 20 : 1, 80 : 20 : 2, 70 : 30 : 3) to give 150 fractions which were combined into nine fractions, A—J. The isolation studies on the fractions D, E, F, G, I, and J afforded iridoid, lignan and flavonoid glycosides.[2)] The subfractions E1, G2—G3 and J2, remained after the isolation of these compounds, were subjected to repeated chromatography on Sephadex LH-20 (MeOH) and C_{18}-MPLC (0 to 70% MeOH in H_2O) to afford lucidin 3-*O*-β-glucopyranoside (**3**, 7 mg), putorinoside B (**2**, 11 mg), lucidin 3-*O*-primeveroside (**4**, 33 mg), and putorinoside A (**1**, 130 mg), respectively.

Putorinoside A (**1**): ^{1}H-NMR (500 MHz, DMSO-d_6, Table 1), ^{1}H-NMR (500 MHz, CD_3OD): δ 3.40—3.59 (4H, H-2′-H-5′), 3.73 (1H, dd, J=4.0, 11.0 Hz, H-6′a), 3.90 (1H, br d, J=11.0 Hz, H-6′b), 3.86 (3H, s, OMe), 4.70 (d, J=11.0 Hz, H-15a), 4.73 (d, J=11.0 Hz, H-15b), 5.14 (d, J=7.8 Hz, H-1′), 7.20 (d, J=8.0 Hz, H-7), 7.60 (d, J=8.0 Hz, H-8), 7.80 (s, H-4). ^{13}C-NMR (125 MHz, DMSO-d_6, Table 1), ^{13}C-NMR (125 MHz, CD_3OD): δ 53.8 (t, C-15), 62.3 (t, C-6′), 63.6 (q, OMe), 71.1 (d, C-4′), 74.9 (d, C-2′), 77.8 (d, C-3′), 78.5 (d, C-5′), 102.4 (d, C-1′), 110.7 (d, C-4), 116.9 (s, C-11), 122.2 (d, C-8), 122.3 (d, C-7), 122.6 (s, C-2), 126.6 (s, C-12), 132.9 (s, C-14), 137.6 (s, C-13), 151.6 (s, C-5), 153.8 (s, C-6), 162.1 (s, C-1), 162.4 (s, C-3), 181.4 (s, C-9), 188.4 (s, C-10). UV (MeOH) λ_{max} 268, 304 (sh), 345, and 432 nm. IR (KBr) ν_{max} 3400 (OH), 1668 (unchelated C=O), 1641 (chelated C=O), and 1585 cm^{-1} (aromatic ring). Negative ion ESI-MS *m/z* 477 $[M-H]^-$, 315 $[M-163]^-$ and 299 $[M-163-14]^-$. Positive ion ESI-MS *m/z* 501 $[M+Na]^+$, 479 $[M+H]^+$, 317 $[M-162+H]^+$. Positive FAB-MS *m/z* 479 $[M+H]^+$, HR-FAB-MS *m/z* Obsd 479.1183 for $[M+H]^+$, Calcd for $C_{22}H_{23}O_{12}$ 479.1190. $[\alpha]_D^{20}$ −96° (c=0.05, MeOH).

Putorinoside B (**2**): ^{1}H-NMR (500 MHz, DMSO-d_6, Table 1), ^{1}H-NMR (500 MHz, CD_3OD): δ 3.45—3.65 (4H, H-2′-H-5′), 3.79 (1H, dd, J=5.0, 12.0 Hz, H-6′a), 3.92 (3H, s, OMe), 3.94 (1H, dd, J=2.5, 12.0 Hz, H-6′b), 4.73 (d, J=11.4 Hz, H-15a), 4.82 (d, J=11.4 Hz, H-15b), 5.18 (d, J=7.8 Hz, H-1′), 7.11 (dd, J=2.0, 8.4 Hz, H-7), 7.40 (br s, H-5), 7.80 (s, H-4), 8.04 (d, J=8.4 Hz, H-8); ^{13}C-NMR (125 MHz, DMSO-d_6, Table 1). UV (MeOH) λ_{max} 216, 274, 338 and 416 nm. IR (KBr) ν_{max} 3400 (OH), 1668 (unchelated C=O) and 1580 cm^{-1} (aromatic ring); Negative ion ESI-MS *m/z* 461 $[M-H]^-$, 299 $[M-163]^-$. Positive ion ESI-MS *m/z* 485 $[M+Na]^+$. Positive FAB-MS *m/z* 463 $[M+H]^+$, HR-FAB-MS *m/z* Obsd 463.1219 for $[M+H]^+$, Calcd for $C_{22}H_{23}O_{11}$ 463.1240. $[\alpha]_D^{20}$ −56° (c=0.05, MeOH).

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