

Luteolin-7-O-Glycosides from the Leaves of *Centropogon cornutus* (Campanulaceae)

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Abstract

A new luteolin-7-O-glycoside, named Centropogin A (1), together with three known luteolin-7-O-glycosides, Luteolin-7-O-xyloside (2), Luteolin-7-O-[β -apiofuranosyl-(1 \rightarrow 2)] β -xylopyranoside (3) and Luteolin-7-O-[β -glucopyranosyl-(1 \rightarrow 2)- α -rhamnosyl-(1 \rightarrow 6)] β -glucopyranoside (4) were isolated and purified via repeated chromatography of the n-BuOH soluble fraction from the methanol extract of the leaves of *Centropogon cornutus* (Campanulaceae). The structures of these compounds were elucidated by analysis of their 1D-NMR (^1H , ^{13}C) and 2D-NMR (COSY, HSQC, HMBC), MS (HR-ESIMS) spectral data, and by comparisons with the literature data. This is the first report of secondary metabolites from the genus *Centropogon* and the first report of compounds (2)-(4) from the Campanulaceae family.

Keywords: Campanulaceae; *Centropogon cornutus*; Flavonoids; Luteolin-7-O-glycosides; Centropogin A; NMR; HR-ESIMS

Introduction

Campanulaceae, the bellflower family, is a well-known group of plants comprising 84 genera and approximately 2400 species [1]. The family is cosmopolitan in its distribution and present in a wide array of habitats [2]. A few species in the family belonging to the genera *Adenophora*, *Codonopsis*, *Lobelia* and *Platycodon* have previously been analyzed with respect to their secondary metabolites and biological activities, and a diverse range of compounds have been isolated to date. These include pyrrolidine, piperidine and polyhydroxylated alkaloids, steroidal saponins, triterpenoids, phenylpropanoids, flavonoids, xanthenes, polyacetylenes and cerebrosides, some of which exhibited significant anticancer, antimicrobial and anti-inflammatory activities [3-8]. *Centropogon*, the third largest genus of Campanulaceae, comprises of 230 species [9]. *Centropogon cornutus* is the only *Centropogon* species found in Trinidad and Tobago, where it is commonly known as deer meat, deer bush and crepe coq [10]. These showy roadside plants, generally found along moist shady banks and cocoa fields, are distributed throughout Central and South America and the West Indies [10]. *Centropogon cornutus* is a shrub with a milky sap that grows to about 1-3 meters in height. It has curved scarlet flowers, which are suited for pollination by hummingbirds, and produces berries which are consumed by birds [11]. Traditionally, the leaves are used to treat snake and scorpion bites in Trinidad [12]. In Guyana, the entire plant is boiled and the liquid drunk to remedy venereal diseases. In French Guiana, the leaves are used as a tonic and as an abortive decoction, while in NW Guyana it is used to treat urinary tract problems [13]. The whole plant is also traditionally used to treat stomach ulcers in Brazil [14]. Many chemical and pharmacological investigations focusing on a few genera of Campanulaceae have been carried out previously, however, there is limited information on the pharmacological or phytochemical composition of the *Centropogon* genus which has until now not been investigated for its natural products. As a continuation of the search for secondary metabolites of biological importance from Campanulaceae species, herein, the isolation and structural elucidation of one new compound and three known compounds from the n-BuOH soluble fraction from the methanol extract of the leaves of *C. cornutus* is reported. Their structures were elucidated using spectroscopic methods including NMR and HR-ESIMS as the novel Centropogin A (1), together with three known luteolin glycosides, Luteolin-7-O-xyloside (2), Luteolin-7-O-[β -apiofuranosyl-(1 \rightarrow 2)] β -xylopyranoside (3) and Luteolin-7-O-[β -glucopyranosyl-(1 \rightarrow 2)- α -rhamnosyl-(1 \rightarrow 6)] β -glucopyranoside (4) which are all for the first time reported from the Campanulaceae family (Figure 1).

Experimental Section

General experimental procedures

NMR spectra were recorded on a Bruker AVANCE DRX-600 instrument with TMS as internal standard. HRESIMS was carried out on a microTOF-Q (Bruker Daltonics) spectrometer. Column chromatography (CC) was performed on Silica gel 60 Å 70-230 mesh (Sigma-Aldrich) and VLC was performed using Silica gel 60 PF₂₅₄₊₃₆₆ (Merck). Analytical TLC was carried out on aluminium-backed plates precoated with 0.20 mm thick silica gel (Sigma-Aldrich) and visualization of the chromoplates was facilitated by UV light. The solvents used for extraction and chromatographic separations were either of analytical grade or distilled prior to use.

Plant material

The leaves of *Centropogon cornutus* investigated in this study were collected on the Guaico Tamana road, Trinidad in August 2013. Identification and botanical authentication was performed by Ms. Keisha Manuare of the National Herbarium of Trinidad and Tobago and a voucher specimen (TRIN 50133) was deposited.

Extraction and isolation

The leaves of *Centropogon cornutus* were oven-dried for 3 days at 40°C (473 g), pulverized and extracted exhaustively with MeOH. The methanolic solution was filtered and concentrated *in vacuo* to yield 159 g of a viscous, dark-brown extract which was suspended in MeOH/H₂O (4:1) and partitioned sequentially with Petroleum ether, CHCl₃, EtOAc and n-BuOH, respectively. The n-BuOH extract (22 g) was subjected to vacuum liquid chromatography (VLC) on silica gel (Silica gel 60 PF₂₅₄₊₃₆₆) and eluted with EtOAc: MeOH: H₂O gradients of increasing polarity (95:3:2, 90:6:4, 80:12:8) to give 9 fractions (1A-1I). Fraction 1B, a pure compound was assigned as compound 2 (22.1 mg). Fraction 1E (0.9227 g) was purified by isocratic silica gel column

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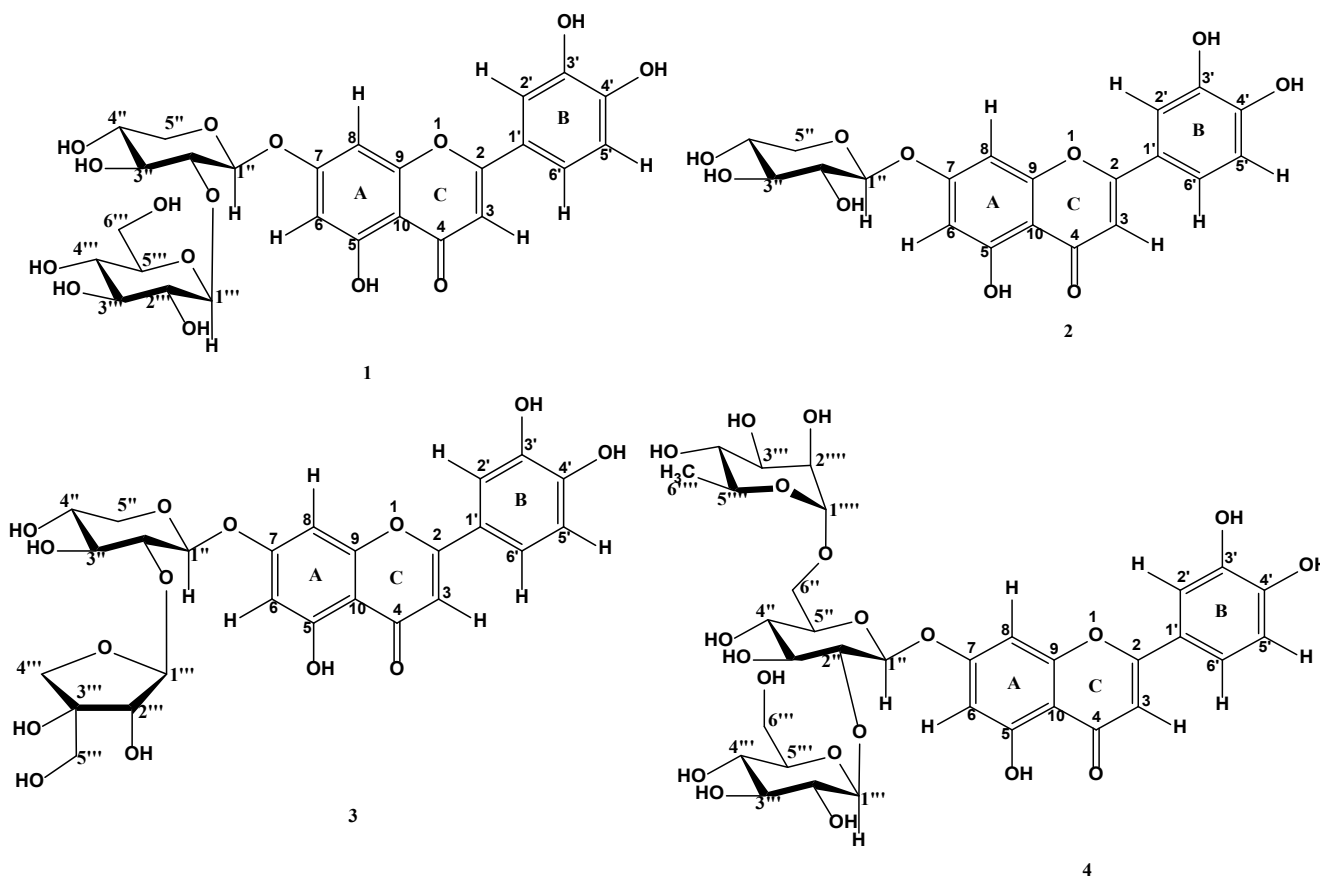


Figure 1: Structures of Centropogin A (1), Luteolin-7-O-xyloside (2), Luteolin-7-O-[[β -apiofuranosyl-(1 \rightarrow 2)] β -xylopyranoside (3) and Luteolin-7-O-[[β -glucopyranosyl-(1 \rightarrow 2)- α -rhamnosyl-(1 \rightarrow 6)] β -glucopyranoside (4).

chromatography using the system EtOAc: MeOH: H₂O (90:6:4) to yield compound **3** (155.9 mg). 1G (0.5025 g) was subjected to isocratic silica column chromatography using the system EtOAc: MeOH: H₂O (90:6:4) to afford compound **1** (12.4 mg) and Fraction II (10.124 g) was chromatographed on a gradient elution silica column chromatography using the mobile phase CHCl₃: MeOH (75:25, 65:35) to give compound **4** (52.9 mg).

Centropogin A (1): Yellow amorphous powder, yielded a positive response to the Shinoda test; HRESIMS (positive mode) m/z 603.1310 [M+Na]⁺ (calcd. for C₂₆H₂₈O₁₅Na, 603.1320); ¹H-NMR (DMSO-*d*₆, 600 MHz) and ¹³C NMR (DMSO-*d*₆, 150 MHz) data (Table 1).

Luteolin-7-O-xyloside (2): Yellow amorphous powder, yielded a positive response to the Shinoda test; HRESIMS (positive mode) m/z 441.1863 [M+Na]⁺ (calcd. for C₂₀H₁₈O₁₀Na, 441.0792); ¹H-NMR (DMSO-*d*₆, 600 MHz) δ : 12.99 (1H, bs, OH-5), 7.46 (1H, dd, $J=8.5, 2.0$, H-6'), 7.42 (1H, d, $J=2.0$, H-2'), 6.88 (1H, d, $J=8.5$ Hz, H-5'), 6.78 (1H, d, $J=2.0$, H-8), 6.74 (1H, s, H-3), 6.41 (1H, d, $J=2.0$, H-6), 5.08 (1H, d, $J=7.0$ Hz, Xyl-H-1), 3.26 (1H, d, $J=6.3$ Hz, Xyl-H-2), 3.77 (1H, d, $J=6.0$ Hz, Xyl-H-4); ¹³C-NMR (DMSO-*d*₆, 150 MHz) δ : 164.5 (C-2), 103.0 (C-3), 181.9 (C-4), 161.1 (C-5), 100.2 (C-6), 162.7 (C-7), 94.5 (C-8), 157.0 (C-9), 105.4 (C-10), 121.1 (C-1'), 113.4 (C-2'), 145.9 (C-3'), 149.8 (C-4'), 116.0 (C-5'), 119.3 (C-6'), 99.5 (Xyl-1), 72.9 (Xyl-2), 76.2 (Xyl-3), 69.2 (Xyl-4), 65.8 (Xyl-5).

Luteolin-7-O-[[β -apiofuranosyl-(1 \rightarrow 2)] β -xylopyranoside (3): Yellow amorphous powder, yielded a positive response to the Shinoda test; HRESIMS (negative mode) m/z 549.1093 [M-H]⁺ (calcd. for

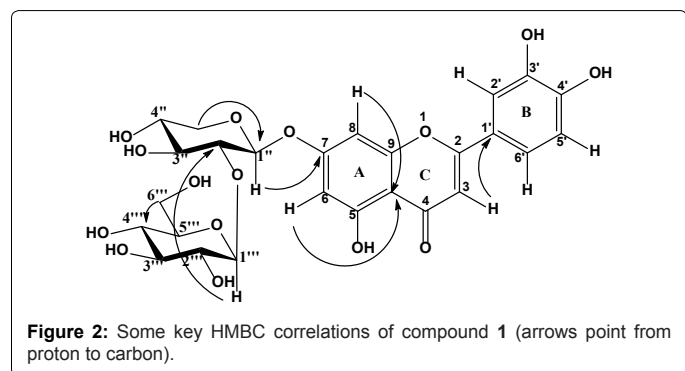
C₂₅H₂₅O₁₄, 549.1239); ¹H-NMR (DMSO-*d*₆, 600 MHz) δ : 12.99 (1H, bs, OH-5), 7.46 (1H, dd, $J=8.0, 2.0$, H-6'), 7.44 (1H, d, $J=2.0$, H-2'), 6.91 (1H, d, $J=8.0$ Hz, H-5'), 6.76 (1H, d, $J=2.0$, H-8), 6.75 (1H, s, H-3), 6.39 (1H, d, $J=2.0$, H-6), 5.19 (1H, d, $J=7.6$ Hz, Xyl-H-1), 5.34 (1H, bs, Api-H-1); ¹³C-NMR (DMSO-*d*₆, 150 MHz) δ : 164.5 (C-2), 103.1 (C-3), 181.9 (C-4), 161.2 (C-5), 99.3 (C-6), 162.4 (C-7), 94.5 (C-8), 156.9 (C-9), 105.4 (C-10), 121.3 (C-1'), 113.6 (C-2'), 145.8 (C-3'), 150.0 (C-4'), 116.0 (C-5'), 119.2 (C-6'), 98.5 (Xyl-1), 75.7 (Xyl-2), 76.6 (Xyl-3), 69.4 (Xyl-4), 65.7 (Xyl-5), 108.9 (Api-1), 76.1 (Api-2), 79.3 (Api-3), 74.0 (Api-4), 64.2 (Api-5).

Luteolin-7-O-[[β -glucopyranosyl-(1 \rightarrow 2)- α -rhamnosyl-(1 \rightarrow 6)] β -glucopyranoside (4): Yellow amorphous powder, yielded a positive response to the Shinoda test; $\alpha_D^{25} = -0.60$ (c 0.1, MeOH); HRESIMS (positive mode) m/z 779.1914 [M+Na]⁺ (calcd. for C₃₃H₄₀O₂₀Na, 779.2005); ¹H-NMR (DMSO-*d*₆, 600 MHz) δ : 13.00 (1H, bs, OH-5), 7.42 (1H, dd, $J=8.0, 2.0$, H-6'), 7.37 (1H, d, $J=2.0$, H-2'), 6.86 (1H, d, $J=8.0$ Hz, H-5'), 6.79 (1H, d, $J=2.0$, H-8), 6.71 (1H, s, H-3), 6.48 (1H, d, $J=2.0$, H-6), 5.22 (1H, d, $J=7.0$ Hz, Glc-H-1), 4.48 (1H, d, $J=7.5$ Hz, Glc'-H-1), 4.55 (1H, bs, Rha-H-1), 1.07 (3H, d, $J=6.1$ Hz, Rha-H-6); ¹³C-NMR (DMSO-*d*₆, 150 MHz) δ : 164.5 (C-2), 103.4 (C-3), 181.8 (C-4), 161.1 (C-5), 99.6 (C-6), 162.6 (C-7), 95.1 (C-8), 156.8 (C-9), 105.4 (C-10), 121.2 (C-1'), 113.9 (C-2'), 146.0 (C-3'), 151.4 (C-4'), 115.3 (C-5'), 119.2 (C-6'), 98.4 (Glc-1), 82.5 (Glc-2), 75.5 (Glc-3), 69.2 (Glc-4), 75.3 (Glc-5), 65.8 (Glc-6), 104.7 (Glc'-1), 74.7 (Glc'-2), 76.2 (Glc'-3), 69.6 (Glc'-4), 76.9 (Glc'-5), 60.6 (Glc'-6), 100.5 (Rha-1), 70.3 (Rha-2),

Position	δ_c	δ_H , multiplicity
Aglycone		
2	164.3	-----
3	103.2	6.74, s
4	181.8	-----
5	161.3	-----
6	99.3	6.46, d (2.0)
7	162.5	-----
8	94.4	6.82, d (2.0)
9	156.8	-----
10	105.3	-----
1'	121.2	-----
2'	113.7	7.42, d (2.0)
3'	145.7	-----
4'	150.1	-----
5'	116	6.89, d (8.0)
6'	119.1	7.46, dd (8.0, 2.0)
5-OH	-----	12.99, bs
7-O-Xylose		
1"	98.3	5.25, d (6.8)
2"	81.8	3.53, m
3"	74.8	3.50, m
4"	68.6	3.47, m
5"	65.2	3.80, d (6.7) 3.45, m
2"-O-Glucose		
1"	104.4	4.46, d (7.9)
2"	74.5	3.00, t (8.4)
3"	76.1	3.20, m
4"	69.4	3.14, m
5"	76.6	3.17, m
6"	60.2	3.43 (CH ₂)

^aData acquired at 600 MHz (¹H) and 150 MHz (¹³C) in DMSO-*d*₆ with TMS as the internal standard.

Table 1: NMR Spectral data for Compound 1 (DMSO-*d*₆, δ in ppm, J in Hz).^a



70.8 (Rha-3), 72.1 (Rha-4), 68.3 (Rha-5), 17.8 (Rha-6).

Results and Discussion

Purification of the n-BuOH soluble fraction of the crude MeOH extract of the leaves afforded one new compound, Centropogin A (1) and three known compounds (2-4). The three known compounds were identified as Luteolin-7-O-xyloside (2) [15,16], Luteolin-7-O-[β-apiofuranosyl-(1→2)]β-xylopyranoside (3) [17], and Luteolin-7-O-[β-glucopyranosyl-(1→2)-α-rhamnosyl-(1→6)] β-glucopyranoside (4) [17], by comparison of their spectral data with those published in the literature.

Compound 1 was obtained as an amorphous, yellow powder which gave a positive response to Shinoda test [18]. The HR-ESIMS exhibited a pseudo-molecular ion peak at *m/z* 603.1310 [M+Na]⁺ (calcd. for C₂₆H₂₈O₁₅Na, 603.1320) corresponding to the molecular formula of C₂₆H₂₈O₁₅, which implies 13 degrees of unsaturation.

The ¹H-NMR spectrum of compound 1 exhibited six aromatic proton signals (Table 1). A one-proton *meta*-coupled doublet at δ 7.42 ppm (*J*=2.0 Hz), a one-proton *ortho*-coupled doublet at δ 6.89 ppm (*J*=8.0 Hz) and a one-proton *ortho*-, *meta*-coupled doublet of doublets at δ 7.46 ppm (*J*=2.0, 8.0 Hz) were ascribed to aromatic protons H-2', H-5' and H-6', respectively, supporting an ABX-type coupling system of ring B. It also showed two *meta*-coupled doublets (*J*=2.0 Hz) at δ 6.82 and 6.46, each integrating for one proton, which were assigned to H-8 and H-6, respectively of ring A of 5,7-dihydroxyflavonoids. A one-proton sharp singlet at δ 6.74 ppm was ascribed to H-3. These spectral data revealed the presence of a 5, 7, 3', 4' tetrahydroxy Flavone aglycone also known as Luteolin. The presence of a downfield chelated hydroxy proton signal at δ 12.99 (1H, bs) confirmed the presence of a hydroxy group at position 5 of ring-A. Two anomeric proton signals were also observed at δ 5.25 (1H, d, *J*=6.8 Hz) and 4.46 (1H, d, *J*=7.9 Hz), suggesting the presence of two sugar moieties. According to the coupling constants of H-1'' (*J*=6.8 Hz) and H-1''' (*J*=7.9 Hz), the sugar moieties were assigned as β-anomers.

The ¹³C NMR spectrum of 1 also exhibited signals due to the flavone aglycone and two sugar moieties. The ¹³C NMR data showed the presence of a ketone carbonyl (δ 181.8), two olefinic carbons (δ 164.3 and 103.2), four hydroxyl carbons (δ 162.5, 161.3, 150.1 and 145.7) and two anomeric carbons (δ 104.4 and 98.3).

A combined interpretation of the ¹H-¹H COSY, ¹³C NMR, HSQC and HMBC spectra and of the coupling constants aided the identification of the sugars as one β-glucose and one β-xylose moiety (Table 1).

The sequence of the sugars and binding site at the aglycone were unambiguously determined in the HMBC experiment. In the HMBC spectrum, significant correlations were observed between H-1'' (δ 5.25) and C-7 (δ 162.5); H-1''' (δ 4.46) and C-2'' (δ 81.8), suggesting the xylose moiety was located at C-7 position of the aglycone, and the glucosyl was connected with C-2'' of the xylosyl, thus establishing the inter-glycosidic linkage of the disaccharide moiety (Figure 2). Thus, compound 1 was identified as Luteolin-7-O-[β-glucopyranosyl-(1→2)] β-xylopyranoside, and was named Centropogin A.

Conclusion

From the leaves of *Centropogon cornutus*, one novel flavone glycoside (1) is reported as well as three flavone glycosides (2-4) are described for the first time in the Campanulaceae family. Luteolin-7-O-xyloside (2) is reported to exhibit antimutagenic activity [16]. This work contributes to the knowledge of the chemistry of the *Centropogon* genus which is non-existent to date. It would be interesting to examine if these flavonoids are responsible for the traditional medicinal uses of this plant.

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Disclosure Statement

The author has declared no conflict of interest.

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