Commicarpiflavinol Glucosides A and B; Two New 5-Deoxyflavonol Glucosides from Commicarpus grandiflorus

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Abstract: The phytochemical investigation of the aerial parts of Commicarpus grandiflorus (Standl.) resulted in the isolation of two new flavonol 3-O-glucosides, commicarpiflavinol glucoside A (1) and commicarpiflavinol glucoside B (2), along with the known compounds β-sitosterol (3) and betulinic acid (4). The structures of the isolated compounds have been elucidated by extensive 1D (¹H, ¹³C) and 2D (COSY, HSQC, HMBC) NMR spectral data analysis, as well as high-resolution mass determinations.

Keywords: Commicarpus grandiflorus; Nyctaginaceae; flavonol glucosides; commicarpiflavinol glucosides. © 2016 ACG Publications. All rights reserved.

1. Introduction

Family Nyctaginaceae includes about 300 species and over 30 genera [1], from which genus Commicarpus is identified. Members of Commicarpus Standl., grown in arid environments, are 30-35 species distributed throughout the tropical and subtropical regions of the world, especially in Africa and western Asia [2]. Phytochemical investigation of the family’s plants is still not very common. Few reports described the presence of betacianins, flavonols and phenolic compounds from plants of genus Bougainvillea [3-5], flavones from Neea theifera [1], tannins and saponins from Boerhavia coccinea and Boerhavia erecta [6], dihydroisofuranoxanthone [7], rotenoids [8] and lignans [9] from Boerhavia diffusa. Saponins were isolated from Colignonia scandens Benth [10] and from Pisonia umbellifera [11].

Nothing could be traced in the literature concerning the chemical composition of genus Commicarpus. The methanolic extracts of the aerial parts of two Commicarpus species growing in Saudi Arabia, including C. grandiflorus Standl. and C. plumbaginaceus Standl. were reported to exhibit strong

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activity against *Trypanosoma cruzi* and *T. b. brucei*, protozoa that cause Chagas disease and sleeping sickness disease, respectively [12].

This is the first phytochemical investigation of *C. grandiflorus*, which describes the isolation and characterization of two new flavonol glucosides, commicarpiflavonol glucosides A and B (1 and 2) and the known β-sitosterol (3) and betulinic acid (4).

2. Materials and Methods

2.1. General Experimental Procedures

An Agilent Technologies 6200 series mass spectrometer was employed for MS, 1D and 2D-NMR experiments (chemical shifts in ppm, coupling constants in Hz) were recorded in DMSO or CDCl$_3$ on Bruker spectrometer at 400 MHz for $^1$H NMR and 100 MHz for $^{13}$C NMR with solvent peaks as internal standard. Column chromatography was performed on Sephadex LH-20 (Sigma, Germany), silica gel H type 60 (Merck, Darmstadt, Germany) and silica gel (230-400 Mesh, Sigma, Germany); medium pressure pre-packed column Lichroprep SiO$_2$ (250 x 10 mm, 40-63 µm, Merck, Darmstadt, Germany) was used for purification of compounds 1 and 2; TLC analyses were conducted on pre-coated silica gel 60 F$_{254}$ (0.2 mm thickness, Merck, Germany).

2.2. Plant Material

The aerial parts of the plant were collected from the western region of Saudi Arabia (Al-Hadda Road) in March 2013. The plant material was kindly identified by members of Plant Taxonomy Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia. Voucher Specimen (CG-1126) was deposited at the herbarium of the Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. The plant material were air-dried in the shade then ground at time of extraction.

2.3. Extraction and Isolation

Dried powdered plant material (600 g) was exhaustively extracted with ethanol 70% (4 x 5 L) by percolation and the combined extracts were concentrated under vacuum to give 50 g dark green residue. The ethanolic extract (33 g) was successively fractionated with chloroform and ethyl acetate (5 x 500 mL, each) to give 10.3 g and 0.43 g, respectively.

A portion of the ethyl acetate fraction (400 mg) was subjected to CC on Sephadex LH-20 and eluted with MeOH. Further purification on MPLC column (25 cm L x 1 cm D, flow rate 1 mL/min with isocratic elution, using 40% MeOH/ H$_2$O afforded compounds 1 (14 mg) and 2 (9 mg).

A portion of the chloroform fraction (8 g) was chromatographed on VLC silica gel column (3 cm L x 10 cm D), eluted in increasing polarity with $n$-hexane/ CH$_2$Cl$_2$ (90-10%) mixtures followed by CH$_2$Cl$_2$, CH$_3$Cl/ EtOAc (90-10%) mixtures, EtOAc and EtOAc/ MeOH (99-95%) mixtures. The subfraction eluted with 60% $n$-hexane/ CH$_2$Cl$_2$ (700 mg) was purified on SiO$_2$ CC (30 cm L x 2 cm D), eluted with a gradient of $n$-hexane/ CH$_2$Cl$_2$ mixtures to afford compounds 3 (24 mg) and compound 4 (10 mg).

2.4. Structural Elucidation of Isolated Compounds

2.4.1. *Commicarpiflavonol glucoside A* (1). Yellow powder; HRESIMS m/z 493.0792 (calcd for C$_{22}$H$_{22}$O$_{13}$, 493.0791 [M – H]$^-$); NMR data: see table 1.

2.4.2. *Commicarpiflavonol glucoside B* (2). Yellow powder; HRESIMS m/z 477.0950 (calcd for C$_{22}$ H$_{22}$ O$_{12}$, 477.0949 [M – H]$^-$); NMR data: see table 1.
2.4.3. β-sitosterol (3). White powder; EIMS: m/z 414 [M+], C_{29}H_{56}O, 1H NMR (400 MHz, CDCl₃): δ_H 5.37 (t, J = 5.4, 2.4, H-6), 3.54 (tt, J = 11.1, 5.5, H-3), 0.97 (s, H-19), 0.94 (d, J = 6.6, H-21), 0.87 (d, J = 7.2, H-27), 0.86 (t, J = 6.6, H-29), 0.84 (d, J = 7.2, H-26), 0.70 (s, H-18); 13C NMR (100 MHz, CDCl₃): δ_C 140.9 (C-5), 121.9 (C-6), 72.0 (C-3), 56.9 (C-14), 56.2 (C-17), 50.3 (C-9), 46.0 (C-24), 42.3 (C-4), 42.3 (C-13), 40.0 (C-12), 37.4 (C-1), 36.7 (C-10), 36.3 (C-20), 34.1 (C-22), 32.1 (C-7), 32.1 (C-2), 31.8 (C-8), 29.3 (C-25), 28.4 (C-16), 26.3 (C-23), 24.5 (C-15), 23.2 (C-28), 21.3 (C-11), 20.0 (C-27), 19.6 (C-19), 19.2 (C-26), 19.0 (C-21), 12.2 (C-29), 12.0 (C-18).

2.4.4. Betulinic acid (4). White powder; EIMS: m/z 456 [M+], C_{30}H_{40}O_{5}, 1H NMR (400 MHz, CDCl₃): δ_H 4.50 (brs, H-29b), 4.37 (brs, H-29a), 3.00 (brt, J = 8, H-3), 1.53 (H-23), 0.92 (s, H-23), 0.80 (H-27), 0.74 (s, H-26), 0.69 (s, H-24), 0.63 (s, H-25); 13C NMR (100 MHz, CDCl₃): δ_C 173.7 (C-28), 150.2 (C-20), 108.9 (C-29), 79.8 (C-3), 54.6 (C-17), 54.6 (C-5), 49.8 (C-9), 47.8 (C-19), 47.2 (C-18), 43.5 (C-14), 40.3 (C-8), 37.9 (C-1), 37.4 (C-4), 35.0 (C-22), 34.1 (C-13), 33.6 (C-10), 31.3 (C-7), 29.2 (C-16), 29.1 (C-15), 28.9 (C-21), 28.7 (C-2), 27.2 (C-23), 24.4 (C-12), 22.1 (C-11), 18.6 (C-6), 17.9 (C-30), 17.0 (C-26), 16.4 (C-25), 15.8 (C-24), 14.0 (C-27).

3. Results and Discussion

From the ethyl acetate fraction two flavonoid glycosides (1 and 2) were isolated by repeated chromatography on sephadex LH-20 followed by MPLC on Si gel column.

Compound 1 (Figure 1) was obtained as yellow powder. Its molecular formula was determined to be C_{22}H_{32}O_{3} on the basis of HRMS with pseudomolecular ion peak at m/z 493.0792 [M – H]. Combined 1D (1H, 13C) and 2D (COSY, HMQC, HMBC) spectral data of 1 indicated its flavonol nature [13,14]. 1H NMR signals (Table 1) detected at δ_H 7.56, d, J = 2.1 Hz (H-2'), δ_H 7.55, dd, J = 8, 2.1 Hz (H-6') and δ_H 6.84, d, J = 8 Hz (H-5'), demonstrated an ABX coupling system with the presence of 3', 4' substitution in ring B. HMQC experiment correlated each of these protons with the corresponding carbons; δ_C 116.5, 121.6 and 115.6 for C-2', C-6' and C-5', respectively. While HMBC correlations (Figure 2) revealed their coupling to two hydroxy-bearing carbons resonating at δ_C 145.1 and 148.8 assigned for C-3' and C-4' (exchangeable). Moreover, 1H NMR spectrum revealed the presence of only one singlet aromatic proton at δ_H 6.52 indicating three substitutions in ring A. A singlet peak resonated at δ_H 3.75 verified the presence of a methoxy-group, the downfield shift of its corresponding carbon (δ_C 60.4), indicated that is ortho-disubstituted [13]. The aromatic singlet signal (δ_H 6.52, s) was then assigned to H-5. This was secured by HMBC cross peaks of H-5/C-4 and was also supported by the absence of any downfield proton signal in the region of 12-13 ppm demonstrating no chelated hydroxyl group [15]. The methoxy-group was in that case assumed to be placed at C-7 resonating at δ_C 131.7 as verified by HMBC correlation (OCH_{3}/C-7) being then flanked between two hydroxy-bearing carbons resonating at δ_C 152.0 (C-6') and 152.6 (C-8).

In addition, 1H NMR and 13CNMR data revealed the presence of a glucose moiety [13,14]. Glucosidation was concluded from HMBC correlation of the anomeric proton of glucose moiety at δ_H 5.42 (J = 8 Hz) with C-3 at δ_C 133.3 and confirmed the position of sugar moiety at C-3. The coupling constant of the anomeric proton of 8 Hz indicated the β-configuration of glucose moiety [14]. Moreover, signals between δ 3.16 and 3.68 in 1H NMR were assigned to other glucose protons. They were aligned to their corresponding carbons through HMQC experiment (Table 1).

To the best of our knowledge, compound 1 was reported here for the first time as a new natural constituent and was named commicarpiflavonol glucoside A.

Compound 2 (Figure 1) was obtained as yellow powder. It showed a molecular formula C_{22}H_{32}O_{2} as deduced from HRMS, with pseudomolecular ion peak at m/z 477.0950 [M – H]. Extensive study of 1D (1H, 13C) and 2D (COSY, HMQC, HMBC) spectral data of 2 (Table 1, Figure 2) suggested its flavonol nature [13,14] and revealed its close similarity to the structure of compound 1, except in the ring B as its 1H NMR spectrum demonstrated AA'/BB' coupling system indicated by the presence of two doublets, each integrated for two equivalent protons, resonating at δ_H 7.99 (J = 8.6 Hz, H-2', H-6') and 6.86 (J = 8.5 Hz,
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H-3', H-5') and corresponding to $\delta_C$ 131.3 (C-2', C-6' overlapped) and 115.5 (C-3', C-5' overlapped), and thus confirming a C-4' hydroxy substitution ($\delta_C$ 160.30).

To the best of our knowledge, compound 2 was reported here as a new natural constituent and was named commicarpiflavonol glucoside B.

Further chromatography of the CHCl$_3$ fraction resulted in isolation of two more compounds (3 and 4). The structures of compounds 3 and 4 (Figure 1) were assigned by interpretation of their 1D and 2D NMR data and EIMS as well as by comparison with literature data, and were thus identified as β-sitosterol (3) [16,17] and betulinic acid (4) [18-20].

Compounds 1 and 2 belong to an unusual group of flavonoids lacking an oxygen in C-5. 5-Deoxyflavonols were reported here for the first time in family Nyctaginaceae and as the only report on the chemical composition of the genus *Commicarpus*. Extensive studies are required for chemotaxonomic consideration.

**Figure 1.** Structures of compounds 1-4

**Figure 2.** Key HMBC correlations of compounds 1 and 2
Table 1. NMR spectral data of compound 1 and 2 (DMSO, 400 & 100 MHz)

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<th>δ_H (mult., J in Hz)</th>
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<td>-</td>
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<td>-</td>
</tr>
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*: multiplicities were deduced from DEPT and multiplicity-edited HSQC; †: exchangeable values; *, #: overlapped

Supporting Information

Supporting Information accompanies this paper on [http://www.acgpubs.org/RNP](http://www.acgpubs.org/RNP)

References


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