

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

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(Received June 8, 2016; Revised September 29, 2016; Accepted October 7, 2016)

Abstract: The phytochemical investigation of the aerial parts of *Commicarpus grandiflorus* (Standl.) resulted in the isolation of two new flavonol 3-*O*-glucosides, commicarpiflavonol glucoside A (**1**) and commicarpiflavonol 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 glycosides; commicarpiflavonol 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 betacyanins, 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. plumbagineus* 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 CDCl₃ on Bruker spectrometer at 400 MHz for ¹H NMR and 100 MHz for ¹³C 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 SiO₂ (250 x 10 mm, 40-63 μ m, Merck, Darmstadt, Germany) was used for purification of compounds **1** and **2**; TLC analyses were conducted on pre-coated silica gel 60 F₂₅₄ (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/ H₂O 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/ CH₂Cl₂ (90-10%) mixtures followed by CH₂Cl₂, CH₂Cl₂/ EtOAc (90-10%) mixtures, EtOAc and EtOAc/ MeOH (99-95%) mixtures. The subfraction eluted with 60% *n*-hexane/ CH₂Cl₂ (700 mg) was purified on SiO₂ CC (30 cm L x 2 cm D), eluted with a gradient of *n*-hexane/ CH₂Cl₂ 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 C₂₂H₂₂O₁₃, 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 C₂₂H₂₂O₁₂, 477.0949 [M – H][–]); NMR data: see table 1.

2.4.3. *β -sitosterol (3)*. White powder; EIMS: m/z 414 [M^+], $C_{29}H_{50}O$, 1H NMR (400 MHz, $CDCl_3$): 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, H₃-19), 0.94 (d, $J = 6.6$, H₃-21), 0.87 (d, $J = 7.2$, H₃-27), 0.86 (t, $J = 6.6$, H₃-29), 0.84 (d, $J = 7.2$, H₃-26), 0.70 (s, H₃-18); ^{13}C NMR (100 MHz, $CDCl_3$): 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^+], $C_{30}H_{48}O_3$, 1H NMR (400 MHz, $CDCl_3$): δ_H 4.50 (brs, H-29b), 4.37 (brs, H-29a), 3.00 (brt, $J = 8$, H-3), 1.53 (H₃-30), 0.92 (s, H₃-23), 0.80 (H₃-27), 0.74 (s, H₃-26), 0.69 (s, H₃-24), 0.63 (s, H₃-25); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{22}H_{22}O_{13}$ on the basis of HRESIMS with pseudomolecular ion peak at m/z 493.0792 [$M - H$]. Combined 1D (1H , ^{13}C) and 2D (COSY, HMQC, HMBC) spectral data of **1** indicated its flavonol nature [13,14]. 1HNMR 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 hydroxyl-bearing carbons resonating at δ_C 145.1 and 148.8 assigned for C-3' and C-4' (exchangeable). Moreover, 1HNMR 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 (OCH₃/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, 1HNMR and $^{13}CNMR$ 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 1HNMR 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 $C_{22}H_{22}O_{12}$ as deduced from HRESIMS, with pseudomolecular ion peak at m/z 477.0950 [$M - H$]. Extensive study of 1D (1H , ^{13}C) and 2D (COSY, HSQC, 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 1HNMR 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,

H-3', H-5') and corresponding to δ_C 131.3 (C-2', C-6' overlapped) and 115.5 (C-3', C-5' overlapped), and thus confirming a C-4' hydroxy substitution (δ_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.

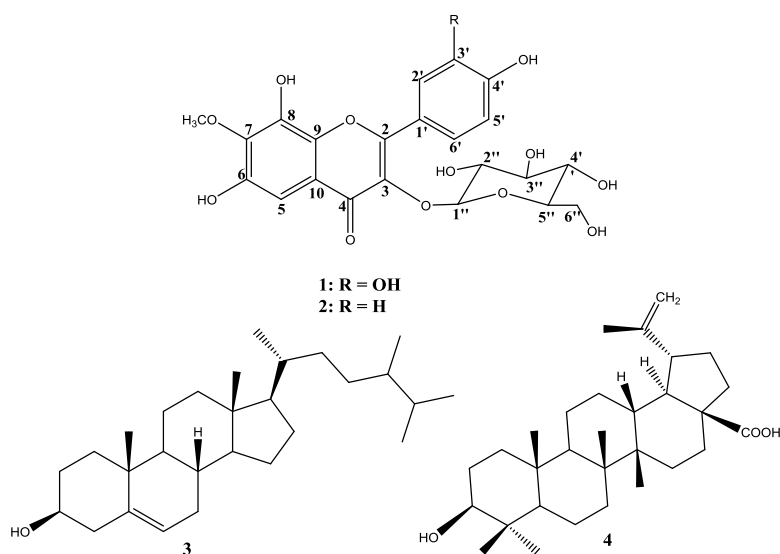


Figure 1. Structures of compounds **1-4**

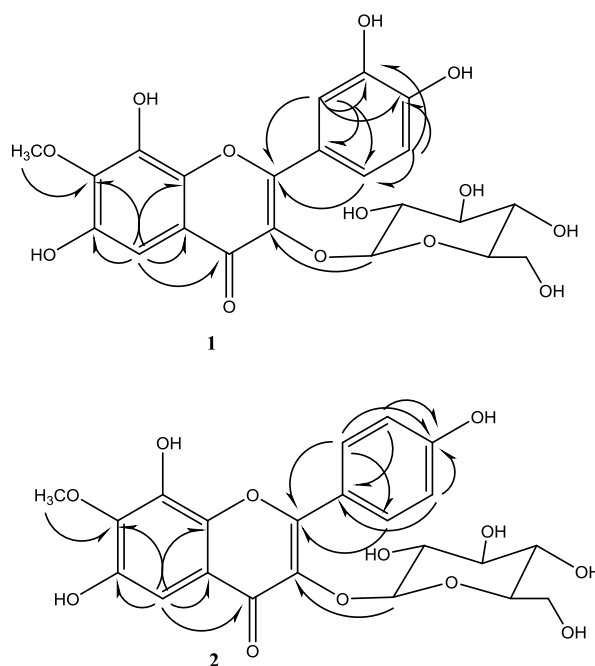


Figure 2. Key HMBC correlations of compounds **1** and **2**

Table 1. NMR spectral data of compound **1** and **2** (DMSO, 400 & 100 MHz)

Position	1		2	
	δ_{H} (mult., <i>J</i> in Hz)	δ_{C} (mult.) ^a	δ_{H} (mult., <i>J</i> in Hz)	δ_{C} (mult.) ^a
1	-	-	-	-
2	-	156.7 C	-	158.3 C
3	-	133.3 C	-	133.2 C
4	-	178.0 C	-	178.0 C
5	6.52 (s)	94.3 CH	6.51 (s)	94.4 CH
6	-	152.0 C	-	152.6 C
7	-	131.7 C	-	131.7 C
8	-	152.6 C	-	152.1 C
9	-	158.0 C	-	156.8 C
10	-	104.6 C	-	104.6 C
1'	-	122.0 C	-	121.3 C
2'	7.56 (d, 2.1)	116.5 CH	7.99 (d, 8.6)	131.3 [#] CH
3'	-	145.1 [†] C	6.86 (d, 8.5)	115.5 [*] CH
4'	-	148.8 [†] C	-	160.3 C
5'	6.84 (d, 8)	115.6 CH	6.86 (d, 8.5)	115.5 [*] CH
6'	7.55 (dd, 8, 2.1)	121.6 CH	7.99 (d, 8.6)	131.3 [#] CH
1''	5.42 (d, 8)	101.2 CH	5.40 (d, 7.4)	101.2 CH
2''	3.32 (brm)	74.4 CH	3.35 (brm)	74.5 CH
3''	3.32 (brm)	76.8 CH	3.35 (brm)	76.7 CH
4''	3.16 (brm)	70.2 CH	3.15 (brm)	70.2 CH
5''	3.16 (brm)	77.7 CH	3.15 (brm)	77.7 CH
6''	3.38 (m)	61.2 CH ₂	3.55 (m)	63.1 CH ₂
	3.68 (m)		3.76 (m)	
O-CH ₃	3.75 (s)	60.4 CH ₃	3.90 (s)	60.4 CH ₃

^a: 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>

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