ORIGINAL ARTICLE

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Rec. Nat. Prod. 11:2 (2017) 141-146

records of natural products

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 (^{1}H, ^{13}C) and 2D (COSY, HSQC, HMBC) NMR spectral data analysis, as well as high-resolution mass determinations.

Keywords: *Commicarpus grandiflorus*; Nyctaginaceae; flavonol glycosides; commicarpiflavonol glucosides.

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. plumbagineus* Standl. were reported to exhibit strong

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 12/24/2016 EISSN:1307-6167

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_2O 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_2Cl_2 (90-10%) mixtures followed by CH_2Cl_2, CH_2Cl_2/ EtOAc (90-10%) mixtures, EtOAc and EtOAc/ MeOH (99-95%) mixtures. The subfraction eluted with 60% *n*-hexane/ CH_2Cl_2 (700 mg) was purified on SiO_2 CC (30 cm L x 2 cm D), eluted with a gradient of n-hexane/ CH_2Cl_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^+], $C_{29}H_{50}O$, ^{1}H 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, H_3-19), 0.94 (d, *J* = 6.6, H_3-21), 0.87 (d, *J* = 7.2, H_3-27), 0.86 (t, *J* = 6.6, H_3-29), 0.84 (d, *J* = 7.2, H_3-26), 0.70 (s, H_3-18); ^{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^+], $C_{30}H_{48}O_3$, ^{1}H 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 (H_3-30), 0.92 (s, H_3-23), 0.80 (H_3-27), 0.74 (s, H_3-26), 0.69 (s, H_3-24), 0.63 (s, H_3-25); ^{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 (^{1}H, ^{13}C) and 2D (COSY, HMQC, HMBC) spectral data of **1** indicated its flavonol nature [13,14]. ^{1}HNMR 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, ^{1}HNMR 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, ^{1}HNMR 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 ^{1}HNMR 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 (^{1}H, ^{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 ^{1}HNMR 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.

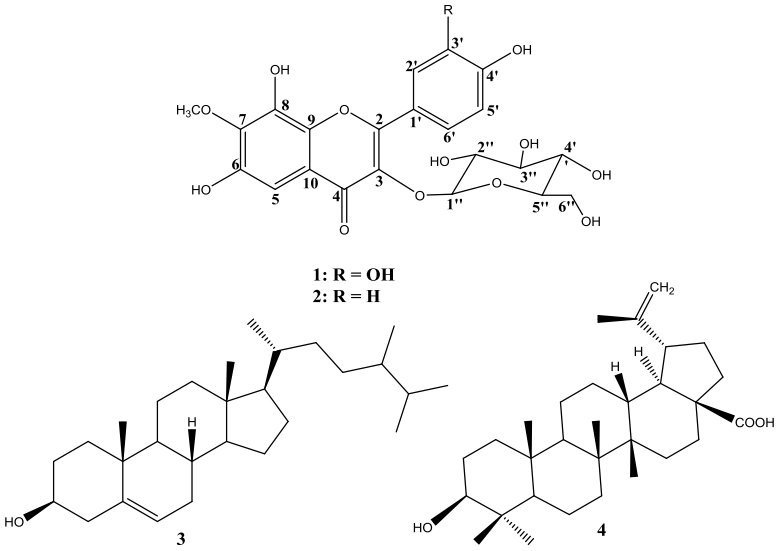

Figure 1. Structures of compounds **1-4**

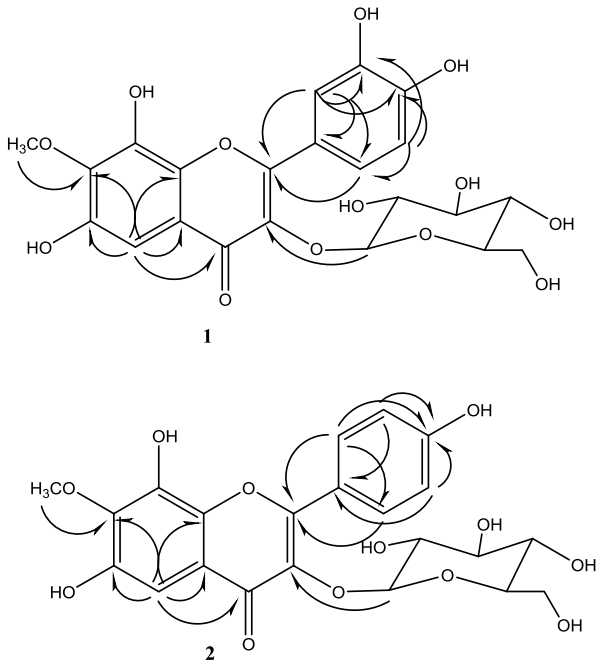

Figure 2. Key HMBC correlations of compounds **1** and **2**

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

Position	**1**		**2**	
	δ_H (mult., *J* in Hz)	δ_C (mult.)[a]	δ_H (mult., *J* 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) 3.68 (m)	61.2 CH_2	3.55 (m) 3.76 (m)	63.1 CH_2
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

References

[1] D. Rinaldo, C.M. Rodrigues, J. Rodrigues, M. Sannomiya, L.C. dos Santos and W. Vilegas (2007). New flavone from the leaves of *Neea theifera* (Nyctaginaceae), *J. Braz. Chem. Soc*. **18**, 1132-1135.

[2] M. Struwig and S.J. Siebert (2013). A taxonomic revision of *Commicarpus* (Nyctaginaceae) in southern Africa, *S. Afr. J. Bot.* **84**, 44–64.

[3] W.S. Chang, Y.J. Lee, F.J. Lu and H.C. Chiang (1993). Inhibitory effects of flavonoids on xanthine oxidase, *Anti-Cancer Res.* **13**, 2165-2170.

[4] W.S. Chang, Y.H. Chang, F.J. Lu and H.C. Chiang (1994). Inhibitory effects of phenolics on xanthine oxidase, *Anti- Cancer Res.* **14**, 501-506.

[5] S. Heuer, S. Richter, J.W. Metzger, V. Wray, M. Nimtz and D. Strack (1994). Betacyanins from bracts of *Bougainvillea glabra*, *Phytochemistry* **37**, 761-767.

[6] H.O. Edeoga and C.I. Ikem (2002). Tannins, saponins and calcium oxalate crystals from Nigerian species of *Boerhavia* L. (Nyctaginaceae), *S. Afr. J. Bot.* **68**, 386-388.

[7] B. Ahmed and C.P. Yu (1992). Borhavine, a dihydroisofuranoxanthone from *Boerhaavia diffusa*, *Phytochemistry* **31**, 4382-4384.

[8] N. Lami, S. Kadota and T. Kikuchi (1991). Constituents of the roots of *Boerhaavia diffusa* L. IV. Isolation and structure determination of boeravinones D, E, and F, *Chem. Pharm. Bull*. **39**, 1863-1865.

[9] N. Lami, S. Kadota, T. Kikuchi and Y. Momose (1991). Constituents of the roots of *Boerhaavia diffusa* L. III. Identification of Ca^{2+} channel antagonistic compound from the methanol extract, *Chem. Pharm. Bull*. **39**, 1551-1555.

[10] V. De Feo, S. Piacente, C. Pizza and R.U. Soria (1998). Saponins from *Colignonia scandens* Benth. (Nyctaginaceae), *Biochem. Syst. Ecol*. **26**, 251-253.

[11] C. Lavaud, S. Beauviere, G. Massiot, L. Lemenolivier and G. Bourdy (1996). Saponins from *Pisonia umbellifera, Phytochemistry* **43**, 189-194.

[12] E. Abdel-Sattar, L. Maes and M.M. Salama (2010). In vitro activities of plant extracts from Saudi Arabia against malaria, leishmaniasis, sleeping sickness and Chagas disease, *Phytother. Res*. **24**, 1322-1328.

[13] P.K. Agrawal (1989). Carbon 13 NMR of flavonoids. Central Ins. of medicinal and aromatic plants, Elsevier, Lucknow.

[14] T.J. Mabry, K.R. Markham and M.B.Thomas (1970). The systematic identification of flavonoids. Springer-Verlag, New York.

[15] P. Charisiadis, V.G. Kontogianni, C.G. Tsiafoulis, A.G. Tzakos, M. Siskos and I.P. Gerothanassis (2014). ^{1}H-NMR as a structural and analytical tool of intra- and intermolecular hydrogen bonds of phenol-containing natural products and model compounds, *Molecules* **19**, 13643-13682.

[16] L.J. Goad and T. Akihisa (1992). Analysis of sterols. Blackie Academic & Professional, London.

[17] L.H. Rasoanaivo, A. Wadouachi, T.T. Andriamampianina, S.G. Andriamalala, E.J. Razafindrakoto, A. Raharisololalao, et al. (2014). Triterpenes and steroids from the stem bark of *Gambeya boiviniana* Pierr, *J. Pharmacogn. Phytochem*. **3**, 68–72.

[18] Md.E. Haque, H.U. Shekhar, A.U. Mohamad, H. Rahman, A.K.M. Islam and M.S. Hossain (2006). Triterpenoids from the stem bark of *Avicennia officinalis*, *Dhaka Univ. J. Pharm. Sci.* **5**, 53-57.

[19] E. Bisoli, W.S. Garcez, L. Hamerski, C. Tieppoand and F.R. Garcez (2008). Bioactive pentacyclic triterpenes from the stems of *Combretum laxum*, *Molecules* **13**, 2717-2728.

[20] G. Uddin, Waliullah, B.S. Siddiqui, M. Alam, A. Sadat, A. Ahmad and A. Uddin (2011). Chemical constituents and phytotoxicity of solvent extracted fractions of stem bark of *Grewia optiva* Drummond ex Burret, *Middle East. J. Sci. Res*. **8**, 85-91.