Vietnam Journal of Chemistry, International Edition, **54**(4): 443-447, 2016
DOI: 10.15625/0866-7144.2016-00344

Flavonoid glycosides from *Viscum album*

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Received 17 March 2016; Accepted for publication 12 August 2016

Abstract

Using combined chromatographic methods, four flavonoid glycosides, (2*S*)-homoeriodictyol-7-*O*-*β*-D-apiofuranosyl-(1→2)-O-*β*-D-glucopyranoside (**1**), (2*S*)-5-hydroxy-7,3′-dimethoxyflavanone-4′-*O*-*β*-D-apiofuranosyl-(1→2)-*O*-*β*-D-glucopyranoside (**2**), homoflavoyadorinin-B (**3**), and 3′-methoxyapiin (**4**) were isolated from the methanol extract of the leaves and twigs of *Viscum album.* Their structures were elucidated by 1D- and 2D-NMR spectra and in comparison with those reported in the literature.

Keywords. *Viscum album*, flavonoid glycoside.

1. INTRODUCTION

Viscum album L. var. *meridianum* Dans. is a hemiparasitic shrub. It has been used as a remedy in traditional oriental medicine to treat swell spleen, wound, tumour and sore ears [1]. *V. album* exerts several biological effects such as antitumor, anticancer [2, 3], and anti-inflammatory activities [4]. It is well established that the extract of *V. album* inhibited tumour angiogenesis and metastasis of haematogenous and non-haematogenous tumour cells in mice [5]. Chemical investigation of *V. album* proved the presence of flavonoids [6], lignans, phenylpropanoids [7], and triterpenes [8]. This paper reported the isolation and structure elucidation of four flavonoid glycosides from the methanol extract of the leaves and twigs of *V. album.*

2. MATERIAL AND METHODS

2.1. Plant materials

The leaves and twigs of *V. album* were collected in Cucphuong, Ninhbinh, Vietnam in October, 2012 and identified by Prof. Dr. Ninh Khac Ban, Institute of Marine Biochemistry, VAST. A voucher specimen (TG1012) was deposited at the Herbarium of the Institute of Marine Biochemistry.

2.2. General experimental procedures

All NMR spectra were on a Bruker AM400 FT-NMR spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR), and chemical shifts (δ) are reported in ppm using TMS as an internal standard. Column chromatography was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) and RP-18 resins. Thin layer chromatography was performed on DC Alufolien Kieselgel 60 F254 (Merck) or RP-18 F_{254s} (Merck) plates. Compounds were visualized by spraying with aqueous 10 % H_2SO_4 and heating for 5 minutes.

2.3. Extraction and isolation

The dried leaves and twigs of *V. album* (2.5 kg) were extracted with hot MeOH three times (3 × 5 L) under reflux for 12 h to yield 320 g extract after evaporation of the solvent. This extract was suspended in H_2O and successively partitioned with $CHCl_3$ and EtOAc to yield the $CHCl_3$ (VA1, 45.0 g), EtOAc (VA2, 9.0 g), and H_2O (VA3, 260.0 g) extracts after removal of the solvents *in vacuo.* The VA2 fraction was chromatographed on a silica gel column eluting with a gradient of $CHCl_3$-MeOH (10:1→2:1, v/v) to give four fractions, VA2A-VA2D. The VA2C fraction was chromatographed on a Sephadex LH-20 column and eluting with MeOH to give compounds **3** (20.0 mg) and **4** (4.1 mg). The VA3 fraction (260 g) was chromatographed on a Diaion HP-20P column eluting with H_2O containing increasing concentrations of MeOH (0, 25, 50, 75, and 100 %, v/v) to yield five sub-fractions, VA3A (120.0 g), VA3B (12.0 g), VA3C (14.0 g), VA3D (20.0 g), and VA3E (15.0 g). The VA3D fraction was chromatographed on a silica gel column eluting with gradient of $CHCl_3$-MeOH (10:1→2:1, v/v) to

give five fractions, VA3D1-VA3D5. The VA3D2 fraction was chromatographed on a RP-18

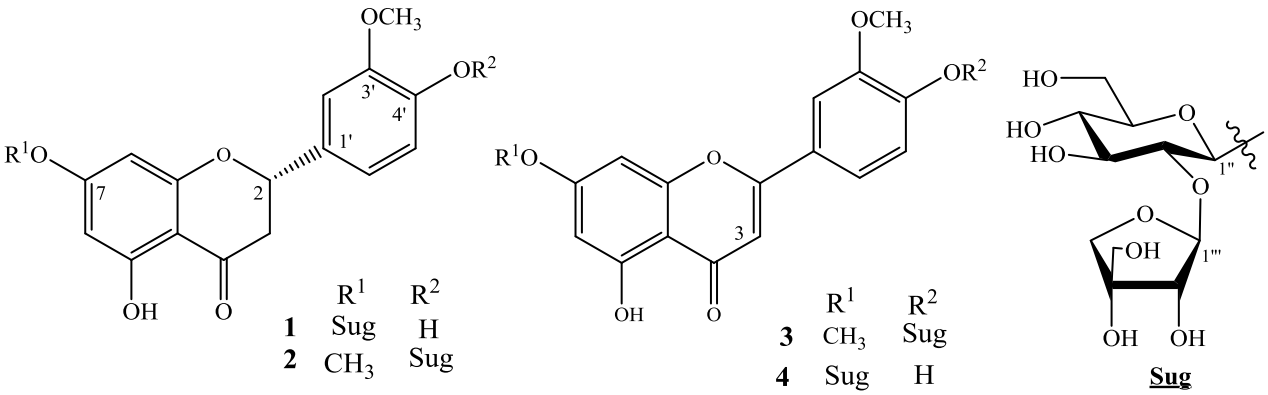

Figure 1: The chemical structures of compounds **1-4**

column eluting with MeOH-H_2O (1:1, v/v) to yield **2** (9.0 mg). The VA3D3 fraction was chromatographed on a RP-18 column eluting with MeOH - H_2O (1:1, v/v) to yield compound **1** (180.0 mg).

(2*S*)-Homoeriodictyol-7-*O*-*β*-D-apiofuranosyl-(1→2)-O-*β*-D-glucopyranoside (1): yellowish powder; mp 143-146 ºC; $[\alpha]_D^{25}$= -46.0 (*c*, 0.1, CH_3OH); $C_{27}H_{32}O_{15}$, ESI-MS *m/z* 597 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data, see table 1.

(2*S*)-5-Hydroxy-7,3′-dimethoxyflavanone-4′-*O*-*β*-D-apiofuranosyl-(1→2)-*O*-*β*-D-glucopyranoside (**2**): yellowish powder; mp 219-221 ºC; $[\alpha]_D^{25}$= -85.0 (*c*, 0.1, MeOH); $C_{28}H_{34}O_{15}$, ESI-MS *m/z* 611 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data, see table 1.

Homoflavoyadorinin-B (**3**): yellowish powder; mp 217-220 ºC; $[\alpha]_D^{25}$= -13.0 (*c* 0.1, CH_3OH); $C_{28}H_{32}O_{15}$, ESI-MS *m/z* 609 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data, see table 1.

3′-Methoxyapiin (**4**): yellowish powder; mp 200-202 ºC; $[\alpha]_D^{25}$= -38.0 (*c* 0.1, CH_3OH); $C_{27}H_{30}O_{15}$, ESI-MS *m/z* 595 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data, see table 1.

3. RESULTS AND DISCUSSION

Compound **1** (figure 1) was yielded as a yellowish powder. The ^{1}H-NMR of **1** showed the signals of one oxymethine proton at δ_H 5.33 (1H, dd, *J* = 2.0, 12.5 Hz), three protons of ABX aromatic system at δ_H 7.05 (1H, s), 6.81 (1H, d, *J* = 8.0 Hz), and 6.90 (1H, d, *J* = 8.0 Hz), suggested the presence of a flavanone moiety; two anomeric protons at δ_H 5.00 (1H, d, *J* = 7.6 Hz) and 5.42 (1H, d, *J* = 2.0 Hz), confirmed two sugar moieties, and one methoxy group at δ_H 3.86 (3H, s). The ^{13}C-NMR and DEPT spectra displayed the signals of 27 carbons, including one methoxy, four methylene, thirteen methine, and nine quaternary carbons. Of which, 16 carbons were assigned to a flavanone with a methoxy group and 11 carbons to two sugar units. The HMBC correlations between methoxy protons (δ_H 3.86) and C-3′ (δ_C 149.04) indicated the methoxy group was located at C-3′. The HMBC correlations between glc H-1″ (δ_H 5.00) and C-7 (δ_C 166.70), api H-1‴ (δ_H 5.42) and glc C-2″ (δ_C 78.53), and between glc H-2″ (δ_H 3.61) and api C-1‴ (δ_C 110.83) indicated the linkage of sugar moiety as *O*-*β*-D-apiofuranosyl (1→2)-*O*-*β*-D-glucopyranoside and this sugar linkage was connected to C-7 of flavanone (Figure 2). All NMR assignments of **1** were confirmed by detailed analyses of HSQC and HMBC spectra (table 1), which are in good agreement with those reported in the literature [6]. Thus compound **1** was identified as (2*S*)-homoeriodictyol-7-*O*-*β*-D-apiofuranosyl-(1→2)-O-*β*-D-glucopyranoside.

The NMR spectra of **2** were similar to those of **1** with the presence of a flavanone skeleton, two sugar moieties but two methoxy groups. The HMBC correlations between methoxy group at δ_H 3.79 (3H, s) and C-7 (δ_C 167.41) confirmed the location of the methoxy group at C-7 (figure 1). The observed HMBC correlations between glc H-1″ (δ_H 4.98) and C-4′ (δ_C 146.51) indicated the position of sugar moiety at C-4′ of the flavanone. The sugar moities were also confirmed by the good agreement of ^{13}C-NMR chemical shifts for sugar moieties previously reported in some flavonoid glycosides from *V. album* [6, 9]. Based on the evidence above and in comparison with those reported in the literature [10], compound **2** was determined to be (2*S*)-5-hydroxy-7,3′-dimethoxyflavanone-4′-*O*-*β*-D-apiofuranosyl-(1→2)-*O*-*β*-D-glucopyranoside.

Table 1: The ^{1}H- and ^{13}C-NMR data for compounds **1-4**

Pos.	**1**			**2**			**3**			**4**	
	δ_C [#]	δ_C [a]	δ_H [a] (*J* in Hz)	δ_C [$]	δ_C [b]	δ_H [b] (*J* in Hz)	δ_C [¥]	δ_C [b]	δ_H [b] (*J* in Hz)	δ_C [a]	δ_H [a] (*J* in Hz)
Aglycon											
2	78.8	80.86	5.33 (dd, 2.0, 12.5)	78.5	78.57	5.53 (d, 12.5)	163.4	163.46	-	166.71	-
3	42.2	44.29	2.70 (m)	42.0	42.04	2.78 (d, 12.5)	104.2	104.29	7.03 (s)	104.50	6.59 (s)
			3.14 (m)			3.36 (m)					
4	197.1	198.51	-	196.6	196.72	-	181.9	182.09	-	184.07	-
5	162.8	164.83	-	163.1	163.12	-	161.1	161.17	-	162.91	-
6	95.2	96.81	6.16 (s)	94.6	94.68	6.15 (s)	98.0	98.09	6.35 (d, 2.4)	101.06	6.37 (s)
7	164.9	166.70	-	167.4	167.41	-	165.2	165.24	-	164.68	-
8	96.4	97.86	6.13 (s)	93.7	93.81	6.09 (s)	92.7	92.83	6.81 (d, 2.4)	96.10	6.73 (s)
9	162.7	164.49	-	162.7	162.72	-	157.2	157.32	-	158.99	
10	103.2	104.84	-	102.5	102.55	-	104.7	104.80	-	107.10	
1′	129.1	131.44	-	131.9	131.95	-	123.9	123.96	-	123.52	
2′	111.2	111.40	7.05 (s)	111.2	111.14	7.16 (s)	110.1	110.00	7.61 (d, 2.4)	110.91	7.42 (s)
3′	147.5	149.04	-	148.8	148.77	-	149.6	149.09	-	149.55	-
4′	147.0	148.14	-	146.5	146.51	-	149.0	149.65	-	152.33	-
5′	115.1	116.13	6.81 (d, 8.0)	114.9	114.89	7.07 (d, 8.0)	114.8	114.79	7.21 (d, 8.4)	116.82	6.85 (d, 8.0)
6′	119.7	120.70	6.90 (d, 8.0)	119.0	119.05	7.00 (d, 8.0)	119.7	119.81	7.66 (dd, 2.4, 8.4)	122.00	7.46 (d, 8.0)
7-OMe				55.7	55.67	3.79 (s)	55.9	59.99	3.86 (s)		
3′-OMe	55.6	56.48	3.86 (s)	55.8	55.86	3.78 (s)	56.0	56.11	3.89 (s)	56.74	3.87 (s)
4′ or 7-*O*-Glc											
1″	97.7	99.67	5.00 (d, 7.6)	98.4	98.34	4.98 (d, 7.5)	98.0	97.95	5.14 (d, 8.4)	100.27	5.06 (d, 7.5)
2″	76.8	78.53	3.61*	77.1	77.18	3.67 (m)	77.0	77.06	3.60*	78.77	3.67*
3″	75.7	78.30	3.60*	75.0	74.91	3.60*	74.8	74.75	3.45*	78.47	3.62*
4″	69.7	71.05	3.40*	69.9	69.97	3.41*	69.9	69.94	3.20 (m)	71.32	3.40 (t, 8.5)
5″	76.6	78.12	3.41*	76.8	76.83	3.46*	77.1	77.23	3.51 (m)	78.35	3.53*
6″	60.4	62.22	3.68 (m)	60.6	60.60	3.55 (m)	60.5	60.60	3.46*	62.46	3.69 (dd, 5.0, 12.0)
			3.85 (m)			3.67 (m)			3.71 (dd, 5.6, 10.0)		3.92 (d, 12.0)
2″-*O*-Apio											
1‴	108.6	110.83	5.42 (d, 2.0)	108.2	108.28	5. 42 (br s)	108.3	108.36	5.44 (s)	110.91	5.35 (s)
2‴	76.0	78.03	3.95 (d, 2.0)	76.0	76.04	3.79 (s)	76.0	76.04	3.78 (s)	78.15	3.95 (s)
3‴	79.1	80.70	-	79.9	79.29	-	79.3	79.44	-	80.69	-
4‴	73.8	75.35	3.77 (d, 9.6)	73.8	73.90	3.59 (d, 9.5)	73.0	74.02	3.62 (d, 9.6)	75.50	3.81 (d, 10.0)
			3.96 (d, 9.6)			4.06 (d, 9.5)			4.07 (d, 9.6)		4.03 (d, 10.0)
5‴	64.1	65.88	3.51 (s)	64.4	64.45	3.30 (s)	64.4	64.53	3.29 (s)	65.89	3.53 (s)

[a]recorded in CD_3OD, [b]DMSO-d_6, [*]overlapped signals, [#]δ_C of (2*S*)-homoeriodictyol-7-*O*-*β*-D-apiofuranosyl-(1→2)-*O*-*β*-D-glucopyranoside [6], [$]$\delta_C$ of (2*S*)-5-hydroxy-7,3′-dimethoxyflavanone-4′-*O*-*β*-D-apiofuranosyl-(1→2)-*O*-*β*-D-glucopyranoside [10], [¥]δ_C of homoflavoyadorinin-B [6].

^{1}H-NMR spectrum of **3** showed characteristic flavone proton signals with three protons at δ_H 7.03 (1H, s, H-3), 6.35 (1H, d, *J* = 2.4 Hz, H-6), 6.81 (1H, d, *J* = 2.4 Hz, H-8), and three protons of ABX aromatic system at δ_H 7.61 (1H, d, *J* = 2.4 Hz, H-2′), 7.21 (1H, d, *J* = 8.4 Hz, H-5′), and 7.66 (1H, dd, *J* = 2.4, 8.4 Hz, H-6′), two anomeric protons of sugar units at 5.14 (1H, d, *J* = 8.4 Hz) and 5.44 (1H, s), and two methoxy groups at δ_H 3.86 and 3.89 (each 3H). The ^{13}C-NMR data showed the presence of a D-glucopyranose moiety and a D-apiofuranose moiety with the chemical shifts of anomeric carbons at δ_C 97.95 (C-1″) and 108.36 (C-1‴). The coupling constant of H-1″ and H-2″ (*J* = 8.4 Hz) indicated the β configuration for the glucopyranose moiety. The HMBC correlations from methoxy groups at δ_H 3.86 and δ_H 3.89 to C-7 (165.24), C-3′ (149.09), respectively, proved the locations of two methoxy groups at C-7 and C-3′. Moreover, the HMBC correlations between glc H-1″ (δ_H 5.14) and C-4′ (δ_C 149.65); api H-1‴ (δ_H 5.44) and glc C-2″ (δ_C 77.06); between glc H-2″ (δ_H 3.60) and api C-1‴ (δ_C 108.36) indicated the sugar moiety of **3** to be [*O*-β-D-apiofuranosyl (1→2)-*O*-β-D-glucopyranoside] and its location at C-4′. All NMR assignments of **3** were confirmed by detailed analyses of HSQC and HMBC spectra, which are in good agreement with those reported in the literature [6]. Thus compound **3** was identified as homoflavoyadorinin-B.

Compound **4** was yielded as yellowish powder and its molecular formula was determined as $C_{27}H_{30}O_{15}$ by the ESI-MS at *m/z* 595 $[M+H]^+$ and ^{13}C-NMR data. Analysis the NMR spectra of **4** indicated that the structure of **4** was very similar to those of **3** except for the position of sugar linkage and methoxy group. The HMBC correlation from

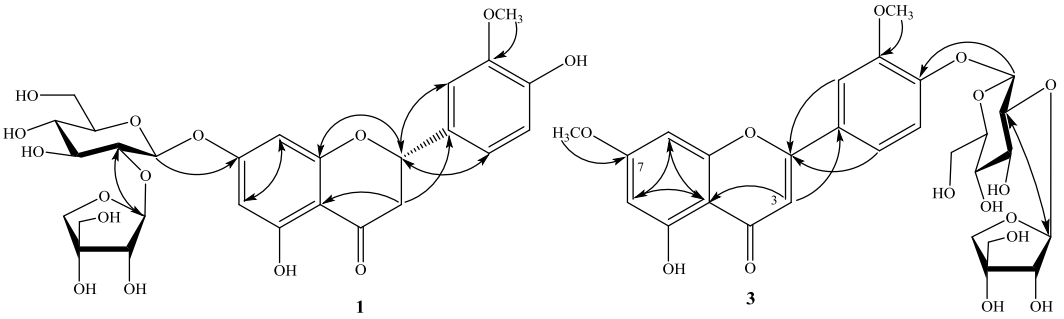

Figure 2: The key HMBC correlations of compounds **1** and **3**

methoxy group (δ_H 3.87) to C-3′ (149.55) proved the location of methoxy group at C-3′. Moreover, the observed HMBC correlations between glc H-1″ (δ_H 5.06) and C-7 (δ_C 164.68), between api H-1‴ (δ_H 5.35) and glc C-2″ (δ_C 78.77), and between glc H-2″ (δ_H 3.67) and api C-1‴ (δ_C 110.91) indicated the sequence of sugar linkages of **4** and the position of sugar moiety at C-7 of the flavone. From all the above evidence, the structure of **4** was determined as 3′-methoxyapiin [11].

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