VIETNAM JOURNAL OF CHEMISTRY 54(2) 184-187 APRIL 2016
DOI: 10.15625/0866-7144.2016-00257

FLAVONES AND LIGNANES FROM *Glochidion obliquum* Decne

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Received 7 August 2015; Accepted for Publication 26 April 2016

Abstract

Using combined chromatographic methods, two flavones, vitexin (**1**) and 6"-*O*-acetylorientin (**2**), and two lignanes, (+)-pinoresinol (**3**) and (+)-syringaresinol (**4**) were isolated from the methanol extract of the leaves of *Glochidion obliquum* Decne. Their structures were elucidated by 1D- and 2D-NMR spectra and in comparison with those reported in the literature.

Keywords. *Glochidion obliquum*, flavone, lignane, vitexin, 6"-*O*-acetylorientin, pinoresinol, syringaresinol.

1. INTRODUCTION

Glochidion obliquum Decne is a small tree distributed throughout Vietnam, India, Malaysia, Indonesia, and Cambodia. In Vietnam, this plant was distributed in Lang Son, Phu Tho, Vinh Phuc, Tay Ninh, Dong Nai, and Kien Giang provinces. The leaves of *G. obliquum* have been used in the folk medicine to treat sarcoptic acariasis [9]. Flavonoids may have beneficial health effects because of their antioxidant properties and their inhibitory role in various stages of tumour development in animal studies [7]. Lignans have many biological properties, such as antioxidant, antitumor, antiviral, antibacterial, insecticidal, fungistatic and anti-platelet activities disease [6, 8]. As a part of our phytochemical studies of *G. obliquum* [10], we report herein the isolation, structural elucidation of two flavones and two lignanes from this plant.

2. MATERIAL AND METHODS

2.1 Plant material

The leaves of *Glochidion obliquum* Decne were collected in Phucyen, Vinhphuc, Vietnam in December, 2012 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (GO1212) was deposited at the Herbarium of the Institute of Marine Biochemistry.

2.2 General experimental procedures

All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR), and chemical shifts (δ) are reported in ppm using TMS as an internal standard. Column chromatography (CC) was performed on silica gel 230-400 mesh (0.040-0.063 mm, Merck) or YMC RP-18 resins (30-50 μm, Fujisilisa Chemical Ltd.). 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 of *G. obliquum* (4.2 kg) were powdered and extracted three times with hot methanol (50 °C) to give the methanol extract (210.3 g), which was then suspended in water and extracted in turn with *n*-hexane, dichloromethane, and EtOAc, giving corresponding extracts: *n*-hexane (87.2 g, GOH), dichloromethane (55.0 g, GOD), and ethyl acetate (12.0 g, GOE), and water layers (35.5 g, GOW).

GOE was chromatographed on a silica gel column using *n*-hexane - acetone (2:1 v/v) to yield two sub-fractions, GOE1 and GOE2. The GOE1 fraction was chromatographed on a silica gel column eluting with chloroform - acetone (3:1, v/v) to give two smaller fractions, GOE1A and GOE1B. The GOE1B fraction was chromatographed on a RP-18

column eluting with acetone - water (1.5:1, v/v) to afford compounds **1** (8 mg) and **2** (10 mg).

GOW was subsequently chromatographed over Diaion HP20 column and then eluted in turn with solvent mixture methanol - water (1:3, 1:1, 2:1, respectively) to yield three fractions GOW1 (3.34 g), GOW2 (13.5 g), and GOW3 (10.7 g). The GOW3 fraction was chromatographed on a silica gel column eluting with ethyl acetate - methanol (10:1, v/v) to yield two fractions, GOW3A and GOW3B. The GOW3A sub-fraction was further separated on a RP-18 column eluting with methanol - water (1:1.5, v/v) to give two smaller fractions, GOW3A1 and GOW3A2. Compound **3** (18 mg) was yielded from GOW3A2 fraction by chromatography on a silica gel column eluting with chloroform - methanol (4:1, v/v). The GOW3B fraction was continued to be chromatographed on a silica gel column using dichloromethane - ethyl acetate (10:1 v/v) to yield sub-fractions, GOW3B1 and GOW3B2. Compound **4** (12 mg) was yielded from GOW3B2 fraction through chromatography on a RP-18 column eluting with methanol - water (1:1, v/v).

Vitexin (**1**): yellow powder; mp 269-270 ºC; $[\alpha]_D^{25}$= +10.0 (*c* 0.1, pyridine); $C_{21}H_{20}O_{10}$, ESI-MS *m/z* 433 $[M + H]^+$; ^{1}H- and ^{13}C-NMR data, see table 1.

6''-*O*-acetylorientin (**2**): yellow powder; mp 190-193 ºC; $[\alpha]_D^{25}$= +27.0 (*c* 0.1, pyridine); $C_{23}H_{22}O_{12}$; ESI-MS *m/z* 491 $[M + H]^+$; ^{1}H- and ^{13}C-NMR data, see table 1.

(+)-Pinoresinol (**3**): white amorphous powder, mp 120-121 ºC; $[\alpha]_D^{25}$= +35.0 (*c* 0.1, $CHCl_3$); $C_{20}H_{22}O_6$; ESI-MS *m/z* 359 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data, see table 1.

(+)-Syringaresinol (**4**): white amorphous powder, mp 174-176 ºC; $[\alpha]_D^{25}$= +50.0 (*c* 0.1, $CHCl_3$); $C_{22}H_{26}O_8$; ESI-MS *m/z*: 441 $[M+Na]^+$; ^{1}H- and ^{13}C-NMR data, see table 1.

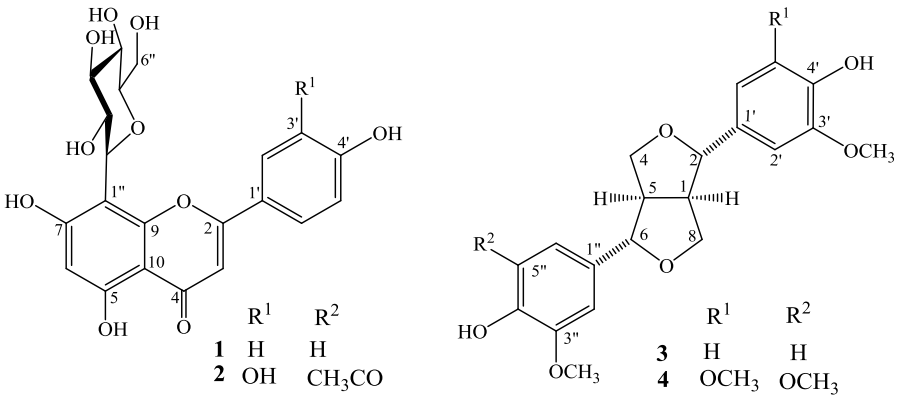

Figure 1: Chemical structures of **1-4**

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow powder and its molecular formula was determined as $C_{21}H_{20}O_{10}$ by the ESI-MS at *m/z* 433 $[M + H]^+$ with a combination of ^{1}H-NMR and ^{13}C-NMR data. The NMR spectrum of **1** is typical for a flavone glycoside. The ^{1}H-NMR showed protons of aromatic AA′BB′-system at δ_H 8.28 (2H, d, *J* = 7.0 Hz, H-2', 6'), and 6.86 (2H, d, *J* = 7.0 Hz, H-3', 5') which are characteristic for 4'-substituted B-ring and two singlet protons at δ_H 6.79 and 6.25. The *C*-glycoside moiety was confirmed by the one anomeric proton signal at δ_H 4.65 (d, *J* = 9.0 Hz). The ^{13}C-NMR and DEPT spectra of **1** revealed the signals of 21 carbons, indicating the presence of one flavonoid moiety, including: one carbonyl at δ_C 182.14, six olefine methine carbons at δ_C 98.18, 101.90, 115.79×2, and 129.72×2, four hydroxyl carbons at δ_C 160.43, 162.86, 104.69, and 161.14, four olefine quaternary carbons at δ_C 121.12, 164.20, 156.11, and 104.69, and the six sugar moiety carbons from δ_C 61.37 to 80.54. From analysis of NMR data, **1** was deduced to be a flavone *C*-glycoside and a sugar moiety was determined to be β (the large coupling constant of two protons, H-1″,H-2″, *J* = 9.0 Hz). The chemical shift value of the anomeric carbon atom at 73.96 indicated that the linkage of glucose was through a *C*-bond. The HMBC correlations from H-1″ δ_H 4.65 to C-7 (162.86), C-8 (104.69) and C-9 (156.11) of the aglycone confirmed the direct linkage from C-1″ of sugar to C-8 of the aglycone (Figure 1). All NMR assignments of **1** were confirmed by detailed analyses of HSQC and HMBC spectra, which are in good agreement with those reported in the literature [2, 3]. Thus compound **1** was identified as vitexin.

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

No.	1		2		No.	3		4	
	δ_C[a]	δ_H[a] (*J*, Hz)	δ_C[a]	δ_H[a] (*J*, Hz)		δ_C[b]	δ_H[b] (*J*, Hz)	δ_C[b]	δ_H[b] (*J*, Hz)
2	164.20	-	164.47	-	1, 5	54.19	3.10 (m)	54.37	3.09 (m)
3	101.90	6.79 (s)	102.09	6.66 (s)	2, 6	85.89	4.73 (d, 4.0)	86.09	4.73 (d, 4.0)
4	182.14	-	182.31	-	4, 8	71.69	3.88 (dd, 4.0, 9.5) 4.24 (dd, 7.0, 9.5)	71.82	3.90 (dd, 4.5, 9.0) 4.28 (dd, 6.5, 9.0)
5	160.43	-	160.74	-	1′, 1″	132.95	-	132.12	-
6	98.18	6.25 (s)	98.38	6.27 (s)	2′, 2″	108.62	6.89 (d, 1.5)	102.75	6.58 (s)
7	162.86	-	162.97	-	3′, 3″	146.72	-	147.18	-
8	104.69	-	104.42	-	4′, 4″	145.26	-	134.34	-
9	156.11	-	156.33	-	5′, 5″	114.28	6.88 (d, 8.0)	147.18	-
10	104.69	-	104.27	-	6′, 6″	118.98	6.81 (dd, 1.5, 8.0)	102.75	6.58 (s)
1′	121.12	-	121.43	-	OCH_3	55.98	3.90 (s)	56.40	3.90 (s)
2′	129.72	8.28 (d, 7.0)	113.51	7.45 (d, 2.0)					
3′	115.79	6.86 (d, 7.0)	145.66	-					
4′	161.14	-	150.10	-					
5′	115.97	6.86 (d, 7.0)	116.37	6.85 (d, 8.5)					
6′	129.72	8.28 (d, 7.0)	121.40	8.03 (dd, 2.0, 8.5)					
1″	73.96	4.65 (d, 9.0)	74.06	4.67 (d, 10.0)					
2″	68.17	4.20*	69.82	3.85*					
3″	75.53	3.35*	77.00	3.78*					
4″	69.29	3.86*	68.10	4.20*					
5″	80.54	3.50*	75.21	3.46*					
6″	61.37	3.57*	65.19	4.18*/4.21*					
6″-Ac			170.85	-					
			20.95	1.97 (s)					

[a]Recorded in DMSO-d_6, [b]$CDCl_3$, Ac, acetyl group *Overlapped signals.

The analytical ^{1}H- and ^{13}C-NMR spectra of **2** indicated the structure of **2** was similar to **1** except for an additional acetyl group at C-6″ which was confirmed by the methyl proton signal at δ_H 1.97 (3H, s) and cacbonyl carbon at δ_C 170.85 and three aromatic protons at δ_H 7.45 (1H, d, *J* = 2.0 Hz), 6.85 (1H, d, *J* = 8.5 Hz), and 8.03 (1H, dd, *J* = 2.0, 8.5 Hz) ascribed to aromatic protons H-2′, H-5′ and H-6′, respectively, supporting ABX-type coupling system of ring B (Figure 1). The position of this acetyl group was further confirmed by the HMBC correlations from H-6″ (δ_H 4.18 and 4.21) to $CH_3\underline{CO}$ (δ_C 170.85). The NMR data of compound **2** were similar to those in the literature data [4]. Therefore, the structure of **2** was identified as 6"-*O*-acetylorientin. To the best of our knowledge, compound **2** was reported from *Glochidion* genus for the first time.

Compound **3** showed a pseudo-ion peak at *m/z* 359 $[M + H]^+$ in the ESI-MS spectrum, which together with ^{13}C-NMR data was consitent with the moleculer formula of $C_{20}H_{22}O_6$. The ^{1}H-NMR spectrum of **3** showed signals for six olefinic methine of two ABX aromatic systems at δ_H 6.88 (2H, d, *J* = 8.0 Hz), 6.89 (2H, d, *J* = 1.5 Hz), and 6.81 (2H, dd, *J* = 1.5, 8.0 Hz); two protons of oxygenated methines at δ_H 4.73 (2H, d, *J* = 4.0 Hz), four protons of oxygenated methylenes at δ_H 4.24 (2H, dd, *J* = 7.0, 9.5 Hz) and 3.88 (2H, dd, *J* = 4.0, 9.5 Hz); two methoxy groups at δ_H 3.90 (6H, s). These data suggested the structure of **3** to be lignan [1, 5]. The ^{13}C-NMR and DEPT spectra showed the presence of 20 carbons, including six nonprotonated carbons at δ_C 132.95×2, 146.72×2, 145.26×2, six olefinic methine at δ_C 108.62×2, 114.28×2, and 118.98×2, two oxygenated methine at δ_C 85.89×2, two methylene at δ_C 71.69×2, two methoxy at δ_C 55.98×2, and two methine carbons at δ_C 54.19×2. All the above data indicated that structure of **3** to be a lignan and their data were similar to those of (+) pinoresinol [1, 5] and elucidated to be (+)

pinoresinol. This compound was isolated from genus *Glochidion* for the first time.

Compound **4** was obtained as a white amorphous powder and its molecular formula was determined as $C_{22}H_{26}O_8$ by the ESI-MS at *m/z* 441 $[M + Na]^+$ and ^{13}C-NMR data. The ^{1}H- and ^{13}C-NMR data of **4** showed similar to those of compound **3** except for an addition of methoxy groups at C-5′ and C-5″ (δ_C 56.40, δ_H 3.90). Thus **4** was defined as syringaresinol. This compound was isolated from *G. obliquum* for the first time.

Acknowledgement. *This research was supported by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2012.24.*

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