Phytochemistry Letters 11 (2015) 316–319

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Two new flavonols from flowers of *Getonia floribunda* Roxb.

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ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form 1 February 2015
Accepted 4 February 2015
Available online 16 February 2015

Keywords:
Getonia floribunda Roxb.
Flavonol
Oral cavity cancer
Cytotoxicity

ABSTRACT

Phytochemical investigation of the EtOAc and MeOH extracts from flowers of *Getonia floribunda* Roxb., a Thai herbal medicine, resulted in the isolation of two new flavonols, 4′-hydroxy-6,7,8,3′-tetramethoxyflavonol (**1**) and 4′-hydroxy-6,7,8-trimethoxyflavonol (**2**), along with a known pachypodol (**3**). Their structures were elucidated by spectroscopic methods including UV, IR, 1D and 2D NMR techniques and MS analysis. Compound **1** showed cytotoxicity against the oral cavity cancer (KB) cell line with an IC_{50} value of 8.99 ± 2.00 μg/ml.

1. Introduction

Getonia floribunda Roxb. (Combretacea) is used in Asian traditional medicine systems, including Ayurveda, Unani and folk medicine (Ali et al., 2008). It is a woody climbing shrub and flowering plant commonly found in many part of the south-east Asia. It is known as "Kradaeng" in Thai, and a water decoction of the stems and/or roots has been used in Thai traditional medicine as a tonic to heal infertile women (Pathommapas et al., 2008–2009). Previous phytochemical studies have revealed the presence of various classes of bioactive constituents: calycopterin with anthelmintic properties (Ratnagiriswaran et al., 1934), biflvonoids, calycopterones exhibiting cytotoxicity against human cancer cell lines (Mayer, 1999; Wall et al., 1994) and calyflorenones (Mayer, 2004), and macrocyclic lactones, combretastatins, with cytotoxicity towards the small cell lung cancer cell line (Vongvanich et al., 2005). In order to search for active flavonoids from flowers of *G. floribunda*, further chemical investigation of this plant was conducted, which led to the isolation of two new flavonols, 4′-hydroxy-6,7,8,3′-tetramethoxyflavonol (**1**) and 4′-hydroxy-6,7,8-trimethoxyflavonol (**2**) and a known flavonol, pachypodol (**3**) (Ali et al., 2008; Sy and Brown, 1998) from EtOAc and MeOH extracts. We report herein the isolation, structural elucidation and biological evaluation including antiplasmodial, antimycobacterial and antioxidant activities, and cytotoxicity against three cancer cell lines of isolated compounds.

2. Results and discussion

Crude EtOAc and MeOH extracts were obtained from dried flowers of *G. floribunda*. Chromatographic separation of these extracts gave two new flavonols, 4′-hydroxy-6,7,8,3′-tetramethoxyflavonol (**1**) and 4′-hydroxy-6,7,8-trimethoxyflavonol (**2**), and a known pachypodol (**3**) (Fig. 1). Their structures were identified on the basis of spectroscopic methods, mass spectrometric techniques and comparison of the data with those reported in the literature.

Compound **1** was obtained as a pale yellow powder and had the molecular formula of $C_{19}H_{18}O_8$ (11 degrees of unsaturation) based on a molecular ion peak at *m*/*z* 397.0983 $[M+Na]^+$ in the HRESIMS. The IR spectrum showed the presence of hydroxy (3419 cm^{-1}) and unsaturated carbonyl (1657 cm^{-1}) functional groups. The UV spectrum displayed absorption bands at λ_{max} 226 and 350 nm. The ^1H and ^{13}C NMR (Table 1) and DEPT spectroscopic data showed 19 carbon signals attributable to four methoxy (δ_C 56.3, 56.5, 60.3 and 61.0), four methine aromatic (δ_C 90.5, 111.1, 114.8 and 122.7), and eleven nonprotonated carbons (δ_C 106.7, 122.5, 132.5, 138.8, 146.6, 148.5, 152.4, 152.9, 156.1, 158.9 and 179.0). These data allowed the formulation of a flavonoid skeleton which was supported by the above IR and UV spectroscopic data (Plazonić et al., 2009). The ^1H NMR spectrum showed a singlet signal at δ_H 6.49 (H-5), which showed HMBC correlations (Fig. 2) with C-4, C-6,

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http://dx.doi.org/10.1016/j.phytol.2015.02.004
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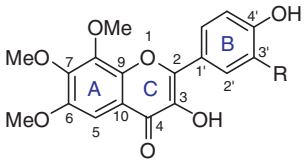

1 : R = OMe
2 : R = H

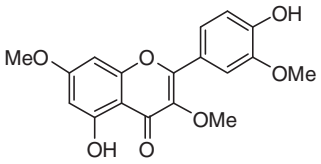

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Fig. 1. Structures of compounds **1**–**3**.

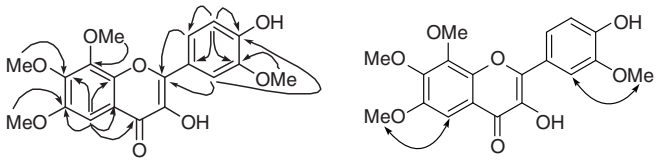

Fig. 2. Key HMBC (left) and NOESY (right) correlations of **1**.

C-7, C-9 and C-10 suggesting the presence of a 1,2,3,4,5-pentasubstitution on the benzene ring A. Ring A contained three methoxy groups at C-6, C-7 and C-8, confirmed by HMBC correlations from methoxy protons at δ_H 3.95 to C-6, methoxy protons at δ_H 3.91 to C-7 and methoxy protons at δ_H 3.85 to C-8 as well as NOESY correlation (Fig. 2) between H-5 and methoxy protons at C-6. The ^{1}H NMR spectrum of ring B exhibited proton resonances at δ_H 7.03 (d, J = 8.4 Hz, H-5′), 7.65 (dd, J = 8.4, 1.4 Hz, H-6′) and 7.69 (d, J = 1.4 Hz, H-2′) as well as the HMBC correlations (Fig. 2), consistent with a 1,3,4-trisubstituted benzene ring. A methoxy group appearing at δ_H 3.98 showed an HMBC correlation with C-3′ (δ_C 146.6) and a NOESY correlation with H-2′, confirming the location of the methoxy group at C-3′. The chemical shift of C-4′ appeared at δ_C 148.5 revealing oxygenation at this carbon. The connectivity of ring B to ring C was established by HMBC correlations from both H-2′ and H-6′ to the oxygenated carbon C-2. Thus, compound **1** was elucidated as a new flavonol, 4′-hydroxy-6,7,8,3′-tetramethoxyflavonol.

Compound **2** was obtained as a pale yellow powder and displayed a $[M+Na]^+$ ion at m/z 367.0769 in the HRESIMS, consistent with a molecular formula $C_{18}H_{16}O_7$ (11 degrees of unsaturation). The UV and IR spectra were similar to those of **1**. The ^{1}H and ^{13}C NMR spectral data (Table 1), and a DEPT experiment, revealed that **2** had a flavonol structure with 18 carbon signals indicating one less methoxy carbon than **1**. The ^{1}H NMR data for **2** was compatible with those of **1**, except for the proton spin patterns of ring B. Ring B of **2** showed two overlapped two-proton doublets at δ_H 7.99 (J = 8.8 Hz, H-2′/6′) and 6.93 (J = 8.8 Hz, H-3′/5′) that were consistent with a 1,4-disubstitution benzene ring. This was confirmed by HMBC correlations from H-2′/6′ to C-2, C-4′, C-6′/2′ and from H-3′/5′ to C-1′, C-4′, C-5′/3′ as well as NOESY correlation between H-2′/6′ and H-3′/5. The chemical shifts of C-4′ (δ_C 160.9) helped establish the attachment of a hydroxy group at this position. Therefore, compound **2** was defined as a new 4′-hydroxy-6,7,8,-trimethoxyflavonol.

Compound **3** was elucidated as pachypodol by comparing its spectroscopic data with those reported in the literature (Ali et al., 2008; Sy and Brown, 1998). All isolated compounds were evaluated for their biological activities. Flavonol **1** displayed moderate cytotoxicity towards the KB cell line with IC_{50} value of 8.99 ± 2.00 μg/ml, while compounds **2** and **3** showed no cytotoxicity. The results indicate that the methoxy at C-3′ and the hydroxy at C-3 of **1** play an important role in enhancing cytotoxicity against the KB cancer cell line, compared to **2** and **3**, respectively. However these compounds were non-cytotoxicity against the MCF7 cell line and were inactive for antimycobacterial, antiplasmodial and antioxidant tests.

3. Experimental

3.1. General experimental procedures

UV spectra were recorded using an Agilent 8453 UV-visible spectrophotometer. IR spectra were obtained using a Bruker Tenser 27 spectrophotometer. NMR spectra were recorded in $CDCl_3$ and CD_3OD, as solvents on a Varian Mercury Plus 400 spectrometer; the internal standards were referenced from the residue of those solvents. HRESITOFMS data were obtained using a Micromass Q-TOF-2 spectrometer. Column chromatography (CC) was carried out on MERCK silica gel 60 (230–400 mesh) and Sephadex LH-20. Preparative TLC was carried out on silica gel

Table 1
^{1}H and ^{13}C NMR (400 and 100 MHz) and HMBC data of compounds **1** and **2** (δ in ppm, J in Hz).

Position	**1**[a]			**2**[b]		
	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC
2	156.1			157.9		
3	152.9			152.8		
4	179.0			179.6		
5	90.5	6.49 s	4, 6, 7, 9, 10	91.4	6.61 s	4, 6, 7, 9, 10
6	158.9			159.6		
7	132.5			132.7		
8	138.8			139.0		
9	152.4			153.2		
10	106.7			106.9		
1′	122.5			121.9		
2′	111.1	7.69 d (1.4)	2, 1′, 3′, 4′, 6′	130.9	7.99 d (8.8)	2, 4′, 6′
3′	146.6			116.2	6.93 d (8.8)	1′, 4′, 5′
4′	148.5			160.9		
5′	114.8	7.03 d (8.4)	1′, 3′, 4′, 6′	116.2	6.93 d (8.8)	1′, 3′, 4′
6′	122.7	7.65 dd (8.4, 1.4)	2, 2′, 4′	130.9	7.99 d (8.8)	2, 2′, 4′
6-OMe	56.5	3.95 s	6	56.8	3.95 s	6
7-OMe	61.0	3.91 s	7	61.2	3.86 s	7
8-OMe	60.3	3.85 s	8	60.5	3.77 s	8
3′-OMe	56.3	3.98 s	3′, 4′			

[a] Recorded in $CDCl_3$.
[b] Recorded in a mixture of CD_3OD and $CDCl_3$.