Bioorganic & Medicinal Chemistry Letters 26 (2016) 3425–3428

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Anti-inflammatory polyphenol constituents derived from *Cissus pteroclada Hayata*

Li Yi-jie [†], Xu Cheng-ting [†], Lin Dan-dan, Qin Jiang-ke [*], Ye Gao-jie, Deng Qing-hua

Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, School of Chemistry and Pharmaceutical Sciences, Guangxi Normal University, Guilin 541004, China

ARTICLE INFO

Article history:
Received 30 January 2016
Revised 16 June 2016
Accepted 20 June 2016
Available online 21 June 2016

Keywords:
Cissus pteroclada Hayata
Bergenin
Anti-inflammatory
NF-κB

ABSTRACT

A new bergenin derivative, bergenin-11-*O*-α-D-galactopyranoside (compound **1**), together with seven known polyphenolic compounds, were isolated from the stem of *Cissus pteroclada Hayata*. The structures of the 8 compounds were elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Moreover, the in vitro anti-inflammatory effects of compounds (**1**–**8**) in LPS-stimulated murine macrophage RAW 264.7 cells were also investigated. Our results revealed that compound **1** inhibited the production of pro-inflammatory mediators NO and PGE_2 and the expression of NF-κB, TNF-α, IL-1β, iNOS and COX-2.

Cissus pteroclada Hayata, a genus of perennial vine plant belonging to the family *Vitaceae*, is widely distributed in southern China, Indo-China peninsula, and Southeast Asia. *Cissus pteroclada Hayata* is popularly known as 'Sifang-teng' in Chinese, commonly named after its particularly ribbed stem. Traditional Chinese medicine literature recorded that the stem of *Cissus pteroclada Hayata* could activate circulation to remove blood stasis. Extracts from the *Cissus pteroclada Hayata* had been used for treating various aseptic inflammation, such as rheumatism, cramping, contusions, and bruising,[1] which may cause the production of inflammatory mediators and cytokines.

Inflammation is a central immune response of many pathophysiological conditions such as tissue injury, and host defenses against invading microbes.[2] As an important feature of the innate immune system, beneficial acute inflammation can progress into a chronic state, which if remains unresolved, is the pathological basis of various diseases.[3]

In the present work, we isolated, characterized the structure, and examined the anti-inflammatory potential of a new compound, bergenin-11-*O*-α-D-galactopyranoside (**1**), and seven other known polyphenolic compounds: bergenin (**2**), norbergenin (**3**), 11-*O*-galloylbergenin (**4**), purpurogallin (**5**), resveratrol (**6**), myricetin (**7**), and gallic acid (**8**) (Fig. 1) from the water fraction of 60% EtOH extract from the aerial portion of *Cissus pteroclada Hayata*. This study focused on the structural elucidation of bergenin-11-*O*-α-D-galactopyranoside and the anti-inflammatory activities of the bergenin derivatives (**1**–**4**).

The water fraction of *Cissus pteroclada Hayata* extract was separated by repeated chromatography over MCI gel, sephadex LH-20 and semi-preparative HPLC, that yielded 8 polyphenol compounds.

Compound **1** was obtained as a light brown amorphous powder. Its molecular formula was determined as $C_{20}H_{26}O_{14}$ from the $[M-H]^-$ peak at *m*/*z* 507.1363 in the HR-ESI-MS. The ^{13}C NMR spectrum (Table 1) showed 20 signals among which 14 were assigned to the aglycone of bergenin, the remaining six signals, including an anomeric carbon at δ 99.1, were indicative of the presence of a sugar unit. The sugar composition of **1** was determined by pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP)-HPLC of the acid hydrolysates of compound **1**.[4] Subsequently, the analysis showed that D-galactose was the sugar unit of **1**. The ^1H NMR spectrum of **1** showed two anomeric protons' signals at 4.74 (d, *J* = 3.6 Hz) and 4.99 (d, *J* = 10.5 Hz), which belonged to D-galactose and aglycone moiety (bergenin is a C-glycosides that contains D-glucose), respectively. Signals at δ 4.74 (d, *J* = 3.6 Hz) of ^1H NMR and δ 99.1 of ^{13}C NMR indicated that the configuration of D-Gal*p* was α type. Additionally, ring protons of each monosaccharide were assigned starting from the anomeric protons using TOCSY and H–H COSY spectra. The correlation observed in the HMBC spectrum between the proton at δ 4.74 (H-1′) and the carbon at δ 67.7 (C-11) indicated that galactose was attached to C-11 of bergenin (Fig. 2).

* Corresponding author. Tel.: +86 18807730696; fax: +86 0773 2120958.
E-mail address: jiangkeq@sina.com (J.-k. Qin).
† These authors share the first authorship.

http://dx.doi.org/10.1016/j.bmcl.2016.06.054
0960-894X/

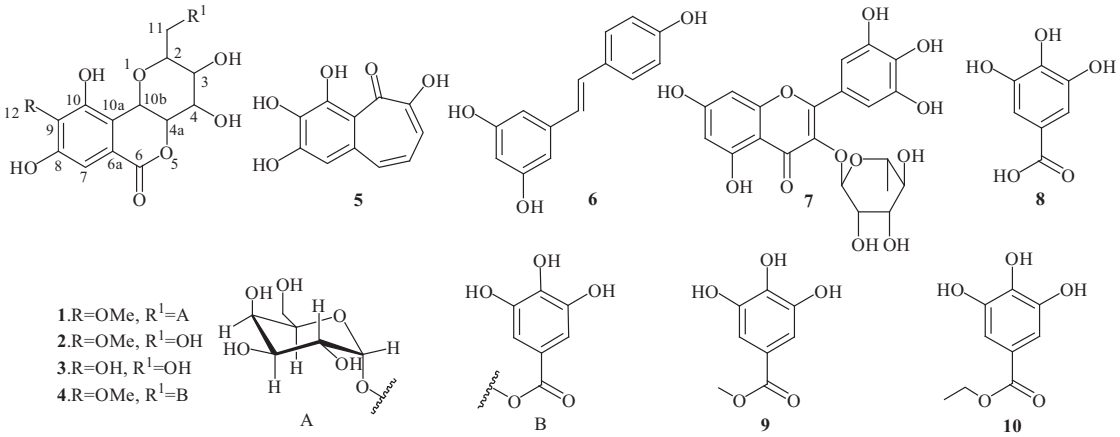

Figure 1. Structures of compounds **1**–**8** isolated from *Cissus pteroclada Hayata.*

Table 1
^{1}H and ^{13}C NMR data of compounds 1 (DMSO-d_6, 600, 150 MHz)[a]

Position	^{13}C NMR	^{1}H NMR	Position	^{13}C NMR	^{1}H NMR
2	79.6	4.02 (t, *J* = 9.9 Hz)	1′	99.1	4.74 (d, *J* = 3.6 Hz)
3	73.6	3.72–3.62(m)	2′	71.9	3.26–3.22(m)
4	70.7	3.26–3.22(m)	3′	73.1	3.51–3.43(m)
4a	79.5	3.83–3.76(m)	4′	72.9	3.51–3.43(m)
6	163.5		5′	70.3	3.11(t, *J* = 9.2 Hz)
6a	118.1		6′	60.9	3.72–3.62(m), 3.51–3.43(m)
7	109.4	7.01	3-OH		5.59
8	140.6		4-OH		5.76
9	148.1		8-OH		8.32
10	150.9		10-OH		9.83
10a	115.9		2′-OH		4.79
10b	71.9	4.99 (d, *J* = 10.5 Hz)	3′-OH		4.88
11	67.7	3.83–3.76(m), 3.72–3.62(m)	4′-OH		4.93
12	59.8	3.79(s)	6′-OH		4.51

[a] Full assignments of the protons and carbons were accomplished by analysis of H–H COSY, TOCSY, HSQC and HMBC spectra, chemical shifts are in ppm and coupling pattern and coupling constants (*J* in Hz) are in parentheses.

Based on the above evidence, the structure of compound **1** was established as bergenin-11-*O*-α-D-galactopyranoside, a bergenin derivative that has not been previously identified.

The cytotoxic effects of the isolated compounds **1**–**8** were evaluated using MTT assay. None of the compounds affected the cell viability of RAW 264.7 macrophage cells in either the absence or presence of LPS, even following 24 h stimulation at 50 μmol/L (data not shown).

Because of their similar structure, we evaluated the in vitro anti-inflammatory activity of compounds **1**–**4** and further examined the preliminary structure–functional activity relationship of the bergenin derivatives. The anti-inflammatory activity of compounds **5**–**8** are shown in Supplementary data. Nuclear factor-κB (NF-κB), an important transcription factor in the inflammatory response and a key regulator of multiple pro-inflammatory signaling pathways, is sequestered in the cytoplasm as an inactive precursor, and complexed with an inhibitor κB (IκB) protein.[5] Inhibition of NF-κB signaling becomes a potential target for either the treatment or prevention of inflammation. Therefore, we examined the effect of the four bergenin derivatives on NF-κB signaling in LPS-stimulated macrophages.

As shown in Figure 3A, treatment with LPS increased the expression of NF-κB, while compounds **1**–**4** inhibited the NF-κB signaling pathway in a concentration-dependent manner. NF-κB is known to play a critical role in the regulation of cell survival genes and induction of inflammatory enzymes and cytokine expression.[6,7] Moreover, blocking the NF-κB transcriptional activity in the RAW 264.7 nucleus can suppress the expression of iNOS and pro-inflammatory cytokines such as TNF-α, IL-1β, PGE_2, and NO.[8] Hence, we investigated the effects of compounds **1**–**4** on these

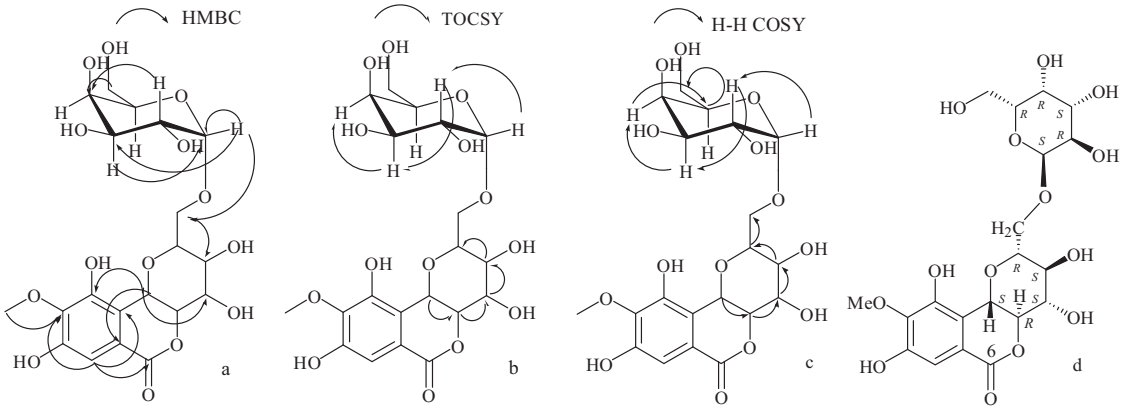

Figure 2. Key HMBC (a), TOCSY (b), H–H COSY (c) correlations and the absolute configuration (d) of **1**.