Phytochemistry Letters 22 (2017) 255–260

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Investigation of chemical constituents from *Spiraea prunifolia* var. *simpliciflora* and their biological activities

Chung Sub Kim[a,b], Joonseok Oh[a,b], Won Se Suh[c], Sung Wan Jang[c], Lalita Subedi[d,e], Sun Yeou Kim[d,e], Sang Un Choi[f], Kang Ro Lee[c,*]

[a] *Department of Chemistry, Yale University, New Haven, CT 06520, United States*
[b] *Chemical Biology Institute, Yale University, West Haven, CT 06516, United States*
[c] *Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea*
[d] *Gachon Institute of Pharmaceutical Science, Gachon University, Incheon 21936, Republic of Korea*
[e] *College of Pharmacy, Gachon University, #191, Hambakmoero, Yeonsu-gu, Incheon 21936, Republic of Korea*
[f] *Korea Research Institute of Chemical Technology, Daejeon 34114, Republic of Korea*

ARTICLE INFO

Keywords:
Spiraea prunifolia var. *simpliciflora*
Phenolic glycosides
GIAO NMR chemical shift calculations
Cytotoxicity
Anti-inflammation
Neuroprotection

ABSTRACT

Quantum mechanics (QM)-based calculations for elucidating full structures of natural compounds are growing in importance and reliability. Two new phenolic glycosides (**1** and **2**) and 11 known compounds were isolated from the twigs of *Spiraea prunifolia* var. *simpliciflora*. The chemical structures of the new compounds (**1** and **2**) were initially established through different NMR techniques (^{1}H and ^{13}C NMR, COSY, HSQC, and HMBC), HRMS data analysis, and chemical hydrolysis. These structure assignments were further verified by QM-based NMR chemical shift calculations. All of the purified compounds (**1–13**) were evaluated for their cytotoxicity against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and BT549). Those phytochemicals were also evaluated for both anti-inflammatory activity through the measurement of nitric oxide (NO) production levels in lipopolysaccharide (LPS)-stimulated murine microglia BV-2 cell lines and neuroprotective effects via induction of nerve growth factor (NGF) in C6 glioma cells.

1. Introduction

Spiraea prunifolia var. *simpliciflora* Nakai (Rosaceae), commonly called “bridal wreath,” is a deciduous shrub widely distributed in Korea. The roots of this plant have been used as Korean traditional medicine to treat malaria, fever, and emetic conditions, and its young leaves have been consumed as a salad (Oh et al., 2001). Previous investigation reported that the extracts of *S. prunifolia* var. *simpliciflora* show anti-inflammatory and antipyretic activities (So et al., 1999), and possess terpenoids, flavonoids, and phenolic compounds (Oh et al., 2001; Park et al., 2013; Yean et al., 2014; Youn and Chung, 1987). On the contrary to numerous studies on the roots, there have only been a few investigations of bioactive phytochemicals of the twigs.

Quantum mechanics (QM)-based predictions, for the identification and/or verification of full structures of natural product-originating compounds, are growing in significance and reliability (Lodewyk et al., 2011). These approaches are of particular use in verifying structural assignments, considering erroneous assignment due to a high degree of molecular complexity, human errors, and NMR correlation ambiguities (Grimblat et al., 2015). Despite the utility of synthesis and X-ray crystallography in structural validation and corrections (Nicolaou et al., 2000), such methods can be impractical in terms of costs and unfavorable molecular properties. Alternatively, QM-based calculations of NMR properties, using gauge-including atomic orbitals (GIAO) NMR chemical shift calculations, have been spotlighted as effective protocols for the verification of chemical structure assignments made based upon conventional methods (Grimblat et al., 2015; Kim et al., 2017a; Lodewyk et al., 2011).

In a continuing search for bioactive scaffolds from Korean medicinal plants, we recently reported cytotoxic phenolic compounds from *S. prunifolia* var. *simpliciflora* (Jang et al., 2015). In this study, we isolated and identified two new phenolic compounds, *trans*- and *cis*-pruspirides (**1** and **2**), along with 11 known compounds from this plant (Fig. 1). The chemical structures of purified compounds were determined by 1D and 2D NMR (^{1}H and ^{13}C NMR, COSY, HSQC, and HMBC), HRMS, and LC/MS data analysis. Notably, the initial structural assignment of compound **1** was verified utilizing GIAO-based NMR chemical shift calculations. These phytochemicals (**1–13**) were also assessed for their cytotoxic, anti-inflammatory, and neuroprotective activities employing relevant bioassay approaches.

* Corresponding author.
E-mail address: krlee@skku.edu (K.R. Lee).

http://dx.doi.org/10.1016/j.phytol.2017.09.014
Received 3 July 2017; Received in revised form 11 September 2017; Accepted 22 September 2017

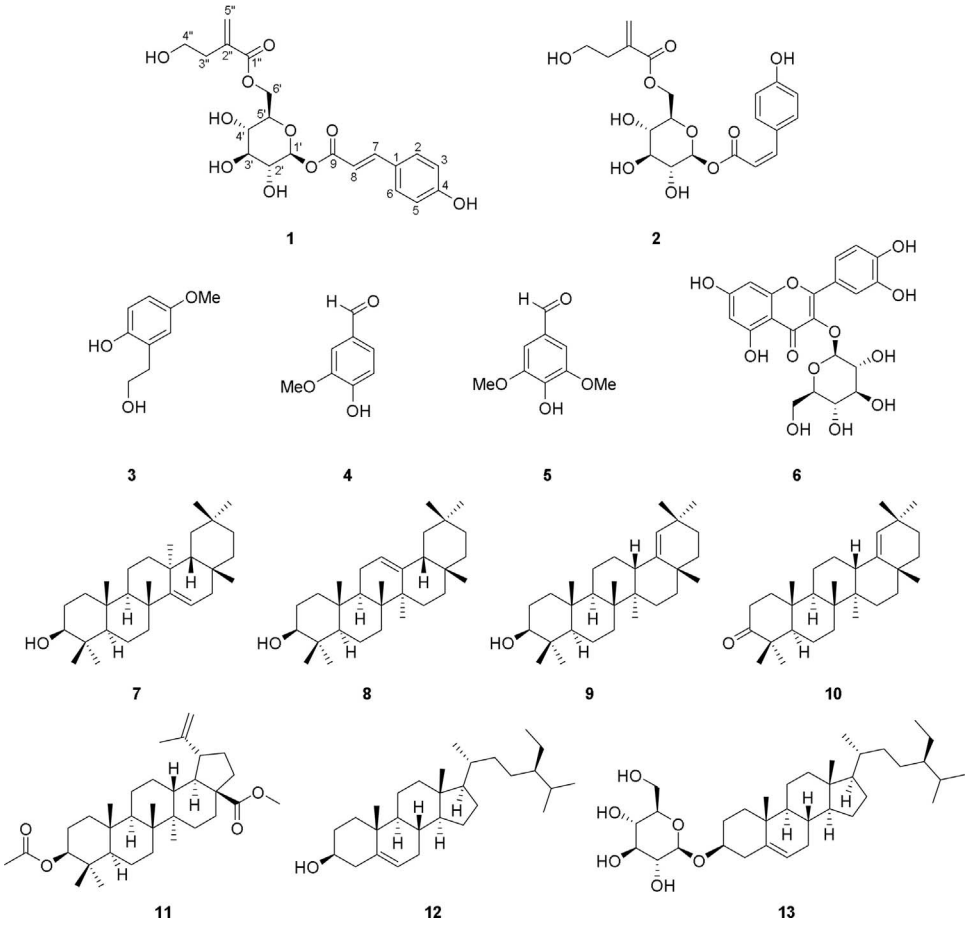

Fig. 1. Chemical structures of compounds **1–13**.

2. Results and discussion

Compound **1** was purified as a colorless gum and its molecular formula was confirmed as $C_{20}H_{24}O_{10}$ based on the protonated HRFABMS molecular ion $[M + H]^+$ at *m/z* 425.1450 (calcd for $C_{20}H_{25}O_{10,}$ 425.1448). The ^{1}H NMR spectrum of **1** exhibited the presence of a *trans*-coumaroyl group [(δ_H 7.76 (1H, d, J = 15.9 Hz), 7.51 (2H, d, J = 8.7 Hz), 6.84 (2H, d, J = 8.7 Hz), and 6.40 (1H, d, J = 15.9 Hz)], a glucopyranosyl moiety [δ_H 5.50 (1H, d, J = 8.1 Hz), 4.51 (1H, dd, J = 12.1, 2.1 Hz), 4.31 (1H, dd, J = 12.1, 5.7 Hz), 3.69 (1H, ddd, J = 9.7, 5.7, 2.1), 3.50 (1H, t, J = 9.0 Hz), 3.46 (1H, dd, J = 9.1, 8.1 Hz), and 3.43 (1H, dd, J = 9.7, 9.0 Hz)], two olefinic protons [δ_H 6.27 (1H, d, J = 1.5 Hz) and 5.72 (1H, dd, J = 1.5, 1.1 Hz)], and two methylenes [δ_H 3.69 (2H, t, J = 6.6 Hz), 2.56 (2H, td, J = 6.6, 1.1 Hz)]. The ^{13}C NMR spectrum of **1** showed 20 resonances characteristic for an ester-like carbonyl (δ_C 168.4), an 1,4-disubstituted aromatic ring [δ_C 161.8, 131.5 (×2), 127.1 (×2), and 117.0], two double bonds (δ_C 148.1, 138.8, 127.7, and 114.6), a glucopyranosyl group (δ_C 95.9, 78.2, 76.5, 74.2, 71.3, and 64.9), an oxygenated carbon (δ_C 61.7), and a methylene carbon (δ_C 36.4). These spectroscopic data (Table 1) were similar to those of 1-caffeoyl-6-tuliposide A (Park et al., 2013), except for the presence of resonances for an 1,4-disubstituted aromatic ring in **1** (see above) instead of those for an 1,2,4-trisubstituted aromatic ring [δ_C 148.2, 145.4, 126.3, 121.5, 115.1, and 113.8; δ_H 7.06 (1H, d, J = 1.8 Hz), 6.97 (1H, dd, J = 8.4, 1.8 Hz), and 6.78 (1H, d, J = 8.4 Hz)] in 1-caffeoyl-6-tuliposide A.

The 2D structure of **1** was established by analyses of 2D NMR data including COSY, HSQC, and HMBC, and the connectivities among the *trans*-coumaroyl group, glucopyranose, and 4-hydroxy-2-methylenebutanoyl moiety were established via the HMBC cross peaks of H-1′/C-9 and H-6′/C-1″ (Fig. 2). The monosaccharide unit was assumed to be β-glucopyranose by the associated chemical shifts of the ^{1}H and ^{13}C resonances, which was confirmed by the relatively large coupling constants between H-1′/H-2′ (8.1 Hz), H-2′/H-3′ (9.1 Hz), H-3′/H-4′ (9.0 Hz), and H-4′/H-5′ (9.7 Hz). To establish the absolute configuration of the glucopyranosyl motif, compound **1** was hydrolyzed and

Table 1
^{1}H [ppm, mult., (J in Hz)] and ^{13}C NMR spectroscopic data of compounds **1** and **2** in methanol-d_4 and calculated ^{13}C NMR data of **1**.

position	**1**			**2**	
	δ_H	δ_C (exp.)	δ_C (cal.)	δ_H	δ_C
1		127.1	123.4		127.5
2/6	7.51, d (8.7)	131.5	129.1	7.72, d (8.7)	134.4
3/5	6.84, d (8.7)	117.0	111.4	6.77, d (8.7)	116.0
4		161.8	156.8		160.7
7	7.76, d (15.9)	148.1	144.3	6.97, d (12.8)	147.4
8	6.40, d (15.9)	114.6	108.0	5.84, d (12.8)	115.6
9		167.9	165.5		166.6
1′	5.50, d (8.1)	95.9	99.9	5.55, d (8.2)	95.7
2′	3.46, dd (9.1, 8.1)	74.2	77.1	3.39, dd (9.1, 8.2)	74.1
3′	3.50, t (9.0)	78.2	79.9	3.48, t (9.1)	78.3
4′	3.43, dd (9.7, 9.0)	71.3	71.2	3.41, dd (9.7, 9.1)	71.3
5′	3.69, ddd (9.7, 5.7, 2.1)	76.5	76.9	3.67, ddd (9.7, 5.8, 2.1)	76.3
6′a	4.51, dd (12.1, 2.1)	64.9	66.9	4.52, dd (12.0, 2.1)	64.9
6′b	4.31, dd (12.1, 5.7)			4.31, dd (12.0, 5.8)	
1″		168.4	166.0		169.1
2″		138.8	140.4		138.8
3″	2.56, td (6.6, 1.1)	36.4	40.6	2.57, td (6.7, 1.1)	36.5
4″	3.69, t (6.6)	61.7	66.6	3.69, t (6.7)	61.7
5″a	6.27, d (1.5)	127.7	127.7	6.28, d (1.5)	127.7
5″b	5.72, dd (1.5, 1.1)			5.72, dd (1.5, 1.1)	