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Two new phenolic compounds from the white flower of *Impatiens balsamina*

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ABSTRACT

During the course of our continuing search for biologically active compounds from Korean medicinal sources, we investigated the white flower of *Impatiens balsamina*. From the MeOH extract, two new phenolic compounds (**1**–**2**) containing a nitrile group and eleven known phenolic compounds (**3**–**13**) were isolated. The chemical structures of new compounds (**1**–**2**) were determined through NMR, HRMS, and CD data. We tested the isolated compounds (**1**–**13**) for their cytotoxic activities by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) *in vitro* using the sulforhodamine B (SRB) assay. We also investigated their neuroprotective activity by determining their effects on nerve growth factor (NGF) secretion in C6 cells, and anti-neuroinflammatory activity by measuring nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated BV-2 cells.

1. Introduction

Impatiens balsamina L., also known as Garden balsam or Rose balsam, is an annual herbaceous plant of the Balsamineaceae family grown as an ornamental garden plant. *I. balsamina*has been used in traditional Chinese medicine, and in some areas of China is used as a vegetable or anticancer herb (Su et al., 2012). The aerial parts of *I. balsamina*have been used for the treatment of articular rheumatism, bruises, and beriberi (Imam et al., 2012), whereas the seeds have been used to treat lumps, puerperal pain, and cancer (Lei et al., 2010). The flower of this plant also has been used to treat dermititis, lumbago, neuralgia, burns, and scalds (Imam et al., 2012). Previous studies have suggested that flavonoids and naphthoquinones from the flower of *I. balsamina*are associated with antipruritic, antianaphylactic, and anti-inflammatory properties (Fukumoto et al., 1996; Ishiguro and Oku, 1997; Oku and Ishiguro, 2002). As part of the searching for bioactive constituents of Korean medicinal plant sources, we investigated the active constituents of *I. balsamina*. In the present study, we report the isolation and structural elucidation of two new phenolic compounds (**1** and **2**) and eleven known compounds (**3**–**13**) (Fig. 1), from the white flower of*I. balsamina*. We also determined their anticancer effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) *in vitro* using the sulforhodamine B (SRB) assay, their neuroprotective activity by determining their effects on nerve growth factor (NGF) secretion in C6 cells, and their anti-neuroinflammatory activity by measuring nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated BV-2 cells.

2. Results and discussion

Compound **1** was isolated as a colorless gum. The molecular formula was determined as $C_{10}H_7NO_3$ from the pseudomolecular ion peak $[M+H]^+$ at *m/z*190.0557 (calcd for $C_{10}H_8NO_3$, 190.0504) in the HRESIMS. The IR spectrum of **1** displayed absorption bands at 3433 (hydroxyl) and 2243 (nitrile) cm^{-1}. The ^1H NMR spectrum of **1** exhibited the presence of a 1,2-disubstituted aromatic ring [δ_H 7.57 (1H, d, *J* = 7.5 Hz), 7.35 (1H, t, *J* = 7.5 Hz), 7.13 (1H, t, *J* = 7.5 Hz), and 6.96 (1H, d, *J* = 7.5 Hz)] and a methylene group [δ_H 3.10 (1H, d, *J* = 16.6 Hz) and 2.90 (1H, d, *J* = 16.6 Hz)]. The ^{13}C NMR spectrum of **1** showed 10 carbon signals including a carbonyl carbon (δ_C 179.4), six

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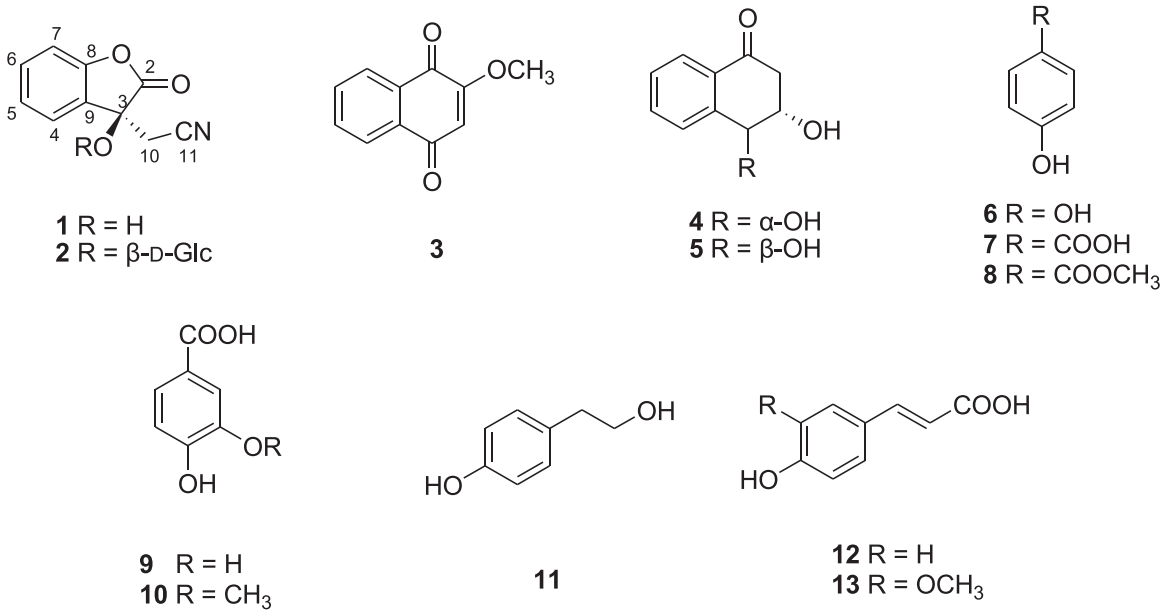

Fig. 1. Chemical structures of compounds **1**–**13**.

aromatic carbons [δ_C 142.9, 131.7 (×2), 125.5, 124.1, and 111.7], a nitril carbon (δ_C 117.4), an oxygenated carbon (δ_C 74.1), and an alkane carbon (δ_C 27.4). These NMR data of **1** (Table 1) were very similar to those of (−)-(*S*)-2-(4-hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile (Zhang et al., 2014), except for the presence of four aromatic proton signals and an oxygenated carbon signal in **1** instead of three aromatic proton signals [δ_H 7.09 (1H, dd, *J* = 8.4, 7.8 Hz), 6.50 (1H, d, *J* = 8.4 Hz), and 6.44 (1H, d, *J* = 7.8 Hz)] and a methine carbon (δ_C 42.3) signal in (−)-(*S*)-2-(4-hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile. The ^{1}H–^{1}H COSY correlations from H-4 to H-7 and the HMBC cross-peaks of H-5/C-9, H-6/C-8, and H-7/C-9 confirmed the substructure of unit A (Fig. 2). The HMBC cross-peaks of H-10/C-2, C-3, and C-11 suggested two possible substructures, unit B1 and B2 (Fig. 2). However, in the case of unit B2, the chemical shift of C-10 was expected to be at δ_C 40 from the inspection of related compounds (Xie and Stahl, 2015; Yuan et al., 2009), which was quite different from that of the experimental value (δ_C 27.4). Therefore, the only possible substructure was unit B1. Direct linkage between C-3 and C-9 was confirmed through the HMBC correlations of H-4/C-3 and H-10/C-9, and the relatively downfield shifted chemical shift of C-8 (δ_C 142.9) corroborated that C-8 was connected to the oxygen atom adjacent to C-2. The absolute configuration at C-3 was determined through the CD spectrum of **1** (Fig. 3). The strong negative Cotton effect at 236 nm in **1** was opposite to that of (−)-(*S*)-2-(4-hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile (Zhang et al., 2014), which confirmed the absolute configuration at C-3 in **1** to be the *S*form. Therefore, the structure of **1** was established as (*S*)-2-(3-hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile, named balsamitril.

Table 1
1H (700 MHz) and ^{13}C (175 MHz) NMR data of compounds **1** and **2** in CD_3OD (δin ppm, *J*values in parentheses).

Pos.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2		179.4		176.5
3		74.1		79.8
4	7.57, d (7.5)	125.5	7.72, d (7.5)	127.9
5	7.13, t (7.5)	124.1	7.12, t (7.5)	124.0
6	7.35, t (7.5)	131.7	7.37, t (7.5)	132.5
7	6.96, d (7.5)	111.7	6.96, d (7.5)	112.0
8		142.9		143.8
9		131.7		126.4
10a	3.10, d (16.6)	27.4	3.33, d (16.6)	27.0
10b	2.90, d (16.6)		3.14, d (16.6)	
11		117.4		117.0
1′			4.24, d (7.5)	101.3
2′			3.24, overlap	74.9
3′			3.21, overlap	78.0
4′			3.26, overalp	71.4
5′			2.97, ddd (9.5, 5.7, 2.5)	78.1
6′a			3.71, dd (11.9, 2.5)	62.7
6′b			3.57, dd (11.9, 5.7)	

Compound **2** was obtained as a colorless gum. The HRESIMS data (*m*/*z*352.1049 [M + H]$^+$, calcd for $C_{16}H_{18}NO_8$, 352.1032) of **2** indicated that this molecule possessed the molecular formula $C_{16}H_{17}NO_8$. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**, except for the presence of a D-glucosyl unit [δ_H 4.24 (1H, d, *J* = 7.5 Hz), 3.71 (1H, dd, *J* = 11.9, 2.5 Hz), 3.57 (1H, dd, *J* = 11.9, 5.7 Hz), 3.26 (1H, overlap), 3.24 (1H, overlap), 3.21 (1H, overlap), and 2.97 (1H, ddd, *J* = 9.5, 5.7, 2.5 Hz); δ_C 101.3, 78.1, 78.0, 74.9, 71.4, and 62.7]. The location of the glucosyl unit was confirmed at C-3 through the HMBC cross-peak of H-1′/C-3 (Fig. 2). Enzymatic hydrolysis of **2** yielded the aglycone **2a**, which was identified as **1** by comparing the ^{1}H NMR and ESIMS data with those of **1**. Identification of D-glucose was performed through the acid hydrolysis of **2**, followed by co-TLC confirmation with authentic sample, specific optical rotation {([α]^{25}D + 59.8) (*c*0.05, H_2O)}, and GC/MS analysis. The absolute configuration at C-3 in **2** was determined to be *S*by the same method as **1** (see Supplementary data). Thus, the structure of **2** was elucidated as (*S*)-2-(3-hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile-3-*O*-β-D-glucoside, named balsamitril -3-*O*-β-D-glucoside.

The known compounds were identified as 2-methoxy-1,4-naphthoquinone (**3**) (Ding et al., 2008), (3*S*,4*R*)-3,4-dihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (**4**) (Husain et al., 2012), *trans*-(3*S*,4*S*)-3,4-dihydroxy-1-tetralone (**5**) (Husain et al., 2014), hydroquinone (**6**) (Bernini et al., 2005), *p*-hydroxybenzoic acid (**7**) (O'Connor et al., 1987), *p*-hydroxybenzoic acid methyl ester (**8**) (Kwak et al., 2009), protocatechuic acid (**9**) (Kwak et al., 2009), vanilic acid (**10**) (Prachayasittikul et al., 2009), tyrosol (**11**) (Takaya et al., 2007), *trans*-*p*-coumaric acid (**12**) (Salum et al., 2010), and *trans*-ferulic acid (**13**) (Prachayasittikul et al., 2009) by comparison with NMR and MS data in the literature.