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A New Antioxidant Monoterpene Glycoside, α-Benzoyloxypaeoniflorin from *Paeonia suffruticosa*

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α-Benzoyloxypaeoniflorin (**1**), a new antioxidant monoterpene α-glycoside anomer was isolated from *Paeonia suffruticosa* along with known compounds, β-benzoyloxypaeoniflorin (**2**), paeonolide, paeoniflorin and mudanpioside H. The structure of **1** has been determined by comparing spectral data with those of β-benzoyloxypaeoniflorin(**2**). Compound **1** exhibited moderately potent radical scavenging activity on DPPH radical.

Key words: *Paeonia suffruticosa*, Antioxidative activity, Anomer, α-Benzoyloxypaeoniflorin

INTRODUCTION

The root cortex of *Paeonia suffruticosa* Andrews (Korean name: mokdanpi) (Paeoniaceae) is one of the most important crude drugs having analgestic, sedative and antiinflammatory activities, and used as a remedy for cardiovascular extravasated blood and female genital diseases in Oriental traditional medicine (Morita *et al.*, 1991). Through the chemical component studies on Paeonia Cortex, the presence of various monoterpene glycosides has so far been reported (Shibata *et al.*, 1996; Kitakawa *et al.*, 1979; Murakami *et al.*, 1996; Ding *et al.*, 1999). During the course of searching for bioactive compounds from the Korean herbal medicines, we found antioxidative activity in the methanolic extract of the medicinal plant. By monitoring radical scanvenging effect on α,α-diphenylpicryhydrazyl (DPPH) radical, we isolated a new monoterpene glycoside anomer, α-benzoyloxypaeoniflorin (**1**) along with known compounds (Kitakawa *et al.*, 1979; Kariyone *et al.*, 1956; Shibata *et al.*, 1996; Ding *et al.*, 1999), β-benzoyloxypaeoniflorin (**2**), paeonolide, paeoniflorin and mudanpioside H.

MATERIALS AND METHODS

General experimental procedures

The UV spectrum was recorded with a HP8453 UV/VIS spectrophotometer. IR spectrum was obtained on a Perkin-Elmer model 1750 FT-IR spectrophotometer. MS data were measured on JEOL JMX-SX 102 mass spectrometer. Exact mass measurements were obtained on a JEOL AX-505H mass spectrometer at high resolution using NBA as matrix. ^{1}H-NMR and ^{13}C NMR spectra were recorded in CD_3OD at 25°C on a Bruker ARX-400 NMR spectrometer, using either a standard 5 mm direct or indirect probe. Chemical shifts (δ) are given relative to the solvent peaks at δ 3.32 (for ^{1}H spectra) and δ 49.5 (for ^{13}C spectra) as the internal standards. Proton-proton correlation spectra (COSY) were recorded using the standard pulse sequence. Proton-carbon correlations were obtained using indirect detection methods, HMQC (with $^1J_{XH}$=140 Hz) and HMBC. The HMBC spectra were optimized for a $^nJ_{CH}$ of 8.0 Hz. NOESY experiments were performed with mixing times of 0.7, 0.5 and 0.3 sec.

Plant materials

The root cortex of *Paeonia suffruticosa* was given from Hankook Sinyak Pharmaceutical Co., LTD, Taejon, Korea in September, 1988. A specimen of the plant (KM-96055) has been deposited at the Laboratory of Natural Products Chemistry, Hanbat National University.

DPPH antioxidative assay

Radical scavenging activity was determined using the modified Uchiyama's method (Uchiyama *et al.*, 1968) as

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follows. To the 1.0×10^{-4} M ethanol solution of DPPH, a testing sample dissolved in EtOH was added, and the final sample concentration was 20 mM. Then the solution with a testing sample was shaked vigorously and kept in the dark for 30 min at 25°C. The absorbance of a sample was measured on a spectrophotometer (Hewlett Packard HP8453) at 517 nm against a blank of EtOH without DPPH. A blank sample of DPPH without a testing sample was also measured under the same condition. All tests were run in triplicate. Inhibition percentage was calculated according to the formula (Yen *et al.*, 1994):

$$\% \text{ Inhibition} = [(A_B - A_A)/A_B]*100$$

where A_B is the absorbance of the blank sample and A_A is the absorbance of the tested sample after 15 min.

Extraction and isolation

The dried root cortex (1.0 kg) were extracted with 90% MeOH at room temperature for a day. The extract was concentrated under reduced pressure, and the residue was suspended in H_2O and partitioned with $CHCl_3$. The aqueous phase was further partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was subjected to ODS flash chromatography with aqueous MeOH (0, 20, 40, 60, 90, 100% MeOH). The 60% MeOH fraction was further fractionated by gel filtration on Sephadex LH-20 with MeOH/$CHCl_3$ (9:1) to afford an antioxidative fraction. This was resubjected to silica column chromatography

Table I. ^1H and ^{13}C NMR spectral data of compounds **1** and **2** in CD_3OD

Position	**1**		**2**	
	$\delta^{13}C$	δ^1H (multi., *J* Hz)	$\delta^{13}C$	δ^1H (multi., *J* Hz)
1	89.2		89.2	
2	87.0		87.0	
3	44.3	1.74 (H, d, 12.1)	44.3	1.61 (H, d, 12.2)
		1.87 (H, d, 12.1)		1.79 (H, d, 12.2)
4	105.4		105.4	
5	43.8	2.52 (H, d, 6.6)	43.8	2.40 (H, d, 6.7)
6	23.2	1.40 (H, d, 10.4)	23.3	1.38 (H, d, 10.3)
		1.64 (H, dd, 10.4, 6.6)		1.59 (H, dd, 10.3, 6.7)
7	71.9		72.0	
8	61.4	4.70 (2H, s)	61.2	4.56 (2H, s)
9	102.1	5.39 (H, s)	102.1	5.26 (H, s)
10	19.6	1.26 (3H, s)	19.6	1.24 (3H, s)
1	97.2	4.95 (H, br s, <2.0)	99.9	4.47 (H, d, 7.8)
2	74.2	3.27 (H, m)	74.9	3.19 (H,m)
3	77.4	3.38 (H. m)	77.9	3.24 (H, m)
4	71.8	3.32 (H, m)	72.0	3.28 (H. m)
5	72.8	3.59 (H, dd, 7.2, 5.6)	75.1	3.52 (H, dd, 7.7, 6.0)
6	64.7	4.45 (H, dd, 11.0, 5.6)	65.2	4.32 (H, dd, 11.2, 6.0)
		5.05 (H, d, 11.0)		4.98 (H, 11.2)
1	121.4		121.5	
2	132.8	7.89 (H, d, 8.0)	132.9	7.79 (H, d, 8.1)
3	116.6	6.83 (H, d, 8.0)	116.5	6.72 (H, d, 8.1)
4	164.8		164.5	
5	116.6	6.83 (H, d, 8.0)	116.5	6.72 (H, d, 8.1)
6	132.8	7.89 (H, d, 8.0)	132.9	7.79 (H, d, 8.1)
7	168.0		168.1	
1	131.3		131.3	
2	130.5	8.04 (H, d, 7.9)	130.5	7.95 (H, d, 7.8)
3	129.6	7.49 (H, t, 7.9)	129.6	7.39 (H, t, 7.8)
4	132.7	7.62 (H, t, 7.9)	132.9	7.52 (H, t, 7.8)
5	129.6	7.49 (H, t, 7.9)	129.6	7.39 (H, t, 7.8)
6	130.5	8.04 (H, d, 7.9)	130.5	7.95 (H, d, 7.8)
7	167.7		167.6	

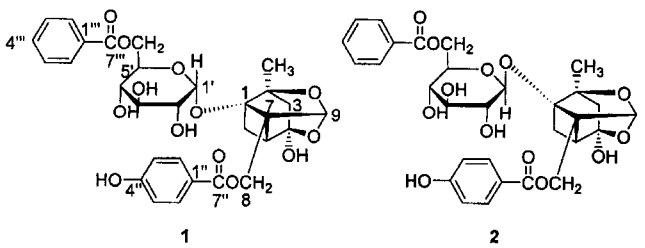
with $CHCl_3$/MeOH [95:5 (fraction I), 90:10 (fraction II), 80:20 (fraction III) and MeOH (fraction IV)], stepwisely. Final purification of fraction I on reversed-phase ODS HPLC with 48% MeOH gave α-benzoyloxypaeoniflorin (**1**, 14 mg) and β-benzoyloxypaeoniflorin (**2**, 16 mg). In the same manner, fraction II using 45% MeOH as eluent furnished paeonolide (27 mg), and fraction III (40% MeOH as eluent) afforded paeoniflorin (42 mg) and mudanpioside (58 mg).

α-Benzoyloxypaeoniflorin (1)

Colorless amorphous solid; $[\alpha]_D^{23}$ + 6.2° (*c* 0.8, MeOH); UV (MeOH) $\lambda_{max}(\varepsilon)$ 210 (17,000), 231 (14,000), 259 (17,000), 273 (11,500) nm; IR (film) ν_{max} 3450 (OH), 1760 and 1710 (ester), 1610 and 1520 (aromatic C=C), 1270 (C-O) cm^{-1}; FABMS *m/z* 601 $[M+H]^+$; HRFABMS (pos) *m/z* 601.1923 $[M+H]^+$, calcd for $C_{30}H_{33}O_{13}$, 601.1921; ^1H NMR (400 MHz, CD_3OD) see Table I; ^{13}C NMR (100 MHz, CD_3OD) see Table I.

RESULTS AND DISCUSSION

The structure of α-benzoyloxypaeoniflorin (**1**) was readily determined by spectroscopic analysis. Compound **1** was obtained as a colorless amorphous solid. Absorption bands at 3450, 1760, 1510, and 1270 cm^{-1} in the IR spectrum were attributable to hydroxyl, carbonyl, aromatic C=C, and C-O funtionalities, respectively. The FABMS data of **1** exhibited a quasimolecular $(M+H)^+$ ion peak at *m/z* 601 and the molecular formula was determined as $C_{30}H_{32}O_{13}$ by HRFABMS [*m/z* 601.1923 $(M+H)^+$, Δ+0.2 mmu]. ^1H NMR spectrum (Table I) of **1** was very similar to that of β-benzoyloxypaeoniflorin (**2**). Detailed analysis of 2D NMR [COSY, HMQC (Bax and Subramanian, 1986) and HMBC (Bax and Sommers, 1986)] data for **1** revealed the same planar structure as **2**. The specific rotation of **1** $\{[\alpha]_D^{23}$ + 6.2° (*c* 0.8, MeOH)$\}$ is different from that of **2** $\{[\alpha]_D^{23}$ - 9.7° (*c* 0.6, MeOH)$\}$, which suggests that **1** does not have the same stereochemistry as **2**. To determine the stereochemistry of **1**, NOESY experiments were carried out to give the relative configuration of monoterpene skeleton of **1** in accordance with **2** (Fig. 1). However, compound **2** showed a weak NOESY cross peak between H1′ and H5′, while compound **1** showed no cross peak between them. Analysis of coupling constants ($J_{H1',H2'}$) of



1 2

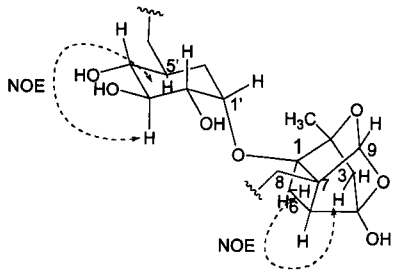


Fig. 1. NOESY correlations obtained from α-benzoyloxypaeoniflorin (**1**)

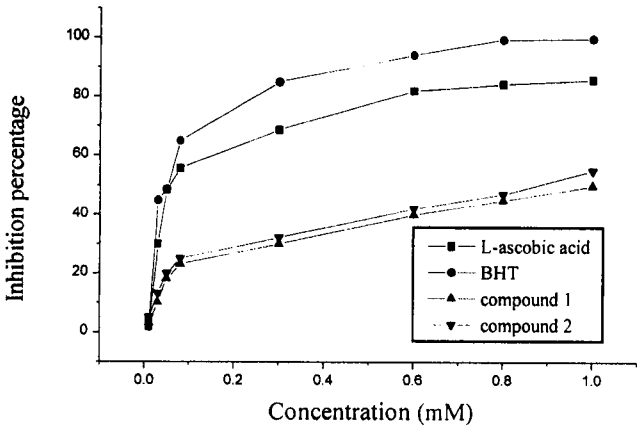


Fig. 2. Radical scanvenging effect of antioxidants on DPPH radical

1 and **2** unambiguously confirmed that **1** and **2** are α- and β-anomers, respectively (Bock *et al.*, 1973). From these results, compound **1** was determined as α-benzoyloxy-paeoniflorin.

As given in Fig. 2, both compounds **1** and **2** showed similar radical scavenging activity, which were less potent than that of L-ascorbic acid and BHT.

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