Arch Pharm Res Vol 28, No 10, 1127-1130, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

A Phenolic Glucoside Isolated from *Prunus serrulata* var. *spontanea* and its Peroxynitrite Scavenging Activity

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(Received June 22, 2005)

A new phenolic glucoside (**1**), pursargentoside, was isolated from the leaves of *Prunus serrulata* var. *spontanea,* along with three other known compounds, orobol 7-*O*-glucoside (**2**), 1β, 2α, 3α, 24-tetrahydroxy-urs-12-en-28-oic acid (**3**), and chlorogenic acid (**4**). The structure of pursargentoside (**1**) was identified by spectroscopic data analysis including 1D and 2D NMR spectroscopy, as 2-*O*-β-(6'-benzoyl)-glucopyranosyl *o*-(*Z*)-coumaric acid. Compounds **1**, **2**, and **4** exhibited $ONOO^-$ scavenging activity, whereas compound **3** was determined to be virtually inactive.

Key words: *Prunus serrulata* var. *spontanea*, Rosaceae, Phenolic glucoside, Pursargentoside, Peroxynitrite scavenging activity

INTRODUCTION

Prunus serrulata var. *spontanea* (Rosaceae), a member of the *Prunus* species, is a large tree which is found throughout Korea (Kritikar and Basu, 1974). This species possess interesting biological properties, possibly exhibiting sedative, anti-inflammatory, anti-hyperlipidemic, anti-tumor, and antioxidant activities (Sang *et al.*, 2002; Donovan *et al.*, 1998; Nakatani *et al.*, 2000; Kayano *et al.*, 2002; Wang *et al.*, 1999a, 1999b). The red fruits of this plant are edible, and have been used in traditional folk medicine for the treatment of heart failure from beriberi, dropsy, mastitis, toothache, and as an emmenagogue (Kim, 1996). In a previous study, 11 flavonoids and 5 triterpenoids were isolated from this species (Jung *et al.*, 2002, 2004). Further investigations into the properties of this plant led to the isolation of a phenolic glucoside (**1**), along with three other known compounds, orobol 7-*O*-glucoside (**2**), 1β, 2α, 3α, 24-tetrahydroxy-urs-12-en-28-oic acid (**3**), and chlorogenic acid (**4**). This paper elucidates the structure of this new compound **1**. In addition, these compounds were evaluated with regard to their ability to scavenge authentic $ONOO^-$.

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MATERIALS AND METHODS

General experimental procedures

^1H-, ^{13}C-NMR, and DEPT spectra were recorded on a Varian UNITY-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C). The HMBC and HMQC experiments were performed using pulsed field gradients. EI-MS data were carried out on a JEOL JMS-700 spectrometer. Positive-ion LR and HR FAB-MS data were collected on a JEOL JMS-HX110/110A Tandem mass spectrometer (JEOL). Column chromatography was performed with silica (Si) gel (Merck, 70-230 mesh) and Sephadex LH-20 and reversed phase C_{18} (RP-18). Thin layer chromatography (TLC) was performed on pre-coated Merck Kieselgel 60 F_{254} plate (0.25 mm) and 50% H_2SO_4 was used as spray reagent. Chemical shifts were referenced to the respective residual solvent peaks expressed in ppm. Multiplicities of ^1H- and ^{13}C-NMR signals are indicated as s (singlet), d (doublet), brs (broad singlet), and m (multiplet).

Chemicals

The DL-Penicillamine (DL-2-amino-3-mercapto-3-methylbutanoic acid) and DHR 123 (dihydrorhodamine 123) were purchased from Molecular Probes (Eugene, OR., U.S.A.), and $ONOO^-$ was purchased from Cayman Chemicals Co. (Ann Arbor, MI, U.S.A.).

Plant materials

The leaves of *P. serrulata* var. *spontanea* were collected on Gumjung Mountain, in Busan, Korea in April 1999, and were authenticated by Dr. Maeng Ki Kim, at Korea Environmental and Ecological Services, in Busan, Korea. A voucher specimen has been deposited in the Herbarium of the Medicinal Plant Garden, at the College of Pharmacy, Pusan National University, in Busan, Korea.

Extraction, fractionation, and isolation of *Prunus serrulata* var. *spontanea* leaves

The dried leaves of *P. serrulata* var. *spontanea* (1.8 kg) were refluxed with MeOH for three hours. The total filtrate was then concentrated to dryness *in vacuo* at 40°C, in order to render the MeOH extract (471 g). This extract was then suspended in H_2O, and partitioned with CH_2Cl_2, EtOAc and *n*-BuOH, successively, which yielded a CH_2Cl_2 extract (144 g), EtOAc extract (77 g), *n*-BuOH extract (105 g), and an H_2O residue (145 g). The EtOAc extract (77 g) was chromatographed on a Si gel column (12 × 60, Silica gel 60, Merck, 2 kg) and eluted with EtOAc-MeOH (8:1 to 1:1), which produced 14 fractions (Fr. 1-Fr. 14). The filtrate of fraction 4 (5.14 g) was then chromatographed on a Si gel column with EtOAc (to pure MeOH), which yielded 20 sub-fractions (Fr. 4-1 to 4-20). Fraction 4-20 was then further purified with Sephadex LH-20 eluted with MeOH, which yielded compound **1** (10 mg). Fraction 5 (1.66 g) was purified consecutively with Sephadex LH-20 eluted with MeOH, and yielded compound **3** (40 mg). Fraction 13 (2.82 g) was subjected to column chromatography over RP-18, and eluted with H_2O-MeOH (0, 20%, 40%, 60%, 80%, and 100% MeOH), which produced 6 subfractions. Fraction 13-2 (20% MeOH fraction) was further purified with Sephadex LH-20 eluted with MeOH and yielded compound **2** (15 mg). Fraction 14 (2.29 g) was repeatedly subjected to a Sep-Pak C_{18} with H_2O-MeOH (0%, 10%, 30%, 50%, 100% MeOH), which produced 5 fractions (Fr. 14-1 to Fr. 14-5). Subfraction 14-1 (1.9 g) was subjected to further chromatography with EtOAC-MeOH-H_2O on a Si gel column, which produced 12 fractions (Fr. 14-1-1 to Fr. 14-1-12). Subfraction 7 (Fr. 14-1-7, 0.6 g) was purified with Sephadex LH-20 and 50% MeOH, and yielded compound **4** (25 mg).

Pursargentoside (1)

Amorphous white powder, $[\alpha]_D^{20}$ −3.70° (c=0.015, MeOH); IR ν_{max} cm^{-1}: 3417, 1723, 1701, 1634, 1597, 1488, 1451, 1317, 1280, 1235, 1071, 836, 750, 705; UV λ_{max} (MeOH) nm (log ε): 223 (4.27), 264 (3.96); + NaOMe 214 (4.30), 255 (4.12), 299sh (3.83); HR-FAB-MS *m/z* 453.1161 $[C_{22}H_{22}O_9 + Na]^+$ (Calc. for 453.1162); ^{1}H-NMR and ^{13}C-NMR (see Table I).

1β, 2α, 3α, 24-Tetrahydroxy-urs-12-en-28-oic acid (3)

Amorphous white powder, EIMS *m/z* 504 $[C_{30}H_{48}O_6]^+$, 248, 203; LR-FABMS *m/z* 527 $[C_{30}H_{48}O_6 + Na]^+$; HR-FABMS *m/z* 527.3344 $[C_{30}H_{48}O_6 + Na]^+$ (Calc. for 527.3348); ^{1}H-NMR (400 MHz, pyridine-d_5) δ: 5.56 (s, H-12), 4.67 (d, J = 2.8, H-3), 4.29 (dd, J = 2.2, 9.4, H-2), 4.19 (d, J = 9.0, H-1), 4.17 (d, J = 10.9, H-24b), 3.80 (d, J = 11.0, H-24a), 2.59 (d, J = 11.2, H-18), 1.65 (s, H-23), 1.28 (s, H-25), 1.17 (s, H-27), 1.12 (s, H-26), 0.95 (d, J = 6.0, H-30), 0.90 (d, J = 6.4, H-29); ^{13}C-NMR data (100 MHz, pyridine-d_5) δ: 179.8 (C-28), 138.2 (C-13), 127.0 (C-12), 81.0 (C-1), 74.5 (C-3), 71.5 (C-2), 65.2 (C-24), 53.4 (C-18), 49.3 (C-5), 49.1 (C-9), 48.0 (C-17), 44.8 (C-4), 43.7 (C-10), 42.4 (C-14), 40.8 (C-8), 39.4 (C-19), 39.4 (C-20), 37.4 (C-7, 22), 28.7 (C-15), 31.1 (C-21), 27.7 (C-11), 24.9 (C-16), 23.8 (C-23), 23.8 (C-27), 21.3 (C-29), 18.9 (C-6), 17.8 (C-26), 17.4 (C-30), 13.5 (C-25).

Measurement of $ONOO^-$ scavenging activity

Peroxynitrite scavenging was evaluated by monitoring the oxidation of DHR 123, using a slightly modified version of the method described by Kooy *et al* (1994). DHR 123 (5 mM) in dimethylformamide, which was purged with nitrogen, was stored as a stock solution at -20°C. A working solution with 5 μM DHR 123 diluted from the stock solution was placed on ice in the dark immediately prior to the experiment. A buffer (90 mM sodium chloride, 50 mM sodium phosphate, and 5 mM potassium chloride at pH 7.4) was purged with nitrogen and placed on ice prior to use. Immediately before use, 5 mM diethylenetriaminepentaacetic acid (DTPA) was added. $ONOO^-$ scavenging ability, by the oxidation of DHR 123, was measured at room temperature on a microplate fluorescence reader (FL_X 500, Bio-Tek Instruments) with excitation and emission wavelengths of 485 nm and 530 nm, respectively. The background and final fluorescent intensities were determined 5 minutes after treatment with and without authentic 10 μM $ONOO^-$ in 0.3N sodium hydroxide (NaOH). Authentic $ONOO^-$ easily oxidized DHR 123 with its final fluorescent intensity being stable over time. Penicillamine was used as a positive control.

Statistical analysis

The data is expressed as a mean ± standard error of three experiments.

RESULTS AND DISCUSSION

The EtOAc-soluble portion of the MeOH extract made from the leaves of *P. serrulata* var. *spontanea* was consecutively chromatographed over Si gel, Sephadex LH-20, and RP-18, which yielded a phenolic glucoside (**1**), pursargentoside, along with three other known compounds,

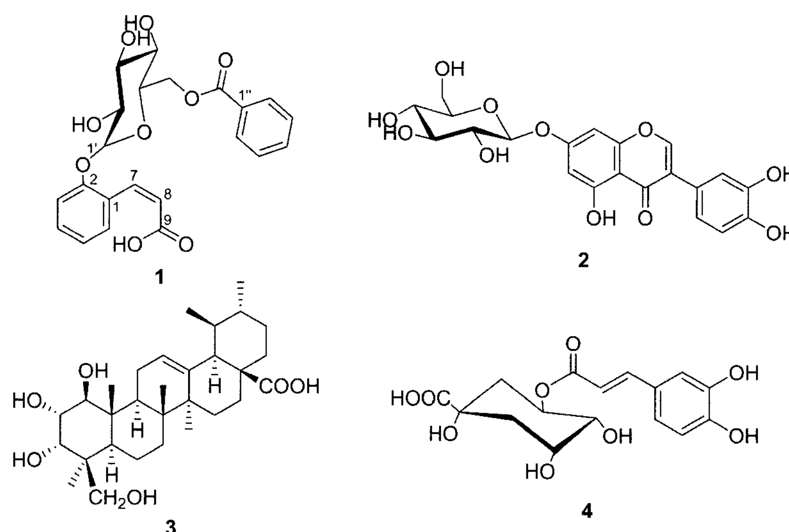

Fig. 1. Structures of isolated compounds **1-4** from *P. serrulata* var. *spontanea*

orobol 7-*O*-glucoside (**2**) (Anhut *et al.*, 1984), 1β, 2α, 3α, 24-tetrahydroxy-urs-12-en-28-oic acid (**3**) (Lahlou *et al.*, 1999), and chlorogenic acid (**4**) (Kim *et al.*, 1997) (Fig. 1). Although the NMR spectral data of compound **3** in CD_3OD has been reported previously, our data regarding pyridine-d_5 are presented, since the value of this solvent in NMR studies of triterpenoids has been discussed at length.

Compound **1** was isolated as a white amorphous powder with the molecular formula $C_{22}H_{22}O_9$, which was deduced from a $[M + Na]^+$ peak at *m/z* 453.1161 on HR FAB-MS, and confirmed by analysis of the ^{13}C-NMR spectrum. The UV spectrum in MeOH exhibited typical aromatic absorption bands at 223 and 264 nm. Furthermore, the IR of this compound exhibited absorptions of the hydroxyl (3417 cm^{-1}), ester (1723 cm^{-1}), acidic (1701 cm^{-1}), aromatic (1634 & 1597 cm^{-1}), and glycosidic (1071 cm^{-1}) groups. The ^1H- and ^{13}C-NMR spectra (See Table I) exhibited signals suggesting a hydroxy cinnamic acid with glycosidic and benzoyl groups. The ^1H-NMR signals at δ5.95 (1H, d, J = 12.6 Hz, H-8), 6.91 (1H, dd, J = 7.5 Hz, H-5), 7.03 (1H, dd, J = 7.5 Hz, H-4), 7.11 (1H, d, J = 7.7 Hz, H-3), 7.24 (1H, d, J = 12.6, H-7), 7.47 (1H, d, J = 7.8 Hz, H-6), due to the aglycon, displayed the characteristic pattern of *o*-hydroxy cinnamic acid derivatives. This finding was supported by analyses of the ^{13}C-NMR spectrum (Table I), that signals of an *o*-coumaroyl moiety at δ 171.48, 157.20, 139.33, 132.30, 131.80, 127.77, 123.70, 122.58, and 117.59. The *cis*-configuration of the C7-C8 double bond was evident from the small coupling constant (J = 12.6 Hz) detected between H-7 and H-8. The ^1H-NMR spectrum also revealed the presence of one glycosyl moiety, an anomeric proton at δ 4.93 (1H, d, J = 7.5 Hz), and benzoyl groups at δ 7.45 (2H, dd, J = 7.6 Hz, H-3" and H-5"), 7.61 (1H, d, J = 7.5 Hz, H-4"), and 8.01 (2H, d, J = 7.9 Hz, H-2" and H-6"). The results of the ^1H-^1H COSY and 2D HMBC experiments, as compared with the results of the ^{13}C-NMR spectrum, allowed for the identification of the sugar moiety as β-glucopyranoside. In the ^{13}C-NMR spectrum, the downfield shift of a methylene

Table I. ^1H- and ^{13}C-NMR data for compound **1** (CD_3OD)[a]

Position	^1H	^{13}C
1		127.77(C)
2		157.20(C)
3	7.11 (1H, d, J = 7.7 Hz)	117.59(CH)
4	7.03 (1H, dd, J = 7.5 Hz)	131.80(CH)
5	6.91 (1H, dd, J = 7.5 Hz)	123.70(CH)
6	7.47 (1H, d, J = 7.8 Hz)	132.30(CH)
7	7.24 (1H, d, J = 12.6 Hz)	139.33(CH)
8	5.95 (1H, d, J = 12.6 Hz)	122.58(CH)
9		171.48(C)
1'	4.93 (1H, d, J = 7.5 Hz)	103.50(CH)
2'	3.50 (1H, m)	78.79(CH)
3'	3.78 (1H, dd, J = 8.0, 8.5 Hz)	76.31(CH)
4'	3.54 (1H, m)	75.65(CH)
5'	3.46 (1H, m)	72.72(CH)
6'	4.43 (1H, dd, J = 7.5, 11.8 Hz) 4.69 (1H, dd, J = 2.0, 11.8 Hz)	66.09(CH_2)
1"		132.06(C)
2", 6"	8.01 (1H, d, J = 7.9 Hz)	131.42(CH×2)
3", 5"	7.45 (1H, dd, J = 7.6, 7.9 Hz)	130.38(CH×2)
4"	7.61 (1H, d, J = 7.5 Hz)	135.13(CH)
7"		168.55(C)

[a]Assignments were confirmed by DEPT, ^1H-^1H COSY, and ^1H-^{13}C COSY experiments.

carbon (C-6' at δ 66.09), along with the upfield shift of a neighboring carbon (C-5' at δ 72.72) of a glucose suggested that the benzoyl group was attached at the C-6 of the glucose. In the HMBC spectrum, the relevant correlations between the benzoyl carbonyl group (δ 168.55) and the H-6' signal, and between C-2 (δ 157.20) and H-1' (δ 4.93), revealed that the benzoyl group was located at C-6' and the β-glucopyranosyl moiety was located at C-2. These spectral data established that compound **1** was a new natural compound, 2-*O*-β-(6'-benzoyl)-glucopyranosyl *o*-(*Z*)-coumaric acid. This compound was given a trivial name, prusargentoside. To our knowledge, this is the first report of the isolation and identification of an *o*-(*Z*)-hydroxy cinnamic acid containing benzoylated glucose, in spite of the abundance of hydroxy cinnamic acid glycosides in nature, particularly in the *Prunus* species (Shimomura *et al.*, 1987a, 1987b, 1988; Takaishi, 1968).

$ONOO^-$ has been associated with a variety of diseases, including cancer, as well as a host of cardiovascular and neurological diseases (Ronson *et al.*, 1999; Good *et al.*, 1998). With regard to their $ONOO^-$ scavenging effects, compounds **2** and **4** exhibited good inhibitory activity, with IC_{50} values of 8.06 ± 0.40 and 2.78 ± 0.10 μM, respectively, which are comparable to penicillamine (IC_{50} 7.24 ± 0.24 μM). Compound **1** exhibited moderate $ONOO^-$ scavenging activity, with an IC_{50} values of 15.34 ± 0.35 μM, whereas compound **3** was determined to be virtually inactive.

ACKNOWLEDGEMENTS

This research was supported by a grant (PF0320801-00) from the Plant Diversity Research Center of 21st Century Frontier Research Program funded by the Ministry of Science and Technology of Korean government. The FAB-MS spectra were provided by the KBSI.

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