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Antioxidant phenolic compounds from rhizomes of *Polygonum paleaceum*

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Abstract

Polygonum paleaceum Wall. ex Hook. f. is a small herb in Polygonaceae family. In southwest of China, the rhizomes of this plant were used as folk drug for the treatment of chronic gastritis, duodenal ulcers, dysentery, wound, pain, hemorrhage and irregular menstruation. The crude aqueous acetone extract exhibited high antioxidant activity (SC_{50} = 16.72 μg/ml) in 1,1-diphenyl-2-picryldydrazyl (DPPH) radical-scavenging assay. With bioactivity-guided fractionations, 14 antioxidant phenolic compounds **1–14** were obtained from the air-dried rhizomes of this plant. Of them, compound **1**, a new phenol named paleaceolactoside, was determined to be 3,5-dihydroxyl hexanoic acid 1,5-lactone 3-(6′-*o*-galloyl)-*o*-β-D-glucopyranoside by detailed spectroscopic analysis. The radical-scavenging activity of all the isolated compounds was also described.

Keywords: *Polygonum paleaceum*; Polygonaceae; Antioxidant phenols; Paleaceolactoside

1. Introduction

The free radicals and reactive oxygen species have been proposed to induce cellular damage and to be involved in several human diseases, such as cancer, arteriosclerosis inflammatory disorders, as well as in aging processes (Halliwell, 1994; Aviram, 2000). Of various kinds of natural antioxidants, phenolic compounds have received much attention (Espin et al., 2000; Luo et al., 2002).

The genus *Polygonum* (Polygonaceae), comprising of about 300 species, is distributed worldwide, of which most are in the north temperate regions. In China, 120 species are found especially in the southwest area, and more than 80 species, *Polygonum multiforum* Thunb., *Polygonum cuspidatum* Sieb. et Zucc., *Polygonum orientale* L., *Polygonum bistorta*, *Polygonum tinctorium* Lour and *Polygonum aviculare* L. were recorded to have uses in traditional Chinese medicine, concerning anti-inflammation, promoting blood circulation, dysentery, diuretic and hemorrhage etc. It is noticed that the plants of genus *Polygonum* provided many kinds of polyphenols, some of which showed interesting biological activities. (Gong et al., 2002).

Polygonum paleaceum Wall. ex Hook. f., as a small herb, is mainly distributed in the southwest of China, where the local people, such as Yi, Miao, Bai and Tibetan people, use its rhizome as a folk medicine for the treatment of chronic gastritis, duodenal ulcers, dysentery, wound, pain, inflammation, hemorrhage and irregular menstruation (Jiangsu New Medical College, 1977). Recently, a few pharmacological studies showed that extract of *Polygonum paleaceum* has anti-inflammatory effect on inflammatory rates and anti-tumor activity to K_{562}, HL_{60} in vitro and S_{180}, Hep A in vivo, which are related to oxygen free radical-scavenging and anti-lipid peroxidation activity of *Polygonum paleaceum* (Li et al., 1998, 2001, 2002). Though the rhizomes of this plant were reported rich in mixture of tannins, so far, there is no detailed chemical study on this plant. Our preliminary experiment showed that the extract of the rhizomes of *Polygonum paleaceum* exhibited considerable antioxidant activity on 1,1-diphenyl-2-picryldydrazyl (DPPH) radical-scavenging assay. This observation promoted us to perform a detailed bioassay-guided

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chemical investigation on this plant, which led to the isolation of a new phenol, named paleaceolactoside (**1**), together with 13 known phenolic compounds. This paper describes herein the structure determination of the new compound **1** on the basis of spectroscopic method. The antioxidant activities of these compounds on DPPH radical-scavenging assay are also reported.

2. Materials and methods

2.1. Plant material

The air-dried rhizome of *Polygonum paleaceum* was bought from Kunming herbal medicinal market and identified by Prof. C.R. Yang, Kunming Institute of Botany, Chinese Academy of Sciences.

2.2. General experimental procedures

$[\alpha]_D$ was carried out on JASCO-20 polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were recorded on a UV 210A Shimadzu spectrometer. 1D- and 2D-NMR spectra were run on Bruker AM-400 and DRX-500 instruments with TMS as internal standard. The MS data were recorded on a VG Auto Spec-3000 spectrometer. DPPH radical-scavenging assay was performed on Emax precision microplate reader.

2.3. Chemicals

DPPH radical (Aldrich Chem. Co.). Column chromatography was performed on Diaion HP20SS (Mitsubishi Chemical Co.), MCI-gel CHP-20P (75–150 μm, Mitsubishi Chemical Co.), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co. Ltd.), Chromatorex ODS (100–200 mesh, Fuji Silysia Chemical Co. Ltd.), and Toyopearl HW-40F (37–70 μm, Tosoh Co.). TLC was carried on silica gel G precoated plates (Qingdao Haiyang Chemical Co.) with benzene–ethyl formate–formic acid (3:6:1). Spots were detected by spraying with ferric chloride ($FeCl_3$) and 10% sulfuric acid reagents followed by heating.

2.4. Extraction and isolation

The air-dried and powdered rhizome of *Polygonum paleaceum* (8.0 kg) was extracted with 60% acetone–H_2O (20 l × 3) at room temperature. After removal of the organic solvent, the extracts were concentrated to a small volume (2 l), and then partitioned with EtOAc and *n*-BuOH sequentially to yield EtOAc, *n*-BuOH and aqueous fraction. The crude extract, EtOAc, *n*-BuOH and aqueous fractions displayed SC_{50} value as 16.72, 10.64, 30.64 and 1223.93 μg/ml, respectively, according to the DPPH assay; so the further isolation were focused on EtOAc and *n*-BuOH fraction.

The EtOAc fraction (168 g) was fractionated on Diaion HP20SS eluted with H_2O–MeOH (1:0–0:1) to give nine fractions (A_1–A_9). Fraction A_1 (19.5 g) was subjected on Sephadex LH-20, MCI and ODS eluted with H_2O–MeOH (1:0–0:1) to yield **3** (20 mg), **5** (23 mg), **7** (20 mg), **14** (200 mg); fraction A_2 (15.9 g) was subjected on Sephadex LH-20, MCI and ODS eluted with H_2O–MeOH (1:0–0:1) to yield **4** (24 mg), **6** (16 mg), **13** (16 mg), **8** (26 mg), **9** (18 mg), **10** (12 mg); fraction A_3 (8.4 g) was subjected on Sephadex LH-20 and MCI with H_2O–MeOH (1:0–0:1) to give **12** (10 mg); fraction A_6 (6.3 g) was subjected on Sephadex LH-20 with H_2O–MeOH (1:0–0:1) to give **11** (46 mg).

n-BuOH fraction (200 g), of which 180 g was fractionated on Diaion HP20SS eluted with H_2O–MeOH (1:0–0:1) to give 10 fractions (B_1–B_{10}). Fraction B_2 (16.5 g) was subjected on Sephadex LH-20 eluted with H_2O–MeOH (1:0–0:1) to give 12 subfractions (B_{2-1}–B_{2-12}), of which B_{2-2} was subjected on Sephadex LH-20 eluted with EtOH to give **1** (10 mg) and **2** (40 mg).

2.4.1. Paleaceolactoside

White amorphous powder, mp 159–161 °C, $[\alpha]_D^{21}$ +6.81° (c 0.005, MeOH); UV_{max} (MeOH): 217 (log ε, 4.21), 276 (3.80); IR bands (KBr) ν_{max} 3411, 2926, 1704, 1235, 1037 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6): δ 2.66 (2H, d, *J* = 3.95 Hz, H-2), 4.26 (1H, m, H-3), 1.61 (1H, ddd, *J* = 2.67, 11.35, 14.50 Hz, H-4a), 2.19 (1H, ddd, *J* = 3.30, 3.50, 14.50 Hz, H-4b), 4.68 (1H, m, H-5), 1.14 (3H, d, *J* = 5.06 Hz, CH_3), 7.14 (2H, s, galloyl H), Glc: 4.48 (1H, d, *J* 7.82 Hz, H-1′), 3.23 (1H, dd, *J* = 7.82, 8.92 Hz, H-2′), 3.44 (1H, d, *J* = 8.92, 9.09 Hz, H-3′), 3.40 (1H, d, *J* = 9.09, 9.42 Hz, H-4′), 3.64 (1H, ddd, d, *J* = 2.10, 5.50, 9.42 Hz, H-5′), 4.57 (1H, dd, *J* = 2.10, 11.80 Hz, H-6′a), 4.29 (1H, dd, *J* = 5.50, 11.80 Hz, H-6′b); ^{13}C NMR (125 MHz, acetone-d_6) see Table 1. Negative ion HRFAB-MS *m/z* 443.1183 $[M-H]^-$, calcd. for $[C_{19}H_{23}O_{12}]^-$: 443.1189.

2.5. DPPH radical-scavenging assay

The DPPH assay was performed as described (Yoshida et al., 1989). In this assay, ascorbic acid was used as positive control, reaction mixtures containing an ethanolic solution

Table 1
^{13}C NMR (125 MHz, CD_3OCD_3) spectral data of **1**

Carbon	^{13}C Chemical shift	No.	^{13}C Chemical shift
1	171.2 s	5′	74.6 d
2	36.1 t	6′	64.4 t
3	72.4 d	Galloyl 1″	121.0 s
4	36.3 t	2″	109.6 d
5	73.5 d	3″	145.9 s
Methyl group	21.3 q	4″	138.9 s
Glc-1′	103.4 d	5″	145.9 s
2′	74.2 d	6″	109.6 d
3′	77.0 d	7″	167.1 s
4′	70.9 d		

of 200 μM DPPH (100 μl) and two-fold serial dilutions of sample (dissolved in 100 μl ethanol, with amounts of sample ranging from 2 to 1000 μg/ml) were placed in a 96 well microplate and incubated at 37 °C for 30 min. After incubation, the absorbance was read at 517 nm, and mean value was obtained from three duplicated readings. Scavenging activity was determined by following equation: % scavenging activity = $[A_{control} - A_{sample}] / A_{control} \times 100$. The SC_{50} value was obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals.

3. Results and discussion

The 60% aqueous acetone extract of air-dried rhizomes of *Polygonum paleaceum* was suspended into H_2O and then partitioned successively with EtOAc and *n*-BuOH. All of the crude extract, EtOAc and *n*-BuOH fractions exhibited obvious antioxidant activity (SC_{50} = 16.72, 10.64 and 30.65 μg/ml, respectively) on DPPH radical-scavenging assay, which has been widely used to measure the radical-scavenging ability of various plant extracts and constituents (Soares et al., 1997; Dapkevicius et al., 2002). Further purification of the EtOAc and *n*-BuOH fractions were carried out with Diaion HP20SS, MCI-gel CHP-20P, Sephadex LH-20, Chromatorex ODS and Toyopearl HW-40F column chromatography. This led us to obtain a new compound **1** together with 6-*o*-galloyl-D-glucose (**2**) (Nonaka and Nishioka, 1983) from *n*-BuOH fraction. In addition, (+)-catechin (**3**) (Nonaka et al., 1983), (−)-epicatechin (**4**) (Nonaka and Nishioka, 1982), procyanidins B-1 (**5**), B-2 (**6**) (Nonaka et al., 1981) and C-1 (**7**) (Nonaka et al., 1982), chlorogenic acid (**8**) (Kelley et al., 1976), methyl 5-*o*-caffeoylquinate (**9**), 3-*o*-caffeoyl quinic acid (**10**) (Machida and Kikuchi, 1992; Ida et al., 1994), rutin (**11**) (Wenkert and Gottlieb, 1977), quercetin-3-*o*-β-D-glucuronide (**12**) (Urushibara et al., 1992), as well as caffeic acid (**13**) and gallic acid (**14**), were yielded from the EtOAc fraction. The structural elucidations of all the compounds were based on the spectroscopic evidences and comparing with literature data (Fig. 1).

Compound **1** was obtained as a white amorphous powder. The negative ion high resolution FAB mass spectrum showed a quasi-molecular ion at *m/z* 443.1183 $[M - H]^-$, consistent with the molecular formula $C_{19}H_{24}O_{12}$ (calc. for $C_{19}H_{24}O_{12}$: *m/z* 443.1189). The IR spectrum gave absorptions at 3411, 2926, 1704, 1235 and 1037 cm^{-1}. The ^1H and ^{13}C NMR spectra exhibited typical signals rising from a galloyl group

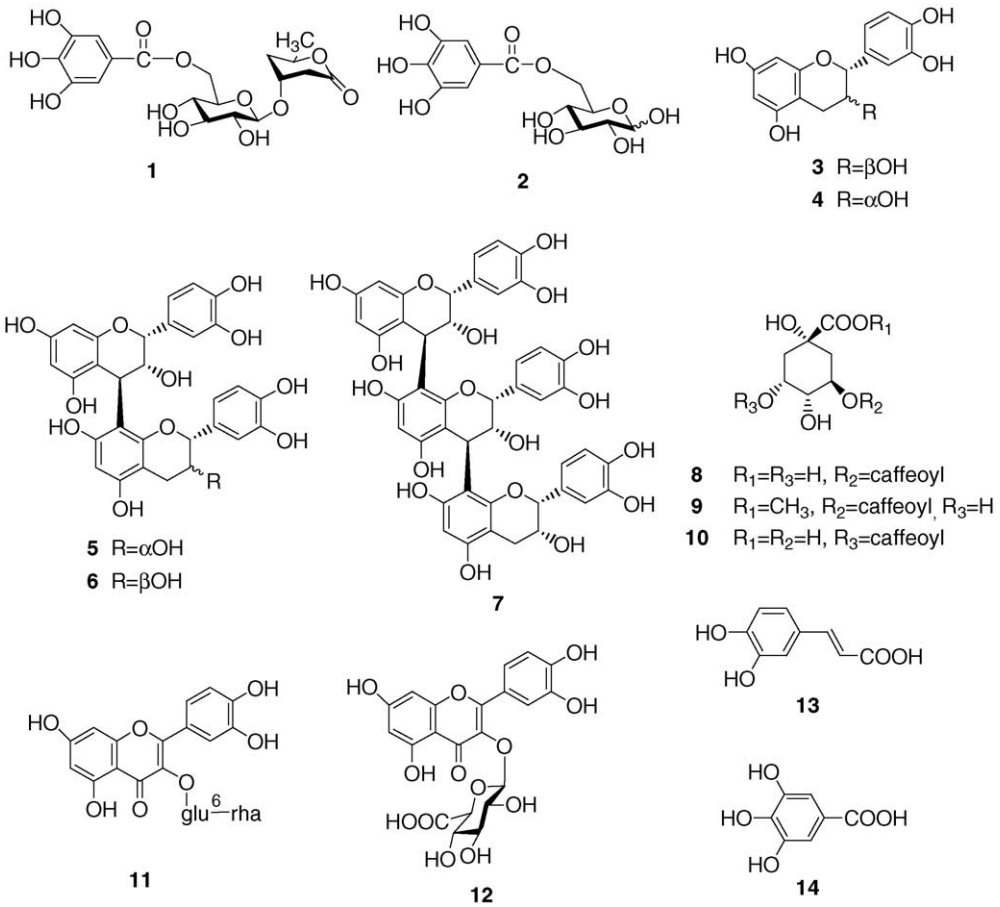

Fig. 1. Structures of phenolic antioxidants from *Polygonum paleaceum*.

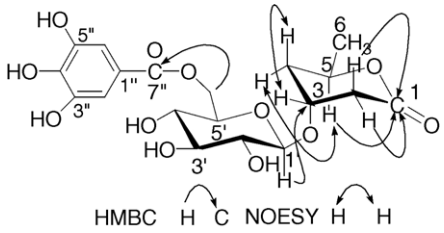

Fig. 2. Important HMBC and NOESY correlations of compound **1**.

and a β-D-glucopyranosyl unit (Table 1). In addition, six carbon signals including a methyl (δ 21.3), two methylene (δ 36.1, 36.3), two oxygenated methine (δ 72.4, 73.5), and a carboxyl (δ 171.2) groups were observed at the DEPT spectrum. Detailed analysis of HMQC and ^{1}H–^{1}H COSY spectra suggested that the six carbon signals reformed a fragment of CH_3–CH(O)–CH_2–CH(O)–CH_2–CO–(C_6–C_5–C_4–C_3–C_2–C_1). In the HMBC experiment (Fig. 2) of **1**, correlations of δ 4.68 (H-5) and δ 2.66 (H-2) with δ 171.2 (C-1) revealed the presence of 1,5-lactone form in **1**. The NOESY spectrum (Fig. 2) clearly showed correlations between δ 4.26 (H-3) and δ 1.61 (Hax-4), δ 4.68 (H-5) and δ 2.19 (Heq-4), however, no cross peak between H-3 and H-5 was found. These observations as well as the proton–proton coupling constants of Hax-4 [δ 1.61 (ddd, J 2.67, 11.35, 14.50 Hz)] and Heq-4 [δ 2.19 (ddd, J 3.30, 3.50, 14.50 Hz)], suggested that H-3 was equatorially orientated while H-5 was axially orientated in a $^4C_1$ chair conformation of **1**. The locations of the glucopyranosyl moiety and the galloyl group were revealed by the HMBC correlations of δ 4.48 (H-1′, anomeric H of glucose) with δ 72.4 (C-3), and δ 4.29, 4.57 (H-6′) with δ 167.1 (galloyl C-7″), that is, at C-3 of the 1,5-lactone aglycone and the C-6′ position of the glucosyl moiety, respectively. On the basis of the above evidence, the structure of **1** was determined to be 3,5-dihydroxy hexanoic acid 1,5-lactone 3-(6′-*o*-galloyl)-*o*-β-D-glucopyranoside. As far as we know, compound **1** is a new natural phenol found for the first time in plant material. We gave it the name of 'paleaceolactoside'.

The DPPH radical-scavenging activities of compounds **1–14** were detected (Table 2). It is noticed that most of the isolated compounds showed obvious scavenging activity on DPPH radicals. Comparing with those of flavonoid glycosides (**11** and **12**), gallic acid and caffeic acid derivatives (**1**, **2**, **8**, **9** and **10**), all of the flavan-3-ol derivatives displayed stronger activities than that of ascorbic acid. In which, procyanidin C-1 (**7**) displayed the strongest activity (SC_{50} = 9.89 μM). And the order of the activities of flavan-3-ol derivatives were showed to be as **7** > **6** > **4** > **5** > **3** (Table 2), that is, the oligomers showed much higher antioxidant activities than those of monomers. It is well known that the flavan-3-ol derivatives are rich in tea, and may act as potential antioxidants in plasma to modulate cellular oxidative stress and prevent the oxidation of low-density lipoprotein (LDL) (Mangiapane et al., 1992; Tijburg et al., 1997). Epidemiological observation also indicated that the consumption of tea could reduce the risk of cardiovascular diseases (Yang and Landau, 2000). The flavonoidal glycosides **11** and **12** were reported to play an important role in the prevention of lipid peroxidation and cardiovascular disease (Hertog and Holmann, 1996; Lekse et al., 2001), however, both of them did not show distinguished scavenging activity on DPPH radicals in our work.

Table 2
DPPH radical-scavenging activity of samples from *Polygonum paleaceum*

Sample	SC_{50} (μM)	Sample	SC_{50} (μM)
Ascorbic acid	30.79	Chlorogenic acid (**8**)	34.57
Paleaceolactoside (**1**)	38.42	Methyl 5-*o*-caffeoyl quinate (**9**)	30.07
6-*o*-Galloyl-D-glucose (**2**)	25.17	3-*o*-Caffeoyl quinic acid (**10**)	55.66
(+)-Catechin (**3**)	22.64	Rutin (**11**)	42.49
(−)-Epicatechin (**4**)	17.39	Quercetin-3-*o*-β-D-glucuronide (**12**)	28.91
Procyanidin B-1 (**5**)	18.40	Caffeic acid (**13**)	63.43
Procyanidin B-2 (**6**)	15.31	Gallic acid (**14**)	12.10
Procyanidin C-1 (**7**)	9.89		

SC_{50}: radical-scavenging activity (concentration in μM required for 50% reduction of DPPH radical).

Gallic acid (**14**) as a well-known natural antioxidant found widely from plants, was showed to have the antiapoptotic potential in normal human peripheral blood lymphocytes (PBL) and protect the cell from damage induced by H_2O_2 in vivo (Sohi et al., 2003). It is the main constituents isolated from *Polygonum paleaceum* and may play an important role for the antioxidant activity of this plant.

Finally, the above results will provide the evidences to evaluate the biological functions of *Polygonum paleaceum* and promote the reasonable usage of this herb.

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