Phenolic Compounds from the Mongolian Medicinal Plant *Scorzonera radiata*

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Z. Naturforsch. **67c**, 135–143 (2012); received May 11/November 30, 2011

Chromatographic separation of a crude extract obtained from aerial parts of the Mongolian medicinal plant *Scorzonera radiata* yielded fifteen natural compounds, including two new flavonoids and one new quinic acid congener, as well as four flavones and eight quinic acid derivatives, all of which are known natural compounds. The structures of the isolated compounds were elucidated on the basis of NMR (^{1}H, ^{13}C, COSY, HMBC, ROESY, and TOCSY) and mass spectrometric data. The antioxidant activities of the quinic acid derivatives were evaluated by the DPPH assay.

Key words: Scorzonera radiata, Flavonoid, Quinic Acid

Introduction

The genus *Scorzonera* (family Asteraceae) which comprises over 150 species is distributed in the temperate zones of Eurasia (Tulin *et al.*, 1976; Malyschev and Peschkova, 1979; Mabberley, 1997). Eleven species of *Scorzonera* are found on the Mongolian plateau, two of which are endemic including *S. radiata* (Grubov, 1982; Gubanov, 1996; Liu *et al.*, 2001). Most of the Mongolian *Scorzonera* spp. are used in traditional medicine and as forage for livestock, especially in desert regions (Sancher *et al.*, 2003). *S. radiata* Fisch. is a typical mesophyte and a perennial herbaceous rosette plant, which is widely distributed in Hangai, Douria, Kobodo, Mongolia-Altai, and East-Mongolia (Liu *et al.*, 2002). It grows at an elevation between 900 and 1800 m above sea level, on rubble slopes, underbrush, forest fringe, meadow, and the gravel zone of floodplains. All parts of the plant are used in Mongolian folk medicine for the treatment of poisonous ulcers, fever caused by bacterial and viral infections, as well as for its diuretic and galactagogue properties (Ligaa, 1996; He, 2004).

Previous phytochemical studies on this genus indicated it is rich in phenolic compounds and yields stilbene derivatives, sesquiterpene lactones, lignans, phenolic acids, flavonoids, dihydroisocoumarins, in addition to triterpenes (Tolstikhina and Semenov, 1998; Tolstikhina *et al.*, 1988, 1999; Öksüz *et al.*, 1990; MacLeod and Ames, 1991; Bryanskii *et al.*, 1992a, b; Menichini *et al.*, 1994; Zidorn *et al.*, 2000a, b, 2002, 2003, 2005; Paraschos *et al.*, 2001; Li *et al.*, 2004; Sari *et al.*, 2007; Tsevegsuren *et al.*, 2007). *S. radiata* has not been investigated as yet. In this paper, we report the isolation and structure elucidation of three new natural compounds from this species, two flavonoids and one quinic acid congener, and twelve known phenolic constituents. Furthermore we report the antioxidant activities of the nine isolated quinic acid derivatives.

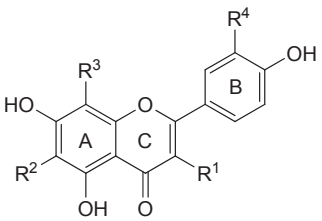
Results and Discussion

Structure elucidation

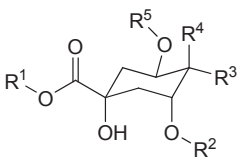
Analysis of a crude MeOH extract of aerial parts of *S. radiata*, by HPLC-DAD and LC-MS, indicated the presence of several unknown phe-

nolic compounds in addition to known derivatives such as flavonoids and quinic acid congeners (Fig. 1). By chromatographic separation the new flavonoids, scorzonerin A (**1**), scorzonerin B (**2**), and the likewise new 4,5-dicaffeoyl-*epi*-quinic acid (**3**) were obtained.

Compound **1** was isolated as a yellow amorphous solid. Its molecular formula was determined as $C_{27}H_{30}O_{14}$ by HRESIMS (*m*/*z* 579.1708 $[M + H]^+$). The UV spectrum of **1** showed absorption maxima at 335, 272, and 215 nm, and closely resembled that of isovitexin. The ^1H and ^{13}C NMR spectra of **1** (Table I) confirmed the presence of a flavone glycoside characterized by an AA′BB′ system with signals at δ_H 6.97 ppm (d, J = 8.0 Hz, H-3′ and H-5′)/δ_C 117.2 ppm (C-3′ and C-5′) and δ_H 7.88 ppm (d, J = 8.0 Hz, H-2′ and H-6′)/δ_C 129.5 ppm (C-2′ and C-6′), and a further aromatic proton signal at δ_H 6.62 ppm (s, H-3)/δ_C 103.6 ppm (C-3). The identity of the two sugar units followed from the magnitude of the vicinal coupling constants determined from the 1D ^1H NMR and connectivities from the 2D COSY and TOCSY spectra. In the ^1H-^1H COSY spectrum, a cross-peak was observed between the anomeric proton at δ_H 4.90 ppm (d, J = 9.8 Hz) and the broad triplet at δ_H 4.57 ppm, corresponding to H-2″, which was further coupled to the double doublet of H-3″



Compound	R^1	R^2	R^3	R^4
Scorzonerin A (**1**)	H	*C*-*β*-galactosyl	*C*-*α*-rhamnosyl	H
Violanthin	H	*C*-*β*-glucosyl	*C*-*α*-rhamnosyl	H
Isoorientin	H	*C*-*β*-glucosyl	H	OH
Scorzonerin B (**2**)	*C*-*α*-rhamnosyl	H	*C*-*β*-glucosyl	H
Kaempferol 3-*O*-rutinoside	*O*-*β*-glucosyl-6″-*O*-*α*-rhamnosyl	H	H	H
Rutin	*O*-*β*-glucosyl-6″-*O*-*α*-rhamnosyl	H	H	OH



Compound	R^1	R^2	R^3	R^4	R^5
(–)-Quinic acid	H	H	OH	H	H
4,5-Dicaffeoyl-*epi*-quinic acid (**3**)	H	H	H	*O*-caffeoyl	caffeoyl
4,5-Dicaffeoylquinic acid	H	H	*O*-caffeoyl	H	caffeoyl
3,5-Dicaffeoyl-*epi*-quinic acid	H	caffeoyl	H	OH	caffeoyl
3,5-Dicaffeoylquinic acid	H	caffeoyl	OH	H	caffeoyl
Macroantoin F	CH_3	H	*O*-caffeoyl	H	caffeoyl
Macroantoin G	CH_3	caffeoyl	OH	H	caffeoyl
Chlorogenic acid	H	H	OH	H	caffeoyl
5-*p*-Coumaroylquinic acid	H	H	OH	H	*p*-coumaroyl

Fig. 1. Compounds isolated from the MeOH extract of the aerial parts of *S. radiata*. Known compounds were elucidated by comparison of their NMR and MS data with reported values.

Table I. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of scorzonerin A (**1**) in CD_3OD.

No.	δ_H (mult., *J* in Hz)	δ_C	No.	δ_H (mult., *J* in Hz)	δ_C
Apigenin			6-*C*-galactosyl		
2		165.7	1″	4.90 (1H, d, *J* = 9.8)	75.6
3	6.62 (1H, s)	103.6	2″	4.57 (1H, br. t)	70.0
4		183.9	3″	3.60 (1H, dd, *J* = 9.5, 2.4)	77.0
5		161.5	4″	4.00 (1H, d, *J* = 2.4)	71.3
6		109.9	5″	3.67 (1H, t, *J* = 5.5)	80.8
7		166.8	6″	3.78 (1H, m[a]), 3.76 (1H, m[a])	62.8
8		104.4	8-*C*-rhamnosyl		
9		155.9	1‴	5.33 (1H, s)	77.4
10		104.1	2‴	4.10 (1H, br. s)	74.0
1′		123.2	3‴	3.72 (1H, br. d, *J* = 9.0)	76.3
2′	7.88 (1H, d, *J* = 8.0)	129.5	4‴	3.63 (1H, t, *J* = 9.0)	73.9
3′	6.97 (1H, d, *J* = 8.0)	117.2	5‴	3.56 (1H, dq, *J* = 8.8, 5.8)	79.1
4′		163.1	6‴	1.44 (3H, d, *J* = 5.8)	18.5
5′	6.97 (1H, d, *J* = 8.0)	117.2			
6′	7.88 (1H, d, *J* = 8.0)	129.5			

[a] Overlapping signals assigned by ^{1}H-^{1}H COSY, HMBC, and TOCSY spectra without designating multiplicity.

at δ_H 3.60 ppm. Hence all three protons were in axial positions of a *β*-galactopyranose ring system that was evident from the small vicinal couplings to H-4″ that clearly distinguished it from a *β*-glucopyranose system found in violanthin (Carnat *et al.*, 1998). Similarly the magnitude of the vicinal coupling constants and chemical shifts of the second sugar unit, with the anomeric proton at δ_H 5.33 ppm, indicated this was a rhamnopyranose system. We assumed that the rhamnose had an *α*-anomeric configuration and the absolute configuration of the galactose and rhamnose units were D and L, respectively. The linkage of the sugar moieties to the flavone aglycone were established from HMBC data (Fig. 2) which allows unambiguous assignment of the position of the sugar substituents. This is possible as the ^{13}C chemical shift of C-6 was to low field of C-8 in di-C6,C8-glycosyl flavanoids (Markham and Chari, 1982; Carnat *et al.*, 1998). The anomeric proton

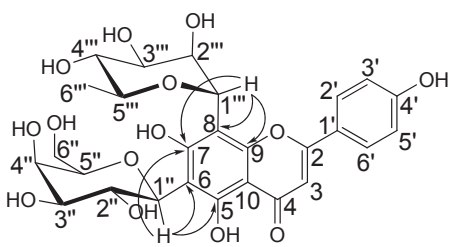


Fig. 2. Key HMBC correlations of **1**.

at δ_H 4.90 ppm of the galactose moiety showed a HMBC correlation with C-6 (δ_C 109.9 ppm) in ring A of the flavone and two hydroxylated carbon atoms C-5 at δ_C 161.5 ppm and C-7 at δ_C 166.8 ppm, respectively, thus establishing the *C*-glycosidic linkage at C-6 of the flavone nucleus. The second anomeric proton at δ_H 5.33 ppm belonging to the rhamnose moiety gave a HMBC cross-peak with the hydroxylated carbon atom at δ_C 166.8 ppm (C-7), the oxygen-bearing carbon atoms C-9 (δ_C 155.9 ppm) and C-8 (δ_C 104.4 ppm), respectively, indicating that the rhamnose moiety was bound to C-8 via a *C*-glycosidic linkage, similar to violanthin (Carnat *et al.*, 1998). Thus **1** was considered to be apigenin-6-*C*-*β*-D-galactopyranosyl-8-*C*-*α*-L-6-rhamnopyranoside and given the trivial name scorzonerin A.

Compound **2** was isolated as a yellow amorphous solid with a molecular formular of $C_{27}H_{30}O_{14}$ by HRESIMS (*m*/*z* 579.1708 $[M + H]^+$). The UV spectrum of **2** showed absorption maxima at 335, 272, and 215 nm similar to **1**. In the aromatic region of the ^{1}H NMR spectrum of **2** (Table II), an AA′BB′ system with signals at δ_H 7.83 ppm (d, *J* = 8.8 Hz, H-2′ and H-6′) and 6.94 ppm (d, *J* = 8.8 Hz, H-3′ and H-5′) and one singlet signal at δ_H 6.53 ppm (H-6) was observed, characteristic of an apigenine moiety. In the upfield region, two anomeric protons were presented at δ_H 4.92 ppm (d, *J* = 9.9 Hz) and 5.29 ppm (br. s), which differed from the chemical shifts of anomeric protons of *O*-glycosides that are usually observed at

δ_H 5–6 ppm (Hesse *et al.*, 1997). Along with the molecular weight and the residual resonances of the ^1H and ^{13}C NMR spectra, respectively, the presence of two carbon-bound sugar units was inferred. The connections between the flavone aglycone and the sugar moieties were determined by ROESY and HMBC spectra (Fig. 3). The aromatic proton at δ_H 7.83 ppm (H-2′ and H-6′) gave a ROESY cross-peak with the anomeric proton of the rhamnose moiety at δ_H 5.29 ppm, which further correlated with the oxygen-bearing carbon atom C-2 at δ_C 156.9 ppm and C-3 at δ_C 105.8 ppm in the HMBC spectrum, thus establishing the linkage of the rhamnose moiety at C-3 of the flavone core. Attachment of the glucose moiety was determined from the HMBC cross-peaks of the anomeric proton at δ_H 4.92 ppm (d, J = 9.9 Hz) with the oxygen-bearing carbon atoms C-9 at δ_C 162.0 ppm and C-8 at δ_C 110.8 ppm in ring A, respectively, which indicated that the glucose unit was attached to C-8 via a *C*-glycosidic bond. This was corroborated from the chemical shifts of the ^{13}C NMR spectrum of **2** (Table II) and comparison with the ^{13}C NMR data of reference compounds (Markham and Chari, 1982). Thus the differences in ^{13}C chemical shifts of C-6 and C-8 in mono-*C*-glycosyl ring A-substituted flavonoids were characteristic of the respective linkages. Thus flavanoids with 6-C-sugar substituents, as in isoorientin and isoaffinetin, have a chemical shift difference Δ(6–8) of ca. 15 ppm, while the difference of corresponding 8-C-sugar substituents Δ(8–6), as in vitexin and adonivernith, is smaller and in the range 5–8 ppm. Hence the difference of 6.2 ppm found for **2** was compatible with a C-sugar substituent at C-8. From these data, we concluded **2** is apigenin-3-*C*-*α*-L-6-rhamnopyranosyl-8-*C*-*β*-D-glucopyranoside, to which we assigned the trivial name scorzonerin B.

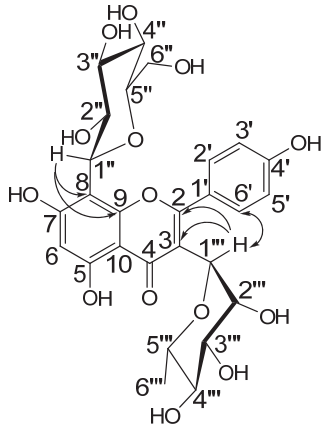


Fig. 3. Key HMBC (→) and ROESY (↔) correlations of **2**.

Compound **3** was obtained as an amorphous solid. The molecular formula was determined as $C_{25}H_{24}O_{12}$ from the HRESIMS data (m/z 517.1341

Table II. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of scorzonerin B (**2**) in CD_3OD.

No.	δ_H (mult., J in Hz)	δ_C	No.	δ_H (mult., J in Hz)	δ_C
Apigenin			8-*C*-glucosyl		
2		156.9	1″	4.92 (1H, d, J = 9.9)	75.5
3		105.8	2″	4.48 (1H, dd, J = 9.9, 9.2)	71.8
4[a]			3″	3.48 (1H, dd, J = 9.2, 9.1)	80.7
5		165.1	4″	3.59 (1H, dd, J = 9.7, 9.1)	71.6
6	6.53 (1H, s)	103.0	5″	3.40 (1H, ddd, J = 9.7, 4.7, 2.2)	82.3
7		165.5	6″	3.86 (1H, dd, J = 12.1, 2.2), 3.78 (1H, dd, J = 12.1, 4.7)	62.8
8		110.8	3-*C*-rhamnosyl		
9		162.0	1‴	5.29 (1H, br. s)	76.9
10		102.3	2‴	4.12 (1H, d, J = 3.0)	74.2
1′		123.0	3‴	3.69 (1H, dd, J = 9.1, 3.0)	77.1
2′	7.83 (1H, d, J = 8.8)	129.2	4‴	3.63 (1H, t, J = 9.1)	74.1
3′	6.94 (1H, d, J = 8.8)	117.6	5‴	3.55 (1H, dq, J = 9.1, 6.1)	79.0
4′		163.9	6‴	1.40 (3H, d, J = 6.1)	18.4
5′	6.94 (1H, d, J = 8.8)	117.6			
6′	7.83 (1H, d, J = 8.8)	129.2			

[a] Due to the low amount of compound, no ^{13}C NMR signal could be obtained.

$[M + H]^+$). The UV spectrum of **3** showed absorption maxima at 325, 243, and 218 nm typical of a caffeic acid derivative. The ^{1}H NMR data were very similar to those of the caffeic acid moieties of 4,5-dicaffeoylquinic acid, but differed from known compounds with regard to the signals of the quinic acid moiety (Pauli *et al.*, 1998; Lin *et al.*, 1999). The ^{1}H NMR spectrum of **3** (Table III) showed two pairs of doublets with coupling constants of 15.9 Hz indicative of *trans* olefinic protons found in hydroxycinnamic acids. In the aromatic region, resonances for two ABX systems [δ_H 7.05 ppm (d, J = 2.1 Hz), 6.78 ppm (d, J = 8.1 Hz), and 6.92 ppm (dd, J = 8.1, 2.1 Hz); and δ_H 7.09 ppm (d, J = 2.2 Hz), 6.80 ppm (d, J = 8.2 Hz), and 6.97 ppm (dd, J = 8.2, 2.2 Hz)] were observed, which were assigned to two 1,3,4-trisubstituted phenyl units. From these observations, along with the analysis of the ^{13}C NMR data (Table III), the presence of two caffeic acid moieties was inferred. The assignments were further supported by analysis of the ROESY spectrum of **3** (Fig. 4). The protons at δ_H 7.05 ppm (H-2′) and 7.09 ppm (H-2″) gave ROESY cross-peaks with the olefinic protons at δ_H 7.55 ppm (H-7′) and 7.62 ppm (H-7″). The presence of the quinic acid moiety was indicated by ^{1}H NMR resonances of three oxymethine protons at δ_H 5.67 ppm (ddd, J = 8.6, 4.1, 3.3 Hz), 5.24 ppm (dd, J = 6.1, 3.0 Hz), and 4.16 ppm (ddd, J = 5.6, 5.4, 5.3 Hz), together with two pairs of sp^3 methylene protons at δ_H 2.22/2.10 ppm and 2.11/2.08 ppm for H_2–6 and H_2–2, respectively. All of the latter are characteristic of a quinic acid unit, with regard to their multiplicity and coupling patterns. The assignments of the protons of the quinic acid nucleus were corroborated by analysis of the ^{1}H-^{1}H COSY and ROESY spectra of **3**. The attachment of caffeoyl moieties at C-4 and C-5, respectively, of the quinic acid part was deduced from the HMBC correlation of H-4 and H-5, respectively, with their ester carbonyl carbon atoms (C-9′ and C-9″) at δ_C 168.3 ppm. The deshielded resonances of two oxymethine protons in the quinic acid nucleus at δ_H 5.67 ppm (H-5) and 5.24 ppm (H-4) implied acylation of the hydroxy group at these positions as earlier reported for other naturally occurring quinic acid derivatives (Pauli *et al.*, 1998; Lin *et al.*, 1999). From these observations, the structure of **3** was initially thought to be that of the known compound 4,5-dicaffeoylquinic acid. However, the ^{1}H NMR spectrum of **3** showed slightly but distinctly different peak patterns of the quinic acid unit compared to the known 4,5-dicaffeoylquinic acid [δ_H 4.29 ppm (dt, J = 3.2, 2.8 Hz, H-3), δ_H 5.09 ppm (dd, J = 10.4, 3.2 Hz, H-4), and δ_H 5.69 ppm (dt, J = 6.6, 10.4 Hz, H-5)], which was also isolated from this plant. The struc-

Table III. ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR data of 4,5-dicaffeoyl-*epi*-quinic acid (**3**) in CD_3OD.

No.	δ_H (mult., J in Hz)	δ_C	No.	δ_H (mult., J in Hz)	δ_C
1		75.5			
2_{ax}	2.11 (1H, m[a])	38.0			
2_{eq}	2.08 (1H, dd, J = 13.1, 5.3)				
3_{eq}	4.16 (1H, ddd, J = 5.6, 5.4, 5.3)	67.9			
4_{eq}	5.24 (1H, dd, J = 6.1, 3.0)	72.8			
5_{ax}	5.67 (1H, ddd, J = 8.6, 4.1, 3.3)	70.0			
6_{eq}	2.22 (1H, dd, J = 13.2, 4.1)	39.7			
6_{ax}	2.10 (1H, m[a])				
C_1–COOH		182.0			
1′		127.8	1″		127.8
2′	7.09 (1H, d, J = 2.2)	115.0	2″	7.05 (1H, d, J = 2.1)	115.0
3′		146.7	3″		146.7
4′		149.6	4″		149.6
5′	6.80 (1H, d, J = 8.2)	116.5	5″	6.78 (1H, d, J = 8.1)	116.5
6′	6.97 (1H, dd, J = 8.2, 2.2)	123.1	6″	6.92 (1H, dd, J = 8.1, 2.1)	123.1
7′	7.62 (1H, d, J = 15.9)	147.1	7″	7.55 (1H, d, J = 15.9)	147.1
8′	6.34 (1H, d, J = 15.9)	115.1	8″	6.28 (1H, d, J = 15.9)	115.1
9′		168.3	9″		168.3

[a] Overlapping signals assigned by ^{1}H-^{1}H COSY and HMBC spectra without designating multiplicity.

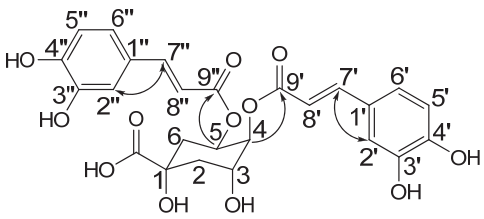


Fig. 4. Key HMBC (→) and ROESY (↔) correlations of **3**.

ture of 4,5-dicaffeoylquinic acid had been ascertained by a detailed comparison of the physical and spectral data with those of the literature (Pauli *et al.*, 1998).

Thus, we assumed that compound **3** is a conformational isomer of 4,5-dicaffeoylquinic acid. To elucidate the conformation of **3**, comprehensive NMR studies were undertaken. Firstly, a ROESY experiment was recorded and the data, together with the magnitude of the coupling constants and data from the COSY spectrum, compared to those of the known 4,5-dicaffeoylquinic acid. No clear ROESY cross-peaks from the oxymethine proton H-4 (δ_H 5.24 ppm) to any of the sp^3 methylene protons H-2 (δ_H 2.11 ppm and 2.08 ppm) or H-6 (δ_H 2.22 ppm and 2.10 ppm) were evident compared to those found for 4,5-dicaffeoylquinic acid, which suggested that H-4 is equatorial compared to its axial position in the known compound. Moreover, the physical properties (solubility, optical rotation) of **3** were different from those of the known 4,5-dicaffeoylquinic acid. Like other *epi*-quinic acid derivatives (Kim and Lee, 2005), **3** was of limited solubility in methanol, while the known derivative is freely soluble in this solvent. Conformational isomers of quinic acid have been investigated thoroughly, and three principal structures have been confirmed, namely (–)-quinic acid, (–)-*epi*-quinic acid and (+)-quinic acid (Kim and Lee, 2005). The negative optical rotation of **3** of $[\alpha]_D^{20}$ –32° eliminated the probability of a (+)-quinic acid derivative, and the magnitude differed from that of the known 4,5-dicaffeoylquinic acid ($[\alpha]_D^{20}$ –74°). Taken together these data indicate **3** contains the *epi*-isomer of quinic acid and is 4,5-dicaffeoyl-*epi*-quinic acid.

However the coupling constants in the ^{1}H NMR spectrum of **3** measured at 600 MHz were not in agreement with a single chair-like conformation as shown in Fig. 1. Detailed studies (Flores-Parra *et al.*, 1989; Eliel and Ramirez, 1997) had disclosed that the quinic acid moiety exists as two conformers in rapid equilibrium. Therefore the ^{1}H NMR spectrum of **3** was measured at low temperature (300 K, 273 K, and 253 K). Although the spectra recorded for **3** were not absolutely unambiguous, it did appear that lowering the temperature from 300 K to 253 K caused a broadening of the signals of H-4 and H-3 of the major isomer. This implied that the molecule exists as an equilibrium mixture of various (approximately 3) conformers at room temperature. This would rationalize the unusual couplings observed for H-3 (ddd, J = 5.6, 5.4, 5.3 Hz).

Antioxidant activity

Free radicals can participate in unwanted side reactions resulting in cell damage inducing atherosclerosis and cancers. Phenolic compounds from plants are effective antioxidant constituents, which can prevent those oxidative stress-related diseases. The main mechanism of action of phenolic antioxidants is considered to be the scavenging of free radicals by hydrogen atom donation, although other mechanisms may be involved (Nenadis and Tsimidou, 2002; Balasundram *et al.*, 2006). Radical scavenging activity of the quinic acid derivatives, including compound **3**, isolated from *S. radiata* was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. As a reference compound, the well known naturally occurring antioxidant resveratrol was included. For each of the compounds, IC_{50} values were determined (Table IV). The caffeoyl quinic acid congeners were considerably more active than 5-*p*-coumaroylquinic acid, since phenolic compounds with vicinal OH groups have a higher radical scavenging activity than monohydroxylated isomers (*p*-coumaric

Table IV. IC_{50} values of quinic acid derivatives in the DPPH assay.

Compound	IC_{50} [μM]
4,5-Dicaffeoyl-*epi*-quinic acid (**3**)	40.6
4,5-Dicaffeoylquinic acid	42.2
3,5-Dicaffeoyl-*epi*-quinic acid	40.4
3,5-Dicaffeoylquinic acid	41.5
Macroantoin F	40.5
Macroantoin G	41.3
Chlorogenic acid	48.7
5-*p*-Coumaroylquinic acid	378.2
Resveratrol	149.5

acid) (Rice-Evans *et al.*, 1996). The antioxidant efficiency of chlorogenic acid (monocaffeoyl quinic acid) was found to be weaker than those of the dicaffeoylquinic acids. 4,5-Dicaffeoyl-*epi*-quinic acid (**3**) and 3,5-dicaffeoyl-*epi*-quinic acid exhibited slightly stronger antioxidant activities compared to 4,5-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid, respectively. Macroantoins F and G (Fig. 1) had IC_{50} values in the DPPH assay similar to those of 4,5-dicaffeoyl-*epi*-quinic acid and 3,5-dicaffeoylquinic acid, thus methoxylation of the carboxyl group of the quinic acid moiety did not decrease the radical scavenging activity.

The solutions of the two new flavonoids **1** and **2** were found to show very strong UV-visible absorption. The UV absorption intensity of **2** in 25 μM solution was even higher than that of 100 μM DPPH solution, which was prepared as a negative control. Hence the method we used for the DPPH assay does not allow an assessment of their activities. However, when a qualitative analysis of the antioxidant activity of the isolated flavonoids was performed on TLC plates, only isoorientin and rutin showed activity, suggesting only compounds with the *ortho*-dihydroxy structure in the flavonoid B-ring of flavonoids are active (Rice-Evans *et al.*, 1996; Balasundram *et al.*, 2006).

Experimental

General

Optical rotations were recorded on a Perkin-Elmer (Überlingen, Germany) 241 MC polarimeter. 1D and 2D NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on Bruker (Rheinstetten, Germany) ARX 500 and DMX 600 NMR spectrometers using the standard Bruker software and CD_3OD as solvent. NMR spectra were referenced to the solvent signal. ESI mass spectra were obtained on a ThermoFinnigan (Egelsbach, Germany) LCQ DECA mass spectrometer coupled to an Agilent (Waldbronn, Germany) 1100 HPLC system that included an on-line photodiode array detector (DAD). HRESIMS spectra were determined on a ThermoFinnigan LTQ-Orbitrap FT-ESIMS instrument. For HPLC analysis, 20-μl samples were injected into an HPLC system (Dionex, Munich, Germany) equipped with a DAD, employing a linear gradient from 0.1% phosphoric acid to MeOH (HPLC grade; Merck, Darmstadt, Germany) for 35 min. Routine detection was at 254 nm. The separation column (125 × 4 mm, i.d.) was prefilled with 5 μm Eurospher-100 C18 (Knauer, Berlin, Germany). The temperature of the column oven was set at 20 °C. Semipreparative HPLC was performed on a Merck-Hitachi (Darmstadt, Germany) instrument (Eurospher-100 C18, L-7100 pump, and L-7400 UV detector). TLC was performed on TLC plates precoated with Si 60 F_{254} (Merck) using EtOAc/HCOOH/H_2O (85:10:5, v/v/v) as solvent system. The compounds were detected from their UV absorbance and by spraying the TLC plates with anisaldehyde reagent. Radical scavenging activities by DPPH were measured on a Perkin-Elmer Lambda 25 UV/VIS spectrometer.

Plant material

Aerial parts of *S. radiata* Fisch. were collected in July 2004 in Khandgait am forest, Ulaanbaatar region, Mongolia. The plant was identified by Prof. Sc. D. Sh. Darijmaa (Mongolian State University of Education). Voucher specimens (ts-15–02072004-khandgaitUB) have been deposited in the herbarium section of the Department of Organic and Food Chemistry, National University of Mongolia, Ulaanbaatar, Mongolia.

Extraction and isolation

The air-dried, powdered plant material of *S. radiata* (300 g) was extracted exhaustively by maceration with MeOH (3 × 400 ml) at room temperature. The total extract was concentrated to dryness *in vacuo*. The concentrated gum (32.0 g) was reconstituted with 100 ml of MeOH/H_2O (3:7, v/v) and then partitioned successively with *n*-hexane (5 × 100 ml), EtOAc (5 × 100 ml), and *n*-BuOH (5 × 100 ml) to give the *n*-hexane, EtOAc, *n*-BuOH, and aqueous fractions. Solvents (technical grade) were distilled prior to use, and spectral grade solvents (Merck) were used for spectroscopic measurements.

The *n*-BuOH fraction of the MeOH extract derived from the aerial parts of *S. radiata* (4.20 g) was separated by Sephadex LH-20 column chromatography (CC) using MeOH as mobile phase to afford 16 fractions. Scorzonerin A (**1**, 9.2 mg, 0.002% yield) and scorzonerin B (**2**, 2.2 mg, 0.0005% yield) were isolated from fraction 6 by reversed-phase CC (RP-18) using gradient elu-

tion with MeOH/H_2O as eluent. 4,5-Dicaffeoyl-*epi*-quinic acid (**3**, 3.1 mg, 0.0007% yield) was purified from fraction 15 by semipreparative HPLC utilizing RP-18 as stationary phase and mixtures of MeOH/H_2O as solvent.

Scorzonerin A (**1**): Amorphous solid. – $[\alpha]_D^{20}$ +5° (*c* 0.10, MeOH). – UV (MeOH/H_2O): λ_{max} = 215, 272, 335 nm. – ^1H and ^{13}C NMR: see Table I. – (+) ESIMS: *m/z* = 579.1 $[M + H]^+$. – (–)ESIMS: *m/z* = 577.3 $[M - H]^-$. – HRESIMS: *m/z* = 579.1708 $[M + H]^+$ (579.1714 calcd. for $C_{27}H_{31}O_{14}$).

Scorzonerin B (**2**): Amorphous solid. – $[\alpha]_D^{20}$ –22° (*c* 0.10, MeOH). – UV (MeOH/H_2O): λ_{max} = 215, 272, 335 nm. – ^1H and ^{13}C NMR: see Table II. – (+)ESIMS: *m/z* = 579.3 $[M + H]^+$. – (–)ESIMS: *m/z* = 577.4 $[M - H]^-$. – HRESIMS: *m/z* = 579.1708 $[M + H]^+$ (579.1714 calcd. for $C_{27}H_{31}O_{14}$).

4,5-Dicaffeoyl-epi-quinic acid (**3**): Amorphous solid. – $[\alpha]_D^{20}$ –32° (*c* 0.10, MeOH). – UV (MeOH/H_2O): λ_{max} = 218, 243, 325 nm. – ^1H and ^{13}C NMR: see Table III. – (+)ESIMS: *m/z* = 516.9 $[M + H]^+$. – (–)ESIMS: *m/z* = 515.4 $[M-H]^-$. – HRESIMS: *m/z* = 517.1341 $[M + H]^+$ (517.1346 calcd. for $C_{25}H_{25}O_{12}$).

DPPH assay

Qualitative analysis of the radical scavenging activity of the extracts and fractions was carried out by spraying the TLC plates after development in an appropriate solvent system [EtOAc/HCOOH/H_2O (85:10:5)] with 1% DPPH reagent. Active components were observed as yellow bands against a violet background.

To quantify the antioxidative capacity, absorption at 517 nm was determined after a test sample dissolved in 10 μl of MeOH had reacted with 490 μl DPPH solution (100 μM) at room temperature. Incubation time was 5 min. Prior to measurement, the difference in absorption between a DPPH blank solution and the positive control (propylgallate, 100 μM) was determined. This difference was then taken as 100% antioxidative activity. The percent antioxidative activity was calculated from the difference in absorption between the test sample at 100 μM and the DPPH blank as follows (Tsevegsuren *et al.*, 2007):

$$a_A\,(\%) = [(A_B - A_P)/(A_B - A_{Pos})] \cdot 100,$$

where a_A is the percent antioxidative activity compared to the positive control, A_B is the absorption of the DPPH blank solution, A_P is the absorption of the test sample, and A_{Pos} is the absorption of the positive control (propylgallate). Measurements were performed in triplicate, and IC_{50} values were calculated by linear regression.

Acknowledgements

We are grateful to Prof. Sc. D. Sh. Darijmaa from the Mongolian State University of Education for collection and identification of the plant material. We also thank C. Kakoschke and B. Jaschok-Kentner for NMR data (Helmholtz Centre for Infection Research, Braunschweig, Germany). Financial support of this project by grants of the BMBF and of MOST to P. P. and W. L. is gratefully acknowledged. N. T. thanks the Humboldt Foundation, the ARC-NUM project, and for a DFG scholarship.

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