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Biologically Active Natural Products from Mongolian Medicinal Plants *Scorzonera divaricata* and *Scorzonera pseudodivaricata*

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Chromatographic separation of a crude extract obtained from the aerial parts of the Mongolian medicinal plant *Scorzonera divaricata* yielded the two new quinic acid derivatives feruloylpodospermic acids A and B. Both compounds feature a feruloyl group and two dihydrocaffeoyl substituents. For feruloylpodospermic acid A, the dihydrocaffeic acid substituents were found esterified at positions 1 and 5 of the quinic acid moiety, while the feruloyl group was attached at position 3. For feruloylpodospermic acid B, the substituents were linked at positions 1, 3, and 4. The aerial parts of *S. pseudodivaricata* that are likewise used in Mongolian traditional medicine yielded two further new natural products, for which the names scorzoneric acid and scorzonerin are proposed. Scorzoneric acid is an unusual phenolic compound featuring a central tetrasubstituted phenyl ring to which a glucose unit is bound, which in turn is substituted by an esterified acyl side chain. Further substituents of the central phenyl ring system include a butan-2-one group, which is linked to a second *para*-substituted phenyl ring system. Scorzonerin is a matricarin-based sesquiterpene lactone that carries an esterified dihydrocoumaric acid moiety, which in turn is glycosidically bound to glucose. The structures of all new compounds were unambiguously established from NMR (^{1}H, ^{13}C, COSY, HMBC) spectroscopic and mass spectrometric data. The new quinic acid derivatives feruloylpodospermic acids A and B exhibited strong antioxidative activity when analyzed in the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

About 170 species of the genus *Scorzonera* (family Asteraceae) are distributed around the world.[1,2] Chemically, the genus, like many other members of the tribe Lactuceae, is characterized by the accumulation of terpenoids, consisting mainly of polyisoprenes, triterpenes, and diterpenoid resins deposited in parenchymatic cells of roots and stems in amounts up to 20−30% of the dry matter.[3] Some species of the genus have been widely utilized as food and medicinal supplements. *S. hispanica*, known as vipers grass, is used as a nutritional condiment to stimulate appetite and as an herbal medicine, especially against pulmonary diseases, as well as colds. Furthermore, it was described to be a stomachic, diuretic, and antipyretic. Its roots are edible and may be used as a coffee substitute. Another species, *S. humilis*, known as humble vipers grass, was even exploited to adulterate *Arnica*, while its young leaves are consumed as vegetables. Of industrial importance are *S. kirghisorum* and *S. tau-saghyz*, which are considered as valuable sources of natural rubber. [1]

Eleven species of *Scorzonera* are found in Mongolia, one of which is endemic, while five are subendemic.[4] Two of the latter, *S. divaricata* and *S. pseudodivaricata*, are used in folk medicine in Mongolia. Both species are widely distributed in Middle Khalkha, East Mongolia, Depression of Great Lakes, Valley of Lakes, East Gobi, Govi Altai, Transaltai Gobi, and Alashan Gobi. They grow on thin sandy desert steppes, rubble slopes, and terrains of mountains and knolls, or at the bottom of dry riverbeds. *S. divaricata* Turcz is a perennial herbaceous plant reaching 15−30 cm in height. Its root and herbs are reported to have antipyretic and antidote activity and are used in traditional medicine for treatment of poisonous ulcers as well as malignant stomach neoplasia.[5] *S. pseudodivaricata* Lipsch is also a perennial herbaceous rosette plant, 50 cm in height with yellow flower heads. It is also widely used in Mongolian traditional medicine for the treatment of diarrhea, parasitic diseases, lung edema, and fever caused by bacterial and viral infections, as well as for its diuretic properties.[6]

No phytochemical studies have been recorded for *S. divaricata* and *S. pseudodivaricata*. For the genus *Scorzonera*, previous chemical investigations have been carried out on *S. austriaca*, *S. hispanica*, *S. humilis*, *S. tomentosae*, *S. cretica*, and *S. columnae*, resulting in the isolation of sesquiterpenes,[7−10] lignans,[11,12] neolignans,[13] phenolic acids, triterpene derivatives,[9,14] stilbene derivatives,[15] dihydroisocoumarins,[16] and flavonoids.[17] In this paper, we report the isolation, structure elucidation, and biological activity of four new natural products from the aerial parts of *S. divaricata* and *S. pseudodivaricata*.

Results and Discussion

Analysis of the EtOAc extract of a MeOH extract of the respective aerial parts of *S. divaricata* and *S. pseudodivaricata*, using HPLC-DAD and LC-MS, indicated the presence of several unknown natural products in addition to known compounds such as scopoletin, simple quinic acid congeners, and flavonoid derivatives as shown in Table 1. Our interest was focused on the isolation of new biologically active compounds, which were monitored by a bioassay-guided isolation scheme in parallel with the analysis of the HPLC-DAD/MS chromatograms of the fractions. Further chromatography of the EtOAc extract of *S. divaricata* yielded two new DPPH-active feruloylquinic acid derivatives, for which we propose the names feruloylpodospermic acids A (**1**) and B (**2**). From *S. pseudodivaricata*, a novel phenolic compound and a new sesquiterpene lactone glycoside were isolated, for which we designated the trivial names scorzoneric acid (**3**) and scorzonerin (**4**), respectively.

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Table 1. Known Compounds[a] from the EtOAc Extracts of the Aerial Parts of *S. divaricata* and *S. pseudodivaricata*

S. *divaricata*	S. *pseudodivaricata*
Quinic acid derivative	Quinic acid derivative
• chlorogenic acid	• 3,5-caffeoyl quinic acid
Flavonoids	Flavonoids
• apigenin	• isovitexin-2″-*O*-xyloside
• isovitexin-4′-*O*-glucoside	• luteolin
• isovitexin-2′-*O*-xyloside	• luteolin-7-glucoside
• kaempferol-3-*O*-rutinoside	• luteolin-5-glucoside
Coumarin derivative	Coumarin derivative
• scopoletin	• scopoletin
	Other phenolic compounds
	• platyphylloside

[a] Known compounds were elucidated by comparison of their NMR and MS data with reported values. Flavonoids were also identified by matching the UV spectra of the known compound previously recorded in the HPLC-DAD database library and by coelution studies with reference standards.

Compound **1**, obtained as an amorphous solid, was shown to have the molecular formula $C_{35}H_{36}O_{15}$ as determined by HRESIMS data (*m*/*z* 719.1950 $[M + Na]^+$). The UV spectrum of **1** showed absorption maxima at 329, 286 (sh), and 220 nm. The ^{1}H NMR data in DMSO-d_6 were comparable to those of podospermic acid[18] with the exception of the presence of *trans*-olefinic protons. However, the solvent was changed to MeOD, which gave better signal resolution.[19] The ^{1}H NMR spectrum of **1** in CD_3OD (Table 2) showed a pair of doublets with coupling constants of 16.0 Hz due to the *trans* olefinic protons of H-2″ and H-3″ as found in cinnamic acid. In the aromatic region, resonances for three ABX systems [δ_H 7.06 (d, $J = 2.0$ Hz), 6.98 (dd, $J = 8.2$ and 2.0 Hz), and 6.77 (d, $J = 8.2$ Hz); δ_H 6.64 (d, $J = 2.0$ Hz), 6.66 (d, $J = 8.2$ Hz), and 6.53 (dd, $J = 8.2$ and 2.0 Hz); and δ_H 6.52 (d, $J = 2.0$ Hz), 6.46 (d, $J = 7.9$ Hz), and 6.17 (dd, $J = 7.9$ and 2.0 Hz)] were observed, which were assigned to three 1,3,4-trisubstituted phenyl units. A methyl resonance at δ 3.71 indicated an *O*-methyl function. From these observations, along with the analysis of the ^{13}C NMR data (Table 2), a caffeic or ferulic acid unit and two dihydrocinnamic acid moieties were inferred to be present. The assignments were further supported by analysis of the ^{1}H−^{1}H COSY and HMBC spectra of **1** (Figure 2). The attachment of the *O*-methyl group was shown from the HMBC cross-peak of the methyl singlet at δ_H 3.71 with δ_C 151.2, which further correlated with the *ortho*- and *meta*-protons at δ 6.77 and 7.06 of the ABX system, respectively. The proton at δ 7.06 gave a cross-peak with the olefinic methine at $\delta_{C-3''}$ 147.7, and this correlation unequivocally indicated the presence of a feruloyl substituent rather than a caffeic acid unit. The presence of two dihydrocaffeic acid moieties was evident from the deshielded carboxylic carbon resonances at δ_C 173.7 and 174.5, which are usually shielded at δ 169. In the HMBC spectrum, carbons C-1′ and C-1‴ correlated with methylene protons H-3′ and H-3‴ at δ 2.89, which in turn showed HMBC connectivities with C-5′/C-5‴ and C-9′/C-9‴ at ca. δ 117 and 121, respectively. These HMBC correlations allowed the carbon and proton assignments of the dihydrocaffeoyl substituents in compound **1**.

The quinic acid moiety was indicated by ^{1}H NMR resonances of three oxymethine protons at δ_H 5.40, 5.37, and 3.89, together with two pairs of sp^3 methylene protons at δ_H 2.39/2.95 and 1.92/2.58 for H_2-2 and H_2-6, respectively, as shown in Table 2. By inspection of the ^{13}C NMR spectrum, these resonances were in agreement with three oxymethine resonances at δ_C 72.1, 73.1, and 74.2; two sp^3 methylenes at δ_C 34.1 and 38.9; an oxygenated quaternary carbon at δ_C 83.5; and a carboxyl resonance at δ_C 174, all of which are characteristic of a quinic acid unit. The assignments for the quinic acid nucleus were further corroborated by analysis of the ^{1}H−^{1}H COSY and HMBC spectra of **1**. The deshielded resonances of two oxymethine protons in the quinic acid nucleus at δ_H 5.37 (H-3) and 5.40 (H-5) implied acylation of the hydroxy group at these positions as earlier reported for other natural quinic acid derivatives.[19,20] The attachment of the feruloyl moiety at C-3 was deduced from the HMBC correlation of H-3 with its ester carbonyl carbon (C-1″) at δ_C 168.6. Similarly, a cross-peak was observed between the dihydrocaffeoyl ester carbonyl carbon (C-1‴) at δ_C 174.5 and H-5 that indicated its connectivity at C-5. The remaining 1,3,4-trisubstituted phenyl unit, which is the second dihydrocaffeic acid group, was linked to the tertiary hydroxy group at C-1. This was evident from the ^{13}C NMR chemical shift of the C-1-oxygenated quaternary carbon, which was deshielded at δ_C 83.5 compared to that of quinic acid derivatives with free OH groups at C-1, for example in 3,5-di-*O*-caffeoyl-4-*O*-coumaroylquinic acid (δ_C 74.7).[21] A low-field shift was also previously observed for 1-acylquinic acid derivatives (e.g., δ_C 80.7, C-1 of 1,4,5-tri-*O*-caffeoylquinic acid).[22,23] From these results, the structure of compound **1** was concluded to be 3-*O*-feruloyl-1,5-di-*O*-dihydrocaffeoylquinic acid, for which we propose the name feruloylpodospermic acid A.

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Feruloylpodospermic Acids A (**1**) and B (**2**) in CD_3OD

1			**2**		
no.	δ_H, m, *J* (Hz)	δ_C, m	no.	δ_H, m, *J* (Hz)	δ_C, m
1		83.5 s	1		82.9 s
2 ax	2.39 bd 16.0	34.1 t	2	2.40 bd 12.0	34.0 t
2 eq	2.95 bd 16.0[a]			2.78 m	
3	5.37 m	73.1 d	3	5.53 m	72.5 d
4	3.89 dd 3.1 10.8	74.2 d	4	4.89[b]	71.6 d
5	5.40 m	72.1 d	5	4.25 m	73.7 d
6 ax	1.92 brt 12.5	38.9 t	6	1.98 dd 16.0, 9.6	38.9 t
6 eq	2.58 brd 12.0[a]			2.45 bd 16.0	
7		174.0 s	7		175.1 s
1′		173.7 s	1′		174.5 s
2′	2.70 m	38.3 t	2′	2.52 m	38.2 t
3′	2.89 m	31.5 t	3′	2.75 m	31.8 t
4′		134.1 s	4′		134.0 s
5′	6.64 d 2.0	117.0 d	5′	6.57 d 2.0	116.9 d
6′		146.6 s	6′		146.6 s
7′		145.1 s	7′		145.0 s
8′	6.66 d 8.2	116.9 d	8′	6.60 d 8.2	116.8 d
9′	6.53 dd 2.0, 8.2	121.1 d	9′	6.48 dd 2.0, 8.2	121.0 d
1″		168.6 s	1″		168.6 s
2″	6.37 d 16.0	116.7 d	2″	6.26 d 15.8	116.6 d
3″	7.66 d 16.0	147.7 d	3″	7.61 d 15.8	147.7 d
4″		128.0 s	4″		127.9 s
5″	7.06 d 2.0	111.9 d	5″	7.05 d 1.5	115.9 d
6″		149.9 s	6″		149.8 s
7″		151.2 s	7″		151.1 s
8″	6.77 d 8.2	116.0 d	8″	6.75 d 7.9	116.8 d
9″	6.98 dd 2.0, 8.2	124.9 d	9″	6.96 dd 1.5, 8.0	124.8 d
OMe	3.71 s	56.8 q	OMe	3.73 s	55.9 q
1‴		174.5 s	1‴		173.7 s
2‴	2.70 m	37.9 t	2‴	2.52 m	37.9 t
3‴	2.89 m	31.9 t	3‴	2.75 m	31.5 t
4‴		134.0 s	4‴		133.9 s
5‴	6.52 d 2.0	116.9 d	5‴	6.57 d 1.5	116.8 d
6‴		146.7 s	6‴		146.5 s
7‴		144.9 s	7‴		144.8 s
8‴	6.46 d 7.9	116.8 d	8‴	6.46 d 7.9	116.9 d
9‴	6.17 dd 2.0, 7.9	120.8 d	9‴	6.30 dd 1.5, 8.0	120.8 d

[a] A long-range planar *W*-coupling was observed in the 2D COSY spectrum. [b] Resonance is under the water signal and was detected in the 2D COSY spectrum.

Congener **2** had the same molecular formula of $C_{35}H_{36}O_{15}$ by HRESIMS (*m*/*z* 719.1950 $[M + Na]^+$) as **1**. The UV spectrum of **2** showed absorption maxima at 330, 286 (sh), and 220 nm, which are also comparable to those of **1**. Compound **2** is a positional isomer of **1**, which was deduced from inspection of the ^{1}H and ^{13}C NMR spectra of **2** (Table 2). As in **1**, three ABX systems were also present in **2**. The presence of a ferulolyl unit was also implied by an *O*-methyl resonance at δ_H 3.73/δ_C 55.9 and a *trans*-olefinic pair of doublets at δ_H 6.26 and 7.61. The occurrence of two dihydrocaffeic acid units was evident from the two pairs of

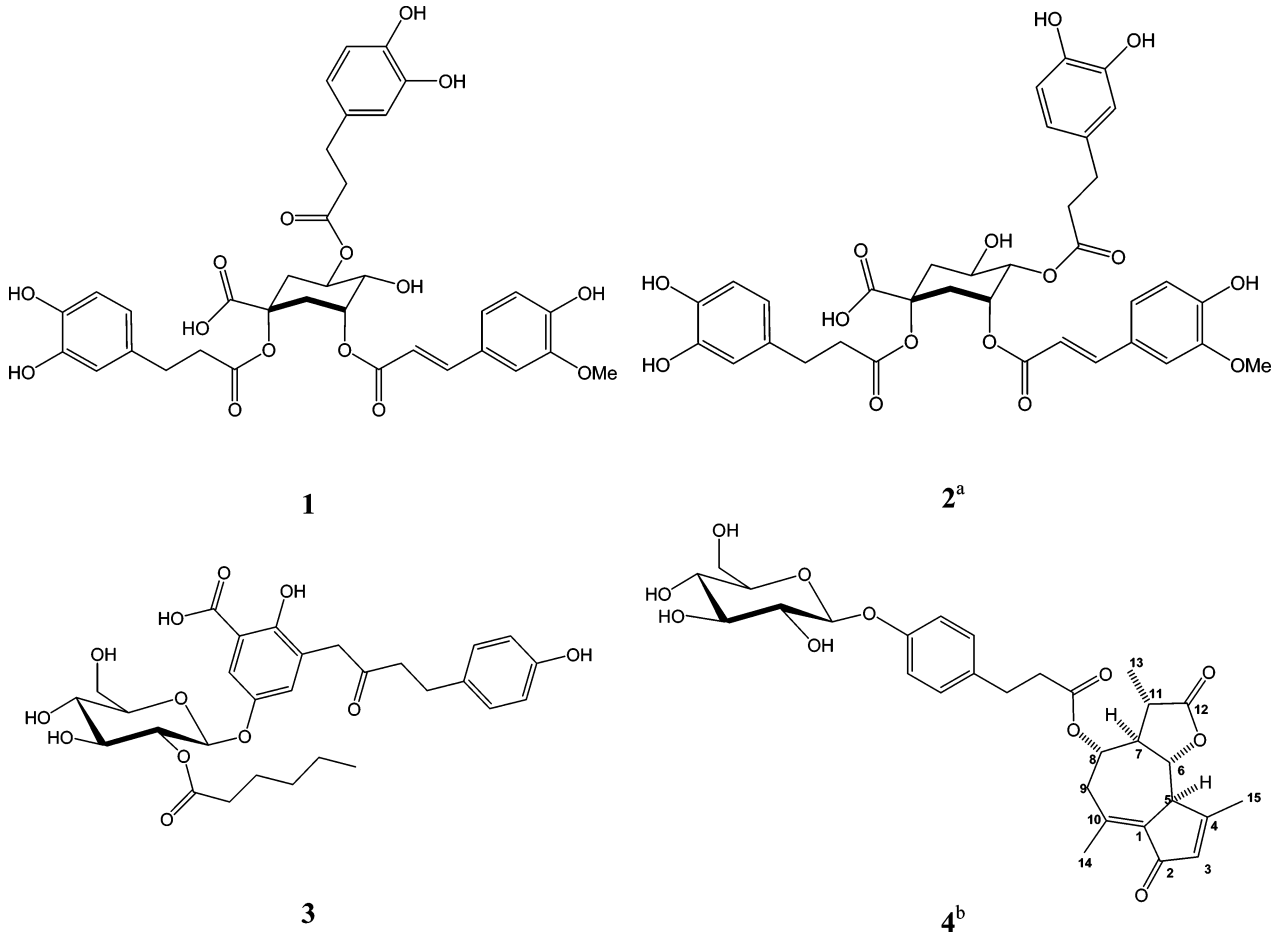


Figure 1. New compounds isolated from the aerial parts of *S. divaricata* and *S. pseudodivaricata.* [a]Structure is tentatively assigned. [b]Only relative configuration is shown.

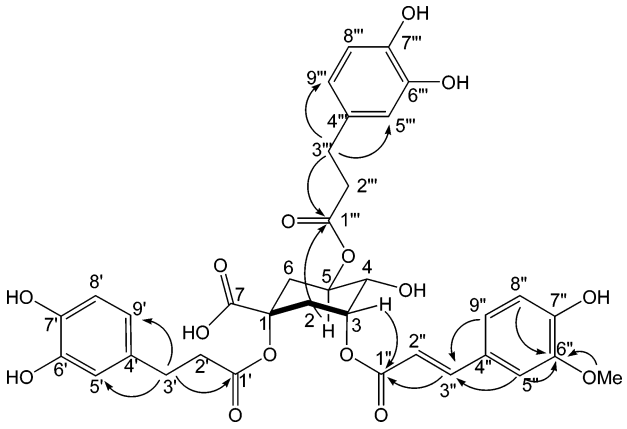


Figure 2. Key HMBC correlations in feruloylpodospermic acid A (**1**).

methylene proton resonances at δ 2.52 and 2.75, which exhibited direct correlations to triplet carbons at δ_C 31.8, 31.5, 37.9, and 38.2, as shown in the HMQC spectrum of **2**. Obvious differences in the NMR data between congeners **1** and **2** were observed for the quinic acid moiety, particularly for the oxymethine protons resonating at δ 4.89 and 4.25. The spin−spin coupling for the protons belonging to the quinic acid moiety was further established from the ^{1}H−^{1}H COSY spectra of **2**. Acylation of the hydroxy functionalities at C-3 and C-4 instead of C-3 and C-5 as in **1** was suggested by the downfield shifts of H-3 and H-4 by +1.45 and +1.50, respectively, compared to that of the ^{1}H NMR data of quinic acid. [19] Accordingly, when the ^{1}H NMR data of **2** were compared to those of **1**, H-5 was shifted upfield by −1.15 ppm while H-4 was shifted downfield by +1.0 ppm, which implied that the acylation was occurring at the C-4 hydroxy group instead of C-5.

However, positions of the aromatic substituents on the quinic acid nucleus cannot be unambiguously assigned by analysis of the HMBC spectrum of **2**. Due to a very diluted sample of compound **2**, no cross-peaks from H-3 (δ 5.53) or H-4 (δ 4.89) to the carbonyl units of any of the dihydrocaffeoyl units or the feruloyl moiety could be observed as found in **1**. Since the ^{1}H and ^{13}C NMR data as well as the HMBC spectrum of **2** were almost superimposable and similar to those of **1**, we assumed that the feruloyl group was also attached to the C-3 hydroxy group, while the dihydrocaffeoyl units could be plausibly attached at positions 1 and 4. Thus, **2** was tentatively assigned as 3-*O*-feruloyl-1,4-di-*O*-dihydrocaffeoylquinic acid, for which we propose the trivial name feruloylpodospermic acid B (**2**). Regarding the structure of compounds **1** and **2**, it cannot be completely ruled out that **2** could be an acyl migration product of **1** formed during the isolation procedure. However, both congeners could be clearly detected in the crude extract of *S. divaricata* by LC-MS.

Compound **3** was isolated from the aerial parts of *S. pseudodivaricata* as an amorphous solid with the molecular formula $C_{29}H_{36}O_{12}$, from HRESIMS (*m*/*z* 599.2110 $[M + Na]^+$). The UV spectrum of **3** showed absorption maxima at 350, 291, and 235 nm in MeOH. The ^{13}C NMR spectrum displayed 29 carbon resonances. One carboxylic and one ester carbon were observed at δ 167.0 and 173.0, respectively, while a carbon resonance at δ 206.5 suggested the presence of a keto function in an aliphatic system. By assessing the ^{13}C, DEPT, and HMQC NMR spectra, a further six quaternary carbons, 11 methines, eight methylenes, and one methyl group were identified (Table 3). Inspection of the ^{1}H NMR spectrum of **3** revealed the presence of at least four spin systems.

Table 3. ^{1}H (400 MHz) and ^{13}C (100.6 MHz) NMR Data of Scorzoneric Acid (**3**) in DMSO-d_6

no.	δ_H, m, J (Hz)	δ_C, m	no.	δ_H, m, J (Hz)	δ_C, m
1		155.0 s	1′	5.05 d 8.1	98.0 d
2	6.63 "d" 8.5	114.9 d	2′	4.74 dd 9.5, 8.0	73.0 d
3	6.93 "d" 8.5	128.8 d	3′	3.49 m	73.5 d
4		132.0 s	OH-3′	5.24 d 5.4	
5	6.93 "d" 8.5	128.8 d	4′	3.26[a]	70.0 d
6	6.63 "d" 8.5	114.9 d	OH-4′	5.14 bd 4.9	
7	2.68−2.60 m	26.5 t	5′	3.40 ddd 9.7, 5.2, 2.0	77.0 d
8	2.68−2.60 m	43.5 t	6′A	3.69 ddd 11.9, 5.1, 1.8	60.5 t
9		206.5 s	6′B	3.51 m	
10	3.80 d 17.0	49.0 t	OH-6′	4.60 t 5.9	
	3.92 d 17.0				
11		114.0 s	1″		173.0 s
12	5.88 d 2.6	108.0 d	2″	2.32 t 7.0	34.0 t
13		158.0 s	3″	1.51 m	24.5 t
14	6.15 d 2.6	103.0 d	4″	1.22 m	30.0 t
15		114.9 s	5″	1.22 m	22.5 t
16		141.0 s	6″	0.79 t 7.0	14.0 q
17		167.0 s			

[a] Resonance is under the water signal and was detected in the 2D COSY spectrum.

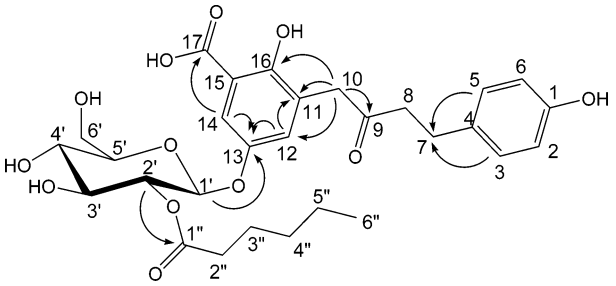
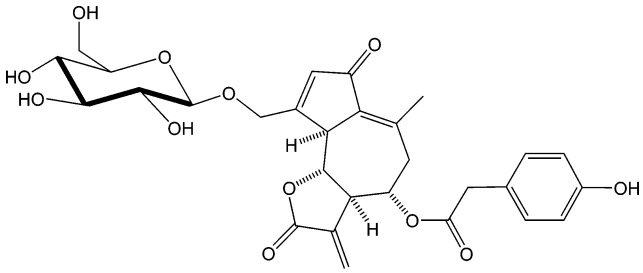


Figure 3. Key HMBC correlations in scorzoneric acid (**3**).

In the aromatic region, the occurrence of a *para*-substituted benzene ring and of a 1,3,4,5-tetrasubstituted phenyl unit was evident on the basis of the AA′BB′ coupling pattern and the *meta*-coupled proton doublets, respectively. Multiplicity and spin−spin coupling constants of the oxymethine protons observed between δ 3.20 and 5.05 inferred the presence of a sugar unit, while signals in the region δ 0.79 to 2.32 implied the presence of an acyl side chain. These spin systems were clearly assigned from the COSY spectrum of **3** particularly for the acyl side chain, which commenced at the methylene triplet at $\delta_{H-2''}$ 2.32 (J = 7.0 Hz) and ended at the methyl triplet at $\delta_{H-6''}$ 0.79 (J = 7.0 Hz). The β-glucopyranose system was evident from the vicinal coupling constants, and the presence of glucose was further confirmed by methylation analysis.[24] In addition, methylene resonances adjacent to a keto function were observed at δ 3.80 and 3.92 with a coupling constant of 17 Hz as part of an AB system, as well as further resonances between δ 2.60 and 2.68, which gave direct correlations with carbons at δ 49.0 and 43.5, respectively, as determined by HMQC. The COSY spectrum of **3** indicated the latter proton to couple with another methylene proton in the same chemical shift range, which suggested the occurrence of a butan-2-one group.

An HMBC experiment was used to determine the connectivity of the different spin systems (Figure 3). The carbonyl resonance at δ 173.0 (C-1″) correlated with the oxymethine proton H-2′ (δ 4.72) of the glucose unit. Acylation of the C-2′ hydroxy function was also evident through the deshielded resonance of H-2′. Furthermore, the anomeric proton H-1′ (δ 5.05) of the glucose unit exhibited a cross-peak with the resonance at δ_{C-13} at 158.0 of the 1,3,4,5-tetrasubstituted phenyl unit. Three other functional groups attached to the tetrasubstituted phenyl unit were inferred from the HMBC correlation of the carboxylic carbon at δ_{C-17} 167.0 with the *meta*-doublet proton at δ_{H-14} 6.15, which additionally gave a cross-peak with δ_{C-16} 141.0, a hydroxy bearing carbon that is *ortho* to the carboxyl substituent. The fourth substituent was indicated by the correlations of the AB methylene doublet pairs at δ_{H-10} 3.80 and 3.92 (J = 17.0 Hz) with the methine carbon at δ_{C-12} 108.0 (which bears the *meta*-proton at δ 5.88), with the hydroxy-bearing carbon at δ 141.0, and with the keto carbon resonances at δ 206.5. The HMBC data confirmed that the hydroxy group is at C-16 and a butan-2-one group at C-11. Attachment of the *para*-substituted benzene ring to the butan-2-one unit was based on the HMBC cross-peak of the AA′BB′ doublet proton at δ 6.93 with the methylene carbon at δ_{C-7} 26.5, which corresponds to the proton at δ_{H-7} 2.60. From the above 1D and 2D NMR data, the structure of **3** could be unambiguously elucidated as 5-(1-(2-*O*-hexanoyl)-β-D-glucopyranosyloxy)-2-hydroxy-3-[4-(4-hydroxyphenyl)-2-oxobutyl]benzoic acid, to which we assigned the trivial name scorzoneric acid.



lactucopicriside

Compound **4** was isolated as an amorphous solid from the MeOH extract of the aerial parts of *S. pseudodivaricata*. Its molecular formula was determined as $C_{30}H_{36}O_{11}$ by HRESIMS. The ^{1}H and ^{13}C NMR data of **4** were comparable to those of lactucopicriside,[25] a sesquiterpene lactone glucopyranoside, $C_{29}H_{32}O_{12}$, obtained from the roots of *Lactuca laciniata*. However, in spite of the obvious similarities, apparent differences between the known compound and the new congener were also observed in their ^{1}H and ^{13}C NMR spectra. In the ^{13}C and DEPT spectra of **4**, besides the occurrence of two additional methyl resonances in the upfield region, no exomethylene triplet in the δ 100−120 region but an extra triplet at δ 31.1 was observed. The ^{1}H and ^{13}C NMR spectra of **4** showed the presence of a sesquiterpene lactone nucleus identical to that of matricarin[26] isolated from the flowers of *Matricaria chamomilla*. The COSY spectrum of **4** indicated the occurrence of spin systems similar to those found in lactucopicriside, which included a glucose unit and a *p*-substituted aromatic moiety. Their connectivities were established from the HMBC data. The C-α carbonyl at δ_C 173.4 showed a correlation with the methylene protons at δ 2.70 and 2.90 belonging to the two methylene carbons at δ_C 37.2 and 31.1, respectively, while the latter correlated with the overlapping AA′BB′ system at δ_H 7.03 and 7.14 of the *p*-substituted aromatic moiety. A correlation of the oxygen-bearing carbon at δ_C 157.8 with the anomeric proton at δ_H 4.82 (d, J = 7 Hz) established the glycosidic linkage of the glucose unit with the *p*-hydroxyphenylpropanoate moiety. In lactucopicriside, the aromatic moiety is connected to the guaiadienenolide at C-8, while the glucose unit is attached to the methylene group at C-4 via a glycosidic linkage. This is the first reported guaianolide glucoside where the sugar unit is not attached directly to the sesquiterpene nucleus but rather to the *p*-hydroxyphenylpropanoate unit. The relative configuration of the sesquiterpene lactone nucleus of **4** is identical to those of matricarin as revealed by their almost identical NMR data,[26c] and its ROESY spectra exhibited the expected NOE responses (Figure 4) associated with the single-crystal X-ray diffraction data[26d] of matricarin and its congeners. Compound **4** was elucidated as 8α-matricarinyl 3-[4-(1-β-D-glucopyranosyloxy)phenyl]propanoate and assigned the trivial name scorzonerin.

Since the feruloylquinate derivatives, **1** and **2**, were isolated from the DPPH-active fraction of the aerial parts of *S. divaricata*, radical-scavenging activity of feruloylpodospermic acids A (**1**) and B (**2**) was assessed using the DPPH assay. For comparison, the known

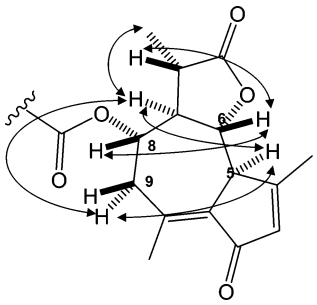


Figure 4. Key NOEs observed for the matricarin nucleus of scorzonerin (**4**).

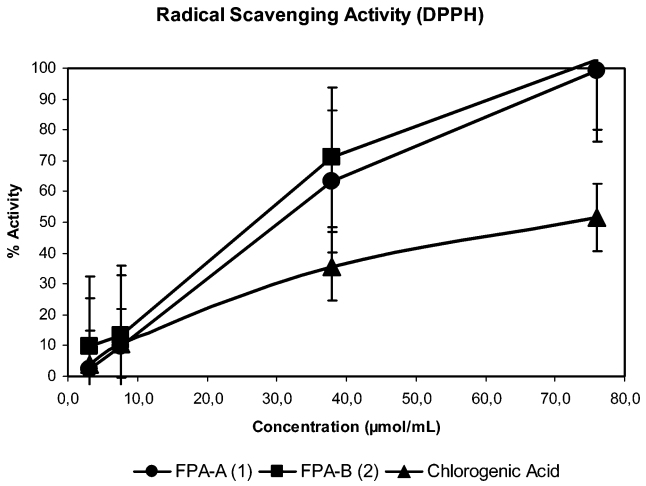


Figure 5. DPPH assay of feruloylpodospermic acids A and (FPA-A, **1**) and B (FPA-B, **2**) in comparison to chlorogenic acid.

naturally occurring antioxidant chlorogenic acid was included. The new congeners were found to be more active than the reference compound. Feruloylpodospermic acids A (**1**) and B (**2**) gave IC_{50} values of 36.36 and 34.24 μmol/mL, respectively, while chlorogenic acid had an IC_{50} value of 67.92 μmol/mL. Compounds **3** and **4** showed no antioxidative activity. From the cytotoxic fraction of *S. pseudodivaricata*, the isolated compounds platyphylloside (a known compound), scorzoneric acid (**3**), and scorzonerin (**4**) were tested for their cytotoxicity toward the mouse lymphoma cell line (L5175Y). Only the known compound platyphylloside[27] was found to be active and exhibited over 90% cell growth inhibition at a concentration of 22 mM.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter. 1D and 2D NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on Bruker ARX 400, 500, and/or DMX 600 NMR spectrometers using standard Bruker software and DMSO-d_6 or CD_3OD as solvents. NMR spectra were referenced to the solvent signal. ESI mass spectra were obtained on a Thermofinnigan LCQ DECA mass spectrometer coupled to an Agilent 1100 HPLC system that included an on-line photodiode array detector. HRESIMS spectra were determined on a Micromass Q-Tof 2 mass spectrometer. For HPLC analysis, 20 μL samples were injected into an HPLC system (Dionex, Munich, Germany) equipped with a photodiode-array detector, employing a linear gradient from 5% MeOH (against Nanopure H_2O adjusted to pH 2 with 0.1% phosphoric acid) to 100% MeOH (HPLC grade, Merck) at 35 min. Routine detection was at 254 nm. The separation column (125 × 4 mm, i.d.) was prefilled with 5 μm Eurospher-100 C_{18} (Knauer, Berlin, Germany). The temperature of the column oven was set at 20 °C. The compounds were eluted at 20.99 min for feruloylpodospermic acid A (**1**), 22.62 min for feruloylpodospermic acid B (**2**), 23.14 min for scorzonerin (**4**), and 26.02 min for scorzoneric acid (**3**). Semipreparative HPLC was performed on Merck-Hitachi Eurospher-100-C18, L-7100 pump, and L-7400 UV detector. TLC was performed on TLC plates precoated with Si 60 F_{254} (Merck, Darmstadt, Germany) using EtOAc/HCO_2H/H_2O, 85:10:5, as solvent system. The compounds were detected from their UV absorbance and by spraying the TLC plates with anisaldehyde reagent.

Plant Material. Aerial parts of *S. divaricata* and *S. pseudodivaricata* were collected in August 2004 in Burgastain am, Ikh Nart, Dalanjargalan sum, in the region of Dornogovi aimag, Mongolia. The plants were taxonomically identified by Prof. Sc. D. Sh. Darijmaa and Miss T. Baynmunkh from the Mongolian State University of Education. Voucher specimens 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 materials of *S. divaricata* (256 g) and *S. pseudodivaricata* (400 g) were extracted exhaustively by maceration with MeOH (3 × 400 mL) at room temperature. The total extract was concentrated to dryness *in vacuo*. The concentrated solids (46.9 and 82.0 g, respectively) were reconstituted with 100 mL of MeOH/H_2O (3:7), then partitioned successively with hexane (5 × 100 mL), EtOAc (5 × 100 mL), and n-BuOH (5 × 100 mL) to give the respective 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.

Aliquot amounts of the EtOAc fractions of the MeOH extracts derived from the aerial parts of *S. divaricata* (1.03 g) and *S. pseudodivaricata* (0.97 g) were separated by Sephadex LH-20 CC (4 × 75 cm) using MeOH as mobile phase to afford 21 and 17 fractions, respectively. The fractions were qualitatively analyzed for their antioxidant activity by spraying the thin-layer chromatography plates with 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent (1%). Fractions were also submitted to a cytotoxicity assay using the leukemia mouse lymphoma cell line L5178Y. Feruloylpodospermic acids A (**1**) and B (**2**) were isolated from the DPPH-active fractions 13 (221 mg) and 15 (85.5 mg), respectively, of *S. divaricata*. Feruloylpodospermic acid A (**1**, 21 mg, 0.008% yield) was separated by silica gel CC by gradient elution using CH_2Cl_2 and MeOH as solvents. Feruloylpodospermic acid B (**2**, 6 mg, 0.002% yield) was purified by semipreparative HPLC utilizing RP-18 as column and mixtures of MeOH/H_2O as solvent. Other DPPH-active fractions were also investigated and were determined to contain 3,5-caffeoylquinic acid and luteolin glycosides. However, no further chemical or biological analyses were done on the known compounds or fractions containing them, as sufficient data are already available regarding their antioxidant activities.

From the Sephadex fractions of *S. pseudodivaricata*, fraction 4 afforded the new guiainolide scorzonerin (**4**, 104 mg, 0.026% yield) as its major component. The cytotoxic fraction 7 (78 mg) was subjected to silica gel column chromatography using the solvent system CH_2Cl_2/MeOH (8:2), from which a further 15 fractions were obtained. The known active compound platyphylloside[27] (23.7 mg, 0.006% yield) and the new phenolic glycoside **3** (scorzoneric acid, 5.8 mg, 0.0014% yield) were isolated and purified from the nonpolar fractions 8 and 14, respectively, by reversed-phase column chromatography using MeOH/H_2O (65:35) as eluent.

Feruloylpodospermic acid A (1): amorphous solid; $[\alpha]^{20}_D$ −22.0 (*c* 0.109, EtOH); UV (MeOH/H_2O) λ_{max} 220, 286.5, 328.9 nm; EIMS *m/z* $[M + 1]^+$ 697; HRESIMS *m/z* 719.1950 $[M + Na]^+$ (719.1952 calc for $C_{35}H_{36}O_{15}Na$); ^1H and ^{13}C NMR data are given in Table 2.

Feruloylpodospermic acid B (2): amorphous solid; $[\alpha]^{20}_D$ −89.6 (*c* 0.05, EtOH); UV (MeOH/H_2O) λ_{max} 220, 286.3, 329.9 nm; EIMS *m/z* 697 $[M + 1]^+$; HRESIMS *m/z* 719.1950 $[M + Na]^+$ (719.1952 calc for $C_{35}H_{36}O_{15}Na$), *m/z* 697.2140 $[M + H]^+$ (697.2140 calc for $C_{35}H_{37}O_{15}$); ^1H and ^{13}C NMR data are given in Table 2.

Scorzoneric acid (3): amorphous solid; $[\alpha]^{20}_D$ −17.3 (*c* 0.10, EtOH); UV (MeOH) λ_{max} 235, 291, 350 nm; (−)ESIMS *m/z* 575 $[M - H]^-$; (+)ESIMS *m/z* 577; HRESIMS *m/z* 599.2110 $[M + Na]^+$ (599.2104 calc for $C_{29}H_{36}O_{12}Na$); ^1H and ^{13}C NMR data are given in Table 3.

Scorzonerin (4): amorphous solid; $[\alpha]^{20}_D$ −16.3 (*c* 0.14, EtOH); UV (MeOH) λ_{max} 235, 291, 350 nm; HRFABMS *m/z* 595.2160 $[M + Na]^+$ (595.2155 calc for $C_{30}H_{36}O_{11}Na$); ^1H and ^{13}C NMR data are given in Table 4.

Table 4. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Scorzonerin (**4**) in CD_3OD

no.	δ_H, m, *J* (Hz)	δ_C, m	no.	δ_H, m, *J* (Hz)	δ_C, m
1		134.7 s	α		173.4 s
2		197.7 s	*β*	2.70 m	37.2 t
3	6.25 s	136.0 d	*γ*	2.90 m	31.1 t
4		173.4 s	1′		135.6 s
5	3.62 bd 10.0	52.4 d	2′	7.14 “d” 8.6	130.5 d
6	3.78 t 10.0	82.7 d	3′	7.03 “d” 8.6	117.8 d
7	2.51 m	59.5 d	4′		157.8 s
8	4.85[a]	72.0 d	5′	7.03 “d” 8.6	117.8 d
9A	2.73 dd 13.5, 10.2	45.2 t	6′	7.14 “d” 8.6	130.5 d
9B	2.22 dd 13.5, 1.7				
10		147.6 s	1″	4.82 d 7.0	100.6 d
11	2.56 m	41.6 d	2″	3.43 m	74.9 d
12		179.3 s	3″	3.43 m	78.1 d
13	1.25 d 7.0	15.4 q	4″	3.44−3.35 m	71.4 d
14	2.40 s	21.4 q	5″	3.39 m	78.0 d
15	2.35 s	19.9 q	6″A	3.87 dd 12.0, 1.9	62.5 t
			6″B	3.68 dd 12.0, 5.0	

[a] Resonance is under the water signal and was detected in the 2D COSY spectrum.

Cytotoxicity Assay. Antiproliferative activity was examined against the cell line L1578Y and was determined by an MTT assay as described earlier.[28] L5178Y mouse lymphoma cells were grown in Eagle’s minimal essential medium supplemented with 10% horse serum in roller tube culture. The cell culture media contained 100 units/mL penicillin and 100 μg/mL streptomycin and was changed twice per week. The cells were maintained in a humidified atmosphere at 37 °C with 5% CO_2.

L1578Y was plated on a 96-well plate with 50 000 cells per well. The cells were allowed to attach for 24 h and were then treated with different concentrations of the test samples. After this treatment the medium was changed and the cells were incubated for 3 h under cell culture conditions with 20 μg/mL MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide). The conversion of the tetrazolium salt MTT to a colored formazan by mitochondrial dehydrogenases was determined as a marker of cell viability according to Mosmann, 1983.[29] After this incubation the cells were fixed on the plate with an aqueous solution containing 1% formaldehyde and 1% $CaCl_2$ and then lysed with 95% 2-propanol/5% HCO_2H. The concentration of reduced MTT as a marker for cell viability was measured photometrically at 560 nm. Fractions and pure compounds dissolved in DMSO were screened at a concentration of 10 μg per well.

Measurement of Radical-Scavenging Activity by DPPH. Qualitative analysis of radical-scavenging or antioxidant activity of the extracts and fractions was carried out by spraying the TLC plates after development in the appropriate solvent system ($EtOAc/HCO_2H/H_2O$, 85:10:5) with 1% 2,2-diphenyl-1-picrylhydrazyl (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 in 10 μL of DMSO had reacted with a 490 μL DPPH solution (4.5 mg/100 mL). Incubation time was 2 min. Prior to the measurement, the difference in absorption between a DPPH blank solution and the positive control (propylgallate, 76 μM) was determined. This difference was then taken as 100% antioxidative activity. The percent antioxidative activity could be calculated from the difference in absorption between the test sample at 76 μM and the DPPH blank as follows:[30]

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

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

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