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Podospermic acid, 1,3,5-tri-*O*-(7,8-dihydrocaffeoyl)quinic acid from *Podospermum laciniatum* (Asteraceae)

Christian Zidorn,[a,*] Bent O. Petersen,[b] Vedrana Udovičić,[a] Thomas O. Larsen,[c] Jens Ø. Duus,[b] Judith M. Rollinger,[a] Karl-Hans Ongania,[d] Ernst P. Ellmerer[d] and Hermann Stuppner[a]

[a]*Institut für Pharmazie der Universität Innsbruck, Abteilung Pharmakognosie, Josef-Moeller-Haus, Innrain 52, A-6020 Innsbruck, Austria*
[b]*Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark*
[c]*Center for Microbial Biotechnology, BioCentrum-DTU, Søltofts Plads, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark*
[d]*Institut für Organische Chemie der Universität Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria*

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Dedicated to Professor Dr. Sigmar Bortenschlager (Institut für Botanik der Universität Innsbruck, Austria) on the occasion of his 65th birthday

Abstract—A phytochemical investigation of *Podospermum laciniatum* (L.) DC. (Asteraceae) yielded the new quinic acid derivative podospermic acid (1,3,5-tridihydrocaffeoylquinic acid), which was named after the genus it was isolated from. The structure was established by HR mass spectrometry and extensive 1D and 2D NMR spectroscopy. Podospermic acid is the first naturally occurring dihydrocaffeoylquinic acid derivative. The chemosystematic impact and the radical scavenging activity of the new compound are discussed briefly.

Podospermum laciniatum (L.) DC. (synonym: *Scorzonera laciniata* L.) is an annual or biennial herb of 20–70 cm height with yellow ligules, which is native to Northwest Africa, France, Mediterranean and Central Europe as well as the Caucasus region and scattered localities in Turkey and South-East Europe.[1] In the present communication, we report the isolation and structure elucidation of a new naturally occurring polyphenolic compound with pronounced radical scavenging activity.

P. laciniatum was collected in May 2000 near Trasacco SE Avezzano/L'Aquila/Abruzzo/Italy [altitude: 735 m (above m.s.l.), N 41° 56′, E 13° 32′]. A voucher specimen (CZ-20000517A-2) is preserved at the Institut für Pharmazie/Innsbruck.

1,3,5-Tridihydrocaffeoylquinic acid **1** (37.6 mg) was isolated from the EtOAc phase (2.73 g) of the methanolic extract (16.9 g) of subaerial parts (105 g) of *P. laciniatum* by silica gel 60 (230–400 mesh) column chromatography (CC) using a gradient of CH_2Cl_2 and MeOH and two successive Sephadex LH-20 CCs using MeOH as an eluant. HRMS[2] of compound **1** revealed a molecular formula of $C_{34}H_{36}O_{15}$. ^{1}H NMR and ^{13}C NMR data (Table 1) indicated that **1** consisted of a quinic acid moiety[3] and three dihydrocaffeoyl moieties.[4] ^{1}H NMR data of the quinic acid moiety (Table 1) also proved that two of the three CHOH moieties (in positions 3, 4, and 5) in the cyclohexane ring were acylated as evidenced by pronounced downfield shifts of the geminal proton signals [δ_H = 5.25 (H-3) and 5.05 ppm (H-5), respectively], while one of these groups was unsubstituted [δ_H = 3.75 ppm (H-4)]. The HHCOSY spectrum showed crosspeaks from the signal assignable to the proton of the unsubstituted CHOH moiety to both signals assignable to the protons of the acylated CHOH moieties. Thus, the acylation of positions 3 and 5 was established. Comparison of ^{13}C NMR shift values observed for C-1

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* Corresponding author. Tel.: +43 512 507 5302; fax: +43 512 507 2939; e-mail: christian.h.zidorn@uibk.ac.at

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Table 1. NMR data of podospermic acid (**1**)[a,12]

Position	^{1}H NMR	^{13}C NMR
Quinic acid moiety		
1		79.7
2 ax.	2.34 1H, m	32.0
2 eq.	2.30 1H, m*	
3	5.24 1H, q (*J* 4.0 Hz)	70.9
4	3.74 1H, m	68.9
5	5.05 1H, td (*J* 9.5, 4.0 Hz)	69.9
6 ax.	1.82 1H, m	35.9
6 eq.	2.30 1H, m*	
1-COOH		171.8[b]
7,8-Dihydrocaffeoyl moieties		
1′		131.3
1″		131.1
1‴		131.3
2′	6.59 1H, m*	115.6[b]
2″	6.58 1H, m*	115.6[b]
2‴	6.59 1H, m*	115.6[b]
3′/3″/3‴		145.0[b]
4′/4″/4‴		143.5[b]
5′/	6.59 1H, m*	115.6[b]
5″	6.58 1H, m*	115.6[b]
5‴	6.61 1H, m*	115.6[b]
6′	6.40 1H, dd (*J* 8.0, 2.0 Hz)	118.6
6″	6.37 1H, dd (*J* 8.0, 2.0 Hz)	118.6
6‴	6.45 1H, dd (*J* 8.0, 2.0 Hz)	118.7
7′	2.66/2.66 m*	29.5[b]
7″	2.65/2.63 m*	
7‴	2.67/2.67 m*	
8′	2.52/2.42 m	35.6[b]
8″	2.51/2.43 m	
8‴	2.50/2.50 m	
9′		171.0
9″		171.8[b]
9‴		171.8[b]

[a] Measured in DMSO-d_6 at 799.63 and 201.01 MHz, respectively. Signals were referenced to solvent residual and solvent signals at $\delta_H = 2.50$ and $\delta_C = 39.5$ ppm, respectively.
[b] Broad signals, assignable to more than one carbon atom.
* Overlapping signals.

($\delta_C = 79.5$ ppm) with C-1 shift values reported for 3,5-dicaffeoylquinic acid ($\delta_C = 74.7$ ppm)[5] and 1,3,5-tricaffeoylquinic acid ($\delta_C = 80.8$ ppm)[5] verified the assumption that the OH group of position-1 of the quinic acid moiety was also acylated. Conclusively, the structure of compound **1** was established as 1,3,5-tridihydrocaffeoylquinic acid (Fig. 1), a new natural compound to which we assign the name podospermic acid. Shift values observed for the three dihydrocaffeoyl moieties differed only marginally (Table 1). However, a combination of HMBC (Fig. 2) and HSQC experiments conducted at high frequencies (^{1}H NMR: 800 MHz, ^{13}C NMR: 201 MHz) enabled us to individually assign most signals observed for the acyl moieties. The differentiation between axial and equatorial protons at C-2 and C-6 of the quinic acid moiety was possible by a NOE experiment. The spectrum showed correlations between the signal assignable to H-4 of the quinic acid moiety

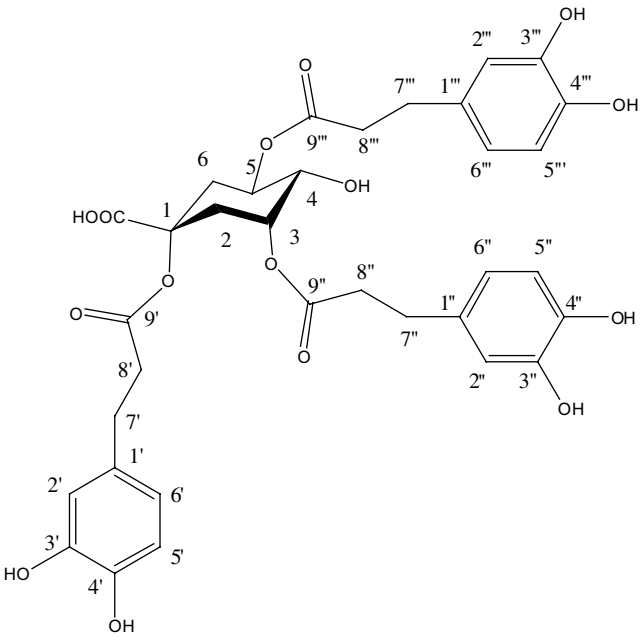

Figure 1. Structure of 1,3,5-tri-*O*-(7,8-dihydrocaffeoyl)quinic acid (**1**) from *Podospermum laciniatum*.

and the signals assignable to the axial protons in position 2 and 6 of the quinic acid moiety.

As other esters of quinic acid and dihydrocaffeic acid are not known yet as natural compounds[6–8] the compound at hand is considered to be a representative of a whole new class of naturally occurring polyphenolic compounds, dihydrocaffeoylquinic acid derivatives. This new class of natural compounds might be of considerable pharmaceutical interest, because the semi-synthetically manufactured dihydrochlorogenic acid (5-dihydrocaffeoyl quinic acid) has been demonstrated to possess inhibitory activity on hepatic glucose-6-phosphate translocase[6] and has been claimed to possess lipolytic and antiobesity effects.[7] Furthermore, semi-synthetically prepared dihydrochlorogenic acid and 3,5-di-*O*-(7,8-dihydrocaffeoyl)quinic acid have been claimed to exhibit cosmetically relevant antioxidant activity.[8] Interestingly, two other new classes of plant polyphenolics have been reported from members of the Lactuceae tribe of the Asteraceae family recently: *Taraxacum linearisquameum* Soest yielded a new class of *p*-hydroxyphenylacetylinositol derivatives[9] and lettuce (*Lactuca sativa* L.) was the source of 2,3,4-tri-(*p*-hydroxyphenylacetyl)-β-glucopyranose.[10]

Radical scavenging activity of **1** was assessed using the DPPH assay in comparison to ascorbic acid, caffeic acid, and chlorogenic acid. The results are summarized in Table 2. All four compounds have comparable radical scavenging activity when comparing IC_{50} values in μg/ml. However, taking into account the high molecular mass of the new compound, podospermic acid (**1**) is by far the most potent radical scavenger on a molar basis. The radical scavenging activity of podospermic acid is also of interest for human nutrition, because

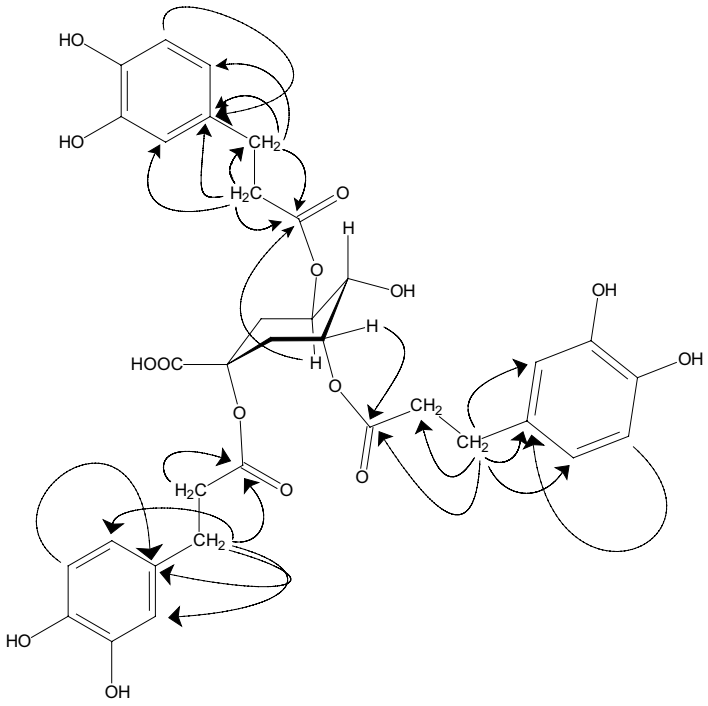

Figure 2. Important HMBC correlations observed for compound **1**.

Table 2. Radical scavenging activity in a DPPH[13] assay (c_{DPPH} = 40 mg/l) of podospermic acid compared to known radical scavengers (standard deviations are indicated in brackets)

Compound	IC_{50} (μg/ml)	IC_{50} (μmol/l)
Podospermic acid	4.8 (0.9)	7.0 (1.3)
Ascorbic acid	5.0 (1.1)	28.2 (6.2)
Caffeic acid	2.8 (0.5)	15.7 (3.0)
Chlorogenic acid	4.2 (0.4)	11.8 (1.1)

Podospermum taxa are used as vegetables in the Italian Abruzzo region.[11]

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2. Podospermic acid **1**—Colorless amorphous substance; glass transition 123–129 °C; $[\alpha]_D^{20}$ − 18° (*c* 0.006 g/ml, MeOH); UV (MeOH) λ_{max} (log ε) 283 (3.79) nm; FTIR : 3400 (br), 2950, 1722, 1714, 1608, 1522, 1447, 1367, 1284, 1195, 1113, 947, 866, 817, 785; HRFABMS: *m/z* = 685.21090 $[M + H]^+$ (85.4), calculated for $[C_{34}H_{37}O_{15}]^+$: 685.21270; 707.18842 $[M + Na]^+$ (16.7), calculated for $[C_{34}H_{36}O_{15}Na]^+$: 707.19464 (16.7); 684.20609 $[M]^+$ (85.0), calculated for $[C_{34}H_{36}O_{15}]^+$: 684.20487; 667.20528 $[M - H_2O + H]^+$ (100.0), calculated for $[C_{34}H_{35}O_{14}]^+$: 667.20213. NMR data are described in Table 1.
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12. NMR chemical shifts were assigned using 1D and 2D homo- and heteronuclear experiments at 799.96 MHz for proton and 201.12 MHz for carbon. For 2D experiments

the Varian standard programs tndqcosy, tnnoesy (mixing time 600 ms) and tntocsy (spinlock 80 ms) were used. The heteronuclear experiments were performed using the pulse field gradient programs gHSQC and gHMBC.

13. Methanolic solutions of test compounds were mixed with a methanolic solution of DPPH (Sigma–Aldrich, Steinheim, Germany). The final DPPH concentration was 40 mg/l. Compounds were tested in final concentrations of 1, 2, 4, 8, 16, and 32 μg/ml, respectively. After incubation in 96 well-plates, the reaction mixture (250 μl) was kept in the dark at ambient temperature (25 °C) for 30 min. Then, the optical density of the test mixtures in comparison to DPPH and pure methanol was measured using a Hidex Chameleon plate reader. IC_{50} values for each replicate were calculated using the following formula: $IC_{50} = [(50 - LP)/(HP - LP)*(HC - LC)] + LC$.
LP = low percentage, that is, highest percent inhibition less than 50%; HP = high percentage, that is, lowest percent inhibition greater than 50%; HC = high concentration, that is, concentration of test substance at the high percentage, LC = low concentration, that is concentration of test substance at the low percentage. All compounds and concentrations were assayed in triplicate and mean values were calculated for each compound. Ascorbic acid (Merck, Darmstadt, Germany), caffeic acid (Fluka, Buchs, Switzerland), chlorogenic acid (Roth, Karlsruhe, Germany), and DPPH (Sigma–Aldrich, Steinheim, Germany) were obtained commercially.