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SPINACETIN, A NEW CAFFEOYLGLYCOSIDE, AND OTHER PHENOLIC COMPOUNDS FROM *Gnaphalium uliginosum*

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*The new flavonoid gnaphaloside C, which was identified based on UV, MS, and NMR spectral data as spinacetin-7-*O*-(6″-*O*-caffeoyl)-β-D-glucopyranoside, and 24 known compounds including for the first time from this species quercetagetrin, quercetagetin-7-*O*-(6″-*O*-caffeoyl)glucoside, patulitrin, tinctoside, and spinacetin-7-*O*-glucoside were isolated from the aerial part of* Gnaphalium uliginosum. *HPLC determined that* G. uliginosum *contained mainly caffeoylquinic acids (10.54–48.48 mg/g). The flavonoid content was 1.20–16.55 mg/g.*

Keywords: *Gnaphalium uliginosum*, Asteraceae, spinacetin-7-*O*-(6′′-*O*-caffeoyl)-*β*-D-glucopyranoside, gnaphaloside C, HPLC.

Gnaphalium uliginosum L. [*Filaginella uliginosa* (L.) Opiz] (Asteraceae) is a medicinal plant that is used in medical practice as a hypotensive and wound-healing agent for hypertension, stomach ulcers, and difficultly healed wounds [1]. Chemical investigations found in *G. uliginosum* flavones, flavonols [2, 3], caffeoylquinic [4] and caffeoylglucaric acids [5], and carotinoids [6]. Early research on the chemical composition used raw material collected in central Russia [2, 4], Finland [3], and Austria [5]. Considering the abundant resources of *G. uliginosum* in Siberia, it seemed interesting to study the chemistry of raw material growing in the Republics of Buryatia and Sakha (Yakutia) in order to determine if it could be used in practice.

Preliminary studies compared the quantitative contents of separate groups of phenolic compounds (PC) [flavonoids (FL) and phenylpropanoids (PP)] in the aerial part of *G. uliginosum* collected in two regions of Siberia with those of commercial samples. It was found that the FL/PP contents in batches of raw material from the Republics of Buryatia (4.84–6.28/19.73–21.14 mg/g) and Sakha (Yakutia) (7.02–9.36/27.61–29.36 mg/g) were significantly greater than that in the commercial samples (1.83–3.78/11.38–15.24 mg/g).

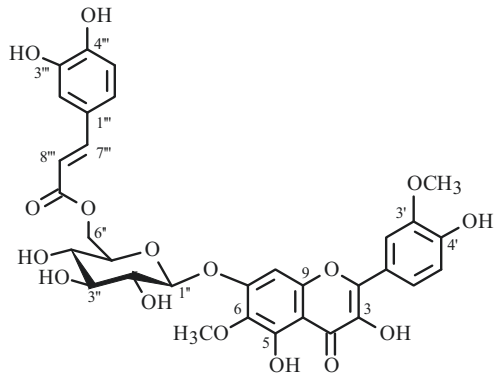

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TABLE 1. PMR (500 MHz) and ^{13}C NMR (125 MHz) Spectra of **1** and **11** (MeOH-d_4, δ, ppm, J/Hz)

C atom	Gnaphaloside C (**1**)		Spinacetin-7-*O*-glucoside (**11**)*	
	δ_H	δ_C	δ_H	δ_C
		Spinacetin		
2		148.7		148.8
3		135.9		135.8
4		178.2		178.4
5		148.5		148.4
6		131.2		131.2
7		152.9		152.8
8	6.77 (1H, s)	93.5	6.74 (1H, s)	93.4
9		155.0		155.2
10		105.4		105.3
1′		121.5		121.6
2′	7.52 (1H, d, J = 2.1)	115.7	7.49 (1H, d, J = 2.0)	114.6
3′		145.3		145.1
4′		147.3		147.5
5′	6.85 (1H, d, J = 8.1)	115.2	6.82 (1H, d, J = 8.1)	114.1
6′	7.40 (1H, dd, J = 8.1, 2.1)	120.8	7.41 (1H, dd, J = 8.1, 2.0)	120.9
6-OCH_3	3.72 (3H, s)	59.8	3.74 (3H, s)	59.9
3′-OCH_3	3.81 (3H, s)	55.9	3.80 (3H, s)	55.6
		7-*O*-β-D-Glucopyranose		
1″	5.14 (1H, d, J = 7.5)	101.7	5.11 (1H, d, J = 7.4)	101.5
2″	3.57 (1H, dd, J = 9.0, 7.5)	74.2	3.54 (1H, dd, J = 9.0, 7.4)	74.3
3″	3.52 (1H, t, J = 9.0)	77.8	3.47 (1H, t, J = 9.0)	77.6
4″	3.43 (1H, m)	71.9	3.41 (1H, m)	71.9
5″	3.87 (1H, m)	73.4	3.80 (1H, m)	73.8
6″	4.25 (1H, dd, J = 12.1, 2.1, H_B), 4.82 (1H, dd, J = 12.1, 7.8, H_A)	64.5	3.71 (1H, dd, J = 12.2, 2.0, H_B), 3.90 (1H, dd, J = 12.2, 5.2, H_A)	61.4
		6″-*O*-Caffeic acid		
1‴		126.4		
2‴	6.59 (1H, d, J = 1.5)	114.9		
3‴		145.9		
4‴		147.0		
5‴	6.40 (1H, d, J = 8.0)	115.9		
6‴	6.45 (1H, dd, 8.0, 1.5)	122.3		
7‴	7.48 (1H, d, J = 15.9)	146.4		
8‴	6.02 (1H, d, J = 15.9)	114.2		
9‴		168.2		

*PMR and ^{13}C NMR data are presented for the first time; the compound was characterized earlier using chemical transformations and UV spectroscopy [9, 10].

The new compound **1** and 21 known compounds were isolated after extraction and chromatographic separation over polyamide, SiO_2, RP-SiO_2, Sephadex LH-20, and preparative HPLC during a study of the constituent composition of PC from the aerial part of *G. uliginosum* collected in the Republic of Sakha (Yakutia). UV, MS, PMR, and ^{13}C NMR spectral data identified the known constituents as nepetin-7-*O*-glucoside (**2**); jaceosidin-7-*O*-glucoside (**3**); gnaphaloside A (**4**); gnaphaloside B (**5**); quercimeritrin (**6**); quercetagetrin (**7**); patulitrin (**8**); quercetagetin-7-*O*-(6″-*O*-caffeoyl)glucoside (**9**); tinctoside (**10**); spinacetin-7-*O*-glucoside (**11**); caffeic acid (**12**); 3-*O*- (**13**), 4-*O*- (**14**), 1,3-di-*O*- (**15**), 1,5-di-*O*- (**16**), 3,4-di-*O*- (**17**), 3,5-di-*O*- (**18**), 4,5-di-*O*- (**19**), 1,3,5-tri-*O*- (**20**), 3,4,5-tri-*O*- (**21**) caffeoylquinic acids; and leontopodic acid (**22**). Compounds **2**–**6**, **12**–**20**, and **22** were found previously in *G. uliginosum* [2–5]; **7**–**11** and **21** were detected for the first time.

The HR-ESI-MS data for **1** agreed with the formula $C_{32}H_{30}O_{16}$ {*m*/*z* 669.601 $[M - H]^-$ (calcd 669.578)}. UV spectroscopy indicated that the compound was a flavonol. Total hydrolysis products of **1** included glucose, caffeic acid, and a flavonoid aglycon that was identified using MS and ^{13}C NMR spectral data as spinacetin (6,3′-dimethoxy-3,5,7,4′-tetrahydroxyflavone). Glucose was assigned as the D- or L-isomer after derivatization of the hydrolysate with 1-(trimethylsilyl)-

imidazole followed by GC, which detected D-glucose. Absorption spectra in the presence of ionizing additives suggested that spinacetin was substituted on the C-7 hydroxyl whereas the C-3, C-5, and C-4′ hydroxyls were unsubstituted. The principal products from alkaline hydrolysis of **1** were identified as caffeic acid and spinacetin-7-*O*-glucoside (**11**). The results indicated that caffeic acid was bonded to the carbohydrate part of the glycoside, i.e., **1** was spinacetin-7-*O*-caffeoylglucoside. This conclusion was confirmed by ESI-MS/MS spectroscopy. The spectrum showed ions of characteristic fragments with *m*/*z* 323 and 179 that were assigned to deprotonated residues of an acylhexose (caffeoylglucose) and caffeic acid, respectively, in addition to ions with *m*/*z* 507 and 345 that were formed after **1** lost caffeic acid {[M – H – caffeoyl]$^-$} and caffeoylglucose {[M – H – caffeoylglucose]$^-$} [7].

The PMR spectrum of **1** was similar to that of spinacetin-7-*O*-glucoside (**11**) with the exception of additional characteristic resonances for a 1,2,4-trisubstituted aryl group in the aromatic region [δ 6.59 (1H, d, J = 1.5 Hz), 6.45 (1H, dd, J = 8.0, 1.5 Hz), 6.40 (1H, d, J = 8.0 Hz)] and *trans*-olefinic protons [δ 7.48 (1H, d, J = 15.9 Hz), 6.02 (1H, d, J = 15.9 Hz)] that were due to the influence of the acyl group (caffeic acid) (Table 1). Weak-field shifts of the H-6″$_A$ and H-6″$_B$ glucopyranose proton resonances (δ 4.25 and 4.82) compared with those of **11** (δ 3.71 and 3.90) and of the C-6″ resonance in the ^{13}C NMR spectrum (δ 61.4→64.5) indicated that the glucose C-6″ hydroxyl was substituted. Correlations in the HMBC spectrum between resonances of H-6″ (δ 4.25 and 4.82) and the caffeic acid carbonyl C atom (δ 168.2) indicated that the acylhexose residue in **1** was 6″-*O*-caffeoylglucose. The chemical shifts of glucose H-1″ and C-1″ resonances [δ_H 5.14 (1H, d, J = 7.5 Hz); δ_C 101.7] showed that the carbohydrate residue was *β*-glucopyranose. Thus, the results determined the structure of **1** as spinacetin-7-*O*-(6″-*O*-caffeoyl)-*β*-D-glucopyranoside, which was called gnaphaloside C.

Until now, the only known spinacetin-7-*O*-glycoside was spinacetin-7-*O*-glucoside (**11**), which was observed in the aerial part of *Anacyclus radiatus* Loisel. [8], *Lepidophorum repandum* (L.) DC. [9], and several *Clibadium* L. sp. (Asteraceae) [10]. Gnaphaloside C (**1**) is the second known spinacetin-7-*O*-glycoside.

Twenty compounds including **1**, **2**, **4**, **5**, **8**, **10**, and **12**–**22** in addition to cosmosiin (**23**), plantaginin (**24**), and isoquercitrin (**25**) were isolated from the aerial part of *G. uliginosum* collected in the Republic of Buryatia. We isolated 14 compounds (**4**–**6**, **12**, **13**, **15**–**22**, and **24**) by chromatographic separation of PC from a commercial sample of *G. uliginosum* from Moscow District. Flavonol glycosides **1** and **7**–**11** were not detected. The differences found in the constituent compositions of the samples were probably due to environmental factors.

According to HPLC, the dominant group of compounds in *G. uliginosum* herb was PP (10.54–48.48 mg/g) with di-*O*-caffeoylquinic acids having the greatest contents (8.50–28.42 mg/g) (Table 2). The principal constituent was 3,5-di-*O*-caffeoylquinic acid (**18**), the content of which was 6.11–15.20 mg/g. The flavonoid contents were much lower (1.20–16.55 mg/g). The dominant compounds in Siberian populations of *G. uliginosum* were gnaphaloside B (**5**, 0.65–9.21 mg/g) and tinctoside (**10**, 0.55–3.60 mg/g). The principal flavonoid in commercial samples was gnaphaloside A (**4**, 0.65–2.16 mg/g). Gnaphaloside C (**1**) was not detected. A comparison of the chemical compositions of *G. uliginosum* herb collected in the various regions of Russia indicated that raw material growing in Siberia characteristically had higher PC contents (23.29–65.03 mg/g). Thus, the FL and PP contents in samples from Sakha (Yakutia) exceeded those in the commercial raw material by 13.8 and 4.6 times, respectively. These results indicated that Siberian populations were promising for harvesting high-quality *G. uliginosum* raw material.

The compounds responsible for the hypotensive activity of *G. uliginosum* have not yet been identified despite the long history of using its preparations as hypotensive agents [1]. However, caffeoylquinic acids (**17**, **18**, **21**), which were observed in this plant species, possess pronounced antihypertensive activity [11]. Conversely, the hypotensive activity of the dominant flavonoids in *G. uliginosum* (**1**, **4**, **5**, **10**) has not been reported. The definitive class of compounds for standardizing *G. uliginosum* herb in Russia is FL [12]. Considering the obtained information about the quantitative PC contents in *G. uliginosum* herb, the adequacy of this approach must be questioned because the contents of caffeoylquinic acids were 2.9–8.8 times greater than those of FL. Therefore, we recommend that PP be examined as the active ingredients in this plant raw material.

TABLE 2. Quantitative Contents of Phenolic Compounds (PC) in Commercial and Wild Samples of *G. uliginosum*, mg/g (± SD)

Compound	Commercial samples			Buryatia		Yakutia	
	C-01	C-02	C-03	B-01	B-02	Y-01	Y-02
Flavonoids (FL)							
1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.37 ± 0.00	0.52 ± 0.01	1.27 ± 0.03	1.58 ± 0.05
4	0.65 ± 0.01	1.32 ± 0.04	2.16 ± 0.06	0.88 ± 0.02	1.63 ± 0.04	1.92 ± 0.07	2.16 ± 0.06
5	0.55 ± 0.01	1.18 ± 0.03	1.63 ± 0.04	3.04 ± 0.07	6.29 ± 0.16	7.67 ± 0.21	9.21 ± 0.22
10	Tr.	Tr.	Tr.	1.16 ± 0.03	1.12 ± 0.03	1.39 ± 0.04	3.60 ± 0.09
Phenylpropanoids (PP)							
Mono-*O*-caffeoylquinic acids (MCQA)							
13	1.07 ± 0.02	1.57 ± 0.04	1.62 ± 0.04	2.57 ± 0.06	4.29 ± 0.12	5.62 ± 0.17	5.91 ± 0.17
14	Tr.*	Tr.	Tr.	0.20 ± 0.00	0.54 ± 0.01	0.79 ± 0.02	1.12 ± 0.03
Di-*O*-caffeoylquinic acids (DCQA)							
15	0.09 ± 0.00	Tr.	0.31 ± 0.00	0.17 ± 0.00	0.37 ± 0.00	0.53 ± 0.01	0.59 ± 0.01
16	0.10 ± 0.00	Tr.	0.20 ± 0.00	0.40 ± 0.00	0.61 ± 0.02	0.94 ± 0.02	1.32 ± 0.04
17	0.45 ± 0.01	0.65 ± 0.01	0.93 ± 0.02	0.68 ± 0.01	0.53 ± 0.01	1.22 ± 0.03	1.39 ± 0.04
18	6.11 ± 0.14	7.63 ± 0.21	9.51 ± 0.26	6.84 ± 0.18	9.63 ± 0.28	12.15 ± 0.31	15.20 ± 0.42
19	1.75 ± 0.04	1.29 ± 0.03	2.15 ± 0.06	2.50 ± 0.06	4.27 ± 0.12	7.67 ± 0.22	9.92 ± 0.28
Tri-*O*-caffeoylquinic acids (TCQA)							
20	0.05 ± 0.00	0.21 ± 0.00	0.32 ± 0.00	0.57 ± 0.01	0.91 ± 0.02	1.63 ± 0.04	1.77 ± 0.04
21	0.92 ± 0.02	1.67 ± 0.04	1.59 ± 0.04	3.91 ± 0.09	6.37 ± 0.19	9.31 ± 0.26	11.26 ± 0.27
Σ_{PC}	11.51	15.52	20.42	23.29	37.08	52.11	65.03
Σ_{FL}	1.20	2.50	3.79	5.45	9.56	12.25	16.55
Σ_{PP}	10.54	13.02	16.63	17.84	27.52	39.86	48.48
Σ_{MCQA}	1.07	1.57	1.62	2.77	4.83	6.41	7.03
Σ_{DCQA}	8.50	9.57	13.10	10.59	15.41	22.51	28.42
Σ_{TCQA}	0.97	1.88	1.91	4.48	7.28	10.94	13.03

*Tr.: trace (< 0.05 mg/g).

EXPERIMENTAL

Plant raw material (aerial part) of *G. uliginosum* was collected in the Republic of Buryatia at Il′inka village (Zaigrayevsky District, 20 Jun., 2013; 51°71′02″ N, 108°46′80″ E; sample B-01) and Severobaikalsk (Severo-Baikalsky District, 15 Jul., 2013; 55°73′96″ N, 109°42′93″ E; sample B-02); in the Republic of Sakha (Yakutia) at Solyanka village (Olyokminsky District, 20 Jul., 2013; 60°46′27″ N, 120°66′83″ E; sample Y-01) and Tomtor (Verkhoyansky District, 25 Jul., 2013; 67°11′04″ N, 134°73′63″ E; sample Y-02); and through the pharmacy chains (commercial samples) OOO Leks+ (Khimki, Moscow Oblast, batch No. 010413; sample C-01), OOO Avita-K (Moscow, Moscow Oblast, batch No. 012013; sample C-02), and ZAO Ivan-Chai (Moscow, Moscow Oblast; batch No. 010113; sample C-03). The species was determined by Dr. T. A. Aseeva (IGEB, SB, RAS). Specimens of the wild raw material were preserved in the IGEB, SB, RAS herbarium (No. HAs/ae-12/08-14/0613, HAs/ae-12/14-27/0713, HAs/ae-12/16-39/0713Y, and HAs/ae-12/19-40/0713Y).

Column chromatography (CC) used SiO_2, RP-SiO_2, polyamide (Sigma Aldrich), and Sephadex LH-20 (Amersham Biosciences). Spectrophotometric studies used an SF-2000 spectrophotometer (Spektr). MS analysis was performed in a MAT 8200 high-resolution mass spectrometer (Finnigan). NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian). Preparative HPLC was carried out on a Summit liquid chromatograph (Dionex) with a LiChrospher RP-18 column (250 × 10 mm, ∅ 10 μm; Merck). HPLC-MS/MS analysis used an Agilent 1100 liquid chromatograph connected to an LC-MSD-Trap-SL mass spectrometer. Electrospray ionization (ESI) used N_2 gas, drying temperature 300°C, drying-gas flow rate 8 L/min, spray-gas pressure 5 bar, capillary end potential –500 V, fragmentation potential –30 to –80 V, and *m*/*z* range 100–900 amu. Analytical HPLC used a Milikhrom A-02 microcolumn liquid chromatograph (EkoNova) with a UV detector (analytical HPLC). The total contents of FL and PP in the raw material were determined using spectrophotometry [13, 14].

Extraction and Fractionation. A weighed portion of dried and ground raw material (1.4 kg, sample Y-02) was extracted twice with EtOH (70%, 1:20) in an ultrasonic bath (100 W, 35 kHz) at 45°C for 90 min. The resulting extract was filtered and concentrated *in vacuo* to an aqueous residue that was extracted with hexane, EtOAc, and *n*-BuOH to produce hexane (16.8 g), EtOAc (134.4 g), *n*-BuOH (204.6 g), and aqueous (222.1 g) fractions. The EtOAc fraction (120 g) was

separated over polyamide (CC, 10 × 40 cm) with elution by H_2O (fraction E-01, 30.7 g), EtOH (40%, E-02, 10.7 g), EtOH (90%, E-03, 2.4 g), NH_3 (0.1% in 90% EtOH, E-04, 26.1 g), and NH_3 (0.5% in 90% EtOH, E-05, 46.7 g). Fraction E-02 (10 g) was separated using flash chromatography over SiO_2 (CC, 6 × 50 cm) and a hexane–EtOAc gradient (100:0→0:100) to produce subfractions E-02/01–E-02/10. Subfractions E-02/04, E-02/05–E-02/06, E-02/08, and E-02/09 were rechromatographed over RP-SiO_2 (CC, 4 × 40 cm, H_2O–MeCN eluent, 100:0→0:100) to isolate four compounds that were identified as quercetagetrin (quercetagetin-7-*O*-glucoside, 36 mg, **7**), patulitrin (patuletin-7-*O*-glucoside, 12 mg, **8**) [15], quercimeritrin (quercetin-7-*O*-glucoside, 10 mg, **6**) [16], and spinacetin-7-*O*-glucoside (18 mg, **11**). CC of fraction E-03 (2 g) over RP-SiO_2 (4 × 40 cm, H_2O–MeCN eluent, 100:0→0:100) and SiO_2 (5 × 30 cm, hexane–Me_2CO eluent, 100:0→80:20) isolated nepetin-7-*O*-glucoside (eupafolin-7-*O*-glucoside, 11 mg, **2**) [3] and jaceosidin-7-*O*-glucoside (16 mg, **3**) [2]. Fraction E-04 (22 g) was separated by CC over RP-SiO_2 (4 × 60 cm, H_2O–MeCN eluent, 100:0→0:100) and Sephadex LH-20 (4 × 60 cm, MeOH–H_2O eluent, 100:0→0:100) and by preparative HPLC (conditions 1) to produce five compounds including quercetagetin-7-*O*-(6″-*O*-caffeoyl)glucoside (27 mg, **9**) [15], tinctoside [patuletin-7-*O*-(6″-*O*-caffeoyl)glucoside, 39 mg, **10**] [17], gnaphaloside A [jaceosidin-7-*O*-(6″-*O*-caffeoyl)glucoside, 26 mg, **4**] [2, 3], gnaphaloside B [nepetin-7-*O*-(6″-*O*-caffeoyl)glucoside, 79 mg, **5**] [2], and **1** (39 mg). Fraction E-05 was chromatographed over Sephadex LH-20 (4 × 80 cm, MeOH–H_2O eluent, 100:0→0:100) and by preparative HPLC (conditions 2) to produce 11 compounds that were identified as caffeic acid (10 mg, **12**); 3-*O*- (102 mg, **13**), 4-*O*- (24 mg, **14**), 1,3-di-*O*- (16 mg, **15**), 1,5-di-*O*- (27 mg, **16**), 3.4-di-*O*- (14 mg, **17**), 3,5-di-*O*- (114 mg, **18**), 4,5-di-*O*- (29 mg, **19**), 1,3,5-tri-*O*- (11 mg, **20**), 3,4,5-tri-*O*- (57 mg, **21**) caffeoylquinic acids [5, 18]; and leontopodic acid (8 mg, **22**) [19].

The compositions of another two samples of the aerial part of *G. uliginosum* were studied analogously. Sample B-02 (2.5 kg) yielded **1** (29 mg), **2** (10 mg), **4** (31 mg), **5** (85 mg), **8** (14 mg), **10** (35 mg), **12** (19 mg), **13** (86 mg), **14** (22 mg), **15** (15 mg), **16** (26 mg), **17** (14 mg), **18** (127 mg), **19** (26 mg), **20** (9 mg), **21** (61 mg), **22** (10 mg), cosmosiin (apigenin-7-*O*-glucoside, 7 mg, **23**) [20], plantaginin (scutellarein-7-*O*-glucoside, 11 mg, **24**) [21], and isoquercitrin (quercetin-3-*O*-glucoside, 5 mg, **25**) [22]. Sample C-03 (0.9 kg) produced **4** (18 mg), **5** (10 mg), **6** (5 mg), **12** (5 mg), **13** (22 mg), **15** (10 mg), **16** (9 mg), **17** (5 mg), **18** (29 mg), **19** (11 mg), **20** (4 mg), **21** (9 mg), **22** (4 mg), and **24** (9 mg).

Gnaphaloside C (1). $C_{32}H_{30}O_{16}$. HR-ESI-MS, *m*/*z* 669.601 $[M - H]^-$; calcd 669.578. ESI-MS/MS, *m*/*z*: 669 $[M - H]^-$, 507 $[M - H -$ caffeoyl$]^-$, 345 $[M - H-$ caffeoyl-glucose$]^-$, 323 [caffeoyl-glucose – H]$^-$, 179 [caffeic acid – H]$^-$. UV spectrum (EtOH, λ_{max}, nm): 254, 270 sh, 331, 370; +$AlCl_3$ 265, 287 sh, 335, 427; +$AlCl_3$/HCl 263, 288 sh, 334, 425; +NaOAc 255, 333, 376; +NaOAc/H_3BO_3 255, 330, 370; +NaOMe 269, 338 sh, 397. ^{1}H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm) see Table 1, ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm) see Table 1.

Acid Hydrolysis of 1. Compound **1** (5 mg) was dissolved in TFA (10 mL, 5%) and heated to 110°C (2 h). The hydrolysate was concentrated *in vacuo*. The residue was dissolved in MeOH. The resulting hydrolysate was chromatographed over polyamide (CC, 30 g) with elution by H_2O (100 mL, eluate 1), EtOH (90%, 250 mL, eluate 2), and NH_3 (0.5% in 90% EtOH, 150 mL, eluate 3). HPLC of eluate 1 detected glucose (conditions 3, t_R 14.10 min); of eluate 3, caffeic acid (conditions 4, t_R 7.22 min). Eluate 2 was separated by preparative HPLC (conditions 1) to isolate spinacetin (2 mg) that was identified using MS and ^{13}C NMR spectroscopy.

Spinacetin. $C_{17}H_{14}O_8$. HR-ESI-MS, *m*/*z* 345.295 $[M - H]^-$; calcd 345.288. ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm, DEPT): 55.9 (CH_3, 3′-O<u>C</u>H_3), 60.1 (CH_3, 6-O<u>C</u>H_3), 92.4 (CH, C-8), 103.9 (C, C-10), 114.0 (CH, C-5′), 114.8 (CH, C-2′), 120.9 (CH, C-6′), 121.3 (C, C-1′), 130.5 (C, C-6), 135.8 (C, C-3), 145.4 (C, C-3′), 147.3 (C, C-4′), 148.2 (C, C-5), 148.9 (C, C-2), 154.7 (C, C-7), 156.4 (C, C-9), 178.0 (C, C-4) [23].

Alkaline Hydrolysis of 1. Compound **1** (5 mg) was dissolved in NaOH solution (1 mL, 0.4%) and heated at 50°C (30 min). The hydrolysate was neutralized with HCl (0.4%) and chromatographed over polyamide (CC, 40 g) with elution by H_2O (150 mL, eluate 1), EtOH (60%, 300 mL, eluate 2), and NH_3 (0.5% in 90% EtOH, 200 mL, eluate 3). HPLC of eluate 3 detected caffeic acid (conditions 4, t_R 7.22 min). Eluate 2 was separated by preparative HPLC (conditions 1) to produce spinacetin-7-*O*-β-D-glucopyranoside (2.5 mg) that was identified using UV, MS, PMR , and ^{13}C NMR spectroscopy.

Spinacetin-7-*O*-β-D-glucopyranoside (11). $C_{23}H_{24}O_{13}$. HR-ESI-MS, *m*/*z* 507.422 $[M - H]^-$; calcd 507.432. ESI-MS/MS, *m*/*z*: 507 $[M - H]^-$, 345 $[M - H -$ glucose$]^-$, 330 $[M - H -$ glucose $- CH_3]^-$. UV spectrum (EtOH, λ_{max}, nm): 255, 270 sh, 369; +$AlCl_3$ 264, 285 sh, 425; +$AlCl_3$/HCl 265, 287 sh, 424; +NaOAc 256, 375; +NaOAc/H_3BO_3 255, 371; +NaOMe 270, 395. ^{1}H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm) and ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm) see Table 1.

HPLC. Conditions 1: preparative HPLC, mobile phase H_2O (A) and MeCN (B), gradient mode (%B) 0–90 min 40–100%, flow rate 2 mL/min, column temperature 30°C, UV detector at 270 nm. Conditions 2: preparative HPLC, mobile phase H_2O (A) and MeCN (B), gradient mode (%B) 0–100 min 0–70%, flow rate 2 mL/min, column temperature 30°C,

UV detector at 330 nm. Conditions 3: analytical HPLC, Separon 5-NH_2 column (2 × 75 mm, ∅ 5 μm, TESSEK Ltd.), mobile phase 75% MeCN, isocratic mode (0–20 min), flow rate 100 μL/min, column temperature 35°C, UV detector at 190 nm. Conditions 4: analytical HPLC, ProntoSIL-120-5-C18 AQ column (2 × 75 mm, ∅ 5 μm, Metrohm AG), mobile phase $LiClO_4$ (0.2 M) in $HClO_4$ (0.006 M) (A) and MeCN (B), gradient mode (%B) 0–20 min 5–45%, flow rate 150 μL/min, column temperature 35°C, UV detector at 330 nm.

Plant raw material was analyzed quantitatively using analytical HPLC by placing raw material (40 mg) in an Eppendorf tube (2 mL), adding EtOH (1 mL, 70%), treating with ultrasound (50 kHz, 30 min, 40°C), and centrifuging (6000 g, 20 min). The resulting extract was filtered through a 0.45-μm filter and used for the analysis (1 μL). Conditions: ProntoSIL-120-5-C18 AQ column (2 × 75 mm, ∅ 5 μm, Metrohm AG), mobile phase $LiClO_4$ (0.2 M) in $HClO_4$ (0.006 M) (A) and MeCN (B), gradient mode (%B) 0–16 min 10–40%, 16–20 min 100%, flow rate 150 μL/min, column temperature 35°C, UV detector at 330 (caffeoylquinic acids, gnaphaloside C, tinctoside) and 340 nm (gnaphalosides A and B). The contents of pure constituents were calculated from calibration curves that were constructed using commercial samples of standard compounds (3-*O*-, 4-*O*-, 1,3-di-*O*-, 1,5-di-*O*-, 3,4-di-*O*-, 3,5-di-*O*-, 4,5-di-*O*-caffeoylquinic acids, all Sigma Aldrich) and isolated compounds of purity ≥95% (1,3,5-tri-*O*- and 3,4,5-tri-*O*-caffeoylquinic acids; gnaphalosides A, B, and C; and tinctoside).

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