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NEW BENZOFURAN LIGNANS FROM *Nepeta multifida*

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*The new benzofuran lignans nepetamultin A (**1**) and B (**2**) were isolated from flowers of* Nepeta multifida *L. [*Schizonepeta multifida *(L.) Briq.; Lamiaceae]. UV and NMR spectroscopic and mass spectrometric data indicated **1** was the 9′9′′′-di-*O*-methyl ester of schizotenuin A; **2**, the 9′-*O*-methyl ester of schizotenuin C_1. Biological studies revealed that **1** and **2** possessed antioxidant and antihyaluronidase activity.*

Keywords: *Nepeta multifida*, Lamiaceae, benzofuran lignans, antioxidants, hyaluronidase inhibitors.

Nepeta multifida L. [*Schizonepeta multifida* (L.) Briq.] is a perennial herbaceous species in the family Lamiaceae and is typical of steppe and meadow communities of Siberia. The herb of *N. multifida* is used in Tibetan medicine for allergic skin maladies and as an insecticide and antitussive [1]. Previously, extracts of *N. multifida* were found to possess stress-protective and anxiolytic activity [2]. The chemical composition of this plant species is poorly studied, despite the practical interest in it. The principal constituents of *N. multifida* essential oil were phellandrene, pulegone [3], limonene [4], β-ocimene, 1,8-cineol, and terpinolene [5]. In continuation of research on metabolites of the family Lamiaceae [6–8], phenolic compounds from flowers of *N. multifida* growing in the Republic of Buryatia were studied.

Separation of the MeOH extract from *N. multifida* flowers by column chromatography (CC) over polyamide, normal- and reversed-phase silica gel, and Sephadex LH-20 and preparative HPLC isolated known compounds **3**–**24** and two new benzofuran lignans, nepetamultin A (**1**) and B (**2**).

Compound **1** (nepetamultin A) had molecular formula $C_{38}H_{32}O_{16}$ (HR-ESI-MS, *m/z* 745.4271 $[M + H]^+$; calcd 745.6062) and a UV spectrum typical of caffeic acid derivatives (λ_{max} 228, 290, 320 nm). It gave after hydrolysis with NaOH two compounds that were identified as schizotenuin D (**18**) [9] and the methyl ester of α-hydroxyhydrocaffeic acid (oresbiusin A, danshensu methyl ester, **19**) [10]. The ESI-MS spectrum contained peaks for the protonated ion (*m/z* 745) and deacylated fragments with *m/z* 551 and 357 due to successive loss of α-hydroxyhydrocaffeic acids [9]. NMR spectra were similar to those of schizotenuin A (**16**), which is the 9,9′′-di-*O*-α-hydroxyhydrocaffeic ester of schizotenuin D [9], except for additional resonances for two methoxyls (δ_H 3.65, 3.75; δ_C 52.0, 52.2) (Table 1).

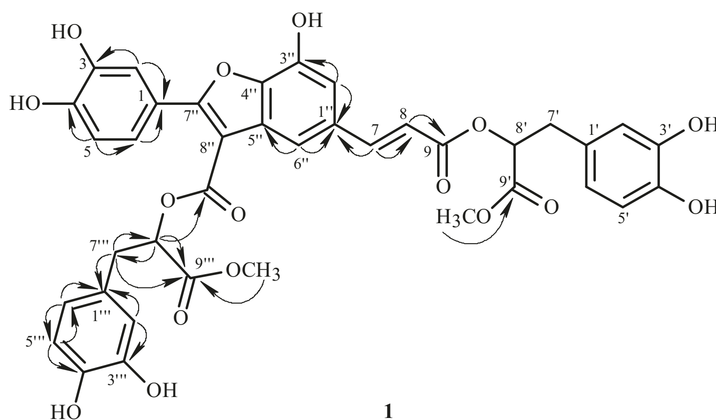

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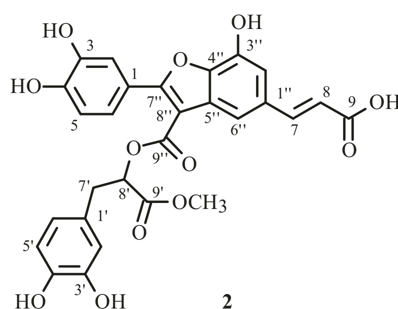
TABLE 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectra of **1** and **2** (DMSO-d_6, 298 K, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	–	120.1	–	119.9
2	7.50 (1H, d, J = 2.0)	116.8	7.51 (1H, d, J = 2.0)	116.5
3	–	145.4	–	145.6
4	–	148.5	–	148.2
5	6.93 (1H, d, J = 8.1)	115.0	6.89 (1H, d, J = 8.0)	115.0
6	7.39 (1H, dd, J = 2.0, 8.1)	122.1	7.42 (1H, dd, J = 2.0, 8.0)	122.4
7	7.59 (1H, d, J = 16.0)	146.4	7.62 (1H, d, J = 16.0)	144.6
8	6.22 (1H, d, J = 16.0)	116.6	6.25 (1H, d, J = 16.0)	117.5
9	–	165.3	–	168.2
1′	–	127.8	–	128.0
2′	6.69 (1H, d, J = 2.0)	117.0	6.78 (1H, d, J = 2.0)	117.2
3′	–	145.8	–	146.0
4′	–	144.6	–	145.0
5′	6.57 (1H, d, J = 8.1)	115.8	6.60 (1H, d, J = 8.1)	115.8
6′	6.45 (1H, dd, J = 2.0, 8.1)	120.4	6.48 (1H, dd, J = 2.0, 8.1)	120.3
7′	3.42 (1H, dd, J = 4.7, 14.0)	38.2	3.45 (1H, dd, J = 4.8, 14.2)	38.3
	3.12 (1H, dd, J = 8.1, 14.0)		3.15 (1H, dd, J = 8.2, 14.2)	
8′	5.22 (1H, dd, J = 4.7, 8.1)	75.1	5.48 (1H, dd, J = 4.8, 8.2)	75.0
9′	–	171.5	–	171.5
9′-OCH_3	3.65 (3H, s)	52.0	3.70 (3H, s)	52.1
1″	–	131.3	–	131.6
2″	7.11 (1H, s)	110.3	7.15 (1H, s)	110.0
3″	–	142.1	–	142.5
4″	–	143.2	–	143.0
5″	–	129.4	–	129.5
6″	7.65 (1H, s)	114.0	7.63 (1H, s)	114.0
7″	–	161.8	–	161.5
8″	–	106.9	–	106.5
9″	–	162.7	–	162.5
1‴	–	128.3		
2‴	6.75 (1H, d, J = 1.9)	117.4		
3‴	–	146.1		
4‴	–	145.2		
5‴	6.62 (1H, d, J = 7.9)	116.2		
6‴	6.50 (1H, dd, J = 1.9, 7.9)	120.6		
7‴	3.47 (1H, dd, J = 5.1, 13.8)	38.7		
	3.15 (1H, dd, J = 8.0, 13.8)			
8‴	5.53 (1H, dd, J = 5.1, 8.0)	75.3		
9‴	–	172.3		
9‴-OCH_3	3.75 (3H, s)	52.2		

The positions of the methoxyls were determined by analyzing HMBC spectrum in which resonances of protons at 3.65 and 3.75 ppm correlated with resonances of carbonyl C atoms C-9′ (δ 171.5) and C-9‴ (δ 172.3), respectively. The absolute configurations of C-8′ and C-8‴ were determined as *R* based on the retention time of the amide with (*S*)-2-phenylglycine methyl ester for α-hydroxyhydrocaffeic acid, which was obtained after alkaline hydrolysis of **1**. The studies showed that 1 was the 9′,9‴-di-*O*-methyl ester of schizotenuin A.

The molecular formula of nepetamultin B (**2**) was $C_{28}H_{22}O_{12}$ (HR-ESI-MS, *m/z* 551.2630 $[M + H]^+$; calcd 551.4362), which was 194 amu less than that of **1**. Alkaline hydrolysis also gave schizotenuin D (**18**) and oresbiusin A (**19**). NMR spectra of **2** were similar to those of schizotenuin C_1 (9″-*O*-α-hydroxyhydrocaffeic ester of schizotenuin D, **17**) [9] with an additional methyl (δ_H 3.70; δ_C 52.1) (Table 1). The C-9 resonance (δ 168.2) in the ^{13}C NMR spectrum was located at weaker field than that in **1** (δ 165.3), which was possible if this carboxyl was unsubstituted [11]. The HMBC spectrum exhibited mutual correlations

between resonances for H-8′ (δ 5.48) of an α-hydroxyhydrocaffeic acid and C-9″ of a substituted carbonyl (δ 162.5) and for H-8 (δ 6.25) and C-9 (δ 168.2), which indicated that the C-8′ carboxylic group of the benzofuran skeleton was esterified. If the C-8 carboxylic group of the propanoid moiety were substituted, then cross-peaks would be observed between resonances of H-8 (δ ~6.2)/H-8′ (δ ~5.2) and C-9 of the substituted carbonyl (δ ~165.5) with the simultaneous lack of correlations for the C-8″ unsubstituted carbonyl [1, 19]. Thus, **2** (nepetamultin B) was the 9′-*O*-methyl ester of schizotenuin C_1.



Known compounds were identified as pyracanthoside (**3**) [12], cynaroside (**4**) [13], diosmetin-7-*O*-glucoside (**5**) [13], eriocitrin (**6**) [13], scolimoside (**7**) [13], diosmin (**8**) [13], luteolin-7-*O*-glucuronide (**9**) [14], apigenin-7-*O*-glucuronide (**10**) [14], diosmetin-7-*O*-glucuronide (**11**) [14], luteolin-7-*O*-(6″-*O*-acetyl)-glucoside (**12**) [15], apigenin-7-*O*-(6″-*O*-acetyl)glucoside (**13**) [15], rosmarinic acid (**14**) [9], lithospermic acid (**15**) [9], schizotenuin A (**16**) [9], schizotenuin C_1 (**17**) [9], schizotenuin D (**18**) [9], oresbiusin A (**19**) [10], danshensu (**20**), 4-*O*-caffeylquinic acid (**21**) [16], 5-*O*-caffeylquinic acid (**22**) [16], caftaric acid (**23**) [17], and chicoric acid (24) [17]. Previously, flavones **4**, **5**, 7, **9**, and **10** and caffeic acid derivatives **14** and **22** were reported from *N. tenuifolia* Benth. [*Schizonepeta tenuifolia* (Benth.) Briq.] [9, 18, 19], *N. annua* Pall. [*S. annua* (Pall.) Schischk.] [20], and other *Nepeta* species [21] while benzofuran lignans **16**–**18** were observed only in *N. tenuifolia* [9].

Studies of the biological activity of **1** and **2** showed pronounced antiradical activity against DPPH˙ radical with 50% inhibition IC_{50} values of 58.11 and 49.18 μg/mL, respectively. This was close to the activities of known antioxidants rosmarinic acid (**14**, 25.32 μg/mL), schizotenuin A (**16**, 53.72 μg/mL), and schizotenuin C_1 (**17**, 44.49 μg/mL) [11]. Both compounds were inhibitors of hyaluronidase (IC_{50} 167.10 μg/mL for **1** and 171.60 μg/mL for **2**). This was characteristic of known benzofuran lignans **16** (152.11 μg/mL), **17** (165.08 μg/mL), and **14** (95.16 μg/mL) [22]. Antioxidants and hyaluronidase inhibitors are known to be capable of suppressing degranulation of mast cells [23]. Therefore, it can be assumed that the new compounds from *N. multifida* are promising antiallergic agents.

EXPERIMENTAL

Flowers of *N. multifida* were collected in the Republic of Buryatia (Mukhorshibirsky District, Aug. 3, 2020, 51°8′32.4″ N, 107°7′40″ E, 638 m above sea level) and dried in air in the shade (moisture <9%). The species was determined by Dr. N. K. Chirikova. A specimen of the raw material is preserved in the Herbarium of the IGEB, SB, RAS (No. BU/LAM-0820/37-209). CC used polyamide, normal- (SiO_2) and reversed-phase silica gel (RP-SiO_2), and Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO, USA). Spectrophotometric studies were performed on an SF-2000 spectrophotometer (OKB Spectr, St. Petersburg, Russia). Mass spectra were recorded on an LCMS-8050 TQ-mass-spectrometer (Shimadzu, Columbia, MD, USA) under the previously described conditions [24]. NMR spectra were taken on a VXR 500S spectrometer (Varian, Palo Alto, CA, USA). Preparative HPLC used an LC-20 Prominence liquid chromatograph (Shimadzu) equipped with a Shim-pak PREP-ODS column (20 × 250 mm, ∅ 15 μm) and an SPD-M30A diode-array detector (Shimadzu) at flow rate 1.0 mL/min and column temperature 20°C.

Extraction and Isolation of Compounds from *N. multifida*. Ground *N. multifida* raw material (1.4 kg) was extracted (2×) with MeOH (70%, 1:8, 60°C, US bath). The extract was concentrated and transferred to polyamide for CC (6 kg) with elution by H_2O (50 L), EtOH (70%, 25 L, fraction B), and NH_3 (0.5%) in EtOH (90%) (40 L, fraction C). Fraction B (27 g) was separated using CC over SiO_2 (2 × 40 cm, EtOAc–Me_2CO, 100:0→60:40), RP–SiO_2 (1 × 20 cm, H_2O–MeCN, 95:5→50:50), and Sephadex LH-20 (1 × 60 cm, MeOH–H_2O, 80:20→30:70) to isolate pyracanthoside (eriodictyol-7-*O*-glucoside, 20 mg, **3**) [12],

cynaroside (38 mg, **4**) [13], diosmetin-7-*O*-glucoside (7 mg, **5**) [13], eriocitrin (eriodictyol-7-*O*-rutinoside, 6 mg, **6**) [13], scolimoside (luteolin-7-*O*-rutinoside, 26 mg, **7**) [13], and diosmin (diosmetin-7-*O*-rutinoside, 5 mg, **8**) [13]. Fraction C (47 g) was separated using CC over SiO_2 (2 × 60 cm, EtOAc–Me_2CO–MeOH, 100:0:0→60:30:10), RP-SiO_2 (1 × 20 cm, H_2O–MeCN, 100:0→20:80), and Sephadex LH-20 (1 × 60 cm, MeOH–H_2O–AcOH, 90:5:5→20:75:5) and preparative HPLC (eluent A, MeOH; eluent B, H_2O; gradient of B: 0–30 min, 5–15%; 30–45 min, 15–38%; 45–90 min, 38–58%; 90–120 min, 58–85%) to afford **1** (29 mg), **2** (10 mg), luteolin-7-*O*-glucuronide (108 mg, **9**) [14], apigenin-7-*O*-glucuronide (22 mg, **10**) [14], diosmetin-7-*O*-glucoronide (7 mg, **11**) [14], luteolin-7-*O*-(6″-*O*-acetyl)glucoside (53 mg, **12**) [15], apigenin-7-*O*-(6″-*O*-acetyl)glucoside (5 mg, **13**) [15], rosmarinic acid (420 mg, **14**) [9], lithospermic acid (7 mg, **15**) [9], schizotenuin A (311 mg, **16**) [9], schizotenuin C_1 (75 mg, **17**) [9], schizotenuin D (21 mg, **18**) [9], oresbiusin A (methyl ester of α-hydroxyhydrocaffeic acid, 9 mg, **19**) [10], danshensu (α-hydroxyhydrocaffeic acid, 53 mg, **20**), 4-*O*-caffeylquinic acid (6 mg, **21**) [16], 5-*O*-caffeylquinic acid (5 mg, **22**) [16], caftaric acid (7 mg, **23**) [17], and chicoric acid (3 mg, **24**) [17].

Nepetamultin A (1). $C_{38}H_{32}O_{16}$. UV (MeOH, λ_{max}, nm): 228 sh, 290, 320. HR-ESI-MS *m/z* 745.4271 $[M + H]^+$ (calcd for $C_{38}H_{33}O_{16}$, 745.6062). ESI-MS, *m/z* (%): 767 $[M + Na]^+$ (100), 745 $[M + H]^+$ (52). ESI-MS^2 [745]: 551 $[(M + H) - 194]^+$ (100), 533 $[(M + H) - 212]^+$ (23), 357 $[(M + H) - 2 \times 194]^+$ (100), 339 $[(M + H) - 194 - 212]^+$ (11), 321 $[(M + H) - 2 \times 212]^+$ (5), 213 (35). ESI-MS^3 [213]: 199 (100), 195 (33), 181 (58). Table 1 lists the ^{1}H NMR (500 MHz, DMSO-d_6, 298 K, δ, ppm) and ^{13}C NMR spectra (125 MHz, DMSO-d_6, 298 K, δ, ppm) see in Table 1.

Nepetamultin B (2). $C_{28}H_{22}O_{12}$. UV (MeOH, λ_{max}, nm): 220 sh, 238 sh, 290 sh, 317. HR-ESI-MS *m/z* 551.2630 $[M + H]^+$ (calcd for $C_{28}H_{23}O_{12}$, 551.4362). ESI-MS, *m/z* (%): 573 $[M + Na]^+$ (100), 551 $[M + H]^+$ (58). ESI-MS^2 [551]: 357 $[(M + H) - 194]^+$ (100), 339 $[(M + H) - 212]^+$ (11), 213 (28). ESI-MS^3 [213]: 199 (100), 195 (35), 181 (52). Table 1 lists the ^{1}H NMR (500 MHz, DMSO-d_6, 298 K, δ, ppm) and ^{13}C NMR (125 MHz, DMSO-d_6, 298 K, δ, ppm).

Hydrolysis of 1 and 2 by NaOH. A weighed portion of compound (10 mg) was dissolved in NaOH solution (1 mL, 10%). The mixture was incubated at 20°C for 1 h, neutralized with AcOH (10%), placed onto polyamide (20 g) preconditioned with H_2O, and eluted with H_2O (200 mL) and EtOH (70%). The EtOH eluate was concentrated and separated by prep. HPLC (eluent 45% MeOH, isocratic mode, 0–60 min, flow rate 1 mL/min). Compound **1** (10 mg) gave schizotenuin D (4 mg, **18**) [9] and oresbiusin A (5 mg, 19) [10]; compound **2** (10 mg), **18** (6 mg) and **19** (3 mg).

Determination of Absolute Configuration of C-8′/C-8‴. A weighed portion of **19** (2 mg) obtained after hydrolysis by NaOH was demethylated in anhydrous TFA (1 mL) with heating (100°C, 8 h) [25] to produce α-hydroxyhydrocaffeic acid (~1 mg) [26]. A weighed portion of the latter (1 mg) and (*S*)-2-phenylglycine methyl ester (5 mg) were dissolved in DMF (500 μL). The mixture was treated with benzotriazol-1-yloxy-*tris*-pyrrolidinophosphonium hexafluorophosphate (10 mg), 1-hydroxybenzotriazole (3 mg), and *N*-methylmorpholine (15 μL); left for 12 h with constant stirring at 20°C; treated with EtOAc (1 mL); and washed with HCl solution (5%) and saturated $NaHCO_3$ and NaCl solutions. The organic layer was separated by prep. HPLC (eluent 0.05% TFA and 80% MeCN, isocratic mode, 0–60 min, flow rate 1 mL/min) to afford the (*S*)-amide of α-hydroxyhydrocaffeic acid (0.3 mg) [22], the retention time of which (15.6 min, anal. HPLC; eluent A, MeCN, eluent B, H_2O; gradient of B: 0–20 min, 15–60%; 20–30 min, 60–100%) coincided with that of the (*S*)-amide of α-hydroxyhydrocaffeic acid obtained from rosmarinic acid with the (*R*)-configuration of C-8.

Biological Activity. The antiradical activity of the compounds against 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\cdot$; Sigma-Aldrich) was determined by spectrophotometry using a microplate method [27]. The effects of the compounds on hyaluronidase were studied by spectrophotometry [22] using hyaluronidase (3.2.1.35; type IV-S, 750-3000 U/mg; Sigma-Aldrich).

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