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NEW FLAVONOIDS FROM *Nonea rossica* AND *Tournefortia sibirica*

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The chemical composition of two species in the family Boraginaceae, Nonea rossica *Steven and* Tournefortia sibirica *L., in which alkaloids, hydroxycinnamates, and flavonoids, including two new acylglycosides **1** and **2** were observed, was studied. UV and NMR spectroscopy and mass spectrometry found that the new compounds were quercetin 3-*O*-(2″-O-caffeoyl-6″-O-acetyl)-β-D-glucopyranoside (noneaside, **1**; from* N. rossica*) and kaempferol 3-*O*-(2″-O-caffeoyl-6″-O-acetyl)-β-D-glucopyranoside (tournefoside, **2**; from* T. sibirica*). Both flavonoids possessed antiradical activity.*

Keywords: *Nonea rossica*, *Tournefortia sibirica*, Boraginaceae, flavonoids, quercetin, kaempferol, antioxidants.

The family Boraginaceae is represented in Siberia by 28 genera including >100 species. Despite the broad distribution, the chemical compositions of most representatives have not been reported or are incomplete [1]. In continuation of research on this family [2, 3], two broadly distributed species in the region, *Nonea rossica* Steven [*N. pulla* subsp. *pulla*, *N. pulla* subsp. *rossica* (Steven) Soo] and *Tournefortia sibirica* L. [*Arguzia rosmarinifolia* Steven, *Messerschmidia sibirica* (L.) L.], were studied. The chemical composition of *N. rossica* is unknown while essential oil [4], flavones [5], the alkaloid tournesibirin [6], and cembrane diterpenoids [7] were observed in *T. sibirica* of Chinese origin. Both species were used in traditional Buryat medicine under the name gyer-shing-pa as antipyretic and antibacterial agents [8]. Herein, results of a chemical study of the aerial parts of *N. rossica* and *T. sibirica* growing in Baikal District are reported.

The EtOH extract of *N. rossica* was separated by column chromatography (CC) over polyamide, Al_2O_3, normal and reversed-phase silica gel, and Sephadex LH-20 and by preparative HPLC to afford 27 compounds including the alkaloids intermedine (**3**) [9], lycopsamine (**4**) [9], intermedine *N*-oxide (**5**) [9], and lycopsamine *N*-oxide (**6**) [9]; the flavonoids kaempferol 3-*O*-galactoside (**7**) [10], kaempferol 3-*O*-glucoside (*8*) [10], kaempferol 3-*O*-rutinoside (**9**) [10], kaempferol 3-*O*-neohesperidoside (**10**) [10], quercetin 3-*O*-glucoside (**11**) [10], quercetin 3-*O*-galactoside (**12**) [10], quercetin 3-*O*-rutinoside (**13**) [10], quercetin 3-*O*-neohesperidoside (**14**) [11], kaempferol 3-*O*-gentiobioside (**15**) [10], quercetin 3-*O*-gentiobioside (**16**) [10], quercetin 3-*O*-(2″-*O*-acetyl)-glucoside (**22**) [12], quercetin 3-*O*-(6″-*O*-acetyl)-glucoside (**23**) [12], and quercetin 3-*O*-(2″,6″-di-*O*-acetyl)-glucoside (**24**) [12]; hydroxycinnamates 2-*O*-caffeoylthreonic acid (**17**) [13], 3-*O*-caffeoylthreonic acid (**18**) [13], 2-*O*-caffeoylglyceric acid (**19**) [13], 3-*O*-caffeoylglyceric acid (**20**) [13], globoidnan B (**21**) [13], rosmarinic acid (**25**) [13], and salvianolic acids B (**26**) [13] and L (**27**) [14]; in addition to new compound **1**.

The molecular formula $C_{32}H_{28}O_{16}$ for **1** was determined using mass spectrometry (HR-ESI-MS, *m/z* 669.4083 $[M + H]^+$, calcd for $C_{32}H_{29}O_{16}$, 669.5174) and ^{13}C NMR spectroscopy. The shape of the absorption spectrum of **1** was characteristic of flavonoids acylated by a caffeic acid moiety, which was observed after hydrolysis of **1** with trifluoroacetic acid (TFA, 2 M) together with quercetin and D-glucose. Daughter ions of the protonated ion in the mass spectrum appeared at *m/z* 507, 465, and 303, indicating loss of fragments with masses of 162 (caffeoyl), 42 (acetyl), and 162 amu (glucose), respectively.

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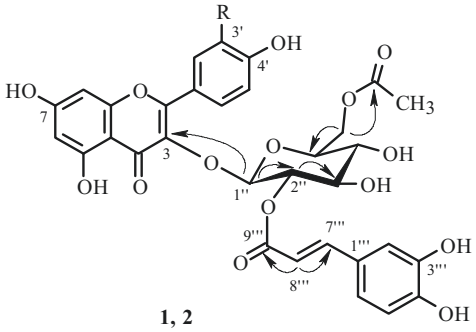
TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectra of **1** and **2** (DMSO-d_6, 298 K, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2	–	157.0	–	157.2
3	–	134.8	–	134.5
4	–	178.0	–	177.8
5	–	161.1	–	161.0
6	6.14 (1H, d, J = 2.0)	99.0	6.42 (1H, d, J = 2.0)	99.3
7	–	163.4	–	163.0
8	6.40 (1H, d, J = 2.0)	94.5	6.84 (1H, d, J = 2.0)	94.2
9	–	156.1	–	155.8
10	–	104.2	–	104.1
1′	–	120.3	–	119.4
2′	7.31 (1H, d, J = 2.1)	116.5	8.02 (2H, d, J = 9.0)	130.5
3′	–	145.4	6.99 (2H, d, J = 9.0)	115.1
4′	–	148.3	–	160.3
5′	6.91 (1H, d, J = 8.0)	115.9	6.99 (2H, d, J = 9.0)	115.1
6′	7.24 (1H, dd, J = 2.1, 8.0)	122.3	8.02 (2H, d, J = 9.0)	130.5
1″	5.01 (1H, d, J = 7.2)	102.1	4.95 (1H, d, J = 7.0)	101.8
2″	5.14 (1H, m)	74.9	5.16 (1H, m)	74.6
3″	3.68 (1H, m)	76.3	3.70 (1H, m)	76.5
4″	3.45 (1H, m)	71.6	3.47 (1H, m)	71.9
5″	3.61 (1H, m)	75.4	3.60 (1H, m)	75.2
6″	4.54 (1H, dd, J = 2.1, 11.8), 4.29 (1H, dd, J = 5.4, 11.8)	65.3	4.51 (1H, dd, J = 2.0, 12.0), 4.33 (1H, dd, J = 5.2, 12.0)	65.2
1‴	–	127.3	–	127.2
2‴	7.03 (1H, d, J = 2.0)	115.3	7.00 (1H, d, J = 1.9)	114.7
3‴	–	148.8	–	148.6
4‴	–	146.0	–	145.7
5‴	6.73 (1H, d, J = 8.1)	117.2	6.70 (1H, d, J = 8.0)	117.1
6‴	6.84 (1H, dd, J = 2.0, 8.1)	123.2	6.85 (1H, dd, J = 1.9, 8.0)	123.0
7‴	7.55 (1H, d, J = 15.8)	147.2	7.52 (1H, d, J = 16.0)	147.4
8‴	6.30 (1H, d, J = 15.8)	114.8	6.25 (1H, d, J = 16.0)	114.5
9‴	–	168.1	–	168.0
6″-$\underline{C}H_3CO$	2.03 (3H, s)	21.3	2.01 (3H, s)	21.0
6″-$CH_3\underline{C}O$	–	169.3	–	169.1

Work up of **1** with NaOH solution (0.5%) formed quercetin-3-*O*-glucoside (**11**) [10], which indicated acyl fragments were present in the carbohydrate part of the compound. A comparative analysis of ^{1}H NMR spectra of **1** and **11** revealed weak-field shifts of resonances in **1** for C-2″ (δ_C 73.4→74.9) and C-6″ (δ_C 61.4→65.3) (Table 1). The HMBC spectrum showed correlations between resonances of H-2″ and the carbonyl C atom of caffeic acid (δ_H/δ_C 5.14/168.1) and H-6″ and the carbonyl C atom of acetyl (δ_H/δ_C 4.29, 4.54/169.3). As a result, compound **1** was elucidated as quercetin 3-*O*-(2″-*O*-caffeoyl-6″-*O*-acetyl)-*β*-D-glucopyranoside, which we called noneaside.

Chromatographic separation of the EtOH extract of *T. sibirica* isolated **1**, **4**, **6**, **8**, **9**, **11**, **13**, **23**–**25**, 7-*O*-acetyllycopsamine (**28**) [15], 7-*O*-acetyllycopsamine *N*-oxide (**29**) [15], piperonal (**30**) [16], lithospermic acid (**31**) [13], rosmarinic acid 9-*O*-methyl ester (**32**) [17], kaempferol 3-*O*-(6″-*O*-acetyl)-glucoside (**33**) [10], and a new flavonoid **2**. All compounds were observed in *T. sibirica* for the first time.

Compound **2** ($C_{32}H_{28}O_{15}$, HR-ESI-MS, *m/z* 653.3004 $[M + H]^+$, calcd for $C_{32}H_{29}O_{15}$, 653.5184) gave kaempferol, D-glucose, and caffeic acid after hydrolysis by TFA (2 M). Work up with NaOH solution (0.5%) gave kaempferol-3-*O*-glucoside (**8**) [10]. UV and NMR spectroscopic (Table 1) and mass spectrometric data indicated that **2** was an analog of **1** containing kaempferol as the aglycon or kaempferol 3-*O*-(2″-*O*-caffeoyl-6″-*O*-acetyl)-*β*-D-glucopyranoside, which was named tournefoside.



1, 2

→ HMBC

1: R = OH; **2:** R = H

A study of the antiradical activity against DPPH radical showed the greatest activity (IC_{50} 7.43 μM) for **1**, which exceeded those of the reference compound Trolox (IC_{50} 30.08 μM) and **2** (IC_{50} 35.71 μM).

According to the results, *N. rossica* and *T. sibirica* contained dehydropyrrolizidine alkaloids, caffeic acid esters, rosmarinic acid and its derivatives, and quercetin and kaempferol *O*-glycosides. These compound groups were encountered in other *Boraginaceae* species [18, 19]. However, flavonoid acylglycosides were found for the first time in the family.

EXPERIMENTAL

The aerial part of the flowering plants was collected in the Republic of Buryatia and air-dried in the shade (humidity < 5%): *N. rossica*, near Mukhorshibir (Mukhorshibirsky District, Jun. 20, 2020; 51°01′58.9″ N, 107°49′15.1″ E, 650 m above sea level); *T. sibirica*, near Gusinozersk (Selenginsky District, Jul. 15, 2019; 51°12′39.2″ N, 106°31′47.3″ E, 420 m above sea level). The species were determined by Dr. N. K. Chirikova. Specimens of raw material are preserved in the herbarium of the IGEB, SB, RAS (No. BU/BOR-0620/51-062, BU/BOR-0719/92-114). Column chromatography (CC) used polyamide, Al_2O_3, normal (SiO_2) and reversed-phase silica gel (RP-SiO_2), and Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO, USA). Spectrophotometric studies used an SF-2000 spectrophotometer (OKB Spectr, St. Petersburg, Russia). Mass spectra were recorded in an LCMS-8050 TQ-mass spectrometer (Shimadzu, Columbia, MD, USA) [20]. 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, d 15 μm) and an SPD-M30A diode array detector (Shimadzu) using flow rate 1.0 mL/min and column temperature 20°C. Antiradical activity of the compounds against 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH, Sigma-Aldrich) was determined by a microplate spectrophotometric method [21].

Extraction and Isolation of 1 and 3–27 from *N. rossica*. Ground raw material (3.5 kg) was extracted with EtOH [70%, 1:10, 50°C, 3 ×, ultrasound (US) bath]. The EtOH extract was concentrated to dryness. The dry solid was exhaustively extracted with hexane and water-saturated BuOH. The BuOH fraction (630 g) was separated by CC (1:10) over polyamide with elution by H_2O (fraction A), EtOH (60%, faction B), and NH_3 (0.5% in 95% EtOH, fraction C). Fraction A (320 g) was separated by CC over Al_2O_3 (eluent $CHCl_3$–EtOH–NH_3, 99:0:1→59:40:1) and RP-SiO_2 (1 × 30 cm, eluent H_2O–MeCN, 90:10→50:50) and by preparative HPLC (eluent A, 0.2% HCOOH in H_2O; eluent B, 0.2% HCOOH in MeCN; isocratic mode, 15% B) to isolate intermedine (10 mg, **3**) [9], lycopsamine (5 mg, **4**) [9], intermedine *N*-oxide (35 mg, **5**) [9], and lycopsamine *N*-oxide (30 mg, **6**) [9].

Fraction B (80 g) was chromatographed over SiO_2 (3 × 40 cm, eluent hexane–EtOAc, 100:0→70:30, EtOAc–Me_2CO, 100:0→80:20), Sephadex LH-20 (2 × 80 cm, eluent EtOH–H_2O, 90:10→50:50), and RP-SiO_2 (1 × 30 cm, eluent H_2O–MeCN, 95:5→20:80) and by preparative HPLC (eluent A, H_2O; eluent B, MeCN; gradient mode, %B: 0–60 min, 5–50%, 60–80 min, 50–60%) to afford kaempferol 3-*O*-galactoside (trifolin, 10 mg, **7**) [10], kaempferol 3-*O*-glucoside (astragalin, 50 mg, **8**) [10], kaempferol 3-*O*-rutinoside (nicotiflorin, 60 mg, **9**) [10], kaempferol 3-*O*-neohesperidoside (30 mg, **10**) [10], quercetin 3-*O*-glucoside (isoquercitrin, 25 mg, **11**) [10], quercetin 3-*O*-galactoside (hyperoside, 20 mg, **12**) [10], quercetin 3-*O*-rutinoside (rutin, 15 mg, **13**) [10], quercetin 3-*O*-neoherperidoside (calendoflavobioside, 8 mg, **14**) [11], kaempferol 3-*O*-gentiobioside (5 mg, **15**) [10], and quercetin 3-*O*-gentiobioside (9 mg, **16**) [10]. Fraction C (200 g) was separated over Sephadex LH-20 (2 × 80 cm, eluent EtOH–H_2O, 50:50→0:100) and RP-SiO_2 (1 × 30 cm, eluent H_2O–MeCN, 95:5→50:50) and by preparative HPLC

(eluent A, 0.5% AcOH in H_2O; eluent B, 0.5% AcOH in MeCN; gradient mode, %B: 0–20 min, 5–10%, 20–90 min, 10–40%, 90–150 min, 40–50%) to isolate 12 compounds including **1** (30 mg), 2-*O*-caffeoylthreonic acid (20 mg, **17**) [13], 3-*O*-caffeoylthreonic acid (25 mg, **18**) [13], 2-*O*-caffeoylglyceric acid (55 mg, **19**) [13], 3-*O*-caffeoylglyceric acid (70 mg, **20**) [13], globoidnan B (25 mg, **21**) [13], quercetin 3-*O*-(2″-*O*-acetyl)-glucoside (30 mg, **22**) [12], quercetin 3-*O*-(6″-*O*-acetyl)-glucoside (40 mg, **23**) [12], quercetin 3-*O*-(2″,6″-di-*O*-acetyl)-glucoside (65 mg, **24**) [12], rosmarinic acid (1.4 g, **25**) [13], salvianolic acid B (30 mg, **26**) [13], and salvianolic acid L (45 mg, **27**) [14].

Noneaside (1), $C_{32}H_{28}O_{16}$. UV (MeOH, λ_{max}, nm): 255, 267 sh., 300 sh., 341; +$AlCl_3$ 271, 296 sh., 348 sh., 422; +$AlCl_3$ + HCl 265, 296 sh., 340, 400; +NaOAc 260, 296 sh., 388; +NaOAc + H_3BO_3 260, 360. HR-ESI-MS, *m/z* 669.4083 $[M + H]^+$ (calcd for $C_{32}H_{29}O_{16}$, 669.5174). ESI-MS, *m/z* (%): 669 $[M + H]^+$ (100). ESI-MS^2 [669]: 507 $[(M + H) – 162]^+$ (73), 465 $[(M + H) – 162 – 42]^+$ (14), 303 $[(M + H) – 2 \times 162 – 42]^+$ (100). ^1H NMR (500 MHz, DMSO-d_6, 298 K) and ^{13}C NMR (125 MHz, DMSO-d_6, 298 K), see Table 1.

Extraction and Isolation of 1, 2, 4, 6, 8, 9, 11, 13, 23–25, and 28–33 from *T. sibirica*. The above scheme was used to isolate from *T. sibirica* herb (1 kg) **1** (10 mg), **2** (35), **4** (15), **6** (40), **8** (10), **9** (25), **11** (20), **13** (10), **23** (140), **24** (35), **25** (220), 7-*O*-acetyllycopsamine (20 mg, **28**) [15], 7-*O*-acetyllycopsamine *N*-oxide (5 mg, **29**) [15], piperonal (heliotropin, 15 mg, **30**) [16], lithospermic acid (40 mg, **31**) [13], rosmarinic acid 9-*O*-methyl ester (35 mg, **32**) [17], and kaempferol 3-*O*-(6″-*O*-acetyl)-glucoside (25 mg, **33**) [10].

Tournefoside (2). $C_{32}H_{28}O_{15}$. UV (MeOH, λ_{max}, nm): 270, 328; +$AlCl_3$ 272, 298, 340, 387 sh.; +$AlCl_3$ + HCl 275, 298, 333, 388 sh.; +NaOAc 269, 332; +NaOAc + H_3BO_3 261, 350. HR-ESI-MS, *m/z* 653.3004 $[M + H]^+$ (calcd for $C_{32}H_{29}O_{15}$, 653.5184). ESI-MS, *m/z* (%): 653 $[M + H]^+$ (100). ESI-MS^2 [653]: 491 $[(M + H) – 162]^+$ (70), 449 $[(M + H) – 162 – 42]^+$ (10), 287 $[(M + H) – 2 \times 162 – 42]^+$ (100).^1H NMR (500 MHz, DMSO-d_6, 298 K) and ^{13}C NMR (125 MHz, DMSO-d_6, 298 K), see Table 1.

Hydrolysis. Acid hydrolysis was performed in TFA (2 M) [22] followed by analysis of the monosaccharide composition (HPLC after derivatization with 3-methyl-1-phenyl-2-pyrazolin-5-one) [23], determination of the D/L-series type (HPLC after reductive amination with L-tryptophan) [24], and determination of the aglycons by HPLC-MS [25]. The hydrolysis products contained quercetin (**1**), kaempferol (**2**), D-glucose (**1**, **2**), and caffeic acid (**1**, **2**). Alkaline hydrolysis used NaOH solution (0.5%) as before [22]. The hydrolysis products of **1** and **2** were identified using UV, NMR, and mass spectrometry as quercetin 3-*O*-glucoside (**11**) [10] and kaempferol 3-*O*-glucoside (**8**) [10], respectively.

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