Chemistry of Natural Compounds, Vol. 47, No. 6, January, 2012 [Russian original No. 6, November-December, 2011]

CHEMICAL INVESTIGATION OF *Caragana spinosa* RUNNERS

D. N. Olennikov,* L. M. Tankhaeva, and V. V. Partilkhaev

UDC 582.738:547.99:547.913

Caragana spinosa (L.) DC is a highly branched spiny bush of the family Fabaceae that is widely distributed in the Republic of Buryatiya. Flowering runners and flowers of *C. spinosa* (*ci yang hwa*, *ching yang ho*) were used in Tibetan medicine [1]. Information on the chemical composition of *C. spinosa* runners is limited. The presence of quercetin, kaempferol, isoquercitrin, quercitrin, rutin, and narcissin was established previously [2]. The goal of the present work was to investigate the chemical composition of *C. spinosa* runners.

Runners of *C. spinosa* were collected during flowering near Nizhnii Ubukun (Selengin Region, Republic of Buryatiya, June 23, 2010; 51°52′54″N, 106°89′35″E). The species was defined by Cand. Pharm. Sci. G. V. Chekhirova (IGEB, SB, RAS). Specimens of the plant are stored in the herbarium of the IGEB, SB, RAS (No. Fb/s-24/17-02/1006). Ground raw material (420 g) was extracted with EtOH (70%, 1:15, 5×) on a boiling-water bath. The alcohol extract was concentrated to an aqueous residue that was subjected to liquid-phase extraction by hexane, $CHCl_3$, EtOAc, and *n*-BuOH to produce five fractions. These were hexane (16.31 g), $CHCl_3$ (1.11), EtOAc (3.96), BuOH (11.23), and aqueous (75.70).

Column chromatography (CC) over SiO_2 (3 × 40 cm) using a C_6H_{14}:EtOAc gradient (100:0→70:30) with subsequent CC over Sephadex LH-20 (1 × 45 cm, $CHCl_3$:EtOH, 100:0→0:100) and TLC (solvent system 1) were used to separate the $CHCl_3$ fraction (1 g). The EtOAc (2.5 g) and BuOH (6 g) fractions were chromatographed using CC over Sephadex LH-20 (4 × 30 cm) and an EtOH:H_2O gradient (96:4→0:100) with subsequent CC over polyamide (5 × 40 cm, EtOH:H_2O, 96:4→0:100), SiO_2 (2 × 50 cm, $CHCl_3$:EtOH, 100:0→70:30), and TLC (solvent systems 1 and 2). The separation isolated 22 compounds that were identified using mp, UV, IR, and ^{13}C NMR spectroscopy, and MS. The $CHCl_3$ fraction afforded β-sitosterol (**1**, 65 mg) [3], umbelliferone (**2**, 6 mg), scopoletin (**3**, 4 mg), xanthotoxin (**4**, 11 mg) [4], apigenin (**5**, 3 mg), and luteolin (**6**, 2 mg) [5]; the EtOAc fraction, kaempferol (**7**, 3 mg), quercetin (**8**, 16 mg) [6], myricetin (**9**, 2 mg) [7], avicularin (quercetin-3-*O*-arabinoside, **10**, 4 m), quercitrin (quercetin-3-*O*-rhamnoside, **11**, 4 mg), isoquercitrin (quercetin-3-*O*-glucoside, **12**, 3 mg), hyperoside (quercetin-3-*O*-galactoside, **13**, 11 mg) [8], and *p*-coumaric (**14**, 2 mg), caffeic (**15**, 3 mg), 3-caffeylquinic (**16**, 4 mg), 5-caffeylquinic (**17**, 3 mg) [6], ferulic (**18**, 8 mg) [9], and gallic (**19**, 24 mg) acids [6]; the BuOH fraction, rutin (quercetin-3-*O*-rutinoside, **20**, 46 mg), narcissin (isorhamnetin-3-*O*-rutinoside, **21**, 12 mg) [10], and (–)-epigallocatechin (**22**, 5 mg) [11]. Compounds **7**, **8**, **11**, **12**, **20**, and **21** were isolated previously from *C. spinosa*. Compounds **1–6**, **9**, **10**, **13–19**, and **22** were observed for the first time in this plant species. The phenylpropanoids (**14–18**), gallic acid (**19**), and (–)-epigallocatechin (**22**) were found for the first time in the genus *Caragana* Lam.

According to HPLC data, the phenolic compounds were evenly distributed in *C. spinosa* runners (Table 1). The compositions of flavonoids and phenylpropanoids of leaves and flowers were similar and differed from that of stems. Flavonoid glycosides accumulated in leaves and flowers, 14.43 and 11.57 mg/g, respectively. The content in stems was 1.57 mg/g. Aglycons of flavonoids, which were found in trace quantities in leaves and flowers, and phenylpropanoids concentrated in stems (6.64 and 1.69 mg/g, respectively).

The dominant flavonoids of stems and leaves were rutin (**20**) and narcissin (**21**); of stems, quercetin (**8**). A comparison of the compositions of phenolic compounds of epidermis and stem phloem showed that the epidermal layers were sites of localization for this group of compounds. The concentration could reach from 4.2 (for 3-caffeylquinic acid, **16**) to 13.7 times (for quercitrin, **11**). Aglycons of flavonoids (**7**, **8**) and isoquercitrin (**13**) were not observed in phloem. Umbelliferone (**2**) and caffeic acid (**15**) were present only in this plant part.

Institute of General and Experimental Biology, Siberian Branch, Russian Academy of Sciences, 670047, Ulan-Ude, ul. Sakh′yanovoi, 6, fax: (3012) 43 30 34, e-mail: oldaniil@rambler.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 6, November–December, 2011, pp. 861–863. Original article submitted April 28, 2011.

 0009-3130/12/4706-0988

TABLE 1. Content of Phenolic Compounds in *C. spinosa* Runners, mg/g of Air-Dried Raw Material

Compound, group of compounds	Morphological group					
	leaves	flowers	stems	stem epidermis	stem phloem	spines
2	–	–	–	–	Tr.	Tr.
7	Tr.	–	1.55	2.68	–	0.46
8	0.15	Tr.	5.09	7.25	–	0.73
11	1.01	0.68	0.15	1.51	0.11	0.57
12	2.26	1.12	0.59	1.06	0.18	0.40
13	2.16	1.60	–	–	–	0.56
15	–	–	Tr.	–	0.25	–
16	0.93	0.53	1.69	2.67	0.63	0.30
20	4.67	4.81	0.45	0.66	0.06	1.00
21	4.33	3.36	0.38	0.51	0.06	1.13
Flavonoids including	14.58	11.57	8.21	13.67	0.41	4.85
aglycons	0.15	Tr.	6.64	9.93	–	1.19
glycosides	14.43	11.57	1.57	3.74	0.41	3.66
Phenylpropanoids	0.93	0.53	1.69	2.67	0.88	0.30

–, not observed; Tr., trace (<0.01 mg/g).

The study of the composition of phenolic compounds in *C. spinosa* spines indicated that these metamorphoses were intermediate between stems and leaves. Algycons of flavonoids (**7**, **8**) that were characteristic of stems and relatively high contents of quercetin biosides (**20**, **21**) were observed in them. This indicated that this organ was similar to leaves. According to early information, *C. spinosa* spines are altered leaf petioles that remained on runners after the autumn shedding of leaves, became woody, and converted into spines [12]. This was confirmed by the chemical analysis.

Considering information on the biological activity of β-sitosterol as an anticholesterolemic agent [13], *C. spinosa* organs were analyzed quantitatively using an HPTLC-densitometric method [14]. It was found that the β-sitosterol contents in stems, leaves, flowers, and spines were 2.51 ± 0.07, 3.61 ± 0.11, 9.22 ± 0.28, and 1.03 ± 0.03 mg/g of air-dried raw material, respectively. Epidermal layers of stems typically had higher accumulations of β-sitosterol (3.36 ± 0.10 mg/g) than phloem (2.27 ± 0.06 mg/g).

Essential oil (EO) of flowering *C. spinosa* runners was isolated from ground raw material (150 g) by steam distillation in a Clevenger apparatus for 90 min. EO was a thick yellow liquid with a weak specific aroma (0.15% yield). A total of 16 constituents were identified in the EO. These were:

Compound	*%*	*Compound*	*%*
α-Pinene	0.2	β-Bisabolene	3.0
1,8-Cineol	0.2	β-Sesquiphellandrene	0.4
α-Terpineol acetate	0.6	(*E*)-Nerolidol	0.3
cis-α-Bergamotene	0.3	Caryophyllene oxide	24.4
β-Caryophyllene	13.6	Caryophylla-4(12),8(13)-dien-5α-ol	1.4
trans-α-Bergamotene	15.8	Caryophylla-4(12),8(13)-dien-5β-ol	4.8
α-Humulene	5.3	*trans*-α-Bergamotol	10.8
(*E*)-β-Farnesene	0.5	Unidentified	81.7
ar-Curcumene	0.1		

Sesquiterpene compounds including caryophyllene oxide (24.4%), *trans*-α-bergamotene (15.8), β-caryophyllene (13.6), and *trans*-α-bergamotol (10.8) dominated. The EO composition of *C. spinosa* was studied for the first time.

TLC was performed on Sorbfil PTSKh-AF silica gel plates (Imid Ltd.) using solvent systems toluene:EtOAc:HCOOH (5:4:1, 1) and EtOAc:CH_2Cl_2:AcOH:HCOOH:H_2O (10:2.5:1:1:1, 2). The detectors were anisaldehyde in EtOH:AcOH:H_2SO_4 (8.5:1.0:0.5) (0.5%, triterpenes) and 2-aminoethyldiphenylborinate in MeOH (1%, phenolic compounds). Spectrophotometric studies were carried out on an SF-2000 spectrophotometer (OKB Spektr). IR spectra were recorded from films on ZnSe window-substrates on an FT-801 IR-Fourier spectrometer (Simeks) in the range 4000–600 cm^{-1}. GC/MS analysis of EO was conducted on a 5973N GC/MS (Agilent Technologies) with a MSD 5793N mass-selective detector (Agilent Technologies) with a diffusion pump using an HP-5ms capillary column (30 m/250 μm/0.25 μm), He carrier gas (1 mL/min), vaporizer temperature 280°C, column 50°C (2 min), 50-200°C (4°C/min), 200–280°C (20°C/min), 280°C (5 min isothermal), ion source

170°C, interface between GC and MS detector 280°C, ionizing-electron energy 70 eV, sample volume 1 μL of solution with 20:1 flow separation. ^{13}C NMR spectra were recorded from 1% solutions in DMSO-d_6 on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz. MS analysis was performed in a high-resolution MAT 8200 mass spectrometer (Finnigan); HPLC, on a Summit liquid chromatograph (Dionex) using a Prodigy ODS 3 column (Phenomenex, 250 × 4.6 mm, 5 μm), mobile phase H_2O:MeOH:AcOH (14:6:1), flow rate 1 mL/min, 20°C, UVD 170S UV-detector at λ 330 nm.

ACKNOWLEDGMENT

The work was supported financially by SB, RAS, Project No. VI.52.1.3.

REFERENCES

1. S. Pradjnya, *Sman Sbyar Tshad Kyi Skor Rnams Che Long Tsham Phyogs Gcig tu Bkod pa Kun Phan Bdud Rtsi'i Snying po*, Ksilograf Aginskogo Datsana [in Tibetan].
2. G. A. Shpekina, *Chem. Nat. Comp.*, **26**, 95 (1990).
3. T. Tomimatsu and M. Hashimoto, *Yakugaku Zasshi*, **88**, 1357 (1968).
4. V. M. Malikov and A. N. Saidkhodzhaev, *Chem. Nat. Comp.*, **34**, 202, 345 (1998).
5. V. M. Malikov and M. P. Yuldashev, *Chem. Nat. Comp.*, **38**, 358, 473 (2002).
6. D. N. Olennikov, L. M. Tankhaeva, and S. V. Agafonova, *Appl. Biochem. Microbiol.*, **47**, 411 (2011).
7. S. A. M. Hussein, A. N. M. Hashem, M. A. Seliem, U. Lindequist, and M. A. M. Nawwar, *Phytochemistry*, **64**, 883 (2003).
8. J. S. Choi, H. S. Young, J. C. Park, J.-H. Choi, and W. S. Woo, *Arch. Pharm. Res.*, **9**, 233 (1986).
9. V. A. Kurkin, *Chem. Nat. Comp.*, **39**, 123 (2003).
10. S. H. R. Alavi, N. Yassa, R. Hajiaghaee, M. M. Yekta, N. R. Ashtiani, Y. Ajani, and A. Hadjiakhondi, *Iran. J. Pharm. Res.*, **8**, 71 (2009).
11. C. Ma, B. Li, Q. Xu, and G. Zhang, *Chin. J. Appl. Environ. Biol.*, **12**, 487 (2006).
12. *Guide to Plants of Buryatiya* [in Russian], Ulan-Ude, 2001.
13. A. M. Lees, H. Y. I. Mok, R. S. Lees, M. A. McCluskey, and S. M. Grundy, *Atherosclerosis*, **28**, 325 (1977).
14. M. Starek, J. Krzek, and S. Michnik, *J. Planar Chromatogr. Mod. TLC*, **20**, 327 (2007).