Phenylpropanoid Glycosides from *Rhodiola rosea*

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***Rhodiola rosea* L. (Golden Root) has been used for a long time as an adaptogen in Chinese traditional medicine and is reported to have many pharmacological properties. Along its known secondary metabolites tyrosol (1), salidroside (rhodioloside) (2), rosin (3), rosarin (4), rosavin (5), sachaliside 1 (6) and 4-methoxy-cinnamyl-*O*-*β*-D-glucopyranoside (7), four compounds were isolated from aqueous methanol extract of the plant and identified as cinnamyl-(6′-*O*-*β*-xylopyranosyl)-*O*-*β*-glucopyranoside (8), 4-methoxy-cinnamyl-(6′-*O*-*α*-arabinopyranosyl)-*O*-*β*-glucopyranoside (9), picein (10) and benzyl-*O*-*β*-glucopyranoside (11) by UV, MS and NMR methods. Compounds 8 and 9 are new natural compounds whereas compounds 10 and 11 were isolated first time from *R. rosea*. Also the compounds 6 and 7 are isolated earlier only from the callus cultures of the plant but not from the differentiated plant.**

Key words *Rhodiola rosea* L.; Crassulaceae; phenylpropanoid; glycoside

Golden root (Roseroot, *Rhodiola rosea* L., Crassulaceae) has been used in Chinese traditional medicine to enhance the body's resistance against fatigue and to extend human life.[1)] The plant is distributed globally in arctic regions, including Northern Asia, Alaska and northern parts of Europe, and it has been connected to number of biological activities, such as effects on prolyl endopeptidase inhibition,[2)] antiallergic effects,[3)] effect to memory and learning,[4)] antidepressant and anti-inflammatory effects[5)] and effects in cancer therapy.[6—10)] The main compounds responsible for these activities are believed to be phenylpropanoid tyrosol (**1**), its glucoside salidroside (*p*-hydroxyphenylethyl-*O*-*β*-D-glucopyranoside) (**2**) and phenylpropenoids rosin (cinnamyl-*O*-*β*-D-glucopyranoside) (**3**), rosarin (cinnamyl-(6′-*O*-*α*-L-arabinofuranosyl)-*O*-*β*-D-glucopyranoside) (**4**) and rosavin (cinnamyl-(6′-*O*-*α*-L-arabinopyranosyl)-*O*-*β*-D-glucopyranoside) (**5**), which are reported to be pharmacologically active as antioxidants and neurostimulants.[11,12)] Also some other phenylpropenoids, such as sachaliside 1, (4-hydroxycinnamyl-*O*-*β*-D-glucopyranoside) (**6**) and 4-methoxy-cinnamyl-*O*-*β*-D-glucopyranoside (**7**) have been isolated from the callus cultures of the plant.[13)] This paper reports the isolation of compounds **1**—**7** along the four compounds **8**—**11**, of which **8** and **9** are novel natural compounds and **10** and **11** are not isolated from *R. rosea* be-

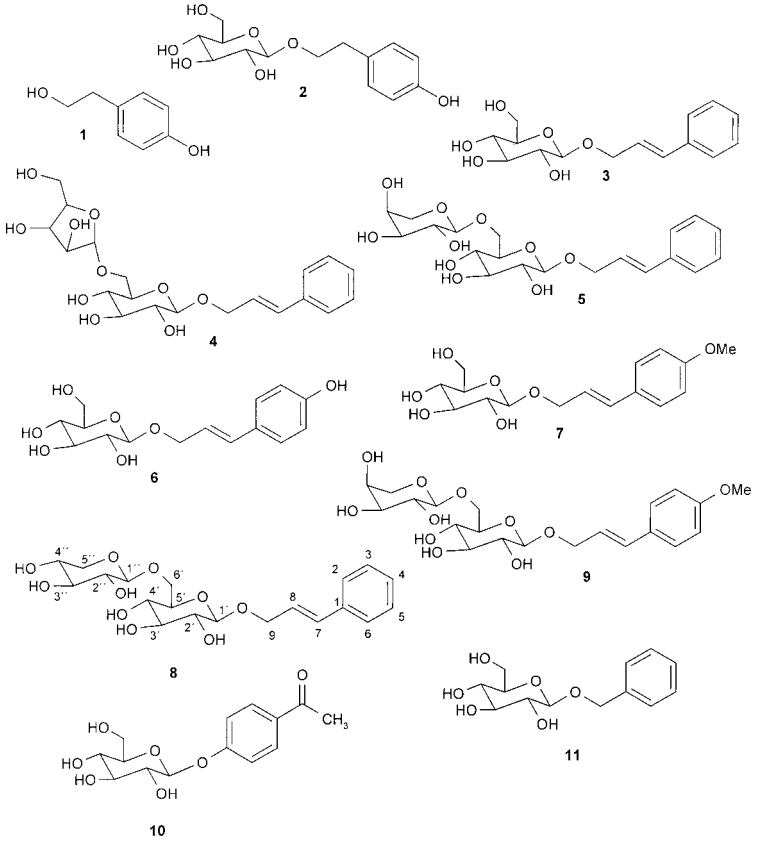

Fig. 1. Isolated Compounds

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Table 1. Mass- and NMR Spectrometric Data Obtained from Compounds **8** and **9**

	ESI^+/MS/MS (*m/z*, rel. int. %)	ESI^-/MS/MS (*m/z*, rel. int. %)	^{1}H-NMR, 500 MHz (δ=ppm) (*J*=Hz) in D_2O	^{13}C-NMR, 125 MHz (δ=ppm) in D_2O
8	CID of $[M+Na]^+$, 30 eV	CID of $[M-H]^-$, 15 eV	7.43 ppm (dd, 8.2, 1.0 Hz, H-2, H-6)	137.0 ppm (C-1)
	451 $[M+Na]^+$ (60)	427 $[M-H]^-$ (85)	7.32 ppm (dd, 8.2, 7.6 Hz, H-3, H-5)	134.2 ppm (C-7)
	333 $[M+Na-118]^+$ (70)	293 $[M-H-134]^-$ (45)	7.25 ppm (tt, 7.6, 1.0 Hz, H-4)	129.2 ppm (C-3, C-5)
	319 $[M+Na-132]^+$ (25)	233 $[M-H-194]^-$ (7)	6.66 ppm (d, 17.3 Hz, H-7)	128.9 ppm (C-4)
	275 $[M+Na-176]^+$ (6)	191 $[M-H-236]^-$ (35)	6.32 ppm (ddd, 17.3, 7.8, 6.5 Hz, H-8)	126.9 ppm (C-2, C-6)
	201 $[M+Na-250]^+$ (20)	161 $[M-H-266]^-$ (100)	4.46 ppm (d, 8.5 Hz, H-1′)	125.2 ppm (C-8)
	155 $[M+Na-296]^+$ (6)	149 $[M-H-278]^-$ (85)	4.43 ppm (dd, −12.7, 6.5 Hz, H-9a)	103.9 ppm (C-1″)
	117 $[M+Na-334]^+$ (100)	131 $[M-H-296]^-$ (25)	4.34 ppm (d, 8.7 Hz, H-1″)	101.6 ppm (C-1′)
	CID of $[M+NH_4]^+$, 15 eV	125 $[M-H-302]^-$ (30)	4.33 ppm (dd, −12.7, 7.8 Hz, H-9b)	76.1 ppm (C-3″)
	446 $[M+NH_4]^+$ (15)	113 $[M-H-314]^-$ (15)	4.04 ppm (dd, −12.8, 1.7 Hz, H-6′a)	75.9 ppm (C-3′)
	117 $[M+NH_4-329]^+$ (100)	101 $[M-H-326]^-$ (30)	3.82 ppm (dd, −12.4, 6.0 Hz, H-5″ax)	75.1 ppm (C-5′)
			3.74 ppm (dd, −12.8, 6.2 Hz, H-6′b)	73.4 ppm (C-2′)
			3.50 ppm (dd, 9.7, 6.0 Hz, H-4″)	73.2 ppm (C-2″)
			3.50 ppm (m, 9.1, 6.2, 1.7 Hz, H-5′)	70.7 ppm (C-9)
			3.38 ppm (dd, 9.8, 9.3 Hz, H-3′)	69.7 ppm (C-4′)
			3.36 ppm (dd, 9.3, 9.1 Hz, H-4′)	69.2 ppm (C-4″)
			3.32 ppm (dd, 9.7, 9.6 Hz, H-3″)	68.9 ppm (C-6′)
			3.22 ppm (dd, 9.8, 8.5 Hz, H-2′)	65.5 ppm (C-5″)
			3.20 ppm (d, 12.4 Hz, H-5″eq)	
			3.18 ppm (dd, 9.6, 8.7 Hz, H-2″)	
9	CID of $[M+Na]^+$, 30 eV	CID of $[M-H]^-$, 15 eV	7.39 ppm (d, 9.3 Hz, H-2, H-6)	159.3 ppm (C-4)
	481 $[M+Na]^+$ (30)	457 $[M-H]^-$ (20)	6.91 ppm (d, 9.3 H-3, H-5)	133.7 ppm (C-7)
	333 $[M+Na-148]^+$ (25)	293 $[M-H-164]^-$ (65)	6.61 ppm (d, 17.3 Hz, H-7)	129.9 ppm (C-1)
	275 $[M+Na-206]^+$ (6)	233 $[M-H-224]^-$ (20)	6.19 ppm (ddd, 17.3, 6.9, 6.0 Hz, H-8)	128.5 ppm (C-2, C-6)
	201 $[M+Na-280]^+$ (5)	191 $[M-H-266]^-$ (25)	4.46 ppm (d 8.8 Hz, H-1′)	123.3 ppm (C-8)
	147 $[M+Na-334]^+$ (100)	161 $[M-H-296]^-$ (20)	4.41 ppm (dd, −12.7, 6.0 Hz, H-9a)	114.8 ppm (C-3, C-5)
	CID of $[M+NH_4]^+$, 15 eV	149 $[M-H-308]^-$ (100)	4.30 ppm (dd, −12.7, 6.9 Hz, H-9b)	104.0 ppm (C-1″)
	476 $[M+NH_4]^+$ (10)	131 $[M-H-326]^-$ (50)	4.29 ppm (d, 8.4 Hz, H-1″)	101.6 ppm (C-1′)
	147 $[M+NH_4-329]^+$ (100)	125 $[M-H-332]^-$ (30)	4.05 ppm (dd, −12.6, 1.8 Hz, H-6′a)	76.0 ppm (C-3′)
		113 $[M-H-344]^-$ (15)	3.82 ppm (dd, 4.5, 2.9 Hz, H-4″)	75.3 ppm (C-5′)
		101 $[M-H-356]^-$ (10)	3.80 ppm (dd, −13.1, 2.9 Hz, H-5″ax)	73.5 ppm (C-2′)
			3.75 ppm (s, −OMe)	72.6 ppm (C-3″)
			3.72 ppm (dd, −12.6, 5.6 Hz, H-6′b)	70.8 ppm (C-9)
			3.54 ppm (dd, 9.3, 4.5 Hz, H-3″)	70.1 ppm (C-2″)
			3.53 ppm (d, −13.1 Hz, H-5″ eq)	69.5 ppm (C-4′)
			3.49 ppm (dd, 11.5, 5.6 Hz, H-5′)	68.7 ppm (C-6′)
			3.48 ppm (dd, 9.3, 8.4 Hz, H-2″)	68.5 ppm (C-4″)
			3.38 ppm (dd, 11.5, 8.8 Hz, H-4′)	66.5 ppm (C-5″)
			3.38 ppm (dd, 8.8, 8.7 Hz, H-3′)	55.7 ppm (–OMe)
			3.22 ppm (dd, 8.8, 8.7 Hz, H-2′)	

fore.

Results and Discussion

Compounds were identified according their UV spectra, high-resolution mass spectra, fragment ion mass spectra (collision induced dissociation, CID) obtained by triple quadrupole instrument and one- and two-dimensional NMR experiments (total correlation spectroscopy (TOCSY), correlation spectroscopy (COSY), heteronuclear single quantum coherence-distortionless enhancement by polarization transfer (HSQC-DEPT) and heteronuclear multiple bond correlation (HMBC)). Collision induced dissociation experiments were performed from 10 mM ammonium acetate solution, colliding $[M+Na]^+$ and $[M+NH_4]^+$ ions in positive ion mode and $[M-H]^-$ ion in negative ion mode with argon gas. The proton–proton coupling constants were obtained from the ^{1}H spectrum and phase-sensitive COSY spectra and refined with PERCH spectral analysis software.[14] Chemical shifts for ^{13}C were obtained from HSQC and HMBC spectra.

Spectroscopic data obtained from compounds **1**—**2**,[15] **3**—**5**,[16] **6**,[13,17] **7**,[13] **10**[18] and **11**[19] were in very good agreement with the structures and the earlier measurements.

For compound **8** the high resolution ESI/TOF mass spectrum showed $[M+Na]^+$ at *m/z* 451.1600, that was consistent with molecular formula $C_{20}H_{28}O_{10}$ (calculated mass for $C_{20}H_{28}O_{10}Na$ 451.1580 amu). In all CID experiments the results were very similar to those of **5**, showing only same fragment ions, indicating similar structure. In fragmentation of $[M+NH_4]^+$ at *m/z* 446 only an ion at *m/z* 117 $[M+NH_4-329]^+$ due to cleavage of both sugar moieties with ammonium cation was seen. In fragmentation of $[M+Na]^+$ ion at *m/z* 451 the most intense fragment-ion peak was also at *m/z* 117, while spectrum also showed peaks at *m/z* 333 $[M+Na-118]^+$ due to loss of phenylpropenyl group, at *m/z* 319 $[M+Na-132]^+$ due to expulsion of xyloside moiety without the *O*-linkage oxygen atom ($C_5H_8O_4$), at *m/z* 201 $[M+Na-250]^+$, that could be due to simultaneous expulsion of phenylpropenyl group and xyloside residue, and at *m/z* 155 $[M+Na-296]^+$, that may be due to the simultaneous cleavage of both sugar units without *O*-linkage oxygen atom between glucose and aglycon or from the cleavage of cinnamyl moiety and glucose residue. In negative ion mode the main fragment ions in collision of $[M-H]^-$ ion at *m/z* 427 were at *m/z* 293 $[M-H-134]^-$ due to expulsion of cinnamyl alcohol

($C_9H_{10}O$), and at *m/z* 149 due to the cleavage of xyloside moiety with the *O*-linkage oxygen, retaining the negative charge. Also peaks at *m/z* 233, 191, 161 and 131 were seen and can be interpreted with more complete breaking of sugar moieties as suggested by Domon and Costello.[20] In ^{1}H-NMR spectrum (in D_2O) of **8** the signals for cinnamyl moiety were identical with those of **4** and **5**, showing signals at 7.43 ppm (dd, J=8.2, 1.0 Hz, H-2, H-6), 7.32 ppm (dd, J=8.2, 7.6 Hz, H-3, H-5), 7.25 ppm (tt, J=7.6, 1.0 Hz, H-4), 6.66 ppm (d, J=17.3 Hz, H-7), 6.32 ppm (m, J=17.3, 7.8, 6.5 Hz, H-8), 4.43 ppm (dd, J=−12.7, 6.5 Hz, H-9a) and 4.33 ppm (dd, J=−12.7, 7.8 Hz, H-9b). Coupling constant 17.3 Hz between olefinic protons H-7 and H-8 suggests clearly them being in *trans* geometry to each others. Chemical shifts and coupling constants of sugar residues showed that they were β-glucopyranose and β-xylopyranose. The absolute configurations were not determined, but most probably the both glucose and xylose are in D-configuration, as commonly in nature. A doublet signal of glucose anomeric proton H-1′ appeared at 4.46 ppm with the coupling constant 8.5 Hz and the doublet signal of xylose anomeric proton H-1″ had a chemical shift 4.34 ppm with coupling constant 8.7 Hz. HMBC correlations from H-9a/b to C-1′ and from H-1′ to C-9 confirmed the attachment of glucose unit to aglycon, and the position of xylose unit was confirmed in a similar manner by correlations from H-1″ to C-6′ and from H-6′a/b to C-1″.

For compound **9** the high resolution ESI/TOF mass spectrum showed $[M+Na]^+$ at *m/z* 481.1661, that was consistent with molecular formula $C_{21}H_{30}O_{11}$ (calculated mass for $C_{21}H_{30}O_{11}Na$ 481.1686 amu). In positive ion mode ESI/CID two molecular ions $[M+Na]^+$ at *m/z* 481 and $[M+NH_4]^+$ at *m/z* 476 were formed. Similarly to compounds **5** and **8**, the main fragment ion peak for both of these originated from loss of both sugar moieties with sodium cation/ammonium ion, resulting to peak at *m/z* 147 $[M+Na-334/329]^+$. The CID of $[M+Na]^+$ showed also a peak at *m/z* 333 $[M+Na-148]^+$ due to loss of 4-methoxy-phenylpropenyl group. In negative ion mode the colliding of $[M-H]^-$ ion at *m/z* 457 resulted to peaks with same *m/z*-ratios as in case of **5** and **8**. In ^{1}H-NMR spectrum compound **9** showed peaks for 4-methoxy cinnamyl moiety at 7.39 ppm (d, J=9.3 Hz, H-2 and H-6), 6.91 ppm (d, J=9.3 Hz, H-3 and H-5), 6.61 ppm (d, J=17.3 Hz, H-7), 6.19 ppm (ddd, J=17.3, 6.9, 6.0 Hz, H-8), 4.41 ppm (dd, J=−12.7, 6.0 Hz, H-9a) and 4.30 ppm (dd, J=−12.7, 6.9 Hz, H-9b). A singlet signal for methoxide protons was observed at 3.75 ppm. Coupling constant 17.3 Hz between olefinic protons H-7 and H-8 suggests the *trans* conformation of the double bond. Chemical shifts and coupling constants of sugar residues were practically identical with those of **5**, and showed that the sugars were β-glucopyranose and α-arabinopyranose. Similarity of the NMR data with that of **5** gives also a reason to assume that the glucose occurs probably as a D-form and arabinoside as a L-form, as in **5**. A doublet signal of glucose anomeric proton H-1′ appeared at 4.46 ppm with the coupling constant 8.8 Hz and the doublet signal of arabinose anomeric proton H-1″ had a chemical shift 4.29 ppm with coupling constant 8.4 Hz. HMBC correlations from H-1′ to C-9 confirmed the attachment of glucose unit to aglycon, and the position of arabinose unit was confirmed by correlations from H-1″ to C-6′ and from H-6′ b to C-1″.

Experimental

The CID fragmentation mass spectra were measured with Micromass Quattro II triple quadrupole mass spectrometer and exact mass measurements with Micromass LCT time-of-flight instrument. In both instruments electrospray ionization source was used. The samples were introduced into instrument using syringe pump at flow rate of 10 μl/min. The capillary voltages used were 3.1 kV and sample cone voltages 25—35 V. The source temperatures used were 120 °C and the desolvatation temperatures 150 °C. Nitrogen was used as both drying and nebulizing gas with flow rates of 600 l/h and 200 l/h, respectively. In CID measurements the collision energy used was 25—40 eV and the collision gas used was argon in 1.6×10^{-3} mbar pressure. The NMR spectra were acquired at 11.7 T on a Bruker DRX 500 spectrometer. The samples were dissolved in D_2O and a 70—100 μl sample volume was used together with Shigemi NMR tubes (susceptibility matched to water) and a BBI probe head equipped with z-axis gradient coils. Typically a 0.4—0.8 s acquisition time with 2 s relaxation delay was used for ^{1}H experiments, with or without water suppression that was performed by excitation sculpting method by Hwang and Shaka.[21] For TOCSY and COSY experiments a 0.4 s acquisition times and 1.6 s relaxation delays were used together with 128 or 256 increments and varying number of experiments. Mixing time used for TOCSY were 120—160 ms. For HSQC-DEPT and HMBC experiments 128 increments and varying number of experiments were acquired with 0.5 s acquisition times and 1.4 s relaxation delays, optimizing J_{CH} couplings for 145 Hz and 4—10 Hz, respectively. The ^{1}H chemical shifts were referred to water signal at 4.72 ppm. ^{1}H-NMR spectra were iterated using PERCH spectral analysis software.[14]

Plant Material The plant material of *R. rosea* was grown on Botanical Gardens at University of Oulu.

Extraction and Isolation Fifteen grams of freeze-dried root of the plant were powdered and extracted with 1 l of methanol/water 3/2 (v/v) solution for 60 min in ultrasonic bath. The extract was centrifuged for 5 min in 7000 rpm and the supernatant was dried and reconstructed to 100 ml of 10% aqueous methanol. The non-soluble precipitate was filtered off with Buchner filter and the solution was concentrated to 15 ml with rotary evaporator. This solution was filtered with Gelman GHP acrodisc 0.45 μm syringe filters before fractionating the solution to three parts with Waters 2690 Alliance HPLC system using Waters Symmetry300 C18 3.9×150 mm column with 5 μm particle size. The fractions were collected from 150 HPLC runs with 100 μl injections with Isco Foxy 200 fraction collector. HPLC gradient consisted of laboratory purified water (A) and acetonitrile (B). The initial gradient conditions for isolation were 98% A: 2% B, that chanced linearly to 97% A: 3% B in 8 min, then linearly to 93% A: 7% B in next 11 min, then to 88% A: 12% B in one minute, and finally to 87% A: 13% B in next 22 min. The first fraction was collected at retention time 6—9 min, second at 15—18 min and third at 25—29 min. The fractions were purified with semipreparative Waters Xterra RP18 4.2×250 mm column with 5 μm particle size. For fraction one isocratic eluent system containing 3% acetonitrile in water was used, yielding compounds **1**—**2** and **10**. For fraction two eluent system used consisted of 6% acetonitrile in water during 0—6 min and then linear gradient of 6—22% acetonitrile during 6—48 min, which yielded the compounds **6** and **11**. For the fraction three an isocratic eluent system consisting of 13% acetonitrile in water was used, yielding the compounds **3**—**5** and **7**—**9**. Flow rate used for all eluent systems in purification was 0.85 ml/min at 35 °C. The purified compounds were dried under nitrogen atmosphere at 80 °C.

Cinnamyl-(6′-*O*-β-xylopyranosyl)-*O*-β-glucopyranoside (**8**): A colorless substance (from MeOH–H_2O). UV (in 12% aqueous acetonitrile): λ_{max} nm (log ε): 251 (3.34), 282 (2.22), 292 (1.91). High resolution MS 451.1600 Da, $C_{20}H_{28}O_{10}Na$ (Calcd 451.1580). See MS/MS and ^{1}H-, ^{13}C-NMR data in Table 1.

4-Methoxy-cinnamyl-(6′-*O*-α-arabinopyranosyl)-*O*-β-glucopyranoside (**9**): A colorless substance (from MeOH–H_2O). UV (in 12% aqueous acetonitrile): λ_{max} nm (log ε): 261 (3.36), 292 (2.58), 302 (2.40). High resolution MS 481.1661 Da, $C_{21}H_{30}O_{11}Na$ (Calcd 481.1686). See MS/MS and ^{1}H-, ^{13}C-NMR data in Table 1.

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References

1) Saratikov A. S., Krasnov E. A., Chnika L. A., Duvidson L. M., Sotova M. I., Marina T. F., Nechoda M. F., Axenova R. A., Tscherdinzeff S. G., *Pharmazie*, **23**, 392—395 (1968).
2) Fan W., Tezuka Y., Komatsu K., Namba T., Kadota S., *Biol. Pharm.*

Bull., **22**, 157—161 (1999).
3) Yoshikawa M., Shimada H., Shimoda H., Murakami T., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **44**, 2086—2091 (1996).
4) Petkov V. D., Yonkov D., Mosharoff A., Kambourova T., Alova L., Petkov V. V., Todorov I., *Acta Physiol. Pharmacol. Bulg.*, **12**, 3—16 (1986).
5) Maslova L. V., Kondratyev B., Maslov L. N., Lishmanov B., *Exp. Clin. Pharm.*, **57**, 61—63 (1994).
6) Bocharova O. A., Serebriakova R., Bodrova N. B., *Vestn. Ross. Akad. Med. Nauk.*, **5**, 41—43 (1994).
7) Bocharova O. A., Matveev B. P., Baryshnikov A. I., Figurin K. M., Serebriakova R., Bodrova N. B., *Urol. Nefrol.*, **2**, 46—47 (1995).
8) Udintsev S. N., Shakov V. P., *Eur. J. Cancer*, **27**, 1182 (1991).
9) Udintsev S. N., Shakov V. P., *Neoplasma*, **38**, 323—331 (1991).
10) Razina T. G., Zueva E. P., Amosova E. N., Krylova S. G., *Eksp. Klin. Farmakol.*, **63**, 59—61 (2000).
11) Sokolov S., Ivashin V. M., Zapesochnaya G., Kurkin V. A., Shchavlinskii A. N., *Khim. Farm. Zh.*, **19**, 1367—1371 (1985).
12) Furmanowa M., Skopinska-Rozewska E., Rogala E., Hartwich M., *Acta Soc. Bot. Pol.*, **67**, 69—73 (1998).
13) Kurkin V. A., Zapisochnaya G. G., Dubichev A. G., Vorontsov E. D., Aleksandrova I. V., Panova R. V., *Khim. Prir. Soedin.*, **4**, 481—490 (1991).
14) Laatikainen R., Niemitz M., Weber U., Sundelin J., Hassinen T., Vepsäläinen J., *J. Magn. Reson. A*, **120**, 1—10 (1996).
15) La Londe R. T., Wong C., Tsai A. I. M., *J. Am. Chem. Soc*., **98**, 3007—3013 (1976).
16) Zapesochnaya G. G., Kurkin V. A., *Chem. Nat. Compd.*, **18**, 685—688 (1982).
17) Mizuno M., Kato M., Hosoi N., Iinuma M., Tanaka T., Kimura A., Ohashi H., Sakai H., Kajita T., *Heterocycles*, **31**, 1409—1412 (1990).
18) Wang M., Kikuzaki H., Lin C. C., Kahayaoglu A., Huang M. T., Nakatani N., Ho C. T., *J. Agric. Food Chem.*, **47**, 1911—1914 (1999).
19) De Rosa S., De Giulio A., Tommonaro G., *Phytochemistry*, **42**, 1031—1034 (1996).
20) Domon B., Costello C., *Glycoconjucate J.*, **5**, 397—409 (1988).
21) Hwang T. L., Shaka A. J., *J. Magn. Reson. A*, **112**, 275—279 (1995).