Chemistry of Natural Compounds, Vol. 42, No. 4, 2006

COMPARATIVE ANALYSIS OF ESSENTIAL OIL COMPOSITIONS FROM LEAVES AND STEMS OF *Rhododendron adamsii*, *R. aureum*, AND *R. dauricum*

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UDC 547.913

Chemical compositions of essential oils (EO) from leaves and stems of Rhododendron adamsii, R. aureum, *and* R. dauricum *were studied by GC/MS. The analysis demonstrated that the EO of these plants contained mainly mono- and diterpene hydrocarbons. A large amount of alkanes was also found in the EO of* R. aureum.

Key words: *Rhododendron*, essential oil, terpenoids, *β*-farnesene.

The genus *Rhododendron* is the largest (about 800 species) in the family Ericaceae [1] and comprises 19 species in Russia, the majority of which grow in Siberia and the Far East. The use of certain rhododendrons as anti-inflammatory, fungicidal, stimulatory, tonic, diuretic, and antimicrobial agents in addition to others has been reported [2]. Such applications require detailed studies of the plant chemical composition in order to determine the principal classes of compounds in them.

Essential oils (EO), which are rather simply isolated from plants and possess broad spectra of biological activities, are medicinal agents that are widely used in medicine. The chemical composition of EO from runners of four rhododendron species growing in Russian has been previously reported. These were *R. dauricum* L., *R. ledebouri* Pojark., *R. sichotense* Pojark., and *R. mucronulatum* (Turcz.) Worosch. [3]. The present article supplements existing information with previously unreported data on the EO composition of *R. adamsii* and *R. aureum*. Furthermore, the EO compositions are compared with that of *R. dauricum*.

Components from EO of leaves and stems of *R. adamsii* were isolated in our studies (Table 1). These included both *α*- and *β*-pinene, *β*-myrcene, *cis*-*β*-ocimene, isoledene, aromadendrene, humulene, *β*-farnesene, *δ*-cadinene, *trans*-nerolidol, spathulenol, *β*-elemenone, and germacrone. EO from stems of this plant (samples 1b, 2b, and 3b) contained germacrene D and germacrene B, which were not found in leaves (1a, 2a, and 3a). In addition, the content of EO components varied in different samples of *R. adamsii* depending on the collection and storage times. For example, the fraction of *trans*-nerolidol in EO from leaves (from 18.2 to 29.4%) and stems (from 9.6 to 15.7%) increased markedly for raw material stored in a warm place. Simultaneously, the relative content of such major EO of components as *α*-pinene, humulene, *β*-farnesene, and others decreased. This fact may indicate that *trans*-nerolidol is probably a conversion product of other components present in the plant whereas the content of the other compounds decreases as a result of their transformations or volatility.

All EO samples from leaves and stems of *R. adamsii* contained 4-phenyl-2-butanone, the content of which was from 3 to 13%, and also the related 4-phenyl-2-butanol (from 1.9 to 7.4%, absent in EO of sample 1b). The structure of 4-phenyl-2-butanone is similar to that of the so-called "raspberry ketone" 4-(4-hydroxyphenyl)-2-butanone, which is used in the food and fragrance industries [4].

Components from EO of *R. dauricum* stems were completely identified. Compounds making up ~92% of the EO from leaves of this plant were determined. In contrast with *R. adamsii*, the major components of EO from *R. dauricum* leaves were different from those of stems. *α*-Pinene and limonene dominate the EO from stems of the freshly collected plant (6b). The fraction of each of these was >19%. These and certain other compounds with a content in EO of 6b greater than 1% were absent in EO from leaves (6a), the main components of which were caryophyllene (28.3%), humulene (15.0%), caryophyllene-*α*-oxide (6.1%), and other compounds at the 2-3% level. Such results indicate that leaves and stems of *R. dauricum* may differ markedly in chemical composition. Therefore, the raw material must be sorted into separate plant parts when it is studied.

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TABLE 1. Content of Essential Oil Components Relative to Total Ion Current

Compound[a]	*R. adamsii*						*R. aureum*				*R. dauricum*		
	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	[3][b]
n-Hexanal		0.629						2.920					
α-Pinene	5.206	0.987	0.745	3.997	4.583	4.642			5.416			19.757	10.5
Camphene					0.127							0.720	1.3
β-Pinene	0.959	0.483	0.304	1.918	1.806	2.665			2.661			8.133	0.8
β-Myrcene	0.590	0.692	0.352	1.376	0.771	1.290			0.723			5.991	2.2
Hexanoic acid[c]						0.263		6.295	9.992	7.802			
p-Cymene												1.501	9.4
Limonene			0.216	0.258	0.366	0.227					0.218	19.179	34.5
cis-*β*-Ocimene	1.060	0.511	0.631	1.102	1.032	1.343						2.162	
γ-Terpinene												8.839	1.3
n-Nonanal								1.032	1.238				
Phenylethyl alcohol	2.045		1.924		1.877	0.734							
Terpineol-4						0.173			0.603		0.662	4.132	
α-Terpineol					0.206		1.151		3.078	0.590	3.585	0.977	0.8
4-Phenyl-2-butanone[c]	6.565	2.836	12.635	3.593	7.546	3.270							
Bornylacetate						0.124					1.713	2.549	1.8
4-Phenyl-2-butanol[c]	7.439		3.341	2.332	3.762	1.943							
Nonanoic acid							0.413	2.671	1.944	2.353			
Carvacrol									7.768	8.096			
Bicycloelemene[c]		1.329				2.216							
Isoledene		0.524	0.250	0.443	0.241	0.328							
α-Copaene		0.500	0.233				0.235				0.884		0.6
α-Gurjunene		0.441	0.282	0.427	0.278	0.380							
1,6-Dimethylnaphthalene									1.020	1.372			
Caryophyllene		0.502									28.328	3.526	6.1
Calarene[c]							5.596		1.420				
γ-Elemene	0.984	6.211		3.340	0.528	4.131						0.741	
Aromadendrene	6.027	10.550	3.233	8.172	3.083	5.963							0.6
Humulene	2.274	1.481	1.790	1.085	1.503	0.548					15.004	1.736	7.8
β-Farnesene	17.106	32.757	6.932	16.345	9.394	20.588				0.648	1.894	0.183	0.8
allo-Aromadendrene									1.055		0.120		
γ-Murolene	0.634	1.184	0.454	0.432	0.347	0.316					3.416	0.397	1.4
Germacrene D		1.364		0.279		0.687						0.536	
β-Selinene	0.972	0.814	0.675	0.592	0.705	0.514	0.648				1.222	0.305	1.9
α-Selinene[c]							0.413						2.6
Ledene	3.354	4.914	2.093	6.330	2.494								
Bicyclogermacrene		2.822				9.127						0.679	
γ-Cadinene			0.196			0.203		1.065	0.769		1.677	0.377	0.6
α-Farnesene	2.322	1.246	0.560	0.346	0.294	0.325							
Calamene							0.339[d]	2.143[d]	0.877	0.503	0.417		
δ-Cadinene	0.949	1.151	0.706	0.430	0.658	0.476	0.339[d]	2.143[d]			2.804	0.504	1.6
Selina-4(15),7(11)-diene	5.980	2.441			5.036								
α-Calacorene							0.226	1.983	0.438	0.673	0.304		
Selina-3,7(11)-diene	7.853	2.994			6.871								
Germacrene B		1.163		0.875		1.567						0.192	
trans-Nerolidol	18.178	9.572	29.445	15.692	28.970	18.588					0.651		
Spathulenol[c]		1.745	2.191	4.273	0.796	2.808						0.140	
Caryophyllene-*α*-oxide											6.122	0.554	
Globulol			~1.27	2.091	0.747	1.893							
Dehydroolopanone									2.229	0.411			

TABLE 1. (continued)

Compound	*R. adamsii*						*R. aureum*				*R. dauricum*		
	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	[3][b]
Humulen-6,7-epoxide											3.726	0.213	
β-Elemenone	1.291	1.191	1.451	1.508	0.891	1.179							0.9
α-Cadinol								10.518				0.261	
α-Eudesmene[c]					0.281								1.4
Germacrone	2.511	1.286	1.805	1.587	1.183	1.245							1.4

Also: sabinene, 6.245 (6b); thujylacetate, 5.094 (6b); β-copaene, 2.515 (5a); dehydroaromadendrene, 1.692 (5a); γ-amorphene[c], 2.291 (6a); γ-selinene[c], 1.125 (3b); cariolan-1-ol, 1.385 (6a); betuligenol[c] (rhododendrol), 1.871 (4a); *trans*-longipinocarveol, 2.621 (5a); β-panasensen-5-α-ol, 2.714 (5b); α-copaen-11-ol, 1.020 (5b); cadalene[c], 1.732 (5b); epi-α-bisabolol, 2.979 (6a); caryophylla-3,18-dien-5-(β)-ol, 2.383 (6a); longifolol, 1.545 (3b).

[a] Compounds listed in order of retention time; [b] in addition to components shown in columns, *p*-cymenene (0.6%) was determined; [c] compound determined only by mass spectrum; [d] both compounds elute in a single gas-chromatographic peak.

TABLE 2. Compounds Detected in *R. aureum* Essential Oil (content relative to total ion current)

Compound[a]	4a	4b	5a	5b
Caur-16-ene[b]			4.604	2.024
n-Heneicosane			3.167	1.538
n-Docosane				2.019
Tricosane	0.379		2.401	
Squalene	5.227	3.350	1.169	1.322
Pentacosane	0.874		0.975	
Bakharis oxide[b]	4.214	4.639		13.519
Nonacosane	6.571		4.553	6.390
Hentriacontane	6.988		3.241	5.304
Friedelin[b]	12.115		4.156	2.319

Also: 6,10,14-trimethylpentadecan-2-one, 2.012 (5b); stachene, 1.618 (5a); eicosane, 0.686 (5a); hexacosane, 0.999 (4a); heptacosane, 1.613 (4a); octacosane, 2.416 (4a); triacontane, 3.269 (4a); dotriacontane, 2.629 (4a); tritriacontane, 2.466 (4a); tetratriacontane, 2.074 (4a); stigmast-4-en-3-one, 2.732 (4a); pentatriacontane, 1.779 (4a); hexatriacontane, 10.412 (4a).

[a] Compounds listed in order of retention time; [b] compound determined only by mass spectrum.

Practically all components that had been previously reported [3] were determined by us in EO from leaves and stems of freshly collected *R. dauricum*. However, their contents were different. Apparently this was due to the following reasons. First, we investigated the EO composition from freshly collected raw material whereas that from dried material was analyzed previously [3]. Second, we sorted the raw material into leaves and stems and analyzed the EO composition of each part separately. EO from the whole plant was obtained previously [3]; therefore, the calculations were made for a mixture in which compounds isolated from both leaves and stems were present. Third, the previously studied sample of *R. dauricum* was collected in Amur region whereas the EO obtained by us was isolated from the plant growing in Irkutsk.

The compositions of EO from leaves and stems of *R. aureum* differed markedly from each other. Nevertheless, components present in the EO from both leaves and stems could be isolated. These were nonanoic acid, calamene, α-calacorene, and squalene. The EO of samples 5a and 5b contained compounds absent in EO of samples 4a and 4b. These were carvacrol, 1,6-dimethylnaphthalene, *ar*-curcumene, dehydroolopanone, *n*-heneicosane, and others. The EO of sample 4a (leaves) also contained a large amount of high-molecular-weight compounds, most of which was alkanes with 23-26 C atoms (Table 2). The major components were calarene, carvacrol, squalene, bakharis oxide, friedelin, and certain of the aforementioned alkanes.

TABLE 3. Characteristics of *Rhododendron* Raw Materials

Sample	Conditions	Preparation time of EO	EO content[a], %
		R. adamsii	
1a* (leaves)	-	March 2005	0.48
1b* (stems)	-	"	0.48
2a* (leaves)	Samples stored at room temperature	November 2005	1.1
2b* (stems)	before EO prepared	"	1.0
3a* (leaves)	July-August 2005	"	1.9
3b* (stems)	July-August 2005	"	1.8
		R. aureum	
4a* (leaves)	Samples stored in a dry place	October 2005	0.05
4b* (stems)	"	November 2005	0.02
5a* (leaves)	Samples dried at elevated temperature	"	0.09
5b* (stems)	"	"	0.03
		R. dauricum[b]	
6a** (leaves)	-	November 2005	0.11
6b** (stems)	-	"	0.11

Collected: *July 2004, Tunkin valley near Arshan, Rep. Buryatia; **near Irkutsk, end of October 2005.
[a]Oil content relative to air-dried raw material (except *R. dauricum*); [b]EO isolated from freshly collected raw material.

The EO from stems of this plant (4b) contained mainly hexanoic acid (6.3%), α-cadinol (10.52%), squalene (3.4%), and bakharis oxide (4.64%). The EO composition from samples 5a and 5b differed markedly from that of 4a and 4b, respectively. Components that were present in EO from leaves of one sample (4a) were absent in that from another (5a). The opposite was also found. EO of 5a contained α- and β-pinene, β-myrcene, terpineol-4, and certain other compounds. These were not observed in EO of 4a.

EO from *R. aureum* stems gave a similar picture. Such a large scatter in the data is probably explained by the different temperature regimes for storing the material (see Experimental) and the very low EO content in them (0.02 to 0.09%). This introduces into them a large uncertainty or makes it impossible to determine them.

The comparision of the chemical compositions of EO obtained by us with those reported for *R. dauricum*, *R. ledebouri*, *R. sichotense*, and *R. mucronulatum* [3] demonstrated that they are similar to each other with respect to the set of principal components but differ in macrocomponents. Thus, a large part of EO from *R. adamsii* was β-farnesene, aromadendrene, and *trans*-nerolidol; *R. aureum*, carvacrol, α-cadinol, and certain alkanes; and *R. dauricum*, limonene. The major components of EO from *R. mucronulatum* were caryophyllene and humulene; *R. ledebouri*, bornylacetate and germacrene D; *R. sichotense*, palustrol. These data indicate that interspecies differences in the qualitative and quantitative compositions of EO are possible within the single genus *Rhododendron*.

EXPERIMENTAL

Plant Material. Material for chemical studies was collected within the natural distribution of each species and dried in air (except *R. dauricum*). Table 3 lists the characteristics of the raw materials and the EO content in them. All studied samples were identified by S. E. Kalinovich, biologist at the Botanical Garden of Irkutsk State University.

EO Isolation. EO were prepared by steam distillation using the standard method [5] as oily liquids from light yellow to yellowish-green (samples 2b and 3b) in color with a sharp odor. The amount of raw material was 35-50 g. The steam distillate was 350-400 mL. Portions (50 mL each) of the distillate were extracted with diethylether (2 × 20-25 mL). The ether extracts were combined and dried over anhydrous $MgSO_4$. The diethylether was carefully removed in vacuo. The EO was stored at 4°C before analysis.

EO Analysis. Chemical compositions of EO were investigated by GC/MS in a Hewlett—Packard 5890/II GC with a quadrupole MS (HP MSD 5971) as the detector using a HP-5MS quartz capillary column (30-m) with 0.25 mm internal diameter and 0.25 μm film thickness. The injector temperature was 280°C; thermostat held 2 min at 50°C, increased to 300°C at 4°C/min, and held at that temperature for 30 min. The carrier gas was He, flow rate 1.0 mL/min, separation coefficient 1:20. The component contents (Tables 1 and 2) were calculated from areas of GC peaks without using correction coefficients. Compounds were identified by the agreement of retention times and complete mass spectra of the studied compounds with the corresponding ones of MS catalogs at the Inst. Org. Chem., Sib. Div. RAS [6]. Instances where only mass spectra were compared (for example, from Wiley7N and NIST02 catalogs) are noted separately (see the footnotes in Tables 1 and 2).

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