Chemistry of Natural Compounds, Vol. 45, No. 5, 2009

BRIEF COMMUNICATIONS

n-ALKANES IN NEEDLE WAXES OF *Picea omorika* VAR. *vukomanii*

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UDC 547.91

Serbian spruce, *Picea omorika* (Pancic) Purkyne (Pinaceae family), is a relic, endemic, and vulnerable tree species [1], naturaly distributed in fragmented areas in Serbia and Bosnia and Herzegovina [2]. Its variety, (var. *vukomanii* Pavlovic et Matovic), is represented by a small (up to 30 trees) and geographically isolated natural population in the Milesevka canyon (Serbia). In habitus, branches, and needles, this variety differs clearly from other natural populations of a typical variety of Serbian spruce, *Picea omorika* var. *omorika* (syn. var. *serbica*) [3, 4]. The composition and variability of needle essential oil of Vukoman′s Serbian spruce have been recently investigated [5].

Variability in profile and concentration of *n*-alkanes are very often used in chemotaxonomic investigations of many conifers and flowering plants [6–15]. The epicuticular wax composition of needles of Serbian spruce and numerous other *Picea* species have already been studied [16–18], but almost all listed researches dealt with a small number of samples of artificial origin. To the best of our knowledge, this is the first report of the composition and variability of *n*-alkanes in the needles of *P. omorika* natural populations. This is also the first report of needle epicuticular wax composition of its variety *vukomanii*.

n-Alkanes in epicuticular waxes of two-year old needles of Vukoman′s Serbian spruce ranged from C_{18} to C_{35} (Table 1). The most abundant alkane is C_{31} (18.58% on average), rarely C_{29} (14.10% on average). C_{27} is also present in significant amounts (10.87% on average). The variation of each alkane hydrocarbon is listed as the value of the standard deviation (SD) (Table 1). The most variable alkanes are C_{33} and C_{31} (SD values are 16.17 and 10.05, respectively), while the variability of C_{27} is quite low (SD = 2.64).

The carbon preference index values (CPI) of *n*-alkanes of *P. omorika* var. *vukomanii*, calculated by the formula of Bray and Evans, ranged from 0.34 to 4.78 (2.48 on average) (Table 2). Average chain length values (ACL) for all *n*-alkanes, calculated by the formula of Poynter and Eglington, ranged from 24.80 to 31.18 (27.91 on average) (Table 2). ACLs of *n*-alkanes with odd carbon numbers show a smaller variation (SD = 1.35) but a higher mean value (29.01) compared to those of even carbon numbers (SD = 1.78, mean value: 26.57).

Vukoman′s Serbian spruce has a wider range of carbon numbers of *n*-alkanes (18–35) than typical Serbian spruce: 19–33 [17] and 18–33 [18]. Vukoman′s Serbian spruce also has a wider range of *n*-alkane compounds comparing with other *Picea* species [16–18]. In comparison with literature results [17], Vukoman′s Serbian spruce is more similar to *Picea wilsonii* from section Eupicea, according to the classification of Krusmann [2], and less similar to the same species, *Picea omorika*, where only one of these compounds dominated: C_{31} = 17% after [17] or C_{33} = 15.7% after [18]. C_{31} and C_{29} also dominate in two species from section Casicta (*P. yezoenzis* and *P. likiangensis*) [17]. However, in wax needles of *P. likiangensis* only C_{31} (53.85%) strongly dominates [18].

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0009-3130/09/4505-0697

TABLE 1. Chemical Composition of Needle *n*-Alkanes of *Picea omorika* var. *vukomanii*

n-Alkanes	Statistical parameters		*n*-Alkanes	Statistical parameters	
	*X±S_X	**SD±S_{SD}		*X±S_X	**SD±S_{SD}
C_{18}	3.42±1.72	7.29±1.22	C_{27}	10.87±0.62	2.64±0.44
C_{19}	0.99±0.55	2.31±0.39	C_{28}	6.14±0.43	1.84±0.31
C_{20}	1.75±0.83	3.52±0.59	C_{29}	**14.10±1.08**	4.58±0.76
C_{21}	0.42±0.29	1.21±0.20	C_{30}	6.43±0.45	1.89±0.31
C_{22}	3.41±0.55	2.31±0.39	C_{31}	**18.58±2.37**	10.05±1.68
C_{23}	4.45±0.54	2.27±0.38	C_{32}	1.15±0.48	2.02±0.34
C_{24}	4.02±0.63	2.67±0.44	C_{33}	9.82±3.81	16.17±2.69
C_{25}	6.68±0.61	2.57±0.43	C_{34}	1.75±0.65	2.78±0.46
C_{26}	5.38±0.27	1.13±0.19	C_{35}	0.63±0.36	1.53±0.26

*Mean and mean error of the mean; **standard deviation and mean error of standard deviation.

TABLE 2. CPI and ACL Values for Needle *n*-Alkanes of *Picea omorika* var. *vukomanii*

Statistical parameters	CPI (C_{18}–C_{35})	ACL all (C_{18}–C_{35})	ACL odd (C_{18}–C_{35})	ACL even (C_{18}–C_{35})
Range (min–max)	0.34–4.78	24.80–31.18	27.13–31.74	23.21–29.68
*X±S_X	2.48±0.27	27.91±0.41	29.01±0.32	26.57±0.42
**SD±S_{SD}	1.16±0.19	1.74±0.29	1.35±0.22	1.78±0.42

*Mean and mean error of the mean; **standard deviation and mean error of standard deviation.

In spite of the CPI and ACL values of *n*-alkanes, Vukoman′s Serbian spruce is more similar to *Picea sitchensis*, section Casicta (2.8 and 28.0, respectively), and less similar to *P. breweriana*, section Omorika (4.7 and 27.3 or 6.7 and 25.6, respectively) or to *P. abies*, section Eupicea (5.9 and 33.3) [16]. The differences between our data and literature results for *Picea omorika* or between other *Picea* species might be the consequence of differences in sampling procedures, environmental factors, origin (natural or artificial), and/or number of processed samples.

We suggest further detailed population analysis of epicuticular wax *n*-alkanes of *Picea omorika,* which could lead to a better insight into the variability of alkanes and their taxonomic significance within this species, and consequently to understanding the relationships of this and related species within the genus *Picea*.

Plant Material, Extraction, and Isolation. Twigs with needles from the lowest third of the tree crown were collected in autumn from 18 selected trees. The collected twigs were stored at –20°C. The total wax of each sample was extracted by immersing 1 g of leaves in 5 mL of hexane for 45 sec. After extraction the solvent was removed under vacuum at 60°C and the remaining wax dissolved in 1.0 mL hexane. These wax samples were stored at –20°C until further analysis.

GC and GC-MS Analysis. The GC analysis was performed using a gas chromatograph HP 5890 equipped with a flame ionization detector (FID) and a split/splitless injector. The separation was achieved using an HP-5 (5% diphenyl and 95% dimethylpolysiloxane) fused silica capillary column, 30 m × 0.25 mm i.d., 0.25 μm film thickness. GC oven temperature was programmed from 50°C (6 min) to 285°C at a rate of 4.3°C/min. Hydrogen was used as carrier gas; flow rate: 1.6 mL/min at 45°C. Injector temperature: 250°C; detector temperature: 280°C. Injection mode: splitless. The injection volume was 1.0 μL.

The GC-MS analysis was performed using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 Network mass selective detector (MSD) in the positive ion electron impact (EI) mode. The separation was achieved using an Agilent 19091S-433 HP-5MS fused silica capillary column, 30 m × 0.25 mm i.d., 0.25 μm film thickness. GC oven temperature was programmed from 60°C to 285°C at a rate of 4.3°C/min. Helium was used as carrier gas; inlet pressure was 25 kPa; linear velocity: 1 mL/min at 210°C. Injector temperature: 250°C. Injection mode: splitless. MS scan conditions: source temperature, 200°C; interface temperature, 250°C; E energy, 70 eV; mass scan range, 40–350 amu. Identification of the components was done on the basis of the retention index and comparison with reference spectra (Wiley and NIST databases). The percentage (relative) of the identified compounds was computed from GC peak area.

ACKNOWLEDGMENT

This research was supported by a grant from the Ministry of Science of Serbia (projects 143049B and 142053).

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