Comparative Investigation of Douglas Fir Headspace Samples, Essential Oils, and Extracts (Needles and Twigs) Using GC-FID and GC–FTIR–MS

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The headspace, the essential oil, and the petroleum ether extract of Austrian Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] needles and twigs were analyzed by GC-FID and GC-FTIR–MS. More than 60 compounds, mainly monoterpenes, were identified in the samples. The main constituents of the investigated samples were β-pinene, sabinene [both dominate samples of needles (headspace, ca. 19%/22%; essential oil, ca. 13%/16%; extract, ca. 21%/23%) and twigs (headspace, ca. 20%/20%; essential oil, ca. 20%/15%, extract, ca. 21%/20%)], terpinen-4-ol, α-pinene, δ-3-carene, limonene, terpinolene, α-terpineol, α-terpinene, γ-terpinene, and myrcene. In the essential oils, bornyl acetate and citronellyl acetate were also found. The odor of these samples has been characterized, and some attractive effects on animals (deer and wild boar) are given.

Keywords: *Douglas fir; essential oil; GC–FTIR–MS; GC sniffing techniques; headspace; odor*

INTRODUCTION

The odor of Douglas fir samples, like that of the essential oil of needles or that of various geographic origins, was analyzed by many authors in years past (Laver et al., 1977; Maarse and Kepner, 1970; Sakai et al., 1967). Moreover, variations in the composition of the volatiles depending on seasonal aspects (Gildemeister and Hoffmann, 1961; Wagner et al., 1989 and 1990) determine the antimicrobial activity of Douglas fir samples (Bastide et al., 1987; Chalchal et al., 1986, 1987, 1989, 1991) as well as deer browsing and Douglas fir beetle pheromone effects (Dickens et al., 1983; Radwan, 1972; Radwan and Crouch, 1978) of this valuable natural product.

A comparative investigation of the odorous compounds of Austrian (cultivars) Douglas fir needle and twig samples using GC-FID, GC–FTIR–MS, and GC sniffing technique methods has not yet been carried out. Therefore, it was our aim to analyze and identify the volatiles of the six samples: headspace, essential oil, and petroleum ether extract of both the needles and the twigs.

Independently from these analytical results, further were observed and reported attractive effects on different species of animals.

EXPERIMENTAL PROCEDURES

Materials. Douglas fir needles and twigs [*Pseudotsuga menziesii* (Mirb.) Franco] from cultivars (~50 year old trees; origin, imported layers from the western Rocky Mountains of the United States) were collected in Lower Austria in October 1993. The headspace samples were trapped according to the dynamic method of Bicchi and Joulain (1990) and Brunke et al. (1992) for 3 h each in charcoal tubes (SKC Inc.) using a commercial pumping–trapping system (Brey Co.) and analyzed immediately after extraction from the charcoal tubes with dichloromethane (200 μL each). The essential oils were produced by exhaustive distillation of 12.0 g of needles (yield of oil, 0.83%) or 23 g of twigs (yield of oil, 0.87%). The petroleum ether extracts were obtained by the Soxhlet method from 10.5 g of needles (70 mL of petroleum ether, 24 h extraction time, 0.61% yield) or 17g of twigs (100 mL of petroleum ether, 24 h extraction time, 0.72% yield).

Organoleptic Testings. Ten microliters of each sample was placed on an odor strip, and the odor was characterized by perfumers.

GC Sniffing Technique. A Fractovap 2101 GC with split system and an LT Programmer 230, an Electrometer 160 (Carlo Erba Co.), and a Kompensograph III recorder (Siemens Co.) were used. The GC column was a 30 m low-polarity FSOT-RSL-200 fused silica column (0.32 mm i.d., 0.2 μm film thickness, Bio-Rad Co.). Conditions were as follows: detector temperature (FID), 320 °C; injector temperature, 250 °C; sniffing capillary heating, 250 °C; temperature program, 40 °C/5 min at a rate of 10 °C/min to 200 °C/20 min; H_2 carrier gas (2 mL/min); splitless mode, sniffing split ratio, 1:50 (FID detector:nose). The peak/odor impression correlations were performed by two perfumers and four fragrance chemists.

Gas Chromatography. The volatiles were separated in a GC 14A with a C-R6A integrator (both Shimadzu Co.). For other parameters, see GC Sniffing Technique.

Gas Chromatography–Fourier Transform Infrared Spectroscopy–Mass Spectrometry. An HP-5890A GC in connection with an HP-5965B IRD (MCT detector) and an HP-5970C MSD (Hewlett-Packard Co.) were used. This system was under the control of the data systems HP-9000/340 (IRD) and HP-9000/300 (MSD). Operation parameters for spectra registration were as follows: IR range from 4000 to 850 cm^{-1}; mass range from 35 to 450 amu (EI mode, 70 eV; acquisition cyclus times, IRD 0.15 s, MSD 0.35 s); carrier gas, helium; interface heating, 220 °C. For other parameters, see GC Sniffing Technique. IR spectra and MS spectra of the detected compounds were identified by correlation with EPA and ROBERTET (on-line) IR library and NBS and WILEY (on-line) or FOOD and NIST (off-line) MS library. Many nonterpenic constituents (labeled in Table 1) could be identified also by GC-FID by co-injection of pure substances and correlation of their Kovats indices according to the method of Jennings and Shibamoto (1980).

RESULTS AND DISCUSSION

More than 60 compounds in the headspace, the essential oil, and the petroleum ether extract of both Douglas fir needles and twigs were detected by GC-FID,

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Table 1. Volatiles in the Headspace (HSN), Essential Oil (EON), and Petroleum Ether Extract (PEN) of *P. menziesii* (Austria) Needles and Twigs (HST, EOT, and PET)

compound	% peak area GC-FID					
	HSN	EON	PEN	HST	EOT	PET
Monoterpenes						
santene	tr[a]	0.01	tr	0.03	0.02	tr
tricyclene	tr	0.01	tr	0.02	0.03	tr
α-thujene	0.18	0.04	0.27	0.21	0.04	2.61
α-pinene	8.22	6.19	9.14	7.16	5.32	9.31
camphene	4.18	1.03	1.22	4.22	1.17	1.17
β-pinene	19.36	13.38	21.31	20.32	19.97	21.16
sabinene	22.37	15.39	23.48	19.77	15.44	20.23
δ-3-carene	8.12	4.27	2.17	9.16	4.12	9.19
myrcene	2.17	0.12	3.18	4.11	1.24	2.19
α-terpinene	6.18	4.12	3.98	4.02	2.61	0.22
α-phellandrene	tr	0.12	tr	tr	0.03	tr
limonene	2.12	3.22	4.12	1.27	2.12	3.34
β-phellandrene	tr	0.33	tr	tr	0.03	tr
1,8-cineole	tr	0.01	tr		tr	tr
cis-β-ocimene		0.14	tr		tr	tr
γ-terpinene	6.33	4.23	2.14	5.28	2.72	2.37
trans-β-ocimene	tr	0.31	tr		0.01	tr
p-cymene	tr	0.44	0.36		0.14	tr
terpinolene	10.18	7.29	9.17	11.18	7.82	10.48
p-cymenene	tr	0.02	tr		tr	tr
fenchol	tr	tr	tr		0.05	tr
linalol		0.14	tr		1.02	tr
terpinen-4-ol	4.22	9.17	6.61	5.27	9.11	3.01
α-terpineol	3.89	1.44	2.17	5.44	2.96	1.16
citronellol	tr	1.33	0.22	tr	1.31	0.14
nerol	tr	0.12	0.11		0.07	tr
geraniol	tr	0.31	0.08		0.08	tr
borneol		0.26	0.11		tr	tr
citronellal		0.04	tr		tr	
camphor		0.01	tr		tr	
anethole		0.76	tr		0.42	
carvone		0.18	tr		0.01	
fenchone		tr	tr		0.02	
piperitone		tr	tr		tr	
thymol	tr	0.03			tr	0.18
carvacrol		0.06			0.02	
linalyl acetate		0.02			0.05	
bornyl acetate	tr	3.11	tr	tr	1.94	0.22
citronellyl acetate	tr	5.12	tr	tr	4.61	0.48
neryl acetate	tr	0.86	tr		1.33	tr
geranyl acetate	tr	3.64	tr		3.01	tr
Sesquiterpenes						
α-guaiene		0.03			0.04	0.29
farnesene		0.17			0.01	0.48
β-caryophyllene	tr	0.28	tr	tr	0.12	tr
longifolene		0.01			tr	tr
germacrene D		0.21	tr		0.01	0.27
muurolene		0.14			0.03	tr
elemene		0.22	tr		0.02	tr
humulene		0.18	tr		0.04	0.19
cadinene	tr	0.78		tr	0.71	0.24
elemol	tr	1.02	tr	tr	0.01	1.22
β-caryophyllene epoxide		tr			0.03	tr
Others						
butanal	tr	tr	0.09	tr	0.14	tr
ethyl 2-methylbutyrate	tr	0.02	0.17	tr	0.27	tr
2-hexenal	tr	tr	0.27		0.31	0.29
hexen-3-ol	tr	0.17	1.13	tr	0.54	1.16
eugenol		tr	tr	tr	0.25	
isoeugenol		0.22		tr	0.16	
methylisoeugenol		0.46			0.31	tr
benzyl benzoate		1.36	0.76	tr	tr	0.88
dodecanol		1.02	0.22		tr	tr
isoamyl cinnamate		1.14	tr		tr	tr
fatty acids/esters	0.98	4.16	6.33	0.79	5.29	6.11
unidentified compounds ca.	1	1	1	1	1	1

[a] Trace compound less than 0.01%.

and the structures of 62 constituents identified by GC–FTIR–MS (Table 1). The main components (higher than 4%, excluding fatty acids and their esters) are as follows: in the needles headspace, sabinene, β-pinene, terpinolene, α-pinene, δ-3-carene, γ-terpinene, α-terpinene, terpinen-4-ol, and camphene; in the twigs headspace, β-pinene, sabinene, terpinolene, δ-3-carene, α-pinene, α-tepineol, γ-terpinene, terpinen-4-ol, camphene, myrcene, and α-terpinene; in the essential oil of the needles, sabinene, β-pinene, terpinen-4-ol, terpinolene, α-pinene, citronellyl acetate, δ-3-carene, and α-terpinene; in the essential oil of the twigs, β-pinene, sabinene, terpinen-4-ol, terpinolene, α-pinene, citronellyl acetate, and δ-3-carene; in the needles extract, sabinene, β-pinene, terpinolene, α-pinene, terpinen-4-ol, and limonene; and finally in the twigs extract, β-pinene, sabinene, terpinolene, α-pinene, and δ-3-carene.

Table 2. Odor Characterization of *P. menziesii* (Austria) Needle and Twig Samples

needles	
headspace	weak, woody-floral, cedarwood-like
essential oil (steam distillate)	spicy, nutmeg- and leather-like, weak caraway with camphoraceous side-note, green, weak fir needle-like
petroleum ether extract	green, fir needle extract, balsamic sweet, beautiful, fruity (strawberry), smoky (olibanum)
twigs	
headspace	at the beginning spicy, weak sweet, coriander-like; later rose- and geranium-like
essential oil (steam distillate)	nutmeg, weak fir needle-like, woody with linalool side-note
petroleum ether extract	typical fir odor, clean, natural, weak sweet

The chemical compositions of each sample are very similar, but there are significant changes in the concentrations of aforementioned main compounds. From the data shown in Table 1, sabinene is the dominating terpenic constituent of the needles, while that of the twig samples is β-pinene.

The headspace samples of both the needles and twigs show a total concentration of the mentioned main compounds of more than 90% (ranging as a single compound from ca. 4% to ca. 22%) and only a few side and trace compounds. Contrary to this the essential oils consist of more than 25% of side and trace compounds, while, in correlation to the other samples, the main constituents are less dominating (highest ca. 15%). The extracts seem to be similar to the corresponding headspace samples but differ in a higher number of side and trace compounds. These analytic results are a good comparison with olfactoric data. The petroleum ether extract showed the highest correlation with the genuine odor of Douglas fir (Table 2). The olfactoric characterization by perfumers allows the conclusion that for the typical *P. menziesii* odor the concentration of main compounds (β-pinene, sabinene, terpinolene, α-pinene, δ-3-carene) should be high (ca. 9–21%) in comparison to the great number of side and trace compounds (range of less than 0.01% to ca. 3%).

Deer (wiping their tails and fraying their heads) and wild boars (brushing) show interesting affinity effects to Douglasia (E. Karamat, personal communication, 1993). Among other conifers in a light forest, only the Douglasia trees are preferred. Therefore, the presented investigation on volatiles of *P. menziesii* may, in addition, give basic information for scientists working in the field of semiochemicals and could be important for the research of attractive effects by Douglas fir on different species of animals in the future.

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