Chemical Composition of Essential Oils from Needles and Twigs of Balkan Pine (*Pinus peuce* Grisebach) Grown in Northern Greece

P. K. Koukos,*,† K. I. Papadopoulou,† D. Th. Patiaka,‡ and A. D. Papagiannopoulos†

National Agricultural Research Foundation, Forest Research Institute, Vassilika, Thessaloniki, GR-570 06 Greece, and Chemical Process Engineering Research Institute, P.O. Box 361, Thermi, Thessaloniki, GR-570 01 Greece

The composition of essential oils from twigs and needles of Balkan pine (*Pinus peuce* Gris.) grown in northern Greece was investigated. The compounds were identified by using GC-MS analysis. The twig oil was rich in α-pinene (7.38%), β-pinene (12.46%), β-phellandrene (26.93%), β-caryophyllene (4.48%), and citronellol (12.48%), and the needle oil was rich in α-pinene (23.07%), camphene (5.52%), β-pinene (22.00%), β-phellandrene (6.78%), bornyl acetate (9.76%), β-caryophyllene (3.05%), and citronellol (13.42%). The mean oil yield was 2.85% for twigs and 0.57% for needles.

Keywords: *Pinus peuce; Pinaceae; essential oil composition*

INTRODUCTION

The Balkan pine (*Pinus peuce* Gr. Pinaceae), a rare pine of southeastern Europe, grows in a few places in the mountains of southern Yugoslavia (formerly) and in the adjacent parts of Albania, Bulgaria, and Greece (Mirov, 1967; Tsoumis, 1972). In northern Greece, the Balkan pine grows naturally in mixed stands with beech (*Fagus silvatica*), other pines (*Pinus silvestris*, *P. nigra*), and fir (*Abies*) in two different places, namely, Aridea and Rodopi, as shown in Figure 1 (Papadopoulou and Koukos, 1996; Tsoumis, 1972). Although numerous studies dealing with the composition and variation of the essential oils of various *Pinus* species have been published during the past 30 years (Gorunovic et al., 1992; Hanus and Pensar, 1973; Henning et al., 1994; Kolesnikova, 1985; Latish et al., 1983; Stepen and Klimova, 1985; Udarov et al., 1984; Vidrich et al., 1987a,b, 1989), a limited number of studies have been done on the essential oils of Balkan pine, and only a few of them refer to its natural stands (Gorunovic et al., 1992; Henning et al., 1994; Kolesnikova, 1985; Latish et al., 1983; Papadopoulou and Koukos, 1996). The purpose of this study was to investigate the composition of the essential oils extracted from needles and twigs of the Balkan pine from a natural population, as part of a project aiming to discover potential uses of the forestry biomass.

MATERIALS AND METHODS

Plant Material and Extraction. Because the amount of oil extracted from needles and twigs was found to be higher in the autumn than in the spring (Papadopoulou and Koukos, 1996), needle samples (200 g) and twigs (diameter < 15 mm) were collected in the autumn of 1997 (November) from 25−100-year-old trees that were growing in a natural stand in Rodopi (Xanthi). The needle and twig samples were cut from all sides of the trees up to the height of 4 m. Ten trees were selected at random, and one sample was collected from each tree. The samples were immediately placed in plastic bags and were transported to the Forest Research Institute the same day. The needles and twigs were separated and stored in plastic bags in a freezer at −20 °C. Aliquots (1−5 g) of the fresh needles and twigs were weighed, and the moisture content was determined at 100−105 °C (Papadopoulou and Koukos, 1996; Von Rudloff, 1975). Ninety grams of each sample was steam distilled in a Vapodest (Buchi) apparatus incorporating a separate steam-producing system for 3 h. The oil was collected in a lighter than water oil graduated trap and stored in the freezer at −20 °C until analyzed.

Chemical Analysis. Mass spectra were recorded with a gas chromatograph−mass spectrometer, Hewlett-Packard model GC5890 series II/5989 MS engine. The GC-MS was operated under the following conditions: mode, EI; El energy, 70 eV; mass range, 50−500 amu; scan/min, 0.98; (MS zones) source, 200 °C; quad, 100 °C; (GC parameters) column type, HP-5MS (5% diphenyl, 95% dimethylsiloxane); column length, 30.00 m; column diameter, 0.250 mm; gas, He; flow, 0.7 mL/min; linear velocity, 30.2 cm/s; split ratio, 1:100; (GC zone temperatures) injector, B, 240 °C; transfer line, 280 °C; (oven program) initial temperature, 40 °C; initial time, 0.00 min; rate, 4.0 °C/min; final temperature, 220 °C; final time, 20.00 min; solvent delay, 2.0 min; injection, 1 μL. The pure compounds were dissolved in CH_2Cl_2 to produce a solution 2% w/w. Deuterated toluene was added as internal standard (ISTD). The relative response factor (RRF) for each compound was estimated, and the same RRFs were used for the isomers that were not available from market. The oil samples were also dissolved in CH_2Cl_2 at a ratio of 1:4 to achieve peak abundance as in artificial solutions used for calibration. Internal standard was added and analyzed as above.

The oil components were identified by comparing their retention times and MS with those of the authentic samples (Aldrich) and by the use of a computerized MS data bank (Wiley and NIST).

RESULTS AND DISCUSSION

The mean moisture contents of the twigs and needles were 54.4 and 50.0%, respectively, and the mean yields of the volatile oil were 2.85 and 0.60% (dry weight basis), respectively. The compounds identified in the oil are shown in Table 1.

* Author to whom correspondence should be addressed (telephone +30 31 461171; fax +30 31 461341; e-mail pkoukos@fri.gr).

† National Agricultural Research Foundation.

‡ Chemical Process Engineering Research Institute.

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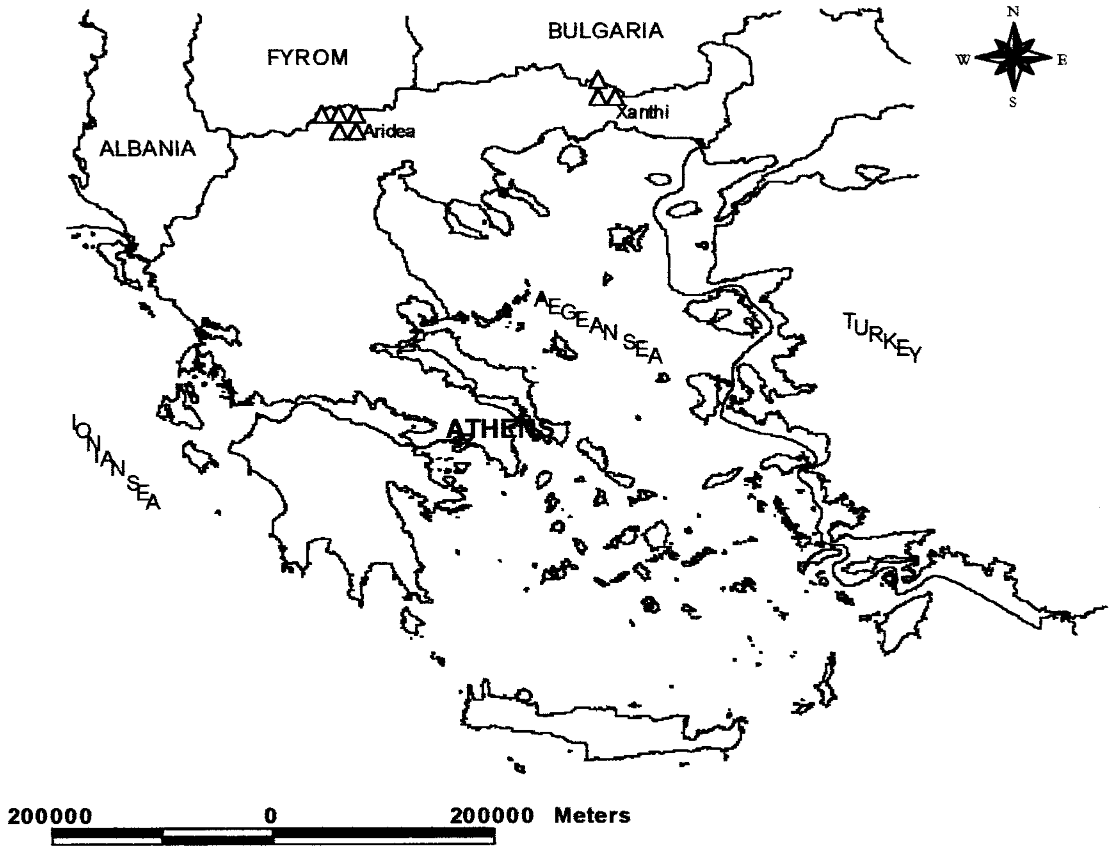

Figure 1. Area of collection of Balkan pine samples in Greece.

Table 1. Percentage Composition of the Twig and Needle Oils of *P. peuce*

peak	compound	twigs	needles	retention time (min)
1	trycyclene		0.36	7.35
2	α-pinene	7.38	23.07	7.75
3	camphene	0.24	5.52	8.20
4	β-pinene	12.46	22.00	9.13
5	α-myrcene	3.41	2.04	9.58
6	α-phellandrene	0.09	0.20	9.99
7	δ-3-carene	2.58	0.46	10.20
8	*p*-cumene		0.29	10.70
9	β-phellandrene	26.93	6.78	10.99
10	α-terpinolene	0.26	0.33	12.89
11	α-terpineol	0.11		16.53
12	myrtenol	0.18	0.21	16.74
13	linalyl acetate	0.10	0.24	18.72
14	bornyl acetate	0.57	9.76	19.79
15	α-terpinyl acetate	0.56	2.02	21.85
16	neryl acetate	0.12		22.33
17	α-copaene	0.20		22.60
18	geranyl acetete	0.07		22.96
19	2,4-diisopropenyl-1-methyl-1-vinylcyclohexene	0.23	0.36	23.18
20	junipene	0.12		23.58
21	β-caryophyllene	4.48	3.05	24.08
22	α-humulene	0.97	0.53	25.10
23	citronellol	12.48	13.42	26.05
24	isocaryophyllene	0.94		26.38
25	α-muurolene	0.64	0.27	26.52
26	γ-cadinene	1.16	0.36	26.94
27	δ-cadinene	1.60	0.65	27.21
	total	78.99	91.91	

Twenty-six compounds were identified in the oil, which accounted for 79% of the total twig oil composition, and 21 compounds were identified in the needle oil, which accounted for 92% of the total needle oil. It is evident that there are large quantitative differences in the oil compositions between needle oil and twig oil, with the latter consisting of more compounds. The ion chromatograms of the oil samples are shown in Figure 2.

The major compounds in the twig oil were β-phellandrene (26.93%), citronellol (12.48%), β-pinene (12.46%), α-pinene (7.38%), β-caryophyllene (4.48%), α-myrcene (3.41%), and δ-3-carene (2.58%), representing 66% of the total oil. Minor compounds were δ-cadinene (1.60%) and γ-cadinene (1.16%). On the other hand, the major compounds in the needle oil were α-pinene (23.07%), β-pinene (22.00%), citronellol (13.42%), bornyl acetate (9.76%), α-phellandrene (6.78%), camphene (5.52%), β-caryophyllene (3.05%), α-myrcene (2.04%), and terpinyl acetate (2.02%), representing 87.7% of the total oil. Minor compounds were δ-cadinene (0.65%), α-humulene (0.53%), and δ-3-carene (0.46%).

In comparison with the published data on another natural Greek population of *P. peuce* (Papadopoulou and Koukos, 1996), the twig oil had much lower amounts of α-pinene (7.38 versus 27.59%) and α-myrcene (3.41 versus 9.69%) and higher amounts of limonene (26.93 versus 15.79%), citronellol (12.48 versus 8.93%), β-caryophyllene (4.48 versus 7.22%), and δ-cadinene (1.60 versus 0.33%). However, β-pinene (12.46 versus 12.04%) and δ-3-carene (2.58 versus 1.39%) contents were rather similar. Also, α-phellandrene was present (2.58%).

This investigation has shown that although the essential oil composition varied between the twigs and needles in two Greek natural populations of Balkan pine, the quantity and quality of the essential oils isolated from twigs in comparison with the published data (Gorunovic et al., 1992; Latish et al., 1983; Papadopoulou and Koukos, 1996) are equal or better and have potential commercial value, being richer in β-pinene, limonene, and citronellol.

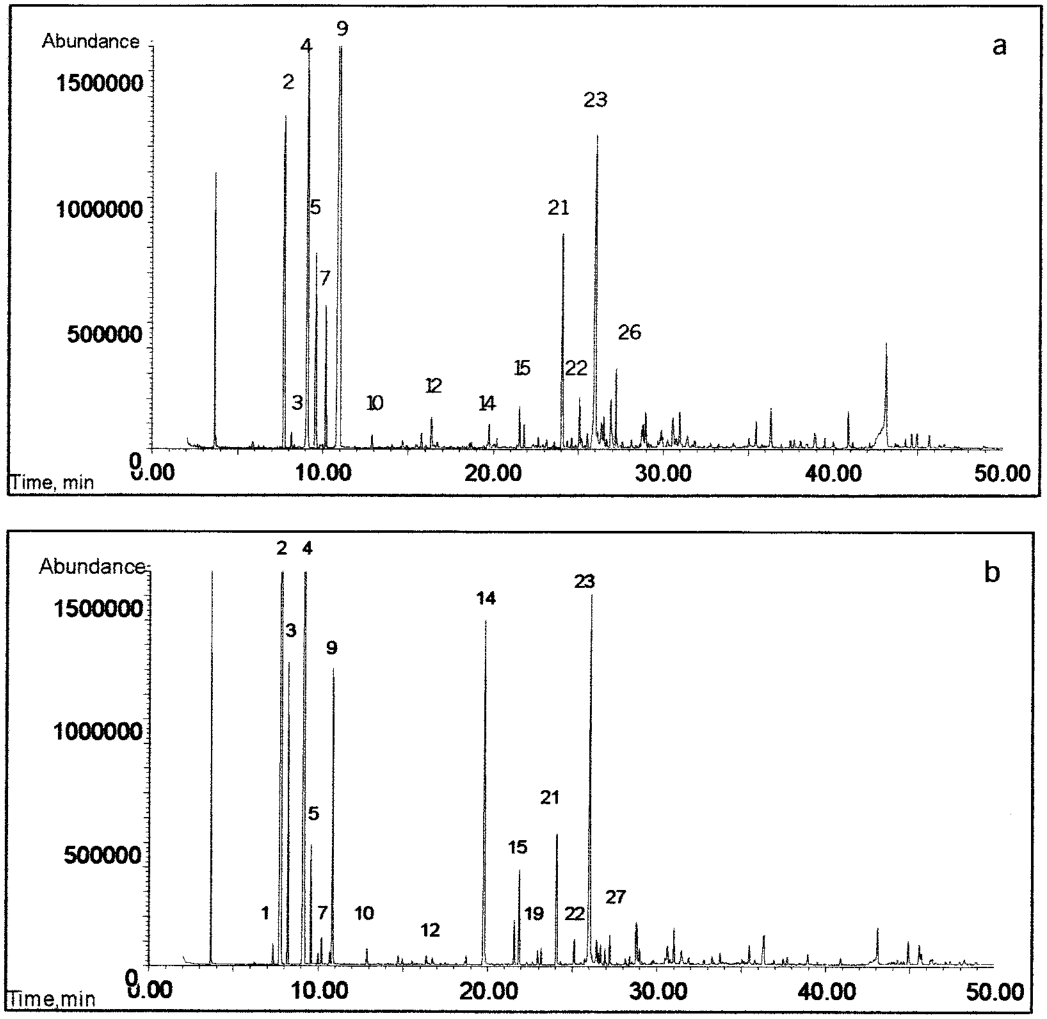

Figure 2. Chromatogram of essential oil from twigs (a) and needles (b) of Balkan pine.

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