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VARIATION IN COMPOSITION OF EXTRACTIVES FROM WOOD OF *PINUS NIGRA* VARIETIES

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Key Word Index—*Pinus nigra* var. *pallasiana*; *P. nigra* var. *pyramidata*; Pinaceae; extractives; unsaponifiables; sterols; diterpenes; fatty acids; resin acids.

Abstract—The wood of *P. nigra* vars *pallasiana* and *pyramidata* was analysed with regard to the main components lignin and holocellulose, as well as extractives. The lignin content was unusually low. Most of the extractives were fatty and resin acids. The var. *pyramidata* is richer in resin acids and differs also in the ratio of abietane- and pimarane-type acids from var. *pallasiana*. Some differences were also found in the sterol- and neutral diterpene-containing fractions.

INTRODUCTION

For several decades, extractives of the genus *Pinus* have been at the centre of interest because of their chemotaxonomic and genetic significance [1]. From 1970 onwards, the ease and speed of gas-chromatographic techniques have simplified the analyses of extractives. Amongst other things, attention has been paid to the xylem oleoresin for chemotaxonomy. Interest has, however, increasingly shifted to needles and cortex resin acids, because of the greater diversity of components. Zinkel [2] emphasized the major advantages of diterpene resin acids in chemotaxonomic investigations: they are nonvolatile and the acid pool seems to be quite stable in seasonal and tree variability. Regarding foliage analyses of various pine species, valuable studies have been done by Zinkel and co-workers in the course of the past 15 years [3–6].

It is more difficult, however, to differentiate between pine species according to wood extractives. Hafizoglu [7] reviewed the wood extractives and oleoresins of *P. sylvestris*, *P. nigra* and *P. brutia* with regard to their fatty and resin acids, as well as to monoterpenic hydrocarbons and unsaponifiables. Considering the data from various sources, he came to the conclusion that *P. brutia* is, in many ways, clearly different in its composition from other species, whereas *P. nigra* and *P. sylvestris* appear to be closely related taxonomically.

Black pine (*P. nigra* Arnold) is divided into five subspecies according to its natural occurrence in Europe, North Africa and Anatolia. Of the Anatolian black pine, four of five varieties are recognized [8, 9]. The most widespread variety is *P. nigra* Arn. ssp. *pallasiana* var. *pallasiana*. Another variety is *P. nigra* ssp. *pallasiana* var. *pyramidata*; the name derives from the growth habit of the tree with its thin ascending branches almost parallel to the stem.

The variety *pyramidata* occurs only in a single stand, mixed with the variety *pallasiana*, in one region of the world near the province of Tavsanli (Kütahya). The small forest area (235 ha) known as 'Vakif Ormani' is well protected. The present paper describes an attempt to characterize the varieties *P. nigra* ssp. *pal.* var. *pallasiana* and var. *pyramidata* with regard to the chemical composition of their woods.

RESULTS AND DISCUSSION

The yields obtained from extractions and basic analyses of the *P. nigra* vars *pallasiana* and *pyramidata* are summarized in Table 1. Both contain similar amounts of extractives, with slightly higher yields generally being found in var. *pyramidata*. When comparing the extractive data, the differences are less in the case of polar solvent or polar solvent-containing extraction medium (0.03–0.07%) than with non-polar solvents (0.2–0.5%). This finding may be an indication of a somewhat higher content of neutral compounds in var. *pyramidata*.

The unusually low lignin contents of both pine varieties are rather unusual. *Pinus nigra* var. *pallasiana* grown in other regions of Turkey has much higher lignin values, e.g. black pine from Belgrad forest (Istanbul) contains 27.2% lignin (unpublished results). The lignin determination of var. *pyramidata* was carefully checked in parallel with wood from an Angiosperm species (*Quercus* sp.). No similar tendency can be deduced from the holocellulose values because the holocellulose of var. *pallasiana* has a slightly higher content of residual lignin.

Table 1. Yield of extractives and main components from the wood of *P. nigra* varieties

Extraction	Var. *pallasiana*		Var. *pyramidata*	
	Freeze-dr. wt%	Air-dried wt%	Freeze-dr. wt%	Air-dried wt%
Cyclohexane	3.97	—	4.23	4.28
Ethanol (after cyclohexane)	0.70	—	0.65	0.66
Petrol	4.02	—	4.51	—
Acetone	4.47	—	4.73	—
Cyclohexane–ethanol (2:1)	4.55	—	4.58	—
Ethanol (after cyclohexane–ethanol)	0.19	—	0.26	—
Lignin*	—	24.67	—	23.08
Holocellulose*	—	85.09	—	84.66

*Based on extractive-free wood.

The cyclohexane extractives from both varieties were separated into neutral and acidic fractions. The var. *pallasiana* contains 6.9% neutrals and 93.1% saponifiables, whereas var. *pyramidata* contains 11.3% neutrals and 88.7% saponifiables. Thus, the var. *pyramidata* is richer in unsaponifiable neutral compounds which confirms the assumption made above (Table 1).

Gas chromatogram analysis of the silylated neutral fractions of both varieties revealed that sterols were the major components: 74% (*pallasiana*) and 68% (*pyramidata*). Up to 65% of sterols were found in *P. nigra* ssp. *pallasiana* grown in the Kastamonu district, with sitosterol as the main component constituting over 80% of the total sterols [10]. In our case, the composition of sterols is rather different with five sterols being detected. The first three peaks in the sterol region were identified as campesterol (24α-methylcholest-5en-3β-ol), stigmasterol (24α-ethylcholest-5,22dien-3β-ol) and sitosterol (24α-ethylcholest-5en-3β-ol) by comparing retention times and mass spectra of reference substances. The other sterol peaks belong presumably to cycloartenol and another sterol derivative. On the other hand, the distribution of sitosterol and its dehydroderivative, stigmasterol, in the neutrals from var. *pyramidata* differs somewhat from that from var. *pallasiana* in favour of stigmasterol.

Part of the neutral fraction contained diterpenes and diterpenoids. Some differences between the varieties were apparent, but because of their low contents these components were not investigated further.

The composition of the acidic fractions are given in Table 2. Eight fatty acids, 10:0, 16:0, 17:1, 18:0, 18:1, 18:2 and 18:3 were found in detectable amounts and identified. Furthermore, the presence of eight common diterpenic resin acids (pimaric, sandaracopimaric, isopimaric, levopimaric, palustric, dehydroabietic, abietic and neoabietic acid) was established. The ratio of fatty acids to resin acids is between 5.8 and 6.6 for var. *pallasiana* and between 3.2 and 3.5 for var. *pyramidata*. The proportion of fatty acids to resin acids for var. *pallasiana* is in good agreement with the earlier results of Hafizoglu [7]. The wood of the var. *pyramidata* contains markedly more resin acids.

The sensitivity of both unsaturated fatty acids and resin acids to oxidation is a well-known phenomenon. With extension of storage time the ratio of fatty acids to resin acids dropped to 5.0 for var. *pallasiana* and 2.7 for var. *pyramidata*; obviously the unsaturated fatty acids are degraded faster than the resin acids. The amounts of 18:2 and 18:3 also decreased noticeably which supports this concept.

The quantitative data reveal that palustric acid is the major resin acid in the black pine varieties. It is followed by isopimaric acid in var. *pallasiana* and by dehydroabietic acid in var. *pyramidata*. According to Zinkel and Han [11], the concerted oxidation–dehydration of methyl levopimarate and palustrate form methyl dehydroabiate during storage in a non-polar solvent. This effect could be avoided in our case because derivatization was carried out in the injection port. The relatively high amounts of palustric and levopimaric acid may contribute to the high dehydroabietic acid content in natural conversion in var. *pyramidata*.

Furthermore, another distinguishing feature between the varieties is when the resin acids are divided into the pimarane and abietane types from which they are derived. Thus, pimaric, isopimaric and sandaracopimaric acid belong to the pimarane-type, whereas the remaining five acids belong to the abietane-type. In this way, the estimated ratios of abietane- to pimarane-type are *ca* 3 for var. *pallasiana* and *ca* 4 for var. *pyramidata*.

EXPERIMENTAL

In Vakif Ormani, province Tavsanli (Kütahya, Turkey), 2 neighbouring trees of average quality (*ca* 0.25 m diameter at breast height, 14 m long) were felled in July 1993. Although the trees had almost identical diameters, the var. *pyramidata* was 103 years old, whereas the var. *pallasiana* was only 50 years old. The amounts of heartwood in both trees were very small with *ca* 4–5 cm in

Table 2. Composition of acidic fraction from extracts of *P. nigra* varieties

Peak No.	RR_t	Acid	Var. *pallasiana* %	Var. *pyramidata* %
1	0.13	8:0	+	+
2	0.20	9:0?	0.16	0.20
3	0.28	10:0	0.32	0.48
4	0.41	12:0	+	+
5	0.53	14:0	+	+
6	0.64	16: 1	+	+
7	0.67	16:0	1.41	1.25
8	0.73	17:1	0.38	0.39
9	0.75	17:0	+	+
10	0.80	18:3*	3.31	4.59
11	0.81	18:3*	0.45	0.55
12	0.83	18:2	36.65	32.36
13	0.84	18:1	41.51	34.51
14	0.87	18:0	0.51	0.50
15	0.92	20:1*?	0.32	0.33
16	1.0	Pimaric	0.89	1.74
17	1.02	Sandaracopimaric	0.20	0.33
18	1.04	Isopimaric	2.22	2.46
19	1.05	20:1*?	0.36	0.36
20	1.07	Levopimaric	1.34	2.26
21	1.08	Palustric	4.31	7.38
22	1.11	Dehydroabietic	1.15	3.54
23	1.13	20:0	+	+
24	1.18	Abietic	1.80	2.82
25	1.25	?	+	0.21
26	1.26	Neoabietic	1.51	2.18
27	1.35	?	0.20	0.56
28	1.43	22:0	+	+
29	1.67	24:0	+	+
Sum			99.0	99.0

+ < 0.1%.
*Cis–trans isomers.
Based on the results of four separate analyses.

diameter at the bottom with a stem diameter of 30 cm. The heartwood disappeared above one third of the entire stem length. Within *ca* 30 min, 4 discs were taken at 30 cm from the ground and at one third, half and two thirds of the length of each tree. Discs were packed in tightly closed plastic bags and brought to Istanbul within 6 hr. Bark was removed and the fr. wood was frozen by keeping at − 20° for 2 days. Subsequently, frozen samples were flown to Munich for analysis.

About half the material was chipped into matchstick-size pieces with the exclusion of heartwood. From the well-mixed chips, samples were taken at random. For analyses of main wood components, air-dried sticks were ground, sieved and particles sized between 60 and 100 mesh were used. Extractions were carried out with the 0.5 mm fr. obtained by grinding freeze-dried chips.

Lignin was determined by hydrolysis of polysaccharides with H_2SO_4–HBr according to ref. [12]. Holocellulose was prepd by delignifying the extracted wood with acidified $NaClO_2$ soln. Extractions were carried out in a Soxhlet apparatus using Me_2CO, petrol (bp 40–60°) and cyclohexane (8 hr each). Additionally, an extraction with cyclohexane–EtOH (2:1, 6 hr) followed by EtOH (6 hr) was carried out.

Cyclohexane extractives were saponified with EtOH–KOH soln. The unsaponifiable neutral components were isolated by partition into petrol. The saponified acids were obtained from their alkali salts by neutralizing the soln with HCl. Isolation of the free acids was achieved by extraction into petrol.

For GC analysis, neutrals were converted into their corresponding TMSi ether derivatives by treating with the mixt. of trimethylchlorosilane and bis-(trimethylsilyl)-trifluoracetamide (1:3). Acid frs were injected together with trimethylsulphonium hydroxide (TMSH) which efficiently methylated the fatty and resin acids in the injection port.

Neutral and acidic frs were analysed on a 25 m HP-1 nonpolar capillary column using FID. GC-MS was carried out on an Ultra-1 column with properties very similar to those of the HP-1 column. The temp. progression for the sepn of neutrals was 100° for 5 min, then

10° min^{-1} to 200°, 4° min^{-1} to 275° and 5 min at 275°. The temp. progression for the acidic fr. was 125° for 4 min, then 12° min^{-1} to 205°, 2° min^{-1} to 245°, 10° min^{-1} to 275° and 5 min at 275°. Injection and detector temp. were maintained at 280° and 250°, respectively.

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