Chemistry of Natural Compounds, Vol. 41, No. 5, 2005

A COMPARATIVE STUDY ON THE CHEMICAL COMPOSITION OF THE ORIENTAL SPRUCE WOODS *Picea orientalis* FROM PLANTED AND NATURAL FORESTS

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UDC 547.915

The main constituents and cyclohexane extractives of wood obtained from planted and native oriental spruce, P. orientalis, *were comparatively investigated. The plantation stand in Belgrad Forest near the city Istanbul is about 1000 km west to the original provenance in northeast Turkey with somewhat different environmental factors. The distribution of the main components and the total extractive contents in the woods were affected insignificantly. Only few differences were notable in the amount of sugar monomers of the main polyoses, mannan and xylan, when the native and planted woods were subject to total hydrolyses. The yield of lipophilic extractives in native and planted woods was almost the same. The composition of lipophilic extractives of the woods, determined after proper derivatization by GC-MS, were also not different qualitatively, but some constituents exhibited quantitative changes. Although the planted wood contained significantly more resin acids, sterols, and alcohols, the natural samples had more unsaturated fatty acids.*

Key words: Main wood constituents, HPLC, lipophilic wood extractives, GC-MS, *Picea orientalis*.

Picea orientalis is a commercially important wood species in Turkey. Therefore, many successful plantations of oriental spruce exist in many areas, one of which is in Belgrad Forest lying on the northwest part of Istanbul. Contrary to European spruce, oriental spruce has a limited distribution, growing in the eastern Black Sea region of Anatolia and Georgia [1]. Its wood is utilized in the furniture industry and in the building sector and this species serves as the raw material for the production of mechanical pulp in Aksu SEKA mill.

Wood and bark of oriental spruce grown in natural stands were examined with regard to the main cell wall components [2, 3]. Some works also dealt with the composition of lipophilic extractives from wood or oleoresin obtained from bark and cones [3–5].

The soxhlet and supercritical gas extracts of oriental spruce wood were investigated in order to determine the composition of terpene hydrocarbons [6]. Demirbas [7] has also determined the yields and composition of fatty and resin acids in supercritical gas- and soxhlet extracts.

Oriental spruce afforestation in Belgrad Forest lies about 1000 km west to the natural distribution areas of this species in northeast Turkey. This large distance and somewhat different environmental factors would make themselves felt in the chemical composition of the wood. Since there was no reported data in this context, the objective of the present study was to check whether both the main components of wood and its extractive composition differ in oriental spruce trees from the afforestation and natural stands. For this purpose, standard solubility and main component analyses of woods, as well as, following the total wood hydrolysis, quantitative saccharifications were performed. Lipophilic extractives isolated with nonpolar solvent extraction from wood were separated into neutral and saponifiable fractions, which were in turn analyzed by GC-MS after proper derivatization.

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 0009-3130/05/4105-0494

TABLE 1. Comparison of Main Components and Solubilities of Spruce Woods from Natural and Planted Stands (wt % of oven-dry original and *extract-free wood, n.d. not determined)

Analysis	Planted	Natural
Ethanol-benzene solubility	1.3	1.5
Ethanol solubility	0.3	0.2
Hot water solubility	2.9	2.6
1% NaOH solubility	12.1	10.5
Klason lignin*	26.8	26.1
Acid soluble lignin*	0.3	0.3
Holocellulose*	N.d.	80.4
α-Cellulose*	N.d.	50.5
Pentosan*	9.9	9.2

TABLE 2. Sugar Composition of Oriental Spruce Woods (wt % Based on EtOH/benzene and EtOH extracted Material)

Substance	Planted	Natural
Glucose	49.54	49.80
Xylose	8.1	7.50
Arabinose	2.58	2.20
Mannose	13.86	14.90
Galactose	2.06	2.50
Σ (Monosaccharide)	76.14	76.90
Σ (Polysaccharide)[a]	68.31	69.02
Lignin (Klason + acid soluble)[b]	27.06	26.38
Sum [a] + [b]	95.37	95.40

Table 1 shows the cell-wall components and solubility characteristics of spruce wood from natural and plantation stands. The results are averaged values of three trees for native and planted specimens respectively. The low amounts organic solvent- and hot water solubilities of the woods are quite close to each other, and the small differences observed here should be considered as acceptable between trees of the same stand (usual tree to tree differences). However, a slightly higher dissolution of the planted specimen in 1% sodium hydroxide solution is noticeable. Both wood samples from planted and natural stands have almost equal proportions of klason and acid oluble lignin. Similarly, a comparison of pentosan contents of the samples shows that the differences between both woods are negligible. It can be concluded that the woods of both provenances are not distinguishable with regard to the amount of the total main components and extractives.

The results of quantitative saccharification of wood from planted and natural trees are given in Table 2. The literature survey shows that the sugar composition of oriental spruce wood has not been determined to date; thus the present work provides the first data. No significant differences were observed between the glucose content of planted and natural woods. The amount of xylose in wood from plantation was slightly higher compared to natural wood. However, a lower amount of mannose occurred in this spruce, and, its galactose content was also lower than that innatural wood. Assuming that the ratio of mannose and glucose units in galactoglucomannans of most softwoods is about 3:1, the glucose originating from this polyoses is approximately 3.5% in *Picea orientalis* woods.

The yield of lipophilic extractives in planted and natural woods was 0.7%, 0.8% respectively. This is about the same value reported in earlier works for *P. orientalis* wood of natural origin [3, 7, 13]. Although the amount of saponifiables and neutrals in the extract of planted wood was estimated to be 57 and 43%, these values were determined to be 75% and 25% in the extract of natural wood. Thus, the natural oriental spruce produces more acids than planted one. In Table 3 the major groups of extracts belonging to acid and neutral fractions are shown in percentages based on whole extracts. From Table 3 is apparent that unsaturated fatty acids are the majority in natural wood, whereas resin acids dominate in the planted one. Furthermore, higher percentages of alcohols and sterols are present in planted spruce.

TABLE 3. Major Groups of Extracts in Saponifiable (%) and Neutral Fractions

Substance	Planted	Natural
Unsat. acids	18.8	46.3
Alcohols	13.9	5.8
Sat. acids	14.8	11.1
Sterols	26.3	16.3
Resin acids	23.4	17.7
Others	2.8	2.8

TABLE 4. Composition of Saponifiables from Lipophilic Wood Extractives of *P. orientalis*

Compound	R. Tm	Planted	Natural	Compound	R. Tm	Planted	Natural
Octanoic	7.55	0.2	Tr.	**Pimaric**	26.14	**2.0**	0.6
Nonanoic	9.65	0.1	Tr.	**Sandracopimaric**	26.40	**3.9**	**1.4**
Dodecanoic	15.36	0.2	Tr.	C20-Trienoic	26.50	**2.4**	**3.2**
Me-Dodecanoic	16.58	0.1	Tr.	C20-Enoic	26.71	0.1	0.2
Tetradecanoic	18.65	0.3	0.1	Eicosadienoic	26.84	0.1	0.3
Me-Tetradecanoic	19.75	0.4	0.4	**Isopimaric**	26.97	**3.0**	**2.8**
Pentadecanoic	20.17	0.2	0.2	**Palustric&Levopimaric**	27.11	**11.3**	**8.7**
Me-Pentadecanoic	21.10	0.1	0.0	Unid. Alkenoic acid	27.23	0.4	0.3
Hexadecenoic	21.23	0.8	0.6	**Eicosanoic**	27.39	**1.2**	0.8
Hexadecanoic	21.66	**4.9**	**5.2**	**Dehydroabietic**	27.44	**10.5**	**3.1**
Heptadecenoic	22.28	0.2	0.1	**Abietic**	28.25	**3.3**	**1.9**
14Me-Hexadecanoic	22.66	**3.1**	**3.1**	Heneicosanoic	28.94	0.5	0.2
Heptadecanoic	23.01	0.5	0.3	**Neoabietic**	29.12	**1.8**	**2.3**
Octadecatrienoic	23.68	**3.0**	**8.4**	Hydroxy Abietatrienoic?	29.74	0.4	0.3
Octadecadienoic	23.75	**1.1**	0.6	Abietarienoic	29.96	0.3	0.3
Octadecadienoic	24.01	**12.8**	**28.0**	Hydroxy Abietatrienoic	30.12	0.9	0.4
Octadecenoic	24.12	**9.5**	**15.4**	**Docosanoic**	30.58	**6.3**	**1.4**
C18enoic isomer	24.15	0.5	0.8	Me-Dehydroabietic	30.87	**1.5**	0.6
Unid. Alkenoic	24.26	0.2	0.2	Unid. Diterpene acid	31.41	0.4	0.2
Octadecanoic	24.43	0.7	**1.0**	**Me-Docosanoic**	31.78	**1.6**	0.1
C19 Dienoic	24.77	0.2	0.4	Tricosanoic	32.23	0.9	0.2
Nonadecenoic	25.05	0.4	0.7	**Tetracosanoic**	33.96	**2.5**	0.5
Nonadecanoic ME	25.47	0.5	0.4	Pentcosanoic	35.91	0.3	0.0
C19Alkanoic isomer?	25.87	0.3	0.2	Hexacosanoic	38.25	0.1	0.0
				Sum		**95.9**	**96.0**

Bold: major compounds. I.e. > 1%. Tr.: trace < 0.05%.

The chemical composition of saponifiables for both samples is depicted in Table 4. The most abundant fatty acids in the woods are palmitic, octadeca-tri-, -di (linoleic), and -monoenic (oleic) and docosanoic acids. To the abundance of unsaturated acids in native wood contribute the linolenic and oleic acids most. Among diterpene acids, palustric, levopimaric, and dehydroabietic acid were pronounced compounds. A part of the high amount of dehydroabietic acid in the Belgrad specimen might be considered as isomerized product.

In the work of Hafizoglu et al. [3], the very low percentage of resin acids in a fresh native wood (2% of total extracts) is probably an exception. The lowest percentage of resin acids found in analyzed woods was around 15% of total extracts in the present study.

TABLE 5. Composition of Neutrals from Lipophilic Wood Extractives of *P. orientalis*

Compound	Planted	Natural	Compound	Planted	Natural
Alcohols			**Sterols**		
Dodecanol	0.6	Tr.	Colestenol	0.1	0.1
Tetradecanol	0.3	0.1	**Campesterol**	**9.3**	**12.1**
Pentadecanol	0.1	0.1	**Campestanol**	**1.9**	**2.1**
Hexadecanol	0.2	0.2	Ergostenol	0.1	0.2
Octadecanol	0.3	0.1	Stigmasterol	0.2	0.1
Eicosanol	**2.7**	**1.4**	**Sitosterol**	**32.2**	**37.7**
Henicosanol	0.2	0.3	**Sitostanol**	**10.7**	**10.7**
Docosanol	**13.5**	**10.0**	Cycloartenol	0.4	1.0
Tricosanol	0.6	0.4	Cyclolaudenol	0.1	0.3
Tricosanol isomer	0.6	0.3	Unid. Sterols	6.2	1.0
Tetracosanol	**5.9**	**3.1**	**Sum of sterols**	**61.1**	**65.3**
Pentacosanol	Tr.	Tr.	**Others**		
Docosandiol	1.0	0.4	Hydrocarbons (C14-C28)	1.5	1.8
Hexacosanol	Tr.	Tr.	α-Copaene	Tr.	0.2
Sesquiterpn. alcohols	1.2	1.2	β-Caryophyllene	0.2	0.5
Pimarol	0.5	0.6	α-Humulene	Tr.	0.1
Isopimarol	0.7	0.7	Cadinene isomers	0.2	0.4
Sandaracopimarol	0.2	0.1	Caryophyllene oxide	0.1	0.2
Dehydroabietol	1.0	0.4	Diterpn. HC (MW:272)	0.2	0.6
Neoabietol	0.4	0.7	Manoyl oxides	1.0	1.1
Unid. Diterpn. Alcohols	2.2	3.4	Dehydroabietane	0.2	0.1
Labdadienol	0.2	0.1	Diterpn. aldehydes	1.2	2.0
Sum of alcohols	**32.3**	**23.4**	Dehydroabietal	0.2	0.5
			Squalene	0.1	0.4
			Unident. compounds	1.5	3.2
			Sum of others	**6.5**	**11.3**

The neutral fraction of extracts contained some methylated resin acids and other long-chain saponifiable acids in amounts such as 10–12%. This small part of the neutral fraction should be considered as carryover substances. Therefore, the compounds belonging to the unsaponifiable fraction are listed in Table 5 in the manner that they were grouped in alcohols, sterols, and others. The percentage of true neutrals (total > 87%) was then corrected by a factor, so that the sum of compounds resulted in 100%.

The neutrals were composed mainly of sterols and alcohols. While more alcoholic compounds were present in planted wood (32% vs. 23% of neutrals or 14% vs. 6% of total extracts), diterpene and sesquiterpene alcohols constituted 1/3 of them, being about the same in both specimens. Among long-chain primary alcohols, the docosanol was predominant, following by tetracosanol and eicosanol. Apart from unidentified diterpene alcohols, an interesting one, presumably a labdadienol isomer, was detected in woods. This compound was found in volatile needle and wood extracts of oriental spruce in noticeable amounts [14].

Although the sterol composition of woods did not differ from each other, with some 60 to 65%, sterols were the main components of neutral extracts. Three of them, campesterol, b-sitosterol, and sitostanol, were present in majority, while some members of the sterols remained unidentified. They reached an appreciable amount in planted spruce wood. On the other hand, as minor constituents, colestanol, ergostenol, and stigmasterol were identified for the first time in *P. orientalis* wood.

From C14 to C28, long-chain hydrocarbons were detected in spruce woods. Their members with C numbers between 20–25 were predominant. Some sesqui- and di-terpene and -terpenoids, as well as two manoyl oxide isomers, were found in woods. There are no reports on the existence of these compounds in oriental spruce to date.

Evaluating all results together, the differences found in the composition of lipophilic extracts can rather be considered as variations in the chemical composition of the trees, which occur due to the different geographic growth locations and genetic factors.

EXPERIMENTAL

Samples of planted oriental spruce (*Picea orientalis* L. Carr.) were obtained from Belgrad Forest at an altitude of 100 m. Sufficient number of trees (40 years old, 20–25 cm diameter at the bottom of the trunk) were felled and three discs were taken from the bottom, center and top of each stem. Natural wood samples were obtained from Trabzon (60–70 years old, 30–35 cm diameter at the bottom of the trunk). Fresh wood of all discs from each tree was chipped and mixed homogeneously, and about 50 g chips were dried in a vacuum desiccator for 24 h in the dark before they were ground (particle size < 1 mm). After redrying in the desiccator for 24 h, ground material was used for soxhlet extraction. Another 50 g of chips, which was air dried, was ground in a Wiley mill and sieved to a size between 40–100 mesh [8] for the solubility determinations and standard wood analyses.

The solubilities in ethanol-benzene, ethanol, hot water, and 1% NaOH were performed according to standard methods (Tappi T 204 om-88, Tappi T 207 om-88, Tappi T 212 om-88) [9]. The pentosan content was determined according to the ISO method [10] (absorbance measurement in Shimadzu UV-1601 Spectrophotometer) and klason lignin and acid soluble lignin determinations were conducted in accordance with Tappi T 222 om-88 and Tappi UM 250 [11].

For estimation of lipophilic extractives, about 20 g of the wood meal was extracted in a Soxhlet apparatus with cyclohexane for 8 h. The solvent was evaporated and the dry extract was saponified with 0.5 N KOH solution in 90% ethanol at 70°C for 4 h. After separation of saponifiables, the neutral fraction was silylated with a mixture of trimethylchlorosilane and bis-(trimethylsilyl)-trifluoracetamide (1:3) [5]. The acidic fraction was methylated by diazomethane. On a 30 m DB-1 nonpolar capillary column the acidic and neutral fractions were analyzed in GC-MS (Shimadzu, QP 5050 A). The temperature program for the separation of acids was 80°C for 3 min, then 8°C min^{-1} to 240°C, 4°C min^{-1} to 280°C, and 10 min at 280°C. The temperature progression for the neutrals was 90°C for 0.75 min, 8°C min^{-1} to 250°C, 5 min at 250°C, 10°C min^{-1} to 280°C, 5 min at 280°C, 5°C min^{-1} to 300°C, and 12 min at 300°C. The He-flow rate was maintained at 1.2 mL min^{-1} for acids and 1.5 mL min^{-1} for neutrals with a split ratio of 1/10. The commercial libraries Nist 21, Nist 107, and Wiley 229 were used for the identification of the compounds.

In order to determine the polysaccharide composition, the extracted wood meal was hydrolyzed with 77% H_2SO_4 [12]. Then the hydrolyzate was neutralized with $Ba(OH)_2$ and the monosaccharides were analyzed on an Aminex column (Aminex HPX 87P) in the HPLC system (Waters Associates, 600 system controller, 717 plus autosampler, 410 refractive index detector, 746 data module).

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