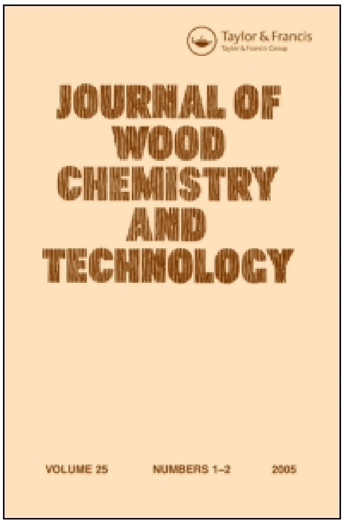

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CHEMICAL DIFFERENCES BETWEEN THE WOOD EXTRACTS OF JACK PINE (*Pinus banksiana*), BLACK SPRUCE (*Picea mariana*) AND BALSAM FIR (*Abies balsamea*) FROM EASTERN CANADA

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ABSTRACT

Differences in the chemical composition of the wood of balsam fir (*Abies balsamea* (L.)), jack pine (*Pinus banksiana* Lamb.) and black spruce (*Picea mariana* (Mill.)) were determined by GC analysis of their hexane extracts. Certain constituents such as juvabione, (E)-α-atlantone and δ-cadinene were shown to be present only in the heartwood of balsam fir. Analysis of the sapwood of the three species also revealed the presence of certain constituents such as juvabione, dehydrojuvabione and (E)-α-atlantone, all found only in balsam fir and an unidentified compound found only in jack pine. The chromatograms of the hexane extracts of both sapwood and heartwood showed characteristic patterns for each of the three species of conifers.

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INTRODUCTION

The differentiation of various wood species has been the subject of several publications in the past few years.[1-6] Some of the studies were aimed at applying these differences to the development of a method of sorting lumber.[7-9] Paper Mills are particulary interested in differentiating conifers as their proportions in wood chips affect the quality of the pulp and paper produced. However a prerequisite to developing a method of wood sorting is to determine whether the three species of conifer used in pulping operations in eastern Canada can be differentiated. Most of the aforementioned methods result in very high accuracy levels when applied to heartwood compared to the less accurate identification of species when sapwood[1,8] was analysed. Species sorting with these methods is based on the assumption that some natural compounds present in wood are specific to a particular species or, at least are much more plentiful in one of the species. Based on this assumption and work previously published on the differences in western canadian conifers chemical compositions,[10-11] we thought it would be interesting and useful to determine whether there are differences in the chemical composition of three conifer species, jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* (Mill.)) and balsam fir (*Abies balsamea* (L.)), by analyzing natural products from the hexane extracts of the heartwood and sapwood using capillary gas-phase chromatography (GC) and GC in conjonction with mass spectrometry (GC-MS).

RESULTS AND DISCUSSION

Heartwood Analyses

Results of the heartwood analyses are shown in Table 1. Three species specific natural products (markers) are observed only in balsam fir. Two of these compounds, δ-cadinene and (E)-α-atlantone, are minor constituents and the third, juvabione, a major component, was isolated from the wood of balsam fir by Bowers,[12] Cerny[13] and Manville.[14] In spite of the absence of specific markers in black spruce and jack pine, the hexane extracts of the heartwood contain abundant

TABLE 1

Chemical Composition of Heartwood Hexane Extracts of the Conifers

		Composition (%)		
Kovats indices	Identification	Jack Pine	Black Spruce	Balsam Fir
937	α-pinene	64.75	12.99	0.31
979	β-pinene	3.02	6.36	0.16
1191	α-terpineol	5.73	1.95	0.12
1525	δ-cadinene	0.00	0.00	1.71
1772	(E)-α-atlantone	0.00	0.00	2.90
1982	manoyl oxide	0.81	1.40	0.18
2002	manoyl oxide epi-13	1.08	2.41	0.00
2017	juvabione	0.00	0.00	55.44
2031	unknown 1	0.63	9.10	0.00
2053	manool	1.46	21.43	23.53
2089	dehydrojuvabione	0.10	0.00	15.64
2107	isoabienol	0.19	1.94	0.00
2144	neoabienol	2.61	18.79	0.00
2150	(Z)-abienol	3.99	21.43	0.00
2158	pimarane diterpene	6.43	0.74	0.00
2175	unknown 2	2.77	0.87	0.00
2189	unknown 3	6.42	0.59	0.00

natural products some of which could be considered specific to one of the three conifers as they are either absent or present in very small amounts in the other two species. For example, unknown 1 is abundant in black spruce but absent in balsam fir and present only in small amounts in jack pine. Unknown 3 and a pimarane diterpene are fairly abundant in jack pine whereas they are absent in balsam fir and present in small amounts in black spruce.

The chemical constituents of the conifer extracts display characteristic chromatographic patterns which could eventually be used to differentiate the heartwood of the three species. It can be seen in Figure 1 that 94% of the balsam

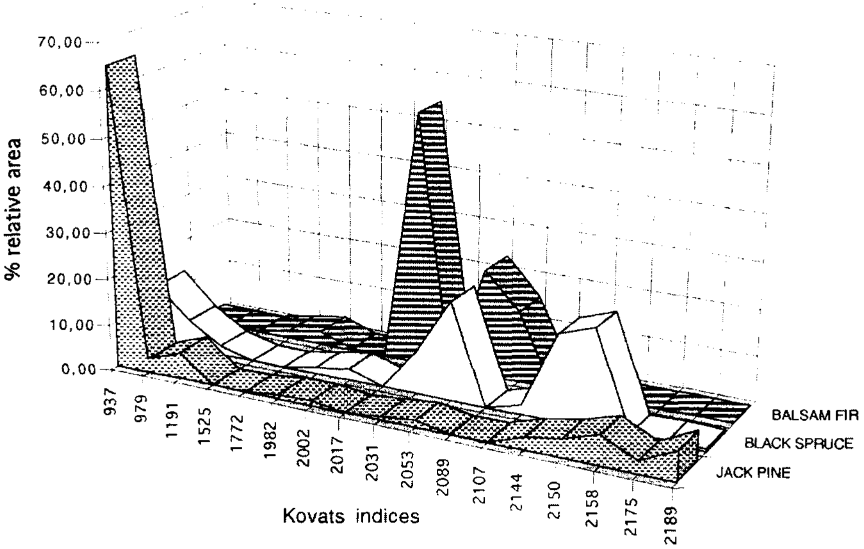

FIGURE 1. Chromatographic profiles of the extracts of heartwood.

fir heartwood extract is mainly composed of three major products with high retention indices, two sesquiterpenes (dehydrojuvabione and juvabione) and one diterpene (manool). This pattern contrasts sharply with that observed for black spruce extracts, composed mainly of four compounds with high retention indices, unknown 1, and three diterpenes (manool, neoabienol and (Z)-abienol) representing 70% of the extract and jack pine extracts composed of several minor products eluting in the same area of the chromatogram (neoabienol, (Z)-abienol, a pimarane diterpene and unknowns 2 and 3) and representing only 21% of the extract. In addition, the monoterpene content of the extracts of the balsam fir is less than 1% whereas the extracts of black spruce and jack pine are composed of 21% and 73% respectively of the same monoterpenes (α-pinene, β-pinene and α-terpineol).

It would therefore be possible to differentiate the heartwood of balsam fir from the two other conifers by the presence of juvabione in the balsam fir. Black spruce and jack pine heartwoods could be differentiated by comparing the chromatographic patterns of their extracts.

TABLE 2

Chemical Composition of Sapwood Hexane Extracts of the Conifers

		Composition (%)		
Kovats indices	Identification	Jack Pine	Black Spruce	Balsam Fir
937	α-pinene	39.38	9.71	1.13
979	β-pinene	4.37	5.92	0.74
1191	α-terpineol	1.40	0.32	0.37
1772	(E)-α-atlantone	0.00	0.00	1.08
1982	manoyl oxide	1.48	1.26	0.43
2002	manoyl oxide epi-13	2.34	2.09	0.75
2017	juvabione	0.00	0.00	15.44
2031	unknown 1	2.57	4.06	0.00
2053	manool	4.95	11.11	71.48
2089	dehydrojuvabione	0.00	0.00	8.58
2107	isoabiénol	0.44	1.84	0.00
2144	neoabienol	6.20	28.43	0.00
2150	(Z)-abienol	14.11	33.08	0.00
2158	pimarane diterpene	9.66	0.77	0.00
2168	unknown 4	4.47	0.00	0.00
2175	unknown 2	4.10	0.63	0.00
2189	unknown 3	4.54	0.78	0.00

Sapwood Analyses

The analyses of the sapwood hexane extracts of the conifers are shown in Table 2. Species specific constituents are observed for balsam fir and jack pine. Balsam fir extracts contain three specific compounds which are, in decreasing order of abundance, juvabione, dehydrojuvabione and (E)-α-atlantone. Unknown 4 is only found in the jack pine sapwood hexane extracts.

As is the case in the heartwood extracts, the chromatographic patterns of the sapwood extracts of the species are very characteristic (Figure 2). The balsam fir

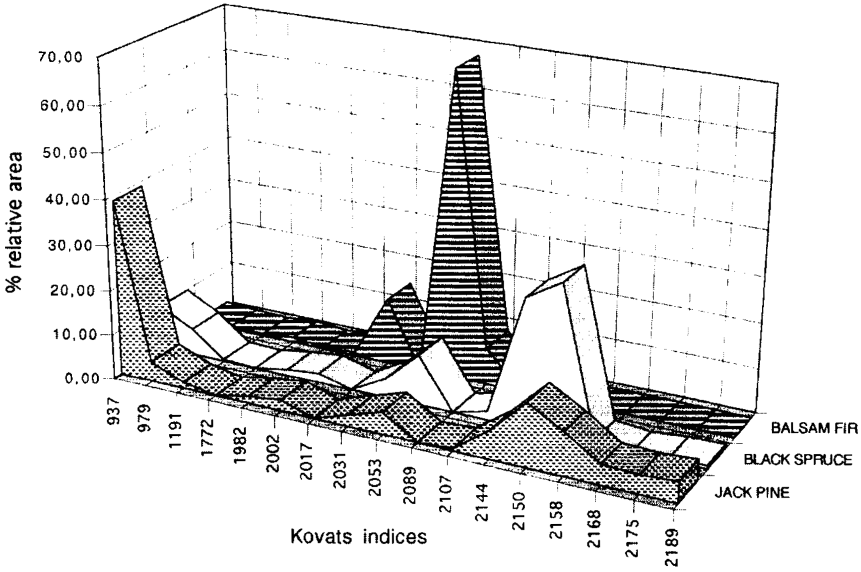

FIGURE 2. Chromatographic profiles of the extiacts of sapwood.

sapwood extracts are composed mainly of the same sesquiterpenes (juvabione and dehydrojuvabione) and the same diterpene (manool) as was found in the heartwood extracts. Black spruce extracts are composed of abundant diterpenes of high retention times (neoabienol, (Z)-abienol) whereas those of jack pine are composed of several minor products also eluted later in the temperature program (neoabienol, (Z)-abienol, a pimarane diterpene and unknowns 2, 3 and 4). On the other hand, monoterpene content (α-pinene, β-pinene and α-terpineol) of the jack pine sapwood extracts (45%) are greater than that of black spruce (16%) and balsam fir (2%) as was the case in the heartwood extracts. The results for sapwood show that it is possible to differentiate these three conifers due to the presence of markers specific to balsam fir and jack pine and also due to the chromatographic signatures characteristic for each of the three species.

The results observed for heartwood and sapwood are relatively similar within a particular species. However, certain differences are noteworthy with regard to the abundance of certain major components in the extracts. For example, the main difference between the heartwood and sapwood extracts of balsam fir is the identity of the major compound; juvabione (54.6%) is the most important compound in the heartwood whereas manool (71.5%) is the major product in the sapwood. The composition of the black spruce heartwood and sapwood extracts are almost identical, however (Z)-abienol appears as the major constituent in the sapwood whereas it is present in equal amounts to manool in the heartwood. The major product in both heartwood and sapwood jack pine extracts is α-pinene while unknown 4 is present only in the sapwood.

Identification of the Pimarane Diterpene

The pimarane diterpene was isolated by high performance liquid chromatography (HPLC) and studied by high resolution NMR and mass spectrometry. The proton and carbon-13 NMR spectra indicated the presence of near equimolar amounts of two stereoisomers. Careful examination of 2D proton-proton spectra, carbon-13 DEPT experiments and spectral patterns showed a high degree of similarity to the tricyclic pimarane-type diterpenes, particularly to the chemical shifts reported for isopimara-7,15-dien-18-oic acid and its endocyclic isomer, isopimara-8(14),15-dien-18-oic acid.[15] However, signals at 9.24 and 9.22 ppm in the proton spectrum and at 206.56 and 206.36 ppm in the carbon-13 spectrum indicated the presence of an aldehyde function at position 18 rather than the carboxylic acid. Grant et al.[16] have reported the presence of isopimara-7,15-dien-18-al and its endocyclic isomer, isopimara-8(14),15-dien-18-al in other *Pinus* spp.

Chromatographic and Spectroscopic Data of the Unidentified Minor Constituents

Although four minor constituents were not identified (unknowns 1 to 4), the Kovats indices of unknowns 1 and 2 on a polar column in addition to the mass spectra of the four constituents shown in Table 3 provide some information as their structure.

TABLE 3

GC-MS Data of Unknowns

	KI (Spwax)	m/z (%)*
unknown 1	2610	272(M^+, 0.5), 121(8.0), 109(8.2), 107(19.2), 95(16.0), 93(34.4), 91(10.4), 81(40.0), 79(16.0), 71(20.0), 69(100), 67(24.0), 55(22.0), 53(15.2), 43(32.4), 41(70.4)
unknown 2	2466	272(M^+, 20.0), 257(20.8), 239(60.2), 197(17.6), 187(44.8), 157(54.4), 143(18.4), 141(24.8), 129(32.8), 128(32.0), 117(22.4), 115(21.6), 71(21.6), 58(25.6), 43(100), 41(28.0)
unknown 3	not available	287(3.2), 241(43.2), 145(47.2), 121(40.0), 119(33.6), 107(48.0), 105(60.0), 95(35.2), 93(69.6), 91(100), 81(35.2), 79(81.6), 77(53.6), 55(48.8), 43(35.2), 41(72.0)
unknown 4	not available	286(M^+, 12.0), 187(42.4), 138(52.8), 131(39.2), 123(44.0), 121(44.0), 119(43.2), 117(44.6), 107(48.0), 105(43.2), 93(40.0), 91(100), 77(65.6), 67(49.6), 55(64.0), 43(96.0), 41(84.0)

* MS-data were obtained with DB-5 column.

The Kovats indices of the unknowns on both apolar and polar columns are characteristic of diterpenes. The mass of the parent ions of unknowns 1 and 2 as well as the fragmentation pattern correspond to those recorded for resin diterpene dienes such as pimaradiene.[17-18] Interpretation of the mass spectrum of unknown 3 is difficult due to the probable absence of the parent ion. A comparison of the mass spectrum of unknown 4 with those of the diterpene aldehyde identified in this study, levopimaral[17] and pimara-8(14), 15-dien-18-al[19] suggests a pimaradiene type diterpene aldehyde structure for this unidentified minor constituent.

CONCLUSION

The results show that the three aforementioned conifers can be differentiated chemically due either to the presence of specific markers or due to species specific chromatographic patterns of their hexane extracts. Eventually, these differences could be used to differentiate these three species whether heartwood or sapwood is analysed. Further work will be carried out in blind tests on several individual trees of these three species to verify the differentiation capability and precision of this method of analysis. Concurrently, it would be important to verify the degree of variability of the chemical composition of individual trees for each of the species to ensure the practicability of such a method of differentiation. Studies are underway to analyse extracts using more polar solvents in order to identify new species specific compounds (phenols, fatty and resin acids, etc.) present in the wood of these three conifers. In addition work is in progress to complete the identification of some of the unknown natural products shown in Tables 1, 2 and 3 by mass spectrometry and various high resolution NMR experiments.

EXPERIMENTAL

Extraction

The sapwood and heartwood of 12 jack pines, 12 balsam firs and 10 black spruces were analysed. Wood disks (1 cm) were cut from debarked logs of each tree and the sapwood and heartwood were carefully separated using chisels. Pieces of heartwood and sapwood were dried overnight at 22 °C and ground with an industrial grinder equipped with a 2 mm sieve. The particles (50 g) were extracted with 295 mL of hexane and 5 mL of a solution of an internal standard (1.5 g of tetradecane in 500 mL of hexane). This mixture was slowly stirred for 10 min using a mixer and an aliquot of 20 mL was dried over anhydrous $MgSO_4$, filtered, evapored at room temperature to 0.5 mL and analysed by GC and GC-MS.

GC and GC-MS Analyses

Gas chromatographic analyses were performed on a Hewlett-Packard 5890 gas chromatograph equipped with a polar Supelcowax column (30 m x 0.25 mm) and an apolar DB-5 column (30 m x 0.25 mm) and a split-splitless injection port (split mode). The temperature program was 60°C for 2 min, then 2°C/min to 140°C, then 1°C/min to 190°C, then 2°C/min to 210°C and this temperature was held constant for 28 min. The compounds were identified by their Kovats indices[20] on both columns and by GC-MS using a Hewlett-Packard MSD 5972 mass spectrometer at 70 eV coupled to an HP 5890GC equipped with a DB-5 column (30 m x 0.25 mm).

Evaluation of GC Chromatograms

The GC analytical results presented were those obtained using the non polar capillary column (DB-5). The chromatograms were simplified by excluding all constituents with Kovats indices greater than 2200 and those constituents present in 0.5% or less. Following this selection, the peak area percentage were recalculated by standardizing the injection volumes of each analysis using the peak area of the internal standard (tetradecane).

Identification of Juvabione, Dehydrojuvabione and a Pimarane Type of Diterpene

Juvabione and dehydrojuvabione were identified by comparaison of their GC Kovats indices and mass spectra with authentic samples. The pimarane diterpene was isolated from the hexane extract by flash chromatography on silica gel. Six fractions were obtained using an ether-hexane gradient. The second fraction was further purified by HPLC using a Supelco column LC-18 (5 µm, 10 mm x 25 cm), acetonitrile:water (70:30) and a UV detector (214 nm) to give a mixture of two compounds identified as isomers of pimarane or isopimarane diterpene aldehydes based on NMR spectra.

NMR Experiments

Proton and carbon-13 NMR spectra were recorded at 300°K on a Bruker AM400 spectrometer operating at a standard frequency of 400.13 and 100.61 MHz for proton and carbon-13 respectively.

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