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Antioxidant Activity of Knotwood Extractives and Phenolic Compounds of Selected Tree Species

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The antioxidant potency and the radical scavenging capacity of superoxide and peroxyl radicals were assessed for 13 hydrophilic knotwood extracts of commercially important wood species, or fractions thereof, as well as for five pure wood-derived lignans and the flavonoid taxifolin. The chemical composition of the knotwood extracts was determined by gas chromatography combined with mass spectrometry. Most of the investigated wood species were rich in hydrophilic extractives (10−20% of the dry wood) with one or a few compounds dominating in each extract. All extracts had a high antioxidative potency and/or radical scavenging capacity as compared to the well-known antioxidants Trolox and butylated hydroxyanisole. The pure wood-derived lignans and taxifolin also had a high antioxidative potency and/or radical scavenging capacity. However, the antioxidant potency and/or radical scavenging capacity of several of the hydrophilic knotwood extracts were higher than that of the dominating compounds in pure form.

KEYWORDS: Knots; branch base; knotwood; phenolic compounds; lignans; pinosylvins; flavonoids; oligolignans; juvabiones; antioxidant potency; radical scavenging

INTRODUCTION

Knots (i.e., the branch stubs encased in the tree stem) of *Picea abies* and *Pinus sylvestris* contain exceptionally large amounts of bioactive phenolic compounds (*1*, *2*). The amount of extractable phenolic compounds in *P. abies* knot heartwood, or knotwood for short, can be close to 30% (w/w), but is on average around 15% (w/w) (*1*). The amount of phenolic compounds in the knotwood is usually 50−100 times that in the stemwood. In *P. sylvestris* knotwood, the amount of extractable phenolic compounds can be as large as 10%, which is several times more than that in the stemwood (*2*). *Salix caprea* knotwood has also been shown to contain 2−10 times the amount of phenolic compounds found in the stemwood (*3*). In recent research at our laboratory, we have found that knotwood of several other wood species, both softwood and hardwood species, follow the same pattern.

Tree materials such as heartwood, foliage, phloem, bark, and cork of several species have been found to be sources of natural phenolic antioxidants, also including tannins (*4*−*8*). However, the extract yield obtained from such materials is low, and the extracts usually contain a large variety of different phenolic and nonphenolic compounds, both as glycosides and as free aglycones. The degree of glycosylation affects the antioxidant properties of phenolic compounds. For example, the antioxidant activity was found to be lower for quercetin and myricetin glycosides than for their corresponding aglycones (*9*). The hydrophilic compounds in softwood knots contain mainly free aglycones of lignans, oligolignans, stilbenes, and flavonoids (*1*, *2*, *10*). One or a few phenolic compounds dominate in the knotwood extracts of most softwood species. For example, more than half of the hydrophilic extractives of *P. abies* knotwood are lignans, the rest being mainly oligolignans, while the two isomers of hydroxymatairesinol constitute over 70% of the lignans (*1*, *10*). Hydroxymatairesinol, extracted and purified from *P. abies* knotwood, has been found to be a very strong antioxidant in vitro (*11*).

It is possible to separate most of the knotwood from the oversized chip fraction in a pulp mill (*12*). This could be done to utilize the extractives found in the knotwood, and at the same time, increase the pulp quality, because wood knots are detrimental during pulping and papermaking. The phenolic compounds could be extracted (*13*), and if necessary, purified by chromatographic methods. The phenolic compounds in wood knots constitute a valuable resource, with a potential for use as active ingredients in technical antioxidants, biological antioxi-

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dants in foodstuffs, functional foods, pharmaceuticals, and natural biocides such as fungicides, pesticides, bactericides, and other applications.

In this investigation, we have applied three different antioxidant tests to 13 well-defined hydrophilic knotwood extracts of commercially important wood species, as well as to six pure wood-derived phenolic compounds.

MATERIALS AND METHODS

Chemicals. Taxifolin ((±)-3,3′,4′,5,5-pentahydroxyflavanone) was obtained from Sigma-Aldrich Chemie Gmbh. (Steinhem, Germany); Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and butylated hydroxyanisole (BHA) were from Aldrich Chemical Co. (Milwaukee, WI.). All other chemicals used were of the highest quality available.

Wood Material. Knotwood was sampled from full-grown trees of eleven wood species: *Picea abies* (Norway spruce, 71 growth rings at 1.5 m, several knots sampled at 14.5 m heigth), *Betula pendula* (silver birch, 28 growth rings, one knot at 4.2 m), and *Pinus sylvestris* (Scots pine, several knots) grown in Ekenäs, Finland; *Abies sibirica* (Siberian fir, 30 growth rings, one knot at 2.5 m) and *Pinus sibirica* (Siberian stone, 20 growth rings, four knots at 1.1 m) grown in the St. Petersburg region, Russia; *Abies balsamea* (balsam fir, 41 growth rings, one knot at 1.4 m) and *Thuja occidentalis* (northern white-cedar, 144 growth rings, two knots at 1.7 m) grown in Itasca County, Blandin Land, USA; *Pinus cembra* (Siberian stone, several knots), *Pseudotsuga menziesii* (Douglas fir, 55 growth rings, 6 knots at 16 m (tree 1); 54 growth rings, 6 knots at 20 m (tree 2)), and *Larix decidua* (European larch, 62 growth rings, two knots at 15 m) grown in Solböle, Bromarf, Finland; and *Pinus contorta* (lodgepole pine, 22 growth rings, two knots at 1.9 m) grown in Sävar, Sweden. *P. sibirica* and *P. cembra* are essentially the same species. One to six knots from one tree, taken at the same height, were sampled and pooled for each species, except for *P. menziesii*, where two different trees were chosen. All knots were normal and healthy, except the knots of *B. pendula*, which contained a black inner part that was sampled. The heartwood of the knots was splintered, freeze-dried, and ground.

Preparation of Knotwood Extracts. Sequential extraction was carried out in an accelerated solvent extractor apparatus (Dionex Corp., Sunnyvale, CA) according to Willför et al. (*1*). The lipophilic extractives were first extracted with hexane, and thereafter the hydrophilic extractives were ectracted with an acetone/water (95:5 v/v) mixture. The extracts were stored at −18 °C. The gravimetrically determined total amount of hydrophilic extractives, expressed as % of dry wood, was 20% for *Abies balsamea*, 18% for *A. sibirica*, 17% for *Picea abies*, 10% (tree 1) and 15% (tree 2) for *Pseudotsuga menziesii*, 11% for *Thuja occidentalis*, 3% for *Pinus contorta*, 16% for *P. sibirica*, and 20% for *Larix decidua*.

Preparation of *Picea abies* Oligolignans. A *P. abies* knotwood extract was fractionated according to Willför et al. (*10*) on normal-phase silica gel columns using flash chromatography and medium-performance liquid chromatography, giving an extract containing mostly oligolignans with some lignans as the main impurity. The oligolignans consisted mainly of sesquilignans and dilignans. The oligolignans were dried and stored cold.

Preparation of *Araucaria* Extract Fraction. The *Araucaria* extract fraction was the residue after the isolation of secoisolariciresinol from *A. angustifolia* knotwood. The extract fraction was dried and stored cold.

Preparation of *Pinus cembra* Stilbenes. The *P. cembra* stilbenes were prepared from *P. cembra* knotwood by extraction with acetone in a large Soxhlet apparatus. The lipophilic extractives were then removed by refluxing the dry acetone extract with hexane. The residue containing mainly stilbenes was dried and stored cold.

Preparation of *Betula pendula* Extract Fraction. The *B. pendula* extract fraction was prepared from a hydrophilic extract of the black inner part of knotwood by flash chromatography on normal-phase silica gel columns (15 cm × 40 mm i.d.), using dichloromethane/ethanol as eluent. The extract fraction was dried and stored cold.

Preparation of Hydroxymatairesinol. Hydroxymatairesinol was prepared from *P. abies* knotwood by extraction with acetone in a large Soxhlet apparatus. Hydroxymatairesinol was fractionated from the hydrophilic extract by flash chromatography on normal-phase silica gel columns, using dichloromethane/ethanol as eluent.

Preparation of Secoisolariciresinol. Secoisolariciresinol was prepared from *A. angustifolia* knotwood by extraction with acetone in a large Soxhlet apparatus. Secoisolariciresinol was fractionated from the hydrophilic extract by flash chromatography on normal-phase silica gel columns, using dichloromethane/ethanol as eluent. The purified secoisolariciresinol was further recrystallized from dilute 2-propanol.

Preparation of Nortrachelogenin. Nortrachelogenin was prepared from *P. sylvestris* knotwood by extraction first with hexane and then with acetone in a large Soxhlet apparatus. Nortrachelogenin was fractionated from the hydrophilic extract by flash chromatography on normal-phase silica gel columns, using dichloromethane/ethanol as eluent.

Preparation of Lariciresinol. Lariciresinol was prepared from *A. balsamea* knotwood by extraction with acetone in a large Soxhlet apparatus. Lariciresinol was fractionated from the hydrophilic extract by flash chromatography on normal-phase silica gel columns, using dichloromethane/ethanol as eluent. The purified lariciresinol was recrystallized from cyclohexane/ethanol.

Preparation of Matairesinol. Matairesinol was prepared from isolated hydroxymatairesinol according to Eklund et al. (*14*).

Preparation of Extracts and Compounds for Antioxidant Tests and Analysis. The extracts and pure compounds were dissolved in 5−12 mL of ethanol, giving a concentration of 3−26 mg dry extract/mL and then filtered using 0.2-μm syringe filters. Aliquots (300 μL) were taken for chemical characterization.

Analysis by Gas Chromatography (GC) and GC-Mass Spectrometry (MS). Lignans, stilbenes, flavonoids, and juvabiones were analyzed on a 25-m × 0.20-mm i.d., 0.11-μm HP-1 capillary column coated with cross-linked methyl polysiloxane (Agilent Technologies, Palo Alto, CA). The gas chromatograph was a Perkin-Elmer Auto-System XL instrument (Perkin-Elmer, Boston, MA). Column oven, 120 °C − 6 °C/min − 300 °C (10 min); carrier gas, H_2 (20 mL/min); split injector, (1:20) 260 °C; FID detector, 300 °C; injection volume, 1 μL. The ethanol was evaporated and the extractives were silylated by addition of 80 μL of bis-(trimethylsilyl)-trifluoroacetamide, 20 μL of trimethylchlorosilane, and 20 μL of pyridine. The reaction was completed by keeping the test tubes in an oven at 70 °C for 1 h. Heneicosanoic acid and betulinol were used as internal standards. The method used was that according to Ekman and Holmbom (*15*). A correction factor of 1.2 was used for the lignans that were calculated against betulinol (*1*). Oligolignans were quantified on a short 6-m × 0.53-mm i.d., 0.15-μm HP-1 column, using cholesteryl heptadecanoate and 1,3-dipalmitoyl-2-oleyl glycerol as internal standards (*16*). The gas chromatograph was a Varian 3400 instrument (Varian Inc., Palo Alto, CA). Column oven, 100 °C (1.5 min), 12 °C/min − 340 °C (5 min); carrier gas, H_2 (20 mL/min); SPI (septum equipped programmable injector), 80 °C (0.5 min) − 200 °C/min − 340 °C (18 min); FID detector, 340 °C; injection volume, 0.4 μL. Identification of individual components was performed by GC-MS analysis of the silylated components with an HP 6890−5973 GC-quadrupole- MSD instrument. Both a similar 25 m HP-1 GC column as above and a 15-m × 0.25-mm i.d., 0.1-μm MXT-65TG column (Restek Corp., USA), which allowed elution of the silylated oligolignans (*10*), were used.

Estimation of Antioxidant Potency. The antioxidant properties of the extracts and the pure compounds were estimated by their potency to inhibit *tert*-butylhydroperoxide (*t*-BuOOH) induced lipid peroxidation in rat liver microsomes in vitro (*17*). The lipid peroxidation was detected by luminol-enhanced chemiluminescence. Test compounds or extracts were added to incubation mixtures in a small volume (2% of incubation volume), and the lipid peroxidation potency was compared to that of the vehicle (ethanol). Assays for the t-BuOOH-initiated lipid peroxidation was performed as follows. The buffer (50 mM sodium carbonate, pH 10.2, with 0.1 mM EDTA) was pipetted in a volume of 0.8 mL in the luminometer cuvette. 20 μL of diluted rat liver microsomes (final concentration 1.5 μg protein/mL) was added, followed by 6 μL of luminol (0.5 mg/mL) and test chemicals. The reaction was initiated by

Table 1. Main Component Groups and Compounds in the Hydrophilic Knotwood Extracts and Fractions of Extracts

wood species	composition[a] (% of gravimetric extract)		wood species	composition[a] (% of gravimetric extract)	
Abies balsamea	lignans	33	*Pinus contorta*	lignans	10
	secoisolariciresinol	18		nortrachelogenin	5
	lariciresinol	9		liovil	3
	oligolignans	19		oligomers	3
	juvabiones	2		flavonoids	20
				pinocembrin	15
Abies sibirica	lignans	33		pinobanksin	7
	secoisolariciresinol	21		stilbenes	15
	lariciresinol	7		pinosylvin monomethyl ether	9
	oligolignans	31		pinosylvin	6
	juvabiones	3			
			Pinus sibirica	lignans	26
Picea abies	lignans	53		lariciresinol	19
	hydroxymatairesinol	41		isolariciresinol	3
	secoisolariciresinol	3		secoisolariciresinol	2
	α-conidendrin	3		oligolignans	6
	oligolignans	12		flavonoids	7
				pinocembrin	6
Pseudotsuga menziesii 1[b]	lignans	9		stilbenes	46
	nortrachelogenin	4		pinosylvin monomethyl ether	25
	lariciresinol	2		dihydropinosylvin monomethyl ether	15
	oligomers	6		pinosylvin	3
	flavonoids	42		dihydropinosylvin	2
	taxifolin	41			
	juvabiones	3	*Larix decidua*	lignans	40
				secoisolariciresinol	24
Pseudotsuga menziesii 2[b]	lignans	45		lariciresinol	7
	isolariciresinol	31		isolariciresinol	6
	secoisolariciresinol	9		oligolignans	18
	oligomers	7		flavonoids	17
	flavonoids	1		taxifolin	14
	taxifolin	1		dihydrokaempferol	3
Thuja occidentalis	thujalignans[c]	25	*Pinus cembra* stilbenes	pinosylvins	68
	oligomers	2		pinosylvin monomethyl ether	43
				dihydropinosylvin monomethyl ether	24
Araucaria extract fraction	lignans	48			
	dimethyl pinoresinol	15	*Picea abies* oligolignans	oligolignans	70
	lariciresinol	15		lignans	16
	pinoresinol	9		liovil + lignan a	15
	monomethyl pinoresinol	3			
	hinokiresinol (norlignan)	3			
	monomethyl lariciresinol	2			
	dimethyl lariciresinol	1			

[a] Compound groups and major compounds present in 1% or more. [b] Two different trees. [c] Several unidentified lignans, probably related to thujaplicatin and 4-O-demethylyatein + small amounts of matairesinol and lignan A.

0.05 mL of 0.9 mM t-BuOOH at 33 °C. The chemiluminescence was measured for 45 min at 1 min cycles. The tests were repeated two to four times on separate days. Variation between assays was <10%.

Estimation of Free Radical Trapping Capacity. The capacity of the extracts and the pure compounds to trap superoxide (oxygen radical) and peroxyl radicals was estimated by chemiluminescence-based methodology (*17*). Superoxide anions were produced by xanthine−xanthine oxidase system as follows: 20 μL of xanthine oxidase (420 mU/mL), 0.02 mL of 5 mM lucigenin, 0.02 mL of 200 mM linoleic acid dissolved in 50 mM KOH, 0.78 mL of 50 mM potassium phosphate buffer, pH 10.0, and test samples were pipetted in cuvettes. The reaction was initiated by the automated dispensing of 0.11 mL of 1.45 mM xanthine (final volume 1.0 mL). Chemiluminescence in duplicate samples at 35 °C was measured for 6 min in 1-min cycles. Peroxyl radicals were generated by thermal decomposition of 2,2′-azobis(2-amidinopropane)-hydrochloride as follows: 0.45 mL of 0.1 M sodium phosphate buffer, pH 7.4, containing 0.9% NaCl; 0.02 mL of 120 mM linoleic acid; 0.05 mL of luminol (0.5 mg/mL); and test compounds were mixed in the cuvette. The assay was initiated by 0.05 mL of ABAP (83 mg/mL). Chemiluminescence in triplicate cuvettes at 37 °C was measured until a peak value for each sample was detected. The half-peak time point defined the peroxyl radical trapping capacity.

RESULTS AND DISCUSSION

Total Amount of Hydrophilic Extractives. The total amount of hydrophilic knotwood extractives was considerable, being 10−20% of the dry wood, from most of the investigated wood species. The amount was smaller from the *Pinus contorta* knotwood, being only 3%. It should be kept in mind that these numbers represent only a few knots in a tree. However, it has been found that knotwood in general contains exceptionally large amounts of hydrophilic extractives, even though the natural variation between different knots and trees can be large (*1*−*3*).

Chemical Composition of the Hydrophilic Extracts and Fractions. Lignans and oligolignans (mainly sesquilignans and dilignans) were the main compounds in the *Abies*, *Picea*, *and Larix* extracts and in the *Araucaria* extract fraction, constituting at least half of the extracts (**Table 1**). The lignans also dominated

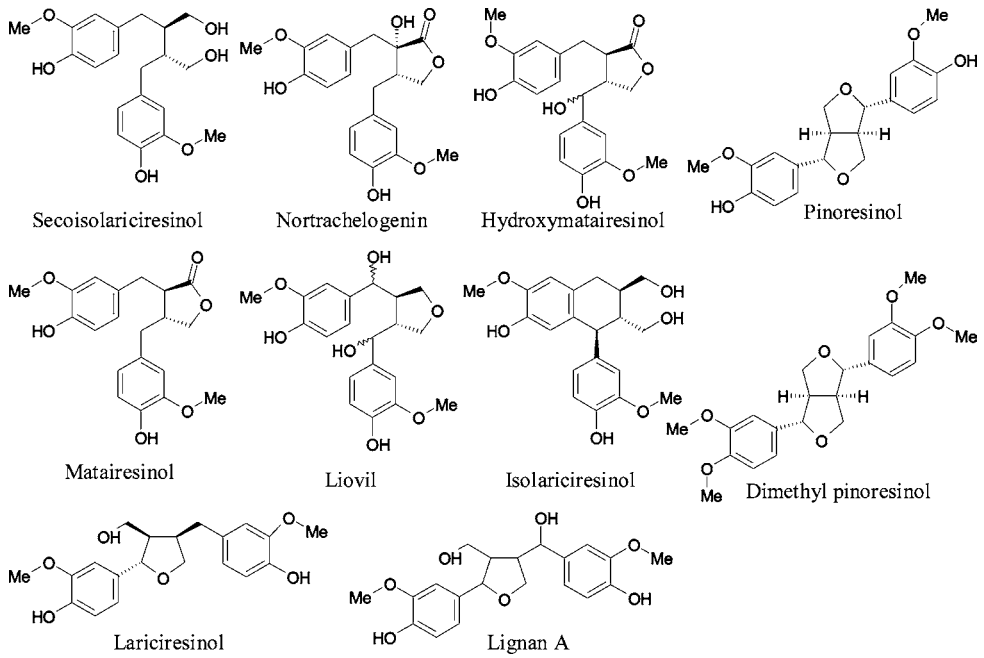

Figure 1. Structures of the isolated and synthesized lignans and of the predominant lignans found in the knotwood extracts.

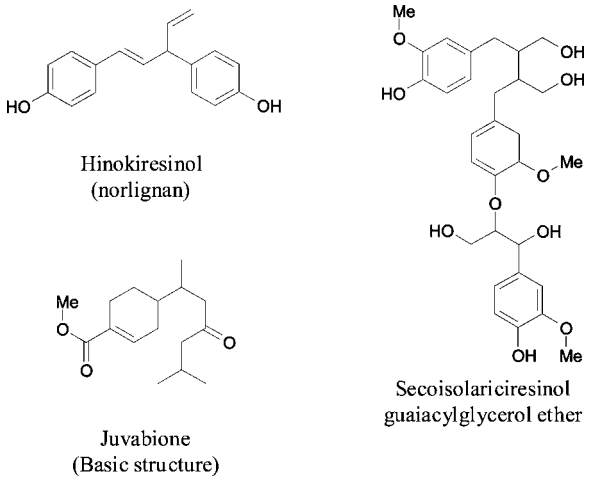

Figure 2. Structures of the norlignan hinokiresinol, as well as an example of an oligolignan (sesquilignan) and the basic structure of the juvabiones found in the knotwood extracts.

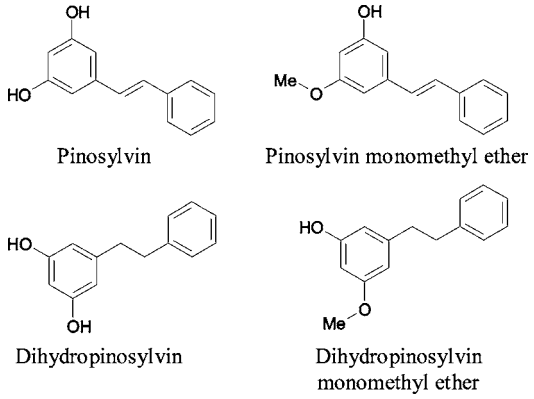

Figure 3. Structures of the predominant stilbenes found in the knotwood extracts.

in one of the two *Pseudotsuga* extracts, and the *Thuja* extract, while flavonoids or stilbenes dominated in the other extracts. Only the *Pinus* extracts contained stilbenes.

A few lignans dominated in all extracts (**Table 1**, **Figure 1**). The *Picea abies* knotwood contained mainly hydroxymatairesinol, about 7% (w/w), while the *Abies* and *Larix* knotwood contained much secoisolariciresinol. The main lignans in *Thuja occidentalis* knotwood were slightly different, because they have additional methoxyl and hydroxyl groups in their structure (*18*), compared to the lignans in the other extracts. The oligolignans were a group of compounds consisting mainly of guaiacylglycerol ethers of the main lignans in the *Abies*, *Picea*, *Larix*, and *Pinus sibirica* extracts. An example of an oligolignan is shown in **Figure 2**. Such compounds have recently been characterized in *Picea abies* and *Pinus sylvestris* knotwood extracts (*1*, *2*, *10*). However, no such compounds were detected in the *Pseudotsuga* or *Pinus contorta* extracts, even though the GC elution time suggested the presence of oligomeric substances in the extracts. The *Thuja* oligomers were not identified either, but these were probably oligolignans derived from the thujalignans. The oligomeric substances should be further characterized in these extracts.

A few pinosylvins dominated among the stilbenes in the *Pinus* extracts and in the *P. cembra* fraction (**Table 1**, **Figure 3**). Pinosylvin monomethyl ether and dihydropinosylvin monomethyl ether were the most abundant stilbenes. Taxifolin and pinocembrin, both representing the flavanone type of compounds, were the main flavonoids (**Table 1**, **Figure 4**). Some juvabiones were present in the *Abies* extracts and in one of the *Pseudotsuga* extracts (**Table 1**, **Figure 2**). The *Araucaria* extract fraction also contained a norlignan, hinokiresinol, which was not found in any other extract. The *Betula pendula* extract fraction contained stilbene-derived compounds. However, the exact structure of this compound group is still to be determined. No substantial amounts of polymeric compounds were present in the extracts.

Composition of the Isolated and Synthesized Phenolic Compounds. Two batches of the lignans hydroxymatairesinol, lariciresinol, secoisolariciresinol, and nortrachelogenin were isolated, while one batch of matairesinol was synthesized. The chemical composition of the isolated or synthesized lignans, as well as of the purchased taxifolin, was determined by GC analysis. The GC-purity of the compounds was over 95%, except for the second batch of secoisolariciresinol, which had a GC-

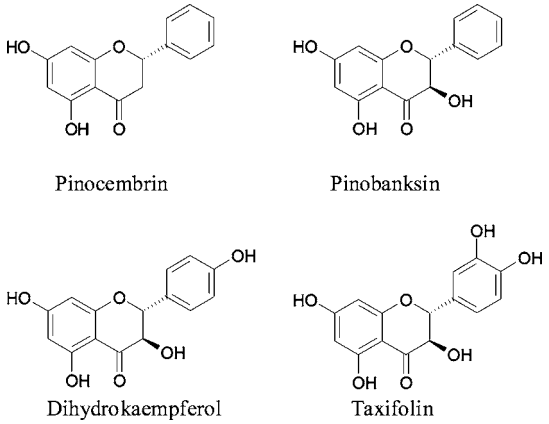

Figure 4. Structures of the predominant flavonoids found in the knotwood extracts.

purity of about 92%. The main impurity, constituting about 4% of the extract, was monomethyl secoisolariciresinol. The isolated hydroxymatairesinol consisted of two epimers, *7S,8R,8′R*-hydroxymatairesinol (92%) and *7R,8R,8′R-allo*-hydroxymatairesinol (8%). The structure of these epimers was recently unambiguously proven (*19*).

Inhibition of Lipid Peroxidation in Vitro. The antioxidative potency of the extracts was estimated on the basis of its potency to inhibit t-BuOOH-induced lipid peroxidation in rat liver microsomes in vitro. Eleven of the 13 tested knotwood extracts had a higher antioxidative potency than the synthetic antioxidant BHA (IC_{50} 198 μg/L), while only the *Thuja* and one of the *Pseudotsuga* extracts had a lower antioxidative potency (**Table 2**). The *Pinus contorta* knotwood extract especially, but also the two *Abies* and one of the *Pseudotsuga* extracts, had an antioxidative potency similar to that of Trolox (IC_{50} 5.0 μg/L). The isolated lignans and taxifolin all had a high antioxidative potency similar to that of Trolox and higher than that of BHA. The antioxidant potency of hydroxymatairesinol was well in the range of the earlier work of Saarinen et al. (*11*). The differences in the obtained antioxidative potency between the lignans in series 1 and 2 are probably due to the facts that the assays were done on two different batches of rat liver microsomes and that the time between the assays was about 6 months.

The high antioxidant potency of some of the knotwood extracts may indicate synergistic effects between the different phenolic compounds present, because the antioxidant potency of several of the knotwood extracts was higher than that of the predominant pure compounds (**Table 2**). However, it is possible that small amounts of nonanalyzed and nonidentified compounds contributed much to the antioxidant potency. The *Pinus contorta* knotwood extract contained the flavanone-type compounds pinocembrin and pinobanksin, as well as pinosylvin monomethyl ether and pinosylvin, as the main compounds (**Table 1**). Flavanone type compounds, such as taxifolin, and pinosylvin and related compounds have been shown to be good inhibitors of lipid peroxidation in vitro (*20*−*22*). The main compound in the *Pseudotsuga menziesii* 1 extract was taxifolin, while the *Abies* extracts contained the lignans secoisolariciresinol and lariciresinol and some oligolignans. These three extracts were also the only ones that contained juvabiones. Even though the amounts of juvabiones were small, it cannot be ruled out that these compounds contributed much to the antioxidant potency. It is interesting to note the relatively low antioxidant potency of the *Pinus cembra* stilbenes and the *P. sibirica* extract (**Table 2**). This indicates that the pinosylvin type compounds were not effective inhibitors of lipid peroxidation in this specific test, even though this type of compounds has been shown earlier to be good a inhibitor of lipid peroxidation in vitro (*23*). Neither the thujalignan type compounds nor the lignan isolariciresinol, dominating the *Thuja occidentalis* and *Pseudotsuga menziesii* 2 extracts, respectively, were good inhibitors of lipid peroxidation in this test.

Table 2. Inhibition of Lipid Peroxidation in Vitro, Expressed as IC_{50} Values (i.e., Concentration Extract that Inhibits Lipid Peroxidation by 50%), by the Knotwood Extracts, Fractions, Two Different Series of Lignans, Taxifolin, and Reference Compounds

sample	IC_{50} (μg/L)	IC_{50} (μM)
Trolox (reference)	5.0	
Pinus contorta	8.1	
Abies sibirica	16	
Abies balsamea	18	
Pseudotsuga menziesii 1	18	
Betula pendula extract fraction	32	
Picea abies	45	
Picea abies oligolignans	52	
Larix decidua	57	
Araucaria extract fraction	97	
Pinus cembra stilbenes	132	
Pinus sibirica	132	
BHA (reference)	198	
Pseudotsuga menziesii 2	219	
Thuja occidentalis	447	
series 1[a]		
Trolox (reference)	5.0	0.02
Secoisolariciresinol	37	0.10
Taxifolin	46	0.15
Nortrachelogenin	53	0.14
Hydroxymatairesinol	58	0.15
Matairesinol	99	0.28
Lariciresinol	126	0.35
BHA (reference)	198	1.1
series 2[a]		
Trolox (reference)	46	0.18
Secoisolariciresinol	54	0.15
Lariciresinol	63	0.17
Hydroxymatairesinol	67	0.18
Nortrachelogenin	70	0.19

[a] Different test series.

Scavenging of Superoxide Radicals in Vitro. The capacity of the extracts and the pure compounds to scavenge superoxide radicals was estimated by chemiluminescence-based methodology. All extracts, except that of *Pinus sibirica* and the *Araucaria* extract fraction, were at least fairly good scavengers of superoxide radicals in this test (**Table 3**). The *Pinus cembra* stilbenes (IC_{50} 0.84 μg/L) and the *Picea abies* oligolignans (IC_{50} 0.93 μg/L) were even more effective scavengers of superoxide radicals than both BHA (IC_{50} 2.7 μg/L) and Trolox (IC_{50} 6.3 μg/L). Of the pure compounds, taxifolin, nortrachelogenin, and secoisolariciresinol were more effective scavengers than both BHA and Trolox. Only hydroxymatairesinol (IC_{50} 81 μg/L) showed a very ineffective scavenging of the superoxide radicals.

The synergistic effect that was suggested for the inhibition of the lipid peroxidation was not observed for the scavenging of superoxide radicals. On the contrary, pure compounds and fractions containing mainly stilbene and oligolignan type compounds were the most effective scavengers (**Table 3**). However, the scavenging capacity of the *Pinus sibirica* extract was lower than expected (IC_{50} 171 μg/L), considering that the main compounds in the extract were stilbenes similar to the *Pinus cembra* stilbenes. The large difference in the superoxide radical scavenging capacity of the lignans hydroxymatairesinol

Table 3. Scavenging of Superoxide Radicals in Vitro, Expressed as IC_{50} Values (i.e., Concentration Extract Required for Scavenging of 50% of the Radicals), by the Knotwood Extracts, Fractions, Lignans, Taxifolin, and Reference Compounds

sample	IC_{50} (μg/L)	IC_{50} (nM)
Pinus cembra stilbenes	0.84	
Picea abies oligolignans	0.93	
BHA (reference)	2.7	
Trolox (reference)	6.3	
Abies sibirica	15	
Pseudotsuga menziesii	22	
Picea abies	23	
Pseudotsuga menziesii	31	
Thuja occidentalis	33	
Betula pendula extract fraction	34	
Larix decidua	35	
Pinus contorta	51	
Abies balsamea	57	
Araucaria extract fraction	74	
Pinus sibirica	171	
series 1		
Taxifolin	0.16	0.51
Nortrachelogenin	0.53	1.4
soisolariciresinol	1.8	4.8
BHA (reference)	2.7	15
Trolox (reference)	6.3	25
Lariciresinol	13	35
Matairesinol	14	40
Hydroxymatairesinol	81	217

and nortrachelogenin and matairesinol is also surprising, because the structures of these compounds are quite similar (**Figure 1**).

Scavenging of Peroxyl Radicals in Vitro. The capacity of the extracts and the pure compounds to scavenge peroxyl radicals was estimated by chemiluminescence-based methodology. The *Pinus contorta* knotwood extract was an effective scavenger of peroxyl radicals (trapping capacity 47 mmol/g) compared to Trolox (trapping capacity 8.0 mmol/g), while the capacity of most of the other extracts was similar to that of Trolox (**Table 4**). Only the *Thuja occidentalis* and the *Pseudotsuga menziesii* 2 extracts had a low peroxyl scavenging capacity (trapping capacity 2.4 mmol/g and 1.1 mmol/g, respectively). Of the pure compounds, taxifolin was the most effective scavenger of peroxyl radicals (stoichiometric factor 4.7 mole/mole), while also secoisolariciresinol was an effective scavenger (stoichiometric factor 3.1−4.0 mole/mole). The effect of the other lignans was similar to that of Trolox (stoichiometric factor 1.7−2.0 mole/mole).

The relative effect of the different extracts in the peroxyl radical scavenging test was similar to the relative potency to inhibit t-BuOOH induced lipid peroxidation, with the *Pinus contorta* extract as the most effective one in both tests (**Tables 2** and **4**). The *Pinus cembra* stilbenes and the stilbene-rich *Pinus sibirica* extract had a slightly lower scavenging capacity compared to Trolox (**Table 4**). The pinosylvin-related stilbenes resveratrol and pterostilbene have been reported to be more effective peroxyl radical scavengers than Trolox (*23*).

The present study showed that the hydrophilic extracts of knotwood of selected industrially important softwood and hardwood species, or fractions of the same, have a high antioxidative potency and/or radical scavenging capacity compared to the well-known antioxidant Trolox and to the synthetic antioxidant BHA. It was also shown that pure wood-derived lignans and the flavonoid taxifolin had a high antioxidative potency and/or radical scavenging capacity. The lignans have not yet been recognized as natural compounds with antioxidant properties. The hydrophilic knotwood extracts were also quite

Table 4. Scavenging of Peroxyl Radicals in Vitro, Expressed as the Trapping Capacity (i.e., mmoles Peroxyl Radicals Scavenged per Gram of Extract) and as the Stoichiometric Factor (i.e., mmoles Peroxyl Radicals Scavenged per Mole of Compound), by the Knotwood Extracts, Fractions, Different Series of Lignans, Taxifolin, and Reference Compounds

sample	trapping capacity (mmol/g)	stoichiometric factor (mole/mole)
Pinus contorta	47	
Abies sibirica	15	
Pseudotsuga menziesii 1	12	
Abies balsamea	9.6	
Betula pendula extract fraction	8.2	
Trolox (reference)	8.0	
Larix decidua	6.4	
Araucaria extract fraction	5.9	
Picea abies	4.8	
Pinus cembra stilbenes	4.2	
Picea abies oligolignans	4.2	
Pinus sibirica	3.2	
Thuja occidentalis	2.4	
Pseudotsuga menziesii 2	1.1	
series 1[a]		
Taxifolin	16	4.7
Secoisolariciresinol	8.5	3.1
Trolox (reference)	8.0	2.0
Nortrachelogenin	5.9	2.2
Hydroxymatairesinol	5.6	2.1
Matairesinol	2.9	1.0
Lariciresinol	2.7	1.0
series 2[a]		
Secoisolariciresinol	11	4.0
Hydroxymatairesinol	7.3	2.7
Lariciresinol	7.3	2.6
Trolox (reference)	6.8	1.7
Nortrachelogenin	5.3	2.0

[a] Different test series.

pure in the sense that only a few compounds, belonging to the groups of lignans, oligolignans, pinosylvins, or flavonoids, strongly dominated in each extract. The extracts may have synergistic effects, because the antioxidant potency and/or radical scavenging capacity of several of the knotwood extracts were higher than that of the predominant compounds in these extracts. However, it cannot be ruled out that nonanalyzed compounds present in small amounts contributed much to the antioxidative potency and radical scavenging capacity. Knotwood is available on a large scale in pulp mills in the oversized chip fraction (*12*), and the hydrophilic extracts can be obtained from this knotwood by simple extraction (*13*). The next step in the evaluation of the potentially valuable knotwood extracts is to perform application tests with the extracts or purified substances as active ingredients in, for example, technical antioxidants, functional foods, pharmaceuticals, natural biocides, and wood preservatives. In conclusion, knots in trees have not previously been recognized as a rich source of natural antioxidants, even though knots may be the richest source in all of nature.

ABBREVIATIONS USED

BHA, butylated hydroxyanisole; ASE, accelerated solvent extractor, t-BuOOH, *tert*-butylhydroperoxide

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