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Phenolic extractives in *Salix caprea* wood and knots

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Abstract

Salix caprea stemwood and knots were found to contain the phenolic extractives vanillic acid, 3-*p*-coumaryl alcohol, coniferyl alcohol, sinapylaldehyde, dihydrokaempferol, catechin, naringenin, gallocatechin, dihydromyrcetin and taxifolin. The knots contained larger quantities of flavonoids than did stemwood of the same tree.

Keywords: Salicaceae; *Salix caprea*; Wood; Knots; Phenolic extractives

1. Introduction

A knot is the part of a branch that is embedded in the tree stem. When a branch is broken close to the stem, an open wound is formed, making the knot susceptible to attack by fungi and other micro-organisms.

In recent studies, knots of softwoods (*Picea abies* and *Pinus sylvestris*) have been found to contain exceptionally large amounts of various phenolic substances, especially lignans and stilbenes (Willför et al., 2003a,b; Ekman et al., 2002). The concentration of lignans in spruce knots can be 100–500 times higher than in the stemwood (Willför et al., 2003a). Lignans have antimicrobial and antioxidative properties and they are involved in the defence against fungi and other micro-organisms. However, no studies have so far been reported on extractives in the knots of any hardwoods.

Salix caprea is a common tree in the agricultural landscape of northern Europe and has been used as a wind shield bush growing in ditches between fields. The economic value of *S. caprea* is however small. As a fast-growing tree it may be of interest for bioenergy production.

Willows are one of the most taxonomically diverse plant genera in the Northern Hemisphere (Argus, 1997). The extractives in bark, wood, leaves and pollen of different *Salix* species have been studied to some extent.

According to Lönnberg (1975), gravimetrical amounts of hydrophilic extractives in *S. caprea* stemwood vary between 2.0 and 3.1 mg g^{-1} and the amounts of lipophilic extractives between 1.5 and 1.7 mg g^{-1}. Larger amounts were found in young stems than in older ones.

Flavonoids present in *S. caprea* wood were reported to have antifungal properties (Malterud et al., 1985). Of six identified flavonoids [dihydrokaempferide, naringenin, aromadendrin, taxifolin, prunin and (+)-catechin], naringenin was found to be the most effective one against both fungi and microbes. Naringenin-5-glucoside has been found in the bark of *Salix*-species (Rowe, 1989).

Catechin is a common constituent in plants and trees (Rowe, 1989). It has been shown to have antioxidant (Choi et al., 2001), as well as antifungal and antimicrobial (Mantani et al., 2001) properties.

Astralgin, quercimeritrin and quercitin-3,7-di-*O*-glucoside were found in pollen of *S. caprea* (Gorobets et al., 1982), while salicin, saligenin, (±)-gallocatechin, rutin, cynaroside, quercitin and luteolin were found in the leaves (Sagareishvili et al., 1990).

Here we present the results from analysis of the hydrophilic extractives of stemwood and knots from two *S. caprea* trees.

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2. Results and discussion

The heartwood samples of *S. caprea* stemwood and knotwood contained 5–20 times more hydrophilic extractives, as determined by GC, than did the sapwood samples (Table 1). The concentrations were 2–10 times higher in the knots than in the stemwood. Red coloured wood present in two of the four knots was found to contain similar concentrations of extractives as the heartwood of the dead knots. The most abundant component in both stemwood and knots was catechin. Different flavonoid glycosides were also present in large amounts.

Catechin dominated in the extracts with concentrations of 16–24 mg g^{-1} o.d. wood. The amount of catechin was larger in the knots than in the stemwood. Catechin is a well-known flavonoid with antioxidative properties. Other flavonoids found were naringenin, naringenin-enol, dihydrokaempferol, gallocatechin, taxifolin and dihydromyrcetin. A glycoside of dihydrokaempferol was also identified. All aglycones of the identified phenolic compounds are presented in Fig. 1.

Small amounts of the simple phenols coniferyl alcohol, vanillic acid, 3-*p*-coumarylalcohol and sinapylaldehyde were found in the extracts. Their concentrations were lower than 1.1 mg g^{-1} o.d. wood. The amounts of both low-molar-mass phenolic extractives, as well as fatty acids, such as linoleic acid, increased and some new phenolic compounds were found after alkaline hydrolysis of the acetone extracts. The dominating low-molar-mass phenolic compound was *p*-salicylic acid with a concentration of ca. 2 mg g^{-1} in the heartwood of knots. Small amounts of vanillin, syringylaldehyde, 4-hydroxycinnamic acid and ferulic acid were also observed. These phenolic compounds may also occur esterified with fatty alcohols (Paasonen, 1967) or as glycosides (Fengel and Wegener, 1983). The linoleic acid may have been esterified to phenolics prior to hydrolysis. Pearl et al. (1959) found *p*-hydroxybenzoic, vanillic, syringic and ferulic acid, as well as vanillin and syringaldehyde in the stemwood of three *Salix* species: *S. nigra*, *S. babylonica* and *S. eriocephala*. Recently we found vanillic acid, coumaryl alcohol, coniferyl alcohol and sinapyl alcohol in knots and stemwood of *S. viminalis* (Pohjamo et al., 2002).

The heartwood of knots contained flavonoid glycosides with total concentrations of 17–39 mg g^{-1}. These were also present in the sapwood of the knots, although their total amount varied there between 1.6 and 2.8 mg g^{-1}. More studies are needed to identify these glycosides.

No lignans were detected in the *S. caprea* extracts. Lee et al. (1993, 1994) identified syringaresinol and a lignan ester, as well as three neolignan-related compounds in *S. sachalinensis*. Pohjamo et al. (2002) identified liovil, hydroxymatairesinol and lariciresinol in

Table 1
Phenolic compounds in *Salix caprea* acetone extracts analyzed by GC[a]

	Tree A							Tree B					
	Stem		Living knot[b]		Dead knot[c]			Stem		Living knot			Dead knot[i]
	HW[d]	SW[e]	HW	SW	HW	SW	RW[f]	HW	SW	HW	SW	RW	
Vanillic acid	–[g]	–	0.1	–	0.1	–	0.1	0.1	–	–	–	–	–
3-*p*-Coumaryl alcohol	–	0.1	–	0.1	–	0.1	–	–	0.3	–	0.1	–	–
Coniferyl alcohol	0.1	0.4	0.3	0.7	0.2	0.6	0.3	0.3	–	0.4	1.1	0.3	0.2
Sinapylaldehyde	–	–	–	–	–	–	–	–	0.1	–	0.1	–	–
Catechin	2.2	0.4	21	3.7	16	1.9	12	15	0.7	24	3.1	8.8	13
Gallocatechin	–	–	1.2	–	0.7	–	0.3	0.9	–	2.3	0.1	0.3	1.4
Naringenin[h]	–	–	0.5	–	0.7	–	0.5	0.8	–	1.1	–	0.9	1.5
Dihydrokaempferol	0.1	–	0.9	–	2.1	–	0.9	1.0	–	1.0	–	0.7	1.6
Taxifolin	–	0.1	0.4	0.4	0.2	0.2	0.2	0.2	–	0.3	0.4	0.5	0.2
Dihydromyrcetin	–	–	1.5	–	1.2	–	0.4	0.5	–	2.0	–	0.2	1.2
Unknown compounds (5 peaks)	–	–	1.3	0.3	2.0	0.1	1.2	1.5	–	1.7	0.5	1.5	2.3
Glycoside of dihydrokaempferol	–	–	0.7	–	0.5	–	0.2	0.3	–	0.6	–	0.2	0.1
Total glycosides (short column)	2.3	0.5	26	2.8	17	1.6	7.9	11	1.2	39	2.4	11	20

[a] All amounts given in mg g^{-1} of dry wood.
[b] A knot with a living branch attached.
[c] A knot with a living branch attached.
[d] Heartwood.
[e] Sapwood.
[f] Red-colored wood.
[g] <0.1 mg/g.
[h] Incl. naringenin-enol.
[i] No sapwood observed.

S. viminalis. This suggests a large diversity of phenolic extractives in the *Salix* plant genera.

No polymeric material was detected by HPSEC for any of the *S. caprea* extracts.

The difference in concentrations of phenolic extractives in knots and stemwood is evident in *S. caprea*. The large variations in the concentrations in stemwood and knotwood samples between trees cannot be explained by environmental factors, because the sample trees grew at the same site. Also the fact that the two trees are of different age can not give rise to this phenomenon because both trees were full-grown and in the middle of their life cycle. Evidently there are natural variations between knots. Similar large variations for phenolic extractives were observed in knots of the softwood species *P. abies* (Willför et al., 2003a) and *P. sylvestris* (Willför et al., 2003b; Erdtman et al., 1951).

The reason for the difference in concentrations between stem and knots is still unclear. A plausible reason is that trees need antimicrobial substances in the knots to prevent microbes from invading the stem through open wounds that are formed when a branch is broken, e.g., because of mechanical stress caused by snow, wind, etc. The stemwood requires protection because the fibers of the tension wood in hardwoods contain a gelatinous layer that can easily be degraded by brown-rot fungi (Zenker, 1962). It has also been suggested that phenolic extractives can be formed as a response to external stress (Ekman, 1979; Hart, 1981), which certainly is the situation at the base of a branch.

3. Experimental

3.1. General experimental procedures

Sequential extraction was carried out in an ASE-apparatus (ASE 200 Accelerated Solvent Extractor, Dionex Inc.), first with hexane and thereafter with acetone:water (95:5). Qualitative analysis was performed with a HP6890 Series GC using a HP5973 mass selective detector.

Quantitative analysis of individual separate phenolic components was performed with a Perkin-Elmer Auto-System XL gas chromatograph equipped with a HP-1 column (25 m×0.20 mm) and a FID detector. The temperatures for the injector and the detector were 260 °C and 290 °C, respectively. The volume of the split injection (20:1) was 1 μl. The carrier gas (hydrogen) flow was 1 ml min^{-1}.

Analysis of component groups was performed with a Varian 3400 gas chromatograph equipped with a short HP-1 column, (6 m×0.53 mm) and a FID detector. The temperatures for the injector and the detector were 80–340 °C and 340 °C, respectively. The injection was on-column type and the injection volume was 0.4 μl. The carrier gas (hydrogen) flow was 18 ml min^{-1}.

The distribution of the hydrophilic substances according to molecular size in the acetone extracts was studied by high-pressure size exclusion chromatography (HPSEC). The system was as follows: TSK G3000, TSK G2500, and TSK G1500HXL columns and a guard column and a Pharmacia LKB 2142 differential refractometric detector. The solvent (THF) flow was 1 ml min^{-1} and the injection volume 100 μl.

3.2. Wood material

The two healthy trees of *S. caprea* were felled in August 2001 in Houtskär in the SW archipelago of Finland. Wood samples were sawn out immediately after felling and put into storage at −24 °C within 1 h.

S. caprea A was 25 years old and *S. caprea* B 20 years old (Table 2), counted as annual rings at the height of 1.5 m. Stemwood was sampled at 1.5 m height. One living and one dead knot were taken from each tree. The living knots had a living branch, whereas the branch of the dead knots had fallen off. The knot material was easily distinguished from the normal wood material as a change in the annual ring direction.

Heartwood was present in both trees. One of the dead and one of the living knots contained strongly red-coloured wood material, which was separated from the normal knotwood and analyzed separately.

Segments of wood were cut out from each stem disk. The sapwood and heartwood parts were separated in both knots and stemwood except for the *S. caprea* B dead knot, where sapwood was not observed. All samples were splintered manually and then freeze-dried. The dried wood samples were ground in a CycloTec mill,

Table 2
Samples of *Salix caprea*

	Tree A				Tree B			
	Height (cm)	Diameter (cm)	Angle		Height (cm)	Diameter (cm)	Angle	
Stem	140	10			150	9.5		
Living knot	310		70°	Red part	220		28°	
Dead knot	150				120			Red part

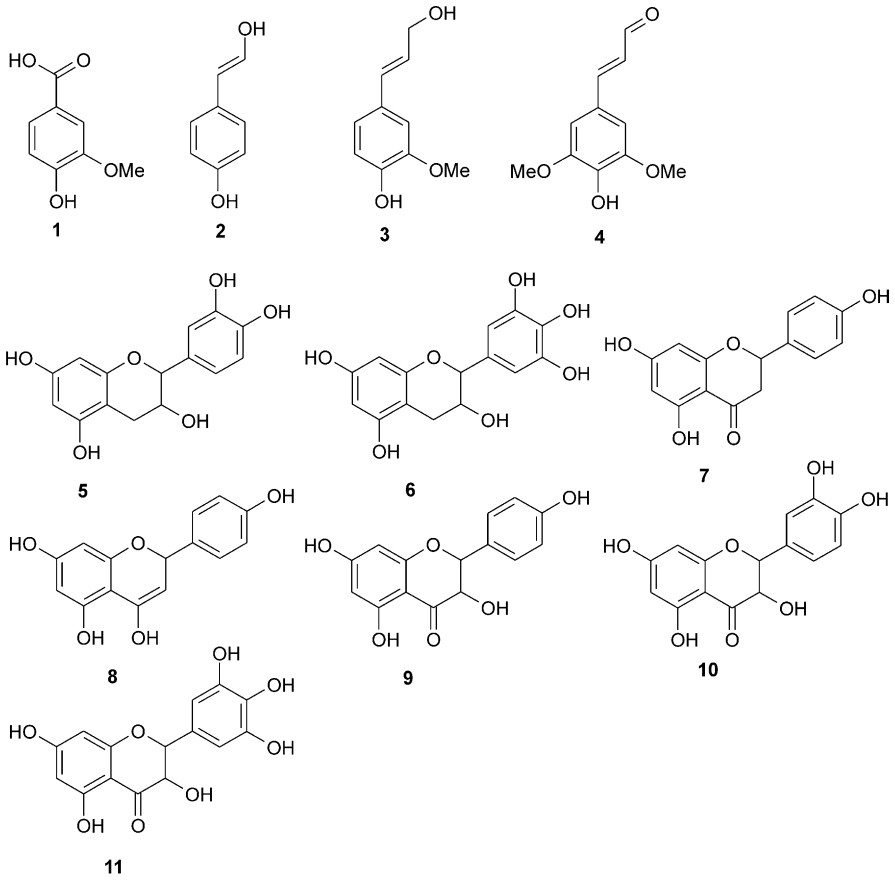

Fig. 1. Phenolic compounds identified in *Salix caprea*: **1** vanillic acid, **2** 3-*p*-coumaryl alcohol, **3** coniferyl alcohol, **4** sinapylaldehyde, **5** (+)-catechin, **6** gallocatechin, **7** naringenin, **8** naringenin-enol, **9** dihydrokaempferol, **10** taxifolin, **11** dihydromyrcetin.

producing particles passing a 10-mesh screen. A second freeze-drying step after the milling ensured almost complete removal of the volatile compounds, including some phenols with low boiling point.

3.3. Extraction and analysis

Sequential extraction of lipophilic and hydrophilic extractives was performed as described by Willför et al. (2003a).

The hydrophilic acetone extracts were analyzed by gas chromatography as described by Örså and Holmbom (1994), and by HPSEC (Willför et al., 2002a,b).

Esterified phenols and fatty acids were analyzed after alkaline hydrolysis using a 0.5 M KOH solution in 90% EtOH. The solutions were kept at 70 °C for 5 h. After this, distilled water and a drop of bromocresol green was added. The solution was acidified to pH ca. 3 with a 30% phosphoric acid solution. The acid and neutral components were extracted with three portions of MTBE. The organic fractions were combined, the solvent evaporated and the extracts silylated.

Acid hydrolysis was carried out as described by Harborne (1965). The extracts were analyzed by GC after silylation.

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