Preliminary

The Role of Extractives Involved in the Natural Durability of Domestic Softwood*[1]

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It is generally known that natural durability of heartwood is different among wood species. It has been explained mainly by the presence of antifungal extractives in durable wood and many researchers have isolated and identified the relevant extractives. However, all isolated extractives did not always exhibit the high antifungal activity in vitro against test fungi, and the involvement of some other factors such as synergistic or additive effect of few substances and/or their hydrophobic effect to inhibit the fungal growth in heartwood[1)]. Also, Rudman reported that heartwood extractives from durable species were not always toxic against broad spectrum of wood-decay fungi but rather specific to limited species[2)]. In this study, growth-inhibiting effect of extractives, water uptake of wood after extraction, decay resistance and behavior of fungal hyphae in wood after extraction were investigated in several parts from outer sapwood to inner heartwood, using sugi and hinoki as typical domestic softwood species.

Test specimens, 10 mm $(T)\times 25$ mm $(R)\times 5$ mm (L) in size, were taken from sapwood and heartwood of sugi (*Cryptomeria japonica* D. Don) and hinoki (*Chamaecyparis obtusa* Endl.) logs in Kyoto University Experimental Forest at different radial directions. For bio-assay tests, *Fomitopsis palustris* (Berk. et Curt.) Murr. as brown-rot fungus and *Trametes versicolor* (L. ex Fr.) Quél. as white-rot fungus were used.

Six specimens from each part of wood were extracted in soxhlet with ethanol-benzene (1 : 2) for 15 hours at 60°C. The extract solutions were evaporated and concentrated to 5 ml. The inhibition of mycelial growth was evaluated on PDA (potato dextrose agar) media containing 0.05% of concentrated extractive solution. The extent of inhibition was determined by the difference of mycelium diameter from control agar plate as percentage. One hundred % means the complete inhibition of fungal growth and 0% means non-effect. As shown in Table 1, amount of extractives was highest in the outermost heartwood in both woods. All extractives of sugi wood exhibited the poor antifungal activities against both fungi, while those of hinoki heartwood had the higher activities than sugi.

Water uptake of wood placed on the agar solid in Petri dish was measured and was compared before and after extraction. Percentage water uptake was not significantly different in hinoki but outer heartwood of sugi absorbed more water after extraction. This suggests that more amounts of sugi heartwood extractives acts to retard the penetration of water into wood.

Conventional laboratory decay test was made for three months by exposing of extracted and unextracted specimens to the two test fungi. When exposing to *T. versicolor,* all extracted specimens of sugi were severely attacked by this fungus (Table 2). Therefore, heartwood

Table 1. The amount of extractives and the antifungal activity against wood decay fungi.

Species	Zone of sample		Distance from pith (cm)	Amount of extractive (%)	Antifungal activity	
					F. palustris	*T. versicolor*
C. japonica sugi	(inner)	A	1.5	5.81	20	27
		B	4.0	5.26	20	29
	heartwood	C	7.0	5.97	28	25
		D	9.5	6.71	29	27
	(outer)	E	12.5	7.07	22	23
	sapwood	F	15.5	0.62	35	26
C. obtusa hinoki	(inner)	A	1.5	2.58	72	68
	heartwood	B	4.0	3.43	62	47
	(outer)	C	7.0	4.57	60	49
	sapwood	D	10.0	1.00	17	−8

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Table 2. The radial variation in decay resistance after 3 months.

Species	Zone of sample		Percentage mass loss after three months (%)*							
			F. palustris				*T. versicolor*			
			Unextracted		Extracted		Unextracted		Extracted	
			AV.	S.D.	AV.	S.D.	AV.	S.D.	AV.	S.D.
C. japonica sugi	(inner)	A	44.6	5.6	39.6	14.5	14.8	11.3	26.6	3.8
		B	51.0	7.0	42.5	16.5	12.9	8.7	26.8	4.9
	heartwood	C	45.5	7.6	36.9	13.0	7.7	5.3	29.9	4.2
		D	44.1	5.3	45.9	9.2	5.2	5.1	31.0	5.0
	(outer)	E	42.8	5.8	47.2	10.4	11.4	7.9	35.4	3.5
	sapwood	F	57.0	2.9	40.8	11.7	35.9	8.0	33.0	5.2
C. obtusa hinoki	(inner)	A	44.5	5.2	50.6	11.6	33.1	5.2	41.7	3.3
	heartwood	B	43.3	4.5	48.9	8.4	28.5	2.7	33.2	5.6
	(outer)	C	42.8	4.0	44.2	12.5	27.9	4.4	34.8	4.6
	sapwood	D	44.2	6.1	40.8	13.4	35.2	5.3	36.7	2.7

* Averages of nine replicates.

extractives of sugi might contribute the relatively higher decay resistance against this fungus by some other role than direct antifugal activity. However, these extractives might not affect the wood-rotting ability of *F. palustris*. Unexpectedly, extraction of hinoki specimens did not influence on the decay resistance against both fungi, although heartwood extractives of this wood had some extent of antifungal activity as shown in Table 1.

The behaviors of fungal hyphae in the extracted and unextracted specimens were observed for thin transverse sections (2 μm) prepared by conventional procedure of light microscopy. In extracted hinoki heartwood, many numbers of *F. palustris* hyphae were observed after one month exposure, although the extraction did not affect the mass loss of wood by this fungus after three-month exposure as shown above. Therefore, heartwood extractives of hinoki have a possible effect to prevent the invasion of this fungus at some extent. Hyphal sheath of *T. versicolor* was observed in unextracted specimens of sugi heartwood but not in extracted ones. Some researchers suggested the protective barrier from toxic substances as one of the possible functions of fungal sheath[3–6]. Isolated extractives of sugi heartwood had poor activity to *T. versicolor* on agar (Table 1) but these substances might affect the behavior of this fungus in wood structure.

From these results, it was demonstrated that heartwood extractives of sugi and hinoki also affect in different manner to decay fungi in vitro and in situ conditions and that antifungal activities of these extractives were rather specific.

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