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Static Defense Components for Sugi Butt-rot Disease

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Sound sugi wood and butt-rot sugi woods, which were sampled from artificial forests in Kumamoto Prefecture, were divided into ten fractions along the diameter. Each fraction of wood sample was analyzed. Holocellulose contents of the butt-rot portions were considerably decreased and adversely lignin contents were increased. It means that this might be caused by brown wood rot fungi. Methanol extractive content of each fraction in the butt-rot wood was largely different, the seriously decayed portion showed the lowest extractive content, on the other hand, the portions surrounding the decayed portion showed extremely high extractive contents compared to those of the sound wood. Especially, contents of acidic and neutral substances were much higher in the butt-rot wood than in the sound wood, but their components were enormously different. Ethyl acetate insoluble fractions in methanol extracts from sound wood were greatly decreased by butt-rot disease. Phenolic substances, especially norlignans in the butt-rot woods were also largely decreased and were converted into highly acidic substances. Norlignan contents depend on clone varieties of sugi, and may be important for anti-fungal properties.
Keywords: sugi, butt-rot disease, extractives, norlignan, terpen

1. INTRODUCTION

Sugi (*Cryptomeria japonica* D. Don) extensively used for construction is a most popular afforesting tree in Japan. Artificial forests of sugi have often incurred damages by several diseases. Among them, wood boring insects cause the discolored woods [1], which lose the economical value for construction materials and are left in the forests even after felling. Many works on the wood discoloration of sugi have been made in connection with the phenolic compounds, such as norlignans [2], but most of them were related to the sapwood. Butt-rot diseases are popular for many kinds of tree, which are caused by wood rot fungi *via* the injured roots, and initially discolor and subsequently decay the heartwood. In this case, the sapwoods are usually not damaged at all. Sugi has been known as a tree resistant against butt-rot disease due to high concentration and anti-fungal characteristics of heartwood extractives [3]. But, recent researches elucidated quite high percentage (ca. 83%) of butt-rot woods in the woods felled from some artificial forests [4]. This disease has often been found in rather older trees and lowers the wood value seriously. Its detail is still unknown and therefore at the beginning of our researches on this problem the chemical aspects of butt-rot woods were precisely investigated.

2. EXPERIMENTAL

Sugi sound wood (70 years old) and butt-rot woods (70 and 83 years old) were sampled from Aso district of Kumamoto Prefecture in 2002. They are all Aya sugi (clone variety). For the comparison, Yanase sugi (35 years old) from Kochi University's Forest was also used.

Sugi sound wood and butt-rot wood (83 years old) are illustrated in Figs. 1 and 2, respectively. Ten fractions were obtained as shown in Figs. 1 and 2.

Each fraction was pulverized by Wiley-mill, the wood meals passed through 42 mesh of sieve were extracted with methanol by Soxhlet apparatus. Holocellulose and Klason lignin contents of each wood meal were determined by conventional methods [5]. Methanol extracts were divided into solubles and insolubles in ethyl acetate. The solubles were further divided into neutral and acidic fractions by conventional method. The acidic fractions include not only organic acids but also phenolic substances.

The neutral fraction was analyzed by gas chromatography-mass spectrometry, GC/MS QP5050 (SIMADZU Co.). GC condition: Capillary column (TC-1701, 0.25mm x 30m, GL Science), temperature; 100-250℃, 4℃/min, carrier gas; helium, flow rate; 20.6ml/min. MS condition;

electron impact at 70eV.

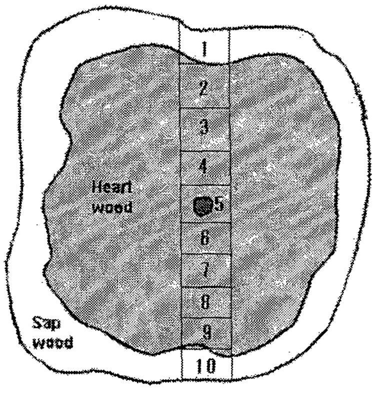

Fig. 1 Diagram of sugi sound wood and sampling positions.

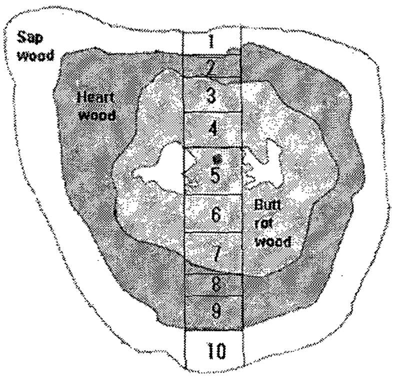

Fig. 2 Diagram of sugi Butt-rot wood and sampling positions.

The acidic fraction was analyzed by high performance liquid chromatography (HPLC). HPLC condition: Octadecyl silylated silica gel (ODS) column; COSMOSIL 5C18, 4.6 x 250 mm (Nacalai Tesque), solvent; methanol, flow rate; 0.5 ml/min, detector; reflex index detector.

The methanol extracts mixed with syringic acid as internal standard were silylated with N,O-bis(trimethylsilyl)-acetamide and N-(trimethylsilyl)-imidazole, then analyzed by GC. GC condition: Capillary column (TC-1 0.25 x 15 m, GL Science), temperature; 150-270℃, 4℃/min, carrier gas; nitrogen, split ratio=1:30.

Typical norlignan, sequirin-C was isolated from the methanol extracts of Yanase sugi heartwood according to the method described by Takahashi et.al. [2], and purified by recrystalization with mixed solvent of acetone and benzene. Agatharesinol was also isolated, but not crystallized. These compounds were used for determination of GC factors of the norlignans to the internal standard, syringic acid.

3. RESULTS AND DISCUSSION

3.1 Changes of main wood components by butt-rot disease

Holocellulose and lignin contents, of sound wood and butt-rot wood are shown in Fig. 3 and Fig. 4, respectively.

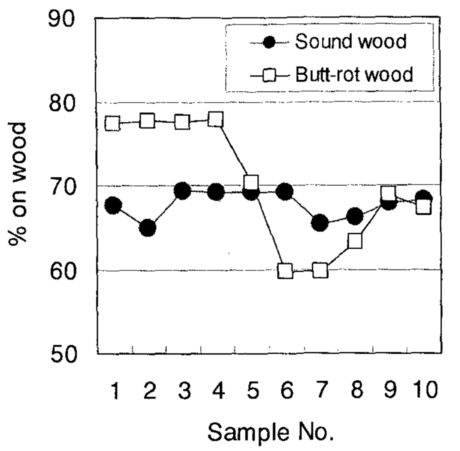

Fig. 3 Holocellulose content of each fraction obtained from sound and butt-rot woods.

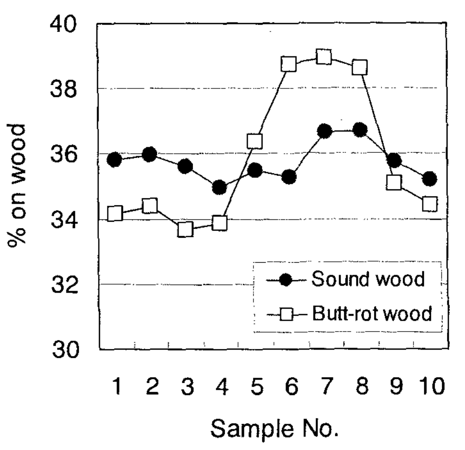

Fig. 4 Klason lignin content of each fraction obtained from sound and butt-rot woods.

Sample No. 5, 6. 7 are seriously decayed as shown in Fig. 2. These samples have lower holocellulose and higher lignin contents than the corresponding samples of sound wood do. It means that sugi butt-rot may be caused by brown wood rot fungi which mainly decompose cellulose and hemicellulose [6]. Isolation and identification of butt-rot fungi are being conducted, therefore, fungal information will be reported elsewhere.

3.2 Influence of butt-rot on methanol extracts, and their neutrals and acidics and ethyl acetate insolubles

Methanol extract content of each fraction from sound wood and butt-rot wood are shown in Fig. 5.

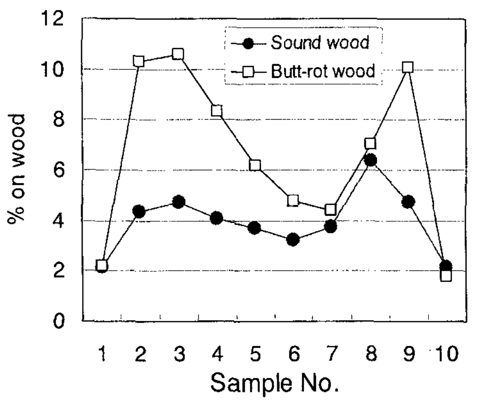

Fig. 5 Methanol extract contents of sugi sound wood and butt-rot wood.

Extract contents are usually low in sapwood and high in heartwood. The extract contents of the heartwoods (No.2,3 and 9) outer from the decayed portions (No. 5-7) of butt-rot wood are extremely high compared to the sound wood. Sugi butt-rot trees are considered to exude a lot of extracts as defense substances against fungal attacks [3].

Contents of neutral, acidic and ethyl acetate insoluble fractions of methanol extracts are shown in Fig. 6 and 7.

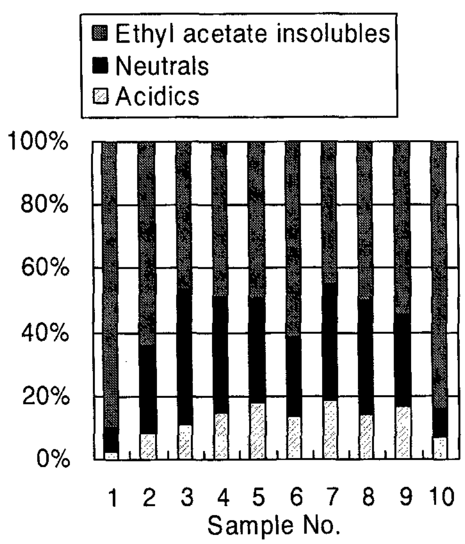

Fig. 6 Composition of methanol extract from sugi sound wood.

Although methanol extracts from sound wood are abundant in ethyl acetate insoluble fractions, those from butt-rot wood are greatly

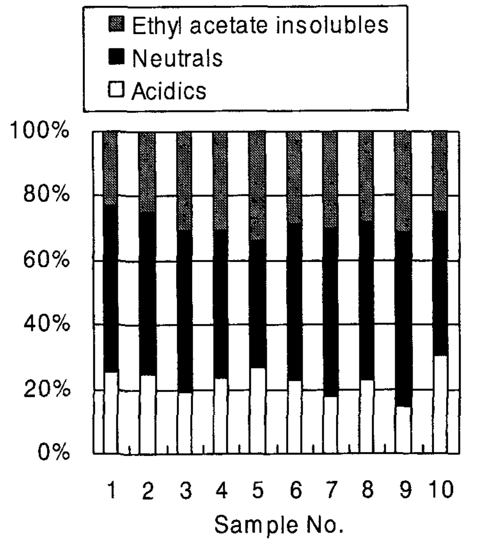

Fig. 7 Composition of methanol extract from sugi butt-rot wood.

decreased. Ethyl acetate insoluble fractions are thought to mainly consist of glucosides and be easily hydrolyzed by fungal attacks, and consequently the neutral and acidic substances may be liberated.

HPLC indicated that the acidic fractions, perhaps phenolic substances were further oxidized by butt-rot fungi.

3.3 Change of dominant terpens and norlignans by butt rot disease

Extractives from sugi wood have been well investigated [7]. Major terpens and norlignans detected this time are illustrated in Fig. 8 and Fig.9, respectively.

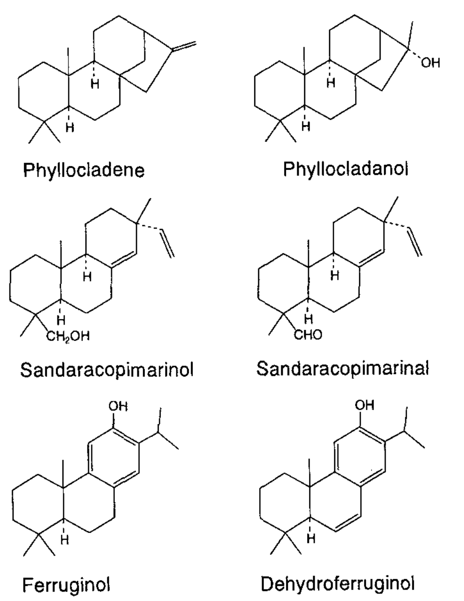

Fig. 8 Major terpens detected in sugi methanol extracts.

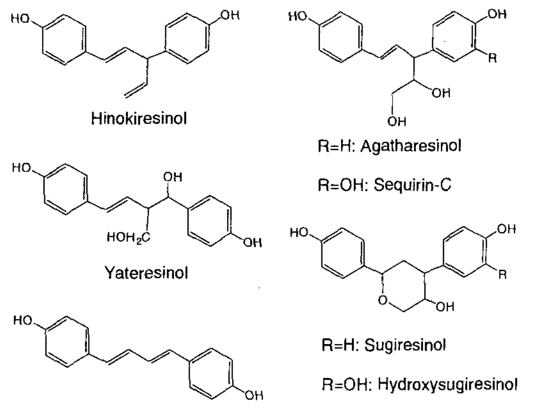

Fig. 9 Major norlignans detected in sugi methanol extracts.

Terpens, like ferruginol etc. are known to have an antifungal activity against white wood rot fungi, *Lentinula edodes* etc. [8]. Norlignans have also anti-pathogenic activities [3]. Norlignans described in Fig. 9 are typical phenolic compounds of sugi wood and their contents have wide variations, Ogiyama et.al.. have classified sugi clones into five chemotypes based on norlignan constitutions [9].

Contents of terpens and norlignans from Yanase sugi and Aya sugi are shown in Table 1. Norlignans, especially agatharesinol and sequirin-C are highly contained in Yanase sugi On the other hand, Aya sugi contains a lot of terpens, but much less norlignans than Yanase sugi does. Butt-rot disease has not been reported in Yanase sugi. It means that these phenolics may play an important role in resistant activities against sugi butt-rot disease.

4. CONCLUSION

Sugi butt-rot seems to be caused by brown rot fungi. Compositions of sugi wood extracts are changed largely by butt-rot disease. Norlignan content is much higher in Yanase sugi than in Aya sugi, and may be a crucial factor for anti-fungal activity against butt-rot disease.

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Table 1 Contents of terpens and norlignans in the methanol extracts from Yanase sugi and Aya sugi (% on methanol extracts)

	Yanase sugi sapwood	Yanase sugi heartwood	Aya sugi rot wood sapwood	Aya sugi rot heartwood *1)	Aya sugi rot heartwood *2)	Aya sugi sound sapwood	Aya sugi sound heartwood
Phyllocladene	1.75	2.27	2.45	13.43	6.25	5.04	11.15
Sandaracopimarinol	1.81	7.22	6.84	18.79	20.09	12.75	23.46
Ferruginol	1.06	4.32	2.82	7.32	13.77	3.79	9.42
Hinokiresinol	0.18	0.33	0.07	0.36	0.28	0.53	0.50
Yateresinol	0.07	1.42	0.98	2.19	2.94	2.37	2.92
1,4-Bis (p-hydroxyphenyl)-butadiene	2.63	0.00	0.15	0.16	1.30	0.37	0.27
Agatharesinol	2.63	4.55	3.10	1.04	0.66	7.58	1.46
Sugiresinol	1.69	0.20	0.72	2.66	1.90	3.32	2.13
Sequirin-C	0.32	14.47	0.45	2.78	1.57	4.07	3.38
Hydroxysugiresinol	1.40	0.15	0.90	0.36	0.51	2.42	1.19
Total major terpens	4.62	13.81	12.11	39.54	40.10	21.58	44.03
Total norlignans	8.92	21.13	6.39	9.54	9.16	20.65	11.84

*1 : Sound part of butt rot wood, *2 : Discolored part of butt rot wood

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