Characterization and Degradation Mechanisms of Wood Components by Steam Explosion and Utilization of Exploded Wood*1

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CONTENTS

Key words: steam explosion, degradation mechanisms, wood components, lignin model compounds, utilization of steam-exploded wood

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INTRODUCTION

Woody biomass is the most abundant organic resource on the earth and the total amount of wood is estimated to 1.8×10^{12} ton which almost corresponds to the estimated amount of fossil deposits[1]. Besides, the biomass is renewable and especially forest fixes most effectively the energy of the sunlight. The amount of the fixed material is 7.4×10^{10} ton a year which corresponds to almost ten times of the consumption of oil or five times of the consumption of wood a year[2]. While, recent annual consumption wood in Japan is about one hundred million m^3 of which 70 % is imported. We have entered the energy shortage age and the importance of wood as a renewable resource of energy and chemicals has increased. Thus, developments of useful chemicals, cattle feed, and energy from wood residue are keenly demanded.

Woody biomass is a conglomeration of cell wall constituted with polysaccharisdes (cellulose and hemicelluloses) and an aromatic polymer (lignin) which could be converted to foods, feeds, liquid fuels and raw materials for chemical industry. Recently, chemical industries of wood saccharification[3~6], preparation of cattle feeds[7] and wood-refinery[8~10] have promoted the development of technology converting wood to energy and chemical raw materials. However, cellulose and hemicelluloses are strongly associated with lignin in wood, and therefore delignification has been recognized as the most important step for chemical utilization of wood.

Steam explosion process which was first introduced by DELONG[11] to defibrate wood into fiber fragments or even single fibers[12] has been developed as a useful pretreatment of woody materials for enzymatic saccharification[13], preparation of cattle feeds[7,14~16], and wood refinery. The process would be employed as a useful technique for total utilization of wood in the near future[17].

The process consists of a combined reaction of chemical degradation and mechanical deformation of wood to result in the separation of main wood components, cellulose, hemicelluloses and lignin. By steam explosion hemicelluloses become water soluble, lignin methanol soluble, and cellulose becomes very accessible to hydrolytic enzymes[18]. Incidentally, lignin as one of the most abundant polymers, has usually been used as fuel to recover chemicals from waste liquor in Kraft pulp industry. Little attention has been focused on the use of lignin as chemicals or conversion of lignin to valuable products since these could be produced from inexpensive petroleum. However, the recent energy crisis and the scarcity of crude oil have prompted research activities to develop alternative and renewable feedstock for polymers and chemicals.

Chemical degradation of lignin has been performed mainly to elucidate its structure[19,20]. It is important to elucidate the reaction mechanism of lignin degra-

dation for understanding chemical conversion of wood and its process. Little work has been conducted the reaction mechanism of lignin degradation by steam explosion although chemical characterization of steam-exploded lignin has been performed by several workers[21~25].

The purpose of this work is to elucidate the degradation mechanism of lignin by steam explosion for possible control of the degradation reaction toward useful utilization of steam-exploded lignin. The structure and physical properties of steam-exploded wood have been elucidated in relation to the utilization of woody biomass[26], and the chemical properties of main components in steam-exploded woods are discussed related to the mechanism of chemical changes of cellulose, hemicelluloses and lignin by the process[27,28] (Chapter 1).

The reaction mechanism for lignin degradation by steam explosion has been elucidated. Comparatively large amounts of syringaresinol and coniferyl alcohol were obtained from steam-exploded lignin of Sirakanba (white birch, *Betula platyphylla* Sukatchev var. *japonica* Hara), and the results suggested that the homolytic cleavage of β-O-4 ether linkages of lignin by steam explosion occurred[27,29]. The steam explosion and steam treatment of lignin substructure model dimers, DHP and LCC have also been made to elucidate the degradation mechanism of lignin by steam explosion[30~34]. For the synthesis of highly polymerized DHP and LCC, new dehydrogenative polymerization method (dialysis tube method) has been also developed[35~38] (Chapter 2).

Utilization of steam-exploded wood, enzymatic saccharification, and preparation of ruminant feed from the steam-exploded wood, were described[16,17] (Chapter 3).

1. CHARACTERIZATION OF STEAM-EXPLODED WOOD

1. 1 Introduction

Since we have entered the energy shortage age, the importance of wood as a renewable resource of energy and chemicals, is increasing. For the continuously maintained utilization of wood, development of useful chemicals, cattle feed, and energy from wood residues is keenly demanded.

Steam-explosion process which was developed by STAKE Technology, and IOTEC in Canada has attracted attention in utilization of woody biomass[1]. The present investigation was carried out to characterize the structure and physical properties of steam-exploded wood in relation to the utilization of woody biomass.

1. 2 Structure and Physical Properties of Steam-Exploded Wood

1. 2. 1 Steam explosion of wood

The process contains a physical rupture of wood structure by adiabatic expan-

Table 1. Distribution of the size of fragments of explosion woods.

sample	kgf/cm²	meshes min	meshes mm	Weight % of fragment on sieves 7 on 2.830	14 on 1.190	28 on 0.590	42 on 0.350	80 on 0.117	80 pass	average size of EXWs
Shirakanba	28	1		14.0	54.7	21.1	5.1	3.1	2.0	1.74(mm)
	28	2		8.9	55.0	25.8	6.0	2.7	1.6	1.64
	28	4		4.1	60.4	16.3	10.7	4.6	3.9	1.55
	28	8		0.7	12.5	22.9	20.1	20.4	23.4	0.65
	28	16		0.0	4.2	8.9	17.9	18.9	50.1	0.34
	20	16		8.7	40.9	22.9	12.6	8.9	6.0	1.38
	24	16		2.3	23.0	25.5	18.3	18.9	12.1	0.91
	28	16		0.0	4.2	8.9	17.9	18.9	50.1	0.34
Karamatsu	28	1		16.1	49.4	22.7	6.8	3.8	1.3	1.72
	28	2		12.5	55.4	22.7	5.3	2.5	1.6	1.72
	28	4		5.2	43.4	31.1	9.4	5.5	5.4	1.37
	28	8		2.0	27.1	32.3	13.9	11.2	13.5	1.00
	28	16		2.2	28.3	30.4	14.2	10.9	14.0	1.01
	20	16		9.8	47.3	31.6	7.2	2.8	1.3	1.57
	24	16		4.6	34.8	29.9	12.1	11.6	7.0	1.20
	28	16		2.2	28.3	30.4	14.2	10.9	14.0	1.01

sion of water in small pores in wood tissues, and autohydrolysis of cell wall components. General aspects of the steam-explosion process of wood have been reported by MARCHESSAULT[12,18]. However no detailed investigation has been reported on the effect of processing conditions. For development of the utilization of steam-exploded wood and understanding of the process it is required to characterize steam-exploded woods under different conditons in pressure, temperature and time of the treatment. In this work, morphological structure and physical properties of steam-exploded wood were investigated. One of the most important characteristics of explosion process is that wood chips were finely ruptured to fibers and/or powder. Table 1 shows the effect of explosion conditions on the destruction of wood chips. Distribution of the size of fragments of steam-exploded wood indicates the effect of temperature in explosion process. The average size of the fragments and the whiteness of steam-exploded wood decreased with increase of the reaction time.

1.2.2 Morphological characteristics

Fig. 1 shows the appearance of the steam-exploded woods of Shirakanba and Karamatsu (Japanese larch, *Larix leptalepis* Gordon) examined by optical microscopy. In Shirakanba steam-exploded wood, when the steam pressure was lower and reaction time was shorter (i.e. 20 kgf/cm², 2 min), shivers were frequently observed. At the

higher pressure (28 kgf/cm^2, 2 min) wood chips were mostly defibrillated to single fibers (Fig. 1), and brown colored oily substances were frequently detected both inside and outside of exploded cell walls (Fig. 1C-a and 1A-a). These substances were insoluble in water but soluble in MeOH, and considered to be derived from lignin, resinous extractives and/or polyphenols. Production of these substances is one of the important characteristics of steam-exploded wood, because they were hardly detected in thermomechanical pulp (TMP) and ground pulp (GP). It seems that lignin both in middle lamellae and secondary walls could be liberated considerably from cell wall polysaccharides by steam explosion. It is concluded consequently that when steaming time is longer (28 kgf/cm^2, 16 min) fibers are almost fibrillated (Fig. 1B).

In Karamatsu steam-exploded wood, which is different from Shirakanba steam-exploded wood, single fibers could scarcely be produced by these conditions. Karamatsu steam-exploded wood did not form fiber but particles, and the particle size decreased with the longer steaming time. However, the microscopic structure of Karamatsu steamexploded wood at different conditions changed a little. Most of tracheids were not disintegrated to fiburs but ruptured to small particles (Fig. 1D and 1E). Lignin was scarcely eluted from tracheids cell walls. Tracheids crossed with ray tracheids were particularly difficult to be exploded and remained as a block.

To investigate the fibers of Shirakanba steam-exploded wood in detail, observations by scanning electron microscopy was performed (Fig. 2). In Fig. 2A, vessels (a), fibers (b) and amorphous substances (c) which are considered to be formed by freeze-drying from hydrolyzed hemicelluloses, and/or lignin were observed. As shown in Fig. 2A intact vessels (a) were scarcely found. Most of vessels were found to be destroyed to small fragments. Most of fibers (b) suffered from some damage, as found in a buckling (Fig. 2A-B), a cleft along the fiber axis (Fig. 2B), rupture at the end and middle of a fiber (Fig. 2C), expansion in a dome shape (Fig. 2D) and tear at middle lamellae and S1 layers[26,39]. Thus, the exploded fibers were so deformed and different from fibers in GP and KP[40].

A preliminary investigation showed that the filtrate of the water extract of steam-exploded wood by analytical filter paper contains cellulose[41]. Then the water extract (suspended fine fibrils) was observed by a transmission electron microscope (TEM). A similar observation of steam-exploded wood by TEM has recently been carried out by Marchessault[42]. When the explosion condition were weaker than 28 kgf/cm^2, 8 min, a few microfibrils were detected. However, when woods were steam exploded at the conditions of 28 kgf/cm^2, 8 and 16 min many microfibrils were observed as shown in Fig. 3A. Observation at a high magnification showed that microfibrils

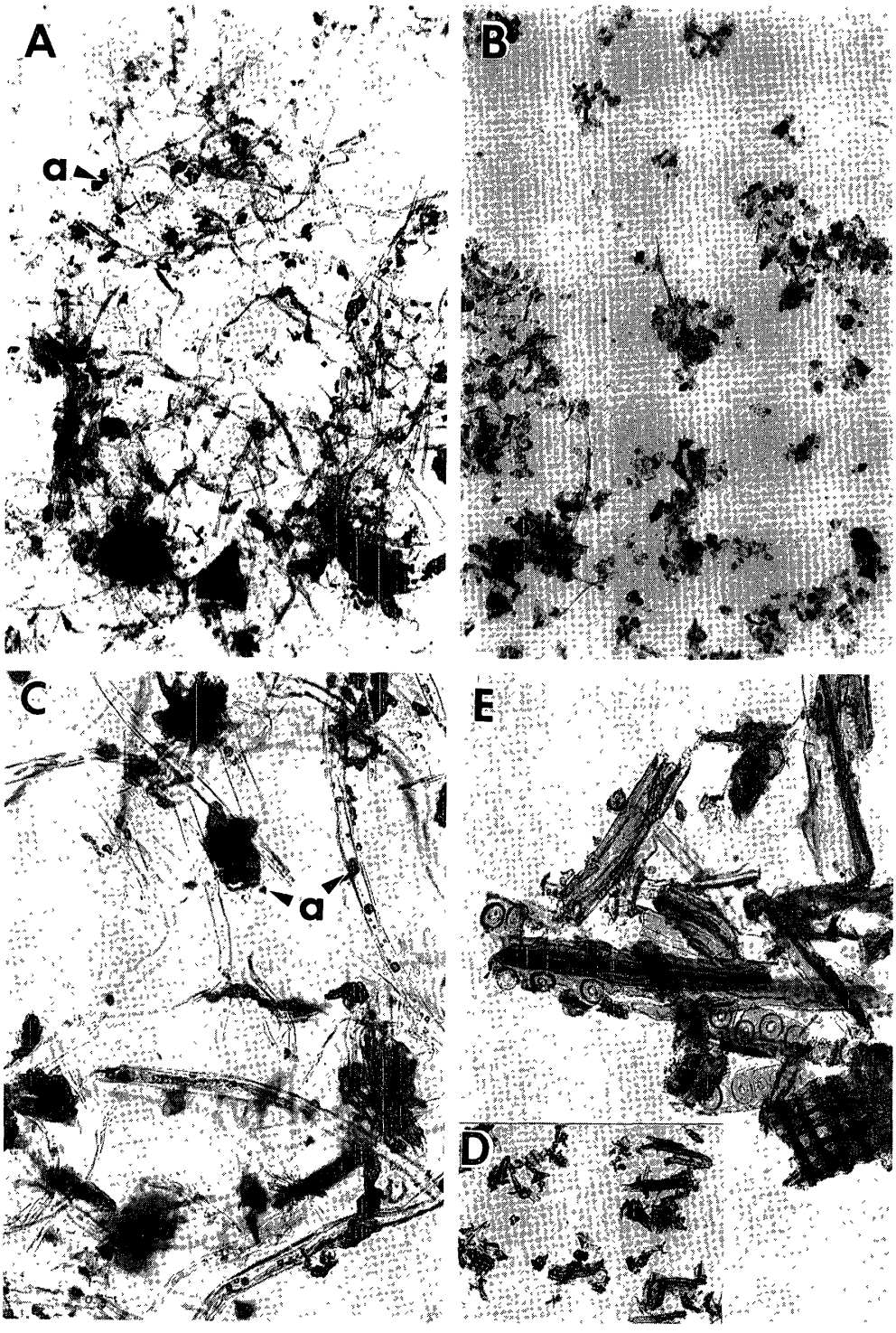

Fig. 1. Observation of explosion woods by an optical microscope.
A: Shirakanba EXW (treated at 28 kgf/cm^2 for 2 min.) (a) Lignin-like oily substance released from fibers. B: Shirakanba EXW (treated at 28 kgf/cm^2 for 16 min.) C: Enlargement of A D: Karamatsu EXW (treated at 28 kgf/cm^2 for 2 min.) E: Enlargement of D

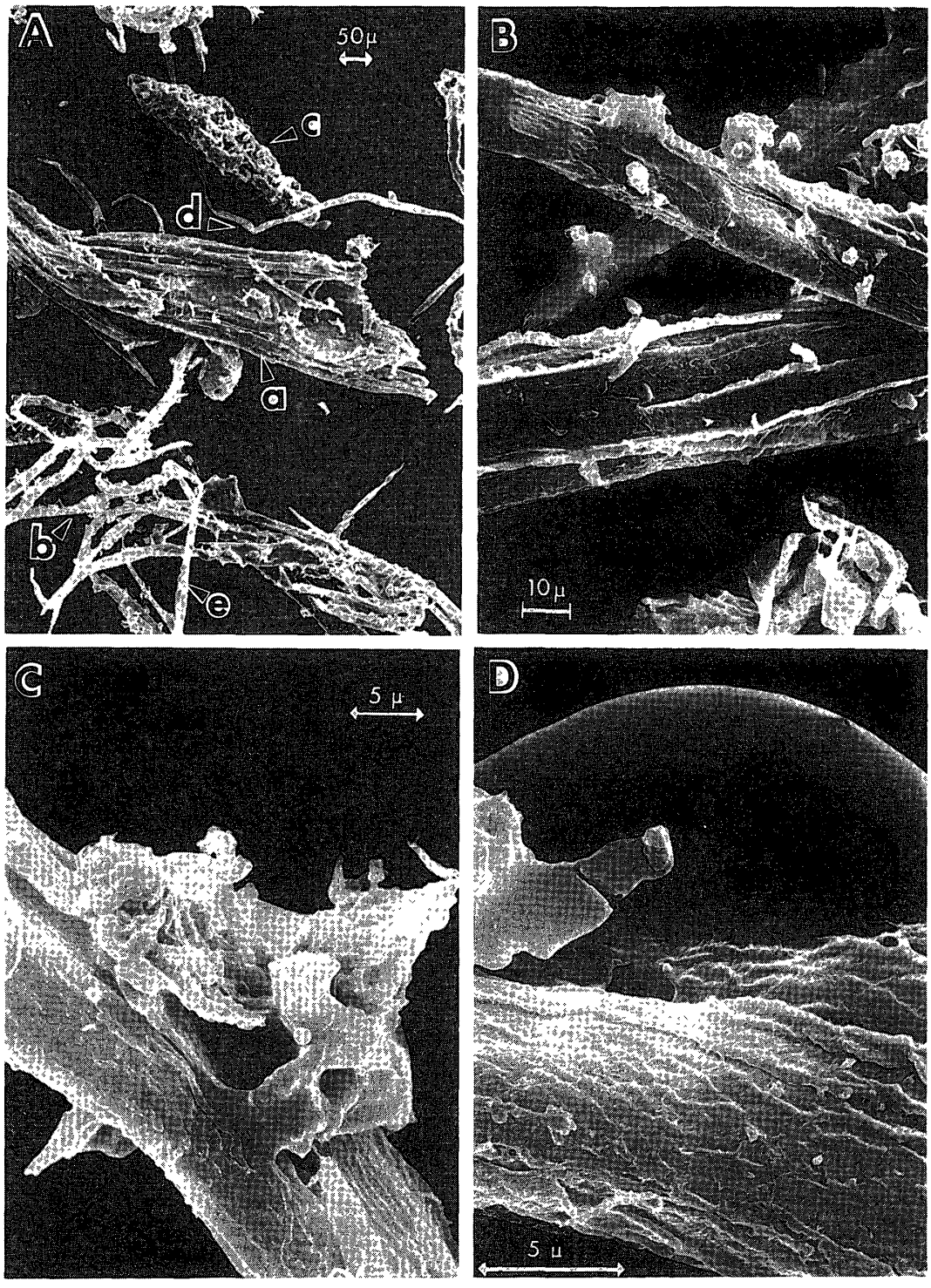

Fig. 2. Observation of Shirakanba exploion wood (treated at 28 kgf/cm^2 for 2 min.) by a scanning electron microscope.
A: Observation of EXW at the lower magnification (a) vessels, (b) fibers, (c) Amorphous substances, (d) Buckling and (e) Expansion of a fiber B: A cleft along a fiber C: Explosion at a middle of a fiber D: Expansion of a fiber

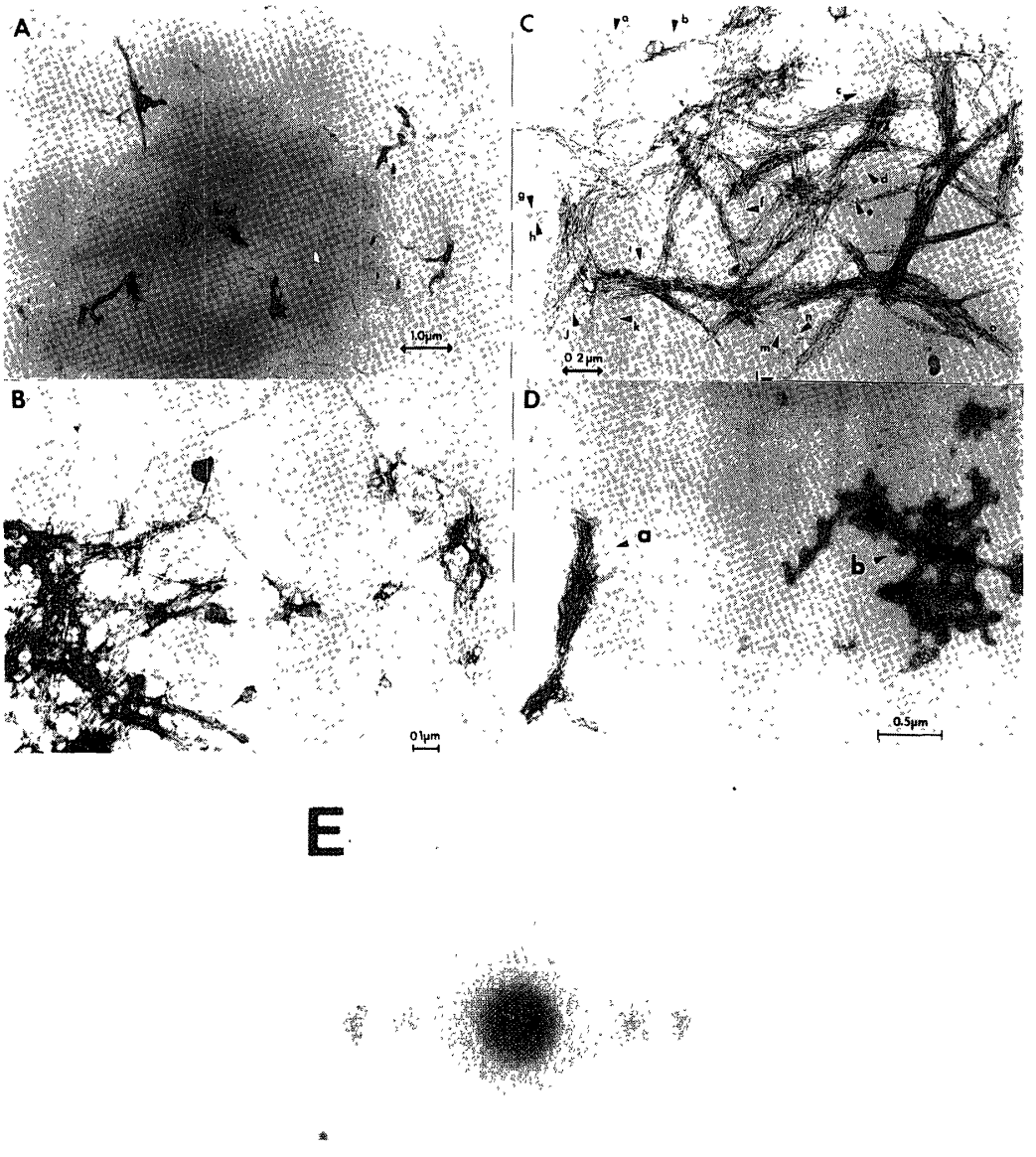

Fig. 3. Observation of EXW by a transmission electron microscope.
A. Observation of Karamatsu EXW at the lower magnification
B. Shirakanba EXW (treated at 28 kgf/cm² for 1 min.)
C. Shirakanba EXW (treated at 28 kgf/cm² for 8 min.)
Width of microfibrils (Å)

Width	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o
Å	66	53	40	66	40	26	79	46	92	66	105	53	92	66	33

D. Karamatsu EXW (treated at 28 kgf/cm² for 16 min.)
(a) Cellulose microfibrils and (b) Lignin-like substances
E. Electron diffraction diagram of microfibrils from Shirakanba EXW

were completely separated to each other (Fig. 3B, C and D). Microfibrils were wider and shorter with increase of the steaming time (Fig. 3C as compared with B). In Karamatsu steam-exploded wood, the same characteristics were observed (Fig. 3D). Such fibrillation hardly occurs in the process of GP and KP manufacturing, and such liberated microfibrils have not been obtained by other methods. It is noteworthy that fibrillation of cellulose fibers occurs easily for a very short time by steam explosion. Small particles as shown in Fig. 3D-b were observed in some cases. They were stained negatively and insoluble in water, suggesting that particle is lignin-like substance but not sugar. To confirm this, the methanol soluble fraction of steam-exploded wood was added dropwise into the excess of water, and a drop of the mixture solution was observed by TEM. Similar particles were again detected, suggesting that the particles as shown in Fig. 3D-b were lignin-like substances. Further investigations are in progress to characterize the substance. The microfibrils were confirmed to be cellulose I by electron diffraction (Fig. 3E).

1. 2. 3 Crystallinity and micelle width

MARCHESSAULT and co-workers reported that X-ray diffraction analysis of aspen steam-exploded wood showed little or no loss in the degree of crystallinity of steam-exploded wood cellulose, and that the cellulose retains its basic crystalline structure[12]. However, the present investigation is not consistent with their conclusion. Fig. 4 shows the X-ray diffraction curves of exploded (28 kgf/cm^2, 16 min) and untreated woods, both for Shirakanba and Karamatsu. The diffraction patterns showed that the steam-exploded woods are composed of cellulose I. However, the peak of (002) diffraction by steam-exploded woods was sharper than that of untreated woods, and the degree of crystallinity and micelle width increased by explosion treatment. Fig. 5 shows the effect of steaming time at 28 kgf/cm^2, on the degree of crystallinity and micelle width. Within 4 min of explosion the degree of crystallinity increased rapidly with increase of steaming time, attaining crystallinity after 4 min (Crystallinity ratios in the steam-exploded wood over untreated wood (E/U) were 1.39 for Shirakanba (50.2%→69.8%) and 1.50 for Karamatsu (45.1%→67.7%), and then decreased slowly. Micelle width of cellulose rapidly increased, attaining maximum width after about 8 min (ratios of micelle width in E/U were 1.39 for Shirakanba (34.3 Å→57.9 Å) and 1.82 for Karamatsu (27.0 Å→49.1 Å)). The effect of steaming pressure on wood was tested at 2 min steaming, it was indicated that the degree of crystallinity and micelle width increased with the higher steam pressure.

HARADA and GOTO found that the width distribution of uranyl acetate-stained microfibrils observed by TEM was correlated with micelle width of corresponding sample determined by X-ray diffraction[43]. The observation of steam-exploded wood by TEM in the present investigation showed that the average width of microfibrils

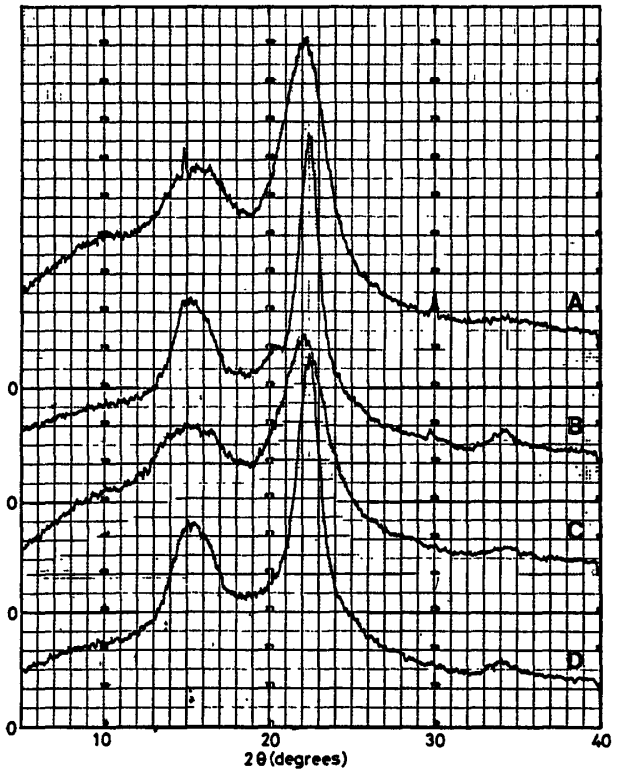

Fig. 4. X-ray diffraction curves of EXW.
A. Shirakanba untreated wood powder
B. Shirakanba EXW (treated at 28 kgf/cm² for 1 6 min.)
C. Karamatsu untreated wood powder
D. Karamatsu EXW (28 kgf/cm², 16 min.)

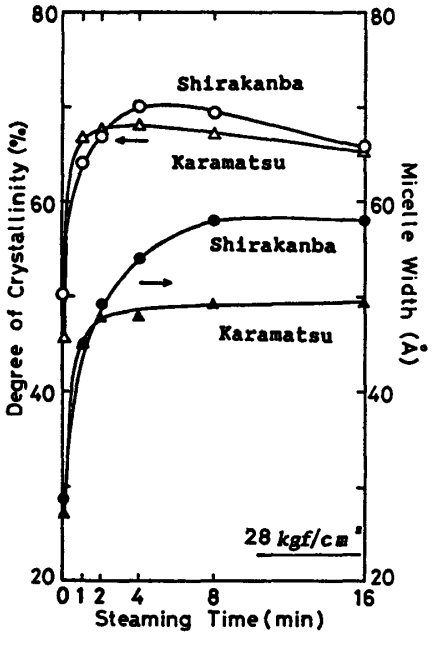

Fig. 5. The effect of steaming time on crystallinity and micelle width of EXW.

of steam-exploded wood treated at the conditions of 28 kgf/cm² for 8 min was about 63 Å. Thus, the degree of the crystallinity and width of micelle increased about 1.5 and 2.0 times, respectively by the explosion. These results suggest that most of amorphous region of cellulose transformed to crystalline region by the explosion, resulting in the increase of the crystallinity and micelle width of the explosion wood.

1. 2. 4 Thermal softening property

Fig. 6 shows the effect of steaming time on the thermal softening behavior of Karamatsu and Shirakanba steam-exploded woods at 28 kgf/cm² of steam pressure, and Fig. 7 shows the differential thermoanalysis curves. In the Shirakanba untreated wood, there is a shoulder around 200~300°C which is probably attributed to L.C.C.[37]. In the steam-exploded wood, on the other hand, the shoulder disappeared and a new peak at about 125°C was observed (Fig. 7): the methanol extract, which mostly composed of guaiacyl-syringyl lignin gave the softening point (120°C) corresponding to the new peak. In the Karamatsu steam-exploded wood, the same

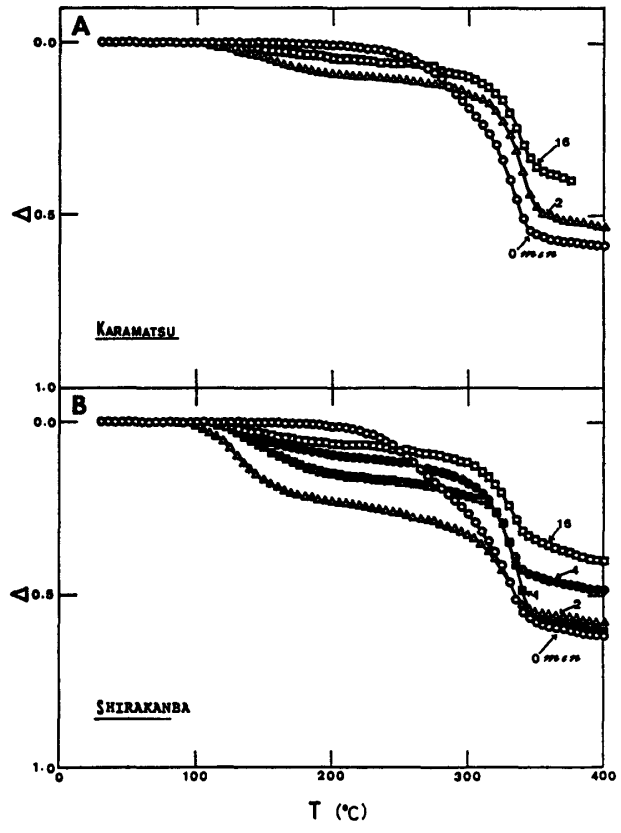

Fig. 6. The effect of steaming time at 28 kgf/cm² of the steam pressure on the thermal softening process
A. Karamatsu EXW
B. Shirakanba EXW
Δ: Normalized deformation function $\left(\frac{\Delta l}{l_0}\right)$

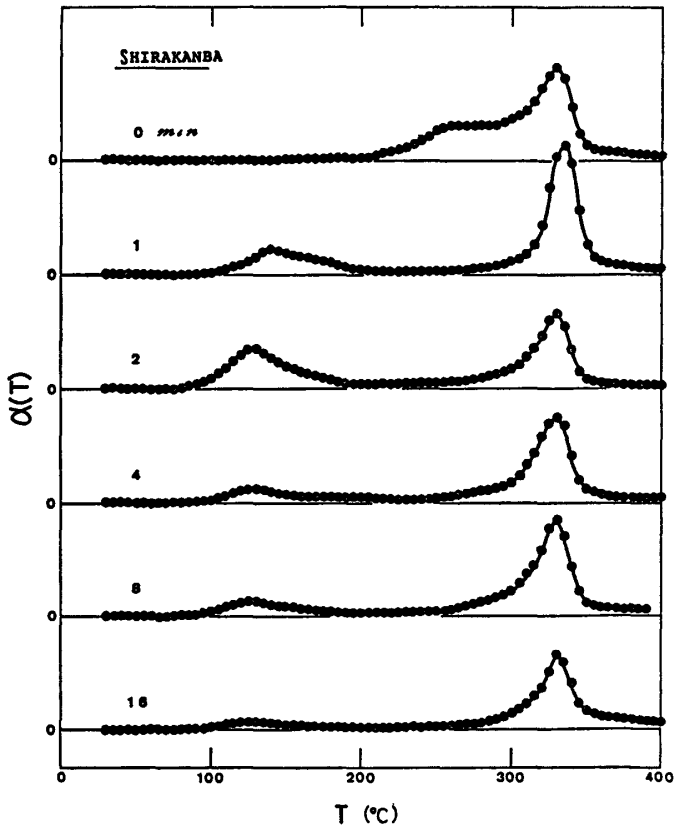

Fig. 7. The differential curves of thermal softening process for shirakanba EXW at 28 kg/cm² of the steam pressure.
α: Rate of deformation at each temperature T $\left(\frac{d\Delta}{dT}\right)$

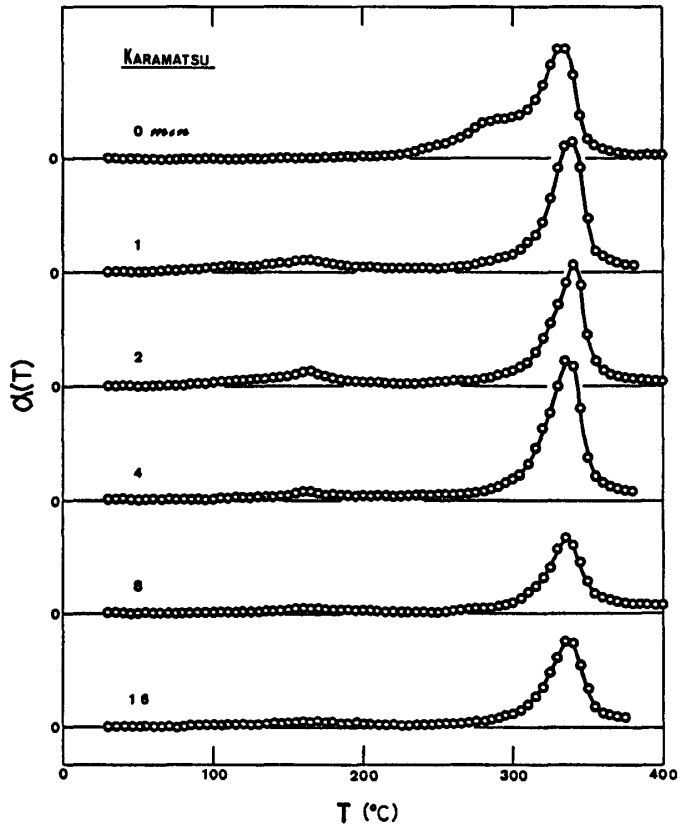

Fig. 8. The differential curves of thermal softening process for Karamatsu EXW at 28 kgf/cm² of the steam pressure.

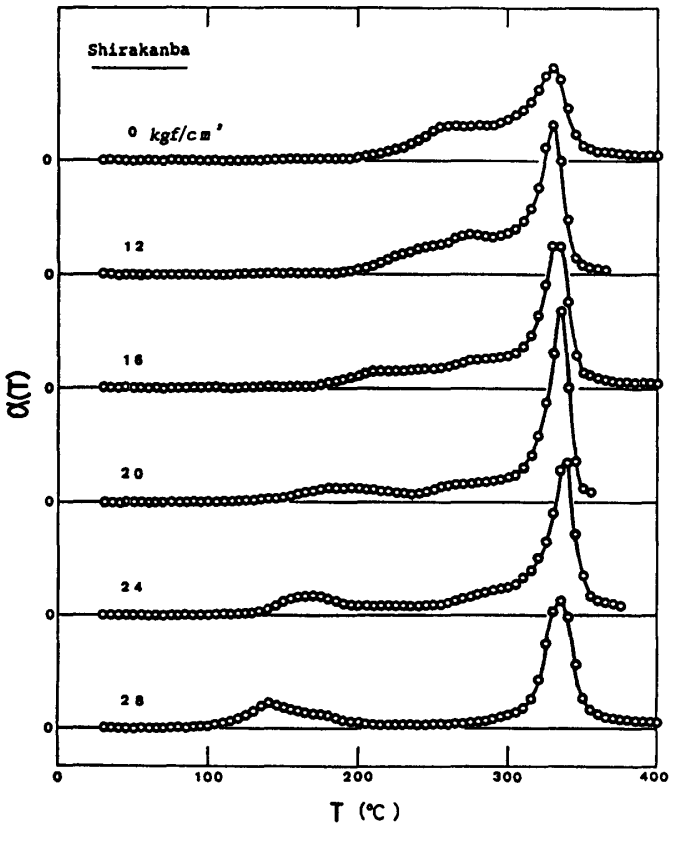

Fig. 9. The effect of steam pressure on the thermal softening process of Shirakanba EXW for one min. steaming.

tendency as in Shirakanba was observed, except that the new peak was shifted at about 165°C which may correspond to guaiacyl lignin (Fig. 8).

These results suggested that hemicelluloses and lignins were decomposed to low molecular weight fragments by steam explosion[35,36]. However, the height of the new peak decreased with the increase of the steaming time (Fig. 7 and 8). It seems that the depolymerized substance was repolymerized and transformed to the unsoftened product. In agreement with this view, the rate of deformation at 350°C decreased with the increase of the explosion time as shown in Fig. 6. These results conclusively indicated that the best conditions of delignification from wood is 28 kgf/cm^2, 2 min both for Shirakanba and Karamatsu in the present investigation. However, a considerable amount of lignin remained in Karamatsu steam-exploded wood, and the total amount of eluted lignin was lower than that of Shirakanba.

In Shirakanba steam-explosed wood, treated at 28 kgf/cm^2 for 1 min, the thermal softening temperature of the new peak slightly shifted to higher temperature than that of steam-exploded wood treated for 2 min (Fig. 7). The pattern of the new peak of steam-exploded wood (at lower temperature) under different pressure for 1 min showed that the shoulder which probably attributed to the lignin connected to hemicelluloses was gradually shifted to the lower temperature up to 130°C corresponding to that of liberated lignin[36,37]. The amount of the dissolved substances increased with the increased steam pressure (Fig. 9).

1. 3 Changes in Chemical Structures of Wood Components by Steam Explosion

1. 3. 1 Separation of wood components

Steam-exploded wood was separated to hemicelluloses, lignin and cellulose fractions by two methods to characterize the chemical properties of EXW. Fig. 10 shows the procedure for separation of main components of EXW. The yields of the separated fractions are shown in Table 2. Water extractives (EXS) and methanol soluble fractions (EXL) were mainly composed of hemicelluloses and lignin, respectively. Dioxane-water (9 : 1 v/v) extractives (EXD) were a mixture of hemicelluloses and lignin which were separated by precipitation into water and subsequent extraction of the water solution with ethyl acetate to high molecular lignin (DL), water soluble lignin (DWL) and hemicelluloses (DW). Hemicelluloses in wood were easily hydrolyzed by steaming to oligosaccharides and converted into almost soluble materials (27.9% of wood) in water by only 1 min treatment at 20 kgf/cm^2. Severe treatments such as 8 min and 16 min steaming at 28 kgf/cm^2 decreased the yields of water soluble fractions and increased those of furfural and 5-hydroxymethylfurfural. Lignin was more resistant than hemicelluloses (Table 2) and was gradually degraded by steaming. The maximum yield of lignin was given at 8 min treatment at 28

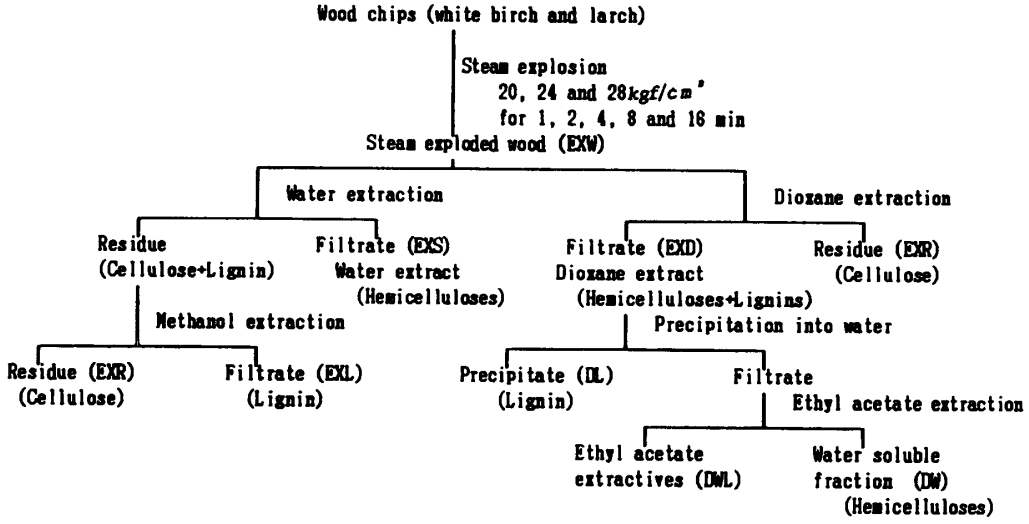

Fig. 10. Fractionation of steam-exploded wood (EXW).

Table 2. Contents of extractives and residual lignin in EXW

Samples			Extractives/EXW			Contents/dioxane extractives			Residual ignin
Species	Pressure kgf/cm²	Time min	Water EXS (%)	Methanol EXL (%)	Dioxane EXD (%)	DL (%)	DWL (%)	DW (%)	in EXR (%)
	20—	1	27.9	8.5	27.1	9.6	1.6	88.8	15.1
	24—	1	25.8	10.8	41.2	12.2	1.2	86.6	12.8
	28—	1	29.3	13.7	47.1	19.4	1.2	78.4	12.2
Shirakanba	28—	2	29.2	18.3	58.7	25.7	1.5	72.8	9.7
	28—	4	29.4	23.7	58.1	40.1	1.9	58.0	5.0
	28—	8	18.8	29.2	56.0	48.0	1.8	50.2	5.0
	28—	16	22.0	26.2	54.8	44.9	1.9	53.2	2.7
	0	0	—	—	—	—	—	—	22.3
	20	1	18.7	4.2	15.8	—	1.9	—	—
	24	1	21.4	6.8	28.9	—	1.8	—	—
	28	1	25.2	9.1	32.8	—	2.0	—	30.1
Karamatsu	28	2	27.0	10.3	36.3	19.4	2.1	79.5	27.2
	28	4	23.1	11.0	35.9	25.8	2.7	71.5	17.7
	28	8	18.8	11.3	33.2	26.3	2.8	70.9	11.5
	28	16	22.4	10.4	31.9	—	3.0	—	8.7
	0	0	—	—	—	—	—	—	34.8

kgf/cm² and then the yield was decreased by condensation reaction. Dioxane soluble fractions corresponded to the combined yield of EXL and EXS.

1.3.2 Changes of hemicelluloses

At a weak steam explosion condition (20 kgf/cm², 1 min) oligosaccharides were

main components of dioxane extractives but by enhancing steaming conditions the contents of lignin and monosaccharides were increased (Fig. 11). By 28 kgf/cm^2

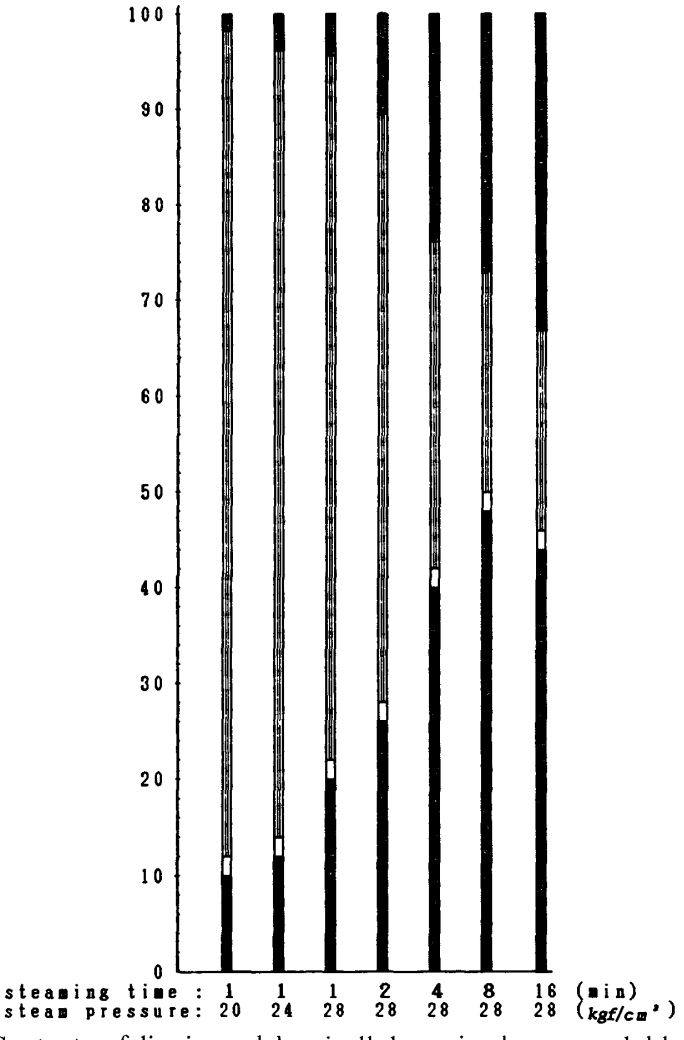

Fig. 11. Contents of lignin and hemicelluloses in dioxane soluble fraction of steam-exploded Shirakanba. ■; lignin, □; water soluble lignin, ▤; oligosaccharides, ▦; monosaccharides.

Table 3 Composition of monosaccharides in water soluble fraction of steam-exploded Shirakanba wood (EXW)

Sample	Unknown	Ara.	Xyl.	Man.	Gal.	Glc.	Total
20—1	—	0.8	1.1	—	—	—	1.9
24—1	—	1.0	3.4	—	—	—	4.4
28—1	—	1.5	3.9	—	—	—	5.5
28—2	1.0	2.6	8.8	0.6	0.7	0.7	14.4
(%)	(6.9)	(18.1)	(61.1)	(4.2)	(4.9)	(4.9)	(100)
28—4	1.6	0.3	28.8	1.9	2.5	5.7	40.8
(%)	(3.9)	(0.7)	(70.6)	(4.7)	(6.1)	(14.0)	(100)
28—8	2.1	2.6	37.5	2.5	3.0	6.0	53.7
(%)	(3.9)	(4.8)	(69.8)	(4.6)	(5.6)	(11.2)	(100)

for 8 min steaming more than a half of hemicelluloses were converted to monosaccharides (Table 3). In 2 min steaming at 28 kgf/cm^2 61.1% of monosaccharides was composed of D-xylose and the content of D-glucose was only 4.9% of monosaccharides. The result showed that cellulose in the exploded wood was hardly degraded to glucose (Table 3)[27,44].

1.3.3 Changes of lignin in wood

Residual lignin content of EXR was decreased with increasing steam pressure and reaction time. In 8 min steaming at 28 kgf/cm^2 the content of lignin was decreased to 2.7% of original wood which was caluculated from the data in Table 2 (residual lignin content was 5.0% of residual wood and the yield of residual wood was 44% of Shirakanba EXW). Then more than 94% of lignin in wood could be extracted by dioxane. In the case of a softwood (Karamatsu) the yield of extracted lignin was lower than in hardwood (Shirakanba) (Table 2). Recondensation of

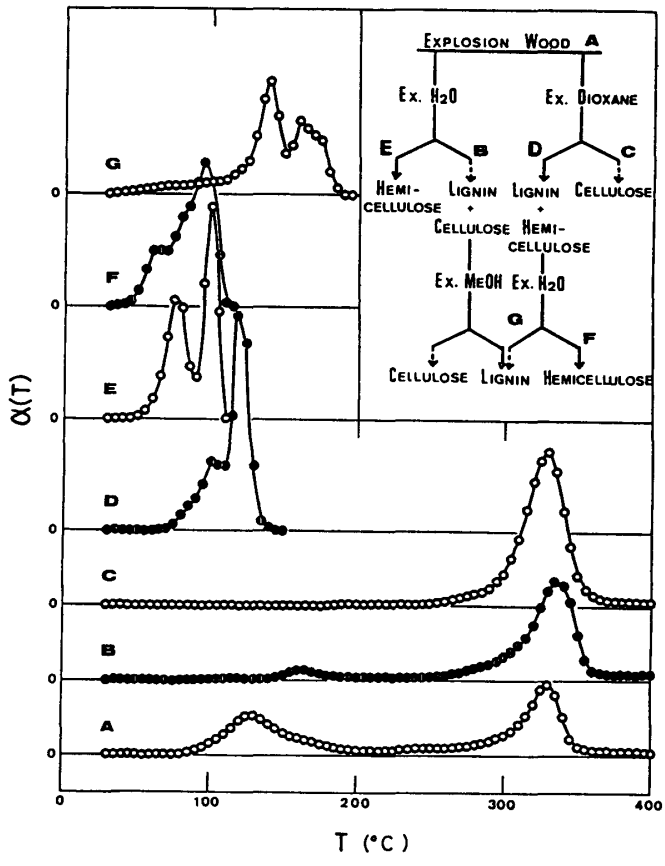

Fig. 12. Thermal softening analysis of the fractions of steam-exploded shirakanba (28 kgf/cm^2, 2 min. steaming).
A to G on respective curves denote the fractions separated from EXW shown in the upper right figure. α(T): rate of deformation at each temperature (T)

Table 4. Average molecular weight of steam-expoded lignin (EXL)

Sample	Pressure kgf/cm²	Time min.	$\overline{Mw}$	$\overline{Mn}$	$\overline{Mw}/\overline{Mn}$
Shirakanba	28	1	2200	780	2.8
	28	2	2110	800	2.6
	28	4	1890	880	2.1
	28	8	1870	900	2.1
	28	16	1130	780	1.5
	20	4	2300	860	2.7
	24	4	1900	730	2.6
	28	4	1890	880	2.1
Karamatsu	20	4	1630	740	2.2
	24	4	1330	690	1.9
	28	4	1460	690	1.2
	28	16	1220	630	1.9

the degraded softwood lignin would be the cause to decrease the yield of methanol and dioxane-water extractives and to disturb enzyme saccharification of the steam-exploded softwood.

The extracted EXWs with water, methanol and dioxane were subjected to the analysis of thermal softening properties (Fig. 12). The softening points of EXW (28 kgf/cm², 2 min) which was a mixture of steam-exploded cellulose, lignin and hemicelluloses appeared at 328°C, 160°C and 123°C, respectively (Fig. 12-A)[37,45]. By water extraction hemicelluloses (E) were extracted and the residue was composed of cellulose and lignin (B). By dioxane extraction a mixture of hemicelluloses and lignin (D) were extracted, and cellulose remained as residue (C). Lignin fraction (G) was extracted with methanol from water extracted residue (B), and hemicelluloses fraction (F) was separated from dioxane extractives (D) by precipitation from water. The fractions (D, E, F and G) gave two peaks of softening and melting points. The softening point (Ts) and melting point (Tm) of exploded lignin were 138°C and 169°C, respectively and these of exploded hemicelluloses were 77°C and 100°C, respectively. However, the fraction (D) which was a mixture of exploded hemicelluloses and lignin, was melted at 100°C. Molecular motion of lignin seems to become easy in melted hemicelluloses solution, and the softening and melting points of lignin would be shifted to lower temperature at 123°C. If both hemicelluloses and lignin were more higher molecular weight polymers and not melted, these lower shift of softening points would not be observed[37].

Molecular weight distribution of steam-exploded lignin (EXL) was measured by GPC, and weight average molecular weight (Mw), number average molucular weight (Mn) and a factor of dispersion (Mw/Mn) were calculated using a series of poly-

styrene standards. Molecular weights (Mw) of exploded lignin were decreased with increasing steam pressure and increasing period (20 kgf/cm^2: Mw=2300, 28 kgf/cm^2: Mw=1900, in constant steaming time for 4 min, and 1 min: Mw=2200, 4 min: Mw =1900, 16 min: Mw=1100, in constant steam pressure at 28 kgf/cm^2) (Table 4). The molecular weight of softwood exploded lignin was lower than hardwood exploded lignin in contrast to that of their native lignin and MWL. It seems that in the case of hardwood almost all exploded lignins were extracted by methanol but the extraction of exploded softwood lignins was rather difficult, and probably only lower molecular weight fraction of the exploded lignin was extracted with methanol.

^{13}C-NMR spectra of EXLs were shown in Fig. 13. EXL of 1 min steaming at 28 kgf/cm^2 of Shirakanba gave a similar spectrum to that of MWL. However, ether linkages of lignin (152, 110, 86, 72, 60 ppm) were gradually degraded with increasing steaming time and the spectrum of 16 min steamed EXL showed that aryl ether bonds were almost degraded but intensity of the carbonyl groups in the spectrum of EXL was very weak. If lignin degradation by steam explosion occurred through acidolysis reaction, carbonyl groups would be more increased followed by increasing of phenolic hydroxyl groups in EXW. The spectrum of the lignin from 16 min steamed EXW showed the increase of resinol (Cα; 86.9 ppm and Cβ; 54.8 ppm) and phenylcoumarane structures (Cα: 88.1 ppm and Cβ: 51.1 ppm) compared with the amounts of both structures in 1 min steamed EXL.

Water soluble lignin (DWL) was a mixture of low molecular weight lignin

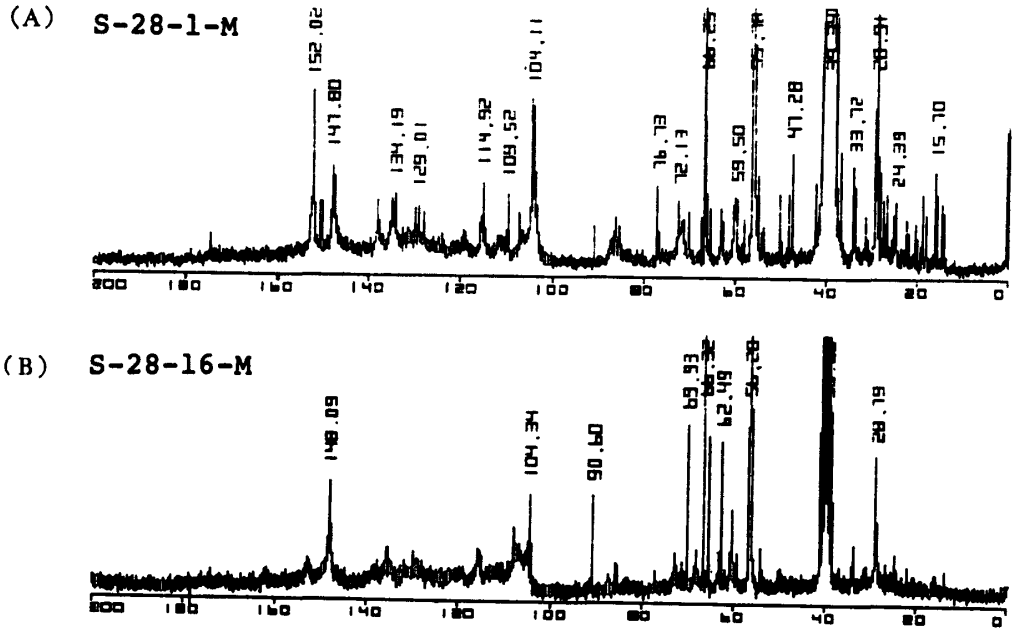

Fig. 13. ^{13}C-NMR spectra of methanol soluble fractions (EXL) from steamexploded Shirakanba. (A): 1 min, and (B): 16 min steaming at 28 kg/cm^2.

degradation products, phenolic extractives, 5-hydroxymethylfurfural and organic acids. From this fraction vanillin, syringaldehyde, vanillic acid, syringic acid, coniferyl aldehyde, sinapaldehyde, coniferyl alcohol, sinapyl alcohol and a mixture of *d*, l-epi- and *d*, *l*-syringaresinols were separated by TLC and determined by ^{1}H-NMR. However, acidolysis monomers were not detected from DWL, and therefore the degradation of lignin by steam explosion would not occur through acidolysis[46~49]. We supposed that the degradation of lignin by steam explosion occurs through homolytic cleavage of aryl ether linkage of lignin. Detailed study of the degradation mechanism of lignin by steam explosion will be discussed in chapter 2 with results on the steam explosion of lignin substructure model compounds (guaiacylglycerol- and syringylglycerol-β-guaiacyl ethers)[30].

1.4 Transformation of Cellulose Crystals and Changes of Crystallinity by Steam Explosion

1.4.1 Microfibril width and length

After the steam explosion of the wood, individual microfibrils of cellulose could be observed clearly by TEM (Fig. 14-1, 2, 4, 8 and 16), whereas cellulose microfibrils of untreated wood could be seen only after some mechanical treatment such as homogenization (Fig. 14-0). It was observed that the microfibrils were cut longitudinally for the widths to be shortened by the steam explosion process. The values of microfibril widths are summarized collectively in Fig. 15. The increases of the widths, judged by the mean values of microfibril widths, were similar to those of the micelle widths by X-ray diffraction. Fig. 16 shows an electron micrograph of large microfibrils of exploded wood as indicated by the arrow heads. Large microfibrils more than 100 Å in width compared to those of untreated wood (20~40 Å) were observed, and some microfibrils were observed to be fused together as indicated by the double arrow head. In most cases, the microfibrils were cut short in length, but their widths rather increased.

The steam explosion process could be divided into three stages: 1) the elevation of the temperature of the digester, 2) holding steamed conditions of high temperature and pressure, and 3) the release of the pressure (explosion). The present investigation focused on the effect of the pressure release (the third stage) on the transformation of cellulose. Thus, Shirakanba wood was heated to 230°C under a pressure of 28 kgf/cm^2; the pressure was held for 8 min, and then the digester was cooled overnight to room temperature without opening the valve. Samples obtained by this process were referred to as being "anneeled". As an experiment to eliminate hydrolysis at a high temperature and an explosion, wood chips were treated as in the annealing process, and the digester was cooled rapidly by the slow release of

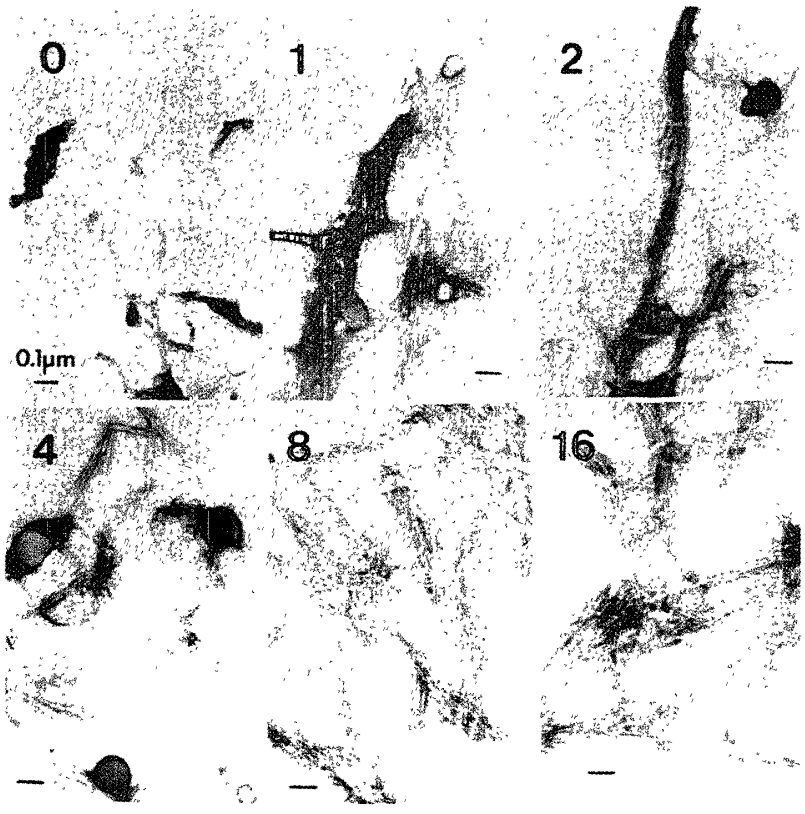

Fig. 14. Electron micrographs of cellulose microfibrils of steam-exploded white birch (Shirakanba).

Note: 0, homogenized white birch. The numbers indicate the steaming times (min) at 230°C.

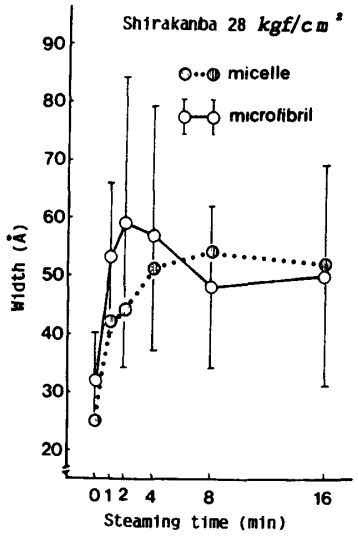

Fig. 15. Changes of micelle width and microfibril width of exploded white birch (Shirakanba) with steaming time.

Note: 0 min, unexploded original wood.

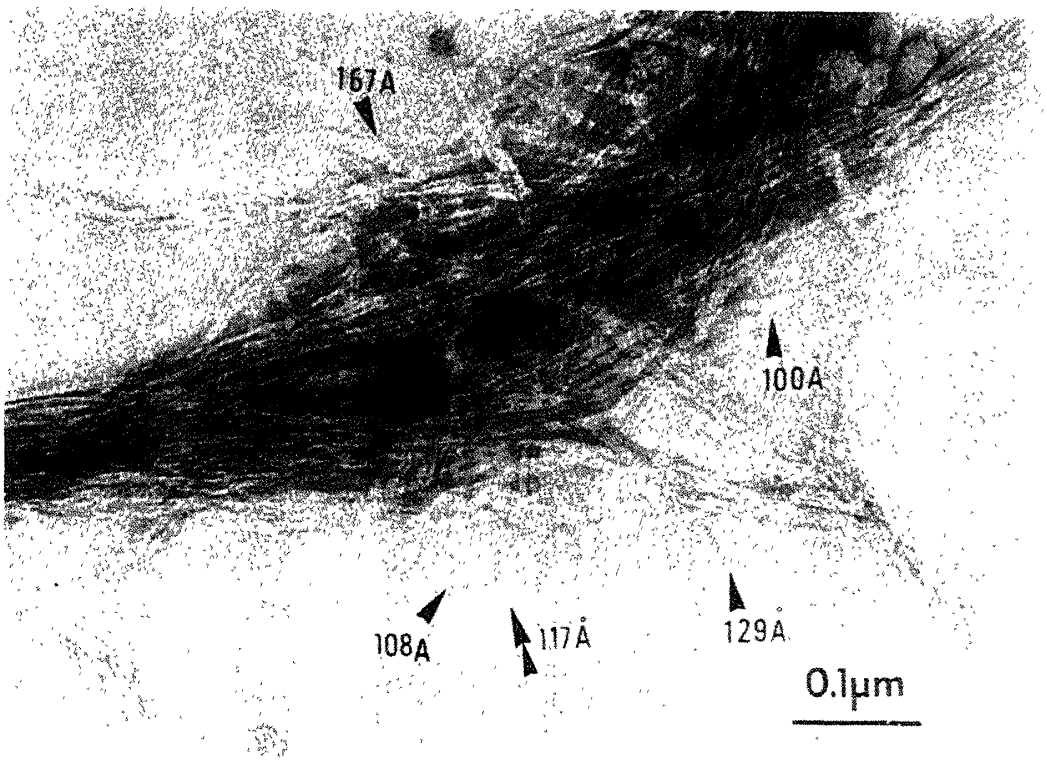

Fig. 16. Electron micrograph of steam-exploded white birch (Shirakanba) (28 kgf/cm², 2 min steaming).

Note: Arrowheads indicate large microfibrils, and some fibrils are fused with other microfibrils (double arrowhead).

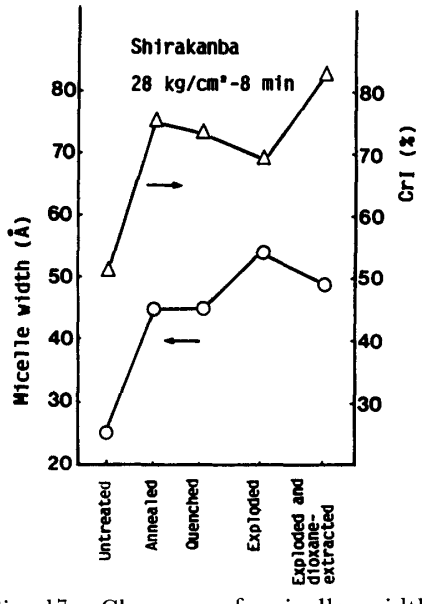

Fig. 17. Changes of micelle widths and crystallinity indices of steam-exploded white birth (Shirakanba) caused by the explosion process.

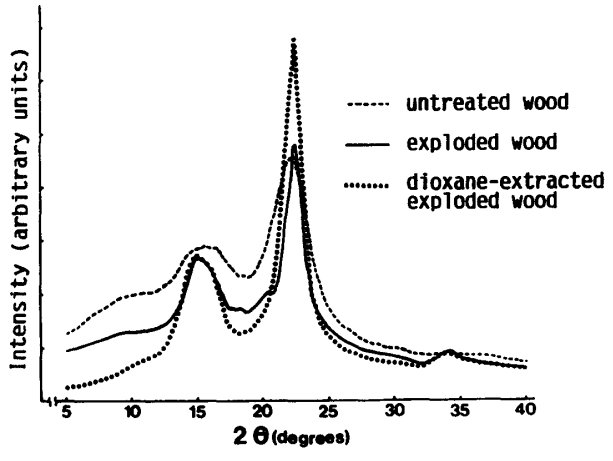

Fig. 18. X-ray diagrams of steam-exploded white birch (Shirakanba) compared with untreated wood.

the steam for 10 min. The samples produced were referred to as being "quenched". The micelle widths and crystallinity indices of the exploded wood, the above treated wood, dioxane-extracted exploded-wood, and untreated wood were compared (Fig. 17). The results indicated that the increases of micelle widths and cellulose crystallinity were caused only by the high temperature and steam pressure independently of the explosion.

1. 4. 2 Crystallinity and micelle width

X-ray diagrams of Shirakanba wood before and after the explosion process and of dioxane-extracted exploded-wood are shown in Fig. 18. The peak of (002) spacing indexed by a Meyer-Misch model was remarkably sharper after the explosion, and this feature was strengthened further after the dioxane extraction.

Changes of micelle widths were observed with varying steaming times at a constant steam pressure (Fig. 15). The micelle widths changed with increasing steaming times, and the maximum value was observed after 8 min of steaming. The micelle width of the exploded wood (52 Å) was more than twice that of the original materials (25 Å).

Thus, to examine the possible influences of other wood constituents on the increased crystallinity of cellulose, several cellulose materials were subjected to steam explosions. Figs. 19 and 20 show the differences of crystallinity indices and of micelle width, respectively, of Shirakanba, Karamatsu, NBKP, LBKP and filter paper before and after the explosion process. Cellulose of the wood preparations (Shirakanba and Karamatsu) were increased in both crystallinity and micelle widths, whereas the crystallinity and micelle widths of the pulps and filter paper were

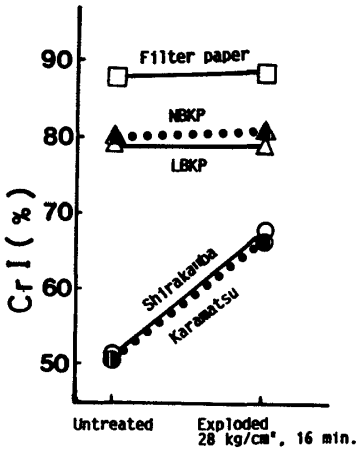

Fig. 19. Crystallinity indices of steam-exploded cellulose materials before and after the explosion treatment.

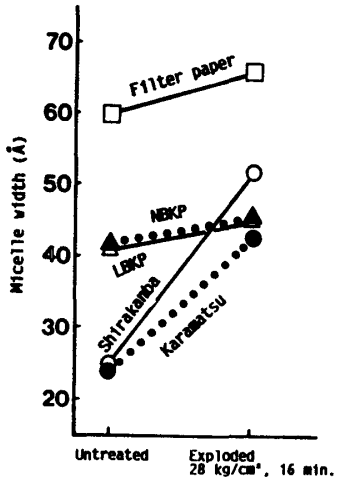

Fig. 20. Micelle widths of steam-exploded cellulose materials before and after the explosion treatment.

constant or increased only slightly.

1.4.3 Changes of cellulose crystalline form

For both hardwood (Shirakanba, white birch) and softwood (Sugi, Japanese cypress, *Cryptomeria japonica* D. Don), CP/MAS ^{13}C-NMR spectra before and after steam-explosion are shown in Figs. 21 and 22, respectively. Cellulose crystallinity was calculated by measuring each crystalline and noncrystalline area of carbons C_4 and C_6. The C_4 and C_6 areas at 82~93 ppm and 61~69 ppm, respectively, of the entire spectra in Fig. 21-(1) show two peaks, but these areas in the crystalline component spectra show only one lower field peak. Then it was decided to be separated crystalline and noncrystalline components, of both C_4 and C_6, into lower and upper fields, respectively[50]. Crystallinity of the wood polysaccharide was calculated from these peaks in the entire spectra. After steaming, the noncrystalline areas of C_4 and C_6 in the entire spectra decreased (Fig. 22). Because the signals from the hemicelluloses overlapped in these spectra, crystallinities were expressed as relative values for the effect of the steam explosion treatment. However, from the results of X-ray and ^{13}C-NMR analysis, the crystallinity of cellulose was seemed to have been increased by the steam explosion.

There are two types of crystalline forms in ^{13}C-NMR spectra for native cellulose: a cotton-ramie type (Cellulose Ia) (Fig. 23) and a bacteria-valonia type (Cellulose

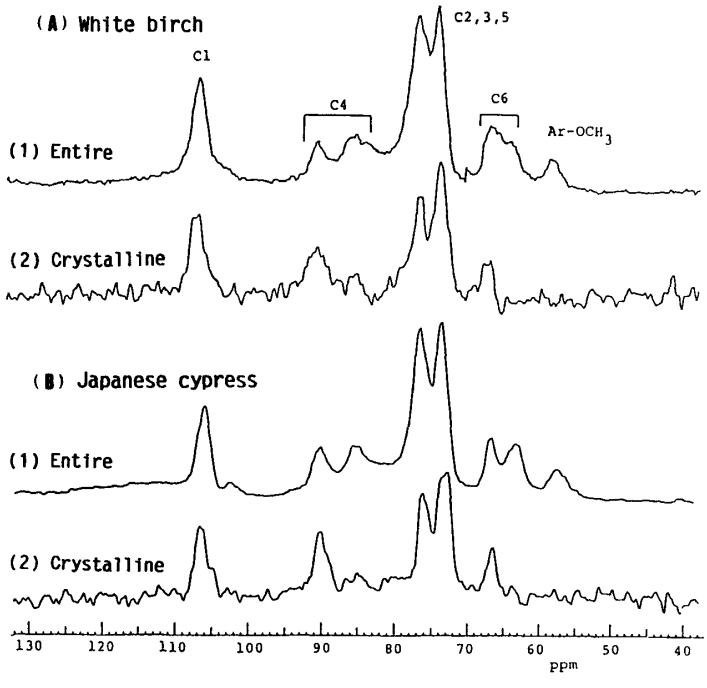

Fig. 21. CP MAS ^{13}C-NMR spectra of wood cellulose, (A): white birch (Shirakanba). (B): Japanese cypress (Sugi), (1): Entire spectra, (2): crystalline component spectra.

Ib) (Fig. 24)[51]. However, wood cellulose could not be assigned to Types Ia or Ib because the spectra of the crystalline component of the original celluose have broad peaks in the respective species' carbon regions (Fig. 21-(2)). From a comparison of the peak widths of C_1, C_4, and C_6 in the crystalline component spectra of the woods with those of valonia and cotton cellulose, the crystalline form of intact wood-cellulose would be identical with Cellulose Ib rather than Ia. However, the spectra of the crystalline component showed that the crystalline form clearly changed after the steam explosion. Steam-exploded cellulose had fine, doublet peaks (C_1: 104～109, C_4: 87～90, and C_6: 64～68 ppm, Fig. 22-(2)), and these spectra were similar to cotton-cellulose crystalline (Cellulose Ia). Horii and others showed the transformation of the cellulose crystalline form by a high-pressure saturated-steam treatment at a high temperature by CP/MAS ^{13}C-NMR[51]. The crystalline form, Type Ib, of valonia and bacteria cellulose was transformed to Cellulose Ia' which was almost identical to Cellulose Ia, by increasing the steam temperature. For valonia cellulose, 30 min of steaming at 260°C was required for complete transformation, and for 30 min of steaming at 230°C, only half of the transformation occurred (Fig. 24). However, the present investigation showed that only 4 min of

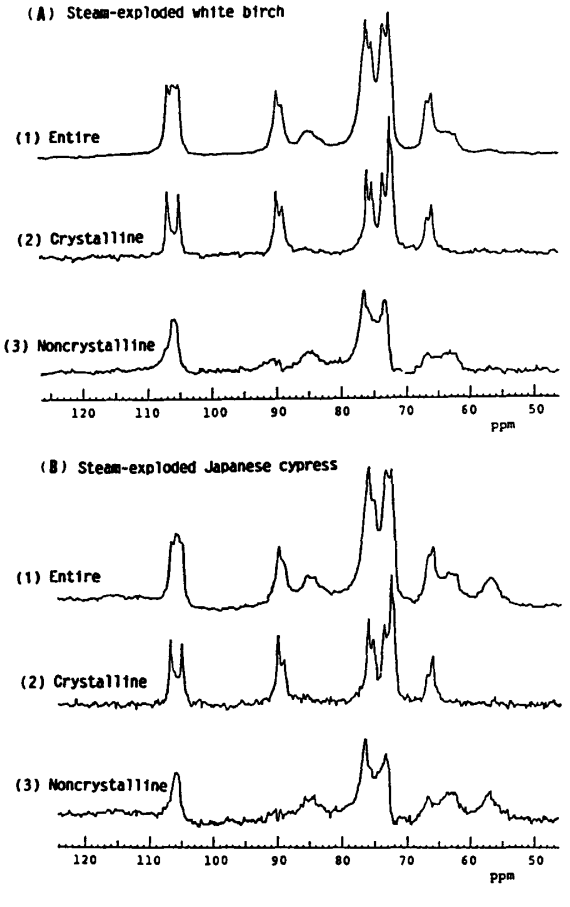

Fig. 22. CP/MAS ^{13}C-NMR spectra of steam-exploded white birch (Shirakanba) and Japanese cypress (Sugi) woods. (A): steam-exploded white birch (Shirakanba), (B): steam-exploded Japanese cypress (Sugi), (1): Entire spectra, (2): Crystalline component spectra, (3): Noncrystalline component spectra.

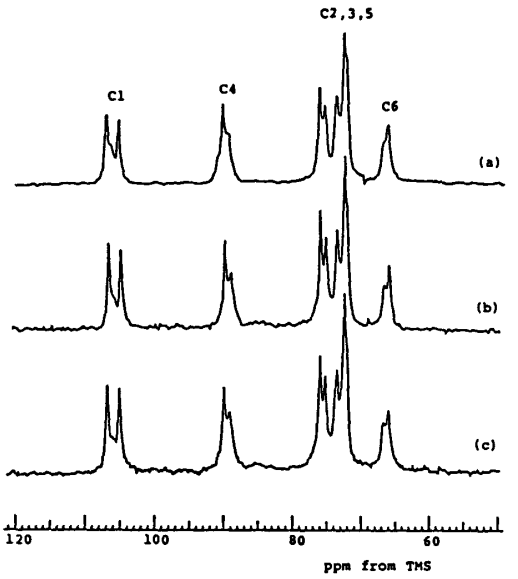

Fig. 23. CP/MAS ^{13}C-NMR spectra, 50 MHz, of the crystalline components of cotton cellulose treated with steam at different temperatures: (a) original, (b) 230 C, (c) 260 C.

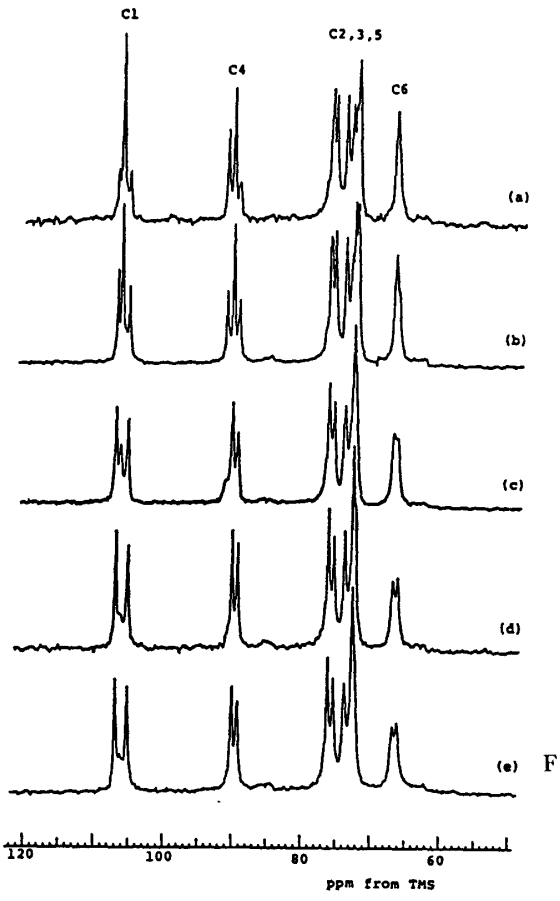

Fig. 24. CP/MAS ^{13}C-NMR spectra, 50 MHz, of valonia cellulose treated with steam at different temperatures: (a) original; (b) 230°C; (c) 245°C; (d) 260° C; (e) 280°C.

steaming is enough for complete transformation of the crystalline form of wood cellulose to Cellulose Ia'. Thus, the crystalline form of original wood-cellulose was considered to be of a less-ordered orientation and was tranfsormed to Cellulose Ia'

by increasing the order of orientation and crystallinity by the steam explosion. In the case of the filter paper which was made of cotton linter, the crystallinity of the cellulose increased in the entire spectra after washing it with water, and the crystalline form changed only slightly from Ia to Ia′.

1.4.4 Thermostability of cellulose crystals

Thermal softening or the degrading temparatures of original cellulose in Shira kanba and Karamatsu woods were 330°C and 332°C, respectively. On the other hand, those of steam-exploded woods were shifted to higher temparatures in the case

Table 5. Crystalline structure of native and steam-exploded cellulose.

Sample	Crystallinity (%)			Microfibril Width(Å)		Microfibril Length(Å)	Crystalline form	Thermal softening temperature (°C)
	X-ray	13C-NMR		X-ray	TEM	TEM		
	CrI	C4	C6	(002)				
Shirakanba								
(original)	51	43	58	25	32	∞	? (Ib)	330
28 kgf/cm², 1 min	64	—	—	42	53	—	—	335
2	67	—	—	44	59	—	—	330
4	70	66	69	51	58	1900	Ia′	330
8	70	—	—	54	48	2000	—	329
16	67	68	64	52	50	2000	—	330
Karamatsu								
(original)	50	—	—	24	—	—	—	332
28 kgf/cm², 1 min	65	—	—	42	—	—	—	338
2	68	—	—	41	—	—	—	340
4	69	—	—	45	—	—	—	337
8	69	—	—	44	—	—	—	336
16	65	—	—	43	—	—	—	337
Sugi								
(original)	48	51	46	—	—	—	? (Ib)	332
28 kgf/cm², 4 min	63	62	59	—	—	—	Ia′	336
Filter paper	88	75	67	60	—	∞	Ia	—
28 kgf/cm², 16 min	89	83**	74**	67	80	1000	Ia′	—
Cotton*	77	72	70	47	—	∞	Ia	337
49 kgf/cm², 30 min*	88**	70**	61**	62	76	1200	Ia′	334
Valonia*	90	87	90	143	—	—	Ib	—
28 kgf/cm², 30 min*	90**	89**	—	—	—	—	Ia+Ib	—
49 kgf/cm², 30 min*	95**	90**	99**	108	140	1400	Ia′	—

* : These data were taken partially from 11) and the reaction conditions were only steaming without explosion.

**: These data were observed on the sample after washing it with water.

of short-time steaming (335°C and 340°C, respectively). However, in the case of longer steaming times, they were shifted to lower temparatures again. These data are summarized in Table 5. The maximum softening temperature (338°C) of exploded Shirakanba was obtained with 1 min of steaming at 24 kgf/cm^2 of steam pressure.

1.4.5 Discussion

The increases of microfibril or micelle widths of cellulose caused by a steam explosion can be explained by the following three main reactions: 1) rearrangment or reorientation of cellulose molecules inside and near the crystalline region of microfibrils by relaxation caused by high temparatures and pressures or 2) by removal of other components such as hemicelluloses and lignin, and 3) crystalline fusion with adjacent microfibrils by removal of hemicelluloses and lignin.

Steam at ligh temperatures and pressures is greatly ionized to H^+ and OH^- ($[H^+][OH^-]/[H_2O]=10^{-7}$)[52]. The activated steam reacts rapidly with polysaccharides and hydrolyzes them to smaller molecular-weight sugars. In addition, acetic acid formed from the acetyl groups of hemicelluloses, and levulinic and formic acids partially formed by degradation of the hemicelluloses, catalyze the hydrolysis of carbohydrates. On the other hand, lignin is degraded by steam explosion mainly through the homolytic cleavage reaction of the aryl ether linkage as discribed in Chapter 2. By these reactions the wood constituents were degraded partly to become mobile, and then the inner stresses in the crystalline region of cellulose would be loosened. Under such a condition, the crystallinity of wood cellulose could be increased by rearrangement or reorientation of the cellulose molecules of the paracrystalline regions during steaming. On the other hand, in relatively pure cellulose materials such as NBKP, LBKP, or filter paper, almost constant crystallinity was observed independent of steam explosions. This is ascribed to the fact that original materials do not contain hemicelluloses which affect the rearrangement of paracrystalline regions.

The fusion of microfibrils to become greater fibrils observed by TEM (Fig. 16) can be ascribed to the fact that lignin in intermicrofibril spaces becomes soluble or mobile by heating and is removed. Softening temparatures of native lignin and hemicelluloses complexes are 220~300°C[26,37,53] and those of isolated lignin and hemicelluloses are 153~186°C[36] and 167~181°C[54], respectively. However, the appatrent melting point of steam-exploded lignin is 150~190°C[27] in a dried condition and is assumed to be less in a wet condition. Steam-exploded lignin was observed to be eluted from its original inter-microfibril location as oil droplets[26,30]. On the other hand, hemicelluloses were hydrolyzed rapidly and their bondings with cellulose or lignin could be cleaved, and then steam-exploded hemicelluloses were almost

soluble in water. The mobility of these components caused by steaming make cellulose free from other constituents in wood. The free cellulose can be fused together under high pressure to make larger microfibrils or crystallines (Fig. 16).

The fact that the width of cellulose crystallites of steam exploded wood increased much greater than that of pure cellulose (Fig. 19 and Fig. 20) suggests that the latter contains less paracrystalline region, and that the increase of crystallinity may depend on the quantity of amorphous cellulose. The quenching and annealing experiments (Fig. 17) indicated that the increase of crystalline width was caused by heating up at steam pressure but not by the explosion process. The present experiment showed that microfibril width has a maximum peak at a steaming time of 2 min, and that further steaming causes a decrease of the crystalline widths of microfibrils by a gradual hydrolysis of the cellulose at the surface of the crystallites. The same result was obtained in the thermal softening temperature of cellulose. The softening temparature produced a mobility of the molecule which is related to molecular weight and the strength of the hydrogen bond of the crystallites. The increase in the softening temperature of cellulose suggests that the width of the crystallites increased in the early steaming, and the softening temperature gradually decreased with the decrease of crystalline size.

On the other hand, the lengths of the cellulose microfibrils were decreased by the steam explosion. The lengths of original cellulose microfibrils of woods and other materials were to long to be measured. However, the lengths of steamed cellulose microfibrils were almost 1000~2000 Å under TEM. The earlier decrease in thermal softening temperature than the decreases in micelle width and in crystallinity would be caused by the decrease in the molecular weight of the cellulose.

The crystalline form was changed gradually from Cellulose Ib to Ia′ in valonia cellulose during steam treatment. However, the cellulose crystallites of the original wood was of a less-ordered orietation and easily transformed to Cellulose Ia′ crystalline form by the steam explosion (Fig. 21 and Fig. 22).

These results suggested that there were three stages in the reaction of cellulose to a steam explosion. In the first stage of steaming, hemicelluloses and paracrystalline cellulose were hydrolyzed partially, and the inner stress in the crystalline region of the cellulose was loosened. Then paracrystalline cellulose was relocated to the crystalline region, and the widths of cellulose microfibrils increased. In the second stage, microfibrils were cut at some nodes of the cellulose crystallites to give microcrystalline cellulose, and the lengths of the microfibrils decreased to 1000 ~2000 Å. In the third stage, the surfaces of cellulose crystallites gradually were hydrolized. Then the microfibril widths and crystallinity of the cellulose decreased. In addition to these reactions of cellulose during steaming, transformations from

Cellulose Ib or Ia to Ia' of cellulose crystalline form were accomplished.

1.5 Summary

Wood chips of Shirakanba and Karamatsu were treated with a high pressure steam (12～28 kgf/gm^2) for 1～16 min, and the steam pressure was released instantaneously to result in steam-exploded wood. When the treating time was longer more fibrillation of cell walls of Shirakanba occurred. Fibers of the exploded woods were observed to be vigorously ruptured.

Chemical changes of main components in wood (cellulose, hemicelluloses and lignin) by steam explosion process have been elucidated by ^{1}H- and ^{13}C-NMR, gas chromatography, GPC and thermal softening property. By steam explosion hemicelluloses were rapidly hydrolyzed to lower molecular weight products. Almost all hemicelluloses (27.9%) in Shirakanba wood were hydrolyzed to oligosaccharides to be extracted with water by only one min steaming at 20 kgf/cm^2, and by 8 min steaming at 28 kgf/cm^2 53.7% of hemicelluloses were converted to monosaccharides. Monosaccharides obtained by 2 min steaming of Shirakanba wood were composed of 61.1% of xylose and only 4.9% of glucose, and the yields were in acord with original composition of hardwood hemicelluloses. Lignin was degraded slower than hemicelluloses. The yield of lignin was 29.2% in maximum by 8 min steaming at 28 kgf/cm^2, and the molecular weights of lignins obtained were decreased to Mw= 2100 and 1100 by 2 min and 16 min steaming, respectively. A mechanism of lignin degradation by steam explosion was presumed to be homolytic cleavage of aryl ether linkage. Chemical changes of cellulose caused by steam explosion were examined by X-ray diffraction, transmission electron microscopy, and CP/MAS ^{13}C-NMR spectroscopy. Cellulose in non-crystalline area was partially hydrolyzed, and micelle length was decreased to about 2000 Å by 8 min steaming at 28 kgf/cm^2. However, cellulose was not hydrolyzed to glucose, and non-crystalline cellulose would be annealed and transformed to crystalline cellulose. Thus the crystallinity and micelle width of cellulose were increased by steam explosion. By a steam explosion (28 kgf/cm^2, 230°C, 16 min) cellulose in Shirakanba wood was increased in crystallinity (CrI: 51% to 67%), micelle width (25 Å to 52 Å), and microfibril width (32 Å to 50 Å). The crystalline form of cellulose clearly was changed by the steam explosion: broad peaks in the CP/MAS ^{13}C-NMR spectrum of the crystalline component of the wood cellulose assigned at C_1, C_4, and C_6 of the pyranose ring changed to fine double peaks of crystal form, Cellulose Ia. It also was found that the crystallinity of cellulose is increased by steaming the wood at high temperatures and pressures without explosion. However, purified, greatly crystalline cellulose, such as filter paper, was influenced less in crystallinity by steaming, and the results suggested that other constituents accompanying cellulose were involved in

the incease of crystallinity of the cellulose by the steam explosion.

2. MECHANISM OF LIGNIN DEGRADATION BY STEAM EXPLOSION

2.1 Introduction

Morphology, physical properties, and chemical changes of steam-exploded wood (EXW) were discussed in chapter 1. Carbon 13 nuclear magnetic resonance (^{13}C-NMR) studies of steam-exploded lignin (EXL), and solid state cross-polarization magic-angle spinning (CP/MAS) ^{13}C-NMR studies of steam-exploded woods were reported by MARCHESSAULT and others[13], BARDET and others[54], HEMMINGSON[22~24], and TEKELY and VIGNON[25]. These studies partly characterized chemical and physical structures of EXL. However, the mechanism of lignin degradation by steam explosion has not been well elucidated.

The present author showed in the previous chapter that comparatively large yields of syringaresinol and coniferyl alcohol were obtained from the ether-soluble fraction of EXL of Shirakanba possibly through the homolytic cleavage of β-O-4 ether linkages of lignin by steam explosion[27].

This chapter describes the mechanism of lignin degradation by steam explosions using β-O-4 lignin substructure model compounds[30].

2.2 Degradation Products of Lignin and β-O-4 Lignin Substructure Model Dimers

2.2.1 Chemical and morphological changes of lignin in wood

By steam explosions (230°C, 28 kgf/cm^2), rapid chemical degradation of wood components occurs accompanied by physical ruptures of wood by the adiabatic expansion of water in wood and by machanical destruction of the wood chips when passed through the narrow nozzle of a blow valve. By the explosion, wood chips were defibrillated mostly to single fibers. An electron micrograph (Fig. 25) showed that cellulose and lignin were oriented alternately parallel to lumen surfaces, and that lignin droplets were arranged parallel between the cellulose lamellae, although the lamella structure was enlarged twice to three times the thickness of the original fibers by swelling of the cell walls in the steam explosion. Lignin in the secondary walls of fibers was degraded easily to low-molecular weight fractions by cleavage of the aryl ether linkages after only 4 min of steaming, and melted by high-temperature steam. While, middle-lamella lignin was resistant to steaming, the major part of the lignin melted and transfered from the middle lamellae and secondary walls to give small oily droplets. Differences of reactivities of the lignins can be ascribed to the differences of the chemical structures and the concentrations of lignins between the secondary walls and middle lamellae.

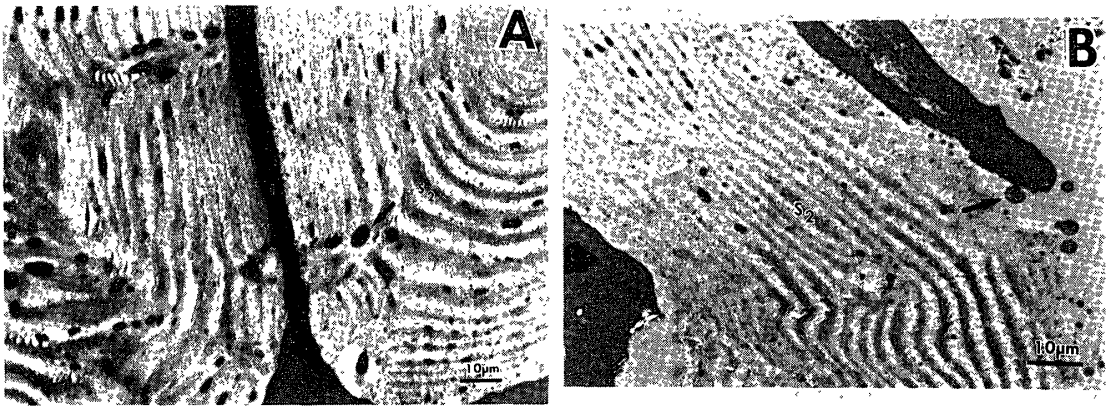

Fig. 25. Photograph of steam exploded Shirakanba wood under a transmission microscope.
Notes: Steam explosion (28 kgf/cm², 16 min.). $KMnO_4$-stained.

Table 6. Yields of extractives from exploded wood.

Fractions	Weight (g)	%/dry wood (%/fractions)
Exploded wood (oven dry) (EXW)	192.5	100
Waer extract (EXS)	42.6	22.1
Dioxane extract (EXL)	56.0	29.0
Ether insoluble fraction (EXL-EP)	34.1	17.7 (60.9)
Ether soluble fraction (EXL-ES)	21.9	13.4 (39.1)
Water soluble (EXL-ESW)	4 64	2.41 (41.7)
Acid fraction (EXL-ESA)	1.46	0.76 (13.1)
Phenolic fraction (EXL-ESP)	3.25	1.69 (29.2)
Neutral fraction (EXL-ESN)	1.78	0.92 (16.0)

The EXSs were composed mainly of hemicelluloses and a small amount of water-soluble lignin degradation products, EXLs were mainly lignin, and residual products after these extractions (EXRs) were mainly cellulose. The yields of these fractions are shown in Table 6.

2.2.2 Characteristics of steam-exploded lignin

The degradation rate of the lignin by steam explosion was smaller than that of hemicelluloses[27]. Dioxane extracted lignin (EXL) was analyzed by ^{13}C-NMR. The ether linkages of lignin (67～73 ppm: Cα; 82～86 ppm: Cβ; 61～64 ppm: Cr; 105 ppm: $S_{2,6}$; 112～115 ppm: $G_{2,5}$; 138 ppm: $S_{1,4}$; and 152 ppm: $S_{3,5}$) were degraded gradually with an increase in steaming time and were degraded mostly by 8 min steaming. The amounts of resinol and phenylcoumarane substructures (56 and 54 ppm: Cb; respectively) and free phenolic hydroxyl groups (147～148 ppm) in the lignin increased. ^{1}H-NMR spectrum of the ether-soluble phenolic fraction of the

Table 7. Contents of phenolic hydroxyl groups and conjugated carbonyl groups in exploded wood lignin (EXL) and acidolyzed MWL.

Sample	Phenolic hydroxyl (%/C_6-C_3)	Conjugated carbonyl (%/C_6-C_3)		
		Nonphenolic	Phenolic	Total
MWL	8.7	5.6	2.2	7.8
MWL (after acidolysis)	32.3	11.0	8.0	19.0
EXL-EP	22.1	1.3	2.7	4.0
EXL-EP (after acidolysis)	11.6	3.8	3.5	7.3
EXL-ES	43.5	1.0	4.6	5.0
EXL-ES (after acidolysis)	30.9	1.6	4.4	6.0

lignin showed that α- and β-protons of *d,l*-syringaresinol (4.7 ppm: α-CH; and 3.2 ppm: β-CH), *d,l*-episyringaresinol (4.3 and 4.9 ppm: α-CH; and 2.9 and 3.4 ppm: β-CH, respectively), α-proton of phenylcoumarane (5.5 ppm), and the double bond (Cα-Cβ) structure of the side chain increased during the steam explosion (6.2~6.7 ppm). The result suggested that syringaresinol, phenylcoumarane, cinnamyl alcohol, and cinnamyl aldehyde structures in steam-exploded lignin could be produced from the β-O-4 ether bond of the original lignin. The molecular weight of the THF-soluble fraction of EXL decreased to about 2000 by the explosion at 28 kgf/cm^2, 16 min. More than 90% of the lignin in the wood was converted to the dioxane soluble fraction. The fraction was separated into ether soluble (EXL-ES) and insoluble (EXL-EP) fractions, and the phenolic hydroxyl groups of these fractions were estimated to be 44 and 22%/C6-C3, respectively. The EXL-ES fraction amounted to 40% lignin. The average value of free phenolic hydroxyl groups of EXL was about 30%/C6-C3, almost the same as that of the acid degradation products of MWL (Table 7). These results suggested that the degradation of lignin by steam explosion is apparently similar to acidolysis reaction which includes cleavage of the α- and β- ether linkages followed by an increase of phenolic hydroxyl groups. However, the result showed that the content of carbonyl groups of the steam-exploded lignin were very much smaller (4 and 6%/C6-C3 in the EXL-ES and EXL-EP fractions, respectively) than in the acidolysis of MWL (19%/C6-C3) (Table 7). Therefore, the cleavage reaction of lignin by explosion is different from that in acidolysis.

2.2.3 Separation of steam-exploded lignin

The EXL-ES was separated into the four ESW, ESA, ESP, and ESN fractions. The yields of these fractions are shown in Table 6. ESP was the main fraction of EXL-ES. From the ESP fraction, vanillin (5), syringaldehyde (5'), coniferyl alcohol

Table 8. Degradation products in the ether-soluble fraction (EXL-ES) of explodeed Shirakanba.

Fractions	Products
Phenolic (EXL-ESP)	*d,l*-syringaresinol (9'), *d,l*-episyringaresinol (10'), dehydrodiconiferyl alcohol (11), sinapaldehyde (4'), coniferyl aldehyde (4), coniferyl alcohol (2), sinapyl alcohol (2'), vanillin (5), and syringaldehyde (5').
Acid (EXL-ESA)	vanillic acid (7), and syringic acid (7').
Water sol. (EXL-ESW)	furfural (29), and 5-hydroxymethylfurfural (30).
Neutral (EXL-ESN)	betulin (31).

(2), coniferaldehyde (4), sinapyl alcohol (2'), sinapaldehyde (4'), *d,l*-syringaresinol (9'), *d,l*-episyringaresinol (10') and dehydrodiconiferyl alcohol (11), from the acid fraction (ESA) vanillic acid (7) and syringic acid (7'), and from EXL-ESW furfural (29) and 5-hydroxymithylfurfural (30) were isolated and identified by ^{1}H-NMR, ^{13}C-NMR, and GC-MS. The NMR and GC-MS spectra of isolated compounds were identical to those of authentic compounds. Betulin (31) was crystallized from the neutral fraction (ESN) (Table 8). These degradation products indicated that the mechanism of lignin degradation accompanied by steam explosion is different from acidolysis but similar to mild hydrolysis.

2. 2. 4 Steam explosion of guaiacylglycerol-β-guaiacyl ether

Guaiacylglycerol-β-guaiacyl ether (1) was treated under the same explosion conditions described above: namely, 28 kgf/cm^2 for 16 min. However, 80% of the starting material remained intact, and the major degradation products were coniferyl alcohol (2), its γ-methyl ether (13), and guaiacol (3). Coniferyl aldehyde (4), vanillin (5), vanillyl alcohol (6), vanillic acid (7), dehydrodiconiferyl alcohol (11), *d,l*-pinoresinol (9), and *d,l*-epipinoresinol (10) were separated by TLC from chloroform extractives and identified by NMR in comparison with the spectra of authentic compounds (Fig. 26). Guaiacylglycerol (8) was identified from the water-soluble fraction. A large amount of 5-hydroxymethylfurfural (30) derived from the cellulose used as a matrix for model compound (1) by a steam explosion was detected. Large amounts of cellulose (100~200 g for 200 mg samples) as a matrix were required for the steam explosion by using a 2 *l* digester for our explosion device. A sample compound was set at the top of the cellulose matrix. Because the lower part of the digester was filled with drained water during steaming, the degradation reaction of the sample by the steam hardly was effected. Nevertheless, 80% of the starting

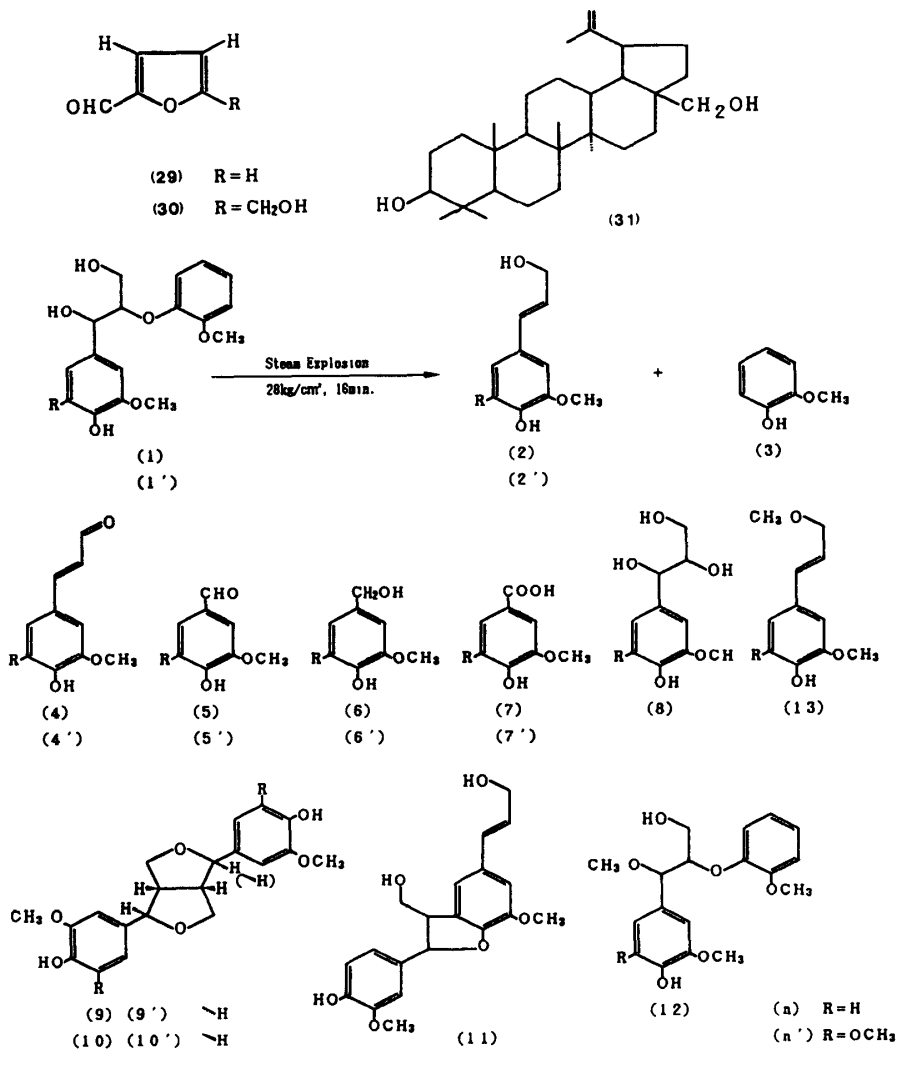

Fig. 26. Degradation products of guaiacylglycerol- and syringylglycerol-β-guaiacyl ethers resulting from a steam explosion.

material remained without degradation because the sample was dissolved in hot water condensed from steam. The methyl derivative of the starting material (12) and γ-methyl coniferyl alcohol (13) were produced when methanol was used as a solvent for the starting material. The result suggested that the degradation reaction occurred via the quinonemethide intermediate (21), which could act effeciently in the degradation of the β-ether linkage by a resonance effect.

Mainly coniferyl alcohol and guaiacol were obtained by the steam explosion of guaiacylglycerol-β-guaiacyl ether, but acidolysis monomers were scarcely detected. The acidolysis products of the compound (1) mainly were composed of β-oxyconiferyl alcohol (14), which was separable into keto (14a) and enol types (14b) by acetylation, 1-propanone (16), 2-propanone (15), guaiacylacetone (18), vanilloyl methyl ketone (17), and guaiacol (3) (Fig. 27). However, coniferyl alcohol (2),

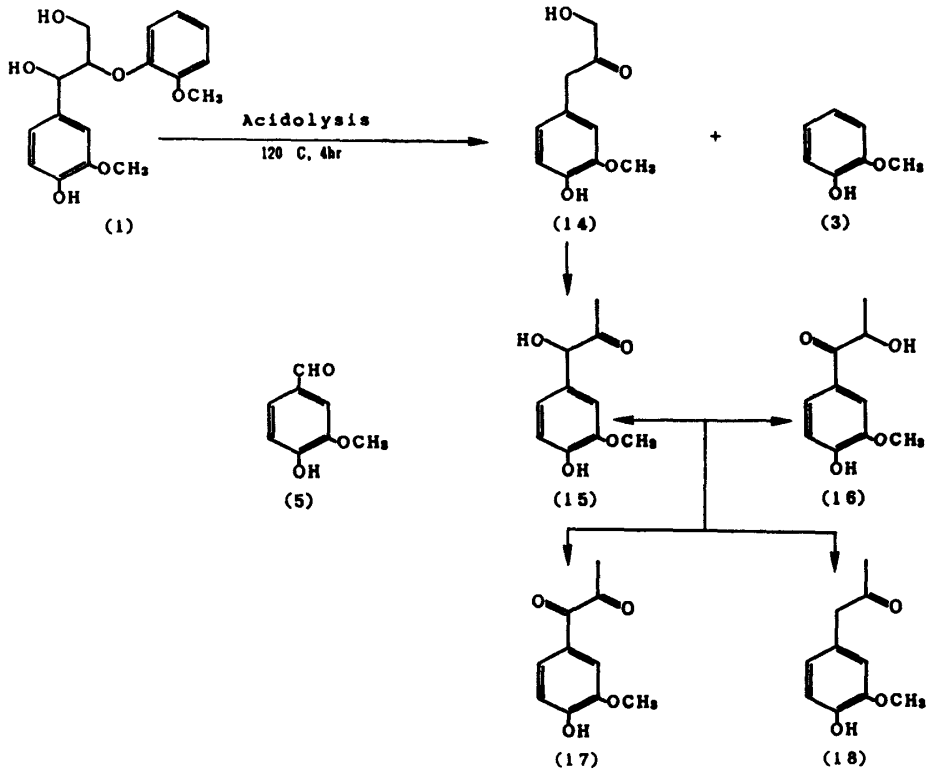

Fig. 27. Acidolysis products of guaiacylglycerol-β-guaiacyl ether.

dehydrodiconiferyl alcohol (11), and pinoresinol (9, 10) which were the main products by steam explosion, were hardly detected in the acidolysis products of Compound (1). Thus, the mechanism of lignin degradation acccomanying steam explosion is entirely different from acidolysis.

Coniferyl alcohol could be produced by a one-electron reduction of the coniferyl alcohol radical derived from the β-ether linkage of the structure (1) by homolytic cleavage or a two-electron reduction of β-ether linkage by enediol forms of reducing sugars derived from polysaccharides as in alkaline pulping. However, it has been known that phenylcoumarane and resinol are not detected by alkaline pulping of the compound (1). Based on these results, the present author proposed that by steam explosion, lignin is cleaved mainly homolytically to produce cinnamyl alcohol radicals which couple to give Cβ-Cβ or Cβ-C5 linkages, that a disproportionation of the radical produces cinnamyl alcohol and cinnamyl aldehyde, and that cinnamyl alcohol radical also can be reduced by sugar to give cinnamyl alcohol.

2. 2. 5 Steam explosion of syringylglycerol-β-guaiacyl ether

Syringylglycerol-β-guaiacyl ether (1′) was subjected to a steam explosion under the same conditions. Forty percent of the starting material remained intact, and major degradation products were identified as sinapyl alcohol (2′), *d,l*-syringaresinol

(9′), and *d,l*-episyringaresinol (10′). Syringaldehyde (5′), syringic acid (7′), and sinapaldehyde (4′) also were identified (Fig. 26). These results well agreed with the previous experiments with compound (1) and hardwoods. Parts of *d,l*-syringaresinol and *d,l*-episyringaresinols obtained from steam-exploded hardwood lignins can be derived from the original resinol substructure in lignin but mainly from β-ether of syringyl-type lignin by homolytic cleavage to give synapyl alcohol radicals, which are coupled to give Cβ-Cβ and Cβ-C5 linkages.

2. 2. 6 Degradation mechanism of β-O-4 type lignin substructure model compounds.

Syringaresinol (9′, 10′) is a symmetrical compound linked by the Cβ of the side chains of two molecules of the sinapyl alcohol radical (22′). These couplings can occur only by radical reaction. Although dehydrodiconiferyl alcohol (11) is not a symmetrical compound, it can be formed by the coupling of the Cβ (22) and C-5 radicals (24) derived from the coniferyl alcohol radical (22). The pairs of sinapyl alcohol (2′) and sinapaldehyde (4′), and of coniferyl alcohol (2) and coniferyl aldehyde (4) could be formed by dismutations of sinapyl alcohol radicals (22′) and coniferyl alcohol radicals (22), respectively. γ-Methyl ether of coniferyl alcohol (13) can be formed by the addition of methanol used as solvent to the quinonemathide intermediate (27) derived by the disproportionation of coniferyl alcohol

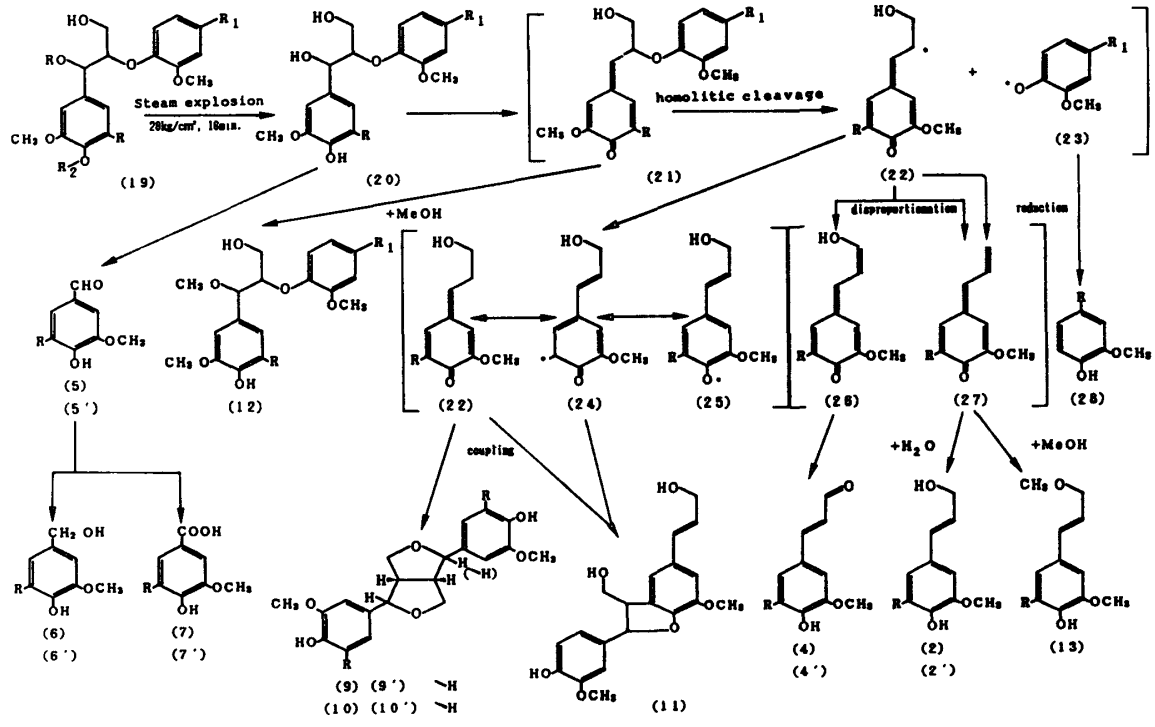

Fig. 28. Possible degradation mechanism of lignin resulting from a steam explosion.

radicals (22). Guaiacylglycerol (8) can be formed by the addition of water to the quinone methide intermediate derived from coniferyl alcohol radical. Thus, we proposed the possible mechanism of lignin degradation by steam explosion as shown in Fig. 28. It is conceivable that under the current steaming conditions, the ionization of water is 10^{-7} mol which is about 10^{7} times greater than under normal conditions[52]. Therefore, hydroxyl groups or ether linkage of the α-position of lignin side-chains could be protonated easily and converted to the quinone methide structure (21). Thus, β-O-4 ether linkage can be homolytically degraded to produce coniferyl alcohol- and sinapyl alcohol radicals (22, 22′). These radicals then could react as described in Fig. 28.

It is very likely that syringaresinol (9′, 10′) is produced by the conversion of the β-O-4 syringyl substructure (19′). In hardwood lignin the amount of syringyl unit is estimated to be more than half of that of monomeric units, and that the β-O-4 substructure is the major linkage of syringyl units followed by the β-β linkage in hard-wood lignin. Therefore, we consider that a remarkable amount of syringaresinol could be formed from these syringyl units by steam explosion.

The present investigation indicated that the steam explosion of wood is a good method for converting lignocelluloses to useful products.

2.3 High-Pressure Steam Treatments of Guaiacylglycerol-β-Guaiacyl Ether and Shirakanba Wood

2.3.1 Comparison of the degradation products by steaming at different conditions

Degradation products of guaiacylglycerol-β-guaiacyl ether (1) by steam explosion was described in section 2.2. Homolytic cleavage of β-ether bond was proposed as the main degradation reaction of lignin by steam explosion based on the identified degradation products. However, a considerable amount of degradation products of cellulose used as matrix were contained in the degradation products of model compound, and hampered the determination of quantitative analysis. Therefore, guaiacylglycerol-β-guaiacyl ether was steamed in a small autoclave without "explosion", and degradation products were analyzed by GC-MS.

Steam treatments were carried out by two methods, (1) gradual heating of the reactor by an electric heater from outside, and (2) high pressure steam injection into the reactor. In the former method, about 20~30 min was required to increase the temperature and the pressure, and a part of sample would have already reacted at the lower temperature. Under such a condition β-ether could be cleaved by a mixed reaction of hydrolysis and homolysis and the degradation products were more complicated than the steam-exploded products.

Acidolysis reaction was proposed by WAYMAN and others and MARCHESSAULT and others for the degradation of lignin by steam explosion[12,55,56]. Acidolysis would be involved in the cleavage of β-ether by steam and/or drained water at lower temperature. However, in the steam injection, temparature and pressure were immediately reached to 230°C and 28 kgf/cm², and the sample was degraded by high temparature and high pressure steam to produce mainly coniferyl alcohol (2) and guaiacol (3) in agreement with steam explosion.

2. 3. 2 Steam treatment of guaiacylglycerol-β guaiacyl ether

In steam explosion, the yield of guaiacol was smaller than that in steaming, suggesting that a part of guaiacol was steam-evaporated. In steam treatment, guaiacol (3) was produced mainly and the amount was comparable to that of coniferyl alcohol (2). The result indicated the β-O-4 ether linkage was cleaved homolytically to produce coniferyl alcohol radical (22) and guaiacol radical (34). These radicals formed were converted to coniferyl alcohol (2) and guaiacol (3) by one electron reduction, respectively. The reducing reagent could be reducing end groups of glucose derived from cellulose used as matrix of model compound. Small amounts of pinoresinols (9, 10) and phenylcoumaranes (11, 32) which could be produced by radical coupling reaction were also produced by steam treatment of compound (1). Pinoresinols (9, 10) were only produced by homo-coupling of

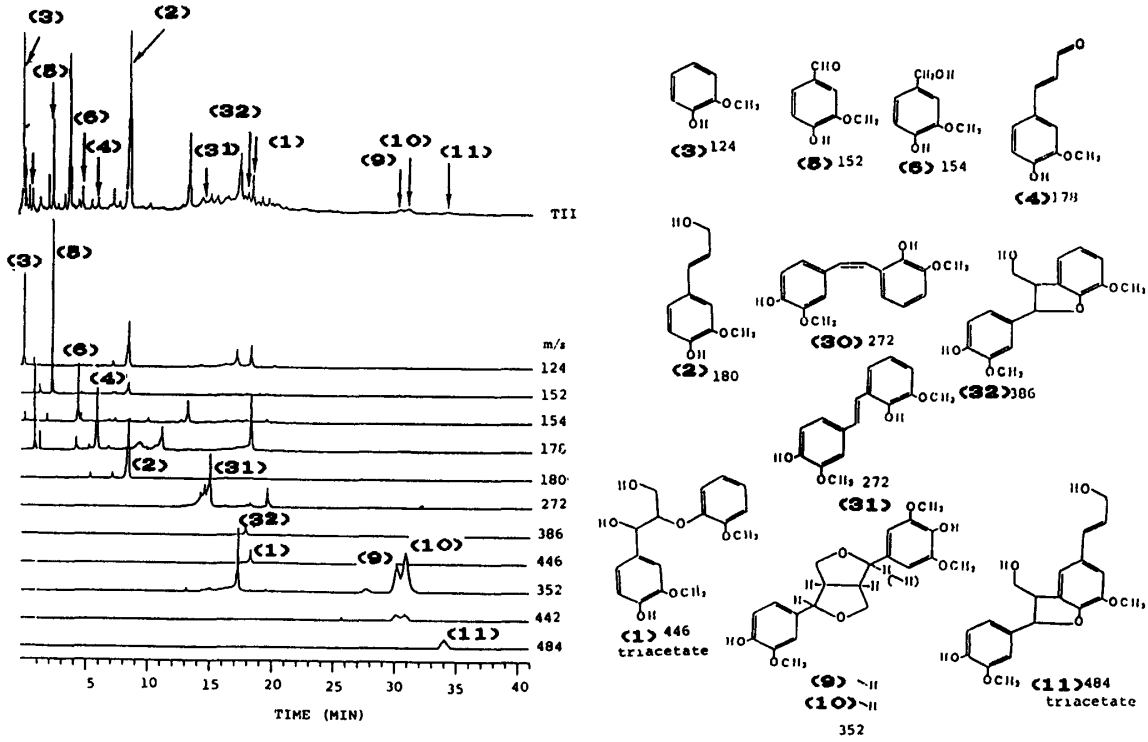

Fig. 29. GC-MS chromatography of degradation products of guaiacylglycerol-β-guaiacyl ether by steam treatment.

Cβ-radicals of coniferyl alcohol (22). The results showed that radical reaction occurred during steam treatment of lignin substructure model compound. Other degradation products were vanillin (5), vanillyl alcohol (6), and coniferyl aldehyde (4). However, acidolysis products such as β-oxyconiferyl alcohol (keto and enol forms) and other Hibbert's ketones could not be detected (Fig. 29). Thus, it is evident that the reaction of steam treatment of lignin was neither acidolysis nor hydrolysis, but homolytic cleavage.

The compound (32) could be produced by two mechanisms, rearrangement of ether oxygen to quinonemethide (33) or coupling of coniferyl alcohol radical (22) and C_5 radical of guaiacol after homolytic cleavage of β-O-4 ether. The compound (32) could be converted to trans and cis stilbenes (31, 30) by elimination of formaldehyde. On the other hand, the formation of α-methyl etherated compound (12) suggested that quinonemethide structure (33) is an essential intermediate of degradation pathway, and that methanol remained as solvent was added to the quinonemethide. In the same manner, the formation of γ-methyl etherated coniferyl alcohol (13) suggested the involvement of an extended quinonemethide intermediate (27) derived from coniferyl alcohol radical (22) by disproportionation or one electron reduction. Vanillin could be formed by one electron oxidation of *p*-hydroxyphenyl alcohol to its phenoxy radical and followed by Cα-Cβ cleavage of side chain (Fig. 30).

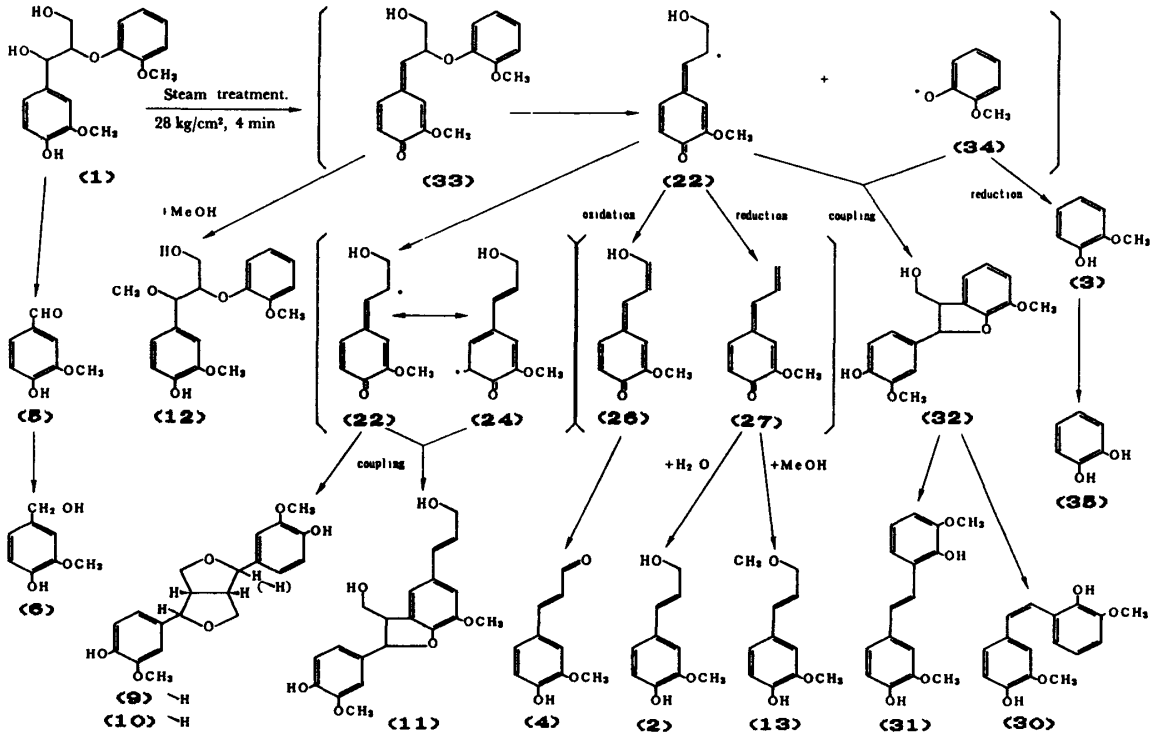

Fig. 30. Degradation mechanism of guaiacylglycerol-β-guaiacyl ether by steam treatment.

2. 3. 3 Effect of concentration of a model compound

Among the degradation products of lignin from steam-exploded Shirakanba wood, a considerable amount of syringaresinol was yielded[30]. However, from steam-exploded or steam-treated model compound (1), only a small amount of pinoresinol and phenylcoumarane were detected (Fig. 31-a, b). The results suggest that the low yield of pinoresinol and phenylcoumarane depends on the concentration of model compound used. In steam explosion of guaiacylglycerol-β-guaiacyl ether, when the ratio of model compound / cellulose was 3/1000 pinoresinol was hardly detected in the degradation products (Fig. 31-a). In steam treatment of sample at low concentration (1/20), only a small amount of pinoresinol was detected (about 1/7 of the amount of coniferyl alcohol) (Fig. 31-b). And in steam treatment of

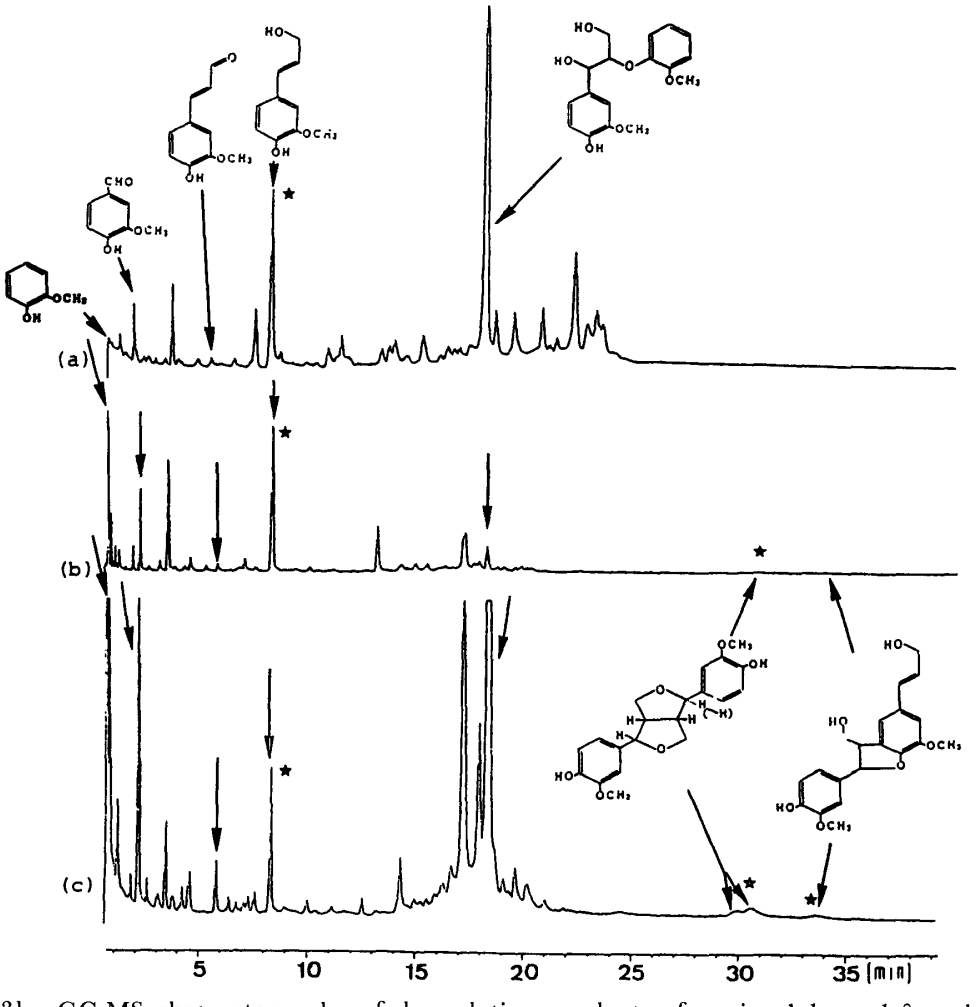

Fig. 31. GC-MS chromatography of degradation products of guaiacylglycerol-β-guaiacyl ether by steam explosion and by steam treatment.
a: steam explosion (200 mg of substrate/100 g of linter cellulose), b: steam treatment (20 mg of substrate/400 mg of linter cellulose), c: steam treatment (200 mg of substrate/ 400 mg of linter cellulose).

sample at high concentration (1/2), the yield of pinoresinol was increased to a twice amount of coniferyl alcohol (Fig. 31-c). These results indicate that the yield of pinoresinol was dependent on the concentration of model compounds used, and the coupling reaction competed with reduction of radicals by sugar or other reducing reagents.

It seems that even if the concentratiotion of model compound was increased to that of lignin in wood, a partial concentration of radical formed in the lignin would be considerably higher than that of model compound, because many radicals would be formed closely in a certain area of lignin polymer. Thus, we concluded that homolytic cleavage is the major reaction in the degradation of lignin and model compound by steam treatment. The difference in the yield of degradation products was ascribed to the differences of molecular weight, oxidation level and solubility of the substrates, model compound and lignin.

2. 3. 4 Steam treatment of Shirakanba wood

The results of steam treatment of Shirakanba wood chips were shown in Fig. 32. The yield of syringaresinol was almost 50% of analyzable degradation product by gas chromatography, and amounted to 9.6% of phenolic fraction by using ligno-

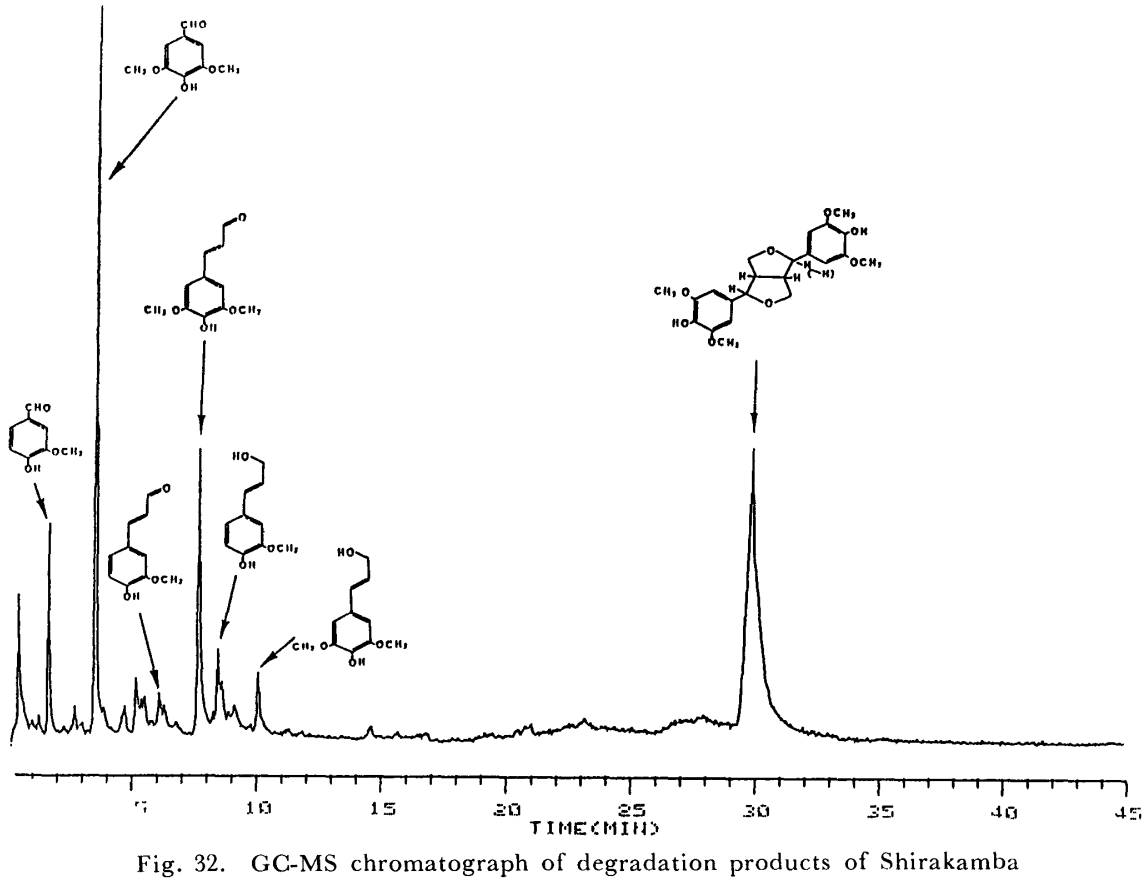

Fig. 32. GC-MS chromatograph of degradation products of Shirakamba wood by steam treatment

lide diacetate as an internal standard. The yield corresponded to about 0.93% of extractive free wood. The phenolic fraction contained vanillin (1.4%), syringalde dyhe (4.3%), coniferyl aldehyde (0.4%), sinapaldehyde (2.5%), coniferyl alcohol (0.8%), sinapyl alcohol (0.7%) etc. The fact that hard wood lignin which contains less than 5% of syringaresinol substructure (Fig. 33) gave a considerable amount of syringaresinol by steam treatment suggested that coupling of sinapyl alcohol radical obviously occurred during steaming. Cinnamyl alcohols were mainly obtained from model compounds, whereas by steam treatment of lignin, more cinnamyl aldehydes were produced than cinnamyl alcohols. Such difference in the amounts of the degradation compounds could be derived from the difference of oxidation levels at C_6-C_3 unit of model compounds and lignin. The present investigation showed that the degradation reaction by steam explosion mainly occurred during steaming but not at the explosion process. The results indicat that β-O-4 ether linkage is cleaved homolytically to give cinnamyl alcohol radicals which is reduced by sugar to cinnamyl alcohols. On the other hand, cinnamyl alcohol radical is coupled to give resinols and phenylcoumaranes, and that the reaction depends on

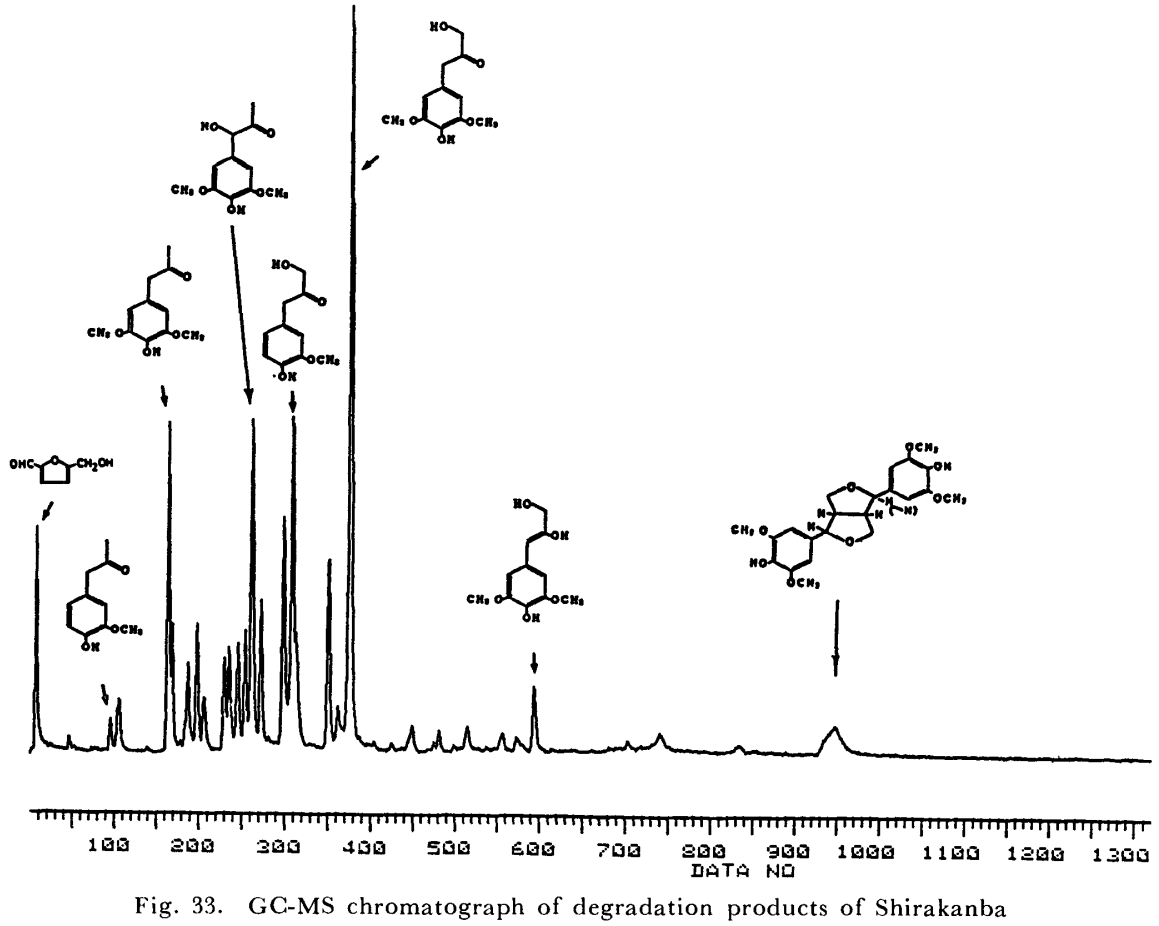

Fig. 33. GC-MS chromatograph of degradation products of Shirakanba wood by acidlysis

the concentration of radical. In these reactions, quinonemethide structures might be involved as an essential intermediate and contributed to a stability of radicals.

Lignin and phenolic low molecular weight products of lignin were easily extracted and separated from exploded wood. Purification of syringaresinol was easy, and a half of acetone soluble fraction of acetylated phenolic fraction was syringaresinol. Syringaresinol glucoside contained in the root of eleuterococus is known to be a stimulant and tonic substance, and syringaresinol itself has some physiological activities such as an inhibitor for blood-coagulation.

Syringaresinol is contained only 0.02% in special plants such as eleuterococus and tulip tree, but hard woods generally contain about 20% of guaiacyl-syringyl lignin and syringyl moiety is mainly connected by β-O-4 ether linkage. The β-O-4 ether substructure is easily cleaved by steam explosion homolytically to produce sinapyl alcohol radical which could be coupled to give syringaresinol. Thus, medical use of syringaresinol will open up total utilization of wood by biomass conversion.

2.4 Steam Treatments of DHP and LCC

2.4.1 Preparation of high molecular weight coniferyl alcohol DHP

In lignification of plant cell walls, dehydrogenation of monolygnols occurs not in a dilute aueous solution which is usually used in the preparation of DHP by the "Zutropfverfahren" methods but proceeds on a matrix of cellulose and hemicelluloses by the cell wall bound peroxidase. Lignin formed in the matrix cannot be diffused from the vicinity of peroxidase. When coniferyl alcohol and hydrogen peroxide are supplied continuously to the cell walls on which previously formed DHP and peroxidase are present together, the DHP would grow up to high molecular weight materials such as natural lignin. High concentrations of enzyme and intermediary DHP should be necessary for the formation of a highly polymerized DHP. For this purpose a cellulose dialysis tube was used in the present investigation.

Coniferyl alcohol and H_2O_2 can pass through membrane of the cellulose tube, but peroxidase and oligolignols should be impenetrable. When the sealed cellulose dialysis tube containing a concentrated peroxidase was put into the solution of coniferyl alcohol and H_2O_2, these compounds penetrate into the tube and react with peroxidase to form phenoxy radicals. These monomeric radicals are coupled rapidly to give oligomers inside the tube which cannot pass through the membrane to the outer solution. In fact, precipitated DHP was found only inner side of the tube. The amount of the precipitate in the tube increased in the early stage of the reaction, but after 24 hr the formation of the precipitate was scarcely observed in spite of the presence of coniferyl alcohol and H_2O_2 in outside solution of the

dialysis tube. The stop reaction seems to be ascribed to that the inner surface of the membrane was coated with the precipitate of DHP formed and that penetration of substrates was interfered. When a new tube containing peroxidase was replaced in the same solution reaction was continued. The amounts of the DHPs obtained in the first and the second tubes were 58 mg and 32 mg, respectively and total yeld in the two reactions was 30%. When the reaction was continued further using new tubes in the same solution more precipitates were produced in each time, and when five tubes were put into the solution at once, total yield was amounted over 50% for 48 hr. The DHP obtained by this method was almost insoluble in any solvents of lignin (e.g. dioxane, THF, acetone, methanol, ethyl acetate, acetic

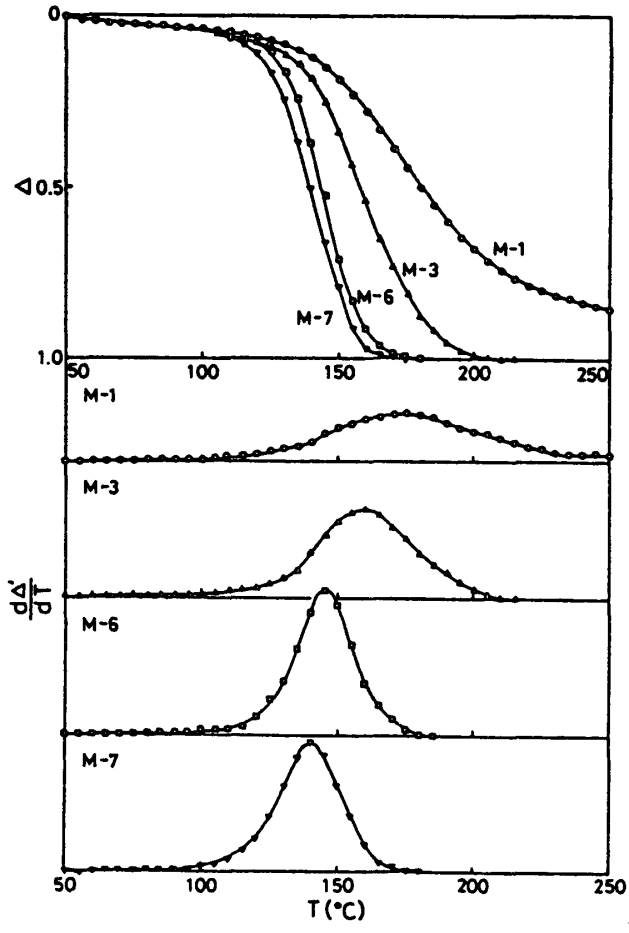

Fig. 34. Thermal softening curves for dialysis membrane method DHPs
○: peroxidase content=10.0 mg (M-1)
△: peroxidase content= 1.0 mg (M-3)
□: peroxidase content= 0.1 mg (M-6)
▽: peroxidase content= 0.05 mg (M-7)
The deformation function △′ was normalized in such a way that a maximum deformation=1.

acid, 2 % NaOH and aqueous dioxane). In highly polar solvents such as formamide, DMF, DMSO and pyridine, a part of the DHP was dissolved but major part was only swelled about three to five times of the initial volume without dissolving.

As the dialysis method DHPs were insoluble in any organic solvents usual methods used in determination of molecular weight could not be applied. Then thermal softening temperature (Ts) of the DHP was measured to determine the molecular weight. Ts of the DHPs were measured to elucidate the effect of enzyme concentration in preparation on the molucular weight of insoluble DHPs. The Ts raised when enzyme concentration was increased as shown in Fig. 34. The presu-

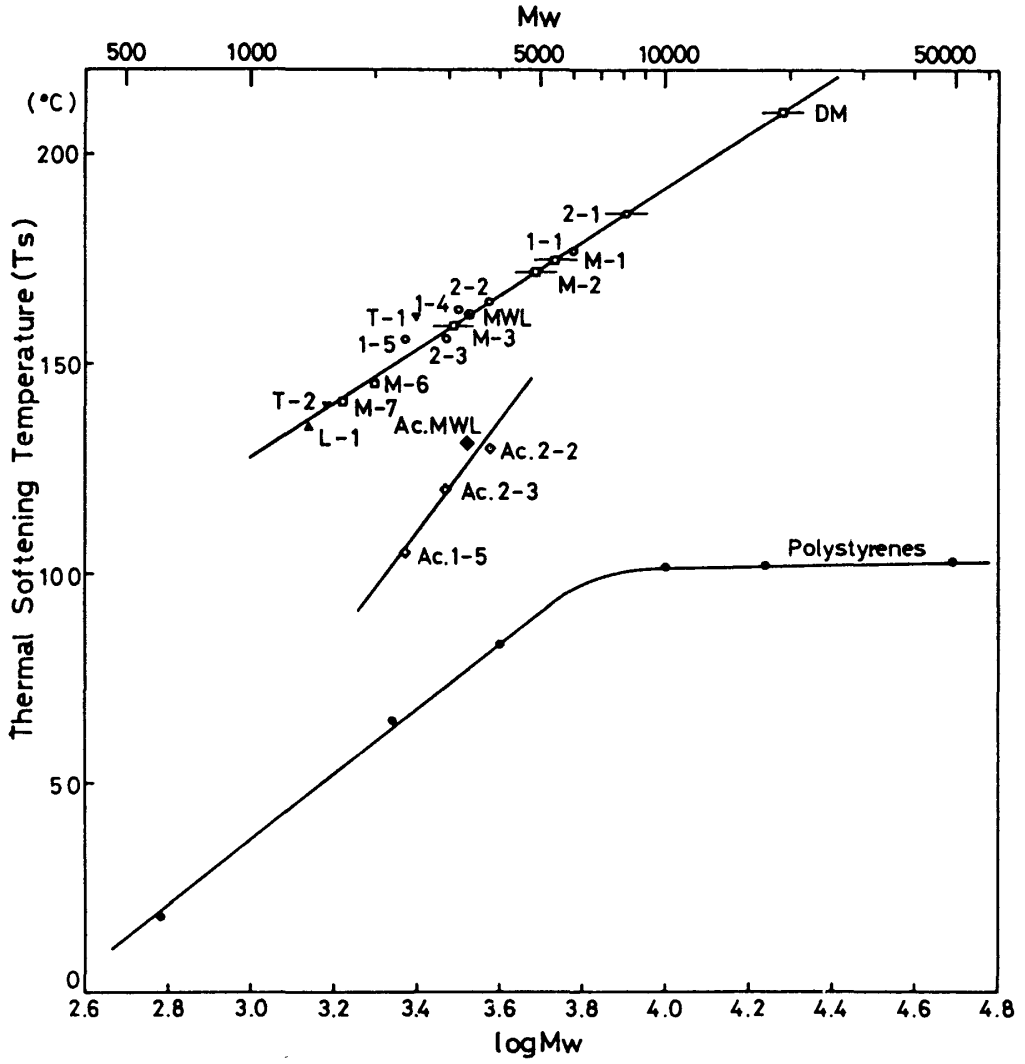

Fig. 35. Calibration curves for thermal softening temperature (Ts) against log Mw determined by GPC using standard polystyrenes
●: polystyrenes, ◎: MWL, ○: MWL fractions, ▽: Zutropfverfahren DHPs, △: Zulaufverfahren DHP and □: dialysis membrane method DHPs.
—○— and —□—: insoluble MWL fractions and insoluble dialysis membrane method DHPs.

med molecular weight of the DHP from the Ts peak at 175°C was 83,000 as compared with Goring's data of dioxane lignin[53]. The molecular weight of MWL calculated by the same method was about 50,000. These facts indicated that C-DHP (dialysis method) is a highly polymerized and widely dispersed material than MWL.

For determination of the molecular weight and the molecular weight distribution of soluble lignins, gel permeation chromatography has usually been used[57~60]. However, for insoluble lignins no good methods have hitherto been established. Thermal softening behavior is related to the micro-Brownian motion which reflects molecular weight and structure of the molecules. This experiments to measure the Ts was applicable to determine the molecular weight of insoluble lignins. In order to prepare the calibration curve of Ts vs. molucular weight, the molecular weights of soluble fractions of MWL and DHPs were measured by GPC with μ-styragel 500 Å using standard polystyrenes. DMF was used as a solvent and the solution was diluted twice with THF. The results obtained from GPC well agreed with those of gel filtration chromatography on Sephadex LH-60 with DMF as a solvent[35]. However, for the insoluble lignin samples, Ts was measured to determine the relation between Ts and the molecular weight. The Ts of polystyrenes linearly went up to the high temperature range with increasing of the molecular weight (log Mw).

The results of these investigations are summarized in Fig. 35. For polystyrenes, an approximately linear relationship was found between the log Mw (up to 10^4) and the Ts, and the curve was levelled off in further higher molecular weight, which is a general characteristic of macromolecules. All lignin preparations used in the present investigation gave linear plots for this relation. However, different from linear polystyrene, the calibration curve of lignin related compounds shifted to higher Ts side, which may reflect a three dimensional structure of lignin polymer, connecting by hydroxyl groups via hydrogen bonds. Ts of lignin did not give the saturation in the present experimental conditions, suggesting that Ts saturation of lignin would occur at the more higher temperature range. The symbols with horizontal line show insoluble fractions of MWL (2-1; 186°C) and dialysis membrane method DHPs (M-1; 175°C, M-2; 172°C and M-3; 158°C), the molecular weights of which cannot be measured by GPC method. The precipitate (2-1) of a DMF soluble fraction of lignin in dialysis against water became insoluble in DMF. The molecular weights of these preparations were estimated to be 8000, 5600, 5000 and 3000, respectively from the calibration line (Fig. 35). Since the molecular weights were calculated from GPC calibration curve for polystyrenes the values would be lower than the true value. Actually, they were estimated to be 160,000, 84,000, 70,000 and 30,000 by calculating on the basis of Goring's experiment with dioxane lignin[53] as shown in Fig. 36. The DHP obtained by double dialysis membrane method with

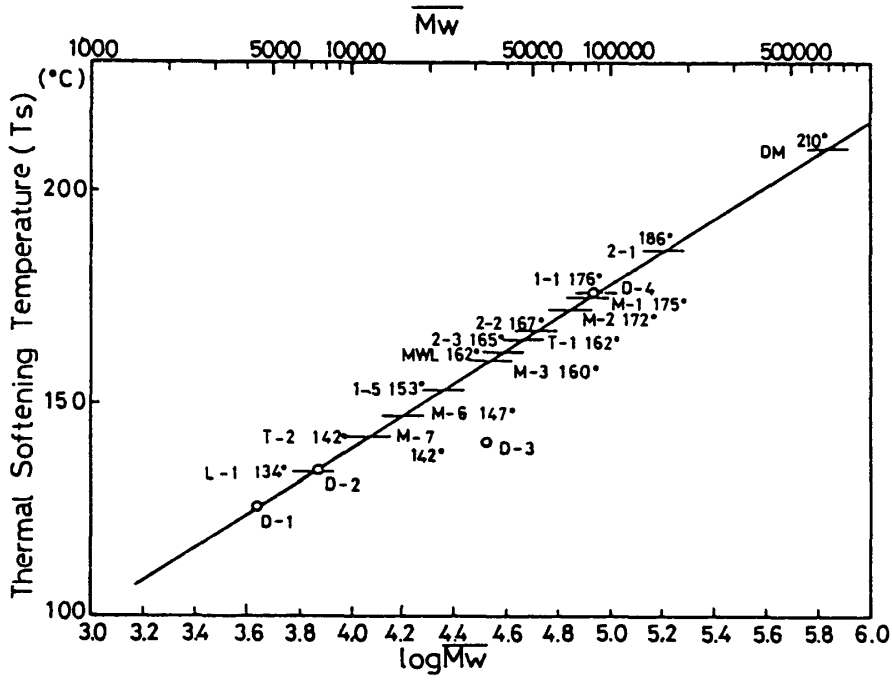

Fig. 36. Calibration curve for thermal softening temperature (Ts) against log Mw prepared from from Goring's data (1963)
○: Goring's data for spruce dioxane lignins (D-1, D-2, D-3 and D-4)

a high speed supplying of substrate (DM-1) showed a Ts at 210°C. By extrapolation of the calibration curve, the molecular weight of the DHP was estimated to be 20,000 (on the basis of polystyrene's calibration) and 670,000 (on the basis of Goring's data), respectively. The molecular weights of DHPs (M-1 and M-2) would be higher than that of MWL. The result indicated that highly polymerized DHPs can be synthesized by dialysis tube method. In spite of lower Ts than those of MWL fractions (1-1 and 2-1), DHPs (M-1, M-2 and M-3) were insoluble in organic solvents. It could be ascribed to the more branched and/or more bridged structures.

The peaks of the acetylated lignin preparations, by GPC slightly shifted to higher molecular side, but the peaks of Ts shifted to the lower temperature (30~50°C). This may be due to that hydrogen bonds via hydroxyl groups in lignin were broken resulting lower Ts by acetylation. The result suggested that Ts is remarkably affected by hydrogen bonding via hydroxyl groups in lignin.

2. 4. 2 Steam treatment of C DHP

The C-DHP degradation product (88%) was mainly obtained from DMF insoluble fraction. However, in this study water soluble fraction, which was a major fraction of soluble degratation products of steamed C-DHP, was mainly analyzed. These degradation products were analyzed by GC-MS, separated by preparative TLC and identified by ^{1}H-NMR and GC-MS.

The major products of the fraction were coniferyl aldehyde (2) and pinoresinol (6). Other products identified were vanillin (1), dihydroconiferyl alcohol (3), coniferyl alcohol (4) and phenylcoumarane (γ-aldehyde, (5)) (Fig. 37).

In addition to the C-DHP degradation products described here, similar products on degradation of lignin substructure model compound were also found. However, the GC-MS data showed obvious differences in the formation of coniferyl alcohol (4), coniferyl aldehyde (2) and pinoresinol (6). In the degradation of lignin substructure model compound, coniferyl alcohol (4) was the major degradation product and the amount of pinoresinol was much smaller than that from degraded C-DHP. On the other hand, the major product obtained from steamed C-DHP was coniferyl aldehyde (2). The major difference of the degradation product is possibly due to the difference in oxidation level of C_6–C_3 unit of the substrate molecule. In the steaming of C-DHP, the oxidation level of the C_6-C_3 units deduced from the elementary composition is equivalent to that of coniferyl aldehyde. By the steam treatment, the cleavage of interunit linkage in lignin occurred to give a high concentration of radicals which resulted in the coupling to form dimers. This hypothesis was confirmed by the experiment to change the amount of sample in the treatment (Fig. 31, 37-B). Fig. 31-C and Fig. 37-B showed experimental results with the same amount of guaiacylglycerol-β-guaiacyl ether and C-DHP (400

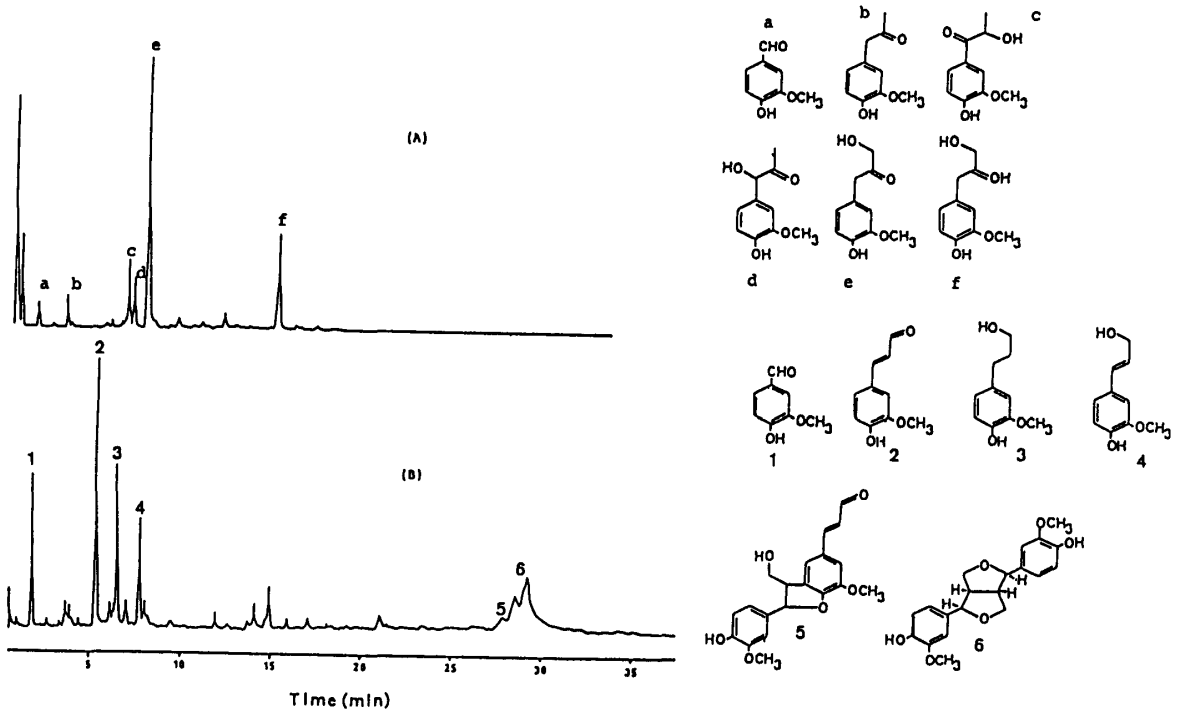

Fig. 37. GC-MS chromatogram of the degradation products (acetates) of C-DHP by (A): acidolysis (B): steaming (20 kgf/cm², 230°C, 4 min)

mg). Pinoresinol peak, compared with others, became more intense in spectra of steamed C-DHP than that of steamed guaiacylglycerol-β-guaiacyl ether, suggesting that the amount of pinoresinol increased by coupling of the radicals formed.

On the other hand, the oxidation level of C_6-C_3 unit in lignin substructure model compound was similar to that of coniferyl alcohol. In production of coniferyl alcohol in high yield, two reductive reactions might be important. In the steaming of lignin substructure model compound, β-aryl ether linkage was homolytically cleaved with the formation of coniferyl alcohol radical. Disproportionation of this radical gave rise to coniferyl alcohol and coniferyl aldehyde respectively. However, it is conceivable that glucose derived from cellulose, reduced coniferyl alcohol radical to coniferyl alcohol.

On acidolysis of lignin (reflux temperature in dioxane/2N HCl 9:1, 4 hr), the β-ether linkage was cleaved leading to the formation of β-oxyconiferyl alcohol (e) which converted to the corresponding γ-methyl isomers, 1-propanone (c) and 2-propanone (d) (Fig. 37-A). These acidolysis monomers were scarcely detected in degradation products of C-DHP by steam treatment. On the other hand, coniferyl aldehyde and pinoresinol which were main products in the steam treatment, were not detected in acidolysis product of C-DHP. Because resinol was not detected in the acidolysis products, it is evident that the proportion of resinol component in the C-DHP was low, and that most of the pinoresinol was produced by conversion of β-O-4 linkage of guaiacyl unit in C-DHP by steam treatment.

As for the production of pinoresinol from β-ether linkage of C-DHP by steam treatment the homolytic cleavage mechanism of β-ether linkage was rationalized. However, the yield of ether soluble fraction from steamed C-DHP was very low, suggesting that phenoxyl radical formed by the steam treatment, repolymerized to form more condensed polymer which was insoluble in lignin solvent. It was difficult to determine differentially the amounts of pinoresinol newly produced and originally existed in lignin.

Then, S-DHP which will scarcely be repolymerized and mainly produced syringaresinol as a dimeric product. However, on the normal dehydrogenative polymerization method (Zutropfverfahren) of sinapyl alcohol, syringaresinol content in the S-DHP was high. To elucidate the degradation mechanism, S-DHP which is composed mainly of β-O-4 linkage and contain less syringaresinol substructure was needed. Hence, in the next section several new synthetic methods of S-DHP were developed and their steam treatment was carried out.

2.4.3 Preparation of sinapyl alcohol-DHPs and LCC

The membrane method was performed using a dialysis tube (Fig. 38). By this procedure the syringaresinol mainly formed in early stage was excluded from the

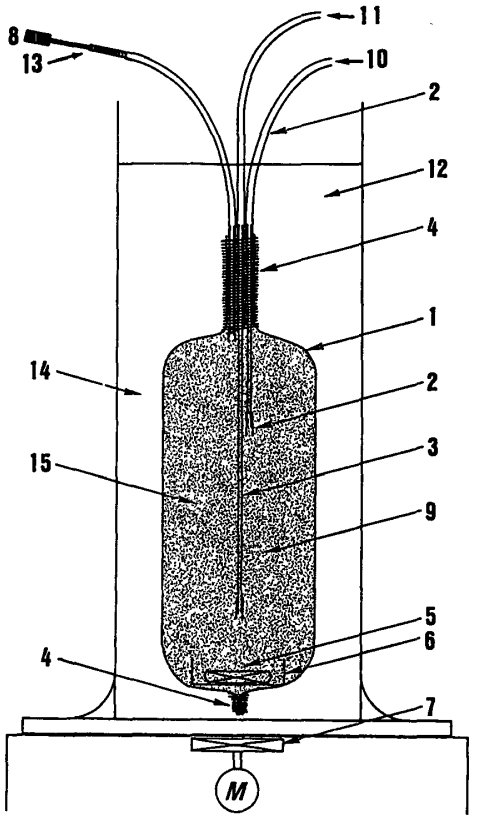

Fig. 38. Synthesis of S-DHP and S-LCC by the dialysis-membrane method.
Legend; 1: dialysis tube, 2: silicon microtube, 8: Teflon tube, 4: binding with a thread, 5: rotor, 6: petri dish, 7: stirrer, 8: stopper, 9: horseradish peroxidase solution or mixed solution of mannan and peroxidase, 10: H_2O_2 solution, 11: sinapyl alcohol solution, 12: distilled water, 13: additional peroxidase solution, 14: low molecular weight products, 15: S-DHP or S-LCC, M: moter.

reaction system through the dialysis membrane, and the DHP with higher molecular weights mainly connected by β-aryl ether linkage remained in the tube. The intermediary low molecular DHPs were oxidized to their radicals and gradually converted to a high polymer by their couplings and the couplings with monomer radicals derived from newly added sinapyl alcohol. The DHP thus produced was mainly composed of β-aryl ether linkage with a less amount of syringaresinol substructure. In polar solvent (water) the electron density at Cβ of sinapyl alcohol radical was increased by the E-effect of the methoxyl group[61]. Hence, the produced dimers in dehydrogenation of sinapyl alcohol in aqueous solution (polar solvent) were composed of syringaresinol 91% and the β-aryl ether 9% indicating the electronic effect of methoxyl group on the coupling reaction[62]. FREUDENBERG and others proposed the effect of steric hindrance on polymerization[55]. However, native hard wood lignin is composed of higher amount of β-O-4 ether linkage of syringyl

unit with a small amount of syringaresinol substructure, in disagreement with the steric effect on polymerization.

Our previous investigation showed that the dehydrogenation of sinapyl alcohol in dioxane (non-polar solvent) gave dimers, the ratio of syringaresinol to the syryngylglycerol-β-sinapyl alcohol ether was 4:96[62] (Table 9). While, in non-polar solvent ionization of a lone pair of methoxyl group would be limited, and then the E-effect was suppressed by the I-effect of the methoxyl group to result in the increased electron density at the phenoxyl group than at Cβ position. Then the amount of β-aryl ether increased on dehydrogenation of sinapyl alcohol in non-polar solvent. Besides the intermediary DHP was soluble in dioxane and their radical polymerization continued easily to lead a higher molecular DHP of sinapyl alcohol through couplings with sinapyl alcohol radical.

We investigated the dehydrogenation of sinapyl alcohol with $FeCl_3$ in dioxane solution by Zutropfverfahren method and the dialysis tube method, respectively. The DHPs formed were analyzed by ^{13}C-NMR and by chemical degradation such as acidolysis. The spectra showed a very low content of syringaresinol substructure in the DHPs, and this was confirmed by the formation of very little syringaresinol in acidolysis.

It has been known that syringyl lignin in hardwoods mostly consist of β-aryl ether substructure and that the amount of syringaresinol substructure is less than 5 %[56,63]. The present experiment suggested that the polymerization of sinapyl alcohol to give syringyl lignin occurs in non-polar and hydrophobic reaction site in plant cell wall.

It is generally considered that hemicelluloses have hydrophilic but not hydrophobic property. However, it has been known that the inside of cyclodextrin[64~66] and helical amylose[67] is hydrophobic, and easily incorporate hydrophobic compounds. While cellulose also forms inclusion compounds with some hydrophobic compounds such as cyclohexane[68]. In the hydrophobic region I-effect of the methoxyl group is dominant as in non-polar solvent. This suggests that in plant cell wall monolignols would be introduced into hydrophobic region of hemicellulose molecules, and dehydrogenatively polymerized by cell wall bound peroxidase there. In order to examine this hypothesis we studied the dehydrogenative polymerization of monolignols in the presence of hemicelluloses.

The obtained S-LCC was dissolved in *d*6-DMSO and analyzed by ^{13}C-NMR. The spectrum showed that the content of syringaresinol was remarkably low, and sinapyl alcohol LCC formed was mainly composed of β-aryl ether linkage.

The analysis of low molecular weight fraction in the dialyzate showed that 1) the low molecular weight fraction in dehydrogenation of sinapyl alcohol in water

Table 9. Substructures in various S-DHPs and low molecular products formed by different dehydrogenative polymerization of sinapyl alcohol

Dehydrogenative oxidation methods	Solvent (pH or ratio)	Oxidant	Polymerized products Mw	Polymerized products main substructure	Dimeric products (resinol: β-O-4)	By-products
Zuraufverfahren	phosphate buffer (pH 6.5)	peroxidase /H_2O_2	—	—	91: 7	—
	water / acetone (40:3)	$FeCl_3$	—	—	73:27	—
	dioxane / water (5:2)	$FeCl_3$	—	—	15:85	2,6-dimethoxyquinone
	dioxane	$FeCl_3$	—	—	4:96	2,6-dimethoxyquinone
Zutropverfahren	phosphate buffer (pH 6.0)	peroxidase /H_2O_2	Low	resinol>β-O-4	syringaresinol	—
	dioxane	$FeCl_3$	medium	β-O-4	—	2,6-dimethoxyquinone
Dialysis method	water	peroxidase /H_2O_2	high	β-O-4>resinol	syringaresinol	—
	dioxane	$FeCl_3$	high	β-O-4	—	2,6-dimethoxyquinone
	mannan / water (2 g/5 ml)	peroxidase /H_2O_2	high	β-O-4	—	2,6-dimethoxyquinone

was mostly composed of syringaresinol, 2) the low molecular weight fraction in dehydrogenation of the alcohol in dioxane, mainly contained 2, 6-dimethoxybenzoquinone, and 3) the low molecular weight fractionin dehydrogenation of sinapyl alcohol with mannan in water also gave 2, 6-dimethoxybenzoquinone but not syringaresinol (Table 9). The result suggested that 1) the reaction in aq. solution of hemicelluloses proceeded as did in dioxane and 2) dehydrogenation of the alcohol in the presence of hemicelluloses in water was favorite to the formation of β-aryl ether linkage to lead high polymers. The analytical results of the DHP by ^{13}C-NMR and acidolysis were in good agreement with this hypothesis.

It is therefore concluded that dehydrogenative polymerization of monolignols in plant cell walls could occur in hydrophobic region of hemicelluloses, by cell wall bound peroxidases. Hence, syringyl lignin is composed mainly of β-O-4 ether linkage and that the hemicelluloses are easily connected to α-position of lignin via quinonemethide intermediates to give LCC[37].

2.4.4 Steam treatment of sinapyl alcohol DHP[33]

In order to elucidate the reaction mechanism of lignin degradation by steam explosion, β-O-4 lignin substructure model compounds were used in the previous study. The result of the study clearly indicated that β-aryl ether linkage, which is the most abundant linkage connecting the phenylpropane units in lignin, was cleaved with the formation of *p*-hydroxycinnamyl alcohol radicals. In addition, the concentration of guaiacylglycerol-β-guaiacyl ether subjected to the steam treatment, significantly influenced on the yield of dimers formed.

In the present study a comparison was made with the experiment for lignin polymer in wood. S-DHP resembles to syringyl lignin in secondary wall of hardwood. For this reason, S-DHP was synthesized and used to elucidate the mechanism of lignin degradation by steam treatment. In the degradation of S-DHP by steam treatment, sinapyl alcohol, sinapaldehyde and syringaresinol were formed as major products (Fig. 39-B). Syringaresinol obtained from the steamed S-DHP could be derived from β-ether of syringyl type lignin. The β-ether linkage could be homolytically cleaved to give sinapyl alcohol radical which was coupled to give Cβ-Cβ linkage.

On the other hand, the acid hydrolysis of S-DHP with dioxane - HCl (9:1) in reflux temperature for 4 hr, mainly yielded syringyl acetone, syringyl methyl ketone, and syringyl-1-hydroxy-2-propanone which were not found in the degradation products of S-DHP by steam treatment (Fig. 39-A). This result suggested that the degradation of lignin by steam treatment is different from acid hydrolysis reaction.

These results were in good agreement with the degradation mechanism of lignin by steaming proposed in section 2.3.5.

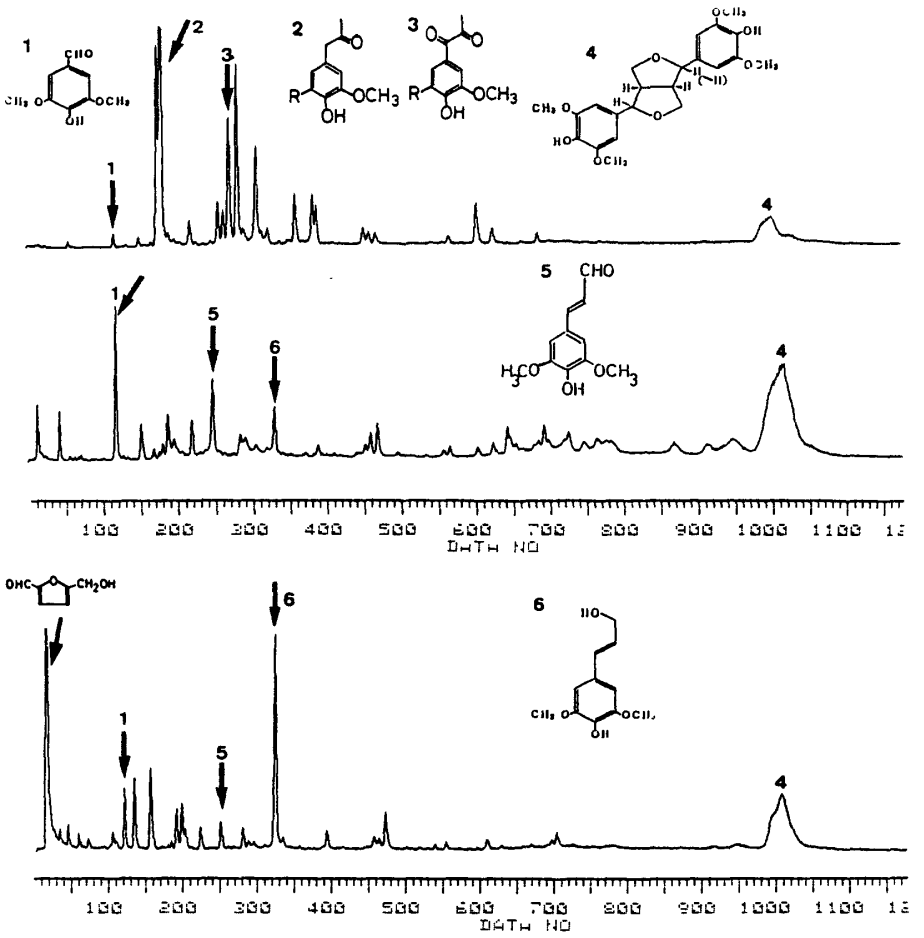

Fig. 39. GC-MS chromatogram of degradation products (acetates) of sinapyl alcohol DHP and LCC by steam treatment
A: acidolysis of S-DHP (membrane method)
B: steam treatment of S-DHP (membrane method)
C: steam treatment S-LCC/mannan

2. 4. 5 Steam treatment of sinapyl alcohol LCC

The presant study was carried out in order to confirm, whether hemicelluloses reduce sinapyl alcohol radicals formed by steam treatment to sinapyl alcohol. Lignin carbohydrate complexes (LCCs) which represent the association between lignin and carbohydrate in plant cell walls were synthesized and subjected to the steam treatment. S-LCC was prepared by dehydrogenative polymerization of sinapyl alcohol with peroxidase and H_2O_2 in concentrated solution of glucomannan. Modified dialysis membrane method was employed in order to obtain the highly polymerized DHP.

Degradation products formed from the steamed S-LCC were also identified by ^{1}H-NMR and GC-MS. The GC-MS analysis was conducted by monitoring the mass chromatogram of the products. The major products of steamed S-LCC detected were sinapyl alcohol, syringaresinol and 5-hydroxymethylfurfural. Other products

identified were syringaldehyde and sinapaldehyde.

It was shown that a large amount of 5-hydroxymethylfurfural was formed from the steamed S-LCC. By steam treatment, mannan of LCC was degraded to mannose, which was converted to 5-hydroxymethylfurfural by loss of two molecules of water. Under the conditions used, the homolytic cleavage of β-ether linkage of LCCs to produce sinapyl alcohol radical occurred. Mannose derived from mannan possibly reduced sinapaldehyde to sinapyl alcohol radical and the radical to sinapyl alcohol. Then the yield of sinapaldehyde was decreased, and sinapyl alcohol and syringaresinol were increased (Fig. 39-C). The idea was confirmed by the ^{13}C-NMR spectrum of the steamed DHP. The ^{13}C-NMR data of S-LCC showed that a small peak corresponding to syringaresinol. In dehydrogenative polymerization of sinasyl alcohol in water, syringaresinol was formed in a large amount by the β-β coupling. However, in mannan solution, the formation of syringaresinol was restricted by the presence of mannan, and only a small amount of syringaresinol was found in the S-LCC. While, the ^{13}C-NMR of steamed S-LCC showed the intense peaks of syringaresinol 54.8 ppm (Cβ), 72.4 ppm (Cγ), 86.4 ppm (Cα), respectively. The results suggested that the increase of syringaresinol by steam treatment of S-LCC mannan is ascribed to homolytic cleavage of β-ether of syringyl type lignin to sinapyl alcohol radical, which coupled to give Cβ-Cβ linkage

2. 4. 6 Degradation machanism of lignin by steam explosion

Based on the product identified, the present author proposed Fig. 30 for degradation mechanism of lignin by steam treatment. By steaming conditions at 28 kgf/cm^2-230°C for 4 minutes, the hydroxyl group or the ether linkage of α-position of lignin side chain could be protonated and loses a molecule of water or phenol to give the quinonemethide-like structure. Then β-aryl ether linkage could be homolytically cleaved to produce its intermediate radicals. Reduction and oxidation of these intermediates occurred and followed by the homolytic cleavage of the ether linkage at 4-position, to give sinapyl alcohol and coniferyl alcohol radicals, respectively. Disproportionation of these radicals produced cinnamyl alcohol (2, 2') and cinnamaldehyde (4, 4'). On the other hand these radicals could be coupled together at Cβ of the side chain to produce syringaresinol and phenylcoumaranes. The homolytic cleavage of α-β side chain could produce vanillin.

2. 5 Summary

The ether soluble fraction of steam-exploded wood lignin of Shirakanba mainly contained *d*,*l*-syringaresinol, *d*,*l*-episyringaresinol, dehydrodiconiferyl alcohol, coniferyl alcohol, sinapyl alcohol, vanillin, syringaldehyde, vanillic acid, syringic acid, furfural, 5-hydroxymethylfurfural, and betulin.

By steam explosion, guaiacylglycerol- and syringylglycerol-β-guaiacyl ethers gave coniferyl and sinapyl alcohols, respectively, as main products with small amounts of dehydrodiconiferyl alcohol and syringaresinol, respectively. The results indicated that the β-O-4 ether linkage of the substrates mainly was cleaved homolytically to produce cinnamyl alcohol radicals which were converted to cinnamyl alcohol and their dimers.

A lignin substructure model compound (guaiacylglycerol-β-guaiacyl ether and syringylglycerol-β-guaiacyl ether), DHPs (coniferylacohol DHP and sinapylalcohol DHP) and sinapylalcohol-mannan LCC were treated with high pressure steam (at 28 kgf/cm^2, 230°C for 4 min). The degradation products obtained were identified and compared with those by steam explosion to characterize the degradation mechanism of lignin by steam explosion. The degradation products of guaiacylglycerol-β-guaiacyl ether by steam treatment were almost the same as those by steam explosion. Coniferyl alcohol and guaiacol were major products, and followed by vanillin, vanillyl alcohol, coniferyl aldehyde, *d,l*-pinoresinol, *d,l*-epipinoresinol, and dehydrodiconiferyl alcohol. In addition, 2-(3-methoxy-4-hydroxyphenyl)-7-methoxycoumarane, *trans*-3-methoxy-4-hydroxy-2′-hydroxy-3′-methoxystilbene, *cis*-3-methoxy-4-hydroxy-2′-hydroxy-3′-methoxystilbene, catechol, *cis*-coniferyl alcohol, dihydroconiferyl alcohol, homovanillyl alcohol, 1-(2-hydroxy)ethoxy-2-methoxybenzene were formed. The formation of pinoresinol and phenylcoumarane from guaiacylglycerol-β-guaiacyl ether was consistent with the homolytic cleavage of Cβ-O ether linkage to produce coniferyl alcohol radical and guaiacol radical followed by their coupling to derive the dimers. The formation of coniferyl alcohol and coniferyl aldehyde suggested that disproportionation of coniferyl alcohol radical occurred. Furthermore one electron reduction of the radical by sugars led to a higher yield of coniferyl alcohol than coniferyl aldehyde.

On the other hand, by steam treatments of DHPs and LCCs coniferyl aldehyde and sinapaldehyde were produced in larger amounts than coniferyl alcohol and sinapyl alcohol, and that the yields of dimer fractions were larger than these in the steaming of dimeric lignin substructure model compounds.

It was thus concluded that a homolytic cleavage reaction of β-O-4 ether linkage occurred as main degradation reaction of lignin in wood by steam treatment and steam explosion.

By dialysis membrane mathod coniferyl and sinapyl alcohols were dehydrogenated to highly polymerized DHPs which were insoluble in any solvents. The molecular weights of insoluble lignin-related polymers could be determined by measurement of their softening point (Ts) which has a linear relation with the log Mw. This method is very useful to estimate the molecular weight of insoluble polymers.

Dehydrogenative polymerization of monolignols in plant cell wall would occur in hydrophobic region of hemicelluloses by cell wall bound peroxidases, and in the hydrophobic condition sinapyl alcohol could be polymerized mainly through β-O-4 ether linkage connected to hemicelluloses at α-position.

3. UTILIZATION OF STEAM-EXPLODED WOOD FOR ENZYMATIC SACCHARIFICATION AND RUMINANT FEED

3.1 Introduction

Wood including bamboo is by far the most abundant biomass on the earth, and it can be endlessly renewed. The need to develop renewable alternatives to petroleum, coupled with the world's growing requirements for fuel and food, will guarantee the more wide-spread use, and more efficient conversion of wood. However, because of chemical and structural heterogeneity of wood the wood-conversion process is still not economically feasible.

Several papers on the conversion of wood to ruminant feed have been reported[69,70]. It has been known that for enzymatic saccharification and digestion of wood by ruminant, delignification or cleavage of the lignin-carbohydrate linkages

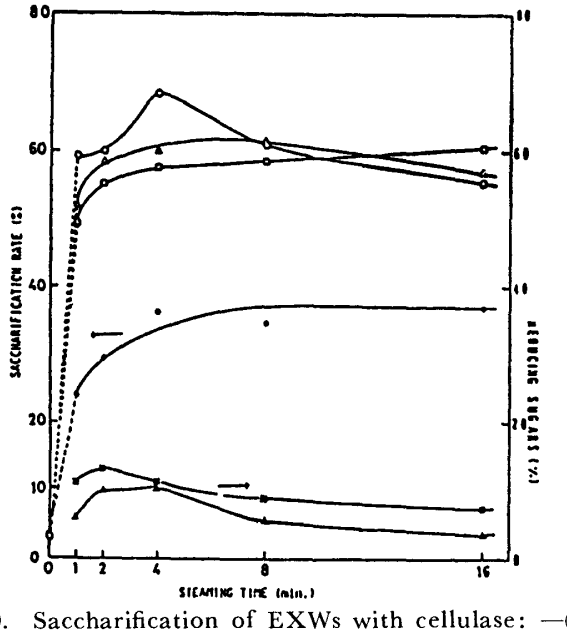

Fig. 40. Saccharification of EXWs with cellulase: —○—: Shirakanba EXW (steaming pressure: 28 kg/cm^2); —△—: Shirakanba EXW (steaming pressure: 24 kg/cm^2); —□—: Shirakanba EXW (steaming pressure: 20 kg/cm^2); —●—: Karamatsu EXW (steaming pressure: 28 kg/cm^2); —▲—: Reducing sugars (%) of Shirakanba EXWs (28 kg/cm^2) incubated in the same condition without cellulase; —■—: Reducing sugars (%) of Karamatsu EXWs (28 kg/cm^2) incubated in the same condition without cellulase.

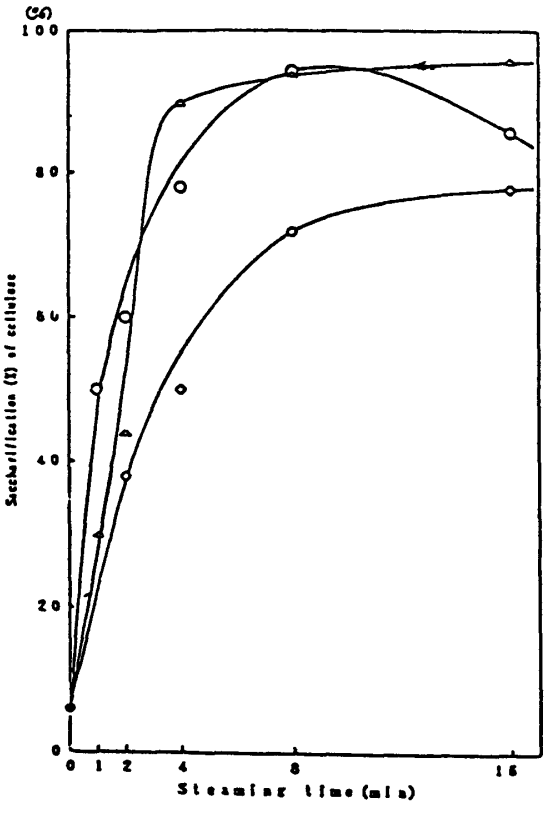

Fig. 41. Saccharification (%) of cellulose of Mosochiku EXB
○: steam pressure, 28 kg/cm²
△: steam pressure, 24 kg/cm²
◇: steam pressure, 20 kg/cm²
●: unexploded bamboo powder

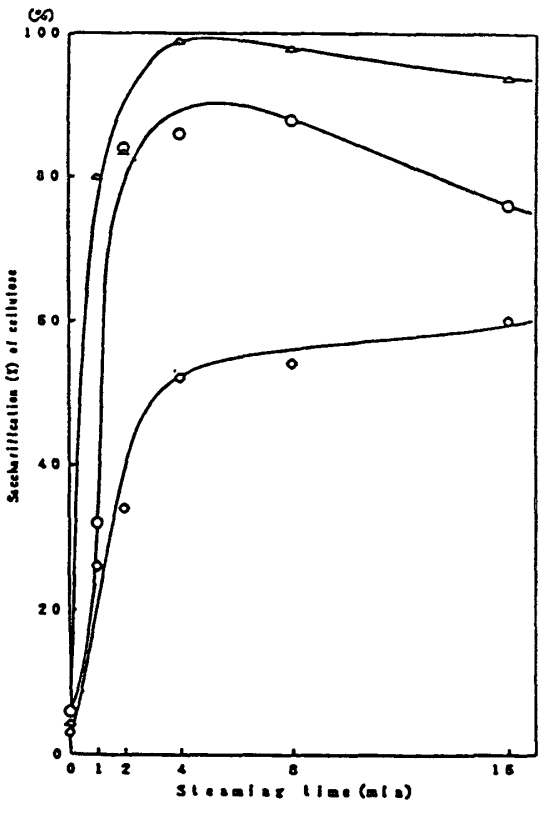

Fig. 42. Saccharification (%) of cellulose of exploded materials
○: Chishimazasa (steam pressure, 28 kg/cm²)
△: Shirakanba (steam pressure, 28 kg/cm²)
◇: Karamatsu (steam pressure, 28 kg/cm²)

is prerequisite[71]. We have investigated the steam explosion of woods and bamboos to accomplish economically feasible separation of cellulose, hemicelluloses and lignin of woody materials for chemicals, pulp and enzymatic saccharification[17,26,40,42].

This section reports on the enzymatic saccharification and ruminant digestion of the steam-exploded wood (EXW) and bamboo (EXB), and nutritional improvement of EXW for ruminant feed by microbial protein of *Peacilomyces varioti*[8,9].

3. 2 Enzymatic Saccharification

EXWs of Shirakanba and Karamatsu, and Mosochiku and Chishimazasa EXBs, were subjected to enzymatic hydrolysis using a *Trichoderma* cellulase (Meiselase). The results are shown in Figs. 40, 41 and 42. Untreated wood and bamboo powder gave less than 5 % saccharification with the enzyme, whereas Mosochiku EXB, Chishimazasa EXB and Shirakanba EXW gave 59%, 44%, and 68% saccharification of the sample, corresponding to 95%, 87%, and 98% of cellulose, respectively. However, Karamatsu EXW (28 kgf/cm², 4 min.) gave 37% saccharification of the sampple.

The poor saccharification of the Karamatsu EXW with the cellulase could bu ascribed to anatomical differences of wood, higher lignin content, and structural differences of lignin: coniferous woods are composed of mainly tracheids, and the lignin is composed mainly of guaiacyl lignin which is more condensed than guaiacyl-syringyl lignin in hardwoods and grasses. However, we recently found that pretreatment of electron beam irradiation of conifer wood chips or after ball milling of conifer EXWs gave almost the same saccharification as in hardwood EXWs[72]. Hence, it was indicated that EXBs and Shirakanba EXW were suitable for enzymatic saccharification and ruminant feed.

3. 3 Characterization and Nutritional Improvement as Ruminant Feed

3. 3. 1 *In vitro* digestibility of steam-exploded woods

Bamboo, wood and rice straw contain large amounts of cellulose and hemicelluloses but are low value as ruminant feed, because of their low digestibility due to physical and chemical linkages between polysaccharides and lignin in the materials. However, by steam explosion these linkages were cleaved and these materials were converted to easily digestible materials in a mixture of the rumen fluid fo a sheep and artificial saliva of McDougall. Steam-exploded Mosochiku and rice straw gave 50% and 75% digestibility of organic matter (OM) in comparison with 8.0% and 44% of those in the unexploded samples, and 39% and 64% digestibility of organic cell wall (OCW) (unexploded samples, 3.2% and 21%), respectively.

Thus, digestibility of Shirakanba EXW and Mosochiku EXB comparable to that of standard feeds, orchard-timothy mixed hay (68.5%) and alfalfa (64.4%) (Table 10, 11 and 12).

In vitro digestibility of Shirakanba EXW by cattle and goats in a preliminary investigation also showed better value (90%) than that of hay cube (73%). Body weight of the goats fed with Shirakanba EXW were the same to those of control[73].

Table 10. Nutritional analysis and digestibility of white birch exploded woods

Wood sample (Shirakanba)	Steaming time	Nutritional analysis					*in vitro* digestibility		
		DM (%)	OM %	CM of	OCW DM	Ash	DM (%)	OM (%)	OCW (%)
Unexploded Wood powder	0 min.	94. 9	93. 7	85. 2	83. 5	1. 2	13. 3	13. 4	2. 8
	1 min.	95. 4	94. 9	61. 5	60. 6	0. 5	40. 0	40. 1	6. 3
Exploded	2 min.	91. 4	90. 4	55. 2	54. 4	0. 7	57. 3	57. 3	29. 1
	4 min.	87. 4	96. 8	51. 0	51. 4	0. 6	76. 5	76. 5	60. 6
woods	8 min.	90. 0	89. 4	54. 3	53. 6	0. 6	78. 8	78. 8	65. 0
	16 min.	89. 9	89. 1	56. 4	55. 7	0. 7	75. 9	76. 0	61. 6

Table 11. Nutritional analysis and digestibility of exploded Mosochiku

Samples (Mosochiku)	Steaming time (min)	Nutritional analysis DM (%)	OM	CW	OCW	Ash	*in vitro* Digestibility DM (%)	OM	OCW
			% of DM				(%)	(%)	(%)
Unexploded	0	92.4	98.0	94.1	93.2	2.0	9.6	8.0	3.2
	1	93.3	98.0	73.0	72.4	2.0	32.6	31.8	7.1
	2	94.5	98.8	72.3	71.6	1.2	33.8	33.5	8.1
Exploded	4	91.8	99.0	71.1	70.3	1.0	47.8	47.3	25.8
	8	91.9	98.9	81.0	80.1	1.1	48.6	48.7	36.6
	16	91.2	98.9	82.3	81.5	1.1	50.0	49.8	39.1

Table 12. Nutritional analysis and *in vitro* digestibility of exploded materials

	Nutritional analysis DM (%)	OM	Crude protein	Crude fat	OCW	*In vitro* Digestibility DM (%)	OM	OCW
			(%) of DM			(%)	(%)	(%)
Mixed hey of orchardgrass and timothy	85.8	90.9	18.1	2.0	61.9	70.2	68.5	53.7
Alfalfa	86.5	89.9	20.1	2.4	46.4	64.0	64.4	31.1
Rice straw (unexploded)	86.1	85.1	5.2	1.8	60.9	43.6	43.7	20.9
Rice straw (exploded) (22 kgf/cm^2 4 min)	90.2	84.6	4.5	2.2	56.8	69.1	75.0	64.2
Bagasse (unexploded)	92.0	97.2	—	—	93.7	41.6	41.8	39.6
Bagasse (exploded) (27 kgf/cm^2 2 min)	91.9	96.9	—	—	77.5	63.3	65.8	57.2
Karamatsu (unuxploded)	90.3	99.8	—	—	88.4	11.6	12.1	0.6
Karamatsu (exploded) (28 kgf/cm^2 4 min)	92.3	99.7	—	—	72.1	29.1	29.4	2.4
Shirakanba (unexploded)	94.9	93.7	—	—	83.5	13.3	13.4	2.8
Shirakanba (exploded) (28 kgf/cm^2 4 min)	87.4	86.8	—	—	51.4	76.5	76.5	60.6
Shirakanba (exploded) (26 kgf/cm^2 4 min)	25.2	99.8	0.6	3.4	75.8	52.7	54.0	41.0
EXW with *P. valioti* (26 kgf/cm^2 4 min, 40°C, 14 days)	40.7	97.2	7.2	0.9	82.7	52.4	52.7	45.4

In addition, we recently found[16] that the culture of *Paecilomyces varioti* which was developed by FORSS et al.[74] to produce microbial protein, with Shirakanba EXW considerably improved nutritional quality of EXW (crude protein content increased to 7.2%, Table 12) as ruminant feed.

Sugars derived from hemicelluloses, phenolic compounds from lignin, and 5-hydroxymethylfurfural in water soluble fraction of the white birch EXW were almost completely catabolized by the culture.

It is thus concluded that EXB and EXW are suitable for fermentation and ruminant feed. It was also shown that the steam explosion process is one of the best pretreatment of bamboo and woody residues for enzymatic saccharification, and preparation of ruminant feed and wood chemicals.

3.3.2 Mycelial growth of *Paecilomyces varioti*

Both strains (IFO-4855 and 5674) of *P. varioti* grew very well in the four media (A, B, C, and D) as shown in Fig. 43. The mycelial growth in B media for which carbon source, glucose is substituted with water extract of EXW is almost the same. It was further shown that the mycelial growth of the fungus in C medium, for which yeast extract and some of minerals were omitted from the standard medium A, was not significantly different from the growth in A medium. The result indicated that the sugars in the water extract of EXW were good carbon source for the mycelial growth of *P. varioti*. pH of the cultures in A and B media graudually decreased to about 3.2 and then increased to about 6.5 after 5 days culture (Fig. 44). However, in the cultures in C and D pH decreased to about 2 after 4 days culture and then slowly increased to about 3. The lower pH in the cultures of C and D media might be ascribed to relatively poor uptake of nitrate released by assimilation of ammonium ion from ammonium nitrate. This could be improved

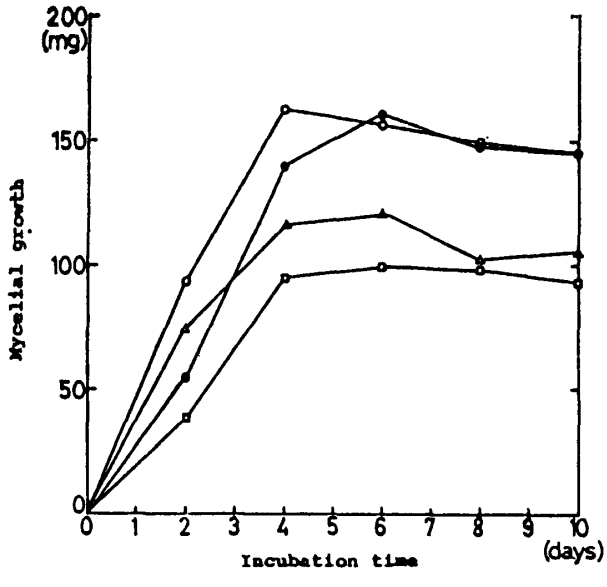

Fig. 43. Mycelial growth of *Paecilomyces varioti* IFO 4855: A: Glucose+standard medium: —●—; B: Water extract of EXW+standard medium; —○—; C: Water extract of EXW +the medium composed of NH_4NO_3 2 g, K_2HPO_4 1 g and $MgSO_4$ 0.5 g/1000 ml: —△— D. The medium C+NH_4NO_3 3 g/1000 ml: —□—.

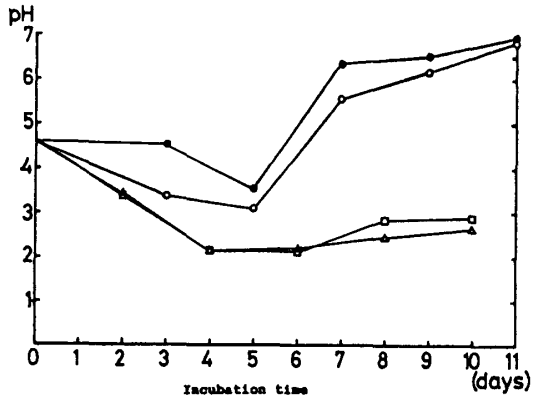

Fig. 44. Change of pH in cultures of *P. varioti* IFO-4855; A: Glucose+standard medium: —●—; B: Water extract of EXW +standard medium: —○—; G: Water extract of EXW+the medium composed of NH_4NO_3 2 g, K_2HPO_4 1 g and $MgSO_4$ 0.5g/1000 ml: —△—; D: The medium C+NH_4NO_3 3 g/1000 ml: —□—.

to use ammonium carbonate as nitrogen source.

3. 3. 3 Assimilation of sugars in the water extract by *P. varioti*

Sugars in the water extract of EXW before and after culture of *P. varioti* were analyzed by GLC. As shown in Fig. 45 arabinose, xylose, mannose, galactose and glucose in the extract were all consumed after 9 days culture. The process of sugar consumption in the water extract of EXW is shown in Table 13. It is obvious that xylose, glucose and galactose were consumed faster than other sugars. In addition,

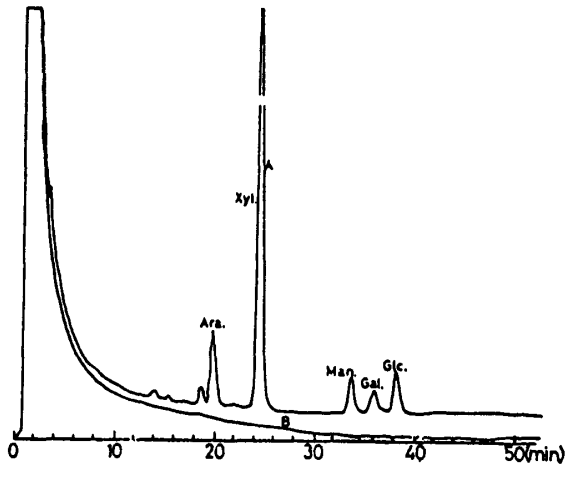

Fig. 45. GLC analysis of assimilation of monosaccharides in water extract of EXW by *P. varioti*: A: The original monosaccharides; B: After 9 days culture.

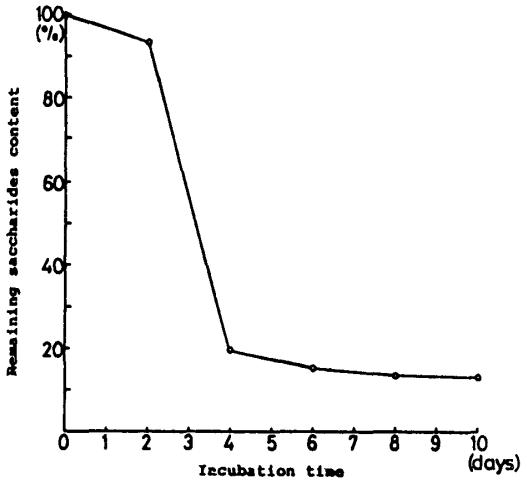

Fig. 46. Assimilation of oligosaccharides in water extract of EXW by *P. varioti*.

Table 13. Assimilation of sugars in the water extract of EXW by *P. varioti*

	Cont	3 days	5 days	9 days
Ara. (%)	9.5	6.0	0.2	0
Xyl. (%)	57.7	24.3	0.2	0
Man. (%)	6.8	3.9	1.0	0.6
Cal. (%)	6.6	1.6	0	0
Glc. (%)	8.0	1.0	0	0
Other compounds (%)	11.3	2.9	0.4	1.6
Total (%)	100	39.8	1.9	2.3
(% of water extract)	(30.3)	(12.1)	(0.5)	(0.7)

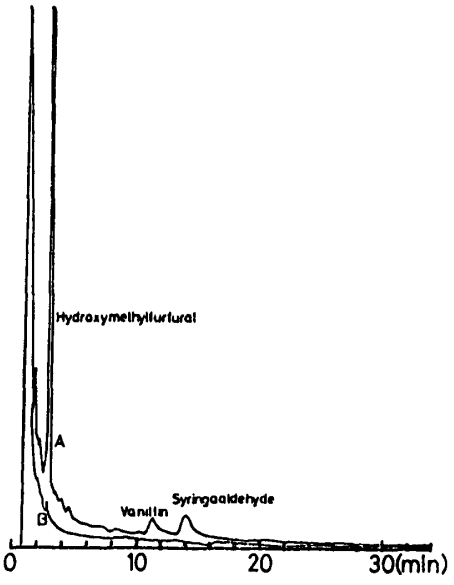

Fig. 47. Degradation of phenolic compounds and hydroxymetylfurfural by *P. varioti*.

the consumption of total sugars including oligosaccharides in the water extract during fungal growth were measured by phenol-sulfuric acid method as shown in Fig. 46. The results showed that oligosaccharides which were not analzed by GLC was slowly assimilated and still remained after 10 days culture.

3. 3. 4 Degradation of phenolic compounds and hydroxymethylfurfural by *P. varioti*

By steam explosion of wood both α-O-4 and β-O-4 linkages of lignin were cleaved to give various phenolic compounds and low molecular weight lignin. While a part of hexosan and pentosan gave hydroxymetylfurfural and furfural, respectively. However, furfural and some volatiles were taken out of the water extract during freeze-drying. These phenolic compounds and hydroxymetylfurfural which are partly soluble in water were separated by TLC and identified by NMR analysis to be vanillin, syringaldehyde, *d,l*-syringaresinol, *d,l*-episyringaresinol and coniferyl aldehyde. *P. varioti* catabolized these phenolic compounds and hydroxymethylfurfural as shown in Fig. 47. It was suggested that hydroxymethylfurfural and phenolic lignin degradation compounds in EXW might be dislikable substances for cattle. We found that the EXW cultured with *P. varioti* did not give a stimulative odor or bitter taste found in original EXW.

3. 3. 5 Nutritional analysis and *in vitro* digestibility of cultured steam-exploded woods

The nutritional analysis of the EXW cultured with *P. varioti* showed that crude protein whose amount in the original EXW was 0.6% was increased to 7.2% in the cultured EXW. The protein amount was a little lower than that in orchard-timothy and alfalfa but much higher than that in rice straw (4.5%). *In vitro* digestibility of EXW and cultured EXW was not different but much higher than that of rice straw. These results indicated EXW is a suitable feed for ruminant, and that mycelial protein cultured with EXW considerably improved nutritional quality of the EXW.

It is thus concluded that EXW is well digested by ruminants and hydrolyzed by cellulase: *in vitro* and *in vivo* digestibilities were 50~80% of OM, and saccharification, 80~90% of the polysaccharides. Culture of *P. varioti* with EXW remarkably improved nutritional quality of EXW, and phenolic compounds and hydroxymethylfurfural which may be dislikable compounds for ruminants were completely catabolized by the fungus. Thus, utilization of wood residues for ruminant feed became promising by steam-explosion of wood. It was also shown that the EXW process is one of he best pretreatments of woody residues for enzymatic saccharification.

3.4 Summary

The steam-exploded wood (EXW) (28 kgf/cm^2, 4 min) of Shirakanba was easily hydrolyzed by cellulase and digested by ruminants as in alfalfa: saccharification was about 87% of the polysaccharides of EXW, *in vitro* digestibility about 77% of EXW. These values of steam-exploded bamboos (saccharification: 95% of cellulsoe and *in vitro* digestibility: 50% of dry matter (DM) for the Mosochiku EXB) are comparable to those of steam-exploded wood (EXW) of Shirakanba but better than those of Karamatsu EXW. Culture of *P. varioti* with EXW considerably improved nutritional quality of EXW (crude protein, 7.2%) as a ruminant feed. Sugars derived from hemicelluloses, phenolic compounds, and 5-hydroxymethylfurfural in water soluble fraction of EXW were almost completely metabolized by the culture. The results indicated that utilization of wood residues for ruminant feed and fermentation become promising by the steam-explosion.

CONCLUSION

Wood chips of Shirakanba (white birch, *Betula platyphylla* Sukatchev var. *japonica* Hara) and Karamatsu (Japanese larch, *Larix leptolepis* Gordon) were treated with a high pressure steam (12~28 kgf/cm^2) for 1~16 min, and the steam pressure was released instantaneously resulting in steam-exploded wood. When the treating time was longer more fibrillation of cell walls of Shirakanba occurred. Fibers of the exploded woods were observed to be vigorously ruptured.

Chemical changes of main components in wood (cellulose, hemicelluloses and lignin) by steam explosion have been elucidated by ^{1}H- and ^{13}C-NMR, gas chromatography, GPC, X-ray diffraction, transmission electron microscopy, thermal softening property and CP/MAS ^{13}C-NMR (cross polarization and magic-angle spinning carbon 13 nuclear magnetic resonance) spectroscopy. By steam explosion hemicelluloses were rapidly hydrolyzed to lower molecular weight products. Almost all hemicelluloses (27.9%) in Shirakanba wood were hydrolized to oligosaccharides extractable with water by only one min steaming at 20 kgf/cm^2, and by 8 min steaming at 28 kgf/cm^2 53.7% of hemicelluloses were converted to monosaccharides. Monosaccharides obtained by 2 min steaming of Shirakanba wood were composed of 61.1% of xylose and only 4.9% of glucose, and the yields were in accord with original composition of hardwood hemicelluloses. Cellulose in non-crystalline area was partially hydrolyzed, and micelle lingth was decreased to about 2000 Å by 8 min steaming at 28 kgf/cm^2. However, cellulose was not completely hydrolyzed to give glcose, and non-crystalline cellulose would be annealed and transformed to crystalline cellulose.

By a steam explosion (28 kgf/cm^2, 230°C, 16 min) cellulose in Shirakanba wood

was increased in crystallinity (CrI: 51% to 67%), micelle width (25 A to 52 Å), and microfibril width (32 Å to 50 Å), whereas the langth of cellulose microfibrils was decreased (to about 2000 Å). The crystalline form of cellulose clearly was changed by the steam explosion: broad peaks in the CP/MAS ^{13}C-NMR spectrum of the crystalline component of the wood cellulose assigned at C_1, C_4, and C_6 of the pyranose ring changed to fine doublet peaks of crystal form, Cellulose Ia. It also was found that the crystallinity of cellulose is increased by steaming the wood at high temperatures and pressures without explosion. However, purified, crystalline cellulose, such as filter paper, was influenced less in crystallinity by steaming, and the results suggested that other constituents accompanied by cellulose were responsible for the increase of crystallinity of the cellulose by the steam explosion.

However, when reaction time was increased, the yields of furfural and 5-hydroxymethylfurfural derived from polysaccharides were increased. Lignin was degraded slower than hemicelluloses. The yield of lignin was 29.2% in maximum by 8 min steaming at 28 kgf/cm^2, and the molecular weight of lignins obtained were decreased to Mw=2100 and 1100 by 2 min and 16 min steaming, respectively.

The ether soluble fraction of steam-exploded wood lignin of Shirakanba mainly contained *d,l*-syringaresinol, *d,l*-episysingaresinol, dehydrodiconiferyl alcohol, coniferyl alcohol, sinapyl alcohol, vanillin, syringaldehyde, vanillic acid, syringic acid, furfural, 5-hydroxymethylfurfural, and betulin.

By steam explosion, guaiacylglycerol- and syringylglycerol-β-guaiacyl ethers gave coniferyl and sinapyl alcohols, respectively, as main products with small amounts of dehydrodiconiferyl alcohol and syringaresinol, respectively. The results suggested that the β-O-4 ether linkage of the substrates mainly was cleaved homolytically to produce cinnamyl alcohol radicals which are converted to cinnamyl alcohol and their dimers

Hence, we proposed a radical reaction as the main degradation mechanism of lignin by steam explosion.

To confirm the radical degradation theory of lignin by steam explosion, a lignin substructure model compound, guaiacylglycerol-β guaiacyl ether was treated with high pressure steam (at 28 kgf/cm^2, 230°C for 4 min). The degradation prosducts obtained were identified and compared with those by steam explosion to characterize the degradation mechanism of lignin by steam explosion. The degradation products of guaiacylglycerol-β-guaiacyl ether by steam treatment were almost the same as those by steam explosion. Coniferyl alcohol and guaiacol were major products followed by vanillin, vanillyl alcohol, coniferyl aldehyde, *d,l*-pinoresinol, *d,l*-epipinoresinol, and dehydrodiconiferyl alcohol. In addition, 2-(3-methoxy-4-hydroxyphenyl)-7-methoxycoumarane, *trans*-3-methoxy-4-hydroxy-2′-hydroxy-3′-methoxystilbe-

ne, *cis*-3-methoxy-4-hydroxy-2′-hydroxy-3′-methoxystilbene, catechol, *cis*-coniferyl alcohol, dihydroconiferyl alcohol, homovanillyl alcohol, 1-(2-hydroxy)ethoxy-2-methoxybenzene were identified. The formation of pinoresinol and phenylcoumarane from guaiacylglycerol-β-guaiacyl ether was consistent with the homolytic cleavage of Cβ-O ether linkage to produce coniferyl alcohol radical and guaiacol radical followed by their coupling to derive the dimers. The formation of coniferyl alcohol and coniferyl aldehyde suggested that disproportionation of coniferyl alcohol radical occurred. Furthermore one electron reduction of the radical led to a higher yield of coniferyl alcohol than coniferyl aldehyde.

In steaming of guaiacylglycerol-β-guaiacyl ether, coniferyl alcohol was produced in a larger amount than coniferyl aldehyde, however, sinapaldehyde was produced in a larger amount than sinapyl alcohol in steaming S-DHPs. This may be caused by the difference in the oxidation level of the starting material used. In the steaming of lignin substructure model compound syringylglycerol-β-guaiacyl ether, the oxidation level of C_6-C_3 unit of the substrate is equivalent to that of sinapyl alcohol radical, while the oxidation level of C_6-C_3 unit of S-DHPs is equivalent to that of sinapaldehyde. Compared to the steaming of syringylglycerol-β-guaiacyl ether with that of S-DHP, the yield of dimer fractions (e.g. syringaresinol) increased in latter case. It seems that by steam treatment, the cleavage of β-O-4 linkage in S-DHPs occurred to maintain a high concentration of radicals which coupled to the increase of the dimers formation.

The increased yield of sinapyl alcohol in steamed S-LCC, indicated that sinapyl alcohol radical produced by the steam treatment was reduced by the mannose derived from mannan linked to S-DHP. The results obtained in this study indicated that a remarkable amount of resinols are produced from β-aryl ether units during steam treatment.

It is thus concluded that a homolytic cleavage reaction occurred as main degradation reaction of lignin in wood by steam treatment and steam explosion.

References

1) C.R. WILKE: "**Cellulose as a Chemical and Energy Resource**", John Wiley & Sons, New York, London, Sydney, Toront (1975)
2) D.O. HALL: *Solor Energy*, **22**, 307 (1979)
3) H.E. GRETHLEIN: *Biotechnol. Bioeng.* **20**, 503 (1978)
4) B. RUGG, P. ARMSTRONG and R. STANTON: *Dev. Ind. Microbiol.*, **22**, 131 (1981)
5) J.A. CHURCH and D. WOOLDRIGE: *Ind. Eng. Chem. Prod. Res. Dev.*, **20**, 371 (1981)
6) Nuclear Assurance Corp., Report No. DOE/RA 50322-TI-V.**1**, 2, (1981)
7) W. GUENTER and N. FORSBERG: *Nutritional Release,* No. **4**, Stake Tchnology LTD., Ottawa, Canada (1972)
8) T.N. KLEINET: *Tappi*, **57**(8), 99 (1974)
9) K.V. SARKANEN: NSF Report, 7708979 (1978)

10) A. SAKAKIBARA, Y. EDASHIGE and H. TAKEYAMA: *Japan Tappi*, **37**(5), 423 (1983)
11) E.A. DELONG: *Can. Pat.*, 1 096 374
12) R.H. MARCHESSAULT and J. St-PIERRE: in "**Future sources of organic raw material-CHEMRAWN I**", Proceedings of CHEMRAWN Conferrence, Toronto, Canada, July 1978. Edited by L.E. St-Pierre and G.R. Brown eds.) Pergamon Press, 613 (1980)
13) R.H. MARCHESSAULT, S. COULOMB, H. MORIKAWA and D. ROBERT: *Can. J. Chem.*, **60**, 2372 (1982)
14) Y. TOGAMURA, A. MIYAZAKI and R. KAWASHIMA: *Jpn. J. Zootech. Sci.*, **58**, 505 (1986)
15) Y. TOGAMURA, A. MIYAZAKI, K. KAWASHIMA, T. HIGUCHI, M,TANAHASHI and K. KIYOTO: *Jpn. J. Zootech. Sci.*, **54** , 206 (1983)
16) M. TANAHASI, T. HIGUCHI, Y. TOGAMURA and M. SIMADA: *Cell. Chem. Technol.*, **19**, 687 (1985)
17) M. TANAHASHI: *Wood Res. and Technical Notes*, No. **18**, 34 (1983)
18) R.H. MARCHESSAULT, S. COULOMB, T. HANAI and H. MORIKAWA: *Transactions Tech. Sec. CPPA*, **6**, TR52-TR56 (1980)
19) A. SAKAKIBARA: **Recent Advances in Phytochemistry**, vol. 11, Frank A. LOEWUS, ed., Plenum Press, 117 (1977)
20) E. ADLER: *Wood Sci. Technol.*, **11**, 169 (1977)
21) M. WAYMAN and M.G.S. CHUA: *Can. J. Chem.*, **57**, 2599 (1979)
22) J.A. HEMMINGSON: *J. Wood Chem. Technol.*, **5**, 513 (1985)
23) J.A. HEMMINGSON: *ibid.*, **5**, 159 (1985)
24) J.A. HEMMINGSON: *ibid.*, **7**, 229 (1987)
25) P. TEKELY and M.R. VIGNON: *ibid.*, **7**, 215 (1987)
26) M. TANAHASH, S. TAKADA, T. AOKI, T. GOTO, T. HIGUCHI and S. HANAI: *Wood Research*, No. **69**, 36 (1983)
27) M. TANAHASHI, K, TAMABUCHI, T. GOTO, T. AOKI, M. KARINA and T. HIGUCHI: *ibid.* No. **75**, 1 (1988)
28) M. TANAHASHI, T. GOTO, F. HORII, A. HIRAI and T. HIGUCHI: *Mokuzai Gakkaishi* **35**, 654 (1989)
29) M. TANAHASHI, M. KARINA and T. HIGUCHI: *Proc. Fourth Intern. Symp. Wood Pulp Chem.* (Paris), Vol. 2, 343 (1986)
30) M. TANAHASHI, M. KARINA, K. TAMABUCHI and T. HIGUCHI: *Mokuzai Gakkaishi*, **35**, 135 (1989)
31) M. TANAHASHI, M. KARINA and T. HIGUCHI: *ibid.*, **36**, 380 (1990)
32) M. KARINA, M. TANAHASHI and T. HIGUCHI: *ibid.*, **36**, 466 (1990)
33) M. TANAHASHI, M. KARINA and T. HIGUCHI: *Cellulose Chem. Technol.*, to be published.
34) M. KARINA, M. TANAHASHI and T. HIGUCHI: *Mokuzai Gakkaishi*, to be published.
35) M. TANAHASHI and T. HIGUCHI: *Wood Research*, No. **67**, 29 (1981)
36) M. TANAHASHI, T. AOKI and T. HIGUCHI: *Holzforschung*, **36**, 117 (1982)
37) M. TANAHASHI, T. AOKI and T. HIGUCHI: *Mokuzai Gakkaishi*, **27**, 116 (1981)
38) M. TANAHASHI and T. HIGUCHI: *ibid.*, **36**, 424 (1990)
39) M. TANAHASHI: *Symp. Biomas & Biotechnology*, '83, Japan Management Accociation, Session **4**, 1 (1983)
40) M. TANAHASHI and T. HIGUCHI: *Japan Tappi J.*, **39**, 118 (1985)
41) M. TANAHASHI and T. HIGUCHI: *Polymer Applications*, **32**, 595 (1983)
42) R.H. MARCHESSAULT, S.L. MALHOTRA, A.Y. JONES and A. PEROVIC: "**Wood and Agricultural Residues**; Research on Use for Feed, Fuels and Chemicals" ed. by J. Soltes, Academic Press, 401 (1983)
43) H. HARADA and T. GOTO: "**Celllulose and Other Natural Polymer Systems**" ed. by R.M. BRAWN Jr., Plenum Press, New York, 383 (1982)
44) T. HIGUCHI, M. TANAHASHI and T. UMEZAWA: "**Research on Energy from Biomass**"

36, 151 (1984)
45) T. AOKI, N. SHIRAISHI, M. TANAHASHI and T. YAMADA: *Wood Research and Technical Notes*, No. **15**, 61 (1980)
46) M. TANAHASHI and T. HIGUCHI: in "**Methods in Enzymology**", Academic Press, Vol. 161 Biomass Part B, Lignin, Pectin, and Chitin, 101 (1988)
47) T. HIGUCHI, M. TANAHASHI and A. SATO: *Mokuzai Gakkaishi*, **18**, 183 (1972)
48) F. NAKATSUBO, M. TANAHASHI and T. HIGUCHI: *Wood Research*, No. **53**, 9 (1983)
49) T. HIGUCHI, M. TANAHASHI and F. NAKATSBO: *ibid.*, No. **54**, 9 (1972)
50) F. HORII, A. HIRAI and R. KITAMARU: *Macromolecules*, **19**, 930 (1986)
51) F. HORII, H. YAMAMOTO, R. KITAMARU, M. TANAHASHI and T. HIGUCHI: *Macromolecule*, **20**, 2946 (1987)
52) K. TODHEID: *in* "**Water-A Comprehensive Treatise**" (F. FRANKS, ed.) Plenum Press, New York, Vol. **1**, Chapter 13, 463 (1972)
53) D.A.I. GORING: *Pulp Paper Mag. Can.*, **64**, T-517 (1963)
54) M. BARDET, D. ROBERT and K. LUNDQUIST: *Svensk Papperstidn.*, **6**, R61 (1985)
55) K. FREUDENBERG, J.M. HARKIN, M. RECHERT and T. FUKUZUMI: *Chem. Ber.*, **91**, 581 (1958)
56) S. LARSSON and G.E. MIKSCHE: *Acta Chem. Scand.*, **25**, 647 (1971)
57) T.I. OBIAGA and M. WAYMAN: *Svensk Papperstidn.*, **76**, 699-703 (1973)
58) W.J. CONNORS, S. SARKANEN and J. L. McCARTHY: *Holzforschung* **34**, 80 (1980)
59) O. FAIX, W. LANGE and O. BEINHOFF: *ibid.*, **34**, 174 (1980)
60) M. WAYMAN and T.I. OBIAGA: *TAPPI*, **57**, 123 (1974)
61) D.H. McDANIAL and H.C. BRAWN: *J. Org. Chem.*, **23**, 420 (1958)
62) M. TANAHASHI, H. TAKEUCHI and T. HIGUCH: *Wood Research*, No. **61**, 44 (1976)
63) H. NIMZ: *Angew. Chem.*, **13**, 313 (1974)
64) D. FRENCH, M. LEVINE, J. PAZUR and E. NORBERG: *J. Am. Chem. Soc.*, **71**, 354 (1949)
65) F. CRAMER: *Chem. Ber.*, **84**, 851 (1951)
66) F. CRAMER, W. SAENGER and H.C. SPATZ: *J. Am. Chem. Soc.*, **89**, 14 (1967)
67) K. NAKAMURA T. SHIMIZU and FUNABASHI: *Bull. Agric. Chem. Soc. Japan*, **22**, 324 (1958)
68) H. STAUDINGER and W. DOHLE: *Macromol. Chem.*, **9**, 188 (1953)
69) M.G. JACKSON: *Feed Sci. Technol.*, **2**, 105 (1977)
70) T. KLOPFENSTEIN: in "**Upgrading Residues and Byproducts for Animal**", ed. by J H. HUBER, CRC Press Inc., Florida, 40 (1981)
71) G.T. TSAO, M, LADISCH, C. LADISCH, T.A. HSU, B. DALE and T. CHUO: in "**Fermentation Processes**" Ed. by D. PERLMAN, Academic Press, New York (1978)
72) M. TANAHASHI and T. HIGUDHI: unpubulished data
73) F. TERADA, S. HORII, A. TAKIGAWA, T. HARYUU, R. TANO, K. IWASAKI, K. KAMEOKA, S. NAGASAWA, T. MATSUDA, Y. TOMIMURA, K. SHIMIZU, M. TANAHASHI and T. HIGUCHI: *Bull. National Inst. Animal Ind.*, No. **44**, 55 (1986)
74) K. FORSS and K PASSINEN: *Paperi Ja Puu*, **9**, 608 (1976)