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Gas chromatographic and mass spectrometric (GC-MS) analysis of lignin-derived products from *Cryptomeria japonica* treated in supercritical water

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Abstract Sugi (*Cryptomeria japonica* D. Don) wood was treated with supercritical water (>374°C, >22.1 MPa), and fractionated into the water-soluble portion, the methanol-soluble portion, and the methanol-insoluble residue. The methanol-soluble portion mainly consisted of the lignin-derived products. To characterize the compounds in the methanol-soluble portion, gel permeation chromatographic (GPC) and gas chromatographic-mass spectrometric (GC-MS) analyses were performed. The GPC analysis indicated that the methanol-soluble portion contained lignin-derived monomeric and dimeric products. GC-MS analysis detected 31 products which were expected to be monomeric compounds, and 18 of these were identified to be guaiacol, methylguaiacol, ethylguaiacol, vinylguaiacol, eugenol, propylguaiacol, vanillin, *cis*-isoeugenol, homovanillin, *trans*-isoeugenol, acetoguaiacone, propioguaiacone, guaicylacetone, 2-methoxy-4-(1-hydroxypropyl)phenol, homovanillic acid, 2-methoxy-4-(prop-1-en-3-one)phenol, coniferyl aldehyde, and ferulic acid. In addition, 22 dimeric products were detected, and 4 of these were believed to be compounds with biphenyl type (β -5), diphenylethane type (β -1), stilbene type (β -1), and phenylcoumaran type (β -5) structures. These results clearly indicated that the methanol-soluble portion included various monomeric and dimeric compounds produced as a result of the cleavage of ether linkages and propyl chains of lignin.

Key words Chemical conversion · Supercritical water · Lignin · β -O-4 linkage · Condensed type linkage · GC-MS

Introduction

In the face of current worldwide depletion of fossil resources, special attention has been directed toward utilization of biomass resources such as wood (lignocellulosics) for the production of energy and useful chemicals. The features of biomass are that it is renewable, carbon-neutral, and abundant. However, efficient technology for conversion to energy and fuels is needed for utilization of biomass resources.

In our laboratory, some research on biomass conversion has been carried out using supercritical water (>374°C, >22.1 MPa)¹⁻⁴ and supercritical methanol (>239°C, >8.1 MPa).⁵⁻⁸ It was reported that cellulose was converted to glucose and its derivatives using our batch type and flow type supercritical water biomass conversion systems.^{1,4} It was also reported that main cell wall components of wood such as cellulose, hemicelluloses, and lignin could be separated into water-soluble and methanol-soluble portions after supercritical water treatment.² The former portion, which mainly consists of cellulose- and hemicellulose-derived products, may have potential as a source of ethanol fermentation. The latter portion mainly consists of lignin-derived products.

In our previous report, lignin-derived products in the methanol-soluble portion were partly characterized.³ As a result, it was revealed that the lignin-derived products mainly consisted of condensed type linkages of lignin due to the preferential degradation of the ether linkages of lignin that occurred during supercritical water treatment. However, more detailed characterization and identification of products in the methanol-soluble portion are necessary for its efficient utilization.

The purpose of this study is, therefore, to obtain further information on the methanol-soluble portion regarding its potential for efficient utilization. Therefore, gel permeation chromatography (GPC) and gas chromatographic-mass spectrometry (GC-MS) analyses of the methanol-soluble portion were conducted. From the mass spectral data obtained by GC-MS analysis, lignin-derived monomeric and dimeric products were identified.

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Materials and methods

Samples and chemicals

Sugi (*Cryptomeria japonica* D. Don) wood flour (80 mesh pass) was used for supercritical water treatment. Distilled water for the supercritical water treatment and methanol for fractionation were HPLC grade. Other chemicals were reagent grade and were used without purification. 2,2'-Dihydroxy-3,3'-dimethoxy-5,5'-dimethyl biphenyl was prepared according to the method of Kratzl and Vierhapper.⁹

Supercritical water treatment and fractionation of the methanol-soluble portion

Supercritical water treatment and fractionation were conducted in the same manner as described in our previous report.³ Figure 1 shows the flowchart of supercritical water treatment and fractionation of the methanol-soluble portion. Water (4.9 ml) and 150 mg of wood flour were added to a batch type reaction vessel made of Inconel-625, and it was quickly heated by immersing it in a molten tin bath that was preheated to 500°C. To quench the reaction, the reaction vessel was immersed in a water bath. During this treatment, the temperature and pressure in the reaction vessel were monitored by a thermocouple and pressure gauge. In this study, samples were treated under supercritical water conditions of 380°C and 100 MPa for 8 s.

After supercritical water treatment, water-soluble and water-insoluble portions were retrieved. The water-insoluble portion was then treated with 10 ml of methanol for 10 min by sonication and separated by centrifugation to give a methanol-soluble portion and a methanol-insoluble residue. The methanol-soluble portion was used for analysis.

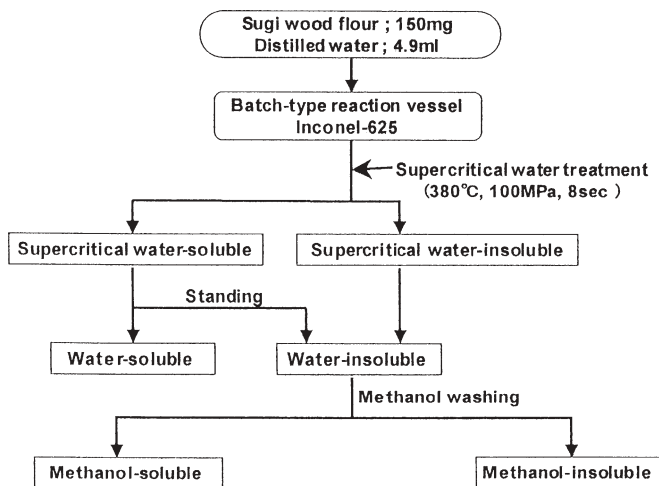


Fig. 1. Flowchart of supercritical water treatment and fractionation of the methanol-soluble portion

Analytical methods

The molecular weight (MW) distribution of the sample was estimated by GPC (Shimadzu LC-10A) through Shodex KF-801, KF-802, KF-802.5, and KF-803 columns (Showa Denko, Tokyo, Japan) by UV detector at 280 nm with a solvent of tetrahydrofuran, and a flow rate of 0.6 ml/min.

Hitachi G-7000M and M-9000 gas chromatograph 3-dimensional quadrupole mass spectrometer was used to analyze the methanol-soluble portion. The capillary column was a CP-Sil 8 (30 m long, 0.25 mm diameter, 0.25 μm film thickness). The temperature program was 1.0 min at 40°C, 5.0°C/min to 300°C, and 1 min at 300°C. Helium carrier gas was used at a flow rate of 1.5 ml/min. Injector and detector temperatures were both 230°C. One microliter of the methanol-soluble portion was directly injected unless otherwise noted. Lignin-derived monomeric products were identified from their mass spectra by comparing their retention times with those of authentic compounds as described in the literature.¹⁰⁻¹⁸ The trimethylsilylated samples were prepared with *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) in pyridine at 70°C for 30 min.

Results and discussion

Molecular weight distribution of the methanol-soluble portion

Figure 2 shows the gel permeation chromatogram of the methanol-soluble portion, which eluted from 45 min to

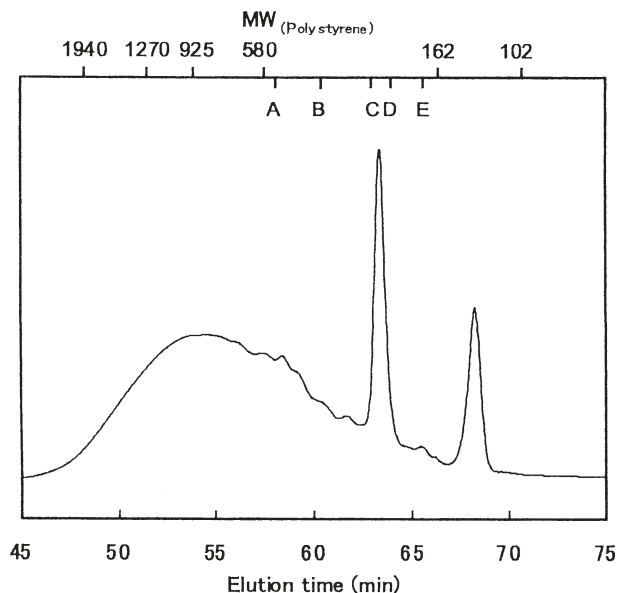
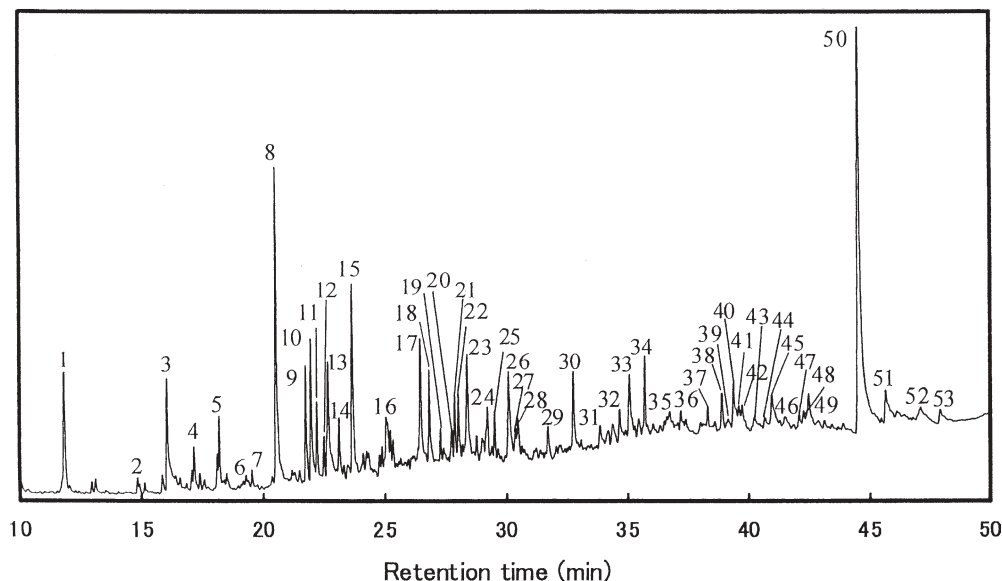


Fig. 2. Gel permeation chromatogram of the methanol-soluble portion from sugi wood after treatment in supercritical water. A, B, C, D, and E represent the elution times of 2,2',3,3'-tetramethoxy-5,5'-dimethylbiphenyl (MW 302), coniferylalcohol (MW 180), acetoguaiacone (MW 166), vanillin (MW 152), and guaiacol (MW 124), respectively

Fig. 3. Total ion chromatogram of the methanol-soluble portion from sugi wood after treatment in supercritical water



70 min. Polystyrene standards with MWs of 1940, 1270, 925, 580, 162, and 102 were eluted at 48.5, 51.7, 54.0, 57.2, 66.0, and 70.5 min, respectively, suggesting that the MW of the methanol-soluble portion must be less than decameric phenylpropane units. However, lignin model compounds such as 2,2', 3,3'-tetramethoxy-5,5'-dimethyl biphenyl, coniferylalcohol, acetoguaiacone, vanillin, and guaiacol were eluted at 58.3, 60.6, 63.0, 63.9, and 65.6 min, respectively. Interestingly, the elution times of vanillin and guaiacol with MWs of 152 and 124, respectively, were shorter than that of a polystyrene standard with a MW of 162, indicating that the lignin model compounds tend to elute earlier than polystyrene standards. Thus, the products eluted before 58 min are likely to be trimeric and oligomeric lignin-derived products. This result indicated that the methanol-soluble portion consists of lignin-derived monomeric, dimeric, trimeric, and some oligomeric products. Therefore, we tried to characterize and identify these low molecular weight lignin-derived products by using GC-MS.

Identification of lignin-derived monomeric products

Figure 3 shows the total ion chromatogram of the methanol-soluble portion from sugi wood treated in supercritical water. This chromatogram could be categorized into two different regions in the retention time; one in the first 34 min and the other between 34 and 50 min. The lignin-derived monomeric and dimeric products existed in the former and latter regions, respectively. Unfortunately, lignin-derived trimeric and oligomeric products could not be detected in the GC-MS analysis because of their high boiling points.

Table 1 shows the list of lignin-derived monomeric products in the methanol-soluble portion and their retention time (RT), structure, and characteristic ions. All identified

products, except for **3** [5-hydroxymethylfurfural (5-HMF)], had guaiacyl nuclei (2-methoxyphenol). 5-HMF was a contaminant derived from cellulose and separated predominantly into the water-soluble portion.¹⁻³ The major compounds found in the methanol-soluble portion are guaiacol (**1**), vanillin (**8**), isoeugenol (**9**, **11**), homovanillin (**10**), acetoguaiacone (**13**), guaiacylacetone (**15**), 2-methoxy-4-(1-hydroxypropyl)phenol (**17**), homovanillic acid (**18**), and coniferylaldehyde (**23**), as shown in Fig. 3.

Products **6**, **7**, **9**, **11**, **14**, **15**, **17**, **21**, **23**, and **26**, which are eugenol, propylguaiacol, *cis*-isoeugenol, *trans*-isoeugenol, propioguaiacone, guaiacylacetone, 2-methoxy-4-(1-hydroxypropyl)phenol, 2-methoxy-4-(prop-1-en-3-one)phenol, *trans*-coniferylaldehyde, and ferulic acid, respectively, were composed of phenylpropane units (C_6-C_3). In our previous study, it was demonstrated that the β -*O*-4 linkage of lignin was easily cleaved under supercritical water conditions.³ Thus, these products would be derived through the cleavage of ether linkages of lignin.

In addition to C_6-C_3 units, C_6-C_2 and C_6-C_1 units were also detected. The C_6-C_2 units were ethylguaiacol (**4**), vinylguaiacol (**5**), homovanillin (**10**), acetoguaiacone (**13**), and homovanillic acid (**18**). The C_6-C_1 units were methylguaiacol (**2**) and vanillin (**8**). In our previous study of lignin model compounds in supercritical water, the cleavage between C_β and C_γ (C_β/C_γ) in the C_6-C_3 unit was observed.³ Therefore, the existence of these products suggests that the cleavage of C_β/C_γ and C_α/C_β linkages of lignin take place in supercritical water.

Products **12**, **16**, **19**, **20**, **22**, **24**, **25**, **27**, **28**, **29**, **30**, and **31** could not be identified. However, it has been already clarified that the methanol-soluble portion mainly consisted of lignin-derived products.^{2,3} Furthermore, these products showed fragment peaks typical of monomeric lignin compounds such as **123**, **138**, **150**, **152**, **164**, and **178**. Therefore, it is reasonable to assume that these compounds are also lignin-derived monomeric compounds.

Table 1. Lignin-derived monomeric products in the methanol-soluble portion and their retention time (RT), structure, and characteristic ions

No.	Product	RT (min)	Structure	Characteristic ions (m/z)
1	Guaiacol	11.8	G	124, 109, 82
2	Methylguaiacol	14.8	G—C	138, 123, 95
3	5-Hydroxymethylfurfural (5-HMF)	16.0		126, 97, 69
4	Ethylguaiacol	17.2	G—C—C	152, 137, 122
5	Vinylguaiacol	18.2	G—C=C	150, 135, 108
6	Eugenol	19.3	G—C—C=C	164, 149, 103
7	Propylguaiacol	19.5	G—C—C—C	166, 137, 122
8	Vanillin	20.5	G—CHO	152, 121, 109
9	<i>cis</i> -Isoeugenol	21.8	G—C=C—C	164, 150, 104
10	Homovanillin	22.0	G—C—CHO	166, 137, 123
11	<i>trans</i> -Isoeugenol	22.2	G—C=C—C	164, 149, 104
12	Unknown	22.5		162, 147, 130, 119, 92
13	Acetoguaiacone	22.7	G—CO—C	166, 151, 123
14	Propioguaiacone ^a	23.1	G—CO—C—C	180, 151, 92
15	Guaiacylacetone ^a	23.7	G—C—CO—C	180, 137, 123
16	Unknown	25.2		176, 161, 152, 134, 123
17	2-Methoxy-4-(1-hydroxypropyl)phenol	26.5	G—C—C—C—OH	182, 138, 124
18	Homovanillic acid	26.8	G—C—COOH	182, 138, 122
19	Unknown	27.3		184, 183, 174, 160, 106
20	Unknown	27.7		190, 178, 176, 162, 150
21	2-Methoxy-4-(prop-1-en-3-one)phenol ^b	27.9	G—CO—C=C	178, 151, 123
22	Unknown	28.0		192, 164, 150, 134, 123
23	<i>trans</i> -Coniferyl aldehyde	28.4	G—C=C—CHO	178, 164, 136
24	Unknown	29.2		188, 174, 159, 146, 123
25	Unknown	29.5		188, 174, 160, 146, 123
26	Ferulic acid	30.1	G—C=C—COOH	194, 167, 139
27	Unknown	30.3		208, 163, 151, 138, 123
28	Unknown	30.4		202, 188, 179, 173, 146
29	Unknown	31.7		202, 188, 175, 160, 146
30	Unknown	32.8		206, 190, 152, 150, 134
31	Unknown	33.8		220, 192, 178, 163, 150

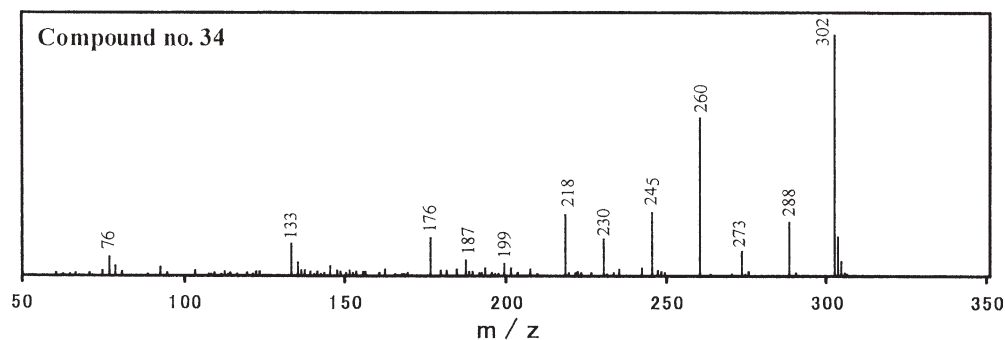
Products were identified from their mass spectra and by comparing their retention times with those of authentic compounds

G, guaiacyl (2-methoxyphenol)

^a Product identified with help from literature¹⁰

^b Product identified with help from literature¹²

Fig. 4. Mass spectrum of compound **34** believed to be a biphenyl type dimer



Identification of lignin-derived dimeric products

Table 2 shows mass spectral data of compounds in the methanol-soluble portion assigned as lignin-derived dimeric products. Because authentic compounds could not be obtained from commercial sources, the chemical structures were assigned from spectral data.

Figure 4 shows the mass spectrum of **34** with both the molecular ion (MI) peak and base peak (BP) of m/z 302. There are some fragments indicating methyl group detachments and a fragment ion of m/z 245 which is thought to originate from the guaiacol-guaiacol (biphenyl) structure. This fragment pattern was similar to that of 5,5'-

dehydrodiguaiacylthane.¹⁶ Therefore, this compound was believed to have a biphenyl type structure.

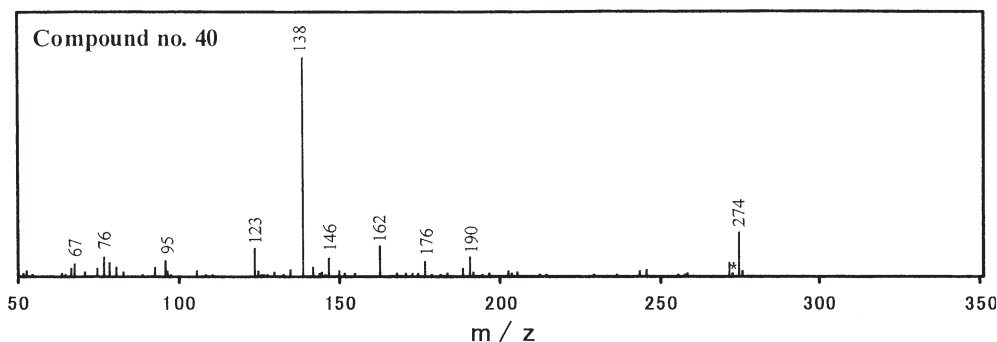
In our previous study using lignin model compounds, biphenyl type dimers, such as 2,2'-dihydroxy-3,3'-dimethoxy-5,5'-dimethyl biphenyl and 2,2',3,3'-tetramethoxy-5,5'-dimethyl biphenyl, were very stable during supercritical water treatment.³ Therefore, biphenyl type (5-5) linkages in protolignin were presumed to be stable in supercritical water, and consequently those in lignin-derived dimeric products would be included in the methanol-soluble portion.

Figure 5 shows the mass spectrum of **40** with an MI and BP of m/z 274 and m/z 138, respectively. There were very

Table 2. Mass spectral data of lignin-derived dimeric products in the methanol-soluble portion

No.	RT (min)	Mass spectral data (m/z)
32	34.6	256, 228, 213, 198, 181, 170, 162, 148, 142, 122, 117, 90, 78, 64
33	35.1	288, 273, 245, 233, 219, 213, 198, 188, 176, 170, 162, 148, 131, 123, 118, 106, 80, 78, 76, 66, 52
34	35.7	302, 288, 273, 260, 245, 230, 218, 199, 187, 176, 133, 76
35	36.6	302, 293, 287, 273, 259, 246, 231, 229, 214, 209, 188, 186, 174, 160, 148, 136, 129, 122, 106, 90, 80, 63
36	37.2	316, 301, 273, 258, 247, 229, 217, 204, 202, 200, 177, 161, 150, 137, 130, 123, 104, 77, 76, 66
37	38.3	330, 315, 299, 287, 272, 260, 245, 230, 214, 203, 187, 176, 158, 137, 123, 104, 95, 80, 78, 76, 65
38	38.9	272, 256, 243, 226, 212, 198, 187, 176, 170, 159, 138, 122, 92, 78, 76
39	39.2	328, 312, 301, 286, 272, 260, 245, 228, 212, 200, 189, 176, 163, 159, 138, 132, 123, 106, 92, 77, 66, 52
40	39.4	274, 271, 190, 176, 162, 146, 138, 123, 95, 76, 67
41	39.6	344, 315, 272, 259, 242, 240, 226, 208, 198, 175, 172, 162, 140, 137, 125, 117, 108, 102, 78, 76
42	39.7	274, 256, 243, 227, 208, 189, 171, 159, 146, 138, 123, 95, 76
43	40.2	344, 314, 284, 272, 258, 255, 245, 215, 212, 200, 182, 169, 161, 148, 138, 130, 120, 104, 96, 77, 64, 52
44	40.7	328, 314, 299, 288, 266, 255, 245, 243, 229, 209, 200, 176, 169, 158, 152, 137, 132, 123, 96, 92
45	40.9	330, 315, 287, 271, 258, 245, 226, 218, 185, 179, 162, 151, 139, 132, 123, 104, 80, 76
46	41.5	344, 342, 328, 315, 299, 269, 257, 245, 225, 214, 208, 194, 176, 168, 161, 151, 139, 129, 123, 103, 96, 89
47	42.1	344, 329, 301, 286, 271, 252, 244, 228, 212, 201, 184, 167, 158, 135, 129, 123, 106, 80, 78
48	42.2	286, 270, 256, 238, 227, 211, 196, 188, 182, 162, 148, 137, 132, 104, 90, 77
49	42.5	358, 315, 272, 260, 252, 211, 176, 168, 150, 134, 105, 80, 78
50	44.5	272, 241, 211, 197, 181, 168, 141
51	45.7	344, 315, 284, 268, 244, 239, 208, 187, 181, 162, 146, 132, 78, 76
52	47.1	324, 314, 299, 285, 270, 266, 254, 225, 222, 212, 199, 182, 162, 149, 132, 118, 106
53	47.9	344, 326, 312, 297, 283, 267, 255, 239, 220, 213, 211, 209, 194, 175, 162, 148, 138, 135, 106, 95

Fig. 5. Mass spectrum of compound **40** believed to be a diphenylethane type dimer. The asterisk corresponds to the [M-2] peak



few patterns typical of lignin-derived compounds between mass fragment of m/z 138 and m/z 274. Nimz¹⁷ reported that the mass fragments of bivanillyl [1,2-(3',4'-dimethoxyphenyl)ethane] were m/z 274, 137, 122, 105, 94, 77, and 66. The fragment pattern of **40** proposed good homology to his report. Furthermore, it is known that diphenylethanes provide an [M-2] peak which indicates the multiple loss of hydrogen atoms.¹⁸ Such a peak was observed in the mass spectral pattern of **40**. Thus, **40** was believed to have a diphenylethane type structure.

Figure 6 shows mass spectra of **38** and its silylated derivative. This compound had an MI and BP of m/z 272. The mass fragment of m/z 245 which indicates the biphenyl type dimeric compounds of guaiacol was not detected. Nimz¹⁹ reported that the mass fragments of 4,4'-dihydroxy-3,3'-dimethoxy stilbene were m/z 272, 262, 239, 225, 211, 207, 202, 181, 169, 157, 152, 139, and 136, a pattern to which the fragmentation of **38** was similar. In addition, after derivatization of this product, it was revealed that two hydroxyl groups were trimethylsilylated as shown in Fig. 6b. Therefore, this compound was believed to have a stilbene type structure.

Figure 7 shows the mass spectra of **50** before and after trimethylsilylation. The MI of this product was m/z 272, and fragments typical of monomeric lignin compounds, such as m/z 123 (guaiacol) and m/z 137 (methylguaiacol) were not found in Fig. 7a. Evans et al.²⁰ proposed three plausible structures for a parent ion incorporating diarylpropane (stilbene), the β -O-4-alkyl-arylether bond, and the phenylcoumaran linkage. However, the MI of this product was m/z 344 after trimethylsilylation as shown in Fig. 7b, indicating that only one hydroxyl group was trimethylsilylated. Furthermore, it is already known that the ether linkage is easily broken down in supercritical water.³ Thus, this product was expected to be a phenylcoumaran type dimer. The phenylcoumaran type compound contains not only an α -O-4 type linkage but also a β -5 type linkage. Essentially, this means the structure is a cyclic ether or furan. In our previous study, furan compounds such as furfural and 5-HMF, which are derived from cellulose and separate predominantly to water-soluble portions, were comparatively stable under supercritical water treatment.^{1,4} Thus, the phenylcoumaran type structure would possibly be stable under the supercritical condition.

Fig. 6. Mass spectra of compound **38**, believed to be a stilbene type dimer, before (a) and after (b) trimethylsilylation

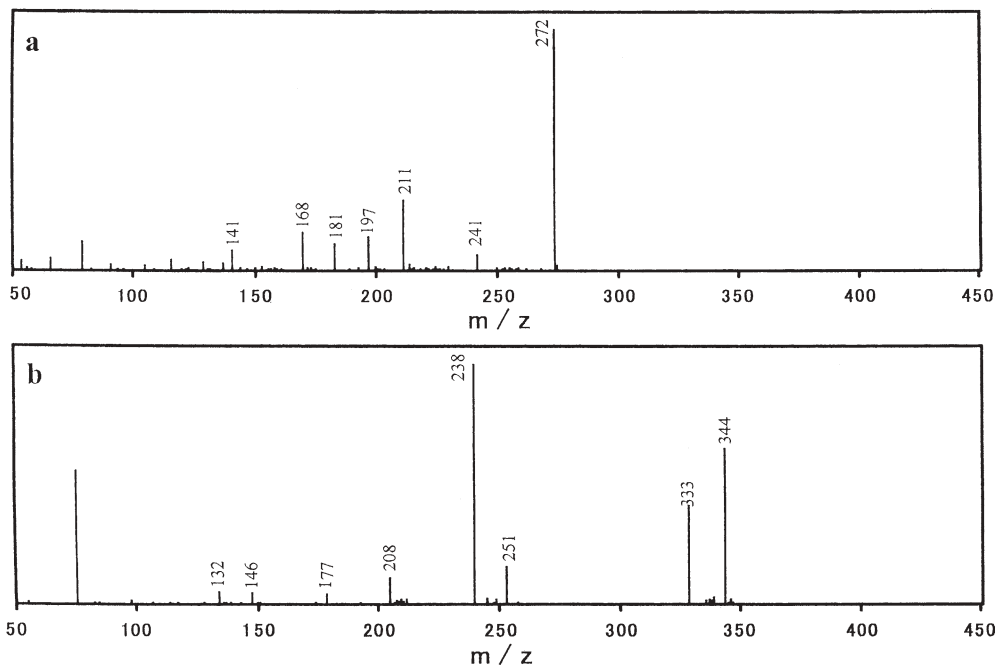
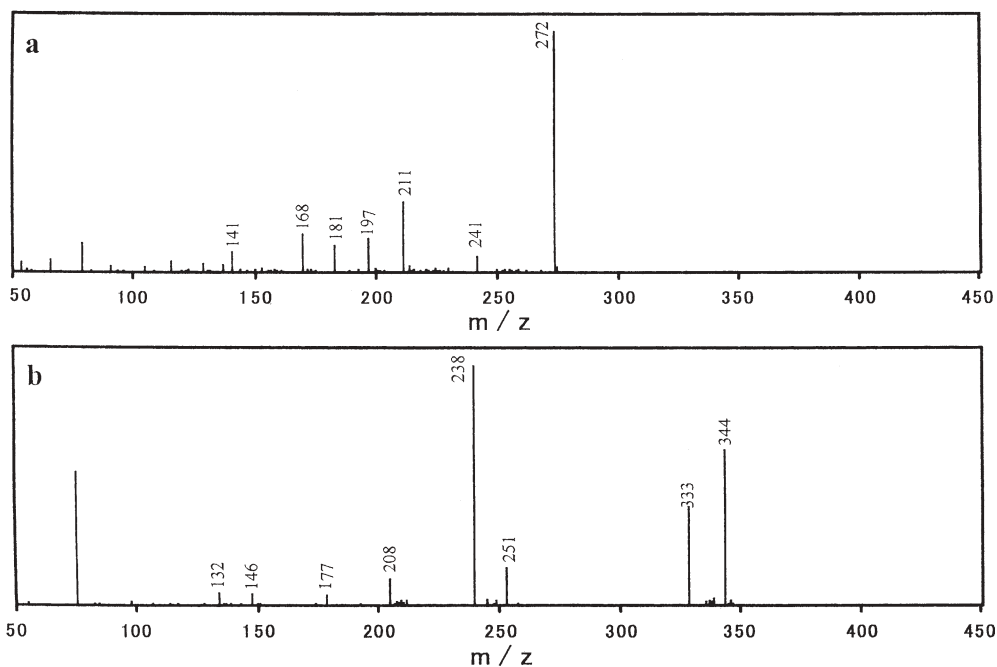


Fig. 7. Mass spectra of compound **50**, believed to be a phenylcoumaran type dimer, before (a) and after (b) trimethylsilylation



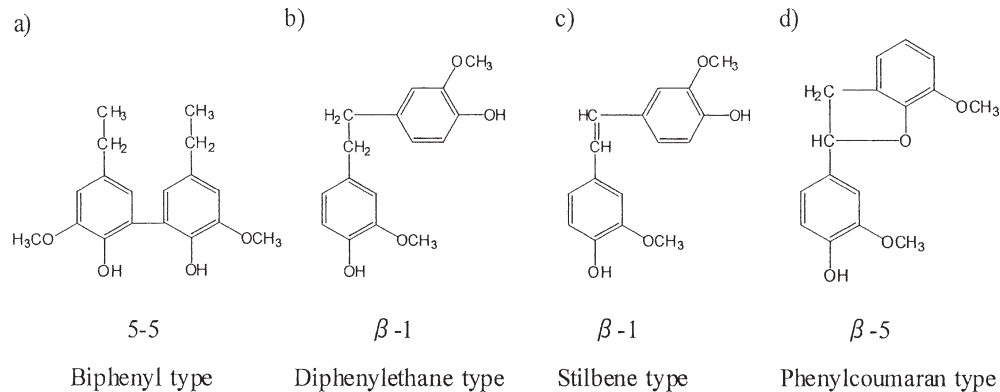
From these lines of data, four dimeric lignin-derived products were determined to be biphenyl type (β -5), diphenylethane type (β -1), stilbene type (β -1), and phenylcoumaran type (β -5) compounds as shown in Fig. 8. These products were considered to be more stable than those that have ether linkages such as β -O-4 and α -O-4 because preferential degradation of ether linkages of lignin occurs during supercritical water treatment. Therefore, it is possible that other dimeric lignin-derived products listed in Table 2 also have these condensed-type linkages.

In future, more detailed study, such as isolation and synthesis of the above-mentioned products, will be necessary to

identify the dimeric structures. Such work, including characterization of the trimeric and larger lignin-derived products, will provide important clues for the efficient utilization of lignin-derived products.

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Fig. 8a-d. Speculative structures of some lignin-derived dimeric products. **a** biphenyl type (5-5), **b** diphenylethane type (β -1), **c** stilbene type (β -1), and **d** phenylcoumaran type (β -5)



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