ELSEVIER

Spectrochimica Acta Part A 53 (1997) 1621–1628

SPECTROCHIMICA ACTA PART A

Fourier transform Raman assignment of guaiacyl and syringyl marker bands for lignin determination

Miyuki Takayama, Toru Johjima, Takeshi Yamanaka, Hiroyuki Wariishi *, Hiroo Tanaka

Department of Forest Products, Kyushu University, Fukuoka 812-81, Japan

Received 6 January 1997; accepted 21 March 1997

Abstract

A near infrared fourier transform Raman (NIR-FTR) spectroscopic technique was utilized to characterize lignin in wood. The Raman bands for C=C stretching derived from 4-hydroxy-3-methoxyphenyl (guaiacyl) nuclei and from 3,5-dimethoxy-4-hydroxyphenyl (syringyl) nuclei exist independently. The NIR-FTR analysis of a series of lignin model compounds indicated that a syringyl band was shifted to a lower frequency compared to a guaiacyl band. This shift was also observed in chemically synthesized lignin (DHP). Syringyl DHP, in which all the aromatic nuclei consist of syringyl type, exhibited a C=C stretching band at 1594 cm^{-1}, while guaiacyl DHP exhibited the band at 1599 cm^{-1}. These bands were designated as syringyl and guaiacyl marker bands, respectively. Chemical and physical treatment of hardwood and softwood exhibited different characteristics. One of the reasons is the chemical structure of lignin. Softwood mainly contains only guaiacyl lignin, while hardwood contains both guaiacyl and syringyl lignin, and the syringyl/guaiacyl (S/G) ratio varies among species. Under high-resolution conditions (1 cm^{-1}), the NIR-FTR spectra of 10 hardwoods (wood meal samples) revealed that both syringyl and guaiacyl marker bands existed. On the other hand, the spectra of softwoods contained only a guaiacyl marker bands existed. On the other hand, the spectra of softwoods contained only a guaiacyl marker band. The S/G ratio in hardwood calculated from the peak area intensity ratio of two marker bands shows a linear relationship with the S/G ratio obtained from conventional nitrobenzene oxidation analysis with the correlation factor > 0.96. Furthermore, if peak component separation analysis was combined, low-resolution spectral data gave a similar S/G ratio. Either syringyl of guaiacyl marker bands can be assigned in the NIR-FTR spectra of wood blocks (saw-cut surface). This spectral technique may provide an easy-handling and non-destructive analytical method for lignin determination. © 1997 Elsevier Science B.V.

Keywords: FT Raman; Guaiacyl lignin; Syringyl lignin; Marker band; Non-destructive analysis

1. Introduction

A growing demand for wood and the preservation of natural resources strongly pertains to tree plantations. A lot of work has been directed

Abbreviations: FTR, Fourier transform Raman; G, guaiacyl; NIR, near-infrared; S, syringyl; DHP, dehydrogenated polymerizate (synthetic lignin).

* Corresponding author. Tel.: +81 92 642 2993; fax: +81 92 642 3078; e-mail: hirowari@agr.kyushu-u.ac.jp

PII S1386-1425(97)00100-5

towards the improvement of the planting stock via breeding, selection, and clonal propagation of elite genotypes [1]. In tree plantation, rapid non-destructive screening methods are of great importance, since huge numbers of progenies should be analyzed annually over several years. Recent efforts have been devoted to quantifying the content of wood components, such as cellulose and lignin, using a micro scale sample [2,3]. Furthermore, for chemical treatment of wood, especially for pulping, not only quantity but also quality of lignin, i.e., the structure of aromatic nuclei, strongly affects the properties of the products.

Vibrational studies such as infrared and UV-vis spectroscopies have been providing the structural information of plant materials. Among them, the Raman spectroscopic technique was advantageous since solid or powder samples can be measured directly. However, conventional Raman spectroscopy using the excitation laser of the visible region causes a fluorescent emission interfering with the Raman measurement and causes sample damage due to high energy of the visible laser. Using near-infrared excitation laser dramatically reduced these problems [4]. The variation of samples was enhanced and no complicated procedures were required to obtain near-infrared Fourier transform Raman (NIR-FTR[1]) spectra of lignocellulosic materials [5–7]. NIR laser does not excite ν (O–H) in water much, so that the moisture in samples gives little effect on spectra [4]. Furthermore, due to the difference in the selection rule for infrared and Raman spectroscopies, ν (C=C) vibration is only active in Raman scattering, which is specific for lignin-derived aromatic skeleton [7].

From these advantages, we have utilized the NIR-FTR spectroscopic technique to characterize a series of lignin model compounds and DHPs as well as 12 different wood samples. The minimum amount of wood sample required for NIR-FTR spectral determination was only 3–5 mg. The aromatic C=C vibration band was shifted when the aromatic ring was substituted by methoxyl group. Either high-resolution spectra or low-resolution spectra treated with the curve-fit technique, C=C vibrations of guaiacyl (4-hydroxy-3-methoxyphenyl) and syringyl (3,5-dimethoxy-4-hydroxyphenyl) skeletons were assigned to individual Raman bands, designated as guaiacyl and syringyl markers, respectively. In DHP samples, an S-marker and a G-marker bands were observed at 1594 and 1599 cm^{-1}, respectively. The S/G ratio was calculated from the area intensity of these markers, which exhibited a good accordance with the values obtained from the conventional chemical analysis. It was also shown that these G- and S-maker bands exist clearly not only in the spectra of wood meals but also in those of wood blocks, indicating that the NIR-FTR spectroscopic method could be an easy-handling technique for non-destructive and quantitative determination of lignin structure.

2. Materials and methods

2.1. Samples

The wood samples (sapwood) were all obtained from the experimental forest of Kyushu University. The hardwoods (angiosperms) used were: *Actinodaphne lancifolia* Meisn., *Albitia julibrissin* Durazz., *Cinamomum japonicum* Sieb., *Cornus controversa* Hemsel., *Fagus crenata* Bl., *Kalopanax pictus* Nak., *Platycarya strobilacea* S. and Z., *Quercus monogolica* Fisch. ver *grosseserrata* Rahd. and Wils., *Quercus myrsinaefolia* Bl., and *Zelkova serrata* Mgk. The softwoods (gymnosperms) used were *Cryptomeria japonica* D. Don and *Pinus densiflora* S. and Z. They were all grained and fractionated using 200, 100, 48 and 16 mesh screens. Extract-free wood meals were prepared by treating ground woods with ethanol-toluene (1:2 v/v) for 6 h in a Soxlet apparatus. Holocellulose and α-cellulose were prepared from extract-free wood meals as previously reported [8,9].

4-Hydroxy-3-methoxybenzyl (vanillyl) alcohol, 4-hydroxy-3-methoxybenzaldehyde (vanillin), 4-hydroxy-3-methoxybenzoic (vanillic) acid, 3,5-dimethoxy-4-hydroxybenzaldehyde (syrnigaldehyde), 3,5-dimethoxy-4-hydroxybenzoic (syringic) acid were purchased from Wako Pure Chem. They were purified by recrystallization or by column chromatography before use.

3,5-Dimethoxy-4-hydroxybenzyl (syringyl) alcohol was synthesized by reducing syringaldehyde with $NaBH_4$. Syringyl alcohol was purified by recrystallization and identified using GCMS as a ditrimethylsilyl ether (m/z, M^+; 328). 4-Hydroxy-3-methoxyacetophenone and 3,5-dimethoxy-4-hydroxyacetophenone were purchased from Aldrich. Both acetophenones were reduced with $NaBH_4$, obtaining 1-(4-hydroxy-3-methoxyphenyl)ethane-1-ol (m/z, M^+; 168) and 1-(3,5-dimethoxy-4-hydroxy)ethane-1-ol (m/z, M^+; 198). These compounds were purified using flash chromatography (hexane/ethyl acetate, 3/1).

Coniferyl alcohol (Aldrich) was polymerized using H_2O_2 and horseradish peroxidase (Sigma), forming G-DHP [10]. Sinapyl alcohol was prepared by the condensation of syringaldehyde and malonate as previously reported [11], which was polymerized as described above to obtain S-DHP.

2.2. Instrumentation

NIR-FTR spectra were obtained using a Perkin Elmer System 2000R spectrometer. The laser for excitation was Nd: YAG, operating at 1064 nm. The detector was InGaAs, averaging 100 scans with a resolution of 4–8 cm^{-1} and the laser power was 200 mW, or otherwise indicated. FTIR spectra of wood meals and lignin model compounds were recorded using a Perkin Elmer System 2000 FTIR spectrometer, averaging 100 scans with a resolution of 4 cm^{-1} by KBr pellet method. FTIR spectra of wood meals were also obtained by the diffuse reflectance method.

A GCMS analysis was performed using a QP-1000 system (Shimadzu) equipped with fused silica column (NB-5; 30 m, GL Science); 70 eV, 120–300°C (8°C min^{-1}). Samples were derivatized as previously reported [12].

2.3. Chemical analysis of wood components

To chemically determine the S/G (S/V) ratio of each extract-free wood sample, the nitrobenzene oxidation method was applied [13]. Extract-free wood meals (50 mg) were treated with 2 M KOH (4 ml) and nitrobenzene (0.24 ml) in a stainless tube at 160°C for 2 h. The products were extracted with chloroform, and then analyzed using HPLC (20% acetonitrile in 0.05% phosphate to 100% acetonitrile) on the yield of syringaldehyde and vanillin, obtaining the S/G (S/V) ratio.

3. Results and discussion

3.1. Measurement conditions for NIR-FTR spectra of wood meals and blocks

Measurement conditions for NIR-FTR spectra of wood meals and blocks were optimized based on the factors listed herewith; the effect of fluorescent emission, the measuring time, the shape of peaks, and the amount of samples. To take NIR-FTR spectra of wood meals, the laser power of 200 mW, the resolution of 4–8 cm^{-1}, the scan number of 200, and wood meal samples of 3–5 mg were employed. The effect of the particle size of wood meal on NIR-FTR spectra was also examined and 48 mesh-pass/100 mesh-nonpass particle (0.10–0.23 mm diameters) was chosen for determination. Surface characteristics of wood blocks did not affect NIR-FTR spectra much, sot that the saw-cut surface was laser-irradiated for spectral determination.

3.2. Assignment of peaks

Fig. 1 shows the NIR-FTR spectra of untreated wood meal, extract-free wood meal, holocellulose, and α-cellulose prepared from *F. crenata* (hardwood). As delignification proceeded, several peaks reduced their peak intensity or completely disappeared. These peaks observed at 3070, 2938, 1745, 1668, 1602, 1332, 1189, and 379 cm^{-1} in wood meal spectra could be derived from lignin. When the same sample was treated with $NaBH_4$. the peak at 1668 cm^{-1} was dramatically reduced and the peak at 1602 cm^{-1} was intact (data not shown), indicating that ν (C=O) is assigned to the peak at 1668 cm^{-1}. Both NIR-FTR and FTIR spectra of monosaccharide, disaccharaides, cellulose, lignin models, and DHPs were measured and referred for peak assignment. NIR-FTR characterization of polysaccharides was recently reported [5–7,14–17], supporting our assignment of

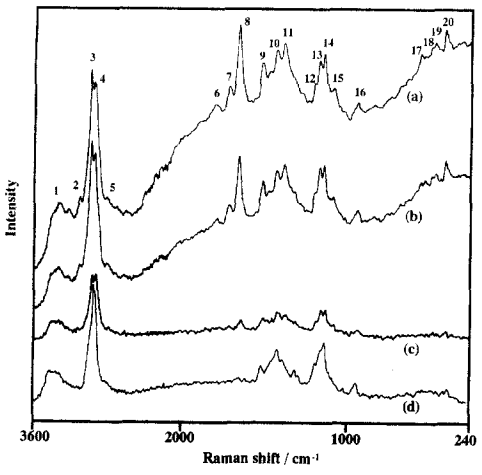

Fig. 1. NIR-FTR spectra of (a) wood meal (untreated), (b) extract-free, (c) holocellulose, (d) α-cellulose, prepared from *F. crenata*. The spectra were obtained with 8 cm^{-1} resolution, 200 scans, and 200 mW laser.

lignin-derived Raman bands. The peak assignment for *F. crenata* was summarized in Table 1. Previously assigned FTIR group frequencies and visible-laser excited Raman bands for lignin are also shown in Table 1.

Hardwoods consist of guaiacyl and syringyl lignin, while softwoods mainly consist of guaiacyl lignin. It has been known that chemical treatment causes different behaviours on S- and G-lignin. This is crucial for the pulping process and elite

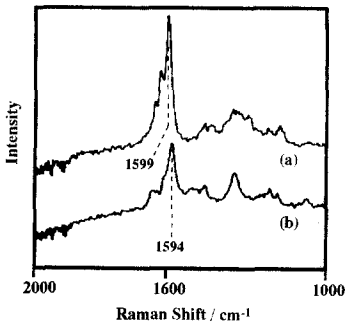

Fig. 2. NIR-FTR spectra of (a) guaiacyl DHP and (b) syringyl DHP, with 4 cm^{-1} resolution, 200 scans, and 200 mW laser.

tree selection. A more practical method for S/G determination than a conventional chemical method has been demanded, which may lead to the development of in situ analysis. It has been reported that this structural difference would be monitored on NIR-FTR spectra [18,19] but the quantitative measurement was not mentioned. Among the NIR-FTR peaks derived from lignin, the band at 1745 cm^{-1} was seen only in the hardwood sample, suggesting that this could be a specific syringyl lignin marker. However, it was recently reported to be derived from holocellulose [18]. The band at 1745 cm^{-1} has not yet been convincingly assigned. Then, the peak appearing around 1600 cm^{-1} was chosen for quantitative analysis because of its specificity for aromatic ring (C=C stretching) and intensity (Fig. 1).

Table 1
Lignin-derived Raman bands found in the NIR-FTR spectra of *F. creneta*

Peak No.[a]	Raman shift (cm^{-1})[b]	FTIR (cm^{-1})[c]	Assignment	References
1	3320	3320	ν (O–H)	[5,6,14]
2	3070	—	ν (=C–H)	[15,18,21]
3	2983	2905	ν (C–H)	[6,14,15,18,21]
7	1668	1737	ν (C=O)	[5,14,15,18,20,21]
8	1602	—	ν (C=C)	[5–7,14,15,20,21]
11	1332	1051	δ (C–H)	[14,20]
12	1189	—	δ ($C–H_2$)	[14]
20	379	—	ν and breathing (aromatics)	[14]

[a] Peak Numbers are from Fig. 1.
[b] NIR-fTR Raman shift found in this study (Fig. 1).
[c] Peaks found in this study using FTIR equipped with the diffuse reflection appratus.

3.3. NIR-FTR spectra of DHPs and lignin model compounds

Fig. 2 shows the Raman spectra of G- and S-DHPs, clearly indicating that ν (C=C) for guaiacyl and syringyl nuclei exhibited different Raman shifts at 1599 and 1594 cm^{-1}, respectively. To further confirm spectral differences between guaiacyl and syringyl nuclei, a series of lignin model compounds were applied to the NIR-FTR measurement. Several lignin model compounds exhibited the ν (C=C) for syringyl nuclei shifted to lower frequencies (Table 2). Then, the 1599 and 1594 cm^{-1} Raman shifts found in G- and S-DHPs (Fig. 2), were designate as guaiacyl (G) and syringyl (S) maker bands, respectively.

3.4. G- and S-maker bands observed in NIR-FTR spectra of hardwoods

Fig. 3 shows the spectral region from 1580 to 1620 cm^{-1} of *C. japonica* (softwood mainly containing G-lignin) and *F. crenata* (hardwood containing both G- and S-lignin). The non-symmetrical shape of peaks (Fig. 3) at 1600 cm^{-1} suggested that each peak may consist of 2 or more components such as G- and S-maker bands. To investigate whether there are differences in peak components between softwood and hardwood, a peak component analysis based on the Gauss/Lorentz curve-fit using Galactic GRAMS/386 software (Parkin Elmer) was attempted. In softwood, the peak was separated to 5 components and one of them was observed at 1598 cm^{-1} as the G-marker, while in hardwood, the peak was separated to 6 components and two lignin marker bands were observed at 1592 cm^{-1} as the S-marker and 1599 cm^{-1} as the G-marker (Fig. 3). The positions of these marker bands showed a good correlation with the spectral data of DHPs (Fig. 2). The same peak analysis was applied to other wood samples. All other hardwood samples revealed both G- and S-marker bands. The other softwood sample revealed the G-marker band but not the S-marker band. The presence of wood extract did not affect the NIR-

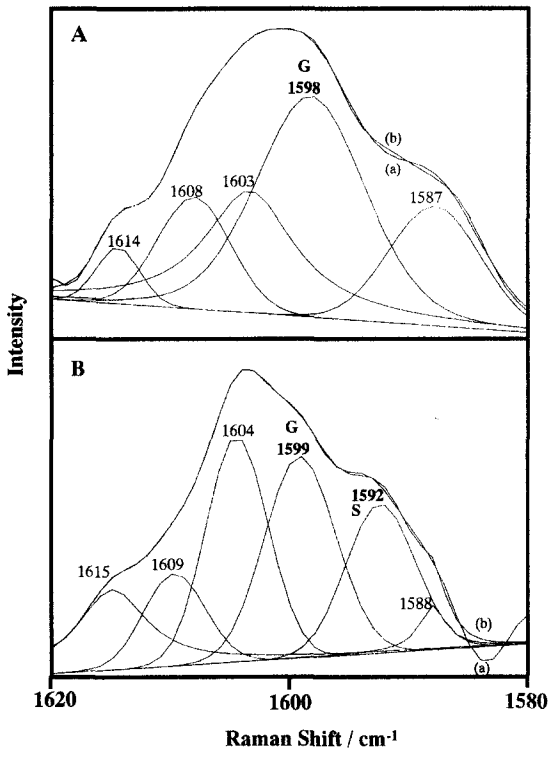

Fig. 3. Component-fitting in the ν (C=C) spectral region. A; *C. japonica* (softwood) and B; *F. crenata* (hardwood). Spectra exhibit (a) experimental data and (b) calculated data from resolved spectra. Number show peak position for each resolved peaks. Proposed guaiacyl- and syringyl-markers are indicated as G and S, respectively. The experimental spectra were obtained with 1 cm^{-1} resolution, 100 scans, and 200 mW laser using wood meals.

Table 2
Comparison of ν (C=C) Raman bands for G- and S-type lignin medel compounds

	Type of aromatic skelton (Raman Shift cm^{-1})	
	4-hydroxy-3-methoxy-(G)	3,5-dimethoxy-4-hydroxy-(S)
Benzyl alcohol	1610	1606
Benzaldehyde	1592	1586
Benzoic acid	1600	1592
1-Hydroxy-ethylbenzene[a]	1610	1604

[a] Shown as 1-(4-hydroxy-3-methoxyphenyl)ethane-1-ol and 1-(3,5-dimethoxy-4-hydroxyphenyl)ethane-1-ol in the text.

FTR spectral data, since almost identical spectra were obtained from either untreated wood meal or extract-free wood meal (data not shown).

3.5. S/G ratio determination based on S- and G-marker bands

For more detailed analysis of S- and G-marker bands, the NIR-FTR spectra of hardwoods and softwoods were taken with higher resolution of 1 cm^{-1}. Fig. 4 shows the spectra of *P. densiflora* (softwood) and *P. strobilacea* (hardwood). To develop a novel S/G ratio determination method, the relationship between peak area intensity ratio of S- and G-marker bands and S/G (S/V) ratio calculated from nitrobenzene oxidation data were investigated. The peak area intensity ratio was calculated as shown in Fig. 4 (shaded area). Fig. 5 clearly indicates that there is a linear relationship between peak area intensity ratio and S/V ratio with the correlation factor > 0.96. S- and G-marker bands assigned in this study could also be used to calculate the S/G ratio in hardwood lignin.

The relationship between S/G ratio calculated from nitrobenzene oxidation method ($R_{S/V}$) and

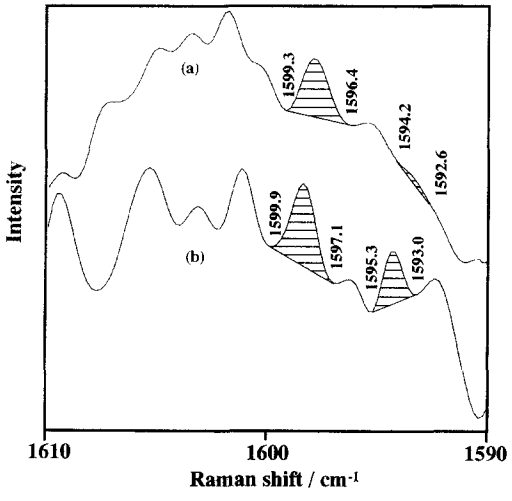

Fig. 4. NIR-FTR spectra of (a) *P. densiflora* (softwood) and (b) *P. strobilacea* (hardwood) in the *v* (C=C) region. The spectra were obtained with 1 cm^{-1} resolution, 100 scans, and 200 mW laser using wood meals. The area strength of the shaded area were utilized to estimate S/G ratio.

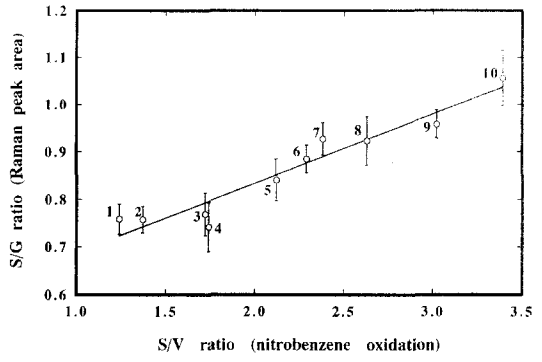

Fig. 5. The relationship between S/V ratio (nitrobenzene oxidation method) and S/G ratio (NIR-FTR method). Wood meals used are listed below. 1. *A. julibrissin* D., 2. *P. strobilacea* S. and Z., 3. *K. pictus* N., 4. *Q. mongolica* Fisch. ver *grosseserrata* R. and W., 5. *Z. serrata* M., 6. *C. controversa* H., 7. *F. crenata* B., 8. *C. japonica* S., 9. *Q. myrsinaefolia* B., 10. *Actinodophene l* M.

S/G ratio calculated from the NIR-FTR method ($R_{S/G}$) could be expressed by the formula;

$$R_{S/G} = 0.15\, R_{S/V} + 0.54$$

It is too early to rationalize the formula shown above, but at least, it has been shone that the NIR-FTR *v* (C=C) intensity would be good probe to quantify the S/G ratio (Fig. 5). More detailed quantitative analysis, however, may also be affected by the benzylic carbonyl group on the band intensity of *v* (C=C), since this type of carbonyl group is conjugated with the aromatic structure. A study on the NIR-FTR characterization of carbonyl compounds is now under way.

An S/G ratio calculation based on low-resolution (4 cm^{-1}) spectra with curve-fitting (Fig. 3) was also attempted, showing similar results to the ones shown in Fig. 5 (data not shown).

As a model for in situ analysis of wood components, the NIR-FTR of wood blocks was attempted. Fig. 6 shows hardwood, *K. pictus* spectra at 4 cm^{-1} resolution. After curve-fit treatment, the spectra exhibited the presence of S- and G-marker bands. The S/G ratio of 10 hardwood blocks determined from FTR spectra were compared with the S/V ratio obtained from nitriben-zene method, showing a larger deviation than the data shown in Fig. 5. Since wood meals are much

more homogeneous than wood blocks, the former would give less deviations in FTR spectra. Furthermore, the diameter of NIR laser irradiation is only a few μm, resulting in the larger deviation with the spectra of wood blocks. Statistical treatment of the data may help obtain more reliable results. But, even using wood blocks, at least rough estimation of S/G ratio would be determined. The advantage of the NIR-FTR analysis over chemical analysis is a much shorter time required for measurement. Ten samplings for each tree would be achieved in less than 1 h.

The aim of this study was to demonstrate the NIR-FTR spectroscopy as a new tool to determine wood composition using micro scale samples (mg order) and under non-destructive conditions. The assignment of S- and G-marker bands were successfully achieved. Furthermore, the S/G ratio of hardwood lignin could be determined using the NIR-FTR technique without any chemical treatment of wood. Very recently, we have also reported that NIR-FTR information on a series of hemoproteins in aqueous solution could be obtained without complicated procedure [22]. Thus arises the advantages of this system: short analysis time; and giving clear spectra independent of sample shapes.

In addition, the ν (C=O) band can be seen as an isolated sharp band with reasonable intensity of NIR-FTR spectra (Fig. 1 and Table 1), which has been known to give a broad absorption with huge intensity on FTIR spectra. It is said that the carbonyl group in lignin may be a good monitoring probe following wood deterioration. The quantitative determination of a carbonyl band using NIR-FTR spectroscopy is now under investigation.

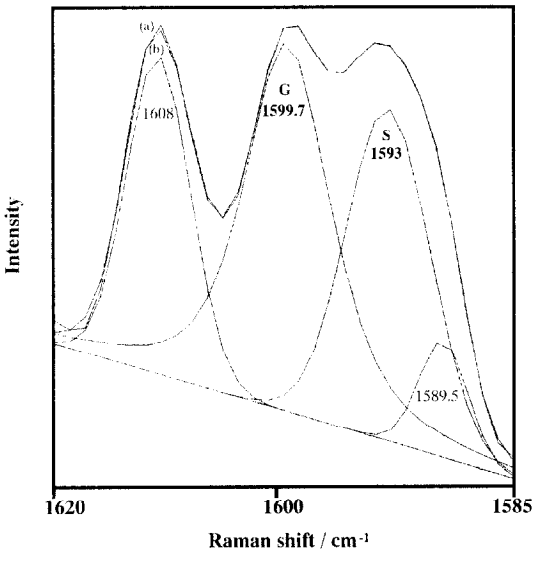

Fig. 6. NIR-FTR spectra of wood block (*K. pictus*) and resolved spectra for component-fitting. Spectra exhibit (a) experimental data and (b) calculated data from resolved spectra. Numbers show peak positions for each resolved peaks. Proposed guaiacyl- and syringyl-makers are indicated as G and S, respectively. The experimental spectra were obtained with 4 cm^{-1} resolution, 200 scans, and 200 mW laser.

References

[1] K.G. Eldridge, J. Davidson, C. Hardwood. Eucalypt Domestication and Breeding, Clarendon Press, Oxford, UK, 1993.
[2] T. Ona, T. Sonoda, M. Shibata, K. Fukazawa, Tappi J. 79 (1995) 121.
[3] H.D. Erickson, Tappi J. 45 (1962) 710.
[4] R.P. Carey, Biochemical Applications of Raman and Resonance Raman Spectroscopies, Academic Press, New York, 1982.
[5] R.H. Atalla, U.P. Agarwal, Science 227 (1985) 636.
[6] U.P. Agarwal, R.H. Atalla, Planta 169 (1986) 325.
[7] U.P. Agarwal, R.H. Atalla, in: Proceedings of the 8th International Symposium on Wood and Pulping Chemistry, Helsinki, Vol. II and III, 1995, pp. 67.
[8] J.D. Wilson, Forest Prod. J. 11 (1961) 260.
[9] Tappi Standards T203 os-61, Technical Association of the Pulp and Paper industry, Atlanta, GA.
[10] M. Wayman, T.I. Obiaga, Can. J. Chem. 52 (1974) 2102.
[11] T. Yamasaki, K. Hata, T. Higuchi. Mokuzai Gakkaishi 19 (1973) 299.
[12] H. Wariishi, K. Valli, M.H. Gold, Biochemistry 28 (1989) 6017.
[13] S.Y. Lin, C.W. Dence, Methods in Lignin Chemistry, Springer Verlag, New York, 1992.
[14] R.H. Atalla, J. Wood Chem. Technol. 7 (1987) 115.
[15] J. Goral, V. Zichy, Spectrochim. Acta 46A (1990) 253.
[16] H.G.M. Edwards, D.W. Farwell, Spectrochim. Acta 50A (1994) 807.
[17] H.G.M. Edwards, E. Ellies, D.W. Farwell, R.C. Janaway, J. Raman Spectrosc. 27 (1996) 663.
[18] P.A. Evans, Spectrochim. Acta 47A (1991) 1441.
[19] T. Takei, N. Katao, T. Iijima, M. Higaki, Mokuzai Gakkaishi 41 (1995) 229 (in Japanese).

[20] U.P. Agawal, R.H. Atalla, J. Wood Chem. Technol. 14 (1994) 227.
[21] I.A. Weinstock, R.H. Atalla, U.P. Agarwal, J.L. Minor, Spectrochim. Acta 49A (1993) 819.
[22] T. Johjima, H. Wariishi, H. Tanaka, Biochem. Biophys Res. Commun. 226 (1996) 601.