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Lignin characteristics of *Abies beshanzuensis*, a critically endangered tree species

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Abstract As one of the major components of plant cell walls, lignin structural features are closely related with taxonomical and genetic classification of plants. In this study, the structural features of lignin of *Abies beshanzuensis* were investigated. *Abies firma*, which is genetically the closest species to *A. beshanzuensis*, *Cryptomeria japonica*, a typical gymnosperm tree species, and *Phyllostachys pubescens* (bamboo), which includes *p*-hydroxyphenyl nuclei in arylglycerol-β-aryl intermonomer linkages, were also analyzed to compare lignin features with those of *A. beshanzuensis*. The lignin content of *A. beshanzuensis* (39.2%) was significantly higher than that of *A. firma* (33.7%). The high value may be due to the adaptation of *A. beshanzuensis* to environmental stresses in surviving the Riss glacial period. Alkaline nitrobenzene oxidation, ozonation, acidolysis, and ^{1}H nuclear magnetic resonance spectra showed that the structural features of lignin of *A. beshanzuensis* were similar to those of *A. firma*, which is genetically the closest species of *A. beshanzuensis*. The results of this study suggest that *A. firma* would be a suitable mother tree species for grafting *A. beshanzuensis* on the basis of their lignin characteristics.

Key words *Abies beshanzuensis* · Endangered tree species · Graft · Lignin characteristics

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Introduction

Lignin is a major structural component of secondary plant cell walls and is closely related to the evolution of plants. Chemical analyses (alkaline nitrobenzene oxidation, potassium permanganate oxidation, hydrogenolysis, acidolysis, thioacidolysis, etc.) and spectroscopic analyses [ultraviolet (UV), infrared, nuclear magnetic resonance (NMR), etc.] have shown that pteridophyte and gymnosperm lignin is an aromatic polymer in which the monomeric guaiacyl propane units are the main components (>90%). Angiosperm lignin is composed of syringyl (S) and guaiacyl propane (G) units, and the S/G ratio varies with species from 1 to 4. Besides syringyl and guaiacyl propane units, Monocotyledon lignin contains small amounts of *p*-hydroxyphenylpropane units in addition to syringyl and guaiacyl propane units. In monocotyledon lignin *p*-coumaric acid is esterified to the side chains of the lignin polymer.[1]

Research on *Abies concolor*,[2] *Abies fraseri*,[3] *Abies balsamea*,[4] *Abies sibirica*,[5] and *Abies sachalinensis*[6] has indicated that lignin of *Abies* genus is characterized by the presence *p*-hydroxyphenyl nuclei, and the yield of *p*-hydroxybenzaldehyde by alkaline nitrobenzene oxidation is similar to that for monocotyledon lignin. However, whether the *p*-hydroxyphenyl nuclei are involved in arylglycerol-β-aryl ether intermonomer linkages or are esterified to lignin macromolecules has not been sufficiently confirmed.

Abies beshanzuensis, a geographically and morphologically distinctive tree species in China, has grown thickly in the middle to south coastal mountainous areas of Zhejiang, Jiangsu, Anhui, and Fujian Provinces in China during the Riss (contemporaneous with Illinoian or Saale periods: 125–200 thousand years ago) glacial period.[7] However, the population of *Abies beshanzuensis* reduced drastically due to climate change, natural disaster, and human activities after the last (the Würm or Wisconsin periods: 15–70 thousand years ago) glacial period. *A. beshanzuensis* was rediscovered at about 1700 m elevation on Baishanzu Mountain in Qingyuan, Zhejiang Province, China, in 1963, and at that time there were only seven individual trees. It was reduced

to three individuals in 1988. In 1987, *A. beshanzuensis* was listed as one of 12 critically endangered plant species in the world by the Species Survival Commission (SSC) of International Union for Conservation of Nature and Natural Resources (IUCN).[8]

Research on phylogeny and divergence times of Pinaceae confirmed that *A. firma* is genetically the closest species of *A. beshanzuensis*.[9] A significant rejuvenation effect has been found after grafting *A. beshanzuensis* by using *A. firma* as a stock tree. The aim of this study was to clarify the rejuvenation phenomenon by lignin structure analyses. In this study, the lignin structural features of *A. beshanzuensis* were investigated. The lignin structural features of *A. firma*, the closest species of *A. beshanzuensis*; *Cryptomeria japonica*, a typical gymnosperm tree species; and *Phyllostachys pubescens* (bamboo), which contains *p*-hydroxyphenyl units in arylglycerol-β-aryl intermonomer linkages, were also analyzed to compare lignin characteristics with *A. beshanzuensis*.

Materials and methods

Materials

Branches of *Abies beshanzuensis* were collected at Baishanzu Mountain (27.7° N, 117.8° E) in Qingyuan, southwest Zhejiang Province, China. Branches of *A. firma* of similar size to that of *A. beshanzuensis* were collected at the University Forest of the University of Tokyo in Chichibu (36.0° N, 139.5° E). Trunks of *Cryptomeria japonica* and *Phyllostachys pubescens* were collected at the Chiba Forest (35.4° N, 140.1° E) and the Tanashi Experimental Station (35.7° N 137.7° E) of the University of Tokyo, respectively. The samples of each species were freeze-dried and ground in a Wiley mill to pass a 420-μm sieve. The ground meal was extracted with ethanol–benzene (1:2, v/v) under reflux for 6 h with a Soxhlet apparatus. Finer wood meals were prepared from the extracted wood meal using a vibratory ball mill (Retsch type MM200) for 15 min with vibration of 30 Hz, and then were subjected to alkaline nitrobenzene oxidation, ozonation, and acidolysis analysis.

Preparation of Björkman lignin

The extracted meal was ground finely with a vibratory ball mill VS-2 (Irie Shokai, Tokyo, Japan) for 72 h with cooling provided by flowing tap water. Dispersing solvent was not used during milling.[10] Björkman lignin was extracted from the finely ground sample with dioxane–water (9:1, v/v) and purified according to the procedure of Björkman.[11]

Chemical analyses of lignin

Lignin content of the extracted meal was determined with the Klason procedure according to Tappi Standard T222 om-98. Acid-soluble lignin content was determined by measuring the UV absorption at 205 nm using an extinction coefficient of 110 $l \cdot g^{-1} \cdot cm^{-1}$ of H_2SO_4 hydrolysate.[12]

Aromatic composition of lignin in the extracted fine meal and Björkman lignin was analyzed with an alkaline nitrobenzene oxidation.[13] The reaction products were separated and quantified with gas chromatography after being trimethylsilylated with *N*,*O*-*bis*(trimethylsilyl)acetamide using a Shimadzu GC-17A gas chromatograph with an NB1 capillary column (25 m × 0.25 mm i.d.) equipped with a flame ionization detector (FID). Both injector and detector temperatures were 280°C. The column temperature was kept at 150°C for 10 min, and then programmed to rise at 5°C/min to 250°C. Ethyl vanillin was used as an internal standard.

Ozonation analysis was carried out according to the procedure of Akiyama et al.[14] for the extracted fine meal and Björkman lignin. The ozonation products, erythronic and threonic acids, were analyzed by a Shimadzu GC-17A gas chromatograph using an NB1 capillary column (25 m × 0.25 mm i.d.) equipped with a FID. Both injector and detector temperatures were 280°C. The column temperature was kept at 120°C for 5 min, and then programmed to rise at 4°C/min to 170°C and then by 10°C/min to 280°C.

Acidolysis of the extracted fine meals and Björkman lignin was performed according to the procedure of Lapierre et al.[15] Acidolysis products were identified as trimethylsilyl derivatives using *N*,*O*-*bis*(trimethylsilyl)acetamide by gas chromatography-mass spectrometry (GC-MS) (Shimadzu GC-17A coupled with a Shimadzu QP-5000 mass spectrometer). The NB1 capillary column (25 m × 0.25 mm i.d.) was used. The injector temperature was 250°C. The column temperature was kept at 150°C for 10 min, and then programmed to rise at 5°C/min to 250°C. In addition, the reaction products were quantified by gas chromatography as trimethylsilyl derivatives using a Shimadzu GC-17A gas chromatograph with NB1 capillary column (25 m × 0.25 mm i.d.) equipped with a FID. Both injector and detector temperatures were 280°C. The column temperature was kept at 150°C for 10 min, and then programmed to rise at 5°C/min to 250°C. Ethyl vanillin was used as internal standard.

The yields of alkaline nitrobenzene oxidation and ozonation were calculated on the basis of the monomeric phenylpropane unit of lignin, for which the average molecular weight is 200, in units of millimoles per 200 g of lignin [$mmol \cdot (200\,g\ lignin)^{-1}$]. All chemical analyses were replicated three times, and average values are presented in this article.

Spectroscopic analyses

The Björkman lignin (around 100 mg) was acetylated with 15 ml of acetic anhydride with 0.1 ml of pyridine as a catalyst at room temperature overnight with stirring. The acetylated sample was dissolved in chloroform-*d* and the ^{1}H NMR spectrum was recorded using a Jeol JNM-A 500 spectrometer (Jeol, Japan).

Results and discussion

The total lignin content of *Abies beshanzuensis* and *Abies firma* was 39.2% and 33.7%, respectively (Table 1). The value for *A. beshanzuensis* was higher than those of gymnosperms, which range from 25% to 35%.[16,17] Lignins synthesized under stress usually have different chemical compositions from those synthesized under normal secondary growth conditions. The concentration of phenylpropane units of the *p*-hydroxyphenyl type in Douglas fir compression wood lignin was almost three times that in normal wood lignin.[18] Lignin content of *A. beshanzuensis* (39.2%) was significantly higher than that of *A. firma* (33.7%). The high value may be due to the adaptation of *A. beshanzuensis* to environmental stresses in surviving from the Riss glacial period.

By alkaline nitrobenzene oxidation, *A. beshanzuensis* lignin gave 244.5 mmol·(200 g lignin)$^{-1}$ of vanillin and 15.1 mmol·(200 g lignin)$^{-1}$ of *p*-hydroxybenzaldehyde. The values for *A. firma* were similar to those of *A. beshanzuensis* (Table 1). The ratio of *p*-hydroxybenzaldehyde to total yield of alkaline nitrobenzene oxidation products was 5.7 and 7.5 for *A. beshanzuensis* and *A. firma*, respectively. A trace of syringaldehyde was also identified among the oxidation products. The alkaline nitrobenzene oxidation products obtained from Björkman lignin of *A. beshanzuensis* and *A. firma* were comparable with those obtained from the extracted fine meals (Table 2). The total yield of alkaline nitrobenzene oxidation products of extracted meal and Björkman lignin of *A. beshanzuensis* and *A. firma* showed typical gymnosperm wood lignin characteristics. The total yield of aromatic aldehydes in the alkaline nitrobenzene oxidation products serves as an index of the presence of uncondensed units in lignin. The lower yield of total alkaline nitrobenzene oxidation products of *A. beshanzuensis* and *A. firma* indicates the high level of condensed units when compared with *Phyllostachys pubescens* (bamboo) (Table 2).

The yields of *p*-hydroxybenzaldehyde obtained from extracted fine meal of *A. beshanzuensis* and *A. firma* were similar to those obtained from Björkman lignin (Tables 1 and 2). Alkaline nitrobenzene oxidation analysis on *Abies concolor*,[2] *Abies fraseri*,[3] *Abies balsamea*,[4] *Abies sibirica*,[5] and *Abies sachalinensis*[6] also showed the existence of *p*-hydroxybenzaldehyde. The ratio of *p*-hydroxybenzaldehyde to total yield of alkaline nitrobenzene oxidation products of Björkman lignin was 5.6, 7.1, and 7.0 for *A. beshanzuensis*, *A. firma*, and *P. pubescens* (bamboo), respectively (Table 2). In bamboo lignin, 5% to 10% of the lignin is esterified to *p*-coumaric acid through lignin side chains and about two thirds of the *p*-hydroxybenzaldehyde yielded by alkaline nitrobenzene oxidation is derived from the esterified *p*-coumaric acid of the lignin.[19]

The presence of arylglycerol-β-aryl ether intermonomer linkage is essential to distinguish lignin from other polyphenols.[20] The yields of acidolysis products from lignin are usually lower than those of alkaline nitrobenzene oxidation products. However, the advantage of acidolysis over nitrobenzene oxidation is that acidolysis can give C_6-C_3 monomeric structures, which are specifically produced from arylglycerol-β-aryl ether intermonomer linkages in lignin. Namely, if *p*-hydroxyphenyl propanones (H lignin) are produced from a sample by acidolysis, it is safe to conclude that *p*-hydroxyphenyl nuclei are incorporated in the lignin macromolecule. The acidolysis products of the

Table 1. Klason lignin contents and yields of alkaline nitrobenzene oxidation and ozonation products from *Abies beshanzuensis* and *Abies firma*

Species	Lignin content (% of extract-free sample)			Alkaline nitrobenzene oxidation [mmol·(200 g lignin)$^{-1}$]								Ozonation products [mmol·(200 g lignin)$^{-1}$]			
	KR	ASL	Total	H	HA	V	VA	S	SA	Total	H/Total[a] (%)	E	T	Total	E/T[b]
A. beshanzuensis	38.8	0.4	39.2	15.1	1.8	244.5	10.3	Trace	Trace	264.9	5.7	77.5	67.8	145.2	1.14
A. firma	33.3	0.4	33.7	21.1	2.4	246.1	11.0	Trace	Trace	280.6	7.5	85.1	75.1	160.2	1.13

KR, Klason residue; ASL, acid-soluble lignin; H, *p*-hydroxybenzaldehyde; HA, *p*-hydroxybenzoic acid; V, vanillin; VA, vanillic acid; S, syringaldehyde; SA, syringic acid; E, erythronic acid; T, threonic acid

[a] Molar ratio of *p*-hydroxybenzaldehyde to total yield of alkaline nitrobenzene oxidation products

[b] Molar ratio of erythronic acid to threonic acid of ozonation products

Table 2. Alkaline nitrobenzene oxidation products from Björkman lignin of *A. beshanzuensis*, *A. firma*, *and Phyllostachys pubescens*

Species	Alkaline nitrobenzene oxidation products [mmol·(200 g lignin)$^{-1}$]							H/Total[a] (%)	H_{acid}/H_{nb}[b]
	H	HA	V	VA	S	SA	Total		
A. beshanzuensis	14.7	1.7	232.9	14.1	Trace	Trace	263.5	5.6	0.18
A. firma	18.5	2.1	224.2	15.3	Trace	Trace	260.1	7.1	0.19
P. pubescens	22.8	1.4	112.3	5.3	169.7	13.6	325.1	7.0	0.12

[a] Molar ratio of *p*-hydroxybenzaldehyde to total yield of alkaline nitrobenzene oxidation products

[b] Ratio of *p*-hydroxyphenyl propanones in acidolysis products to *p*-hydroxybenzaldehydes in alkaline nitrobenzene oxidation products

Björkman lignins were identified by GC-MS as trimethylsilyl derivatives (Fig. 1). Two peaks assigned to *p*-hydroxyphenyl propanones (H_α: *m*/*z* 310, 179, 103, 73; H_β: *m*/*z* 310, 193, 117, 73) were detected in the Björkman lignins of *A. beshanzuensis*. Similar results were also obtained from *A. firma* and *P. pubescens* lignin, while those peaks assigned to *p*-hydroxyphenyl propanones could not be detected from *Cryptomeria japonica* lignin (Fig. 2). These results suggest that the lignins of *A. beshanzuensis*, *A. firma*, and *P. pubescens* contain *p*-hydroxyphenylglycerol-*β*-aryl ether intermonomer linkages. The relative intensities of *p*-hydroxyphenyl propanones of *P. pubescens* Björkman lignin were smaller than those of *A. beshanzuensis* and *A. firma*, which demonstrates that lignin of *A. beshanzuensis* and *A. firma* is characterized by the presence of *p*-hydroxyphenylglycerol-*β*-aryl ether intermonomer linkages with higher ratio compared than *P. pubescens* lignin (Table 2).

The proportion of *p*-hydroxyphenyl lignin among *p*-hydroxybenzaldehyde in the low molecular weight products of alkaline nitrobenzene oxidation was also confirmed by calculating the molar ratio of *p*-hydroxyphenyl type acidolysis products to the corresponding alkaline nitrobenzene oxidation products (H_{acid}/H_{nb}). The ratios of H_{acid}/H_{nb} of *A. beshanzuensis* and *A. firma* were higher than that of *P. pubescens* (Table 2). These results are in agreement with the observations that *p*-hydroxybenzaldehyde produced by alkaline nitrobenzene oxidation of bamboo lignin is mostly ascribed to the esterified *p*-coumaric acid and the amount of *p*-hydroxyphenyl compounds as lignin degradation product is quite small.[21]

The arylglycerol-*β*-aryl ether intermonomer linkage can be either *erythro*- or *threo*-form, and the ratio of *erythro*- to *threo*-form of arylglycerol-*β*-aryl ether intermonomer linkage (E/T ratio) is an important characteristic of lignin.[22,23] By ozonation, erythronic and threonic acids are obtained from the *erythro*- and *threo*-forms of arylglycerol-*β*-aryl ether structures, respectively, and gymnosperm lignin contains approximately equal amounts of *erythro*- and *threo*-forms of the linkage.[14,24,25]

The total yields of erythronic and threonic acids were 145.2 and 160.2 mmol (200 g lignin)$^{-1}$ for the extracted fine meal of *A. beshanzuensis* and *A. firma*, respectively (Table 1). The molar ratio of erythronic acid to threonic acid (E/T ratio) of *A. beshanzuensis* was exactly the same as that of *A. firma*, suggesting that the side chain lignin character of *A. beshanzuensis* is similar to that of *A. firma*.

The presence of *p*-hydroxybenzaldehyde and syringaldehyde in *A. beshanzuensis* lignin was also confirmed by ^{1}H NMR spectroscopy (Fig. 3). Acetylated Björkman lignins of *A. beshanzuensis* exhibited a signal at δ 7.4, which is assigned to the aromatic protons of *p*-hydroxyphenyl nuclei, in addition to a strong signal corresponding to the aromatic protons of guaiacyl nuclei at *δ* 6.9, and a shoulder corresponding to syringyl nuclei at *δ* 6.6. *Abies firma* lignin showed similar signals to those of *A. beshanzuensis* (Fig. 3).

Alkaline nitrobenzene oxidation, ozonation, acidolysis, and ^{1}H NMR spectra showed that the structural features of lignin of *A. beshanzuensis* are similar to those of *A. firma*. A significant rejuvenation phenomenon has been found by grafting *A. beshanzuensis* to *A. firma*, which may be ascribed to the similarity of lignin structure.

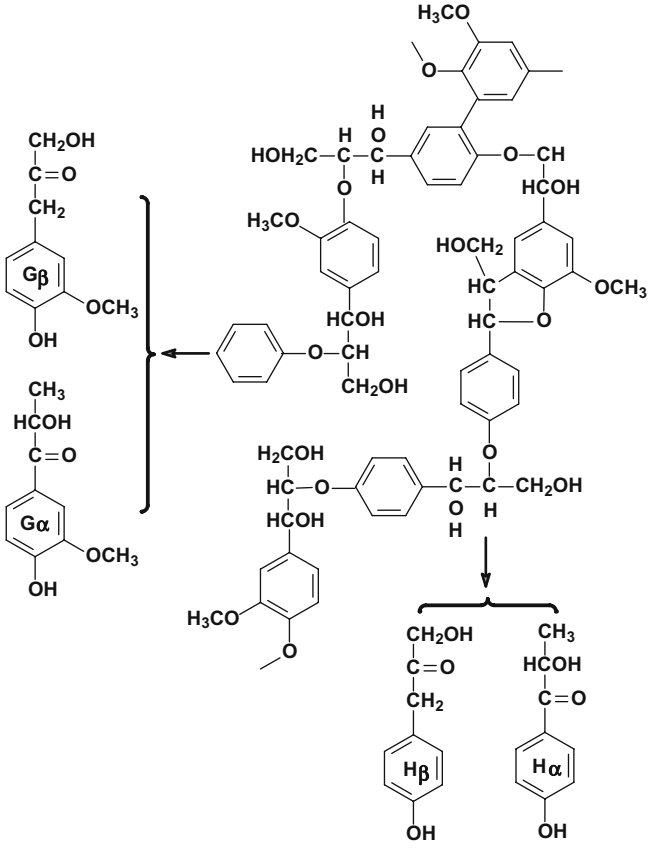

Fig. 1. Production of phenylpropanone derivatives (H_α, H_β, G_α, and G_β) from arylglycerol-*β*-aryl ether intermonomer linkages of lignin during acidolysis

Conclusions

The lignin content of *Abies beshanzuensis* was found to be 39.2%, and this high value suggests the remarkable ability of *A. beshanzuensis* to adapt to environmental stresses after having survived the Riss glacial period. Acidolysis, alkaline nitrobenzene oxidation, ozonation, and ^{1}H NMR spectroscopy showed that the structural features of lignin of *A. beshanzuensis* are similar to those of *Abies firma*, which is genetically the closest species to *A. beshanzuensis*. *Abies firma* would be a suitable species for the mother tree for grafting *A. beshanzuensis* on the basis of the similarities in lignin characteristics.

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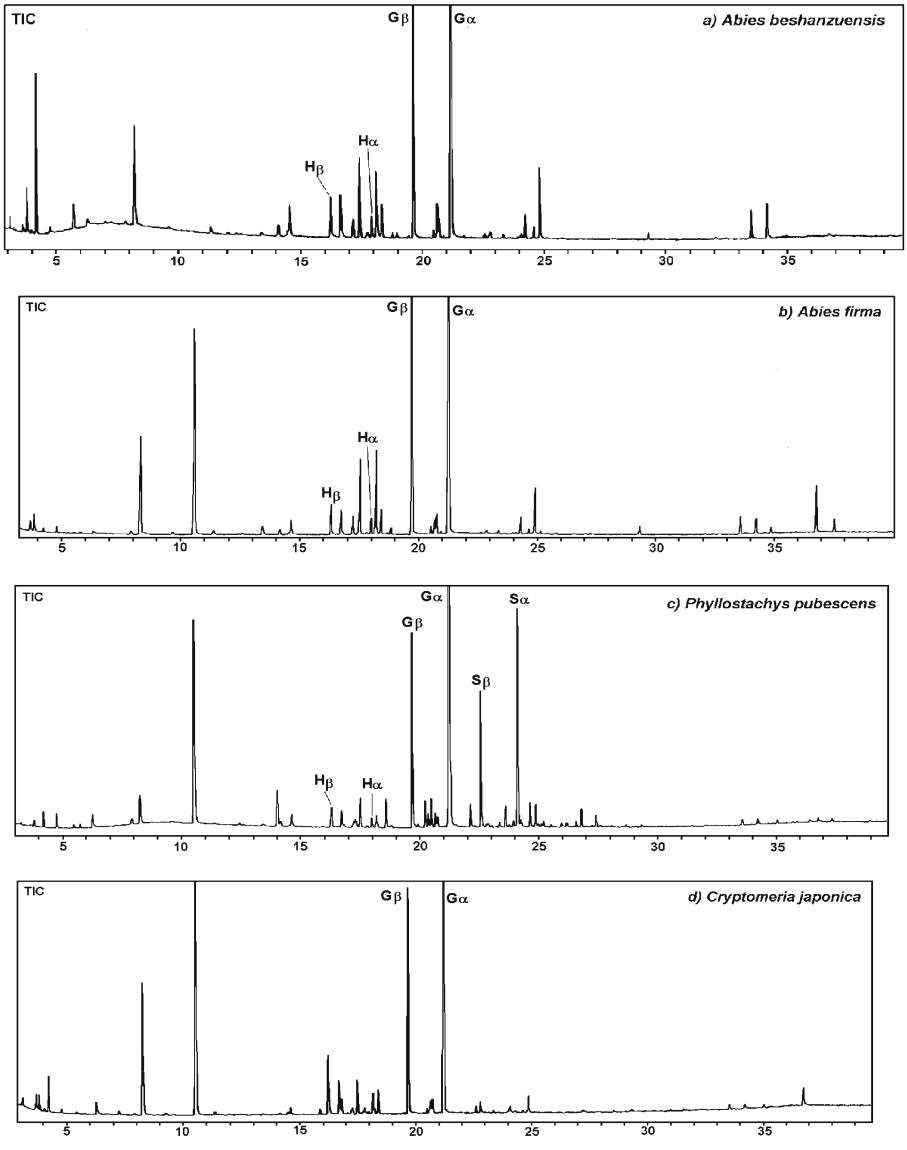

Fig. 2a–d. Gas chromatograms of acidolysis products of the extract-free samples of **a** *Abies beshanzuensis*, **b** *Abies firma*, **c** *Phyllostachys pubescens*, and **d** *Cryptomeria japonica.* Mass fragmentation: H_{α}: *m*/*z* 310, 179, 103, 73; H_{β}: *m*/*z* 310, 193, 117, 73; G_{α}: *m*/*z* 340, 209, 103, 73; G_{β}: *m*/*z* 340, 223, 117, 73; S_{α}: *m*/*z* 370, 239, 103, 73; S_{β}: *m*/*z* 370, 253, 117, 73

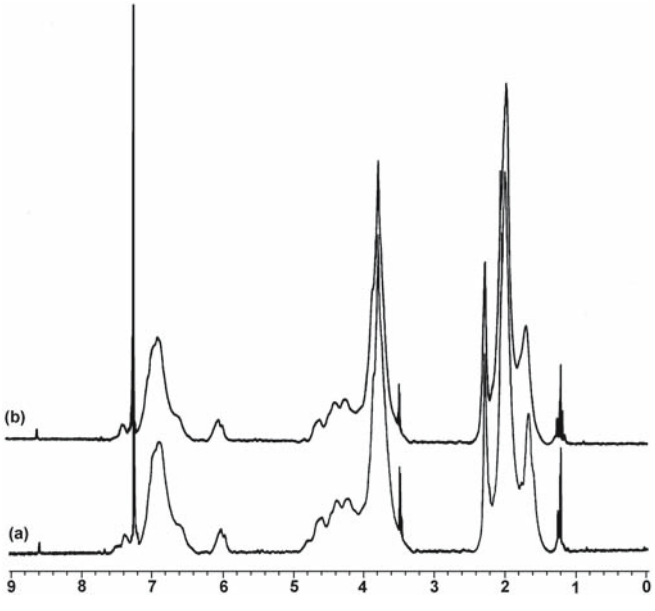

Fig. 3a, b. ^{1}H Nuclear magnetic resonance spectra of Björkman lignins isolated from *A. beshanzuensis* (**a**) and *A. firma* (**b**)

References

1. Higuchi T (2000) The present state and problems in lignin biosynthesis. Cellul Chem Technol 34:79–100
2. Bicho JG, Zavarin E, Brink DL (1966) Oxidative degradation of wood. II. Products of alkaline nitrobenzene oxidation by a methylation-gas chromatographic technique. TAPPI 49:218–226
3. Balakshin MY, Capanema EA, Goldfarb B, Frampton J, Kadla JF (2005) NMR studies on Fraser fir *Abies fraseri* (Pursh) Poir. Lignins. Holzforschung 59:488–496
4. Donaldson LA (2001) Lignification and lignin topochemistry – an ultrastructural view. Phytochemistry 57:859–873
5. Kuznetsov BN, Efremov AA, Levdanskii VA, Kuznetsova SA, Polezhayeva NI, Shilkina TA, Krotova IV (1996) The use of non-isobaric pre-hydrolysis for the isolation of organic compounds from wood and bark. Bioresource Technol 58:181–188
6. Ozawa S, Sasaya T (1991) Extractives of todomatsu *Abies sachalinensis* masters. IX. Brauns lignin of todomatsu *Abies sachalinensis* (2). Mokuzai Gakkaishi 37:847–851
7. Sun X, Li X (1999) A pollen record of the last 37 ka in deep sea core 17940 from the northern slope of the South China Sea. Mar Geol 156:227–244
8. Conifer Specialist Group (1998) *Abies beshanzuensis*. IUCN red list of threatened species. http://www.iucnredlist.org/search/details.php/32318/all. Cited 12 August 2006
9. Wang XQ, Tank DC, Sang T (2000) Phylogeny and divergence times in Pinaceae: evidence from three genomes. Mol Biol Evol 17:773–781
10. Koshijima T, Taniguchi T, Tanaka R (1972) Lignin–carbohydrate complex. I. Influences of milling of wood upon the Björkman LCC. Holzforschung 26:211–217
11. Björkman A (1956) Studies on finely divided wood. Part 1. Extraction of lignin with neutral solvents. Svensk Papperstidn 59:477–485
12. Schöning AG, Johansson G (1965) Absorptiometric determination of acid-soluble lignin in semichemical bisulfite pulp and in some wood and plants. Svensk Papperstidn 68:607–613
13. Iiyama K, Lam TBT (1990) Lignin in wheat internodes. Part 1. The reactivities of lignin units during alkaline nitrobenzene oxidation. J Sci Food Agric 51:481–491
14. Akiyama T, Sugimoto T, Matsumoto Y, Meshitsuka G (2002) *Erythro/threo* ratio of *β-O*-4 structures as an important structural characteristic of lignin. Part 1. Improvement of ozonation method for the quantitative analysis of lignin side-chain structure. J Wood Sci 48:210–215
15. Lapierre C, Rolando C, Monties B (1983) Characterization of poplar lignins acidolysis products: capillary gas–liquid and liquid–liquid chromatography of monomeric compounds. Holzforschung 37:189–198
16. Creighton RHJ, Gibbs RD, Hibbert H (1944) Studies on lignin and related compounds. LXXV. Alkaline nitrobenzene oxidation of plant materials and application to taxonomic classification. J Am Chem Soc 66:32–37
17. Chen CL (1992) Nitrobenzene and cupric oxide oxidations. In: Lin SY, Dence CW (eds) Methods in lignin chemistry. Springer, Berlin Heidelberg New York, pp 301–367
18. Sarkanen KV, Hergert L (1971) Classification and distribution. In: Sarkanen KV, Ludwig CH (eds) Lignins – occurrence, formation, structure and reactions. Wiley, New York, pp 43–94
19. Higuchi T (1987) Chemistry and biochemistry of bamboo. Bamboo J 4:132–145
20. Adler E (1977) Lignin chemistry – past, present and future. Wood Sci Technol 11:169–218
21. Higuchi T, Tanahashi M, Sato A (1972) Acidolysis of bamboo lignin. I. Gas–liquid chromatography and mass spectrometry of acidolysis monomers. Mokuzai Gakkaishi 18:183–189
22. Brunow G, Karlsson O, Lundquist K, Sipilä J (1993) On the distribution of the diastereomers of the structural elements in lignins: the steric course of reactions mimicking lignin biosynthesis. Wood Sci Technol 27:282–286
23. Akiyama T, Goto H, Nawawi DS, Syafii W, Matsumoto Y, Meshitsuka G (2005) *Erythro*/*threo* ratio of *β-O*-4 structures as an important structural characteristic of lignin. Part 4: variation in the *erythro*/*threo* ratio in softwood and hardwood lignins and its relation to syringyl/guaiacyl ratio. Holzforschung 59:276–281
24. Matsumoto Y, Ishizu A, Nakano J (1986) Studies on chemical structure of lignin by ozonation. Holzforschung 40(Suppl):81–85
25. Matsumoto Y, Minami K, Ishizu A (1993) Structural study on lignin by ozonation. The *erythro* and *threo* ratio of the *β-O*-4 structure indicates how lignin polymerizes. Mokuzai Gakkaishi 39: 734–736