

NOTE

Masahiro Matsunaga · Keiko Sakai
Hiroshi Kamitakahara · Kazuya Minato
Fumiaki Nakatsubo

Vibrational property changes of spruce wood by impregnation with water-soluble extractives of pernambuco (*Guilandina echinata* Spreng.) II: structural analysis of extractive components

Received: May 12, 1999 / Accepted: July 19, 1999

Abstract Heartwood of pernambuco (*Guilandina echinata* Spreng. syn *Caesalpinia echinata* Lam.), which has been used as material for violin bows, was extracted by soaking in water, and the obtained extractives were analyzed. The main components of the extractives were identified to be protosappanin B and brazilin. In particular, protosappanin B occupied about 40% of the pernambuco extractives. The loss tangent ($\tan\delta$) of spruce wood impregnated with protosappanin B decreased markedly, the same as that of specimens impregnated with extractives before being purified. It is expected that protosappanin B can make a contribution to the decrease in $\tan\delta$ due to impregnation with extractives.

Key words Pernambuco · Extractives · Protosappanin B · Brazilin · Vibrational property

Introduction

The heartwood of pernambuco (*Guilandina echinata* Spreng. syn *Caesalpinia echinata* Lam.), a Brazilian hardwood, is the most suitable material for violin bows. In previous studies we found that the loss tangent ($\tan\delta$) of pernambuco was exceptionally low among many wood species examined and proposed that low $\tan\delta$ is a necessary factor for bow material.^{1,2} We also reported that a low $\tan\delta$

is attributed to the extractive components contained in pernambuco because the $\tan\delta$ of pernambuco increased drastically after extraction with water.¹ Although the $\tan\delta$ of wood may also vary depending on the mean microfibril angle,^{3,4} the main factor that lowers the $\tan\delta$ was the extractive components, as far as pernambuco is concerned.⁵

Impregnation of extractive components into other wood species significantly decreased the $\tan\delta$ of these specimens.⁶ Generally, the $\tan\delta$ of wood increases after impregnation of any reagents or chemical treatment, although in peculiar cases we can find some instances where the $\tan\delta$ decreases after some chemical treatments.^{7,8} This finding is supported by the fact that these treatments cause cross-linkages to form between neighboring hydroxyl groups of the wood components. In our case, however, the impregnated components could not form covalent bonds with wood components, because the extractives were impregnated at room temperature and dried at a mild condition. Therefore, it is plausible that the extractive components bound to the wood components by some secondary intermolecular force, such as hydrogen bonds, and that the $\tan\delta$ consequently decreased. To clarify the mechanism, it is necessary to determine the chemical structure of the main components in the extractives.

In this study, we determined the chemical structure and the ratio of the water-soluble major components in the extractives of pernambuco. Moreover, we examined the vibrational property changes in the wood specimens impregnated with the main extractive components of pernambuco. It is important for elucidation of the behavior of extractives in wood to know the relation between the chemical structure of the impregnated reagent and the resulting vibrational property changes of wood.

Materials and methods

General methods

Thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed on silica

M. Matsunaga (✉)
Forestry and Forest Products Research Institute, Ibaraki 305-8687,
Japan
Tel. +81-298-73-3211; Fax +81-298-74-3720
e-mail: cla@ffpri.affrc.go.jp

K. Sakai · H. Kamitakahara · K. Minato · F. Nakatsubo
Graduate School of Agriculture, Kyoto University, Kyoto 606-8502,
Japan

Part of this work was presented at the 48th annual meeting of the Japan Wood Research Society, Shizuoka, April 1998; and the 49th annual meeting of the Japan Wood Research Society, Tokyo, April 1999

gel plates (Kieselgel 60 F₂₅₄; Merck), and the spots were detected under ultraviolet (UV) light (254nm). ¹H-NMR spectra were recorded with a Bruker AC300 FT-NMR (300MHz) spectrometer in chloroform-*d* or deuterium oxide with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) and coupling constants (J) are given in δ values (ppm) and hertz, respectively. The following abbreviations are used: s, singlet, d, doublet, dd, double doublet. Optical rotations were measured at 28°C using a JASCO Dip-1000 digital polarimeter. Electron impact-mass spectroscopy (EI-MS) was performed on a Shimadzu GC-MS QP-1000.

Analysis of water-soluble extractives of pernambuco

The wood meal of pernambuco (2.96g) was extracted by soaking in water (20ml) with stirring at ambient temperature for 24h. The supernatant was filtered and freeze-dried to give extractives (76.9mg) as a red-brown powder. The extractives were checked by TLC developed with MeOH/CHCl₃ (1:4, v/v). Two major spots were detected [R_f values: 0.51 (compound **1**); 0.33 (compound **2**)] under UV light. Then the crude extractives were purified by a silica gel column (Wakogel C-200, 200mm in length and 25mm inside diameter) eluted with ethyl acetate/*n*-hexane/methanol (11:8:1, v/v/v) to afford fractions **1** (7.8mg) and **2** (44.0mg). Fraction **1** contained compound **1** as almost pure, checked by TLC. Fraction **2** consisted of compound **2** as a main compound and other impurities. These fractions were acetylated with acetic anhydride (0.5ml) and pyridine (0.5ml) at room temperature overnight; and acetylated fractions **3** (compound **3**, 12.4mg) and **4** (61.6mg) were obtained from fractions **1** and **2**, respectively. Fraction **4** was chromatographed on TLC developed with MeOH/CHCl₃ (1:19, v/v), and two spots were observed [R_f values: 0.49 (compound **4**); 0.35 (compound **5**)]. These compounds were purified by PTLC developed with MeOH/CHCl₃ (1:19, v/v) to afford compounds **4** (4.5mg) and **5** (25.9mg) as pure compounds.

Measuring the proportions of main compounds

The other new extractives were separated into two fractions – compounds **1**- and **2**-predominant fractions – by a silica gel column (Wakogel C-200) developed with ethyl acetate/*n*-hexane/methanol (11:8:1, v/v/v); each fraction was then analyzed by high-performance liquid chromatograph (HPLC). A Shimadzu liquid chromatograph injector (LC-10ATvp), Shimadzu column oven (CTO-10Avp), Shimadzu UV-VIS detector (SPD-10Avp), Shimadzu refractive index detector (RID-10A), Shimadzu communication bus module (CBM-10A), Shimadzu LC workstation (CLASS-LC10), and a column (nacalai tesque COSMOSIL 5C₁₈-MS) were used. The elution condition was as follows: eluent, MeOH/0.02M phosphate buffer solution (pH 7.0) (95:5, v/v); flow rate 1.0ml/min; column temperature 40°C.

Compound 1. EI-MS m/z (%): 318 (M^+ , 30.4), 286 (100), 268 (47.5), 229 (28.2). ¹H-NMR (300MHz, D₂O): δ 2.87

(1H, d, J = 16.08; C₇-H) and 3.07 (1H, d, J = 16.14; C₇-H), 3.68 (1H, d, J = 11.72; C₆-H) and 4.00 (1H, d, J = 11.86; C₆-H), 4.05 (1H, s; C₁₂-H), 6.41 (1H, d, J = 2.02; C₄-H), 6.65 (1H, dd, J = 2.08, 8.37; C₂-H), 6.78 (1H, s; C₁₁-H), 6.82 (1H, s; C₈-H), 7.35 (1H, d, J = 8.28; C₁-H).

Compound 2 (fraction 2). $[\alpha]^{28}$ -10.6° (c = 2.14, in methanol). EI-MS m/z : 304 (M^+ , 100), 243 (25.8), 229 (48.2). The ¹H-NMR spectrum was complicated because of impurities.

Compound 3 (acetylated compound 1). ¹H-NMR (300MHz, CDCl₃): δ 2.08 (3H, s; OAc), 2.26 (6H, s; OAc \times 2), 2.29 (3H, s; OAc), 3.45 (2H, s; C₇-H₂), 3.73 (1H, d, J = 12.29; C₆-H) and 4.76 (1H, dd, J = 1.84, 12.31; C₆-H), 4.47 (1H, s; C₁₂-H), 6.66 (1H, d, J = 2.31; C₄-H), 6.80 (1H, dd, J = 2.32, 8.32; C₂-H), 7.05 (1H, s; C₁₁-H), 7.09 (1H, s; C₈-H), 7.36 (1H, d, J = 8.62; C₁-H).

Compound 4 (acetylated compound 2). $[\alpha]^{28}$ -59.3° (c = 1.59, in chloroform). EI-MS m/z : 514 (M^+ , 56.0), 472 (100), 430 (91.5), 388 (38.1), 310 (35.0), 268 (37.2), 229 (32.1). ¹H-NMR (300MHz, CDCl₃): δ 2.03 (3H, s; OAc), 2.10 (3H, s; OAc), 2.30 (9H, s; OAc \times 3), 2.71 (1H, d, J = 14.11) and 3.49 (1H, d, J = 14.09), 3.99 (1H, d, J = 12.78) and 4.51 (1H, d, J = 12.76), 4.43 (1H, d, J = 11.97) and 4.84 (1H, d, J = 11.96), 6.85 (1H, d, J = 2.11), 6.88 (1H, dd, J = 2.31, 8.41), 7.09 (1H, s), 7.19 (1H, s), 7.24 (1H, d, J = 8.46).

Compound 5 (acetylated compound 2). $[\alpha]^{28}$ -38.7° (c = 2.57, in chloroform). ¹H-NMR (300MHz, CDCl₃): δ = 2.12 (3H, s; OAc), 2.31 (9H, s; OAc \times 3), 2.74 (2H, s), 3.91 (1H, d, J = 12.29) and 4.33 (1H, d, J = 12.28), 4.14 (2H, s), 6.88 (1H, d, J = 2.24), 6.92 (1H, dd, J = 2.30, 8.35), 7.16 (1H, s), 7.18 (1H, s), 7.28 (1H, d, J = 8.44).

Treatments of spruce wood with isolated extractives of pernambuco

Sitka spruce (*Picea sitchensis* Carr.) specimens were made 150mm (longitudinal direction) \times 12mm (radial direction) \times 2mm (tangential direction). Before impregnation with extractive components, vibrational properties were measured by a free-free flexural vibration method. The specific dynamic Young's modulus (E'/γ , where E' is dynamic Young's modulus, and γ is specific density) in the longitudinal direction was calculated from the resonant frequency using the Euler-Bernoulli equation and $\tan \delta$ from the decremental curve of the vibration at the resonant frequency. The measurements were carried out in a chamber maintained at 20°C and 65% relative humidity (RH).

Approximately 20g of extractive was obtained from the wood meal of pernambuco (150g), by a procedure previously reported.⁶ The extractives were then purified by silica gel column (Wakogel C-200) developed with ethyl acetate/*n*-hexane/methanol (11:8:1, v/v/v) to afford compound **2**-predominant fraction (12g).

Four pieces of Sitka spruce specimens were soaked in H₂O/MeOH (9:1, v/v) solutions of compound **2**-predominant fraction whose concentrations were 5, 10, 20, and 40g/l for 8 days with occasional evacuation using an aspirator. After drying under ambient conditions and

subsequent drying at 60°C under vacuum for 2 days, the weight percent gain (WPG) of the specimens was measured. The specimens were then conditioned at 20°C and 65% RH for 2 weeks, and the vibrational properties were determined again.

Results and discussion

Analysis of water-soluble extractives of pernambuco

The $^1\text{H-NMR}$ spectrum of compound **3** coincided with that of the brazilin tetraacetate⁹ thoroughly. Moreover, the molecular weight (286 kDa) of compound **1** coincided with that of brazilin ($\text{C}_{16}\text{H}_{14}\text{O}_5$) (Fig. 1). From these results, **1** was identified with brazilin, which has been reported to be contained in Brazil wood, species *Caesalpinia*.^{10,11}

The $^1\text{H-NMR}$ spectrum and molecular weight (514 kDa) of compound **4** coincided with that of protosappanin B pentaacetate.¹² The optical rotation $\{[\alpha]^{28} - 59.3^\circ (\text{CHCl}_3)\}$ of **4** was almost the same as that of protosappanin B pentaacetate $\{[\alpha]_{\text{D}}^{25} - 62.5^\circ (\text{CHCl}_3)\}$.¹² Compound **5** has four acetyl groups and showed the same $^1\text{H-NMR}$ spectrum as compound **4** after acetylation at 60°C overnight. The optical rotation of **5** $\{[\alpha]^{28} - 38.7^\circ (\text{CHCl}_3)\}$ was almost equal to that of protosappanin B tetraacetate $\{[\alpha]_{\text{D}}^{25} - 36.8^\circ (\text{CHCl}_3)\}$.¹² Therefore, **5** was found to be protosappanin B tetraacetate. From these results, compound **2** was identified with protosappanin B (Fig. 1), which has been reported to be contained in the heartwood of sappan.¹² Although compound **2** contained a small quantity of impurities, the optical

rotation $\{[\alpha]^{28} - 10.6^\circ (\text{MeOH})\}$ and molecular weight (304 kDa) of **2** almost coincided with those of protosappanin B $\{[\alpha]_{\text{D}}^{15} - 11.4^\circ (\text{MeOH})\}$.¹²

Protosappanin B and brazilin were quantitatively analyzed by HPLC. It was roughly estimated that the amount of protosappanin B and brazilin were 40% and 10% of the whole extractives, respectively. However, the exact contents of the main components were not determined because the peaks of impurities could not be separated thoroughly from that of the main component.

Influence of protosappanin B on vibrational properties

It was found that the extractive components of pernambuco lowered the $\tan \delta$ of wood, and that the main component of the extractives was protosappanin B. However, it cannot be concluded from these facts alone that protosappanin B has great influence on lowering the $\tan \delta$ of wood. Therefore, the change in vibrational properties of spruce wood impregnated with almost pure protosappanin B was considered.

Figure 2 shows the relation between WPG and percent change of $\tan \delta$. The data for the vibrational property changes of spruce wood impregnated with unpurified extractives from pernambuco are taken from a previous study.⁶ The $\tan \delta$ of the specimen impregnated with protosappanin B decreased markedly, the same as that of the specimen impregnated with extractives from pernambuco. Figures 3 and 4 show the percent changes of E'/γ and E' , respectively. The change in the properties of specimen impregnated with protosappanin B exhibited almost the same tendency as those impregnated with unpurified

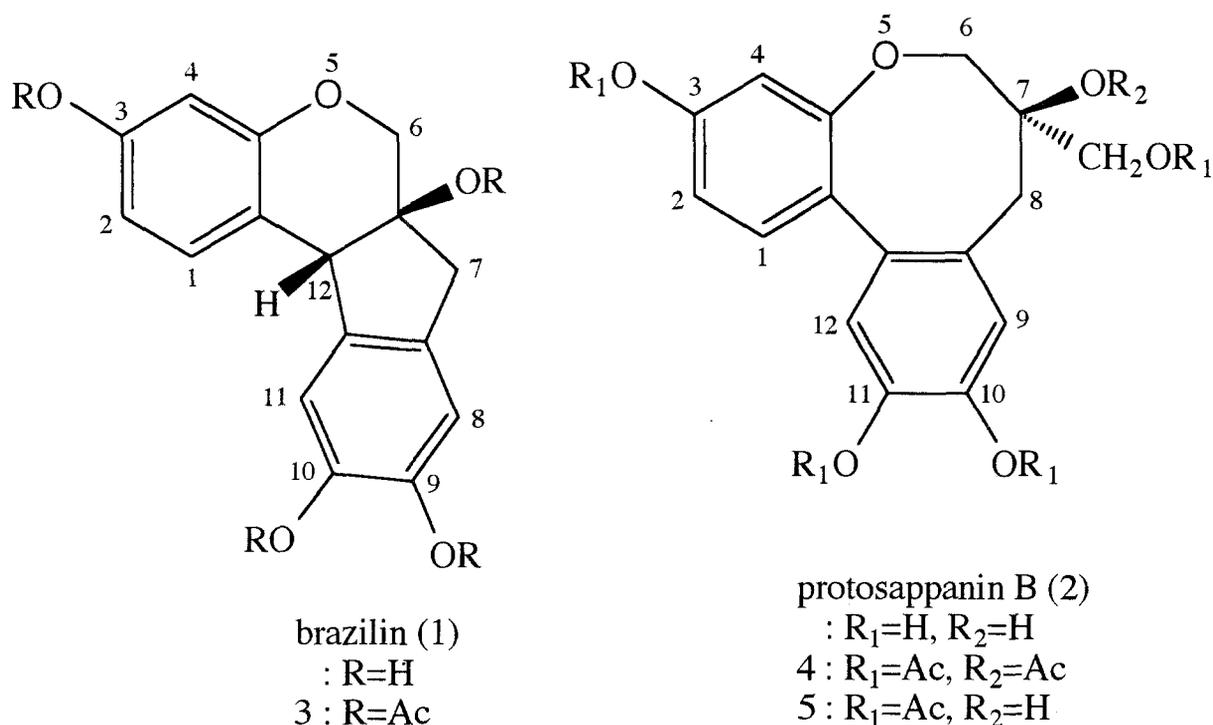


Fig. 1. Main extractives of pernambuco and their acetylated compounds

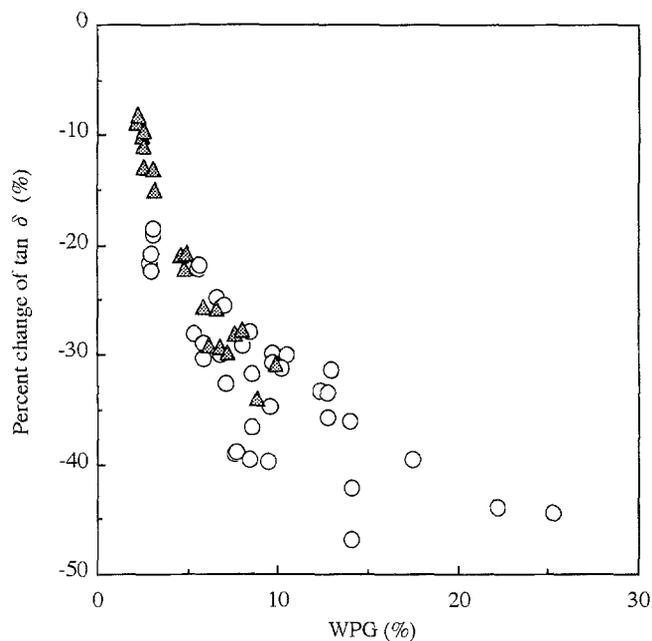


Fig. 2. Relation between weight percent gain (WPG) and percent change of $\tan \delta$. Filled triangles, protosappanin B; open circles, extractives of pernambuco

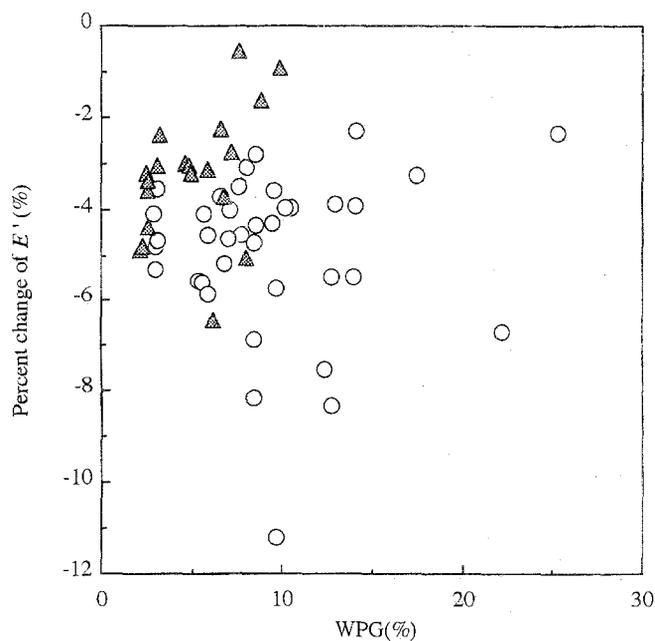


Fig. 4. Relation between weight percent gain (WPG) and percent change of E' . Symbols are the same as in Fig. 2

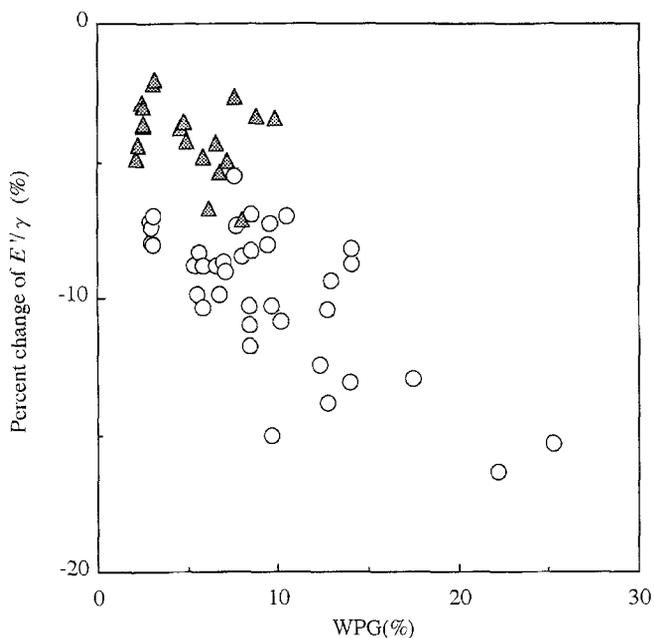


Fig. 3. Relation between weight percent gain (WPG) and percent change of E'/γ . Symbols are the same as in Fig. 2

extractives. From these results, it was clear that protosappanin B predominantly influences the change of vibrational properties due to impregnation with extractives of pernambuco.

It is thought that the extractive components formed secondary bonds between matrix substances, resulting in the observed decrease in $\tan \delta$. Protosappanin B has five hydroxyl groups, so it is proposed that protosappanin B forms

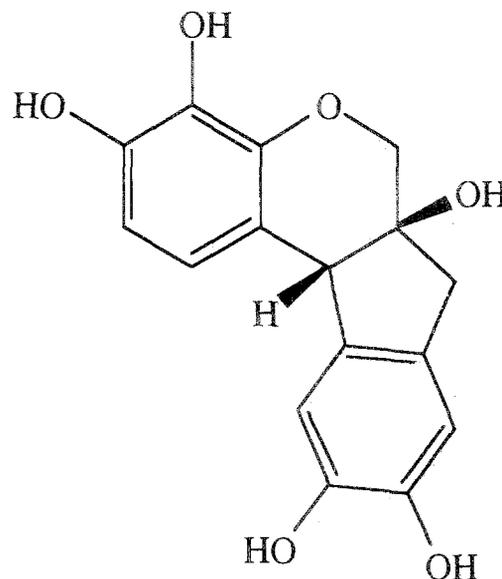


Fig. 5. Chemical structure of hematoxylin

hydrogen bonds between neighboring hydroxyl groups of the wood components.

On the other hand, the amount of brazilin contained in extractives of pernambuco is so small sufficient amount of brazilin could not be obtained to impregnate several specimens. However, impregnation of hematoxylin (Fig. 5), whose chemical structure is similar to that of brazilin, brought about an extreme decrease of $\tan \delta$.^{13,14} It is thought

that brazilin is also one of the components that contribute to a decreasing $\tan \delta$ of wood by impregnation.

Conclusions

The heartwood of pernambuco was extracted by soaking it in water, and the obtained extractives were analyzed. Two main components of extractives, protosappanin B and brazilin, were found. Protosappanin B comprised about 40% of the extractives of pernambuco. The $\tan \delta$ of spruce wood impregnated with protosappanin B decreased markedly, the same as that of specimens impregnated with extractives before being purified. It is expected that protosappanin B can make a great contribution to decreasing the $\tan \delta$ during impregnation with extractives.

Acknowledgments The authors are grateful to Associate Professor Dr. T. Umezawa, Wood Research Institute, Kyoto University, for determining the molecular weights of the isolated compounds. This investigation was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (10660164).

References

1. Sugiyama M, Matsunaga M, Minato K, Norimoto M (1994) Physical and mechanical properties of pernambuco (*Guilandina echinata* Spreng) used for violin bows (in Japanese). *Mokuzai Gakkaishi* 40:905–910
2. Matsunaga M, Sugiyama M, Minato K, Norimoto M (1996) Physical and mechanical properties required for violin bow materials. *Holzforschung* 50:511–517
3. Ono T, Norimoto M (1983) Study on Young's modulus and internal friction of wood in relation to the evaluation of wood for musical instruments. *Jpn J Appl Phys* 22:611–614
4. Norimoto M, Tanaka F, Ohgama T, Ikimune T (1986) Specific dynamic Young's modulus and internal friction of wood in the longitudinal direction. *Wood Res Technol Notes* 22:53–65
5. Matsunaga M, Minato K (1998) Physical and mechanical properties required for violin bow materials. II. Comparison of the processing properties and durability between pernambuco and substitutable wood species. *J Wood Sci* 44:142–146
6. Matsunaga M, Minato K, Nakatsubo F (1999) Vibrational property changes of spruce wood by impregnation with water-soluble extractives of pernambuco (*Guilandina echinata* Spreng.). *J Wood Sci* 45:470–474
7. Yano H, Yamada T, Minato K (1986) Change in acoustical properties of sitka spruce due to reaction with formaldehyde. *Mokuzai Gakkaishi* 32:984–989
8. Akitsu H, Norimoto M, Morooka T (1991) Vibrational properties of chemically modified wood (in Japanese). *Mokuzai Gakkaishi* 37:590–597
9. Craig JC, Naik AR, Pratt R, Johnson E, Bhacca NS (1965) Nuclear magnetic resonance spectra and stereochemistry of the antibacterial principle from *Haematoxylon brazilletto*. *J Org Chem* 30:1573–1576
10. Gilbody AW, Perkin WH, Yates T (1901) Brazilin and haematoxylin. Part I. *J Chem Soc* 79:1396–1411
11. Perkin WH, Ray JN, Robinson R (1928) Experiments on the synthesis of brazilin and haematoxylin and their derivatives. Part III. *J Chem Soc* 1504–1513
12. Nagai M, Nagumo S (1986) Protosappanin B, a new dibenzoxocin derivative from sappan lignum. *Heterocycles* 24:601–605
13. Minato K, Sakai K, Matsunaga M, Nakatsubo F (1997) The vibrational properties of wood impregnated with extractives of some species of Leguminosae. *Mokuzai Gakkaishi* 43:1035–1037
14. Sakai K, Matsunaga M, Minato K, Nakatsubo F (1999) Effects of impregnation of simple phenolic and natural polycyclic compounds on physical properties of wood. *J Wood Sci* 45:227–232