Eurasian J. For. Res. 16-1: 63-66 , 2013

Raman and Infrared Spectroscopic Marker Bands for Rapid Detection of Cyanomaclurin

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Abstract

Methanol extracts from *Artocarpus heterophyllus*, which is a tropical polyphenol-rich tree species, were separated by column chromatography and six fractions were obtained as powder. Raman and infrared (IR) spectra of all the fractions were recorded, and it was found that cyanomaclurin, which was one of the fractions and a flavonoid available for biomedical research, showed two characteristic Raman and IR bands in the 750-700 cm^{-1} wavenumber region. These spectroscopic results show the high potential of these vibrational bands as a marker for rapid detection of cyanomaclurin.

Key words: *Artocarpus heterophyllus*, Cyanomaclurin, Flavonoid, IR spectroscopy, Raman spectroscopy

Introduction

Artocarpus heterophyllus is a species of tropical evergreen tree in the mulberry family (Moraceae), which is native to rainforests in India and Bangladesh. This tall tree is distributed over Southeast and Southern Asia, Africa, and South America. A botanical feature of *Artocarpus heterophyllus* is that it bears the largest tree-borne and edible fruit (Jack fruit), so this plant is widely grown as a fruit tree. *Artocarpus heterophyllus* has hard heartwood which shows good preservative performance and is thus frequently utilized for construction and furniture.

We have explored weathering mechanisms of various tropical wood species using infrared (IR) spectroscopic techniques (Sudiyani *et al*. 2003, Yamauchi *et al*. 2004) in order to improve wood properties. It was suggested that *Artocarpus heterophyllus* heartwood, given that yellow heartwood is a rich source of many kinds of polyphenols, shows high weathering resistance. In addition, we previously reported on the characteristic Raman bands of *Artocarpus heterophyllus* heartwood and concluded that the bands are attributable to flavonoids contained in the heartwood (Yamauchi *et al*. 2003).

The polyphenols isolated from *Artocarpus heterophyllus*, such as flavonoids, have been widely studied from biochemical and medical perspectives (Nomura *et al*. 1998, Wei *et al*. 2005, Arung *et al*. 2005, Arung *et al*. 2006, Fang *et al*. 2008, Zheng *et al*. 2008A, Zheng *et al*. 2008B, Zhu *et al*. 2009, Loizzo *et al*. 2010, Baliga *et al*. 2011). In recent years, it was confirmed that several of these polyphenols show strong tyrosinase inhibitor activity (Arung *et al*. 2005, Arung *et al*. 2006, Zheng *et al*. 2008A, Zheng *et al*. 2008B) and the extracts from *Artocarpus heterophyllus* play a role as antibrowning agents (Zheng *et al*. 2008A). Moreover, Zhu *et al*. (2009) provided evidence that polyphenols such as flavan-3-ols, theaflavins, cyanomaclurin, and dihydrochalcones effectively trap acrolein and 4-hydroxy-trans-2-nonenal, lipid-derived unsaturated aldehydes, which have been implicated as contributors to carbonyl stress.

Our aim here is to find Raman or IR spectral features available for identification of valuable compounds contained in extracts from *Artocarpus heterophyllus*. Our finding in this study that cyanomaclurin, which is a useful flavonoid in biomedical research, shows two characteristic vibrational bands in the 750-700 cm^{-1} region provides a rapid procedure for its detection.

Materials and Methods

Sample preparation

Heartwood of *Artocarpus heterophyllus* planted in Indonesia was used in this study. The heartwood was ground in a cutting-mill and then the powder that passed through a 1-mm-mesh sieve was utilized as a methanol extraction sample. The heartwood powder was sequentially extracted using organic solvents in the order of *n*-hexane, ethyl acetate, and methanol. The amount (v/w) of each solvent was ten times greater than that of the wood powder. The powder was extracted using each solvent twice and each extraction time was 24 h. The extraction solvent was removed using a rotary evaporator and then the residues were solidified by a freeze-drying technique.

The methanol extracts were passed through a Sephadex LH-20 column (ϕ 30 × 430 mm) as an ethanol solution and the effluent was obtained using a fraction collector. The effluent was then subjected to silica gel thin-layer chromatography (Silica gel 60 F_{254}, 1.05554, Merck Co., Darmstadt, Germany) with a benzene/acetone (1/1) solvent. The methanol extracts

(Received; Oct. 5, 2012: Accepted; Dec. 11, 2012)
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were separated into six fractions named from a to f according to the Rf value. Each fraction was solidified by freeze-drying after removal of the developing solvent. Fraction b was further purified by ODS column chromatography (Lobar RP-18, ϕ 25 × 310 mm, Merck co. Darmstadt, Germany) with acetonitrile/water (10/90), (15/85), and (20/80) solvents.

Instruments

Fourier transform (FT)-Raman spectra in the 4000-400 cm^{-1} region of the fractions in the methanol extracts were recorded using a JEOL JIR7000W spectrometer connected to an RS-RSU-200 Raman module. The Raman spectra were excited with radiation of 1064.1 nm from a Nd^{3+}:YAG laser and the excitation power was about 200 mW. All the FT-Raman spectra were obtained in back-scattering geometry. FT-IR spectra in the 4000-400 cm^{-1} region of the fractions diluted in KBr powder were obtained using the same spectrometer by a diffuse reflectance technique. All the Raman and IR measurements were performed at room temperature with 4 cm^{-1} spectral resolution. ^{13}C-NMR spectra were recorded using CD_3COCD_3 solutions on a JEOL Lambda 400 instrument at 100 MHz.

Results and Discussion

Raman and IR spectra

Figure 1 depicts the FT-Raman spectra in the 1800-600 cm^{-1} region of the six fractions from the methanol extracts. All the spectra show a strong broad band around 1610 cm^{-1} attributable to a skeletal in-plane vibration (ν_8) of a benzene molecule. This broadening suggests that all the fractions contain no less than two kinds of substituted benzene rings. For the Raman spectra of fractions b, c, and d, several medium-intensity bands assignable to C-C-O or C-O-C stretching vibrations are observed in the wavenumber range from 1050 to 950 cm^{-1}.

We previously reported characteristic Raman bands at 1247 and 745 cm^{-1} of *Artocarpus heterophyllus* heartwood and its methanol extracts (Yamauchi *et al*. 2003). Here, the Raman band at 1247 cm^{-1} is clearly observed only in the spectrum of fraction a, while all the spectra show a sharp line at 745 cm^{-1}. It is interesting to note that a significant Raman band can be seen at about 720 cm^{-1} only in the spectrum of fraction b.

The FT-IR spectra in the 1800-600 cm^{-1} range of the six fractions are shown in Fig. 2. The strong broad lines due to the skeletal in-plane vibration (ν_8) of the benzene ring also appear around 1610 cm^{-1} in all the IR spectra as well as the Raman spectra. As for the IR spectra of fractions a, c, and d, a few peaks attributable to C=O stretching vibrations are observed from 1700 cm^{-1} to 1640 cm^{-1}. Additionally, the IR spectrum of fraction b shows strong absorption at about 1004 cm^{-1}.

Although an IR band is observed at about 1240 cm^{-1} for fraction a, the peak intensity is not as prominent as the Raman band at 1247 cm^{-1}. The IR spectra of fractions b, c, d, and f only have a shoulder at the same wavenumber. The IR band corresponding to the Raman band at 745 cm^{-1} can be seen at 740 cm^{-1} as a medium-intensity absorption peak in the spectrum of fraction b and the spectra of fractions a, c, e, and f also show a weak band at the same position. Furthermore, it should be noted that the IR spectrum of fraction b possesses an absorption band at about 717 cm^{-1} equivalent to the Raman band at 720 cm^{-1}. The absorption intensity at 717 cm^{-1} is comparable to that at 740 cm^{-1}, although it is not strong.

Hereafter, the Raman bands at 745 and 720 cm^{-1} (IR bands at 740 and 717 cm^{-1}) are referred to as bands A and B, respectively. These results from the Raman and IR measurements indicate that this set of vibrational bands, A and B, can be used as a spectroscopic marker of fraction b.

Identification of purified fraction b

There were no significant differences in ^{13}C-NMR spectrum between purified and unpurified fraction b. ^{13}C-NMR chemical shifts of purified fraction b is as follows (see Fig. 3).

^{13}C-NMR chemical shifts: δ 63.7 (C8), 66.4(C9), 72.6(C7), 95.2(C1), 96.1(C3), 100.3(C12), 103.5(C14), 109.2(C16), 114.0(C5), 132.9(C17), 155.6(C13), 155.7(C6), 159.4(C15), 160.1(C4), 160.3(C2).

Comparison with the NMR data from earlier works (Lin *et al*. 1995, Zheng *et al*. 2008A) lead us to the conclusion that purified fraction b is cyanomaclurin. Therefore, unpurified fraction b is most likely to be crude cyanomaclurin. Cyanomaclurin is a flavonoid that was isolated from the Jack fruit tree by Perkin and Cope (1895) and its molecular structure was determined from NMR investigations (Nair *et al.* 1966). Moreover, it has been shown recently that cyanomaclurin acts as a direct trapping agent of cytotoxic lipid-derived unsaturated aldehyde (Zhu *et al*. 2009).

Assignment of vibrational bands A and B

As mentioned above, a Raman or IR spectroscopic feature of fraction b, crude cyanomaclurin, is that the vibrational bands A and B appear clearly. As shown in Fig. 3, the cyanomaclurin molecule contains two polysubstituted benzene rings. Thus, the molecular structure allows us to presume that vibrational bands A and B are due to a vibrational mode induced by the coupling between the skeletal in-plane vibration of the benzene ring (ν_1, ν_6, or ν_{12}) and substituted group-benzene stretching vibrations (Hamaguchi and Hirakawa 1988).

Conclusion

Two Raman and IR bands A and B in the 750-700 cm^{-1} region were observed for cyanomaclurin, which is a medically important compound. We propose that the set of vibrational bands A and B can be used as a marker for rapid screening of cyanomaclurin because only cyanomaclurin among the methanol extracts from *Artocarpus heterophyllus* heartwood exhibits both A and B bands. Raman or IR measurements will provide valuable information about the content of cyanomaclurin in extracts from tropical vegetation.

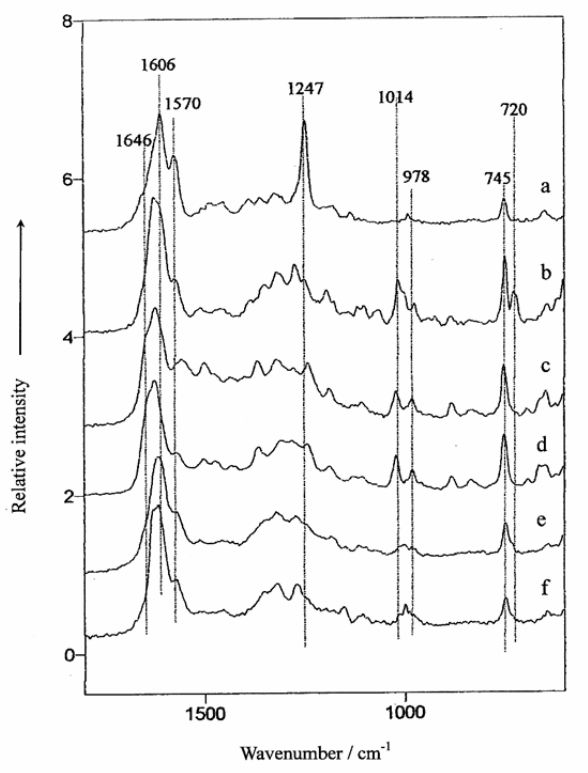

Fig. 1. FT-Raman spectra of six fractions in the methanol extracts from *Artocarpus heterophyllus* heartwood.
a, fraction a; *b*, fraction b; *c*, fraction c; *d*, fraction d; *e*, fraction e; *f*, fraction f

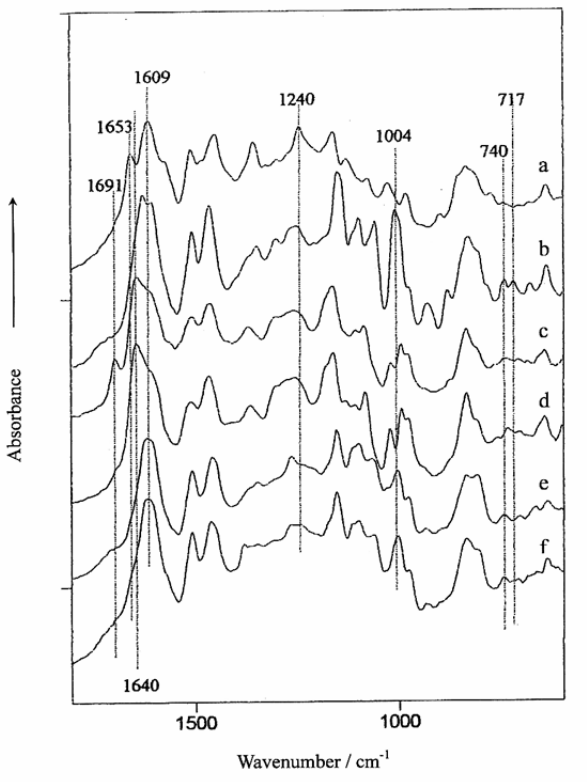

Fig. 2. FT-IR spectra of six fractions in the methanol extracts from *Artocarpus heterophyllus* heartwood.
a, fraction a; *b*, fraction b; *c*, fraction c; *d*, fraction d; *e*, fraction e; *f*, fraction f

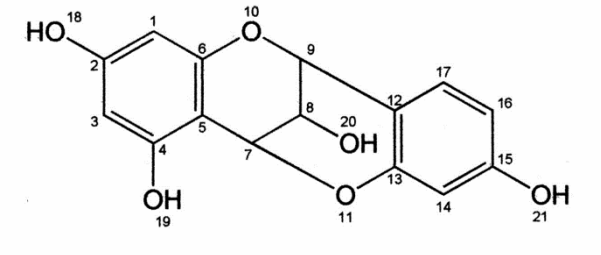

Fig. 3. Chemical structure of cyanomaclurin.

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