Ionic Liquid-Assisted Extraction of Essential Oils from Thujopsis dolobrata (Hiba)

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ABSTRACT: Aomori hiba (Thujopsis dolobrata) has been used as a building material for centuries because of the wood’s resistance against fungi and termites. In addition, the essential oil of hiba wood has a relaxing woody fragrance and antifungal properties and is composed mainly of (−)-thujopsene, cedrol, and hinokitiol. Steam distillation is the main industrial method for obtaining the oil but requires large amounts of water and heating energy. The present study describes the use of a cellulose-dissolving [C2mim][MeO(H)PO2] ionic liquid (IL) for efficient extraction of hiba oil. As a control method, organic solvent extraction using either hexane, Et2O, or EtOAc was conducted at 80 °C for 1 h. The method developed in this study using an IL was conducted at the same temperature and time as the control method and relied on partial dissolution of the wood using the IL before partitioning the hiba oil into the organic solvent for analysis. Quantitative analysis was performed using gas chromatography−mass spectrometry in selected ion-monitoring mode. The results showed that extraction using the [C2mim][MeO(H)PO2] IL/hexane biphasic system improved the yields of (−)-thujopsene, cedrol 1.2- and 2.3-fold, when compared with the control method. Furthermore, extraction at a lower temperature (30 °C) did not significantly change the yield. The IL could be recovered with high purity, while the cellulose-rich material and lignin were regenerated. This study demonstrates the ability to effectively extract wood essential oil more quickly and under milder conditions than the conventional method, with subsequent separation and regeneration of wood biopolymers using an IL.

1. INTRODUCTION

Hiba (Thujopsis dolobrata), or Aomori Asunaro, is a cypress (Cupressaceae) endemic to Japan. It has been used as a building material for centuries because of its resistance to fungi and termites. Recently, hiba wood has gained popularity as a natural room deodorant and antifungal material. The wood oil has a characteristic sweet and slightly smoky fragrance and is used in aromatherapy for its insect-repellant, antibacterial, antifungal, and relaxing properties. As with other wood essential oils, hiba tree essential oil is obtained commercially by distillation, which requires large amounts of water and heating energy. The main constituents in hiba oil are (−)-thujopsene, cedrol, and tropolones such as hinokitiol, as shown in Figure 1.1,2,3

Wood provides structural support to trees and is a sustainable source of biopolymers. In general, woody tissue contains a greater cellulose content than other tissues. As a comparison, woody tissues contain 40−50% cellulose,4 while citrus peels contain approximately 13%.5 Wood is also rich in lignin, which provides rigidity, support, and protection against pathogens.

Ionic liquids (ILs) are substances comprised solely of ions and are liquid at temperatures below 100 °C. Because of their low volatility, low flammability, and reusability, ILs are sometimes considered environmentally friendly (“green”) solvents. The anion and cation pairs can also be designed to adjust the polarity, viscosity, and electrochemical conductivity and even to reduce the toxicity of the IL. The ionic nature of ILs results in their ability to accept hydrogen bonds that bind biopolymers such as cellulose, lignin, and chitin together. As a result, ILs can dissolve these biopolymers that generally are

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Figure 1. Main constituents of hiba essential oil.
considered waste products.\textsuperscript{5–8} Dissolution of these materials enables more time-efficient and milder methods of biorefinery into valuable and sustainable materials. For comparison, the conventional method for dissolving cellulose is the viscose process, which requires large amounts of carbon disulfide, sodium hydroxide, sulfuric acid, and fresh water. In addition, this process generates large quantities of waste water, alkali, and acidic pollution.\textsuperscript{9} For lignin extraction (delignification), the Kraft method is the most commonly implemented but has several disadvantages including generation of pollution, odor, and waste water.\textsuperscript{9,10}

The IL 1-ethyl-3-methylimidazolium methylphosphonate $[\text{C}_2\text{mim}]\[(\text{MeO})(\text{H})\text{PO}_2\]$ possesses excellent cellulose solvating properties under mild conditions\textsuperscript{10} and increases the essential oil extraction yield from leaves and flowers.\textsuperscript{11,12} To develop a more environmentally benign method for wood essential oil extraction with subsequent recovery of the IL and wood biopolymers, $[\text{C}_2\text{mim}]\[(\text{MeO})(\text{H})\text{PO}_2\]$ was used to extract hiba oil. Since this is the first study to employ $[\text{C}_2\text{mim}]\[(\text{MeO})(\text{H})\text{PO}_2\]$ for wood dissolution, the dissolution capacity of this IL was investigated.

2. EXPERIMENTAL SECTION

2.1. General. All reagents were obtained from commercial suppliers and were used without further purification unless mentioned otherwise. The $[\text{C}_2\text{mim}]\[(\text{MeO})(\text{H})\text{PO}_2\]$, (R)-(+)-limonene, and cedrol were purchased from Kanto Chemical Co. Ltd. (Tokyo, Japan). Hinokitiol was obtained from TCI Co. Ltd. (Tokyo, Japan). Aomori hiba wood powder was purchased from Cul de Sac (Tokyo, Japan) and was stored at $-24\,^\circ\text{C}$ prior to use. The particle size in the wood powder ranged from 50 $\mu$m to 2 mm. Gas chromatography–mass spectrometry (GC–MS) analysis was conducted using Shimadzu GCMS-QP2010 SE instruments with an Agilent DC-WAX capillary column (30 m length, 0.25 mm internal diameter, and 0.25 $\mu$m film thickness). Infrared (IR) spectra were recorded on a JASCO FT-IR 4100 spectrometer and plotted by wavenumber (cm$^{-1}$). $^1\text{H}$ NMR spectra were obtained on a JEOL JNM-ECA 500 spectrometer.

2.2. Organic Solvent Extraction. Organic solvent extraction was conducted for the control method (Scheme 1) using 0.3 g of hiba wood powder that was placed into a 15 mL centrifugation tube. Then, 8 mL of organic solvent was added, the cap was sealed with parafilm, and extraction was conducted at 80 $^\circ\text{C}$ and 1200 rpm for 1 h. After removing the stirring chip, the mixture was centrifuged for 5 min and the supernatant was collected for analysis. Each extraction was performed twice.

2.3. IL-Assisted Extraction. As shown in Scheme 2, extraction using $[\text{C}_2\text{mim}]\[(\text{MeO})(\text{H})\text{PO}_2\]$ IL was performed as follows: 0.3 g of hiba wood powder was placed in a 30 mL round-bottom flask and 4 mL of IL was added. After closing the mouth of the flask with a septum and parafilm, extraction of the hiba oil and partial dissolution of the wood was conducted at 30 or 80 $^\circ\text{C}$ and 1200 rpm for 40 min. After cooling to room temperature, 6 mL of organic solvent was added and extraction of the oil was allowed to continue for 20 min at 1200 rpm. This mixture then was transferred into 15 mL centrifugation tubes and centrifuged for 5 min. After collecting the organic layer, the IL/wood layer was washed twice with 1.5 mL of organic solvent. The combined organic layers were used for analysis. Each extraction was performed in triplicate.

2.4. GC–MS Quantification. The GC–MS analysis was performed as follows: the oven temperature was held at 50 $^\circ\text{C}$ for 2 min, then raised at a rate of 10 $^\circ\text{C}$/min until it reached 230 $^\circ\text{C}$, and held at this temperature for another 10 min. The conditions were as follows: carrier gas: helium; flow rate: 1.7 mL/min; pressure: 100 kPa; injector temperature: 230 $^\circ\text{C}$; splitting ratio: 26:1; interface temperature: 240 $^\circ\text{C}$; EI-MS source temperature: 230 $^\circ\text{C}$; solvent: hexane, Et$_2$O, or EtOAc; injection volume: 1.0 $\mu$L.

Calibration curves were obtained as follows: standard solutions of (−)-thujopsene and cedrol were prepared. A (−)-limonene internal standard (IS) was added to each solution of IS ($X_i$), (−)-thujopsene ($X_t$), and cedrol ($X_c$). Analysis of the injected solution gave the peak areas for IS ($Y_i$), (−)-thujopsene ($Y_t$), and cedrol ($Y_c$). These values were used to determine the ratio of the analyte and IS concentration $x = X_i/\sum X_i$, and the ratio of analyte and IS peak areas $y = Y_i/\sum Y_i$. The calibration equations are

\begin{align*}
\text{Cedrol (hexane): } & y = 0.6906x - 0.0125 \\
& (R^2 = 0.9745) \\
\text{Cedrol (Et}_2\text{O)}: & y = 0.7084x - 0.0125 \\
& (R^2 = 0.9922) \\
\text{Cedrol (EtOAc): } & y = 0.7301x - 0.0138 \\
& (R^2 = 0.9992)
\end{align*}

A (−)-thujopsene standard is commercially unavailable, so the calibration curves for cedrol were also used to calculate the amount of cedrol in the extract. Quantitative analysis was
conducted in selected ion-monitoring mode. A fixed concentration of the IS was added to the analyte. Injection of this solution gave peak areas $Y_1$, $Y_2$, and $Y_3$, to allow calculation of $y$. Using this value in the calibration equations gave $x$, from which the concentrations of $(-)$-thujopsene ($X_1$) and cedrol ($X_2$) could be determined. The amount of analyte (mg) in the extract solution could be determined using 8 mL of total extract solution and an aliquot of 500 μL before diluting twice using an IS solution, with an injection amount of 1.0 μL. The wt % extraction yield then was calculated as the percentage ratio of analyte in the extract (mg) over the original amount of wood powder (mg). The IS solution was prepared by placing 3 μL of limonene inside a vial. The accurate weight of the limonene was used to measure the IS concentration. A typical chromatogram of hiba oil extract spiked with limonene is shown in Figure S1.

2.5. Biopolymer and IL Recovery. The procedure for regeneration of the biopolymers and recovery of IL was based on that reported by Rogers and co-workers. Briefly, the IL/wood mixture was collected after hiba oil extraction. Any dissolved cellulosic material was precipitated by addition of 1:1 (v/v) acetone/water followed by stirring at room temperature for 1 h. The supernatant containing lignin was collected. The cellulosic-rich residue was washed with water and vacuum-filtered. The filtered solids containing cellulose were thoroughly dried at 60 °C for at least 4 h. The filtrate, containing the remaining lignin, was combined with the previous supernatant, and after evaporating the acetone by stirring at room temperature overnight, the precipitated lignin was vacuum-filtered and dried at 60 °C for 4 h. Meanwhile, as much water as possible was removed from the IL/water filtrate by evaporation at reduced pressure, to recover the IL. The cellulosic-rich material and lignin were analyzed by FT-IR spectroscopy, while the purity of IL was determined using $^1$H NMR spectroscopy.

2.6. Wood Dissolution Capacity Measurement. The measurement of wood dissolution capacity was based loosely on the method of Argyropoulos and co-workers. Wood powder (0.01 g) was placed inside a 20 mL round-bottom flask, and 1 g of IL was added. The mixture was stirred at 80 °C until the wood was dissolved. When the wood powder was fully solubilized in the IL (as indicated by the disappearance of the fibrous appearance of the wood), more wood powder was added in 1% (w/w) increments. When no more wood could be dissolved, the remaining powder was recovered by vacuum filtration and dried at 60 °C for 6 h. The total weight of powder that could not be dissolved was measured, and the wood dissolution capacity was reported as the total amount of wood dissolved in the IL (wt %).

3. RESULTS AND DISCUSSION

3.1. Comparison of $(-)$-Thujopsene and Cedrol Yields. The results of the IL-assisted extraction are summarized in Table 1, where the yields are compared with the control. A schematic depiction of the results is shown in Figure 2. Using the current IL method, IL/hexane gave the greatest yields for both $(-)$-thujopsene and cedrol, 2.3-fold and 1.2-fold greater, respectively, than when using organic solvent extraction. Extraction at 30 °C was similar to that at 80 °C, even showing a slightly improved yield of thujopsene.

The greatest yields of $(-)$-thujopsene occurred when IL/hexane and hexane alone were used for extraction, perhaps due to better hexane solubility compared to that in the other organic solvents. For cedrol, control experiments suggested that EtOAc would have better solvating ability and should give a better extraction yield. However, when IL was used, the yield of cedrol on average was slightly greater in IL/hexane than in IL/EtOAc. One reason for this may be that the IL promotes dissolution of the slightly polar cedrol. For hiba wood, IL/hexane promoted both dissolution of the lignocellulosic biomass and separation of the desired cedrol and the lignocellulosic material.

3.2. Recovered Biopolymers and IL. The FT-IR spectra of regenerated lignin and cellulose-rich material are shown in Figure S2. Characteristic bands for lignin indicating aromatic breathing at 1500–1700 cm$^{-1}$ were detected, and the alcohol band was confirmed for the cellulose-rich material. For both, a strong band was found at 2365 cm$^{-1}$ corresponding to CO$_2$ stretching, which appeared even though blank measurements were conducted to remove background noise.

To determine the purity of the IL after being used for extraction, the $^1$H NMR spectrum after extraction was compared with that of fresh IL (Figure S3). The spectrum of the recovered IL contained no impurity peaks. However, some of the peaks in the spectrum of the IL after extraction were shifted relative to those for the fresh IL, which may be because of inadequate dilution using the deuterated solvent for recording the fresh IL spectrum. In addition, even after 4 h of water evaporation, a significant amount of water remained in the recovered IL. This may reduce the ability of the IL to dissolve cellulose and lignin because of competing hydrogen bonding between the IL and the water or the lignocellulosic hydroxyl groups, although previous work found that the extraction yield of the target compound did not decrease significantly, even after 8 runs.

3.3. Wood Dissolvability Using C$_2$mim][(MeO)(H)-PO$_3$]. The efficacy of the C$_2$mim][(MeO)(H)PO$_3$] IL for wood dissolution was investigated. Dissolution of 0.01 g of hiba wood powder in 1 g of IL at 80 °C was monitored for 3 days (Figure 3). A slight amount of the wood appeared to dissolve at 6 h. However, the appearance of the mixture did not change, even after stirring for 3 d, after which the weight of the undissolved wood was measured. Only about 20% of the wood was dissolved in 1 g of IL, indicating a dissolution capacity for C$_2$mim][(MeO)(H)PO$_3$] of 0.2% at 80 °C. Increasing the treatment temperature or using microwave/ultrasonic radiation may increase the solubility of the wood.

4. CONCLUSIONS

Wood dissolution and wood essential oil extraction using the C$_2$mim][(MeO)(H)PO$_3$] IL was reported for the first time. The wood used was one of the three Japanese precious woods, hiba (T. dolabrata), which contains an essential oil with a characteristic woody fragrance and antifungal properties. As

| Table 1. Extraction Yields for $(-)$-Thujopsene and Cedrol |
|-----------------------------------------------------------|
| **average yield (% w/w)**                               |
| **extraction solvent** | $(-)$-thujopsene | cedrol |
| hexane                  | 0.65097 ± 0.01477 | 0.03139 ± 0.00090 |
| Et$_2$O                 | 0.56744 ± 0.05925 | 0.03018 ± 0.00115 |
| EtOAc                  | 0.35656 ± 0.03004 | 0.05131 ± 0.00342 |
| IL/hexane              | 0.74909 ± 0.11338 | 0.11861 ± 0.01488 |
| IL/Et$_2$O            | 0.60524 ± 0.03409 | 0.10954 ± 0.00462 |
| IL/EtOAc             | 0.57640 ± 0.03544 | 0.10962 ± 0.00372 |
| IL/hexane (30 °C)       | 0.78742 ± 0.05241 | 0.10033 ± 0.01004 |
target compounds for quantitative analysis, (−)-thujopsene and cedrol were chosen. As controls, organic solvent extractions using hexane, EtOAc, and Et2O were performed. The results showed that hexane extraction produced the greatest (−)-thujopsene yield, while EtOAc was best for cedrol extraction among the control methods. The IL-assisted extraction method relies first on partial wood dissolution in the IL, then partitioning of the oil into an organic solvent, and finally separation by centrifugation. This study revealed that IL/hexane gave the best yields of (−)-thujopsene and cedrol, with 1.2- and 2.3-fold increases, respectively, when compared with the organic solvent extraction controls. Extraction at room temperature gave similar or improved yields.

Wood dissolution using the [C2mim][MeO(H)PO2] IL was investigated and resulted in dissolution of 0.2% of the wood in the IL at 6 h. Furthermore, lignocellulosic biopolymers recovered by the IL extraction procedure were analyzed by FT-IR spectroscopy. To address possible recycling, the recovered IL was also examined by 1H NMR spectroscopy and the results were compared to those for fresh IL.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04860.

GC−MS chromatograph; FT-IR spectra; and 1H NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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