

Article

Characterization of an Oligomeric Lignin-Derived UV Absorber Produced from Hardwood Beech

Chen Qu,* Keigo Mikame, Yasunori Ohashi, Hiroshi Nishimura, Kenzo Koike, and Takashi Watanabe*



III Metrics & More

ABSTRACT: The damage caused by ultraviolet (UV) radiation to human skin and various materials, such as solar panels, has been extensively documented. Lignin-derived polymers have demonstrated excellent UV-protective properties; however, the uncertainty of their chemical structures and properties has hindered their further utilization. In this study, hardwood beech was degraded by CuO with the assistance of microwave heating. Several oligomeric lignin fractions were obtained from the degradation products by sequential extraction of the precipitates using diethyl ether, ethyl acetate, and acetone. The ethyl acetatesoluble lignin fraction and acetone-soluble lignin fraction were characterized by UV spectroscopy, heteronuclear single quantum coherence nuclear magnetic resonance (HSQC NMR), and gel permeation chromatography (GPC). The ethyl acetate-soluble lignin fractions exhibited UV absorbance comparable to that



Article Recommendations

of technical lignins. HSQC NMR quantitative analysis results demonstrated an increase of conjugated structures involving aldehyde carboxylic acid, and α -ketone groups, and α,β -conjugated cinnamic acid structure end groups in the acetone-soluble fraction (side chain region: 19.4%; aromatic region: 16.8%) and the ethyl acetate-soluble fraction (side chain region: 28.1%; aromatic region: 22.0%), which may be attributed to the increase of UV absorbance. The average molecular weights of the ethyl acetate- and acetone-soluble lignin fractions were 940 and 1200, respectively. These results highlighted the potential of the oligomeric lignin-derived fractions obtained in this study as ideal natural UV absorbers. Moreover, the characterization method described herein may serve as a standardized protocol for evaluating UV-protective compounds derived from various biomasses through different processing techniques.

1. INTRODUCTION

Ultraviolet (UV) spectrum is categorized into UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (100-280 nm) based on the wavelength. The overexposure of human skin to UV radiation, particularly UV-A and UV-B, poses risks, such as premature aging and even skin cancer. Additionally, materials like food packaging and solar panels exposed to UV radiation from the sun may experience color changes (yellowing) and degradation.^{1,2} Synthetic UV absorbers are effective; however, their photoinstability is a significant drawback.³ Lignin, one of the main components of wood, is a heterogeneous biopolymer that naturally protects plants from UV damage due to its chemical structure, which includes guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) units that inherently absorb UV radiation. Various studies have explored modifying lignin into a natural UV protection ingredient.^{4,5} Qian et al. demonstrated that UV-absorbing performance could be enhanced by blending alkali lignin and Kraft lignin with commercial creams.^{6,7} Zhang et al. further reported that sunscreen ingredients could be developed from acetylated Kraft lignin via solvent fractionation.8 However, Kraft lignin, recovered from black liquor produced by the NaOH and Na₂SO₃ pulping process, poses risks due to the presence of sulfite. The poorly

understood nature of its chemical structure has also hindered its future use, especially in cosmetics. In addition to the chemical structure, the molecular weight of lignin significantly impacts its application as a UV absorber.⁹ Studies suggest that oligomeric lignin-like compounds with molecular weights ranging from 1790 to 2230 Da enhance UV shielding performance.¹⁰ Moreover, higher molecular weights may improve stability and reduce risk of penetration into human skin.

In 2021, Mikame et al. developed a method to produce a series of lignin-derived monomeric and dimeric compounds through cupric oxide (CuO) oxidation of wood under alkaline conditions with microwave heating from the soluble fraction. The resulting lignin-derived monomeric and dimeric compounds exhibited excellent UV-A and UV-B absorbing

Received:January 22, 2025Revised:May 11, 2025Accepted:May 20, 2025Published:May 30, 2025







Figure 1. Process flow for wood decomposition and sequential solvent extraction.

performance.¹¹ Additionally, their detailed structures were confirmed through NMR and mass spectrometry analyses, marking the first reported data to define the chemical structures of natural lignin-derived UV-absorbing compounds. The formation mechanism was also proposed in the study. The soluble fraction produced by this method from wood was a mixture of monomeric, dimeric, and oligomeric lignins. The oligomeric lignin was not utilized in Mikame's study, which raises two key questions. First, can the oligomeric lignin fractions maintain the same UV absorbance performance as lignin monomeric and dimeric compounds? Second, what are their chemical structures? In this study, oligomeric lignin fractions were obtained from the soluble lignin fraction, followed by acidification to induce precipitation. The resulting precipitate was then subjected to sequential solvent extraction with diethyl ether, ethyl acetate, and acetone.¹¹ The UV-A absorbance performance of the obtained diethyl ether-soluble lignin fraction, ethyl acetate-soluble lignin fraction, and acetone-soluble lignin fraction was lower than that of the monomeric and dimeric compounds isolated from the soluble fraction. These oligomeric lignin fractions showed competitive UV absorbance with Kraft lignin. The chemical structures responsible for UV-A and UV-B absorbance in the oligomeric lignin fractions were quantitatively and qualitatively characterized by HSQC NMR analysis. The molecular weight distribution was confirmed by GPC analysis. The lignin fractions obtained from ethyl acetate extraction in this study exhibited significant potential as safe UV-absorbing ingredients for cosmetics and various polymeric materials. Notably, the characterization method reported herein may also provide a

reliable protocol for evaluating various UV-protective ingredients obtained through different methods from biomass.

2. MATERIALS AND METHODS

2.1. Preparation of the Wood Samples. Beech wood (*Fagus crenata* Blume) powders were ground by using a microfine grinding mill equipped with a cutting grinding head (MF-10, IKA Works, USA) and then fractionated with sieves. The fractionated plant cell walls (~0.35 mm) were extracted with acetone for 6 h and dried under vacuum over P_2O_5 . Copper(II) oxide (99.9%) was purchased from Fujifilm Wako, Japan. All other chemicals were also obtained from Fujifilm Wako Chemical Industries, Osaka, Japan.

2.2. Microwave-Assisted Wood Decomposition and Sequential Solvent Fractionation of the Acid-Insoluble Fraction. The prepared wood samples were degraded by CuO under alkaline conditions with microwave assistance at 180 °C for 30 min, following a reported method.¹¹ In a cap-sealed vial, wood powder (1 g), 1 M/L sodium hydroxide solution (20 mL), and copper oxide (0.5 g) were mixed heterogeneously and heated by microwave (Biotage Initiator 60 Exp, Biotage, Japan), which was equipped with a magnetic stirring system and an air-cooled condenser, at 180 $^\circ C$ for 30 min at 2.45 MHz. The temperature was controlled by automatically adjusting the microwave power. The soluble fraction was acidified with hydrochloric acid (HCl), and the pH was adjusted to 1.5. Precipitates formed during acidification are termed the acid-insoluble (ACIS) fraction in this study. The yield was approximately 9.6% of the whole wood. ACIS lignin (100 mg) was sequentially extracted with diethyl ether (5 mL),



Figure 2. UV absorbance data for the following samples were analyzed: (A) acetylated ACIS, (B) ACIS, (C) ACIS acetone-soluble fraction, (D) ACIS ethyl acetate-soluble fraction, and (E) ACIS diethyl ether-soluble fraction. The acetylated samples were measured at a concentration of 300 mg/L in acetonitrile, while the nonacetylated samples were measured at a concentration of 25 mg/L in 2-methoxyethanol.

ethyl acetate (5 mL), and acetone (5 mL), each solvent used three times for 2 h per extraction. The diethyl ether-soluble fraction, ethyl acetate-soluble fraction, and acetone-soluble fraction were collected, and the solvents were evaporated by an evaporator. The ethyl acetate- and acetone-soluble fractions were further washed with diethyl ether three additional times each to remove low molecular weight fractions. The extracted lignin samples were named the ACIS-diethyl ether-soluble fraction, the ACIS-ethyl acetate-soluble fraction, and the ACISacetone-soluble fraction, respectively (Figure 1). The yields of each fraction, based on the original ACIS, were ACIS-beechether-soluble fraction: 14.2%, ACIS-beech-ethyl acetate fraction: 37.9%, ACIS-beech-acetone: 22.5%, and residue: 18.2%, respectively.

2.3. UV Absorbance Analysis. UV absorbance spectra were recorded using a HITACHI U-3310 spectrophotometer with 1 cm path-length quartz cells. The ACIS sample was acetylated following the literature method and then measured at wavelengths ranging from 200 to 600 nm in acetonitrile at a concentration of 300 mg/L.¹² In contrast, the UV absorbance of the non-acetylated ACIS, along with the ACIS-ether, ACIS-ethyl acetate, and ACIS-acetone fractions, was measured over the same wavelength range (200–600 nm) in 2-methoxyethanol at a concentration of 25 mg/L.

2.4. NMR Analysis. NMR spectra were acquired at 313 K by using a Bruker AVANCE 600 MHz spectrometer (Bruker BioSpin, Germany) equipped with a cryogenic probe. HSQC experiments were performed using the Bruker hsqcetgpsisp2.2 standard pulse program with spectral widths of 5000 Hz for the ¹H dimension and 20 843 Hz for the ¹³C dimension. The ¹H dimension included 2048 complex points, with a recycle delay

of 2.0 s, and 12 transients were collected. For the ¹³C dimension, 256 increments were recorded. The coupling constant ${}^{1}J_{\rm CH}$ was set to 145 Hz. Data processing was conducted by using Bruker Topspin 4.0.8 software. For analysis, acetylated ACIS samples (30 mg) were dissolved in 0.5 mL of CDCl₃, while the ACIS-acetone and ACIS-ethyl acetate fractions (10 mg each) were dissolved in 0.5 mL of DMSO- d_6 . Chemical shifts were referenced to the respective NMR solvent peak (DMSO: $\delta C/\delta H$ 39.6/2.49; CHCl₃: $\delta C/\delta H$ 77.0/7.27 ppm). The signal assignments were performed by comparison with previously reported data, and NMR quantifications were determined based on peak intensities.¹³⁻¹⁷

2.5. GPC Analysis. Molecular weight analysis was performed using GPC on a Shimadzu liquid chromatography system equipped with a UV detector (SPD-M20A, Prominence diode array detector) at 40 °C. Tetrahydrofuran (THF) was used as the eluent at a flow rate of 0.8 mL/min. Sample solutions were prepared at a concentration of 1 mg/mL, filtered through a 0.45- μ m PTFE syringe filter, and 10 μ L of the filtered solution was injected into Shodex GPC columns (KF804 + KF802, 30 cm \times 8 mm) with a guard column. Molecular weight calibration was performed using guaiacol (124 Da), syringaldehyde (182 Da) (both from TCI, Japan), and polystyrene standards (Sigma-Aldrich, Japan) with molecular weights of 500, 2430, 4000, 13 700, and 216 000 Da. Sample molecular weights were calculated based on absorbance at 280 nm. Number-average molecular weight (M_n) , weight-average molecular weight (M_w) , polydispersity $(M_{\rm w}/M_{\rm p})$, and peak molecular weight $(M_{\rm p})$ were determined using the LC Solution software (Shimadzu).

2.6. Gas Chromatography–Mass Spectrometry (GC-MS). GC-MS analysis was performed using a Shimadzu GCMS-QP5050A system (Shimadzu Co., Ltd., Kyoto, Japan) with an ionization voltage of 70 eV. The instrument featured an electron multiplier and a transfer line set to 280 °C, utilizing a DB-5MS column (30 m × 0.25 mm i.d., 0.25- μ m film thickness; J&W Scientific). The temperature program was as follows: the initial temperature was set at 50 °C and held for 3 min, then ramped at 8 °C/min to 300 °C and held for 20 min. Additional parameters included an injection temperature of 250 °C, a detector temperature of 300 °C, helium as the carrier gas at a flow rate of 1.6 mL/min, an injection volume of 1 μ L, a split ratio of 1:10, and a mass range of m/z 40–600. Tetracosane (C₂₄) at a concentration of 1 mg/mL was used as the internal standard.

3. RESULTS AND DISCUSSION

3.1. Microwave-Assisted Copper Oxide Wood Degradation and Sequential Solvent Extraction for the UV-Absorbing Ingredients. Hardwood beech powder was decomposed by CuO under alkaline conditions with the assistance of microwave heating at 180 $^\circ \mathrm{C}$ for 30 min. 11 The soluble fraction was then acidified to form a precipitate, which was separated into an acid-soluble (ACSL) fraction and an acid-insoluble (ACIS) fraction. It is regarded that the ACSL fraction basically consists of monomers and dimers of phenolic derivatives. ACIS is a mixture of lignin monomers, dimers, and oligomer compounds, which are secondary products formed during wood degradation. The ACIS yield was approximately 10% of the original wood. A higher reaction temperature was also tested for wood degradation to obtain UV absorbers. However, excessive decomposition led to the conversion of lignin into mainly lower molecular weight compounds (data not shown). After acidification, instead of forming a precipitate, an emulsion layer appeared between the aqueous and organic layers during extraction with ethyl acetate. It is presumed that the polysaccharide fractions were also gradually degraded at the elevated temperature and subsequently formed lignin-carbohydrate complexes in the emulsion layer.

UV absorbance measurements were conducted on ACIS and its acetylated sample (Figure 2A,B). The ACIS lignin sample was sequentially extracted with diethyl ether, ethyl acetate, and acetone. The ACIS-ethyl acetate and acetone-extracted samples were further washed with diethyl ether to remove monomers and dimers. While ACIS was not fully soluble in acetonitrile, both ACIS and its extracted fractions dissolved completely in 2-methoxyethanol. UV absorbance data for ACIS and each isolated lignin fraction are presented in Figure 2B-E. All extracted samples exhibit strong absorbance at around 280 nm, attributed to the basic lignin phenylpropane unit. The UV absorbance gradually decreases from the UV-B to UV-A ranges across all samples. UV-A and UV-B rays can penetrate the Earth's surface, while UV-C rays are mostly blocked by the ozone layer.¹⁻³ Therefore, higher UV-A and UV-B absorbances are crucial for evaluating lignin as a UVprotective ingredient. To precisely assess UV-A and UV-B absorbance in various lignin samples in different solvents, the UV absorbance at 340 and 300 nm was selected as the characteristic peaks for UV-A and UV-B, respectively. The UV-A (340 nm) to UV (280 nm) ratio was calculated to assess UV-A absorption strength, while the UV-B (300 nm) to UV (280 nm) ratio was calculated for UV-B absorption strength (Table 1). These ratios were chosen because all lignin samples

Table 1. UV-A (340 nm)/UV (280 nm) and UV-B (300 nm)/UV (280 nm) Ratios of Various Lignin Samples

No.	Lignin sample	UV-A (340 nm)/UV (280 nm)	UV-B (300 nm)/UV (280 nm)
1	Ac-ACIS	0.36	0.73
2	ACIS	0.39	0.76
3	ACIS-acetone fraction	0.21	0.49
4	ACIS-ethyl acetate fraction	0.35	0.76
5	ACIS-ether fraction	0.27	0.76
6	softwood Kraft lignin ²¹	0.36	0.50
7	hardwood Kraft lignin ²²	0.40	0.77
8	softwood milled wood lignin ²²	0.25	0.55
9	hardwood milled wood lignin ²³	0.19	0.54
10	grass milled wood lignin ²⁴	0.31	0.63

containing aromatic rings absorb at 280 nm. The relative ratios allow for evaluating UV-A and UV-B absorbance across lignin samples independent of solvent and concentration effects. Additionally, the UV-A and UV-B absorption strengths of the sample were compared with various technical lignin samples.^{18–24} ACIS (UV-A: 0.39; UV-B: 0.76) showed slightly higher UV absorbance at both UV-A and UV-B ranges compared to Ac-ACIS samples (UV-A: 0.36; UV-B: 0.73). Zhang et al. reported that Kraft lignin's UV absorbance significantly decreased after acetylation, as acetyl groups block free phenolic hydroxyls.¹⁹ In this study, ACIS with free phenolic hydroxyls acts as an electron donor, enhancing benzene ring conjugation and UV absorbance.

Milled wood lignin represents the natural lignin structure found in wood.²⁰ The UV absorbance strengths of softwood milled wood lignin (UV-A: 0.25; UV-B: 0.55), hardwood milled wood lignin (UV-A: 0.19; UV-B: 0.54), and grass milled wood lignin (UV-A: 0.31; UV-B: 0.63) were calculated by the same method for comparison with our samples, based on reported data (Table 1). ACIS demonstrated higher UV absorbance in both the UV-A and UV-B ranges compared with all species of milled wood lignin. Additionally, ACIS exhibited slightly stronger absorbance than softwood Kraft lignin (UV-A: 0.36; UV-B: 0.50) and comparable absorbance to hardwood Kraft lignin (UV-A: 0.40; UV-B: 0.77). These results confirm that Kraft lignin generally has greater UV absorbance than milled wood lignin, with hardwood Kraft lignin showing higher absorbance than softwood Kraft lignin. In this study, ACIS derived from hardwood displayed UV absorbance levels that were competitive with those of hardwood Kraft lignin. However, UV-A absorbance decreased in the fractions extracted from ACIS using diethyl ether, ethyl acetate, and acetone, while UV-B absorbance was retained in both the ACIS-ethyl acetate- and ACIS-ether-soluble fractions. These findings suggest that the oligomeric ACIS-ethyl acetate fraction holds significant potential for use as a UV-protective ingredient in various applications.

3.2. HSQC NMR Analysis of the Lignin-Derived UV-Absorbing Ingredients. Extensive studies on the use of lignin as a UV-protective ingredient have been reported; however, research on the chemical structures responsible for UV absorption and their quantification remains very limited.^{25–27} Understanding the chemical structures of UV-



Figure 3. Heteronuclear single quantum coherence (HSQC) spectra of the acid-insoluble (ACIS) fraction (NMR solvent: CDCl₃).

absorbing components is crucial for future applications. The acetylated ACIS sample was used for NMR analysis because the original ACIS lignin sample could not fully dissolve in NMR solvents. Figure 3 presents the HSQC NMR spectrum of acetylated ACIS derived from hardwood beech. Signals from lignin substructures, including the methoxyl group (-MeO), β -aryl ether (β -O-4, A), phenylcoumaran (β -5, B), resinol $(\beta - \beta, C)$ and cinnamyl alcohol (F), were clearly observed. These signals were assigned based on reported data.¹³⁻¹⁷ Strong signals corresponding to aromatic structures from S, S', G, and G' units were also detected in the acetylated ACISbeech sample. The HSQC spectrum confirmed the presence of lignin side-chain structures, including β -O-4, β - β , and β -5 linkages. Furthermore, signals attributed to polysaccharide structures, such as glucose and xylose, were also identified in the spectrum (Figure 3).

The beech ACIS-ethyl acetate and acetone extraction samples were fully dissolved in DMSO-d₆ for HSQC NMR analysis without acetylation. The HSQC NMR spectra of the ACIS-beech acetone-soluble fraction and the ACIS-beech ethyl acetate-soluble fraction were remarkably similar, indicating that these two fractions contained nearly identical components (Figure 4A,B). In the side-chain region (Figure 4A), signals for lignin β -O-4 (A) and polysaccharide residues from glucose and xylose were absent. However, a signal corresponding to the oxidized β -O-4 (A') structure at the β position (δ C/ δ H: 85.4/ 5.33 ppm) was detected. Strong signals for lignin β -5 (**B**), β - β (C), and cinnamyl alcohol (F) were also observed. The chemical structures detected in this study are illustrated in Figure 5. In the high-carbon magnetic field region ($\delta C/\delta H$: 27.6/9.76 ppm), a signal was assigned to the aldehyde structure (X), which correlated with the low magnetic field signal at $\delta C/\delta H$: 196.6/9.76 ppm reflection during the NMR measurement. Additionally, benzoic acid (X') peaks were strongly detected in the aromatic region of both the ACISacetone and ACIS-ethyl acetate fractions (Figure 4). Furthermore, the α position of $\alpha_{,\beta}$ -conjugated methyl acrylate (\mathbf{Y}) or cinnamic acid (\mathbf{Y}') end-group structures was tentatively assigned by comparison with reported model compound data

at $\delta C/\delta H$: 147.1/7.18 ppm for the α position and $\delta C/\delta H$: 116.0/6.44 ppm for the β position (data not shown).^{28,29} In our previous study, we isolated a dehydrozingerone monomeric compound from the acid-soluble (ACSL) fraction, supporting the hypothesis that similar end groups may also form in the ACIS-ethyl acetate and acetone-soluble fractions.¹¹ The formation of aldehyde and carboxylic acid structures under alkaline conditions by microwave heating has been reported to occur via the formation of epoxide or quinone methide intermediates, followed by $C_{\alpha}-C_{\beta}$ bond cleavage and the generation of aldehydes or carboxylic acids.^{30,31} Another possible route is that the secondary alcohol at the α position of the β -O-4 side-chain structure is first oxidized to form the A' structure, which is then followed by C–O bond cleavage to yield the X'' structure³² (Figure 6).

The UV absorbance mechanism is explained as the electron transitions, including electron jumps from π -bonding orbitals to π -antibonding orbitals, from nonbonding orbitals to π antibonding orbitals, or from nonbonding orbitals to σ antibonding orbitals. Free and etherified hydroxyl groups in lignin contribute to UV absorption at 240 and 282 nm, while lignin's syringyl and guaiacyl groups are responsible for absorption around 280 nm. UV-A absorption is attributed to $\pi - \pi^*$ and $n - \pi^*$ transitions in lignin's aromatic rings, indicating that double bonds (CH=CH) conjugated with aromatic ring structures have both UV-A and UV-B absorption. Newly formed oxidized hardwood beech lignin components are considered to be the fractions that absorb UV irradiation at longer wavelengths. The chemical structures with both UV-A and UV-B absorbance are highlighted in the black square in Figure 5. The extent of UV absorption is influenced by the number of conjugated structures in the samples. In this study, the ratio of conjugated structures in the Ac-ACIS fraction, the ACIS-acetone-soluble fraction, and the ACISethyl acetate-soluble fraction was quantified based on peak intensities. For the side chain region, the intensities of signals corresponding to structures A (β), B (β), C (β), A' (β), X'' (β), **F** (γ), and **Y**+**Y**' (α) were regarded as 100%. The relative ratios of UV-A and UV-B-absorbing structures, specifically A'



Figure 4. Heteronuclear single quantum coherence (HSQC) spectra of the A) ACIS-ethyl acetate-soluble fraction and B) ACIS-acetone soluble fraction (NMR solvent: DMSO- d_6).

(β), X'' (β), F (γ), and Y+Y' (α), were calculated for each sample (Figure 7A). Similarly, in the aromatic region, the intensities of G5/6, S2/6, S'2/6, G'6, X6, and X'6 peaks were regarded as 100%. Conjugated structures, including S'2/6, G'6, X6, and X'6, were identified as UV-A and UV-B-absorbing components, and their relative ratios were calculated (Figure 7B). The relative ratios of conjugated structures showed an increasing trend across the samples: 7.7%, 19.4%, and 28.1% for the side chain region and 8.5%, 16.8%, and 22.0% for the aromatic region in the Ac-ACIS, ACIS-acetone-soluble fraction, and ACIS-ethyl acetate-soluble fraction, respectively. These results demonstrate that the ACIS-ethyl acetate-soluble fraction contains the highest ratio of UV-absorbing structures. Furthermore, the NMR quantification data are also consistent with the UV data.

3.3. Molecular Weight Characterization of the Oligomeric Lignin UV-Absorbing Fractions. Another

critical intrinsic factor influencing UV absorbion properties is the molecular weight. Lim et al. synthesized a lignin-like oligomer and explored the relationship between molecular weight and UV absorbance in lignin samples. They found that molecular weights ranging from 1790 to 2230 Da benefit UV absorption.¹⁰ In this study, the molecular weight distributions of ACIS-beech-diethyl ether-soluble, ACIS-beech-ethyl acetate-soluble, and ACIS-beech-acetone-soluble fractions and ACIS samples were analyzed using GPC (Figure 8). A gradual increase in the molecular weight was observed across the fractions, from diethyl ether to ethyl acetate and then to acetone-soluble fractions. The average molecular weight (M_w) of the ethyl acetate- and acetone-soluble fractions was approximately 1000, indicating that these fractions primarily contained higher-order oligomers (Table 2). Polydispersity values of 8.5 and 6.2, respectively, suggested the presence of



Figure 5. Structures detected in this study (structures that have both UV-A and UV-B absorption are highlighted in black squares).



Figure 6. Proposed mechanism of lignin degradation for the generation of UV-active compounds.

low-molecular-weight lignin compounds in the extracted samples.

UV-absorbing ingredients for cosmetic use comply with stringent safety assessments. There is a 500 Da (Dalton) rule for chemical compounds and drugs, which states that molecules larger than 500 Da cannot penetrate the skin and cannot act as allergens in humans.^{33,34} Consequently, the ethyl



Figure 8. GPC analysis of the beech wood decomposition fraction ACIS and each lignin fraction.

acetate and acetone fractions were washed three times with diethyl ether to remove monomers and dimers from these fractions. GC-MS analysis was conducted to confirm the



Figure 7. Quantification of the structure ratio, which has both UV-A and UV-B absorbance in the acetylated ACIS, ACIS-acetone soluble fraction, and ACIS-ethyl acetate-soluble fraction, by nuclear magnetic resonance (NMR) analysis.

Table 2. GPC Analysis of ACIS and Each Solvent-Extracted Fraction Measured by Guaiacol, Syringaldehyde, and Polystyrene as Standards

Sample	$M_{ m p}$	$M_{\rm n}$	$M_{ m w}$	$M_{ m w}/M_{ m n}$
ACIS	1130	140	1220	8.8
ACIS-diethyl ether fraction	230	120	430	3.6
ACIS-ethyl acetate fraction	910	110	940	8.5
ACIS-acetone fraction	1140	190	1200	6.2

removal of monomers and dimers from each fraction (Figure 9). In a comparison of the GC-MS spectra of the extracted samples, low-molecular-weight compounds were detected in the ether-soluble fraction, primarily vanillin (152 Da), acetovanillone (166 Da), syringaldehyde (182 Da), acetosyringone (196 Da), and syringic acid (198 Da) in the monomeric fraction. The structures were confirmed by comparison with authentic compounds. Additionally, various unidentified dimeric compounds were detected. Almost no peaks were observed in the acetone-soluble fraction, confirming the efficient removal of low-molecular-weight compounds through washing. Monomeric compounds such as vanillin, syringaldehyde, and acetosyringone were detected in the ethyl acetate-soluble fraction, but nearly all of the dimeric peaks disappeared. These results demonstrate that the oligomeric lignin-derived samples produced in this study are well-suited for use as UV-protective ingredients in various applications. However, a safety evaluation is also needed for further use in cosmetic production.

4. CONCLUSION AND PROSPECTIVE

In conclusion, UV absorbers derived from oligomeric lignin were obtained through sequential extraction with diethyl ether, ethyl acetate, and acetone from wood CuO degradation products via microwave heating. The UV-absorbing chemical structures were characterized using HSQC NMR analysis, which revealed that the enhanced UV-A and UV-B absorbances resulted from increased aldehyde, carboxylic acid, and α -ketone groups, and α_{β} -conjugated cinnamic acid structure end groups in the ethyl acetate-soluble fraction. The average molecular weights of the ACIS ethyl acetate-soluble and ACIS acetone-soluble fractions were confirmed to be 940 and 1200, respectively. The oligomeric lignin-derived samples produced in this study demonstrate their suitability as UVprotective ingredients for various applications. Furthermore, this method may be applicable to a broad range of biomass sources for the production of lignin-based UV absorbers. However, several challenges remain. Notably, the UV absorbance of these lignin oligomers is currently lower than that of conventional synthetic UV absorbers. Additionally, for potential cosmetic applications, key factors such as photoinstability, biodegradability, and toxicity must be thoroughly evaluated to ensure safe use in consumer products.

AUTHOR INFORMATION

Corresponding Authors

- Chen Qu Advanced Institute for Materials Research (WPI-AIMR), Tohoku University, Sendai 980-8577, Japan;
 orcid.org/0000-0002-8833-0156; Phone: +81 22-217-6383; Email: qu.chen.e4@tohoku.ac.jp; Fax: +81 22-217-5129
- Takashi Watanabe Laboratory of Biomass Conversion, Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan; Biomass Product Tree Industry-Academia Collaborative Research Laboratory, Kyoto University, Uji, Kyoto 611-0011, Japan; Ocrid.org/ 0000-0003-0220-4157; Phone: +81 774 38 3640; Email: twatanab@rish.kyoto-u.ac.jp; Fax: +81 774 38 3681



Figure 9. GC-MS analysis of each solvent fraction of the degraded beech lignin.

Authors

- Keigo Mikame Laboratory of Biomass Conversion, Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan
- Yasunori Ohashi Laboratory of Biomass Conversion, Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan
- Hiroshi Nishimura Laboratory of Biomass Conversion, Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan; Biomass Product Tree Industry-Academia Collaborative Research Laboratory, Kyoto University, Uji, Kyoto 611-0011, Japan

Kenzo Koike – Kao Corporation, Tokyo 131-8501, Japan

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.5c00654

Author Contributions

C.Q., T.W., and K.K. contributed to conceptualization of this study; C.Q. and T.W. contributed to the methodology, draft writing, and editing; C.Q., K.M., and Y.O. contributed to the experiments and analyses; H.N. contributed to the NMR measurements.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by JSPS KAKENHI Grant Number JP 22K05932.

ABBREVIATIONS

ACIS, acid-insoluble fraction; AISL, alkali-insoluble fraction; ACSL, acid-soluble fraction; HSQC, heteronuclear single quantum coherence; NMR, nuclear magnetic resonance; DMSO, dimethyl sulfoxide; β -Glcp, β -D-glucopyranose; β -D-Xylp, β -D-xylopyranose; G, guaiacyl; S, syringyl

REFERENCES

(1) Chisvert, A.; Salvador, A. UV filters in sunscreens and other cosmetics. Regulatory aspects and analytical methods. In *Analysis of Cosmetic Products*, Salvador, A.; Chisvert, A., Eds.; Elsevier: Amsterdam, The Netherlands, 2007; pp. 83–85.

(2) González, S.; Fernández-Lorente, M.; Gilaberte-Calzada, Y. The latest on skin photoprotection. *Clin. Dermatol.* **2008**, *26*, 614–626.

(3) Damiani, E.; Astolfi, P.; Giesinger, J.; Ehlis, T.; Herzog, B.; Greci, L.; Baschong, W. Assessment of the photo-degradation of UV-filters and radical-induced peroxidation in cosmetic sunscreen formulations. *Free Radical Res.* **2010**, *44*, 304–312.

(4) Tran, M.H.; Phan, D.P.; Lee, E.Y.; Y, E. Review of lignin modifications toward natural UV protection ingredient for lignin-based sunscreens. *Green Chem.* **2021**, *23*, 4633–4646.

(5) Zhang, Y.; Naebe, M. Lignin: A review on structure, properties, and applications as a light-colored UV absorber. *ACS Sustainable Chem. Eng.* **2021**, *9*, 1427–1442.

(6) Qian, Y.; Qiu, X.; Zhu, S. Lignin: A nature-inspired sun blocker for broad-spectrum sunscreens. *Green Chem.* **2015**, *17*, 320–324.

(7) Qian, Y.; Qiu, X.; Zhu, S. Sunscreen performance of lignin from different technical resources and their general synergistic effect with synthetic sunscreens. *ACS Sustainable Chem. Eng.* **2016**, *4*, 4029–4035.

(8) Zhang, H.; Liu, X.; Fu, S.; Chen, Y. High-value utilization of kraft lignin: Color reduction and sunscreen ingredient. *Int. J. Biol. Macromol.* **2019**, *133*, 86–92.

(9) Zhang, Y.; Naebe, M. Lignin: A review on structure, properties, and applications as a light-colored UV absorber. *ACS Sustainable Chem. Eng.* **2021**, *9*, 1427–1442.

(10) Lim, J.; Sana, B.; Krishnan, R.; Seayad, J.; Ghadessy, F. J.; Jana, S.; Ramalingam, B. Laccase-catalyzed synthesis of low-molecularweight lignin-like oligomers and their application as UV-blocking materials. *Chem. - Asian J.* **2018**, *13*, 284–291.

(11) Mikame, K.; Ohashi, Y.; Naito, Y.; Nishimura, H.; Katahira, M.; Sugawara, S.; Koike, K.; Watanabe, T. Natural organic ultraviolet absorbers from lignin. *ACS Sustainable Chem. Eng.* **2021**, *9*, 16651– 16658.

(12) Lu, F.; Ralph, J. Non-degradative dissolution and acetylation of ball-milled plant cell walls: High-resolution solution-state NMR. *Plant J.* **2003**, *35*, 535–544.

(13) Kim, H.; Ralph, J. Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO- d_6 /pyridine- d_5 . Org. Biomol. Chem. 2010, 8, 576–591.

(14) Qu, C.; Kishimoto, T.; Kishino, M.; Hamada, M.; Nakajima, N. Heteronuclear single-quantum coherence nuclear magnetic resonance (HSQC NMR) characterization of acetylated fir (*Abiessachallnensis* MAST) wood regenerated from ionic liquid. *J. Agric. Food Chem.* **2011**, *59*, 5382–5389.

(15) Rahimi, A.; Azarpira, A.; Kim, H.; Ralph, J.; Stahl, S. S. Chemoselective metal-free aerobic alcohol oxidation in lignin. *J. Am. Chem. Soc.* **2013**, *135*, 6415–6418.

(16) Qu, C.; Kishimoto, T.; Hamada, M.; Nakajima, N. Dissolution of ball-milled plant cell walls in ionic liquid systems at room temperature: Application to NMR analysis. *Holzforschung* **2013**, *67*, 25–32.

(17) Ralph, J.; Landucci, L. L. NMR of lignins. In *Lignin and Lignan. Advances in Chemistry*, Heitner, C.; Dimmel, D. R.; Schmidt, J. A., Eds.; CRC Press: Boca Raton, FL, USA, 2010; pp. 137–244.

(18) Guo, Y.; Tian, D.; Shen, F.; Yang, G.; Long, L.; He, J.; Song, C.; Zhang, J.; Zhu, Y.; Huang, C.; et al. Transparent cellulose/technical lignin composite films for advanced packaging. *Polymers* **2019**, *11*, 1455.

(19) Zhang, H.; Liu, X.; Fu, S.; Chen, Y. High-value utilization of kraft lignin: Color reduction and evaluation as sunscreen ingredient. *Int. J. Biol. Macromol.* **2019**, *133*, 86–92.

(20) Lin, S. Y. Ultraviolet spectrophotometry. In *Methods in Lignin Chemistry*, Lin, S. Y.; Dence, C. W., Eds.; Springer: Berlin, Heidelberg, 1992; pp. 222–223.

(21) Goliszek, M.; Podkościelna, B.; Rybiński, P.; Klapiszewska, I.; Klepka, T.; Masek, A.; Klapiszewski, Ł. Toward a green economy: Lignin-based hybrid materials as functional additives in flameretardant polymer coatings. J. Polym. Res. 2024, 31, 316.

(22) Lu, F.; Wang, C.; Chen, M.; Yue, F.; Ralph, J. A facile spectroscopic method for measuring Lignin content in the lignocellulosic biomass. *Green Chem.* **2021**, *23*, 5106–5112.

(23) Ruwoldt, J.; Tanase-Opedal, M.; Syverud, K. Ultraviolet spectrophotometry of lignin revisited: Exploring solvents with low harmfulness, lignin purity, Hansen solubility parameter, and determination of phenolic hydroxyl groups. *ACS Omega* 2022, *7*, 46371–46383.

(24) Higuchi, T.; Ito, Y.; Shimada, M.; Kawamura, I. Chemical properties of milled wood lignin of grasses. *Phytochemistry* **1967**, *6*, 1551–1556.

(25) Kim, Y.; Suhr, J.; Seo, H.-W.; Sun, H.; Kim, S.; Park, I.-K.; Kim, S.-H.; Lee, Y.; Kim, K.-J.; Nam, J.-D. All biomass and UV protective composite composed of compatibilized lignin and poly (lactic-acid). *Sci. Rep.* **2017**, *7*, 43596.

(26) Shikinaka, K.; Nakamura, M.; Otsuka, Y. Strong UV absorption by nanoparticulated lignin in polymer films with reinforcement of mechanical properties. *Polymer* **2020**, *190*, 122254.

(27) Xiong, F.; Wu, Y.; Li, G.; Han, Y.; Chu, F. Transparent nanocomposite films of lignin nanospheres and Poly(vinyl alcohol) for UV-absorbing. *Ind. Eng. Chem. Res.* **2018**, *57*, 1207–1212.

(28) Förster, H.UV/VIS spectroscopyCharacterization I. Molecular Sieves – Science and TechnologySpringerBerlin, Heidelberg20044337– 426

(29) Ralph, S. A.; Ralph, J.; Lu, F. NMR database of lignin and cell wall model compounds; GLBRC, 2024, www.glbrc.org/databases_and_software/nmrdatabase/.

(30) Qu, C.; Ito, K.; Katsuyama, I.; Mitani, T.; Kashimura, K.; Watanabe, T. Directly Microwave-Accelerated Cleavage of C–C and C–O Bonds of Lignin by Copper Oxide and H_2O_2 . *ChemSuschem* **2020**, *13*, 4510.

(31) Tao, J.; Qu, C.; Kawamoto, H. Catalytic conversion of woody biomass into monomeric chemicals by a microwave mediated metalorganic-framework 818. *Ind. Crops Prod.* **2024**, *222*, 119864.

(32) Bosque, I.; Magallanes, G.; Rigoulet, M.; Karkas, K. D.; Stephenson, C. R. J. Redox catalysis facilitates lignin depolymerization. *ACS Cent. Sci.* **2017**, *3*, 621–628.

(33) Nohynek, G. J.; Antignac, E.; Re, T.; Toutain, H. Safety assessment of personal care products/cosmetics and their ingredients. *Toxicol. Appl. Pharmacol.* **2010**, *243*, 239–259.

(34) Bos, J. D.; Meinardi, M. M. H. M. The 500 Da rule for the skin penetration of chemical compounds and drugs. *Exp. Dermatol.* **2000**, *9*, 165–169.