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Article

Structural Elucidation of Suberin from the Bark of Cultivated Willow (*Salix* sp.)

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ABSTRACT: Although extractives have been symbolized as major bioactive pharmacological compounds from *Salix* (Salicaceae) bark, we speculated that these pharmaceutical effects cannot be solely attributed to phenolic components and their derivatives, but the long-chain suberin acids also contribute to their therapeutic effects. Hence, isolation and deconstruction of suberin were conducted, for the first time, to enrich our knowledge about the macromolecular components at the cell wall of willow bark. Saponification was adopted to obtain suberin extracts at a yield of approximately 5 wt % based on the bark of the studied hybrids. Gas chromatography−mass spectrometry allowed qualification and quantification of 23 compounds from the released suberin monomers, from which fatty acids represented majority of the isolated suberin, namely, fatty acid methyl esters (C17−C19); mono-carboxylic acid (C7−C16); alpha, omega-dicarboxylic acid (C7−C16); and omega-hydroxy long-chain fatty acids (C16−C22). Additionally, the lipophilic extractive was dominated by piceol, heptacosane, *β*-sitosterol, and fatty acids (C16−C28) from the studied hybrids. These findings could boost our integrative approach toward full valorization of willow bark.

KEYWORDS: *lipophilic extractive, omega-hydroxy fatty acid, suberin, willow bark*

INTRODUCTION

The concept of bark biorefinery[1] is envisioned to produce high-value products out of the often-underappreciated bark that is abundantly available from the forest and agricultural industry. In particular, the traditional energy use could be upgraded into the production of biologically active compounds,[2] colorants,[3] and functional nanomaterials[4] based on uniqueness and singularity of bark's chemical constituents. This transformation could be envisioned to benefit pulp manufacturers, biochemical industries, and color and textile industries.

The fast-growing willow (*Salicaceae*) biomass is traditionally cultivated for energy use. Well-managed willow crops can be 8−30% higher than the growth of other Finnish forest species (i.e., downy birch, silver birch, and gray alder) on the same marginal land.[5] Peatland restoration by planting energy willow can significantly mitigate greenhouse emissions into the environment better than other trees. Willow bark has been identified as a potential source of high-value pharmaceuticals,[6,7] colorants,[8] and functional fibers.[9] However, not all bioactive substances have been fully elucidated out of willow bark.

Suberin, a highly hydrophobic substance, was speculated contributing to significant hydrophobicity features[4] of willow bark-derived nanofilms. Suberin, present at the root of *Salix martiana* and rich in aromatic moieties, was reported to limit oxygen diffusion as an adaptation to the flooding environment where the willow traditionally grows.[10] These earlier reports inspired us to explore the chemical characteristics of suberin from willow bark.

Suberin is a lipophilic polyester macromolecule arising from three building units, that is, long-chain fatty acids (poly-aliphatic domain), ferulic acid (polyaromatic domain), and glycerol, which was supposed to interlink the domains together.[11,12] Suberin is considered as a macromolecular structural component that cannot be extracted by simple organic solvents. Harsh treatments, like alkaline methanolysis with sodium methoxide (saponification),[13] can be used to deconstruct suberin macromolecules into their monomers.

Suberin has been reported to be present in the cell walls in trees' outer bark or their wounded external tissues, including *Salix*,[14] *Quercus suber* L.,[15] and birch[16] in which suberin provides a protective barrier between the organisms and the surrounding environment. Suberin-derived chemicals have gained great interest from the cosmetics and pharmaceutical industries, including the use in the treatment of cancer-related diseases (absorbent of carcinogens and antimutagenicity)[17] and wound therapy.[18] Therefore, the objective of this study was to characterize the isolated macromolecular components of suberin from willow bark.

MATERIALS AND METHODS

Materials and Chemicals. All the chemicals and solvents were from Sigma-Aldrich unless described separately. Four-year old willow hybrid "Karin" (SalixEnergi Europa AB) was harvested from a plantation of VTT Technical Research Center of Finland (Kyyjärvi, Finland) on May 13, 2015. Two-year old "Klara" was harvested from

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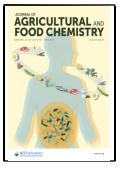

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the field of Carbons Oy (Kouvola, Finland) on May 8, 2018. The bark was manually stripped off and dried at 40 °C in an oven. The dried bark was ground into fine particles (<1 mm mesh) (Wiley mill, USA) that were kept in a desiccator until further use.

Experimental Flow. *Suberin Extraction (Figure 1).* The ground dry bark was extracted first with dichloromethane (DCM) and then with hot water to capture both lipophilic and hydrophilic compounds following Tappi T 204 om-88.[19] The yield of the extracts was determined based on their weight after drying under a vacuum oven. The extract-free bark (8 g) was then refluxed in 300 mL 2.5 wt % sodium methoxide in absolute methanol for 3 h. Then, the upper phase of the suspension was first filtered through a crucible with a pore size of 10−16 μm. Approximately 50 mL methanol was used to rinse and collect any solubilized components from the remaining residues before a second round of filtration. Hot water was used here to neutralize the eluents. Aqueous HCl (10 wt %) was used to acidify (pH 5) the combined methanol extracts which were further concentrated with a rotary evaporator to bring the volume below 100 mL. The filtrate was then transferred into a separatory funnel and washed three times with 100 mL chloroform. Anhydrous sodium sulfate was used to remove any remaining water from the combined chloroform solution. The solvent was removed in a vacuum oven to obtain the product, suberin.

Hydrolyzable "Tannin-Like" Substance. Diethyl ether (100 mL) was used three times to collect the hydrolyzable substance via liquid/liquid extraction, which remained at the liquid effluents after chloroform extraction (Figure 1). Anhydrous sodium sulfate was used to eliminate any remaining water, and most of the solvents were removed under vacuum in a rotavapor evaporator. The concentrated fraction was vacuum-dried overnight before further analysis.

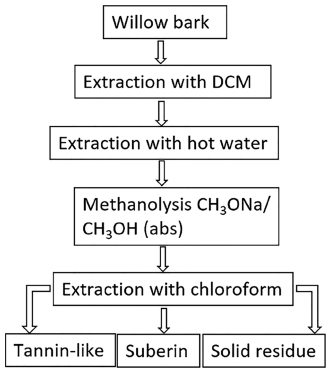

Figure 1. Suberin and hydrolyzable "tannin-like" substance extraction through alkaline methanolysis with sodium methoxide.

Acid-Insoluble Residue and Ash. The acid-insoluble residue of bark and solid residues from both schemes was determined by the Klason lignin method according to Tappi standard T 222 om-88.[20] The ash content was determined according to standard procedure Tappi T 211 om-93.[21]

Characterization. *Gas Chromatography−Mass Spectrometry (GC−MS).* Klara (9.6 mg) and Karin (12.3 mg) of isolated suberin were solubilized in 500 μL pyridine with tetracosane as the internal standard (1 mg mL^{-1}). *N*,*O*-Bis(trimethylsilyl) trifluoroacetamide (300 μL) was then added into the mixture which was kept at room temperature for 12 h. The specific temperature program of GC−MS was described previously.[15] Additionally, the lipophilic extracts (7.2 and 11.7 mg for Karin and Klara, respectively) and hydrolyzable tannin-like substances (unquantified) were also characterized. Their mass fragments were referenced with NIST Chemistry WebBook, MassBank of North America, and the literature.

Solid-State Nuclear Magnetic Resonance Spectroscopy. Solid-state ^{13}C cross polarization with magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectroscopy was performed on a Bruker AVANCE III spectrometer operating at 400 MHz for protons using a double-resonance CP-MAS probe head. Samples were packed into 4 mm ZrO_2 rotors and plugged with KEL-F endcaps and spun at a spinning frequency of 12 kHz. The contact time for cross polarization was 1 ms, and at least 20 k scans were collected with a 5 s relaxation delay.

RESULTS AND DISCUSSION

Chemical Characteristics of Acid-Insoluble Residue and Lipophilic Extracts. The acid-insoluble residue after removal of suberin with the NaOMe/MeOH treatment led to an acid-insoluble residue content (7 ± 0.2%, Table 1) that was significantly lower than the previously reported Klason lignin content (24.7 ± 0.1%).[7] Similar behavior was observed for hybrid Klara bark. According to the literature, the bark lignin content has been frequently overestimated because of the presence of proteins and tannins.[22] The water-soluble extract content determined according to Tappi T 204 om-88[19] was lower than the hot water extract content obtained under milder conditions.[7] Possibly, the higher temperature and longer reflux time in the standard method led to partial precipitation or condensation of the hydrophilic substances.

Table 1. Yield Loss of Extracts and Acid-Insoluble Residue from Willow Bark Based on Figure 1[a]

	Karin	Klara
DCM extracts %	1.89 (0.09)	1.84 (0.04)
water extracts %	12.1 (0.87)	9.8 (1.95)
acid-insoluble residue %	7 (0.2)	10 (0.6)
suberin	4.5 (0.4)	5.3 (0.7)
hydrolyzable tannin	not known	not known
others	74.5	73.1

[a]Others include cellulose, hemicellulose, pectin, ash, and others. The numbers in the parenthesis are standard deviations.

The $^{13}C-CP/MAS$ spectrum of the acid-insoluble residue after the NaOMe/MeOH treatment revealed strong signals of cellulose and hemicelluloses (62−105 ppm) while the aromatic and methoxyl signals, characteristic of lignin, were weak (Figure 2a), which indicates that ester bonds of lignin might be removed by de-esterification (i.e., saponification); this may partly reflect the fact that the methylene peaks (30 and 33 ppm) of suberin involve linkages between lignin and carbohydrates[23] within the matrix of the cell wall.[11] The detailed peak assignment is summarized in Table S1 (Supporting Information). Figure S1 presented a similar spectrum profile of the Klara hybrid.

The yield of DCM extracts varied between 1.89 ± 0.09% (w/w) in Karin and 1.84 ± 0.04% (w/w) in Klara, which was slightly lower than that reported for *Salix* viminalis (2.66%) and *Salix* atrocinerea Brot (3.94%). GC−MS allowed quantification of 18.7 and 14.3%, respectively, for DCM extracts of Karin and Klara bark. More than 80 wt % DCM extracts, remained as unquantified, were speculated as high-molecular-weight condensed compounds that were hard to solubilize in pyridine. The identified principal components were saturated fatty acids, long-chain alkanes, sterols, and aromatic compounds as shown in Figures 3 and 4, and Table S2.

Saturated fatty acids, that is, hexadecanoic acid (C16), tetracosanoic acid (C24), hexacosanoic acid (C26), and octacosanoic acid (C28), represented a major class of identified DCM extracts from Karin (7.8 wt %) and Klara (9.3 wt %). These fatty acids were observed from other parts of

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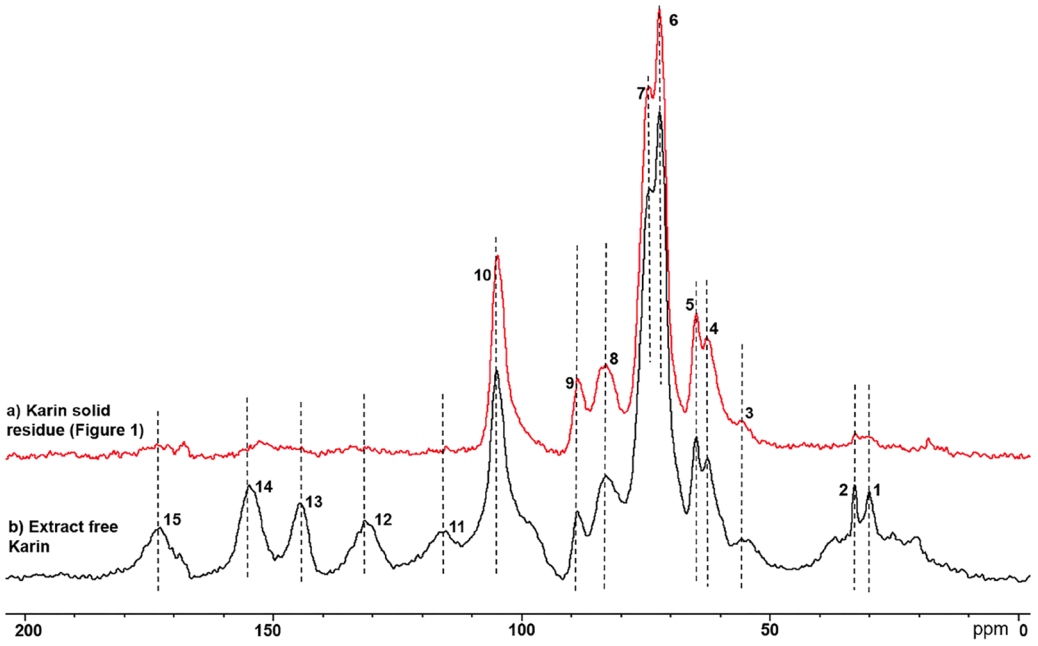

Figure 2. Solid-state ^{13}C CP/MAS spectrum of (a) Karin bark solid residue (Figure 1) and (b) extract-free Karin.

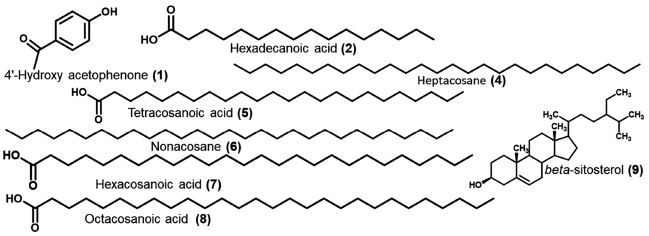

Figure 3. Detected **DCM** extracts from Karin and Klara bark based on the GC−MS total-ion chromatogram. Mass spectra of their trimethylsilylated profile are summarized in Figure S2.

Salix, including bark,[24] leaf,[25] and root.[10] The Klara bark DCM extracts contained 3.2 wt % alkanes with carbon atom numbers from C27 (heptacosane) to C29 (nonacosane). These alkanes were earlier detected from the leaf of *Salix*.[25] Piceol, as the aglycons of picein (principal water-soluble extractives at Karin bark),[7] represented up to 3.8 wt % of Karin DCM extracts. This compound has been suggested as a potential anti-inflammatory agent[24] and shown to be active against spruce budworm.[26] *beta*-Sitosterol accounted for roughly 2 wt % from DCM extracts of studied hybrids, and this sterol possesses pharmaceutical effects in inhibiting the growth of *Escherichia coli* bacteria.[27] These insights here suggest us to utilize these lipophilic extracts as potential agents for antibacterial uses. The hypothesis was that lipophilic substances present at inner bark can be extracted using DCM, and those suberin-derived lipophilic components located within the macromolecule

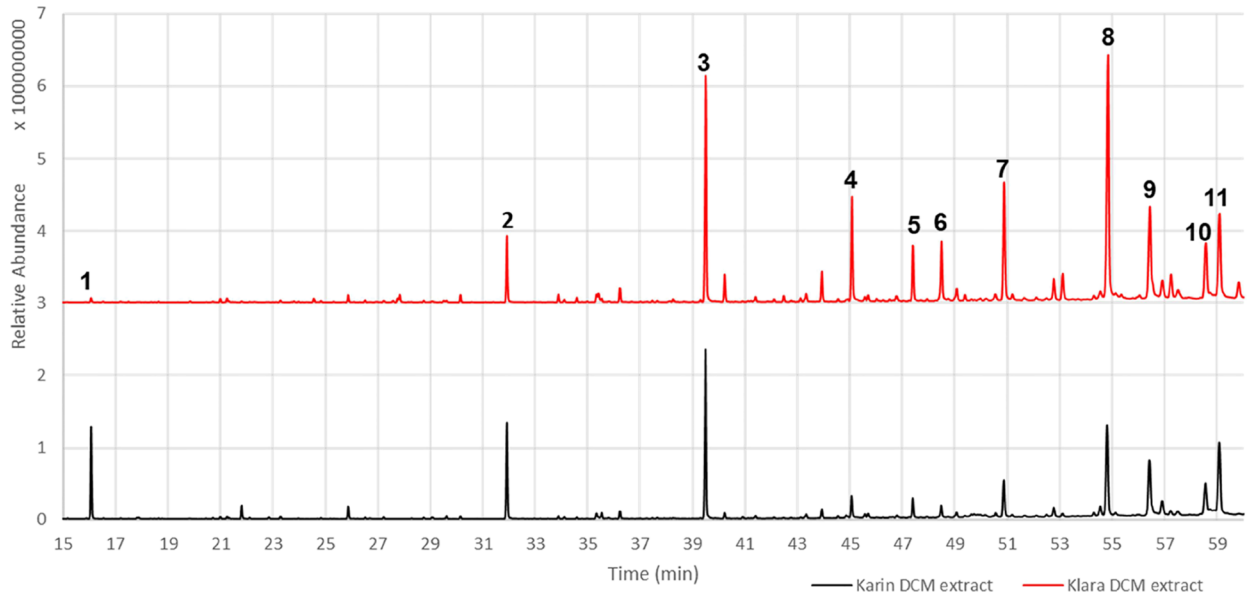

Figure 4. GC−MS total-ion chromatogram of the major peaks from Karin/Klara DCM extracts.

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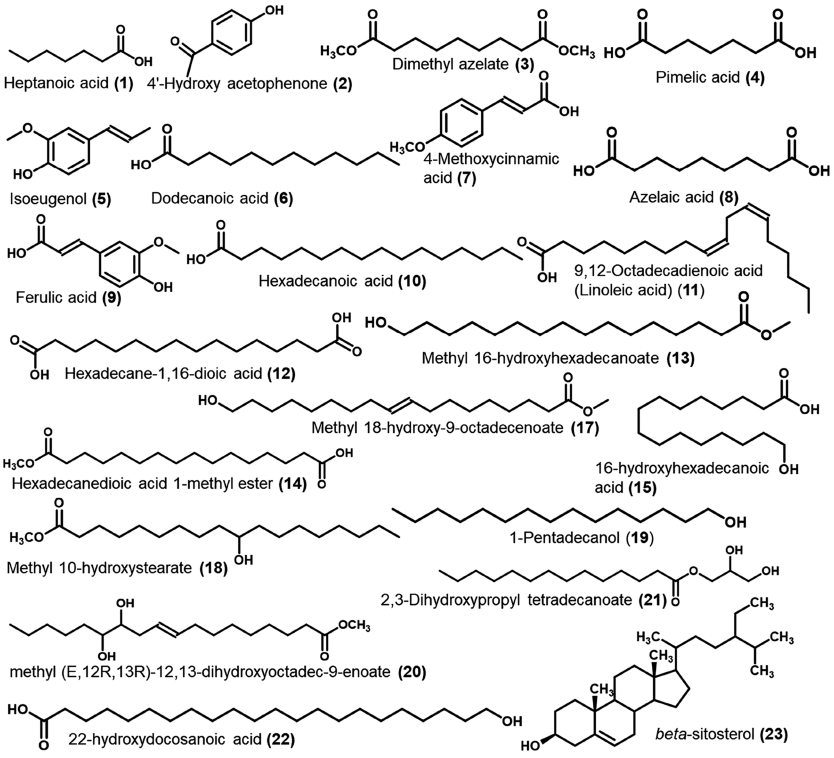

Figure 5. Detected suberin monomers from Karin and Klara bark based on the GC−MS total-ion chromatogram.

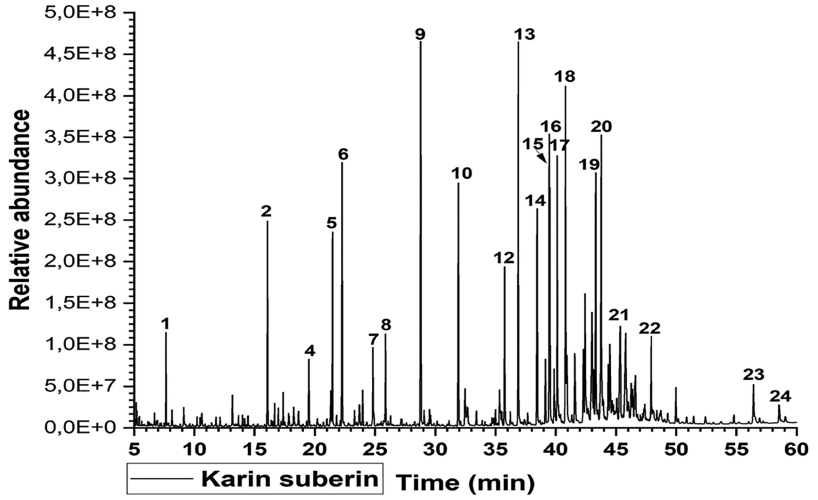

Figure 6. GC−MS total-ion chromatogram of the major peaks of suberin obtained from Karin bark by alkaline methanolysis (Figure 1). Mass spectra of their trimethylsilylated (**TMS**) profiles are summarized in Figure S4.

matrix of outer bark require harsh conditions to liberate their monomers out.

Monomeric Composition of Suberin (Chloroform-Soluble). Suberin was successfully isolated from the matrix as indicated by the disappearing aliphatic methylene peaks (30.0 and 32.9 ppm) of suberin[28] in Figure 2a and Figure S1. Isolated suberin accounted for 4.5 ± 0.4 and 5.3 ± 0.7% from the bark of Karin and Klara, respectively. Suberin was speculated as the major compound present at the outer bark of studied *Salix* hybrids as its layer represents less than 10 wt % of whole bark depending on the age. GC−MS allowed the quantification of 70.7 and 55.4% based on the extracted suberin of Karin and Klara, respectively. The majority of Karin suberin compounds were fatty acids, aromatic compounds, long-chain aliphatic alcohols, and sterol compounds as shown in Figures 5 and 6, and Table 2. Detailed assignment for Klara suberin is included in Table S3 and Figure S3. Mass spectra of trimethylsilylated suberin compounds are summarized in Figure S4.

Aromatic Compounds. Four aromatic compounds were identified and quantified in suberin of Karin (13.7 wt %) and Klara (19.2 wt %), which suggested a function of suberin in pathogen defense.[11] Ferulic acid accounted for approximately 46.7 wt % of total identified aromatic compounds from Karin suberin, which has been reported to possess pharmaceutical activities, including antimicrobial, anti-inflammatory, antithrombosis, and anticancer.[36] Moreover, it was speculated as the connection points between lignin units with the identified

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Table 2. Chemical Composition of Suberin (70.71 wt % Detected) Obtained from Karin Bark by Alkaline Methanolysis as Weight Percentage (wt %) of Analyzed Suberin[a]

retention time, min	peak number	compounds, detected as methyl ester (Me) and/or TMS ester/ether (TMS) derivatives	amount, % (w/w)	characteristic fragments m/z	M_w of TMS derivatives	DB/ literature
aromatic compound			**13.7**			
16.07	2	4'-hydroxy acetophenone (Me; TMS ether)	3.0	208; 193; 151; 133; 89	208	[N]
21.48	5	isoeugenol (Me; TMS ether)	3.3	236; 205; 189; 177; 115; 73	236	[N]
24.82	7	4-methoxycinnamic acid (Me; TMS ester)	1.0	250; 235; 203; 175; 102; 73	250	[N]; [TI]
28.78	9	ferulic acid (Me; TMS ether; TMS ester)	6.4	280; 270; 250; 227; 171; 143; 129; 87	338	[N]; [M]; [15]
fatty acids			**48.4**			
saturated fatty acids (mono-carboxylic acid)			**9.7**			
7.64	1	heptanoic acid (TMS ester)	1.2	189; 173; 129; 89; 73	202	[N]; [TI]
22.27	6	dodecanoic acid (TMS ester)	4.2	259; 201; 171; 152; 117; 73	272	[N]; [TI]
31.92	10	hexadecanoic acid (TMS ester)	4.3	328; 313; 269; 145; 132; 117; 75; 73	328	[N]; [15]
saturated fatty acids (alpha, omega-dicarboxylic acid)			**5.3**			
19.51	4	pimelic acid (di TMS ester)	1.0	245; 187; 169; 159; 117;75	304	[N]; [TI]
25.86	8	azelaic acid (di TMS ester)	1.2	317; 217; 204; 201; 149; 129; 117; 75; 73	332	[N]
35.77	12	hexadecane-1,16-dioic acid (diTMS ester)	3.1	283; 241; 209; 191; 154; 112; 98; 97	430	[N]; [29]
omegahydroxy-long-chain fatty acids			**4.8**			
39.47	15	16-hydroxyhexadecanoic acid (TMS ether; TMS ester)	3.1	401; 385; 311; 217; 204; 147; 117; 75; 73	416	[30]
47.93	22	22-hydroxydocosanoic acid (TMS ether; ester)	1.6	427; 395; 204; 159; 146; 75	500	[15; 31]
fatty acids methyl esters			**28.6**			
36.9	13	methyl 16-hydroxyhexadecanoate (Me; TMS ether)	7.4	343; 311; 146; 103; 75	358	[32]
38.46	14	hexadecanedioic acid 1-methyl ester (Me; TMS ester)	4.2	357; 313; 307; 269; 159; 117; 98; 73	372	[29]
40.13	17	methyl 18-hydroxy-9-octadecenoate (Me; TMS ether)	5.2	384; 369; 353; 337; 262; 159; 129; 95; 75	384	[33]
40.81	18	methyl 10-hydroxystearate (Me; TMS ether)	6.3	371; 339; 273; 244; 185; 146; 103; 73	386	[33]
43.77	20	methyl (*E*,12R,13R)-12,13-dihydroxyoctadec-9-enoate (diTMS ether; TMS ester)	5.6	315; 275; 270; 185; 147; 129; 95; 73	472	[34;35]
monoglyceride						
45.35	21	2,3-dihydroxypropyl tetradecanoate (diTMS ether)	2.4	382; 343; 329; 243; 187; 129; 87; 73	446	[N]
long-chain aliphatic alcohols						
43.32	19	1-pentadecanol (TMS ether)	5.0	285; 181; 135; 129; 75; 73	300	[N]
Sterols						
56.41	23	beta-sitosterol (TMS ether)	0.8	486; 396; 357; 207; 129; 119; 107; 73	486	[N]
unknown compound						
58.54	24	unknown compound	0.3	604; 589; 487; 397; 273; 205; 191; 109; 73		

[a]MS spectra were referenced with the literature[15,29−35] and publicly available databases [DB]: NIST Chemistry WebBook [N]; MassBank of North America [M]. Some compounds were tentatively identified [TI] according to their mass fragments and molecular weight (M_w)

aliphatic compounds of the suberin.[15] Isoeugenol, represented approximately 56.7 wt % of identified aromatic from Klara suberin, has shown its role in anticholinergic and antidiabetic effects.[37] Additionally, the glyceridic ester of the 4-methoxycinnamic acid has been traditionally used in the formulation of UV-B sun screening.[38] 4′-Hydroxy acetophenone (piceol) was identified here at suberin from both hybrids.

Fatty Acids. Together, 16 fatty acids (C7−C22) were detected from suberin monomers of Karin and Klara bark, symbolized as typical suberin markers. Fatty acid methyl esters (**FAMEs**) accounted for more than half of the total identified fatty acids from Karin (59.1 wt %) and Klara (68.0 wt %), which can be further isolated for biodiesel use.[39] Dodecanoic acid, hexadecanoic acid, and hexadecane-1,16-dioic acid were detected as major saturated fatty acids. 9,12-Octadecadienoic acid (linoleic acid) was the only detected diunsaturated fatty acid present at Klara suberin, which is known for inhibiting the ultraviolet-induced pigmentation of the skin.[40] Furthermore, 16-hydroxyhexadecanoic acid and 22-hydroxydocosanoic acid represented approximately 10 wt % based on fatty acids of Karin suberin. These long-chain ω-hydroxy fatty acids (omega HFAs) are rare in nature and known as important apoplastic substances protecting plants from radiation and pathogen infections,[41] which can be applied as potential anticancer agents.[42]

2,3-Dihydroxypropyl tetradecanoate, as a monoglyceride, accounted for 2.4 wt % from the Karin suberin, which was known as a promising emulsifier to improve the physical stabilities of the protein-stabilized emulsions.[43] 1-Pentadecanol was identified as the only aliphatic alcohol from the suberin substances, which accounted for 5.0 and 2.5 wt % based on Karin and Klara suberin, respectively. β-Sitosterol was the only

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sterol found in the studied *Salix*, which was also observed from its lipophilic extracts.

Chemical Characteristics of Hydrolyzable (Diethyl Ether-Soluble) Tannin. GC−MS analyzed the chemical composition of the hydrolyzable substances qualitatively. Figure 7, Table S4, and Figure S5 demonstrated that major

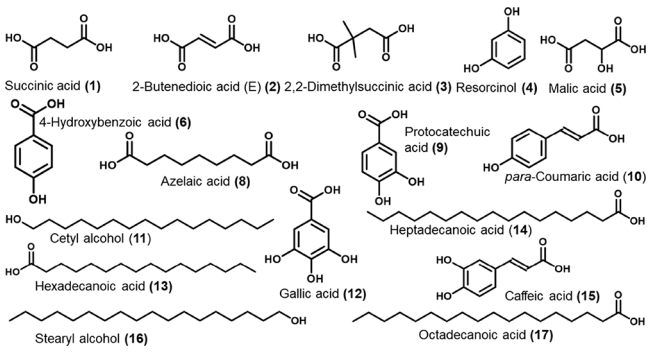

Figure 7. Detected diethyl ether-soluble components from Karin and Klara bark (Figure 1) based on the GC−MS total-ion chromatogram. Mass spectra of these trimethylsilylated compounds are summarized in Figure S6.

substances were suberin-derived fatty acid (succinic acid; 2-butanedioic acid and azelaic acid), 2-hydroxyacid (malic acid), and aromatic compounds (protocatechuic acid). Interestingly, the protocatechuic acid can be applied as a pharmaceutical agent for reducing the risks of cancer and diabetes.[44] *para*-Coumaric acid[45] and caffeic acid[46] were identified and reported to be the intermediate in the biosynthesis of lignin. Gallic acid, identified here, has been traditionally symbolized as hydrolyzable tannins. Resorcinol (insoluble in chloroform) can be used in the production of resins.[47] Fatty acids (heptadecanoic acid, 2,2,-dimethylsuccinic acid, and octadecanoic acid) and saturated long-chain aliphatic alcohols (cetyl alcohol and stearyl alcohol) were identified from both hybrids. Especially cetyl alcohol (C16) can be used for the treatment of eczema.[48] Furthermore, hexadecanoic acid was identified both from the chloroform-soluble suberin and diethyl ether-soluble fractions.

In conclusion, Willow bark suberin had a significantly higher amount of fatty acid (FAME enriched) moiety than aromatic compounds, which suggested a function of suberin not only in pathogen defense but also providing energy for various metabolic mechanisms at the cell wall of willow bark. The identified catechol and piceol might partly be linked with the water-soluble extractives (i.e., catechin and picein) of the studied hybrids. Catechol and gallic acid, symbolized as hydrolyzable tannins, were identified from the hydrolyzable fractions. Overall, these promising insights evidenced that suberin, lipophilic extractives, and its hydrolyzable tannin-like substances could be further applied as a source of pharmaceuticals and cosmetics. Willow outer and inner bark can be physically separated through the water flotation mechanism.[49] The hydrophilic inner bark absorbs water and can sink into the water, while the hydrophobic outer bark floats.[49] Therefore, the willow bark biorefinery concept[50] in Figure 8 is suggested here for the first time. The inner bark can be processed for extractives, sugars, and functional fibers,[9,51] whereas outer bark can be used as a source of suberin-derived pharmaceutical compounds. The extractives can be further

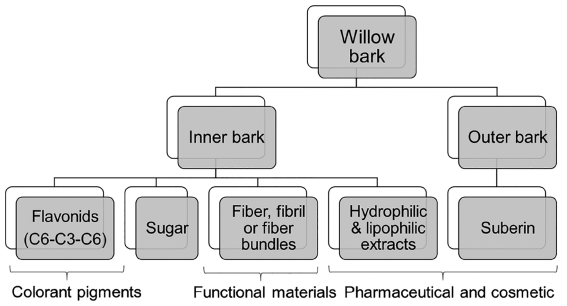

Figure 8. Fractionation scheme of a conceptual willow bark biorefinery.

chromatographically purified[52] for pharmaceutical[6] and colorant use.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c04112.

Section of the assignments for the solid-state ^{13}C CP/MAS spectra assignments and all the associated mass spectra (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) le Normand, M.; Edlund, U.; Holmbom, B.; Ek, M. Hot-water extraction and characterization of spruce bark non-cellulosic polysaccharides. *Nord. Pulp Pap. Res. J.* **2012**, *27*, 18−23.

(2) Devappa, R. K.; Rakshit, S. K.; Dekker, R. F. H. Forest biorefinery: Potential of poplar phytochemicals as value-added co-products. *Biotechnol. Adv.* **2015**, *33*, 681−716.
(3) Yazaki, Y. Wood Colors and their Coloring Matters: A Review. *Nat. Prod. Commun.* **2015**, *10*, 505−512.
(4) Dou, J.; Bian, H.; Yelle, D. J.; Ago, M.; Vajanto, K.; Vuorinen, T.; Zhu, J. Y. Lignin containing cellulose nanofibril production from willow bark at 80°C using a highly recyclable acid hydrotrope. *Ind. Crops Prod.* **2019**, *129*, 15−23.
(5) Hytönen, J.; Saarsalmi, A. Long-term biomass production and nutrient uptake of birch, alder and willow plantations on cut-away peatland. *Biomass Bioenergy* **2009**, *33*, 1197−1211.
(6) Kesari, K. K.; Dhasmana, A.; Shandilya, S.; Prabhakar, N.; Shaukat, A.; Dou, J.; Rosenholm, J. M.; Vuorinen, T.; Ruokolainen, J. Plant-Derived Natural Biomolecule Picein Attenuates Menadione Induced Oxidative Stress on Neuroblastoma Cell Mitochondria. *Antioxidants* **2020**, *9*, 552.
(7) Dou, J.; Xu, W.; Koivisto, J. J.; Mobley, J. K.; Padmakshan, D.; Kögler, M.; Xu, C.; Willför, S.; Ralph, J.; Vuorinen, T. Characteristics of Hot Water Extracts from the Bark of Cultivated Willow (*Salix* sp.). *ACS Sustainable Chem. Eng.* **2018**, *6*, 5566−5573.
(8) Lohtander, T.; Arola, S.; Laaksonen, P. Biomordanting willow bark dye on cellulosic materials. *Color. Technol.* **2020**, *136*, 3−14.
(9) Dou, J.; Paltakari, J.; Johansson, L.-S.; Vuorinen, T. Novel Insight into the Separation and Composite Utilization of Sclerenchyma Fiber Bundles of Willow Bark. *ACS Sustainable Chem. Eng.* **2019**, *7*, 2964−2970.
(10) de Simone, O.; Haase, K.; Müller, E.; Junk, W. J.; Hartmann, K.; Schreiber, L.; Schmidt, W. Apoplasmic Barriers and Oxygen Transport Properties of Hypodermal Cell Walls in Roots from Four Amazonian Tree Species. *Plant Physiol.* **2003**, *132*, 206−217.
(11) Kolattukudy, P. E. Biopolyester membranes of plants: cutin and suberin. *Science* **1980**, *208*, 990−1000.
(12) Graça, J. Suberin: the biopolyester at the frontier of plants. *Front. Chem.* **2015**, *3*, 62.
(13) Graça, J.; Pereira, H. Methanolysis of Bark Suberins: Analysis of Glycerol and Acid Monomers. *Phytochem. Anal.* **2000**, *11*, 45−51.
(14) Trockenbrodt, M. Light and electron microscopic investigations on wound reactions in the bark of *Salix caprea* L. and *Tilia tomentosa* MOENCH. *Flora* **1994**, *189*, 131−140.
(15) Branco, D. G.; Campos, J. R.; Cabrita, L.; Evtuguin, D. V. Structural features of macromolecular components of cork from *Quercus suber* L. *Holzforschung* **2020**, *74*, 625−633.
(16) Heinämäki, J.; Pirttimaa, M. M.; Alakurtti, S.; Pitkänen, H. P.; Kanerva, H.; Hulkko, J.; Paaver, U.; Aruväli, J.; Yliruusi, J.; Kogermann, K. Suberin Fatty Acids from Outer Birch Bark: Isolation and Physical Material Characterization. *J. Nat. Prod.* **2017**, *80*, 916−924.
(17) Križková, L.; Lopes, M. H.; Polónyi, J.; Belicová, A.; Dobias, J.; Ebringer, L. Antimutagenicity of a suberin extract from *Quercus suber* cork. *Mutat. Res.* **1999**, *446*, 225−230.
(18) Tamm, I.; Heinämäki, J.; Laidmäe, I.; Rammo, L.; Paaver, U.; Ingebrigtsen, S. G.; Škalko-Basnet, N.; Halenius, A.; Yliruusi, J.; Pitkänen, P.; Alakurtti, S.; Kogermann, K. Development of Suberin Fatty Acids and Chloramphenicol-Loaded Antimicrobial Electrospun Nanofibrous Mats Intended for Wound Therapy. *J. Pharm. Sci.* **2016**, *105*, 1239−1247.
(19) TAPPI - Technical Association Of The Pulp And Paper Industry. 1996b. *T 204 om-88: solvent extractives of wood and pulp*. Atlanta.
(20) TAPPI - Technical Association Of The Pulp And Paper Industry. 1999. *T 222 om-02: Acid − insoluble lignin in wood and pulp*. Atlanta.
(21) TAPPI - Technical Association Of The Pulp And Paper Industry. 1993. *T 211 om-93: Ash in wood, pulp, paper and paperboard: combustion at 525 °C*. Atlanta.
(22) Kim, H.; Padmakshan, D.; Li, Y.; Rencoret, J.; Hatfield, R. D.; Ralph, J. Characterization and Elimination of Undesirable Protein Residues in Plant Cell Wall Materials for Enhancing Lignin Analysis by Solution-State Nuclear Magnetic Resonance Spectroscopy. *Biomacromolecules* **2017**, *18*, 4184−4195.
(23) Dou, J.; Kim, H.; Li, Y.; Padmakshan, D.; Yue, F.; Ralph, J.; Vuorinen, T. Structural Characterization of Lignins from Willow Bark and Wood. *J. Agric. Food Chem.* **2018**, *66*, 7294−7300.
(24) Ramos, P. A. B.; Moreirinha, C.; Santos, S. A. O.; Almeida, A.; Freire, C. S. R.; Silva, A. M. S.; Silvestre, A. J. D. Valorisation of bark lipophilic fractions from three Portuguese *Salix* species: A systematic study of the chemical composition and inhibitory activity on *Escherichia coli*. *Ind. Crops Prod.* **2019**, *132*, 245−252.
(25) Szafranek, B.; Tomaszewski, D.; Pokrzywińska, K.; Gołębiowski, M. Microstructure and chemical composition of leaf cuticular waxes in two *Salix* species and their hybrid. *Acta Biol. Crac. Ser. Bot.* **2008**, *50*, 49−54.
(26) Mageroy, M. H.; Parent, G.; Germanos, G.; Giguère, I.; Delvas, N.; Maaroufi, H.; Bauce, É.; Bohlmann, J.; Mackay, J. J. Expression of the *β*-glucosidase gene *Pgβglu-1* underpins natural resistance of white spruce against spruce budworm. *Plant J.* **2015**, *81*, 68−80.
(27) Kukić, J.; Popović, V.; Petrović, S.; Mucaji, P.; Ćirić, A.; Stojković, D.; Soković, M. Antioxidant and antimicrobial activity of *Cynara cardunculus* extracts. *Food Chem.* **2008**, *107*, 861−868.
(28) Lopes, M. H.; Gil, A. M.; Silvestre, A. J. D.; Neto, C. P. Composition of Suberin Extracted upon Gradual Alkaline Methanolysis of *Quercus suber L. Cork. J. Agric. Food Chem.* **2000**, *48*, 383−391.
(29) Järvinen, R.; Silvestre, A. J. D.; Holopainen, U.; Kaimainen, M.; Nyyssölä, A.; Gil, A. M.; Pascoal Neto, C.; Lehtinen, P.; Buchert, J.; Kallio, H. Suberin of Potato (*Solanum tuberosum* Var. Nikola): Comparison of the Effect of Cutinase CcCut1 with Chemical Depolymerization. *J. Agric. Food Chem.* **2009**, *57*, 9016−9027.
(30) Kallio, H.; Nieminen, R.; Tuomasjukka, S.; Hakala, M. Cutin Composition of Five Finnish Berries. *J. Agric. Food Chem.* **2006**, *54*, 457−462.
(31) Marques, G.; Gutiérrez, A.; del Río, J. C. Chemical Characterization of Lignin and Lipophilic Fractions from Leaf Fibers of Curaua (*Ananas erectifolius*). *J. Agric. Food Chem.* **2007**, *55*, 1327−1336.
(32) Nicolaides, N.; Soukup, V. G.; Ruth, E. C. Mass Spectrometric Fragmentation Patterns of the Acetoxy and Trimethylsilyl Derivatives of all the Positional Isomers of the Methyl Hydroxypalmitates. *Biomed. Mass Spectrom.* **1983**, *10*, 441−449.
(33) Xia, W.; Budge, S. M. GC-MS Characterization of Hydroxy Fatty Acids Generated From Lipid Oxidation in Vegetable Oils. *Eur. J. Lipid Sci. Technol.* **2018**, *120*, No. 1700313.
(34) Oliw, E. H. Analysis of 1,2-diols of linoleic, *α*-linolenic and arachidonic acid by gas chromatography—mass spectrometry using cyclic alkyl boronic esters. *J. Chromatogr.* **1983**, *275*, 245−259.
(35) Street, J. M.; Evans, J. E.; Natowicz, M. R. Glucuronic Acid-conjugated Dihydroxy Fatty Acids in the Urine of Patients with Generalized Peroxisomal Disorders. *J. Biol. Chem.* **1996**, *271*, 3507−3516.
(36) Ou, S.; Kwok, K.-C. Ferulic acid: pharmaceutical functions, preparation and applications in foods. *J. Sci. Food Agric.* **2004**, *84*, 1261−1269.
(37) Topal, F. Anticholinergic and antidiabetic effects of isoeugenol from clove (*Eugenia caryophylata*) oil. *Int. J. Food Prop.* **2019**, *22*, 583−592.
(38) Freitas, Z. M. F.; Gonçalves, J. G. S.; Santos, E. P.; Vergnanini, A. Glyceridic esters of *p*-methoxycinnamic acid. A new sunscreen of the cinnamate class. *Int. J. Cosmet. Sci.* **2001**, *23*, 147−152.
(39) Vyas, A. P.; Verma, J. L.; Subrahmanyam, N. A review on FAME production processes. *Fuel* **2010**, *89*, 1−9.
(40) Ando, H.; Ryu, A.; Hashimoto, A.; Oka, M.; Ichihashi, M. Linoleic acid and *α*-linolenic acid lightens ultraviolet-induced hyperpigmentation of the skin. *Arch. Dermatol. Res.* **1998**, *290*, 375−381.
(41) Liu, J.; Zhang, C.; Lu, W. Biosynthesis of Long-Chain *ω*-Hydroxy Fatty Acids by Engineered *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* **2019**, *67*, 4545−4552.

https://doi.org/10.1021/acs.jafc.1c04112
J. Agric. Food Chem. 2021, 69, 10848−10855

(42) Abe, A.; Sugiyama, K. Growth Inhibition and Apoptosis Induction of Human Melanoma Cells by Omega-Hydroxy Fatty Acids. *Anti-Cancer Drugs* **2005**, *16*, 543−549.
(43) Loi, C. C.; Eyres, G. T.; Birch, E. J. Effect of mono- and diglycerides on physical properties and stability of a protein-stabilised oil-in-water emulsion. *J. Food Eng.* **2019**, *240*, 56−64.
(44) Kakkar, S.; Bais, S. A Review on Protocatechuic Acid and Its Pharmacological Potential. *ISRN Pharmacol.* **2014**, *2014*, No. 952943.
(45) Ralph, J.; Hatfield, R. D.; Quideau, S.; Helm, R. F.; Grabber, J. H.; Jung, H. J. G. Pathway of *p*-Coumaric Acid Incorporation into Maize Lignin As Revealed by NMR. *J. Am. Chem. Soc.* **1994**, *116*, 9448−9456.
(46) Boerjan, W.; Ralph, J.; Baucher, M. Lignin biosynthesis. *Annu. Rev. Plant Biol.* **2003**, *54*, 519−546.
(47) Arbenz, A.; Avérous, L. Chemical modification of tannins to elaborate aromatic biobased macromolecular architectures. *Green Chem.* **2015**, *17*, 2626−2646.
(48) Soga, F.; Katoh, N.; Kishimoto, S. Contact dermatitis due to lanoconazole, cetyl alcohol and diethyl sebacate in lanoconazole cream. *Contact Dermatitis* **2004**, *50*, 49−50.
(49) Eckerman, C.; Ekman, R. Comparison of solvents for extraction and crystallisation of betulinol from birch bark waste. *Pap. Puu* **1985**, *67*, 100−106.
(50) Dou, J.; Galvis, L.; Holopainen-Mantila, U.; Reza, M.; Tamminen, T.; Vuorinen, T. Morphology and Overall Chemical Characterization of Willow (Salix sp.) Inner Bark and Wood: Toward Controlled Deconstruction of Willow Biomass. *ACS Sustainable Chem. Eng.* **2016**, *4*, 3871−3876.
(51) Dou, J.; Rissanen, M.; Ilina, P.; Mäkkylä, H.; Tammela, P.; Haslinger, S.; Vuorinen, T. Separation of fiber bundles from willow bark using sodium bicarbonate and their novel use in yarns for superior UV protection and antibacterial performance. *Ind. Crops Prod.* **2021**, *164*, No. 113387.
(52) Dou, J.; Heinonen, J.; Vuorinen, T.; Xu, C.; Sainio, T. Chromatographic recovery and purification of natural phytochemicals from underappreciated willow bark water extracts. *Sep. Purif. Technol.* **2021**, *261*, No. 118247.

https://doi.org/10.1021/acs.jafc.1c04112
J. Agric. Food Chem. 2021, 69, 10848−10855