



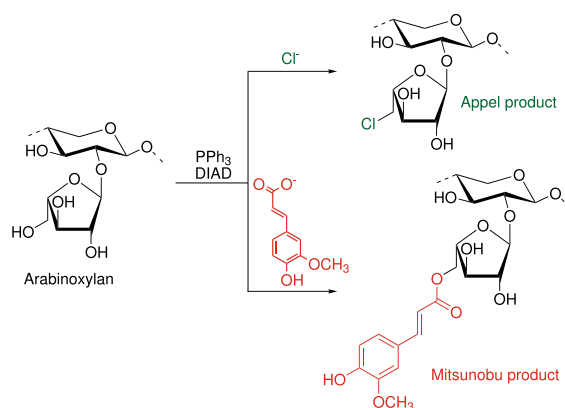
Overcoming challenges in the synthesis of a lignin-carbohydrate complex (LCC) model: Mitsunobu versus Appel product

Thomas Elschner · Erica Brendler ·
Steffen Fischer

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Abstract Arabinoxylan ferulate representing a macromolecular LCC model valid for annual plants is synthesized under Mitsunobu conditions. The content of ferulic acid ester is tuned by the reaction conditions achieving degree of substitution values from 0.09 to 0.45. Utilization of the chloride-free solvent N-methyl-2-pyrrolidone allows the design of pure Mitsunobu products without occurrence of deoxychloro moieties arising from Appel type reaction. 2D NMR experiments reveal nature-identical structure of ferulate moieties present at position 5 of the arabinose side chain. Enzymatic dehydrogenation polymerization of coniferyl alcohol on ferulate anchor groups under homogeneous conditions lead to β -O-4, β -5, and Hibbert ketone structures identified by Py-GC-MS. The results are valuable to study structure-property relationships within the formation of natural and non-native lignins.

Graphical abstract



Keywords Arabinoxylan ferulate · Biomass · Carbohydrates · Mitsunobu reaction · Synthetic methods

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T. Elschner (✉) · S. Fischer
Institute of Plant and Wood Chemistry, Technische Universität Dresden, Piennner Str. 19, 01737 Tharandt, Germany
e-mail: thomas.elschner@tu-dresden.de

E. Brendler
Institute of Analytical Chemistry, TU Bergakademie Freiberg, Leipziger Str. 29, 09599 Freiberg, Germany

Introduction

Lignocellulosic biomass, a sustainable resource for biomaterials and biofuel, is predestined to substitute fossil prospects. However, the interesting main components cellulose, hemicellulose, and lignin are locked in the structural complexity of the plant cell walls (Giummarella et al. 2019). In practice, there is an unpleasant recalcitrance of lignocellulose regarding selective fractionation and digestibility, i.e. unresolved challenges in biorefining. One approach to

make annual plants (e.g. grasses) more accessible to bioconversion is the targeted genetic engineering of cell wall polysaccharides (Bhatia et al. 2017). Those efforts include remodelling of the chemical structure and substitution patterns. However, the chemical synthesis of cell wall polysaccharides is complementing a very promising concept, yielding model compounds independent of genetic information.

Approaches to discover the secrets of lignin-carbohydrate complexes (LCCs) are based on solid-state nuclear magnetic resonance on intact plant cells (Kirui et al. 2022) or isolation and analysis of LCCs including model compound studies (Balakshin et al. 2014; Tarasov et al. 2018). Several types of so called LCC models have been employed. To mimic the covalent linkage between hemicellulose and lignin in forage plants, typical fragments composed of hydroxycinnamic acid esters and arabinoxylan oligosaccharides were synthesized by protecting group chemistry of sugars (Ralph and Helm 1993).

Focusing on macromolecular approaches, the cell wall polysaccharide xylan decorated with hydroxycinnamates is a predestinated model compound to perform lignification experiments, i.e. enzymatic dehydrogenation polymerization of monolignols (Barakat et al. 2007b, a). Similar experiments were carried out directly on natural plant cell walls (Grabber et al. 1996, 1998, 2002). Moreover, cell wall models composed of honeycomb-patterned films were applied as supports for artificial lignification (Li et al. 2015). Dehydrogenation polymerization on 2D model films (Lyu et al. 2021; Elschner et al. 2022) allows a discovery with surface sensitive analytical techniques such as quartz crystal microbalance with a dissipation monitoring (QCM-D).

In the light of all macromolecular LCC models, it is advancing to synthesize tailorable polymer structures, e.g. with varying degree of substitution (DS) and regioselective substitution pattern. A first attempt was the esterification of cellulose with hydroxycinnamic acids to investigate the digestibility by rumen fluid (Jung and Sahlu 1986). However, in nature hydroxycinnamates are not bound directly to cellulose. Therefore, improved model compounds of phenolic acid-hemicellulose esters were synthesized to study digestibility (Jung et al. 1991). Within this work we focus on esterification of arabinoxylan with ferulic acid demonstrating a powerful tool to adjust the amount of hydroxycinnamic acids including

regiochemistry at the primary hydroxyl group of position 5 of the arabinose side chain. Thus, we mimic the typical structural motive in plant cell walls of ferulic acid acting as cross-linker between lignin and hemicellulose (Hartley 1972). Ferulic acid is the key component in grass lignocellulose causing recalcitrance to enzyme hydrolysis (de Oliveira et al. 2015).

An early method for esterification of polysaccharides with ferulic acid chloride yield less defined products (Jung and Sahlu 1986; Jung et al. 1991) since phenolic hydroxyl groups might be converted, too. Therefore, a two-step procedure was established using acetylated ferulic acid chloride. However, the protecting group has to be removed by sodium borohydride in a long time reaction (up to 100 h, Wrigstedt et al. 2010). For the synthesis of starch ferulate the well established reagent *N,N'*-carbonyldiimidazole (CDI) was applied (Wen et al. 2016), but sensitive double bonds of cinnamic acids may cause an undesired addition reaction of released imidazole. Steglich esterification of cellulose (Trombino et al. 2008, 2009a, b) leads to products with poor solubility and the byproduct dicyclohexyl urea is very hard to remove. Moreover, all previous reagents for esterification of polysaccharides with hydroxycinnamic acids are not selective regarding specific hydroxyl groups. In the present work, authors come up with a regioselective one-step procedure.

Recently, we found an innovative synthesis route for ferulic acid esters of cellulose applying Mitsunobu conditions (Elschner et al. 2021). This method is insensitive regarding double bonds and phenolic hydroxyl groups present in phenolic acids. Protecting group chemistry was not necessary and moreover, side products could be removed. Cellulose ferulates obtained by Mitsunobu chemistry were found to be soluble in dimethyl sulfoxide (DMSO). Since the reaction is selective to primary hydroxyl groups, Mitsunobu reaction seems to be excellently suited to modify the arabinose side chain of xylan at position 5 to synthesize natural structures. Therefore, we adapted the reaction conditions to arabinoxylan, but surprisingly found the occurrence of deoxychloro moieties next to ferulate in the polymer backbone.

In this work, we focus on the competing reactions forming Mitsunobu type and Appel type products of arabinoxylan. Critical analysis of results in non-innocent solvents containing chloride ions lead us to a convenient procedure to yield nature-identical

LCCs, present in annual plants. The structures could be obtained without deoxychloro moieties, but adjustable amount of ferulate at position 5 of the arabinose side chain.

Experimental

Materials

Arabinoxylan from wheat was provided by Jäckering Mühlen- und Nahrungsmittelwerke GmbH (Hamm, Germany) and possessed a \overline{M}_w of 1315 g mol⁻¹ and a PDI of 1.49. The molar ratio of monosaccharides after acidic hydrolysis (Fengel et al. 1978) applying trifluoroacetic acid (TFA) was found to be 44 % xylose, 39 % arabinose, 10 % galactose, and 7 % glucose by HPLC. Accordingly, the molar mass of one average repeating unit is 137.31 g mol⁻¹ and the amount of primary hydroxyl groups is 4.0 mmol g⁻¹. Arabinoxylan was dried at 60 °C in vacuum before use. Lithium chloride (98.5 %, Carl Roth) was dried at 100 °C in vacuum. ABTS (diammonium salt of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) was received from Roche Diagnostics (Mannheim, Germany). Horseradish peroxidase (HRP) from Merck (Darmstadt, Germany) possessed an activity of 59 U mg⁻¹ in NMP/ H₂O (1:10) according to ABTS assay. Miscellaneous chemicals were purchased and were used without further purification as listed below.

N-Methyl-2-pyrrolidone (99.5 %, extra dry, Acros); N,N-Dimethylformamide (99.8 %, extra dry, Acros); N,N-Dimethylacetamide (99.8 %, anhydrous, Sigma-Aldrich) Chloroform-d₁ (deuteration degree > 99.8 %, VWR); DMSO-d₆, deuteration degree 99.80 %, VWR); Coniferyl alcohol (98 %, Alfa Aesar); Triphenylphosphine (99.5 %, Carl Roth); Diisopropyl azodicarboxylate (98 %, Sigma-Aldrich); trans-Ferulic acid (99 %, Sigma-Aldrich); Pyridine (99.8 %, anhydrous, Sigma-Aldrich); Acetic anhydride (99 %, Grüssing); Hydrogen peroxide (35 %, Carl Roth); Sodium hydrogencarbonate (99 %, Carl Roth)

Measurements

NMR spectra were recorded with a Bruker Avance III 600 MHz spectrometer and a Bruker Avance Neo 700 MHz SB instrument applying up to 80 mg mL⁻¹

sample in DMSO-d₆ or CDCl₃. Up to 10000 scans were applied for ¹³C NMR measurements. For ¹H NMR experiments, relaxation delay was set to 10 s for quantitative evaluation.

FTIR measurements were carried out on a FTIR spectrometer Tensor27 (Bruker Optics GmbH).

UV spectroscopy was performed with a UV-Vis spectrometer V-650 (Jasco).

HPLC analysis of arabinoxylan hydrolyzate was performed on a HPLC/UHPLC unit Azura (Knauer). Measurements were run at a temperature of 80 °C and a flow rate of 0.3 mL min⁻¹ (H₂O) using Agilent Met-aCarb87P columns (50 × 4.6 mm and 300 × 7.8 mm) and the RID2.1L detector (Knauer). Glucose, arabinose, xylose, and galactose were used as standards for calibration, applying a concentration of 5 mg mL⁻¹. Samples were pressed through a syringe filter (0.45 μm, CA membrane) before injection (volume of 15 μL).

SEC was measured with a HPLC/UHPLC unit Azura (Knauer) with RID2.1L detector (Knauer) using DMSO with 0.1 % NaNO₃ and a sample concentration of 1 mg mL⁻¹. The temperature was set to 60 °C and the flow rate was 0.3 mL min⁻¹. Separation was achieved by the columns PolarGel-M (Agilent) and ABOA DMSO-phiL-P-250 (AppliChrom). Dextran standards (180–225000 g mol⁻¹) were used for calibration. The samples were filtered through a 0.45 μm CA membrane (syringe filter) before injecting 30 μL.

Elemental analyses were carried out with an UNICUBE device (Elementar, Langensfeld, Germany).

Py-GC-MS was performed on an Agilent system (GC 7890 B/MSD 5977). Samples were pyrolyzed by a Multi-Shot Pyrolyzer EGA/PY-3030D (Frontier Lab) at 450 °C. A ZB-5MS capillary column (30 m × 0.25 mm) with a temperature program of 50–240 °C at 4 K min⁻¹ was used for separation.

Synthesis of cellulose hydroxycinnamates, general procedure

Reactions were carried out under nitrogen atmosphere using dry solvents. Arabinoxylan (1 g, 7.28 mmol, 4.0 mmol primary OH) was dissolved in 15 mL of the corresponding amide and heated to 80 °C for 1 h. For examples **3a-c**, LiCl (0.75 g) was added and the mixture was allowed to cool down yielding a clear solution. Ferulic acid (up to 1.55 g, 8.0 mmol) was

dissolved in the amide solvent (3 mL) and added to the xylan solution. Afterwards, triphenylphosphine (up to 2.10 g, 8.0 mmol) was added and the solution was cooled with an ice bath to 0 °C. Diisopropyl azodicarboxylate (DIAD, up to 1.6 mL, 8.0 mmol) was added dropwise under stirring. The solution was allowed to warm up to room temperature and stirred for 2 d. The product was obtained by precipitation into methanol (350 mL) and filtration. It was washed four times with methanol (150 mL). The solid was dried at 40 °C in vacuum. For further purification, reprecipitation from DMSO in methanol was carried out.

Arabinoxylan ferulate (**3a**) Yield: 73 %, DS_{FA} 0.11, DS_{Cl} 0.10; ^{13}C NMR (175 MHz, DMSO- d_6): δ [ppm] = 165.1, 164.7 (C=O), 151.8, 150.4, 149.8, 148.5 (C), 147.9, 146.7, 145.9 (CH), 142.0, 133.3, 126.0 (C), 123.9, 122.7 (CH), 117.5, 116.0 (CH), 114.6, 113.4, 112.7, 112.3, 111.9, 111.5 (CH), 109.5 (C1-Ara), 102.2 (C1-Xyl), 87–72 (C2–4), 63.7 (C5-Xyl), 61.5 (C5-Ara), 56.5, 56.2 (OCH₃), 45.5 (CH₂Cl).

Lignification

Dehydrogenation polymerization was carried out following the bulk method. Arabinoxylan ferulate (10 mg) was dissolved in N-methyl-2-pyrrolidone (NMP, 1 mL) and added to an aqueous solution of coniferyl alcohol (10 mg in 10 mL) dropwise under stirring. Horseradish peroxidase (HRP, 1 mg) was dissolved in the reaction mixture followed by the addition of H₂O₂ (500 μ L, 0.1 M) at once. After 1 h and after 20 h stirring at room temperature, the same amounts of H₂O₂ were added at once again. The clear solution was concentrated by a rotary evaporator after 24 h total reaction time. The residue was precipitated into ethanol (5 mL) to isolate the product. The precipitate was separated by centrifugation and washed three times with ethanol. The product was dried in vacuum at 40 °C. The procedure was repeated without arabinoxylan ferulate yielding colloidal particles, which were isolated from diethyl ether.

Determination of enzyme activity

The enzyme activity of HRP in NMP/water (1:10) was determined by the ABTS assay. The oxidation product [ABTS*]⁺ was quantified by UV-Vis spectroscopy at 405 nm. For recording the reaction

kinetics, ABTS (5 mM), H₂O₂ (5 mM), and HRP (2.5 *10⁻⁵ to 5*10⁻⁶ mg mL⁻¹) in ultra pure water were used. HRP activity was calculated from the slopes of the time-dependent absorption with Lambert-Beer law. The extinction coefficient ϵ of [ABTS*]⁺ is 27.5 L mmol⁻¹cm⁻¹ and was taken from literature (Obst et al. 2019).

Determination of DS values

Arabinoxylan ferulate (150 mg) was dissolved in N,N-dimethylformamide (DMF, 3 mL) at 60 °C. The solution was allowed to cool to room temperature and pyridine (2.5 ml) as well as acetic anhydride (2.5 mL) were added dropwise under stirring. Subsequently, the reaction mixture was stirred at 60 °C over night. After cooling to room temperature, impurities were centrifuged off. The clear solution was precipitated into deionized water (150 mL) containing NaHCO₃ (0.5 g). The product was isolated by filtration, washed four times with deionized water (150 mL) and dried in vacuum at 40 °C. Complete acetylation, i.e. absence of OH groups, was proved by FTIR spectroscopy.

DS values of ferulate (DS_{FA}) were calculated from integral intensities (I) of ¹H NMR signals (Figure S5) of CH-protons (8.0–6.0 ppm) and CH₃-protons (2.5–1.0 ppm) according to Equation 1. n_{OH} = 2.17 is the number of hydroxyl groups per average repeating unit and was determined from sugar composition by HPLC. DS_{Cl} was determined from the chlorine content of elemental analysis.

$$DS_{FA} = \frac{3(n_{OH} - DS_{Cl})I_{CH}}{5I_{CH_3}} \quad (1)$$

Results and discussion

Mitsunobu versus Appel product

Conventional synthesis of xylan ferulates with acetyl-protected ferulic acid chloride yields products possessing ferulate moieties also at the secondary hydroxyl groups of the polymer backbone. However, nature-identical structures are only decorated with ferulate at primary position 5 of the arabinose side chain. With respect to yield natural structures, we found a novel synthesis path and obtained this

lignin-carbohydrate complex (LCC) model of annual plants for the first time by organic synthesis.

Recently, the synthesis of cellulose ferulate under Mitsunobu conditions in *N,N*-dimethylacetamide (DMA)/LiCl was performed (Elschner et al. 2021). This reaction was found to be highly selective including preferred modification of primary hydroxyl groups and tolerance regarding double bonds and phenolic hydroxyl groups. Thus, innovative Mitsunobu chemistry on polysaccharides overcomes utilization of protecting groups and drawbacks of Steglich esterification, i.e. hardly removable dicyclohexylurea and poor solubility of derivatives. With respect to the synthesis of LCC models, similar conditions were adapted to design nature-identical ferulic acid esters of arabinoxyylan.

In first experiments, *N,N*-dimethylformamide (DMF)/LiCl was used as reaction medium to enable proper dissolution of arabinoxyylan. Ferulic acid and triphenylphosphine (PPh_3) were added to yield a clear reaction mixture. Diisopropyl azodicarboxylate (DIAD) was added at 0°C to follow the standard Mitsunobu protocol. However, next to the occurrence of ferulate groups, deoxychloro moieties appeared in the product, indicated by a ^{13}C NMR signal at 45 ppm and a significant chlorine content. Obviously, chloride ions from the solvent system were involved in the $\text{S}_{\text{N}}2$ reaction (Fig. 1). A detailed reaction mechanism is depicted in Scheme S1.

The primary hydroxyl group at position 5 of the arabinose side chain is converted into the triphenylphosphonium ion (2) by PPh_3 and DIAD in a first step. Subsequent $\text{S}_{\text{N}}2$ reaction with ferulate as well as

chloride ions from the solvent system takes place to form the products (3). In fact, we found a mixture of Mitsunobu (DS_{FA} 0.11) and Appel (DS_{Cl} 0.10) product (Table 1, 3a).

Although we did not observe this Appel product during Mitsunobu reaction on cellulose, this synthesis path is known for carbohydrates. The typical Appel reagent, that is PPh_3 and a carbon tetrahalide, converts primary hydroxyl groups of e.g. kestose into halides via $\text{S}_{\text{N}}2$ reaction (Tachrim et al. 2018). This reagent was already used to synthesize 6-azido-6-deoxy polysaccharides in DMA/ LiN_3 (Cimecioglu et al. 1997; Shey et al. 2006). Nevertheless, the Appel product is also obtained under Mitsunobu conditions in presence of lithium salts (Manna et al. 1985). Dar

Table 1 Synthesis of arabinoxyylan ferulate

Solvent ^a	Molar ratio ^b	No	$\text{DS}_{\text{FA}}^{\text{c}}$	$\text{DS}_{\text{Cl}}^{\text{d}}$	Yield [%]
DMF/LiCl	1.0	3a	0.11	0.10	73
DMA/LiCl	1.0	3b	0.12	0.06	71
DMA/LiCl	1.5	3c	0.24	0.07	93
DMSO/THF	1.0	3d	0.03	–	63
NMP	1.0	3e	0.09	–	69
NMP	1.5	3f	0.23	–	80
NMP	2.0	3g	0.45	–	96

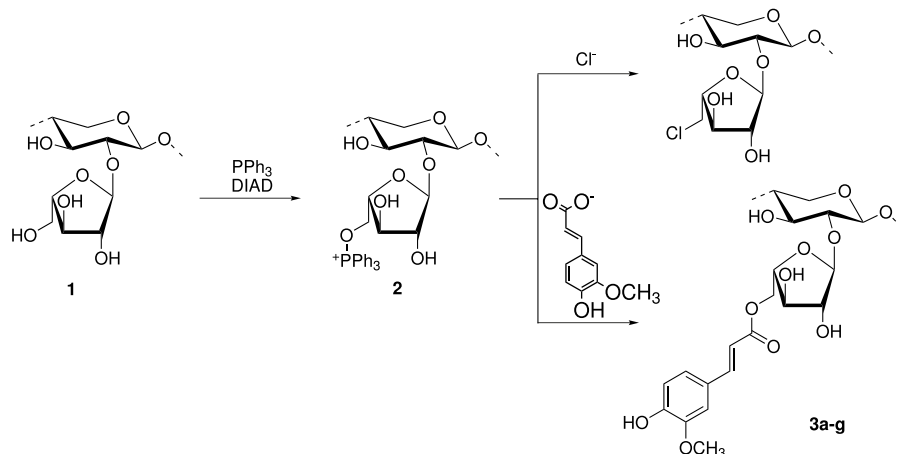
^a DMF: *N,N*-Dimethylformamide, DMSO: Dimethyl sulfoxide, THF: Tetrahydrofuran, DMA: *N,N*-Dimethylacetamide, NMP: *N*-Methyl-2-pyrrolidone

^b Mole reagents per mole primary OH groups

^c Degree of substitution (DS) of ferulate

^d DS of deoxychloro moieties

Fig. 1 Competing reactions: Triphenylphosphonium ion of arabinose side chain (2) is formed under Mitsunobu conditions from arabinoxyylan (1) and reacts via $\text{S}_{\text{N}}2$ not only with ferulate, but also with chloride ions of the solvent system



et al. (2013) were investing the Mitsunobu reaction applying PPh_3 and DIAD with trimethylsilyl (TMS) chloride. According to their proposed mechanism, we also suggest for arabinoxylan (Fig. 1), in sugars and sterically hindered non-sugars, only the primary hydroxyl group is substituted by chlorine.

Further experiments were carried out in DMA/LiCl. Within a first hypothesis, we assumed a lower reactivity of chloride ions, since the complex DMA/LiCl is more stable compared to DMF/LiCl (Morgenstern and Berger 1993). Stability of amide solvent/LiCl complexes was determined by ^{13}C NMR spectroscopy (El-Kafrawy 1982) and a cation-sensitive glass electrode (Nakamura 1975) in the past. Indeed, a lower DS value of deoxychloro moieties (DS_{Cl} 0.06, **3b**) is observed in DMA compared to DMF (DS_{Cl} 0.10, **3a**) at the same molar ratio of 1.0, i.e. the reactivity of Cl^- was decreased. Up to now, the different behavior of cellulose and arabinoxylan under Mitsunobu conditions could not be explained unambiguously. Probably, there are effects arising from different supramolecular structures or organo-catalysts present as impurities in arabinoxylan.

In the light of the synthesis of natural LCC models, the incorporation of chloro moieties should be avoided and thus, chlorine-free solvents were used. A solution of arabinoxylan in dimethyl sulfoxide (DMSO) was diluted with tetrahydrofuran (THF) to decrease the melting point of the solvent, when performing the reaction at 0°C . However, the DS value of the product was very low when applying a molar ratio of 1.0 (**3d**, DS_{FA} 0.03, Table 1). Although it was possible to adjust a volume ratio of DMSO/THF of 3:1 to keep arabinoxylan in solution, freezing of DMSO at the wall of the flask was unavoidable. It can be assumed that solvent polarity and occurring inhomogeneity are not beneficial to perform efficient Mitsunobu reactions.

Finally, we overcame the challenges with non-innocent solvents and used N-methyl-2-pyrrolidone (NMP) without LiCl, which is able to dissolve wheat arabinoxylan. Varying the molar ratio, the DS_{FA} could be adjusted from 0.09 to 0.45 (**3e-g**). Based on the sugar composition obtained from HPLC, a maximum DS value of 0.55 could be achieved by modification of all primary hydroxyl groups. Thus, a molar ratio of 2.0 (mole reagents per mole primary OH groups) lead to functionalization of about 82 % of theoretically available moieties. In general, an increasing yield was

observed with increasing DS, which can be explained by an improved precipitation behavior of higher functionalized products compared to pure arabinoxylan.

In comparison with literature data, the DS values of the products obtained within this work are in a similar range, but theoretically limited to 0.55 for used arabinoxylan, since only primary hydroxyl groups are reactive under Mitsunobu conditions. Wrigstedt et al. (2010) used oat spelt xylan and birchwood xylan that was allowed to react with the acetyl-protected acid chloride to yield ferulates with DS values between 0.05 and 0.89 (values of deprotected products). The authors showed by ^{13}C NMR spectroscopy that secondary positions 2 and 3 of the anhydroxylose unit as well as the arabinose side chain could be functionalized (Wrigstedt et al. 2010). In contrast, Mitsunobu reaction lead to selective esterification of the primary hydroxyl group of the arabinose side chain, as revealed by NMR spectroscopy below.

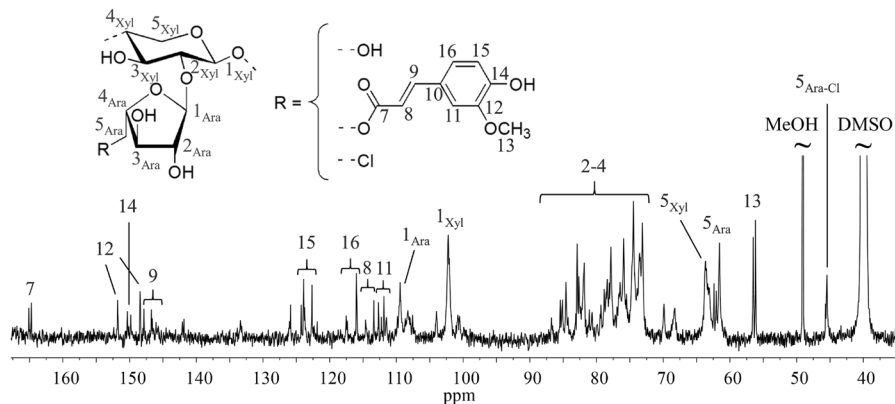
The content of ferulate in natural sources may vary from 0.53 % or 1.34 % for wheat bran xylan up to 3.1 % for maize bran, i.e. maize bran xylan could contain more than 5 % of ferulic acid ester (Rudjito et al. 2019; Pazo-Cepeda et al. 2021; Mathew and Abraham 2004). In comparison, our synthetic samples possess 3.8 % to 36.5 % of ferulate. Thus, organic synthesis may yield arabinoxylan ferulates from low up to non-natural high degree of functionalization.

Proof of molecular structure

FTIR spectroscopy revealed typical signals of the aliphatic $-\text{C}-\text{H}$ stretches (2900 cm^{-1}) and the $-\text{C}=\text{O}$ vibration of the ester (1724 cm^{-1}). Aromatic structural motives are indicated at 1635, 1598, and 1513 cm^{-1} (Figure S1).

^{13}C NMR spectroscopy of arabinoxylan ferulate (Fig. 2) allowed a clear evaluation of molecular structure. The resonances arising from position 1 and 5 of the arabinose side chain and the anhydroxylose unit could be assigned according to literature (Savitha Prashanth and Muralikrishna 2014). The CH_2Cl signal of deoxychloro moieties is visible at 45 ppm as proved by the DEPT 135 experiment and reference value (Heinze et al. 2006). The resonances of the ferulate substituent could be assigned by means of 2D NMR spectroscopy. Combining information from HSQC and TOCSY experiment (Fig. 3), CH signals could be clarified. TOCSY with short mixing time

Fig. 2 ^{13}C NMR spectrum of arabinoxylan ferulate **3a** recorded in DMSO- d_6



shows coupling of protons 8 and 9 along the double bond. Moreover, correlation from proton 9 to position 16 (ring) is visible. The connection from adjacent CH moieties within the ring (15 and 16) could be detected. HMBC experiment (Figure S2) reveals the correlation between methoxy protons (13) and quaternary carbon 12 of the ring. The $\text{C}=\text{O}$ signal at 165 ppm evidence the linkage of ferulate to the polymer backbone. Moreover, HMBC indicates the ester at position 5 of arabinose side chain as present in nature (Figure S3). Two cross-peaks show coupling via 3 bonds of $\text{C}=\text{O}$ to CH_2 group (position 5) and a further cross-peak is visible that might arise from correlation of $\text{C}=\text{O}$ to CH moiety (position 2) via 4 bonds.

Lignification of arabinoxylan ferulate under homogeneous conditions

Nature-identical arabinoxylan with adjustable content of ferulic acid ester that was synthesized, is predestinated for mimicking of the lignification process. While enzymatic dehydrogenation polymerization of monolignols in aqueous solution usually yields colloidal particles of dehydrogenation polymer (DHP), our attempt is based on homogeneous lignification in NMP/ H_2O (1:10). Polymerization is starting from ferulate groups of arabinoxylan. The LCC was kept dissolved during the reaction with coniferyl alcohol. The blank experiment without arabinoxylan ferulate resulted in the formation of a particle dispersion.

The molecular structure of formed DHP was characterized by Py-GC-MS (Hoffmann et al. 2019). Figure 4 shows chromatograms of DHP-arabinoxylan (top) and pure DHP (bottom). Broad signals arising from carbohydrates are visible but not marked.

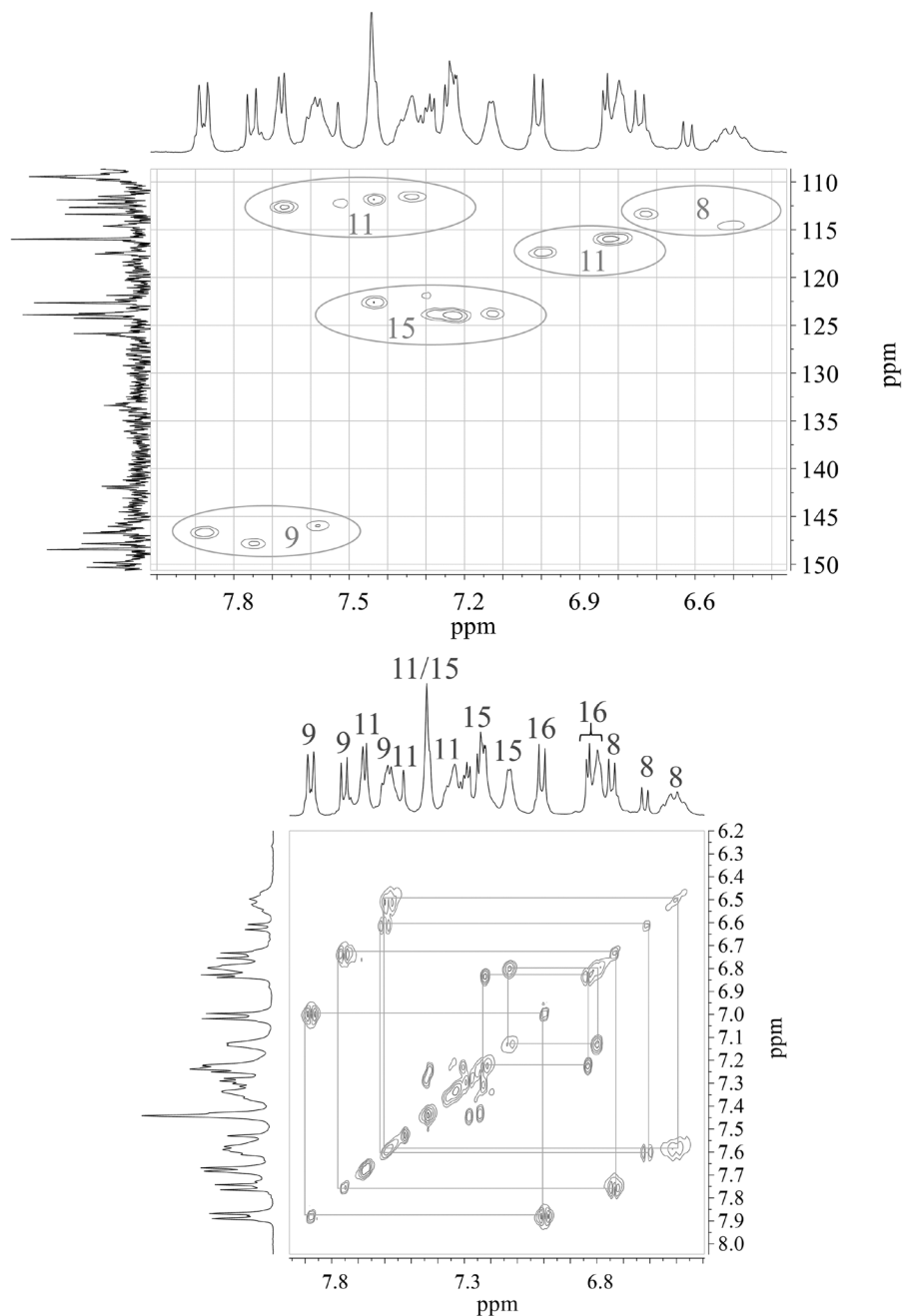
Coniferyl alcohol (o) is a typical compound from the primary pyrolysis reaction of β -O-4 lignin models under H-donation, which occurs between 200 and 400 °C (Kawamoto 2017). Side chain conversions of coniferyl alcohol arising also from primary pyrolysis reactions, leading to coniferyl aldehyde (n), dihydroconiferyl alcohol (l), isoeugenol (g), and 4-vinylguaiacol (d), are typical for lignin (Kotake et al. 2013). Moreover, other pyrolysis products such as guaiacylacetone (k), acetovanillone (j), vanillin (f), eugenol (e), 4-ethylguaiacol (c), 4-methylguaiacol (b), and guaiacol (a) were found (O'Neill et al. 2014). As previously evidenced for DHP on thin films (Elschner et al. 2022) β -5 linkages are visible from pyrolysis product (i) based on phenylcoumaran units.

A very interesting peak that could be assigned to a Hibbert ketone (m) (Kulka and Hibbert 1943), is visible from the pyrolysis of lignified arabinoxylan ferulate. This signal was not detected in the blank experiment. Non-native Hibbert ketone structures are formed by acidolysis of two adjacent β -O-4 units and can be detected in technical lignins (Miles-Barrett et al. 2016). Therefore, dehydrogenation polymerization on ferulate anchor groups of nature-identical arabinoxylans may extend the knowledge towards the formation of natural and non-native lignins.

Conclusions

In order to synthesize nature-identical LCC models occurring in annual plants, highly selective Mitsunobu reaction was applied to perform esterification at position 5 of the arabinose side chain with ferulic acid. This structure could be obtained by selective

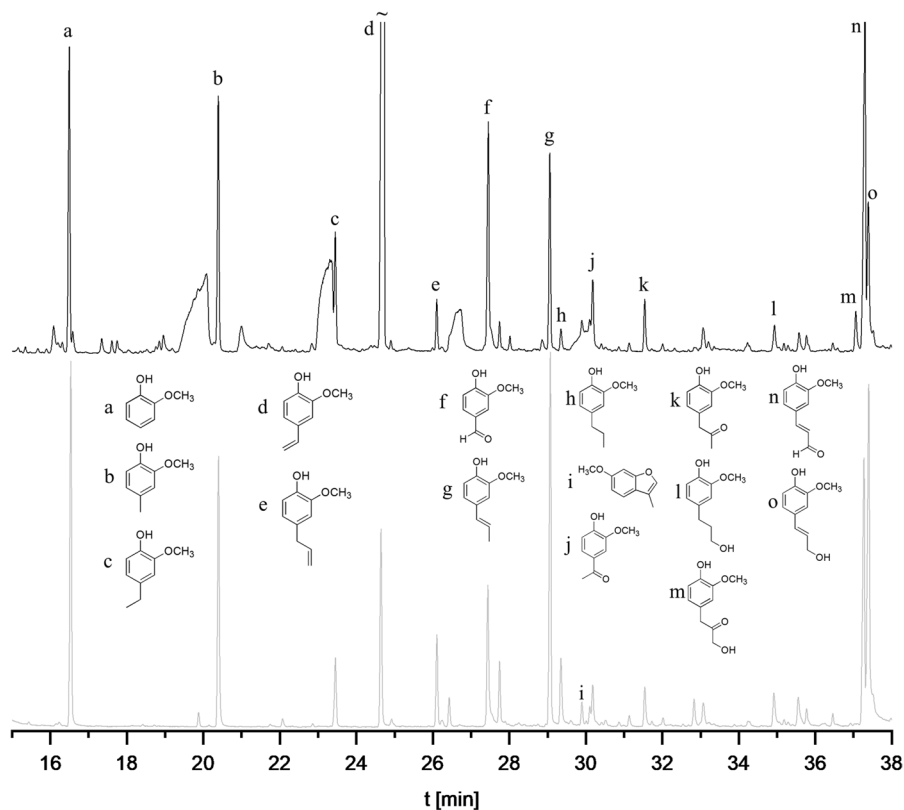
Fig. 3 Sections of HSQC (top) and TOCSY (bottom) NMR spectra of arabinoxylan ferulate **3a** recorded in DMSO-d₆



chemical synthesis for the first time. Previous reagents for esterification of xylan with ferulic acid, e.g. the (protected) acid chloride, lead also to a modification of the secondary hydroxyl groups of the polymer backbone. In this article, we described how the challenges of organic synthesis were tackled. Due to the solvent systems containing LiCl, a competition between Mitsunobu and Appel pathway occurred.

Thus, ferulate as well as deoxychloro moieties were found in the products. We overcame this obstacle by the synthesis of arabinoxylan ferulate in pure NMP. The amount of ferulic acid ester could be tuned without the formation of chloro substituents. DS values from 0.09 up to 0.45 could be achieved applying moderate excesses of reagents.

Fig. 4 Chromatogram of pyrolysis products from DHP-arabinoxylan (top) and pure DHP (bottom) obtained by Py-GC-MS. Lignin-derived compounds **a–o** are marked



A preliminary study showed that our synthetic LCC model can be used for mimicking dehydrogenation polymerization in annual plants. Within a first proof of principle, arabinoxylan ferulate was used for artificial lignification with coniferyl alcohol under homogeneous conditions. Next to β -O-4 and β -5 linkages, a Hibbert ketone structure was observed by Py-GC-MS. Thus, biomimetic polymerization on ferulate anchoring groups of this LCC model could provide new insights in the formation of natural and non-native lignins. Structure-property relationships between DS of ferulate, lignification conditions, and resulting DHP will be a subject of further studies.

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Data availability Information or data are available in the Supplementary Information (SI) and moreover, on reasonable request from the corresponding author.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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