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ACCELERATED LIGHT-INDUCED AGING OF α-, β-, AND γ-^{13}C-ENRICHED CELL WALL-DEHYDROGENATION POLYMERS STUDIED WITH SOLID STATE ^{13}C NMR SPECTROSCOPY

Jim Parkås, Magnus Paulsson
Chalmers University of Technology
Department of Forest Products and Chemical Engineering
SE-412 96 Göteborg, Sweden

David L. VanderHart
National Institute of Standards and Technology
Gaithersburg, MD 20899, USA

Ulla Westermark
Luleå University of Technology
Department of Wood Material Science, Skeria 3
SE-931 87, Skellefteå, Sweden

ABSTRACT

Light-induced aging of lignocellulosic materials has been studied with a new technique involving selectively α-, β-, and γ-^{13}C-enriched cell wall-dehydrogenation polymers (CW-DHP:s) and solid state ^{13}C NMR spectroscopy. The results from cross-polarization magic angle spinning (CP/MAS) ^{13}C NMR experiments of unirradiated and irradiated CW-DHP have revealed mainly a decrease in the amount of end-groups of both coniferaldehyde and coniferyl alcohol type. The results suggest that these end-groups become saturated and that the terminal functionalites, *i.e.*, γ-aldehyde and γ-hydroxymethyl groups, at least to some extent, are retained. The results indicate further that no detectable cleavage of the β-O-4 bonds occurs in the examined lignocellulosic model. In terms of proposed mechanisms of yellowing, there is marginal evidence that up to 2 % of the •-labeled sites are converted by irradiation to •-carbonyls (aldehyde

or ketones); moreover, we cannot dismiss the possibility that the precursor structures giving rise to these few •-carbonyls are •-O-4 structures.

The ^{13}C-enriched CW-DHP was formed directly on spruce (*Picea abies*) wood tissue (differentiating xylem) by administering selectively ^{13}C-labeled coniferin at pH 6.0 in the presence of glucose oxidase and β-glucosidase, *i.e.*, no phenol-oxidizing enzyme was added and the wood cells' own enzymes polymerized the precursor.

INTRODUCTION

The production of mechanical pulp is an efficient way of using the world's natural wood resources, because of the high pulp yield and the relatively low environmental impact of the production itself. Furthermore, the optical and mechanical properties of mechanical pulp are good enough to justify its use as the main constituent in high-quality paper products, had it not been for its poor brightness stability. When paper made from mechanical pulp is subjected to ultraviolet radiation, chromophores which absorb light in the blue-green region are formed, thus, leading to a decrease in brightness and a yellow tone. This phenomenon is called light-induced yellowing, and if this aging problem could be solved, the market for mechanical pulp would increase significantly.[1,2]

Light-induced yellowing is mainly ascribed to the lignin part of the pulp and paper material, which explains why chemical pulps do not yellow to the same extent. Although significant progress has been made in recent years, the exact mechanisms which cause light-induced yellowing are not fully known (*e.g.*, the extent of cleavage of β-aryl ether bonds and the structure of the formed colored chromophores). At this point, no economically and technically feasible method exists that effectively inhibits the brightness reversion reactions. If the mechanisms behind yellowing were better known, the task of finding a preventive method would be more straightforward.

Studies of lignin reactions, including yellowing reactions, in pulp and paper have been obstructed by the fact that it is difficult to analyze lignin in the solid

matrix. A new way to studying light-induced aging has been introduced in a previous paper.[3] The technique is based on solid state ^{13}C nuclear magnetic resonance (NMR) analysis of irradiated and unirradiated ^{13}C-enriched CW-DHP (cell wall-dehydrogenation polymer). With this technique, using β-^{13}C-enriched CW-DHP, it was possible to identify a reduction in the amount of end-groups after prolonged irradiation directly in the solid sample. Other possible changes that may have occurred could not be elucidated when ^{13}C-enrichment in the β-position was studied alone. The combination of selectively ^{13}C-enriched lignocellulosic material and the use of differentiating xylem as a matrix for DHP-formation provided a unique possibility to use the material as a model to study lignin reactions in solid state. The CW-DHP is formed in a natural carbohydrate environment and, compared with samples with natural ^{13}C abundance, the ^{13}C-enrichment results in better sensitivity to small chemical changes using solid state ^{13}C NMR spectroscopy analysis. This method provides the potential to obtain difference spectra between ^{13}C-enriched and unenriched materials both before and after irradiation.

Light-induced yellowing has historically been studied mainly by using lignin preparations like milled wood lignin and dioxane lignin, low molecular-weight lignin model compounds, lignin isolated from mechanical pulp, and pulp and paper material in general.[4-8] Although these methods have given essential and valuable information about the fundamental principles of light-induced yellowing, the difficulties of fully explaining the yellowing reactions based on these approaches include the fact that only parts of the lignin have been studied (model compounds, lignin preparations); moreover, solid state investigations normally have limited sensitivity and resolution. The difficulties of isolating lignin from wood or pulp in a quantitative and unchanged form make solid state investigations, where the lignin remains in the lignocellulosic material, a preferred method. For example, cross-polarization, magic-angle-spinning (CP/MAS), ^{13}C NMR spectra have been used to study the light-induced aging of newsprint.[9] However, solid state investigations

of natural wood material with spectroscopic methods are, in general, characterized by limited performance. The use of solid state ^{13}C NMR spectroscopy is restricted by the low natural abundance of the ^{13}C-isotope (1.1 %) and by overlapping lignin and carbohydrate signals. The performance can be improved by the administration of selectively ^{13}C-enriched precursors of lignin to plants. Several successful investigations with selectively ^{13}C-enriched lignin and subsequent ^{13}C NMR spectroscopy analysis, in solution or solid state, have been performed using this technique on several species of plants and trees.[10-19] The ^{13}C-enriched lignins had structures closely resembling protolignin, but the enrichment degrees were generally quite low. One way to get higher enrichment factors is to produce dehydrogenation polymers (DHP) from selectively ^{13}C-enriched precursors, such as coniferyl alcohol or coniferin. Investigations using DHP selectively ^{13}C-enriched at α-, β-, or γ-positions of the phenylpropane side-chain, as well as at the methoxyl carbon and at position 4 in the aromatic ring, prepared from coniferyl alcohol by enzymatic dehydrogenation, have been published.[20-24] The preparation of DHP from coniferin has also been described and the structure determined and compared with DHP from coniferyl alcohol.[25,26] Recently, a new method for the formation of DHP in a naturally lignifying carbohydrate matrix (differentiating xylem from spruce (*Picea abies*)) from ^{13}C-enriched coniferin, with an enzyme system consisting of β-glucosidase and glucose oxidase, was suggested.[3,27] The β-glucosidase releases coniferyl alcohol and glucose from the coniferin and hydrogen peroxide is formed locally from the oxidation of glucose by the added glucose oxidase. With this method, no external peroxidase or hydrogen peroxide is needed, *i.e.*, the wood cells' own phenol-oxidizing enzymes polymerize the liberated coniferyl alcohol. The formed CW-DHP has a structure similar to the structure of traditional DHP, *i.e.*, a lignin model with more C-C linkages (β-β, β-5) and end-groups (coniferaldehyde, coniferyl alcohol) and less β-O-4 substructures than in native lignin. As with all lignin preparations (milled wood lignin, enzymatically liberated lignins, *etc*.), it is not possible to be absolutely sure that all substructures

(photosensitive or not) present in native lignin also are present in CW-DHP. However, all major types of linkages between phenylpropane units seem to be present in this high-molecular weight lignocellulosic model. In the previously published paper,[3] experiments using CW-DHP with ^{13}C-enrichment of the β-carbon were presented. The material was irradiated for 4 h and 100 h, but no chemical changes could be identified after the shorter irradiation time. Substantially more information could be obtained if also α- and γ-^{13}C-enriched CW-DHP could be prepared and irradiated. Results from such experiments are presented in this paper.

EXPERIMENTAL

Isolation of Differentiating Xylem from Spruce

Trees of the species *Picea abies*, grown in the southern parts of Sweden (approximately 20 cm to 30 cm in diameter at the base), were cut down in mid July. The bark was removed and the newly formed, soft differentiating xylem was collected by careful scraping with a blunt tool. The material was subsequently frozen and stored in a deep freezer.

Coniferin Syntheses

Coniferin (β-glucoside of coniferyl alcohol), ^{13}C-enriched (α, β, and γ-position) and unenriched, was synthesized according to the methods published by Terashima *et al.*[28] As judged by solution state ^{1}H NMR, the ^{13}C-enrichment degrees were approximately 99 %, 88 %, and 86 % for α, β, and γ-labeled coniferin, respectively.

Formation of Cell Wall-Dehydrogenation Polymer (CW-DHP) on Differentiating Xylem

The thawed, differentiating xylem was washed with water to ensure that no bark remained in the sample and, thereafter, was shaken with 200 mM $CaCl_2$ once and with water three times. For a typical experiment, 400 mg (calculated dry mass) xylem was transferred to a glass test tube (75 ml, inner diameter approximately 25 mm) and 172 units of β-glucosidase (from almonds, Sigma-Aldrich Sweden AB[1]) and 172 units of glucose oxidase (from *Aspergillus niger*, Sigma-Aldrich Sweden AB) were added and the sample was diluted to a total volume of 32 ml with a 0.1 M phosphate buffer, pH 6.0. A total of 200 mg coniferin, unenriched or ^{13}C-enriched, was added in small portions during a period of 42 h. The total time for CW-DHP formation was 62 h. All experiments were performed at room temperature (•20° C). After the CW-DHP-formation was complete, the samples were collected on filter paper and washed thoroughly with water.

Preparation of Sheets

From each batch of CW-DHP (originally 400 mg calculated dry mass), 4 small sheets were formed (basis mass •200 g/m^2, diameter approximately 25 mm). The sheets were formed on filter paper (Munktell analytical filter paper no. 5) using a vacuum filtration assembly, pressed (0.62 MPa), and thereafter dried at room temperature.

Lignin Determination

[1] Certain commercial companies are named in order to specify adequately the experimental procedure. This in no way implies endorsement or recommendation by the authors or their agencies.

The lignin content was determined according to the improved acetyl bromide method described by Iiyama and Wallis.[29] An absorptivity value of 20.0 $dm^3/(g\ cm)$ at 280 nm was used.

Accelerated Light-Induced Aging

The sheets were irradiated on both sides in a SUNTEST CPS (Hereus HANAU) equipped with a xenon lamp and filters (ultraviolet and window glass) excluding light with a wavelength shorter than 310 nm. The spectral characteristics of the light source have been described elsewhere.[30] The irradiation effect was approximately 400 W/m^2 and the temperature was kept close to room temperature with a fan. Both ^{13}C-enriched and unenriched sheets were irradiated for a total time of 100 h, 50 h on each side.

Solid State ^{13}C NMR Spectroscopy

^{13}C NMR spectra, utilizing the techniques of magic angle spinning (MAS) and cross-polarization (CP), were taken at ambient temperature on a non-commercial spectrometer operating at 2.35 T (25.2 MHz). The probe is non-commercial also except that the 7-mm-OD rotor and stator were manufactured by Doty Scientific of Columbia, South Carolina, USA. All CP/MAS spectra were collected under the same conditions, namely, 1 ms CP time, 4 s repetition time, 4.00 kHz MAS frequency and rf field strengths corresponding to nutation frequencies of 68 kHz for ^{13}C nuclei and 64 kHz for protons. No techniques for the suppression of spinning sidebands were employed. All spinning sidebands were very small; moreover, there was minimal overlap between centerbands and sidebands owing to the substantial 160-ppm separation between them. MAS frequencies were held constant so that whatever small spinning sidebands there were would not contribute to the difference spectra. Accumulations typically extended at least one day, and were in the range from 20,000 to 25,000. No linebroadening was

applied. The same amount of linear phase correction was applied to each spectrum after Fourier transformation. This choice of linear phase correction is important since, in the spectra considered herein, there is continuous intensity over a range of about 170 ppm; thus, it is difficult to choose the correct linear phase correction. Our linear correction was based on calibrations using spectra with widely separated, narrow lines.

Samples, equilibrated with ambient humidity, were cut into small pieces, of the order of 1 mm in largest dimension. These were packed into the rotor, having initially been pressed into a cylindrical overall shape under mild pressure in a separate die. Total overall sample height was held close to 9 mm in order to provide reproducible filling factors so that rf matching conditions, which inevitably vary slightly across the sample, would give negligible contributions to the difference spectra. Since difference spectra play a key role in this study, careful control of spinning frequencies, rf matching conditions, spectral phasing, and sample filling factors all help to minimize artefacts in these spectra.

Uncertainies

Uncertainties that are assigned to measured quantities are standard uncertainties unless otherwise indicated.

RESULTS AND DISCUSSION

Formation of CW-DHP in Differentiating Xylem

The lignin content in the differentiating xylem was measured with the acetyl bromide technique and was found to be 5.9 % before and 7.3 % after CW-DHP-formation. The characteristics of the differentiating xylem were determined in an analogous study of β-^{13}C-enriched CW-DHP.[3] However, it has to be stressed that the differentiating xylem used in this investigation might be in a different degree of

differentiation as is indicated by the somewhat lower lignin content compared to the material used in the previous study. Generally, the nitrogen content of differentiating xylem is higher and the contents of arabinose, galactose, and galacturonic acid are also higher than in mature spruce wood.[3] The carbohydrate composition indicates a substantial content of pectic substances, most likely due to a high portion of primary wall and middle lamella material in the isolated differentiating xylem. The relatively high nitrogen content of the differentiating xylem is probably due to the presence of proteins, which has been reported before.[31,32] The relatively high protein content makes lignin determination with the Klason method unreliable, due to condensation reactions between proteins and lignin during the acid treatment which leads to an over-estimation of the lignin content (*cf.* References 32 and 33). Therefore, the acetyl bromide method was used in this study, a method that has been used for a wide variety of plant materials and should reflect the lignin content better than the amount of lignin determined by the Klason lignin method.

Solid State CP/MAS ^{13}C NMR Spectroscopy

Figure 1 shows the solid state spectra of unirradiated α-, β-, and γ-^{13}C-enriched as well as unenriched CW-DHP. Corresponding spectra for irradiated CW-DHP are given in Figure 2. Increases in total intensity of about (30 ± 6)% are observed for the enriched materials compared to the unenriched materials. Moreover, fluctuations about this 30 % increase were not correlated with irradiated/unirradiated pairs of samples; hence, there was no indication of a systematic loss of label during irradiation. We can also use this 30 % increase to estimate a 0.025 fractional mass gain of lignin during the time when the coniferin was administered. In relating the 30 % increase to the mass gain, we assume the following: a) a ratio of 1.48 for the carbon density of lignin versus the polysaccharides, b) a lignin repeating unit with 10 carbons, one of which is 90 % labeled, c) natural abundance (0.011) ^{13}C levels at all unlabeled sites, and d)

administered coniferin is the sole source of "lignin" formed during the labeling period. The change in mass fraction of lignin, as judged by the acetyl bromide method is 0.014, or about 56 % of that expected based on label incorporation. This suggests that some of the material seen by NMR is not recognized in the acetyl bromide assay. On the other hand the idea that the administered coniferin is the dominant source of "lignin" formed during the CW-DHP formation is well supported.

In our previous investigation, the material was irradiated for 4 h and 100 h, but no chemical changes could be discerned after the short irradiation time.[3] Therefore, the larger irradiation time was chosen for this study. Difference spectra were produced by subtracting the spectrum of the unenriched sample from the spectrum of the ^{13}C-enriched sample, and normalization was based on minimizing the polysaccharide-carbon resonance contributions. Ideally, in the difference spectrum only the chemical environment of the ^{13}C-enriched side-chain carbon is represented.

α-^{13}C-Enriched Cell-Wall Dehydrogenation Polymer

The solid state ^{13}C NMR spectra of unirradiated, α-^{13}C-enriched and unenriched CW-DHP are shown in Figure 1, and the difference spectrum (enriched - unenriched) is included in Figure 3. Assignments of the signals are based on data published by Terashima and coworkers.[19] The broad complex of signals in the region of 69 ppm to 95 ppm in the difference spectrum (see Figure 3) corresponds to: C_α in β-O-4 (72 ppm to 73 ppm), C_α in (β-O-4)-5-5-(β-O-4) (74 ppm to 75 ppm), C_α in β-O-4/α-O-R (79 to 83 ppm), C_α in β-1 (75 ppm), and C_α in β-β and β-5 (87 and 88 ppm, respectively). Furthermore, it is possible to discern signals corresponding to C_α in coniferyl alcohol end-groups (131 ppm) and C_α in coniferaldehyde end-groups (~155 ppm). As judged from the spectra, it seems that the structure of the CW-DHP is similar to the structure of traditional DHP, *i.e.*, with more β-β and β-5 moieties than β-O-4 and with a substantial amount of end-

groups, especially of the coniferyl alcohol type. Integrals associated with the different spectral regions of all the difference spectra can be found in Table 1.

The spectra corresponding to irradiated (100 h), α-^{13}C-enriched and unenriched CW-DHP are presented in Figure 2, together with the difference spectra in Figure 3. For easier comparison of the chemical changes that occur during irradiation, the difference (irradiated - unirradiated) of the two difference spectra, scaled to the same total intensities, *i.e*. areas, is included in Figure 3 (bottom). This means that a negative response in the "difference of difference spectra" corresponds to a decrease in intensity in this region upon irradiation and *vice versa*. While the assignment of the different structures is relatively straightforward in the original CW-DHP, problems may arise when the sample has been treated in some way (for example irradiated) since many new structures can form in such a complex substance. Irradiation (100 h) of the α-^{13}C-enriched CW-DHP gave rise to notable changes in the appearance of the difference spectra. It was concluded in the study with β-^{13}C-enriched CW-DHP[3] that the relative signal area corresponding to the unsaturated β-carbons in coniferaldehyde and coniferyl alcohol end-groups decreased after irradiation for 100 h, but whether this decrease were related to end-groups of the coniferyl alcohol or coniferaldehyde type, or both, could not be determined. However, upon closer examination of the integrals in Table 1 and the difference spectra in Figure 3 it can be seen that both the amount of coniferaldehyde end-groups (~155 ppm) and the amount of coniferyl alcohol end-groups (131 ppm) decreased. The relative signal area in the region of C_α associated with β-O-4, β-1, β-β, and β-5 substructures (69 to 95 ppm) seems to be essentially unchanged even after prolonged irradiation. A broader resonance, comprising about 3 % of the total intensity and centered at about 196 ppm (assigned to •-carbonyls of ketones or an aldehyde[34]), is visible in the difference spectrum of the irradiated sample in Figure 3. That feature is not as prominent in the spectrum of the unirradiated sample; however, the latter intensity is harder to assess accurately because of the poorer signal-to-noise ratio. Nevertheless, the data support the

likelihood that irradiation induces a growth of such •-carbonyl intensity corresponding to between 1 % and 2 % of the •-labeled sites. One new, not yet conclusively assigned, broad resonance at 25 ppm to 60 ppm was also introduced in the difference spectrum of irradiated α-^{13}C-enriched CW-DHP. In this region of the spectrum, mainly tetrahedrally bonded carbons not bound to oxygen appear. With the previously suggested yellowing reactions it is, at present, hard to explain the resonance in this region. However, since the main changes upon irradiation involve the end-groups, one possible explanation is that a part of the end-groups loose their unsaturation in such a way that additions across the double bonds do not involve the formation of carbon-oxygen bonds. Further studies to explain these observed changes are underway.

β-^{13}C-Enriched Cell-Wall Dehydrogenation Polymer

Solid state ^{13}C NMR spectra of β-^{13}C-enriched and unenriched, unirradiated CW-DHP are presented in Figure 1 and the difference spectrum (^{13}C-enriched – unenriched) is included in Figure 4. Corresponding spectra for irradiated (100 h) β-^{13}C-enriched and unenriched CW-DHP are placed in Figures 2 and 4. In the difference spectrum of unirradiated CW-DHP (β-^{13}C-enriched – unenriched) three well separated peaks can be discerned at chemical shifts of 40 ppm to 60 ppm (C_β in β-β, β-5, and β-1 substructures), 70 ppm to 95 ppm (C_β in β-O-4 derived substructures), and 120 ppm to 135 ppm (C_β in coniferaldehyde and coniferyl alcohol end-groups). From the integrated peak areas (Table 1) in the difference spectrum of the unirradiated sample, the signals in the above-mentioned region corresponded to (35 ± 2)%, (36 ± 2)%, and (29 ± 2)% respectively. These numbers are fairly close to those reported in the previous article[3] (42 %, 36 %, and 22 %), indicating that the CW-DHP used in this study, as judged by the bonding pattern in β-enriched CW-DHP, is comparatively close in structure to that previously analyzed, considering that it is not the same differentiating xylem and that some changes have been made in the preparative

method (*cf*. Experimental section). The main differences in the structure of the CW-DHP's used in the two investigations are the larger amount of end-groups and the smaller amount of β-β, β-5, and β-1 structures in the material used in this investigation.

When examining the difference spectrum corresponding to irradiated (100 h) CW-DHP (β-^{13}C-enriched - unenriched) and the "difference of the difference spectra" (*cf*. the previous discussion regarding the α-^{13}C-enriched CW-DHP) in Figure 4, it can clearly be seen that the amount of end-groups of the type coniferaldehyde and coniferyl alcohol decreases substantially and that the signal area in the region 30 ppm to 60 ppm increases. The relative signal area in the region of C_β in β-O-4 substructures (80 ppm to 89 ppm) seems to be essentially unchanged, although the material has been irradiated for a long period of time. However, the minor, but new peaks that appeared near 37 ppm, 70 ppm, and 102 ppm after prolonged irradiation in the previous article[3] are not clear in this investigation. A broadening of the peak corresponding to β-β, β-5, and β-1 substructures can be seen after prolonged irradiation which might be equivalent to the peak at around 37 ppm reported before. At the same time, the peak near 70 ppm is only weakly displayed in the "difference of the difference spectra" and there is no identifiable peak at 102 ppm in this study. The reasons behind these remaining discrepancies in the difference spectra are not obvious. It is possible that the 102 ppm peak seen previously arose from a phasing problem in one of the parent spectra since the peak is in the vicinity of the C1 resonance of cellulose. The 70 ppm peak cannot be explained that way since it is nearer a minimum in the cellulose spectrum. In principle, there is a possibility of a different carbohydrate environment in the two investigations, and this may affect the outcome of the irradiation. However, the preparative procedures for the two studies are very similar. At least, in the present study, the signal-to-noise ratio in the difference spectra is better and great care has been taken to reproduce the instrumental conditions for each spectrum. Hence, if any of the features noted

before arise from artefacts, we believe that the difference spectra in this study are less prone to such artefacts.

γ-^{13}C-Enriched Cell-Wall Dehydrogenation Polymer

Figure 1 displays the solid state spectra of unirradiated γ-^{13}C-enriched CW-DHP together with the unirradiated unenriched CW-DHP and Figure 5 includes the difference spectrum (enriched – unenriched). Based on the assignments given by Terashima and coworkers,[19] signals corresponding to the following substructures can be discerned: C_γ in β-O-4, β-1, and β-5 (50 ppm to •65 ppm), C_γ in β-β (•65 ppm to 80 ppm) and C_γ in coniferaldehyde end-groups (centered at 197 ppm). Based on solution-state NMR-data, the γ-carbon in coniferyl alcohol end-groups should appear in the same region as the β-O-4, β-1, and β-5 substructures. In Figure 2, the spectra of γ-^{13}C-enriched and unenriched CW-DHP, after irradiation with UV/VIS light, are presented. The corresponding difference spectrum can be seen in Figure 5 together with the "difference of the difference spectra". The most notable change that can be discerned in the difference spectra (γ-^{13}C-enriched) is that the amount of coniferaldehyde end-groups has decreased. This confirms the results from the experiments with α-^{13}C-enriched CW-DHP, regarding the end-groups. In the difference spectrum of the irradiated sample, there is also a spreading of the 197 ppm intensity to lower field. Such a spreading is consistent[34] with a portion of the end-group retaining its aldehyde functionality while the α- and β-carbons become tetrahedrally bonded. Furthermore, the peak at 50 ppm to •65 ppm (γ-carbon in β-O-4, β-1, β-5, and coniferyl alcohol substructures in unirradiated CW-DHP) decreases most probably as a result of a decrease in the amount of coniferyl alcohol end-groups, as was noted based on Figures 3 and 4. At the same time the peak at ~65-80 ppm (γ-carbon in β-β substructures in unirradiated CW-DHP) increases modestly; however since there is no evidence for an increase in the β-β structures based on Figures 3 and 4, we look for another

explanation for the downfield shift of a portion of this 50 ppm to 65 ppm intensity. Given the strong suggestion that some double bonds are becoming saturated upon irradiation, such a downfield shift is also consistent with the loss of unsaturation of the side-chain, provided that a C-C bond, as opposed to a C-H bond is formed at the β-position and that the terminal hydroxymethyl group is retained. If the carbon in β-position were simply bound to hydrogen atoms in the disappearance of the double bond, the resonance would not move downfield enough.[34] These results, for the γ-enriched materials, give support to the notion that a portion, but not all, of the coniferyl alcohol and coniferaldehyde groups maintain their terminal functionalities while undergoing a saturation of the double bonds. In the difference spectrum of the irradiated sample in Figure 5, there is also a weak, diffuse build-up of intensity in the 85 ppm to 110 ppm range. Since this region strongly overlaps the saccharide resonances, we cannot be absolutely sure that the intensity in this region truly arises from lignin differences rather than from differences in saccharide crystallinity. However, to the extent that these weak resonances are associated with the lignin component, there may be a clue here as to some minor, unidentified product involved in photoaging.

Comments on the Mechanism of Light-Induced Yellowing

The behavior of end-groups of the type coniferaldehyde and coniferyl alcohol during yellowing has been studied earlier.[35-38] The suggested mechanisms for the photoxidation of coniferaldehyde and coniferyl alcohol end-groups result in the generation of end-groups of the vanillin and vanillic acid type. The α-carbonyl carbon of a vanillin end-group would appear at around 192 ppm to 194 ppm, and the carboxylic α-carbon in a vanillic acid end-group at around 173 ppm.[39] No peak can be discerned at around 173 ppm in the difference spectrum of irradiated α-^{13}C CW-DHP (Figure 3) indicating that vanillic acid is not present in detectable amounts in the sample. There was a suggestion that the α-carbonyls increased by

1 % to 2 % of the •-labeled sites and vanillin is a candidate structure consistent with the observed chemical shift. So the production of vanillin remains a possibility for the yellowing reaction. The changes we observed in the difference spectra during irradiation mainly involved the decrease in signal areas corresponding to double bonded α- and β-carbons in coniferaldehyde and coniferyl alcohol end-groups (*cf.* Figures 3 and 4) and the appearance of new peaks at high field (25 ppm to 60 ppm for irradiated α-^{13}C CW-DHP and 35 ppm to 60 ppm for irradiated β-^{13}C CW-DHP). A self-consistent explanation for these changes is that the side-chains of the end-groups become saturated. Furthermore, it seems as if at least part of the terminal functionalities, *i.e.* the aldehyde group and the hydroxymethyl group in the original coniferaldehyde and coniferyl alcohol end-groups, respectively, are retained as judged by the shifting of the corresponding peaks in the difference spectrum of irradiated γ-^{13}C CW-DHP to lower field (*cf.* Figure 5).

The task of finding a method preventing light-induced yellowing has proven to be difficult. The reason for this can be that new reactive structures form as a result of the irradiation. The cleavage of β-O-4 bonds would be such a reaction. It has been suggested that such a cleavage can occur, for example via the "ketyl radical pathway"[40] which should be a source of new phenolic groups. The "ketyl radical pathway" should also give rise to new α-carbonyls. There is evidence that 1 % to 2 % of the •-labels get converted to •-carbonyls based on intensity changes for the weak, isolated resonance near 196 ppm in Figure 3. However, an attempt to verify this mechanism, say, via a corresponding reduction in the •-O-4 intensity (=1.6% • 4.0% from Table 1) in the •-labeled samples of Figure 4, is thwarted by the fact that the •-O-4 peak is much more intense than the •-carbonyl peak and the integration errors are correspondingly too large to verify the mechanism. Thus, while we argue that no *detectable* change in •-O-4 population occurs upon irradiation, the transformation of less than 2 % of the labels at •-O-4 sites is still a possibility.

In the present investigation, the observed chemical changes have not been coupled to assays of the extent of discoloration. Furthermore, since only the chemical changes after prolonged irradiation were examined, one must be cautious when drawing general conclusions concerning color-forming reactions. However, unpublished data show that traditionally formed DHP absorbed on filter paper discolors already after 4 h of irradiation in the accelerated testing equipment used in this study. Further work in line with this investigation, in which the chemical changes will be coupled to color change after both a short and prolonged irradiation time, is underway.

CONCLUSIONS

This study, which is a continuation of a previous study of β-^{13}C-enriched cell wall-dehydrogenation polymer[3], has shown that α- and γ-^{13}C-enriched CW-DHP can also be used as models for lignocellulosic materials during light-induced yellowing, using solid state ^{13}C NMR spectroscopy to track chemical changes. The ^{13}C-enriched CW-DHP was subjected to accelerated light-induced aging by UV/VIS-light for 100 h and the difference spectra (α-, β-, and γ-^{13}C-enriched) have revealed mainly a decrease in the relative amount of both coniferaldehyde and coniferyl alcohol end-groups. A new, broad peak at 25 ppm to 60 ppm was observed in the difference spectrum of the α-^{13}C-enriched CW-DHP, and the peak appearing at 40 ppm to 60 ppm in the difference spectrum of the β-^{13}C-enriched CW-DHP was broadened to higher field (30 ppm to 60 ppm). These peaks represent tetrahedrally bonded, non-oxygenated carbons; however, the mechanism of their formation remains elusive. The results imply that a part of the end-groups lose their unsaturation during photodegradation. Furthermore it seems as if at least part of the terminal functionalities, *i.e.* the aldehyde group and the hydroxymethyl group in the original coniferaldehyde and coniferyl alcohol end-groups, respectively, are retained in the saturated side-chains as judged by shifting of the

corresponding peaks in the difference spectra of γ-^{13}C CW-DHP to lower field. The results also indicated no detectable cleavage of β-O-4 bonds in the investigated material even after prolonged irradiation. On the other hand, there was an indication of a growth of •-carbonyl (aldehyde or ketone) intensity upon irradiation involving only 1 % to 2 % of the labeled sites. In terms of proposed mechanisms of yellowing, we cannot dismiss the possibility that such a low-level growth of •-carbonyl intensity could have a •-O-4 precursor. It is important to note that this study has focused on chemical changes that can be tracked by solid state ^{13}C NMR spectroscopy. No complementary quantitative assays of color formation upon irradiation were made.

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TABLE 1. Integrals associated with different spectral regions in the difference spectra, before and after irradiation (100 h), for the three ^{13}C-enriched CW-DHP's.

α-^{13}C		β-^{13}C		γ-^{13}C	
Shift range (ppm)	Relative intensities (%)	Shift range (ppm)	Relative intensities (%)	Shift range (ppm)	Relative intensities (%)
Unirradiated samples					
20-57	3.0 ± 1.2	38-66	34.8 ± 2.0	40-87	88.5 ±2.0
57-69	6.5 ± 1.5	68-98	36.2 ± 2.0	50-68	60.2 ± 3.0
69-80	23.0 ± 3.0	114-140	29.0 ± 2.0	50-70	69.0 ± 3.0
80-100	41.5 ± 3.0			68-87	28.3 ± 3.0
25-100	74.1 ± 3.0			70-87	19.5 ± 3.0
120-144	18.1 ± 2.0			87-112	2.1 ± 0.7
145-166	6.4 ± 1.5			164-184	1.7 ± 0.7
190-210	1.5 ± 1.0			188-206	7.7 ± 1.0
Irradiated samples, 100 hours					
20-57	15.0 ± 2.0	22-66	47.4 ± 2.5	40-87	84.6 ± 2.5
57-69	6.2 ± 1.5	68-98	34.6 ± 2.0	50-68	51.6 ± 3.0
69-80	18.8 ± 3.0	114-140	17.9 ± 1.5	50-70	58.3 ± 3.0
80-100	41.8 ± 3.0			68-87	33.0 ± 3.0
25-100	81.9 ± 2.5			70-87	26.3 ± 3.0
120-144	13.3 ± 1.5			87-112	7.2 ± 1.5
145-166	1.6 ± 1.0			164-184	3.2 ± 1.0
190-210	3.1 ± 1.0			188-206	5.0 ± 1.0

FIGURE 1. Solid state ^{13}C NMR spectra of unirradiated α-, β-, and γ-^{13}C-enriched CW-DHP and unenriched CW-DHP (top to bottom).

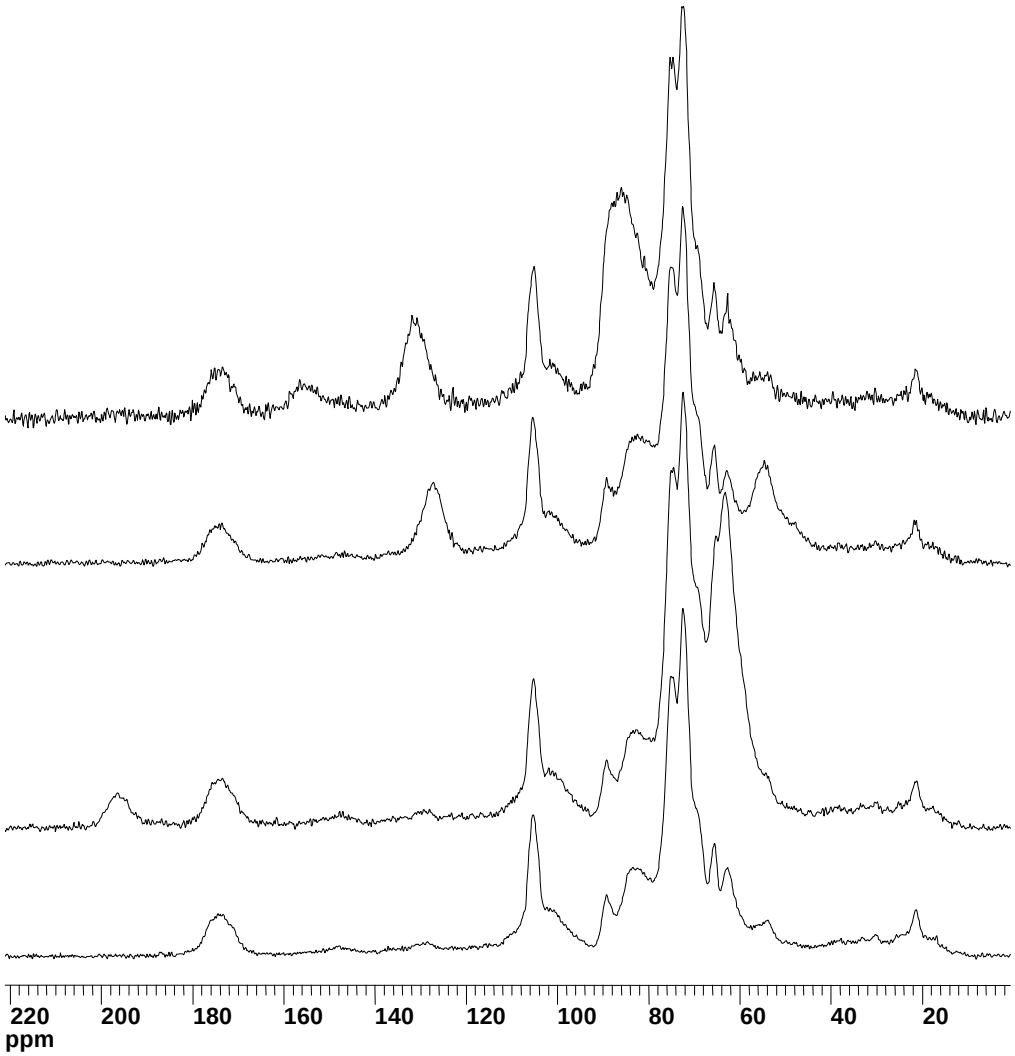

FIGURE 2. Solid state ^{13}C NMR spectra of irradiated (100 h) α-, β-, and γ-^{13}C-enriched CW-DHP and unenriched CW-DHP (top to bottom).

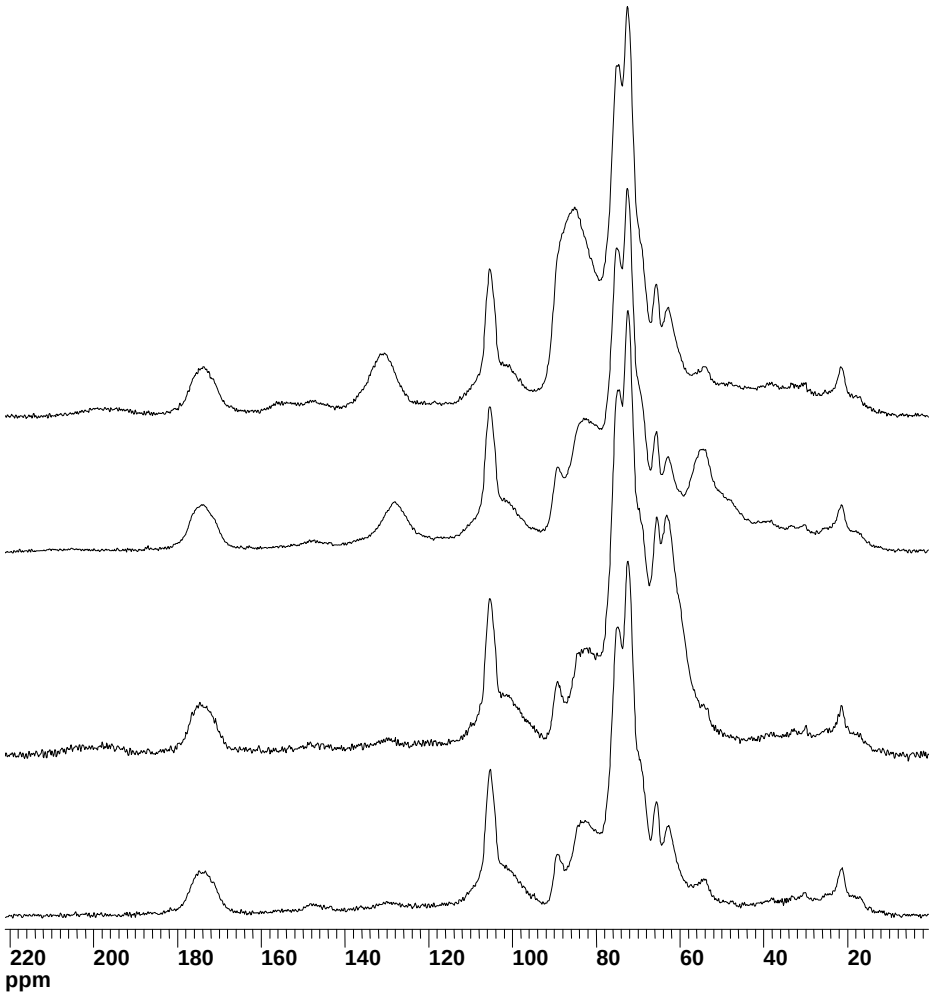

FIGURE 3. Difference spectra corresponding to unirradiated (middle) and irradiated (100 h, top) α-^{13}C-enriched CW-DHP. The “difference of the difference spectra” (top spectrum - middle spectrum) is placed at the bottom.

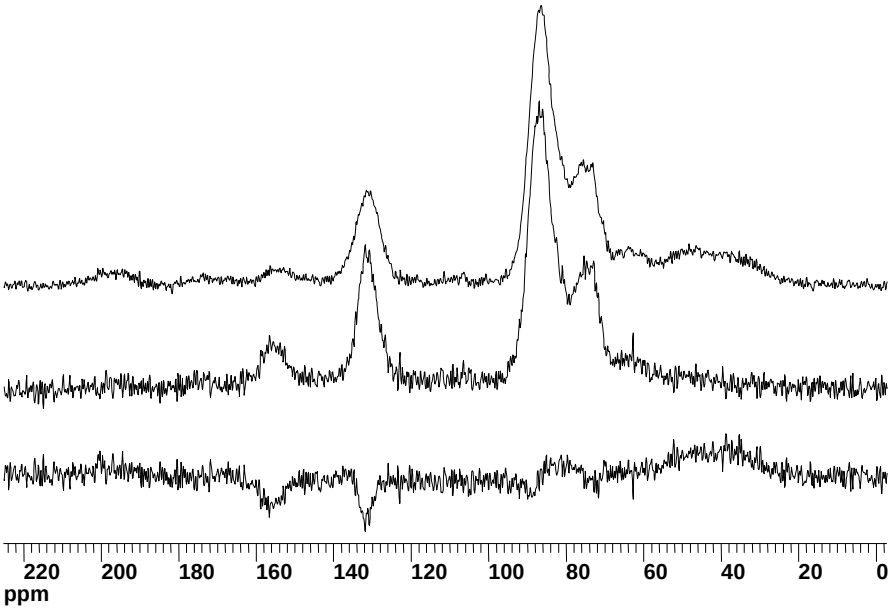

FIGURE 4. Difference spectra corresponding to unirradiated (middle) and irradiated (100 h, top) β-^{13}C-enriched CW-DHP. The “difference of the difference spectra” (top spectrum - middle spectrum) is placed at the bottom.

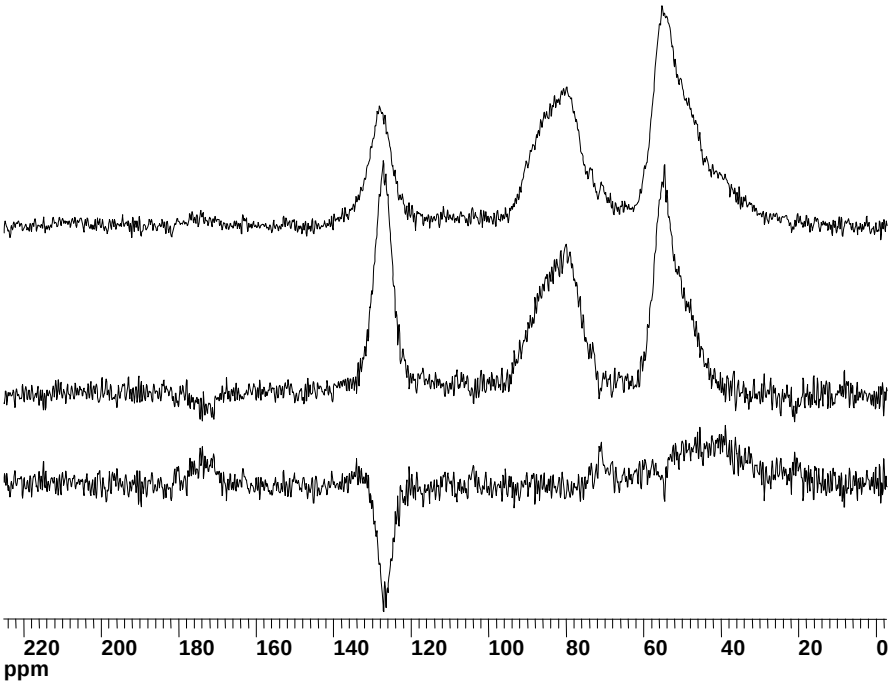

FIGURE 5. Difference spectra corresponding to unirradiated (middle) and irradiated (100 h, top) γ-^{13}C-enriched CW-DHP. The “difference of the difference spectra” (top spectrum - middle spectrum) is placed at the bottom.