

Light-induced yellowing of selectively ^{13}C -enriched dehydrogenation polymers (DHPs). Part 2. NMR assignments and photoyellowing of aromatic ring 1-, 3-, 4-, and 5- ^{13}C DHPs

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SUMMARY: Light-induced yellowing of lignocellulosic materials has been studied using ^{13}C -enriched DHP (dehydrogenation polymer), selectively ^{13}C -enriched at positions 1, 3, 4, and 5 in the aromatic ring, and quantitative solution state ^{13}C NMR spectroscopy. The NMR study confirmed the results of previous studies using side-chain labeled DHP, mainly that coniferyl alcohol end groups are degraded with the subsequent formation of α -carbonylic structures, e.g. vanillin end groups. The developed technique renders the potential to follow chemical changes of aromatic carbons 1, 3, 4, and 5, independently from each other. Although a drastic photoyellowing of the sheets was observed, definitive chemical shifts corresponding to quinoid carbons could not be discerned in the spectra. The reasons for this may be that the amount of quinoid carbons formed was too low to be detected, with the experimental setup used, or that the quinoid photo-products, if formed, were not extractable with the solvent used or had reacted further forming non-quinoid structures.

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Light-induced yellowing is the main reason why mechanical pulp has limited use in higher-quality paper grades. The lignin in mechanical pulps undergoes photooxidation when subjected to ultraviolet and visible light causing a brightness loss and a yellow tone in the paper product, leading to an unacceptable aging performance. There could be both economic and environmental benefits if the yellowing could be inhibited or if the yellowing rate could be slowed down so that mechanical pulp could be used to a larger extent even in high-quality paper products. Although recent research has given valuable insight into the mechanism of light-induced yellowing (Gratzl 1985; Heitner 1993; Leary 1994; Davidson 1996; Forsskåhl 2000; Lanzalunga, Bietti 2000), there is still some controversy, for instance regarding the nature of the initially formed colored structures (*ortho*- and/or *para*-quinones, see below), and the contribution of β -aryl ether

cleavage to color forming reactions.

It was proposed early on that the chromophores initially formed during irradiation were of the quinone, quinone methide, and cyclohexadienone type (Leary 1968a; Leary 1968b). The observation that a sodium borohydride treatment of photo-aged paper made from mechanical pulp almost completely removed the formed chromophores also indicates that these chromophores contain carbonyl structures (like quinones, ketones, and aldehydes) (Hemmingson, Morgan 1990; Ragauskas 1993; Capretti et al. 1994; Schmidt, Heitner 1995; Pan, Ragauskas 1997). Lin and Kringstad (1971) have irradiated lignin model compounds and have suggested that both *o*-quinones and *p*-quinones can be formed, although the latter to a lesser extent. Photoexposure of TMP and CTMP produced chromophores with absorption maxima at 350 and 420 nm (Heitner, Min 1987). These chromophores were considered to be a methoxy-*p*-benzoquinone and an *o*-quinone with an aliphatic substituent in the 2-position. Others have stated that hydroquinones (*p*-quinones) are the main leucochromophores (chromophores) formed during photoyellowing (Gellerstedt, Pettersson 1977; cf. Forsskåhl et al. 1991; Hirashima, Sumimoto 1994; Agarwal 1998). Agarwal (1998) did not find detectable amounts of *o*-quinones in photoyellowed TMP by Raman spectroscopy, but assigned a band at 1675 cm^{-1} to formed *p*-quinone groups. Photochemically generated *o*-quinones have been detected in several high-yield hydrogen-peroxide-bleached pulps by fluorescence spectroscopy (Zhu et al. 1995). Lebo et al. (1990) have detected increased amounts of *o*-quinones after photoyellowing of mechanical pulp using ^{31}P NMR spectroscopy. Based on experiments using photoyellowed mechanical pulp and solid state ^{31}P NMR spectroscopy, Argyropoulos et al. (1995), have reported that during the early stages of photoyellowing there is a rapid formation of *o*-quinones that subsequently react further to create more complex chromophores of a non-quinoid nature. This may be the reason why no quinones were detected after irradiation of milled wood lignin (Sjöholm et al. 1992). Argyropoulos et al. (1995) have also shown that the *o*-quinones formed in hydrogen-peroxide-bleached groundwood pulp (GWP) during photoyellowing are converted more slowly than those formed in the corresponding unbleached pulp. This may be due to the higher content of stilbenes in the bleached pulp. Structures that are known to form stilbene *ortho*-quinones (cf. Zhang, Gellerstedt 1994a) during irradiation and

these may need more time to be converted into non-quinonoid structures. It has been suggested that monomeric o-quinones are the major chromophores formed during light-induced yellowing of lignin-rich pulps based on UV/VIS reflectance spectroscopy of hydrogen-peroxide-bleached GWP, CTMP, and quinoid lignin model compounds (Zhang, Gellerstedt 1994b, 1998). It is important to remember that quinoid structures are themselves photosensitizers and that they can contribute to further chromophore formation.

Dehydrogenation polymers (DHPs) can be seen as high-molecular weight lignin model compounds. It is generally accepted that the structure of DHP differs from that of milled wood lignin, which is considered to be the lignin preparation best representative of native lignin. The amount of end groups (especially of the coniferyl alcohol type) and β - β and β -5 type structures are higher and the amount of β -O-4 structures is lower in DHP than in milled wood lignin (Nimz, Lüdemann 1976; Brunow, Lundquist 1980; Terashima et al. 1995, 1996a). However, most of the linkages in native lignin are also found in DHP, although not necessarily in the same proportions, making DHP suitable as a model for lignin.

In this study, dehydrogenation polymers, ^{13}C -enriched in positions 1, 3, 4, and 5 of the aromatic ring, have been prepared and applied to filter paper (for denotations of the carbons in the phenylpropane units, see *Formula Fig 1*). The DHP-impregnated sheets have been subjected to accelerated light-induced yellowing and the structure of the extractable photodegraded DHP have subsequently been studied with quantitative ^{13}C NMR spectroscopy in solution state. Carbon-13 enrichment of aromatic carbons in the phenylpropane-units in DHP is a method for tracking small-scale changes (for example, the formation of quinoid structures) during photoyellowing with higher sensitivity than when using unenriched material.

This study is a continuation of a previous investigation of light-induced yellowing using specifically α -, β -, and γ - ^{13}C -enriched dehydrogenation polymers (DHPs) and ^{13}C NMR spectroscopy (Parkås et al. 2004), which means that there is a potential to label all but two carbons in the phenylpropane units.

Experimental

Coniferin synthesis

Aromatic ring 4- and 5- ^{13}C -enriched coniferins were prepared according to the method published by Terashima and coworkers (2003). ^{13}C -Enrichment of position 3 in the aromatic ring was achieved after a slight modification of the above-mentioned synthesis, i.e. by reacting ^{13}C -labeled formaldehyde with sodium cyanide yielding hydroxyacetonitrile-[2- ^{13}C]. The hydroxyacetonitrile was methylated with dimethylsulfate to form methoxyacetonitrile-[2- ^{13}C] which was isolated (Terashima et al. 2002). The rest of the synthesis was conducted according to the method described by Terashima et al. (2003). Ring-1- ^{13}C coniferin was synthesized by reacting ^{13}C -enriched triformylmethane [$^{13}\text{CH}(\text{CHO})_3$] with methoxyacetone in

an alkaline solution, which yielded about 8.8% of pure vanillin-1- ^{13}C calculated from triformylmethane (Terashima et al. 2002). The synthesis from vanillin to coniferin is analogous with that published for side-chain ^{13}C -labeling (Terashima et al. 1996a). Unlabeled coniferin was prepared according to the method by Terashima and coworkers (Terashima et al. 1996a). The enrichment degrees at the labeled sites of the ^{13}C -coniferin were in the region of 10-20%.

DHP preparation

The dehydrogenation polymers, ^{13}C -labeled as well as unlabeled, were prepared from coniferin mainly according to Method A described by Terashima and coworkers (1996b). In a typical experiment, 400 mg of coniferin (400 mg unenriched or 200 mg ^{13}C -enriched + 200 mg unenriched) was dissolved in 40 ml phosphate buffer of pH 6 in an Erlenmeyer flask. To the coniferin solutions; 80 u of β -glucosidase (from almonds, Sigma-Aldrich, Sweden), 94 u of glucose oxidase (from *Aspergillus niger*, Sigma-Aldrich, Sweden), and 92 u of peroxidase (from horseradish, Sigma-Aldrich, Sweden) were added. The flasks were put in a water bath and the temperature was maintained at 31-32°C. After 24 hours the pH was adjusted to 6 with a dilute solution of NaOH and the same amount of the three enzymes was added again. After this step, the pH was adjusted to 6 each 24 hours throughout the DHP formation period of 77 h. After DHP formation, the solutions were centrifuged and freeze-dried. The DHP was purified by precipitating the part soluble in 1,2-dichloroethane (DCE)/ethanol (EtOH, 99.5%) (2/1 v/v, 3 ml) in dry ether (45 ml). The precipitated DHP was collected by centrifugation, the supernatant was decanted off and a small amount of petroleum ether was added. After centrifugation and decantation of the supernatant, the DHP was dried. The yield of purified DHP averaged 106.1 mg, corresponding to about 50% of the coniferyl alcohol moiety in the added coniferin. The structure of the formed DHP was subsequently studied with solution state ^{13}C NMR spectroscopy.

Application of DHP to filter paper

Air dried, previously extracted (1,2-dichloroethane/ethanol; 2:1) filter papers (Analytical grade 00A, diameter 5.5 cm, Munktell Filter AB Sweden) were impregnated with the DHPs dissolved in a small amount of DCE/EtOH (2:1) until the desired weight increase was achieved. The amount of DHP on the filter papers was determined by weighing the sheets in the air-dry state. The amount of applied DHP to each filter paper averaged 55 mg and one filter paper for each labeled position was used. The sheets were kept in the dark when not handled.

Accelerated light-induced yellowing and determination of optical properties

The sheets were subjected to accelerated light-induced aging in a SUNTEST CPS (Hereus HANAU) equipped with a xenon lamp and filters (ultraviolet and window glass) that exclude light with a wavelength shorter than 310 nm. The spectral characteristics of the light source

Table 1. Optical properties of unirradiated and irradiated DHP-impregnated sheets. UE = unenriched.

Irradiation time on each side (h)	Brightness(% ISO)					b*				
	UE	1- ¹³ C	3- ¹³ C	4- ¹³ C	5- ¹³ C	UE	1- ¹³ C	3- ¹³ C	4- ¹³ C	5- ¹³ C
0	53.9	55.6	54.7	50.2	53.5	22.8	23.2	23.2	25.3	23.6
1	56.7	53.9	55.6	52.8	55.0	19.7	21.2	20.3	21.9	20.5
4	52.9	52.6	53.5	51.2	51.8	21.2	21.9	21.4	22.8	22.2
21	37.3	36.4	37.2	36.1	36.4	29.4	30.0	29.6	30.4	30.0

have been described elsewhere (Paulsson, Ragauskas 1998). The sheets were irradiated for equal times on both sides and the optical properties measured after fixed times. ISO-brightness and color changes according to the CIELAB color scale (L*, a*, and b*-values) were measured after 0, 1, 4, and 21 hours of irradiation on each side with an Elrepho 2000 spectrophotometer. For the measurement of R_∞, an opaque stack of unextracted filter papers was used as the background for all samples. Optical properties were measured on both sides of the sheets after each irradiation period.

Extraction of the photo-aged DHP

The sheets were cut into smaller pieces and extracted with DCE/EtOH (211, 5 ml) for 2x24 h + 1x48 h with new extraction solvent after each period. The combined extracts were evaporated to dryness on a rotary evaporator and the weight of the extracted DHP was determined. On the average, 46 mg of photooxidized DHP was isolated. This constitutes approximately 84% of the applied weight of the DHP (the molecular weight could, however, differ from the original). Experiments with unenriched DHP showed that before irradiation, all of the DHP was extractable from the filter paper (Parkis et al. 2004), i.e. the failure to recover the all of the applied DHP after irradiation is due to reactions that take place during the accelerated photoexposure.

Solution state ¹³C NMR spectroscopy

The ¹³C NMR data for DHPs were obtained with a Bruker DPX-250 spectrometer (62.9 MHz carbon) fitted with a 5 mm quadrupole probe. The samples were dissolved in 400 μl of DMSO-d₆. Quantitative ¹³C NMR spectra were obtained with a pulse width of 6.5 microseconds corresponding to a tip angle of 90°. The relaxation delay was 15 seconds and sample temperature was 300K. 7000 transients (16K data points) were collected over a period of 30 hours.

Results

Optical properties of DHP-impregnated filter paper before and after irradiation

The optical properties of the DHP-impregnated sheets before and after irradiation are shown in Table 1. The optical properties are given as the average values of both sides of the sheets. The brightness decreased during the irradiation from an average of 54% before irradiation to 37% after irradiation for 21 hours on each side corresponding to an average PC-number (Post Color-Number)

due to irradiation of approximately 34. The variation in initial brightness is probably an effect of difficulties in applying the DHPs completely even on the filter papers (especially pronounced for the sheet with 4-¹³C DHP, as indicated by the lower brightness and the higher b*-value). During the same irradiation time (2x21 h), the b*-value increased on the average from 24 to 30, i.e. the sheets did yellow upon irradiation. Furthermore, during irradiation, the a*-values increased somewhat and the L*-values decreased to some extent (not included in Table 1). The optical properties (ISO-brightness and b*-values) of extracted filter paper without DHP, aged for the same amount of time, did not change significantly during irradiation. The observed changes in optical properties are, on the average, very similar to the values reported for the side-chain labeled experiment, in which case the brightness also decreased from, an average of, 54% to 37% and the b*-value increased from 22 to 29 (Parkås et al. 2004). The pieces of filter paper were still colored after extraction for 2x24 + 1x48 hours, the approximate amount of retained color was determined in a previous paper (cf. Parkås et al. 2004).

¹³C NMR analysis

Fig 1 shows the ¹³C NMR spectra of unirradiated ring-1, ring-3, ring-4, and ring-5-¹³C-enriched as well as unenriched DHP before irradiation and Fig 2 shows the corresponding spectra of the extractable part of the DHP after irradiation for 21 hours on each side of the filter paper. The difference spectra presented in the following figures have been produced by subtracting the spectra of unenriched DHP from the spectra of ¹³C-enriched DHP (¹³C-enriched - unenriched). It should be noted that, as not all of the DHP applied to the filter papers was extractable after irradiation, the NMR-analysis is of the soluble part only (cf. Experimental section). Studies aimed at analyzing the non-extractable part of the DHP by solid state ¹³C NMR spectroscopy are underway. The assignments of the signals are mainly based on the database published by Ralph and coworkers (1998), but also on other published material [e.g. Nishibe et al. 1984; Deyama et al. 1987a,b; Drumond et al. 1989, 1992; Miyakoshi, Chen 1992; Sjöholm et al. 1992 (and references therein); Pan et al. 1994 (and references therein); Alves et al. 2000]. However, there is a need to conduct more model studies to be able to completely assign all peaks individually with certainty.

Ring-1-¹³C-enriched DHP

Fig 3 presents the difference spectra corresponding to unirradiated (top) and irradiated ring-1-¹³C-enriched

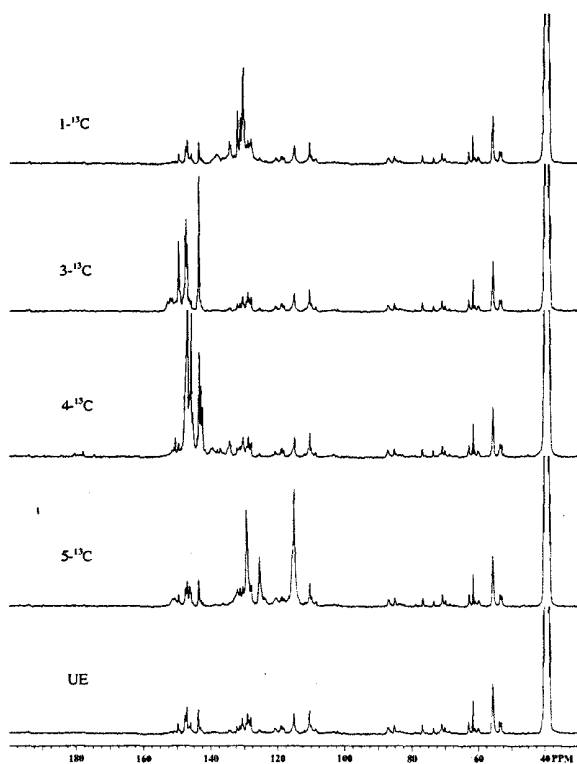


Fig 1. Quantitative ^{13}C NMR spectra of unirradiated 1-, 3-, 4-, and 5- ^{13}C DHP and unenriched (UE) DHP.



Fig 2. Quantitative ^{13}C NMR spectra of irradiated (2x21 h) 1-, 3-, 4-, and 5- ^{13}C DHP and unenriched (UE) DHP.

DHP. Examples of structures with chemical shifts that correspond to the signals in the spectra are included in the figure. Conclusive assignment of all peaks is difficult due to the narrow shift region and the limitations of the

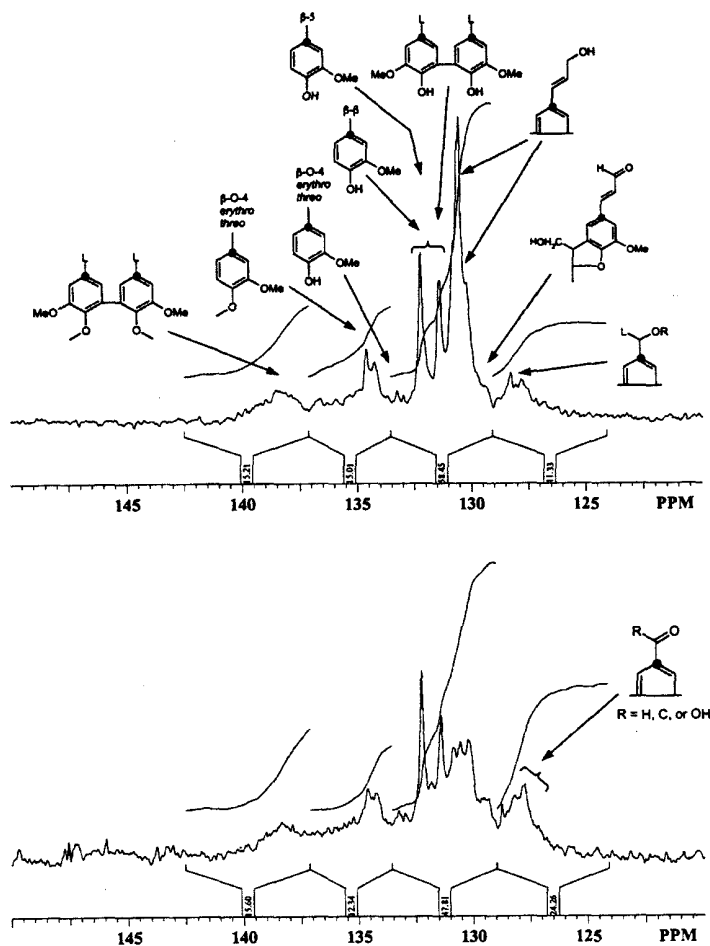
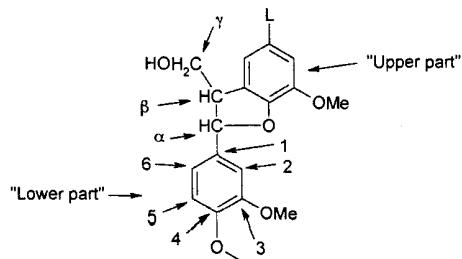


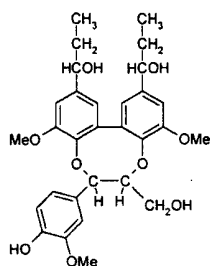
Fig 3. Difference spectra (^{13}C -enriched - unenriched) of unirradiated (top) and irradiated (2x21 h, bottom) 1- ^{13}C DHP. L = a general part of the structure.

model compound NMR data available. The difficulties are especially pronounced for ring-1- ^{13}C -enriched DHP since the chemical shift of the carbon-1 is very dependent on the side-chain structure. There is a need for more NMR data, for instance, of model compounds of β -5 and 5-5 type structures, with appropriate side-chains. In a paper by Miyakoshi and Chen (1992), substituent effects on the ^{13}C NMR shifts of phenylcoumaran models were studied. When changing the side-chain of the "upper part" (see *Formula Fig 1*) of a phenylcoumaran model from $-\text{CH}=\text{CH}-\text{CH}_3$ to $-\text{CH}(\text{OH})-\text{CH}_3$, the shift of the attached carbon 1 changed from 131.6 ppm to 141.1 ppm.

The major resonance in the narrow region that corresponds to the carbon 1 in the aromatic ring of the DHP mainly corresponds to coniferyl alcohol end groups



Formula Fig 1.



Formula Fig 2.

attached, for instance, by a β -5 or a β -O-4 linkage to the rest of the structure (big peak centered at 130.6 ppm). Other relatively clear signals are those of the etherified β -O-4 moieties (arylglycerol- β -aryl ether structures) at 134.3 ppm (*threo*) and 134.6 ppm (*erythro*). There are also small peaks that correspond to the C1 in phenolic β -O-4 structures at 133.0 ppm (*threo*) and 133.3 ppm (*erythro*). Phenolic biphenyls (5-5') appear at around 131-133 ppm (Ralph et al. 1998; Drumond et al. 1989, 1992; Alves et al. 2000) and etherified analogues appear at around 136-138 ppm (Drumond et al. 1989, 1992; Alves et al. 2000). Dibenzodioxocins (etherified biphenyls that form an 8-membered ring through α - and β -etherification with a side-chain in another unit) occur in lignin (Karhunen et al. 1995) and the C1 in these structu-

res (the 5-5' part), according to model compound data (a phenolic trimer with hydroxypropyl side-chains, see *Formula Fig 2*), appears at around 132 ppm, i.e. not in the same region as etherified biphenyls generally appear. It has to be stressed that in analogy with previous discussions, the exact location of this chemical shift is dependent on the exact structure of the side-chains. The C1 in the rest of the structure (i.e. the C1 attached to the α - and β -etherified side-chain) appears at 129.6 ppm (phenolic aromatic unit). The presence of dibenzodioxocins in unirradiated DHP was confirmed in the study of side-chain labeled DHP (Parkäs et al. 2004). The chemical shift of the C1 in phenolic parts of structures of the β - β type is around 132.2-132.4 ppm according to model data. The chemical shift of etherified β - β units will probably be around 134-134.5 ppm (a change in shift of around +2 ppm compared to the phenolic analogue), so they may overlap, at least in part, with the signal that corresponds to etherified arylglycerol- β -aryl ether structures. As mentioned above, due to the lack of model compound data it is more difficult to satisfactorily predict the shift of the C1 in the upper part of the β -5 type structures with side-chains other than that of the unsaturated type (e.g. $-\text{CH}=\text{CH}-\text{CH}_2\text{OH}$) satisfactorily. The C1 in the lower part of phenolic β -5 structures would, appear at around 132-133 ppm (Ralph et al.

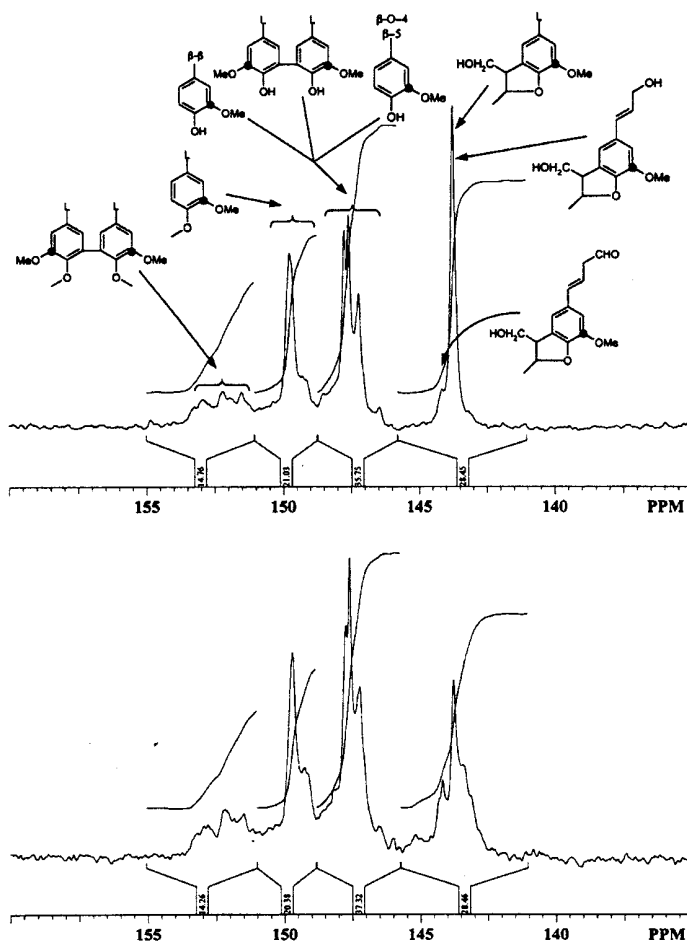


Fig 4. Difference spectra (^{13}C -enriched - unenriched) of unirradiated (top) and irradiated (2x21 h, bottom) $3\text{-}^{13}\text{C}$ DHP. L = a general part of the structure.

1998). The shift of the C1 in the lower part of etherified β -5 structures will be shifted downfield (i.e. to a higher ppm value) +1-2 ppm from the signal of the lower C1 in phenolic β -5 moieties, much like in the etherified β - β units. To some extent they might also overlap with the peak corresponding to etherified β -O-4 structures. The presence of β -5 linked coniferaldehyde end groups is indicated by a small shoulder at around 129.5 ppm. The C1 in these end groups, if β -O-4-linked would, according to Deyama et al. (1987a), appear at 126.7 ppm. The broad high-field region between 126-129 ppm mainly holds structures in which the carbon 1 is attached to α -carbons involved in α -ether bonds.

After irradiation for 2x21 hours (bottom spectrum in *Fig 3*) the most conspicuous changes are the decrease in the signal area that corresponds to the carbon 1 in coniferyl alcohol structures (peak centered at 130.6 ppm) and the increase in the high-field (lower ppm value) region. This observation can be explained by photo-oxidation of coniferyl alcohol end groups concomitant with the formation of mainly vanillin end groups, and perhaps also vanillic acid end groups. This is consistent with the results of analogous experiments with side-chain ^{13}C -labeled DHP (Parkäs et al. 2004). A moderate increase in the amount of coniferaldehyde end groups (129.5 ppm) can also be discerned, an observation also consistent with the previous study. The exact nature of the α -carbonyl structures formed is not

fully known. In experiments with α - ^{13}C -labeled DHP (Parkås et al. 2004), irradiation caused the formation of new α -carbonyls (apart from vanillin and vanillic acid end groups) possibly related to α -carbonylic β -ethers and to a small extent to α -carbonylic photoproducts [possibly from β -ether cleavage, i.e. of the 1-aryl-3-hydroxy-1-propanone type ($\text{Ar-CO-CH}_2\text{-CH}_2\text{OH}$)]. Clear identifications may perhaps be obtained by detailed 2-D NMR studies of ring-1- ^{13}C - and α - ^{13}C -labeled DHP in the future.

No very low-field signals that can be attributed to carbonylic C1s (e.g. in *para*-quinones) can be discerned in the difference spectrum of irradiated ring-1- ^{13}C -DHP (out of the chemical shift range in Fig 3). Possible reasons for the absence of quinone-peaks are discussed in a section below. A new peak is introduced at 131.8 ppm, the exact structure behind this peak remains elusive at the moment.

Ring-3- ^{13}C -enrichedDHP

Fig 4 shows the difference spectra that correspond to unirradiated (top) and irradiated (bottom) ring-3- ^{13}C -enriched DHP. The large peak centered at 143.8 ppm corresponds to C3 in the upper part of a β -5-unit (Miyakoshi, Chen 1992; Okusa et al. 1996; Ralph et al. 1998), most commonly with a coniferyl alcohol type unsaturated side-chain. The small peak at 144.2 ppm corresponds to the upper C3 of β -5-linked coniferaldehyde structures as indicated in Fig 4. The peak complex at 146.5-148 ppm holds C3s in phenolic structures, including β - β , β -5, β -O-4, and biphenylic structures. The position 3 aromatic ring carbons in etherified units appear in the region of the peak centered around 149.8 ppm. The broad, ill-defined region at low field (151-153.5 ppm) possibly originates from C3-carbons in etherified biphenyls and in diaryl ether structures (cf. Pan et al. 1997; Sjöholm et al. 1992; Alves et al. 2000). The model compound NMR data suggest that the C3 in the 5-5 part of dibenzodioxocins appears at around 152 ppm. The C3 of the lower part of this structure (that holds the etherified side-chain) in phenolic units appears in the same region as the phenolic structures in general (at around 147 ppm).

The integrations in Fig 4 (top unirradiated, bottom irradiated) show that the photoyellowing does not induce changes that drastically move the peak positions that correspond to the aromatic carbon 3; the integrated regions have essentially the same relative area before and after irradiation. The most conspicuous change is the broadening of the peak centered at 143.8 ppm, probably due to the degradation of coniferyl alcohol end groups (β -5-bonded) and the formation of vanillin and vanillic acid end groups (β -5-bonded), as previously observed (Parkås et al. 2004). Furthermore, it can be seen that the amount of β -5-bonded coniferaldehyde end

groups (the peak at 144.2 ppm) increases somewhat as was the case for ring-1-labeled and side-chain-labeled (Parkås et al. 2004) DHPs. Another change is that the relative signal area of the higher-field shoulder on the peak at 149.8 ppm increases in comparison to the main peak. No peaks that correspond to carbon 3 in quinone structures (ortho-quinones) can be observed after irradiation (this region excluded from Fig 4 for clarity).

Ring-4- ^{13}C -enrichedDHP

Fig 5 illustrates the difference spectra that correspond to unirradiated (top) and irradiated ring-4- ^{13}C -labeled DHP. The peaks at 142.5-144 ppm correspond to phenolic biphenyl moieties. The small peak at 145.5 ppm corresponds to carbon 4 in phenolic β -O-4 units and the region between 145.9-146.6 holds C4 in phenolic β -5 and β - β units. Etherification of biphenyls causes the shift of C4 to move downfield by about 2 ppm, which makes it probable that the peaks corresponding to these kinds of structures appear in the same region as phenolic β -5, β - β and β -O-4 structures (cf. Drumond et al. 1989; Sjöholm et al. 1992; Alves et al. 2000). The large peak centered at 147.1 ppm corresponds to carbon 4 in coniferyl alcohol end groups (e.g. β -5 and β -O-4 linked). The broad resonance down field from the coniferyl alcohol peak (147.1 ppm) corresponds to, for instance, etherified β -O-

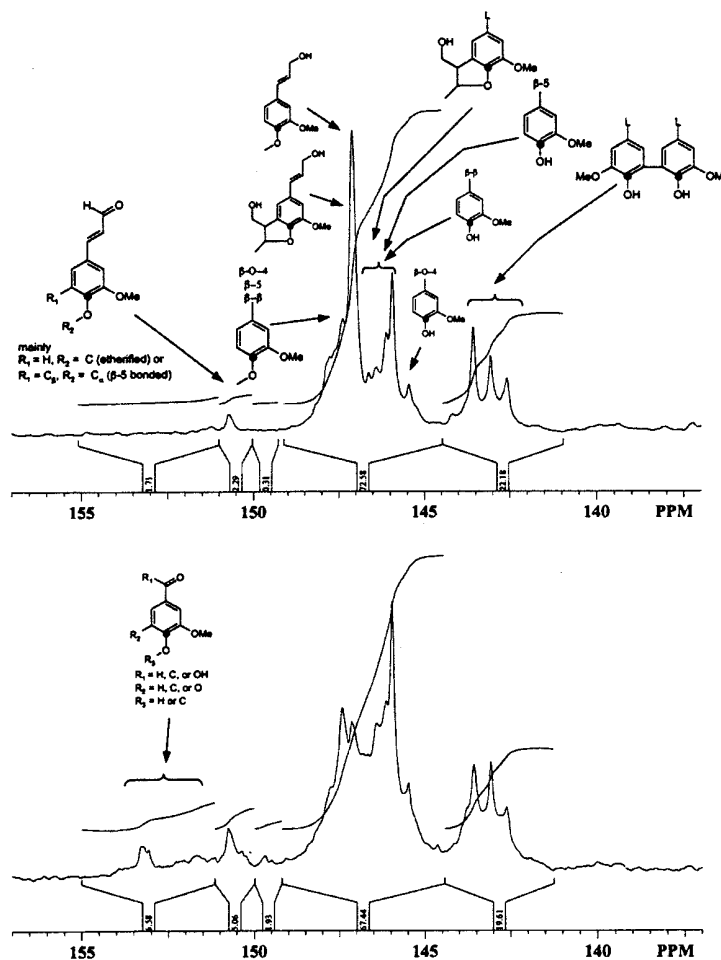


Fig 5. Difference spectra (^{13}C -enriched - unenriched) of unirradiated (top) and irradiated (2x21 h, bottom) 4- ^{13}C DHP. L = a general part of the structure.

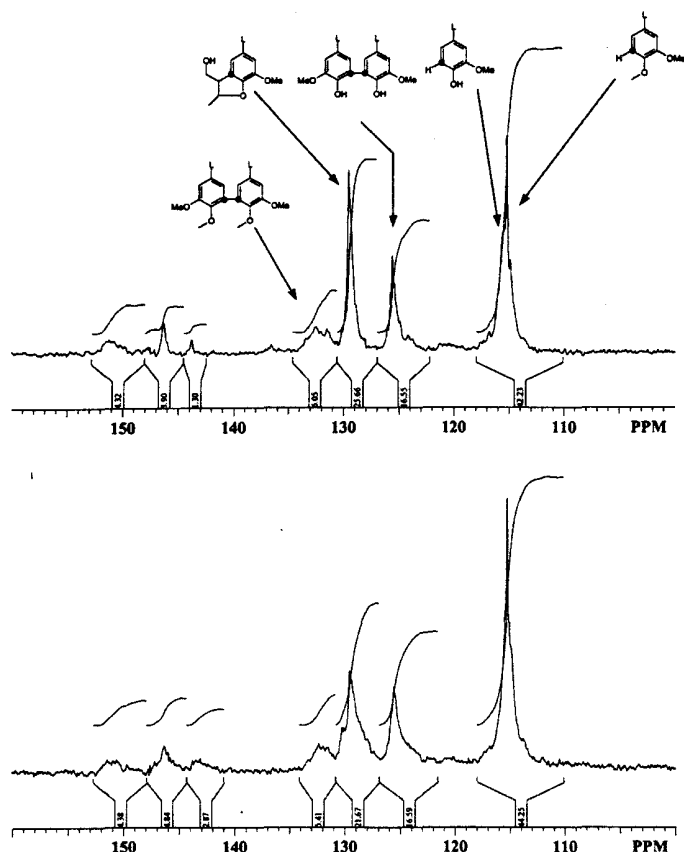


Fig 6. Difference spectra (^{13}C -enriched - unenriched) of unirradiated (top) and irradiated (2x21 h, bottom) 5- ^{13}C DHP. L = a general part of the structure

4, β - β , and β -5 units. It is difficult to predict the chemical shift of etherified C4 in some structures due to the lack of appropriate model compound data. There is often large differences in the C4-shift of models that are methylated or that have other types of etherification (glucosylated or etherified to, for instance, the β -carbon of another unit), cf. Landucci, Ralph (1997). For instance, the chemical shift of the C4 in pinoresinol is 145.9 ppm, when methylated the chemical shift changes to 148.1 ppm but glucosylation causes no change in the chemical shift of the C4, according to Nishibe et al. (1984). The carbon 4 in the upper part of a phenylcoumaran (β -5) structure without the unsaturated side-chain as in coniferyl alcohol should appear at around 146-147 ppm, i.e. in approximately the same region as the lower part C4 of phenolic β -5 structures (Miyakoshi, Chen 1992). The small peak at 150.7 ppm is assigned to coniferaldehyde end-group structures connected to the rest of the DHP mainly via β -O-4 or β -5 linkages, as indicated in Fig 5. The assignment of C4 in the diarylether structures is somewhat tentative, due to limited model compound data. Model data indicate that the C4 in the 5-5 part of dibenzodioxocin linkages appears at around 145 ppm (hydroxypropyl side-chain) and the C4 of the lower part of a phenolic structure (the C4 in the α - and β -etherified C9-unit) at around 146 ppm, i.e. in the region of etherified biphenyls and phenolic β -ethers, respectively.

After irradiation it can be concluded that the relative

signal area of the peak at 147.1 ppm decreases considerably together with an increase in the lower-field region. Again, this is consistent with the degradation of coniferyl alcohol moieties with the formation of α -carbonylic degradation products like vanillin and vanillic acid end groups (β -O-4 or β -5 bonded). Other structures that could give rise to new lower-field peaks are α -carbonylic β -ether structures and α -carbonylic β -ether cleavage products as indicated in the photoyellowing of side-chain labeled DHP (Parkås et al. 2004). It can also be seen that the amount of coniferaldehyde structures increases as a result of irradiation, as shown before. New, small, inconclusively assigned peaks appear at 144.6 ppm and at around 149.7 ppm after irradiation. No peaks that could be attributed to carbon 4 in quinones (para- and/or ortho-quinones) were observed.

Ring-5- ^{13}C -enriched DHP

Fig 6 shows the difference spectra that correspond to unirradiated (top) and irradiated (bottom) ring-5- ^{13}C -enriched DHP. Three clearly distinct regions of signals can be discerned in the spectra of ring-5- ^{13}C -labeled DHP, arising from aromatic rings with C5 bound to H, R (and Ar), and O, respectively, in order from high to low field. The signal centered at 115.2 ppm corresponds to the C5 on uncondensed (C5-H) guaiacyl (G, 4-hydroxy-3-methoxyphenyl) units, with the C5 in phenolic G-units generally at a lower field than the C5 in etherified G-units. The peak between 123-127 ppm corresponds to phenolic biphenyls. The carbon 5 in phenylcoumaran rings appears centered at 129.5 ppm and the broad resonance at 130.5-134 ppm is assigned to etherified biphenyls. The biphenyl structures could, of course, also be partially etherified and have one C5 at around 125 and the other at around 132 ppm. The C5 of the 5-5 part in a dibenzodioxocin model (with hydroxypropyl side-chain) appears at 142.5 ppm. The shift of this type of carbon in a DHP/lignin structure may be somewhat different due to a different side-chain environment. The small peaks at lower field are candidates that might correspond to the C5 of dibenzodioxocins. The low-field region is, somewhat tentatively, also assigned to C5 in diarylether (4-O-5) Structures. Pan et al. (1994) and Sjöholm et al. (1992) assign peaks at 152.6 ppm and 152.3 ppm, respectively, to C3/C5 in 4-O-5 type structures.

Irradiation leads to a broadening of the peak at 129.5 ppm, due to the degradation of β -5-linked coniferyl alcohol moieties to vanillin and/or vanillic acid type end groups (β -5-linked). Also, the peak that corresponds to uncondensed G-units at around 115 ppm is shifted slightly towards higher field, an indication of the formation of α -carbonylic structures and/or increase in β -O-4-linked coniferaldehyde end groups. There is no indication of an increase in condensed units in the extractable part of the DHP due to irradiation.

Discussion

The results from the present study of 1-, 3-, 4-, and 5-¹³C-labeled DHP have, to a large extent, confirmed the chemical changes traced by side-chain labeled DHP (Parkås et al. 2004). These changes include the degradation of coniferyl alcohol end groups and the formation of vanillin and vanillic acid type end groups. One of the main objectives of the present study was to, if possible, confirm the formation of *ortho*- and/or *para*-quinones during light-induced yellowing. ¹³C-Labeling of aromatic carbons is suited for this since the formation of quinones yields aromatic carbonyls with very low-field chemical shifts distinct from the normal aromatic region and because of the ¹³C-labeling, detection of small amounts of formed quinones should be easier than with unenriched material. We found no evidence for the presence of quinones after irradiation for 2x21 h (21 hours on each side of the filter paper), an observation that can be explained in several ways:

1 No quinones were formed. This is unlikely, since many studies have indicated the formation of quinones during photoyellowing, cf. e.g. the references cited in the introduction part of the paper.

2 Quinones were formed, but reacted further to form unidentified and complex non-quinoid structures, as proposed by Argyropoulos et al. (1995).

3 Quinones were formed, but the quinoid products were not extractable with the solvent used (1,2-dichloroethane/ethanol, 2/1).

4 The amount of formed quinones was under the detection limit with the present experimental setup, i.e. with a rather low ¹³C-enrichment degree. Using DHP with a higher ¹³C enrichment degree will increase the sensitivity of the NMR analysis.

The specific labeling of aromatic carbons of lignin precursors provides unique potential to study lignin and DHP chemistry. The administration of ring-labeled coniferin to living plants, e.g. ginkgo (*Ginkgo biloba*), will give new and valuable information on the fine structure of native lignin after solid state ¹³C NMR analysis of wood meal and solution state ¹³C NMR of milled wood lignin. High-molecular weight lignin models of the DHP-type, as in this study, can also be used for studying lignin reactions during many kinds of technical processes, not only light-induced yellowing. This study has shown that many of the signals in the difference spectra that correspond to ¹³C-enrichment of carbons in the aromatic ring can be assigned with certainty, although there is need for complementary studies; model compound NMR studies. At the present, we have the potential to ¹³C-label all three side-chain carbons together with aromatic ring carbons 1, 3, 4, and 5, selectively (cf. Terashima et al. 1996a, 2002, 2003).

In the future, important information could be obtained if the DHP-impregnated sheets are studied with solid state ¹³C NMR spectroscopy prior to extraction. It would also be interesting to try to obtain a time-scale for the chemical changes that occur, i.e. to see how the structure of the ¹³C-enriched DHP changes after both shorter and longer periods of irradiation. Moreover, extensive 2-D

NMR studies may help to assign additional structures prior to irradiation, as well as to identify some of the photo-induced changes in more detail.

Conclusions

The present study has shown that DHP, selectively ¹³C-enriched in positions 1, 3, 4, and 5 in the aromatic ring, can be used as high-molecular weight lignin models during light-induced yellowing and, additionally, as lignin models in studies of other types of technical processes. Most of the signals can be confidently assigned and only a few peaks have tentative assignments. The chemical changes observed have confirmed the results of previous studies using side-chain ¹³C-enriched DHP, i.e. a decrease in the amount of coniferyl alcohol end groups and a formation of α -carbonylic structures. The α -carbonylic structures are likely, to a large extent, to be of the vanillin and vanillic acid end-group type, and perhaps, to a lesser extent, α -carbonylic structures of the β -ether type or products of β -ether cleavage. In the present study, we could not identify quinones among the photoproducts. There could be several explanations for this; that no quinones were formed, although yellowing was observed, that quinones were formed initially and are only present in the extract at sub-detectable levels, or that initially formed quinones reacted further to form complicated structures of a non-quinoid character. It is also possible that the quinoid products, if formed, were unextractable with the solvent used and/or in some way physically or chemically combined with the substrate.

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