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A COMPARISON OF LIGNIN POLYMER MODELS (DHPs) AND LIGNINS BY ^{31}P NMR SPECTROSCOPY

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Key Word Index—*Picea abies*; *Prunus*; Pinaceae; Rosaceae; lignin analysis; dehydrogenation polymer; DHP; hydroxyl groups; *erythro*; *threo*; molecular weight; nuclear magnetic resonance; phosphorus NMR spectroscopy.

Abstract—Fractionated guaiacyl (G) and guaiacyl/syringyl (GS) DHPs prepared by continuous (Zutropf, ZT) and discontinuous (Zulauf, ZL) dehydrogenation schemes were subjected to quantitative ^{31}P NMR spectroscopy and their various hydroxyl groups determined. Two milled wood lignin samples from softwood and hardwood species were also examined. It was found that GS-DHPs resemble more GS milled wood lignins than G-DHPs resemble G milled wood lignins. The total phenolic-OH contents of ZT-DHPs are always lower than those of ZL-DHPs, in good agreement with the theory which predicts more β-O-4 linkages in the former. Furthermore, in agreement with the dehydrogenation theory, the bulk character of G-DHPs results from the formation of condensed units. In GS-DHPs, the S units are etherified early in the polymerization process, while the bulk character of such samples is mainly due to G-units. Regarding the secondary hydroxyl groups the results obtained for the G-DHPs are extremely low. This underlines again the principal differences of G-DHPs compared with GS-DHPs and MWLs. The *erythro* to *threo* ratio of the G-type samples was found to vary between 1 and 1.5, indicating only minor dependence on molar mass or mode of preparation. The *erythro* to *threo* ratio in GS-type samples was found to vary from 1.6 to 4.3, showing the highest value for low molar mass ZL-DHPs and lowest values for the cherry tree MWL. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Lignins are enzyme-initiated natural polymers emerging from random radical coupling reactions of at least two precursors. Coniferyl alcohol gives rise to a guaiacyl (G) lignin in the case of softwoods and a mixture of coniferyl and sinapyl alcohol yields a guiaiacyl/syringyl (GS) lignin in hardwoods [1]. A major milestone in lignin chemistry was Freudenberg's success in polymerizing coniferyl alcohol to a lignin-like dehydrogenation polymer (DHP) using fungal laccase and other oxidative enzymes [2].

In subsequent years, the synthesis of DHPs was refined. For example, peroxidase/H_2O_2 was used as phenol dehydrogenase instead of laccase [3]. By adding the precursors in a slow and continuous fashion ('*Zutropf*' method, ZT) it was found that the products structurally resembled lignins more than when adding the precursors in a batch-wise mode ('*Zulauf*' method, ZL), in which all precursors were allowed to polymerize at the same time [4].

A considerable body of literature has accumulated on DHPs and lignins since those early developments. Sarkanen [5] pointed out in 1971 that DHPs are lignin-like but not identical to lignins formed *in vivo*. He coined the notion 'lignin polymer models' for DHPs and developed a clear theoretical model for the structural differences obtained by the continuous (ZT) and batch-wise (ZL) modes of polymerization. According to his nomenclature, the former leads to an 'end-wise' and the latter to a 'bulk' polymer. In practice, there is no distinct border between these polymer types. For the short description of a dehydrogenation polymer it is more correct to state that the 'end-wise' or 'bulk' character is prevailing. Figure 1 shows the relevant intermonomeric linkages of lignins, which also includes the recently discovered octogonal structure (no. 6, Fig. 1) [6].

According to Sarkanen [5] a typical end-wise polymer contains a lot of β-O-4 linkages and consequently many α-hydroxyl and γ-hydroxyl groups and a few phenolic ones. Some β-1 linkages and structures with displaced side-chains containing α-hydroxyl and non-

This paper is dedicated to Professor Dr H. H. Nimz on the occasion of his 65th birthday and his retirement.

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conjugated aldehyde groups are also relevant in an end-wise polymer. Conversely, only a few β-5 (i.e. phenylcoumaran) and β-β (of the 'resinol' and tetrahydrofuran type structures are present in this polymer, in which the genuine α-hydroxy and γ-hydroxyl groups are etherified by furan-ring closure. Furthermore, only low amounts of conjugated double bonds are seen because the formation of the cinnamic alcohol 'end-groups', which also maintain their original γ-hydroxyl groups, is not favoured during polymerization. The oxygen content of end-wise polymers is usually high, with values around 270 for 100 phenylpropane units [5, 7] (a phenylpropane unit has a C_9-skeleton, thus the data presented based on 100 units will be designated in the following as data/C_{900}). The high oxygen value is a consequence of the hydroxyl ion addition to α-carbon atoms during the formation of a β-O-4 dimer. The dehydrogenation degree (DD), which is a measure of hydrogen loss during the polymerization, is lower than 50/C_{900}, while the double bound equivalents (DBE), which are a measure of unsaturated structures and ring closures, are around 450/C_{900}, or lower (the DD and DBE value are calculated based on the formulae presented by Sarkanen [5]).

Sarkanen [5] also pointed out that in a typical bulk polymer, β-O-4 linkages occur in low amounts, while the β-5, β-β linkages are predominant. Consequently, most of the oxygen atoms in the α- and γ-positions are etherified. The β-5 and β-β units, formed in the dimerization stage, undergo further polymerization via 5-O-4 and 5-5 coupling. Therefore, a higher amount of phenolic hydroxyl groups can be expected in polymers of that kind. An abundance of double bonds in the side-chains is also characteristic. These features are manifested in the C_{900} formula because the oxygen content of such polymers is low (around 250). The DD values (*ca* 100) and DBE values (*ca* 500) are high [5].

These conceptual models and the differences between DHPs and lignins are supported by experimental observations. Nimz and co-workers [8, 9] compared a spruce milled wood lignin (MWL) with G-ZL- and G-ZT-DHPs using ^{13}C NMR spectroscopy and found the following binding frequencies: β-O-4: ZT > ZL; β-5: $ZL_{low\ Mw} > ZL_{high\ Mw} > ZT$; β-β: ZL > ZT; cinamyl end-groups: $ZL_{low\ Mw} > ZL_{high\ Mw} > ZT$. Lai and Sarkanen [7] confirmed that the C_{900} formula of a G-ZT-DHP contains more oxygen and hydrogen than that of a G-ZL-DHP. The infrared spectrum of a G-ZL-DHP revealed the presence of units linked by β-5, 4-O-5, or 5-5 bonds. Several, subsequent, papers reported on differences between G-ZL-DHPs, G-ZT-DHPs and G milled wood lignins, respectively [9–15]. However, the differences between ZT- and ZL-DHPs are not always as clear as dictated by the theory [16, 17]. Sipilä and Brunow [18], and Brunow and co-workers [19] recently called attention to the fact that the polymerization mode of coniferyl alcohol at pH 4 favours the formation of benzylic alcohols and resembles MWLs more closely than coniferyl alcohol polymerized at pH values between 5.5 and 7.0. Under these conditions, almost no formation of benzyl aryl ethers occurs [18–20]. The question of noncyclic benzyl alkyl ethers is controversial [21–24]. Quideau and Ralph [20] recently pointed out that these structures are not formed under either alkaline or acidic conditions when silver (I) oxide oxidation of coniferyl alcohol is performed [20].

Although DHP research has paved the way towards a deeper understanding of lignin chemistry, modelling and investigating the molecular architecture of these lignin-like polymers is still instructive [25]. This work is a further contribution to this topic. Various ZT- and ZL-DHPs were prepared from guaiacyl and syringyl type precursors and compared with to a softwood and a hardwood lignin, respectively. The DHPs were fractionated on a Sephadex 60 column before analysis and the molecular weight of the fractions was determined. Because various structural features and the hydroxyl group contents in lignins are closely related (Fig. 1), and since ^{31}P NMR spectroscopy can quantitatively determine the various hydroxyl groups in lignins [26–29], we used this analytical tool for comparison of DHPs and lignins.

RESULTS AND DISCUSSION

Sample characterization

The molecular weights, C_{900} formulae, dehydrogenation degrees (DDs), and double bound equivalents (DBEs) of the fractionated lignin polymer models (DHPs) and milled wood lignins (MWLs) are shown in Table 1. Unless indicated otherwise, the analytical data will be discussed on a C_{900} basis. The five G-ZT-DHPs, whose molecular weights vary between 9000 and 1600 g mol^{-1} (dispersities between 2.3 and 1.3), do not show any systematic differences. Therefore, their key data can be averaged: 256 O, DDs 161, and DBEs 484. This indicates that there is no systematic impact of the molecular weight on the end-wise or bulk character of these polymers. Surprisingly, the oxygen contents, DD and DBE values of the G-ZL-DHPs are arranged in a similar order of magnitude as for the G-ZT-DHPs. Only the low molecular weight fraction at 860 M_w shows a low oxygen content (228) and a lower DD (138). This finding suggests, that for the G-DHPs the difference of ZT and ZL mode at pH 6.8 is not as large as proposed by the theoretical models of Sarkanen [5] described in the introduction. The OMe content of the spruce MWL (94) is lower than those of the G-DHPs (around 101) owing to the participation of 4-hydroxyphenylpropanes in its structure [1]. Its oxygen content (269) is similar to those of the G-DHPs while its DBE (446) is slightly lower. The DD of spruce MWL (88) is approximately half of that of the DHPs.

The OMe content of the GS-DHPs (175) is high, which indicates an S content of around 75 mol%, and refers to a preferred incorporation of S units during the polymerisation procedure. In contrast to results for

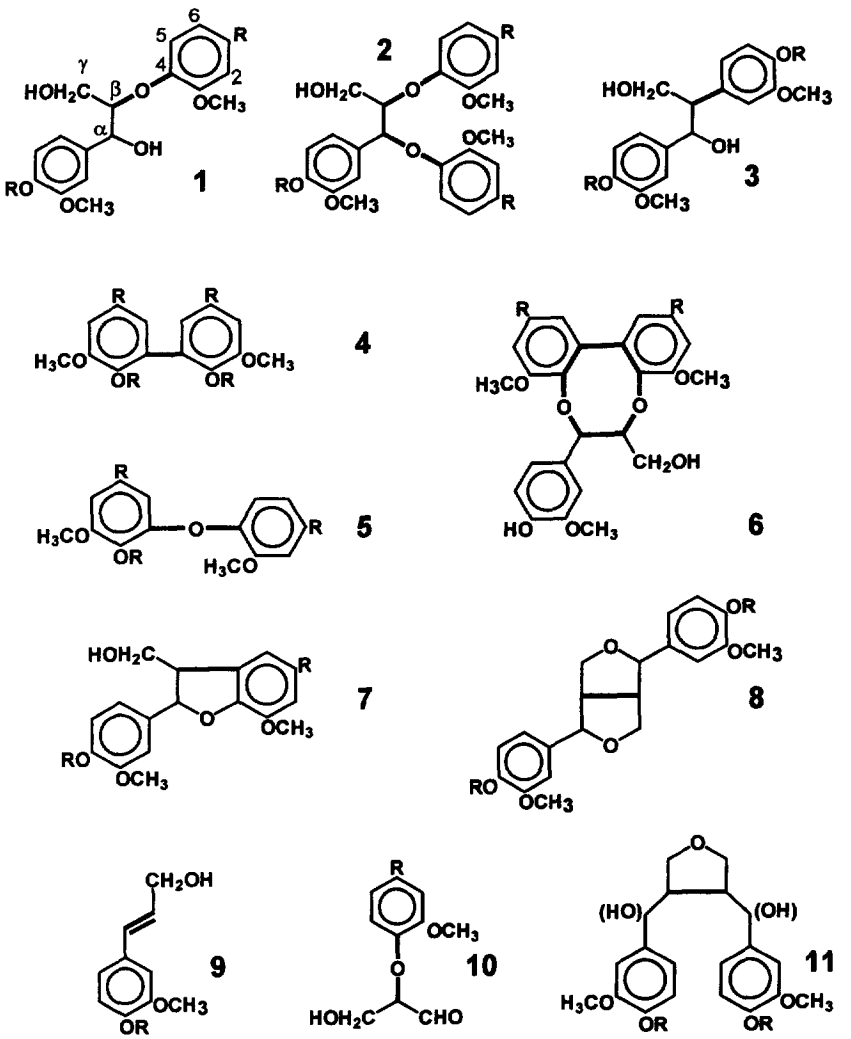

Fig. 1. Typical dimeric structures of lignins. R in position 1 of the aromatic ring: propane side-chain linked to the lignin: R on position of phenolic oxygen: H or lignin. **1**: β-O-4; **2**: α-O-4; β-O-4; **3**: β-1; **4**: 5-5; **5**: 5-*O*-4; **6**: (5-5) + (α-O-4, β-O-4) octogonal; **7**: β-5 coumaran; **8**: β-β pinoresinol; **9**: cinnamic alcohol end-group; **10**: displaced side chain; **11**: β-β tetrahydrofuran.

G-DHPs, the data of GS-DHPs show clear dependencies on molecular weights, i.e. the oxygen content decreases from 318 (at M_w 5100 g mol^{-1}) to 260 (at M_w 1100 g mol^{-1}), demonstrating that their bulk character is increasing. In general, their DD values are low, decreasing from 81 in the first fraction to 41 in the last one. Their DBE values are also decreasing from 442 to 422. Once again, there is a discrepancy between the analytical data and the theoretical model of Sarkanen [5]. Nevertheless, the C_{900} formulae of GS-DHPs demonstrate that their end-wise character, as a whole, is more pronounced than those of the G-DHPs. For example, a closer inspection of samples of similar molecular weight indicates that the DD values of the GS-DHPs are approximately half those of G-DHPs. The DD value (120) of the GS-ZL-DHP fraction with M_w 1790 g mol^{-1} is remarkably high. The OMe content of the cherry MWL (151) is typical for hardwood lignins [28]. Its DD (81) is similar to those of GS-ZT-DHPs in the M_w range between 3000 and 5000 g mol^{-1}.

Signal assignment of the ^{31}P NMR spectra

The ^{31}P NMR spectra of two G-DHPs and GS-DHPs and of sprue MWL and cherry MWL are depicted in Fig. 2. Signal assignments and the integration limits were carried out according to previous publications [26–29].

The spectra including the internal standard were integrated between 136.4 and 128 ppm. The range 136.4–133.8 ppm can be assigned mainly to the α-hydroxyl groups. For the samples in question the proper selection of the upper limit of this range requires attention. Signals above 136 ppm are mainly due to carbohydrate impurities. Therefore, the upper integration limit for MWLs was set at 136 ppm [26, 27]. The

Table 1. Molecular weights and C_{900} formulae of fractionated DHPs and milled wood lignins

Samples	Molecular weight*		Dispersity	C_{900} formula			DD†	DBE‡
	M_w	M_n	M_w/M_n	H	O	OCH_3		
G-ZT DHPs	9020	3990	2.3	742	261	102	156	489
	8610	3480	2.5	724	257	101	175	489
	4500	2560	1.8	752	259	101	147	475
	2720	1820	1.5	732	256	101	167	485
	1630§	1200	1.4	739	261	102	159	481
G-ZL DHPs	2500	1670	1.5	729	255	102	169	486
	1510§	1150	1.3	736	260	103	161	482
	860	710	1.2	762	228	100	138	471
GS-ZT DHPs	5110	3330	1.5	749	318	170	81	442
	3010	2280	1.3	749	291	173	78	441
	1790§	1440	1.2	763	279	176	61	432
	1440	1220	1.2	773	263	175	52	428
	1070	1030	1.1	784	260	175	41	422
GS-ZL DHPs	1790§	1440	1.2	706	272	174	120	462
	1170	1030	1.1	738	253	173	89	446
	960	840	1.2	755	255	165	80	442
G-MWL (*Picea abies*)	4190§	1380	3.0	818	269	94	88	446
GS-MWL (*Prunus* sp.)	4850§	2290	2.1	768	285	151	81	442

*Polystyrene standards.

†Degree of dehydrogenation = $1000 - (H/C_{900} + OMe/C_{900})$, according to Freudenberg (1968).

‡Double bond equivalents in $C_aH_bO_c = ((2a + 2) - b):2$.

§Samples are those whose ^{31}P NMR spectra are displayed in Fig. 2.

same integration boundary was used for G-DHPs. Tailing can be observed above 136 ppm for ZT-GS-DHPs. Therefore, the integration limit for these samples was extended to 136.4 ppm (Fig. 2).

In previous studies, it was shown that the α-$OH_{erythro}$ configuration is in an upfield region (above 134.4 ppm), whereas the *threo* configurations can be detected below this limit [26]. It is noteworthy that the G-DHPs show only weak signals for α-hydroxyl groups which makes the differentiation of the *erythro* and *threo* configurations less important (Fig. 2). The deficiency of α-hydroxyl groups might be partly a consequence of phenylcoumaran and/or pinoresinol structures. In addition, the high pH used during DHP synthesis favours the formation of benzyl aryl ethers, according to recent publications [18–20].

In contrast to these observations, the spectra of the GS-DHPs show a large signal with a maximum at 135.4 ppm in the *erythro* domain. Because the syringyl content of these DHPs is high, it is likely that this signal can be assigned to the α-hydroxyls of the *S* units. As a supporting evidence to this assumption, the cherry MWL also exhibits this feature, albeit the corresponding signal centred at 135.5 ppm is broad. The high molecular weight and dispersity of the cherry MWL may explain the broadening.

The question arising in this context about the chemical shifts for G α-OH_{threo} and S α-OH_{threo} configurations may be rationalized as follows. The small, sharp signal at 134.3 ppm in GS-DHPs originates probably from the S α-OH_{threo} configuration because it is unlikely that the G units with *ca* 30% abundance give rise to a discernible signal here. However, the featureless character of this spectral region in G-DHPs does not allow the determination of the chemical shift of G α-OH_{threo} structures. The spectrum of spruce MWL shows a maximum at 134.1 ppm which can be due to the G α-OH_{threo} groups. In the spectrum of cherry MWL, the maximum is also at 134.1 but this signal shows a shoulder around 134.3 ppm (indicated by an arrow in Fig. 2). This corresponds to the signal of GS-DHPs and thus might have the same origin (i.e. α-OH_{threo} structures of S units).

For G-DHPs and the G lignin, the signals of the primary hydroxyls (γ-hydroxyls) appear between 133.8 and 131.6 ppm. However, for GS-DHPs and the GS lignin, this range overlaps with the signal of the phenolic-hydroxyl groups in syringyl units, as manifested in a pronounced signal with a maximum at 131.9 ppm. There are several ways to deal with this overlap in order to derive quantitative information. In this paper, the S-OH_{phen} signal was treated in analogy with GC data, i.e. as if it was a 'rider' peak. More specifically, a baseline was drawn (hatched areas in Fig. 2) to separate the S-OH_{phen} signal from the primary hydroxyl moieties of this region. Notably, there is a distinct difference between the primary hydroxyl region of spruce MWL and G-DHPs. The latter exhibit a strong signal at 132 ppm, the assignment of which is not yet proven by model compounds. Because DHPs are known to be rich in phenylcoumaran (β-5) structures, the primary hydroxyls of such units could be the origin of this signal [26].

The range between 132.3 and 129 ppm is the domain of phenolic hydroxyl groups (the overlap of phenolic syringyl hydroxyls and primary hydroxyls has been discussed previously). The G-OH_{phen} groups in 5-5 and 4-O-5 or β-5 units are positioned between 131.6 and

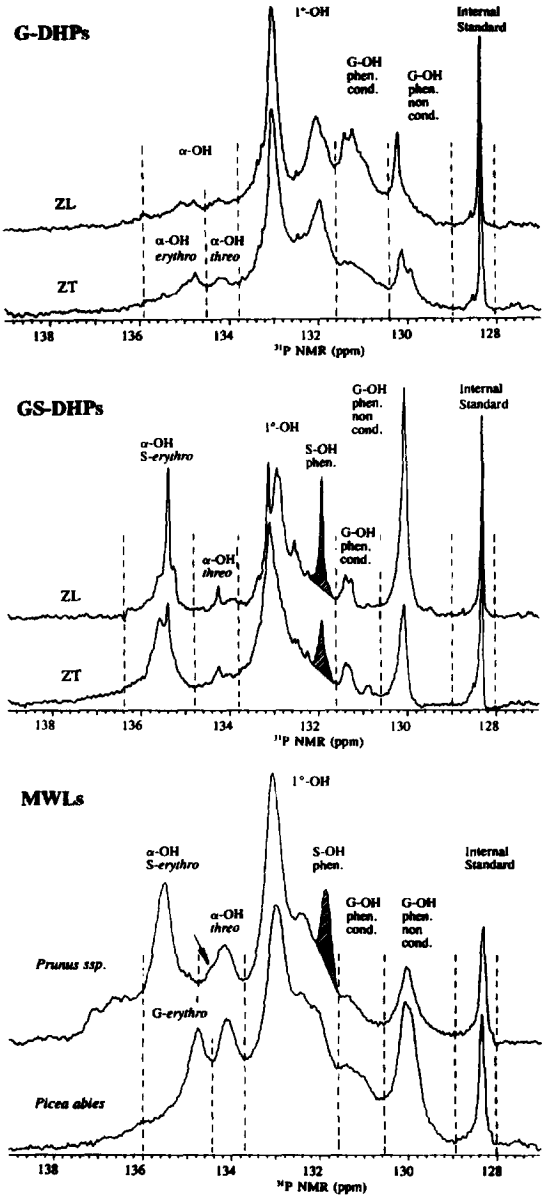

Fig. 2. ^{31}P NMR spectra of GS-DHPs, G-DHPs and MWLs. The DHP samples are of similar weight average molecular weights i.e. 1790–1510 (g mol^{-1}). ZT, continuous '*zutropf*' mode; ZL, discontinuous '*zulauf*' mode; *Prunus* sp, cherry; *Picea abies*, spruce; phen., phenolic; cond., condensed.

130.4 ppm while the other phenolic G-units generate the major signal at 130.1 ppm.

Quantitative determination of phenolic hydroxyl groups

The total phenolic hydroxyl groups (total OH_{phen}), presented in Fig. 3 as a function of molar mass, are the results of summed sub-integrals with the integration boundaries displayed in Fig. 2. The quantitative evaluation of the specific phenolic hydroxyl groups are presented in Fig. 4.

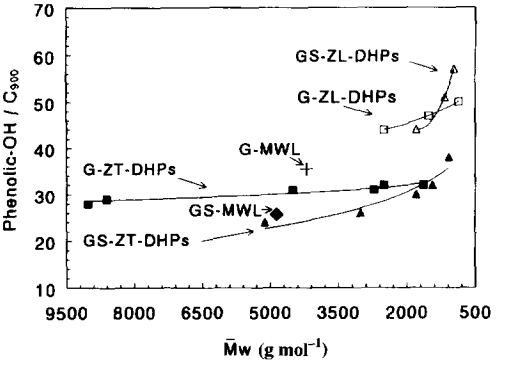

Fig. 3. Total phenolic hydroxyl content as a function of weight average molecular weight for DHPs and MWLs. ZT, continuous '*zutropf*' mode; ZL, discontinuous '*zulauf*' mode; GS-MWL, *Prunus* sp.; G-MWL, *Picea abies*.

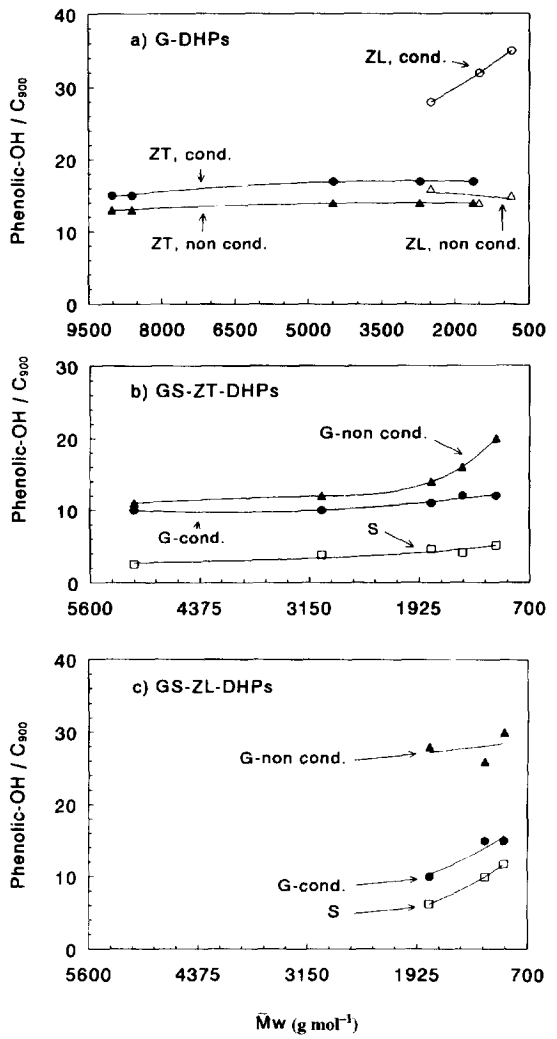

Fig. 4. Guaiacyl (G), syringyl (S) and C5-condensed (C5-cond.) phenolic hydroxyl content as a function of weight average molecular weight for the different DHPs. ZT, continuous '*zutropf*' mode; ZL, discontinuous '*zulauf*' mode.

Figure 3 is in agreement with the theoretical considerations outlined in the introduction, i.e. ZT-DHPs contain less total OH_{phen} groups (*ca* 30) than the ZL-DHPs (*ca* 50). Apparently, the higher the molecular weight, the lower the total phenolic hydroxyl group content. As expected, this trend is less pronounced for the G-ZT-DHPs as their data in Table 1 do not show a dependence on molecular weight. Conversely, the total OH_{phen} values of GS-DHPs are clearly decreasing for the higher molecular weight fractions.

The value of cherry MWL was found to be 26 (GS-ZT-DHPs 24-38). Selective aminolysis according to Mansson [31] on the same MWL resulted in 25 and the FTIR-PLS approach in 24 total OH_{phen} [32, 33]. Accordingly, the phenolic hydroxyl contents of GS-DHPs and cherry MWL are in a similar range. For the spruce MWL, 36 OH_{phen} were obtained while the average of G-ZT-DHPs was found to be somewhat lower (30). Robert and Brunow [13] used ^{13}C NMR spectroscopy and determined 37 OH_{phen} for a G-ZT-DHP and 19 OH_{phen} for a spruce MWL.

In view of the wide variety of possible structural variations between the samples under discussion, the congruence of these data can be considered as fair.

The plots of the specific OH_{phen} data for G-DHPs (Fig. 4a) reveals that the OH_{phen} groups in condensed units of G-ZL-DHPs show elevated values ranging between 28 and 35. All other OH_{phen} contents are between 13 and 16 and do not change significantly with molecular weight. This finding is in agreement with the bulk polymerization theory of DHPs. The diminishing OH_{phen} group contents in condensed units of ZL-DHPs in the higher molecular mass region can be interpreted as a combination of dimeric and/or oligomeric intermediates through 4-O-5 linkages. The OH_{phen} group content in spruce MWL amounts to 21 in noncondensed and 15 in condensed units. These values are similar to those of corresponding G-DHPs.

In GS-ZT-DHPs (Fig. 4b), the S-OH_{phen} and the G-OH_{phen} groups in condensed units show only a minor dependence on molecular weights. It is obvious that the S units are etherified at early stages of radical coupling. Therefore, their degree of etherification cannot develop any further during polymerization. As the macromolecule grows, more phenolic G-units are involved in ether linkages, i.e. through β-O-4, β-5, non-cyclic α-O-4, or 4-O-5 bonds. The G-OH_{phen} content thus declined from 20 to 12. Accordingly, the change of the end-wise or bulk character of the GS-ZT-DHPs, as discussed in the previous paragraph, is on account of G units. The corresponding values of cherry MWL fit well into this general picture (S-OH_{phen} 5; G-OH_{phen} in condensed units 8; G-OH_{phen} 12). In the case of GS-ZL-DHPs, all types of phenolic OH groups were found to be lower in the higher molecular weight range (Fig. 4c).

Quantitative determination of primary and secondary hydroxyl groups

One-hundred phenylpropane units linked by β-O-4 bonds in an idealized end-wise polymer should contain

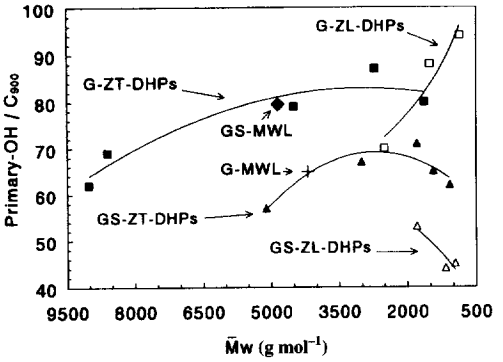

Fig. 5. Total primary hydroxyl content of different DHPs and MWLs as a function of weight average molecular weight. ZT, continuous '*zutropf*' mode; ZL, discontinuous '*zulauf*' mode; GS-MWL, *Prunus* sp.; G-MWL, *Picea abies*.

100 γ-OHs. Primary hydroxyl groups (γ-hydroxyls) are mainly etherified in β-β units by formation of furan rings in resinol structures (Fig. 1). The simultaneous examination of the primary (Fig. 5) and the secondary groups (Fig. 6) increases the clarity of the results.

The plots of γ-OHs versus molecular weight (Fig. 5) reveal the following. The molecular weights, G-ZL and ZT-DHPs do not differ significantly as far as their primary hydroxyl group contents are concerned. In general, G-DHPs contain more of these functional groups (62–94) than the GS-DHPs (54–79). For the latter samples, the ZL polymers showed significantly lower γ-hydroxyl contents (45) than those produced by the ZT polymerization mode (55–65). The γ-hydroxyls of G-DHPs are dependent on the molecular weights. Lower molecular weight fractions showed primary hydroxyl group contents ranging between 80 and 90, while for the higher molecular weight range, the primary OH content was between 60 and 80. The γ-hydroxyl group content of spruce MWL (65) was lower than the results found by ^{13}C NMR for a spruce MWL (76) [13]. The cherry MWL contains 80 γ-hydroxyls per 100 C_9 units.

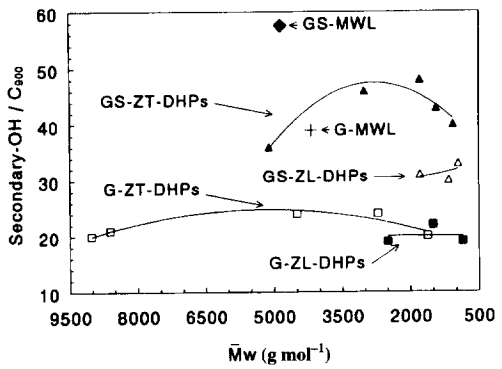

Fig. 6. Total secondary hydroxyl content of different DHPs and MWLs as a function of weight average molecular weight. ZT, continuous '*zutropf*' mode; ZL, discontinuous '*zulauf*' mode; GS-MWL, *Prunus* sp.; G-MWL, *Picea abies*.

These γ-hydroxyl values, which were lower than the maximal possible value of 100, are difficult to interpret as being exclusively the result of β-β linkage formation. In order to explain the etherification of 35 γ-hydroxyl units, about 17 pinoresinol structures should be invoked for spruce MWL. According to Freudenberg [1] only four pinoresinol dimers should be present in every 100 C_9 units of spruce lignin. The nearly identical amounts of α-hydroxyl groups in the G-ZL- and G-ZT-DHPs (around 20), which are independent of the molecular weight of the fraction (Fig. 6), do not support the presence of noncyclic α-O-γ linkages, a finding which is in accordance with recent results. Due to the high pH used in synthesis, the α-position seems to be preferentially involved in α-O-4 linkages [18–20]. Accordingly, the low amount of γ-hydroxyl groups in the higher molecular weight fraction of G-DHPs, defies explanation. Karhunen *et al.* [6] demonstrated by NMR studies the presence of octogonal structures in pine lignin. These structures would result in lower amounts of α-OH and phenolic-OH. However, the number of those structures in DHPs has not yet been investigated.

The interpretation of the corresponding data of the GS-DHPs is clearer. For GS-ZT-DHPs, both primary and secondary hydroxyl groups show curves with a maximum between 2000 and 3500 g mol^{-1} (Figs 5 and 6). The secondary hydroxyl group content of the GS-ZL-DHPs is lower than that of GS-ZT-DHPs, in agreement with the corresponding primary hydroxyl group contents. Therefore, these data support the idea of etherification between α and γ carbons. The same consideration holds true regarding the α and γ-hydroxyls of the MWLs. Cherry MWL is richer in both α- and γ-hydroxyl groups (58 and 80, respectively) than the spruce MWL (39 and 65, respectively).

Erythro/threo *ratios*

The *erythro/threo* ratios of both ZT- and ZL-G-DHPs were found to fluctuate between 1.1 and 1.4 and show only minor dependence on molecular weight. The *erythro/threo* ratio of spruce MWL has been estimated to be 1.2. Hauteville and co-workers [34] determined the same feature for several softwood MWLs by ^{1}H NMR as ranging between 1 and 1.5. It is remarkable that similar results were obtained by Taneda and co-workers [35] using ozonation, despite the fact that ozonation also determines the stereoisomers of the α-O-4 bonds, which are not detected by ^{31}P NMR. Nimz and co-workers [36] investigated the stereochemical configuration of an acetylated G-ZT-DHP and found that the ^{13}C NMR signals for *erythro* and *threo* configurations were of a similar order of magnitude. The low amount of α-hydroxyl groups in G-DHPs investigated in this paper make the results less representative but they are in agreement with the literature.

In contrast to the G-DHPs, the *erythro/threo* ratios of GS-DPHs are highly dependent on the molecular weight and the method of synthesis. As shown in Fig. 7, the *erythro/threo* ratio of the ZL-DHPs diminishes dramatically from 4.3 (M_w 960 g mol^{-1}) to 2.4 (M_w 1790 g mol^{-1}). The corresponding ratios of the ZT-DHPs also decrease in a linear fashion, although less precipitously, i.e. from 2.7 (M_w 1070 g mol^{-1}) to 1.9 (M_w 5110 g mol^{-1}).

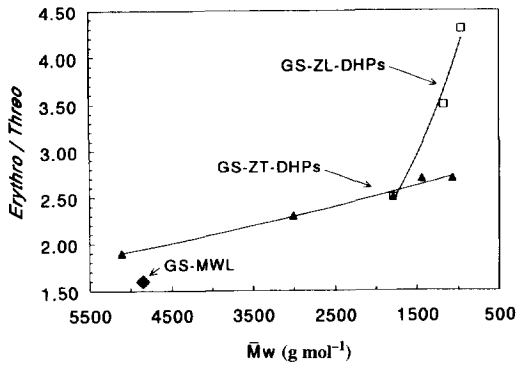

Fig. 7. *Erythro/threo* ratios obtained for the different GS-DHPs and cherry MWL as a function of weight average molecular weight. ZT, continuous '*zutropf*' mode; ZL, discontinuous '*zulauf*' mode; GS-MWL, *Prunus* sp.

Cherry MWL yielded an *erythro/threo* ratio of 1.6 at M_w 4010 g mol^{-1}. It is well documented in the literature that in hardwood lignins the erythro configuration is favoured more [36–38]. Matsumoto and co-workers [39] found that the erythro/threo ratios of hardwood MWLs, as determined by ozonation, may vary between 1.7 and 2.4, depending on the milling time, the morphological origin, and molecular weight.

CONCLUSION

Based on quantitative ^{31}P NMR and elemental analytical data, it can be confirmed that GS-DHPs more resemble GS type milled wood lignins than G-DHPs resemble G type milled wood lignins. The hydroxyl groups of GS-DHPs show distinct dependence on the molecular weight, while for the case of G-DHPs only the primary-OH content showed such an effect. The minor impact of the molecular weight on the G-DHPs could also be confirmed from the results of elemental analysis. In good agreement with the theory, which predicts more β-O-4 linkages in ZT-DHPs, lower phenolic OH content was detected in ZT-DHPs than in ZL-DHPs. The OH_{phen} group content of GS-DHPs is in agreement with that of the cherry MWL while G-DHPs show lower values than spruce MWL. In harmony with the dehydrogenation theory, the bulk character of G-DHPs results from the formation of condensed units in which position 5 of the aromatic ring is involved. In GS-DHPs, the S units are etherified from the very beginning of the polymerization while the G-units form preferentially β-5, β-β (in resinols), 4-O-4 and 5-5 linkages.

The G-DHPs contain extremely low amounts of secondary hydroxyl groups. Samples investigated in this paper have been synthesized at a pH of 6.8. Under these conditions, the formation of α-hydroxyl groups is

less favoured while the noncyclic α-O-4 ethers are probably predominant [18–20]. Interestingly, this effect is less pronounced for the GS-DHPs, which always show much higher α-hydroxyl content than the G-DHPs. The G-DHPs have in general a more pronounced bulk character. Even in GS-DHPs, the bulk character can be ascribed mainly to the G-units. It is possible that the G-DHP investigated here resembles more a lignin from the compound middle-lamella (ML) than a MWL, which is extracted from the secondary wall [40, 41]. ML-lignin is described as highly condensed G-type lignin, with higher proportion of noncyclic α-O-4, β-β and 5,5 structures [42–44].

The fractionation of G-ZT-DHP yielded fractions with higher molar mass than for the other samples. It might be possible that the branching of the polymer by non cyclic α-O-4 ethers enables the formation of higher molar masses.

The *erythro* to *threo* ratios of the G-type samples was found to vary between 1 and 1.5, indicating only a minor relationship between molar masses or the preparation modes of DHPs. In GS-type samples, the variation was higher (1.6 to 4.3) showing the highest value for low molar mass ZL-DHPs and lowest values for the cherry MWL.

EXPERIMENTAL

Preparation of ZT-DHPs. For the synthesis of G-DHPs, 2.7 g (15 mmol) of coniferyl alcohol were used or an equimolar mixture of coniferyl and sinapyl alcohol for the case of GS-DHPs. The precursors were dissolved in 3 l of phosphate buffer (0.01 M, pH 6.8) and kept at 1°C under N_2. This soln was then pumped over 120 hr at a rate of 25 ml hr^{-1} into 1 l of phosphate buffer containing 40 mg of horseradish peroxidase with a specific activity of 250 U g^{-1} (Boehringer Mannheim, FRG). Concurrently, 2 l of a H_2O_2 soln (0.025% w/w) in phosphate buffer were introduced into the reaction mixt. at 17.7 ml hr^{-1}. A magnetic stirrer was used to agitate the mixt. Twenty mg of peroxidase were added during the reaction at 48, 120, and 144 hr. After 168 hr the mixt. was evapd, in a rotary evaporator, to a volume of 400 ml. The ppt. was centrifuged, washed two times with distilled water and dried over P_2O_5 in a vacuum desiccator.

Fractionation. The DHP sample was dissolved in a few ml of methylcellosolv (MCS) and fractionated through a Sephadex LH60 column (35 × 2 cm) with MCS as eluent. Ten frs, each in 100 ml MCS, were collected. The collected frs were then evapd in a rotary evaporator under red. pres. to dryness. The solid residue was dissolved in dichlorethane–EtOH (1:1) and the soln was filtered through a membrane filter (1 μm). The polymer was precipitated by adding this solution dropwise into a sixfold (by volume) of peroxide-free diethyl ether. The precipitate was centrifuged and washed two times with diethyl ether and once with petroleum ether (bp 40–60°).

Preparation of ZL-DHPs. For the synthesis of G-DHPs, 2.7 g (15 mmol) of coniferyl alcohol were used or an equimolar mixt. of coniferyl and sinapyl alcohol for GS-DHPs. The precursors were dissolved in 4 l of phosphate buffer (0.01 M, pH 6.8) together with 40 mg peroxidase. H_2O_2 (0.025% w/w) was pumped into the agitated mixt. over a period of 120 hr, while the increments of addition of peroxidase were the same as per the synthesis of the ZT-DHPs. Similarly, the fractionation and purification procedures were identical to those described previously.

Preparation of milled wood lignins. Milled wood lignins (MWLs) were prepared form spruce (*Picea abies*) and cherry (*Prunus* sp.) after 1 week milling of the pre-extracted (cyclohexane–ethanol, 1:1) and dried wood in a Retsch (FRG) planetary ball mill and by using the standard purification method of ref. [30].

Molecular weight determinations. For this purpose, acetylated samples were examined using a high-press. liquid chromatograph equipped with Microgel™ columns with THF as the mobile phase. The calibration was based on monodisperse polystyrene standards. Detail of the technique have been published previously [14].

Quantitative ^{31}P *NMR.* The protocol described in ref. [27] was followed. All samples were thoroughly dried for 48 hr at room temp. over silica gel *in vacuo* prior to phosphitylation. Approximately 30 mg dry sample were dissolved in 800 μl of a solvent mixt. composed of pyridine and deuterated $CDCl_3$ (1.6:1). After 20 min of stirring, 100 μl of the solvent mixture containing the internal standard (5.5×10^{-6} mol of isopropylidenediphenol) and the relaxation agent (1.7×10^{-3} mole of chromium acetylacetonate) were added. Finally, 100 μl 1,3,2-diaxaphospholanyl chloride (Fluka) were added. The sample was stirred again for a short period of time and transferred into a 5 mm NMR tube. The NMR spectra were obtained approximately 5–10 min after the phosphitylation regent was added using a Varian XL 300 FT-NMR spectrometer at 121.5 MHz. As indicated in Fig. 2, where the integration limits are illustrated, the phenolic OH groups in syringyl units in GS-DHPs and hardwood lignins were integrated from a baseline.

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