Chapter 14

Comparative Analysis of the Lignins of Cork from *Quercus suber L.* and Wood from *Eucalyptus globulus L.* by Dry Hydrogen Iodide Cleavage

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Lignin from cork (*Quercus suber L.*) was isolated by two procedures: organosolv extraction and dioxane-water (9:1) extraction in presence of HCl. These lignins were characterized using a mild hydrogen iodide-cleavage method followed by ^{1}H NMR and GPC analysis. The results were compared with those for eucalyptus lignins (*Eucalyptus globulus*) isolated by the same procedures. The method used provided syringyl/guaiacyl ratios for the linear parts of the macromolecules and the degrees of crosslinking. The prevalence of guaiacyl units was demonstrated for cork lignin. Syringyl units were found to be minor components and present mainly in the linear parts of macromolecules. *p*-Hydroxyphenyl units were mainly condensed. Cork lignin was found to be significantly more cross-linked than eucalyptus lignin.

Lignin represents about 20% of cork, the outer bark of *Quercus suber* L. (1). Unfortunately, both the determination of lignin content and the analysis of its structure are hindered by the presence of suberin and extractive compounds. This is a general problem in bark lignin analysis (2). Unlike suberin, the major cork component responsible for the characteristic mechanical properties of cork, lignin has attracted little attention (3, 4). The chemical structure of cork lignin is still under discussion being chemically close and difficult to distinguish from suberin. Lignin, however, plays an important role in cork-adhesive interactions during industrial bonding processes and therefore the search for new polymeric compositions requires a sound knowledge of the structure of cork lignin.

This work is a continuation of previously published studies (5, 6) in which cork lignin was isolated and characterized using conventional procedures, such as

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quantitative ^{13}C NMR, FTIR, nitrobenzene and permanganate oxidation. In our previous papers, it was shown that cork lignin posses some peculiar structural features which differ from those of traditional lignins. In this paper, we characterize cork lignin using a recently developed method of selective mild cleavage with dry hydrogen iodide. A characterization of a hardwood lignin from *Eucalyptus globulus* isolated using the same procedure is included for comparison.

The method of HI-cleaving (depolymerization with dry hydrogen iodide) was developed a few years ago (7, 8) and has proven to be a powerful tool in the study of lignin structure. This is a mild analytical method in which samples are subjected to a short treatment with dry hydrogen iodide that leads to a mixture of polymeric, oligomeric and monomeric products. The monomeric products can be characterized by NMR spectroscopy (9) and the mixture of oligolignols by GPC and ^{13}C NMR methods (8, 10). The data thus obtained gives information about the degree of lignin cross-linking, i.e. the ratio between linear parts and branching points of the macromolecular structure. In this study, we applied this method also to evaluate the guaiacyl/syringyl ratio in the linear chains of lignin.

Materials and Methods

Isolation of lignins. High quality reproduction cork from *Quercus suber L.* was milled in a cross-beater mill and sieved to 20 mesh. The cork powder was then sequentially soxhlet-extracted with methylene chloride, ethanol and water (8 hrs. for each solvent) and dried at 40°C. Eucalyptus (*Eucalyptus globulus* L.) wood was milled to 40-60 mesh, extracted with ethanol-toluene (1:2, v/v) in a soxhlet extractor for 4 hours, water for 4 hours, and then was air dried.

The cork organosolv lignin (COL) was isolated with a yield of 2.6% (o.d. cork) from solvent extracted cork powder by an acid catalyzed ethanol-water (1:1, v/v) organosolv method (170°C, 4 hrs.) as previously described (5). The same procedure was applied to solvent extracted eucalyptus sawdust, to obtain eucalyptus organosolv lignin (EOL) with a yield ranging from 10 to 12 % (o.d. wood).

The cork dioxane lignin (CDL) was prepared according to the procedure described in (11). Cork powder, extracted as described above, was suspended in dioxane-water (9:1) containing 0.2 N HCl. The mixture was refluxed for 1 hour, cooled, and filtered. The filtrate was vacuum evaporated to a syrup, which was then poured into water, precipitating the lignin. The precipitated lignin was centrifuged, dried under vacuum, and purified by dissolution in a 1,2-dichloroethanol:ethanol (2:1,v/v) mixture followed by precipitation in ethyl ether. The purified precipitated lignin was centrifuged, washed with ethyl ether, and dried yielding 1. % of o.d. cork. The eucalyptus dioxane lignin (EDL) was isolated using the same procedure, except for a 4 hours reflux time. The yield of EDL was 9.3% (o.d. wood).

Analytical methodology. Elemental microanalyses were performed at the laboratories of the Service Central d'Analyse of CNRS (Vernaison, France). The methoxy group content was determined by the modified Zeisel method (12).

The hydrogen iodide treatment was conducted by suspending 10-mg samples in 0.5 ml $CDCl_3$ in 0.5-mm (O.D.) NMR tubes. Dry HI was bubbled in an argon flow through the suspension for 1.5 hours. The ^{1}H NMR spectra were recorded using

a Bruker AC-300 instrument at ambient temperature; spectra of the reaction mixture were recorded every 30 minutes. Before recording the last spectrum, a coaxial capillary containing a solution of known amount of 1,1,2,2-tetrachloroethane in $CDCl_3$ was inserted in the tube as an external standard for the quantitative measurements. The amount of diiodides was determined based on the intensities of the NMR signals to the 1,1,2,2-tetrachloroethane standard. The signals for OCH_3, H_α and aromatic protons could be distinguished and integrated. Under the chosen conditions, only monomeric products remained in solution; the other minor products formed a colloidal suspension giving no signal in the NMR spectrum. The G/S ratio was determined from the relative intensities of the corresponding OCH_3, H_a and aromatic proton signals of 1,3-diiodo-1-(4-hydroxy-3-methoxyphenyl)propane (guaiacyl product) and 1,3-diiodo-1-(3,5-dimethoxy-4-hydroxyphenyl)propane (syringyl product). After recording of the last ^1H NMR spectrum, the suspension was filtered and the residue washed five times with $CHCl_3$, dried *in vacuo* and dissolved in DMSO before GPC analysis. Gel permeation chromatography of the lignins was performed using an 840x10 mm Sephadex LH-20 column with. DMSO + 0.03M H_3PO_4 + 0.03M LiBr as the eluent. Chromatograms were monitored by UV light at a wavelength of 280 nm.

^1H NMR spectrum of 1,3-diiodo-1-(4-hydroxy-3-methoxyphenyl)propane **I** (d, ppm; J, Hz): 2.45 (H_β, m), 2.78 ($H_{\beta'}$, m), 3.16 (H_γ, t, $J_{\gamma-\beta}$ 6.7), 3.92 (OCH_3, s), 5.25 (H_α, t, $J_{\alpha-\beta}$ 7.5), 6.83 (H_5, d, J_{5-6} 8.1), 6.90 (H_2, d, J_{2-6} 2.1), 6.95 (H_6, dd, J_{5-6} 8.1, J_{6-2} 2.1).

^1H NMR spectrum of 1,3-diiodo-1-(3,5-dimethoxy-4-hydroxyphenyl)propane **II** (d, ppm; J, Hz): 2.47 (H_β, m), 2.74 ($H_{\beta'}$, m), 3.13 (H_γ, d, $J_{\gamma-\beta}$ 6.7), 3.88 (OCH_3, s), 5.20 (H_α, $J_{\alpha-\beta}$ 7.6), 6.63 ($H_{2,6}$, s).

Results and Discussion

The method used to isolate lignin from lignocellulosics while preserving its chemical structure, involves milling the material in a vibrating or rotary ball mill, followed by solvent extraction of the released lignin (13, 14). When these methods are applied to cork, some problems may arise because of its particular chemical composition and mechanical properties. The Poisson ratio of cork is extremely low due to its cellular microstructure and the high elasticity of its cell walls (15). Significant recovery of linear dimensions takes place even after strong compression (16). Therefore, cork is a material that is not easy milled, and, consequently, the yields of the "milled cork lignin" are very low (3). In addition, the isolated lignin-like polymer is highly contaminated with aliphatic suberin fragments and carbohydrates (3). This occurs because lignin is a minor component of cork and is strongly associated with other cork components. This drawback may be overcome if cork is previously desuberized (17). However, desuberization by strong alkaline solutions may induce structural changes in the lignin macromolecule. These are the reasons for choosing other methods of lignin isolation that avoid the mechanical treatment stage and provide higher selectivity and increased yield and purity of cork lignin.

The first of these methods is mild organosolv extraction in ethanol-water solution (1:1) catalyzed by 0.1M acetic acid (5). The yield of lignin (COL) thus

obtained, 2.6%, is higher than that from milled cork lignin (3). This lignin is not contaminated with carbohydrates and contains only small amounts of aliphatic structures, which were assumed to be covalently bound to the phenylpropane units of lignin. Analysis of the permanganate oxidation products of intact cork and COL (6) suggested that this lignin was representative of the structure and composition of the "in situ" lignin of cork. The acidolysis method was chosen because it was reported to be a convenient method for the isolation of lignin from barks (18, 19). Cork dioxane lignin (CDL) was obtained in 1.6% yield.

Table I. Elemental Analyses and Molecular Formulae of Cork and Eucalyptus Lignins.

Sample	Elemental Analysis, %				Molecular Formula
	C	H	O	OCH_3	
COL	61.9	5.9	32.2	12.0	$C_9H_{8.91}O_{3.07}(OCH_3)_{0.73}$
CDL	62.1	6.4	31.5	6.8	$C_9H_{10.40}O_{3.18}(OCH_3)_{0.40}$
EOL	59.6	6.0	34.4	21.3	$C_9H_{8.25}O_{3.08}(OCH_3)_{1.44}$
EDL	59.3	5.9	34.8	18.2	$C_9H_{8.52}O_{3.28}(OCH_3)_{1.21}$

The results of elemental and methoxyl content analyses of the lignins are given in Table I. Empirical molecular formulas calculated based on this data are also presented. The main difference between cork and eucalyptus wood lignins is the methoxyl content which is much lower for both cork lignins compared to eucalyptus. This obviously results from a low content of syringyl structures in cork lignins. Permanganate oxidation of intact cork and COL has previously shown that cork lignin is a guaiacyl-rich HGS-type lignin with H:G:S molar ratios of 2:93:5 for OCL and 3:93:4 for the "in situ" lignin of cork (6). The methoxyl content of CDL is particularly low. This may by attributed to the fact that this lignin, contrary to COL, is highly contaminated with suberinic aliphatic chains, as evidenced by its FTIR spectrum (not shown) which exhibits strong absorption bands at 2854 and 2930 cm^{-1}. The eucalyptus wood lignins (EOL and EDL) gave high methoxyl contents in agreement with their high syringyl contents. Previous results of permanganate oxidation of *Eucalyptus globulus* dioxane lignin had shown that it is a syringyl-rich lignin with H:G:S ratios of 3:36:67 (20). Both cork and eucalyptus organosolv lignins (COL and EOL) displayed methoxyl contents higher than those in the corresponding dioxane lignins (CDL and EDL). This may be explained by the formation of ethoxy structures during the organosolv treatment in ethanol-water system. The methoxyl group calculations have been adjusted accordingly.

Hydrogen iodide cleavage. The treatment of the lignin suspension in chloroform with dry hydrogen iodide led to extensive destruction of the polymer. Because of the low solubility of the polymeric and oligomeric products in solvents such as chloroform, only monomeric products can be registered in the ^1H NMR spectrum of the resulting solution.

Previously, it was demonstrated that only one type of monomeric product is released after HI-cleavage of guaiacyl-type lignins and lignin model polymers, namely 1,3-diiodo-1-(4-hydroxy-3-methoxyphenyl)propane **I** (9). Due to the specific mechanism of its formation (Fig. 1), the yield of this compound corresponds to the amount of structural units in the linear parts of the lignin macromolecule and reflects the degree of cross-linkage of the polymer. On the other hand, α-ethers could provide branching, but the abundance of non-cyclic α-O-4 ethers is very low (21).

The treatment of eucalyptus syringyl-rich lignins released the second monomeric product, i.e. the syringyl analog of **I**, 1,3-diiodo-1-(3,5-dimethoxy-4-hydroxyphenyl)propane **II** (Figures 2 and 3). Compound **II** was also found in the products of the cork lignin treatment. Monomers **I** and **II** come from coniferyl/sinapyl alcohol end-groups and linear end-wise parts of the polymer (9). The amount of diiodides formed corresponds to the degree of cross-linking in the polymer. Obviously, the guaiacyl/syringyl (G/S) monomer ratio must correspond to the G/S ratio in the linear parts of the macromolecules. No monomeric compound resulting from *p*-hydroxyphenyl structural units was identified in the spectra of the decomposition products.

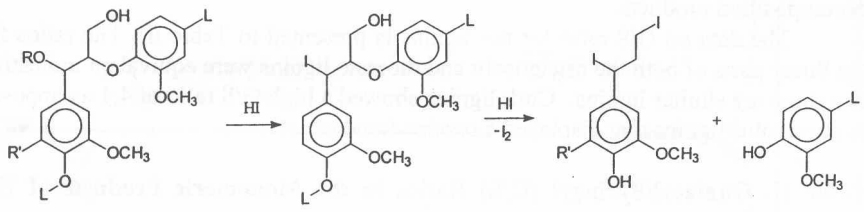
The data on G/S ratio for the lignins is presented in Table II. The ratios for the linear parts of both the organosolv and dioxane lignins were equivalent indicating that these are similar lignins. Cork lignins showed a high G/S ratio of 4:1 as opposed to eucalyptus lignins that displayed a reverse trend of 1:5.

Table II. Guaiacyl/Syringyl (G/S) Ratios in the Monomeric Products of HI-Cleavage

Sample	G/S ratio
COL	4:1
CDL	4:1
EOL	1:5
EDL	1:5

The presence of products **I** and **II** in the spectra of the decomposition products of cork lignins in a 4:1 ratio supports the idea that cork lignin is guaiacyl rich lignin which confirms our previous results (5, 6). Although cork comes from a hardwood tree, the G/S value of cork lignin is similar to a G/S value for a softwood lignin. Generally, many bark lignins display higher G/S ratios in comparison with wood lignins (2).

The G/S values for organosolv cork lignins were found to be quite different from the 19:1 ratio obtained by the permanganate oxidation (6) and 56:1 ratio obtained by nitrobenzene oxidation. Even when the inaccuracies caused in the three techniques by the low syringyl contents are taken into account, the general tendency is that the G:S ratio in the products of HI cleavage is lower than in the products of the permanganate or nitrobenzene oxidation. This suggests that the linear end-wise fragments of cork lignin were enriched with syringyl units. The same tendency was



Where: L = lignin; R = H, lignin; R' = H, OCH_3

Figure 1. Chemical reactions involved in the HI-cleavage of an β-O-4 linkage in lignin.

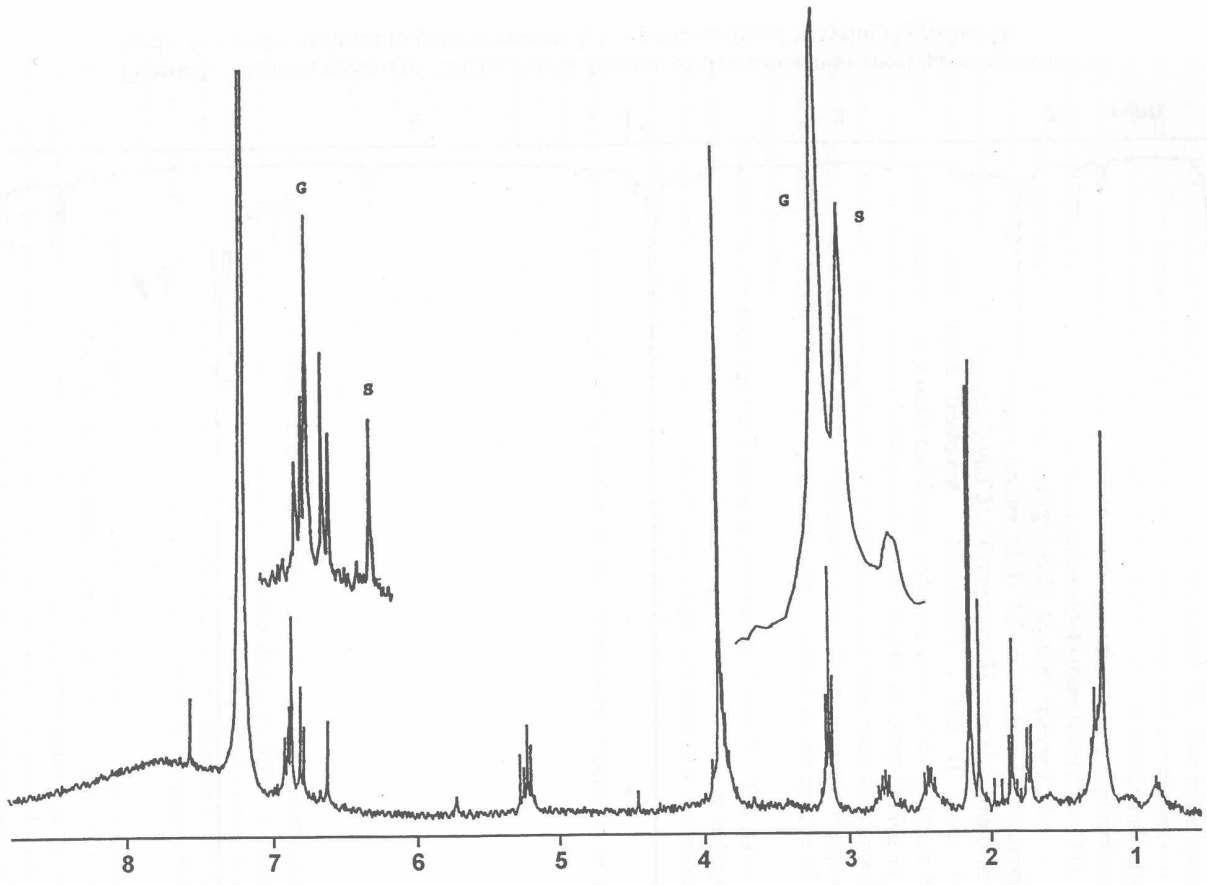


Figure 2. ^{1}H NMR spectra of $CDCL_3$ soluble fraction of HI-treatment of cork organosolv lignin. G – peaks assigned to guiacyl product **I**, S – peaks assigned to syringyl product **II.**

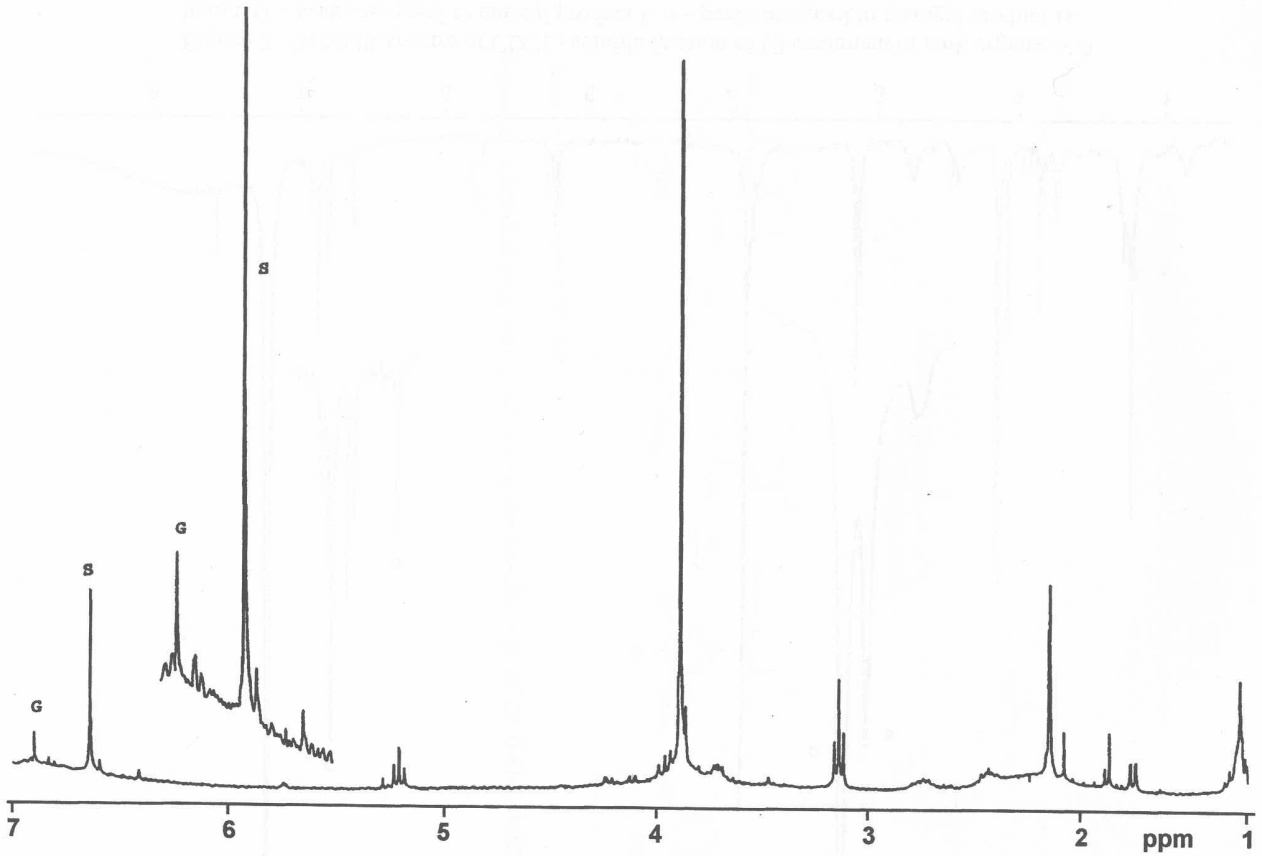


Figure 3. ^{1}H NMR spectra of $CDCL_3$ soluble fraction of HI-treatment of eucalyptus organosolv lignin. G – peaks assigned to guiacyl product **I**, S – peaks assigned to syringyl product **II.**

observed for the eucalyptus wood lignin. The G/S value obtained for eucalyptus dioxane lignin from permanganate oxidation was approximately 1:2 (20), whereas that obtained from the HI cleavage was 1:5. We believe, therefore, that the linear end-wise fragments in eucalyptus lignin consist mainly of syringyl units. The guaiacyl moieties are mainly present in branching sites of the lignin macromolecule due to their ability to form β-5 and 5-5' bonds.

Decomposition products from cork and eucalyptus wood lignins arising from *p*-hydroxyphenylpropane units were not detected. Since *p*-hydroxyphenyl units were previously observed in both types of lignin by other techniques (20, 6), it can be concluded that almost all the *p*-hydroxyphenyl units are condensed and incorporated into the branching points of the macromolecule instead of the linear portions. This would preclude the formation of the corresponding diiodides. This conclusion is in agreement with our previous results on the characterization of COL by ^{13}C NMR (5). It should also be added that unpublished results on some grass lignins (*p*-hydroxyphenyl type of lignin) confirm this conclusion.

Polymeric/oligomeric/monomeric distribution of lignin cleavage products. Data on the distribution of different types of decomposition products after HI-splitting is presented in Table III. Because cork organosolv lignin (COL) was found to be representative of the "in situ" cork lignin and mostly free of carbohydrates and suberin, it was chosen for the experiments on the distribution of depolymerization products as was an equivalent sample of eucalyptus organosolv lignin. The polymer/oligomer ratio was determined from the area measurements of gel-permeation chromatograms (Fig. 4). The amount of monomeric fraction was calculated from ^{1}H NMR spectra.

Table III. Ratios of Polymers/Oligomers/Monomers in Products of Lignin HI-Cleavage

Sample	Polymeric Fraction (%)	Oligomeric Fraction (%)	Monomeric Fraction (%)
EOL	15	65	20
COL	25	65	10

In both samples the amounts of oligomeric structures were almost equal and the main difference rested in the ratio of polymeric and monomeric fractions. The yield of monomeric species in eucalyptus lignin was twice as high as that of cork lignin. This suggests that cork lignin contains significantly less linear fragments or that these fragments are much shorter. The increasing amount of polymeric species indicates that the number of hydrogen iodide unsplittable carbon-carbon and Ar-O-Ar bonds was much higher in cork lignin and that the polymeric matrix was more cross-linked. The high degree of condensation of cork lignin was already proposed based

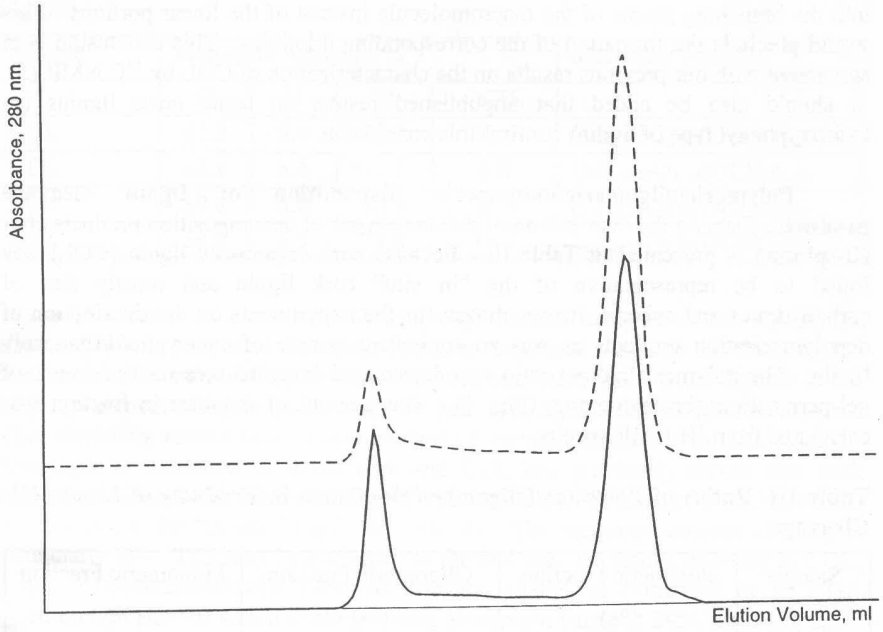


Figure 4. Gel-permeation chromatograms of cork and eucalyptus organosolv lignins depolymerized with dry hydrogen iodide in $CDCl_3$: α - COL; β - EOL.

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