Reprint

Matrix-Assisted Laser Desorption Mass Spectrometry of Lignins**

By *Jürgen O. Metzger,* Christine Bicke, Oskar Faix,
Wilfried Tuszynski, Raimond Angermann, Michael Karas,*
and *Kerstin Strupat*

As component of the lignocelluloses, the skeletal substance
of terrestrial plants, lignin is the most common biopolymer
besides the polysaccharides cellulose and hemicelluloses. As
side product of the cellulose recovery over 50 million tons of
technical lignins is produced yearly worldwide, and its use as
renewable raw material with a view to protecting the envi-
ronment is a challenge for chemists. Knowledge about lignins
is still fragmentary.[1-3] It is certain that its monomeric pre-
cursor in conifers is predominantly coniferyl alcohol (1a); in
deciduous trees sinapyl alcohol (1b) is an additional precur-
sor, as is *p*-cumaryl alcohol (1c) in grasses and herblike di-
cotyledons.[1] According to Freudenberg,[4] these alcohols
polymerize to give lignin, by a radical mechanism. However,
the biosynthesis path from the monomers to the macro-
molecule has not yet been unambiguously elucidated.[2] At
any rate lignin is fundamentally different from other bio-
polymers like proteins, nucleic acids, and polysaccharides, in

$$HO-\underset{R^2}{\overset{R^1}{\diamondsub}}-CH=CH-CH_2OH \quad 1$$
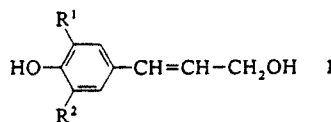
a: $R^1 = OCH_3$, $R^2 = H$; b: $R^1 = R^2 = OCH_3$; c: $R^1 = R^2 = H$.

[*] Prof. Dr. J. O. Metzger
   Fachbereich Chemie der Universität
   Carl-von-Ossietzky-Strasse 9–11, D-W-Oldenburg (FRG)

   Dipl.-Chem. C. Bicke, Priv. Doz. Dr. O. Faix
   Bundesforschungsanstalt für Forst- und Holzwirtschaft, Hamburg (FRG)

   Dr. W. Tuszynski, Dipl.-Phys. R. Angermann
   Fachbereich Physik der Universität Oldenburg

   Dr. M. Karas, K. Strupat
   Institut für Medizinische Physik der Universität Münster

which the type of linkage and order of the units is stipulated; in lignin at least ten types of binding are found to different extents linking the phenylpropane units into a completely irregular three-dimensional polymer.[5]

To determine the constitutional makeup of lignin by chemical degradation methods is extremely labor-intensive and time-consuming and nevertheless gives no clear answers. In the ^{13}C NMR spectrum, about forty different carbon atoms can be assigned.[5] The molecular weight determination is problematic, because lignins tend strongly toward molecular aggregation in solution.[2] Mass spectrometry has hitherto been used as analytical method for the study and characterization of lignins only in the form of pyrolysis mass spectrometry, in which only monomeric, and to a small extent dimeric, pyrolysis products are detected.[6]

The study of polymers in general, and biopolymers in particular, by mass spectrometry has, however, made enormous advances in recent years with the development of new "soft" ionization techniques such as secondary ion (SI),[7] fast atom bombardment (FAB),[8] and ^{252}Cf plasma desorption (PD) mass spectrometry,[9] as well as various spray processes like ion spray,[10] and in particular, matrix-assisted laser desorption (MALD).[11] The accessible mass range has been steadily extended. Thus MALD mass spectrometry has provided proof of proteins of at least 250000 Da.[12] The protonated quasi-molecular ion was detected, but fragmentation was not observed with this method. MALD-MS was also used successfully for oligosaccharides like maltodextrins and dextrans. The mass spectrum showed peaks for the individual oligomers at well-defined spacings corresponding to the monomeric units, and enabled the determination of the molecular weight distribution up to about 10000 Da.[13] A series of linear polymers like polystyrene and polyethyleneglycol of average molecular weight $\bar{M}_n < 10000$ Da were studied mainly with SIMS.[14]

The lack of a rapid, sensitive, and powerful method for characterizing small amounts of lignins prompted us to investigate the potential of the soft ionization techniques for lignins. Although FAB-MS and PDMS brought no success, MALD-MS provided the breakthrough in the application of mass spectrometry to lignins.

Milled-wood lignin from birch (*Betula alba*), which was well-characterized by classical methods of lignin chemistry,[15] was mixed in the mass ratio 1:15 without further purification with 2,5-dihydroxybenzoic acid as matrix in acetone/water (9:1). A little of the solution (1 μL corresponding to 50 ng of lignin) was placed onto a silver support, dried, and mounted in a time of flight mass spectrometer, as described in reference [12]. A nitrogen laser with a wavelength of 337 nm and an impulse duration of less than 5 ns was used for desorption. The resolution is relatively low ($m/\Delta m = 50$), and the signals thus relatively broad. The peak centroids can, however, be determined with quite good precision (standard deviation ± 0.1 %).[12]

The obtained mass spectrum (Fig. 1) shows a wide molecular weight distribution of several hundred to larger than 16000 Da. The center of gravity of the distribution lies around 2600 ± 100 Da, which agrees quite well with the number average of the molecular weight $\bar{M}_n$ of birch lignin determined by gel chromatography.[15] The broad distribution curve (Fig. 1) has a pronounced fine structure up to about 2000 Da (Fig. 2), which may be assigned to oligomeric lignin molecules. These oligomers are not all the same, but themselves show a broad molecular weight distribution. Clear groups of oligomers—from trimers of ca. 600 Da to nonamers of ca. 1800 Da—can be distinguished. The center of gravity of the peaks of oligomeric groups yields the number average of
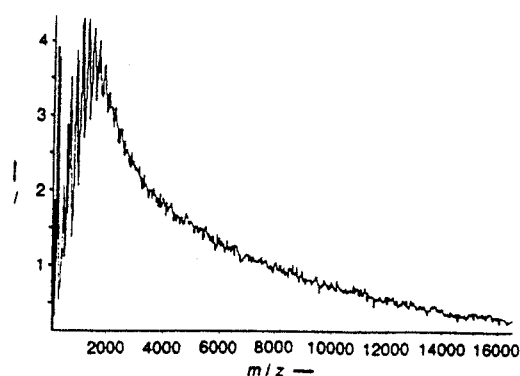

Fig. 1. MALD mass spectrum of birch lignin. Sum of twenty single spectra. I = relative intensity in arbitrary units, molecular weight M, in Da (m/z).

their molecular weight $\bar{M}_n$. This number average divided by the degree of oligomerization gives an average formula weight of between 214 and 204 Da for one phenylpropane unit of these oligomers, which agrees well with the chemically determined average formula weight of birch lignin (209 Da). The conclusion is that the ionized lignin molecules are probably protonated, since a cationization with Na^+, as observed for sugars,[13] would lead to distinctly higher average molecular weights.
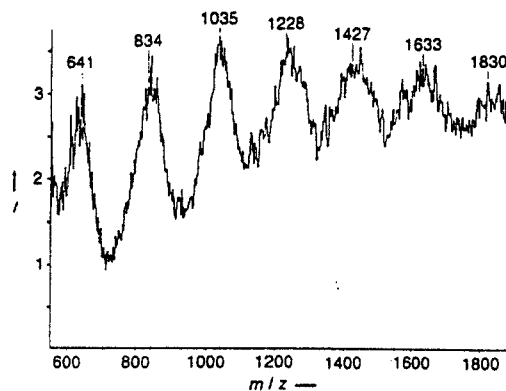

Fig. 2. Fine structure of the MALD mass spectrum in the range 600–1800 Da for birch lignin (section of Fig. 1). The numbers over the peak maxima give the center of gravity of the distribution of the oligolignol signals. I in arbitrary units. M, in Da.

Figure 2 shows that superimposed on the fine structure of the oligomers (Fig. 1) is a hyperfine structure, which is depicted, expanded, for tetramers in Figure 3. Many tetramers with different molecular weights can be recognized. Two sequences with $\Delta m = 30$ Da (826, 856, 886; and 776, 805, 834, 866) confirm the existence of guaiacyl and syringyl propane building blocks in birch lignin, which differ only by the mass of a methoxy group ($\Delta m = 30$), and indicate that tetramers with four guaiacyl units, three guaiacyl and one syringyl unit, etc. to four syringyl units are present. The center of gravity of the molecular weight distribution of the tetramers lies between m/z 826 (two syringyl and two guaiacyl units) and m/z 856 (three syringyl and one guaiacyl units), which shows that the statistical distribution of the monomers in lignin (syringyl:guaiacyl ≈ 1.4) is already present in the low oligomers. The distribution of the different linkages in lignin identified by chemical degradation[5] also seems to correspond in the low oligomers and in the polymer, as a molecu-
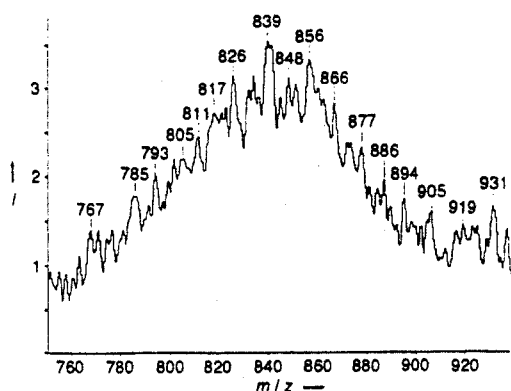
Fig. 3. Hyperfine structure of the MALD mass spectrum in the range for tetramers of birch lignin (section of Fig. 2).

lar mass analysis of the many different tetramers shows. The hyperfine structure of the MALD mass spectrum contains the coded information that enables an increasingly detailed analysis of the complex construction of lignin.

Apart from birch lignin the lignin from a conifer (Chinese redwood, *Metasequoia glyptostroboides*)[17] was studied. The MALD mass spectrum revealed a broad distribution of several hundred to larger than 12000 Da and again directly yielded the number average molecular weight $\bar{M}_n = 2300 \pm 100$. The fine structure of the peaks up to 2000 Da is assigned to oligomers from trimers to decamers (Fig. 4). In contrast to
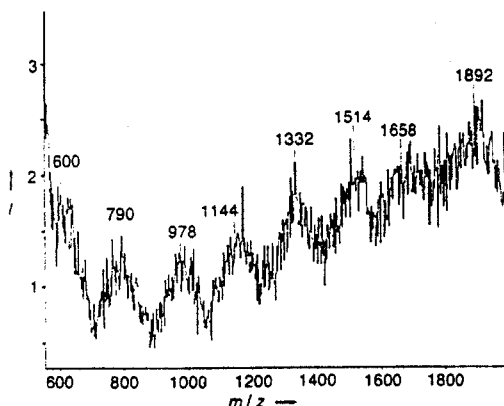

Fig. 4. Fine structure of the MALD spectrum of a conifer lignin (Chinese redwood) from 600 to 2000 Da. Section of the complete spectrum. The numbers above the peak maxima give the center of gravity of the molecular weight distribution for each oligomer range.

birch lignin (Fig. 2), the chemically determined average formula weight of a phenylpropane unit is 190 Da, because conifer lignin consists almost exclusively of guaiacyl propane units. This value is also confirmed by the MALD mass spectrum. As for birch lignin, the hyperfine structure reveals several different oligolignols of the same degree of oligomerization in which the monomers are linked by various binding types.

The results presented here show that MALD mass spectrometry is a fast, sensitive, and extremely powerful micromethod for the characterization of lignins, which yields the molecular weight distribution directly. Up to nonamers (birch lignin) or decamers (conifer lignin) were detected and their average molecular weights determined. In addition, the technique revealed that many different oligomers with the same degree of oligomerization are present in lignin, which reflect the variety and statistical distribution of binding types. It can

be expected that the development of MALD mass spectrometry will extend the accessible mass range to lignins of substantially higher molecular weight. An improvement in the spectrometric resolution will enable the fine structure of even the high polymers and the hyperfine structure of the middle range polymers to be analyzed. It remains to be seen whether a subsequent collision-induced fragmentation and application of MS/MS techniques will allow sequencing. Furthermore our results show that mass spectrometry can be used on cross-linked and irregular polymers, which is of significance for many technically important polymers.

[1] *Lignins – Occurrence, Formation, Structure and Reactions* (Eds.: K. V. Sarkanen, C. H. Ludwig), Wiley-Interscience, New York, 1971.
[2] D. A. I. Goring in *Lignin – Properties and Materials* (Eds.: W. G. Glasser, S. Sarkanen), *ACS Symp. Ser.* 1989, 397.
[3] D. Fengel, G. Wegener, *Wood – Chemistry, Ultrastructure, Reactions*, Gruyter, Berlin, New York, 1989.
[4] K. Freudenberg, A. C. Neish, *Constitution and Biosynthesis of Lignin*, Springer, Berlin, New York, 1968.
[5] H. Nimz, *Angew. Chem.* 1974, *86*, 336; *Angew. Chem. Int. Ed. Engl.* 1974, *13*, 313; G. Krüger, *Chemie Unserer Zeit* 1976, *10*, 21.
[6] J. O. Metzger, *Fresenius Z. Anal. Chem.* 1979, *295*, 45, and references cited therein.
[7] A. Benninghoven, *Chem. Phys. Lett.* 1970, *6*, 616.
[8] M. Barber, R. S. Bordoli, R. D. Sedgwick, A. N. Tyler, *J. Chem. Soc. Chem. Commun.* 1981, 325.
[9] R. D. Mac Farlane, D. F. Torgersen, *Science* 1976, *191*, 920.
[10] J. W. Metzger, W. Beck, G. Jung, *Angew. Chem.* 1992, *104*, 235; *Angew. Chem. Int. Ed. Engl.* 1992, *31*, 226, and references cited therein.
[11] M. Karas, F. Hillenkamp, *Anal. Chem.* 1988, *60*, 2299.
[12] M. Karas, U. Bahr, A. Ingendok, F. Hillenkamp, *Angew. Chem.* 1989, *101*, 805; *Angew. Chem. Int. Ed. Engl.* 1989, *28*, 760.
[13] B. Stahl, M. Steup, M. Karas, F. Hillenkamp, *Anal. Chem.* 1991, *63*, 1463.
[14] I. V. Bletsos, D. M. Hercules, D. van Leyen, B. Hagenhoff, E. Niehuis, A. Benninghoven, *Anal. Chem.* 1991, *63*, 1953.
[15] Elementary analysis: 60.05% C; 5.79% H; 34.16% O; 21.50% MeO: MeO per phenylpropane unit 1.41; average formula mass of one phenylpropane unit 208.8; $\bar{M}_w \approx 9700$; $\bar{M}_n \approx 2600$ [16].
[16] O. Faix, *Das Papier*, 1976 (10A), V 1.
[17] Elementary analysis: 62.91% C, 5.88 H, 32.21% O, 15.98 MeO; MeO per phenylpropane unit 0.96; average formula mass of one phenylpropane unit 190.0; $\bar{M}_w \approx 7800$; $\bar{M}_n \approx 2300$ [16].