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Distribution of *erythro* and *threo* forms of different types of β-O-4 structures in aspen lignin by ^{13}C NMR using the 2D INADEQUATE experiment

Michel Bardet,[1] Danielle Robert,[1]† Knut Lundquist[2]* and Sverker von Unge[2]

[1] Département de Recherche Fondamentale sur la Matière Condensée, SCIB, CEA-Grenoble, F-38041 Grenoble Cedex 9, France
[2] Department of Organic Chemistry, Chalmers University of Technology, S-41296 Gothenburg, Sweden

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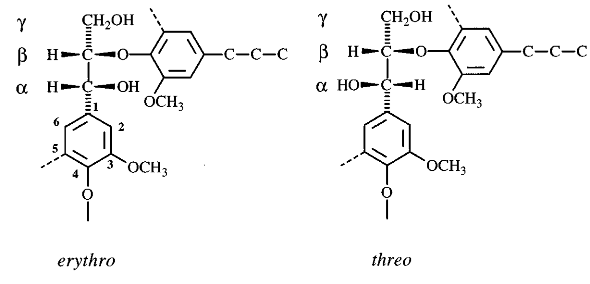
ABSTRACT: Carbon–carbon connectivity spectra of ^{13}C-enriched aspen lignin recorded using the 2D INADEQUATE experiment revealed cross peaks which can be assigned to four types of arylglycerol β-aryl ethers (β-O-4 structures): *erythro* forms of arylglycerol β-syringyl ethers, *threo* forms of arylglycerol β-syringyl ethers, *erythro* forms arylglycerol β-guaiacyl ethers and *threo* forms of arylglycerol β-guaiacyl ethers. The intensities of the cross peaks suggest larger amounts of β-syringyl ethers than β-guaiacyl ethers. The *erythro* isomers dominate among the β-syringyl ethers. *Erythro* and *threo* forms of β-guaiacyl ethers are present in similar amounts. © 1998 John Wiley & Sons Ltd.

KEYWORDS: NMR; ^{13}C NMR; 2D INADEQUATE; lignins; lignin models; diastereomer distribution

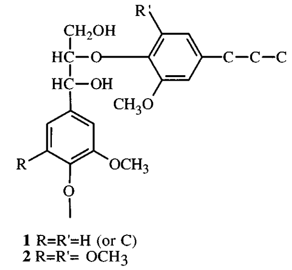
INTRODUCTION

Arylglycerol β-aryl ethers constitute the most important type of structural elements in lignins.[1] In hardwood lignins there are substantial amounts of guaiacylpropane units in addition to syringylpropane units. Therefore, four types of arylglycerol β-aryl ethers (**1**–**4**) have to be considered in studies of such lignins. In addition, there is a structural variation due to stereoisomerism since structural elements of the β-aryl ether type can exist as *threo* or *erythro* forms (Fig. 1). The distributions of *erythro* and *threo* forms of β-O-4 structures in hardwood lignins have been studied by NMR spectroscopic methods. ^{1}H NMR studies of birch lignin suggest an overall predominance of *erythro* forms, although substantial amounts of *threo* forms also are present.[2–4] In ^{13}C NMR studies of beech lignin, it could be concluded that *erythro* forms of β-syringyl ethers (**5**) are prevalent among the different types of β-ethers present.[5]



erythro *threo*

Figure 1. The *erythro* and *threo* forms of arylglycerol β-aryl ethers.



1 R=R'=H (or C)
2 R=R'= OCH_3
3 R=OCH_3, R'=H (or C)
4 R=H (or C), R'=OCH_3

Previous ^{13}C NMR studies of ^{13}C-enriched aspen lignin included the application of the 2D INADEQUATE experiment.[6,7] These studies revealed that some of the cross peaks in the ^{13}C NMR 2D INADEQUATE spectrum could be attributed to carbon atoms in the side-chains of four classes of β-O-4 structures, namely *threo* and *erythro* forms of β-O-4 structures of the syringyl ether (**5**, **6**) and guaiacyl ether (**7**, **8**) types. On the basis of comparisons with model compound data, it could be shown that *erythro* forms of β-O-4 structures dominated as a consequence of a prevalence of *erythro* forms of arylglycerol β-syringyl ethers (**5**). A prevalence of β-ethers of type **5** in hardwood lignins is in accordance with current views on the biosynthesis of lignins.[8] In the present work, we extended and corroborated the 2D INADEQUATE studies[7] of

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* *Correspondence to*: K. Lundquist, Department of Organic Chemistry, Chalmers University of Technology, S-41296 Gothenburg, Sweden
† Present address: CERMAV/CNRS, Domaine Universitaire, BP 53, F-38041 Grenoble Cedex 9, France.

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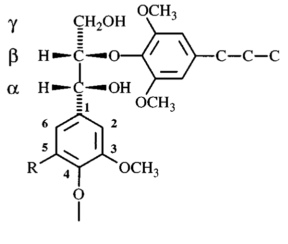
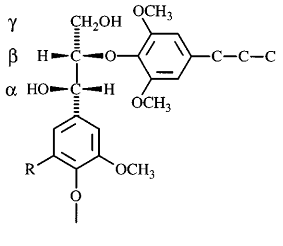
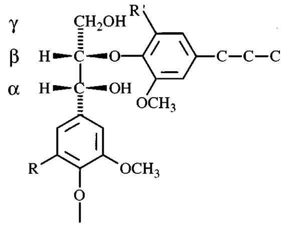
the distribution of diastereomeric forms of β-ethers in aspen lignin.



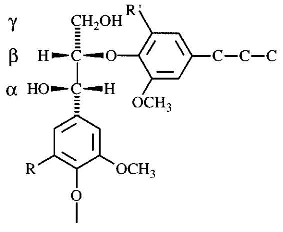
erythro
5



threo
6

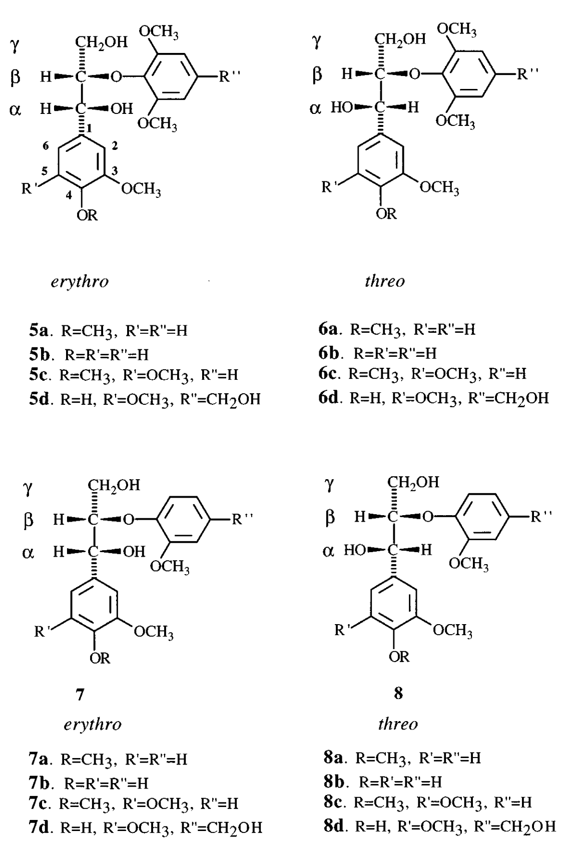


erythro
7



threo
8

R=H, C or OCH3
R'= H or C



erythro *threo*

5a. R=CH3, R'=R''=H
5b. R=R'=R''=H
5c. R=CH3, R'=OCH3, R''=H
5d. R=H, R'=OCH3, R''=CH2OH

6a. R=CH3, R'=R''=H
6b. R=R'=R''=H
6c. R=CH3, R'=OCH3, R''=H
6d. R=H, R'=OCH3, R''=CH2OH

7 *erythro* **8** *threo*

7a. R=CH3, R'=R''=H
7b. R=R'=R''=H
7c. R=CH3, R'=OCH3, R''=H
7d. R=H, R'=OCH3, R''=CH2OH

8a. R=CH3, R'=R''=H
8b. R=R'=R''=H
8c. R=CH3, R'=OCH3, R''=H
8d. R=H, R'=OCH3, R''=CH2OH

Figure 2. Lignin model compounds representative of *erythro* and *threo* forms of lignin structures of the arylglycerol β-syringyl ether (**5**, **6**) and arylglycerol β-guaiacyl ether (**7**, **8**) types.

Table 1. ^{13}C NMR chemical shifts for side-chain carbons and methoxy carbons in lignin model compounds **5a–d** (*erythro* forms of β-syringyl ethers), **6a–d** (*threo* forms of β-syringyl ethers), **7a–d** (*erythro* forms of β-guaiacyl ethers) and **8a–d** (*threo* forms of β-guaiacyl ethers)

	δ (ppm)			
Compound	C_α	C_β	C_γ	OCH_3
5a	72.2	85.9	59.9	55.6, 55.7, 56.0 (2C)
5b	72.2	86.1	59.8	55.7, 56.0 (2C)
5c	72.5	85.9	59.9	55.9(2C), 56.0 (2C), 59.9
5d	72.4	86.2	59.8	56.0 (2C), 56.1 (2C)
6a	71.6	86.9	60.3	55.6, 55.7, 56.0 (2C)
6b	71.7	87.0	60.3	55.7, 56.0 (2C)
6c	71.6	86.4	60.3	55.9 (2C), 56.0 (2C), 59.9
6d	71.8	87.1	60.3	56.0 (2C), 56.1 (2C)
7a	71.8	84.0	60.3	55.5, 55.7, 55.8
7b	71.9	84.1	60.3	55.7, 55.8
7c	72.1	83.5	60.3	55.7, 55.8 (2C), 59.9
7d	72.1	84.0	60.3	55.7, 56.0 (2C)
8a	71.1	84.6	60.2	55.5, 55.7, 55.8
8b	71.3	84.8	60.3	55.6, 55.8
8c	71.1	84.1	60.2	55.7, 55.8 (2C), 59.9
8d	71.2	84.7	60.2	55.7, 56.0 (2C)

RESULTS AND DISCUSSION

^{13}C NMR data for the side-chain carbon atoms in a series of lignin model compounds (Fig. 2) representing the diastereomers of two classes of β-O-4 structures, namely arylglycerol β-syringyl ethers [*erythro* (**5**) and *threo* forms (**6**)] and arylglycerol β-guaiacyl ethers [*erythro* (**7**) and *threo* forms (**8**)], are given in Table 1 (for the assignment of signals to C_α, C_β and C_γ, see Ref. 5 and preceding work). The selection of model compounds includes a variety of models representative of arylglycerol β-aryl ether structures of types **1**–**4** in order to elucidate the influences of the structure of the aromatic groups on the chemical shifts of side-chain carbon atoms. The stereochemistry of the model compounds has recently been verified by X-ray crystallography of a series of model compounds.[9]

Figure 3 shows the part of the INADEQUATE spectrum where most of the lignin signals from aliphatic carbons are found and includes the cross peaks caused by structural elements of the β-O-4 type. Figure 4 shows the region of the 2D INADEQUATE spectrum where the C_α/C_β cross peaks caused by various types of β-O-4 structures are located. Four cross peaks can be clearly discerned (Fig. 4), corresponding to *erythro* forms arylglycerol β-syringyl ethers (**5**) (72.5/86.0), *threo* forms of arylglycerol β-syringyl ethers (**6**) (71.9/87.2), *erythro* forms of arylglycerol β-guaiacyl ethers (**7**) (72.2/83.5) and *threo* forms of arylglycerol β-guaiacyl ethers (**8**) (71.4/84.5).

The positions of C_α/C_β cross peaks from model compounds (derived from the model compound data in Table 1) are included in Fig. 4 to facilitate direct comparisons. It is evident from Fig. 4 that the model com-

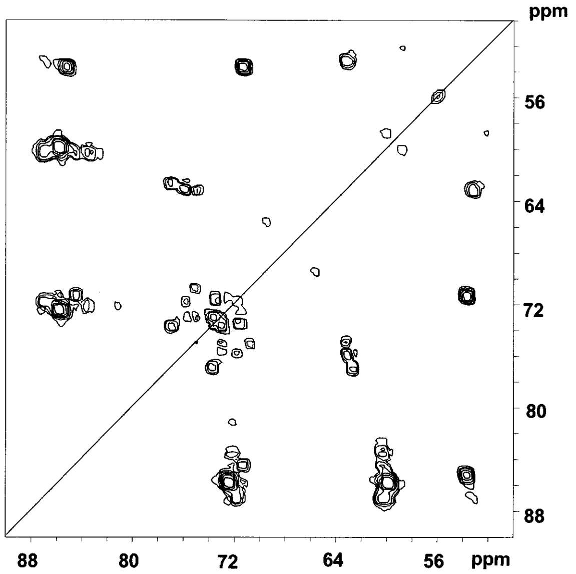


Figure 3. The region of the 2D INADEQUATE ^{13}C NMR spectrum of ^{13}C-enriched aspen lignin that includes signals from aliphatic carbon atoms in lignin. The complete spectrum is given in Ref. 7.

pound data provide convincing evidence for the assignments of the cross peaks given above. Moreover, quantitative analysis of the different structures can be carried out directly by comparing the intensities of cross peaks. Such analysis can be performed, since correlations between carbons with similar ^1J(CC) and T_1 are compared. The high intensity of the 72.5/86.0 cross peak suggests a predominance of *erythro* forms of arylglycerol β-syringyl ethers (**5**). The intensities of the cross peaks suggest larger amounts of β-syringyl ethers than β-guaiacyl ethers. The *erythro* isomers dominate among the β-syringyl ethers. It seems that the *erythro* and *threo* forms of β-guaiacyl ethers are present in almost equal proportions. Rough estimates of the distribution of the different types of β-ethers could be made based on integrations of the signals. Such estimates suggest that the proportion of β-ethers of type **5** is 55% and that the proportions of the other types of β-ethers are about equal (*ca.* 15%). It is obvious from the present study that the 2 D INADEQUATE experiment is a powerful technique for studies of the stereochemistry of lignin. The use of ^{13}C-enriched lignin samples makes it possible to overcome the problems related to low sensitivity.

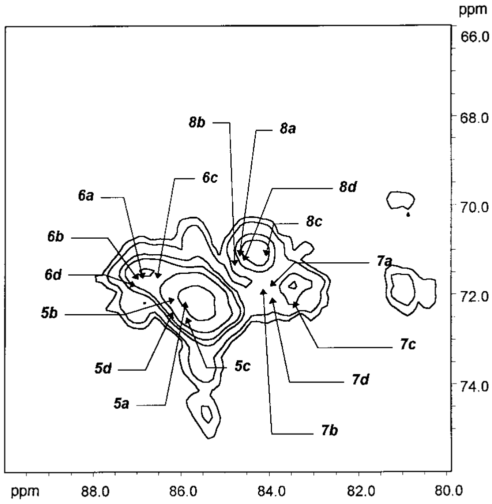


Figure 4. Enlargement of the cross peak in Fig. 3 centred at 85/72 ppm. The peak is assigned to the C_α/C_β correlation in arylglycerol β-aryl ethers and four sub-peaks that are attributed to structures **5–8** are discerned. Positions (derived from the chemical shifts in Table 1) corresponding to C_α/C_β correlations of model compound are shown: *erythro*-arylglycerol β-syringyl ether type (**5**), *threo*-arylglycerol β-syringyl ether type (**6**), *erythro*-arylglycerol β-guaiacyl ether type (**7**) and *threo*-arylglycerol β-guaiacyl ether type (**8**).

EXPERIMENTAL

Materials

Sterically defined[9] model compounds were synthesized according to procedures described in the literature.[3,10] Milled wood lignin was obtained from aspen (*Populus euramericana*) statistically ^{13}C enriched with an overall enrichment of 11%.[11]

Spectra

^{13}C NMR spectra were recorded at 100 MHz on a Bruker AM 400 instrument using dimethyl-d_6 sulfoxide as solvent and TMS as internal reference (temperature, 323 K). The solution examined was prepared by dissolving 360 mg of lignin in 2 ml of the solvent (10 mm tube). The 2D spectra of the ^{13}C-enriched lignin were recorded using the homonuclear ^{13}C–^{13}C 2D INADEQUATE experiment,[12] which gives a COSY-like symmetry representation.[13,14] It was performed using the standard pulse sequence (INADSYM.AU) given by Bruker. To improve the signal-to-noise ratio, we added (before the 2D transformation) three different 2D data sets. These were obtained by setting the delay in the pulse sequence allowing the double quantum coherence transfer to 3, 4 and 6 ms, respectively. For each experiment, 256 transients were acquired for each of the 128 increments in F_1. The recycle delay was set to 1.5 s. The spectral width was set to 12 500 Hz (number of data points, 1024). The sum matrix was multiplied by shifted sine-bell squared filter functions in both dimensions before Fourier transformation and zero filled to 512 W in the F_1 dimension. No quadrature detection was used in F_1. The data are therefore displayed in magnitude mode. The spectrum in Fig. 3 was obtained by symmetrization of the transformed 2D data. This process is known to suppress artefacts[13] but the intensities of the cross peaks may be slightly changed. Therefore, the enlargement shown in Fig. 4 is taken from a non-symmetrized 2D map.

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