Magn. Reson. Chem., 31(4), 357-363 (1993)

^{1}H NMR of Acetylated β-Ether/β-Ether Lignin Model Trimers

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Detailed examination of the ^{1}H NMR spectra of acetylated β-ether/β-ether lignin model trimers, particularly those synthesized with partially defined stereochemistry, revealed that all of the eight chemically distinct isomers (sixteen optical isomers from four optical centers) are detectable. These compounds provide excellent models for both internal (etherified) and terminal (free-phenolic) units in lignin and clearly delineate the chemical shifts for each isomer. High-field two-dimensional TOCSY or relayed coherence transfer NMR experiments allow correlation of all the side-chain protons in each isomer, while *J*-resolved experiments clearly show the presence of all expected isomers. The degree of complexity already encountered with these simple compounds points to the overwhelming improbability of finding even small crystalline regions in lignin.

KEY WORDS TOCSY *J*-resolved β-Aryl ether Lignin model compounds Stereoisomers Trimer Relayed coherence transfer

INTRODUCTION

The use of trimeric lignin model compounds is a natural exension toward more complex and accurate modeling of lignin for structural and reactivity studies.[1–7] In the NMR spectra of dimers such as the peracetate of guaiacylglycerol-β-guaiacyl ether (**1**) (Fig. 1), it has long been recognized that, while the modeling of the side-chain and free-phenolic end of the molecule is accurate and gives ^{1}H and ^{13}C chemical shifts which are in good agreement with higher oligomers and the lignin polymer itself, the B ring is only poorly modeled since it possesses no side-chain. In particular, the carbons of this ring, although they have been completely and unambiguously assigned,[8] are of little value for the case of true lignins. Etherified units, internal to the lignin structure, are often simply modeled by using methylated (or veratryl) analogues (cf. **2**, Fig. 1), and this is also an imperfect model. Trimeric compounds allow accurate modeling of the free-phenolic end and the internal unit; the trimer C ring suffers from the same inadequacies as the B ring in dimers, but such trimers allow NMR parameters to be accurately determined for both the free-phenolic and the internal units of β-aryl ether moieties in lignin chains.

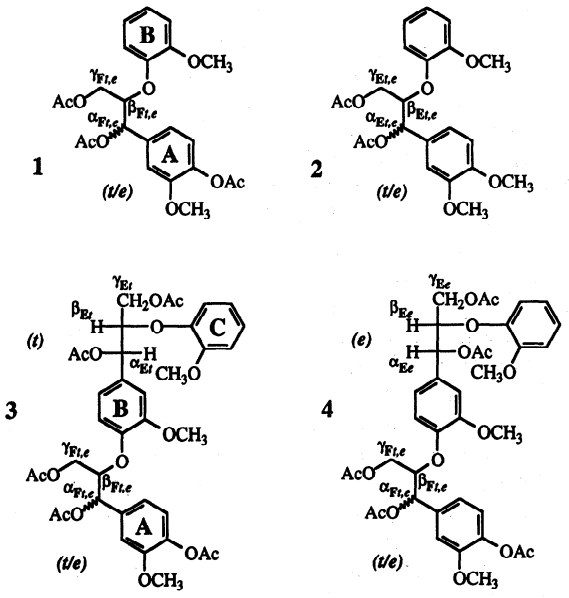

Figure 1. Dimeric β-aryl ethers **1** and **2** and trimer isomers **3** (*t/e–t*) and **4** (*t/e–e*). The *t/e–e* nomenclature, for example, implies that the B/C ring moiety has *erythro* stereochemistry (having been derived from a pure *erythro* dimer), whereas the A/B ring moiety has both *threo* and *erythro* isomers resulting from non-stereoselective reduction of an α-ketone by $NaBH_4$.[2]

In early studies, the dimer guaiacylglycerol-β-guaiacyl ether (**1**) (Fig. 1) was not the β-ether used ubiquitously for model compound work because it could not easily be synthesized as a single isomer. Unlike the simpler guaiacylglycol-β-guaiacyl ether, which has only one optical center and therefore one physical form, compound **1** has two optical centers and four optical isomers, and is synthesized generally as a mixture of *threo* (*RR/SS*) and *erythro* (*RS/SR*) forms.[9] There are now methods available to synthesize predominantly the *threo* or the *erythro* isomer, and each is relatively easy to obtain in pure crystalline form.[10–13] For the trimeric models, however, it is soon apparent that there are sixteen (2^4) possible optical isomers due to the four optical centers. The number of distinct (non-optical) compounds is eight ($2^4/2$ or 2^{4-1}). During our initial synthesis of the trimeric β-ether/β-ether model compounds **3** and **4**,[2] we halved this number of possibilities by constraining the dimeric compound used in the linear synthesis to be either the *threo* or *erythro* form. The other unit was produced non-selectively via ketone reduction and was of mixed *threo/erythro* stereochemistry. It is tempting to think that fixing the stereochemistry of one unit of the trimer will leave just two

isomeric possibilities; for example, if the fixed unit is *threo*, both *threo–threo* and *erythro–threo* possibilities seem logical. However, this represents only half of the possibilities since fixing the second unit to *threo* constrains only the relative stereochemistry of the two optical centers in that unit, it could still be *RR* or *SS*. Consequently, there are still eight possible optical products or four physical isomers. Fixing the stereochemistry of the second unit has halved the number of possibilities.

The study of the high-field ^{1}H NMR spectra of acetylated β-ether/β-ether lignin model trimers **3** and **4** is the major emphasis of this paper. A subsequent paper will address detailed ^{13}C NMR assignments and current efforts at futher reducing the number of isomers by stereoselective reactions. The notion of finding chemically ordered regions in lignin is also addressed.

EXPERIMENTAL

Compounds **1–4** were synthesized as described previously.[2,10,11] Proton NMR spectra of acetone-d_6 solutions (2–5 mg of sample in *ca.* 0.4 ml of solvent in a 5 mm NMR tube) were determined at 298–303 K with Bruker AM instruments at either 500 or 600 MHz. The central peak of the residual acetone-d_5 proton was used as internal reference (2.04 ppm). Spectral widths of 5376 Hz were collected into 64K data blocks, zero filled to 256K points and resolution enhanced by Gaussian multiplication. The resulting accuracy of coupling constants and chemical shifts is estimated to be at least 0.1 Hz. TOCSY[14] spectra (Figs 5 and 7) at 600 MHz were obtained with Bruker's standard MLEV17PH.AU experiment, with a 2.5 ms spin-lock pulse and attenuation sufficient to give a 22 μs 90° pulse; the hard 90° pulse was 6 μs. The number of 2K increments acquired was 256 with 16 transients each. These were processed with squared cosine-bell apodization, and zero-filling gave a final real matrix size of 1K × 512 (3.1 and 6.2 Hz per point in t_2 and t_1). The 600 MHz two-step relayed coherence transfer (magnitude mode) COSY[15] spectrum (Fig. 6) was acquired with Bruker's COSYRCT2.AU micropogram with delays of 56.8 ms, and 26.3 ms for the 0.5/(1.6*J*) coherence periods (corresponding to coupling constants of 5.5 and 11.9 Hz). Sixteen transients of 2K data points were acquired in t_2; 200 of 512 increments in t_1 were zero-filled to 1K for processing. Unshifted sine-bell apodization was used in each dimension. The 500 MHz *J*-resolved spectrum (Fig. 4) was obtained with the JRES.AU microprogram. Eight transients of 8K data points were acquired in t_2 and 32 zero-filled to 64 increments in t_1. Unshifted sine-bell apodization was used in each dimension. COSY and long-range COSY spectra (not shown) were obtained with standard pulse programs and parameters. Plots were edited and annotated in Claris MacDraw Pro on a Macintosh.

RESULTS AND DISCUSSION

Figure 2 shows selected regions of the 600 MHz 1D spectra of the acetates of guaiacylglycerol-β-guaiacyl ether (**1**), veratrylglycerol-β-guaiacyl ether (**2**), the four isomeric *threo/erythro–threo t/e–t*) trimers **3** and the four isomeric *threo/erythro–erythro* (*t/e–e*) trimers **4** (see Fig. 1 for nomenclature). Some of the assignments were made from the 2D spectra, as discussed below. In order to discuss the features of these spectra, the following conventions will be used. In describing a proton, the first letter or letter pair refer to the atom, i.e. α, β, γ_1 or γ_2 for each of the four protons on the side-chain. The second term, a subscripted bold capital letter, refers to the position of the unit within the structure, **E** for etherified (internal unit), **F** for free (terminal free-phenolic end, protected by an acetate group in these peracetates), and the final term, italic and subscripted, refers to the isomer, either *t* or *e* for *threo* or *erythro*. Thus $\alpha_{\mathbf{F}e}$ refers to an α-proton at the terminal or free-phenolic end of the molecule in a unit with *erythro* stereochemistry.

It is the α-proton resonances of acetylated models that are often used to determine stereochemistry, and to determine *erythro*:*threo* ratios.[10,11,16–19] They are well dispersed in the acetylated dimers, for both etherified **2** and free-phenolic **1** models, even at relatively moderate field strengths (200 MHz). In the α-proton region of trimer **4** (Fig. 2), the etherified proton resonances ($\alpha_{\mathbf{E}e}$) are well dispersed from the free-phenolic ones, and the free-phenolic *threo* ($\alpha_{\mathbf{F}t}$) and *erythro* ($\alpha_{\mathbf{F}e}$) resonances are also well dispersed. Clearly, each of the free-end α-protons show both possible sets of resonances. For example, the pair of $\alpha_{\mathbf{F}t}$ doublets arise from a *threo* (*RR/SS*) A/B moiety and an *erythro* (*RS/SR*) B/C moiety resulting in four optical isomers (*RR–RS*, *RR–SR*, *SS–RS*, *SS–SR*) and two physical stereoisomers. In the etherified unit (peaks $\alpha_{\mathbf{E}e}$), all four isomers (from an *erythro* B/C moiety plus either an *erythro* or *threo* A/B moiety) are distinguishable (Table 1). The fine 'splitting' of these peaks (barely visible in Fig. 2) is not due to coupling; examination of a *J*-resolved spectrum in this region (not shown) reveals that each peak of each doublet is in fact a small-*J* triplet, but the major 'splitting' seen here is due to chemical shift differences between each pair of doublets. The alpha region of trimer **3** (the *t/e–t* compound) is different. Here, both *threo* free-end (*t–e*) isomers have magnetically similar resonances and a single doublet is observed (peak $\alpha_{\mathbf{F}t}$) although this is resolvable into two very closely spaced doublets (Table 1) with sufficient resolution enhancement. The $\alpha_{\mathbf{F}e}$ peaks are disperse and overlap with the etherified peaks, $\alpha_{\mathbf{E}t}$.

The β-region is informative and disperse. In the dimers, these peaks are well resolved at 600 MHz (Fig. 2). In trimer **3**, all three β-proton possibilities ($\beta_{\mathbf{F}e}$, $\beta_{\mathbf{F}t}$ and $\beta_{\mathbf{E}t}$) are fully resolved. With contributions from four isomers (*t–t* and *e–t*), the internal etherified unit $\beta_{\mathbf{E}t}$ is more disperse and complex than the free-end peaks ($\beta_{\mathbf{F}e}$ and $\beta_{\mathbf{F}t}$) which arise from two isomers each. In trimer **4**, the dispersion of the β-proton classes is less, and $\beta_{\mathbf{F}e}$ and $\beta_{\mathbf{F}t}$ overlap severely, whereas $\beta_{\mathbf{F}e}$ is almost fully resolved at lower field (as will be evident from TOCSY spectra described below).

The gamma region is the most complex. In the dimers, each proton in each isomer is well resolved. There are four γ-protons in each trimer isomer, two on each unit. The *threo*-γ_1 protons in both the dimers and trimers are well resolved in the 3.9–4.1 ppm region, and

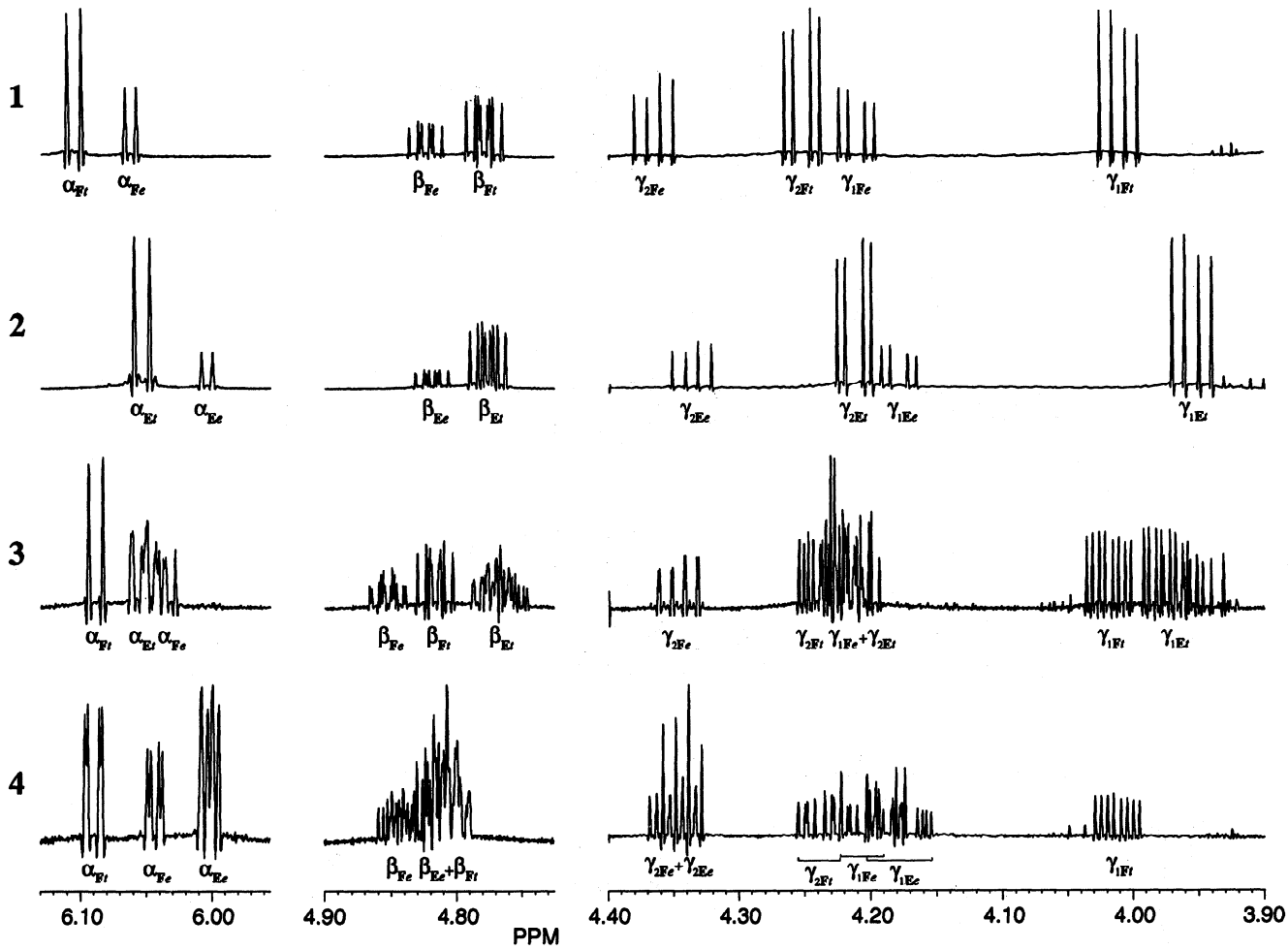

Figure 2. Partial 600 MHz ^{1}H NMR spectra of **1–4** resolution enhanced, showing the α-, β- and γ-regions. The peak labeling follows that shown in Fig. 1. Thus α_{Ft}, for example, identifies an α-proton of a free phenolic end unit with *threo* stereochemistry.

the *erythro*-γ_2 protons are also well resolved at 4.3–4.4 ppm (Fig. 2). In the *t/e–t* trimer **3**, both of the γ_{2Fe} protons are resolved, as are both of the γ_{1Ft} protons. The γ_{1Et} protons clearly show all 16 peaks from the dds of each of the four isomers. This is more clearly illustrated in Fig. 3, an expansion of the region. At this point, we are not attempting to refine further the assignment of these sets of isomeric protons. In trimer **4**, the γ_{1Ft} peak is fully resolved, revealing the two isomers. The γ_{2Fe} and γ_{2Ee} peaks overlap and it is not possible to assign them from these data. However, the assignments

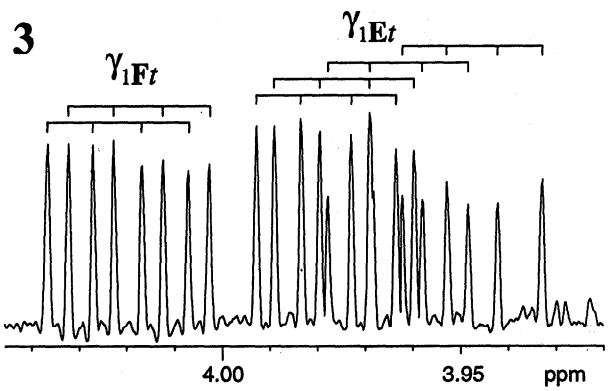

Figure 3. Expansion of the *threo*-γ_1 region of trimer **3** at 600 MHz showing γ_{1Et} dd s for each of the four isomers.

in Table 1 have been made from ^{13}C–^{1}H correlations and the synthesis of more stereochemically constrained trimers (to be reported elsewhere). These assignments are consistent with the *J*-resolved spectrum of Fig. 4 (the 1D projection appears slightly different to the spectrum in Fig. 2 owing to the difference in instrument field strength, 500 *vs.* 600 MHz). The 500 MHz *J*-resolved spectrum in Fig. 4 (deliberately not tilted or symmetrized so that the peak positions can be correlated) clearly shows the dd nature of each resonance.

The aromatic, methoxyl and acetate regions of these spectra are also interesting but not as completely interpretable and are not shown here.

COSY and long-range COSY spectra of these compounds are also informative, especially for assigning some of the aromatic protons, but it is experiments which are capable of correlating all protons within a spin system that are the most valuable. These experiments define those protons belonging to each of the four isomers for each trimer, and allow unambiguous assignments because it is possible to make at least one unequivocable side-chain proton assignment. The prime experiment of this type at present is the HOHAHA or TOCSY experiment,[14,20] but older instruments are often not capable of performing them. A less efficient, but remarkably useful and surprisingly flexible alternative is the relayed coherence transfer experiment.[15] In this experiment, magnetization is passed sequentially

Table 1. Chemical shifts (ppm) and coupling constants (Hz); for side-chain protons of compounds 3 and 4

Compound	α				β				γ_2				γ_1			
	Ft	Et	Fe	Ee	Ft	Et	Fe	Ee	Ft	Et	Fe	Ee	Ft	Et	Fe	Ee
3	6.087 (6.5) 6.086 (6.5)	6.055 (6.7) (0.4) 6.053 (6.6) (0.4) 6.047 (6.7) (0.4) —	6.038 (5.1) (0.6) 6.030 (5.2) (0.5)		4.817	4.767	4.853		4.239 (11.9) (4.1) 4.235 (11.9) (4.1)	4.211 (11.9) (3.8) 4.204 (11.9) (3.8) 4.213 (11.9) (3.8) —	4.347 (11.9) (6.0) 4.346 (11.9) (6.0)		4.018 (11.95) (5.7) 4.014 (11.95) (5.7)	3.975 (11.95) (5.6) 3.971 (11.95) (5.7) 3.959 (11.95) (5.7) 3.943 (11.95) (5.6)	4.224 (11.9) (3.8) 4.222 (11.9) (3.8)	
Nom. δ Nom. J Nom. J	6.09 (6.5)	6.05 (6.7) (0.4)	6.03 (5.2) (0.5)		4.82	4.77	4.85		4.24 (11.9) (4.1)	4.22 (11.9) (3.8)	4.35 (11.9) (6.0)		4.01 (11.95) (5.7)	3.96 (11.95) (5.7)	4.20 (11.9) (3.8)	
4	6.089 (6.5) (0.5) 6.087 (6.5) (0.5)		6.043 (5.2) (0.5) 6.040 (5.2) (0.5)	6.003 (5.2) (0.4) 6.002 (5.2) (0.4) 5.998 (5.1) (0.4) 5.997 (5.1) (0.4)	~4.81[a]		4.845	~4.81[a]	4.239 (11.95) (4.1) 4.234 (11.95) (4.1)		4.349 (11.9) (6.0) 4.343 (11.95) (6.1)	4.353 (11.95) (6.1) 4.348 (11.9) (6.1) 4.343 (11.95) (6.1) —	4.012 (11.95) (5.7) 4.007 (11.95) (5.7)		4.207 (11.9) (4.1) 4.202 (11.9) (4.1)	4.188 (11.9) (3.9) 4.185 (12.0) (4.0) 4.169 (11.9) (3.9) 4.165 (12.0) (3.9)
Nom. δ Nom. J Nom. J	6.09 (6.5) (0.5)	6.06 (6.2)	6.04 (5.1) (0.5)	6.00 (5.1) (0.4)	4.81		4.85	4.81	4.24 (12.0) (4.1)		4.35 (11.9) (6.1)	4.35 (11.95) (6.1)	4.01 (11.95) (5.7)		4.20 (11.9) (4.1)	4.18 (11.9) (3.9)
Lit.[21] Nom. δ Nom. J Nom. J	6.1 6.5	6.05 7	6.07 5.3	6.01 5.5					4.27 —	4.27 —	4.39 (11.9) (5.8)	4.39 (11.9) (5.8)			4.22 (10.3) (6.8)	

[a] Overlapping (unresolved) peaks.

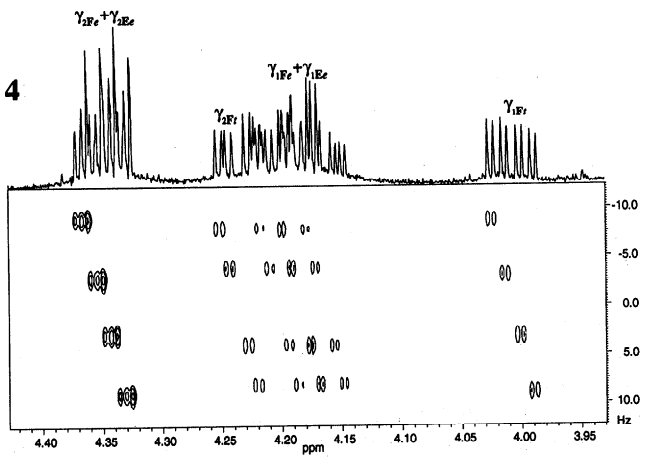

Figure 4. Partial 500 MHz J-resolved spectrum of the t/e–e trimer **4**, showing the dd nature of all γ-protons. The spectrum is deliberately not tilted or symmetrized so that individual peaks in the 1 D spectrum can be matched.

from one proton in a molecule to an adjacent, coupled proton. The coupling constants are chosen to maximize this transfer, and the spectra are often completely detailed before the full complement of increments is taken.

The side-chain region of a TOCSY spectrum of the t/e–e trimer **4** is shown in Fig. 5. Because of the excellent dispersion of the three classes of α-protons (α_{Ft}, α_{Fe} and α_{Ee}) in this compound, the assignment of all side-chain proton groups is particularly easy. The most informative region is the vertical band of contours (on the left-hand side of Fig. 5) correlating the α-protons. Thus, for example, it is clear that the low-field β-resonances are from the erythro free unit (β_{Fe}). Similarly, the very high-field γ resonances belong to the *threo* isomer of the free-phenolic end unit (γ_{1Ft}). From Fig. 5, other assignments noted on Fig. 2 can be confirmed. The instrumentally less demanding two-step relayed coherence transfer experiment can give equally informative data. Figure 6 shows a small section showing clear correlations between each α-proton group and the β- and γ-protons within the same coupling network. Again, the dispersion and assignability of the α-resonances allow the assignment of all other side-chain protons via this experiment.

The TOCSY spectrum of the t/e–t trimer **3** (Fig. 7) is equally informative, although not so trivial to analyze. Since the α-protons are not fully resolved, there is no band of contours that gives all the information, but it is possible to draw unambiguous lines (shown dashed) for various isomer-unit combinations and, again, confirm the assignments made in Fig. 2. Assignment guides are shown schematically on Figs 5 and 7.

The chemical shift assignments for each isomer group are summarized in Fig. 8 and Table 1. Two principles

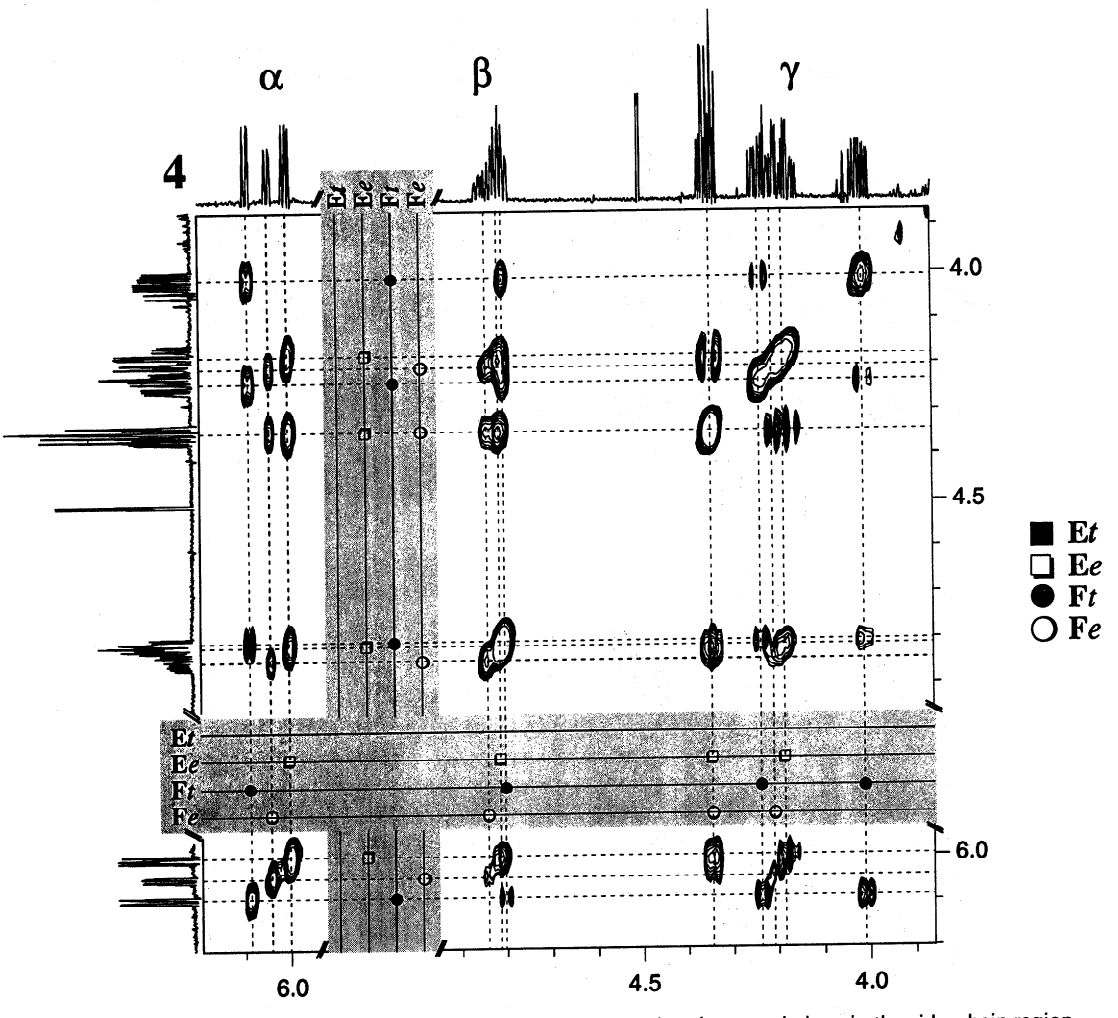

Figure 5. Partial 600 MHz TOCSY spectrum of the t/e–e trimer showing correlations in the side-chain region.

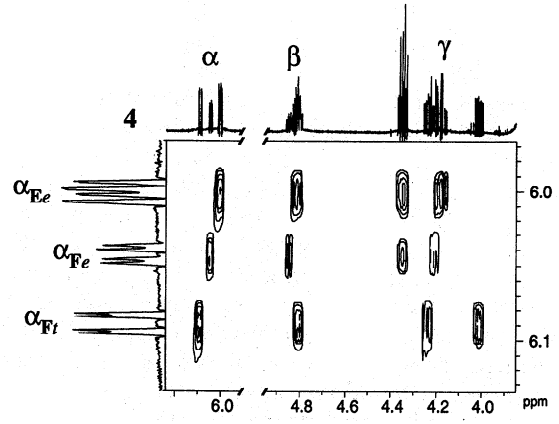

Figure 6. Partial 600 MHz COSYRCT2 spectrum of the *t/e–e* trimer **4** showing the complete α–β–γ_1–γ_2 coupling networks. Only 200 of the 512 increments were processed.

are evident from Fig. 8. First, **F***t* or **F***e* resonances have similar chemical shifts whether the attached unit is *threo* or *erythro*, indicating the difficulties that would be encountered in trying to make assignments from a stereochemically random trimer mixture containing all eight isomers. Second, comparing **E***t* and **F***t* or **E***e* and **F***e* resonances, etherification causes all side-chain resonances to move to higher field. It is additionally evident from Fig. 2 that the veratryl models **2** are surprisingly good predictors of side-chain proton data in these etherified units, whereas the dimeric models **1** for the free-end are not particularly good predictors for the side-chain data in the trimers. Table 1 also lists chemical shifts and coupling constant data determined with exceptional clarity from a *J*-resolved spectrum of an isolated milled wood lignin.[21] With the exception of the γ_{1Fe} data, all agree remarkably well, showing that these trimers make exceptionally good models for assignment of polymer resonances. It also indicates that the ^{1}H NMR chemical shifts in lignins are dictated by the bonded environment and are primarily independent of more remote structures and of the tertiary structure of the polymer. These assignments also corroborate those made earlier from work using model compounds and isolated lignins.[18,19]

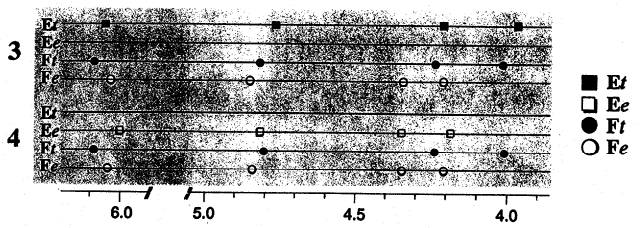

Figure 8. Chemical shifts of **E***t*, **E***e*, **F***t* and **F***e* protons in trimers **3** and **4**.

Figures 8 and 2 also give some insight into why the ^{1}H NMR spectra of lignins look so broad and featureless. Much of this can be attributed to broadening due to the polymeric nature of the material, but an important contributor is the wide range of chemical shifts possible for each of these resonances. Thus, even with the narrow (in ppm) multiplets detected at 600 MHz, just the *threo*-γ_1 resonances in the simple trimers **3** cover 0.11 ppm (Fig. 2) and the β-resonances cover 0.12

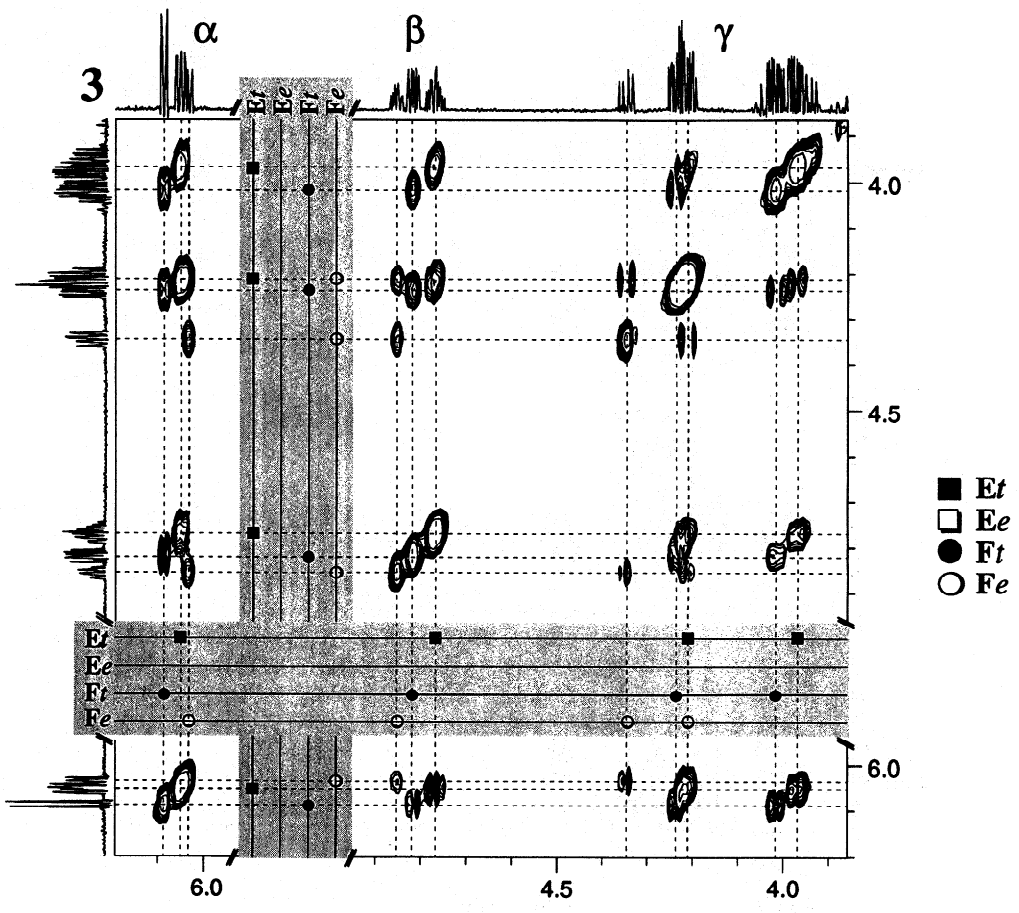

Figure 7. Partial 600 MHz TOCSY spectrum of the *t/e–t* trimer **3** showing correlations in the side-chain region.

ppm. Lignin is a great deal more complex than the trimers examined here.

CRYSTALLINE REGIONS IN LIGNIN?

It becomes obvious from studying these relatively simple trimeric compounds that there is a great deal of overlooked complexity in lignin structures. For each phenylpropanoid unit that is added to a structure, two more optical centers are added, and four times as many physical isomers become possible. What does this mean for polymeric lignin? From time to time researchers speculate on the possibility of crystallinity in lignin.[22] Is the occurrence of crystalline regions likely, and can we even expect to crystallize small lignin fragments? It should be clear already that the number of isomer possibilities is likely to become huge for a molecule of even mild complexity.

In order to put the magnitude of these possibilities into a relevant perspective, consider a relatively small, totally regular and simplified 'lignin' polymer **5** (Fig. 9) made up of only β-ether units. If the degree of polymerization (DP) is 100 (MW $\approx$ 19 600), there are *ca.* 1.6×10^{60} ($2^{100 \times 2} = 2^{200}$) optical isomers and, consequently, of the order of 10^{60} distinct physical compounds. If there is no optical regularity, the existence of crystalline regions in this polymer is astronomically improbable. We have not even been successful in separating or crystallizing any of the four isomers from trimers **3** or **4**, although current efforts at stereoselectively synthesizing discrete isomers are promising. Unless there is some evidence that there are biological templates for forming regular and isomerically identical chains of units into a lignin polymer, the idea of crystallinity or even just crystalline regions in lignin is highly improbable. This is not to say that other aspects of order[23] in the polymer are impossible.

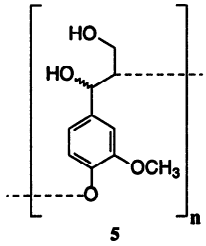

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Figure 9. A regular β-ether polymer of degree of polymerization *n*.

CONCLUSION

Trimeric lignin model compounds can be used very effectively to provide more representative compounds for determining NMR parameters appropriate to lignin structures. The spectra illustrate how any peak in the lignin spectrum will be broadened by the diversity of chemical shifts, and not solely as a result of its slow tumbling owing to its molecular size. In addition, the data from molecules such as these allow the determination of exact chemical shifts for both terminal free-phenolic end and etherified internal structures, and potentially allow for a more detailed analysis of lignin spectra by two- or three-dimensional spectroscopy. Finally, the difficulties associated with the purification and spectral interpretation of these relatively simple compounds document further evidence for the extraordinary isomeric complexity of lignin.

Acknowledgements

The author gratefully acknowledges the use of NMR instrumentation at the University of Wisconsin–Madison Chemistry Department and at the National Magnetic Resonance Facility at Madison; equipment in the facility was purchased with funds from NIH grant RR02301, the University of Wisconsin, the NSF Biological Instrumentation Program (grant DMB-8415048), the NIH shared Instrumentation Program (grant RR02781) and the US Department of Agriculture. The author is also grateful to Dr Lawrence L. Landucci (US Forest Products Laboratory, Madison, WI) for his careful critique of the data in this paper, Dr Charles Rodger (Bruker Spectrospin, Canada), who enthusiastically ran some early spectra of these interesting compounds, and Professor John C. Vederas, Chemistry Department, University of Alberta, Edmonton, Alberta, Canada, for the Macintosh program NMR-Plot. This work was supported in part by grants from the USDA Competitive Grants Program (Plant Growth and Development Nos 90-37261-5617 and 92-37304-8057).

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