A new method for the analysis of phenolic groups in lignins by ^{1}H NMR spectrometry

Shiming Li and *Knut Lundquist*, Department of Organic Chemistry, Chalmers University of Technology and University of Gothenburg, Sweden

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SUMMARY: Phenolic groups in lignins were analysed by ^{1}H NMR spectrometry at 400 and 500 MHz (DMSO-d_6 solution, temperature 300 K). Under these conditions the majority of the signals from protons in phenolic hydroxyl groups are found in the spectral range δ 8.0-9.3. Most carbonyl-conjugated phenols give signals at δ values >9.3; the corresponding types of lignin units constitute only a very small fraction of the phenolic groups. The number of phenolic groups in milled wood lignin (MWL) from spruce was estimated as 0.24/phenylpropane unit; phenolic groups in biphenyl and diaryl ether structures constituted about 20% of the total number of phenolic groups. Spectra of MWL from birch exhibited separate peaks for phenols in guaiacyl units ($\delta \approx 8.8$) and syringyl units ($\delta \approx 8.2$); the total number of phenolic groups was estimated as 0.18/phenylpropane unit. The proportion of phenols in guaiacyl units was larger than that of syringyl units.

ADDRESS OF THE AUTHORS: Department of Organic Chemistry, Chalmers University of Technology, S-41296 Gothenburg, Sweden.

The total number of hydroxyl groups in lignins have frequently been determined by ^{1}H NMR spectroscopy of their acetate derivatives using a method developed by Ludwig et al. (1964) or modifications thereof (Lundquist 1992). ^{1}H NMR spectroscopy of lignin samples in DMSO-d_6/CF_3COOD has also been applied for this purpose (Gagnaire, Robert 1968). Acetylated lignins give signals from acetate groups at $\delta \approx 2.0$ (aliphatic acetate) and $\delta \approx 2.3$ (phenolic acetate). It is difficult to obtain accurate figures for the number of phenolic groups on the basis of the $\delta \approx 2.3$ peak since it is not very well separated from the peak due to aliphatic acetate. Furthermore signals from phenolic acetate in biphenyl structures appear at ca. δ 2.10 and are not included in estimates of phenolic groups on the basis of the δ 2.3 peak (Lundquist 1991). This paper describes an ^{1}H NMR spectrometric method for the analysis of the phenolic groups in lignins which does not suffer from the above-mentioned drawbacks. It is based on examinations of spectra of non-derivatized lignins in DMSO-d_6 solution.

Fig. 1 shows a series of spectra recorded at 500 MHz (solvent, DMSO-d_6). The spectra of milled wood lignin (MWL) from spruce and a synthetic dehydrogenation polymer of coniferyl alcohol (DHP) in fig. 1 exhibit a number of peaks in the range δ 8.0-9.3 which on the basis of results from model compound studies could be attributed to phenolic hydroxyl groups. Fig. 2 shows the spectral range δ 8.0—10.5 of the spruce lignin spectrum; a detailed interpretation of the peaks in this spectral range is given in a separate section of this paper. Results from examinations of samples of DHP, MWL from birch and kraft lignin from pine (fig. 3) are also treated in this context.

Besides the information about phenolic groups discussed below, the DMSO spectra provided a confirmation of earlier results regarding the presence of cinnamyl alcohol groups in MWL from spruce and DHP: it appears in fig. 1 that only trace amounts of cinnamyl alcohol units are present in the MWL sample while there are substantial amounts of such units in the DHP sample (cf. Brunow, Lundquist 1980, Lundquist 1991; see also Nimz, Lüdemann 1976). It is also noteworthy that there is a small peak at $\delta \approx 2.8$ in the MWL spectrum (fig. 1) which can be attributed to H_β in ß-1 structures (Lundquist 1987). Any peak at $\delta \approx 2.8$ could not be discerned in the DHP spectrum (fig. 1).

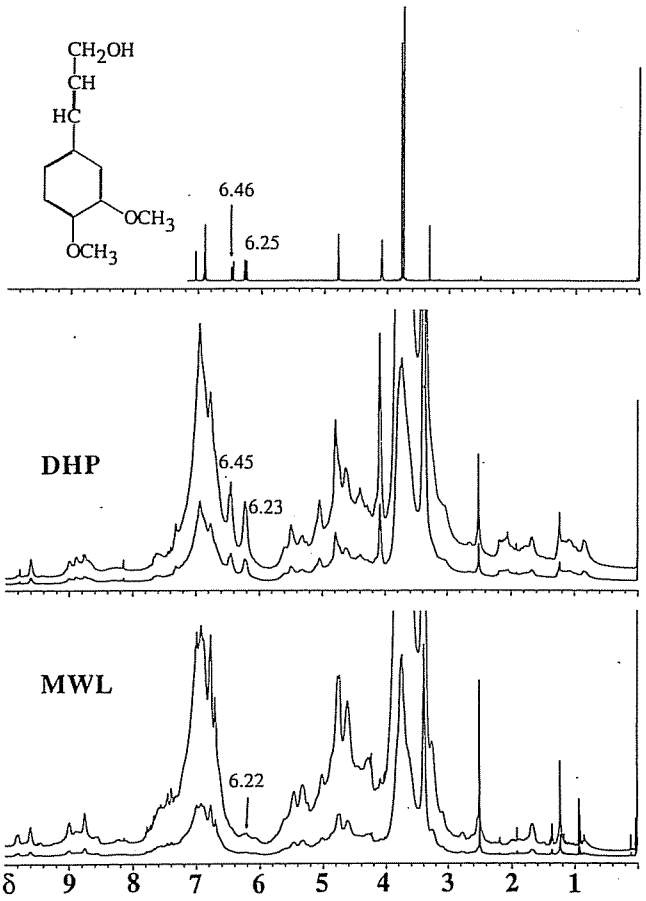


Fig. 1. ^{1}H NMR spectra (500 MHz, solvent DMSO-d_6) of MWL from spruce and a dehydrogenation polymer of coniferyl alcohol (DHP). Peaks with $\delta > 8$ are due to phenolic hydroxyl and formyl groups. The spectrum of the methyl ether of coniferyl alcohol is included in the figure as a reference for signals of cinnamyl alcohol units in the samples.

Examination of model compounds

To obtain a basis for the interpretation of the peaks in the spectral range shown in fig. 2, a number lignin model compounds were examined (400 MHz). Table 1 summarizes results obtained with a number of model compounds representative of guaiacylpropane (**G**), *p*-hydroxyphenylpropane (**H**) and syringylpropane (**S**) units.

The approximate positions of the signal from the phenolic hydroxyl group in models representative of

Fig. 2. The spectral range δ 8.0-10.5 (400 MHz, solvent DMSO-d_6) of MWL from spruce. The assignments of the peaks are indicated in the figure (the peak at δ 8.14 is due to an unidentified contaminant). The dashed lines indicate the baselines used in connection with quantitative estimates.

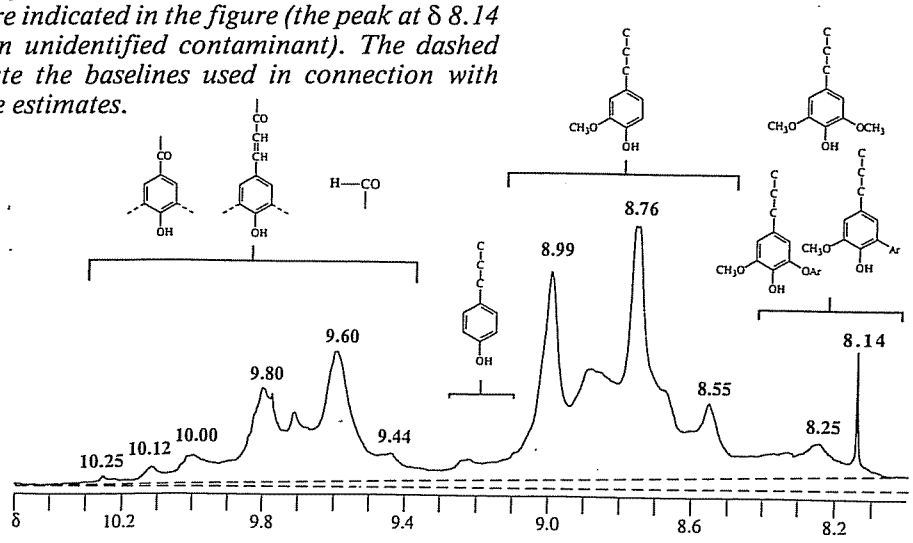
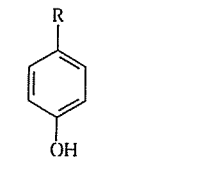


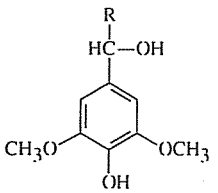
Table 1. Position of the signal from phenolic hydroxyl in ^{1}H NMR spectra of lignin model compounds representative of lignin units of the guaiacylpropane (G), p-hydroxyphenylpropane (H) and syringylpropane (S) types.

Compound	ArOH (δ units/ppm)
G1	8.74
G2[a]	8.71 (broad, 35 Hz at half-height)
G3 (threo)	8.75
G3 (erythro)	8.72
G4	8.52 and 8.62
G5	9.02
G6	8.97
G7	8.06
G8	8.21
G9	8.08
G10	8.35
G11	8.38
(+)-pinoresinol	8.89
H1	9.07
H2	9.21
H3	9.20
H4[b]	9.12 (broad, 40 Hz at half-height)
S1	8.06
S2 (threo)	8.11
S2 (erythro)	8.13

[a] The signal from the carboxylic group was found at δ 12.10 (broad).
[b] The signal from the carboxyl group was found at δ 12.01 (broad).

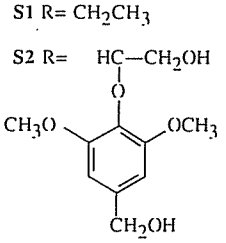


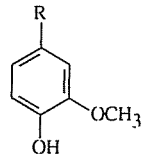
H1 R= CH_2CH_3
H2 R= CH_2OH
H3 R= $CH(OH)CH_2CH_3$
H4 R= CH_2CH_2COOH



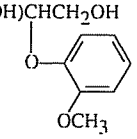
S1 R= CH_2CH_3

S2 R= $HC(OH)CH_2OH$

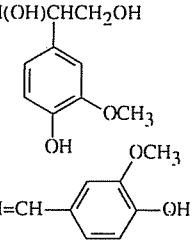




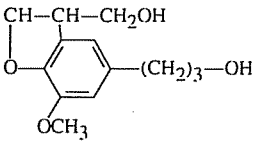
G1 R= CH_2OH
G2 R= CH_2CH_2COOH
G3 R= $CH(OH)CHCH_2OH$



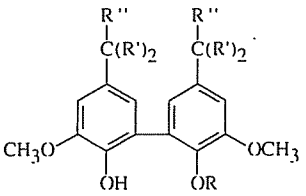
G4 R= $CH(OH)CHCH_2OH$



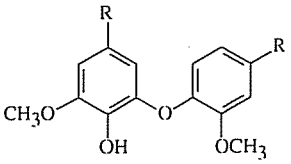
G5 R= CH=CH-



G6 R= $CH{-}CH{-}CH_2OH$



G7 R=R'=R''= H
G8 R=R'= H, R''= OH
G9 R=R'=R''= CH_3



G10 R= CH_3
G11 R= $CH_2CH_2CH_3$

various types of lignin units are shown in fig. 2. The peaks in fig. 2 in the range δ 9.4—10.0 are mainly due to formyl groups. To obtain a basis for the assignments of these peaks the model compounds **ALD 1-9** were examined (table 2). Besides providing information about the formyl protons, this study revealed that the signals from the phenolic groups in the aldehydic models appeared at comparatively high δ values (table 2). This was assumed to be related to the presence of a carbonyl group conjugated with the aromatic nucleus. To elucidate the effect of CO-group conjugation a number of arylconjugated carboxylic acids, esters and ketones (**CO 1-15**) were examined. The results are summarized in table 3. Comparisons of data in table 1 with those in table 3 show that CO-conjugation leads to shifts of the phenol signal positions to lower field. The location of signals from carboxylic acid groups are also of some interest in this context. Signal positions for such groups are given in table 3 (see also the footnotes in Table 1).

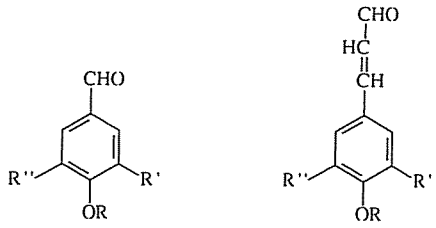
Table 2. Position of the signal from phenolic hydroxyl and formyl protons in ^1H NMR spectra of lignin model compounds representative of benzaldehyde and cinnamaldehyde units in lignin.

Compound	δ units/ppm	
	ArOH	-CHO
ALD1	10.58	9.80
ALD2	—	9.88
ALD3	10.23	9.78
ALD4	—	9.85
ALD5	9.58	9.78
ALD6	—	9.90
ALD7	9.79	9.59
ALD8	—	9.63
ALD9	9.15	9.60

Table 3. Position of the signal from phenolic hydroxyl and carboxyl groups in ^1H NMR spectra of lignins models with arylconjugated carboxyl, ester or keto groups.

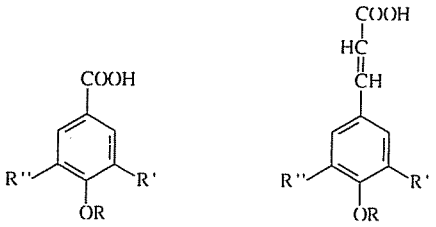
Compound	δ units/ppm	
	ArOH	COOH[a]
CO1	10.20[b]	12.39
CO2	10.22[b]	12.42
CO3	—	12.65
CO4	9.18[b]	12.58
CO5	9.94[b]	12.10
CO6	9.58[b]	12.15
CO7	—	12.19
CO8	10.32	—
CO9	10.01	—
CO10	9.56	—
CO11	8.93	—
CO12	10.28	—
CO13	9.98	—
CO14	9.59	—
CO15	10.06	—

[a] Broad signal.
[b] Broad signal.



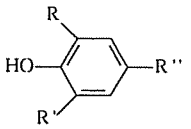
ALD1 R=R'=R''= H
ALD2 R= CH_3, R'=R''= H
ALD3 R=R'= H, R''= OCH_3
ALD4 R= CH_3, R'= H, R''= OCH_3
ALD5 R= H, R'=R''= OCH_3
ALD6 R= CH_3, R'=R''= OCH_3

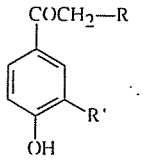
ALD7 R=R'= H, R''= OCH_3
ALD8 R= CH_3, R'= H, R''= OCH_3
ALD9 R= H, R'=R''= OCH_3



CO1 R=R'=R''= H
CO2 R=R'=H, R''= OCH_3
CO3 R= CH_3, R'=H, R''= OCH_3
CO4 R= H, R'=R''= OCH_3

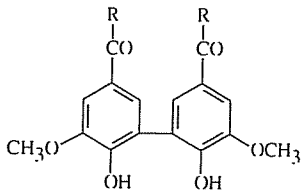
CO5 R=R'=R''= H
CO6 R=R'= H, R''= OCH_3
CO7 R= CH_3, R'= H, R''= OCH_3





CO8 R=R'= H , R''= $COOCH_3$
CO9 R=R'= H , R''= $CH{=}CHCOOCH_3$
CO10 R= H, R'= OCH_3, R''= $CH{=}CHCOOC_2H_5$
CO11 R=R'= OCH_3, R''= $CH{=}CHCOOC_2H_5$

CO12 R= CH_3, R'= H
CO13 R= H, R'= OCH_3



CO14 R= $CH_3CH(OH)$
CO15 R= CH_3CO

The spectra were run at 300 K. A lowering of the temperature to 292 K shifted the phenol peaks slightly (≈0.05 δ unit/10°C) to lower field and the peaks became somewhat sharper.

An increase of the concentration of model **G1** (from 2% to 8% w/v) resulted in a very slight move of signal position to lower field (≈0.01 δ units) and the signal became somewhat broader (width, 6 Hz at half-height). A similar shift of signal position was observed in an analogous study with vanillin (**ALD3**). The appearance of the spectra of phenolic acids (**CO4, CO5**) depended rather much on sample concentration. An increase of the sample concentration from 2% to 8% shifted the phenol group signal downfield by ≈ 0.1 δ units and the signal became broad. Similar changes of the phenol group signal occurred when acetic acid was added to a solution of **G3** in DMSO-d_6.

Comparisons of spectra of model **S2** in DMSO-d_6 and DMSO-d_6/water (50:1) showed that the presence of water caused a downfield shift of the phenol group signal by 0.05 δ units and also resulted in a slight broadening of the signal (width, 6 Hz at half-height). Downfield shifts of the same magnitude were also observed when water was added to samples of **CO**-compounds, phenolic aldehydes (**ALD3, ALD5**), **G2** as well as the biphenyls **G7** and **G8**. The broadening of the signals was rather pronounced in these instances.

It should be noted in this context that the signals from carboxyl groups were always rather broad; the addition of water or other hydroxyl-containing compound caused a substantial broadening of the carboxyl group signals and also a shift of the signal positions to higher field.

The addition of small amounts of bases (sodium hydroxide or sodium acetate) to the sample solutions leads to extensive proton exchange and separate peaks from different types of hydroxyl groups could not be observed.

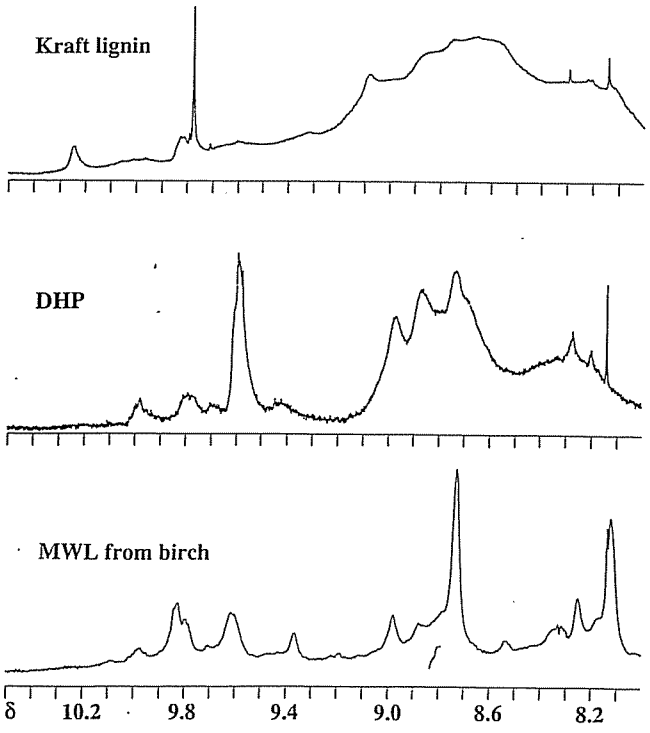


Fig. 3. The spectral ranges δ 8.0-10.5 of ^{1}H NMR spectra (400 MHz, solvent DMSO-d_6) of MWL from birch, DHP and a purified kraft lignin sample. The peaks at δ 9.78 and δ 10.23 in the kraft lignin spectrum are due to the presence of traces of vanillin in the sample.

In conclusion our model compound studies suggest that it is possible to analyse lignin samples for phenolic groups by ^{1}H NMR spectrometry in DMSO-d_6 solution provided the following conditions are fulfilled: a) the amount of water present is small b) no acids are present except for a limited number of carboxyl groups in the sample c) no bases are present.

Analysis of lignin samples

The signals with δ values above 9.4 in fig. 2 are largely due to formyl groups; this could be concluded from studies of the temperature dependence of the signal positions [signals due to phenolic groups are shifted to higher field on an increase of the temperature ($\approx$ 0.05 δ units/10°C) while the signals due to formyl protons are practically unaffected by temperature changes]. Examinations of spectra recorded after addition of trifluoroacetic acid to the sample (cf. Gagnaire, Robert 1977) as well as examinations of borohydride reduced lignin samples confirmed the results. The small peaks discerned at δ 10.12 and δ 10.25 are caused by phenolic groups. It could also be concluded that the inflexion at δ 9.4 and the peak at δ 10.0 partly are due phenolic groups. The phenolic signals located at $\delta \approx 10$ are attributed to phenols conjugated with CO groups (tables 2 and 3). The total of these phenolic groups was estimated as 0.01-0.02/phenylpropane unit. This result agrees very well with what could be expected from studies of the carbonyl groups in MWL from spruce (Adler, Marton 1959). The total number of phenolic groups was estimated as 0.24/phenylpropane unit assuming 2.7H/aromatic ring (the values obtained were in the range 0.22-0.26). Calculations based on recordings with internal standard added to the sample gave approximately the same result. The proportion of phenolic units in biphenyl and diarylether structures was found to be approximately 20% (15—25%) (the contribution from syringyl units is neglected in this estimate).

Fig. 3 shows the region δ 8.0-10.5 of the spectra of a birch lignin sample, a DHP sample (Kirk, Brunow 1988) and a sample of purified kraft lignin (Lundquist, Kirk 1980). The number of phenol groups was estimated as 0.18/aromatic ring in the birch lignin sample assuming 2.3 H/aromatic ring. The **G**-type phenol units/**S**-type phenol units ratio was determined as 3/2. The peaks due to **S**-units (found in the range δ 8.0-8.5) include minor contributions of signals from **G**-units of biphenyl or diaryl ether type. In spite of this the peak caused by **G**-units is larger than that caused by **S**-units (together with certain types of **G**-units, see above). Birch lignin consists of nearly equal amounts of **G**-units (phenolic and non-phenolic) and **S**-units (phenolic and non-phenolic). Accordingly, the ratio **G**-units (phenolic) /**G**-units (non-phenolic) is larger than the ratio **S**-units (phenolic)/ **S**-units (non-phenolic) (cf. Lundquist 1992). A calculation based on the assumption that there are 2.7H/ aromatic ring in the DHP gives a total of 0.28 phenolic OH/phenylpropane unit and the proportion of phenolic groups in biphenyls and diaryl ethers was estimated as 30% (approximately). The kraft lignin sample (2.5 H/aromatic ring) contained 0.58 phenolic group/aromatic ring (figures in the range 0.54-0.62 were obtained). In this case there is no resolution of the peak caused by phenolic hydroxyl (fig. 3); this is related to the structural complexity of kraft lignin.

A method for the analysis of different types of hydroxyl groups in lignins based on ^{31}P NMR spectroscopy of oxyphosphorylated lignin samples has recently been developed (Argyropoulus et al. 1993). The ^{1}H NMR spectrometric method presented in this paper provides an alternative method for the analysis of various types of phenolic hydroxyl groups in lignins.

Experimental

Milled wood lignin samples were prepared according to procedures described by Björkman (1956) and Lundquist (1992c).

^{1}H NMR spectra of lignins and model compounds were in most cases recorded at 400 MHz with a Varian XL-400 (VXR-5000) instrument. Some spectra were recorded at 500 MHz with a Varian Unity 500. DMSO-d_6 was used as solvent if not stated otherwise. Temperature was 300 K if not stated otherwise. TMS was used as internal reference. When lignin spectra were recorded the number of scans was 1000 and the pulse interval was $\approx$7 s (examinations of the lignin signals showed that T_1 was <1 s). To facilitate phase correction methyl methanoate was added to the sample; this compound gives a signal at δ 8.22 (-CHO). Correct phasing, satisfactory sweepwidth (>15 δ units) and high sample concentration (>100 mg/ 0.5 ml) are crucial in connection with quantitative estimations (baseline corrections were not used). It seems that somewhat better results were obtained on lowering the temperature to 292 K.

Quantitative estimates of phenolic groups in lignins were based on the assumption that the peak at $\delta \approx 7$ corresponds to 2.7 H/phenylpropane unit in the case of MWL from spruce, 2.3 H/phenylpropane unit in the case of MWL from birch, 2.7H/phenylpropane unit in

the case of DHP and 2.5H/aromatic ring in the case of kraft lignin (cf. Lundquist 1991 and Lundquist 1992b). Phenolic groups in MWL from spruce was also estimated using internal standards [1-decanol (the calculations were based on the signal from the methylene protons at δ 1.24) and 1,3,5-trimethylbenzene (the calculations were based on the signal from the methyl protons at δ 2.21)]. A suitable amount of the internal standard was injected into the sample solution using a syringe. The pulse interval was 15 s in the experiments with internal standard added.

*Biphenyl **CO14*** was obtained in low yield as a mixture of diastereomers (m.p. 199—207°C) on persulfate oxidation (Elbs, Lerch 1916) of α-hydroxypropiovanillone. ^{1}H NMR (400 MHz; solvent, $CDCl_3$) of the purified acetate derivative (the NMR data for the diastereomers were very similar, one of them dominated): δ 1.53 (6H, d, J= 7.0 Hz; $CH_3C<$), 2.14 (6H, s; CH_3CO), 2.15 (6H, s; CH_3CO), 3.93 (6H, s; CH_3O), 5.85 (2H, $\approx$ q, J=7.0; -CH<), 7.48 (2H, d, J=1.8 Hz) and 7.64 (2H, d, J=1.8 Hz) (aromatic protons).

*Biphenyl **CO15*** (m.p. 197-198°C) was obtained as a by-product in connection with the synthesis of vanilloyl methyl ketone according to Brickman et al. (1940). ^{1}H NMR (400 MHz) of **CO15** : δ 2.46 (6H, s; CH_3CO), 3.91 (6H, s; CH_3O), 7.40 (2H, d, J=2.0 Hz; aromatic protons), 7.48 (2H, d, J=2.0 Hz; aromatic protons), 10.06 (2 H, s (broad); OH).

*Diaryl ether **G10*** was separated in low yield from the reaction mixture obtained on oxidation (H_2O_2/horseradish peroxidase, cf. Bergbom et al. 1981) of creosol. ^{1}H NMR (400 MHz) of the acetate derivative (solvent, $CDCl_3$): δ 2.22 (3H, s; CH_3-Ar), 2.26 (3H, s; CH_3CO), 2.34 (3H, s; CH_3-Ar), 3.81 (3H, s; CH_3O), 3.82 (3H, s; CH_3O), 6.47 (1H) and 6.78 (1H) (d, J=1.4 Hz; aromatic protons at the ring carrying an acetoxy group), 6.20 (1H, m) 6.69 (1H, m) and 6.85 (1H, m) (aromatic protons).

Acknowledgements

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