

Coupling P-31 NMR with the Mannich reaction for the quantitative analysis of lignin

Zhi-Hua Jiang and Dimitris S. Argyropoulos

Abstract: The reactivity of lignin model compounds under Mannich conditions with piperidine and formaldehyde was studied. The piperidinomethyl group was confirmed to be introduced quantitatively at sterically unhindered positions *ortho* to a phenolic hydroxyl when the substrate was allowed to react under ambient conditions. The sequential application of the Mannich reaction followed by quantitative ^{31}P NMR on a variety of lignin samples allowed the amounts of various aromatic groups bearing free phenolic hydroxyls to be determined. More specifically, the technique allowed the quantification of *para*-hydroxyl phenols, catechols, guaiacols, and phenols bearing C_5 or C_6 substituents. The quantitative reliability of the technique was also examined with a series of dissolved kraft lignins, isolated at various degrees of delignification. The data were found to favourably compare to those obtained by permanganate oxidation.

Key words: lignin, lignin model compounds, Mannich reaction, nuclear magnetic resonance (NMR), phenols, phosphorus, piperidine, quantitative analysis.

Résumé : On a étudié la réactivité de composés modèles de la lignine dans la réaction de Mannich avec de la pipéridine et du formaldéhyde. Il a été confirmé que, lorsque le substrat réagit dans des conditions ambiantes, le groupe pipéridinométhyle s'introduit quantitativement dans les positions stériquement non empêchées en *ortho* de l'hydroxyle phénolique. L'application séquentielle de la réaction de Mannich sur divers échantillons de lignine, suivie d'une étude quantitative RMN du ^{31}P , permet de déterminer les quantités de divers groupes aromatiques portant des hydroxyles phénoliques. D'une façon plus spécifique, la technique permet de quantifier les *para*-hydroxyphénols, les catéchols, les guaiacols et les phénols portant des substituants en C_5 et en C_6 . On a aussi évalué la fiabilité quantitative de la technique avec une série de lignines kraft dissoutes, isolées à divers degrés de délignification. On a trouvé que les données se comparent favorablement à celles obtenues par oxydation avec le permanganate.

Mots clés : lignine, composés modèles de la lignine, réaction de Mannich, résonance magnétique nucléaire (RMN), phénols, phosphore, pipéridine, analyse quantitative.

[Traduit par la rédaction]

1. Introduction

Softwood lignin contains, amongst others, a variety of phenolic units that may be classified (1, 2), based on their substitution patterns, as shown in Fig. 1. The relative frequencies of these moieties have been determined by a variety of degradative techniques, i.e., oxidative degradation (3–5) and thioacidolysis–desulfuration (6). Amongst the nondegradative methods of analysis, advanced ^{13}C NMR spectroscopy may offer a quantitative estimate for some of these units (7). Its execution, however, requires acetylation, independent methoxyl analyses, and lengthy acquisition protocols. Therefore, at present, there is no facile, nondegradative means to determine these units.

The potential and unique features of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane as an additional phosphitylation reagent used in quantitative ^{31}P NMR analysis of lignin

have been demonstrated by Jiang et al. (8) and Granata and Argyropoulos (9). This reagent offers some distinct advantages compared to 2-chloro-1,3,2-dioxaphospholane, i.e., better resolution of the various phenolic moieties (8, 9), permitting the accurate calculation of ^{31}P chemical shifts for various phenolic compounds (8), and better stability of the phosphitylated moieties (9).

However, the spectral resolution obtained using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane as the phosphitylation reagent is still not adequate for quantifying all types of phenolic structures. The ^{31}P NMR signals belonging to types (B), (C), and (D) phenolic units overlap in the region of 138.3–140.2 ppm as observed by Granata and Argyropoulos (9). The information about the amount of each type of phenolic units (B), (C), and (D) cannot therefore be determined using quantitative ^{31}P NMR alone. Data reported elsewhere by Jiang et al. (8) demonstrated that *ortho* substitution onto the aromatic ring of phenols has a considerably higher effect on the magnitude of the ^{31}P NMR chemical shifts than that of *para* and *meta* substitution patterns. As such it became of interest to investigate the potential of coupling quantitative ^{31}P NMR with the Mannich reaction, aimed at obtaining further structural information for lignin.

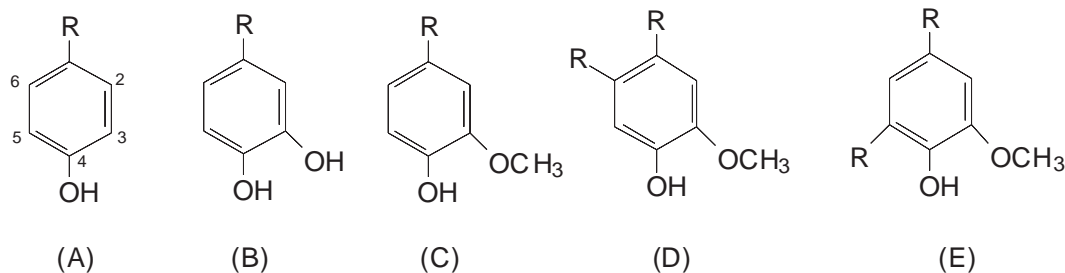
The Mannich reaction on phenols is a classic organic reaction. The essential feature of the reaction is the replacement of

Received December 4, 1997.

Z.-H. Jiang and D.S. Argyropoulos,¹ PAPRICAN and Department of Chemistry, McGill University, Pulp and Paper Research Centre, 3420 University St., Montreal, QC H3A 2A7, Canada.

¹ Author to whom correspondence may be addressed. Telephone: (514) 398-6178. Fax: (514) 398-8254.

Fig. 1. The major types of phenolic units in softwood lignin, where R represents a lignin interunit [1,2].



the active hydrogen atoms in phenols, especially active hydrogen atoms in positions *ortho* and *para* to phenolic hydroxyl groups, by aminomethyl or substituted aminomethyl groups (10–15).

The reactivity of various lignin model phenols toward the Mannich reaction with dimethylamine and (or) piperidine was first investigated by Mikawa et al. (17). They demonstrated that a dimethylaminomethyl or piperidinomethyl group was introduced quantitatively to free C₅ positions of lignin-related phenolic units (17). The application of the Mannich reaction to lignin, followed by nitrogen analysis, allowed Mikawa et al. (17) to quantify the phenolic units with free C₅ positions in lignin. Their data showed that about 25–40% of the phenolic hydroxyl groups in a dissolved softwood kraft lignin belong to this type of phenolic unit. The Mannich reaction has also been applied in the preparation of lignin derivatives with ion-exchange properties by Brezny et al. (18) and Rusina et al. (19).

This study examines the potential of coupling the Mannich reaction with quantitative ³¹P NMR, to provide further fine structural information of lignin. Initially, the course and conditions of the Mannich reaction with piperidine were examined on 4-hydroxy-3-methoxybenzoic acid, and then the Mannich reaction with piperidine was carried on a variety of lignin model compounds. Finally the Mannich reaction was coupled with quantitative ³¹P NMR for quantifying various types of phenolic units in lignin. The determined values were compared to those reported in the literature for similar samples.

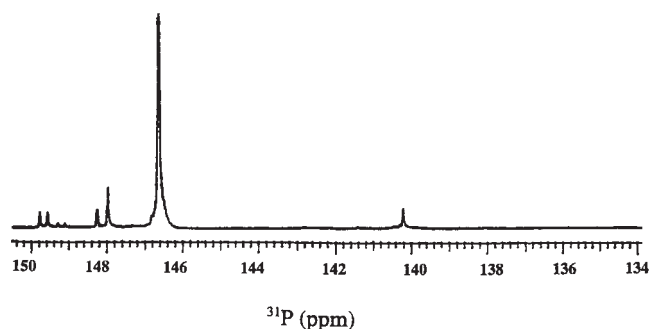
2. Results and discussion

2.1 Studies on lignin model compounds

Although dimethylamine is the most frequently reported secondary amine for use in the Mannich reaction of phenols, piperidine was chosen in this study since the former also causes substitution at available methylol groups (17). Piperidine is more selective in this respect (unreactive with methylol groups) (17), yet of comparable reactivity to dimethylamine (15).

The work of Mikawa et al. (17), using either dimethylamine or piperidine as bases, has shown that type (C) lignin model compounds (see Fig. 1) give, without exception, C₅ substituted Mannich bases at very high yields. When the C₁ position of type (C) is a carboxyl group, it forms the Mannich base with simultaneous decarboxylation and substitution at the C₅ and C₁ positions. Other C₁ groups remain intact when piperidine is used. Mikawa et al. (17) also confirmed that type (E) moieties do not react, and also the Mannich reaction does not occur in all cases, when the phenolic hydroxyl group is etherified. These conclusions offered the basis for interpreting the ³¹P

Fig. 2. The ³¹P NMR spectrum, phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, of blank Mannich reaction medium, a mixture of equivalent amounts of piperidine and formaldehyde with 3.3 equiv. of ethanol.

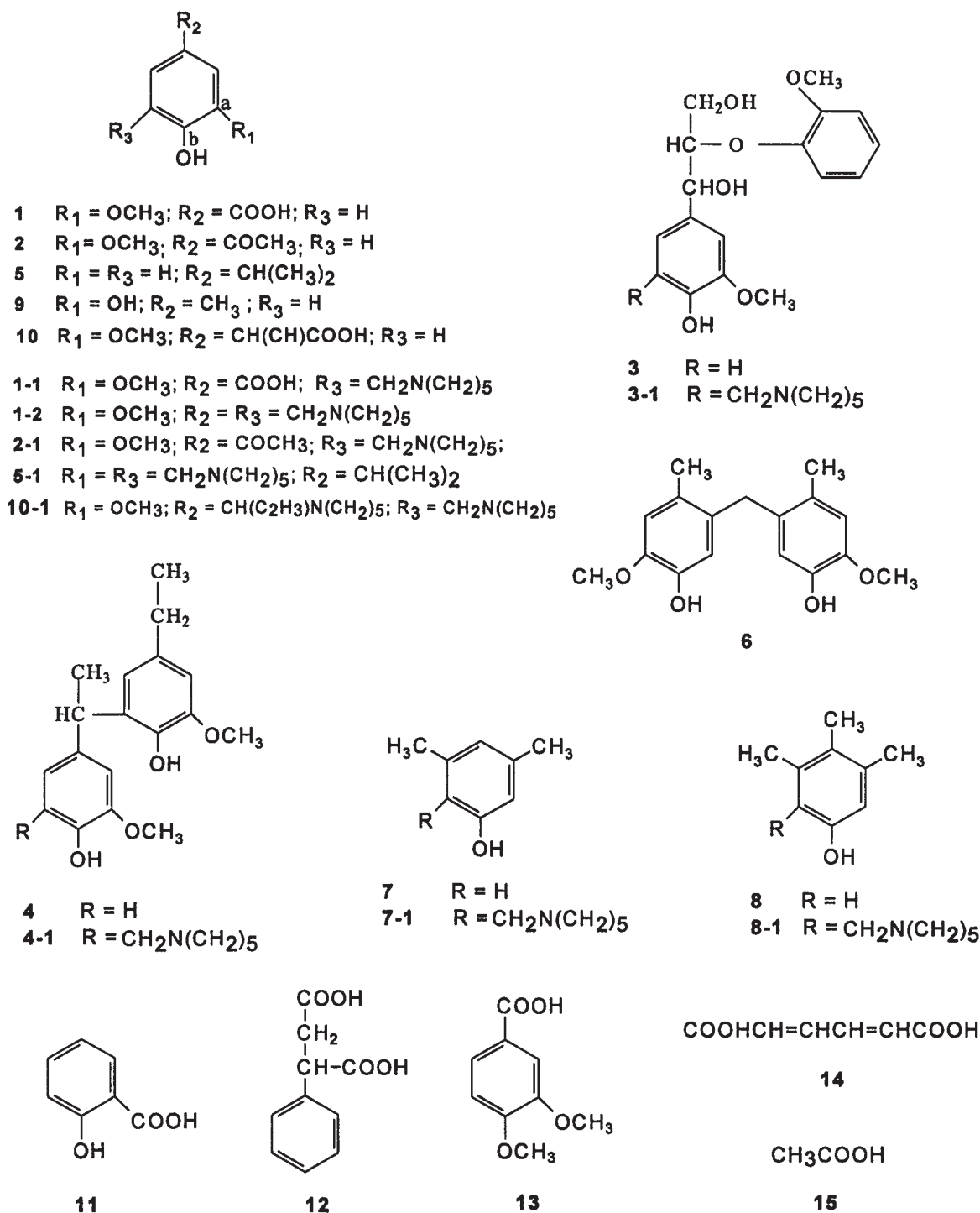


NMR spectra obtained with the following lignin model compounds, using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane as a phosphitylation reagent. Since the ³¹P chemical shifts of phenolic hydroxyls with various substitution patterns strongly depend on the nature of the substituents, they can be calculated using the parameters developed by Jiang et al. (8). All lignin-related phenols with *ortho*-mono substituents have ³¹P chemical shifts within the range 138.3–140.5 ppm. These signals are completely separated from those of *ortho*-disubstituted phenols, including phenols with a substitution of a piperidinomethyl group at C₅ positions, which are located in the range 141.1–144.5 ppm (8).

Furthermore, in the early phases of this study, it was found that the ³¹P NMR technique could be applied to identify the Mannich reaction products even if the reaction products are not completely pure. A ³¹P NMR spectrum of the Mannich reaction medium used in this work (a mixture of equivalent amounts of piperidine and formaldehyde with 3.3 equiv. of ethanol) is shown in Fig. 2. The major signals of the reaction medium are located in the downfield (146–150 ppm) and are well separated from those signals of phenols and carboxylic acids (8, 9).

The course and conditions of the Mannich reaction with piperidine were first reexamined on 4-hydroxy-3-methoxybenzoic acid **1** (see Fig. 3) as a lignin model compound. Figure 4 shows the ³¹P NMR spectra of 4-hydroxy-3-methoxybenzoic acid under Mannich conditions as a function of time. The following progressive changes can be visualized from these spectra: (i) reduction of signal intensity at 139.1 and 135.1 due to the ³¹P derivatives of the phenolic hydroxyl and the carboxylic acid of 4-hydroxy-3-methoxybenzoic acid respectively; (ii) increase of signal intensity at 143.7, which is assigned to the ³¹P derivative of the phenolic hydroxyl of the Mannich

Fig. 3. The structure of model compounds examined in this work and their Mannich reaction products with piperidine and formaldehyde.

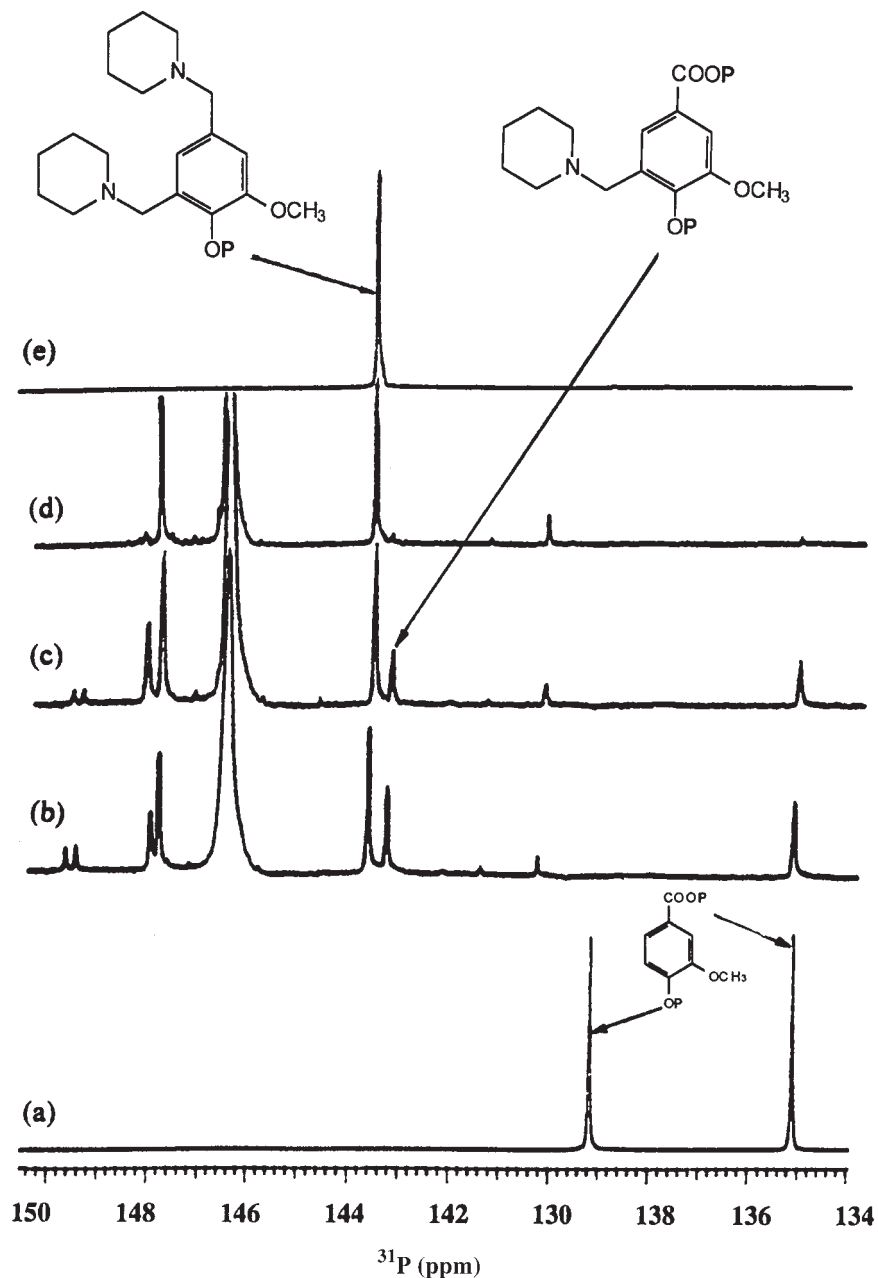


base, 2,4-dipiperidinomethyl-6-methoxyphenol **1-2** (see Fig. 3); (iii) increase over the first day and reduction thereafter of signal intensity at 143.4, which is assigned to the ^{31}P derivative of the phenolic hydroxyl of the Mannich base, 5-piperidinomethyl-4-hydroxy-3-methoxybenzoic acid **1-1** (see Fig. 3). Figure 5 shows further the results of this kinetic investigation.

As anticipated, the active hydrogen position *ortho* to the phenolic hydroxyl in 4-hydroxy-3-methoxybenzoic acid **1** was

completely substituted to form the Mannich base **1-1** within 24 h. However, the displacement of the carboxyl group, which results in product **1-2**, was considerably slower as evidenced from quantitative ^{31}P NMR studies, which showed that the carboxyl groups reacted completely only after a 5-day reaction period. When the temperature was increased to 45°C, more than 50% of the carboxyl groups in model **1** were substituted within 2 h and about 85% of them were reacted after 6 h. Besides, the reaction of model **1** was found to be considerably

Fig. 4. The ^{31}P NMR spectra, phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (shown as P), of 4-hydroxy-3-methoxybenzoic acid under the Mannich reaction conditions as a function of time: (a) before reaction; (b), (c), and (d) after reaction for 1, 3, and 5 day(s), respectively; (e) after reaction for 5 days followed by purification. The unassigned signals belong to the Mannich reaction medium (see Fig. 2).



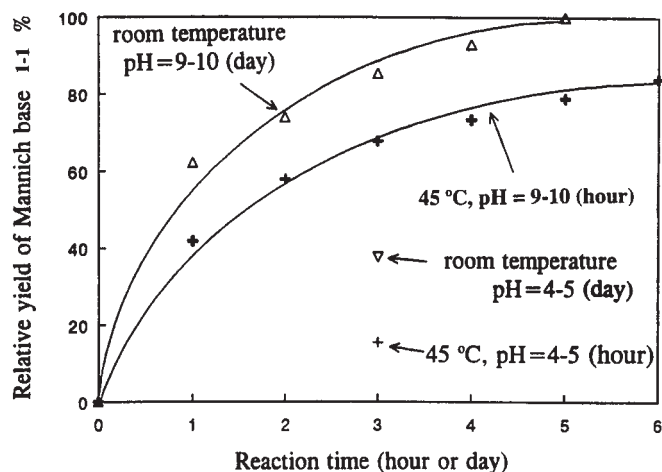
slower under acidic conditions. At pH 4–5 and after 3 days of reaction, only about 38% of the carboxylic groups and 92% of the active hydrogen position *ortho* to the phenolic hydroxyl were replaced. At the same pH and at 45°C, 11.5% of the carboxylic groups and 23.5% of the active hydrogen position *ortho* to the phenolic hydroxyl were replaced after 3 h.

The Mannich reaction was then carried out at room temperature on a variety of lignin-related model phenols. These model compounds were such chosen so as to represent all types of phenolic units in softwood lignin as shown in Fig. 1. The structure of these model compounds and their Mannich reaction products are shown in Fig. 3.

Acetovanillone **2** and lignin model dimer **3** over a period of 5 days gave the Mannich bases **2-1** and **3-1**, respectively. The active hydrogen positions *ortho* to the phenolic hydroxyl were substituted, however, the side chains of models **2** and **3** remained intact, in agreement with Mikawa et al. (17). Lignin model dimers **4**, over a period of 24 h, gave the Mannich base **4-1** with a 5-piperidinomethyl substituent at the C₅ position as expected.

p-Isopropylphenol **5**, which represents type (A) phenolic units, containing two active hydrogen positions *ortho* to the phenolic hydroxyl, was found to yield the Mannich base **5-1** within 5 days. Similar observations have also been reported

Fig. 5. The course of the Mannich reaction with piperidine on 4-hydroxy-3-methoxybenzoic acid under various conditions.



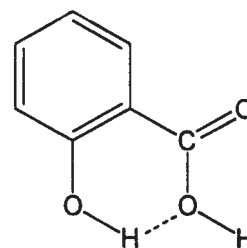
when unhindered phenols were allowed to react with dimethylamine and morpholine (15). No changes in terms of ^{31}P NMR chemical shifts were observed for model 6, after the Mannich reaction was carried out for 5 days at room temperature. This seems to indicate that both unsubstituted positions *ortho* to the phenolic hydroxyls were unreactive because the substitution of the piperidinomethyl group at one of these positions is expected (8, 20) to result in a very significant change in the ^{31}P NMR chemical shift (downfield shift of about 4 ppm).

After the Mannich reaction, the ^{31}P NMR chemical shifts of both models 7 and 8 were found to shift downfield by about 0.7 ppm. For a similar reason as described above, these data seem to indicate that the reaction products were Mannich bases bearing a substituent *ortho* to the phenolic hydroxyl.

Although this information seems to be adequate as far as the main objective of this study is concerned, these products were further purified and identified. It was found that only one unsubstituted position *ortho* to the phenolic hydroxyl reacted. This is very likely due to steric effects exerted by the peri positions, inhibiting Mannich disubstitution. Steric factors are known to significantly affect the course of the Mannich reaction (15). For example, hydroquinone treated with 3 equiv. each of dimethylamine and formaldehyde gave 2,5-dimethylhydroquinone not tri-(dimethylaminomethyl)-hydroquinone (21). Also, 5,7-bis(piperidinomethyl)-8-quinolinol could not be prepared by means of the Mannich reaction with 8-quinolinol under usual conditions, since only monosubstituted Mannich base (7-piperidinomethyl-8-quinolinol) was formed (16).

When the Mannich reaction was carried out on model 9 (*m*-methylcatechol), using 3 equiv. of piperidine, a mixture of several Mannich bases was obtained. No attempts were made to identify these products. However ^{31}P NMR analyses of the products seem to indicate that the positions *ortho* to the phenolic hydroxyl (C_2 and C_5) were not simultaneously substituted, and one of them was substituted by the piperidinomethyl group, as evidenced by quantitative ^{31}P NMR analysis of the mixtures of the Mannich base products, which showed that the signal intensity in the range of 138.4–139.0 ppm is the same compared to those in the range of 143.0–144.4 ppm. The signals at around 138.4–139.0 and 143.0–144.4 ppm are known

Fig. 6. A possible six-membered ring in salicylic acid formed through hydrogen bonding.



to be due to the phenolic hydroxyl groups with *ortho*-mono and *ortho*-disubstituents, respectively (8).

Similar to model 3, 4-hydroxy-3-methoxycinnamic acid 10 gave Mannich base 10-1 with simultaneous decarboxylation. However, such decarboxylation reactions were not observed when the Mannich reaction was carried out, under similar conditions, on model acid compounds salicylic acid 11, phenylsuccinic acid 12, 3,4-dimethoxybenzoic acid 13, muconic acid 14, and acetic acid 15, as evidenced by ^{31}P NMR analyses, which showed that the ^{31}P NMR signals of acid groups in these models did not change in terms of position and intensity. These observations seem to indicate that the decarboxylation reaction, during the Mannich reaction, may occur only to the carboxyl groups that link (i.e., model 1) or are conjugated with (i.e., model 10) aromatic units bearing a *para* hydroxyl substituent.

The Mannich reaction did not seem to occur on the positions *ortho* and *para* to the phenolic hydroxy of salicylic acid 11 because the ^{31}P NMR signal position due to the phenolic hydroxy group did not change after the Mannich reaction. Similar results were reported previously by Regnier et al. (22). A possible explanation for this phenomenon is that intramolecular interactions cause the formation of a six-membered ring and may consequently reduce the acidity of the phenol and prevent Mannich substitution as shown in Fig. 6.

2.2 Summary of studies on lignin model compounds

The results of the model compound studies described above confirmed and complemented the results reported by Mikawa et al. (17). These results showed that: (i) model compounds of type (A) gave Mannich bases with the substituents at both the C_3 and C_5 positions; (ii) a type (B) model compound gave a mixture of the Mannich bases with the substituent at the C_2 or C_5 position; (iii) model compounds of type (C) gave Mannich bases with the substituent at the C_5 position; (iv) those of type (D) and (E) do not react; (v) carboxyl groups that link or are conjugated with aromatic units bearing a phenolic hydroxyl group in the *para*-position gave Mannich bases with simultaneous decarboxylation; however, all other carboxyl groups do not react; (vi) all other groups attached to phenolic aromatic units at C_1 positions including methylol and methyl groups remain intact.

2.3 Summary of ^{31}P NMR chemical shifts of Mannich base products

A methodology for accurate prediction of the ^{31}P chemical shifts of lignin-related phenolic compounds was developed in our previous effort as a result of correlation analysis using the Hammett principles (8). This methodology has recently been

Table 1. The ^{31}P NMR chemical shifts of phenolic hydroxyls in various lignin model compounds and their Mannich reaction products. The calculations were carried out using the method and parameters previously described (8).

Lignin model compounds			Mannich reaction products		
No. ^a	^{31}P chemical shift, ppm		No.	^{31}P chemical shift, ppm	
	Observed	Calculated		Observed	Calculated
1	139.1	139.1	1-1	143.4	143.6
				1-2	143.7
2	139.3	139.4	2-1	143.5	143.9
3	139.5	139.6	3-1	144.2	144.1
4	OH ^a 143.9	143.9	4-1	143.9	143.9
	OH ^b 139.8	139.8			144.4
5	138.1	138.1	5-1	144.3	— ^b
6	139.8	139.8	6-1	139.8	139.8
7	137.8	137.8	7-1	138.5	— ^b
8	138.1	138.1	8-1	138.8	— ^b
9	OH ^a 139.2 (d)	139.2	M ^c	138.4(d);138.7(d);	— ^b
	OH ^b 138.9 (d)	139.0		139.0 (d); 143.7(d)	
10	139.4	139.4	10-1	144.4(d)	
11^d	OH ^a 137.4 (d)	137.2		143.8	— ^b
	OH ^b 139.2 (d)	— ^b			
12-15^d	137.7	137.7			

^a Roman number designations refer to Fig. 3.

^b Parameters not available for calculation.

^c A mixture of several Mannich products.

^d For all these compounds, no changes in terms of signal positions were observed after the Mannich reaction.

successfully applied for calculating or assigning the ^{31}P chemical shifts of phenolic units in lignin (9, 20, 23). In the present study the methodology was also applied to calculate the changes of the ^{31}P chemical shifts occurring on model phenols before and after the Mannich reaction (Table 1). These changes were further generalized as shown in Table 2, using the parameters previously developed (8, 20) and taking the changes of the lignin structure after the Mannich reaction into account.

2.4 The Mannich reaction on lignin

2.4.1 Kinetics of the Mannich reaction with piperidine on lignin

Prior to embarking at an extensive investigation of the Mannich reaction on lignin, a kinetic study was carried out on a dissolved softwood kraft lignin isolated at a degree of delignification of 92.9%. When this lignin sample was treated with formaldehyde and piperidine, the nitrogen content of the lignin sample was found to increase as the reaction time was prolonged (Table 3). The increase was most pronounced during the first 2 days, which seems to reflect the rapid replacement of the active hydrogens in phenolic units by the piperidino-methyl group. This increase is unlikely to be attributed to piperidine contamination because the excess piperidine seems to be effectively removed by the work-up procedure applied (see relevant experimental section for more details). The nitrogen content was found to remain constant after 3 days. Very similar results were also reported by Mikawa et al. (17) with

dimethylamine. The Mannich reaction on lignin was therefore carried out at room temperature for 5 days.

2.4.2 The determination of the various phenolic moieties in kraft lignin

A series of dissolved and well characterized (24) softwood kraft lignins, isolated at various degrees of delignification, were subjected to the Mannich reaction with piperidine at room temperature for 5 days. Quantitative ^{31}P NMR analyses prior to and after the Mannich reaction quantified the various phenolic units in the lignin samples. Figure 7 shows typical quantitative ^{31}P NMR spectra of such a lignin sample and its Mannich reaction product.

By integrating the region between 144.4–140.3 ppm and 138.2–137.3 ppm in the spectrum before the Mannich reaction (Fig. 7a), the amounts of type (E) and type (A) phenolic nuclei can be quantified. Integration of the region 140.2–138.3 ppm in the same spectrum allows the determination of the sum of phenolic types (B), (C), and (D) to be made. Furthermore, integration over from 144.4 to 137.3 ppm allows the determination of the amount of all phenolic moieties. These integration ranges applied are based on previous model compound (see Table 2 for details) and lignin studies by Granata and Argyropoulos (9). Such integration ranges have recently been adopted by Sun and Argyropoulos (25, 26), Froass et al. (27), and Senior et al. (28).

On the other hand, the ^{31}P NMR signals at 139.1–138.0, 140.4–139.1, and 144.8–138.0 ppm, in the spectrum after the Mannich reaction (Fig. 7b), could be assigned to the corresponding Mannich base products of phenolic (B), (D), and the sum of all types of phenolic units, respectively. These integration ranges are based on the results of early calculations (Table 2). Integrating the ranges between 139.1–138.0 and 140.0–139.1 ppm and dividing these integrals to the integral obtained by integrating the region 144.8–138.0, respectively, gives rise to the relative frequencies of type (B) and (D) phenolic units, expressed in terms of their percentages in the total phenolic hydroxyls. These relative frequencies should remain unchanged prior to and after the Mannich reaction. The absolute amounts of type (B) and (D) phenolic units in a given sample can therefore be obtained by multiplying their relative frequencies with the absolute amount of all phenolic hydroxyls obtained by integrating the region 144.4–137.3 ppm in the spectrum (a) of Fig. 7. By subtracting the absolute amounts of type (B) and (D) phenolic units from the sum of (B), (D), and (C), obtained by integrating the region 140.2–138.3 ppm in the spectrum (a) of Fig. 7, the amount of type (C) phenolic units in a given sample was calculated.

Using the method described above, the absolute amounts of all types of phenolic units shown in Fig. 1 could be quantified. This method when applied to a series of softwood dissolved kraft lignin samples revealed the data of Table 4. These data can now be compared to those reported in the literature with similar samples. The relative frequencies of individual phenolic units (A)–(E) were calculated and expressed as a percentage of the total phenolic hydroxyl content. The major phenolic units were then compared to those obtained by Gellerstedt and Gustafsson (29) using permanganate degradation analysis (Fig. 8). The agreement shown between the two sets of data is apparent, bearing in mind that different wood species and techniques were used to detect these moieties. In addition, a

Table 2. The ^{31}P chemical-shift ranges for various Mannich base products phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane. (For details see ref. 8.)

Type of phenolic unit ^a	Ranges of calculated ^{31}P chemical shift (ppm)		Detailed calculations for the ranges after the Mannich reaction
	Before ^b	After ^c	
(A)	137.3–138.2	143.8–144.4	Up-field: $138.02^d - 0.37^e + 6.2^f$; downfield: $138.02^d + 0.13^g + 6.2^f$
(B)	OH ^a 138.9–139.5 OH ^b 138.3–139.2	138.0–139.0; ^h 143.3–144.4	Up-field: $138.4^i - 0.37^e$; downfield: 139.0^j ; up-field: $143.7^k - 0.37^e$; downfield: 144.4^l
(C)	139.1–140.0	143.8–144.4	Up-field: $138.02^d + 1.76^m - 0.37^e + 4.45^n$; downfield: $138.02^d + 1.76^m + 0.13^g + 4.45^n$
(D)	139.0–140.5	139.3–140.5	Up-field: $138.02^d + 1.76^m - 0.37^e - 0.11^o$; downfield: $138.02^d + 1.76^m + 0.13^g + 0.50^p$
(E)	141.1–144.0	141.3–144.0	Up-field: $138.02^d + 1.76^o - 0.37^e + 1.96^q$; downfield: $138.02^d + 1.76^o + 0.13^g + 4.03^r$

^a For structures see Fig. 1.^b Before the Mannich reaction.^c After the Mannich reaction.^d Value for phenol.^e Value for *p*-COCH₃.^f Value for *ortho* disubstitution of piperidinomethyl groups (from the chemical-shift difference between model **5** and its Mannich base **5-1**, Table 1).^g Value for *p*-CH₃.^h Range for one of the two phenolic hydroxyls.ⁱ Up-field limit value of model **9** after the Mannich reaction (for signals in the range of 138.4–139.0 ppm, Table 1).^j Downfield limit value of model **9** after the Mannich reaction (for signals in the range of 138.4–139.0 ppm, Table 1).^k Up-field limit value of model **9** after the Mannich reaction (for signals in the range of 143.7–144.4 ppm, Table 1).^l Downfield limit value of model **9** after the Mannich reaction (for signals in the range of 143.7–144.4 ppm, Table 1).^m Value for *o*-OCH₃.ⁿ Value for second *ortho* substitution by a piperidinomethyl group.^o Value for *m*-CH₂Ph.^p Value for *m*-COCH₃.^q Up-field limit value selected for second *ortho* disubstitution.^r Downfield limit value selected for second *ortho* disubstitution. Values from ref. 8 if unspecified.

comparison of the sum of types (A), (B), (C), and (D) phenolic units can also be made for a sample of softwood milled wood lignin. When these moieties are expressed per methoxyl group, Alder and Lundquist (30) obtained values of 0.15–0.18 using Fremy's salt oxidation, while the methodology of this work showed a value of 0.16.

2.4.3 The reproducibility of the Mannich – ^{31}P NMR analysis

Quantitative ^{31}P NMR analysis of lignin using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane as the phosphitylation reagent was found to have a high reproducibility by Sun and Argyropoulos (25). For all functional groups, the maximum error obtained between duplicate acquisitions was found to be below 1% (25). This is probably due to the better stability of the moieties phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane than with 2-chloro-1,3,2-dioxaphospholane moieties (9).

In an effort to examine the reproducibility of the Mannich – ^{31}P NMR technique, the dissolved softwood lignin with a degree of delignification of 92.9% was subjected to three Mannich – ^{31}P NMR analyses (Table 5). The relative standard deviation obtained for the major types of phenolic units (types (C) and (E)) was found to be less than 6.0%.

Table 3. Nitrogen contents of a dissolved softwood kraft lignin after the Mannich reaction with piperidine as a function of time.

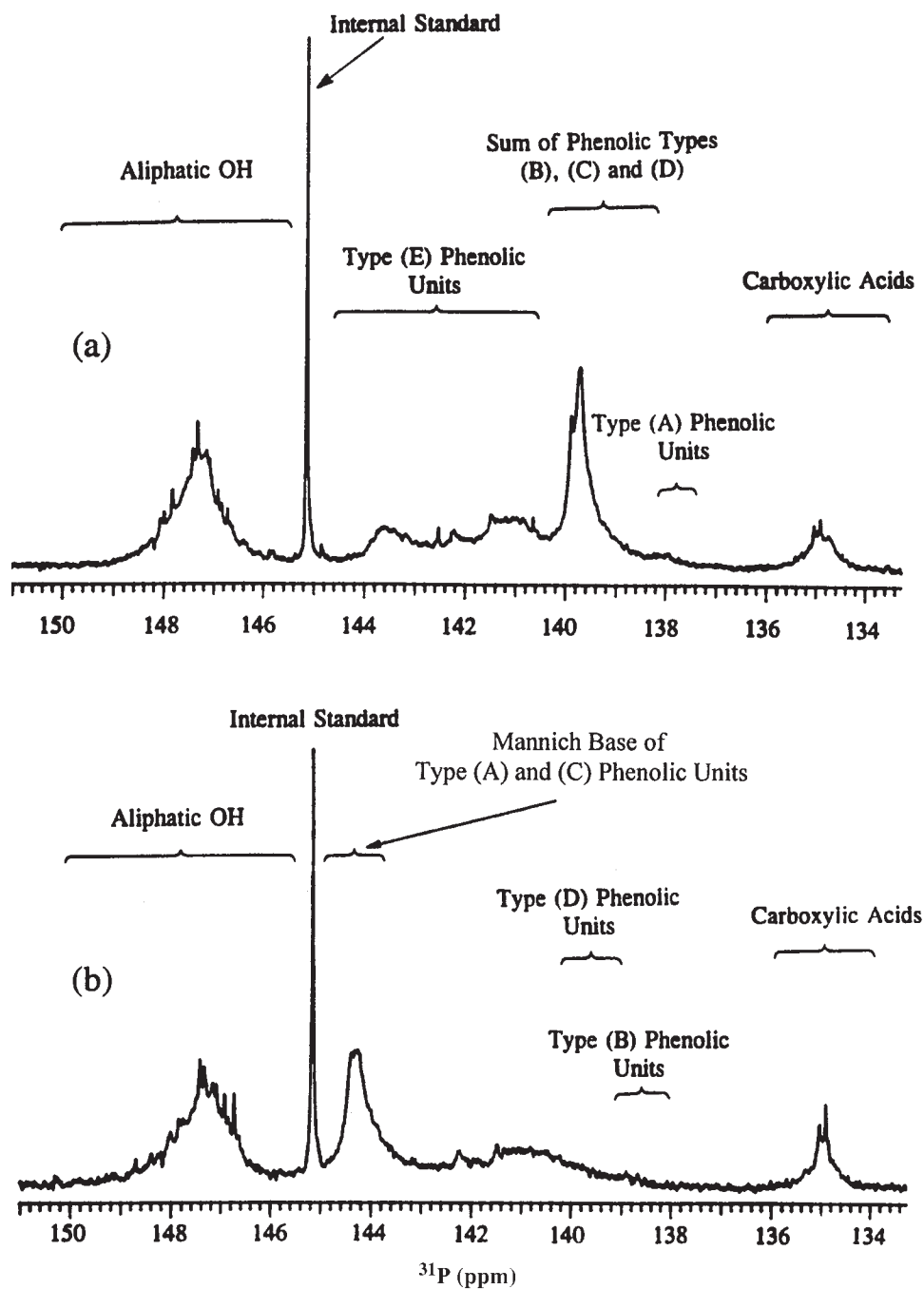
Reaction time (days)	Nitrogen content (%)
Control	<0.01
1	1.64
2	1.87
3	2.02
4	2.18
5	2.15

3. Experimental

3.1 ^{31}P NMR spectroscopy

The lignin sample (about 40 mg), predried overnight in a vacuum oven set at 40°C, was dissolved with 800 μL of solvent (pyridine – deuterated chloroform 1.6:1 v/v) at room temperature in a 5 mL flask with magnetic stirring. Internal standard cyclohexanol (about 1.3 mg) and relaxation reagent chromium acetylacetonate (about 0.4 mg) were added as 200 μL in the same solvent mixture, and the solution was stirred until the lignin was fully dissolved. The phosphitylation reagent

Fig. 7. Quantitative ^{31}P NMR spectra, phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, and signal assignment of (a) a softwood kraft dissolved lignin sample and (b) its Mannich reaction product with piperidine. The chemical shifts of type (E) phenolic units do not change after the Mannich reaction.



2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphosphalane (about 100 μL), was then added and the flask was sealed, stirred to ensure thorough mixing, and this slightly exothermic reaction was complete in about 20 min.

Quantitative ^{31}P NMR analysis was obtained in 5 mm sample tubes on a Varian XL-300 spectrometer at 125.5 MHz following the procedures described by Granata and Argyropoulos (9).

3.2 ^1H NMR spectroscopy

The ^1H NMR spectra were obtained in 5 mm sample tubes on a Varian XL-200 or Varian XL-300 NMR spectrometers,

operating at 200.1 and 299.9 MHz, respectively. Deuterated chloroform was used as solvent and tetramethylsilane was used as internal reference. No relaxation delay was used during acquisition.

3.3 Gas chromatography – mass spectroscopy

The GC–MS analyses were carried out on a Hewlett–Packard 5792 mass spectrometer interfaced to a Hewlett–Packard 5890A gas chromatography with a 30 m \times 0.25 mm packed silica capillary column DB-5. The injection port temperature was 280°C, and the oven temperature was varied from 100 to

Table 4. Phenolic hydroxyl contents of types (A)–(E) phenolic units present in softwood kraft solubilized lignin isolated at various degrees of delignification, determined by the Mannich – ^{31}P NMR technique.

Lignin sample ^a	Degree of delignification (%)	Various types of phenolic OH (mmol/g)					Total
		(A)	(B)	(C)	(D)	(E)	
MWL ^b	—	0.02	0.05	0.62	0.06	0.35	1.15
KSL ^c 1	18.1	0.07	0.04	1.35	0.08	0.86	2.44
KSL 2	22.1	0.06	n.d. ^d	n.d.	n.d.	0.96	2.49
KSL 3	26.7	0.05	n.d.	n.d.	n.d.	1.02	2.4
KSL 4	39.1	0.05	0.01	1.56	0.12	1.31	3.06
KSL 5	59.9	0.05	0.01	1.57	0.12	1.46	3.22
KSL 6	84.6	0.05	0.01	1.62	0.13	1.54	3.36
KSL 7 ^e	92.9	0.04	0.04	1.49	0.19	1.80	3.6

^a Lignin sample from black spruce (*Picea mariana*), see ref. 24 for their elemental composition, methoxyl, and total lignin contents.

^b Milled wood lignin.

^c Kraft solubilized lignin.

^d Not determined.

^e Average of triplicate results.

Table 5. The reproducibility of the Mannich – ^{31}P NMR analysis carried out on a softwood kraft dissolved lignin sample isolated at a degree of delignification of 92.9%.

Type of phenolic units	Mean value (mmol/g)	Standard error	Standard deviation	90% confidence limit
(A)	0.04	3.3×10^{-3}	5.8×10^{-3}	9.7×10^{-3}
(B)	0.04	5.8×10^{-3}	1.0×10^{-2}	1.7×10^{-2}
(C)	1.49	4.7×10^{-2}	8.1×10^{-2}	1.4×10^{-1}
(D)	0.2	1.5×10^{-2}	2.5×10^{-2}	4.2×10^{-2}
(E)	1.8	3.0×10^{-2}	5.1×10^{-2}	8.7×10^{-2}

250°C, with a gradient of 10°C/min. Samples were analyzed after silylation with chlorotrimethylsilane.

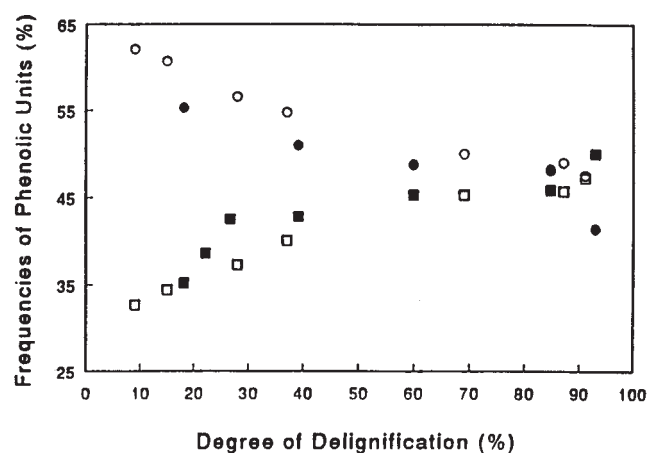
3.4 Elemental analyses

Elemental analyses were carried out by the Schwarzkopf microanalytical laboratories, Woodside, N.Y., in a Perkin–Elmer 2400 carbon, hydrogen, and nitrogen analyzer.

3.5 Mannich reaction with piperidine on 4-hydroxy-3-methoxybenzoic acid

4-Hydroxy-3-methoxybenzoic acid **1** (20 mmol) was dissolved in a mixture of 99% piperidine (60 mmol), aqueous formaldehyde (37%) (60 mmol), and absolute ethanol (20 mL). The reaction mixture was kept at a preset temperature for the required time with occasional shaking. When necessary, the solution was acidified to pH 4–5 with acetic acid prior to the onset of the reaction. After the reaction, a few drops of the solution were used for the ^{31}P NMR analyses without purification. The work-up prior to obtaining the spectrum of Fig. 1(d) was as follows: the solution was concentrated repeatedly in order to expel residual Mannich reagents by adding distilled water and freeze-dried. The solid product obtained was subjected to ^{31}P NMR and GC–MS. ^{31}P NMR δ (ppm): 143.7; MS m/z : 390 (M^+ , 6), 375 (11), 307 (48), 232 (9), 222 (100), 207 (32), 191 (64), 149 (9), 98 (23), 73 (65), 55 (8). ^1H NMR (200 MHz, TMS, CDCl_3) δ (ppm): 1.30–1.80 (m, 12H),

Fig. 8. A comparison of the relative frequencies of type (C), circles, and type (E) phenolic units, squares, as obtained by this work, closed symbols, and those reported by Gellerstedt and Gustafsson (29), open symbols.



2.25–2.90 (m, 8H), 3.75 (s, 4H), 3.86 (s, 3H), 6.68 (s, 1H), 6.94 (s, 1H).

3.6 Mannich reaction with piperidine on other model compounds

Acetonevanillone 2

Acetonevanillone **2** (10 mmol), 99% piperidine (20 mmol), aqueous formaldehyde (37%) (20 mmol), and absolute ethanol (10 mL) were mixed and allowed to stand at room temperature for 5 days. After the reaction, the reaction mixture was concentrated repeatedly by adding distilled water and freeze-dried to give the Mannich base product 5-piperidinomethyl-acetonevanillone **2-1** in a quantitative yield based on ^{31}P NMR analysis. ^{31}P NMR δ (ppm): 143.5; ^1H NMR (200 MHz, TMS, CDCl_3) δ (ppm): 1.50–1.75 (m, 6H), 2.40–2.80 (m, 7H), 3.76 (s, 2H), 3.92 (s, 3H), 7.28 (s, 1H), 7.43 (s, 1H). MS m/z : 335 (M^+ , 22), 320 (15), 292 (11), 252 (42), 221 (57), 194 (19), 179 (8), 144 (11), 98 (39), 84 (100), 73 (66), 55 (8). For crystallization, 5-piperidinomethyl-acetonevanillone (6 mmol) was dissolved in absolute ethanol (5 mL), and concentrated hydrochloric acid (6.2 mmol) was added. The product precipitated as crystals when the mixture was cooled in a fridge. It was finally recrystallized from absolute ethanol, yield 59%. No correct elemental analysis results were obtained.

1-(4-Hydroxy-3-methoxyphenyl)-2-(2-hydroxy-3-methoxyphenyl)-1,3-propanediol 3

To 1-(4-hydroxy-3-methoxyphenyl)-2-(2-hydroxy-3-methoxyphenyl)-1,3-propanediol **3** (5 mmol) was added 99% piperidine (10 mmol), aqueous formaldehyde (37%) (10 mmol), absolute ethanol (5 mL). The reaction mixture was stirred and allowed to stand at room temperature for 5 days. After the reaction, the reaction mixture was concentrated repeatedly by adding distilled water and freeze-dried to give the Mannich base 1-(5-piperidinomethyl-4-hydroxy-3-methoxyphenyl)-2-(2-hydroxy-3-methoxyphenyl)-1,3-propanediol **3-1** in a quantitative yield based on ^{31}P NMR analysis. ^{31}P NMR δ (ppm): 148.2 (1OH, OH_γ), 147.4 (1OH, OH_α), 144.2 (1OH, phenolic OH). MS m/z :

633 (M⁺, 5), 618 (5), 460 (34), 394 (83), 311 (100), 281 (10), 247 (9), 149 (31), 123 (16), 73 (76).

1-(4-Hydroxy-3-methoxybenzyl)-1'-(4-ethyl-3-methoxy-2-phenyl)-ethane 4

To 1-(4-hydroxy-3-methoxybenzyl)-1'-(4-ethyl-3-methoxy-2-phenyl)-ethane **4** was added 99% piperidine (10 mmol), aqueous formaldehyde (37%) (10 mmol), absolute ethanol (5 mL). The reaction mixture was stirred and allowed to stand at room temperature for 1 day. After the reaction, the reaction mixture was concentrated repeatedly by adding distilled water and freeze-dried to give the Mannich base 1-(5-piperidinomethyl-4-hydroxy-3-methoxybenzyl)-1'-(4-ethyl-3-methoxy-2-phenyl)-ethane **4-1** in a quantitative yield based on ³¹P NMR analysis. ³¹P NMR δ (ppm): 143.9 (1OH^a), 144.4 (1OH^b). MS *m/z*: 543 (M⁺, 5), 528 (7), 460 (34), 429 (11), 399 (7), 251 (100), 220 (31), 205 (11), 84 (13), 73 (94).

p-Isopropylphenol 5

To *p*-isopropylphenol **5** (10 mmol) was added 99% piperidine (30 mmol), aqueous formaldehyde (37%) (30 mmol), and absolute ethanol (10 mL). The reaction mixture was stirred and kept at room temperature for 5 days. After the reaction, the reaction mixture was concentrated repeatedly by adding distilled water and freeze-dried to give the Mannich base 2,6-dipiperidinomethyl-4-isopropylphenol **5-1** in a quantitative yield based on ³¹P NMR analysis. ³¹P NMR δ (ppm): 144.3. ¹H NMR (200 MHz, TMS, CDCl₃) δ (ppm): 1.00–1.24 (m, 6H), 1.30–1.80 (m, 12H), 2.20–2.90 (m, 9H), 3.61 (s, 4H), 6.60–6.84 (m, 1H), 6.90–7.00 (m, 1H). MS *m/z*: 402 (M⁺, 5), 387 (11), 319 (83), 276 (34), 235 (55), 219 (53), 161 (24), 98 (42), 73 (100), 55 (15).

3,3'-(6,6'-Dimethoxy-4,4'-dimethyl)-diphenylmethane 6

To 3,3'-(6,6'-dimethoxy-4,4'-dimethyl)-diphenylmethane **6** (5 mmol) was added 99% piperidine (15 mmol), aqueous formaldehyde (37%) (15 mmol), and absolute ethanol (5 mL). The reaction mixture was stirred and kept at room temperature for 1 day. After the reaction, the reaction mixture was concentrated repeatedly by adding distilled water and freeze-dried. The product, obtained in a quantitative yield based on ³¹P NMR analysis, has an identical structure as starting material 3,3'-(6,6'-dimethoxy-4,4'-dimethyl)-diphenylmethane **6**. ³¹P NMR δ (ppm): 139.8; MS *m/z*: 432 (M⁺, 84), 402 (47), 372 (12), 329 (12), 193 (13), 73 (100), 45 (17); ¹H NMR (200 MHz, TMS, CDCl₃) δ (ppm): 2.20 (s, 6H), 3.90 (s, 6H), 6.50 (s, 2H), 6.53 (s, 2H). The crystallization of the product was carried out from absolute ethanol, yield 48%. Anal. calc. for C₁₇H₂₀O₄: C 70.8, H 6.99; found: C 71.0, H 6.77.

3,5-Dimethylphenol 7

To 3,5-dimethylphenol **7** (10 mmol) was added 99% piperidine (20 mmol), aqueous formaldehyde (37%) (20 mmol), and absolute ethanol (10 mL). The reaction mixture was stirred and kept at room temperature for 1 day. After the reaction, the reaction mixture was concentrated repeatedly by adding distilled water and freeze-dried to give the Mannich base 2-piperidinomethyl-3,5-dimethylphenol **7-1** in a quantitative yield based on ³¹P NMR analysis. ³¹P NMR δ (ppm): 138.5; ¹H NMR (200 MHz, TMS, CDCl₃) δ (ppm): 1.40–1.75 (m, 6H), 2.19 (s, 3H), 2.23 (s, 3H), 2.40–2.70 (m, 4H), 3.65 (s, 2H), 6.46

(s, 1H), 6.51 (s, 1H). MS *m/z*: 291 (M⁺, 11), 217 (21), 206 (79), 191 (20), 135 (27), 84 (100), 73 (27). The crystallization of 2-piperidinomethyl-3,5-dimethylphenol was carried out from absolute ethanol, yield 48%. Anal. calc. for C₁₄H₂₀ON: C 77.1, H 9.24, N 6.42; found: C 76.6, H 9.40, N 6.58.

3,4,5-Trimethylphenol 8

To 3,4,5-dimethylphenol **8** (10 mmol) was added 99% piperidine (20 mmol), aqueous formaldehyde (37%) (20 mmol), and absolute ethanol (10 mL). The reaction mixture was stirred and kept at room temperature for 1 day. After the reaction, the reaction mixture was concentrated repeatedly by adding distilled water, diluted with distilled water, and freeze-dried to give the Mannich base 2-piperidinomethyl-3,4,5-trimethylphenol **8-1** in a quantitative yield based on ³¹P NMR analysis. ³¹P NMR δ (ppm): 138.8 (s); ¹H NMR (200 MHz, TMS, CDCl₃) δ (ppm): 1.40–1.70 (m, 6H), 2.12 (s, 3H), 2.17 (s, 3H), 2.24 (s, 3H), 2.40–2.70 (m, 4H), 3.72 (s, 2H), 6.55 (s, 1H). MS *m/z*: 305 (M⁺, 18), 290 (6), 220 (100), 205 (27), 191 (6), 149 (6), 105 (6), 73 (22).

m-Methylcatechol 9

To 3-methylcatechol **9** (10 mmol) was added 99% piperidine (30 mmol), aqueous formaldehyde (37%) (30 mmol), and absolute ethanol (10 mL). The reaction mixture was stirred and kept at room temperature for 5 days. After the reaction, the reaction mixture was concentrated repeatedly by adding distilled water and freeze-dried to give a mixture of Mannich bases. ³¹P NMR δ (ppm): 138.4–139.0 (d, 1OH), 143.0–144.4 (d, 1OH).

4-Hydroxy-3-methoxycinnamic acid 10

4-Hydroxy-3-methoxycinnamic acid **10** (10 mmol) 99% piperidine (20 mmol), aqueous formaldehyde (37%) (20 mmol), and absolute ethanol (10 mL) were mixed and allowed to stand at room temperature for 1 day. After the reaction, the reaction mixture was concentrated repeatedly by adding distilled water and freeze-dried to give the Mannich bases 3,5-dimethoxyl-4-hydroxycinnamic acid and 5-piperidinomethyl-4-hydroxy-3-methoxycinnamic acid **10-1** in a quantitative yield based on ³¹P NMR analysis. ³¹P NMR δ (ppm): 143.8 (1OH). MS *m/z*: 416 (M⁺, 5), 401 (8), 333 (94), 248 (92), 233 (26), 217 (63), 110 (63), 98 (47), 73 (100).

Mannich reaction on model carboxylic acids 11–15

Individual model carboxylic acids (5 mmol) 99% piperidine (10 mol), aqueous formaldehyde (37%) (10 mol), and absolute ethanol (5 mL) were mixed and kept for 5 days at room temperature with stirring. After the reaction, the solution was concentrated under reduced pressure at room temperature and freeze-dried. The solid products were subjected to ³¹P NMR analysis with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane as the phosphitylation reagent. In all cases, the spectra obtained for the products were identical with corresponding starting materials.

3.7 Mannich reaction with piperidine on lignin

The procedures used by Mikawa et al. (17) were essentially followed. Approximately 200 mg of lignin was dissolved in 2 mL of a mixture solution composed of 99% piperidine, aqueous formaldehyde (37%), and absolute ethanol in the following

molar proportions of 1.00:1.05:3.30. It was then kept at room temperature for 5 days with stirring or occasional shaking. After the reaction, the reaction mixture was concentrated repeatedly by adding distilled water and freeze-dried. The solid lignin was washed with diethyl ether three times, centrifuged, and dried in a vacuum oven at room temperature.

4. Conclusions

The absolute amounts of various phenolic nuclei such as, *para*-hydroxylphenyls, catechols, guaiacols, and phenols bearing C₅ or C₆ substituents in lignin, can be determined by using the combination of the Mannich reaction and quantitative ³¹P NMR. The Mannich – ³¹P NMR analysis is a facile and nondestructive technique that may provide detailed structural information of lignin samples, with minimum experimental complexity.

5. Acknowledgements

This research was supported by the Canadian Pulp and Paper Industry and the Natural Sciences and Engineering Research Council of Canada.

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