N-Hydroxy Compounds as New Internal Standards for the $^{31}$P-NMR Determination of Lignin Hydroxyl Functional Groups

M. Zawadzki and A. Ragauskas

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Keywords: $^{31}$P-NMR, residual lignin, hydroxyl and phenolic group determination, N-hydroxy compounds.

Introduction
TMDP (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane) is a very powerful reagent for tagging hydroxyl groups as the $^{31}$P NMR active phosphite derivative (Figure 1). TMDP derivatization/$^{31}$P-NMR spectroscopy has been effectively applied to analyze lignin structural changes during kraft pulping (Ahvazi, Pageau et al. 1998; Froass, Ragauskas et al. 1998) and various bleaching operations (Froass, Ragauskas et al. 1996; Wang, Jiang et al. 1997).

![Figure 1. Phosphitylation of a hydroxyl group by TMDP.](image)

Usually, cyclohexanol is selected as an internal standard for the TMDP/$^{31}$P-NMR method (Granata and Argyropoulos 1995). In our experience, we have found that occasionally the cyclohexanol-phosphite product overlaps with derivatized aliphatic and phenolic lignin structures (Figure 2, also see Zawadzki, Ragauskas 1999). Cholesterol has also been used as an internal standard (Smit, Suckling et al. 1997), but unfortunately, the chemical shift for the phosphite product is very similar to that of cyclohexanol (Table 1).

![Figure 2. $^{31}$P-NMR spectrum of residual lignin (brownstock, kappa = 47) containing cyclohexanol (IS #1) and N-hydroxy-5-norborene-2,3-dicarboximide (IS #2) derivatized with TMDP.](image)

Method
TMDP derivatization and phosphorus-NMR analysis was performed according to the literature (Granata and Argyropoulos 1995; Jiang, Argyropoulos et al. 1995) using ~30 mg lignin or ~30 μmole model compounds. Spin-lattice ($T_1$) relaxation parameters were determined by the standard inversion recovery experiment (Braun, Kalinowski et al. 1996). All chemicals were purchased commercially and used without further purification.

Results and Discussion
Experiments were focused on the development of a new internal standard that was baseline resolved from lignin-derived adducts. Chemical shift and spin-lattice relaxation ($T_1$) values for the studied compounds are given in Table 1. The structures of the compounds studied are illustrated in Figures 3–5.

$\text{ROH} + \text{Cl}_2\text{P} = \text{RO-PC(O)CH}_2\text{CDCl}_3 + \text{P} = \text{Ph} + \text{HCl}$

Piperidine (VIII, Figure 5) was studied, and rejected, as a potential internal standard because the chemical shift of its TMDP product ($\delta$ 138.7 ppm) overlaps with lignin phenolic adducts. Potentially stable triarylphosphites may be used as internal standards. One triarylphosphite studied was hindered tris(2,4-dl-tert-butylphenyl)phosphite (VII, Figure 5).
The $^{31}$P-NMR chemical shift ($\delta$ 130.7 ppm) was found to occur in a region where occasionally TMDP derivatization products are observed ($\delta$ ~130 ppm, Figure 2).

![Figure 3. Commonly used internal standards for $^{31}$P-NMR/TMDP analysis.]

<table>
<thead>
<tr>
<th>#</th>
<th>Compound</th>
<th>$\delta^{31}$P (ppm)</th>
<th>$T_1$ (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>cyclohexanol</td>
<td>145.1 $^a$</td>
<td>4.6 $^a$</td>
</tr>
<tr>
<td>II</td>
<td>cholesterol</td>
<td>144.9 $^b$</td>
<td>&lt;5 $^c$</td>
</tr>
<tr>
<td>III</td>
<td>N-hydroxy-phthalimide</td>
<td>150.7 nd</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>1-hydroxy-7-azabenzotriazole</td>
<td>150.6 1.9</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>N-hydroxy-5-norborene-2,3-dicarboximide</td>
<td>151.9 1.3</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>N-hydroxy-1,8-naphthalimide</td>
<td>153.6 1.5</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>tris(2,4-di-tert-butylphenyl) phosphite</td>
<td>130.7 0.5</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>Piperidine</td>
<td>138.7 nd</td>
<td></td>
</tr>
</tbody>
</table>

nd = not determined  
$^a$ ref. (Granata and Argyropoulos 1995)  
$^b$ ref. (Smit, Suckling et al. 1997)  
$^c$ ref. (Suiit, Suckling et al. 1997), not stated explicitly.

The most promising candidates for new internal standards are the N-hydroxy compounds (III–VI, Table 1). After TMDP derivatization, these compounds were found to give phosphate products with $^{31}$P-NMR chemical shifts in the $\delta$ 150.7–153.6 ppm region.

For a preliminary investigation, N-hydroxy-5-norborene-2,3-dicarboximide (V, Table 1, Figure 4) was selected as a potential new internal standard. After TMDP derivatization, the N-hydroxy internal standard was found to be nearly baseline resolved from the lignin-derived resonances (Figure 1).

![Figure 4. N-hydroxy compounds.]

A reproducibility study was performed on a softwood kraft (brownstock, kappa = 47) residual lignin isolated by acidolysis (Pepper, Daylis et al. 1959). Integration regions used were consistent with the literature (Granata and Argyropoulos 1995; Jiang and Argyropoulos 1995; Jiang, Argyropoulos et al. 1995; Smit, Suckling et al. 1997; Crestini, Sermanni et al. 1998). Integration measurement results for residual lignin samples treated in triplicate are given in Table 2 and 3. Both cyclohexanol and N-hydroxy-5-norborene-2,3-dicarboximide were used as internal standards.

Referring to Figure 1, the reader will notice that cyclohexanol is not baseline resolved from lignin-derived resonances. Therefore, for the residual lignin samples studied, when using cyclohexanol as an internal standard, the baseline was "locally" corrected about the cyclohexanol peak inorder to establish the proper integration areas. Nevertheless, the OH functional group reproducibility of measured lignin functional groups was very good when either cyclohexanol or N-hydroxy-5-norborene-2,3-dicarboximide was used as the internal standard.
Table 2. Functional group values for a softwood kraft (kappa = 47) residual lignin treated in triplicate using cyclohexanol as an internal standard.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Average</th>
<th>Std. Dev.</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliphatic OH</td>
<td>2.81</td>
<td>0.052</td>
<td>0.30</td>
</tr>
<tr>
<td>Phenolic</td>
<td>3.53</td>
<td>0.044</td>
<td>0.25</td>
</tr>
<tr>
<td>Acid</td>
<td>0.49</td>
<td>0.010</td>
<td>0.06</td>
</tr>
</tbody>
</table>

All values in mmol/g lignin
LSD = least significant digit

Table 3. Functional group values for a softwood kraft (kappa = 47) residual lignin treated in triplicate using N-hydroxy-5-norborene-2,3-dicarboximide as an internal standard.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Average</th>
<th>Std. Dev.</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliphatic OH</td>
<td>2.55</td>
<td>0.04</td>
<td>0.24</td>
</tr>
<tr>
<td>Phenolic</td>
<td>3.20</td>
<td>0.04</td>
<td>0.26</td>
</tr>
<tr>
<td>Acid</td>
<td>0.44</td>
<td>0.002</td>
<td>0.01</td>
</tr>
</tbody>
</table>

All values in mmol/g lignin
LSD = least significant digit

Previous reports have defined the T1 relaxation time range of TMDP phosphitylated lignin as 0.5-2.0 seconds (Granata and Argyropoulos 1995). Phosphites derived from TMDP/N-hydroxy compounds were found to display shorter T1 relaxation times relative to TMDP/cyclohexanol-phosphite (Table 1). Therefore, a shorter pulse delay may potentially be selected if an N-hydroxy internal standard is used instead of cyclohexanol. A shorter pulse delay will allow for the more rapid analysis of TMDP phosphitylated lignin. Further work is in progress determining the generality of N-hydroxy internal standards for routine lignin analysis.

Summary
A series of commercially available N-hydroxy compounds were studied by 31P-NMR spectroscopy after TMDP derivatization. The 31P-NMR chemical shifts of phosphitylated N-hydroxy compounds (δ 150.7-153.6 ppm) were found to be well separated from lignin derived components. These compounds show promise as new internal standard for the 31P-NMR analysis of lignin hydroxyl groups.

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References


