



*Institute of Paper Science and Technology
Atlanta, Georgia*

IPST Technical Paper Series Number 811

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

M. Zawadzki and A. Ragauskas

July 1999

Submitted to
Holzforschung

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N-HYDROXY COMPOUNDS AS NEW INTERNAL STANDARDS FOR THE ^{31}P -NMR DETERMINATION OF LIGNIN HYDROXYL FUNCTIONAL GROUPS.

By Michael Zawadzki and Arthur Ragauskas
Institute of Paper Science and Technology
500 10th St., N.W., Atlanta, GA 30318.

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).

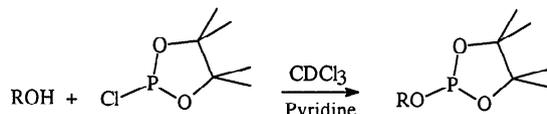


Figure 1. Phosphitylation of a hydroxyl group by TMDP.

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).

Clearly, an internal standard that is baseline resolved would be useful when investigating lignin samples displaying disperse aliphatic- and phenolic-phosphite regions. TMDP is known to react with a variety of heteroatom functional groups containing labile hydrogen; for example, OH, CO₂H, NH, and SH (Wroblewski, Lensink *et al.* 1988). Because of the range of TMDP reactivity, we have investigated the phosphite reaction products arising from a variety of substrates. In this paper, we propose that N-hydroxy compounds may be useful new internal standards for TMDP/ ^{31}P -NMR analysis of isolated lignins.

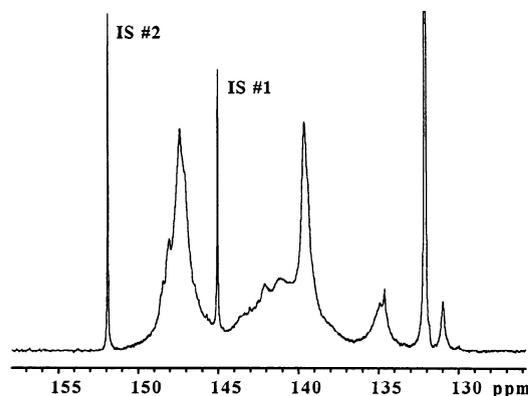


Figure 2. ^{31}P -NMR spectrum of residual lignin (brownstock, kappa = 47) containing cyclohexanol (IS #1) and N-hydroxy-5-norborene-2,3-dicarboximide (IS #2) derivatized with TMDP.

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.

Piperidine (VIII, Figure 5) was studied, and rejected, as a potential internal standard because the chemical shift of its TMDP product (δ 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-di-*tert*-butylphenyl)phosphite (VII, Figure 5).

The ^{31}P -NMR chemical shift (δ 130.7 ppm) was found to occur in a region where occasionally TMDP derivatization products are observed (δ ~130 ppm, Figure 2).

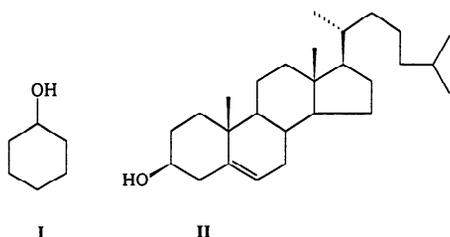


Figure 3. Commonly used internal standards for ^{31}P -NMR/TMDP analysis.

Table 1. Phosphorous-NMR parameters for TMDP treated compounds.

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

nd = not determined

^a ref. (Granata and Argyropoulos 1995)

^b ref. (Smit, Suckling *et al.* 1997)

^c ref. (Smit, 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 phosphite products with ^{31}P -NMR chemical shifts in the δ 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).

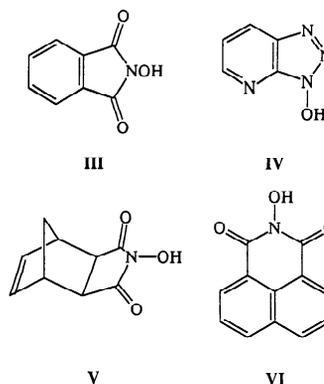


Figure 4. N-hydroxy compounds.

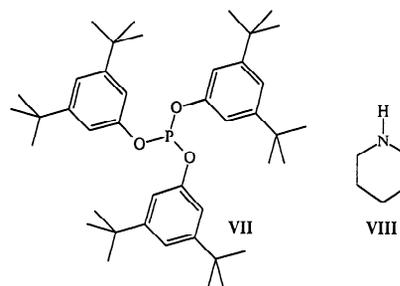


Figure 5. Additional compounds.

A reproducibility study was performed on a softwood kraft (brownstock, kappa = 47) residual lignin isolated by acidolysis (Pepper, Baylis *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 in order 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.

| Structure | Average | Std. Dev. | LSD |
|--------------|---------|-----------|------|
| Aliphatic OH | 2.81 | 0.052 | 0.30 |
| Phenolic | 3.53 | 0.044 | 0.25 |
| Acid | 0.49 | 0.010 | 0.06 |

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.

| Structure | Average | Std. Dev. | LSD |
|--------------|---------|-----------|------|
| Aliphatic OH | 2.55 | 0.04 | 0.24 |
| Phenolic | 3.20 | 0.04 | 0.26 |
| Acid | 0.44 | 0.002 | 0.01 |

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

Previous reports have defined the T_1 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 T_1 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 ^{31}P -NMR spectroscopy after TMDP derivatization. The ^{31}P -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 ^{31}P -NMR analysis of lignin hydroxyl groups.

Acknowledgements

The authors wish to thank Drs. McDonough, Dimmel, and Lucia for guidance. Financial support from the Institute of Paper Science and Technology (IPST) and its member companies is gratefully acknowledged. Portions of this work

were used by M. Z. as partial fulfillment of the requirements for the Ph.D. degree at IPST.

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