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Investigating the Stereochemistry of α-Carbon in Lignin Preparations and Lignin Model Compounds Using ^{77}Se NMR

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Studying the stereochemistry of lignin as a natural chiral polymer may have scientific and technical importance regarding the behavior of lignin and delignification processes. In the present study, the hydroxyl groups of α-carbon (chiral center) in phenyl propane structural units of lignins and two model compounds were selenated. Then, the ^{77}Se nuclear magnetic resonance (NMR) spectra of the selenated samples were examined in order to determine the ratio of diastereomers (erythro and threo). The results revealed that lignins are a mixture of different ratios of two diastereomers. This finding may have scientific importance and practical impacts on the chemistry of delignification processes and other related phenomena in the domain of pulp and paper sciences.

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INTRODUCTION

Nowadays, various techniques are being used in the structural studies of different lignin preparations and lignin model compounds, regarding the origin of lignins and the methods used for their separation. Among them, it seems that nuclear magnetic resonance (NMR) spectroscopy techniques are among the most efficient, precise, and accountable. Many successful studies have been conducted in lignin chemistry by using ^{1}H NMR (Lundquist 1992), ^{13}C NMR, and ^{31}P NMR (Ahvazi 1998) techniques, and a great deal of valuable information has been acquired about the detailed structural features of lignins. From these studies, many questions about the phenyl propane structure of lignins, methoxyl groups (contents and locations), and hydroxyl (phenolic and alcoholic) groups have been examined and answered (Ämmälahti 1999).

However, ^{13}C NMR spectroscopy has been found to be an especially useful technique for counting and determining the mean value of the number of carbon atoms in the structure of lignins as polymers. From these observations, certain conclusions may be drawn about the formula and structure in laboratory-prepared and technical lignins. Such information have been quite helpful in elucidating and explaining many scientific and technical observations, such as the mechanism of delignification, bleaching processes, yellowing of papers, *etc*.

A number of questions about the stereochemistry of α- and β- carbons in the phenyl propane skeleton repeating units of lignin polymers are worthwhile topics for research. In fact, α- and β-carbons in the structural units of lignin polymers or lignin model compounds are chiral centers; therefore, it is expected that lignins are a mixture of diastereomers (erythro or threo) (Fig. 1).

This question may be quite important in studying the biochemical synthesis pathway of lignins in different plant species (woods and non-woods) and most probably a factor which affects the mechanism and kinetics of chemical delignification.
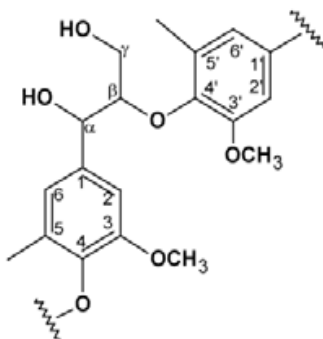

Fig. 1. α and β- Carbons are chiral centers in phenyl propane structural units of lignins

In this study, efforts were made to use a selective selenation at the α-carbon of different lignins and in two suitable lignin model compounds in order to conduct some ^{77}Se NMR experiments and therefore evaluate the stereochemistry of α-carbon atoms and the ratio of diastereomers. The experiments demonstrated that, fortunately, the method is responsive towards the objectives and goals explained above.

EXPERIMENTAL

Materials

Poplar hardwood (*Populus nigra*) was obtained from the city of Tehran. Depithed bagasse (Gramineae: *Saccharum officinarum* L.) was received from Pars Pulp and Paper Company (Shush, Khuzestan Province, Iran); and Cypress softwood (*Cupressus sempervirens*) was received from northern Iran (Noshahr, Mazandaran Province, Iran).

Chemicals were purchased from Merck Co., Germany, and Sigma-Aldrich Co., USA, and used as received. The NMR spectras were obtained using a 400 MHz NMR spectrometer manufactured by Bruker, Germany.

Methods
Preparation of dioxane lignins

The samples (poplar, cypress, and bagasse chips*)* were ground (using a Wiley Mill), sieved at 140 μm, and extracted with a Soxhlet apparatus using water, ethanol, and benzene, respectively. The extractive-free flours were oven-dried at 100 °C overnight. Dioxane lignins were prepared according to a modified method reported by Partovi *et al.* (2012). ^{13}C NMR data of acetylated lignins are shown in Table 1 (Oghbaie *et al.* 2014)

Synthesis of 4-hydroxy-α-methyl benzyl alcohol (Model compound 1)

2.5 mmol of KBH_4 was added to the 4-hydroxyacetophenone (2.5 mmol) in 5 mL of ethanol. After 10 min, the reduction was interrupted by adding 5 mL of water. The mixture was heated for 5 min. Then, 10 mL of additional water was added until the solution turned turbid. The turbid solution was left overnight for the product to be crystallized. After filtering, the reaction product was washed with diethyl ether three

A solution of 2.5 mmol of dioxane lignins (poplar, cypress, and bagasse) in 60 mL of chloroform was prepared. Then, bromotrimethylsilane (0.5 mL) was added under nitrogen atmosphere while stirring. After 20 min, the solution was poured into a separatory funnel and treated with a saturated solution of sodium bicarbonate. Then the non-aquaes (chloroform) layer was separated and dried over magnesium sulfate and the filterated solution was immediately used in the next step.

A solution of 2.5 mmol of benzeneselenol in 10 mL of THF was added to the freshly prepared solution of lignin quinonemethide, and 5 drops of tetrabuthyl ammonium hydroxide was added. Then, the mixture was stirred vigorously under nitrogen atmosphere for 20 min. Then, solution was taken up into the flask of an evaporator and the solvents were distilled off, leaving an oily brown residue. The yield of selenation of lignins was about 90%.

The selenated lignins was taken for NMR spectroscopy. The results of ^{77}Se NMR of selenated dioxane lignins are shown in Table 3.

Table 3. ^{77}Se NMR of Model Compounds 1, 2, and Dioxane Lignins

Samples	Chemical Shift (ppm)	Assignment
Model compound 1	439.2 and 441.03	2s, ^{77}Se of erythro and threo isomers
Model compound 2	436.9 and 440.6	2s, ^{77}Se of erythro and threo isomers
DL, bagasse	459.9 and 469.2	2s, ^{77}Se of erythro and threo isomers
DL, *P. nigra*	459.9 and 469.9	2s, ^{77}Se of erythro and threo isomers
DL, *C. sempervirens*	460.1 and 469.9	2s, ^{77}Se of erythro and threo isomers

RESULTS AND DISCUSSION

In the present study, 4-hydroxy-α-methylbenzyl alcohol and 1-(3-methoxy-4-hydroxyphenyl)-2-(2-methoxyphenoxy)-3-propanol served as lignin model compounds, and dioxane lignins of three plant species (poplar, cypress, and bagasse) were employed as representatives of hardwoods, softwoods, and non-wood lignocellulosic materials; the samples were prepared and converted into α-seleno derivatives. The ^{77}Se NMR of the model compounds and lignin samples revealed the following results:

Lignin model compounds and lignin preparations are a mixture of stereoisomers with different ratios, initiating from the chirality of α- carbon of model 1 and α and β-carbons in phenyl propane skeletons in model 2 and the lignins. (Table 4).

Table 4. Ratio of Diastereomers Initiating from the Stereochemistry of α, β-Carbons

Samples	Ratio of stereoisomers
Model compound 1	68:32%
Model compound 2	51:49%
DL, bagasse	52.6:47.4%
DL, Poplar	96.6:3.4%
DL, Cypress	62.7:37.3%

These observations indicate that the biochemical pathways in biosynthesis of lignins and chemical procedures used to synthesize model compounds 1 and 2 are not stereospecific and they are a mixture of stereoisomers (mixture of enantiomers in model 1 and mixture of diastereomers in model compound 2 and lignins).

The questions still to be considered now include: (1) Does this stereochemistry affect the mechanisms and kinetics of delignification processes of different plant species? (2) What may be the probable behavior of pure diastereomers of lignins regarding technically important phenomena such as delignification, bleaching, brightness, reversion, *etc.*? Additional questions may be raised, and their answers and further analysis of the related phenomena may have scientific or technical importance.

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