FORAGE LIGNINS: ISOLATION, CHARACTERIZATION AND DEGRADATION IN THE GASTROINTESTINAL TRACT OF RUMINANTS

QUIROZ, ROBERTO ABDIEL

DEGREE DATE: 1987

INFORMATION TO USERS

While the most advanced technology has been used to photograph and reproduce this manuscript, the quality of the reproduction is heavily dependent upon the quality of the material submitted. For example:

- Manuscript pages may have indistinct print. In such cases, the best available copy has been filmed.
- Manuscripts may not always be complete. In such cases, a note will indicate that it is not possible to obtain missing pages.
- Copyrighted material may have been removed from the manuscript. In such cases, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, and charts) are photographed by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each oversize page is also filmed as one exposure and is available, for an additional charge, as a standard 35mm slide or as a 17"x 23" black and white photographic print.

Most photographs reproduce acceptably on positive microfilm or microfiche but lack the clarity on xerographic copies made from the microfilm. For an additional charge, 35mm slides of 6"x 9" black and white photographic prints are available for any photographs or illustrations that cannot be reproduced satisfactorily by xerography.

Forage Lignins: Isolation, Characterization and Degradation in the Gastrointestinal Tract of Ruminants

by

Roberto Abdiel Quiroz

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Department of Animal Science

Raleigh

1 9 8 6

Approved by:

Roger H. Nickenberger

Joseph C. Brown
Co-Chairman of Advisory Committee

R. W. Harvey

Kevin R. Pond
Co-Chairman of Advisory Committee

ABSTRACT

QUIROZ, ROBERTO ABDIEL. Forage Lignins: Isolation, Characterization and Degradation in the Gastrointestinal Tract of Ruminants. (Under the direction of Joseph C. Burns and Kevin R. Pond.)

A series of experiments were conducted to investigate the structural composition of forage lignins, structural changes of lignins in the gastrointestinal tract, alkali delignification kinetics and the use of core lignin components as an internal marker. Three species were selected to represent different forage types; alfalfa (Medicago sativa L.), a temperate perennial legume, tall fescue (Festuca arundinacea Schreb.), a temperate perennial grass and coastal bermudagrass (Cynodon dactylon L. Pers.), a tropical perennial grass. Alkaline lignins soluble in 1,4-dioxane, from forage and feces, were isolated and characterized using ^{13}C nuclear magnetic resonance spectroscopy and nitrobenzene oxidation. Core lignins from all three forages were guaiacyl-syringyl-p-hydroxyphenyl type. Guaiacyl was the predominant monomer unit constituting between 55-60% of the uncondensed lignin in the three forages. The proportions of uncondensed syringyl- and p-hydroxyphenylpropane units varied among forages. Hemicellulose, mainly xylans, seemed to be linked to lignin through alkali resistant linkages, presumably ether and(or)

carbon-carbon types. This carbohydrate moiety of the lignin-carbohydrate complex was extensively degraded in the gastrointestinal tract. The aromatic ring of the arylpropanoid units was not altered during the digestion process, whereas the γ-carbon atom of the aliphatic side chain appeared to be susceptible to degradation.

Delignification of forages with strong alkali and high temperature conditions followed first order kinetic with respect to lignin concentration. The delignification rate for tall fescue was faster than for coastal bermudagrass, which in turn was faster than for alfalfa. The extraction of lignin resulted in a linear increase in in vitro dry matter disappearance (IVDMD). The increment change in percentage units of IVDMD per percentage unit of lignin extracted was 0.28 and 0.44 for the legume and the grasses, respectively.

Comparison between in vivo dry matter digestibility, voluntary intake, fecal output and these values estimated from vanillin concentration showed that this core lignin component might be successfully used as an internal marker.

BIOGRAPHY

Roberto Abdiel Quiroz, son of Máximo Quiroz and Buenaventura Quiroz was born in David, Panamá on March 6, 1957. He received his high school diploma in science from the Instituto Centroamericano Adventista in Alajuela, Costa Rica in November 1974. He attended the Universidad de Panamá beginning in January 1975. In May 1979 he received the degree of Licenciado en Quimica. The author has served as chemist in the Instituto de Investigación Agropecuaria de Panamá since September 1979. In May 1982 the author moved to Raleigh, North Carolina to begin his graduate studies at North Carolina State University. He received his Master of Science degree with a major in Nutrition and a minor in Crop Science in 1984.

The author is married to Aracelly Itzel and they have a 5 year old son, Esvan Roberto.

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to Dr. Joseph C. Burns and Dr. Kevin R. Pond for their advice and guidance. He also thanks Dr. R. Harvey and Dr. R. Crickenberger for serving on his committee.

The author also gives special thanks to Dr. Chen-Loung Chen for providing his expertise in lignin chemistry, which made this study possible.

Sincere appreciation is expressed to the Instituto de Investigación Agropecuaria de Panamá and the International Development Research Center for their financial support.

The author also gives thanks to E. Leonard, D. Hudnall, D. Pezo and T. Goodwin for their assistance in the laboratory.

The author expresses his deepest thanks and love to his wife, Aracelly, and his son, Esvan, for their love, support and understanding. He also expresses his love to his parents, brothers and sisters for their encouragement.

The author also expresses his gratitude to the Lord for all the blessings received.

TABLE OF CONTENTS

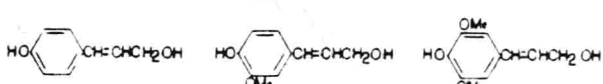
Chapter 1

Literature Review

1.1 Lignin definition and nomenclature

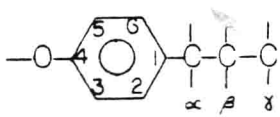
The word "lignin" is derived from the latin word "lignum", meaning wood and, indeed, lignins form an essential component of the woody stems of arborescent plants. However, most vascular terrestrial plants also contain at least some lignins (Harkin, 1973). As cell wall constituents, lignins not only act as encrusting materials but also perform multiple functions essential to the life of the plant. First, lignins play an important role in the intricate internal transport of water, nutrients and metabolites by decreasing the permeation of water across the cell walls in the conducting xylem tissues. Secondly, lignins impart rigidity to the cell walls and finally, lignified tissues effectively resist attacks by microorganisms by impeding penetration of destructive enzymes into the cell wall (Sarkanen and Ludwig, 1971).

Lignins are defined as polymeric natural products arising from an enzyme-initiated dehydrogenative polymerization of three primary precursors: p-coumaryl (1), coniferyl (2), and sinapyl (3) alcohols (Higuchi, 1980).



1 2 3

This definition pertains to what is called the "lignin core." Lignins, as they exist in the cell wall, are always associated with hemicelluloses, not only in intimate physical admixture but also anchored to the hemicellulose by covalent bonds. Further, most lignins contain varying amounts of certain aromatic carboxylic acids in ester-like combinations. These acids are probably not generated from the three primary precursors in the dehydrogenative polymerization process (Sarkanen and Ludwig, 1971) but probably arise from deaminated aromatic amino acids. The notation commonly accepted for the carbon atoms in the basic phenylpropane (C_6C_3-) unit is shown below:



The major broad classification of lignins include: a) gymnosperm or softwood lignins, b) angiosperm or hardwood lignins and c) grass lignins (Sarkanen and Hergert, 1971). These three types of lignins are best differentiated on the basis of nitrobenzene oxidation products. Gibbs (cited by Sarkanen and Hergert, 1971) introduced the division of lignins into two major classes, namely "guaiacyl lignins" and "guaiacyl-syringyl lignins." Guaiacyl lignins include those present in the majority of gymnosperms, while all

angiosperm lignins, including grasses, belong to guaiacyl-syringyl lignins. For convenience and based on the high concentration of p-hydroxyphenylpropane units in the core lignin of grasses, we will classify forage lignins as guaiacyl-syringyl-p-hydroxyphenyl lignins. The abbreviation system for the structures present in lignins (Sarkanen and Ludwig, 1971), is G, S and H representing the aromatic groups of guaiacyl, syringyl and p-hydroxyphenyl units, respectively, and will be used hereafter.

1.2 Lignin biosynthesis

Tracer studies have established that lignin is formed exclusively via the shikimic acid pathway (Freudenberg and Neish, 1968; Sarkanen, 1971; Higuchi, 1980; Goodwin and Mercer, 1983). Thus, lignin shares the same biosynthetic pathways with essential amino acids such as phenylalanine (Phe) and tyrosine (Tyr) (figure 1.1). L-Phe, which is widely distributed in plants as an essential amino acid, is converted to trans-cinnamic acid in a reaction catalyzed by phenylalanine ammonia lyase (PAL). It has been found that PAL is the key enzyme in the initiation of phenolic metabolism in plant cells and that the enzyme is synthesized de novo during xylem differentiation (Higuchi, 1980). L-Tyr, another aromatic amino acid, can be converted to trans-p-coumaric acid only by grasses, which characteristically contain tyrosine ammonia lyase (TAL), in addition to PAL

(Goodwin and Mercer, 1983). Accordingly, gymnosperms and angiosperms can synthesize lignin from L-Phe only, with the exception that grasses can do so from both L-Phe and L-Tyr. Cinnamic acid thus formed is hydroxylated to p-coumaric and caffeic acids successively by specific hydroxylases. The conversion of caffeic acid to ferulic acid is catalyzed by O-methyltransferase (OMT), which is widely distributed in higher plants (Higuchi, 1981). Ferulic acid thus formed may be hydroxylated to 5-hydroxyferulic acid which is methylated again to sinapic acid. 5-hydroxy-cinnamic acid is presumed, on the basis of tracer experiments, to be the intermediate between ferulic and sinapic acids. The compound, however, has not been found in nature (Higuchi, 1980). P-coumaric, ferulic and sinapic acids are reduced to the corresponding cinnamyl alcohols by the successive mediation of three enzymes: hydroxycinnamate Coenzyme A (C_oA) ligase, hydroxycinnamyl C_oA reductase and hydroxycinnamyl alcohol reductase. P-coumaryl, coniferyl and sinapyl alcohols are the building blocks of lignins (Adler, 1977; Higuchi, 1980).

The enzymatic dehydrogenation by the action of peroxidase (Harkin and Obst, 1973) is a one-electron transfer resulting in the formation of a resonance-stabilized phenoxy radical (figure 1.2). Stabilization occurs by the coupling of one radical to another in any of

the positions of the unpaired electron shown in resonance structures a through d (Adler, 1977).

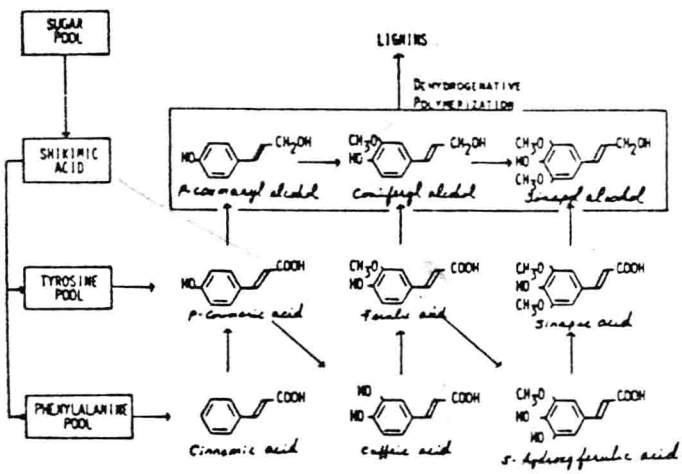


Figure 1.1. Biosynthetic pathway for primary lignin precursors.

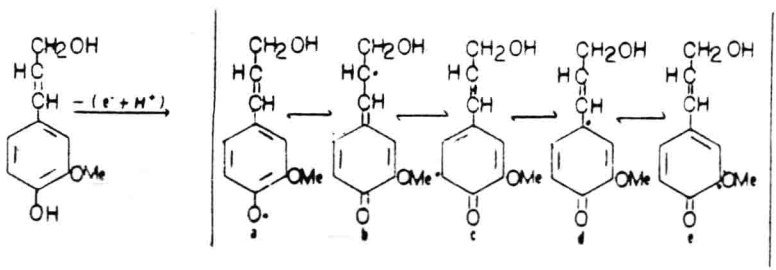


Figure 1.2. Dehydrogenation of coniferyl alcohol.

The formation of a lignin molecule will begin with a dimerization of the radical to give dilignols (figure 1.3). The continued growth of the molecule will take place primarily by what has been called "end-wise" polymerization (Sarkanen, 1971). In the lignifying cell there will be a low stationary concentration of the monomer, for instance, coniferyl alcohol. Therefore, dimerization of the monomer radicals will be less favored than their cross-coupling with phenoxy radicals formed by dehydrogenation of the phenolic end groups of dilignols or larger polymers (Adler, 1977). The end product is the polymer called lignin (figure 1.4).

The estimated frequencies of the different bond types interconnecting arylpropane units in spruce lignins (table 1.1) shows β-O-4 type to predominate. The corresponding dilignols are shown in figure 1.3.

1.3 Analysis of forage lignins

The common gravimetric analyses for lignins are 72% sulfuric acid (H_2SO_4) and potassium permanganate (KM_nO_4). These methods and a brief discussion on nitrobenzene oxidation and ^{13}C nuclear magnetic resonance (^{13}C NMR) follows.