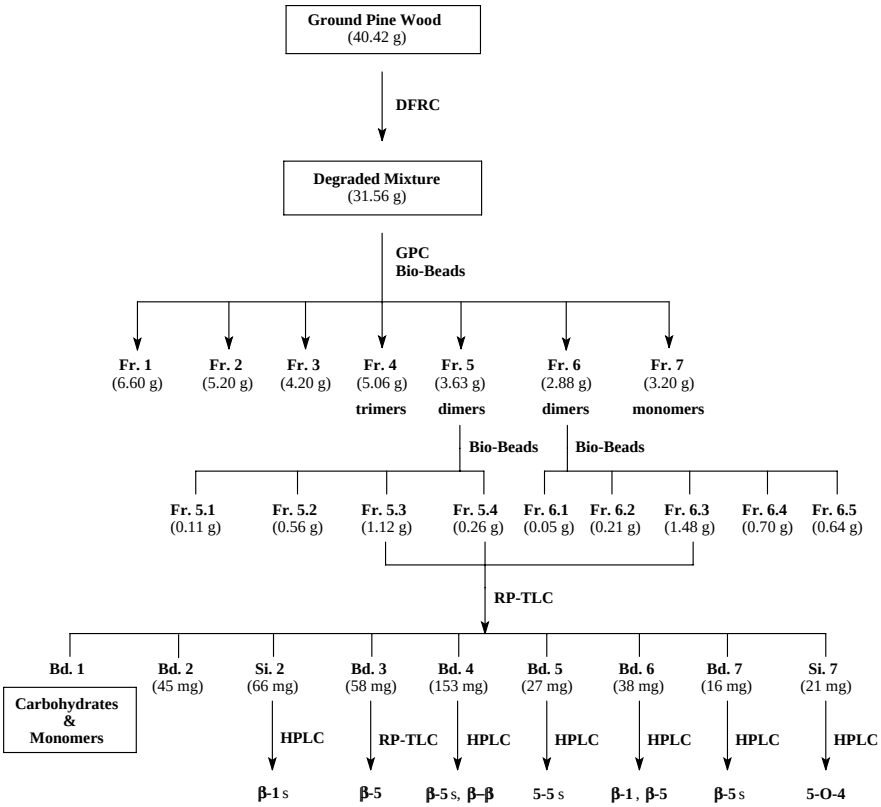

Figure 1. Separation scheme for lignin dimeric products released from DFRC-degraded pine wood.

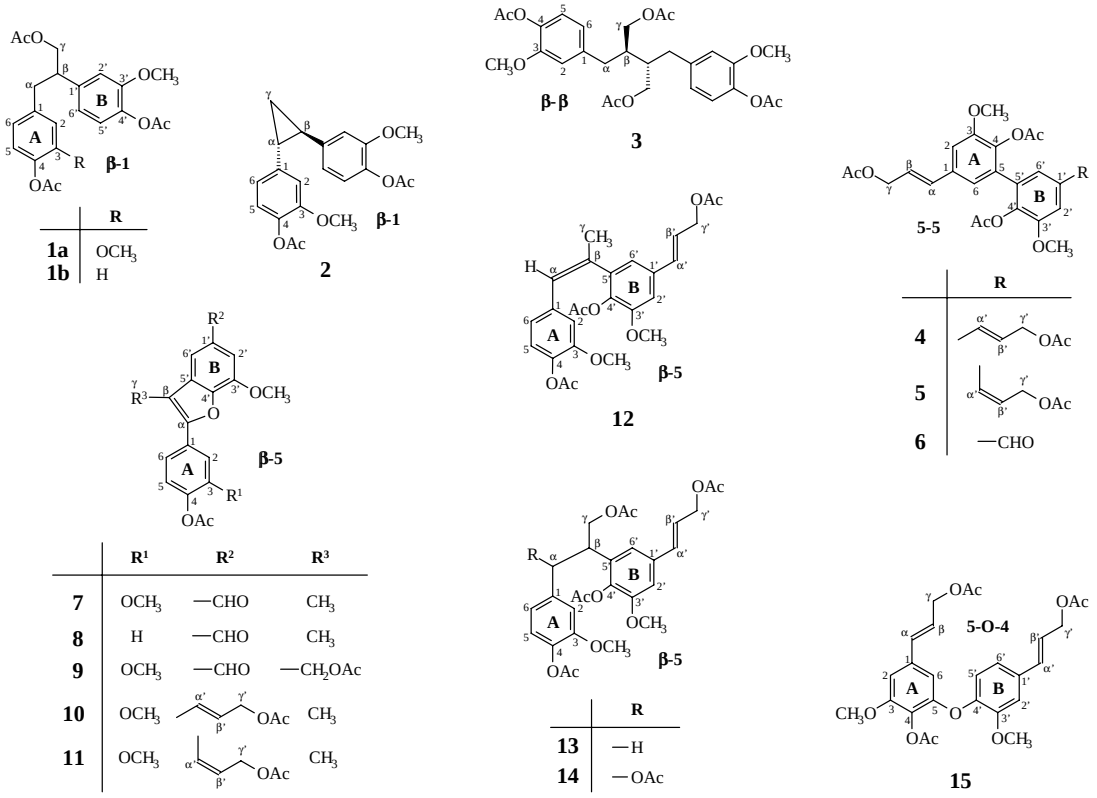

Figure 2. Structures of 16 lignin dimeric products isolated from DFRC-degraded pine wood. All of the common interunit linkages known in lignin are found in the 16 dimers.

wood mixture (about 4% of the lignin) and individual dimers accounted for from 0.01% to 0.1% of the wood.

Although there may be other minor dimers, the structures identified here provide insights into details of the pine lignin structure. Intact sidechains on most dimeric products provide information about the original linkages and where the ether linkages occur; the corresponding dimeric compounds produced by hydrogenolysis or thioacidolysis may lose such information. In the DFRC method, an unsaturated sidechain arises only if that sidechain was originally a b–ether, whereas any endgroup sidechain (–CH=CH–CH_2OH) produces quite different products following DFRC treatment. The 5–5-dimers **4-5** and the 5–O–4 dimer **15** can only come from two b–ether units which have coupled, i.e., from coupling of two pre-formed b–ether dimers or higher oligomers. Products from 5–5-dimerization of coniferyl alcohol could not be found — it is well known that coniferyl alcohol prefers coupling at its b–site, so such products are unlikely.

Aldehydes are well known for the famous phloroglucinol lignin staining reaction. Whether they are present in native lignins or are a product of the isolation procedure has been debated. Aldehydes are not created during the DFRC procedure. Therefore, the aldehydes in dimers **6-9** must have been present in the original lignin or created during the minimal preparation of the wood. We have found that cinnamaldehyde endgroups react reasonably efficiently under the DFRC procedures to give diagnostic products that can be located in small amounts in DFRC product chromatograms of lignins. Benzaldehydes come through the procedure intact or as benzyl acetates. Aldehydes appear in dimers from which they are reasonable products of radical coupling of the pre-formed aldehyde, but they could also arise from post-coupling oxidations. It is well established that vanillin favors coupling at the 5–site; 5,5-dehydrodivanillin can be prepared almost quantitatively from vanillin with peroxidase and hydrogen peroxide. The dimers isolated here contain the vanillin unit coupled at the 5–position. However, the identification of vanillin itself and vanillyl acetate in the monomers implies that it (or its cinnamaldehyde or coniferyl alcohol precursor) can also be 4–O–etherified.

Unusual benzofuran dimers **7-11** are also intriguing. Analogous compounds arise from ozonation reactions. While they clearly arise from b–5 units, the exact mechanism of their formation is not clear.

Currently, there is still some debate on the source of b–1 units; they can scarcely be found in NMR spectra of milled wood lignins, yet can be significant products in solvolysis dimers from the same lignins. The DFRC procedure appears to give b–1 products at similar levels to thioacidolysis.

Conclusion

The DFRC method can play a role in identifying traditional and novel or new lignin constituents. It has recently been invaluable in screening CAD-deficient pine mutants for increased levels of dihydroconiferyl alcohol-derived units. It produces extraordinarily clean products for b–aryl ether units in lignin, cleaving them efficiently, yielding low molecular weight lignin fragments. Other inter-unit structures in lignin are also altered by the DFRC conditions and, regrettably, each linkage type does not give a single product. However, the major, 5–5, b–b, b–1, b–5 and 5–O–4 structures are readily identified in the dimeric fraction, as are several minor b–5 structures with unusual benzofuran skeletons. As with the extended thioacidolysis method, quantification of these released dimers will provide useful insight into lignin structure and structural changes occurring under various chemical and biological treatments. Additionally, the minor products observed here and in the monomer fraction are interesting indicators of minor structural elements in lignins.

Now that dimers have been identified and have been shown to be reasonable products from the chemistry involved, we will optimize conditions for quantitation of major dimers from small samples. A combination of normal and reversed solid-phase extraction followed by GC-FID or GC-MS detection should allow development of a convenient method for dimers in small samples (~30-50 mg wood sample), once response factors are determined. Preliminary trials suggest that the DFRC-degraded products of loblolly pine wood, with the originally overwhelming carbohydrates and monomers removed by solid-phased extraction, give a clean profile with most dimers separated on the GC chromatogram. Hopefully, this emerging method can be utilized for identification, quantification, and comparison of dimers in wide-ranging plant materials.