

Identification of Tannins in *Quercus acutissima* Leaves and Acorns

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ABSTRACT

Hydrolysable and condensed tannins are representatives of a large group of polyphenolic compounds found in plants. There is great speculation as to the importance and function of these compounds in plant-predator relationships. The identification of and characterization of these molecules from plant materials could greatly increase the likelihood of elucidating a role and mechanism of action. Sawtooth Oak (*Quercus acutissima*) is a non-native oak tree found in Missouri. It is native to Korea, China, and Japan and while growing in Missouri it is not subjected to the same predation as native oak trees. This seeming immunity is intriguing and raises speculation to the presence of a biochemical mechanism. Cotyledons and embryo of the *Quercus acutissima* acorn were dried and then pulverized with a mortar and pestle after removing the seed coat. Leaves were dried and homogenized in the extraction solvent. Tannins were extracted from these materials and subjected to LC/ESI/MSn. The tannins were identified based upon the fragmentation patterns and comparison with prior work. Several tannins previously identified in our work on other oak species were found in the Sawtooth oak acorns and leaves, but unique tannins were also discovered in this exotic oak species.

INTRODUCTION

Quercus acutissima, mainly distributed in subtropical regions of Asia, is well known as a rich source of high Mr polyphenols (tannins). Several tannins (both hydrolysable and condensed types) from this plant have been isolated and their chemical structures elucidated. Other work in this area has characterized the small molecular weight tannins in the bark of this oak. Since the tannins serve no physiological purpose for the oak trees, there is considerable speculation as to the role of the tannins in plant defense. Several tannins have been shown to have oxidative properties and even inhibitory actions against DNA polymerases. This inhibitory compound acutissima A (flavono-ellagitannin) is generated from oak-derived vescalagin and grape-derived catechin during the aging of wine in oak barrels. Additionally, the compounds have been implicated as anti-microbial, anti-feedants, and as potential medicinal agents.

SAMPLE PREPARATION FOR MASS SPECTROSCOPY

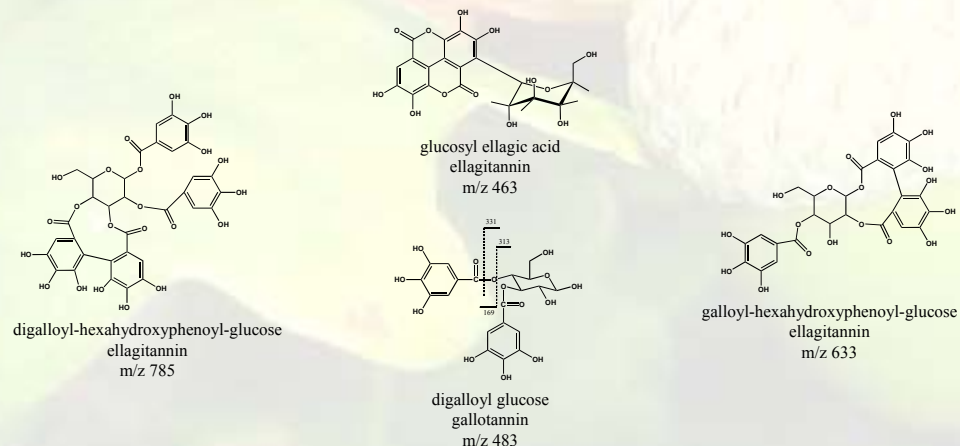
Cotyledons and embryo of *Quercus acutissima* (Sawtooth Oak) were treated with the following method. The cotyledons and embryo were removed from the seed coat and pulverized with a mortar and pestle. One gram of the dry material was added to ten mL of a solution of MeOH/water (80:20 v/v) containing 0.8 mM NaF to prevent sample oxidation. The solution was shaken on a Glas-Col bench top shaker for one hour and allowed to settle. The leaves were treated similarly with the exception that they were homogenized in the solution with a Brinkmann homogenizer or a standard kitchen blender. The supernatant was removed and filtered with a 0.2 µm hydrophilic nylon membrane filter. The filtered extracts were analyzed using LC/ESI/MS AND LC/ESI/MS.

INSTRUMENTATION – HPLC/DAD/ESI-MS/MS Analyses

LC/ESI/MS/MS experiments were performed on an Agilent MSD XCT ion trap mass spectrometer (Palo Alto, CA) equipped with an electrospray ionization (ESI) interface, 1100 HPLC, a DAD detector, and Chemstation software. The column used was a 150 x .5 mm i.d., Zorbax XDB-C18 3.5 µm (Agilent, Palo Alto, CA). Flow rate was 5.00 µL/min, injection volume was 0.5µL, and column temperature was 25 °C. The ESI parameters were as follows: nebulizer, 15 psi; dry gas (N₂), 5.00 L/min; dry temperature, 325 °C; trap drive, 78.0; skim 1, -40 V; lens 1, 5.00 V; octopole RF amplitude, 200.0 Vpp; capillary exit, -200 V. The ion trap mass spectrometer was operated in negative ion mode scanning from m/z 50 to m/z 2200 at a scan resolution of 13000 amu/s. Trap ICC was 70000 units and maximal accumulation time was 200000 µs. MS-MS was operated at a fragmentation amplitude of 1.0 V, and threshold ABS was 20,000 units.

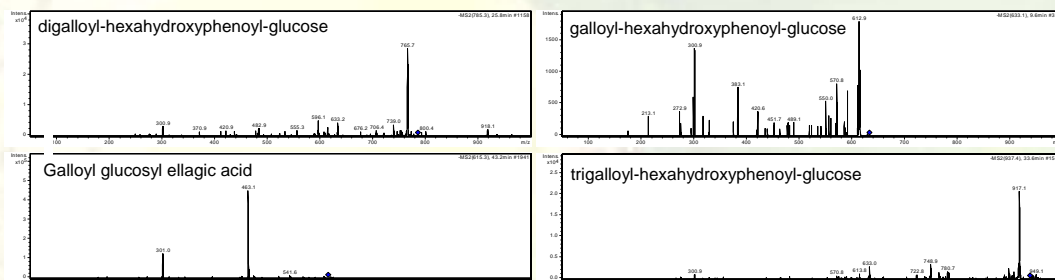
LIQUID CHROMATOGRAPHIC SEPARATION

The constituents were separated using a water (A) and methanol (B) gradient (each containing 0.1% formic acid). Initial conditions were 3% methanol increasing to 25% methanol at 6 minutes increasing to 35% at 25 minutes increasing to 90 % at 35 minutes holding at 90% to 40 minutes and returning to starting conditions at 45 minutes. The detection wavelength was 254nm. This separation method was utilized on both the ESI.



RESULTS

MASS SPECTRAL ANALYSIS



REPRESENTATIVE DATA (ALL TANNINS IDENTIFIED NOT SHOWN)

	RT (min)	Parent (m/z)	Parent Compound	MSMS lon	MSMS lon	MSMS lon	MSMS lon
A	18.0	633.2	galloyl-hexahydroxydiphenoyl-glucose	613.9	300.9		
B	20.8	784.9	digalloyl-hexahydroxyphenoyl-glucose	763.2	633.9	480.9	300.9
C	23.8	633.2	galloyl-hexahydroxydiphenoyl-glucose	613.9	480.8	300.9	
D	25.9	785.4	digalloyl-hexahydroxyphenoyl-glucose	765.5	633.0	482.9	300.9
E	29.9	785.3	digalloyl-hexahydroxyphenoyl-glucose	765.0	596.0	300.9	
F	32.1	467.3	caffeoyl galloyl glucose	416.9	313	169	
G	34.5	937.1	trigalloyl-hexahydroxyphenoyl-glucose	917.1	749.0	632.9	300.9
H	41.5	463.6	Glucosyl-ellagic acid	300.5			
I	43.6	615.2	Galloyl glucosyl ellagic acid	463.0	301.0	254.1	
J	45.0	599.2	Unknown Tannin	592.7	446.9	312.9	168.7
K	45.8	629.4	Unknown Tannin	579.2	462.9	300.9	

CONCLUSIONS

The work done in our laboratory on *Quercus acutissima* (Sawtooth Oak) leaves utilizing the capillary LC/ESI/MS was able to separate approximately ~35 constituents from the methanol:water extract. When this separation was coupled with the ESI mass spectrometer, data was obtained in the negative mode quite successfully. About 30 constituents (11 listed above) in the ESI analysis have been tentatively identified as tannins by comparison to previously published accounts in the literature and also by deduction. The comparison of the mass spectral analysis of the Sawtooth Oak (exotic oak) to the native oak trees (Bur Oak, Pin Oak, Chinkapin, Red Oak) has revealed several similarities as would be expected of trees within the same family. To this point several tannins have been identified possessing the same parent ion mass, but differing daughter ion formation and fragmentation. Collectively, the oak trees are synthesizing similar molecular weight tannins, but it is apparent that within a species and between species these same m/z compounds exist as more than one isomeric form. These compounds possess different retention times in the LC indicating a difference in the attachment of the identified residues to the core structures. Additional research work is being conducted to examine the anti-feedant and insect repellency activities of the tannins from each of the tissue sources and tree species. Preliminary results from bioassays based on the inhibition of meal worm growth and pupation utilizing the crushed and powdered Bur Oak acorns conducted by Dr. Chad Scholes (Rockhurst University Biology Department) have yielded interesting results.

FUTURE WORK

We are currently working on isolating and extracting individual tannins in order to examine their molecular structure. We are also looking to utilize the individual tannins in bioassays to study the predator-prey relationship. We are isolating and examining the predatory insects that feed on the native oaks and that infest the acorns to study the effects of the isolated tannins from the Sawtooth Oak.

REFERENCES

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