UTILIZATION OF CHESTNUT TANNINS BY ENDOTHIA PARASITICA

John Rush Elkins, Zane Lawhorn, and Eric Weyand

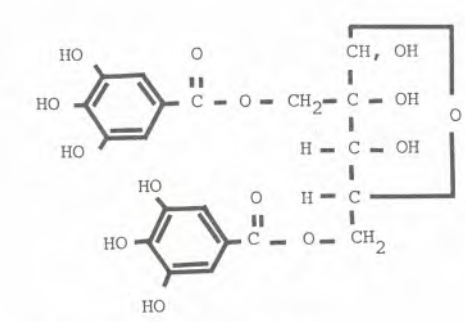
Division of Natural Sciences
Concord College
Athens, West Virginia 24712

ABSTRACT.-- *As an extension of previous work on the utilization of hamamelitannin from the bark of blight-susceptible American and European chestnuts by Endothia parasitica, four other tannins were purified by semi-preparative high performance liquid chromatography and included, at a concentration comparable to that in the bark, as the only carbon source in a minimal medium inoculated with spores of E. parasitica. Two of the tannins, castalagin and vescalagin, had previously been shown to be present in susceptible and resistant (Chinese and Japanese) chestnuts. Two other unknown, late-eluting, and presumably higher molecular weight tannins from the bark and blight-resistant root bark of American chestnut were collected because of their high concentrations. The later eluting unknown tannin had a retention volume similar to that of a known procyanidin dimer of catechin and gave a colorimetric test for condensed tannins. All of the tannins were completely utilized by E. parasitica within four to ten days. Therefore it is concluded that chestnut tannins do not inhibit growth of E. parasitica but instead apparently support growth.*

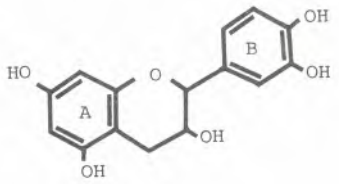
The utilization of tannins from the extracts of American chestnut *Castanea dentata* by the blight fungus *Endothia parasitica* was first observed by Cook and Wilson (1916). We have extended that work by first modifying the standard hide powder method for the analysis of tannins (Elkins and Wright 1977). The hide powder method is time consuming and requires large quantities of extracts to provide enough tannin to be weighed by difference upon adsorption onto hide powder (collagen). Our modification depends on the reduction of ultraviolet absorption following treatment of the extracts with polyamide (nylon) to remove the tannin. Polyamide worked better than other tannin-precipitating agents--hide powder, gelatin (soluble collagen), polyclar (cross-linked polyvinylpyrrolidone). Using the polyamide precipitation method, we have shown (Elkins, Pate, and Porterfield 1978) that *E. parasitica* utilizes tannins from the American chestnut, European chestnut *C. sativa*, and Chinese chestnut *C. mollisima*. There was greater utilization of tannins and greater mycelial weights of *E. parasitica* with the susceptible American chestnut and lesser utilization and mycelial weights with the resistant Chinese chestnut.

Nienstaedt (1953) and Hebard and Kaufman (1978) demonstrated a correlation between blight resistance and the qualitative differences in chestnut tannins. Extracts from the resistant Chinese chestnut contain only pyrogallol

tannins whereas extracts from the susceptible American and the resistant Japanese *C. crenata* chestnuts contain mixtures of catechol and pyrogallol tannins. Pyrogallol and catechol tannins are best described as hydrolyzable tannins and condensed tannins respectively. Hydrolyzable tannins such as hamamelitannin (1) (2-C-hydroxymethylribose) contain ester links which can be hydrolyzed by acid to simpler compounds (sugars and gallic acid). Their designation as pyrogallol tannins comes from the three adjacent phenolic OH's on gallic acid (as in pyrogallol). Condensed tannins are polymers of catechin (2) which further condense (polymerize) on treatment with acid. Their designation as catechol tannins comes from the two adjacent phenolic OH's on the Bring of catechin (2) (as in catechol).



Hamamelitannin (1)



Catechin (2)

Nienstaedt's characterization of pyrogallol and catechol tannins depended on the reaction of each with formaldehyde and acid (Stiasny test). The activated center on the catechol tannins form gels (insoluble three-dimensional polymers) upon treatment with formaldehyde and acid whereas the deactivated center on pyrogallol tannins do not.

Condensed tannins are probably best characterized by the vanillin-sulfuric acid test (Ribereau-Gayon 1972) which gives a colorimetric reaction based on the interaction of vanillin with the highly activated phloroglucinol nucleus (A-ring) in catechin. We (Elkins and Lewis 1978) have used the vanillin-sulfuric acid test for condensed tannins in conjunction with the polyamide precipitation method for total tannins. The concentration of hydrolyzable tannins is determined by difference.

Our work has concentrated on the isolation of those specific tannin constituents which may either promote or inhibit the growth of *E. parasitica.* The tannin constituents are isolated from the lead tannins prepared by precipitation of the tannins from the extracts with basic lead acetate (Ribereau-Gayon 1972).

We (Elkins and Drumm 1980) have established a correlation between the presence of large quantities of hamamelitannin in the blight susceptible American and European chestnuts and its absence in the blight resistant Chinese and Japanese chestnuts. Two other complex hydrolyzable tannins, castalagin and vescalagin (Mayer 1971), were common to all four chestnut species. We have also shown that hamamelitannin is utilized by *E. parasitica* as a carbon source (Elkins, Graham, and Pate 1980). Thus it appears as if hamamelitannin promotes the growth of *E. parasitica.* Therefore, not only does *E. parasitica*

overcome any inhibitory effects of hamamelitannin, but it compounds the problem by putting the hamamelitannin to its own use.

Our recent work has focused on those high performance liquid chromatographic peaks found in the bark of large, surviving American chestnuts and in blight-resistant American chestnut root bark but absent from the bark of susceptible American chestnuts. Since one such unknown peak had previously been observed (Elkins and Drumm 1980), the chromatogram was expanded in the region of the unknown peak. A semi-preparative column was used to collect enough of each peak to inoculate with *E. parasitica.* The first two peaks were castalagin and vescalagin as determined by their retention volumes and ultraviolet spectra. The third peak was an unknown and the fourth peak was an unknown which gave a positive vanillin-sulfuric acid test for a condensed tannin. The retention volume for the unknown condensed tannin was in the same range as a known dimer of catechin, procyanidin B-2 (Thompson et al. 1972).

Castalagin, vescalagin, and the two unknown peaks were all rapidly utilized by *E. parasitica* (four to ten days) and supported growth of the fungus when they were the only carbon source in a minimal medium. Therefore we are forced to conclude that there is probably not an inhibitor to the growth of *E. parasitica* in the tannin fraction from large, surviving American chestnut trees.

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