# *Poria weirii*-inhibiting and other phenolic compounds in roots of red alder and Douglas-fir

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**Abstract**

*Alnus rubra*, which resists infection by the root pathogen *Poria weirii*, and *Pseudotsuga menziesii*, a highly susceptible species, were compared for root content of phenolic compounds by thin-layer chromatography. The phenolics were detected only in bound forms, probably glycosides and esters. Hydrolysed extracts of *Alnus* roots yielded only two *Poria*-inhibiting compounds: ferulic and syringic acids. Only *Pseudotsuga* extracts contained *p*-hydroxybenzoic acid. Both species yielded vanillic and *p*-coumaric acids. Though these compounds appear to be associated with host resistance or susceptibility, an understanding of specific relationships requires research on the hydrolytic mechanisms of both pathogen and hosts.

## Introduction

Phenolic compounds play a role in resistance of some tree species to wood rotting organisms (Barton, 1962; Anderson, 1964). *Poria weirii* (Murr.) Murr., a destructive root pathogen of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] and other conifers in western North America, is inhibited by several phenolics *in vitro* (Li *et al.*, 1969). Red alder resists infection by *P. weirii* (Wallis and Reynolds, 1965), possibly because of its phenolic content. To evaluate this possibility, we studied the specific compounds in roots of red alder compared with those of the *Poria*-susceptible Douglas-fir.

## Materials and methods

### Extraction procedure

Woody roots were collected from several trees of both species at the Cascade Head Experimental Forest on the Oregon coast. The roots were washed, lyophilized, then ground to pass a 32-mesh screen. Twenty-five g of each sample were exhaustively extracted with 300 ml 95% ethanol in Soxhlet apparatus. The extracts were filtered, the ethanol removed by a flash evaporator operated at 40–50°C, and the residue dissolved in boiling water.

The purified extracts of each sample were divided into three subsamples for (1) a neutral fraction, obtained by neutralizing the extract with concentrated HCl; (2) an acid hydrolysate, obtained by refluxing the extract in 2N HCl on a steam bath for an hour; and (3) an alkaline hydrolysate, obtained by leaving the extract in 2N NaOH overnight at room temperature in a nitrogen atmosphere (Ibrahim and Towers, 1960; Bohm and Towers, 1962).

All fractions were then extracted with ethyl ether in a continuous liquid-liquid extractor for at least 12 hr. In the case of the alkaline hydrolysis, the hydrolysate was first acidified to pH 2 with HCl, ether-extracted, the extract washed with 5% $NaHCO_3$, and the alkaline portion re-acidified to pH 2 and

re-extracted. Final ether extracts were evaporated to a few ml for thin-layer chromatography.

### Qualitative chromatographic analyses

Ascending thin-layer chromatography was used for initial identification of compounds in the extracts. Fifteen g of microcrystalline cellulose (Sigma cell type 19, Sigma Chemical Co., St. Louis, Missouri, U.S.A.) were mixed with 90 ml distilled water and homogenized at high speed in a Waring blender for one min. The suspension was spread over 20 × 20 cm plates with a Desaga applicator set at 250 $\mu$. The plates were dried overnight at room temperature.

The ether extracts were spotted on the plates alongside these reagent-grade authentic compounds: *p*-hydroxybenzoic, vanillic, and ferulic acids (Sigma Chemical Co., St. Louis, Missouri, U.S.A.); *p*-coumaric acid (Mann Research Lab., New York, U.S.A.); and syringic acid (Eastman Organic Chemicals, Rochester, N.Y., U.S.A.). Plates were then developed with benzene:acetic acid:water (82·5:17:0·5), dried, sprayed with diazotized *p*-nitro-aniline and 2N NaOH, and observed under UV light, 254 and 366 m$\mu$.

### Spectral and quantitative analyses

Ether extracts and authentic compounds were streaked on plates prepared and developed as for the qualitative analyses, except that syringic acid did not separate well enough from either ferulic or vanillic acids for quantitative analysis. Excellent separation was obtained by two other methods, however: syringic acid from ferulic, by coating plates with silica gel H and developing with ethyl formate:formic acid:water (50:40:10); and syringic from vanillic, by coating plates with silica gel G and developing with ethyl acetate:isopropyl alcohol:water (65:24:11). Methodology was otherwise identical to that used for the other compounds.

After development, part of each plate was covered with glass so that only the uncovered portions of ascended streaks were exposed to spraying reagents.

**Table 1** Phenolic compounds in hydrolyzed extracts from roots of *Alnus rubra* and *Pseudotsuga menziesii*: qualitative analyses by thin-layer chromatography and confirmed by spectrophotometry; quantitative analyses by optical density and expressed as concentration per unit of lyophilized root weight

| Phenolic acid | hRf* | Colour reaction** | Phenolic concentration in roots from two types of hydrolysis***: *Alnus* ($\mu$g/g): Acid | *Alnus* ($\mu$g/g): Alkaline | *Pseudotsuga* ($\mu$g/g): Acid | *Pseudotsuga* ($\mu$g/g): Alkaline |
|---|---|---|---|---|---|---|
| **Vanillic** | 45·3 | Strong reddish purple | 31 | — | 68 | 13 |
| **Ferulic** | 44·7 | Very pale blue | — | 154 | — | — |
| **Syringic** | 41·2 | Vivid purplish blue | 20 | 61 | — | — |
| ***p*-coumaric** | 20·6 | Light blue | — | 76 | — | 79 |
| ***p*-hydroxybenzoic** | 17·1 | Deep pink | — | — | — | 81 |

* Plates of microcrystalline cellulose developed with benzene : acetic acid : water.

** Sprayed with diazotized *p*-nitro-aniline and 2N NaOH; colour terminology according to the Inter-Society Colour Council (Kelly and Judd, 1955).

*** Lyophilized weight basis of the phenolic ($\mu$g) per g of lyophilized root.

After the ascended streaks were located by spraying and UV examination of the exposed parts, their remaining unsprayed part was marked. It was then removed by a vacuum line connected to the narrow end of a microcolumn fitted with a porous, polythene support (Micro Column Chromatography Unit, Quickfit and Quartz Ltd, Stone, Staffordshire, England). An analogous technique has been described by Millett *et al.* (1964).

The compounds were eluted from the column with 95% ethanol and qualitatively analyzed in a Beckman DB Spectrophotometer in the 240 to 360 mμ range. For quantitative analysis, the eluant was evaporated to dryness and dissolved in 1·0 ml double-distilled $H_2O$ to which 1·0 ml Folin-Denis reagent was then added. After 3 min, 1·0 ml of 1 N $NaCO_3$ was added. Optical density of the mixture was determined one hr later at 725 mμ with a Spectronic 20 equipped with a red-sensitive phototube and an infrared filter. Ferulic acid was used for a standard curve (Feldman and Hanks, 1966).

## Results

No phenolic compounds could be identified from neutral extracts. Acid hydrolysates, however, yielded vanillic acid from both red alder and Douglas-fir (at about half the concentration in the alder as in the fir) and syringic acid in red alder only. Alkaline hydrolysates yielded syringic, ferulic, and *p*-coumaric acids in red alder, and vanillic, *p*-hydroxybenzoic, and *p*-coumaric acids in Douglas-fir (Table 1). All of these compounds belong to the hydroxylated benzoic or cinnamic acid groups.

In all cases, spectrophotometric curves of extracted compounds closely matched those of the authentic compounds.

## Discussion

The absence of phenolic compounds from the neutral extract and their presence in hydrolysates suggests that most exist in a bound form in roots of both tree species. Acid hydrolysable compounds could occur as glycosides and alkaline hydrolysable as esters or alkali-sensitive glycosides (Ballou, 1954; Guenzi and McCalla, 1966).

Ferulic and syringic acids, detected only in the hydrolysates of red alder root extracts, have been shown to inhibit growth of *P. weirii in vitro* (Li *et al.*, 1969). These compounds thus may be associated with resistance of red alder to *Poria* infection. Another inhibitor of *P. weirii* (Li *et al.*, 1969), vanillic acid, was found in both red alder and Douglas-fir, so in itself would appear to have little effect on resistance to the pathogen. Neither *p*-coumaric nor *p*-hydroxybenzoic acids affected growth of *P. weirii in vitro* (Li *et al.*, 1969). However, the bound forms of the compounds in the hosts and the hydrolytic mechanisms of both pathogen and host must be learned before firm conclusions on resistance mechanisms can be reached. Moreover, synergistic and antagonistic relationships between all of the compounds detected could multiply or reduce their individual effects in host-parasite relationships.

Thin-layer chromatography with microcrystalline cellulose and benzene:

acetic acid:water as the developer proved ideal for qualitative analysis of the five phenolic compounds studied. Separation of syringic from ferulic and vanillic acids by this method was inadequate for quantitative analyses, however, so other methods must be substituted for that purpose.


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## References


**Anderson A. B.** 1964. On the chemistry of wood durability and decay. Structure on fungicidal components in some cedars. *In* Symposium on Phytochemistry (1961). Edited by Arthur H. R. pp. 101–16. University of Hong Kong Press.

**Ballou C. E.** 1954. Alkali sensitive glycosides. *Adv. Carbohyd. Chem.* **9** 59–95.

**Barton G. M.** 1962. The phenolics of three western Canadian conifers. *In* Plant phenolics and their industrial significance. Plant Phenolics Group N. Amer. Symp. Proc., pp. 59–80. Corvallis, Ore.

**Bohm B. A.** and **Towers G. H. N.** 1962. A study of phenolic compounds in *Impatiens. Can. J. Bot.* **40** 677–83.

**Feldman A. W.** and **Hanks R. W.** 1966. *Identification and quantification of phenolics in the leaves and roots of healthy exocortis-infected Citrus.* Florida Agr. Exp. Sta. J. Ser. 2423, 11 pp.

**Guenzi W. D.** and **McCalla T. M.** 1966. Phenolic acids in oats, wheats, *Sorghum*, and corn residues and their phytotoxicity. *Agron. J.* **58** 303–4.

**Ibrahim R. K.** and **Towers G. H. N.** 1960. The identification, by chromatography, of plant phenolic acids. *Arch. Biochem. Biophys.* **87** 125–8.

**Kelly K. L.** and **Judd D. B.** 1955. *The ISCC-NBS method of designating colors and a dictionary of color names.* U.S. Dep. Com. Nat. Bur. Stand. Circ. 553, 158 pp.

**Li C. Y., Lu K. C., Nelson E. E., Bollen W. B.** and **Trappe J. M.** 1969. Effect of phenolic and other compounds on growth of *Poria weirii in vitro. Microbios* **1** (3) 305–11.

**Millett M. A., Moore W. E.** and **Saeman J. F.** 1964. Techniques for quantitative thin-layer chromatography. *Analyt. Chem.* **36** 491–94.

**Wallis G. W.** and **Reynolds G.** 1965. The initiation and spread of *Poria weirii* root rot of Douglas-fir. *Can. J. Bot* **43** 1–9.


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