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Conversion of *p*-Coumaric Acid to Caffeic Acid and of *p*-Hydroxyphenylacetic Acid to 3,4-Dihydroxyphenylacetic Acid by *Alnus rubra*

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Sato et al. (1) reported that an enzyme of phenolase type in chloroplasts of some plants converts monohydric phenolic substances to the corresponding *o*-dihydric compounds and plays a role in the biosynthesis of polyphenolic phenols. The present investigation was designed to determine if some phenolic compounds occurring in roots of red alder (*Alnus rubra* Bong.) or Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (2) are similarly transformed in various tissues of these species. *p*-Hydroxyphenylacetic acid was also included in the examination because it is readily derived from tyrosin, which occurs in soils under both conifer and red alder stands.

MATERIALS AND METHODS

EXTRACTION PROCEDURE.—Fresh leaves, roots, and root nodules of *Alnus rubra* were each homogenized in cold acetone (−30°) at high speed in a Waring blendor[1]. The homogenates were filtered; the residues rehomogenized in cold acetone and filtered several times until the filtered acetone solution became colorless. The fine powder thus obtained was air dried under the hood and further dried in a desiccator. Fresh needles and roots of *Pseudotsuga menziesii* were each similarly treated.

INCUBATION AND ISOLATION.—The following procedure was carried out at room temperature (25°) with continuous shaking, using *p*-coumaric, ferulic, *p*-hyroxybenzoic, *p*-hydroxyphenylacetic, syringic, and vanillic acids as substrates. Twenty-five mg substrate and 50 mg acetone-extracted plant tissue powder were added to 50 ml 0.05M potassium phosphate buffer, pH 6.5. Two kinds of controls were used: (1) the reaction mixture that contained substrate only and (2) acetone powder only. After 3 hr, the mixture was filtered to remove the acetone powder. The filtrate was acidified to pH 2.0 with 1.0N HCl and extracted three times with 100 ml ethyl ether. The combined ether extract was brought to dryness by a flash evaporator; the residue was dissolved in a few milliliters of ethyl alcohol.

IDENTIFICATION OF COMPOUNDS.—Each sample was spotted on plates that had been coated with 250 μ of MN-cellulose powder 300 (Machery, Nagel, and Co., Düren, Germany) plus 4% Leuchtstoff ZS-Super, an inorganic fluorescent indicator. The plates were developed with ascending unidimensional technique in the following solvent systems: 10% acetic acid, isopropyl alcohol-NH_4OH-water (8:1:1), 20% KCl, 2% formic acid, benzene-acetic acid-water (4:5:1), and *n*-butanol-acetic acid-water (4:1:2). Following development, the plates were examined under ultraviolet light (254 and 366 mμ). The plates were sprayed with diazotized *p*-nitroaniline followed by 2N NaOH; the unsprayed spots on the plates were eluted by methanol for spectrophotometric determination. Identification of compounds was based on comparison with authentic compounds for hR_F values in different solvent systems, spot colors, and uv absorption spectrum.

RESULTS AND DISCUSSION

For the three *Alnus* tissues, caffeic acid was isolated as an oxidation product of *p*-coumaric acid and 3,4-dihydroxyphenylacetic acid as a product of *p*-hydroxyphenylacetic acid (table 1). Completeness of conversions was not determined. No reaction products could be detected from the mixtures of ferulic, *p*-hydroxybenzoic, syringic, or vanillic acids with any *Alnus* tissue or from any of the phenolic substrates with either *P. menziesii* tissue. The results indicate that leaves, roots, and nodules of *A. rubra* contain a phenolase-type enzyme probably similar to that found by Sato (3) and Sato et al. (1), whereas needles and roots of *P. menziesii* lack such type of enzyme.

p-Coumaric acid stimulates *in vitro* growth of *Phellinus weirii* (Murr.) Gilb., a fungus that rots

[1]Mention of products or companies by name does not constitute an endorsement by the U.S. Department of Agriculture.

TABLE 1. *Comparative hRf values, color reactions, and ultraviolet absorption spectra of oxidation products of phenolic compounds incubated with acetone-extracted leaves, roots, and root nodules of* Alnus rubra.

Compounds	Solvent systems						Color reactions to diazotized *p*-nitroaniline and 2N NaOH[a]	λ max in methanol
	10% acetic acid	isopropyl alcohol NH_4OH–H_2O (8:1:1)	20% KCl	2% formic acid	Benzene-acetic acid-water (4:5:1)	*n*-Butanol-acetic acid-water (4:1:2)		
Substrate 1								
(*p*-hydroxyphenylacetic acid)	64	23	67	64	41	—	Brilliant purplish blue	283, 275
Product 1	62	1	64	62	12	—	Light grayish brown	280
3,4-dihydroxyphenyl-acetic acid	62	1	64	62	12	—	Light grayish brown	280
Substrate 2								
(*p*-coumaric acid)	35	18	27	24	—	79	Light blue	307, 295
Product 2	27	2	16	15	—	68	Light orange-yellow	320, 290, 240
Caffeic acid	27	2	16	15	—	68	Light orange-yellow	320, 290, 240

[a]Color determined from Ridgway (12) and designated as the Intersociety Color Council, National Bureau of Standards synonym (13).

roots of coniferous tree species in western North America but is resisted by *A. rubra* (4). This compound occurs in roots of *A. rubra* and *P. menziesii* and in soil under *A. rubra* (2, 5). Soils from conifer and *Alnus* stands contain tyrosine (J. L. Young, Oregon State University, personal communication). Tyrosine readily decomposes in the uninoculated sterile cultural medium to other compounds, including *p*-hydroxphenylacetic acid, which does not affect growth of *P. weirii*. However, the oxidation products of *p*-coumaric and *p*-hydroxphenylacetic acids in the *A. rubra* mixture, caffeic acid and 3,4-dihydroxyphenylacetic acid, respectively, both inhibit the fungus (6).

The phenolic compounds detected in hydrolysates of *P. menziesii* and *A. rubra* roots could have been glycosides in their original nonhydrolyzed state. The release of phenolics could be from glycosides through enzymatic hydrolysis by glycosidases released by plant cells in response to invasion by a pathogen or produced by the pathogen itself (7, 8). It, therefore, is possible that the phenolic substances in *A. rubra* form a defense mechanism against *P. weirii*. Upon invasion to the roots, *p*-coumaric acid released could be oxidized to caffeic acid by the phenolase enzyme in the host. The roots then could contain four inhibitory compounds, the post-infectional caffeic acid and three other phenolic acids that were previously found in *A. rubra;* ferulic, syringic, and vanillic acids (2). Synergism among the compounds could impede further penetration by *P. weirii*.

Similarly, the soil environment immediate to *A. rubra* roots could contain caffeic and 3,4-dihydroxyphenylacetic acids in addition to *p*-hydroxybenzoic, syringic, and vanillic acids as previously found (5). However, the failure to detect measurable amounts of caffeic and 3,4-dihydroxyphenylacetic acids could have been due to degradation of the compounds by soil microorganisms under *A. rubra*. Soil microorganisms that are able to metabolize these two compounds have been reported (9, 10).

Because *P. menziesii* does not contain an enzyme of the type demonstrated for *A. rubra,* the infection of its roots by *P. weirii* would not activate a host response of transformation of existing phenolic substances in the infecting tissues. In fact, the *p*-coumaric, *p*-hydroxybenzoic, and vanillic acid combination associated with *P. menziesii* roots may even stimulate growth of the pathogen (2, 11).

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