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BIOSYNTHESIS OF *p*-HYDROXYBENZOIC ACID IN POPLAR LIGNIN

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Key Word Index—*Populus nigra*; Salicaceae; poplar lignin; *p*-hydroxybenzoic acid; shikimic acid; phenylalanine; biosynthesis.

Abstract—Biosynthetic pathways to *p*-hydroxybenzoic acid in polar lignin were examined by tracer experiments. High incorporation of radioactivity to the acid was observed when shikimic acid-[1-^{14}C], phenylalanine-[3-^{14}C], *trans*-cinnamic acid-[3-^{14}C], *p*-coumaric acid-[3-^{14}C] and *p*-hydroxybenzoic acid-[COOH-^{14}C] were administered, while incorporation was low from shikimic acid-[COOH-^{14}C], phenylalanine-[1-^{14}C], phenylalanine-[2-^{14}C], tyrosine-[3-^{14}C], benzoic acid-[COOH-^{14}C], sodium acetate-[1-^{14}C] and D-glucose-[U-^{14}C]. Thus *p*-hydroxybenzoic acid in poplar lignin is formed mainly *via* the pathway: shikimic acid → phenylalanine → *trans*-cinnamic acid → *p*-coumaric acid → *p*-hydroxybenzoic acid.

INTRODUCTION

Liberation of *p*-hydroxybenzoic acid by alkaline hydrolysis from poplar wood and from poplar lignin has been reported [1–3], and the acid is thought to be incorporated in the lignin structure mainly by ester or by ether linkages [1–5]. The biogenetic origin of this acid, however, has not yet been elucidated. Biosynthetic pathways to this acid were therefore examined by tracer experiments. A preliminary report of a part of the work has been published [6].

RESULTS AND DISCUSSION

Three pathways have been established for biosynthesis of hydroxybenzoic acids in higher plants and microorganisms [7–10]: (1) the acetate pathway, (2) direct conversion from shikimic acid or closely related compounds retaining the C_6–C_1 carbon skeleton, and (3) formation by removal of a two-carbon fragment from the side-chain of a phenylpropanoid.

After radioactive precursors were administered to a young shoot of poplar, *p*-hydroxybenzoic acid was isolated from the extractive-free xylem by hydrolysis with alkali. The incorporation of radioactivity into the acid from phenylalanine-[3-^{14}C] was very high as compared with those from phenylalanine-[1-^{14}C], phenylalanine-[2-^{14}C], sodium acetate-[1-^{14}C] and D-glucose-[U-^{14}C] (Table 1) and the acetate pathway can thus be excluded. If this acid is formed *via* pathway (2), no significant difference in incorporation of activity between shikimic acid-[1-^{14}C] and shikimic acid-[COOH-^{14}C] should be observed. From the results shown in Table 2, it is clear that shikimic acid administered is converted to *p*-hydroxybenzoic acid mainly *via* prephenic acid which is decarboxylated to produce phenylpyruvic acid or *p*-hydroxyphenylpyruvic acid, causing marked differences in incorporation of activity between two labelled shikimic acids. Pathway (2) is thus excluded and (3) must be the main route.

In grasses and in *Catalpa* [11, 12] *p*-hydroxybenzoic acid can be formed *via* a modification of pathway (3), as follows: prephenic acid → *p*-hydroxyphenylpyruvic acid → tyrosine → *p*-coumaric acid → *p*-hydroxybenzoic acid. The low incorporation of activity from tyrosine-[3-^{14}C] indicates that this modified pathway is not involved in poplar. It has been shown that several plants contain hydroxylating systems converting benzoic acid to *p*-hydroxybenzoic acid [12–14]. In poplar, however, such a hydroxylation of benzoic acid was not observed.

From these results, it can be concluded that *p*-hydroxybenzoic acid in poplar lignin is synthe-

Table 1. Incorporation of radioactivity in *p*-hydroxybenzoic acid in poplar lignin

Compound administered	Specific activity (dpm/mmol) Compound administered $(\times 10^6)$	 *p*-Hydroxybenzoic acid $(\times 10^6)$	Specific incorporation ratio (%) $(\times 10^{-3})$
Phenylalanine-[1-^{14}C]*	869*	0·107	12
Phenylalanine-[2-^{14}C]*	878*	0·058	7
Phenylalanine-[3-^{14}C]*	944*	6·81	722
Sodium acetate-[1-^{14}C]	435	0·016	4
Glucose-[U-^{14}C]	892	0·276	31

* Phenylalanine administered was racemic and specific activity was calculated on effective L-phenylalanine.

Table 2. Incorporation of radioactivity in *p*-hydroxybenzoic acid in poplar lignin

Compound administered	Specific activity (dpm/mmol) Compound administered $(\times 10^6)$	 *p*-Hydroxybenzoic acid $(\times 10^6)$	Specific incorporation ratio (%) $(\times 10^{-3})$
Shikimic acid-[1-^{14}C]	622	0·986	159
Shikimic acid-[COOH-^{14}C]	434	0·083	19
Phenylalanine-[3-^{14}C]*	255*	2·684	658
trans-Cinnamic acid-[3-^{14}C]	447	0·758	170
p-Coumaric acid-[3-^{14}C]	43	0·321	744
p-Hydroxybenzoic acid-[COOH-^{14}C]	425	12·61	2966
Benzoic acid-[COOH-^{14}C]	298	9·233	78
Tyrosine-[3-^{14}C]*	417*	0·126	30

* Amino acid administered was racemic and specific activity was calculated on effective L-stereomer.

sized mainly *via* pathway (3), which is a common route for biosynthesis of C_6–C_1 acids in higher plants [7–15].

EXPERIMENTAL

Plant material and administration of precursors. Fresh young shoots were obtained from a Kamabuchi poplar (*Populus nigra* L. × *Populus maximowiczii* A. Henry) grown on the campus of Nagoya University. Cut shoots (15 × 0·7 cm) were held in small vials containing radioactive soln (about 5 μCi, 2 mg in 0·5 ml) under light at 25°. After the soln was absorbed, addition and absorption of dist. H_2O was repeated and incubated for 24 hr. In the expt shown in Table 2, tracers (0·5–1 mg) were administered to short shoots (5 × 0·7 cm).

Isolation of p*-hydroxybenzoic acid and determination of radioactivity.* The newly formed xylem part was collected and extracted thoroughly with EtOH, EtOH–C_6H_6 (1:2) and hot H_2O successively. Hydrolysis of the extracted wood meal with 1 N NaOH for 1 hr at 100° and subsequent separation yielded *p*-hydroxybenzoic acid as the main component of the acid fraction of the hydrolysate. The acid was purified by recrystallization or preparative TLC, and the radioactivity was determined by the scintillation counting method.

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