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THE BIOGENESIS OF CATECHOL IN *GAULTHERIA*

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Abstract—Tracer studies in *Gaultheria adenothrix* have shown salicylic acid to be the best precursor of catechol.

INTRODUCTION

CATECHOL has been detected only infrequently in higher plants,[1-4] but evidence is accumulating which suggests that it is common in the Salicaceae. A chromatographic survey of unhydrolyzed leaf extracts of seven species of *Populus* showed catechol to be present in all.[5] Three species of *Gaultheria* (Ericaceae) have been shown to accumulate catechol-β-D-glucoside in relatively large quantities.[2] Since catechol is a short-lived intermediate in some aromatic ring-cleavage pathways used by micro-organisms and because nothing is known concerning its metabolism in higher plants we were prompted to examine its biogenesis in *Gaultheria.*

RESULTS AND DISCUSSION

Catechol is known to be a frequent intermediate in microbial phenolic metabolism. It can be formed by hydroxylation of phenol,[6] oxidative decarboxylation of salicylic acid[7] or decarboxylation of 2,3-dihydroxybenzoic acid.[8] The latter can arise from anthranilic acid during tryptophan degradation[8] or from chorismic acid.[9] While decarboxylation of 3,4-dihydroxybenzoic acid is also a possibility, micro-organisms have consistently been shown to oxidize the aromatic ring of this acid instead.[10, 11]

The only reference to catechol formation by higher plants is the isolation of an enzyme system from *Tecoma stans* (Bignoniaceae) which converted anthranilic acid to catechol via *ortho*-aminophenol.[12] No catechol could be detected in extracts of the plant,[13] however, and no *in vivo* studies were carried out to determine if catechol is a normal metabolite in *Tecoma.*

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In *Gaultheria* the best precursor for catechol was found to be salicylic acid (twelve replicates) (Table 1). As both phenylalanine and benzoic acid served as precursors the pathway involved may be cinnamic acid → benzoic acid → salicylic acid.[14,15] The large variations in incorporation of benzoic acid (five replicates) may be an indication that the conversion cinnamic acid → *ortho*-coumaric acid → salicylic acid plays a dominant role at times.[14] The age of the leaf tissue determined the extent of conversion of salicylic acid to catechol, young leaves possessing activity at least ten times that of mature leaves. All reported work was carried out on leaves less than 2 weeks old. A similar effect of age has been noted previously in *in vivo* studies on hydroxylation of benzoic acids[15] and formation of hydroquinone.[16,17]

TABLE 1. THE INCORPORATION OF RADIOACTIVE PRECURSORS INTO CATECHOL IN LEAF DISCS OF *Gaultheria adenothrix* AFTER 24 hr

Compound fed	Activity fed (μc)	Activity taken up (μc)	Specific activity of catechol (μc/mM)	Dilution
Phenylalanine-U-^{14}C, 495 μc/uM	2	1·53	2·06	$2{\cdot}4 \times 10^5$
Benzoic acid-1-^{14}C, 2·54 μc/uM	2	1·80	3·48	$7{\cdot}30 \times 10^2$
Benzoic acid-1-^{14}C, 2·54 μc/uM	2	1·94	0·12	$2{\cdot}1 \times 10^3$
Salicylic acid-*ring*-UL-^{14}C, 0·95 μc/uM	2	1·68	5·88	$1{\cdot}61 \times 10^2$
Salicylic acid-*ring*-UL-^{14}C, 0·95 μc/uM	2 young leaves*	1·96	5·90	$1{\cdot}61 \times 10^2$
	2 mature leaves†	1·83	0·15	$6{\cdot}33 \times 10^3$
2,3-Dihydroxybenzoic acid-^{3}H, 0·27 μc/uM	0·114	0·10	nil	
4-Hydroxybenzoic acid-^{3}H, 0·75 μc/uM	1	0·80	nil	
Tryptophan-benzene-*ring*-UL-^{14}C, 52 mc/mM	4	2·99	nil	
Tryptophan-GL-^{3}H, 2·7 c/mM	50	21	0·14	$1{\cdot}93 \times 10^7$

* Less than 2 weeks old.
† More than 6 weeks old.

The possibility that decarboxylation of 2,3-dihydroxybenzoic acid or 3,4-dihydroxybenzoic acid is involved in catechol biosynthesis was checked by feeding the former compound (three replicates) and by feeding 4-hydroxybenzoic acid (three replicates), a likely precursor of the latter; in neither case was any label detected in catechol. Salicylic acid is converted in part to 2,3-dihydroxybenzoic acid, however, since after feeding 20 μC of salicylic GL-^{3}H (sp. act. 238 mc/mM) for 24 hr, $9{\cdot}3 \times 10^5$ dpm were found in catechol (sp. act. 475 uc/mM) and $1{\cdot}34 \times 10^5$ dpm were found in 2,3-dihydroxybenzoic acid.

Tryptophan degradation by *Aspergillus niger* leads to catechol[8] but there was no clear evidence for this in *Gaultheria*. The small amount of activity in catechol after feeding 50 μc of tryptophan-GL-^{3}H had probably been recycled through the shikimate pathway from loss of the tryptophan side-chain but the possibility of a small amount of degradation to the aromatic nucleus cannot be excluded.

Unlike catechol, salicylic acid is quite common in *Gaultheria*, appearing in some species in relatively large quantities as a glycoside of methyl salicylate.[2] The subgroup Amblyandra,

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however, would seem to have evolved a system for converting this salicylic acid to catechol which is then stored as its *beta*-glucoside. This is clearly analogous to the oxidative decarboxylation of 4-hydroxybenzoic acid, a common phenolic constituent in higher plants, to hydroquinone in *Pyrus*, *Grevillea* and *Bergenia*.[16, 17]

Weak salicylate decarboxylating activity can be detected in the supernatant of buffered homogenates of young leaves when incubated with reduced coenzymes and FAD but insufficient material has been available for a more detailed study as yet. The "salicylate hydroxylase" isolated by Katagiri *et al.*[7] from a soil bacterium converted salicylate to catechol when FAD and $NADH_2$ were added.

EXPERIMENTAL

Gaultheria adenothrix (Miq.) Maxim. was grown in flats in the departmental greenhouses under fluorescent lighting on a 16 hr day. The young leaves were used as they appeared throughout the year.

Radioactive Compounds

Phenylalanine-U-^{14}C, benzoic acid-1-^{14}C, salicylic acid-*ring*-UL-^{14}C, salicylic acid-GL-^{3}H, tryptophan-GL-^{3}H and tryptophan-*benzene ring*-UL-^{14}C were purchased from commercial sources. Tritiation of the ring of 4-hydroxy and 2,3-dihydroxybenzoic acids was carried out by refluxing 1 mM of the compound in 10 ml of CF_3COO^3H (prepared from ^3H_2O and trifluoroacetic anhydride) with 10 mg of Pd on charcoal for 48 hr. Labile tritium was removed by successive solution and evaporation and the compound twice crystallized from water. The 2,3-dihydroxybenzoic acid and the 4-hydroxybenzoic acid had specific activities of 266 μc/mM and 752 μc/mM respectively.

All the acids were administered as their ammonium salts in aqueous solution. Ten 1·2-cm discs cut from washed leaves were infiltrated with the radioactive solution (5 ml) using water aspirator vacuum. The discs were then floated on the residual solution in small Petri dishes under constant illumination at 20° for 24 hr.

Isolation of Catechol-β-D-Glucoside and Catechol

The rinsed discs were extracted with hot 95% ethanol. The solvent was evaporated and the residue extracted with hot water followed by filtration through Celite. The filtrate was concentrated to a few milliliters and run on to a column of Avicel (microcrystalline cellulose) which was then eluted with 2% formic acid. The fractions containing catechol glucoside were taken to dryness and purified by preparative TLC on 1 mm Avicel layers, using successively as solvents *n*-butanol:acetic acid:water (4:1:2·2), *n*-butanol:pyridine:water (75:15:10) and ethyl acetate:formic acid:water (75:10:10). The glucoside was then dissolved in 2 ml water and incubated with emulsin (β-glucosidase) for 24 hr. The hydrolysate was banded on Avicel plates and developed in benzene:acetic acid:water (10:7:3—organic phase). The catechol band was eluted and the concentration measured spectrophotometrically at 276 nm. The radioactivity was determined by liquid scintillation counting. Further chromatography left the specific activity essentially unchanged.

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