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BIOSYNTHESIS OF EUGENOL AND CINNAMIC ALDEHYDE IN *CINNAMOMUM ZEYLANICUM*

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Key Word Index—*Cinnamomum zeylanicum*; Lauraceae; cinnamon; biosynthesis; eugenol; phenylalanine; methionine.

Abstract—Incorporation of [^{14}C]-phenylalanine and [^{14}C]-methionine into cinnamon cuttings suggests that synthesis of eugenol from phenylalanine involves exchange of the terminal carbon in the side chain with that from a donor molecule such as methionine whereas synthesis of cinnamic aldehyde incorporates phenylalanine *in toto*.

INTRODUCTION

Cinnamomum zeylanicum Nees (Cinnamon) produces various oils that are of considerable commercial importance. The major constituent of oil derived from the leaf is eugenol while the major component of stem bark oil is cinnamic aldehyde [1]. The biosynthesis of eugenol and cinnamic aldehyde in cinnamon has not been reported in the literature. Studies on other plant tissues [2–6] have shown phenylalanine to be a precursor of eugenol and cinnamic aldehyde but the reaction sequence involved in the syntheses varies in different tissues. This paper describes the feeding of [^{14}C]-phenylalanine and [^{14}C]-methionine to cinnamon cuttings and the determination of the activity incorporated into eugenol and cinnamic aldehyde.

Table 1. Incorporation of [^{14}C] phenylalanine and methionine into cinnamon leaf oil, cinnamic aldehyde and eugenol after 5 hr feeding through the cut ends of cinnamon cuttings

Precursor	Incorporation (%)		
	Leaf oil	Cinnamic aldehyde	Eugenol
DL-Phenyl-[1-^{14}C]-alanine	0.015	0.009	0.003
DL-Phenyl-[2-^{14}C]-alanine	0.69	0.010	0.047
DL-Phenyl-[3-^{14}C]-alanine	0.86	0.009	0.098
L-[Methyl-^{14}C]-methionine	0.70	0.016	0.092
DL-Phenyl-[3-^{14}C]-alanine + L-[Methyl-^{14}C]-methionine	1.36	0.017	0.12

Values are means of two experiments

RESULTS AND DISCUSSION

Table 1 shows that the incorporation of label from phenyl-[2-^{14}C]-alanine and phenyl-[3-^{14}C]-alanine into eugenol was much greater than that from phenyl-[1-^{14}C]-alanine; and that relatively substantial incorporation occurred from [methyl-^{14}C]-methionine. This suggests that the terminal carbon of phenylalanine is removed during its conversion to eugenol and replaced with a —Me from a donor molecule. Degradation of eugenol derived from incubation with [methyl-^{14}C]-methionine to methyleugenol and oxidation to homoveratric acid cleaves the terminal carbon in the allyl group. Homoveratric acid retained very little activity and confirmed that [methyl-^{14}C]-methionine only contributed to the terminal carbon in the allyl chain (Table 2). It was possible that methionine could have also donated the methyl group for OMe on Carbon-3 in the aromatic ring, but if this had occurred to any extent then homoveratric acid would have retained more activity. A relatively small loss in specific activity occurred in methyleugenol relative to eugenol which was probably due to uncontrolled oxidation of the side chain during the reaction and extraction steps. Table 1 shows that the activity incorporated into cinnamic aldehyde was similar for all forms of phenylalanine, indicating that the whole phenylalanine structure was incorporated into cinnamic aldehyde. The biosynthetic pathway pro-

Table 2. Radioactivity of eugenol, labelled by methionine, during degradation to methyleugenol and homoveratric acid

Compound	Yield (mg)	Total radioactivity (dpm)	Specific activity (dpm/mg)
Eugenol	225	38 850	173
Methyleugenol	200	24 450	122
Homoveratric acid	45	135	3

posed for eugenol and cinnamic aldehyde is similar to that suggested by Manitto *et al.* [2–4] for *Ocimum basilicum*.

EXPERIMENTAL

Materials and methods. Fresh cinnamon cuttings were obtained from Royal Botanic Gardens, Sydney. They were divided into 5 lots each of 25 ± 2 g. The stems of the selected cuttings (*ca* 15–20 cm long), each with 5–8 attached leaves were then recut. The cut ends were immediately immersed in glass vials containing the solns with labelled substrate. The substrate solns were 10 μCi of DL-phenyl-[1-^{14}C]-alanine, DL-phenyl-[3-^{14}C]-alanine, 5 μCi of DL-phenyl-[2-^{14}C]-alanine, 10 μCi of L-[^{14}C-Me]-methionine and 10 μCi of L-[^{14}C-Me]-methionine with 7 μCi DL-phenyl-[3-^{14}C]-alanine, each lot with 4 mmol and 1 mg glucose. The total vol. of soln in each vial was 0.1 ml. The cuttings were held in the glass house under normal daylight condition, at a constant temp. (25 ± 1°). Most of the substrate solns were adsorbed in about 30 min; after which the cuttings were maintained on H_2O.

Extraction and isolation procedure. After 5 hr each lot was removed, cut into small pieces and steam distilled immediately. The radioactivity of the distilled oil was determined using toluene based scintillation soln and a scintillation counter.

Isolation of eugenol and cinnamic aldehyde. These two compounds were isolated by preparative GLC on a (3 × 6.4 m o.d.) glass column of 20% Carbowax 20 M on Gas-chrom Q (80–100 mesh). The operating conditions were: column temp. 75–235° at 2°/min; injector 210°; detector 235°; carrier gas N_2 45 ml/min; stream splitter 1 :10; sample size 30 μl. The eluant samples were collected in a glass tube (7 × 0.25 i.d.) packed with Si gel 50–200 mesh with either end plugged with glass wool. The relative abundance of each compound was calculated by peak areas. The radioactivity in eugenol and cinnamic aldehyde was measured using toluene based scintillation soln (10 ml) containing 40% Cab-o-sil.

Isolation and degradation of labelled eugenol. Eugenol was isolated from the distilled leaf oil by method of ref. [1]. Labelled cinnamon leaf oil (460 μl) was dissolved in Et_2O (5 ml) and extracted by shaking with 10% KOH (3 × 5 ml). Eugenol was regenerated by acidifying with excess H_2SO_4, extracted with Et_2O and concd under a gentle stream of N_2, when its radioactivity was determined. GLC showed only one peak corresponding to eugenol. Eugenol labelled with L-[^{14}C-Me]-methionine was subjected to degradation to homoveratric acid by a modification of the method described in ref. [3]. Labelled eugenol (225 μl) mixed with inactive eugenol (25 μl) and methylated by refluxing for 5 hr with Me_2SO_4 (0.7 ml) and 10% KOH (0.7 ml) in dioxane (12.5 ml). The methyleugenol was extracted with ether, dried and concd over a gentle stream of N_2. Methyleugenol was degraded to homoveratric acid (3,4-dimethoxyphenylacetic acid) as follows. A soln of methyleugenol (100 μl), $KMnO_4$ (2.5 g) and K_2CO_3 (0.8 g) in 100 ml H_2O was refluxed for 3 hr, cooled and filtered. Homoveratric acid was extracted with $CHCl_3$ (25 ml × 2) and Et_2O (25 ml). The combined soln was evapd and the homoveratric acid was recrystallized from hot H_2O to constant mp (81.5–82.5°). Its radioactivity was determined using toluene based scintillation solution.

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TWO NEW COUMARINS FROM *RUTA PINNATA**

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INTRODUCTION

Ruta pinnata L.fil, endemic to the Canary Islands, is extremely rich in coumarins, of which we have already isolated more than forty, nine being reported for the first time [1–4]. We have already reported on the coumarins from the leaves of this plant [4–6]; this paper deals with secondary coumarins from the same source.

RESULTS AND DISCUSSION

From the alcohol extract of the leaves of *Ruta pinnata*,

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