Isolation of Possible Intermediate of Chlorogenic Acid Biosynthesis in Sweet Potato Root Tissue

Sir:

Chlorogenic acid is one of the most ubiquitous polyphenols in the plant kingdom,^{1,2)} it plays important roles in some physiological functions of plants. For example, its involvement in the defense action in plants has been shown in our laboratory.^{3~5)} Radioisotopic experiments^{6~9)} revealed that *t*-cinnamic acid is the source of the aromatic moiety of chlorogenic acid. The pathway from *t*-cinnamic acid to chlorogenic acid which involves hydroxylation of the benzen ring and esterification, however, remains obscure, although several theories conflicting with each other have been proposed on the biosynthetic pathway. According to Levey and Zucker⁷⁾ and Runeckles,⁹⁾ the first ester intermediates of chlorogenic acid biosynthesis are 3-*O*-cinnamoylquinic acid and 3-*O*-*p*-coumaroylquinic acid, respectively. On the other hand, El-Basyouni *et al.*¹⁰⁾ suggested that the intermediates of phenylpropanoids in lignin biosynthesis are possible protein binding

esters of cinnamic acid derivatives. In the preceding paper,¹¹⁾ we suggested the operation of the two pathways in sweet potato root tissue: phenylalanine $\rightarrow$ *t*-cinnamic acid $\rightarrow$ *t*-cinnamoyl derivative $\rightarrow$ *p*-coumaroyl derivative $\rightarrow$ chlorogenic acid, and phenylalanine $\rightarrow$ *t*-cinnamic acid $\rightarrow$ *p*-coumaric acid $\rightarrow$ *p*-coumaroyl derivative $\rightarrow$ chlorogenic acid.

As reported previously,^{3~5)} polyphenolic compounds, mainly composed of chlorogenic acid and isochlorogenic acid, are markedly synthesized in sweet potato root tissue in response to slicing. It has also been demonstrated that some enzymes involved in the biosynthesis of the compounds are formed prior to their prominent synthesis.^{12~15)} This communication deals with the finding of a possible intermediate in chlorogenic acid biosynthesis in sweet potato root tissue and some of its properties.

t-Cinnamic acid-2-¹⁴C was prepared in a pure form by the Perkin reaction modified by Doebner.¹⁶⁾ One ml of 5 mM acetate buffer, pH 5.5, containing 12.5 μ moles of *t*-cinnamic acid-2-¹⁴C, 70.5 m μ Ci per μ mole,

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FIG. 1. Paper Radiochromatograms of Ethanol Extracts of Sweet Potato Root Discs Administrated with Cinnamic Acid-2- ^{14}C .

The ethanol extracts were subjected to paper chromatography (5% acetic acid). After development, the paper strips were cut into 1 cm pieces, which were assayed for radioactivity in a liquid scintillation spectrometer. Arrows on chromatograms represent the migrating positions of the individual compounds in the solvent system (5% acetic acid). A, B, C, D and E: radiochromatograms after 2.0 hr-, 5.0 hr-, 8.0 hr-, 10.5 hr-, and 14.0 hr-incubation, respectively.

was administrated to 24 discs (2×19 mm) of tissue, which were incubated at 30°C for appropriate periods. After incubation, the discs were washed with 100 ml of water, boiled in 30 ml of ethanol and homogenized. The homogenate was boiled in a hot water bath for 30 min, and filtered. The resultant ethanol extract was subjected to silica gel column chromatography developed by Hanson and Zucker¹⁷⁾ and paper chromatography (5% acetic acid) to investigate the time-course changes in distribution of radioactivity during incubation. As shown in Fig. 1, radioactivity of *t*-cinnamic acid-2- ^{14}C was distributed in compound V (tentatively called) first, in 2 hr

after administration, then appeared in chlorogenic acid, and finally in isochlorogenic acid. Thin-layer chromatography as well as paper chromatography indicated that compound V consisted of a single component. The results strongly suggest that the compound is an intermediate of chlorogenic acid biosynthesis. Furthermore, time-course analysis with the column chromatography indicated that specific radioactivity of compound V increased prior to that of the fraction containing chlorogenic acid and isochlorogenic acid and decreased prior to that of the latter. This implies the precursor-product relationship. When the radioactive compound V isolated from the discs after administration of *t*-cinnamic acid-2- ^{14}C was fed to other discs, the radioactivity was efficiently incorporated into chlorogenic

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acid and isochlorogenic acid, as shown in Table I. This supports the above view.

Compound V is an acidic substance show-

TABLE I. DISTRIBUTION OF RADIOACTIVITY OF VARIOUS FRACTIONS IN SWEET POTATO ROOT DISCS AFTER ADMINISTRATION OF LABELED COMPOUND V

Twenty eight discs (2×19 mm) were administrated with radioactive compound V (3.57×10^5 dpm, 430 as O.D₂₇₈ in 1 ml) in 1 ml of 5 mM acetate buffer, pH 5.5 and incubated at 30°C for 18 hr.

	Radioactivity dpm	Radioactivity %	Relative %
Administrated	3.57×10^5	100	
Unabsorbed	0.96×10^4	3	
Insoluble Fraction	1.36×10^5	38	
CO ₂	1.08×10^5	30	
EtOH extract ^{a1}	1.03×10^5	29	100
Chlorogenic acid	0.21×10^5	6	20
Isochlorogenic acid	0.57×10^5	16	55

^{a1} Ethanol extract was subjected to paper chromatography (5% acetic acid) to obtain the fractions of chlorogenic acid and isochlorogenic acid.

ing maximum absorption at 278 m μ in either ethanol or water. The UV spectrum without alkaline shift suggested the absence of phenolic hydroxyl group(s) in its structure. Compound V is unstable in alkaline solution and hydrolyzed to two components easily (Fig. 2). Radioactivity of compound V was strictly confined to one of the two components (the spot of *R_f* 0.75 or 0.58 on thin-layer or paper chromatogram in Fig. 2), which was proved to be *t*-cinnamic acid based on paper and thin-layer chromatographies, and UV, IR and Mass-spectrometries. On the other hand, another component of compound V is presumed to be a substance carrying a carboxyl group and alcoholic hydroxyl groups, which is neither quinic acid nor shikimic acid, based on the data on IR spectrum and positive acidic reaction. Taken altogether, compound V is thought to be an intermediate of chlorogenic acid biosynthesis and be an ester of *t*-cinnamic acid with a substance carrying a carboxyl group and alcoholic hydroxyl groups different from 3-*O*-cinnamoylquinic acid and

FIG. 2. Chromatograms of Compound V, Hydrolyzate of Compound V, and *t*-Cinnamic Acid.

Compound V was kept in 1N sodium hydroxide at room temperature for 12 hr, and acidified with 5N hydrochloric acid. An aliquot of hydrolyzate of compound V, compound V or *t*-cinnamic acid was subjected to silica gel thin-layer chromatography (I. toluene-ethyl formate-formic acid, 5:4:1) and paper chromatography (II. 5% acetic acid). A, B and C: compound V, hydrolyzate of compound V and *t*-cinnamic acid, respectively.

3-*O*-*p*-coumaroylquinic acid.

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