A SURVEY OF CATECHOL RING-CLEAVAGE BY STERILE PLANT TISSUE CULTURES

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1. Introduction

Interest has been growing in the fate of aromatic compounds absorbed by plant tissues under sterile conditions. It has been demonstrated that in the absence of microorganisms plant cells are capable of catabolizing flavonoid structures by degradation to cinnamic and benzoic acids [1, 2]. Further degradation by ring-cleavage reactions forms a critical point in the catabolism of the wide range of aromatic compounds found in plants. The ability of plant tissue cultures to degrade the ring of aromatic amino acids [3] and phenolic acids [4] to carbon dioxide has recently been established, and similar studies have been carried out with phenolic acids using wheat plants grown under sterile conditions [5, 6]. Agal cultures have been shown to ring-cleave phloroglucinol [7] and phenylalanine [8]. In general, appreciable ring cleavage has only been reported [4] when dihydroxy phenolic compounds have been metabolized and it is possible that ^{14}C-phenylpropanoic and benzoic acids are only slowly metabolized to form ring-cleavage substrates within the cell. Very high levels of decarboxylation of monohydroxy benzoic acids have been observed [4] but it appears that the products of such decarboxylation are either unsuitable substrates for ring-cleavage or that they are isolated from sites of ring-cleavage activity within the cell.

Very few species have been examined up to now for ring-cleavage ábility and it was deemed interesting to examine the distribution of such activity in a wide variety of tissues. Catechol-U-^{14}C was chosen as chief substrate because it is an important ring-cleavage substrate in known pathways of microbial metabolism [9] and furthermore, the relationship between the ability of a tissue to ring-cleave catechol, to form catechol by oxidative decarboxylation of salicylic acid [10] and to ring-cleave salicylic acid could be examined.

2. Materials

Cultures of *Agave toumeyana* were obtained from Dr. B. Fritig, Strasbourg, *Petunia hybrida* from Dr. Binding, Köln, and *Pimpinella anisum* and *Ruta graveolens* from Dr. H. Becker, Karlsruhe. A second strain of *Ruta graveolens* and the culture of *Melilotus alba* have been described previously [3]. All other cultures were started from surface-sterilized seeds or plant sections. All cultures were maintained on Gamborg's B5 medium [11], in some cases supplemented with casein hydrolysate or coconut milk. Tissues were used for experiments 1–3 weeks after inoculation. All experiments were checked for contamination and radioactive substrates were administered as previously described [3] except that the carbon dioxide was isolated and counted as barium carbonate.

Salicylic acid-7-^{14}C was purchased from the Radiochemical Centre, Amersham and salicylic acid-*ring*-U-^{14}C was obtained from Mallinckrodt Nuclear, St. Louis. Catechol-U-^{14}C and protocatechuic acid-1,2-^{14}C were synthesized [12] and purified.

3. Results and discussion

The ability to split aromatic rings is clearly widespread in the plant kindom (table 1). All tissues to which catechol-U-^{14}C were fed degraded the aromatic ring with as much as 5.5% of the radioactivity appearing in carbon dioxide within the first 24 hr. A con-

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Table 1
Percent of radioactivity recovered as $^{14}CO_2$ within 24 hr of the administration of the radioactive compound to the culture. Cultures were shaken in 40 ml of medium in a 250 ml center-well (KOH) Erlenmeyer flask.

Species	Family	Catechol-U-^{14}C 1.49 μCi/μmole 10^6 dpm added	Salicylic acid	
			carboxyl-^{14}C 31.4 μCi/μmole 1 μCi added	*ring*-^{14}C 1.61 μCi/μmole 1 μCi added
Agave toumeyana Trel.	Agavaceae	0.47	0.5	< 0.1
Agrostemma githago L.	Caryophyllaceae	0.33	0.18	< 0.1
Coleus blumei Benth.	Labiatae	1.26	< 0.1	< 0.1
Daucus carota L.	Umbelliferae	2.18		< 0.1
Galium spurium L.	Rubiaceae	0.32	0.21	< 0.1
Glycine max (L.) Merr.	Leguminosae	2.15	0.30	< 0.1
Malus coronaria (L.) Mill	Rosaceae	4.23	0.14	
Melilotus alba Medik.	Leguminosae	1.59	< 0.1	< 0.1
Nicotiana sylvestris Spegazz. et Comes	Solanaceae	1.90	0.15	
Nicotiana tabacum var. xantha L.	Solanaceae	1.54	0.24	< 0.1
Petunia hybrida Vilm.	Solanaceae	0.25	0.23	
Pimpinella anisum L.	Umbelliferae	1.43	2.32	< 0.1
Phaseolus angularis (Willd.) W.F. Wight	Leguminosae	5.56		
Phaseolus aureaus Roxb.	Leguminosae	5.55	0.30	< 0.1
Phaseolus vulgaris v. nanus (L.) Aschers	Leguminosae	2.03		
Ruta graveolens L.	Rutaceae	0.12	0.30	< 0.1
Ruta graveolens L.	Rutaceae	0.77	0.25	
Rheum palmatum L.	Polygonaceae	1.30		
Sinapis alba L.	Cruciferae	2.92		< 0.1
Solanum tuberosum L.	Solanaceae	2.56	0.16	< 0.1

siderable degradation of catechol by *Phaseolus aureus* and *Glycine max* has also been reported by others [4]. The observed levels of ^{14}C in respiratory carbon dioxide are probably indicative of considerably more radioactivity circulating in the general metabolic pathways. Chromatography of the ethanol extracts of tissues fed catechol-^{14}C showed the presence of a remarkably large number of radioactive compounds. A more detailed study of the short-term fate of catechol in cells of *Phaseolus aureus* showed that the most of the soluble activity appeared rapidly in two compounds, one of which could be chromatographically identified as catechol-β-D-glucoside. Notably, more than 50% of the absorbed radioactivity was not extractable with 80% ethanol which suggests that phenol oxidases in the cell were converting the catechol to polymeric

substances. This phenomenon has been observed by others when feeding phenolics to plant tissue cultures [2] or sterile plants [6].

Catechol itself has been reported only infrequently in higher plants and may therefore not represent a common natural substrate for ring-cleavage in plant cells. It is known to be formed in plants by decarboxylation of salicylic acid [10, 13] and it was therefore of interest to examine the extent of degradation of salicylic acid in the same tissues. While most tissues showed some ability to decarboxylate salicycic acid (table 1) there was no indication of any degradation of the aromatic ring. Assuming the product of the decarboxylation to be catechol, the results suggest that this internally-formed catechol remains isolated from the sites of ring-cleavage in the cell.

Many of the tissues were also tested with protocatechuic acid *ring*-^{14}C, and again ring-cleavage was frequently observed. The highest values were obtained with *Melilotus alba* (9.43% in 24 hr) and *Phaseolus aureus* (5.17%).

The results of the present work with catechol and protocatechuic acid and of other work with a wide range of phenolic acids show that few of the aromatic "secondary metabolites" can be considered end-products since there is clearly a widespread ability in plants to convert the aromatic structures to aliphatic metabolites and carbon dioxide.

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