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DIFFERENCES IN THE INCORPORATION PATTERN OF LABELLED CINNAMIC ACID AND L-PHENYLALANINE INTO THE ANTHOCYANINS OF *PETUNIA HYBRIDA*

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Key Word Index—*Petunia hybrida*; Solanaceae; cinnamic acid-[^{14}C]-incorporation; phenylalanine-[^{14}C]-incorporation; anthocyanin biosynthesis.

Abstract—The incorporation of phenylalanine-[^{14}C] into anthocyanins of petals of *Petunia hybrida* is greater than that of cinnamic acid-[^{14}C]. Moreover, there is a preferential incorporation of phenylalanine-[^{14}C] into delphinidin 3-monoglucoside, as compared with the incorporation into cyanidin and peonidin 3-monoglucosides.

INTRODUCTION

When variously substituted hydroxy-cinnamic acids are fed to petals of *Petunia hybrida* or *Campanula medium*, they are incorporated preferentially into anthocyanins of the same or related B-ring substitution pattern [1–3]. The same should not happen when phenylalanine or cinnamic acid are fed, since these two precursors are further removed biosynthetically from the flavonoid end-products [cf. 4]. However recently, differences in the incorporation pattern of cinnamate and shikimate were found in *Petunia* petal anthocyanins in spite of the fact that shikimate is a precursor of cinnamate via the shikimic acid pathway; metabolic channelling by compartmentation and catalytic facilitation by multienzyme complexes were possibly involved [5]. Because such mechanisms are implicated in comparative feeding experiments with cinnamic acid and phenylalanine as precursors in studies on phenylpropanoid metabolism [6–9], and because anthocyanin biosynthesis evidently takes place partly or completely on membranes [10], cinnamic acid-[^{14}C] and phenylalanine-[^{14}C] were fed to petals of *Petunia hybrida* in order to compare their incorporation into cyanidin (=cya), peonidin (=peo), and delphinidin 3-monoglucosides (=del).

RESULTS

The influence of cold cinnamic acid and phenylalanine on anthocyanin accumulation.

Feeding experiments were carried out with solutions of 0.02 mM cinnamic acid and 0.1 mM phenylalanine. During an incubation period of 25 hr, neither of these substances had any influence on the rate of anthocyanin accumulation. The values (nmol per petal) for cya, peo, and del were in the case of 0.1 mM cinnamic acid 243, 25, 45 with a water control of 232, 26, 42 $[\bar{x}, n = 5]$ and in the case of 0.1 mM phenylalanine 171, 16, 23 with a water control of 169, 17, 21 $[\bar{x}, n = 6]$.

Comparison of cinnamic acid-[^{14}C] *and phenylalanine-*[^{14}C] *incorporation.*

The results are summarized in Table 1. From the averages, the following dilution values are calculated for cinnamic acid-[^{14}C] feeding: cya 1484, peo 934, del 409; and for phenylalanine-[14C] feeding: cya 418, peo 269, del 82. Following phenylalanine-[^{14}C] feeding, the dilution values are lower by factors of 3.6, 3.5 and 5.0 for cya, peo, and del, respectively. The ratios of the relative proportions of the sp. act. cya:peo:del were after cinnamic acid-[^{14}C] feeding 100:160:365, and after phenylalanine-[^{14}C] feeding 100:157:509. In 7 of the 8 experiments, the incorporation of phenylalanine-[^{14}C] into del was higher than that of cinnamic acid-[^{14}C]; the difference is significant for $\alpha < 0.025$.

DISCUSSION

The experiments show clearly that the incorporation of phenylalanine-[^{14}C] into the anthocyanins is higher than that of cinnamic acid-[^{14}C] and also that there is preferential incorporation of phenylalanine-[^{14}C] into del. The results with phenylalanine closely resemble those obtained earlier with shikimic acid-[^{14}C] [5]. These results argue against the existence of alternative pathways for the synthesis of phenylpropanoid derivatives [cf. 4, 5].

It is well established in phenylpropanoid metabolism that sequentially acting enzymes are bound to particulate cell fractions and that compartmentation occurs with respect to different phenylpropane derivatives [cf. 11, 12]. Metabolic channelling and the storage of metabolites in different pools is particularly evident in comparative feeding experiments with cinnamic acid and phenylalanine that have been carried out on the formation of various soluble and bound hydroxy benzoic and cinnamic acids in *Triticum aestivum*, *Mentha arvensis*, *Astilbe chinensis*, *Solanum tuberosum* [6–9]. The present results extend these observations beyond the level of

Table 1. The incorporation of cinnamic acid-[^{14}C] and phenylalanine-[^{14}C] into the 3-monoglucosides of cyanidin, peonidin and delphinidin in isolated petals of *Petunia hybrida*

Number of expt*	Anthocyanin content (μmol)			Specific activity (dpm/nmol)		
	cya	peo	del	cya	peo	del
Incubation with cinnamic acid-[^{14}C]						
1	13.2	1.42	1.45	69	89	347
2	12.1	1.55	1.99	90	102	256
3	15.1	1.27	1.38	89	100	474
4	11.6	1.45	1.40	68	66	230
5	12.0	1.45	1.72	62	81	188
6	10.7	1.23	1.62	103	259	397
7	15.1	1.27	1.38	88	208	275
8	9.8	1.25	1.40	82	133	202
Average	12.4	1.36	1.54	81	130	296
Incubation with phenylalanine-[^{14}C]						
1	15.9	1.53	1.62	48	64	432
2	10.4	1.47	1.43	54	77	295
3	11.8	1.09	1.57	55	71	312
4	12.0	1.53	1.35	51	70	142
5	11.5	1.29	1.76	46	64	184
6	12.2	1.25	1.82	46	87	195
7	14.8	1.69	1.38	63	113	417
8	9.7	1.24	1.31	62	115	181
Average	12.3	1.39	1.53	53	83	270

* In each expt, 140 petals of flowers 26–30 mm in length were equally distributed according to their various lengths in 2 parallel sets. The lower, weakly pigmented halves were cut off and discarded and the upper halves symmetrically bisected before incubation in Petri dishes containing a 6 ml aq. soln of 6,4 ± 9 % μCi cinnamic acid-[^{14}C] in one dish and phenylalanine-[^{14}C] in the other. The incubation period was 5 hr at 5000 lx fluorescent white light and 25°. Values are given per Petri dish with 70 upper petal halves.

hydroxy cinnamic acid esters towards individual anthocyanin pigments in flower petals.

EXPERIMENTAL

The plant material, the analytical methods, and the experimental controls have been described [13]. Specific experimental conditions are given in Table 1. Cinnamic acid-[^{14}C]-3, sp. act. 54.5 Ci mol^{-1}, was from the Département des Radioéléments, Gif-Sur-Yvette. L-Phenylalanine-[^{14}C]-(U), sp. act. 10 Ci mol^{-1}, from The Radiochemical Centre, Amersham. Significance was calculated using the Wilcoxon matched pairs signed rank test [14].

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