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BIOSYNTHESIS OF RADIOLABELLED ALKALOIDS FROM ^{14}C-TYROSINE IN *ERYTHRINA CRISTA-GALLI*

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Key Word Index—*Erythrina crista-galli*; Leguminosae; alkaloids; 8-oxoerythraline; crystamidine; tyrosine; biosynthesis.

Abstract—Alkaloid biosynthesis has been demonstrated in the young expanding tissues of *Erythrina crista-galli* shoots, the stem part of which elaborated, from [2-^{14}C]-tyrosine, alkaloid of specific radioactivity 2 μCi mmol^{-1}, 10-fold greater than previously reported.

INTRODUCTION

Erythrina alkaloids are presumed to be biosynthesised from tyrosine, via condensation of two molecules of DOPA [1], contrary to an earlier proposition [2] that they are derived directly from shikimic acid. Some subsequent steps in the pathway have since been demonstrated experimentally [3] using ^{14}C-labelled intermediates. Thus, while there is no mechanistic reason to doubt the tyrosine origin of *Erythrina* alkaloids, the experimental finding of ref. [1], that β-erythroidine (**1**) was radiolabelled in *E. berteroana* to a specific activity of the order of only 0.1 μCi mmol^{-1} from [2-^{14}C]-tyrosine, might well be improved.

Although competition from primary biosynthetic pathways to protein and lignin would be expected to allow only a small proportion of administered ^{14}C-tyrosine to be available for alkaloid biosynthesis, it ought to be possible to enhance the incorporation of precursor especially if the radiolabel could be directed more efficiently to the sites of alkaloid biosynthesis. *Erythrina* alkaloid biosynthesis has previously been generally attributed to shoot tissues without any consideration whether, for example, the alkaloids are formed in very young tissues or only as leaves mature.

RESULTS AND DISCUSSION

The relative distribution of ^{14}C-tyrosine into alkaloid in various regions of an *E. crista-galli* shoot showed (Table 1, Expt. 1) that most of the radiolabelled alkaloid was located distal to the point of administration of the precursor into the stem. Alkaloid from the youngest leaves had the highest specific activity amongst these tissues, suggesting biosynthesis in the growing region. The stem was also a source of a small amount of alkaloid, though having relatively more radioactivity than that in leaves.

Prior administration of cold tyrosine (Table 1, Expt. 2) appeared to increase the yield of alkaloid several-fold, while not causing a proportionate reduction in specific radioactivity.

The experimental model was therefore modified to administer tyrosine only to young leaves via the stem (Table 1, Expt. 3) and resulted in 8-oxoerythraline (**2**) and crystamidine (**3**) with specific radioactivities of an order similar to that of **1** [1]. Much higher values were found in **2** and **3** extracted from the stem. Radiolabelled **2** from stem tissue was processed through preparative HPLC and the eluate corresponding only to **2** had a specific activity of 2.2 μCi mmol^{-1}, an order of magnitude greater than that found previously [1] and therefore providing compelling experimental evidence for the biosynthetic origin of these aromatic conjugated 1,6-diene alkaloids from tyrosine. These experiments show the particular value of young stem tissue as a site of alkaloid biosynthesis to which radiolabelled precursors may be administered via the transpiration stream advantageously, in spite of the competing pathways of primary metabolism.

EXPERIMENTAL

Radiolabelling of plants. Seven-month-old *E. crista-galli* plants, in which the principal alkaloids are **2** and **3** [4] were fed by a cotton wick [1, 3] with L-[U-^{14}C]-tyrosine (specific activity 522 mCi mmol^{-1} or cold L-tyrosine. Several days later shoots were subdivided into regions (see Table 1) from which the alkaloids were extracted.

The ratio of radiolabelled tyrosine to plant shoot fresh weight in Experiment 1 was 3-fold greater than previously used [1]. The ratio was again increased by a factor of two in the second experiment and by a further factor of four in the third experiment. The relative total amount of tyrosine given in experiment 3 was ten-fold greater than in [1]. Additionally, use of HPLC for separation and quantification has enabled biosynthetic experiments to be performed on 12 g (fresh weight) of intact plant tissue rather than the 700 g previously used [1].

Alkaloid extraction. The method was essentially that used previously [4], scaled down for the smaller amounts of fresh plant tissue, giving 2 and 3 resolved by PLC.

Alkaloid assay. Quantification. Previous studies on *Erythrina* alkaloids have relied on gravimetric determination of extracts of rather large amounts of plant tissue and so the literature contains no assay appropriate to small amounts of experimental material. Thus the HPLC system used to isolate **2** and **3** was calibrated to measure in the linear range 10–100 μg, in which reproducible

Table 1. Incorporation of ^{14}C-tyrosine into alkaloids and the structural and water-soluble components of *E. crista-galli*

Plant region	No of leaves	Fresh wt (g)	8-oxo-Erythraline		Crystamidine		% incorporation of ^{14}C tyrosine measured in:	
			Plant content ($\mu g\,g^{-1}$)	Specific activity ($dpm\,mg^{-1}$)	Plant content ($\mu g\,g^{-1}$)	Specific activity ($dpm\,mg^{-1}$)	Water extract fraction	Water-insoluble fraction
Experiment 1*								
Top leaves	3	1.07	288	127	46	221		
Middle leaves†	3	4.23	345	75	47	169		
Lower leaves†	7	8.04	468	47	68	57		
Stem	—	3.71	17	2166	11	4610		
Tissues proximal to feed point	2	7.41	6	242	9	727		
Experiment 2‡								
Top leaves	1	0.77	1304	120	105	236	0.13	0.40
Middle leaves†	3	2.91	828	96	82	289	0.33	0.37
Lower leaves†	8	4.93	810	95	125	102	0.40	0.40
Stem	—	2.68	117	589	52	742	8.20	44.55
Tissues proximal to feed point	2	0.87	116	142	47	193	7.61	2.26
Experiment 2§								
Top leaves	3	1.98	374	194	48	1339		
Lower leaves	6	1.84	1056	524	60	5860		
Stem	—	1.20	83	21786	22	15980		

*10 μCi ^{14}C-tyrosine (3 μg) fed via 13th node. Label taken up in 1 day. Shoot harvested after 3 days.

†Fully expanded at harvest.

‡10 μCi ^{14}C-tyrosine fed via 13th node one day after 14th node fed with unlabelled tyrosine (335 μg). Administered tyrosine taken up in 1 day. Shoot harvested 3 days after radiolabel fed.

§20 μCi ^{14}C-tyrosine fed via the terminal nodes of 3 shoots on the same plant, 3 hr after feeding unlabelled tyrosine (total 1.0 mg) into the second nodes. Leaves below the second node had been excised. Tyrosine uptake complete after 3–4 days. Shoots harvested 7 days after feeding commenced.

results could be obtained, as detected spectrophotometrically at 270 nm. The mean ratios between **2** and **3** isolated in these experiments were, respectively, 6:1.

Specific radioactivity. The radioactivity of one half of the **2** or **3** from each extraction, dissolved in MeOH, was measured by scintillation counting. Specific radioactivity was calculated following HPLC quantification of **2** or **3** in the other half of each extract.

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