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SHORT COMMUNICATION

BIOSYNTHESIS OF INDICAXANTHIN IN *OPUNTIA FICUS-INDICA* FRUITS*

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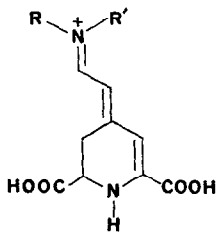
Key Word Index—*Opuntia ficus-indica*; Cactaceae; biosynthesis; betalains; indicaxanthin.

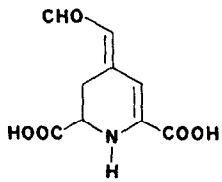
Abstract—The result of the incorporation of doubly labelled tyrosine into indicaxanthin in the fruits of *Opuntia ficus-indica* shows that the dihydropyridine moiety of the pigment originates from dopa by extradiol cleavage of the aromatic ring.

INTRODUCTION

BETALAINS (I), water-soluble nitrogenous plant pigments which include the red–violet betacyanins (glycosides of betanidin and its C-15 diastereoisomer isobetanidin) and the yellow betaxanthins, are characterized by the same dihydropyridine moiety and can be formally considered as immonium derivatives of betalamic acid (II).[1] This compound II was proposed as an intermediate in the biosynthesis of betacyanins and betaxanthins, which would be formed on condensation with appropriate amino acids or amines,[2] and moreover the suggestion was made that it might originate from 3,4-dihydroxyphenylalanine (dopa)



(I)



(II)

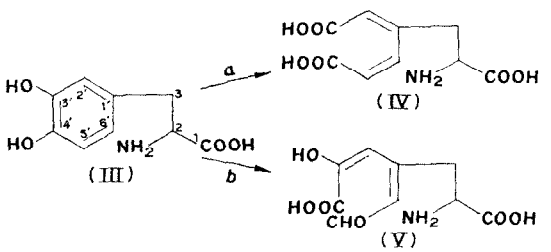
(III) by oxidative cleavage of the aromatic ring and subsequent closure to a dihydropyridine system.[3] Whether or not betalamic acid lies on the pathway to betalains is still an open question, but evidence has been obtained that dopa (and also tyrosine, but less efficiently)

* Part XVI in the series "Pigments of Centrospermae". For part XV see S. SCIUTO, G. ORIENTE and M. PIATTELLI, *Phytochem.* **11**, 2259 (1972). This work was supported by the Consiglio Nazionale delle Ricerche Italy.

[1] T. J. MABRY and A. S. DREIDING, in *Recent Advances in Phytochemistry* (edited by T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES), Vol. I, p. 145, Appleton-Century-Crofts, New York (1968).
[2] H. WYLER, M. E. WILCOX and A. S. DREIDING, *Helv. Chim. Acta* **48**, 361 (1965).
[3] T. J. MABRY, in *Taxonomic Biochemistry and Serology* (edited by C. A. LEONE), p. 239, Ronald Press, New York (1964).

is indeed incorporated in the dihydropyridine moiety of these pigments.[4,5] Conceivably, the C_9N skeleton of this ring could be derived from dopa in either of two ways: (a) cleavage of the aromatic ring between the two hydroxylated carbon atoms followed by bonding of nitrogen to carbon 5'; or (b) cleavage between carbons 4' and 5' and subsequent ring closure by bonding of nitrogen to carbon 3'.

Oxygenases which catalyze the cleavage of dihydroxyaromatic compounds are known to occur in bacteria and is not impossible that analogous enzymes exist in higher plants. Pyrocatechase type enzymes cleave the aromatic ring between the two hydroxylated carbon atoms ('intradiol' cleavage) resulting in the formation of two carboxyl groups, while meta-pyrocatechase type enzymes catalyze the incorporation of two atoms of oxygen into the substrate between a hydroxylated carbon atom and an adjacent carbon atom carrying hydrogen, forming an α-hydroxy dicarboxylic acid semialdehyde ('extradiol' cleavage).[6,7] With dopa as substrate, intradiol cleavage (a) or extradiol cleavage distal with respect to the side chain (b) would be expected to yield 6-amino-4-(carboxymethylene)-2-heptendioic acid (IV) and 6-amino-4-(formylmethylene)-2-hydroxy-2-heptendioic acid (V), respectively, both of which could cyclize to betalamic acid and this would subsequently react with amino acids or amines to give betalains (Scheme 1).



SCHEME 1.

RESULTS

In an attempt to distinguish between these two possibilities, we have studied the incorporation of doubly labelled tyrosine (L-1-^{14}C,3',5'-^{3}H-tyrosine) into indicaxanthin (VI) in the fruits of *Opuntia ficus-indica* Mill. (orange–yellow variety). Since it has been shown that when 3',5'-^{3}H-tyrosine is enzymically hydroxylated one tritium atom is lost from a position *ortho* to the hydroxyl group resulting in the formation of 5'-^{3}H-dopa (3',5'-^{3}H-tyrosine → dopa, 55% tritium retention),[8] isolation of ^{14}C-labelled indicaxanthin free of tritium from the tracer experiments with doubly labelled tyrosine would indicate that the conversion of dopa into the dihydropyridine moiety takes place via an intradiol cleavage, while isolation of labelled pigment with a ratio of tritium to ^{14}C(T/C) halved with respect to that of the administered precursor would be consistent with the pathway involving a distal extradiol cleavage of the aromatic ring of dopa.

[4] L. MINALE, M. PIATTELLI and R. A. NICOLAUS, *Phytochem.* **4**, 593 (1965).
[5] H. E. MILLER, H. RÖSLER, A. WOHLPART, H. WYLER, M. E. WYLCOX, H. FROHOFER, T. J. MABRY and A. S. DREIDING, *Helv. Chim. Acta* **51**, 1470 (1968).
[6] H. FUJISAWA and O. HAYAISHI, *J. Biol. Chem.* **243**, 2673 (1968).
[7] M. NOZAKI, K. ONO, T. NAKAZAWA, S. KOTANI and O. HAYAISHI, *J. Biol. Chem.* **243**, 2682 (1968).
[8] G. GUROFF, J. W. DALY, D. M. JERINA, J. RENSON, B. WITKOP and S. UDENFRIEND, *Science* **157**, 1524 (1957).

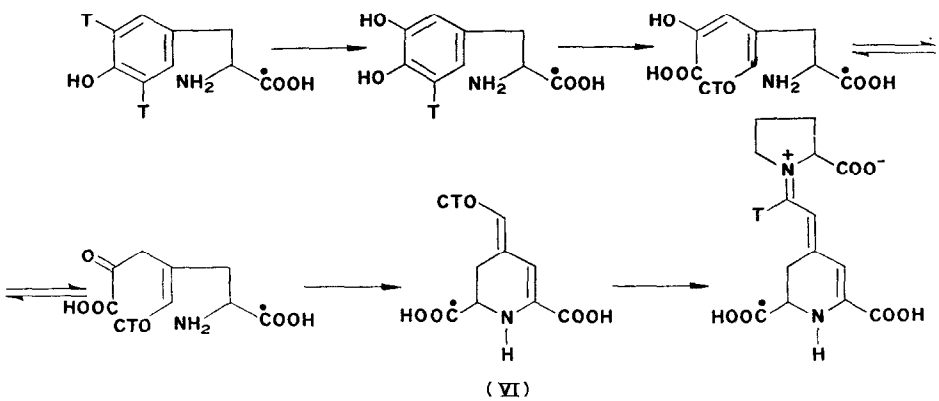
The results of the experiments carried out with different T/C ratios in the precursor are summarized in Table 1 and agree with the latter alternative. Degradation of the radioactive pigment gave ^{14}C-labelled pyridine-2,4,6-tricarboxylic acid free of tritium and proline

TABLE 1. INCORPORATION OF DOUBLY LABELLED TYROSINE INTO INDICAXANTHIN BY *Opuntia ficus-indica*

Expt. No.	Radioactive L-tyrosine fed (μCi)		T/C‡ in the substrate	Indicaxanthin isolated			
				Wt (mg)	Incorporation§ ^{3}H	Incorporation§ ^{14}C	T/C‡
1	1-^{14}C*	4·5	1·04	7·8	0·21	0·38	0·57
	3′,5′-^{3}H†	4·7					
2	1-^{14}C*	3·5	2·46	4·3	0·24	0·45	1·31
	3′,5′-^{3}H†	8·6					
3	1-^{14}C*	2·7	3·70	11·1	0·31	0·57	2·01
	3′,5′-^{3}H†	10·0					
4	1-^{14}C*	2·3	4·00	12·8	0·23	0·42	2·19
	3′,5′-^{3}H†	9·2					
5	1-^{14}C*	2·2	10·10	17·4	0·12	0·23	5·26
	3′,5′-^{3}H†	22·3					

* Specific activity 50 mCi/mmol.
† Specific activity 25 Ci/mmol.
‡ Ratio of tritium to ^{14}C.
§ $\frac{\text{Total dpm in indicaxanthin} \times 100.}{\text{total dpm fed}}$

completely devoid of radioactivity, in agreement with a specific incorporation of tyrosine without noticeable scrambling of the label.



SCHEME 2. PROPOSED PATHWAY FOR THE BIOSYNTHESIS OF INDICAXANTHIN IN *Opuntia ficus-indica*.

Accordingly, a scheme for the biogenesis of indicaxanthin can be proposed (Scheme 2), but the possibility that the closure of the dihydropyridine ring occurs after the step of

condensation with proline must be taken into consideration. Replacement in this scheme of proline by different amino acids or amines gives a probable general route of biogenesis of all the betalains.

EXPERIMENTAL

Plant material. Mature fruits of *Opuntia ficus-indica* Mill. (orange yellow variety) were used immediately after collection.

Administration of L-1-^{14}C, 3′5′-^{3}H-*tyrosine and isolation of radioactive indicaxanthin.* In a typical run, three fruits were injected with an aq. solution of doubly labelled tyrosine (mixture of L-1-^{14}C-tyrosine and L-3′,5′-^{3}H-tyrosine, see Table 1). At the end of the incubation period (24 hr) the radioactive indicaxanthin was isolated according to a previously described procedure[9] and purified to constant specific activity by recrystallization from water.

Degradation of radioactive indicaxanthin. (1) *Pyridine-2,4,6-tricarboxylic acid from indicaxanthin.* A sample of doubly labelled indicaxanthin (47 mg; SA $5{\cdot}1 \times 10^5$ dpm/mmol (^{14}C) $15{\cdot}6 \times 10^5$ dpm/mmol (^{3}H) obtained from a larger scale incubation experiment was oxidized with H_2O_2 as described elsewhere[9] and the isolated pyridine-2,4,6-tricarboxylic acid crystallized from water to constant specific activity. Yield 3·2 mg, SA (^{14}C) $4{\cdot}95 \times 10^5$ dpm/mmol. Tritium activity was negligible. (2) *Proline from indicaxanthin.* Doubly labelled indicaxanthin (11 mg) was subjected to acid degradation and proline isolated as described previously.[9] The amino acid was completely devoid of radioactivity.

Counting of radioactivity. Radioactivity was measured using a Picker Ansitron II liquid scintillation spectrometer. Insta-gel (Packard) was used as the scintillant solution. Prior to counting, coloured samples were bleached with H_2O_2 in alkaline solution. All counts were corrected.

[9] M. PIATTELLI, L. MINALE and G. PROTA, *Tetrahedron* **20**, 2325 (1964).