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BIOSYNTHESIS OF AMARANTHIN IN *CELOSIA PLUMOSA**

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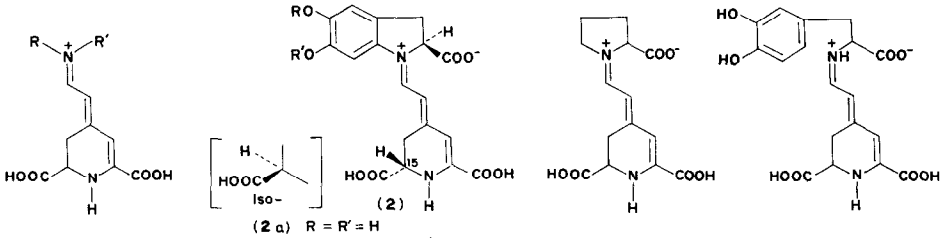
Abstract—Seedlings of a yellow betaxanthin-producing variety of *Celosia plumosa* when fed with appropriate precursors are capable of synthesizing the red-violet pigment normally present in red varieties of the same species, namely amaranthin. Synthesis of amaranthin occurs in seedlings following administration of betanidin and betanin but much greater accumulation was observed after feeding cycloDOPA and its 5-*O*-β-D-glucoside. Possible pathways in the biosynthesis of amaranthin are discussed.

INTRODUCTION

BETALAINS, the nitrogenous pigments of the Centrospermae, have general formula **1** and can be formally considered as immonium derivatives of betalamic acid (**4**). Betacyanins, the red-violet betalains, range in structure from the parent aglycone betanidin (**2a**) to glycosides such as betanin (**2b**), gomphrenin (**2c**) or amaranthin (**2d**) and their C-15 epimers, which can also occur as acyl derivatives, while the yellow betaxanthins, e.g. indicaxanthin (**3**), have an amine or amino acid other than cyclodopa (5,6-dihydroxy-2,3-dihydroindole-2-carboxylic acid) (**5**) attached at the dihydropyridine system. It has been shown by radioactive feeding experiments on the incorporation of DOPA (3,4-dihydroxyphenylalanine) into betanin in the fruits of *Opuntia* ssp. that a minor proportion of the radioactivity appears in the dihydroindole unit and a major proportion in the



dihydropyridine moiety.[1] More recent work has shown that the latter originates from DOPA by extradiol cleavage of the aromatic ring and subsequent closure to the heterocyclic

* Part XIX of the series "Pigments of Centrospermae". For part XVIII see (1973) *Phytochemistry* **12**, 2295. This work was supported by the Consiglio Nazionale delle Ricerche.

[1] MILLER, H. E., RÖSLER, H., WOHLPART, A., WYLER, H., WILCOX, M. E., FROHOFER, H., MABRY, T. J. and DREIDING, A. S. (1968) *Helv. Chim. Acta* **51**, 1470.

system.[2,3] Furthermore, evidence has been reported[4] that in the fruit of *Opuntia dillenii* betanidin is incorporated into betanin, a result which seems to indicate that glycosylation occurs late in the biosynthesis of betacyanins.

To gain a better understanding of the biosynthetic pathway of betacyanins, we fed various presumptive precursors to seedlings of a betaxanthin-producing yellow variety of *Celosia plumosa*. This plant was selected by virtue of its ability to produce, when fed suitable precursors, the betacyanin normally synthesized by red varieties of the same species, namely amaranthin. This made it possible to investigate betacyanin synthesis using readily available unlabelled precursors. The present paper reports the data obtained from these experiments.

METHODS AND RESULTS

Presumptive precursors of amaranthin were administered to seedlings of *C. plumosa* cv. Golden Feather in the presence of ascorbic acid, which was added to prevent losses due to aerial oxidation, particularly severe in the case of cycloDOPA. All compounds were fed in equimolar amounts. Large seasonal differences in the efficiency of incorporation were observed, the best results being obtained in the February–April period.

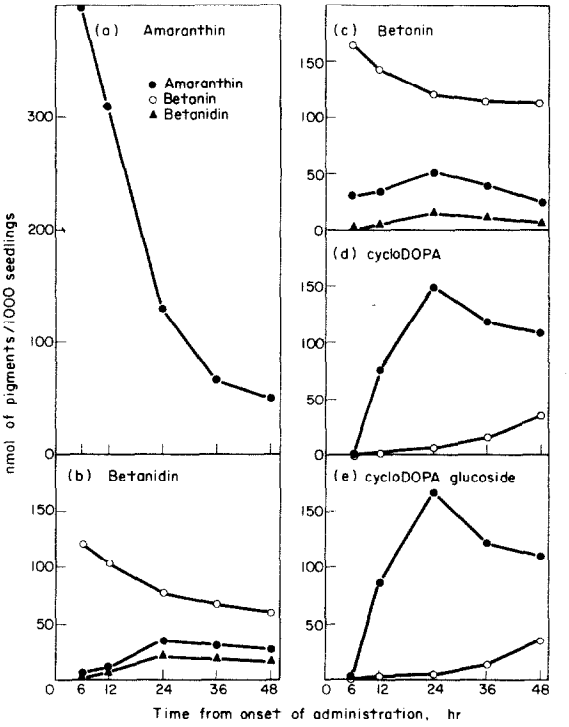


FIG. 1. PATTERN OF BETACYANIN CONTENT IN *Celosia plumosa* SEEDLINGS FOLLOWING ADMINISTRATION OF AMARANTHIN, BETANIDIN, BETANIN, CYCLODOPA, CYCLODOPA GLUCOSIDE.
Points are means of three replicate determinations.

[2] FISCHER, N. and DREIDING, A. S. (1972) *Helv. Chim. Acta* **55**, 649.
[3] IMPELLIZZERI, G. and PIATTELLI, M. (1972) *Phytochemistry* **11**, 2499.
[4] SCIUTO, S., ORIENTE, G. and PIATTELLI, M. (1972) *Phytochemistry* **11**, 2259.

In a preliminary experiment, amaranthin was administered to the seedlings. During the subsequent metabolic period (48 hr) the concentration of the pigment fell sharply with no corresponding increase in betanin or betanidin thus indicating that the aglycone moiety was being metabolized (Fig. 1a).

Since, on the basis of previous evidence,[4] it was reasonable to assume the intermediacy of the aglycone in the biosynthesis of amaranthin, betanidin was then supplied to *Celosia* seedlings. The results indicate (Fig. 1b) that this potential precursor was extensively glucosylated to betanin, which was the main betacyanin present at the end of the feeding period, but further conversion to amaranthin took place to a rather limited extent. A similar relatively poor conversion to amaranthin was also observed when betanin was supplied to the seedlings (Fig. 1c).

Following administration of *S*-cycloDOPA, the amaranthin content increased rapidly until 24 hr after feeding and concomitantly a very slow accumulation of betanin was observed. After a further 24 hr *ca* 25% of the maximum amount of amaranthin had been turned over (Fig. 1d). In order to demonstrate that cycloDOPA was indeed converted into amaranthin and the pigment accumulation was not due to some indirect mechanism, this feeding experiment was replicated using carboxyl-labelled *S*-cycloDOPA. The isolation of radioactive amaranthin with the same specific activity of the precursor clearly showed that this was specifically incorporated into amaranthin. Further experiments with *S*-cycloDOPA-5-*O*-*β*-D-glucoside (**6**) gave results (Fig. 1e) exactly similar to those obtained with *S*-cycloDOPA.

Administration of proline did not result in the formation of indicaxanthin. Finally, there was no evidence for the production of betacyanin when seedlings were fed with DOPAxanthin (**7**).

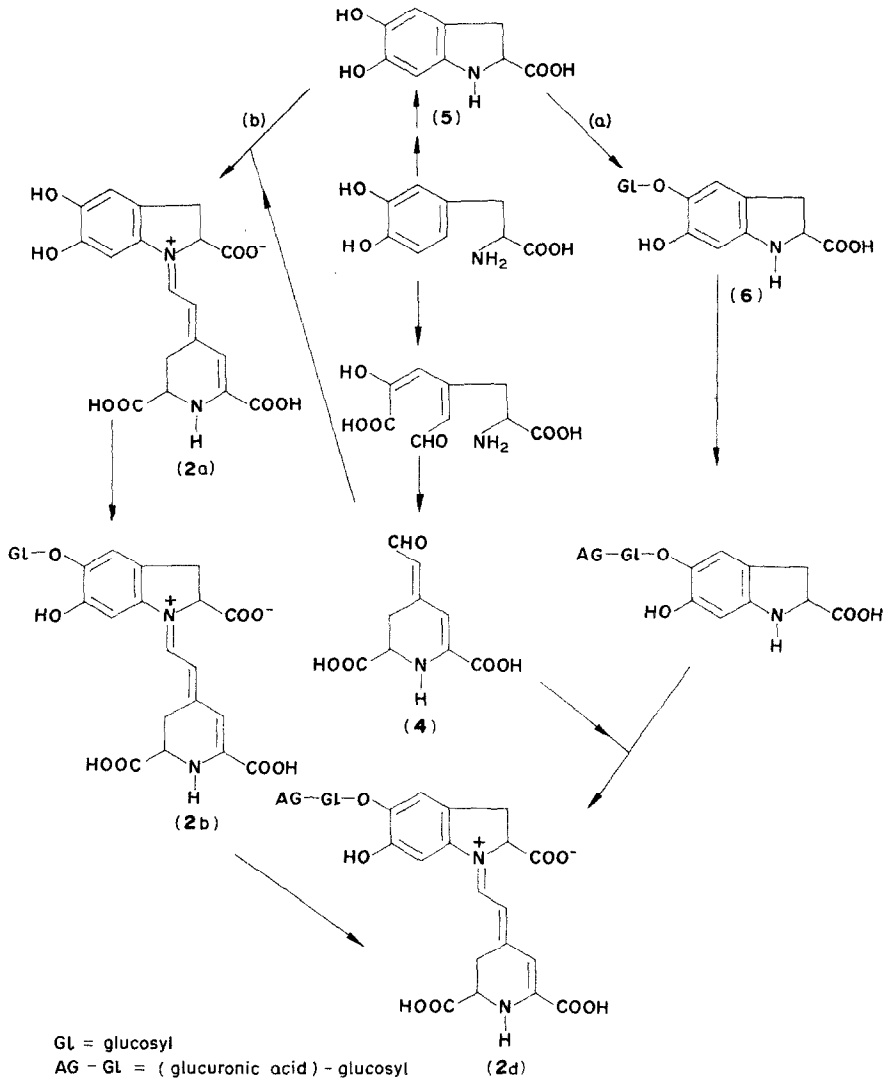
DISCUSSION

The simplest explanation of the results above, in particular the much higher rate of utilization of cycloDOPA as compared to that of betanidin, is that in the plant material investigated the route cycloDOPA → cycloDOPA glucoside → cycloDOPA (glucuronic acid)-glucoside → amaranthin (Scheme 1, a) constitutes the major pathway for amaranthin synthesis while the alternate pathway involving aglycone formation as an intermediate step (b) seems to have far less importance. One cannot exclude that the limited amounts of betanin formed following administration of cycloDOPA originate by way of glucosylation of betanidin resulting from the spontaneous reaction between betalamic acid, the likely key intermediate of betalain biosynthesis, and cycloDOPA. In fact, betalamic acid is known to undergo *in vitro* chemical condensation with this amino acid to give betanidin, as well as with other amino acids or amines to give a range of betalains. However, in view that proline, which *in vitro* reacts readily with betalamic acid,[5] failed to give *in vivo* the relevant betalain (i.e. indicaxanthin), it is reasonable to assume that in the plant such a condensation does not take place to any extent. As *C. plumosa* seedlings were unable to form betacyanin following administration of DOPAxanthin, pathways involving the intermediacy of this betaxanthin can be ruled out.

The relative importance of the pathways leading to amaranthin are, of course, only valid if all the exogenous precursors reach the site of biosynthesis with approximately the same efficiency. Also, it must be pointed out that although *C. plumosa* seedlings possess

[5] KIMLER, L., LARSON, R. A., MESSENGER, L., MOORE, J. B. and MABRY, T. J. (1971) *Chem. Commun.* 1329.

a biosynthetic potential for synthesizing amaranthin, they normally produce only betaxanthin.



SCHEME 1. POSSIBLE ROUTES TO THE BIOSYNTHESIS OF AMARANTHIN.

EXPERIMENTAL

Materials. Samples of betanidin, betanin, amaranthin and DOPAxanthin were available from earlier studies.[6-8] *S*-CycloDOPA hydrochloride was synthesized according to the method of Wyler *et al.*[9] Essentially the same method was followed to prepare *S*-cycloDOPA-[1-^{14}C] hydrochloride (sp. act. $4{\cdot}24 \times 10^8$ dpm/mM) from L-DOPA-[1-^{14}C]. *S*-CycloDOPA-5-*O*-β-D-glucoside was obtained from betanin according to Sciuto *et al.*[4]

[6] PIATTELLI, M., MINALE, L. and PROTA, G. (1964) *Ann. Chim.* **54**, 963.
[7] MINALE, L., PIATTELLI, M., DE STEFANO, S. and NICOLAUS, R. A. (1966) *Phytochemistry* **5**, 1037.
[8] IMPELLIZZERI, G., PIATTELLI, M. and SCIUTO, S. (1973) *Phytochemistry* **12**, 2293.
[9] WYLER, H. and CHIOVINI, J. (1968) *Helv. Chim. Acta* **51**, 1476.

Plants. Seeds of *Celosia plumosa* cv. Golden Feather were surface sterilized in sodium hypochlorite soln, repeatedly washed with sterile tap H_2O and germinated under sterile conditions on moist filter paper in the dark at 28° for 56 hr.

Feeding. Seedlings were selected on the basis of uniform size and, after removal of the radicle, the requisite precursor was allowed to absorb through the cut end for 6 hr. All the compounds used were administered in 5×10^{-4} M aq. soln containing an equimolar amount of ascorbic acid. During the feeding period the seedlings were illuminated with fluorescent white light (5000 lx) in a ventilated growth chamber. At the end of feeding period the seedlings were placed on moist filter paper and allowed to metabolize in darkness at 28° for various lengths of time (0, 6, 18, 30 and 42 hr). At the end of each defined time period a sample group (1000 seedlings) was extracted; the growth of the seedlings which had absorbed the precursor appeared essentially the same as that of the controls.

Isolation, identification and quantitative determination of betacyanins. Plant material was homogenized in 50% aq. MeOH and the homogenate, clarified by centrifugation, was used for the analysis of betacyanins by column chromatography on polyamide powder, according to a previously reported procedure.[10] Identification of pigments was based on retention volumes on the polyamide column, co-chromatography and co-electrophoresis with reference samples. Quantitative analyses were made by comparison to standard curves obtained with pure compounds, using integrated areas of peaks.

Administration of labelled S-cyclodopa and isolation of radioactive amaranthin. *S*-CycloDOPA-[1-^{14}C] hydrochloride was administered in aq. soln to 2000 seedlings of *C. plumosa*, in the experimental conditions reported above for the feeding of non-radioactive precursors. At the end of the metabolic period (18 hr) the seedlings were homogenized with 50% aq. MeOH and after centrifugal removal of the insoluble matter, the betacyanin fraction was isolated from the extract by column chromatography on Dowex 50W-X2 (H^+). From the crude betacyanin fraction, amaranthin was isolated by polyamide column chromatography followed by chromatography on Dowex 50W-X2 (H^+) and purified by high-voltage electrophoresis (0·05 M pyridinium formate pH 4·5). Further purification by recrystallization after addition of non-radioactive amaranthin did not affect significantly, allowed for dilution, the specific radioactivity which was essentially the same ($4{\cdot}32 \times 10^8$ dpm/mM) as that of *S*-cycloDOPA.

Measurements of radioactivity. Radioactivity was measured with a scintillation spectrometer using Insta-gel (Packard) as the scintillant soln. Prior to counting, coloured samples were bleached with H_2O_2 in alkaline soln.

[10] PIATTELLI, M. and MINALE, L. (1964) *Phytochemistry* **3**, 547.