Phytochemistry, 1975, Vol. 14, pp. 479–481. Pergamon Press. Printed in England.

LIGHT CONTROL OF AMARANTHIN SYNTHESIS IN ISOLATED *AMARANTHUS* COTYLEDONS*

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(*Received* 10 *July* 1974)

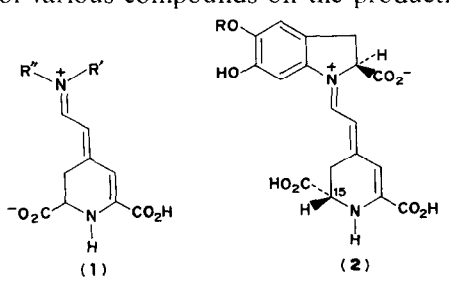
Key Word Index—*Amaranthus tricolor*; *A. caudatus*; Amaranthaceae; biosynthesis; light; phytochrome; betalains; amaranthin.

Abstract—The effect of light on the amaranthin synthesis stimulated by exogenous precursors has been studied in isolated cotyledons of *Amaranthus tricolor* and *A. caudatus*. The results indicate that light acts at the level of the formation of the dihydropyridine moiety of the pigment.

INTRODUCTION

Betalains (**1**), the characteristic pigments of Centrospermae, may be divided into two groups, the red–violet betacyanins (e.g. **2**a) and the yellow betaxanthins, in which the dihydropyridine moiety is bound to an amine or amino acid other than *S*-cycloDOPA (5,6-dihydroxy-2,3-dihydro-2*S*-indole-2-carboxylic acid). Tracer experiments [1–4] have shown that the dihydropyridine unit originates, possibly via betalamic acid, from DOPA (L-3,4-dihydroxyphenylalanine); this amino acid is also precursor of the dihydroxydihydroindole moiety of betacyanins [5].

Early studies by Garay and Towers [6] on the efficacy of various compounds on the production of amaranthin (**2**b) in illuminated seedlings of *Amaranthus tricolor* showed, however, that DOPA was less effective than tyrosine. To explain this unexpected result, it was supposed that any DOPA fed was largely oxidized by phenoloxidases to melanin-like black compounds. In the presence of ascorbic acid, blackening of DOPA-treated seedlings was inhibited, but amaranthin production was not enhanced. In fact, administration of ascorbic acid together with DOPA or tyrosine resulted in a decreased synthesis of amaranthin. Further experiments by French *et al.* [7] indicated that in seedlings of *A. caudatus* var. *viridis* exogenously supplied DOPA elicits a moderate stimulation of dark synthesis of amaranthin, and tyrosine a still smaller promotion, while in illuminated seedlings the accumulation of pigment is considerably greater and of similar magnitude in the presence of either of these precursors. On the basis of this and related observations these authors suggested that light promotes at least two reactions in the biosynthetic pathway, one between tyrosine and DOPA and a second between DOPA and amaranthin.



(1)

(2)

(1a) R′ = H ; R″ = $HO_2C-CH_2-CH_2-CH-CO_2H$

(1b) R′ = H ; R″ = $HO_2C-CH_2-CH-CO_2H$

(1c) R′ = H ; R″ = $(OH)_2C_6H_4-CH_2-CH_2-CH-CO_2H$

(2a) R = H

(2b) R = (glucuronic acid)-glucosyl

This communication describes a study on the amaranthin synthesis in the dark and in the light by isolated cotyledons of *A. caudatus* and *A. tricolor*, in the presence of exogenous precursors. The results show that formation of the dihydropyridine moiety from DOPA is light controlled, but do not confirm or contradict the assumption [7] that the reaction tyrosine → DOPA is stimulated by light.

* This work was supported by the Consiglio Nazionale delle Ricerche.

RESULTS AND DISCUSSION

In addition to tyrosine and DOPA, the effect of cycloDOPA has been examined. When in preliminary experiments intact seedlings of *A. tricolor* cv. Illumination and *A. caudatus* were used, the precursors being administered in aqueous solution via the roots, formation of melanin-like pigment was observed both inside the seedlings and in the incubation medium, particularly with DOPA and cycloDOPA. Blackening was also observed when precursors were supplied to de-rooted seedlings through the cut end. However, melanin formation was not visually detectable using cotyledons isolated from etiolated 2-day-old seedlings, which were therefore utilized throughout this work. Since it was observed that crude extracts obtained from cotyledons fed with precursors contained large amounts of DOPAchrome, it proved necessary to purify by chromatography the amaranthin formed in each individual experiment before its quantitation by spectrophotometry. In experiments with light, the illumination period was no longer than 6 hr, to prevent complications due to the onset of photosynthetic activity.

The results, summarized in Table 1, clearly show that in both *A. tricolor*, which has an absolute light requirement for pigment synthesis, and in *A. caudatus*, which is capable to synthesize in darkness, DOPA has a stimulating effect greater than that of tyrosine under all the experimental conditions employed. From the fact that these amino acids are apt to stimulate the dark synthesis it can be deduced that cotyledons, also these from the species with compulsory light requirement, possess the complete biochemical apparatus necessary for their utilization in the amaranthin synthesis. The activity of this apparatus is strongly enhanced by a 6 hr irradiation with white light, as shown by the fact that in illuminated cotyledons fed with tyrosine or DOPA the amaranthin accumulation is much greater than in dark controls supplied with the same precursors. This and observation that cycloDOPA failed to enhance amaranthin production in the dark indicate that light acts on the reaction(s) leading from DOPA to the dihydropyridine moiety.

Table 1. Effect of tyrosine, DOPA and cycloDOPA on amaranthin synthesis in isolated cotyledons of *Amaranthus caudatus* and *A. tricolor*

Treatment compound (concn in mg/ml)	Amaranthin (10^{-11} mol/seedling)	
	A. caudatus	*A. tricolor*
Dark		
Control	2·75	0·00
Tyrosine (0·5)	4·42	0·95
DOPA (0·5)	7·34	2·43
CycloDOPA (0·5)	2·70	0·00
15 min white light	4·06	1·21
Tyrosine (0·5)	5·95	2·36
DOPA (0·5)	10·37	4·91
CycloDOPA (0·5)	4·10	1·24
6 hr white light	10·85	8·75
Tyrosine (0·05)	11·56	10·90
Tyrosine (0·1)	14·28	12·34
Tyrosine (0·5)	16·83	15·37
DOPA (0·05)	13·46	12·24
DOPA (0·1)	20·40	17·85
DOPA (0·5)	27·20	23·71
CycloDOPA (0·5)	10·75	8·70

Considering that a 15 min illumination period, which is in general sufficient to the photoactivation of phytochrome, has a much smaller stimulatory effect, it appears reasonable to assume, in agreement with previous findings [8], that phytochrome is not the only photoreceptor mediating this action of light.

In the course of amaranthin isolation it was observed that small amounts of a yellow pigment were present in cotyledons illuminated and/or fed with DOPA. This pigment was shown to be a mixture of a number of betaxanthins, among them vulgaxanthin II (**1a**), and miraxanthin II (**1b**) [9, 10]; DOPAxanthin (**1c**) [11] was entirely absent, even when cotyledons were supplied with DOPA, and this demonstrates that *in vivo* a spontaneous condensation of DOPA with betalamic acid or a chemical equivalent does not take place.

In conclusion, the results presented here suggest that the photocontrol of amaranthin synthesis occurs at the level of the formation of the dihydropyridine portion of the molecule, and that the light requirement is not related to the low-energy phytochrome system alone. There is no evidence that light influences other points on the biosynthetic pathway, e.g. the reaction tyrosine → DOPA as claimed by French *et al.* [7], neither do the data reported in the present paper deny this possibility.

EXPERIMENTAL

Plant material. Seeds of *Amaranthus caudatus* and *A. tricolor* cv. Illumination were germinated in darkness at 28° in Petri

dishes on 2 layers of filter paper moistened with tap water and used when 2-days-old.

Chemicals. Chemicals were obtained commercially, except for *S*-cycloDOPA.HCl which was synthesized according to the method of Wyler and Chiovini [12].

Feeding. Cotyledons were isolated from seedlings selected on the basis of uniform size and allowed to absorb the requisite compound (L-tyrosine, L-DOPA or *S*-cycloDOPA) for 6 hr. At the end of the feeding period cotyledons were, where necessary, illuminated with fluorescent white light (5000 lx) for the desired length of time and returned to darkness. Pigment extraction was performed 24 hr after the onset of the experiment.

Methods of analysis. At the end of each treatment an equivalent number of cotyledons were homogenized in acetate buffer pH 4·5 and the homogenate, clarified by centrifugation (10000 *g*), was passed through a column of Dowex 50W-X2 (H^+). After washing with 0·1% HCl, pigment was eluted with H_2O and amaranthin estimated by UV spectrophotometry. Chromatographic analysis of the eluate showed it to contain, in addition to amaranthin, small amounts of betaxanthins; the major yellow pigments were identified as vulgaxanthin II and miraxanthin II by comparison (co-TLC, co-electrophoresis and degradation experiments) with authentic samples. DOPAxanthin was not present in the pigment mixture.

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