Phytochemistry, 1972, Vol. 11, pp. 3195 to 3198. Pergamon Press. Printed in England.

EFFECT OF DIPHENYLAMINE ON CAROTENOID SYNTHESIS IN *DICTYOCOCCUS CINNABARINUS*

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(*Received* 2 *March* 1972. *Accepted* 23 *June* 1972)

Key Word Index—*Dictyococcus cinnabarinus*; chlorophyceae; biosynthesis; keto-carotenoids; diphenylamine.

Abstract—The effect of diphenylamine has been studied on carotenoid synthesis in *Dictyococcus cinnabarinus* grown in submerged culture. At low concentration diphenylamine inhibits the synthesis of keto-carotenoids and stimulates that of two xanthophylls, *iso*-cryptoxanthin and *iso*-zeaxanthin, with hydroxyl groups in the same position as the keto groups of the keto-carotenoids. At higher concentrations, diphenylamine inhibits the desaturation of phytoene.

INTRODUCTION

DESPITE numerous studies on keto-carotenogenesis, several steps are still far from being clear. The most debated points concern the insertion of ketonic groups.[1-8] *Dictyococcus cinnabarinus* seems an excellent organism for the study of keto-carotenogenesis; it is capable of large metabolic variations and produced β-carotene, echinenone, 3,4-dioxo-β-carotene, canthaxanthin, astacene and neoxanthin when it is heterotrophically grown in the presence of glucose in submerged culture. Under these conditions, keto-carotenoids account for 90% of total carotenoids, and belong to the same type.[9,10]

The present work was undertaken to investigate a possible pathway of keto-carotenoid formation.

RESULTS AND DISCUSSION

iso-*Cryptoxanthin*

The pigment had absorption spectra in different solvents and a position on chromatographic columns identical to those of *iso*-cryptoxanthin obtained by reduction of echinenone with $NaBH_4$.[9] The visible absorption spectrum showed a shift to longer wavelengths on treatment with acidic chloroform. This behaviour is consistent with the introduction of an extra conjugated double bond formed by the loss of a water molecule, and points to the allylic character of the hydroxyl with respect to the first ethylenic bond of the conjugated

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chain. Identity with *iso*-cryptoxanthin was confirmed by co-chromatography with an authentic *iso*-cryptoxanthin sample (R_f 0·25 on kieselguhr paper, 2% acetone-light petrol.), and by the similarity of IR spectra.

iso-*Zeaxanthin*

This pigment had absorption spectra identical to those of zeaxanthin in various solvents. On alumina and magnesium oxide-celite chromatographic columns it had the same position as an authentic sample of zeaxanthin. It also had all the characteristics of *iso*-zeaxanthin obtained from canthaxanthin by reduction with $NaBH_4$; for example, it reacted with $CHCl_3$–HCl, indicating the allylic character of the hydroxyl groups with respect to the first ethylenic bond of the conjugated chain. These results indicate that the pigment studied could be *iso*-zeaxanthin with the hydroxyls in position 4,4'. Identity with *iso*-zeaxanthin was confirmed by co-chromatography on TLC with authentic *iso*-zeaxanthin and zeaxanthin samples. On TLC on silica gel G with starch, with *n*-hexane-Et_2O (3:7) as solvent, it had the same R_f as synthetic *iso*-zeaxanthin (0·63); R_f for synthetic zeaxanthin was 0·17. IR spectra were nearly superimposable.

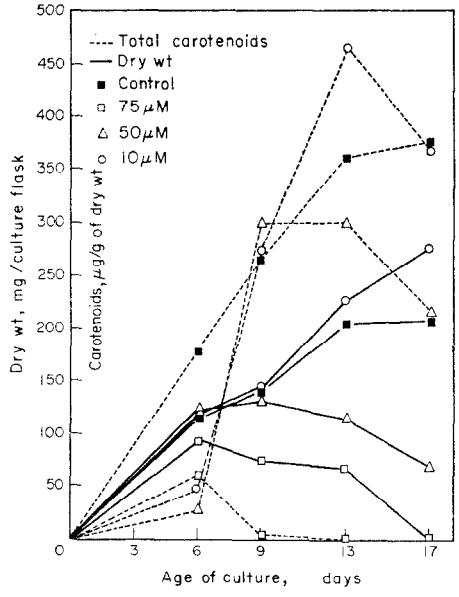

FIG. 1. EFFECT OF DIPHENYLAMINE ON GROWTH AND SYNTHESIS OF CAROTENOIDS IN *Dictyococcus cinnabarinus*.
The algal dry weight is expressed as mg/100 ml of culture fluid.

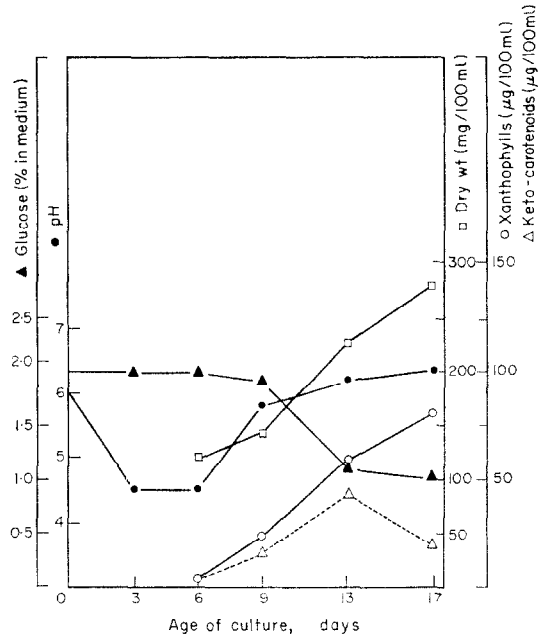

FIG. 2. EFFECT OF 10 μM DIPHENYLAMINE ON THE SYNTHESIS OF KETO-CAROTENOIDS AND XANTHOPHYLLS IN *Dictyococcus cinnabarinus*.

Effects of Diphenylamine

Figure 1 shows that 10 μM diphenylamine caused an increase in the dry wt of the alga after the 9th day, whereas 50 and 75 μM diphenylamine had an inhibitory effect on growth. Diphenylamine reduced the initial velocity of carotenoid synthesis at all the concentrations studied. At 75 μM, it was completely inhibitory.

Diphenylamine at a concentration of 10 and 50 μM changed the pattern of carotenod levels (Table 1). At 10 μM, it stimulated the formation of β-carotene, whereas at 50 μM only trace amounts of this pigment were detected. The total amount of keto-carotenoids (echinenone, 3,4-dioxo-β-carotenene, canthaxanthin and astacene) represented 97% of total carotenoids in cultures without diphenylamine, but only about 30% in cultures with 10 μM diphenylamine; with 50 μM diphenylamine keto-carotenoids were not detected. Moreover, in the presence of diphenylamine, xanthophylls appeared, although they could not be found in control cultures of the alga.

TABLE 1. EFFECT OF DIPHENYLAMINE ON CAROTENOID PRODUCTION IN *Dictyoccocus cinnabarinus*

Carotenoid	Control (μg/g dry wt)	(%)	10 μM (μg/g dry wt)	(%)	50 μM (μg/g dry wt)	(%)
Phytoene	—	—	12·6 ± 1·4	2·6	260·0 ± 2·1	86·2
Phytofluene	—	—	—	—	5·2 ± 0·3	1·8
Zeacarotene	—	—	—	—	14·3 ± 1·5	5·0
β-Carotene	10·8 ± 1·1	2·8	47·6 ± 5·2	10·2	Traces	—
Echinenone	184·2 ± 2·0	50·6	44·2 ± 2·3	9·6	—	—
3,4-Dioxo-β-carotene	12·3 ± 1·4	3·4	Traces	—	—	—
Canthaxanthin	111·1 ± 10·1	30·8	78·7 ± 5·3	16·9	Traces	—
Astacene	45·2 ± 3·2	12·4	19·8 ± 2·2	4·3	—	—
iso-Cryptoxanthin	—	—	53·2 ± 4·1	11·5	—	—
iso-Zeaxanthin	—	—	185·4 ± 8·5	39·7	3·8 ± 0·2	1·3
Lutein	—	—	19·8 ± 0·9	4·3	16·9 ± 1·5	5·7
Neoxanthin	Traces	—	4·1 ± 0·3	0·9	Traces	—
Total carotenoids	363·6	100·0	465·4	100·0	300·2	100·0

The levels of the various carotenoid pigments were measured on the 13th day of growth in submerged culture with 0–50 μM diphenylamine. Means of four experiments are presented. The average dry wt of cells per culture flask (100 ml) was 205·5 mg for controls, 227·2 mg for 10 μM diphenylamine and 115 mg for 50 μM diphenylamine.

The cultures containing 10 μM diphenylamine were analyzed in detail (Fig. 2). Absorption of glucose was low initially and the pH of the culture medium fell to 4·5. The pH returned to its original value after 9 days and glucose uptake increased. After 9 days, keto-carotenoids decreased and xanthophylls increased.

In *Dictyococcus cinnabarinus* diphenylamine blocks phytoene dehydrogenation. Lycopene was not detected in any of the experimental conditions tested, but consistent amounts of zeacarotene were found in cultures containing 50 μM diphenylamine (Table 1).

In conclusion, at low concentrations, diphenylamine inhibits the insertion of a keto-group in the carotenoid molecule whilst at higher concentrations, the well-known effect of inhibition of phytoene desaturation occurs. Since *iso*-cryptoxanthin and *iso*-zeaxanthin have the hydroxyl groups in the same position as the keto groups of the keto-carotenoids, we think, therefore, that these xanthophylls might belong to the same synthetic pathway as the keto-carotenoids in *Dictyococcus cinnabarinus*.

EXPERIMENTAL

Strain. The strain used was *Dictyococcus cinnabarinus* 280 (Kol. Chodat) Fischer, obtained from the algal collection of the Botanical Institute of the University of Geneva (Switzerland). Cultural conditions, the method of extraction and of chromatographic purification of pigments has been described previously.[9]

Diphenylamine (10, 50, 75 μM) was added at the time of the inoculum in a 95% EtOH solution. The final concentration of EtOH in the culture medium did not exceed 0·15 ml/100 ml. In control cultures the same amounts of EtOH were added. Carotenoid concentrations were measured spectrophotometrically using the values given by Davies.[11]

Acknowledgements—The author is most grateful to Professor T. W. Goodwin for valuable suggestions, to Miss A. Contenti and Mr. G. Nusdorfi for technical assistance and to Mr. A. Adriani for the preparation of algae cultures.

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