Bucher, N. L. R. (1953). *J. Amer. chem. Soc.* **75**, 498.
Goodwin, T. W. (1958*a*). *Proc. 4th int. Congr. Biochem., Vienna*, **11**, 54.
Goodwin, T. W. (1958*b*). *Biochem. J.* **70**, 612.
Goodwin, T. W. (1959). In *Biosynthesis of Terpenes and Sterols*, p. 279. Ed. by Wolstenholme, G. E. W. & O'Connor, M. London: J. and A. Churchill Ltd.
Modi, V. V. (1955). Ph.D. Thesis. University of Liverpool.
Shenour, E. A. & Zabin, I. (1959). *J. biol. Chem.* **234**, 770.
Wolf, D. E., Hoffman, C. H., Aldrich, P. E., Skeggs, H. R., Wright, L. D. & Folkers, K. (1957). *J. Amer. chem. Soc.* **79**, 1486.
Wolstenholme, G. E. W. & O'Connor, M. (1959). *Biosynthesis of Terpenes and Sterols*, p. 311. London: J. and A. Churchill Ltd.

Biochem. J. (1960) **76**, 197

Studies in Carotenogenesis

28. THE EFFECT OF ILLUMINATION ON CAROTENOID SYNTHESIS IN FRENCH-BEAN (*PHASEOLUS VULGARIS*) SEEDLINGS

BY T. W. GOODWIN* AND S. PHAGPOLNGARM
Department of Biochemistry, The University, Liverpool 3

(*Received* 19 *November* 1959)

It has clearly been established that seeds of many plants, if germinated under the most stringent conditions of light-exclusion, synthesize carotenoids (see Goodwin, 1952). Xanthophylls predominate and, in maize, wheat and barley, are similar to those present in green leaves (Strain, 1938). However, investigators have not been unanimous about the changes undergone on illuminating etiolated seedlings. Xanthophylls were said to develop more quickly than carotenes by Beck (1937), Seybold & Egle (1938) and Nagel (1940), whereas Barrenscheen, Pany & Srb (1942) and Blaauw-Jansen, Komen & Thomas (1950), using wheat seedlings, claimed that carotenes increased more quickly than xanthophylls. More recently it has been stated that illumination of etiolated maize seedlings brought about a specific stimulation of β-carotene (Kay & Phinney, 1956). This observation was followed up by Goodwin (1958), who considered that illuminated etiolated maize seedlings might be a good system for studying the biosynthesis of β-carotene in higher plants. He confirmed that the etiolated seedlings produced mainly xanthophylls, with only a trace of β-carotene, but found that on illumination of the seedlings β-carotene and xanthophylls were synthesized together in the same relative proportions as exist in chloroplasts. Indeed the carotenoids synthesized on illumination are merely a manifestation of the formation of functional chloroplasts. As this is a fundamental change undergone by all etiolated seedlings on illumination, it would appear probable that the type of increase in carotenoids observed by Goodwin (1958) was a general phenomenon, and that the contradictory results previously recorded were due to extraneous factors; in the earlier investigations inadequate analytical methods may well have been the cause.

However, it was decided to carry out similar experiments to those reported for maize on a dicotyledon; the french bean (*Phaseolus vulgaris*) was chosen as suitable material.

EXPERIMENTAL

Seedlings. The variety of French bean (*Phaseolus vulgaris*) used was Canadian Wonder. The seedlings were grown and illuminated under the same conditions as those described for maize by Goodwin (1958).

Extraction, purification, identification and determination of carotenoids. The cotyledons and true leaves were separately examined. The techniques used for the extraction, purification, identification and determination of the constituent carotenoids have been previously described in detail (Goodwin, 1955, 1958).

RESULTS

Carotenoids present in cotyledons of etiolated bean seedlings. The pigments from the etiolated cotyledons were chromatographed on columns of icing sugar and a typical result is given in Table 1. Fraction A, which ran straight through the column when light petroleum was used, was purified on alumina and shown to be β-carotene by chromatographic and spectroscopic comparison with an authentic specimen of synthetic β-carotene.

Fraction B was hypophasic in the light petroleum–aqueous 90% (v/v) methanol test and had an absorption spectrum similar to that of lutein. This

---

* Present address: Department of Agricultural Biochemistry, University College of Wales, Aberystwyth.

provisional identification was confirmed by cochromatography with an authentic specimen of lutein prepared from grass. No separation was achieved.

Fraction C had the general properties of either chrysanthemaxanthin or flavoxanthin. These two pigments are both 5:8-epoxylutein, probably differing only in the spatial disposition of the two oxygen atoms at positions 3 and 5 in the molecule (Karrer & Jucker, 1951). After some difficulty, because it rapidly undergoes changes on manipulation, fraction C was completely purified, as indicated by the fact that the absorption maxima at 420 and 448 mμ had the same *E* values (Karrer & Jucker, 1951). A zinc carbonate–Celite mixture (4:1, w/w) was more effective than icing sugar in the final stages of purification. On cochromatographing fraction *C* with an authentic (crystalline) specimen of chrysanthemaxanthin on either icing sugar or on zinc carbonate–Celite mixture no separation could be achieved; on the other hand, a mixture of fraction *C* and authentic (crystalline) flavoxanthin was easily separated into two fractions. It was concluded that fraction C was chrysanthemaxanthin.

Fraction D had all the properties of auroxanthin (5:8–5:8-diepoxyzeaxanthin) and its identity was confirmed by chromatographic and spectrographic comparison with authentic auroxanthin isolated from pansies (*Viola tricolor*) according to the method of Karrer & Rutschmann (1942).

Fraction E, which occurred only in comparatively small amounts, had the same spectral maxima as flavoxanthin and chrysanthemaxanthin but was much more strongly adsorbed. No attempt at further characterization was made, but this fraction is probably a polyhydroxy derivative of either flavoxanthin or chrysanthemaxanthin.

Quantitative determinations show that auroxanthin is the predominating cotyledon pigment. The total concentration of pigments in the cotyledons, about 1 mg./g. (dry wt.), is about one-tenth of that in the true leaves.

Carotenoids present in leaves of etiolated bean seedlings. The pigments from the etiolated leaves of bean seedlings were analysed in the same way as the cotyledon extracts. A typical result is given in Table 2. The three main pigments were shown by the usual criteria to be β-carotene (F), lutein (G) and neoxanthin (K); these are the characteristic pigments found in all etiolated leaves (Strain, 1938; Goodwin, 1958). Fraction (H) occurred only in traces and could not be unequivocally identified; it probably represented traces of chrysanthemaxanthin. The quantitative pigment distribution is also characteristic of etiolated leaves, although the β-carotene value is somewhat higher than in many previously reported cases (Goodwin, 1952). The total concentration of pigments is 10–15 mg./g. dry wt. This is some ten times as great as that in the cotyledons but only one-tenth of that in green leaves (Goodwin, 1952, 1958).

Effect of light on etiolated bean seedlings. The effect of illumination on the carotenoid content of the cotyledons of etiolated seedlings is given in Table 3; the effect was found to be essentially the same in

Table 1. *Pigments in the cotyledons of etiolated bean seedlings*

The adsorbent was icing sugar and the developer was light petroleum containing increasing amounts of acetone; fractions are listed in order of increasing adsorptive power.

Fraction	Description	Absorption maxima (mμ) in light petroleum	Acetone (%, v/v) in light petroleum required for elution	Identification	Percentage of total pigments present
A	Yellow	448, 475	0	β-Carotene	10·7
B	Deep yellow	420, 442, 470	1	Lutein	8·3
C	Pale yellow	420, 448	3–4	Chrysanthemaxanthin	23·6
D	Pale yellow	400, 424	4–5	Auroxanthin	46·0
E	Yellow*	420, 448	10	Unknown	11·4

* Determined by assuming $E^{1\%}_{1\,\text{cm.}}$ to be that of β-carotene (2500).

Table 2. *Pigments in the true leaves of etiolated bean seedlings*

The adsorbent was icing sugar and the developer was light petroleum containing increasing amounts of acetone; fractions are listed in order of increasing adsorptive power.

Fraction	Description	Absorption maxima (mμ) in light petroleum	Acetone (%, v/v) in light petroleum required for elution	Identification	Percentage of total pigments present
F	Yellow	448, 475	0	β-Carotene	10·8
G	Yellow	442, 471	0·5–0·7	Lutein	38·4
H	Yellow	420, 440, 468	5	Unknown	Trace
K	Yellow	438, 465	10	Neoxanthin	50·7

both 5- and 8-day seedlings. There is relatively a very small increase in the chrysanthemaxanthin and auroxanthin concentrations, but a very marked synthesis of β-carotene, lutein and neoxanthin; that is, the main increase is in the synthesis of 'plastid carotenoids' (Goodwin, 1959) and reflects the stimulation by light of the formation of functional chloroplasts (Goodwin, 1958).

Table 3. *Changes in carotenoid pigments of the cotyledons of etiolated bean seedlings on illumination*

5-day seedlings were illuminated at 28° for 24 hr.

	Concn. of pigment (μg./g. dry wt.)		
Pigment	Before illumination	After illumination	Increase on illumination
β-Carotene	1·45	6·57	5·12
Lutein	1·12	5·93	4·81
Chrysanthemaxanthin	3·18	3·67	0·49
Auroxanthin	6·21	8·77	2·56
Unidentified	1·54	2·46*	0·92*

* Probably includes traces of neoxanthin.

Table 4. *Changes in carotenoid pigments of the true leaves of etiolated bean seedlings on illumination*

5-day seedlings were illuminated at 28° for 24 hr.

	Concn. of pigment (μg./g. dry wt.)		
Pigment	Before illumination	After illumination	Increase on illumination
β-Carotene	12	109	97
Lutein	41	142	101
Violaxanthin	Absent	65	65
Neoxanthin	54	98	44

The results obtained on illuminating etiolated leaves are given in Table 4. The changes observed are quantitatively much greater than with cotyledons; this again is a reflexion of the formation of functional chloroplasts, which are much more numerous in leaves than in cotyledons. The relative amounts of pigment synthesized in the light are characteristic of chloroplasts and, as with maize (Goodwin, 1958), there is no preferential synthesis of β-carotene on illumination. The stimulation which at first sight appears to take place is due to the fact that β-carotene synthesis in the dark is much less, relative to xanthophylls, than it is in the light. Violaxanthin, a normal component of leaf carotenoids, is also rapidly synthesized in the light, although it is not detectable in etiolated leaves.

DISCUSSION

The investigations reported here have revealed a different distribution of carotenoids in cotyledons and true leaves of etiolated French bean seedlings; this is the first time that such a differentiation has been noted. The xanthophylls in the etiolated leaves are typical of those reported in many other etiolated seedlings, but the predominating pigments in the cotyledons are chrysanthemaxanthin and auroxanthin, pigments previously considered characteristic of carotenogenic flower petals (see Goodwin, 1952). The reason why the cotyledons synthesize such highly oxygenated xanthophylls is unknown, but they must be synthesized afresh because no significant amounts of carotenoids can be found in ungerminated seeds. Extracts of cotyledons may prove to be useful in studying the mechanism of conversion of hydrocarbon carotenoids into xanthophylls.

SUMMARY

1. The main carotenoid pigments in the cotyledons of etiolated French bean (*Phaseolus vulgaris*) are β-carotene, lutein, chrysanthemaxanthin and auroxanthin.

2. The carotenoids in the etiolated leaves of the same plant are β-carotene, lutein and neoxanthin.

3. Illumination of etiolated bean seedlings results in the synthesis in both the cotyledons and the true leaves of the carotenoids associated with chloroplasts; this is a consequence of the formation of functional chloroplasts.

Part of this investigation was made possible only through the generosity of Professor P. Karrer, who very kindly gave us small amounts of crystalline flavoxanthin and chrysanthemaxanthin from his reference collection of carotenoids.

REFERENCES

Barrenscheen, K. H., Pany, P. & Srb, E. (1942). *Biochem Z.* **310**, 335.
Beck, W. A. (1937). *Plant Physiol.* **12**, 885.
Blaauw-Jansen, G., Komen, J. G. & Thomas, J. B. (1950). *Biochem. biophys. Acta*, **5**, 179.
Goodwin, T. W. (1952). *The Comparative Biochemistry of the Carotenoids.* London: Chapman and Hall Ltd.
Goodwin, T. W. (1955). In *Modern Methods of Plant Analysis*, vol. 2, p. 272. Ed. by Paech, K. & Tracey, M. V. Heidelberg: Julius Springer.
Goodwin, T. W. (1958). *Biochem. J.* **70**, 612.
Goodwin, T. W. (1959). *Advanc. Enzymol.* **21**, 296.
Karrer, P. & Jucker, E. (1951). In *Carotenoids*, Ed. by Braude, E. A. Amsterdam: Elsevier.
Karrer, P. & Rutschmann, J. (1942). *Helv. chim. acta*, **25**, 624.
Kay, R. E. & Phinney, B. (1956). *Plant Physiol.* **31**, 226.
Nagel, W. (1940). *Bot. Arch.* **40**, 1.
Seybold, A. & Egle, K. (1938). *Planta*, **28**, 87.
Strain, H. H. (1938). *Publ. Carneg. Instn*, no. 490.