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CAROTENOID PIGMENTS IN THE STEM OF *CUSCUTA AUSTRALIS*

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Abstract—*Cuscuta australis* was found to contain β- and γ-carotene, α-carotene-5,6-epoxide and two xanthophylls, taraxanthin and lutein. Two unknown carotenes in small amounts are also present. The pigment ratios are of the same order as the non-parasitizing plants.

INTRODUCTION

IT WAS well known from 1883[1] that the normal yellow–orange coloration in dodder is due to an unusually high content of carotenoids, but only in 1935[2] was the composition of the carotenoid system exactly determined in *Cuscuta subinclusa* and in *Cuscuta salina*. In these Mackinney[2] found a considerable amount of γ-carotene, some α- and β-carotene, and traces of lycopene and rubixanthin: the presence of chlorophyll was reported earlier[1–3]. During an investigation on the changes of chlorophyll *a*/*b* ratio and photosynthesis in *Cuscuta australis*,[4–5] it was decided to examine also the carotenoid composition and chlorophyll–carotenoid ratio in this plant, in view of their possible significance.

RESULTS

When the epiphase pigments obtained from an acetone extract of the dodder were chromatographed in *n*-hexane on a MgO:celite (1:1) column, five bands appeared (Tables 1 and 2). Fraction 1 (orange), eluted with hexane, showed all the properties of β-carotene, and could not be separated from it on rechromatography on a MgO–celite column. Fraction 2 (orange–pink) was, eluted by 2% acetone in *n*-hexane; the absorption spectra of this fraction in *n*-hexane, chloroform and carbon disulphide suggested that it was γ-carotene, and this was confirmed on co-chromatography with an authentic sample. Fraction 3, (yellow) was eluted by 8% acetone in *n*-hexane and very closely resembled α-carotene-5-6-epoxide. When dissolved in chloroform containing a trace of hydrogen chloride it exhibited the large shift of the absorption maxima to shorter wavelengths previously reported.[6] The remaining two fractions, strongly adsorbed, were eluted by 20% acetone in *n*-hexane and pure acetone respectively, but since they were present in very small amounts could not be identified with any known carotenoid.

When the hypophase pigments dissolved in *n*-hexane were chromatographed on a

[1] F. TEMME (1883) cited by G. J. PIERCE, *Ann. Bot.* **8**, 53 (1894).
[2] G. MACKINNEY, *J. Biol. Chem.* **112**, 421 (1935).
[3] G. J. PIERCE, *Ann. Bot.* **8**, 53 (1894).
[4] F. BERTOSSI, A. BACCARINI and N. BAGNI, *Nuovo giorn. botan. ital.* (In press).
[5] F. BERTOSSI, A. BACCARINI and N. BAGNI, *Nuovo giorn. botan. ital.* (In press).
[6] P. KARRER, *Helv. Chim. Acta* **28**, 474 (1945).

MgO:celite (1:1) column, using increasing concentrations of methanol in *n*-hexane as the developing solvent, only two bands appeared. The former (orange) eluted by methanol in *n*-hexane (49:1) closely resembled taraxanthin or tareoxanthin, in absorption spectra, adsorptive properties and hyposocromic shift after acidification.[7] Owing to lack of material it was impossible for us to distinguish between these two possibilities. The latter (orange-pink) was presumed to be lutein, since it had the same absorption spectra and adsorptive properties, but it has not been unequivocally identified as such.

TABLE 1. THE CHROMATOGRAPHIC SEPARATION OF CAROTENOIDS IN THE STEM OF *Cuscuta australis*

Fraction*	Solvent for elution†	Colour	Relative abundance (% of total)	Identification
Epiphasic				
1	H	Orange	26	β-Carotene
2	H:A (49:1)	Orange-pink	19	γ-Carotene
3	H:A (46:4)	Yellow	21	α-Carotene-5-6-epoxide
4	H:A (40:10)	Yellow-orange	7	Unknown
5	A	Pink	Trace	—
Hypophasic				
1	H:M (49:1)	Orange	27	Taraxanthin or tareoxanthin
2	M	Orange-pink		Lutein?

* Absorbent MgO:celite (1:1); pigment in order of increasing absorptivity.
† H = *n*-hexane, A = acetone, M = methanol.

TABLE 2. ABSORPTION SPECTRA OF CAROTENOID FRACTIONS IN THE STEM OF *Cuscuta australis* (IN VARIOUS SOLVENTS)

Fraction	Wavelength of maximal absorption (mμ) in		
	n-Hexane	Chloroform	Carbon disulphide
Epiphase			
1	~425, 450, 477	465, 496	~448, 482, 515
2	433, 460, 490	443, 470, 502	462, 493, 525
3	438, 465	450, 477	465, 495
4	418, 445, 480	—	—
Hypophase			
1	418, 440, 470	452, 480	442, 470, 498
2	425, 448, 470	—	448, 478, 503

~ Denotes an inflection.

Quantitative determination of the pigments (chlorophylls and carotenoids) enabled the chlorophyll/carotenoid (1·5) and carotenes/xantophylls (2·5) ratios to be calculated. The chlorophyll *a*/*b* ratio was about 3·5–4.

There are very few reports about the carotenoid pigment of Cuscutaceae and of the closely related family of Convolvulaceae so that no taxonomic conclusions can be made. This present investigation has shown that *Cuscuta australis* does not contain α-carotene, lycopene

[7] H. H. STRAIN, *Arch. Biochem. Biophys.* **48**, 458 (1954).

and rubixanthin, found by Mackinney[2] in *Cuscuta subinclusa* and *Cuscuta salina*, and that β-carotene, and not γ-, is the major component as in most plants. Also the presence of lutein is in accordance with this.[9] Besides, the pigment ratios are also of the same order as found in non-parasitic plants and this suggests that the carotenoids may be associated, if not actually involved, in the function of the chloroplasts.

EXPERIMENTAL

Stems of *Cuscuta australis*, detached from the host (*Medicago sativa*), were collected before the floral-bud formation and homogenized in 80% acetone to extract the carotenoid pigments. The pigments were then transferred from the acetone extract to ethyl ether for the chlorophyll determination.[8] This extract was dried *in vacuo* under nitrogen and dissolved in *n*-hexane. The *n*-hexane was diluted with an equal quantity of 90–95% methanol and sufficient water added to form two layers.[9] Pigments were present in both the layers. The epiphase, washed several times with water, was dried over anhydrous Na_2SO_4 overnight and reduced to small bulk ready for chromatography. The hypophase was transferred in *n*-hexane using the same method. The fractions were examined in an Optica CF4 photoelectric spectro-photometer from 350 to 550 mμ. The $E^{1\%}_{1cm.}$ of β-carotene at 450 mμ was taken as 2650, that of γ-carotene at 460 mμ as 2720, and that of mixed xanthophylls at 445 mμ as 2500.[10]

[8] J. H. C. SMITH and A. BENITEZ, in *Modern Method of Plant Analysis*, Vol. 4 (Edited by K. PEACH and M. V. TRACEY), Springer Verlag, Heidelberg (1955).
[9] T. W. GOODWIN, in *Modern Method of Plant Analysis*, Vol. 3 (Edited by K. PEACH and M. V. TRACEY), Springer Verlag, Heidelberg (1955).
[10] E. M. BICKOFF, L. M. WHITE, A. BEVENNE and K. T. WILLIAMS, *J. Agr. Food Chem.* **2**, 563 (1954).