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THE CAROTENOID PIGMENTS IN THE PETALS OF *MIMULUS CUPREUS* AND *MIMULUS TIGRINUS*

T. W. GOODWIN and D. M. THOMAS

Department of Agricultural Biochemistry, University College of Wales, Aberystwyth

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Abstract—β-Carotene constitutes over 80 per cent of the carotenoids in *Mimulus cupreus* (v. Red Emperor), and small amounts of three xanthophylls, one of which is probably taraxanthin are also present. Only about 1 per cent of the total carotenoids in *M. tigrinus* is β-carotene, the remainder being a complex mixture of xanthophylls; taraxanthin was the major component and cryptoxanthin was also detected. In neither species was γ-carotene or pro-γ-carotene found. The rich dark red colour of *M. cupreus* is due mainly to a water-soluble pigment.

INTRODUCTION

ZECHMEISTER and Schroeder[1] in 1942 reported that considerable amounts of γ-carotene and some lycopene were present in extracts of mature flower petals of *Mimulus longiflorus* which grows wild in California. If, however, stems with flower buds were cut and placed in water in diffuse light for several days, the opened flowers contained the poly *cis* compounds, pro-γ-carotene and prolycopene, instead of the all *trans* forms encountered in flowers which had been allowed to open on the plant.[2] In the light of recent work on carotenoid formation, it was decided to reinvestigate these observations and to assess their significance to the problem. The species of *Mimulus* originally used by Zechmeister and Schroeder was not readily available, so two others *M. cupreus* (Red Emperor) and *M. tigrinus* were examined. It was found that neither contained γ-carotene or lycopene or any of their poly *cis* isomers.

RESULTS

Pigments in M. Cupreus

The pigment extract in light petroleum was separated into four main bands on a column of $ZnCO_3$: celite (3:1) (Table 1). Fraction A, which represented some 80 per cent of the total pigment, had all the properties of β-carotene. It was rechromatographed on a column of magnesia, but no α-carotene was present, and its identity with β-carotene was confirmed by mixed chromatography with an authentic specimen on columns of magnesia and alumina.

The three xanthophylls present were very strongly adsorbed and pigments B and C could only be separated after prolonged development of the column with ethyl ether containing 2% (v/v) ethanol. B was not identified but showed some similarities with zeaxanthin. However, on co-chromatography with an authentic specimen, it was found to be much more strongly adsorbed than the latter. C was almost certainly taraxanthin;[3,4] it has the same

[1] L. ZECHMEISTER and W. A. SCHROEDER, *Arch. Biochem.* **1**, 231 (1942).
[2] W. A. SCHROEDER, *J. Amer. Chem. Soc.* **64**, 2510 (1942).
[3] R. KUHN and E. LEDERER, *Z. physiol. Chem. Hoppe-Seyler's* **200**, 108 (1931).
[4] P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLIN, *Helv. Chim. Acta* **28**, 1146 (1945).

spectrum as an authentic specimen isolated from the petals of *Taraxacum officinale*, and could not be separated from it on co-chromatography on a $ZnCO_3$:celite (3:1) column. However, because of the technical difficulties encountered in dealing with such strongly

Table 1. The chromatographic separation of carotenoids of petals of *Mimulus cupreus* (v. red emperor)

Fraction*	Solvent for elution†	Colour	Absorption max. (mμ) in light petroleum (b.p. 60–80°)‡	Relative abundance, % of total	Identification
A	P	Yellow	~ 430, 453, 482	82	β-Carotene
B	P:E(1:9)	Orange–yellow	~ 425, 447·5, 475	5	Unknown
C	E:M(49:1)	Bright yellow	417·5, 442, 472	11	Taraxanthin
D	E:M(20:1)	Yellowish-green	418, 443, 472	2	Unknown

* Adsorbent $ZnCO_3$:Celite (4:1); pigments in order of increasing adsorptivity.
† P = light petroleum (b.p. 60–80°): E = ethyl ether: M = Methanol.
‡ ~ Denotes an inflexion.

adsorbed pigments, it must be concluded that pigment C very closely resembles taraxanthin rather than that it has been unequivocally identified as such. Pigment D could not be identified as any known carotenoid. Three minor pigments could be detected running before pigment B, but they were present in such minute amounts that they could not be examined.

Pigments in M. Tigrinus

In contrast to *M. cupreus*, the carotenoids of *M. tigrinus* separated into 9 zones (Table 2). Fraction A was identified as β-carotene by the criteria described in the previous section; it represented only about 1 per cent of the total pigment extract. Fractions G, H, I, on the basis of co-chromatography appeared to be identical with fractions B, C, D from the *M. cupreus*

Table 2. The chromatographic separation of carotenoids of petals of *Mimulus tigrinus*

Fraction*	Solvent for elution†	Colour	Absorption max. (mμ) in light petroleum (b.p. 60–80°)‡	Relative abundance, % of total	Identification
A	P	Yellow	~ 430, 452, 481	1	β-Carotene
B	P:E(3:2)	Yellow–orange	~ 430, 453, 484	7	Cryptoxanthin
C	P:E(11:9)	Yellow	450, 480	7	Unknown
D	P:E(11:9)	Pale yellow	444, 475	2	Unknown
E	P:E(2:3)	Pale yellow	422·5, 443, 473	Trace	Unknown
F	P:E(2:3)	Pale yellow	—	Trace	—
G	P:E(1:9)	Orange–yellow	~ 425, 447·5, 475	33	Unknown
H	E:M(49:1)	Bright yellow	417·5, 442, 472	46	Taraxanthin
I	E:M(20:1)	Yellowish-green	418, 443, 472	4	Unknown

* Adsorbent $ZnCO_3$:celite (4:1); pigments in order of increasing adsorptivity.
† P = light petroleum (b.p. 60–80°): E = ethyl ether: M = methanol.
‡ ~ Denotes an inflexion.

(Table 1). That is, the major xanthophyll in *M. tigrinus* is almost certainly taraxanthin. The remaining fractions, with the exception of B, were strongly-adsorbed xanthophylls none of which could be identified with any known carotenoid. B closely resembled cryptoxanthin (3-hydroxy-β-carotene) in absorption spectrum and adsorptive properties; however no authentic cryptoxanthin was available for direct comparison.

DISCUSSION

Zechmeister and Schroeder[1] found that *M. longiflorus* synthesized, in addition to γ-carotene and lycopene, β-carotene, cryptoxanthin and zeaxanthin. The marked differences between the pigment distribution in this species and those examined here are (*a*) neither of the latter produces γ-carotenes or lycopene, and (*b*) they both produce a number of strongly-adsorbed carotenoids, such as taraxanthin, which are not found in *M. longiflorus*. A consideration of previous work on carotenoid distribution in flower petals[5] does not reveal other examples of such marked differences in xanthophylls between species of the same genus. On the other hand the absence of γ-carotene and lycopene fits into the general pattern observed with other taraxanthin-producing species, e.g. *Helianthus annuus*, *Impatiens noli me tangere*, *Ranunculus acer*, *Ulex* spp. and *Viola tricolor*.[5] Qualitative differences in the carotene fraction of species of the same genus have been previously reported; for example, Taha[6] found that the flowers of the tropical *Tecoma stans* (Bignoniaceae) contain β-carotene only, whilst those of the related *T. capensis* contain, in addition, γ-carotene and lycopene. This situation is similar to that observed between *M. longiflorus* and *M. cupreus* and *M. tigrinus*; however, in the case of the *Tecoma* spp. zeaxanthin was the main xanthophyll in both cases. Lycopene is absent from the deep yellow strains of *Calendula officinalis*[7] although it is present in large amounts in the deep orange strain;[7,8] considerable amounts of lycopene are also present in orange pansies.[9] β-Carotene is essentially absent from yellow narcissi, but present in high concentration in the deep orange red fringes of many cultivated strains, e.g. *Narcissus poeticus recurvis*.[10] The situation in *M. cupreus* (high β-carotene level) and *M. tigrinus* (traces of β-carotene) are very similar to those just described. There is, however, one difference; in all the examples cited the presence or absence of the carotenoid hydrocarbon plays a predominant part in the coloration of the flowers; the deep red of *M. cupreus* however is apparently due to the presence of a presumed anthocyanin.

EXPERIMENTAL

Plants

Mimulus seeds obtained from Messrs. Unwin (Histon, Cambridge) were germinated in John Innes Seed Compost No. 2 in a greenhouse and planted out when about 1 in. high. The flowers of *M. tigrinus* varied in tint from white to deep yellow, whilst those of *M. cupreus* were always a rich dark red. The flowers were collected when mature and the petals separated from other parts and homogenized in acetone to extract the carotenoid pigments. The acetone was diluted with an equal quantity of light petroleum (b.p. 60–80°) and sufficient

[5] T. W. Goodwin, *The Comparative Biochemistry of the Carotenoids*, London, Chapman and Hall (1952).
[6] M. M. Taha, *Biochem. J.* **58**, 413 (1954).
[7] L. Zechmeister and L. von Cholnoky, *Z. physiol. Chem. Hoppe-Seyler's* **208**, 27 (1932).
[8] T. W. Goodwin, *Biochem. J.* **58**, 90 (1954).
[9] C. O. Chichester, P. S. Wong and G. Mackinney, *Plant Physiol.* **29**, 238 (1954).
[10] V. H. Booth, *Biochem. J.* **65**, 660 (1957).

water added to form two layers. The hypophase was discarded, and the epiphase washed twice with water, dried over anhydrous Na_2SO_4 overnight and taken to small bulk ready for chromatography.

Separation, Purification and Identification of Pigments

The methods used have been previously described in detail.[11,12]

[11] M. B. ALLEN, T. W. GOODWIN and S. PHAGPOLNGARM, *J. gen. Microbiol.* **23**, 93.
[12] B. H. DAVIES, *Phytochemistry*, **1**, 25 (1962).