Carotenoids in Floral Parts of a Narcissus, a Daffodil and a Tulip

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(Received 3 July 1967)

1. The qualitative and quantitative distribution of carotenoids of the floral parts of three monocotyledons, the narcissus Scarlet Elegance, the daffodil King Alfred and the tulip Golden Harvest, were studied. β -Carotene, lutein or epoxy- β -carotenes were usually the main pigments, depending on the floral part and on the flower. When β -carotene was the major pigment there were only small amounts of, or sometimes no, epoxycarotenes. 2. Anthers, stigmas and styles of the three flowers did not possess any specific carotenoids but in some cases contained appreciable amounts of epoxycarotenoids. The possibility that these take part in reproduction is discussed. 3. The generalization that yellow flowers contained large amounts of xanthophylls and only traces of carotenes, whereas deep-orange flowers seemed to be characterized by the presence of large amounts of one carotene, was not always the correct one. It is suggested that the floral parts are yellow or orange depending on what carotenoids are present, which is the major one and the amount of total carotenoids, and also on the presence of other non-carotenoid pigments. 4. Two new probable isomers of 5,6:5',6'-diepoxy- β -carotene were isolated and found together in various floral parts of the tulip Golden Harvest.

The floral parts of a number of plants are known to contain carotenoids (Goodwin, 1952), but no comparative study of parts of the same flower was undertaken until Jungalwala & Cama (1962) carried out their investigation into *Delonix regia* (family Leguminosae) to gain information about the biogenesis and role of carotenoids in this flower. They came to the conclusion that the presence of considerable amounts of specific carotenoids in the anthers suggested that 'oxygenated carotenoids' may have some role in the reproduction of the plant under consideration. As far as we are aware, no similar observations have as yet been made on monocotyledons, and therefore it was decided to investigate the floral parts of a narcissus, a daffodil and a tulip.

Karrer & Jucker (1950) reported that the flowers of 19 different species of Liliaceae and Amaryllidaceae contained carotenoids. Most of the work they mentioned was carried out before 1930 and not many carotenoids were in fact characterized; a yellow variety of *Tulipa* contained violaxanthin (Bidgood, 1905; Schunck, 1903) and daffodil *Narcissus pseudonarcissus* contained xanthophyll (Kuhn & Winterstein, 1930). Booth (1957), on examining several varieties of *Narcissus*, formed the opinion that it was expedient to use popular and varietal names as deeply and less deeply pigmented varieties of what are commonly known as daffodils

and narcissi appeared together in more than one class. We therefore use the popular varietal names.

EXPERIMENTAL

Materials. *Narcissus poeticus* L., the narcissus Scarlet Elegance (lemon-yellow perianth and orange corona), *Narcissus pseudonarcissus* L., the daffodil King Alfred (yellow perianth and corona), and *Tulipa* sp. L., the tulip Golden Harvest (deep-yellow perianth), were obtained from the garden of L. R. G. Valadon, Frimley, Surrey. The perianths, coronae (when present), anthers, filaments and styles and stigmas together (narcissus and daffodil) and stigmas alone (tulip) were collected separately.

Extraction of pigments. The flower parts were extracted several times with methanol in a Waring Blendor and then usually with methanol-diethyl ether (1:1, v/v) until no more colour was extracted. Partition with diethyl ether was performed and all the colour was transferred to the epiphasic layer, which was concentrated under reduced pressure at about 35°. Procedures for the saponification of the extracts, the removal of some sterols, the phase-partition of carotenoids between hexane and aq. 90% (v/v) methanol and the separation and identification of carotenoids by column chromatography were as described by Valadon & Mummery (1967a,b). Purified carotenoids from column fractions were identified by co-chromatography with known standards by the reversed-phase thin-layer chromatography system of Egger (1962). Plates of kieselguhr G (Merck) impregnated with 7% coconut oil in *n*-hexane were used and eluted with methanol-acetone-water (20:4:3, by vol.).

The *cis-trans* configuration of the carotenoids was

Table 1. Quantitative distribution of carotenoids in perianths and coronae of three flowers

Values given are percentages of total carotenoids.

Carotenoid	Corona		Perianths		
	Scarlet Elegance (orange)	King Alfred (yellow)	Scarlet Elegance (lemon yellow)	King Alfred (yellow)	Golden Harvest (deep yellow)
Phytoene	2.2	6.8	1.1	10.4	1.9
Phytofluene	2.8	—	—	—	1.1
α -Carotene	0.6	—	—	—	—
β -Carotene	50.7	1.8	3.4	3.6	3.4
β -Zeaxanthene	1.1	—	—	—	—
ζ -Carotene	2.1	—	—	—	1.4
5,6-Monoepoxy- β -carotene	—	7.3	0.8	6.8	2.8
<i>cis</i> -5,6:5',6'-Diepoxy- β -carotene	—	4.1	—	7.3	18.6
<i>trans</i> -5,6:5',6'-Diepoxy- β -carotene	—	3.6	0.8	24.8	24.4
5,6:5',6'-Diepoxy- β -carotene II	—	9.6	—	—	24.2
Mutatochrome	3.2	—	—	5.7	5.9
Rubixanthin-like	5.2	—	—	—	—
Cryptoxanthin	—	2.7	—	4.9	—
Lutein epoxide	3.7	7.5	38.2	7.6	1.8
Antheraxanthin	—	—	1.4	—	—
Lutein	20.7	34.1	49.1	15.2	2.5
Zeaxanthin	1.2	—	0.6	1.2	—
Chrysanthemaxanthin	1.1	0.5	2.0	1.7	2.6
Flavoxanthin	3.3	7.4	2.6	10.8	8.1
Auroxanthin	2.1	14.6	—	—	1.3
Total carotenoids (mg./g.)	17.7	2.8	3.4	2.4	6.1

established by the iodine isomerization test (Jungalwala & Cama, 1962). Isomerization of *cis*-carotenoids leads to slightly higher $\lambda_{\max}$ values, whereas with *trans* forms the shift is to lower $\lambda_{\max}$ values after addition of iodine.

Carotenoids having epoxy groups were characterized by a modified conc. HCl-ether test. 5,6-Monoepoxy- β -carotene and 5,6:5',6'-diepoxy- β -carotene were converted into mutatochrome and aurochrome respectively on addition of traces of methanolic HCl (Jungalwala & Cama, 1962).

When *trans*-5,6:5',6'-diepoxy- β -carotene (absorption maxima at 418, 438 and 468m μ in *n*-hexane) was subjected to the iodine isomerization test at room temperature for 30 min., it gave $\lambda_{\max}$ at 418, 436 and 466m μ in *n*-hexane. This was then chromatographed on a magnesium oxide-Celite (1:1, v/v) column with 10% (v/v) ether in *n*-hexane as developer. Three bands were obtained, two very narrow yellow bands on either side of a broad orange band. The orange band was identified as *trans*-5,6:5',6'-diepoxy- β -carotene; the lowest of the three bands had absorption maxima at 415, 437 and 468m μ in *n*-hexane. This band could have been *cis*-diepoxy- β -carotene, but was found in too small a quantity to be identified with certainty. The third band was very faint and separated with difficulty from the main orange band. It was not possible to take an absorption reading of this band.

RESULTS

Carotenoids from perianths. Carotenoids from the perianths of the three varieties of *Narcissus* and *Tulipa* are recorded in Table 1. The total amounts

in each were of the same order and the deep-yellow perianth of Golden Harvest had about twice as much total carotenoid as the other two. Further, in all three there was a small proportion of carotenoids in relation to xanthophylls and epoxycarotenoids: in Scarlet Elegance, the major pigment was lutein (49%); in King Alfred, there were two predominant pigments (diepoxy- β -carotene, 25%; lutein, 15%); and in Golden Harvest, the epoxycarotenoids made up 73% of the total carotenoids. Phytoene was found in all three varieties whereas the other C₄₀ colourless polyene, phytofluene, was found only in the tulip. β -Carotene was found in small quantities in all three varieties, forming about 3.5% of the total in each.

Two unknown carotenoids were obtained in Golden Harvest. The first produced a yellow band on the column that migrated above ζ -carotene and below 5,6-monoepoxy- β -carotene. It had absorption maxima at 415, 437 and 468m μ in *n*-hexane and had a very similar absorption spectrum to that of 5,6:5',6'-diepoxy- β -carotene. It was shown to be a *cis*-carotenoid, and after addition of methanolic hydrochloric acid it was converted into aurochrome. It therefore seems that this band was a *cis*-isomer of 5,6:5',6'-diepoxy- β -carotene. This pigment was also found in the King Alfred variety of daffodil.

The second unknown carotenoid gave an orange band that was adsorbed above *trans*-5,6:5',6'-

Fig. 1. Absorption spectra of 5,6:5',6'-diepoxy- β -carotene II in *n*-hexane (—) and of the unknown xanthophyll from styles and stigmas of Scarlet Elegance in *n*-hexane (----).

diepoxy- β -carotene. It had absorption maxima at 420, 442 and 470 $m\mu$ in *n*-hexane (Fig. 1) and was shown to be a *trans*-carotenoid. After iodine catalysis at room temperature, it gave λ_{max} at 418, 438 and 468 $m\mu$ in *n*-hexane. This was chromatographed on a magnesium oxide–Celite (1:1, v/v) column with 10% (v/v) ether in *n*-hexane as developer. Three bands were obtained, a very narrow yellow band, probably *cis*-diepoxy- β -carotene, at the lower end followed by a wide orange band that could not be separated from *trans*-5,6:5',6'-diepoxy- β -carotene by thin-layer chromatography. This band was therefore identified as 5,6:5',6'-diepoxy- β -carotene. The top band was identified as the unknown starting material. Further, after addition of methanolic hydrochloric acid, the unknown substance was converted into aurochrome (λ_{max} at 380, 400 and 425 $m\mu$). It seems likely that this band was another isomer of *trans*-5,6:5',6'-diepoxy- β -carotene, which we refer to as *trans*-5,6:5',6'-diepoxy- β -carotene II.

Carotenoids from coronae. The carotenoids of the coronae of the narcissus Scarlet Elegance and of the daffodil King Alfred are recorded in Table 1. The corona of Scarlet Elegance (bright orange) contained 50% (w/w) of β -carotene out of a total of 17.7 mg. of carotenoids/g. dry wt. The total concentration of carotenoids in the yellow King Alfred (2.8 mg./g.) was much lower; β -carotene made up only 1.8% and lutein, the main pigment, 34.1% of the total. Further, epoxycarotenes (15%) and epoxyxanthophylls (20%) were present. Scarlet Elegance,

however, did not contain any epoxycarotenes but had some epoxyxanthophylls (11.4%). The *cis*-isomer of 5,6:5',6'-diepoxy- β -carotene already identified in the perianth of King Alfred was also present in the corona of this daffodil.

Carotenoids from anthers. The concentrations of carotenoids from the anthers of the three varieties of flowers were very much of the same order (0.1–0.3 mg./g. dry wt.) and are recorded in Table 2. β -Carotene was once again the main pigment of a floral part of Scarlet Elegance, even though the anthers of this flower are yellow. The epoxycarotenes (21.5%) and epoxyxanthophylls (14.2%) made up a large part of the remaining carotenoids. Diepoxy- β -carotene II was found for the first time in Scarlet Elegance. In the anthers of King Alfred the main single pigment was cryptoxanthin (25.9%) and the epoxycarotenes (26.9%) were more abundant than the epoxyxanthophylls (3.9%). In the anthers of the tulip the epoxycarotenes were the most abundant pigments (69.8%) and the epoxyxanthophylls (26.3%) made up the second largest group. The two isomers of 5,6:5',6'-diepoxy- β -carotene that were found in the perianths of this tulip were also present in the anthers. Antheraxanthin (5,6-monoepoxylutein) was found in all three flowers.

Carotenoids from filaments. The filaments were a different colour from the anthers and the carotenoids present (Table 2) were different from those of the anthers. The deep-orange filaments of Golden Harvest had twice as much total carotenoid as the orange filaments of Scarlet Elegance and seven times as much as the yellow ones of King Alfred. Lutein was the main pigment of Scarlet Elegance (53.9%) and King Alfred (39.5%), but in Golden Harvest the main pigment was diepoxy- β -carotene (30.5%). The ratios of epoxycarotenes to epoxyxanthophylls were approx. 1:1.7 in Scarlet Elegance, 1:2.4 in King Alfred and 12.5:1 in Golden Harvest. The two isomers of 5,6:5',6'-diepoxy- β -carotene were once more identified in King Alfred.

Carotenoids from styles and stigmas. The ovaries of these three flowers contained mostly chlorophylls and little carotenoid at the stage when they were examined. A yellow band that adsorbed strongly above auroxanthin on the magnesium oxide–Celite column had shoulders at 398 and 450 $m\mu$ and a peak at 424 $m\mu$ (Fig. 1). It was found in too small an amount to be identified further and was present only in the styles and stigmas of Scarlet Elegance. There is no style in the tulip Golden Harvest, so the concentrations of the carotenoids of both styles and stigmas of Scarlet Elegance and King Alfred and stigmas only of Golden Harvest are presented in Table 2. There was 4.9 mg./g. dry wt. in the orange styles and stigmas of Scarlet Elegance, but only 0.5 mg./g. in the yellow King Alfred, and only

Table 2. *Quantitative distribution of carotenoids in various floral parts of three flowers*

Values given are percentages of total carotenoids.

Carotenoid	Anthers				Filaments				Styles and stigmas				Stigmas of Golden Harvest (yellow)
	Scarlet Elegance (yellow)	King Alfred (yellow)	Golden Harvest (yellow)		Scarlet Elegance (orange)	King Alfred (yellow)	Golden Harvest (deep orange)		Scarlet Elegance (orange)	King Alfred (yellow)			
Phytoene	—	—	—		—	—	2.6		—	—			—
Phytofluene	—	—	—		—	—	2.4		—	—			—
α -Carotene	2.0	—	—		—	—	—		—	—			—
β -Carotene	60.6	15.0	2.7		10.1	2.7	1.2		39.5	11.7			9.1
β -Zeaxanthin	—	3.1	—		—	—	1.1		—	—			9.2
ζ -Carotene	—	—	—		—	—	7.6		—	—			10.0
5,6-Monoeipoxy- β -carotene	4.2	7.5	7.7		2.9	7.2	13.4		—	9.3			20.6
<i>cis</i> -5,6:5',6'-Diepoxy- β -carotene	—	—	26.6		—	—	9.9		—	—			—
<i>trans</i> -5,6:5',6'-Diepoxy- β -carotene	7.4	19.4	18.4		10.2	9.9	30.5		—	7.1			24.2
5,6:5',6'-Diepoxy- β -carotene II	9.9	—	17.1		—	—	17.2		—	—			—
Mutatochrome	—	—	—		—	—	6.6		—	—			—
Cryptoxanthin	—	25.9	—		—	—	—		—	—			—
Lutein epoxide	—	4.1	—		15.2	25.6	—		20.9	27.2			—
Antheraxanthin	6.9	3.4	13.4		—	5.2	—		3.2	—			—
Lutein	1.7	15.1	1.2		53.9	39.5	1.8		31.7	34.4			26.9
Chrysanthemaxanthin + flavoxanthin	7.3	6.5	12.9		7.7	9.5	5.7		1.1	10.3			—
Yellow band (unknown)	—	—	—		—	—	—		3.6	—			—
Total carotenoids (mg./g.)	0.3	0.1	0.3		2.2	0.6	4.3		4.9	0.5			0.2

0.2mg./g. in the yellow stigmas of Golden Harvest. The major pigment in Scarlet Elegance was β -carotene, followed closely by lutein, and there were no epoxycarotenes. In King Alfred the xanthophylls were more abundant, with lutein the major pigment; this was also the case with Golden Harvest. There seemed to be very few different carotenoids in these parts and the two isomers of diepoxy- β -carotene were not observed even in the Golden Harvest, where monoepoxy- and diepoxy- β -carotene made up about half the total carotenoids.

DISCUSSION

Previous workers have shown that narcissi may contain β -carotene (Booth, 1957) and lycopene (Werckmeister, 1960). It has been shown that all the different floral parts of the narcissus Scarlet Elegance contain β -carotene, sometimes as the major pigment; however, no trace of lycopene was ever detected. It is known that different flower varieties may have different carotenoid composition, especially flowers from different parts of the world (Goodwin, 1966; Valadon & Mummery, 1967a). This may be the reason why our results do not agree with those of Werckmeister (1960). Pigment composition is also known to vary with maturity and growing conditions and it is to be expected that amounts of carotenoids also vary. Our results, however, compare very closely with those of Booth (1957); the highest concentration he obtained for carotenoids in corona of narcissi was 20.4mg./g. dry wt., whereas our total concentration of carotenoids in the narcissus Scarlet Elegance was 17.7mg./g. Further, his yellow daffodils had a total carotenoid content of about 0.87mg./g. and a β -carotene content of 0.013mg./g. fresh wt. compared with our total of 2.8mg./g. and a β -carotene content of 0.05mg./g. dry wt. in the daffodil King Alfred. These values represent the same percentage of β -carotene to total carotenoids (1.81%, Booth, 1957; 1.78%, present study), even though the total concentration of carotenoids in this case varied slightly. It was not possible to compare values obtained from the tulip as no thorough study of carotenoids of other tulips seems to have been undertaken.

Karrer, Eugster & Faust (1950) studied the distribution of carotenoids in pollens and anthers of a number of flowers and found, on the whole, that esterified lutein or antheraxanthin was the principal carotenoid, together with traces of carotene. Among the pollens they studied was that of *Narcissus exsertus*, which was found to have very little unidentified carotenoid. Our results do not compare with these, as it was shown that anthers of both the narcissus and the daffodil contained a certain amount of carotenoids and that xanthophylls

were never present in higher concentration than the carotenes; in the anthers of Scarlet Elegance there was 60.6% of β -carotene. Jungalwala & Cama (1962), working with the dicotyledon *Delonix regia*, showed that zeaxanthin (90%) was the principal carotenoid in the anthers and that large amounts of 'oxygenated carotenoids' all having the β -ionone ring structure were also present. These results led to the suggestion that the presence of specific carotenoids in the anthers of *D. regia* may have some role in the reproduction of this plant. The presence of specific carotenoids would not necessarily mean that they play a part in reproduction and, further, as is known, a number of carotenoids other than those with β -ionone ring structure had been identified in pollens and anthers of a number of flowers. No carotenoids specific to anthers or to styles were observed in the present study and it is therefore not likely that specific carotenoids play a part in the reproduction of the three monocotyledon plants.

Other suggestions have been put forward for the functions of epoxycarotenoids: Cholnoky, Györgyfy, Nagy & Pánczél (1955) suggested that epoxycarotenoids may be intermediates in the transfer of oxygen and formation of xanthophylls, and Karrer, Jucker, Rutschmann & Steinlin (1945) proposed that as the epoxides revert to carotenoids by loss of oxygen they may act as oxygen carriers in plants. These epoxycarotenoids were thought to be specific to flower petals (Goodwin, 1952), yet they are found in a number of apple varieties (Valadon & Mummery, 1967b) and also in various floral parts in the present study. The function of these epoxides in the floral parts of the three monocotyledon flowers is unknown.

It had been suggested that yellow flowers contained large amounts of xanthophylls and only traces of carotenes, whereas deep-orange flowers seemed to be characterized by the presence of large amounts of one carotene (Goodwin, 1965). In the orange corona of Scarlet Elegance β -carotene formed 50.7% of the total carotenoids, but in the yellow corona of King Alfred 67.8% was xanthophylls. However, Valadon & Mummery (1967a) showed that this generalization was not always the correct one and that the difference between yellow and orange forms of the African marigold *Tagetes erecta* was due to the greater total amount of carotenoids in the darker flower. It may be suggested therefore that the anthers of the three flowers are yellow because they contain approximately the same low total carotenoid content, though the ratios of carotenes to xanthophylls varied from 5.3:1 in Scarlet Elegance to 1:1.2 in King Alfred. Similarly, the orange filaments of both Scarlet Elegance and Golden Harvest contained a large amount of total carotenoids compared with the

yellow ones of King Alfred. All the yellow floral parts of the three flowers under study seemed to have less than 3.5 mg. of total carotenoids/g. dry wt., and the deeper-yellow and orange parts seemed to have more. However, the deep-orange filaments of Scarlet Elegance had only 2.2 mg. of total carotenoids/g. dry wt. and the major pigment was lutein (53.9%). This shows the danger of generalization. Floral parts are orange or yellow depending on what carotenoids are present, which is the main one and the amount of total carotenoids, and sometimes on the presence of other non-carotenoid pigments.

We thank Dr O. Isler and F. Hoffmann-La Roche and Co. Ltd., Basle, Switzerland, for gifts of authentic carotenoids. A grant from the Central Research Fund of the University of London is gratefully acknowledged.

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