Carotenoids in *Delonix regia* (Gul Mohr) Flower

BY F. B. JUNGALWALA AND H. R. CAMA
Department of Biochemistry, Indian Institute of Science, Bangalore 12, India

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Delonix regia (Gul Mohr) (family Leguminosae) is a striking ornamental medium-sized tree grown in most tropical countries. It produces clusters of orange–red flowers in April–May. The carotenoids present in a variety of different flowers have been studied by several workers (e.g. Goodwin, 1952). A comparative study of the carotenoids present in various floral parts of *D. regia* has been made to gain information about the biogenesis and role of carotenoids in the flower.

EXPERIMENTAL

Materials. Fully opened fresh flowers were obtained from trees and the petals, sepals, anthers and filaments were collected separately.

Light petroleum (b.p. 40–60°) was left over $KMnO_4$, washed, distilled, dried over $CaCl_2$ and redistilled before use. Diethyl ether was freshly distilled over reduced iron to remove any peroxides. Commercial acetone, methanol and ethanol were twice distilled before use. Hexane (b.p. 60–80°) and acetone-free methanol were used for the phase-partition and iodine-isomerization tests. Ethanol, for spectroscopic use, was refluxed with zinc dust and KOH and distilled.

Alumina (E. Merck) for chromatography was used either as such or after partial deactivation with water or methanol as required (Goodwin & Srisukh, 1949; Taha, 1954). Magnesium oxide (for chromatographic analysis, British Drug Houses Ltd.) was mixed with Celite (no. 545, Johns–Manville, U.S.A.) in suitable mixtures.

Extraction of pigments. The flower parts (1 kg. of petals and sepals, 0·5 kg. of filaments and 50 g. of anthers) were soaked in ethanol for 2 days in the dark. The plant residues were filtered off and blended in a Waring Blendor successively with ethanol, acetone and light petroleum–diethyl ether (1:1, v/v). The filtered extracts were combined and washed with water until free of acetone and ethanol. The remaining organic layer containing the carotenoids was dried over anhydrous Na_2SO_4, filtered and concentrated at reduced pressure.

Saponification of extracts and removal of sterols. The oil obtained was dissolved in ethanol and saponified under N_2 with ethanolic 10% (w/v) KOH at 60° for 0·5 hr., and then left overnight at room temperature. Unsaponifiable material was extracted with diethyl ether, washed free of alkali, dried over anhydrous Na_2SO_4, filtered and evaporated. Chlorophylls present in sepal extracts were removed by this treatment.

The unsaponifiable material was redissolved in light petroleum and left overnight under N_2 at −30°; the precipitated sterols were removed by filtration.

Phase-partition of carotenoids between solvents. The carotenoid extracts were subjected to phase-partition between light petroleum and aq. 90% (v/v) methanol. Carotene hydrocarbons, monohydroxy xanthophylls, and mono- and di-epoxides of carotene hydrocarbons and of monohydroxy xanthophylls are found in the upper layer, whereas the di- and poly-hydroxy xanthophylls and their epoxy derivatives remain in the lower layer.

Column chromatography. The upper- and lower-phase carotenoids were dissolved in light petroleum before chromatography. The former group of carotenoids were chromatographed on an alumina column (3 cm. × 35 cm.) which had been partially deactivated by treatment with 5% (v/w) of water. Carotene hydrocarbons did not separate well on this column and were eluted with light petroleum. The remaining carotenoids were well resolved when the column was eluted (gradient elution) with light petroleum containing increasing amounts of diethyl ether. Fractions (15–20 ml.) were collected and their u.v. and visible spectra were determined. Fractions showing similar spectra were combined and concentrated. Individual pigments were further purified by rechromatography on more active alumina columns (2 cm. × 30 cm.). The order of band elution of monohydroxy xanthophylls and epoxides of carotene hydrocarbons and of monohydroxy xanthophylls obtained from petals of *D. regia* is given in Table 2.

The carotene hydrocarbons eluted from these columns were rechromatographed as above on an alumina column (3 cm. × 40 cm.) which had been partially deactivated by treatment with 1% (v/w) of water. Colourless substances like phytoene and phytofluene were located by their u.v. fluorescence. The fractions collected were chromatographed again on fully active alumina. The identification of carotene hydrocarbons from petals of *D. regia* separated on alumina partially deactivated with 1% of water is given in Table 1. The separation of β-carotene, pigment X and ζ-carotene was difficult and longer columns (2 cm. × 45 cm.) were employed for satisfactory resolution.

The lower-phase carotenoids were adsorbed on a methanol-deactivated alumina column (3 cm. × 35 cm.) and were resolved by elution with light petroleum containing increasing amounts of acetone. The chromatographic procedure was repeated three or four times before pure pigments were obtained. The order of elution of these carotenoids obtained from petals of *D. regia* is listed in Table 3.

Better resolution of the pigments was obtained by using a magnesium oxide–Celite (1:1, w/w) column (3 cm. × 35 cm.) eluted with light petroleum containing increasing amounts of acetone. Partially resolved pigments were purified further by rechromatography on magnesium oxide–Celite (3:2 and 2:1, w/w) columns (2·5 cm. × 35 cm.). Their elution from a magnesium oxide–Celite (3:2, w/w) column is illustrated in Table 4. The *cis–trans* isomers, chrysanthe-

maxanthin and flavoxanthin, were eluted as a single band from these columns.

The alteration in the order of elution of these pigments from magnesium oxide–Celite columns compared with that from methanol-deactivated alumina was verified by chromatography of authentic carotenoid samples.

Separation of carotenoids from sepals, filaments and anthers of *D. regia* was performed similarly.

Identification of carotenoids. Individual carotenoids were identified by comparing their chromatographic properties and u.v. and visible spectra with those of authentic carotenoid samples (Tables 1, 2 and 4).

Phase-partition of the carotenoids between hexane and aq. methanol (95, 85 and 75%, v/v) according to the method of Petracek & Zechmeister (1956) also helped in their identification.

The *cis–trans* configuration of the carotenoids was ascertained by the iodine-isomerization test (Zechmeister, 1960). The test was performed by the addition of 0·5 ml. of 0·001% iodine in hexane to 10 ml. of a solution of carotenoid in hexane in a test tube and exposure of this mixture to light from an incandescent lamp for periods of 5, 15 and 30 min. The u.v. and visible spectra of the carotenoid in hexane were recorded before and after exposure to light. Isomerization of *cis*-carotenoids leads to slightly higher $\lambda_{max.}$ values; with *trans*-carotenoids the shift is to lower $\lambda_{max.}$ values.

Carotenoids having epoxy groups were characterized by a modified conc. HCl–ether test (Curl & Bailey, 1954; Karrer & Jucker, 1950). The isomerization of 5,6-epoxide into 5,8-epoxide was studied by observing the change in the spectrum of the carotenoid in light petroleum or ethanol before and after addition of a drop of ethanolic 0·05N-HCl. Hypsochromic (lower-wavelength) shifts of 15–20 and 35–45 mμ were shown by 5,6-monoepoxy and 5,6-diepoxy carotenoids respectively, with an associated decrease in the extinction due to the formation of 5,8-epoxy (furanoid) carotenoids (Table 4).

Melting points and mixed melting points were determined for crystalline pigments.

Quantitative determination. Weights of flower components were determined after drying at 80°.

Total carotenoids of different flower parts were determined by dissolving the unsaponifiable materials in a known volume of light petroleum and measuring E values at 445 mμ (Table 5), assuming $E^{1\%}_{1\,cm.}$ for the crude mixture to be that of β-carotene [2500 (Goodwin, 1955)].

The concentrations of individual carotenoids (Table 6) were determined similarly by measuring $E_{max.}$ and comparing it with known $E^{1\%}_{1\,cm.}$ values at $\lambda_{max.}$ for pure pigments (Goodwin, 1955). For unknown pigments $E^{1\%}_{1\,cm.}$ at $\lambda_{max.}$ was assumed to be 2500 as suggested by Goodwin (1954).

Spectrophotometric measurements. The u.v. and visible spectra of the carotenoids were measured with a Beckman spectrophotometer (model DU).

RESULTS

Carotenoids from petals

Carotene hydrocarbons. Carotene hydrocarbons isolated from petals of *D. regia* are listed in Table 1.

Two phytofluene bands (blue–green fluorescence), with similar absorption spectra, have been observed during the chromatography of extracts of tomatoes and petals of *Calendula officinalis* (Koe & Zechmeister, 1953; Goodwin, 1954). Only one phytofluene band was present in the extracts of petals of *D. regia* and this was identical with the lower phytofluene band from tomatoes. Petracek & Zech-

Table 1. *Chromatographic-adsorption analysis and identification of carotene hydrocarbons from petals of* Delonix regia

The pigments were chromatographed on alumina (deactivated by treatment with 1% of water) by using light petroleum containing varying amounts of ether as the developing solvent. The pigments are listed in the order in which they were eluted from the column.

Band	Absorption maxima in light petroleum (mμ)	Concn. (%, v/v) of ether required for elution of band	Iodine-isomerization test	Co-chromatography with authentic sample	Identification
1	275, 285, 296	—	—	From tomatoes (Rabourn, Quackenbush & Porter, 1954)	Phytoene
2	332, 348, 367	2	—	From tomatoes (Koe & Zechmeister, 1953)	Phytofluene
3	425, 450, 478	3–5	*trans*	Synthetic (Hoffmann–La Roche)	β-Carotene
4	~380, 405, 425, 450	5–7	*trans*	—	Pigment X
5	380, 400, 425	8	*trans*	From tomatoes (Porter & Zscheile, 1946)	ζ-Carotene
6	427, 455, 485	9	*trans*	—	δ-Carotene
7	432, 460, 490	10–13	*trans*	From carrots (Kuhn & Brockmann, 1933)	γ-Carotene
8	440, 468	13–15	*cis*	—	Prolycopene
9	440, 465, 490	15–17	*cis*	From tomatoes (Porter & Zscheile, 1946)	Neolycopene
10	445, 470, 503	20	*trans*	From tomatoes (Porter & Zscheile, 1946)	Lycopene

meister (1952) have suggested that the normal naturally occurring phytofluene is probably a *cis*-isomer. β-Carotene formed the major hydrocarbon of petals and was crystallized (m.p. 181–182°) from light petroleum. Pigment X isolated from petals of *D. regia* had chromatographic and light-absorption properties (Fig. 1*A*) similar to those of a pigment isolated from *Phycomyces blakesleeanus* (Goodwin & Osman, 1953) and *C. officinalis* (Goodwin, 1954). Petzold, Quackenbush & McQuistan (1959) have isolated two similar pigments, α- and β-zeacarotene, from corn, and pigment X may be identical with β-zeacarotene. Lycopene occurred as a mixture of *cis–trans* isomers, and *trans*-lycopene was crystallized (m.p. 168–169°) from light petroleum. Prolycopene and neolycopene, after isomerization with iodine in light, showed a bathochromic shift, and a new peak at about 500 mμ appeared with prolycopene (Goodwin, 1956).

Monohydroxy xanthophylls and epoxides of carotene hydrocarbons and of monohydroxy xanthophylls. These carotenoids isolated from the petals of *D. regia* are listed in Table 2. 5,6-Monoepoxy-β-carotene and 5,6-diepoxy-β-carotene were characterized by their conversion into mutatochrome ($\lambda_{max.}$ 402, 425, 452 mμ) and aurochrome ($\lambda_{max.}$ 380, 400, 425 mμ) respectively on the addition of traces of ethanolic hydrochloric acid (Karrer & Jucker, 1945*b*). Mutatochrome itself was present in the extracts and differs from flavochrome in having slightly higher $\lambda_{max.}$ values. Cryptoxanthin formed a very faint band on the column. Unidentified-428, situated above cryptoxanthin on alumina columns (Table 2, band 5), showed a single broad band at 428 mμ (Fig. 1*B*) and its spectrum did not change on the addition of ethanolic hydrochloric acid. However, it gave a faint blue colour on the addition of ether and hydrochloric acid, indicating the presence of a 5,8-epoxy group. The partition ratio between hexane and 95% (v/v) methanol (88:12) suggests that it may contain a single hydroxyl group. Unidentified-420 (Table 2, band 6) was found to be the *cis*-isomer of unidentified-425 (Table 2, band 7); the absorption spectrum of unidentified-425 is given (Fig. 1,*C*). Chromatographic behaviour, partition ratio, absorption spectrum and the ethanolic hydrochloric acid test suggest that unidentified-425 is a 5,8-epoxy-monohydroxy carotenoid, perhaps *trans*-cryptoflavin (Karrer & Jucker, 1946). The absorption spectrum of *trans*-rubixanthin was indistinguishable from that of γ-carotene (Mackinney, 1935) and thus differed from rubixanthin-like pigments reported by Goodwin (1954) and Taha (1954) in flower petals of *C. officinalis* and *Tecoma capensis* respectively. Unidentified-425a (Table 2, band 10; Fig. 2,*A*), present in petals, showed the presence of a 5,8-epoxy group with a single hydroxyl group as suggested by

Table 2. *Chromatographic-adsorption analysis and identification of monohydroxy xanthophylls and epoxides of carotene hydrocarbons and of monohydroxy xanthophylls from petals of* Delonix regia

The pigments were chromatographed on alumina (deactivated by treatment with 5% of water) by using light petroleum containing varying amounts of ether as the developing solvent. The pigments are listed in the order in which they were eluted from the column.

Band	Absorption maxima in light petroleum (mμ)	Concn. (%, v/v) of ether required for elution of band	Iodine-isomerization test	Partition ratio of hexane–95% (v/v) methanol	Colour on treatment with ethanolic HCl	Co-chromatography with authentic sample	Identification
1	420, 445, 472	5	*trans*	99:1	Faint bluish-green	Synthesized (Karrer & Jucker, 1945*b*)	5,6-Monoepoxy-β-carotene
2	402, 425, 453	7	*trans*	98:2	Faint green	Synthesized (Karrer & Jucker, 1945*b*)	Mutatochrome
3	415, 438, 468	8–10	*trans*	98:2	Blue	Synthesized (Tsukida & Zechmeister, 1958)	5,6-Diepoxy-β-carotene
4	450, 480	12–15	*trans*	84:16	—	From egg yolk (Gillam & Heilbron, 1935)	Cryptoxanthin
5	428	17	—	88:12	Faint green	—	Unidentified-428
6	~400, 420, 445	20	*cis*	86:14	Faint green	—	Unidentified-420
7	403, 425, 452	22	*trans*	86:14	Faint green	—	Unidentified-425
8	430, 455, 485	25	*cis*	83:17	—	—	*cis*-Rubixanthin
9	432, 460, 493	30	*trans*	82:18	—	—	Rubixanthin
10	405, 425, ~455	35–40	—	86:15	Faint green	—	Unidentified-425a

chromatographic behaviour, partition ratio, absorption spectrum and ethanolic hydrochloric acid test.

Di- and poly-hydroxy xanthophylls and their epoxides. Di- and poly-hydroxy xanthophylls and their epoxides characterized are given in Tables 3 and 4. Though the absorption spectra of 5,6-monoepoxylutein, antheraxanthin, violaxanthin, lutein, zeaxanthin and neoxanthin are very similar, these pigments could be differentiated easily by their chromatographic behaviour on different adsorbants (magnesium oxide–Celite and methanol-deactivated alumina) and by their reactions with ethanolic hydrochloric acid (Tables 3 and 4). The significant difference in the partition ratios observed for members of this group of carotenoids (Table 4) also aided their identification.

Chrysanthemaxanthin and flavoxanthin are *cis–trans* isomers. Auroxanthin occurred as a mixture of *cis–trans* isomers. Neoxanthin, a leaf carotenoid, tentatively identified as 5,6-epoxy-6′(or 5′)-hydro-3,3′,5′(or 6′)-trihydroxy-β-carotene (Goldsmith & Krinsky, 1960), was present in the petals of *D. regia*.

The adsorption affinity on alumina columns of these carotenoids increases with the increase in their hypophasic character (Tables 3 and 4). No such relationship was found between partition ratios and adsorption affinity on magnesium oxide–Celite columns.

Xanthophylls having one or more 5,6-epoxy groups (e.g. 5,6-monoepoxylutein, antheraxanthin and violaxanthin) are less strongly adsorbed on magnesium oxide–Celite columns than the corresponding parent xanthophylls (lutein and zeaxanthin), and an increase in the number of 5,6-epoxy groups also results in decreased adsorption [in the order: violaxanthin, antheraxanthin, zeaxanthin (Table 3)]. Similar observations were made by Tsukida & Zechmeister (1958) on the adsorption of β-carotene, 5,6-monoepoxy-β-carotene and 5,6-diepoxy-β-carotene on magnesium oxide–Hyflo-Supercel columns. Further, neoxanthin, with three hydroxyl groups and one 5,6-epoxy group, is adsorbed less strongly than chrysanthemaxanthin, which has only two hydroxyl groups and one 5,8-epoxy group (Table 3).

Carotenoids from sepals

Carotenoids present in sepals of *D. regia* were characterized according to the methods described for petals and are listed in Table 6.

Phytofluene in sepals was identical with the lower phytofluene band from tomatoes (Petracek & Zechmeister, 1952). α-Carotene ($\lambda_{max.}$ 420, 443 and 474 mμ) could be distinguished from β-carotene and its *cis*-isomer by its absorption spectrum and the iodine-isomerization test. Unidentified-428 was identical with that from petals.

Carotenoids from filaments

Carotenoids present in filaments of *D. regia* are given in Table 6. The unidentified-428 (Fig. 2, *B*) was identical with that from petals and sepals.

Carotenoids from anthers

Carotenoids in anthers of *D. regia* were few in number compared with other parts of the flower (Table 6). β-Carotene and its *cis*-isomer, neo-β-carotene, were the only carotene hydrocarbons present in anthers. Two unknown carotenoids (unidentified-430 and unidentified-435), with adsorption affinities slightly greater than that of cryptoxanthin on alumina columns, had $\lambda_{max.}$ at 410, 430 and 455 mμ and at 410, 435 and 460 mμ (Fig. 2, *C*) respectively. Chromatographic behaviour, partition ratio, absorption spectra and the ethanolic hydrochloric acid test suggest that unidenti-

Table 3. *Chromatographic-adsorption analysis of di- and poly-hydroxy xanthophylls and their epoxides from* Delonix regia

The developing solvent used was light petroleum containing varying amounts of acetone. The pigments are listed in the order in which they were eluted from the column.

Order of adsorption of pigments on methanol-deactivated alumina column	Concn. (%, v/v) of acetone to elute band from the column	Order of adsorption of pigments on magnesium oxide–Celite (3:2) column	Concn. (%, v/v) of acetone to elute band from the column
Lutein (3,3′-dihydroxy-α-carotene)	10–15	5,6-Monoepoxylutein	5–10
Zeaxanthin (3,3′-dihydroxy-β-carotene)	15–17	Violaxanthin	10–15
5,6-Monoepoxylutein	17–20	Lutein	15–20
Chrysanthemaxanthin (5,8-monoepoxylutein)	20–23	Antheraxanthin	20–25
Flavoxanthin (5,8-monoepoxylutein)	20–23	Zeaxanthin	25–30
Antheraxanthin (5,6-monoepoxyzeaxanthin)	25–30	Neoxanthin	30–35
Violaxanthin (5,6-diepoxyzeaxanthin)	30–40	Chrysanthemaxanthin	35–40
Auroxanthin (5,8-diepoxyzeaxanthin)	50–60	Flavoxanthin	35–40
Neoxanthin (5,6-monoepoxy-6′-hydro-5′-hydroxyzeaxanthin?)	70–80	Auroxanthin	40–50

Table 4. *Characterization of di- and poly-hydroxy xanthophylls and their epoxides from petals of* Delonix regia

The pigments were chromatographed on a magnesium oxide–Celite (3:2) column by using light petroleum containing varying amounts of acetone as the developing solvent. The pigments are listed in the order in which they were eluted from the column.

Band	Absorption maxima in ethanol (mμ)	Iodine-isomerization test	Partition ratio: Of hexane–85% (v/v) methanol	Partition ratio: Of hexane–75% (v/v) methanol	Colour on treatment with ethanolic HCl	Absorption maxima in ethanol after conversion of 5,6- into 5,8-epoxide (mμ)	Co-chromatography with authentic sample	Identification
1	417, 442, 471	*trans*	24:76	65:35	Faint blue	400, 420, 450	Synthesized (Karrer & Jucker, 1945*a*)	5,6-Monoepoxylutein
2	418, 440, 470	*trans*	12:88	33:67	Blue	380, 402, 427	Synthesized (Karrer & Jucker, 1945*a*)	Violaxanthin
3	420, 445, 475	*trans*	44:56	91:9	—	—	From egg yolk (Gillam & Heilbron, 1935)	Lutein
4	421, 443, 473	*trans*	14:86	64:36	Blue	405, 428, 453	Synthesized (Karrer & Jucker, 1945*a*)	Anthraxanthin
5	428, 451, 482	*trans*	40:60	87:13	—	—	From *Zea mays* (Zechmeister & Cholnoky, 1930)	Zeaxanthin
6	417, 437, 465	*trans*	0:100	0:100	Blue	400, 422, 450	—	Neoxanthin
7	400, 421, 448	—	21:79*	72:28	Faint blue	—	From *Ranunculus acer**	Chrysanthemaxanthin + flavoxanthin
8	375, 400, 425	*cis*	12:88	27:73	Blue	—	—	*cis*-Auroxanthin
9	380, 402, 428	*trans*	8:92	25:75	Blue	—	Synthesized (Karrer & Jucker, 1945*a*)	Auroxanthin

* Determined on a sample of chrysanthemaxanthin supplied by Professor P. Karrer.

fied-435 is a 5,8-epoxy-monohydroxy carotenoid and unidentified-430 is its *cis*-isomer. Zeaxanthin was crystallized from light petroleum containing methanol and had m.p. 205° unchanged by admixture with an authentic sample supplied by Hoffmann–La Roche. All other carotenoids, except β-carotene and its derivatives, were absent in anthers.

Quantitative experiments

Total carotenoids present in petals, sepals, filaments and anthers of *D. regia* are given in Table 5. Relative amounts of the individual carotenoids present in these parts of the flower are given in Table 6.

In petals, carotene hydrocarbons predominate over 'oxygenated' carotenoids (hydroxy and epoxy carotenoids) (7:3). β-Carotene is present in the largest amount. γ-Carotene, rubixanthin and lycopene isomers are other major pigments in petals.

The ratio of carotene hydrocarbons to 'oxygenated' carotenoids is higher in sepals than in petals (21:4). Sepals contain large amounts of colourless carotenoids (phytoene and phytofluene) together with β-carotene.

Filaments contain considerable amounts of 'oxygenated' carotenoids (65% of total carotenoids), mainly lutein, zeaxanthin and epoxy carotenoids. Phytoene and β-carotene are the major carotene hydrocarbons present.

The total carotenoids present in anthers of *D. regia* (570 mg./100 g. dry wt.) are significantly higher than values recorded for anthers of other flowers. The bulk of the total carotenoids present are 'oxygenated' carotenoids (98%), mainly zeaxanthin (90%). β-Carotene and its *cis*-isomer are present in very small amounts.

DISCUSSION

Although many investigators have studied the distribution of carotenoids in petals and anthers of different flowers, no systematic qualitative and quantitative data are available on carotenoids in different parts of the same flower. The present investigation describes a detailed study of carotenoids in different parts of *D. regia* flower.

The distribution of carotenoids in flower petals varies considerably; some contain few or no caro-

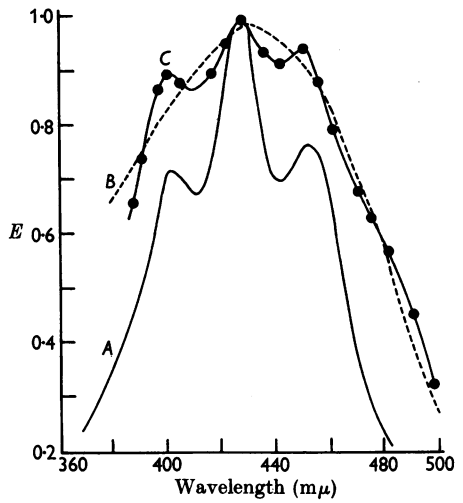

Fig. 1. Absorption spectra in light petroleum of carotenoids from petals. *A*, Pigment X, ——; *B*, unidentified-428 (Table 1, band 5), - - - - -; *C*, unidentified-425 (Table 1, band 7), •—•—•.

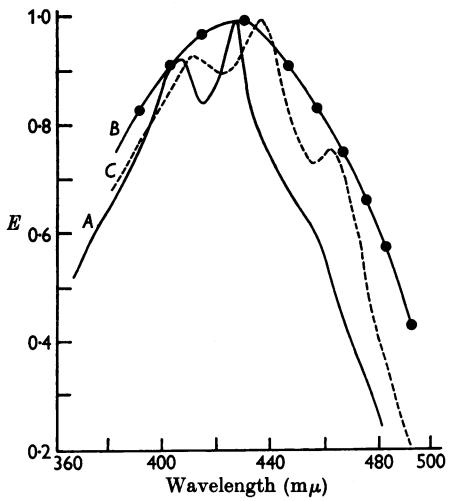

Fig. 2. Absorption spectra in light petroleum of carotenoids. *A*, Unidentified-425a (from petals, Table 1, band 10), ——; *B*, unidentified-428 (from filaments), •—•—•; *C*, unidentified-435 (from anthers), - - - - -.

Table 5. *Quantitative distribution of total carotenoids in* Delonix regia

	Total carotenoids		$E^{1\%}_{1cm.}$ at 445 mμ after saponification	Ratio of carotene hydrocarbons to 'oxygenated' carotenoids
	mg./100 g. fresh wt.	mg./100 g. dry wt.		
Petals	18·8	129·0	315·0	70:30
Sepals	15·5	97·5	75·2	84:16
Filaments	5·1	36·0	42·5	35:65
Anthers	190·4	570·1	408·0	2:98

Table 6. *Relative amounts of individual carotenoids in petals, sepals, filaments and anthers of* Delonix regia

A dash indicates the absence of the carotenoid.

Carotenoid	Percentage by wt. of total carotenoids present			
	In petals	In sepals	In filaments	In anthers
Carotene hydrocarbons				
Phytoene	7·66	36·64	11·79	—
Phytofluene	2·48	7·59	1·35	—
α-Carotene	—	0·54	—	—
Neo-β-carotene	—	—	1·49	0·12
β-Carotene	34·05	32·85	12·55	1·73
Pigment X	0·88	—	—	—
ζ-Carotene	1·49	1·34	1·61	—
δ-Carotene	1·65	—	—	—
γ-Carotene	11·21	1·64	3·03	—
Prolycopene	4·61	0·76	—	—
Neolycopene	2·07	1·65	1·6	—
Lycopene	4·27	1·42	2·0	—
Monohydroxy xanthophylls and epoxides of carotene hydrocarbons and of monohydroxy xanthophylls				
5,6-Monoepoxy-β-carotene	0·27	—	0·11	—
Mutatochrome	1·0	—	0·5	0·15
5,6-Diepoxy-β-carotene	0·17	—	1·16	—
Cryptoxanthin	0·07	1·21	5·7	2·21
Unidentified-428	0·19	0·45	0·9	—
Unidentified-420	0·8	—	—	—
Unidentified-425	1·13	—	—	—
Unidentified-430	—	—	—	0·05
Unidentified-435	—	—	—	0·22
cis-Rubixanthin	5·02	—	—	—
Rubixanthin	10·05	—	—	—
Unidentified-425a	1·60	—	—	—
Di- and poly-hydroxy xanthophylls and their epoxides				
Lutein	2·48	3·36	21·7	—
Zeaxanthin	1·82	2·59	15·2	89·82
5,6-Monoepoxylutein	0·82	—	3·7	—
Chrysanthemaxanthin + flavoxanthin	1·5	2·77	3·95	—
Antheraxanthin	0·44	0·55	6·15	5·24
Violaxanthin	0·85	1·77	4·43	0·29
cis-Auroxanthin	0·28	—	—	—
Auroxanthin	0·94	2·47	1·08	0·18
Neoxanthin	0·2	0·4	—	—

tenoids, whereas others contain mainly epoxy carotenoids specific to petals (Goodwin, 1952; Karrer, Jucker & Krause-Voith, 1947). The petals of *D. regia* contain nine well-characterized epoxy carotenoids and a few unidentified carotenoids which appear to be epoxides, in addition to various other carotenoids. The role of epoxy carotenoids in petals is not yet known. However, they may be intermediates in the transfer of oxygen and formation of xanthophylls (Cholnoky, Györgyfy, Nagy & Pánczél, 1955).

Certain carotenoids are present only in a particular part of *D. regia* flower, e.g. pigment X, δ-carotene and rubixanthin are only found in petals, whereas α-carotene is confined to sepals.

Vivino & Palmer (1944) observed that mixed pollen gathered by bees contains traces of carotene (4–15 mg./100 g.), but considerable amounts of xanthophylls (14–41 mg./100 g.). Karrer, Eugster & Faust (1950), while studying the distribution of carotenoids in pollen and anthers, found that esterified lutein is the principal carotenoid component of pollen, whereas β-carotene is present in traces. In agreement with these observations, xanthophylls represent 98 % of the pigments present in anthers of *D. regia*. However, instead of lutein, zeaxanthin is the principal carotenoid and represents 90 % of the total carotenoids. Large amounts of 'oxygenated' carotenoids, all having the β-ionone ring structure, are present in anthers of *D. regia*, whereas comparatively small quantities of carotenoids of varied ring structure (α-, β- and γ-ionone) are present in petals, sepals and filaments. β-Carotenoids may be synthesized from β-carotene by oxidation and hydroxylation *in situ* in anthers of *D. regia* or they may be formed from various carotenoids present in other parts of the flower, only being transferred to the anthers after synthesis is completed.

Indirect evidence indicates that carotenoids play a part in reproduction of cryptogams and various

animal species (Goodwin, 1952). It is not yet known whether they play a similar role in the phanerogams. The constituents of anthers are transferred in part to seeds, but petal, sepal and filament constituents are discarded by plants. The presence of considerable amounts of specific carotenoids in the anthers of *D. regia* suggests that 'oxygenated' carotenoids may have some role in the reproduction of this plant.

SUMMARY

1. The qualitative and quantitative distribution of carotenoids in different parts of *Delonix regia* (Gul Mohr) flower was studied by chromatographic, spectrophotometric and other methods. The partition ratios, hitherto not reported, of a number of carotenoids between different solvents are recorded.

2. The petals of *D. regia* contained 29 carotenoids. The major pigments identified are: phytoene, phytofluene, β-carotene, γ-carotene, lycopene isomers, rubixanthin, lutein, zeaxanthin and several epoxy carotenoids.

3. The sepals contained 18 carotenoids. Phytoene, phytofluene, β-carotene, γ-carotene, lycopene isomers, lutein, zeaxanthin and carotenoid epoxides were the major carotenoids.

4. Filaments contained 20 carotenoids, including phytoene, β-carotene, γ-carotene, cryptoxanthin, lutein, zeaxanthin, antheraxanthin, violaxanthin, chrysanthemaxanthin, flavoxanthin and other epoxy carotenoids.

5. The highest concentrations of total carotenoids were found in anthers. Of the total carotenoids, 90% is zeaxanthin. The only other carotenoids present were β-carotene derivatives with traces of two uncharacterized carotenoids.

6. The probable physiological significance of such a complex mixture of carotenoids in different parts of the flower is discussed.

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REFERENCES

Cholnoky, L., Györgyfy, C., Nagy, E. & Pánczél, M. (1955). *Acta chim. Acad. Sci. hung.* **6**, 143.
Curl, A. L. & Bailey, G. F. (1954). *J. agric. Fd Chem.* **2**, 685.
Gillam, A. E. & Heilbron, I. M. (1935). *Biochem. J.* **29**, 1064.
Goldsmith, T. H. & Krinsky, N. I. (1960). *Nature, Lond.*, **188**, 491.
Goodwin, T. W. (1952). *The Comparative Biochemistry of the Carotenoids*. London: Chapman and Hall.
Goodwin, T. W. (1954). *Biochem. J.* **58**, 90.
Goodwin, T. W. (1955). In *Modern Methods of Plant Analysis*, vol. 3, p. 272. Ed. by Paech, K. & Tracey, M. Heidelberg: Springer Verlag.
Goodwin, T. W. (1956). *Biochem. J.* **62**, 346.
Goodwin, T. W. & Osman, H. G. (1953). *Arch. Biochem. Biophys.* **47**, 215.
Goodwin, T. W. & Srisukh, S. (1949). *Biochem. J.* **45**, 263.
Karrer, P., Eugster, C. H. & Faust, M. (1950). *Helv. chim. acta*, **33**, 300.
Karrer, P. & Jucker, E. (1945*a*). *Helv. chim. acta*, **28**, 300.
Karrer, P. & Jucker, E. (1945*b*). *Helv. chim. acta*, **28**, 427.
Karrer, P. & Jucker, E. (1946). *Helv. chim. acta*, **29**, 229.
Karrer, P. & Jucker, E. (1950). *Carotenoids*. Translated by Braude, E. A. London: Elsevier.
Karrer, P., Jucker, E. & Krause-Voith, E. (1947). *Helv. chim. acta*, **30**, 537.
Koe, B. K. & Zechmeister, L. (1953). *Arch. Biochem. Biophys.* **46**, 100.
Kuhn, R. & Brockmann, H. (1933). *Ber. dtsch. chem. Ges.* **66**, 407.
Mackinney, G. (1935). *J. biol. Chem.* **112**, 421.
Petracek, F. J. & Zechmeister, L. (1952). *J. Amer. chem. Soc.* **74**, 184.
Petracek, F. J. & Zechmeister, L. (1956). *Analyt. Chem.* **28**, 1484.
Petzold, E. N., Quackenbush, F. W. & McQuistan, M. (1959). *Arch. Biochem. Biophys.* **82**, 117.
Porter, J. W. & Zscheile, F. P. (1946). *Arch. Biochem.* **10**, 537.
Rabourn, W. J., Quackenbush, F. W. & Porter, J. W. (1954). *Arch. Biochem. Biophys.* **48**, 267.
Taha, M. M. (1954). *Biochem. J.* **58**, 413.
Tsukida, K. & Zechmeister, L. (1958). *Arch. Biochem. Biophys.* **74**, 408.
Vivino, A. E. & Palmer, L. S. (1944). *Arch. Biochem.* **4**, 129.
Zechmeister, L. (1960). *Progr. Chem. org. nat. Products*, **18**, 233.
Zechmeister, L. & Cholnoky, L. V. (1930). *Liebigs Ann.* **481**, 42.