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CAROTENOIDS OF CERTAIN COMPOSITAE FLOWERS

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Abstract—Eleven species and varieties of flowers of Compositae have been investigated for their carotenoid contents. Epoxy-carotenes and xanthophylls were found in fairly large amounts and were the main pigments in some cases. Carotenoid compositions are reported for the first time for flowers of *Gerbera jamesonii*, *Hypochoeris radicata* and *Senecio scandens*. Yellow flowers tended to have more xanthophylls while orange ones had a large amount of one carotene, except in *Tagetes erecta* where the only difference between the yellow and orange seemed to be in the total carotenoids. Lutein, universally present in leaves, was not always found in the flowers studied, while β-carotene was always found in fairly small quantities; flavoxanthin and chrysanthemaxanthin were usually present in fairly large amounts.

INTRODUCTION

KARRER and Jucker[1] reported that the flowers of no less than forty-five different species of Compositae possess carotenoids. Some of the very early workers used inadequate techniques for purifying and identifying carotenoids and also tended to be only interested in carotenoids found in large amounts and did not usually record those found in small quantities. Another problem that faced them (and still faces modern workers) was that the same species of flower showed differences in carotenoid composition in different parts of the world. For example, Kuhn and Lederer[2] obtained large amounts of a pigment they termed taraxanthin from *Taraxacum officinale* whereas Karrer and Rutschmann[3] failed to detect this pigment in their samples. Also, *Gazania rigens* of Portuguese[4] and of California origin[5] had β-, γ-carotene, gazaniaxanthin and lutein in common but the former had rubixanthin and a pigment of unknown constitution (possibly neo-gazaniaxanthin) which were lacking in the American species which had cryptoxanthin and lycopene instead. To obtain a more up-to-date picture of the distribution of carotenoids in composites, a study was undertaken of the flowers of eleven species and varieties. It has been suggested that yellow flowers contained large amounts of xanthophylls and only traces of carotenes whilst deep orange flowers seemed to be characterized by the presence of large amounts of one carotene: e.g. lycopene in *Calendula officinalis*.[6] Therefore, in the present study we examined yellow, orange and reddish flowers to find out if the same generalized picture could be obtained.

RESULTS AND DISCUSSION

The carotenoid pigments present in the eleven species of flowers of Compositae studied are given in Table 1.

[1] P. KARRER and E. JUCKER, *Carotenoids*. Elsevier, Amsterdam (1950).
[2] R. KUHN and E. LEDERER, *Z. Physiol. Chem.* **200**, 108 (1931).
[3] P. KARRER and J. RUTSCHMANN, *Helv. Chim. Acta* **25**, 1144 (1942).
[4] K. SCHON, *Biochem. J.* **32**, 1566 (1938).
[5] L. ZECHMEISTER and W. A. SCHROEDER, *J. Am. Chem. Soc.* **65**, 1535 (1943).
[6] T. W. GOODWIN, *The Chemistry and Biochemistry of Plant Pigments*. Academic Press, New York (1965).

TABLE 1. THE QUANTITATIVE DISTRIBUTION OF CAROTENOIDS IN SOME COMPOSITAE. THE VALUES GIVEN ARE A PERCENTAGE OF TOTAL CAROTENOIDS

	Phytoene	Phytofluene	α-Carotene	β-Carotene	β-Zeacarotene	ζ-Carotene	γ-Carotene	Isomer 5,6-monoepoxy-β-carotene	5,6-Monoepoxy-β-carotene	5,6-Diepoxy-β-carotene	Rubixanthin-like	Lycopene	Mutatochrome	Flavochrome	Gazaniaxanthin	Cryptoxanthin	Zeaxanthin	Lutein	Lutein epoxide	Chrysanthemaxanthin	Flavoxanthin	Antheraxanthin	Neoxanthin	Auroxanthin	Total carotenoids (mg/g)
Dimorphotheca aurantiaca	3·20	0·33	0·02	2·79	0·18	0·13	8·54	0·22	2·92	3·87	8·36	64·57	—	—	—	—	—	—	—	—	—	—	4·87	—	7·50
Gazania rigens	2·75	0·12	—	0·49	0·04	0·33	3·73	—	—	3·40	—	64·15	—	—	18·9	—	—	0·86	—	0·99	1·39	—	—	2·85	2·91
Gerbera jamesonii (yellow–orange)	—	1·2	—	4·9	0·5	0·5	—	1·8	4·8	1·8	—	—	—	—	—	11·3	—	—	—	11·7	23·7	—	—	37·8	0·65
G. jamesonii (red–orange)	—	0·4	—	8·5	0·5	0·4	—	2·0	4·7	4·7	—	—	—	—	—	18·1	—	—	—	12·9	26·9	—	—	20·9	0·78
Hieracium aurantiacum	2·1	—	—	1·8	—	—	—	—	11·3	4·4	—	—	11·8	—	—	2·5	—	—	—	25·0	27·1	—	—	14·0	4·88
H. pilosella	—	—	—	3·8	—	—	—	—	1·2	—	—	—	12·7	—	—	+	—	—	—	30·0	39·4	—	—	12·9	1·11
Hypochoeris radicata	—	1·1	0·2	0·5	0·2	0·4	—	0·7	12·0	9·8	—	—	—	—	—	—	—	19·5	—	22·8	27·8	—	—	5·0	3·87
Senecio scandens	—	+	0·8	3·9	0·8	+	—	2·7	37·3	6·5	—	—	21·7	—	—	—	1·6	1·8	—	3·0	7·7	—	—	12·2	0·67
Tagetes erecta (yellow)	4·7	—	—	1·1	0·9	0·5	—	0·3	55·4	4·4	—	—	0·4	—	—	2·8	—	7·6	2·2	1·2	5·8	—	—	12·7	1·15
T. erecta (orange)	0·9	11·4	0·5	1·0	0·2	0·2	—	5·0	39·8	20·8	—	—	0·5	0·8	—	1·4	2·4	3·7	2·0	2·2	2·4	2·2	—	1·2	3·23
T. patula	3·3	—	—	0·7	0·1	0·3	—	0·6	39·4	26·2	—	—	—	—	—	5·6	—	—	—	3·5	8·4	—	—	11·9	6·31

+ Indicates a trace.

The total amount of carotenoids in any one flower varied from 0·6 mg/g dry weight in the yellow *Gerbera* to as much as 6·3 mg/g in the French marigold (*Tagetes patula*). Phytoene and phytofluene, the colourless C_{40} polyenes, precursors of carotenoids[7] were found in a few species and together in only two cases. The following intermediates in the Porter and Anderson[7] pathway for carotene biosynthesis were identified: α-, β- γ-, ζ-carotene, β-zeacarotene and lycopene, but not neurosporene, α-zeacarotene and δ-carotene. Further, two epoxides of β-carotene were also identified as were a number of xanthophylls, mainly epoxy carotenoids which are specific to petals.[8] The presence of large amounts of epoxy carotenoids in flowers is rather puzzling. They may be intermediates in the transfer of oxygen and formation of xanthophylls;[9] or as the epoxides easily revert to carotenoids by loss of oxygen, may act as oxygen carriers in plants;[10] or may play some part in the reproduction of plants.[11]

Lutein, the principal xanthophyll of green leaves, was not universally present in the flowers studied and only occurred in fairly small amounts as did lutein epoxide in the two cases where it was found. Karrer and Jucker[1] stated that flavoxanthin occurred fairly widely in plants, but only in low concentrations and not as the main pigment; and that chrysanthemaxanthin, the *cis*-isomer of flavoxanthin, did not usually accompany it. In a few cases in the present study, these compounds were the main pigments and were always found together.

The orange flowers of *Dimorphotheca aurantiaca* (Cape marigold) were shown by Karrer and Notthafft[12] to contain lycopene which was absent in yellow flowers. Other carotenoids were not characterized, but we found the orange flowers of this species to contain lycopene (64·5 per cent) as the main pigment. γ-Carotene (8·54 per cent) and a rubixanthin-like pigment (8·36 per cent) then followed in order of concentration. The absorption spectrum of the rubixanthin-like pigment was very similar to that obtained by Goodwin[13] in the pot marigold (*Calendula officinalis*). Another composite with lycopene as the major pigment (64·15 per cent) was the orange-coloured *Gazania rigens*. Recent work had shown that lycopene was present in a Californian[5] variety but not in a Portuguese[4] variety, while the pigment gazaniaxanthin seems to be restricted to this genus only. The pigment composition of our species is very similar to the Californian[5] variety.

As far as we are aware, the pigment composition of the flowers of *Gerbera jamesonii* (Transvaal daisy) had not been reported previously. We obtained a yellow–orange and a red–orange variety which contained exactly the same pigments, namely phytofluene, β- and ζ-carotene, β-zeacarotene, 5,6-monoepoxy-β-carotene, 5,6-diepoxy-β-carotene, cryptoxanthin, chrysanthemaxanthin, flavoxanthin and auroxanthin. There was a slightly higher amount of total carotenoid pigments in the red–orange vaiety (0·78 mg/g) as opposed to the yellow–orange variety (0·65 mg/g) and the difference in appearance between the two varieties may be due to an anthocyanin present in the redder variety.

Hieracium pilosella (mouse-ear hawkweed) has a yellow flower while *H. aurantiacum* (fox and cubs) has a red one. They both contained the same carotenoids, but the red species contained over four times as much total carotenoids (4·8 mg/g compared to 1·1 mg/g) as the yellow species. Chrysanthemaxanthin and flavoxanthin formed the major carotenoids

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[8] T. W. Goodwin, *The Comparative Biochemistry of the Carotenoids*. Chapman and Hall, London (1952).
[9] L. Cholnoky, C. Gyorgyfy, E. Nagy and M. Panczel, *Acta Chim. Acad. Sci. Hung.* **6**, 143 (1955).
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[11] F. B. Jungalwala and H. R. Cama, *Biochem. J.* **85**, 1 (1962).
[12] P. Karrer and A. Notthafft, *Helv. Chim. Acta* **15**, 1195 (1932).
[13] T. W. Goodwin, *Biochem. J.* **58**, 90 (1954).

(52·1 per cent red; 69·4 per cent yellow) and the difference in colour may be due to the higher amounts of monoepoxy-β-carotene in the red species and also to the presence of an anthocyanin which is not found in the yellow one.

Hypochoeris radicata (cat's ear) has a yellow flower whose carotenoids had not previously been reported. It contained phytofluene, α-, β- and ζ-carotene, β-zeacarotene, 5,6-monoepoxy-β-carotene and probably a *cis*-isomer of this epoxy-isomer, 5,6-diepoxy-β-carotene, lutein, chrysanthemaxanthin, flavoxanthin and auroxanthin. Another yellow flower, *Senecio scandens* (Chinese groundsel), had monoepoxy-β-carotene as its major pigment (37·3 per cent) and the range of carotenoids was similar to that of *H. radicata* except that it also contained zeaxanthin and lutein. Karrer and Notthafft[12] showed that *S. doronicum* contained zeaxanthin and Kuhn and Brockmann[14] that *S. vernalis* had flavoxanthin, both pigments being found in *S. scandens*.

Two varieties of *T. erecta* (African marigold) were investigated (lemon-yellow and orange). In the orange variety, which contained about three times as much total carotenoids as the yellow one, phytofluene, α-carotene, zeaxanthin and antheraxanthin were present as well as those pigments that were common to both. One point worth noting was that the orange-coloured flowers did not contain a high percentage of any one carotene (as had been suggested) compared to the yellow flowers. There was no anthocyanin present to produce the darker colour in this case and one can only assume that the difference in colour was due solely to the greater total amount of carotenoids in the orange variety. The mono- and di-epoxy-β-carotene formed the main pigments in both varieties as they did in the related French marigold (*T. patula*). Kuhn *et al.*[15] found xanthophyll (lutein) in both the species, while a more thorough investigation by Karrer *et al.*[16] showed that *T. patula* contains lutein, lutein epoxide, rubixanthin, rubichrome, α- and β-carotene, and two unidentified carotenoids. Rubichrome and rubixanthin were obtained by the latter workers but we failed to detect any in our specimens. One of our fractions identified as cryptoxanthin showed a very similar absorption spectrum to rubichrome, as did unpurified monoepoxy-β-carotene. This difference and also the absence of both lutein and lutein epoxide in our specimens is undoubtedly due to varietal differences.

In the eleven varieties and species of the Compositae studied, no new carotenoids were observed. Epoxy-carotenes and xanthophylls were found in fairly large amounts and were the main pigments in some cases. The range of carotenoids varied quite appreciably and there were as many as eighteen different compounds in the orange African marigold and only seven in the yellow mouse-ear hawkweed *Hieracium pilosella*. Yellow flowers on the whole tended to have more xanthophylls while orange ones had a large amount of one carotene. However, when the yellow and orange varieties of the African marigold were compared, it seemed that the only difference was due to the greater total amount of carotenoids in the darker flowers. Red–orange flowers tended to have more or less the same pigments as their yellow counterparts but in these cases, had anthocyanins as well. Finally, lutein is not universally present in flowers as it is in leaves.

EXPERIMENTAL

Hieracium pilosella, L. (yellow), *H. aurantiacum*, L. (red), *Senecio scandens* Buch.-Ham. (yellow), *Hypochoeris radicata*, L. (yellow), *Tagetes erecta*, L. (yellow and orange varieties) and *T. patula*, L. (orange) were obtained from the grounds of Royal Holloway College; *Gazania rigens*, R. Br. (yellow orange) and

[14] R. KUHN and H. BROCKMANN, *Z. Physiol. Chem.* **213**, 192 (1932).
[15] R. KUHN, A. WINTERSTEIN and E. LEDERER, *Z. Physiol. Chem.* **197**, 141 (1931).
[16] P. KARRER, E. JUCKER and K. STEINLIN, *Helv. Chim. Acta* **30**, 531 (1947).

TABLE 2. CHROMATOGRAPHIC-ADSORPTION ANALYSIS AND IDENTIFICATION OF CAROTENE HYDROCARBONS, THEIR EPOXIDES AND MONOHYDROXYXANTHOPHYLLS. THE PIGMENTS WERE CHROMATOGRAPHED ON MgO–CELITE (1:1, v/v) USING *n*-HEXANE CONTAINING ETHER AS THE DEVELOPING SOLVENT. BANDS 1–11 ARE IN ORDER OF INCREASING ADSORPTIVE POWERS

Band	Absorption maxima in *n*-hexane (nm)	Absorption maxima in ethanol after addition of traces of ethanolic HCl (nm)	Co-chromatography with authentic sample	Identification
1	~272, 284, ~294	—	—	Phytoene
2	332, 348, 367	—	From tomatoes	Phytofluene
3	420, 442, 472	—	—	α-Carotene
4	~425, 450, 480	—	Synthetic (Hoffmann-La Roche)	β-Carotene
5	400, 425, 450	—	—	β-Zeacarotene
6	380, 400, 425	—	—	ζ-Carotene
7	435, 460, 490	—	From *Calendula officinalis*	γ-Carotene
8	~420, 441, 472	~400, 423, 450	—	5,6-Monoepoxy-β-carotene (isomer)
9	420, 445, 475	400, 425, 450	—	5,6-Monoepoxy-β-carotene
10	416, 436, 466	380, 400, 425	—	5,6-Diepoxy-β-carotene
11	444, 471, 500	—	Synthetic (Hoffmann-La Roche)	Lycopene
12	397, 422, 450	—	—	Mutatochrome
13	435, 460, 491	—	—	Gazaniaxanthin
14	~426, 447, 473	—	From *Physalis alkekengi*	Cryptoxanthin
15	456, ~480	—	—	Rubixanthin-like

TABLE 3. CHROMATOGRAPHIC ADSORPTION ANALYSIS AND IDENTIFICATION OF DI- AND POLY-HYDROXY-XANTHOPHYLLS AND THEIR EPOXIDES. THE PIGMENTS WERE CHROMATOGRAPHED ON MgO–CELITE (1:2, v/v) USING *n*-HEXANE WITH INCREASING CONCENTRATIONS OF ETHER AS THE DEVELOPING SOLVENT

Band	Absorption maxima in *n*-hexane (nm)	Absorption maxima in ethanol after addition of traces of ethanolic HCl (nm)	Co-chromatography with authentic sample	Identification
1	420, 445, 470	400, 422, 450	From nettle leaves	5,6-Monoepoxy-lutein
2	421, 445, 473	—	From nettle leaves	Lutein
3	~421, 443, 470	405, 426, 452	—	Antheraxanthin
4	~426, 450, 480	—	Synthetic (Hoffmann-La Roche)	Zeaxanthin
5	415, 435, 462	400, 420, 449	—	Neoxanthin
6	400, 420, 446	—	From dandelion	Chrysanthemaxanthin
7	400, 420, 447	—	From dandelion	Flavoxanthin
8	380, 400, 425	—	—	Auroxanthin

Dimorphotheca aurantiaca, Hort. (orange) from the Botanical Supply Unit of the University of London; and *Gerbera jamesonii*, Bolus (yellow and red–orange) from Bentall's, Kingston, Surrey.

Extraction of Pigments

Fully opened fresh flowers 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 came out in the solvent. 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°. The oil obtained was dissolved in methanol and saponified with 60% aqueous KOH overnight[17] and extracted with ethyl ether. The ethereal layer was washed with tepid water, freeze-dried and the pigments taken up in *n*-hexane. The unsaponifiable material was left overnight at -10° and the precipitated sterols were removed by centrifuging in the cold.

The carotenoid extracts were subjected to phase-partition between hexane and aq. 90% methanol. Di- and polyhydroxy xanthophylls and their derivatives remained in the lower hypophasic layer while carotenes and other xanthophylls were found in the upper epiphasic layer.

The upper phase was washed with water to remove the methanol, dried (Na_2SO_4) and was then ready for column chromatography. The lower phase was partitioned with diethyl ether until all the colour was transferred to the upper layer and this was washed free of methanol, reduced to dryness, taken up in *n*-hexane and dried (Na_2SO_4). The two fractions were chromatographed separately on a magnesium oxide–celite (1:2 v/v) column and eluted with hexane containing increasing amounts of diethyl ether. The first colourless fraction from the carotene layer was eluted with purified hexane and contained sterols which were removed according to the method of Mercer *et al.*[18] Phytoene was identified in this fraction by means of its spectrum in *n*-hexane ($\sim$272, 284, $\sim$294 nm). Phytofluene (blue–green fluorescence under u.v. light) had the following absorption spectrum in *n*-hexane (332, 348, 368 nm) and could not be separated from authentic *cis*-phytofluene from tomatoes.[19] The coloured bands eluted with ether in *n*-hexane were evaporated to dryness, taken up in *n*-hexane and the spectra estimated using a Unicam SP 500 spectrophotometer. Individual pigments were further purified by rechromatography on magnesium oxide–celite (1:1, v/v) and the order in which they appear are given in Tables 2 and 3. To identify purified bands, a pure pigment was mixed with the unknown, co-chromatographed on magnesium oxide–celite (1:1, v/v) eluted with ether in *n*-hexane and was shown to be one and the same pigment if they ran as one band. β-Zeacarotene and ζ-carotene were purified and identified according to the method of Simpson *et al.*[20] The *cis–trans* isomers, chrysanthemaxanthin and flavoxanthin were extremely difficult to separate and the values for these two compounds were only approximate.

The *cis-trans* configuration of the carotenoids was established by the iodine-isomerization test.[11] Isomerization of *cis*-carotenoids leads to slightly higher λ_{max} values, while with *trans*-forms the shift is to lower λ_{max} values after addition of the iodine.

Carotenoids having epoxy groups were characterized by a modified conc. HCl–ether test as used by Jungalwala and Cama.[11] 5,6-Monoepoxy-β-carotene and 5,6-diepoxy-β-carotene were characterized by their conversion into mutatochrome and aurochrome respectively on addition of traces of methanolic hydrochloric acid. The small yellow band which appeared below 5,6-monoepoxy-β-carotene was shown to be a *cis*-carotenoid and could well be an isomer of 5,6-monoepoxy-β-carotene, but was found in too small a quantity to be identified further. In certain cases, however, it was not easy to separate it from the *trans*-5,6-monoepoxy-β-carotene.

Quantitative determination. The concentrations of individual carotenoids were determined by measuring E_{max} and comparing it with known $E^{1\%}_{1\,cm}$ values at λ_{max} for pure pigments.[17] For those pigments whose $E^{1\%}_{1\,cm}$ values were not known, λ_{max} was assumed to be 2500.[13]

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