# Carotenoid Constituents of Pyrethrum Flowers *(Chrysanthemum cinerariaefolium)*

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The carotenoid composition of flower heads of *Chrysanthemum cinerariaefolium* at different stages of maturity was investigated. More than 90% of the carotenoid fraction was shown to be related to cis and trans isomers of the xanthophyll lutein, which occur mainly as diesters but are also present as monoesters and the free diols. In the immature flower head, at least 38% of the carotenoids present were in association with pollen, which was rich in *cis*-lutein. The carotenoid composition of pyrethrum oleoresin extract, refined extract, and pyrethrum residue was recorded.

The flower head of *Chrysanthemum cinerariaefolium* is the source of the natural insecticide pyrethrum extract, which is produced by extracting ground dry flowers with a petroleum solvent. The flower head is typical of the Compositae, a collection of disk florets, set on a slightly convex receptacle and surrounded by an outer ring of ray florets. As flowers mature, the outer disk florets open and development proceeds progressively across the disk towards the center. For optimum yields of "pyrethrins," combined with high pyrethrins content, only mature flower heads having horizontal petals are harvested (Head, 1963).

Carotenoid and chlorophyll components present in the flower head are responsible for the color of crude pyrethrum oleoresin extract. The presence of $\beta$-carotene in pyrethrum extract was reported by Subbaratnam and Parsameswaran Pillay (1947) and the use of a by-product from refining processes as a source of xanthophyll for addition to chickenfeed has also been described (Head, 1966; Plebluda *et al.*, 1966). The composition of pyrethrum extract has been the subject of a recent review (Head, 1973).

This paper identifies the major carotenoids present in the pyrethrum flower head and shows how carotenoid composition changes as the flower head matures. The carotenoid content of various pyrethrum products is also reported.

## MATERIALS AND METHODS

Flowers were harvested from clone 194 obtained from the Kenya Government Pyrethrum Research Station at Molo, Kenya.

**Pigment Extraction and Saponification.** Sixty grams of freshly picked flowers were macerated in a hand-operated household mincer (¼-in. screen) and immediately added to 200 ml of a mixture of acetone in light petroleum (3:1 by volume) in an atmosphere of nitrogen. After standing overnight at room temperature, the extract was decanted and the residue extracted with a further 100 ml of the same solvent mixture and finally 100 ml of hexane. The aqueous acetone layer was washed with water (25 ml × 1) and saturated salt solution (25 ml × 3). The petroleum layer was then dried over magnesium sulfate and the solvent removed *in vacuo* at 40°. A portion of the residue in light petroleum was saponified by shaking under nitrogen with 12% KOH in methanol for 1 hr. Solvents were removed under reduced pressure and the ether extract of the residue was washed free of alkali with water and dried over magnesium sulfate. The term "light petroleum" refers to the fraction boiling between 40 and 60°.

**Chromatography.** The unsaponified carotenoid extract (equivalent to 8 g of fresh flowers) was separated into five fractions by column chromatography on alumina (Woelm, neutral, grade II), the fractions being eluted with increasing proportions of acetone in light petroleum (Table I). Each fraction was then further examined by tlc (Table I).

**Identification.** All the isolated carotenoids gave a negative response to the epoxide test (Davies, 1965) and were unchanged after treatment with sodium borohydride in ethanol (Curl, 1962). Column chromatography of the saponified extract showed that fractions 2 and 4 were esters, the free xanthophylls were eluted in fraction 5, while fractions 1 and 3 remained unchanged.

Carotenoids 1a and 1b cochromatographed on tlc with $\alpha$- and $\beta$-carotene, respectively, isolated from carrots. Both pyrethrum carotenes contained large amounts of wax-like impurities, which could account for the relatively low absorption maxima; shapes of the spectral absorption curves were identical with the authentic materials. Carotenoid 1c separated clearly from lycopene (Table I, tlc system c, $R_f$ 0.24); its chromatographic and spectral absorption characteristics were similar to those of neurosporene but no sample was available for comparison.

Fraction 3 was separated into four components by tlc. Carotenoids 3a and 3b had spectral absorption curves very similar to flavoxanthin, but separated from the diester of flavoxanthin isolated from *Taraxacum officinale* (Table I, tlc system d, $R_f$ 0.20). Carotenoids isolated as 3c and 3d were impure and were not further investigated.

Fraction 5 was separated into two components by tlc, 5a and 5b. Fractions 2 and 4 on saponification each gave two products with the same $R_f$ and spectral characteristics as 5a and 5b, which suggested that they were fully and partially esterified forms of the same xanthophylls. The fully esterified compounds could be clearly separated by tlc on alumina (Merck, neutral type T) into two components, 2a and 2b; partially esterified xanthophylls could not be thus separated. Carotenoid 2a was derived from xanthophyll 5b, and 2b was from 5a. Isomerization studies showed 5a and 2b to have a trans configuration, while 5b and 2a were cis. Tlc of each of the isomerized xanthophylls gave three major components identical in all respects, showing 5a and 5b to be the trans and cis isomers of the same xanthophyll. The change in chromatographic sequence between isomeric xanthophyll esters and free xanthophylls was in keeping with previous observations (Zechmeister, 1962). The absorption maxima of the trans form (440 and 470 nm in light petroleum and 470 and 500 nm in carbon disulfide) suggested the parent carotenoid was either taraxanthin or lutein. 5a was shown to cochromatograph with a sample of lutein isolated from elm leaves, and the products of stereomutation were identical. Final confirmation of the identity of 5a with *trans*-lutein was achieved by its isolation in a crystalline form (mp 180–181° evacuated tube, uncorrected) which gave satisfactory carbon–hydrogen analyses and ir identical with that of the authentic material. The mass spectrum showed the parent peak at *m/e* 568.4305; calculated for $C_{40}H_{56}O_2$, 568.4280. The nmr spectrum agreed with published data (Barber *et al.*, 1960).

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**Table I. Carotenoid Composition of the Pyrethrum Flower Head**

| Fraction no.[a] | % acetone for elution | Tlc reference | $R_f$, tlc | Absorption maxima, nm, in light petroleum | Identity | % carotenoid,[b] dry weight | % distribution |
|---|---|---|---|---|---|---|---|
| 1 | 1/2 | 1a | 0.52[c] | (414), 435, 466 | α-Carotene | 0.0002 | 0.3 |
| | | 1b | 0.44[c] | (420), 445, 470 | β-Carotene | 0.0015 | 2.8 |
| | | 1c | 0.24[c] | (419), 440, 469 | Neurosporene-like | 0.0005 | 0.9 |
| 2 | 2 | 2a | 0.45[d] | (417), 436, 466 | Neolutein B diester | 0.0233 | 44.6 |
| | | 2b | 0.40[d] | (419), 441, 470 | *trans*-Lutein diester | 0.0123 | 23.6 |
| 3 | 4 | 3a | 0.36[d] | 397, 419, 445 | Flavoxanthin-like | 0.0008 | 1.7 |
| | | 3b | 0.33[d] | 399, 420, 445 | Flavoxanthin-like | 0.0005 | 0.9 |
| | | 3c | 0.14[d] | 420–421, 439–440, 468–470 | Unknown | 0.0007 | 1.4 |
| | | 3d | 0.12[d] | Indistinct | Unknown | 0.0002 | 0.4 |
| 4 | 10 | 4a | 0.07[d] | 418–421, 440–443, 469–471 | Neolutein B monoester[e] | 0.0065 | 12.4 |
| | | 4b | 0.07 | | *trans*-Lutein monoester[e] | 0.0020 | 3.9 |
| 5 | 30 | 5a | 0.24[f] | (418), 439, 469 | *trans*-Lutein | 0.0018 | 3.4 |
| | | 5b | 0.19[f] | (418), 438, 466.5 | Neolutein B | 0.0019 | 3.7 |
| | | | | | | 0.0522 | |

[a] Column chromatography on alumina (Woelm neutral, grade II, 5 in. × 1/2 in.). [b] Calculated using $E_{1cm}^{1\%}$ of 2500, esters expressed as free diols. [c] Adsorbent silica gel–calcium hydroxide (1:6 w/w), solvent system 2% benzene in light petroleum. [d] Adsorbent alumina (Merck, neutral type T), solvent system 2% ethyl acetate in light petroleum. [e] Proportions determined by tlc of free diols. [f] Adsorbent as in *d* above, solvent system 50% ethyl acetate in light petroleum.

**Table II. Carotenoid Composition of the Pyrethrum Flower Head at Different Stages of Development**

| Stage no. | Description | Approx time for development, days | Avg dry wt/flower head, mg | μg of carotenoid/flower head: α- and β-carotene | Neurosporene-like | Neolutein B diester | *trans*-Lutein diester | Flavoxanthin-like | Lutein monoesters | Free lutein | Total carotenoids | % Total carotenoids, dry wt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Closed buds | 0 | 35.2 | 1.4 | a | 0.5 | 0.7 | a | 1.2 | 3.1 | 6.9 | 0.020 |
| 2 | Ray florets vertical | 12 | 70.5 | 2.2 | 1.9 | 44.3 | 14.7 | 1.6 | 27.1 | 4.3 | 96.1 | 0.136 |
| 3 | Ray florets horizontal, first row of disk florets open | 16 | 78.0 | 3.3 | 1.8 | 45.9 | 13.7 | 2.4 | 25.6 | 5.1 | 97.8 | 0.125 |
| 4 | Approx three rows of disk florets open | 19 | 103.8 | 2.6 | 1.4 | 34.0 | 13.2 | 4.4 | 15.6 | 5.1 | 76.3 | 0.074 |
| 5 | All disk florets open, fully mature | 21 | 132.5 | 2.5 | a | 12.3 | 7.3 | 3.2 | 8.2 | 5.0 | 38.5 | 0.029 |
| 6 | Early overblown condition, color of disk florets diminishing but ray florets still intact | 31 | 147.9 | 2.2 | a | 5.5 | 4.5 | 3.6 | 2.5 | 7.6 | 25.9 | 0.018 |
| 7 | Late overblown condition, little color remaining in disk florets but still intact, ray florets dried out | 43 | 157.9 | 1.5 | a | 2.0 | 1.7 | 1.1 | 0.9 | 5.9 | 13.1 | 0.008 |

[a] Insufficient for determination.

## RESULTS AND DISCUSSION

Up to 90% of the carotenoids present in the pyrethrum flower head are related to cis and trans isomers of the xanthophyll lutein. These occur mainly as diesters but are also present as monoesters and free diols (Table I). Attempts to purify the diester fraction so as to identify the acid component were unsuccessful, but lutein frequently occurs as the dipalmitate (Kuhn and Winterstein, 1930). The *cis*-lutein isomer is identical with that described by Zechmeister and Tuzson (1939) as neolutein B. Traces of the other major lutein isomer, neolutein A, were observed in saponified extracts, but could well be formed during the alkali treatment and subsequent manipulation. Naturally occurring cis isomers of lutein have been observed by Nitsche and Egger (1969), but in their work neoluteins V and U are synonymous with the neoluteins A and B, respectively, reported here. The figure for neolutein B (Table I) includes a minor component (approximately 5% of the diester fraction) that appeared as a front in tlc separation. This component was shown to be of cis configuration and appeared to be another isomer of lutein but differed from the diester of neolutein A, with which it cochromatographed. The diester of neolutein B is fairly common in flower heads of Compositae (Head, 1969).

The carotenoid composition of a single flower head at different stages of development is shown in Table II. There is a rapid increase in the level of carotenoids between stages 1 and 2. This level is maintained at stage 3 but is followed by a steady loss, mainly confined to the mono and diester fractions. During the rapid increase of the diester fraction, the cis components are formed preferentially over the trans. As the flower head develops, the ratio of cis to trans is reduced from more than 3 to almost 1. The main features of this pattern of development can be explained by observation of the maturing flower head. A cross-section of a bud (stage 1) shows that carotenoid formation in the disk florets occurs only in the outer ring of florets, which can not be observed in a very young bud. As the flower develops to stage 2 and ray florets emerge, carotenoids are observed in all disk florets. Dissection of fused anthers at this stage reveals that much of the carotenoid fraction is associated with the then fully developed

**Table III. Carotenoid Analyses of Pyrethrum Extract and Pyrethrum Residue**

| Carotenoid | Pyrethrum extract | | Pyrethrum residue | |
|---|---|---|---|---|
| | wt % | % of total | wt % | % of total |
| $\alpha$- and $\beta$-carotene | 0.01 | 1.2 | 0.03 | 1.9 |
| Neurosporene-like | 0.02 | 2.4 | 0.04 | 2.5 |
| Lutein diesters[a] | 0.57 | 69.5 | 1.18 | 72.8 |
| Flavoxanthin-like | 0.05 | 6.1 | 0.09 | 5.5 |
| Lutein monoester[a] | 0.14 | 17.1 | 0.27 | 16.7 |
| Lutein | 0.03 | 3.7 | 0.01 | 0.6 |
| Total carotenoids | 0.82 | | 1.62 | |

[a] Expressed as the free xanthophyll.

pollen. At stage 3 pollen formation in all florets is complete and the first row of disk florets opens. The rapid rise in carotenoid content is therefore associated with formation of carotenoids in the corolla of the disk florets and development of the carotenoid-rich pollen. As each disk floret develops, the corolla opens and the pollen is released. Loss of the bulk of pollen is surprisingly rapid and occurs within a few hours. Further carotenoid loss occurs with the fading of the open disk floret corolla; thus, as each disk floret matures, carotenoids are reduced by both physical loss of pollen and photolysis–oxidation of the corolla.

By collecting and analyzing pollen collected from flower heads grown from buds in 2% sucrose solution, it was found that at least 38% of the carotenoids present in the flower head are associated with pollen. Further, the lutein diester fraction isolated from pollen dissected from immature flower heads was found to contain more than 80% of cis isomer. The relative stability of the *cis*- and *trans*-lutein diesters was compared by exposing the separate compounds, impregnated on filter papers (approximately 0.5 $\mu g/cm^2$), to sunlight. Overall rates of decomposition were similar, 85 to 90% being decomposed after 30 min exposure. However, examination of the degradation products by tlc showed that the pattern of degradation differed between the two isomers. The cis isomer undergoes a roughly 50% conversion to the trans form; interconversion of the trans to the cis form is not nearly so marked. The effect of this difference in degradation in a mixture of the two isomers would be a differential loss of the cis component over the trans, similar to that observed in the developing flower head.

As the flower head matures, its pyrethrins content increases and is at a maximum when approximately half the florets are open (stage 4, Table II). It is now clear that inclusion of immature flower heads (stages 2 and 3, Table II) not only reduces the yield of pyrethrins but increases the carotenoid content of the oleoresin.

The carotenoid content of pyrethrum oleoresin ranges between 1 and 2% w/w, typical analyses of these materials being recorded in Table III. Refined extract containing 50% pyrethrins contains approximately 0.04% carotenoids. Pyrethrum residue, which is the material remaining after methanol extraction of pyrethrum oleoresin, contains increased proportions of carotene and lutein diesters compared with oleoresin. The residue, which is readily available, represents a valuable source of lutein for carotenoid studies.

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# An Investigation of the Essential Oil of *Hibiscus syriacus* L.

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The essential oil of *Hibiscus syriacus* L., an alternate host plant of the boll weevil (*Anthonomus grandis* Boheman), the major pest of cotton (*Gossypium hirsutum* L.) was obtained by steam distillation of buds and flowers and analyzed with an integrated gas chromatography–mass spectrometry system. Structures for 65 components were proposed which included 12 hydrocarbons, 6 esters, 24 carbonyls, 10 alcohols, and 13 miscellaneous compounds. Significant differences were apparent between cotton essential oil and the essential oil of *H. syriacus*.

Until the discovery by Coad (1914) of the malvaceous plant *Hibiscus syriacus* L. (rose-of-Sharon) on which the boll weevil (*Anthonomus grandis* Boheman) could feed, oviposit, and develop, this insect was considered monophagous on cotton (*Gossypium hirsutum* L.). Since that time, several natural infestations of the boll weevil on *H. syriacus* have been reported (Bondy and Rainwater, 1935; Gaines 1933, 1934). For example, rose-of-Sharon near cotton fields has been observed to be infested in times of stress when cotton has matured or when weevil population pressures are high and food is in short supply.

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