The Carotenoids of the Berries of Lonicera japonica

By T. W. GOODWIN

Department of Biochemistry, The University of Liverpool

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Although a great deal is known concerning the distribution and occurrence of carotenoids in fruit (see Karrer & Tucker, 1949; Goodwin, 1952a), there are still many gaps in our knowledge; these will have to be filled if a rationale of carotenoid distribution in fruit is eventually to be achieved. Furthermore, studies on the minor carotenoid components of fruit may well throw considerable light on the mode of biogenesis of carotenoids. Only one such investigation on tomatoes has been carried out (Porter & Lincoln, 1950); the results permitted the authors to suggest a working hypothesis for carotenogenesis in this fruit.

No precise information is available on the carotenoids of the berries of the family Caprifoliaceae, although early workers had indicated the presence of lipochromes (carotenoids) in Lonicera tartarica (Schimper, 1885), L. zylosteum (Schimper, 1885; Molisch, 1896; Kohl, 1902; Nowak & Zellner, 1921); Sambucus nigra (Nowak & Zellner, 1921), Viburnum opulus and V. lantana (Wisselingh, 1914; Nowak & Zellner, 1921; Kryz, 1919). When a small crop of ripe berries of a cultivated climbing honeysuckle (Lonicera japonica) became available an investigation was undertaken into the nature of the carotenoids present, with special emphasis on the minor components.

EXPERIMENTAL

Materials. The fully ripened berries were obtained from a garden in north-west Cheshire. Two portions of about 200 g. each were examined separately with identical results.

REFERENCES


Extraction and separation of the carotenoids

The fresh berries were ground to a fine powder with Na₂SO₄ and the powder extracted with successive portions of diethyl ether (freshly distilled over reduced iron) until no further colour was extracted. The combined ether extracts were taken to dryness in vacuo at room temperature and the lipids saponified by adding to the residue 1 ml. of 60% (w/v) aqueous KOH and 5 ml. of ethanol, mixing and allowing to stand overnight at room temperature. The unsaponifiable matter was extracted into ether as described by Goodwin & Morton (1946) and the ether was removed in vacuo; the residue was dissolved in a small volume of light petroleum (b.p. 40–60°) and examined chromatographically. Petroleum, b.p. 40–60°, was used throughout this investigation.

Separation 1. A preliminary separation of the pigment mixture was first carried out on alumina (Spence, Grade 'O') deactivated with methanol (Goodwin, 1952a) using light petroleum containing 10% (v/v) of ether as developer. The resulting chromatogram is described in Table 1.

Separation 2. Fraction 1 (Table 1), which eventually percolates through the column, was collected in the filtrate, the mixed solvents removed in vacuo and the residue redissolved in light petroleum and chromatographed on activated alumina (Spence, Grade 'O'). Five fractions were obtained as recorded in Table 2.

Separation 3. Zone 6 (Table 1) was eluted with ethanol. The ethanol was removed in vacuo at 30° and the residue dissolved in a few drops of ether and made to approx. 10 ml. with light petroleum. This treatment is necessary because this fraction will not dissolve directly in light petroleum. Chromatography was carried out on a column of ZnCO₃ using benzene as developer. Four zones were obtained, two of which eventually moved down the column to be collected.
in the filtrate (Table 3). On treatment of the column with benzene containing 5% (v/v) of ethanol, fraction 6C moved off the column and fraction 6D, originally adsorbed at the top, separated into two zones which moved slowly down the column. The faster moving pigment was designated 6DA and the slower moving one 6DB.

Table 1. The first separation of Lonicera carotenoids on a column of deactivated alumina (Goodwin, 1952a), using light petroleum containing 10% (v/v) ether as developer

(Zone 3. The zones are numbered in order of increasing adsorptive power.)

<table>
<thead>
<tr>
<th>Zone no.</th>
<th>Description</th>
<th>Absorption spectrum maximum (solvent, light petroleum) (mμ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Orange, diffuse; greenish blue fluorescent*</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Narrow, orange-khaki</td>
<td>490, 460, 440</td>
</tr>
<tr>
<td>3</td>
<td>Pink, slight blue fluorescent*</td>
<td>471, 445</td>
</tr>
<tr>
<td>4</td>
<td>Orange</td>
<td>479, 450</td>
</tr>
<tr>
<td>5</td>
<td>Trace of khaki</td>
<td>449</td>
</tr>
<tr>
<td>6</td>
<td>Orange-red, major fraction: strongly adsorbed</td>
<td>452</td>
</tr>
</tbody>
</table>

* In ultraviolet light.

Table 2. The separation of fraction 1 (Table 1) on a column of activated alumina (Spence, Grade ‘O’), using light petroleum containing 20% (v/v) ether as developer

(The zones are numbered in order of increasing adsorptive power.)

<table>
<thead>
<tr>
<th>Zone no.</th>
<th>Description</th>
<th>Absorption spectrum maximum (solvent, light petroleum) (mμ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Trace of blue-green fluorescent*</td>
<td>348, 367</td>
</tr>
<tr>
<td>1B</td>
<td>Lemon-yellow, diffuse, slight blue fluorescent*</td>
<td>399, 425</td>
</tr>
<tr>
<td>1C</td>
<td>Colourless, bright green-blue fluorescent*</td>
<td>531, 348, 367</td>
</tr>
<tr>
<td>1D</td>
<td>Diffuse-orange: major zone</td>
<td>450, 474</td>
</tr>
<tr>
<td>1E</td>
<td>Small, narrow, pale lemon</td>
<td>381, 401, 427†</td>
</tr>
</tbody>
</table>

* In ultraviolet light. † In ether.

Table 3. The separation of fraction 6 (Table 1) on a column of ZnCO₃, using benzene as developer

(The zones are numbered in order of increasing adsorptive power.)

<table>
<thead>
<tr>
<th>Zone no.</th>
<th>Description</th>
<th>Absorption spectrum maximum (mμ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6A</td>
<td>Orange-red, major fraction</td>
<td>464, 491*</td>
</tr>
<tr>
<td>6B</td>
<td>Small zone, khaki</td>
<td>438, 463*</td>
</tr>
<tr>
<td>6C</td>
<td>Small brick-red zone</td>
<td>453, 479†</td>
</tr>
<tr>
<td>6D</td>
<td>Lemon-yellow</td>
<td>429 (broad)†</td>
</tr>
</tbody>
</table>

* Solvent benzene. † Solvent ethanol.

Examination of the fractions

Fractions 2, 3 (Table 1), 1C, 1D, 1E (Table 2), 6A, 6C, 6DB (Table 3). Examination of these fractions, after further chromatographic purification on appropriately activated alumina, indicated that in all probability they were γ-carotene (2), lycopene (3), phytofluene (1C), β-carotene (1D), ß-carotene (1E), cryptoxanthin (6A), zeaxanthin (6C) and auroxanthin (6DB). In each case, except the last, the berry carotenoid was compared chromatographically and spectroscopically with an authentic and chromatographically homogeneous sample of the corresponding pigment. Samples of all the carotenoids were obtained from the fungus Phycomyces blakesleeanus (Goodwin, 1952a). Cryptoxanthin and zeaxanthin were obtained from a commercial sample of maize meal.

In the case of fraction 6DB no authentic specimen of auroxanthin was available, but, as demonstrated later, the properties of this pigment (Karrer & Butschartmann, 1942) are so distinctive, that it is virtually impossible to confuse it with any other carotenoid.

Fractions 1A, 1B (Table 2), 6B and 6DA (Table 3). 1A appears to be closely related to phytofluene, whilst 1B is considered to be a carotene not previously described, it is proposed to call it ß-carotene. Fraction 4 appears to be very similar to the unidentified pigment reported in human milk (Kon & Mawson, 1950), whilst fractions 5, 6B and 6DA remain unidentified.

Quantitative experiment

In order to obtain information on the relative amounts of the constituent pigments present in the berries, in one experiment the fractions obtained, as described above, were dissolved in known volumes of light petroleum and the extinctions of the solutions measured at the wavelengths of maximal absorption of the pigments concerned. Using the

Table 4. The wavelengths and E₁%cm. values (in light petroleum) used for quantitative determination of Lonicera carotenoids

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Wavelength (mμ)</th>
<th>E₁%cm.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytofluene</td>
<td>348</td>
<td>1200</td>
<td>Porter &amp; Lincoln (1950)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>450</td>
<td>2980</td>
<td>Zechele et al. (1942)</td>
</tr>
<tr>
<td>ß-Carotene</td>
<td>422</td>
<td>2500</td>
<td>Porter &amp; Lincoln (1950)</td>
</tr>
<tr>
<td>γ-Carotene</td>
<td>469</td>
<td>2760*</td>
<td>Zechmeister (1944)</td>
</tr>
<tr>
<td>Lycopene</td>
<td>469</td>
<td>3460*</td>
<td>Zechmeister (1944)</td>
</tr>
<tr>
<td>Cryptoxanthin</td>
<td>451</td>
<td>2460</td>
<td>Zechele et al. (1942)</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>462</td>
<td>2480</td>
<td>Zechele et al. (1942)</td>
</tr>
<tr>
<td>Auroxanthin</td>
<td>425</td>
<td>1780*</td>
<td>Karrer &amp; Jucker (1945)</td>
</tr>
</tbody>
</table>

* Calculated from graphs.
RESULTS

A. The carotenes

The following carotenes were identified unequivocally: phytofluene, \( \beta \)-carotene, \( \zeta \)-carotene, lycopene and \( \gamma \)-carotene. They were chromatographically indistinguishable from authentic specimens of the corresponding compounds, and the shape and position of their absorption bands in the region 380–500 m\( \mu \). (320–400 m\( \mu \) in the case of phytofluene) were also identical with those of the known polyenes. Full details have recently been given of the chromatographic and spectral properties of these pigments (Goodwin, 1952a), and further elaboration is unnecessary here.

**Fraction 1A (Table 2).** This fraction, which only occurred in very small amounts, had an absorption spectrum very similar to that of phytofluene, but appeared to be rather less strongly adsorbed on alumina than this polyene. Insufficient material was available to examine it in any detail, but these preliminary observations do suggest that it is quite distinct from phytofluene.

**Fraction 1B (Table 2).** This fraction runs down an alumina column as a diffuse dull orange band just ahead of phytofluene and \( \alpha \)-carotene. When these chromatographic properties are considered in conjunction with its spectral properties (Fig. 1), there is no doubt that this is a new carotene. The only carotene with similar chromatographic behaviour is \( \epsilon \)-carotene obtained by Strain & Manning (1943) from the diatoms *Nitzschia closterium* and *Navicula torquatum*. A comparison of the spectrum of \( \epsilon \)-carotene and that of the *Lonicera* carotene given in Fig. 1 shows that they are quite distinct. The only carotene already reported with an absorption spectrum at all similar to that of the pigment in fraction 1B is \( \xi \)-carotene (Porter & Lincoln, 1950). This pigment is, however, easily separable from the *Lonicera* carotene on a chromatogram on alumina (4 parts activated; 1 part deactivated); \( \xi \)-carotene is much more strongly adsorbed, for it forms a zone above \( \beta \)-carotene whilst the new carotene travels down the column well in front of \( \beta \) (and even \( \alpha \)) carotene. Furthermore, \( \xi \)-carotene (1 \( E \)) and the new carotene occur together in *Lonicera*, and are easily separable. The uniqueness of this carotene being so apparent, it is suggested that it be named \( \eta \)-carotene. This nomenclature follows the recommendations of the ‘Union Internationale de Chimie’ as drawn up by Karrer (1948). A full investigation into this pigment must await the availability of larger amounts of ripe *Lonicera* berries at a time when it is feasible to examine them. The discovery of a more potent source of this pigment would also be useful in this connexion, for, as will be seen later (Table 6), \( \eta \)-carotene is only a minor component (1-3 \%) of the total carotenoids of *Lonicera*.

![Absorption spectrum of new carotene (\( \eta \)-carotene) obtained from *Lonicera* berries compared with that of \( \epsilon \)-carotene from the alga *Navicula torquata*. —, \( \eta \)-carotene in light petroleum (b.p. 40–60°); ——, \( \epsilon \)-carotene in ethanol (from Strain & Manning, 1943). Note. (i) The change in the position of absorption maxima of carotenes in light petroleum and ethanol is only slight (2–4 m\( \mu \)); (ii) the \( E \) values are arranged so that \( E_{\text{max}} \) is the same for both pigments.](image)

*Fraction 4.* This fraction is epiphasic to 90 and 95 \% (v/v) aqueous methanol, has an absorption spectrum almost identical with that of \( \beta \)-carotene, and is adsorbed on alumina to very much the same degree as is free vitamin A. It is probable that this is the unidentified pigment found in human blood serum and milk by Kon & Mawson (1950). Their pigment showed absorption bands at 450 and 476 m\( \mu \), was adsorbed more strongly than lycopene and could not be separated chromatographically from vitamin A.

*Fraction 5.* This fraction occurred only in minute traces. It exhibited an absorption spectrum similar to that of \( \beta \)-carotene; insufficient amounts were available for further study.

B. The xanthophylls

*Fraction 6A.* This fraction was identified as cryptoxanthin; it had the same absorption spectrum (Fig. 2) and the same chromatographic properties as authentic cryptoxanthin. Furthermore, in the partition test, it was not extracted from light petroleum by shaking with 90 \% (v/v) aqueous methanol; when, however, 95 \% (v/v) aqueous
methanol was used the pigment was equally distributed in the two phases; this is a characteristic property of cryptoxanthin.

Fig. 3. A comparison of the absorption spectrum of the pigment (zone 6A, Table 3) obtained from Lonicera berries with that of authentic cryptoxanthin obtained from maize. The E values are so arranged that $E_{\text{max}}$ is the same for both pigments. ───, Lonicera pigment; ——, zeaxanthin. Solvent, light petroleum.

A comparison of the spectrum of the berry pigment with that of authentic zeaxanthin is recorded in Fig. 3.

Fraction 6DB. This fraction appears to be auroxanthin. No authentic auroxanthin was available for comparison, but the very characteristic properties of this pigment leave little, if any, doubt as to its identification. The position and shape of its absorption spectrum is shared by only two other carotenoids, $\zeta$-carotene and aurochrome (and to a lesser degree by $\eta$-carotene). A consideration of other properties of these four pigments (Table 5) indicates that the present pigment can only be auroxanthin. A comparison of the spectra of pigment 6DB and that of auroxanthin is recorded in Fig. 4.

Fractions 6B, 6DA. The spectra are recorded in Fig. 4, but could not be identified with any known xanthophylls. The compounds are completely hypophasic. The poor persistence of their spectra suggests that they might be neoxanthophylls (compare the spectral persistence of the neofucoxanthins (Strain, Manning & Hardin, 1943)). Because of the well-known lability of xanthophylls, much further work is necessary before the possibility that these pigments are not merely oxidative artifacts can be completely disproved.

Natural occurrence of the xanthophylls

Partition experiments before saponification showed that the pigments in the crude extract of the berries were almost completely epiphasic using both 90 and 95% (v/v) aqueous methanol, thus indicating that the xanthophylls occurred naturally almost entirely as esters.

The quantitative distribution of the component pigments

The relative amounts of the pigments present in ripe Lonicera berries are recorded in Table 6. It will be clearly seen that cryptoxanthin is the predominant pigment.

DISCUSSION

A great deal of the classical work on the isolation of carotenoids for determination of structure has been carried out on fruit of various species (see Karrer & Jucker, 1949, for a complete survey) and, quite naturally, with the emphasis being on isolation of large amounts, minor components were often ignored. Now that, as a result of these investigations, the properties of so many carotenoids are accurately known, it is possible, by utilizing improved chromatographic and spectrographic techniques, to separate and identify carotenoids without the necessity of isolating them in crystalline form. In this way it has recently been shown that tomatoes
Table 5. A comparison of the properties of pigment 6DB (Table 3) with those of auroxanthin, aurochrome, \( \zeta \)-carotene and \( \eta \)-carotene

<table>
<thead>
<tr>
<th>Property</th>
<th>Pigment 6DB</th>
<th>Auroxanthin</th>
<th>Aurochrome</th>
<th>( \zeta )-Carotene</th>
<th>( \eta )-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption maxima in light petroleum (m( \mu ))</td>
<td>400, 425</td>
<td>400, 425</td>
<td>428*</td>
<td>400, 426</td>
<td>399, 425</td>
</tr>
<tr>
<td>Partition between light petroleum and 90% (v/v) aqueous methanol</td>
<td>Hypophasic</td>
<td>Hypophasic</td>
<td>Epiphasic</td>
<td>Epiphasic</td>
<td>Epiphasic</td>
</tr>
<tr>
<td>Colour with conc. HCl</td>
<td>Stable blue</td>
<td>Stable blue</td>
<td>Stable blue</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Position on chromatogram</td>
<td>Strongly adsorbed above zeaxanthin</td>
<td>Strongly adsorbed above zeaxanthin</td>
<td>Can be developed on ( \text{Ca(OH)}_2 ) with light petroleum; hydroxyxarotenoids are not developed with this solvent</td>
<td>Adsorbed above ( \beta )-carotene but below lycopene</td>
<td>Adsorbed below ( \alpha )-carotene</td>
</tr>
</tbody>
</table>

References

Present work | Karrer & Rutschmann (1942) | Karrer & Jucker (1945, 1949) | Goodwin (1952a) | Present work |

* The lower wave band in light petroleum is not recorded by Karrer & Jucker probably for technical reasons. Its existence is, however, obvious from a reference to the curve for aurochrome in CS\( _2 \).

contain in addition to lycopene (the major component) very small amounts of a series of polyenes, each differing from the next in the series by four hydrogen atoms (Porter & Lincoln, 1950). From this investigation it has been postulated that these pigments represent successive intermediate steps in the synthesis of the fully unsaturated carotenoids (lycopene, \( \beta \)-carotene, etc.). The present work indicates that a very similar series of pigments are present in Lonicera berries and this points to a pathway of synthesis similar to that occurring in tomatoes. As such a series of pigments has never been demonstrated in leaves, this strongly suggests that the route of carotenoid biogenesis in fruit is fundamentally different from that in green leaves.

Recent work has revealed the presence of a very similar series of polyenes in the fungus Phycomyces blakesleeanus (Goodwin, 1952a), and earlier work suggests that most of the components of the series also exists in other carotenogenic fungi; the synthetic route in fruit and fungi may thus be very similar. As yet, no such polyene series has been demonstrated in algae, flower petals or bacteria.
Phytofluene, a member of this series, has, however, been observed in a number of flower species (Zechmeister & Sandoval, 1945), and in one bacterium, Mycobacterium phlei (Goodwin, 1952a). In the photosynthetic bacterium, Rhodospirillum rubrum, however, preliminary experiments suggest that phytofluene is not present (Goodwin & Osman, 1951).

The nature of \( \eta \)-carotene

The position of the absorption spectrum of \( \eta \)-carotene, which is very similar to that of \( \zeta \)-carotene, suggests that, like \( \zeta \)-carotene, it contains seven conjugated double bonds. As it is less strongly adsorbed on a column than is \( \zeta \)-carotene, and as its position on the column is the same relative to \( \beta \)-carotene as that of \( \zeta \)-carotene is to lycopene, it is possible that \( \eta \)-carotene bears the same structural relationship to \( \beta \)-carotene as \( \zeta \)-carotene does to lycopene. Thus, it might well be octahydro-\( \beta \)-carotene, with the double bonds symmetrically placed about the centre of the molecule. If either of the double bonds in the \( \beta \)-ionone residues were concerned in the chromophoric system, then one would expect the position of the absorption bands of \( \zeta \)-carotene to be different from those of \( \eta \)-carotene, in the same way as those of lycopene are different from those of \( \beta \)-carotene.

The small amount of material with a spectrum similar to that of phytofluene, which has been observed to be adsorbed below \( \eta \)-carotene may be, on similar reasoning, dodecahydro-\( \beta \)-carotene, i.e. the \( \beta \)-carotene derivative corresponding to phytofluene, which is probably dodecahydrolycopene (Porter & Lincoln, 1950).

It will be seen from Table 6 that Lonicera berries fall into one of the two main categories of carotenoid-containing fruit: those having cryptoxanthin as their major component. The other group tends to accumulate large amounts of lycopene.

The occurrence in the berries of a pigment (fraction 4, Table 1) which appears to be the 'unidentified pigment' observed in human blood serum and milk by Kon & Mawson (1950) is important because this is the first time it has been reported in plant tissue. Willstaedt & With (1938), who observed a similar pigment in blood serum, considered it to be an 'oxidation product'. This possibility remains, but now the further possibility exists that it occurs in human blood serum and milk as a result of its ingestion in the food.

Two final points of interest may be mentioned: (1) neither \( \alpha \)-carotene nor any of its derivatives occurs in the berries; and (2) this is the first time that auroxanthin has been observed in berries, although it is widespread in flower petals (Karrer & Jucker, 1949).

**SUMMARY**

1. The following known carotenoids have been found in the ripe berries of the climbing honeysuckle (Lonicera japonica): phytofluene, \( \beta \)-carotene, \( \zeta \)-carotene, \( \gamma \)-carotene, lycopene, cryptoxanthin, zeaxanthin and auroxanthin; an unidentified pigment present in human blood and milk (Kon & Mawson, 1950) also appears to be present.

2. Some spectral and adsorption properties of a new carotene (\( \eta \)-carotene) occurring in the berries are described. Small amounts of a polyene very similar to, but distinct from, phytofluene were also observed.

3. Three pigments occurring in small amounts were not identified; their spectra are recorded.

4. The relative amounts of the pigments present have been determined; cryptoxanthin is the major component. The xanthophylls exist almost exclusively as esters.

5. This work provides additional evidence to support the suggestion that the route of carotenogenesis in fruit is different from that in green leaves.

Thanks are due to Prof. R. A. Morton, F.R.S., for his interest in this work.

**REFERENCES**


