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The Carotenoids of the Berries of *Lonicera japonica*

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Although a great deal is known concerning the distribution and occurrence of carotenoids in fruit (see Karrer & Jucker, 1949; Goodwin, 1952*b*), 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. xylosteum* (Schimper, 1885; Molisch, 1896; Kohl, 1902; Nowak & Zellner, 1921); *Sambucus nigra* (Nowak & Zellner, 1921), *Viburnum opulis* 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.

Extraction and separation of the carotenoids

The fresh berries were ground to a fine powder with Na_2SO_4 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, 1952*a*) 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 $ZnCO_3$ 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 6*C* moved off the column and fraction 6*D*, originally adsorbed at the top, separated into two zones which moved slowly down the column. The faster moving pigment was designated 6*DA* and the slower moving one 6*DB*.

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*

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

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

* 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.)

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

* In ultraviolet light. † In ether.

Table 3. *The separation of fraction* 6 *(Table* 1*) on a column of* $ZnCO_3$*, using benzene as developer*

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

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

* Solvent benzene. † Solvent ethanol.

Examination of the fractions

Fractions 2, 3 *(Table* 1*)*, 1*C*, 1*D*, 1*E (Table* 2*)*, 6*A*, 6*C*, 6*DB* (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 (1*C*), β-carotene (1*D*), ζ-carotene (1*E*), cryptoxanthin (6*A*), zeaxanthin (6*C*) and auroxanthin (6*DB*). 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 carotenes were obtained from the fungus *Phycomyces blakesleeanus* (Goodwin, 1952*a*). Cryptoxanthin and zeaxanthin were obtained from a commercial sample of maize meal.

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

Fractions 1*A*, 1*B (Table* 2*), 6B and 6DA (Table* 3*).* 1*A* appears to be closely related to phytofluene, whilst 1*B* is considered to be a carotene not previously described, it is proposed to call it η-carotene. Fraction 4 appears to be very similar to the unidentified pigment reported in human milk (Kon & Mawson, 1950), whilst fractions 5, 6*B* and 6*DA* 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* $E^{1\%}_{1\,cm.}$ *values (in light petroleum) used for quantitative determination of* Lonicera *carotenoids*

Carotenoid	Wavelength (mμ.)	$E^{1\%}_{1\,cm.}$	Reference
Phytofluene	348	1200	Porter & Lincoln (1950)
β-Carotene	450	2580	Zscheile *et al.* (1942)
ζ-Carotene	422	2500	Porter & Lincoln (1950)
γ-Carotene	459	2760*	Zechmeister (1944)
Lycopene	469	3460*	Zechmeister (1944)
Cryptoxanthin	451	2460	Zscheile *et al.* (1942)
Zeaxanthin	452	2480	Zscheile *et al.* (1942)
Auroxanthin	425	1780*	Karrer & Jucker (1945)

* Calculated from graphs.

extinction values of the pigments recorded in Table 4, the amount of each pigment could be calculated. For this present purpose the $E^{1\%}_{1\,cm.}$ (max.) values for the new carotene and the unidentified xanthophylls were assumed to be 2500.

RESULTS

A. *The carotenes*

The following carotenes were identified unequivocally: phytofluene, β-carotene, ζ-carotene, lycopene and γ-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 mμ. (320–400 mμ. 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, 1952*a*), and further elaboration is unnecessary here.

Fraction 1*A* (*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 1*B* (*Table* 2). This fraction runs down an alumina column as a diffuse dull orange band just ahead of phytofluene and α-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 ε-carotene obtained by Strain & Manning (1943) from the diatoms *Nitzschia closterium* and *Navicula torquatum*. A comparison of the spectrum of ε-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 1*B* is ζ-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); ζ-carotene is much more strongly adsorbed, for it forms a zone above β-carotene whilst the new carotene travels down the column well in front of β- (and even α-) carotene. Furthermore, ζ-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 η-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), η-carotene is only a minor component (1·3 %) of the total carotenoids of *Lonicera*.

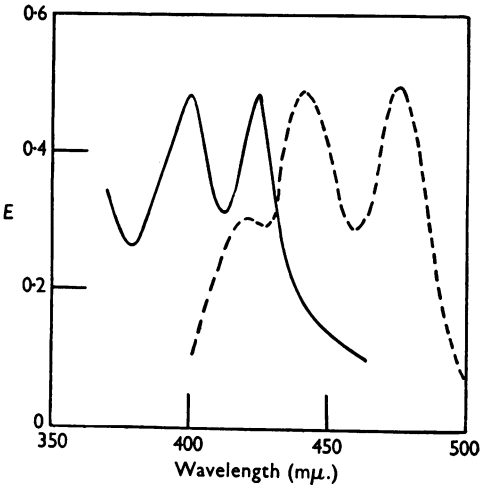

Fig. 1. Absorption spectrum of new carotene (η-carotene) obtained from *Lonicera* berries compared with that of ε-carotene from the alga *Navicula torquata*. ——, η-carotene in light petroleum (b.p. 40–60°); – – – –, ε-carotene in ethanol (from Strain & Manning, 1943). Note. (i) The change in the position of absorption maxima of carotenes in light petroleum and ethanol is only slight (2–4 mμ.); (ii) the *E* values are arranged so that $E_{max.}$ is the same for both pigments.

Fraction 4. This fraction is epiphasic to 90 and 95 % (v/v) aqueous methanol, has an absorption spectrum almost identical with that of β-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 mμ., 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 β-carotene; insufficient amounts were available for further study.

B. *The xanthophylls*

Fraction 6*A*. 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.

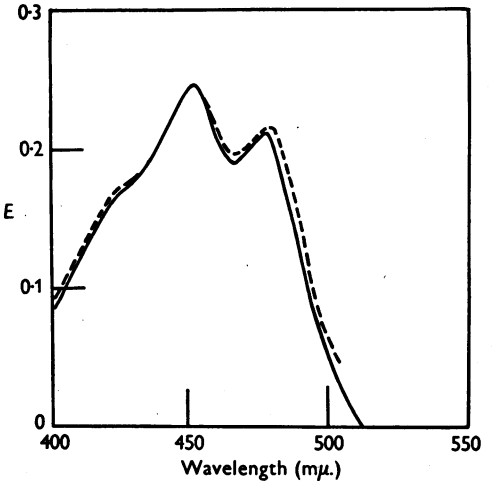

Fig. 2. A comparison of the absorption spectrum of the pigment (zone 6*A*, Table 3) obtained from *Lonicera* berries with that of authentic cryptoxanthin obtained from maize. The *E* values are so arranged that $E_{max.}$ is the same for both pigments. ----, *Lonicera* pigment; ——, cryptoxanthin. Solvent, light petroleum.

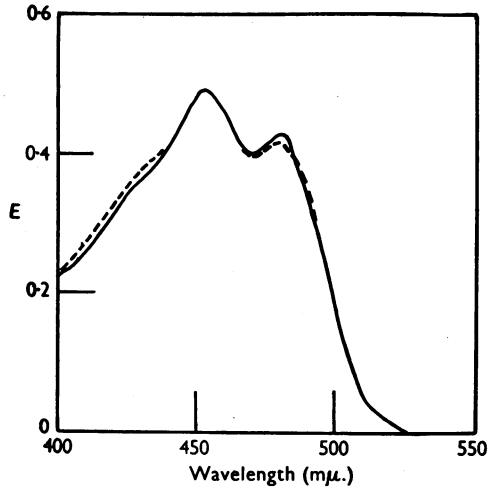

Fig. 3. A comparison of the absorption spectrum of the pigment (zone 6*C*, Table 3) obtained from *Lonicera* berries with that of authentic zeaxanthin obtained from maize. The *E* values are so arranged that $E_{max.}$ is the same for both pigments. ----, *Lonicera* pigment; ——, zeaxanthin. Solvent,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

Fraction 6*C*. A fraction which was completely hypophasic in the partition tests was identified as zeaxanthin by comparison in the usual ways with a specimen of zeaxanthin obtained from maize. A comparison of the spectrum of the berry pigment with that of authentic zeaxanthin is recorded in Fig. 3.

Fraction 6*DB*. 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, ζ-carotene and aurochrome (and to a lesser degree by η-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 6*DB* and that of auroxanthin is recorded in Fig. 4.

Fractions 6*B*, 6*DA*. 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, ζ-carotene and η-carotene*

Property	Pigment 6*DB*	Auroxanthin	Aurochrome	ζ-Carotene	η-Carotene
Absorption maxima in light petroleum (mμ.)	400, 425	400, 425	428*	400, 426	399, 425
Partition between light petroleum and 90% (v/v) aqueous methanol	Hypophasic	Hypophasic	Epiphasic	Epiphasic	Epiphasic
Colour with conc. HCl	Stable blue	Stable blue	Stable blue	None	None
Position on chromatogram	Strongly adsorbed above zeaxanthin	Strongly adsorbed above zeaxanthin	Can be developed on $Ca(OH)_2$ with light petroleum; hydroxycarotenoids are not developed with this solvent	Adsorbed above β-carotene but below lycopene	Adsorbed below α-carotene
References	Present work	Karrer & Rutschmann (1942)	Karrer & Jucker (1945, 1949)	Goodwin (1952*a*) and present work	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

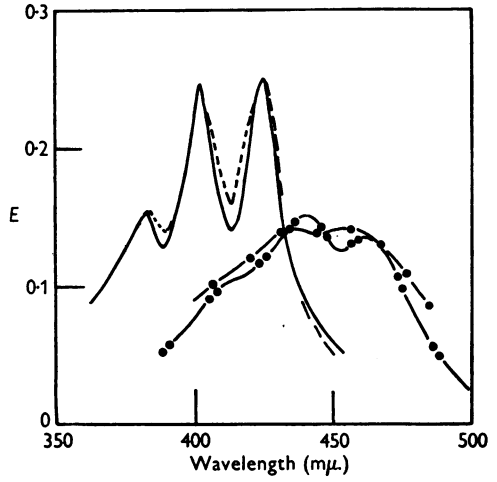

Fig. 4. The absorption spectra of the pigment 6*DB* (Table 3), authentic auroxanthin, and unidentified xanthophylls 6*B* and 6*DA* (Table 3). ——, authentic auroxanthin in ethanol (redrawn from Karrer & Rutschmann, 1942); - - - - -, pigment 6*DB* in ethanol; –·–·–, pigment 6*B* in benzene; ··–··–, pigment 6*DA* in ethanol. The *E* values for pigment 6*DB* and auroxanthin are so arranged that $E_{max.}$ is the same for both pigments. The *E* values for the other two pigments are those observed in solutions of unknown strength. They bear no relationship to each other or to the *E* values of 6*DB* and auroxanthin.

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, β-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.

Table 6. *The quantitative distribution of the polyene components present in* Lonicera *berries, measured as percentages of the total amount of pigments present*

Carotenoid	Amount
Phytofluene-like (Fraction 1*A*, Table 2)	0·45
η-Carotene	1·9
Phytofluene	1·3
β-Carotene	8·95
ζ-Carotene	0·6
γ-Carotene	0·6
Lycopene	2·5
Human milk pigment (Fraction 4, Table 1)	2·9
Unknown (Fraction 5, Table 1)	Trace
Cryptoxanthin	65·2
Unknown (Fraction 6*B*, Table 3)	1·9
Zeaxanthin	3·5
Unknown (Fraction 6*DA*, Table 3)	5·2*
Auroxanthin	5·0

* By difference.

Recent work has revealed the presence of a very similar series of polyenes in the fungus *Phycomyces blakesleeanus* (Goodwin, 1952*a*), 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, 1952*a*). In the photosynthetic bacterium, *Rhodospirillum rubrum*, however, preliminary experiments suggest that phytofluene is not present (Goodwin & Osman, 1951).

The nature of η-carotene

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

The small amount of material with a spectrum similar to that of phytofluene, which has been observed to be adsorbed below η-carotene may be, on similar reasoning, dodecahydro-β-carotene, i.e. the β-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 α-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, β-carotene, ζ-carotene, γ-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 (η-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.

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