(31%), *m*-nitrophenol (9%), *p*-nitrophenol (9%), *m*-aminophenol (4%), *o*-aminophenol (3%), 4-nitrocatechol (0.7%), aniline (0.3%) and *o*-nitrophenol (0.1%). Two new metabolites, nitroquinol (0.1%) and *p*-nitrophenylmercapturic acid (0.3%), were found in the urine.

4. The denitration products, phenol, catechol, L-phenylmercapturic acid, and *trans-trans*-muconic acid, did not occur in the urine.

5. Nitrosobenzene, azoxybenzene, azobenzene and benzidine, the presence of which would have substantiated the occurrence of nitrosobenzene and phenylhydroxylamine as intermediates in the formation of *p*-aminophenol, were not detected in the urine.

6. The total excretion of radioactivity in the expired air, urine and faeces accounts for nearly 70% of the dose of nitrobenzene in 4–5 days after dosing. The remainder is present in the tissues and is eliminated slowly as metabolites in the urine, which is still radioactive 10 days after dosing, and probably also as carbon dioxide.

7. Two days after dosing, about 54% of the administered radioactivity was found in the tissues, particularly in the fat and the intestinal tract. Unchanged nitrobenzene was present in the tissues of this animal. Eight days after dosing, in another experiment, 8% of the radioactivity was found in the tissues, the fat being the most active. No free nitrobenzene was detected in this animal.

8. The faeces contain about 9% of the administered radioactivity, of which about 6% is present as *p*-aminophenol.

9. These experiments account for 85–90% of the administered nitrobenzene.

I am indebted to Professor R. T. Williams for his constant interest in this work. The expenses of this work were in part defrayed by a grant to Professor Williams from the Medical Research Council.

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Studies in Carotenogenesis

19. A SURVEY OF THE POLYENES IN A NUMBER OF RIPE BERRIES*

By T. W. GOODWIN

Department of Biochemistry, The University, Liverpool 3

(Received 19 May 1955)

During the past few years a number of new C_{40} polyenes have been found in plants (Karrer & Jucker, 1950; Goodwin, 1952*a*, 1955*a*). Perhaps the most important of these from the viewpoint of carotenogenesis is the series of hydrocarbons, chemically related to lycopene, consisting of tetrahydrophytoene, phytofluene, ζ -carotene, neurosporene and lycopene. (The first three members of this series are colourless; hence the term 'carotene' will be used only in discussing the coloured members of the series, and the term 'polyene' will be used as a general term including both coloured

and colourless members.) As a result of a study of the distribution of these polyenes in the fruit of various tomato crosses, Porter & Lincoln (1950) concluded that the biosynthesis of lycopene probably occurred via this series by sequential dehydrogenation of the almost completely saturated tetrahydrophytoene.

One of the aims of the present survey was to discover, within the limits of the species available, how widespread was the distribution of the 'Porter-Lincoln' series in fruit and berries.

Goodwin (1952*a*, 1955*a*, *b*) lists the polyenes found in the fruit of some 100 plant species. Most of these species, however, were investigated from

* Part 18: Goodwin & Jamikorn (1956).

a chemical point of view, and only the major components were isolated and examined; the minor components, which, from the trend of modern developments in carotenoid biochemistry, might be of considerable importance, were rarely examined. The second aim of this investigation was, therefore, to begin the accumulation of information on all polyenes occurring in various fruit and berries. It is interesting that since the experimental work reported here was completed, a number of workers have concerned themselves with the distribution of the minor components of carotenoids in plants materials (e.g. Haxo, 1952; Turian & Haxo, 1954; Taha, 1954; Curl & Bailey, 1954).

Twenty-three species, representing eight families, have been examined, most of them for the first time. In the few species which have been re-examined, the minor components have not been considered previously.

EXPERIMENTAL

Materials. The species investigated, together with their trivial names and the localities where they were collected, are listed in Table 1. All specimens were collected during August–September 1952; a second sample of *Arum maculatum* was collected during the same period of 1953.

Extraction of polyenes. The crushed berries were ground to a fine dry powder with anhydrous Na_2SO_4 and acid-washed silver sand, and the pigments extracted in the cold by stirring with successive portions of diethyl ether until no further colour was removed; usually three to four treatments were sufficient. Each extract was filtered through a sintered-glass filter ($G \times 16$) and the combined extracts were evaporated to dryness *in vacuo* at 25° . In

order to remove neutral fat and chlorophyll, which might interfere with the chromatographic separation of the polyenes, the residue was saponified by boiling for 2 min. with a suitable volume (approx. 10 ml.) of 20% (w/v) ethanolic KOH. The unsaponifiable matter (containing the polyenes) was extracted by the method of Goodwin & Morton (1946) and dissolved in light petroleum (10–20 ml.) (light petroleum b.p. $40\text{--}60^\circ$ was used throughout this investigation), ready for chromatographic separation. In some cases the extracts were first dissolved in a few drops of ethyl ether before addition of the light petroleum. This was necessary only when petroleum-insoluble xanthophylls were present.

Chromatographic separation of polyenes. The polyene mixtures were first separated into hydrocarbons and xanthophylls on a column of deactivated alumina, prepared by treating activated alumina (Grade 'O', P. Spence and Co., Widnes) with methanol (Goodwin & Srisukh, 1949). The hydrocarbon polyenes were removed with light petroleum containing 5% (v/v) of diethyl ether, and collected in the filtrate. The solvent was then removed *in vacuo* at $25\text{--}30^\circ$, the residue redissolved in light petroleum and separated into its components by chromatography on a 3:1 mixture of activated and deactivated alumina, light petroleum containing various amounts of diethyl ether as developer being used (Goodwin, 1952*b*). If any fractions overlapped on the chromatogram, they were rechromatographed under slightly different experimental conditions (e.g. with small variations in either the ratio of activated to deactivated alumina or the concentration of ether in the developer) until homogeneous. The polyenes were then identified by their position on the chromatogram relative to other known polyenes and by the shape and position of their absorption spectra. These are sufficient criteria for the common polyenes because: (a) considerable experience in this and other Laboratories (Haxo, 1952; Porter & Lincoln, 1950; Trombly & Porter, 1953; Turian & Haxo, 1954; Goodwin, 1952*b, c*, 1953*a*, 1954, 1955*b*) has shown that

Table 1. *Details of the various species of berries examined*

Scientific name	Family	Trivial name	Place collected
<i>Arum maculatum</i>	Araceae	Cuckoo Pint, Lords and Ladies	N. Wales, near Chester
<i>Atropa belladonna</i>	Solanaceae	Deadly Nightshade	N. Wales
<i>Berberis barbarossa</i>	Berberidaceae	Barberry	Somerset
<i>Berberis</i> sp. (Pirate King)			
<i>Capsicum annuum</i>	Solanaceae	Red pepper	Bought dried from shop
<i>Cotoneaster bullata</i>	Rosaceae	Cotoneaster	Somerset
<i>C. frigida</i>			
<i>C. hebeophylla</i>			
<i>Crataegus oxyacantha</i>	Rosaceae	Hawthorn	N. Wales
<i>C. pratensis</i>			
<i>Hippophaë rhamnoides</i>	Elaeagnaceae	Sea buckthorn	Somerset
<i>Lonicera periclymenum</i>	Caprifoliaceae	Honeysuckle	N. Wales
<i>Pyracantha flava</i> *	Rosaceae	Firethorn	Somerset
<i>P. rogersiana</i> *			
<i>Rosa canina</i>	Rosaceae	Rose (hips)	N.W. Cheshire
<i>R. moyesii</i>			Somerset
<i>R. rubrifolia</i>			N. Wales
<i>Sambucus nigra</i>	Caprifoliaceae	Elderberry	N. Wales
<i>Solanum dulcamara</i>	Solanaceae	Woody nightshade	N.W. Cheshire
<i>Sorbus aucuparia</i>	Rosaceae	Rowan; mountain ash	N. Wales
<i>Tamus communis</i>	Dioscoreaceae	Black bryony	N.W. Cheshire
<i>Taxus baccata</i>	Taxaceae	Yew	N.W. Cheshire
<i>Viburnum opulus</i>	Caprifoliaceae	Gelder rose	Somerset

* These are cultivars of *P. crenulata*.

they always separate on a column in the order given in Table 2; (b) the absorption spectra maxima of adjacently adsorbed polyenes are quite distinct and characteristic. Whenever unusual polyenes, such as *cis* isomers, have been observed these have been examined in greater detail.

Table 2. *Adsorption sequence and absorption maxima of the common hydrocarbon polyenes**

Adsorbent, Al_2O_3 ; polyenes in order of increasing adsorption. Chromatographic methods are given in the Experimental section.

Polyene	E_{max} in light petroleum (m μ .)
Phytoene	275, 285, 296
Phytofluene	332, 348, 367-368
α -Carotene	420, 443, 474
β -Carotene	424, 450, 481
ζ -Carotene	378, 400, 419
δ -Carotene	428, 458, 490
γ -Carotene	431, 461, 494
Neurosporene	410, 433, 468
Lycopene	445, 491, 504

* For a complete list in sequence see Goodwin (1955b).

Table 3. *Adsorption sequence and absorption maxima of the common xanthophylls*

Adsorbent, deactivated Al_2O_3 ; developer, light petroleum containing various concentrations of ethanol; xanthophylls in order of increasing adsorption.

Xanthophyll	E_{max} in light petroleum (m μ .)
Flavochrome } Mutachrome }	427, 456
Cryptoxanthin	420, 451, 482
Rubixanthin	432, 463, 495
Lycioxanthin	443, 471, 502
Rubichrome	472, 501*
†	.....
Lutein	418, 442, 477
Zeaxanthin	424, 451, 483
Chrysanthemaxanthin	421, 448
Flavoxanthin	421, 449
Lycophyll	443, 471, 502
Violaxanthin	442, 471
Neoxanthin	437, 466

* In CS_2 .

† A considerable gap in the chromatogram occurs here because of the change from mono- to di-hydroxy carotenoids.

The xanthophyll fraction. The pigments remaining on the deactivated alumina after the removal of the hydrocarbon polyenes are fractionated by development with light petroleum containing increasing amounts of ethyl ether. Some of the more strongly adsorbed carotenoids are not eluted even with pure ether; in these cases development is continued with ethyl ether containing increasing amounts of ethanol. As with the hydrocarbons the well-described xanthophylls can be identified by their relative adsorptive properties and the shape and position of their absorption spectra, together with ancillary tests, such as partition between light petroleum and methanol containing either 5 or 10% (v/v) of water and interaction with HCl in ethereal solution.

Table 3 shows the relative positions, found in this Laboratory, of the commoner xanthophylls on a column of alumina. Contrary to the experience with the hydrocarbon polyenes, care must be taken not to extrapolate this order to other adsorbents, or even to alumina used with other developing solvents (Strain, 1948). Xanthophylls that are not included in Table 3 are considered in detail in the appropriate section.

Quantitative determination of the polyenes. Good estimates of the total carotenoid content of the crude extracts were made by measuring $E_{1\text{ cm}}$ at 450 m μ . of the extract and assuming $E_{1\text{ cm}}^1\%$ for the mixture to be 2500, the value for β -carotene. For these determinations, the presence of the colourless polyenes was ignored. The various purified fractions were dissolved in known volumes of light petroleum and the E_{max} values measured in a 1 cm. cell. By comparing these with the $E_{1\text{ cm}}^1\%$ values for the pure pigments (Karrer & Jucker, 1950; Goodwin, 1952a) the amounts of the pigments present could be calculated. If the $E_{1\text{ cm}}^1\%$ value of a pure pigment was not known, it was assumed to be 2500. No allowance was made for losses on chromatography; for the hydrocarbons these are negligible, but with the highly oxygenated xanthophylls appreciable losses, up to 25%, may occur.

Spectrophotometric measurements. These were carried out with either a Beckman DU or Unicam SP. 500 instrument.

RESULTS

Carotenoid concentration and relative amounts of hydrocarbons and xanthophylls in the berries

Table 4 shows that the concentration of carotenoids varies from almost zero (0.2 $\mu\text{g./g.}$) to 600 $\mu\text{g./g.}$, and that the relative amounts of hydrocarbons also

Table 4. *Total carotenoid content and relative amounts of hydrocarbon polyenes in berries of various species*

Species	Carotenes (as % of total carotenoids)	Amount of carotenoids ($\mu\text{g./g. wet wt.}$)
<i>Arum maculatum</i>	92, 89	200
<i>Atropa belladonna</i>	23	18
<i>Berberis barbarossa</i>	—	0.25
<i>Berberis</i> sp. (Pirate King)	—	0.2
<i>Capsicum annuum</i>	35	127
<i>Cotoneaster bullata</i>	10	12
<i>C. frigida</i>	37	1.5
<i>C. hebeophylla</i>	7	16
<i>Crataegus oxyacantha</i>	40, 41	4, 25
<i>C. pratensis</i>	57	5
<i>Hippophaë rhamnoides</i>	3	52
<i>Lonicera periclymenum</i>	15	18
<i>Pyracantha flava</i>	59, 27	9, 15
<i>P. rogersiana</i>	—	1
<i>Rosa canina</i>	26	25
<i>R. moyesii</i>	61	224
<i>R. rubrifolia</i>	49	88
<i>Sambucus nigra</i>	19	16
<i>Solanum dulcamara</i>	61	90
<i>Sorbus aucuparia</i>	39	120
<i>Tamus communis</i>	85	96
<i>Taxus baccata</i>	5	10*
<i>Viburnum opulus</i>	32.5	607

* Calculated on whole berry, not just the aril.

Table 5. Qualitative and quantitative distribution of the common hydrocarbon polyenes in various berries

A dash indicates the absence of a polyene; the failure of the percentages of the constituents to add up to 100 indicates the presence of the uncommon pigments discussed in the text.

Berries	Percentage of various components						
	Phytoene*	Phytofluene	β -Carotene	ζ -Carotene	γ -Carotene	Prolycopenes	Lycopene†
<i>Arum maculatum</i> ‡	—, 0.4	3.8, 1.6	25.2, 7.9	0.3	0.2	39.1, 41.8	31.4, 49.3
<i>Atropa belladonna</i>	—	—	100	—	—	—	—
<i>Berberis barbarossa</i>	—	—	100	—	—	—	—
<i>Berberis</i> sp. (Pirate King)	—	—	100	—	—	—	—
<i>Capsicum annuum</i>	19.9	16.4	63.7	—	—	—	—
<i>Cotoneaster bullata</i>	—	Trace	100	Trace	—	—	—
<i>C. frigida</i>	—	—	100	—	—	—	—
<i>C. hebeophylla</i>	—	—	100	—	—	—	—
<i>Crataegus oxyacantha</i>	—	—	100	—	—	—	—
<i>C. pratensis</i>	—	?Trace	100	Trace	—	—	—
<i>Hippophaë rhamnoides</i>	—	Trace	100	3.7	3.7	—	15.4
<i>Lonicera japonica</i> §	2.8	8.0	55.0	3.4	—	—	45.3
<i>L. periclymenum</i>	—	?Trace	51.3	—	—	—	—
<i>Pyracantha flava</i>	—	Trace, 2.1	90.8, 100	—	—	—	—
<i>P. rogersiana</i>	—	—	100	—	—	—	—
<i>Rosa canina</i>	Trace	1.4	66.3	0.9	5.3	2.2	24.9
<i>R. moyesii</i>	7.6	4.6	24.7	5.6	—	21.6	36.1
<i>R. rubrifolia</i>	5.0	5.3	57.2	Trace	?	—	32.5
<i>Sambucus nigra</i>	—	—	100	—	—	—	—
<i>Solanum dulcamara</i>	—	Trace	Trace	—	—	Trace	100
<i>Sorbus aucuparia</i>	—	—	100	Trace	—	—	—
<i>Tamus communis</i>	4.0	1.5	4.7	—	—	—	89.8
<i>Taxus baccata</i>	15.0	4.6	76.5	—	—	—	—
<i>Viburnum opulus</i>	—	13.9	4.1	8.0	8.9	—	56.2

* In many cases *trans*-phytofluene was also observed in traces. In view of the observation of Koe & Zechmeister (1953) that phytofluene is extremely heat-labile, it is probable that the traces of *trans*-phytofluene in our extracts were artifacts.

† Includes neolycopene.

‡ The two values are for specimens collected in different years.

§ Previous observations (Goodwin, 1952c) recorded here for comparison.

|| The two values are for two samples of the same crop.

Fig. 1. Absorption spectra in light petroleum of prolycopene II before (full line) and after I_2 treatment (broken line). $E_{\max}$ has been adjusted to the same value for each pigment.

specimens examined were 'ripe', but the exact degree of ripeness was not known.

Constituent hydrocarbon polyenes

In order to save space the individual protocols for each species are not given; the occurrence and quantitative distribution of the well-characterized hydrocarbons are given in Table 5.

Prolycopenes. The first fractionations of the polyenes of *Arum maculatum* revealed a large brown zone between γ -carotene and lycopene. On rechromatographing this zone on active alumina, three fractions were obtained, with spectral maxima corresponding with those of prolycopenes I, II and III (Zechmeister & Pinckard, 1947). The shapes were not, however, completely identical with those of Zechmeister & Pinckard. This is probably due to the difficulty of obtaining them chromatographically homogeneous, but iodine isomerization convincingly demonstrated their prolycopene nature, for immediately on the addition of iodine all underwent a marked shift in absorption maxima to give the characteristic curve of the equilibrium mixture of *cis-trans*-lycopenes (Zechmeister, 1944); this is illustrated in Fig. 1 for the pigment considered to be prolycopene II.

vary widely from 3 to 92%. These values must be taken only as a general guide, because the concentrations and relative amounts of the constituents, especially the former, can vary considerably with the degree of ripeness (Goodwin, 1952a). All the

Table 6. *Distribution of xanthophylls in various berries*

Amounts in per cent of total xanthophylls present; * a gap indicates the absence of a pigment. *Pyracantha rogersiana* and *Tamas communis* are not included, since only traces of unexamined xanthophylls were present.

Species	Mutato-chrome	Cryptoxanthin	Zeaxanthin	Lutein	Flavoxanthin or chryxanthemaxanthin†	Others‡
<i>Arum maculatum</i>	—	Present	—	—	—	—
<i>Atropa belladonna</i>	—	25.0	—	—	—	—
<i>Berberis</i> spp.§	—	—	14.2	44.6	—	Capsanthin, 43.3
<i>Capsicum annuum</i>	—	11.7	16.7	—	8.5	Capsorubin, 17.3
<i>Cotoneaster bullata</i>	16.0	13.4	Trace	—	—	Aurochrome, 54.0
<i>C. frigida</i>	9.5	13.8	21.4	—	32.1	—
<i>C. hebeophylla</i> ¶	5.6	5.1	—	—	41.1	—
<i>Crataegus oxyacantha</i>	20.6	27.7	—	24.4	—	Auroxanthin, 14.9
<i>C. pratensis</i>	9.2	12.3	—	—	—	—
<i>Hippophaë rhamnoides</i>	—	Trace	57.1	13.9	—	Aurochrome, 2.1
<i>Lonicera japonica</i> * ¶ **	—	77.9	4.2	6.6	—	Auroxanthin, 6.0
<i>L. periclymenum</i>	—	44.6	40.5	—	—	Auroxanthin, 13.0
<i>Pyracantha flava</i>	12.7	—	23.7	48.2	14.6	Luteochrome, trace
<i>Rosa canina</i>	—	2.3	8.1	—	—	Rubixanthin, 56.5
<i>R. moyesii</i>	2.5	28.6	11.5	16.2††	Trace	Rubixanthin, 34.9
<i>R. rubrifolia</i>	—	7.9	5.6††	—	4.5	Rubixanthin, 82.0
<i>Sambucus nigra</i>	—	Trace	—	76.8	11.2	Neoxanthin, 11.2
<i>Solanum dulcamara</i>	Trace (?)	—	—	70.4	—	Lycoxanthin, 29.6
<i>Sorbus aucuparia</i>	59.6	—	Trace	14.3	—	—
<i>Taxus baccata</i>	3.5	—	—	16.9	Trace	Rhodoxanthin, 78.6
<i>Viburnum opulus</i>	95	—	—	—	—	—

* Failure of the figures to add up to 100 indicates presence of pigments discussed further in text or indefinite oxidation products.

† It is very difficult to separate these stereoisomers (Karrer & Jucker, 1950); no attempt was made to do so in this survey.

‡ Additional pigments also probably present in traces but the total amounts of xanthophylls were too small to confirm this.

§ The same results in all species examined; only traces of xanthophylls were present.

|| *Cis* isomers present.

¶ Data from Goodwin (1952c).

** Pigment X (Goodwin & Osman, 1953) also present.

†† This fraction was rather more strongly adsorbed than is usual for lutein; it might be lutein 5:6-epoxide.

‡‡ Also contains traces of lutein.

Fig. 2. Absorption spectra of rhodoxanthin in light petroleum (full line) and benzene (broken line). $E_{\max}$ has been adjusted to the same value for each pigment.

Polycopenes, with polycopene II predominant, were also observed in smaller amounts in *Rosa* spp. and *Solanum dulcamara*, but were not detected in any other species.

Xanthophylls

The distribution of the common xanthophylls in the berries examined is given in Table 6. Apart from *Cotoneaster hebeophylla* (discussed below) a characteristic feature is the complete absence of new pigments.

Cotoneaster hebeophylla. Two pigments present in traces occurred adsorbed between aurochrome and flavoxanthin. These pigments have absorption spectra identical with those of flavoxanthin and aurochrome respectively. From their adsorptive properties they are probably monohydroxy derivatives of the two parent pigments.

Rhodoxanthin. The absorption spectra in two solvents obtained with chromatographically pure rhodoxanthin obtained from the arils of *Taxus baccata* are given in Fig. 2. It will be seen that although the main maxima correspond well with

those of Kuhn & Brockmann (1933) (500 and 502 m μ . in benzene and 484 and 487 m μ . in light petroleum) there was little sign of the two subsidiary bands reported by Kuhn & Brockmann. As it is known now that ketocarotenoids rarely have more than one absorption band (Goodwin, 1952a), it is possible that the earlier observations with less accurate spectroscopic instruments were incorrect in detail. Two compounds closely related to rhodoxanthin, but with absorption maxima at slightly lower wavelengths, were also observed in our extracts; they are probably *cis* isomers of rhodoxanthin. Lubimenko (1920) also noted a number of different modifications of rhodoxanthin.

DISCUSSION

As long ago as 1914 Lubimenko (1914) noted that lycopene was present in *Arum italicum* only in small amounts, together with large amounts of a 'Lykopin erster Ordnung'; this Kylin (1927) observed in *A. italicum* and *A. orientale* and named arumin. *A. maculatum* has been examined only once previously when lycopene was isolated (Karrer & Wherli, 1930); the presence of other pigments was not mentioned. It is now obvious that 'Lykopin erster Ordnung' and arumin are mixtures of polycopenes. 'Arumin' and 'Lykopin erster Ordnung' were also noted in *Rosa canina* and *Solanum dulcamara* (Lubimenko, 1920; Kylin, 1927); in the present work polycopenes have also been identified in these species.

It is difficult to identify Kylin's sorbusin, which he found in his specimens of *S. aucuparia*, with any pigments observed in the present investigation.

Capsicum annuum and *Rosa* spp. have been studied many times previously (see e.g. Goodwin, 1952a), and the only novel feature of the present investigation is the detection for the first time of phytoene in *C. annuum*.

The *Cotoneaster* spp. examined here have not previously been investigated, although lutein and violaxanthin, but not β -carotene, were detected in traces in *C. occidentalis* (Karrer & Rutschmann, 1945); 'lipochromes' were reported in an unnamed *Cotoneaster* sp. by Kohl (1902). Similarly, the only previous report of carotenoids in *Crataegus* spp. is of 'lipochromes' in *C. crusgalli* (Thudichum, 1869).

Hippophaë rhamnoides has been previously investigated by Karrer & Wherli (1930), who reported the presence of traces of zeaxanthin. *Lonicera periclymenum* closely resembles the related *L. japonica* (Goodwin, 1953a), differing only in producing less phytofluene and no η -carotene.

Pyracantha rogersiana berries are similar to *P. coccinea* (Karrer & Rutschmann, 1945) in producing only traces of carotenoids. *P. flava*, on the other hand, synthesizes considerably more, with the

hydrocarbon fraction (about 50% of the total pigments) consisting exclusively of β -carotene with a minute trace of phytofluene. This is again in contrast to another species, *P. angustifolia*, in which, although β -carotene remains the main hydrocarbon component, large amounts of polycopenes I–VI and phytofluene are found (Zechmeister & Sandoval, 1945). Further evidence that there is considerable inter-species variation in the genus *Pyracantha* comes from the observation that *P. yunnanensis* produces much less phytofluene (0.04 mg./kg. fresh wt.) than does *P. angustifolia* (15–28 mg./kg. fresh wt.) (Zechmeister & Sandoval, 1945).

Lycopene has been confirmed as the major hydrocarbon of *Solanum dulcamara* (Zechmeister & Cholnoky, 1936) and *Tamus communis*; phytofluene, phytoene and β -carotene were, however, observed for the first time in *T. communis*. The presence of lycoxanthin and lycophyll was also confirmed in *S. dulcamara*, but, owing to the very low percentage of xanthophylls in our specimens of *T. communis*, and the small amounts of berries available, it was not possible to look for them in this species.

The presence of rhodoxanthin in the arils of *Taxus baccata* has been confirmed (Kuhn & Brockmann, 1933), and a number of *cis* isomers also appeared on the column; it is not known whether these are artifacts or not, but would explain Lubimenko's (1914) observation of five different modifications of rhodoxanthin in his specimens. The hydrocarbons in *T. baccata*, in which β -carotene predominates, have not been previously described.

General conclusions

The following general conclusions can be drawn from these and previous investigations on berries (see Goodwin, 1952a). (a) β -Carotene is probably always present in berries to some extent; the failure to record β -carotene in some previous experiments (see Goodwin, 1952a, pp. 36–37) is almost always associated with the presence of lycopene; when lycopene is present β -carotene can often be a minor constituent (Table 5) and this may well have been missed or ignored in earlier work. (b) Lycopene, often considered a characteristic polyene of fruit, is often absent, but when it is present one or more members of the phytofluene series are also present, although tetrahydrolycopene (neurosporene) is conspicuous by its absence. (c) When β -carotene is the only hydrocarbon present, it is accompanied by the xanthophylls characteristic of green leaves and also by chlorophyll; in these cases the total carotenoid content of the berries is rather low. (d) Chlorophyll is not detectable in fully ripened lycopene-containing berries. (e) Crypto-

xanthin is almost always present. (f) Lutein, the main carotenoid of green leaves, is often absent.

The present investigation further emphasizes the view (Goodwin, 1952a) that the distribution of carotenoids in berries appears at the present time to have no obvious taxonomic significance. The best example in support of this is the great species differences observed in the genus *Pyracantha* (discussed above). The situation is so complex that many more surveys of the present type will be necessary to reveal possible taxonomic correlations.

The failure to observe tetrahydrolycopene (neurosporene) along with the other members of the Porter-Lincoln series in most lycopene-containing berries further suggests that this series is synthesized in parallel fashion rather than sequentially (Goodwin, 1953b).

SUMMARY

The complete polyene distribution has been determined for berries of twenty-three species representing eight families.

Thanks are due to the Medical Research Council for a grant towards laboratory expenses.

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