A Novel Sequence for Phytoene Dehydrogenation in *Rhodospirillum rubrum*

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Differences between the absorption spectra of ζ -carotene (7,8,7',8'-tetrahydrolycopene) and the corresponding conjugated heptaene from diphenylamine-inhibited cultures of *Rhodospirillum rubrum* have been rationalized by the identification of the latter compound as the unsymmetrical isomer, 7,8,11,12-tetrahydrolycopene. The structures of the other conjugated polyene hydrocarbons, phytoene, phytofluene and neurosporene, have been confirmed and a novel pathway for the dehydrogenation of phytoene to lycopene in these bacteria is described.

The only pathway of coloured carotenoid formation that has been demonstrated, and that is generally assumed to operate in all carotenogenic organisms, is the sequence of reactions first proposed by Porter & Lincoln (1950) on the basis of their observations on the carotenoids of tomato fruit. Any uncertainties of the structures of the intermediates in this sequence were eliminated by the unambiguous synthesis of phytoene (I), phytofluene (II), ζ -carotene (III) and neurosporene (IV) and the comparison of the synthetic compounds with natural samples isolated from carrot oil (Davis, Jackman, Siddons & Weedon, 1961, 1966). The structures of these intermediates indicate that phytoene undergoes a series of stepwise dehydrogenations through phytofluene, ζ -carotene and neurosporene to lycopene (V). At each stage of this sequence (Scheme 1) a previously isolated double bond is brought into conjugation, thus increasing the length of the chromophore by two conjugated double bonds. The dehydrogenation steps occur alternately to the left and to the right of the chromophore, since ζ -carotene (7,8,7',8'-tetrahydrolycopene) is a symmetrical conjugated heptaene.

A similar conjugated heptaene has been isolated from diphenylamine-inhibited cultures of the purple non-sulphur photosynthetic bacterium *Rhodospirillum rubrum* by Goodwin & Osman (1954). Jensen, Cohen-Bazire, Nakayama & Stanier (1958) showed that this compound, together with other carotene precursors, disappears from such cultures when the diphenylamine is removed, and deduced from kinetic studies that it takes part in the biosynthesis of lycopene, which is then converted into spirilloxanthin. Although this conjugated heptaene was referred to by both groups of workers as ' ζ -carotene', they described

its absorption spectrum as having maxima at 376, 396 and 418nm in light petroleum, whereas the corresponding values quoted for ζ -carotene isolated from carrot oil, tomato fruit and the fungus *Phycomyces blakesleeanus* are slightly higher, the absorption maxima in light petroleum being at 378, 400 and 425nm (Nash, Quackenbush & Porter, 1952; Nash & Zscheile, 1945; Goodwin, 1952). Nakayama (1958) isolated a conjugated heptaene from a mutant of *Rhodospseudomonas spheroides*; this had an absorption spectrum ($\lambda_{\max}$ at 377, 398 and 422nm) similar to that of the *R. rubrum* heptaene, but was referred to as θ -carotene. This is the name given by Haxo (1955) to a pigment resembling ζ -carotene that he isolated from *Neurospora crassa*.

The purpose of the studies described here was to ascertain whether the apparent difference between the absorption spectra reported for ζ -carotene from higher plants and *P. blakesleeanus* and for the conjugated heptaene from diphenylamine-inhibited cultures of *R. rubrum* is a genuine one and, if so, to explain the anomaly. The results of some of the investigations have already been reported (Davies & Holmes, 1969a).

MATERIALS AND METHODS

Organism. The culture of *Rhodospirillum rubrum* used in these studies was strain C; this strain has been used by earlier workers (Goodwin & Osman, 1953; Davies & Goodwin, 1959; Davies, Goodwin & Mercer, 1961). The cultures were obtained from Professor J. Glover and originated from Dr C. B. van Niel's collection via Dr M. Kamen's laboratory.

Culture conditions. The bacteria were grown anaerobically in 201 batches in completely filled Roux bottles, fitted with aluminium caps, in the light for 7 days at

Scheme 1. Dehydrogenation of phytoene in higher plants (Porter & Lincoln, 1950; Davis *et al.* 1966).

29°C on a medium that was a modification (Davies, 1961) of that used by Goodwin & Osman (1953). It contained, per l: L-glutamic acid, 4.0g; DL-malic acid, 3.5g; NaOH, 3.05g; sodium citrate ($C_6H_5Na_2O_7 \cdot 2H_2O$), 0.8g; bacteriological yeast extract (Difco), 0.25g; $MgSO_4 \cdot 7H_2O$, 0.2g; K_2HPO_4 , 0.18g; KH_2PO_4 , 0.12g; $CaCl_2$, 37.5mg. The pH of the medium was adjusted to 6.8 with 2M-NaOH. Diphenylamine was added at inoculation as an ethanolic solution (2.5mg/ml) to a final concentration of 65 μM .

Determination of bacterial dry weight. As the chromoproteins of *R. rubrum* do not absorb light of a wavelength greater than 900nm (van Niel, 1944) it is reasonable to assume that a measurement of the light scattered by a bacterial suspension at 1000nm is related to the growth of the organism; this relationship is linear (Goodwin & Osman, 1953). The dry weight of bacteria in each culture was estimated from the extinction at 1000nm of the suspension in a 1 cm glass cell; this was determined with a Unicam SP. 600 spectrophotometer. The dry weight (mg/ml) is given by $1.55 \times E_{1000}$ (Davies, 1961).

Solvents. The solvents used were light petroleum (A.R., b.p. 40–60°C and 60–80°C), diethyl ether (A.R.), acetone (A.R.) and benzene (A.R.), all obtained from British Drug Houses Ltd., Poole, Dorset, and methanol (B.P.) from James Burrough Ltd., London S.E.11. The

light petroleum and diethyl ether were dried over sodium wire and redistilled from reduced iron powder before use. Benzene was dried over sodium wire and redistilled. Methanol was distilled from KOH.

Extraction of carotenoids. The bacterial cells were collected from the cultures by centrifugation on a Sharples continuous-flow centrifuge, washed once with 66 mM-potassium phosphate buffer, pH 6.8, centrifuged again (at 8000g for 15 min on a Sorvall RC-2B centrifuge fitted with a GSA head) and extracted by homogenization in methanol. The methanolic mixture was centrifuged (8000g for 15 min) to sediment the cell residue and the supernatant methanol extract was decanted. Extraction was repeated until all the bacteriochlorophyll had been removed from the bacterial residue, which was then extracted with benzene and the extract evaporated to dryness on a hot plate under a stream of N_2 . The combined methanol extract was saponified either by leaving in the cold overnight, or by boiling for 10 min with aq. 60% (w/v) KOH (1 ml/15 ml of extract), and the unsaponifiable fraction was extracted with diethyl ether, washed free of alkali and dried for at least 1 h over anhydrous Na_2SO_4 . The unsaponifiable fraction, dissolved in light petroleum (b.p. 40–60°C), was combined with the residue from the benzene extract before chromatography.

Table 1. *Properties of the carotenoids isolated from diphenylamine-inhibited cultures of R. rubrum by chromatography of the unsaponifiable fraction on alumina columns*

Order of elution	Colour	Percentage of diethyl ether in light petroleum used to elute carotenoid from alumina		Absorption maxima in light petroleum (nm)				No. of conjugated double bonds
		Grade III	Grade II					
1	Colourless	0	0	275	285	296		3
2	Colourless, fluorescent	0	0	331	347	367		5
3	Pale yellow	0	1	(354)	374	394.5	418.5	7
4	Yellow	0-1	2	(394)	414	440	467	9
5	Pale yellow	1	5	(354)	374	394.5	418.5	7
6	Yellow	1-2	5-10	(370)	392	414	440	8
7	Yellow	2	10	(394)	414	439	467	9
8	Orange-pink	2-5	10-20	(414)	446	473	505	11

Preparative chromatography of carotenoids. Each batch of unsaponifiable material (approx. 100 mg) from the bacteria was chromatographed on a column (30 cm × 5 cm) of alumina (Woelm neutral, Brockmann activity grade III), developed with light petroleum (b.p. 40–60°C) containing increasing concentrations of diethyl ether (up to 5%, v/v). Although most of the pigment remained adsorbed on the column, a number of carotenoids were eluted by this procedure and individual bands from the column were, as far as possible, collected as individual fractions. The individual carotenoids were isolated by rechromatographing these fractions from the first column on separate columns (20 cm × 2 cm) of alumina (Woelm neutral, Brockmann activity grade II) with light petroleum (b.p. 40–60°C) containing increasing concentrations of diethyl ether (up to 20%, v/v) as the developing solvent. The eluate fractions were examined spectrophotometrically, and samples from different columns that had the same absorption spectrum and chromatographic behaviour were combined. These individual carotenoids were subjected to a final purification by chromatography on columns (20 cm × 2 cm) of alumina (neutral, Brockmann activity grade II) developed with the appropriate mixture of light petroleum and diethyl ether (Table 1).

Absorption spectra. Visible- and u.v.-absorption spectra of the polyenes were recorded from solutions in light petroleum (b.p. 40–60°C) contained in 1 cm-light-path quartz cells in either a Unicam SP. 800 or a Beckman DK-2A recording spectrophotometer. The wavelength calibration of each instrument was checked with a holmium oxide filter. Quantitative determinations of the polyenes were carried out on solutions in light petroleum of known volume by using the following values for the $E_{1\%}^{1\text{cm}}$: phytoene, 1250 at 285 nm; phytofluene, 1350 at 348 nm; conjugated heptaenes (e.g. ζ -carotene), 2270 at 400 nm (or at 394.5 nm for the bacterial pigment); neurosporene, 2990 at 440 nm; lycopene, 3450 at 472.5 nm (Davies, 1965).

Configuration of polyenes. Determinations of whether carotenoids had all-*trans* or *cis* configurations were carried out by following the changes in their absorption spectra on iodine-catalysed photoisomerization (Zechmeister, 1962). Isomerizations were carried out on light-petroleum solutions of the carotenoids contained in 1 cm quartz spectrophotometer cuvettes. Iodine (1–2% of the

quantity of carotenoid) was added, and spectra were recorded before and after illumination for periods of up to 20 min with two parallel fluorescent lamps (Philips MCFE 65 W, 4400°, Warm White), at a distance of 40 cm.

Thin-layer chromatography. Comparisons of the adsorption characteristics of the conjugated heptaenes from different sources were carried out on thin layers (250 μ m) of Silica Gel G (Merck) with 10% (v/v) benzene in light petroleum (b.p. 60–80°C) as the developing solvent. Chromatograms were developed in the dark in a saturation chamber (Davies, 1963).

Mass spectra. Samples of carotenoids to be analysed by mass spectrometry were evaporated under N_2 from light-petroleum solutions in small vials, which were then evacuated and sealed and the samples sent for analysis. The mass-spectrometric analyses were carried out in the Department of Chemistry, Queen Mary College, London, through the kind co-operation of Professor B.C.L. Weedon, Dr T. P. Toube and Mr D. E. Loeber. The spectra were determined, by using the direct-insertion technique, with an associated Electrical Industries MS 902 instrument at an ionization potential of 70 eV. High-resolution measurements were made relative to heptacosafuorotributylamine.

Isolation of ζ -carotene from carrot root. Carrots (2 kg) were obtained locally and were washed with water and extracted with acetone. The lipid extract was saponified and the unsaponifiable fraction was isolated (Davies, 1965). Chromatography on a column (25 cm × 5 cm) of a 1:1 (w/w) mixture of MgO (British Drug Houses Ltd.) and Hyflo-Super-Cel (Johns-Manville, from Koch-Light Laboratories Ltd) developed with light petroleum (b.p. 40–60°C) containing increasing concentrations of acetone (up to 3%, v/v) yielded impure fractions containing phytoene, phytofluene, α -carotene, β -carotene and ζ -carotene; several other zones, which were not examined, remained adsorbed on the column. The fractions containing ζ -carotene, contaminated with β -carotene, were combined and rechromatographed on a column (20 cm × 1.5 cm) of alumina (Woelm neutral, Brockmann activity grade II) with 1% (v/v) diethyl ether in light petroleum (b.p. 40–60°C) to separate the β -carotene and the ζ -carotene and to elute them in that order.

Isolation of ζ -carotene from Phycomyces blakesleeanus.

Phycomyces blakesleeanus Burgeff (-), obtained from the Centralbureau voor Schimmcultures, Baarn, The Netherlands, was grown on a medium containing, per l: glucose, 25 g; L-asparagine, 1.25 g; L-leucine, 1.25 g; KH_2PO_4 , 1.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; thiamin hydrochloride, 250 μg . The medium was adjusted to pH 5.0 and, to encourage the accumulation of the polyene hydrocarbons (Goodwin, 1952), diphenylamine was added at inoculation as an ethanolic solution (2.5 mg/ml) to give a final concentration of 0.1 mM. The fungus was grown for 7 days in batches of 16 conical flasks (250 ml), each containing 50 ml of the medium (inoculated from a spore suspension), at 27°C on a rotary shaker in an illuminated incubation cabinet.

The mycelium was harvested and the unsaponifiable material extracted by standard methods (Davies, 1965) and dissolved in light petroleum (b.p. 60–80°C). Sterols were precipitated from this solution by cooling to –20°C. The sterol-free solution was chromatographed on a column (25 cm $\times$ 3 cm) of a 3:1 (w/w) mixture of calcium hydroxide (British Drug Houses Ltd.) and Hyflo-Super-Cel with light petroleum (b.p. 60–80°C) as the developing solvent. Phytoene and phytofluene were eluted from the column and, after extrusion of the column, the zone of the column containing ζ -carotene (between β -carotene and neurosporene) was cut out and the impure ζ -carotene extracted with ether. Rechromatography of this fraction on a column of Silica Gel G (Merck) with light petroleum as the developing solvent separated β -zeacarotene (Williams, Davies & Goodwin, 1965) and ζ -carotene, which were eluted in that order.

RESULTS

Repeated chromatography on columns of alumina of the unsaponifiable fraction from an extract of diphenylamine-inhibited cultures of *R. rubrum* yielded a number of chromatographically pure carotenoids. The first eight of these to be eluted from the column are listed in Table 1. Fractions 1, 2, 3, 4 and 8 represented phytoene, phytofluene, the conjugated heptaene, neurosporene and lycopene respectively. Fractions 5, 6 and 7 were not hydrocarbons, and their identification is dealt with in the accompanying paper (Davies, 1970).

Fraction 1. This colourless compound, eluted from the alumina columns with light petroleum (b.p. 40–60°C), had the characteristic u.v.-absorption spectrum of phytoene (λ_{max} in light petroleum at 275, 285 and 296 nm) and was present at a concentration of 187 $\mu\text{g/g}$ dry wt. of bacteria. Its mass spectrum showed a molecular ion of m/e 544.5012 (Calc. for phytoene, $\text{C}_{40}\text{H}_{64}$: 544.5008). Fragmentations at the 'bis-allylic' bonds [3,4 (or 3',4'), 7,8 (or 7',8') and 11,12 (or 11',12')] were indicated by weak ions at m/e 475 ($M-69$) and at m/e 407 ($M-137$) and a strong ion at m/e 339 ($M-205$) respectively. The structure of phytoene (7,8,11,12,7',8',11',12'-octahydrolycopene) was thus confirmed.

Fraction 2. This colourless polyene, eluted from alumina columns with light petroleum, showed the absorption spectrum (λ_{max} in light petroleum at

331, 347 and 367 nm) characteristic of phytofluene (7,8,7',8',11',12'-hexahydrolycopene). It had a strong greenish fluorescence when irradiated with u.v. light (360 nm) and its mass spectrum showed a molecular ion of nominal mass 542 (Calc. for phytofluene, $\text{C}_{40}\text{H}_{62}$: 542) and a strong ion of m/e 337 ($M-205$), which indicated fission at the 11,12 (or 11',12') bond; only weak ion sat ($M-69$) and ($M-137$) were formed. Quantitative determinations showed its concentration to be 75 $\mu\text{g/g}$ dry wt. of bacteria.

Fraction 3. This light-yellow carotenoid was eluted from the alumina column (Brockmann activity grade II) with 1% diethyl ether in light petroleum (b.p. 40–60°C). It had an absorption spectrum in light petroleum [λ_{max} at 354 (infl.), 374, 394.5 and 418.5 nm] that was consistent with the structure of a conjugated heptaene; it was present at a concentration of 63 $\mu\text{g/g}$ dry wt. of bacteria. Samples of ζ -carotene isolated from carrot root and from diphenylamine-inhibited cultures of *P. blakesleeanus* both had absorption maxima in light petroleum at 360 (infl.), 378, 400 and 425 nm. Thus the conjugated heptaene from *R. rubrum* is different in some way from the ζ -carotene from higher plants and from *P. blakesleeanus*.

One explanation that was considered was the possibility that *R. rubrum*, the carotenoids of which are all acyclic (Jensen, Cohen-Bazire & Stanier, 1961), might form an acyclic conjugated heptaene, whereas higher plants (containing predominantly bicyclic carotenoids, e.g. β -carotene) might form a bicyclic ζ -carotene (7,8,7',8'-tetrahydro- β -carotene). Such a situation would arise if carotene cyclization were to take place at an earlier stage in the biosynthetic pathway than had previously been envisaged (Goodwin, 1965; F. W. Quackenbush, quoted by Chichester, 1967). This possibility was investigated by comparing the properties of the natural conjugated heptaenes with those of synthetic 7,8,7',8'-tetrahydro- β -carotene (kindly provided by Professor B. C. L. Weedon). The synthetic compound had absorption maxima in light petroleum identical with those of the heptaene from carrot root and from *P. blakesleeanus*, but was markedly different in its chromatographic adsorption properties. On chromatography of all the conjugated heptaene samples on a thin layer of Silica Gel G with 10% benzene in light petroleum (b.p. 60–80°C) as the developing solvent, the samples from *R. rubrum*, *P. blakesleeanus* and carrot root all had R_f 0.70, whereas 7,8,7',8'-tetrahydro- β -carotene was much less strongly adsorbed, having R_f 0.95.

The second possibility that was considered was that the difference between the absorption spectra of the conjugated heptaenes from *R. rubrum* and from carrot root might be due to the former being a *cis* isomer of ζ -carotene. Iodine-catalysed photoisomerization of the sample of the conjugated

heptaene from carrot root led to hypsochromic and hypochromic shifts of the absorption bands, together with an increase in extinction in the 'cis-peak' region (285 and 296nm). The stereoisomeric mixture had absorption maxima in light petroleum (b.p. 40–60°C) at 285, 296 (infl.), 377, 398 and 423nm. A similar photoisomerization of the conjugated heptaene from *R. rubrum* also showed a hypsochromic shift of the absorption spectrum, a loss in extinction of the main absorption bands and an intensification of absorption in the 'cis-peak' region. The stereoisomeric mixture was different from that formed from the carrot-root sample, showing absorption maxima in light petroleum (b.p. 40–60°C) at 285, 296, 350 (infl.), 372, 391 and 415nm. Thus the conjugated heptaenes from *R. rubrum* and from carrot root are both all-*trans* isomers, but of different parent carotenoids.

The mass spectrum of the conjugated heptaene isolated from carrot root showed a molecular ion of nominal mass 540 (Calc. for ζ -carotene, $C_{40}H_{60}$: 540). Ions at m/e 471 ($M - 69$) and m/e 403 ($M - 137$) indicated fission of the 3,4 (or 3',4') and 7,8 (or 7',8') bonds respectively. There was no fission at the 11,12 (or 11',12') bond, confirming that the conjugated heptaene from higher plants (and, presumably, from *P. blakesleeanus*) has a symmetrically placed chromophore and is ζ -carotene (7,8,7',8'-tetrahydrolycopenes).

The sample of the conjugated heptaene isolated from diphenylamine-inhibited cultures of *R. rubrum* exhibited a mass spectrum that was different from this (Davies, Holmes, Loeber, Toubé & Weedon, 1969). The heptaene had a molecular ion of mass 540.4699 and was therefore isomeric with ζ -carotene (Calc. for $C_{40}H_{60}$: 540.4695) and the spectrum was similar to that of ζ -carotene in that ions of masses m/e 69 (base peak), m/e 471 ($M - 69$) and m/e 403 ($M - 137$) indicated fission at the 3,4 (or 3',4') and 7,8 (or 7',8') bonds. An additional peak at m/e 335 ($M - 205$), however, showed that fission was taking place at an 11,12 (or 11',12') 'bis-allylic' single bond. This fission was confirmed by the appearance in the mass spectrum of a metastable peak at m/e 208, which corresponds to a transition of the molecular ion of mass 540 to give a fragment with a mass/charge ratio of 335 ($335^2/540 = 207.8$). The conjugated heptaene from *R. rubrum* is therefore an isomer of ζ -carotene that has its chromophore placed asymmetrically in the molecule, and has the structure 7,8,11,12-tetrahydrolycopenes (VI). The absence of a peak in the mass spectrum at ($M - 56$) eliminated the possibility of an α -ionone end group (Schwieter *et al.* 1965), and the structure 7,8,11,12-tetrahydro- γ -carotene (which would also be consistent with the mass-spectral properties) was eliminated by the fact that such a compound would have chromatographic properties markedly different from those of ζ -carotene.

Fraction 4. This yellow carotenoid was eluted from the alumina column (Brockmann activity grade II) with 2% diethyl ether in light petroleum (b.p. 40–60°C). Its absorption maxima in light petroleum at 394 (infl.), 414, 440 and 467nm indicated a chromophore of nine conjugated double bonds and, together with its chromatographic characteristics, was consistent with the identification of this carotenoid as neurosporene (Jensen *et al.* 1958). This identification was confirmed by the mass spectrum, which showed a molecular ion of mass 538.4539 (Calc. for $C_{40}H_{58}$: 538.4538) and ions at m/e 69, m/e 469 ($M - 69$) and m/e 401 ($M - 137$). The assignment of an all-*trans* configuration was supported by hypochromic and hypsochromic shifts in the absorption spectrum and the appearance of a 'cis-peak' at 332nm when the carotenoid was subjected to iodine-catalysed photoisomerization in light petroleum (Zechmeister, 1962). The concentration of neurosporene in the cultures was 30 μ g/g dry wt.

Fraction 8. This orange-pink carotenoid was purified on a column of alumina (Brockmann activity grade III) with 2–5% diethyl ether in light petroleum (b.p. 40–60°C). It had an absorption spectrum in light petroleum with an inflexion at 414nm and maxima at 446, 473 and 505nm that showed hypsochromic shifts and loss of extinction on iodine-catalysed photoisomerization; a 'cis-peak' appeared at 362nm. This carotenoid was identified as all-*trans*-lycopenes. It was present at a very low concentration (6 μ g/g dry wt. of bacteria).

DISCUSSION

From the above results it is clear that, in *R. rubrum*, the asymmetrical conjugated heptaene, 7,8,11,12-tetrahydrolycopenes, replaces ζ -carotene, which is apparently absent, as an intermediate in the dehydrogenation of phytoene to lycopene. This is the first instance of this novel carotenoid occurring in Nature, although the synthetic compound (2,6,10,14,19,23,27,31-octamethyldotriacont-2,6,8,10,12,14,16,18,22,26,30-undecaene) has been prepared (Davis *et al.* 1966) and its electronic spectrum is in good agreement with that of the *R. rubrum* heptaene. The occurrence of this carotenoid is not confined to *R. rubrum*, or even to the photosynthetic bacteria, for conjugated heptaenes isolated from a mutant strain of *Rps. spheroides* (Nakayama, 1958) and from *Flavobacterium dehydrogenans* (O. B. Weeks & A. G. Andrewes, personal communication) both have absorption spectra that correspond to that of 7,8,11,12-tetrahydrolycopenes rather than to that of ζ -carotene.

The mass-spectrometric confirmation of the structures of phytoene, phytofluene and neurosporene isolated from *R. rubrum* and the absence of ζ -carotene mean that the phytoene dehydrogenation

Scheme 2. Dehydrogenation of phytoene in *R. rubrum*.

sequence in these, and possibly other, bacteria must be revised (Scheme 2). Phytoene is dehydrogenated first to phytofluene and then to 7,8,11,12-tetrahydrolycopene, both these dehydrogenations taking place on the same side of the chromophore. These are followed by two dehydrogenation steps on the other side of the molecule, to give neurosporene and lycopene in turn.

The quantitative determinations of the polyene hydrocarbons of diphenylamine-inhibited cultures of *R. rubrum* show that phytoene is present in the greatest quantity and lycopene is the least abundant. In fact, the concentrations fall throughout the biosynthetic sequence so that the proportions of phytoene, phytofluene, 7,8,11,12-tetrahydrolycopene, neurosporene and lycopene are in the proportions 30:12:10:5:1. The cultures also contain a number of methoxy-carotenoids formally derived from 7,8,11,12-tetrahydrolycopene and neurosporene as well as from lycopene (Davies & Holmes, 1969b); even those showing a structural relationship to lycopene may be derived biosynthetically from neurosporene and

7,8,11,12-tetrahydrolycopene (Davies, 1970). The formation of such compounds appears to be a direct result of the inhibition of the polyene dehydrogenation sequence. Once the diphenylamine inhibition is removed, there is a net synthesis of spirilloxanthin and other relatively unsaturated carotenoids but no change in the total carotenoid content (Jensen *et al.* 1958). The specific effect of diphenylamine on carotenoid biosynthesis in *R. rubrum* must be in inhibiting the dehydrogenase enzyme (or enzymes) responsible for the conversion of phytoene, through phytofluene, 7,8,11,12-tetrahydrolycopene and neurosporene, into lycopene.

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