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THE NATURE OF "θ-CAROTENE"

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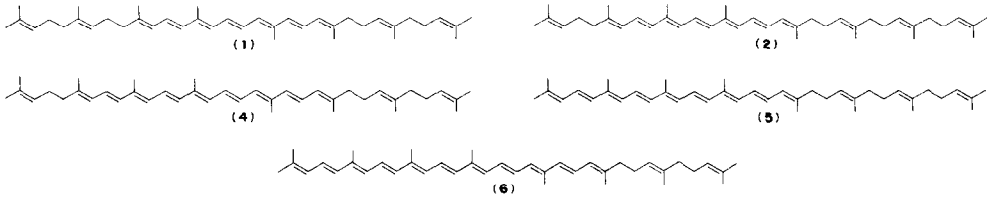
Key Word Index—*Neurospora crassa*; Sphaeriales; structure; biosynthesis; dehydrogenation; carotenoids; "θ-carotene"; ζ-carotene; 7,8,11,12-tetrahydrolycopene; neurosporene.

Abstract—"θ-Carotene" has been isolated from *Neurospora crassa* and resolved into two isometric conjugated heptaene components, ζ-carotene and 7,8,11,12-tetrahydrolycopene. The carotene dehydrogenating sequence operating in *N. crassa* is discussed in the light of these and other observations.

INTRODUCTION

THE NAME "θ-carotene" was first used by Haxo to describe a carotenoid isolated from cultures of *Neurospora crassa*.[1] This pigment closely resembled ζ-carotene (**1**, 7,8,7′,8′-tetrahydro-ψ,ψ-carotene) and was presumably a conjugated heptaene but it had an absorption spectrum in hexane with maxima at 375·5, 397 and 421 nm rather than at the values of 378, 400 and 425 nm which are characteristic of ζ-carotene.[2,3] More recent studies on the conjugated heptaenes of a number of organisms have shown that while higher plants are able to form only the symmetrical ζ-carotene, other organisms such as the purple non-sulphur photosynthetic bacterium, *Rhodospirillum rubrum*, are able to form 7,8,11,12-tetrahydrolycopene (**2**, 7,8,11,12-tetrahydro-ψ,ψ-carotene), an isomer with the chromophore placed unsymmetrically in the molecule.[4,5] This carotene has an absorption spectrum with maxima at significantly lower wavelengths in light petroleum (354 infl., 374, 394·5 and 418·5 nm) than those of ζ-carotene.[4,5] It is in this context that the nature of "θ-carotene" has been reexamined; two possible explanations for the anomalous absorption spectrum that have been investigated are that "θ-carotene" may be a *cis* isomer of ζ-carotene and that "θ-carotene" may represent a mixture of the symmetrical and unsymmetrical conjugated heptaenes.



[1] HAXO, F. T. (1955) *Fortschr. Chem. Org. Naturstoffe* **12**, 169.
[2] NASH, H. A., QUACKENBUSH, F. W. and PORTER, J. W., (1948) *J. Am. Chem. Soc.* **70**, 3513.
[3] DAVIS, J. B., JACKMAN, L. M., SIDDONS, P. T. and WEEDON, B. C. L. (1966) *J. Chem. Soc.* **C**, 2154.
[4] DAVIES, B. H., HOLMES, E. A., LOEBER, D. E., TOUBE, T. P. and WEEDON, B. C. L. (1969) *J. Chem. Soc.* **C**, 1266.
[5] DAVIES, B. H. (1970) *Biochem. J.* **116**, 93.

RESULTS

The absorption spectrum of "*θ*-carotene" in light petroleum has λ_{max} at 285, 296, 358 (infl.), 375, 396 and 420 nm. The ratio of extinctions at the two longest wavebands ($E_{420}/E_{396} = 0{\cdot}959$) is less than unity; in the case of the conjugated heptaenes (*vide infra*), this may be taken to indicate either that the sample is a *cis* isomer or that it is a mixture of different all-*trans* carotenes.

Iodine-catalyzed photoisomerization of a sample of "*θ*-carotene" led to hypsochromic and hypochromic shifts of the absorption bands, together with an increase in extinction in the "*cis*-peak" region (at 285 and 296 nm). The stereoisomeric equilibrium mixture had λ_{max} in light petroleum at 285, 296, 357 (infl.), 374, 394 and 418 nm. Similar photoisomerizations of *ζ*-carotene and 7,8,11,12-tetrahydrolycopene, isolated from carrot root oil and from diphenylamine (DPA)-inhibited cultures of *R. rubrum* respectively, also led to hypsochromic shifts of the absorption maxima, losses in extinction of the main absorption bands and intensifications of absorption in the "*cis*-peak" regions; the stereoisomeric equilibrium mixture from *ζ*-carotene had λ_{max} at 285, 296, 359 (infl.), 377, 398 and 423 nm, while that from 7,8,11,12-tetrahydrolycopene had λ_{max} at 285, 296, 350 (infl.), 372, 391 and 415 nm, both mixtures being in light petroleum. "*θ*-Carotene" is clearly not a *cis*-isomer of *ζ*-carotene because of its failure to show bathochromic and hyperchromic spectral changes on photoisomerization; the evidence from this phase of the investigation is consistent with the view that "*θ*-carotene" is a mixture of *ζ*-carotene and 7,8,11,12-tetrahydrolycopene.

Mass spectra of "*θ*-carotene" (recorded on an MS 12 mass spectrometer at ionization potentials of 12 and 70 eV and using a probe temperature of 220°) showed a molecular ion at *m/e* 540, corresponding to a molecular formula of $C_{40}H_{60}$ (i.e. the same as *ζ*-carotene and 7,8,11,12-tetrahydrolycopene). That a number of different "bis-allylic" fragmentations had occurred was shown by the presence of a weak ion at *m/e* 471 (M-69) and a slightly stronger ion at *m/e* 403 (M-137); a much more prominent ion at *m/e* 335 (M-205), supported by a strong metastable peak at *m/e* 208 ($335^2/540 = 207{\cdot}8$), indicated the "bis-allylic" fission of a saturated 11,12- (or 11′,12′-) bond.[4,5] These fragmentations are consistent with the presence in the sample of 7,8,11,12-tetrahydrolycopene, but its admixture with *ζ*-carotene, which would account for the anomalous electronic absorption spectrum, can neither be proved nor ruled out by such a limited MS analysis. In retrospect, however, the relative intensity of the ion at *m/e* 403 might be taken as an indication of the presence of the symmetrical isomer.

Any proof that "*θ*-carotene" is a mixture of two isomeric conjugated heptaenes can only result from a separation of the two. As their structures are so similar, it was obvious that such a chromatographic separation would be difficult; nevertheless, that "*θ*-carotene" is indeed a mixture is indicated by the diffuseness of its zone on column chromatography and by the relative difficulty, compared with previous experience with *ζ*-carotene,[6] of separating the conjugated heptaene(s) from *β*-zeacarotene (7′,8′-dihydro-*β*,*ψ*-carotene).

The sample of "*θ*-carotene" was subjected to further chromatography on a column (25 × 0·8 cm) of alumina (Woelm neutral, Brockmann activity grade II). Successive development with 250 ml 7% E/P (Et_2O in light petroleum, v/v), 200 ml 10% E/P, 100 ml 12·5% E/P, 100 ml 15% E/P and 200 ml each of 20, 25 and 40% E/P resulted in the isolation of three poorly resolved fractions. The first to be eluted was a minor

[6] WILLIAMS, R. J. H., DAVIES, B. H. and GOODWIN, T. W. (1965) *Phytochemistry* **4**, 759.

component (*vide infra*) and the two major components which followed it (A and B) were present in approximately equal amounts.

The less polar of the two major components (A), eluted with about 12·5% E/P, had λ_{max} in light petroleum at 355 (infl.), 374, 394·5 and 418·5 nm; absorption bands at 285 and 296 nm were weak (E_{296} was less than 10% of $E_{394\cdot5}$). These spectral characteristics are identical with those of all-*trans* 7,8,11,12-tetrahydrolycopene[5] and an all-*trans* configuration was confirmed by the hypochromic and hypsochromic shifts of the main absorption bands and the increase in extinction in the "*cis*-peak" region which occurred on iodine-catalyzed photoisomerization. The relative extinctions of the two longest wavelength maxima ($E_{418\cdot5}/E_{394\cdot5} = 1{\cdot}035$) also indicated that this carotene was an all-*trans* conjugated heptaene. The value of the corresponding ratio for a sample of all-*trans* 7,8,11,12-tetrahydrolycopene isolated from DPA-inhibited cultures of *R. rubrum* was 1·033.

The more polar major component (B) was eluted from the column with 20% E/P and had λ_{max} in light petroleum at 360 (infl.), 378, 400 and 425 nm; these values are identical with those reported for ζ-carotene.[5] Again, absorption in the "*cis*-peak" region (285 and 296 nm) was very weak, the ratio E_{425}/E_{400} was 1·027 (cf. 1·028 for all-*trans* ζ-carotene isolated from carrot root) and iodine-catalyzed photoisomerization led to losses of extinction and shifts of the main absorption bands to lower wavelengths with concomitant increases in extinction at 285 and 296 nm. These observations are consistent with this carotene (B) being all-*trans* 7,8,11,12-tetrahydrolycopene. These identifications were confirmed by MS.

The MS of the *N. crassa* carotenes (A and B), together with that of authentic ζ-carotene isolated from carrot root oil, were recorded on an MS 30 mass spectrometer at an ionization potential of 70 eV and at a probe temp. of 180°. The results of these analyses are recorded in Table 1. The abundances of fragment ions are shown, for convenience, relative to that of the molecular ion; all the spectra had *m/e* 69 as their base peak, but its intensity could not be measured accurately in every case. Also recorded in Table 1 are the corresponding values for an authentic sample of 7,8,11,12-tetrahydrolycopene; as these were recorded at a different time on a different instrument (MS 12), they cannot be regarded as being directly comparable.

The *N. crassa* carotenes, (A) and (B), both show a molecular ion at *m/e* 540, corresponding to a molecular formula of $C_{40}H_{60}$, thus confirming the isomeric relationship between them. Both showed "bis-allylic" fragmentations to yield ions at *m/e* 471 (M-69), *m/e* 403 (M-137) and *m/e* 335 (M-205), but the abundances of these ions relative to the molecular ion were markedly different between the two samples. In the case of carotene (A), the ions corresponding to losses of 69 and 137 m.u. were weak, while the ion at *m/e* 335 (37·5%; M-205) was very strong; this loss of 205 m.u. from the molecular ion, substantiated by a strong metastable peak at *m/e* 208 ($335^2/540 = 207{\cdot}8$), indicated a single bond between carbons 11 and 12 (or 11′ and 12′) and confirms the structure of (A) as 7,8,11,12-tetrahydrolycopene (**2**), which shows the same overall fragmentation pattern.

A further diagnostic feature arising in the mass spectrum from the unsymmetrically-placed conjugated heptaene chromophore of this carotene is the loss of 94 m.u. (1-methylcyclohexa-1,3-diene; Scheme 1, **3**) from the molecular ion to yield an ion at *m/e* 446. The formation of this ion is substantiated, in the case of the *N. crassa* carotene (A), by the appearance of a metastable peak at *m/e* 368·5 ($446^2/540 = 368{\cdot}4$). This loss has also been reported for 3,4,11′,12′-tetrahydrospheroidene (1-methoxy-1,2,7′,8′,11′12′-hexahydro-ψ,ψ-carotene)[4] and for the trimethylsilyl ether of 1-hydroxy-1,2,7′,8′,11′,12′-hexahydro-

lycopene (i.e. 1-trimethylsiloxy-1,2,7′,8′,11′,12′-hexahydro-ψ,ψ-carotene).[7] The further loss of 137 m.u. from *m/e* 446 to yield *m/e* 309 (M-94-137) is also a feature of the MS of both the *N. crassa* carotene (A) and 7,8,11,12-tetrahydrolycopene, and is substantiated in the latter instance by a metastable ion at *m/e* 214 ($309^2/446 = 214{\cdot}1$); these fragmentations are rationalized in Scheme 1.

TABLE 1. MS CHARACTERISTICS OF CONJUGATED HEPTAENES FROM *Neurospora crassa*, *Rhodospirillum rubrum* AND *Daucus carota*

		Relative ion intensities			
m/e	Fragment	7,8,11,12-Tetrahydro-lycopene*	*N. crassa* carotene (A)	*N. crassa* carotene (B)	ζ-Carotene
540	M^+	100†	100†	100†	100†
471	M-69	0·06	4·2	0·7	1·0
448	M-92	0·3	0·7	1·2	3·0
446	M-94	0·8	1·0	0·7	2·0
434	M-106	0·07	1·0	0·2	0·6
403	M-137	0·1	1·2	25·0	23·0
379	M-69-92	0·03	0·5	0·4	3·0
377	M-69-94	0·03	0·2	0·4	3·0
368	M-$C_{13}H_{16}$	3·4	9·0	3·1	91·0
365	M-69-106	0·06	0·7	0·06	5·0
335	M-205	42·9	37·5	3·1	5·0
311	M-92-137	0·2	0·7	3·7	13·2
309	M-94-137	1·4	2·5	1·2	6·3
Metastable assignments		Metastable ions recorded			
$M^+ \rightarrow$ M-137;	$403^2/540 = 300{\cdot}8$	—	—	301	301
$M^+ \rightarrow$ M-205;	$335^2/540 = 207{\cdot}8$	208	208	—	—
$M^+ \rightarrow$ M-69;	$471^2/540 = 410{\cdot}8$	—	411	—	—
$M^+ \rightarrow$ M-94;	$446^2/540 = 368{\cdot}4$	—	368·5	—	—
M-94 → M-94-137;	$309^2/446 = 214{\cdot}1$	214	—	—	—

* The spectrum of 7,8,11,12-tetrahydrolycopene was recorded at 70 eV and 220° (MS 12); all others were determined at 70 eV and 180° (MS 30).

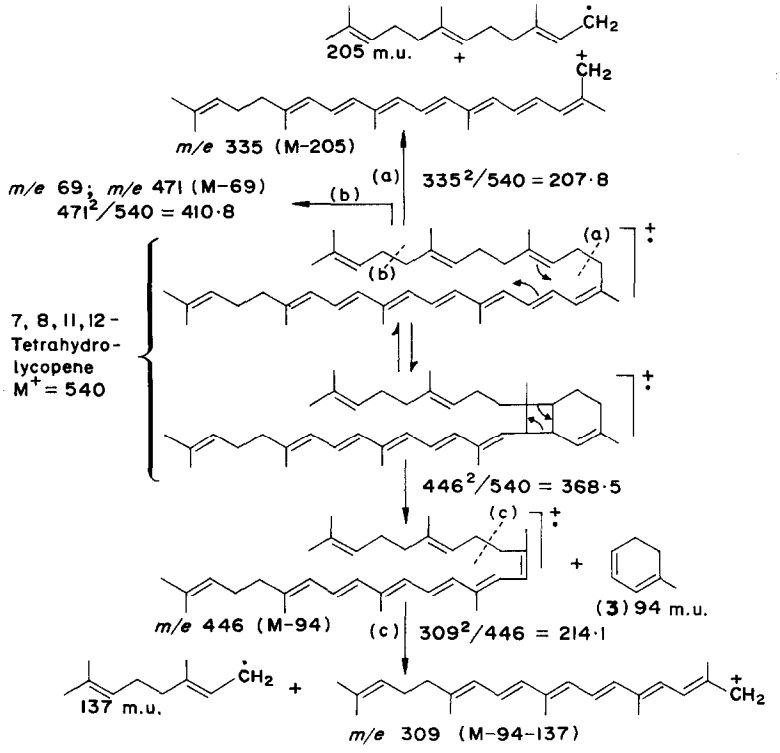
† *m/e* 69 was the base peak of all the spectra, but abundances are shown relative to the molecular ion.

The more polar *N. crassa* carotene (B), like ζ-carotene, shows stronger ions at *m/e* 448 (M-92) and at *m/e* 311 (M-92-137) than for M-94 and M-94-137, indicating that the loss of toluene (92 m.u.) is preferred to that of 1-methylcyclohexa-1,3-diene (94 m.u.). The main diagnostic feature of the MS of the *N. crassa* carotene (B), however, is the evidence of "bis-allylic" fission of the 7,8- (or 7′,8′-) bond provided by the appearance of a very prominent ion at *m/e* 403 (25%; M-137); this fragmentation of the molecular ion is supported by the metastable peak at *m/e* 301 ($403^2/540 = 300{\cdot}8$). These fragmentation characteristics are identical with those of ζ-carotene, and the identity of the *N. crassa* carotene (B) with ζ-carotene is thus amply confirmed. The appearance in all the MS of an ion at *m/e* 368 (M-$C_{13}H_{16}$) reflects a previous report;[4] this ion has not been rationalized with the structures and the apparently random variations in its recorded intensity may indicate that it is due to an impurity.

"θ-Carotene" is therefore proved to be a mixture of ζ-carotene and its unsymmetrical isomer, 7,8,11,12-tetrahydrolycopene. Its chromatographic resolution also yielded a less

[7] DAVIES, B. H. and AUNG THAN (1974) *Phytochemistry* **13**, 209.

polar minor component, eluted from the alumina column with 7% E/P; the absorption spectrum of the latter showed λ_{max} in light petroleum at 285, 296, 354 (infl.), 372, 392 and 416·5 nm. The relative extinctions of the maxima at 285 and 296 nm compared with those of the main absorption bands (E_{296} was some 30% of E_{392}) suggested a *cis* configuration. This was confirmed by iodine-catalyzed photoisomerization which led to hyperchromic and bathochromic shifts of the main absorption bands and a reduction in the extinction of the "*cis*-peaks" (285 and 296 nm). The stereoisomeric equilibrium mixture had λ_{max} in light petroleum at 285, 296, 357 (infl.), 374, 395 and 418 nm, that is, at precisely those wavelengths at which the equilibrium mixture of stereoisomers from "θ-carotene" showed absorption maxima. This minor component is clearly a mixture of *cis* isomers of ζ-carotene and 7,8,11,12-tetrahydrolycopene; these may occur naturally or represent manipulative artifacts.



SCHEME 1. RATIONALIZATION OF MS FRAGMENTATIONS OF 7,8,11,12-TETRAHYDROLYCOPENE

A precise knowledge of the structure of the conjugated nonaene, neurosporene, as isolated from *N. crassa*, is relevant to any discussion of the role of the isomeric conjugated heptaenes in carotenoid biosynthesis in this organism. For reasons outlined below, it must be ascertained whether the structure is genuinely that of neurosporene (**4**, 7,8-dihydro-ψ,ψ-carotene) or whether this is accompanied by the isomeric conjugated nonaene, 3,4-didehydro-7′,8′,11′,12′-tetrahydro-ψ,ψ-carotene (**5**). Accordingly, the MS of a sample of neurosporene from *N. crassa* was determined (at a probe temperature of 180° and an ionization potential of 70 eV) using an MS 30 mass spectrometer. The base peak of the spectrum was at *m/e* 69 and the molecular ion appeared as *m/e* 538 (17·5%; $C_{40}H_{58}$).

Apart from this, the most prominent high mass ions were at *m/e* 401 (2·8%; M-137) and at *m/e* 309 (2·6%; M-92-137). The ion at *m/e* 333 (0·8%; M-205) was much weaker than would be expected if the isomer (**5**) with a terminal chromophore were present. This evidence in itself indicates that the conjugated nonaene has the structure (**4**) normally associated with neurosporene; any formation of the structural isomer (**5**) must be very slight. This view is supported by the apparent preference on the part of the molecular ion for losing toluene (92 m.u.) or *m*-xylene (106 m.u.) rather than 1-methylcyclohexa-1,3-diene (94 m.u., see Scheme 1); ions at *m/e* 444 (0·1%; M-94) and *m/e* 307 (0·2%; M-94-137) were much weaker than the corresponding ions at *m/e* 446 (1·1%; M-92) and *m/e* 309 (2·6%; M-92-137) or at *m/e* 432 (1·8%; M-106) and *m/e* 295 (1·4%; M-106-137). Further ions consistent with the structure (**4**) were recorded at *m/e* 332 (0·9%; M-69-137) and at *m/e* 469 (0·8%; M-69): an ion was again present at *m/e* 368 (1·6%; M-$C_{13}H_{16}$).

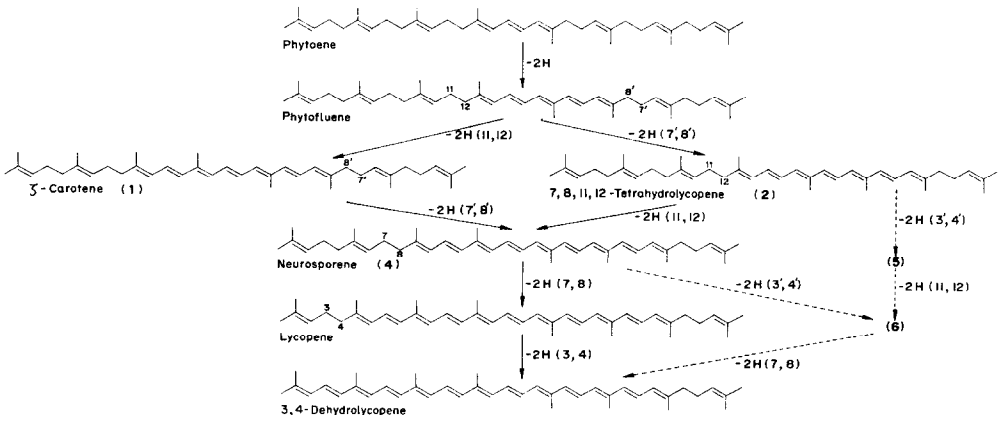
DISCUSSION

The results presented here show conclusively that "*0*-carotene", the conjugated heptaene from *N. crassa*, can be resolved chromatographically to yield, as its major components, the structural isomers ζ-carotene and 7,8,11,12-tetrahydrolycopene. The name "*0*-carotene" should therefore be abandoned. It is clear that the biosynthetic dehydrogenation sequence, which leads from phytoene (7,8,11,12,7′,8′,11′,12′-octahydro-ψ,ψ-carotene) to phytofluene (7,8,11,12,7′,8′-hexahydro-ψ,ψ-carotene) undergoes a bifurcation (Scheme 2). The loss of two hydrogens from the phytofluene molecule can occur either at carbons 11 and 12 to form ζ-carotene (i.e. 7,8,7′,8′-tetrahydro-ψ,ψ-carotene, **1**) or at carbons 7′ and 8′ to yield 7,8,11,12-tetrahydrolycopene (**2**). These conjugated heptaenes are then dehydrogenated to yield neurosporene (i.e. 7,8-dihydro-ψ,ψ-carotene, **4**) with the loss of hydrogens from carbons 7′ and 8′ or from carbons 11 and 12 respectively. Whether these alternative pathways are due to the existence of separate enzymes or to a lack of substrate specificity on the part of one enzyme is not clear at present. The situation in *N. crassa* is in contrast to those in higher plants and in *R. rubrum* where ζ-carotene and 7,8,11,12-tetrahydrolycopene respectively are apparently formed as the exclusive conjugated heptaenes.[5] The existence of parallel pathways through the conjugated heptaene stage of carotene dehydrogenation is also indicated, however, by studies on *Rhodopseudomonas viridis*[8] and *Flavobacterium dehydrogenans*,[9] in mutants of *Phycomyces blakesleeanus*[10] and, in the case of triterpenoid carotene biosynthesis, in *Streptococcus faecium* UNH 564P.[11]

The existence of 3,4-dehydrolycopene (3,4-didehydro-ψ,ψ-carotene) in *N. crassa*, and therefore of an enzyme catalyzing dehydrogenation at carbons 3 and 4 of lycopene (ψ,ψ-carotene), raises the question of whether this enzyme might also utilize 7,8,11,12-tetrahydrolycopene as a substrate. The resulting dehydrogenation would yield 3,4-didehydro-7′,8′,11′,12′-tetrahydro-ψ,ψ-carotene (**5**), a structural isomer of neurosporene (**4**) in which the conjugated nonaene chromophore is displaced further from the centre of the molecule. The MS investigation of the neurosporene fraction from *N. crassa* shows that this cannot occur to any appreciable extent, for the intensities of the appropriate

[8] MALHOTRA, H. C., BRITTON, G. and GOODWIN, T. W. (1970) *Int. Z. Vitaminforsch.* **40**, 315.
[9] WEEKS, O. B., ANDREWES, A. G., BROWN, B. O. and WEEDON, B. C. L. (1969) *Nature* **224**, 879; WEEKS, O. B., personal communication.
[10] DAVIES, B. H. (1973) *Pure Appl. Chem.* **35**, 1.
[11] TAYLOR, R. F. and DAVIES, B. H. (1973) *Trans Biochem. Soc.* **1**, 1091.

diagnostic ions (for M-205, M-94, M-94-137) indicate no significant proportion of such an isomer, although the possibility of trace amounts being formed has not entirely been eliminated. A higher substrate specificity, imposed either by the nature of the enzyme or by its spatial position in a multiplex, is therefore apparent for the removal of hydrogens from carbons 3 and 4 than may be the case for other dehydrogenations. It is worth noting, however, that samples of lycopene from a number of natural sources have MS which, when compared with that of synthetic lycopene (i.e. pure ψ,ψ-carotene), show some evidence of traces of an isomeric polyene (3,4-didehydro-7′,8′-dihydro-ψ,ψ-carotene, **6**) in which the conjugated undecaene chromophore is displaced from the centre of the molecule.[12]



SCHEME 2. CAROTENE DEHYDROGENATIONS IN *Neurospora crassa*

EXPERIMENTAL

Solvents. All solvents used were of AR grade. Light petrol. (b.p. 40–60°) and Et_2O were dried over Na wire or Na/Pb and the Et_2O was distilled from reduced Fe powder prior to use. The dry light petrol. was distilled from reduced Fe powder, passed through a column of silica gel (Woelm) and redistilled again. C_6H_6 was redistilled prior to use.

Growth of organism. The culture of *Neurospora crassa* (Shear and Dodge) was obtained from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands and was maintained on Czapek Dox Agar (Oxoid) slopes. Liquid cultures were on a medium which contained, per litre: sucrose, 50 g; yeast extract (Difco), 25 g; malt extract broth (Oxoid), 5 g; NaCl, 2 g; KNO_3, 1 g; KH_2PO_4, 1 g; $MgSO_4.7H_2O$, 0·5 g; $CaCl_2.2H_2O$, 0·1 g; $FeCl_3.6H_2O$, 5 mg; $ZnSO_4.7H_2O$, 2 mg. Spore suspensions from mature slopes were used to inoculate the 100 ml liquid cultures in Roux bottles; these were maintained at 24° for 3 days in the dark and then illuminated (2000 lx) at the same temperature for 7 days.

Extraction of unsaponifiable lipid. The mycelia were harvested by filtration through muslin, washed with H_2O and squeezed dry by hand. Extraction of the lipid, 2 × with acetone, once with acetone/Et_2O (1 : 1, v/v) and once with Et_2O, was accomplished using an Ultra Turrax homogenizer. Sufficient H_2O was added to the bulked organic extract to transfer the lipid to the Et_2O phase which was washed 3 × with H_2O and, after the Et_2O had been distilled off, the lipid was dissolved in the minimum vol. of EtOH and saponified by boiling for 10 min after the addition of 60% (w/v) aq. KOH (1 ml per 10 ml EtOH). The unsaponifiable fraction (3 g from 1.2 kg mycelium grown in 8 l. medium) was recovered by our standard procedure.[13]

Preparation of "θ-carotene" sample for further analysis. After much of the sterol had been precipitated (overnight at −15°) from a light petrol. soln of the unsaponifiable lipid and had been filtered off and washed with cold light petrol., the combined washings and filtrate were concentrated to a small vol. (*ca* 15 ml). About a third was added to each of three identical chromatography columns. Each was a 30 × 3 cm column of alumina (Woelm neutral, Brockmann activity grade III) and was developed with light petrol. containing increasing concentrations of Et_2O (Table 2). Fractions (100 ml) were collected and their

[12] AUNG THAN and DAVIES, B. H., unpublished work.

absorption spectra in light petrol.[13] indicated the presence of the carotenes shown in Table 2. The conjugated heptaene, heavily contaminated with either β-zeacarotene or γ-carotene (β,ψ-carotene) appeared in fractions 6–10. These fractions from the three columns were bulked, the solvent distilled off and the residue dissolved in light petrol. The "θ-carotene" was completely separated both from the less polar β-zeacarotene and from the more polar γ-carotene by repeated chromatography on 25 × 2 cm columns of alumina (Woelm neutral, grade II) using increasing concentrations of Et_2O in light petrol. (4 to 25%, v/v) as the developing solvent and by TLC on 500 μm layers of MgO (B.D.H., for chromatographic adsorption analysis) using 50% C_6H_6 in light petrol. (v/v) as the solvent. A final chromatography on a column of alumina (Woelm neutral, grade III) yielded a sample of "θ-carotene" which had λ_{max} in light petrol. at 285, 296, 358 (infl.), 375, 396 and 420 nm and which, as there were no remaining traces of absorption peaks at 452 or 489 nm, was contaminated with neither β-zeacarotene nor γ-carotene. A sample of crude neurosporene was also recovered from the initial chromatography; this was rechromatographed on columns of alumina (Woelm neutral, grade III) until spectroscopically pure.

TABLE 2. ABSORPTION MAXIMA (IN LIGHT PETROLEUM) AND CHROMATOGRAPHIC BEHAVIOUR OF *Neurospora crassa* CAROTENES ON ALUMINA (WOELM NEUTRAL, BROCKMANN ACTIVITY GRADE III)

Carotene	Absorption maxima (nm)				100 ml Fraction No.	%E/P*
Phytoene	(264)	275	286	296	2–3	0
Phytofluene	(317)	331	346	368	2–4	0
β-Carotene	(400)	(426)	448	475	3–5	0
β-Zeacarotene	(380)	404	426	452	5–10	0–0·5
"θ-Carotene	(358)	375	396	420	6–10	0–0·5
γ-Carotene	(410)	435	460	489	10–13	0·5–1·0
Neurosporene	(392)	413	438	469	13–17	1–2
Lycopene	(414)	444	468	500	16–20	2–4
3,4-Dehydrolycopene	(430)	452	486	520	21–24	4–7

* % Et_2O in light petrol. (v/v) required for elution.

Authentic samples of conjugated heptaenes. 7,8,11,12-Tetrahydrolycopene was isolated from DPA-inhibited cultures of *Rhodospirillum rubrum* (NCIB 8255) by methods described previously.[5] The isomeric ζ-carotene was isolated from a sample of carrot root oil; 5 ml oil was saponified[14] and the unsaponifiable fraction isolated.[13] Preliminary chromatography of this on alumina columns (Woelm neutral, grade II) developed with increasing concentrations (0 to 50%, v/v) of Et_2O in light petrol. resolved a number of carotene fractions. The crude ζ-carotene fraction, recognized by its characteristic absorption spectrum,[5] was subjected to repeated chromatography with light petrol. on columns of alumina (Woelm neutral, grade III) until it was chromatographically and spectroscopically pure.

Absorption spectra. All electronic absorption spectra were recorded from light petrol. solutions on a Unicam SP 800 recording spectrophotometer, the wavelength scale of which was calibrated for critical measurements by using the appropriate absorption bands (360·9, 418·4 and 453·2 nm) of a holmium oxide filter. Quantitative measurements were made on solutions of known volume in one of a matched pair of 1 cm quartz cuvettes; concentrations of the conjugated heptaenes were calculated using a nominal value of 2270 for their specific extinction coefficient ($E^{1\%}_{1cm}$).[13]

Configuration of conjugated heptaenes. Determinations of whether the carotenes had all-*trans* or *cis* configurations were carried out by following the changes in their absorption spectra on iodine-catalyzed photoisomerization.[13,15] Isomerizations were performed on light petrol. solutions of the carotenes contained in 1 cm quartz spectrophotometer cuvettes; iodine (1–2% of the quantity of carotene) was added in a drop of light petrol. and spectra were recorded before and at intervals during illumination for periods of up to 15 min with two parallel fluorescent lamps (Philips MCFE 65 W, 4400°, warm white) at a distance of a few cm.

MS. Most of the MS were determined on an A.E.I. MS 30 mass spectrometer by Mr. J. Heald of the Department of Botany and Microbiology, U.C.W. The probe temp. was 180° and the ionization potential was 70 eV. Other spectra were determined on an A.E.I. MS 12 mass spectrometer (220°, 12 or 70 eV) at the Department of Biochemistry, University of Liverpool, by Mr. J. Ireland and through the courtesy of Dr. G. Britton.

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