STEREOSPECIFIC STUDIES ON CAROTENOID BIOSYNTHESIS

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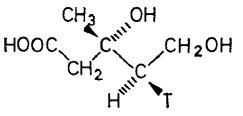
ABSTRACT

Various species of [2-^{14}C] MVA stereospecifically labelled with tritium at C-2, C-4 and C-5 have been used to study details of carotenoid biosynthesis in a variety of preparations including chloroplasts, fruit and root slices, and fungi.

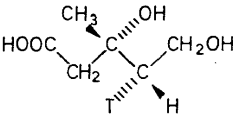
Mechanisms have been proposed for the dimerization of geranylgeranyl pyrophosphate to form phytoene, for the cyclization of acyclic precursors to form α- and β-carotene derivatives, for the formation of 3-hydroxy-carotenoids and for the formation of retro carotenoids. The stereochemistry of the desaturation of phytoene has been partly elucidated.

INTRODUCTION

General reviews of the biosynthesis of carotenoids have recently appeared[12,19] and, in the paper immediately preceding this one[20], Porter has reviewed the enzymology of the various steps in detail and has described the important advances recently made in his laboratory. In this article the progress made in more clearly defining the details of carotenoid biosynthesis with the aid of various species of stereospecifically labelled mevalonic acid (MVA) will be discussed. This approach was initiated in the study of sterol biosynthesis by Popjàk and Cornforth[18]. They synthesized [2-^{14}C-,4*R*,-$^{3}H_1$] MVA [1] and [2-^{14}C-4*S*-$^{3}H_1$] MVA [2] and showed that in liver the isomerization of isopentenyl pyrophosphate (IPP) to dimethylallyl pyrophosphate (DMAPP) occurs with the stereospecific loss of the pro-*R*

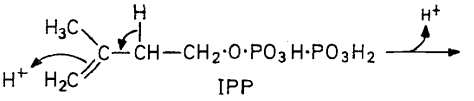


MVA 4-*R*-configuration [1]

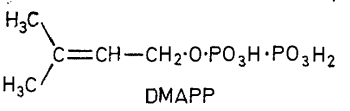


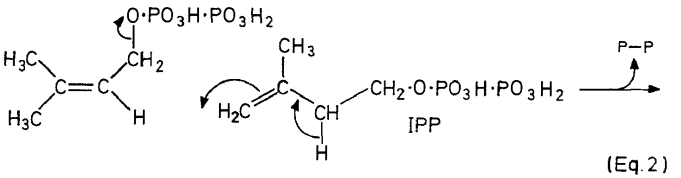
MVA 4-S-configuration [2]

hydrogen from C-2 of IPP, that is the pro-*S*-hydrogen arising from C-4 of MVA (equation 1). The same stereochemical loss of hydrogen occurs when farnesyl pyrophosphate (C-15) is formed by the addition of two further IPP molecules. This is illustrated in equation 2 by the formation of geranyl pyrophosphate. Thus when two molecules of FPP react to form squalene

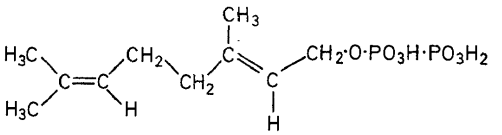


(Eq.1)



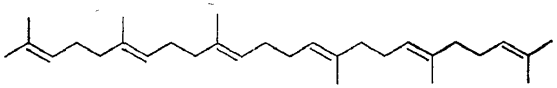


(Eq.2)

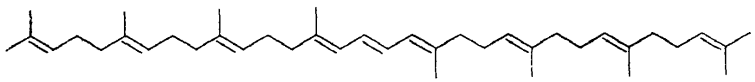


Geranyl pyrophosphate

all six *pro-R* hydrogens from C-4 of the six originating MVA molecules are retained in squalene [3] so that with [2-^{14}C-4*R*-4-^{3}H$_1$] MVA if the ^{14}C:^{3}H ratio of the substrate is normalized to 1:1 the same ratio is found in squalene; on the other hand with [2-^{14}C-4*S*-4-^{3}H$_1$] MVA, all the tritium atoms are



[3] Squalene



[4] Phytoene*

lost[18]. In plants the same stereochemical eliminations were observed in squalene formation (*Table 1*)[13] and in the formation of geranylgeranyl pyrophosphate (GGPP)[4] two molecules of which react to form phytoene [4], the first C-40 precursor of carotenoids[13]. The double bonds formed in squalene and phytoene in which C-4 of MVA is concerned have the *trans*

* Naturally occurring phytoene has its central double bond in the *cis*-configuration, but for simplification in considering changes not involving this double bond, the structure is drawn with the *trans*-configuration.

Table 1. The $^{3}H/^{14}C$ ratios in squalene and phytoene synthesized by isolated carrot root slices from [2-^{14}C-(4*R*)-4-$^{3}H_1$] MVA

Compound	$^{3}H/^{14}C$ *ratio*
MVA	5·99
Squalene	
Sample 1	5·46
Sample 2	5·43
Phytoene	
Sample 1	5·31
Sample 2	5·52

configuration. In rubber they have the *cis*-configuration and it is the 4-*pro-R* hydrogens of MVA which are lost in the biosynthesis of this compound[1].

MECHANISM OF CYCLIZATION OF CAROTENOIDS

In the further metabolism of phytoene to coloured carotenoids in plants it is sequentially desaturated to lycopene (*Figure 1*)[19,20]. In this process no tritium atoms arising from C-4 MVA are lost. When lycopene[26] or a

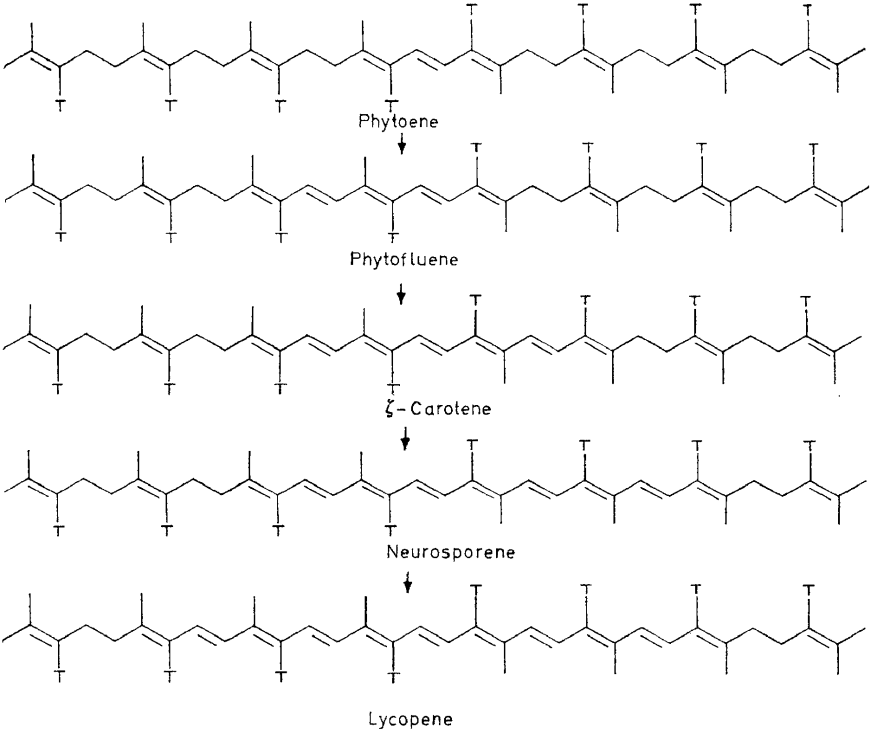


Figure 1. The biosynthetic pathway from phytoene to lycopene. The tritium atoms arising from [4*R*-$^{3}H_1$] MVA are indicated.

related compound such as neurosporene is cyclized then a possible mechanism for the formation of the β-ionone ring is that indicated in *Figure 2* (first route) with loss of a proton from C-6 (carotenoid numbering). In an analogous manner the α-ionone ring would arise by the second route (*Figure 2*) with loss of a proton from C-4. With [2-^{14}C-(4*R*)-^{3}H$_1$] MVA as substrate, the atom lost in forming the β-ionone ring is a tritium atom; so

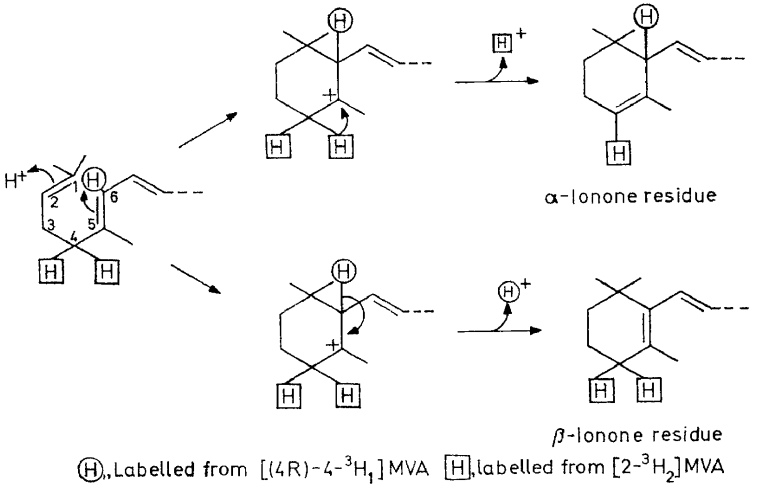


Figure 2. Proposed mechanism for the cyclization of acyclic carotenes to form cyclic carotenes with α- and β-ionone rings.

with the atomic ratio of phytoene normalized to 8:8 that in β-carotene should be 8:6 (β-carotene contains two β-ionone residues). Experiments with *Phycomyces blakesleeanus* showed that the ratio in β-carotene did drop to 8:6[11] and this has now been confirmed numerous times in different tissues. In particular, *Table 2* shows results with slices of fruit of the *del* tomato, a mutant producing various cyclic carotenes and only small amounts of lycopene, which is the preponderant pigment in the normal commercial tomato. In β-carotene the ratio is 8:6; and in γ-carotene (one β-ionone

Table 2. Incorporation of [3*RS*-^{14}C,(4*R*)-4-^{3}H$_1$] MVA into carotenoid polyenes in *del* tomatoes[29]

Polyene	*Radioactivity* (disintegrations/min) (^{3}H)	(^{14}C)	*Mean* ^{3}H/^{14}C *radioactivity ratio*	*Mean* ^{3}H/^{14}C *atomic ratio*
[2-^{14}C-(4*R*)-4-^{3}H$_1$] MVA	23910	4450	5·37	
Squalene	328800	65500	5·02	6:6
Phytoene	8470	1700	4·98	8:8
δ-Carotene	35600	7620	4·70	7·59(±0·04):8
ε-Carotene	529	105	5·04	8·04(±0·33):8
γ-Carotene	5130	1170	4·38	7·05(±0·07):8
α-Carotene	1770	406	4·36	6·95(±0·11):8
β-Carotene	4470	1205	3·71	5·95(±0·33):8
Lycopene	4640	960	4·84	7·76(±0·12):8

ring) it is 8:7. In α-carotene (one α-ionone and one β-ionone ring) it is also 8:7 which means that the α-ionone ring cannot have arisen by isomerization of a preformed β-ionone ring, if so the ratio would have been 8:6. This is confirmed by the observation that the ratios with δ-carotene (one α-ionone ring) and ε-carotene (two α-ionone rings) are the same as that of lycopene.

These results do not however rule out the possibility that a preformed α-ionone ring is isomerized to a β-ionone ring. This problem was tackled with [2-^{14}C-2-$^{3}H_2$] MVA. There is no loss of tritium in the formation of squalene or phytoene from this substrate so that if the atomic ratio of squalene is normalized to 6:12 then that of phytoene should be 8:16; if the α- and β-ionone rings were formed separately, the expected ratios in α-carotene and β-carotene would be 8:11 and 8:12 respectively (*Figure 3*). If the β-ionone ring was formed from the α-ionone ring then the ratio in β-carotene would be 8:10. The results quoted in *Table 3* show that ratios are as expected

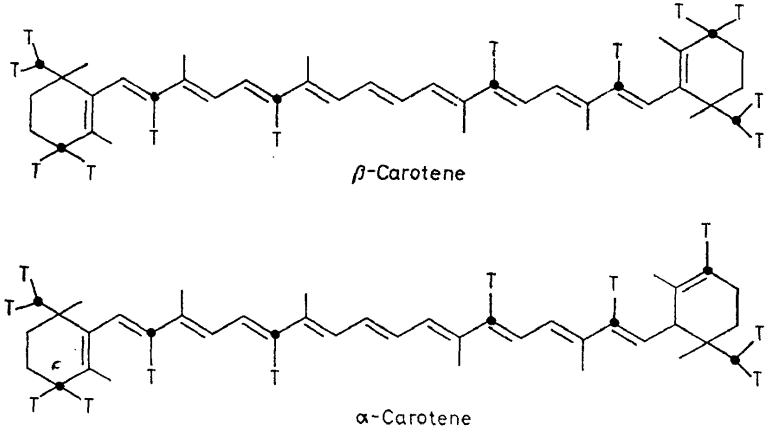


Figure 3. The expected labelling of α-carotene and β-carotene from [2-^{14}C-2-$^{3}H_2$] MVA if the α- and β-ionone rings arose separately (● label from ^{14}C; T label from ^{3}H).

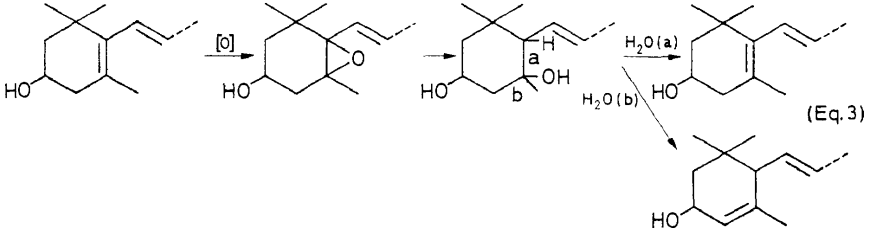
Table 3. Incorporation of (3*RS*)-[2-^{14}C,2-$^{3}H_2$] MVA into squalene, phytoene, β-carotene and α-carotene by slices of carrot root[29]

Polyene	*Radioactivity* (disintegrations/min) (^{3}H)	(^{14}C)	*Mean* ^{3}H/^{14}C *radioactivity ratio*	*Mean* ^{3}H/^{14}C *atomic ratio*
Squalene	85248	5416	15·74	12:8
Phytoene	87028	5568	15·63	15·90(±0·30):8
β-Carotene	55175	4575	12·06	12·25(±0·25):8
α-Carotene	23074	2075	11·12	11·39(±0·10):8

for the separate biogenesis of the two ionone rings. Thus all the experiments quoted support the view that the α- and β-ionone rings in the carotenes are formed by separate pathways probably via a common enzyme-substrate intermediate rather than from a carbonium ion as indicated in *Figure 2*. Further stereochemical problems concerned with this process, such as from

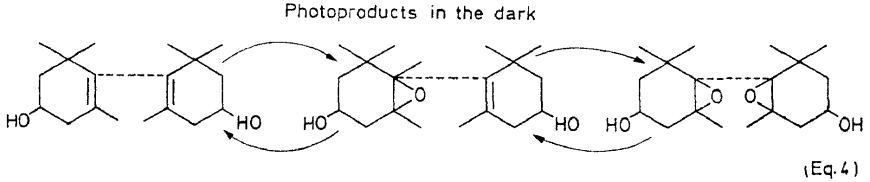
which side of the molecule does H^+ approach and which proton at C-4 is removed in forming the α-ionone ring, remain unsolved. The last point has been investigated with [2-^{14}C-(2*R*)-2-$^{3}H_1$] MVA and [2-^{14}C-(2*S*)-2-$^{3}H_1$] MVA but for reasons discussed later (page 492) satisfactory results have not yet been obtained.

The two major xanthophyll derivatives in green leaves are lutein (3,3'-dihydroxy-α-carotene) and zeaxanthin (3,3'-dihydroxy-β-carotene). There have been reports that these two compounds might be interconvertible via their epoxide (equation 3) (e.g. see [3,6,22]) but although later experiments



(Eq. 3)

indicate that there is, under appropriate physiological conditions, a shuttle of oxygen between violaxanthin, antheraxanthin and zeaxanthin (equation 4) (e.g. see [17,31]), no interconversion of lutein and zeaxanthin could be demonstrated. This conclusion was clearly confirmed in maize seedlings



(Eq. 4)

with the aid of [2-^{14}C-(4*R*)-4-$^{3}H_1$] MVA. The atomic ratios for α- and β-carotene were 8:7 and 8:6 respectively, as already demonstrated in tomatoes and carrots, and the same ratios were found in lutein and zeaxanthin after rigorous purification[14] (*Table 4*).

Table 4. The incorporation of [2-^{14}C-(4*R*)-4-$^{3}H_1$] MVA into carotenes and xanthophylls in maize seedlings[14]

Compound	^{14}C (d.p.m.)	^{3}H (d.p.m.)	^{3}H/^{14}C (d.p.m.)	^{3}H:^{14}C *Atomic ratio*
β-Carotene	24754	126109	4·97	6·00:8
Zeaxanthin	2200	11193	4·93	5·95:8
Zeaxanthin diacetate	741	3727	4·99	6·01:8
α-Carotene	464	2955	6·24	7·52:8
Lutein	6842	39530	5·80	7·00:8
Lutein diacetate	4161	24274	5·90	7·09:8

^{3}H: ^{14}C atomic ratios based on a ratio 6.00:8 for β-carotene.

Formation of phytoene

In the formation of phytoene two hydrogen atoms are lost at the centre of the molecule at C-15 and C-15′. Both these atoms originate from C-5 of MVA. With the use of [2-^{14}C-(5*R*)-5-$^{3}H_1$] MVA and [2-^{14}C-5-$^{3}H_2$] MVA ([2-^{14}C-(5*S*)-5-$^{3}H_1$] MVA has not yet been synthesized) it has been possible to study the stereochemistry of this elimination. Popják and Cornforth[18] showed that one *pro-S* hydrogen from C-1 of FPP (i.e. a *pro-R* hydrogen from C-5 of MVA) was lost in the formation of squalene but that both *pro-R* hydrogens were retained. We examined the formation of phytoene in tomato fruit (tangerine mutant) from the two species of MVA and on the reasonable assumption that squalene in plants is synthesized in the same way as squalene in animals it was shown that the two hydrogens lost from the centre of the phytoene molecule were the two *pro-R* hydrogens originating from C-5 of MVA[29] (*Table 5*).

Table 5. Comparison of incorporation of (3*RS*)-[2-^{14}C-(5*R*)-5-$^{3}H_1$] MVA and [2-^{14}C-5-$^{3}H_2$] MVA into squalene and phytoene by slices of tangerine tomatoes[29]

Substance	*Radioactivity* (d.p.m.) (^{3}H)	(^{14}C)	*Mean* ^{3}H/^{14}C *ratio*	*Mean* ^{3}H/^{14}C *atomic ratio*
[2-^{14}C-(5*R*)-5-$^{3}H_1$] MVA				
MVA	41476	3924	10·57	—
Squalene	312260	26485	11·79	6:6
Phytoene	4001	330	12·12	8·23±0·08:8
[2-^{14}C-5-$^{3}H_2$] MVA				
MVA	43000	3760	11·43	—
Squalene	352950	33550	10·52	11:6
Phytoene	132090	12870	12·26	14·31±0·09:8

In order to eliminate the uncertainty that 'plant squalene' might not be formed in the same stereochemical fashion as 'animal squalene', these experiments have been repeated with chloroplasts isolated in organic media[5] which synthesize geranylgeraniol (GG) and phytoene as the main terpenoid products. Direct comparison of the ^{3}H/^{14}C in phytoene could thus be made with that of GG which loses no hydrogens from C-5 of the four constituent molecules of MVA. *Table 6* shows that all *pro-R* hydrogens are

Table 6. Comparison of incorporation of [2-^{14}C-(5*R*)-$^{3}H_1$] MVA and [2-^{14}C-5-$^{3}H_4$] MVA into geranylgeraniol and phytoene by isolated chloroplasts

Substance	*Radioactivity* (d.p.m.) (^{3}H)	(^{14}C)	^{3}H/^{14}C *ratio*	^{3}H:^{14}C *atomic ratio*
[2-^{14}C-(5*R*)-5-$^{3}H_1$] MVA				
Geranylgeraniol	1877	149	12·59	4:4
Phytoene	17380	1382	12·58	8:8
[2-^{14} C-5-$^{3}H_2$] MVA				
Geranylgeraniol	2176	175	12·44	8:4
Phytoene	1265	121	10·47	13·6:8

retained in phytoene whilst two *pro-S* hydrogens are lost, thus confirming and extending the earlier experiments[4].

In the case of zeaxanthin it would appear that the situation is reversed in maize and the fruit of *Physalis alkekengi*, and that the two *pro-R* hydrogens are lost at the centre of the phytoene molecule and the *pro-S* hydrogens retained (see p. 489). This problem continues to be studied.

These results suggest a mechanism for the formation of phytoene which leads to the *cis* configuration at the centre of the molecule, the configuration of the natural product. The proposal (*Figure 4*), which is only one of a number of possibilities, was made earlier by Cornforth and co-workers[7] in

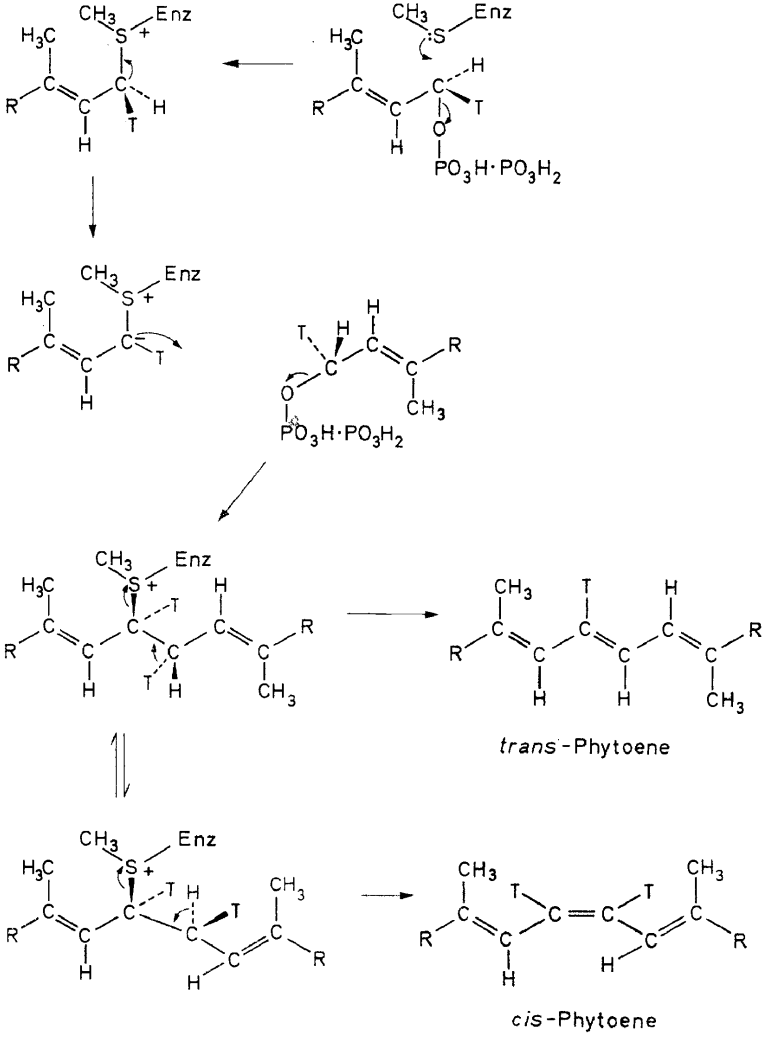


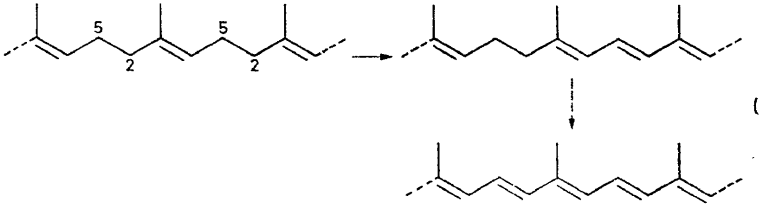
Figure 4. Possible mechanism for the formation of phytoene from geranylgeranyl pyrophosphate.

a slightly different form to explain the formation of squalene. It is assumed that the GG residues are connected via a sulphonium ylide, a mechanism which requires the presence of a thio-ether grouping, e.g. a methionine residue, at the active site of the enzyme. The thio-ether group displaces the pyrophosphate from a molecule of GGPP by an S_N2 substitution reaction which involves inversion of configuration at C-1 of the GG, to give a sulphonium ion. The hydrogens at C-1 situated between a double bond and the S atom tend to ionize with the formation of an ylide. This alkylates a second molecule of GGPP, again with inversion at C-1, to give a lycopersenylsulphonium ion (lycopersene is the C_{40} homologue of squalene). The central double bond is then introduced by normal *trans* elimination of the S-enzyme and a proton from the adjacent methylene group. Retention of both *pro-R* (or both *pro-S*) hydrogens at the centre of the molecule will occur only if the configuration of the newly formed central double bond is *cis*. A *trans* configuration can be obtained only if one *pro-R* and one *pro-S* hydrogen is lost.

This mechanism also accommodates the fact that neither $NADP^+$ nor NAD^+ is involved in the reaction in isolated chloroplasts[5] and tomato plastids[16].

Stereochemistry of desaturation of phytoene

During the stepwise desaturation of phytoene to lycopene one hydrogen



(Eq.5)

Table 7. Comparison of incorporation of [2-^{14}C-(5*R*)-5-$^3H_1$] MVA into carotenes in *del* tomatoes, maize seedlings and fruit of *Physalis alkekengi*[27,30]

Substance	*Radioactivity* (dp..m. (^{3}H)	(^{14}C)	^{3}H/^{14}C *ratio*	^{3}H/^{14}C *atomic ratio*
Del tomatoes				
Phytoene	126940	10500	12·09	8:8
δ-Carotene	120680	16750	6·13	4·05(±0·02):8
γ-Carotene	6573	1050	6·26	4·16(±0·02):8
α-Carotene	8818	1420	6·21	4·11(±0·03):8
β-Carotene	15475	3460	6·29	4·16(±0·01):8
Lycopene	25320	4546	5·57	3·70(±0·03):8
Maize seedlings				
MVA	36402	3173	11·47	1:1
α-Carotene	485	85	5·85	4·09:1
β-Carotene	22126	3780	5·70	3·98:1
Physalis alkekengi				
MVA	9895	1433	6·90	1:1
Phytoene	37918	5466	6·94	8:8
β-Carotene	18779	5442	3·45	4:8

Table 8. Comparison of incorporation of [2-^{14}C-5-$^{3}H_2$] MVA into carotenes in tangerine tomatoes, maize seedlings and fruit of *Physalis alkekengi* [27,30]

Substance	*Radioactivity* (d.p.m.) (^{3}H)	(^{14}C)	^{3}H/^{14}C *ratio*	^{3}H/^{14}C *atomic ratio*
Del tomatoes				
Phytoene	132090	12870	10·26	14:8
β-Carotene	14710	1960	7·50	10·46(±0·24):8
Lycopene	32030	4490	7·13	9·94(±0·24):8
Maize seedlings				
MVA	329155	23638	13·92	2:1
α-Carotene	1737	212	8·21	9·45:8
β-Carotene	41175	4841	8·51	9·79:8
Physalis alkekengi				
Phytoene	31058	3256	9·54	14:8
β-Carotene	21105	2965	7·12	10·2:8

arising from C-5 of MVA and one arising from C-2 MVA are eliminated (equation 5). Experiments with [2-^{14}C-(5*R*)-5-$^{3}H_1$] MVA and [2-^{14}C-5-$^{3}H_2$] MVA have shown that in tomatoes, maize leaves and *Physalis alkekengi* it is the *pro-R* hydrogen which is eliminated during the formation of the four double bonds in converting phytoene into lycopene or the cyclic carotenes (*Tables 7* and *8*)[27,30].

Attempts to define the stereospecificity of the loss of the hydrogens from the other carbon involved in double bonds formed in the phytoene→lycopene transformation have not been particularly successful. These hydrogens originate from C-2 of mevalonic acid and with [2-^{14}C-(2*R*)-2-$^{3}H_1$] MVA and [2-^{14}C-(2*S*)-2-$^{3}H_1$] MVA considerable scrambling of the tritium label

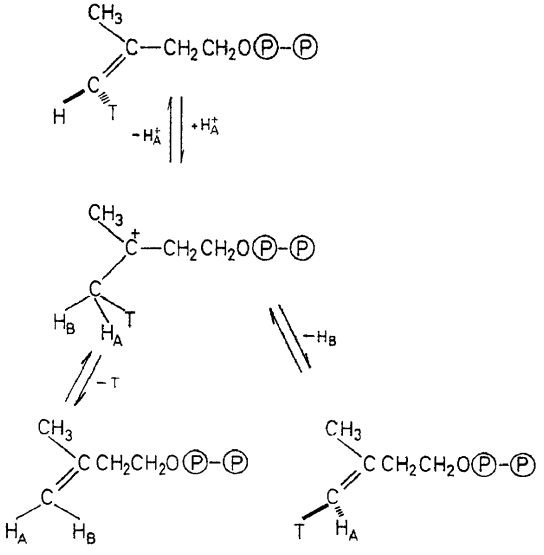


Figure 5. Probable reactions which lead to randomization of the label from [(2*R*)-$^{3}H_1$] MVA and [(2*S*)-$^{3}H_1$] MVA in carotenoids.

takes place. This appears to be due mainly to the isomerase step IPP⇋ DMAPP being freely reversible[15,23] and the enzyme prenyl transferase, which catalyses the reaction DMAPP+IPP→GPP, being insufficiently active to prevent scrambling taking place as indicated in *Figure 5*. Thus starting with [2*R*-$^3H_1$] MVA, both [2S-$^3H_1$] MVA and unlabelled MVA can result. This phenomenon also occurred in our studies on sterol biosynthesis in algae and fungi[2,24] but it is not apparent in liver experiments[1,10].

In preliminary experiments with the fungus *Blakeslea trispora* in which the incubation period has been reduced to 30 minutes, it appears that the 2-*pro*-*R* hydrogens may be eliminated[11] but the problem is by no means settled.

Formation of retro-carotenoids

The Californian poppy, *Eschscholtzia californica* produces the unique retro-carotenoid eschscholtzanthin[25]. Experiments with [2-^{14}C-(4*R*)-4-$^3H_1$] MVA indicated that the tritium content of eschscholtzxanthin was the same as that of β-carotene and allowed a mechanism (*Figure 6*) for the formation of this pigment from antheraxanthin to be proposed[28].

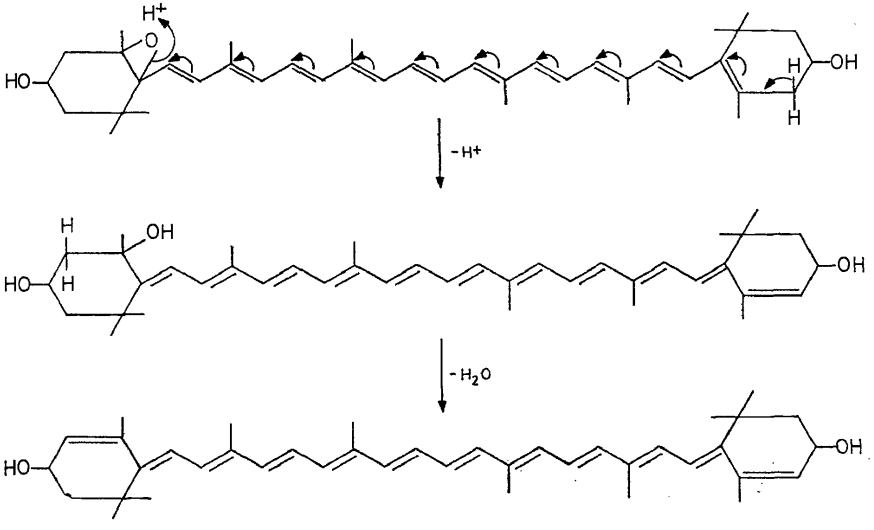


Figure 6. Proposed mechanism for the formation of eschscholtzxanthin from antheraxanthin.

Formation of xanthophylls

There is considerable evidence that the insertion of oxygen into the xanthophylls occurs late in the biosynthetic sequence (e.g. see [19]). All that was known about the formation of major xanthophylls of higher plants was that the oxygen of the hydroxyl groups arises from O_2 and not from the oxygen of water[31,32]. The stereochemistry of hydrogen removal at C-3 and 3′ of lutein and zeaxanthin and C-3 of cryptoxanthin has now been studied. C-3 and 3′ arise from C-5 of MVA, so that with [2-^{14}C-5-$^3H_2$] MVA as substrate either one or two hydrogens should be lost from these

Table 9. Incorporation of [2-^{14}C-(5*R*)-5-$^{3}H_1$] MVA and [2-^{14}C,5-$^{3}H_2$] MVA into carotenes and xanthophylls of maize seedlings and fruit of *Physalis alkekengi*.[30]

Compound	[2-^{14}C(5*R*)-5-$^{3}H_1$] MVA				[2-^{14}C-5-$^{3}H_2$] MVA			
	^{3}H (d.p.m.)	^{14}C (d.p.m.)	$^{3}H/^{14}C$ *ratio*	$^{3}H/^{14}C$ *atomic ratio*	^{3}H (d.p.m.)	^{14}C (d.p.m.)	$^{3}H/^{14}C$ *ratio*	$^{3}H/^{14}C$ *atomic ratio*
Maize seedlings								
MVA	36402	5173	11·47	1:0·98	329155	23638	13·92	2·04:1
α-Carotene	485	85	5·70	3·9:8	1737	212	8·21	9·76:8
β-Carotene	22126	3780	5·85	4·05:8	41175	4841	8·51	10·00:8
Lutein	9130	3197	2·86	1·95:8	6304	917	6·88	8·09:8
Lutein diacetate	83	28	2·92	2·00:8	12535	1859	6·74	7·93:8
Zeaxanthin	584	532	1·10	0·75:8	1196	192	6·22	7·31:8
Zeaxanthin diacetate	95	128	0·74	0·51:8	1276	208	6·14	7·22:8
Physalis alkekengi								
Phytoene	37918	5466	6·94	8·02:8	31058	3256	9·54	13·40:8
β-Carotene	18779	5442	3·45	4·00:8	21105	2965	7·12	10·00:8
β-Cryptoxanthin	2314	855	2·70	3·13:8	27321	4120	6·63	9·32:8
β-Cryptoxanthin acetate	2435	944	2·58	2·98:8	11435	1754	6·52	9·16:8
Zeaxanthin	244	1144	0·22	0·26:8	21890	4033	5·43	7·63:8
Zeaxanthin diacetate	810	3499	0·23	0·27:8	15953	2919	5·46	7·68:8

positions on hydroxylation according to whether or not a keto-compound is an intermediate. If only one hydrogen is lost then studies with [2-^{14}C-(5*R*)-5-$^{3}H_1$] MVA should indicate whether or not the loss of hydrogen was stereospecific and, if so, in what sense. *Table 9* shows that with maize seedlings the ratio with [2-^{14}C-(5*R*)-5-$^{3}H_1$] MVA falls from 8:4 in β-carotene to 8:2 in lutein, whilst corresponding ratios with [2-^{14}C-5-$^{3}H_2$] MVA fall from 8:10 to 8:8. This means that a keto intermediate is not involved and that during the hydroxylation the *pro-R* hydrogen is stereospecifically removed.

As indicated previously, the situation with zeaxanthin is more complex. The experiments with [2-^{14}C-5-$^{3}H_2$] MVA indicate, in agreement with results with lutein, that only two additional atoms are lost during hydroxylation; but with [2-^{14}C-(5*R*)-$^{3}H_1$] MVA as substrate, four atoms are lost compared with β-carotene. The provisional conclusion is that in contrast to lutein and the carotenes zeaxanthin arises from a phytoene which has been formed by elimination of two *pro-S* hydrogens (from C-5 of MVA) at the centre of the molecule. These experiments were repeated on fruit of *Physalis alkekengi*, which is much more effective at synthesizing zeaxanthin. The same results were obtained (*Table 9*), however in β-cryptoxanthin (3-hydroxy-β-carotene) the situation is the same as in the carotenes. Three further conclusions can be made from this series of experiments: (i) if hydroxylation takes place with no change in configuration of the retained hydrogen atom, as happens in sterol hydroxylations, then the absolute configuration around C-3 in zeaxanthin and lutein which emerges from these biochemical studies coincides with the chemical deductions of Weedon[27A] (*Figure 7*); (ii) as the carotenes contain more tritium atoms than the xanthophylls there is no possibility in the systems examined that the

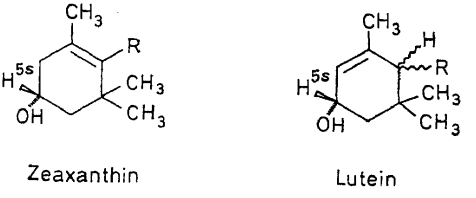


Figure 7. The absolute configuration around C-3 in zeaxanthin and C-3′ in lutein as indicated by biosynthetic experiments.

carotenes arise from the xanthophylls to any significant extent as was recently suggested[9,21] (iii) if our explanation of the unexpected labelling pattern in zeaxanthin is correct, then it possibly means that zeaxanthin cannot arise from β-carotene, via β-cryptoxanthin.

CONCLUSION

The use of stereospecifically labelled MVA's in studies on carotenoid biosynthesis in various tissues has solved a number of problems which would have otherwise been insoluble. Many problems remain and a combination of the use of stereospecific substrates, purified enzyme systems and reliable micro-degradation techniques should result in further progress in the near future.

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