

# SOME RECENT STUDIES ON CAROTENOIDS AND RELATED COMPOUNDS

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## ABSTRACT

Studies on models synthesized by stereochemically controlled routes show that the central triene chromophore in the common *cis* isomer of phytoene has the *trans,cis,trans* configuration, and that the phytoene from *Flavobacterium dehydrogenans* consists mainly of the all-*trans* isomer.

Capsorubin, with the natural configuration at all four chiral centres, has been synthesized from (+)-camphor.

Several routes have been developed for the synthesis of ( $\pm$ )-azafrin.

Mytiloxanthin and isomytiloxanthin, two pigments from *Mytilus edulis*, have been identified as novel acetylenic carotenoids.

## INTRODUCTION

Carotenoids continue to present the chemist with a variety of intriguing problems. In this lecture I shall review progress with some of the problems of structure and synthesis which have of late attracted the attention of the group at Queen Mary College.

## STEREOCHEMISTRY OF PHYTOENE

Phytoene (I) has been detected in many carotenogenic systems, and is generally recognized as the first  $C_{40}$ -conjugated polyene in the biosynthesis of carotenoids<sup>1a</sup>. The main isomer isolated from tomato paste and carrot oil was assigned a central-*cis* configuration on the basis of its inability to form a thiourea adduct, and the observation of an i.r. absorption band near  $760\text{ cm}^{-1}$ , attributed to C—H out-of-plane deformation of the disubstituted *cis* double bond<sup>2</sup>. Our early n.m.r. studies, using phytoene from carrot oil and *Chlorella vulgaris* (mutant G 77) showed that the four unconjugated, non-terminal, trisubstituted double bonds all have the *trans* configuration<sup>3</sup>. Further n.m.r. studies on phytoene and various simple models have now shown that the two remaining double bonds, those at C-13 and C-13', must also have the *trans* configuration<sup>4</sup>.




Identical 100 and 220 MHz n.m.r. spectra were obtained for phytoene from carrot oil, and for samples from *Phycomyces blakesleeanus*, *Rhodospirillum rubrum* (diphenylamine inhibited culture), and tomatoes, which were





A more general route to phytoene models is outlined in *Scheme 2*, and has been applied to the three series indicated<sup>4</sup>. All four isomers were separated in the C<sub>14</sub>-series (R = n-Pr) but the resolution of the pairs of isomers was not achieved in either of the C<sub>20</sub>-series. Nevertheless a comparison of the n.m.r. bands due to the methyl groups at the end of the triene chromophore (*Table 1*) allows two important conclusions to be drawn. First the n.m.r.

*Table 1.* N.m.r. bands ( $\delta$ ) due to the methyl groups at the end of the central triene chromophore

R	RCMe—CH·CH=CH·CH—CMeR			
	<i>t, t, t</i>	<i>t, c, t</i>	<i>t, t, c</i>	<i>t, c, c</i>
	1.72, 1.72	1.72, 1.72	1.72, 1.76	1.72, 1.80
	1.72, 1.72	1.72, 1.72	1.72, 1.76*	1.72, 1.80*
	1.74, 1.74	1.74, 1.74	1.74, 1.76*	1.74, 1.81*
phytoene	1.73, 1.73	1.74, 1.74		

\* From n.m.r. of mixture of isomers

band due to a methyl group attached to a *trans* double bond at the end of the triene unit is independent of the stereochemistry of the rest of the molecule. Second, that a methyl attached to a terminal *cis* bond has a band further downfield, 0.02–0.04 ppm if the neighbouring conjugated double bond is *trans*, and 0.07–0.08 ppm if it is also *cis*. Comparison of the n.m.r. and i.r. spectra of the common natural *cis*-phytoene with those of the models shows that the central triene unit must be *trans,cis,trans*. This is further substantiated by comparison of the coupling constants for the olefinic protons with those calculated for the *trans,cis,trans* and all-*trans* C<sub>14</sub>-models (*Table 2*).

*Table 2.* Coupling constants of olefinic protons in conjugated trienes

	RCMe—CH <sub>B</sub> ·CH <sub>A</sub> —CH <sub>A</sub> ·CH <sub>B</sub> —CMeR				
	$\nu_0 \delta_{AB}$ (Hz)	$J_{AB}$	$J_{AB'}$	$J_{AA'}$	$J_{BB'}$
Phytoene	50.2	12.0	-1.3	10.6	-0.3
<i>t,c,t</i> -model (R = Pr)	48.1	11.5	-1.4	10.8	-0.2
<i>t,t,t</i> -model (R = Pr)	94.2	11.0	-1.1	14.6	0.0

The geometrical configuration of the common *cis* isomer of phytoene is therefore fully represented by (I). (Herber, Granger, Maudinas and Villoutreix have arrived at the same conclusion by 250 MHz and <sup>13</sup>C n.m.r. studies<sup>6</sup>.) It is also evident that the main phytoene isomer which has been isolated by Professor Weeks and his collaborators<sup>7,8</sup> from diphenylamine inhibited cultures of *Flavobacterium dehydrogenans* is the all-*trans* isomer (II)<sup>4</sup>. Small amounts of what may well prove to be the same central-*trans* isomer have also been detected by Dr Davies and his collaborators as minor components of the phytoene fraction from several natural sources<sup>9</sup>. The relevance, if any,

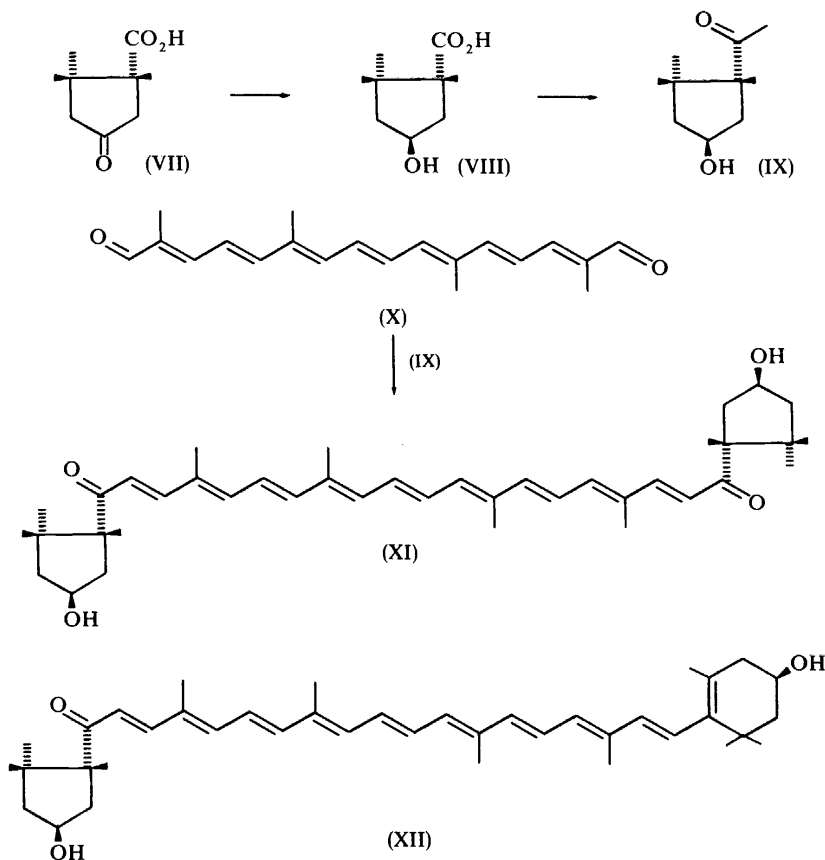
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of these configurational differences to the biosynthesis of carotenoids must await further investigation.

Comparison of the n.m.r. data for the phytoenes and the simple models also reveals that the methyl groups at the end of the triene chromophore are slightly deshielded (c. 0.02 ppm) by the unconjugated double bonds three positions further along the chain. This effect is accompanied by a small, but readily detected, bathochromic shift of 2–3 nm in the u.v. light absorption maxima<sup>4</sup>. Both results are probably due to the influence of the unconjugated double bond in those conformations which bring it close to a terminal double bond of the triene unit.

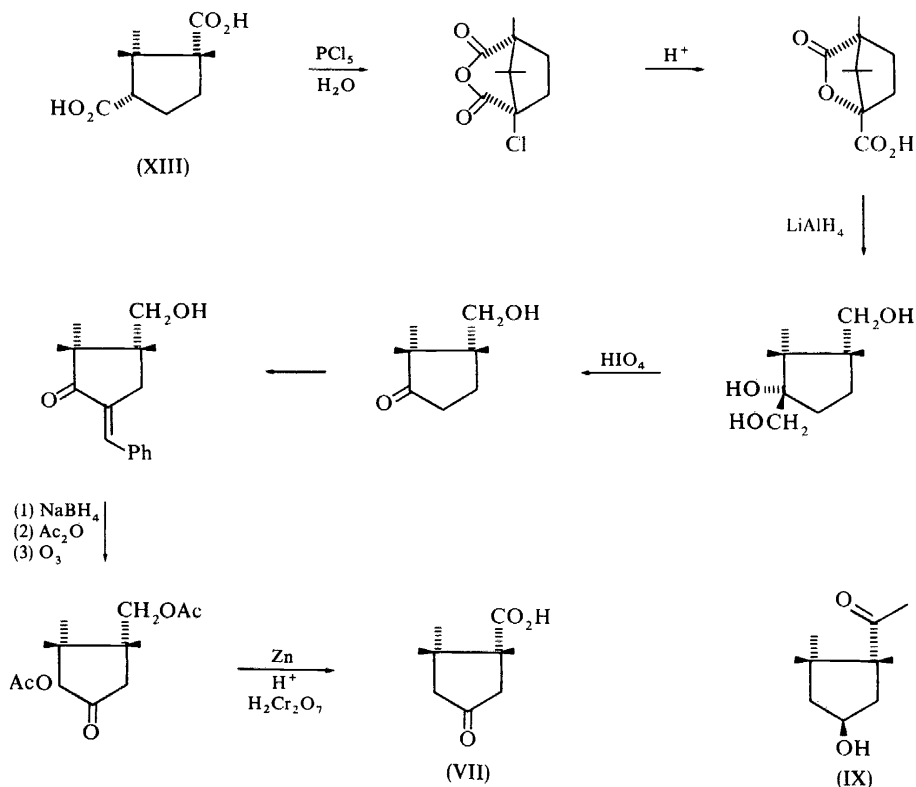
### THE CAPSORUBIN END-GROUP

Studies at Zürich and in our own laboratories have shown that capsorubin has the 3*S*,5*R*,3'*S*,5'*R*-configuration (XI)<sup>10</sup>. We have previously reported the synthesis (Scheme 3) of an optically inactive form of capsorubin by



Scheme 3.

condensation of crocetinial (X) with the racemic *trans*-hydroxy-ketone (IX) derived from the keto-acid (VII) via the hydroxy-acid (VIII)<sup>11</sup>. With the aim of synthesizing capsorubin with the correct optical configuration at all four chiral centres, we have been interested in devising good routes to the 5*R*-isomer of (VII). This enantiomer was first reported by Marquet *et al.*<sup>12</sup> who obtained it in *c.* 0.03 per cent overall yield from (+)-camphor. By an alternative route, also starting from (+)-camphor, we synthesized the same compound in 0.4 per cent overall yield and converted it into the 3*S*,5*R*-hydroxy-acid (VIII) which was identical with a sample prepared by degradation of capsanthin (XII)<sup>1b</sup>.



Scheme 4.

Of the other routes we have examined, the best at the present time is that outlined in *Scheme 4*. This starts with camphoric acid (XIII), a readily available oxidation product of (+)-camphor. Though lengthy it gives the required optically active intermediate (VII) in *c.* 14 per cent overall yield. Further improvements and simplifications are no doubt possible, but this route has now enabled us to complete the synthesis of 'natural' capsorubin.

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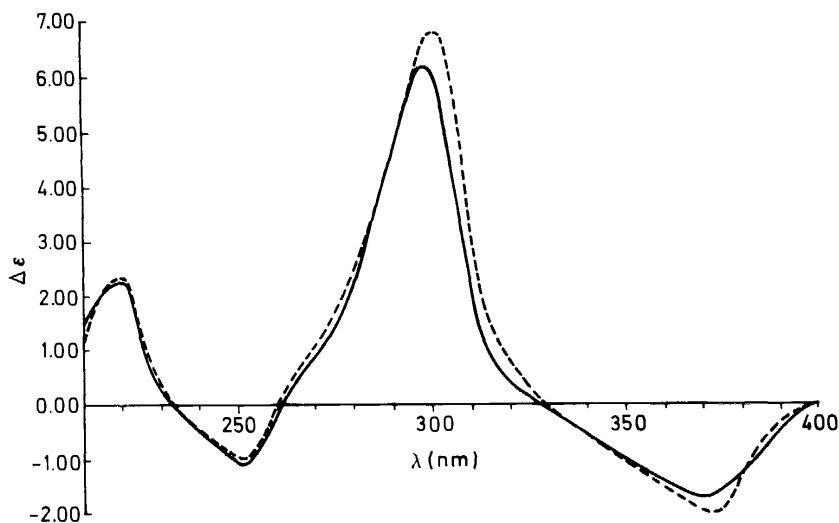


Figure 1. C.d. curve of capsorubin, natural (---) and synthetic (—)

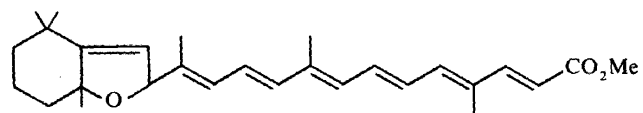
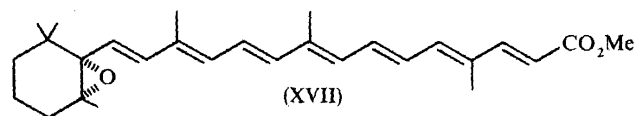
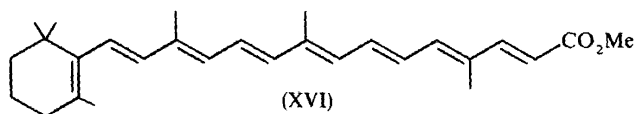
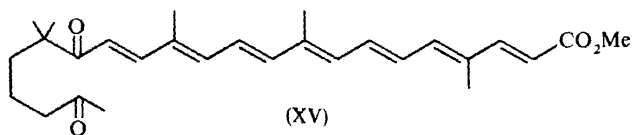
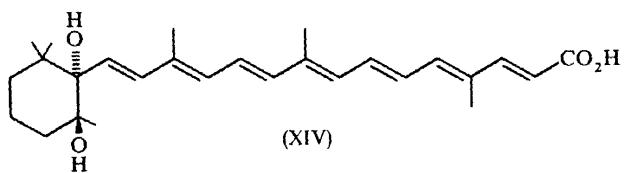
The product is identical with the pigment from *Capsicum annum*. In particular the c.d. properties of the two pigments agree within experimental error (Figure 1).

### SYNTHESIS OF ( $\pm$ )-AZAFRIN

Azafrin (XIV), the major pigment in the roots of *Escobedia scabrifolia*, occupies an important place in the history of carotenoid chemistry. Its degradation by the Kuhn school played a major part in elucidating the structure of carotenoids<sup>13</sup>.

Evidence for the unusual  $\alpha$ -glycol grouping was obtained by oxidative fission of azafrin methyl ester to give the diketone (XV)<sup>14</sup>. Much later we prepared this diketone by chromic acid oxidation of the 10'-apo- $\beta$ -carotenoic acid (XVI), thus confirming the main structural features of azafrin<sup>15</sup>. Moreover, failing to observe in the i.r. spectrum of azafrin methyl ester, any evidence of intramolecular hydrogen bonding between the two hydroxyl groups, we suggested that azafrin has a *threo* structure as shown in (XIV)<sup>15</sup>. Müller and Karrer came to the same conclusion<sup>16</sup>.

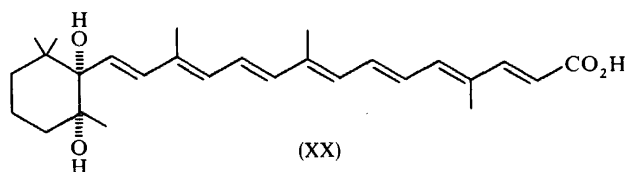
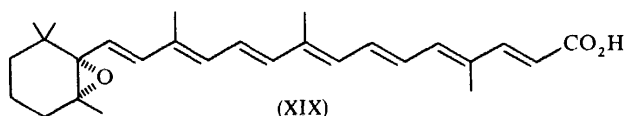
Our attempts to obtain a closely related  $\alpha$ -glycol by acid treatment of the 5,6-epoxide of methyl 15,15'-dehydro-10'-apo- $\beta$ -carotenoate gave only the corresponding furanoid oxide<sup>17</sup>. Recently Schwieter has observed that acid treatment of the corresponding polyene (XVII) gave, in addition to the furanoid oxide (XVIII), small amounts of a glycol ester. Moreover that the epoxy-acid (XIX) gave only the glycol acid (XX)<sup>18</sup>. However, n.m.r. studies clearly showed that in neither synthetic glycol was the relative configuration of the two hydroxyls the same as that of the hydroxyls in azafrin<sup>19</sup>. Since the glycols from epoxides are normally those to be expected from *trans* opening



Scheme 5.



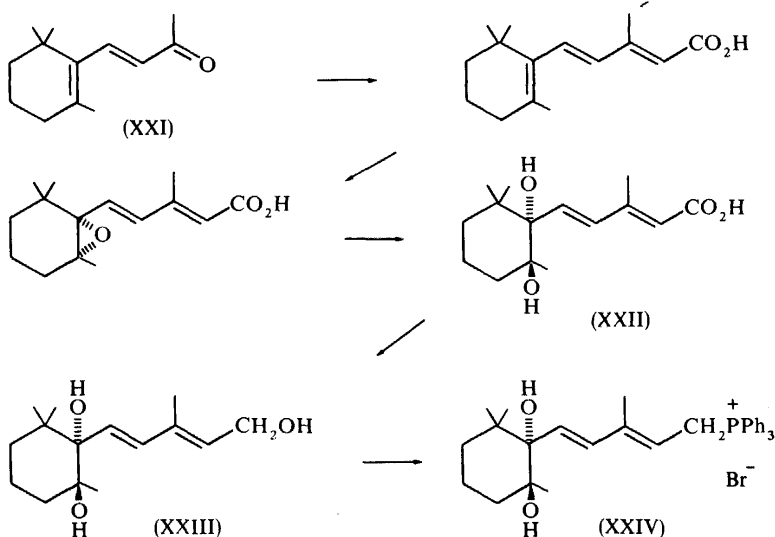
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Ring methyl protons ( $\delta$ )

Me ester	0.89	1.09	1.20
Me azafrin	0.84	1.14	1.19

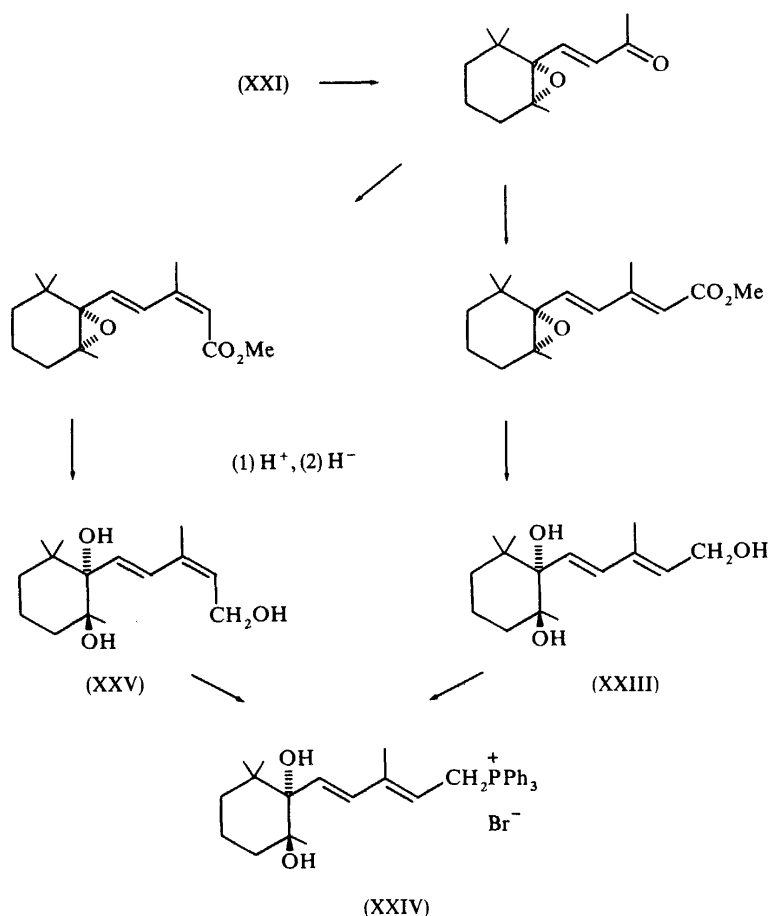
Scheme 6.



Scheme 7.

of the ring, these results obviously cast doubt on the *threo* configuration assigned to azafrin.

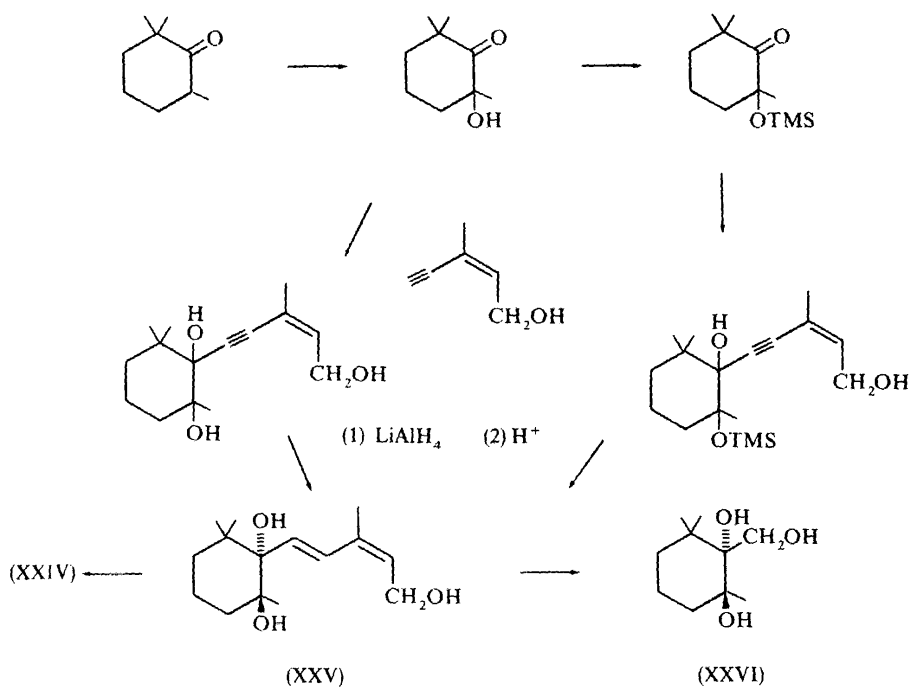
We have now developed the three routes which are outlined in Schemes 7, 8 and 9 to the Wittig salt (XXIII), and used this intermediate to synthesize ( $\pm$ )-azafrin methyl ester (XXVII) as indicated in Scheme 10. In all cases the n.m.r. spectrum of the product showed that the two hydroxyls had the same relative configuration as those in natural azafrin. All these routes to azafrin



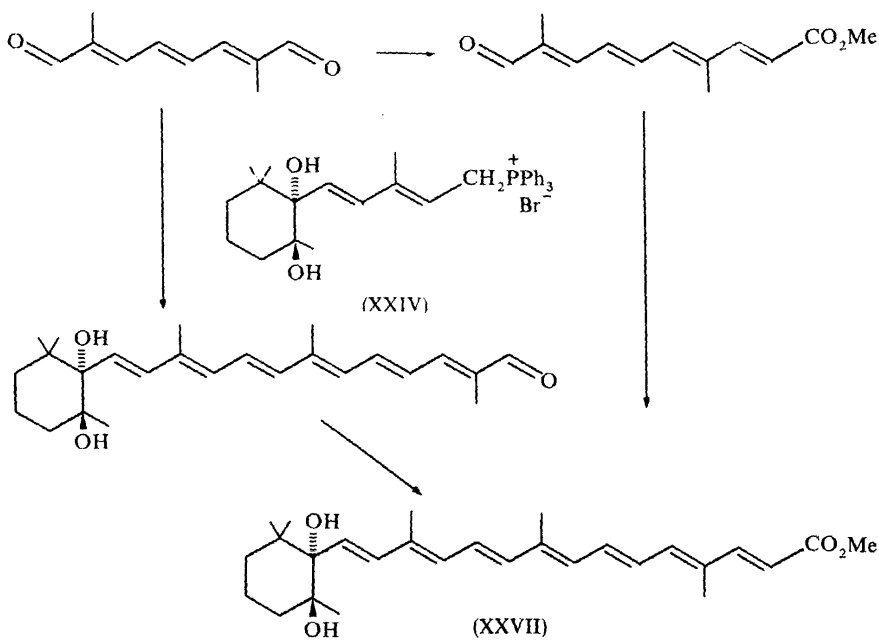
Scheme 8.

methyl ester would be expected to give the *threo* product. However, in view of the results obtained by Schwieter, further confirmation is required. Ozonolysis of the intermediate triol (XXV), and reduction of the resulting ozonide with sodium borohydride, gave a cyclic borate which was hydrolysed to give the crystalline triol (XXVI). This product has previously been obtained in another way by Schwieter *et al.*<sup>20</sup> and was shown to have the *threo* configuration by x-ray crystallography. However, this does not provide unequivocal evidence for the configuration of the triol (XXV), and hence of azafrin, since it is conceivable that an inversion may have occurred during hydrolysis of the cyclic borate. Attempts have therefore been made to settle the question by x-ray crystallography. Satisfactory crystals could not be obtained of either natural azafrin methyl ester or its synthetic racemate. The glycol acid (XXII) gave excellent crystals, but these unfortunately proved to be twinned and therefore unsuitable for our purpose. However, promising progress is now being made with the derived triol (XXIII)<sup>21</sup>.

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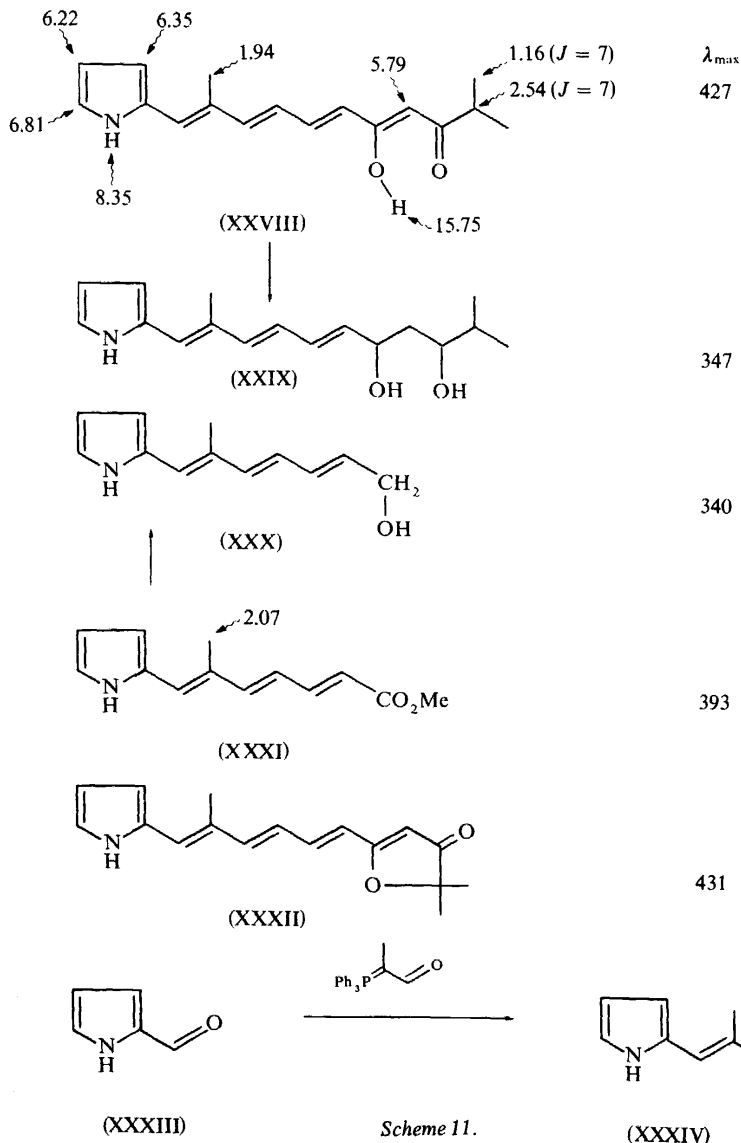
Scheme 9.



Scheme 10.

### PIGMENTS OF *WALLEMIA SEBI*

Compared with the pigments of fungi generally, those of the fungi imperfecti have received little study. Our attention was drawn by Dr G. Valadon (Royal Holloway College) to a bright orange organism of this type, *Wallemia sebi*, which he had obtained from a beehive. We have now identified six pigments in this source. The main ones, A and C, are accompanied by their labile *cis* isomers, B and D respectively, and by their chloro derivatives, E and F respectively. These are not carotenoids, but representatives of a new class of pyrrolic pigments (*Scheme 11*).



Scheme 11.

On the basis of spectroscopic and chemical studies we have formulated A as (XXVIII) and C as its dehydro derivative (XXXII). The main uncertainty in these structures concerns the geometrical configuration about the methylated double bond. This appears to be the opposite of that in the model series of compounds (e.g. XXXI) which we synthesized from pyrrole aldehyde (XXXIII) via (XXXIV), but we do not yet know whether it is the natural or the synthetic series which has a *cis* configuration about the double bond adjacent to the pyrrole ring. However, there seems no doubt that wallcmia A is an enolic  $\beta$ -diketone. Its strongly hydrogen bonded carbonyl group gives rise to an i.r. band at  $1580\text{ cm}^{-1}$ , and, for reasons which will become apparent later, I should like to draw attention to the n.m.r. signals at  $\delta$  15.75 and 5.79 which are due to the enolic proton and the  $C_{\alpha}$ -proton respectively.

### PIGMENTS OF MYTILUS EDULIS

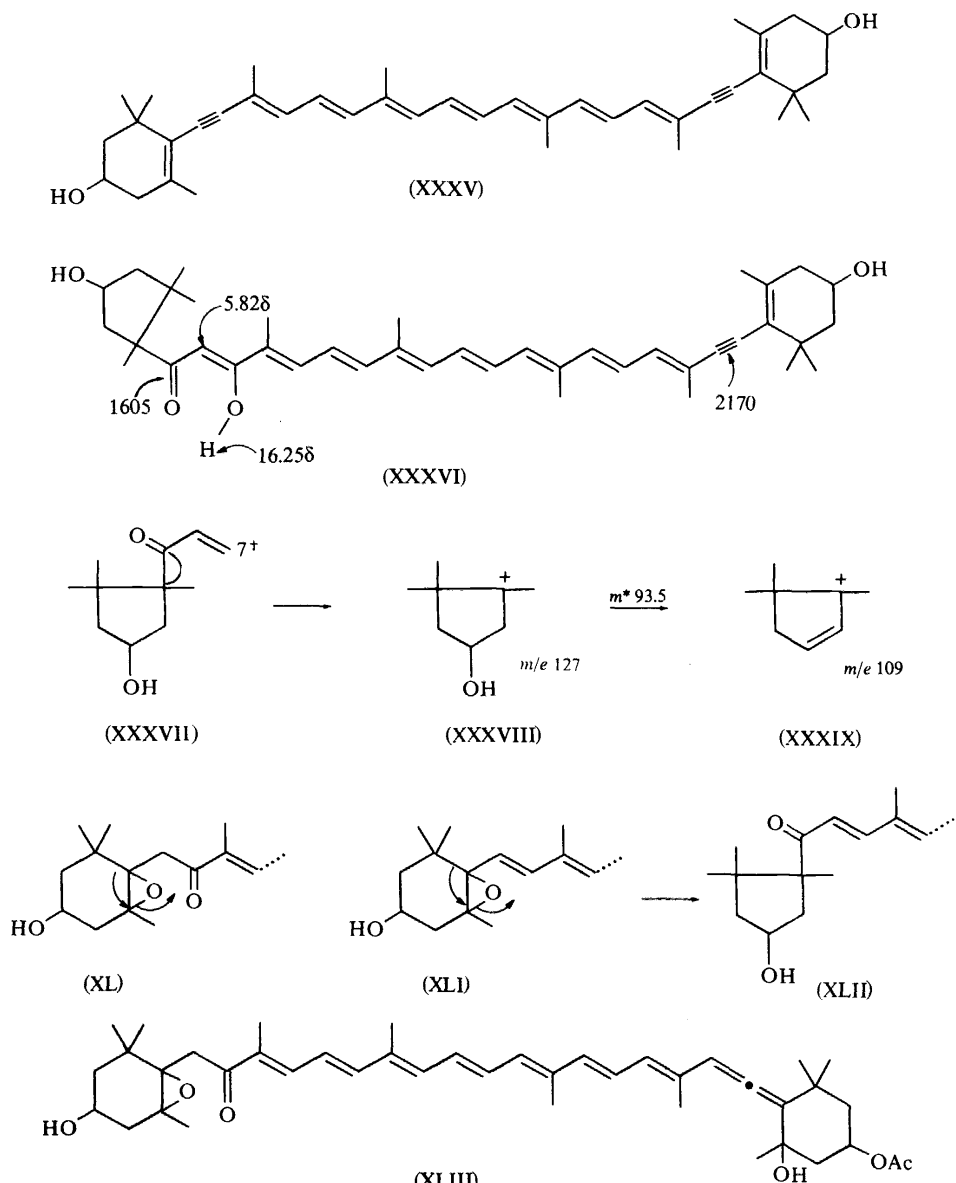
We have previously shown that one of the pigments in the edible mussel, *Mytilus edulis*, is the diacetylene alloxanthin (XXXV)<sup>22</sup>. We have now extended these studies to the other pigments present since Sheer<sup>23</sup> reported that the related species, *M. californianus*, contains a novel acidic carotenoid, mytiloxanthin, and it seemed to us that this might provide a further example of the acetylenic group of carotenoids.

Mytiloxanthin proved to be rather elusive. The amounts present in *M. edulis* varied from very small to negligible, depending on the season of the year. However, from 3000 mussels collected near Southend during July and August (1971) were able to isolate *c.* 14 mg of a pigment with a melting point and visible light absorption properties ( $\lambda_{\text{max}}$  496 nm in  $\text{CS}_2$ ) which were in good agreement with those reported by Sheer for mytiloxanthin. It was shown by mass spectrometry to have the formula  $\text{C}_{40}\text{H}_{54}\text{O}_4$ , and acetylation gave a diacetate,  $\text{C}_{44}\text{H}_{58}\text{O}_6$ . Both exhibited a weak i.r. band near  $2170\text{ cm}^{-1}$ , attributable to an acetylene stretching frequency. Their n.m.r. spectra included bands which were very similar to those of the methyls in the end-groups of alloxanthin and alloxanthin diacetate respectively. We therefore concluded that mytiloxanthin is an unsymmetrical carotenoid in which one end-group is the same as those in alloxanthin (XXXV).

There is a marked hypsochromic shift (25 nm) in the light absorption properties of solutions of mytiloxanthin in alcohol on the addition of alkali. Both mytiloxanthin and its diacetate are tenaciously adsorbed on alumina. Such 'acidic' properties are the result of an enolic  $\beta$ -diketone system similar to that present in wallcmia A. Thus mytiloxanthin has an i.r. band at  $1605\text{ cm}^{-1}$  attributable to a hydrogen bonded carbonyl group, and bands at  $\delta$  16.25 and 5.82 in the n.m.r. spectrum due to the enolic proton and the  $C_{\alpha}$ -proton respectively. Treatment of mytiloxanthin with sodium borohydride reduces the enolic  $\beta$ -diketone system to give a product with light absorption properties in good agreement with those predicted for a 7,8-acetylenic analogue of 8'-apo- $\beta$ -carotenol<sup>24</sup>.

The n.m.r. spectrum of mytiloxanthin also contains three bands (each equivalent to 3H) due to the methyl groups in the 'enolic end-group'. Their positions ( $\delta$  0.85, 1.19 and 1.34) are very similar to those of the methyl groups ( $\delta$  0.84, 1.20, and 1.37) in the end-groups of capsorubin (XI). Moreover, the

base peak in the mass spectrum is  $m/e$  109, and this is related by a metastable peak to a fragment ion  $m/e$  127. These results indicate a fragmentation of the type (XXXVII)→(XXXVIII)→(XXXIX), exactly analogous to those observed with capsorubin (XI) and capsanthin (XII)<sup>25</sup>. We therefore propose structure (XXXVI) for mytiloxanthin. It is conceivable that the novel enolic



Scheme 12.

end-group arises from a pinacollic rearrangement of an epoxide end-group (XL) of the type present in fucoxanthin (XLIII), comparable to that suggested for the conversion of the end-groups (XLI) of violaxanthin into those (XLII) of capsorubin (XI)<sup>1a</sup>.

During the isolation of mytiloxanthin we encountered an isomeric carotenoid which we were unable to crystallize though it was chromatographically homogeneous. This 'isomytiloxanthin' ( $\lambda_{\max}$  504 and 476 nm in CS<sub>2</sub>) has i.r. and n.m.r. spectra which indicate that it too is an unsymmetrical carotenoid with one end-group the same as those in alloxanthin (XXXV). Acetylation gives a monoacetate, though the mass spectrum of this derivative indicates the presence of a further (presumably tertiary) hydroxyl group. The i.r.

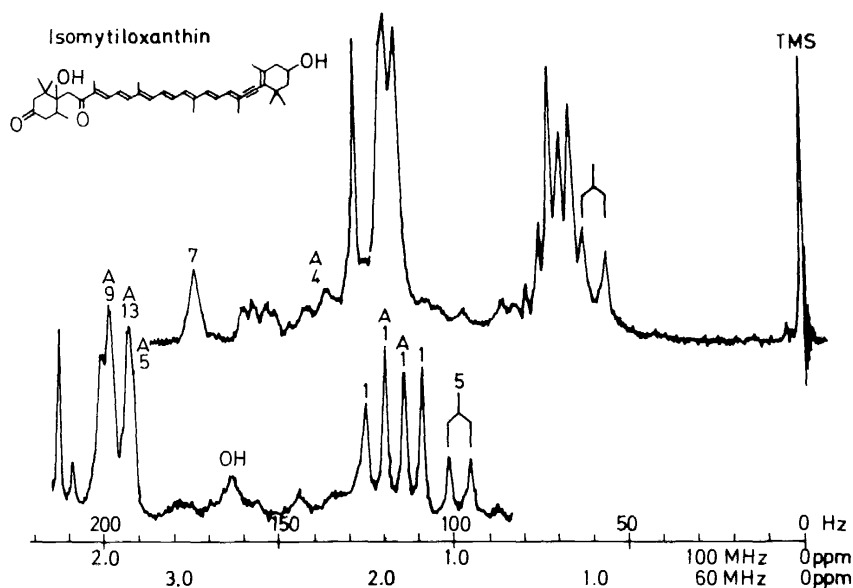


Figure 2. N.m.r. spectra of isomytiloxanthin (in CDCl<sub>3</sub>) at 60 MHz (top curve) and 100 MHz (bottom curve). Assignments of bands are indicated; the bands corresponding to those in alloxanthin are marked A

spectrum of isomytiloxanthin includes bands at 1640 and 1710 cm<sup>-1</sup> indicating the presence of both conjugated and unconjugated keto groups. Reduction with sodium borohydride gives the expected tetrahydro derivative with the same visible light absorption properties as those of the reduction product from mytiloxanthin.

The n.m.r. spectrum of isomytiloxanthin includes three bands (each 3H) attributable to methyl groups in the novel end-group; two are singlets ( $\delta$  1.09 and 1.26) whilst the third ( $\delta$  0.98) is a doublet ( $J$  6.5 Hz) indicating spin-spin coupling of the methyl protons with a single proton on the adjacent carbon atom (Figure 2).

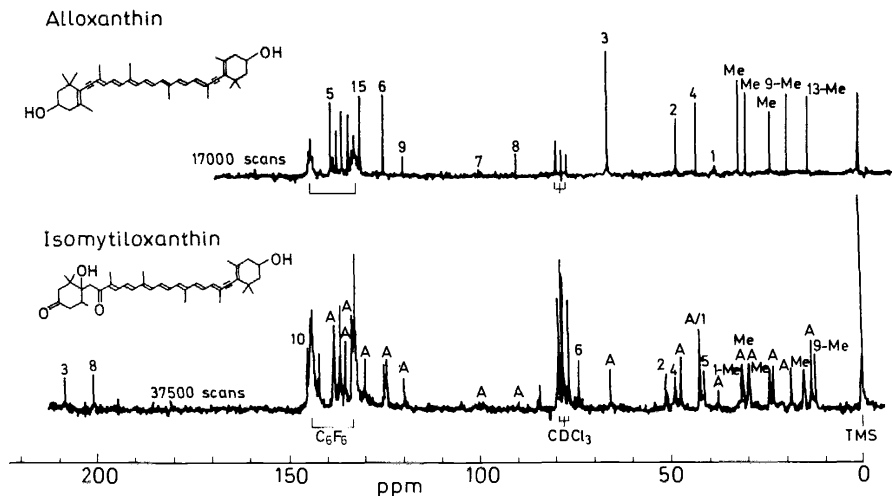
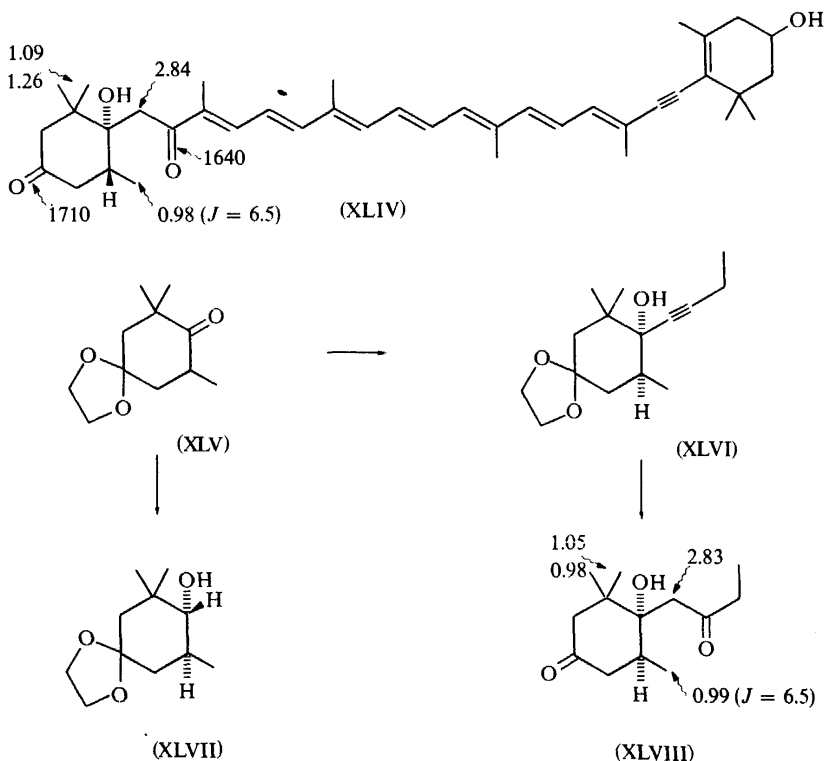


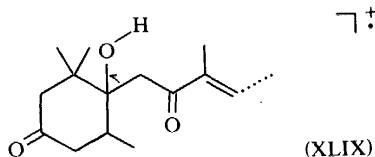
Figure 3.  $^{13}\text{C}$  n.m.r. spectra of alloxanthin (top curve) and isomytiloxanthin (bottom curve). Tentative assignments of lines are indicated; lines in the isomytiloxanthin spectrum which are common to the alloxanthin spectrum are marked A



Scheme 13.



The information summarized above is consistent with formula (XLIV) for isomytiloxanthin. Further support comes from the  $^{13}\text{C}$  n.m.r. spectrum (Figure 3) and also from the mass spectra of isomytiloxanthin and its acetate; both contain a strong line  $m/e$  155 ( $\text{C}_9\text{H}_{15}\text{O}_2$ ) which is attributable to the fragmentation (XLIX).



Ions at  $m/e$  444 and 486 respectively are ascribed to the same fragmentation, with hydrogen transfer, and charge retention on the polyene moiety. There are also ions ( $m/e$  169 and, respectively, 429 and 471) due to the expected cleavage of the single bonds adjacent to the conjugated carbonyl group.

A model (XLVIII) of the novel end-group in isomytiloxanthin has been prepared from (XLV) by the route indicated in Scheme 13. Its mass spectrum has a line at  $m/e$  155 due to a fragmentation similar to that in (XLIX). Its n.m.r. spectrum is in general agreement with corresponding features in the n.m.r. spectrum of isomytiloxanthin. However, the significant difference in the shielding of one of the geminal methyl groups suggests that the relative configuration at C-5 and C-6† in the model is the opposite of that of the corresponding positions in isomytiloxanthin. Since attack of (XLV) by lithium aluminium hydride gives an alcohol in which the C-5 and C-6† protons are *trans* diaxial to one another ( $J = 10$  Hz), it is highly probable that the model end-group has the configuration shown in (XLVIII). It would then follow that the (relative) configuration in isomytiloxanthin is that given in (XLIV). It is thus conceivable that the novel end-group in isomytiloxanthin is formed from an end-group of the type (XL) by a process which involves hydride attack at C-5 on the epoxide, and oxidation of the hydroxyl at C-3.

### ACKNOWLEDGEMENTS

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