

## PHYSICO-CHEMICAL AND SYNTHETIC STUDIES ON CAROTENOIDS

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**Abstract** - A study of the C<sub>30</sub> analogue of  $\beta,\beta$ -carotene at 360 MHz <sup>1</sup>H-n.m.r. and 90.5 MHz <sup>13</sup>C-n.m.r. confirms all of the signal assignments. Staphyloxanthin is identified as a 6-O-ester of  $\alpha$ -D-glucopyranosyl 8'-apo- $\psi$ -carotenoate.

Introduction of an extra methyl group at C-11 (and C-11') is shown to give a 'hindered' 11-trans double bond which is still hindered as the corresponding cis isomer.

The structure of the natural carotenoid, prolycopene is shown to be 7,9,7',9'-tetracis- $\psi,\psi$ -carotene by n.m.r. spectroscopy. The position of the two 'hindered' cis double bonds is confirmed by synthesis.

Two new approaches to the synthesis of astaxanthin have been studied. Preparation of the di-O-methyl ether of astacene is described but after reduction the product gives a complex mixture of compounds when treated with acid. Electrochemical reduction of astacene in the presence of acetic anhydride gives astaxanthin tetra-acetate which is hydrolysed to astaxanthin.

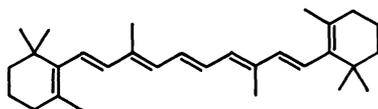
The conformation of fucoxanthin in the solid state has been examined by X-ray crystallography.

### INTRODUCTION

The study of physico-chemical methods may be undertaken either for an intrinsic interest in the techniques or as a adjunct to other studies - such as structure determination, as a check on synthetic intermediates, or as method of studying interactions between molecules. In this lecture I will mainly concentrate on the application of these techniques to carotenoid chemistry.

### N.M.R. SPECTROSCOPY

In my Berne lecture (Ref. 1) I was able to summarise <sup>13</sup>C-n.m.r. data on fifteen carotenoid end groups and several others have been recorded since (Ref. 2). A major problem with this technique is the rather large samples required, although modern instruments using super conducting magnets have reduced the quantity required. With <sup>1</sup>H-n.m.r. spectroscopy spin-spin coupling provides a valuable method of relating one signal with another. In <sup>13</sup>C-n.m.r. the coupling between <sup>13</sup>C-<sup>1</sup>H is normally removed by irradiation of the <sup>1</sup>H region. Using single frequency double resonance unless the appropriate <sup>1</sup>H signal is irradiated a residual one bond <sup>13</sup>C-<sup>1</sup>H coupling is observed. The magnitude of this reduced coupling constant is proportional to the difference in frequency between the <sup>1</sup>H signal and the second irradiating frequency (Ref. 3). This technique was used with the C<sub>30</sub> analogue of  $\beta,\beta$ -carotene to check the assignments of the <sup>13</sup>C-n.m.r. signals. These correlations confirm previous work on  $\beta,\beta$ -carotene and are a useful confirmation of the assignment of C-2 and C-4 (Fig. 1).



(A)

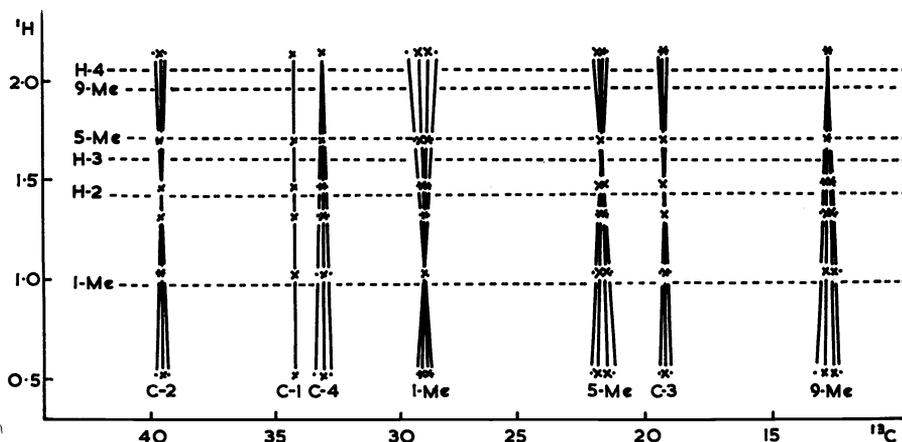
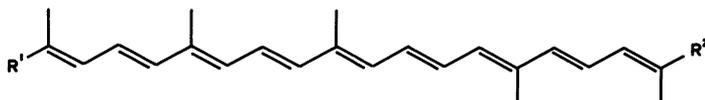


Fig. 1. A plot of the  $^{13}\text{C}$ -n.m.r. signals of  $\text{C}_{30}$   $\beta,\beta$ -carotene (A) versus the  $^1\text{H}$  frequency. Spectra run at 90.5 MHz in  $\text{CDCl}_3$ . Cross = strong signal, dot = weak signal, dashed line =  $^1\text{H}$ -n.m.r. signals (cf. B. Birdsall, N.J.M. Birdsall and J. Feeney, *J.C.S. Chem. Comm.* 316 (1972))

#### STRUCTURE OF STAPHYLOXANTHIN

One of the major uses of n.m.r. spectroscopy in carotenoid chemistry is for structure determination. The advances which permitted routine application of  $^{13}\text{C}$ -n.m.r. have also benefited  $^1\text{H}$ -n.m.r. Fourier-transform techniques permit the measurement of spectra with much smaller samples. As an example we have examined a sample of staphyloxanthin kindly provided by Professor J.H. Marshall (Monash University) (Ref. 4). The  $^1\text{H}$ -n.m.r. on less than 1 mg showed the presence of an acyclic carotenoid end group, and a number of methyl signals at  $\delta$  0.8. Mass spectrometry of an acetylation product showed it was a triacetate ( $\text{C}_{57}\text{H}_{84}\text{O}_{11}$ ) with significant daughter ions at  $m/e$  513 ( $\text{C}_{27}\text{H}_{45}\text{O}_9$ ) and 225 ( $\text{C}_{15}\text{H}_{29}\text{O}$ ). The  $^1\text{H}$ -n.m.r. spectrum of this product showed that the three acetoxy groups were equatorial and from the anomeric proton at  $\delta$  5.75 ( $J=8$  Hz) it was concluded that it was a 6-O-ester of  $\alpha$ -D-glucopyranosyl apo- $\psi$ -carotenoate. The 6-O-ester is probably a 12-methyltetradecanoate since this is the major branched chain fatty acid in *Staphylococcus aureus* (Ref. 21).



- (1)  $\text{R}^1 = \text{Me}_2\text{C}=\text{CHCH}_2\text{CH}_2-$ ,  $\text{R}^2 = -\text{CO}_2\text{Glu}$  (3)  $\text{R}^1 = \text{Me}_2\text{C}=\text{CHCH}_2\text{CH}_2-$ ,  $\text{R}^2 = \text{CH}_2\text{OH}$   
 (2)  $\text{R}^1 = -\text{CO}_2\text{Glu}$ ,  $\text{R}^2 = \text{Me}_2\text{C}=\text{CHCH}_2\text{CH}_2-$  (4)  $\text{R}^1 = \text{CH}_2\text{OH}$ ,  $\text{R}^2 = \text{Me}_2\text{C}=\text{CHCH}_2\text{CH}_2-$

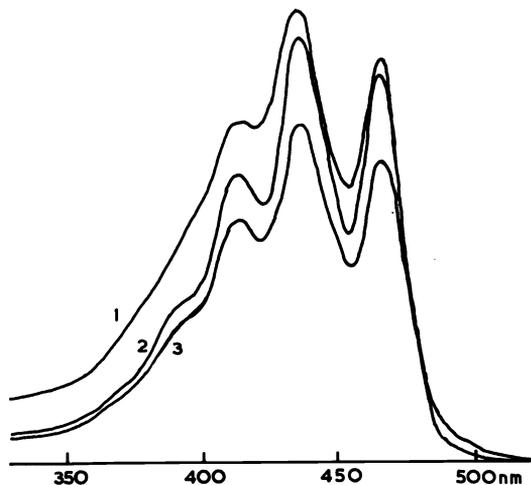
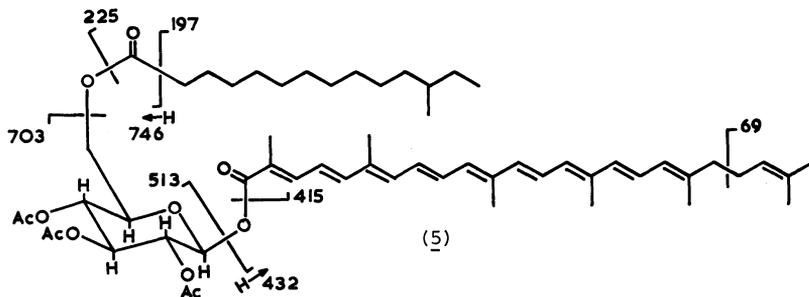


Fig. 2. Electronic spectra of 1. (4), 2. reduced staphyloxanthin, 3. (3).

Two structures are possible for the carotenoidal part - either 8'-apo- $\psi$ -carotenoate (1) or 7',8'-dihydro-4,4'-diapo- $\psi,\psi$ -carotenoate (2). The corresponding primary alcohol (3) and (4) were prepared and shown to have practically identical spectroscopic properties. This is not surprising as they are isomers where the hydroxyl group at one end is interchanged with the isoprenoid unit at the other end. Three differences were noted - slight changes in the relative abundances of the ions at  $m/e$  418, 402, 326 and 312; changes in the shape but not position of the electronic spectrum (Fig. 2); and small differences in the resonant Raman spectrum. When compared with the alcohol produced from staphyloxanthin the spectra corresponded to 8'-apo- $\psi$ -caroten-8'-ol. Thus the structure of staphyloxanthin is suggested as  $\alpha$ -D-glucopyranosyl 1-O-(8'-apo- $\psi$ -carotenoate) 6-O-(12-methyltetradecanoate) (5).



HINDERED CIS AND TRANS DOUBLE BONDS

Pauling (Ref. 5) pointed out that 11-cis double bonds cannot be planar. The interaction between 10-hydrogen and the 13-methyl group results in a 'hindered' cis double bond (Fig. 3). It is perhaps significant that in the crystal structure of 11-cis retinal (Ref. 6) the molecule adopts a s-cis conformation about the 12-13 bond so that the interaction is between the 10-hydrogen and the 14-hydrogen atom. When a methyl group is introduced at C-11 of  $\beta,\beta$ -carotene a similar type of interaction gives two 'hindered' trans double bonds with the 11-methyl group clashing with both the 9-methyl and 13-methyl groups. However either a 9-cis or 11-cis isomer still has a 'hindered' trans double bond and also a 'hindered' cis double bond.

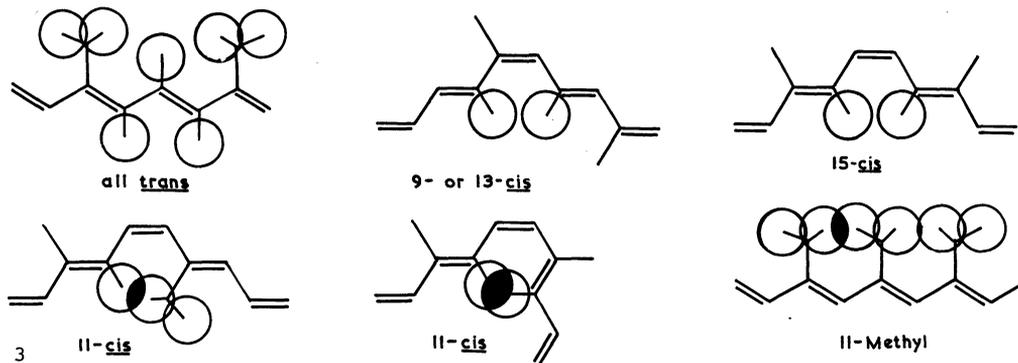
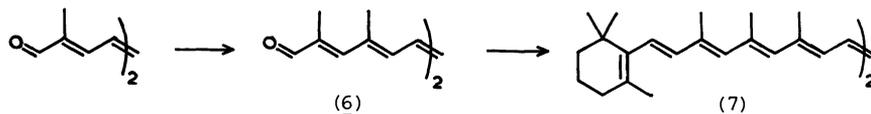


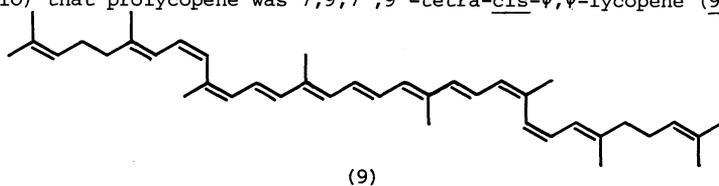
Fig. 3

11,11'-Dimethyl- $\beta,\beta$ -carotene (7) was synthesised by conventional means via the dialdehyde (6). Although the electronic spectrum of (6) is very similar to the corresponding dialdehyde without methyl groups at 11 and 11' the end product (7) had a broad single absorption maximum at 390 nm, 59 nm less than all trans- $\beta,\beta$ -carotene. This difference is similar to that observed with 11,11'-dicis- $\beta,\beta$ -carotene (Ref. 7).



STRUCTURE OF PROLYCOPENE

Prolycopene was first isolated by Zechmeister in 1941 (Ref. 8) from tangerine tomatoes. It was shown to be a cis isomer of lycopene with an absorption maximum at 35 nm shorter wavelength than the corresponding all trans isomer. The number and position of the cis double bonds were not ascertained. We showed that it was a symmetric molecule by  $^1\text{H-n.m.r.}$  spectroscopy at 100 MHz (Ref. 9) but it was only when it was examined by Dr. G. Englert (Hoffmann-la Roche) at 270 (Fig. 4) and eventually at 360 MHz that it was possible to make a complete assignment of all of the n.m.r. signals by means of appropriate decoupling experiments. The spin-spin coupling constants show that the 15(15')- and 11(12)- double bonds are trans and that the 7(8)- double bond is 'hindered' cis. The  $^{13}\text{C-n.m.r.}$  spectrum showed that the 5(6)- and 13(14)- double bonds were trans but that the 9(10)- double bond was cis. Hence it was concluded (Ref. 10) that prolycopene was 7,9,7',9'-tetra-cis- $\psi,\psi$ -lycopene (9).



(9)

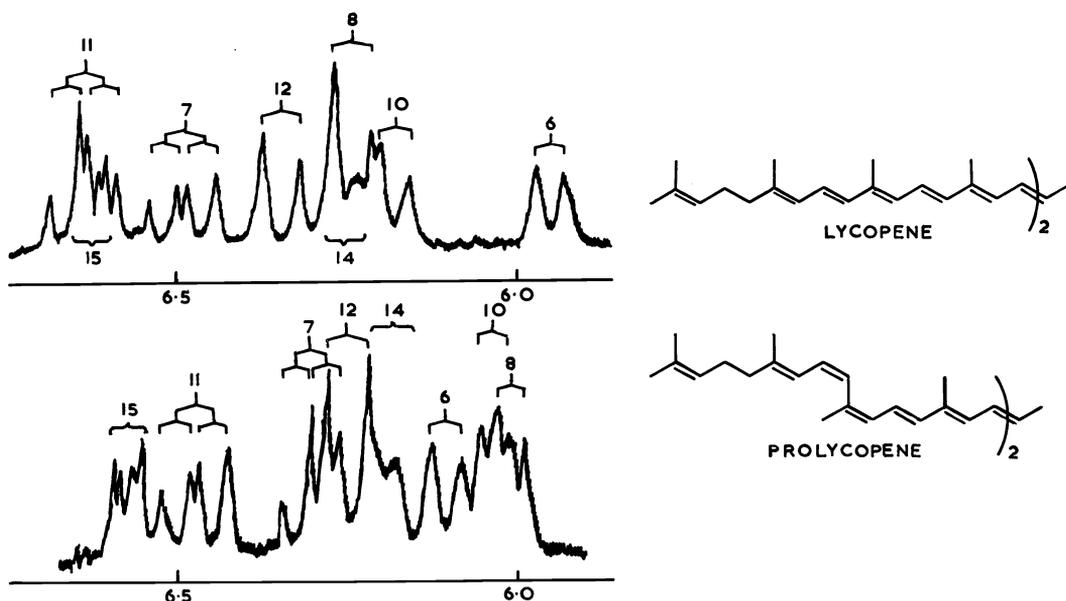
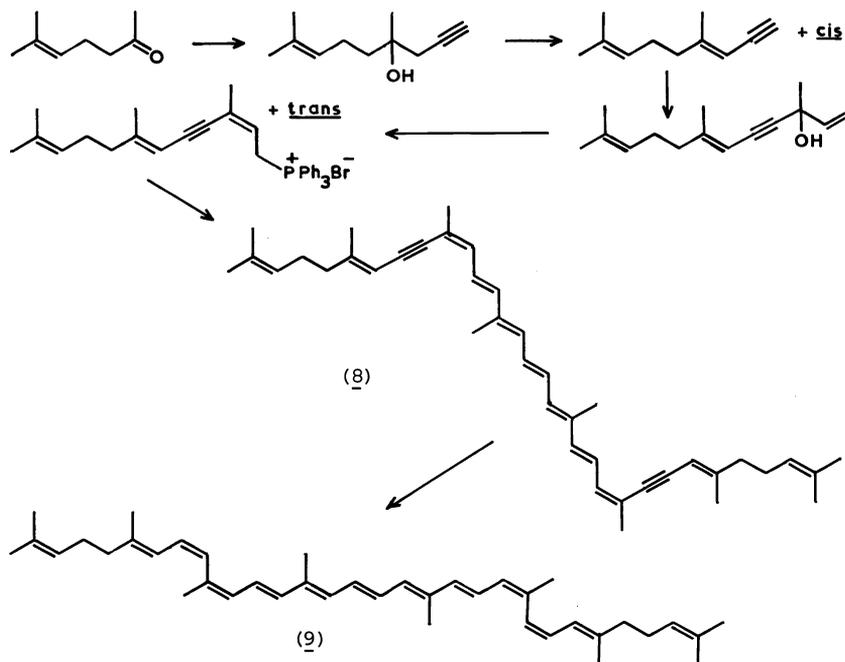


Fig. 4  $^1\text{H}$ -n.m.r. spectra (vinyl region)

#### SYNTHESIS OF PROLYCOPENE

The synthesis of prolycopene was used as a final confirmation of the structure deduced by n.m.r. spectroscopy. Fortunately the symmetric skeleton simplifies the problem, and the 'hindered' *cis* double bond is readily produced by partial reduction of an acetylene. Thus the first objective was 7,8,7',8'-tetrahydro- $\psi,\psi$ -carotene (8). This molecule has an added bonus in that with these two acetylene groups the most stable isomer should have the required 9,9'-*dicis* stereochemistry (cf. alloxanthin Ref. 11). We have developed the route



outlined in Scheme 1. In a trial synthesis without any stereochemical control the product (8) was shown by h.p.l.c. (Fig. 5) to consist of at least fifteen isomers (theoretical maximum 272). However with some stereochemical control the product showed a single broad main peak on h.p.l.c. and a minor pair of peaks. On stereomutation the broad peak becomes sharper and the minor pair of peaks are better resolved (Fig. 6). Partial reduction of this sample gave a mixture of isomers with retention times on h.p.l.c. including that corresponding

to polyycopene. The electronic spectrum of the mixture resembled polyycopene demonstrating that two 'hindered' *cis* double bonds at 7 and 7' are the main reason for the 35 nm difference from all *trans* lycopene. Mass spectrometry confirmed that the required reduction had occurred.

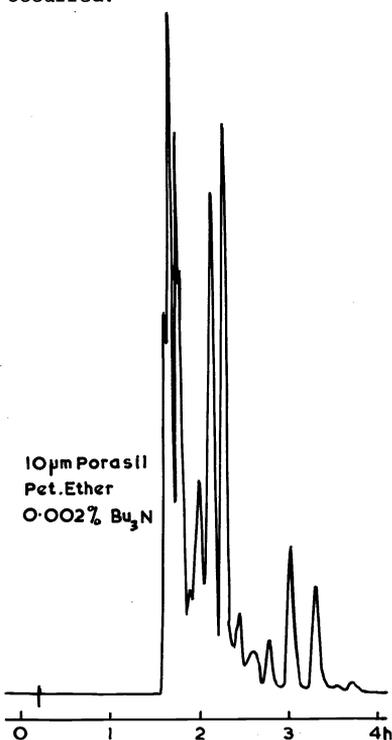


Fig. 5 H.p.l.c. of isomers of (8)

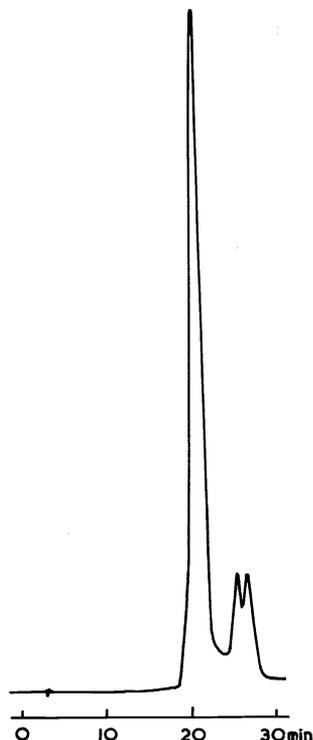


Fig. 6 H.p.l.c. after stereomutation

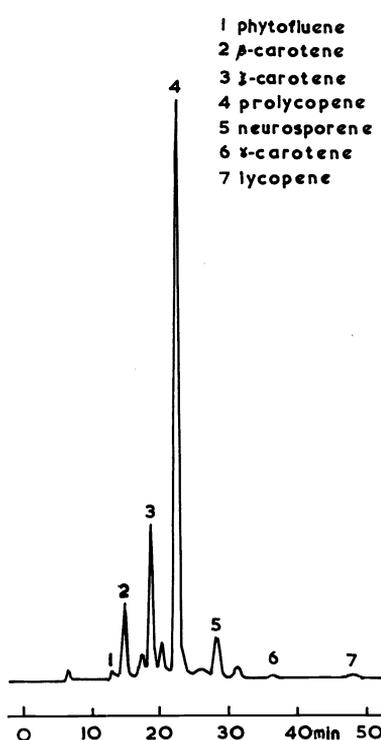


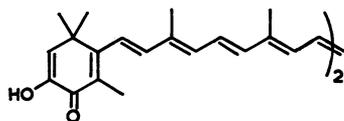
Fig. 7 H.p.l.c. of tomato 'Tangella' extract

H.p.l.c. of an extract from the tomato 'Tangella' showed that the major carotenoid present was polyycopene (Fig. 7). Only traces of all *trans* lycopene were detected and, based on the electronic spectra, the precursors phytofluene, ζ-carotene and neurosporene, as well as the cyclised metabolites, β- and γ-carotene, were present.

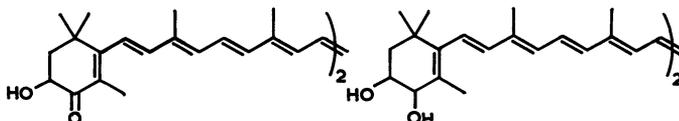
#### ASTACENE ENOL ETHERS

It is normally considered that astacene (10) cannot be methylated (Ref. 12). However a distinction must be made between the case where no reaction occurs, and when the product is so unstable that it rapidly reforms starting material. We have prepared astacene dimethyl ether (13) (as well as its benzyl and allyl analogues) in up to 85% yield. When the reaction is examined on SiO<sub>2</sub> t.l.c. plates only astacene is detected, but the reaction can be followed on Al<sub>2</sub>O<sub>3</sub> t.l.c. plates. As expected the product is quite unstable and is very rapidly hydrolysed by acid - possibly autocatalysed by the astacene formed. An even more unstable compound is astacene di(trimethylsilyl) ether. It was hydrolysed even on Al<sub>2</sub>O<sub>3</sub> t.l.c. plates and could only be characterised by its melting point, i.r. and mass spectra.

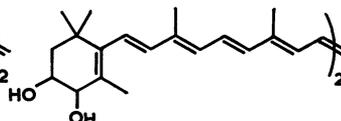
The synthesis of astacene enol ethers opens up a new route to astaxanthin (11) (Ref. 13). Most routes rely on the selective oxidation of crustaxanthin (12); where overoxidation gives astacene. A comparable procedure is the selective reduction of astacene but this is not possible using most reagents (but see below). However reduction of astacene methyl ether with sodium borohydride gives the required oxidation level. Unfortunately treatment of the product (14) with acid only gave a complex mixture of products from which astaxanthin could not be isolated. This is reminiscent of the reaction of isozeaxanthin with acid (Ref. 14).



(10)



(11)

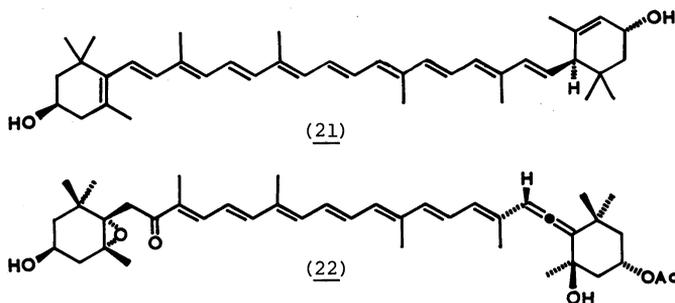


(12)



## X-RAY CRYSTALLOGRAPHY OF CAROTENOIDS

It is often considered that X-ray crystallography has made conventional structure determination methods obsolete. However it is of note that in the carotenoid field apart from the simplest symmetric molecules the only successful crystallographic studies published have required chemical modification of the natural pigment, for example degradation (Ref. 17) or a heavy atom derivative (Ref. 18). The cause of the problem is a combination of several different effects - obtaining suitable crystals, solving the diffraction pattern by direct methods, and pseudo symmetry problems. For example crystals of lutein (21) were studied but it only proved possible to identify the polyene chain. It became apparent that the two different end groups were sufficiently similar to allow the molecule to pack in the crystal randomly either way round. Both orientations had the polyene chain at approximately the same position in the unit cell, but the two end groups were inextricably mixed together in the region at each end of the chain.



The problem of the absolute stereochemistry of fucoxanthin was recently solved by a number of chemical correlations (Ref. 19). Attempts to solve this problem by crystallography failed when the crystal dissolved in the adhesive used to mount it in the capillary. When eventually another suitable crystal was obtained it was barely large enough and gave a poor diffraction pattern indicative of poor crystallinity. It is of note that the crystal was quite stable to X-rays, at least when sealed under nitrogen in the capillary. Poor crystallinity due to defects or poor packing are common in full carotenoids e.g.  $\beta$ , $\beta$ -carotene (Ref. 20). The crystal contains two independent molecules in the unit cell - ninety six carbon or oxygen atoms. With limited data, no heavy atoms and one of the most difficult space groups ( $P_1$ ) the problem is at the limit of feasibility. Dr. G.M. Sheldrick (Cambridge University) with difficulty partially solved the structure to show the two polyene chains. Further refinement was extremely slow, and only possible with constraints on the chain to limit movement. So far the structure has reached  $R=25\%$  with all data included. All of the atoms can just about be recognised although there is some disorder. As expected the conformation of the allenic end group was similar to previous studies (Ref. 17) and the ketone is *s-trans* to the polyene with the epoxide ring oriented away from the main chain (Fig. 8).

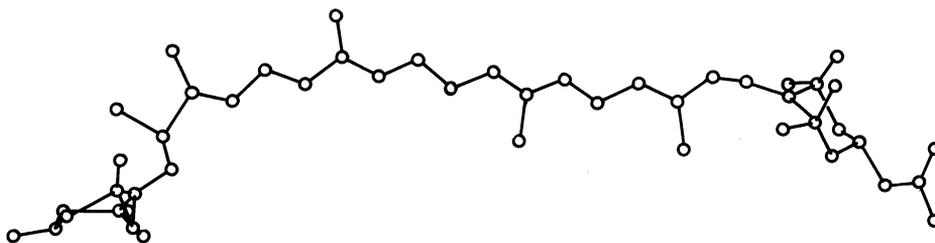


Fig. 8 X-ray crystallographic structure of fucoxanthin (22)

**Acknowledgements** - In conclusion I would like to thank my colleagues associated with the work I have described. In particular I should mention the help and encouragement of Dr. B.C.L. Weedon, and the collaboration with Dr. J.H.P. Utley (Electrochemistry) and Dr. M.B. Hursthouse (X-ray crystallography). The principal contributors to topics I have chosen to discuss are Dr. G.E. Hawkes (n.m.r.), Dr. P.R. Ellis and Dr. J. Wenger (staphyloxanthin and astacene methyl ether), Dr. M. Baranyai ('hindered' *trans* double bond), Dr. S.M. Ali, Dr. M. Hadorn and Mrs. C.A. Robson (prolycopene), Dr. E.A.H. Hall and Dr. V.L. Pardini (electrochemistry), and Dr. T.E. de Ville and Mr. S.C. Naithani (X-ray crystallography). I am also most grateful to Roche Products Ltd. (Welwyn Garden City) and to F. Hoffmann-La Roche and Co. Ltd. (Basle) for their continued support.

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