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CAROTENOID RESEARCH — PAST, PRESENT AND FUTURE

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Abstract — Various features of carotenoid research over the last 50 years are discussed, and attention is drawn to some areas where further significant advances are to be expected in the future.

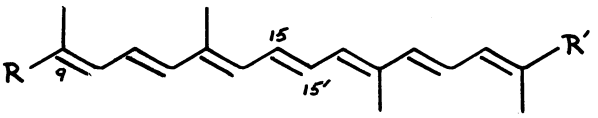
INTRODUCTION

The carotenoids constitute the most widespread class of natural pigments. They are found throughout the plant kingdom, though their presence is often masked by chlorophyll. They are responsible for many of the brilliant yellow and red colours in flowers and fruit. They are found in bacteria, algae, insects, birds and other animals. They occur as the free carotenoid, sometimes as glycosides, more often esterified with one or more long chain fatty acids, and, at least in invertebrates, in stoichiometric combination with proteins as carotenoproteins. Over the years it has become recognised that β-carotene (1), and some related carotenoids, serve as provitamins A in mammals, including man, and evidence has been growing that they fulfil many other important functions, for example in photosynthesis. It is scarcely surprising, therefore, that they have been the subject of serious and continuous study for over half a century, and this meeting in Madison is a good time to take stock of what has been achieved and to speculate on the course of future developments.

SEPARATION

Over 400 carotenoids are now known, and the number is growing year by year as natural materials are screened by the sensitive techniques now available. Although Tswett observed carotenoids in his classical studies on chromatography, his work passed almost unnoticed until the technique was rediscovered by Kuhn and his collaborators specifically to handle these pigments. Thus the Heidelberg school was able to show that "carotene", which had been known as a crystalline substance for about a hundred years, was in fact a mixture of three closely related isomers. Though column chromatography is still widely used for the separation of carotenoids, few people today seem to remember the care that needs to be taken in selecting the adsorbent, and in packing the column in order to achieve the best results. The reason why the 6'S stereoisomer of (6'R-) fucoxanthin (2), differing in configuration about the allene group, and possibly of considerable biosynthetic importance, was not observed until recently was that the stereochemical set from this, the most abundant natural carotenoid, had not previously been examined on a well-packed column of calcium carbonate (1). The same system also permits the separation of the four furanoid oxides (3) derived from lutein (4), and differing only in the configurations at C-5 and C-8, separations which provided the means of determining the absolute configuration of many of the naturally occurring carotenoid epoxides (2).

Thin layer chromatography, introduced by Stahl, received its first major publicity in the carotenoid field, and is now an invaluable aid for monitoring all steps in an isolation or series of reactions. Again, much care must be devoted to the preparation of the plates, and to the selection of the solvent system, if the best results are to be achieved. Only after much trial and error was it possible (3) to separate the three stereoisomers of crustaxanthin (5), a point of some importance in confirming the absolute configuration assigned (4) to astaxanthin (6). Incidentally, this separation can now be achieved comparatively easily by incorporating boric



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6	h	h	25	r	r
7	f	i	26	k	n
9	i	i	27	s	s
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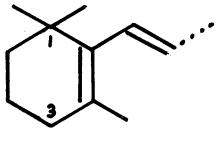

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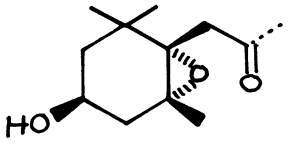

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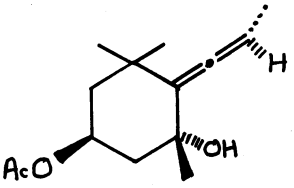

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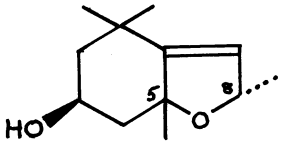

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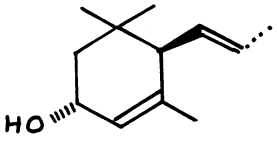

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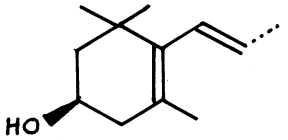

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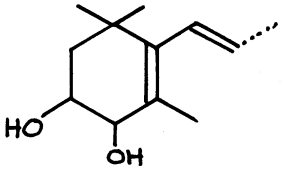

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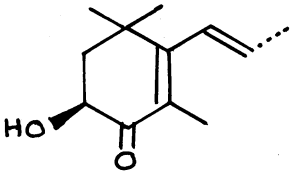

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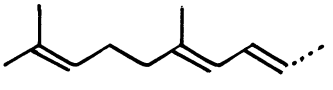
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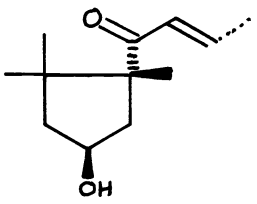

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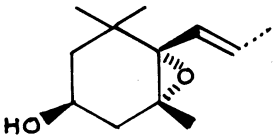

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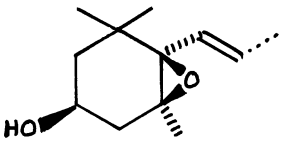

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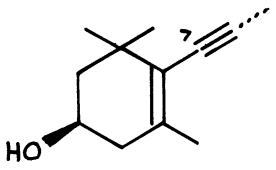

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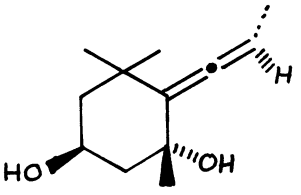

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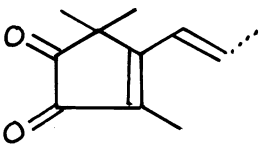

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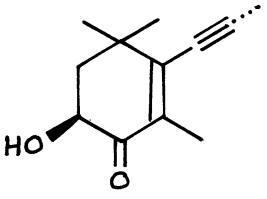

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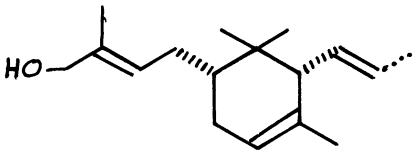

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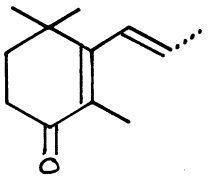

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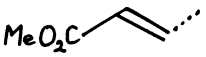

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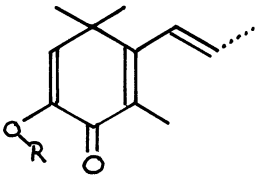

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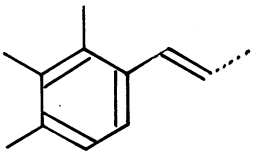

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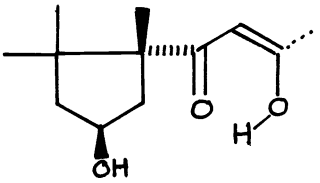

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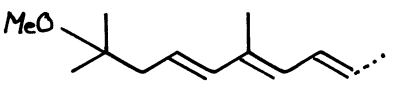

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acid into the stationary phase, which then selectively retards compounds with the threo 3,4-dihydroxy end groups (5).

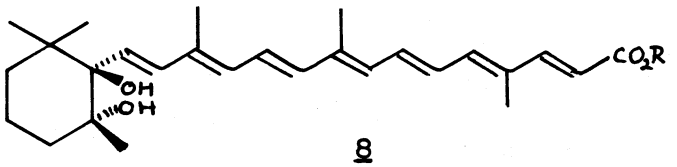
Another major advance has come with the introduction of high performance, or high pressure, liquid chromatography (h.p.l.c.) into the study of carotenoids. Separations can now be readily achieved which would have been impossible, or extremely tedious, before. Thus, rubixanthin (7) can be readily separated (5) from its 5'-cis isomer, gazaniaxanthin, though this presents serious difficulties by other chromatographic techniques (6) despite Schön's early success (7). (Later in this meeting, Dr. Moss will be giving further examples of the separation of geometrical isomers.) Moreover, h.p.l.c. uses very small quantities of material, though the purified products can be collected and are normally adequate for spectroscopic examination and study by the new generation of n.m.r. spectrometers becoming available. Since it is now known (8) that the stereochemical purity of the sample examined is of great significance in the application of many spectroscopic techniques (o.r.d., c.d., ^{1}H and ^{13}C n.m.r.), h.p.l.c. is likely to play an increasingly important rôle in future structural studies.

CHEMICAL EXAMINATION

Though widespread, most carotenoids occur naturally in low concentration, and there are few that can be isolated in sufficient quantities for full structural studies by classical chemical means. Early investigations were, therefore, concentrated on the carotenoids present in rich and convenient sources: β-carotene (1), from carrots, azafrin (8, R=H), from Escobedia scabrifolia, lycopene (9), from tomatoes, bixin (10, R=H), from Bixa orellana, and capsanthin (11), from red peppers (Capsicum annuum) (9). Even so, the isolation of large quantities of these pigments was a substantial undertaking in itself. The subsequent degradations consumed vast quantities of carotenoid, and many of the results obtained would scarcely be regarded as conclusive by modern standards. We now know that attempts to determine the position of the hydroxyl group in β-end groups by permanganate oxidation to yield mixtures of saturated dicarboxylic acids, which were then separated by fractional crystallisation (9), could give a misleading result if the experiment was undertaken with less than 0.5 g of pigment. Well over 40 microanalyses were carried out (10) in an attempt to determine the molecular formula of capsanthin. The photosynthetic purple bacterium, Rhodospirillum rubrum, was grown for two years to collect 900 mg of spirilloxanthin; it was all oxidised to give a product identified (probably wrongly) as bixindial, though the correct conclusion was drawn regarding the chromophore in the bacterial carotenoid (9,11). It is now recognised that all chemical results obtained in the "classical studies" on fucoxanthin were incorrect, except for one (viz. that the pigment contains seven C-Me groups) which no-one seems to have believed at the time, but which contained an important clue as to one of the unusual features in this carotenoid (12). Nevertheless, with limited information, and a good deal of intuition, a number of structures were deduced (9). Outstanding in this respect was Karrer's proposal for β-carotene, involving a reversal of the isoprene units at the centre of the molecule. So-called confirmation of some of these structures were claimed by synthesis of perhydro-compounds (9), but the latter consisted of mixtures of diastereoisomers and any similarity with the perhydro-derivatives of the natural compounds was largely fortuitous, and certainly not conclusive. Proof of the correctness of some of these original proposals was only provided many years later by synthesis, though this in no way detracts from the earlier achievements which were truly remarkable considering the techniques then available. Some of the techniques were specifically developed to deal with this class of compound, for example, the Kuhn-Roth method for C-methyl determination, and quantitative microhydrogenation, and were subsequently adopted in other branches of organic chemistry (8).

One should not leave what I call "the classical period" without mentioning the elegant partial degradations by the schools of Kuhn and Karrer of β-carotene, and the correlations which they achieved between β-carotene (1) and azafrin (8, R=H), between lycopene (9) and bixin (10, R=H), between rhodoxanthin and zeaxanthin (12), and between zeaxanthin and violaxanthin (13), by partial degradation to a common product, or by inter-conversion (9).

Even in recent years, chemical degradation has proved essential in the study of those carotenoids, notably fucoxanthin (12) and peridinin (14)(13), with



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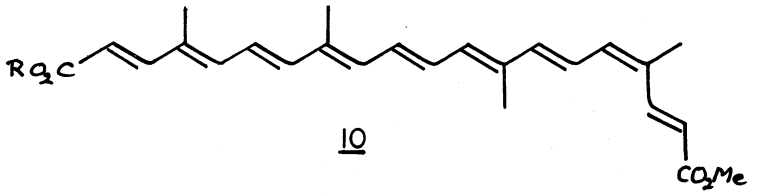

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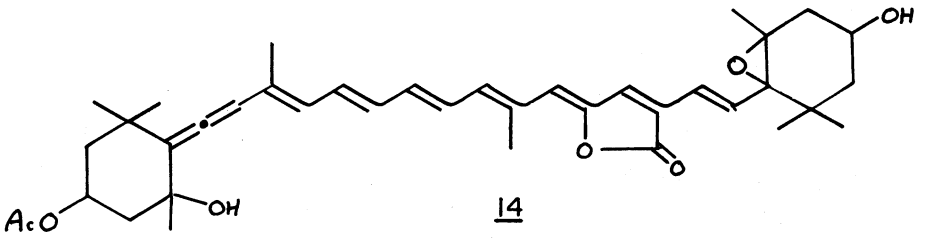

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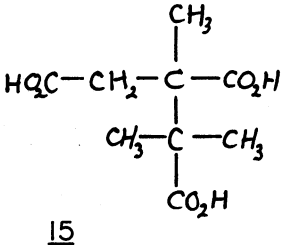

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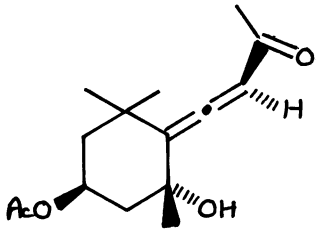

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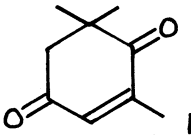

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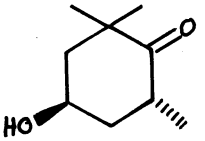

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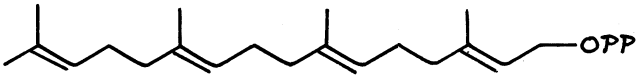

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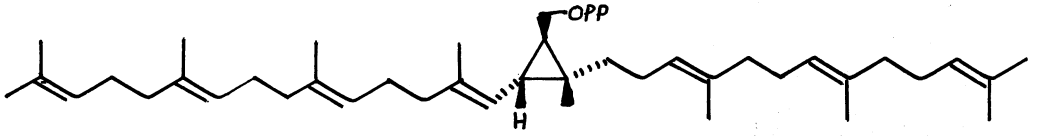

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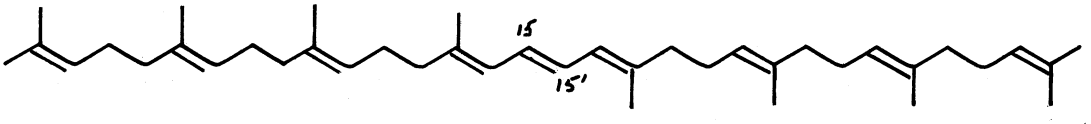

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such novel features that they could not be recognised from the spectroscopic information which is now regularly collected. Furthermore, degradation, such as that of capsanthin (11) to camphoronic acid (15), or of fucoxanthin (2) to the allenic end group (16), and of lutein (4) to derivatives of α-ionone (14), coupled with chemical transformations such as that of fucoxanthin and neoxanthin (26) into zeaxanthin, and of the natural and semi-synthetic 5,6-epoxides (17 and 18) of lutein into four distinct furanoid oxides (2), has proved the essential key to the recent elucidation of the absolute configuration of many natural carotenoids (15).

PHYSICAL EXAMINATION

Melting point

Determination of melting point, perhaps the earliest physical technique, has found little favour in the carotenoid field; the actual value of the m.p. can vary considerably depending on the way in which the determination is carried out. However, it is worth recalling that Chapman (16) first appreciated that alloxanthin (19) was different from zeaxanthin (despite almost identical light absorption properties and chromatographic behaviour) because of a difference in m.p. If others had been equally thorough, the acetylenic carotenoids might have been discovered much earlier. Before h.p.l.c., the only convenient way of distinguishing rubixanthin (7) from gazaniaxanthin was by m.p. (6). For a time neochrome (20) could only be distinguished from an isomeric furanoid oxide derived from fucoxanthin by m.p. and not by t.l.c., light absorption, or n.m.r. (15, 17). In the past, many incorrect identifications would have been avoided had melting points been determined. Despite the practical difficulties in this field, perhaps everyone should be encouraged to determine melting points under standardised conditions (evacuated capillary tube, bath pre-heated to within 10°C of the melting point, and the temperature then raised at 3°C a minute?). At least this would help emphasise the importance of isolating pure samples, and not relying on spectroscopic results obtained with crude materials.

Light absorption spectroscopy

A better appreciated physical technique is, of course, light absorption spectroscopy. This has long been used to follow an isolation and, increasingly in recent years, to monitor reactions carried out on a "spectroscopic scale". Such reactions can provide valuable information and require no more material than is needed to record the main visible light absorption maxima of the initial solution and of that of the product. Examples are the hydride reduction of conjugated carbonyl compounds, the acid promoted dehydration of the resulting allylic alcohols, and the acid catalysed re-arrangement of 5,6-epoxides to 5,8-epoxides (furanoid oxides) (8).

Infra-red light absorption spectroscopy finds its main application in the detection of carbonyl groups (it was particularly helpful in the case of violerythrin (21)(18)), of allene groups, and of geometrical isomers with a cis configuration about the 15,15'-double bond (8). The C≡C stretching frequency is weak in alloxanthin (19) and a number of related compounds, except those such as asterinic acid (22) and pectenolone (23) in which the acetylenic group is conjugated with a carbonyl group (8). Neither is the acetylenic group in alloxanthin readily detected by resonance Raman spectroscopy, though a strong band is seen with 15,15'-dehydro-β-carotene (5).

Nuclear magnetic resonance spectroscopy

Carotenoid studies benefited greatly with the advent of proton n.m.r. as a routine method. I well remember that even with a 40 MHz instrument we were able for a period around 1960 to solve a number of outstanding structural problems in quick succession: phytoene, phytofluene, ζ-carotene, neurosporene, spirilloxanthin, spheroidene, spheroidenone, and the stereochemistry of natural bixin (19). Modern instruments operating at 100 MHz are capable of measuring high resolution spectra on as little as 1 mg, though with the development of pulsed Fourier transform techniques this requirement should be reduced by an order of magnitude or more. A number of spectra have now been recorded at 220 MHz and this, together with spin-spin decoupling techniques means that the olefinic protons are now amenable to study in addition to the methyl protons (8, 20). Already commercial instruments are available which allow these signals to be

studied at 360 MHz. This holds out the prospect that structures, including the geometrical configuration, will be solved on as little as 50 μg of material — assuming, of course, that sufficient reference data are available from compounds of known structure and stereochemistry to interpret the spectra. However, there seems little doubt that high resolution proton n.m.r., coupled with h.p.l.c. to provide the small samples of stereochemically pure material required, will in the future provide an enormously powerful means of studying the structures of carotenoids.

In the last few years a good deal of attention has also been paid to the ^{13}C n.m.r. spectra of carotenoids (8,20,21), As a result, complete assignments have been made to β-carotene and a number of other carotenoids and the technique has been used to show that violeoxanthin is the 9-cis isomer of violaxanthin (13)(22). The potential of the method is illustrated by the fact that in the spectrum of the C_{42}-carotenoid fucoxanthin, no less than 41 separate signals are observed (8). It is only fair to admit that, as yet, these cannot all be assigned. The inherent disadvantage of the method at present is that to determine the spectrum within a reasonable time requires a comparatively large amount of pure material, and this requirement is often difficult to meet. However, ^{13}C n.m.r. studies together with ^{1}H n.m.r. studies at 270 MHz, with spin-spin decoupling, carried out by Dr. G. Englert (Basle) suggest that prolycopene is 7,9,7',9'-tetra-cis lycopene with two hindered and two unhindered cis-double bonds. Further spectroscopic and synthetic studies are in hand to check this provisional solution to a long outstanding problem (23).

Mass spectrometry

In the past, a major problem in many carotenoid studies has been the determination of the molecular formula. The inherent limitation of microanalysis, and the absence of good methods for the accurate determination of molecular weights, led to many uncertainties and inaccurate conclusions. These problems can now be avoided in most cases by precision mass spectrometry. This requires only minute quantities of material, and, provided the molecular ion (or a directly-related major fragment) can be observed, leads to an unambiguous assignment of molecular formula. The characterisation of fucoxanthin as a C_{42}-carotenoid (12) and the recognition of decaprenoxanthin (24) as a C_{50}-carotenoid (24), owe much to this technique. A distinction can now confidently be made between a carotenoid and an acetylenic analogue differing by only two hydrogen atoms (25). Moreover, the fragmentation pattern in the mass spectrum often provides useful structural information (8).

X-ray crystallography

A technique which, in principle, is capable of solving the structure of a carotenoid without any other information is X-ray crystallography — provided, of course, that a suitable crystal is available. Successful, and very informative, studies have been carried out with β-carotene (26), canthaxanthin (25)(27), and their 15,15'-didehydro-analogues, but there has as yet been no successful application of the technique to a carotenoid of unknown structure, or to any (unmodified) optically active carotenoid, though considerable progress has now been made with fucoxanthin (5).

After introducing a heavy atom label into a carotenoid, it should be possible to solve not only the gross structure, but also the absolute configuration. This goal has not yet been realised in practice, though a recent study (28) on capsanthin (11) bis-p-bromobenzoate served to confirm the relative features of the (absolute) configuration assigned (15) to the parent carotenoid on other grounds. Clearly there are considerable practical difficulties in applying X-ray crystallographic methods in this field, but it is reasonable to expect that they will be overcome before long. Meanwhile the assignment of absolute configuration to carotenoids relies primarily on degradation to a comparatively simple product of known configuration, or which is amenable to X-ray crystallographic analysis (8,15). This information is then supplemented by chemically achieved correlations between chiral centres, inter-conversions between carotenoids, and comparisons by optical rotatory dispersion (o.r.d.) or circular dichroism (c.d.) (8,15). However, the switch of just one trans to a cis double bond can change the chiroptical properties of a carotenoid almost completely (8,22). It is essential, therefore, that any comparisons are carried out on pure compounds with the same geometrical configurations.

By these indirect means it has been possible in recent years to establish the absolute configurations of capsanthin (11) and zeaxanthin (12), of the four major natural carotenoids, fucoxanthin (2), lutein (4), violaxanthin (13) and neoxanthin (26), and of several related compounds (2,14,15,29,30).

SYNTHESIS

Many elegant pioneer studies on polyene synthesis were carried out by Kuhn's school in the 1930's. These culminated in the synthesis in 1938 of a ca. 5% concentrate of vitamin A (31). In 1948, Karrer and Schwyzer (32) obtained from vitamin A a crude product containing traces of β-carotene. However, the unambiguous total synthesis of carotenoids in isolatable amounts was first achieved with the hydrocarbons β-carotene (1) and lycopene (9) in 1950 (34-36), and with the oxygenated carotenoid all-*trans* methylbixin (27) in 1952 (37). Since then many other carotenoids have been synthesised (3,38,39).

There are two basic problems in carotenoid synthesis: elaboration of the polyene chain, and preparation of the end groups. The first of these can be solved by the now well-established acetylenic techniques, vinyl ether syntheses, and Wittig-type reactions, including the use of phosphorans, phosphonates and sulphones (38,39). Simpler (and cheaper) methods would no doubt be welcome, and there is a need for better methods for the stereochemically controlled synthesis of some of the naturally occurring *cis* isomers. In this connection it is worth mentioning that 9-*cis* methylbixin (10, R=Me) has been synthesised with the same configuration as natural bixin (40). Good methods are also needed for the synthesis of the natural all-*trans* forms of the acetylenic carotenoids which are thermodynamically unstable (3).

Much remains to be done by way of end group synthesis. Present methods for introducing the 3-hydroxy-5,6-epoxy ("violaxanthin type") end group give predominantly the "wrong" geometrical isomer (15). Only relatively poor methods have yet been published for the synthesis of astaxanthin (41); though the elusive dimethyl ether (28, R=Me) of astacene can now be prepared quantitatively (previous difficulties arose from failure to appreciate the ease with which this derivative is hydrolysed), it does not provide the hoped-for key intermediate (5).

Di- and poly-functional carotenoids pose many problems in stereochemistry, even in the synthesis of the racemates. The (racemic) allenic end group of fucoxanthin and neoxanthin has been prepared (30) with the correct relative configuration of the two chiral centres and the chiral axis, but not as yet in yields which permit further synthetic work. Racemic azafrin methyl ester (8, R=Me) and lutein (4), with the correct relative configuration at C-3' and C-6', have also been prepared (3).

Increasingly, attention will now turn to developing convenient syntheses of chiral carotenoids with the same absolute configuration as that found in the natural pigment. Already "natural" capsorubin (29) has been synthesised (42) from (+)-camphor and "natural" α-carotene from (+)-ionone (43), and "natural" zeaxanthin (44) from an optically active C_9- end group (31) obtained (45) from (30) by microbiological and then chemical reduction. (Incidentally, little, if any, work yet seems to have been done on the microbiological transformation of carotenoids comparable to the extensive studies in the steroid field.) "Natural" trikentriorhodin (32) has been prepared (46), also an enantiomer of (9-*cis*) mytiloxanthin (33) which on genetic grounds probably corresponds to the natural series.

Many steps in carotenoid synthesis involve oxidation or reduction. It is of interest, therefore, to point out that electrochemical methods provide a wider range of oxidation and reduction potentials than those afforded by chemical reagents. Moreover, control of the electrode potential enables greater selectivity to be achieved. Pilot studies have shown that even C_{40}-carotenoids can be handled electrochemically. Astacene (28, R=H) has been reduced to (optically inactive) astaxanthin (6)(47), canthaxanthin (25) converted into some novel derivatives by reductive acetylation (48), and the allylic function in vitamin A acetate hydrogenolysed to give axerophthene almost quantitatively (49).

BIOSYNTHESIS

The combined efforts of many groups, making extensive use of ^{3}H and ^{14}C labelled samples, have now revealed the main outlines of carotenoid biosynthesis, though a number of features and many details still have to be elucidated (50).

Carbon skeleton

The carotenoids are derived from mevalonic acid, and the early steps up to the formation of the C_{20}-geranyl-geranyl pyrophosphate (34) are believed to be identical with, or to resemble, those in the formation of the C_{15}-farnesyl intermediate in the biosynthesis of triterpenes. The analogy appears to proceed one stage further with two of the C_{20}-units combining to give the C_{40}-prephytoene pyrophosphate (35). It then seems likely that the latter is converted directly into the conjugated triene, phytoene (36). Both 15-*cis* and all-*trans* forms of phytoene have been isolated, and there are grounds for believing that at least one of these is the precursor of all other carotenoids. The extension of the conjugated chain involves a series of *trans* β-eliminations of two neighbouring allylic hydrogen atoms.

Cyclisation of the lycopene type end groups to give the common ε- and β-rings can occur at either the lycopene stage, or at the lycopene end of 7,8-dihydro-lycopene (neurosporene). It can be represented formally as resulting from protonation of a 1,2-double bond. Attack of the 1,2-double bond by a C_5-carbonium ion, or related species, affords a rational explanation (51) for the formation of the C_{45}- and C_{50}-carotenoids, such as decaprenoxanthin (24). The carotenoids with aromatic end groups are probably the result of aromatisation of preformed ε- or β-rings.

Oxygen functions

A characteristic feature of many carotenoids is the presence of a hydroxyl group at C-3 and/or C-3' in a cyclic end group. The evidence suggests that these substituents are introduced after the ring has been formed (50).

Experiments on the biosynthesis of zeaxanthin (12) from stereospecifically labelled mevalonic acid clearly demonstrate that no ketonic intermediate is involved in the hydroxylation, and also that hydroxylation occurs by replacement of a hydrogen atom at C-3 (or C-3') with retention of configuration. Comparable experiments on the biosynthesis of lutein (4) suggest that the hydroxyl group at C-3' is similarly derived (50, 52). Since it is now known (14) that the absolute configuration at C-3' in lutein is the opposite of that at the corresponding position in zeaxanthin (12), either the preliminary labelling experiments are misleading, or stereochemical inversion occurs after the introduction of the hydroxyl substituent at C-3'. It may be significant that the natural pigment calthaxanthin is believed (53) to differ from lutein (4) only in the configuration at C-3'.

The 5,6-epoxides of the 3-hydroxy-carotenoids occur widely. Violaxanthin (13), neoxanthin (26) and lutein epoxide (17) all have the same 5*R*, 6*S* configuration (2), indicating that they are formed enzymatically. Experiments with ^{18}O have confirmed that the epoxide oxygen comes from molecular oxygen (54) and not from water as was first thought. There seems little doubt that the cyclopentyl ketone end group in capsanthin (11) and capsorubin (29) are formed from the 3-hydroxy-5,6-epoxides by stereospecific rearrangements of the pinacol type (19, 55).

Many intermediates have been characterised in the conversion of lycopene (9) into spirilloxanthin (37) and related pigments found in photosynthetic bacteria (51). The introduction of the oxygen function at C-1 is an anaerobic process, and probably occurs by hydration of the 1,2-double bond as an alternative to cyclisation. Mechanisms have also been suggested for the introduction of oxygen functions elsewhere in the carotenoid skeleton (50).

Allenes and acetylenes

There has been much speculation (50) on the origin of the allenic end group

found in the two major carotenoids, fucoxanthin (2) and neoxanthin (26). The proposal (56) that they arise from a singlet oxygen type of oxidation of a zeaxanthin end group received a major set back when it was shown that this would give the S- and not the R-allene (30, 57). However, it is now known that the former is unstable and readily converted into the familiar R-form (1). The basic proposal is therefore still a tenable proposition.

There is also great uncertainty concerning the origin of the acetylenic groups found in the 7- and 7'-positions of some algal carotenoids, and in several pigments from marine animals, the commonest being alloxanthin (19). They could arise from dehydration of an allenic end group of the fucoxanthin type (58), and in vitro analogies for this process have been claimed (59). However, the possibility cannot be excluded that they result from a formally simple dehydrogenation of double bonds, even though the positions in question are known to be very hindered sterically.

CONCLUSIONS

Given the methods currently available, or now under development, determination of the structure and configuration of a natural carotenoid should present few difficulties provided a small, but pure, sample can be isolated. The same cannot be claimed with regard to the numerous carotenoproteins, and other carotenoid-protein complexes (60), which remain something of an enigma and a major challenge for the future.

A number of problems in synthesis still await solution and, in view of the industrial interest in some of these compounds as edible colours for food and pharmaceuticals, there will always be a need for better and simpler routes.

A great deal more work needs to be done on the biosynthesis of these compounds, and on their metabolic transformations.

Much still has to be learnt about the biological functions of the carotenoids. Even when a rôle has been identified, little is known about the way in which this rôle is fulfilled.

For these and other reasons, there is no doubt that the carotenoids will continue to excite interest and be the subject of research for many years to come.

REFERENCES

1. K. Bernhard, G. P. Moss, Gy. Tóth and B. C. L. Weedon, Tetrahedron Letters 3899 (1974).
2. D. Goodfellow, G. P. Moss, J. Szabolcs, Gy. Tóth and B. C. L. Weedon, Tetrahedron Letters 3925 (1973)
3. B. C. L. Weedon, Pure & Appl. Chem. 47, 161 (1976).
4. A. G. Andrewes, G. Borch, S. Liaaen-Jensen and G. Snatzke, Acta Chem. Scand. B28, 730 (1974).
5. Queen Mary College Carotenoid Group, unpublished results.
6. B. O. Brown and B. C. L. Weedon, Chem. Commun. 382 (1968).
7. K. Schön, Biochem. J. 32, 1566 (1938).
8. G. P. Moss and B. C. L. Weedon, in "Chemistry and Biochemistry of Plant Pigments", ed. by T. W. Goodwin, 2nd edn., Academic Press, New York (1976).
9. P. Karrer and E. Jucker, "Carotinoide", Birkhäuser, Basle (1948), English translation by E. A. Braude, Elsevier (1950).
10. L. Cholnoky, D. Szabó and J. Szabolcs, Liebig's Annalen 606, 194 (1957).
11. M. S. Barber, L. M. Jackman, P. S. Manchand and B. C. L. Weedon, J. Chem. Soc. C 2166 (1966).
12. R. Bonnett, A. K. Mallams, A. A. Spark, J. L. Tee, B. C. L. Weedon and A. McCormick, J. Chem. Soc. C 429 (1969).
13. H. H. Strain, W. A. Svec, K. Aitzetmüller, M. C. Grandolfo, J. J. Katz, H. Kjøsen, S. Norgård, S. Liaaen-Jensen, F. T. Haxo, P. Wegfahrt and H. Rapaport, J. Amer. Chem. Soc. 93, 1823 (1971).
14. R. Buchecker, P. Hamm and C. H. Eugster, Helv. Chim. Acta 57, 631 (1974).

15. B. C. L. Weedon in "Carotenoids", ed. by O. Isler, Birkhäuser, Basle (1971), chap. V.
16. D. J. Chapman, Phytochem. 5, 1331 (1966).
17. L. Cholnoky, K. Györgyfy, A. Rónai, J. Szabolcs, Gy. Tóth, G. Galasko, A. K. Mallams, E. S. Waight and B. C. L. Weedon, J. Chem. Soc. C 1256 (1969).
18. S. Hertzberg, S. Liaaen-Jensen, C. R. Enzell and G. W. Francis, Acta Chem. Scand. 23, 3290 (1969).
19. B. C. L. Weedon, Chem. in Britain 3, 424 (1967).
20. W. Vetter, G. Englert, N. Rigassi and U. Schwieter, in "Carotenoids", ed. by O. Isler, Birkhäuser, Basle (1971), chap. IV.
21 J. Szabolcs, Pure & Appl. Chem. 47, 147 (1976).
22. G. P. Moss, J. Szabolcs, Gy. Tóth and B. C. L. Weedon, Acta Chim. Acad. Sci. Hung. 87, 301 (1975).
23. G. Englert, B. O. Brown, G. P. Moss, B. C. L. Weedon, G. Britton, T. W. Goodwin and K. L. Simpson, forthcoming publications.
24. S. Liaaen-Jensen, S. Hertzberg, O. B. Weeks and U. Schwieter, Acta Chem. Scand. 22, 1171 (1968).
25. A. K. Mallams, E. S. Waight, B. C. L. Weedon, D. J. Chapman, F. T. Haxo, T. W. Goodwin and D. M. Thomas, Chem. Commun. 301 (1967).
26. C. Sterling, Acta cryst. 17, 1224 (1964).
27. J. C. J. Bart and C. H. MacGillavry, Acta cryst. B24, 1569, 1587 (1968).
28. I. Ueda and W. Nowacki, Z. Krist. 140, 190 (1974).
29. K. Bernhard, G. P. Moss, Gy. Tóth and B. C. L. Weedon, Tetrahedron Letters 115 (1976).
30. J. R. Hlubucek, J. Hora, S. W. Russell, T. P. Toube and B. C. L. Weedon, J.C.S. Perkin I 848 (1974).
31. R. Kuhn and C. J. O. R. Morris, Ber. 70, 853 (1937).
32. P. Karrer and A. Schwyzer, Helv. Chim. Acta 31, 1055 (1948).
33. H. H. Inhoffen, H. Pommer and F. Bohlmann, Liebig's Annalen 569, 237 (1950).
34. H. H. Inhoffen, H. Pommer and F. Westphal, Liebig's Annalen 570, 69 (1950).
35. P. Karrer and C.H. Eugster, Helv. Chim. Acta 33, 1172 (1950).
36. N. A. Milas, P. Davis, I. Belič and D. A. Fleš, J. Amer. Chem. Soc. 72, 4844 (1950).
37. R. Ahmad and B. C. L. Weedon, Chem. & Ind. 882 (1952).
38. H. Mayer and O. Isler, in "Carotenoids", ed. by O. Isler, Birkhäuser, Basle (1971), chap VI.
39. F. Kienzle, Pure & Appl. Chem. 47, 183 (1976).
40. G. Pattenden, J. E. Way and B. C. L. Weedon, J. Chem. Soc. C 235 (1970).
41. R. D. G. Cooper, J. B. Davis, A. P. Leftwick, C. Price and B. C. L. Weedon, J.C.S. Perkin I, 2195 (1975).
42. B. C. L. Weedon, Pure & Appl. Chem. 35, 113 (1973).
43. C. H. Eugster, R. Buchecker, C. Tscharner, G. Uhde and G. Ohloff, Helv. Chim. Acta 52, 1729 (1969)
44. H. Mayer, W. Boguth, H. G. W. Leuenberger, E. Widmer and R. Zell, Abstr. Fourth. Internat. Symp. Carotenoids 43 (1975).
45. H. G. W. Leuenberger, W. Boguth, E. Widmer and R. Zell, Helv. Chim. Acta 59, 1832 (1976).
46. A. K. Chopra, G. P. Moss and B. C. L. Weedon, Chem. Commun. 467 (1977)
47. E. A. H. Hall, G. P. Moss, J. H. P. Utley and B. C. L. Weedon, Chem. Commun. 387 (1978).
48. E. A. H. Hall, G. P. Moss, J. H. P. Utley and B. C. L. Weedon, Chem. Commun. 586 (1976).
49. J.-G. Gourcy, B. Terem, J. H. P. Utley and B. C. L. Weedon, see ref. 3.
50. G. Britton, in "Chemistry and Biochemistry of Plant Pigments", ed. by T. W. Goodwin, 2nd edn., Academic Press, London (1976)
51. S. Liaaen-Jensen, Pure & Appl. Chem. 47, 129 (1976).
52. T. W. Goodwin, G. Britton and T. J. Walton, Pl. Physiol., Lancaster 43 S-46 (1968).
53. A. G. Dabbagh and K. Egger, Z. Pflanzenphysiol. 72, 177 (1974).
54. H. Y. Yamamoto and C.O. Chichester, Biochim. Biophys. Acta 109, 303 (1965).
55. M. S. Barber, L. M. Jackman, C. K. Warren and B. C. L. Weedon, J. Chem. Soc. 4019 (1961).
56. S. Isoe, S. B. Hyeon, H. Ichikawa, S. Katsumura and T. Sekan, Tetrahedron Letters 5516 (1968).
57. T. E. DeVille, J. Hora, M. B. Hursthouse, T. P. Toube and B. C. L. Weedon, Chem. Commun. 1231 (1970).
58. B. C. L. Weedon, Rev. Pure and Appl. Chem. 20, 51 (1970).
59. K. Egger, A. G. Dabbash and H. Nitsche, Tetrahedron Letters 2995 (1969).
60. P. F. Zagalsky, Pure & Appl. Chem. 47, 103 (1976).