MASS SPECTROMETRIC STUDIES OF CAROTENOIDS

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ABSTRACT

Mass spectra of carotenoids are discussed and shown to provide considerable structural information about this class of compounds. Thus, the number of conjugated double bonds in the acyclic polyene chain can often be determined from the intensity ratio of the M-92 and M-106 peaks, which are characteristic of carotenoids. Moreover, most end groups may be identified by significant peaks which are readily associated with structure. Differentiation between oxygenation sites within the end group and in-chain methyl positions is usually feasible, as is distinction between the possible in-chain positions.

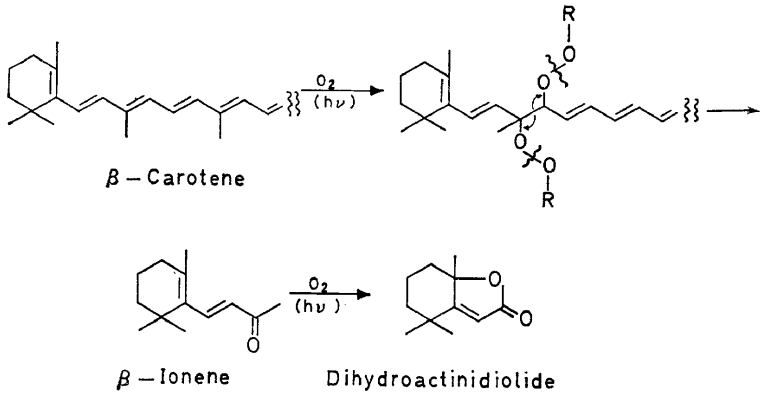
INTRODUCTION

The colour of tobacco is partly determined by carotenoids and is developed in the curing process when the chlorophylls are broken down and the original green colour disappears. Colour is an important factor in judging the quality, and carotenoids are therefore of considerable interest. Although colour and quality are complex conceptions it seems probable that carotenoids constitute one of the links of this empirically established relationship and thus represent a direct connection between the colour of the leaf and its content of terpenoid flavour constituents or precursors.

Support for this hypothesis comes from an investigation by Jeffrey and Griffith[1], which shows that the quantity of carotenoids is considerably higher in the aromatic top leaves than in the lower less aromatic leaves. Although a high carotenoid content may reflect a high biosynthetic activity with respect to all types of terpenoids, it is evident that carotenoids themselves are aroma precursors. A good example of this is found in the recent work of Isoe, Be Hyeon and Sakan[2], demonstrating that β-carotene in the presence of oxygen undergoes oxidative cleavage to give β-ionone and dihydroactinidiolide in the manner indicated below; the reaction is accelerated on irradiation with light of appropriate wavelength.

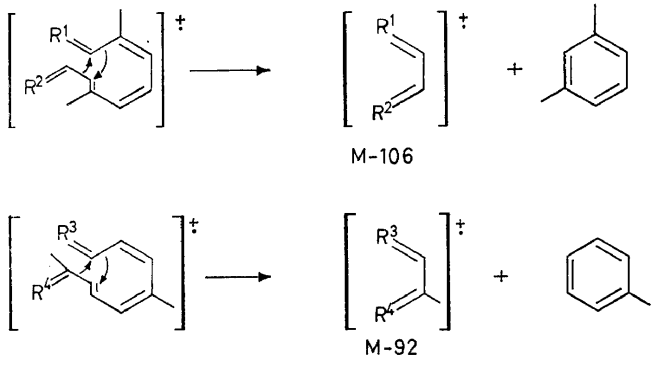
In view of this and the limited knowledge concerning the isoprenoid constituents of tobacco it was of interest to perform a parallel examination of the carotenoids and the more volatile constituents. Since these compounds are present in very low concentrations it was deemed favourable to see what information could be obtained from mass spectra of carotenoids.

A survey of the fragmentation reactions of a number of selected carotenoids, mainly of natural occurrence, was therefore undertaken[3,4] and the results of this study constitute the topic of the present paper.



M-92, M-106 AND RELATED IONS

Previous systematic studies on electron impact induced fragmentation of carotenoids were limited when the present work was initiated to investigations by Schwieter *et al.*[5] on carotenes and to studies on carotenoid epoxides and furanoid oxides by Baldas *et al*[6]. The former workers found that a characteristic feature of the fragmentation of carotenes is the formation of prominent M-92 and M-106 ions. They ascribed the formation of these ions, which were later also encountered in the fragmentation of the carotenoid epoxides and furanoid oxides, to the elimination of part of the polyene chain. These authors also proposed the mechanism shown below to accommodate these losses.



We observed these ions in all spectra of C_{40}-carotenoids possessing a conjugated polyene chain and it seems clear that they are characteristic of carotenoids. In fact they are in many cases more abundant than the molecular ion and frequently provide a useful means of establishing the molecular weight.

It was evident from the spectra that the intensity of these peaks showed considerable variation, and it appeared probable that this observation could be correlated with some structural feature. The intensity ratios of the M-92/M-106 peaks were therefore calculated and listed in decreasing order of magnitude. The results obtained, which are given in *Table 1*, show that these variations are related to the number of double bonds in the acyclic conjugated chain and that the M-92/M-106 ratio decreases as the number of double bonds increases from 9 to 13.

Table 1. Intensity ratios of the (M-92)/(M-106) peaks (*R*) in the mass spectra of carotenoids with a varying number of conjugated carbon-carbon double bounds in the acyclic polyene chain (*DB*)

	Polyene	*R*	*DB*
	Zeaxanthin	10·0	9
	Echinenone	4·46	9
	3-Hydroxy-3′-keto-α-carotene	3·19	9
	3-Hydroxy-3′-methoxy-α-carotene	2·73	9
Bicyclic	Canthaxanthin	2·56	9
	Iso-zeaxanthin	2·17	9
	Lutein diacetate	1·73	9
	Lutein	1·59	9
	Chlorobactene	1·00	10
Monocyclic	Rubixanthin	0·70	10
	Rhodoxanthin*	0·55	10
	Rubixanthin acetate	0·44	10
	Lycophyll	0·36	11
	Lycopene-16-al	0·34	11
	Lycopene	0·34	11
	Lycoxanthin	0·34	11
Acyclic	1,2,1′,2′-Tetrahydro-1,1′-dihydroxy-lycopene	0·34	11
	Rhodopin	0·27	11
	3,4,3′,4′-Tetrahydrospirilloxanthin	0·26	11
	Anhydro-rhodovibrin	0·068	12
	Rhodovibrin	0·057	12
	OH-Spirilloxanthin	0·029	13
	Spirilloxanthin	0·018	13

* Rhodoxanthin is a bicyclic retro-compound, whereas the other compounds having 10 double bonds are monocyclic.

The relationship holds well for all C_{40}-carotenoids containing no more than one oxygen substituent in each end group and hence provides for such compounds information equivalent to that normally obtained from electronic spectra. The compounds having two or more oxygen atoms in one of the end groups do not fit the simple pattern, but when these are grouped according to the type of the oxygen function present the same trend is observed within each set. However, considerably more data has to be collected to clarify the utility in these cases.

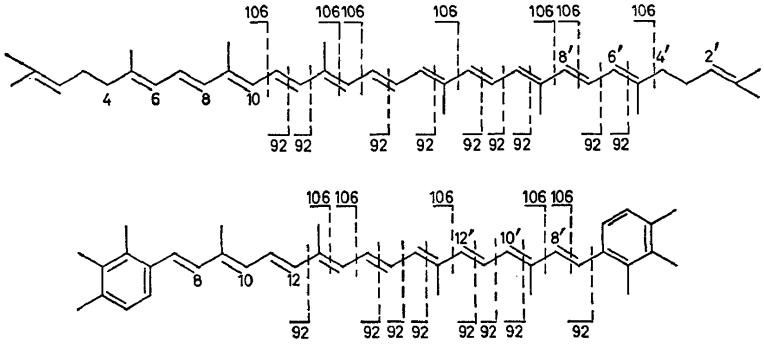
Dependence of the intensity ratio on the type of instrument used, the state of the ion source, and the probe temperature, was shown to be negligible in the cases investigated. Thus, re-examination at different temperatures of several of the compounds on our LKB instrument over a period of two months gave reproducible results. Moreover, comparison with results obtained on an MS-9 instrument and with data available in the literature[7] accord with

those obtained on the LKB instrument, as do the values calculated from the carotene spectra published by Schwieter and co-workers[5], which are given in *Table 2.*

Table 2. Intensity ratios (M-92)/(M-106) for various carotenes from Ref. 5.

	Carotene	*R*	*DB*
	β-Carotene	12·9	9
Bicyclic	α-Carotene	7·5	9
	ε-Carotene	3·1	9
Monocyclic	γ-Carotene	0·85	10
	δ-Carotene	0·43	10
Acyclic	Lycopene	0·30	11

If, following Schwieter *et al*[5]., the reasonable assumption is made that the 92 and 106 fragments are derived from a unit of six consecutive carbons in the polyene chain, it follows from the values for the M-92/M-106 ratio that the elimination of these fragments does not occur to an equal extent from the various possible units within the chain. Evidence for this comes from a study of 7,7′-d_2-renierapurpurin, 7,7′-d_2-isorenierapurpurin and 7,7′-d_2-lycopene.



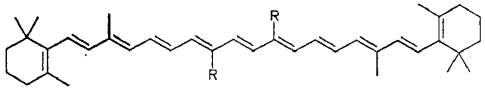
The results obtained for the bicyclic aromatic compounds show that the formation of neither M-92 nor M-106 ions involves the 7- or 7′-hydrogens as these peaks are shifted quantitatively and hence demonstrate that only part of the polyene chain is utilised. In the acyclic dideutero-derivative the M-106 peak is also shifted quantitatively by two mass units, whereas the corresponding shift of the M-92 peak occurs only to the extent of 65 per cent; the remainder is represented by the monodeutero species.

If it is assumed that all trigonally hybridised carbons in the polyene chain of the acyclic compound are incorporated to an equal extent in the smaller fragment, the shift of the M-92 peak by two mass units would occur to the extent of 60 per cent; the corresponding figure for the M-106 peak would be

about 70 per cent. Since the former value is close to that actually observed it seems likely that, in lycopene, the carbons in the 6-6′-range are incorporated to the same extent in the fragment of 92 mass units. With respect to the M-106 species, the results show that cleavage of the bond between the saturated carbon at 4 and the first trigonally hybridised carbon of the polyene chain does not occur and hence also that elimination of the m/e 106 fragment only involves carbons in the 8-8′-range.

In view of the findings for the acyclic compound it seems likely that the main reason for the contraction of the range to positions 10-10′ for the formation of the M-92 ion in the case of the cyclic compounds is steric in nature. Consistent with this are results obtained for carotenoids with oxygenated in-chain substituents, which are summarised in *Table 3*. These show that the formation of species equivalent to the M-92 ion is also limited to losses within the 10-10′-range. Moreover, there is a pronounced preference for the formation of species equivalent to the M-106 ion by eliminations from the central part of the polyene chain.

Table 3. Elimination of in-chain units from some *iso*-carotenoids



R	$C_6H_5CH_3$	C_6H_5R	$C_6H_4CH_3R$	$C_6H_4R_2$
CHO	—	3·5%	—	94%
CH_2OH	—	1·0%	0·3%	100%
CH_2OAc	—	5·0%	—	100%
⟋⟍COOEt	—	6·5%	32%	87%

It may be concluded from these findings that it should be possible to say whether a bicyclic carotenoid is oxygenated in the end group or in one of the in-chain methyl groups and also in the latter case which of the methyl groups is oxidised.

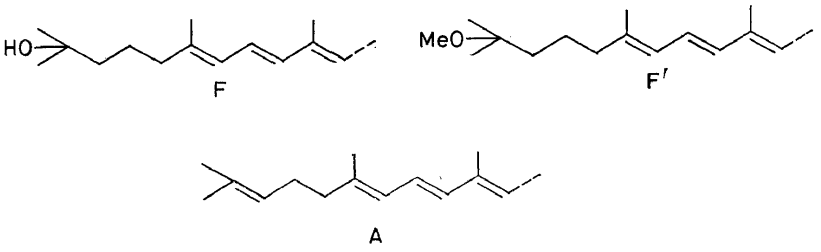
CHARGE LOCALISATION

In the discussion it has so far been assumed that the molecular ion is formed by extrusion of an electron from the conjugated polyene chain and that the charge is localised to this part of the molecule. We have found that this leads to the formation of the M-92 and M-106 ions and that these reactions are not appreciably influenced by the two end groups. It is clear, however, since each compound furnishes a characteristic spectrum, that the end groups influence other fragmentation reactions, which are also initiated by elimination of an electron from the polyene chain.

In compounds having cyclic end groups fragmentation normally occurs in the polyene chain rather than within the end group itself. The particular type of end group present will dictate which of the two fragments so formed

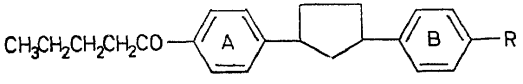
retains the charge preferentially. When the end group is aromatic or furanoid, and hence allows stabilisation of the charge, the smaller fragment is of high abundance, whilst in other cases the charge is preferentially retained by the larger fragment comprising the polyene chain.

When the charge is initially localised to the conjugated chain, carotenoids with acyclic end groups might be expected to undergo cleavage at an allylic saturated bond with retention of the charge by the larger fragment. However, such cleavage is not observed in a number of cases. For instance end groups F and F′, shown below, do not give ions by cleavage of the



allylic 3,4-bond, whilst in contrast end group A, which has a functional group in an appropriate position, undergoes the expected cleavage of this bond.

It may be argued that a reason for this difference is that in the latter case ionisation has occurred at the isolated double bond rather than at the polyene system. However, this is contradicted by the fact that a metastable ion corresponding to a M-92→M-92-69 reaction is seen and that this requires the charge to be localised in the chain of the parent ion. The main reason for the observed difference can be interpreted in one of two ways. The energy requirements may differ for the two reactions, especially when one bears in mind that end group A in contrast to F can give rise to a resonance stabilised neutral species. Alternatively the probability and the effects of charge transfer from the polyene chain to the terminal functional group



may differ in the two cases. Charge transfers over similar distances have recently been demonstrated by Mandelbaum and Biemann[8] for the diphenylcyclopentanes [R = NO_2, NH_2, $CH_2(CH_2)_3CONHCOC_2H_5$, $N(COC_2H_5)_2$] shown above. The results also show that the extent to which these reactions occur is dependent on the difference in ionisation potential between the two aromatic substituents.

Although localisation of the charge to the polyene chain rather than to an isolated functional group is to be expected and receives experimental backing from results obtained on lowering the energy of the impinging

electrons, it is evident that at 70 eV localisation to the end group will also occur to some extent. The extent to which this takes place will primarily be dependent on the ionisation potential of the functional group whether this occurs by direct ionisation or by charge transfer. The end group F′ provides a good example of this and gives rise to a very prominent peak at m/e 73 (100 per cent) corresponding to the smaller fragment formed on cleavage of the 1,2-bond. Similarly, end group A gives rise to a prominent m/e 69 peak showing that the charge in this case is also retained by the smaller fragment. In fact, a general feature in the fragmentation of carotenoids is that the abundance of the smaller fragment is always greater than that of the larger fragment and this may be ascribed principally to the difference in stability between the two fragments.

Having mentioned some general aspects on the fragmentation of carotenoids it seems appropriate to turn to a discussion of the individual end groups. However, prior to this a few words should be said about multiple eliminations and the possibility of detecting both end groups when they differ considerably in stability.

A characteristic feature of carotenoid mass spectra is the occurrence of ions formed by multiple losses. The corresponding peaks often reveal the end group to which a substituent is attached and sometimes provide the only evidence pertaining to the presence of a particular end group. This is because the abundance of the ions due to multiple losses is often much greater than that of ions at higher mass numbers due to simple cleavages. Especially noticeable and useful in this connection are combinations involving extrusion of part of the polyene chain, such as M-92-R, M-106-R, M-158-R, M-92-92-R, etc. A typical example, anhydrorhodovibrin, is illustrated in *Figure 1*.

A large number of combinations is possible and the only restriction, as expected on energetic grounds, is that even electron species do not lose odd electron fragments to give radical ions, e.g. M-92-106 and M-92-69 are observed but not M-69-69. The ions formed by multiple losses may be derived by different paths. Thus, metastable ions are for instance observed for the M→M-69→(M-69)-92 and M→M-92→(M-92)-69 reactions in several spectra. Moreover, in the spectrum of 7,7′d_2-lycopene the d_2/d_1-ratio for the M-92-69 ion (5:1) is higher than that for the M-92 ion (2:1) but lower than that for the M-69 ion (100:1). This would be expected if both ions were precursors and the M-69→(M-69)-92 reaction occurred essentially without loss of deuterium.

The fact that the peaks due to multiple losses provide the same information as those due to simple cleavage makes it possible to omit them from the further detailed discussion.

The possibilities of detecting both end groups in an unsymmetrical carotenoid from the individual fragmentation patterns will largely be dependent on the difference in stability of these groups. The less stable end group can be detected in the upper part of the spectrum for virtually all combinations. However, to identify the other end group when stability differences are large it is sometimes necessary to utilise other information such as the molecular weight, peaks in the lower part of the spectrum, and chemical or other spectroscopic evidence.

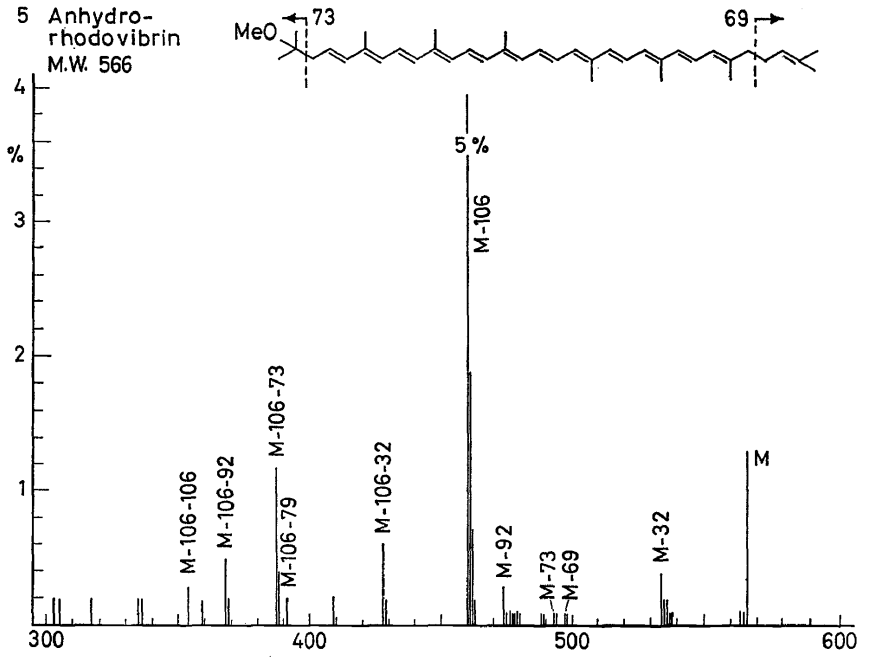


Figure 1. Mass spectrum of anhydrorhodovibrin

ACYCLIC END GROUPS

The fragmentation of the acyclic end groups usually proceeds by the expected routes and the peaks observed are readily associated with the functional groups present. The peaks in the upper part of the spectrum are in nearly all cases due to ions formed by elimination of the functional group together with a hydrogen or by rupture of a bond in an appropriate position relative to two functionalities. The smaller fragment may also retain the charge and when of high stability useful information about the structure of the end group may be obtained from the lower part of the spectrum.

The end group present in lycopene and designated A gives rise to a prominent M-69 peak, as observed previously by Schwieter and co-workers.[5] Results obtained for 7,7′-d_2-lycopene show, as expected, that the corresponding ion is formed by the cleavage of the doubly allylic 3,4-bond. The isopentenyl ion of m/e 69 gives rise to the base peak when both end groups are the same, and to a prominent peak at this mass number in the majority of the other cases.

Reduction of the 7,8-double bond of end group A yields end group B and makes this bond vulnerable to cleavage on electron impact. The two compounds investigated having this end group both show a prominent M-137 peak. Of these lycopersene, which lacks conjugation, also shows a significant M-69 peak. As expected it also gives rise to ions formed by cleavage of the other doubly activated bonds, which are spaced 68 mass units apart.

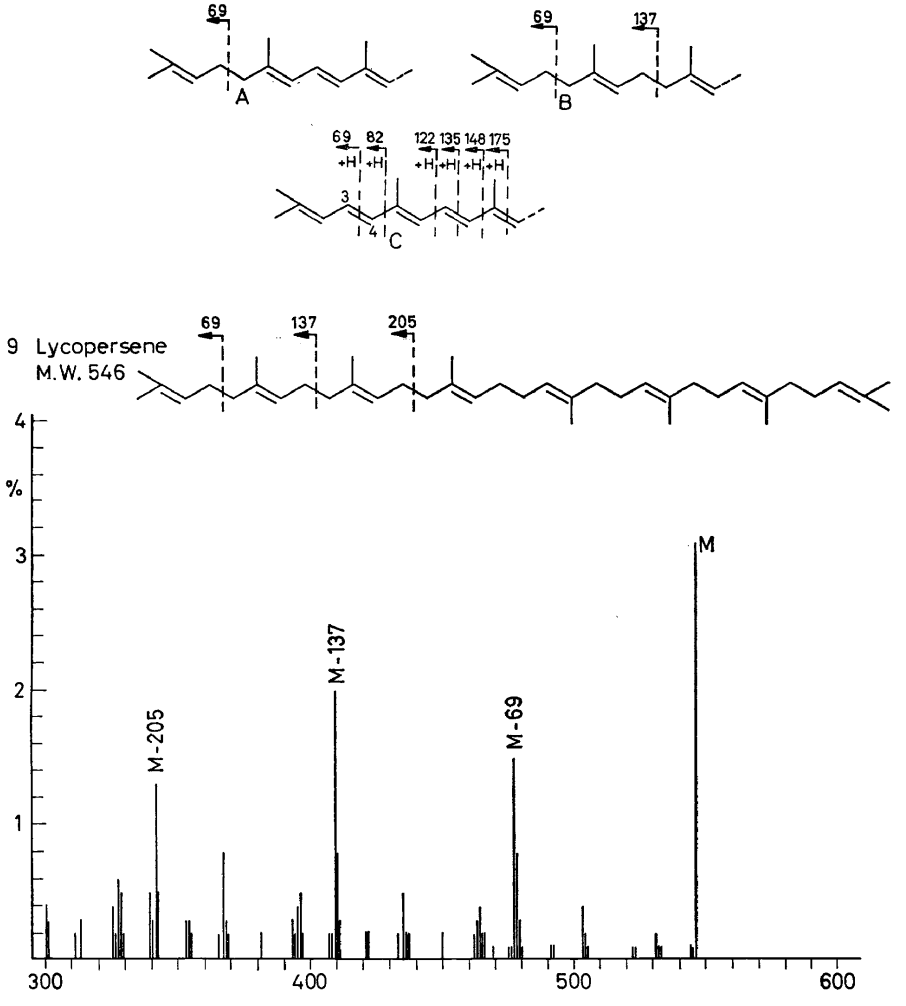
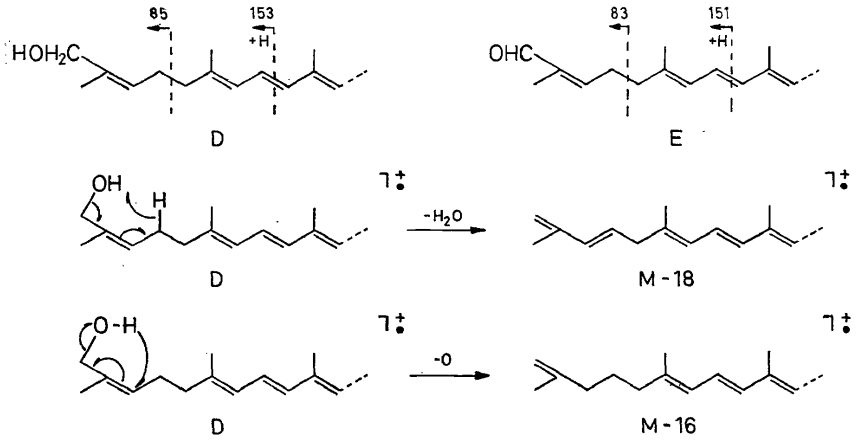


Figure 2. Mass spectrum of hycopersene

Dehydrogenation of the end group A gives the fully conjugated end group C of bisdehydrolycopene, which in addition to prominent M-92, M-106 and M-158 peaks exhibits groups of peaks corresponding to ions formed by cleavage of essentially every bond between position 3 and the centre of the chain. The strongest peak in each group represents a species where the cleavage is accompanied by hydrogen transfer from the larger, charged fragment.

Modification of the end group A by oxygenation of one of the terminal methyl groups giving D and E does not effect the cleavage of the 3,4-bond and thus characteristic M-85 and M-83 peaks are observed for the alcohol and the aldehyde respectively. It seems apparent, however, that introduction of oxygen facilities cleavage of the 7,8-bond with formation of M-153 and

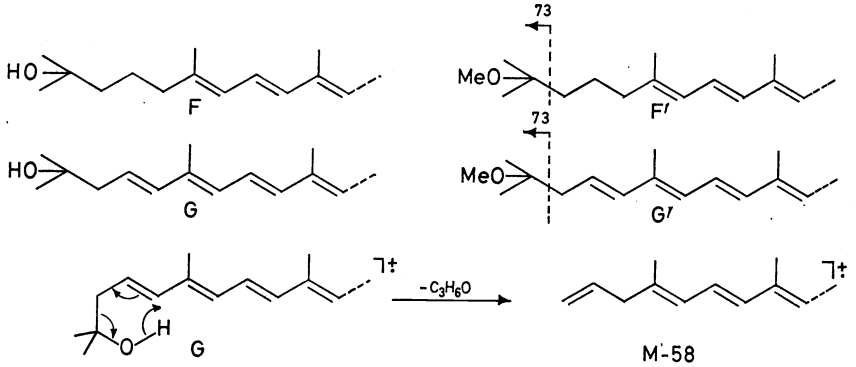


M-151 ions. A reason for this may be that the oxygen allows formation of a terminal, heterocyclic ring and the formation of these ions may occur by an analogous reaction to that discussed later for the cyclic compounds.

The presence of the hydroxyl group in D is evident from the appearance of abundant M-16 and M-18 ions, which are probably formed in the manner shown. The loss of an oxygen atom is unusual and does not seem to have been encountered for allylic alcohols previously. However, confirmation of this process was made by accurate mass measurement. The occurrence of M-28 and M-29 peaks in the spectra of compounds with the end group E provides further indication for the presence of this group.

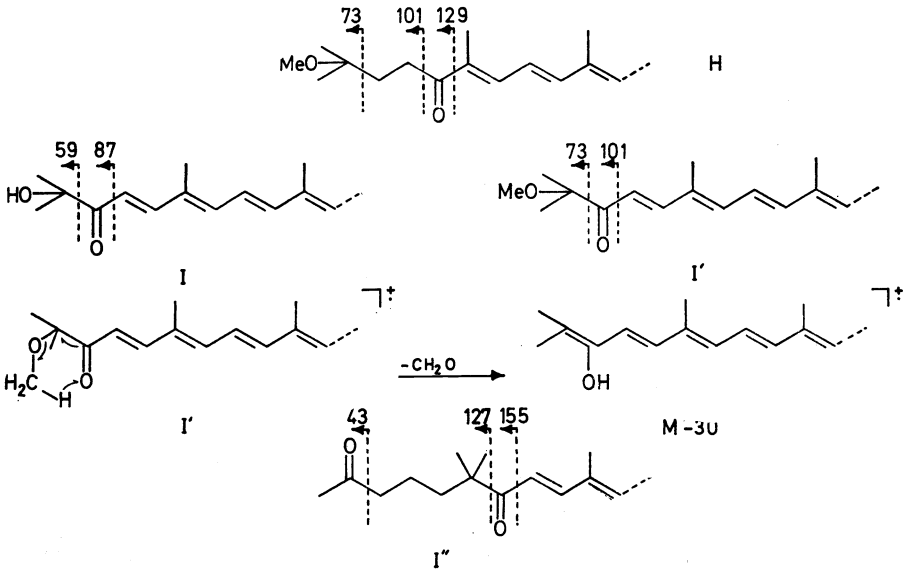
When the end group A is altered by hydration or addition of methanol to the 1,2-double bond to give F and F′, cleavage of the 3,4-bond is no longer favoured and the corresponding ions are absent. The presence of these end groups is therefore only revealed in the upper part of the spectrum by peaks due to the elimination of the functional group, which in the case of F is the M-18 peak and for F′ the M-30 and M-32 peaks. Evidence for the presence of F′ may however be obtained from the lower part of the spectrum since the smaller fragment, on cleavage of the 1,2-bond, gives rise to the base peak at m/e 73. There is no corresponding strong peak at m/e 59 in the case of F, and this may be attributed to the degree of charge localisation to the hetero atom in the two cases and to the different routes of fragmentation of ethers and alcohols.

When a double bond is introduced at the 3,4-position of end groups F and F′, cleavage of the 1,2-bond with retention of the charge by the larger fragment becomes feasible. In the case of end group G the cleavage is accompanied by hydrogen transfer, and the elimination of acetone evidently arises as the result of a McLafferty rearrangement. When the substituent is a methoxyl group, as in end group G′, rupture occurs without hydrogen transfer and a M-73 peak is observed as well as a strong m/e 73 peak. The corresponding peak at m/e 59 for G is prominent when both end groups are identical, but not when the other end group is less stable than G. The



elimination of these substituents together with hydrogen gives rise to prominent M-18 and M-32 peaks and the identification of these end groups is therefore simple.

Compounds with acyclic end groups incorporating an oxo function conjugated with the polyene chain all undergo cleavage of the bonds α to to the carbonyl group with charge retention by the larger fragment[4,9]. The observations agree with results obtained for other α,β-unsaturated ketones[10] and provide information about the position of the oxo group.

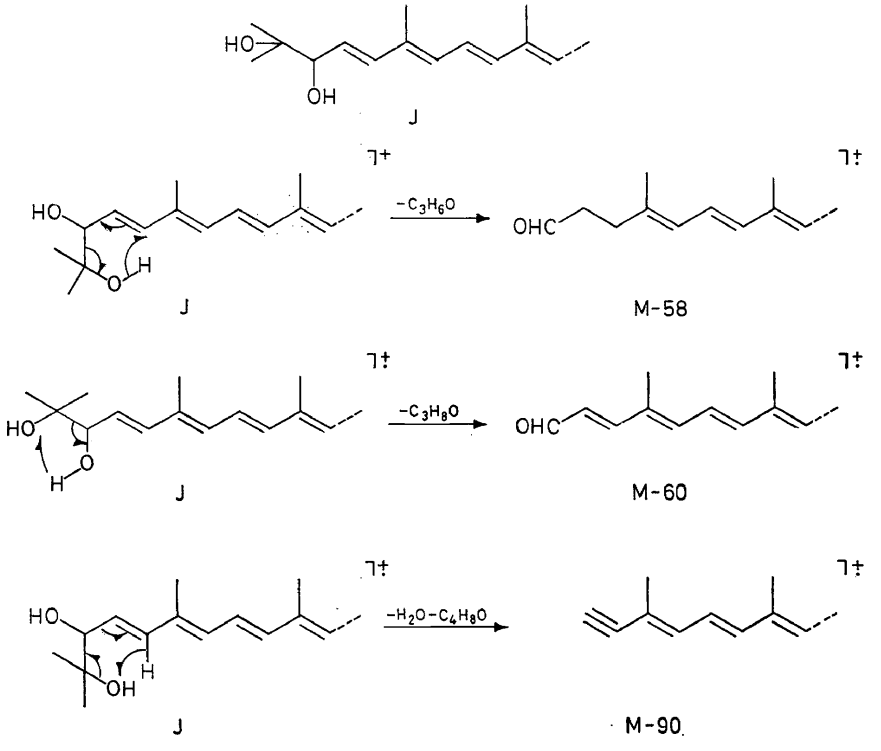


The existence of additional functionalities within the end group usually becomes obvious from the presence of other significant peaks. In the case of H the methoxyl function is revealed by a characteristic M-32 peak and a fairly prominent peak at m/e 73, which also provides evidence for the position of this substituent. The end group I gives rise to prominent M-16

and M-18 peaks, of which the former, as in the case of the allylic acyclic alcohols discussed here, may be associated with a particular position. A further feature of this end group is that cleavage of the 2,3-bond with hydrogen transfer to the smaller fragment giving an M-88 ion, is slightly favoured over the simple cleavage giving the M-87 species.

In end group I′ the presence of the methoxyl substituent is evident from peaks at M-30 and M-32. The former is obviously due to an ion formed through loss of formaldehyde by McLafferty rearrangement and hence indication is also given as to the position of the methoxy group. End group I″ shows a very intense m/e 43 peak which is evidently largely due to elimination of the acetyl fragment from the terminal part of this end group[9]. However, this is of little diagnostic value due to the common occurrence of strong peaks at this mass number. For reasons already mentioned the larger fragment formed on cleavage of the 1,2-bond in end groups H and I″ is not visible. It is also of interest that end group I″ does not give any ions formed by McLafferty rearrangement though the reaction would in this case be feasible from structural considerations.

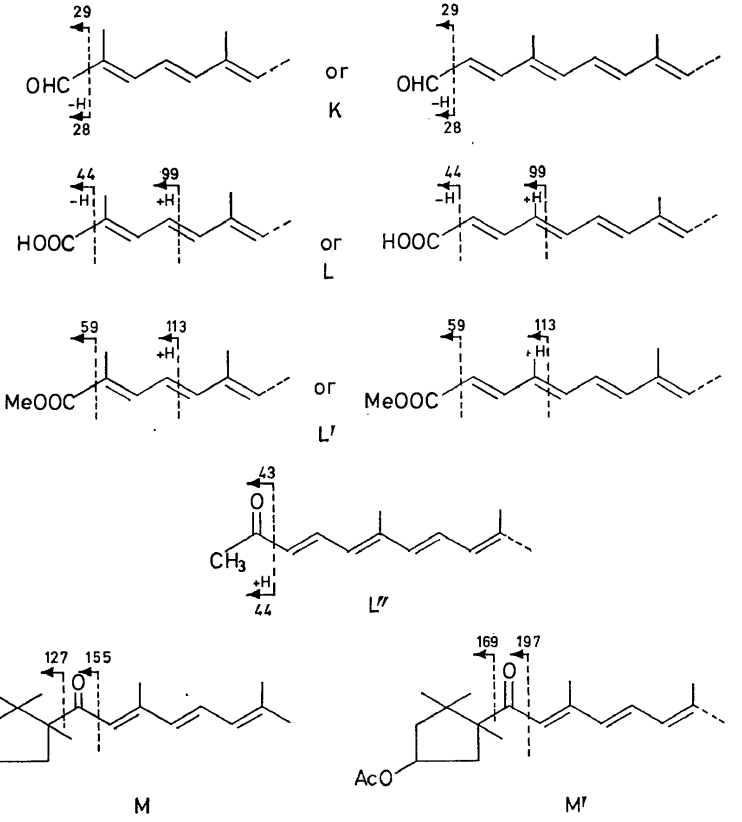
In the 1,2-diol system of end group J, cleavage of the 1,2-bond occurs readily and abundant M-58, M-59 and M-60 ions are formed, probably in the manner illustrated.



A fairly strong M-90 peak and a less intense M-88 peak demonstrate that the 2,3-bond also undergoes cleavage. A plausible mechanism for the loss of 90 mass units is shown above. Prominent M-16 and M-18 peaks are also observed, as one might expect when one of the hydroxyl groups is in an allylic position.

END GROUPS OF APO-CAROTENOIDS

All apo-carotenoids examined have end groups incorporating a carbon-oxygen double bond as the terminal unit of the conjugated system and as anticipated they undergo cleavage of the single bond linking this chromophore to the polyene chain. With the carboxyl group, and to a certain extent with the aldehyde group, cleavage is accompanied by hydrogen transfer to the larger fragment; the neutral fragments lost are respectively carbon dioxide and carbon monoxide. In addition, the acid and the methyl ester groups give rise to ions formed by cleavage of the δ-bond with transfer of hydrogen to the smaller neutral fragment; the mechanism for this reaction is probably similar to the one discussed below for cyclic compounds.

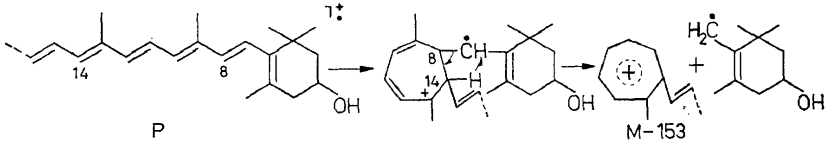


Cleavage of the α-bond on the side opposite to the polyene chain leading to a carbonyl containing ion is only observed for end groups L′, L″, M and M′. Thus, L′ exhibits M-31 and M-32 peaks, L″ a M-15 peak, M a M-127 peak and M′ a M-169 peak[4, 9]. The main reason for the absence of the corresponding ions in the case of the end groups K and L might be associated with difference in stability of the resulting radicals. The presence of the acetate group in M′ is evident from a prominent M-60 peak.

CYCLIC END GROUPS

The cyclic end groups are found to be surprisingly stable and contrary to expectation cleavage of in-chain bonds is favoured over fragmentation within the end group itself. Moreover, the introduction of oxygen substituents usually reinforces this effect.

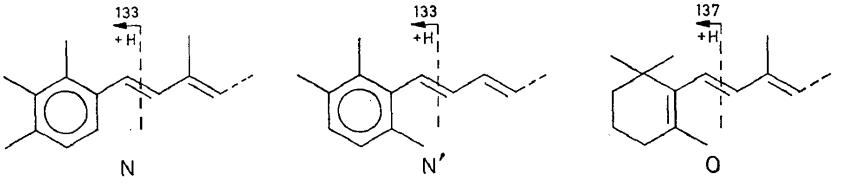
Rupture of the 7,8-, 9,10- and 11,12-bonds accompanied by transfer of a hydrogen to the smaller fragment is of general occurrence though the abundance of the ions so formed varies markedly, partly as a result of the character of the other end group present. A plausible mechanism for these reactions is illustrated by 7,8-bond cleavage in the end group P, as shown below. Although the results obtained for 7,7′-d_2-renierapurpurin are consistent with this mechanism further labelling would be required to establish the origin and degree of specificity of hydrogen transfer in these reactions.



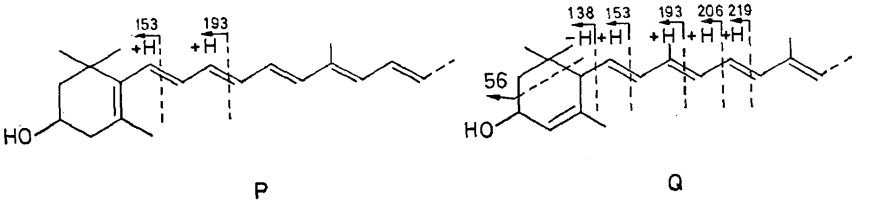
Of diagnostic significance, the only carbon-carbon bond cleavages in the ring are the *retro*-Diels Alder reaction in the case of the 4,5-enes, the loss of 56 mass units from the α,β-unsaturated ketones, and the fragmentation reactions of end group T (see following illustrations). Peaks corresponding to the loss of hetero substituents such as hydroxyl, methoxyl and acetoxyl groups together with hydrogen are always observed and are diagnostically important. However, the loss of oxygen from allylic alcohols, which is of importance in the case of the acyclic end groups, is not significant in the cyclic counterparts studied here.

Though one can not differentiate between the aromatic end groups N and N′ by mass spectrometry, their presence is readily demonstrated by a M-133 peak, and a m/e 133 peak which is the strongest peak in the lower half of the spectra. The corresponding ions are formed by cleavage of the 7,8-bond accompanied by the transfer of a hydrogen to the smaller fragment. The peaks associated with the cleavage of the 9,10- and 11,12-bonds are also observed, but are usually not significant.

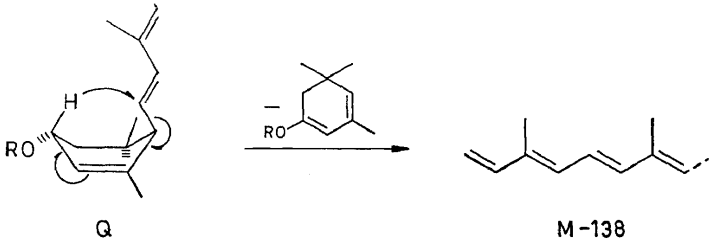
The end group O is clearly the most difficult one to detect directly and though it undergoes the expected cleavages when the complimentary end group is of reasonable stability, it is in many cases only possible to see the fragment due to 7,8-bond cleavage. In fact in one of the six compounds investigated even this peak is invisible. In this case a suitable derivative such as the epoxide must be examined.



The end group P is best recognised in the present set of compounds by the M-153 and M-193 peaks, which in the corresponding acetates are shifted in the expected manner.



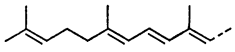
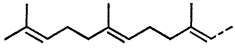
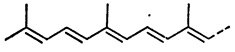
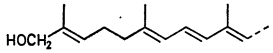
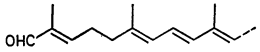
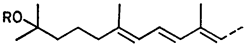
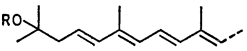
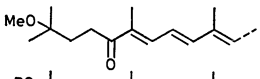
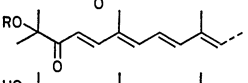
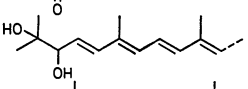
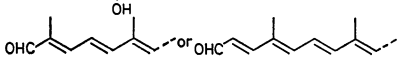
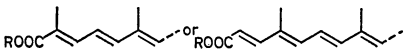
The end group Q and the corresponding methyl ether and acetate undergo the in-chain cleavages depicted above. It is of interest that cleavage of the 6,7-bond occurs with transfer of hydrogen to the larger fragment and thus in a direction opposite to that encountered in the other cleavages. A plausible reason for this may be found by invoking the following tentative mechanism for the reaction:



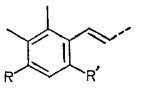
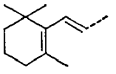
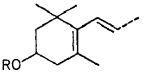
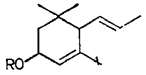
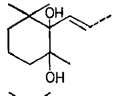
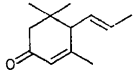
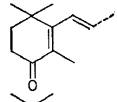
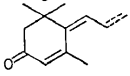
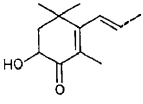
The M-56 ions associated with the *retro*-Diels-Alder fragmentation are observed for the alcohol and the methyl ether, but not for the acetate. This is apparently due to the fact that elimination of the acetate group is strongly favoured.

The end group T gives rise to significant peaks at M-98, M-127, M-155 and m/e 109 (100 per cent). The last three ions are probably formed in the manner shown below and the mechanisms invoked are supported by nearly complete shifts of the M-127 and M-155 peaks by two mass units and by quantitative retention of the m/e 109 peak on exchange of the two hydroxyl hydrogens for deuterium. Moreover, the formation of the m/e 109 ion from

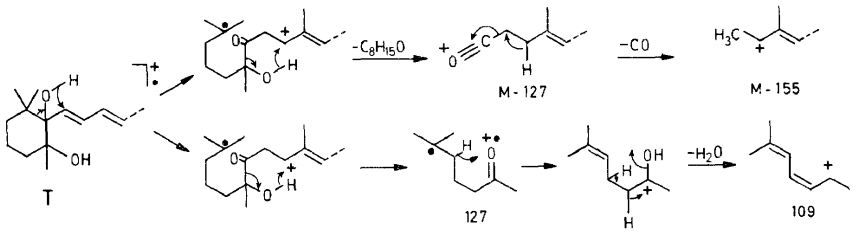
Table 4. Peaks of diagnostic value

End group	*Characteristic peak*
 A	M-69, (69)
 B	M-137, M-69, (69)
 C	M-69, M-82, M-122, M-135, M-148, M-175
 D	M1-6, M-18, M-85, M-153
 E	M-28, M-29, M-83, M-151
 F (R = H) F′ (R = Me)	M-18 M-30, M-32, 73
 G (R = H) G′ (R = Me)	M-18, M-58 M-32, M-73, 73
 H	M-32, M-101, M-129, 73
 I (R = H) I′ (R = Me)	M-16, M-18, M-59, M-87, M-88 M-30, M-32, M-60, M-73, M-101, 73
 J	M-16, M-18, M-58, M-59, M-60, M-88, M-90
 K	M-18, M-28, M-29
 L (R = H) L′ (R = Me)	M-44, M-99 M-31, M-59, M-113

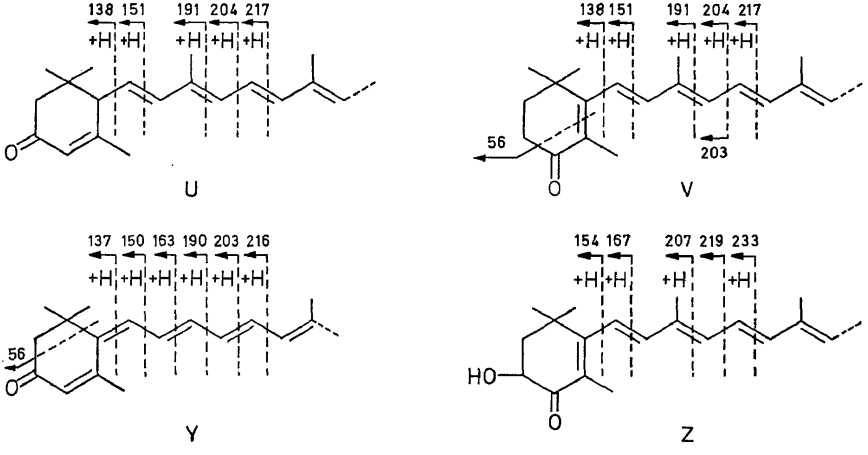
in the mass spectra of carotenoids

End group		*Characteristic peak*
	N (R=Me, R′=H) N′ (R=H, R′=Me)	M-133, 133 M-133, 133
	O	(M-137)
	P (R=H) P′ (R=Ac)	M-18, M-153, M-193 M-60, M-195, M-235
	Q (R=H) Q′ (R=Me) Q″ (R=Ac)	M-18, M-56, M-138, M-153, M-193, M-206, M-219 M-32, M-56, M-152, M-167, M-207, M-220, M-233 M-60, M-180, M-195, M-235, M-248, M-261
	T	M-18, M-36, M-98, M-127, M-155, 109
	U	M-138, M-151, M-191, M-204, M-217
	V	M-56, M-138, M-151, M-191, M-203, M-204, M-217
	Y	M-56, M-137, M-150, M-163, M-190, M-203, M-216
	Z	M-16, M-154, M-167, M-207, M-219, M-233

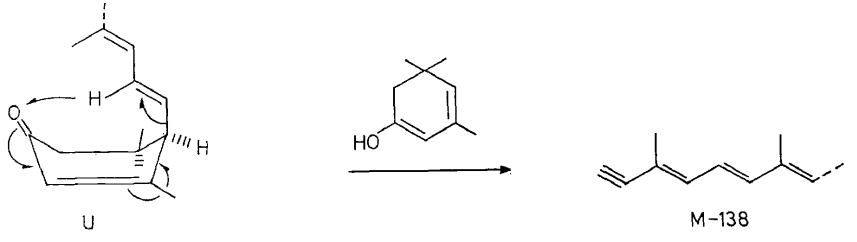
the m/e 127 precursor is substantiated by a metastable ion of appropriate position. The labelling results also show that the formation of the M-18 and M-98 species involves quantitative (M95 per cent) loss of both hydroxyl hydrogens. However, further evidence is required to explain the latter process, which probably involves skeletal rearrangements.



The end groups U, V, Y and Z undergo the cleavages indicated below, and involve in nearly all cases the same bonds as in end group Q.



However, the accompanying hydrogen rearrangements are not the same in all cases and an interesting difference, which may be ascribed to the presence of a keto instead of hydroxyl group, is that cleavage of the 6,7-bond now occurs with transfer of hydrogen to the smaller fragment. A plausible but unsubstantiated mechanism for this reaction in the case of end group U is given below.



The end groups v and y both give rise to a prominent M-56 peak. In the former case the corresponding species may be ascribed to an ion formed by elimination of a C(4)-C(5) fragment in analogy with fragmentation established for simpler cyclohexenones[11], while in the latter case the M-56 ion probably arises by loss of a C(1)-C(2) fragment as in the end group q. A characteristic peak at M-16 is observed for end group z and this is evidently due to the loss of oxygen from the hydroxyl group in a position α to the 4-oxo group.

CONCLUDING REMARKS

In conclusion it may be said that it is possible to obtain information from mass spectra of carotenoids concerning the end groups (*Table 4*) and the length of the polyene chain (*Table 1* and *2*). Moreover, when considering oxygenated carotenoids differentiation can usually be made between end group and in-chain methyl positions and in the latter case the site can often be precisely located. It is evident therefore that in a number of cases the structure of a carotenoid may be determined from its mass spectrum alone and in other cases the information will considerably reduce the number of possibilities that have to be considered.

I would like to emphasise that the work discussed here is the result of a joint study in which I have had the great pleasure of collaborating with Dr Liaaen-Jensen at the Organic Chemistry Department of Norway Institute of Technology, and Mr G. Francis, now working at the same department. Mr H. Kjøsen and Mr F. Imsgaard of this department have synthesised respectively the deuterated carotenes and the compounds with hetero substituents on the polyene chain. The latter were obtained using a cross-conjugated dial supplied by Dr H. Kralt, Phillips-Duphar, Holland.

References

[1] R. N. Jeffrey and R. B. Griffith. *Plant Physiol.* **20**, 34 (1945).
[2] S. Isoe, S. Be Hyeon and T. Sakan. *Tetrahedron Letters* 279 (1969).
[3] C. R. Enzell, G. W. Francis and S. Liaaen-Jensen. *Acta Chem. Scand.* **22** (1968).
[4] C. R. Enzell, G. W. Francis and S. Liaaen-Jensen. *Acta Chem. Scand.* **23** in press (1969).
[5] U. Schwieter, M. R. Bolliger, L. H. Chopard-dit-Jean, G. Englert, M. Kofler, A. König, C. v. Planta, R. Rüegg, W. Vetter and O. Isler. *Chimia* **19** ,294 (1965).
[6] I. Baldas, Q. N. Porter, L. Cholnoky, J. Szabolcs and B. C. L. Weedon. *Chem. Commun.* 852 (1966).
[7] H. Mayer, M. Montavon, R. Rüegg and O. Isler. *Helv. Chim. Acta* **50**, 1606 (1967).
[8] A. Mandelbaum and K. Biemann. *J. Am. Chem. Soc.* **90**, 2975 (1968).
[9] G. W. Francis, unpublished results.
[10] H. Budzikiewicz, C. Djerassi and D. H. Williams. *Mass Spectrometry of Organic Compounds*, Vol. 2 Holden-Day, pp. 5–49. San Francisco (1967).
[11] *ibid.* pp. 143–154.