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Mass Spectrometric Studies of Carotenoids

2. A Survey of Fragmentation Reactions

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The mass spectra of a number of selected carotenoids, mainly of natural occurrence, have been recorded and are given in Figs. 1–41. The majority of the end groups give rise to characteristic peaks by which they may be identified. In the case of cyclic end groups these peaks are due mainly to ions formed by cleavages in the polyene chain, while for acyclic end groups they are due to ions formed chiefly by cleavages in the end group. Mass spectrometry is shown to provide a useful tool for structural elucidation in this series of compounds.

Previous systematic studies on electron impact induced fragmentation of carotenoids have been limited to work by Schwieter *et al.*[1] on carotenes and by Baldas *et al.*[2] on carotenoid epoxides and furanoid oxides. No general survey has appeared in the literature in spite of the considerable amount of information that could evidently be obtained, and the very small amounts of material required. The present investigation was therefore undertaken to fill this gap and to provide a basis for structural elucidation of carotenoids by mass spectrometry. It includes sixteen acyclic, seven monocyclic, nine bicyclic, and nine apo-carotenoids, varying widely in structure and selected mainly from carotenoids of natural occurrence and of well-established structure.

Schwieter *et al.*[1] have found that a characteristic feature of the fragmentation of the carotenes is the formation of prominent M−92 and M−106 ions. They have ascribed the formation of these ions, which have later been encountered in the fragmentation of the carotenoid epoxides and furanoid oxides,[2] to the elimination of part of the polyene chain of the carotenoid skeleton and have proposed a mechanism to account for these losses. These

ions occur in all the C_{40}-carotenoids possessing a conjugated polyene chain which are included in this study and may thus now be regarded as characteristic of this group of compounds. As shown in our preliminary communication [3] the intensities of the peaks at M−92 and M−106 are related to the number of conjugated double bonds in the acyclic polyene chain and the ratio (M−92) (M−106) provides evidence for the chain length of carotenoids containing no more than one oxygen substituent in each end group.

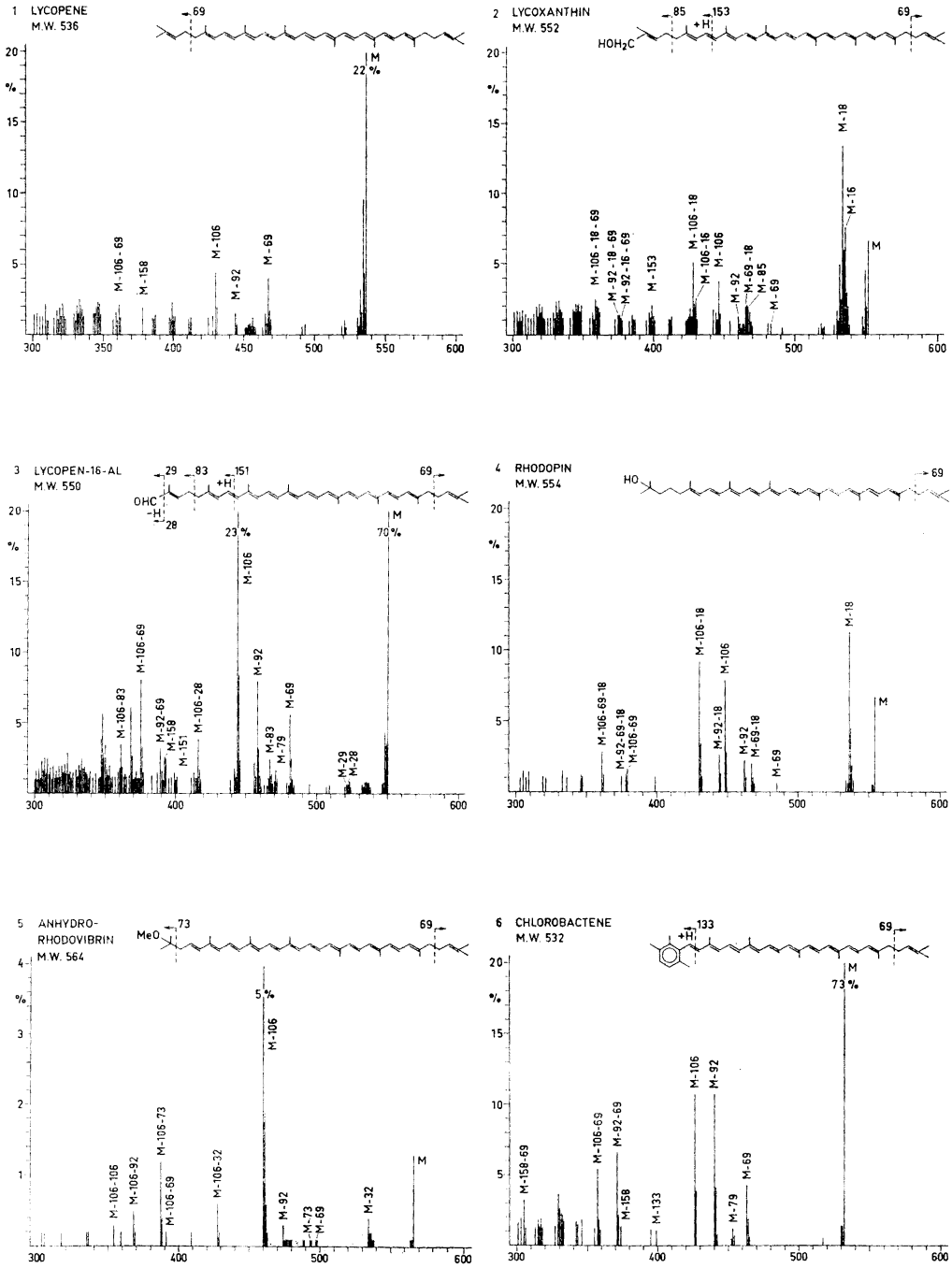
RESULTS

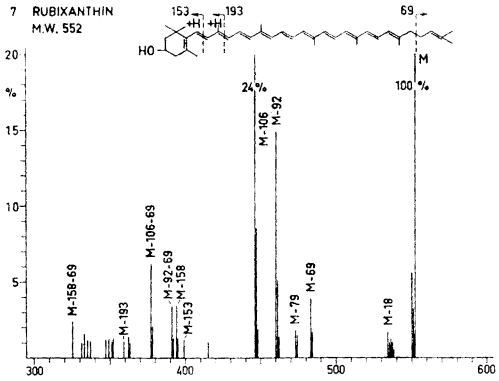
The upper part of the spectra are reproduced in full (the structures of the various compounds are to be found with the corresponding spectra in Figs. 1−41), and the peaks of interest in the lower parts of the spectra are detailed in Table 1. Judging by the information available to date the upper part is sufficient to identify a particular compound in nearly all cases, even when different instruments are used; the spectra are found reproducible over several months on the same machine.

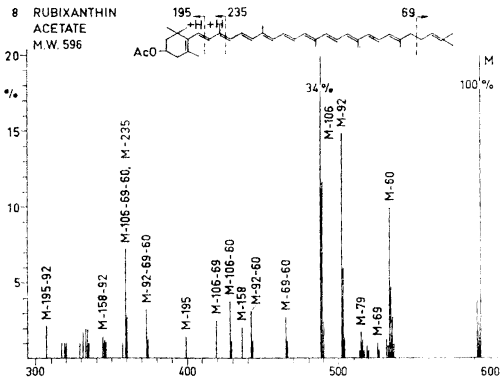
In agreement with the observations of Schwieter *et al.*[1] most of the compounds gave metastable ions corresponding to the loss of 92 mass units from the molecular ion and certain fragment ions, but no metastable peaks were observed for any reaction involving the loss of 106 mass units. Metastable ions were also observed for several other fragmentations but these will be included when of interest in the subsequent discussion.

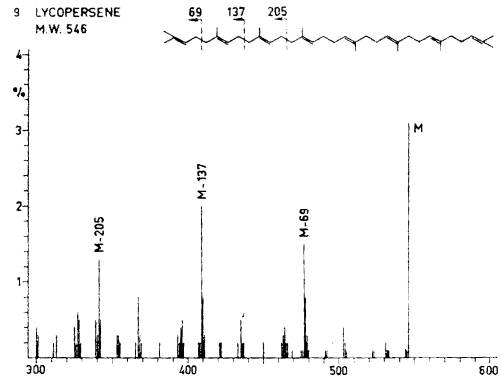
Several of the compounds studied here give rise to prominent M−2 ions formed by dehydrogenation of the molecular ion, and in addition similar losses are observed from other ions. Since there was a very noticeable difference in the intensity of the M−2 peak in the spectrum of lycopene obtained by us and by Schwieter *et al.*[1] this compound was examined on several occasions over a period of two months at various temperatures of the ion-source (250−350°) to determine whether this factor was of importance, and whether variation in the condition of the ion-source itself had any bearing. Since very little variation was observed in this way it appears that the difference must be ascribed to other instrumental factors, *e.g.* different geometry of the ion-source, noticeably the placing of the hot filament. When the intensity of the M peak is high (30−100 %) the intensity of the M−2 peak is usually of the order of one tenth of the M peak, while when the intensity of the M peak is low the (M−2)/M ratio is frequently increased markedly and may on occasion exceed 1. Since on the basis of the compounds investigated so far there is no obvious connection between the occurrence and intensity of the M−2 peak and the molecular structure, this peak will not be further discussed.

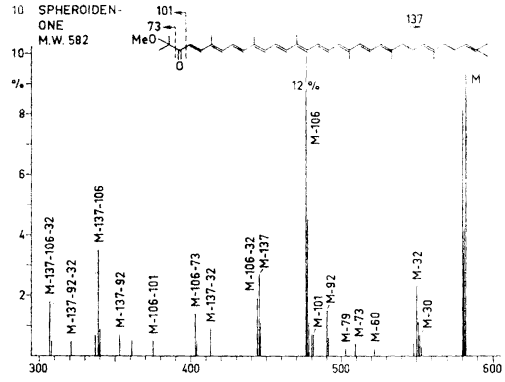
In the subsequent discussion dealing with the fragmentation reactions of the individual end groups and the utility of the various peaks for structural elucidation these groups have been divided into three main categories — acyclic, apo-type, and cyclic.

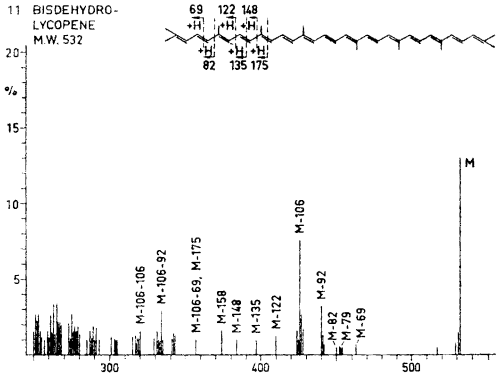

1 LYCOPENE
M.W. 536
2 LYCOXANTHIN
M.W. 552
3 LYCOPEN-16-AL
M.W. 550
4 RHODOPIN
M.W. 554
5 ANHYDRO-
RHODOVIBRIN
M.W. 564
6 CHLOROBACTENE
M.W. 532

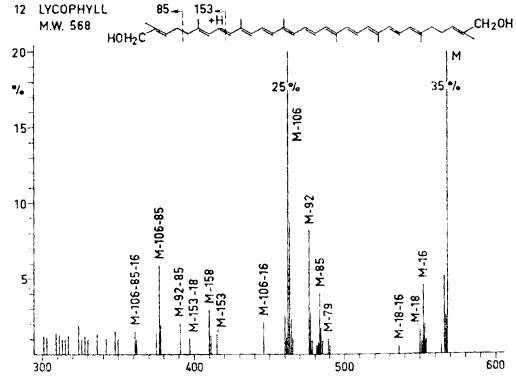

7 RUBIXANTHIN
M.W. 552
153
193
69
HO
M
100 %
24 %
M-106
M-92
M-106-69
M-92-69
M-158
M-153
M-158-69
M-193
M-79
M-69
M-18
%
20
15
10
5
300
400
500
600

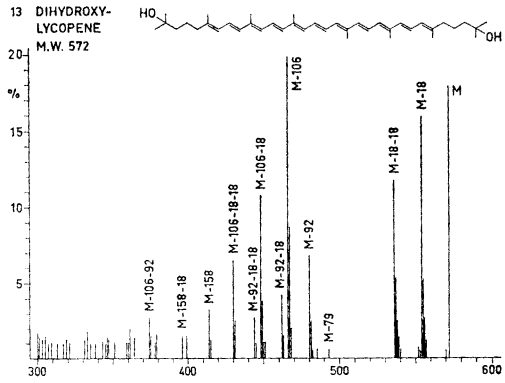

8 RUBIXANTHIN ACETATE
M.W. 596
195
235
69
AcO
M
100 %
34 %
M-106
M-92
M-60
M-105-69-60, M-235
M-92-69-60
M-195-92
M-158-92
M-195
M-106-69
M-106-60
M-158
M-92-60
M-69-60
M-79
M-69
%
20
15
10
5
300
400
500
600

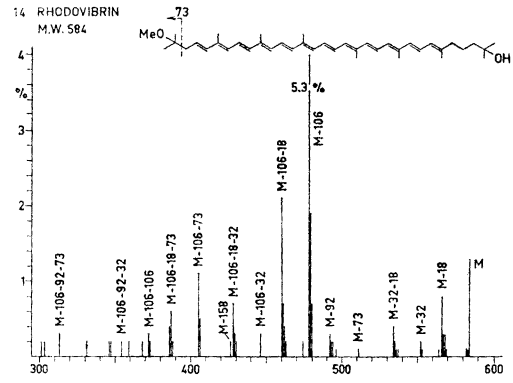

9 LYCOPERSENE
M.W. 546
69
137
205
M
M-137
M-69
M-205
%
4
3
2
1
300
400
500
600

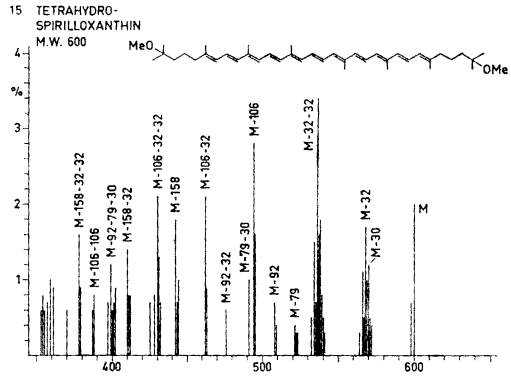

10 SPHEROIDEN-ONE
M.W. 582
101
73
MeO
O
137
M
12 %
M-106
M-137-106-32
M-137-92-32
M-137-106
M-137-92
M-106-101
M-106-73
M-137-32
M-106-32
M-137
M-101
M-92
M-79
M-73
M-60
M-32
M-30
%
10
8
6
4
2
300
400
500
600

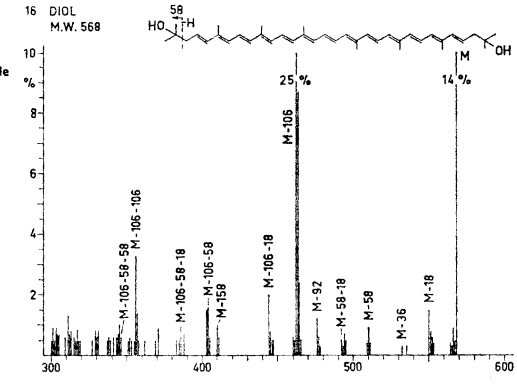

11 BISDEHYDRO-LYCOPENE
M.W. 532
69
122
148
82
135
175
M
35 %
M-106
M-92
M-106-106
M-106-92
M-106-69, M-175
M-158
M-148
M-135
M-122
M-82
M-79
M-69
%
20
15
10
5
300
400
500

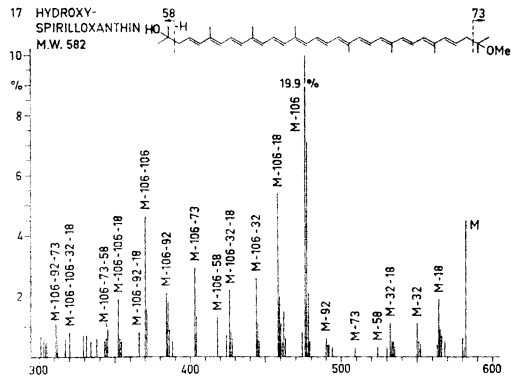

12 LYCOPHYLL
M.W. 568
85
153
HOH2C
CH2OH
M
25 %
35 %
M-106
M-92
M-106-85
M-106-85-16
M-92-85
M-153-18
M-158
M-153
M-106-16
M-85
M-79
M-18-16
M-18
M-16
%
20
15
10
5
300
400
500
600

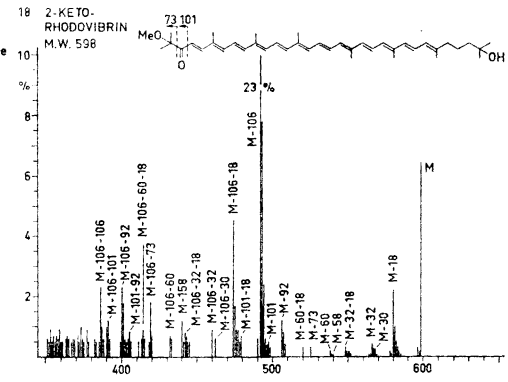

13 DIHYDROXY-
LYCOPENE
M.W. 572
HO
OH
%
M-106-92
M-158-18
M-158
M-106-18-18
M-92-18-18
M-105-18
M-92-18
M-106
M-92
M-79
M-18-18
M-18
M
300
400
500
600

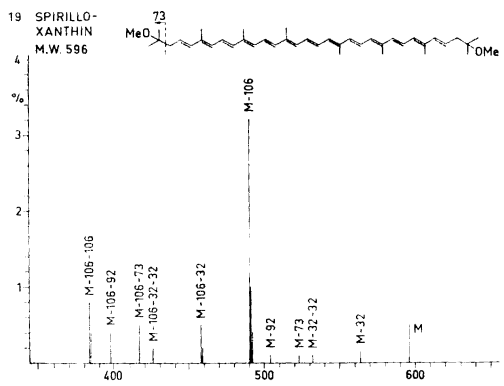

14 RHODOVIBRIN
M.W. 584
MeO
73
OH
5.3 %
M-106-92-73
M-106-92-32
M-106-106
M-106-18-73
M-106-73
M-158
M-106-18-32
M-106-32
M-106-18
M-106
M-92
M-73
M-32-18
M-32
M-18
M
300
400
500
600

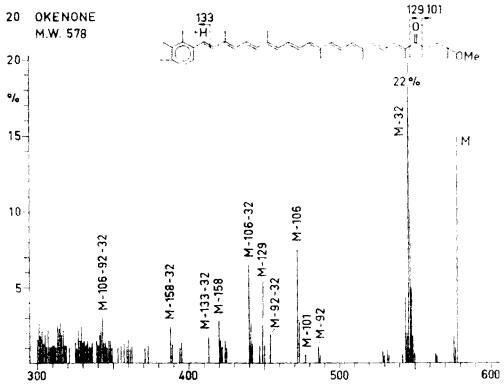

15 TETRAHYDRO-
SPIRILLOXANTHIN
M.W. 600
MeO
OMe
%
M-158-32-32
M-106-106
M-92-79-30
M-158-32
M-106-32-32
M-158
M-106-32
M-92-32
M-79-30
M-106
M-92
M-79
M-32-32
M-32
M-30
M
400
500
600

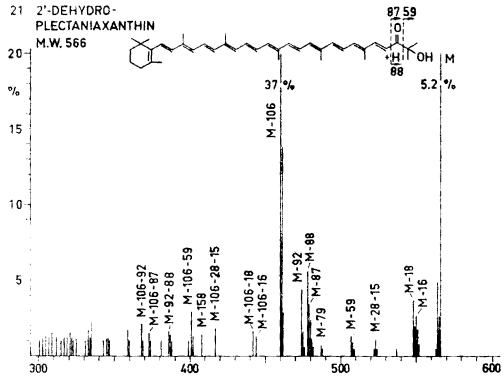

16 DIOL
M.W. 568
58
HO
H
OH
25 %
14 %
M-106-58-58
M-106-106
M-106-58-18
M-106-58
M-158
M-106-18
M-106
M-92
M-58-18
M-58
M-36
M-18
M
300
400
500
600

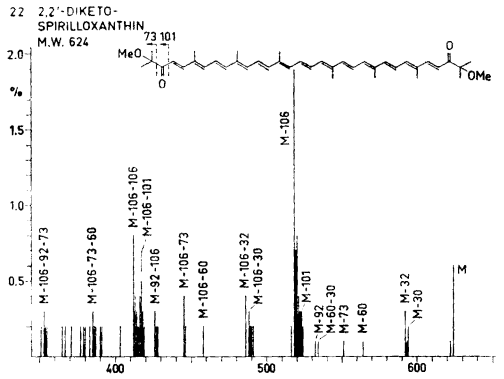

17 HYDROXY-
SPIRILLOXANTHIN
M.W. 582
58
73
HO
H
OMe
19.9 %
M-106-92-73
M-106-106-32-18
M-106-73-58
M-106-106-18
M-106-92-18
M-106-106
M-106-92
M-106-73
M-106-58
M-106-32-18
M-106-32
M-106-18
M-106
M-92
M-73
M-58
M-32-18
M-32
M-18
M
300
400
500
600

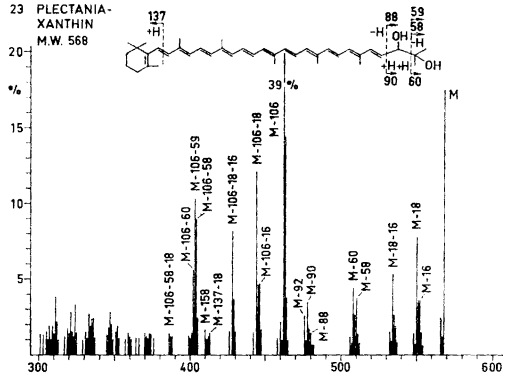

18 2-KETO-
RHODOVIBRIN
M.W. 598
73 101
MeO
O
OH
23 %
M-106-106
M-106-101
M-106-92
M-101-92
M-106-60-18
M-106-73
M-106-60
M-158
M-106-32-18
M-106-32
M-106-30
M-106-18
M-101-18
M-106
M-101
M-92
M-60-18
M-73
M-60
M-58
M-32-18
M-32
M-30
M-18
M
400
500
600

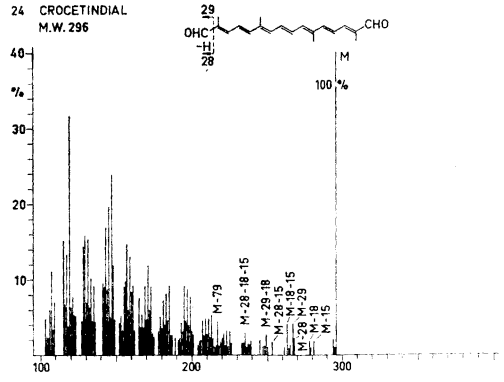

19 SPIRILLO-
XANTHIN
M.W. 596
73
MeO
OMe
%
M-106
M-106-106
M-106-92
M-106-73
M-106-32-32
M-106-32
M-92
M-73
M-32-32
M-32
M

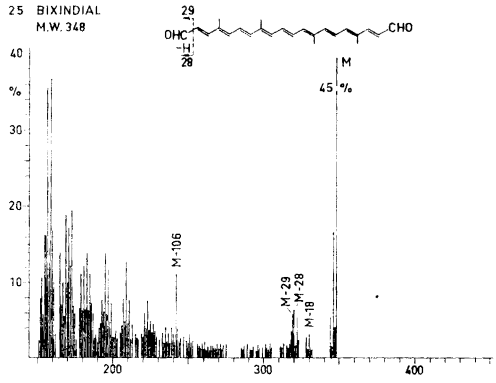

20 OKENONE
M.W. 578
133
129 101
OMe
22 %
M-32
M
%
M-106-92-32
M-158-32
M-133-32
M-158
M-106-32
M-129
M-92-32
M-106
M-101
M-92

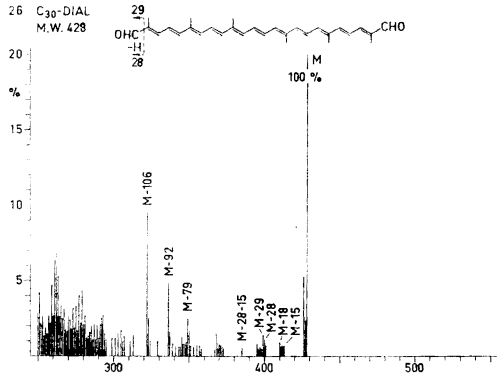

21 2'-DEHYDRO-
PLECTANIAXANTHIN
M.W. 566
87 59
OH
M
88
37 %
5.2 %
%
M-106
M-106-92
M-106-87
M-92-88
M-106-59
M-158
M-106-28-15
M-106-18
M-106-16
M-92
M-87
M-88
M-79
M-59
M-28-15
M-18
M-16

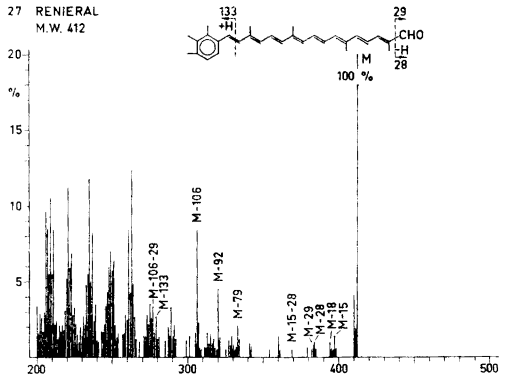

22 2,2'-DIKETO-
SPIRILLOXANTHIN
M.W. 624
73 101
MeO
OMe
%
M-106
M-106-92-73
M-106-73-60
M-106-106
M-106-101
M-92-106
M-106-73
M-106-60
M-106-32
M-106-30
M-101
M-92
M-60-30
M-73
M-60
M-32
M-30
M

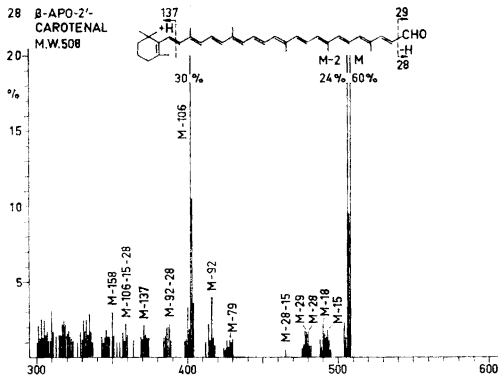

23 PLECTANIA-
XANTHIN
M.W. 568
137
88
59
OH
90
60
39 %
M
%
M-106
M-106-18
M-106-59
M-106-58
M-106-18-16
M-106-60
M-106-58-18
M-106-16
M-158
M-137-18
M-92
M-90
M-88
M-60
M-59
M-18-16
M-18
M-16

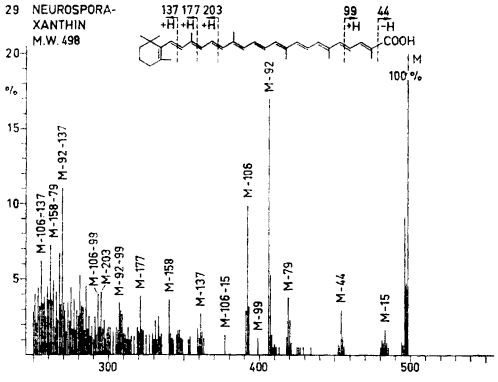

24 CROCETINDIAL
M.W. 296
29
OHC
CHO
28
M
100 %
%
M-79
M-28-18-15
M-29-18
M-28-15
M-18-15
M-28
M-29
M-15

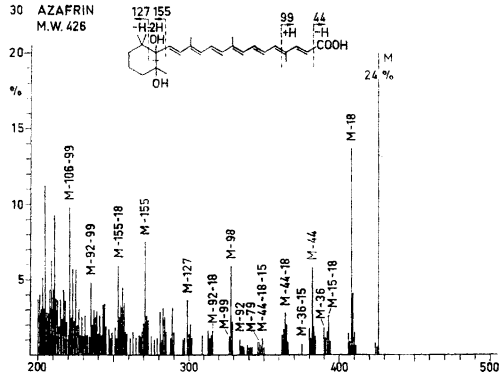

25 BIXINDIAL
M.W. 348
M
45 %
M-106
M-29
M-28
M-18

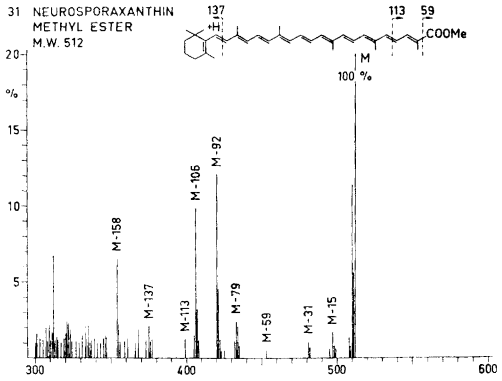

26 C30-DIAL
M.W. 428
M
100 %
M-106
M-92
M-79
M-28-15
M-29
M-28
M-18
M-15

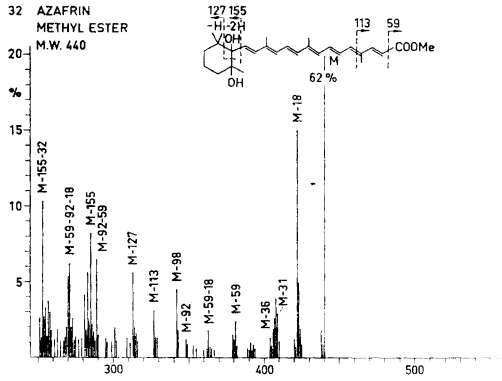

27 RENIERAL
M.W. 412
M
100 %
M-106
M-106-29
M-133
M-92
M-79
M-15-28
M-29
M-28
M-18
M-15

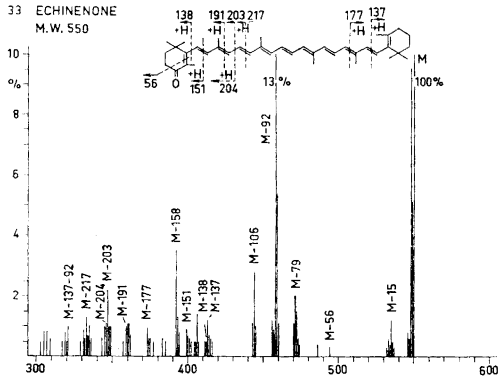

28 β-APO-2'-
CAROTENAL
M.W.508
M-2
M
24 % 60 %
30 %
M-106
M-158
M-106-15-28
M-137
M-92-28
M-92
M-79
M-28-15
M-29
M-28
M-18
M-15

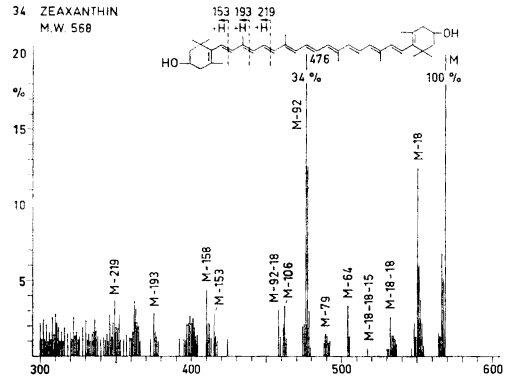

29 NEUROSPORA-
XANTHIN
M.W. 498
M
100 %
M-92
M-92-137
M-106-137
M-158-79
M-106-93
M-203
M-92-99
M-177
M-158
M-137
M-106
M-106-15
M-99
M-79
M-44
M-15

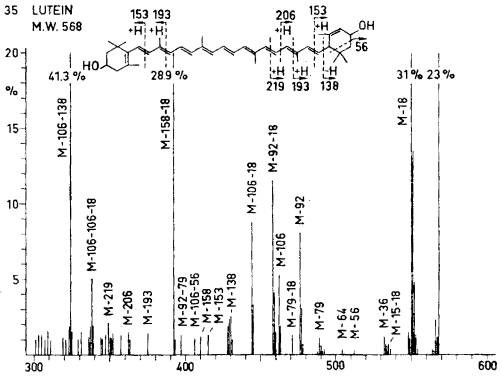

30 AZAFRIN
M.W. 426
M
24 %
M-18
M-106-93
M-92-99
M-155-18
M-155
M-127
M-98
M-92-18
M-99
M-92
M-79
M-44-18-15
M-44-18
M-36-15
M-44
M-36
M-15-18


31 NEUROSPORAXANTHIN
METHYL ESTER
M.W. 512
137
113
59
COOMe
M
100 %
M-158
M-137
M-113
M-106
M-92
M-79
M-59
M-31
M-15


32 AZAFRIN
METHYL ESTER
M.W. 440
127 155
113
59
COOMe
OH
M
62 %
M-18
M-155-32
M-59-92-18
M-155
M-92-59
M-127
M-113
M-98
M-92
M-59-18
M-59
M-36
M-31


33 ECHINENONE
M.W. 550
138
191 203 217
177 137
56
O
151
204
M
100%
13 %
M-92
M-158
M-106
M-79
M-15
M-56
M-137-92
M-217
M-204
M-203
M-191
M-177
M-151
M-138
M-137


34 ZEAXANTHIN
M.W. 568
153 193 219
OH
HO
476
M
34 %
100 %
M-92
M-18
M-219
M-193
M-158
M-153
M-92-18
M-106
M-79
M-64
M-18-15
M-18-18


35 LUTEIN
M.W. 568
153 193
206
153
OH
56
HO
41.3 %
28.9 %
219 193
138
31 % 23 %
M-106-138
M-158-18
M-92-18
M-18
M-106-18
M-106-106-18
M-219
M-206
M-193
M-92-79
M-106-56
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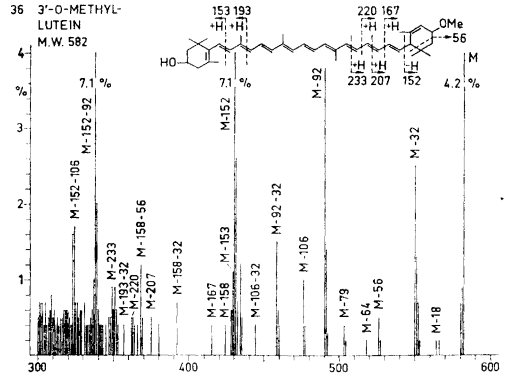
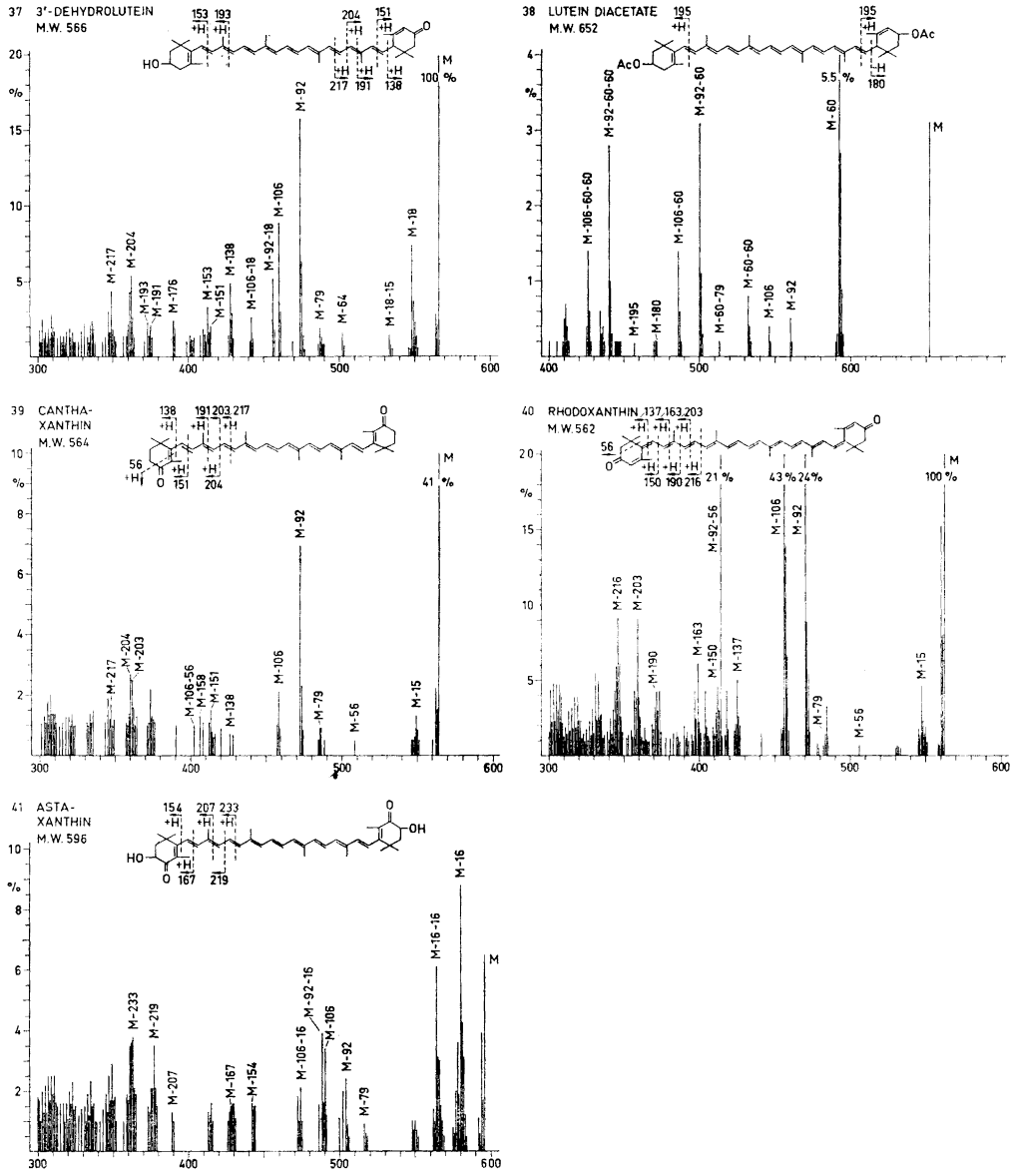
M-92
M-79
M-64
M-56
M-36
M-15-18


36 3'-O-METHYL-
LUTEIN
M.W. 582
153 193
220 167
OMe
56
HO
233 207
152
7.1 %
7.1 %
4.2 %
M-92
M-152-92
M-152-106
M-152
M-32
M-233
M-158-56
M-193-32
M-220
M-207
M-158-32
M-92-32
M-153
M-167
M-158
M-106-32
M-106
M-79
M-64
M-56
M-18



The base peaks in spectra 1—41 occur in most cases in the lower part of the spectra. Peaks that appear as base peak in at least one of the compounds examined and the intensities of these peaks in each spectrum are given in Table 1. Although other peaks of high intensity occur in the lower part of the spectra these are not given here since they appear to be of little diagnostic value.

		Compound Trivial or semisystematic name *	End groups	Relative intensity 43	59	69	73	83	91	109	133	M
Acyclic and Monocyclic	1	Lycopene	$A+A$	16	0	100	1	8	47	19	22	22
	2	Lycoxanthin	$A+D$	30	0	82	1	8	100	24	29	7
	3	Lycopen-16-al	$A+E$	81	3	100	10	40	69	25	31	70
	4	Rhodopin	$A+F$	1	6	100	3	30	30	26	21	7
	5	Anhydrorhodovibrin	$A+G'$	4	0	10	96	1	100	2	2	1
	6	Chlorobactene	$A+N$	10	0	50	1	4	38	7	100	73
	7	Rubixanthin	$A+P$	16	1	65	1	9	34	17	26	100
	8	Rubixanthin acetate	$A+P'$	10	0	19	0	3	20	5	10	100
	9	Lycopersene	$B+B$	4	0	100	0	5	3	12	3	3
	10	Spheroidenone	$B+I'$	5	0	27	100	1	25	2	6	9
	11	Bisdehydrolycopene	$C+C$	2	0	21	1	4	100	16	11	13
	12	Lycophyll	$D+D$	100	1	35	1	8	81	19	6	35
	13	Dihydroxylycopene	$F+F$	27	20	77	1	9	100	25	28	18
	14	Rhodovibrin	$F+G'$	4	2	5	100	1	50	2	3	1
	15	Tetrahydrospirilloxanthin	$F'+F'$	3	3	53	100	9	59	22	23	2
	16	Diol	$G+G$	23	100	15	2	4	67	6	14	14
	17	Hydroxyspirilloxanthin	$G+G'$	5	13	3	100	1	88	2	5	5
	18	2-Ketorhodovibrin	$G+I'$	6	3	1	100	1	85	4	6	6
	19	Spirilloxanthin	$G'+G'$	3	0	1	100	0	16	1	1	1
	20	Okenone	$H+N'$	2	4	51	35	7	80	7	100	15
	21	2,-Dehydroplectaniaxanthin	$I+O$	48	36	37	26	13	100	16	21	52
	22	2,2-Diketospirilloxanthin	$I'+I'$	4	0	2	100	0	30	1	2	0.3
	23	Plectaniaxanthin	$J+O$	30	27	34	1	10	100	15	25	17
Apo-	*24*	Crocetindial	$K+K$	12	0	1	0	3	36	7	10	100
	25	Bixindial	$K+K$	11	0	5	0	4	100	5	17	45
	26	C_{30}-dial	$K+K$	34	1	24	2	13	73	18	23	100
	27	Renieral	$K+N$	11	0	2	1	1	20	2	42	100
	28	β-Apo-2'-carotenal	$K+O$	17	1	31	12	8	100	12	24	60
	29	Neurosporoxanthin	$L+O$	27	1	48	1	14	57	17	31	100
	30	Azafrin	$L+T$	65	32	42	1	6	35	100	18	24
	31	Neurosporoxanthin Me ester	$L'+O$	1	3	17	0	5	23	7	14	100
	32	Azafrin Me ester	$L'+T$	59	9	39	1	5	24	100	19	62
Bicyclic	*33*	Echinenone	$O+V$	4	0	10	1	2	9	4	9	100
	34	Zeaxanthin	$P+P$	95	6	45	2	24	76	37	53	100
	35	Lutein	$P+Q$	36	4	34	1	14	100	28	38	23
	36	3'-*O*-Methyl-lutein	$P+Q'$	100	6	27	6	20	38	21	16	42
	37	3'-Dehydro-lutein	$P+U$									
	38	Lutein diacetate	$P''+Q''$	100	1	32	1	9	82	30	51	4
	39	Canthaxanthin	$V+V$	26	1	15	1	100	19	8	14	41
	40	Rhodoxanthin	$Y+Y$	12	1	12	2	10	89	15	28	100
	41	Astaxanthin	$Z+Z$	52	2	37	2	21	100	24	56	7

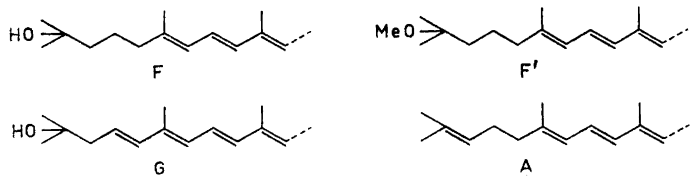
* Structure numbers on the corresponding spectra

DISCUSSION

The carotenoid molecule may be regarded as being made up of a central isoprenoid polyene chain to which are attached two end groups. The fragmentation of these molecules on electron impact may be rationalised in a general manner using the concept of charge localisation.[4] Removal of an electron from the conjugated chain leads to the extrusion of part of this chain giving the M−92 and M−106 ions discussed above and to a less prominent ion at M−158 which according to metastable ions and accurate mass measurements is formed from the molecular ion by loss of a $C_{12}H_{14}$ fragment, a process which may occur by a mechanism similar to that for the M→M−92 reaction. Moreover, there is in the majority of the spectra a significant M−79 peak which is evidently due to an ion formed by elimination of a C_6H_7 fragment, possibly a methyl cyclopentadienyl radical, from the polyene chain.

Localisation of the charge to the polyene chain may also lead to other reactions. In compounds having cyclic end groups fragmentation normally occurs in the polyene chain rather than within the end group itself. Which of the two fragments so formed that will retain the charge preferentially will be dependent on the particular type of end group present. When the end group is furanoid [2] or aromatic, allowing stabilisation of the charge, the smaller fragment becomes of very high abundance, while in other cases the charge is preferentially retained by the larger fragment including the polyene chain.

In C_{40}-carotenoids with acyclic end groups, formation of ions by cleavage of an acyclic saturated bond with retention of the charge by the larger fragment might be expected when the charge is localised to the conjugated chain. However, such cleavage is not observed in cases such as end groups *F* and *F'*, but is observed when there is a functional group present in an appropriate position, *e.g.* end group *A*. While it might be inferred that, in the latter case, the charge is localised to the terminal double bond rather than to the chain, this is contradicted by the fact that a metastable ion corresponding to a M−92→M−92−69 reaction is seen and this requires that the charge must be localised in the chain in the parent ion.



Although localisation of the charge to the polyene chain rather than to an isolated functional group would be expected and receives experimental backing from the results obtained on lowering the energy of the impinging electrons,[5] it is evident that at 70 eV localisation to the end group will also occur to some extent. This is well seen in the end group *F'* where the *m/e* 73 peak, corresponding to the smaller fragment formed on cleavage of the 1,2-bond, is the strongest peak in the spectrum. Localisation of the charge to the isolated double bond in the end group *A*, as well as to the terminal functional

group in similar systems, will give rise to cleavage of the same bond as when the charge is localised to the central chain, *i.e.* α-cleavage.[6] The abundance of the smaller fragment is always greater than that of the larger and this may be ascribed mainly to the difference in stability between the two fragments.

A feature of carotenoid mass spectra is the occurrence of ions resulting from multiple losses. The corresponding peaks observed in this way make it possible to confirm particular fragmentations and may sometimes represent the only evidence for a particular end group as the ions formed from multiple losses are often of much higher abundance than those at higher mass numbers due to the simple cleavages. Especially noticeable and useful in this connection are combinations involving extrusion of part of the polyene chain, *e.g.* M−92−R, M−106−R, M−158−R, M−92−92−R *etc.* The only restriction operating on these multiple fragmentations is, as expected, that once an even electron species has been formed this for energetic reasons no longer loses odd electron fragments to give radical ions,[4] hence (M−92)−R, (M−R)−92, (M−R)−RH *etc.* are observed but not (M−R)−R′. In view of the general occurrence of these peaks due to multiple losses they will, in most cases, not be discussed separately when dealing with the individual end groups.

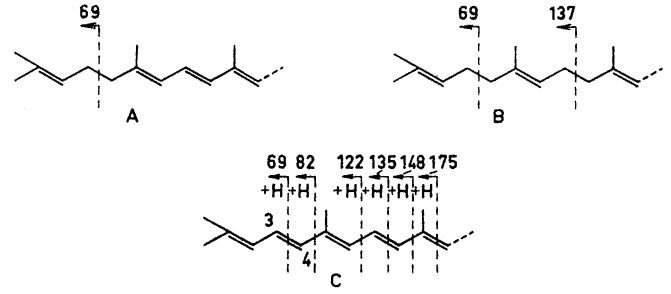
The extent to which the break-down pattern of a particular end group will be apparent is dependent not only on the stability of that end group itself, but also on its stability relative to the other end group in the molecule. In extreme cases all the ions observed in the upper part of the spectrum may be associated with one of the end groups only. However, in such cases it is often possible to deduce the structure of the more stable end group by taking into account other information available such as molecular weight, prominent peaks in the lower part of the spectrum and chemical or other spectral evidence.

Acyclic end groups. In general acyclic end groups (*A*−*I*, *cf.* Table 2) show a straight-forward breakdown pattern with relatively few peaks in the upper part of the spectrum. These peaks can in nearly all cases be ascribed to ions formed either by elimination of a functional group with hydrogen transfer or by cleavage of a bond in appropriate position to two functionalities. The charge may also in part be retained by the smaller fragment, and when the resultant ion is of high stability and abundance, useful information about the structure of the end group may be obtained from the lower part of the spectrum.

The end group *A*, present in the compounds *1*−*8*, undergoes the expected cleavage, previously observed by Schwieter *et al.* for carotenes,[1] of the doubly allylic 3,4-bond. Retention of the charge by the larger fragment gives rise to a significant M−69 peak in all these spectra although the intensity of this peak is sometimes low and peaks resulting from multiple losses involving this cleavage are often of higher intensity. Metastable ions are observed for the M→M−69, M−92→(M−92)−69 and M−69→(M−69)−92 reactions in several spectra, thus showing that ions formed by multiple losses may be derived *via* different paths. The smaller fragment at *m/e* 69 gives rise to the base peak when both end groups are *A* (lycopene, *1*) and to a prominent peak in the majority of the other cases (*cf.* Table 1).

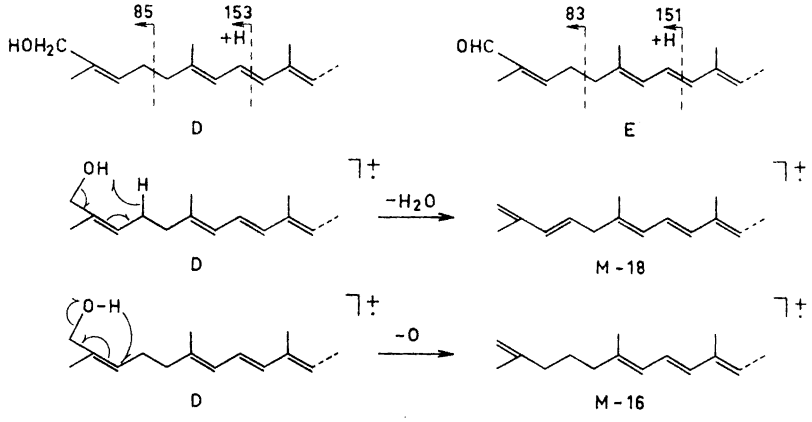
The end group *B* differing from *A* in that the 7,8-bond is reduced, gives rise to a prominent M—137 peak as a result of the cleavage of the 7,8-bond. Lycopersene (*9*), which lacks conjugation, also shows a prominent M—69 peak, while this peak, as might be expected, is insignificant in the spectrum of spheroidenone (*10*). In lycopersene the further doubly activated single bonds present undergo similar facile cleavage with the production of abundant ions spaced 68 mass units (C_5H_8) apart, *i.e.* M—69, M—137, M—205 *etc.*

Bisdehydrolycopene (*11*), the only representative of the end group *C*, exhibits groups of peaks of about equal intensity which correspond to the ions formed by cleavage of essentially every bond from the 3,4-bond towards the centre of the chain, and the strongest peak in each group represents a species where the cleavage is accompanied by hydrogen transfer from the larger charged fragment. Together with the prominent M, M—92, M—106 and M—158 peaks the above peaks describe the essential features of the spectrum.



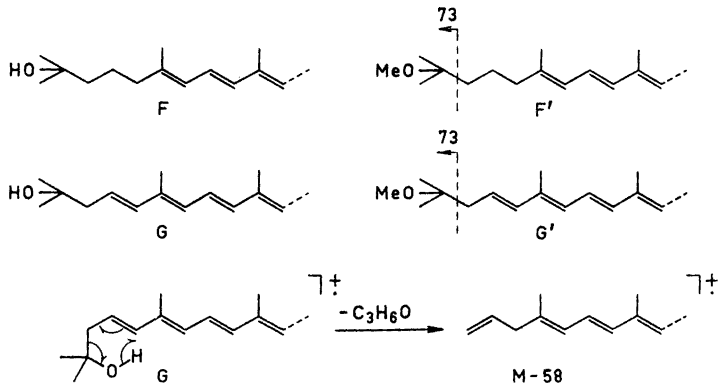
The end group *D*, present in lycoxanthin (*2*) and lycophyll (*12*), differs from *A* in that a hydroxyl group has been introduced at C(16). This end group undergoes the same 3,4-bond cleavage as *A* and the M—85 species so formed is readily recognised, though of moderate relative intensity. Moreover, the introduction of the oxygen substituent apparently facilitates cleavage of the 7,8-bond with hydrogen transfer to the smaller fragment giving a M—153 species. The formation of this species may occur by a process analogous to that discussed below for the cyclic compounds. The presence of the hydroxyl group is evident from the occurrence of prominent peaks at M—16 and M—18, which can be ascribed to ions formed by the tentative mechanisms shown below. Since loss of oxygen in allylic alcohols does not seem to have been encountered previously this loss was confirmed in the case of lycoxanthin (*2*) by accurate mass measurement.

The end group *E* also undergoes 3,4-bond cleavage and an abundant M—83 ion, which according to metastable peaks arises directly from the molecular ion, is present in the spectrum of lycopen-16-al (*3*). The peak at *m/e* 83, corresponding to the smaller fragment, is of fairly high abundance. The M—151 ion associated with cleavage of the 7,8-bond accompanied by transfer of a hydrogen to the smaller fragment is of about the same intensity as the corresponding ion in the case of the end group *D*. Loss of the aldehyde group and of carbon monoxide from the molecular ion is also observed in peaks at M—29



and M—28, but these reactions are evidently less favoured than the loss of carbon monoxide from the M—106 species.

When the end group *A* is modified by hydration or addition of methanol to the 1,2-double bond to give the end groups *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, as shown by inspection of the spectra of rhodopin (*4*), dihydroxylycopene (*13*), rhodovibrin (*14*), and 3,4,3',4'-tetrahydrospirilloxanthin (*15*), is therefore only evident in the upper part of the spectrum from peaks due to the loss of the functional group, *i.e.* M—18 for *F* and M—30 and M—32 for *F'*.

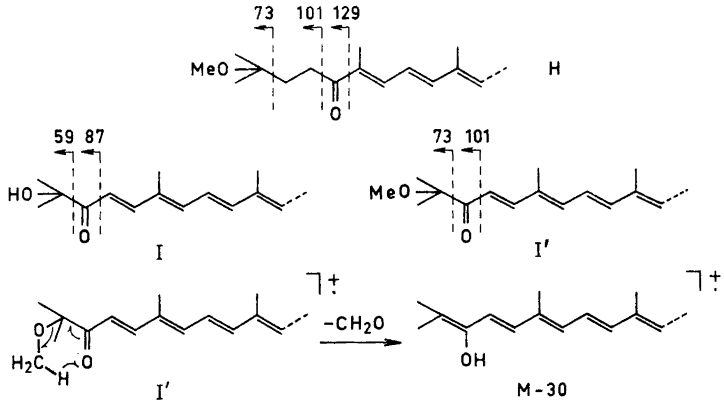


In the case of *F'* the smaller fragment, formed on cleavage of the 1,2-bond, gives rise to the base peak at *m/e* 73, while there is no corresponding strong peak at *m/e* 59 in the case of *F*. This difference may be associated with the degree to which the charge becomes localised to the oxygen atom in the two cases and the different routes of fragmentation of ethers and alcohols.

The introduction of a double bond in the 3,4-position in the end groups *F* and *F'* facilitates the cleavage of the 1,2-bond and allows retention of the

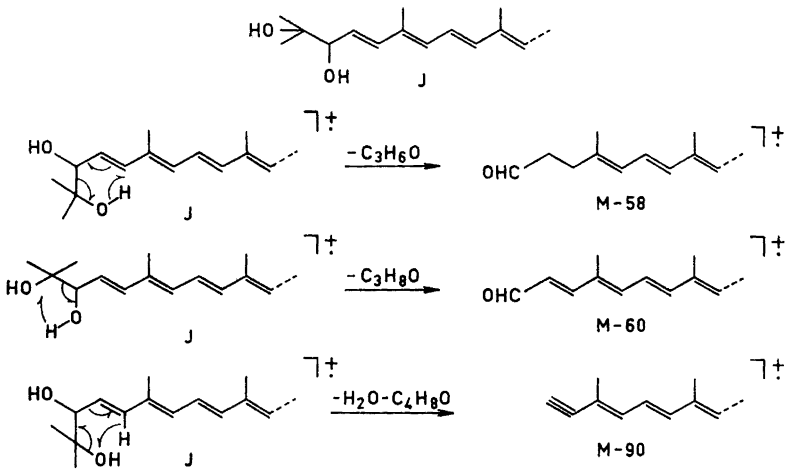
charge also by the larger fragment as this may now be effectively delocalised. When the substituent is a hydroxyl group, as in the end group *G*, this cleavage is accompanied by hydrogen transfer, most probably by McLafferty rearrangement as shown above, and usually gives rise to a significant, but weak M—58 peak as in compounds, *16*, *17*, and *18*. The M—106—58 ion is more abundant and, as shown by metastable ions, originates from the M—106 species. On the other hand, when the substituent is a methoxyl one, as in the end group *G'*, cleavage without hydrogen transfer is favoured and the resulting peak occurs at M—73 as in compounds *5*, *14*, *17*, and *19;* metastable ions demonstrate that the more abundant M—106—73 ion is formed in one step from the M—106 ion. Both end groups *G* and *G'* show the expected loss of the oxygen substituent together with hydrogen, and M—18 and M—32 peaks are observed, respectively. Support for the presence of the end group *G'* may be obtained from the lower part of the spectrum, since in all cases where this occurs the *m/e* 73 peak is strong. Of the compounds possessing end group *G* only the diol *16*, which has this group in both ends, shows the base peak at *m/e* 59.

Okenone (*20*), the sole example of the end group *H*, gives rise to M—101 and M—129 ions resulting from cleavage of the bonds α to the 4-keto group, as might be expected from the results obtained for other α,β-unsaturated ketones.[7] The presence of the methoxyl group is evident from the observation of a prominent M—32 peak. In agreement with expectation there is no M—73 peak as on cleavage of the 1,2-bond the charge is retained by the smaller fragment which gives rise to a fairly prominent peak at *m/e* 73.



The end group *I*, present in 2'-dehydroplectaniaxanthin (*21*), exhibits the expected M—59 and M—87 peaks associated with cleavage of the bonds in the α-position to the 2'-keto group. The M—88 peak is more intense than the M—87 peak indicating that cleavage of the 2,3-bond accompanied by hydrogen transfer to the smaller fragment is preferred. The correctness of these assignments is supported by the lack of shift of these peaks on replacement of the hydroxyl hydrogen by deuterium. Like the allylic acyclic alcohols in the present investigation the end group *I* gives rise to prominent M—16 and M—18 peaks.

The three compounds *10*, *18*, and *22* possessing the end group *I'*, in which the hydroxyl group of *I* has been replaced by a methoxyl group, all show the expected M—73 and M—101 peaks associated with cleavage of bonds in the position α to the keto group. Moreover, the presence of the methoxyl group is evident from peaks at M—30 and M—32, of which the former is most probably due to an ion formed through loss of formaldehyde by McLafferty rearrangement. The base peak occurs at *m/e* 73 in all compounds possessing the end group *I'*. The end group *I'* also gives rise to a significant M—60 peak and although this is probably due to the loss of methanol and carbon monoxide, further experimental evidence is required before discussing the mechanism of this reaction.

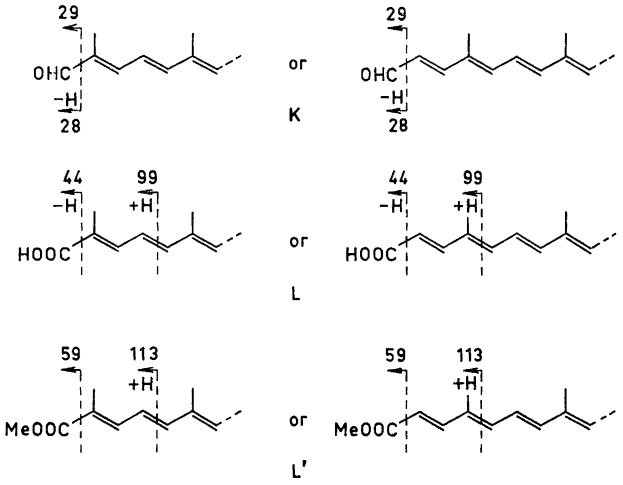


The only example of the end group *J* is that of plectaniaxanthin (*23*). It shows the expected cleavage of the 1,2-bond, and abundant M—58, M—59 (noticeable M—106—59) and M—60 ions are observed. The former ion is most probably formed by McLafferty rearrangement while the formation of the latter involves hydrogen transfer to the smaller neutral fragment. A fairly strong peak is also observed at M—90 and a less intense one at M—88 and these are attributable to cleavage of the 2,3-bond. A plausible but unsubstantiated mechanism for the loss of 90 mass units is shown above. Losses of 16 and 18 mass units and combinations of these are seen in this spectrum, as might be expected on the basis of the presence of a hydroxyl group in allylic position.

End groups of apo-carotenoids. Some of the compounds included in the present study are apo-carotenoids. Five of these, three bis-apo-carotenals (*24*, *25*, and *26*) and two apo-carotenals (*27* and *28*), have in common the aldehydic end group *K*. These compounds in agreement with expectation all show M—18, M—28, and M—29 peaks which can be ascribed to ions formed by loss of water, carbon monoxide and the aldehyde group. In some of these spectra M—15 peaks and peaks due to multiple losses involving extrusion of a methyl group are of similar intensity. It is of interest to note that while both the

M—92 and M—106 peaks are prominent in the spectra of the compounds with 30 or more carbon atoms, neither of these peaks are significant in the spectrum of the C_{20}-derivative (*24*) and only the M—106 peak is characteristic in the spectrum of the C_{24}-derivative (*25*).

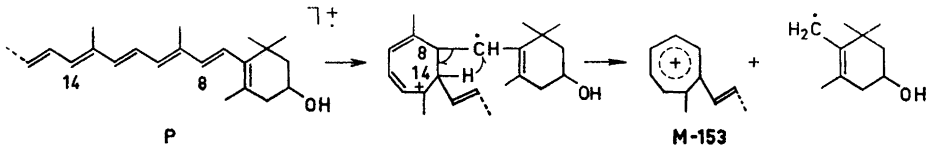
Two of the apo-carotenoids studied, neurosporaxanthin (*29*) and azafrin (*30*), possess the end group *L* and show peaks at M—44 and M—99, which can be associated with this end group. The former of these peaks is due to an ion formed by loss of carbon dioxide from the molecular ion, as expected from analogy with other α,β-unsaturated acids,[8] and the latter corresponds to a species derived by cleavage of the δ-bond with transfer of a hydrogen to the smaller fragment.



The corresponding esters (*31*, *32*) give rise to peaks at M—59 and M—113 which can be associated with the end group *L'*. While the same carbon-carbon bonds are cleaved there is now the difference that cleavage of the α-bond occurs without hydrogen migration, as there is no hydrogen on the ester oxygen. The formation of the M—99 and M—113 species may occur by a process analogous to that discussed below for the cyclic compounds. The end group *L'* also gives rise to a prominent M—32 and/or M—31 peak due to the loss of the ester methoxy group by cleavage of the other α-bond.

Cyclic end groups. Contrary to expectation, in cyclic end groups (*N*—*T*, *cf.* Table 2) cleavage of the in-chain bonds is favoured over fragmentation within the end group itself, and the introduction of oxygen substituents in the ring usually reinforces this effect. Rupture of the 7,8-, 9,10-, and 11,12-bonds, accompanied by transfer of a hydrogen atom to the smaller fragment, is of general occurrence though the abundance of the ions so formed varies to a large extent, partly as a result of the character of the other end group present. While peaks corresponding to the larger fragments derived directly from the molecular ion are normally observed, there are instances when the losses are more readily or only recognised in combinations, *e.g.* from M—32 and M—60

ions. Although deuterium labelling is required to establish the origin and degree of specificity of the hydrogen transfer in these reactions, the evidently low probability of ring cleavage and facile coiling of the acyclic polyene chain in the molecular ion permits the tentative mechanism illustrated below in the case of 7,8-bond cleavage in the end group *P*. Ions corresponding to other less general cleavages in the chain are also present, but these will be discussed along with the fragmentations of the individual end groups.

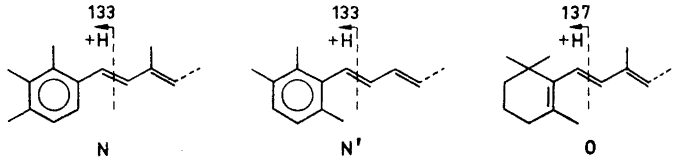


P M-153

Schwieter *et al.*[1] have pointed out that the extrusion of a methyl group from the molecular ion occurs only to a minor extent in carotenes, even when four geminal methyl groups are present in allylic positions. This observation is borne out by the present results, and though of somewhat higher intensity in the compounds possessing a 4-oxo-group in conjugation with the polyene chain, the M—15 peak appears to be of scant diagnostic value. The only other carbon-carbon bond cleavages in the ring of some importance are the *retro*-Diels-Alder reaction in the case of the 4,5-enes, the loss of a fragment of 56 mass units from the α,β-unsaturated ketones and the reactions of end group *I*.

Loss of water from the molecular ion is observed for all alcohols and, as expected, for several other oxygen containing cyclic end groups. When methoxyl and acetoxyl groups are present, diagnostically significant losses of methanol and acetic acid are always observed. The loss of oxygen from allylic alcohols, shown to occur in the case of the acyclic end groups, is not significant in their cyclic counterparts studied here.

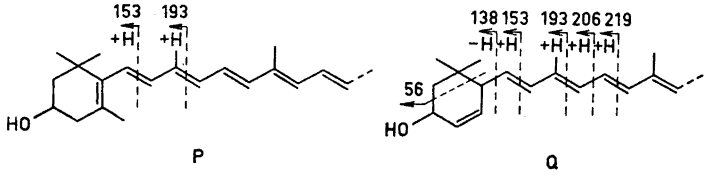
The aromatic end groups *N* and *N'*, present in chlorobactene (*6*, *N*), renieral (*27*, *N*), and okenone (*20*, *N'*), cannot be distinguished on the basis of the present evidence. They are readily recognisable from the fact that the *m/e* 133 peak, corresponding to the smaller fragment formed by cleavage of the 7,8-bond with transfer of hydrogen to this fragment, is the strongest fragment in the lower half of the spectrum and usually the base peak. The M—133 peak is present in all spectra, but in the case of okenone which has a methoxyl group in the other end group it is noticeably weaker than the M—133—32 peak. Corresponding peaks associated with cleavage of the 9,10- and 11,12-bonds are also observed, although they are usually not significant.



N N' O

The apo and bicyclic carotenoids possessing the end group *0* (*28*, *29*, *31*, and *33*) undergo the expected cleavages at the 7,8-, 9,10-, and 11,12-bonds. Of the peaks corresponding to these cleavages that at highest mass number, M—137, is prominent in all spectra and is usually the most readily recognised. However, in the spectra of the monocyclic C_{40}-carotenoids *21* and *23* none of these peaks are significant, except for the M—137—18 peak in the spectrum of plectaniaxanthin (*23*), and it may be concluded that it is difficult or impossible to recognise the end group *O* directly when the fragmentation of the other end group is highly favoured.

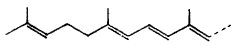
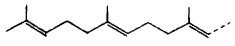
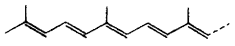
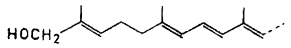
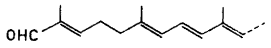
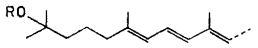
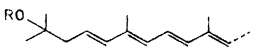
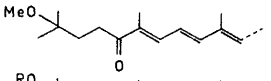
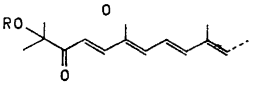
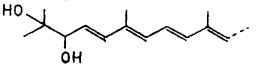
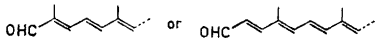
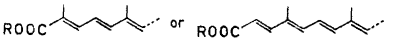
The end group *P* is best recognised in the present set of compounds (*7*, *34*, *35*, *36*, and *37*) by the M—153 and M—193 peaks which correspond to the 7,8- and 9,10-bond cleavages. In the corresponding acetate, the end group *P'*, these peaks are shifted in the expected manner, as is seen in the spectrum of rubixanthin acetate (*8*); the M—18 peak is replaced by the one at M—60 in some cases, when the other end group is readily eliminated, the peaks associated with the end groups *P* and *P'* may be partly or entirely displaced to lower mass number. In all of the bicyclic compounds possessing the end groups *P* or *O* there is a M—64 peak, but this is probably due to an ion formed by elimination of part of the polyene-chain.



The end group *Q* and the corresponding methyl ether *Q'* and acetate *Q''*, which are present in the compounds *35*, *36*, and *38*, respectively, show peaks due to the larger fragments formed on cleavage of the 6,7-, 7,8-, 9,10, 10,11-, and 11,12-bonds in the molecular ion, or in the case of the acetate mainly in the M—60 ion. As indicated above, cleavage of the 6,7-bonds occurs with hydrogen transfer to the larger fragment, a reaction supported by metastable ions, while the other cleavages involve the transfer of a hydrogen in the opposite direction. The expected losses of water, methanol, and acetic acid from the molecular ions are also observed and metastable ions show that ions formed by multiple losses involving the elimination of these may be formed by different paths. While peaks corresponding to *retro*-Diels-Alder fragmentation, M—56 and M—106—56, are observed for the alcohol and the methyl ether, such peaks are not present in the spectrum of the acetate and this may be ascribed to the fact that the elimination of the acetate group is strongly favoured.

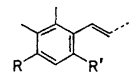
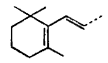
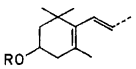
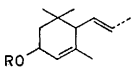
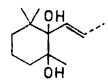
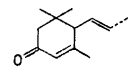
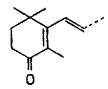
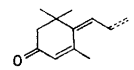
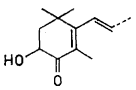
The spectra of azafrin (*30*) and azafrin methyl ester (*32*) show, in addition to peaks due to the loss of water, prominent peaks at M—98, M—127, M—155, and *m/e* 109 (100 %), which can be ascribed to the common end group *T*. The formation of the M—127, M—155, and *m/e* 109 ions can be accounted for as shown below. The mechanisms invoked are supported by nearly complete

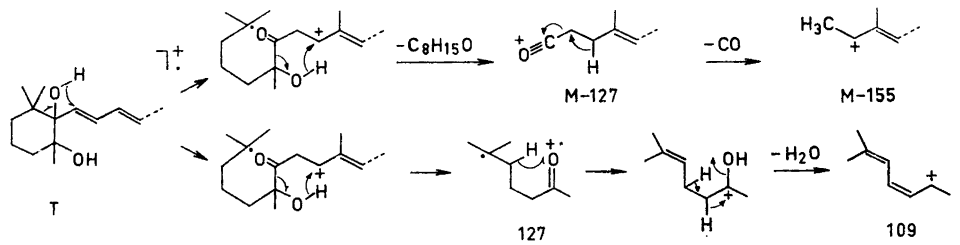
Table 2. Peaks of diagnostic value

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*	M−16, 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,
	*K*	M−60, M−88, M−90 M−18, M−28, M−29
	*L* (R=H) *L′* (R=Me)	M−44, M−99 M−31, M−59, M−113

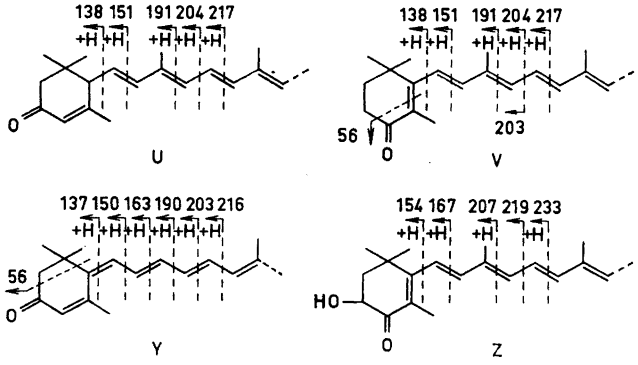
(80 %) shifts of the M−127 and M−155 peaks by two mass units and quantitative retention of the *m/e* 109 peak on exchange of the two hydroxyl hydrogens for deuterium in azafrin methyl ester and by the presence of a metastable ion (*m/e* 93.5) corresponding to the formation of the *m/e* 109 species from the abundant (55—70 % rel. int.) *m/e* 127 precursor. The labelling results also show that the formation of the M−18 and M−98 species involves quantitative (>95 %) loss of both hydroxyl hydrogens, but further evidence required to explain the latter process, which evidently involves skeletal rearrangements, is not available at present.

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 end groups *U*, *V*, and *Y*, which are present in 3′-dehydro-lutein (*37*, *U*) echinenone (*33*, *V*), canthaxanthin (*39*, *V*), and rhodoxanthin (*40*, *Y*), respectively, give rise to peaks which can be ascribed to fragments formed by cleavages of the same bonds as in the end group *Q*, though the accompanying hydrogen rearrangements are not the same in all cases, as indicated below. Thus here, in contrast to end group *Q*, cleavage of the 6, 7 bond occurs with transfer of a hydrogen to the smaller fragment. Moreover, in the compounds *33* and *39* (*V*) where the keto group is conjugated with the polyene chain ions are observed corresponding to cleavage of the 10,11-bond both with and without transfer of hydrogen to the smaller neutral fragment. A metastable ion is observed in the case of canthaxanthin (*39*), corresponding to the loss of a fragment of 203 mass units from the molecular ion, lends support to the former of these reactions. The end group *V* shows a prominent M—56 peak, which in analogy with fragmentation established for simpler cyclohexenones [7] may be ascribed to an ion formed by loss of a C(4)—C(5) fragment, while the M—56 peak observed for the end group *Y* may be associated with loss of a C(1)—C(2) fragment as in the case of the end group *Q*. The end group *Y* also shows a M—163 peak corresponding to cleavage of the 8,9-bond with transfer of a hydrogen to the smaller fragment.



Astaxanthin possesses the end group *Z* which undergoes essentially the same cleavages as the closely related end group *V*. However, it shows in addition a prominent M—16 peak which is evidently due to the loss of oxygen from the 3-hydroxyl group in the α-position to the conjugated 4-keto group.

General remarks. Diagnostically useful information is available in the lower part of the spectrum only from peaks of high intensity which may be associated with a particular end group or functionality. The results given in Table 1 show that prominent peaks at *m/e* 69, 73, 109, and 133 are of diagnostic value while those at the other mass numbers listed here are found to be of little use.

Those compounds having the base peak at *m/e* 69 contain either the end group *A* or *B*. While the intensity is usually high in other compounds with the end group *A* or one likely to give rise to it by dehydration or similar reactions (*F*, *F'*, and *H*), this is not always the case. It follows therefore that it is mainly when it constitutes the base peak that the peak at *m/e* 69 can be used to draw definite conclusions on the end groups present.

The eight compounds possessing end groups *F'*, *G'*, or *I'*, which have in common a tertiary methoxyl group at C(1), all show very high values for the relative intensity of the *m/e* 73 peak; it is the base peak in seven of these spectra and has an intensity of 96 % in the eighth. The corresponding peak in okenone (*20*), the only other compound with a 1-methoxyl group (end group *H*), has an intensity of 35 %. Since the relative intensity of the *m/e* 73 peak in other cases is noticeably lower, usually below 10 %, this peak is of considerable diagnostic value.

The base peak is found to occur at *m/e* 109 in the compounds *30* and *32*, which are the only representatives of the end group *T*. In view of this and the fact that the highest value observed for this peak in any of the other spectra is 37 %, a high intensity peak at *m/e* 109 may be taken as a usable indicator of the end group *T*.

The compounds possessing the aromatic end groups *N* and *N'* all exhibit the *m/e* 133 peak as the most intense peak in the lower half of the spectrum, while this is not the case in any of the other spectra. This peak thus provides a good indication of the presence of an aromatic end group.

In conclusion it may be said that it is possible to obtain information from the mass spectra of carotenoids about the end groups (*cf.* Tables 1 and 2) and the length of the polyene chain,[3] which will either allow the structure to be determined or in more complicated cases, considerably reduce the number of possibilities that have to be considered.

EXPERIMENTAL

The mass spectra were recorded on an LKB 9000 instrument at 70 eV, an ion source temperature of 290—310°, and with the probe heater at the minimum temperature (100—200°). The sample (0.01—0.02 mg) was dissolved in one drop of a suitable solvent, the resulting solution placed in the probe, the solvent removed by streaming with nitrogen and the probe immediately inserted into the machine. Exchange of hydroxyl hydrogens for deuterium was performed in the same manner using deutero methanol, as solvent, when necessary together with another solvent.

The *m/e* 536 (M—16) ion in the spectrum of lycoxanthin (*2*) had the composition $C_{40}H_{56}$ [found (LKB 9020 mass marker) 536.0000; required 536.0000].

A number of the spectra reproduced here have been redetermined since the preliminary communication,[3] and although some variation in the intensity ratio of the M—92 and M—106 peaks is seen, the values are close to those previously reported.

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6. For nomenclature *cf.* Ref. 4, pp. 1—4.
7. Ref. 4, pp. 143—154.
8. Enzell, C. R. and Ryhage, R. *Arkiv Kemi* **23** (1965) 367.

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