Chemical Ionization Mass Spectrometry of Carotenoids

Joseph Carnevale, Edward R. Cole,† Derek Nelson and James S. Shannon
School of Chemistry, The University of New South Wales, Kensington, NSW 2033, Australia

The hydrogen and isobutane chemical ionization mass spectra of a number of carotenoids, with symmetrical and unsymmetrical end groups, have been examined. Similar spectra were obtained with each gas. Loss of fragments, characteristic of both the polyene chain and of the end groups were shown by all compounds. Contrasting with the electron ionization spectra the chemical ionization spectra showed more abundant ions in the high mass region and a simpler fragmentation pattern. However, the diagnostic features of the $[M-92]^+/[M-106]^+$ ratio, established for electron ionization spectra, are retained. A unique fragmentation pattern is shown by the keto derivative, capsanthin, with consecutive losses of xylene and toluene from the $[M+1]^+$ ion. Attention is drawn to the significantly superior sensitivity of the chemical ionization technique over that of the electron ionization procedure. Use of the chemical ionization technique for the identification and structural examination of carotenoids thus offers advantages over the electron ionization and field desorption techniques.

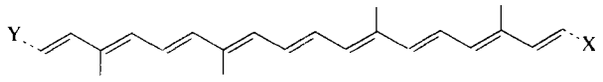
INTRODUCTION

The behaviour of carotenoids in electron ionization mass spectrometry is now well established.[1-9] Discernible patterns of fragmentation arise from the polyene chain and from end groups. Fragmentation of the C_{40} carotenoids is characterized by loss of toluene and xylene and, in some cases, of 2,6-dimethylnaphthalene.[1,5,10] Differences in behaviour of end groups occur, some which are easily fragmented and give dominant peaks while others can be detected only with difficulty.[8]

Problems with EI spectra of carotenoids are mainly related to their low volatility, and the extent to which the $[M-92]^+$ peak (M − toluene) and $[M-106]^+$ peak (M − xylene) result from thermally degraded or isomerized material has not been established. These spectra are further complicated as a result of considerable multiple fragmentation, which leads to crowding of intense peaks in the low mass region of the spectra. Therefore, this part of the spectrum has little structural significance.

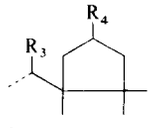
In the CI technique, reaction with the ionizing gas leads to spectra that may be quite distinct from those obtained in the EI technique. Fragmentation is less and the simpler spectra are noteworthy for strong peaks due to $[M+1]^+$ ions.

The value of CIMS in the analysis of isoprenoid hydrocarbons such as pristane and squalene[11] has been noted but the technique does not appear to have been applied to polyene isoprenoids such as the C_{40} carotenoids. The present discussion concerns the extension of the CI technique employing isobutane and hydrogen to a series which includes hydrocarbons, hydroxylated, keto and silylated derivatives, in particular of the paprika pigment capsanthin and its reduction product, capsanthol. Structures of material examined are schematically arranged below.



Carotenoid	X	Y
Lycopene	A	A
β-Carotene	B	B
Canthaxanthin	C	C
4,4'-Dihydroxy-β-carotene	D	D
Capsanthin	E	G
Capsanthol	E	H
bis-Trimethylsilyl capsanthin	F	I

A	B	$R_1 = H$	$R_2 = H$
	C	$R_1 = H$	$R_2 = O$
	D	$R_1 = H$	$R_2 = OH$
	E	$R_1 = OH$	$R_2 = H$
	F	$R_1 = OSi(CH_3)_3$	$R_2 = H$



G	$R_3 = O$	$R_4 = OH$
H	$R_3 = OH$	$R_4 = OH$
I	$R_3 = O$	$R_4 = OSi(CH_3)_3$

EXPERIMENTAL

Materials

β-Carotene and canthaxanthin were commercial materials (Roche). Lycopene was separated from

† Author to whom correspondence should be addressed.

CCC-0306-042X/78/0005-0641$03.00

tomato paste and capsanthin from paprika by standard methods. All pigments were finally purified by thin-layer chromatography on silica gel G plates, taking adequate precautions against oxidation, using the following solvent systems: β-carotene, light petroleum b.p. 40–60 °C + acetone, 99:1; lycopene, light petroleum + methylene chloride, 4:1; canthaxanthin and capsanthin, light petroleum + acetone + benzene + ethanol, 19:6:3:1.

4,4′-Dihydroxy-β-carotene and capsanthol were prepared by sodium borohydride reductions of canthaxanthin (1–3 mg) and capsanthin, respectively, in ethanol. The products obtained by ether extraction of the diluted reaction mixtures were purified as described previously for canthaxanthin. Silylation of capsanthin was performed in *N,N*-dimethylformamide solution using an excess of bis(trimethylsilyl)trifluoroacetamide and the product purified by TLC using light petroleum + acetone, 9:1.

Apparatus

EI spectra were obtained on a GEC-AEI MS 9 spectrometer using an indirectly heated insertion probe with pyrophyllite tip. Source temperatures were in the range 180–220 °C. Operating conditions were: accelerating voltage, 8 kV; electron trap current, 100 μA; electron energy, 70 eV; source pressure 10^{-6} Torr.

The CI spectra were run on a GEC-AEI MS 902 spectrometer, fitted with a dual EICI source (Scientific Research Instruments CIS2). Samples were introduced in a glass holder at the end of a heated direct insertion probe at the lowest probe temperature for volatilization. Source temperatures were in the range 180–200 °C. Operating conditions were: accelerating voltage, 8 kV; electron energy, 450 eV; repeller voltage, zero.

Due to normal machine background the hydrogen spectrum contained a peak due to water $[H_3O]^+$ which was about the same height as that due to hydrogen $[H_3]^+$.

Sensitivity comparisons of the peaks from the CI$[MH]^+$ ion and the EI molecular ion were obtained by placing equal aliquots of a solution in chloroform by way of a capillary tube into glass sample holders. Following removal of the solvent samples were placed on the direct insertion probe then into the spectrometer. The particular ion was monitored on the machine collector meter and readings, taken every minute, were summated as the sample warmed up until no further sample remained. Most of the comparisons were made using the MS 902 instrument fitted with the dual CIEI source. However, careful comparisons were made on a MS 9 and an AEI MS 12 single focusing machine to ensure that EI sensitivity was similar on all machines. The multiplier gain of the three machines was checked and compensated as required. All measurements were made at a resolution of 2000.

RESULTS AND DISCUSSION

A summary of the most important fragments obtained in the CI spectra of the compounds examined is recorded in Table 1.

Similar CI spectra were obtained with hydrogen and isobutane, except for a slightly greater fragmentation pattern with the former gas, especially in the lower mass region. This might be expected from the fact that $[H_3]^+$ is a stronger protonating species than $[C_4H_9]^+$. Use of isobutane also led in each case to ions at $[M+C_4H_9]^+$ and at $[M+C_3H_3]^+$ with the former being generally more abundant. In the spectrum of β-carotene it was a dominant ion. These high mass ions offer similar aid for identifying the parent ion as do the ions at $[M+29]^+$ and $[M+41]^+$ in CI spectra where methane is the reagent gas. When ammonia is the reagent gas diagnostic ions at $[MH]^+$ and $[M+NH_4]^+$ are observed.

CI spectra (hydrogen and isobutane) of β-carotene together with the EI spectrum are shown in Fig. 1. Equivalent spectra for capsanthin are shown in Fig. 2.

The CI spectra of all compounds studied show a fragmentation pattern typical of olefins[12] characterized by multiple fission reactions as illustrated by the pattern from β-carotene (Fig. 3). Hydroxylated and silylated derivatives show similar patterns.

Studies of metastable ions from β-carotene, lycopene and capsanthin indicate that fission occurs at every carbon atom along the polyene chain. Thus, the CI as distinct from the EI spectra show abundant ions in the middle mass range. In addition, the CI spectra have a more simple and useful pattern in the low mass region.

Considerable attention has been given in the EI technique to ions due to loss of toluene and xylene from the molecular ions, and processes by which these losses occur have been well discussed.[10,13] In the CI technique losses of 92 (toluene) and 106 (xylene) from the $[M+1]^+$ ion give rise to relatively abundant ions. Moreover, these losses also occur from the $[M]^{\ddagger}$ and $[M-1]^+$ ions, and when isobutane is the reagent gas, from the $[M+57]^+$ ion.

Although the intensities of these peaks vary widely among the present group, they are much more abundant than the $[M-92]^{\ddagger}$ ion and the $[M-106]^{\ddagger}$ ion appearing in corresponding EI spectra.

The ratio $[M-92]^{\ddagger}/[M-106]^{\ddagger}$ which decreases in EI spectra as the number of double bonds increases from 9 to 13,[6] has been used to determine the length of conjugation in the polyene chain where the molecules contain no more than one oxygen atom. In the CI spectra the ratio $[(M\pm1)-92]^+/[(M\pm1)-106]^+$ obtained in hydrogen and isobutane spectra (Table 2) is similar to the $[M-92]^{\ddagger}/[M-106]^{\ddagger}$ ratio from EI spectra. Presently recorded values for these ratios compare favourably with those quoted for the EI technique.[4,6]

All compounds in the CI technique give rise to the series $[M+1]^+$, $[M]^{\ddagger}$ and $[M-1]^+$ ions, contrasting with only the $[M]^{\ddagger}$ ion of EI spectra. The $[M+1]^+$ ion was the most intense of the group, except with 4,4′-dihydroxy-β-carotene and lycopene where the $[M]^{\ddagger}$ ion predominated. In most cases the $[M\pm1]^+$ and/or $[M]^{\ddagger}$ ion were of greater intensity than the $[M]^{\ddagger}$ ion in EI spectra.

A feature of the hydrogen CI spectrum of lycopene (end group A) is the appearance of the $[M-69]^+$, $[M-92-69]^+$, and $[M-106-69]^+$ ion at *m/e* 467, 375 and 361, respectively, as well as a large peak at *m/e* 69 in the low mass region. In addition there is an abundant peak at *m/e* 43. The formation of the $[M-69]^+$ ion is consistent with scission of the double allylic bond. The diagnostic value of these characteristic losses for the detection of

Table 1. Intensities of characteristic peaks in CI spectra of carotenoids

Compound	Relative Intensity at *m/e* (% base peak)																		Others
	93	107	109	127	M−137	M−153	M−193	M+1 −198 −H_2O	M+1 −198	M+1 −106 −$2H_2O$	M+1 −92 −$2H_2O$	M+1 −106 −H_2O	M+1 −92 −H_2O	M+1 −106	M+1 −92	M+1 −$2H_2O$	M+1 −H_2O	M+1	
Lycopene[a]	28	100	10	3	<1	—	—	—	<1	—	—	—	—	20	6	—	—	18	M(30), M−1(12), M−106−69(6), M−92−69(2), 69(44), 43(55)
β-Carotene[b]	35	30	12	20	3	—	—	—	1	—	—	—	—	6	16	—	—	100	M+57(43), M+57−92(7), M+57−106(2), M(56), M−1(22), M−92(12), M−106(5), 347(6), 281(5), 255(6), 243(5), 203(14), 189(20), 177(23), 149(4), 137(18), 123(14)
β-Carotene[a]	5	14	7	4	3	—	—	—	2	—	—	—	—	7	13	—	—	100	M(26), M−1(31), 347(19), 281(15), 255(11), 243(10), 203(17), 189(23), 177(42), 149(9), 137(42), 123(12)
Canthaxanthin[a]	71	42	7	5	1	—	—	—	2	—	—	—	—	12	18	—	—	100	M+1−16(3), M+1−18(2), M−203(7), M−217(3), M−191(1), M−151(3), 203(32), 189(9)
4,4'Dihydroxy-β-carotene[a]	36	28	16	3	—	3	4	—	—	4	3	5	12	2	12	38	62	40	M(76), M−1(17), M−H_2O(53), M−1−H_2O(28), M−$2H_2O$(8), 361(5), 347(4), 269(5), 257(12), 203(13), 201(10), 189(14), 149(20), 139(13), 123(19)
Capsanthin[b]	3	21	85	14	1	1	7	78	95	22	12	100	16	59	3	82	26	14	M+57(2), M+57−H_2O(3), M+57−92(9), M+57−106(5), M−H_2O(17), M−1−H_2O−92(40), M+1−198−$2H_2O$(10), M−155(3), 155(4), 153(13), 139(3)
Capsanthol[a]	73	100	39	9	—	1	1	10	1	13	5	37	6	9	2	12	29	10	M+1−$3H_2O$(1), M−1−$3H_2O$(2), M−157(4), M+1−198−$2H_2O$(13), M+1−198−$3H_2O$(4), 281(24), 257(37), 223(46), 207(63), 187(11), 157(13), 149(21), 139(32)
Bistrimethylsilyl capsanthin[b]	62	64	21	—	—	—	—	—	51	—	—	—	—	100	5	—	—	15	M+57(2), M+57−90(3), M+57−106(4), M+1−90(16), M+1−180(3), M+1−92−90(4), M+1−106−90(59), M+1−198−90(25), M−227(2), 227(3), 199(5)

[a] Hydrogen CI mass spectrum.
[b] Isobutane CI mass spectrum.

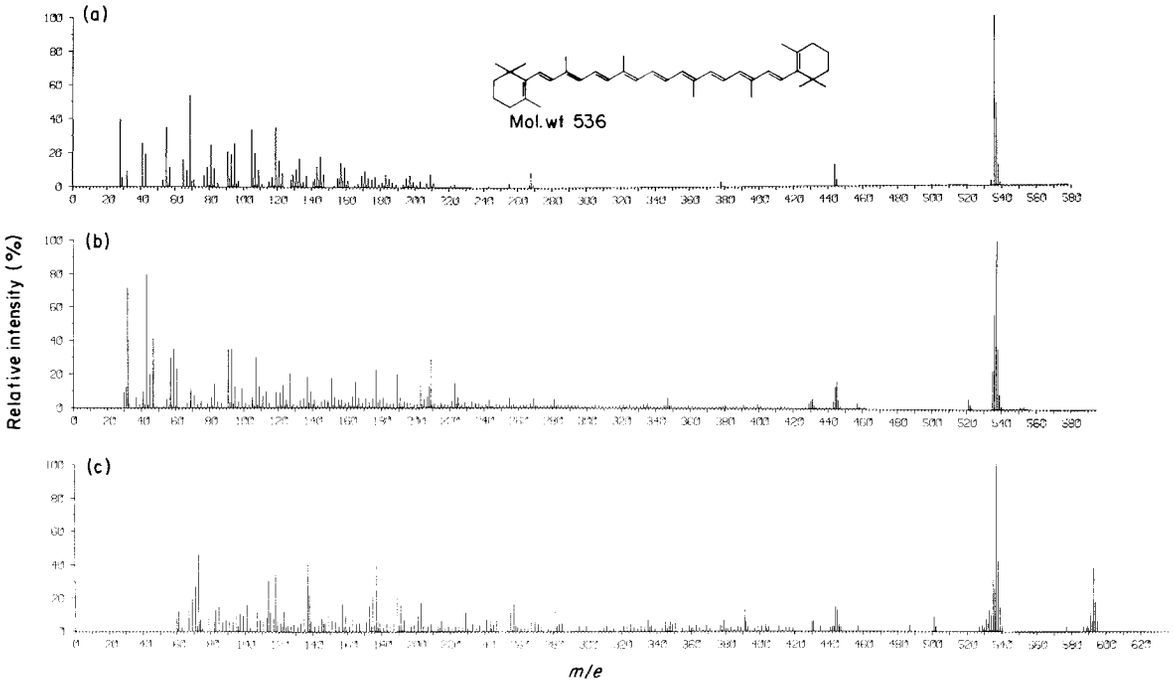


Figure 1. EI and CI spectra (hydrogen and isobutane) of β-carotene. (a) EI; (b) hydrogen CI; (c) isobutane CI.

the end group is enhanced by the fact that the equivalent peak in the EI spectrum appears with much lower intensity [467(3), 375(1), 361(3)].

No special fragmentation features are shown by end group B (β-carotene) in either the EI[8] or CI spectra. However, significant ions at *m/e* 347, 281, 255, 243, 203, 189, 177, 149, 137 and 123 were given by β-carotene which correspond to successive cleavages of the polyene stem as indicated in Fig. 3.

The diagnostic value of these peaks is enhanced by the fact that again the equivalent peaks in the EI spectrum [255(3), 243(1), 203(4), 189(2), 177(6), 137(7) and 123(8)] are much lower in intensity and in some cases (347, 281 and 147) are not present.

Apart from small peaks corresponding to the loss of 16 and 18 mass units from the $[M+1]^+$ ion the fragmentation pattern of 4,4′-diketo-β-carotene(canthaxanthin) is not distinguished by special features. However, significant peaks are observed at *m/e* M−217, M−203, M−191, M−151, 203 and 189, which have been shown to be characteristic of the end group C in EI spectra.[14] Presently recorded spectra show corresponding peaks at *m/e* 347(3), 361(3), 373(2), 413(2), 203(14) and 189(6), which are much lower in intensity than those of the CI spectra. The retro Diels-Alder reaction indicated in other compounds by loss of 56 mass units in their EI spectra[6] is not observed in either the EI or CI spectra in the present instance.

The CI spectra of compounds with hydroxyl-containing end groups (D, E, G, H) are characterized by a predominant fragmentation pattern involving loss of water from the following ions, $[M\pm1]^+$, $[M]^{\ddagger}$, $[M\pm1-92]^+$ and $[M\pm1-106]^+$. A metastable ion for loss of water from the $[M+1-106]^+$ ion of capsanthin (X = E, Y = G) has also been observed. As the number of hydroxyl groups increases, e.g. in the reduction product,

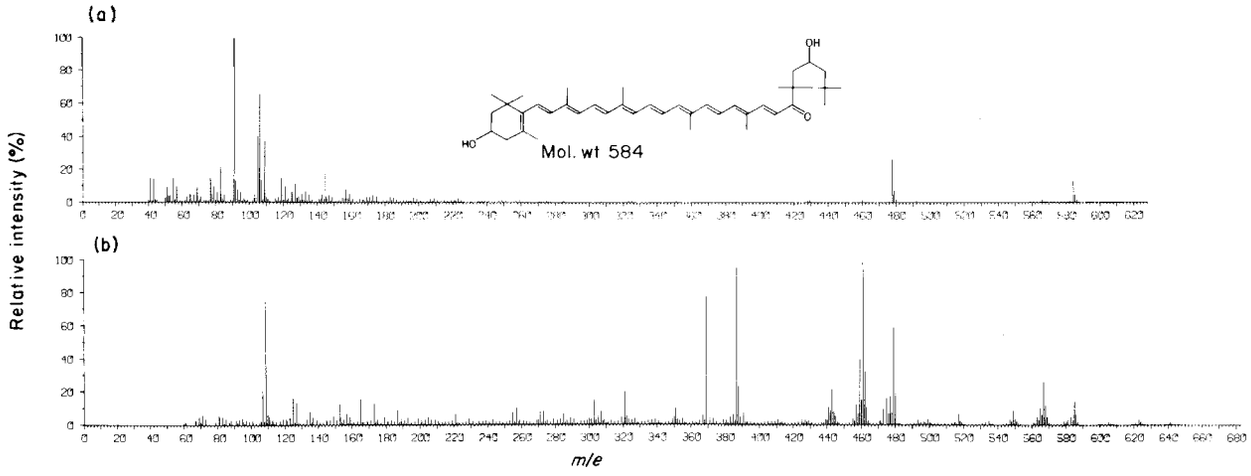


Figure 2. EI and isobutane CI spectra of capsanthin. (a) EI; (b) isobutane CI.

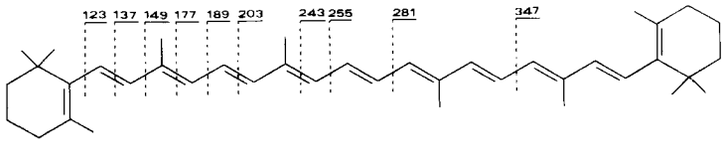


Figure 3. CI fragmentation pattern of β-carotene.

capsanthol (X = E, Y = H) the intensity of the $[M+1]^+$ peak decreases because of the ease with which water is lost.

The $[M\pm1-nH_2O]^+$, $[M\pm1-92-nH_2O]^+$ and $[M\pm1-106-nH_2O]^+$ ions (n = 1–3) usually give rise to intense peaks. Thus, in the capsanthin spectrum the $[M+1-106-H_2O]^+$ ion is responsible for the base peak at m/e 461. In EI spectra peaks due to $[M-nH_2O]^+$ ions are of small intensity so that the EI spectrum of capsanthol shows peaks at m/e M(9), $M-H_2O$(4), $M-2H_2O$(2) and the $M-3H_2O$ peak is not observed. In EI spectra of capsanthin and capsanthol the base peak is at the low mass end at m/e 91. Thus, none of the EI base values are associated with loss of water.

Peaks in the CI spectra associated with the end groups (D,E,G,H) of hydroxylated derivatives contribute greatly to identification of individual carotenoids. For instance, after initial losses of water from 4,4′-dihydroxy-β-carotene (end group D) fragmentation gives ions at m/e 361, 347, 269, 203, 201, 189, 149 and 123 which are characteristic of β-carotene. These peaks are not distinctive in the EI spectrum and only appear as a general fragmentation of the polyene chain. The CI spectra of end group D also show peaks at m/e M − 153 and M − 193 which are not detected in the EI spectrum.

The end groups G and H (of capsanthin and capsanthol respectively) give rise to an intense ion at m/e 109 also derived from a dehydrated precursor noted previously in EI spectra.[3] End group G also leads to peaks at M − 155 and M − 127 as well as ions at m/e 155 and m/e 127 associated with fission reactions flanking the carbonyl group.[4] Small ions at M − 157 and ions at m/e 157 and m/e 127 are given by the reduction product capsanthol (end group H). Similar peaks also appear in the EI spectra.

The CI spectra from bisilylated capsanthin (end groups F and I) are characterized by abundant ions due to losses of 90 mass units (trimethylsilylol) from the $[M+1]^+$, $[M+1-92]^+$ and $[M+1-106]^+$ ions, whereas the EI spectrum has neither abundant ions nor ions with low intensity at high mass [M(6), M − 90 (not observed), M − 92(< 1), M − 106(12), M − 180 (not observed)], but does show a strong ion (base peak) at m/e 197 ($C_{11}H_{21}OSi$, trimethylsilylcyclopentyl group).

Further characteristic ions from this derivative at m/e M − 227, 227 and 199, analogues of M − 155, 155 and 127 of capsanthin, are observed for end group I.

Special features of the CI spectrum of capsanthin are ions at m/e 387, shown by metastable defocusing studies to be due to consecutive losses of xylene and of toluene from the $[M+1]^+$ ion, and at m/e 369 formed by loss of water from the ion at m/e 387. Metastable ions corresponding to the loss of toluene from the $[M+1-\text{xylene}]^+$ and of the loss of 198 [xylene + toluene from the $[M+1]^+$ and $[M+1-H_2O]^+$ ions, were obtained. This consecutive loss of xylene and toluene does not occur in the corresponding EI spectra. These results were confirmed by high resolution measurements which showed that the ions at m/e 387 and m/e 369 corresponded to $C_{25}H_{39}O_3$ and $C_{25}H_{37}O_2$, respectively.

The consecutive loss of xylene and toluene is also observed in the spectrum of bistrimethylsilyl capsanthin and in the spectrum of capsanthol.

A mechanism for the loss of toluene from the $[(M+1)-106]^+$ ion, i.e. succeeding the loss of xylene from capsanthin, is shown in Scheme 1.

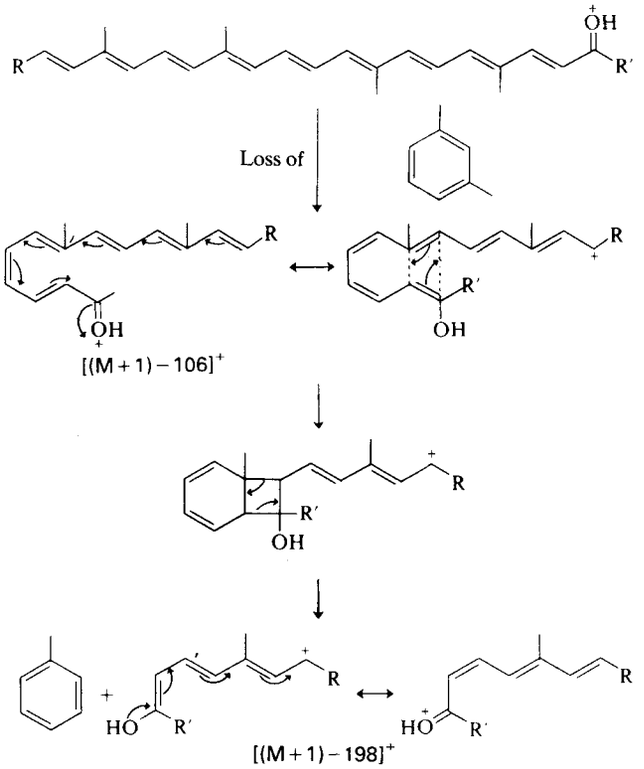
The molecule is assumed to be protonated at the 6′-keto group and xylene is lost from the marked position on the polyene chain according to the Edmunds–Johnstone mechanism.[1] The resulting $[(M+1)-106]^+$ ion can undergo rearrangement making possible elimination of toluene from the chain.

The driving force for this reaction is probably the formation of the highly resonance stabilized $[(M+1)-198]^+$ ion. By the proposed mechanism loss of toluene from the $[(M+1)-106]^+$ ion can occur only if protonation occurs on a polar functional group in the polyene chain. Thus capsanthin, bis-trimethylsilyl capsan-

Table 2. Comparison of intensity ratios of the $[M\pm1-92]^+/[M\pm1-106]^+$ obtained in CIMS with $[M-92]^+/[M-106]^+$ ratios obtained in EIMS

	CI				EI
	Hydrogen		Isobutane		
Polyene	R_1[a]	R_2[b]	R_1[a]	R_2[b]	$\frac{[M-92]^+}{[M-106]^+}$
Lycopene	0.26–0.35	0.28–0.33	0.27	0.82	0.30
β-Carotene	2.8	1.5–4.1	1.2–2.5	0.9–1.7	6.7
Canthaxanthin	1.4	1.1	—	—	—
4,4′-Dihydroxy-β-carotene	6.5	—	—	—	2.7–4.1
Capsanthin	—	—	0.04	0.06	0.03–0.16
Capsanthol	0.10	0.15	0.50	0.40	0.11
Bistrimethylsilylcapsanthin	—	—	0.05	0.14	0.04–0.08

[a] $R_1 = \frac{[(M+1)-92]^+}{[(M+1)-106]^+}$. [b] $R_2 = \frac{[(M-1)-92]^+}{[(M-1)-106]^+}$.



Scheme 1. Fragmentation pattern of capsanthin—loss of xylene followed by loss of toluene.

thin and capsanthol are capable of undergoing this type of fragmentation.

Sensitivity comparisons of the intensity of the CI[MH] ion and the EI molecular ion were made on β-carotene and canthaxanthin (see Experimental). Response of the $[MH]^+$ ion was much greater in each case and the ratios $CI[MH]^+/EI[M]^{\ddagger}$ were in the range 20–200. Limitation on reproducibility is thus apparent and although the greater sensitivity of the CI technique is established the precision was not sufficient to differentiate the sensitivities of the different reagent gases used. Comparison with the recently described FD techniques[15] shows the CI technique to even greater advantage. Spectra of carotenoids obtained by the FD procedure show molecular ions as the base peak but few it any fragment ions, thus eliminating the possibility of chain length measurement.

In addition FD molecular weight determinations, contrasting with CI determinations, are subject to a degree of uncertainty since some foreknowledge of the class identity is often desirable for unequivocal spectral interpretation.

Acknowledgement

The interest and experimental assistance of Dr I. Brown, Department of Organic Chemistry, Sydney University, is gratefully acknowledged.

This work was supported in part by a grant from the Australian Research Grants Committee.

REFERENCES

1. U. Schwieter, H. R. Bolliger, L. H. Chopard-dit-Jean, G. Englert, M. Kofler, A. Konig, C. v. Planta, R. Ruegg, W. Vetter and O. Isler, *Chimia* **19**, 294 (1965).
2. J. Baldas, Q. N. Porter, L. Cholnoky, J. Szabolcs and B. C. L. Weedon, *Chem. Commun.* 852 (1966).
3. J. Baldas, Q. N. Porter, A. P. Leftwick, R. Holzel, B. C. L. Weedon and J. Szabolcs, *Chem. Commun.* 415 (1969).
4. G. W. Francis, *Acta Chem. Scand.* **23**, 2916 (1969).
5. U. Schwieter, G. Englert, N. Rigassi and W. Vetter, *Pure Appl. Chem.* **20**, 365 (1969).
6. C. R. Enzell, *Pure Appl. Chem.* **20**, 497 (1969).
7. H. Budzikiewicz, H. Brzezinka and B. Johannes, *Monatsh.* **101**, 579 (1970).
8. W. Vetter, G. Englert, N. Rigassi and U. Schwieter, in *Carotenoids*, ed. by O. Isler, Chap. 4. Birkhauser Verlag, Basel (1971).
9. H. Budzikiewicz and R. Pesch, *Org. Mass Spectrom.* **9**, 861 (1974).
10. B. Johannes, H. Brzezinka and H. Budzikiewicz, *Org. Mass Spectrom.* **9**, 1095 1095 (1974).
11. E. Gelpi and J. Oro, *Anal. Chem.* **39**, 388 (1967).
12. F. H. Field, *J. Am. Chem. Soc.* **90**, 5649 (1968).
13. G. W. Francis, *Acta Chem. Scand.* **26**, 1443 (1972).
14. C. R. Enzell, G. W. Francis and S. Liaaen-Jensen, *Acta Chem. Scand.* **23**, 727 (1969).
15. C. D. Watts, J. R. Maxwell, D. E. Games and M. Rossiter, *Org. Mass Spectrom.* **10**, 1102 (1975).

Received 23 July 1978