Mass Spectrometric Studies of Carotenoids

3.* The Fragmentation of Some 6-Keto-Carotenoids

G. W. FRANCIS**

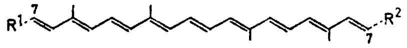
Research Department, Swedish Tobacco Co., Stockholm 17, Sweden

The behaviour of carotenoids on electron impact has been discussed in recent publications and it has been shown that many carotenoid end groups give rise to typical losses. A general study [1] has dealt with a wide variety of carotenoids, and detailed investigations of the carotenes,[2] carotenoid epoxides and furanoid oxides [3,4] have been presented. Carotenoid ketones have been briefly examined by Baldas *et al.*[5]

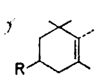
The compounds studied here belong to the group of keto-carotenoids, found in the fruits of higher plants, where the keto group occurs at C6 and is directly conjugated to the central acyclic chain; two of these compounds (*3* and *6*) were examined by Baldas *et al.*[5] whose results accord with those reported here. In the compounds studied, of which six are naturally occurring and the remaining three acetates derived from these, at least one keto group is conjugated with nine double bonds (Table 1). All show typical carotenoid mass spectra with losses of 92 and 106 mass units [1-3,9] together with losses due to fragmentations within the end groups. Some cleavages of the conjugated chain do occur but the ions due to these are of only moderate intensity (*cf.* Ref. 1). Other than the loss of oxygen and water from keto and hydroxy groups and acetic acid from acetoxy groups the fragmentation of 6-keto end groups is limited to cleavages alpha to the keto function.

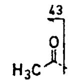
The mechanism normally operating in the α-cleavage of ketones leads to the production of carbonyl ions. In the case of α,β-unsaturated ketones, ions corresponding to the retention of charge by non-carbonyl fragments are also found.[10] An extension of this pattern to aromatic ketones [11,12] produces a situation where the non-carbonyl fragment ions are of comparable intensity to those derived when the carbonyl function retains the charge. Acetophenone has been studied thoroughly [12] and it has been shown that, at least in this case, the non-carbonyl ions are derived by loss of carbon monoxide from the equivalent carbonyl ions. Recently a study [13] has been made of some labelled compounds of the ionone series and this showed that in α- and β-ionone, cleavage alpha to the methyl keto group gives rise to a weak M−43 hydrocarbon ion but not to an M−15 carbonyl ion. Both compounds did, however, give rise to moderately intense acetyl ions due to α-cleavage.

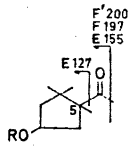
Table 1. Structural formulae of the compounds investigated.



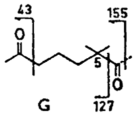
Polyene	R¹	R²
Citranaxanthin (*1*)	A	D
Reticulataxanthin (*2*)	B	D
Capsanthin (*3*)	B	E
Capsanthin diacetate (*4*)	C	F
Capsanthin d_6-diacetate (*5*)	C′	F′
Capsorubin (*6*)	E	E
Capsorubin diacetate (*7*)	F	F
Semi-β-carotenone (*8*)	A	G
β-Carotenone (*9*)	G	G







A R=H D E R=H
B R=OH F $R=COCH_3$
C $R=OCOCH_3$ F′ $R=COCD_3$
C′ $R=OCOCD_3$



G

The situation in the keto-carotenoids corresponds most closely to that found in the ionones,[13] although the non-carbonyl ions are prominent in the case of the carote-

---

* Part 2, see Ref. 1.

** Present address: Organic Chemistry Laboratories, The Technical University of Norway, Trondheim, Norway.

noids while relatively insignificant in the ionones. The parallel extends to the methyl ketones (*1* and *2*) where prominent acetyl ions are observed: these are the only carbonyl ions of any intensity which result from cleavage alpha to the conjugated keto group in the compounds studied here. The stability of the non-carbonyl ions is not surprising since on cleavage of the C6—C7 bond the charge can be readily stabilised in the higher mass fragment by delocalisation throughout the conjugated chain. In the end groups *E*, *F*, *F′* and *G*, fission of the C5—C6 bond results in charge stabilisation at the tertiary C6 atom in the smaller mass fragment. Such stabilisation is not possible in the end group *D* and no M—15 fragment is observed. In the absence of metastable ions it is not possible to say whether the observed non-carbonyl ions are derived from the equivalent carbonyl ions or not.

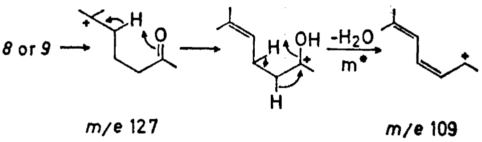
The fragmentations resulting from the presence of the end groups *A*, *B* and *C* have been discussed previously [1,2] and the remarks then made apply to these end groups in the compounds examined here.

The end group *D* (*1* and *2*) gives rise to a prominent M—43 ion which results from α-cleavage with charge retention by the non-carbonyl fragment. In both compounds *1* and *2* the base peak ion is at *m/e* 43 and is the result of the same cleavage where the charge is retained by the carbonyl fragment. An intense M—187 ion is also observed and this is attributable to cleavage of the C15—C15′ bond with hydrogen transfer to the larger non-carbonyl fragment which retains the charge. In addition M—16 and M—18 ions occur but these are of low intensity.

The end group *E* (*3* and *6*) gives rise to an abundant M—155 ion and in the lower mass region to a prominent ion at *m/e* 127, both of which result from α-cleavage where the charge is retained by the non-carbonyl fragment. The base peak ion in both compounds occurs at *m/e* 109 and a metastable ion indicates that this is derived by dehydration of the *m/e* 127 ion. Losses of 16 and 18 mass units are also observed from some of the more prominent ions in the upper part of the spectra.

The end group *F* (*4* and *7*) fragments to produce prominent M—60 and M—197 ions. The expected *m/e* 169 ion is of only average intensity and the base peak occurs at *m/e* 109 and is presumably due to the same species as in the end group *E*. In the end group *F′* the ions in the upper region are shifted to M—63 and M—200 as expected, and the base peak is still at *m/e* 109.

The end group *G* (*8* and *9*) gives an intense M—155 ion as a result of cleavage of the 5—6 bond in the alpha position to the conjugated keto group. Low intensity M—16 and M—18 ions are also observed. The base peak at *m/e* 109 is shown to be due to an ion derived from the abundant *m/e* 127 ion; a mechanism similar to that suggested for the azafrin end group [1] can be written to account for this process. The terminal acyl group gives rise to a very intense *m/e* 43 ion which might prove to be of value when differentiation between the end groups *E* and *G* is required as these are otherwise difficult to distinguish by mass spectrometry. However, this ion is often strong in carotenoid spectra [1] and it would thus seem advisable to undertake derivatisation when these groups are present.



It has been pointed out previously [9] that a qualitative relationship exists between the intensity ratio (*R*) of the M—92/M—106 ions and the length of the conjugated acyclic chain of C_{40}-carotenoids where multiple oxygen functions are absent. At that time it was indicated that this relationship could be extended in modified form when sufficient compounds of a particular type were available; the 6-keto compounds examined here constitute such a set. It is immediately apparent that the presence of the keto function has the same effect as increasing the number of conjugated double bonds in the chain, a result parallel to that found in the visible light absorption of carotenoids. The effect of the first 6-keto function appears to be somewhat larger than that of two double bonds while the second 6-keto function has somewhat less than the effect of two double bonds (see Table 2). Calculation of the ratio (*R*) from the published spectra [1] of other keto-carotenoids shows that this statement can not be extended to those compounds where a keto substituent is situated at a different C-atom.

Table 2. Intensity ratio of M—92/M—106 ions (*R*) as against number of conjugated keto groups (K), conjugated double bonds in the acyclic chain (DB) and (2K+DB).

Polyene	K	DB	2K+DB	*R*
Capsanthin d_6-diacetate (*5*)	1	9	11	0.224
Semi-*β*-carotenone (*8*)	1	9	11	0.190
Citranaxanthin (*1*)	1	9	11	0.162
Capsanthin (*3*)	1	9	11	0.152
Reticulataxanthin (*2*)	1	9	11	0.091
Capsanthin diacetate (*4*)	1	9	11	0.086
Capsorubin diacetate (*7*)	2	9	13	0.039
Capsorubin (*6*)	2	9	13	0.030
β-Carotenone (*9*)	2	9	13	0.026
From Ref. 7				
Acyclic carotenoids	—	11	11	0.27—0.36
Acyclic carotenoids	—	12	12	0.057—0.068
Acyclic carotenoids	—	13	13	0.018—0.029

Experimental. The compounds *1*, *2*, *8*, and *9* were generous gifts from Dr. H. Yokoyama of the Fruit and Vegetable Laboratory, Pasadena, California, USA. Compounds *3* and *6* were isolated from paprika by established procedures [7] and their acetates *4*, *5*, and *7* prepared as described in the original literature.

The mass spectra were obtained on an LKB 9000 instrument as previously described,[1] the only modification being that somewhat lower probe temperatures were required. Accurate mass determinations were carried out on an MS 902 double-focusing instrument on a number of ions whose constitutions were critical to the argument.

Citranaxanthin (*1*) M—43 found 413.320, calc. for $C_{31}H_{41}$ 413.321; M—187 found 269.227, calc. for $C_{20}H_{29}$ 269.227; *m/e* 43 found 43.0185, calc. for CH_3CO 43.0184.

Semi-β-carotenone (*8*) *m/e* 127 found 127.112, calc. for $C_8H_{15}O$ 127.112; *m/e* 109 found 109.102, calc. for C_8H_{13} 109.102; *m/e* 43 found 43.0185, calc. for CH_3CO 43.0184.

Acknowledgements. I am obliged to Docent C. R. Enzell for helpful discussions, Mr. J. Roeraade for valuable assistance in obtaining the spectra and siv.ing. T. Sigmond, The Technical University of Norway, for accurate mass determinations.

1. Enzell, C. R., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand.* **23** (1969) 727.
2. Schwieter, U., Bolliger, H. R., Chopard-dit-Jean, L. H., Englert, G., Kofler, M., König, A., von Planta, C., Rüegg, R., Vetter, W. and Isler, O. *Chimia* **19** (1965) 294.
3. Baldas, J., Porter, Q. N., Cholnoky, L., Szabolcs, J. and Weedon, B. C. L. *Chem. Commun.* **1966** 852.
4. Bonnett, R., Mallams, A. K., Spark, A. A., Tee, J. L., Weedon, B. C. L. and McCormick, A. *J. Chem. Soc.* **1969** 429.
5. Baldas, J., Porter, Q. N., Leftwick, A. P., Holzel, R., Weedon, B. C. L. and Szabolcs, J. *Chem. Commun.* **1969** 415.
6. Yokoyama, H. and White, M. J. *Phytochem.* **5** (1966) 1159; and refs. therein.
7. Cholnoky, L., Szabolcs, J., Cooper, R. D. G. and Weedon, B. C. L. *Tetrahedron Letters* **1963** 1257; and refs. therein.
8. Yokoyama, H. and White, M. J. *Phytochem.* **7** (1968) 1031.
9. Enzell, C. R., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand.* **22** (1968) 1054.
10. Budzikiewicz, H., Djerassi, C. and Williams, D. H. *Interpretation of Mass Spectra of Organic Compounds*, Holden-Day, San Francisco 1964, pp. 155—160.
11. Ref. 8, pp. 191—193.
12. Meyerson, S. and Ryelander, P. N. *J. Am. Chem. Soc.* **79** (1957) 1058.
13. Thomas, A. F., Willhalm, B. and Müller, R. *Organic Mass Spectrometry* **2** (1969) 223.

Received September 27, 1969.