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Mass Spectrometric Studies of Carotenoids

5.* Steric Effects in In-chain Elimination Reactions

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Synthetic carotenoids, having the two central methyl groups oxygenated and shifted from the 13,13′ to the 14,14′ positions, have been examined mass spectrometrically. The fragmentation of these is consistent with the Edmunds-Johnstone mechanism, except for the fact that the $M-C_6H_5CH_3$ ion is absent, and this is ascribed to steric inhibition of the formation of the required intermediate. The influence of the number of conjugated double bonds in the acyclic polyene chain on the intensity ratio of the M − 92 and M − 106 peaks, which has previously been observed, is discussed on the basis of steric factors and recent mechanistic results. The possibilities of determining the position of an oxygenated in-chain substituent in acyclic carotenoids are considered.

Recently, findings on the mechanism of in-chain elimination reactions in normal carotenoids,[1,2] and on the structure and mass spectrometric fragmentation of several naturally occurring carotenoids containing oxygen substituted in-chain methyl groups,[3–7] have been published. It was therefore of considerable interest to examine a set of synthetic model compounds,[8,9] which have the two central methyl groups substituted and shifted from the normal 13,13′ to the 14,14′ positions (*1* – *3*), Fig. 1. The fragmentation reactions of these compounds and aspects of the in-chain eliminations constitute the topic of the present paper.

On the basis of the Edmunds-Johnstone mechanism,[1,10] which involves rupture of double bonds only, it would be expected that the compounds *1* – *3* should give rise to $M-C_6H_5CH_3$, $M-C_6H_5R$, and $M-C_6H_4R_2$ fragments but not to $M-C_6H_4CH_3R$ ions (*cf.* Fig. 1). The spectra show that the fragmen-

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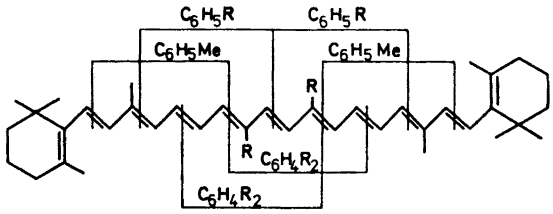
tations take place essentially according to this mechanism, and very strong $M-C_6H_4R_2$ and significant $M-C_6H_5R$ peaks are observed, while peaks due to the $M-C_6H_4CH_3R$ are absent or, in the case of the alcohol, of low intensity. However, no $M-C_6H_5CH_3$ peaks are present, and it may therefore be concluded that elimination of the C(8)–C(13) and C(8′)–C(13′) fragments does not occur, and that this type of elimination is limited to the 10–10′ range. Consequently, a distinction between the two central substituents and those in the 9,9′ positions is easily made, as has previously been demonstrated for the case of bicyclic compounds with the normal substitution pattern.[2,4,10]

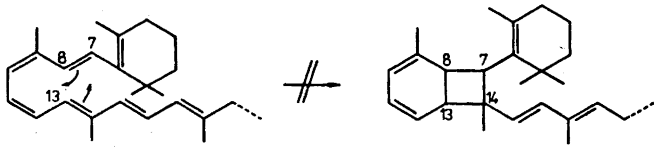
Other features of interest in these spectra are the occurrence of prominent $M-C_{10}H_8R_2$ ions, significant M–16 ions in the case of the aldehyde and the alcohol, and M–58 ions in the case of the acetate. The elimination of oxygen from allylic carotenols on electron impact has been discussed previously.[11,12]



R	$M-C_6H_5CH_3$	$M-C_6H_5R$	$M-C_6H_4CH_3R$	$M-C_6H_4R_2$
1 –CHO	–	3.5 %	–	94 %
2 $-CH_2OH$	–	1.0 %	0.3 %	100 %
3 $-CH_2OCOCH_3$	–	5.0 %	–	100 %

Fig. 1.

In view of the fact that the stage of oxidation of the central in-chain substituents in the compounds *1*–*3* does not appreciably effect the outcome of the in-chain elimination reactions, and also that $M-C_6H_4CH_3CH_2OH$ ions were observed for loroxanthin [4] (19-hydroxylutein), it may be concluded that oxygenation alone cannot satisfactorily explain the observed inhibition of the C(8)–C(13) and C(8′)–C(13′) fragments in compounds *1*–*3*. A more probable cause is that the shift of a substituent from the 13 to the 14 position



I

Fig. 2.

prevents the formation of the cyclic intermediate (I) on steric grounds, since C(14) would here become quaternary, and the vicinal C(7) in the four membered ring would carry the bulky, substituted cyclohexene ring (*cf.* Fig. 2).

Examination of a synthetic analogue of β-carotene (*4*), in which the C(13) methyl group is shifted to C(14), has shown [1] that the intensity ratio of the M − 92 to the M − 106 peaks, I_{M-92}/I_{M-106}, is reduced considerably (0.56) compared with that (12.9) observed for β-carotene (*5*), although the number of structurally possible ways of eliminating toluene and xylene remains the same (*cf.* Fig. 3). Calculations show that the reduction is so great, that it cannot be explained solely by inhibition of elimination of the C(8) − C(13) fragment, and suggest that elimination of xylene by loss of the C(14) − C(12′) fragment is greatly increased. This would be expected on steric grounds as the vicinal carbons C(13) and C(11′) in the four membered ring of the intermediate (II) would be tertiary, while one of these carbons, C(13), on loss of the C(14) − C(12′) fragment from all normally substituted compounds, would be quaternary. Moreover, the cyclohexene substituent on the four membered ring of the intermediate, which is present in the normal case of xylene elimination, is now replaced by part of the conjugated chain with the cyclohexene ring in a remote position.

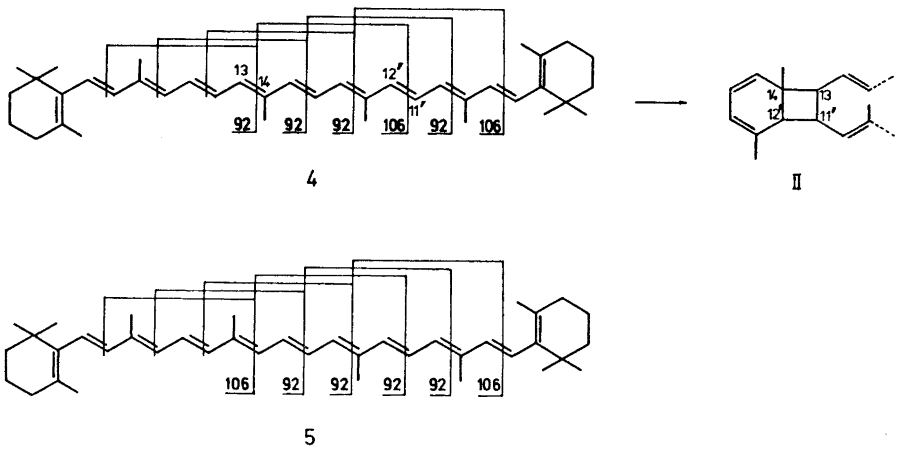
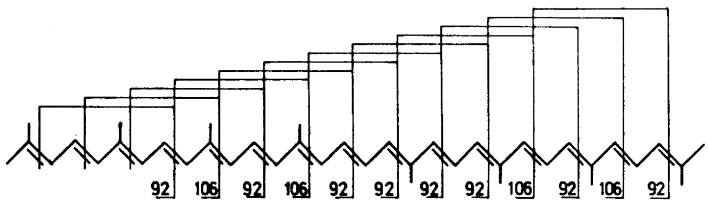


Fig. 3.

Hence, it seems that steric interaction in the intermediate is a dominating factor on the outcome of these elimination reactions, although electronic effects may also be of significance. The previously observed decrease of the M − 92/M − 106 intensity ratio on increasing the number of conjugated double bonds of the acyclic chain from 9 to 13 [13] is consistent with the steric effect. The decrease in this ratio on going from bicyclic compounds with 9 conjugated double bonds in the chain to monocyclic with 10, and from the latter to acyclic compounds with 11 double bonds, is satisfactorily explained by a decrease in

steric hindrance for the elimination of xylene, since the 1,2-interaction in the intermediate decreases as the bulky cyclohexene substituent is replaced by the less space-demanding acyclic substituent. It should be observed that the ratio between the number of structurally possible modes for elimination of toluene and xylene is in fact increased.

The further decrease in the M − 92/M − 106 ratio on increasing the number of conjugated double bonds in the acyclic chain up to 13 may be ascribed to new ways of elimination of xylene and a corresponding increase in the probability of elimination of xylene relative to toluene. Thus, the introduction of a new double bond between carbons 3 and 4 in the saturated acyclic end group, which increases the number of double bonds from 11 to 12, increases the number of structurally possible ways of eliminating xylene from two to three. Insertion of a further double bond between carbons 4′ and 3′ expands the number of possibilities from three to four. In agreement with expectation, the insertion of still further double bonds between C(1) and C(2), and between C(1′) and C(2′) as in bisdehydrolycopene (*6*), causes an increase in the M − 92/M − 106 intensity ratio, as this only allows new ways for the formation of toluene, but not for xylene; no further release of steric strain in the intermediate on elimination of xylene is to be expected.



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In acyclic carotenoids of the lycopene type having an oxygenated methyl group, it is possible, from the presence or absence of $M-C_6H_5R$ and $M-C_6H_4RCH_3$, to say whether it is one of the four central methyl groups or any of the others that have suffered oxygenation. However, contrary to the situation in bicyclic compounds, no distinction between the 19,19′ and 20,20′ methyl groups can be made on this basis.[2,6] In this case, quantitative considerations of the intensity ratios are of interest.

Studies on 7,7′-d_2- and 15,15′-d_2-lycopene have revealed[1,2] that toluene is eliminated to an equal extent from all structurally possible positions of the polyene chain. It may therefore be concluded that the intensity ratio of the $M-C_6H_5R$ to $M-C_6H_5CH_3$ peaks, $I_{M-C_6H_5R}/I_{M-C_6H_5CH_3}$, should be 0.2 if C(19) or C(19′) is substituted, but 0.5 if C(20) or C(20′) is substituted, provided the influence of R and CH_3 on the reaction is the same. Similarly, the $I_{M-C_{10}H_8CH_3R}/I_{M-C_{10}H_8(CH_3)_2}$ ratio should be 2.0 for C(20) or C(20′) substitution, but 0.5 for C(19) or C(19′) substitution, while the $I_{M-C_6H_4CH_3R}/I_{M-C_6H_4(CH_3)_2}$ ratio should be 1, irrespective of which of these four methyl groups have suffered substitution.

Results on the fragmentation of carotenoids of the rhodopinal series, which have been found to be oxygenated in the 20 position, have been published most recently by Francis.[6,7] In the compounds studied, the oxygenated in-chain substituent R represents $-CH_2OH$, $-CHDOH$, $-CH_2OOCCH_3$, $-CH_2OOCCD_3$, and $-CHO$. These studies show that the elimination of C_6H_5R and $C_6H_4RCH_3$ are favoured relative to $C_6H_5CH_3$ and $C_6H_4(CH_3)_2$, and that the $I_{M-C_6H_5R}/I_{M-C_6H_5CH_3}$ values vary between 0.74 – 2.20, $I_{M-C_6H_5CH_3R}/I_{M-C_6H_4(CH_3)_2}$ between 1.9 – 16.6, and $I_{M-C_{10}H_8CH_3R}/I_{M-C_{10}H_8(CH_3)_2}$ between 1.7 – 16.1. These findings therefore indicate that the substituent effect in these cases is too large to permit conclusions to be drawn on the basis of such quantitative data. It should be remarked, however, that these compounds are thermally very unstable, giving mass spectra which exhibit considerable intensity variations.

A satisfactory way for distinction between oxygenation of C(19) and C(20) in acyclic compounds, based on the principle outlined above, should, however, become available on converting the oxygenated methyl group to a deuterium labelled methyl group. The compounds required to test this have, however, not been accessible to date.

The origin of the toluene and xylene species in carotenoids where the polyene chain is extended beyond that of lycopene, *i.e.* comprising 12 – 15 conjugated double bonds, has not yet been studied experimentally. It appears probable from the above considerations (*cf.* structure *6*), however, that in compounds of spirilloxanthin type (*e.g. 7*) or rhodovibrin type (*e.g. 8*) substitution of C(18) should be revealed by the presence of $M-C_6H_4CH_3R$ and absence of $M-C_6H_5R$ ions. This would therefore allow ready distinction between

Table 1. Important peaks in the upper part of the spectra of the compounds *1* – *3*.

Dial *1*			Diol *2*			Diacetate *3*		
Mass number		Relative intensity	Mass number		Relative intensity	Mass number		Relative intensity
M	564	100	M	568	12	M	652	21
M – 2	562	32	M – 2	566	1	M – 2	650	4
M – 16	548	35	M – 16	552	3	M – 58	594	7
M – 18	546	50	M – 18	550	4	M – 60	592	12
						M – 58 – 60	534	5
						M – 60 – 60	532	5
M – 106	458	3.5	M – 108	460	1	M – 150	502	5
			M – 122	446	0.3			
M – 123	441	10						
M – 124	440	7	M – 124	444	0.3			
M – 125	439	8						
M – 134	430	94	M – 138	430	100	M – 222	430	100
M – 148	416	22						
M – 134 – 15	415	10	M – 138 – 15[a]	415	5	M – 222 – 15	415	7
M – 186	378	44	M – 190	378	7	M – 274	378	26

[a] "Metastable" peak at *m/e* 401, corresponding to the reaction $430^+ \rightarrow 415^+ + 15$.

substitution at C(18) and C(19)/C(20). In the fully conjugated pentadecaene (*6*) such distinction should no longer be possible, whereas in-chain and end-of-chain substituents may be distinguishable.

7 Spirilloxanthin, R = CH_3

8 Rhodovibrin, R = CH_3

EXPERIMENTAL

The mass spectra were recorded on an LKB 9000 instrument at 70 eV, with 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 transferred to the probe, the solvent removed with a stream of nitrogen, and the probe immediately inserted into the machine.

The dial *1*[8] was obtained from Dr. Kralt, and the preparation of the derivatives *2* and *3* has been described elsewhere.[9] Significant peaks in the upper part of the spectra of these compounds are given in Table 1.

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