Pure & Appl. Chem., Vol. 57, No. 5, pp. 693–700, 1985.
Printed in Great Britain.

Biodegradation of carotenoids—an important route to aroma compounds

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Abstract - In the group of compounds referred to as degraded carotenoids the majority of the members are volatile compounds comprising 13 to 9 carbon atoms. A fair number of these are important aroma constituents occurring in many higher plants, some of which are widely used and of considerable economic importance. The discussion will be focused on the formation of selected representatives encountered in tobacco, since this is the richest single source of these compounds and also since it comprises a large number of degraded diterpenoids of known structure and stereochemistry providing additional information on possible degradative pathways.

Although degraded carotenoids constitute a large group of compounds, only the function of a few - mainly those which play a vital role in life processes - is known. Many, however, possess highly attractive aroma properties and it is primarily selected members of this group and their formation which will be considered here.

The degraded carotenoids retaining the largest number of original carbon atoms are the nor-carotenoids, which have 39, 38 or 37 carbon atoms. However, these are of little interest here, since neither do they appear to be precursors of any of the aroma compounds under consideration, nor do the reactions leading to their formation have any bearing on the generation of the aroma compounds. In contrast, both the formation and the structures of the apo-carotenoids are of importance, since, being present in higher plants and frequently possessing the same end groups as the four carotenoids predominant here, they are potential precursors of the carotenoid-like aroma compounds. Remarkably little, however, is known about their immediate precursors and the reactions by which they are formed. In fact, it is still unclear whether they result from direct cleavage of the bonds indicated in Fig. 1, or by a stepwise mechanism of the Glover-Redfearn type. Moreover, it is still an open question in virtually all cases whether the cleavage is enzyme-assisted or accomplished by singlet oxygen or autooxidation.

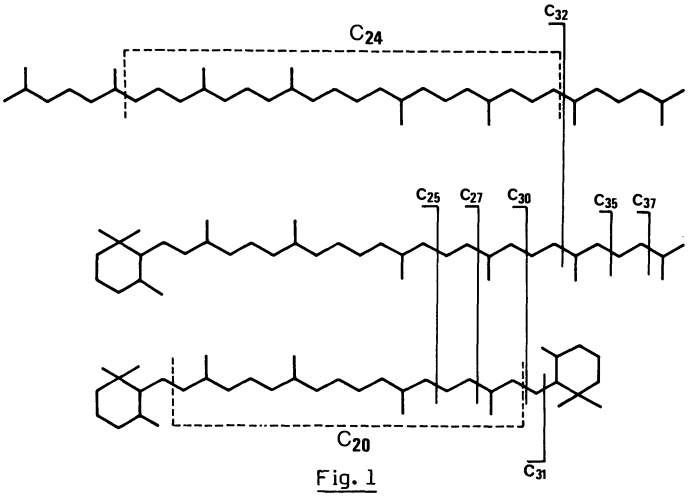


Fig. 1

More detailed information on enzymatic in-chain cleavages is only available for the conversion of β-carotene to retinal, which is postulated to occur as shown in Fig. 2 (Ref. 1). However, it has also been shown that a tea enzyme preparation in the presence of tea flavanols converted ^{14}C-labelled β-carotene into β-ionone, and several other, unidentified volatile substances including some derived exclusively from the central part of the polyene chain (Ref. 2). Furthermore, soy bean lipoxygenase is known to effect the conversion of violaxanthin into 3-hydroxy-β-ionone epoxide (Ref. 3). It seems therefore that there are site-specific as well as non-sitespecific enzymes capable of effecting cleavage of the polyene chain.

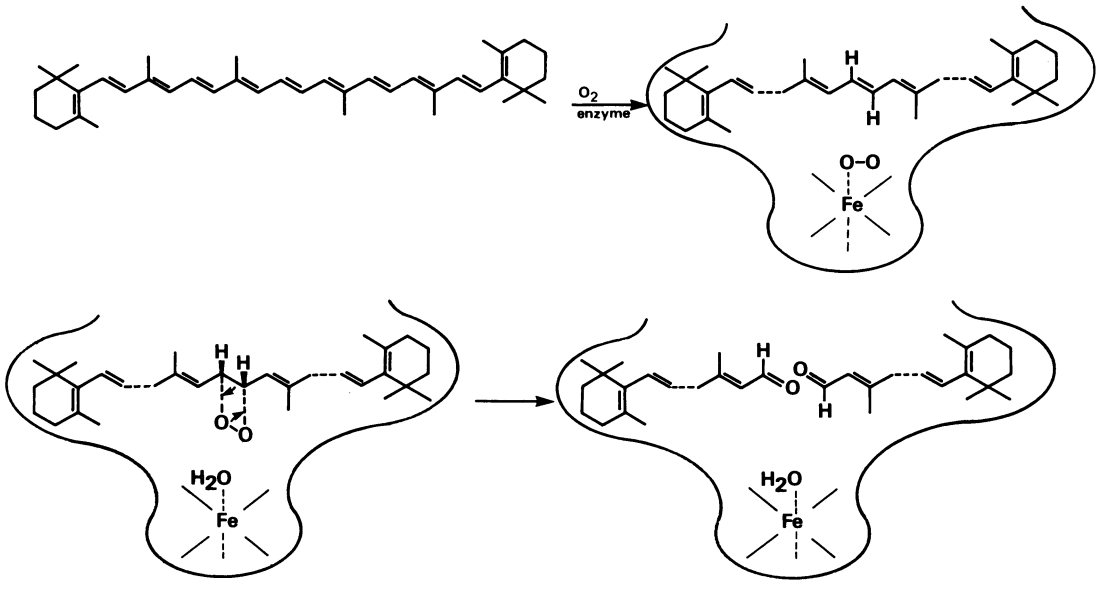
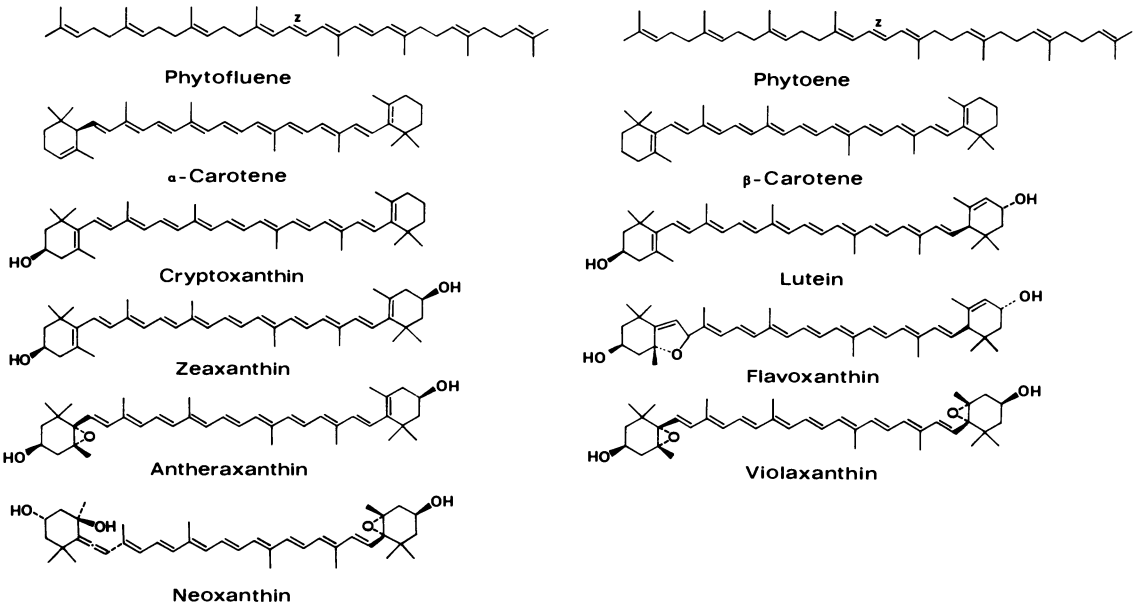


Fig. 2

Attack by non-site specific enzymes resembles, in terms of products, attack by singlet oxygen. Several $^1O_2$ oxidation studies have revealed that products can be obtained from β-carotene by cleavages of the 11,12, 9,10, 8,9 and 6,7 bonds, from zeaxanthin by cleavages of the 9,10, 8,9, 7,8 and 6,7 bonds and from violaxanthin by cleavages of the 11,12, 9,10 and 8,9 bonds (Ref. 4-6). Fairly consistent with these results, perbenzoic acid oxidation of canthaxanthin has been shown to yield the corresponding 9,10-, 11,12- and 13,14-monoepoxides, the last two of which furnish 4-oxo-14'-apo-β-caroten-14'-al and 4-oxo-12'-apo-β-caroten-12'-al on exposure to magnesia (Ref. 7, 8).

Moreover, alkaline permanganate oxidation of 5,6,5',6'- and 5,8,5',8'-epoxides yields mainly the corresponding C_{27} and C_{25} epoxyaldehydes through cleavage of the 9,10 (9',10') and 11,12 (11',12') bonds. Similarly, the 9,10, 9',10', 11,12 and 11',12' bonds are also cleaved in 5,6-epoxy-5,6-dihydro-β,α-carotene, while 5,6-epoxy-5,6-dihydro-β,β-carotene suffers cleavage predominantly of the 7',8' bond to yield the C_{30} epoxyaldehyde (5,6-epoxy-5,6-dihydro-8'-apo-β-caroten-8'-al). Neoxanthin yields the C_{27} and C_{25} epoxy-apo-aldehydes but no allenic aldehydes (Ref. 9). Judging by these results, the functionality pattern in the rings influence both the site and rate of oxidative attack of the in-chain bonds.



In summary therefore, it seems - although all in-chain double bonds are probably vulnerable to attack - that there is a certain preference for attack of the 9,10 double bond and that cleavage of the 6,7 bond is favoured only when C-6 is part of a β-ionone ring structure. Since there is little useful information about the reactions and routes of formation involved in the generation of the apo- and diapo-carotenoids and also in the generation of the trisporic and abscisic acids once they diverge from the established common pathways of terpenoid biosynthesis, we shall turn directly to the carotenoids of tobacco, shown above, and the C_{13} to C_9 degradation products observed here.

On the grounds of the end groups present in the cyclic tobacco carotenoids we would expect to find, as a result of 9,10 double bond cleavage, six C_{13} methyl ketones. Four of these (1-4) have been encountered, while the grasshopper ketone (5) from the allenic end group of neoxanthin and (3R,6R)-3-hydroxy-α-ionone (6) from the end groups of flavoxanthin and lutein are missing (Ref. 10, 11). Not unexpectedly, three of these tobacco constituents are important aroma compounds of common occurrence possessing excellent flavour properties, also when applied at the ppm level (Ref. 12, 13).

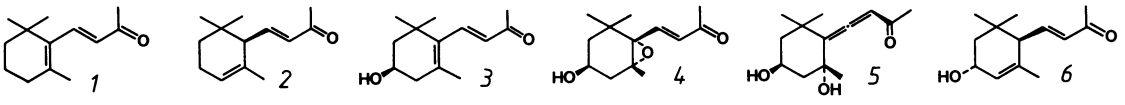


Fig. 3

Whether detected in tobacco or not, it is convenient to regard these primary C_{13} methyl ketones as precursors of the other representatives of the series, now amounting to some fifty compounds. Many of these metabolites can be viewed as arising by simple reactions such as oxidation, reduction, dehydration and cyclisation. An example is given in Fig. 4, which illustrates probable routes of interconversion of a few known tobacco constituents, some of which have outstanding flavour characteristics, e.g. the megastigmatrienones (10, 11) often regarded as the heart of the tobacco aroma.

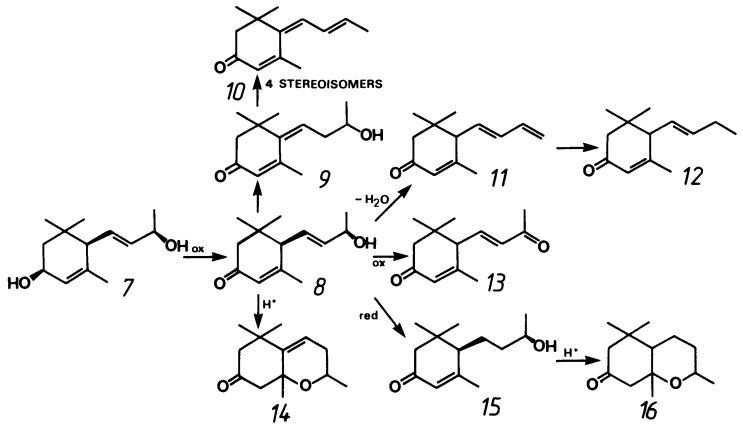


Fig. 4

Inspection of the diol structure (7) shows that the configuration at C-3 is opposite to that of the α-ionone rings present in the known tobacco carotenoids. This difference raises the question whether these compounds are generated by stereospecific reactions at the C_{13} stage or are derived from carotenoids not yet encountered in tobacco, e.g. 3'-epilutein. The latter possibility is rendered likely by the facts that 3'-epilutein is very difficult to separate and distinguish from lutein (Ref. 14), that the 9,10-cleavage product of the major tobacco carotenoid lutein has not been detected in tobacco, and that the 9-epimer of the diol (7), expected on the grounds of non-stereospecific reduction of the primary C_{13} product from 3'-epilutein, also occurs in tobacco.

Many of these C_{13} compounds are clearly formed by more complex routes, which make the precursor-product relationships less obvious, and in the absence of isotopic experiments, leave the stereochemical information and the numerous chemical studies on degraded carotenoids as the sole source of information about these relationships.Thus, of the pathways invoked in Fig. 5 for the formation of the three tobacco decalines (18, 19, 25), those leading to the formation of the two aroma compounds (18, 25) have been carried out experimentally (Ref. 15, 16).

Both of these compounds have been obtained from other sources, the ketone (18) from passion fruit and the dihydronaphthalene (25) from peach and strawberry oils as well as from the "bouquet" of Italian red wine (Ref. 12).

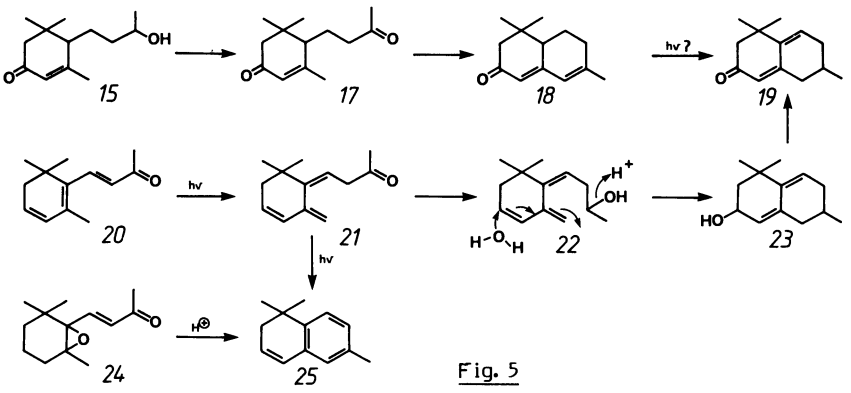


Fig. 5

A most important group of carotenoid-derived aroma compounds are the damascones, which are all oxygenated at C-7. Damascenone (28) as well as some of the other C_{13} tobacco constitutents of this class can on the basis of chemical studies and biogenetic considerations by Ohloff et al. (17) and Isoe et al. (18) be regarded as derived from neoxanthin via the grasshopper ketone (5) and the allenic triol (26), cf. Fig. 6. Although none of these steps have been carried out experimentally, support is obtained from an analogous set of reactions involving the conversion of the allenic diol (30), obtained from β-ionol (29) by $^1O_2$ oxidation, to β-damascone (31) (Ref. 19). An alternative route to the 7-oxo compounds, which is of considerable interest in view of the ready conversion of allenic carotenoids into their acetylenic counterparts (neoxanthin → diadinochrome) (Ref. 20) and the recent isolation of an acetylenic diol from tobacco, is that implied by Ohloff's biomimetic synthesis. This involves the acid catalysed conversion of the trihydroxy-acetylene (33) to damascenone (28) and 3-hydroxy-β-damascone (27) (Ref. 17). Hydride reduction of the latter furnished another tobacco constituent, the hydroxyketone (32) (Ref. 20). It is of interest to note that the levels of damascenone and β-damascone are increased on curing and ageing of tobacco.

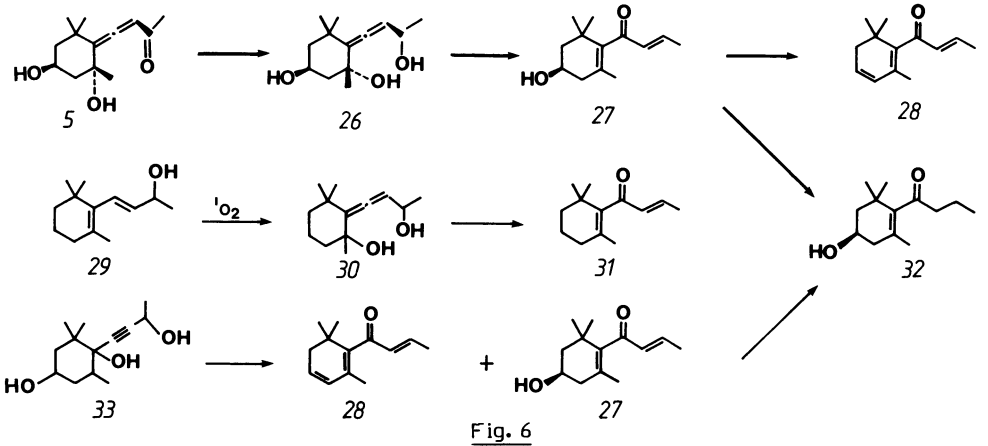


Fig. 6

Related to these compounds are two bicyclodamacenones, bicyclodamascenone A (37) and B (38), which have been obtained from Virginia tobacco by steam-distillation (Ref. 21). These are regarded as being derived from damascenone (28) and were accordingly prepared from this compound by acid catalysed cyclisation, reactions which were rationalised as shown in Fig. 7. Thus, an initial stereospecific cyclisation of the "trans" hydroxyallyl cation (34) furnishes in turn a bicyclic intermediate (35) and a protonated cyclobutanone (36), which on deprotonation accompanied by cleavage of the 5,8-bond or the 5,6-bond produces bicyclodamascenone A (37) and B (38) respectively.

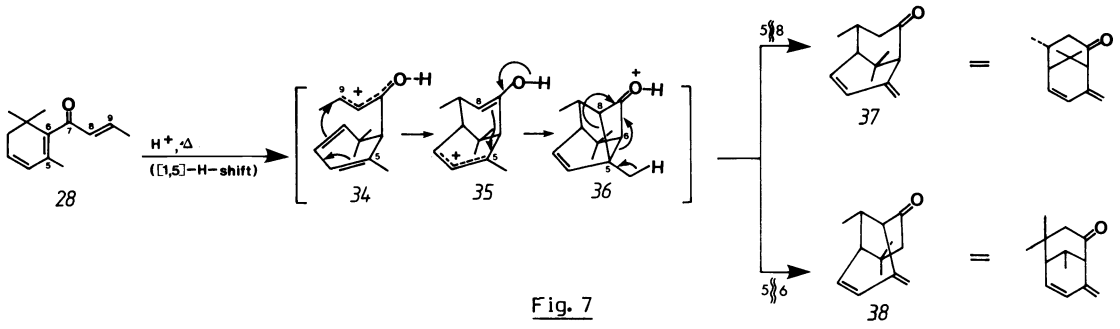


Fig. 7

In addition to 3,6-and 5,6-epoxides, there are four C_{13} compounds in tobacco which have an oxygen in the 6-position. One these constitutes the first tobacco C_{13} representative having an exocyclic 5,13 double bond (5(13),7E-megastigmadiene-6,9-diol) and hence evidently derived directly from β-ionol by singlet oxygen oxidation, a reaction which in our laboratory furnished this compound as the major product (Ref 22). While dehydrovomifoliol (39) and blumenol A (40) could also arise on $^1O_2$ oxidation from 3-oxo-β-ionone and 3-oxo-β-ionol respectively, the chemically verified route from the primary precursor (4) in Fig. 8, is of equal probability. Here, 3-hydroxy-β-ionon-5,6-epoxide (4) is shown to yield dehydrovomifoliol (39) on Sarett oxidation (Ref. 23), which on reduction furnishes blumenol A (40) (Ref. 24). It seems highly probable that blumenol A (40) in turn can give rise to blumenol B (41), not yet found in tobacco, and subsequently to the important tobacco flavour compound theaspirone (44). These latter transformations have been effected synthetically (Ref. 24) and blumenol B is known to yield theaspirone on dehydration (Ref. 25). The other tobacco spiroether (42) may arise in an analogous manner from dehydrovomifoliol (39) after reduction of the two double bonds.

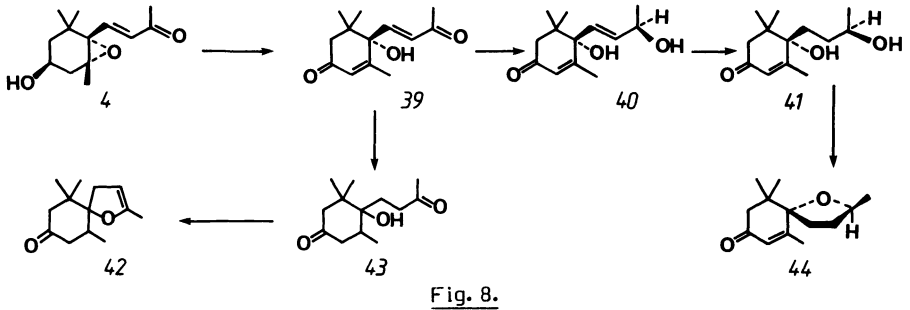
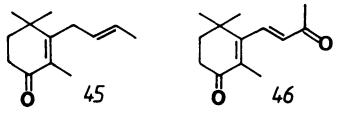


Fig. 8.

Despite their excellent aroma qualities, the two megastigmenes oxygenated in the 4-position (45,46) have only recently been obtained from tobacco. Of these, the diketone (46), which has been isolated earlier from both rabbit urine and the anal gland secretion of the red fox (Ref. 12), is produced on photo-oxygenation of the primary precursor α-ionone (Ref. 26), a route by which it is obviously also formed in tobacco.



Although the diketone (46) might serve as a precursor of the monoketone (45), the latter can also arise, as demonstrated by Demole (27), from one of the four epoxides depicted in Fig. 9. Thus, the 5,6-epoxides (47,48) on protonation of the epoxide group followed by C-4 deprotonation yield two diols (52,53), which exist in an acid catalysed equilibrium with the corresponding secondary diols (51,54). The formation of 5,8E-megastigmadien-4-one (45) and of β-ionone (1) from the diol equilibrium mixture also obtained on acid treatment of two related epoxides (49,50), is explicable by vinylogous dehydration involving two isomeric enols (56,57) as intermediates.

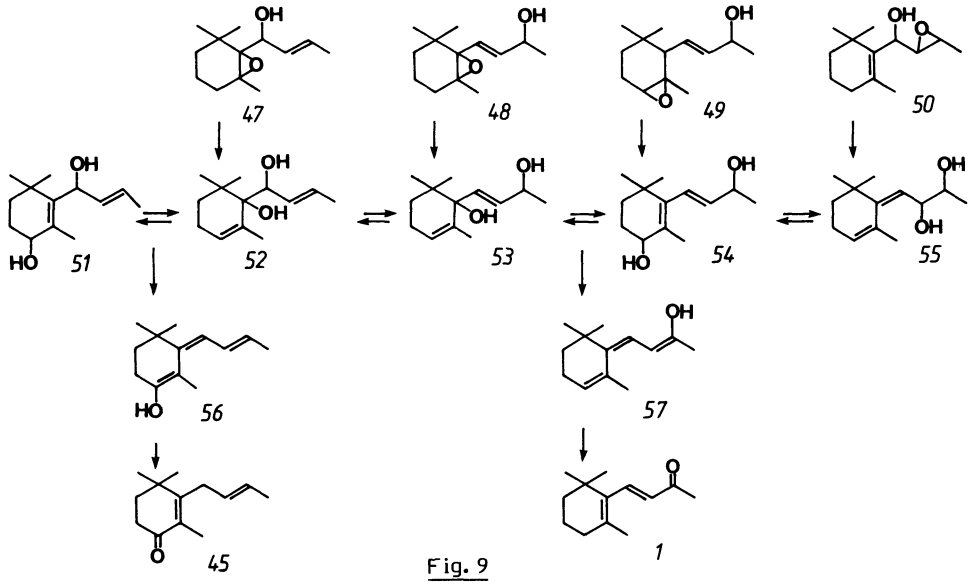
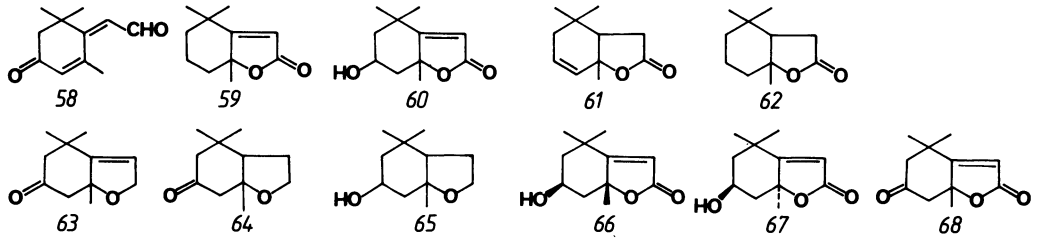


Fig. 9

It is noteworthy that ketonisation of the enol proceeds with proton shift exclusively to C-7 and not to C-9, which would have yielded the conjugated 5,7-megastigmadien-4-one, and also that there is no participation of the proton at C-7, which would have furnished damascone-type end products. However, despite these restrictions it seems probable that these allylic diols (51-55) are important intermediates in the generation of many tobacco megastigmanes, since they are accessible both by $^1O_2$ and peracid oxidation of compounds such as β-ionol and also by epoxidation of hydrocarbons such as 4,6,8-megastigmatriene.

C_{11} constituents



The structures of the C_{11} compounds (58-68) offer little variation beyond that expected on the basis of the presumed carotenoid precursor. However, the number of routes by which they may be formed are numerous and likely to include precursors such as the 5,8-epidioxide (69) and the 5,6-epoxide (70) shown in Fig. 10, which can rearrange to 5,8-epoxides yielding C_{11} lactones on oxidation (Ref. 28-30).

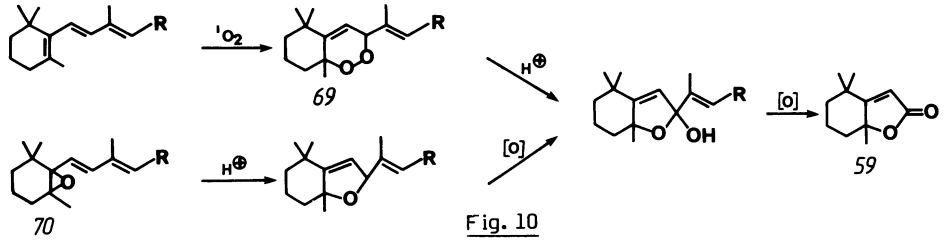


Fig. 10

It is also probable that pyrans such as the one (72) shown in Fig. 11, which arises on irradiation of the ketone (71) and furnishes the diol (73) and the aldehyde (74) on autooxidation (Ref. 31), are involved.

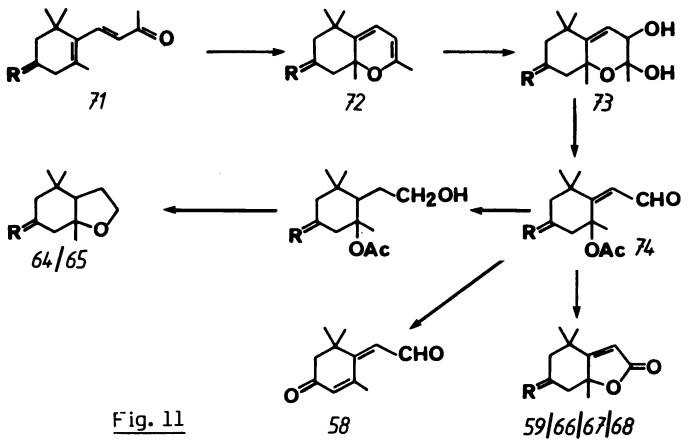
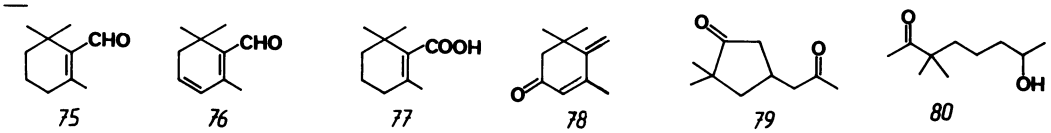


Fig. 11

The flavour properties of three of these compounds (58,59,63) are of interest. Thus, the aldehyde, (58) although seemingly odourless, adds body and enhances the flavour of Burley tobacco, while the ketoether (63) constitutes a mild aroma component. Dihydroactinidiolide (59), which exerts a cooling effect on the mucous membranes of the mouth cavity, occurs widely, e.g. in black tea, tomato, oil of cassie and the anal gland secretion of the red fox (Ref. 12). It has been shown to be formed on photooxygenation from β-carotene as well as from β-ionone and trans-β-ionol.

C_{10} constituents



Two (79,80) of the six C_{10} compounds (75-80), which are formally monoterpenoids, do not possess an unaltered carbon skeleton. A route to the first of these (79), which also accounts for the formation of the related tobacco alkaloid (81) and thereby lends support to it, has been proposed by Demole (32) and is shown in Fig. 12.

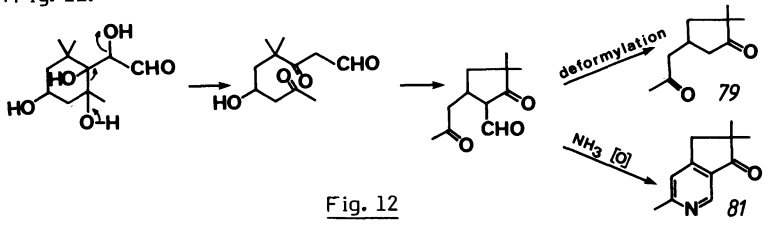
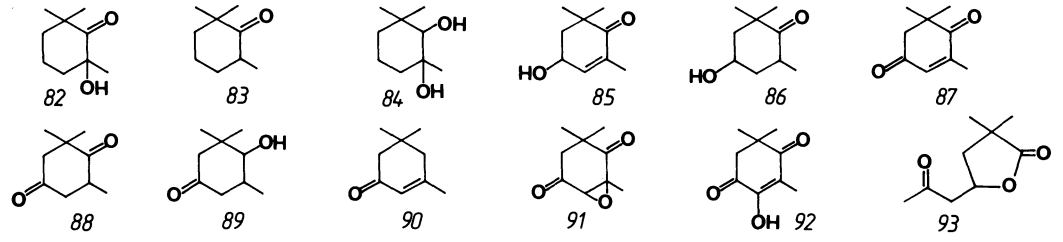


Fig. 12

Although the seco-compound (80) might be expected to be derived in an analogous manner from the corresponding C_{11} triol, attempts to accomplish this cleavage of the triol and of the corresponding primary tosylate proved abortive, a result which suggests that it is formed by other routes (Ref. 33). The most well known of the three aroma compounds (75,76,78) of this group is safranal (76), which has a saffron-like odour and taste. It can be derived from a major component of saffron, the glucoside picrocrocin (Ref. 12,13).

C_9 constituents



A major route to the C_9 compounds of the carotenoid series (82-93) is indicated by the ready conversion of the hydroperoxides (95) and (96), derived from β-damascol (94), to the cyclohexenones (97) and (98) (Ref. 34) (Fig. 13). Support is provided by the fact that stable 6-hydroperoxides have also been obtained from methyl β-ionylidenacetate and the corresponding 3-methoxy derivative (Ref. 29). Subsequent reactions such as oxidation, reduction and hydration serve to explain the structural variation observed for the C_9 tobacco compounds. Trimethyl-5-cyclohexene-1,4-dione (87), which also occurs in saffron, seems to be the most important of the aroma compounds of this group (82,87) and produces an odor impression similar to that of dried leaves or straw, capable improving the flavour of infusions and fermented drinks (Ref. 12).

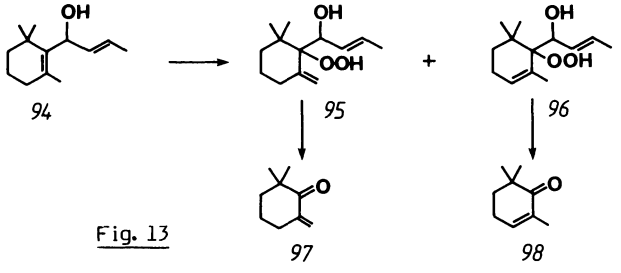


Fig. 13

Finally, it should be made clear that although most of the carotenoid metabolites of tobacco are most abundant at and subsequent to the time of cell wall degradation, many are present in the green plant prior to and during the time of most rapid growth (Ref. 6, 35). It is obvious therefore that they can be produced in the intact cell and the recent isolation of glucosides such as the β-glucosides of 3-oxo-α-ionol and 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionol (Ref. 36) supports this and shows that glucosidation must occur after cleavage of the 9,10 double bond in the precursor. Since glucosidation provides a means of transportation within the plant, it seems pertinent to ask where to and why.

Acknowledgements

I am grateful to Dr. I. Wahlberg for valuable discussion, and to Mrs. K. Askling and Mr. T. Öhman who assisted in preparing this manuscript.

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