Tobacco isoprenoids – precursors of important aroma constituents

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Dedicated to Dr Gunther Ohloff on his 65 birthday

Much of the aroma of tobacco can be viewed as arising by way of biodegradation of tobacco isoprenoids, predominantly of the cembranoid, labdanoid and carotenoid groups. The oxygenation patterns and relative abundance of these diterpenoids imply that most of them are derived from (Z)-abienol and two cembratrienediol epimers (1 and 2) by a series of reactions often initiated by singlet oxygen and/or analogous enzymatic systems - assumptions verified by biomimetic synthesis of a large number of them. Further transformations of these C20 tobacco constituents furnish a series of degraded diterpenoids having 19 to 8 carbon atoms, many of which contribute to the characteristic aroma of tobacco.

Here, the interest will be focused on the transformations of the cembranoids. Evidence for the presence of several new cembrane-derived tobacco constituents and of their stereostructures will be presented along with the major biogenetic pathways adopted to explain the formation of most cembranoid compounds known in tobacco to date.

The biosynthesis of the two major groups of isoprenoids of tobacco, the cembranoids and the labdanoids, occurs in the glandular heads of the trichomes present on the surface of the leaves and in the flowers (ref. 1 and 2). The genetic background as well as the edafic conditions determine the abundance of these constituents and, along with the postharvest treatment, govern their degradation to lower molecular weight compounds. Many of these degraded constituents are important flavour substances giving significant contribution to the aroma of commercial tobacco.

Scheme 1

The major cembranoid components are the two epimeric diols 1 and 2 (ref. 3), which possess growth (ref. 3) and tumor inhibiting (ref. 4) as well as insect repelling properties (ref. 5). According to recent studies (ref. 6) these diols seem to arise from geranylgeraniol (3) via ent-casbene (4), cembrene (5) and 2,4,7-cembratetraen-6-ol (6), cf. Scheme 1. Our present knowledge about the subsequent metabolism is mainly based on results from biomimetic studies of the two diols 1 and 2, which are regarded as the major precursors of more than fifty C20 cembranoids (ref. 3). Some of these give in turn rise to a large number of degraded cembranoids. The latter can be viewed as being derived via six key metabolites, which have 12, 13, 14, 15, 18 or 19 carbon atoms and are generated by ruptures of the bonds in the cembrane skeleton indicated in scheme 2. Since many of the degraded cembranoids are present in green leaves and fresh flowers, it is concluded that these reactions also occur in the growing plant (ref. 3).
Cleavage of the 11,12 double bond, required for the formation of the C_{12}, C_{13}, C_{14}, and C_{15} key metabolites, probably occurs in an oxidative manner either by an enzyme-assisted reaction or one involving singlet oxygen. Evidence for this comes from the isolation of tobacco flowers of five hydroperoxides having the HOO-group attached to the 11- or 12-position, e.g. 7, 8 (ref. 3), from photooxygenations studies revealing preferential attack on the 11,12-bond (ref. 3) and from compounds isolated after incubating either diol 1 or 2 with a Cantharidin roseus cell culture (ref. 7).

The 7,8 double bond is less vulnerable to oxidation than is the 11,12 double bond and only two compounds having the 7,8 but not the 11,12 double bond oxidised have so far been isolated from tobacco, i.e. the 7,8-epoxides 9 and 10 (ref. 3). Chemically this regiospecificity can be achieved using t-C_{4}H_{9}OOH/VO(acac)_{2}.

The rupture of the 6,7 bond was initially proposed to occur by acid-catalysed rearrangement of the 7,8 epoxides, but lack of success in carrying out this reaction experimentally and the recent isolation of the new tetraols 11 and 12 (ref. 8) suggest the existence of an alternative pathway involving the corresponding 7-hydroperoxides as intermediates.

The fragmentation of the 5,6 bond can be viewed as the result of an acid-catalysed 1,3-diol rearrangement furnishing in the case of the diols 1 and 2 the seco-aldehyde 13, a reaction easily mimicked using dilute H_{2}SO_{4} in dioxane/water (ref. 3).

As verified experimentally, the opening of the 4,5 bond can readily be accounted for by a retro-aldol fragmentation of the tobacco ketols 14 and 15. The latter, which derive from the diols 1 and 2 by oxidation of their secondary hydroxyl group, yield the seco-diketone 16 (ref. 3).

The cleavage of the 12,20 bond leading to the C_{19} key metabolite 17, recently isolated from tobacco flowers, might occur by the biogenetic pathway shown in Scheme 3 (ref. 3).

The C_{18}, C_{15}, C_{14}, C_{13} and C_{19} key metabolites are recognised by their irregular isoprenoid skeleton comprising an isopropyl group of S-configuration. They undergo further chemical degradation, including loss of carbon atoms, to yield a large number of tobacco constituents of which nearly 70 are known to date. Most of these compounds have only been obtained from tobacco, which makes them unique to this plant (ref. 3).
The C18 key metabolite prenylsolanone (18), probably formed by a retro-aldol condensation from 13, is the sole C18 representative encountered to date (ref. 3).

We have recently isolated a new C17 tobacco constituent (19), which does not fit the general fragmentation pattern shown in Scheme 2 since it seems to arise by cleavages of the 8,9 and 11,12 bonds (ref. 9). One possible route would involve intermediate 20, readily obtainable from the hydroperoxide 7 on acid treatment (ref. 10).

The C15 key metabolite (21) may arise as shown in Scheme 4. The closely related diols (22, 23), derivable by reduction of the oxo groups followed by dehydration, have been isolated from Japanese SUIFU tobacco (ref. 3). Four other C15 tobacco compounds having the same skeleton are probably formed from thunbergol by the same set of reactions (ref. 3). The new C15 compound (24), isolated from Greek tobacco (ref. 9), is apparently derived in a different way, i.e. from the 11-hydroperoxide 25, the corresponding ketotriol of which has recently been obtained from tobacco (ref. 8).

The predicted C14 key metabolite (26), like its C15 counterpart, is an aldehyde not yet encountered in tobacco. It may be generated as illustrated in Scheme 5, which also shows the five daughter compounds (27-31) isolated from tobacco (refs. 3 and 9).

Cleavage of the 5,6 and 11,12 bonds furnishes the abundant and important C13 key metabolite solanone (32), itself a major aroma constituent and the parent of a series of compounds contributing to the tobacco aroma (ref. 3). Some of these are shown in Scheme 6.

It is suggested from the structure of newly isolated C13 ketoaldehyde 33 that it is derived from the parent diols 1 and/or 2 in a different fashion (ref. 9), i.e. by cleavage of the 4,5 and 10,11 bonds as shown in Scheme 7.
The C12 key metabolite, norsolanadione (34), is postulated to arise by cleavage of the 4,5 and 11,12 bonds of parent cembranoids (ref. 3). Not only an important aroma constituent per se, norsolanadione is obviously an important precursor of many other C12 constituents (Scheme 8) and also of several of C8-C11 tobacco compounds, such as the recently encountered degradation products 35 and 36 (ref. 9).

Scheme 8

REFERENCES

7. J. Kutney, private communication.