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Tobacco Cembranoids*

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SUMMARY

The discovery of the first diterpenoids of the cembrane type in tobacco dates back to the early 1960's. Since then some forty tobacco cembranoids have been encountered. Most of these have a hydroxyl substituent at C-4 and are commonly divided into two series: those having a 4*R*- and those having a 4*S*-configuration. Additional oxygenation is found at C-6, C-7, C-8, C-11 or C-12.

These compounds, which are present in the gummy exudate of the tobacco leaf and flower, are susceptible to biodegradation thus accounting for the presence of the large number of odoriferous norcembranoids in tobacco. They are also reported to include representatives having growth inhibiting and insect resistance properties.

A considerable insight into the biological transformations of the tobacco cembranoids has been obtained by isolation and determination of the stereostructures of new compounds and by biomimetic experiments. The latter have involved singlet oxygen reactions, epoxidations and acid- and base-induced rearrangements. The results obtained support the importance of the (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)- and (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diols, the major tobacco cembranoids, as key metabolites in the biogenesis of the other cembranic compounds. An account of these biogenetic reactions will be given and the isolation of a few new cembranoids will be reported.

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ZUSAMMENFASSUNG

Seit der Entdeckung der ersten Cembran-Diterpenoide im Tabak in den frühen 1960er Jahren hat man im Tabak ungefähr vierzig Cembranoide nachgewiesen, die in der Mehrzahl an der Position C-4 eine Hydroxylgruppe haben und im allgemeinen in zwei Gruppen eingeteilt werden: Cembranoide mit 4*R*- und Cembranoide mit 4*S*-Konfiguration. Zusätzlich wurde eine Oxidation an den Positionen C-6, C-7, C-8, C-11 oder C-12 beobachtet.

Die Cembranoide befinden sich in dem gummiartigen Exsudat des Blattes und der Blüte der Tabakpflanze. Sie sind biologischen Abbauprozessen unterworfen und daher für die große Zahl geruchstragender Norcembranoide im Tabak verantwortlich. Sie sollen auch Stoffe mit insektiziden und wachstumshemmenden Eigenschaften enthalten.

Wichtige Einblicke in die biologischen Veränderungen, die Cembranoide im Tabak durchlaufen, konnten durch Isolierung und stereochemische Untersuchung neuer Verbindungen sowie durch biomimetische Versuche gewonnen werden. Letztere umfaßten Reaktionen mit Singulettsauerstoff, Epoxidierungen sowie säure- und baseinduzierte Umlagerungen. Die Untersuchungsergebnisse machten erneut die wichtige Rolle deutlich, die die im Tabak hauptsächlich vorkommenden Cembranoide, (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)- und (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-Cembratrien-4,6-diole, als Schlüsselmetaboliten bei der Entstehung der anderen cembranartigen Verbindungen in der Pflanze spielen. Über diese biogenetischen Reaktionen und über die Isolierung einiger neuer Cembranoide wird berichtet.

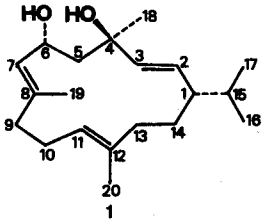
RÉSUMÉ

Depuis la découverte des premiers cembrane-diterpénoïdes dans le tabac, au début des années 60, on est parvenu à mettre encore environ 40 cembranoïdes en évidence; la majorité d'entre eux possèdent un groupe hydroxyle à la position C-4 et ils se divisent généralement en deux groupes : cembranoïde à configuration 4*R* et cembranoïde à configuration 4*S*. De plus, on observa une oxydation aux positions C-6, C-7, C-8, C-11 ou C-12.

Les cembranoïdes se trouvent dans l'exsudat gommeux de la feuille et de la fleur du tabac. Ils sont soumis à un processus de bio-dégradation de sorte qu'ils sont à l'origine du grand nombre de norcembranoïdes odorants du tabac. On suppose qu'ils contiennent également des substances à propriétés de résistance aux insectes et inhibitrices de croissance.

Un isolement et une étude stéréochimique de nouvelles combinaisons ainsi que des tests bio-mimétiques ont donné d'importantes informations concernant les modifications biologiques subies par les cembranoïdes du tabac. Les essais bio-mimétiques ont mis en œuvre des réactions avec l'oxygène monoatomique, des époxydations et des recombinaisons induites par acides et bases. Les résultats de l'analyse firent à nouveau apparaître le rôle important joué par les cembranoïdes du tabac, essentiellement (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*) et (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratrien-4,6-diole, en tant que métabolites clés pour la naissance des autres combinaisons de type cembranes dans la plante. L'article expose ces réactions bio-génétiques et l'isolement de quelques nouveaux cembranoïdes.

The presence of diterpenoids of the cembrane type in tobacco was disclosed in 1962, when *Roberts* and *Rowland* (1) reported the isolation and determination of the gross structures of diols *1* and *2*. Later work, which has included X-ray analysis (2) and ozonolytic degradation (3), has allowed the formulation of diol *1* as (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol. Diol *2* has been identified as (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol, i. e. the 4*S*-epimer of diol *1*, by chemical correlation with a 2,7,12(20)-cembratriene-4,6,11-triol [*8*] (4).



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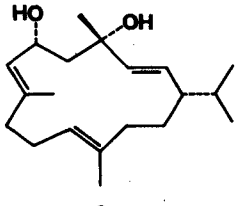

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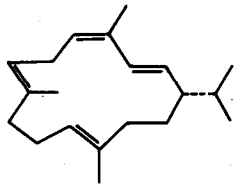
These two diols [*1*, *2*], which have been found in most tobacco varieties (5) and which possess growth inhibiting (2) and insect resistance properties (6), are the major tobacco cembranoids. They are present in the cuticular wax of the leaf and flower and are prone to undergo biodegradation during the post-harvest treatment of the leaf (7) thus offering an explanation for the occurrence of the large number of norcembranoids in tobacco (8).

There is no literature report on the biosynthesis of diols *1* and *2*. It seems highly probable, however, that they arise in tobacco by oxidation of cembrene [*3*], which is a tobacco constituent (9). If this route proceeds via formation of monools, then 2,4,7,11-cembratetraen-6-ol [*4*] is, in our opinion, a more plausible intermediate than thunbergol [*5*] and its C-4 epimer *6*, all of which are also tobacco constituents (10, 11). It cannot be excluded, however, that the monool *4* is formed in tobacco by dehydration of diols *1* and *2*.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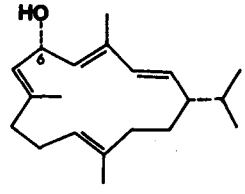

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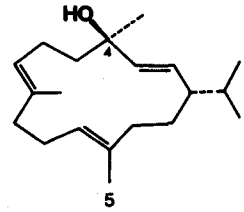

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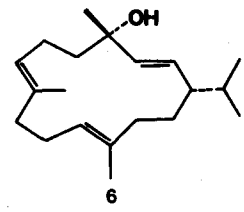

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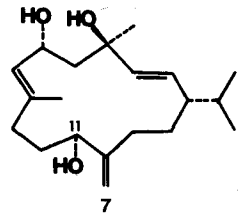
Our insight into the subsequent biotransformations of diols *1* and *2* is more extensive. In fact, the results obtained by determination of the stereostructures of new tobacco cembranoids and by biomimetic experiments suggest that diols *1* and *2* are key metabolites in the biogenesis of the majority of the tobacco cembranoids.



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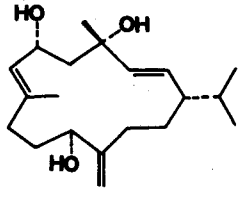

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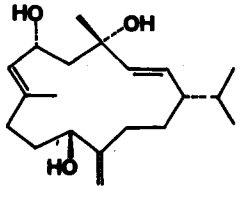

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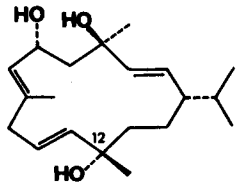
This review will survey the cembranoids of tobacco, the biomimetic experiments which have interlinked them as well as the plausible routes for their bioformation.

An examination of Greek tobacco (4, 12) has revealed the presence of three 2,7,12(20)-cembratriene-4,6,11-triols [*7—9*] and four 2,7,10-cembratriene-4,6,12-triols [*10—13*]. Following determination of stereochemistries by the use of X-ray analysis, spectral methods and chemical correlations, the 4,6,11-triols have been formulated as the (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-, (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)- and (1*S*,2*E*,4*S*,6*R*,7*E*,11*R*)-2,7,12(20)-cembratriene-4,6,11-triols [*7—9*].

The 4,6,12-triols [*10—13*] all have 1*S*,2*E*,6*R*,7*E*,10*E*-configurations and are diastereoisomers with respect to the configurations of C-4 and C-12.



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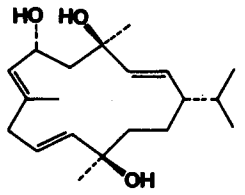

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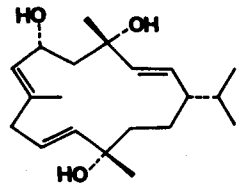

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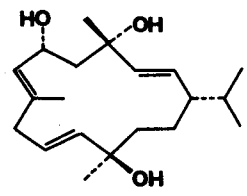

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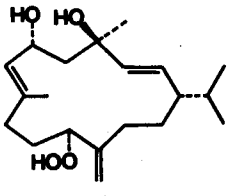
A plausible biogenetic route to these triols would involve attack of oxygen on the 11,12 double bond in the 4,6-diols [*1, 2*] either by a singlet oxygen reaction, i. e. an ene reaction, or an enzyme-assisted reaction. The ene reaction proceeds, as shown in Scheme 1, by attachment of singlet oxygen to an sp^2 carbon atom, rearrangement of an allylic hydrogen atom and double bond migration. The disubstituted 2,3 double bond in the 4,6-diols [*1, 2*] is expected to be less reactive toward singlet oxygen than the trisubstituted 7,8 and 11,12 double bonds and, due to the deactivating effect of the hydroxyl group at C-6, the 11,12 double bond should react more readily than the 7,8 double bond.

In agreement with this, the 4*S*,6*R*-diol [*2*] reacted smoothly with singlet oxygen at the 11,12 double bond giving, after reduction of the initially generated hydroperoxides using triethyl phosphite, two 4,6,11-triols [*8, 9*] and two 4,6,12-triols [*12, 13*] in the ratio 63:1:31:5 (12).

The generation of both major triols [*8, 12*] may be accounted for by reactions taking place with conformer *a* of the 4*S*,6*R*-diol [*2*] (Scheme 3). Thus oxygen attachment to C-11 and rearrangement of a hydrogen from C-20 would yield the hydroperoxide precursor of the 4*S*,6*R*,11*S*-triol, whereas the hydroperoxide precursor of the 4*S*,6*R*,12*S*-triol would arise by oxygen attachment to C-12 and migration of the *pro-R*-hydrogen from C-10. The hydroperoxide precursors of the two minor triols are formed by reactions occurring with conformer *b*, the *pro-S*-hydrogen at C-10 being involved in the generation of the 12*R*-hydroperoxide. It can be concluded, therefore, that conformer *a* is more populated or reacts more rapidly than conformer *b*.

All four products [*8, 9, 12, 13*] are formed by *syn* ene reactions, i.e. hydrogen abstraction occurs solely from the 1,2-disubstituted side of the trisubstituted double bond (Scheme 4). A similar mode of reaction has previously been found for acyclic compounds and compounds having other cyclic systems, cyclohexenes being exceptions (13).

Additional experimental support for the view that the 4,6,11- and 4,6,12-triols [*7—13*] arise by biotransformations of the 4,6-diols [*1, 2*] has most recently been provided by the isolation of the first hydroperoxides from tobacco. They have been identified as the (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)- and (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diols [*14, 15*] and the (1*S*,2*E*,4*R*,6*R*,7*E*,10*E*,12*S*)-, (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)- and (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-12-hydroperoxy-2,7,10-cembratriene-4,6-diols [*16—18*] (14). Their presence in tobacco does not, however, exclusively favour the operation of singlet oxygen reactions, since enzyme-assisted reactions are also expected to proceed via hydroperoxide intermediates.



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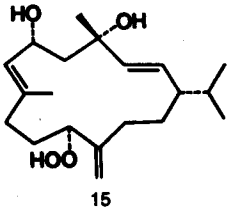

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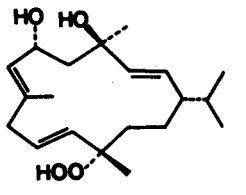

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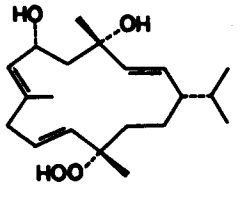

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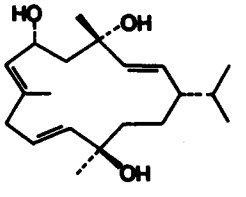

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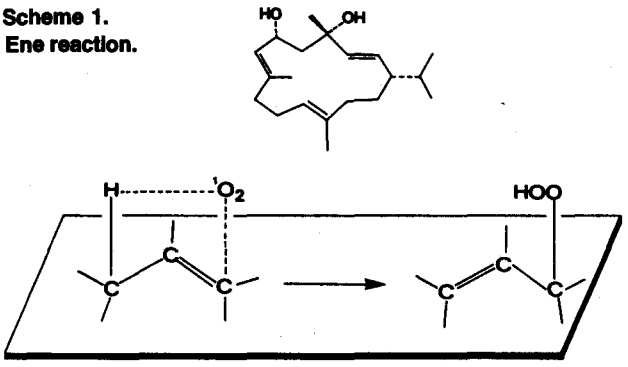
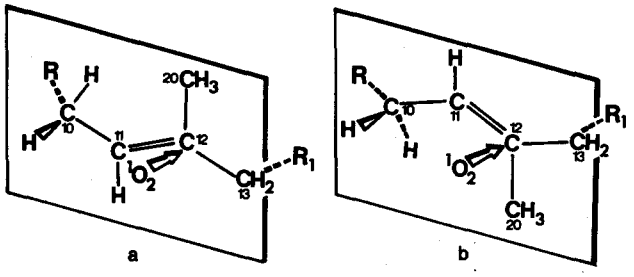
The isolation from Greek tobacco of two 2,8(19),12(20)-cembratriene-4,6,7,11-tetrols [*19, 20*]

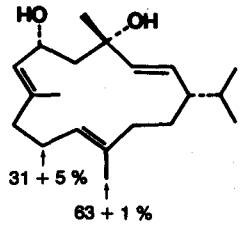
Scheme 1.
Ene reaction.



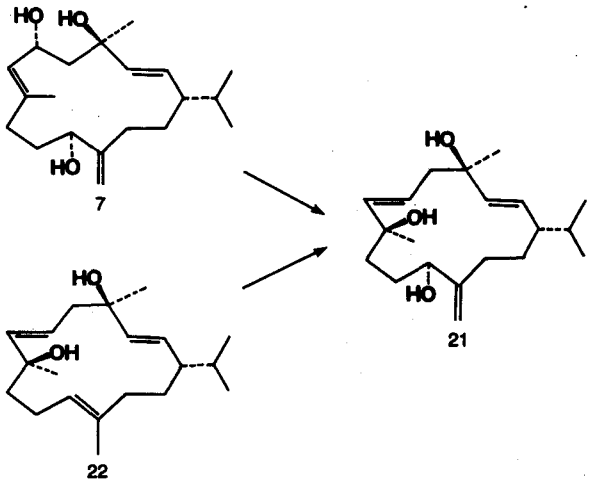
Scheme 3. Proposed mechanism for the singlet oxygen reaction.



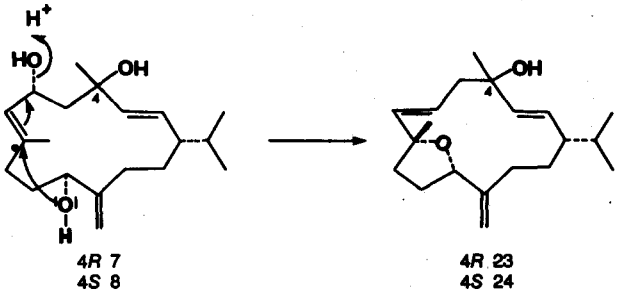
Scheme 4. Sites of hydrogen abstraction in the reaction of the 4*S*,6*R*-diol *2* with singlet oxygen.



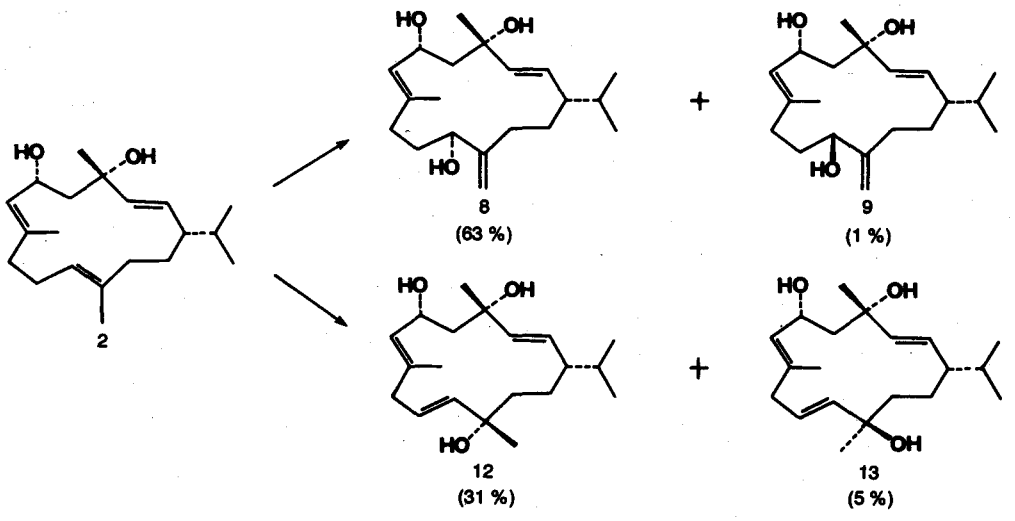
Scheme 5. Suggested pathways for the generation of the 4*R*,8*S*,11*S*-triol *21*.



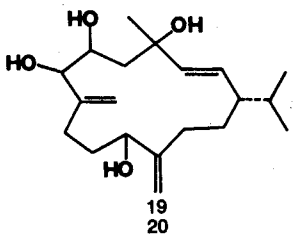
Scheme 6. Formation of the 8*R*,11*S*-epoxides *23* and *24* by treatment of the 4*R*,6*R*,11*S*- and 4*S*,6*R*,11*S*-triols *7* and *8*, respectively, with acid.



Scheme 2. Products obtained from the 4*S*,6*R*-diol *2* by sensitized photooxygenation followed by reduction.



(15), which have the same gross structure and apparently differ with respect to stereochemistry, suggests that the tobacco triols are susceptible to oxidation at the 7,8 double bond. This may again take place either by the action of singlet oxygen or by an enzyme-catalyzed reaction.



Treatment of the 4*R*,6*R*,11*S*-triol [*7*] with weak acid yielded as the minor product (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*)-2,6,12(20)-cembratriene-4,8,11-triol [*21*], a compound recently discovered in tobacco (Scheme 5) (2). Its generation evidently occurs by an allylic rearrangement and is analogous to the conversion of the 4*R*,6*R*-diol [*1*] to the 4*R*,8*S*-diol ([*22*], cf. Scheme 13). An alternative pathway, validated by photooxygenation experiments, involves oxidation of the 11,12 double bond in the 4*R*, 8*S*-diol [*22*] (12).

The major product obtained by treatment of the 4*R*, 6*R*,11*S*-triol [*7*] with acid was identified as (1*S*,2*E*, 4*R*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol [*23*], a compound which was first reported present in tobacco in 1964 (16). The corresponding 4*S-epimer* [*24*], also a tobacco constituent (3, 16), is obtainable by an acid-induced cyclization of the 4*S*,6*R*,11*S*-triol [*8*] (cf. Scheme 6) (4).

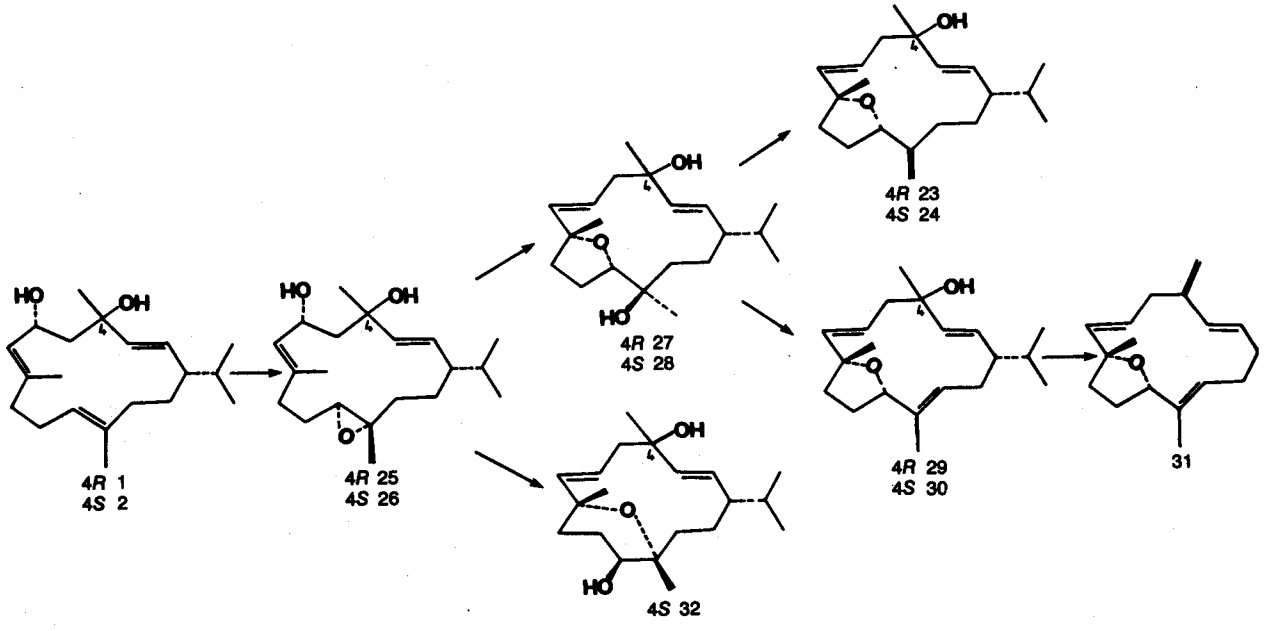
This route to *23* and *24* may represent a biological alternative to the main route, which is depicted in Scheme 7 and which likewise has received experimental support by isolation of postulated intermediates and by biomimetic reactions.

In this pathway, the 4,6-diols [*1, 2*] are initially converted to the (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*S*)- and (1*S*,2*E*, 4*S*,6*R*,7*E*,11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diols *25* (17) and *26* (4), respectively. These undergo acid-induced rearrangements to give the (1*S*,2*E*,4*R*,6*E*,8*R*, 11*S*,12*R*)- and (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diols [*27, 28*] (18). The generation of *23, 24, 29* (17, 19), *30* (16) and *31* (19) is then accounted for by subsequent dehydration. All these compounds as well as the 8*R*,12*R*-epoxide *32* (20), which is another product arising by rearrangement of the 11*S*,12*S*-epoxide *26*, have been found in various tobaccos.

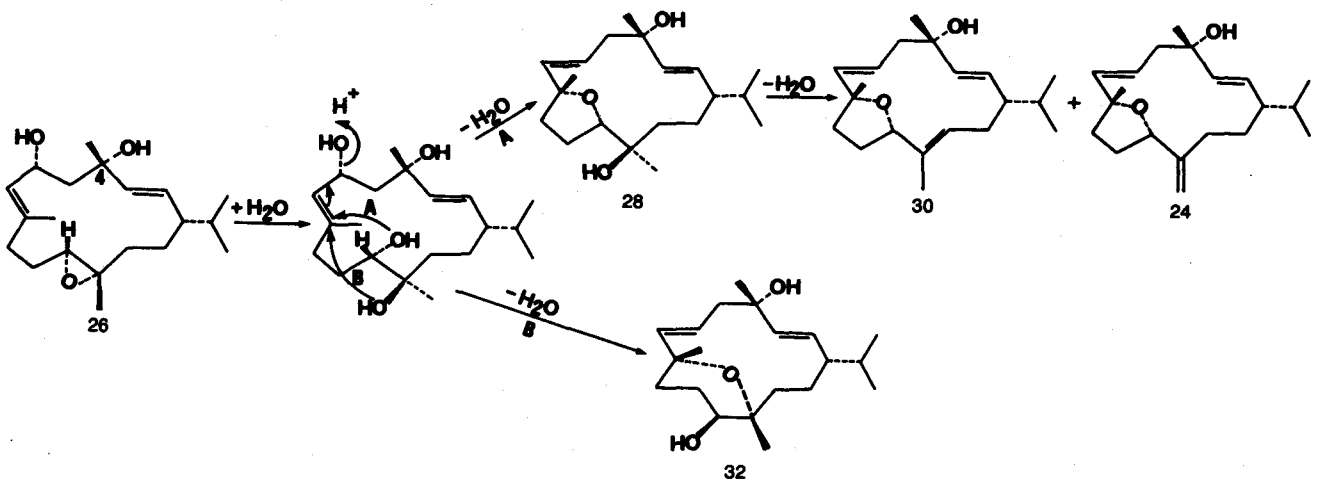
The validity of this biogenetic pathway has been reinforced by treatment of epoxides *25* and *26* with dilute hydrochloric acid in dioxane-water (17). The results obtained for epoxide *26* are summarized in Scheme 8. Four major products were isolated and identified as the 8*R*,11*S*-epoxides *28, 30* and *24* and the 8*R*,12*R*-epoxide *32*. The generation of *28* is explicable, as shown in route A, by the anti-addition of water to the 11,12-epoxide group followed by the attack of the newly formed 11*S*-hydroxyl group on the 7,8 double bond and the simultaneous elimination of the hydroxyl group at C-6. An analogous attack of the 12-hydroxyl group on the 7,8 double bond would be involved in the reaction leading to the 8*R*,12*R*-epoxide *32* (route B).

Only one 8*S*,11*R*-epoxy bridged cembranoid, (1*S*,2*E*, 4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol [*33*], has so far been isolated from tobacco (21). This may arise by the route shown in Scheme 9 which is analogous to that described in Scheme 7 and which involves the 11*R*,12*R*-epoxide *34* as an intermediate. Consistent with this is the fact that epoxide *34*, which is a minor product obtained by epoxidation of the 4*S*,6*R*-

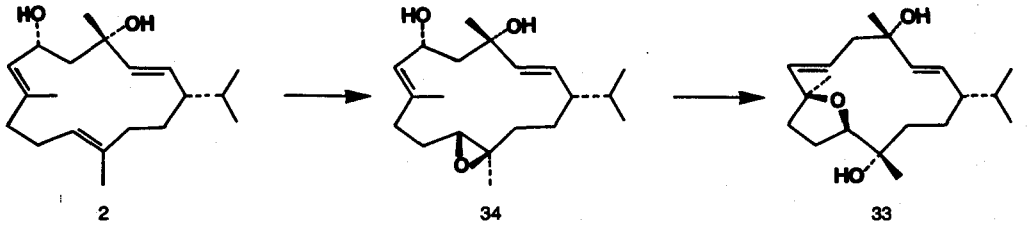
Scheme 7. Probable biogenesis of the 8*R*,11*S*- and 8*R*,12*R*-epoxy bridged tobacco cembranoids.



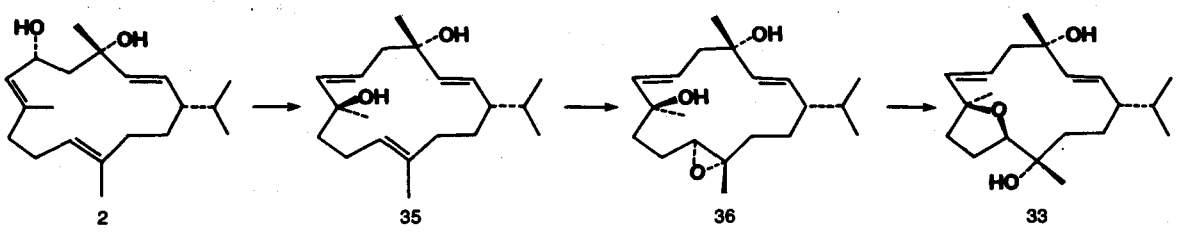
Scheme 8. Proposed mechanism and products for the acid-induced rearrangement of the 11*S*,12*S*-epoxide *26*.



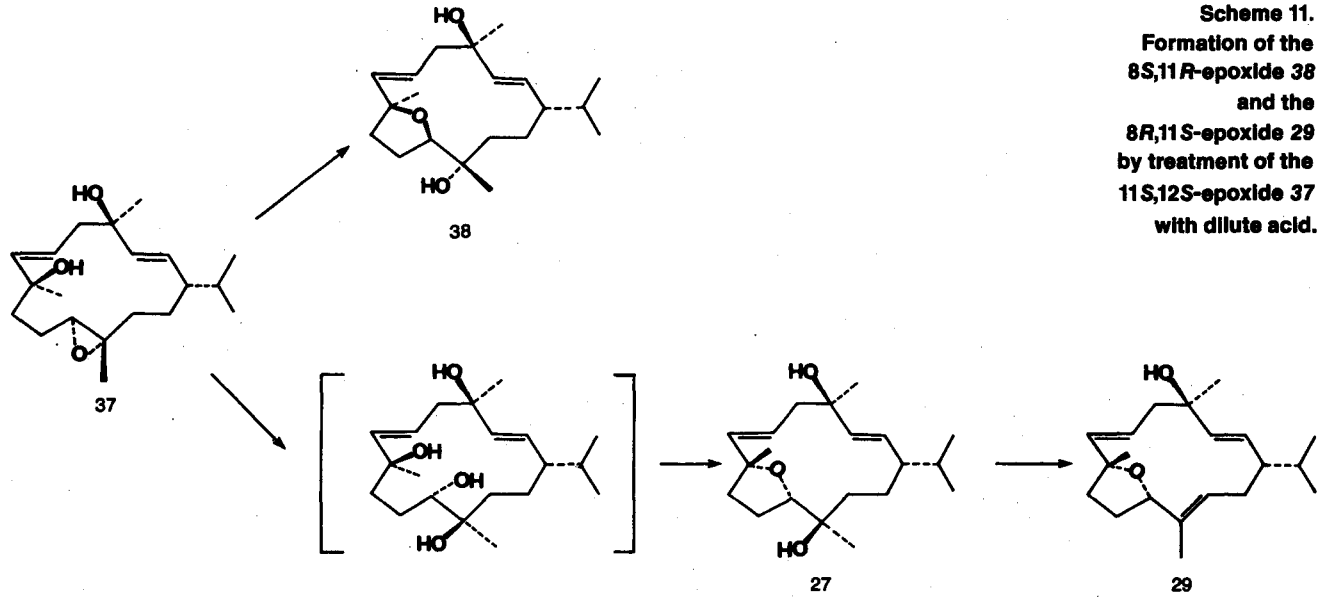
Scheme 9. Formation of the 8*S*,11*R*-epoxide *33* via acid-induced rearrangement of the 11*R*,12*R*-epoxide *34*.



Scheme 10. Formation, and probable biogenesis, of the 8*S*,11*R*-epoxide *33* via the 4*S*,8*S*-diol *35*.



Scheme 11. Formation of the 8*S*,11*R*-epoxide *38* and the 8*R*,11*S*-epoxide *29* by treatment of the 11*S*,12*S*-epoxide *37* with dilute acid.



diol 2 (17) and as yet not detected in tobacco, gave the 8*S*,11*R*-epoxide *33* on treatment with dilute sulphuric acid in dioxane-water (21).

In a biogenetically more plausible pathway, the existence of which has been borne out by model experiments (21), the 4*S*,6*R*-diol *2* is initially converted to the 4*S*,8*S*-diol [*35*]. The latter is then epoxidized affording the 11*S*,12*S*-epoxide *36* as the predominant product. This when treated with a trace of aqueous hydrochloric acid in chloroform undergoes an S_N2 type of attack of the 8-hydroxyl group on C-11 to give the 8*S*,11*R*-epoxide *33*.

Chemical results (21) also infer that a pathway exists between the 4*R*,8*S*- and 4*S*,8*S*-diols [*22*, *35*] and 8*R*, 11*S*-epoxy bridged cembranoids. Thus, treatment of (1*S*, 2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4, 8-diol [*37*] with dilute sulphuric acid in dioxane-water, afforded, besides the 8*S*,11*R*-epoxide *38*, the 8*R*,11*S*-epoxide *29*. Its generation evidently proceeds by hydroxylation at C-12 and a proton-induced loss of the hydroxyl group at C-8. Whether the reaction involves an intermediate tetrol, as indicated in Scheme 11, or occurs in one step via protonation of the hydroxyl group at C-8, is unclear.

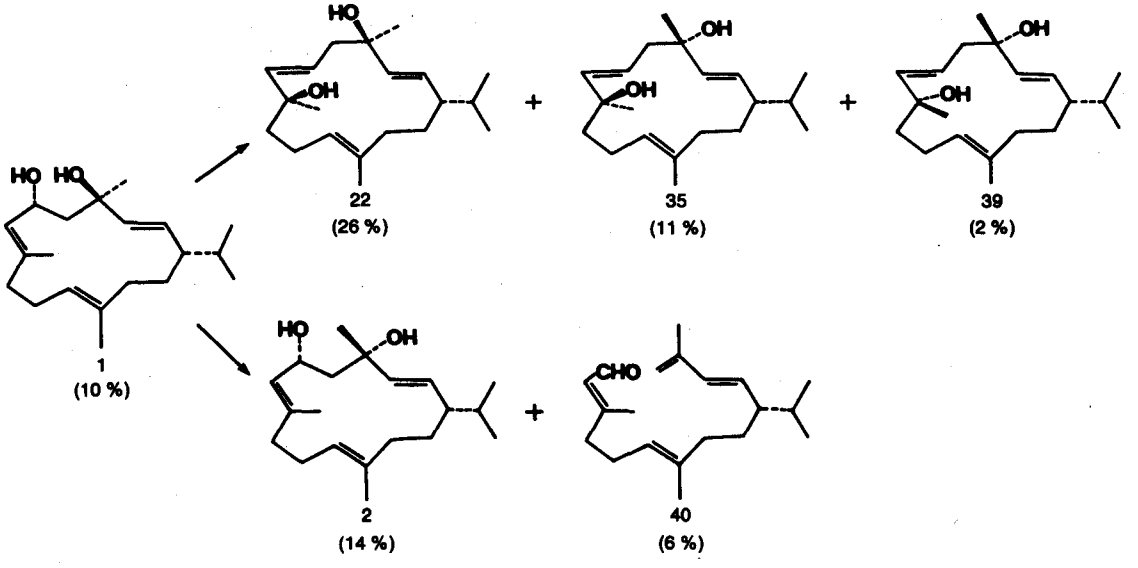
With the aid of X-ray analyses (23), 8*S*-configuration has recently been assigned to the 4*R*,8*S*- and 4*S*,8*S*-diols *22* and *35*, which have long been known as tobacco constituents (22). It is likely that these are formed in tobacco from the 4*R*,6*R*- and 4*S*,6*R*-diols *1* and *2*, respectively, by allylic rearrangement reactions. In order to get some insight into these conversions, each of the 4,6-diols [*1*, *2*] was treated with dilute sulphuric acid in dioxane-water (23). Typical results obtained for the 4*R*,6*R*-diol [*1*] are summarized in Scheme 12. It can be seen that, besides starting material, five major products, which were identified as the 4*R*,8*S*-, 4*S*,8*S*-, 4*S*,8*R*- and 4*S*,6*R*-diols [*22*, *35*, *39*, *2*] and the secoaldehyde *40*, are formed. Analogous results were obtained for the 4*S*,6*R*-diol *2*.

It can be concluded, therefore, that under weakly acidic conditions a 4,6-diol will be subjected to competing allylic rearrangement, fragmentation and epimerization reactions.

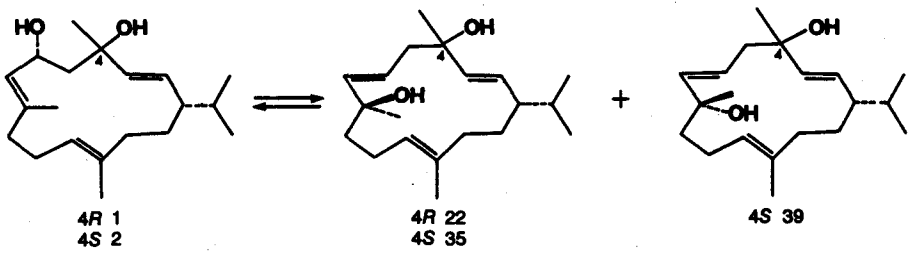
The results reveal that in the allylic rearrangement reactions, i.e. the interconversions of the 4,6- and 4,8-diols, the equilibrium positions favour the formation of the 4,8-diols (cf. Scheme 13) (23).

Inspection of *Dreiding* models shows that the formation of the 4*R*,8*S*- and 4*S*,8*S*-diols [*22*, *35*] may be explained by conformer *a* of the 4*R*,6*R*- and 4*S*,6*R*-diols *1* and *2*, respectively, undergoing an S_N2' type of reaction involving *cis*-stereochemistry (Scheme 14). Conversely, the 4*R*,8*S*- and 4*S*,8*S*-diols [*22*, *35*], when existing in conformer *b*, are amenable to S_N2' reactions

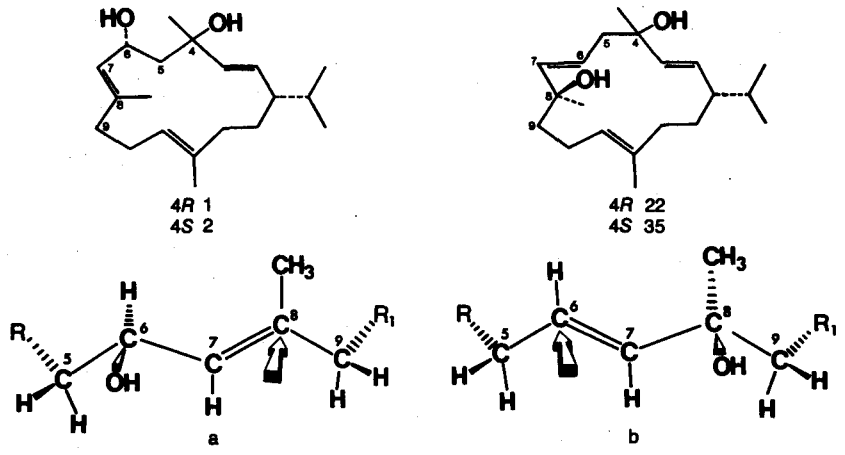
Scheme 12. Products obtained by treatment of the 4*R*,6*R*-diol [*1*] with dilute acid.



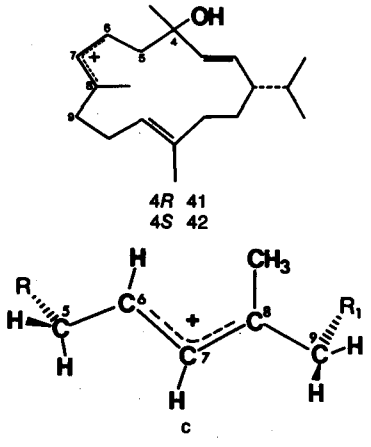
Scheme 13. Interconversions of the 4,6- and 4,8-diols *1*, *2*, *22*, *35* and *39*.



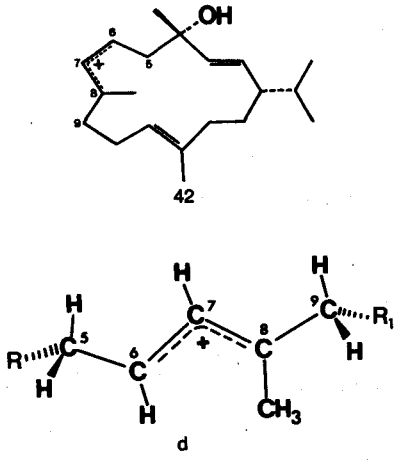
Scheme 14. Proposed S_N2' mechanism for the interconversions of diols *1* and *22* and of diols *2* and *35*.



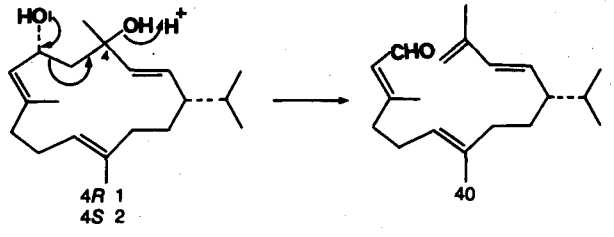
Scheme 15.



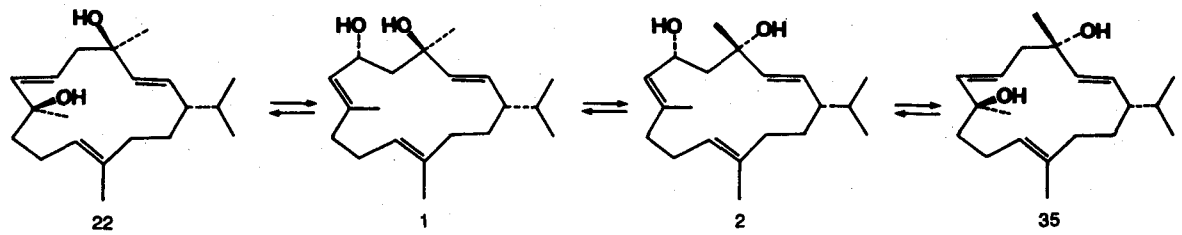
Scheme 16.



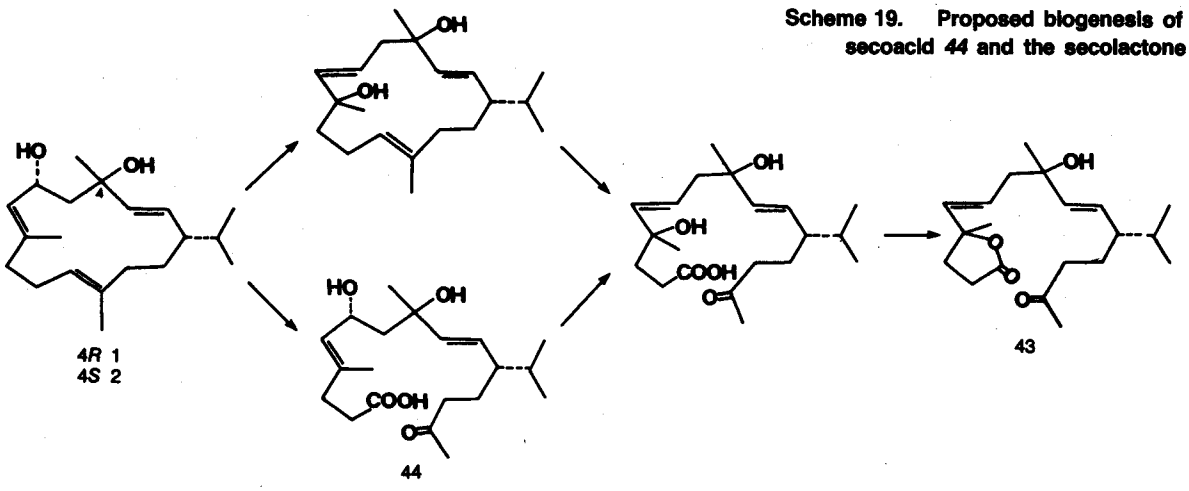
Scheme 17. Formation of the secoaldehyde *40* by acid-induced fragmentation of the 4*R*,6*R*- and 4*S*,6*R*-diols *1* and *2*.



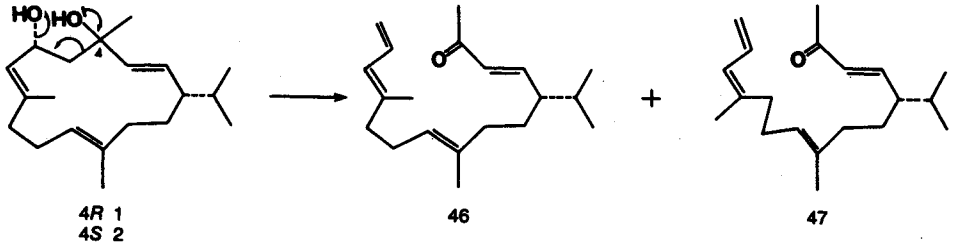
Scheme 18. Epimerization and allylic rearrangement reactions taking place with the 4,6- and 4,8-diols *1*, *2*, *22* and *35*.



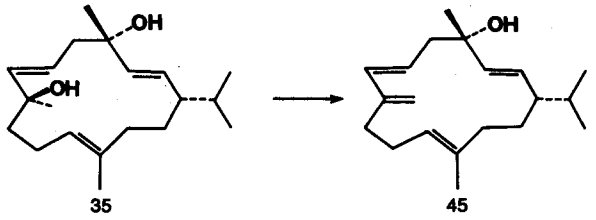
Scheme 19. Proposed biogenesis of the secoacid *44* and the secolactone *43*.



Scheme 21. Formation of the secoketones *46* and *47* by a dehydrative ring cleavage of the 4*R*,6*R*- and 4*S*,6*R*-diols *1* and *2*.



Scheme 20. Formation of compound *45* by dehydration of the 4*S*,8*S*-diol [*35*].



giving rise to the 4*R*,6*R*- and 4*S*,6*R*-diols [*1*, *2*] (23).

The interconversions of the 4,6- and 4,8-diols [*1*, *2*, *22*, *35*] may, however, also be rationalized by an S_N1 mechanism, in which hydroxylation occurs at C-8 in conformer *c* of carbonium ions *41* and *42* would yield the 4*R*,8*S*- and 4*S*,8*S*-diols *22* and *35*, respectively (Scheme 15). The 4*R*,6*R*- and 4*S*,6*R*-diols [*1*, *2*] would arise by hydroxylation at C-6 (23).

The formation of the 4*S*,8*R*-diol *39* is not consistent with an S_N2' mechanism and can be explained by an S_N1 reaction taking place with conformer *d* of carbonium ion *42* (Scheme 16) (23).

The secoaldehyde *40*, first reported as a constituent of tobacco flowers (24), arises on acid treatment from the 4,6-diols [*1*, *2*] by a fragmentation reaction (Scheme 17), which is essentially irreversible (23).

The chemical results also show that the 4*R*,6*R*- and 4*S*,6*R*-diols [*1*, *2*] are prone to undergo epimerization at C-4 on treatment with weak acid (23). This process is probably also part of the reaction sequence, in which the 4*R*,8*S*- and 4*S*,8*S*-diols [*22*, *35*] are interconverted, i.e. *22* ⇄ *1* ⇄ *2* ⇄ *35* (cf. Scheme 18).

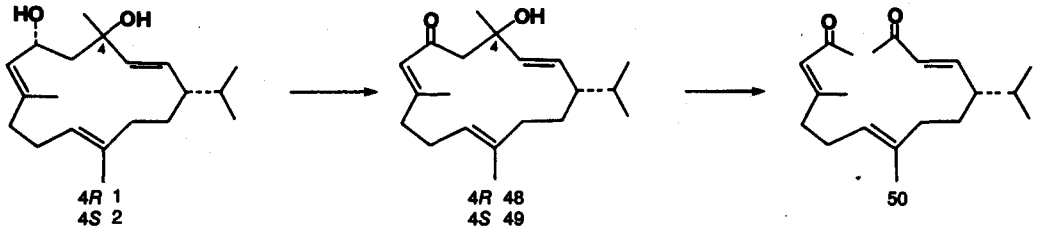
The formation of the 8*S*,11*R*-epoxide *33* from the 4*S*,8*S*-diol ([*35*], cf. Scheme 10) and of the 4*R*,8*S*,11*S*-triol [*21*] from the 4*R*,8*S*-diol ([*22*], cf. Scheme 5) suggests that the 11,12 double bond in the 4,8-diols [*22*, *35*] is susceptible to oxidation. This view is borne out by the recent isolation of the secolactone *43* from Burley tobacco (11). The generation of this can be explained, as shown in Scheme 19, by oxidative cleavage of the 11,12 double bond in a 4,8-diol and subsequent lactonization.

Alternatively, the secoacid *44*, which has been found as a mixture of diastereoisomers in Burley tobacco (25), could be involved as an intermediate. Allylic rearrangement and esterification would complete the formation of the secolactone [*43*].

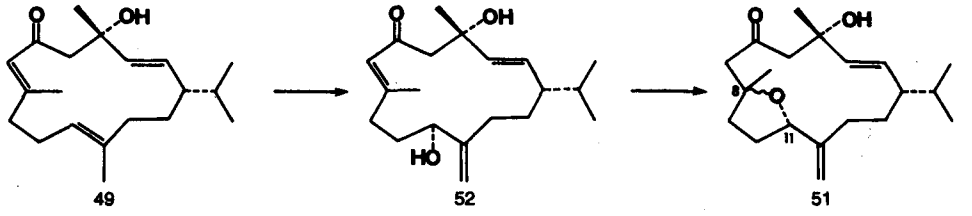
The metabolism of the 4,8-diols [*22*, *35*] also evidently proceeds by dehydration reactions. Thus, (1*S*,2*E*,4*S*,6*E*,11*E*)-2,6,8(19),11-cembratetraen-4-ol [*45*], which is a constituent of flue-cured tobacco (19), is a product derived from the 4*S*,8*S*-diol ([*35*], cf. Scheme 20).

The secoketone *46* and its *Z*-isomer *47* have recently been isolated from cigarette smoke (26) but have as yet not been found in tobacco. They are likely to arise, as illustrated in Scheme 21, directly from the 4,6-diols [*1*, *2*] by a dehydrative ring cleavage reaction.

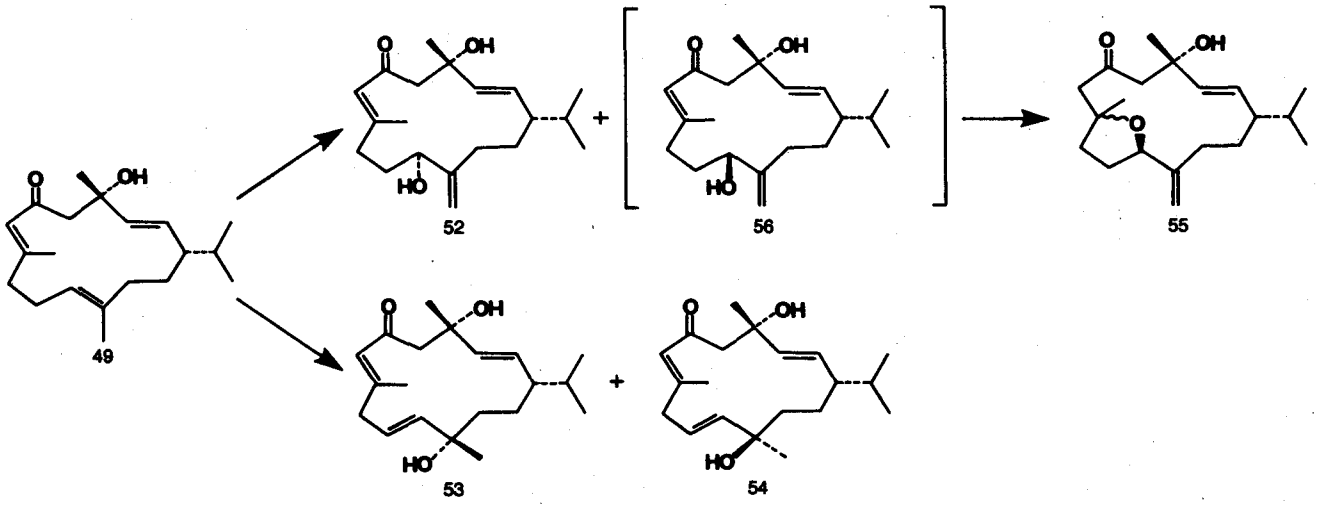
Scheme 22. Probable biogenesis of ketols *48* and *49* and secodiketone *50*.



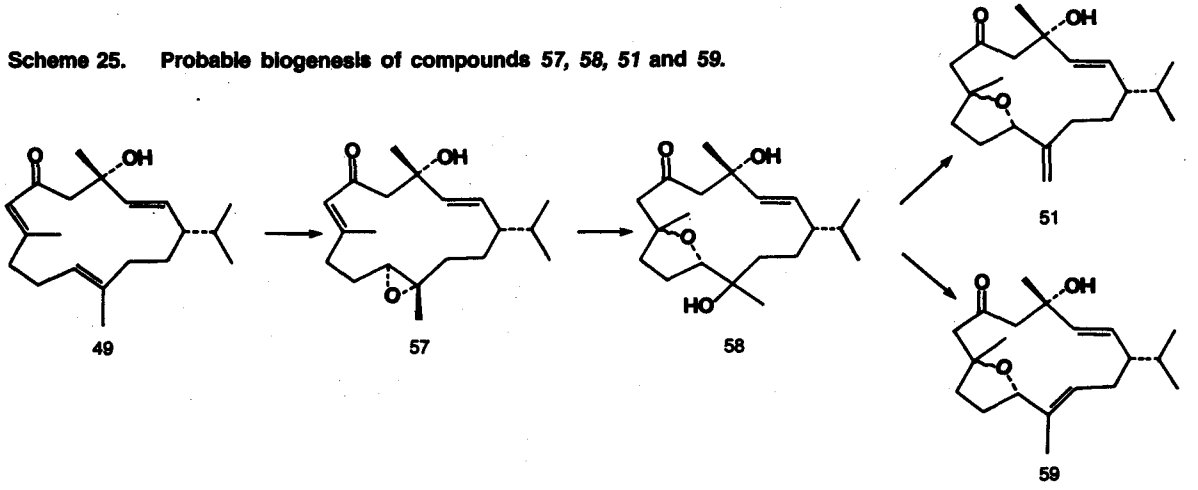
Scheme 23. Probable biogenesis of compound *51*.



Scheme 24. Products obtained from ketol *49* by sensitized photooxygenation followed by reduction.



Scheme 25. Probable biogenesis of compounds *57*, *58*, *51* and *59*.



The 4*R*,6*R*- and 4*S*,6*R*-diols [*1, 2*] are evident precursors of ketols *48* and *49*, respectively, both of which give rise to the secodiketone *50* by a retroaldol type of reaction (Scheme 22). All three ketones [*48*–*50*] are tobacco constituents, the secodiketone *50* having been obtained as a *cis,trans*-mixture from dark-fired tobacco (27).

The recent isolation of (1*S*,2*E*,4*S*,8ξ,11*S*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one [*51*] from Greek tobacco indicates that like the 4,6- and 4,8-diols [*1, 2, 22, 35*], the ketols *48* and *49* are susceptible to oxidation of the 11,12 double bond. This was readily verified, since photooxygenation of the 4*S*-ketol *49* followed by reduction and an acid-induced cyclization of the resulting (1*S*,2*E*,4*S*,7*E*,11*S*)-4,11-dihydroxy-2,7,12(20)-cembratrien-6-one [*52*] proved to be a viable biomimetic route to the 8,11-epoxy bridged ketol *51* (Scheme 23) (28).

In addition to the 4*S*,11*S*-ketodiol [*52*], the reaction of the 4*S*-ketol [*49*] with singlet oxygen was found to afford two of the expected ene products, i.e. (1*S*,2*E*,4*S*,7*E*,10*E*,12*S*)-4,12-dihydroxy-2,7,10-cembratrien-6-one [*53*] and its 12*R*-epimer *54*. The isolation of an 8,11*R*-epoxy bridged compound [*55*] from the reaction mixture infers, however, that the 4*S*,11*R*-ketodiol [*56*], the fourth ene product, is formed as well but that it cyclizes spontaneously (Scheme 24) (28).

Another route to *51*, the existence of which has also been reinforced by chemical methods, is initiated by epoxidation of the 4*S*-ketol *49* to yield the 11*S*,12*S*-epoxide *57*. A subsequent acid-induced rearrangement results in the formation of an 8,11*S*-epoxy bridged ketodiol [*58*], which on dehydration produces both *51* and *59* (Scheme 25). This pathway is also attractive from another point of view since intermediates *57* and *58* as well as compound *59* have, most recently, been isolated from tobacco (28).

Although more than forty tobacco cembranoids are known, the vast majority of these can be prepared from the 4,6-diols [*1, 2*] by relatively simple reactions such as epoxidations, singlet oxygen reactions, dehydrations and acid- and base-induced rearrangements. These processes may also occur in the tobacco leaf and flower and it is reasonable to assume that the 4,6-diols [*1, 2*] are the key intermediates in the biogenesis of most tobacco cembranoids. In the biodegradation of parent cembranoids to norcembranoids, however, more extensive chemical alterations are involved.

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