Tobacco Chemistry. 62. Five New Cembranoids from Tobacco[1a]

Inger Wahlberg,* Ingrid Forsblom, Carmen Vogt, Ann-Marie Eklund, Toshiaki Nishida,* Curt R. Enzell,* and Jan-Eric Berg[1b]

Research Department, Swedish Tobacco Company, S-104 62, Stockholm, Sweden

Received February 15, 1985

Five new cembranoids have been isolated from Greek tobacco. They have been identified as (1*S*,2*E*,4*S*,7*E*,11*S*,12*S*)-11,12-epoxy-4-hydroxy-2,7-cembradien-6-one (**1**), (1*S*,2*E*,4*S*,7*E*,10*E*,12*S*)-4,12-dihydroxy-2,7,10-cembratrien-6-one (**2**), (1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-epoxy-4-hydroxy-2,12-cembradien-6-one (**3**), (1*S*,2*E*,4*S*,8*R*,11*S*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one (**4**), and (1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one (**5**) by chemical and spectral methods and by X-ray analysis (**3**). Two-dimensional NMR spectroscopy has been used extensively, and an example of the applicability of high-resolution COSY spectra for structural studies is given. The biogenesis of the new compounds (**1**–**5**) is discussed on the basis of results obtained from biomimetic syntheses, which have, inter alia, involved acid-induced rearrangement of compound **1** and sensitized photooxygenation of ketol **6**. Comments are made on the assignment of ^{13}C NMR spectra of tobacco cembranoids through the combined use of ^1H–^1H and ^1H–^{13}C shift correlation spectroscopy together with selective proton flip 2D *J* experiments. Characteristic features in the mass spectra of some 8,11-epoxy-bridged 6-oxocembranoids are presented.

The cuticular wax of the leaf and flower of most tobacco varieties contains substantial amounts of diterpenoids of the cembrane class, the (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diols (**7** and **8**) being the main components. These two diols are also the postulated precursors of most of the other tobacco cembranoids,[2] among which are two ketols epimeric at C-4 (**6**, **9**).[3] We now report the isolation from Greek tobacco of five new cembranoids, which are likely to arise by oxidative biotransformations of ketol **6**.

Results

Structure Determination. The presence of an α,β-unsaturated oxo group in the first new compound (**1**), $C_{20}H_{32}O_3$ (high-resolution mass spectrometry), was disclosed by the IR spectrum, which contains absorption bands at 1665 and 1605 cm^{-1}, and by the ^1H NMR spectrum, which exhibits a broad singlet at δ 6.08. The remaining two oxygen atoms are accommodated by an epoxide group extending from a methine to a fully substituted carbon atom [^1H NMR signal at δ 2.62; ^{13}C NMR signals at δ 58.6 (d) and 60.1 (s)] and by a tertiary hydroxyl group [OH absorption in the IR spectrum; ^{13}C NMR signal at δ 72.2 (s)].

Of the five methyl groups present in compound **1**, two form part of an isopropyl substituent (methyl doublets at δ 0.81 and 0.84; IR bands at 1385 and 1370 cm^{-1}). A third methyl group, resonating as a narrowly split doublet at δ 2.20, is attached to the β-carbon atom of the enone system. This assignment is reinforced by the ^1H–^1H shift correlation spectrum (COSY 45, Figure 1a),[1,4] which includes a cross-peak due to coupling between this methyl group and the vinylic proton at δ 6.08. A fourth methyl group, which gives rise to a singlet at δ 1.35, is correlated with the hydroxyl hydrogen, leaving the methyl group resonating at δ 1.27 to be assigned to the fully substituted carbon atom bearing the epoxide. Furthermore, since the ^1H and ^{13}C NMR spectra provide evidence for the presence of an additional double bond, which is *E* disubstituted ($J_{A,B}$ = 15.5 Hz), it was concluded that the new compound (**1**) is a carbomonocyclic diterpene, most likely of the cembrane class.

Although the information present in the low-resolution COSY 45 spectrum (Figure 1a) did not suffice alone, use of results from the high-resolution COSY 45 spectrum in Figure 1b together with results from COSY spectra, which were recorded under conditions to enhance the detection of long-range couplings,[5] allowed the identification of **1** as an 11,12-epoxy-4-hydroxy-2,7-cembradien-6-one.

Thus, in contrast to the low-resolution COSY 45 spectrum, the high-resolution one contains cross-peaks correlating, inter alia, H-1 with H-14a, H-14b, and H-15. Moreover, the cross-peaks arising from coupling between H-9a and H-10a and between H-10a and H-10b, which are partially overlapping in the low-resolution spectrum, are now better resolved into their components. Due to the higher resolving power it is also easier to observe the tilts of certain cross-peaks in Figure 1b than in Figure 1a. These tilts (indicated by arrows) reflect the relative signs of the couplings and can hence be used to distinguish geminal and vicinal couplings.[4]

COSY spectra recorded with a delay time of 0.35 or 0.40 s inserted in the basic pulse sequence and a mixing pulse of 56° or 60° displayed cross-peaks due to long-range coupling between, inter alia, H-5b and H-9b and between H-11 and H-13b. These findings were a prerequisite to combine the structural fragments formulated with the aid of results from the COSY 45 spectra in Figures 1a and 1b into structure **1**.

The structure assigned to **1** was reinforced and a 4*S* configuration was suggested by a comparison of the ^{13}C NMR spectrum with those of the 4(*S*)- and 4(*R*)-ketols **6** and **9** (Table I). Conclusive evidence was then readily obtained by chemical means. Oxidation using chromium trioxide converted the diol epoxide **10**[6,7] into (1*S*,2*E*,4*S*,7*E*,11*S*,12*S*)-11,12-epoxy-4-hydroxy-2,7-cembradien-6-one, which was identical with the naturally occurring **1** (Chart I).

The second new compound (**2**), $C_{20}H_{32}O_3$, is also an α,β-unsaturated ketone (IR bands at 1666 and 1605 cm^{-1}; ^1H NMR signal at δ 6.06). However, in contrast to **1**, **2**

(1) (a) For part 61, see: Nishida, T.; Wahlberg, I.; Nordfors, K.; Vogt C.; Enzell, C. R. *Tetrahedron Lett.* **1984**, *25*, 1299. (b) Department of Structural Chemistry, Arrhenius Laboratory, University of Stockholm, Sweden.
(2) Wahlberg, I.; Enzell, C. R. *Beitr. Tabakforsch.* **1984**, *12*, 93.
(3) Zane, A. *Phytochemistry* **1973**, *12*, 731.
(4) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542.
(5) Steffens, J. C.; Roark, J. L.; Lynn, D. G.; Riopel, J. L. *J. Am. Chem. Soc.* **1983**, *105*, 1669.
(6) Wahlberg, I.; Wallin, I.; Narbonne, C.; Nishida, T.; Enzell, C. R.; Berg, J.-E. *Acta Chem. Scand. Ser B* **1982**, *B36*, 147.
(7) Wahlberg, I.; Arndt, R.; Wallin, I.; Vogt, C.; Nishida, T.; Enzell, C. R. *Acta Chem. Scand., Ser. B* **1984**, *B38*, 21.

0022-3263/85/1950-4527$01.50/0

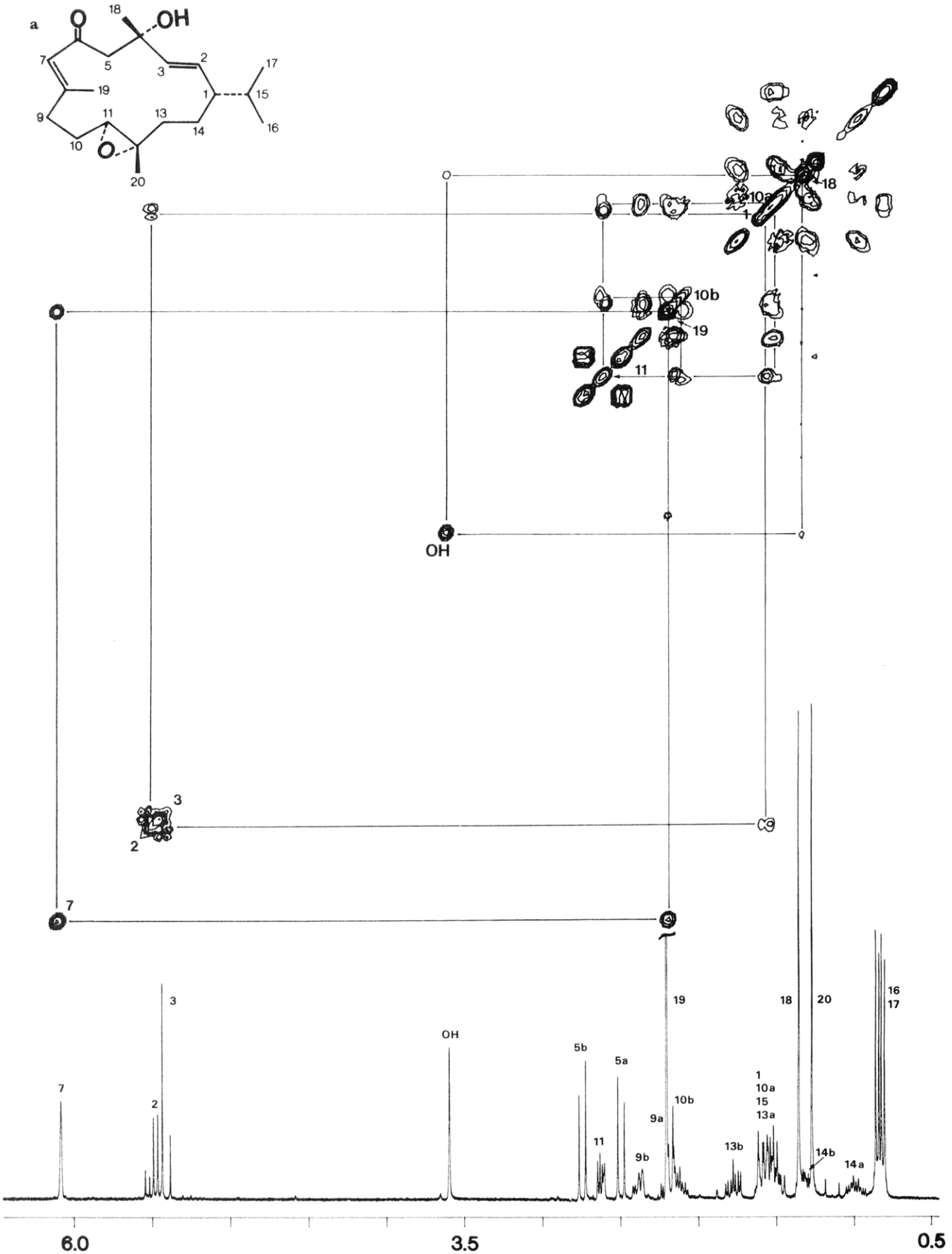
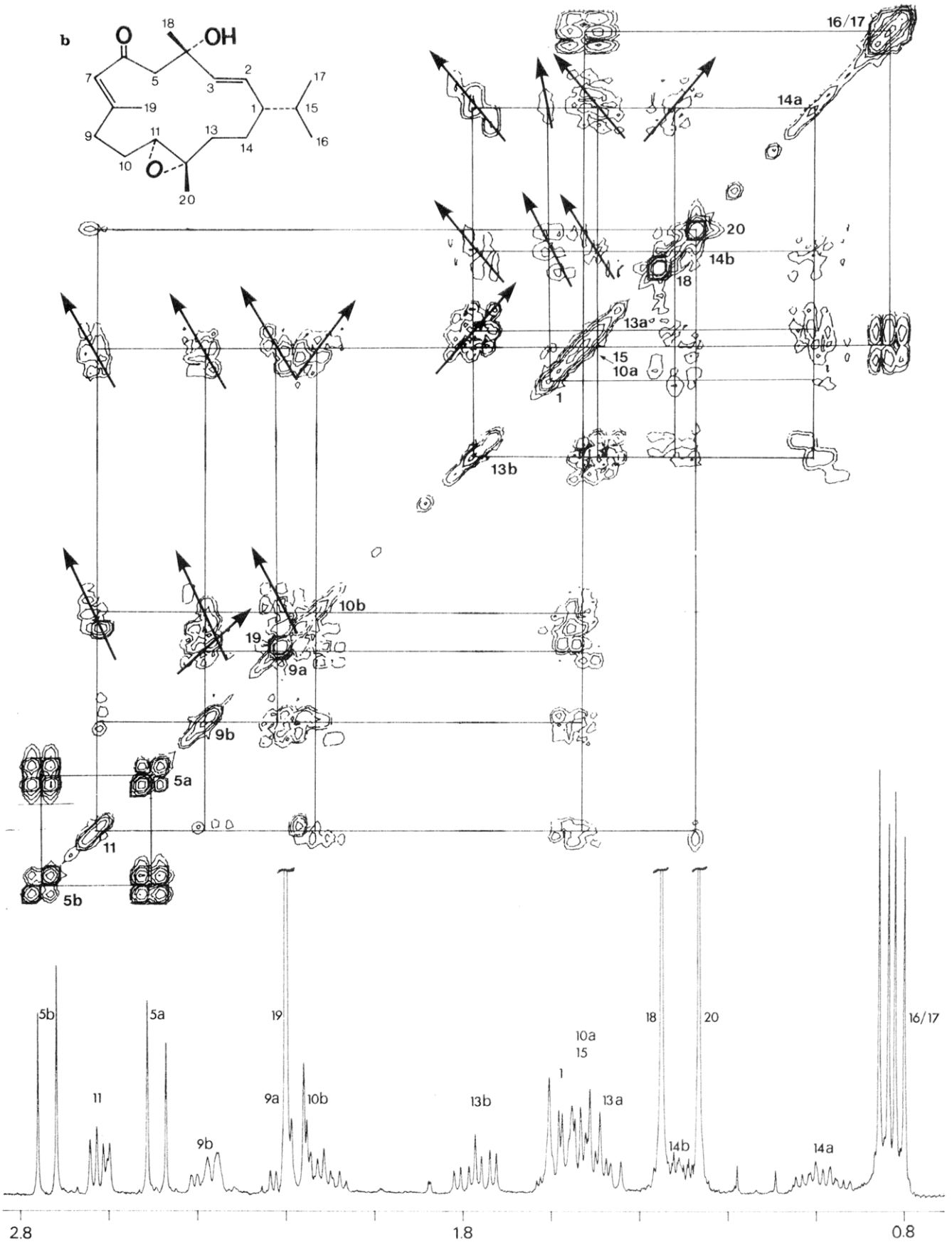


Figure 1. (a) Contour plot of the COSY 45 spectrum of **1** run in $CDCl_3$ (300 MHz). (b) Contour plot of the upfield region of the

contains two tertiary hydroxyl groups [OH absorption in the IR spectrum; ^{13}C NMR signals at δ 71.9 (s) and 73.3 (s)] and two *E* disubstituted double bonds ($J_{A,B}$ = 15.6 Hz and 15.8 Hz) but no epoxide group. These results and a comparison of the ^{13}C NMR spectra of **2**, the 4(*S*)-ketol **6** and the 4(*S*),6(*R*),12(*S*)-triol **11**[6,7] allowed a tentative formulation of **2** as a 4(*S*),12(*S*)-dihydroxy-2(*E*),7(*E*),10-(*E*)-cembratrien-6-one.



high-resolution COSY 45 spectrum of **1** (300 MHz).

Consistent with this, oxidation of the 4(*S*),6(*R*),12(*S*)-triol **11** using pyridinium dichromate yielded as the major product (1*S*,2*E*,4*S*,7*E*,10*E*,12*S*)-4,12-dihydroxy-2,7,10-cembratrien-6-one, which was indistinguishable from the second new tobacco constituent (**2**).

The IR and NMR spectra revealed that the third new compound (**3**), $C_{20}H_{32}O_3$, contains an oxo group, which is flanked on each side by a methylene group. Each of these

Table I. ^{13}C NMR Chemical Shifts and Assignments for Compounds 1–6, 9, 11–14, 16, 18–21, 23, and 24[a]

no.	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
1[d]	48.0	130.6	136.1	72.2	54.9	200.9	126.4	159.3	38.3	24.7	58.6	60.1	33.8	26.2	31.5	19.9	20.4	30.9	19.1	18.2
2[d]	49.9	130.7	135.8	71.9	57.4	200.6	124.8	159.3	43.1	123.9	140.3	73.3	41.5	27.3	30.3	18.6	21.2	30.1	20.8	29.3
3[d]	50.6	129.5	137.8	72.2	55.4	210.9	52.1	82.5	35.0	30.9	81.4	134.8	122.9	29.1	32.1	20.1	20.7	30.1	28.2	14.9
4[d]	46.4	128.8	137.3	72.2	54.6	212.2	51.2	82.8	36.5	28.7	83.6	147.5	23.2	27.6	32.0	20.2	20.7	29.3	27.6	112.2
5[d]	49.5	130.9	137.1	72.1	55.0	212.2	50.6	82.9	34.9	26.2	86.1	75.6	28.2	26.0	32.4	20.2	20.8	29.3	27.6	22.8
6	47.1	130.2	136.1	72.7	56.3	200.6	127.0	157.9	40.4	23.9	122.9	134.8	37.2	28.7	32.6	19.3	20.5	30.6	18.7	15.7
9	46.4	130.3	136.4	72.5	54.7	202.9	127.8	158.0	40.2	23.5	123.0	134.7	36.5	27.6	32.5	19.3	20.2	27.3	18.2	15.5
11	50.8	127.3	138.3	74.3[b]	47.2	69.4	128.4	134.5	40.7	124.6	138.9	74.0[b]	40.1	26.5	30.1	17.8	21.8	31.6	18.0	30.0
12	49.7	130.5	136.3	72.3	54.0	201.8	125.8	162.4	36.2	31.7	73.3	153.0	34.6	30.9	32.4	19.4	20.4	30.9	21.6	110.3
13[d]	49.0	132.0	136.4	72.8	60.4	211.7	51.8	82.5	36.5	33.3	83.3	150.9	32.0	36.0	32.7	19.2	20.6	30.4	31.9	110.3
14[d]	47.9	128.4	138.4	72.1	54.2	212.2	51.3	83.0	35.6	28.6	80.4	62.2	58.8	30.1	32.2	19.7	20.6	29.8	28.7	14.4
16[d]	176.1	28.7	32.8	83.7	53.7[b]	208.6	53.9[b]	71.7	137.5	129.9	48.6	26.2	42.0	209.4	30.0	26.5	28.9	32.0	19.1	20.7
18	48.5	132.9	135.1	73.6	60.5	211.6	51.5	83.4	36.3	29.1[b]	78.7	135.2	124.9	28.9[b]	31.4[b]	20.9	22.0	30.8[b]	29.9[b]	17.0
19[d]	47.0	132.5	136.3	72.0	57.0	210.0	52.2	82.0	37.9	25.8	82.5	73.2	34.9	26.7	30.7	20.5	21.0	29.4	29.0	22.3
20[d]	51.0	130.0	137.6	71.9	55.4	215.9	50.4	82.2	36.4	25.2	85.8	74.0	32.7	23.1	32.2	19.9	20.9	29.4	28.5	22.3
21[d]	49.2	129.3	138.1	72.2	54.9	212.1	50.7	83.6	35.0	26.2	83.2	58.3	21.2	24.7	32.1	20.3	20.7	29.3	27.4	53.1
23	49.0	130.5	135.8	71.9	56.6	200.7	125.0	159.3	42.5[b]	124.5	140.3	73.5	42.2[b]	28.3	30.6	18.7	21.0[c]	30.9	20.9[c]	27.1
24[d]	50.2	131.3	135.8	72.4	52.3	210.4	55.0	80.7	40.0	30.6	85.2	149.3	25.9	29.4	32.2	19.8	20.7	29.6	26.2	110.8

[a] δ Values in $CDCl_3$ relative to Me_4Si. [b,c] Assignment may be reversed. [d] Assignment of the ^{13}C NMR spectrum has been made with the aid of ^1H–^1H and ^1H–^{13}C shift correlation spectroscopy.

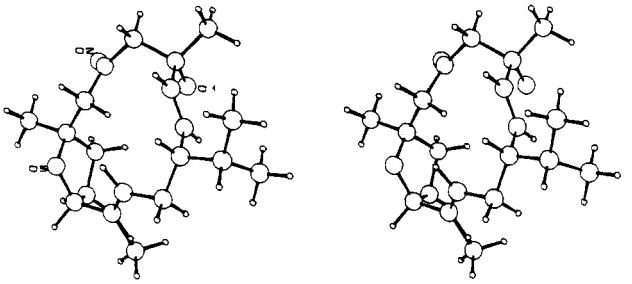


Figure 2. A stereoscopic view of (1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-epoxy-4-hydroxy-2,12-cembradien-6-one (**3**).

is, in turn, attached to a fully substituted carbon atom. The remaining two oxygen atoms are present as a tertiary hydroxyl group [OH absorption in the IR spectrum; ^{13}C NMR signal at δ 72.2 (s)] and an ether oxygen. The latter is linked to a methine and a fully substituted carbon atom [^{13}C NMR signals at δ 81.4 (d) and 82.5 (s)] and was deduced from the ^1H–^1H shift correlation spectrum to be part of a five-membered ring structure.

Since the spectral data were also consonant with the occurrence of an isopropyl group, three methyl groups and two double bonds, **3** was preliminary identified as a cembranoid having structural features hitherto not encountered among the tobacco cembranoids. An X-ray analysis using a direct phase procedure was therefore undertaken.

Compound **3** formed crystals of the monoclinic space group $P2_1$. The crystal data, obtained on a computer-controlled Philips PW 1100 spectrometer, are a = 14.130 Å, and b = 6.193 Å, and c = 11.292 Å, β = 100.60°, Z = 2. The present R value, including anisotropic thermal parameters for all non-hydrogens, is 0.123; location of the hydrogen atoms and further refinement is underway.[8] A stereoscopic view, which summarizes the X-ray results and demonstrates that **3** is (1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-epoxy-4-hydroxy-2,12-cembradien-6-one (relative stereochemistry) is shown in Figure 2.

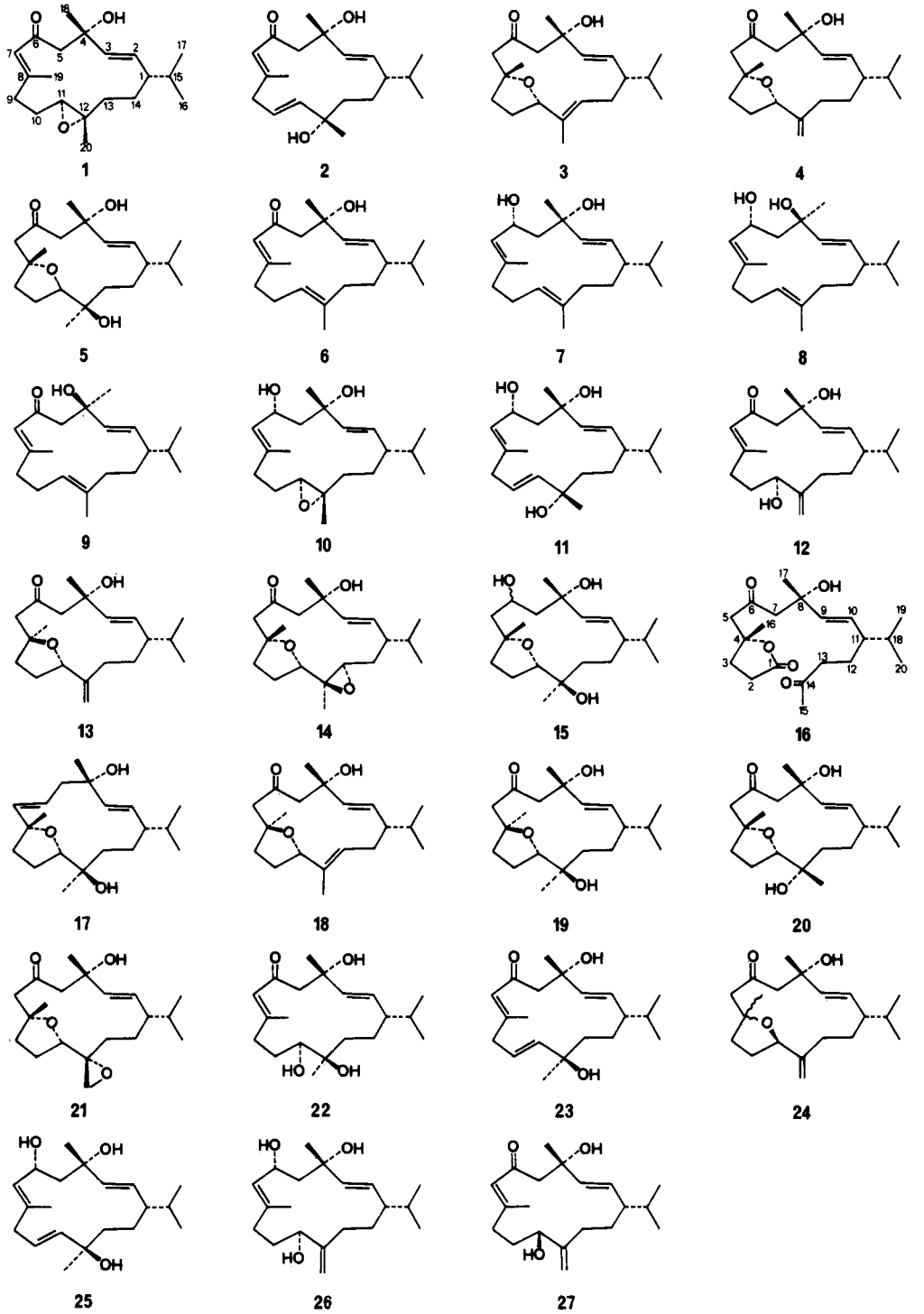
The absolute stereochemistry was resolved by treatment of the 11(*S*),12(*S*)-epoxide **1** with a trace of hydrochloric acid in chloroform. The product thus obtained had optical rotation and spectral data identical with those of the naturally occurring **3** (vide infra).

The fourth tobacco isolate (**4**), $C_{20}H_{32}O_3$, having an oxo group, a tertiary hydroxyl group, and a five-membered ether ring, is evidentally structurally closely related to the 8,11-epoxide **3**. In contrast to **3**, however, **4** contains an exocyclic methylene group [^1H NMR signals at δ 4.77 and 5.08; ^{13}C NMR signals at δ 147.5 (s) and 112.2 (t)] and is devoid of the vinylic methyl group. These results allowed a provisional identification of **4** as an 8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one. In harmony with this assignment the ^1H–^1H shift correlation spectrum contains cross-peaks corresponding to coupling between, inter alia, the hydroxyl hydrogen and H-18, between one of the protons at C-9 and H-19, and between the two protons at C-13 and those at C-20.

Additional supporting evidence was provided by treatment of (1*S*,2*E*,4*S*,7*E*,11*S*)-4,11-dihydroxy-2,7,12(20)-cembratrien-6-one (**12**, vide infra) with a trace of hydrochloric acid in chloroform. The major product proved to be identical with tobacco constituent **4**. Its formation occurs via an attack of the hydroxyl group at C-11 on C-8, a reaction that is probably intiated by protonation of the oxo group. Since a minor product (**13**), which was deduced from the spectral data to be isomeric to **4**, was also ob-

(8) Berg, J.-E., to be published.

Chart I



tained, it can be concluded that the cyclization process takes place with the reacting species existing in conformers of type a as well as type b (Figure 3). This correlation is consonant with 1*S*,2*E*,4*S*,11*S* stereochemistries in **4** and **13**; the assignment of 8*R* and 8*S* chiralities to **4** and **13**, respectively, will be described below.

In addition to an oxo group and a five-membered ether ring, the fifth new compound (**5**), $C_{20}H_{34}O_4$, contains two tertiary hydroxyl groups [OH absorption in the IR spectrum; ^{13}C NMR signals at δ 72.1 (s) and 75.6 (s)]. Dehydration using thionyl chloride in pyridine converted **5** into **3** (95%) and **4** (5%), a result which demonstrates that **5** is a (1*S*,2*E*,4*S*,8*R*,11*S*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one and that the chirality of C-8 is *R* in **4** and hence *S* in **13**.

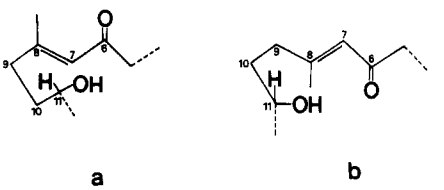
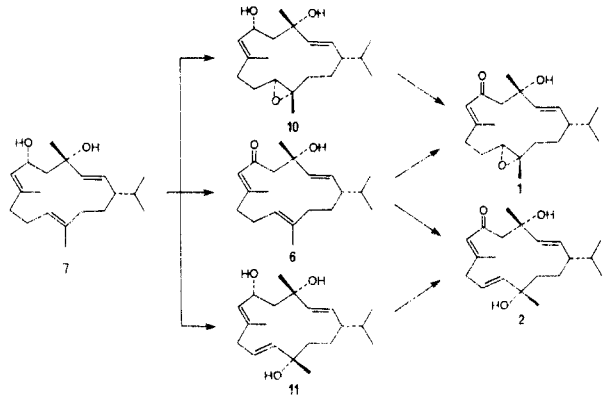


Figure 3. Reacting conformers in the conversion of **12** to **4** and **13** and of **22** to **5** and **19**.

Information on the stereochemistry at C-12 in **5** was sought by chemical methods. Treatment of **3** with *m*-chloroperbenzoic acid proceeded in a stereoselective manner, giving one 12,13-epoxide (**14**) [^1H NMR signal at δ 2.97 (dd); ^{13}C NMR signals at δ 62.2 (s) and 58.8 (d)].

Scheme I. Proposed Biogenesis of 1 and 2



This was reduced to **15** and subsequently oxidized by using pyridinium dichromate to give a major product, which was identical with **5**. This result suggests that **5** has a 12*R* configuration, since the intermediate 12,13-epoxide **14**, if generated by a reaction occurring with a conformer of **3** similar to that existing in the crystalline state, would have a 12*S*,13*S* stereochemistry.

A minor product of the oxidation reaction was assigned a (4*R*,8*S*,9*E*,11*S*)-4,8-dimethyl-6,14-dioxo-8-hydroxy-11-isopropyl-9-pentadecen-4-olide (**16**) structure [IR band at 1784 cm^{-1} (five-membered ring lactone); three-proton singlet at δ 2.13 (methyl ketone)]. Its formation demonstrates that the bond connecting the oxygen-carrying C-11 and C-12 in the 8,11-epoxy 4,6,12-triol **15** is amenable to oxidative cleavage. This result is of interest from a biogenetic point of view, since cleavage of the 11,12 bond in tobacco cembranoids having such structural features, e.g., **5** and **17**,[9] may be a step in the biotransformations leading to odoriferous degraded cembranoids.[10]

Biogenesis. There are two plausible routes for the formation of the new 11,12-epoxide **1** in tobacco, both of which originate from the 4,6-diol **7**. One proceeds via oxidation of diol epoxide **10** and the other via epoxidation of ketol **6** (Scheme I). Support for this view is provided by the fact that both **6** and **10** are tobacco constituents.[3,6]

By analogy with the formation of (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diol (**17**) from the 4,6-diol 11,12-epoxide **10**,[6,11] it seemed likely the 6-oxo 11,12-epoxide **1** would, in turn, be a precursor of some of the new 6-oxo-8,11-epoxy-bridged tobacco cembranoids. To verify this assumption, **1** was treated with dilute sulfuric acid in dioxane/water (3:1). Six products were isolated, the major one being identical with **5**, and two of the minor ones with **3** and **4**, respectively.

The three remaining products were formulated as (1*S*,2*E*,4*S*,8*S*,11*S*,12*E*)-8,11-epoxy-4-hydroxy-2,12-cembradien-6-one (**18**) and the (1*S*,2*E*,4*S*,8*S*,11*S*,12*R*)- and (1*S*,2*E*,4*S*,8*R*,11*S*,12*S*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-ones (**19** and **20**) on the basis of the following evidence.[12]

It was concluded from the ^1H NMR spectra of **18**–**20**, which all display two AB quartets in the region δ 2.3–3.5, that the oxo group at C-6 is adjacent to methylene groups, C-5 and C-7, and that C-4 and C-8 are fully substituted.

The presence of the 8,11-epoxide group and one (**18**) or two (**19**, **20**) tertiary hydroxyl groups was suggested by the ^{13}C NMR (cf. Table I) and IR spectra (OH absorption) and by the ^1H NMR spectra, which exhibit the H-11 signal at δ 4.59, 3.85, and 3.75, respectively. Furthermore, since the ^1H NMR spectra contain signals due to two (**18**) or three (**19**, **20**) methyl groups attached to fully substituted carbon atoms and one vinylic methyl group (**18**), **18** was formulated as an 8,11-epoxy-4-hydroxy-2,12-cembradien-6-one and **19** and **20** as 4,12-dihydroxy-8,11-epoxy-2-cembren-6-ones.

Spectral results were also used to provide a clue to the stereochemistry of **18**. Firstly, the chemical shift value of C-20, δ 17.0, is consistent with a 12*E* geometry only.[7] Secondly, the epoxide group is assigned an 8*S*,11*S* stereochemistry, since the C-2, C-3, and C-5 signals are present at virtually invariant positions in the spectra of **18** and the 8*S*,11*S*-epoxide **13** but at significantly different positions in the spectrum of **18** as compared with the spectra of the 8*R*,11*S*-epoxides **3** and **4**. In agreement with this assignment, H-2 and H-3 resonate at δ 5.5–5.7 in the ^1H NMR spectra of **13** and **18** but at slightly higher fields in the spectra of **3** and **4**. Moreover, the H-9b signal obtained from **13** and **18** is characteristically downfield from the corresponding signal for **3** and **4**, δ 2.85 (**13**) and 3.00 (**18**) vs. δ 2.38 (**3**) and 2.46 (**4**).

Diol **19** was converted into (1*S*,2*E*,4*S*,8*S*,11*S*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one (**13**) by dehydration using thionyl chloride, hence demonstrating that its epoxide group has an 8*S*,11*S* stereochemistry. This assignment accords with the observation that H-2 and H-3 resonate at δ 5.6–5.9, while the C-2 and C-3 signals appear at δ 132.5 and 136.3, respectively (cf. Table I). The remaining chiral center in diol **19**, C-12, is provisionally assigned an *R* stereochemistry solely on the basis of mechanistic arguments (vide infra).

Since diol **20** failed to undergo dehydration at C-12, a different method was used to resolve its stereochemistry. This involved an initial treatment of the 8(*R*),11(*S*)-epoxide **4** with *m*-chloroperbenzoic acid in chloroform. The reaction proceeded in a stereoselective manner giving one 12,20-epoxide (**21**), which was subsequently reduced. The resultant 4,6,12-triol was oxidized by using pyridinium dichromate to give a product identical with diol **20**. This result is consonant with an 8*R*,11*S* stereochemistry in diol **20**. Furthermore, since **20** is not identical with diol **5**, which has an 8*R*,11*S*,12*R* stereochemistry, it can be concluded that the chirality of C-12 is *S*.

As expected, the difference in stereochemistry between diols **5** and **20** is reflected in the shieldings of certain atoms close to C-12. Besides C-12 (Table I), it follows from a comparison of the ^1H–^{13}C chemical shift correlation spectra in Figure 4 that H-1, C-14, and H-14b are shielded, while C-1, C-9, H-10a, H-10b, C-13, and H-13b are deshielded on going from the 12*R* configuration in **5** to the 12*S* configuration in **20**.

It is noteworthy that C-6 resonates at fairly divergent positions in the spectra of **5** and **20**, δ 212.2 as against 215.9. This result cannot be directly associated with the configurational difference at C-12 but may be explained by a different degree of intramolecular hydrogen bonding between the oxo group and the C-4 hydroxyl hydrogen in **5** and **20**.

The generation of these products (**3**–**5**, **18**–**20**) may be envisaged to take place as shown in Scheme II. An anti addition of water to the 11,12-epoxide group in **1** would produce (1*S*,2*E*,4*S*,7*E*,11*S*,12*R*)-4,11,12-trihydroxy-2,7-

(9) Behr, D.; Wahlberg, I.; Aasen, A. J.; Nishida, T.; Enzell, C. R.; Berg, J.-E.; Pilotti, A.-M. *Acta Chem. Scand, Ser. B* **1978**, *B32*, 221.

(10) Enzell, C. R.; Wahlberg, I. *Recent Adv. Tob. Sci.* **1980**, *6*, 64.

(11) Behr, D.; Wahlberg, I.; Nishida, T.; Enzell, C. R.; Berg, J.-E.; Pilotti, A.-M. *Acta Chem. Scand., Ser. B* **1980**, *B34*, 195.

(12) Treatment of (1*S*,2*E*,4*R*,7*E*,11*S*,12*S*)-11,12-epoxy-4-hydroxy-2,7-cembradien-6-one, i.e., the 4*R* epimer of **1**, with dilute acid led to the formation products which are different from **3**–**5** and **18**–**20**. This result then excludes the possibility that the acid-induced rearrangement of **1** occurs with epimerization at C-4.

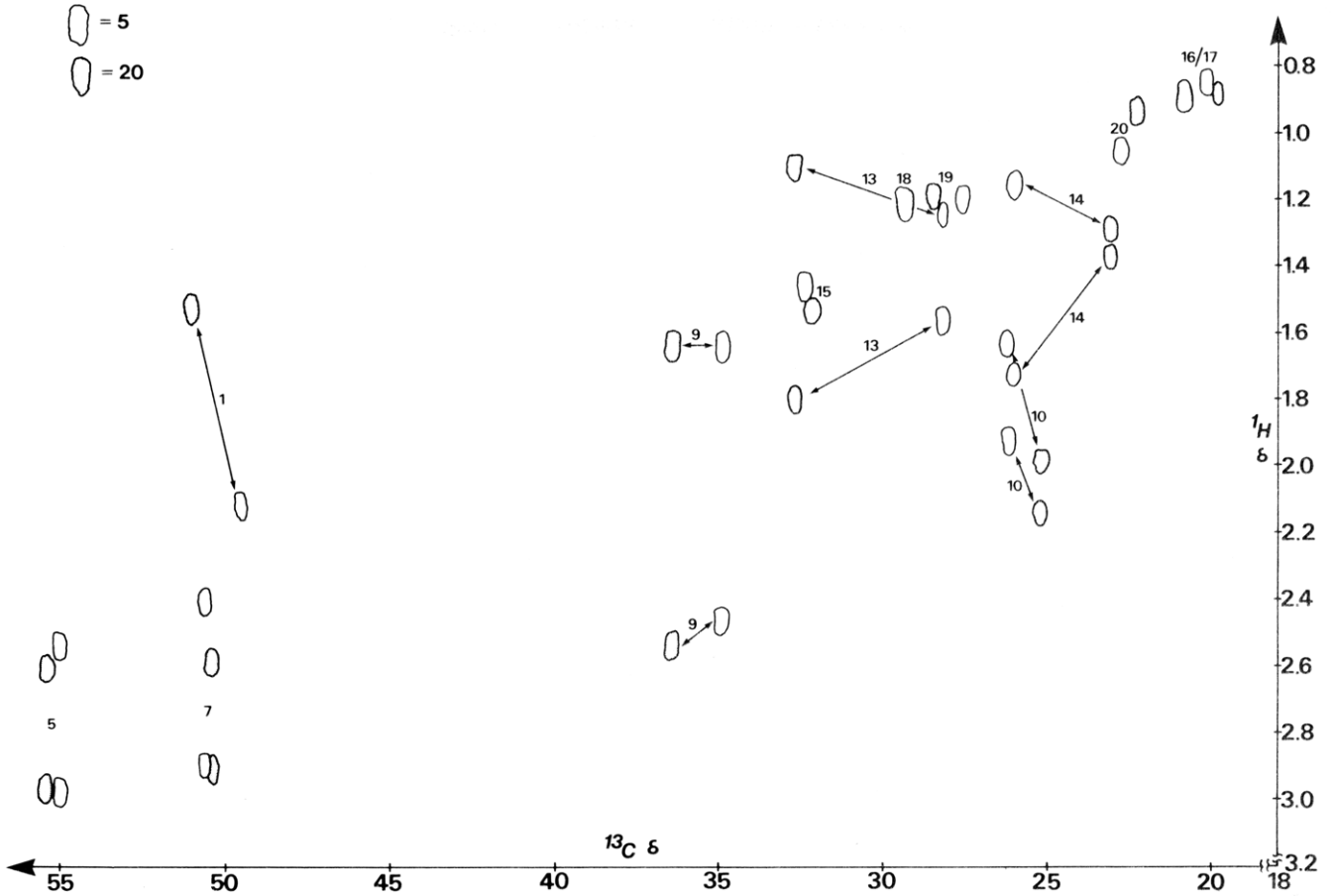
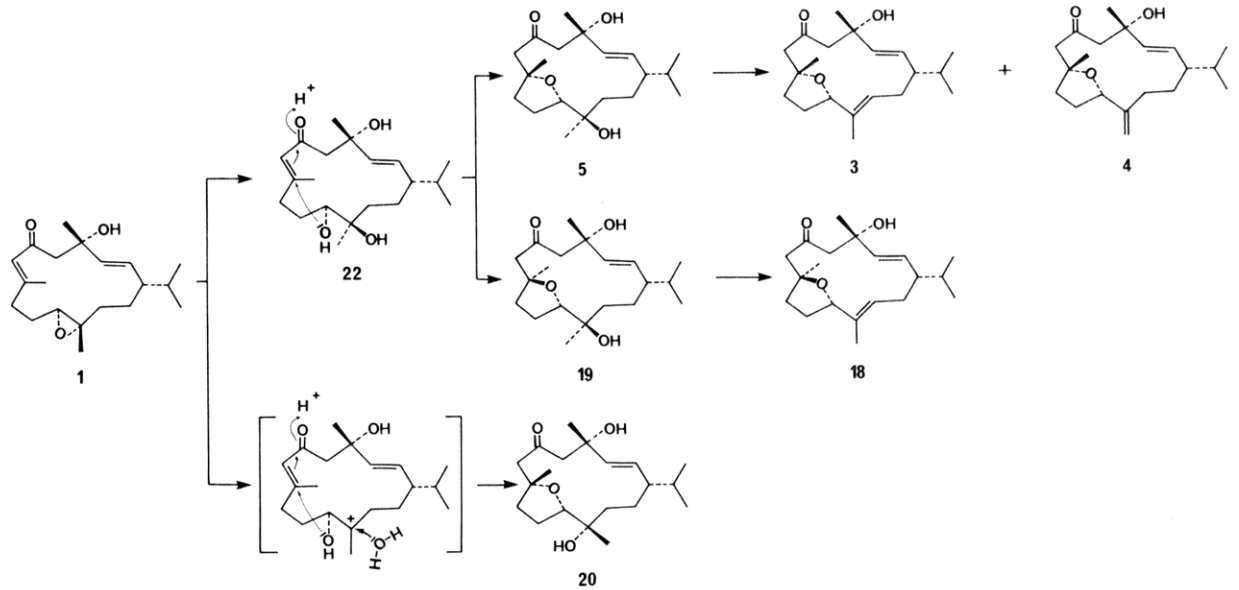


Figure 4. ^1H–^{13}C chemical shift correlation map for the resonances of **5** and **20**.

Scheme II. Proposed Mechanism for the Acid-Induced Rearrangement of 1

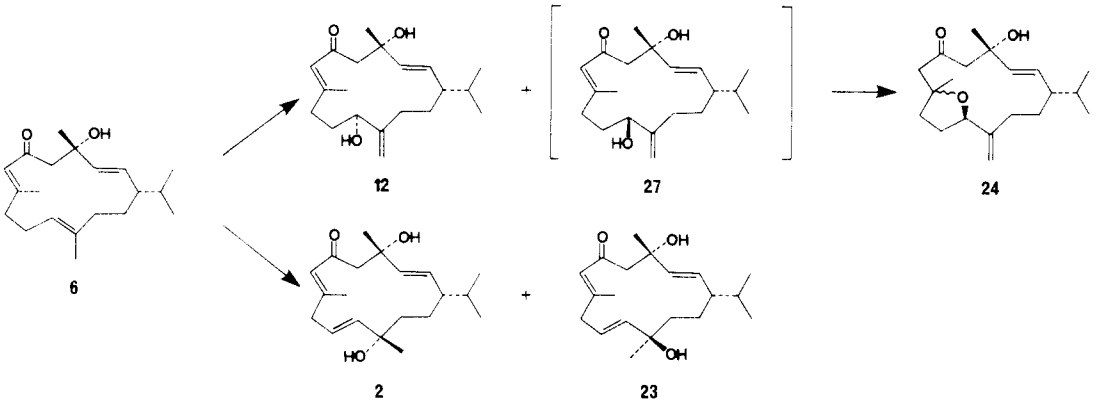


cembradien-6-one (**22**). This undergoes protonation of the oxo group, double-bond migration and, attack of the 11-hydroxyl group on C-8. If the reacting species then exits as a conformer of type a, the 8(*R*),11(*S*)-epoxide **5** is formed, whereas the reaction sequence when taking place with a conformer of type b would yield the 8(*S*),11(*S*)-epoxide **19** (cf. Figure 3). As a result, both **5** and **19** would have 12*R* stereochemistries. These two diols (**5**, **19**) are, in turn, the plausible precursors of **3**–**4** and **18**, respectively.

Support for the validity of this pathway is provided by the isolation of the major polar intermediate, which was shown by TLC to be formed prior to the appearance of **5** and **19**. This compound was assigned a 4,11,12-trihydroxy-2,7-cembradien-6-one structure but could not be conclusively identified as **22** on the basis of its ^1H NMR data. On treatment with dilute sulfuric acid in dioxane/water, however, this triol yielded the 8(*R*),11(*S*)-epoxide **5** as the major and the 8(*S*),11(*S*)-epoxide **19** as the minor product.

The 8(*R*),11(*S*)-epoxide **20**, which has a 12*S* stereochemistry, must be formed from the 11,12-epoxide **1** by a mechanism involving a C-12 carbonium ion as an in-

Scheme III. Products Obtained from 6 by Sensitized Photooxygenation Followed by Reduction



termediate. It is also evident that the reaction of **1** with hydrochloric acid in chloroform, which is rapid and yields **3** exclusively, proceeds via a mechanism different from that shown in Scheme II. One possible pathway involves the addition of hydrochloric acid to the 11,12-epoxide group in **1**, ether ring formation, and loss of hydrochloric acid.

While acid-induced rearrangement of the 11,12-epoxide **1** is a means of accounting for the occurrence of the 8-(*R*),11(*S*)-epoxides **3**–**5** in tobacco, the formation of the new 4,12-diol **2** must take a different course. One possible route (Scheme I) would involve oxidation of the 4(*S*),6(*R*),12-(*S*)-triol **11**, which is a tobacco constituent and a plausible metabolite of the 4(*S*),6(*R*)-diol **7**.[6,7]

Another route would comprise oxygen attack on the 11,12 double bond in ketol **6** either by sensitized photooxygenation or an enzyme-assisted reaction. This possibility was rendered likely in view of previous findings for the 4,6-diols **7** and **8**[6,7] and was explored experimentally by treatment of ketol **6** with singlet oxygen. The reaction proceeded smoothly and, after reduction of the initially generated hydroperoxides by using triethyl phosphite, four products (**2**, **12**, **23** and **24**) were isolated in the ratio 60:33:3:4 (Scheme III). The major one, giving rise to triol **11** on reduction using $NaBH_4$, was identified as (1*S*,2*E*,4*S*,7*E*,10*E*,12*S*)-4,12-dihydroxy-2,7,10-cembratrien-6-one and proved to be indistinguishable from tobacco constituent **2**. It is evidently formed via the expected ene reaction at the 11,12 double bond.

A structurally related minor product (**23**), whose ^1H NMR spectrum was reminiscent of that of **2** and whose ^{13}C NMR spectrum differed mainly from that of **2** with respect to the shielding of C-20, δ 27.1 vs. 29.3, was formulated as (1*S*,2*E*,4*S*,7*E*,10*E*,12*R*)-4,12-dihydroxy-2,7,10-cembratrien-6-one, i.e., the 12*R* epimer of **2**. This assignment was verified by direct comparison with an authentic sample obtained by oxidation of the 4(*S*),6(*R*),12-(*R*)-triol **25**[7] with manganese dioxide.

The second major product, also obtained via an ene reaction, was identified as (1*S*,2*E*,4*S*,7*E*,11*S*)-4,11-dihydroxy-2,7,12(20)-cembratrien-6-one (**12**), since it gave rise to the known 4(*S*),6(*R*),11(*S*)-triol **26**[6,7] on reduction using $NaBH_4$. As described above, **12** undergoes a facile ring closure to the new 8(*R*),11(*S*)-epoxide **4** on exposure to acid, hence implying that there exists an alternative route to the latter compound in tobacco.

The fourth product (**24**) was ascribed an 8,11-epoxy-4-(*S*)-hydroxy-2(*E*),12(20)-cembradien-6-one structure from its spectral data. Since this compound is not identical with the 8(*R*),11(*S*)-epoxide **4** nor with the 8(*S*),11(*S*)-epoxide **13**, it must have an 11*R* configuration. It would then arise by spontaneous cyclization of the 4(*S*),11(*R*)-diol **27**. The latter would be the fourth ene product but could not be isolated, as such, from the reaction mixture.

It can be concluded from the results described above that the reaction of ketol **6** with singlet oxygen occurs with a high degree of stereoselectivity, the hydroperoxides corresponding to the 11(*S*)- and 12(*S*)-diols **12** and **2** being formed in preference to their 11*R* and 12*R* epimers (93:7). This effect may be accounted for by conformational arguments similar to those presented previously for the 4,6-diols **7** and **8**.[6,7]

As expected, all four products (**2**, **12**, **23**, and **24**) arise via syn-ene additions. Any anti-ene products formed must be present in minute amounts, a result which infers that photooxygenation is not a viable route leading to the 8-(*R*),11(*S*)-epoxide **3**.

^{13}C NMR Spectra. The ^{13}C NMR spectra, which have been published by us[1,6,7,9,11,13-15] in connection with previous structural studies on tobacco cembranoids, have been assigned by application of simple chemical shift arguments and multiplicity information and by comparison of data for structurally related compounds. However, since the chemical shift values are strongly affected by spatial factors and since the conformation of a given cembranoid in solution is seldom known or predictable,[7] the assignment of the ^{13}C NMR spectrum is often left with some ambiguity.

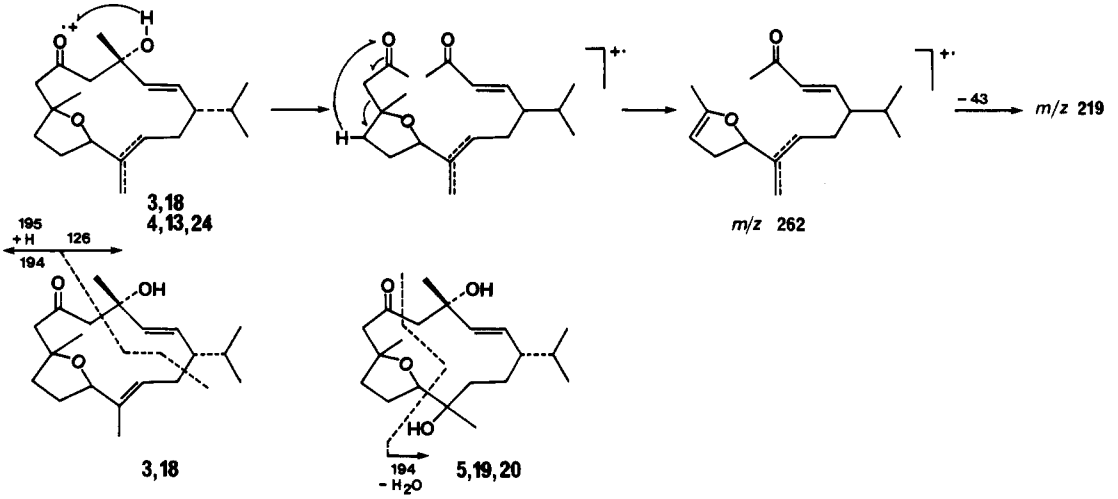
We have now found that a combined use of ^1H–^1H and ^1H–^{13}C shift correlation spectroscopy is a powerful tool, which has allowed an unequivocal assignment of most of the spectra compiled in Table I.

The C-5 and C-7 signals in the spectra of the 8,11-epoxy-bridged compounds **3**–**5**, **13**, **14**, and **18**–**21**, which could not be distinguished by using this method, were identified with the help of LIS experiments. These were carried out on compounds **13** and **21** and resulted, as expected, in preferential complex formation between the shift reagent, Eu $(dpm)_3$, and the hydroxyl group at C-4. The effect on the two pertinent AB quartets in the ^1H NMR spectra was measured, and the quartet undergoing the largest induced shift was attributed to the protons at C-5. The C-5 and C-7 signals were then readily assigned via the ^1H–^{13}C shift correlation spectra.

The assignment of the C-13 and C-14 signals in the spectrum of **13** was not straightforward, since the signals due to the protons at C-13 and C-14 could not be unambiguously identified because of overlap and low intensities of certain cross-peaks in the COSY 45 spectrum. These difficulties were circumvented using selective proton flip

(13) Wahlberg, I.; Behr, D.; Eklund, A.-M.; Nishida, T.; Enzell, C. R.; Berg, J.-E. *Acta Chem. Scand., Ser. B* **1982**, *B36*, 37.
(14) Wahlberg, I.; Behr, D.; Eklund, A.-M.; Nishida, T.; Enzell, C. R.; Berg, J.-E. *Acta Chem. Scand., Ser. B* **1982**, *B36*, 443.
(15) Wahlberg, I.; Nordfors, K.; Vogt, C.; Nishida, T.; Enzell, C. R. *Acta Chem. Scand., Ser. B* **1983**, *B37*, 653.

Scheme IV. Proposed Routes for the Mass Spectral Fragmentation of 3-5, 13, 18-20, and 24



2D J spectroscopy.[16] Thus, four doublets are observed as a result of long-range CH coupling to H-11 (δ 4.02). Three of these, centered at δ 33.3 ($^2J_{C\text{-}10,H\text{-}11}$ = 2.0 Hz), 110.3 ($^3J_{C\text{-}20,H\text{-}11}$ = 4.4 Hz), and 150.9 ($^2J_{C\text{-}12,H\text{-}11}$ = 3.1 Hz), were ascribed to C-10, C-20, and C-12, respectively. The remaining doublet, present at δ 32.0 ($^3J_{C\text{-}13,H\text{-}11}$ = 2.2 Hz), must hence have arisen through coupling to C-13. This assignment was confirmed by a second selective proton flip experiment involving H-20b (δ 5.15). Three doublets are now visible, these reflecting long-range coupling to C-13 ($^3J_{C\text{-}13,H\text{-}20b}$ = 10.3 Hz), C-12 ($^2J_{C\text{-}12,H\text{-}20b}$ = 2.5 Hz), and C-11 (δ 83.3, $^3J_{C\text{-}11,H\text{-}20b}$ = 4.9 Hz).

Mass Spectra. The 8,11-epoxy-4-hydroxy-2,12-cembradien-6-ones **3** and **18** and the 8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-ones **4**, **13**, and **24** give mass spectra containing ions of diagnostic value at m/z 262 ($C_{17}H_{26}O_2$), 219 ($C_{14}H_{19}O_2$), 195 ($C_{12}H_{19}O_2$), and 194 ($C_{12}H_{18}O_2$).

The m/z 262 species is likely to arise as shown in Scheme IV via an initial conversion to a *seco*-diketone, which undergoes a McLafferty type of rearrangement with elimination of a molecule of acetone. Subsequent elimination of a propyl radical from the m/z 262 ion may account for the generation of the m/z 219 ion.

The generation of the m/z 195 and 194 ions probably involves ruptures of the 4,5 and 1,14 bonds with charge retention on the larger fragment. In compounds **3** and **18**, which have a 12,13 double bond, charge retention also occurs with the smaller fragment of mass 126. Consistent with this is the presence of a m/z 126 species in the mass spectra of 8,11-epoxy-2,6,12-cembratrien-4-ols.[17]

The formation of the m/z 262, 219, and 195 ions is suppressed in the fragmentation of the 4,12-dihydroxy-8,11-epoxy-2-cembren-6-ones **5**, **19**, and **20**. The spectra of these compounds do contain a peak at m/z 194, but this is due to a $C_{13}H_{22}O$ ion. Its formation may be triggered by cleavage of the 11,12 bond and proceed by dehydration and rupture of the 5,6 bond (Scheme IV).

Experimental Section

Instruments. Melting points were measured on a Leitz Wetzlar instrument and are uncorrected. Optical rotations and infrared and NMR spectra were recorded on Perkin-Elmer 141, Perkin-Elmer 983, and Varian XL-300 instruments, respectively. Mass spectra were obtained on a Kratos MS 50 Stereo DS 55 SM-DS 55 S mass spectrometer–computer system. Large-scale high performance liquid chromatography was performed on a Waters Prep LC/System 500 chromatograph, while for small-scale HPLC a Waters 6000 A solvent delivery system, a U6K injector, and an R-401 differential refractometer was used.

NMR Experiments. ^1H–^1H shift correlation spectra for survey purposes were recorded of compounds **1–5**, **13**, **14**, **16**, **18–21**, and **24**. The map was composed of 512 × 512 data point spectra, each consisting of 16 transients. The evolution period, t_1, was incremented in 256 steps. The mixing pulse was 45° in most cases, and a pseudoecho shaping function was applied in both dimensions.[4,18]

For high-resolution COSY 45 spectra a data matrix of 1024 × 1024 data points was used and 512 increments were made.

In order to emphasize long-range coupling in the COSY spectra delay times of 0.35, 0.40, and 0.60 s and mixing pulses of 56° and 60° was applied.[5]

^1H–^{13}C shift correlation spectra were recorded of compounds **1–5**, **13**, **14**, **16**, **19–21**, and **24** using the pulse sequence: 90° (^1H)–0.5t_1–180° (^{13}C)–0.5t_1–D_3–90°(^1H)90°(^{13}C)–D_4–Acq. (t_2) [^1H BB decoupling]. The data matrix was composed of 2048 × 512 data points and 64 or 128 increments were made.[19]

Selective long-range ^1H–^{13}C spin coupling constants were measured by using the pulse sequence described by Bax and Freeman.[16] The following parameters were used: spectral width in the F_1 dimension = 20 Hz, spectral width in the F_2 dimension = 16583.8 Hz, data points for F_1 = 256 and for F_2 = 16 K; number of increments; 32. The selective proton pulse was obtained by a decoupler soft pulse ($\gamma B_1/2\pi$ = 25 Hz; 20 ms).

Isolation. An extract obtained by immersing flowers of Greek *Nicotiana tabacum* (Basma Drama) in chloroform was initially separated into five fractions A (12.7 g), B (4.7 g), C (8.0 g), D (30 g), and E (3.6 g) by flash chromatography over silica gel using a gradient of hexane/ethyl acetate/methanol as the eluent. Part of the fraction C (6.2 g) was separated further into eight fractions, C1–C8, by using a PrepPak-500/C_{18} cartridge and methanol/water (65:35) as the eluent.

Fractions C5 (221 mg) and C6 (326 mg) were combined and separated by flash chromatography over silica gel (hexane/ethyl acetate gradient) and subsequent HPLC using columns packed with Spherisorb 5 Nitrile and Spherisorb 5 to give 3.8 mg of (1*S*,2*E*,4*S*,7*E*,11*S*,12*S*)-11,12-epoxy-4-hydroxy-2,7-cembradien-6-one (**1**).

In another workup, fraction C (8.0 g) was separated by flash chromatography over silica gel into five fractions, C1A–C5A. Repeated HPLC of fraction C4A using columns packed with Spherisorb 5, Spherisorb 5 Nitrile and Spherisorb 5 ODS allowed the isolation of 3.0 mg of (1*S*,2*E*,4*S*,7*E*,10*E*,12*S*)-4,12-dihydroxy-2,7,10-cembratrien-6-one (**2**).

Flash chromatography (silica gel; hexane/ethyl acetate, 30:70) was used to separate fraction D (30 g) into six fractions, D1–D6.

(16) Bax, A.; Freeman, R. *J. Am. Chem. Soc.* **1982**, *104*, 1099.
(17) Enzell, C. R.; Wahlberg, I.; Ryhage, R. *Mass Spectrom. Rev.* **1984**, *3*, 395.
(18) Bax, A.; Freeman, R.; Morris, G. A. *J. Magn. Reson.* **1981**, *43*, 333.
(19) Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501.

Fraction D5 (5.7 g) was subject to repeated HPLC using columns packed with PrepPak-500/C_{18}, Spherisorb 5, Spherisorb 5 Nitrile, and Spherisorb 5 ODS to give 1.6 mg of (1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one (**5**).

(1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-Epoxy-4-hydroxy-2,12-cembradien-6-one (**3**, 3 mg) and (1*S*,2*E*,4*S*,8*R*,11*S*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one (**4**, 13 mg) were isolated from fraction B7 of a diethyl ether extract of 295 kg of sun-cured Greek tobacco (Serres)[20] by column chromatography over silica gel followed by HPLC using columns packed with Partisil-PAC and μ-Bondapak/CN.

(1*S*,2*E*,4*S*,7*E*,11*S*,12*S*)-11,12-Epoxy-4-hydroxy-2,7-cembradien-3-one (1): mp 67.0–67.5 °C; $[\alpha]_D$ +85° (*c* 0.60, $CHCl_3$); exact mass found $M^{+\cdot}$ 320.2340, calcd for $C_{20}H_{32}O_3$, 320.2351, IR (CCl_4) 3610, 3470, 1665, 1605, 1385, and 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (d, *J* = 6.5 Hz)/0.84 (d, *J* = 6.5 Hz) (H-16/H-17), 1.27 (s, H-20), 1.35 (s, H-18), 2.20 (d, *J* = 1.2 Hz, H-19), 2.49 (d, *J* = −12.6 Hz, H-5a), 2.62 (dd, *J* = 4.5 and 9.0 Hz, H-11), 2.74 (d, *J* = −12.6 Hz, H-5b), 3.60 (br s, OH), 5.42 (d, *J* = 15.5 Hz, H-3), 5.49 (dd, *J* = 8.0 and 15.5 Hz, H-2) and 6.08 (br s, H-7); MS [*m*/*z* (relative intensity, composition)], 320 (M, 0.4), 305 (0.6, $C_{19}H_{29}O_3$), 302 (0.3, $C_{20}H_{30}O_2$), 287 (0.5, $C_{19}H_{27}O_2$), 277 (3, $C_{18}H_{29}O_2$), 259 (2, $C_{17}H_{23}O_2$), 241 (0.5, $C_{17}H_{21}O$), 223 (1), 201 (1, $C_{15}H_{21}$ and $C_{14}H_{17}O$), 180 (7), 137 (41, $C_9H_{13}O$ and $C_{10}H_{17}$), 123 (13, C_9H_{15} and $C_8H_{11}O$), 109 (23, C_7H_9O and C_8H_{13}), 97 (26, C_6H_9O and C_7H_{13}), 81 (35, C_6H_9 and C_5H_5O), 71 (17, C_4H_7O), 55 (15, C_4H_7 and C_3H_3O), and 43 (100, C_2H_3O and C_3H_7).

(1*S*,2*E*,4*S*,7*E*,10*E*,12*S*)-4,12-Dihydroxy-2,7,10-cembratrien-6-one (2): mp 140–141 °C; $[\alpha]_D$ +59° (*c* 0.61, $CHCl_3$); exact mass found $M^{+\cdot}$ 320.2353, calcd for $C_{20}H_{32}O_3$, 320.2351; IR (CCl_4) 3615, 3452, 1666, 1605, 1387, and 1369 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (d, *J* = 6.7 Hz)/0.86 (d, *J* = 6.6 Hz) (H-16/H-17), 1.32 (s, H-18 and H-20), 2.19 (d, *J* = 1.2 Hz, H-19), 2.53 (d, *J* = −11.4 Hz, H-5a), 2.71 (d, *J* = −11.4 Hz, H-5b), 2.83 (d, *J* = 6.6 Hz, H-9a and H-9b), 5.46 (d, *J* = 15.6 Hz, H-3), 5.51 (d, *J* = 15.8 Hz, H-11), 5.61 (dd, *J* = 8.2 and 15.6 Hz, H-2), 5.64 (dt, *J* = 6.6 and 15.8 Hz, H-10), and 6.06 (m, H-7); MS *m*/*z* (relative intensity, composition)], 320 (M, 0.1), 302 (1, $C_{20}H_{30}O_2$), 287 (0.5, $C_{19}H_{27}O_2$), 259 (2, $C_{17}H_{23}O_2$ and $C_{18}H_{27}O$), 241 (1, $C_{17}H_{21}O$ and $C_{18}H_{25}$), 217 (1, $C_{16}H_{25}$, $C_{14}H_{17}O_2$ and $C_{15}H_{21}O$), 201 (1, $C_{14}H_{17}O$ and $C_{15}H_{21}$), 151 (13), 126 (18, $C_8H_{14}O$), 109 (30, C_7H_9O and C_8H_{13}), 95 (13, C_6H_7O and C_7H_{11}), 81 (14, C_6H_9 and C_5H_5O), 71 (16, C_4H_7O), 55 (13, C_4H_7), and 43 (100, C_2H_3O and C_3H_7).

(1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-Epoxy-4-hydroxy-2,12-cembradien-6-one (3): mp 102–103 °C; $[\alpha]_D$ +26° (*c* 0.49, $CHCl_3$); exact mass found $M^{+\cdot}$ 320.2360, calcd for $C_{20}H_{32}O_3$, 320.2351; IR (CCl_4) 3496, 1706, 1384, and 1368 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (d, *J* = 6.8 Hz)/0.91 (d, *J* = 6.8 Hz) (H-16/H-17), 1.24 (s)/1.25 (s) (H-18/H-19), 1.55 (d, *J* = 0.7 Hz, H-20), 2.38 (m, H-9b), 2.48 (d, *J* = −15.4 Hz, H-7a), 2.50 (d, *J* = −17.0 Hz, H-5a), 2.90 (d, *J* = −17.0 Hz, H-5b), 3.00 (d, *J* = −15.4 Hz, H-7b), 4.20 (br s, O*H*), 4.30 (m, H-11) and 5.3–5.6 (overlapping signals, H-2, H-3 and H-13); MS [*m*/*z* (relative intensity, composition)], 320 (M, 1), 302 (1, $C_{20}H_{30}O_2$), 277 (2), 262 (3, $C_{17}H_{26}O_2$), 250 (1), 219 (4, $C_{14}H_{19}O_2$), 195 (23, $C_{12}H_{19}O_2$), 194 (26, $C_{12}H_{18}O_2$), 181 (5), 153 (15, $C_{10}H_{17}O$), 137 (11, $C_9H_{13}O$), 126 (8, $C_8H_{14}O$), 111 (11, $C_7H_{11}O$), 95 (21, C_7H_{11} and C_6H_7O), 83 (13, C_5H_7O and C_6H_{11}), 69 (21, C_5H_9 and C_4H_5O), 55 (22), and 43 (100).

(1*S*,2*E*,4*S*,8*R*,11*S*)-8,11-Epoxy-4-hydroxy-2,12(20)-cembradien-6-one (4): mp 58–59 °C; $[\alpha]_D$ +92° (*c* 0.63, $CHCl_3$); exact mass found M^+ 320.2363, calcd for $C_{20}H_{32}O_3$, 320.2351; IR ($CHCl_3$) 3480, 3090, 1705, 1650, 1390, and 1375 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (d, *J* = 6.7 Hz)/0.90 (d, *J* = 6.7 Hz) (H-16/H-17), 1.17 (d, *J* = 1.0 Hz, H-18), 1.25 (s, H-19), 2.44 (dd, *J* = 1.0 and −13.1 Hz, H-7a), 2.44 (d, *J* = −17.8 Hz, H-5a), 2.46 (m, H-9b), 2.83 (d, *J* = −13.1 Hz, H-7b), 3.03 (dd, *J* = 1.0 and −17.8 Hz, H-5b), 4.45 (dd, *J* = 5.8 and 8.3 Hz, H-11), 4.77 (m, $W_{1/2}$ = 4.0 Hz, H-20a), 4.80 (br s, OH), 5.08 (m, $W_{1/2}$ = 4.0 Hz, H-20b), 5.25 (d, *J* = 15.4 Hz, H-3), and 5.34 (dd, *J* = 8.4 and 15.4 Hz, H-2); MS [*m*/*z* (relative intensity, composition)], 320 (M, 1), 302 (2, $C_{20}H_{30}O_2$), 277 (2, $C_{17}H_{25}O_3$), 262 (12, $C_{17}H_{26}O_2$), 219 (25, $C_{14}H_{19}O_2$), 195 (3, $C_{12}H_{19}O_2$), 194 (11, $C_{12}H_{18}O_2$), 181 (10, $C_{11}H_{17}O_2$), 161 (8, $C_{11}H_{13}O$ and $C_{12}H_{17}$), 149 (9, $C_{10}H_{13}O$), 139 (11, $C_9H_{15}O$ and $C_8H_{11}O_2$), 123 (18 $C_8H_{11}O$ and C_9H_{15}), 109 (24, C_7H_9O and C_8H_{13}), 95 (26, C_7H_{11} and C_6H_7O), 81 (22, C_6H_9 and C_5H_5O), 69 (22, C_5H_9 and C_4H_5O), 55 (21, C_4H_7 and C_3H_3O), and 43 (100).

(1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-4,12-Dihydroxy-8,11-epoxy-2-cembren-6-one (5): mp 141–143 °C; $[\alpha]_D$ +44° (*c* 0.85, $CHCl_3$); exact mass found $M^{+\cdot}$ 338.2459, calcd for $C_{20}H_{34}O_4$, 338.2457; IR (CCl_4) 3587, 3504, 1703, 1384, and 1366 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (d, *J* = 6.7 Hz)/0.89 (d, *J* = 6.7 Hz) (H-16/H-17), 1.05 (s, H-20), 1.20 (s, H-19), 1.22 (s, H-18), 2.41 (dd, *J* = 0.7 and −13.9 Hz, H-7a), 2.54 (d, *J* = −18.1 Hz, H-5a), 2.90 (d, *J* = −13.9 Hz, H-7b), 2.98 (dd, *J* = 1.0 and −18.1 Hz, H-5b), 3.86 (m, H-11), 4.93 (br s, OH), 5.27 (d, *J* = 15.5 Hz, H-3), and 5.40 (dd, *J* = 9.4 and 15.5 Hz, H-2); MS [*m*/*z* (relative intensity, composition)], 338 (M, 1), 320 (4, $C_{20}H_{32}O_3$), 302 (1, $C_{20}H_{30}O_2$), 277 (2), 223 (3, $C_{17}H_{19}$), 205 (1, $C_{14}H_{21}O$), 194 (25, $C_{13}H_{22}O$), 181 (12, $C_{11}H_{17}O_2$), 136 (47, $C_{10}H_{16}$), 125 (21, $C_8H_{13}O$ and $C_7H_9O_2$), 109 (17, C_8H_{13} and C_7H_9O), 93 (16, C_7H_9), 81 (23, C_6H_9 and C_5H_5O), 71 (21, C_4H_7O), 55 (16, C_4H_7 and C_3H_3O), and 43 (100).

Oxidation of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-11,12-Epoxy-2,7-cembradiene-4,6-diol (10). To a solution of 157 mg of **10**[6] in 10 mL of pyridine was added 240 mg of chromium trioxide in 10 mL of pyridine. The reaction mixture was left at room temperature for 2 h and then diluted with water and extracted with ether. The organic phase was washed with aqueous H_2SO_4 (5%) and water, dried, and concentrated. The residue was separated by flash chromatography over silica gel using hexane/ethyl acetate (75:25) as an eluent into 17.5 mg of starting material (**10**) and 110 mg of (1*S*,2*E*,4*S*,7*E*,11*S*,12*S*)-11,12-epoxy-4-hydroxy-2,7-cembradien-6-one, which was identical (mp, optical rotation, IR, ^1H NMR, and MS) with the naturally occurring **1**.

Oxidation of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-2,7,10-Cembratriene-4,6,12-triol (11). To a solution of 16 mg of **11**[6,7] in 0.3 mL of dry dimethylformamide was added 32 mg of pyridinium dichromate. After being stirred at room temperature for 6 h, the reaction mixture was diluted with water, extracted with ether, dried, and concentrated. The residue was separated by HPLC using columns packed with Spherisorb 5 Nitrile and Spherisorb 5 (hexane/ethyl acetate, 60:40) to yield as the major product 2.3 mg of (1*S*,2*E*,4*S*,7*E*,10*E*,12*S*)-4,12-dihydroxy-2,7,10-cembratrien-6-one, whose physical and spectral data were indistinguishable from those of tobacco isolate **2**.

Conversion of 1 to (1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-Epoxy-4-hydroxy-2,12-cembradien-6-one (3). To a solution of 190 mg of **1** in 30 mL of chloroform was added 8 mL of a solution of hydrochloric acid in chloroform (1%). The reaction mixture was stirred at room temperature and under nitrogen for 40 min. Workup and purification by HPLC (Spherisorb 5, hexane/ethyl acetate, 60:40) gave 111 mg of (1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-epoxy-4-hydroxy-2,12-cembradien-6-one, whose mp, optical rotation, and IR, ^1H NMR, and mass spectra were identical with those of tobacco isolate **3**.

Treatment of (1*S*,2*E*,4*S*,7*E*,11*S*)-4,11-Dihydroxy-2,7,12(20)-cembratrien-6-one (12) with Acid. To a solution of 313 mg of **12** in 5 mL of chloroform was added 5 mL of a solution of hydrochloric acid in chloroform (1%). The reaction mixture was kept at room temperature and under nitrogen for 30 min, washed with aqueous $NaHCO_3$ and water, dried, concentrated, and flash chromatographed over silica gel to give 155 mg of (1*S*,2*E*,4*S*,8*R*,11*S*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one, whose mp, optical rotation, IR, ^1H NMR, and MS were indistinguishable from those of the naturally occurring **4**, and 41 mg of (1*S*,2*E*,4*S*,8*S*,11*S*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one (**13**): mp 47–49 °C; $[\alpha]_D$ +86° (*c* 0.95, $CHCl_3$); IR (CCl_4) 3395, 1698, 1385, and 1367 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.76 (d, *J* = 6.8 Hz)/0.83 (d, *J* = 6.7 Hz) (H-16/H-17), 1.28 (s)/1.29 (s) (H-18/H-19), 2.33 (d, *J* = −13.2 Hz, H-7a), 2.46 (d, *J* = −11.9 Hz, H-5a), 2.85 (ddd, *J* = 7.1, 12.6 and −12.6 Hz, H-9b), 2.91 (d, *J* = −11.9 Hz, H-5b), 3.26 (d, *J* = −13.2 Hz, H-7b), 4.02 (dd, *J* = 4.0 and 11.3 Hz, H-11), 4.90 (br s, H-20a), 4.98 (br s, OH), 5.15 (br s, H-20b), 5.62 (dd, *J* = 8.4 and 15.8 Hz, H-2), and 5.70 (d, *J* = 15.8 Hz, H-3); MS [*m*/*z* (relative intensity)], 320 (M, 12), 302 (3), 277 (1), 262 (8), 245 (2), 219 (11), 194 (10), 181 (12), 139 (13), 123 (16), 109 (21), 95 (24), 81 (16), 69 (23), 55 (19), and 43 (100).

Dehydration of (1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-8,11-Epoxy-4,12-dihydroxy-2-cembren-6-one (5). To a solution of 16.6 mg of

(20) Kimland, B.; Aasen, A. J.; Enzell, C. R. *Acta Chem. Scand.* **1972**, *26*, 2177.

5 in 1 mL of pyridine, kept at 0 °C, was added 20 μL of thionyl chloride. The reaction mixture was kept at 0 °C for 30 min, poured into ice–water, and extracted with ether. The ether solution was washed with aqueous sulfuric acid (10 %) and water, dried, and concentrated. Separation by HPLC (Spherisorb 5 Nitrile, hexane/ethyl acetate, 70:30) afforded 3.7 mg of (1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-epoxy-4-hydroxy-2,12-cembradien-6-one and 0.8 mg of (1*S*,2*E*,4*S*,8*R*,11*S*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one, which were identical with the naturally occurring **3** and **4**, respectively.

Preparation of (1*S*,2*E*,4*S*,8*R*,11*S*,12*S*,13*S*)-8,11;12,13-Diepoxy-4-hydroxy-2-cembren-6-one (14). To a cooled (0 °C) solution of 63 mg (0.20 mmol) of (1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-epoxy-4-hydroxy-2,12-cembradien-6-one (**3**) in 6 mL of chloroform was added a solution of 40 mg (0.23 mmol) of *m*-chloroperbenzoic acid in 2 mL of chloroform. After 1.5 h, the reaction mixture was washed with aqueous $NaHCO_3$ and water, dried, and concentrated. Flash chromatography over silica gel using a hexane/ethyl acetate gradient as the eluent furnished 45 mg of (1*S*,2*E*,4*S*,8*R*,11*S*,12*S*,13*S*)-8,11;12,13-diepoxy-4-hydroxy-2-cembren-6-one (**14**): mp 97–99 °C; $[\alpha]_D$ +34° (*c* 0.73, $CHCl_3$); IR ($CHCl_3$) 3475, 1702, 1386, and 1369 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.889 (d, *J* = 6.8 Hz)/0.894 (d, *J* = 6.8 Hz) (H-16/H-17), 1.207 (s)/1.219 (s)/1.222 (s) (H-18/H-19/H-20), 2.41 (d, *J* = −15.0 Hz, H-7a), 2.50 (d, *J* = −18.0 Hz, H-5a), 2.90 (d, *J* = −15.0 Hz, H-7b), 2.93 (d, *J* = −18.0 Hz, H-5b), 2.97 (dd, *J* = 4.2 and 6.8 Hz, H-13), 3.89 (dd, *J* = 6.8 and 7.7 Hz, H-11), 4.88 (br s, OH) and 5.4–5.5 (overlapping signals, H-2 and H-3); MS [*m*/*z* (relative intensity)], 336 (M, 0.1), 321 (0.5), 292 (1), 279 (1), 260 (0.2), 237 (0.3), 219 (0.6), 211 (0.8), 168 (9), 141 (9), 126 (21), 111 (32), 95 (21), 81 (32), 71 (22), 55 (23), and 43 (100).

Preparation of (1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-4,12-Dihydroxy-8,11-epoxy-2-cembren-6-one (5). To a solution of 14.5 mg of **14** in dry ether was added an excess of LAH, and the reaction mixture was refluxed for 5.5 h. Workup and purification by HPLC (Spherisorb 5, hexane/ethyl acetate, 40:60) gave 9.3 mg of a major product (**15**), which was dissolved in 1 mL of dry dimethylformamide and stirred at room temperature with 86 mg of pyridinium dichromate. The reaction mixture was diluted with water and extracted with ether. The ether extract was washed with water, dried, and concentrated. The residue was separated by HPLC (Spherisorb 5 Nitrile, hexane/ethyl acetate, 40:60) to give 2.8 mg of (1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one, which was identical (mp, optical rotation, ^1H NMR and MS) with the naturally occurring **5**, and 1.7 mg of (4*R*,8*S*,9*E*,11*S*)-4,8-dimethyl-6,14-dioxo-8-hydroxy-11-isopropyl-9-pentadecen-4-olide (**16**), which was an oil: IR (CCl_4) 3522, 1784, 1711, 1380, and 1367 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (d, *J* = 6.8 Hz)/0.87 (d, *J* = 6.7 Hz) (H-19/H-20), 1.29 (s, H-17), 1.45 (s, H-16), 2.13 (s, H-15), 2.63 (m, H-2a and H-2b), 2.66 (d, *J* = −17.1 Hz, H-7a), 2.77 (d, *J* = −17.1 Hz, H-7b), 2.85 (d, *J* = −16.9 Hz, H-5a), 2.89 (d, *J* = −16.9 Hz, H-5b), 3.57 (br s, OH), 5.34 (dd, *J* = 9.0 and 15.7 Hz, H-10), and 5.45 (d, *J* = 15.7 Hz, H-9); MS [*m*/*z* (relative intensity)], 135 (2), 126 (3), 121 (3), 111 (2), 97 (12), 81 (3), 71 (6), 55 (8), and 43 (100).

Treatment of (1*S*,2*E*,4*S*,7*E*,11*S*,12*S*)-11,12-Epoxy-4-hydroxy-2,7-cembradien-6-one (1) with Acid. A. A solution of 55 mg of **1** in 10 mL of dioxane/water (3:1) and 0.8 mL of aqueous sulfuric acid (5%) was kept at room temperature and under nitrogen for 24 h. Workup and HPLC using a column packed with Spherisorb 5 Nitrile (hexane/ethyl acetate, 60:40) furnished 1.1 mg of (1*S*,2*E*,4*S*,8*S*,11*S*,12*E*)-8,11-epoxy-4-hydroxy-2,12-cembradien-6-one (**18**), 3.3 mg of (1*S*,2*E*,4*S*,8*R*,11*S*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one (**4**), 6.5 mg of (1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-epoxy-4-hydroxy-2,12-cembradien-6-one (**3**), 5.4 mg of (1*S*,2*E*,4*S*,8*S*,11*S*,12*R*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one (**19**), 2.4 mg of (1*S*,2*E*,4*S*,8*R*,11*S*,12*S*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one (**20**), and 24.3 mg of (1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one (**5**).

Of these, (1*S*,2*E*,4*S*,8*R*,11*S*,12*E*)-8,11-epoxy-4-hydroxy-2,12-cembradien-6-one, (1*S*,2*E*,4*S*,8*R*,11*S*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one, and (1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one were identical (mp, optical rotation, IR, ^1H NMR and MS) with the naturally occurring **3**, **4**, and **5**, respectively.

(1*S*,2*E*,4*S*,8*S*,11*S*,12*E*)-8,11-Epoxy-4-hydroxy-2,12-cembradien-6-one (18): IR (CCl_4) 3400 and 1702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.77 (d, *J* = 6.6 Hz)/0.95 (d, *J* = 6.6 Hz) (H-16/H-17), 1.22 (s, H-19), 1.27 (s, H-18), 1.66 (br s, H-20), 2.31 (d, *J* = −13.4 Hz, H-7a), 2.31 (ddd, *J* = 5.0, 11.8 and −13.2 Hz, H-14b), 2.34 (d, *J* = −10.9 Hz, H-5a), 2.94 (d, *J* = −10.9 Hz, H-5b), 3.00 (ddd, *J* = 7.2, 11.8, and −11.8 Hz, H-9b), 3.43 (d, *J* = −13.4 Hz, H-7b), 4.59 (dd, *J* = 4.5 and 11.5 Hz, H-11), 5.38 (dd, *J* = 6.3 and 11.8 Hz, H-13), 5.54 (dd, *J* = 8.6 and 15.4 Hz, H-2), and 5.60 (d, *J* = 15.4 Hz, H-3); MS [*m*/*z* (relative intensity)], 320 (M, 5), 302 (0.5), 277 (3), 262 (1), 259 (1), 219 (1), 205 (2), 195 (1), 194 (1), 139 (10), 126 (13), 109 (15), 95 (35), 81 (15), 69 (20), 55 (31), and 43 (100).

(1*S*,2*E*,4*S*,8*S*,11*S*,12*R*)-4,12-Dihydroxy-8,11-epoxy-2-cembren-6-one (19): mp 58–60 °C; $[\alpha]_D$ +23° (*c* 0.27, $CHCl_3$); IR (CCl_4) 3620, 3477, 1710, 1385, and 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (d, *J* = 6.7 Hz)/0.88 (d, *J* = 6.6 Hz) (H-16/H-17), 1.12 (s, H-20), 1.29 (s, H-19), 1.36 (s, H-18), 2.56 (d, *J* = −14.6 Hz, H-7a), 2.65 (d, *J* = −15.3 Hz, H-5a), 2.75 (d, *J* = −14.6 Hz, H-7b), 2.79 (d, *J* = −15.3 Hz, H-5b), 3.30 (br s, OH), 3.85 (dd, *J* = 6.3 and 8.9 Hz, H-11), and 5.6-5.9 (overlapping signals, H-2 and H-3); MS [*m*/*z* (relative intensity)], 338 (M, 0.3), 320 (3), 302 (1), 277 (2), 262 (2), 223 (3), 205 (2), 194 (21), 136 (41), 121 (20), 109 (21), 95 (23), 81 (29), 71 (30), 55 (24), and 43 (100).

(1*S*,2*E*,4*S*,8*R*,11*S*,12*S*)-4,12-Dihydroxy-8,11-epoxy-2-cembren-6-one (20): mp 175–177.5 °C; $[\alpha]_D$ +38° (*c* 0.18, $CHCl_3$); IR (CCl_4) 3529, 1702, 1381, and 1369 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (d, *J* = 6.6 Hz)/0.89 (d, *J* = 6.4 Hz) (H-16/H-17), 0.93 (s, H-20), 1.19 (s, H-19), 1.21 (s, H-18), 2.59 (d, *J* = −14.9 Hz, H-7a), 2.61 (d, *J* = −18.1 Hz, H-5a), 2.91 (d, *J* = −14.9 Hz, H-7b), 2.97 (d, *J* = −18.1 Hz, H-5b), 3.75 (m, H-11), 4.68 (br s, OH), 5.29 (d, *J* = 15.4 Hz, H-3), and 5.44 (dd, *J* = 9.2 and 15.4 Hz, H-2); MS [*m*/*z* (relative intensity)], 338 (M, 0.5), 320 (6), 302 (2), 281 (4), 223 (3), 205 (2), 194 (33), 180 (13), 141 (30), 136 (51), 127 (31), 121 (30), 109 (22), 93 (26), 81 (37), 71 (30), 55 (20), and 43 (100).

B. A solution of 22 mg of **1** in 4 mL of dioxane/water (2:1) and 0.06 mL of aqueous hydrochloric acid (5%) was kept at room temperature and under nitrogen for 24 h. Workup and separation by HPLC (Spherisorb 5 Nitrile; hexane/ethyl acetate, 60:40) afforded 6.5 mg of starting material (**1**), 3.1 mg of **5**, and 2.2 mg of (1*S*,2*E*,4*S*,7*E*,11*S*,12*R*)-4,11,12-trihydroxy-2,7-cembradien-6-one (**22**): ^1H NMR ($CDCl_3$) δ 0.84 (d, *J* = 6.4 Hz)/0.88 (d, *J* = 6.4 Hz) (H-16/H-17), 1.23 (s, H-18 and H-20), 1.90 (d, *J* = 1.2 Hz, H-19), 2.12 (m, H-9a), 2.58 (d, *J* = −16.3 Hz, H-5a), 2.65 (d, *J* = −16.3 Hz, H-5b), 3.31 (m, H-9b), 3.39 (m, H-11), 5.31 (d, *J* = 15.4 Hz, H-3), 5.56 (dd, *J* = 9.6 and 15.4 Hz, H-2), and 5.95 (br s, H-7).

Treatment of (1*S*,2*E*,4*S*,7*E*,11*S*,12*R*)-4,11,12-Trihydroxy-2,7-cembradien-6-one (22) with Acid. A solution of 1.4 mg of **22** in 0.6 mL of dioxane/water (3:1) and 0.04 mL of aqueous sulfuric acid (5%) was kept at room temperature for 4 h. Workup and separation by HPLC (Spherisorb 5 Nitrile; hexane/ethyl acetate, 60:40) gave 0.3 mg of (1*S*,2*E*,4*S*,8*S*,11*S*,12*R*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one (**19**) and 1.3 mg of (1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one (**5**).

Dehydration of (1*S*,2*E*,4*S*,8*S*,11*S*,12*R*)-4,12-Dihydroxy-8,11-epoxy-2-cembren-6-one (19). To a solution of 2.4 mg of **19** in 0.5 mL of pyridine was added 10 μL of thionyl chloride. The reaction mixture was kept at 0 °C for 30 min, poured into ice–water, and extracted with ether. The ether solution was washed with dilute sulfuric acid (10%) and water, dried, and concentrated. Separation by HPLC (Spherisorb 5 Nitrile, hexane/ethyl acetate, 75:25) afforded 0.3 mg of a product, whose IR, ^1H NMR, and mass spectra were identical with those of (1*S*,2*E*,4*S*,8*S*,11*S*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one (**13**).

Preparation of (1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-8,11;12,20-Diepoxy-4-hydroxy-2-cembren-6-one (21). To a cooled (0 °C) solution of 34.5 mg of **4** in 4 mL of methylene chloride was added a solution of 26.6 mg of *m*-chloroperbenzoic acid in 1 mL of methylene chloride. The reaction mixture was kept at 0 °C for 1 h and at room temperature for 4 h, washed with aqueous $NaHCO_3$ and water, dried, and concentrated. The residue was separated by flash chromatography over silica gel into 14.4 mg of starting material (**4**) and 9.8 mg of (1*S*,2*E*,4*S*,8*R*,11*S*,12*R*)-8,11;12,20-diepoxy-4-hydroxy-2-cembren-6-one (**21**): mp 87–89 °C; $[\alpha]_D$ +49° (*c* 0.65, $CHCl_3$); IR (CCl_4) 3503, 1704, 1384, and 1367 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (d, *J* = 6.7 Hz)/0.86 (d, *J* =

6.7 Hz) (H-16/H-17), 1.19 (s, H-19), 1.22 (s, H-18), 2.46 (d, J = −14.0 Hz, H-7a), 2.51 (d, J = −17.7 Hz, H-5a), 2.62 (d, J = −4.5 Hz, H-20a), 2.82 (d, J = −4.5 Hz, H-20b), 2.88 (d, J = −14.0 Hz, H-7b), 2.98 (dd, J = 1.0 and −17.7 Hz, H-5b), 3.44 (t, J = 7 Hz, H-11), 4.69 (br s, OH), 5.33 (d, J = 15.3 Hz, H-3), and 5.39 (dd, J = 8.4 and 15.3 Hz, H-2); MS [m/z (relative intensity)], 318 (M − 18, 1), 279 (4), 275 (3), 248 (2), 235 (2), 217 (2), 203 (2), 197 (8), 141 (20), 123 (14), 109 (22), 95 (28), 81 (40), 69 (20), 55 (23), and 43 (100).

Preparation of (1*S*,2*E*,4*S*,8*R*,11*S*,12*S*)-4,12-Dihydroxy-8,11-epoxy-2-cembren-6-one (20). A solution of 4.5 mg of diepoxide **21** in 2.5 mL of dry ether was reacted with an excess of LAH at room temperature for 0.5 h. The reaction mixture was worked up in usual manner. Without further purification the crude product obtained was dissolved in 1 mL of dry dimethylformamide and stirred at room temperature for 0.5 h with 10 mg of pyridinium dichromate. Workup and purification by HPLC using a column packed with Spherisorb-5 gave 0.6 mg of diepoxide **21** and 0.7 mg of a product, which was identical with (1*S*,2*E*,4*S*,8*R*,11*S*,12*S*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one (**20**).

Photooxygenation of (1*S*,2*E*,4*S*,7*E*,11*E*)-4-Hydroxy-2,7,11-cembratrien-6-one (6). A solution of 935 mg of **6**[3] and 40 mg of Rose Bengal in 25 mL of methanol in a tube cooled by a water jacket was irradiated with a 400-W sodium high pressure lamp placed outside the tube, while oxygen was bubbled through the reaction mixture. After 1.5 h, 700 μL of triethyl phosphite was added. The reaction mixture was kept at room temperature for 1 h and then concentrated at reduced pressure. The residue was filtered through alumina using ethyl acetate as the solvent and separated by flash chromatography over silica gel using a hexane/ethyl acetate gradient and subsequently by HPLC using columns packed with Spherisorb and Spherisorb 5 Nitrile to give 31 mg of (1*S*,2*E*,4*S*,8ξ,11*R*)-8,11-epoxy-4-hydroxy-2,12(20)-cembradien-6-one (**24**), 280 mg of (1*S*,2*E*,4*S*,7*E*,11*S*)-4,11-dihydroxy-2,7,12(20)-cembratrien-6-one (**12**), 26 mg of (1*S*,2*E*,4*S*,7*E*,10*E*,12*R*)-4,12-dihydroxy-2,7,10-cembratrien-6-one (**23**), and 500 mg of (1*S*,2*E*,4*S*,7*E*,10*E*,12*S*)-4,12-dihydroxy-2,7,10-cembratiren-6-one (**2**).

While (1*S*,2*E*,4*S*,7*E*,10*E*,12*S*)-4,12-dihydroxy-2,7,10-cembratrien-6-one was indistinguishable from tobacco isolate **2**, the other products are characterized as follows.

(1*S*,2*E*,4*S*,7*E*11*S*)-4,11-Dihydroxy-2,7,12(20)-cembratrien-6-one (12): oil; $[\alpha]_D$ −20° (c 0.48, $CHCl_3$); exact mass found $M^{+\cdot}$ 320.2329, calcd for $C_{20}H_{32}O_3$, 320.2351; IR (CCl_4) 3617, 3457, 3083, 1664, 1603, 1386, and 1367 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (d, J = 6.6 Hz)/0.86 (d, J = 6.6 Hz) (H-16/H-17), 1.32 (s, H-18), 2.19 (d, J = 1.1 Hz, H-19), 2.37 (d, J = −12.2 Hz, H-5a), 2.82 (d, J = −12.2 Hz, H-5b), 3.89 (t, J = 6.8 Hz, H-11), 4.02 (br s, OH), 4.90 (m, $W_{1/2}$ = 3.0 Hz, H-20a), 5.08 (m, $W_{1/2}$ = 3.0 Hz, H-20b), 5.3–5.6 (overlapping signals, H-2 and H-3), and 6.12 (br s, H-7); ^1H NMR (C_6D_6) δ 5.16 (d, J = 15.3 Hz, H-3) and 5.56 (dd, J = 9.3 and 15.3 Hz, H-2); MS [m/z (relative intensity)], 320 (M, 0.4), 302 (0.8), 287 (0.7), 277 (0.7), 262 (2), 259 (2), 241 (1), 219 (3), 201 (3), 177 (4), 161 (6), 149 (7), 139 (9), 121 (11), 109 (19), 95 (21), 81 (13), 71 (17), 55 (18), and 43 (100).

(1*S*,2*E*,4*S*,7*E*,10*E*,12*R*)-4,12-Dihydroxy-2,7,10-cembratrien-6-one (23): mp 83–85 °C; $[\alpha]_D$ +12° (c 1.3, $CHCl_3$); exact mass found $M^{+\cdot}$ 320.2336, calcd for $C_{20}H_{32}O_3$, 320.2351); IR (CCl_4) 3609, 3479, 1670, 1608, 1385, and 1367 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (d, J = 6.7 Hz)/0.86 (d, J = 6.6 Hz) (H-16/H-17), 1.29 (s, H-20), 1.34 (s, H-18), 2.17 (d, J = 1.2 Hz, H-19), 2.49 (d, J = −11.4 Hz, H-5a), 2.63 (br s, OH), 2.77 (d, J = −11.4 Hz, H-5b), 2.82 (d, J = 6.5 Hz, H-9a, and H-9b), 2.84 (br s, OH), 5.48 (d, J = 15.5 Hz, H-3), 5.56 (d, J = 15.9 Hz, H-11), 5.57 (dd, J = 8.3 and 15.5 Hz, H-2), 5.68 (dt, J = 6.5 and 15.9 Hz, H-10), and 6.06 (q, J = 1.2 Hz, H-7); MS [m/z (relative intensity)], 320 (M, 0.1), 302 (1), 287 (0.8), 259 (2), 250 (0.9), 241 (1), 217 (1), 201 (2), 167 (12), 151 (19), 126 (14), 109 (29), 95 (15), 81 (12), 71 (14), 55 (13), and 43 (100).

(1*S*,2*E*,4*S*,8ξ,11*R*)-8,11-Epoxy-4-hydroxy-2,12(20)-cembradien-6-one (24): mp 98–101 °C; $[\alpha]_D$ −49° (c 0.37, $CHCl_3$); exact mass found $M^{+\cdot}$ 320.2343, calcd for $C_{20}H_{32}O_3$, 320.2351; IR (CCl_4) 3606, 3481, 3081, 1710, 1648, 1386, and 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (d, J = 6.8 Hz)/0.89 (d, J = 7.0 Hz) (H-16/H-17), 1.26 (s, H-18), 1.44 (s, H-19), 2.36 (d, J = −14.6 Hz, H-5a), 2.57 (d, J = −18.6 Hz, H-7a), 2.68 (d, J = −14.6 Hz, H-5b), 2.76 (d, J = −18.6 Hz, H-7b), 4.48 (m, H-11), 4.58 (br s, OH), 4.79 (br s, $W_{1/2}$ = 4.0 Hz, H-20a), 4.95 (br s, $W_{1/2}$ = 4.0 Hz, H-20b), and 5.2–5.6 (overlapping signals, H-2 and H-3); ^1H NMR (C_6D_6) δ 5.30 (d, J = 15.0 Hz, H-3) and 5.53 (dd, J = 9.4 and 15.0 Hz, H-2); MS [m/z (relative intensity)], 320 (M, 2), 302 (3), 277 (2), 262 (10), 219 (20), 194 (6), 177 (6), 161 (6), 149 (7), 137 (9), 121 (13), 109 (17), 95 (19), 81 (15), 69 (14), 55 (14), and 43 (100).

Oxidation of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-2,7,10-Cembratriene-4,6,12-triol (25). To a solution of 4.8 mg of **25**[7] in 2 mL of acetone was added 20 mg of active manganese dioxide. The reaction mixture was stirred for 30 h at room temperature, concentrated, and chromatographed over silica gel to give 2.1 mg of (1*S*,2*E*,4*S*,7*E*,10*E*,12*R*)-4,12-dihydroxy-2,7,10-cembratrien-6-one, whose mp, optical rotation, IR, ^1H NMR, and MS were indistinguishable from those of the photooxygenation product **23**.

Reduction of (1*S*,2*E*,4*S*,7*E*,10*E*,12*S*)-4,12-Dihydroxy-2,7,10-cembratrien-6-one (2). A solution of 29 mg of **2** in 5 mL of methanol was stirred with 25 mg of $NaBH_4$ at room temperature for 1 h. Workup in the usual manner and separation by HPLC using columns packed with Spherisorb 5 Nitrile and Spherisorb 5 gave 4.8 mg of a major product, whose mp, optical rotation, IR, ^1H NMR, and MS were identical with those of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-2,7,10-cembratriene-4,6,12-triol (**11**).

Reduction of (1*S*,2*E*,4*S*,7*E*,10*E*,11*S*)-4,11-Dihydroxy-2,7,12(20)-cembratrien-6-one (12). A solution of 20 mg of **12** in 4 mL of methanol was stirred with 15 mg of $NaBH_4$ at room temperature for 1 h. Workup in the usual manner and separation by HPLC using columns packed with Spherisorb 5 Nitrile and Spherisorb 5 ODS gave 2.5 mg of a major product, whose optical rotation, IR, ^1H NMR, and MS were identical with those of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (**26**).

Acknowledgment. We are grateful to Dr. Petra Ossowski-Larsson and Mr. Jacek Bielawski for recording the mass spectra, to Dr. Ray Freeman, Oxford University, and Dr. Gareth Morris, University of Manchester, for generous gifts of NMR software and for advice, and to Professor Peder Kierkegaard, University of Stockholm, for his stimulating interest in the X-ray work.

Registry No. 1, 98064-73-4; **2**, 98064-74-5; **3**, 98064-75-6; **4**, 98064-76-7; **5**, 98064-77-8; **6**, 57760-49-3; **10**, 75281-99-1; **11**, 82003-46-1; **12**, 98064-78-9; **13**, 98167-30-7; **14**, 98064-79-0; **16**, 98064-80-3; **18**, 98167-31-8; **19**, 98167-32-9; **20**, 98167-33-0; **21**, 98064-82-5; **22**, 98064-81-4; **23**, 98167-35-2; **24**, 98167-34-1; **25**, 89362-10-7; **26**, 89362-08-3.