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Tobacco Chemistry. 51.* New Cembranic Diterpenoids from Greek Tobacco

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The isolation of (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diol** (*1*), the (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*E*)- and (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ols (*2*, *3*) and (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*4*) from Greek tobacco is reported. Of these *1* is a new natural product, whereas *2*–*4*, previously found in other tobacco varieties, are new to Greek tobacco. The structure of *1* has been determined by synthesis and X-ray analysis of the corresponding mono-acetate *5*. Compounds *2* and *3* have been correlated by dehydration and compound *4* has been used as the starting material for a synthesis which confirms that the structure of a previously isolated tobacco diterpenoid is (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*6*).

The acid rearrangement of *1*, a reaction which is proposed to mimic the biogenesis of some of the 8,11- and 8,12-epoxy bridged tobacco cembranoids, is discussed.

Recent studies have shown that depending on their genetic origin tobacco cultivars produce cembranes, labdanes or both.[3,4] These diterpenoids, which are present in the gummy exudate of the tobacco leaf and flower, are prone to biodegradation thus accounting for the presence of the unusually large number of odoriferous nor-diterpenoids encountered in tobacco.[5] We now report the isolation of four cembranoids from Greek tobacco, which is a producer of both cembranes and labdanes. Of these, one is a new natural product, whereas the other three are new to this tobacco variety.

RESULTS

The elemental composition, $C_{20}H_{34}O_3$, and the presence of an isopropyl group (methyl doublets at δ 0.81 and 0.86; ν_{max} at 1375 and 1390 cm^{-1}) and three methyl groups, of which one was vinylic and two attached to fully substituted oxygen-carrying carbon atoms implied that the first tobacco isolate (*1*) was a diterpenoid.

A secondary (one-proton multiplet at δ 4.85 in *1* and at δ 5.82 in the monoacetate *5*) and a tertiary hydroxyl group (ν_{max} at 3620 and 3500 cm^{-1} in *5*) accommodated two of the oxygen atoms. The remaining oxygen atom was evidently present as an epoxide group extending from a methine to a fully substituted carbon atom (doublet of doublets at δ 2.83; ^{13}C NMR signals at δ 61.0 (d) and 59.3 (s), *cf.* Table 1). Furthermore, since the ^{13}C NMR spectrum was consistent with the presence of a di- and a trisubstituted double bond, it followed that *1* was carbomonocyclic and a cembrane structure seemed most plausible.

A spectral comparison, which showed that fifteen signals in the ^{13}C NMR spectrum of *1* were of appropriate multiplicities and had chemical shift values close to those assigned to the C-1 to C-8

* For part 50 see Ref. 1.

** We have abandoned the thunberganoid nomenclature and adopted the nomenclature and structural representation recommended in a recent review on naturally occurring cembranes.[2] This representation, although advantageous in most instances, has also certain drawbacks as is evident from the illustration of the formation of the 8,12-epoxy bridged compounds in Scheme 1.

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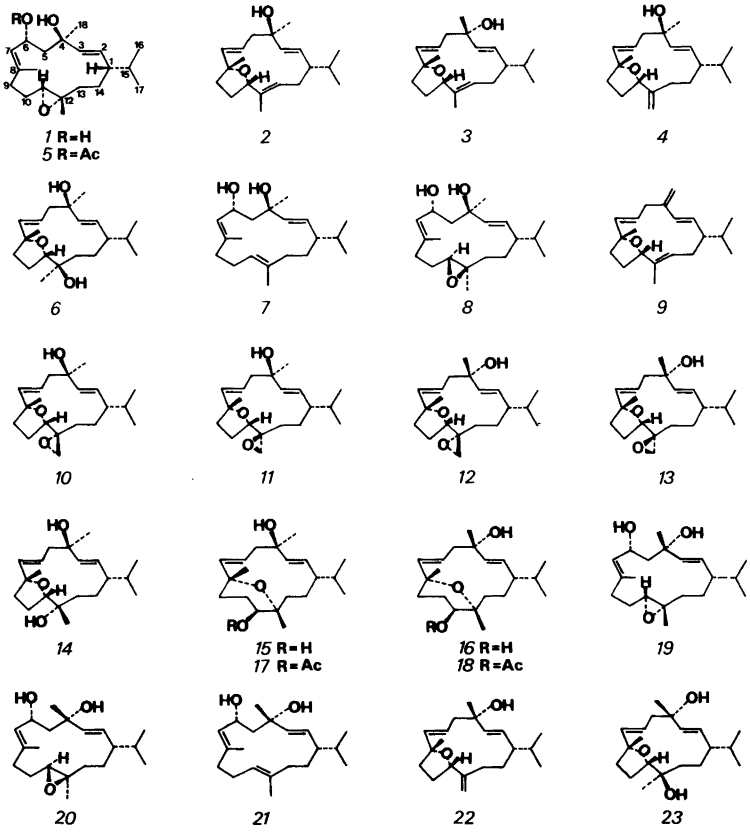


and C-13 to C-19 signals for (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*7*), corroborated this view and provisionally identified *1* as an 11,12-epoxy-2,7-cembradiene-4,6-diol.

This assignment was verified by chemical means. Thus, treatment of the 4,6-diol 7 with *m*-chloroperbenzoic acid afforded the two diastereomeric epoxides *1* and *8*, the most polar of which was

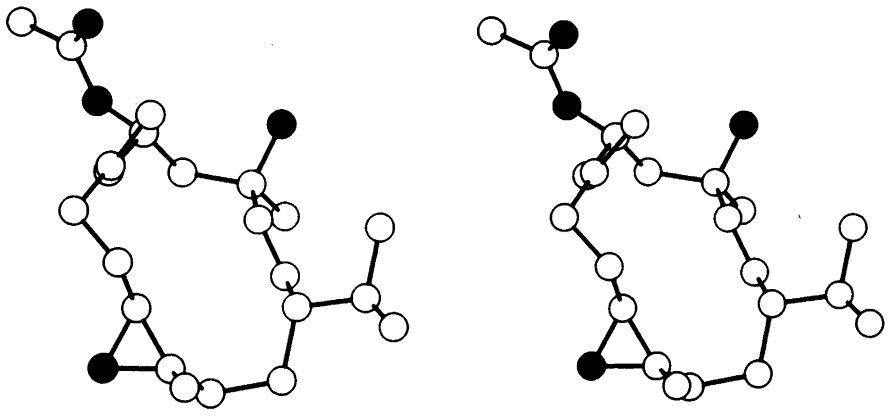


Fig. 1. Stereoscopic view of (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*S*)-6-acetoxy-11,12-epoxy-2,7-cembradien-4-ol (*5*).

identical in all respects to the new tobacco constituent. This result settled the structure and the (1*S*,2*E*,4*R*,6*R*,7*E*)-configuration but left the chiralities at C-11 and C-12 to be accounted for. An X-ray analysis of acetate *5* using a direct phase determination procedure was therefore undertaken.

Acetate *5* formed orthorhombic crystals of space group $P2_12_12_1$. The crystal data, obtained on a computer-controlled Philips PW 1100 diffractometer, were: $a = 21.501$ (3), $b = 12.882$ (3) and $c = 8.032$ (3) Å, $Z = 4$. The present R-value including anisotropic thermal parameters for all non-hydrogen atoms is 0.115, location of the hydrogen atoms and further refinement being under way. A stereoscopic view of acetate *5*, which summarizes the X-ray results and demonstrates that *5* has the 11*S*,12*S*-configuration, is shown in Fig. 1.

The second isolate from Greek tobacco was identified as (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ol (*2*). A compound of this structure has previously been reported as a constituent of flue-cured tobacco.[6] However, since its physical or spectral data were not included in that report, a brief account for our structural assignment is given here.

It followed from the ^{1}H and ^{13}C NMR spectra that *2*, $C_{20}H_{32}O_2$, contained an isopropyl group, two methyl groups linked to fully substituted oxygen-carrying carbon atoms, one vinylic methyl group and three double bonds, of which one was tri- and two disubstituted. One of the oxygen atoms was accommodated by a hydroxyl group and the other by an ether group (ν_{max} 3600 and 3400 cm^{-1}; ^{13}C NMR signals at δ 73.3 (s), 82.1 (d) and 83.0 (s)). These results inferred that *2* was a diterpenoid of the cembrane type. Moreover, a comparison of the ^{13}C NMR spectrum of *2* with that of (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ol (*3*), now isolated from Greek tobacco but previously known as a constituent of other tobacco varieties,[6-8] suggested that *2* was the 4*R*-epimer of *3*.

Conclusive evidence for this assignment was obtained by chemical means. Thus, treatment of *2* with $KHSO_4$ yielded a product, which was identical in all respects to (1*S*,2*E*,6*E*,8*R*,11*S*,12*E*)-2,4(18),6,12-cembratetraen-8,11-epoxide (*9*),[7] the major dehydration product obtained from *3*.

The isolation from Greek tobacco of the fourth compound, (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12-(20)-cembratrien-4-ol (*4*),[7,8] offered a possibility to confirm chemically the structure of another tobacco

Table 1. Carbon-13 chemical shifts and assignments for compounds *1*–*4*, *7*–*9*, *14*–*16* and *19*–*21*.[a]

Compound	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	46.8	130.4	137.1	71.4	52.4	64.4	133.0	135.4	35.7[b]	24.9	61.0	59.3	36.7[b]	28.0	33.1	20.4/19.0	28.8	16.1	16.1
2	50.0	131.9	137.9	73.3	46.3	122.7	139.6	83.0	34.1	31.1	82.1	134.7	121.8	29.2	32.2	20.6/19.9	24.4	28.5	14.2
3	50.0	129.5	138.5	73.7	45.5	123.4	140.0	83.1	34.3	30.9	82.4	134.6	122.2	29.5	32.3	20.7/19.9	29.5	28.7	14.3
4	50.5	131.5	138.1	73.6	46.5	121.1	140.2	83.1	33.5	31.7	85.7	150.0	35.7	26.9	32.0	20.7/19.8	24.6	28.6	113.0
7	46.3	130.5	136.1	71.7	52.7	64.5	131.3	136.5	38.9	23.1	124.5	133.0	36.5	27.7	33.0	20.5/19.4	28.7	15.9	15.0
8	46.4	128.9	135.7	71.3	51.1	64.7	130.4	137.8	36.0[b]	24.3	62.1	62.0	36.7[b]	26.7	32.7	19.7/19.1	29.6	15.2	16.8
9	49.9	132.1	137.6[b]	145.2	37.7	124.2	135.9[b]	82.6	34.9	31.3	81.9	135.2	121.0	29.8	32.3	20.7/20.3	112.2	27.7	14.2
14	51.4	132.7	137.5	73.3	46.1	122.5	140.2	83.5	34.0	24.7	87.5	74.8	35.7	26.9	32.4	20.8/19.9	24.7	29.5	24.7
15	51.6	131.9	139.2	73.1	47.2	119.8	143.8	74.5	37.3	23.4	67.9	78.3	24.9	26.0	31.4	20.8/20.8	24.1	32.2	25.0
16	51.4	128.5	139.5	73.1	46.2	121.1	142.9	75.6	37.3	23.5	67.8	78.4	24.8	25.9	31.5	20.8/20.6	30.8	32.3	25.3
19	47.1	127.6	138.7	72.2	53.3	65.9	133.2	134.5	35.8	25.0	61.3	60.2	36.7	28.7	33.2	20.9/19.1	30.0	16.0[b]	16.3[b]
20	45.5	127.8	136.2	72.7	49.6	66.8	130.6	136.2	37.0[b]	24.3	62.8	62.0	37.2[b]	27.0	33.1	19.4/19.3	29.2	15.1	16.2
21	46.5	127.7	137.7	72.5	52.5	66.2	131.0	135.9	38.9	23.4	124.6	133.2	36.9	28.1	33.1	20.7/19.4	30.1	16.1	15.0

[a] δ-Values in $CDCl_3$ relative to TMS. [b] Assignment may be reversed.

constituent, which had been formulated as (1*S*,2*E*,-4*R*,6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*6*) on the basis of spectral evidence.[9]

Treatment of *4* with *m*-chloroperbenzoic acid furnished two 12,20-epoxides (*10*,*11*), which were assigned the 12*R*- and 12*S*-configurations, respectively, by a comparison of their ^{1}H NMR spectra with those of the (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*R*)- and (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*S*)-8,11;12,20-diepoxy-2,6-cembradien-4-ols (*12*, *13*).[9] Thus, the most polar epoxide of each pair, *i.e.* *11* and the 4*S*,12*S*-isomer *13*, exhibited the signals due to H-20a and H-20b as one-proton doublets at δ 2.46 and 2.79 (J = 4.5 Hz) and at δ 2.48 and 2.80 (J = 4.5 Hz), respectively, whereas the corresponding signals for the least polar epoxides, *10* and the *4S*,12*R*-isomer *12*, appeared as two one-proton doublets at δ 2.65 and 2.71 (J = 5 Hz) and a two-proton singlet at δ 2.68, respectively.

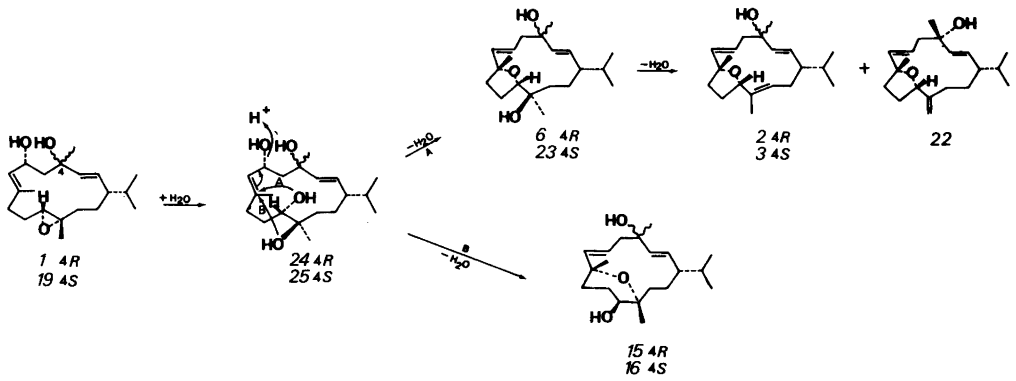
Reduction using LAH converted the 12*S*-epoxide *11* to (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diol, which proved to be identical to the tobacco diol *6*, and the 12*R*-epoxide *10* to the epimeric 4*R*,12*S*-diol *14*, which has hitherto not been encountered in nature.

The 11,12-epoxide *1* is a plausible intermediate in the biogenesis of some of the 8,11- and 8,12-epoxy bridged tobacco cembranoids from the 4,6-diol *7*. This hypothesis was reinforced experimentally by treatment of *1* with acid, which yielded as isolable products (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ol (*2*) and (1*S*,2*E*,4*R*,6*E*,8*R*,-11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*6*). A third product, as yet not known as a natural product, was also obtained. It was tentatively identified as (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*R*)-8,12-epoxy-2,6-cembradiene-4,11-diol (*15*) on the basis of the following evidence.

Its ^{13}C NMR spectrum differed from that of (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*R*)-8,12-epoxy-2,6-cembradiene-4,11-diol (*16*)[10] solely with respect to the shieldings of C-2 and C-18 (*cf.* Table 1), a result which implied that *15* is the 4*R*-epimer of *16*. This conclusion was in harmony with their ^{1}H NMR spectra, which displayed the signals due to the isopropyl group, the methyl groups at C-8 and C-12 and the proton attached to the hydroxyl-carrying carbon atom at virtually invariant positions but showed divergent chemical shift values for the signals assigned to the methyl group at C-4, 1.41 as against 1.35 ppm for *15* and *16*, respectively. An analogous correspondence was found for the ^{1}H NMR spectra of acetates *17* and *18*.

Acid rearrangement of the (11*S*,12*S*)-epoxide *19*, epimeric to *1* at C-4 and obtained together with the (11*R*,12*R*)-epoxide *20* on epoxidation of (1*S*,2*E*,-4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*21*),* gave analogous results and lead to the isolation of (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ol (*3*), (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*22*), (1*S*,2*E*,4*S*,6*E*,8*R*,-11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*23*) and (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*R*)-8,12-epoxy-2,6-cembradiene-4,11-diol (*16*), all of which are tobacco constituents.[6-11] The formation of these com-

* It should be pointed out that conclusive evidence of the assignments of the 6*R*-configurations to compounds *19*–*21*, and *25* is, as yet, not available.



Scheme 1. Proposed mechanisms for the acid-induced rearrangements of compounds *1* and *19*.

pounds may be envisaged to proceed as shown in Scheme 1.

An *anti* addition of water to the 11,12-epoxide group of the 4*R*,6*R*- and 4*S*,6*R*-diols *1* and *19* would produce the (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*R*)- and (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*R*)-2,7-cembradiene-4,6,11,-12-tetrols (*24*, *25*), which suffer an attack of the 11- or 12-hydroxyl group on the 7,8 double bond and a concomitant elimination of the hydroxyl group at C-6 to yield the 8*R*,11*S*-epoxy bridged 4*R*,12*R*- and 4*S*,12*R*-diols *6* and *23*, formal precursors of the dehydration products *2*, *3* and *22*, or the 8*R*,12*R*-epoxy bridged 4*R*,11*S*- and 4*S*,11*S*-diols *15* and *16* respectively.

EXPERIMENTAL

With the exception of accurate mass measurements, which were carried out on a Kratos MS 50-Stereo DS 50 SM/DS 50S mass spectrometer-computer system, the instruments specified in Ref. 12 were used.

Isolation. (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*S*)-11,12-Epoxy-2,7-cembradiene-4,6-diol (*1*) was isolated from fraction A 3,[13] (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ol (*3*) from fraction B 5,[13] and (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ol (*2*) and (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*4*) from fraction B 7[13] of an extract obtained from 295 kg of sun-cured Greek *Nicotiana tabacum* L. by column chromatography over silica gel followed by HPLC using columns packed with Partisil-PAC (Whatman) and μ-Bondapak/CN (Waters).

(1*S*,2*E*,4*R*,7*E*,11*S*,12*S*)-11,12-Epoxy-2,7-cembradiene-4,6-diol (*1*, 28 mg) was an oil, $[\alpha]_D$ +66.4° (*c* 1.1 $CHCl_3$) (Found: $[M-18]^{\cdot+}$ 304.2389. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR (film) bands at 3400, 1390 and 1375 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.81 (3 H, d, *J* = 6 Hz), 0.86 (3 H, d, *J* = 6 Hz), 1.20 (3 H, s), 1.38 (3 H, s), 1.72 (3 H, d, *J* = 1.3 Hz), 2.83 (1 H, dd, *J* = 2 and 7.5 Hz), 4.85 (1 H, dt, *J* = 2 and 9 Hz), 5.20 (1 H, dd, *J* = 7.5 and 16 Hz), 5.35 (1 H, d, *J* = 8 Hz) and 5.48 (1 H, d, *J* = 16 Hz); MS [*m/z* (%)]: 322 (M, 1), 304 (7), 286 (6), 261 (9), 243 (7), 233 (3), 205 (4), 163 (18), 150 (17), 136 (54), 121 (41), 107 (43), 95 (51), 81 (70), 69 (57), 55 (58) and 43 (100).

(1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*E*)-8,11-Epoxy-2,6,12-cembratrien-4-ol (*2*, 10 mg) was an oil, $[\alpha]_D$ +63.6° (*c* 0.47, $CHCl_3$); (Found: $M^{\cdot+}$ 304.2398. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$) bands at 3600, 3400, 1380 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87 (3 H, d, *J* = 6.5 Hz), 0.91 (3 H, d, *J* = 6.5 Hz), 1.29 (3 H, s), 1.31 (3 H, s), 1.49 (3 H, broad s), 4.42 (1 H, m, $W_{\frac{1}{2}}$ = 14 Hz), 5.02 (1 H, dd, *J* = 8.5 and 16 Hz), 5.43 (1 H, d, *J* = 16 Hz), 5.65 (1 H, d, *J* = 16 Hz) and 4.8–5.6 (2 H, obscured signals); MS [*m/z* (%)]: 304 (M, 1), 286 (5), 261 (1), 243 (7), 228 (3), 209 (5), 179 (6), 161 (27), 160 (30), 145 (14), 126 (45), 111 (28), 93 (29), 81 (43), 71 (38), 55 (36) and 43 (100).

(1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*E*)-8,11-Epoxy-2,6,12-cembratrien-4-ol (*3*, 70 mg) had m.p. 93–94 °C, $[\alpha]_D$ +83.8° (*c* 0.42, $CHCl_3$) (reported m.p. 95–96 °C; $[\alpha]_D$ +86°)[7] (Found: $M^{\cdot+}$ 304.2447. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR (KBr) bands at 3440, 1385 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (3 H, d, *J* = 6 Hz), 0.90 (3 H, d, *J* = 6 Hz), 1.27 (3 H, s), 1.31 (3 H, s), 4.45 (1 H, m, $W_{\frac{1}{2}}$ = 14 Hz), 5.13 (1 H, dd, *J* = 9 and 15.5 Hz), 5.42 (1 H, d, *J* = 15.5 Hz). 5.44 (1 H, m, $W_{\frac{1}{2}}$ = 11 Hz) and 5.6–5.8 (2 H, overlapping signals); MS [*m/z* (%)]: 304 (M, 1), 286 (10), 243 (10), 228 (3), 209 (8), 179 (9), 161 (55), 160 (75), 145 (28), 126 (100), 111 (46), 93 (41), 81 (71), 71 (47), 55 (48) and 43 (88).

(1*S*,2*E*,4*R*,6*E*,8*R*,11*S*)-8,11-Epoxy-2,6,12(20)-cembratrien-4-ol (*4*, 14 mg) had m.p. 103–108 °C (hexane); $[\alpha]_D$ +73.6° (*c* 0.67 $CHCl_3$) (reported m.p. 108–109 °C; $[\alpha]_D$ +72.5°);[7] IR ($CHCl_3$) bands at 3600, 3430, 1385 and 1375 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (3 H, d, *J* = 6.5 Hz), 0.89 (3 H, d, *J* = 6.5 Hz), 1.32 (3 H, s), 1.37 (3 H, s), 4.44 (1 H, m), 4.89 (1 H, m, $W_{\frac{1}{2}}$ = 4 Hz), 5.00 (1 H, m, $W_{\frac{1}{2}}$ = 4 Hz), 5.11 (1 H, dd, *J* = 9 and 16 Hz), 5.28 (1 H, dt, *J* = 6.5 and 16 Hz), 5.42 (1 H, d, *J* = 16 Hz), 5.52 (1 H, d, *J* = 16 Hz) and MS [*m/z* (%)]: 304 (M, 2), 286 (4), 271 (2), 261 (3), 243 (11), 225 (5), 209 (10), 203 (9), 185 (11), 159 (13), 133 (25), 123 (21), 109 (21), 95 (30), 81 (39), 71 (38), 55 (34) and 43 (100).

Preparation of the (1S,2E,4R,6R,7E,11S,12S) and (1S,2E,4R,6R,7E,11R,12R)-11,12-epoxy-2,7-cembradiene-4,6-diols (1 and 8). To a cooled (0 °C) solution of 226 mg (0.74 mmol) of (1*S*,2*E*,4*R*,6*R*,-7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*7*) in $CHCl_3$ was added a solution of 153 mg (0.88 mmol) of *m*-chloroperbenzoic acid in $CHCl_3$. The reaction mixture was kept at room temperature for 1 h, washed with aqueous $NaHCO_3$ and water, dried and evaporated. The residue was separated by column chromatography over silica gel and HPLC using a column packed with μ-Bondapak/CN (Waters) into two diastereomeric epoxides, the most most polar of which, (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diol (128.4 mg), gave $[\alpha]_D$, IR, ^1H NMR, ^{13}C NMR and mass spectra indistinguishable from those of the new tobacco epoxide (*1*).

The least polar epoxide, (1*S*,2*E*,4*R*,6*R*,7*E*,11*R*,-12*R*)-11,12-epoxy-2,7-cembradiene-4,6-diol (*8*, 4.0 mg) had m.p. 120–125 °C; $[\alpha]_D$ +18.8° (*c* 0.17, $CHCl_3$) (Found: $[M-18]^{\cdot+}$ 304.2411. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$) bands at 3600, 3450, 1380 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$):

δ 0.83 (3 H, d, $J=6$ Hz), 0.84 (3 H, d, $J=6$ Hz), 1.27 (3 H, s), 1.40 (3 H, s), 1.86 (3 H, broad s), 2.68 (1 H, broad d, $J=10$ Hz), 4.85 (1 H, dt, $J=3.5$ and 10 Hz), 5.36 (1 H, d, $J=8$ Hz), 5.44 (1 H, dd, $J=6$ and 16 Hz) and 5.70 (1 H, d, $J=16$ Hz); MS [*m/z* (%)]: 304 (M−18, 3), 286 (3), 263 (2), 243 (3), 203 (6), 163 (12), 136 (20), 121 (28), 109 (28), 95 (38), 81 (43), 71 (32), 55 (35) and 43 (100).

Preparation of (1S,2E,4R,6R,7E,11S,12S)-6-acetoxy-11,12-epoxy-2,7-cembradien-4-ol (5). Acetylation using acetic anhydride in pyridine converted *1* into (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*S*)-6-acetoxy-11,12-epoxy-2,7-cembradien-4-ol (*5*), which had m.p. 103−105 °C; $[\alpha]_D$ +75.0° (*c* 0.22 $CHCl_3$); IR (CCl_4) bands at 3620, 3500, 1740 and 1720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (3 H, d, $J=6$ Hz), 0.87 (3 H, d, $J=6$ Hz), 1.21 (3 H, s), 1.37 (3 H, s), 1.88 (3 H, d, $J=1$ Hz), 2.04 (3 H, s), 2.82 (1 H, dd, $J=2$ and 7.5 Hz), 5.20 (1 H, dd, $J=7.5$ and 16 Hz), 5.32 (1 H, m), 5.46 (1 H, d, $J=16$ Hz) and 5.82 (1 H, ddd, $J=2$, 7.5 and 10 Hz); MS [*m/z* (%)]: 304 (M−60, 1), 286 (3), 268 (2), 243 (3), 225 (3), 215 (1), 145 (12), 132 (26), 118 (28), 106 (45), 93 (21), 81 (44), 69 (20), 55 (28) and 43 (100).

Dehydration of the (1S,2E,4S,6E,8R,11S,12E) and (1S,2E,4R,6E,11S,12E)-8,11-epoxy-2,6,12-cembratrien-4-ols (3 and 2). To a solution of 10 mg of *3* in dioxane was added 20 mg of $KHSO_4$, and the mixture was refluxed for 6 h. Work-up and HPLC using a column packed with μ-Porasil (Waters) furnished as the major component (1*S*,2*E*,6*E*,8*R*,-11*S*,12*E*)-2,4(18),6,12-cembratetraen-8,11-epoxide (*9*, 1.6 mg), which had $[\alpha]_D$ +12.9° (*c* 0.14 $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.86 (3 H, d, $J=6$ Hz), 0.93 (3 H, d, $J=6$ Hz), 1.31 (3 H, s), 1.50 (3 H, broad s), 2.88 (2 H, t, $J=5$ Hz), 4.45 (1 H, m, $W_{\frac{1}{2}}=14$ Hz), 4.82 (1 H, m), 4.99 (1 H, m), 5.1−5.5 (3 H, overlapping signals), 5.56 (1 H, d, $J=15.5$ Hz) and 5.84 (1 H, d, $J=15.5$ Hz); MS [*m/z* (%)]: 286 (M, 7), 243 (8), 203 (8), 189 (18), 173 (19), 159 (18), 145 (72), 133 (24), 119 (28), 105 (42), 91 (48), 81 (67), 69 (29), 55 (100), 45 (58) and 43 (63).

Using the same procedure *2* (4 mg) was dehydrated to a compound (0.2 mg), which gave $[\alpha]_D$, ^1H NMR and mass spectra identical to those of (1*S*,2*E*,6*E*,8*R*,11*S*,12*E*)-2,4(18),6,12-cembratetraen-8,11-epoxide (*9*).

Preparation of the (1S,2E,4R,6E,8R,11S,12S)- and (1S,2E,4R,6E,8R,11S,12R)-8,11-epoxy-2,6-cembradiene-4,12-diols (6 and 14). To a cooled solution (0 °C) of 13.3 mg (0.044 mmol) of (1*S*,2*E*,4*R*,6*E*,8*R*,-11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*4*) in 2 ml of $CHCl_3$ was added a solution of 8.6 mg (0.050 mmol) of *m*-chloroperbenzoic acid in 1 ml of $CHCl_3$. The reaction mixture was kept at room temperature for 7 h. Work-up and HPLC using a column packed with μ-Bondapak/CN (Waters) furnished two diastereomeric epoxides, the least polar of which, (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*R*)-8,11;12,20-diepoxy-2,6-cembradien-4-ol (*10*, 1.5 mg) had $[\alpha]_D$ +11.3° (*c* 0.15 $CHCl_3$); IR ($CHCl_3$) band at 3600 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (3 H, d, $J=6.5$ Hz), 0.87 (3 H, d, $J=6.5$ Hz), 1.33 (3 H, s), 1.40 (3 H, s), 2.65 (1 H, d, $J=5$ Hz), 2.71 (1 H, d, $J=5$ Hz), 3.48 (1 H, m), 5.15 (1 H, dd, $J=8.5$ and 15 Hz), 5.32 (1 H, dt, $J=6.5$ and 15.5 Hz), 5.43 (1 H, d, $J=15$ Hz) and 5.55 (1 H, d, $J=15.5$ Hz); MS [*m/z* (%)]: 320 (M, 1), 302 (3), 284 (2), 259 (3), 241 (3), 225 (3), 207 (5), 189 (4), 161 (5), 137 (13), 121 (13), 109 (22), 95 (28), 81 (30), 71 (26), 55 (27) and 43 (100).

The most polar epoxide, (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,-12*S*)-8,11;12,20-diepoxy-2,6-cembradien-4-ol (*11*, 1.6 mg) had m.p. 135−137.5 °C; $[\alpha]_D$ +36.7° (*c* 0.24 $CHCl_3$); IR ($CHCl_3$) band at 3600 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (3 H, d, $J=6.5$ Hz), 0.86 (3 H, d, $J=6.5$ Hz), 1.34 (3 H, s), 1.38 (3 H, s), 2.46 (1 H, d, $J=4.5$ Hz), 2.79 (1 H, d, $J=4.5$ Hz), 3.52 (1 H, m), 5.10 (1 H, dd, $J=8.5$ and 15.5 Hz), 5.32 (1 H, dt, $J=6.5$ and 15.5 Hz), 5.45 (1 H, d, $J=15.5$ Hz) and 5.59 (1, H, d, $J=15.5$ Hz); MS [*m/z* (%)]: 320 (M, 1), 302 (3), 284 (2), 259 (3), 241 (4), 225 (5), 207 (5), 189 (4), 161 (6), 137 (18), 121 (19), 109 (22), 95 (30), 79 (33), 71 (27), 55 (30) and 43 (100).

A solution of 2.8 mg of *10* in ether was reacted with LAH at room temperature for 1 h. Work-up in the usual manner and purification by chromatography over silica gel afforded 0.8 mg of (1*S*,2*E*,4*R*,-6*E*,8*R*,11*S*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*14*), which was an oil and had $[\alpha]_D$ −4.0° (*c* 0.05 $CHCl_3$); (Found: $M^{\cdot+}$ 322.2548. Calc. for $C_{20}H_{34}O_3$: 322.2508); IR ($CHCl_3$) bands at 3600 and 3500 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (3 H, d, $J=6.5$ Hz), 0.90 (3 H, d, $J=6.5$ Hz), 1.12 (3 H, s), 1.28 (3 H, s), 1.34 (3 H, s), 3.89 (1 H, m), 5.19 (1 H, dd, $J=8.5$ and 15.5 Hz), 5.48 (1 H, d, $J=15.5$ Hz) and 5.5−5.7 (2 H, overlapping signals); MS [*m/z* (%)]: 304 (M−18, 8), 286 (2), 261 (4), 243 (3), 217 (3), 206 (5), 177 (12), 159 (22), 133 (17), 121 (27), 109 (26), 95 (25), 81 (29), 71 (34), 55 (28) and 43 (100).

Reduction of 2.4 mg of *11* using LAH in ether gave 2.0 mg of (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diol, which was identical ($[\alpha]_D$, IR, ^1H NMR) to tobacco diol *6*.[9]

Treatment of (1S,2E,4R,6R,7E,11S,12S)-11,12-epoxy-2,7-cembradiene-4,6-diol (1) with acid. A solution of 111 mg of *1* in 10 ml of dioxane−H_2O (2:1) and 0.5 ml of aqueous HCl (5 %) was kept at room temperature for 3 h. Work-up and chromatography over silica gel followed by HPLC using columns packed with Partisil 10 PAC (Whatman) and μ-Bondapak/CN (Waters) furnished (1*S*,2*E*,4*R*,-6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ol (*2*, 13 mg) and (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6 cembradiene-4,12-diol (*6*, 6 mg),[9] which were identified by direct comparison with authentic samples, and (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*R*)-8,12-epoxy-

2,6-cembradiene-4,11-diol (*15*), which had m.p. 179–184 °C; $[\alpha]_D$ +49.1° (*c* 0.33 $CHCl_3$) (Found: $M^{\cdot+}$ 322.2519. Calc. for $C_{20}H_{34}O_3$: 322.2508); IR ($CHCl_3$) bands at 3600 and 3450 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (3 H, d, J=6 Hz), 0.90 (3 H, d, J=6 Hz), 1.18 (6 H, s), 1.41 (3 H, s), 2.33 (2 H, m), 2.67 (1 H, m), 3.50 (1 H, m), 4.8–5.3 (2 H, overlapping signals), 5.41 (1 H, d, J=15.5 Hz) and 5.54 (1 H, d, J=16 Hz). Irradiation at the frequency of the two-proton multiplet at δ 2.33 ppm (H-5a and H-5b) converted a signal, assigned to H-6 and centered at δ 5.15, to a doublet (J=16 Hz) and uncovered a doublet of doublets (J=5.5 and 15.5 Hz) at δ 5.06 which is due to H-2; MS [*m/z* (%)]: 322 (M, 1), 304 (4), 286 (2), 261 (3), 243 (3), 227 (8), 209 (4), 197 (13), 179 (7), 161 (15), 139 (17), 121 (36), 109 (27), 95 (25), 81 (27), 71 (37), 55 (19) and 43 (100).

Preparation of (1S,2E,4R,6E,8R,11S,12R)-11-acetoxy-8,12-epoxy-2,6-cembradien-4-ol (17). Acetylation using acetic anhydride in pyridine converted *15* into (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*R*)-11-acetoxy-8,12-epoxy-2,6-cembradien-4-ol (*17*) which had IR ($CHCl_3$) bands at 3600, 1725 and 1220 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.85 (3 H, d, J=6.5 Hz), 0.89 (3 H, d, J=6.5 Hz), 1.08 (3 H, s), 1.18 (3 H, s), 1.40 (3 H, s), 2.11 (3 H, s), 2.33 (2 H, m), 2.74 (1 H, m), 4.80 (1 H, m), 4.9–5.3 (2 H, overlapping signals; pattern virtually identical to that in the spectrum of *15*), 5.40 (1 H, d, J=15.5 Hz) and 5.54 (1 H, d, J=16 Hz); MS [*m/z* (%)]: 346 (M-18, 1), 304 (1), 286 (2), 268 (1), 243 (4), 225 (2), 159 (9), 145 (10), 119 (17), 106 (26), 93 (51), 81 (16), 71 (15), 55 (16) and 43 (100).

Preparation of the (1S,2E,4S,6R,7E,11R,12R)- and (1S,2E,4S,6R,7E,11S,12S)-11,12-epoxy-2,7-cembradiene-4,6-diols (20 and 19). Treatment of 330 mg (1.08 mmol) of (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*21*) with 220 mg (1.28 mmol) of *m*-chloroperbenzoic acid in $CHCl_3$ afforded, after work-up and chromatography, two diastereomeric 11,12-epoxides. The least polar of these, (1*S*,2*E*,4*S*,6*R*,7*E*,11*R*,12*R*)-11,12-epoxy-2,7-cembradiene-4,6-diol (*20*, 16 mg) had m.p. 105–107 °C; $[\alpha]_D$ +28.7° (*c* 0.62, $CHCl_3$); (Found: $[M-18]^{\cdot+}$ 304.2386. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR (KBr) band at 3350 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (6H, d, J=6.5 Hz), 1.23 (3 H, s), 1.27 (3 H, s), 1.79 (3 H, broad s), 2.61 (1 H, broad d, J=11 Hz), 4.67 (1 H, m), 5.48 (1 H, d, J=9 Hz) and 5.6–5.9 (2 H, overlapping signals); MS [*m/z* (%)]: 322 (M, 1), 304 (6), 286 (8), 261 (4), 243 (9), 225 (5), 215 (3), 203 (4), 177 (6), 159 (15), 145 (19), 123 (34), 107 (38), 95 (49), 81 (71), 69 (37), 55 (52) and 43 (100).

The most polar epoxide, (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diol (*19*, 117 mg) had m.p. 99–103 °C (reported 103–105 °C);[7] $[\alpha]_D$ +113.9° (*c* 0.62, $CHCl_3$); (Found: $M^{\cdot+}$ 322.2510. Calc. for $C_{20}H_{34}O_3$: 322.2508); IR (KBr) band at 3430 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80 (3 H, d, J=6 Hz), 0.86 (3 H, d, J=6 Hz), 1.20 (3 H, s), 1.37 (3 H, s), 1.78 (3 H, d, J=1 Hz), 2.88 (1 H, dd, J=2.5 and 7 Hz), 4.48 (1 H, dt, J=3 and 8.5 Hz), 5.2–5.5 (3 H, overlapping signals); MS [*m/z* (%)]: 322 (M, 1), 304 (6), 286 (6), 261 (10), 243 (14), 203 (5), 177 (9), 163 (21), 136 (42), 123 (60), 109 (58), 95 (80), 81 (94), 69 (66), 55 (72) and 43 (100).

Treatment of (1S,2E,4S,6R,7E,11S,12S)-11,12-epoxy-2,7-cembradiene-4,6-diol (19) with acid. A solution of 78 mg of *19* in 5 ml of dioxane–H_2O (2:1) and 1 ml of aqueous HCl (5 %) was kept at room temperature for 6 h. Work-up and chromatography over silica gel gave a series of products of which (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ol (*3*, 2.0 mg), (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*22*, 0.8 mg), (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*23*, 5.4 mg) and (1*S*,2*E*,4*S*,6*E*,8*R*,-11*S*,12*R*)-8,12-epoxy-2,6-cembradiene-4,11-diol (*16*, 5.4 mg) were identified by direct comparison with the corresponding authentic samples.[6–11]

16 was an oil; $[\alpha]_D$ +81.9 (*c* 0.32, $CHCl_3$); IR (film) bands at 3400, 1390 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.86 (3 H, d, J=6 Hz), 0.89 (3 H, d, J=6 Hz), 1.18 (6 H, s), 1.35 (3 H, s), 3.03 (1 H, m), 3.54 (1 H, m), 5.12 (1 H, dd, J=8 and 16 Hz), 5.33 (1 H, d, J=16 Hz) and 5.4–5.5 (2 H, overlapping signals); MS [*m/z* (%)]: 322 (M, 1), 304 (7), 286 (2), 261 (4), 243 (3), 227 (9), 197 (14), 161 (18), 139 (17), 121 (38), 109 (27), 95 (24), 81 (33), 71 (40), 55 (22) and 43 (100).

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