Tobacco Chemistry. 72.* Five New Cembratrienetriols from Tobacco

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Five new diterpenoids of the cembrane class have been isolated from flowers of Greek tobacco. They have been identified as (1*S*,2*E*,4*S*,6*E*,8*S*,11*S*)-2,6,12(20)-cembratriene-4,8,11-triol (**1**), the 12*S*- and 12*R*-epimers of (1*S*,2*E*,4*S*,6*E*,8*S*,10*E*)-2,6,10-cembratriene-4,8,12-triol (**2**, **3**) and the 12*S*- and 12*R*-epimers of (1*S*,2*E*,4*R*,6*E*,8*S*,10*E*)-2,6,10-cembratriene-4,8,12-triol (**4**, **5**) by spectral methods and biomimetic syntheses. The biogenesis of the new compounds is discussed.

The cembranic diterpenoids present in the cuticular wax of the leaf and flower of tobacco include as major components the two 2,7,11-cembratriene-4,6-diols **6** and **7**. As suggested by results from biomimetic experiments, these two diols are the principal precursors of most of the other tobacco cembranoids.[2] We now report the isolation, from an extract of flowers of Greek tobacco, of five new cembratrienetriols (**1–5**), which are all plausible metabolites of the 4,6-diols **6** and **7**.

Results

Structure determination. The first new compound (**1**), $C_{20}H_{34}O_3$, has three double bonds of which one is 1,1-disubstituted [IR band at 3085 cm^{-1}; ^{13}C NMR: δ 110.3 (t) and 150.8 (s)] and two are 1,2-disubstituted [^{13}C NMR: δ 123.5(d), 129.4 (d), 138.1 (d) and 139.2 (d)]. The oxygen atoms are accommodated by one secondary and two tertiary hydroxy groups [OH-absorption in the IR spectrum; ^1H NMR signal at δ 4.27; ^{13}C NMR signals at δ 73.1 (s), 73.3 (s) and 74.0 (d)]. These results demonstrated that triol **1** is carbomonocyclic.

The presence of an isopropyl group and two methyl groups that are attached to the fully substituted carbon atoms carrying the tertiary hydroxy groups (methyl doublets at δ 0.86 and 0.88 and methyl singlets at δ 1.26 and 1.32 in the ^1H NMR spectrum) suggested that the triol **1** is a diterpenoid of the cembrane class.

This proposion was supported, and the triol **1** was tentatively formulated as a 2,6,12(20)-cembratriene-4,8,11-triol isomeric to the known (4*R*,8*S*,11*S*)-triol **8**[3] with further use of spectral data. Thus, eighteen signals in the ^{13}C NMR spectrum of the triol **1** were of appropriate multiplicities and had chemical shift values close to those of the C-1, C-3 to C-17, C-19 and C-20 signals for the (4*R*,8*S*,11*S*)-triol **8** (cf. Table 1). Provided that triols **1** and **8** are conformationally similar, this result is only consistent with the triol **1** having an 8*S*,11*S*-configuration and being the 4*S*-epimer of **8**.

This assignment was readily verified by a biomimetic type of synthesis involving photooxygenation, Rose Bengal being used as the sensitizer. The (4*S*,8*S*)-diol **9**,[4,5] a tobacco constituent of relevant stereochemistry, was chosen as the starting material. As expected, the reaction proceeded with an attack of singlet oxygen on the trisubstituted 11,12-double bond.[3] After reduction of the hydroperoxides initially formed using triethyl phosphite, five triols were isolated. The major one was identical in all respects with the first new triol (**1**), hence confirming that this has the 1*S*,2*E*,4*S*,6*E*,8*S*,11*S*-configuration. A 2,6,12(20)-cembratriene-4,8,11-triol (no stereochemistry given) has previously been isolated from tobacco, and its use as a flavour additive to tobacco has been patented. The physical and spectral data reported in the patent for this compound agree well with those of triol **1**.[6]

One of the minor products (**10**) was identified as the 11*R*-epimer of **1**. Its ^1H NMR spectrum displayed the H-11 signal as a triplet at δ 4.09 and the H-20a and H-20b signals as broad singlets at δ 4.87 and 4.98. Furthermore, the shieldings of C-11 and C-20, δ 76.6 and 111.3, respectively, for **10** as against δ 74.0 and 110.3 for **1** and δ 74.2 and 110.4 for **8** were consistent with triol **10** having an 11*R*-configuration.

The (1*S*,2*E*,4*S*,6*E*,8*S*,10*E*)-2,6,10-cembratriene-4,8,12-triols **2** and **3**, expected *syn*-ene products[3] and identical with two of the new tobacco isolates, were also obtained. Their ^1H and ^{13}C NMR spectra were consistent with the presence of three methyl groups each attached to a fully substituted carbon atom also carrying a tertiary hydroxy group. The magnitudes of the relevant vicinal coupling constants, which could be measured for triol **2**, were used to confirm that all three 1,2-disubstituted double bonds have *E*-geometries.

*For part 71, see Ref. 1.

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Table 1. ^{13}C NMR chemical shift values and assignments for compounds **1–5**, **8**, **10**, **14**, **16** and **17**.[a]

Compound	Carbon																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	49.9	129.4	138.1	73.1[b]	45.3	123.5	139.2	73.3[b]	36.3	28.6	74.0	150.8	29.8	29.3	32.4	19.6	20.6	30.7	29.9	110.3
2	49.0	131.3	136.3	72.8[b]	44.8[c]	124.4[d]	139.0[e]	72.9[b]	45.6[c]	124.5[d]	139.2[e]	72.9[b]	37.2	28.2	30.6	20.7	20.9	28.4[f]	29.2[f]	30.3[f]
3	48.7	130.9	136.3	72.5[b]	45.2[c]	124.3[d]	139.2	72.7[b]	45.3[c]	124.8[d]	139.2	73.0[b]	37.6	28.1	30.7	20.3	20.5	29.8	28.5	29.0
4	48.6	132.2	135.8	72.7[b]	45.1[c]	124.1[d]	139.1[e]	72.8[b]	45.3[c]	124.7[d]	139.4[e]	73.1[b]	38.4	27.3	31.0	20.3	20.4	28.0[f]	28.7[f]	29.4[f]
5	48.5	131.4	136.1	72.7[b]	45.2[c]	124.2[d]	139.1[e]	72.8[b]	45.4[c]	124.8[d]	139.2[e]	72.9[b]	39.8	27.7	31.5	19.9	20.5	28.5	28.1	27.9
8	49.5	131.1	137.5	73.3	46.2	123.8	139.2	73.1	36.8	28.9	74.2	150.8	29.6	28.6	32.3	19.6	20.4	26.3	29.3	110.4
10	49.2	130.2	138.2	73.0[b]	45.7	123.6	139.3	73.2[b]	37.0	29.4	76.6	151.4	29.1	31.2	32.2	19.7	20.6	30.7	30.6	111.3
14	50.0	129.6	138.3	73.1[b]	45.2	123.8	139.2	73.2[b]	32.2	38.8	75.9	136.8	125.0	30.0	32.7	19.5	20.7	30.8	28.3	15.4
16	49.3	131.7	137.3	73.3[b]	46.3	123.7	139.3	72.9[b]	37.2	28.8	76.3	151.1	29.4	30.7	32.1	19.8	20.4	26.4	30.1	111.8
17	49.8	131.3	137.7	72.9[b]	45.9	124.2	138.9	73.0[b]	32.3	38.6	75.9	137.0	124.9	29.5	32.5	19.6	20.4	26.7	27.9	15.7

[a]δ values in $CDCl_3$ relative to Me_4Si. [b–f]Assignment may be interchanged.

Ozonolytic degradation was applied to resolve the chiralities of C-12 in triols **2** and **3**. Thus, treatment of triol **2** with ozone followed by reductive work-up gave a product that was indistinguishable from an authentic sample of (2*S*,5*S*)-5-hydroxymethyl-2,6-dimethyl-1,2-heptanediol (**11**) obtained by ozonolytic degradation of the (4*S*,6*R*,12*S*)-triol **12**.[3,7] Triol **3** gave rise to a 5-hydroxymethyl-2,6-dimethyl-1,2-heptanediol (**13**), which, as concluded from the spectral data, was isomeric with **11**. These results are only consistent with a 12*S*-configuration in the triol **2** and 12*R*- and 2*R*-configurations in triols **3** and **13**, respectively.

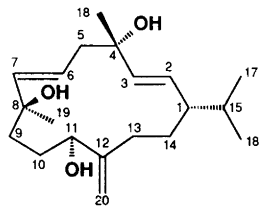
The fifth product (**14**), obtained via photo-oxygenation of the (4*S*,8*S*)-diol **9**, was identified as a 2,6,12-cembratriene-4,8,11-triol from its spectral data, 2D NMR being a particularly useful tool. H-11 resonates as a multiplet at δ 4.01, H-13 as a broad triplet at δ 5.36 and H-20 as a narrowly split three-proton doublet at δ 1.68. C-20 gives rise to a signal at δ 15.4, a value consistent with an *E*-geometry of the 12,13-double bond.[8] The configuration at C-11 remains unsettled.

The remaining two tobacco isolates (**4**, **5**, both $C_{20}H_{34}O_3$) gave rise to ^1H and ^{13}C NMR spectra reminiscent of those of the two (4*S*,8*S*,12*S*)- and (4*S*,8*S*,12*R*)-triols **2** and **3**; each has three 1,2-disubstituted double bonds, which were confirmed by analysis of the ^1H NMR spectrum of triol **5** to have *E*-geometries, and three methyl groups each attached to a fully substituted carbon atom also linked to a tertiary hydroxy group. They were hence identified as 4,8,12-triols. Since the (4*R*,8*S*)-diol **15** is a tobacco constituent,[4,5] it seemed most likely from a biogenetic point of view that triols **4** and **5** have 4*R*,8*S*-configurations and differ with respect to the chirality of C-12.

To validate this suggestion, the diol **15** was treated with singlet oxygen. The hydroperoxides formed were reduced to give, as in the case of the (4*S*,8*S*)-diol **9**, five triols. Two of these proved to be 4,8,12-triols identical with the new tobacco isolates **4** and **5**. Ozonolytic degradation of the triol **4** gave a product indistinguishable from (2*S*,5*S*)-5-hydroxymethyl-2,6-dimethyl-1,2-heptanediol (**11**). These results allowed the identification of triols **4** and **5** as the 12*S*- and 12*R*-epimers of (1*S*,2*E*,4*R*,6*E*,8*S*,10*E*)-2,6,10-cembratriene-4,8,12-triol, respectively.

The major product obtained from the (4*R*,8*S*)-diol **15** via sensitized photo-oxygenation was identical with the (4*R*,8*S*,11*S*)-triol **8**, previously isolated from tobacco.[3] The fourth product (**16**) was identified as the 11*R*-epimer of **8**, the shieldings of C-11 and C-20, δ 76.3 and 111.8, respectively, thereby being diagnostic (cf. Table 1).

The remaining product (**17**) was assigned a (1*S*,2*E*,4*R*,6*E*,8*S*,12*E*)-2,6,12-cembratriene-4,8,11-triol structure from its spectral data. A multiplet at δ 3.96 was assigned to H-11, a broad triplet at δ 5.34 to H-13 and a three-proton doublet at δ 1.69 to H-20. A comparison of the ^{13}C NMR spectra of triols **14** and **17** proved informative and suggested that both compounds have the same configuration at C-11 and differ solely with respect to the configuration at C-4. Thus, while the signals due to C-2 and C-18 show



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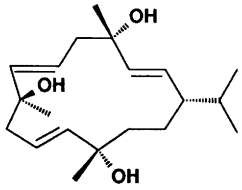

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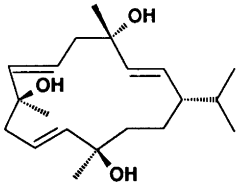

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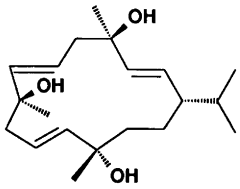

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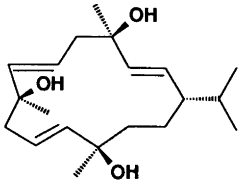

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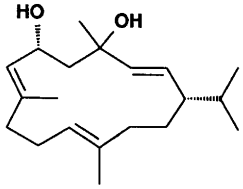
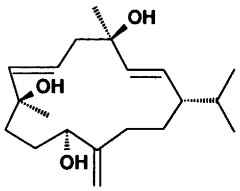


6 4S
7 4R



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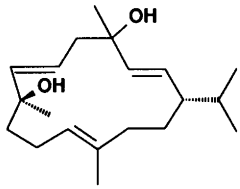
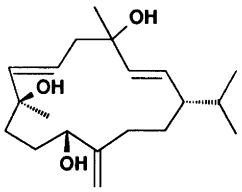
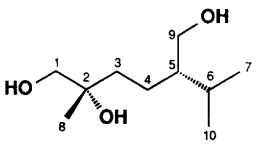


9 4S
15 4R



10 4S
16 4S



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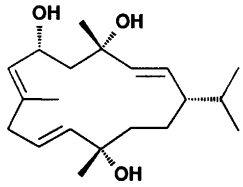

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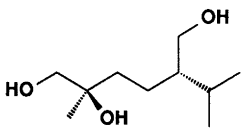

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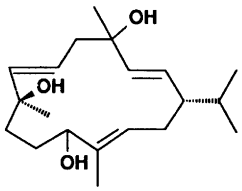
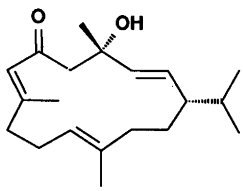


14 4S
17 4R



18

divergent chemical shift values, all other signals are present at virtually invariant positions in the spectra of the triols (Table 1).

The outcome of the photo-oxygenation reactions is summarized in Table 2. It can be seen that the formation of the *syn*-ene products, which involves hydrogen abstraction from the 1,2-disubstituted side of the trisubstituted 11,12-double bond, is highly favoured over the formation of the *anti*-ene products, 97:3 in both the 4*S*- and the 4*R*-series. No *anti*-ene products were detected when the 4,6-diols **6** and **7** or the 6-oxo compound **18** were treated with singlet oxygen.[3,9] These results concur with previous findings for acyclic and most cyclic compounds, cyclohexenes being exceptions.[10–12]

Of the *syn*-ene products, the 4,8,11-triols are formed in preference to the 4,8,12-triols, the ratio being 66:31 in the 4*S*-series and 51:46 in the 4*R*-series. If corrected for the number of hydrogen atoms available at each site, those at C-10 and those at C-20 then appear to have roughly equal reactivities. Similar results were obtained for the 4,6-diols **6** and **7**, while in the case of the 6-oxo compound **18**, the hydrogen atoms at C-10 are more reactive than those at C-20.[3,9]

Both the 4*S*,8*S*- and 4*R*,8*S*-diols **9** and **15** react with singlet oxygen with a certain stereoselectivity, the 11*S*- and 12*S*-triols being formed in preference to their 11*R*- and 12*R*-counterparts; the ratio is 79:18 in the 4*S*-series and 73:24 in the 4*R*-series. Higher stereoselectivities were observed for the 4,6-diols **6** and **7** and the 6-oxo compound **18** (Table 2).[3,9]

Biogenesis. As proposed in Scheme 1, there are two plausible routes for the formation of the new 4,8,11- and 4,8,12-triols **1–5** in tobacco. Both of these originate from the

Table 2. Relative yields, as determined by integration of the HPLC traces, of the products obtained by sensitized photo-oxygenation of the 4,8-diols **9** and **15**, the 4,6-diols **6** and **7**, and the 6-oxo compound **18** and subsequent reduction.

Starting material	*syn*-Ene product (%)				*anti*-Ene product (%)
	11*S*	11*R*	12*S*	12*R*	11ζ
9	57	9	22	9	3
15	43	8	30	16	3
6[3]	63	1	31	5	–
7[3]	58	2	31	9	–
18[9]	33	4[a]	60	3	–

[a]Undergoes spontaneous cyclization.

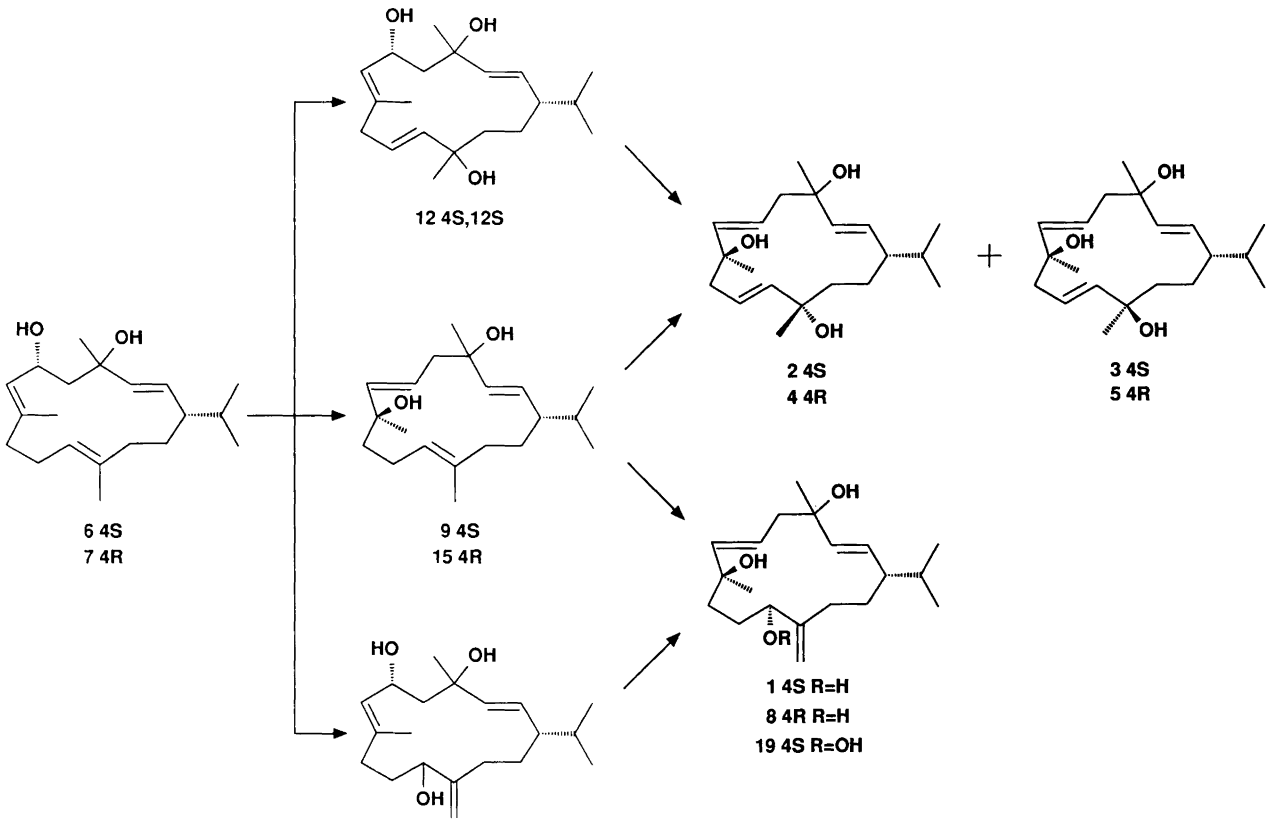
4,6-diols **6** and **7**. One involves an acid-induced conversion of **6** and **7** into the 4,8*S*-diols **9** and **15** and subsequent oxygenation of the 11,12-double bond by the action of singlet oxygen or with the assistance of an appropriate enzyme. Alternatively, the 4,6-diols **6** and **7** are initially converted into 4,6,11- and 4,6,12-triols (e.g. **12**), which then undergo the prerequisite allylic rearrangement.

The validity of these pathways is reinforced by results of biomimetric studies involving photo-oxygenation of the 4,8*S*-diols **9** and **15** (*vide supra*) and the 4,6*R*-diols **6** and **7**[3] and acid-induced transformations of the latter two diols.[5] Moreover, the (4*S*,8*S*)- and (4*R*,8*S*)-diols **9** and **15** as well as some of the 4,6,11- and 4,6,12-triols, are present in a fair amount in tobacco.[3-5,7] It is also noteworthy that the 11-hydroperoxide **19**, a plausible intermediate in the formation of the (4*S*,8*S*,11*S*)-triol **1**, has recently been detected in tobacco as an inhibitor of indole-3-acetic acid.[13] Its use as a flavour additive to tobacco has been patented.[14]

Experimental

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter, IR spectra on a Perkin-Elmer FT-IR 1725X spectrometer, some of the ^{1}H NMR spectra on a Bruker 400 MHz instrument and part of the high performance liquid chromatography work was carried out using a Waters Delta Prep 3000 solvent delivery system, a Waters U6K injector and a Waters R-403 differential refractometer. For other instrumental details see Ref. 15.

Isolation. Fraction D (128 g), obtained from a extract of flowers of Greek *Nicotiana tabacum* (Basma), was separated by flash chromatography over silica gel into 8 fractions, D1–D8. Fraction D4 (17.1 g) was separated by HPLC (Spherisorb 5, EtOAc) into 5 fractions. Of these, fraction D43 (3.6 g) was separated by HPLC (Spherisorb 5CN, hexane/EtOAc 60:40) into 10 fractions. Repetitive HPLC of fraction D435 (577 mg) (Lichrosorb Diol, hexane/



Scheme 1. Proposed biogenesis of compounds **1–5**.

EtOAc 30:70; Spherisorb 5, hexane/EtOAc 20:80) gave 9.7 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*S*)-2,6,12(20)-cembratriene-4,8,11-triol (**1**). Further separation of fraction D433 (84 mg) by HPLC (Lichrosorb Diol, hexane/EtOAc 40:60; Spherisorb 5, hexane/EtOAc 20:80) gave 3.5 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,10*E*,12*R*)-2,6,10-cembratriene-4,8,12-triol (**3**) and 1.7 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,10*E*,12*S*)-2,6,10-cembratriene-4,8,12-triol (**4**).

Fraction D7 (10.5 g) was separated into 8 fractions by HPLC (Spherisorb 5, EtOAc). Repetitive HPLC of fraction D72 (0.8 g) (Spherisorb 5CN, hexane/EtOAc 1:1; Lichrosorb Diol, hexane/EtOAc 20:80) afforded 4.6 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,10*E*,12*R*)-2,6,10-cembratriene-4,8,12-triol (**5**). Further separation of fraction D74 (1.0 g) by HPLC (Lichrosorb Diol, hexane/EtOAc 10:90; Spherisorb 5CN, hexane/EtOAc 1:1) gave 4.7 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,10*E*,12*S*)-2,6,10-cembratriene-4,8,12-triol (**2**).

(1*S*,2*E*,4*S*,6*E*,8*S*,11*S*)-2,6,12(20)-Cembratriene-4,8,11-triol (**1**) had m.p. 137.0–138.5 °C; $[\alpha]_D$ +66° (*c* 0.65, $CHCl_3$); (Found: $[M-18]^{+\cdot}$ 304.2394. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$): 3601, 3431, 3085, 1646, 1386, 1371 and 979 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (d, *J* 6.8 Hz)/0.88 (d, *J* 7.0 Hz) (H-16/H-17), 1.26 (s, H-19), 1.32 (s, H-18), 2.22 (dd, *J* 8.6 and −14.0 Hz, H-5a), 2.37 (ddd, *J* 1.3, 5.3 and −14.0 Hz, H-5b), 4.27 (m, H-11), 4.87 (br s, H-20a), 4.98 (br s, H-20b), 5.2–5.4 (overlapping signals, H-2 and H-3), 5.54 (dd, *J* 1.3 and 15.7 Hz, H-7) and 5.66 (ddd, *J* 5.3, 8.6 and 15.7 Hz, H-6); MS [*m/z* (%)]: 304 (0.2, *M*−18), 286 (1), 268 (0.7), 243 (2), 225 (2), 203 (2), 185 (3), 173 (3), 159 (5), 145 (8), 133 (10), 119 (11), 105 (17), 91 (21), 81 (29), 69 (25), 55 (34) and 43 (100).

(1*S*,2*E*,4*S*,6*E*,8*S*,10*E*,12*S*)-2,6,10-Cembratriene-4,8,12-triol (**2**) had m.p. 137.0–141.0 °C; $[\alpha]_D$ +38° (*c* 0.39, $CHCl_3$); (Found: $[M-36]^{+\cdot}$ 286.2292. Calc. for $C_{20}H_{30}O$: 286.2296); IR ($CHCl_3$): 3598, 3430 and 978 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (d, *J* 6.6 Hz)/0.90 (d, *J* 6.6 Hz) (H-16/H-17), 1.22 (s)/1.26 (s)/1.31 (s) (H-18/H-19/H-20), 2.26 (dd, *J* 8.0 and −14.0 Hz)/2.28 (dd, *J* 6.4 and −13.2 Hz) (H-5a/H-9a), 2.30 (dd, *J* 6.4 and −13.2 Hz)/2.31 (dd, *J* 6.5 and −14.0 Hz) (H-5b/H-9b), 5.40 (d, *J* 15.5 Hz, H-3), 5.50 (d, *J* 15.9 Hz)/5.56 (d, *J* 16.0 Hz) (H-7/H-11), 5.50 (ddd, *J* 6.4, 6.4 and 16.0 Hz)/5.55 (ddd, *J* 6.5, 8.0 and 15.9 Hz) (H-6/H-10) and 5.60 (dd, *J* 9.1 and 15.5 Hz, H-2); MS [*m/z*(%)]: 286 (0.7, *M*−36), 268 (0.5), 243 (1), 221 (1), 203 (2), 177 (3), 162 (4), 145 (6), 133 (9), 126 (19), 119 (10), 109 (12), 95 (30), 81 (24), 69 (34), 55 (25) and 43 (100).

(1*S*,2*E*,4*S*,6*E*,8*S*,10*E*,12*R*)-2,6,10-Cembratriene-4,8,12-triol (**3**) had m.p. 141.5–142.0 °C; $[\alpha]_D$ +36° (*c* 0.35, $CHCl_3$); (Found: $[M-36]^{+\cdot}$ 286.2294. Calc. for $C_{20}H_{30}O$: 286.2296); IR ($CHCl_3$): 3599, 3452, 1666 and 979 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (d, *J* 6.7 Hz)/0.89 (d, *J* 6.6 Hz) (H-16/H-17), 1.25 (s, H-20), 1.28 (s, H-19), 1.31 (s, H-18), 2.2–2.4 (overlapping signals, H-5a, H-5b, H-9a and H-9b), 5.41 (d, *J* 15.6 Hz, H-3), 5.54 (dd, *J* 8.9 and 15.6 Hz, H-2) and 5.4–5.6 (overlapping signals, H-6, H-7, H-10 and H-11); MS [*m/z* (%)]: 304 (0.1, *M*−18), 286 (1), 268 (3), 243 (2), 225 (3), 203 (2), 183 (4), 177 (4), 169 (6), 157 (6), 145 (14), 133 (16), 126 (27), 119 (18), 105 (24), 95 (45), 81 (37), 69 (47), 55 (39) and 43 (100).

(1*S*,2*E*,4*R*,6*E*,8*S*,10*E*,12*S*)-2,6,10-Cembratriene-4,8,12-triol (**4**) had m.p. 146.5–148.5 °C; $[\alpha]_D$ +34° (*c* 0.29, $CHCl_3$); (Found: $[M-18]^{+\cdot}$ 304.2387. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$): 3600, 3437, 1665 and 979 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (d, *J* 6.5 Hz)/0.87 (d, *J* 6.4 Hz) (H-16/H-17), 1.25 (s)/1.28 (s)/1.36 (s) (H-18/H-19/H-20), 2.20–2.50 (overlapping signals, H-5a, H-5b, H-9a and H-9b) and 5.35–5.65 (overlapping signals, H-2, H-3, H-6, H-7, H-10 and H-11); MS [*m/z* (%)]: 304 (0.1, *M*−18), 286 (1), 268 (2), 243 (1), 225 (3), 203 (2), 183 (4), 169 (6), 157 (6), 145 (12), 133 (12), 126 (14), 119 (16), 105 (24), 91 (33), 79 (33), 69 (50), 55 (41) and 43 (100).

(1*S*,2*E*,4*R*,6*E*,8*S*,10*E*,12*R*)-2,6-10-Cembratriene-4,8,12-triol (**5**) had m.p. 157.5–158.5 °C; $[\alpha]_D$ +17° (*c* 0.46, $CHCl_3$); (Found: $[M-36]^{+\cdot}$ 286.2282. Calc. for $C_{20}H_{30}O$: 286.2296); IR ($CHCl_3$): 3599, 3424 and 979 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (d, *J* 6.7 Hz)/0.87 (d, *J* 6.7 Hz) (H-16/H-17), 1.24 (s, H-20), 1.32 (s, H-19), 1.34 (s, H-18), 2.30 (dd, *J* 6.4 and −14.0 Hz, H-5a), 2.32 (dd, *J* 8.2 and −13.8 Hz, H-9a), 2.37 (dd, *J* 5.5 and −13.8 Hz, H-9b), 2.40 (dd, *J* 6.4 and −14.0 Hz, H-5b), 5.38 (dd, *J* 7.7 and 16.0 Hz, H-2), 5.45 (d, *J* 16.0 Hz, H-3), 5.52 (d, *J* 15.8 Hz, H-7), 5.53 (ddd, *J* 5.5, 8.2 and 15.8 Hz, H-10), 5.56 (ddd *J* 6.4, 6.4 and 15.8 Hz, H-6) and 5.62 (d, *J* 15.8 Hz, H-11); MS [*m/z* (%)]: 286 (0.8, *M*−36), 268 (2), 243 (1), 225 (2), 203 (2), 183 (2), 169 (3), 159 (4), 145 (7), 133 (9), 126 (16), 105 (12), 95 (25), 81 (20), 69 (31), 55 (21) and 43 (100).

*Photo-oxygenation of (1S,2E,4S,6E,8S,11E)-2,6,11-cembratriene-4,8-diol (**9**).* A solution of 158 mg of **9**[4,5] and 10 mg of Rose Bengal in 25 ml of MeOH in a tube cooled with a water jacket was irradiated with a 400 W sodium high pressure lamp, placed outside of the tube, while oxygen was bubbled through the reaction mixture. After 1.5 h, when TLC showed that all of the starting material had been consumed, 300 μl of triethyl phosphite were added, and the reaction mixture was kept at room temperature for 1 h. The solvent was removed under reduced pressure, and the residue was filtered through alumina using EtOAc and EtOAc/MeOH (90:10) as the solvents. Subsequent separation by HPLC (Spherisorb 5 CN, hexane/EtOAc 1:1; Spherisorb 5, hexane/EtOAc 20:80) gave 8.8 mg of (1*S*,2*E*,4*S*,6*E*,8*S*, 10*E*,12*R*)-2,6,10-cembratriene-4,8,12-triol (**3**), 14 mg of the corresponding 12*S*-epimer (**2**), 50 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*S*)-2,6,12(20)-cembratriene-4,8,11-triol (**1**), 6.8 mg of the corresponding 11*R*-epimer (**10**) and 2.2 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11ζ,12*E*)-2,6,12-cembratriene-4,8,11-triol (**14**). The product ratio of **1**:**10**:**2**:**3**:**14** was measured to be 57:9:22:9:3 by integration of the HPLC traces (RI-detection).

Of these, (1*S*,2*E*,4*S*,6*E*,8*S*,11*S*)-2,6,12(20)-cembratriene-4,8,11-triol, (1*S*,2*E*,4*S*,6*E*,8*S*,10*E*,12*S*)-2,6,10-cembratriene-4,8,12-triol and the 12*R*-epimer thereof were identical (m.p., optical rotation, IR, ^1H NMR and mass spectra) with the new triols **1**, **2** and **3**, respectively.

(1*S*,2*E*,4*S*,6*E*,8*S*,11*R*)-2,6,12(20)-Cembratriene-4,8,11-triol (**10**) had m.p. 104.0–107.0 °C; $[\alpha]_D$ +51° (*c* 0.68, $CHCl_3$); IR ($CHCl_3$): 3602, 3420, 3074, 1646, 1386, 1371 and 980 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (d, *J* 6.6 Hz)/0.87 (d *J* 6.6 Hz) (H-16/H-17), 1.26 (s, H-19), 1.31 (s, H-18), 2.24 (ddd, *J* 0.7, 8.9 and −13.8 Hz, H-5a), 2.36 (ddd, *J* 1.5, 5.6 and −13.8 Hz, H-5b), 4.09 (t, *J* 6.2 Hz, H-11), 4.87 (br s, H-20a), 4.98 (br s, H-20b), 5.3–5.4 (overlapping signals, H-2 and H-3), 5.45 (ddd, *J* 0.7, 1.5 and 15.7 Hz, H-7) and 5.64 (ddd, *J* 5.6, 8.9 and 15.7 Hz, H-6); MS [*m/z* (%)]: 286 (1, *M*−36), 261 (1), 243 (4), 225 (2), 203 (3), 185 (5), 161 (5), 147 (10), 133 (16), 123 (13), 105 (16), 95 (24), 81 (30), 71 (34), 55 (28) and 43 (100).

(1*S*,2*E*,4*S*,6*E*,8*S*,11ζ,12*E*)-2,6,12-Cembratriene-4,8,11-triol (**14**), had m.p. 130.0–132.0 °C; $[\alpha]_D$ +50° (*c* 0.21, $CHCl_3$); IR ($CHCl_3$): 3600, 3453 and 978 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (d, *J* 6.8 Hz)/0.90 (d, *J* 6.7 Hz) (H-16/H-17), 1.25 (s, H-19), 1.31 (s, H-18), 1.68 (d, *J* 1.2 Hz, H-20), 2.24 (dd, *J* 8.5 and −14.4 Hz, H-5a), 2.36 (dd, *J* 5.5 and −14.4 Hz, H-5b), 4.01 (m, H-11), 5.33 (dd, *J* 8.0 and 15.4 Hz, H-2), 5.36 (br t, H-13), 5.36 (d, *J* 15.4 Hz, H-3), 5.62 (d, *J* 15.7 Hz, H-7) and 5.67 (ddd, *J* 5.5, 8.5 and 15.7 Hz, H-6); MS [*m/z* (%)]: 304 (0.4, *M*−18), 286 (2), 277 (2), 268 (0.5), 243 (1), 225 (1), 201 (1), 189 (2), 175 (2), 161 (5), 145 (4), 135 (5), 126 (11), 111 (11), 95 (18), 81 (21), 69 (20), 55 (32) and 43 (100).

*Photo-oxygenation of (1S,2E,4R,6E,8S,11E)-2,6-11-cembratriene-4,8-diol (**15**).* A solution of 242 mg of **15**[4,5] and 10 mg of Rose Bengal in 25 ml of MeOH was reacted with singlet oxygen for 1.5 h using the apparatus described. After addition of 350 µl of triethyl phosphite, the reaction mixture was kept at room temperature for 2 h. The solvent was removed under reduced pressure, and the residue was filtered through alumina using EtOAc and EtOAc/MeOH 90:10 as the solvents. Subsequent separation by HPLC (Spherisorb 5, hexane/EtOAc 20:80; Spherisorb 5 ODS, MeOH/H_2O 70:30; Spherisorb 5 CN, hexane/EtOAc 1:1) gave 88 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*)-2,6,12(20)-cembratriene-4,8,11-triol (**8**), 15 mg of the corresponding 11*R*-epimer (**16**), 43 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,10*E*,12*S*)-2,6,10-cembratriene-4,8,12-triol (**4**), 27 mg of the corresponding 12*R*-epimer (**5**) and 2.7 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11ζ,12*E*)-2,6,12-cembratriene-4,8,11-triol (**17**). The product ratio of **8**:**16**:**4**:**5**:**17** was measured to be 43:8:30:16:3 by integration of the HPLC traces (RI-detection).

Of these, (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*)-2,6,12(20)-cembratriene-4,8,11-triol was identical with a triol (**8**) previously isolated from tobacco.[3] The (1*S*,2*E*,4*R*,6*E*,8*S*,10*E*,12*S*)- and (1*S*,2*E*,4*R*,6*E*,8*S*,10*E*,12*R*)-2,6,10-cembratriene-4,8,12-triols were indistinguishable (m.p., optical rotation, IR, ^1H NMR and mass spectra) from the new tobacco triols **4** and **5**, respectively.

(1*S*,2*E*,4*R*,6*E*,8*S*,11*R*)-2,6,12(20)-Cembratriene-4,8,11-triol (**16**) had m.p. 136.5–138.0 °C; $[\alpha]_D$ +28° (*c* 0.59, $CHCl_3$); IR ($CHCl_3$): 3602, 3425, 1646 and 979 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (d, *J* 6.6 Hz)/0.88 (d, *J* 6.7) (H-16/H-17), 1.27 (s, H-19), 1.40 (s, H-18), 2.33 (dd, *J* 8.2 and −13.3 Hz, H-5a), 2.40 (dd, *J* 5.1 and −13.3 Hz, H-5b), 4.10 (m, H-11), 4.89 (br s, H-20a), 4.99 (br s, H-20b), 5.33 (dd, *J* 8.1 and 15.9 Hz, H-2), 5.42 (d, *J* 15.9 Hz, H-3), 5.47 (d, *J* 15.5 Hz, H-7) and 5.54 (ddd, *J* 5.1, 8.2 and 15.5 Hz, H-6); MS [*m/z* (%)]: 286 (8, *M*−36), 268 (6), 243 (9), 225 (11), 201 (5), 145 (29), 133 (32), 119 (37), 105 (51), 91 (54), 81 (51), 69 (38), 55 (48) and 43 (100).

(1*S*,2*E*,4*R*,6*E*,8*S*,11ζ,12*E*)-2,6,12-Cembratriene-4,8,11-triol (**17**) had m.p. 139.5–145.0 °C; $[\alpha]_D$ +43° (*c* 0.20, $CHCl_3$); IR ($CHCl_3$): 3600, 3428 and 978 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (d, *J* 6.8 Hz)/0.90 (d, *J* 6.8 Hz) (H-16/H-17), 1.27 (s, H-19), 1.37 (s, H-18), 1.69 (d, *J* 1.2 Hz, H-20), 2.32 (dd, *J* 7.3 and −13.4 Hz, H-5a), 2.39 (dd, *J* 4.9 and −13.4 Hz, H-5b), 3.96 (m, H-11), 5.23 (dd, *J* 8.7 and 15.8 Hz, H-2), 5.34 (br t, *J* 5.9 Hz, H-13), 5.42 (d, *J* 15.8 Hz, H-3), 5.56 (ddd, *J* 4.9, 7.3 and 15.6 Hz, H-6) and 5.62 (d, *J* 15.6 Hz, H-7); MS [*m/z* (%)]: 286 (3, *M*−36), 268 (2), 243 (4), 225 (3), 203 (2), 160 (21), 145 (25), 133 (11), 126 (39), 119 (18), 105 (28), 93 (33), 81 (51), 71 (26), 55 (42) and 43 (100).

*Ozonolysis of (1S,2E,4S,6R,7E,10E,12S)-2,7,10-cembratriene-4,6,12-triol (**12**).* A solution of 35 mg of **12**[3,7] in 25 ml of methanol was treated with ozone at −78 °C for 5 min. The reaction mixture was then stirred at 0 °C with an excess of $NaBH_4$ for 3 h. The mixture was made neutral using aqueous HCl (5 %), and the solvent was removed under reduced pressure. The residue was filtered through a small column packed with silica gel using ethyl acetate as the solvent. The eluate obtained was concentrated and separated by HPLC (Spherisorb 5 CN, EtOAc) to give 8.0 mg of (2*S*,5*S*)-5-hydroxymethyl-2,6-dimethyl-1,2-heptanediol (**11**), which was an oil and had $[\alpha]_D$ −3.6° (*c* 0.25, $CHCl_3$); IR ($CHCl_3$): 3623, 3420, 1389 and 1373 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.900 (d, *J* 6.8 Hz)/0.922 (d, *J* 6.9 Hz) (H-7/H-10), 1.18 (s, H-8), 3.43 (d, *J* −10.9 Hz, H-1a), 3.50 (d, *J* −10.9 Hz, H-1b), 3.59 (dd, *J* 6.4 and −10.9 Hz, H-9a) and 3.70 (dd, *J* 5.0 and −10.9 Hz, H-9b); ^{13}C NMR ($CDCl_3$): δ 19.8 (C-7 and C-10), 21.6 (C-4), 23.3 (C-8), 28.4 (C-6), 35.7 (C-3), 46.7 (C-5), 63.6 (C-9), 69.8 (C-1) and 73.1 (C-2); MS [*m/z* (%)]: 159 (17, *M*−31), 141 (14), 123 (57), 105 (4), 95 (5), 83 (56), 75 (48), 71 (29), 55 (46) and 43 (100).

*Ozonolysis of (1S,2E,4S,6E,8S,10E,12S)-2,6,10-cembratriene-4,8,12-triol (**2**).* A solution of 14 mg of **2** in 25 ml of methanol was treated with ozone at −78 °C for 5 min. The reaction mixture was then stirred at 0 °C with an excess of $NaBH_4$ for 2 h. Work-up and separation as described above gave 2.9 mg of a product which was identical (optical rotation, IR, ^1H NMR and mass spectra) with (2*S*,5*S*)-5-hydroxymethyl-2,6-dimethyl-1,2-heptanediol (**11**).

*Ozonolysis of (1S,2E,4R,6E,8S,10E,12S)-2,6,10-cembratriene-4,8,12-triol (**4**).* Using the conditions described above 24 mg of **4** were ozonolyzed to give 3.2 mg of a product

which was identical in all respects with (2*S*,5*S*)-5-hydroxymethyl-2,6-dimethyl-1,2-heptanediol (**11**).

*Ozonolysis of (1*S*,2*E*,4*R*,6*E*,8*S*,10*E*,12*R*)-2,6,10-cembratriene-4,8,12-triol (**5**).* Using the conditions described above 20 mg of **3** were ozonolyzed to give 5.1 mg of (2*R*,5*S*)-5-hydroxymethyl-2,6-dimethyl-1,2-heptanediol (**13**), which was an oil and had $[\alpha]_D$ −4.0° (*c* 0.35, $CHCl_3$); IR ($CHCl_3$): 3627, 3392, 1388 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.908 (d, *J* 6.9 Hz)/0.912 (d, *J* 6.9 Hz) (H-7/H-10), 1.17 (s, H-8), 3.41 (d, *J* −11.0 Hz, H-1a), 3.51 (d, *J* −11.0 Hz, H-1b), 3.58 (dd, *J* 5.8 and −10.9 Hz, H-9a) and 3.69 (dd, *J* 4.9 and −10.9 Hz, H-9b); ^{13}C NMR ($CDCl_3$): δ 19.8/19.9 (C-7/C-10), 21.9 (C-4), 23.2 (C-8), 28.6 (C-6), 35.9 (C-3), 46.6 (C-5), 63.6 (C-9), 69.6 (C-1) and 73.2 (C-2); MS [*m*/*z* (%)]: 159 (13, *M*−31), 141 (11), 123 (47), 109 (3), 95 (5), 83 (48), 75 (41), 71 (27), 55 (43) and 43 (100).

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