Tobacco Chemistry. 68.* Structure Determination and Biomimetic Syntheses of Two New Tobacco Cembranoids

Rolf Arndt,[a] Inger Wahlberg,[a] Curt R. Enzell[a] and Jan-Eric Berg[b]

[a]Research Department, Swedish Tobacco Company, P.O. Box 17007, S-104 62 Stockholm and [b]Department of Structural Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

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Two new diterpenoids of the cembrane class have been isolated from Greek tobacco. They have been identified as (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-8,11-epoxy-2,12(20)-cembradiene-4,6,7-triol (**1**) and (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-8,11-epidioxy-2,12(20)-cembradiene-4,6,7-triol (**2**) by spectroscopic methods, X-ray analysis of the 6-benzoate (**14**) of **2**, and biomimetic syntheses.

The acid-induced rearrangement of hydroperoxide **7**, which leads to the generation of **1** and **2** (approximate ratio 2:1), has been studied under different experimental conditions. It is suggested, and supported by experimental results, that the formation of **1**, which formally involves loss of hydroxyl, proceeds with participation of a peroxonium ion.

The cuticular wax of the leaf and flower of most tobacco varieties contains large amounts of diterpenoids of the cembrane class. The two (1*S*,2*E*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diols epimeric at C-4 (**3**, **4**) are the major components and, as suggested by results from biomimetic studies, also the precursors of most other tobacco cembranoids.[2] We now report the isolation from flowers of Greek tobacco of two new tobacco cembranoids (**1**, **2**), which are likely to arise via oxidation of the 4*S*,6*R*-diol **3**.

Results

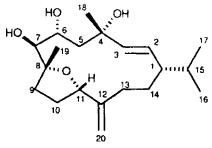
The first tobacco isolate (**1**), $C_{20}H_{34}O_4$, is a triol having two secondary and one tertiary hydroxyl group [^{13}C NMR: δ 68.6 (d), 72.6 (s) and 74.7 (d); ^{1}H NMR: δ 3.60 and 4.28]. The two secondary hydroxyl groups apparently form part of a 1,2 glycol system. This conclusion was based on the presence of an appropriate cross-peak in the ^{1}H–^{1}H shift correlation spectrum and on the fact that the signals arising from the protons under the secondary hydroxyl groups are broadened because of chemical exchange.

The remaining oxygen atom in **1** is accomodated by an epoxide group extending from a methine to a fully substituted carbon atom [overlapping ^{13}C NMR signals at δ 85.1 (d) and (s)]. Since the ^{1}H and ^{13}C NMR spectra also demonstrated the presence of two double bonds, of which one is *E*-1,2-disubstituted (J = 15.4 Hz) and one is 1,1-disubstituted, it followed that triol **1** is carbomonocyclic.

These results, and the occurrence of an isopropyl group and two methyl groups which are attached to the fully substituted oxygen-carrying carbon atoms, suggested that **1** is a diterpenoid of the cembrane class, and an 8,11-epoxy-2*E*,12(20)-cembradiene-4,6,7-triol structure seemed most plausible from a biogenetic point of view.

This assignment was readily verified by treatment of the 4*S*,6*R*,11*S*-triol **5**[3,4] with *t*-butyl hydroperoxide and a catalytic amount of vanadyl acetylacetonate in benzene. One product, identical in all respects to the naturally occurring **1**, was isolated. Its formation may be rationalized by epoxidation of the 7,8 double bond in **5** and a subsequent attack by the hydroxyl group at C-11 on C-8, thereby causing an opening of the epoxide group. This result would then be consistent with 1*S*,2*E*,4*S*,6*R*,11*S*-stereochemistry in **1**.

*For part 67, see Ref. 1.



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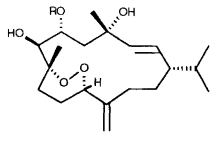
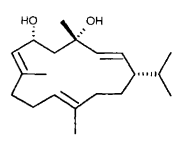


2 R= H
14 R= C_6H_5CO-



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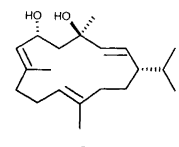

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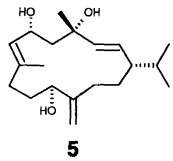

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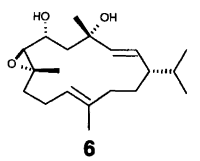

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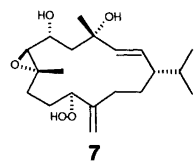

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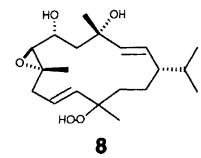

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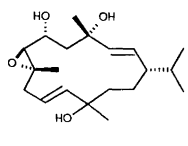

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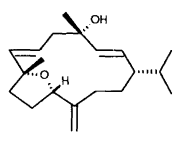

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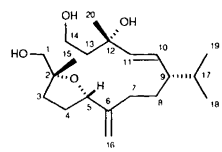

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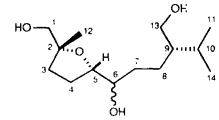
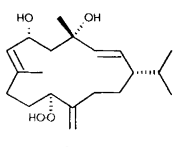


12 6-S1
13 6-S2



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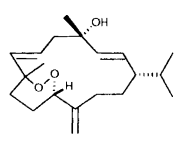

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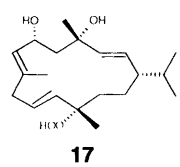

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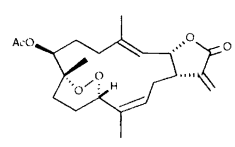

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In order to determine the configurations of C-7 and C-8, the 7*S*,8*S*-epoxide **6**[5] was subjected to photo-oxygenation with the use of Rose Bengal as the sensitizer. Two major products were obtained. They were identified as an 11-hydroperoxide (**7**) [^{1}H NMR: δ 3.77 (m, H-11), 5.04 and 5.13 (broad s, H-20a and H-20b); ^{13}C NMR: δ 88.0 (d, C-11)] and a 12-hydroperoxide (**8**) [^{1}H NMR: δ 5.31 (ddd, H-10) and 5.41 (dd, H-11); ^{13}C NMR: δ 83.8 (s, C-12)], both being expected syn-ene products.[3,4] Reduction of the hydroperoxide group at C-11 in **7** by using triethyl phosphite led to a spontaneous cyclization reaction, presumably taking place by the mechanism suggested above, and affording a product identical to **1**. Compound **1** would consequently be ascribed a 7*S*,8*R* stereochemistry and compound **7** an 11*S* stereochemistry. Reduction of the hydroperoxide group at C-12 in **8** gave the corresponding alcohol (**9**) [^{13}C NMR: δ 73.4 (s, C-12)]. No attempt was made, however, to determine the configuration at C-12 in these two compounds.

Independent evidence of the configuration at C-8 and C-11 in **1** was obtained by chemical correlation of **1** with (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (**10**).[6,7] This involved the conversion of **1** to the *seco*-triol **11** via $NaIO_4$ cleavage of the 6,7 bond and subsequent reduction of the resultant unstable dialdehyde with the use of $NaBH_4$. The *seco*-triol **11** was subjected to ozonolysis and reductive work-up. The product formed was identical to one (**12**) of the two C-6 epimers of (2*R*,5*S*,9*S*)-2,10-dimethyl-2,5-epoxy-9-hydroxymethyl-1,6-undecanediol (**12**, **13**) obtained by ozonolysis of **10**. These results conclusively identify **1** as (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-8,11-epoxy-2,12(20)-cembradiene-4,6,7-triol.

The spectral data indicated that the second new compound (**2**) is structurally closely related to triol **1**. Thus, its ^{1}H NMR spectrum retains the signals due to the isopropyl group, the methyl groups attached to C-4 and C-8 (H-18 and H-19), H-11, the exocyclic methylene group (H-20a and

Tabel 1. ^{13}C NMR chemical shift values and assignments for compounds **1**, **2**, **7**, **8**, **9**, **11** and **16**[a].

Compound	Carbon atom																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	50.0	130.3	137.0	72.6	47.2	68.1	74.7	85.1	39.3	31.6	85.1	149.1	34.0	26.2	31.7	20.4	20.8	32.7	22.9	113.5
2	48.9	130.2	136.7	72.7	46.5	68.2	70.7	81.6	34.2	25.5[b]	85.4	147.3	31.5	24.4[b]	30.7	20.5	20.5	32.4	21.6	116.1
7	47.7	129.4	139.8	73.7	43.9	70.8	63.5	62.9	30.2	33.4	88.0	146.6	29.1	27.1	31.8	18.9	21.0	34.2	17.5	112.9
8	50.7	129.3	138.3	73.8	43.3	71.7	62.5	62.8	39.2	122.8	136.1	83.8	34.5	26.1	30.4	17.9	21.7	32.4	18.9	23.8
9	50.6	128.9	137.9	73.8	43.7	72.4	62.1	60.9	38.5[b]	120.8	139.8	73.4	39.7[b]	26.1	30.2	17.7	21.8	32.5	19.0	29.5
11	69.2	83.3	31.8[b]	30.7[b]	82.2	149.3	34.2	29.1	49.1	130.3	138.0	73.9	42.6	60.3	23.5	109.7	32.2	19.4	20.7	29.1
16	49.7	128.8	138.3	73.5	45.4	125.9	136.2	80.1	34.3	26.7	85.8	147.5	30.3	24.2	32.0	19.6	20.6	31.6	27.0	115.7

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

H-20b), and the 2,3 double bond. The signals assigned to H-6 and H-7, assigned with the aid of spin decoupling experiments, are present at somewhat lower fields as compared with the corresponding signals in the ^1H NMR spectrum of **1**.

The EI (high resolution) mass spectrum exhibited an $[M-15]^+$ peak at *m/z* 339 corresponding to a $C_{19}H_{31}O_5$ species, and the CI mass spectrum obtained with the use of ammonia as the reagent gas included intense peaks at *m/z* 372 and 354, which were ascribed to $[M+NH_4]^{+\cdot}$ and $[M+NH_4-H_2O]^{+\cdot}$ ions. These results are consonant with **2** having the composition $C_{20}H_{34}O_5$, i.e. one oxygen more than **1**.

A clue to the location of this oxygen atom in **2** was provided by the ^{13}C NMR spectrum. This displayed the C-20 signal at δ 116.1, a chemical shift value indicative of the presence of an 8,11-epidioxide group in **2**.[8] In order to confirm this assignment and to determine the stereochemistry, the 6-benzoate (**14**) obtained from **2** was subjected to X-ray analysis.

Compound **14** formed orthorhombic crystals of space group $P2_12_12_1$. The crystal data, obtained on a Siemens/Stoe AED 2 diffractometer, were $a = 9.1809$, $b = 11.6288$ and $c = 25.2030$ Å, $Z = 4$. The present R value including anisotropic thermal parameters for all non-hydrogen atoms and isotropic thermal parameters for all hydrogen atoms except those of the hydroxyl groups is 0.073. Work to locate the remaining hydrogen atoms and further refinement is under way.[9] A stereoscopic view, which summarizes the X-ray results, is shown in Fig. 1. It can be concluded from this study that the second new tobacco constituent (**2**) is (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-8,11-epidioxy-2,12(20)-cembradiene-4,6,7-triol (relative stereochemistry).

The absolute stereochemistry was resolved by a biomimetic type of synthesis involving treatment of the 11*S*-hydroperoxide **7** (*vide infra*) with aqueous HCl in $CHCl_3$. Two products were obtained, the minor one of which had spectral data and optical rotation indistinguishable from those of the naturally occurring **2**. The major product proved to be identical to triol **1**. Since its formation formally involves loss of hydroxyl, we decided to study the acid-induced reaction of **7** under different experimental conditions.

In a series of experiments, **7** was treated with aqueous $HCl/CHCl_3$ in the presence of 2,6-di-*tert*-butyl-4-methylphenol, with *p*-toluenesulfonic

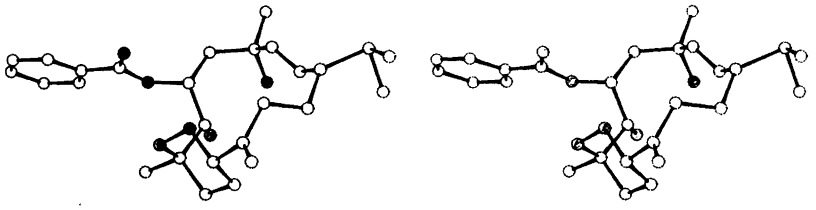
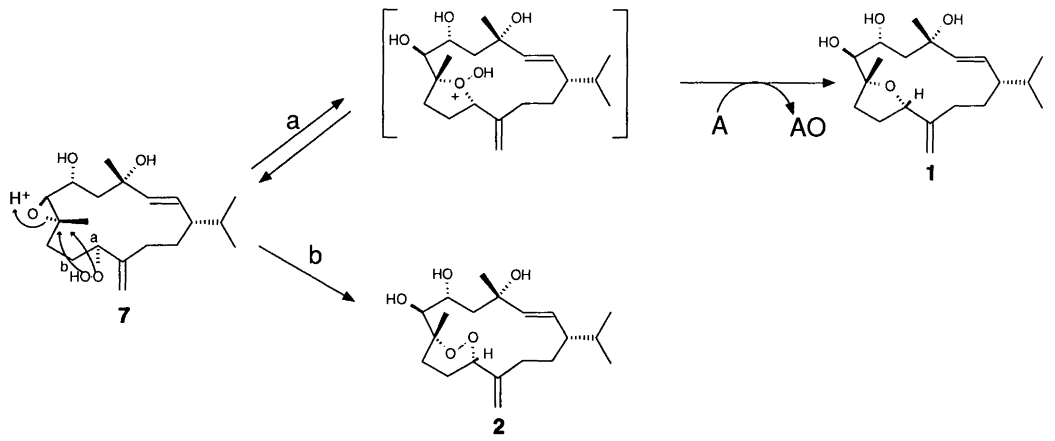


Fig. 1. A stereoscopic view of (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-6-benzoyloxy-8,11-epidioxy-2,12(20)-cembradiene-4,7-diol (**14**).

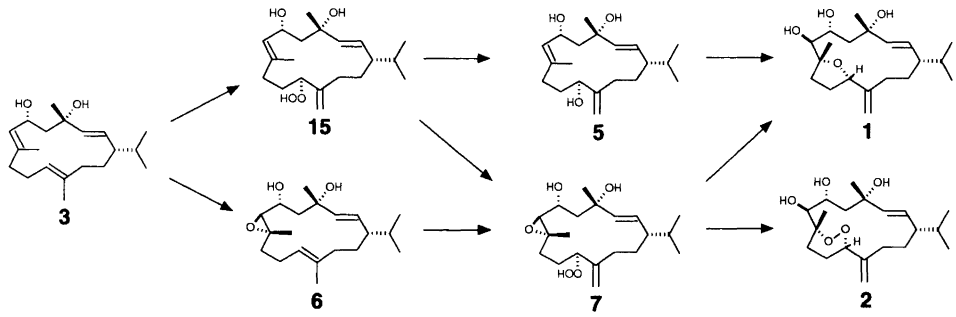
acid (PTSA) in toluene, with dry pyridine hydrochloride in toluene, and with dry trifluoroethanol in toluene. Compounds **1** and **2**, approximate ratio 2:1, were invariably the sole products isolated. These results exclude the possibility that a radical type of reaction or a displacement reaction[10] between the hydroperoxide group and water are involved in the formation of **1**. Nor is the presence of the 7,8-epoxide group in **7** a prerequisite, since the reaction of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (**15**)[11] with PTSA in toluene yielded the 8,11-epoxide **10** and the 8,11-epidioxide **16** [mol. wt. 320; ^{13}C NMR signals at δ 80.1 (C-8), 85.8 (C-11) and 115.7 (C-20)] in the ratio 1:2.

In order to obtain further insight into the mechanism, **7** was next treated with aqueous HCl in $CHCl_3$ in the presence of 3 molar equiv. of methyl phenyl sulfoxide, an agent known to be oxidized by peroxonium ions.[12] Methyl phenyl sulfone was indeed isolated from the reaction mixture. Compounds **1** and **2** were obtained in the ratio 4:1 as compared to a ratio of 2:1 in a control experiment carried out without sulfoxide present. Furthermore, since the 12*S*-hydroperoxide **17**, which does not form an ether on treatment with weak acid, failed to oxidize methyl phenyl sulfoxide, we suggest that the formation of **1** from **7** proceeds through the intermediacy of a peroxonium type of ion (Scheme 1).

It is reasonable to assume that the biogenesis of the two new tobacco consitutents (**1**, **2**) involves the 4*S*,6*R*-diol **3** as the precursor. Thus, as outlined in Scheme 2, **3** may undergo oxygenation of the 11,12 double bond, either by the action of singlet oxygen or through the assistance of a relevant enzyme, to form the 11*S*-hydroperoxide **15**, and regiospecific epoxidation to form the 7,8-epoxide **6**. Compounds **6** and **15** are converted to **7**, which is an intermediate in the generation of both **1** and **2**. An alternative route to **1** would involve reduction of **15** with formation of the 4*S*,6*R*,11*S*-triol **5** and subsequent epoxida-



Scheme 1. Proposed mechanism for the formation of **1** and **2** from **7**.



Scheme 2. Proposed biogenesis of **1** and **2**.

tion. Support for the validity of the biogenetic pathway shown in Scheme 2 is provided by the fact that, in addition to **3**,[13] compounds **5**, **6**, and **15** have previously been found in tobacco.[3,5,11]

To our knowledge, compound **2** is the second cembranic epidioxide found in nature, the first representative being denticulatolide (**18**) isolated from the soft coral *Lobophytum denticulatum*.[14]

Experimental

With the exception of optical rotations, which were recorded on a Perkin-Elmer 241 polarimeter, the instruments specified in Ref. 15 were used.

Isolation. Fraction D (50 g),[1] obtained from an extract of flowers of Greek *Nicotiana tabacum* (Basma), was separated by flash chromatography over silica gel, using a gradient of hexane/EtOAc as the eluent, into eight fractions, D1–D8. Fraction D3 (3 g) was separated further into four fractions, D31–D34, by HPLC using a column packed with Spherisorb 5 CN and with hexane/EtOAc (30:70) as the eluent. Repetitive HPLC of fraction D33 (590 mg) (Lichrosorb-diol; hexane/EtOAc 30:70) gave 16 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-8,11-epoxy-2,12(20)-cembradiene-4,6,7-triol (**1**). Recrystallization of fraction D32 (436 mg) from hexane/Et_2O afforded 18 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-8,11-epidioxy-2,12(20)-cembradiene-4,6,7-triol (**2**).

1 had m.p. 188–189 °C; $[\alpha]_D$ −23° (*c* 0.76, EtOH); (Found: $[M-18]^{+\cdot}$: 320.2376. Calc. for $C_{20}H_{32}O_3$: 320.2351); IR ($CHCl_3$): 3603, 3429, 3077, 1645, 1385, and 1368 cm^{-1}; ^1H NMR ($CDCl_3$):δ 0.86 (d, *J* = 6.6 Hz)/0.91 (d, *J* = 6.6 Hz) (H-16/H-17), 1.34 (s, H-18), 1.40 (s, H-19), 2.66 (d, *J* = 7.5 Hz, −O*H*), 3.60 (d, *J* = 7.5 Hz, H-7), 4.28 (q, *J* = 7.5 Hz, H-6), 4.46 (dd, *J* = 5.5 and 9.9 Hz, H-11), 4.89 (broad s, H-20a), 5.03 (broad s, H-20b), 5.38 (dd, *J* = 8.8 and 15.4 Hz, H-2), and 5.47 (d, *J* = 15.4 Hz, H-3); MS [*m/z* (%, composition)]: 338 (0.2, M), 320 (2), 302 (0.9, $C_{20}H_{30}O_2$), 277 (1, $C_{18}H_{29}O_2$), 263 (5, $C_{17}H_{27}O_2$), 245 (3), 221 (1, $C_{14}H_{21}O_2$ and $C_{15}H_{25}O$), 217 (5, $C_{15}H_{21}O$), 203 (5, $C_{14}H_{19}O$ and $C_{15}H_{23}$), 163 (10, $C_{11}H_{15}O$), 147 (10, $C_{11}H_{15}$), 135 (14, $C_{10}H_{15}$ and $C_9H_{11}O$), 121 (15, C_9H_{13} and C_8H_9O), 109 (17, C_7H_9O and C_8H_{13}), 95 (21, C_7H_{11} and C_6H_7O), 81 (22, C_5H_5O), 69 (23, C_5H_9 and C_4H_5O), 55 (21, C_4H_7 and C_3H_3O), and 43 (100).

2 had m.p. 171–174 °C; $[\alpha]_D$ −52° (*c* 0.32, EtOH); (Found: $[M-15]^+$: 339.2155. Calc. for $C_{19}H_{31}O_5$: 339.2171); IR ($CHCl_3$): 3605, 3468, 3080, 1385 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (d, *J* = 7.4 Hz)/0.89 (d, *J* = 6.5 Hz) (H-16/H-17), 1.26 (s)/1.36 (s) (H-18/H-19), 4.12 (broad s, H-7), 4.40 (m, H-6), 4.59 (m, H-11), 4.94 (broad s, H-20a), 5.09 (broad s, H-20b), and 5.4–5.7 (overlapping signals due to H-2 and H-3); ^1H NMR (C_3D_6O): δ 5.56 (dd, *J* = 8.7 and 15.2 Hz, H-2) and 5.67 (d, *J* = 15.2 Hz, H-2); MS,EI [*m/z* (%, composition)]: 354 (0.1, M), 339 (O.3), 319 (O.1, $C_{20}H_{31}O_3$), 262 (0.4, $C_{17}H_{26}O_2$), 247 (1, $C_{16}H_{23}O_2$), 222 (2, $C_{14}H_{22}O_2$), 219 (2, $C_{15}H_{23}O$ and $C_{14}H_{19}O_2$), 201 (2, $C_{15}H_{21}$), 179 (6, $C_{11}H_{15}O_2$), 161 (3), 121 (10, C_9H_{13} and C_8H_9O), 109 (15), 95 (16, C_7H_{11} and C_6H_7O), 81 (17, C_5H_5O), 71 (20, C_4H_7O), 55 (19, C_4H_7 and C_3H_3O), and 43 (100, C_2H_3O and C_3H_7); MS, CI (NH_3) [*m/z* (%)]: 372 (40, M+NH_4), 354 (100), 338 (26), 298 (70), 251 (61), and 229 (80).

Epoxidation of (1S,2E,4S,6R,7E,11S)-2,7,12(20)-cembratriene-4,6,11-triol (**5**). To a stirred and cold (0 °C) solution of 65 mg of **5** and a catalytic amount of vanadyl acetylacetonate in 5 ml benzene was added a solution of 50 µl of *t*-butyl hydroperoxide (72.5 %) in 3 ml of benzene. After having been stirred for 6 h at room temperature, the reaction mixture was diluted with water and extracted with EtOAc. The organic phase was washed with a saturated aqueous solution of ferrous sulphate, with water and dried. The solvent was removed under reduced pressure and the residue was separated by HPLC (Spherisorb 5 ODS; methanol/water 80:20) to give 21 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-8,11-epoxy-2,12(20)-cembradiene-4,6,7-triol, which was identical (m.p., optical rotation, IR, ^{1}H NMR and mass spectra) to the naturally occurring **1**.

Sensitized photo-oxygenation of (1S,2E,4S,6R,7S,8S,11E)-7,8-epoxy-2,11-cembradiene-4,6-diol (**6**). A solution of 88 mg of **6** and 15 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 5 h, the reaction mixture was concentrated *in vacuo* and separated by HPLC (Spherisorb 5 ODS; methanol/water 80:20) to give 18 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,8*S*,11*S*)-7,8-epoxy-11-hydroperoxy-2,12(20)-cembradiene-4,6-diol (**7**) and 23 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,8*S*,10*E*,12ξ)-7,8-epoxy-12-hydroperoxy-2,10-cembradiene-4,6-diol (**8**).

7 had m.p. 118–121 °C and $[\alpha]_D$ +40.0 ° (*c* 1.35, EtOH); IR ($CHCl_3$): 3604, 3509, 1386 and 1369 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.86 (d, *J* = 7.0 Hz)/0.89 (d, *J* = 6.6 Hz) (H-16/H-17), 1.31 (s)/1.32 (s) (H-18/H-19), 1.82 (dd, *J* = 2.4 and −14.9 Hz, H-5a), 2.27 (dd, *J* = 5.3 and −14.9 Hz, H-5b), 3.21 (d, *J* = 7.5 Hz, H-7), 3.77 (m, H-11), 4.36 (ddd, *J* = 2.4, 5.3, and 7.5 Hz, H-6), 5.04 (broad s, H-20a), 5.13 (broad s, H-20b), 5.47 (d, *J* = 15.4 Hz, H-3), 5.63 (dd, *J* = 9.4 and 15.4 Hz, H-2), and 8.12 (broad s, −OO*H*); MS [*m/z* (%)]: 336 (1, M−18), 318 (1), 263 (1), 217 (1), 203 (3), 163 (4), 135 (11), 121 (12), 109 (15), 95 (24), 81 (20), 69 (23), 55 (26) and 43 (100).

8 was an oil and had $[\alpha]_D$ +33.2 ° (*c* 2.35, EtOH); IR ($CHCl_3$): 3675, 3398, 1603, 1385 and 1371 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.82 (d, *J* = 6.6 Hz)/(0.87 (d, *J* = 6.6 Hz) (H-16/H-17), 1.23 (s)/1.32 (s)/1.33 (s) (H-18/H-19/H-20), 2.40 (dd, *J* = 9.1 and −15.3 Hz, H-9a), 2.54 (ddd, *J* = 1.9, 4.1 and −15.3 Hz, H-9b), 3.58 (d, *J* = 7.9 Hz, H-7), 3.87 (m, H-6), 4.14 (broad s, −O*H*), 4.86 (broad s, −O*H*), 5.31 (ddd, *J* = 4.1, 9.1 and 15.6 Hz, H-10), 5.36 (d, *J* = 5.6 Hz, H-3), 5.41 (dd, *J* = 1.9 and 15.6 Hz, H-11), 5.68 (dd, *J* = 9.6 and 15.6 Hz, H-12), and 9.14 (broad s, −OO*H*); MS [*m/z*, %]: 339 (0.1, M-15), 303 (1), 276 (0.3), 259 (0.8), 221 (1), 203 (2), 177 (3), 163 (4), 149 (5), 137 (10), 123 (14), 109 (19), 95 (24), 81 (21), 71 (23), 55 (23) and 43 (100).

Reduction of (1S,2E,4S,6R,7S,8S,11S)-7,8-epoxy-11-hydroperoxy-2,12(20)-cembradiene-4,6-diol (**7**). A solution of 3.1 mg of **7** and 20 µl of triethyl phosphite in 2 ml of methanol was kept at room temperature for 7 h. The reaction mixture was concentrated *in vacuo* and separated by HPLC (Spherisorb 5 ODS, methanol/water 80:20; Spherisorb 5 CN, hexane/EtOAc 20:80) to give 1.1 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-8,11-epoxy-2,12(20)-cembradiene-4,6,7-triol, which was indistinguishable (optical rotation, IR, ^{1}H NMR and MS) from the naturally occurring **1**.

Reduction of (1S,2E,4S,6R,7S,8S,10E,12ξ)-7,8-epoxy-12-hydroperoxy-2,10-cembradiene-4,6-diol (**8**). A solution of 18 mg of **8** and 75 µl of triethyl phosphite in 3 ml of methanol was stirred at room temperature overnight. Concentration *in vacuo* and flash chromatography over silica gel (hexane/EtOAc 30:70) gave 12 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,8*S*,10*E*,12ξ)-7,8-epoxy-2,10-cembradiene-4,6,12-triol (**9**), which was an oil and had $[\alpha]_D$ +63 ° (*c* 0.53, EtOH); IR ($CHCl_3$): 3599 and 3433 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.81 (d, *J* = 6.6 Hz)/0.87 (d, *J* = 6.6 Hz) (H-16/H-17), 1.25 (s)/1.26 (s)/1.33 (s) (H-18/H-19/H-20), 1.71 (dd, *J* = 1.8 and −14.9 Hz, H-5a), 2.25 (dd, *J* = 6.2 and −14.9 Hz, H-5b), 2.45 (dd, *J* = 9.3 and −15.5 Hz, H-9a), 2.56 (ddd, *J* = 1.7, 3.5 and −15.5 Hz, H-9b), 3.51 (d, *J* = 7.9 Hz, H-7), 3.85 (ddd, *J* = 1.8, 6.2 and 7.9 Hz, H-6), 5.36 (d, *J* = 15.1 Hz, H-3), 5.41 (ddd, *J* = 3.5, 9.3 and 15.7 Hz, H-10), 5.51 (dd, *J* = 1.7 and 15.7 Hz, H-11), and 5.63 (dd, *J* = 9.7 and 15.1 Hz, H-2); MS [*m/z* (%)]: 323 (0.1, M-15), 320 (0.2), 305 (0.4), 287

(0.4), 259 (0.9), 241 (0.5), 219 (0.8), 203 (2), 189 (1), 177 (2), 163 (3), 151 (3), 137 (6), 123 (13), 109 (16), 95 (19), 81 (19), 69 (17), 55 (19) and 43 (100).

*Preparation of (2*R*,5*S*,9*S*,12*S*)-2,12-dimethyl-2,5-epoxy-9-isopropyl-6-methylene-10-tetradecene-1,12,14-triol* (**11**). To a stirred and cold (0 °C) solution of 27 mg of **1** in 2 ml of methanol was added a solution of 32 mg of sodium periodate in 2 ml of water. After 5 h at (°C the reaction mixture was worked up in the usual manner. The crude product obtained was dissolved in 4 ml of methanol, cooled (0 °C), and reacted with 9.5 mg of $NaBH_4$ for 3 h. The reaction mixture was acidified using aqueous HCl (5 %), and the solvent was removed at reduced pressure. The residue was filtered through a column packed with silica gel using EtOAc as the solvent, and was subsequently purified by HPLC (Spherisorb 5 CN; hexane/EtOAc 30:70) to give 15 mg of (2*R*,5*S*,9*S*,12*S*)-2,12-dimethyl-2,5-epoxy-9-isopropyl-6-methylene-10-tetradecene-1,12,14-triol (**11**), which was an oil and had $[\alpha]_D$ −30° (*c* 0.49, $CHCl_3$); IR (CCl_4): 3405, 3090 and 1643 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (d, *J* = 6.8 Hz)/0.89 (d, *J* = 6.7 Hz) (H-18/H-19), 1.23 (s)/1.32 (s) (H-15/H-20), 3.42 (d, *J* = 11.2 Hz, H-1a), 3.51 (d, *J* = −11.2 Hz, H-1b), 3.7–3.9 (overlapping signals due to H-14a and H-14b), 4.44 (m, H-5), 4.85 (broad s, H-16a), 5.06 (broad s, H-16b), 5.44 (dd, *J* = 8.3 and 15.6 Hz, H-10), and 5.51 (d, *J* = 15.6 Hz, H-11); MS [*m/z* (%)]: 340 (0.2, M), 322 (1), 291 (5), 279 (4), 236 (4), 193 (11), 155 (11), 135 (9), 123 (13), 109 (16), 95 (21), 81 (31), 69 (37), 55 (32) and 43 (100).

*Ozonolysis of (2*R*,5*S*,9*S*,12*S*)-2,12-dimethyl-2,5-epoxy-9-isopropyl-6-methylene-10-tetradecene-1,12,14-triol* (**11**). A solution of 15 mg of **11** in 20 ml of methanol was treated with ozone at −78 °C for 15 min. The reaction mixture was stirred at 0 °C for 3 h with an excess of $NaBH_4$. After addition of a few drops of aqueous HCl (5 %), the solvent was removed under reduced pressure and the residue was filtered through a column packed with silica gel using EtOAc as the solvent. The eluate obtained was concentrated and separated by HPLC (Spherisorb 5 CN; hexane/EtOAc 30:70) to give 1.2 mg of a product which was identical (optical rotation, IR, ^1H NMR, MS) to (2*R*,5*S*,6ξ_1,9*S*)-2,10-dimethyl-2,5-epoxy-9-hydroxymethyl-1,6-undecanediol (**12**).

*Ozonolysis of (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol* (**10**). A solution of 20 mg of **10** in 20 ml of methanol was treated with ozone at −78 °C for 20 min. The reaction mixture was then stirred at 0 °C for 3 h with an excess of $NaBH_4$. Work-up as described above and separation by HPLC (Spherisorb 5 CN; hexane/EtOAc 20:80) afforded two C-6 epimers of (2*R*,5*S*,9*S*)-2,10-dimethyl-2,5-epoxy-9-hydroxymethyl-1,6-undecanediol (**12**, **13**). The least polar epimer (**12**, 3.2 mg) was an oil and had $[\alpha]_D$ −8.3° (*c* 0.30, $CHCl_3$); IR ($CHCl_3$): 3622 and 3392 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (d, *J* = 6.9 Hz)/0.90 (d, *J* = 6.8 Hz) (H-11/H-14), 1.19 (s, H-12), 3.46 (d, *J* = −11.1 Hz, H-1a), 3.53 (dd, *J* = 6.7 and −10.6 Hz, H-13a), 3.60 (d, *J* = −11.1 Hz, H-1b), 3.67 (dd, *J* = 4.6 and −10.6 Hz, H-13b) and 3.87 (m)/4.03 (m) (H-5/H-6); MS [*m/z* (%)]: 229 (9, M−31), 211 (14), 153 (10), 145 (11), 135 (10), 127 (23), 115 (73), 97 (40), 85 (45), 69 (74), 57 (58) and 43 (100).

The most polar epimer (**13**, 1.5 mg) was an oil and had $[\alpha]_D$ −5.3° (*c* 0.15, $CHCl_3$); IR ($CHCl_3$): 3438 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90 (d, *J* = 6.8 Hz)/0.91 (d, *J* = 6.9 Hz) (H-11/H-14), 1.19 (s, H-12), 3.43 (d, *J* = −11.9 Hz, H-1a), 3.56 (d, *J* = −11.9 Hz, H-1b), 3.59 (dd, *J* = 6.0 and −10.9 Hz, H-13a), 3.66 (dd, *J* = 5.4 and −10.9 Hz, H-13b), and 3.45 (m)/3.94 (ddd, *J* = 5.2, 6.8 and 7.2 Hz) (H-5/H-6); MS [*m/z* (%)]: 229 (5, M−31), 211 (18), 153 (11), 145 (11), 135 (10), 127 (26), 115 (64), 97 (37), 85 (48), 69 (68), 57 (55) and 43 (100).

*Preparation of (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-6-benzoyloxy-8,11-epidioxy-2,12(20)-cembradiene-4,7-diol* (**14**). A solution of 3.0 mg of **2** and 1.4 mg of benzoyl chloride in 2 ml of pyridine was heated under reflux for 2 h. Work-up and flash chromatography over silica gel using a hexane/EtOAc gradient gave 3.5 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-6-benzoyloxy-8,11-epidioxy-2,12(20)-cembradiene-4,7-diol (**14**), which had m.p. 185–187 °C; $[\alpha]_D$ −84° (*c* 0.35, EtOH); IR ($CHCl_3$): 3606, 3503, 1711 and 1278 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (d, *J* = 6.7 Hz)/0.92 (d, *J* = 6.7 Hz) (H-16/H-17), 1.22

(s)/1.37 (s) (H-18/H-19), 2.12 (dd, J = 5.3 and −14.1 Hz, H-5a), 2.22 (dd, J = 10.8 and −14.1 Hz, H-5b), 3.43 (d, J = 6.1 Hz, −O*H*), 4.50 (dd, J = 2.2 and 6.1 Hz, H-7), 4.55 (m, H-11), 4.89 (broad s, H-20a), 5.02 (dd, J = 0.3 and 1.6 Hz, H-20b), 5.67 (ddd, J = 2.2, 5.3 and 10.8 Hz, H-6), 5.67 (dd, J = 8.7 and 15.3 Hz, H-2), 5.72 (d, J = 15.3 Hz, H-3), and 7.2–8.1 (overlapping signals due to the benzoyloxy group); MS [*m/z* (%)]: 443 (0.2, M−15), 425 (1), 397 (0.2), 318 (0.2), 275 (0.3), 247 (1), 229 (1), 201 (1), 122 (9), 105 (100), 95 (12), 83 (15), 77 (23), 55 (13) and 43 (52).

Treatment of (1S,2E,4S,6R,7S,8S,11S)-7,8-epoxy-11-hydroperoxy-2,12(20)-cembradiene-4,6-diol (7) with acid.

A. To a solution of 11 mg of **7** in 1.5 ml of $CHCl_3$ was added 0.2 ml of aqueous HCl (5 %). After 6 h at room temperature the reaction mixture was passed through a column packed with silica gel using EtOAc as the solvent. The crude product (10.2 mg) was separated by HPLC (Spherisorb 5 CN; hexane/EtOAc 50:50) into two products. The minor product (3.1 mg) was identical (m.p., optical rotation, IR, ^{1}H NMR and MS) to (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-8,11-epidioxy-2,12(20)-cembradiene-4,6,7-triol (**2**), and the major one (5.8 mg) to (1*S*,2*E*,4*S*,6*R*,7*S*,8*R*,11*S*)-8,11-epoxy-2,12(20)-cembradiene-4,6,7-triol (**1**).

B. A solution of 10 mg of **7** and 10 mg of 2,6-di-*tert*-butyl-4-methylphenol (TBA, 1.6 molar equiv.) in 0.2 ml of aqueous HCl (5 %) and 1.5 ml of $CHCl_3$ was stirred at room temperature for 4 h. Work-up and separation by HPLC gave 4.5 mg of **1** (^{1}H NMR, MS) and 2.0 mg of **2** (^{1}H NMR, MS).

C. A solution of 10 mg of **7** and 3.0 mg of *para*-toluenesulfonic acid in 5 ml of toluene was stirred at 0 °C for 3 h. Work-up and separation by HPLC gave 4.0 mg of **1** (^{1}H NMR, MS) and 2.5 mg of **2** (^{1}H NMR, MS).

D. To a stirred solution of 11 mg of **7** in 2 ml of dry toluene was added 0.4 mg of pyridine hydrochloride in the form of a dried stock solution. After 8 days at room temperature the reaction mixture was worked up and separated by HPLC to give 5.5 mg of **1** (^{1}H NMR, MS) and 2.9 mg of **2** (^{1}H NMR, MS).

E. A solution of 8.5 mg (0.024 mmol) of **7** and 10.1 mg (0.072 mmol) of methyl phenyl sulfoxide in 1.5 ml of $CHCl_3$ and 0.2 ml of aqueous HCl (5 %) was stirred at room temperature for 8 h. Work-up and separation by flash chromatography (SiO_2; hexane/EtOAc gradient) gave 1.8 mg of methyl phenyl sulfone, 8.1 mg of unreacted methyl phenyl sulfoxide and 6.2 mg of a mixture of **1** and **2** (ratio 3.9:1.0 as determined by integration of the corresponding HPLC peaks; differential refractometer). The mixture was separated by HPLC (Spherisorb 5 CN; hexane/EtOAc 1:1) to give 4.0 mg of **1** (^{1}H NMR, MS) and 1.0 mg of **2** (^{1}H NMR, MS).

A control experiment employing 8.5 mg of **7** and using the conditions described under E but with no methyl phenyl sulfoxide present was also carried out. A mixture (5.9 mg) of **1** and **2** (ratio 2.3:1.0 as determined by integration of the corresponding HPLC peaks) was obtained. This was separated into 3.1 mg of **1** (^{1}H NMR, MS) and 1.5 mg of **2** (^{1}H NMR, MS).

Treatment of (1S,2E,4S,6R,7S,8S,11S)-7,8-epoxy-11-hydroperoxy-2,12(20)-cembradiene-4,6-diol (**7**) *with trifluoroethanol.* To a stirred and dry solution of 8 mg of **7** in 2 ml of dry toluene was added a dry solution of 250 µl of trifluoroethanol in 250 µl of benzene. After 72 h at room temperature the reaction mixture was worked up and separated by HPLC to give 2.5 mg of **1** (^{1}H NMR, MS) and 1.2 mg of **2** (^{1}H NMR, MS).

Treatment of (1S,2E,4S,6R,7E,11S)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (**15**) *with PTSA.* A solution of 100 mg of **15** and 6 mg of PTSA in 3.5 ml of toluene was stirred at −15 °C for 3 h. Work-up and separation by HPLC (Spherisorb 5; hexane/EtOAc 70:30 and Spherisorb 5 CN; hexane/EtOAc 90:10) gave two products, of which the minor one (5.0 mg) was identical (IR, ^{1}H NMR, MS) to (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (**10**). The major product (10 mg), (1*S*,2*E*,4*S*,6*E*,8ξ,11*S*)-8,11-epidioxy-2,6,12(20)-cembratrien-4-ol (**16**), had m.p. 119–120 °C; $[\alpha]_D$ + 32° (*c* 0.40, $CHCl_3$); IR (CCl_4): 3612 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.85 (d, J = 6.6 Hz)/0.87 (d, J = 6.6 Hz) (H-16/H-17), 1.13 (s)/1.34 (s) (H-18/H-19), 4.50 (dd, J = 2.9 and 11.4 Hz, H-11), 4.94 (broad s, H-20a), 5.09 (broad s, H-20b), and 5.3–5.7 (overlapping sig-

nals due to H-2, H-3, H-6, and H-7); ^{1}H NMR (C_6D_6): δ 5.19 (d, J = 15.4 Hz, H-3), 5.40 (dd, J = 9.3 and 15.4 Hz, H-2), 5.65 (ddd, J = 3.0, 9.4 and 16.4 Hz, H-6), and 5.74 (dt, J = 1.7 and 16.4 Hz, H-7); MS [*m/z* (%)]: 302 (0.4, M−18), 263 (0.4), 259 (0.4), 245 (0.4), 231 (0.3), 219 (0.5), 209 (2), 191 (3), 173 (3), 163 (3), 149 (5), 133 (8), 121 (9), 107 (9), 95 (19), 81 (17), 71 (37), 55 (21) and 43 (100).

Treatment of (1S,2E,4S,6R,7E,10E,12S)-12-hydroperoxy-2,7,10-cembratriene-4,6-diol (**17**) *with acid and methyl phenyl sulfoxide.* To a stirred solution of 5.0 mg of **17** and 30 mg of methyl phenyl sulfoxide in 1.5 ml of $CHCl_3$ was added 0.2 ml of aqueous HCl (5 %). After 24 h at room temperature the reaction mixture was worked-up and separated into unreacted methyl phenyl sulfoxide and **17** (4.7 mg).

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