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Tobacco Chemistry. 55.* Three New Cembranoids from Greek Tobacco. The Stereochemistry of (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-Cembratriene-4,6-diol

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Three new diterpenoids have been isolated from tobacco. The first was shown to be (1*S*,2*E*,4*S*,6*R*,-7*E*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (*3*) by X-ray analysis and chemical correlation, the second was formulated as (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-2,7,10-cembratriene-4,6,12-triol (*4*) by spectral and chemical methods, while the third, (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,-12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diol (*5*), previously described as a synthetic product, is now reported as a tobacco constituent.

The configuration at C-6 in (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*2*) has been resolved by chemical correlation with triol *3*.

The biogenesis of the two new triols (*3*,*4*) is discussed in the light of results obtained by singlet oxygen reactions.

More than twenty cembranoids have so far been isolated from the cuticular wax of different tobacco varieties. Most of these have a hydroxyl group at C-4 and are conveniently divided into two series, one comprising compounds having a 4*R*- and the other, compounds having a 4*S*-configuration. Additional oxygenation is commonly found at C-6, C-8, C-11 or C-12.[2]

Two diols, originally isolated by Roberts and Rowland in 1962[3] and characterized as (2*E*,7*E*,11ξ)-cembratriene-4,6-diols having different configurations at C-4[3] and possibly also at C-6,[4] are the major tobacco cembranoids. While the relative stereochemistry and absolute configuration of one of these have later been determined to be (1*S*,2*E*,-4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*1*) by X-ray analysis[4] and ozonolytic degradation,[5] the chirality at C-6 in the other diol (*2*) has remained unknown.

This structural uncertainty has now been resolved in conjunction with the structure determination of two new cembratrienetriols (*3*,*4*), which have been isolated from a wax extract of Greek tobacco. The present communication describes these results as well as the isolation of a third new tobacco constituent (*5*).

RESULTS

The first tobacco isolate (*3*), $C_{20}H_{34}O_3$, is a triol having two secondary hydroxyl groups (signals at δ 4.07, dd, and δ 4.67, ddd, both shifted downfield in the ^{1}H NMR spectrum of diacetate *6*) and one tertiary hydroxyl group (^{13}C NMR signal at δ 74.1 (s), *cf.* Table 1; OH-absorption in the IR spectrum of *6*). Furthermore, since the ^{1}H and ^{13}C NMR spectra were consistent with the presence of three double bonds, of which one is attached to an exocyclic methylene group, one is a di- and one a trisubstituted double bond, it followed that triol *3* is carbomonocyclic.

The occurrence of two methyl groups, one of which is vinylic and one attached to the fully substituted carbon atom carrying the tertiary hydroxyl group, and an isopropyl group demonstrated that the carbocyclic ring is fourteen-

*For part 54 see Ref. 1.

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Table 1. Carbon-13 chemical shifts and assignments for compounds *3*, *4*, *6* and *8*–*11*.[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
3	49.0	127.4	139.8	74.1	46.5	68.5	128.5	137.8	29.8	32.9	74.8	151.6	34.6	29.3	32.2	19.2/	20.8	33.4	16.0	111.2
4	50.8	127.3	138.3	74.3[b]	47.2	69.4	128.4	134.5	40.7	124.6	138.9	74.0[b]	40.1	26.5	30.1	17.8/	21.8	31.6[c]	18.0	30.0[c]
6[e]	48.2	129.5	138.3	72.0	47.8	69.5	124.4	140.2	30.4	30.4	75.5	147.7	34.5	30.1	31.9	19.2/	20.7	30.4	16.8	112.2
8[f]	49.5	128.0	138.0[b]	72.0	48.6	69.8	124.1	139.7	40.9	124.3	138.5[b]	73.7	40.1	26.1	30.2	18.0/	21.4	29.7[c]	18.3	29.4
9[g]	46.3	127.8	137.1	72.3	50.7	68.8	126.6	139.2	38.8	23.1	124.3	133.4	36.6	27.8	32.9	19.4/	20.7	29.6	16.1	14.8
10[h]	48.3	130.0	137.8	72.1	48.1	69.5	124.4	140.9	30.9	32.0	72.9	152.1	34.9	29.9	31.9	19.2/	20.7	30.7	16.6	110.5
11[i]	48.6	128.4	137.6[b]	72.3	48.0	69.9	123.9	140.3	41.5	125.7	138.2[b]	74.1	39.7	27.5[d]	29.9[c]	18.2/	21.3	29.8[c]	18.2	27.6[d]

[a] δ-Values in $CDCl_3$ relative to TMS. [b,c,d] Assignment may be reversed. [e] $OCOCH_3$ 170.4 and 169.8;$OCOCH_3$ 21.4 and 21.3. [f] $OCOCH_3$ 169.9;$OCOCH_3$ 21.4. [g] $OCOCH_3$ 170.2; $OCOCH_3$ 21.4. [h] $OCOCH_3$ 170.0;$OCOCH_3$ 21.4. [i] $OCOCH_3$ 169.8; $OCOCH_3$ 21.4.

membered and suggested that triol *3* is a cembratrienetriol.

Treatment of triol *3* with weakly acidified chloroform, which resulted in the formation of (1*S*,2*E*,4*S*,-6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*7*),[6] verified this assignment and allowed the formulation of triol *3* as a (1*S*,2*E*,4*S*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol. The geometry of the 7,8 double bond was determined to be *E* from the characteristic chemical shift value[7] of the C-19 methyl group, δ 16.0, in the ^{13}C NMR spectrum of triol *3*, thereby leaving the chirality at C-6 to be accounted for. An X-ray analysis of triol *3* using a direct phase determination procedure was therefore undertaken.

Triol *3* formed orthorhombic crystals of space group $P2_12_12_1$. The crystal data, obtained on a Philips PW 1100 diffractometer, were: $a=16.047$, $b=21.247$ and $c=6.229$ Å, $Z=4$. The present *R*-value including anisotropic thermal parameters for all non-hydrogen atoms is 0.122; location of the hydrogen atoms and further refinement being under way.[8] A stereoscopic view, which summarizes the X-ray results and demonstrates that triol *3* has a 6*R*-configuration, is shown in Fig. 1.

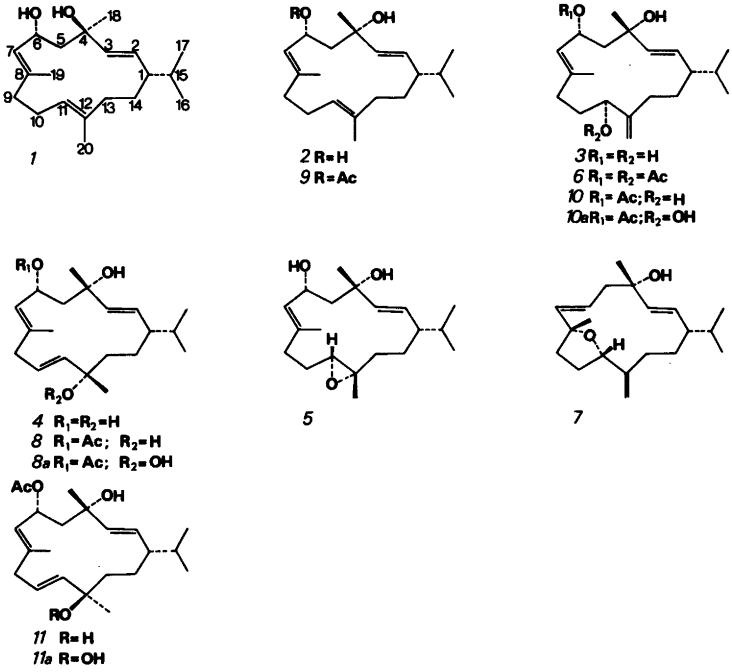
The spectral data indicated that the second tobacco isolate (*4*), $C_{20}H_{34}O_3$, is a cembratrienetriol and structurally closely related to triol *3*. However, in contrast to triol *3*, triol *4* incorporates one secondary hydroxyl group (a signal at δ 4.80 ddd, shifted to δ 5.74 in the ^1H NMR spectrum of monoacetate *8*) and two tertiary hydroxyl groups (^{13}C NMR signals at δ 74.0 (s) and 74.3 (s); OH-absorption in the IR spectrum of *8*). Of the double bonds, two are disubstituted and one trisubstituted.

Spin decoupling and spin simulation experiments carried out on both triol *4* and monoacetate *8* allocated the secondary hydroxyl group, the trisubstituted double bond and an *E*-disubstituted double bond ($J_{AB}=15.9$ Hz) to partial structure A (Fig. 2).

Since the remaining groups comprised one *E*-disubstituted double bond ($J_{AB}=15.5$ Hz), one isopropyl group, one sp^3 methine and two sp^3 methylene groups, triol *4* was provisionally identified as a (2*E*,10*E*)-2,7,10-cembratriene-4,6,12-triol.

With this result at hand, it could be inferred from the chemical shift value of the signal due to the C-19 methyl group in triol *4*, δ 18.0, that the geometry of the 7,8 double bond is *E*.

Additional structural information was obtained by chemical means. Thus, (1*S*,2*E*,4*S*,6ξ,7*E*,11*S*,12*S*)-



11,12-epoxy-2,7-cembradiene-4,6-diol (5)[6,9] was rearranged *via* an intermediate hydroxy selenide and an unstable selenoxide[10] to (1*S*,2*E*,4*S*,6ξ,7*E*,10*E*,-12*S*)-2,7,10-cembratriene-4,6,12-triol (*4*). The latter proved to be identical in all respects to the new tobacco diol.

A plausible biogenetic route to the new triols (*3*, *4*) would involve attack of oxygen on the 11,12 double bond in (1*S*,2*E*,4*S*,6ξ,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (*2*). In harmony with this view, acetate *9*, which in contrast to diol *2* has a 7,8 double bond not susceptible to singlet oxygen reactions, proved to undergo facile ene reactions at the 11,12 double bond. Reduction of the reaction mixture using triethyl phosphite and separation by HPLC yielded two major and one minor product (*10*, *8* and *11*; ratio according to HPLC: 54:43:3).

The ^{1}H NMR spectrum demonstrated that the least polar, major product (*10*) retained the 2,3 and

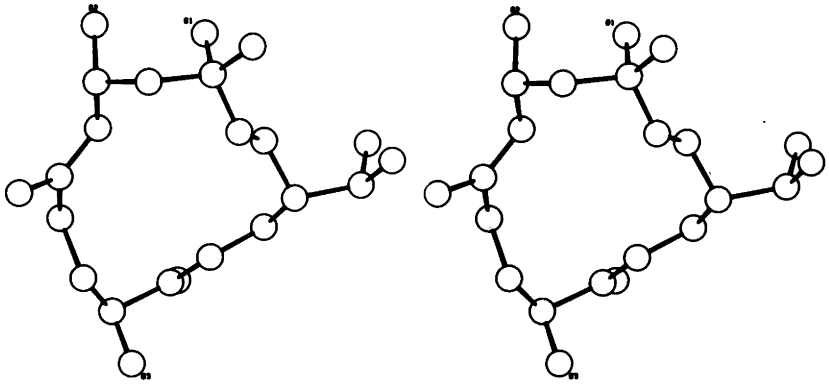
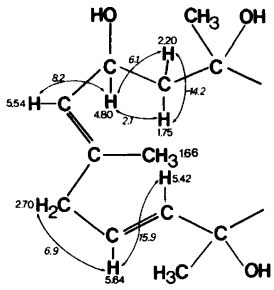


Fig. 1. Stereoscopic view of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (*3*).



A

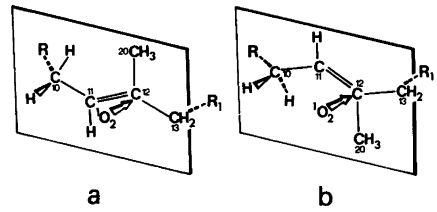
Fig. 2. Partial structure A. Chemical shift values (δ) are in Roman, coupling constants (Hz) are in italic type.

7,8 double bonds and the 6-acetoxyl group and contained a newly introduced secondary hydroxyl group (one-proton triplet at δ 4.03) and an exocyclic methylene group (one-proton signals at δ 4.92 and 5.07). Monoacetate *10* was converted by treatment with acid into (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*7*) and by acetylation into a diacetate (*6*), which proved to be identical to the diacetate derived from triol *3*. These results allowed the assignment of *10* as (1*S*,2*E*,4*S*,6*R*,7*E*,-11*S*)-6-acetoxy-2,7,12(20)-cembratriene-4,11-diol.

The most polar product (*8*), being indistinguishable from the acetate obtained from triol *4*, was hence identified as (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-6-acetoxy-2,7,10-cembratriene-4,12-diol, whereas the minor product (*11*), whose ^{13}C NMR spectrum differed mainly with respect to the shielding of C-20, δ 29.4 as against δ 27.6, was formulated as (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-6-acetoxy-2,7,10-cembratriene-4,12-diol.

As a consequence of the chemical correlations described above, diol *2*, which is a synthetic precursor of *10*, is now conclusively identified as (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol. Likewise, it can be concluded that triol *4* as well as epoxide *5*, which has previously been described as a synthetic product[6,9] and is now reported as the third new tobacco isolate, have 6*R*-configurations.

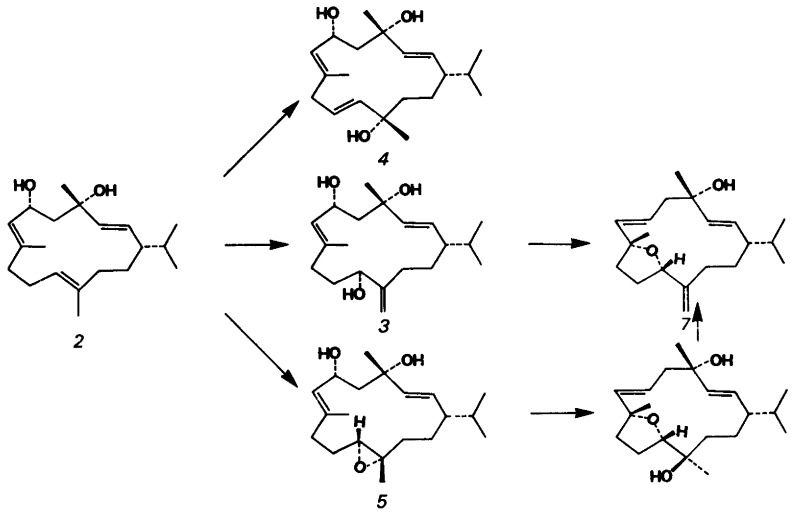
The outcome of the photo-oxygenation reaction deserves comment. Thus, in keeping with the *cis*-cyclic mechanism[11] the generation of both the major hydroperoxides *8a* and *10a* (43 % and 54 %) may be rationalized by ene reactions involving the 11,12 double bond in conformer *a* of acetate *9*. Compound *8a* arises by oxygen attachment to C-12 and migration of the *pro*-*R*-hydrogen at C-10 and compound *10a* by oxygen attachment to C-11 and migration of a hydrogen from C-20. The minor hydroperoxide *11a* (3 %) would be formed by attack of singlet oxygen at C-12 in conformer *b* and abstraction of the *pro*-*S*-hydrogen at C-10. Conformer **a** may then exist in preference to conformer **b** or react with a higher rate constant than the latter, a conclusion which accords with the fact that peracid oxidation of diol *2* yields predominantly the 11*S*,12*S*-epoxide *5*.[9]



a b

It is noteworthy that all three products arise by *syn* ene additions, *i.e.* singlet oxygen abstracts hydrogen from the 1,2-disubstituted side of the trisubstituted double bond.[12] This result indicates that 2*E*,7*E*,11*E*-cembratrienes having a fourteen-membered ring system react with singlet oxygen in the same fashion as has previously been found for acyclic and other cyclic systems, cyclohexenes being exceptions.[13]

The two new triols (*3*, *4*), being present in the cuticular wax of the leaf may well be generated in tobacco by sensitized photo-oxygenation of the 4*S*,6*R*-diol *2* (Scheme 1). Another possibility, which is currently exploited, is that the 4*S*,6*R*-diol *2* is converted to triols *3* and *4* by an enzyme-assisted reaction.



Scheme 1. Probable biogenesis of compounds *3*–*5* and *7*.

Triol *3*, in turn, may be an intermediate in the biogenesis of (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*7*), whose alternative path of bioformation would involve a rearrangement of epoxide *5* and a subsequent dehydration.[9]

EXPERIMENTAL

With the exception of accurate mass measurements, which were carried out on a Kratos MS 50 Stereo DS 55 SM/DS 55 S mass spectrometer-computer system and the ^{1}H NMR spectra, which were recorded on a Varian XL-200 spectrometer, the instruments specified in Ref. 14 were used.

Isolation. An extract (24 g) obtained by immersing green leaves of Greek *Nicotiana tabacum* (Basma Drama) in chloroform was distributed between hexane and methanol–water (80:20). The polar material obtained (16 g) was chromatographed over silica gel using a gradient of hexane–ethyl acetate as eluent to give three fractions, of which the least polar one (1 g) was a complex mixture and fraction 2 (8 g) was a mixture of the (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)- and (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diols (*1*, *2*). Fraction 3 (6 g) was separated further by chromatography over silica gel followed by HPLC using columns packed with μ-Porasil and μ-Bondapak/CN and gradients of hexane–ethyl acetate as eluents to yield 50 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,-11*S*)-2,7,12(20)-cembratriene-4,6,11-triol (*3*), 20 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-4,6,12-triol (*4*) and 105 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diol (*5*).

(1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-2,7,12(20)-Cembratriene-4,6,11-triol (*3*) had m.p. 106–107 °C; $[\alpha]_D$ +48° (*c* 1.2, EtOH); (Found: $[M-18]^{\cdot+}$ 304.2374. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$) bands at 3605, 3440, 3090, 1650, 1390 and 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.87 (d, *J* = 6.7 Hz)/0.89 (d, *J* = 6.6 Hz) (H-16/H-17), 1.28 (s, H-18), 1.67 (d, *J* = 1.2 Hz, H-19), 1.80 (dd, *J* = 2.7 and −14.4 Hz, H-5a), 2.22 (dd, *J* = 5.5 and −14.4 Hz, H-5b), 4.07 (dd, *J* = 4.5 and 8.5 Hz, H-11), 4.67 (ddd, *J* = 2.7, 5.5 and 9.1 Hz, H-6), 4.94 (m, $W_{1/2}$ = 3 Hz, H-20a), 5.07 (broad s, H-20b), 5.46 (d, *J* = 15.1 Hz, H-3), 5.55 (dd, *J* = 8.2 and 15.1 Hz, H-2) and 5.59 (broad d, *J* = 9.1 Hz, H-7); MS[*m*/*z* (%, composition)]: 304 (M−18, 3), 286 (5, $C_{20}H_{30}O$), 261 (4), 243 (8, $C_{14}H_{27}O_3$), 225 (3, $C_{17}H_{21}$), 205 (7, $C_{14}H_{21}O$, $C_{13}H_{17}O_2$ and $C_{15}H_{25}$), 177 (9, $C_{12}H_{17}O$ and $C_{13}H_{21}$), 159 (12, $C_{12}H_{15}$), 147 (23, $C_{11}H_{15}$ and $C_{10}H_{11}O$), 133 (27, $C_{10}H_{13}$ and C_9H_9O), 123 (30, C_9H_{15} and $C_8H_{11}O$), 109 (31), 95 (40, C_7H_{11} and C_6H_7O), 81 (54, C_5H_5O), 69 (36, C_5H_9 and C_4H_5O), 55 (39, C_4H_7 and C_3H_3O) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-2,7,10-Cembratriene-4,6,12-triol (*4*) had m.p. 140–143 °C; $[\alpha]_D$ +158° (*c* 0.3, $CHCl_3$); (Found: $[M-18]^{\cdot+}$ 304.2379. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR (CCl_4) bands at 3605, 3380, 1390 and 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.80 (d, *J* = 6.7 Hz)/0.85 (d, *J* = 6.7 Hz) (H-16/H-17), 1.21 (s, H-20), 1.29 (s, H-18), 1.66 (broad s, H-19), 1.75 (dd, *J* = 2.1 and −14.2 Hz, H-5a), 2.20 (dd, *J* = 6.1 and −14.2 Hz, H-5b), 2.70 (dd, *J* = 0.8 and 6.9 Hz, H-9a and H-9b), 4.80 (ddd, *J* = 2.1, 6.1 and 8.2 Hz, H-6), 5.40 (d, *J* = 15.5 Hz, H-3), 5.42 (d, *J* = 15.9 Hz, H-11), 5.54 (d, *J* = 8.2 Hz, H-7), 5.54 (dd, *J* = 8.5 and 15.5 Hz, H-2) and 5.64 (dt, *J* = 6.9

and 15.9 Hz, H-10); MS [*m/z* (%, composition)]: 304 (M−18, 3), 286 (5, $C_{20}H_{30}O$), 268 (5, $C_{20}H_{28}$), 243 (6), 225 (5, $C_{17}H_{21}$), 203 (3, $C_{15}H_{23}$ and $C_{14}H_{19}O$), 185 (5, $C_{14}H_{17}$), 159 (10, $C_{12}H_{15}$), 145 (19, $C_{11}H_{13}$), 133 (21, $C_{10}H_{13}$), 119 (22, C_9H_{11}), 105 (27, C_8H_9), 93 (35, C_7H_9), 81 (38, C_5H_5O), 69 (27, C_5H_9 and C_4H_5O), 55 (28) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-11,12-Epoxy-2,7-cembradiene-4,6-diol (*5*) was identified by direct comparison with a synthetic sample (optical rotation, IR, ^{1}H NMR and MS).[9]

Acetylation of (1S,2E,4S,6R,7E,11S)-2,7,12(20)-cembratriene-4,6,11-triol (3). Treatment of 8.2 mg of *3* with acetic anhydride in pyridine at room temperature for 4 h followed by work-up and HPLC using a column packed with μ-Porasil gave 6.5 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-6,11-diacetoxy-2,7,12(20)-cembratrien-4-ol (*6*), which was an oil and had $[\alpha]_D+31°$ (*c* 0.21, $CHCl_3$); IR (CCl_4) bands at 3600, 3480, 3090, 1735, 1652 and 1245 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.86 (d, *J* = 6.5 Hz)/0.88 (d, *J* = 6.5 Hz) (H-16/H-17), 1.32 (s, H-18), 1.72 (d, *J* = 1.2 Hz, H-19), 2.03 (s, OCOC*H*$_3$), 2.05 (s, OCOC*H*$_3$), 4.97 (m, $W_{1/2}$ = 3 Hz, H-20a), 5.09 (broad s, H-20b), 5.14 (broad t, *J* = 6 Hz, H-11), 5.36 (broad d, *J* = 10 Hz, H-7) and 5.5−5.8 (overlapping signals, H-2, H-3 and H-6); MS [*m/z* (%)]: 328 (M−18−60, 3), 304 (1), 286 (6), 268 (8), 253 (3), 243 (6), 225 (14), 197 (5), 185 (10), 159 (11), 145 (19), 133 (21), 119 (19), 105 (37), 93 (36), 79 (23), 69 (17), 60 (22), 55 (19) and 43 (100).

Treatment of (1S,2E,4S,6R,7E,11S)-2,7,12(20)-cembratriene-4,6,11-triol (3) with acid. To a solution of 12 mg of *3* in 2 ml of chloroform was added 0.1 ml of aqueous HCl (10 %). The reaction mixture was kept at room temperature for 30 min, washed with water, dried and evaporated. The residue was separated by HPLC using a column packed with μ-Porasil and ethyl acetate−hexane (20:80) as solvent to give 1.6 mg of a product, whose m.p., optical rotation, IR, ^{1}H NMR and mass spectra were indistinguishable from those of (1*S*,2*E*,4*S*,6*E*,-8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*7*).[6]

Acetylation of (1S,2E,4S,6R,7E,10E,12S)-2,7,10-cembratriene-4,6,12-triol (4). Acetylation of 8.4 mg of *4* using acetic anhydride in pyridine at room temperature for 1 h followed by chromatography over silica gel gave 5.8 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,-12*S*)-6-acetoxy-2,7,10-cembratriene-4,12-diol (*8*), which had m.p. 105−107 °C, $[\alpha]_D+118°$ (*c* 0.56, $CHCl_3$); IR ($CHCl_3$) bands at 3590, 3450, 1720, 1670 and 1230 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.80 (d, *J* = 6.7 Hz)/0.84 (d, *J* = 6.8 Hz) (H-16/H-17), 1.24 (s, H-20), 1.29 (s, H-18), 1.76 (broad s, H-19), 1.91 (dd, *J* = 3.4 and −13.6 Hz, H-5a), 2.05 (s, OCOC*H*$_3$), 2.07 (dd, *J* = 7.6 and −13.6 Hz, H-5b), 2.67 (dd, *J* = 8.1 and −18 Hz, H-9a), 2.76 (dd, *J* = 5.4 and −18 Hz, H-9b), 5.29 (d, *J* = 9.7 Hz, H-7), 5.41 (d, *J* = 15.5 Hz, H-3), 5.44 (d, *J* = 15.4 Hz, H-11), 5.55 (dd, *J* = 8.2 and 15.5 Hz, H-2), 5.67 (ddd, *J* = 5.4, 8.1 and 15.4 Hz, H-10) and 5.74 (ddd, *J* = 3.4, 7.6 and 9.7 Hz, H-6); MS [*m/z* (%)]: 286 (M−60−18, 3), 268 (7), 253 (2), 243 (3), 225 (7), 183 (8), 169 (8), 157 (8), 145 (20), 131 (19), 119 (18), 105 (29), 91 (32), 81 (25), 69 (18), 60 (12), 55 (24) and 43 (100).

Conversion of (1S,2E,4S,6R,7E,11S,12S)-11,12-epoxy-2,7-cembradiene-4,6-diol (5) to (1S,2E,4S,-6R,7E,10E,12S)-2,7,10-cembratriene-4,6,12-triol (4). To a stirred suspension of 27 mg of diphenyl diselenide in 5 ml of dry ethanol was added, under nitrogen, 7 mg of $NaBH_4$. After addition of 45 mg of *5* the reaction mixture was refluxed for 26 h. The solution was cooled and 2.5 ml of tetrahydrofuran was added, followed by dropwise addition of 0.3 ml of hydrogen peroxide (30 %). The temperature was kept below 20 °C while cooling the mixture in an ice-bath. After 3 h the elimination was complete by TLC. The resulting slurry was diluted with water and extracted with ether. The organic phase was washed with aqueous sodium carbonate (10 %), brine and water, dried and concentrated. The residue, 65 mg, was chromatographed over silica gel to give 30.1 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-2,7,10-cembratriene-4,6,12-triol, whose m.p., optical rotation, IR, ^{1}H and ^{13}C NMR and mass spectra were identical with those of the naturally occurring triol (*4*).

Acetylation of (1S,2E,4S,6R,7E,11E)-2,7,11-cembratriene-4,6-diol (2). Acetylation using acetic anhydride in pyridine converted *2* into (1*S*,2*E*,4*S*,6*R*,-7*E*,11*E*)-6-acetoxy-2,7,11-cembratrien-4-ol (*9*), which was an oil and had $[\alpha]_D+139°$, (*c* 1.37, $CHCl_3$); IR ($CHCl_3$) bands at: 3600, 3460, 1725, 1670 and 1255 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.79 (d, *J* = 6.7 Hz)/0.83 (d, *J* = 6.8 Hz) (H-16/H-17), 1.38 (s, H-18), 1.51 (broad s, H-20), 1.72 (d, *J* = 1.2 Hz, H-19), 1.97 (dd, *J* = 8.0 and −13.0 Hz, H-5a), 2.02 (dd, *J* = 3.2 and −13.0 Hz, H-5b), 2.03 (s, OCOC*H*$_3$), 5.03 (m, $W_{1/2}$ = 10 Hz, H-11), 5.24 (broad d, *J* = 10.2 Hz, H-7), 5.32 (dd, *J* = 8 and 16 Hz, H-2), 5.35 (d, *J* = 16 Hz, H-3) and 5.54 (ddd, *J* = 3.2, 8.0 and 10.2 Hz, H-6); MS [*m/z* (%)]: 288 (M−60, 2), 270 (27), 255 (10), 245 (4), 227 (36), 199 (6), 185 (11), 171 (17), 159 (32), 145 (38), 133 (40), 119 (43), 107 (50), 91 (52), 81 (74), 69 (40), 55 (46) and 43 (100).

Photo-oxygenation of (1S,2E,4S,6R,7E,11E)-6-acetoxy-2,7,11-cembratrien-4-ol (9). A solution of 110 mg of *9* and 10 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 80 min when TLC showed that all starting material had been consumed, 100 μl of triethyl phosphite was added, and the reaction mixture was kept at room tem-

perature for 45 min. The solvent was removed under reduced pressure. The residue was filtered through silica gel and subsequently separated by HPLC using a column packed with Spherisorb 5 Nitrile to afford 8.7 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-6-acetoxy-2,7,10-cembratriene-4,12-diol (*11*), 52.2 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-6-acetoxy-2,7,12(20)-cembratriene-4,11-diol (*10*) and 36.1 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-6-acetoxy-2,7,10-cembratriene-4,12-diol (*8*).

(1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*R*)-6-Acetoxy-2,7,10-cembratriene-4,12-diol (*11*) was an oil and had $[\alpha]_D$ +102° (*c* 0.23, $CHCl_3$); IR ($CHCl_3$) bands at 3590, 3440, 1725, 1670 and 1245 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80 (d, J=6.8 Hz)/0.86 (d, J=6.7 Hz) (H-16/H-17), 1.26 (s, H-20), 1.30 (s, H-18), 1.78 (d, J=1.2 Hz, H-19), 1.94 (dd, J=3.6 and −13.4 Hz, H-5a), 2.04 (s, $OCOCH_3$), 2.05 (dd, J=8.8 and −13.4 Hz, H-5b), 2.68 (dd, J=6.6 and −16.2 Hz, H-9a), 2.79 (dd, J=6.1 and −16.2 Hz, H-9b), 5.27 (broad d, J=9.2 Hz, H-7) 5.45 (d, J=15.4 Hz, H-3), 5.50 (d, J=15.7 Hz, H-11), 5.57 (dd, J=8.0 and 15.4 Hz, H-2), 5.62 (ddd, J=6.1, 6.6 and 15.7 Hz, H-10) and 5.70 (ddd, J=3.6, 8.8 and 9.2 Hz, H-7); MS [*m/z* (%)]: 304 (M−60, 2), 286 (4), 268 (3), 243 (8), 225 (4), 203 (3), 185 (6), 159 (11), 145 (20), 133 (23), 119 (12), 105 (22), 93 (26), 81 (28), 74 (27), 59 (40) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-6-Acetoxy-2,7,12(20)-cembratriene-4,11-diol (*10*) was an oil and had $[\alpha]_D$ +61° (*c* 0.57, $CHCl_3$); IR ($CHCl_3$) bands at 3590, 3430, 1720, 1665, 1645 and 1245 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (d, J=6.6 Hz)/0.88 (d, J=6.5 Hz) (H-16/H-17), 1.32 (s, H-18), 1.73 (d, J=1.3 Hz, H-19), 1.99 (dd, J=3.3 and −13.8 Hz, H-5a), 2.04 (s, $OCOCH_3$), 2.09 (dd, J=7.5 and −13.8 Hz, H-5b), 4.03 (t, J=6.5 Hz, H-11), 4.92 (m, $W_{1/2}$=3 Hz, H-20a), 5.07 (broad s, H-20b), 5.35 (broad d, J=9.5 Hz, H-7), 5.46 (d, J=15.4 Hz, H-3), 5.59 (dd, J=8.3 and 15.4 Hz, H-2) and 5.65 (ddd, J=3.3, 7.5 and 9.5 Hz, H-6); MS [*m/z* (%)]; 304 (M−60, 2), 286 (10), 243 (10), 225 (7), 185 (9), 173 (7), 159 (11), 147 (16), 133 (23), 119 (22), 105 (32), 93 (32), 79 (42), 69 (26), 60 (38), 55 (32) and 43 (100).

The third product, (1*S*,2*E*,4*S*,6*R*,7*E*,10*E*,12*S*)-6-acetoxy-2,7,10-cembratriene-4,12-diol (*8*) proved to be identical in all respects (m.p., optical rotation, IR, ^1H NMR and MS) to the acetate derived from the naturally occurring 4*S*,6*R*,12*S*-triol.

Treatment of (1S,2E,4S,6R,7E,11S)-6-acetoxy-2,7,11-cembratriene-4,11-diol (10) with acid. To a solution of 27 mg of *10* in 2 ml of chloroform was added 0.1 ml of aqueous HCl (10 %). The reaction mixture was kept at room temperature for 72 h, washed with aqueous Na_2CO_3 (10 %) and water, dried and evaporated. The residue (18 mg) was chromatographed over silica gel to give 3 mg of starting material (*10*) and 3 mg of a product, whose m.p., optical rotation, IR, ^1H NMR and mass spectra were indistinguishable from those of (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*)-8,11-epoxy-2,6,12(20)-cembratrien-4-ol (*7*).[6]

Acetylation of (1S,2E,4S,6R,7E,11S)-6-acetoxy-2,7,12(20)-cembratriene-4,11-diol (10). Acetylation of 10 mg of *10* using acetic anhydride in pyridine gave, after work-up and HPLC over μ-Porasil, 6.2 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-6,11-diacetoxy-2,7,12(20)-cembratrien-4-ol (*6*), whose optical rotation, IR, ^1H NMR and mass spectra were identical to those of the diacetate obtained from the naturally occurring 4*S*,6*R*,11*S*-triol.

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