

Tobacco Chemistry. 71.* Structure Determination and Biomimetic Studies of Five New Tobacco Cembranoids

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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*R*,7*R*,11*S*)-2,8(19),12(20)-cembratriene-4,6,7,11-tetraol (**1**) and the corresponding (7*S*)-epimer (**2**), (1*S*,2*E*,4*S*,8*S*,11*S*)-4,8,11-trihydroxy-2,12(20)-cembradien-6-one (**3**) and the corresponding (8*R*)-epimer (**4**) and (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*R*)-2,7-cembradiene-4,6,11,12-tetraol (**5**) by spectral methods and biomimetic syntheses (**1**–**4**). The outcome of the reaction of the (4*S*,6*R*,11*S*)-triol **10** and the 6-oxo-(4*S*,8*S*)-diol **14** with singlet oxygen is discussed as is the biogenesis of the new compounds (**1**–**5**). The crystal structures of the tetraol **1** and oxotriol **16** have been determined and are described.

More than sixty diterpenoids of the cembrane class have hitherto been reported as tobacco constituents. These compounds are present in the cuticular wax of the leaf and flower, where they co-occur with compounds such as aliphatic hydrocarbons, fatty alcohols, wax esters and sucrose esters.²

The two C-4 epimers of (1*S*,2*E*,6*R*,7*E*,11*E*)-2,6,11-cembratriene-4,6-diol (**6**, **7**),³ which have certain physiological functions⁴ and antitumour properties,^{5,6} are the major tobacco cembranoids. They are also 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 cembranoids (**1**–**5**), which are likely to arise via oxidation of the (4*S*,6*R*)-diol **6**.

Results

Structure determination of 1–4. The first new tobacco constituent (**1**), C₂₀H₃₄O₄, gave rise to a triacetate (**8**) (three-proton singlets at δ 2.01, 2.08 and 2.16 in the ¹H NMR spectrum) on treatment with acetic anhydride in pyridine. Since **8** contains a tertiary hydroxy group [IR: 3612 and 3522 cm⁻¹; ¹³C NMR: δ 72.0 (s)] compound **1** is evidently a tetraol. **1** also possesses three double bonds, of which two are 1,1-disubstituted [¹³C NMR: δ 110.2 (t), 113.0 (t), 147.7 (s) and 150.0 (s), cf. Table 1] and one is *E*-1,2-disubstituted (*J*_{1,2} = 15.4 Hz). Hence, it was concluded that tetraol **1** is carbomonocyclic.

The presence of an isopropyl group (three-proton doublets at δ 0.86 and 0.90 in the ¹H NMR spectrum) and a

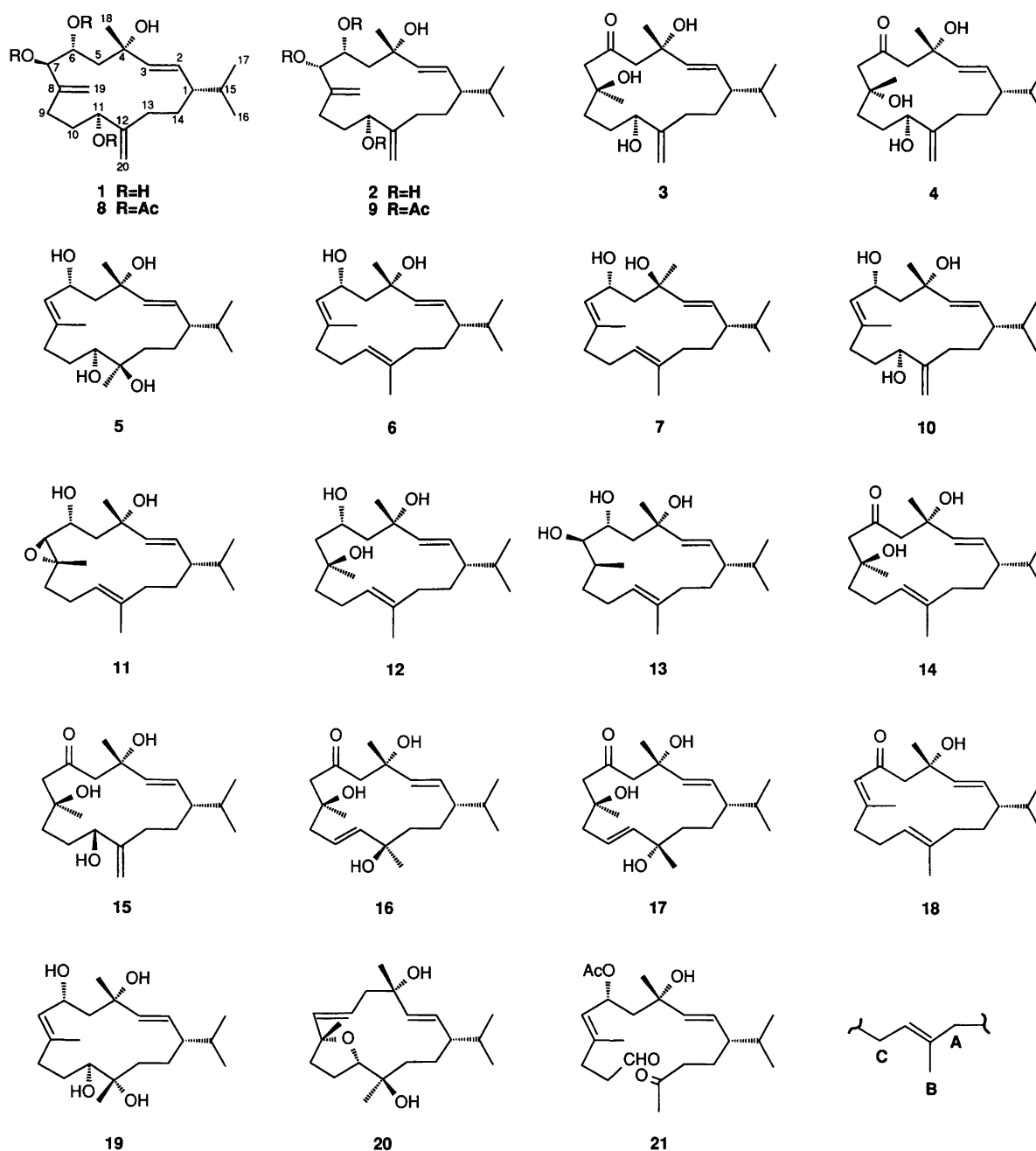
methyl group that is attached to the carbon atom carrying the tertiary hydroxy group [¹H NMR: δ 1.38 (s)] suggested that **1** is a diterpenoid of the cembrane class. This proposition was supported and a (2*E*),8(19),12(20)-cembratriene-4,6,7,11-tetraol structure was provisionally formulated with the aid of results obtained from 1D and 2D NMR experiments. In order to confirm this assignment and to determine the relative stereochemistry crystals of tetraol **1** were subjected to X-ray analysis. The result obtained showed that **1** has the structure proposed and that the relative stereochemistry is 1*S*, 4*S*, 6*R*, 7*R* and 11*S* (*vide infra*).

The second new compound (**2**), C₂₀H₃₄O₄, which formed a triacetate (**9**) on acetylation, was identified as a stereoisomer of **1** from its spectral data. Thus, the signals present at δ 5.15, 5.22 and 5.43 in the ¹H NMR spectrum of triacetate **9** are ascribed to H-6, H-11 and H-7, respectively. H-16 and H-17 resonate as three-proton doublets at δ 0.85 and 0.88, H-18 as a three-proton singlet at δ 1.29, while H-19 and H-20 give rise to characteristic signals at δ 4.94, 4.98, 5.05 and 5.06. H-2 and H-3 are present as a doublet of doublets at δ 5.54 and as a doublet at δ 5.49, respectively. A comparison of the ¹³C NMR spectra of triacetates **8** and **9** (cf. Table 1) suggested that tetraols **1** and **2** both have *S*-configuration at C-11 and differ with respect to the configuration at C-4, C-6 and/or C-7, but no assignment was made at this stage (*vide infra*).

The third new compound (**3**), C₂₀H₃₄O₄, contains an oxo group [IR: 1691 cm⁻¹; ¹³C NMR: δ 214.4 (s)] and two double bonds, of which one is *E*-1,2-disubstituted (*J*_{1,2} = 15.2 Hz) and one is 1,1-disubstituted [¹H NMR: δ 4.87 and 4.97]. The remaining three oxygen atoms in **3** are accommodated by one secondary and two tertiary hydroxy groups

* For part 70 see Ref. 1.

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[OH-absorption in the IR spectrum; ^{13}C NMR signals at δ 71.6 (s), 72.0 (s) and 77.4 (d)]. The oxotriol **3** also possesses an isopropyl group (three-proton doublets at δ 0.90 and 0.91 in the ^1H NMR spectrum) and two methyl groups that are linked to the carbon atoms carrying the tertiary hydroxy groups (three-proton singlets at δ 1.13 and 1.22). A 4,8,11-trihydroxy-(*2E*),12(20)-cembradien-6-one structure accommodates these structural fragments. The formulation of this structure was also strongly supported by results from ^1H - ^1H and ^1H - ^{13}C shift correlation spectroscopy.

An analysis of the spectral data revealed that the fourth new compound (**4**), $\text{C}_{20}\text{H}_{34}\text{O}_4$, is an oxotriol and contains

the same structural fragments as **3**. Their close structural relationship was further illustrated by a comparison of their ^{13}C NMR spectra (cf. Table 1). Compound **4** was hence tentatively identified as a (*2E*)-4,8,11-trihydroxy-2,12(20)-cembradien-6-one isomeric with **3**. It was also concluded from the shielding of C-18 in the ^{13}C NMR spectra of **3** and **4**, δ 29.5 and 29.6, that both compounds have a (*4S*)-stereochemistry.

A biomimetic strategy was chosen to determine the absolute stereochemistry of **1** and the stereostructures of **2-4**. To this end, the (*4S,6R,11S*)-triol **10**,⁷ which has the prerequisite functionalities and appropriate stereochemistry at

Table 1. ^{13}C NMR chemical shift values and assignments for compounds **1**, **3**–**5**, **8**, **9**, **12**, **14**–**17** and **19**.^a

Com- pound	Carbon																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	49.8	130.0	137.6	72.5	44.0	68.7	73.0 ^b	147.7	33.3	29.5	73.8 ^b	150.0	30.8	27.6	32.3	19.8	20.7	32.6	110.2	113.0
3	50.0	131.1	135.9	71.6 ^b	51.8	214.4	50.9	72.0 ^b	36.2	30.1	77.4	149.7	25.7	27.6	32.3	19.7	20.6	29.5	27.5	112.1
4	49.2	130.9	136.4	71.6 ^b	53.0	211.3	52.6	72.0 ^b	35.1	28.7 ^c	75.9	150.3	27.1 ^c	28.2 ^c	32.4	19.7	20.7	29.6 ^d	29.8 ^d	109.8
5	51.0	129.4	137.6	73.7	47.0	68.0	126.5	138.1	27.7 ^b	37.2	74.9	74.9	33.8	26.7 ^b	32.6 ^c	19.9	21.1	31.6 ^c	17.3	24.6
8^e	49.3	131.1	137.7	72.0	42.1	69.9	75.5	143.6	30.1 ^b	30.3 ^b	74.5	145.2	29.3 ^b	27.8	32.2	19.7	20.6	32.3	112.9	111.2
9^f	47.9	131.4	136.6	72.3	40.6	71.0	75.5	142.6	28.3 ^b	29.6 ^b	75.2	146.5	29.9 ^b	28.2 ^b	32.5	19.6	20.4	29.1	112.4	111.3
12	47.0	129.2	138.9	73.4	48.2 ^b	69.1	45.5 ^b	74.4	43.3	23.0	126.5	133.5	38.3	28.8	33.0	19.0	20.1	34.6	27.8	14.9
14	47.5	131.6	135.8	72.0 ^b	54.9	214.1	53.0	72.1 ^b	40.7	22.9	125.8	134.6	37.4	28.2	33.1	19.5	20.3	30.5	27.8	15.4
15	49.6	131.2	135.9	71.7 ^b	52.2	214.1	51.4	72.0 ^b	32.9	30.0 ^c	73.4	149.9	29.8 ^c	28.1	32.3	19.7	20.6	29.6	27.8	106.7
16	48.6	130.8	134.9	71.7 ^b	53.5	213.6	52.5	71.8 ^b	44.9	122.6	139.7	73.1	40.5	27.1	31.4	19.9	20.4	30.0	28.4	27.6
17	49.2	131.7	135.0	71.7 ^b	54.7	213.4	52.4	71.8 ^b	45.2	124.1	139.6	72.6	39.2	27.0	30.8	20.3	20.5	30.6	28.7	27.5
19	50.6	130.3	137.1	73.7	47.3	67.1	128.3	138.2	28.2	35.3	74.8	75.0	37.9	26.0	31.8	19.7	20.9	31.7	16.3	20.3

^a δ -values in CDCl_3 relative to TMS. ^{b,c,d}Assignment may be reversed. ^e OCOCH_3 170.1, 170.2, 170.4; OCOCH_3 20.9, 21.1, 21.2. ^f OCOCH_3 169.9, 170.3, 170.7; OCOCH_3 20.9, 21.1, 21.3.

C-1, C-4, C-6 and C-11 to serve as a precursor of **1**, was selected for photo-oxygenation using Rose Bengal as the sensitizer. As expected, the reaction proceeded with an attack by singlet oxygen at the trisubstituted 7,8 double bond. After reduction of the hydroperoxides initially formed, four products were isolated. The major one was identical in all respects with the tetraol **1** hence establishing that its absolute stereochemistry is *1S*, *4S*, *6R*, *7R* and *11S*. The remaining three products proved to be indistinguishable from **2**, **3** and **4**, respectively. Consequently, it can be concluded that **2** is the (*7S*)-epimer of **1** and that **3** and **4** are the C-8 epimers of (*1S,2E,4S,11S*)-4,8,11-trihydroxy-2,12(20)-cembradien-6-one.

In order to resolve the chirality of C-8 in **3** and **4**, epoxide **11**,⁸ which has a (*7S,8S*)-stereochemistry, was refluxed with lithium aluminium hydride (LAH) in dioxane. Two products were obtained: the (*4S,6S,8S*)-triol **12** and the (*4S,6R,7R*)-triol **13**. In the ^1H NMR spectrum of **12**, the H-18 and H-19 signals are displayed as three-proton singlets at δ 1.24 and 1.26, while in the spectrum of **13**, H-19 gives rise to a three-proton doublet at δ 0.87 and H-7 to a multiplet at δ 3.36.

Oxidation using pyridinium dichromate converted the (*4S,6S,8S*)-triol **12** into the 6-oxo compound **14** (IR band at 1692 cm^{-1} ; ^1H NMR: two AB doublets at δ 2.5–2.8). With a view to introducing a hydroxy group at C-11, the oxodiol **14** was next treated with singlet oxygen, Rose Bengal being used as the sensitizer. The hydroperoxides generated were treated with triethyl phosphite, four products being obtained after the reduction. One of these was identical with tobacco constituent **3**. We can hence conclude that **3** and **4** have (*8S*)- and (*8R*)-stereochemistry, respectively. Of the remaining three products one was identified as the (*11R*)-epimer of **3** (**15**) from its spectral data. Thus, a multiplet at δ 4.20 and two characteristic broad singlets at δ 4.90 and 5.05 in the ^1H NMR spectrum of **15** are ascribed to H-11, H-20a and H-20b, respectively. The identification of **15** as the (*11R*)-epimer of **3** was also consistent with the ^{13}C NMR data. While the signals due to C-9, C-11, C-13 and C-20 show divergent chemical shift values, the other signals are present at virtually invariant positions in the spectra of the two epimers (**3**, **15**; Table 1).

^1H and ^{13}C NMR data were used to identify the remaining two compounds as the 12-epimers of (*2E,4S,8S,10E*)-4,8,12-trihydroxy-2,10-cembradien-6-one (**16**, **17**), while the assignment of a (*12R*)-configuration to **16** and a (*12S*)-configuration to **17** was based on an X-ray analysis of **16** (*vide infra*).

The outcome of the reaction of the 6-oxo-(*4S,8S*)-diol **14** with singlet oxygen is worthy of further comment. The hydroperoxide precursors of **3**, **15**, **16** and **17** are all formed by *syn*-ene processes, that is hydrogen abstraction occurs from the 1,2-disubstituted side of the trisubstituted 11,12-double bond. This result is consistent with previous findings that *syn*-ene additions are favoured in acyclic and most cyclic systems.^{9–11}

Compounds **3**, **15**, **16** and **17** are obtained in the ratio

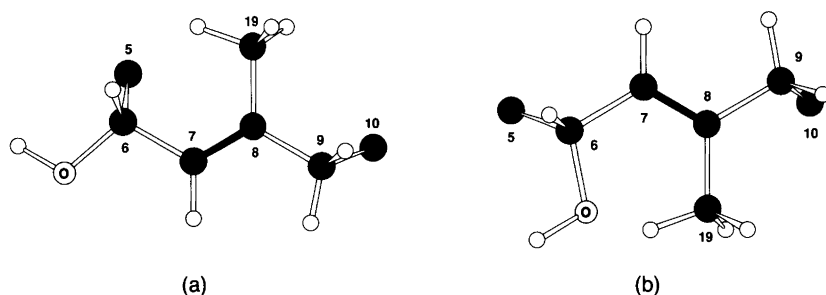


Fig. 1. Reacting conformers in the conversion of **10** into **1–4**.

23:10:21:46, *i.e.* the 4,8,12-triols are formed in preference to the 4,8,11-triols (67:33). This indicates that the hydrogen atoms at C-10 (site C, cf. Formulae) are more reactive than those at C-20 (site B) during the ene addition. Similar results have previously been obtained for the 6-oxo compound **18**,¹² while roughly equal reactivities were observed when the 4,6-diols **6** and **7** were treated singlet oxygen.¹³ The reason for this discrepancy is not yet fully known. It should be noted, however, that studies involving various trisubstituted acyclic olefins have shown that the reactivities are as follows: site C > site B > site A.^{14,15}

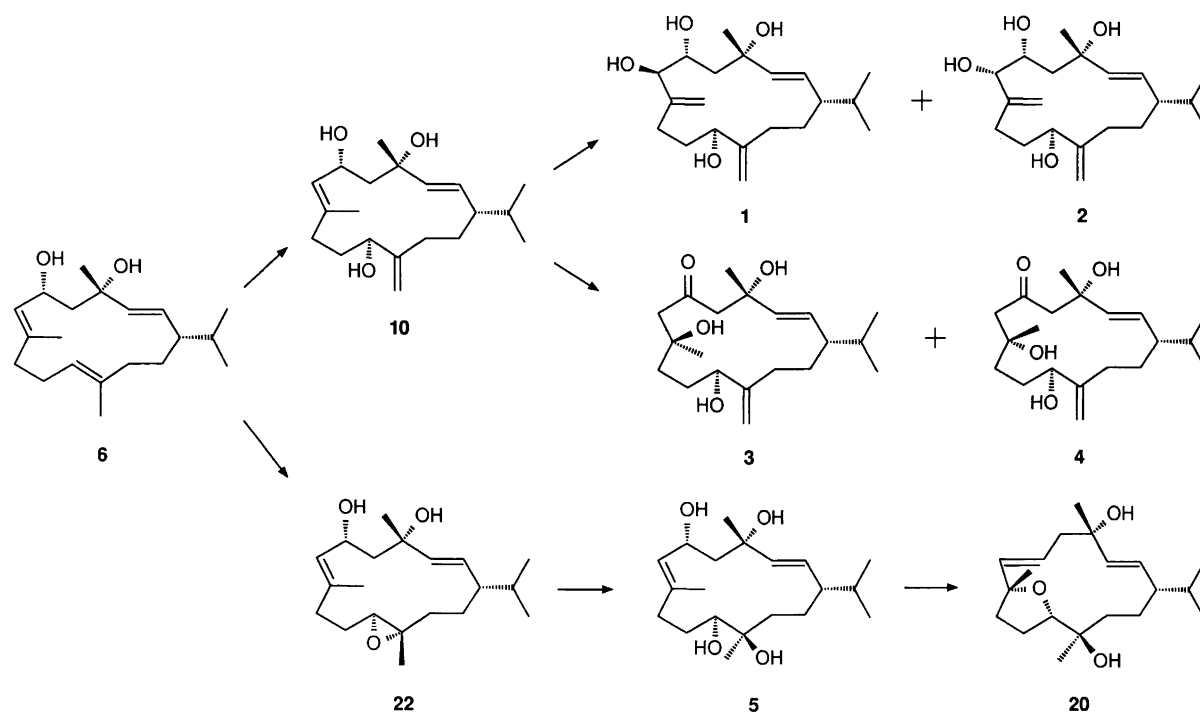
It also follows from the product ratio that the reaction occurs with a slight degree of stereoselectivity, the (11*S*)- and (12*S*)-triols being formed in preference to their (11*R*)- and (12*R*)-counterparts (69:31). In keeping with the *cis*-cyclic mechanism of the ene reaction this effect may be accounted for by conformational arguments. It is noteworthy that the reaction of the (4*S*,6*R*)- and (4*R*,6*R*)-diols **6** and **7** with singlet oxygen takes place with higher stereoselectivity (89:11 and 94:6, respectively).¹³

The (4*S*,6*R*,11*S*)-triol **10** also reacts with singlet oxygen

in a *syn*-ene fashion, compounds **1**, **2**, **3** and **4** being obtained in the ratio 77:17:3:3. Hence, the formation of the hydroperoxide precursors of the 4,6,7,11-tetraols **1** and **2** is highly favoured over the formation of the hydroperoxide precursors of the 6-oxo-4,8,11-triols **3** and **4** (94:6).

The generation of the hydroperoxide precursors of compounds **1** and **3** may be accounted for by reactions taking place with conformer (a) of the (4*S*,6*R*,11*S*)-triol **10**, while the hydroperoxide precursors of **2** and **4** would arise by reactions occurring with conformer (b) (Fig. 1). Since **1** and **3** are formed in preference to **2** and **4** (80:20), it is evident that conformer (a) is more populated or reacts faster than conformer (b). It is worth mentioning that the spatial arrangement of the C-5 to C-8 portion of the (4*S*,6*R*,11*S*)-triol **10** in the solid state is reminiscent of conformer (a).⁷

Structure determination of 5. The fifth new tobacco isolate (**5**), C₂₀H₃₆O₄, is a tetraol having two secondary and two tertiary hydroxy groups [¹H NMR: δ 3.46 (m) and 4.71 (ddd); ¹³C NMR: δ 68.0 (d), 73.7 (s), 74.0 (d), and 74.9 (s)]. The ¹H NMR spectrum also contains signals consistent



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

Table 2. Atom coordinates ($\times 10$) and equivalent isotropic temperature factors ($A \times 100$) for the non-hydrogen atoms and fixed isotropic temperature factor (U_{iso} 5.0) for the hydrogen atoms in the tetraol **1** and oxotriol **16**.

	<i>x</i>	<i>y</i>	<i>z</i>	U_{eq}^a		<i>x</i>	<i>y</i>	<i>z</i>	U_{eq}^a
Tetraol 1					Oxotriol 16				
C(1)	13687(8)	−4(4)	1963(1)	4.49(12)	C(1)	8987(2)	3581	9940(2)	3.13(6)
H(1)	12245(8)	−583(4)	1937(1)		H(1)	9222(2)	2665	10499(2)	
C(2)	13503(7)	1167(3)	1672(1)	4.09(11)	C(2)	7369(2)	3750(3)	9352(2)	3.29(6)
H(2)	14906(7)	1777(3)	1640(1)		H(2)	6960(2)	4731(3)	9018(2)	
C(3)	11758(7)	1490(3)	1457(1)	3.98(11)	C(3)	6445(2)	2782(3)	9227(2)	3.58(6)
H(3)	10400(7)	844(3)	1487(1)		H(3)	6886(2)	1800(3)	9511(2)	
C(4)	11411(6)	2657(3)	1172(1)	3.65(10)	C(4)	4828(2)	2883(3)	8728(2)	3.54(6)
C(5)	10894(6)	2258(3)	705(1)	3.48(10)	C(5)	4142(2)	1973(3)	7491(2)	4.01(7)
H(5A)	12330(6)	1815(3)	575(1)		H(5A)	3053(2)	1754(3)	7432(2)	
H(5B)	10541(6)	3128(3)	529(1)		H(5B)	4746(2)	1057(3)	7622(2)	
C(6)	9013(6)	1323(3)	627(1)	3.31(10)	C(6)	4125(2)	2608(3)	6197(2)	3.66(6)
H(6)	9019(6)	608(3)	879(1)		C(7)	5460(2)	2517(3)	5816(2)	3.49(6)
C(7)	9284(5)	672(3)	190(1)	3.44(10)	H(7A)	6210(2)	3250(3)	6381(2)	
H(7)	9641(5)	1399(3)	−48(1)		H(7B)	5905(2)	1531(3)	6069(2)	
C(8)	11200(6)	−252(3)	157(1)	3.18(9)	C(8)	5245(2)	2756(3)	4344(2)	3.26(6)
C(9)	11491(6)	−1242(3)	513(1)	3.63(10)	C(9)	6698(2)	2743(3)	4108(2)	4.17(7)
H(9A)	11554(6)	−741(3)	817(1)		H(9A)	6516(2)	2917(3)	3071(2)	
H(9B)	10118(6)	−1883(3)	520(1)		H(9B)	7178(2)	1773(3)	4378(2)	
C(10)	13554(6)	−2052(4)	467(1)	3.76(11)	C(10)	7730(2)	3784(3)	4890(2)	4.06(7)
H(10A)	13469(6)	−2567(4)	166(1)		H(10)	7464(2)	4818(3)	4653(2)	
H(10B)	14920(6)	−1405(4)	462(1)		C(11)	8934(2)	3517(3)	5842(2)	3.54(6)
C(11)	13913(7)	−3038(3)	829(1)	3.90(11)	H(11)	9219(2)	2477(3)	6005(2)	
H(11)	15554(7)	−3386(3)	811(1)		C(12)	9965(2)	4507(3)	6736(2)	3.62(6)
C(12)	13808(7)	−2481(3)	1281(1)	3.97(11)	C(13)	9624(2)	4716(3)	8027(2)	3.55(6)
C(13)	15427(7)	−1425(4)	1363(1)	4.83(13)	H(13A)	8568(2)	5147(3)	7782(2)	
H(13A)	17001(7)	−1798(4)	1281(1)		H(13B)	10401(2)	5401(3)	8640(2)	
H(13B)	15031(7)	−636(4)	1151(1)		C(14)	9653(2)	3447(3)	8818(2)	3.54(6)
C(14)	15578(8)	−873(4)	1823(1)	5.37(14)	H(14A)	9072(2)	2684(3)	8145(2)	
H(14A)	17046(8)	−316(4)	1844(1)		H(14B)	10755(2)	3144(3)	9256(2)	
H(14B)	15662(8)	−1674(4)	2043(1)		C(15)	9668(2)	4685(3)	10953(2)	4.00(7)
C(15)	13902(9)	456(4)	2441(1)	5.56(15)	H(15)	9463(2)	5611(3)	10410(2)	
H(15)	15221(9)	1137(4)	2445(1)		C(16)	11282(2)	4528(4)	11570(3)	6.47(10)
C(16)	14484(13)	−642(6)	2749(2)	8.19(23)	H(16A)	11867(2)	4681(4)	10889(3)	
H(16A)	16044(13)	−1055(6)	2678(2)		H(16B)	11490(2)	3537(4)	11982(3)	
H(16B)	13275(13)	−1391(6)	2749(2)		H(16C)	11619(2)	5254(4)	12355(3)	
H(16C)	14532(13)	−188(6)	3061(2)		C(17)	8987(3)	4726(3)	12052(3)	6.12(10)
C(17)	11850(11)	1132(6)	2595(2)	7.71(20)	H(17A)	7839(3)	4806(3)	11616(3)	
H(17A)	11523(11)	2010(6)	2421(2)		H(17B)	9384(3)	5553(3)	12717(3)	
H(17B)	11925(11)	1336(6)	2935(2)		H(17C)	9252(3)	3801(3)	12592(3)	
H(17C)	10578(11)	445(6)	2534(2)		C(18)	4268(2)	2445(3)	9846(2)	5.26(9)
C(18)	9644(8)	3533(4)	1360(1)	5.17(13)	H(18A)	4590(2)	1425(3)	10102(2)	
H(18A)	8102(8)	3066(4)	1405(1)		H(18B)	3115(2)	2510(3)	9515(2)	
H(18B)	9440(8)	4367(4)	1157(1)		H(18C)	4708(2)	3073(3)	10701(2)	
H(18C)	10278(8)	3829(4)	1668(1)		C(19)	4269(2)	1686(3)	3475(2)	4.50(8)
C(19)	12462(6)	−181(4)	−191(1)	3.99(11)	H(19A)	4736(2)	712(3)	3750(2)	
H(19A)	14017(6)	290(4)	−194(1)		H(19B)	4165(2)	1884(3)	2457(2)	
H(19B)	12362(6)	−844(4)	−457(1)		H(19C)	3225(2)	1717(3)	3594(2)	
C(20)	12464(10)	−2955(4)	1573(1)	6.21(16)	C(20)	9978(3)	5845(3)	6061(2)	5.16(9)
H(20A)	12952(10)	−3606(4)	1825(1)		H(20A)	8961(3)	6346(3)	5850(2)	
H(20B)	10942(10)	−2514(4)	1657(1)		H(20B)	10194(3)	5666(3)	5149(2)	
O(1)	13346(5)	3454(2)	1155(1)	4.26(9)	H(20C)	10813(3)	6463(3)	6700(2)	
H(O1)	14158(80)	3162(48)	998(16)		O(1)	4459(2)	4250(2)	8389(2)	4.19(5)
O(2)	6967(4)	2007(3)	634(1)	4.66(9)	H(O1)	3553(28)	4265(24)	8202(24)	
H(O2)	6514(81)	1482(46)	427(15)		O(2)	3055(2)	3186(3)	5505(2)	5.72(6)
O(3)	7288(4)	8(3)	110(1)	4.42(8)	O(3)	4652(2)	4049(2)	3940(2)	4.51(5)
H(O3)	7387(78)	−387(46)	−160(16)		H(O3)	3958(28)	4124(25)	4124(25)	
O(4)	12407(5)	−4083(2)	760(1)	4.44(8)	O(4)	11398(1)	3945(3)	7152(2)	4.98(6)
H(O4)	12848(82)	−4813(44)	900(15)		H(O4)	11515(27)	3808(27)	6494(27)	

$$^a U_{eq} = \frac{1}{3} \sum_i \sum_j U_{ij} \cdot a_i^* \cdot a_j^* \cdot a_i \cdot a_j$$

with the presence of an isopropyl group [δ 0.86 (d) and 0.91 (d)], three methyl groups, of which one is vinylic [δ 1.76 (d)] and two attached to the carbon atoms carrying the tertiary hydroxy groups [δ 1.23 (s) and 1.28 (s)]. Of the two double bonds present one is di- and one is tri-substituted [$^1\text{H NMR}$: δ 5.53 (d), 5.62 (dd) and 5.69 (dd); $^{13}\text{C NMR}$: δ 126.5 (d), 129.4 (d), 137.6 (d) and 138.1 (s)]. These results indicated that tetraol **5** is a cembranoid. Since both the ^1H and ^{13}C NMR spectra are reminiscent of those of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-4,6,11,12-tetraol (**19**),¹⁶ **5** was tentatively identified as a stereoisomer thereof.

This assignment was verified and the stereochemistry was resolved by chemical means. When treated with weakly acidified chloroform, **5** underwent a facile conversion into the (8*R*,11*S*)-epoxide **20**.¹⁷ This product is likely to arise via dehydration involving the hydroxy group at C-6, double-bond migration and a concomitant attack of the hydroxy group at C-11 on C-8. Its formation is then consistent with (1*S*,2*E*,11*S*,12*R*)-stereochemistry in tetraol **5**, leaving the configurations of C-4 and C-6 and the geometry of the 7,8 double bond to be accounted for.

Tetraol **5** was next treated with NaIO_4 , and the seco-compound formed by cleavage of the 11,12 bond was acetylated. Since the acetate obtained was identical with (4*E*,6*R*,8*S*,9*E*,11*S*)-6-acetoxy-8-hydroxy-11-isopropyl-4,8-dimethyl-14-oxo-4,9-pentadecadienal (**21**),¹⁶ **5** is now conclusively identified as (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*R*)-2,7-cembradiene-4,6,11,12-tetraol.

Table 3. Bond lengths (Å) in the tetraol **1** and oxotriol **16**.

Tetraol 1			
C(2)–C(1)	1.521(7)	C(14)–C(1)	1.542(8)
C(15)–C(1)	1.569(8)	C(3)–C(2)	1.315(7)
C(4)–C(3)	1.518(7)	C(5)–C(4)	1.545(7)
C(18)–C(4)	1.539(7)	O(1)–C(4)	1.457(5)
C(6)–C(5)	1.536(7)	C(7)–C(6)	1.530(7)
O(2)–C(6)	1.453(5)	C(8)–C(7)	1.529(6)
O(3)–C(7)	1.437(5)	C(9)–C(8)	1.524(7)
C(19)–C(8)	1.337(6)	C(10)–C(9)	1.536(7)
C(11)–C(10)	1.538(7)	C(12)–C(11)	1.524(7)
O(4)–C(11)	1.447(6)	C(13)–C(12)	1.508(7)
C(20)–C(12)	1.328(7)	C(14)–C(13)	1.546(8)
C(16)–C(15)	1.532(9)	C(17)–C(15)	1.528(10)
Oxotriol 16			
C(2)–C(1)	1.525(4)	C(14)–C(1)	1.553(5)
C(15)–C(1)	1.543(5)	C(3)–C(2)	1.310(4)
C(4)–C(3)	1.516(4)	C(5)–C(4)	1.570(5)
C(18)–C(4)	1.537(5)	O(1)–C(4)	1.435(3)
C(6)–C(5)	1.521(5)	C(7)–C(6)	1.505(5)
O(2)–C(6)	1.222(3)	C(8)–C(7)	1.540(5)
C(9)–C(8)	1.538(4)	C(19)–C(8)	1.538(5)
O(3)–C(8)	1.431(3)	C(10)–C(9)	1.509(5)
C(11)–C(10)	1.319(4)	C(12)–C(11)	1.519(5)
C(13)–C(12)	1.540(5)	C(20)–C(12)	1.527(5)
O(4)–C(12)	1.454(3)	C(14)–C(13)	1.525(5)
C(16)–C(15)	1.524(5)	C(17)–C(15)	1.534(5)

Table 4. Bond angles (deg.) in the tetraol **1** and oxotriol **16**.

Tetraol 1			
C(14)–C(1)–C(2)	110.9(4)	C(15)–C(1)–C(2)	109.1(4)
C(15)–C(1)–C(14)	112.5(5)	C(3)–C(2)–C(1)	124.7(5)
C(4)–C(3)–C(2)	128.2(5)	C(5)–C(4)–C(3)	111.4(4)
C(18)–C(4)–C(3)	110.5(4)	C(18)–C(4)–C(5)	111.7(4)
O(1)–C(4)–C(3)	111.0(4)	O(1)–C(4)–C(5)	106.9(4)
O(1)–C(4)–C(18)	105.2(4)	C(6)–C(5)–C(4)	118.4(4)
C(7)–C(6)–C(5)	109.7(4)	O(2)–C(6)–C(5)	110.4(4)
O(2)–C(6)–C(7)	109.0(4)	C(8)–C(7)–C(6)	115.0(4)
O(3)–C(7)–C(6)	105.7(4)	O(3)–C(7)–C(8)	110.7(4)
C(9)–C(8)–C(7)	117.9(4)	C(19)–C(8)–C(7)	118.2(4)
C(19)–C(8)–C(9)	123.9(4)	C(10)–C(9)–C(8)	113.6(4)
C(11)–C(10)–C(9)	114.6(4)	C(12)–C(11)–C(10)	114.6(4)
O(4)–C(11)–C(10)	107.4(4)	O(4)–C(11)–C(12)	113.2(4)
C(13)–C(12)–C(11)	113.9(4)	C(20)–C(12)–C(11)	121.3(5)
C(20)–C(12)–C(13)	124.8(5)	C(14)–C(13)–C(12)	117.8(5)
C(13)–C(14)–C(1)	115.7(4)	C(16)–C(15)–C(1)	112.7(5)
C(17)–C(15)–C(1)	111.5(5)	C(17)–C(15)–C(16)	110.0(6)
Oxotriol 16			
C(14)–C(1)–C(2)	110.0(3)	C(15)–C(1)–C(2)	111.7(3)
C(15)–C(1)–C(14)	114.1(3)	C(3)–C(2)–C(1)	124.3(3)
C(4)–C(3)–C(2)	127.4(3)	C(5)–C(4)–C(3)	112.0(3)
C(18)–C(4)–C(3)	108.2(3)	C(18)–C(4)–C(5)	109.0(3)
O(1)–C(4)–C(3)	107.7(2)	O(1)–C(4)–C(5)	109.7(3)
O(1)–C(4)–C(18)	110.2(3)	C(6)–C(5)–C(4)	113.6(3)
C(7)–C(6)–C(5)	118.1(3)	O(2)–C(6)–C(5)	120.2(3)
O(2)–C(6)–C(7)	121.7(3)	C(8)–C(7)–C(6)	115.3(3)
C(9)–C(8)–C(7)	110.0(2)	C(19)–C(8)–C(7)	111.2(3)
C(19)–C(8)–C(9)	109.3(3)	O(3)–C(8)–C(7)	110.9(3)
O(3)–C(8)–C(9)	105.7(3)	O(3)–C(8)–C(19)	109.5(3)
C(10)–C(9)–C(8)	113.7(3)	C(11)–C(10)–C(9)	124.5(3)
C(12)–C(11)–C(10)	127.3(3)	C(13)–C(12)–C(11)	110.9(2)
C(20)–C(12)–C(11)	112.6(3)	C(20)–C(12)–C(13)	110.1(3)
O(4)–C(12)–C(11)	109.1(3)	O(4)–C(12)–C(13)	104.7(3)
O(4)–C(12)–C(20)	109.2(3)	C(14)–C(13)–C(12)	114.5(3)
C(13)–C(14)–C(1)	114.9(3)	C(16)–C(15)–C(1)	112.7(3)
C(17)–C(15)–C(1)	111.0(3)	C(17)–C(15)–C(16)	109.2(3)

Biogenesis. Previous studies have suggested that most of the tobacco cembranoids are formed from the 4,6-diols **6** and **7** by reactions such as oxidations, dehydrations, acid- and base-induced rearrangements and eliminations.²

It seems highly probable that the five newly discovered tobacco constituents (**1**–**5**) are all metabolites of the (4*S*,6*R*)-diol **6**. Thus, as proposed in Scheme 1, the biogenetic route to compounds **1**–**4** may involve singlet oxygen reactions, or enzyme-catalysed oxygenation reactions, taking place at the 7,8- and 11,12-double bonds in the (4*S*,6*R*)-diol **6**. Since the 11,12-double bond is more susceptible to oxidation than the 7,8-double bond,¹³ it is highly plausible that the (4*S*,6*R*,11*S*)-triol **10** is an intermediate in the biogenesis of **1**–**4**. This view is substantiated by the facts that 4,6,11-triols (e.g. **10**) are present in a fair amount in tobacco⁷ and that no 2,8(19),11-cembratriene-4,6,7-triols or 4,8-dihydroxy-2,11-cembradiene-6-ones (e.g. **14**) have hitherto been encountered.

The new 4,6,11,12-tetraol **5** is probably formed from the (4*S*,6*R*)-diol **6** by an *anti*-addition of water to the corresponding (11*S*,12*S*)-epoxide **22**. The latter is a tobacco

Table 5. Selected non-bonded distances (Å) and possible hydrogen bonds in the tetraol **1** and oxotriol **16**.

Tetraol 1		Oxotriol 16	
Intramolecular			
O(2)–O(3)	2.649	O(2)–O(1)	3.134
		O(3)–O(2)	2.787
Intermolecular			
C(22)–C(11b)	3.492	O(4)–C(4b)	3.429
O(21)–C(10a)	3.340	O(4)–C(5b)	3.280
O(4)–O(1b)	2.898	O(4)–C(6b)	3.450
O(4)–O(3c)	2.876	O(3)–C(7c)	3.493
O(2)–O(1a)	3.150	O(4)–O(2b)	2.873
		O(4)–O(1b)	2.898

Key to symmetry operations relating designated atoms to reference atoms at (x, y, z) :

- (a) $-1.0+x, y, z$ (a) $1.0-x, 0.5+y, 1.0-z$
 (b) $x, -1.0+y, z$ (b) $1.0+x, y, z$
 (c) $0.5+x, -0.5-y, -z$

constituent⁷ as is the (8*R*,11*S*)-epoxide **20**¹⁷ arising from tetraol **5** by a facile acid-induced rearrangement.

Crystallography. Final fractional coordinates with estimated standard deviations and equivalent isotropic temperature factors for the non-hydrogen atoms of the tetraol **1** and oxotriol **16** are listed in Table 2. Bond lengths and bond angles, both with estimated standard deviations, are found in Tables 3 and 4, while selected non-bonded distances and

possible hydrogen bonds are given in Table 5. Crystal and experimental data are listed in Table 6. Stereoscopic drawings of tetraol **1** and oxotriol **16** are shown in Figs. 2 and 3.

The maximum, minimum and mean values of the sp^3 – sp^3 bonds are 1.528(10), 1.569(8) and 1.540 Å in tetraol **1** and 1.524(5), 1.570(5) and 1.539 Å in oxotriol **16**. The sp^2 – sp^2 bonds are in the range 1.315(7) to 1.337(6) in tetraol **1** and 1.310(4) to 1.319(4) Å in oxotriol **16**. All bond angles are within the expected range. Intermolecular contacts less than 3.5 Å are listed in Table 5, all other intermolecular distances corresponding to normal van der Waals contacts. Possible intermolecular hydrogen bonds are in the range (acceptor–donor) 2.876 to 3.150 Å for tetraol **1** and 2.873 to 2.898 Å for oxotriol **16**. Tetraol **1** has one intramolecular hydrogen bond, O(2)–O(3), the distance between acceptor and donor being 2.649 Å; oxotriol **16** has two: O(2)–O(1) of 3.134 Å and O(3)–O(2) of 2.787 Å.

Experimental

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter and part of the high performance liquid chromatography work was carried out using a Waters Delta Prep 3000 solvent delivery system, a Water U6K injector and a Waters R-403 differential refractometer. For other instrumental details see Ref. 16.

Isolation. Fraction D (128 g),¹⁸ obtained from an extract of flowers of Greek *Nicotiana tabacum* (Basma), was separated by flash chromatography over silica gel (hexane/EtOAc/methanol gradient) into 8 fractions,

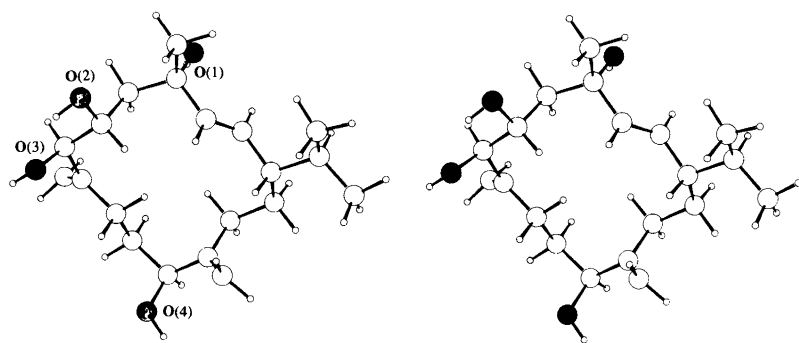


Fig. 2. A stereoscopic view of (1*S*,2*E*,4*S*,6*R*,7*R*,11*S*)-2,8(19),12(20)-cembratriene-4,6,7,11-tetraol (**1**).

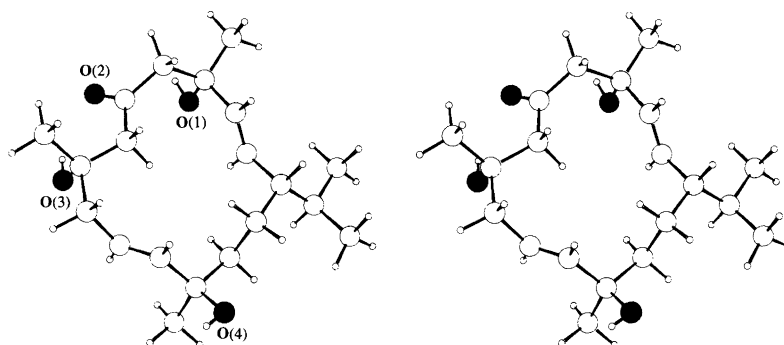


Fig. 3. A stereoscopic view of (1*S*,2*E*,4*S*,8*S*,10*E*,12*R*)-4,8,12-trihydroxy-2,10-cembradien-6-one (**16**).

D1–D8. Fraction D3 was separated into 5 fractions by HPLC (Spherisorb 5 CN; hexane/EtOAc 40:60). Repetitive HPLC of fraction D33 (3.6 g) using columns packed with Lichrosorb Diol (hexane/EtOAc 10:90) and Spherisorb 5 ODS (acetonitrile/water 70:30) gave 4.0 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,11*S*)-2,8(19),12(20)-cembratriene-4,6,7,11-tetraol (**2**). The (7*R*)-epimer of **2** (**1**, 5.6 mg) was isolated from fraction D32 (595 mg) by HPLC using columns packed with Spherisorb (EtOAc) and Lichrosorb Diol (hexane/EtOAc 10:90). Fraction D7 (10.5 g) was separated further into 8 fractions D71–D78 by HPLC (Spherisorb 5; EtOAc). Repetitive HPLC of fraction D75 (721 mg) (Lichrosorb Diol; hexane/EtOAc 10:90, Spherisorb 5 CN; hexane/EtOAc 30:70) afforded 8.0 mg of (1*S*,2*E*,4*S*,8*S*,11*S*)-4,8,11-trihydroxy-2,12(20)-cembradien-6-one (**3**) and 9.3 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*R*)-2,7-cembradiene-4,6,11,12-tetraol (**5**). Further separation of fraction D74 (1.0 g) by HPLC (Lichrosorb Diol; hexane/EtOAc 10:90, Spherisorb 5 CN; hexane/EtOAc 30:70, Spherisorb 5; EtOAc) gave 0.8 mg of (1*S*,2*E*,4*S*,8*R*,11*S*)-4,8,11-trihydroxy-2,12(20)-cembradien-6-one (**4**).

Compound **1** had m.p. 121–123°C; $[\alpha]_D^{+21}$ (c 0.30, EtOH); (Found $[M-18]^+$: 320.2306. Calc. for $C_{20}H_{32}O_3$: 320.2351); IR (KBr): 3482, 3397, 3104 and 1645 cm^{-1} ; 1H NMR ($CDCl_3$): δ 0.86 (d, J 6.7 Hz)/0.90 (d, J 6.7 Hz) (H-16/H-17), 1.38 (s, H-18), 1.90 (dd, J 5.5 and -14.3 Hz, H-5a), 1.99 (dd, J 8.9 and -14.3 Hz, H-5b), 3.82 (ddd, J 2.5, 5.5, and 8.9 Hz, H-6), 4.32 (dd, J 4.3 and 5.6 Hz, H-11), 4.43 (br s, H-7), 4.93 (quintet, J 1.4 Hz, H-20a), 5.04 (tt, J 0.9 and 1.5 Hz, H-20b), 5.11 (br s, H-19a), 5.20 (dt, J 0.5 and 1.7 Hz, H-19b), 5.43 (dd, J 8.8 and 15.4 Hz, H-2) and 5.50 (d, J 15.4 Hz, H-3); MS $[m/z (\%)]$: 320 (0.5, $M-18$), 302 (2), 284 (1), 259 (5), 241 (4), 231 (3), 215 (3), 205 (4), 189 (8), 175 (7), 163 (10), 147 (12), 135 (16), 121 (38), 109 (33), 95 (50), 81 (45), 69 (49), 55 (45) and 43 (100).

Compound **2** had m.p. 185–187°C; $[\alpha]_D^{+41}$ (c 0.22, EtOH); (Found $[M-36]^+$: 302.2304. Calc. for $C_{20}H_{30}O_2$: 302.2246); IR (KBr): 3506, 3407, 3108 and 1644 cm^{-1} ; 1H NMR (CD_3OD): δ 0.90 (d, J 6.7 Hz)/0.93 (d, J 6.8 Hz) (H-16/H-17), 1.30 (s, H-18), 3.85 (br s, H-6 and H-7), 4.12 (t, J 6.7 Hz, H-11), 4.88 (br s, obscured by a solvent peak)/4.97 (d, J 2.0 Hz)/5.00 (br s)/5.03 (d, J 1.9 Hz) (H-19a/H-19b/H-20a/H-20b), 5.44 (dd, J 9.0 and 15.4 Hz, H-2) and 5.53 (d, J 15.4 Hz, H-3); MS $[m/z (\%)]$: 320 (0.4, $M-18$), 302 (2), 284 (0.8), 259 (4), 241 (3), 231 (2), 215 (3), 205 (3), 189 (6), 175 (5), 163 (7), 147 (9), 135 (11), 121 (27), 109 (24), 95 (35), 81 (34), 69 (40), 55 (43) and 43 (100).

Compound **3** had m.p. 139–141°C; $[\alpha]_D^{-41}$ (c 0.80, $CHCl_3$); (Found $[M-15]^+$: 323.2203. Calc. for $C_{19}H_{31}O_4$: 323.2222); IR ($CHCl_3$): 3603, 3496, 1691 and 1644 cm^{-1} ; 1H NMR ($CDCl_3$): δ 0.90 (d, J 6.6 Hz)/0.91 (d, J 6.5 Hz) (H-16/H-17), 1.13 (s, H-19), 1.22 (s, H-18), 2.51 (d, J -15.7 Hz)/2.51 (d, J -20.9 Hz) (H-5a/H-7a), 2.73 (d, J -15.7 Hz)/2.73 (d, J -20.9 Hz) (H-5b/H-7b), 3.45 (br s, $-OH$), 4.09 (dd, J 4.0 and 9.8 Hz, H-11), 4.65 (br s, $-OH$), 4.87 (q, J 1.6 Hz, H-20a), 4.97 (q, J 1.4 Hz, H-20b), 5.29

(d, J 15.2 Hz, H-3) and 5.51 (dd, J 9.0 and 15.2 Hz, H-2); MS $[m/z (\%)]$: 320 (0.1, $M-18$), 302 (0.5), 287 (0.2), 284 (0.2), 269 (0.1), 262 (2), 219 (3), 201 (2), 191 (1), 177 (3), 161 (9), 149 (9), 135 (16), 122 (22), 109 (36), 97 (18), 81 (17), 71 (13), 55 (16) and 43 (100).

Compound **4** was obtained as an oil and had $[\alpha]_D^{+16}$ (c 0.08, $CHCl_3$); (Found $[M-18]^+$: 320.2329. Calc. for $C_{20}H_{32}O_3$: 320.2351); IR ($CHCl_3$): 3603, 3475, 3090, 1702 and 1645 cm^{-1} ; 1H NMR ($CDCl_3$): δ 0.87 (d, J 6.8 Hz)/0.90 (d, J 6.7 Hz) (H-16/H-17), 1.23/1.31 (H-18/H-19), 2.48 (d, J -17.7 Hz)/2.50 (d, J -17.5 Hz) (H-5a/H-7a), 2.77 (d, J -17.7 Hz)/2.92 (d, J -17.5 Hz) (H-5b/H-7b), 4.10 (dd, J 4.3 and 7.2 Hz, H-11), 4.39 (br s, $-OH$), 4.85 (q, J 1.7 Hz, H-20a), 4.99 (quintet, J 1.3 Hz, H-20b), 5.32 (d, J 15.2 Hz, H-3) and 5.48 (dd, J 9.6 and 15.2 Hz, H-2); MS $[m/z (\%)]$: 320 (0.1, $M-18$), 305 (0.1), 302 (0.2), 287 (0.2), 277 (0.3), 263 (2), 245 (1), 219 (3), 201 (3), 179 (8), 161 (7), 149 (5), 135 (8), 123 (12), 109 (22), 97 (24), 81 (19), 71 (27), 55 (21) and 43 (100).

Compound **5** had m.p. 137–139°C; $[\alpha]_D^{+25}$ (c 0.90, $CHCl_3$); (Found $[M-36]^+$: 304.2423. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR (KBr): 3557, 3431 and 975 cm^{-1} ; 1H NMR ($CDCl_3$): δ 0.86 (d, J 6.7 Hz)/0.91 (d, J 6.7 Hz) (H-16/H-17), 1.23 (s)/1.28 (s) (H-18/H-20), 1.76 (d, J 1.1 Hz, H-19), 1.81 (dd, J 2.4 and -14.4 Hz, H-5a), 2.17 (dd, J 7.0 and -14.4 Hz, H-5b), 3.46 (m, H-11), 4.71 (ddd, J 2.4, 7.0, and 9.2 Hz, H-6), 5.53 (d, J 15.6 Hz, H-3), 5.62 (dd, J 9.5 and 15.6 Hz, H-2) and 5.69 (dd, J 1.1 and 9.2 Hz, H-7); MS $[m/z (\%)]$: 304 (0.4, $M-36$), 289 (0.3), 286 (0.1), 279 (0.2), 271 (0.1), 261 (0.9), 243 (0.7), 227 (0.6), 207 (0.7), 197 (1), 177 (3), 161 (4), 149 (3), 136 (11), 121 (17), 109 (17), 95 (21), 81 (28), 71 (25), 55 (23) and 43 (100).

Acetylation of (1S,2E,4S,6R,7R,11S)-2,8(19),12(20)-cembratriene-4,6,7,11-tetraol (1). Treatment of 10 mg of **1** with 0.5 ml of acetic anhydride in 2 ml of pyridine for 24 h at room temperature followed by work-up and purification by flash chromatography (SiO_2 ; hexane/EtOAc 70:30) afforded 11 mg of (1*S*,2*E*,4*S*,6*R*,7*R*,11*S*)-6,7,11-triacetoxy-2,8(19),12(20)-cembratrien-4-ol (**8**), which was an oil and had $[\alpha]_D^{+66}$ (c 1.1, $CHCl_3$); IR (CCl_4): 3612, 3522, 3095, 1741, 1646 and 1229 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-18), 1.71 (dd, J 4.2 and -14.2 Hz, H-5a), 1.97 (dd, J 9.1 and -14.2 Hz, H-5b), 2.01 (s, $-OCOCH_3$), 2.08 (s, $-OCOCH_3$), 2.16 (s, $-OCOCH_3$), 4.93 (m, H-19a, H-20a and H-20b), 4.96 (t, J 1.4 Hz, H-19b), 5.24 (ddd, J 3.1, 4.2, and 9.1 Hz, H-6), 5.42 (m, H-11), 5.50 (dd, J 9.3 and 15.4 Hz, H-2), 5.52 (dt, J 1.4 and 3.1 Hz, H-7) and 5.61 (d, J 15.4 Hz, H-3); MS $[m/z (\%)]$: 344 (0.3, $M-120$), 326 (0.3), 302 (0.8), 284 (3), 269 (2), 241 (5), 223 (3), 199 (3), 183 (4), 157 (7), 145 (9), 131 (10), 121 (11), 105 (18), 91 (22), 79 (19), 69 (17), 55 (18) and 43 (100).

Acetylation of (1S,2E,4S,6R,7S,11S)-2,8(19),12(20)-cembratriene-4,6,7,11-tetraol (2). Treatment of 8 mg of **2** with 0.5 ml of acetic anhydride in 2 ml of pyridine for 24 h at

room temperature followed by work-up and purification by HPLC (Spherisorb 5; hexane/EtOAc 60:40) gave 8 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,11*S*)-6,7,11-triacetoxy-2,8(19),12(20)-cembratrien-4-ol (**9**), which was an oil and had $[\alpha]_D +0.9^\circ$ (*c* 0.8, CHCl₃); IR (CCl₄): 3607, 3472, 3093, 1744, 1646 and 1243 cm⁻¹; ¹H NMR (CDCl₃): δ 0.85 (d, *J* 6.8 Hz)/0.88 (d, *J* 6.8 Hz) (H-16/H-17), 1.29 (s, H-18), 1.76 (dd, *J* 1.4 and -14.6 Hz, H-5a), 2.05 (dd, *J* 10.8 and -14.6 Hz, H-5b), 2.05 (s, -OCOCH₃), 2.08 (s, -OCOCH₃), 2.14 (s, -OCOCH₃), 4.94 (q, *J* 1.3 Hz, H-20a), 4.98 (quintet, *J* 1.2 Hz, H-19a), 5.05 (m, H-19b), 5.06 (quintet, *J* 1.1 Hz, H-20b), 5.15 (ddd, *J* 1.4, 2.9, and 10.8 Hz, H-6), 5.22 (t, *J* 6.1 Hz, H-11), 5.43 (dt, *J* 1.5 and 2.9 Hz, H-7), 5.49 (d, *J* 15.7 Hz, H-3) and 5.54 (dd, *J* 7.7 and 15.7 Hz, H-2); MS [*m/z* (%): 446 (0.2, *M*-18), 404 (0.4), 386 (0.2), 344 (1), 329 (0.3), 326 (0.8), 302 (4), 284 (6), 266 (3), 251 (2), 241 (9), 223 (6), 201 (4), 183 (6), 159 (7), 145 (10), 131 (11), 121 (12), 105 (14), 91 (17), 79 (15), 69 (13), 55 (12) and 43 (100).

Sensitized photo-oxygenation of (1S,2E,4S,6R,7E,11S)-2,7,12(20)-cembratriene-4,6,11-triol (10). To a solution of 400 mg of **10** in 10 ml of CH₂Cl₂, kept in a tube cooled by a water jacket, were added 40 mg of Rose Bengal B bound to polystyrene. The reaction mixture was irradiated with a 400 W sodium high pressure lamp, while oxygen was bubbled through. After 7 h, the reaction mixture was filtered and the solvent was removed *in vacuo*. The crude material was redissolved in 1 ml of CH₂Cl₂, cooled (0°C) and 1.1 ml of freshly distilled triethyl phosphate was added. After 4 h at room temperature, the solvent was removed under reduced pressure. The residue was separated by flash chromatography (SiO₂; hexane/EtOAc gradient) into three fractions, 1 (185 mg, Et₃PO₄), 2 (552 mg) and 3 (48 mg). Fraction 2 was separated further by HPLC (Spherisorb 5 CN; hexane/EtOAc 20:80) to give 30 mg of (1*S*,2*E*,4*S*,6*R*,7*S*,11*S*)-2,8(19),12(20)-cembratriene-4,6,7,11-tetraol (**2**) and 134 mg of the corresponding *R*-epimer (**1**). Fraction 3 afforded, after separation by HPLC (Lichrosorb 5 Diol; EtOAc), 4.8 mg of (1*S*,2*E*,4*S*,8*S*,11*S*)-4,8,11-trihydroxy-2,12(20)-cembradien-6-one (**3**) and 4.0 mg of the corresponding *8R*-epimer (**4**). The product ratio of **1**:**2**:**3**:**4** was measured to be 77:17:3:3 by integration of the HPLC traces (RI-detection).

(1*S*,2*E*,4*S*,6*R*,7*R*,11*S*)-2,8(19),12(20)-Cembratriene-4,6,7,11-tetraol had m.p. 124–126°C and $[\alpha]_D +22.4^\circ$ (*c* 1.03, EtOH). Its IR, ¹H NMR and mass spectra were identical with those of the naturally occurring **1**.

(1*S*,2*E*,4*S*,6*R*,7*S*,11*S*)-2,18(19),12(20)-cembratriene-4,6,7,11-tetraol had m.p. 187–190°C; $[\alpha]_D +43^\circ$ (*c* 0.32, EtOH). The IR, ¹H NMR and mass spectra were identical with those of the naturally occurring **2**.

(1*S*,2*E*,4*S*,8*S*,11*S*)-4,8,11-Trihydroxy-2,12(20)-cembradien-6-one had $[\alpha]_D -43^\circ$ (*c* 0.40, CHCl₃). Its m.p., IR, ¹H NMR and mass spectra were identical with those of the naturally occurring **3**.

(1*S*,2*E*,4*S*,8*R*,11*S*)-4,8,11-Trihydroxy-2,12(20)-cembra-

dien-6-one had $[\alpha]_D +19^\circ$ (*c* 0.21, CHCl₃). Its IR, ¹H NMR and mass spectra were identical with those of the naturally occurring **4**.

Reduction of (1S,2E,4S,6R,7S,8S,11E)-7,8-epoxy-2,11-cembradiene-4,6-diol (11). A solution of 67 mg of **11** and 32 mg of LAH in 5 ml of dioxane was heated under reflux for 9 h. Work-up and separation by HPLC (Spherisorb 5; hexane/EtOAc 30:70) gave 17 mg of (1*S*,2*E*,4*S*,6*S*,8*S*,11*E*)-2,11-cembradiene-4,6,8-triol (**12**) and 20 mg of (1*S*,2*E*,4*S*,6*R*,7*R*,8*S*,11*E*)-2,11-cembradiene-4,6,7-triol (**13**).

Compound **12** was an oil and had $[\alpha]_D +3.8^\circ$ (*c* 0.87, CHCl₃); IR (CHCl₃): 3605 and 3434 cm⁻¹; ¹H NMR (CDCl₃): δ 0.81 (d, *J* 6.9 Hz)/0.83 (d, *J* 6.7 Hz) (H-16/H-17), 1.24 (s)/1.26 (s) (H-18/H-19), 1.58 (br s, H-20), 4.25 (m, H-6), 5.16 (m, H-11), 5.37 (d, *J* 15.5 Hz, H-3) and 5.50 (dd, *J* 8.1 and 15.5 Hz, H-2); MS [*m/z* (%): 306 (0.4, *M*-18), 288 (0.6), 273 (0.5), 270 (0.4), 255 (0.6), 245 (2), 227 (2), 189 (3), 161 (7), 136 (10), 121 (17), 107 (17), 95 (25), 81 (36), 69 (25), 55 (29) and 43 (100).

Compound **13** was an oil and had $[\alpha]_D +19^\circ$ (*c* 0.28, CHCl₃); IR (CHCl₃): 3600 and 3433 cm⁻¹; ¹H NMR (CDCl₃): δ 0.82 (d, *J* 6.8 Hz)/0.84 (d, *J* 6.9 Hz) (H-16/H-17), 0.87 (d, *J* 6.8 Hz, H-19), 1.37 (s, H-18), 1.56 (br s, H-20), 3.36 (m, H-7), 3.62 (m, H-6), 4.93 (m, H-11), 5.44 (dd, *J* 7.1 and 15.5 Hz, H-2) and 5.49 (d, *J* 15.5 Hz, H-3); MS [*m/z* (%): 306 (0.2, *M*-18), 288 (0.2), 270 (0.4), 245 (0.5), 227 (0.7), 205 (3), 189 (1), 177 (1), 163 (2), 149 (3), 135 (4), 121 (8), 107 (8), 95 (20), 81 (20), 69 (17), 55 (21) and 43 (100).

Preparation of (1S,2E,4S,8S,11E)-4,8-dihydroxy-2,11-cembradien-6-one (14). Treatment of 7 mg of **12** with 12 mg of pyridinium dichromate in 1 ml of DMF for 3 h at room temperature followed by work-up and purification by flash chromatography (SiO₂; hexane/EtOAc gradient) gave 3 mg of (1*S*,2*E*,4*S*,8*S*,11*E*)-4,8-dihydroxy-2,11-cembradien-6-one (**14**), which had m.p. 117–118°C; $[\alpha]_D +7.1^\circ$ (*c* 0.31, CHCl₃); IR (CHCl₃): 3501, 1692 and 982 cm⁻¹; ¹H NMR (CDCl₃): δ 0.82 (d, *J* 6.6 Hz)/0.85 (d, *J* 6.7 Hz) (H-16/H-17), 1.17 (s)/1.28 (s) (H-18/H-19), 1.55 (s, H-20), 2.52 (d, *J* -14.8 Hz, H-5a), 2.68 (d, *J* -18.0 Hz, H-7a), 2.73 (d, *J* -18.0 Hz, H-7b), 2.74 (d, *J* -14.8 Hz, H-5b), 5.01 (m, H-11), 5.42 (d, *J* 15.6 Hz, H-3) and 5.56 (dd, *J* 8.6 and 15.6 Hz, H-2); MS [*m/z* (%): 304 (0.3, *M*-18), 289 (0.1), 286 (0.2), 271 (0.1), 261 (0.6), 243 (0.6), 221 (1), 203 (3), 189 (2), 179 (2), 163 (6), 149 (3), 139 (9), 121 (11), 109 (13), 97 (24), 81 (23), 71 (15), 55 (15) and 43 (100).

Sensitized photo-oxygenation of (1S,2E,4S,8S,11E)-4,8-dihydroxy-2,11-cembradien-6-one (14). To a solution of 210 mg of **14** in 10 ml of CH₂Cl₂, kept in a tube cooled by a water jacket, were added 40 mg of Rose bengal B bound to polystyrene. The reaction mixture was irradiated with a 400 W sodium high pressure lamp, while oxygen was bubbled through. After 1.5 h, the reaction mixture was filtered and

the solvent was removed *in vacuo*. The crude material was redissolved in 2 ml of CH_2Cl_2 and 500 μl of triethyl phosphite was added. After 12 h at room temperature, the solvent was removed under reduced pressure. The residue was separated by flash chromatography (SiO_2 ; hexane/EtOAc gradient) and subsequent HPLC (Spherisorb 5; hexane/EtOAc 20:80 and Spherisorb 5 CN; hexane/EtOAc 30:70) to give 12.4 mg of (1*S*,2*E*,4*S*,8*S*,11*S*)-4,8,11-trihydroxy-2,12(20)-cembradien-6-one, the m.p., optical rotation, ^1H NMR and mass spectra of which were identical with those of naturally occurring **3**, 9.1 mg of the corresponding 11*R*-epimer **15**, 26.0 mg of (1*S*,2*E*,4*S*,8*S*,10*E*,12*R*)-4,8,12-trihydroxy-2,10-cembradien-6-one (**16**) and 32.3 mg of the corresponding 12*S*-epimer (**17**). The product ratio of **3**:**15**:**16**:**17** was measured to be 23:10:21:46 by integration of the HPLC trace (RI-detection).

Compound **15** had m.p. 125–127 °C; $[\alpha]_{\text{D}} -32^\circ$ (*c* 0.20, CHCl_3): 3602, 3491, 3095 and 1692 cm^{-1} ; ^1H NMR (CDCl_3): δ 0.89 (d, *J* 6.5 Hz)/0.90 (d, *J* 6.4 Hz) (H-16/H-17), 1.13 (s)/1.22 (s) (H-18/H-19), 2.52 (d, *J* –19.0 Hz, H-7a), 2.53 (d, *J* –17.3 Hz, H-5a), 2.67 (d, *J* –19.0 Hz, H-7b), 2.72 (d, *J* –17.3 Hz, H-5b), 3.50 (br s, –OH), 4.20 (br s, H-11), 4.57 (br s, –OH), 4.90 (br s, H-20a), 5.05 (br s, H-20b), 5.28 (d, *J* 15.3 Hz, H-3) and 5.53 (dd, *J* 9.3 and 15.3 Hz, H-2); MS [*m/z* (%): 302 (0.1, *M*–36), 262 (0.3), 219 (0.8), 201 (0.5), 179 (2), 161 (2), 123 (4), 109 (9), 97 (9), 81 (9), 71 (13), 55 (19) and 43 (100).

Compound **16** had m.p. 168–170 °C; $[\alpha]_{\text{D}} +46^\circ$ (*c* 1.02, EtOH); IR (KBr): 3515, 3459 and 1666 cm^{-1} ; ^1H NMR (CDCl_3): δ 0.84 (d, *J* 6.4 Hz)/0.87 (d, *J* 6.4 Hz) (H-16/H-17), 1.20 (s, H-19), 1.22 (s, H-20), 1.26 (s, H-18), 2.21 (dd, *J* 5.3 and –12.9 Hz, H-9a), 2.44 (d, *J* –15.2 Hz,

Table 6. Crystal and experimental data for the tetraol **1** and oxotriol **16**.

	Tetraol 1	Oxotriol 16
Formula	$\text{C}_{20}\text{H}_{34}\text{O}_4$	$\text{C}_{20}\text{H}_{34}\text{O}_4$
Formula weight	338.49	338.49
Space group	$P2_12_12_1$	$P2_1$
Unit-cell dimension / Å	<i>a</i> = 6.1914(10) <i>b</i> = 10.3895(20) <i>c</i> = 31.1388(27)	<i>a</i> = 9.8847(23) <i>b</i> = 10.0312(35) <i>c</i> = 10.7108(43) β = 108.97(76)°
Unit-cell volume $V/\text{Å}^3$	2003.02(53)	1004.33(58)
Formula units per unit cell <i>Z</i>	4	2
Calculated density $D_x/\text{g cm}^{-3}$	1.12	1.12
Radiation	Cu K_α	Mo K_α
Wavelength, $\lambda/\text{Å}$	1.54184	0.71073
Linear absorption coefficient/ cm^{-1}	5.75	0.71
Temperature <i>T</i> /K	293(1)	293(1)
Crystal shape	Prismatic	Prismatic
Crystal size/mm	0.10×0.08×0.16	0.50×0.25×0.35
Diffractometer	Siemens/Stoe AED 2	Siemens/Stoe AED 2
Determination of unit cell		
Number of reflections used	16	9
θ -range/°	12.5 to 25.0	10.0 to 25.0
Intensity data collection		
Maximum $[\sin(\theta)/\lambda]/\text{Å}^{-1}$	0.56	0.65
Range of <i>h</i> , <i>k</i> and <i>l</i>	0 to 7 0 to 12 0 to 35	–13 to 13 –13 to 13 0 to 14
Standard reflections	3	3
Intensity instability (%)	< 3	< 4
Number of unique reflections	1798	3438
Number of observed reflections	2017	4491
Criterion for significance	$F > 2.0 \cdot \sigma(F)$	$F > 6.06 \cdot \sigma(F)$
Structure refinement		
Minimization of	$\sum w \cdot \Delta F^2$	$\sum w \cdot \Delta F^2$
Anisotropic thermal parameters		All non-hydrogen atoms
Isotropic thermal parameters		Hydrogen atoms
Number of refined parameters	239	241
Weighting scheme	$[\sigma^2(F) + 0.0050 F ^2]^{-1}$	$[\sigma^2(F) + 0.0007 F ^2]^{-1}$
Final <i>R</i> for observed refls.	0.058	0.038
Final <i>wR</i> for observed refls.	0.083	0.051
Final <i>wR</i> for all observed refls.	0.087	0.053
Final $(\Delta/\sigma)_{\text{max}}$	0.13	0.04
Final $\Delta\rho_{\text{min}}$ and $\Delta\rho_{\text{max}}$	–0.43 and 0.33 e Å^{-3}	–0.32 and 0.34 e Å^{-3}

H-5a), 2.52 (d, J -19.2 Hz, H-7a), 2.64 (d, J -19.2 Hz, H-7b), 2.68 (d, J -15.2 Hz, H-5b), 2.72 (dd, J 9.4 and -12.9 Hz, H-9b), 3.15 (br s, -OH), 4.09 (br s, -OH), 5.38 (d, J 15.4 Hz, H-3), 5.47 (ddd, J 5.3, 9.4 and 15.3 Hz, H-10), 5.59 (dd, J 8.7 and 15.4 Hz, H-2) and 5.63 (d, J 15.3 Hz, H-11); MS [m/z (%): 302 (0.1, M -36), 260 (0.3), 219 (0.5), 201 (0.7), 177 (1), 161 (3), 135 (7), 121 (4), 109 (16), 95 (9), 81 (10), 69 (6), 55 (8) and 43 (100).

Compound **17** had m.p. 115–117°C; $[\alpha]_D$ -13° (c 0.70, CHCl₃); IR (KBr): 3462, 3395, 1701 and 1681 cm⁻¹; ¹H NMR (CDCl₃): δ 0.84 (d, J 6.5 Hz)/0.89 (d, J 6.5 Hz) (H-16/H-17), 1.20 (s, H-19), 1.27 (s, H-20), 1.28 (s, H-18), 2.13 (dd, J 6.9 and -13.2 Hz, H-9a), 2.48 (d, J -14.4 Hz, H-5a), 2.53 (d, J -18.7 Hz, H-7a), 2.62 (dd, J 7.2 and -13.2 Hz, H-9b), 2.63 (d, J -18.7 Hz, H-7b), 2.71 (d, J -14.4 Hz, H-5b), 5.39 (d, J 15.2 Hz, H-3), 5.49 (ddd, J 6.9, 7.2 and 15.7 Hz, H-10), 5.59 (d, J 15.7 Hz, H-11) and 5.66 (dd, J 8.9 and 15.2 Hz, H-2); MS [m/z (%): 302 (0.1, M -36), 262 (0.2), 219 (0.5), 201 (0.6), 177 (1), 161 (2), 135 (3), 126 (7), 109 (20), 95 (10), 81 (11), 69 (8), 55 (10) and 43 (100).

Conversion of 5 into (1S,2E,4S,6E,8R,11S,12R)-8,11-epoxy-2,6-cembradiene-4,12-diol (20). To a solution of 3 mg of **5** in 5 ml of CHCl₃, was added 2 ml of CHCl₃ saturated with HCl. After 24 h at room temperature, the reaction mixture was worked-up. The crude product was separated by HPLC (Spherisorb 5; hexane/EtOAc 1:1) to give 1 mg of a product, which was identical (optical rotation, IR, ¹H NMR and MS) to (1S,2E,4S,6E,8R,11S,12R)-8,11-epoxy-2,6-cembradiene-4,12-diol (**20**).¹⁷

Conversion of 5 into (4E,6R,8S,9E,11S)-6-acetoxy-8-hydroxy-11-isopropyl-4,8-dimethyl-14-oxo-4,9-pentadecadienal (21). To a solution of 5 mg of **5** in 3 ml of methanol/water (1:1) were added 10 mg of sodium periodate. After 5 h at room temperature, the reaction mixture was worked-up. The crude product obtained was treated with acetic anhydride/pyridine (1:6) at room temperature overnight. Work-up and chromatography over silica gel (EtOAc) gave 3 mg of a product which was indistinguishable (optical rotation, IR, ¹H NMR, and MS) from (4E,6R,8S,9E,11S)-6-acetoxy-8-hydroxy-11-isopropyl-4,8-dimethyl-14-oxo-4,9-pentadecadienal (**21**).¹⁶

X-Ray crystallography study. The intensities of 3 test reflections (1 0 2̄, 1 1̄ 3, 0 1̄ 6̄ for the tetraol **1** and 1̄ 1 2, 0 2 0, 2 0 0 for the oxotriol **16**), remeasured every 60 min, showed < 3% and < 4% intensity instability, respectively, during the data collection. The space-group symmetry was determined from systematic absences and by the unit-cell parameters found by the least-squares method from 16 (for **1**) and 9 (for **16**) centred reflections. The intensities were corrected for Lorentz and polarization effects, but no correction was made for absorption.

In the case of the tetraol **1**, 17 of the 24 non-hydrogen atoms were found by direct methods using the SHELXS

program,¹⁹ while the 7 remaining non-hydrogen atoms were located from electron-density difference maps. All non-hydrogen atoms in the oxotriol **16** were found by direct methods. Except for the hydroxy hydrogens, which were located from electron-density difference maps, all hydrogen atoms were geometrically placed and refined. The structures were refined by the full-matrix least-squares technique using the SHELX76 program.²⁰ Anisotropic temperature factors were introduced for all non-hydrogen atoms, while all hydrogen atoms were refined with all isotropic temperature factors constrained to a common value. The three strongest reflections (2 4 3, 0 2 7, 1 4 12) for the tetraol **1** were omitted due to extinction effects. The atomic scattering factors used for the non-hydrogen atoms and the hydrogen atoms were those included in the SHELX76 program.²¹

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