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Eight New Cembranoids from Tobacco - Structural Elucidation and Conformational Studies[1]

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Abstract: Eight new cembranoids have been isolated from an extract of flowers of Greek tobacco. They have been identified as (1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*E*)-2,7,11-cembratriene-4,6,10-triol (**1**), the corresponding (10*S*)-, (4*R**)- and (4*R**,10*S**)-diastereomers (**2-4**), (1*S**,2*E*,4*R**,6*R**,7*E*,10*S**,11*Z*)-2,7,11-cembratriene-4,6,10-triol (**5**), (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-4,6-dihydroxy-2,7,11-cembratrien-10-one (**6**), the corresponding (4*R**)-epimer (**7**) and (1*S**,2*E*,4*S**,7*E*,10*S**,11*E*)-4,10-dihydroxy-2,7,11-cembratrien-6-one (**8**) with the aid of chemical and spectral methods, 2D-NMR techniques being particularly helpful The crystal structure of the diacetate **11** has been determined and is described. The solution conformations of triols **1** and **3** have been studied by using NMR methods in conjunction with molecular mechanics calculations (MM3). The biogenesis of the new compounds is discussed.

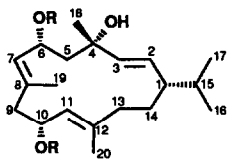
In addition to aliphatic hydrocarbons, fatty alcohols, wax esters and sucrose esters the cuticular wax of the leaf and flower of tobacco contains a wide array of cembranic diterpenoids.[2] The two 2,7,11-cembratriene-4,6-diols **9** and **10** are the major cembranoids and, as suggested by biomimetic studies, the principal precursors of most of the other tobacco cembranoids.[3] We now report the isolation by HPLC of eight new cembratrienetriols (**1-8**) from an extract of flowers of Greek tobacco and the elucidation of their structures The solution conformations of two of the new compounds (**1**, **3**) have also been studied

RESULTS AND DISCUSSION

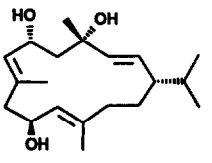
Structural Elucidation.

The first new compound (**1**), $C_{20}H_{34}O_3$, is a triol having two secondary and one tertiary hydroxy group [IR: 3600 cm^{-1} and 3426 cm^{-1}; ^1H NMR: δ 4.42 (ddd) and 4.53 (ddd); ^{13}C NMR: δ 65.0 (d), 65.8 (d) and 72.3 (s) (Table 1)]. This assignment was confirmed by conversion of triol **1** to a diacetate (**11**) on treatment with acetic anhydride in pyridine (IR· 3597, 3475, 1724 and 1250 cm^{-1}; ^1H NMR. methyl singlets at δ 2.02 and 2 04 and ddd's at δ 5.48 and 5 67) Triol **1** also possesses three double bonds, of which two are trisubstituted [δ 128.0 (d), 132.8 (d), 133 8 (s) and 140.2 (s)] and one is 1,2-disubstituted [δ 127.8 (d) and 137.4 (d)]. These results are consistent with triol **1** being carbomonocyclic.

The occurrence of one isopropyl group (methyl doublets at δ 0.80 and 0 83 in the ^1H NMR spectrum), one methyl group attached to the fully substituted carbon atom carrying the tertiary hydroxy group and two vinylic methyl groups (one methyl singlet at δ 1.36 and two methyl singlets at δ 1.68 in the ^1H NMR spectrum) suggested that triol **1** is a diterpenoid of the cembrane class



1 R=H
11 R=Ac



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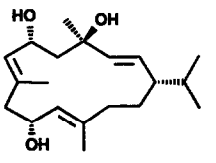

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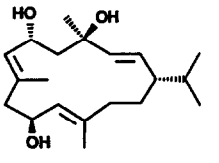

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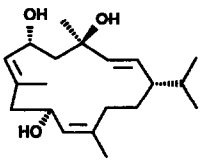

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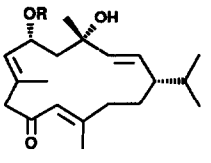

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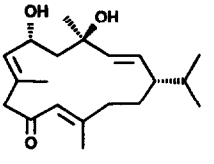
13 R=Ac



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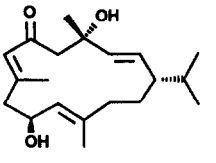

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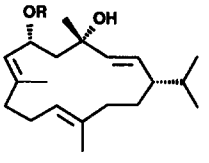

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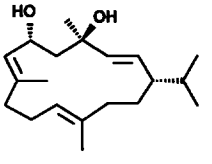

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Four structural fragments were formulated by using DQ COSY and HMQC experiments. These were linked into a 2,7,11-cembratriene-4,6,10-diol structure with the aid of information from an HMBC experiment, crucial three bond correlations being those observed between C-3 and H-5a, H-5b and H-18, between C-5 and H-18, between C-9 and H-7 and H-19 and between C-13 and H-11 and H-20 (Table 2).

All three double bonds in triol **1** have *E*-geometries. This conclusion was based on the magnitude of the vicinal coupling constant of the 1,2-disubstituted double bond (J=15.3 Hz) and the chemical shift values of the signals due to the carbon atoms of the vinylic methyl groups (δ 14.7 and 16.5)[4] and is supported by results from a NOESY experiment (Table 3) The relative stereochemistry of triol **1** was determined as 1*S*, 4*S*, 6*R*, 10*R* by X-ray analysis of the diacetate **11**, which in contrast to triol **1** formed single crystals (see below).

The absolute configuration of triol **1** was established by chemical means The acetate (**12**) of the (4*S*,6*R*)-diol **9** was chosen as the starting material since it has relevant stereochemistry at C-1, C-4 and C-6. Treatment of the acetate **12** with pyridinium dichromate and *tert.*-butyl hydroperoxide resulted in allylic oxidation to form the 10-oxo compound **13** [IR 1685, 1666 and 1616 cm^{-1}, ^{13}C NMR. δ 197 8 (s)] in a small amount. Reduction of **13** with $LiAlH_4$ gave two products of which one was identical in all respects to the new triol (**1**). Hence, compound **1** is conclusively identified as (1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*E*)-2,7,11-cembratriene-4,6,10-triol.

The other reduction product was indistinguishable from the second new tobacco isolate (**2**), which was hence identified as the (10*S*)-epimer of triol **1**. In agreement with this assignment the shieldings of ten of its carbon atoms were close to those of C-1 to C-6 and C-15 to C-18 of triol **1** (cf Table 1)

The third and fourth new tobacco constituents (**3**, **4**), both $C_{20}H_{34}O_3$, were identified as the (4*R**)-isomers of triols **1** and **2**, respectively, with the use of the ^1H and ^{13}C NMR spectra in conjunction with DQ COSY and HMQC experiments for proton and carbon assignment Of particular diagnostic importance in the determination of the gross structures were the correlations between H-10 and H-9a, H-9b and H-11 ($^3J_{HH}$) and between H-19 and H-9a and H-9b ($^4J_{HH}$) in the DQ COSY spectra of both **3** and **4**, which allowed the

Table 1. ^{13}C NMR Chemical Shift Values and Assignments for Compounds **1-8**, **11** and **13**.[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	46.4	127.8	137.4	72.3	52.3	65.8	132.8	133.8	48.5	65.0	128.0	140.2	36.3	27.3	33.0	19.4	20.6	30.2	16.5	14.7
2	47.5	128.4	137.9	72.8	50.1	67.2	129.8	134.7	45.6	66.8	127.7	138.7	39.2	28.8	34.0	19.2	20.4	30.9	19.0	16.8
3	46.4	130.3	136.2	71.4	52.5	64.1	133.4	133.2	48.8	64.6	128.0	140.4	36.2	27.1	33.0	19.4	20.4	28.6	16.3	14.6
4	47.0	130.3	136.1	71.4	52.2	64.4	130.9	135.5	45.5	66.6	127.4	138.5	38.2	28.0	33.4	19.4	20.3	29.3	19.4	16.9
5	47.8	131.2	137.1	72.9	51.6	65.3	131.2	137.0	47.2	71.3	129.4	138.3	28.0	29.1	31.3	20.1	20.3	30.9	18.8	23.0
6	46.6	127.8	137.7	72.4	52.9	66.1	135.2	132.2	57.5	198.0	121.7	159.2	38.8	27.8	32.9	19.1	20.8	30.4	16.9	17.7
7	46.4	130.4	136.4	71.3	52.5	64.3	135.7	132.2	57.6	198.3	121.5	158.7	38.7	27.4	32.8	19.1	20.6	28.6	16.8	17.7
8	49.2[b]	131.6	134.8	72.5	54.9	201.6	127.2	156.7	49.3[b]	66.0	125.9	141.1	39.5	30.8	33.0	19.4	20.3	29.7	20.2	16.4
11[c]	46.5	128.2	136.9	72.2	50.3	68.2	129.0	135.5	44.8	67.4	123.5	142.6	36.3	27.3	32.9	19.5	20.5	29.8	16.5	14.8
13[d]	46.5	127.8	137.4	72.4	51.0	68.3	131.0	134.7	57.5	197.8	121.6	159.3	38.8	27.8	32.9	19.2	20.8	30.0	17.1	17.6

[a] δ-values i $CDCl_3$ relative to TMS.
[b] Assignment may be reversed.
[c] $OCOCH_3$ 170.0, 170.4; $OCOCH_3$ 21.3, 21.4.
[d] $OCOCH_3$ 169.9; $OCOCH_3$ 21.4.

Table 2. Selected HMBC Correlations for Compounds **1, 2, 5, 7** and **8**.[a]

Atom	Compound 1	2	5	7	8
C - 1	H-16/17	H-16/17	H-16/17	H-15, H-16/17	H-15, H-16/17
C - 3	H-5a, H-5b, H-18	H-5a/b, H-18	H-5a/b, H-18	H-5b, H-18	H-5a, H-5b, H-18
C - 4	H-2, H-3, H-5a, H-5b, H-18	H-2, H-3, H-5a/b, H-18	H-2, H-5a/b, H-6, H-18	H-2, H-5b, H-6, H-18	H-2, H-3, H-5a, H-5b, H-18
C - 5	H-18	H-18	H-18	H-18	H-18
C - 6	H-5a, H-5b	H-5a/b	H-5a/b	H-5a, H-5b	H-5a, H-5b, H-7
C - 7	H-9a, H-9b, H-19	H-9a/b, H-19	H-9a, H-9b, H-19	H-9a, H-9b, H-19	H-9a, H-9b, H-19
C - 8	H-9a, H-9b, H-19	H-9a/b, H-19	H-6, H-9a, H-9b, H-19	H-6, H-9a, H-9b, H-19	H-9a, H-9b, H-19
C - 9	H-7, H-19	H-7, H-19	H-7, H-19	H-7, H-19	H-7, H-19
C - 10	H-9a, H-9b, H-11	H-9a/b	H-9a, H-9b	H-9a, H-9b, H-11	H-9a, H-9b
C - 11	H-20	H-20	H-20	H-13a, H-13b, H-20	H-13a, H-13b, H-20
C - 12	H-20	H-20	H-13b, H-20	H-13a, H-13b, H-20	H-13a, H-13b, H-20
C - 13	H-11, H-20	H-11, H-20	H-11, H-20	H-11, H-20	H-11, H-20

[a] The HMBC spectra were recorded in $CDCl_3$ at 300 MHz .

allocation of one of the hydroxy groups to C-10. Useful stereochemical information was provided by a comparison of the ^1H and ^{13}C NMR spectra of triols **1-4**. Thus, H-6 and H-10 resonate at δ 4.77 and 4.50 for **3** as against δ 4.42 and 4.53 for the (4*S*,10*R*)-triol **1** and at δ 4.84 and 4.66 for **4** as against δ 4.5-4.7 for the (4*S*,10*S*)-triol **2**. Furthermore, the signal due to C-4 is present at δ 72.3 and 72.8 for the (4*S*)-triols **1** and **2** and at δ 71.4 for the triols **3** and **4**, while the signal due to C-10 is present at δ 65.0 and 64.6 for triols **1** and **3** and at δ 66.8 and 66.6 for triols **2** and **4**.

The fifth new compound (**5**), $C_{20}H_{34}O_3$, was conclusively identified as a 2,7,11-cembratriene-4,6,10-triol with the aid of the DQ COSY, NOESY, HMQC and HMBC spectra Particularly helpful were the correlations between H-18 and C-3 ($^3J_{CH}$), C-4 ($^2J_{CH}$) and C-5 ($^3J_{CH}$), between H-7 and C-9 ($^3J_{CH}$) and between H-11 and C-13 ($^3J_{CH}$) in the HMBC spectrum, since they linked the structural fragments identified by using the DQ COSY spectrum through the intervening quarternary C-4, C-8 and C-12. The 7,8 double bond was deduced to have *E*-geometry because of the chemical shift value of C-19 (δ 18.8), while the 11,12-double bond was ascribed *Z*-geometry because of the chemical shift value of C-20 (δ 23.0).[4] In accordance with this an NOE was observed between H-20 and H-11 but not between H-19 and H-7 (Table 3). The 2,3-double bond has *E*-geometry as concluded from the magnitude of the vicinal coupling constant, *J*=15 9 Hz. The NOESY spectrum was also used to suggest that triol **5** has a (1*S**,4*R**,6*R**,10*S**)-configuration (see below).

The new tobacco isolate **6**, $C_{20}H_{32}O_3$, possesses one α,β-unsaturated oxo group [IR: 1683, 1665 and 1615 cm^{-1}; ^{13}C NMR: δ 121 7 (d), 159.2 (d), and 198 0 (s)] and two hydroxy groups of which one is secondary and one is tertiary [IR: 3599 and 3453 cm^{-1}; ^1H NMR δ 4.48 (ddd), ^{13}C NMR δ 66.1 (d) and 72 4 (s)]. Treatment of the oxodiol **6** with acetic anhydride in pyridine gave a monoacetate that was identical in all respects to the 10-oxo compound **13** formed by allylic oxidation of acetate **12**. Hence, compound **6** is (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-4,6-dihydroxy-2,7,11-cembratrien-10-one. On reduction using $NaBH_4$, **6** was converted to the (4*S*,6*R*,10*R*)- and (4*S*,6*R*,10*S*)-triols **1** and **2**, respectively.

The seventh new compound (**7**), $C_{20}H_{32}O_3$, was identified as the (4*R**)-epimer of **6** using spectral data. While C-2 to C-4, C-6 and C-18 showed divergent chemical shift values, all other carbon atoms in the ^{13}C NMR spectra of **6** and **7** had virtually identical shieldings (Table 1) Further support for the assignment of a (4*R**)-configuration to the oxodiol **7** was provided by the shieldings of H-6: δ 4 48 for **6** and δ 4.88 for **7**

The remaining new tobacco constituent (**8**), $C_{20}H_{32}O_3$, contains an α,β-unsaturated oxo group [IR: 1667 and 1604 cm^{-1}, ^{13}C NMR δ 127.2 (d), 156.7 (d) and 201 6 (s)] and two double bonds of which one is *E*-1,2-disubstituted (*J*=15 5 Hz) and one is trisubstituted [^{13}C NMR. δ 125 9 (d), 131.6 (d), 134.8 (d) and 141 1 (s)] The remaining oxygen atoms are accommodated by one secondary and one tertiary hydroxy group [^1H NMR. δ 4.65 (m), ^{13}C NMR· δ 66 0 (d) and 72.5 (s)]. Of the methyl groups, two are vinylic, one is attached to the carbon atom carrying the tertiary hydroxy group and two form part of an isopropyl group. These structural units were incorporated into a 4,10-dihydroxy-2,7,11-cembratrien-6-one structure with the aid of results from the DQ COSY, HMQC and HMBC spectra Thus, the oxo group was allocated to C-6, since H-5a and H-5b showed long range correlations to C-3, C-4 and C-18, and since C-9 had a three-bond correlation to H-7 in the HMBC spectrum Further support for the structural assignment was provided by the observation of three-bond correlations between C-13 and H-11 and H-20 (Table 2) The NOESY spectrum was then used to propose that the oxodiol **8** has 1*S**, 4*S**,10*S**, 11*E* stereochemistry (see below)

NOE Results for 1-5, 7 and 8.

The results from the NOESY experiments, compiled in Table 3, were used to study the solution conformations of triols **1-4** and to confirm the configurational assignments made. All four triols exhibit NOEs between H-1 and H-3, between H-3 and H-6 and between H-6 and H-19 indicating that all these protons are located on the β–face of the macrocyclic ring. The NOE response between H-2 and H-16/H-17 would then allocate these protons to the α–face, and all four triols (**1-4**) are suggested to have a similar backbone arrangement of the C-1 to C-8 portion of the molecule. The configurational differences are disclosed by the NOE responses between H-18 and H-3 and H-6 for the (4*S*)-triols **1** and **2** (conformation **a**) and by the NOE responses between H-18 and H-2 for the (4*R*)-triols **3** and **4** (conformation **b**, see Fig. 1). It seems therefore

Table 3. Selected NOESY Data for Compounds **1-5, 7** and **8**.[a]

Irradiated signal	Compound 1	2	3	4	5	7	8
H - 1	H-3, H-20	H-3	H-3, H-20	H-3	H-3	H-3	H-3
H - 2	H-14a, H-16/17	H-16/17, H-18(weak)	H-14a, H-16/17, H-18	H-16/17, H-18	H-14b, H-18	H-16/17, H-18	H-16/17 (weak)
H - 3	H-1, H-6, H-18, H-19	H-1, H-6, H-18	H-1, H-6, H-19	H-1, H-6, H-19	H-1, H-6, H-18 (weak)	H-1, H-6, H-18 (weak), H-19	H-1, H-5b, H-18
H - 6	H-3, H-5b, H-18, H-19	H-3, H-5a/b, H-18, H-19	H-3, H-5b, H-19	H-3, H-5b, H-19	H-3, H-5a/b, H-19	H-3, H-5b, H-19	—
H - 7	H-5a, H-9a, H-11	H-5a/b, H-9a/b, H-10	H-5a, H-9a, H-11	H-5a, H-9b, H-10	H-5a/b, H-9b, H-10	H-5a, H-9b, H-11	H-5b, H-9a
H - 10	H-9b, H-20	H-7, H-9a/b, H-20	H-9b, H-20	H-7, H-9b, H-20	H-7, H-9b, H-13b	—	H-9b, H-19, H-20
H - 11	H-7, H-9a, H-13a	H-9a/b, H-13a	H-7, H-9a, H-13a	H-9a, H-13a	H-9a, H-20	H-7, H-9b, H-13a, H-14a	H-9a, H-13a
H - 18	H-3, H-5b, H-6	H-2 (weak), H-3, H-5a/b, H-6	H-2, H-5a, H-5b	H-2, H-5a, H-5b	H-2, H-3 (weak), H-5a/b,	H-2, H-3 (weak), H-5a, H-5b	H-3, H-5a, H-5b
H - 19	H-3, H-6, H-9b	H-6, H-9a/b	H-3, H-6, H-9b	H-3, H-6, H-9a	H-6, H-9a	H-3, H-6, H-9a	H-9b, H-10
H - 20	H-1, H-10, H-13b	H-10, H-13b	H-1, H-10, H-13b, H-14b	H-10	H-11		H-10

[a] The NOESY spectra were recorded in $CDCl_3$ at 600 MHz for **1** and **3** and at 300 MHz for **2, 4, 5, 7** and **8**

that reversal of the configuration at C-4 does not affect the rotation about the 5,6 bond in triols **1-4**. This result differs from previous findings that 2,7,12(20)-cembratriene-4,6,11-triols and 2,7,10-cembratriene-4,6,12-triols of the (4*S*)- and (4*R*)-series have different conformations about the 5,6 bond.[5]

The NOE data for triol **5** are consonant with its C-1 to C-8 portion populating a conformation of type **b**. As a consequence triol **5** is assigned a (1*S**,4*R**,6*R**)-configuration. The (4*R**,6*R**)-oxodiol **7** is also proposed to have conformation **b** from its NOE data.

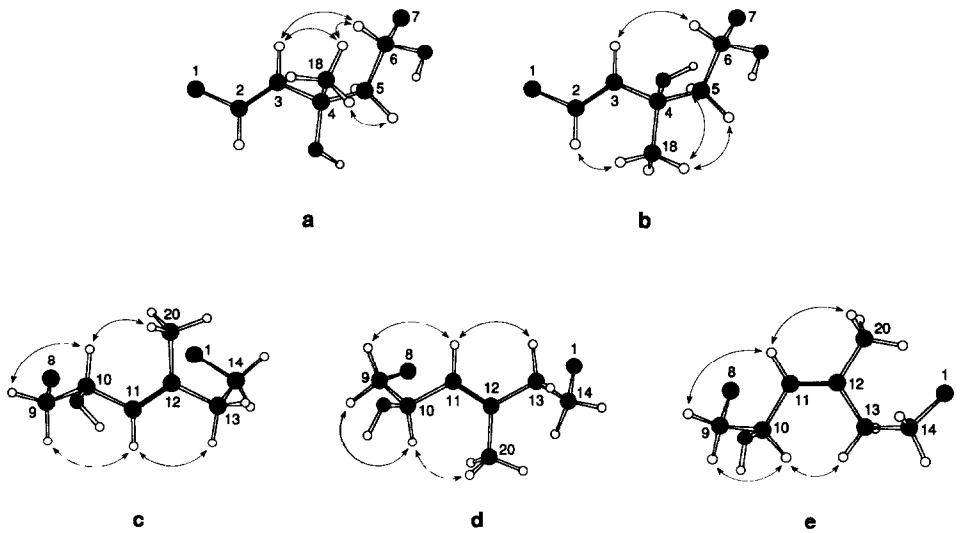


Fig. 1 Drawings of conformations **a-e**. The arrows illustrate NOE responses observed.

Let us now turn to the C-8 to C-14 portion of the macrocyclic ring of triols **1-4**. NOEs are observed between H-1 and H-20, between H-10 and H-20 and between H-7 and H-11 in triols **1** and **3** suggesting that H-10 and H-20 are situated on the β–face and H-11 on the α–face. These results along with the additional NOE data, which are listed in Table 3 and visualized by arrows in Fig. 1, are consonant with a backbone arrangement of type **c** and a (10*R*)-configuration in triols **1** and **3**.

The (10*S*)-triols **2** and **4** exhibit NOEs between H-7 and H-10 and between H-10 and H-20. These protons are then allocated to the α–face of the macrocyclic ring, and triols **2** and **4** are suggested to have a backbone arrangement of type **d**. It can be concluded therefore that the (10*R*)- and (10*S*)-triols have different conformations about the 10,11 bond.

Triol **5**, whose 11,12 double bond is of *Z*-geometry, is assigned a backbone arrangement of type **e** and a (10*S*)-configuration. This conclusion is based on the NOE enhancements listed in Table 3, that between H-7, which is α–oriented, and H-10 being particularly diagnostic.

The NOESY spectrum of the 6-oxo-4,10-diol **8** contains cross-peaks corresponding to interactions between H-1 and H-3, between H-3 and H-5b and H-18 and between H-5b and H-7, all these protons are allocated to the β–face and the configuration is 1*S**,4*S**. It is noteworthy that H-19, i. e. the methyl group at C-8, must be situated on the α–face. The rotation about the 6,7 bond is hence different in **8** and in compounds **1-5** and **7** The spectrum of **8** also includes an NOE response between H-19 and H-10. The latter is consequently α–oriented and the C-8 to C-14 backbone arrangement is of type **d**. The configuration of C-10 is *S**.

Crystallography.

The diacetate **11** crystallizes with two molecules, A and B, in the asymmetric unit. Both of these have disordered isopropyl groups. Although peaks were found in the $\Delta\rho$ map, no refinement of the disordered groups was possible.

Intramolecular bond lengths and bond angles, both with estimated standard deviations, are listed in Tables 4 and 5, respectively. Crystal and experimental data are given in Table 9. Fig. 2 is a drawing of the molecules A and B. There is one long intermolecular hydrogen bond O(1)-O(1a) of 3.042(10) Å, the label (a) representing the symmetry operation: $1+x, y, z$. The shortest intramolecular non-bonded distance of 2.281(14) Å is between O(4A)-O(5A).

Table 4. Bond Lengths (Å) in Diacetate **11**.

Molecule A					
C(2A)-C(1A)	1.491(19)	C(14A)-C(13A)	1.525(20)	C(8A)-C(7A)	1.316(17)
C(15A)-C(1A)	1.646(35)	C(17A)-C(15A)	1.585(36)	C(19A)-C(8A)	1.509(17)
C(4A)-C(3A)	1.487(18)	O(2A)-C(21A)	1.297(17)	C(11A)-C(10A)	1.476(17)
C(18A)-C(4A)	1.535(19)	C(24A)-C(23A)	1.505(20)	C(12A)-C(11A)	1.321(19)
C(6A)-C(5A)	1.510(17)	O(5A)-C(23A)	1.154(20)	C(20A)-C(12A)	1.506(19)
O(2A)-C(6A)	1.454(14)	C(14A)-C(1A)	1.575(19)	C(16A)-C(15A)	1.215(38)
C(9A)-C(8A)	1.513(17)	C(3A)-C(2A)	1.298(18)	C(22A)-C(21A)	1.469(20)
C(10A)-C(9A)	1.553(18)	C(5A)-C(4A)	1.518(18)	O(3A)-C(21A)	1.232(19)
O(4A)-C(10A)	1.471(15)	O(1A)-C(4A)	1.447(15)	O(4A)-C(23A)	1.338(19)
C(13A)-C(12A)	1.530(19)	C(7A)-C(6A)	1.503(17)	O(1X)-C(1X)	1.111
Molecule B					
C(2B)-C(1B)	1.513(20)	C(14B)-C(13B)	1.486(18)	C(8B)-C(7B)	1.296(17)
C(15B)-C(1B)	1.567(26)	C(17B)-C(15B)	1.371(33)	C(19B)-C(8B)	1.495(17)
C(4B)-C(3B)	1.509(19)	O(2B)-C(21B)	1.372(20)	C(11B)-C(10B)	1.473(16)
C(18B)-C(4B)	1.505(20)	C(24B)-C(23B)	1.482(18)	C(12B)-C(11B)	1.304(19)
C(6B)-C(5B)	1.550(18)	O(5B)-C(23B)	1.196(20)	C(20B)-C(12B)	1.521(20)
O(2B)-C(6B)	1.466(14)	C(14B)-C(1B)	1.514(19)	C(16B)-C(15B)	1.518(29)
C(9B)-C(8B)	1.497(15)	C(3B)-C(2B)	1.299(19)	C(22B)-C(21B)	1.448(23)
C(10B)-C(9B)	1.544(17)	C(5B)-C(4B)	1.534(18)	O(3B)-C(21B)	1.190(22)
O(4B)-C(10B)	1.460(14)	O(1B)-C(4B)	1.432(16)	O(4B)-C(23B)	1.338(18)
C(13B)-C(12B)	1.542(19)	C(7B)-C(6B)	1.489(16)		

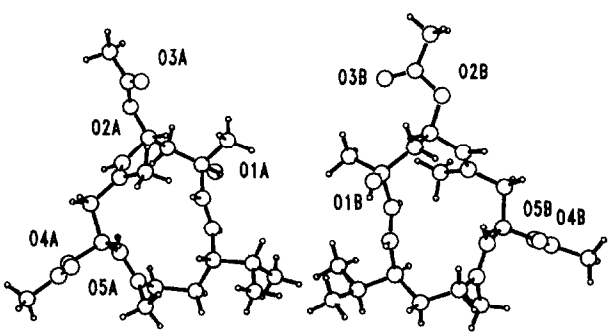


Fig. 2. A drawing of the two molecules (A and B) in the asymmetric unit of the diacetate **11**.

Table 5. Bond Angles (deg.) in Diacetate **11**.

Molecule A			
C(14A)-C(1A)-C(2A)	112.5(11)	C(15A)-C(1A)-C(2A)	104.4(13)
C(15A)-C(1A)-C(14A)	121.4(13)	C(3A)-C(2A)-C(1A)	124.9(12)
C(4A)-C(3A)-C(2A)	128.4(12)	C(5A)-C(4A)-C(3A)	114.4(11)
C(18A)-C(4A)-C(3A)	107.5(11)	C(18A)-C(4A)-C(5A)	109.2(10)
O(1A)-C(4A)-C(3A)	108.2(10)	O(1A)-C(4A)-C(5A)	108.7(10)
O(1A)-C(4A)-C(18A)	108.6(10)	C(6A)-C(5A)-C(4A)	117.6(10)
C(7A)-C(6A)-C(5A)	114.8(10)	O(2A)-C(6A)-C(5A)	105.8(9)
O(2A)-C(6A)-C(7A)	106.9(9)	C(8A)-C(7A)-C(6A)	129.3(11)
C(9A)-C(8A)-C(7A)	120.4(11)	C(19A)-C(8A)-C(7A)	125.3(11)
C(19A)-C(8A)-C(9A)	114.1(11)	C(10A)-C(9A)-C(8A)	115.2(10)
C(11A)-C(10A)-C(9A)	111.0(10)	O(4A)-C(10A)-C(9A)	106.9(9)
O(4A)-C(10A)-C(11A)	108.0(10)	C(12A)-C(11A)-C(10A)	124.6(12)
C(13A)-C(12A)-C(11A)	117.6(12)	C(20A)-C(12A)-C(11A)	124.3(12)
C(20A)-C(12A)-C(13A)	118.0(12)	C(14A)-C(13A)-C(12A)	113.7(11)
C(13A)-C(14A)-C(1A)	112.3(10)	C(16A)-C(15A)-C(1A)	102.0(23)
C(17A)-C(15A)-C(1A)	103.8(19)	C(17A)-C(15A)-C(16A)	95.7(22)
O(2A)-C(21A)-C(22A)	114.0(13)	O(3A)-C(21A)-C(22A)	122.0(13)
O(3A)-C(21A)-O(2A)	123.8(13)	O(4A)-C(23A)-C(24A)	106.6(13)
O(5A)-C(23A)-C(24A)	127.1(15)	O(5A)-C(23A)-O(4A)	126.3(14)
C(21A)-O(2A)-C(6A)	119.6(10)	C(23A)-O(4A)-C(10A)	117.8(11)
Molecule B			
C(14B)-C(1B)-C(2B)	113.6(11)	C(15B)-C(1B)-C(2B)	112.5(12)
C(15B)-C(1B)-C(14B)	112.7(13)	C(3B)-C(2B)-C(1B)	128.9(12)
C(4B)-C(3B)-C(2B)	128.2(12)	C(5B)-C(4B)-C(3B)	110.6(10)
C(18B)-C(4B)-C(3B)	109.4(11)	C(18B)-C(4B)-C(5B)	113.0(11)
O(1B)-C(4B)-C(3B)	110.1(10)	O(1B)-C(4B)-C(5B)	104.2(10)
O(1B)-C(4B)-C(18B)	109.4(10)	C(6B)-C(5B)-C(4B)	116.6(10)
C(7B)-C(6B)-C(5B)	113.8(10)	O(2B)-C(6B)-C(5B)	109.5(10)
O(2B)-C(6B)-C(7B)	104.8(9)	C(8B)-C(7B)-C(6B)	130.6(11)
C(9B)-C(8B)-C(7B)	121.7(11)	C(19B)-C(8B)-C(7B)	122.9(11)
C(19B)-C(8B)-C(9B)	115.4(10)	C(10B)-C(9B)-C(8B)	113.3(9)
C(11B)-C(10B)-C(9B)	112.9(10)	O(4B)-C(10B)-C(9B)	107.1(9)
O(4B)-C(10B)-C(11B)	109.5(9)	C(12B)-C(11B)-C(10B)	124.7(12)
C(13B)-C(12B)-C(11B)	118.6(12)	C(20B)-C(12B)-C(11B)	126.3(12)
C(20B)-C(12B)-C(13B)	115.0(12)	C(14B)-C(13B)-C(12B)	113.9(11)
C(13B)-C(14B)-C(1B)	114.1(11)	C(16B)-C(15B)-C(1B)	113.3(18)
C(17B)-C(15B)-C(1B)	110.1(17)	C(17B)-C(15B)-C(16B)	105.1(17)
O(2B)-C(21B)-C(22B)	111.0(15)	O(3B)-C(21B)-C(22B)	127.7(17)
O(3B)-C(21B)-O(2B)	121.0(16)	O(4B)-C(23B)-C(24B)	110.2(13)
O(5B)-C(23B)-C(24B)	125.0(13)	O(5B)-C(23B)-O(4B)	124.8(12)
C(21B)-O(2B)-C(6B)	116.0(11)	C(23B)-O(4B)-C(10B)	117.5(10)

Molecular Mechanics Calculations.

In order to gain further insight into the conformational properties of two of the compounds (**1**, **3**), molecular mechanics calculations (MM3)[6-8] were performed. To this end, the acetoxy groups in the energy-minimized solid state structure of the diacetate **11** was replaced by hydroxy groups to give a three-dimensional structure of **1** (structure **X**). This was submitted to the Ringmaker program[9] for a systematic conformation

search. By using an angle increment of 20°, some 3000 primary conformations were obtained. Of these, 1263 were unique. These were then energy-minimized, the relative permittivity, ε, being set to 4.8 to simulate the effect of the solvent, $CDCl_3$, used in the NMR experiments. The resultant conformations had relative steric energies ranging from 0 to 23 kcal. Those falling within an energy range of 0-1.9 kcal, in all 23, were selected for further study. They converged into four groups, **A** (0 kcal), **B** (0.7 kcal), **C** (0.9 kcal) and **D** (1.5 kcal). Together they comprise 87 % of the Boltzmann population of the conformation space.

It can be seen from Fig. 3 and the endocyclic torsion angles listed in Table 6 that the best fit with structure **X** and the corresponding energy-minimized structure **X*** is found for conformation **B**. Conformations **A** and **D** differ mainly from conformation **B** with respect to the C-1 to C-8 portion of the molecule. The main difference between conformation **C** on one hand and conformations **A**, **B** and **D** on the other hand resides in the C-8 to C-14 portion of the molecule.

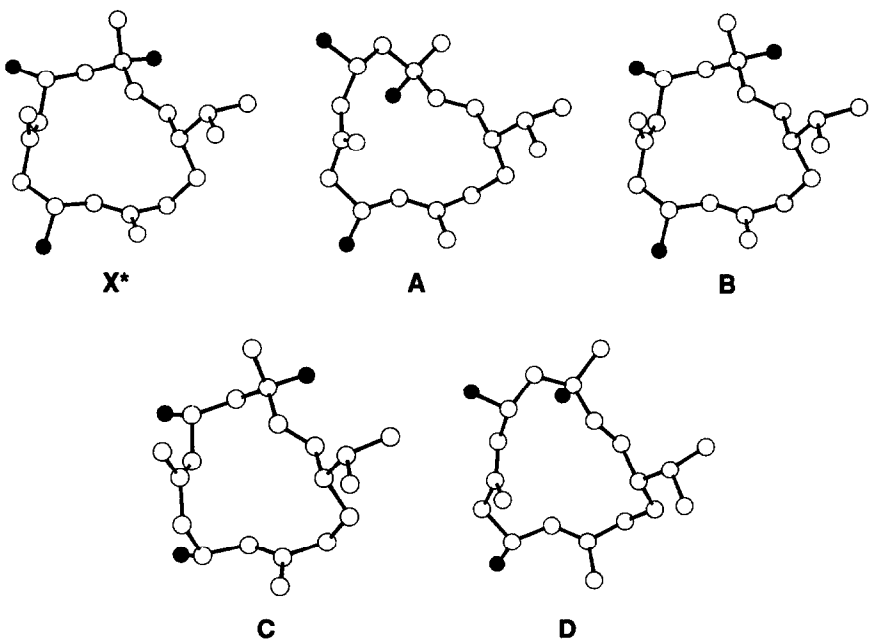


Fig. 3. Plots of conformations **X*** and **A-D** (triol **1**).

The NOE data are consistent with the solution conformation of triol **1** being of type **B**. In conformations **A** and **D**, H-6 is too distant from H-18 to account for the NOE response observed between these protons and in **C**, H-7 and H-11 are too distant from H-9a.

A generalized Karplus equation[10,11] was used to estimate the vicinal ^1H-^1H coupling constants for conformations **X**, **X*** and **A-D**. The values obtained are compared with those measured for triol **1** in Table 7. It is evident that conformations **X**, **X*** and **B** show the best agreement with the observed coupling constants, hence substantiating the view that these are reminiscent of the solution conformation of triol **1**.

Plausible conformations of triol **3** were generated in a similar manner. In this case the input to the Ringmaker program, structure **Y**, was obtained by reversal of the configuration of C-4 in structure **X** Some 1900 initial geometries were obtained, of which 796 were unique. They were energy-minimized (relative steric energy range 0-19 kcal), those conformations having energies below 1 9 kcal being subjected to further study. These formed four groups, **E** (0 kcal), **F** (0.2 kcal), **G** (0.3 kcal) and **H** (1.2 kcal) together accounting for 93 % of the Boltzmann population of the conformation space.

Table 6. Endocyclic Torsion Angles (deg.) in Conformations **X**, **X***, **Y**, **Y*** and **A-H**.[a,b]

Torsion angle	Conformation											
	X	**X***	**A**	**B**	**C**	**D**	**Y**	**Y***	**E**	**F**	**G**	**H**
1-2-3-4	176.3	170.7	-174.2	169.7	172.4	-173.7	176.3	171.0	172.7	171.0	178.8	174.5
2-3-4-5	119.9	116.0	-142.3	120.8	108.2	159.1	120.0	118.9	111.3	119.0	116.2	97.8
3-4-5-6	61.9	58.0	-41.2	62.2	60.1	-37.7	61.9	58.6	58.2	58.3	54.9	57.2
4-5-6-7	-96.0	-95.9	-47.0	-89.8	-78.6	-52.5	-96.0	-97.5	-80.4	-97.6	-82.9	-104.4
5-6-7-8	123.9	129.5	133.2	122.3	122.8	143.2	123.9	128.1	126.5	127.0	-71.7	125.0
6-7-8-9	-179.2	176.9	-171.5	175.7	-179.6	176.6	-179.1	179.0	-178.9	177.3	180.0	175.1
7-8-9-10	107.4	107.3	121.4	107.6	6.3	122.3	107.4	108.9	5.9	110.1	-121.3	113.3
8-9-10-11	-56.1	-60.2	-59.0	-58.3	60.5	-58.7	-56.1	-59.9	60.7	-57.5	51.4	-55.4
9-10-11-12	166.2	166.8	150.9	169.4	86.5	147.2	166.2	167.9	85.4	163.6	115.7	158.2
10-11-12-13	-179.5	-174.1	-179.8	-174.9	-177.2	-179.5	-179.4	-175.1	-177.3	-175.6	177.7	-172.0
11-12-13-14	120.6	118.5	123.9	118.2	120.1	120.0	120.6	118.1	120.2	120.1	117.0	116.1
12-13-14-1	-53.8	-56.7	-59.1	-55.7	-54.6	-58.1	-53.7	-56.8	-55.0	-57.4	-58.7	-56.7
13-14-1-2	71.9	65.2	59.1	65.5	60.6	55.6	72.0	65.6	60.8	64.6	60.1	65.5
14-1-2-3	131.7	139.7	113.2	130.1	143.2	157.4	131.6	136.8	142.6	136.6	153.4	158.9

[a] Conformation **X** was obtained by replacing the acetoxy groups in the energy-minimized (MM3) solid state structure of diacetate **11** with hydroxy groups. Conformation **X*** represents the structure obtained by energy-minimization of structure **X**.

[b] Conformation **Y** was obtained from the energy-minimized solid state structure of diacetate **11** by replacement of the acetoxy groups with hydroxy groups and reversal of the configuration at C-4. Conformation **Y*** represents the structure obtained by energy-minimization of structure **Y**.

Table 7. Observed Vicinal Coupling Constants (Hz) for Triol 1 and Calculated Values for Conformations X, X* and A-D.[a]

J_3	Observed[b]	Calculated Conformation					
		X	X*	A	B	C	D
H_5-H_6	8.6	9.0	10.4	9.7	11.1	11.6	10.2
	1.9	1.2	1.1	6.3	1.4	0.9	5.7
H_9-H_{10}	4.8	4.5	4.1	4.6	4.4	2.2	4.6
	10.2	11.3	11.3	11.1	11.1	4.6	11.1
H_{13}-H_{14}	4.2	2.2	3.1	3.5	3.0	2.8	3.4
	4.2	4.2	3.9	3.5	4.0	4.3	3.7
	4.2	4.2	3.7	3.6	3.9	4.2	3.7
	12.9	13.7	13.6	13.6	13.5	13.4	13.6
H_{14}-H_1	11.3	12.0	12.3	12.4	12.2	12.4	12.3
	2.5	1.9	2.1	2.6	2.0	2.6	3.1

[a] Conformation X was obtained by replacing the acetoxy groups in the energy-minimized (MM3) solid state structure of diacetate 11 with hydroxy groups. Conformation X* represents the structure obtained by energy-minimization of structure X.

[b] Coupling constants (accuracy ± 0.2 Hz) were obtained from a 600 MHz spectrum recorded in $CDCl_3$.

It is evident from Fig. 4 and the endocyclic torsion angles listed in Table 6 that conformations **F** and **H** show the best agreement with **Y** and the energy-minimized **Y*** and that **E** and **G** differ from these mainly with respect to the C-8 to C-14 portion of the molecule. Moreover, the vicinal coupling constants calculated for conformations **F** and **H** agree well enough with those observed in solution to suggest that these conformations are similar to the solution conformation of triol **3** (Table 8). This view is supported by the NOE results. Thus conformation **E** is excluded because the through-space distances between H-7 and H-9a and between H-11 and H-9a are too large. In conformation **G**, H-19 is too distant from H-6 to account for the NOE observed between these protons. The distances between relevant atoms in conformations **F** and **H**, on the other hand, are compatible with the NOE data.

Table 8. Observed Vicinal Coupling Constants (Hz) for Triol 3 and Calculated Values for Conformations Y, Y* and E-H.[a]

J_3	Observed[b]	Calculated Conformation					
		Y	Y*	E	F	G	H
H_5-H_6	8.3	9.0	10.0	11.5	10.0	11.2	8.9
	1.3	1.2	1.1	0.9	1.1	1.5	1.3
H_9-H_{10}	5.1	4.6	4.2	2.1	4.6	3.1	4.8
	10.8	11.3	11.2	4.6	11.1	3.4	10.9
H_{13}-H_{14}	4.2	2.2	3.2	2.9	3.2	3.5	3.2
	4.2	4.1	3.9	4.2	3.8	3.6	3.9
	4.2	4.2	3.7	4.2	3.7	3.6	3.7
	13.1	13.7	13.6	13.4	13.6	13.6	13.6
H_{14}-H_1	11.5	12.0	12.2	12.4	12.3	12.4	12.2
	2.6	1.9	2.0	2.6	2.1	2.5	2.0

[a] Conformation Y was obtained from the energy-minimized (MM3) solid state structure of diacetate 11 by replacement of the acetoxy groups with hydroxy groups and reversal of the configuration at C-4. Conformation Y* represents the structure obtained by energy-minimization of structure Y.

[b] Coupling constants (accuracy ± 0.2 Hz) were obtained from a 600 MHz spectrum recorded in $CDCl_3$.

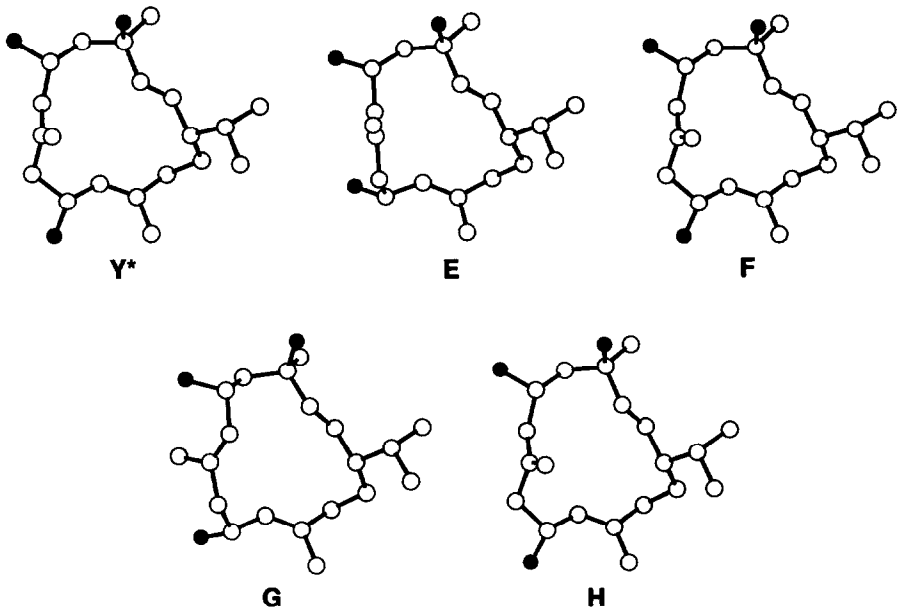
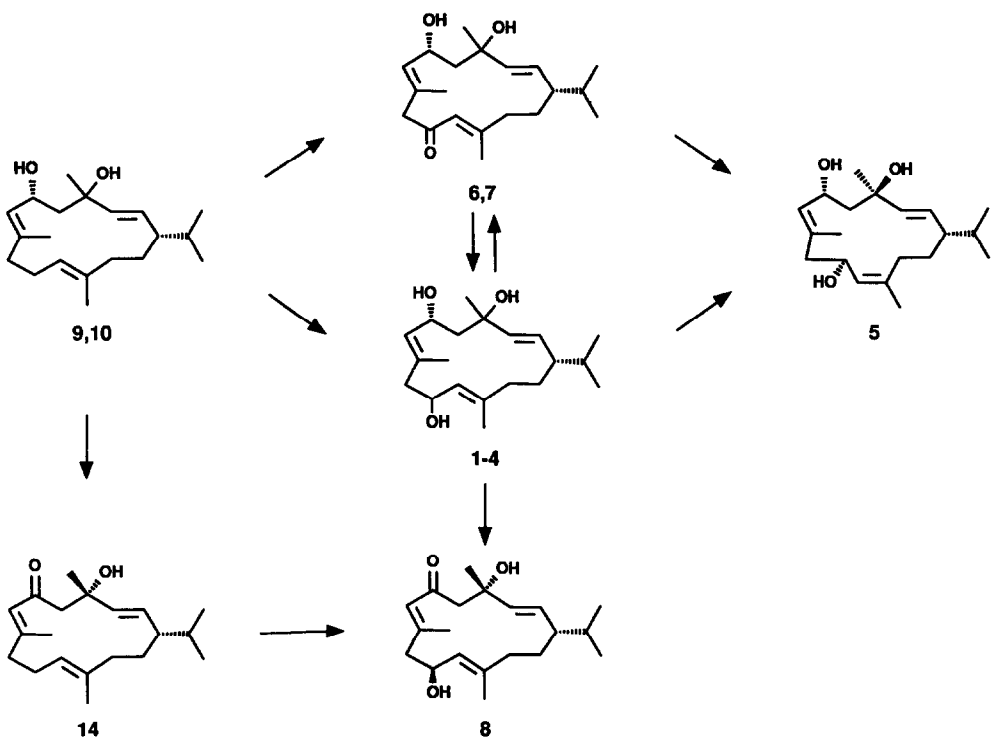


Fig.4. Plots of conformations **Y*** and **E-H** (triol **3**)



Scheme 1. Proposed biogenesis of compounds **1-8**.

Biogenesis.

It is reasonable to assume that the new compounds **1-8** are metabolites of the 4,6-diols **9** and **10**. The triols **1-4** and the oxodiols **6** and **7** would then be formed by allylic oxidation at C-10 resulting in the introduction of a hydroxy group or an oxo group (Scheme 1). Consonant with this is a patent describing the preparation of (a) 2,7,11-cembratriene-4,6,10-triol(s) from (a) 4,6-diol(s) with the aid of bacteria.[12] Triol **5** may arise by isomerization of the 11,12-double bond in **4** or by isomerization and reduction of the oxo group in **7**. Oxidation of the hydroxy group at C-6 in **9** and **10** followed by allylic oxidation at C-10 is a plausible route to the oxodiol **8**. The existence of this route is supported by the fact that the 6-oxo compound **14** is present in tobacco.[13] Another possible route to **8** involves the oxidation of the hydroxy group at C-6 in triol **2**.

EXPERIMENTAL

Instruments. High performance liquid chromatography was carried out using a Waters 6000A or Delta Prep 3000 solvent delivery system, a Waters 6UK injector and a Waters R-401 or R-403 differential refractometer. Melting points, optical rotations and infrared spectra were recorded on a Leitz Wetzlar instrument, a Perkin-Elmer 241 polarimeter and a Perkin-Elmer FT-IR 1725X spectrometer, respectively. NMR spectra were obtained on a Varian XL-300 instrument and mass spectra on a Kratos MS 50 Stereo DS 55 SM / DS 55 S mass spectrometer-computer system.

2D-NMR Experiments. The NOESY experiments were carried out using a 1.00 sec. mixing time. The HMQC and HMBC experiments were obtained with delay times set to 3.45 msec. and 50 msec., respectively.

Isolation. Fractions C (70.9 g) and D (128 g), obtained from an extract of flowers of Greek *Nicotiana tabacum* (Basma),[14] were separated by flash chromatography over silica gel into 6 fractions, C1-C6, and 8 fractions, D1-D8, respectively. Further separation of fraction C4 (14.7 g) by HPLC (Spherisorb 5, hexane/EtOAc 1:1; Spherisorb 5 ODS, MeOH/H_2O 90:10; Lichrosorb Diol, hexane/EtOAc 40:60, Spherisorb 5 ODS, MeOH/H_2O 80:20; Spherisorb 5 CN, hexane/EtOAc 60:40) gave 2.2 mg of (1*S**,2*E*,4*S**,7*E*,10*S**,11*E*)-4,10-dihydroxy-2,7,11-cembratrien-6-one (**8**).

Fraction D4 (17.1 g) was separated by HPLC (Spherisorb 5, EtOAc) into 5 fractions. Of these, fraction D43 (3.6 g) was separated further by HPLC (Spherisorb 5 CN, hexane/EtOAc 60:40; Lichrosorb Diol, hexane/EtOAc 30:70) to give 75.5 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-4,6-dihydroxy-2,7,11-cembratrien-10-one (**6**). Repetitive HPLC of fraction D44 (1.8 g) (Spherisorb 5 CN, hexane/EtOAc 60:40; Spherisorb 5 ODS, MeOH/H_2O 70:30) gave 24.6 mg of (1*S**,2*E*,4*R**,6*R**,7*E*,11*E*)-4,6-dihydroxy-2,7,11-cembratrien-10-one (**7**).

Fraction D7 (10.5 g) was separated into 8 fractions using HPLC (Spherisorb 5, EtOAc). Separation of fraction D74 (1.0 g) by HPLC (Lichrosorb Diol, hexane/EtOAc 10:90) gave 128 mg of a crude sample of (1*S*,2*E*,4*S*,6*R*,7*E*,10*S*,11*E*)-2,7,11-cembratriene-4,6,10-triol (**2**). Purification of part of this material by HPLC (Spherisorb 5 ODS, MeOH/H_2O 75:25) gave 26.2 mg of an analytical sample of triol **2**.

Further separation of fraction D75 (721 mg) by HPLC (Lichrosorb Diol, hexane/EtOAc 10:90; Spherisorb 5 CN, hexane/EtOAc 40:60) gave 159 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*E*)-2,7,11-cembratriene-4,6,10-triol (**1**). Repetitive HPLC of fraction D76 (211 mg) (Spherisorb 5 CN, hexane/EtOAc 1:1; Spherisorb 5, hexane/EtOAc 20:80) afforded 8.2 mg of (1*S**,2*E*,4*R**,6*R**,7*E*,10*S**,11*E*)-2,7,11-cembratriene-4,6,10-triol (**4**). Further separation of fraction D77 (437 mg) by HPLC (Lichrosorb Diol, EtOAc; Spherisorb 5 CN, hexane/EtOAc 40:60) afforded 104 mg of (1*S**,2*E*,4*R**,6*R**,7*E*,10*R**,11*E*)-2,7,11-cembratriene-4,6,10-triol (**3**) Repetitive HPLC of fraction D78 (471 mg) (Spherisorb 5 CN hexane/EtOAc 1·1; Spherisorb 5 ODS, acetonitrile/acetone/H_2O 4:3.4) gave 8.0 mg of (1*S**,2*E*,4*R**,6*R**,7*E*,10*S**,11*Z*)-2,7,11-cembratriene-4,6,10-triol (**5**).

(1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*E*)-2,7,11-Cembratriene-4,6,10-triol (**1**) was obtained as an oil, $[\alpha]_D$ +111 ° (*c* 0 92, $CHCl_3$), (Found: $[M\text{-}36]^{+\cdot}$ 286 2303 Calc. for $C_{20}H_{30}O$: 286.2296); IR ($CHCl_3$): 3600, 3426, 1664, 1385, 1370 and 974 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80 (d, *J* 6 5 Hz) / 0.83 (d, *J* 6.4 Hz) (H-16 / H-17), 1.36 (s, H-18), 1.68 (broad s, H-19 and H-20), 1.95 (dd, *J* 8.1 and -13 4 Hz, H-5a), 2.02 (dd, *J* 2.3 and -13.4 Hz, H-5b), 2.15 (dd, *J* 10.3 and -12.4 Hz, H-9a), 2.54 (ddq, *J* 0.6, 4.8 and -12.4 Hz, H-9b), 4.42 (ddd, *J* 2.3, 8.1 and

9.3 Hz, H-6), 4.53 (ddd, *J* 4.8, 7.7 and 10.3 Hz, H-10), 5.21 (dquintet, *J* 1.3 and 7.7 Hz, H-11), 5.28 (d, *J* 15.3 Hz, H-3), 5.34 (dd, *J* 8.7 and 15.3 Hz, H-2) and 5.42 (dquintet, *J* 1.2 and 9.3 Hz, H-7); MS [*m/z* (%)]: 304 (0.5), 287 (8), 286 (1), 269 (2), 243 (1), 223 (20), 203 (11), 187 (3), 177 (4), 165 (23), 147 (15), 137 (17), 123 (15), 109 (18), 97 (39), 84 (39), 69 (45), 55 (39) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,10*S*,11*E*)-2,7,11-Cembratriene-4,6,10-triol (**2**) had m.p. 92.0-93.5 °C; $[\alpha]_D$ +36 ° (*c* 0.63, $CHCl_3$); (Found: [*M*-18]$^{+\cdot}$ 304.2379. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$): 3602, 3466, 1666, 1385, 1369 and 977 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80 (d, *J* 6.8 Hz) / 0.84 (d, *J* 6.7 Hz) (H-16 / H-17), 1.28 (s, H-18), 1.70 (d, *J* 1.1 Hz, H-20), 1.77 (d, *J* 1.2 Hz, H-19), 2.3-2.4 (overlapping signals, H-9a and H-9b), 4.5-4.7 (overlapping signals, H-6 and H-10), 5.16 (dq, *J* 1.2 and 8.6 Hz, H-7), 5.28 (dq, *J* 1.1 and 8.9 Hz, H-11), 5.36 (d, *J* 15.3 Hz, H-3) and 5.41 (dd, *J* 8.7 and 15.3 Hz, H-2); MS [*m/z* (%)]: 322 (0.2), 304 (0.6), 286 (2), 260 (2), 243 (2), 223 (13), 203 (6), 189 (4), 177 (5), 165 (17), 147 (12), 137 (16), 123 (22), 109 (22), 97 (34), 81 (38), 69 (44), 55 (42) and 43 (100).

(1*S**,2*E*,4*R**,6*R**,7*E*,10*R**,11*E*)-2,7,11-Cembratriene-4,6,10-triol (**3**) was obtained as an oil; $[\alpha]_D$ +102 ° (*c* 0.85, $CHCl_3$); (Found· [*M*-36]$^{+\cdot}$ 286.2330. Calc. for $C_{20}H_{30}O$: 286.2296); IR ($CHCl_3$): 3673, 3602, 3436, 1664, 1385, 1369 and 976 cm^{-1}; ^1H NMR ($CDCl_3$). δ 0.80 (d, *J* 6.6 Hz) / 0.83 (d, *J* 6.5 Hz) (H-16 / H17), 1.39 (s, H-18), 1.68 (s) / 1.69 (s) (H-19 / H-20), 1.86 (dd, *J* 8.2 and -14.4 Hz, H-5a), 1.93 (dt, *J* 4.1 and -13.0 Hz, H-13a), 2.04 (dd, *J* 1.5 and -14.4 Hz, H-5b), 2.10 (dd, *J* 10.8 and -12.2 Hz, H-9a), 2.58 (dd, *J* 5.2 and -12.2 Hz, H-9b), 4.50 (ddd, *J* 5.2, 7.5 and 10.8 Hz, H-10), 4.77 (ddd, *J* 1.5, 8.2 and 9.8 Hz, H-6), 5.18 (dquintet, *J* 1.3 and 7.5 Hz, H-11), 5.22 (dd, *J* 8.7 and 15 6 Hz, H-2), 5.33 (dquintet, *J* 1.3 and 9.8 Hz, H-7) and 5.35 (d, *J* 15.6 Hz, H-3); MS [*m/z* (%)]· 304 (0.5), 286 (6), 268 (4), 243 (4), 223 (12), 205 (7), 187 (5), 177 (5), 165 (18), 147 (17), 133 (16), 123 (21), 105 (25), 95 (37), 81 (53), 69 (56), 55 (46) and 43 (100).

(1*S**,2*E*,4*R**,6*R**,7*E*,10*S**,11*E*)-2,7,11-Cembratriene-4,6,10-triol (**4**) had m.p. 107.0-108.0 °C; $[\alpha]_D$ +33 ° (*c* 0.83, $CHCl_3$); (Found: [*M*-18]$^{+\cdot}$ 304.2395. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$): 3603, 3433, 1666, 1385 and 979 cm^{-1}; ^1H NMR ($CDCl_3$); δ 0.80 (d, *J* 6.8 Hz) / 0 84 (d, *J* 6.7 Hz) (H-16 / H-17), 1.38 (s, H-18), 1.69 (d, *J* 1.3 Hz, H-20), 1.73 (dd, *J* 9.9 and -13.8 Hz, H-5a), 1 86 (d, *J* 1.4 Hz, H-19), 2.06 (dd, *J* 1.9 and -13.8 Hz, H-5b), 2.26 (dd, *J* 8.5 and -15.5 Hz, H-9a), 2.41 (ddd, *J* 1 5, 3.3 and -15.5 Hz, H-9b), 4.66 (dt, *J* 3.3 and 8.5 Hz, H-10), 4.84 (ddd, *J* 1.9, 9.2 and 9.9 Hz, H-6), 5.06 (dsextet, *J* 1.4 and 9.2 Hz, H-7), 5.23 (dd, *J* 9.2 and 15.6 Hz, H-2), 5.30 (dsextet, *J* 1.3 and 8.5 Hz, H-11) and 5.41 (d, *J* 15.6 Hz, H-3); MS [*m/z* (%)]: 286 (7), 268 (5), 243 (4), 223 (6), 203 (5), 187 (4), 165 (11), 159 (16), 145 (17), 133 (22), 119 (22), 105 (32), 91 (40), 81 (58), 69 (39), 55 (41) and 43 (100).

(1*S**,2*E*,4*R**,6*R**,7*E*,10*S**,11*Z*)-2,7,11-Cembratriene-4,6,10-triol (**5**) was obtained as an oil; $[\alpha]_D$ +102 ° (*c* 0.87 $CHCl_3$); (Found· [*M*-18]$^{+\cdot}$ 304 2365. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$): 3602, 3425, 1662, 1380, 1369 and 979 cm^{-1}; ^1H NMR ($CDCl_3$)· δ 0 80 (d, *J* 6.6 Hz) / 0.87 (d, *J* 6.6 Hz) (H-16 / H-17), 1.36 (s, H-18), 1.69 (d, *J* 1.4 Hz) / 1.70 (d, *J* 1.4 Hz) (H-19 / H-20), 2.50 (ddd, *J* 1.1, 3.8 and -12.4 Hz, H-9b), 4.34 (ddd, *J* 3.8, 9 2 and 10.0 Hz, H-10), 4 56 (ddd, *J* 4 1, 5.2 and 9.2 Hz, H-6), 5 18 (broad d, *J* 9.2 Hz, H-11), 5.23 (dd, *J* 8 6 and 15.9 Hz, H-2), 5 42 (broad d, *J* 9.2 Hz, H-7) and 5.49 (d, *J* 15.9 Hz, H-3); MS [*m/z* (%)]: 304 (0.4), 286 (1), 243 (1), 223 (20), 205 (8), 187 (4), 177 (3), 165 (10), 147 (16), 137 (24), 121 (18), 109 (17), 97 (39), 81 (36), 69 (42), 55 (38) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-4,6-Dihydroxy-2,7,11-cembratrien-10-one (**6**) was obtained as an oil; $[\alpha]_D$ +383 ° (*c* 0.83, $CHCl_3$); (Found: [*M*-18]$^{+\cdot}$ 302.2275 Calc. for $C_{20}H_{30}O_2$: 302.2246); IR ($CHCl_3$): 3599, 3453, 1683, 1665, 1615, 1387, 1369 and 977 cm^{-1}; ^1H NMR ($CDCl_3$). δ 0.78 (d, *J* 6.5 Hz) / 0.81 (d, *J* 6.5 Hz) (H-16 / H-17), 1.40 (s, H-18), 1.70 (d, *J* 1.2 Hz, H-19), 2.10 (s, H-20), 2.85 (d, *J* -12.8 Hz, H-9a), 3.15 (d, *J* -12.8 Hz, H-9b), 4 48 (ddd, *J* 2.2, 7.9 and 9.2 Hz, H-6), 5.27 (d, *J* 15.3 Hz, H-3), 5.36 (dd, *J* 8.2 and 15.3 Hz, H-2), 5.51 (broad d, *J* 9.2 Hz, H-7) and 6.23 (broad s, H-11); MS [*m/z* (%)]: 320 (0.1), 302 (1), 259 (1), 237 (2), 221 (26), 193 (5), 175 (4), 151 (3), 135 (12), 123 (13), 109 (9), 95 (24), 84 (25), 69 (25), 61 (8), 55 (16) and 43 (100).

(1*S**,2*E*,4*R**,6*R**,7*E*,11*E*)-4,6-Dihydroxy-2,7,11-cembratrien-10-one (**7**) was obtained as an oil; $[\alpha]_D$ +307 ° (*c* 0.98, EtOH); (Found: [*M*-18]$^{+\cdot}$ 302.2237. Calc. for $C_{20}H_{30}O_2$: 302.2246); IR ($CHCl_3$): 3601, 3431, 1684, 1666, 1617, 1387, 1368 and 979 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.79 (d, *J* 6.6 Hz) / 0.82 (d, *J* 6.5 Hz) (H-

16 / H-17), 1.43 (s, H-18), 1.74 (dt, *J* 0.5 and 1.3 Hz, H-19), 1.93 (dd, *J* 8.6 and -14.4 Hz, H-5a), 2.10 (dd, *J* 1.2 and -14.4 Hz, H-5b), 2.11 (dd, *J* 0.6 and 1.2 Hz, H-20), 2.87 (d, *J* -13.0 Hz, H-9a), 3.14 (d, *J* -13.0 Hz, H-9b), 4.88 (broad t, *J* 9.0 Hz, H-6), 5.25 (dd, *J* 8.8 and 15.6 Hz, H-2), 5.42 (d, *J* 15.6 Hz, H-3), 5.48 (broad d, *J* 9.6 Hz, H-7) and 6.20 (broad s, H-11); MS [*m/z* (%)]: 320 (0.1), 302 (2), 284 (1), 259 (2), 237 (2), 221 (47), 193 (8), 175 (6), 151 (5), 135 (19), 123 (21), 109 (14), 95 (37), 84 (38), 69 (39), 55 (24) and 43 (100).

(1*S**,2*E*,4*S**,7*E*,10*S**,11*E*)-4,10-Dihydroxy-2,7,11-cembratrien-6-one (**8**) was obtained as an oil; $[\alpha]_D$ -45 ° (*c* 0.22, $CHCl_3$); (Found: [*M*-18]$^{+\cdot}$ 302.2221. Calc. for $C_{20}H_{30}O_2$: 302.2246); IR ($CHCl_3$): 3603, 3461, 1667, 1604, 1386, 1368 and 999 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.81 (d, *J* 6.7 Hz) / 0.84 (d, *J* 6.7 Hz) (H-16 / H-17), 1.31 (d, *J* 0.7 Hz, H-18), 1.43 (d, *J* 3.1 Hz, OH), 1.63 (dd, *J* 0.7 and 1.4 Hz, H-20), 2.10 (dd, *J* 0.7 and 1.3 Hz, H-19), 2.24 (ddd, *J* 0.7, 9.8 and -12.5 Hz, H-9a), 2.42 (d, *J* -12.4 Hz, H-5a), 2.64 (dd, *J* 4.2 and -12.5 Hz, H-9b), 2.77 (d, *J* -12.4 Hz, H-5b), 3.62 (s, OH), 4.65 (m, H-10), 5.07 (broad d, *J* 9.3 Hz, H-11), 5.40 (d, *J* 15.5 Hz, H-3), 5.50 (dd, *J* 8.2 and 15.5 Hz, H-2) and 5.98 (q, *J* 1.0 Hz, H-7); MS [*m/z* (%)]: 320 (0.2), 302 (2), 284 (0.4), 259 (1), 219 (3), 203 (2), 177 (3), 165 (4), 149 (10), 136 (11), 123 (10), 109 (8), 97 (20), 82 (70), 69 (22), 55 (28) and 43 (100).

Acetylation of (1S,2E,4S,6R,7E,10R,11E)-2,7,11-cembratriene-4,6,10-triol (**1**). Treatment of 21 mg of **1** with 0.5 ml of acetic anhydride in 1 ml of pyridine for 5 h at room temperature followed by work-up and purification by HPLC (Spherisorb 5, hexane/EtOAc 60:40) gave 18.3 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*E*)-6,10-diacetoxy-2,7,11-cembratriene-4-ol (**11**), which had m.p. 121.5-122.5 °C; $[\alpha]_D$ +113 ° (*c* 0.88, $CHCl_3$); IR ($CHCl_3$): 3597, 3475, 1724, 1666, 1250 and 976 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.80 (d, *J* 6.6 Hz) / 0.83 (d, *J* 6.5 Hz) (H-16 / H-17), 1.37 (s, H-18), 1.60 (s, H-20), 1.75 (s, H-19), 2.02 (s, -OCOCH$_3$), 2.04 (s, -OCOCH$_3$), 2.16 (dd, *J* 10.7 and -12.6 Hz, H-9a), 2.56 (dd, *J* 5.2 and -12.6 Hz, H-9b), 5.12 (dquintet, *J* 1.3 and 8.4 Hz, H-7), 5.2-5.4 (overlapping signals, H-2 and H-3), 5.33 (broad d, *J* 8.1 Hz, H-11), 5.48 (ddd, *J* 2.6, 8.4 and 9.9 Hz, H-6) and 5.67 (ddd *J* 5.2, 8.1 and 10.7 Hz, H-10); MS [*m/z* (%)]: 406 (0.2), 346 (4), 328 (2), 303 (2), 286 (16), 268 (8), 243 (10), 223 (22), 205 (18), 187 (5), 165 (22), 147 (15), 137 (11), 123 (13), 107 (14), 97 (23), 81 (23), 69 (17), 55 (16) and 43 (100).

Oxidation of (1S,2E,4S,6R,7E,11E)-6-acetoxy-2,7,11-cembratriene-4-ol (**12**). To a stirred solution of 180 mg of **12** in 18 ml of benzene and 1.98 g of Celite were added 388 mg of pyridinium dichromate (PDC) followed by the addition of 133 mg of 70% *tert.*-butyl hydroperoxide.[15] The reaction was stirred for 28 h at room temperature. Diethyl ether (20 ml) was added and the reaction mixture was filtered through a pad of Celite and washed with diethyl ether. The pooled filtrate was taken to dryness and the residue was separated by flash chromatography (SiO_2, hexane/EtOAc 80:20 and 60:40) and HPLC (Spherisorb 5, hexane/EtOAc 60:40) to give as one of the products 8.4 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-6-acetoxy-4-hydroxy-2,7,11-cembratrien-10-one (**13**) which was obtained as an oil; $[\alpha]_D$ +377 ° (*c* 0.69, $CHCl_3$); IR ($CHCl_3$): 3597, 3457, 1728, 1685, 1666, 1616, 1386, 1371 and 1247 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.78 (d, *J* 6.5 Hz) / 0.82 (d, *J* 6.4 Hz) (H-16 / H-17), 1.43 (s, H-18), 1.76 (broad s, H-19), 2.04 (s, -OCOCH$_3$), 2.10 (broad s, H-20), 2.87 (d, *J* -13.0 Hz, H-9a), 3.13 (d, *J* -13.0 Hz, H-9b), 5.25-5 40 (overlapping signals, H-2 and H-3), 5.42 (broad d, *J* 9.6 Hz, H-7), 5.52 (ddd, *J* 3.9, 6.1 and 9.6 Hz, H-6) and 6.20 (broad s, H-11); MS [*m/z* (%)]: 362 (0.2), 302 (4), 284 (2), 259 (2), 221 (82), 193 (14), 175 (8), 159 (5), 151 (5), 135 (22), 123 (18), 109 (12), 95 (32), 82 (21), 69 (14), 55 (13) and 43 (100).

Reduction of (1S,2E,4S,6R,7E,11E)-6-acetoxy-4-hydroxy-2,7,11-cembratrien-10-one (**13**). To a solution of 8.8 mg of **13** in 1 ml of dry diethyl ether was added an excess of $LiAlH_4$. The reaction mixture was stirred under nitrogen at room temperature for 2 h. Work-up followed by HPLC (Spherisorb 5 CN, hexane/EtOAc 30:70) gave 1.0 mg of a product which was identical (optical rotation, IR, ^{1}H NMR and MS) to (1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*E*)-2,7,11-cembratriene-4,6,10-triol (**1**) and 1.8 mg of a product which was identical to (1*S*,2*E*,4*S*,6*R*,7*E*,10*S*,11*E*)-2,7,11-cembratriene-4,6,10-triol (**2**).

Acetylation of (1S,2E,4S,6R,7E,11E)-4,6-dihydroxy-2,7,11-cembratrien-10-one (**6**). Treatment of 5.2 mg of **6** with 0.2 ml of acetic anhydride in 0.4 ml of pyridine for 2.5 h at room temperature followed by work-up gave

4.6 mg of a product which was identical (optical rotation, IR, ^{1}H NMR and MS) with (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-6-acetoxy-4-hydroxy-2,7,11-cembratrien-10-one (**13**).

*Reduction of (1*S*,2E,4*S*,6R,7E,11E)-4,6-dihydroxy-2,7,11-cembratrien-10-one* (**6**). To a solution of 12 mg of **6** in 2 ml of MeOH was added an excess of $NaBH_4$. The reaction mixture was stirred at room temperature for 40 min. Work-up followed by HPLC (Spherisorb 5 CN, hexane/EtOAc 30:70) gave 0.8 mg of a product which was identical (optical rotation, IR, ^{1}H NMR and MS) to (1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*E*)-2,7,11-cembratriene-4,6,10-triol (**1**) and 2.4 mg of a product which was identical to (1*S*,2*E*,4*S*,6*R*,7*E*,10*S*,11*E*)-2,7,11-cembratriene-4,6,10-triol (**2**).

X-Ray Crystallography Study. Single crystals of **11** were obtained by recrystallization from a mixture of benzene and hexane. The space group symmetry was determined as $P2_12_12$ from systematic extinctions and by the unit cell parameters found by the least-squares method from 17 centred reflections. The intensity stability was monitored by measurement of three standard reflections (0 1 7, 3 3 2, 0 4 0) every 60 min. Three crystals were measured until the intensity of the standard reflections of each decreased by 5%. The intensities were scaled and corrected for Lorentz and polarization effects but no correction was made for absorption.

In all, 53 of the 58 non-hydrogen atoms were found by direct methods using the SHELXS86 program.[16] The remaining non-hydrogen atoms were located from difference electron density maps. Except for the hydroxy hydrogens, which were not found, all hydrogen atoms were geometrically placed and refined. Block matrix least-squares anisotropic refinement of the non-hydrogen parameters was applied using the SHELX76 program.[17] The hydrogen parameters were refined with all isotropic temperature factors constrained to a common value and bond lengths restricted to 1.00 Å. The atomic scattering factors[18] used for the non-hydrogen and hydrogen atoms were those included in SHELX76 program (Table 9) Lists of refined coordinates have been deposited at the Cambridge Crystallographic Data Centre.

Table 9. Crystal and Experimental Data for (1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*E*)-6,10-Diacetoxy-2,7,11-cembratriene-4-ol(**11**).

Formula	$2(C_{24}H_{38}O_5)$
Formula weight	811 11
Space group	$P2_12_12$
Unit cell dimensions	a=10.6655(16), b=33.1329(44), c=13.9388(34) Å
Unit cell volume,	4925.7(12.2)Å^3
Formula units per unit cell, Z	4
Calculated density, D_x	1.10 g cm^{-3}
Radiation	Cu Kα
Wavelength, λ	1.54184 Å
Linear absorption coefficient	5.70 cm^{-1}
Temperature, T	293(1) K
Crystal shape	Prismatic
Diffractometer	Siemens/Stoe AED 2
Determination of unit cell:	
Number of reflections used	17
θ-range	10.0 to 25.0°
Intensity data collection:	
Maximum [sin(θ)/λ]	0.59 Å^{-1}
Range of h, k and l	-0 to 12, 0 to 38 and 0 to 16
Standard reflections	3
Intensity instability	< 5 %
Number of collected reflections	4602
Number of unique reflections	3675

Number of observed reflections	1392
Criterion for significance	$F > 6\cdot\sigma(F)$
Structure refinement:	
Minimization of	$\Sigma\omega\cdot\Delta F^2$
Anisotropic thermal parameters	All non-hydrogen atoms
Isotropic thermal parameters	Hydrogen atoms
Number of refined parameters	530
Weighting scheme	$(\sigma^2(F)+0.0008\,\lvert F\rvert^2)^{-1}$
Final R for observed refls.	0.051
Final wR for observed refls.	0.063
Final wR for all 3650 refls	0.14
Final $(\Delta/\sigma)_{max}$	0.09
Final $\Delta\rho_{min}$ and $\Delta\rho_{max}$	-0.15 and 0.17 eÅ^{-3}

Computational Methods. The possible conformers of triols **1** and **3** used in the MM calculations were generated by using the computer program RNGCFM (QCPE No. 510).[9] The following parameters were used: angle increment 20 °, ring closure bond length limits 1.3-1.7 Å, bond closure angle limits 100-130 °, dihedral closure tolerance limits 30 °, vdW radii scale 0.8, no ring constraints. The structures obtained were subjected to energy minimization employing the computer program MM3(89).[6-8]

The vicinal coupling constants, $^3J_{HH}$, were calculated by using an empirically generalized Karplus equation developed by Haasnoot, de Leeuw and Altona[10] and applied in the program 3JHHPC (QCPE No. QCMPO25).[11] All calculations were carried out on a Convex C220 computer.

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