Tobacco Chemistry. 74.* Four New Epoxycembranoids from Tobacco

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The isolation of (1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*R*)-10,11-epoxy-2,7,12(20)-cembratriene-4,6-diol (**1**), the corresponding (10*S*,11*S*)-isomer (**2**), (1*S*,2*E*,4*S*,6*R*,7*E*,11*R*,12*R*)-11,12-epoxy-2,7-cembratriene-4,6-diol (**3**) and the corresponding (4*R*)-epimer (**4**) from tobacco flowers is reported. All four compounds are new natural products, **3** and **4** having previously been described as synthetic products. The structures of **1** and **2** have been determined by spectral methods and, in the case of **1**, by X-ray analysis. A full account of the X-ray work is given. The solution conformation of **1** has been studied with the aid of molecular mechanics calculations and NMR coupling information.

Since the discovery of the first tobacco cembranoids in the early 1960s the group has expanded considerably and now comprises some seventy compounds. The major constituents and also the principal precursors of most of the other tobacco cembranoids are the C-4 epimers of (1*S*,2*E*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (**5**, **6**).[2] We now report the isolation, from an extract of flowers of Greek tobacco, of four new cembranoids (**1**–**4**). They are plausible metabolites of the 4,6-diols **5** and **6**.

Results

Structure elucidation. The first new compound (**1**), $C_{20}H_{32}O_3$, is a diol having one secondary and one tertiary hydroxy group [OH-absorption in the IR spectrum; ^{13}C NMR: δ 68.6 (d) and 73.8 (s)]. The remaining oxygen atom is part of a 1,2-disubstituted epoxide group [^{13}C NMR: δ 60.3 (d) and 60.4 (d)]. Epoxide **1** also possesses three double bonds [^{13}C NMR: δ 106.6 (t), 127.6 (d), 128.7 (d), 133.9 (s), 139.3 (d) and 144.0 (s)] of which one is *E*-1,2-disubstituted ($J_{1,2}$ = 15.3 Hz), one is 1,1-disubstituted [^1H NMR: narrowly split signals at δ 4.79 and 5.12] and one is trisubstituted. These results demonstrate that epoxide **1** is carbomonocyclic.

Furthermore, the presence of an isopropyl group and two methyl groups, of which one is vinylic and one is attached to the carbon atom carrying the tertiary hydroxy group [^1H NMR: three-proton doublets at δ 0.83 and 0.87, a doublet of triplets at δ 1.71 and a singlet at δ 1.23] suggested that epoxide **1** is a diterpenoid of the cembrane class. This proposition was supported and a (2*E*,7*E*)-10,11-epoxy-2,7,12(20)-cembratriene-4,6-diol structure was tentatively formulated with the aid of results obtained from ^1H–^1H and ^1H–^{13}C shift correlation spectroscopy. This assignment was confirmed by X-ray analysis of epoxide **1** (see below). The results obtained, which are illustrated by the stereoscopic drawing in Fig. 1, also show that the relative configuration of epoxide **1** is 1*S*, 4*S*, 6*R*, 10*R* and 11*R*. Horeau's method[3] was then applied to settle the absolute configuration of C-6 as *R*.

Spectral data including results from 2D-NMR experiments were used to identify the second new tobacco isolate (**2**), $C_{20}H_{32}O_3$, as a 10,11-epoxy-2,7,12(20)-cembratriene-4,6-diol isomeric to epoxide **1**. Thus, the ^1H NMR spectrum displays the H-16 and H-17 signals as three-proton doublets at δ 0.83 and 0.88, H-18 as a three-proton singlet at δ 1.27 and H-19 as a narrowly split doublet of triplets at δ 1.70. H-2 and H-3 resonate as a doublet of doublets and a doublet at δ 5.60 and 5.38 (C_6D_6), respectively. Their vicinal coupling constant, $J_{2,3}$, is 15.4 Hz, a value which is consistent with an *E*-geometry of the 2,3 double bond. The remaining signals in the olefinic region of the ^1H NMR spectrum appear at δ 4.88, 5.08 and 5.63; they were ascribed to H-20a, H-20b and H-7, respectively. H-6 gives rise to a multiplet at δ 4.75, while the signals due to H-10 and H-11 are present at δ 2.81 and 3.18, respectively. Their vicinal coupling constant, $J_{10,11}$, is 2.3 Hz, demonstrating that the 10,11-epoxide group is *trans*-disubstituted.

Additional stereochemical information was provided by the ^{13}C NMR spectrum. Thus, the shielding of C-19, δ 18.4, is consonant with an *E*-geometry of the 7,8 double bond.[4] Moreover, the signals due to C-2 to C-9, C-18 and C-19 are present at virtually invariant positions in the spectra of epoxides **1** and **2**, while e.g. those due to C-12, C-13 and

* For part 73 see Ref. 1.

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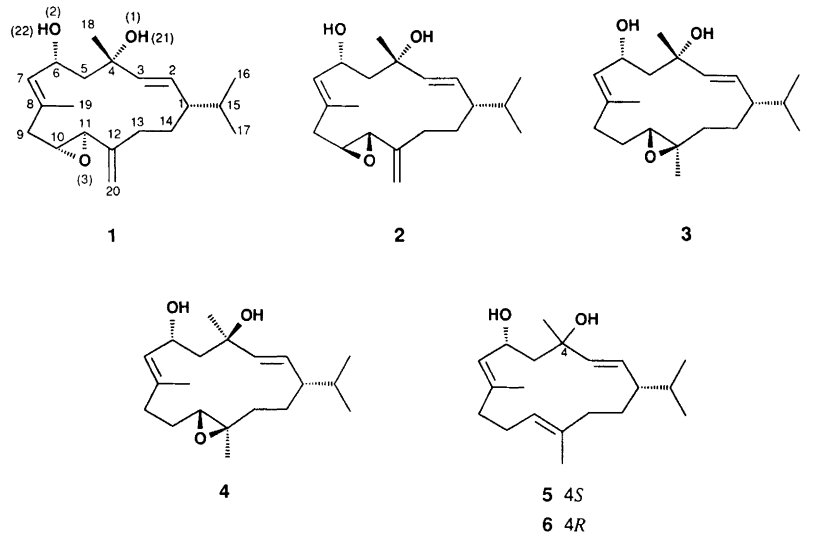


C-20 show divergent chemical shift values. These results suggest that epoxides **1** and **2** differ solely with respect to the chiralities of C-10 and C-11; epoxide **2** is hence identified as (1*S**,2*E*,4*S**,6*R**,7*E*,10*S**,11*S**)-10,11-epoxy-2,7,12(20)-cembratriene-4,6-diol.

We now also report the isolation of (1*S*,2*E*,4*S*,6*R*,7*E*,11*R*,12*R*)-11,12-epoxy-2,7-cembradiene-4,6-diol (**3**) and the corresponding (4*R*)-epimer (**4**) from tobacco. They have previously been reported as synthetic products.[5]

Biogenesis. As proposed in Scheme 1, the new tobacco constituents (**1–4**) are all plausible metabolites of the 4,6-diols **5** and **6**. Thus, the (4*S*,6*R*)-diol **5** is converted, by photo-oxygenation or an enzyme-catalyzed reaction, into the (4*S*,6*R*,12*S*)- and (4*S*,6*R*,12*R*)-triols **7** and **8**, both of which are tobacco constituents.[6,7] Subsequent dehydration and epoxidation account for the formation of the 10,11-epoxides **1** and **2**.

Like the (11*S*,12*S*)-epoxides **9** and **10**,[5,6] their (11*R*,12*R*)-counterparts **3** and **4** are likely to arise in tobacco by epoxidation of the 11,12 double bond in the (4*S*,6*R*)- and (4*R*,6*R*)-diols **5** and **6**, respectively. This assumption is reinforced by the fact that the (11*R*,12*R*)-epoxides **3** and **4** are produced both chemically and in tobacco in considerably smaller amounts than the (11*S*,12*S*)-epoxides **9** and **10**.

Crystallography. Final fractional coordinates with estimated standard deviations and equivalent anisotropic temperature factors for the non-hydrogen atoms of epoxide **1** are listed in Table 1. Intramolecular bond lengths and bond angles, both with estimated standard deviations, are found in Table 2, while selected non-bonded distances and possible hydrogen bonds are given in Table 3. Crystal and experimental data are detailed in Table 7.

All bond lengths and bond angles are within the expected range. A possible intramolecular hydrogen bond is present between the hydroxy groups at C(4) and C(6), the distance between acceptor and donor being 2.651 Å. There is one possible intermolecular hydrogen bond: O(2)–O(1^{i}) of 2.786 Å, the label i representing the symmetry code: $1-x$, $1/2+y$, $2-z$.

Solution conformation of epoxide **1**. Having determined the solid-state structure of **1** by X-ray analysis, it was of interest to explore whether this conformation is retained in solution. To this end, the solid-state structure was initially energy-minimized using the MM2(87) program.[8] In an at-

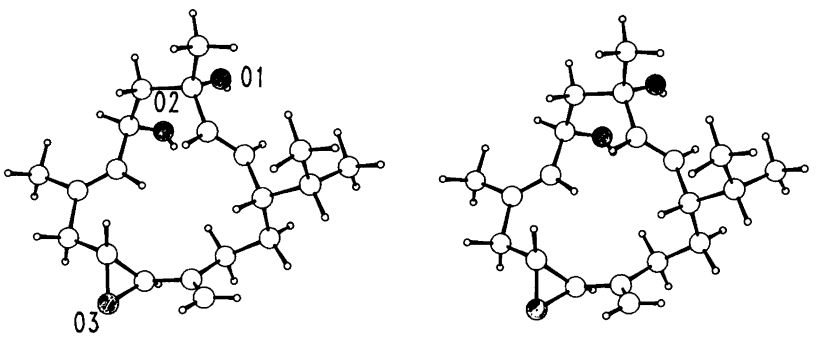
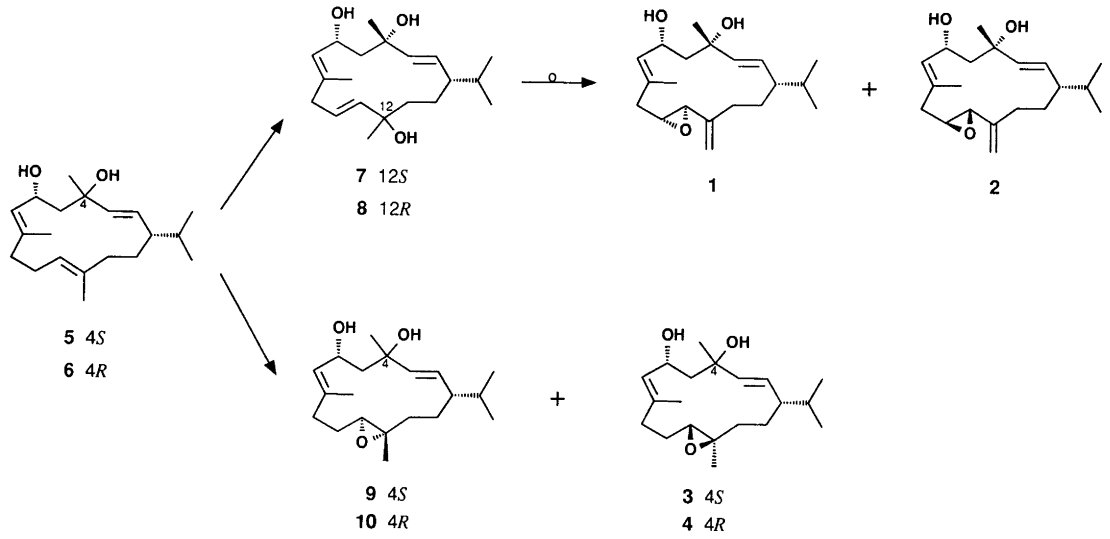


Fig. 1. A stereoscopic view of (1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*R*)-10,11-epoxy- 2,7,12(20)-cembratriene-4,6-diol (**1**).



Scheme 1. Proposed biogenesis of compounds **1–4**.

tempt to simulate the effect of the solvent used in the NMR experiments, C_6D_6, the relative permittivity, ε, was set to 2.28 in all calculations. The energy-minimized solid-state conformer was submitted to the ring-maker program,[9] by which possible conformers are generated in a systematic manner. Of the 8408 primary conformations generated, 91 were considered unique. These were then energy-minimized. The resultant conformations had relative steric energies between 0 and 10.7 kcal. Those being above a population limit of 1 %, in all 18, were selected for further study. Their relative steric energies and Boltzmann distribution are listed in Table 4. Together they comprise 96.4 % of the total population.

Inspection of torsion angles reveals that conformations 1 and 6 are structurally very similar. Conformations 9 and 10 are also essentially identical. The same is true for conformations 11, 12 and 13, for conformations 15 and 16 and for conformations 17 and 18. It can be seen from Table 5 that the best correspondence with the solid-state structure is found for conformation 1, which has the lowest steric energy, and for conformation 6. The main differences between conformations 1–18 reside in the C-4 to C-8 and C-12 to C-14 portions of the molecule. These results are also illustrated by the drawings shown in Fig. 2.

A generalized Karplus equation[10,11] was used to estimate the vicinal proton–proton coupling constants, $^3J_{H,H}$, in the solid-state structure, the corresponding energy-minimized structure and in selected generated conformers. The values obtained are compared with the corresponding observed coupling constants in Table 6. As expected, the solid-state structure and conformations 1 and 6 show the best fit with the observed values. The coupling constants between H-10 and H-11 calculated for conformations 1–18 are erroneous as a consequence of the lack of accurate parameters for the epoxy group in the MM2 program and have not been included in Table 6.

Experimental

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter and IR spectra on a Perkin-Elmer FT-IR 1725X spectrometer. For other instrumental details see Ref. 12.

Isolation. Fraction C (70.9 g), obtained from an extract of flowers of Greek tobacco (Basma),[13] was separated by flash chromatography over silica gel into six fractions, C1–C6. Fraction C4 (14.7 g) was separated further by HPLC (Spherisorb 5, hexane–EtOAc 50:50) into six fractions, C41–C46. Repetitive HPLC of fractions C42 (2.16 g) and C43 (4.20 g) gave 36 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*R*)-10,11-epoxy-2,7,12(20)-cembratriene-4,6-diol (**1**) and 32 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,10*S*,11*S*)-10,11-epoxy-2,7,12(20)-cembratriene-4,6-diol (**2**). Fraction D4 (17.1 g), also derived from the aforementioned extract of flowers of Greek tobacco,[13] was separated by HPLC (Spherisorb 5; EtOAc) into five fractions, D41–D45. Of these, D43 (3.6 g) was fractionated further by repetitive HPLC to give 36 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*R*,12*R*)-11,12-epoxy-2,7-cembradiene-4,6-diol (**3**). Fraction D44 (1.8 g) was separated by HPLC (Spherisorb 5 CN; hexane–EtOAc 60:40) into 10 fractions, D441–D4410. Further fractionation of D446 (36 mg) by HPLC (Lichrosorb Diol; hexane–EtOAc 40:60) yielded 14 mg of (1*S*,2*E*,4*R*,6*R*,7*E*,11*R*,12*R*)-11,12-epoxy-2,7-cembradiene-4,6-diol (**4**).

(1*S*,2*E*,4*S*,6*R*,7*E*,10*R*,11*R*)-10,11-Epoxy-2,7,12(20)-cembratriene-4,6-diol (**1**) had m.p. 143.0–144.0 °C; $[\alpha]_D$ −21° (*c* 0.55, $CHCl_3$); (Found: $[M-18]^{+\cdot}$ 302.2277. Calc. for $C_{20}H_{30}O_2$: 302.2246); IR ($CHCl_3$): 3606, 3481, 1386 and 1369 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (d, *J* 6.8 Hz) / 0.87 (d, *J* 6.8 Hz) (H-16/H-17), 1.23 (s, H-18), 1.71 (dt, *J* 0.7 and 1.5 Hz, H-19), 1.72 (dd, *J* 1.4 and −14.5 Hz, H-5a), 2.26 (dd, *J* 7.3 and −14.5 Hz, H-5b), 2.64 (ddd, *J* 2.1, 2.8 and 10.0 Hz, H-10), 2.97 (br s, H-11), 4.79 (dt, *J* 1.4 and −2.2

Table 1. Atom coordinates ($\times 10^4$) and equivalent isotropic temperature factors (Å $\times 100$) for the non-hydrogen atoms and fixed isotropic temperature factors (U_{iso} 5.0) for the hydrogen atoms in epoxide **1**.

	x	*y*	*z*	U_{eq}[a]
C(1)	8056(4)	2078(7)	7532(4)	5.52(14)
H(1)	7751(4)	2336(7)	6567(4)	
C(2)	6736(4)	1835(7)	8249(4)	5.35(14)
H(2)	6853(4)	1709(7)	9261(4)	
C(3)	5453(4)	1785(7)	7734(4)	4.68(11)
H(3)	5323(4)	2033(7)	6742(4)	
C(4)	4144(4)	1420	8415(4)	4.83(12)
C(5)	2936(4)	2421(7)	8212(4)	5.37(14)
H(5A)	2074(4)	2055(7)	8745(4)	
H(5B)	2639(4)	2408(7)	7216(4)	
C(6)	3160(5)	3914(7)	8582(4)	5.63(16)
H(6)	2149(5)	4389(7)	8664(4)	
C(7)	3869(5)	4745(7)	7610(4)	5.60(16)
H(7)	4872(5)	4384(7)	7318(4)	
C(8)	3383(5)	5866(7)	7059(5)	5.83(17)
C(9)	4207(5)	6636(8)	6089(5)	6.93(17)
H(9A)	4468(5)	7629(8)	6469(5)	
H(9B)	3547(5)	6750(8)	5242(5)	
C(10)	5540(5)	5951(8)	5740(5)	6.47(16)
H(10)	4999(5)	5019(8)	5483(5)	
C(11)	6876(6)	6213(8)	6432(5)	6.93(17)
H(11)	7148(6)	6576(8)	7374(5)	
C(12)	7988(5)	5168(8)	6576(5)	6.82(19)
C(13)	8180(5)	4642(8)	7908(5)	6.73(17)
H(13A)	8797(5)	5383(8)	8440(5)	
H(13B)	7155(5)	4566(8)	8294(5)	
C(14)	8901(5)	3258(8)	8094(5)	6.93(18)
H(14A)	9890(5)	3300(8)	7639(5)	
H(14B)	9087(5)	3077(8)	9093(5)	
C(15)	8952(5)	755(8)	7469(6)	7.3(2)
H(15)	9903(5)	1069(8)	7038(6)	
C(16)	9389(7)	188(11)	8775(7)	9.8(3)
H(16A)	9788(7)	1001(11)	9376(7)	
H(16B)	10170(7)	−602(11)	8724(7)	
H(16C)	8453(7)	−227(11)	9162(7)	
C(17)	8245(7)	−308(9)	6656(8)	9.5(3)
H(17A)	8030(7)	134(9)	5734(8)	
H(17B)	7276(7)	−647(9)	7041(8)	
H(17C)	8943(7)	−1166(9)	6573(8)	
C(18)	3656(5)	13(8)	7972(5)	6.30(17)
H(18A)	3405(5)	146(8)	6975(5)	
H(18B)	2721(5)	−259(8)	8449(5)	
H(18C)	4425(5)	−787(8)	8102(5)	
C(19)	2000(6)	6524(9)	7304(8)	8.7(2)
H(19A)	1473(6)	6019(9)	8054(8)	
H(19B)	1381(6)	6426(9)	6431(8)	
H(19C)	2133(6)	7589(9)	7533(8)	
C(20)	8708(6)	4753(10)	5598(6)	8.9(2)
H(20A)	9468(4)	4116(8)	5808(5)	
H(20B)	8521(5)	5054(7)	4908(4)	
O(1)	4461(4)	1279(6)	9761(3)	5.49(10)
H(21)	4339(49)	1976(60)	10058(50)	
O(2)	3959(4)	3934(7)	9774(3)	6.41(11)
H(22)	4436(49)	4568(63)	9845(44)	
O(3)	6581(5)	6812(7)	5215(4)	8.62(14)

[a] $U_{eq} = \frac{1}{3}\Sigma_i\Sigma_j \cdot U_{ij} \cdot \boldsymbol{a}_j^* \cdot \boldsymbol{a}_j^* a_i \cdot a_j$.

Table 2. Bond lengths (Å) and bond angles (deg) in epoxide **1**.

Bond lengths

C(2)–C(1)	1.520(8)	C(14)–C(1)	1.519(10)
C(15)–C(1)	1.559(10)	C(3)–C(2)	1.321(6)
C(4)–C(3)	1.514(8)	C(5)–C(4)	1.524(8)
C(18)–C(4)	1.525(9)	O(1)–C(4)	1.451(6)
C(6)–C(5)	1.531(10)	C(7)–C(6)	1.498(8)
O(2)–C(6)	1.447(7)	C(8)–C(7)	1.321(8)
C(9)–C(8)	1.552(10)	C(19)–C(8)	1.503(10)
C(10)–C(9)	1.501(9)	C(11)–C(10)	1.467(8)
O(3)–C(10)	1.436(7)	C(12)–C(11)	1.481(9)
O(3)–C(11)	1.434(8)	C(13)–C(12)	1.506(10)
C(20)–C(12)	1.331(10)	C(14)–C(13)	1.533(11)
C(16)–C(15)	1.532(11)	C(17)–C(15)	1.497(11)

Bond angles

C(14)–C(1)–C(2)	111.3(5)	C(15)–C(1)–C(2)	111.1(5)
C(15)–C(1)–C(14)	111.6(5)	C(3)–C(2)–C(1)	125.1(5)
C(4)–C(3)–C(2)	125.7(5)	C(5)–C(4)–C(3)	114.6(5)
C(18)–C(4)–C(3)	108.4(5)	C(18)–C(4)–C(5)	108.7(4)
O(1)–C(4)–C(3)	110.4(4)	O(1)–C(4)–C(5)	109.0(4)
O(1)–C(4)–C(18)	105.3(5)	C(6)–C(5)–C(4)	119.0(5)
C(7)–C(6)–C(5)	114.1(5)	O(2)–C(6)–C(5)	107.4(5)
O(2)–C(6)–C(7)	110.8(5)	C(8)–C(7)–C(6)	126.6(5)
C(9)–C(8)–C(7)	122.0(6)	C(19)–C(8)–C(7)	125.2(6)
C(19)–C(8)–C(9)	112.8(6)	C(10)–C(9)–C(8)	114.4(5)
C(11)–C(10)–C(9)	121.9(6)	O(3)–C(10)–C(9)	116.2(6)
O(3)–C(10)–C(11)	59.2(4)	C(12)–C(11)–C(10)	122.4(6)
O(3)–C(11)–C(10)	59.3(4)	O(3)–C(11)–C(12)	119.3(5)
C(13)–C(12)–C(11)	113.0(6)	C(20)–C(12)–C(11)	121.7(6)
C(20)–C(12)–C(13)	125.3(7)	C(14)–C(13)–C(12)	117.4(6)
C(13)–C(14)–C(1)	113.4(5)	C(16)–C(15)–C(1)	113.2(6)
C(17)–C(15)–C(1)	111.7(5)	C(17)–C(15)–C(16)	111.4(7)
C(11)–O(3)–C(10)	61.5(4)		

Hz, H-20a), 4.82 (ddd, *J* 1.4, 7.3 and 9.7 Hz, H-6), 5.12 (m, H-20b), 5.48 (d, *J* 15.3 Hz, H-3), 5.52 (dd, *J* 7.8 and 15.3 Hz, H-2) and 5.83 (d sextets, *J* 1.5 and 9.7 Hz, H-7); ^{13}C NMR ($CDCl_3$): δ 17.9 (C-19), 18.8/21.0 (C-16/C-17), 28.2/29.3 (C-13/C-14), 31.7 (C-15), 32.2 (C-18), 40.6 (C-9), 45.8 (C-1), 46.2 (C-5), 60.3 (C-11), 60.4 (C-10), 68.6 (C-6), 73.8 (C-4), 106.6 (C-20), 127.6 (C-2), 128.7 (C-7), 133.9 (C-8), 139.3 (C-3) and 144.0 (C-12); MS [*m/z* (%, composition)]; 320 (0.1, *M*), 302 (0.2), 284 (0.4, $C_{20}H_{28}O$), 259

Table 3. Selected non-bonded distances (Å) and possible hydrogen bonds in epoxide **1**.[a]

Intramolecular

O(2)–O(1)	2.651		

Intermolecular

O(2)–O(1^{i})	2.786	O(1)–C(7^{ii})	3.490
O(2)–C(18^{ii})	3.398		

[a] Key to symmetry operations relating designated atoms to reference atoms at (*x*, *y*, *z*): (i) 1.0−*x*, 0.5+*y*, 2.0−*z*; (ii) 1.0−*x*, −0.5+*y*, 2.0−*z*.

Table 4. Relative steric energies ΔE/kcal mol^{-1} and populations (%) for conformations 1–18.

Conformation	ΔE	Population (%)	Conformation	ΔE	Population (%)
1	0.00	16.9	10	0.95	3.4
2	0.15	13.1	11	1.13	2.5
3	0.19	12.2	12	1.13	2.5
4	0.38	8.9	13	1.21	2.2
5	0.48	7.5	14	1.28	2.0
6	0.66	5.6	15	1.31	1.8
7	0.72	5.1	16	1.32	1.8
8	0.75	4.7	17	1.46	1.5
9	0.94	3.5	18	1.58	1.2

(1, $C_{19}H_{25}O$), 241 (1), 219 (2, $C_{15}H_{23}O$), 201 (2), 191 (3), 175 (3), 161 (4), 147 (9), 135 (11, $C_{10}H_{15}$), 121 (18, C_9H_{13}), 109 (21, C_8H_{13} and C_7H_9O), 95 (28), 81 (40, C_5H_5O), 69 (30, C_5H_9 and C_4H_5O), 55 (35, C_4H_7 and C_3H_3O) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,10*S*,11*S*)-10,11-Epoxy-2,7,12(20)-cembratriene-4,6-diol(**2**) had m.p. 135–139 °C; $[\alpha]_D$ +103° (*c* 0.30, $CHCl_3$); (Found: $[M-18]^{+\cdot}$ 302.2256. Calc. for $C_{20}H_{30}O_2$: 302.2246); IR ($CHCl_3$): 3603, 3481, 1385 and 1369; ^{1}H NMR ($CDCl_3$): δ 0.83 (d, *J* 6.8 Hz) / 0.88 (d, *J* 6.7 Hz) (H-16/H-17), 1.27 (s, H-18), 1.70 (dt, *J* 0.7 and 1.4 Hz, H-19), 1.88 (dd, *J* 2.2 and −14.1 Hz, H-5a), 2.18 (dd, *J* 7.3 and −14.1 Hz, H-5b), 2.81 (ddd, *J* 2.3, 3.2 and 8.3 Hz, H-10), 3.18 (dd, *J* 1.0 and 2.3 Hz, H-11), 4.75 (br ddd, *J* 2.2, 7.3 and 8.3 Hz, H-6), 4.88 (ddt, *J* 0.5, 1.0 and 2.0 Hz, H-20a), 5.08 (dd, *J* 1.2 and 2.0 Hz, H-20b), 5.4–5.6 (overlapping signals, H-2 and H-3) and 5.63 (d sextets, *J* 1.4 and 8.3 Hz, H-7); ^{1}H NMR (C_6D_6): δ 5.38 (d, *J* 15.4 Hz, H-3) and 5.60 (dd, *J* 9.4 and 15.4 Hz, H-2); ^{13}C NMR ($CDCl_3$): δ 18.4 (C-19), 19.1/20.9 (C-16/C-17), 31.0/33.8 (C-13/C-14), 31.6 (C-18), 32.1 (C-15), 40.5 (C-9), 48.1 (C-5), 48.2 (C-1), 59.2 (C-10), 59.9 (C-11), 68.4 (C-6), 73.5 (C-4), 111.3 (C-20), 128.8 (C-2), 128.9 (C-7), 133.2 (C-8), 139.3 (C-3) and 146.7 (C-12); MS [*m/z* (%, composition)]: 302 (0.6, *M*−18), 287 (0.8), 259 (2), 241 (2), 219 (3, $C_{15}H_{23}O$), 201 (3), 175 (5), 161 (5), 147 (9, $C_{11}H_{15}$), 135 (12, $C_{10}H_{15}$), 121 (20, C_9H_{13}), 109 (21, C_8H_{13}), 95 (30, C_7H_{11}), 81 (43, C_5H_5O), 69 (32, C_5H_9 and C_4H_5O), 55 (39, C_4H_7 and C_3H_3O) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*E*,11*R*,12*R*)-11,12-Epoxy-2,7-cembradiene-4,6-diol (**3**) and its (4*R*)-epimer (**4**) were identified by direct comparison with corresponding synthetic samples (optical rotation, IR, ^{1}H and ^{13}C NMR and MS).[5]

Determination of the absolute configuration of **1** *by Horeau's method.* To a solution of 4.2 mg of **1** in 1.5 ml of pyridine were added 8.0 µl of 2-phenylbutanoic anhydride. The reaction mixture was stirred for 4 days at 40 °C. The solvent was removed at reduced pressure and the residue was suspended in 5 ml of CH_2Cl_2 and washed with aqueous $NaHCO_3$ (5 %). The organic phase was evaporated to dryness and the residue consisting of unchanged **1** and its ester was analyzed quantitatively by ^{1}H NMR spectroscopy: esterification yield 50 %. The aqueous phase was acidified to pH 5 using dilute HCl (30 %) and extracted with CH_2Cl_2. The solvent was removed and the residue containing the unchanged acid residue was dissolved in benzene: $[\alpha]_D$ +10°; optical yield 11 %.

X-Ray crystallography study. Single crystals of **1** were obtained by recrystallization from a mixture of hexane and ethyl acetate. The intensities of three standard reflections ($\bar{1}\,\bar{1}\,3$, $\bar{3}\,3\,1$ and $3\,\bar{2}\,3$) were monitored every 60 min to show that the total deterioration of intensity was <4 %. The space-group symmetry was determined as $P2_1$ or $P2_1/m$ from systematic extinctions and by the unit-cell parameters

Table 5. Endocyclic torsion angles (deg) in epoxide **1** observed by X-ray diffractometry and calculated by molecular mechanics.

Torsion angle	Observed		Calculated											
	X-ray structure	X-ray structure[a]	Conformation											
			1	2	3	4	5	6	7	8	9	11	14	15	17
1–2–3–4	174.2	174.0	172.8	179.5	173.6	175.6	174.8	174.0	−174.0	174.5	177.3	175.4	173.3	−174.2	171.9
2–3–4–5	130.0	129.3	135.1	155.4	64.1	114.0	151.9	129.2	−67.4	150.7	163.4	67.9	100.1	−57.4	85.4
3–4–5–6	−58.4	−61.6	−59.1	−67.0	82.2	−60.4	−72.4	−60.7	−64.0	−74.6	−73.9	66.5	−61.1	−67.3	−69.8
4–5–6–7	81.9	85.4	79.2	60.4	−58.8	73.6	54.3	89.2	71.9	52.5	49.6	−71.3	75.6	63.9	95.5
5–6–7–8	123.0	126.8	85.3	76.8	165.3	72.5	86.9	157.5	82.1	92.9	84.9	128.7	71.0	86.6	79.2
6–7–8–9	179.4	180.0	176.2	176.0	178.8	179.1	176.5	−179.8	175.5	179.5	174.3	174.1	−178.0	171.1	173.6
7–8–9–10	5.0	1.8	31.0	90.8	17.7	72.4	99.8	−32.2	95.9	98.9	105.9	94.5	75.6	104.3	73.7
8–9–10–11	−89.7	−101.0	−97.6	−114.8	−103.5	−147.8	−156.2	−81.9	−144.6	−168.6	−139.8	−143.6	−155.1	−131.5	−110.1
9–10–11–12	149.2	149.1	158.6	158.4	155.3	156.1	153,7	148.2	155.4	149.9	160.7	155.6	151.8	157.3	156.1
10–11–12–13	−110.0	−100.5	−103.0	−102.9	−103.8	−97.5	−101.5	−108.5	−95.6	−94.6	−114.0	−104.5	−94.5	−105.2	−71.3
11–12–13–14	159.4	164.8	145.3	62.1	159.0	63.3	59.2	165.5	59.7	61.3	53.3	58.6	63.0	53.3	−74.8
12–13–14–1	−66.1	−69.1	−69.2	61.7	−66.9	64.7	67.1	−74.4	66.1	67.7	62.1	66.9	68.7	66.1	175.1
13–14–1–2	−68.1	−68.4	−70.4	−169.3	−61.9	−162.6	−165.9	−64.6	−156.5	−164.0	−165.3	−164.8	−156.3	−159.3	−76.9
14–1–2–3	126.2	127.7	132.2	104.5	126.9	121.0	114.8	131.7	−34.2	114.4	120.1	125.1	125.8	−25.0	104.8

[a]Energy-minimized.

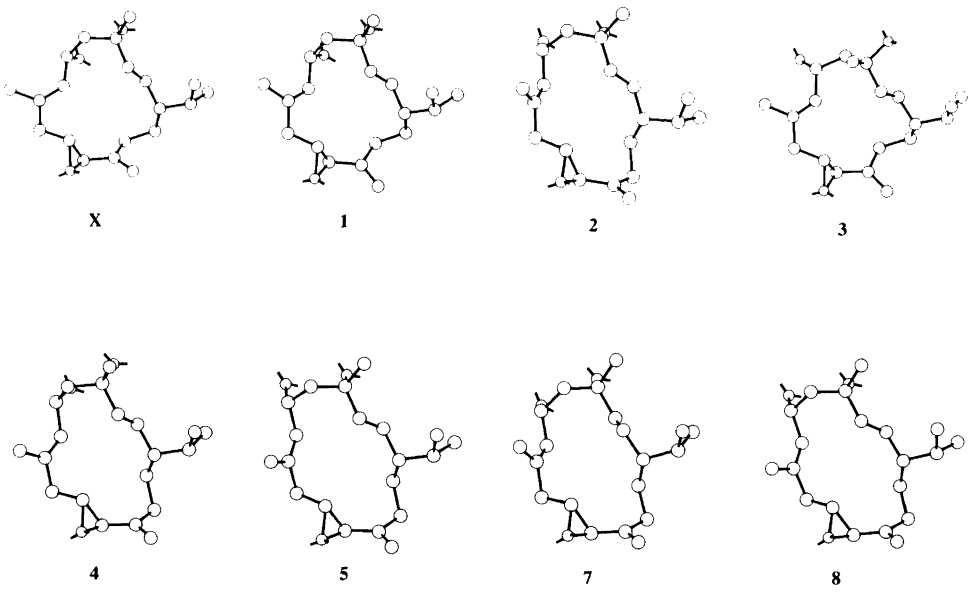


Fig. 2. Drawings of the energy-minimized solid-state conformer (**X**) and of selected generated conformers.

found by the least-squares method from 25 centered X-ray reflections. Lorentz and polarization corrections were applied but no correction was made for absorption.

In all, 22 of the 23 non-hydrogen atoms were found by direct methods using the program SHELXS86,[14] while the remaining non-hydrogen atom and the hydroxy hydrogen atoms were located from difference electron density maps. The other hydrogen atoms were geometrically placed with a distance of 1.08 Å to the adjacent carbon atom and refined. The structure was refined by the full-matrix least-squares technique using the SHELX76[15] program. Anisotropic temperature factors* were introduced for the non-hydrogen atoms, while all hydrogen atoms were refined with all isotropic thermal factors constrained to a common value of 0.05 Å. The atomic scattering factors[16] used for the non-hydrogen and the hydrogen atoms were those included in the SHELX76 program.

Computational methods. The solid-state structure of epoxide **1** was energy-minimized using the molecular mechanics program MM2(87)[8] with the relative permittivity set to 2.28. Except for the angle parameters for the epoxy group, which were set to 0.70 mdynÅ/rad^2, 107.5° ($C_{sp}{}^3$–$C_{sp}{}^3$–O) and 0.77 mdynÅ/rad^2, 108.8° ($C_{sp}{}^3$–O–$C_{sp}{}^3$), default parameters were used. The energy-minimized solid-state structure was then submitted to the RNGCFM system,[9] which includes the programs RCG5, XYZTOD and FILTERD. The program RCG5 opens the ring, sets all dihedral ring angles to 180° and rotates them in increments of 20°. If any atom in any generated conformer has an impossibly short distance to another atom, this conformer is rejected. The program then compares the bond dis-

* Available from J.-E. Berg on request.

Table 6. Observed vicinal coupling constants (Hz)[a] for epoxide **1** (in C_6D_6) and calculated values for the solid-state conformer, the energy-minimized solid-state conformer and selected generated conformations.

J_3	Observed	Calculated													
		X-ray structure	X-ray structure[b]	Conformation											
				1	2	3	4	5	6	7	8	9	11	14	15	17
H_5–H_6	7.3	6.9	8.0	7.1	4.4	10.8	6.2	3.7	8.1	5.9	3.6	3.2	11.5	6.5	4.9	8.6
	1.4	0.9	0.9	0.9	2.2	4.7	1.1	2.8	0.9	1.3	2.9	3.3	3.4	1.0	1.9	1.0
H_{13}–H_{14}	3.6	1.9	2.0	1.9	2.7	2.3	2.2	2.0	1.4	2.1	1.9	2.7	2.0	1.7	2.2	13.2
	6.5	4.5	5.1	5.2	13.3	4.7	13.3	13.3	5.9	13.3	13.3	13.3	13.3	13.3	13.3	1.8
	10.3	13.2	13.3	13.3	4.0	13.3	4.6	4.9	13.1	4.7	5.0	4.0	4.7	5.3	4.6	3.6
	3.4	2.0	1.8	1.8	3.0	2.2	2.5	2.3	1.3	2.4	2.2	3.0	2.3	2.0	2.5	13.2
H_{14}–H_1	4.1	2.0	1.7	1.5	2.3	2.3	1.6	1.8	1.9	1.2	1.6	1.8	1.7	1.1	1.4	11.5
	9.1	12.1	12.0	11.9	12.3	12.3	12.0	12.2	12.2	11.6	12.1	12.2	12.1	11.4	11.8	1.3

[a]The coupling constants were obtained by using spin-simulation techniques. [b]Energy-minimized.

Table 7. Experimental conditions for the crystal structure determination of epoxide **1**.

Formula	$C_{20}H_{32}O_3$
Formula weight	320.47
Space group	$P2_1$
Unit cell dimensions	$a = 9.5589(13)$, $b = 9.8191(12)$ $c = 10.5891(15)$ Å, $\beta = 92.49(1)$
Unit cell volume, V	992.95(20) Å^3
Formula units per unit cell, Z	2
Calculated density, D_x	1.07 g cm^{-3}
Radiation	Mo $K\alpha$
Wavelength, λ	0.71069 Å
Linear absorption coefficient	0.65 cm^{-1}
Temperature, T	293(1) K
Crystal shape	Prismatic
Crystal size	0.09 × 0.06 × 0.27 mm
Diffractometer	Simens/Stoe AED 2
Determination of unit cell	
Number of reflections	25
θ-range	10.0–25.0°
Intensity data collection	
Maximum $\sin(\theta)/\lambda$	0.59 Å^{-1}
Range of h, k and l	−11 to 11, 0 to 11 and 0 to 12
Standard reflections	3
Intensity instability	< 4 %
Internal R value	0.031
Number of unique reflections	1365
Number of observed reflections	1872
Criterion for significance	$F > 6.06 \cdot \sigma(F)$
Structure refinement	
Minimization of	$\Sigma w \cdot \Delta F^2$
Anisotropic thermal parameters	All non-hydrogen atoms
Isotropic thermal parameters	Hydrogen atoms
Number of refined parameters	226
Weighting scheme	$[\sigma^2(F) + 0.0050 \mid F \mid^2]^{-1}$
Final R for observed refls.	0.058
Final wR for observed refls.	0.080
Final wR for all 1872 refls.	0.085
Final $(\Delta/\sigma)_{max}$	0.26
Final $\Delta\varrho_{min}$ and $\Delta\varrho_{max}$	−0.34 and 0.23 e Å^{-3}

tances, bond angles and the dihedral angles of the end atoms with the bond-closure length limits (1.3–1.7 Å), bond-closure angle limits (100–130°) and dihedral closure tolerance limits (30°) in the ring of the conformer generated with the input values. These sets must be accepted for the conformer generated to be saved as a possible alternative to the input structure. No cross-ring distance constraints were applied.

When all dihedral angles have been generated for the carbocyclic ring, the program XYZTOD calculates a set of dihedral angles for each conformer. This is then used as an input to the third program, FILTERD, which compares all generated conformers with each other. If all dihedral angles in a conformer are within ± 30° from the input values, the conformer generated is rejected. The conformers retained are then resubmitted to energy-minimization using the MM2(87) program.

The vicinal coupling constants, $^3J_{H,H}$, were calculated by using an empirically generalized Karplus equation developed by Haasnoot *et al.*[10] and used in the program 3JHHPC by Petillo.[11]

All calculations were carried out on a C220 Convex computer.

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