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Tobacco Chemistry. 54.* (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-Epoxy-2,6-cembradiene-4,12-diol, a New Constituent of Greek Tobacco

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A new diterpenoid has been isolated from Greek tobacco and is formulated as (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*1*) by spectroscopic methods, synthesis and X-ray analysis of its *4R*-epimer (*12*). The biogenesis of diol *1* is discussed on the basis of results obtained from epoxidation and rearrangement reactions.

The cembranic diterpenoids isolated from the cuticular wax of the leaf of certain tobacco varieties include as the major components the (1*S*,2*E*,4*S*,6*R*,-7*E*,11*E*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diols (*2*, *3*), whereas *i.a.* a series of 8,11-epoxy bridged cembranoids, all having an 8*R*,11*S*-stereochemistry, *e.g.* *4*–*7*, is present in a minor amount.[2] We now report the isolation of the first 8*S*,11*R*-epoxy bridged cembranoid from sun-cured Greek tobacco.

RESULTS

The new compound (*1*), $C_{20}H_{34}O_3$, gave a ^{13}C NMR spectrum containing signals due to five methyl, five sp^3 methylene, three sp^3 methine carbon atoms, of which one was oxygen-carrying, three fully substituted oxygen-carrying sp^3 carbon atoms and four sp^2 methine carbon atoms, *i.e.* two disubstituted double bonds. Since the ^1H NMR spectrum displayed two three-proton doublets at δ 0.86 and 0.90 and since the IR spectrum had bands at 1375 and 1390 cm^{-1}, two of the methyl groups were deduced to form part of an isopropyl group. The remaining three methyl groups, giving rise to singlets at δ 1.03, 1.29 and 1.31, are evidently linked to fully substituted oxygen-carrying carbon atoms.

It followed from the characteristic chemical shift values of two of the signals in the ^{13}C NMR spectrum, δ 88.6 (d) and 82.6 (s), and the presence of a signal at δ 4.03 (t) in the ^1H NMR spectrum that one of the oxygen atoms is present as an ether group extending from a methine to a fully substituted carbon atom. The remaining two oxygen atoms are accommodated by tertiary hydroxyl groups (OH-absorption in the IR spectrum). These results indicated that diol *1* is a carbomonocyclic diterpenoid and a cembranic structure seemed most plausible from a biogenetic point of view.

Additional structural information, which reinforced this alternative, was provided by the ^1H and ^{13}C NMR spectra. Thus, spin decoupling and spin simulation experiments allowed the allocation of the double bonds, both of which were found to have *E*-configurations ($^3J = 16$ and 15.5 Hz), to partial structures A and B. These were suggested to be included in diol *1* as the C-1 to C-8 portion of an 8,11-epoxy-2*E*,6*E*-cembradiene-4,12-diol structure by a comparison, which showed that fourteen signals in the ^{13}C NMR spectrum of diol *1* were of appropriate multiplicities and had chemical shift values close to those observed for the C-1 to C-5, C-8, C-11, C-12 and C-15 to C-20 signals for (1*S*,2*E*,4*S*,-6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*4*).[3]

A clue to the chiralities at C-4 and C-8 was obtained by a comparison, which included the ^{13}C

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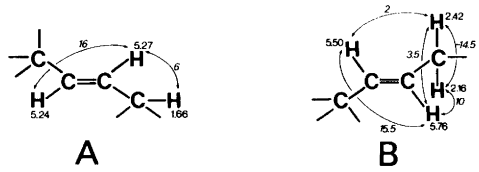
Table 1. Carbon-13 chemical shifts and assignments for compounds *1*, *4* – *7*, *9*, *11* and *12*.[a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
1	51.0	129.7	138.3[b]	73.7	45.8	126.5	138.5[b]	82.6	32.5	24.4	88.6	73.7	39.5	27.0	32.3	20.9	20.1	30.4	28.9	22.4
4	50.9	129.4	138.2	73.6	45.6	122.6	139.6	82.9	35.1	27.0	88.9	74.1	36.7	28.5	32.3	20.7	20.0	30.1	29.2	22.1
5	50.9	132.2	137.4	73.3	46.7	121.5	140.1	83.0	34.6	25.7	89.0	74.2	36.0	28.5	31.9	20.7	20.4	24.8	29.1	21.8
6	51.4	130.1	138.3	73.4	45.2	123.1	140.0	83.5	34.0	25.4	87.5	74.8	36.2	26.8	32.4	20.9	19.8	30.1	29.5	24.8
7	51.4	132.7	137.5	73.3	46.1	122.5	140.2	83.5	34.0	24.7	87.5	74.8	35.7	26.9	32.4	20.8	19.9	24.7	29.5	24.7
9	46.3	129.4	137.5	73.2	46.9	124.2	140.2	73.2	35.9	23.7	63.7	61.4	37.7	28.2	33.8	20.5	19.1	30.9	30.9	15.6
11	46.8	130.5	138.3	73.1	47.2	124.2	138.8	72.0	36.0	23.8	64.4	61.3	38.4	27.2	33.3	20.1	19.4	27.4	26.7	15.6
12	50.9	132.4	138.0	73.2	46.4	125.7	139.2	82.5	32.6	24.4	88.4	73.7	39.2	26.8	32.3	20.8	20.1	25.5	28.7	22.2

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



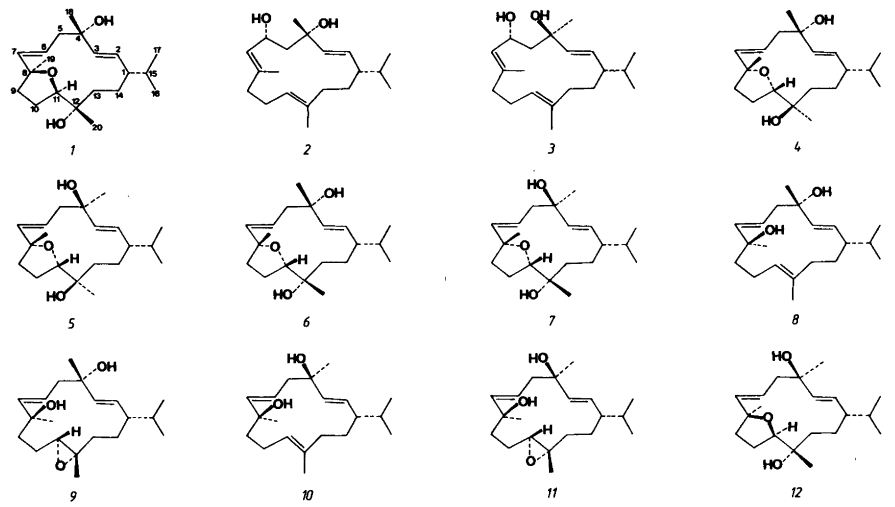
A B

Partial structures A and B. Chemical shift values (δ) are in Roman; coupling constants (Hz) in italic.

NMR spectra of diols *1* and *4* and the (1*S*,2*E*,4*R*,6*E*,-8*R*,11*S*,12*R*)-,[3] (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*S*)-[3] and (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diols[4] (*5* – *7*). Thus, the chemical shift values of the C-2 and C-18 signals, δ 129.7 and 30.4, respectively, are only consistent with a 4*S*-configuration in diol *1*,[5] and the significantly different shielding of C-6 in diol *1* and in the 8*R*,11*S*-epoxy bridged compounds *4* – *7*, δ 126.5 as against δ 121.5 – 123.1, suggested that the configuration at C-8 is *S*.

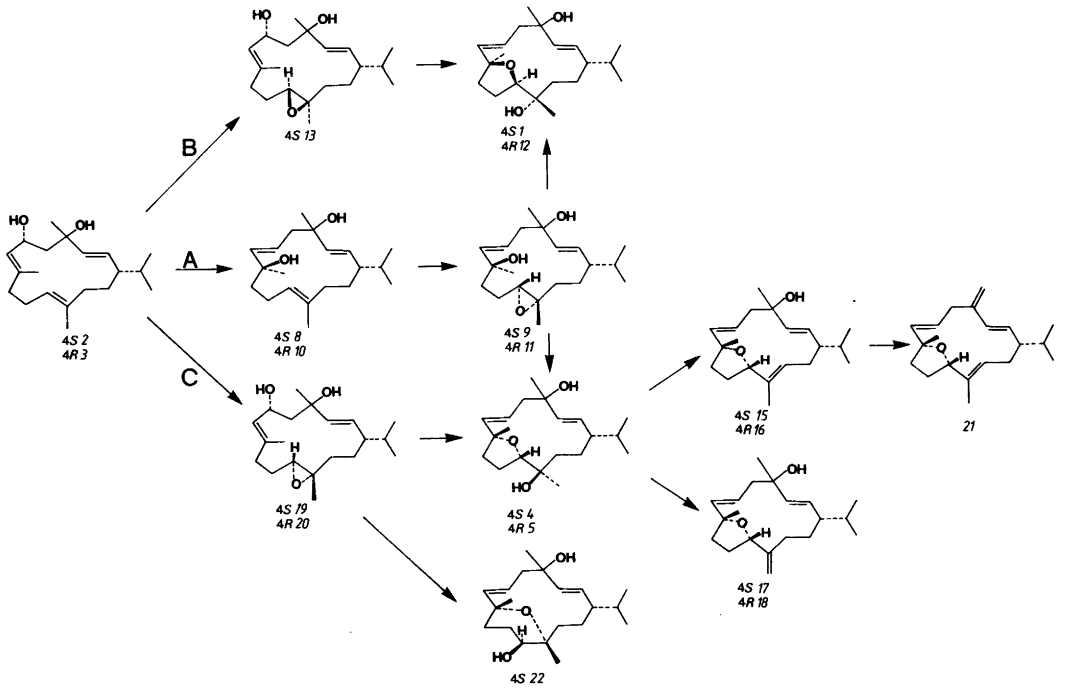
With this information at hand structural elucidation was sought by synthesis. Thus, (1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol (*8*), available through acid-induced rearrangement of the 4*S*,6*R*-diol (*2*)[6] was reacted with *m*-chloroperbenzoic acid to afford an 11,12-epoxide (*9*), whose ^1H NMR spectrum displayed the signal due to H-11 as a doublet of doublets at δ 3.12. By analogy with the formation of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*11*) from the 4*R*,8*S*-diol (*10*),[6] epoxide *9* was assigned an 11*S*,12*S*-stereochemistry. On treatment with weakly acidified chloroform it underwent a facile conversion to a product, which proved to be identical in all respects to the new tobacco constituent (*1*). Since the mechanism involved is most likely an S_N2 type of epoxide opening at the secondary C-11 by attack of the hydroxyl group at C-8, (for other alternatives, *cf.* below) the new compound was tentatively formulated as (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*1*).

Under similar conditions epoxide *11* was converted to a diol (*12*), which in contrast to diol *1*, was amenable to X-ray analysis. Diol *12* formed orthorhombic crystals of space group $P2_12_12_1$. The crystal data, obtained on a computer-controlled Philips PW 1100 diffractometer, were $a=17.843$, $b=10.655$ and $c=10.283$ Å, $Z=4$. The present *R*-value including anisotropic thermal parameters for all non-hydrogen atoms is 0.111, location of the hydrogen atoms and further refinement being under way.[7] A stereoscopic view, which summarizes the X-



ray results and demonstrates that diol *12* is (1*S*,2*E*,-4*R*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol, is shown in Fig. 1. Since the ^{13}C NMR spectrum of diol *12* with the exception of shift differences for the C-2 and C-18 signals reflecting the configurational differences at C-4 was virtually superimposable on that of diol *1*, the latter is conclusively identified as (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol.

Biogenesis. The new tobacco constituent (*1*) is the only 8,11-epoxy bridged cembranoid encountered so far, which has an 8*S*,11*R*-stereochemistry. It may arise in tobacco by a route (A in Scheme 1) similar to the synthetic one described above. This



Scheme 1. Probable biogenesis of the 8*S*,11*R*-, 8*R*,11*S*- and 8*R*,12*R*-epoxy bridged tobacco constituents.

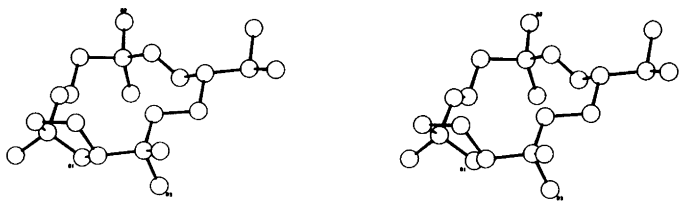
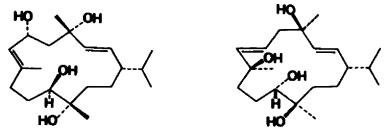


Fig. 1. Stereoscopic view of (1*S*,2*E*,4*R*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*12*).

view is reinforced by the fact that the precursor, *i.e.* the 4*S*,8*S*-diol *8*, has been found present in tobacco.[8]

Another route (B) to compound *1* would involve (1*S*,2*E*,4*S*,6*R*,7*E*,11*R*,12*R*)-11,12-epoxy-2,7-cembradiene-4,6-diol (*13*) as an intermediate and proceed *via* an *anti*-opening of the epoxide group with formation of (1*S*,2*E*,4*S*,6*R*,7*E*,11*R*,12*S*)-2,7-cembradiene-4,6,11,12-tetrol (*14*). The latter would undergo protonation of the hydroxyl group at C-6, migration of the 7,8 double bond and an attack of the 11-hydroxyl group at C-8. In consistence with this view, epoxide *13*, which is a minor product obtained upon epoxidation of the 4*S*,6*R*-diol *2*[4] and as yet not encountered in tobacco, yielded compound *1* on treatment with dilute H_2SO_4 in dioxane–water. It should be noted that pathway B is analogous to pathway C, which describes the generation of the 8*R*,11*S*-epoxy bridged tobacco constituents (*4*, *5* and *15*–*18*) *via* acid-induced rearrangements of the (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)- and (1*S*,2*E*,4*R*,6*R*,7*E*,-11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diols (*19*, *20*) (*cf.* Scheme 1, which also includes plausible modes of formation of compounds *21* and *22*).[4]



14 *23*

Experimental support for the existence of a pathway between the 4,8*S*-diols *8* and *10* and the 8*R*,11*S*-epoxy bridged compounds was provided by the fact that treatment of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*11*) with dilute H_2SO_4 in dioxane–water afforded, besides *12*, also (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratrien-4-ol (*16*). Its generation evidently involves hydroxylation of C-12 and a proton-induced loss of the hydroxyl group at C-8. Whether this reaction occurs in one step initiated by protonation of the hydroxyl group at C-8 or takes place *via* an initial *anti*-opening of the epoxide group with formation of an intermediate tetrol (*23*) is presently unclear.

EXPERIMENTAL

With the exception of accurate mass measurements, which were carried out on a Kratos MS 50 Stereo DS 55 SM/DS 55 S mass spectrometer-computer system and some of the ^{1}H NMR spectra, which were recorded on a Varian XL-200 spectrometer, the instruments specified in Ref. 9 were used.

Isolation. Column chromatography over silica gel of fraction A3[10] obtained from an extract of 295 kg of sun-cured Greek *Nicotiana tabacum* L. followed by HPLC using columns packed with Partisil/PAC, μ-Bondapak/C_{18} and μ-Bondapak/CN gave 3.7 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*1*), which had m.p. 130–132 °C, $[\alpha]_D$ +88° (*c* 0.36, $CHCl_3$) (Found: $M^{\cdot+}$ 322.2498, Calc. for $C_{20}H_{34}O_3$: 322.2507); IR ($CHCl_3$) bands at 3605, 3570, 3450, 1390 and 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.86 (d, J = 6.4 Hz)/0.90 (d, J = 6.4 Hz) (H-16/H-17), 1.03 (s, H-19), 1.29 (s)/1.31 (s) (H-18/H-20), 2.16 (dd, J = 10 and −14.5 Hz, H-5a), 2.42 (ddd, J = 2, 3.5 and −14.5 Hz, H-5b), 4.03 (t, J = 6.5 Hz, H-11), 5.24 (d, J = 16 Hz, H-3), 5.27 (dd, J = 16 and 6 Hz, H-2), 5.50 (dd, J = 2 and 15.5Hz, H-7) and 5.76 (ddd, J = 3.5, 10 and 15.5 Hz, H-6); MS[*m*/*z* (%, composition)]: 322 (M, 1), 304 (17, $C_{20}H_{32}O_2$), 286 (3, $C_{20}H_{30}O$), 261 (10), 260 (6, $C_{18}H_{28}O$), 243 (7, $C_{17}H_{23}O$ and $C_{18}H_{27}$), 227 (10, $C_{13}H_{23}O_3$), 217 (6, $C_{15}H_{21}O$), 206 (14, $C_{14}H_{22}O$), 177 (27, $C_{12}H_{17}O$), 159 (23, $C_{12}H_{15}$), 135 (25, $C_{10}H_{15}$ and $C_9H_{11}O$), 121 (34, C_9H_{13} and C_8H_9O), 109 (33, C_8H_{13} and C_7H_9O), 93 (32, C_7H_9), 81 (40, C_5H_5O), 71 (46), 55 (28) and 43 (100).

Preparation of (1S,2E,4S,6E,8S,11S,12S)-11,12-epoxy-2,6-cembradiene-4,8-diol (9). To a cooled (0 °C) solution of 9.4 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*E*)-2,6,11-cembratriene-4,8-diol (*8*)[6] and 15.4 mg of sodium acetate in 4 ml of chloroform was added 5.9 mg of *m*-chloroperbenzoic acid. The

reaction mixture was kept at 0 °C for 15 min. Work-up and separation by HPLC using a column packed with μ-Bondapak/CN gave 2.1 mg of(1*S*,2*E*,4*S*,6*E*,-8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*9*), which was an oil and had $[\alpha]_D$ +24° (*c* 0.37, $CHCl_3$); IR ($CHCl_3$) bands at 3590, 3420, 1385 and 1370 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.81 (d, J=6.8 Hz)/ 0.86 (d, J=6.6 Hz) (H-16/H-17), 1.18 (s, H-20), 1.30 (s)/1.33 (s) (H-18/H-19), 2.2–2.5 (m, H-5a, H-5b), 3.12 (dd, J=1.5 and 10.5 Hz, H-11), 5.40 (dd, J=8 and 16 Hz, H-2), 5.47 (d, J=16 Hz, H-3), 5.60 (d, J=15.2 Hz, H-7) and 5.80 (ddd, J=5.7, 8.2 and 15.2 Hz, H-6); MS [*m/z* (%)]: 304 (M-18, 5), 286 (10), 268 (5), 243 (7), 225 (6), 215 (4), 145 (18), 123 (23), 109 (23), 95 (33), 81 (50), 69 (24), 55 (30) and 43 (100).

Treatment of (1S,2E,4S,6E,8S,11S,12S)-11,12-epoxy-2,6-cembradiene-4,8-diol (9) with acid. A solution of 13 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*9*) in 1 ml of chloroform was acidified by adding 1 ml of chloroform, which was saturated with aqueous hydrochloric acid. The reaction mixture was kept at room temperature for 5.5 h. Work-up and chromatography over silica gel yielded 7.5 mg of (1*S*,2*E*,4*S*,-6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*1*), which was identical (m.p., $[\alpha]_D$, IR, ^1H NMR and MS) to the naturally occurring compound.

Treatment of (1S,2E,4R,6E,8S,11S,12S)-11,12-epoxy-2,6-cembradiene-4,8-diol (11) with acid. I. A solution of 4.0 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*11*)[6] in 1 ml of chloroform was acidified by adding 1 ml of chloroform, which was saturated with aqueous hydrochloric acid. The reaction mixture was kept at room temperature for 2.5 h. Work-up and chromatography over silica gel furnished 2.2 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*12*), which had m.p. 167–169 °C, $[\alpha]_D$ +69° (*c* 0.28, $CHCl_3$) (Found: M-18 $^+$ 304.2426. Calc. for $C_{20}H_{32}O_2$: 304.2402); IR ($CHCl_3$) bands at 3600 and 3440 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), 1.03 (s, H-19), 1.30 (s)/1.43 (s) (H-18/H-20), 2.27 (dd, J=8 and −13.6 Hz, H-5a), 2.43 (dd, J=3.5 and −13.6 Hz, H-5b), 4.01 (t, J=6.5 Hz, H-11), 5.16 (dd, J=7.5 and 15.5 Hz, H-2), 5.33 (d, J=15.5 Hz, H-3), 5.47 (ddd, J=3.5, 8 and 15.7 Hz, H-6) and 5.56 (d, J=15.7 Hz, H-7); MS [*m/z* (%)]: 304 (M-18, 8), 286 (4), 261(3), 243 (5), 227 (3), 217 (4), 177 (13). 159 (42), 133 (23), 121 (33), 109 (22), 93 (43), 81 (40), 71 (38), 55 (42) and 43 (100).

II. A solution of 33.7 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,-12*S*)-11,12-epoxy-2,6-cembradiene-4,8-diol (*11*) in 8 ml of dioxane-H_2O (3:1) and 0.5 ml of aqueous H_2SO_4 (5%) was stirred at room temperature for 5.5 h. Work-up and chromatography over silica gel gave 8.8 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*12*) and 1.3 mg of (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*E*)-8,11-epoxy-2,6,12-cembratriene-4-ol (*16*), which has m.p. 55–56 °C and was identified by comparison of its IR, ^1H NMR and mass spectra with those of an authentic sample.[4]

Treatment of (1S,2E,4S,6R,7E,11R,12R)-11,12-epoxy-2,7-cembradiene-4,6-diol (13) with acid. A solution of 30 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 (*13*)[4] in 10 ml of dioxane-H_2O (2:1) and 0.5 ml of aqueous H_2SO_4 (5%) was kept at room temperature for 2.5 h. Work-up and separation by column chromatography over silica gel followed by HPLC using a column packed with μ-Bondapak/CN yielded 0.2 mg of(1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (1), whose ^1H NMR and mass spectra were identical to those of the naturally occurring compound (*1*), as well as a series of unidentified products.

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