Note on the Stereostructures of Thunbergol (Isocembrol) and 4-Epiisocembrol

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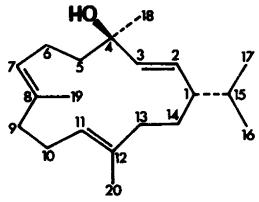
Thunbergol (*1*) was first isolated from the pocket resin of Douglas fir, *Pseudotsuga menziesii*, and characterised as a (1*S*,2*E*)-2,7,11-cembratrien-4-ol.[1] The remaining stereochemical features were later claimed to have been settled by a synthesis, which involved introduction of a 3,4-epoxide *syn* to the 2-hydroxyl group in (±)-epimukulol (*2*) with formation of the 3*S**,4*S**-epoxide *3*, mesylation to *4* and reduction. The resultant alcohol was assigned the structure (1*S**,2*E*,4*S**,7*E*,11*E*)-2,7,11-cembratrien-4-ol (*5*) and proved to be identical (^{1}H NMR) to thunbergol.[2] An analogous reaction sequence converted (±)-mukulol (*6*) to the 4*R**-alcohol *1*.[2]

These assignments were questioned, however, by Weinheimer *et al.*,[3] who in a recent review article suggested that due to a plausible conformational preference about the 2,3-bond, epoxidation of the 3,4-double bonds in (±)-epimukulol (*2*) and (±)-mukulol (*6*) would in contrast yield the 3*R**,4*R**- and 3*S**,4*S**-epoxides *7* and *8*, respectively. As a



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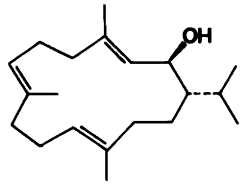

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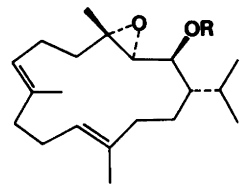
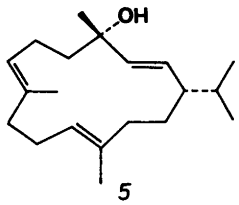


3 R=H
4 R=Mesylate



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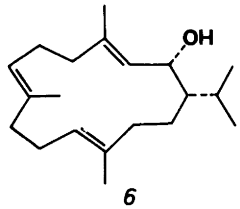

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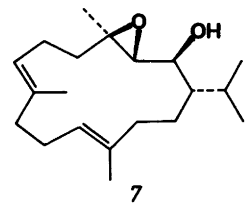

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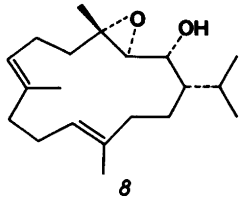

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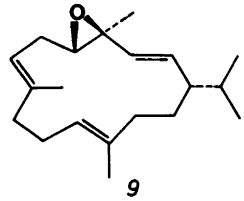

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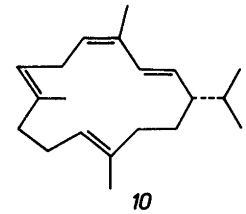

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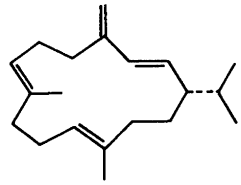

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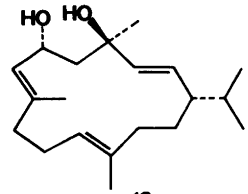

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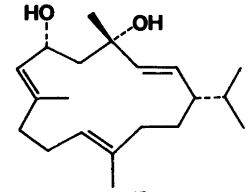

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consequence thunbergol should be reformulated as (1*S*,2*E*,4*R*,7*E*,11*E*)-2,7,11-cembratrien-4-ol (*1*).

The latter structure was recently ascribed to isocembrol, a constituent of certain *Pinus* and *Picea* species.[4-7] This assignment was based on a synthesis, which involved LAH reduction of a mixture containing (4*S*,5*R*)-epoxycembrene (*9*), 7,8- and 11,12-epoxycembrenes and allowed the isolation of an alcohol (*1*) indistinguishable from isocembrol.[8]

Although hexahydrothunbergol and hexahydroisocembrol have proven to be identical by a direct comparison,[1] no conclusive report on the identity of thunbergol and isocembrol is presently at hand.[3] Nor has the minor constituent, which was found to co-occur with isocembrol in *Pinus koraiensis* and *Pinus sibirica* and which was formulated as 4-epiisocembrol (*5*) on account of its spectral data and its dehydration to cembrene (*10*) and isocembrene (*11*),[9] been compared with the synthetic alcohol obtained from (±)-mukulol.

With a view to resolving these structural ambiguities, we now report the isolation of two compounds (*1*, *5*) both from the pocket resin of *Pseudotsuga menziesii* and the gummy exudate of green leaves of *Nicotiana sylvestris*. Both compounds had the composition $C_{20}H_{34}O$ and contained a tertiary hydroxyl group [IR band at 3610 cm^{-1}; ^{13}C NMR singlet at δ 72.5 (*1*) and 73.8 (*5*)], which was attached to the methyl-carrying fully substituted carbon atom, [three-proton singlet at δ 1.36 (*1*) and 1.29 (*5*)]. Furthermore, since both alcohols gave rise to a mixture of cembrene (*10*) and isocembrene (*11*) upon dehydration, they were identified as the 4-epimers of (1*S*,2*E*,7*E*,11*E*)-2,7,11-cembratrien-4-ol.

A detailed comparison showed that the physical and spectral properties of the major alcohol (*1*) agreed sufficiently well with those published for thunbergol, isocembrol and the synthetic alcohol obtained from (±)-epimukulol (*cf.* Experimental) to demonstrate the identity of these compounds. A good congruity was also found between the data of the minor alcohol (*5*) and those reported both for 4-epiisocembrol and the synthetic alcohol derived from (±)-mukulol.

The ^{13}C NMR spectra of the two alcohols (*1*, *5*), which were assigned with the use of single frequency off-resonance decoupling, simple chemical shift characteristics and a comparison with the spectra of a number of other, differently substituted cembranoids,[10] contained useful stereochemical information. Thus, as shown in Table 1, which also includes ^{13}C NMR data for the (1*S*,2*E*,4*R*,6*R*,7*E*,-11*E*)- and (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diols (*12*, *13*) for comparison purpose,[11] the signal assigned to C-2 for the minor alcohol (4-epiisocembrol, *5*) is upfield from the corresponding signal for the major alcohol (thunbergol, isocembrol *1*), whereas the reverse is true for the C-18 signal. An analogous situation has been found for other cembranoids epimeric at C-4[10] and is only consistent with a 4*R*-configuration in thunbergol (*1*) and a 4*S*-configuration in 4-epiisocembrol (*5*). Furthermore, since the chemical shift values of the C-19 and C-20 signals for both alcohols (*1*, *5*), ~δ 15, are diagnostic of *E*-configurations of the 7,8 and 11,12-double bonds,[12] it can be concluded that thunbergol and isocembrol are identified as (1*S*,2*E*,-4*R*,7*E*,11*E*)-cembratrien-4-ol (*1*), whereas 4-epiisocembrol is assigned the structure (1*S*,2*E*,4*S*,7*E*,11*E*)-cembratrien-4-ol (*5*).

Experimental. Preparative (large-scale) high performance liquid chromatography was carried out on a Waters Prep LC/System 500 chromatograph

Table 1. Carbon-13 chemical shifts and assignments for compounds *1*, *5*, *12* and *13*.[a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
1	45.9	129.0	138.3	72.5	43.1	22.6	128.6	132.2	36.8	23.8
5	46.2	126.9	138.9	73.8	44.1	23.5	127.9	132.4	36.9	23.7
12	46.3	130.5	136.1	71.7	52.7	64.5	131.3	136.5	38.9	23.1
13	46.5	127.7	137.7	72.5	52.5	66.2	131.0	135.9	38.9	23.4

Compound	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
1	125.2	132.3	39.2	27.6	33.0	20.4	19.5	28.1	14.7	15.0
5	124.9	132.7	39.1	28.0	33.1	20.7	19.4	29.3	14.8	15.1
12	124.5	133.0	36.5	27.7	33.0	20.5	19.4	28.7	15.9	15.0
13	124.6	133.2	36.9	28.1	33.1	20.7	19.4	30.1	16.1	15.0

[a] δ-Values in $CDCl_3$ relative to TMS.

using a prepPAK-500 Silica column. For other instrumental details see Ref. 13.

Isolation. A fraction containing a mixture of two alcohols (600 mg) was obtained from the neutral portion of the pocket resin of *Pseudotsuga menziesii* (Mirb.) Franco (15 g) by preparative HPLC followed by chromatography over silica gel. Part of this fraction was separated further by HPLC using a column packed with μ-Bondapak/CN to give the (1*S*,2*E*,4*R*,7*E*,11*E*)- and (1*S*,2*E*,4*S*,7*E*,11*E*)-2,7,11-cembratrien-4-ols (*1*, *5*), ratio 7:3.

These two alcohols (*1*, *5*; 8 and 2 mg respectively) were also isolated from a chloroform extract of the gummy exudate of green leaves of *Nicotiana sylvestris* Spegazzini and Comes (33 g) by chromatography over silica gel and subsequent HPLC using columns packed with μ-Bondapak/CN and μ-Porasil.

(1*S*,2*E*,4*R*,7*E*,11*E*)-2,7,11-Cembratrien-4-ol (*1*) was an oil and had $[\alpha]_D$ +75.2° (*c* 1.2, $CHCl_3$); reported for thunbergol $[\alpha]_D$ +75.4°, (*c* 0.6),[1] for isocembrol $[\alpha]_D$ +78.4° (*c* 2.0, $CHCl_3$);[7] (Found: $M^{\cdot+}$ 290.2605. Calc. for $C_{20}H_{34}O$: 290.2609); the IR data agreed well with those published for thunbergol (film) [1] and isocembrol (CCl_4);[5] the ^1H NMR data were identical to those reported for thunbergol ($CDCl_3$)[1], the synthetic alcohol obtained from (±)-epimukulol ($CDCl_3$)[2] and isocembrol (CCl_4),[5] the mass spectrum agreed with that published for thunbergol.[1]

(1*S*,2*E*,4*S*,7*E*,11*E*)-2,7,11-Cembratrien-4-ol (*5*) was an oil and had $[\alpha]_D$ +91.9° (*c* 1.3, $CHCl_3$) and $[\alpha]_D$ +105° (*c* 3.1, $CHCl_3$); reported for 4-epiisocembrol $[\alpha]_D$ +110.5° (*c* 3.35, $CHCl_3$);[9] (Found: $M^{\cdot+}$ 290.2621. Calc for $C_{20}H_{34}O$: 290.2609); the IR and ^1H NMR data were identical to those reported for 4-epiisocembrol (CCl_4)[9] and the ^1H NMR data obtained in $CDCl_3$ solution agreed well with those published for the synthetic alcohol obtained from (±)-mukulol;[2] MS [*m*/*z* (%)]: 290 (M, 2), 272 (20), 257 (10), 229 (28), 215 (3), 204 (10), 189 (17), 173 (12), 161 (95), 147 (25), 133 (38), 121 (53), 107 (80), 93 (91), 81 (100), 69 (78), 55 (83) and 43 (93).

Dehydration of the (1S,2E,4S,7E,11E)- and (1S,2E,4R,7E,11E)-2,7,11-cembratrien-4-ols (5, 1). To a solution of 100 mg of *5* in 1 ml of pyridine kept at 0 °C was added 35 μl of phosphoryl chloride. The reaction mixture was kept at room temperature for 24 h, poured into ice-water and extracted with ether. The ether solution was washed with aqueous H_2SO_4 (10 %) and water, dried and concentrated. The residue was chromatographed on silver nitrate impregnated silica gel using ethyl acetate: hexane (1:99) as an eluent to give two fractions. The first fraction, 14 mg. had m.p. 58 °C and $[\alpha]_D$ +239° (*c* 0.3, $CHCl_3$); reported m.p. 59–60 °C, $[\alpha]_D$ +238° (*c* 1.13, $CHCl_3$)[14]; the UV[14], IR[14], ^1H NMR[1] and mass spectra[1] were indistinguishable from those of authentic cembrene, (1*S*,2*E*,4*Z*,7*E*,11*E*)-2,4,7,11-cembratetraene (*10*).

The second fraction, 6 mg, was an oil and had $[\alpha]_D$ +55° (*c* 0.3, $CHCl_3$); reported $[\alpha]_D$ +60.3° (*c* 2.6, $CHCl_3$);[5] the UV[4] and IR[4] spectra were identical with those of authentic isocembrene, (1*S*,2*E*,7*E*,11*E*)-2,4(18),7,11-cembratetraene (*11*); ^1H NMR ($CDCl_3$): δ 0.83 (3H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 6.5 Hz), 1.58 (6H, overlapping methyl signals), 4.85 (1H, d, *J* = 2 Hz), 5.02 (1H, d, *J* = 2 Hz), 4.8–5.3 (2H, overlapping signals), 5.53 (1H, dd, *J* = 8 and 16 Hz) and 5.92 (1H, d, *J* = 16 Hz); MS [*m*/*z* (%)]: 272 (M, 25), 257 (8), 229 (36), 201 (5), 189 (16), 173 (12), 161 (25), 147 (22), 133 (42), 119 (63), 105 (71), 93 (100), 81 (87), 69 (43), 55 (62) and 41 (44).

Treatment of *1* with phosphoryl chloride in pyridine using the same conditions as described above for *5* yielded, after separation, cembrene (*10*) and isocembrene (*11*) in the ratio 2:1.

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