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STRUCTURE ELUCIDATION OF GERMACRANE ALCOHOLS FROM *JUNIPERUS COMMUNIS* SUBSP. *HEMISPHAERICA*

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ABSTRACT.—Thirty-one constituents have been identified in the neutral part of a *n*-hexane extract from the leaves of *Juniperus communis* subsp. *hemisphaerica*. Among them, three germacrane alcohols were isolated and their structures established. The stereochemistry of germacra-1(10),5-dien-4β-ol [**1**] was established by a combination of chemical transformations and spectral analysis. The stereochemistries of germacra-5,10(14)-dien-1α,4β-diol [**2**] and germacra-5,10(14)-dien-1β,4β-diol [**3**] were established by partial synthesis from **1**.

A study of *Juniperus* species and subspecies (Cupressaceae) growing in the Iberian Peninsula, initiated by our group several years ago, has led to the characterization of a large number of terpenoids, lignans and other components (1–4). Among them, germacrane sesquiterpenoids were found in the essential oil and neutral extracts of leaves and berries of these plants (5,6).

We now communicate the isolation, identification, and chemical transformation of the neutral components of an extract of leaves of *Juniperus communis* L. subsp. *hemisphaerica* (K. Presl.) Nyman. A previous study on the acidic components of the same extract was published recently (7).

The *n*-hexane extract of the leaves of *J. communis* subsp. *hemisphaerica* was defatted and divided into acidic and neutral fractions using aqueous NaOH (4%). On chromatographic separation of the neutral fraction, several known sesquiterpenoid and diterpenoid compounds with different carbon skeletons were isolated and identified by comparison with authentic samples (or by comparison with their spectroscopic properties) isolated previously from other species or subspecies of *Juniperus*: nerolidol, germacrene B, germacrene D, δ-cadinene, γ-cadinene, α-cadinol, τ-cadinol, β-selinene, β-caryophyllene, caryophyllene oxide, humulene epoxide, α-himachalene, oplopanone, phytol, isopimarol, isopimaral, sandaracopimaral, sugiol, $\Delta^{13(16)}$-isocommunal, and 4-*epi*-dehydroabietol, along with geraniol, β-sitosterol, and the lignans, yatein and sesamin (1–7). Three structurally related germacrane alcohols, namely, germacra-1(10),5-dien-4β-ol [**1**], germacra-5,10(14)-dien-1α,4β-diol [**2**], and germacra-5,10(14)-dien-1β,4β-diol [**3**], were also isolated.

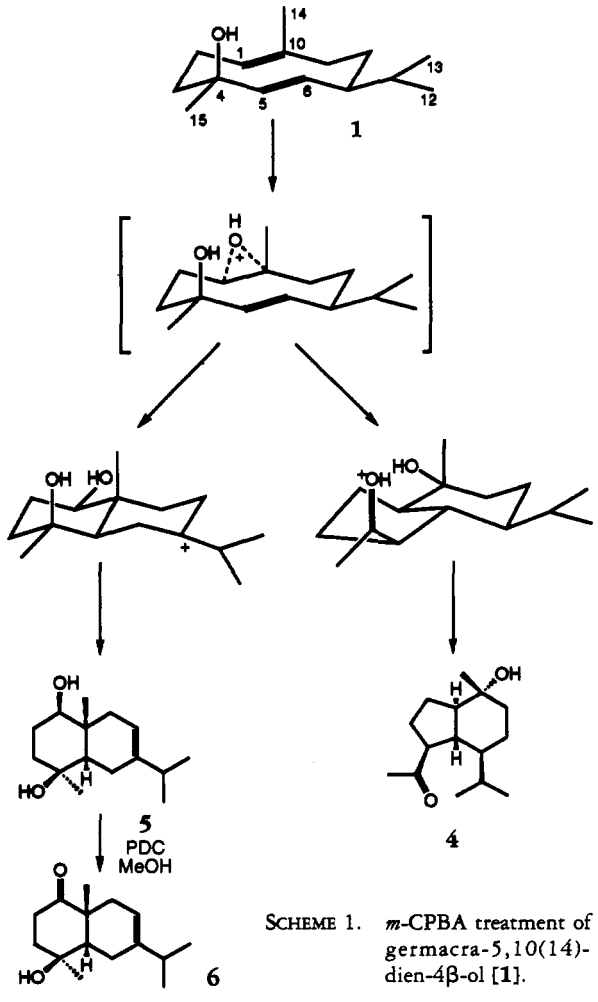
Compound **1**, the major component of the extract, was isolated in 4.3% yield. Ms, ir, and nmr (Table 1) data of **1** were employed for its structural identification, although the configurations at C-4 and C-7 remained to be definitively established. In fact, this compound was previously described by Fenical and Izac (8) and by Bohlmann *et al.* (9,10) and the published spectroscopic properties were in accordance with those found for **1**. The previous investigators assigned different relative stereostructures to **1**, while no chemical proof of the absolute stereochemistry was reported. The first group of investigators used nmr shifts induced by $Eu(fod)_3$ to deduce the relative stereochemistry, while the structural proposal of the second group was based on nOe experiments.

In order to obtain further stereochemical information, we decided to prepare cyclized and more rigid derivatives from **1**. Because the C-1–C-10 epoxide could be a desirable intermediate for the transannular cyclization of germacrane derivatives, the *m*-CPBA treatment of **1**

TABLE 1. ^{1}H-Nmr (200 MHz) Spectral Data for Compounds **1**, **2**, and **3**.

Proton	Compound		
	1	**2**	**3**
H-1	4.95 (br d, J=11.0 Hz)	4.16 (dd, J=7.8, 3.2 Hz)	3.94 (dd, J=9.1, 3.0 Hz)
H-2	2.5 (dddd, J=14.3, 11.0, 11.0, 4.1 Hz)		
H-5	5.25 (d, J=15.0 Hz)	5.29 (d, J=15.5 Hz)	5.31 (d, J=15.0 Hz)
H-6	5.17 (dd, J=15.0, 8.2 Hz)	5.34 (dd, J=15.5, 1.9 Hz)	5.21 (dd, J=15.0, 8.2 Hz)
H-12	0.79 (d, J=6.7 Hz)	0.88 (d, J=6.7 Hz)	0.84 (d, J=6.7 Hz)
H-13	0.83 (d, J=6.7 Hz)	0.84 (d, J=6.7 Hz)	0.89 (d, J=6.7 Hz)
H-14	1.54 (s)	5.10 (br s) 5.22 (br s)	4.85 (br s) 5.12 (br s)
H-15	1.19 (s)	1.28 (s)	1.27 (s)

was carried out under standard conditions. Instead of the simple epoxidation product, two bicyclic compounds were isolated from the reaction mixture (Scheme 1), namely, oplopanone [**4**] (10,11) and oplodiol [**5**] (9,12). The ke-



SCHEME 1. *m*-CPBA treatment of germacra-5,10(14)-dien-4β-ol [**1**].

tone **4** had a carbonyl group at the position equivalent to C-4 of the germacrane precursor, so it had lost the stereochemical information about this center. The other compound was formed from **1**, by C-1–C-10 epoxidation followed by C-10–C-5 cyclization and deprotonation-isomerization, without affecting the configuration of C-4. The relative stereochemistry was unambiguously deduced from solvent shifts (Table 2), induced by pyridine-d_5, and C_6H_6-d_6 on the ^{1}H-nmr signals of the hydroxyketone **6**. The solvent-induced shifts on the signals for Me-4 and Me-10 and the significant displacement of the β-axial H-2 by both solvents, were in agreement with the presence in **6** of a β-axial OH group and an α-equatorial methyl group at C-4. Further, this structure was supported by the absence of nOes between both methyl groups Me-4 and Me-10.

The relative stereochemistry of **1**, deduced in this way from **5** and **6**, corresponds to that proposed by Bohlmann *et al.* (9,10). Finally, the absolute stereochemistry at C-7 must be the same *S*-configuration as (−)-oplopanone [**4**], obtained by cyclization of **1**.

Compounds **2** and **3** had the same M^+ at *m/z* 238 and very similar ^{1}H-nmr signals (Table 1). In comparison with **1**, both compounds contained an additional secondary OH and an olefinic methylene, instead of the trisubstituted double bond with a methyl group. The structures of the epimeric germacra-5,10(14)-dien-1,4-diols **2** and **3** were assigned to these products.

TABLE 2. Shifts Induced by Solvents in the ^{1}H-Nmr Spectrum of **6**.

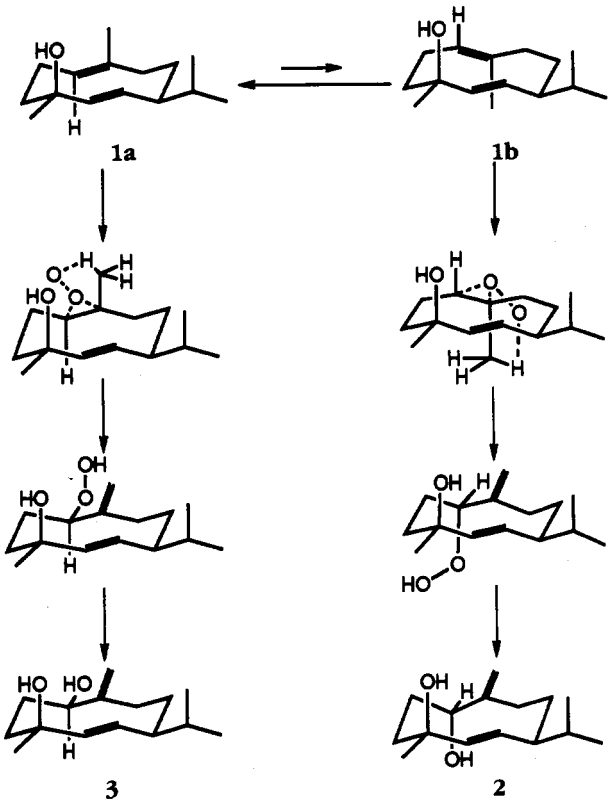
	$CDCl_3$	C_6D_6	C_5D_5N
H-2	3.10	2.94	3.31
Δ.....		−0.16	+0.21
H-12	1.03	1.00	1.00
Δ.....		−0.03	−0.03
H-13	1.01	1.00	1.00
Δ.....		−0.01	−0.01
H-14	1.28	0.92	1.32
Δ.....		−0.36	+0.04
H-15	1.23	1.19	1.42
Δ.....		−0.04	+0.19

This proposal was confirmed by the oxidation of **1** with singlet oxygen. Conversion was very low. Compound **3** was isolated as the major product and compound **2** was detected as a minute reaction product. In this way, the constitution and C-4–C-8 stereochemistries of both compounds were definitively established. The stereochemistry at C-1 for each epimer is generated during oxidation, so it was possible to assign the configuration of the major reaction product at this center as that derived from the most favorable process. As depicted in Scheme 2, the most stable conformation is the chair-chair **1a**, which can only produce a β-OH at C-1, because the other face of the double bond is not accessible. Thus, the configuration (1*R*) was assigned to the major reaction product **3**. The minor product **2** can only be produced after a change to the less stable conformation **1b**, exposing the other face of the double bond to the reagent. The configuration (1*S*) for this minor product corresponds to the α-OH disposition. These stereochemical assignments agree with the small differences observed in the ^{1}H-nmr spectra of both compounds. In the major product, H-1 and H-14a are respectively shielded by the effect of the Δ^5 double bond and the adjacent hydroxyl group, while in the minor product the spatial disposition is not favorable to the appearance of these effects.

Structure **3** was proposed by Fenical and Izac for one product isolated from *Laurencia supposita* (8), which had very similar spectroscopic properties to our isolate. Compound **2** is described for the first time.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were measured on a Beckman AccuLab VIII spectrophotometer in $CHCl_3$ solution. ^{1}H-(200 MHz) and ^{13}C-nmr (50.3 MHz) spectra were recorded in $CDCl_3$ on a Bruker WP 200 SY



SCHEME 2. Singlet oxygen oxidation of compound **1**.

instrument, using TMS as internal standard. Chemical shifts are reported in δ (ppm) values. Ms were obtained with a VG model TS-250 mass spect-rometer operating at 70 eV. Merck 60 (70–230 mesh and 230–400 mesh) Si gels were used for column and flash chromatography, respectively. Precoated plates of Polychrom Si F_{254} were used for tlc.

PLANT MATERIAL.—The plant material was collected in Navacerrada, Madrid, Spain, in September 1990 and identified by Dr. C.J. Valle of the Faculty of Pharmacy, Department of Botany, University of Salamanca, where a specimen was deposited (Ref. SAF 23798).

EXTRACTION AND ISOLATION.—The air-dried leaves (2.9 kg) were extracted with *n*-hexane by the Soxhlet procedure. After cooling the extract (−20°) overnight, an insoluble part (112 g, 3.9% of dry wt) and a *n*-hexane-soluble part (223 g, 7.7%) were obtained. On defatting, the *n*-hexane-soluble part (78 g) was fractionated with 4% NaOH giving acidic (79.5%) and neutral fractions (20.1%) (7).

The neutral fraction (27.0 g) of the soluble part was chromatographed on dry Si gel using a *n*-hexane-Et_2O (1:1) mixture as eluent and divided into six chromatographic fractions. From the less polar fraction (a) and by consecutive chromatographic steps (*n*-hexane/Et_2O) on Si gel and Si gel/20% $AgNO_3$, the following compounds were isolated and identified by comparison of their physical and spectroscopic data with those reported in the literature: δ-cadinene (66 mg) (13), α-himachalene (15 mg) (14), γ-cadinene (351 mg) (15), β-selinene (28 mg) (16), β-caryophyllene (163 mg) (17), germacrene D (45 mg) (18), germacrene B (839 mg) (19), ferruginol (49 mg) (20), caryophyllene oxide (6 mg) (21), humulene oxide (9 mg) (22), **1** (1.04 g), nerolidol (26 mg) (23), **2** (4 mg), **4** (77 mg), **3** (37 mg), α-cadinol (139 mg) (24,25), and sugiol (21 mg) (26).

The following substances were isolated from the other chromatographic fractions:

Fraction b: after acetylation, τ-cadinol (132 mg) (24,27), phytyl acetate (47 mg) (28), and isopimaryl acetate (38 mg) (29).

Fraction c: 4-*epi*-dehydroabietol (84 mg) (30), labda-8(17),13-dien-15-ol (31 mg) (31), and 4-*epi*-dehydroabietyl acetate (60 mg) (30), and labda-8(17),13-dien-15-yl acetate (53 mg) (31), and, after acetylation of several fractions for facilitating isolation, geraniol (57 mg) (32) and sesamin (164 mg) (33,34).

Fraction d: sitosterol (1.1 g) (35) and agatolal (500 mg) (36).

Fraction e: agatolal (340 mg).

Fraction f: agatadiol (844 mg) (37) and yatein (110 mg) (38).

Germacra-1(10),5-dien-4β-ol [**1**].—$[\alpha]^{25}D$ −112° (c=0.25); ir ν max ($CHCl_3$) 3600, 990, 900 cm^{-1}; ^1H-nmr data, see Table 1; ^{13}C nmr δ 129.1, 23.8, 41.4, 73.2, 140.2, 126.1, 53.0, 39.8, 26.1, 132.6, 33.1, 20.6, 19.1, 16.7, 30.7.

Germacra-5,10(14)-dien-1α,4β-diol [**2**].—Ir ν max ($CHCl_3$) 3600, 1100, 990, 900, 650 cm^{-1}; eims m/z M^+ 238 (10), 223 (12), 220 (8), 205 (11), 175 (20), 183 (24), 153 (54), 55 (100); ^1H-nmr data, see Table 1.

Germacra-5,10(14)-dien-1β,4β-diol [**3**].—Mp 84–86° (hexane); $[\alpha]^{25}D$ −147.9° (c=0.3); ir ν max ($CHCl_3$) 3600, 1010, 990, 900 cm^{-1}; eims m/z M^+ 238 (2), 220 (8), 205 (10), 202 (6), 187 (10), 177 (38), 159 (32), 81 (100), 55 (88); ^1H-nmr data, see Table 1; ^{13}C nmr δ 78.7, 28.6, 30.1, 72.3, 137.6, 129.6, 50.1, 38.8, 28.6, 151.0, 32.4, 20.6, 20.6, 111.4, 29.6.

EPOXIDATION OF GERMACRA-1(10),5-DIEN-4β-OL [**1**].—Compound **1** (430 mg) was dissolved in $CHCl_3$ and $NaHCO_3$ (334 mg) was added. A solution of *m*-CPBA (334 mg) in $CHCl_3$ was dropped into the mixture at −40° while stirring. The mixture was allowed to react at room temperature for 4 h. After this time, $NaHSO_3$ (dilute aqueous solution) was added and the organic layer washed with H_2O, dried ($CaCl_2$), and the solvent removed under reduced pressure. The crude material (338 mg) was chromatographed on Si gel (*n*-hexane-EtOAc, 85:15) yielding **4** (27 mg), **5** (61 mg), and unreacted **1** (250 mg)

Oplopanone [**4**].—Colorless crystals, mp 96–98° (hexane); $[\alpha]^{25}D$ −18.3° (c=0.6); ir ν max ($CHCl_3$) 3700, 3450, 1700, 1100, 910, 900 cm^{-1}; ^1H nmr δ 0.68 (3H, d, J=6.8 Hz, H_3-12), 0.89 (3H, d, J=6.8 Hz, H_3-13), 1.19 (3H, s, H_3-14), 2.19 (3H, s, H_3-15), 2.70 (1H, m, H-5); ^{13}C nmr δ 15.6 (q, C-14), 20.2 (q, C-12), 21.8 (q, C-13), 23.0 (t, C-3), 25.3 (t, C-8), 28.6 (t, C-2), 29.2 (d, C-11), 29.4 (q, C-15), 42.1 (t, C-9), 46.7 (d, C-7), 49.5 (d, C-6), 55.8 (d, C-5), 57.1 (d, C-1), 72.7 (s, C-10), 211.2 (s, C-4).

Oplodiol [**5**].—$[\alpha]^{25}D$ −77.7° (c=0.6); ir ν max ($CHCl_3$) 3600, 3580–3400, 1200, 1165, 1000, 950, 900 cm^{-1}; ^1H nmr δ 0.95 (3H, s, H_3-14), 1.01 (3H, d, J=6.8 Hz, H_3-12), 1.04 (3H, d, J=6.8 Hz, H_3-13), 1.18 (3H, s, H_3-15), 3.30 (1H, dd, J=11.7 and 4.0 Hz, H-1), 5.33 (1H, d, J=5.0 Hz, H-8); ^{13}C nmr δ 11.7 (q, C-14), 21.3 (q, C-12), 21.8 (q, C-13), 23.2 (t, C-6), 26.9 (t, C-2), 29.9 (q, C-15), 35.0 (d, C-11), 37.8 (s, C-10), 39.6 (t, C-9), 40.9 (t, C-3), 46.5 (d, C-5), 71.1 (s, C-4), 80.0 (d, C-1), 116.2 (d, C-8), 142.0 (s, C-7).

OXIDATION OF OPLODIOL [**5**] WITH PYRIDINIUM DICHROMATE (PDC).—A solution of PDC in CH_2Cl_2 was dropped into a solution of oplodiol [**5**] (25 mg) in the same solvent. The mixture was left for 2 h at room temperature. The crude product was chromatographed on Si gel using CH_2Cl_2-Me_2CO (98:2) as eluent, yielding 4β-hydroxyeudesm-7-en-1-one [**6**] (10.1 mg): $[\alpha]^{25}D$ +0.1° (c=0.7); ir ν max ($CHCl_3$) 3600, 3570–3500, 1710, 1110, 1090, 910 cm^{-1}; ^1H nmr δ 1.01 (3H, d, J=6.8 Hz, H_3-13), 1.05 (3H, d, J=6.8 Hz, H_3-12), 1.20 (3H, s, H_3-15), 1.30 (3H, s, H_3-14), 3.11 (1H, dt, J=14.2 and 4.0 Hz, H-2β), 5.35 (1H, d, J=5.8 Hz, H-8); ^{13}C nmr δ 18.8 (q, C-14), 21.3 (q, C-12), 21.8 (q, C-13), 23.8 (t, C-6), 29.4 (q, C-15), 34.5 (t, C-2), 35.0 (d, C-11), 35.5 (t, C-9), 41.1 (t, C-3), 46.4 (s, C-10), 48.3 (d, C-5), 70.7 (s, C-4), 116.5 (d, C-8), 141.4 (s, C-7), 216.2 (s, C-1).

SYNTHESIS OF GERMACRA-5,10(14)-DIEN-1α,4β-DIOL [**2**] AND GERMACRA-5,10(14)-DIEN-1β,4β-DIOL [**3**].—In several separate experiments, a total amount of 550 mg of **1** in *i*-PrOH (20 mg/ml) was exposed to natural or artificial (400 watt) sunlight irradiation for 3–30 h in the presence of Rose Bengal at room temperature. The reaction progress was checked by tlc, but only low transformations were observed in all the conditions used. At the end of the reaction, $(CH_3)_2S$ was added and the mixture washed with H_2O. The Rose Bengal was removed by filtration through Si gel and the crude material was dissolved in *n*-hexane for crystallization. The crystalline product (36 mg) was identified as germacra-5,10(14)-dien-1β,4β-diol [**3**], identical to that isolated from the natural source. Germacra-5,10(14)-dien-1α,4β-diol [**2**] (2 mg) was also obtained from the mother liquor after chromatography.

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