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A SESQUITERPENE ALCOHOL FROM *STREPTOMYCES CITREUS* CBS 109.60

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ABSTRACT.—Separation of an extract of *Streptomyces citreus* CBS 109.60 by column chromatography and preparative gas chromatography yielded the novel sesquiterpene alcohol **1**, whose structure and relative conformation were established by spectroscopic means. Comparison of the its spectral data with compounds of known absolute configuration led to assignment of the stereochemistry at C-4 and C-7. Thus, **1** was assigned as 4*S*,7*R*-germacra-1(10)*E*,5*E*-diene-11-ol.

Streptomycetes spp. are well-known for their ability to produce geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) (1). Geosmin has an intense "earthy" or "musty" odor. It may cause off-flavors in drinking water (2) and aquaculture-raised fish (3), but may also act positively by complementing food flavors (such as beetroot or whiskey) and perfumes (4). Therefore, microorganisms producing geosmin are being investigated intensely. *Streptomyces citreus* CBS 109.60 has proved to be a potent producer of geosmin (F.C. Pollak and R.G. Berger, University of Hannover, unpublished data). During the detailed study of other terpenoid secondary metabolites of *S. citreus* CBS 109.60, the novel germacrane-type sesquiterpene alcohol **1** was isolated and identified.

The second most abundant component [**1**], after geosmin, in the terpenoid spectrum of *S. citreus* CBS 109.60 was isolated by adsorption from the fermentation broth, and subsequent cc and prep. gc (see Experimental). Its structure was deduced from its ^{1}H-, ^{13}C-nmr, ir, and mass spectra.

The ms of **1** $[M]^+$ *m/z* 222 ($C_{15}H_{26}O$) exhibited a base peak at *m/z* 59 (C_3H_7O) indicating the presence of a hydroxy-isopropyl group. Consequently, the tertiary alcohol failed to produce an acetylated derivative upon treatment with Ac_2O in pyridine. Fifteen carbon atoms were revealed from the ^{13}C-nmr spectrum with four being ethylenic, one bearing an oxygen atom (71.81 ppm), and four methyl, two methine, and four methylene carbon atoms also occurring (Table 1). Thus, a doubly unsaturated monocyclic compound was present.

^{1}H-Nmr resonances could be readily assigned by analysis of the ^{1}H-^{1}H-COSY spectrum of **1**. From the large coupling constants of H-5 and H-6 (16 Hz) an *E*-configuration of the C-5,C-6 double bond was evident. Because of overlapping (with H-2β and H-9α/H-9β, respectively) the coupling constants of H-4 and H-7 could not be directly assigned from the ^{1}H-nmr spectrum, but the equatorial orientation of H-4 was evident by inspection of the signals of the neighboring protons (Table 2). The H-3α,H-4 coupling should be large for an axially oriented H-4 rather than the observed $J=3.5$ Hz for **1**. This assumes a single conformation in solution for the ring system, which seems justified from the observation of sharp lines and some significantly high coupling constants between the vicinal an-

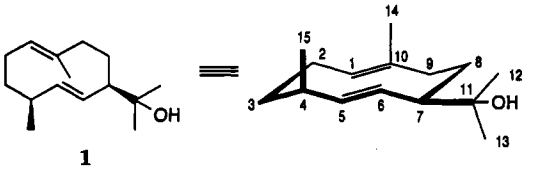

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TABLE 1. ^{13}C-Nmr Data of **1** (75 MHz, $CDCl_3$, internal standard TMS).[a]

Carbon	δ (multiplicity)	Carbon	δ (multiplicity)	Carbon	δ (multiplicity)	Carbon	δ (multiplicity)
1	130.66 (d)	5	143.15 (d)	9	41.37 (t)	13	26.91[c] (q)
2	22.12[b] (t)	6	123.84 (d)	10	131.18 (s)	14	16.77 (q)
3	32.87 (t)	7	58.99 (d)	11	71.81 (s)	15	14.79 (q)
4	33.93 (d)	8	23.80[b] (t)	12	26.37[c] (q)		

[a]Multiplicities were established by the DEPT pulse sequence. The assignments of C-12 to C-15 were made by selective heteronuclear decoupling experiments. Other assignments are tentative and were made by analogy with spectra of compounds of known structure, if available (5,6).
[b,c]Assignments may be interchanged.

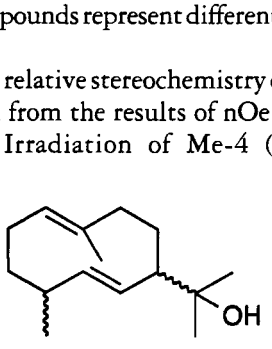
TABLE 2. ^{1}H-Nmr Data of **1** (300 MHz, $CDCl_3$, internal standard TMS).

Proton	δ mult.[a]	Proton	δ mult.[a]	Proton	δ mult.[a]	Proton	δ mult.[a]
1	5.03 br d	4	2.43[b] m	8β	1.25 dddd	14	1.55[d] br s
2α	1.90 dddd	5	5.66 dd	9α	2.21[c] m	15	1.11[d] d
2β	2.41[b] dddd	6	4.97 dd	9β	2.22[c] m		
3α	1.71 dddd	7	2.23[c] m	12	1.07[d] s		
3β	1.53 dddd	8α	1.47 ddt	13	1.16[d] s		

[a]*J* (Hz): 1,2α~3.5; 1,2β=12; 2α/2β=14; 2α/3α=3.5; 2α/3β=3; 2β/3α=12.5; 2β/3β=3.5; 3α3β=13.5; 3α/4=3.5; 3β/4=3.5; 4/5=3.7; 5/6=16; 6/7=10; 7/8α=3.5; 7/8β=11.4; 8α/8β=14; 8α/9α=3.5; 8α/9β=3.5; 8β/9α=11.5; 8β/9β=4; 4/15=7.2.
[b,c]Overlapping signals.
[d]Intensity three protons.

nular protons in the ^{1}H-nmr spectrum (7). Similarly, the axial orientation of H-7 was established by inspection of the signals for H-6 and H-8β. The above results led to the proposal of a germacra-1(10)*E*,5*E*-diene-11-ol structure for **1**. In 1987 Valle *et al.* reported on the identification of the sesquiterpene alcohol allohedycariol [**2**] with the same constitution, but the stereochemistry remained unsettled (8). However, ^{13}C-nmr data as well as the [α]D for **1** were substantially different from those published for allohedycariol [**2**], indicating that the two compounds represent different stereoisomers.

The relative stereochemistry of **1** was deduced from the results of nOe experiments. Irradiation of Me-4 (H_3-15) showed a large nOe on H-6 and smaller effects on H-4, H-2β, and H-14. Because of the absence of an nOe on H-5, the configuration of the double-bond relative to H-15 must be as shown. On the other hand, saturation of Me-10 (H_3-14) gave small effects on H-2β, H-8β, H-6, and H-15, indicating a quasi-axial orientation above the plane. From the ^{13}C-nmr spectrum, *E*-configuration of the C-1(C-10) double bond clearly followed because the chemical shift of C-14 was smaller than 20 ppm (9). Quasi-axial orientation of the vinylic methyl (C-14) was evident from the highfield shift observed for C-8, which can be explained by γ-interactions. Similarly, the shielding of C-6 confirmed the proposed quasi-axial orientation of the allylic methyl (C-15). C-2 was shielded from C-14 as well as from C-15. From the above results a chair-chair-conformation in solution can be deduced for **1** having either 4*S*,7*R*- or 4*R*,7*S*-configuration.



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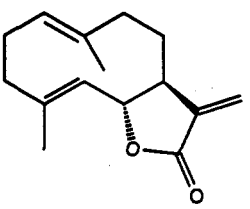
Costunolide **3**, whose absolute configuration is known (10), can be con-

verted into 4*S*,7*S*-germacra-1(10)*E*,5*E*-diene **4** (11). This hydrocarbon is reported to show an $[\alpha]_D$ of $-100°$ (11). Alterations in the isopropyl side-chain (replacing the H-13 hydrogen by OH or COOH) only led to small changes in the magnitude of $[\alpha]_D$ ($-98°$ and $-82°$, respectively) (12). Other examples in the germacradiene class show that replacement of H-11 in the side-chain by OH hardly affected the $[\alpha]_D$ [e.g., dilophol vs. hydroxydilophol (13,14)]. As **1** exhibited an $[\alpha]_D$ of the same sign and of similar magnitude ($-82°$) as the hydrocarbon **4**, it should be of the same rather than of the opposite configuration. Thus, **1** is represented by 4*S*,7*R*-germacra-1(10)*E*,5*E*-diene-11-ol.



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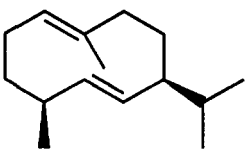

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EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Bruker AC-300 spectrometer at 300 MHz for ^1H and at 75 MHz for ^{13}C with TMS as internal standard. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Ir data were collected on a Perkin-Elmer 1000PC Ft-ir spectrometer. Analytical gc was carried out with a Carlo-Erba HRGC Fractovap 4160 chromatograph equipped with a flame-ionization detector, using a CW 20M fused-silica column (25 m×0.32 mm i.d., film thickness 0.4 μm) [temperature programmed from 40° to 210° at 3° min^{-1} and held at 210° for 10 min]. The temperature of the injector and the detector was kept at 270°. The carrier gas was H_2 and the splitting ratio was 1:15. A retention index for **1** (2127) was calculated according to Kováts (15) with *n*-alkanes as reference compounds. Gc-ms was performed on a Hewlett-Packard 5989A mass spectrometer coupled to a Hewlett-Packard HP 5890 Series II gas chromatograph. Gas chromatographic conditions were the same as for analytical gc (except that He was used as carrier gas) and the ionization energy was 70 eV.

MICROORGANISM.—*Streptomyces citreus* CBS 109.60 was obtained from Centraalbureau for Schimmelcultures (CBS), P.O. Box 273, 3740 AG Baarn, The Netherlands. The microorganisms were cultivated in V1 medium (pH 8.0) containing 3.0 g/liter yeast extract, 1.0 g/liter KH_2PO_4, 1.0 g/liter $MgSO_4$, 2.0 g/liter L-asparagine, 10.0 g/liter D-glucose, 10.0 g/liter malt extract, and 2.0 ml/liter trace element solution (0.4 g/liter $FeCl_3 \times 6 H_2O$, 0.45 g/liter $ZnSO_4 \times 7 H_2O$, 0.125 g/liter $MnSO_4 \times H_2O$, 0.025 g/liter $CuSO_4 \times 5 H_2O$, 2.0 g/liter EDTA; pH 4.6). After 72 h, the fermentation (25°, 1000 rpm, 50% pO_2) was terminated.

EXTRACTION AND ISOLATION.—From 2 liters of fermentation broth compounds were adsorbed onto activated Lewatit® 1064 (final concentration 0.5 g/liter) by gentle shaking overnight (16). After separation of the adsorbent from the cells and the culture medium, adsorbed compounds were desorbed with 3×40 ml pentane-CH_2Cl_2 (2:1). Another 5×2 liters of fermentation broth were treated in the same manner. Combined organic layers were dried over Na_2SO_4 and brought to a final volume of 2 ml (containing 23 major compounds, approximately 36 mg), which were separated by Si gel cc (20×2 cm) using pentane, mixtures of pentane-CH_2Cl_2 (9:1, 3:1, 1:3), and CH_2Cl_2 (200 ml each) as eluent. The pentane-CH_2Cl_2 (1:3)-fraction (R_f [**1**] 0.34; Si gel plates, pentane-CH_2Cl_2, 1:3) was concentrated to 1 ml (seven major compounds, approximately 16 mg) and subjected to prep. gc. Prep. gc was performed on a MCS Gerstel Series II instrument (Gerstel, Mülheim/Ruhr, Germany) with a Gerstel MCS 1.15 control and data acquisition system, a Gerstel KAS-3 cold injection system, and a HP 7673 autosampler. A CW 20M precolumn (1.5 m×0.53 mm i.d., film thickness 2 μm) was connected to a OV-1 fused silica capillary column (25 m×0.53 mm i.d., film thickness 2 μm). Two ports (PC1 and PC2, respectively), allowing the removal of undesired components from the sample, were located between the cold injection system and the pre-column on one side and the pre-column and the main column on the other side. The temperature of the cold injection system (45°) after injection was rapidly raised (10°/sec) to 250° and then held for 1 min. The oven temperature was programed as follows: 130° isothermal for 1 min, linear gradient to 220° at 3° min^{-1}, isothermal at 220° for 10 min. Other temperatures were: FID 200°, supply pipe and distributor 240°, trap cooling 0°. The carrier gas

was H_2 at 5 ml/min, the counter-gas (during blowing out in PC1 and PC2) was H_2 at 10 ml/min, and the auxiliary gas (allowing components of the sample to get onto the pre-column and main column) was H_2 at 1 ml/min. PC1 was switched to blow-out status 1 min after injection, and PC 2 after 18 min. Compound **1** was eluted after 16.5 min and collected in cooled glass traps, total yield 5.9 mg. Recrystallization from pentane afforded colorless needles of **1**, mp 50–53°; $[\alpha]^{20}$ (nm): (c=0.23, $CHCl_3$) −82° (589), −87° (578), −101° (546), −212° (436), −432° (365); ir (CCl_4) ν max 3460 (br), 2980, 2920, 1660 (very weak), 1440, 1430, 1166 cm^{-1}; eims (70 eV) *m/z* 222 (M^+, 6), 204 (9), 189 (12), 164 (26), 161 (17), 149 (37), 135 (16), 121 (24), 107 (43), 93 (43), 82 (91), 67 (47), 59 (100); ^{13}C-nmr data, see Table 1; ^{1}H-nmr data, see Table 2.

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