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Novel Sesquiterpene from Bioactive Fractions of Cultivar Sunflowers[1]

Francisco A. Macías*, Rosa M. Varela, Ascensión Torres and José Mª G. Molinillo
Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz,
Apdo 40, 11510-Puerto Real, Cádiz, Spain.

Frank R. Fronczek
Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana, 70803, U.S.A.

Abstract: From the medium polar active fractions, we have isolated a sesquiterpene heliannuol A. It contains a previously unknown skeleton, heliannuol, whose structural elucidation was made based on spectroscopic techniques and X-Ray diffraction analisys.

The existence of allelopathy has been well documented over the past few decades, particularly in relation to its significance in both natural and agroecosystems.[2] The potentiality of sunflower (*Helianthus annuus*) as source of allelochemicals is well known.[3] Laboratory Bioassays of leaf aqueous extract from different cultivated sunflower varieties from Andalusia region (Spain) have shown strong inhibition on germination and root length of different crop seeds, as well as stimulatory effects on shoot length.

The chemical constitution of the aerial parts of *Helianthus annuus* L. has been investigated previously.[4] In addition to various sesquiterpene lactones, the dominant secondary metabolites of the genus,[5] flavonoids aglycones,[6] diterpenes,[7] coumarins[8] and other derivatives of the phenylpropanoid[9] pathway have been reported. In a recent publication the isolation of three new bisabolen-type compounds located in noncapitate glandular hairs have been described.[10] In continuation of our systematic allelopathic activity studies of the different varieties of cultivar sunflowers, we now report the isolation and structure elucidation of heliannuol A (2), a sesquiterpene that contain a previously unknown skeleton, heliannuol.

Extraction of fresh leaf aqueous extract of *Helianthus annuus* L. var. SH-222 with CH_2Cl_2 afforded, after chromatography the mentioned compound. Heliannuol A was obtained as a colorless crystal from $CHCl_3$, mp 80-81°C, $[\alpha]_D$ -55.4° (*c* 0.3, CH_3OH).[11] The mass spectrum suggested a sesquiterpene with five unsaturations structure with a molecular ion at m/z 250, $C_{15}H_{22}O_3$. The IR spectrum showed absorptions at 3406 (hydroxyl group), 1605 (aromatic C=C) and 1124 cm^{-1} (C-O-C asymmetric stretching). The presence of the additional peak in the mass spectrum m/z 232 $[M - H_2O]^+$ and the observed absorptions in the IR spectrum and the absence of those of a carbonyl group confirm the presence of two hydroxyl groups and an ether function.

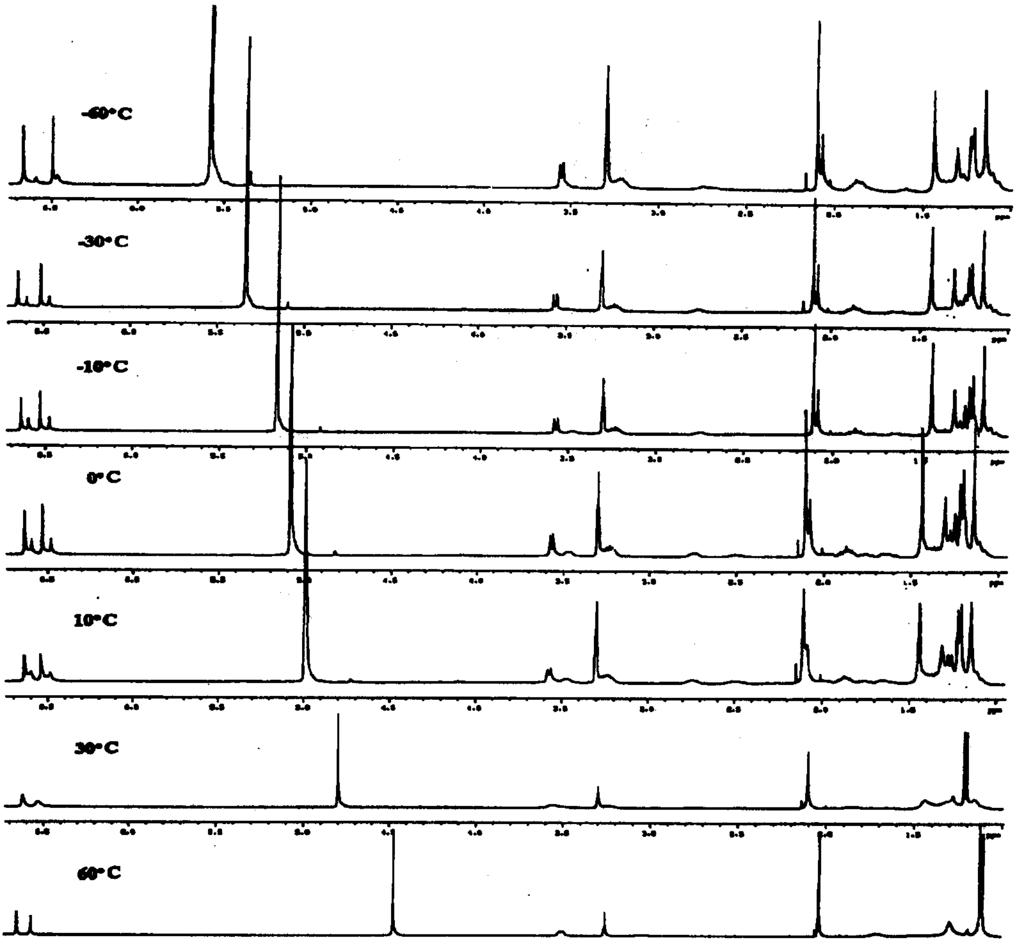


Figure 1. Temperature dependence of ^{1}H-NMR spectrum of heliannuol A.

The room temperature ^{1}H-NMR spectrum in $CDCl_3$ showed broad signals in the aromatic protons area, δ6.71 and δ6.53, corresponding to a 1,2,4,5-tetrasubstituted benzenic ring. A very broad doublet signal appeared at δ3.65 and three singlet signals that integrated for 3 protons at δ2.17, 1.27 and 1.24; corresponding with an aromatic methyl group and a *gem*-dimethyl group on an oxygenated carbon. There are other signals with an undefined multiplicity patterns. The corresponding spectrum in benzene showed a similar resolution. These data suggested the existence of a conformational equilibrium at room temperature. Experiences at different temperatures (-60°C, -30°C, -10°C, 0°C, 10°C, 30°C and 60°C, Fig. 1) using deuterated methanol as solvent, were performed to confirm this fact.

Heliannuol A at 0°C shows a well-resolved spectrum, that remains essentially invariant down to -10°C. Bellow this temperature the system appears to exist as two major conformers. The more abundant one showed two singlet signals (δ6.65 and 6.51) that confirmed the aromatic ring substitution; a broad doublet signal (δ3.56) assigned to a proton attached to a hydroxylic carbon,

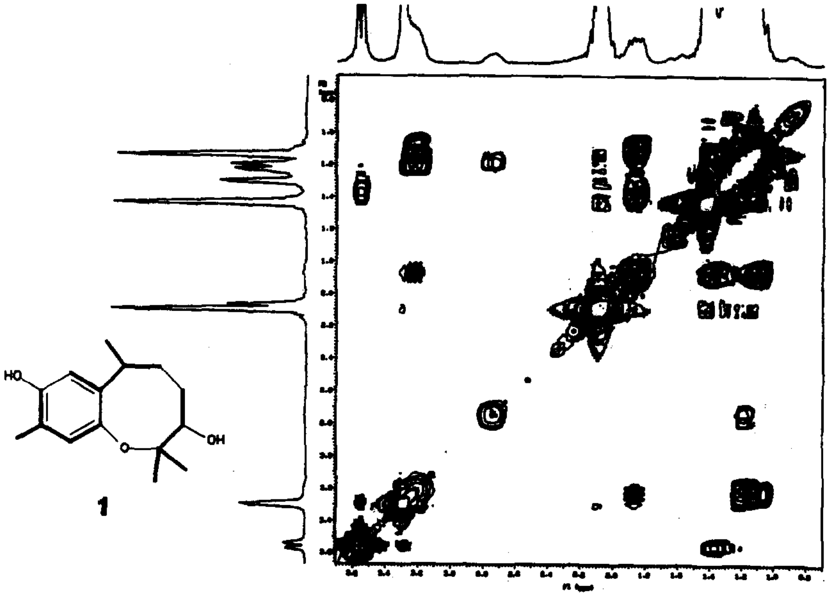
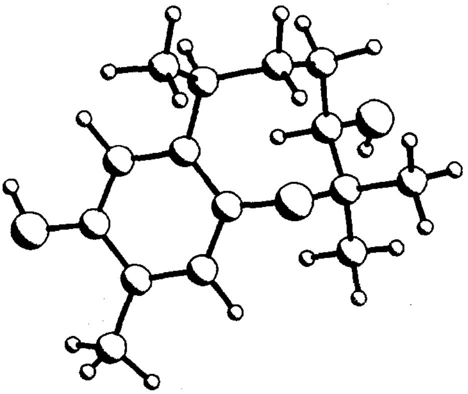
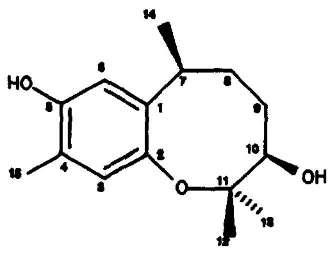


Figure 2. Aliphatic region ^{1}H-NMR COSY 2D spectrum of heliannuol A at -30°C.

coupled with a methylene group (δ1.37, 2H, m); a broad doublet quartet at δ3.25 corresponding to a benzylic proton coupled with a methyl group (δ1.22, 3H, d) and a methylene group that appeared at δ1.87 as a multiplet; and three singlet signals, 3H each, at δ2.11, 1.44 and 1.15.

After a thorough study of ^{1}H-NMR COSY 2D experiment at -30°C (Fig. 2) and application of the isoprene rule, we proposed structure 1 for heliannuol A. It was not possible to assign the relative stereochemistry of carbons 7 and 10 by NMR, and it was established as 7-S, 10-R, by single-crystal X-ray diffraction analysis, structure 2.[12]





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This compound is of particular interest since it is the first member of a new class of bioactive sesquiterpene, for which we suggest the name heliannuol. Based on their bioassay results[3c], it is likely to be significantly involved in the allelopathic action of cultivar sunflower. Its biogenesis may proceed through a bisabolene-type precursor like the recently reported from glandular trichomes of *Helianthus annuus* L.[10]

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11.- Compound 1: ^{1}H-NMR (399.952 MHz, CD_3OD, 30°C) δ: 6.62 (1H, H-3, s); 6.51 (1H, H-6, s); 3.54 (1H, H-10, m); 3.21 (1H, H-7, m); 2.10 (3H, H-15, s); 1.87 (2H, H-8, bs); 1.43 (3H, H-14, bs); 1.30 (2H, H-9, m); 1.22 (3H, H-12, s); 1.20 (3H, H-13, s). ^{1}H-NMR (399.952 MHz, CD_3OD, -30°C) δ: 6.65, 6.60 (1H, H-3, s); 6.51, 6.46 (1H, H-6, s); 3.56 bd, 3.47 m (1H, H-10); 3.25 (1H, H-7, d); 2.10, 2.08 (3H, H-15, s); 1.87, 1.65 (2H, H-8, m); 1.22, 1.27 (3H, H-14, d); 1.36 (2H, H-9, m); 1.44, 1.31 (3H, H-12, s); 1.15, 1.31 (3H, H-13, s). $J_{10,9\beta}=7.4$ Hz; $J_{10,9\alpha}=1$ Hz; $J_{14,7}=7$ Hz, $J_{7,8\beta}=7$ Hz; $J_{7,8\alpha}=1$ Hz. ^{13}C-NMR (100.577 MHz, CD_3OD, -30°C) δ:151.00 (C-2), 139.66 (C-1), 126.21 (C-5), 115.38 (C-4), 115.31 (C-3), 114.71 (C-6), 79.71 (C-11), 74.70 (C-10), 51.57 (C-7), 36.01 (C-8), 26.67 (C-9), 29.64 (C-12*), 29.61 (C-13*), 26.32 (C-14). *May be interchanged.

12.- X-ray data for compound 1:

Bonds lengths (Angström) with e.s.d. 's in parentheses

0_1-C_2	1.401(3)	0_1-C_{12}	1.477(4)	0_2-C_5	1.376(3)	0_2-H_20	0.84(3)	0_3-C_{10}	1.430(4)
0_3-H_30	0.95(4)	C_1-C_2	1.386(4)	C_1-C_6	1.385(4)	C_1-C_7	1.515(4)	C_2-C_3	1.377(4)
C_3-C_4	1.384(4)	C_4-C_5	1.402(4)	C_4-C_{15}	1.501(4)	C_5-C_6	1.381(4)	C_7-C_8	1.472(6)
C_7-C_{14}	1.480(1)	C_8-C_{14}	1.461(8)	C_{14}-C_{10}	1.519(5)	C_{10}-C_{11}	1.523(5)	C_{11}-C_{13}	1.519(5)
C_{11}-C_{12}	1.519(5)								

Bond angles (deg.) with e.s.d. 's in parentheses

C_2-0_1-C_{11}	118.7(3)	C_5-0_2-H_20	106.0(2)	C_{10}-0_3-H_30	114.0(2)	C_2-C_1-C_6	116.4(3)
C_2-C_1-C_7	125.5(3)	C_6-C_1-C_7	117.9(3)	0_1-C_2-C_1	119.8(3)	0_1-C_2-C_3	119.2(3)
C_1-C_2-C_3	120.8(3)	C_2-C_3-C_4	123.1(3)	C_3-C_4-C_5	116.3(3)	C_3-C_4-C_{15}	122.3(3)
C_5-C_4-C_{15}	121.3(3)	0_2-C_5-C_4	118.0(3)	0_2-C_5-C_6	121.8(2)	C_4-C_5-C_6	120.1(3)
C_1-C_6-C_5	123.2(3)	C_1-C_7-C_8	119.6(3)	C_1-C_7-C_{14}	109.2(4)	C_8-C_7-C_{14}	110.1(5)
C_7-C_8-C_9	127.7(5)	C_8-C_9-C_{10}	120.2(3)	0_3-C_{10}-C_9	106.5(3)	0_3-C_{10}-C_{11}	109.7(2)
C_9-C_{10}-C_{11}	116.2(3)	0_1-C_{11}-C_{10}	108.4(2)	0_1-C_{11}-C_{13}	109.7(3)	0_1-C_{11}-C_{12}	104.0(3)
C_{10}-C_{11}-C_{13}	111.3(3)	C_{10}-C_{11}-C_{12}	112.5(3)	C_{13}-C_{11}-C_{12}	110.6(3)		

Complete tables of distances, angles, torsion angles, least-squares planes, H-atom parametres, anisotopic thermal parametres and structure factors have been deposited with the British Library Document Supply Centre as Supplementary Publication. Copies may be obtained through the Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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