Pergamon

Tetrahedron Letters 39 (1998) 427–430

TETRAHEDRON
LETTERS

Heliespirone A. The First Member of a Novel Family of Bioactive Sesquiterpenes[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.

Received 22 September 1997; revised 27 October 1997; accepted 31 October 1997

Abstract: (-)-Heliespinore A, the first member of a novel family of bioactive sesquiterpenes, a potential allelopathic agent, has been isolated from cultivar sunflowers var. SH-222® (*Helianthus annuus L.*). The structure displays an unusual previously unknown spirosesquiterpene skeleton of Heliespirane. The structure elucidation of Heliespinone A was performed by homo- and hetero- nuclear 2D-NMR spectral data. On the basis of combined studies of the theoretical conformations and NOEDIFF data its relative stereochemistry is proposed. © 1997 Elsevier Science Ltd. All rights reserved.

The indiscriminate use of herbicides has resulted in a) increasing incidence of resistance in weeds to some herbicide classes such as triazines and dinitroanilines b) shifts in weed population to species that are more closely related to the crop they infest c) environmental pollution and potential health hazards. Allelopathy, an emerging branch of applied sciences which studies biochemical plant-plant and plant-microorganisms interactions, may help in overcoming such problems through development of crop varieties having greater ability to smother weeds, use of natural phytotoxins from plants or microbes as herbicides and use of synthetic derivatives of natural products as herbicides.[2]

Plants have their own defence mechanisms and allelochemicals are, in fact, natural herbicides. One way to use allelopathy in agriculture is through isolation, identification and synthesis of active compounds from allelopathic plant species.

Sunflower species are allelopathic, indeed some of them, as *Helianthus rigidus*, exhibit autotoxicity. Cultivated sunflower (*Helianthus annuus*) has great allelopathic potential and inhibits weed-seedling growth of velvet leaf, thorn apple, morning glory, wild mustard and other weeds.[3] Field studies have demonstrated that the weed biomass is equally reduced in sunflower plots with or without herbicides.[4]

With this concept in mind and with the notion that exist, a wide diversity of skeletal types of allelopathic compounds we have initiated systematic allelopathic activity studies on cultivar sunflowers in order to evaluate their potential as source of allelopathic agents and consequently as natural herbicide templates. We have

---

* E-mail: famacias@uca.es

0040-4039/98/$19.00 © 1997 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(97)10538-X

previously described and characterized, in addition to simple phenolics, triterpenes and steroids,[5] sesquiterpene lactones, mainly germacranolides and guaianolides,[6] flavonoids,[7] diterpenes[8] and a novel family of sesquiterpenes named heliannuols.[9]

Extraction of fresh leaves aqueous extract of *Helianthus annuus L.*var SH-222 with CH_2Cl_2 afforded 9g of crude extract from 4Kg of fresh plant material, that yield, after chromatography, 3 mg of **1** as a yellowish oil ($[\alpha]_D$= -29.0°; c=0.1, $CHCl_3$ 25°). The HRMS spectrum suggested a sesquiterpene with six unsaturations with a molecular ion at m/z 264.1354 $[M]^+$ (calculated 264.1362), in accord with a molecular formula $C_{15}H_{20}O_4$, plus fragments at m/z 249$[M-CH_3]^+$, and 231$[M-CH_3-H_2O]$ $.^+$The IR spectrum showed absorptions at 3406 cm^{-1} (hydroxyl group), 1693 and 1681 cm^{-1} (two carbonyl groups), 1621 cm^{-1} (double bond) and 1249 cm^{-1} (C-O-C asymmetric stretching).

The ^{1}H-NMR spectrum[10] showed a deshielded singlet (H-3; δ6.61s) corresponding to a proton attached to a double bond conjugated with a carbonyl group, and a doublet (H-15; δ1.96; 3H, d, $J_{3,15}$=1.5 Hz) which was assigned to a methyl group attached to double bond. In the 2D-COSY-^{1}H-NMR spectrum the following correlations were observed: H-14 (δ5.06; ddd, $J_{14,14'}$ = 16.8; $J_{7,14}$ = 1.3; $J_{14,8\alpha}$ = 0.7 Hz) with H-14' (δ4.96; dd, $J_{7,14'}$ = 10.0 Hz) and H-7 (δ5.29; ddd, $J_{7,8\alpha}$ = 9.8 Hz); H-7 showed coupling with H-8α (δ2.91; ddd, $J_{8\alpha,9\alpha}$ = 6.4; $J_{8\alpha,9\beta}$ = 12.7 Hz) and H-8α with H-9α (δ1.96; ddd, $J_{9\alpha,9\beta}$ = 12.7; $J_{9\alpha,10}$ = 5.2 Hz) and H-9β (δ2.14; ddd, $J_{9\beta,10}$ =10.6 Hz) and both showed coupling with the signal corresponding to H-10α (δ4.03; dd). Additionally, ^{1}H-NMR spectrum showed the following signals: two doublets corresponding to two geminal protons, H-6 and H-6' (δ3.23; d and δ2.95; d, J= 15.5 Hz) only coupled with each other and two three-proton singlets assigned to two methyl groups H-12 and H-13 (δ1.32 and δ1.09). These data suggested the structure shown in figure 1.

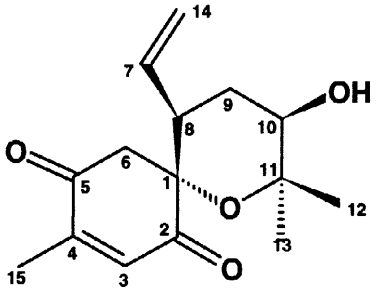

Figure 1. Heliespirone A (**1**).

The ^{13}C-NMR spectrum[11] confirmed this hypothesis with two signals corresponding to two carbonyl carbons C-2 (δ201.5) and C-5 (δ179.5) and four signals in the region of olefinic carbons C-3 (δ137.0), C-4 (δ153.4), C-7 (δ135.3) and C-14 (δ118.4).

Complete assignment of ^{13}C NMR spectrum was made following the observed correlations in the HMQC, HMBC and GHMBC spectra (Figure 2). Those signals corresponding to carbons attached to oxygen have been assigned to C-1 (δ87.6) and C-11 (δ70.1) based on HMBC spectra. These spectra allowed as to correlate the signals belonging to H-6 and H-6' with C-8. These data support the spirane-type nature of C-1.

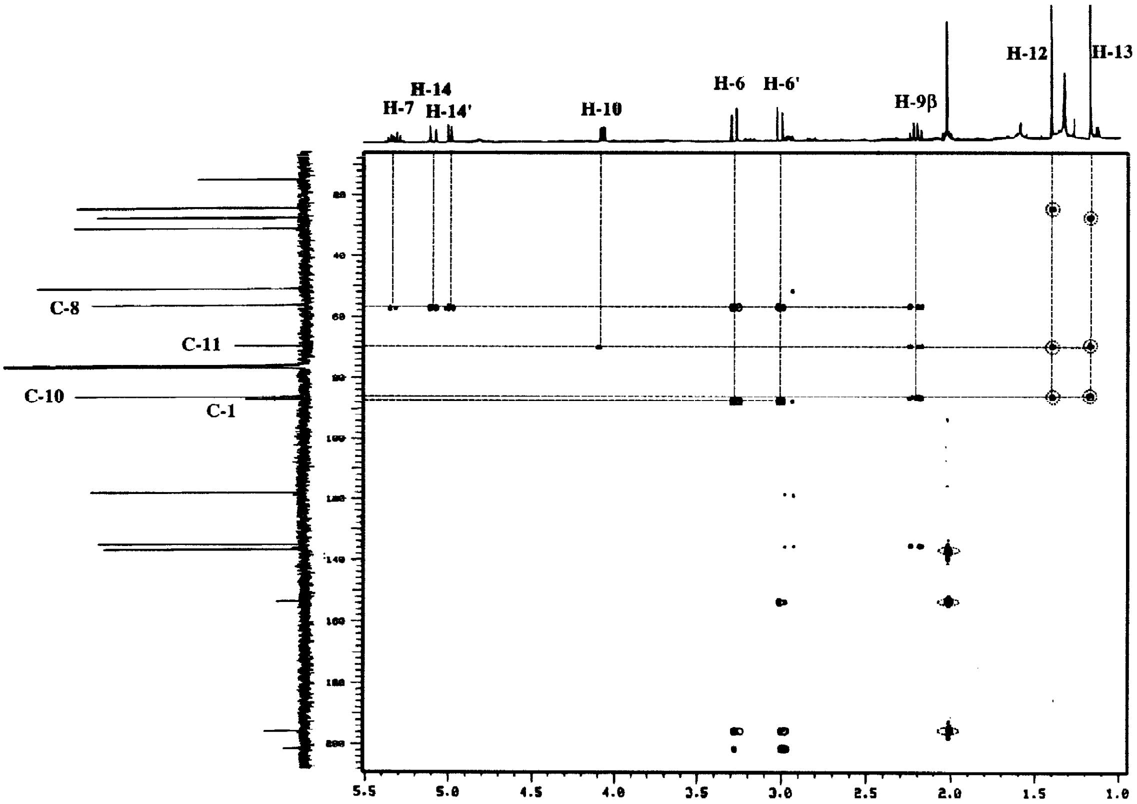

Figure 2. HMBC (60 ms) and GHMBC (110ms, signals with circles) of heliespirone A. H-12 and H-13 may be interchanged.

The relative stereochemistry of the molecule was established by NOE difference experiments. Figure 3 represents the main observed effects by irradiation of signals corresponding to H-7, H-8, H-6 and H-3. These effects are explained by the most stable conformation obtained using semiempirical calculations (PM3)[12] with this stereochemistry. Irradiation of H-8 caused a significant effect on H-10 that indicated that both protons presented the same α-orientation. A clear effect on H-6α was observed when H-10α was irradiated.

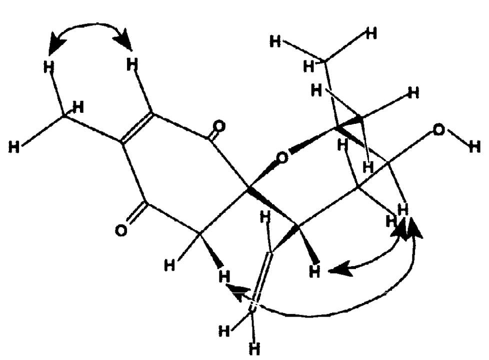

Figure 3. Observed NOEs for the most stable conformer of 1, using PM3 calculations.

This compound is of particular interest since it is the first member of a new class of bioactive sesquiterpenes with potential allelopathic activity, for which we suggest the name Heliespirane. Based on their bioassay results,[13] it is likely to be significantly involved in the allelopathic action of cultivar sunflowers. Its biogenesis may proceed through a bisabolene-type precursor like the recently reported heliannuols,[9] also found in this plant.

Acknowledgements: This research has been supported by the Dirección General de Investigación Científica y Técnica, Spain (DGICYT; Project No. PB95-1231).

REFERENCES AND NOTES

1. Part 11 in the series "Allelopathic studies in cultivar species" for part 10 see Macías, F.A.; Varela, R.M.; Torres, A.; Oliva, R.M. and Molinillo, J.M.G. *Phytochemistry* **1998**, *48*, in press.

2. Macías, F.A.; Varela, R.M.; Torres, A; Molinillo, J.M.G. in Allelopathy in Pests Management for Sustainable Agriculture, Vol 2 (Ed. S.S. Narwal; P. Tauro) Scientific Publishers, Jodhpur, India **1996**, pp. 77-92.

3. Leather, G.R. Plant Soil **1987**, *98*, 17-23.

4. a) Leather, G.R. *J. Chem. Ecol.* **1983**, *9*, 983-989. b) Saggese, E.J.; Foglia,T.A.; Leather, G.; Thompson,M.P.; Bills, D.D.; Hoagland, P.D. in The Chemistry of Allelopathy (Ed. A.C. Thompson) ACS Symposium Series No. 268, Washington D.C., U.S.A. **1985**, pp. 99-112.

5. Macías, F.A.; Varela, R.V.; Torres, A.; Molinillo, J.M.G.; Castellano, D. in Recent Advances on Allelopathy. A Science for The Future, Vol I (Eds. F.A. Macías; J.M.G. Molinillo; J.C.G. Galindo; H.G. Cutler) CAB Publishers, U.K. **1997**, pp. 000-000.

6. a) Macías, F.A.; Varela, R.M.; Torres, A.; Molinillo, J.M.G. *Phytochemistry* **1993**, *34*, 669-674. b) Macías, F.A.; Torres, A., Molinillo, J.M.G.; Varela, R.M.; Castellano, D. *Phytochemistry* **1996**, *43*, 1205-1215.

7. Macías, F.A.; Molinillo, J.M.G.; Torres, A.; Varela, R.M.; Castellano, D. *Phytochemistry* **1997**, *45*, 683-687.

8. Macías, F.A.; Molinillo, J.M.G.; Torres, A.; Varela, R.M. *J. Chem. Ecol* **1997**, *23*, 0000-0000.

9. a) Macías, F.A.; Varela, R.M.; Torres, A.; Molinillo, J.M.G. *Tetrahedron Letters* **1993**, *34*,1999-2002.
   b) Macías, F.A.; Molinillo, J.M.G.; Varela, R.M.; Torres, A. *J. Org. Chem.* **1994**, *59*, 8261-8266.

10. Compound 1: ^{1}H-NMR (399.952 MHz, $CDCl_3$) δ: 6.61(1H, H-3, s); 3.23 (1H, H-6, d); 2.95 (1H, H-6', d); 5.29 (1H, 10H-7, ddd); 2.91 (1H, H-8α, ddd); 1.96 (1H, H-9α, ddd); 2.14 (1H, H-9β, ddd); 4.03 (1H, H-10α, dd); 1.32* (3H, H-12, s); 1.09*(3H, H-13, s); 5.06 (1H, H-14, ddd); 4.96 (1H, H-14', dd); 1.96(3H, H-15, d).* May be interchanged. $J_{3,15}$=1.5 Hz; $J_{6,6'}$=15.5 Hz; $J_{7,8\alpha}$ = 9.8 Hz; $J_{7,14}$= 16.8 Hz; $J_{7,14'}$= 9.8 Hz; $J_{8\alpha,9\beta}$= 12.7 Hz; $J_{8\alpha,9\alpha}$= 6.4 Hz; $J_{9\alpha,9\beta}$= 12.7 Hz; $J_{9\alpha,10\beta}$= 5.2 Hz; $J_{9\beta,10\beta}$ =10.6 Hz; $J_{14,8\alpha}$=0.7 Hz; $J_{14,14'}$=1.3 Hz.

11. Compound 1: ^{13}C-NMR (100.577 MHz, $CDCl_3$) δ: 87.6 (C-1), 201.5 (C-2), 137.0 (C-3), 153.4 (C-4), 179.5 (C-5), 51,8 (C-6), 135.3 (C-7), 57.1 (C-8), 31.9 (C-9), 86.7 (C-10), 70.1 (C-11), 28.3* (C-12), 25.3* (C-13), 118.4 (C-14), 15.9 (C-15). * May be interchanged.

12. Stewart, J.J.P. *J. Comput. Chem.* **1989**, *10*, 209,221. Molecular orbital calculations were carried out using PM3 Hamiltonian as implemented in MOPAC 6.0. Geometries were fully optimized using the PRECISE keyword.

13. Varela, R.M. "Allelochemicals of The Variety Cultivar Sunflower SH-222. Heliannuols and Heliespirones Two Novel Families of Sesquiterpenes". Doctoral Thesis. University of Cádiz, Spain, **1996**.