J Chem Ecol (2008) 34:65–69
DOI 10.1007/s10886-007-9400-4

Helikauranoside A, a New Bioactive Diterpene

Francisco A. Macías · Adriana López · Rosa M. Varela · Ascensión Torres · José M. G. Molinillo

Received: 24 August 2007 / Revised: 10 October 2007 / Accepted: 4 November 2007 / Published online: 11 December 2007

Abstract A new *ent*-kaurane glucoside, named helikauranoside A (**4**), was isolated from the aerial parts of *Helianthus annuus* L. together with three known *ent*-kaurane-type diterpenoids: (−)-kaur-16-en-19-oic acid (**1**), grandifloric acid (**2**), and paniculoside IV (**3**). The structure of 4 was determined by using a combination of 1D (^{1}H-NMR and ^{13}C-NMR) and 2D (COSY, HSQC, and HMBC) NMR techniques. Bioactivity spectra of isolated compounds were tested by using the etiolated wheat coleoptile bioassay in aqueous solutions at concentrations ranging from 10^{-3} to 10^{-6}M. Helikauranoside A (**4**) was the most active (−84%, 10^{-3}M; −56%, 10^{-4}M). These results suggest that this new compound may be involved in defense mechanisms of *H. annuus*.

Keywords Allelopathic agents · Kaurane · Glycoside · Sunflower · Diterpene

Introduction

Chemical studies of *Helianthus annuus* L. (Spring et al. 1981; Melek et al. 1985; Alfatafta and Mullin 1992; Spring et al. 1992; Macías et al. 1993a, b, 1994, 1996) have shown that this species is a rich source of terpenoids, particularly sesquiterpenoids. A wide spectrum of biological activities has been reported for sunflower extracts and components (Beale et al. 1983; Rieseberg et al. 1987; Spring et al. 1991; Ghisalberti 1997). These activities include potential allelopathic effects of extracts from different sunflower cultivars (Macías et al. 1998, 1999a, b).

The isolations of diterpenoids from *H. annuus* L. that have different skeleton types include the following: trachylobanes (Ferguson et al. 1982; Pyrek 1984; Melek et al. 1985); atisane (Morris et al. 2005); gibberellins (Hutchinson et al. 1988); and the kaurenoid carboxilic acids (−)-kaur-16-en-19-oic acid (**1**) (Fig. 1) (Pyrek 1970), grandifloric acid (**2**) (Fig. 1) (Panizo and Rodriguez 1979; Ferguson et al. 1982), angeloylgrandifloric acid, 15β-acetoxy-kaur-16-en-19-oic acid, 15β-*i*-valeroxy-kaur-16-en-19-oic acid, 15β-angeloxy-kaur-16-en-19-oic acid (Ferguson et al. 1982), *ent*-kauran-16α-ol (Morris et al. 2005), 2β,16β-*ent*-kauranediol, and 15α,16α-epoxy-17β-al-*ent*-kaurane-19-oic acid (Suo et al. 2006).

A bioassay-guided fractionation of *H. annuus* L. extracts was carried out to isolate, identify, and characterize the allelopathic constituents. Four kaurene diterpenes were isolated from SH-222 and Stella cultivars of sunflowers. Helikauranoside A (**4**) (Fig. 1) is characterized by a glycosylated kaurane-type skeleton and is described for the first time. The etiolated wheat coleoptile bioassay in aqueous solutions at concentrations between 10^{-3} and 10^{-6}M were used to complete the bioactivity spectrum of the isolated compounds.

F. A. Macías (✉) · A. López · R. M. Varela · A. Torres · J. M. G. Molinillo
Grupo de Alelopatía, Departamento de Química Organica, Facultad de Ciencias, Universidad de Cádiz, C/ República Saharaui s/n, Apdo. 40, 11510 Puerto Real, Cádiz, Spain
e-mail: famacias@uca.es

Methods and Materials

General IR spectra (KBr) were recorded on a Perkin Elmer FT-IR Spectrum 1000, Matton 5020 spectrophotometer. NMR spectra were run on Varian INOVA-400 and Varian INOVA 600 spectrometers. Chemical shifts are given in

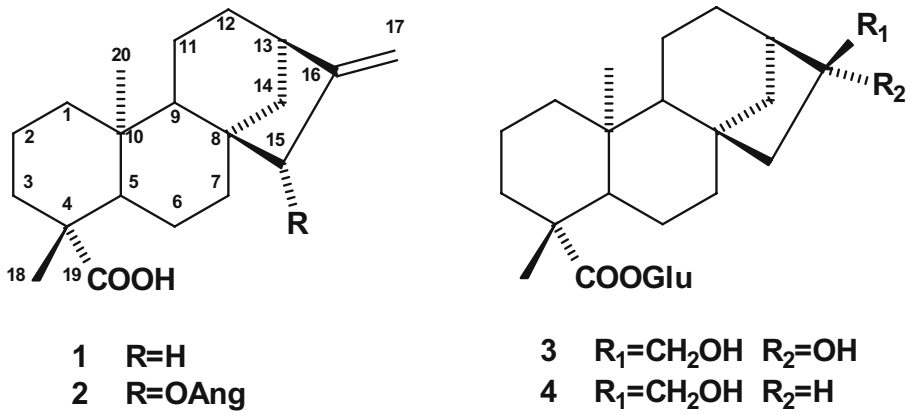

Fig. 1 Isolated diterpenes from *H. annuus* cv. Stella and SH-222

parts per million with respect to residual ^{1}H signals of MeOD-d_4 and py-d_5 (δ 3.30 and 7.55, respectively), as well as ^{13}C with those of the solvent (δ 49.0 and 135.5, respectively). Optical rotations were determined with a Perkin Elmer polarimeter model 241 (on the sodium D line). High-resolution mass spectroscopy (HRMS) was carried out on VG AUTOESPEC mass spectrometer (70eV).

Plant Material Helianthus annuus cv. SH-222 (commercialized by Semillas Pacífico) and Stella were collected during the third plant development stage (Macías et al. 1999c) (i.e., plants were 1.2m tall with flowers, 1month before harvest) and were provided by Rancho de la Merced, Agricultural Research Station (CIFA), Junta de Andalucía, Jerez, Spain.

Extraction and Isolation Fresh leaves of *H. annuus* cv. SH-222 (6kg) were extracted in water (18l) for 24h in the dark at room temperature. The aqueous solution was extracted with CH_2Cl_2 and then with EtOAc at room temperature. The organic layer was removed by reduced pressure evaporation and yielded two extracts of 16g (CH_2Cl_2-W) and 6g (EtOAc-W), respectively. EtOAc-W was chromatographed by using hexane–ethyl acetate mixtures of increasing polarity. The most polar fraction was chromatographed on a reverse-phase column (C-18) with a water–methanol (1:4 to 0:1) solvent system to elute compounds **3** (2mg) and **4** (3mg) (Fig. 1).

Similarly, 4kg of *H. annuus* cv. Stella was extracted and yielded 77g (CH_2Cl_2-W) and 7g (EtOAc-W). CH_2Cl_2-A was chromatographed with hexane–ethyl acetate mixtures of increasing polarity. The fraction eluting with hexane–ethyl acetate (1:1) was chromatographed and yielded two compounds—**1** (28mg) and **2** (1mg).

Known compounds were identified by comparison of their physical and spectroscopical data (m.p., [α], IR, MS, ^{1}H-NMR and ^{13}C-NMR) with those previously reported in the literature. *Helikauranoside A* (**4**) crystalline solid mp 260°C; IR ν_{max} (KBr) cm^{-1}: 3,500 (OH, carboxylic acid) 1,700 (C=O, acid). ^{1}H-NMR data (600MHz) MeOD-d_4: δ 0.95 (s, H-20), 1.20 (s, H-18), 1.91 (m, H-16), 2.17 (m, H-3α), 3.32–3.37 (m, H-4′, H-3′, H-2′), 3.35 (m, 2H, H-17), 3.40 (ddd, 8.0, 1.7, 4.6, H-5′), 3.68 (dd, 11.9, 4.6, H-6′a), 3.81 (dd, 11.9, 1.7, H-6′b), 5.39 (d, 8.1, H-1′); py-d_5: δ 6.26 (d, 7.9, H-1′), δ 4.45 (brd, 11.2, H-6′β), δ 4.38 (brd, 11.2, H-6′α), δ 4.34 (dd, 10, 8.8, H-3′), δ 4.25 (dd, 8.8, 8.8, H-4′), δ 4.22 (dd, 8.1, 7.9, H-2′), δ 3.94 (ddd, 8.8, 4.4, 2.4, H-5′), δ 3.61 (m, 2H, H-17), δ 2.38 (brd, 13.2, H-3a), δ 2.29 (brs, H-13), δ 2.16 (m, H-16), δ 1.79 (brd, 12.3, H-1a), δ 1.61 (dd, 9, 12.1, H-15a), δ 1.44 (m, H-7a), δ 1.27 (s, 3H, H-18), δ 1.22 (s, 3H, H-20), δ 1.08 (dd, 5.1, 12.1, H-15b), δ 0.96 (brd, 8.0, H-3a), δ 0.76 (m, H-7b); ^{13}C NMR data (150MHz): see Table 1; HREIMS *m/s* 482.2884 [M^{+}] (cal. for 482.2880).

Coleoptile Bioassay Wheat seeds (*Triticum aestivum* L. cv. Duro) were sown in Petri dishes (15cm diameter), misted with water, and grown in the dark at 22 ± 1°C for 3days (Hancock et al. 1964). The roots and caryopsis were removed from the shoots. The apical 2mm of the caryopsis was cut off and discarded with a Van der Weij guillotine. The next 4mm of the coleoptiles was removed and used for bioassay. All manipulations were performed under a green safelight (Nitsch and Nitsch 1956). Compounds were predisolved in DMSO and diluted to the final bioassay concentration with a maximum of 0.1% DMSO. Parallel controls treated with water and DMSO were also run at the same concentrations.

Crude extracts, fractions, or pure compounds to be assayed for biological activity were added to test tubes. Assays were run in duplicate. Phosphate–citrate buffer (2ml) containing 2% sucrose (Nitsch and Nitsch 1956) at pH5.6 was added to each test tube. Following the placement of five coleoptiles in each test tube (three tubes per dilution), the tubes were rotated at 0.25rpm in a roller

Table 1 ^{13}C NMR data for compound 4 in deuterated methanol and pyridine

C	$\delta^{13}C$ MeOD-d_4	$\delta^{13}C$ py-d_4
1	42.1 *t*	41.0
2	20.0 *t*	19.3
3	39.1 *t*	38.5
4	45.1 *s*	44.1
5	58.8 *d*	57.5
6	23.5 *t*	22.9
7	43.0 *t*	42.2
8	45.9 *s*	45.0
9	56.9 *d*	55.6
10	40.9 *s*	40.0
11	20.2 *t*	19.6
12	32.5 *t*	
13	39.5 *d*	38.7
14	37.9 *t*	37.2
15	46.3 *d*	45.7
16	44.5 *s*	44.3
17	67.7 *t*	67.1
18	29.1 *q*	28.7
19	178.3 *s*	175.6
20	16.5 *q*	16.0
1′	95.6 *d*	95.8
2′	74.1 *d*	74.1
3′	78.7 *d*	71.1
4′	71.1 *d*	79.2
5′	78.7 *d*	79.4
6′	62.4 *d*	62.1

tube apparatus for 24h in the dark at 22°C. Coleoptiles were measured following digitalization of their images. Data were statistically analyzed by using Welch's test (Martín Andrés and Luna del Castillo 1990). Data are presented as percentage differences from control. Thus, zero represents the control, positive values represent stimulation of the studied parameter, and negative values represent inhibition.

Results and Discussion

Fresh *H. annuus* cv SH-222 leaves were extracted in water in the dark at room temperature. The aqueous solution was extracted with CH_2Cl_2 and then with EtOAc. Following chromatography, the EtOAc extract was separated into eight fractions. Fraction H contained the compounds paniculoside IV (**3**) (2mg) and helikauranoside A (**4**) (3mg) described for the first time in this report (Fig. 1). *Helianthus annuus* cv. Stella was extracted similarly. The fraction eluting with hexane-EtOAc (1:1) yielded compounds **1** (28mg) and **2** (1mg). The spectroscopic data for **1**, (Pyrek 1970; Ohno et al. 1979) **2** (Panizo and Rodriguez 1979; Herz et al. 1983), and **3** (Fig. 1)(Ohno and Mabry 1980) were identical to those previously reported.

Table 2 Selected ^{13}C NMR data for α- and β-isomers of aglycone in $CDCl_3$ and those for compound 4 in methanol

C	α-CH_2OH	β-CH_2OH	4
12	20.0	31.4	32.5
13	36.9	38.1	39.5
15	44.2	45.0	46.3
17	64.2	67.4	67.7

Helikauranoside A (**4**) was isolated as white crystals (mp 260°C) with $[\alpha]_D^{25}$ −6.4 (*c* = 0.14) from fraction H. The molecular formula was deduced to be $C_{26}H_{42}O_8$ on the basis of HRMS that showed an ion peak $[M]^+$ at *m/z* 482.2884. Intense absorption bands at 3,500 and 1,700cm^{-1} were observed in the IR spectrum of **4**, in accordance with the presence of hydroxyl and carbonyl functions, respectively. The ^1H NMR spectrum of **4** was run in methanol (CD_3OD) and pyridine (C_5D_5N). Both spectra exhibited signals for a β-glucopyranosyl moiety in the structure [CD_3OD: δ 5.39 (H-1′, d, *J* = 8.1), δ 3.81 (H-6′β, dd, *J* = 11.9, 1.7), δ 3.68 (H-6′α, dd, *J* = 11.9, 4.6), δ 3.40 (H-5′, ddd, *J* = 8.0, 4.6, 1.7), δ 3.32–3.37 (H-2′, H-3′, H-4′, m); C_5D_5N: δ 6.26 (H-1′, d, *J* = 7.9), δ 4.45 (H-6′β, brd, *J* = 11.2), δ 4.38 (H-6′α, brd, *J* = 11.2), δ 4.34 (H-3′, dd, *J* = 10, 8.8), δ 4.25 (H-4′, dd, *J* = 8.8, 8.8), δ 4.22 (H-2′, dd, *J* = 8.1, 7.9), δ 3.94 (H-5′, ddd, *J* = 8.8, 4.4, 2.4)] (Agrawal 1992; Rakotondraibe et al. 2002; Harinantenaina et al. 2002a, b). This was confirmed by signals in the ^{13}C NMR spectrum [CD_3OD: δ 95.6 (C-1′), δ 78.7 (C-3′), δ 78.7 (C-5′), δ 74.1 (C-2′), δ 71.1 (C-4′), δ 62.4 (C-6′)].

Additionally, the ^1H NMR in methanol exhibited a signal for the protons of two methyl groups at δ 1.20 (3H, s, H-18) and δ 0.95 (3H, s, H-20). The H-17 protons resonate in MeOD at δ 3.35 (2H, m) and suggest that **4** is an ent-kaurane-type diterpene glycoside. The ^{13}C NMR and HSQC spectra of **4** showed the presence of two methyls, 11 methylenes, nine methines, and four quaternary carbons

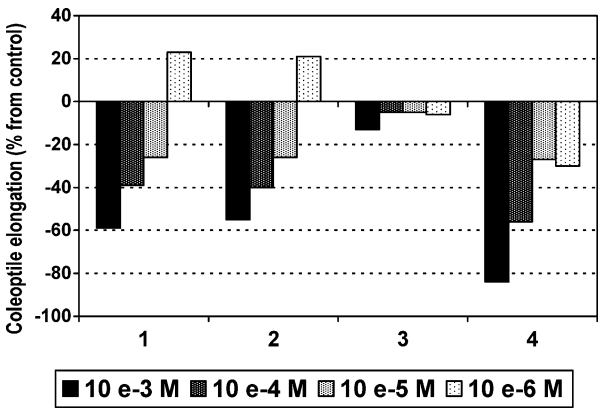

Fig. 2 Bioactivities of compounds **1**–**4** in wheat coleoptile bioassay

containing an ester carbonyl group. We found analogies in the chemical shifts of signals from the ^{1}H NMR and ^{13}C NMR spectra of **4** with those previously described for the epimeric diterpenes at C-16 that correspond to the aglycone (−)-17-hydroxy-kauran-19-oic acid (Table 1). On the basis of the stereochemical studies performed by Yang et al. (2002), the chemical shift of H-17 in methanol (δ 3.35) suggested a β-orientation for the hydroxymethylene moiety (δ 3.40 and 3.72 of the aglycone in $CDCl_3$ for the β and α isomers, respectively). This was confirmed by comparison of the chemical shifts observed in ^{13}C NMR spectrum with those found for the both isomers described by Wu et al. (1996; see Table 2). According to the chemical shift observed for C-17 in the pyridine-d_5 spectrum (δ 67.7), the β-glucopyranosyl moiety must be attached to the carboxylic ester at C-19; otherwise, a glycosylic moiety attached at C-17 should resonate between δ 74 and 75ppm (Shao et al. 1996). These data allow us to propose the structure *O*-β-D-glucopyranosyl-17-hydroxy-16β-kauran-19-oate. This is the first report of this structure, which was named helikauranoside A (**4**).

Bioactivity—Coleoptiles Bioassay There are many antecedents of bioactivity of kaurane diterpenes. Some of them are for compounds described in this report or for structures related to them. Thus, (−)-kaur-16-en-19-oic acid (**1**) has an inhibitory effect on the growth of *Bacillus subtilis* and *Staphylococus aureus* (Phan et al. 2005) and has been tested on the aggregation of Washed Rabbit Platelets, showing complete inhibition induced by collagen at 200μM (Yang et al. 2002). This compound was active in the brine shrimp test (BST LC_{50} 16μg/ml) and was selective for MCF-7 (breast cancer, ED_{50} 1.0μg/ml) cells among six human solid tumor cell lines (Majekodunmi and Oladimeji 1996). Compound **1** and the aglycone of **4** have strong inhibitory effects against cyclooxygenase (COX) (Phan et al. 2005).

The aglycone of **3** gave 46% and was inhibited by HIV reverse transcriptase at a concentration of 33μg/ml (Chang et al. 1998). Epimers at C-16 inhibited HIV replication in H9 lymphocyte cells with EC_{50} of 0.8μg/ml (Wu et al. 1996).

To complete the bioactivity spectrum of the isolated compounds, isolated compounds were tested with the etiolated wheat coleoptile bioassay (Hancock et al. 1964) in a range of 10^{-3}–10^{-6}M. This bioassay has been used to estimate plant growth regulation, herbicide, or phytotoxic activities (Cutler 1984).

The growth of etiolated wheat coleoptiles (Fig. 2) was inhibited ($P<0.01$) by solutions of **1** (−59%, 10^{-3} M; −39%, 10^{-4} M), **2** (−55%, 10^{-3} M; −40%, 10^{-4} M), and **4** (−84%, 10^{-3} M; −56%, 10^{-4} M). The most active compound was **4**, whereas compounds **1** and **2** presented similar activities, but **3** did not show any significant effect.

Chemical differences among diterpenes **1**, **2**, **3**, and **4**, are centered at C-16. Therefore, the differences in the activity must be explained by the different substitutions in this position. Compound **4** has a hydroxymethylene group at C-16 and is highly active, whereas the presence of an additional hydroxyl group (compound **3**) decreases activity dramatically. On the other hand, if a double bond (between C-16 and C-17) is present instead of the hydroxymethylene group, activity also decreases (compounds **1** and **2**). Other positions in the kaurane skeleton seem to be less important in relation to activity, such as those compounds that present an angelate at C-15 or a sugar at C-19. The presence of angelate at C-15 only changes the activity from slightly stimulatory to inhibitory at the lowest concentration (compound **2** vs. compound **1**).

Although diterpenoids are not usually reported as phytotoxic agents, in this case, the most active compound is the glycosylated diterpenoid **4**. The ecological role of these compounds has been associated more with antifeedant, insecticidal, and deterrent activity. Moreover, diterpenoids as giberrellins act as important plant hormones involved in growth regulation (Macías et al. 2007). In contrast, the glycosidic form of active natural products is less active than the aglycones (Macías et al. 2005). Unfortunately, the lack of sufficient quantities of compound did not allow us to hydrolyze it and to bioassay the aglycone to compare their activities.

These results suggest that compound **4** should be involved in defense mechanisms of *H. annuus*. The shown activity indicates that its role may be related with the allelopathic behavior shown by this species.

Acknowledgement This research was supported by the *Ministerio de Ciencia y Tecnología*, Spain (MCYT; Project No. AGL2005–05190). A.L. acknowledges a fellowship from *La Agencia Española de Cooperación Internacional*, *Ministerio de Asuntos Exteriores*, Spain.

References

AGRAWAL, P. K. 1992. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. *Phytochemistry* 31:3307–3330.

ALFATAFTA, A. A., and MULLIN, C. A. 1992. Epicuticular terpenoids and an aurone from flowers of *Helianthus annuus*. *Phytochemistry* 31:4109–4113.

BEALE, M. H., BEARDER, J. R., MACMILLAN, J., MATSUO, A., and PHINNEY, B. O. 1983. Diterpene acids from *Helianthus* species and their microbiological conversion by *Gibberella fujikurol* mutant B1-41 a. *Phytochemistry* 22:875–881.

CHANG, F., YANG, P., LIN, J., LEE, K., and WU, Y. 1998. Bioactive kaurane diterpenoids from *Annona glabra*. *J. Nat. Prod.* 61:437–439.

CUTLER, H. G. 1984. Fresh look at the wheat coleoptile bioassay, pp. 1–9, in Proceedings of the 11th Annual Meeting of the Plant Growth Regulator Society of America. PGRSA, Boston, MS.

FERGUSON, G., MCCRINDLE, R., MURPHY, S. T., and PARVEZ, M. 1982. Further diterpenoid constituents of *Helianthus annuus* L. Crystal and molecular structure of methylent-15b-hydroxytrachyloban-19-oate. *J. Chem. Res. Synop.* 8:200–201.

GHISALBERTI, E. L. 1997. The biological activity of naturally occurring kaurene diterpenes. *Fitoterapia* 68:303–325.

HANCOCK, C. R., BARLOW, H. W., and LACEY, H. J. 1964. The east malling coleoptiles straight growth test method. *J. Exp. Bot.* 15:166–176.

HARINANTENAINA, R., KASAI, R., and YAMASAKI, K. 2002a. A new e*nt*-kaurane diterpenoid glycosides from the leaves of *Cussonia bojeri*, a Malagasy endemic plant. *Chem. Pharm. Bull.* 50:1122–1123.

HARINANTENAINA, R., KASAI, R., and YAMASAKI, K. 2002b. *Ent*-kaurane diterpenoid glycosides from a Malagasy endemic plant, *Cussonia vantsilana*. *Phytochemistry* 61:367–372.

HERZ, W., KULANTHAIVEL, P., and WATANABE, K. 1983. *Ent*-kauranes and other constituents of three *Helianthus* species. *Phytochemistry* 22:2021–2025.

HUTCHINSON, M., GASKIN, P., MACMILLAN, J., and PHINNEY, B. O. 1988. Gibberellins in seeds of *Helianthus annuus*. *Phytochemistry* 27:2695–2701.

MACÍAS, F. A., VARELA, R. M., TORRES, A., and MOLINILLO, J. M. G. 1993a. Novel sesquiterpenes from bioactive fractions of cultivar sunflowers. *Tetrahedron Lett.* 34:1999–2002.

MACÍAS, F. A., VARELA, R. M., TORRES, A., and MOLINILLO, J. M. G. 1993b. Potential allelopathic guaianolides from cultivar sunflowers leaves var. SH-222. *Phytochemistry* 34:669–674.

MACÍAS, F. A., MOLINILLO, J. M. G., VARELA, R. M., and TORRES, A. 1994. Structural elucidation and chemistry of a novel family of bioactive sesquiterpenes: heliannuols. *J. Org. Chem.* 59:8261–8266.

MACÍAS, F. A., TORRES, A., MOLINILLO, J. M. G., VARELA, R. M., and CASTELLANO, D. 1996. Potential allelopathic sesquiterpene lactones from sunflower leaves. *Phytochemistry* 43:1205–1215.

MACÍAS, F. A., VARELA, R. M., TORRES, A., OLIVA, R. M., and MOLINILLO, J. M. G. 1998. Bioactive norsesquiterpenes from *Helianthus annuus* with potential allelopathic activity. *Phytochemistry* 48:631–636.

MACÍAS, F. A., MOLINILLO, J. M. G., GALINDO, J. C. G., VARELA, R. M., TORRES, A., and SIMONET, A. M. 1999a. Terpenoids with potential use a natural herbicide templates, pp. 15–31, in H. G. Cutler, and S. J. Cutler (eds.). Biologically Active Natural ProductsAgrochemicals CRC Press LLC, Boca Raton, FL.

MACÍAS, F. A., VARELA, R. M., TORRES, A., and MOLINILLO, J. M. G. 1999b. Bioactive compounds from the genus *Helianthus*, pp. 121–134, in F. A. Macías, J. C. G. Galindo, J. M. G. Molinillo, and H. G. Cutler (eds.). Recent Advances in Allelopathy, vol I. A Science for the FutureServicio de Publicaciones, Universidad de Cádiz, Cádiz.

MACÍAS, F. A., VARELA, R. M., TORRES, A., and MOLINILLO, J. M. G. 1999c. Potentiality of cultivar sunflowers (*Helianthus annuus* L.) as source of natural herbicide templates, pp. 531–550, in Inderjit, K. M. M. Dakshini, and C. L. Foy (eds.). Principles and Practices in Plant Ecology. Allelochemical InteractionsCRC Press LLC, Boca Raton, FL.

MACÍAS, F. A., MARIN, D., OLIVEROS-BASTIDAS, A., CASTELLANO, D., SIMONET, A. M., and MOLINILLO, J. M. G. 2005. Structure–Activity Relationships (SAR) studies of Benzoxazinones, their degradation products and analogues. Phytotoxicity on Standard Target Species (STS). *J. Agric. Food Chem.* 53:538–548.

MACÍAS, F. A., MOLINILLO, J. M. G., VARELA, R. M., and GALINDO, J. C. G. 2007. Allelopathy—a natural alternative for weed control. *Pest Manag. Sci.* 63:327–348.

MAJEKODUNMI, O. F., and OLADIMEJI, T. A. 1996. Bioactive *ent*-kaurane diterpenoids from *Annona senegalensis*. *J. Nat. Prod.* 59:301–303.

MARTÍN ANDRÉS, A., and LUNA DEL CASTILLO, J. DE D. 1990. Bioestadística para las Ciencias de la Salud, . rd ed. 3rd edn. Norma, Madrid.

MELEK, F. R., GAGE, D. A., GERSHENZON, J., and MABRY, T. J. 1985. Sesquiterpene lactones and diterpene constituents of *Helianthus annuus*. *Phytochemistry* 24:1537–1539.

MORRIS, B. D., FOSTER, S. P., GRUGEL, S., and CHARLET, L. D. 2005. Isolation of the diterpenoids, *ent*-atisan-16α-ol, from sunflowers, as oviposition stimulants for the banded sunflower moth, *Cochylis hospes*. *J. Chem. Ecol.* 31:89–102.

NITSCH, J. P., and NITSCH, C. 1956. Studies on the growth of coleoptiles and first internodes sections. A new sensitive, straight-growth test for auxins. *Plant Physiol.* 31:94–111.

OHNO, N., and MABRY, J. T. 1980. Sesquiterpene Lactones and diterpene carboxylic acids in *Helianthus* niveussubspecies *Canescens*. *Phytochemistry* 19:609–614.

OHNO, N., MABRY, J. T., ZABEL, V., and WATSON, W. H. 1979. Tetrachirin a new rearraged kaurenoid lactone, and diterpene acids from *Tetrachyron orizabaensis* and *Helianthus debilis*. *Phytochemistry* 18:1687–1689.

PANIZO, F. M., and ODRIGUEZ, B. 1979. Algunos componentes diterpenicos del girasol. *An. Quím.* 75:428–430.

PHAN, V. K., MINH, V., NGUYEN, T. D., LEE, J. J., and KIM, Y. H. 2005. Bioactivities of compounds isolated from *Acanthopanax trifoliatus*. *Tap Chi Hoa Hoc* 43:51–55.

PYREK, J. S. 1970. New pentacyclic diterpene acid. Trachylobane-19-oic acid from sunflowers. *Tetrahedron* 26:5029–5032.

PYREK, J. S. 1984. Neutral diterpenoids of *Helianthus annuus*. *J. Nat Prod.* 47:822–827.

RAKOTONDRAIBE, L., HARINANTENAINA, R., KASAI, R., and YAMASAKI, K. 2002. *Ent*-kaurane diterpenoid glycosides from the leaves of *Cussonia racemosa*, a Malagasy endemic plant. *Chem. Pharm. Bull.* 50:268–271.

RIESEBERG, L. H., SOLTIS, D. E., and ARNOLD, D. 1987. Variation and localization of flavonoid aglycones in *Helianthus annuus* (Compositae). *Am. J. Bot.* 74:224–233.

SHAO, Y., BAI, N., and ZHOU, B. 1996. Kaurane glycosides from *Inula britannica*. *Phytochesmitry* 42:783–786.

SPRING, O., ALBERT, K., and GRADMANN, W. A. 1981. A new biologically active germacranolide from *Helianthus annuus*. *Phytochemistry* 20:1883–1885.

SPRING, O., BENZ, A., and FAUST, V. 1991. Impact of downy mildew (*Plasmopara halstedii)* infection on the development and metabolism of sunflower. *J. Plant Dis. Protect.* 98:597–604.

SPRING, O., ODON, U., and MACÍAS, F. A. 1992. Sesquiterpenes from noncapitate glandular trichomes of *Helianthus annuus*. *Phytochemistry* 31:1541–1545.

SUO, M. R., YANG, J. S., LU, Y., WU, L., and ZHENG, Q. T. 2006. Two new diterpenes from *Helianthus annuus* L. *Chin. Chem. Lett.* 17:45–48.

WU, Y., HUNG, Y., CHANG, F., COSENTINO, M., WANG, H., and LEE, K. 1996. Identification of *ent*-16β,17.dihydroxykauran-19-oico acid an anti-HIV principle and islation of the new diterpenoids annosquamosins A and B from *Annona squamosa*. *J. Nat. Prod.* 59:635–637.

YANG, Y., CHANG, F., WU, C., WANG, W., and WU, Y. 2002. New *ent*-kaurane diterpenoids with anti-platelet aggregation activity from *Annona squamosa*. *J. Nat. Prod.* 65:1462–1467.