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Determination of root growth inhibitory activity produced by diterpene isolated from *Azorrella biloba*

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ABSTRACT

Bioassay-guided fractionation of a hexane extract obtained from the leave of *Azorellabiloba* led to the isolation of the diterpene natural product mulen-11,13-dien-20-oico **(1)**. Inhibitory activity root growth was tracked using a *Ipomea purpurea* and *zea mais*.

KEYWORDS

Azorella biloba;
Apeaceae;
Inhibitory activity root growth;
Falcarindiol;
Mulen-11;
13-dien-20-oico;
Ipomea purpurea;
Zeamais.

INTRODUTION

Maximizing the world's agricultural efficiency depends largely on controlling a variety of diseases and pests- especially weeds. The weeds control research over the last 50 year has been focused almost exclusively on synthetic herbicides[7]. The development of genetically engineered herbicide resistant crops has further expanded the use of herbicides. Widespread use of synthetic herbicides has resulted in herbicide-resistant weeds, and public concerns over the impact synthetic herbicides have on human health and the environments are increasing[10]. These concerns are shifting attention to alternative weed control technologies based on natural products[1].

Plants are known to produce secondary metabolites that affect germination and growth of other plants. This is one of a variety of ways in which certain plant can reduce interspecies competition in their natural habitats. Some of these compounds may play an important role in chemical mediation of growth and development of natural plant communities[2-4,14,15].

Allelopathy is commonly defined as any direct or indirect effect by one plant, including microorganisms, on another through the production of chemical compounds released into the environment. It includes both inhibitory and stimulative reciprocal biochemical interactions.Nowadays, allelopathic agents have received a considerable amount of attention due to the agricultural potential of these compounds as environmentally-benign herbicides[13,16].

The weed control technologies based on natural

products meets with a wide variety of compound. Many of these they don't present an evaluation of their allelopathic potential.

Among them we find the compoundsditerpene, of which, there are not many references about on yours activity[5,6,11,12]

Quassinoioids are an example of naturally occurring diterpenephytotoxins. These compounds have been associated with anticancer, anti malarial, antileukemic, antitubercular, antiviral, insecticidal, fungicidal, and herbicidal activities[4]. Hailanthone, a chaparrinone-type quassinoid, has broad weed spectrum activity when applied either as preemergence or postemergence herbicide and provides 100% weed control of green foxtail (*Setariaviridis*) and sicklepod (*Cassia obtusifolia*) at rates of 0.125 kg/ha Duke *et al.*, 2000b[4].

In our study in search of new native substances with biological activity especially in front of plagues that affect the cultivations, carry out the bioassay-guided purification of the metabolites of *Azorella biloba* (Apiaceae).

Azorella is a South American genus of the Apiacea (Umbellifereae) represented by 26 species growing in Andean Mountains and Patagonia, Argentina, respectively. *Azorella biloba* is one of the species that grow in the county of Córdoba.

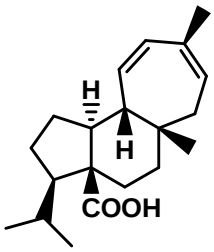
Two metabolites were isolated and identified. Falcarindiol[8] characteristic poliacetilene in the members of the family Apiacea and whose biological activity has been broadly studied.

Mulen-11,13-dien-20-oico **(1)** isolated previously of *A. compact*[9]. **(1)** showed phytotoxic differentiated activity against dicotyledoneous overgrowths

RESULTS AND DISCUSSION

Dry leave of *Azorella biloba* were extracted with EtOH. The water suspension of the original EtOH extract was subjected to liquid-liquid partition to obtain hexane, dichloromethane and EtOAc. Comparison of the inhibitory activity on *Ipomeapur purea* and *zeamais* root growth (total activity) revealed that the major activity of the original extract was fractionated into the hexane fractions (Figure 1A, B).

The bioactive hexane fraction was subjected to column chromatography and radial chromatography.



(1)

Two metabolites were isolated. Falcarindiol, a poliacetilene, whose biological activity has been broadly studied and mulen-11,13-dien-20-oico **(1)**. The full proton and carbon NMR spectral assignments for thismetabolites were performed using a combination of ^{1}H, ^{13}C-NMR 1D experiments and COSY, HETCORR and NOESY 2D experiments.

The compound **(1)** accounted of the activity ob-

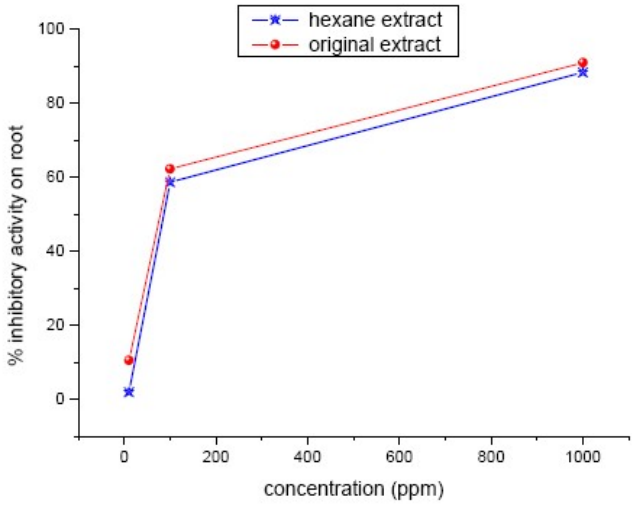


Figure 1A

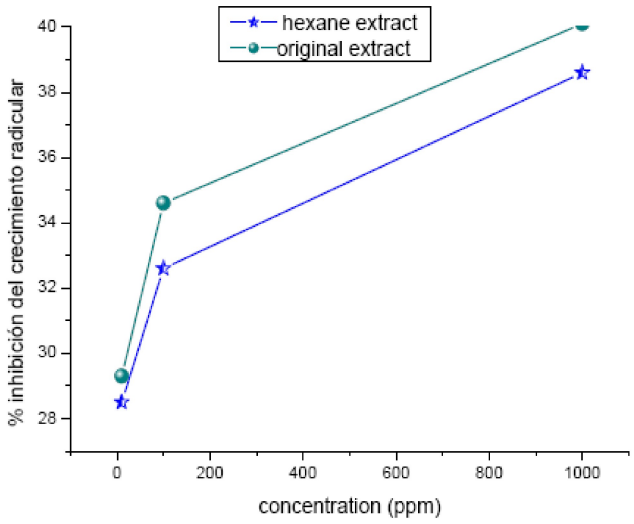


Figure 1B

served in the original hexane fraction (Figure 1C). Therefore, it was revealed that compound **(1)** was the cause of the inhibitory activity of the hexane fraction.

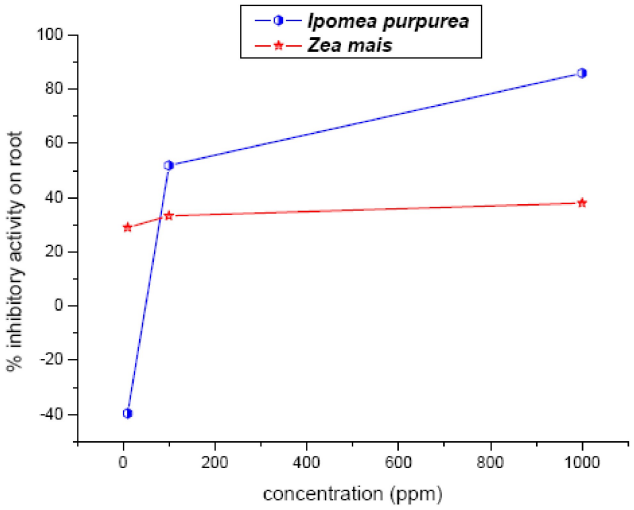


Figure 1C

EXPERIMENTAL

General

The 1D and 2D NMR spectroscopic experiments were recorded on a Bruker AC-200 spectrometer, using $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts are given en δ downfield from TMS and coupling constants are measured in Hz. COSY; DEPT, HETCORR and NOESY experiments were obtained using standard Briker software.IR spectra were recorded on a NIICOLET 5-SXC-FTIR spectrophotometer, optical rotations were determined on a Jasco P1010 polarmeter. EIMS were collected on a Finnigan 3000 F-100 at 70 eV by direct inlet. CC was performed on silica gel 60 (70-230 mesh ASTM) (Merck), silica gel 60 (230-400 mesh ASTM)(Merck) and prep. TLCand TLC were performed on silica gel 60 GF_{254} (Merck).

Plant material

Azorella biloba was collected in Córdoba Province, Argentina, and identified by L. Ariza Espinar. Voucher specimens are deposited in the MuseoBotánico Córdoba.

Measurement of phytotoxic activity

A filter paper was placed in a glass Petri dish (27 mm ∅). Test solution was added to the filter paper in the petri dish and dried completely in vacuum at 40ºC. After addition of distiller water (3 ml), ten seedlings were placed on the filter paper, and incubated for 48 h at 30ºC with light periods. The inhibitory activity on root was detected by measuring the length of the root and comparing the data obtained with that of untreated controls.

Extraction and isolation

Finely cut whole fresh aerial parts (645 g) of *A. biloba* were extracted three times with EtOH at room temp., 48 h each. The combined EtOH extracts were evaporated to give 35.4g of a gummy residue. This residue was suspended in EtOH:H_2O (7:3) mixture, and partitioned successively with hexane (6.5g), Cl_2CH_2 (3.7 g) and EtOAc (1.8g). The hexane extract was subjected to chromatography column in vacuum on silica gel, eluting with gradient mixtures of hexane: EtAcO of increasing polarity and combined according to their TLC profiles.

The bioactive hexane fraction was subjected to CC eluting with gradient mixtures of hexane:Cl_2CH_2 (7:3) and combined according to their TLC profiles to give 5 fractions, 1 through 5. The fraction5 was subjected to prep. TLC with hexane:EtAcO (9:1) yielded 25.6 mg of **(1)**.

Mulin-11,13-dien-20-oic acid (1)

Needles; ^1H NMR and ^{13}C NMR data and EIMS were in good agreement with those reported in lit[9].

Falcarindiol

Viscous pale yellow oil; spectroscopic were in good agreement with those reported in lit[4].

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REFERENCES

[1] F.Dayan, J.Romagni, M.Tellez, A.Rimando, S.Duke; Pest.Outlook, 185-188 **(1999)**.

[2] F.E.Dayan, J.G.Romagni, S.O.Duke; Investigating the mode of action of natural phytotoxin,

J.Chem.Ecol., **26**, 2079–2094 **(2000)**.

[3] S.O.Duke, F.E.Dayan, J.G.Romagni, A.M.Rimando; Natural products as sources of herbicides: current status and future trends, Weed Res., **10**, 99–111 **(2000a)**.

[4] S.O.Duke, J.G.Romagni, F.E.Dayan; Natural products as sources for new mechanisms of herbicidal action, Crop.Protect., **19**, 583–589 **(2000b)**.

[5] B.J.M.Jansen, A.de Groot; The occurrence and biological activity of drimane sesquiterpenoids. Nat.Prod.Rep., **8**, 309-318 **(1991)**.

[6] B.S.Kennedy, M.T.Nielsen, R.F.Severson, V.A.Sisson, M.K.Stephenson, D.M.Jackson; Leaf surface chemicals from Nicotiana affecting germination of Peronosporatabacina (ADAM) sporangia. J.Chem.Ecol., **18**, 1467–1479 **(1992)**.

[7] M.J.Kropff, H.Walter; EWRS and the challenges for weed research at the start of a new millennium. Weed Res., **40**, 7-10 **(2000)**.

[8] D.Lechner, M.Stavri, M.Oluwatuyi, R.Pereda-Miranda, S.Gibbons; The anti-staphylococcal activity of *Angelica dahurica* (BaiZhi). Phytochemistry, **65**, 331-335 **(2004)**.

[9] L.A.Loyola, J.Bórquez, G.Morales, A.San Martin; Diterpenoids from Azorellacompacta. Phytochemistry, **58**, 649-51 **(1997)**.

[10] F.A.Macías; In *Allelopathy: Organisms, Processes and Applicarions*; Inderjit, K.M.M.Dakshini, F.A.Einhellig, (Eds); *ACS Symposium Series 582*, American Chemical Society: Washigton, DC, 310-329 **(1995)**.

[11] F.A.Macías, J.M.G.Molinillo, J.C.G.Galindo, R.M.Varela, A.Torres, A.M.Simonet; In *Biologically Active Natural Products: Agrochemicals*. H.G.Cutler, S.J.Cutler, (Eds); CRC Press, Boca Raton, London, New York, Washigton, DC, 23-24 **(1999)**.

[12] M.L.Menetrez, H.W.Spurr Jr., D.A.Danchower, D.R.Lawson; Influence of tobacco leaf surface chemicals on *Peronospora tabacina* Adam sporangia. J.Chem.Ecol., **16**, 1565-1576 **(1990)**.

[13] E.L.Rice; In: *Allelopathy* (2nd Edition), New York, Academic, 1–7 **(1984)**.

[14] D.S.Seigler; Chemistry and mechanisms of allelopathic interactions, Agronomy J., **88**, 876–885 **(1996)**.

[15] J.R.Vyvyan; Allelochemicals as leads for new herbicides and agrochemicals, Tetrahedron, **58**, 1631–1646 **(2002)**.

[16] J.R.Vyvyan; Allelochemicals as leads for new herbicides and agrochemicals.Tetrahedron, and references cited therein, **58(9)**,1631-46 **(2002)**.