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Phytogrowth-inhibitory activities of a clerodane from *Viguiera tucumanensis*

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Abstract

The phytotoxicity of clerod-14-ene-3α,4β,13ξ-triol, obtained from *Viguiera tucumanensis* (Hook. et Arn.) Griseb, was examined in Petri dish bioassays. This clerodane inhibited both germination and root growth of *Sorghum halapense* and *Chenopodium album*, and also slightly inhibited *Ipomoea purpurea*. Crop species also showed inhibitory activity: the growth and germination of *Avena sativa* was strongly inhibited. Germination of *Brassica napus* was unaffected. The strongest plant growth inhibitory activity of the clerodane was on *C. album*, and has been expressed as the p*I*50.

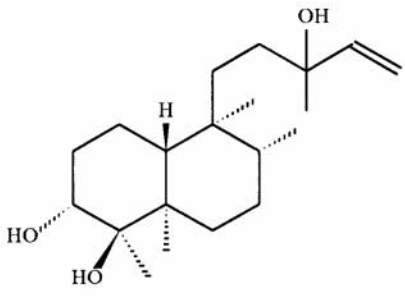
Keywords: Viguiera tucumanensis; *Asteraceae*; Phytogrowth; p*I*50; Clerodane

1. Introduction

Allelopathy is a harmful chemical effect by one species upon another (Rice, 1984) and has frequently been suggested as an important factor in regulating the structure of plant communities (Rutherford & Powrie, 1993; Smith & Martin, 1994). Plants produce a wide spectrum of secondary metabolites in relation to their defense, some of which are active allelopathic agents (Reese, 1979). These natural products are an attractive source of potential leads to new agrochemicals, because of the diversity and novelty of chemical structures produced by living organisms and the potential specificity of biological action and the reduced likelihood of harmful bioaccumulation on soil and ground water residues (Cardellina, 1992).

The genus *Viguiera* includes approximately 150 species which occur exclusively in warm and tropical America (Mabberley, 1990). Sesquiterpene lactones, including germacranolide, heliangolide, and 3,10-furanoheliangolide skeletal-types, and diterpenes, mainly kauranoic type, have been found to the major constituents in the genus. In a previous work in *Viguiera tucumanensis*, two of us reported the first clerodane in this genus, clerod-14-ene-3α,4β,13ξ-triol (**1**) (Meragelman, Ariza Espinar, Sosa, Uriburu, & de la Fuente, 1996).

Until now, no work has been done on the phytogrowth-inhibitory activity of the clerodane-type compounds. Therefore, in this work, we report the germination and growth inhibitory activities of clerod-14-ene-3α,4β,13ξ-triol (**1**).



Clerod-14-ene-3α,4β,13ξ-triol (**1**).

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Table 1
Inhibitory effects of clerodane **1** of *Viguiera tucumanensis* on germination of weed and crop species at a concentration of 6×10^{-4} M[a]

Species	Germination inhibition (%)	Radicle growth inhibition
Dicotyledonous		
Chenopodium album	70[b]	85.5[b]
Brassica napus	0[c]	50[b]
Ipomoea purpurea	17[c]	29.5[b]
Monocotyledonous		
Sorghum halepense	28.6[b]	62.7[b]
Avena sativa	73.3[b]	72.7[b]

[a]Values are expressed as percentage from the control. Growth was slowest in seeds soaked in the concentration of 6×10^{-4} M of clerodane **1**.
[b]Differences statistically significant ($p < 0.05$) with respect to control using a ANOVA test.
[c]Differences statistically no significant ($p > 0.05$) with respect to control using a ANOVA test.

2. Results and discussion

In a screening of *V. tucumanensis* extracts, in preflowering, flowering and fructification stages, showed phytotoxicity effect against crop and weed species. Clerod-14-ene-3α,4β,13ξ-triol (**1**) was isolated from the three extracts and it was assayed for phytogrowth inhibitory activity. The bioassay testing the selectivity of **1** (6×10^{-4} M) on the germination determined that the inhibitory activity was stronger (70%) on *Chenopodium album* L. and *Avena sativa* L. while, on *Brassica napus* L., *Ipomoea purpurea* (L.) Roth, and *Sorghum halapense* L. ranged about 20%. The root inhibitory effect was stronger (85–50%) on *C. album*, *A. sativa*, *S. halapense* and *B. napus* and slightly (29%) on *I. purpurea* (Table 1). At higher concentrations of this compound, greater inhibition of *C. album* root growth was observed, as shown in Fig. 1.

The phytogrowth inhibitory activity of clerodane **1** has been expressed as the p*I*50 on the weed species. As shown in Table 2 the inhibitory activity was higher on *C. album* and *S. halapense* (p*I*50 = 4.12 and 3.15, respectively) than *I. purpurea*.

3. Experimental

3.1. General

CC: Silica gel 60 (70–230 mesh) (Merck); TLC: precoated Silica gel 60 F_{254} plates Merck; Vacuum Liquid Chromatography: Silica gel 60G (Merck). Detection was achieved by irradiation with UV light and spraying with H_2SO_4 in EtOH as reagent followed by heating. ^{1}H-NMR and ^{13}C-NMR were recorded at 200 and 50 MHz, respectively (TMS) in a Bruker AC-200. $[\alpha]_D$ was measured with a Jasco Model DIP-370 digital polarimeter.

3.2. Plant material

The three materials of *V. tucumanensis* were collected near Toledo, Córdoba Province, Argentina, in three different plant development stages (PF: preflowering, F: flowering, and FR: fructification), in April 1995, and identified by Dr Luis Ariza Espinar. Voucher specimens are deposited in the Museo Botánico, Córdoba (CORD 309, CORD 310, and CORD 311, respectively).

3.3. General extraction procedure

Fresh aerial parts were exhaustively extracted with EtOH. Each residue obtained after evapn of the solvent was dissolved in hot EtOH and a soln of 4% $Pb(AcO)_2$ was added. After standing overnight, the ppt. was filtered off, the organic solvent evaporated and the aq. soln extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, the solvent evaporated under reduced pressure yielding a gummy residue.

V. tucumanensis (PF: 1.1 kg, F: 1.2 kg, and FR: 754 g) plant material processed as described above yielded 8.22, 6.95, and 6.18 g, respectively, of a dark extract. ^{1}H-NMR spectra of the extracts showed that all extracts contained the clerodane **1**. Each one was divided into several frs by vacuum liquid chromatography using hexane containing increasing amounts of EtOAc and Me_2CO as eluant. All frs obtained that contained the clerodane **1** were collected. Further purification by CC, and recrystallization from benzene yielded 427 mg of clerod-14-ene-3α,4β,13ξ-triol (**1**).

Clerod-14-ene-3α,4β,13ξ-triol (**1**): $[\alpha]_D^{25^\circ}$: −9.9 ($CHCl_3$, *c* 0.71).

3.4. Weeds and other species seed germination material

Seeds of *S. halapense* L., *C. album* L. and *I. purpurea* L. (Roth) were obtained from Laboratorio de Semillas, in Facultad de Agronomía, Universidad Nacional de Córdoba, Córdoba, Argentina. Seeds of *B. napus* L. and *A. sativa* L. were purchased from Semillería LaMoratta in Córdoba, Argentina. All undersized and damaged seeds were discarded and the assay seeds were selected by uniformity of size.

3.5. Bioassays

To a filter paper (Whatman No. 1, 6 cm i.d.) in a Petri dish (6 cm i.d., 2 cm depth) was added a known amount of the test compound in 100% EtOH. Control consisted of EtOH in place of the test solns. The

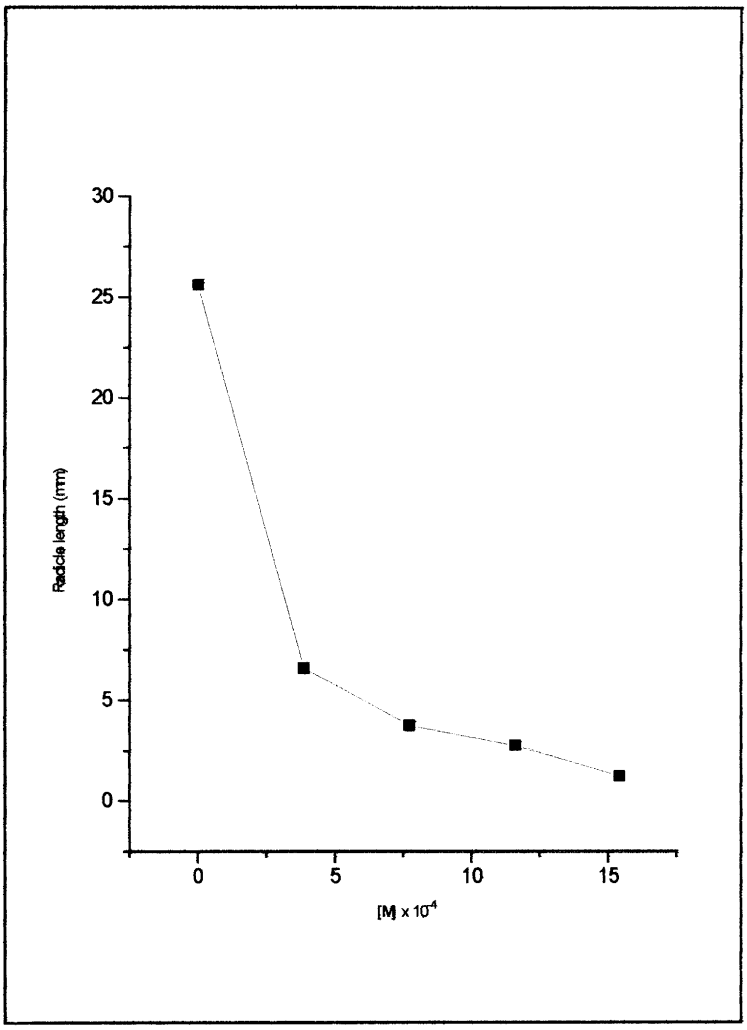

Fig. 1. Radicle length inhibitory activity of clerodane **1** against *Ch. album*.

solvent was evapd in a desiccator under red. pres. There were 3 replicates for the weeds and crop species of each treatment, and parallel controls. After addition of H_2O (3 ml) to the dish, plant seeds (25 in *S. halepense* and in *C. album*, 20 in *I. purpurea* and in *A. sativa*, and 15 in *B. napus*) were sown on the paper. They were incubated at $25 \pm 2°C$ under 12–12 h light dark cycle. After 4 days for *S. halapense*, *C. album*, and *B. napus*; and 3 days for *I. purpurea*, and *A. sativa*; inhibition of seed germination was judged by comparing the treated plant with that of the control experiment. The radicle protrusion was taken as parameter for the test.

Table 2
Comparison of pI50 values of three weed species treated by clerod-14-ene-3α,4β,13ξ-triol (**1**)

Species	pI50[a]
Chenopodium album	4.12
Sorghum halapense	3.15
Ipomoea purpurea	< 3.23

[a]pI50 defined as the negative logarithm of the molar concentration required for the 50% inhibition of root elongation after 5 days of cultivation. $R^2 > 0.90$ was considered and the equation were justified up to 99.5% level by *t*-test.

The growth inhibition test, in which the elongation of the roots and a total plant elongation were measured, was a length of 6 days in *C. album*, and *S. halapense*, 5 days in *I. purpurea*, and *A. sativa*; and 7 days in *B. napus*. The growth inhibitory activity was expressed as the p*I*50 in the weed species, defined as the negative logarithm of the molar concentration required for the 50% inhibition of root elongation after cultivation.

3.6. Statistical treatment

The germination, total growth and radicle length values were analyzed using analysis of variance testing for the extract and clerodane **1** effects on weeds and

crop species, followed by ANOVA significant difference test at $P = 0.05$. The pI50 values were calculated using regression analyses and justified up to 99.5% level by t-test.

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