BLACPMA ISSN 0717 7917

Plant Growth Inhibitory Activity of N,N´-di-(4-*R*-phenyl)-alkanediamides

[Actividad inhibitoria de crecimiento de plantas de N,N'-di-(4-R-phenyl)-alcanodiamidas]

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Abstract

Diamides are a class of metabolites that occurring in some Meliaceae plants, in *Aglaia spp* for example, with an ample body of biological activities, being insecticidal and herbicidal two of the most important. In our program of search for botanical pesticides, a series of N,N´-di-(4-*R*-phenyl)-alkanediamides was evaluated for its herbicidal activity. Many of the analogues tested exhibited moderate to good herbicidal activity both pre-emergence and post-emergence and have been found to inhibit energetic metabolism of pre-emergence weeds. The structure-activity relationships were probed by substitution on the benzene ring. Among the variations investigated, it was found that maximal herbicidal activity was obtained by substitution of –F, -CN and -Br at the aromatic portion and by n=2 of the aliphatic long chain. This last number of carbons (n=2) substitution was the key for the inhibitory activity.

Keywords: Aglaia plants, diamides, alkanediamides, plant-growth-regulation, mitochondrial respiration inhibitor.

Resumen

Diamidas son una clase de metabolitos que estan presentes en plantas perteneciente a la familia de la Meliaceas, en Aglaia por ejemplo, poseen un amplio cuerpo de actividades biologicas, siendo la insecticida y la herbicida dos de las mas importantes. En nuestro programa para la busqueda de pesticidas botanicos, una serie de N,N'-di-(4-R-phenyl)-alkanodiamidas se evaluo para su actividad herbicida. Muchos de los analogos exhibieron desde buenas a moderadas actividades, tanto como pre-emergentes como post-emergentes y ademas se encontro que inhiben el metabolismo pre-emergente energetico de malezas. La relacion estructura-actividad fue probada por sustitución sobre al anillo aromatico. Entre las variaciones investigadas, se encontro que la maxima actividad herbicida se obtuvo por sustitución de F, CN y Br en la porcion aromatica y por n=2 del largo de la cadena alifatica. Este ultimo numero de carbonos de sustitución (n=2) fue clave para la actividad inhibitoria.

Palabras Clave: Aglaia, Meliaceae, herbicida, alelopatia, pre-emergencia.

List of abbreviations: ASB – albumina sérica bovina; LPS – lipopolisacárido.

Recibido | Received: November 24, 2009.
Aceptado en versión corregida | Accepted in revised form: May 17, 2010.
Publicado en línea | Published online: July 31, 2010.
Declaración de intereses | Declaration of interests: the authors have no competing interests.
Financiación | Funding: C.L. Céspedes acknowledges an internal grant from Departamento de Ciencias Basicas, Universidad del Bio-Bio, Chillan, Chile. This work was partially supported by Internal Grant of Instituto de Química-UNAM to R. Martínez.
This article must be cited as: Carlos L. CÉSPEDES[*], María E. RODRÍGUEZ, J. Gustavo AVILA, Roberto MARTÍNEZ. 2010. Plant Growth Inhibitory Activity of N,N´-di-(4-*R*-phenyl)-alkanediamides. Bol Latinoam Caribe Plant Med Aromat 9(4): 246 – 257. {EPub M 2010}.

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INTRODUCTION

From recent years *Aglaia* species have been introduced into America for ornamental, aromatic, timber, and pesticide botanical sources purposes. Often these trees are used as control of weeds and insect pest. The genus *Aglaia* (Meliaceae: order Sapindales) consists of about 130 species that are dioecious trees or shrubs and the plants of this genus are mainly distributed in tropical and subtropical regions. During the past few years, the genus has received increasing scientific focus due to its bioactivity potential such insecticide (Legocki *et al*., 2008) and as aromatic and cosmetic value (Tilaar, *et al*., 2008). Mainly, these plant species contain Flavaglines, especially cyclopenta[*b*]benzofurans, were shown to be potent insecticides (Brader *et al*., 1998; Bacher *et al*., 1999; Nugroho *et al*., 1999; Dreyer *et al*., 2001; Greger *et al*., 2001). In addition to that, cytotoxicity (Cui *et al*., 1997) and antifungal (Engelmeier *et al*., 2000) effects were found for these compounds, so far only known from *Aglaia* species.

One class of very interesting natural products occurring in *Aglaia* include lignans and bisamides (Brader *et al*., 1998; Greger *et al*., 2000, 2001; Inada *et al*., 2001; Wang *et al*., 2001), the bisamides also only known from *Aglaia*; some of these exhibit strong pesticides, cytotoxicity and antiviral properties (Saifah *et al*., 1993, 1999). Several novel amides or diamides containing a cyclopenta-[*bc*]benzopyran moiety (5aglain-type) and a cyclopenta-[*b*]benzofuran moiety (5rocaglamide-type) as the acid part have been characterized from this genus, some of which were shown to exhibit cytotoxicity, insecticidal and antifungal activities (Inada *et al*., 2001, Muellner *et al*., 2005).

In our series of studies about herbicidal and pesticidal activities of Meliaceae species (Céspedes *et al*., 2000a) we have investigated the chemical synthesis and hemisynthesis of some diamides derivatives constituents from *Aglaia spp.* A major problem with herbicides is the evolution of resistance in weeds. This phenomenon has been a constant challenge in the search for new herbicides. New resistant biotypes appear. A logical consequence of this phenomenon has been a renewed search for new herbicides with new modes of action.

There is a good body of investigation about new herbicides more potent and selective towards monocot and dicot weeds and with new sites and modes of action. From the introduction of 2,4-dichlorophenoxy acetic acid (2,4-D), the agrochemical industry has developed a large range of selective herbicides. New EPA US regulations have caused the pesticide industry to focus on protection of the environment and in the investigation of new types of herbicides.

Transport through cellular membranes is essential for herbicides to reach the site of action (Bromilow and Chamberlain, 2000). Physicochemical properties of herbicides can strongly influence their efficacy by influencing uptake and translocation (Inoue *et al*., 1998).

Our group has focused considerable effort in this field (Céspedes *et al*., 1999; 2000a; 2000b; 2001a; 2001b; 2002; 2003). In this paper we report the effects of a series of 1,2-N,N´-di-(4-*R*-phenyl)-alkanediamides (Figure 1) (Martinez *et al*., 2000; Chacon-Garcia and Martinez, 2001; Chacon-Garcia *et al*., 2003), with strong or partial herbicidal activity against several crops and weeds. Some authors have found that compounds of this type are active against *Mycobacterium tuberculosis* (Dave *et al*., 1985), Gram-positive bacteria (Al-Arab *et al*., 1990), plants (Stauffer, 1975), citotoxic activity (Chacon-Garcia and Martinez, 2001; Chacon-Garcia *et al*., 2003), Plasmodium spp. (Raynes *et al*., 1995), inhibiting oxygen evolution in spinach chloroplasts (Kubicoba *et al*., 1998; 2000a), growth and chlorophyll production in *Chlorella vulgaris* (Kubicova *et al*., 2000b) and acting as ryanodine receptor modulators (Legocki *et al*., 2008). The substitution pattern of the aromatic ring has shown to influence the biological activities.

In this work, we describe the results of the plant growth regulatory (PGR) activity of a new series of these compounds, having the general structure shown in Figure 1. But, the main objective of this work was focused to establish a correlation between structure-activity relationships on germination, respiration, and oxygen uptake from mitochondria, several of the main macroscopic parameters of the physiology of development and growth of seeds of weedy plants (Macias *et al*., 1999). In addition, our data indicate that it is possible to correlate some antioxidant

activities (i.e. crocin, DPPH) against germination and respiration; these data are important for herbicide studies (Céspedes *et al*., 2002). On the other hand, these parameters are accepted as indirect measures of other different physiological processes (Macias *et al*., 2000) affected by the assayed chemicals.

MATERIALS AND METHODS

Chemicals and solvents.

All reagents used were either analytical reagent grade or chromatographic grade and were purchased from Sigma-Aldrich Química, S.A. de C.V., Toluca, Mexico. Methanol, CH_2Cl_2, $CHCl_3$, KCl, $CuSO_4$, NH_4Cl, $MgCl_2$, silica gel GF_{254} analytical chromatoplates, silica gel grade 60, (70-230, 60A°) for column chromatography, n-hexane, and ethyl acetate were purchased from Merck-Mexico, S.A., Mexico.

Instruments.

^{1}H-NMR spectra were recorded at 300 and 500 MHz, ^{13}C-NMR at 75 and 125 MHz respectively, on Varian VXR-300S and VXR-500S spectrometers. Chemical shifts (ppm) are related to $(CH_3)_4Si$ as an internal reference, $CDCl_3$ and acetone-d_6 (Aldrich Chemical Co.) were used as solvents, coupling constants are quoted in Hz. IR spectra were obtained in $CHCl_3$ with Perkin Elmer 283-B and FT-IR Nicolet Magna-IR 750 spectrophotometers. Biological activities were determined with a Spectronic model Genesys 5 spectrophotometer. Optical rotation was measured with a JASCO DIP-360 spectropolarimeter. Melting points were recorded with a *Melt-temp II* and were uncorrected. Oxygen evolution (uptake) was determined with a Clark type electrode connected to YSI oxygraph (Model 5300). Centrifugation was carried out in a Sigma B. Braun Model 2-15 rotor.

Chemical synthesis.

The synthesis of N,N´-di-(4-R-phenyl)-alkanediamides (**1 - 12**), was carried out through condensation of an acid chloride and an amine (Chacon-Garcia *et al*., 2003). The series were:

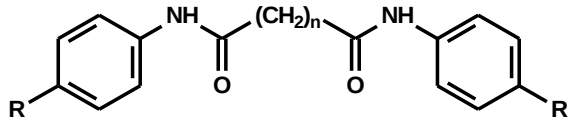


Figure 1. Chemical structure of alkanediamide series.

General methods.

All starting reagents were used without further purification and solvents were dried and distilled prior to use. The physical data are shown in Table 1.

Table 1. Physical data of a series of N,N´-di-(4-R-phenyl)-alkanediamides (See Fig. 1)

Compound number	R	n	Formulae	Molecular Weight	m.p. (ºC)
1	Cl	2	$C_{16}H_{14}N_2O_2Cl_2$	337	287 – 290
2	Br	2	$C_{16}H_{14}N_2O_2Br_2$	426	281 – 282
3	Me	2	$C_{18}H_{20}N_2O_2$	296	273 – 275
4	OMe	2	$C_{18}H_{20}N_2O_4$	328	255 – 256
5[#]	CN	2	$C_{18}H_{14}N_4O_2$	318	248 – 250 (*)
6[#]	F	4	$C_{18}H_{18}N_2O_2F_2$	332	230 – 233
7	Cl	4	$C_{18}H_{18}N_2O_2Cl_2$	365	255 – 256
8	Br	4	$C_{18}H_{18}N_2O_2Br_2$	454	287 – 288
9[#]	I	4	$C_{18}H_{18}N_2O_2I_2$	548	268 – 270 (*)
10	Me	4	$C_{20}H_{24}N_2O_2$	324	258 – 259
11	OMe	4	$C_{20}H_{24}N_2O_4$	356	233 – 235
12	CN	4	$C_{20}H_{18}N_4O_2$	346	272 – 273

[#] New compounds. * Decomposition Temperature

The initial phytotoxic activity of the compounds was evaluated for its effects on germination and growth of *Lolium multiflorum* (monocot) and *Physalis ixocarpa* (dicot), as model of selectivity, these weeds are spread throughout Latin-America, using the Petri dish bioassay described previously (Céspedes *et al*., 2000b).

After a preliminary trial, potent compounds were tested for activity at shorter intervals of concentrations. All compounds were analyzed and characterized by their R_f, IR, UV, ^{1}H NMR and ^{13}C NMR data as reported previously (Chacon-Garcia *et al*., 2003).

Seed germination and growth bioassays.

L. multiflorum var. Gulf, and *P. ixocarpa* were purchased from semillas "COBO" S. A. de C. V. Mexico D.F., México. Twenty five seeds of *L. multiflorum* or *P. ixocarpa* were placed on filter paper (Whatman No.1) in Petri dishes (85-mm diameter). Seeds were selected for uniformity of size; the damaged ones were discarded (Céspedes *et al*., 2003). The number of seeds used for each experiment was selected so an appreciable change in O_2 uptake could be detected. The paper was wet with 8 mL deionized water or test solution (MeOH less than 1%). The dishes were wrapped with Parafilm (laboratory film) and incubated at 28°C in the dark. Germination rates were counted at 72 and 120 h later for root and shoot growth measurements.

The number of germinated seeds was determined according to the criteria of 1-mm extrusion of the radical. There were three replications for each germination assay. Control dishes contained the same amount of seeds, volume of water and methanol as the test solutions.

Coleoptiles or hypocotyls and root lengths only for germinated seeds were measured after 120 h, the seedlings were dried to constant weight at 40°C (Céspedes *et al*., 2000b). The I_{50} for plant development of the pure compounds and CH_2Cl_2 extract were obtained by determining the concentration that induced 50% of growth inhibition of development of roots and shoots (Table 2).

Seed respiration

Seed respiration was measured polarographically as oxygen uptake during the germination process in a separate experiment with the same species assayed in seedling growth. The oxygen uptake, in the presence of different concentrations of tested compounds, was evaluated over 5 and 10 min, in a non-illuminated cell. The requirement for oxygen was plotted as percentage, taking the control as 100% (Céspedes *et al*., 2001b).

Determination of mitochondrial oxygen consumption

The procedure for the isolation of bean root mitochondria is based on previously described protocols (Céspedes *et al*., 2003; Winning *et al*., 1995; Bradford, 1976; Estabrook, 1967; Chance and Williams, 1955) with minor modifications. Approximately 700 g of *Phaseolus vulgaris* seeds were sterilized in a 10% (v/v) solution of sodium hypochlorite for 30 min and then washed with sterilized water thoroughly. Seeds were grown in a sterile sphagnum Peat-Moss™ mixed with vermiculite (1:1) (Hummert de Mexico, S.A. de C.V., Cuernavaca, Mexico) and grown in complete darkness for 72 h at 28°C. Roots were obtained from the coleoptile all subsequent procedures were carried out as in previously reported paper (Céspedes *et al*., 2002).

Statistical analysis

Data shown in the figures and tables are the means of three replicates of seedling growth, crocin and DPPH and are presented with mean ± standard errors. Data were subjected to analysis of variance (ANOVA) with significant differences between means identified by GLM Procedures. The results are given in the text as probability values, with $p < 0.05$ adopted as the criterion of significance. Differences between treatment means were established with a Student-Newman-Keuls (SNK) test. The necessary amount for 50% of inhibition of germination (GI_{50}), root elongation (RI_{50}) and shoot elongation (SI_{50}) values for each activity were calculated by probit analysis (Table 2). I_{50} is the concentration producing 50% inhibition. Statistical analyses were done via the MicroCal Origin 8.0 statistical and graphs PC program.

RESULTS AND DISCUSSION

Inhibition of Seed Germination and Seedling Growth.

Table 2 summarizes the seed germination activity of the compounds and the GI_{50} values of growth-inhibitor activities are shown. Compound **12** showed the highest inhibition activity at 4.0 μM for *L. multiflorum* and *P. ixocarpa*, respectively. In addition, compound **5** showed a potent germination inhibitory activity against monocot *L. multiflorum* GI_{50}=4.4 μM and a good inhibition against dicot *P. ixocarpa* with GI_{50} value of 9.9 μM.

Figure 2 shows the seed germination inhibition effects of compounds **1 – 10** and **12** on *L. multiflorum* and *P. ixocarpa*. The greatest inhibition was observed with the cyanide moieties in **12** and **5**, than with **8** and **4**. In general, monocot seeds (*L. multiflorum*)

were more sensitive to these compounds given in all cases 100% inhibition at 50 μM. In addition to **12** and **5,** the low germination I_{50} values indicate that **2**, **3, 8**, and **10** are the most powerful inhibitors for seed germination of *L. multiflorum* with I_{50} values lower than 10 μM ($GI_{50's}$ are 5.9, 7.4, 9.2, and 6.4 μM, respectively; Table 2). According to Hatfield and Karlen (1994) and Mohr and Schopfer (1995); we have applied preemergence selective inhibitors after planting; but before emergence of weeds.

Table 2. I_{50} values of compounds on physiological activities of *L. multiflorum* and *P. ixocarpa* [a, b].

Weed spp			*Lolium multiflorum*			*Physalis ixocarpa*		
Compound [e]	*n*	*X*	Germination (GI_{50}) [c]	Root (RI_{50})	Shoot (SI_{50})	Germination (GI_{50})	Root (RI_{50})	Shoot (SI_{50})
6	4	F	10.7	17.1	6.2	N. D. [d]	N. D.	N. D.
7	4	Cl	35.0	10.8	6.2	N. D.	N. D.	N. D.
8	4	Br	9.2	13.8	5.2	39.6	24.1	6.0
9	4	I	11.1	35.2	16.0	N. D.	N. D.	N. D.
10	4	Me	6.4	5.7	5.0	38.4	34.8	7.7
11	4	OMe	15.0	10.0	9.0	N. D.	N. D.	N. D.
12	4	CN	4.0	5.9	5.9	4.0	11.6	4.5
1	2	Cl	24.5	32.6	25.0	23.2	14.8	15.1
2	2	Br	5.9	7.6	5.2	N. D.	N. D.	N. D.
3	2	Me	7.4	5.8	5.8	28.5	8.8	6.1
4	2	OMe	13.5	7.6	6.8	N. D.	N. D.	N. D.
5	2	CN	4.4	7.4	7.7	9.9	9.9	5.5
2,4-D	-		1.2	0.9	0.7	0.85	0.63	0.39

[a] Means of three experiments.
[b] Each value corresponds to concentration that inhibits 50% of either root or coleoptile/hypocotyl development growth during seedling stage in length at 72 hours, and was calculated as the dose corresponding to midpoint between complete inhibition (100 % of control) and no effect by PROBIT analysis ($P< 0.05$).
[c] Each value corresponds to concentration that inhibits 50% of germination of seeds during previous seedling stage at maximum of 72 h, and was calculated as the dose corresponding to midpoint between complete inhibition (100 % of control) and no effect by PROBIT analysis ($P< 0.05$).
[d] Concentration of inhibition not determined due to lack of response.
[e] Values in μM.

Compounds **1**, **4**, **6**, **7**, **9** and **11** have the lowest potency against seed germination, being between 10 – 50 μM for *L. multiflorum* and not determined for *P. ixocarpa* (>100 μM being required for 100% inhibition of the dicot), whereas the other chemicals were 10x more active (<10 μM). Length of chain in **1** to **5** play an important role in the inhibition activities, which may be either due to their lipophilicity, or to the fact that the hydrophilicity of the **5** and **12** (at physiological pH) makes it easy for these compounds to reach the target.

Monocot and Dicot Growth.

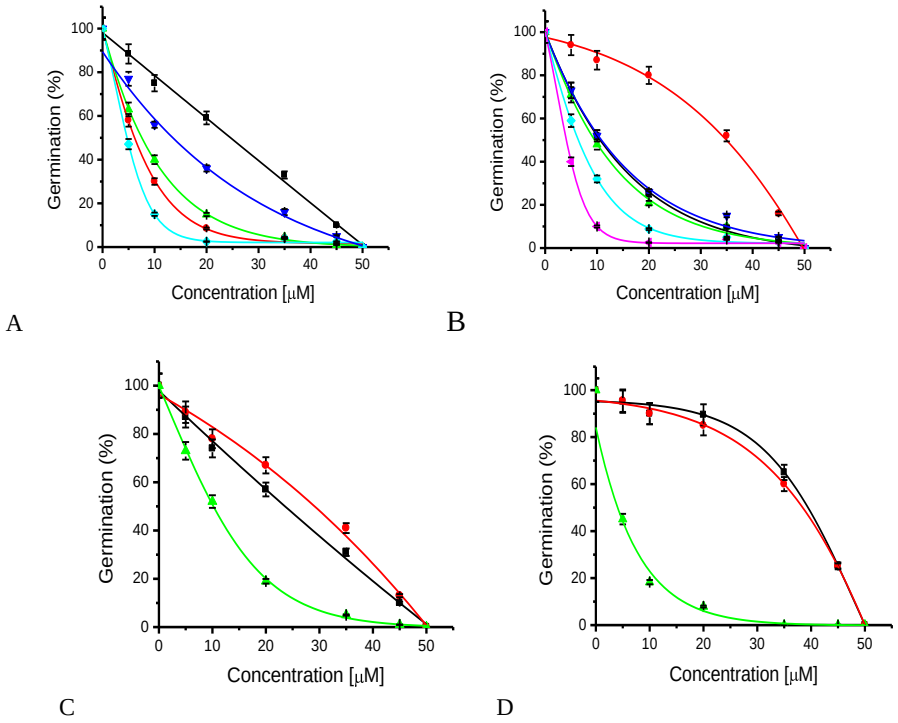
Figures 3 and 4 show the inhibitory effects of the compounds on the shoot and root length of monocot and dicot seeds.

Shoot development is more sensitive to a larger extent based on I_{50} values, as compared with coleoptile or hypocotyl development. Growth of the dicot apparently was less sensitive to inhibition by the bioassayed compounds as based on I_{50} values. Only **1**, **3**, **5**, **8**, **10** and **12** showed partial inhibitory effect on dicot with I_{50} between 10 to 50 μM (Table 2) and reached the complete inhibitory activity upon 50 μM (Figs 2, 3 and 4).

The I_{50} values are indicators of resistance of *P. ixocarpa* mainly towards the application of the compounds **7**, **8** and **10**, respectively.

The results showed the differences in behavior of the cyanide, bromide, chloride, methyl and methoxyl derivatives indicating that the mechanisms of action of these compounds may be different for growth and germination (Cespedes *et al*., 2002). 2,4-D shows a pronounced effect on root and shoot length, as well as on the germination of seeds. For

Figure 2. Effects of alkanediamides on seed germination of *L. multiflorum* (graphs A and B) and *P. ixocarpa* (graphs C and D).



Graph A: **1** (■), **2** (●), **3** (▲), **4** (▼), and **5** (◆); graph B: **6** (■), **7** (●), **8** (▲), **9** (▼), **10** (◆) and **12** (◀); graph C: **1** (■), **3** (●), and **5** (▲) and graph D: **8** (■), **10** (●) **12** (▲), expressed as percent of control germination. Each value represents mean ± SE (*N*=5), all graphs are the results of three measurements.

this compound the 100% of inhibition was obtained at 70 μM, but the values for I_{50} of 0.4 and 0.5 μM for root and shoot, respectively, shows that the inhibition have and exponential effect. Anaya *et al*., (1995) has also reported similar results.

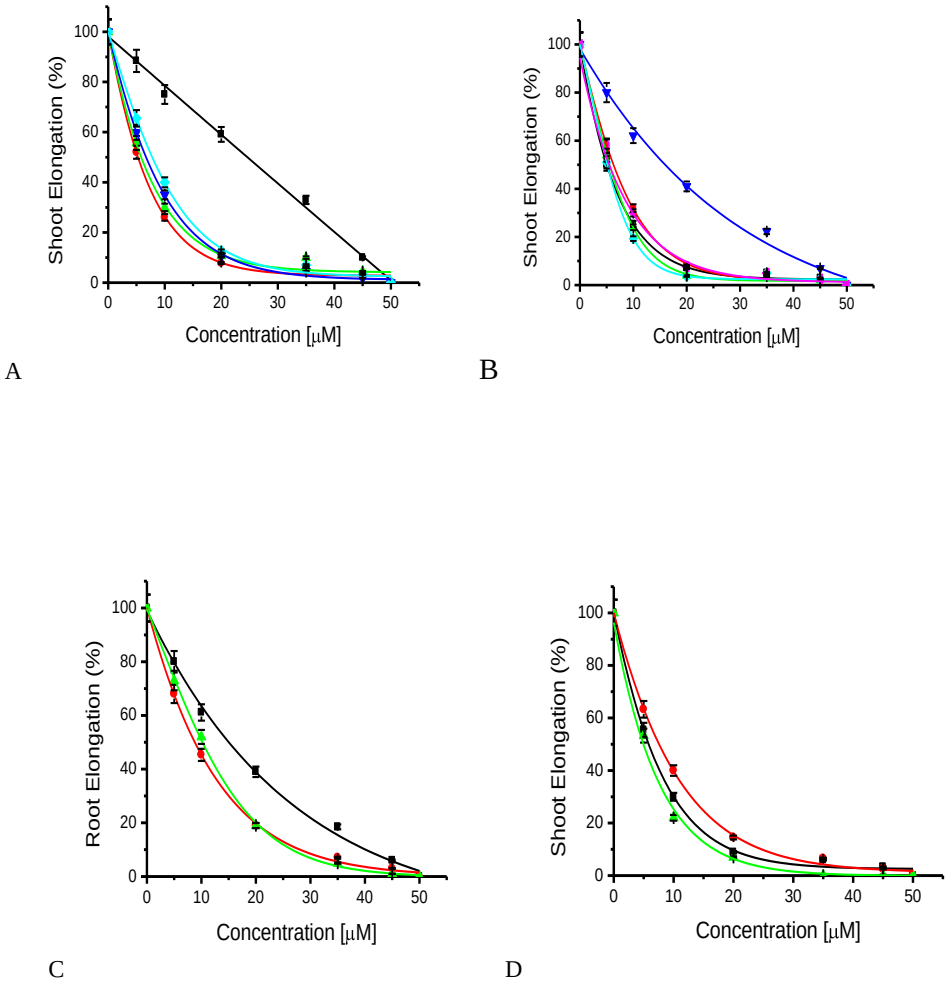
Seeds Respiration During Seed Germination.

The respiratory rates of all seeds decrease with exposure to the compounds in a concentration-dependent manner (Table 3, Figure 5 and 6). The only exceptions are for the compounds **1**, **6**, **7**, **9**, **10** and **11** on both species where the oxygen evolution not showed response above of 100 μM, as the time of imbibitions increases. However, with compounds **2**, **3**, **4**, **5**, **8** and **12** at lower concentrations of 50 μM, respiration was inhibited similarly to germination and growth, as shown by these compounds at the lowest concentrations (Table 3). These results suggest that those compounds with nucleophilic moieties may act as uncouplers of phosphorylation at low concentrations, inhibiting energy transduction or the respiration redox enzymes. Table 3 shows the RI_{50} values for the all compounds tested. According to their RI_{50} values, monocot seed (*L. multiflorum*) is more sensitive to inhibition. On the other hand, *Physalis ixocarpa* seeds showed the highest resistance to respiration inhibition.

Mitochondrial Respiration.

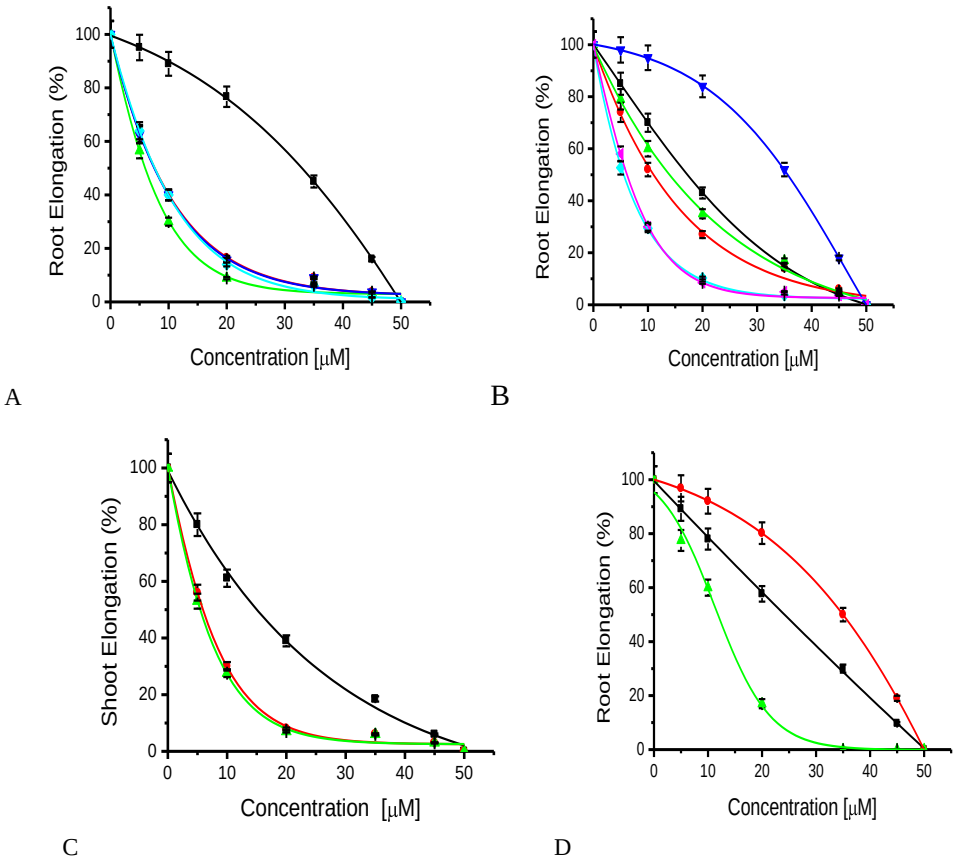
The effect of active compounds on mitochondrial respiration at 50 μM is shown in Figure 7. The I_{50} values of **2, 3, 4, 8, 12** and **5** are 8.4, 8.5, 7.9, 6.5, 5.9 and 3.8 μM, respectively. These data corroborate the results obtained with seed respiration. As in the seed respiration compound **5** was found with the lowest value and therefore the most potent.

Figure 3. Effects on seedling growth inhibitory activity by alkanediamides on development of shoot elongation of *L. multiflorum* (graphs A and B) and *P. ixocarpa* (graphs C and D) seedlings



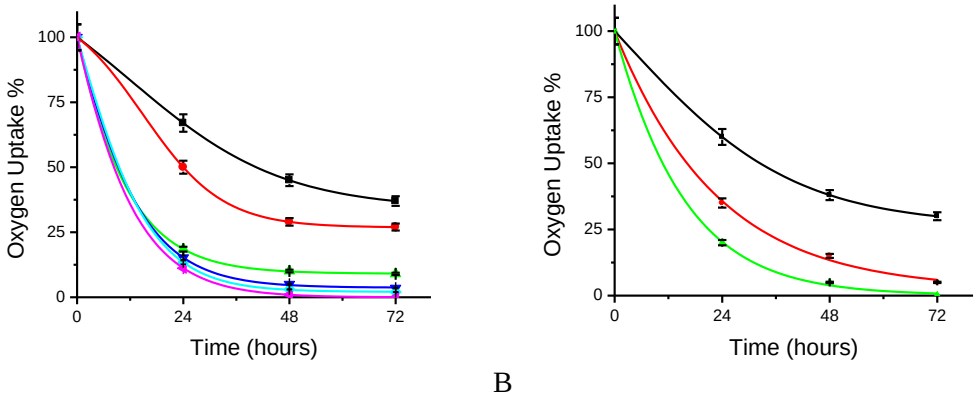
Graph A: **1** (■), **2** (●), **3** (▲), **4** (▼), and **5** (◆); graph B: **6** (■), **7** (●), **8** (▲), **9** (▼), **10** (◆) and **12** (◀); graph C: **1** (■), **3** (●), and **5** (▲) and graph D: **8** (■), **10** (●) **12** (▲), expressed as percent of control germination. Each value represents mean ± SE (*N*=5), all graphs are the results of three measurements.

Figure 4. Effects on seedling growth inhibitory activity by alkanediamides on development of root elongation of *L. multiflorum* (graphs A and B) and *P.ixocarpa* (graphs C and D) seedlings.



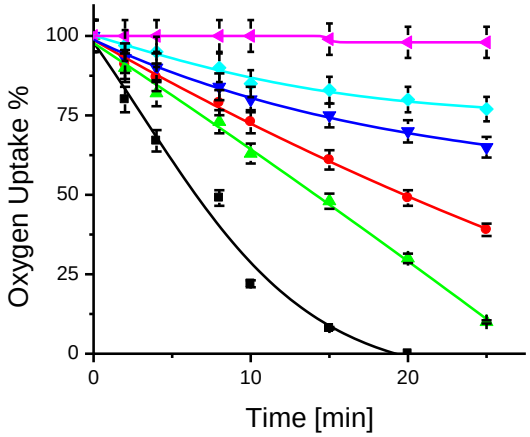
Graph A: 1 (■), 2 (●), 3 (▲), 4 (▼), and 5 (◆); graph B: 6 (■), 7 (●), 8 (▲), 9 (▼), 10 (◆) and 12 (◀); graph C: 1 (■), 3 (●), and 5 (▲) and graph D: 8 (■), 10 (●) 12 (▲), expressed as percent of control of root elongation. Each value represents mean ± SE (*N*=5), all graphs are the results of three measurements.

Figure 5. Effect of alkanediamides on seed respiration of *L. multiflorum* (monocot) and *P. ixocarpa* (dicot).



L. multiflorum (monocot). **3** (■), **2** (●), **4** (▲), **8** (▼), **5** (◆), **12** (◀). **B)** *P. ixocarpa* (dicot). **4** (■), **5** (●), **12** (▲). Each value represents mean ± SE (*N*=5), means of three experiments. Values correspond to the concentration that inhibits seed respiration during germination. Values at 72 hours.

Figure 6. Oxygen uptake of mitochondrial respiration in presence of **12** and **5** (◀), **8** (◆), **4** (▼), **3** (●), **2** (▲), at 50 μM, and control (■).



Each value represents mean ± SE (*N*=5), means of three experiments (conditions in Material and Methods). Compounds **12** and **5** are on the same line, since both showed almost identical values.

Cleaning mitochondria with buffer suspensions, recovered full respiratory activity that was inhibited by the compounds. Our results showed that compounds **12** and **5** are powerful inhibitor of mitochondria respiration, with similar concentrations to those reported by Moreland and Novitzky (1987). The molecular target site and mechanism of action is being studied.

Table 3. Mean concentrations of RI_{50} values of assayed compounds expressed as concentration that inhibit 50% of O_2 uptake as function of control seed respiration of *L. multiflorum* and *P. ixocarpa*.

Compound	*L.multiflorum*	*P.ixocarpa*
1	N. D. [c]	N. D.
2	12.6	35.1
3	21.4	78.9
4	4.8	17.2
5	4.1	5.4
6	N. D.	N. D.
7	N. D.	N. D.
8	4.5	21.3
9	N. D.	N. D.
10	N. D.	N. D.
11	N. D.	N. D.
12	4.1	8.5

Means of three experiments. Each value corresponds to the concentration (μM) that inhibits 50% of seed respiration during germination. Values at 72 h.

[a] I_{50} was undeterminated due to lack of seed respiration response at 24 hours.

CONCLUSIONS

Interestingly **6** to **12** do not have the n=2 in their chemical structure. Additionally, it was not possible to make the synthesis of compounds with n=2 and F-I substituted, because when we did not have the availability of the necessary reagents for the synthesis, these derivatives have already been synthesized and are being reported in a next work. The twelve compounds examined in this study have similar molecular structures, the differences resting in the position of the substituents and the length of intermedial chain (Fig. 1). Of the 12 compounds tested for growth inhibition of two model species, **2**, **3**, **5**, **8**, **10** and **12** showed the greatest effects on the monocot weed (*L. multiflorum*). On the other hand, **1**, **3**, **5**, **8**, **10**, and **12** showed similar inhibitory effects on the shoot growth of *P. ixocarpa* (Fig. 3); whereas, 12 and 5 showed a greater inhibitory effect on root growth than the effect showed by other compounds (Fig. 4). At amounts greater than 30 μM of **12** and **5** (data not shown), the shoots were severely malformed with a corkscrew-like appearance and were significantly smaller ($P < 0.05$ ANOVA) than controls. In addition to inhibiting growth, a bleaching of leaves was observed. These effects are currently under study.

In conclusion, our data indicate that **2**, **3**, **4**, **5**, **8** and **12** are more selective and potent respiratory inhibitors towards monocots than to dicots. Respiration processes are involved in the interference action, as these processes were inhibited in a parallel manner by these compounds assayed. At the same doses, a higher inhibition was observed on seed germination than on seed respiration and it was concluded that these extract and compounds have more than one target of interference.

The treatment concentrations for the compounds that reduced seedling growth were low (1-50 μM) compared to allelopathic chemicals that have been previously studied under laboratory conditions (Galindo *et al*., 1999; Dayan *et al*., 1999). The reported herbicidal compounds potency is in the range from 10-100 μM for growth reduction by many phenolic acids, or around 10 μM for sorgoleone, or at the micromolar level for juglone (Einhellig and Souza, 1992; Rietveld, 1983).

Whatever the mechanism(s) of action of these compounds, they have proved to be good inhibitors of plant growth. They show very good pre-emergent phytotoxic properties by inhibiting germination and growth. They also show some degree of selectivity on monocotyledonous species. We are determining the site and mechanism of action of the most active compounds. Finally, this study demonstrated that the diamides compounds type that occurring in the Aglaia plant species are promissory weedy plant growth regulatory compounds.

ACKNOWLEDGMENTS

Taken in part from B. Sc. Thesis of María E. Rodríguez. CL Céspedes acknowledges an internal grant from Departamento de Ciencias Básicas, Universidad del Bio-Bio, Chillan, Chile. This work was partially supported by Internal Grant of Instituto de Química-UNAM to R. Martinez. We thank Luis Velasco, Alejandrina Acosta, Rocío Patiño for spectral data, I.Q.-UNAM.

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