PERGAMON

Phytochemistry 61 (2002) 141–148

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Phytotoxicity and ultrastructural effects of gymnopusin from the orchid *Maxillaria densa* on duckweed (*Lemna pausicostata*) frond and root tissues☆

N.A. Valencia-Islas[a], R.N. Paul[b,*], W.T. Shier[c], R. Mata[a], H.K. Abbas[d,*]

[a]*Facultad de Química, Universidad Nacional Autónoma de México, Coyoacán 04510, México D.F., Mexico*
[b]*USDA-ARS, Southern Weed Research Unit, Stoneville, MS 38776, USA*
[c]*College of Pharmacy, University of Minnesota, Minneapolis, MN 55455, USA*
[d]*USDA-ARS, Crop Genetics and Production Research Unit, PO Box 345, Stoneville, MS 38776, USA*

Received 16 April 2002; received in revised form 5 June 2002

Abstract

Two phenanthrene derivatives, characterized as erianthridin (9,10-dihydro-2,7-dihydroxy-3,4-dimethoxyphenanthrene) and gymnopusin (2,7-dihydroxy-3,4,9-trimethoxyphenanthrene), were isolated from an extract of the orchid *Maxillaria densa*, using phytotoxicity with amaranth (*Amaranthus hypochondriacus*) to guide fractionation. Gymnopusin and erianthridin inhibited radicle elongation of *A. hypochondriacus* seedlings with IC_{50} values of 330 and 58.2 μM, respectively. The phytoxicity of the two phenanthrene derivatives was also assessed on duckweed (*Lemna pausicostata*), and compared with mammalian toxicity estimated in vitro with four mammalian cell lines. On duckweed, both phenanthrene derivatives caused electrolyte leakage, chlorophyll loss and photobleaching. Ultrastructural examination of duckweed frond and root tissues treated with gymnopusin (100 μM) revealed membrane damage to the tonoplast after 12 h of exposure. Effects on membrane integrity followed a time course similar to that of electrolyte leakage. Both phenanthrene derivatives exhibited moderate cytotoxicity to all mammalian cells tested, which precludes their use as a bioherbicide.

Keywords: Gymnopusin; *Lemna pausicostata*; Lemnaceae; Erianthridin; Orchid; Phenanthrene derivatives; Phytotoxicity; Mammalian cytotoxicity; Ultrastructure; Membrane leakage

1. Introduction

Maxillaria densa Lindley (Orchidaceae) is an epiphytic orchid widely distributed in Mexico and Guatemala (Hietz et al., 1994). Previous chemical investigation of this species allowed the isolation and structure elucidation of several phenanthrene derivatives (Estrada et al., 1999). During our search for potential herbicide agents from Mexican plants, an extract of *M. densa* was identified as possessing phytotoxicity against (duckweed) (*Lemna pausicostata* L.) and amaranth (*Amaranthus hypochondriacus* L). This paper reports the isolation of the major phytotoxic principles from *M. densa*, which were shown to be the phenanthrene derivatives (Fig. 1) erianthridin **1** (9,10-dihydro-2,7-dihydroxy-3,4-dimethoxyphenanthrene) and gymnopusin **2** (2,7-dihydroxy-3,4,9-trimethoxyphenanthrene). Both compounds have previously been isolated from several orchids, including *M. densa* (Estrada et al., 1999; Majumder and Joardar, 1985; Majumder and Banerjee, 1989), but the phytotoxic properties of gymnopusin and erianthridin have not been previously reported. However, it has been reported that a structurally-related compound, batatasin I, inhibited the growth of liverworts, algae and oat coleoptiles. Batatasin I also inhibited the CO_2-dependent O_2 evolution and the flow of electrons from water to methylviologen in spinach chloroplasts (Gorham, 1995), and it inhibited the succinate-dependent O_2 uptake in potato tuber mitochondria (Gorham, 1995). Other phenanthrenes such as orchinol, which has a free hydroxyl at the 7-position, inhibit indole-3-acetic acid (IAA) oxidation catalyzed by horseradish peroxidase

☆ Taken in part from the PhD thesis of N.A. Valencia-Islas.

* Corresponding author. Tel.: +1-662-686-5313; fax: +1-662-686-5218.
E-mail address: habbas@msa-stoneville.ars.usda.gov (H.K. Abbas).

* Deceased 15 February 2002. This paper is dedicated to his memory.

0031-9422/02/$ - see front matter
PII: S0031-9422(02)00220-0

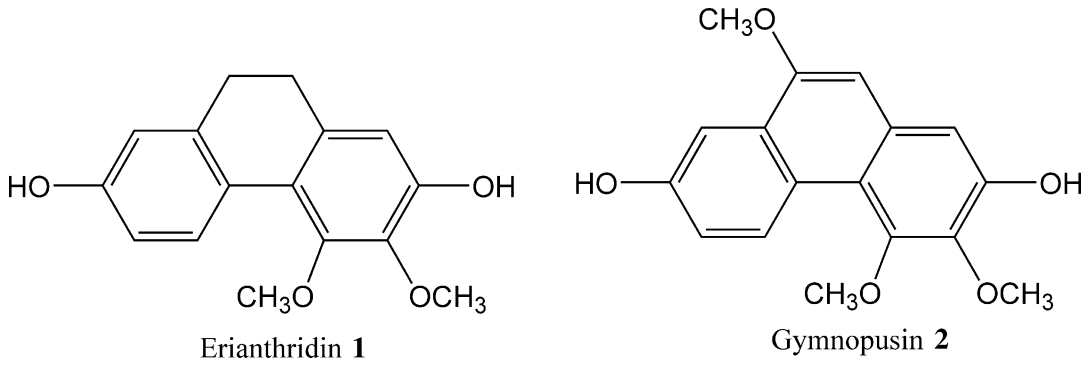

Fig. 1. Structures of erianthridin **1** and gymnopusin **2** from *Maxillaria densa*.

(Lee et al., 1978). The objectives of this research were (i) to better understand the phytotoxic properties of gymnopusin and erianthridin, (ii) to compare the phytotoxicity and the mammalian cytotoxicity of these compounds; and (iii) to investigate the ultrastructural effects of gymnopusin on frond and root tissues of duckweed.

2. Results and discussion

2.1. Bioassay-guided isolation of erianthridin *1* and gymnopusin *2*

A MeOH:$CHCl_3$ (1:1) extract of *M. densa* (whole plant) inhibited radicle growth of *A. hypochondriacus* (IC_{50} = 271 μg/ml), when tested in a germination and radicle elongation assay (Mata et al., 1998). In addition, the extract induced marked growth inhibition, chlorophyll reduction and electrolytic leakage (Table 1) after 72 h in duckweed cultures (Tanaka et al., 1993). This extract was fractionated using column chromatography on silica gel guided by a bioautographic phytogrowth inhibitory assay using *A. hypochondriacus* seedlings to monitor phytotoxicity (Mata et al., 1998). Phytotoxicity in the extract was limited to the fractions with the phenanthrene derivatives (Fig. 1) erianthridin **1** and gymnopusin **2**. Both were identified by comparing spectroscopic and mass spectrometric properties with values previously reported in the literature (Majumder and Joardar, 1985; Majumder and Banerjee, 1989).

2.2. Toxic effects of erianthridin and gymnopusin on duckweed and *A. hypochondriacus*

Erianthridin **1** (200 μM) was highly phytotoxic to duckweed as measured by increasing cellular leakage, complete growth inhibition and significant chlorophyll reduction (Table 1). Gymnopusin **2** was more potent than erianthridin **1**. The electrolyte leakage induced by 100 μM gymnopusin **2** began after 12 h (Fig. 2). The level of electrolyte leakage was proportional to the concentration of the phytotoxin with poor leakage at 25 μM, moderate leakage at 50 μM and massive leakage at 100 μM. The effect was more pronounced after 48 h of treatment. Gymnopusin **2** also caused a significant decrease in chlorophyll content at concentrations ranging from 50 to 200 μM (Table 1). Finally, gymnopusin **2** and erianthridin **1** inhibited radicle elongation of *A. hypochondriacus* seedlings with IC_{50} values of 330 and 58.2 μM, respectively.

2.3. Ultrastructural effects of gymnopusin *2*

Since several phytotoxins cause cellular electrolyte leakage and chlorophyll loss by disruption of cell membranes in treated tissues (Abbas et al., 1994), the ultrastructural effects of gymnopusin **2** were investigated in duckweed frond and root tissues. Gymnopusin **2** caused membrane disruption in frond tissue since the samples viewed through the transmission electron microscope showed ruptured tonoplasts, free-floating organelles and loss of cytoplasm in treated relative to control tissues. In treated frond tissue there was a gradual loss of cytoplasm and its component organelles. In the early controls (Fig. 3A), the cytoplasm was dense with ribosomes and distinct organelles. The chloroplasts were relatively starch free, although some small starch inclusions were visible. At 12 h of treatment (Fig. 3B), some damage attributable to the phytotoxin was observed. This result occurred at the time electrolyte leakage began to increase (Fig. 2). This micrograph also revealed that adjacent cells might show different responses to the toxin. At 24 h (Fig. 3C), free-floating organelles were common occurrences due to ruptured tonoplasts and cytoplasmic leakage. The tonoplast may be the primary target for gymnopusin **2** toxicity, which represents an unusual, if not unique toxic mechanism among phytotoxins. The plastids contained a buildup of starch inclusions and plastoglobuli which owe their electron density to osmium accumulation during fixation, and are thought to be thylakoid remnants. The accumulation of starch in chloroplasts suggests that the chloroplasts are functioning normally, but carbohydrate

Table 1
Effect of crude extract, erianthridin **1** and gymnopusin **2** from *Maxillaria densa* on *Lemna pausicostata* cultures[a] at 72 h

Treatment	Concentration (ppm or μM)	Conductivity leakage (μmho/cm)	Growth inhibition (%)	Chlorophyll reduction (%)
Media (M)	–	0.0±51.6	0.0±2.1	0.0±13.1
M + DMSO	–	16.3±14.1	5.3±4.2	0.0±18.2
Extract	31.3 ppm	188.5±25.0	29.8±2.8	29.4±6.7
	62.5 ppm	169.3±28.0	36.8±2.8	62.6±1.6
	125 ppm	218.5±14.1	52.6±2.1	64.5±9.7
	250 ppm	303.5±35.0	100.0±0.0	100.0±0.0
	500 ppm	303.5±7.1	100.0±0.0	100.0±0.0
	1000 ppm	361.5±35.4	100.0±0.0	100.0±0.0
Erianthridin **1**	25 μM	130.2±8.5	19.6±2.7	6.9±5.2
	50 μM	121.7±3.5	33.3±5.5	14.2±9.2
	100 μM	184.2±3.5	56.9±5.5	35.8±19.1
	200 μM	245.2±4.2	100.0±0.0	60.9±18.3
Gymnopusin **2**	25 μM	8.8±2.1	13.7±0.0	23.7±19.4
	50 μM	77.2±1.4	19.6±2.7	70.7±5.9
	100 μM	214.2±11.3	100.0±0.0	69.7±9.8
	200 μM	238.2±0.0	100.0±0.0	85.0±18.6

[a] Results are the means of three replicates±standard deviation. All samples exhibited significantly ($P<0.005$, Student's unpaired *t*-test) higher phytotoxicity than the controls at all concentrations tested.

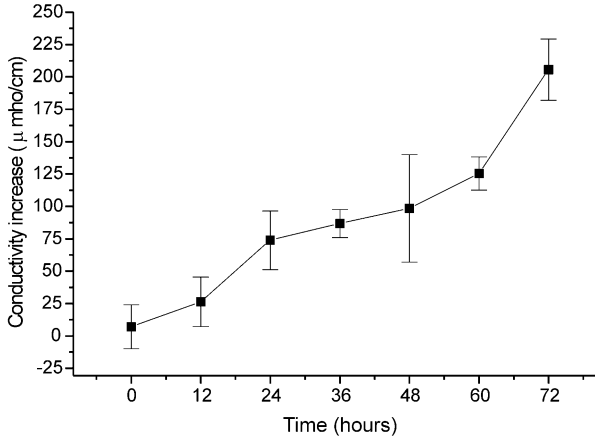

Fig. 2. Electrolyte leakage from duckweed exposed to gymnopusin **2** (100 μM) for 72 h. Electrolyte leakage was measured by the change in electrical conductivity in the bathing medium relative to untreated control duckweed. Values are the means of three replicates±standard deviation at each time.

transport from the cell has been disrupted, possibly because metabolite-utilizing cells and tissues have been more severely affected by gymnopusin than mature photosynthetic cells. At 48 h (Fig. 3D), the tissue appears very similar to that observed at 24 h, with intact organelles floating in a diluted cytoplasm. Ruptured chloroplasts became more evident at 60 h (Fig. 3E, Arrows), and although they lacked part of their plastid envelope, they still retained cohesion.

Alternatively, a light micrograph of the control root tip (Fig. 4A) showed a 72 h root tip with an intact root cap. Electron micrographs of the root tissue (Fig. 4B and C) exhibited cells with dense cytoplasm and well-formed organelles. Small cytoplasmic vacuoles (V) appeared proximal to the tip, and increased in number distally. The chloroplasts (Ch) had well formed membranes (thylakoids), dense stroma, and small starch inclusions. A light micrograph of the 72 h treated root tip showed cells similar in appearance to the apical cells of the control root tip. The root cap was missing, which is typical of a treated root apex at longer incubation times. The cells of the root tip became more vacuolated and differed in appearance from cells a similar distance from the apex in the controls. Ultrastructurally (Fig. 4E) apical cells of the treated tissue appeared not to differ from the control root tip tissue, although the vacuoles became more irregular in shape and the chloroplasts had larger starch inclusions. It would appear that in the presence of the toxin, the apical meristematic tissue continues to divide, but the cells die sooner than they do in the control tissue.

2.4. Cytotoxic effects of erianthridin *1* and gymnopusin *2* against normal and oncogenically transformed mammalian cell lines

The potential use of natural products as bioherbicides is limited by concerns about safety for animals and humans. Because ideal candidates for commercially viable herbicides should have strong phytotoxicity to susceptible weeds and low mammalian toxicity, we evaluated erianthridin **1** and gymnopusin **2** for in vitro toxicity against four cultured mammalian cell lines representing undifferentiated normal and tumor cells and differentiated kidney and liver lines. In vitro toxicological evaluation of this type is routinely conducted before a full toxicological evaluation *in vivo*. The results obtained revealed that both phenanthrene derivatives showed moderate cytotoxicity to all the mammalian cell lines tested (Table 2).

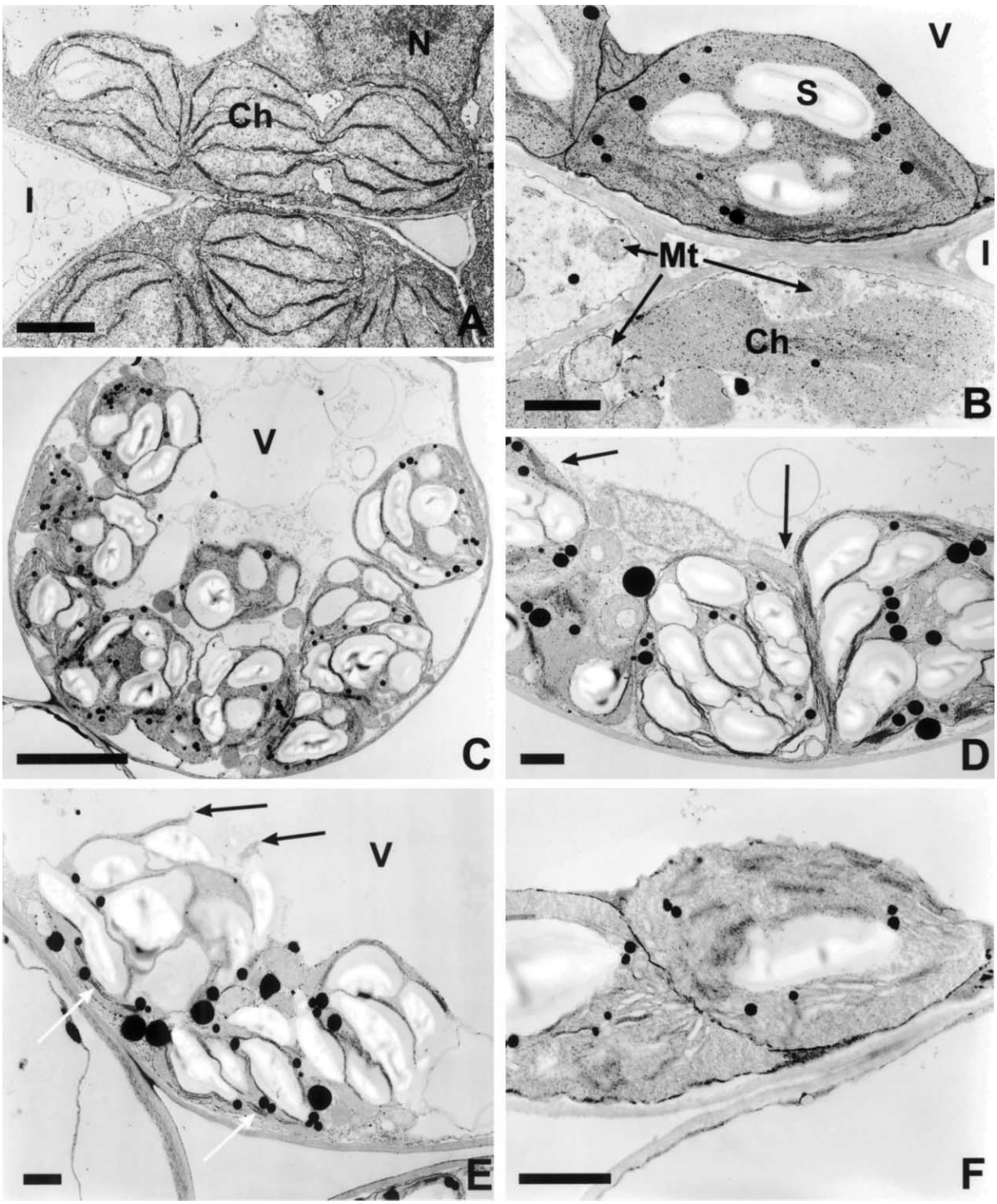

Fig. 3. Transmission electron micrographs of duckweed frond tissue. (A) Control, zero time. The chloroplasts have fully developed thylakoids with small grana stacks. Some small starch grains are present. Ch = chloroplast; I = intercellular space; N = nucleus. Bar = 1 μm. (B) Treated with 100 μM gymnopusin **2** for 12 h. Some damage appears at this time. Pictured here are three adjacent cells, of which the top cell is relatively undamaged. The damaged cells appear to have ruptured tonoplasts, since there are organelles floating freely in the cells. Mt = mitochondrion; S = starch grain; V = vacuole. Bar = 1 μm. (C) Treated with 100 μM gymnopusin **2** for 24 h. Some cells at this time exhibit loss of cytoplasm content. The relatively intact chloroplasts in this example appear to float freely within the cell, again indicating a ruptured tonoplast. Bar = 5 μm. (D) Treated with 100 μM gymnopusin **2** for 48 h. The rate of deterioration of cells exposed to the toxin varies greatly within each sample time. Here, the heavily starch-laden chloroplasts are still contained within a relatively intact, though diffuse cytoplasm. There is evidence, however, that the tonoplast is not intact (arrows). Bar = 1 μm. (E) Treated with 100 μM gymnopusin **2** for 60 h. In this cell, large starch grains and lipid bodies, which may be a sign of membrane deterioration, are present in the chloroplasts and the cytoplasm (black arrows). Grana stacks (white arrows, lower center and center left) are still present in the thylakoids. No tonoplast is evident. Bar = 1 μm. (F) Treated with 100 μM gymnopusin **2** for 72 h. The chloroplasts are showing thylakoid disruption. Lamellae are beginning to swell. Bar = 1 μm.

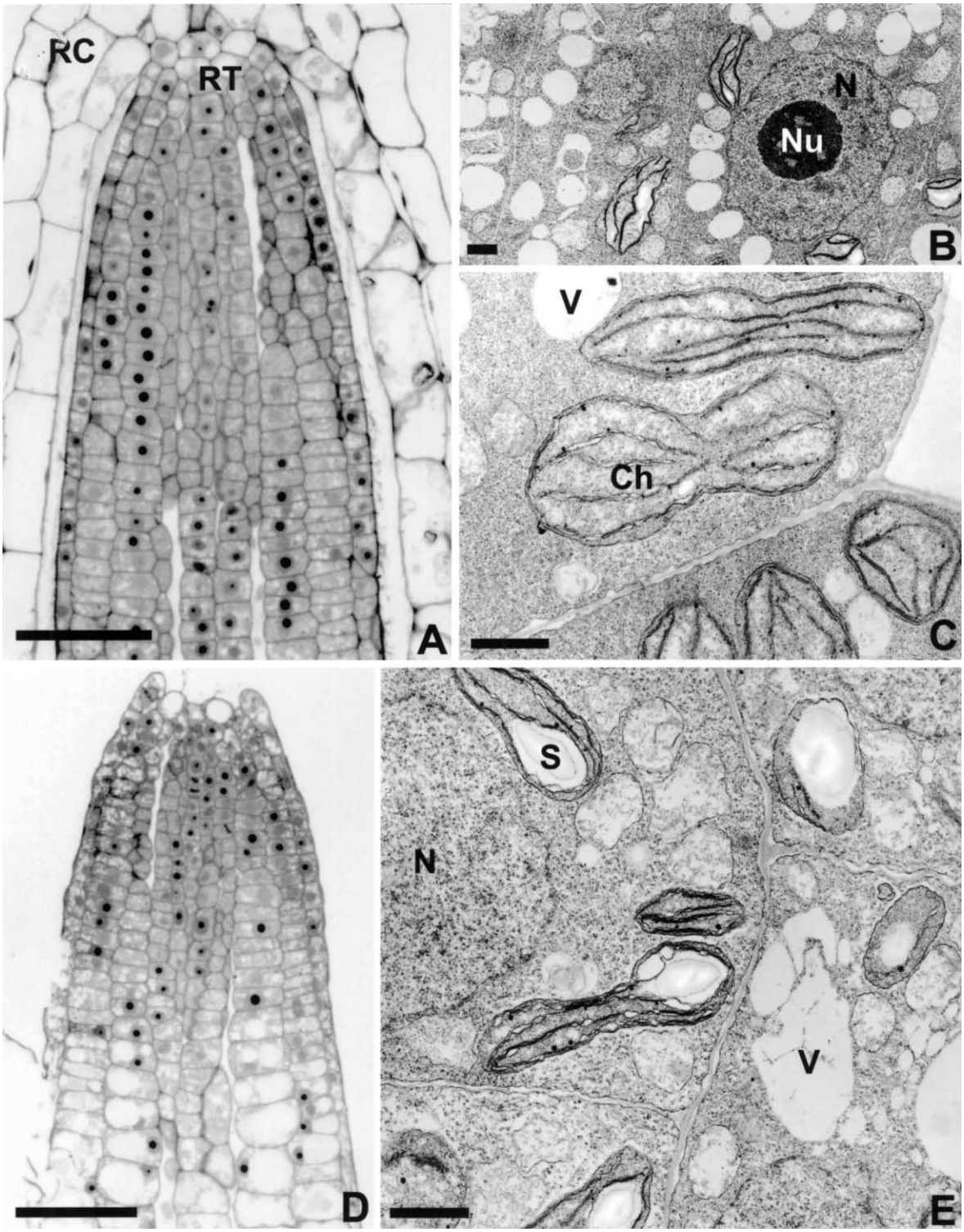

Fig. 4. Light (LM) and transmission electron (TEM) micrographs of duckweed root tissue. (A) Light micrograph of control tissue after 72 h in culture. This photograph shows the root tip and the intact root cap. RC = root cap. RT = root tip. Bar = 50 μm. (B) Transmission electron micrograph of control tissue after 72 h in culture. This micrograph shows the nucleus, chloroplasts and numerous small vesicles of adjacent root tip cells. The chloroplasts have small starch inclusions. Nu = nucleolus. Bar = 1 μm. (C) Transmission electron micrograph of control tissue after 72 h in culture. At high magnification, chloroplasts are shown to have small starch inclusions and thylakoids with small grana. Ch = chloroplast. Bar = 1 μm. (D) Light micrograph of tissue treated for 72 h with 100 μM gymnopusin **2**. This root tip, shows typical damage caused by the toxin. Root tip cells at the apex manage to grow and divide but rapidly become highly vacuolated and distinct from comparable cells in the control (Fig. 4A). The root cap has been lost, which is a characteristic of toxins on root tips. Bar = 50 μm. (E) Transmission electron micrograph of tissue treated for 72 h with 100 μM gymnopusin **2**. A micrograph of root tip cells in the treated tissue appears similar to the control (compare to Fig. 4C) with the exception of large starch grains within the chloroplast stroma. Vacuoles become more irregular, which may be due to tonoplast disruption. Bar = 1 μm.

Table 2
Cytotoxicity of erianthridin **1** and gymnopusin **2** in cultured mammalian cell lines

Compound	IC_{50} (μM)[a]			
	H4TG	MDCK	NIH3T3	KA31T
Erianthridin **1**	20.0±1.1	10.2±0.5	19.3±0.9	13.0±0.3
Gymnopusin **2**	13.0±0.9	11.0±0.5	12.0±1.0	21.0±0.5

[a] The concentration of toxin which causes a 50% reduction in cell-bound dye after 5 days in culture. Cell lines used were H4TG, thioguanine-resistant rat hepatoma cells; MDCK, Madin-Darby canine kidney cells; NIH3T3, NIH Swiss mouse embryo fibroblasts and KA31T, Kirsten strain of Moloney sarcoma virus-transformed 3T3 cells.

2.5. Conclusion

The results of the present investigation indicate that gymnopusin **2** and erianthridin **1**, secondary metabolites synthesized by several orchids, possess weak phytotoxic properties, and they are responsible for the bulk of the phytotoxic activity present in extracts of *M. densa*. On duckweed tissues gymnopusin **2** induced various ultrastructural changes consistent with membrane destruction at the same time as electrolyte leakage and chlorophyll reduction were occurring. The occurrence of toxic effects in both plant and animal cells is consistent with gymnopusin **2** and erianthridin **1** acting non-specifically on membranes. Both have molecular weights and calculated octanol/water coefficients in the range that cause non-specific phytotoxic effects (Giroux and Miller, 2001). However, the preferential lysis of the tonoplast membrane in duckweed tissue exposed to gymnopusin **2** is consistent with the latter also acting by a more specific and possibly novel mechanism. Similarly, differences in toxicity of gymnopusin **2** in fronds *versus* roots of duckweed are not consistent with purely non-specific toxic effects. Nevertheless, gymnopusin **2** appears to be 5–10 times more toxic to mammalian cells in vitro, suggesting it is not very promising as a bioherbicide.

3. Experimental

3.1. Plant material

Whole plants of *M. densa* Lindley (Orchidaceae) were collected in July 1996 from Ejido Ruíz Cortines, Catemaco, State of Veracruz, México. A voucher specimen (Carmona 96–1) was deposited at the Instituto de Ecología Herbarium (XAL), Xalapa, Veracruz.

3.2. Extraction and isolation of erianthridin **1** and gymnopusin **2** from M. densa

Air-dried and ground plant material (2.2 kg) was extracted by maceration at room temperature with a mixture of MeOH:$CHCl_3$ (1:1). The resulting extract was evaporated and the residue (161.2 g) was fractionated by column chromatography on silica gel (775 g). The column was eluted with hexane, followed by a gradient of hexane:EtOAc (1:0→0:1) and finally with a gradient of EtOAc:MeOH (1:0→1:1) as previously described (Estrada et al., 1999). Altogether, 215 fractions (600 ml each) were collected and combined according to their TLC profiles to yield seven primary fractions (F_I to F_{VII}). The bioautographic method employed for monitoring the phytotoxic activity was carried out as previously reported (Mata et al., 1998) and revealed that fraction F_{VI} was the active pool. Active fraction F_{VI} (17.3 g) was further subjected to silica gel column chromatography (759 g), and eluted with a gradient of hexane:EtOAc (1:0→0:1). This process led to four secondary fractions (F_{VI-A} to F_{VI-D}). Once more, the bioautographic method showed two active pools, namely, F_{VI-B} and F_{VI-C}. Extensive preparative TLC [silica gel plates, $CHCl_3$:EtOAc (8:2)] of active fraction F_{VI-B}, eluted with hexane:EtOAc (7:3), allowed the isolation of erianthridin **1** (1.3 g). When active fraction F_{VI-C} was eluted with hexane:EtOAc (6:4), 1.8 g of gymnopusin **2** crystallized spontaneously.

3.3. Inhibition of radical elongation of Amaranthus hypochondriacus

The growth inhibitory activity of the extract and pure compounds on seedlings of *A. hypochondriacus* was evaluated using the Petri dish radicle elongation and germination bioassay at 28 °C as previously described (Mata et al., 1998). The results were analyzed by ANOVA ($P<0.05$), and IC_{50} values were calculated by Probit analysis based on percent of radicle growth or gemination inhibition. The extract was evaluated at four concentrations (1, 10, 100 and 1000 μg/ml), whereas fractions and pure compounds were tested at three concentrations (10, 100 and 1000 μg/ml), and 2,4-D was used as the positive control (data not shown).

3.4. Duckweed bioassay

The duckweed bioassay was carried out in triplicate essentially as described by Tanaka et al. (1993). Briefly, 20 colonies of three fronds each were incubated 3 days in 3.5 cm polystyrene petri dishes containing 3 ml of half-strength Hunter's medium (Becerril et al., 1992) with the toxin dissolved in 1% (vol/vol) DMSO at 0, 25, 50, 100 and 200 μM. A conductivity meter was used to determine electrolyte leakage by sampling and returning the bathing medium to each dish at intervals of 12 h. Data are expressed as a percentage increase in conductivity over the control. In all the experiments, chlorophyll content was expressed on the basis of fr. wt by first removing duckweed from the bathing media, blot-

ting with paper towels, weighing, then soaking in 5 ml of DMSO at room temperature in the dark for 24 h, at which time chlorophyll extraction was complete. The total chlorophyll was assayed by ultraviolet/visible spectroscopy according the method of Hiscox and Israelstam (1979). Growth inhibition was measured by comparing duckweed fresh weight at the beginning and end of the experiment.

3.5. Microscopy

Gymnopusin **2**-treated duck weed tissue that exhibited toxic effects as indicated by electrolyte release was compared to solvent-treated control tissue. The tissues were placed in a drop of 0.4% glutaraldehyde fixative in 0.01 M cacodylic acid buffer at pH 7.0. The tissue was chopped in the fixative with a single edge razor blade and transferred to a vial containing more of the buffered fixative. The tissue was fixed for 1.5 h, then rinsed for 1 h with 6 changes of glutaraldehyde-free buffer. The tissue was then post-fixed for 1 h in 1% osmium tetroxide in 0.05 M cacodylate buffer pH 7.0. After rinsing in distilled water for a period of 1 h, (6 changes) the tissue was dehydrated in a graded series of acetone to 100% acetone, from which the tissue was embedded and polymerized in Spurr's medium. Semi-thin sections obtained with a diamond knife in a Leica Ultracut E ultramicrotome were mounted on glass slides, stained with toluidine blue and observed using a light microscope. Thin sections were stained with uranyl acetate and Reynolds lead citrate, then observed and photographed in a Zeiss EM10 CR transmission electron microscope.

3.6. Cytotoxicity assay

Four permanent mammalian cell lines were used. Untransformed 3T3 Swiss mouse fibroblasts (strain NIH3T3) were obtained from A. Aaronson, National Cancer Institute, Bethesda, MD, USA, and oncogenically transformed 3T3 mouse fibroblasts (strain KA31T, transformed by the Kirsten strain of Moloney sarcoma virus) were obtained from R. Pollack, Columbia University, New York. Rat hepatoma cell line H4TG and dog kidney cell line MDCK were purchased from the American Type Culture Collection, Rockville, MD, USA. The cells were cultured and the cytotoxicity assays were conducted as described previously (Abbas et al., 1995; Shier et al., 1991).

Briefly, cytotoxicity bioassays were carried out for each cell line in triplicate 200 μl cultures in 96-well microtiter trays [Nunc MicroWell (untreated)] with gymnopusin **2** and erianthridin **1** at final treatment concentrations of 0, 1, 2, 5, 10, 20, 50, 100 and 200 μM in Dulbecco's modified Eagle's medium containing 5% (vol/vol) calf serum. The wells were inoculated with 10^4 cells from an actively-growing culture and cultured for 5 days at 37 °C in a humidified CO_2-containing atmosphere. Growth was evaluated by fixing washed cultures with 3.7% (wt./vol) formaldehyde in saline, staining with two drops of 0.05% (wt./vol) crystal violet in 20% (vol/vol) aqueous methanol, washing away unbound dye with tap water, drying, extracting bound dye by addition of 200 μl DMSO and measuring absorbance of each well at 562 nm using a microplate photometer (SPECTRAmax PLUS Instrument). Analysis of variance was used to compare percent inhibition values to solvent controls at each concentration for each cell line. IC_{50} values were estimated from straight lines fitted by the least squares method to the mean percent inhibition values.

Acknowledgements

This investigation was partially supported by grants from DGAPA-UNAM IN-207300 (awarded to R. Mata) and DGEP-UNAM. N.V. acknowledges the fellowship awarded by CONACyT and DGEP-UNAM to carry out graduate studies.

References

Abbas, H.K., Tanaka, T., Shier, W.T., 1995. Biological activities of synthetic analogues of *Alternaria alternata* toxin (AAL-toxin) and fumonisin in plant and mammalian cell cultures. Phytochemistry 40, 1681–1689.

Abbas, H.K., Tanaka, T., Duke, S.O., Porter, J.K., Wray, E.M., Hodges, L., Session, A.E., Wang, E., Merrill Jr., A.H., Riley, R.T., 1994. Fumonisin- and AAL-toxin induced disruption of sphingolipid metabolism with accumulation of free sphingoid bases. Plant. Physiol. 106, 1085–1093.

Becerril, J., Duke, M.V., Nandihalli, U.B., Matsumoto, H., Duke, S.O., 1992. Light control of porphyrin acumulation in acifluorfen-methyl-treated *Lemna pausicostata*. Physiologia Plantarum 86, 6–16.

Estrada, S., Toscano, R., Mata, R., 1999. New phenanthrene derivatives from *Maxillaria densa*. J. Nat. Prod. 62, 1175–1178.

Giroux, P.Y., Miller, J.R., 2001. Phytotoxicity of pheromonal chemicals to fruit tree foliage: chemical and physiological characterization. J. Economic Entomology 94, 1170–1176.

Gorham, J., 1995. The Biochemistry of Stilbenoids. Chapman and Hall, London.

Hietz, P., Hietz-Seifert, V., de Veracruz, E., 1994. Guía Ilustrada para las Regiones de Xalapa y los Tuxtlas, Veracruz. Instituto de Ecología, A. C. Veracruz, México, p. 48.

Hiscox, J.D., Israelstam, G.F., 1979. A method for the extraction of chlorophyll from leaf tissues without maceration. Can. J. Bot. 57, 1332–1334.

Lee, T.T., Rock, G.L., Stoessl, A., 1978. Effects of orchinol and related phenanthrenes on the enzymic degradation of indole-3-acetic acid. Phytochemistry 17, 1721–1726.

Majumder, P.L., Banerjee, S., 1989. Revised structure of gymnopusin, a ring BB oxygenated phenanthrene derivative isolated from the orchid *Bulbophyllum gymnopus*. Indian J. Chem. 28B, 1085–1088.

Majumder, P.L., Joardar, M., 1985. Erianthridin, a new 9,10-dihydrophenanthrene derivative from the orchids *Eria carinata* and *Eria stricta*. Indian J. Chem. 24B, 1192–1194.

Mata, R., Macías, M., Rojas, S., Lotina-Hensen, B., Toscano, R., Anaya, A., 1998. Phytotoxic compounds from *Esembeckia yaxhoob*. Phytochemistry 49, 441–449.

Shier, W.T., Abbas, H.K., Mirocha, C.J., 1991. Toxicity of the mycotoxins fumonisins B1 and B2 and *Alternaria alternata* f. sp. *lycopersici* toxin (AAL) in cultured mammalian cells. Mycopathologica 116, 97–104.

Tanaka, T., Abbas, H.K., Duke, S.O., 1993. Structure-dependent phytotoxicity of fumonisins and related compounds in a duckweed bioassay. Phytochemistry 33, 779–785.