

## In Vitro Sensitivity of *Botrytis cinerea* to Anthraquinone and Anthrahydroquinone Derivatives

LEONORA MENDOZA,<sup>\*,§</sup> RAMIRO ARAYA-MATURANA,<sup>#</sup> WILSON CARDONA,<sup>#</sup>  
TOMÁS DELGADO-CASTRO,<sup>#</sup> CAROLINA GARCÍA,<sup>†</sup> CAROL LAGOS,<sup>†</sup> AND  
MILENA COTORAS<sup>†</sup>

Departamento de Ciencias Biológicas y Departamento de Química de los Materiales, Facultad de Química y Biología, Universidad de Santiago de Chile, and Departamento de Química Orgánica y Físicoquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla 40-Correo 33, Santiago, Chile

The effect on mycelial growth of the fungus *Botrytis cinerea* of a set of structurally related tricyclic hydroquinones [9,10-dihydroxy-4,4-dimethyl-2,3,5,8-tetrahydroanthracen-1(4*H*)-one and 9,10-dihydroxy-4,4-dimethyl-5,8-dihydroanthracen-1(4*H*)-one derivatives] and tricyclic quinones [4,4-dimethylantracene-1,9,10(4*H*)-trione derivatives] was studied. In general, the anthraquinones presented higher activity than the anthrahydroquinones. Anthraquinone and anthrahydroquinone derivatives with methyl groups on the A ring showed higher antifungal activity than the unsubstituted ones, 4,4,6,7-tetramethyl-(4*H*)-anthracene-1,9,10-trione being the most active compound of this set. The presence of a polar group such as hydroxymethyl reduced the activity. The effect of two anthrahydroquinones and two anthraquinones on the conidia germination of the fungus was also determined. Anthrahydroquinones did not affect the germination. The most active compound was 4,4-dimethylantracene-1,9,10(4*H*)-trione, with 100% inhibition of germination at 7 h of incubation. These results again suggest that the structure of the anthraquinones is important in exerting an antifungal effect on *B. cinerea*. Furthermore, possible mechanisms of action of compound 4,4-dimethylantracene-1,9,10(4*H*)-trione were studied. This compound did not produce lipoperoxidation of membrane and did not induce the formation of oxygen reactive species, but it was able to permeabilize the plasmatic membrane of *B. cinerea*, increasing the phosphorus concentration in the intracellular medium.

**KEYWORDS:** *Botrytis cinerea*; anthraquinones; anthrahydroquinones; antifungal activity

### INTRODUCTION

*Botrytis cinerea* is a very common phytopathogenic fungus in nature, which causes diseases on a variety of unrelated crops. Climatic conditions such as high relative humidity and low temperatures lead to a high incidence of diseases caused by this fungus. Losses result from the rotting of fruit in the field or in storage (1).

In Chile, *B. cinerea* has traditionally been controlled by commercial fungicides (dicarboximides and benzimidazoles). The use of these fungicides has caused serious problems such as the appearance of highly resistant strains and the contamination of soil and water (2).

The benzimidazoles are specific inhibitors of microtubule assembly, and the dicarboximides cause significant cellular leakage and lipid peroxidation on the fungi (2–4).

New classes of fungicides including anilinopyrimidines, phenoxyquinolines, oxazolidinediones, spiroketalamines, phenylpyrroles, and strobilurins have been reported (5). These compounds act by diverse and novel modes of action. Among them, the anilinopyrimidines pyrimethanil and mepanipyrim are especially active against gray mold (*B. cinerea*). These compounds prevent the fungal secretion of hydrolytic enzymes such as proteases, cellulases, lipases, or cutinases, which play an important role in the infection process (6).

The phenylpyrroles fenpiclonil and fludioxonil have been used against *B. cinerea*. The antifungal spectrum of activity of these phenylpyrroles is similar to that of dicarboximides. In *B. cinerea*, they induced similar morphological alterations of the germ tubes, and their fungitoxicity is reversed by  $\alpha$ -tocopherol and piperonyl butoxide. The antagonistic effect of free radical scavenger  $\alpha$ -tocopherol reported in *B. cinerea* treated with fenpiclonil suggests that this fungicide stimulates the formation of reactive oxygen products, and as a consequence it could induce peroxidation (7).

There are no studies on the antifungal activity of synthetic anthrahydroquinones and anthraquinones. It has been reported

\* Corresponding author (telephone 52-2-681-1644; fax 56-2-681-2108; e-mail lmendoza@lauca.usach.cl).

<sup>†</sup> Departamento de Ciencias Biológicas, Universidad de Santiago de Chile.

<sup>§</sup> Departamento de Química de los Materiales, Universidad de Santiago de Chile.

<sup>#</sup> Universidad de Chile.

that anthraquinones isolated from *Cassia tora*, *Rheum emodi*, and *Drechslera avenae* presented antifungal activity against fungi (8–10). The aim of the present study was to evaluate the effect of a structurally related series of synthetic anthraquinone and anthrahydroquinone derivatives on mycelial growth and conidial germination of *B. cinerea* and to determine a possible mechanism of action.

## MATERIALS AND METHODS

**Chemicals.** Malt extract was obtained from Cramer Co., Ltd. (Santiago, Chile). Yeast extract was obtained from Sigma Chemical Co. (St. Louis, MO). Agar was obtained from Difco Laboratories (Detroit, MI). Technical grade fungicide iprodione [3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioximidazolidine-1-carboxamide] was a gift from INIA (Santiago, Chile). The organic solvents were obtained from Merck Química Chilena (Santiago, Chile).

The melting point uncorrected was determined on a Kofler hot-stage apparatus. The NMR spectra were acquired using a Bruker Avance DRX 300 spectrometer operating at 300.13 MHz ( $^1\text{H}$ ) or 75.47 MHz ( $^{13}\text{C}$ ). Measurements were carried out at a probe temperature of 300 K, using  $\text{CDCl}_3$  containing tetramethylsilane (TMS) as an internal standard.

**Anthrahydroquinone and Anthraquinone Derivatives.** The compounds used in this study are shown in Figure 1. The anthrahydroquinones 2–9 and the anthraquinones 10–13 were synthesized as described previously (11–14). The new compound 1 was obtained as follows.

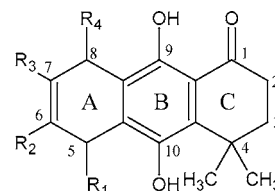
**9,10-Dihydroxy-4,4,6,7-tetramethyl-3,4,5,8-tetrahydro-1(2*H*)-anthracenone (1).** A solution of 8,8-dimethyl-6,7-dihydro-1,4,5(8*H*)-naphthalenetrione (14) (157 mg, 0.77 mmol) and 2,3-dimethylbutadiene (65 mg, 0.78 mmol) in toluene (10 mL) was left at room temperature for 7 days, then silica gel (2 g) was added, and the mixture was stirred overnight at room temperature. The solution was filtered, and the solid was washed repeatedly with ethyl acetate. Removal of the solvent afforded a yellow solid (210 mg, 95%) of a mixture of tricyclic hydroquinone 1 and the product of reduction of starting quinone in a 91:9 ratio. Column chromatography on silica gel with an 80:20 light petroleum/EtOAc mixture allowed the anthracenone to be purified.: mp 140–150 (d);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.51 (s, 6H, 4- $\text{CH}_3$ ), 1.79 (s, 6H, 6- and 7- $\text{CH}_3$ ), 1.93 (t, 2H,  $\text{CH}_2$ ,  $J = 6.7$  Hz), 2.67 (t, 2H,  $\text{CH}_2$ ,  $J = 6.7$  Hz), 3.09–3.27 (m, 4H,  $\text{CH}_2$ ), 4.34 (s, 1H, OH), 12.96 (s, 1H, OH);  $^{13}\text{C}$  NMR  $\delta$  17.47, 17.57, 26.19, 29.34, 30.70, 32.85, 33.97, 37.68, 112.33, 119.05, 121.47, 122.67, 130.91, 131.66, 141.63, 153.73, 204.16; IR (KBr) 1361, 1615, 2950, 3455  $\text{cm}^{-1}$ . Anal. found: C, 75.33; H, 7.27. Calcd. for  $\text{C}_{18}\text{H}_{22}\text{O}_3$ : C, 75.50; H, 7.74.

**Fungal Strains and Culture Conditions.** The strain G29 of *B. cinerea* isolated from infected grapes was used in this study. This is an isolate resistant to dicarboximide because the  $\text{ED}_{50}$  is  $>10$   $\mu\text{g/mL}$  (Table 1) (15). The fungus was maintained in Petri dishes on malt–yeast extract agar (1.5% agar, 2% malt extract, and 0.2% yeast extract) at 4 °C. The fungus was grown in the dark in the following culture media.

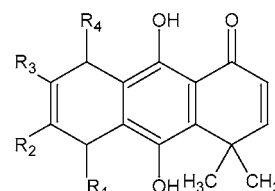
**Solid Media.** Two solid media were used: malt–yeast extract agar medium and soft agar medium (2% malt extract, 0.2% yeast extract and 0.6% agar).

**Liquid Medium.** The fungus was cultured in malt–yeast extract medium (2% malt extract, 0.2% yeast extract) (16).

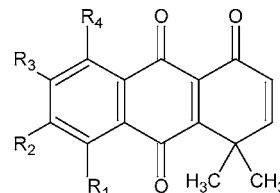
**Antifungal Activity of Anthraquinone and Anthrahydroquinone Derivatives on *B. cinerea*.** Effect on Mycelial Growth of *B. cinerea* in Solid Medium. Fungitoxicity of the compounds and the fungicide iprodione was assessed using the radial growth test on malt–yeast extract agar (17). The compounds or iprodione dissolved in methanol was added at different final concentrations (20, 40, 80, 160, and 200  $\mu\text{g/mL}$ ) to Petri dishes containing 5 mL of soft agar medium. The final methanol concentration was identical in control and treatments. Dishes were left open in a laminar-flow hood for 30 min to remove methanol. After evaporation of the solvent, the culture medium was inoculated with 0.5 cm agar disks from an actively growing culture of *B. cinerea*. Cultures were incubated in the dark at 22 °C for 7 days. Mycelial growth



Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
1	H	CH <sub>3</sub>	CH <sub>3</sub>	H
2	CH <sub>3</sub>	H	H	CH <sub>2</sub> OH
3	CH <sub>2</sub> OH	H	H	CH <sub>3</sub>



Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
4	H	H	H	H
5	H	CH <sub>3</sub>	H	H
6	H	H	CH <sub>3</sub>	H
7	H	CH <sub>3</sub>	CH <sub>3</sub>	H
8	CH <sub>3</sub>	H	H	CH <sub>2</sub> OH
9	CH <sub>2</sub> OH	H	H	CH <sub>3</sub>



Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
10	H	H	H	H
11	CH <sub>3</sub>	H	H	H
12	H	CH <sub>3</sub>	H	H
13	H	CH <sub>3</sub>	CH <sub>3</sub>	H

Figure 1. Chemical structures of anthrahydroquinones (1–9) and anthraquinones (10–13) used in this study.

Table 1. Effect of Anthrahydroquinone and Anthraquinone Derivatives on in Vitro Mycelial Growth of *B. cinerea*

compound	$\text{ED}_{50}^a \pm \text{SD}$ ( $\mu\text{g/mL}$ )
anthrahydroquinones	
1	>200
2	>200
3	>200
4	114.3 $\pm$ 33.5
5	107 $\pm$ 0
6	82.4 $\pm$ 15.5
7	49.3 $\pm$ 0.2
8	>200
9	>200
anthraquinones	
10	70.1 $\pm$ 1.5
11	38.5 $\pm$ 0.6
12	40.4 $\pm$ 1.8
13	35.5 $\pm$ 1.4
iprodione	35.0 $\pm$ 1.5

<sup>a</sup> Estimation of median effective doses ( $\text{ED}_{50}$ ) was based on colony diameter measurements after 4 days of incubation.

diameters were measured daily. Results were expressed as effective concentration ( $\text{ED}_{50}$ ) (the concentration that reduced mycelial growth

by 50%) determined by regressing the inhibition of radial growth values (percent control) against the values of compounds concentration. Each experiment was done at least in triplicate.

**Effect on Mycelial Growth of *B. cinerea* in Liquid Medium.** The 4,4-dimethylanthracene-1,9,10(4H)-trione (**10**) was added dissolved in methanol at different concentrations (1, 5, 10, 20, or 40  $\mu\text{g/mL}$ ) to 125 mL Erlenmeyer flasks containing 20 mL of malt–yeast extract medium. The final methanol concentration was identical in control and treatments assays. After evaporation of solvent, the medium with or without test compound was inoculated with 1.6 mg (dry weight) of a suspension of ground mycelium of *B. cinerea*. After 3 days of incubation in static condition at 22 °C, the mycelium was filtered and dried at 100 °C for 1 h. Each experiment was done at least in triplicate.

**Effect on Germination of *B. cinerea* Conidia.** Conidial germination assays were carried out on microscope slides coated with soft agar medium (2 mm thickness). Compounds were added dissolved in methanol at a final concentration of 40  $\mu\text{g/mL}$ . Methanol was allowed to evaporate prior to inoculation. The slides were inoculated with dry conidia obtained from sporulated mycelia (1 week old), placed in a humid chamber (90% relative humidity), and incubated in the dark at 22 °C for 7 h. Conidial germination was determined directly on the slides at hourly intervals. The percentage of germination was estimated by counting the number of germinated conidia in five microscope fields each containing ~40 conidia. Conidia were judged to have germinated when the germ tube length was equal to or greater than conidial diameter. Each experiment was done at least in triplicate.

**Mechanism of Action of 4,4-Dimethylanthracene-1,9,10(4H)-trione (**10**) on *B. cinerea*.** **Lipid Peroxidation.** The degree of lipid peroxidation was estimated by the rate of malondialdehyde (MDA) production, as described previously by Lee et al. (4). The *B. cinerea* mycelia were treated for 2 or 48 h at 22 °C with compound **10** at different concentrations (40, 80, and 100  $\mu\text{g/mL}$ ) or with the commercial fungicide iprodione at 40  $\mu\text{g/mL}$ . The treated mycelia were collected by centrifugation at 5000g for 10 min. The fungal mycelium was pulverized in a mortar and pestle using liquid nitrogen. The MDA concentration was determined in the mycelium and in the incubation medium as described by Lee et al. (4).

**Determination of Free Radical Formation.** The formation of oxygen reactive species was assessed following a chemiluminescence technique (18). Ground mycelium of *B. cinerea* was added to a potassium phosphate buffer (20 mM, pH 7.0) containing compound **10** dissolved in methanol or control solutions (methanol or *tert*-butylhydroperoxide). The final concentration of compound **10** was 80  $\mu\text{g/mL}$ . The final methanol concentration was identical in control and treatment assays. The chemiluminescence emission was measured in a luminometer (Beckman LS 6500).

**Effect of 4,4-Dimethylanthracene-1,9,10(4H)-trione (**10**) on Cellular Leakage.** To analyze the cellular leakage, the efflux of phosphorus after treatment of the mycelium with compound **10** was determined.

Pregrown mycelia were incubated in 5 mM Tris-HCl buffer (pH 7.0) with compound **10** to a final concentration of 40  $\mu\text{g/mL}$  at 22 °C. Cellular leakage from the fungal mycelium was determined after 6 h of incubation at 22 °C by detection of phosphorus in the bathing medium, using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Optimo 200 DV ICP-OES, Perkin-Elmer).

## RESULTS AND DISCUSSION

**Effect of Anthraquinone and Anthrahydroquinone Derivatives on *B. cinerea*.** In this work we have characterized the effect of a series of synthetic anthraquinones and anthrahydroquinones on *B. cinerea*. The basic structural features of these compounds consist of a carbonyl group in the ortho position (C-1) with regard either to the phenolic function of hydroquinones or to the carbonyl group in the corresponding quinones. These compounds also incorporate a *gem*-dimethyl group at C-4, which resembles the substitution pattern of *tert*-butylquinone, a well-known compound that exhibits interesting biological activities (19).

In a previous paper we reported the activities of 4,4-dimethyl-5,8-dihydroxynaphthalene-1-one and a series of derivatives on the growth of mouse carcinoma TA3 and TA3-MTX-R cell lines (20). We suggested that the phenoxy radicals derived from these compounds, which show the structural characteristics mentioned above, remain inside the tumor cells in a range of concentrations sufficient to inhibit oxygen uptake. Considering that alkylation of the hydroquinone moiety should stabilize the semiquinone free radical presumably involved in the inhibition of cellular respiration, we screened another series that incorporated a third ring in the molecular structure, blocking the free positions of the aromatic ring.

To evaluate the antifungal activity of these compounds and the corresponding quinones, the effect on the mycelial growth on solid was determined (Table 1).

The most active compounds were the anthraquinones **11**–**13**. The ED<sub>50</sub> values of 38.5, 40.4, and 35.5, respectively, were comparable to those obtained with iprodione fungicide used as control.

The compounds having methyl and hydroxymethyl groups at ring A had no activity (compounds **2**, **3**, **8**, and **9**). On the contrary, anthraquinones and anthrahydroquinones that have the unsubstituted ring A or one or two methyl groups in the absence of a hydroxymethyl group (compounds **4**–**7** and **10**–**13**) showed antifungal activity. Therefore, the presence of a hydroxymethyl group seems to increase the polarity of the compounds, and this characteristic would reduce the activity of the molecules. The only exception was the anthrahydroquinone **1**, with two methyl groups in ring A, which had no activity.

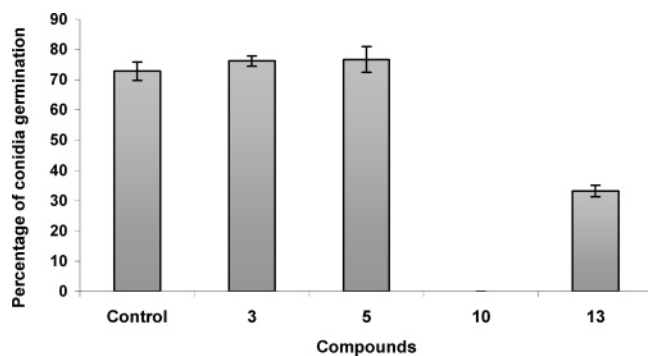
In general, the anthraquinones presented higher activity than the anthrahydroquinones of identical substitutions. Examples of this behavior are the pairs anthrahydroquinone **1** and anthraquinone **13**, anthrahydroquinone **7** and anthraquinone **13**, anthrahydroquinone **4** and anthraquinone **10**, and anthrahydroquinone **5** and anthraquinone **12**.

These results suggest that the structure of the anthraquinone is more adequate as antifungal agent against *B. cinerea*. It has been reported that quinones exhibit biological activities dependent on their structure, such as some natural quinones with anticancer activity (21) or anthraquinones with at least two phenolic groups in peri positions that acts as antitumor agents (22). Also, it has been shown that the type and position of the substitutions at the C ring of the anthraquinones isolated from *C. tora*, *R. emodi*, and *D. avenae* have an important role in the expression of antifungal activity (8–10).

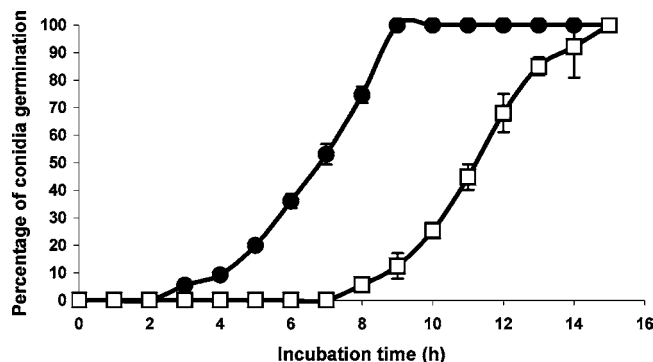
The effect of compounds **3**, **5**, **10**, and **13** on the germination of *B. cinerea* conidia after 7 h of incubation was also determined (Figure 2). Anthrahydroquinones **3** and **5** did not affect the germination, whereas anthraquinones **10** and **13** showed 100 and 67% inhibition, respectively. These results again suggest that the structure of the anthraquinones is important in exerting an antifungal effect on *B. cinerea*.

Additionally, the effect of compound **10** on the germination of the *B. cinerea* conidia was evaluated during 15 h (Figure 3). In the presence of compound **10**, germination started 5 h later than in the control, and after 15 h of incubation, 100% germination was attained. It was observed that compound **10** did not produce morphological changes in the germ tube (data not shown).

These results show that compound **10** affects the germination of the *B. cinerea* conidia significantly. Because germination is a very important early event in the infection process of this fungus (1), the following studies were carried out with this compound.



**Figure 2.** Effect of methanolic solutions (40  $\mu\text{g/mL}$ ) of compounds **3**, **5**, **10**, and **13** on germination of *B. cinerea* conidia. The control contained methanol at the same concentration as treatments. Each bar represents the mean of at least three independent experiments  $\pm$  standard deviation.



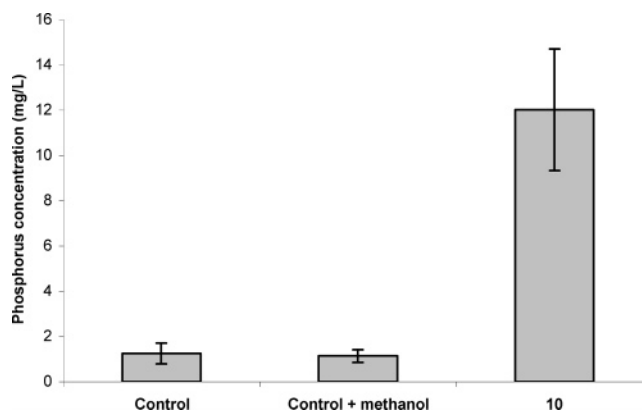
**Figure 3.** Effect of compound **10** on germination of *B. cinerea* conidia: control with methanol ( $\bullet$ ); in the presence of 40  $\mu\text{g/mL}$  of compound **10** ( $\square$ ). Compound was added dissolved in methanol. The solvent was allowed to evaporate prior to inoculation. The slides were inoculated with dry conidia, placed in a humid chamber, and incubated at 22  $^{\circ}\text{C}$ . Conidia germination was determined directly on the slides at hourly intervals. Each point represents the mean of at least three independent experiments  $\pm$  standard deviation.

The effect of different concentrations of compound **10** in liquid medium was also evaluated. In this medium, this compound produced a higher effect on mycelial growth than in solid medium. The  $\text{ED}_{50}$  was 28  $\mu\text{g/mL}$ .

The results of the present work show that anthraquinone compounds do not completely inhibit the mycelial growth and conidial germination of *B. cinerea*. These results could be explained by the ability of *B. cinerea* to biotransform the compounds to less active products. In fact, it has been reported that *B. cinerea* biotransforms compounds such as fungistatic agent (*R*)-(+)-1-(4'-chlorophenyl)propan-1-ol (23) and other secondary metabolites as terpenoids, flavonoids, saponins, etc. (17, 24, 25). The main reaction pathways involved hydroxylations of several positions, as well as condensations with secondary metabolites of the fungus (23).

**Mode of Action Experiments.** The mechanism of action of the antifungal anthraquinone is not known yet. To determine the possible mechanism of action of compound **10** on the fungus *B. cinerea*, the production of oxygen reactive species was analyzed. The results show that compound **10** did not produce lipoperoxidation of membrane and did not induce the formation oxygen reactive species (results not shown).

These results were in disagreement with other results obtained with some anthraquinone derivatives, which are an important class of anticancer drugs with peroxidating action and, furthermore, cardiotoxic properties (22, 26). These compounds possess



**Figure 4.** Effect of compound **10** on the phosphorus concentration in the extracellular medium of *B. cinerea*. Pregrowth mycelia were incubated in 5 mM Tris-HCl buffer (pH 7.0) with compound **10** at a final concentration of 40 mg/mL for 6 h at 22  $^{\circ}\text{C}$ . In control experiments, the fungus was maintained in the buffer. Phosphorus concentration was measured in the extracellular medium by ICP-OES. Each bar represents the mean of at least four independent experiments  $\pm$  standard deviation.

the ability to mediate the transfer of one electron to molecular oxygen to form the superoxide anion radical (19). The free radical formation is initiated by three oxidoreductases. The affinity of anthraquinones for these enzymes is an essential factor governing the rate of the one-electron transfer and the generation of oxygen radicals (21). On the other hand, the unsubstituted 4,4-dimethyl-(4*H*)anthracene-1,9,10-trione exhibits potent lytic activity in vitro against *Trypanosoma cruzi* and several *Leishmania* species (unpublished results). Previous studies show that this compound is reduced by microsomal NADPH-cytochrome P-450 reductase. The anion radical was detected by studies of electron spin resonance spectroscopy (ESR) (27).

The reduction of quinones or the oxidation of hydroquinones to semiquinones has been related to biological properties such as quinone cytotoxicity, antitumor activity, the functioning of the antioxidant, and the inhibition of 5-lipoxygenase (21).

Finally, the effect produced by compound **10** on the membrane permeability of *B. cinerea* was tested. To analyze this effect, the efflux of phosphorus from mycelium treated with compound **10** was measured. **Figure 4** shows that in the extracellular medium, the phosphorus concentration was 12 times higher in treated cultures than in the control with or without methanol. Therefore, compound **10** acts by causing changes in the permeability of the cell membrane of *B. cinerea*. This membrane dysfunction could occur by interaction of the compound with some membrane constituents or by interaction with some metabolic process.

On the other hand, germination assays are also very useful for evaluating the mechanism of action of antifungal compounds. For example, members of the strobilurin class of fungicides, which block the electron transport chain, are extremely potent inhibitors of spore germination but much less active as inhibitors of mycelial growth (28). Compound **10** was more active as an inhibitor of germination with a 100% inhibition at 7 h of incubation, whereas this compound was less active as an inhibitor of mycelial growth. These results suggest that compound **10** might act on the electron transport chain, producing mycelium lysis. This hypothesis must be demonstrated.

## LITERATURE CITED

- (1) Jarvis, W. R. Epidemiology. In *The Biology of Botrytis*; Coley-Smith, J. R., Verhoeff, K., Jarvis, W. R., Eds.; Academic Press: London, U.K., 1980; pp 219–250.
- (2) Latorre, B.; Flores, V.; Sara, A. M.; Roco, A. Dicarboximide-resistant strains of *Botrytis cinerea* from table grapes in Chile: survey and characterization. *Plant Dis.* **1994**, *7*, 990–994.
- (3) Yarden, O.; Katan, T. Mutations leading to substitutions at amino acids 198 and 200 of  $\beta$ -tubulin that correlate with benomyl-resistance phenotypes of field strains of *Botrytis cinerea*. *Phytopathology* **1993**, *83*, 1478–1483.
- (4) Lee, H.; Choi, G.; Cho, K. Correlation of lipid peroxidation in *Botrytis cinerea* caused by dicarboximide fungicides with their fungicidal activity. *J. Agric. Food Chem.* **1998**, *46*, 737–741.
- (5) Gullino, M.; Leroux, P.; Smith, C. Uses and challenges of novel compounds for plant disease control. *Crop Prot.* **2000**, *19*, 1–11.
- (6) Milling, R. J.; Richardson, C. J. Mode of action of the anilinopyrimidine fungicide pyrimethanil. 2. Effects on enzyme secretion in *Botrytis cinerea*. *Pestic. Sci.* **1995**, *45*, 43–48.
- (7) Leroux, P.; Lanen, C.; Fritz, R. Similarities in the antifungal activities of fenpiclonil, iprodione and tolclofosmethyl against *Botrytis cinerea* and *Fusarium nivale*. *Pestic. Sci.* **1992**, *36*, 255–261.
- (8) Agarwal, S. K.; Singh, S.; Verma, S.; Kumar, S. Antifungal activity of anthraquinones derivatives from *Rheum emodi*. *J. Ethnopharmacol.* **2000**, *72*, 43–46.
- (9) Chrysai-Tokousbalides, M.; Kastanias, M. A. Cynodontin: a fungal metabolite with antifungal properties. *J. Agric. Food Chem.* **2003**, *51*, 4920–4923.
- (10) Kim, Y. M.; Lee, C. H.; Kim, H. G.; Lee, H. S. Anthraquinones isolated from *Cassia tora* (Leguminosae) seed show an antifungal property against phytopathogenic fungi. *J. Agric. Food Chem.* **2004**, *52*, 6096–6100.
- (11) Valderrama, J. A.; Araya-Maturana, R.; Zuloaga, F. Studies on quinones part 27. The Diels–Alder reaction of 8,8-dimethylnaphthalene-1,4,5(8H)-trione. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1103–1107.
- (12) Araya-Maturana, R.; Cassels, B. K.; Delgado-Castro, T.; Valderrama, J. A.; Weiss-Lopez, B. Regioselectivity in the Diels–Alder reaction of 8,8-dimethylnaphthalene-1,4,5(8H)-trione with 2,4-hexadien-1-ol. *Tetrahedron* **1999**, *55*, 637–648.
- (13) Araya-Maturana, R.; Delgado-Castro, T.; Cardona, W.; Jullian, C. Complete assignment of the  $^{13}\text{C}$  NMR spectra of 4,4-dimethylantracene-1,9,10(4H)-trione and their regioisomeric monomethyl derivatives. *Magn. Reson. Chem.* **2000**, *38*, 135–136.
- (14) Cardona, W. Efecto del Puente de hidrógeno intermolecular en la regio y diastereoselectividad de la cicloadición Diels–Alder de dienos hidroxilados en posición alílica y quinonas bicíclicas 5-oxosustituidas. Ph.D. Thesis, Universidad de Chile, 2004.
- (15) LaMondia, J. A.; Douglas, S. M. Sensitivity of *Botrytis cinerea* from Connecticut greenhouses to benzimidazole and dicarboximide fungicides. *Plant Dis.* **1997**, *81*, 729–732.
- (16) Cotoras, M.; Folch, C.; Mendoza, L. Characterization of the antifungal activity on *Botrytis cinerea* of the natural diterpenoids kaurenoic acid and  $\beta$ 3-hydroxy-kaurenoic acid. *J. Agric. Food Chem.* **2004**, *52*, 2821–2826.
- (17) Aleu, J.; Hanson, J.; Hernández-Galán, R.; Collado, I. Biotransformation of the fungistatic sesquiterpenoid patchoulol by *Botrytis cinerea*. *J. Nat. Prod.* **1999**, *62*, 437–440.
- (18) García-Campaña, A. M.; Baeyens, W. R. G.; Zhang, X.; Alés, F.; Gamiz, L. Unfamiliar though exciting analytical detection in flowing stream: chemiluminescence. *Ars Pharm.* **2001**, *42* (1), 81–107.
- (19) Okubo, T.; Yokoyama, Y.; Kano, K.; Kano, I. Cell death induced by the phenolic antioxidant *tert*-butylhydroquinone and its metabolite *tert*-butylquinone in human monocytic leukemia U937 cells. *Food Chem. Toxicol.* **2003**, *41*, 679–688.
- (20) Araya-Maturana, R.; Delgado-Castro, T.; Garate, M.; Ferreira, J.; Pavani, M.; Pessoa-Mahana, H.; Cassels, B. Effects of 4,4-dimethyl-5,8-dihydroxynaphthalene-1-one and 4,4-dimethyl-5,8-dihydroxytetralone derivatives on tumor cell respiration. *Bioorg. Med. Chem.* **2002**, *10*, 3057–3060.
- (21) Ravelo, A. G.; Estevez-Braun, A.; Chavez-Orellana, H. E.; Pérez-Sacau, D.; Mesa-Siverio. Recent studies on natural products as anticancer agents. *Curr. Top. Med. Chem.* **2004**, *4* (2), 241–265.
- (22) Tarasiuk, J.; Tkaczyk-gobis, K.; Stefańska, B.; Dzieduszycka, M.; Priebe, W.; Martelli, S.; Borowski, E. The role of structural factors of anthraquinone compounds and their quinone-modified analogues in NADH dehydrogenase-catalysed oxygen radical formation. *Anti-Cancer Drug Design* **1998**, *13*, 923–939.
- (23) Bustillo, A.; Aleu, J.; Hernández-Galán, R.; Collado, I. Biotransformation of the fungistatic compound (*R*)-(+)-1-(4'-chlorophenyl)propan-1-ol by *Botrytis cinerea*. *J. Mol. Catal. B: Enzymatic* **2003**, *21*, 267–271.
- (24) Quidde, T.; Osbourn, A.; Tudzynski, P. Detoxification of  $\alpha$ -tomatine by *Botrytis cinerea*. *Physiol. Mol. Plant Pathol.* **1998**, *52*, 151–165.
- (25) Farooq, A.; Tahara, S. Biotransformation of two terpenes,  $\alpha$ -santonin and sclareol by *Botrytis cinerea*. *Z. Naturforsch.* **2000**, *55*, 713–717.
- (26) Jeziorek, D.; Ossowski, T.; Liwo, A.; Dyl, D.; Nowacka, M.; Woznicki, W. Theoretical and electrochemical study of the mechanism of anthraquinone-mediated one electron reduction of oxygen: the involvement of adducts of dioxygens species to anthraquinones. *J. Chem. Soc., Perkin Trans. 2* **1997**, 229–236.
- (27) Olea-Azar, C.; Mendizábal, F.; Alarcón, J.; Briones, R.; Cassels, B.; Delgado-Castro, T.; Araya-Maturana, R. Electron spin resonance studies and theoretical quantum calculations of free radicals generated from anthracenetrione by electrochemical and microsomal reduction. *Spectrochim. Acta Part A* **2001**, *57*, 1889–1895.
- (28) Slawacki, R.; Ryan, E.; Young, D. Novel fungitoxic assay for inhibition of germination-associated adhesion of *Botrytis cinerea* and *Puccinia recondite* spores. *Appl. Environ. Microbiol.* **2002**, *68*, 597–601.

---

Received for review May 20, 2005. Revised manuscript received September 15, 2005. Accepted September 21, 2005. This research was supported by the Departamento de Investigaciones Científicas y Tecnológicas (Dicyt) of the Universidad de Santiago de Chile, by the International Foundation for Science Grants F/3115-1 and C/2807-2, and by FONDECYT 1030916.

JF0511749