DOI:10.1021/jf9037369

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Primary Study on Mode of Action for Macrocyclic Fungicide Candidates (7B3, D1) against *Rhizoctonia solani* Kühn†

Xiaojing Yan,[§,#] Xiaomei Liang,[§] Shuhui Jin,[§] Jinping Lv,[§] Chunxin Yu,[§] Wenyan Qi,[§] Baoju Li,[⊥] Huizhu Yuan,*[,#] Shuhua Qi,[#] Yanxia Shi,[⊥] Jingping Wu,[§] Fuheng Chen,[§] and Daoquan Wang*[,§]

[§]Key Laboratory of Pesticide Chemistry and Application Technology, Department of Applied Chemistry, China Agricultural University, Beijing 100193, People's Republic of China, [#]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, People's Republic of China, and [⊥]Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China

A novel macrolactam fungicide candidate (**7B3**) and a novel aza-macrolactone fungicide candidate (**D1**) were designed and synthesized, and the bioassay showed that both displayed excellent fungicidal activity against *Rhizoctonia solani* Kühn. To elucidate the biochemical mode of action of the two compounds against *R. solani* and illustrate the similarities and differences of action mechanism resulting from subtle differences in structure of the two compounds, the effects of the two compounds on the ultrastructure of hyphae, electrolyte leakage, and respiration of mycelia cell suspension caused by **7B3** or **D1** were studied. The results showed that the two compounds had very similar modes of action. Both induced irregular swelling of hyphae, vacuolation of cytoplasm, and thickening of cell wall. The conductivity of mycelia cell suspension increased in the presence of **7B3** or **D1**, which indicated that the two compounds had a similar effect on cell membrane permeability. In addition, both **7B3** and **D1** were insufficient in inhibiting the respiration of mycelia.

KEYWORDS: 7B3; D1; *Rhizoctonia solani* Kühn; mode of action; ultrastructure; conductivity; respiration

INTRODUCTION

In the search for potential pesticides, more than 10 series of compounds have been designed and synthesized by taking the natural macrolactams or macrolactones (*1*−*3*) as templates, and their biological activities have been evaluated in our laboratory. Relationships between structure and activity were evaluated, and the results showed that the compounds with high biological activity possessed the same structural characteristics (*4*−*7*): (a) coexistence of two polarizable groups on the ring, one polarizable group being a hydrogen-bonding donor (NH or −CONH−) and the other polarizable group being a hydrogen-bonding acceptor (C═O or ═N—O−); (b) having two or three methylenes' distance between the two polarizable groups; (c) nonexistence of any groups greater than a hydrogen atom or an active hydrogen between the two polarizable groups.

In previous papers (*5*, *7*), we reported two series of macrolactam and macrolactone derivatives that showed good fungicidal activity against *Rhizoctonia solani*. Among them, **7B3** (12-propyloxyimino-1,15-pentadecanlactam) (*7*, *8*) and **D1** (13-aza-1,15-cyclopentadecanolide fluoroborate) (*5*, *9*)) had the best fungicidal activities (**Figure 1**). **7B3** was prepared from 12-oxopentadecanlactam by reaction with hydroxylamine hydrochloride and subsequent alkylation using 1-chloropropane; **D1** was prepared from cyclododecanone by reaction with 2-azidoethanol in the solution of boron trifluoride etherate. **7B3** has high fungicidal activities against *Alternaria kikuchiana*, *Phyllospicpa physaleos* Sacc., *R. solani*, and *Botrytis cinerea* Pers. with EC_{50} values of 1.2, 1.9, 4.6, and 8.6 μg mL^{-1}, respectively (*7*). The inhibition rate of **D1** against *R. solani*, *Fulvia fulva* (Cooke) Ciferri, *Colletotrichum orbiculare* (Berk. et Mont.) Arx, *Verticillium dahliae* Kleb., *Sclerotinia sclerotiorum* (Lib.) de Bary, and *Alterneria kikuchiarna* Tanaka in vitro were 96, 89.3, 73.2, 86.9, 85.7, and 85.7%, respectively (*5*).

In two-year−four-place field efficacy trials, the prevention of *R. solani* on cotton by **7B3** reached 64−92% at a rate of 140 g of ai ha^{-1} and was better than or comparable to the commercial fungicide carbendazin at the same dose (*10*); meanwhile, **D1** was more effective than the commercial fungicide carbendazim against *R. solani* both in pot culture experiments and in field efficacy trials (*5*). Furthermore, the acute toxicity of **7B3** is low (LD_{50} > 5000 mg kg^{-1} for acute oral and LD_{50} > 2000 mg kg^{-1} for acute dermal), and the teratogenesis, mutagenesis, and carcinogenesis tests were negative(*10*). The acute toxicity of **D1** is also low (LD_{50} = 3160 mg kg^{-1} for acute oral and LD_{50} > 2000 mg kg^{-1} for acute dermal), and the Ames tests were all negative (*5*). Therefore, both **7B3** and **D1** were low-toxicity compounds and safe to human beings on the basis of the classification standard procedure of the People's Republic of China (*5*, *11*), and they could be further developed to practical fungicides. With the growing resistance of phytopathogens to commercially available fungicides, the continuing need for further research to discover new classes of fungicides, especially those with novel modes of action, is more and more urgent. Consequently, it is imperative to study the biological mode of action of novel fungicides. Therefore, the preliminary mode of action of **7B3** and **D1** against *R. solani*

†Part of the ECUST-Qian Pesticide Cluster.

*Authors to whom correspondence should be addressed [e-mail (D.W.) wangdq@cau.edu.cn and (H.Y.) hzhyuan@gmail.com].

 Published on Web 01/05/2010

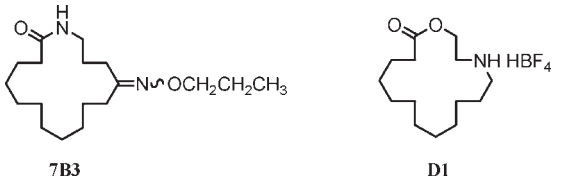

Figure 1. Chemical structures of **7B3** and **D1**.

and the similarities and differences of their action mechanisms resulting from subtle differences in structure of the two compounds were studied for this paper.

MATERIALS AND METHODS

Chemicals and Reagents. **7B3** and **D1** were synthesized in our laboratory (with a purity of >99% (HPLC-MS)). Triadimefon (with a purity of 95%) was provided by Shanghai Pesticide Factory Co., Ltd. Azoxystrobin (with a purity of 95%) was provided by Syngenta Co., Ltd. (Beijing, China). All other chemicals and reagents were of analytical reagent grade and purchased from Beijing Chemical and Reagent Ltd., Beijing, China. All aqueous solutions were prepared using distilled water.

Pathogens and Cultures. *R. solani* was provided by Shandong Academy of Agricultural Sciences, Shandong, China, and maintained on potato dextrose agar (PDA) (potato infusion from 200 g L^{-1}, 20 g L^{-1} dextrose, and 18 g L^{-1} agar) in the dark at 25 °C.

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). *SEM*. *R. solani* mycelia tips (5 mm) from the margins of an actively growing colony on PDA medium amended with 0 and 50 μg mL^{-1} **7B3** or **D1** were cut from the edge of the colony after being cultured for 3 days and treated with 4% of glutaraldehyde at 4 °C, then rinsed with 0.1 M phosphate buffer (pH 7.3), and fixed with 1% w/v osmium tetraoxide solution. After being rinsed with 0.1 M phosphate buffer three times, the mycelium block was dehydrated using a series of acetone solutions in the following order of concentration: 30, 50, 70, 80, and 90% and anhydrous acetone. The processes of drying at critical point, mounting, and gold spraying were completed last and examined in a scanning electron microscope S-3400N (Hitachi, Nissei Sanyo, Japan) with an accelerating voltage of 18−20 kV.

TEM. The mycelial blocks were prepared according to the method given under SEM. After dehydrating and embedding in Epon 112, thin sections were cut and double-stained with uranyl acetate and lead citrate. The grids were examined with a JEOL-1230 (JEOL, Japan) transmission electron microscope.

Electrolyte Leakage. Mycelial disks (5 mm in diameter) of *R. solani* grown on PDA plates were cut from the margins of the colony and were placed in PD liquid medium with 200 rpm shaking for 3 days. The mycelia were collected in culture medium and washed three times with sterile distilled water and subsequently filtered and weighed. Stock solutions of **7B3**, **D1**, and triadimefon were diluted with sterile distilled water to 50 μg mL^{-1}, respectively. After the addition of 1 g of fresh mycelia into the different solutions mentioned above, the conductivity of the solutions was measured using a DDS-11C model conductivity detector (Beijing Hua Rui Bo Yuan S&T Development Co. Ltd., Beijing, China) at 0, 1, 4, 8, 16, 22, 24, 36, 48, and 60 h after treatment. The experiment was repeated twice with three replicates per treatment.

Respiration Measurement. Mycelia plugs of *R. solani* from 3-day-old colony margins were transferred to conical flasks containing 150 mL of PD liquid medium for shake culture (25 °C, 150 rpm). After 5 days, mycelia were washed three times with 50 mM potassium phosphate buffer (pH 7.2) and resuspended in 0.1 M phosphate buffer (pH 7.2), containing 2% glucose (50 mg fresh weight of mycelia mL^{-1}) (*12*). The mycelia suspension was treated with 10 or 100 μg mL^{-1} **7B3** or **D1** and 10 μg mL^{-1} azoxystrobin, respectively. All measurements were carried out at room temperature (25 ± 1 °C). The ratio of the mycelia oxygen consumption was tested by oxygraphy (DW1, Hansatech Instruments Ltd., U.K.) and the inhibition of respiration was calculated using the formula

$$I_R = (R_0 - R_1)/R_0 \times 100$$

where I_R is the inhibition of respiration (%) and R_0 and R_1 are the ratios of mycelial oxygen uptake pre- and post-addition of fungicides (nmol of O_2/min/mg of mycelia). The experiment was repeated twice with three replicates per treatment. Results were analyzed statistically using the Statistical Program for Social Science (SPSS).

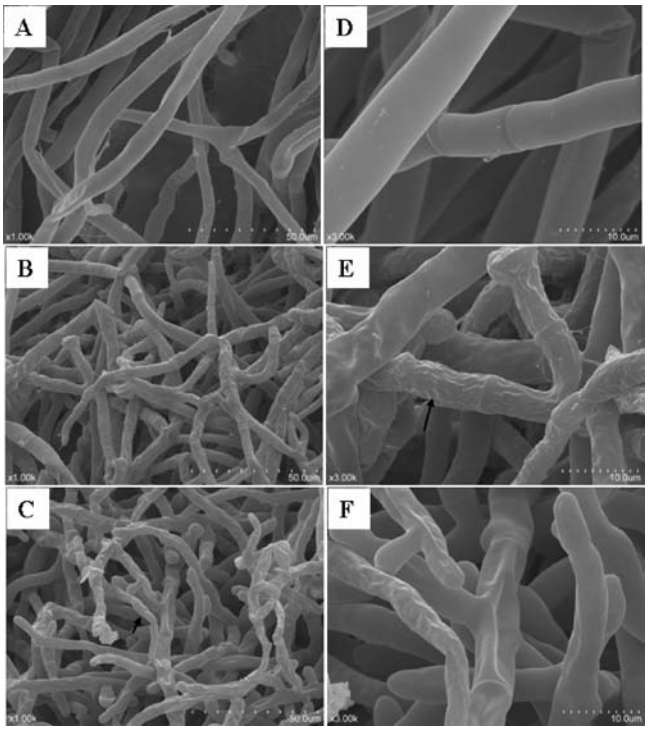

Figure 2. Scanning electron micrographs of the hyphae from the colony of *R. solani*: (**A**, **D**) 1000 (**A**) and 3000 (**D**) sections of *R. solani* hyphae grown on PDA medium in the absence of **7B3** or **D1** (control) (the mycelium was low density and fresh and had a fine structure); (**B**, **E**) 1000 (**B**) and 3000 (**E**) sections of *R. solani* hyphae grown on PDA medium containing 50 μg mL^{-1} **7B3** (the mycelium was comparatively high density of colony, and the surface of the mycelium was rough (arrowhead)); (**C**, **F**) 1000 (**C**) and 3000 (**F**) sections of *R. solani* hyphae grown on PDA medium containing 50 μg mL^{-1} **D1** (the amount of ramification increased and formed irregular ramification or abnormal configuration ("beaded" morphology) on the mycelium tip (arrowhead)). Bars: (**A**−**C**) 50.0 μm; (**D**−**F**) 10.0 μm.

RESULTS AND DISCUSSION

Effect of 7B3 and D1 on Morphology and Ultrastructure Transformation of *R. solani*. Mycelia of *R. solani* grew smoothly along the surface of culture media without **7B3** or **D1**, and the shape of the whole colony appeared to radiate from its central point. The fringe of the colony was round and regular. However, the growth of mycelia was seriously depressed during culture in media with 50 μg mL^{-1} **7B3** or **D1**. The fringe of the colony was concavo-convex and irregular and is not as smooth and regular as that of control mycelia.

SEM images indicated that the mycelial grew well in control media (*13*, *14*), that it was of low density and fresh and had a fine structure, and that most of the mycelial ramification occurred in a right angle (**Figure 2A**,**D**). However, there were similar morphology changes in mycelia of *R. solani* when mycelia were cultured in culture media with **7B3** or **D1** of 50 μg mL^{-1}. Mycelia grew abnormally with comparatively high density of colony, the ramification was not in a right angle any more, the distance between ramifications decreased, and some mycelia were entangled with each other (**Figure 2B**,**C**). In the presence of **7B3** or **D1**, the surface of the mycelia was rough (**Figure 2B**,**E**) and mycelia were irregularly ramified and formed irregular ramification or abnormal configuration,

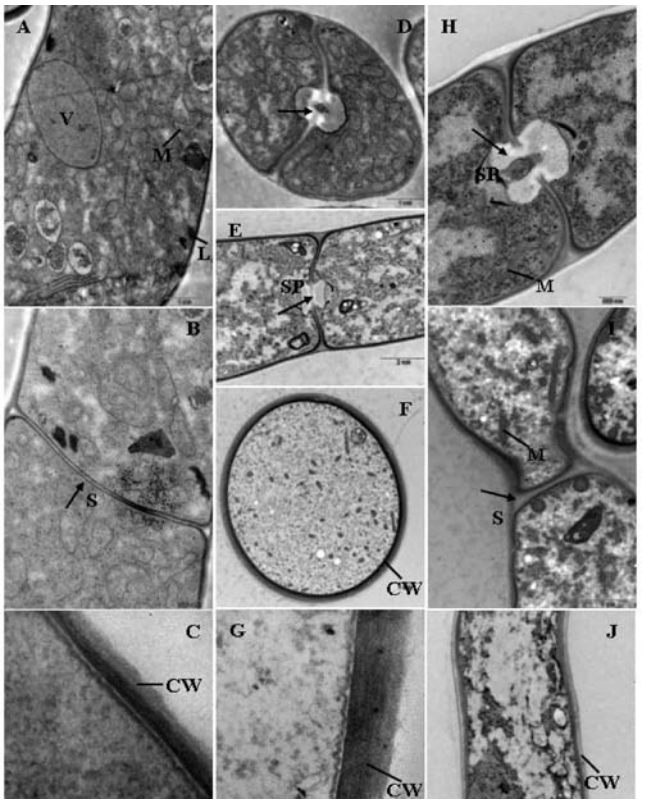

Figure 3. Transmission electron micrographs of *R. solani* hyphae: (**A**−**D**) TEM of the hyphae of *R. solani* in the untreated control ((**A**) longitudinal of control hyphae; many organelles were observed such as vacuole (V), mitochondria (M), and lipid body (L)); (**B**) uniform spectra (S) of control hyphae; (**C**) cell wall of control hyphae; (**D**) transverse of control hyphae and septal pore caps was visible; (**E**, **F**, **I**, **J**) TEM of the hyphae of *R. solani* treated with 50 μg mL^{-1} **D1** ((**E**) longitudinal of **D1**-treated hyphae (the septal pore caps isappeared); (**F**) transverse of **D1**-treated hyphae (cell wall thickening); (**I**) longitudinal of **D1**-treated hyphae (septum of hyphae thickening); (**J**) longitudinal of **D1**-treated hyphae (cell wall thickening)); (**G**, **H**) TEM of the hyphae of *R. solani* treated with 50 μg mL^{-1} **7B3** ((**G**) cell wall thickening of **7B3**-treated hyphae; (**H**) longitudinal of **7B3**-treated hyphae (the septal pore caps were almost unaffected). Bars: (**A**, **D**, **E**, **F**, **I**, **J**) 1.0 μm; (**B**, **H**) 500 nm; (**C**, **G**) 100 nm.

producing a "beaded" morphology with some parts of the mycelia contracted and some parts swelled (**Figure 2C**).

R. solani mycelial tips (5 mm) from the margins of an actively growing colony on PDA medium were examined by TEM (**Figure 3**). The cell walls and septa of the hyphae from the untreated control were uniform (**Figure 3B**,**C**). There were abundant organelles in cytoplasm such as vacuole (V), mitochondria (M), and lipid body (L) (**Figure 3A**). The dolipore septa and septal pore caps (SPCs) were obviously visible in control mycelia (**Figure 3D**). Following fungicide treatment, different ultrastructural modifications occurred in the hyphae (**Figure 3G**,**H** ultrastructure treated with **7B3**; **Figure 3E**,**F**,**G**,**I**,**J** ultrastructure treated with **D1**). The cell walls of the hyphae became considerably thicker following exposure to either **7B3** or **D1** (**Figure 3F**,**G**). The walls of the septa were also abnormally thickened (**Figure 3I**).

Under treatment with **7B3** and **D1**, the organelles became disorganized and decreased in the hyphae cytoplasm (**Figure 3F**,**J**). Another striking characteristic was the disappearance of septal pore caps of **D1**-treated hyphae (**Figure 3E**), whereas the septal pore caps were almost unaffected in **7B3**-treated hyphae (**Figure 3H**).

SEM and TEM observations revealed that growth inhibition of *R. solani* as a response to **7B3** and **D1** was accompanied by marked morphological and cytological changes, including irregular ramification and a "beaded" morphology, excessive branching, irregular thickening of hyphae cell walls, and necrosis or degeneration of hyphae cytoplasm. These changes were very similar to those occurring in some other fungi treated with chitosan and antibiotics, which inhibited fungi cell wall (*15*, *16*). The cell wall of fungi is a sturdy structure providing physical protection and osmotic support, which is considered as that complex of macromolecules with chitin, glucan, and mannose interconnected by covalent bonds. Hyphae growth, branching, cell fusion, and other morphogenetic events all depended on a balance between decomposition and extension of the hyphae wall, as well as on synthesis and incorporation of new wall material (*17*, *18*). In the present study, the hyphae walls of *R. solani* were thickened irregularly and there was excessive branching of the hyphae, which were very similar to the phenomena induced by ergosterol biosynthesis inhibitor (EBI) fungicides (*18*), although there are great differences in chemical structure between **7B3** or **D1** and EBI fungicides. Therefore, it was also assumed that the thickness of the wall and excessive branching might result from the changes in the activity of enzymes involved in wall synthesis. Another phenomenon observed in TEM study was the thickness of septa and disappearance of septal pore caps of **D1**-treated hyphae. More studies should be done to interpret whether the phenomenon was in relation to cell wall associated enzymes.

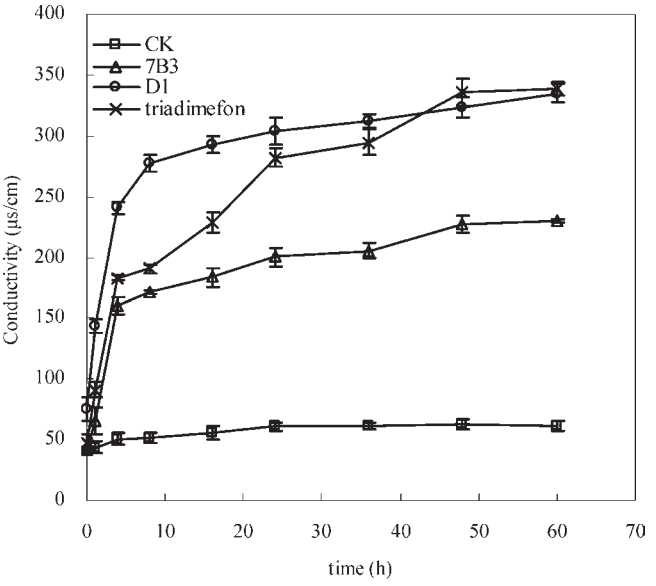

Figure 4. Electrolyte leakage from *R. solani* suspensions during different time exposures to different fungicides: blank control (□); **7B3** (△); **D1** (○); triadimefon (×). The conductivity of the solutions was measured using a DDS-11C model conductivity detector at different treatment times after the addition of 1 g of fresh mycelia into 50 μg mL^{-1} **7B3**, **D1**, and triadimefon solution, respectively.

Electrolyte Leakage. To examine the effect of **7B3** and **D1** on cell membrane, electrical conductivity of mycelia suspension was measured using the commercial fungicide triadimefon as a positive control (**Figure 4**). The conductivity of mycelia suspension treated with **7B3**, **D1**, and triadimefon all increased extremely compared with the conductivity of control mycelia during the entire time of treatment. **D1** induced more significant electrolyte leakage from hyphae than **7B3** and a change similar to that with triadimefon. Thus, it was proposed that **7B3** and **D1** were related to the impairment of cell membrane.

Electrolyte leakage was used as an indicator of cell membrane permeability of hyphae exposed to various fungicides. The

Table 1. Respiratory Inhibition of Intact Mycelia of *R. solani* by **7B3** and **D1**[a]

inhibitor	concentration (μg mL^{-1})	R_0 (μmol of O_2 g^{-1} min^{-1})	R_1 (μmol of O_2 g^{-1} min^{-1})	inhibition rate[b] (%)
7B3	10	27.03 ± 1.39	26.30 ± 0.85	3.39 ± 0.62
	100	27.03 ± 1.39	23.95 ± 7.49	12.05 ± 5.50
D1	10	27.03 ± 1.39	26.77 ± 1.39	1.67 ± 1.02
	100	27.03 ± 1.39	26.41 ± 2.45	3.00 ± 1.80
azoxystrobin	10	27.03 ± 1.39	4.12 ± 1.80	84.86 ± 1.33

[a] The mycelia respiration of *R. solani* was determined by treating the mycelia suspension with different concentration inhibitors. [b] Mean and SD of inhibition of respiration (n = 3).

alteration of conductivity induced by **7B3** and **D1** resembled the alteration caused by triadimefon, one of a class of azole compounds that can inhibit ergosterol biosynthesis and damage the permeability of cell membrane (*19*). These results indicated that both **7B3** and **D1** caused damage to the mycelia cell membrane system and induced electrolyte leakage from the cell and, as a result, the conductivity of the solution was increased. Sterols are required for growth and reproduction of eukaryotic organisms and serve as architectural components of membranes. Thus, the thickening of hyphae walls was most likely to be associated with biochemical changes in the plasmalemma induced by **7B3** and **D1**. In addition, the phenomenon that **D1** induced greater morphological, cytological, and conductivity alterations than **7B3** probably can be explained by the solubility difference between **D1** and **7B3**, because **D1** was a tetrafluoroborate possessing better solubility in water than **7B3**.

Effect on Respiration of Intact Mycelia. The effects of **7B3**, **D1**, and azoxystrobin on oxygen consumption of intact mycelia are shown in **Table 1**. Both **7B3** and **D1** almost did not affect oxygen consumption of intact mycelia, with 3.39% inhibition at 10 μg mL^{-1} and 12.05% inhibition at 100 μg mL^{-1} of **7B3** and 1.67% inhibition at 10 μg mL^{-1} and 3.00% inhibition at 100 μg mL^{-1} of **D1**, whereas azoxystrobin, a respiration inhibitor, exhibited a strong effect on oxygen consumption of intact mycelia with 84.86% inhibition at 10 μg mL^{-1}. These results proved that neither **7B3** nor **D1** was a respiration inhibitor and indicated that they did not disturb the energy generation system of *R. solani*.

In conclusion, the result of our research showed that both **7B3** and **D1** caused marked changes of hyphae with a "beaded" morphology, excessive branching, irregular thickening of hyphae cell walls, and necrosis or degeneration of hyphae cytoplasm and electrolyte leakage of membrane. In addition, neither **7B3** nor **D1** affected the respiration of mycelia. These results suggested that **7B3** and **D1** had similar modes of action against *R. solani* relating to the impairment of biosynthesis of cell wall or membrane and further supported our initial proposal of structure−activity relationship. This primary study set a good stage for further research, which should be done to elucidate the specific action site of **7B3** and **D1** against *R. solani* and the subtle difference between **7B3** and **D1** in mode of action.

ACKNOWLEDGMENT

We thank Prof. Zhengsheng Kang of College of Plant Protection Northwest A&F University for analyzing the figures of SEM and TEM.

LITERATURE CITED

(1) Gunasekera, S. P.; Gunasekera, M.; McCarthy, P. Discodermide: a new bioactive macrocyclic lactam from the marine sponge *Discodermia dissoluta*. *J. Org. Chem.* **1991**, *56*, 4830–4833.

(2) Jakobi, M.; Winkelmann, G. Maltophilin: a new antifimgal compound produced by *Stenotvophomonas maltophilia* R3089. *J. Antibiot.* **1996**, *49* (11), 1101–1104.

(3) Kupchan, S. M.; Komoda, Y.; Court, W. A.; Thomas, G. J.; Smith, R. M.; Karim, A.; Gilmore, C. J.; Haltiwanger, R. C.; Bryan, R. F. Tumor inhibitors. LXXIII. Maytansine, a novel antileukemic ansa macrolide from *Maytenus ovatus*. *J. Am. Chem. Soc.* **1972**, *94*, 1354–1356.

(4) Meng, X. Q.; Zhang, J. J.; Liang, X. M.; Zhu, W. J.; Dong, Y. H.; Wu, X. M.; Huang, J. X.; Rui, C. H.; Fan, X. L.; Chen, F. H.; Wang, D. Q. Synthesis and herbicidal activity of 12-(aryloxyacyloxyimino)-1,15-pentadecanlactone derivatives. *J. Agric. Food Chem.* **2009**, *57*, 610–617.

(5) Dong, Y. H.; Liang, X. M.; Yuan, H. Z.; Chen, F. H.; Wang, D. Q. Potential green fungicide: 16-oxo-1-oxa-4-azoniacyclohexadecan-4-ium tetrafluoroborate. *Green Chem.* **2008**, *10* (9), 990–994.

(6) Zhu, W. J.; Wu, P.; Liang, X. M.; Dong, Y. H.; Zhang, J. J.; Yuan, H. Z.; Qi, S. H.; Meng, X. Q.; Wu, J. P.; Chen, F. H.; Wang, D. Q. Design, synthesis, and fungicidal activity of macrolactones and macrolactams with a sulfonamide side chain. *J. Agric. Food Chem.* **2008**, *56*, 6547–6553.

(7) Huang, J. X.; Jia, Y. M.; Liang, X.; Zhu, W.; Zhang, J.; Dong, Y.; Yuan, H.; Qi, S.; Wu, J.; Chen, F.; Wang, D. Synthesis and fungicidal activity of macrolactams and macrolactones with an oxime ether side chain. *J. Agric. Food Chem.* **2007**, *55*, 10857–10863.

(8) Wang, D. Q.; Liang, X. M.; Li, C. S.; Xu, Z. T.; Wu, J. P.; Huang, J. X.; de Yuan, K.; Li, L.; Hou, C. J.; Jia, Y. M.; Wang, R. G. Method for preparation of macrolactam fungicide. CN 1939908, 2007.

(9) Wang, D. Q.; Liang, X. M.; Dong, Y. H.; Chen, F. H.; Zhng, L. P.; Li, C. S.; Xu, Z. T.; Li, L. Fungicidal 13-aza-1,15-cyclopentadecanolide fluoborate. CN 101027992, 2007.

(10) Huang, J. X.; Liang, X. M.; Zhang, J. J.; Yan, X. J.; Dong, Y. H.; Li, C. S.; Zhang, L. P.; Xu, T. Z; Li, L.; Yuan, H. Z.; Qi, S. H.; Chen, F. H.; Wang, D. Q. Fungicidal activity of 12-propoxyimino-1,15-pentadecanlactam on selected crops. *Crop Prot.* **2009**, *28*, 947–951.

(11) China, S. A. O. T. Standard GB 15670-1995, Toxicological test methods of pesticides for registration in Beijing, 1995.

(12) Chen, Y.; Jin, L. H.; Zhou, M. G. Effect of azoxystrobin on oxygen consumption and cyt b gene expression of colletotrichum capsici from chilli fruits. *Agric. Sci. China* **2009**, *8* (5), 628–631.

(13) Mu, K. G.; Zhao, X. Q.; Hu, L.; Zhang, F. S.; Zhang, W. J.; Cui, J. Y. Toxicity of lanthanum to pathogenic fungi and its morphological characteristics. *J. Rare Earths* **2006**, *24*, 607–612.

(14) Butler, E. E.; Bracker, C. E. *Morphology and Cytology of R. solani*; University of California: Berkeley, CA, 1970; pp 32−50.

(15) Vesentini, D.; Steward, D.; Singh, A. P.; Ball, R.; Daniel, G.; Franich, R. Chitosan-mediated changes in cell wall composition, morphology and ultrastructure in two wood-inhabiting fungi. *Mycol. Res.* **2007**, *111* (8), 875–890.

(16) Debono, M.; Gordee, R. S. Antibiotics that inhibit fungal cell wall development. *Annu. Rev. Microbiol.* **1994**, *48* (1), 471–497.

(17) Wessels, J. G. H. Tansley Review 45: wall growth, protein excretion and morphogenesis in fungi. *New Phytol.* **1993**, *123* (3), 397–413.

(18) Kang, Z.; Huang, L.; Krieg, U.; Mauler-Machnik, A.; Buchenauer, H. Effects of tebuconazole on morphology, structure, cell wall components and trichothecene production of *Fusarium culmorum in vitro*. *Pest Manag. Sci.* **2001**, *57* (6), 491–500.

(19) Yoshida, M.; Kawasaki, A.; Yukimoto, M.; Nose, K. Detection of the effects of fungicides on the cell membrane by proton nuclear magnetic resonance spectroscopy. *Pestic. Biochem. Phys.* **1990**, *38* (2), 172–177.

Received for review July 14, 2009. Revised manuscript received December 17, 2009. Accepted December 22, 2009. We acknowledge financial support of this investigation by the National Basic Research Program of China (2003CB114407) and the Foundation of Key Laboratory of Pesticide and Chemical Biology (Central China Normal University), Ministry of Education, China (200803A03).