Oocydin A, a chlorinated macrocyclic lactone with potent anti-oomycete activity from *Serratia marcescens*

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A unique chlorinated macrocyclic lactone, termed oocydin A, was isolated from a strain of *Serratia marcescens* growing as an epiphyte on *Rhyncholacis pedicillata*, an aquatic plant native to the Carrao river of the Venezuelan-Guyanan region of South America. The lactone has a molecular mass of 470 Da, and contains one atom of chlorine, a carboxyl group and a tetrahydrofuran ring internal to a larger macrocyclic ring. MICs of approximately 0·03 µg ml^{-1} were noted for oocydin A against such phytopathogenic oomycetes as *Pythium ultimum, Phytophthora parasitica, Phytophthora cinnamomi* and *Phytophthora citrophora*. With regard to the true fungi, oocydin A had either minimal or no effect against certain Fungi Imperfecti (including several pathogens of humans), two ascomycetes and a basidiomycete. Oocydin A may have potential as an antimycotic in agricultural applications and especially for crop protection.

INTRODUCTION

Plants, especially those growing in freshwater environments, must have disease resistance mechanisms to cope with aquatic plant-pathogenic fungi that may invade and destroy them. Some of the most commonly known plant pathogens preferring aquatic environments are the oomycetes. This group of organisms (known as the water moulds) has recently been characterized as belonging to an entirely new biological kingdom known as the Stramenophila (Alexopoulos *et al.*, 1996). The Stramenophila include such genera of phytopathogens as *Phytophthora*, *Aphanomyces*, *Bremia*, *Pernospora*, *Pythium* and *Plasmopara*. The presence of free water or high humidity is an absolute prerequisite for the pathogenicity of these organisms (Buczacki, 1983).

The oomycetes can be devastating to terrestrial plants; however, the appropriate moisture requirements must be met (Buczacki, 1983). Yet, conceivably these organisms could also attack and infect plants normally existing in strictly aquatic environments but nevertheless, aquatic plants seem to thrive and are relatively disease-free in their respective ecosystems. This biological conundrum may be related to either intrinsic or extrinsic factors controlling plant disease resistance, a phenomenon little understood or studied in aquatic plants. With regard to the possibility of extrinsic factors controlling disease, certain epiphytic or endophytic microbes may associate with aquatic plants and produce anti-oomycetous compounds. This may contribute to the defence of the plant by killing, inhibiting or warding off invading oomycetes. This rationale served as a basis for a search for microbes participating in associations with aquatic plants in which antimycotics may be produced by epiphytes or endophytes. If such compounds exist, they may prove agriculturally applicable to plant disease control situations in which one or more phytopathogenic oomycetes are a potential problem.

Rivers arising and flowing in the Venezuelan-Guyana of South America make up the region's primary bodies of fresh water. *Rhyncholacis pedicillata* is a small highly specialized aquatic plant of the family Podostemaceae that grows in colonies and thrives in some of the brown-black rivers of the Venezuelan-Guyana (Steyermark *et al.*, 1995). These plant colonies are comprised of hundreds of individuals that grow firmly attached to rock surfaces and prefer swift water currents. *R. pedicillata* is 0·1–0·5 m in size and it has a slightly enlarged bulbous-like base and an extensive root system that anchors the plant to rock. Its stems are multi-branched, are lace-like and covered with numerous small leaves. Close examination of individual plants in

the Carrao river revealed animal or environmentally inflicted wounds on the stems. Normally, it would seem that such wounds would serve as entry points for one or more pathogenic oomycetes. However, little or no disease symptoms on the plants were observed.

Small stem pieces were examined for their associated microbes and each of those recovered was assayed against *Pythium ultimum*. The most commonly observed microbe was a strongly red-pigmented bacterium that was identified as *Serratia marcescens*. It colonized the surface of the stems of *R. pedicillata*, rather than internally. In culture, this bacterium produced a novel macrocyclic chlorinated lactone termed oocydin A that demonstrated selective toxicity (antimycotic) towards the oomycetes with extremely low MICs (e.g. 0·03 μg ml^{-1} for various *Phytophthora* spp.). This report deals primarily with the isolation of *S. marcescens* and the chemical characterization of oocydin A. Some specific details are also given on the biological activity of the compound. Finally, its relationship to the biology of the plant–microbe interaction is discussed, along with its potential usefulness to agriculture.

METHODS

Isolation of *S. marcescens* and demonstration of antimycotic activity. Several small stem fragments of *R. pedicillata* were removed from the plant growing in its river environment and cut into pieces about 5·0 mm long. These tissue pieces were then soaked overnight in 0·1 M sodium phosphate buffer (pH 6·8), 0·1 M NaCl. After 12 h, the saline solution was collected, left at 23 °C for 1 h and then streaked onto a semi-selective medium (King's B; KB) according to the procedures previously outlined (Miller *et al.*, 1998). After 2 d, the most commonly appearing colonies consisted of bacilliform bacteria that produced a bright-red pigment. Each of these colonies was transferred back onto KB medium and incubated for several days prior to being transferred as individual colonies onto potato dextrose agar (PDA) plates. These plates were incubated for at least 3–5 d at 23 °C prior to being co-inoculated (as 7 × 7 mm infested agar plugs) with a rapidly growing culture of *P. ultimum*. The plates were then incubated at 23 °C and examined for antimycotic activity, detected as a zone of inhibition of fungal growth around the bacterial colony. The red-pigmented, antimycotic-producing bacterial strain was identified as *S. marcescens* by Dr J. van der Toorn of the Identification Service of the Kluyver Laboratory for Biotechnology, Delft, The Netherlands. Our isolate of *S. marcescens* is stored in the bacterial culture collection of Montana State University, Bozeman, MT, USA, as acquisition no. 97 (MSU-97).

Fungal strains. All strains of *Phytophthora* used in bioassays were the generous gift of Dr John Menge, University of California, Riverside, CA, USA. *P. ultimum* and all other plant-pathogenic fungi used in bioassays were a generous gift of Dr Don Mathre, Montana State University. Standard isolates of *S. marcescens*, used for comparative purposes, were obtained from the Montana State University bacterial culture collection.

Bioassays. A simple plate bioassay was used to detect bioactivity of various fractions during the purification of oocydin A. Aliquots (10–20 μl) of sample were placed on a PDA plate and dried. The plates were then inoculated with four 7 × 7 mm plugs of agar infested with *P. ultimum*, one in each quadrant, and incubated for 36–48 h at 23 °C. Antimycotic activity was apparent as a zone of growth inhibition. The antimycotic activity of pure oocydin A was also tested against a series of plant pathogens by dissolving 50 μg of the compound in 100 μl methanol, spotting 10 μl portions onto PDA plates and allowing the droplets to dry. Each plate was overlaid or sprayed with an aqueous suspension of the test fungus in water (containing mycelial fragments and/or spores), sealed with a piece of Parafilm and then incubated at 23 °C for 4–5 d. In each case, a positive control test was also conducted (the residue obtained from the methylene-chloride-extracted medium without the bacterium). MICs of oocydin A against various phycomycetes were determined by preparing a stock solution of the compound (1 mg ml^{-1} in methanol). This was dispensed, by serial twofold dilution, into a 24-well plate. Each well of the plate contained 500 μl potato dextrose (PD) broth. A small plug of the test fungus was placed into each well and the plate incubated for 48–72 h at 23 °C. The MIC was taken as the concentration of oocydin A in the well where no growth was detected visually after either 48 or 72 h.

MIC tests were also conducted for several fungal pathogens of humans using the microbroth dilution assay as recommended by the subcommittee on antifungal susceptibility testing of the US National Committee for Clinical Laboratory Standards (NCCLS). The effects of the well-established antimycotics metalaxyl, pseudomycin B and amphotericin B were determined concomitantly.

Oocydin A was tested against human cancer cell lines BT-20, MCF 7 (both from ATCC, Manassas, VA, USA) and a normal human mammary cell line, cc2551 HMEC (from Clonetics, San Diego, CA, USA). These cells were exposed to serial dilutions of oocydin A. After 3 d, they were stained with neutral red and the absorbance measured at 540 nm. For non-adherent cells, a modification of the staining protocol was used (Berent *et al.*, 1986). The results were recorded as median inhibitory concentrations (IC$_{50}$ values).

Oocydin A isolation procedure. Cells from a single colony of *S. marcescens* MSU-97 were used to inoculate 15 ml PD broth and the culture was grown overnight with shaking at 23 °C. This culture was then used to inoculate two 2·0 litre flasks, each containing 1 l of medium consisting of 24 g PD; 6 g soytone; 4 g yeast extract and 100 mg NaCl in H$_2$O. The cultures were incubated at 23 °C for 15 d. The micro-organism was removed by centrifugation and the culture fluid extracted twice with two equal volumes of methylene chloride. This solvent was taken to dryness by flash evaporation at 45–50 °C under vacuum. Approximately 2·1 g of residue was present after evaporation of the solvent. This material was dissolved in chloroform (5 ml) and placed on a 3·0 × 20·0 cm column of silica gel. The column was eluted with a chloroform/methanol (10:1, v/v) solution. The first 100 ml to elute from the column was discarded and the next 50–100 ml contained the antimycotic activity. This material was placed onto another silica gel column (the same size as initially used) and eluted with methylene chloride/methanol (10:1, v/v) solution. In this case, the first 50 ml of eluate was discarded and the next 80 ml contained the bioactivity (approx. 50–60 mg). The mixture of compounds was then subjected to a series of preparative TLC steps on 20 × 20 cm plates to yield a pure product. The solvent systems used in succession were as follows: solvent A, chloroform/methanol/acetic acid (12:1: 0·1, by vol.); solvent B, chloroform/methanol/ammonium hydroxide (6:2:0·1, by vol.); and solvent C, methylene chloride/methanol (6:2, v/v). After each successive TLC step, each band on the plate was eluted with methanol and the material possessing bioactivity

(see bioassay procedure above) was reapplied to the next plate prior to separation. Once obtained and authenticated, a small amount of oocydin A was applied at the margin to the TLC plate as an appropriate reference for larger scale preparations. It appeared as a brownish spot with the vanillin-sulfuric reagent after gentle heating (Cardellina, 1991). Approximately 15–18 mg oocydin A was recovered per litre of culture fluid. It was obtained as a whitish powder by dissolving it in a methanolic-aqueous solution, which was then frozen and subjected to lyophilization. This was the preparation that was subjected to physical, chemical and biological characterization, once the purity of the sample was firmly established.

HPLC. The putative TLC-purified sample of oocydin A (20 μg) was subjected to HPLC using an Altima C-18 column (7·0 × 250 mm) (Alltech) and eluting with a linear gradient starting with a solution of 0·1% TFA/30% acetonitrile in water and finishing with a solution of 0·1% TFA/50% acetonitrile in water, over the course of 30 min. Detection was performed with a Waters variable wavelength detector at 208 nm.

Spectroscopic analyses. After the purity of oocydin A had been established it was subjected to a series of analyses using standard spectroscopic techniques. Electrospray mass spectroscopy was performed by dissolving the sample in methanol/water/acetic acid (50:50:1, by vol.). The sample was then injected into Montana State University's custom-built mass spectrometer with a spray flow of 2 μl min^{-1} and a spray voltage of 2·2 kV via the loop injection method. Oocydin A was also subjected to laser desorption mass spectroscopy on a Perspectives Biosystems instrument. The sample was incorporated into a matrix of 3,5-dihydroxybenzoic acid and scanned accordingly. The following parameters were used: accelerating voltage 30000 V, grid voltage 70%, mirror ratio 1·06, laser 260 and cytochrome *c* to standardize the instrument. Negative high resolution FAB mass spectroscopy was done to acquire empirical formula data. Elemental analysis was done by degradative techniques at Atlantic Microlab, Norcross, GA, USA. Infrared spectroscopy was done on a Perkin Elmer instrument with oocydin A embedded in a matrix of anhydrous KBr and pressed into a pellet. An average of 16 scans was taken on the sample. The UV absorption spectrum of oocydin A was determined in 1 ml 100% methanol (1·0 cm light path, Beckman DU 50 spectrophotometer). Its optical rotation was determined in a sample dissolved in 100% methanol and analysed in a JASCO P1010 instrument.

Oocydin A was subjected to NMR techniques after being dissolved in 100% deuterated methanol. Initially, the ^{1}H spectrum was obtained on a Bruker DRX 500 instrument with 64 scans and a delay cycle of 2 s, and collected as 32k real-time domain points using a transmitter frequency of 500·13 MHz. The ^{1}H spectrum was referenced to the MeOD (deuterated methanol) signal at 3·3 p.p.m. For acquisition of the ^{13}C spectrum, 4096 scans were made with a recycle delay of 10 s and collected as 32k real-time domain points using a transmitter frequency of 125·77 MHz. The spectrum was referenced to the residue MeOD signal at 49·0 p.p.m.

Oocydin A was also analysed by 2D INADEQUATE analysis on a 500 MHz Varian Inova spectrometer operating at 125·892 MHz and 26 °C. Oocydin A (58 mg) was dissolved in 200 μl CDCl$_3$ and the analysis performed using a Varian 5 mm probe and a Shigemi microtube whose susceptibility was matched to the solvent used. Analysis parameters included a 10 μs [13]C 90° degree pulse and a pulse sequence delay optimized for detection of 55 Hz carbon–carbon scalar coupling constants. A total of 64 evolution increments of 800 transients each were used for an analysis time of 4·7d. Digital

resolution of the acquisition and evolution dimensions were 0·2 and 176·1 Hz per point, respectively. The gross structure of oocydin A was deduced from the various NMR data, particularly gradient DQF-COSY, gradient HMQC, gradient HMBC and difference 1D-NOE spectra in a JEOL JNM-alpha 600 spectrometer. Stereochemistry of oocydin A was primarily determined by 1D-NOE and selective NOE experiments. INADEQUATE spectral processing and signal assignments were done in a near automated fashion using software described by Dunkel *et al*. (1990, 1992) and Harper *et al*. (1996).

Scanning electron microscopy. Materials to be examined were placed in 2% glutaraldehyde in 0·1 M sodium cacodylate buffer (pH 7·2–7·4) (Upadhyay *et al*., 1991). The samples were critical-point-dried, gold-coated with a sputter coater, and observed and photographed with a JEOL 6100 scanning electron microscope. Bacterial preparations were supported on dried γ-irradiated carnation leaves.

Materials. All solvents used for HPLC and TLC were HPLC grade. Solvents used for extraction of oocydin A were ACS grade. All TLC was conducted on EM-Merck precoated glass silica gel plates at a thickness of 0·25 mm.

RESULTS AND DISCUSSION

Identity, isolation and biology of the bacterium

The bacterium (MSU-97) used in this study was identified as *Serratia marcescens* based on a series of biochemical tests conducted at the Kluyver Laboratory for Biotechnology, Delft, The Netherlands. This bacterium, based on its deep-red pigmentation, was the most commonly observed bacterial isolate obtained from *R. pedicillata*, an aquatic plant native to rapidly flowing freshwater rivers of the Venezuelan-Guayana region of South America (Fig. 1a). Examination of the surface of some small stems of *R. pedicillata* by scanning electron microscopy revealed that bacterial colonies were in abundance. Hundreds of individual cells seemed to constitute each colony (Fig. 1b). Individual bacterial cells also appeared on the plant surface. The identity of the bacteria could not be firmly attributed to *S. marcescens* by scanning electron microscopy, but the surface characteristics of the bacteria on the plant (Fig. 1c) were identical to those of bacteria in pure cultures of authenticated *S. marcescens* (isolate 97) (Figs 1c, d). These included multiple small projections and a generally relatively rough cell surface. Such surface characteristics do not appear to be common in bacteria and may serve as a means to help locate and identify *S. marcescens* on the surface of its host. In addition, the size and shape of the bacterial cells on the plant and in the authenticated culture appeared to be identical. This bacterium was labelled as MSU-97, and stored both in 15% glycerol and at −70 °C. Since *S. marcescens* was primarily recovered from the plant surface and not from internal tissues, it was considered more epiphytic than endophytic in its relationship to the plant.

Purification of oocydin A from *S. marcescens*

Several antimycotic-producing bacteria were isolated from *R. pedicillata*, but *S. marcescens* was by far the most prevalent. Plate bioassays demonstrated that *S.*
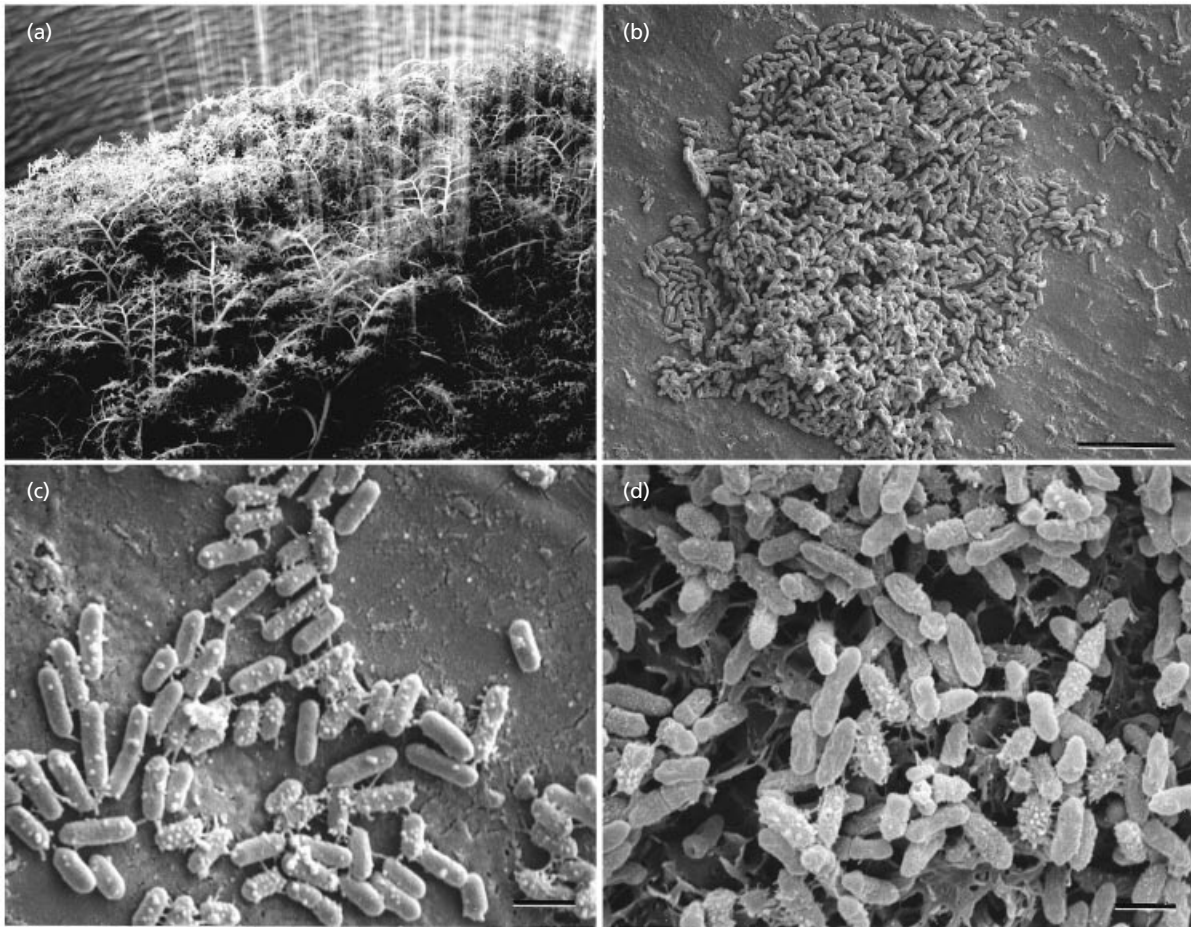
Fig. 1. *S. marcescens* and its relationship to *R. pedicillata*. (a) The plant (*R. pedicillata*) from which *S. marcescens* was originally isolated growing beneath the Sappo waterfall in the Carrao river of Venezuela. (b) A scanning electron micrograph of a colony of *S. marcescens* attached to the surface of the stem of *R. pedicillata*. Bar, 10 μm. (c) A scanning electron micrograph at higher magnification of *S. marcescens* illustrating its size and surface characteristics on the plant surface. Bar, 1 μm. (d) The bacterium that was positively identified as *S. marcescens* grown in pure culture and then examined by scanning electron microscopy. Bar, 1μm.
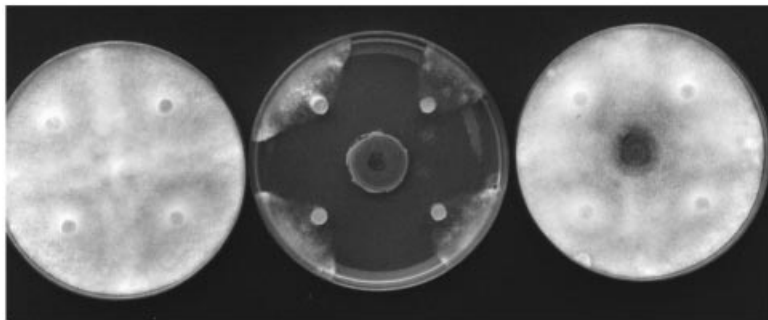
Fig. 2. Effect of *S. marcescens* MSU-97 (centre), MSU-69 (left) and ATCC 1009 (right) on *P. ultimum* 3 d after inoculation of plates with the oomycete.

marcescens MSU-97 is a potent inhibitor of growth of *P. ultimum* (Fig. 2). Inhibition zones persisted on the plates at 23 °C until the agar in the plate desiccated (after 4–6 weeks). Since *S. marcescens* is a relatively common micro-organism, standard isolates were also screened in the antifungal plate test. Neither ATCC 1009 nor MSU-69 demonstrated any antifungal activity diffusing from the culture after 3 d of exposure to *P. ultimum* (Fig. 2). Following the purification procedure described in Methods, the antimycotic from *S. marcescens* was subjected to a series of chromatographic tests to check its purity. In solvent systems A and B, the compound
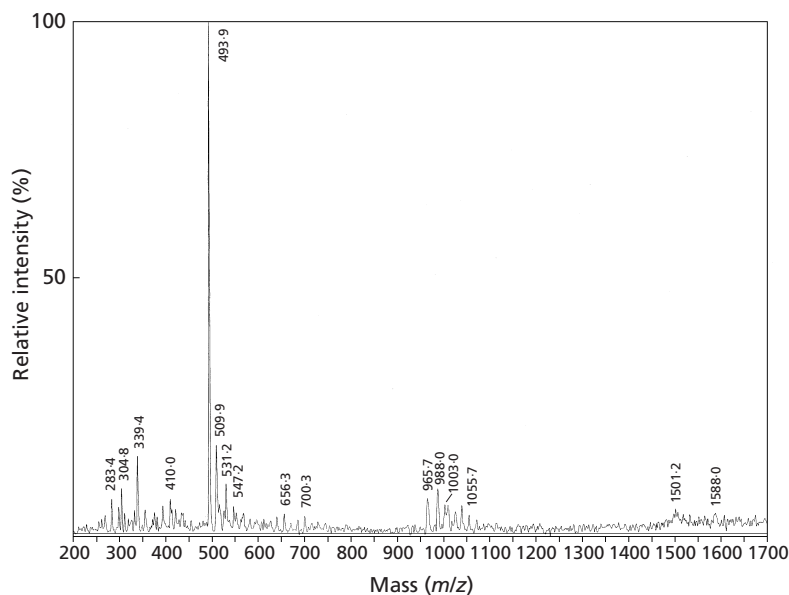
appeared at R_F values of 0·49 and 0·34, respectively. In solvents consisting of methylene chloride/methanol (6:2, v/v) and chloroform/methanol (7:1, v/v), the R_F values were 0·65 and 0·27, respectively. In each case, after spraying with the vanillin-sulfuric acid reagent, only one spot appeared on the plate, which was coincident with antimycotic activity. Furthermore, no other UV-absorbing or UV-fluorescent spots appeared on the plates after examination using long- or short-wavelength UV. After HPLC analyses, under the conditions described, only one sharp symmetrical peak absorbing at 208 nm appeared and it had a retention time of 19·7 min. When the fractions corresponding to this peak were combined and subjected to the bioassay test the pool of fractions inhibited the growth of *P. ultimum*. Collectively, these chromatographic data suggested that the antimycotic agent of *S. marcescens* MSU-97 was homogeneous and ready for spectroscopic and chemical analyses.

Characterization of oocydin A

The uncorrected melting point of oocydin A was 106–108 °C. The optical rotation, measured on 2·2 mg of the compound dissolved in 1 ml methanol at 589 nm was + 18·2°.

Electrospray mass spectroscopic analysis of oocydin A produced a major peak at 493·9 m/z. This is consistent with a single charged species of $(M + \mathrm{Na})^+$ (Fig. 3). The laser desorption spectrum also produced a major peak at 493·3 consistent with $(M + \mathrm{Na})^+$. However, there was also a significant $M + 2$ peak at 495·3, which could be accounted for by oocydin A possessing a chlorine atom. This may be true because of a high abundance of the isotope $^{37}\mathrm{Cl}$. Negative HRFAB data yielded $(M - \mathrm{H})^+$ of 469·1640, which accounted for the empirical formula $C_{23}H_{30}O_8Cl_1$. The difference between the negative HRFAB data $[469 = (M - \mathrm{H})^+]$ and the electrospray

laser desorption data $[M = (470 + \mathrm{Na})^+]$ is equivalent to 23 m/z, which is the atomic mass of sodium. Therefore, in both the electrospray and laser desorption mass spectral analysis, oocydin A sequestered a sodium ion, which accounted for its molecular mass plus 23 m/z. In addition, the molecule assumed a net positive charge by picking up H^+. Elemental analysis of oocydin A for its halogen content revealed that it was = 7·1 % (the expected Cl content is 7·4 %). In the elemental analysis, the halogen was not specifically identified as chlorine. Although the negative HRFAB and the laser desorption mass spectral data gave a strong indication for the presence of chlorine in oocydin A, these analyses were not totally definitive. Therefore, further supportive evidence was obtained with an Oxford Instrument energy-dispersive X-ray microanalysis system on a JEOL scanning electron microscope. The test was conducted on about 15–20 µg oocydin A supported on an Al stub. A peak distinctive for chlorine and no other halogen appeared in the spectrum. Thus, the empirical formula for oocydin A is $C_{23}H_{31}O_8Cl_1$.

The chemical structure of oocydin A was ascertained by IR and NMR spectroscopic analyses since it was impossible, in our hands, to crystallize this substance and obtain X-ray analytical data. The IR absorption band at 2982 cm^{-1} with a shoulder suggested alkane and alkene functionalities, whereas the intense band at 1713 cm^{-1} indicated the presence of one or more acetate esters (Silverstein *et al.*, 1991). Weaker bands at 1417 and 1602 cm^{-1} suggested the presence of a carboxylate functionality. Other notable bands occurred at 1640, 1124, 1030, 902 and 835 cm^{-1}. Although the IR spectrum was not that informative, it provided a data set unique to oocydin A and it did suggest the presence of certain functional groups.

The ^{1}H-NMR spectrum was consistent with a compound having methyl, methylene and hydrogen bonded

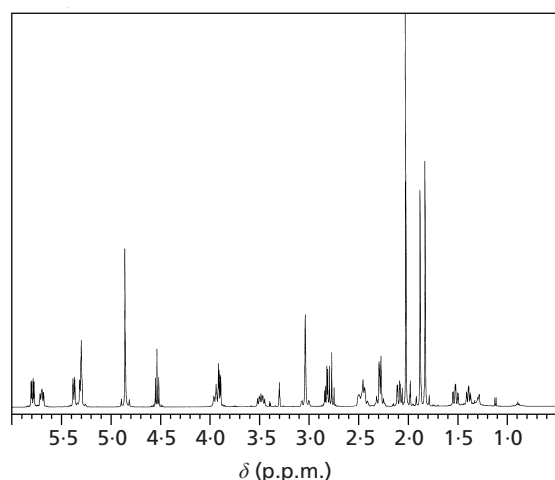Fig. 4. The ¹H-NMR spectrum of oocydin A obtained in 100% deuterated methanol. The peak at 3·3 p.p.m. is the CD₃OH signal.
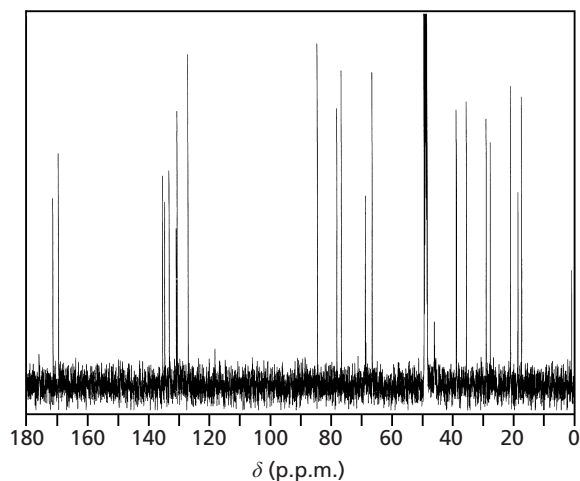

Fig. 5. The ¹³C-NMR spectrum of oocydin A obtained in 100% deuterated methanol. The signal at 49 p.p.m. is from the solvent carbon atom.

Fig. 6. The structure of oocydin A (no absolute stereochemistry is implied).

on carbons bearing oxygen (Fig. 4). In the ¹³C-NMR spectrum, it was possible to account for 23 carbon atoms (Fig. 5). This NMR analysis also showed double-bonded carbons, carbons bearing carbonyl groups and methyl carbons.

Whilst the one-dimensional NMR spectra of oocydin A were instructive, the structure proposed for this compound is primarily based on the use of a collection of sophisticated NMR techniques, each of which provided useful information on the relative positions of the atoms making up this compound. The carbon types were first determined by DEPT analysis (Doddrell et al., 1982). One bond and longer proton–carbon connections were established by HMQC and HMBC (Bax et al., 1983;
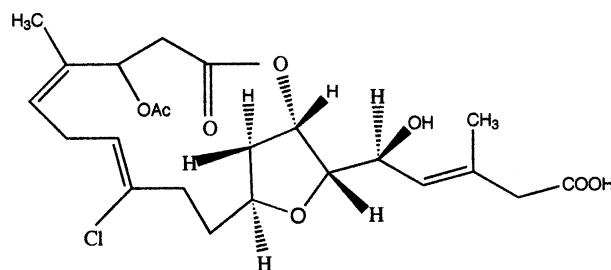
Bax & Summers, 1986). Selective ROESY and NOE difference analyses allowed the relative stereochemical orientations to be established. A 2D INADEQUATE analysis was used to verify the proposed carbon–carbon connections (Bax et al., 1980, 1981). All INADEQUATE connections were determined using previously described data analysis software specifically suited to low signal-to-noise acquisitions (Dunkel et al., 1990, 1992; Harper et al., 1996). The INADEQUATE analysis confirmed all but three bonds at greater than a 99·9% confidence level. Thus, this very powerful, but relatively insensitive experimental technique confirmed all of the structural conclusions. A more complete description of the NMR analysis of oocydin A will be given elsewhere. Thus, oocydin A is a chlorinated macrocyclic lactone having a tetrahydrofuran ring internal to it with a side chain terminated by a carboxylic acid functionality. The proposed structure (Fig. 6) is consistent with all of the spectral information obtained. Although several asymmetric centres are present in the molecule, only the relative stereochemistry is presented and no absolute stereochemistry is implied. Oocydin A is one of only a few macrocyclic lactones that have ever been isolated. Others, including the fijinolides and the laulimalides, have been isolated from marine animals and demonstrate general toxic activity to various biological systems.

Bioactivities of oocydin A

The initial biological screen test showed the potent antimycotic activity of some substance(s) diffusing from the colonies of S. marcescens (Fig. 2). At least one bioactive substance that seemed to be involved in this phenomenon of fungal inhibition is oocydin A. The effect of purified oocydin A on several oomycetes was ascertained. It appeared that Phytophthora spp. were the most sensitive, with MICs of approximately 0·03 μg ml⁻¹ (Table 1). P. ultimum, however, was less sensitive than the Phytophthora spp. When tested on PDA plates (5·0 μg oocydin A applied to a localized agar surface in a droplet of methanol) against a group of organisms representing the other three classes of fungi, oocydin A showed little or no activity. For instance, against Geotrichum candidum (Fungi Imperfecti) it was inactive; likewise only slight inhibition of growth was

Table 1. The effects of oocydin A on various phytopathogenic oomycetes

MIC values were determined for representative oomycetes using diluted purified oocydin A in 24 well plates. A small plug of PDA containing the appropriate micro-organism was added to each well. The plates were incubated for 72 h and evaluated for growth of the test organism (see Methods).

Oomycete tested	MIC of oocydin A ($\mu g\ ml^{-1}$)		MIC of metalaxyl ($\mu g\ ml^{-1}$)
	Test 1	Test 2	
Pythium ultimum	0·11	0·39	ND
Phytophthora parasitica	0·03	0·05	0·06
Phytophthora cinnamomi	0·03	0·02	0·06
Phytophthora citrophora	0·03	0·02	ND

ND, Not determined.

observed for *Sclerotinia sclerotiorum* (Ascomycetes) and *Rhizoctonia solani* (Basidiomycetes). In other plate tests, no activity was noted against either *Verticillum dahliae* or *Alternaria tomato*.

Oocydin A was tested against several important fungal pathogens of humans using the MIC microwell plate assay. At concentrations up to 80 $\mu g\ ml^{-1}$, it had no effect on *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Candida parapsilosis* or *Histoplasma capsulatum*. However, pseudomycin B inhibited growth of all of these organisms except *A. fumigatus* with MICs of $< 1.0\ \mu g\ ml^{-1}$ and amphotericin B inhibited them all with MICs of $< 1.0\ \mu g\ ml^{-1}$.

Other biological considerations

Oocydin A is a novel bioactive compound possessing some unique biological and chemical properties. Overall, it appears that it has selective lethal activity against oomycetes at MICs lower or similar to those noted for metalaxyl (Table 1). Metalaxyl is well established as the fungicide to which the oomyctes are the most sensitive (Lyr, 1995). However, in the past 10 years, increasing resistance to metalaxyl is developing in certain oomycete populations and this fungicide is being rendered useless or less effective (Lyr, 1995). Thus, a tremendous need exists for new compounds to combat these plant pathogens. These observations suggest that oocydin A may have potential as a novel antimycotic against some of the most important plant pathogens – the oomycetes. The cursory examination of the biological selectivity of oocydin A described here suggests that it targets one or more unique structural sites in the oomycetes. However, no mode of action studies on the compound have yet been done to confirm this suggestion. Oocydin A appears to have selective toxicity against various human cell lines. In preliminary studies, IC_{50} values of 0·2 $\mu g\ ml^{-1}$ against BT-20 (breast cancer cell line), 0·42 $\mu g\ ml^{-1}$ against MCF-7 (breast cancer cell line) and 0·6 $\mu g\ ml^{-1}$

against a normal mammary cell line were noted. In future it may be possible to chemically modify oocydin A so that the compound is more selectively toxic to cancer cells. This would increase the likelihood that an oocydin A derivative could be more seriously considered as an anticancer drug candidate. However, a more immediate goal should be the exploration of oocydin A as a candidate compound for agricultural applications. This would necessitate the acquisition of large-scale amounts of the compound for plant testing and perhaps chemical modification to reduce any toxicity problems that may arise if the compound is to be used in controlling diseases of crops caused by oomycetes.

In natural settings, *S. marcescens* may play a role in the *R. pedicillata* relationship by first establishing itself on the plant surface and then producing oocydin A. This may eventually diffuse into the plant and/or, due to its solubility and charge characteristics, it may adhere to the plant surface. The presence of oocydin A in the area of the bacterial colony may prevent oomyceteous pathogens from causing rot or decay of the plant by virtue of the selective toxicity of this novel compound.

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