ORGANIC LETTERS

2004
Vol. 6, No. 24
4615−4617

Chemical Mediators: The Remarkable Structure and Host-Selectivity of Depsilairdin, a Sesquiterpenic Depsipeptide Containing a New Amino Acid

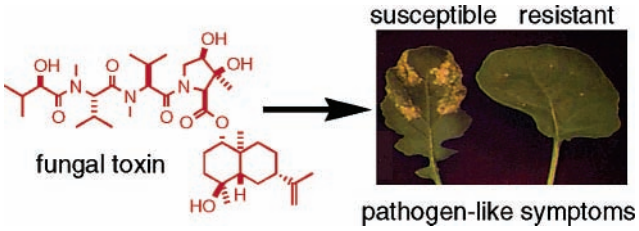
M. Soledade C. Pedras,* Paulos B. Chumala, and J. Wilson Quail

Department of Chemistry, University of Saskatchewan, 110 Science Place, Saskatoon SK, S7N 5C9, Canada

s.pedras@usask.ca

Received October 13, 2004

ABSTRACT



The chemical structure determination of depsilairdin, a highly selective phytotoxin produced by the plant pathogenic fungus *Leptosphaeria maculans*/*Phoma lingam*, is described. The elucidation of the unusual chemical structure used a combination of NMR spectral data and X-ray crystallography. The absolute configuration was established using chemical degradation and synthesis of (3*S*,6*R*)-3,6-diisopropyl-2,5-morpholinedione and its (3*R*,6*S*) and (3*R*,6*R*) stereoisomers. Similar to the fungal pathogen, depsilairdin caused strong lesions only on brown mustard leaves but not on related species.

The chemical mediators of molecular interactions between pathogenic fungi and their host plants are of great significance to the understanding and control of plant fungal diseases.[1] Yet, the chemical structures and biological effects of potential mediators of many fungal diseases remain to be discovered. The task is indeed daunting, as fungi appear to possess a complex chemical arsenal that enables them to elude the plants' counterattack weaponry.

In this context, the "blackleg" fungus [*Leptosphaeria maculans* (Desm.) Ces. et de Not., asexual stage *Phoma lingam* (Tode ex Fr.) Desm] has been a major player in defeating plants designed to resist its invasion.[2,3] The host-range of the fungus includes some of the most economically important cruciferous oilseed crops (e.g., canola, *Brassica napus*). Our studies of blackleg fungal isolates[4] revealed that this plant pathogen produces various phytotoxic metabolites, including phomalirazine (**1**),[5] sirodesmins (**2**),[6] phomalide (**3**),[7] and phomalairdenone (**4**).[8] Importantly, **3** was toxic only to plants that host fungal isolates producing it, e.g., canola

* Telephone: 1-306-966-4772. Fax: 1-306-966-4730.

(1) For reviews on plant−pathogen interactions, see for example: (a) Jackson, A. O.; Taylor, C. B. *Plant Cell* **1996**, *8*, 1651−1668. (b) Knogge, W. *Plant Cell* **1996**, *8*, 1711−1722.

(2) Gugel, R. K.; Petrie, G. A. *Can. J. Plant Pathol.* **1992**, *14*, 36−45.

(3) Howlett, B. J.; Idnurm, A.; Pedras, M. S. C. *Fungal Genet. Biol.* **2001**, *33*, 1−14.

(4) For a recent review, see: Pedras, M. S. C. *Rec. Res. Dev. Phytochem.* **2001**, *5*, 109−117.

(5) Pedras, M. S. C.; Abrams, S. R.; Séguin-Swartz, G.; Quail, J. W.; Jia, Z. *J. Am. Chem. Soc.* **1989**, *111*, 1904−1905.

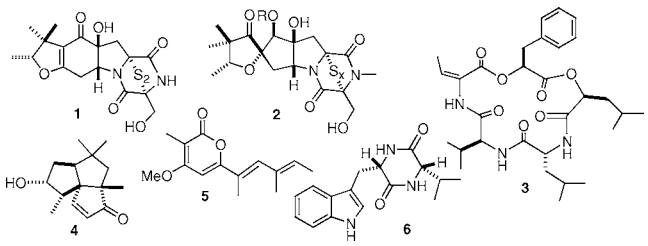
(6) Pedras, M. S. C.; Séguin-Swartz, G.; Abrams, S. R. *Phytochemistry* **1990**, *29*, 777−782.

(7) Pedras, M. S. C.; Taylor, J. L.; Nakashima, T. T. *J. Org. Chem.* **1993**, *58*, 4778−4780.

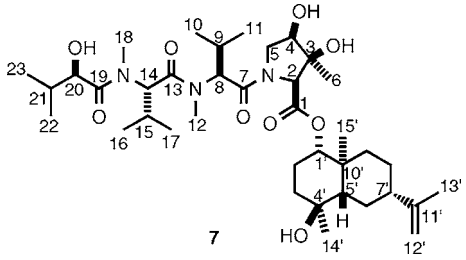
(8) Pedras, M. S. C.; Erosa-López, C. C.; Quail, J. W.; Taylor, J. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3291−3294.

10.1021/ol0478786 CCC: $27.50
Published on Web 10/29/2004

type.[7,9] By contrast, a new group of blackleg fungal isolates able to produce **4**, caused disease only on brown mustard but not on canola.[10] This finding was rather disturbing because brown mustard was known to be resistant to blackleg disease. The leaf lesions observed on blackleg-infected mustard suggested that colonization by these "new" blackleg isolates (Laird 2 and Mayfair 2) would be mediated by a rather potent metabolite. However, none of the metabolites isolated from these new isolates, **4**−**6**,[8] appeared to be involved in causing the disease symptoms observed on brown mustard.



A comprehensive metabolite search for new phytotoxic compounds produced by isolates Mayfair 2 and Laird 2 led to the discovery of a highly selective phytotoxic metabolite that we named depsilairdin (**7**). Here we describe the structure elucidation and the chemistry involved in the determination of the absolute configuration of this chemical mediator. In addition to the unique structural combination tripeptide−sesquiterpene, **7** contains a (2*S*,3*S*,4*S*)-3,4-dihydroxy-3-methylprolyl residue, which represents an amino acid hitherto undescribed. The remarkable selectivity of **7** to brown mustard was observed over a broad concentration range from μM to mM.



The molecular formula of **7**, $C_{38}H_{65}N_3O_9$ based on MS (HREI-MS) and NMR (^{1}H and ^{13}C NMR and HMQC) data, indicated eight degrees of unsaturation. The ^{1}H NMR spectrum displayed signals for six methyl singlets and six methyl doublets; two of the methyl singlets (δ_H 3.36, H_3-12, and 2.74, H_3-18) could be attributed to *N*-methyl groups. The ^{13}C NMR spectrum displayed four carbon signals at δ_C 171.7, 175.4, 170.1, and 171.1, indicating the presence of amide or ester carbonyl groups, and two signals at δ_C 108.4 and 150.1 attributable to olefinic carbons. These four carbonyl groups and the C−C double bond accounted for

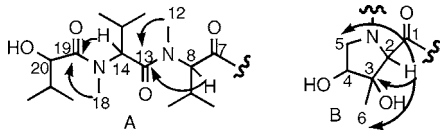


Figure 1. Substructures A and B, and selected HMBC correlations observed for depsilairdin (**7**). A: H-8 (δ_H 5.29) and H_3-12 (δ_H 3.36) to C-13 (δ_C 171.1), and H-14 (δ_H 5.34) and H_3-18 (δ_H 2.74) to C-19 (δ_C 175.4). B: H-2 (δ_H 4.47) to C-3 (δ_C 76.6) and C-5 (δ_C 52.8).

five of the eight degrees of unsaturation of the molecule; the remaining three unsaturations unaccounted for by the NMR data suggested the presence of three rings.

The ^{1}H−^{1}H COSY spectrum displayed several spin systems, as shown in Figures 1 and 2. Three isopropyl spin

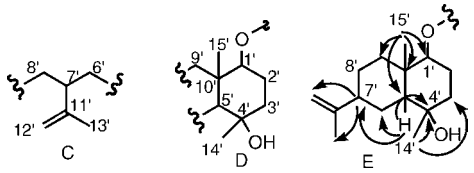


Figure 2. Substructures C, D, and E, and selected HMBC correlations observed for depsilairdin (**7**). E: H_3-14′ (δ_H 1.00) to C-3′ (δ_C 40.5), C-4′ (δ_C 71.3), and C-5′ (δ_C 53.1); H_3-15′ (δ_H 1.09) to C-1′ (δ_C 83.3), C-5′ (δ_C 53.1), C-9′ (δ_C 40.3), and C-10′ (δ_C 38.9).

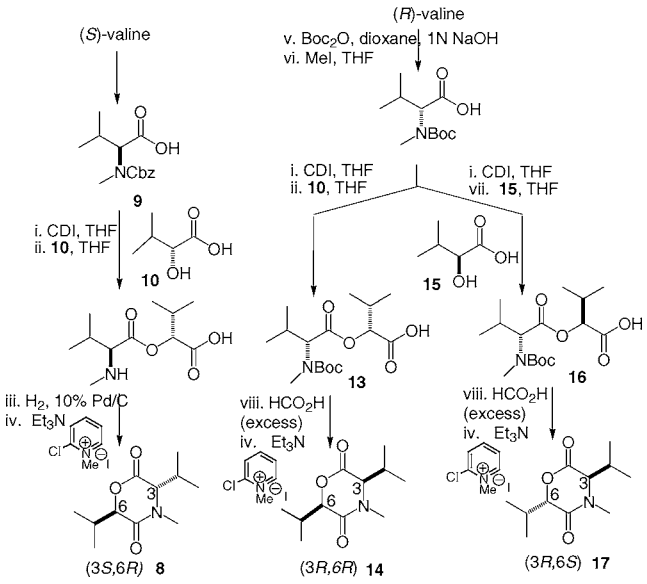
systems coupled to three methine protons at δ_H 5.34 (H-14, d, J = 11 Hz), 5.29 (H-8, d, J = 11 Hz), and 4.22 (H-20, br d, J = 7 Hz) could be connected using HMBC correlations summarized for structural subunit A, Figure 1. Another spin system containing a proton at δ_H 3.68 (H-4), coupled to two methylene protons at δ_H 4.48 and 3.89 (m, H_2-5), showed HMBC correlations summarized for subunit B, Figure 1. Two additional spin systems C and D, Figure 2, were observed through H−H coupling. Selected HMBC correlations are summarized in Figure 2. The connectivity of subunits B (Figure 1) and E (Figure 2) was established on the basis of an HMBC correlation between proton H-1′ (δ 4.89) of E and carbonyl carbon C-1 (δ_C 171.1) of B. Because subunit A did not show a correlation with any other unit, there was only one possibility to connect units A and B and assign the structure of depsilairdin as **7**. Depsilairdin was crystallized to yield crystals, monoclinic system, $P2_1$ space group.[11] The data obtained from X-ray crystallographic analysis of a single-crystal allowed the assignment of the relative but not the absolute stereochemistry of **7**. To establish the absolute stereochemistry of depsilairdin (**7**), a mild degradation

(9) Plants' defenses were found to inhibit the biosynthesis of sirodesmins. Pedras, M. S. C.; Taylor, J. L. *J. Nat. Prod.* **1993**, *56*, 731−738.

(10) Isolates producing sirodesmins (**2**) are classified as group A, but the new isolates Laird 2 and Mayfair 2 remain unclassified, cf. ref 3.

(11) Compound **7** was crystallized from hexane−acetone (7:3). An X-ray crystallographic data file (CIF) can be found in Supporting Information.

Scheme 1. Syntheses of 3,6-Diisopropyl-4-methyl-2,5-morpholinediones **8**, **14**, and **17**



procedure[12] yielded product X in an amount sufficient for spectroscopic analysis. The molecular formula ($C_{11}H_{19}O_3N$, HREI-MS) and the NMR data of X suggested *trans*-3,6-diisopropyl-4-methyl-2,5-morpholinedione.

Next, to determine the absolute configuration of *trans*-morpholinedione X (3*S*,6*R* or 3*R*,6*S*), three stereoisomers were synthesized as summarized in Scheme 1. Analysis of the ^{1}H NMR spectra of **8**, **14**, and **17** confirmed that isomers **8** and **17** displayed proton signals identical to the product X, whereas **14** was clearly different. Subsequently, the ^{1}H NMR spectra of **8**, **17**, and X were obtained separately in the presence of an NMR chiral solvating agent ((*R*)-(−)-TFAE).[13] The ^{1}H NMR spectrum of X was identical to that of **8**. Furthermore, the ^{1}H NMR spectrum of X spiked with **17** was different, showing two signals for H-6, at δ_H 4.55 and 4.52. These results confirmed that X was identical to **8**. Since X was a structural fragment of depsilairdin (**7**), and the relative configurations of **7** were established by X-ray crystallography, the overall assignment of the absolute configuration of all stereogenic centers of **7** could be established as 2*S*, 3*S*, 4*R*, 8*S*, 14*S*, 20*R*, 1′*S*, 4′*S*, 5′*S*, 7′*S*, and 10′*S*.

Depsilairdin (**7**) caused strong necrotic and chlorotic lesions[18] only on brown mustard leaves,[19] whereas no damage was observed on canola or white mustard leaves. The high selectivity of **7** appears to mimic the pathogenicity range of the producing isolates, and the lesions resemble the disease symptoms, which is consistent with a chemically mediated interplay between host and pathogen.[20] It is remarkable that **7** was selective over a wide range of concentrations (μM−mM), whereas most phytotoxins are selective only at much narrower ranges (μM).[19] It is expected that a highly selective mediator such as **7** can be an excellent probe to detect, e.g., genes and/or receptors targeted by the pathogen to defeat the plant.

Acknowledgment. We thank Prof. D. E. Ward, Chemistry, University of Saskatchewan, for a generous gift of **9**. Financial support from the Natural Sciences and Engineering Research Council of Canada (Discovery Grant to M.S.C.P) and the University of Saskatchewan (graduate teaching assistantship to P.B.C.) is gratefully acknowledged.

Supporting Information Available: General experimental, experimental procedures including the synthesis of **8**, X-ray crystallographic data (CIF) for depsilairdin (**7**), characterization data for compounds **7** (copies of ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC spectra) **8**, **14**, and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0478786

(12) Depsilairdin (**7**) was allowed to stand in a solution of DCl in CD_3OD. After completion of the reaction (15 days), the reaction mixture was concentrated to dryness and the residue separated by chromatography.

(13) ^{1}H NMR (0.12 M of (*R*)-(−)-TFAE in $CDCl_3$) for H-6 of (3*S*,6*R*)-enantiomer was at δ_H 4.55, whereas that of the (3*R*,6*S*)-enantiomer was at δ_H 4.52.

(14) Koch, C.; Simonyiová, S.; Pabel, J.; Kärtner, A.; Polborn, K.; Wanner, K. T. *Eur. J. Org. Chem.* **2003**, 1244−1263.

(15) Li., W.; Ewing, W. R.; Harris, B. D.; Joullié, M. M. *J. Am. Chem. Soc*. **1990**, *112*, 7659−7672.

(16) Campbell, A. D.; Raynham, T. M.; Taylor, J. K. *Synthesis* **1998**, 1707−1709.

(17) Cheung, S. T.; Benoiton, N. L. *Can. J. Chem*. **1977**, *55*, 906−910.

(18) Bioassays described in Supporting Information, as previously reported for other toxins: Pedras, M. S. C.; Biesenthal, C. J.; Zaharia, I. L. *Plant Sci.* **2000**, *156*, 185−192.

(19) Depsilairdin (**7**) caused lesions on brown mustard (blackleg susceptible) ranging from 12 mm (10^{-3} M) to 6 mm (5×10^{-6} M) in diameter, whereas canola or white mustard (blackleg resistant) were not affected, even at 10^{-3} M. For a multiauthor review on phytotoxins, see: Graniti, A. *Experientia* **1991**, *47*, 751−755.

(20) We suspect that blackleg isolates similar to Mayfair 2 and Laird 2 are not common because their favorite host is not widely cultivated. Nonetheless, because new oilseed varieties of brown mustard are commercially available, the new blackleg type may soon become widespread.