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

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SUPPLEMENTARY DATA FOR:
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Experimental Section

General

All chemicals were purchased from Sigma-Aldrich Canada Ltd., Oakville, ON except for deuterated chemicals and solvents purchased from Cambridge Isotope Laboratories, Inc., Andover, MA. All solvents were HPLC grade and used as such, except for CH_2Cl_2 and CHCl_3 which were redistilled and THF which was dried over Na and benzophenone. Flash CC: JT Baker WP C-18 reversed phase silica gel 40 μm . Analytical HPLC analysis was carried out with a high performance liquid chromatograph equipped with quaternary pump, automatic injector, and photodiode array detector (wavelength range 190-600 nm), degasser, and a Hypersil ODS column (5 μm particle size silica, 4.6 i.d. $\times$ 200 mm), equipped with an in-line filter. Mobile phase: H_2O - CH_3CN 20:80, 10 min, gradient 20:80 to 60:40, 10 min, 60:40 to 75:25, 10 min, 75:25 to 100 CH_3CN , 10 min, and flow rate 1.0 mL/min.

Fungal Cultures and Isolation of Depsilairdin

Isolates of *L. maculans*/*P. lingam* (Mayfair 2 and Laird 2) solid cultures (to provide spores to inoculate liquid cultures) were grown on V8 agar medium, and liquid cultures were grown in 250 mL Erlenmeyer flasks containing 100 mL of a chemically defined medium, as previously reported.¹ Liquid cultures (122 L) were incubated for six days, were filtered, the broth was concentrated to 22 L by freeze drying, and then was extracted with ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate and concentrated to dryness under reduced pressure. The ethyl acetate extracts (ca. 350 mg per batch, total amount 2.3 g) were subjected to flash column chromatography (silica gel, 2 cm $\times$ 15 cm, 50 mL fractions, CH_2Cl_2 , 200 mL followed by CH_2Cl_2 -MeOH, 97:3, 200 mL, and then CH_2Cl_2 -MeOH, 90:10, 200 mL). Fractions were concentrated to dryness under reduced pressure. The most phytotoxic fractions (F_8 - F_{10}) were combined and subjected to further

¹ Pedras, M. S. C.; Biesenthal, C. J. *Phytochemistry* **2001**, 58, 905-909.

chromatography (C₁₈ column and prep. TLC) to yield depsilairdin (**7**) (22 mg, ca. 0.2 mg/L of culture).

Phytotoxicity Assays

The selective phytotoxicity of depsilairdin (**7**) to plants resistant and susceptible to blackleg isolates Mayfair 2 and Laird 2 (canola, resistant; brown mustard, susceptible; white mustard, tolerant) was evaluated as previously reported for other toxins.² Seeds of canola (*B. napus*) cv. Westar, white mustard (*Sinapis alba*) cv. Ochre, and brown mustard (*B. juncea*) cv. Cutlass were obtained from Plant Gene Resources, Agriculture and Agric-Food Canada Research Station, Saskatoon, SK. Plants were grown in a growth chamber, under controlled environmental conditions, as previously reported.² Phytotoxicity assays were conducted on leaves of two-week-old plants with depsilairdin (**7**) (1.0×10^{-3} – 5.0×10^{-6} M in water-methanol, 1:1, v/v), as previously described for other compounds.² Plants were incubated in a growth chamber for seven days and visual symptoms recorded.

Synthesis of (3*S*,6*R*)-3,6-diisopropyl-2,5-morpholinedione (**8**)

A solution of *N,N'*-carbonyldiimidazole (CDI) (67 mg, 0.4 mmol) in THF (1 mL) was added to a solution of *N*-Me-Cbz-Val (**9**, 110 mg, 0.4 mmol) in THF (1 mL) and the solution was stirred under argon atmosphere at room temperature for 4 h. (*R*)-2-Hydroxy-3-methylbutyric acid (**10**, 24 mg, 0.2 mmol) in THF (1 mL) was added to the reaction mixture, the mixture was stirred for another 30 h at room temperature, and the solvent was removed in vacuum. After chromatography, the product (54 mg, 0.15 mmol) was dissolved in 2 mL of methanol, followed by addition of 10% Pd/C (12 mg) and the mixture was vigorously stirred under an H₂ atmosphere (balloon) for 2 h. Then the mixture was filtered through Celite and the combined filtrate and washings were concentrated to yield the deprotected didepsipeptide **11** quantitatively. Didepsipeptide **11** (16 mg,

² Pedras, M. S. C.; Biesenthal, C.J.; Zaharia, I. L. *Plant Science* **2000**, *156*, 185-192.

0.06 mmol) was suspended in CH₂Cl₂ (4 mL) and treated with 2-chloro-1-methylpyridinium iodide (26 mg, 0.10 mmol). After addition of triethylamine (24 μ L, 0.18 mmol), the reaction mixture was kept under reflux for 4 h. Finally the solvent was removed in vacuum and the residue was chromatographed to yield **8** in 69% overall yield.

Characterization data

Depsilairdin (7). HPLC R_t = 28.4 min. $[\alpha]_D$ = -65 (c 0.90, CH₂Cl₂). ¹H NMR in Table ; ¹³C NMR in Table; FTIR (cm⁻¹): 3449, 2962, 1635, 1455, 1200, 1136. UV λ_{max} (log ϵ) 207 (3.6). HREI-MS: m/z 707.4724, calc. for C₃₈H₆₅N₃O₉ 707.4721. EI-MS: m/z (relative intensity) 707 (11%), 634 (50%), 578 (45%), 521 (39%) 465 (100%).

Table. ¹H NMR (500 MHz), ¹³C NMR (500 MHz), and HMBC data of depsilairdin (**7**) in C₆D₆.

Carbon	δ_C (ppm)	δ_H (ppm), J (Hz)	HMBC
1	171.7	-	-
2	67.8	4.47, br s	1, 3, 5, 6
3	76.6	-	-
4	76.4	4.47, m	3
5	52.8	4.48, m	2, 4
		3.89, dd, 12, 4	4
6	24.8	1.28, s	2, 3, 4
7	170.1	-	-
8	59.4	5.29, d, 11	7, 9, 11, 12, 13
9	28.0	2.47, m	8, 10, 11
10	20.3	1.18, d, 7	8, 9, 11
11	18.8	0.83, d, 7 Hz	8, 9, 10
12	31.0	3.36, s	8, 13
13	171.1	-	-
14	59.5	5.34, d, 11 Hz	13, 15
15	27.8	2.45, m	14, 16, 17
16	19.5	0.92, d 7 Hz	14, 15, 17
17	18.7	0.81, d 7 Hz	14, 15, 16
18	29.8	2.74, s	14, 19
19	175.4	-	-
20	72.6	4.22, br d, 6	19, 21, 22, 23
21	31.0	1.80, m	22, 23

22	19.5	1.18, d, 7 Hz	20, 21, 23
23	15.0	0.99, d, 7	20, 21, 22
1'	83.3	4.89, dd, 12, 4	2', 9', 10', 15', 1
2'	25.8	2.10, m; 1.78, m	1', 3', 4'
3'	41.1	1.64, m; 1.44, m	1', 2', 4', 5'
4'	70.7	-	-
5'	53.5	1.26, m	4', 6', 7', 10', 15'
6'	26.1	2.08, m; 1.28, m	5', 7', 10'
7'	46.1	1.94, m	6'
8'	26.9	1.62, m; 1.41, m	10'
9'	40.7	1.19, m; 2.05, m	5', 8', 10'
10'	38.9	-	-
12'	108.4	4.92, s; 4.93, s	7', 13'
11'	150.1	-	-
13'	21.3	1.80, s	7', 11', 12'
14'	22.7	1.00, s	3', 4', 5'
15'	14.5	1.09, s	1', 5', 9', 10'

(3S,6R)-3,6-Diisopropyl-2,5-morpholinedione (8). HPLC R_t = 19.9 min. $[\alpha]_D$ = 168 (c 0.94, CH_2Cl_2); ^1H NMR (CDCl_3): δ_{H} 0.94 (d, J = 7 Hz), 1.07 (d, J = 7 Hz), 1.14 (d, J = 7 Hz), 1.17 (d, J = 7 Hz), 2.30 (m), 2.64 (m), 3.05 (s), 3.87 (d, J = 6 Hz), 4.73 (d, J = 2 Hz); ^{13}C NMR (CDCl_3): δ_{C} 15.7, 18.6, 19.2, 20.0, 30.8, 32.2, 34.7, 67.8, 82.3, 165.9, 166.3. HREI-MS: m/z 213.1364, calc. for $\text{C}_{11}\text{H}_{19}\text{O}_3\text{N}$ 213.1365, EI-MS: m/z (relative intensity) 213 (8%), 171 (100%).

(3R,6R)-3,6-Diisopropyl-2,5-morpholinedione (14). HPLC R_t = 20.1 min. $[\alpha]_D$ = 31 (c 0.95, CH_2Cl_2); ^1H NMR (CDCl_3): δ_{H} 1.06 (d, J = 7 Hz), 1.08 (d, J = 7 Hz), 1.15 (d, J = 7 Hz), 1.2 (d, J = 7 Hz), 2.30 (m), 2.33 (m), 3.04 (s), 3.85 (d, J = 6 Hz), 4.53 (d, J = 6 Hz); ^{13}C NMR (CDCl_3): δ_{C} 18.6, 19.4, 20.0, 20.4, 32.7, 32.8, 34.7, 66.8, 84.0, 165.4, 165.7. HREI-MS: m/z 213.1371, calc. for $\text{C}_{11}\text{H}_{19}\text{O}_3\text{N}$ 213.1365, EI-MS: m/z (relative intensity) 213 (12%), 171 (100%).

(3R,6S)-3,6-Diisopropyl-2,5-morpholinedione (17). HPLC R_t = 19.9 min. $[\alpha]_D$ = -179 (c 1.15, CH_2Cl_2). ^1H NMR (CDCl_3): δ_{H} 0.94 (d, J = 7 Hz), 1.07 (d, J = 7 Hz), 1.14 (d, J = 7 Hz), 1.17 (d, J = 7 Hz), 2.30 (m), 2.64 (m), 3.05 (s), 3.87 (d, J = 6 Hz), 4.73 (d, J = 2 Hz). ^{13}C NMR (CDCl_3): δ_{C}

15.7, 18.6, 19.2, 20.0, 30.8, 32.2, 34.7, 67.8), 82.3, 165.9, 166.3. HREI-MS: m/z 213.1364, calc. for $C_{11}H_{19}O_3N$ 213.1365, EI-MS: m/z (relative intensity) 213 (8%), 171 (100%).

¹H NMR Spectrum

^{13}C NMR Spectrum

COSY Spectrum

HMQC Spectrum

HMBC Spectrum