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New sesquiterpenic phytotoxins establish unprecedented relationship between different groups of blackleg fungal isolates

M. Soledade C. Pedras,* Paulos B. Chumala and Uppala Venkatesham

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

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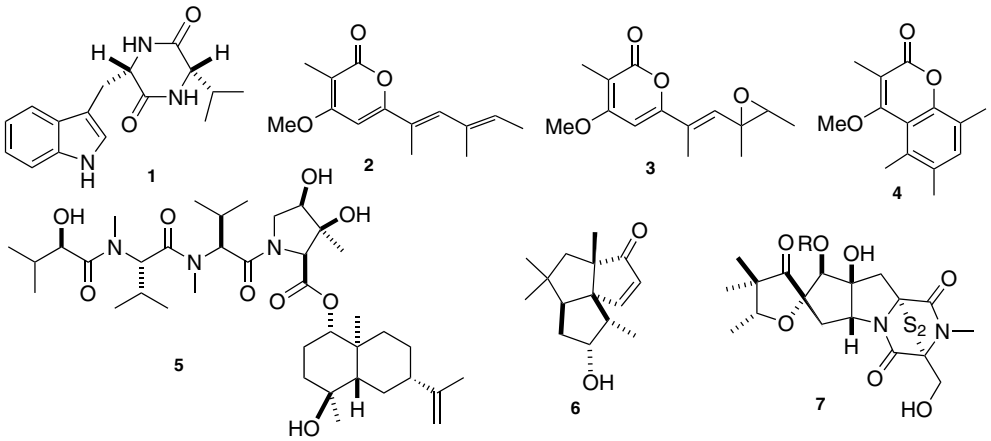
Abstract—A comprehensive search for sesquiterpenic metabolites produced by isolates of the blackleg fungus [*Leptosphaeria maculans* (Desm.)] Ces. et de Not. [asexual stage *Phoma lingam* (Tode ex Fr.) Desm.] revealed that an isolate pathogenic on both canola and brown mustard (IBCN 18) and two isolates pathogenic on brown mustard (Laird 2 and Mayfair 2) produced similar sesquiterpenes. The isolation, chemical structure elucidation, and phytotoxicity of these new sesquiterpenes with silphinene and selinene type skeletons is reported. This is the first time that an isolate virulent on canola and brown mustard is found to produce metabolites characteristic of both virulent (sirodesmins) and avirulent (phomalairdenones) *L. maculans*/*P. lingam*. In the context of grouping the various isolates of *L. maculans*/*P. lingam*, this work suggests an additional pathogenicity group comprising isolates that produce both sirodesmins and phomalairdenones and are virulent on both canola and brown mustard.

1. Introduction

The blackleg fungus (*Leptosphaeria maculans* (Desm.)) Ces. et de Not. [asexual stage *Phoma lingam* (Tode ex Fr.) Desm.] causes blackleg, a plant disease particularly devastating for the oilseed crops rapeseed/canola (*Brassica napus, B. rapa*).[1] Various chemical investigations of blackleg fungal isolates revealed that this plant pathogen produces a great variety of secondary metabolites, some of which are phytotoxic.[2,3] Recently, we reported on the metabolites produced by an unusual group of isolates of *L. maculans*/*P. lingam*, Laird 2 and Mayfair 2,[2] which are virulent on brown mustard (*B. juncea*), a traditionally resistant plant,[4] but not on canola.



Keywords: Fungal metabolites; *Leptosphaeria maculans*; Phomalairdenone; *Phoma lingam*; Sesquiterpenes; Sirodesmin.
* Corresponding author. Tel.: +1 306 966 4772; fax: +1 306 966 4730; e-mail: soledade.pedras@usask.ca

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Interestingly, a few Australian isolates (e.g., isolate IBCN 18) were reported to be virulent on both brown mustard (*B. juncea*) and canola (*B. napus*).[5] Isolates Mayfair 2 and Laird 2 produce polanrazine A (**1**),[6] phomapyrones (e.g., **2**–**4**),[7] depsilairdin (**5**),[8] and phomalairdenone A (**6**),[9] whereas isolate IBCN 18 produces mainly sirodesmins (**7**).[9,10] Both phomalairdenone A (**6**) and depsilairdin (**5**) are host-selective toxins phytotoxic on brown mustard but not on canola, whereas sirodesmins are non-selective phytotoxins.

Detailed secondary metabolite profiles of the various fungal isolates will allow the chemical characterization of the pathogenicity groups of *L. maculans*/*P. lingam* and can provide a reliable basis for isolate differentiation. For example, sirodesmins (**7**) are chemical markers that distinguish isolates virulent on canola (*B. napus* and *B. rapa*).[11] To discover potential chemical markers, we have carried out a comprehensive search for sesquiterpenic metabolites from isolates Laird 2, Mayfair 2, and IBCN 18.[12] As a result of this investigation, we discovered eight new metabolites (**8**–**14** and **16**), and found that isolate IBCN 18, similar to Laird 2 and Mayfair 2, produces in culture phytotoxic sesquiterpenes similar to phomalairdenone A (**6**). Although sirodesmins (**7**) are the main metabolites produced by isolate IBCN 18, this is the first time that an isolate virulent on canola is found to produce both sirodesmins and sesquiterpenes. It is likely that some of the new sesquiterpenes (**8**–**14** and **16**) may become relevant chemical markers for pathogenicity groups of *L. maculans*/*P. lingam* and together with other metabolites provide a basis for a reliable chemical differentiation.

2. Results and discussion

Isolates IBCN 18 and Mayfair 2 were grown in a chemically defined medium, the cultures were extracted, and the extracts were fractionated. Metabolites **6**, **8**, and **9** were isolated from cultures of isolate IBCN 18, whereas **6**, and **10**–**17** were isolated from cultures of Mayfair 2 and Laird 2. Compound **6** was identified as phomalairdenone A ($C_{15}H_{22}O_2$) by comparison of chromatographic and spectroscopic data with those of an authentic sample.[9] The molecular formulas of compounds **8**–**10** were deduced from the HR-EIMS as $C_{15}H_{22}O_2$, identical to that of phomalairdenone A (**6**). The ^1H and ^{13}C NMR spectral data (Table 1) indicated that these compounds were structural isomers of phomalairdenone A (**6**). Further analysis of their NMR data indicated the presence of three methyl singlets, a methyl doublet, two methines, two methylenes, and two olefinic protons. Comparison of the HMQC and HMBC spectral data of **8**–**10** to those of **6** allowed the placement of the hydroxyl groups at C-3, C-5, and C-9, respectively. The relative stereochemistry of each hydroxyl group was determined from analyses of NOE data as shown in Figure 1 for compound **8**.

The foregoing spectral data and a literature search revealed that neither **8** nor **9** were known structures, whereas **10**, though not reported from natural sources, had been semi-synthesized previously.[13] These metabolites were named phomalairdenones B (**8**), C (**9**), and D (**10**) due to the structural similarity to phomalairdenone A (**6**). Metabolites **6**, **8**, and **9** were not previously detected in cultures of isolate IBCN 18 perhaps due to

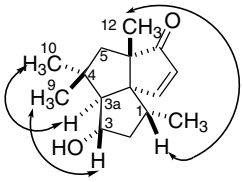
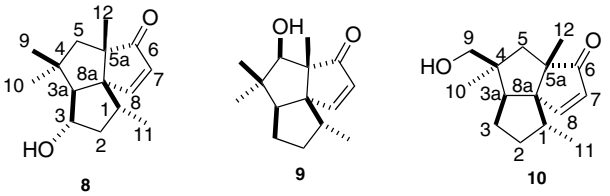


Figure 1. Relevant NOE correlations observed for phomalairdenone B (**8**).

Table 1. ^1H and ^{13}C NMR chemical shifts (ppm) and ^1H multiplicities (*J* in Hz) for phomalairdenones B (**8**), C (**9**), and D (**10**), solvent

C/H	Phomalairdenone B (**8**), CD_2Cl_2		Phomalairdenone C (**9**), C_6D_6		Phomalairdenone D (**10**), C_6D_6	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.18, m	35.3	2.23, m	39.4	1.70, m	38.6
2	2.18, m 1.52, m	46.4	2.02, m 1.96, m	36.8	1.82, m 1.30, m	36.6
3	4.03, m	75.7	1.77 m 1.99, m	26.9,	1.70, m 1.30, m	28.4
3a	1.80, d, 8.5	69.1	1.69, m	58.5	1.82, m	59.1
4	—	43.0	—	47.1	—	45.0
5	1.52, d, 14 1.98, d, 14	51.8	3.64, s	85.3	2.13, d, 13 1.32, d, 13	46.6
5a	—	59.0	—	59.7	—	56.7
6	—	217.0	—	215.4	—	215.1
7	6.04, d, 6	132.1	6.01, d, 6	128.1	5.99, d, 6	130.3
8	7.75, d, 6	170.8	7.58, d, 6	170.2	7.05, d, 6	168.8
8a	—	70.0	—	68.1		67.9
9	1.09, s	28.4	0.95, s	20.3	3.25, d, 10 3.08, d, 10	69.9
10	0.82, s	32.5	0.89, s	29.6	0.99, s	25.1
11	0.96, d, 6	17.2	0.88, d, 7	16.5	0.77, d, 7	16.1
12	1.04, s	22.5	1.18, s	14.3	1.60, s	21.2

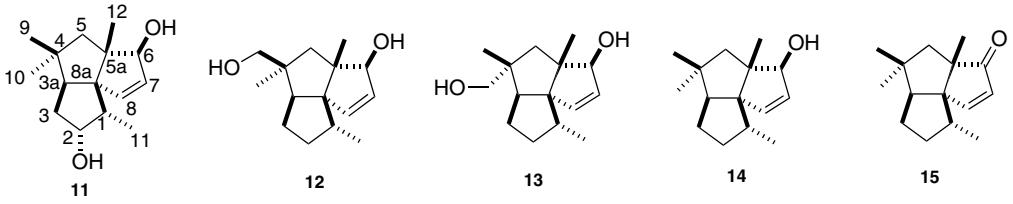
the large amounts of sirodesmin PL (**7**, R = Ac, $n = 2$, ca. 400 mg/L)[14] and small amounts of **6**, **8**, and **9** (0.1–0.2 mg/L) produced in fungal cultures.



Compounds **11**–**13** gave parent ions in HREI-MS consistent with the molecular formula of $C_{15}H_{24}O_2$, indicating four degrees of unsaturation, that is, one less than phomalairdenones **8**–**10**. The ^1H and ^{13}C NMR spectra revealed that the α,β-unsaturated carbonyl group (C-6) was replaced with an allylic hydroxyl group, thus accounting for one less unsaturation. As previously, the ^1H–^1H COSY, HMBC, and HMQC spectral data of compounds **11**–**13** allowed the assignment of the second hydroxyl group to carbons C-2, C-9, C-10, respectively (Table 2). The relative stereochemistry was deduced from NOE spectral data as reported above for **8**. Metabolites **11**–**13** are new compounds that were named phomalairdenols A (**11**), B (12), and C (**13**) due to their structural similarity to phomalairdenone A (**6**).

Compound **14** had a molecular formula of $C_{15}H_{24}O$ (HREI-MS and NMR data), indicating four degrees of unsaturation, and one less oxygen atom phomalairdenols **11**–**13**. Compared to phomalairdenols B (**12**) and C (**13**), the ^1H NMR spectrum of **14** displayed an additional methyl singlet replacing hydroxymethylene proton signals at C-9/10 (Table 2). The relative configuration of the only hydroxyl group was determined by NOE difference experiments as summarized in Figure 2. For example, irradiation of H_3-10 (δ_H 1.04) caused an NOE enhancement on the signals at δ_H 4.31 (H-6) and 1.94 (H-3a), and irradiation of H_3-12 (δ_H 1.18) caused an NOE enhancement on the signal of H_3-9 (δ_H 1.03). These NOE results imply that H-3a, H-6, and H_3-10 are *cis* to each other and *trans* to H_3-9 and H_3-12. Consequently, structure **14** was assigned for the compound we named phomalairdenol D.

An additional silphinene type compound was isolated and the structure was determined to be identical to that of 3-oxosilphinene (**15**), after comparison of the spectroscopic data with those reported previously.[15]



The molecular formula of compound **16** was determined to be $C_{15}H_{26}O_2$ on the basis of HREI-MS and NMR data, indicating three degrees of unsaturation. Signals observed in the ^1H, ^{13}C NMR, and HMQC spectra,

Table 2. ^1H and ^{13}C NMR chemical shifts (ppm) and ^1H multiplicities (J in Hz) for phomalairdenols A (**11**), B (**12**), C (**13**), and D (**14**), solvent

C/H	Phomalairdenol A (**11**), CD_2Cl_2		Phomalairdenol B (**12**), C_6D_6		Phomalairdenol C (**13**), C_6D_6		Phomalairdenol D (**14**), C_6D_6	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.67,	46.4	1.88, m	39.4	1.86	39.6	1.91, m	39.6
2	3.52, ddd, 10, 10, 6	80.3	1.85, m	37.7	1.90	38.4	1.34, m	38.2
			1.23, m		1.40		1.84, m	
3	1.86, ddd, 11, 8, 6	36.1	1.65, m	27.0	1.57, m	27.6	1.60, m	27.1
	1.34, ddd, 11, 11, 10		1.27, m		1.36, m		1.34, m	
3a	1.70, m	57.0	1.95, dd, 10, 8	61.5	2.02, dd, 9, 9	58.5	1.94, dd, 9, 9	63.0
4	—	39.9	—	44.3	—	45.1	—	39.7
5	1.63, d, 13	57.9	1.72, d, 13	53.1	1.79, d, 13	53.6	1.62, d, 13	58.8
	1.57, d, 13		1.55, d, 13		1.46, d, 13		1.73, d, 13	
5a	—	54.0	—	53.8	—	52.8	—	53.3
6	4.24, br s	85.3	4.37, d, 2	86.4	4.21, d, 2	85.4	4.31, d, 2	85.9
7	5.58, dd, 6, 2	128.5	5.50, dd, 6, 2	129.0	5.61, dd, 6, 2	128.7	5.61, dd, 6, 2	128.7
8	5.54, d, 6	143.2	5.71, d, 6	143.3	5.70, d, 6	143.5	5.74, d, 6	143.5
8a	—	70.3	—	72.5	—	72.4	—	73.0
9	0.99, s	32.5	3.36, d, 10	70.0	1.07, s	22.9	1.03, s	27.9
			3.30, d, 10					
10	1.01, s	28.0	1.10, s	26.8	3.17, d, 10	73.0	1.04, s	32.3
					3.11, d, 10			
11	0.93, d, 7	13.7	0.90, d, 7	16.8	0.90, d, 7	16.6	0.90, d, 7	16.7
12	1.16, s	19.4	1.10, s	20.0	1.19, s	19.6	1.18, s	19.4

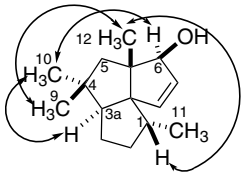
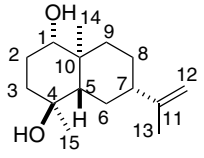


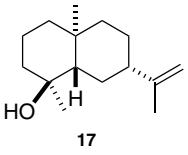
Figure 2. Relevant NOE correlations observed for phomalairdenol D (**14**).

namely three methyls ($\delta_{H/C}$ 0.88/13.3, 1.02/22.9, and 1.82/21.2), olefinic methylene ($\delta_{H/C}$ 4.96/108.8), and methine ($\delta_{H/C}$ 3.11/79.5) groups, indicated a ring system rather different from that of compounds **8**–**15**. These NMR data together with the molecular formula suggested a eudesmane skeleton. Two spin systems (A and B, Fig. 3) were apparent in the ^1H–^1H COSY spectrum. One of the systems was observed through correlations of a proton at δ_H 1.95 (H-7) with methylene protons at δ_H 1.47 and 1.65 (H_2-8) and methylene protons at δ_H 1.30 and 2.03 (H_2-6) with a methine proton at δ_H 1.15 (H-5) (A, Fig. 3). Another system displayed a methine proton at δ_H 3.11 (H-1, dd, $J = 11$, 4 Hz) coupling with methylene protons at δ_H 1.44 and 1.50 (H_2-2), and further coupling with protons at δ_H 1.38 and 1.67 (H_2-3) (B, Fig. 3). In the HMBC spectrum, the methyl singlet at δ_H 1.82 (H_3-13) correlating with olefinic carbons at δ_C 108.8 (C-12) and 150.6 (C-11) was assigned to a vinylic position. A further HMBC correlation of this vinyl methyl group (H_3-13) with the carbon signal at δ_C 46.4 (C-7), and an additional correlation between this carbon (C-7) and the methylene proton at δ 4.96 (H_2-12) indicated the presence of an isopropyledene moiety attached to C-7. HMBC correlations observed from a methyl singlet at δ_H 0.88 (H_3-14) to carbons at δ_C 41.5 (C-3), 71.1 (C-4), and 53.3 (C-5) indicated the connectivity of the quaternary carbon at δ_C 71.1 (C-4) with C-3 and C-5. Further HMBC correlations of a methyl singlet at δ_H 1.02 (H_3-15) to carbons at δ_C 79.5 (C-1), 53.3 (C-5), 41.0 (C-9), and 39.3 (C-10) established the connectivity between C-15 and the quaternary carbon C-10, and also between C-10 and C-5, C-10 and C-1, and C-10 and C-9, as summarized in Figure 3. The chemical shifts of C-1 (δ_C 79.5) and C-4 (δ_C 71.1) suggested that these carbons were connected to oxygen atoms. The structural subunits A and B were connected on the basis of additional long-range correlations between H-5 (δ_H 1.15) and C-4 (δ_C 71.1), C-6 (δ_C 26.3), and C-7 (δ_C 46.4) to establish structure **16**.

The relative configuration of **16** was determined on the basis of coupling constants and NOE difference spectral data. For example, the coupling constants of H-1 (δ_H 3.11, $J = 11$, 4 Hz) was indicative of its orientation as *trans*-diaxial with one of the protons C-2. In agreement with this observation, irradiation of H-1 resulted in an NOE enhancement of axial proton signals at δ_H 1.15 (H-5) and δ 1.05 (H-9), and vice versa. Further irradiation of the methyl proton at δ_H 0.88 (H_3-14) caused an NOE enhancement on the other methyl signal at δ_H 1.02 (H_3-15), and vice versa. These NOE spectral data suggested a *trans*-fused ring as well as *cis* relationship between CH_3-14 and CH_3-15, allowing the assignment of the relative configuration of **16**. Since **16** is a structural fragment of depsilairdin (**7**) and the absolute configuration of **7** was established by chemical methods and X-ray crystallography, it is proposed that the absolute configuration of **16** is identical to that of the corresponding fragment present in **7**. It is not likely that the presence of **16** in culture extracts is due to partial hydrolysis of depsilairdin (**7**) since both metabolites are present in similar amounts (**6**—18 mg and **7**—22 mg) and the fungus produces also the related sesquiterpene **17**. A literature search revealed that the enantiomer of **16**, cyperusol C, was recently isolated from *Cyperus longus*.[16] The $[\alpha]_D$ reported for cyperusol C is −42.3 (*c* 1.10 MeOH), whereas that of **16** is +18 (*c* 0.40 CH_2Cl_2); however, since the specific optical rotation values were obtained in different solvents these values are not comparable. The absolute configuration of cyperusol C was established by a modified Mosher's ester method, and that assignment is in complete agreement with our data. Due to the relationship to depsilairdin (**7**), we named compound **16** as lairdinol A.



16



17

Compound **17** had the molecular formula $C_{15}H_{26}O$, as determined by HREI-MS and NMR data, indicating three degrees of unsaturation and one oxygen less than **16**. In ^1H, ^{13}C NMR, and HMBC spectra, three methyl singlets and olefinic methylene protons indicated a ring system identical to that of compound **16**. The relative configuration of **17** was established using ^1H NMR and NOE difference data, as reported for **16**. Hence, a *trans*-fused stereochemistry at the ring junction and a *cis* relative orientation between CH_3-14 and CH_3-15 was identified. Finally, a literature survey showed that compound **17** was identical to selin-11-en-4α-ol, a sesquiterpenoid reported previously from the essential oil of *Podocarus dacrydioides*.[17]

Phytotoxicity assays using phomalairdenones A (**6**) and D (**10**), phomalairdenols A–C (**11**–**14**), and lairdinol A showed that **6**, **10**, **11**, and **16** caused lesions (3–4 mm diameter) on *B. juncea* but not on *B. napus*.[18] It is particularly interesting to note that among the phomalairdenols **11**–**14**, only A (**11**) showed phytotoxicity, suggesting that the hydroxyl group at C-2 is important,

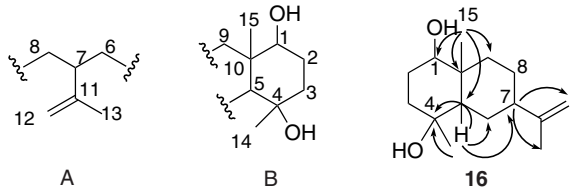


Figure 3. Substructures A and B, and selected HMBC correlations observed for lairdinol (**16**).

whereas the position of the hydroxyl group in phomalairdenones does not appear to have such a critical effect. However, further work is necessary to fully understand the structure–activity relationships among these new metabolites.

In summary, we have established that isolates Mayfair 2, Laird 2, and IBCN 18 produced similar sesquiterpenes, most of which were not known previously. In the context of grouping the various isolates of *L. maculans*/*P. lingam*, it is particularly interesting to note that isolate IBCN 18, a sirodesmin (**7**, markers for isolates virulent on canola) producer, produces also a group of metabolites similar to those produced by isolates Laird 2 and Mayfair 2, non-sirodesmin producing (usually avirulent on canola). The production of similar metabolites by isolates Laird2/Mayfair 2 and IBCN 18 suggests that IBCN 18 may represent an additional pathogenicity group. This additional group might comprise isolates that produce both sirodesmins (**7**) and phomalairdenones (e.g., **6**) and are virulent on both canola and brown mustard (e.g., IBCN 18). Nonetheless, substantial chemical and biological studies need to be carried out to verify this possibility. The fact that this hypothesis can be formulated is in itself of great interest and warrants further work. It is expected that relevant chemical markers for each pathogenicity group of the *L. maculans*/*P. lingam* will provide the chemical basis for a reliable chemical differentiation.

3. Experimental

3.1. General procedures

All chemicals were purchased from Sigma–Aldrich Canada Ltd, Oakville, ON. 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 m particle size silica, 4.6 i.d. × 200 mm), equipped with an in-line filter. Mobile phase: system A—H_2O–CH_3CN, 75:25 to 100% CH_3CN, for 35 min, linear gradient, and 1.0 mL/min flow rate; system B—isocratic elution 80:20 for 10 min, followed by gradient elution 80:20 to 60:40 for 10 min, and 60:40 to 75:25 for 10 min, H_2O–CH_3CN, flow rate 1.0 mL/min.

3.2. Plant material and phytotoxicity bioassays

Canola (*Brassica napus*) cv. Westar, white mustard (*Sinapis alba*) cv. Ochre and brown mustard (*Brassica juncea*) cv. Cutlass plants were grown in a growth chamber at 24 ± 2 °C.[19] The phytotoxicity assays of fractions and isolated compounds from Mayfair 2/Laird 2 cultures were conducted on leaves of two-week-old plants. Samples were prepared in 50% aqueous methanol at concentrations of 5×10^{-4} and 1×10^{-3} M. Leaves were punctured at four places with a needle and inoculated at each spot with a 10 μL sample. Control leaves were treated similarly employing 50% aqueous methanol. Plants were incubated in a growth chamber and the diameter of the lesions was measured after 7 days of the incubation period.

3.3. Fungal cultures and isolation of metabolites

L. maculans/*P. lingam*, isolate IBCN 18 was obtained from the International Blackleg Crucifer Network (IBCN) collection, and isolates Laird 2 and Mayfair 2 from Agriculture & Agri-Food-Canada, Saskatoon, SK collection. Fungal cultures were grown in 250 mL Erlenmeyer flasks containing 100 mL of minimal medium[20] plus thiamine (10^9 spores per flask) and cultures incubated on a shaker at 120 rpm, at 23 ± 1 °C for 6 days (23 L of cultures for IBCN 18, 120 L for Mayfair 2 and 20 L for Laird 2).

3.3.1. Isolate IBCN 18. The broth and mycelium were separated by gravity filtration, the broth (23 L) was concentrated (5 L), extracted with EtOAc, and the solvent removed under vacuum (1.82 g). The EtOAc extract was subjected to flash column chromatography (FCC), eluted with CH_2Cl_2, CH_2Cl_2–EtOAc mixtures and EtOAc, finally washed the column with CH_2Cl_2–MeOH (90:10), to yield 10 fractions, each 200 mL. The least polar fraction F1 contained a mixture of phomalairdenones (55 mg). F1 was further purified by multiple preparative TLC (CH_2Cl_2–EtOAc, 80:20, three developments), to yield a fraction (6.1 mg) further purified by reversed phase (C_{18}) preparative TLC (CH_3CN–H_2O, 55:45, two developments), to yield phomalairdenone C (**9**) (1.5 mg), phomalairdenone A (**6**) (2.4 mg) and impure phomalairdenone B (**8**) (1.8 mg), which was further purified (CH_3CN–H_2O, 9:91) to yield phomalairdenone B (**8**) (1.2 mg).

3.3.2. Isolate Mayfair 2. The broth and mycelium were separated by gravity filtration, the broth (120 L) was concentrated to (22 L), extracted with EtOAc, and the solvent removed under vacuum. The ethyl acetate extracts (350 mg per batch, total amount 2.3 g) was subjected to FCC and eluted first with CH_2Cl_2 (200 mL) followed by CH_2Cl_2–MeOH (97:3, 200 mL) and then with CH_2Cl_2–MeOH (90:10, 200 mL). All fractions collected were concentrated to dryness under reduced pressure. Fractions F3 to F6 were combined, further subjected to prep. TLC (CH_2Cl_2–MeOH, 98:2, three times). This fractionation gave phomalairdenol D (**14**) (12 mg), together with non-homogenous material. This material was further separated by prep. TLC using hexane–EtOAc (90:10, developed four times) to yield 3-oxosilphinene (**15**) (3 mg) and selin-11-en-4α-ol (**17**) (2.5 mg). Fraction F7 was further chromatographed on FCC (CH_2Cl_2–EtOAc, 95:5, 200 mL, CH_2Cl_2–EtOAc, 90:10, 200 mL, a CH_2Cl_2–EtOAc, 80:20, 200 mL). Twelve fractions were collected, concentrated to dryness and fractions F6 to F9 from FCC of F7 were combined, further subjected to preparative TLC and developed with CH_2Cl_2–MeOH (98:2) to yield phomalairdenone A (**6**) (12 mg) and a fraction of non-homogenous material. This non-homogenous fraction was further subjected to preparative TLC (hexane–EtOAc, 60:40) to yield phomalairdenone D (**10**) (8 mg). Fractions F11 to F14 from FCC on ethyl acetate extracts were

combined and subjected to preparative TLC (CH_2Cl_2–MeOH, 97:3, developed three times). The UV-inactive non-homogenous fraction was further chromatographed on preparative TLC and developed with CH_2Cl_2–Et_2O (50:50, four times) to yield two compounds, phomalairdenol C (**13**) (24 mg) and lairdinol A (**16**) (18 mg) together with non-homogeneous material. This material was further chromatographed on preparative TLC (hexane–EtOAc, 70:30, several times) to yield phomalairdenol A (**11**) (9 mg) and phomalairdenol B (8 mg) (**12**). Isolate Laird 2 produced identical metabolites.

3.3.3. Phomalairdenone B (8). HPLC t_R = 9.6 min (solvent system A). $[\alpha]_D$ −18 (*c* 0.10, CH_2Cl_2). ^1H NMR, ^{13}C NMR in Table 1. FTIR (cm^{-1}): 3304, 2958, 2928, 1702, 1457. HREI-MS *m/z* measured 234.1619 (M^+, 234.1620 calcd for $C_{15}H_{22}O_2$). EIMS *m/z* (% relative abundance) 234 (M^+) (100), 219 (38), 192 (22), 178 (42), 159 (49).

3.3.4. Phomalairdenone C (9). HPLC t_R = 17.6 min (solvent system A). $[\alpha]_D$ −32 (*c* 0.12, CH_2 Cl_2). ^1H NMR, ^{13}C NMR in Table 1. FTIR (cm^{-1}): 3460, 2952, 28741693, 1451. HREI-MS *m/z* measured 234.1617 (M^+, 234.1620 calcd for $C_{15}H_{22}O_2$). EIMS *m/z* (% relative abundance) 234 (M^+) (11), 206 (19), 179 (19), 163 (100).

3.3.5. Phomalairdenone D (10). HPLC t_R = 21.7 min (solvent system B). $[\alpha]_D$ −8 (*c* 0.80, CH_2Cl_2). ^1H NMR, ^{13}C NMR in Table 1. FTIR (cm^{-1}): 3454, 2958, 2870, 1697, 1031, 832. HREI-MS: *m/z* 234.1620 (M^+, 234.1620 calcd for $C_{15}H_{22}O_2$). EI-MS: *m/z* (relative intensity) 234 (81%), 203 (100%), 119 (30%).

3.3.6. Phomalairdenol A (11). $[\alpha]_D$ +21 (*c* 0.50, CH_2Cl_2). ^1H NMR, ^{13}C NMR in Table 2. FTIR (cm^{-1}) 3352, 2951, 2871, 1375, 1024, 996. HREI-MS: *m/z* 236.1773 (M^+ calcd for $C_{15}H_{24}O_2$ 236.1776). EI-MS: *m/z* (relative intensity) 236 (19%), 205 (100%), 122 (35%). Mp 136–138 °C.

3.3.7. Phomalairdenol B (12). $[\alpha]_D$ −36 (*c* 1.2, CH_2Cl_2). ^1H NMR, ^{13}C NMR in Table 2. FTIR (cm^{-1}) 3287, 2953, 2868, 1373, 1037. HREI-MS: *m/z* 236.1774 (M^+ calcd for $C_{15}H_{24}O_2$ 236.1776). EI-MS: *m/z* (relative intensity) 236 (27%), 205 (100%), 203 (40%), 91 (48%).

3.3.8. Phomalairdenol C (13). $[\alpha]_D$ +32 (*c* 1.2, CH_2Cl_2). ^1H NMR, ^{13}C NMR in Table 2. FTIR (cm^{-1}) 3323, 2925, 2873, 1373, 1021. HREI-MS: *m/z* 220.1830 (M^+ calcd for $C_{15}H_{24}O_1$ 220.1827). EI-MS: *m/z* (relative intensity) 220 (100%), 205 (62%), 163 (55%), 123 (92%).

3.3.9. Phomalairdenol D (14). $[\alpha]_D$ +45 (*c* 0.40, CH_2Cl_2). ^1H NMR, ^{13}C NMR in Table 2. FTIR (cm^{-1}) 3323, 2952, 1459, 1076, 1025. HREI-MS: *m/z* 236.1773 (calcd for $C_{15}H_{24}O_2$ 236.1776). EI-MS: *m/z* (relative intensity) 236 (60%), 218 (100%), 203 (85.0%), 163 (49%), 107 (61%). Mp 75–77 °C.

3.3.10. Lairdinol A (16). $[\alpha]_D$ +18 (*c* 0.40, CH_2Cl_2). ^1H NMR (C_6D_6): δ 4.96 (d, J = 15 Hz, H_2-12), 3.11 (dd, J = 11, 4 Hz, H-1), 2.03 (m, H_a-6), 1.95 (m, H-7), 1.85 (ddd, J = 13, 3, 3 Hz, H_b-9), 1.82 (s, H_3-13), 1.67 (m, H_a-3), 1.65 (m, H_a-8), 1.50 (m, H_a-2), 1.47 (m, H_b-8), 1.44 (m, H_b-2), 1.38 (m, H_b-3), 1.30 (ddd, J = 12, 12, 12 Hz, H_b-6), 1.15 (dd, J = 12, 2 Hz, H-5), 1.05 (ddd, J = 13, 13, 3 Hz, H_a-9), 1.02 (s, H_3-15), 0.88 (s, H_3-14). ^{13}C NMR (C_6D_6): δ 150.6 (C-11), 108.8 (C-12), 79.5 (C-1), 71.1 (C-4), 53.3 (C-5), 46.4 (C-7), 41.5 (C-3), 41.0 (C-9), 39.3 (C-10), 29.2 (C-2), 27.0 (C-8), 26.3 (C-6), 22.9 (C-15), 21.2 (C-13), 13.3 (C-14). FTIR (cm^{-1}) 3379, 2932, 2856, 1382, 1073. HREI-MS: *m/z* 238.1936 (M^+ calcd for $C_{15}H_{26}O_2$ 238.1932). EI-MS: *m/z* (relative intensity) 238 (27%), 220 (41%), 179 (49%), 162.14 (66%), 72 (100%).

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