Phomalirazine, a Novel Toxin from the Phytopathogenic Fungus *Phoma lingam*

M. Soledade C. Pedras* and Suzanne R. Abrams

Plant Biotechnology Institute
National Research Council Canada
Saskatoon, Saskatchewan S7N 0W9, Canada

Ginette Séguin-Swartz

Agriculture Canada Research Station
Saskatoon, Saskatchewan S7N 0X2, Canada

J. Wilson Quail and Zongchao Jia

Department of Chemistry, University of Saskatchewan
Saskatoon, Saskatachewan S7N 0X2, Canada

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Phoma lingam (perfect stage *Leptosphaeria maculans*) is a fungal pathogen which infects rapeseed/canola (*Brassica napus* and *B. campestris*) and cabbage (*B. oleracea*), causing leaf spots and stem cankering.[1] The "blackleg disease" of the oilseed crops rapeseed and canola is a major agricultural problem.[1] The disease symptoms indicate that the fungus produces phytotoxin(s).[2] Identification of fungal toxins is important for understanding the chemical basis of plant-microbe interactions.[3] Their availability can be of enormous importance in agriculture where the screening of plant material for toxin resistance can be used to provide resistant plants.[4] As part of a plant breeding program we have been studying the toxins produced by *P. lingam*.[5] Here we report the isolation and characterization of an unusual epidithiodioxopiperazine which we have named phomalirazine (**4**). The structure of phomalirazine has important implications on the biogenetic pathway of this broad class of sulfur bridged dioxopiperazines.

Phoma lingam was grown in liquid still culture on minimal medium supplemented with thiamine[6] for 21 days. The broth was separated from the mycelium, concentrated, and extracted with ethyl acetate. The isolation of bioactive metabolites was guided by a simple cotyledon or leaf assay.[7] Flash column chromatography of the broth extract and assay indicated that the activity was mainly due to sirodesmin PL[8] (**5**, ca. 70% of the total extract, w/w); however, more polar fractions were also active. Phomalirazine (**4**) was obtained after fractional crystallization of the most polar of the active fractions, as a white powdery material[9] (<1% of the total extract, w/w). The NMR spectra indicated

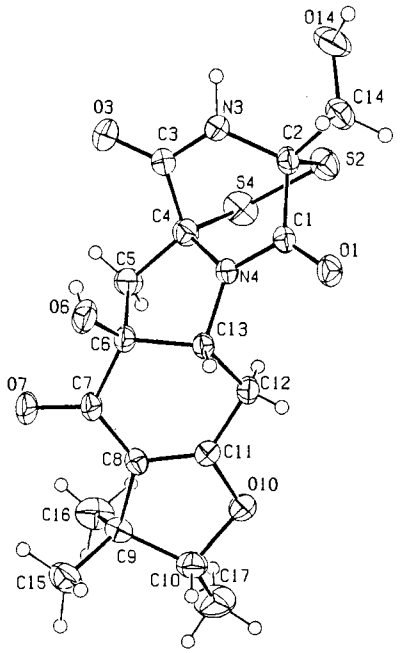

Figure 1. ORTEP drawing of phomalirazine (**4**) with atomic numbering.

the presence of 20 hydrogens and 17 carbons.[10] Proton decoupling experiments revealed a close similarity between the proton spin systems of phomalirazine (**4**) and those of sirodesmin PL (**5**). The ^{1}H NMR of **4** showed two methyl singlets, a methyl doublet, and a methine quartet as observed (within 0.5 ppm) for the ring A fragment of **5**. Similarly, two AB and an ABX spin system were observed, which closely corresponded to those associated with the hydrogens at C5, C14, and C12 to C13, respectively, of **5**. Resonances corresponding to the acetoxy and N-methyl groups of **5** were absent in the spectrum of **4**. The ^{13}C NMR spectra of both compounds revealed common structural features. Fourteen carbons observed in the spectrum of **4** could be related, in terms of chemical shift and multiplicity (J_{CH}), with those due to C1 to C6, C10 to C14, and C16 to C18 in **5**. The remaining three signals [189.60 (s) 172.66 (s) and 118.87 (s) ppm], while clearly not related to C7 to C9 in **5**, suggested the presence of a β-alkoxyenone moiety. Thus the difference in structure between **4** and **5** should reside in ring B. The presence of a six-membered B ring in **4** was suggested by comparing the $^2J_{HH}$ for the C12 hydrogens in **5** with that for the analogous hydrogens in **4** (14.1 vs 18.6 Hz). The spectral data[11] coupled with biogenetic considerations led us to propose structure **4** for phomalirazine. This structure, including the absolute configuration at the asymmetric centers, was confirmed by X-ray diffraction. An ORTEP drawing of phomalirazine is shown in Figure 1. Slow evaporation of an ethyl acetate solution[12] led to the formation of crystals that belonged to space group $P2_12_12_1$ ($Z = 4$) with $a = 7.373$ (1), $b = 12.122$ (3), and $c = 20.921$ (1) Å. The crystal structure was stabilized by an

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(3) Scheffer, R. P.; Briggs, S. P. *Toxins in Plant Disease*; Durbin, R. D., Ed.; Academic: New York, 1981; pp 1-20.

(4) Durbin, R. D. *Toxins in Plant Disease*; Durbin, R. D., Ed.; Academic: New York, 1981; pp 495-505.

(5) Pedras, M. S. C.; Abrams, S. R.; Séguin-Swartz, G. *Tetrahedron Lett.* **1988**, *29*, 3471-3474.

(6) Tinline, R. D.; Stauffer, J. F.; Dickson, J. G. *Can. J. Bot.* **1960**, *38*, 275-282.

(7) The test sample, dissolved in a methanol-water (1:1, v/v) solution, was applied on cotyledons or leaves of plants growing in a growth chamber as described in ref 2. Lesions produced by the broth extract, and lesions produced by the pathogen were similar.

(8) Sirodesmin PL has been isolated previously from *P. lingam*. See: Férézou, J. P.; Rich, C.; Quesneau-Thierry, A.; Pascard-Billy, C.; Barbier, M.; Bousquet, J. F.; Boudart, G. *Nouv. J. Chim.* **1977**, *1*, 327-334.

(9) The material had no definite mp and decomposed at 218 °C. It was very soluble in Me_2SO or in pyridine, but these solvents were not suitable. A pyridine solution of phomalirazine showed decomposition in less than 30 min at room temperature, and recovery from a Me_2SO solution led to some decomposition.

(10) ^{1}H NMR ($CDCl_3$, 360 MHz) δ 6.64 (br s, HN), 4.59 (m, 13-H), 4.51 (q, J = 6.5 Hz, 10-H), 4.26 (dd, J = 12.7, 7.4 Hz, 14-H$_a$), 4.21 (dd, J = 12.7, 6.4 Hz, 14-H$_b$), 3.79 (s, 6-HO), 3.38 (dd, J = 14.3, 1 Hz, 5-H$_a$), 3.20 (dd, J = 18.6, 7.8 Hz, 12-H$_a$), 2.89 (dd, J = 18.6, 8.6 Hz, 12-H$_b$), 2.71 (dd, J = 7.4, 6.4 Hz, 14-HO), 2.53 (d, J = 14.3 Hz, 5-H$_b$), 1.34 (d, 6.5 Hz, 17-H), 1.34 (s, 15/16-H), 1.16 (s, 16/15-H); ^{13}C NMR (2% Me_2SO in $CDCl_3$, 90.5 MHz) δ 189.60 (s, C7), 172.66 (s, C11), 164.77 (s, C1/C3), 162.81 (s, C3/C1), 118.87 (s, C8), 92.75 (d, C10), 79.86 (s, C6), 73.98 (s, C2/C4), 73.35 (s, C4/C2), 62.20 (d, C13), 61.10 (t, C14), 46.12 (s, C9), 42.56 (t, C5), 26.79 (t, C12), 26.20 (q, C15/C16), 20.02 (q, C16/C15), 14.45 (q, C17).

(11) We could not identify the molecular ion in the MS. The CIMS [m/z 445 (9%), 413 (70%), 381 (56%), 363 (38%) 349 (100%)] was consistent with a trisulfur bridge, but the NMR data was not. Epitrithiodioxopiperazines exist in two stable conformations which are distinguishable by NMR spectroscopy. See, for example: Kirby, G. W.; Rao, G. V.; Robins, D. J. *J. Chem. Soc., Perkin Trans 1* **1988**, 301-304, and references therein.

(12) A very dilute ethyl acetate solution of phomalirazine was left in a loosely covered NMR tube till complete evaporation of the solvent occurred at room temperature.

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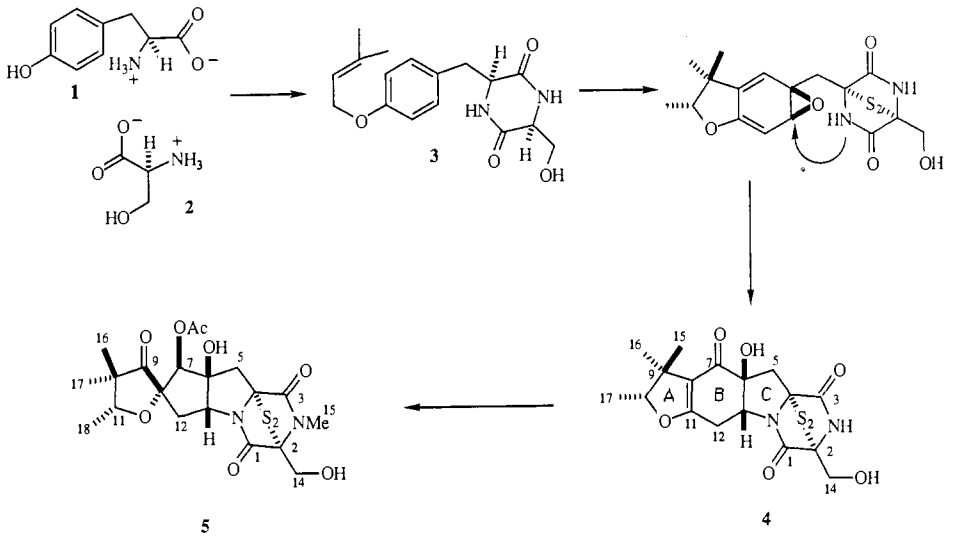
Scheme I



intermolecular hydrogen bond formed between O14 and O1.

Phomalirazine (**4**) possesses a new ring system, and its epidithiodioxopiperazine group is unusual because one of the nitrogen atoms is not alkylated.[13] The biosynthesis of polythiodioxopiperazines has been studied, and a general pathway is acknowledged.[14,15] Cyclic dipeptides act as precursors of epipolythiodioxopiperazines; however, there are very few intermediates which give any clues on the sequence of steps necessary to accomplish the transformation. In particular for "sirodesmins" a pathway was proposed[16] and later partly confirmed by the incorporation of L-tyrosine (**1**), L-serine (**2**), and the cyclic dipeptide **3** into sirodesmin PL (**5**).[17] The sequence of steps required to transform **3** into **5**, namely introduction of the disulfur bridge, N-methylation, and cyclization to form both the A and the C rings, is not known. Phomalirazine (**4**) is a likely intermediate between **3** and **5** (Scheme I). This proposal is consistent with the absolute configurations of compounds **4** and **5**. Carbons 2, 4, 6, 10, and 13 of **4** have absolute configurations identical with carbons 2, 4, 6, 11, and 13, respectively, of sirodesmin PL (**5**). The isolation of **4** indicates that, during the transformation of **3** into **5**, oxidative cyclization through a possible arene oxide intermediate[16] (to form the C ring) occurs prior to the N-methylation step. Oxidative ring contraction of the B ring of phomalirazine can then originate sirodesmin PL (**5**).

Recently there has been a renewed interest in the epipolythiodioxopiperazines due to their inhibitory effect on platelet aggregation and immunosuppressive properties.[18,19] Metabolites containing that group have been isolated from diverse fungal sources,[14] and their biological activity has been associated with the epipolythiodioxopiperazine group.[18,20,21]

Phomalirazine is active at 10^{-5} M in a cotyledon assay, whereas sirodesmin PL is active at 2×10^{-4} M. Further biological studies are under way.

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Supplementary Material Available: Tables of fractional coordinates, thermal parameters, interatomic distances, and interatomic and torsional angles for phomalirazine and an ORTEP drawing of **4** along with a packing diagram illustrating the hydrogen bonding (8 pages). Ordering information is given on any current masthead page.

(13) See, for example: Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Academic: New York, 1983; pp 417-422.

(14) Taylor, A. *Microbial Toxins Vol VII*; Kadis, S., Ciegler, A., Ajl, S. J., Eds.; Academic: New York, 1971; pp 337-376, and references therein.

(15) Kirby, G. W.; Robins, D. J. *The Biosynthesis of Mycotoxins*; Stein, P. S., Ed.; Academic: New York, 1980; pp 301-326.

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(17) Férézou, J. P.; Quesneau-Thierry, A.; Servy, C.; Zissmann, E.; Barbier, M. *J. Chem. Soc., Perkin Trans 1* **1980**, 1739-1746.

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The Ambiphilic Nature of *N*-Acyliminium Ion-Enamide Tautomers. A Novel Annulation to Enantiomerically Pure Polycyclic Frameworks

Stefan Bienz, Carl Busacca, and A. I. Meyers*

Department of Chemistry, Colorado State University
Fort Collins, Colorado 80523

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The recently promoted synthetic utility of *N*-acyliminium ions has been reviewed by Speckamp and Hiemstra.[1] These authors and others have also contributed a vast amount of useful chemistry[2] to this subject by treating succinimides **1** with borohydride to afford the carbinol amides **2A** (R = H). These species are in facile acid-catalyzed equilibrium with the *N*-acyliminium ions **2B** (R = H) which are, in turn, capable of intramolecular capture of a wide variety of nucleophiles (Nuc:alkene, alkyne, aryl, enamine, etc.) producing polycyclic systems such as **3**. In spite of the impressive behavior of acyliminium ions, there is still one member of the family (**2C**) which has shown little, if any, synthetic importance.[3] Thus, deprotonation of **2B** or dehydration of **2A**

(1) Speckamp, W. N.; Hiemstra, H. *Tetrahedron* **1985**, *41*, 4367.

(2) Klaver, W. J.; Moolenaar, M. J.; Hiemstra, H.; Speckamp, W. N. *Tetrahedron* **1988**, *44*, 3805 and earlier references cited.

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