Understanding Host-Selective Phytotoxicity: Synthesis and Biological Discrimination of Phomalide and Its (*Z*)-Isomer

Dale E. Ward,* Alfredo Vazquez, and M. Soledade C. Pedras*

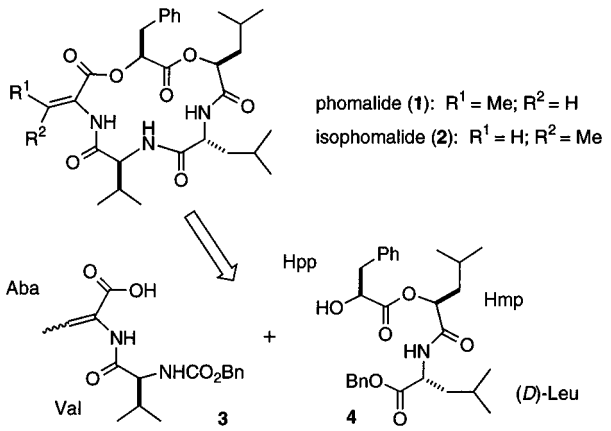
Department of Chemistry, University of Saskatchewan, 110 Science Place, Saskatoon, SK, Canada S7N 5C9

Received August 28, 1996

Numerous fungal pathogens selectively damage host plants via the synthesis and release of host-selective phytotoxins.[1] Although a large number of fungal diseases appear to be mediated by host-selective toxins, the molecular basis for the selectivity of this process is not well understood in the majority of the cases.[1] One such disease, blackleg, affects many economically important brassica crops and can be particularly devastating for the oilseeds canola (*Brassica napus* and *B. rapa*) and rapeseed (*B. napus* and *B. rapa*).[2] Blackleg disease has caused significant crop losses in Canada and worldwide, as there are no disease resistant varieties commercially available. Recently, a better understanding of the blackleg causing fungus [*Leptosphaeria maculans* (Desm.) Ces. et de Not., asexual stage *Phoma lingam* (Tode ex Fr.) Desm.] was achieved with the isolation of phomalide (**1**), a host-selective toxin, which appears to be involved in disease development.[3] Remarkably, phomalide is produced by *P. lingam* in liquid culture only for a short period (24−60 h); older fungal cultures produce only nonselective phytotoxins (epipolythiodioxopiperazines) such as sirodesmin PL.[4] This unusually short production period was attributed to an inhibitory effect of sirodesmin PL on the biosynthesis of phomalide. Because of the small quantities obtained from fungal cultures and difficulties in scaling up production, evaluation of the role of **1** in the development of blackleg disease has been seriously hampered. Therefore, the chemical synthesis of phomalide was an essential prelude to biological studies. We have now synthesized phomalide (**1**) and its (*Z*)-isomer (isophomalide, **2**) and assayed their phytotoxicity to blackleg resistant and susceptible plants. Most noteworthy, we established that blackleg susceptible plants discriminate between **1** and **2**.

Phomalide (**1**) is an unusual cyclic depsipeptide composed of three α-amino acid and two α-hydroxy acid residues. The structure was assigned on the basis of spectroscopic data; the (*E*)-configuration of the 2-amino-2-butenoic (Aba) residue was determined from NOE experiments and the absolute configurations of the four stereogenic centers were determined by acid hydrolysis and comparison of the resulting intact residues with authentic samples.[3] The 15-membered ring, the consecutive ester linkages, and the unusual (*E*)-Aba residue are noteworthy structural features within this class of natural products.[5]

Scheme 1



The synthesis of cyclic depsipeptides (and peptides) typically proceeds by coupling (linear or convergent) of intact hydroxy acid and amino acid fragments followed by cyclization.[6] Such an approach focuses the strategic decisions on the site of cyclization and the order of the residue coupling. Application of this strategy to phomalide leaves the introduction of the Aba residue and control of its stereochemistry as a major concern because α,β-unsaturated amino acids are not normally incorporated into peptides as intact residues.[7] A variety of methods[7] for the synthesis of derivatives of unsaturated amino acids including Aba[8] have been reported but few have been applied to stereocontrolled peptide (or depsipeptide) synthesis.[9] Our retrosynthetic analysis of phomalide is presented in Scheme 1. We selected the Val-D-Leu linkage as the most favorable cyclization site because it is an amide bond between two amino acid residues of opposite configuration; all of these features are precedented to facilitate cyclization.[6,10] The [2 + 3] fragment coupling approach to the acyclic precursor was chosen to provide flexibility in the choice of potential Aba precursors [e.g. D- or L-threonine],[11] an alternative site for cyclization,[12] and convergence. The three component residues of the tridepsipeptide **4** are readily available. We chose to incorporate an intact Aba residue (rather than, for example, a threonine derivative)[8] in the dipeptide fragment for increased efficiency and because this would allow [2 + 3] fragment coupling to proceed without risk of "racemization". We initially sought to utilize an *N*-blocked Val-(*E*)-Aba dipeptide; however, because the stereochemical integrity of the thermodynamically less stable (*E*)-configuration could not be preserved through fragment coupling and cyclization (*vide infra*), the syn-

(1) For a recent multiauthor review on phytotoxins, see: Graniti, A.; et al. *Experientia* **1991**, *47*, 751−826.

(2) (a) For a recent review on the history and control of blackleg disease, see: Gugel, R. K.; Petrie, G. A. *Can. J. Plant Pathol.* **1992**, *14*, 36−45. (b) Canola refers to varieties of rapeseed containing very low amounts of erucic adic and glucosinolates.

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

(4) For a recent review on phytotoxins of the blackleg fungus, see: Pedras, M. S. C. *Rev. Latinoam. Quim.* In press.

(5) (a) Turner, W. B.; Aldrige, D. C. *Fungal Metabolites II*; Academic Press: New York, 1983; pp 436−442. (b) Kleinkauf, H.; von Doheren, H. *Eur. J. Biochem.* **1990**, *192*, 1−15.

(6) (a) Bodanszky, M. *Principles of Peptide Synthesis*, 2nd ed.; Springer-Verlag: Berlin, 1993. (b) Izumiya, N.; Kato, T.; Aoyagi, H.; Waki, M.; Kondo, M. *Synthetic Aspects of Biologically Acitve Cyclic Peptides*; Halsted Press: New York, 1979. (c) Kopple, K. D. *J. Pharm. Sci.* **1972**, *61*, 1345−1356.

(7) Reviews: (a) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1988**, 159−172. (b) Noda, K.; Shimohigashi, Y.; Izumiya, N. In *The Peptides*; Gross, E.; Meienhofer, J., Eds.; Academic Press: New York, 1983, Vol. 5, pp 285−339.

(8) Most methods give the (*Z*)-isomers selectively.[7] The (*E*)-isomers can be prepared by *syn*-elimination of certain threonine derivatives [(a) Rich, D. H.; Tam, J. P. *J. Org. Chem.* **1977**, *42*, 3815−3820] or by *anti*-elimination of *allo*-threonine derivatives: (b) Somekh, L.; Shanzer, A. *Ibid.* **1983**, *48*, 907−908 and cited references.

(9) For a recent example involving (*Z*)-Aba, see: Li, K. W.; Wu, J.; Xing, W.; Simon, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 7237−7238.

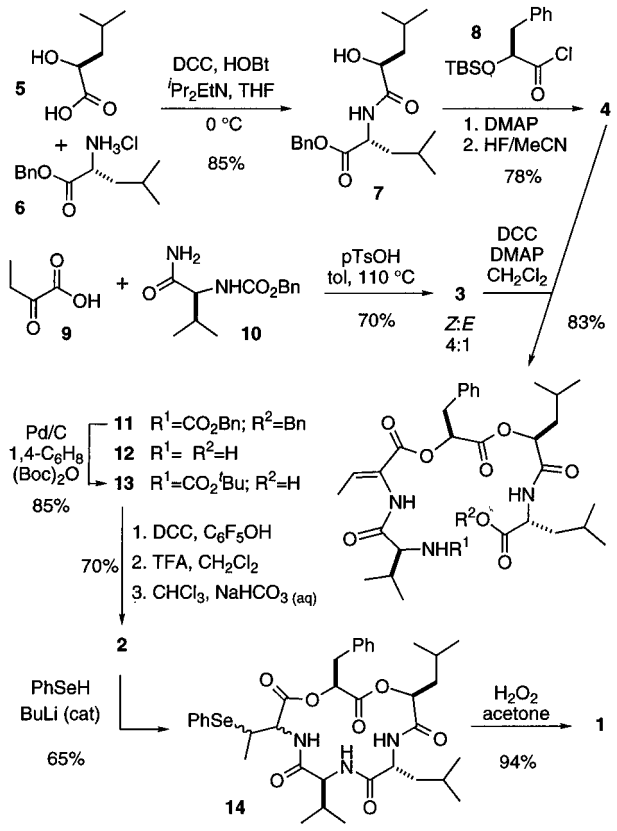
(10) (a) Cavelier-Frontin, F.; Achmad, S.; Verducci, J.; Jacquier, J.; Pèpe, G. *J. Mol. Struct. (THEOCHEM)* **1993**, *286*, 125−130. (b) Ueda, K.; Waki, M.; Izumiya, N. *Int. J. Pept. Protein Res.* **1987**, *30*, 33−39 and cited references.

(11) Changing the configuration of one residue in the linear precursor can have a dramatic effect on cyclization.[6]

(12) The Aba-Hpp linkage is also an attractive cyclization site because it is not subject to "racemization".

S0022-3263(96)01661-1 CCC: $12.00

Scheme 2



thesis proceeded with the more stable (*Z*)-isomer followed by a late-stage stereoselective isomerization.[7,13]

Condensation of Hmp-OH (**5**) with D-Leu-OBn (**6**) mediated by DCC/HOBt gave the didepsipeptide **7** which, in turn, was acylated with TBDMSO-Hpp-Cl (**8**; generated *in situ*)[14] in the presence of DMAP to provide the three-residue depsipeptide fragment Hpp-Hmp-D-Leu-OBn (**4**) after removal of the silyl ether protecting group. Reaction of Cbz-Val-NH_2 (**10**)[15] with 2-oxobutanoic acid gave the dipeptide **3** as a 4:1 mixture of (*Z*)- and (*E*)-isomers, respectively, in analogy to literature precedent (Scheme 2).[16] The adduct resulting from coupling **4** with the mixture of **3** isomers using DCC/DMAP was almost exclusively the (*Z*)-isomer **11**.[17] Hydrogenolysis of **11** gave the corresponding amino acid **12** which failed to cyclize under the influence of EDC/HOBt but gave isophomalide (**2**) in 20% yield after reaction with BOP-Cl/^{i}Pr$_2$EtN. Alternatively, cyclization of **13** via the pentafluorophenyl ester gave **2** in excellent overall yield.[18,19] Other than small differences in the chemical shifts of the signals for the Aba residue, the ^{1}H and ^{13}C NMR spectra of isophomalide were very similar to those of phomalide ($\Delta\delta_H < 0.1$; $\Delta\delta_C < 0.5$). Numerous attempts to isomerize **2** into **1** under acidic, basic, or photochemical conditions failed. Finally, addition of PhSeH to **2** gave three stereoisomeric selenides **14** in a 10:2:1 ratio (65%).[20] Oxidation of the major selenide with H_2O_2 gave phomalide (**1**) uncontaminated by isophomalide (**2**).[20−23] The synthesis of isophomalide (**2**) proceeds in five steps and 30% overall yield from **5** and **6**; the isomerization of **2** into **1** occurs with complete stereoselectivity and represents a rare example of a stereocontrolled synthesis of an (*E*)-dehydroamino acid containing depsipeptide (or peptide).[7]

To evaluate the selective phytotoxicity of phomalide (**1**) and its (*Z*)-isomer (isophomalide, **2**) to plants resistant and susceptible to blackleg, both compounds were assayed *in planta* on leaves of canola (susceptible), mustard (*B. juncea*, resistant), and wasabi (*Eutrema wasabi*, resistant),[24] as previously reported for other toxins.[25] The naturally occurring phomalide (**1**) caused necrotic, chlorotic, and red lesions on canola leaves (10^{-5} M), whereas no damage was observed on mustard or wasabi leaves, even at significantly higher concentrations (10^{-4} M). Thus, the selective phytotoxicity of phomalide (**1**) appears to mimic the pathogenicity range of the blackleg fungus.[25] That is, phomalide causes lesions on plants that are susceptible to blackleg infection (canola), but not on resistant plants (mustard and wasabi). Most importantly, the (*Z*)-isomer **2** did not cause lesions on any of the plant leaves tested, even at 5×10^{-4} M. These results indicate that the configuration of the double bond in phomalide (**1**) is important for phytotoxicity; however, it remains to be determined whether (and how) resistant plants enzymatically convert phomalide to less toxic products. An interesting possibility would be the isomerization of **1** into the nonphytotoxic **2**, a process that would not occur in susceptible plants.

Acknowledgment. Financial support from the Natural Sciences and Engineering Research Council (Canada) in the form of a Research Grant (to D.E.W.) and a Strategic Project Grant (to M.S.C.P. and D.E.W.) is gratefully acknowledged. A.V. thanks the University of Saskatchewan and the Universidad Nacional Autonoma de México for scholarships.

Abbreviations: *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphonic chloride (BOP-Cl); 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (EDC); 2-hydroxy-4-methylpentanoic acid (Hmp); 1-hydroxybenzotriazole (HOBt); 2-hydroxy-3-phenylpropanoic acid (Hpp); leucine (Leu); valine (Val).

Supporting Information Available: Experimental procedures and characterization data for **1**−**14** (9 pages).

JO961661A

(13) (a) Nitz, T. J.; Holt, E. M.; Rubin, B.; Stammer, C. H. *J. Org. Chem.* **1981**, *46*, 2667−2673. (b) Arenal, I.; Bernabe, M.; Fernadez-Alvarez, E. *An. Quim., Ser. C* **1981**, *77*, 56−62. (c) Makowski, M.; Rzezotarska, B.; Kubica, Z.; Pietrzynski, G.; Hetper, J. *Liebigs Ann. Chem.* **1986**, 980−991.

(14) Wissner, A.; Grudzinskas, C. V. *J. Org. Chem.* **1978**, *43*, 3972−3974.

(15) Chen, S.-T.; Wu, S.-H.; Wang, K.-T. *Synthesis* **1989**, 37−38.

(16) (a) Makowski, M.; Rzeszotarska, B.; Kubica, Z.; Wieczorek, P. *Liebigs Ann. Chem.* **1984**, 920−928.(b) Smelka, L.; Rzeszotarska, B.; Pietrznski, G.; Kubica, Z. *Liebigs Ann. Chem.* **1988**, 485−486.

(17) A small amount of the corresponding (*E*)-isomer (ca. 5%) could be isolated. A similar result was obtained starting from either the pure (*E*)- or pure (*Z*)-isomer of **3**.

(18) Schmidt, U.; Weinbrenner, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1003−1004.

(19) Cyclization of the (*E*)-isomer of **13** under the same conditions gave a mixture of **1** and **2** in good yield.

(20) (a) Mazur, R. H.; Pilipauskas, D. R. *Pept.: Synth, Struct., Funct., Proc. Am. Pept. Symp., 7th*; Rich, D. H., Gross, E., Eds.; Pierce Chem. Co.: Rockford, IL, 1981; pp 81−84. (b) Mazur, R. H.; Pilipauskas, D. R. *Pept., Proc. Eur. Pept. Symp., 17th*; *1982*; Blaha, K., Malon, P., Eds.; de Gruyter: Berlin, 1983; pp 319−322.

(21) Similar oxidation of the minor selenide isomer gave a 6:1 mixture of **1** and **2**; oxidation of the other isomer gave only **2**. Thus, assuming a stereoselective *syn* elimination of the selenides (via the corresponding selenoxides), the relative stereochemistry for major and minor isomers should be *lk* (i.e., *syn* PhSe and NH groups) and *ul* for the second major isomer. Phomalide and isophomalide are separable (PTLC), albeit with considerable difficulty and with low efficiency (i.e., mainly mixed fractions are obtained).

(22) For a similar *syn*-elimination of sulfoxide derivatives of threonine, see ref 8a. For other examples of dehydroamino acid syntheses by selenoxide fragmentation, see: (a) Walter, R.; Roy, J. *J. Org. Chem.* **1971**, *36*, 2561−6563. (b) Reich, H. J.; Jasperse, C. P.; Renga, J. M. *Ibid.* **1986**, *51*, 2981−2988. (c) Hashimoto, K.; Sakai, M.; Okuno, T.; Shirahama, H. *Chem. Commun.* **1996**, 1139−1140.

(23) Synthetic phomalide was identical in all respects (^{1}H and ^{13}C NMR, $[\alpha]_D$, TLC and HPLC retention times) with an authentic sample.

(24) Pedras, M. S. C.; Taylor, J. L.; Morales, V. M. *Phytochemistry* **1995**, *38*, 1215−1222.

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