

A convenient synthesis of the cruciferous phytoalexins brassicanal A and brassilexin by mimicry of a fungal detoxification pathway

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The cruciferous phytoalexin brassilexin **3** has been synthesized in four steps from indoline-2-thione *via* 3-(aminomethylene)indole-2-thione **2**, a metabolic intermediate of the detoxification pathway of the phytoalexin cyclobassinin **1**; in addition, the phytoalexin brassicanal A **8** has been synthesized in two steps from 2-indolinethione.

It is now well-recognized that the blackleg fungus [*Phoma lingam* (Tode ex Fr.) Desm., perfect stage *Leptosphaeria maculans* (Desm.) Ces. et de Not.], one of the most destructive fungal pathogens of rapeseed (*Brassica napus*, *B. rapa*), can overcome the plant's induced chemical defenses, *i.e.* phytoalexins, by enzymatic detoxification.^{1–6} Recently, we reported⁶ an unprecedented detoxification of the cruciferous phytoalexin cyclobassinin **1**⁷ *via* the phytoalexins brassilexin **3**⁸ and dioxibassinin **4**^{9,10} by isolates of *P. lingam* (Scheme 1). A detailed analysis of the metabolites involved in that fungal biotransformation of cyclobassinin **1** indicated that 3-(aminomethylene)indole-2-thione **2**, or its thiol tautomer(s), was a precursor of brassilexin **3** (Scheme 1).⁶ In continuation of that work we have now accomplished the synthesis of brassilexin **3** by mimicry of that detoxification pathway, utilizing the key intermediate **2** (Scheme 2). This route provides a very convenient process for preparation of both compounds **2** and **3**,

offering also the possibility of introducing carbon and/or nitrogen isotopes into the indol-3-yl substituents in good yield. Previous syntheses of brassilexin **3** from indole-3-carbaldehyde *via* cyclobassinin **1** (11% overall yield),^{11–13} or directly from indole-3-carbaldehyde (11–30% overall yield),¹⁴ have been reported. However, our route affords the best overall yield to date while following the simplest process in terms of purification and reaction conditions. Utilizing the route shown on Scheme 2, brassilexin **3** was obtained from indolinethione **5**¹⁵ in four steps (typically 50 mg scale reactions) in *ca.* 64% overall yield. In addition, this route provided aldehyde **6**, which on reaction with CH₂N₂ quantitatively yielded brassicanal A **8**, another cruciferous phytoalexin,¹⁶ in *ca.* 92% yield. This synthesis of brassicanal A **8** also presents a great improvement to the previously reported procedure, which employed methylation of indolinethione **5** followed by Vilsmeier formylation of the corresponding 2-thiomethyl ether **9** (Scheme 2, 39% overall yield from **5**).⁴

During previous biotransformation studies,⁶ we observed that brassilexin **3** could be obtained from metabolite **2** after standing in solution or on silica gel TLC plates. Interestingly, this transformation was catalyzed by activated charcoal to afford brassilexin almost quantitatively, demonstrating that metabolite **2** would be a synthetically useful brassilexin precursor if available in reasonable yields. One apparent method for the preparation of **2** was the reduction of amino derivatives of 3-(hydroxymethylene)indole-2-thione (or equivalent tautomer, *e.g.* **6**) similar to the preparation of 3-hydroxymethylene-2-oxindole derivatives.¹⁷ Thus, formylation of thione **5**¹⁵ with EtO₂CH afforded aldehyde **6**, whose ¹H and ¹³C NMR spectroscopic data⁸ indicated that only the thiol tautomeric form was present. Oximation of **6** under standard conditions¹⁸ yielded quantitatively oxime **7**, which was readily reduced to the desired intermediate **2** with NaBH₃CN in the presence of TiCl₃.¹⁹ Finally, treatment of **2** with activated charcoal afforded brassilexin in excellent yield.[¶]

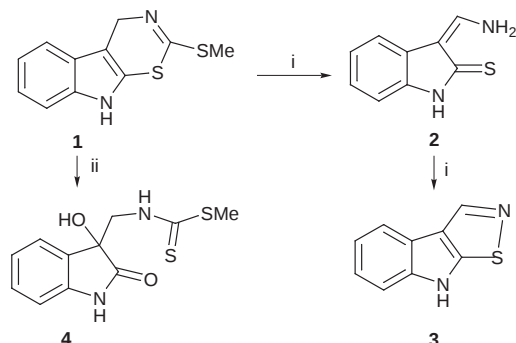
It is worth noting that thione **2** was hypothesized to be a biogenetic precursor of brassilexin **3** *in planta*.⁶ In addition, although thiol **6** does not appear to have been prepared previously, it was proposed as a precursor of brassicanal A *in planta*.²⁰ Our facile synthesis of intermediates **2** and **6** offers the possibility of isotopic labelling and should facilitate future biosynthetic studies on brassilexin **3** and brassicanal A **8**. Particularly because brassilexin **3** appears to be involved in the blackleg disease resistance of several agriculturally important cruciferous oilseeds (*e.g.* *B. juncea*, *B. carinata*),²¹ its biosynthesis has tremendous potential application and is presently under investigation in our laboratory.

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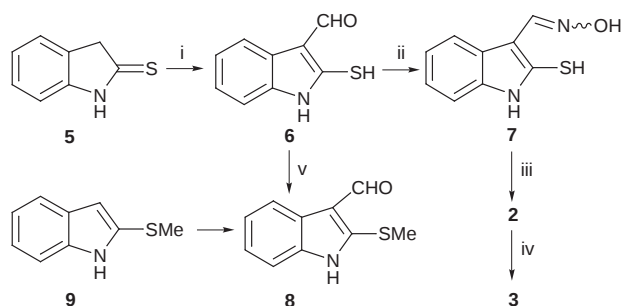
Notes and References

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‡ The fungal species *Phoma lingam* is subdivided in various groups (*cf.* M. S. C. Pedras, J. L. Taylor and V. M. Morales, *Phytochemistry*, 1995, **38**, 1215; J. L. Taylor, M. S. C. Pedras and V. M. Morales, *Trends Microbiol.*,



Scheme 1 Transformation of cyclobassinin by the phytopathogenic fungus *Phoma lingam*: i, 'avirulent' isolate Unity; ii, 'virulent' isolate BJ-125



Scheme 2 Reagents and conditions: i, NaH, HCO₂Et, 25 °C, 92%; ii, NH₂OH·HCl, AcONa, EtOH, reflux, quant.; iii, TiCl₃, NaBH₃CN, MeOH, 25 °C, 85%; iv, activated charcoal, 25 °C, 82%; v, CH₂N₂, Et₂O, 25 °C, quant.

1995, **3**, 202); the so-called 'avirulent' group, *e.g.* isolate Unity, is now considered a species different from that of the 'virulent' group, *e.g.* BJ-125, although no formal reclassification has been carried out.

§ *Selected data for 6*: δ_{H} (500 MHz, CD_3OD) 9.49 (s, CHO), 8.15 (d, J 8.0, 1 H), 7.48 (d, J 8.0, 1 H), 7.37 (dd, J 8.0, 7.5, 1 H), 7.27 (dd, J 8.0, 7.5, 1 H); δ_{H} (500 MHz, $[\text{D}_6]\text{DMSO}$) 12.77 (br s, NH), 9.49 (s, CHO), 8.06 (d, J 8.0, 1 H), 7.50 (d, J 8.0, 1 H), 7.35 (dd, J 8.0, 7.0, 1 H), 7.26 (dd, J 8.0, 7.0, 1 H); δ_{C} (125.5 MHz, $[\text{D}_6]\text{DMSO}$) 184.3, 138.5, 137.1, 125.2, 124.6, 122.9, 120.8, 119.8, 112.3; HRMS: found 177.0249 (calc. for $\text{C}_9\text{H}_7\text{NOS}$: 177.0248); m/z (EI) 177 (M^+ , 100%), 149 (18), 148 (24), 121 (15); m/z (CI) 178 ($\text{M}^+ + 1$, 19%), 164 (32), 150 (24), 146 (100), 132 (50). For **7**: δ_{H} (300 MHz, CD_3CN): δ 9.88 (br s, NH), 8.52 (br s, OH), 8.03 (d, J 8.0, 1 H), 7.95 (s, H-1'), 7.42 (d, J 8.0, 1 H), 7.31 (dd, J 8.0, 7.0, 1 H), 7.21 (dd, J 8.0, 7.0, 1 H); δ_{C} (75.5 MHz, CD_3CN) 145.2, 138.9, 131.1, 126.0, 125.7, 123.5, 122.4, 117.2, 112.6; HRMS: found 174.0251 (calc. for $\text{C}_9\text{H}_8\text{N}_2\text{OS} - \text{H}_2\text{O}$: 174.0252); m/z (EI) 174 ($\text{M}^+ - \text{H}_2\text{O}$, 100%), 149 (51), 142 (24); m/z (CI) 175 ($\text{M}^+ + 1 - \text{H}_2\text{O}$, 100%).

¶ All compounds gave satisfactory spectroscopic data.

- 1 M. S. C. Pedras and J. L. Taylor, *J. Org. Chem.*, 1991, **56**, 2619.
- 2 M. S. C. Pedras, I. Borgmann and J. L. Taylor, *Phytochem. (Life Sci. Adv.)*, 1992, **11**, 1.
- 3 M. S. C. Pedras and J. L. Taylor, *J. Nat. Prod.*, 1993, **56**, 731.
- 4 M. S. C. Pedras and A. Q. Khan, *J. Agric. Food Chem.*, 1996, **44**, 3403.
- 5 M. S. C. Pedras, A. Q. Khan and J. L. Taylor, in *Phytochemicals for Pest Control*, ed. P. A. Hedin, R. M. Hollingworth, E. P. Masler, J. Miyamoto and D. G. Thompson, *ACS Symp. Ser.*, 1997, **658**, 155.
- 6 M. S. C. Pedras and F. I. Okanga, *Chem. Commun.*, 1998, 67.
- 7 First isolation and synthesis: M. Takasugi, N. Katsui and A. Shirata, *J. Chem. Soc., Chem. Commun.* 1986, 1077.
- 8 First isolation: M. Devys, M. Barbier, I. Loiselet, T. Rouxel, A. Sarniguet, A. Kollmann and J. Bousquet, *Tetrahedron Lett.*, 1988, **29**, 6447.
- 9 First isolation: K. Monde, K. Sasaki, A. Shirata and M. Takasugi, *Phytochemistry*, 1991, **30**, 2915.
- 10 Synthesis: K. Monde, M. Takasugi and T. Ohnishi, *J. Am. Chem. Soc.*, 1994, **116**, 6650.
- 11 M. Devys and M. Barbier, *Synthesis*, 1990, 214.
- 12 M. Devys and M. Barbier, *J. Chem. Soc., Perkin Trans. 1*, 1990, 2856.
- 13 M. Devys and M. Barbier, *Z. Naturforsch.*, 1992, **47C**, 318.
- 14 M. Devys and M. Barbier, *Org. Prep. Proced. Int.*, 1993, **25**, 344.
- 15 T. Hino, K. Tsuneoka, M. Nakagawa and S. Akaboshi, *Chem. Pharm. Bull.*, 1969, **17**, 550.
- 16 K. Monde, N. Katsui, A. Shirata and M. Takasugi, *Chem. Lett.*, 1990, 209.
- 17 E. Wenkert, N. K. Bhattacharyya, T. L. Reid and T. E. Stevens, *J. Am. Chem. Soc.*, 1956, **78**, 797.
- 18 E. Wenkert, B. S. Bernstein and J. H. Udelhofen, *J. Am. Chem. Soc.*, 1958, **80**, 4899.
- 19 J. P. Leeds and H. A. Kirst, *Synth. Commun.*, 1988, **18**, 777.
- 20 K. Monde, A. Tanaka and M. Takasugi, *J. Org. Chem.*, 1996, **61**, 9053.
- 21 T. Rouxel, A. Kollmann, L. Boulidard and R. Mithen, *Planta*, 1991, **184**, 271.

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