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

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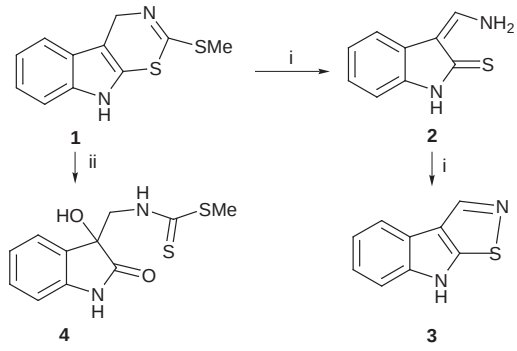
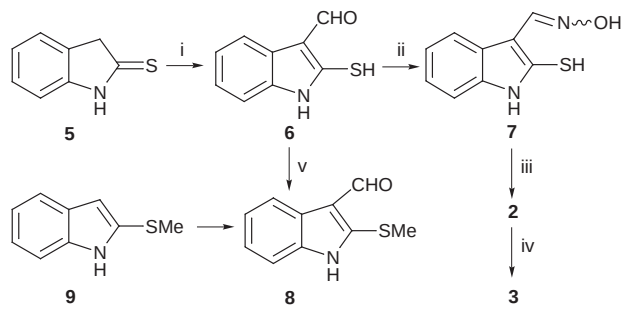
The cruciferous phytoalexin brassilexin 3 has been synthesized in four steps from indoline-2-thione *via* 3-(aminomethylene)indole-2-thione 2, a metabolic intermediate of the detoxification pathway of the phytoalexin cyclobrassinin 1; in addition, the phytoalexin brassicanal A 8 has been synthesized 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 reported[6] an unprecedented detoxification of the cruciferous phytoalexin cyclobrassinin **1**[7] *via* the phytoalexins brassilexin **3**[8] and dioxibrassinin **4**[9,10] by isolates of *P. lingam*‡ (Scheme 1). A detailed analysis of the metabolites involved in that fungal biotransformation of cyclobrassinin **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**,



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



Scheme 2 *Reagents and conditions*: i, NaH, HCO_2Et, 25 °C, 92%; ii, $NH_2OH·HCl$, AcONa, EtOH, reflux, quant.; iii, $TiCl_3$, $NaBH_3CN$, MeOH, 25 °C, 85%; iv, activated charcoal, 25 °C, 82%; v, CH_2N_2, Et_2O, 25 °C, quant.

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* cyclobrassinin **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 CH_2N_2 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-oxoindole derivatives.[17] Thus, formylation of thione **5**[15] with EtO_2CH afforded aldehyde **6**, whose ^1H and ^{13}C NMR spectroscopic data§ indicated that only the thiol tautomeric form was present. Oximination of **6** under standard conditions[18] yielded quantitatively oxime **7**, which was readily reduced to the desired intermediate **2** with $NaBH_3CN$ in the presence of $TiCl_3$.[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.

We gratefully acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada and the University of Saskatchewan.

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.*,

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, [$^2H_6$]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, [$^2H_6$]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 C_9H_7NOS: 177.0248); *m/z* (EI) 177 (M^+, 100%), 149 (18), 148 (24), 121 (15); *m/z* (CI) 178 ($M^+ + 1$, 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 $C_9H_8N_2OS - H_2O$: 174.0252); *m/z* (EI) 174 ($M^+ - H_2O$, 100%), 149 (51), 142 (24); *m/z* (CI) 175 ($M^+ + 1 - H_2O$, 100%).

¶ All compounds gave satisfactory spectroscopic data.

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Received in Corvallis, OR, USA, 8th May, 1998; 8/03485K