Biosynthesis of insecticidal loline alkaloids by epichloë endophytes of grasses

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Epichloë endophytes are protective mutualists of cool-season grasses. Some epichloë endophytes produce high levels of loline alkaloids (up to 2 % plant dry mass), which deter or kill insect herbivores. Lolines have unusual structures, including a saturated 1-aminopyrrolizidine ring system and a unique oxygen bridge. *N*-Formylloline (Fig. 1; cmp **1**) is the most abundant alkaloid in symbiota of tall fescue with *Neotyphodium coenophialum* or meadow fescue with *N. uncinatum*. Biochemical studies identified intermediates in the pathway, and molecular genetic studies identified likely genes for several steps (Fig. 1). The precursors L-proline (L-Pro) and L-homoserine (L-Hse), positionally labelled with stable isotopes (^{13}C, ^{2}H, and ^{15}N), were fed to *N. uncinatum* cultures, which incorporated the labels into the expected positions of **1**. Also labelled were putative intermediates **2** and exo-1-aminopyrrolizidine **3**, both of which were incorporated into **1**, whereas intermediates of hypothetical alternative routes did not incorporate. Genes encoding the likely biosynthetic steps were clustered in endophyte genomes, and the involvement of LolC and LolP in the pathway was demonstrated genetically

Figure 1 Proposed pathway for biosynthesis of *N*-formylloline (**1**). L-Pro and L-Hse are combined to form **2**, which is oxidatively decarboxylated to iminium ion **4**. Decarboxylation gives **5**, and cyclization gives exo-1-aminopyrrolizidine (**3**). Subsequent oxidation/oxygenation (Ox) steps yield norloline (**6**), which is N-methylated twice, and the product is oxidised by the cytochrome P450 enzyme LolP to give 1. Gene products likely to be involved are indicated above arrows. Three *lol* genes (*lol*E, *lol*F, and *lol*O) apparently encode oxidases or oxygenases for the three steps labelled Ox.