Structure elucidation of 10,11 -Epoxyjanthitrem B and isolation of Janithitrem B from *Penicillium janthinellum* cultures

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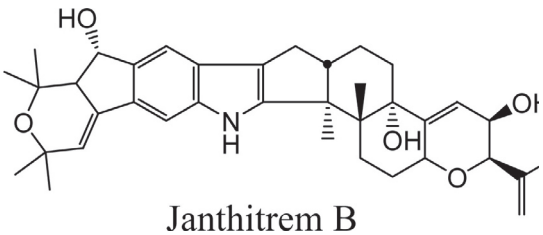
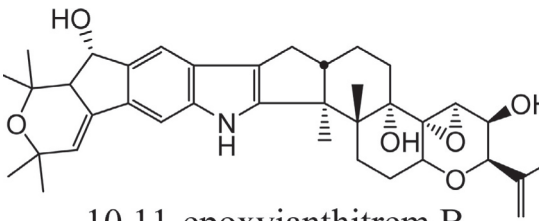
Early investigations into ryegrass staggers were directed towards the characterisation of tremorgen-producing *Penicillium* species (Gallagher *et al.* 1977). Work in the 1980s identified a number of *Penicillium janthinellum* strains isolated from pastures where there had been outbreaks of staggers (Gallagher *et al.* 1980). When these strains were grown in culture, tremorgenic compounds named janthitrems were produced. Research into the role of janthitrems in ryegrass staggers ceased when lolitrems where identified as the probable causative agents. However several novel janthitrems, including 11,12-epoxyjanthitrem G, have recently been identified in ryegrass infected with the AR37 endophyte (Tapper & Lane 2004). These compounds are structurally related to the known janthitrems from *Penicillium* but are difficult to isolate and very unstable. Isolation of janthitrems from *Penicillium* cultures is relatively simple compared to isolation of janthitrems from seed or herbage and allows relatively large amounts of toxin to be collected. We have therefore isolated janthitrems from *Penicillium* cultures, using standard methods (Lauren & Gallagher 1982; Wilkins, Miles *et al.* 1992) for use in the development of biological and chemical assays, as a prelude to similar work with janthitrems from endophyte.

We have isolated and purified Janthitrem B, the predominant janthitrem from *P. janthinellum*, and are using it as a model to investigate the stability of the janthitrem class of compounds, under conditions required to isolate janthitrems and perform bioassays. This information will be used to prevent decomposition of janthitrems isolated from endophytes. The effect of janthitrem B on mice and insects is also being studied. We have also isolated a novel janthitrem compound from *P. janthinellum*, identified as 10,11-epoxyjanthitrem B by 1D- and 2D-NMR spectroscopy (including ^{1}H, ^{13}C, DEPT135, COSY, TOCSY, NOESY, HSQC, and HMBC spectra) and high resolution mass spectrometry.

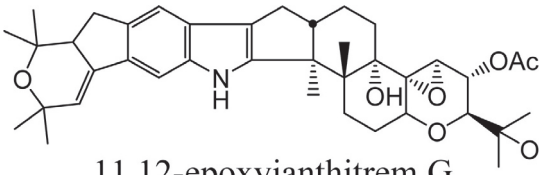


Janthitrem B



10,11-epoxyjanthitrem B

Since janthitrems isolated from ryegrass infected with AR37 also contain a 10,11-epoxide, the more readily available analogue from *Penicillium* will provide an excellent surrogate for use in chemical and biological assays.



11,12-epoxyjanthitrem G

Isolation of other novel janthitrems from *Penicillium* spp. is underway and should provide an insight into the structure-activity of this little-studied group of indole-diterpenoids.

Keywords: endophyte, janthitrems, tremorgen, indole-diterpenoid

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