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TALAROMYCINS C, D, E, and F

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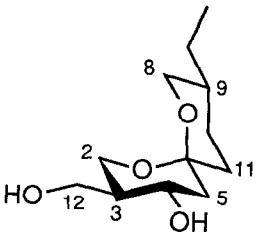
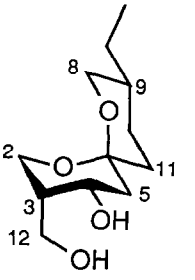
Summary: Four new spiroketal talaromycins have been isolated from *T. stipitatus* and, by equilibration with acid, have been structurally correlated with the previously identified talaromycins **A** and **B**.

The talaromycins, **A** and **B**, represent a new class of spiroketal mycotoxins.[1] As a result of this structural novelty and their potential biological importance,[2] several synthetic studies have focused on the talaromycins.[3] Stereoselective control has been elegantly achieved[3h] utilizing the thermodynamic stability of the spiroketal system.[4] In light of this thermodynamic stability,[1,3] it was striking that talaromycins **A** and **B** appeared to be biosynthetically produced in an equimolar mixture. We have now reinvestigated the minor products produced by *Talaromyces stipitatus* with the hope of laying the groundwork for a better understanding of the biosynthetic production of the talaromycins.



talaromycin B



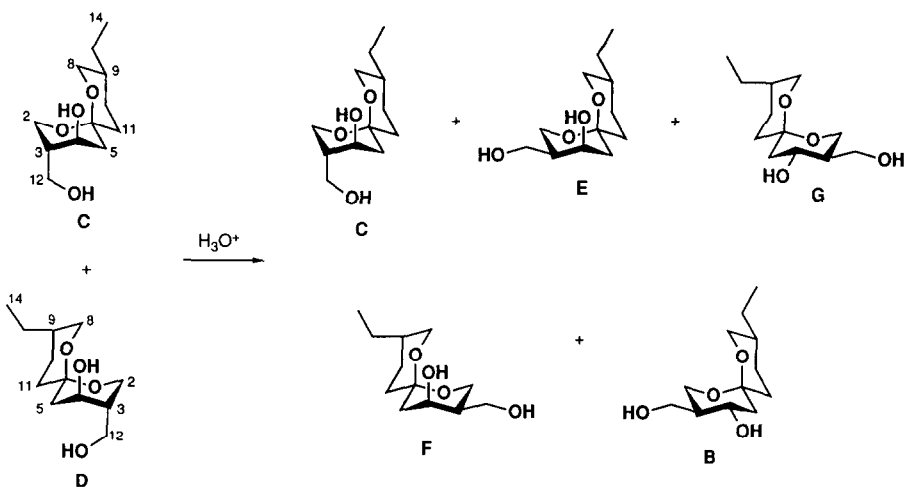
talaromycin A

A crude talaromycin fraction (97 mg), purified as previously reported,[1a] was subjected to droplet counter-current chromatography ($CHCl_3/CH_3OH/H_2O$, 5:6:4, lower phase mobile). Two bands eluting in descending mode just prior to the

talaromycins **A** and **B** (*ca* 7 mg) were found to contain materials structurally related to the talaromycins. The fractions were readily purified on silica gel to give submilligram quantities of talaromycins **C/D** and **E/F** (SiO_2 tlc, 2x $CHCl_3$/ 5% CH_3OH, R_f = 0.41 and 0.61, respectively).

The chemical ionization spectra of the two fractions were very characteristic of the talaromycins,[1a,5] giving CI (CH_4) positive ions at *m/z* 213 $(M+H-H_2O)^+$ and CI (N_2O) negative ions at *m/z* 229 $(M-H)^-$. ^{1}H-NMR, however, revealed that each fraction existed as a mixture of two compounds. While the amount of material precluded detailed analysis, the resonances downfield of δ3.2 were very diagnostic. In particular, a broad singlet centered at δ3.95 in the **C/D** spectrum and one at δ4.16 in the **E/F** spectrum were characteristic of an equatorial C-4 methine proton.[3h] The evidence from both ^{1}H-NMR and MS that these other four metabolites were talaromycins with axially oriented C-4 hydroxyl groups suggested that they might be new spiroketal closure products whose configurations could be correlated through acid-catalyzed equilibration.

Limited exposure of **C/D** to catalytic aqueous HCl in $CDCl_3$ partially isomerized the sample, producing talaromycin **B** (*ca.* 50%) and two other products that eluted on SiO_2 with **B** and **E/F**. The single remaining component of the original **C/D** mixture, compound **C**, was assigned as *epi*-4-OH-talaromycin **A** (δ3.95, bs, H-4e; δ1.86, bs, H-3e; and δ3.34, t, J=11, H-8a). Ketal equilibration of **C** could give *epi*-4-OH-**B** (**E**) and *epi*-9-ethyl- **B** (**G**), but not talaromycin **B**. Therefore, **D** must be an acid-labile compound with the configuration of **B**.

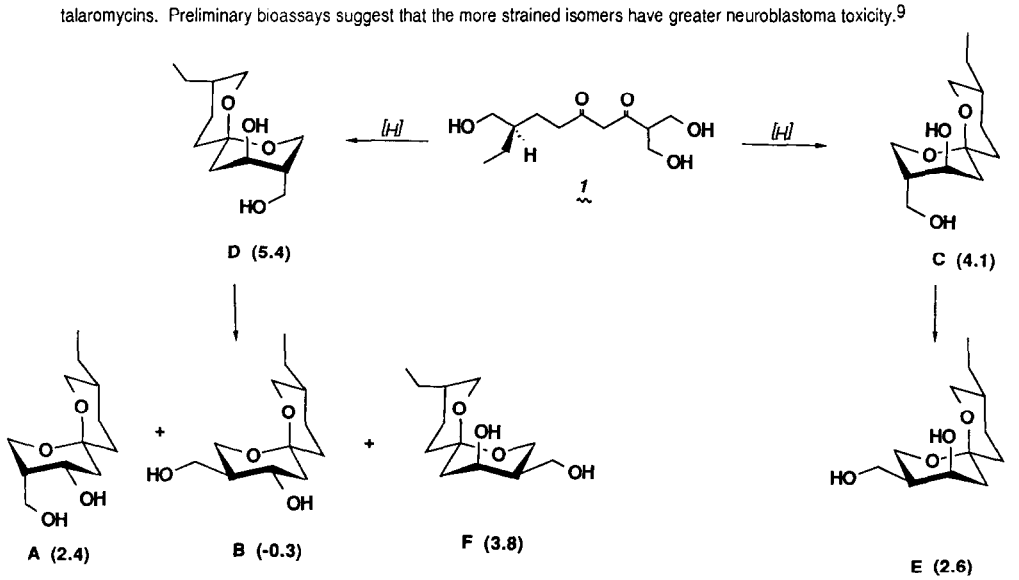


The product chromatographing with **B** was assigned structure **G** (δ3.40, bd, J=11, H-8a) and the product co-migrating with **E/F** was assigned structure **E** (δ 4.16, bs, H-4e; δ3.97, t, J=12, H-2a). Therefore, the four distinct bands on the chromatogram correspond to the four possible configurations at C-3 and 4; talaromycins **A**, **B**, *epi*-4-OH **A** (**C**), and *epi*-4-OH **B** (**E**). The remaining compounds were assigned as epimeric at C-9 from the clearly resolved H-8a protons (δ3.34, t, J=11 in **C** and δ3.52, bd, J=11 in **D**; δ3.36, t, J=11 in **E** and δ3.52, bd, J=11 in **F**). Integrated intensities of these signals in both

mixtures have allowed for the assignment of the relative ratios of talaromycins **E** to **F** (2:1) and **C** to **D** (1:1).

Further proof of these assignments came from a direct comparison with synthetic samples corresponding to the assigned structures for talaromycins **C** and **E**.[6] Equilibration of talaromycin **C** in acid (2% pTSA, THF, r.t.) gave unreacted **C** and talaromycin **G**.[7]

The identification of talaromycins **C**, **D**, **E**, and **F** expands the number of mycotoxins in this family and further highlights the questions about their biosynthetic production. If they originate from a polyketide precursor, **1**, then cyclization and reduction to the axial OH in **C** and **D** would provide the proper stereochemistry for the generation of the remaining talaromycins[8] (Scheme I). The yields of the resulting products are not in the expected thermodynamic ratios. **A** and **B** are the major isomers and exist in an equimolar mixture. The other toxins containing the equatorial hydroxymethyl, **E**/**F**, are isolated in an equivalent amount to **C**/**D**, the axial isomers. Some of the isomers predicted to be more thermodynamically stable, **G** and *epi*-9-ethyl-**A**, are not detected. Whether the product ratio is controlled from a single precursor or whether there are multiple enzymatic steps specifying each ketal must await the definition of *T. stipitatus* culture conditions sufficient for the biosynthesis of the talaromycins. Preliminary bioassays suggest that the more strained isomers have greater neuroblastoma toxicity.[9]



Scheme I. A possible biosynthetic pathway to the talaromycins with the estimated[10] strain energies of each indicated.

References and Notes

1) a. Lynn, D. G.; Phillips, N. J.; Hutton, W. C.; Shabanowitz, J.; Fennell, D. I.; Cole, R. J. *J. Am. Chem. Soc.* **1982**, *104*, 7319.

b. Hutton, W. C.; Phillips, N. J.; Graden, D. W.; Lynn, D. G. *J. Chem. Soc. Chem. Comm.* **1983**, 864.