Characterization of Novel Acidic Sequiterpenoid Phytoalexins in Maize

Sims, J.; Huffaker, A.; Schmelz, E.A. (USDA-CMAVE)

Introduction:

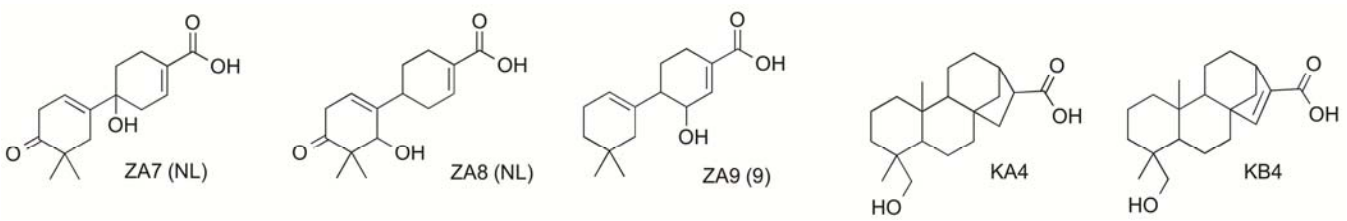
Phytoalexins are reactive antimicrobial low-molecular weight chemicals that accumulate specifically in tissues infected by plant pathogens. Terpenoids constitute dominant classes of phytoalexins in many plants, yet they have long been considered absent from maize (*Zea mays*). We have recently discovered 2 diverse classes of highly reactive acidic terpenoids that collectively accumulate in diseased tissues.[1,2] The predominant sesquiterpenoid phytoalexins, termed zealexins, are based on a 4′,5,5-trimethyl-1,1′-bi(cyclohexane)-1,3′-diene hydrocarbon skeleton with additional oxidations and desaturations resulting in conjugated 1,1′,3′-triene systems. Importantly, Huffaker *et al*. recently elucidated structures for 4 of the 14 related zealexins that are produced following inoculation with *Aspergillus flavus*, a primary causal agent of aflatoxin accumulation in maize. Strong correlations were found with specific zealexins in kernels of maize lines that are highly resistant to *A. flavus* infection and aflatoxin accumulation. The focus of this project is use NMR to elucidate the chemical structures of novel zealexins and kauralexins and gain a better understanding of the biological relevance of phytoalexins. Three additional zealexins and two kauralexins were characterized in this past year bringing the total number of newly identified phytoalexins characterized with AMRIS instrumentation to 22.

Methods:

Infected maize tissue was ground in liq. N_2 and extracted with ethyl acetate. The resulting organic extract was concentrated and purified by silica flash chromatography using a Teledyne silica system. Fractions were screened for the presence of phytoalexins using GC/MS on a DB-35 column and EI mass detector. Compounds of interest were purified by multiple HPLC steps including both normal and reverse phase conditions followed by GC/MS analysis. Purified phytoalexins were structurally characterized by NMR using either a Bruker Avance III 500 console with a 5mm FBBO-Z probe and a Magnex 11.75T/54mm magnet or a Bruker Avance II 600 console with a 5mm TXI cryoprobe and a Magnex 14.1T/54 mm AS magnet. Both ^{1}H and ^{13}C 1D experiments as well as HSQC, HMBC, COSY, NOESY and TOCSY 2D experiments were performed. Carbon chemical shifts were inferred from HSQC and HMBC experiments.

Results:

Zealexins with an m/z of 278 and 264 were isolated by extraction of infected corn tissue and subsequent chromatography. Careful NMR analysis of the purified metabolites confirmed that each molecule is part of the zealexins family of acidic sesquiterpenes, belonging to the zealexin A series. Structures were determined using correlations from 2D experiments. Putative kauralexins A4 and B4 for were also isolated, these are putative biosynthetic precursors to the previously characterized kauralexins.



Conclusions:

We have identified three new zealexins family and two additional kauralexins. Initial investigation in the biological activity of this class of molecules suggests that activity in fungal growth assays include inhibition, induction and no activity. Potential synergism of antimicrobial activity between molecules has been explored.

Acknowledgements

A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490, the State of Florida, and the U.S. Department of Energy. Additional funding was used in the form of AMRIS grant ML-Schmelz-001 and NSF grant 1139329

References

[1] Schmelz, E.A. et al., *Proc. Natl. Acad. Sci.* **108**, 5455-5460 (2011)
[2] Huffaker, A. et al., *Plant Physiol.* **156**, 2082-2097 (2011)