Characterization of Novel Sequiterpenoid Phytoalexins in *Zea Mays*

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Introduction

Phytoalexins are antimicrobial low-molecular weight chemicals that accumulate specifically in tissues infected by plant pathogens and occasionally insects. Terpenoids are common classes of phytoalexins in many plants, yet they have long been considered absent from maize (*Zea mays*). We have recently discovered at least 2 diverse classes of highly reactive acidic terpenoids that accumulate in diseased tissues at concentrations reaching 1 mg g^{-1} FW.[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*. elucidated structures for 4 of at least 14 related zealexins that are produced following inoculation with *Aspergillus flavus*, a primary causal agent of aflatoxin accumulation in maize. Strong correlations exist with specific zealexins in kernels of maize lines that are highly resistant to *A. flavus* infection and aflatoxin accumulation. To date, no low molecular weight biochemical has been previously found to strongly regulate aflatoxin levels in kernels. The focus of this project is use NMR to elucidate the chemical structures of novel zealexins and diterpenoids, termed kauralexins, to gain a better understanding of the interactions between phytoalexins and aflatoxin production. Four novel zealexins were characterized in this past year.

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 (analyzed as methyl esters) with an m/z of 280 and 248 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 zealexin family of acidic sequiterpenes, belonging to either the A or D series. Structures were determined using correlations from 2D experiments. We have identified a new carbon back bone in the zealexin family, based on a β-bisabolene carbon skeleton. Both β-macrocarpene and β- bisabolene can be produce from the same enzyme, namely ZmTps6/11.

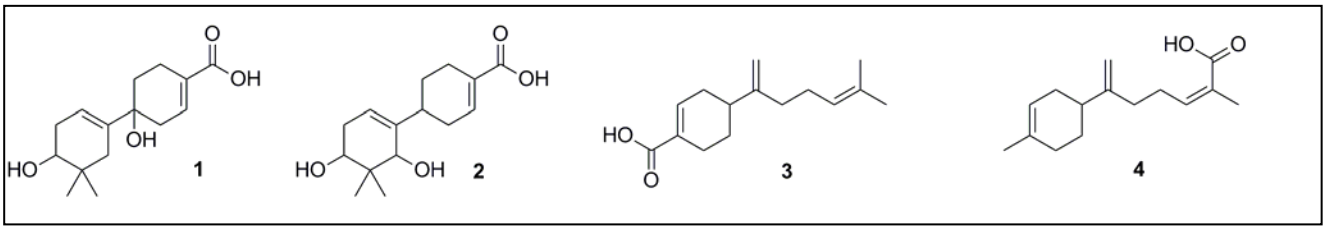

Figure 1. Stuctures of zealexin A5 (1), A6 (2), D1 (3), and D2 (4).

Conclusions

We have identified four new members of the zealexin family. Initial investigation in the biological activity of this class of molecules suggests that some inhibit fungal growth and others induce aflatoxin production.

Acknowledgements

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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).