Compound Discovery and Biosynthesis in Walkingstick Insects (Order Phasmatodea)

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Introduction
Our studies of walkingstick insect (phasmid) chemical defenses [1-7] have greatly benefited from the high sensitivity NMR resources at NHMFL AMRIS, such as the 1mm HTS probe [8] and 5mm Bruker cryoprobe. The chemical components in the defensive secretion of several phasmid species have been identified. This work illustrates the value of phasms as models for studying insect biosynthesis and demonstrates the utility of cryogenic high sensitivity NMR technology.

Experimental
In addition NMR, standard analytical tools have been utilized such as mass spectrometry and gas chromatography. NMR was performed at the MBI-UF AMRIS facility on the Avance 600 MHz system using both the 1mm and 5mm cryoprobes. Insects not native to the United States were reared in captivity in Europe by Oskar V. Conle and Bruno Kneubühler.

Results and Discussion
So far a number of known compounds, and one novel compound which was named parectadial, have been identified from walkingstick chemical defense sprays (Figure 1) [4, 5] (and publications submitted and in preparation). Additionally, glucose has been identified in nearly all of the phasmid defense sprays which we have analyzed by NMR so far [4, 5, 9] (and unpublished results). Recently, through collaboration with the laboratory of Wilhelm Boland at Max Planck in Jena, Germany, we have used NMR and mass spectrometry to demonstrate glucosidase activity (Figure 1) on deuterated geraniol glucoside precursor compounds in aqueous extracts of phasmid chemical defense sprays (unpublished results). Additionally, the 1mm NMR probe was utilized in an in-vivo labeling experiment which demonstrated that A. buprestoides can synthesize its own defensive monoterpene de-novo from 13C glucose [10]. These results suggest a mechanistic explanation for the presence of glucose in phasmid chemical defense sprays in which gluco-conjugate precursors are processed in the gland reservoir of these insects to their final products.

Conclusions
Walkingstick insects produce a wide variety of small molecule toxins for self-defense and at least one species has been shown to have the ability to synthesize its own defense compounds de-novo, likely involving gluco-conjugate precursor(s). Also, NMR is a powerful tool for elucidating biosynthetic pathways in insects.

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References