Chocolate Odorants in the Defensive Spray of *Phyllium westwoodii* (Order Phasmatodea)

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Introduction

The NHMFL 1-mm HTS probe in AMRIS (1) has been a great asset for our studies of walkingstick insect chemical defense (2-6). Using this probe, we have determined that the defensive spray of the phasmid insect *Phyllium westwoodii*. This work illustrates the uniqueness of this insect among the order Phasmatodea, the value of walkingstick insects as models for studying insect chemical defense, provides an analytical framework for identifying such substances, and demonstrates the utility of the 1mm HTS probe for such studies.

Experimental, Results and Discussion

For this project, *P. westwoodii* (Family Phyllidae) were reared in captivity in Italy by M. Gottardo. In addition to the 1mm NMR probe, standard analytical tools have been utilized such as mass spectrometry and gas chromatography. The samples analyzed were all just a few microliters representing several milkings of the insects, thus demonstrating the power of the 1 mm HTS probe. Both NMR and GC-MS results, compared with synthetic standards, that the defensive spray of *P. westwoodii* contained predominantly three alkyl dimethylpyrazines (Figure 1) and glucose. Additionally, A. T. Dossey notes that the defensive spray actually smells like chocolate.

Conclusions

*P. westwoodii*, and likely other Phyllidae, is unique among phasmid insects in their defensive chemistry. The pyrazines a surprise, as walkingsticks were previously only known to produce predominantly monoterpenes. Thus, we hypothesize that the biosynthetic pathways in the defense glands of *P. westwoodii* are novel among phasmids. Also, dimethylpyrazines are well known components of a variety of roasted food, especially chocolate. Considering the utility of dimethyl alkylpyrazines in the flavor and fragrance industry, we believe that this biosynthetic pathway merits further study. Also, the NHMFL 1-mm HTS probe is very useful for analysis of sample-limited natural products. This work has been submitted to the Journal of Natural Products for publication.

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References