SINGLE INSECT NMR: TECHNOLOGY LEADING TO NEW SCIENCE

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Introduction

Due to analytical limitations, multiple animals or plants are typically required to identify natural products. Using a unique 1-mm high-temperature superconducting NMR probe developed at the NHMFL (1), we directly examined the chemical composition of defensive secretions from individual walkingstick insects. We found that the Florida walkingstick *Anisomorpha buprestoides* secretes similar quantities of glucose and mixtures of monoterpene dialdehydes that are stereoisomers of dolichodial. Different individual animals produce different stereoisomeric mixtures, the ratio of which varies between individual animals raised in the same container and fed the same food. Another walkingstick from Peru, *Peruphasma schultei*, also secretes glucose and a single, unique stereoisomer that we named “peruphasmal”.

Experimental

Adult *A. buprestoides* were collected at night in Gulf Hammock, Florida during the fall of 2005. Eggs produced by the insects were hatched in captivity. The young phasmids were fed a diet of only variegated *Ligustrum sinense* purchased from a local plant nursery. We were able to collect single milkings from half-grown males consisting of about 1 μL of a whitish fluid by gently touching the secretory duct with a glass pipette. To this we added 10 μL D2O and without purification or additional preparation, we were able to collect the 1D 1H NMR spectrum in Fig. 1 within about ten minutes following the milking. Complete assignments were done with standard 2D NMR experiments from the same sample.

Results and Discussion

1) We were able to obtain very high quality NMR data from the milking of a single insect (2). Previous studies on the same insect required over 1000 milkings (3).
2) We observed glucose in the defensive secretions (2). This was not previously observed, because samples were extracted into organic solvents.
3) The active component of the defensive spray is present as three different stereoisomers in the Florida insect but only a single isomer in the Peruvian species. Surprisingly, the composition of the stereoisomers changes between individual insects and as a function of time.

Conclusions

Traditionally, natural product studies consist of samples collected from hundreds or thousands of individuals. This was the first study to use NMR to analyze natural products from a single insect. The variability in isomers suggests new fertile ground in chemical biodiversity.

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References