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Application Note

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Golden Gate ATR Study of Poisonous Hairs Obtained from the Crested Rat



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Inside: Find how the Golden Gate® has been applied to the study of Crested Rat hair to yield fundamental knowledge about the chemical coatings of the hair.

Introduction

Plant toxins are used by some invertebrates as a defence against predation. However, this behaviour is not often observed in a mammal [1]. The Crested Rat, *Lophiomys Imhausi*, applies a potent toxin to the fur along its flanks, which it obtains from the poisonous shrub *Acokanthera Schimper*. The poison itself, a cardenolide called ouabain, [2] appears not to affect the rat but causes pain and cardiac dysrhythmia to most vertebrates, with even a small amount capable of killing an elephant. However, in the hands of a skilled medic, this cardiac glycoside is a known treatment for congestive heart failure [3].

The Specac Golden Gate® is the original best selling single reflection diamond ATR accessory. A flagship product for more than two decades, the Golden Gate remains unrivalled for choice of sampling options and rugged durability.

- ▶ Seven sampling top plates
 - ▶ Swap hot for cold, static for flow and other options
- ▶ Wide temperature range (-150 to 300 °C) with pressures up to 6000 psi depending on sampling option
- ▶ Signature bridge design allows the application of a reproducible load up to 180 lbs for optimal sample contact

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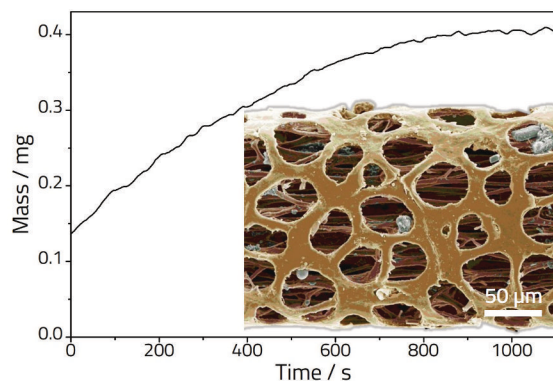


Figure 1: Gravimetric uptake of a rat hair suspended above water. Inset: magnified false colour image of a rat hair demonstrating its porous structure.

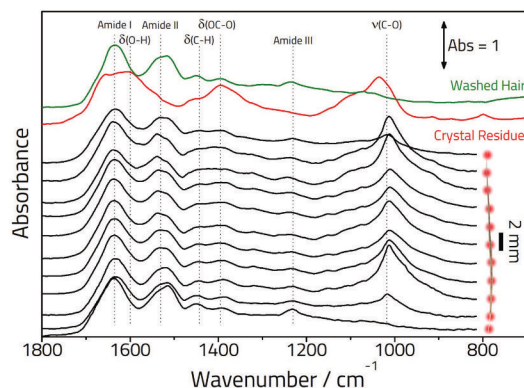


Figure 2: Location dependent ATR spectra recorded along the rat hair from tip (top black spectrum) to root (bottom black spectrum). Also shown is a spectrum recorded from the middle of the rat hair after washing (green) and a spectrum of the ATR crystal after a rat hair was pressed into and subsequently released from it (red).

Experimental

Lateral hairs from the Crested Rat, *Lophiomys Imhausi*, were taken from a skin belonging to the National Museums of Kenya (Catalogue number NMK 180396 Coll Sept. 2010 Field No D. 1).

A commercially available spectrometer was used with a Golden Gate® Diamond ATR allowing the selective probing of the first few microns of the hair's surface at various points along its length. A simple ATR correction was applied to compensate for the penetration depth dependency on the wavelength. The spectra were then normalised with the integral of the 1700–1300 cm^{-1} region. The wicking properties of the hair were

measured with a thermal gravimetric analyser by immersing the first 1 mm of the tip of the hair in water. The mass of the suspended hair was recorded as a function of time as water was absorbed through capillary action.

Results and Discussion

As shown in the background of Figure 1, the hairs consist of a hollow mesh cylinder, 200–250 μm in diameter, encapsulating many fine strands of approximately 10 μm diameter. It is proposed that this open lattice enables capillary uptake and ensures a high storage capacity for the poison payload with rapid release when compressed by biting.

Figure 1 also shows the increase in mass of the hair when its tip is immersed in water. Soon after immersion, the mass increased with time with a water uptake rate of $\sim 0.5 \text{ nL s}^{-1}$. After 15 minutes the uptake reached a plateau at approximately 3 times its initial mass. The ability for the water to spread throughout suggests a highly effective means of uptake, regardless of where the rat applies its saliva.

To locate the presence of the toxin, the local chemical composition was probed by FTIR-ATR at intervals of 2 mm along the hair. Figure 2 illustrates that every spectrum shows protein amide I, II and III absorption bands due to α -keratin (at 1635, 1531 and 1230 cm^{-1} , respectively). The amide I band position and shape is typical of a secondary structure dominated by α -helix structures, which is generally observed for hair [6].

The spectra obtained are highly dependent on the section probed. The main differences are the bands at 1018, 1395 and 1600 cm^{-1} assigned to CO, Ester and OH functional groups, respectively. Underneath the epidermis of the animal, the root of the hair (bottom spectrum) only presents bands characteristic of keratin proteins, with no trace of the additional compounds which are strongest for the mid portion of the hair. When washed with water, these bands disappeared confirming the solubility of these compounds (green spectrum). By pressing a loaded hair against the ATR crystal and removing it, a residue was observed on the crystal surface detectable by IR spectroscopy (red spectrum), demonstrating the release capability of these porous hairs. This demonstrates that both

the force of the bite and the saliva of the predator release toxin from these hairs.

These components are assigned to the ouabain toxin as they have numerous hydroxyl groups. Since the bark and roots of *Acokanthera* contain vast amounts of these active ingredients, their presence in the rat's hairs corroborates visual observations of the animal applying the chewed bark mixture onto its fur.

Conclusions

The porous microstructure on the hair enables efficient distribution for the self-applied toxic plant compounds. Using spectral markers associated with polyphenols, the location dependant presence of *Acokanthera* toxic mixture has been determined. Two modes of release have been identified: by dissolution in saliva and by the force of the bite.



References

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