

MASS SPECTROMETRY IN THE CHARACTERIZATION OF METABOLIC PATHWAYS OF TERPENES IN HERBIVOROUS MARSUPIALS

Rebecca Boyle², Noel Davies¹, Stuart McLean² and Georgia Pass²

1. Central Science Laboratory, University of Tasmania

2. School of Pharmacy, University of Tasmania

Herbivorous marsupials such as koalas, possums and gliders rely to varying extents on foliage of *Eucalyptus* and other native trees as a food source. They mainly deal with the potentially toxic dose of terpenes in the leaves by a variety of biotransformations to enable excretion of water soluble metabolites in their urine. As part of a broad project aimed at obtaining a more detailed understanding of these processes via *in vivo* and *in vitro* experiments, mass spectrometry has been a key tool in understanding the chemistry of these sometimes complex pathways.

We have studied the metabolic fate of 1,8-cineole and p-cymene in several marsupials, and identified a complex range of mono- and di-hydroxylated, carboxylated and multiply oxidized metabolites in both free and conjugated forms, with significant differences between species noted. Of 20 cineole metabolites detected in possum urine only 5 were previously reported compounds, so identification has relied in the first instance on interpretation of mass spectrometric data from first principles, including techniques such as conventional GC-MS of derivatized samples, accurate mass CI GC-MS, APCI LC-MS, MS/MS and the identification of site-specific fragmentations. Compounds initially identified this way include 7-hydroxy-9-cineolic acid (1) and 4-carboxy- α,β -dihydroxycumene (2), the former being the most abundant organic component in normal koala urine.

The use of APCI LC-MS has also for the first time enabled direct characterization, at least to the level of oxidation, of individual peaks for both glucuronic acid conjugated and free metabolites in crude urine samples. This has strongly supported hypotheses on the relationships between the degree of specialisation of the herbivore, the level of conjugation, and the level of terpene oxidation.

