# Identification of high-molecular weight esters in plant cuticles by GC-MS and ESI-MS/MS

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Cuticle is the thin membrane that covers the primary organs of plants, like leaves and fruits. This membrane offers protection to internal tissues, both insulating them against environmental aggressions and preventing water loss. Cuticles include in its composition the polymeric cutin and extractable waxes. The later are accumulated in the outside of the cuticle being the first barrier of plant defense. These waxes are mixtures of low and high-molecular weight molecules. Alkanes, alkanoic acids, alkanols and other alkyl-based compounds are amongst the smaller ones. The higher mass compounds include mainly esters of some of the alcohols and acids found in the free form.

Traditionally, these waxes were hydrolyzed and analyzed afterwards. As a result, higher molecular weight ester molecules were lost. However, without hydrolysis, some of these ester molecules can be analyzed intact, using high temperature GC-MS. More recent techniques like electrospray coupled with mass spectrometry in tandem (ESI-MS/MS) allow the ionization and the structural analysis of much bigger molecules. In this work, we have used both techniques to analyze the extractable waxes from the cuticle of the ivy leaves (*Hedera helix*).

Cuticle was isolated from ivy leaves by enzymatic means, separating it from the remaining epidermis. The isolated cuticle was dried and extracted in

dichloromethane. An aliquot of the extract was derivatized with pyridine/BSTFA for GC-MS analysis. Other aliquots of the dichloromethane extract were diluted in methanol and ionizing agents were added for the ESI-MS/MS analysis. Collision induced dissociation (CID) with argon as collision gas was used in MS/MS. The waxes ester molecules were identified by mass spectrometry and their structure proved by comparing the spectra with model compounds either bought or synthesized.

In the high temperature range of the GS-MS analysis of the ivy leave´s cuticle waxes, alkanyl alkanoates and alkanyl coumarates were identified. The alkanyl alkanoates found were mainly esters of hexadecanoic (palmitic) acid with alkanols ranging from C16 to C26. The other ester waxes found in the GC-MS analysis were alkanyl coumarates. The ones identified included esters of coumaric acid with alkanols ranging from C16 to C24. In the ESI-MS/MS analysis, alkanyl alkanoates were identified with esterified alkanols with chain length up to C34 and alkanyl coumarates with alkanols up to C32. EIMS spectra in the GC-MS analysis and the CID-MS/MS spectra in the ESI analysis were shown to be informative and diagnostic in the structural identification of both alkanyl alkanoates and alkanyl coumarates.

The high temperature GC-MS analysis complemented with ESI-MS/MS was shown to be an improvement in the characterization of the high molecular weight ester molecules of plant waxes.