



¹Ecology & Evolutionary Biology and BIO5: Institute for Collaborative Bioresearch, The University of Arizona; ²Arizona Center for Phytomedicine Research and Department of Pharmacology, The University of Arizona

ABSTRACT

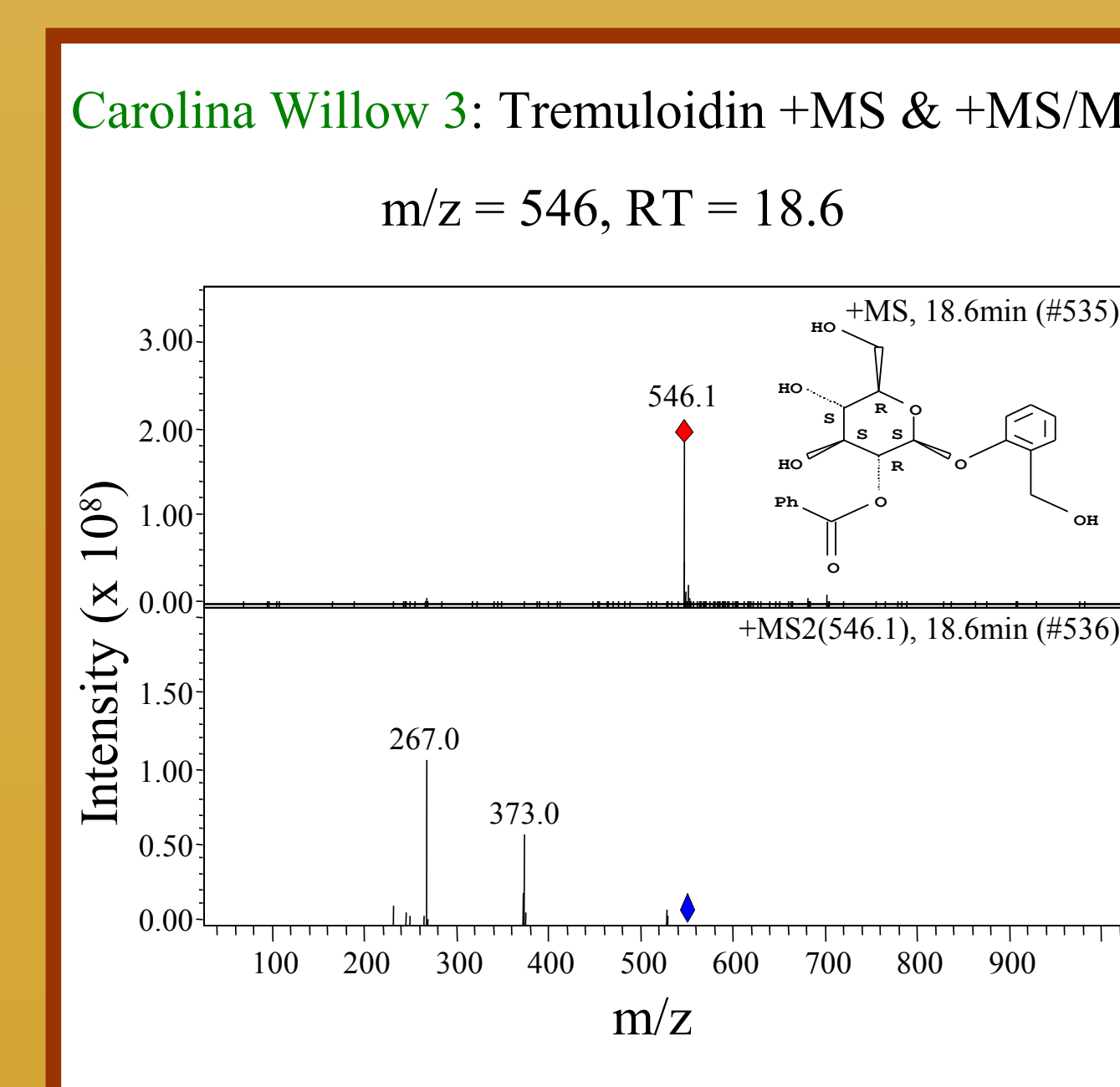
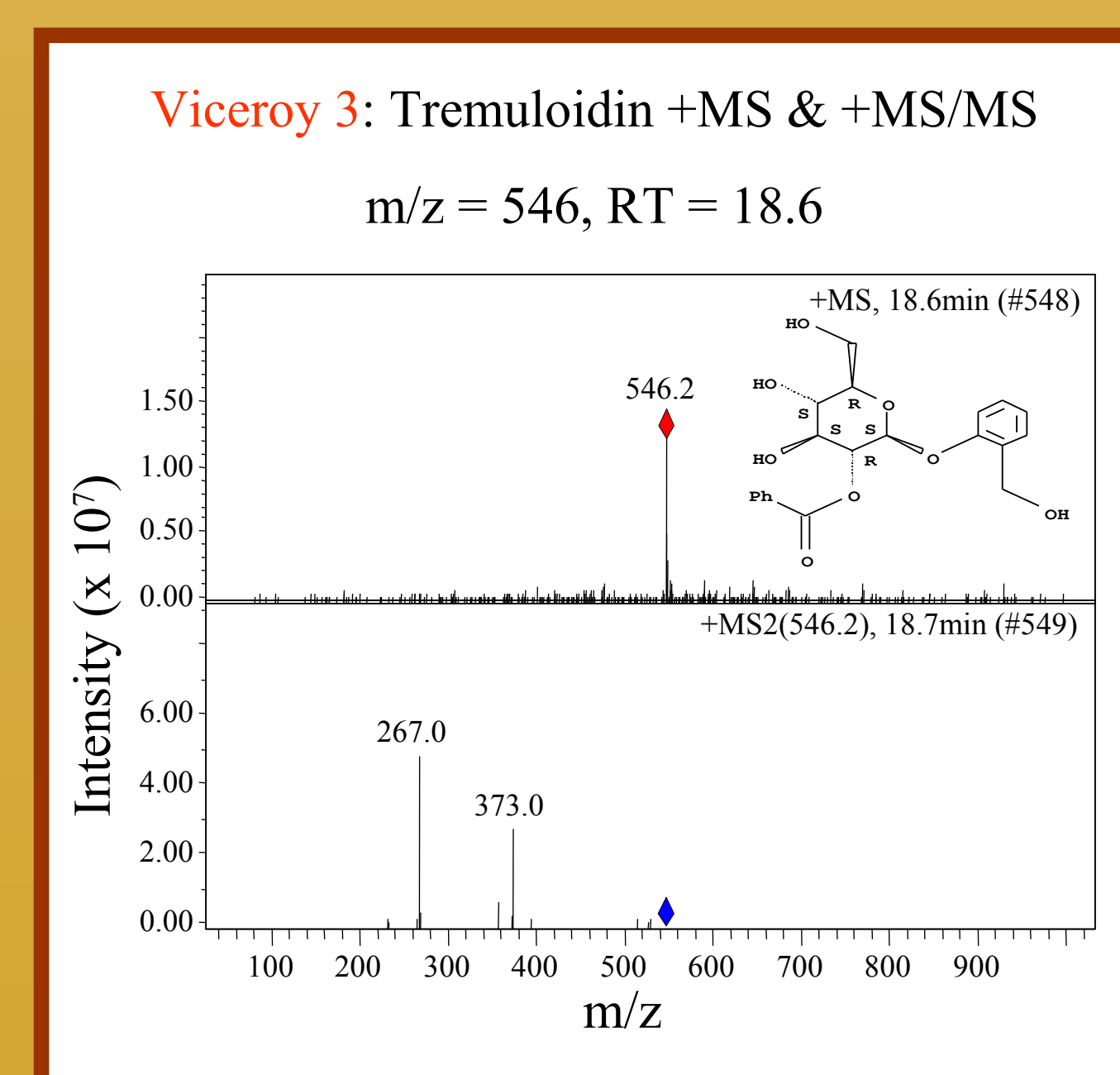
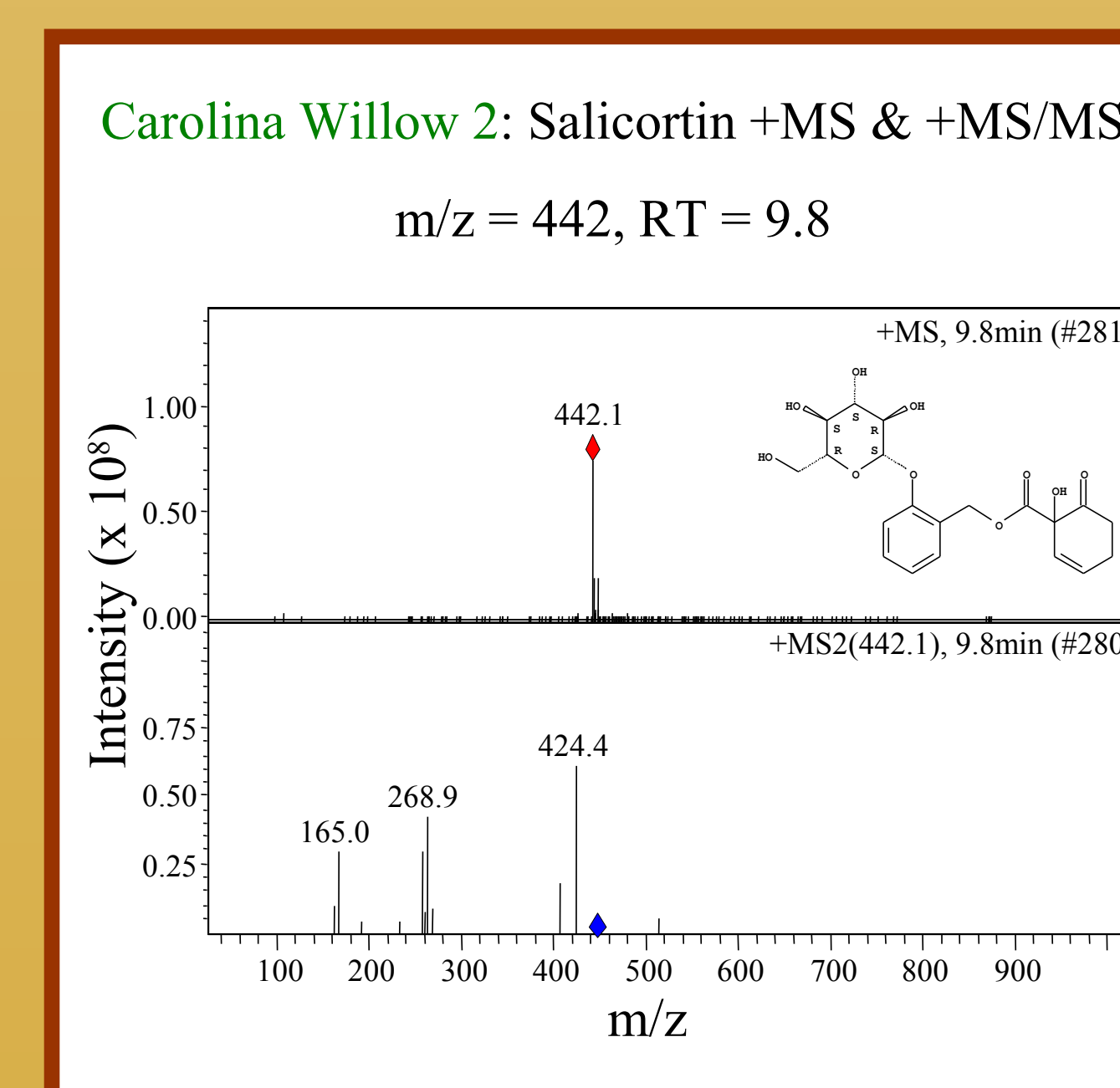
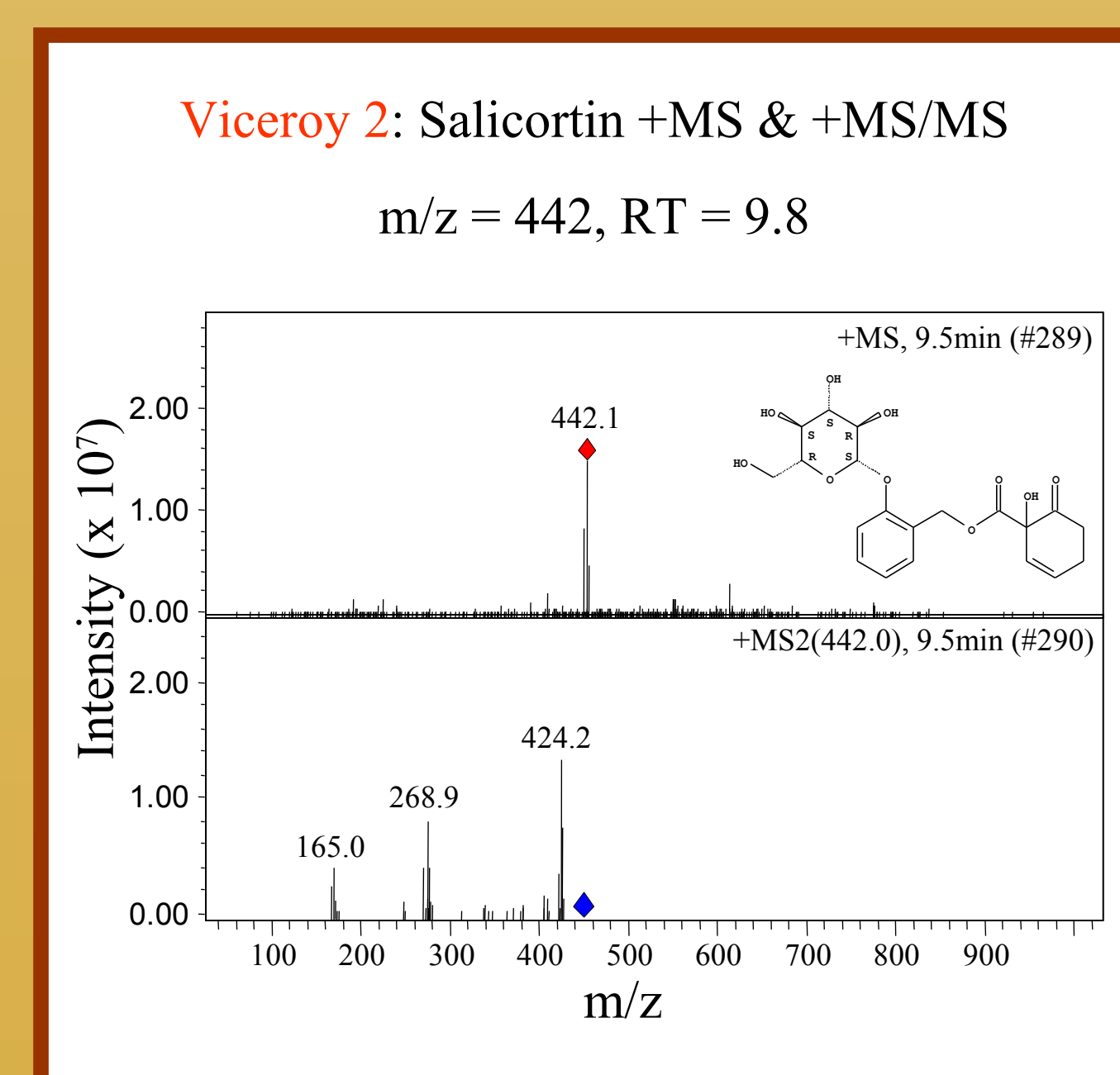
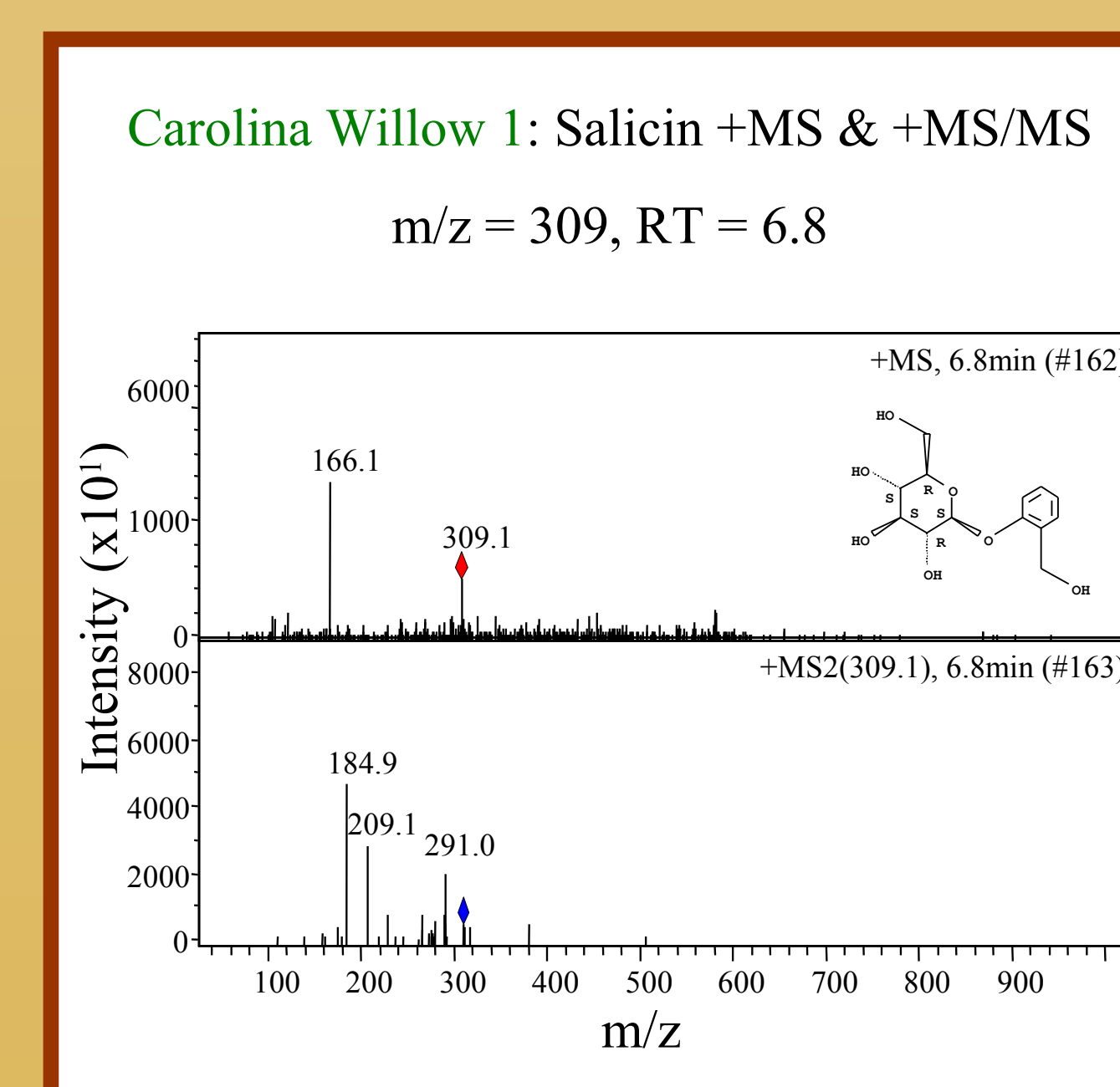
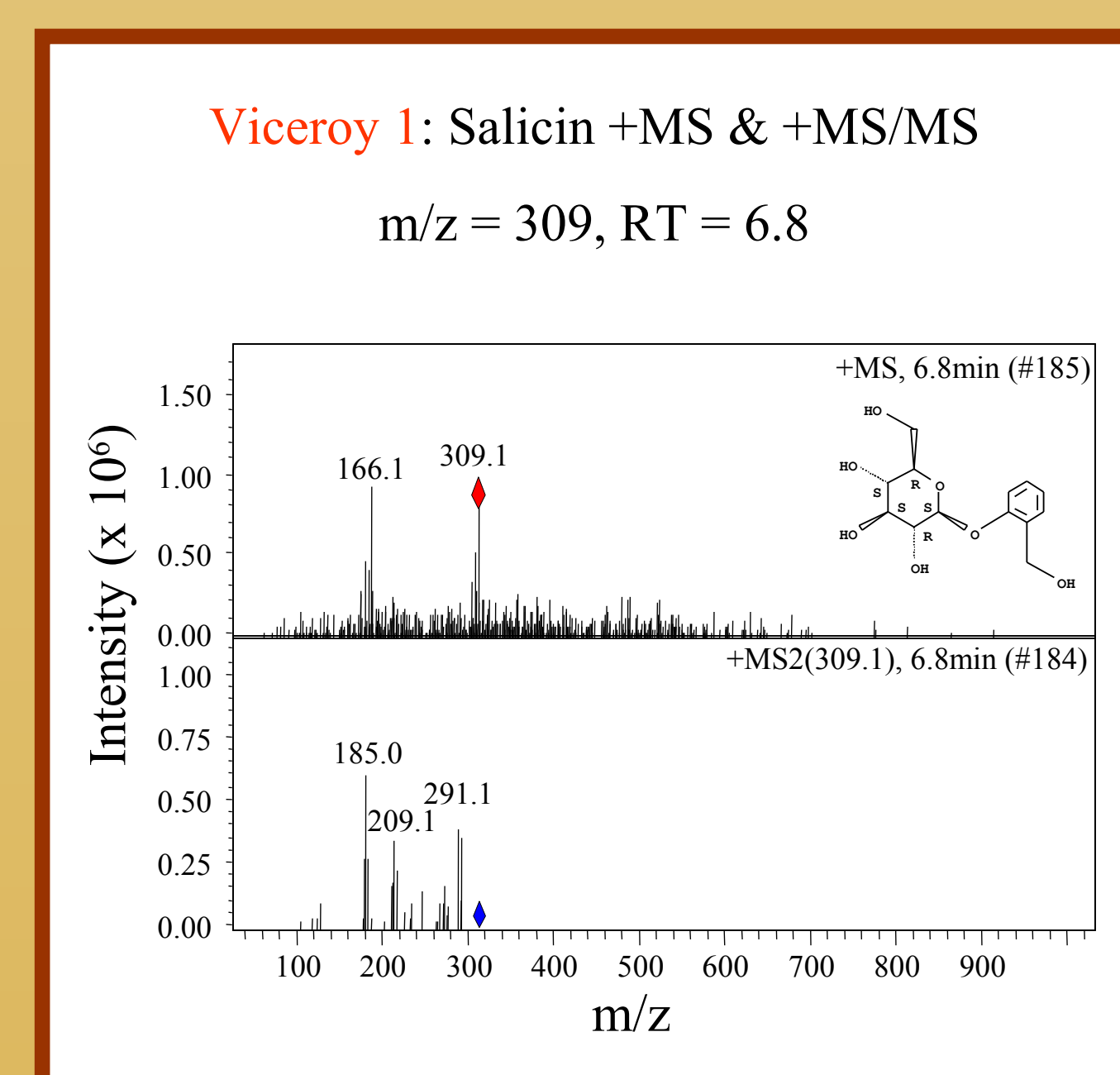
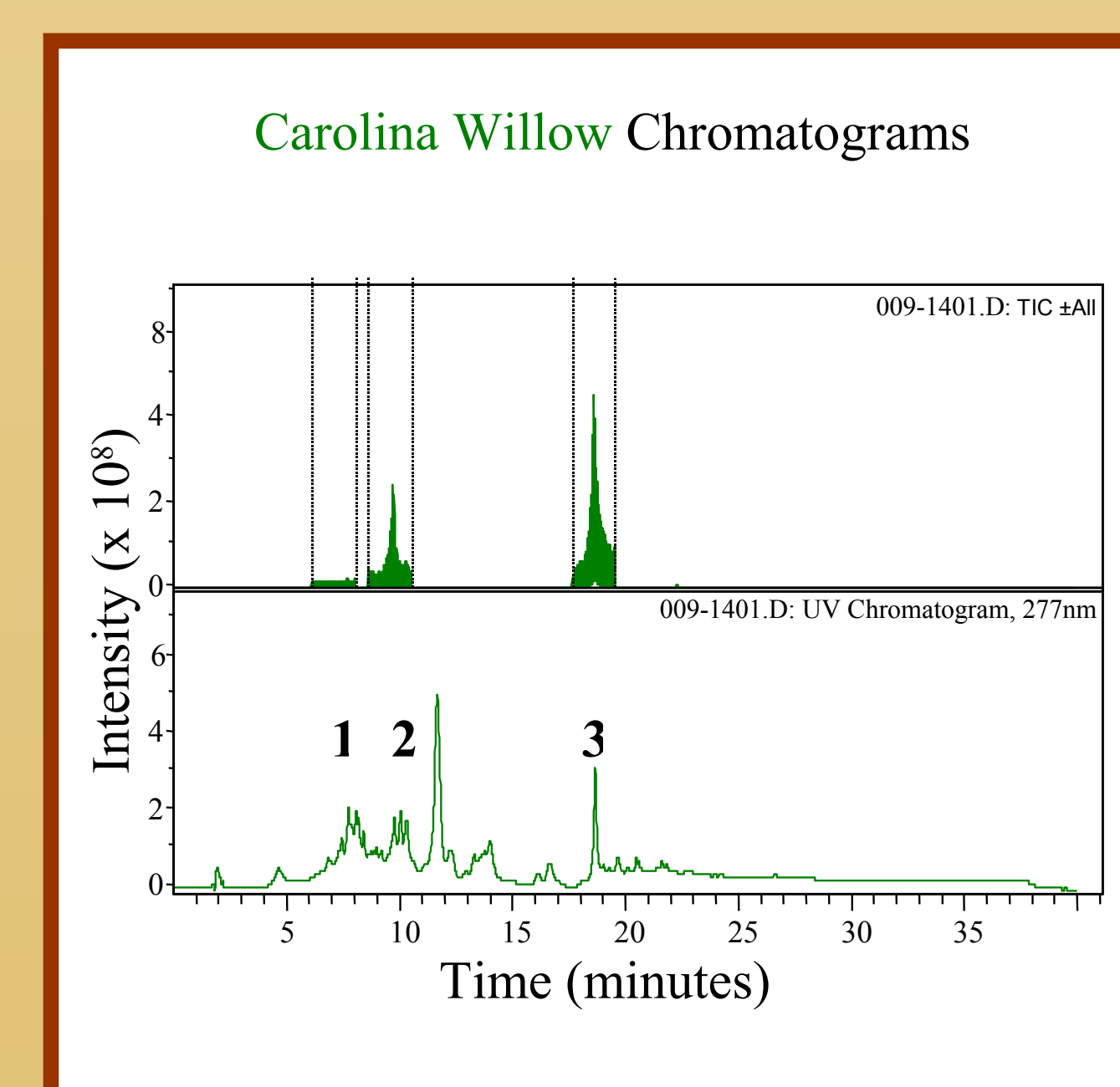
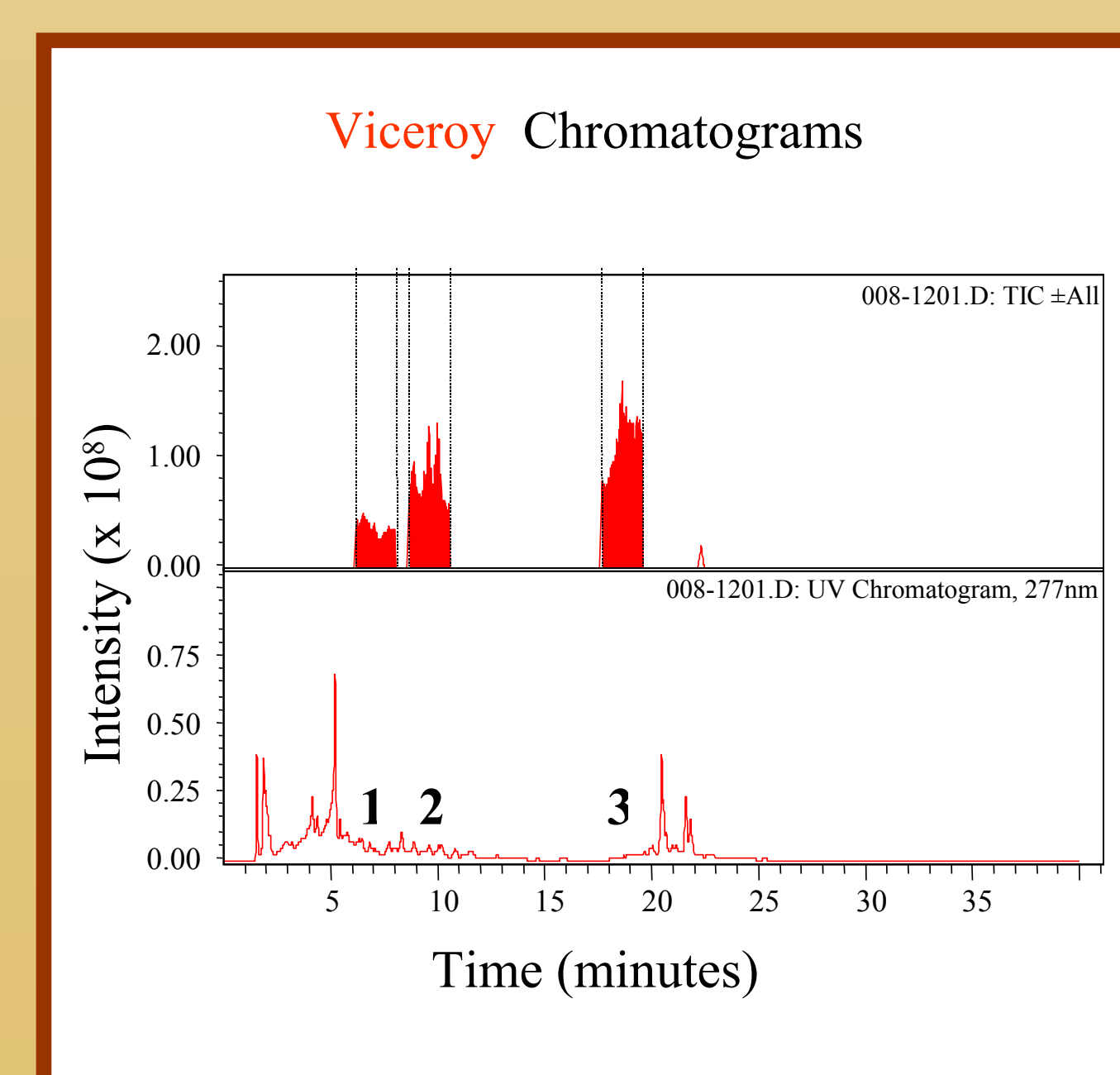
Visual defensive mimicry, defined as strong physical resemblance between unrelated species, is an enduring example of adaptation by natural selection. The viceroy-monarch butterfly association is a classic mimicry example gracing the pages of nearly all introductory biology textbooks. This butterfly mimicry system was originally classified as Batesian but was later reclassified as Müllerian. The reclassification has received mixed scientific acceptance since the defensive chemistry of the viceroy butterfly remained unknown. Here we show the viceroy butterfly (*Limenitis archippus*: Nymphalidae) sequesters known defensive compounds from its larval host-plant, the Carolina willow (*Salix caroliniana*; Salicaceae). We developed LC/MS/MS methods to identify the shared phenolic glycosides between the adult viceroy butterfly and the Carolina willow. To separate the phenolic glycosides from the methanol extracts, we developed a reversed phase HPLC method with diode array detection. Positive electrospray ionization (ESI) mode was used to identify the phenolic glycosides in the willow and the viceroy. The structures of the components were determined based on their mass spectral fragmentation patterns and confirmed with authentic standards. Our results confirm the Müllerian reclassification with the viceroy butterfly possessing different chemical defenses than its monarch counterpart (phenolic glycosides vs. cardiac glycosides, respectively).



Viceroy Butterfly



Carolina Willow



BACKGROUND

Phenolic glycosides were our primary candidate compounds for causing viceroy unpalatability because (1) these compounds are ubiquitous anti-herbivore compounds in the caterpillar host-plant, Salicaceae (willows and poplars)¹; (2) these compounds are sequestered by other insects of the willow-feeding guild, *Phratora* spp. and *Chrysomela* spp. (*Chrysomelidae*: *Coleoptera*)^{2,3}; (3) the presence of these compounds in beetles is known to deter predator and parasitoid attack³.

EXPERIMENTAL PROCEDURE

➤ Dried foliage and adult butterflies were extracted in MeOH with the resulting concentrations: 2.23 mg/ml willow and 2.73 mg/ml viceroy

➤ For HPLC, we used an Agilent 1100 HPLC system (quaternary gradient pump, diode array detector, thermostated column compartment, and autosampler) with a Waters Nova Pak reverse phase C₁₈ column (4.6 x 150 mm) to obtain the chromatograms. We injected 20 µl of sample with a methanol-water gradient flowing at 1 ml/min. UV signals were observed at 277 nm

➤ For LC-MS, we used the Agilent 1100 HPLC system tandem with Agilent MSD-Trip-SL ion trap mass spectrometer in positive ESI mode to obtain the MS spectra

➤ We characterized and verified molecular structures using two methods: comparing experimental spectra to authentic standards and comparing experimental spectra to simulated spectra with ACD/MS Fragmentor⁴

CONCLUSIONS

HPLC

➤ Six peaks were shared between larval host-plant, Carolina willow and adult viceroy butterfly. Three of these corresponded to published retention times of phenolic glycosides frequently found in willow species¹: salicin, salicortin, and tremuloidin

LC-MS (/MS)

➤ Each of the three putative phenolic glycosides exhibited a > 95% spectra assignment using ACD/MS Fragmentor⁴

➤ These characterizations were confirmed with authentic standards

Biological Importance

➤ The 3 compounds we found are known to deter generalist parasitoids and predators in other insect species³

➤ Thus, the monarch-viceroy system is not a classic example of Batesian mimicry, but is actually a Müllerian mimicry example with a completely different ecological and evolutionary dynamic

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ACKNOWLEDGMENTS

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B.N. Timmermann is now the chairperson of the Department of Medicinal Chemistry at University of Kansas.

