HPLC planning

Cucurbitacin: expect ~1 mg/ 100 10-day seedlings of cultivated cucs. Levels decrease after ~6d of seedling growth, and may be largely bred out of cultivated varieties (due to strong bitter flavor) (Balliano et al. 1983).

8e-6 g (8 ug) cucumerin per g fresh tissue (McNally et al. 2003). May be better off assaying p-coumarin or p-came, which are added to cucumerin precursor (McNally et al. 2003).

Methods of extraction (from McNally et al. 2003):

Leaf tissue from each treatment was

then carefully harvested, freeze-dried, and stored in the dark

at - 80 °C until further analysis.

Extraction and Isolation. Freeze-dried leaf tissue from

both resistant and control plants was extracted with 80%

MeOH (80 mL/g of dry mass plant material) for 48 h on a

rotary shaker (100 rpm). Extracts were filtered using a

Buchner apparatus to remove particulate matter, then roto-

evaporated at 38 °C until only water remained. Pigments,

lipids, free phenolics, and other unwanted nonpolar compounds

were eliminated by partitioning with Et2O (5 x 30 mL).

Extracts were then hydrolyzed by adding an equal volume of

4 N HCl to each extract and heating for 90 min at 100 °C under

reflux using an oil bath as heat source. Extracts were

partitioned with Et2O (3 x 30 mL) and EtOAc (3 x 30 mL) to

recover aglycones. Both organic fractions were then combined,

rotoevaporated to dryness, and resuspended in MeOH. Metha-

nolic extracts containing aglycones were then desalted using

Sep-Pak reversed-phase C18 cartridges by rinsing with 10 mL

of H2O prior to elution with 10 mL of MeOH. Comparison of

extracts from both treatments using HPLC revealed induction

of 1 - 8 within resistant plant extracts.