Sheng-wen returned and did some more experiments after turning in her thesis and graduating. Now it is clear that the dissociation when antimycin is added to pure ethanol is not due to "hydrolysis of a weak acid" as treated by Nightingale for example, but to basic impurities in all commercial ethanol. Antimycin is stable for hours in EtOH with a slight excess of NaOH, however spectral changes were seen with excess acid.

Because HCl is a more practical standard than bases such as NaOH, especially at low dilution where CO2 uptake becomes a problem for the latter, it is convenient to treat the antimycin solution with aproximately 0.7 equivalents of base, divide into portions, and add precise amounts of standard acid to back titrate before taking spectra. The extinction coefficient is then calculated from the slope of a plot of absorbance vs concentration of acid, making the end points unnecessary. Better, fittng the spectra as a linear combination of absolute and difference spectra of antimycin, and plotting the amount of difference spectrum vs acid concentration, puts the difference spectrum on an absolute scale allowing the extinction coefficient at any wavelength to be read off. Figure 1 shows preliminary results indicating an extinction coefficient of 5.6 mM^{-1}.

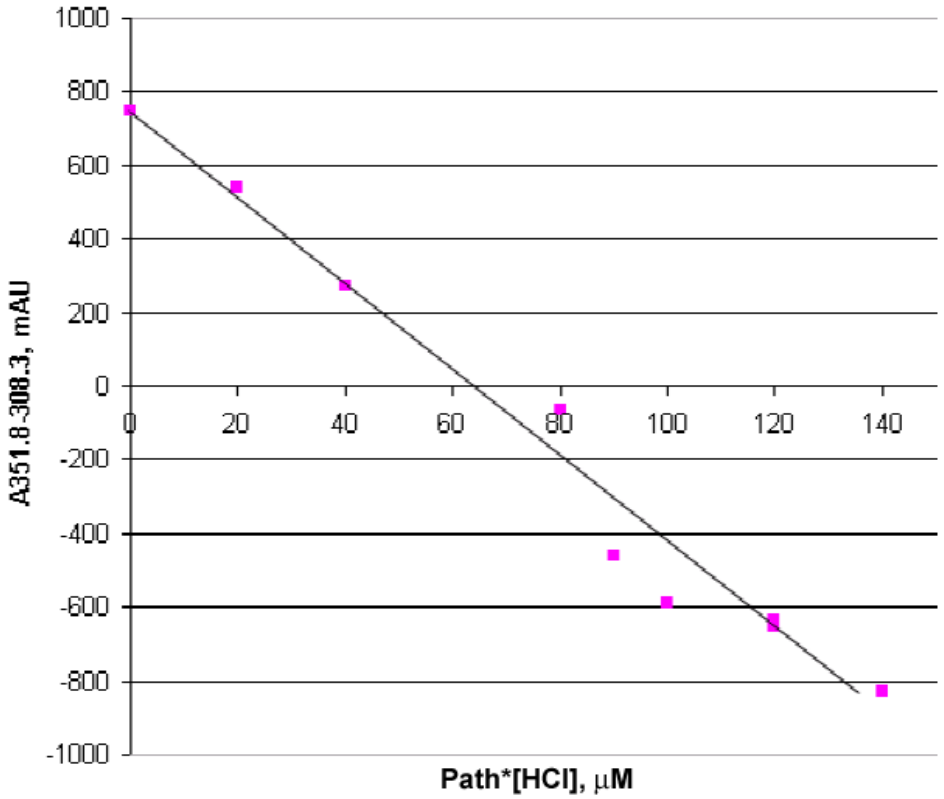

Figure x. Titratin of antimycinate with standard HCl- Absorbance vs conccentration of HCl added, corrected for 0.2 cm path length. The slope indicates an $\Delta\Delta\varepsilon_{352.8\text{-}308.3}$ of 11.3 mM^{-1}, which corresponds to 5.6 mM^{-1} for the acid form at 319 nm**.

*According to the literature, the lactone and ester linkages are readily hydrolyzed in alkali. But these are electronically isolated from the phenolic chromophore, so may not affect the vis-UV spectrum.

**Later NaOH titrations (http://www.cytbc1.com/jordan/thesis.pdf) by Jamie Jordan arrived at a value of 5.47 ±0.24 mM^{-1} for ε_{319}, acid