Isolation and Analysis of Steroidal Alkaloids from *Veratrum californicum*

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**Abstract**

*Veratrum californicum* ("corn lily") grows in the western part of the United States and contains high concentrations of steroid alkaloids with therapeutic value. Several of these steroid alkaloids exhibit antitumor activity by disruption of the Hedgehog (Hh) signaling pathway. Here we describe methods to extract, purify, and characterize cyclopamine, veratramine, jervine, muldamine, and other alkaloids from *V. californicum* biomass. These alkaloids are separated from extraction solution by high performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD) followed by identification using electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance spectroscopy (NMR).

**Background**

*V. californicum* was noticed in the 1950s when sheep grazing on the lily produced offspring with malformations including one central eye (cytologia). The cyclopamine molecule in the plant was found to suppress the hedgehog (Hh) signaling pathway resulting in the malformations. Cyclopamine is a possible anti-cancer treatment, where the Hh pathway is overactive. However, separation of cyclopamine proves difficult because of the similar alkaloids within the lily, depicted in **Figure 1**. The interaction of cyclopamine in the Hh pathway has yet to be characterized.

**Tools for Isolation and Characterization**

- HPLC analysis provides an appropriate technique for purification. Steroidal alkaloid standards originally purified from *V. californicum* and root samples were analyzed using a Gilson HPLC-ELSD. A solvent gradient ranging from 30-100% acetonitrile was used with a C18 reversed phase column having dimensions of 250 x 4.6 mm, 5µ, and 80 Å pore size.
- Following HPLC-ELSD separation of the alkaloids from the *V. californicum* root sample, each peak was collected and then individually infused into a Bruker Ultra High Resolution TOF – the maXis for mass spectral analysis. The ESI-MS data of the individual peaks were compared to ESI-MS data of the alkaloid standards for peak identification.
- Peak collections were dried and re-solubilized in dichloromethane and then analyzed using a Bruker 600 MHz NMR. Proton spectra were used to analyze each peak collection.

**HPLC Separation and Analysis**

**Hypothesis:**
- Methods used to separate steroidal alkaloids from another species of *Veratrum plant* would separate those in *V. californicum*

**Methods and Design:**
- A gradient described in Table 1 was used in an attempt to separate the steroidal alkaloids.
- Pure alkaloid standards were profiled. Cyclopamine, the active Hh signaling inhibitor, has a profile displayed in **Figure 2A** and compared to root samples displayed in **Figure 2C**.
- Collections of peaks were made and separation was confirmed by mass spectral data displayed in **Figure 3**.

**NMR Analysis**

**Methods:**
- Collections were dried and re-solubilized in CD3Cl2-d2 for analysis through NMR.

**Analysis:**
- Absence of the aromatic region in the cyclopamine spectra (**Figure 4A**) and peaks in this same region appearing for veratramine (**Figure 4B**) confirms the separation and success of the method.
- Sharp multiplets support the hypothesis and confirm purity.

**Discussion and Conclusion**

- The steroidal alkaloids have similar structures, making them difficult to separate by HPLC. A slow and more gradual HPLC elution profile was found to separate the alkaloids cleanly.
- MS spectra of separate HPLC peaks displayed in **Figure 3** show the successful separation and purity of the alkaloids extracted from a root sample.
- NMR analysis (**Figure 4**) provided further evidence for successful HPLC separation of the extracted steroidal alkaloids.

**Future Work:**
- Studies will continue to develop an efficient extraction process of alkaloids from roots. Extracts can then be separated by HPLC and quantified by MS to determine abundance.

**References:**

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