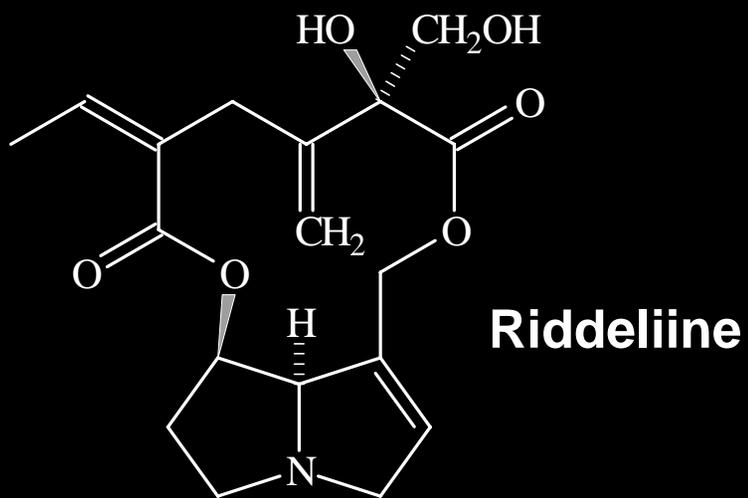


Development of Enzyme-Linked Immunosorbent Assays for Plant Toxins

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OUTLINE

- ▶ **Immune system overview**
- ▶ **Description of enzyme-linked immunosorbent assay (ELISA)**
- ▶ **Synthesis of immunoconjugates for Larkspur alkaloids**
- ▶ **ELISAs for Larkspur Alkaloids**
- ▶ **Measurement of toxins in plant material and feeds**
- ▶ **Conclusions**

Immunogens

Molecules that trigger an immune response and interact with the antibodies produced.

Natural: Foreign macromolecules, cells, or viruses and/or their products.

Artificial: Vaccines, conjugates.

Requirements:

- 1) High Molecular Weight; $MW < 1000$ not immunogenic**
- 2) “Foreign”, Not native**
- 3) Chemical complexity**

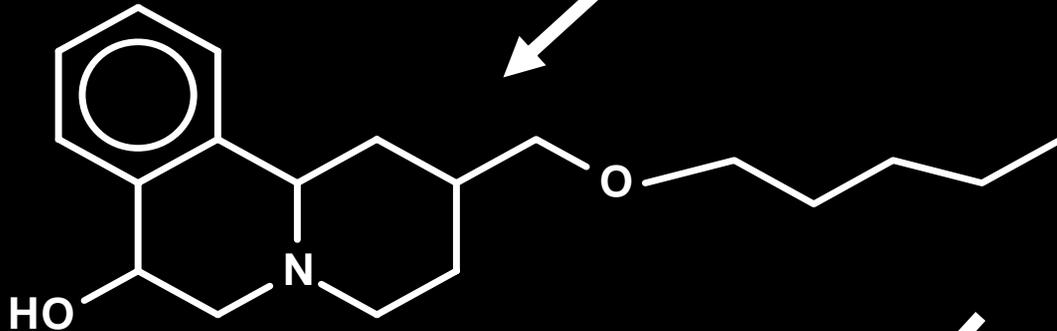
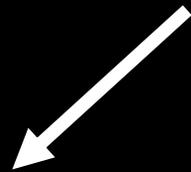
Immunoconjugates



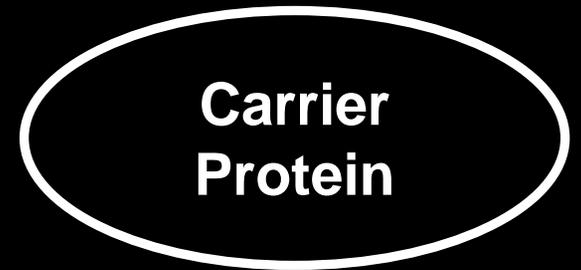
Toxin



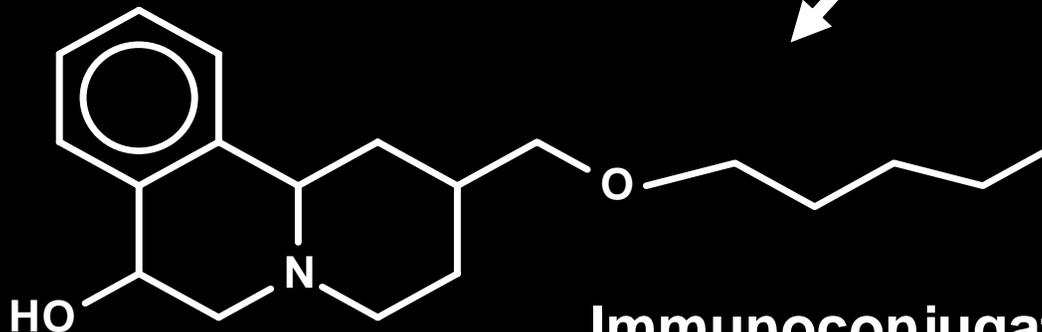
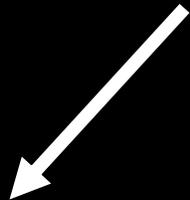
Linker



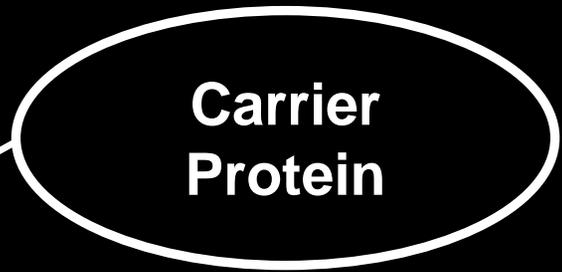
Hapten



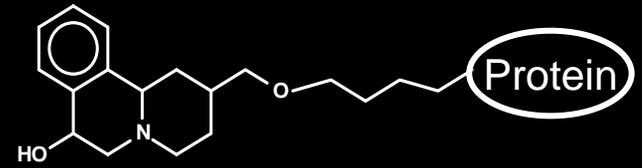
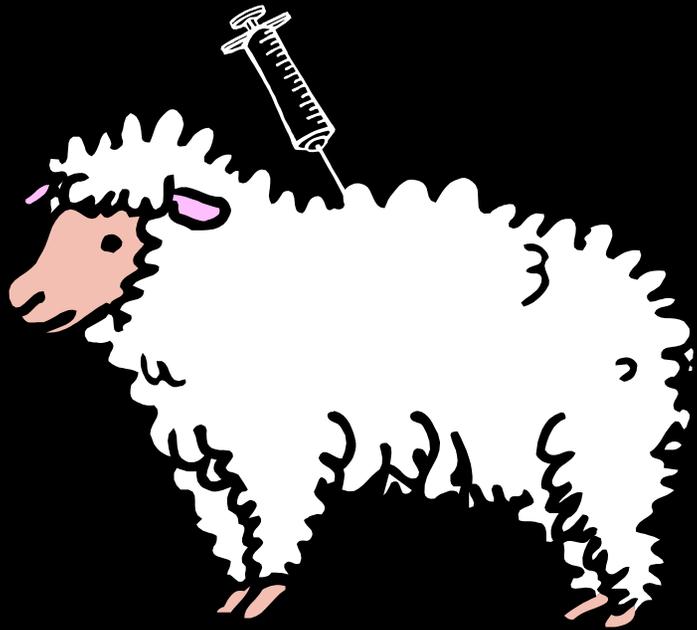
Carrier Protein



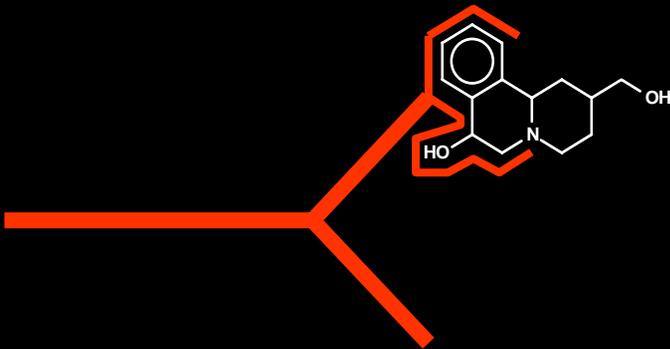
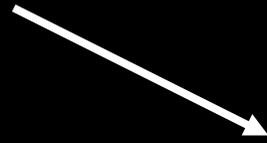
Immunoconjugate



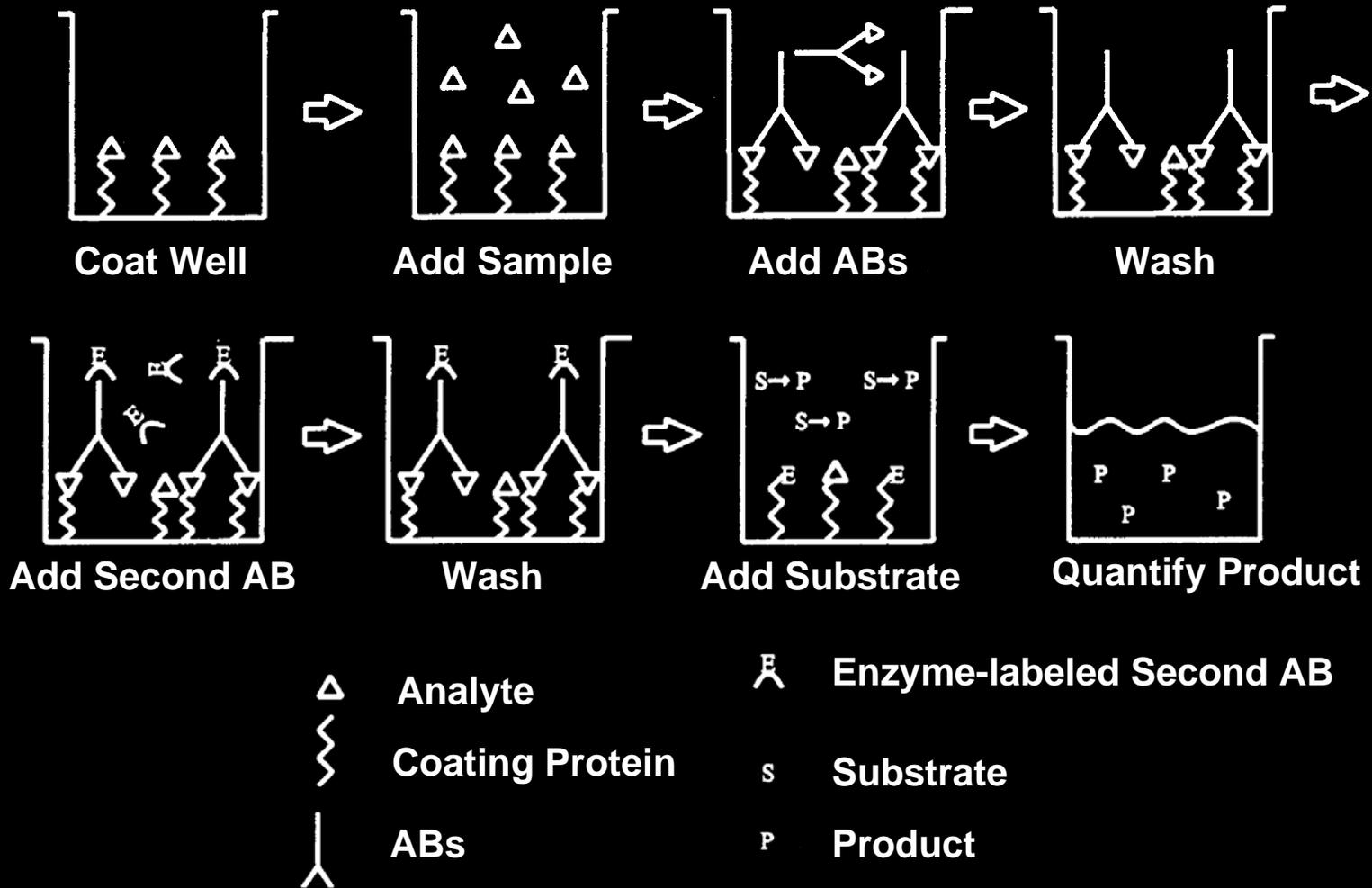
Carrier Protein



2 booster shots
3 months



Indirect Competitive ELISA



ELISA

Advantages:

Wide range of analytes

Low detection limits

Specific

Can analyze small samples

Rapid - high sample throughput

Ease of use

Field use

Extensive use in pharmaceutical and environmental chemistry

Disadvantages:

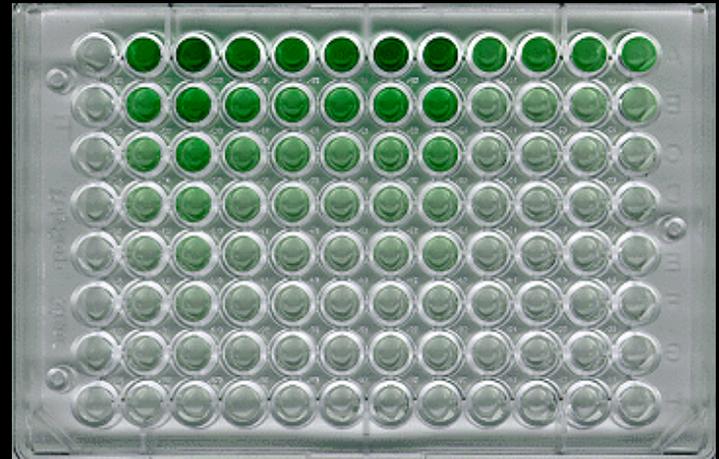
High development costs

Haptens can be difficult to synthesize

Interferences or undesired cross-reactions

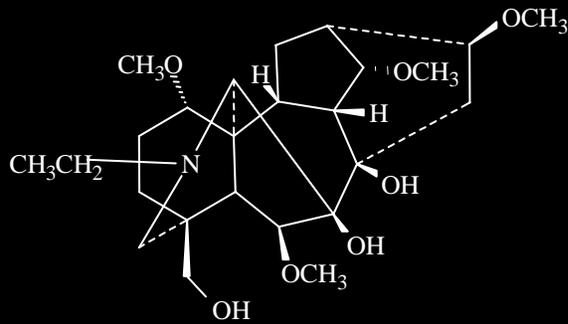
Requires independent confirmation

Not good for small sample loads

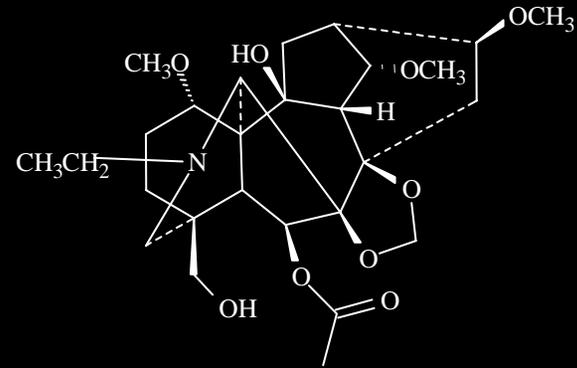




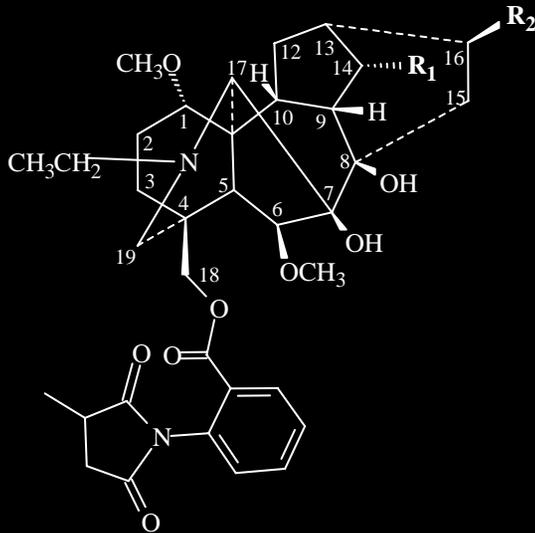
Larkspur Alkaloids



Lycoctonine



Deltaline



MSAL

R₁

R₂

Methyllycaconitine

OCH₃

OCH₃

14-Deacetylindicauline

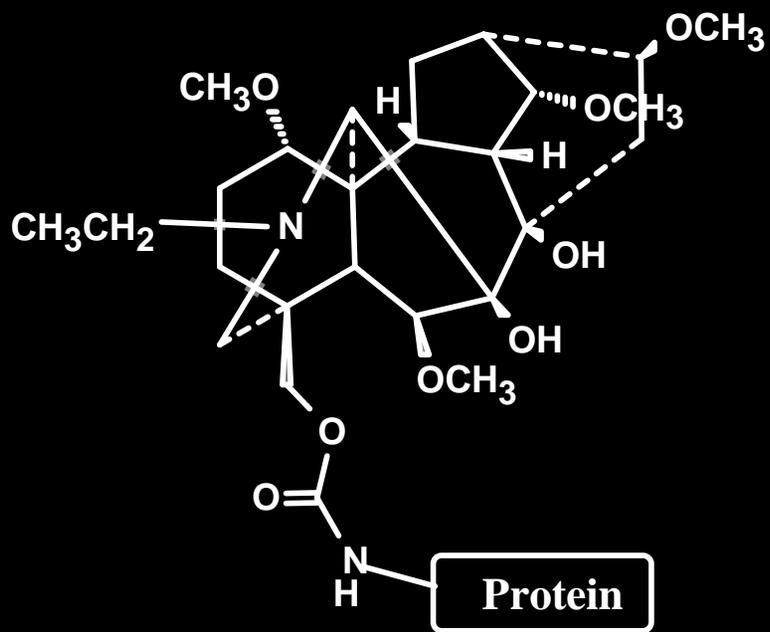
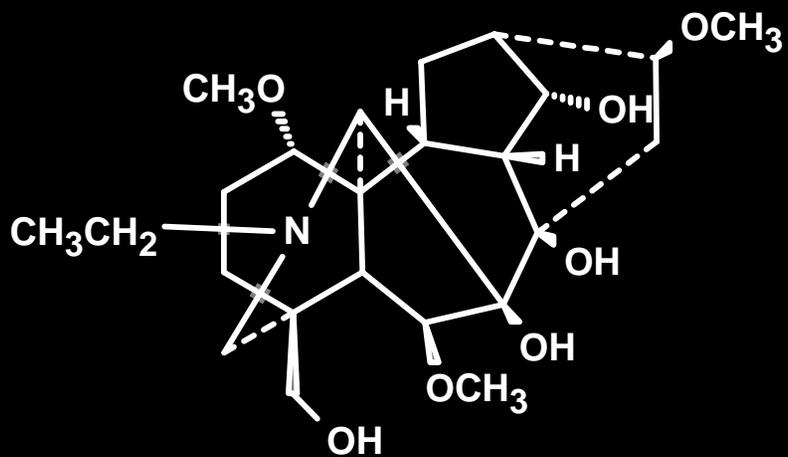
OH

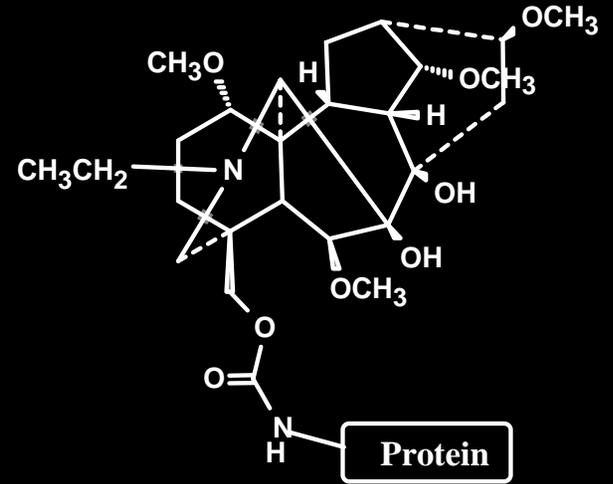
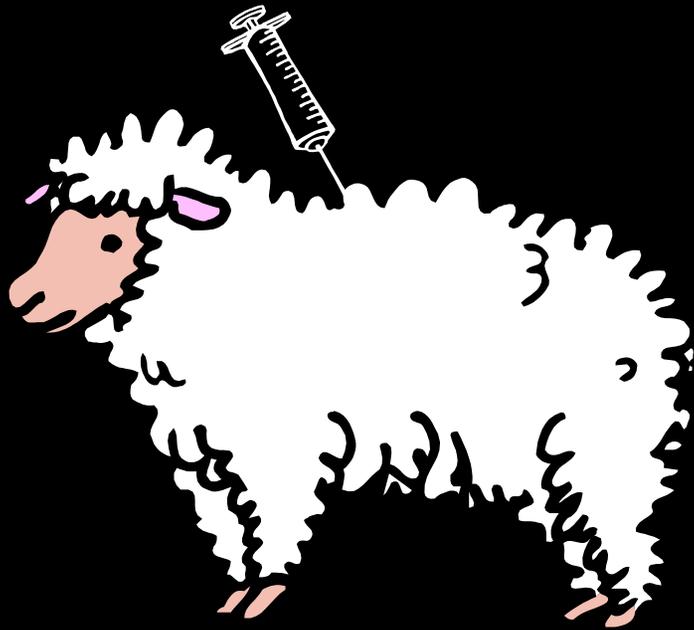
OCH₃

Bearline

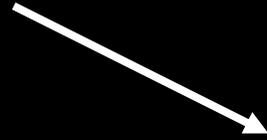
OH

Oac

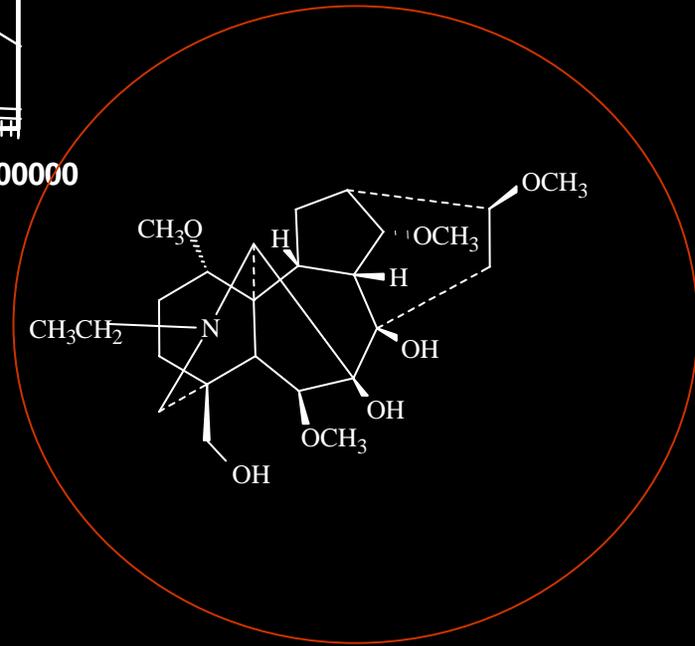
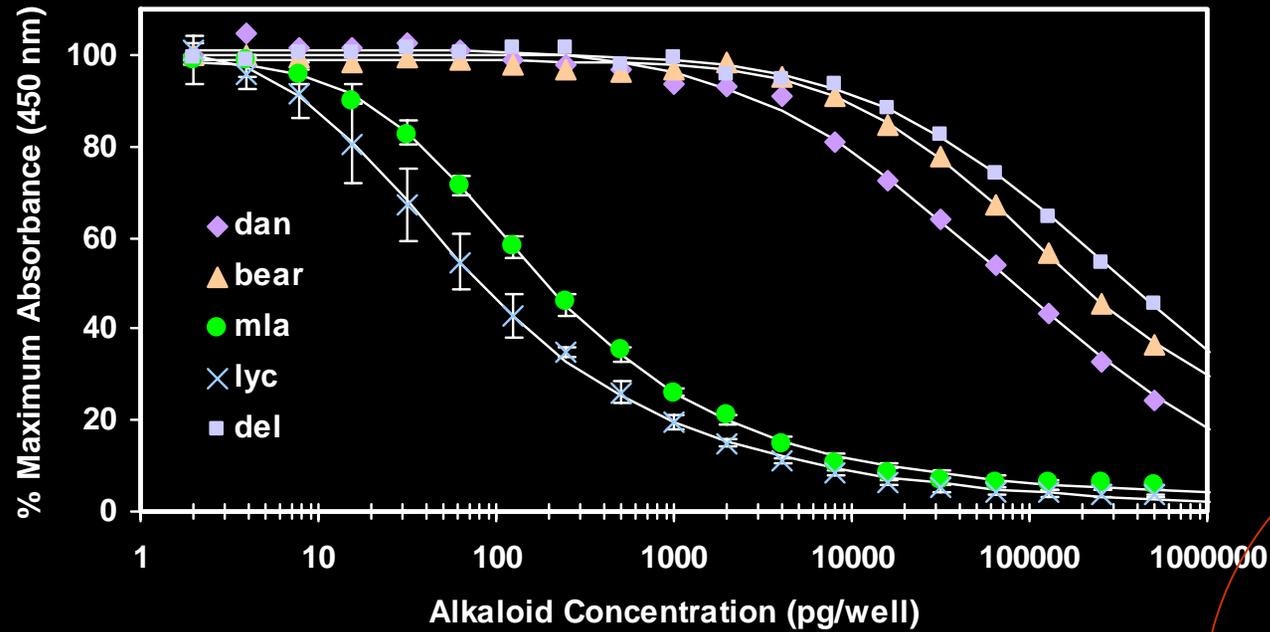




2 booster shots
3 months



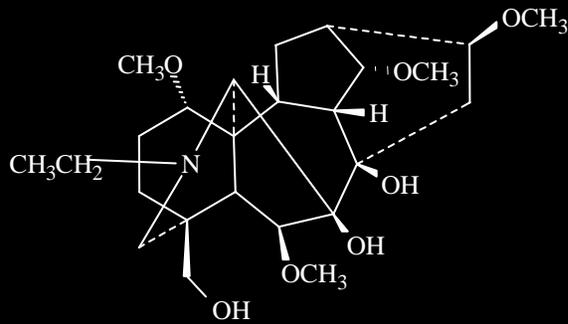
Lycotoxine Assay



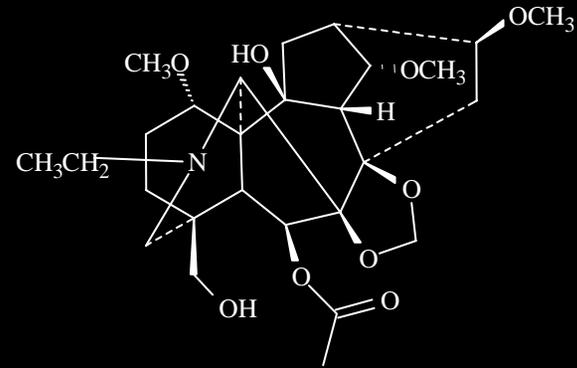
Results - Lycoctonine Assay

- ▶ **Lycoctonine and methyllycaconitine are recognized by antibodies**
- ▶ **Antibodies have reduced affinities to deltaline, 14-deacetyl nudicauline and bearline**
- ▶ **Norditerpenoid backbone structure of lycoctonine is the primary recognition site for the antibodies**
- ▶ **Limit of detection for lycoctonine is 14.3 pg and methyllycaconitine is 30.5 pg**

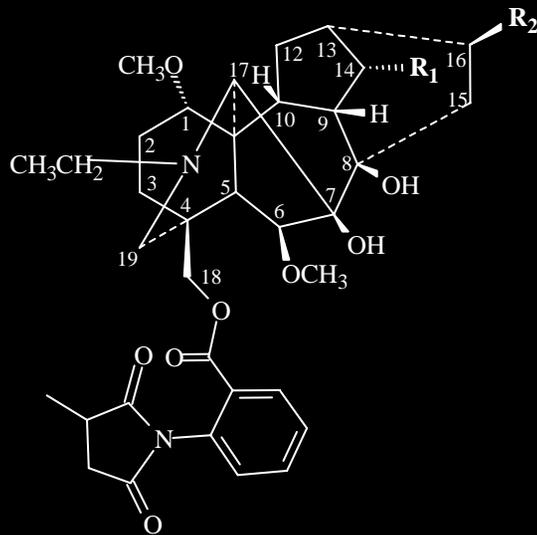
Larkspur Alkaloids



Lycoctonine



Deltaline



MSAL

R₁

R₂

Methyllycaconitine

OCH₃

OCH₃

14-Deacetylindicauline

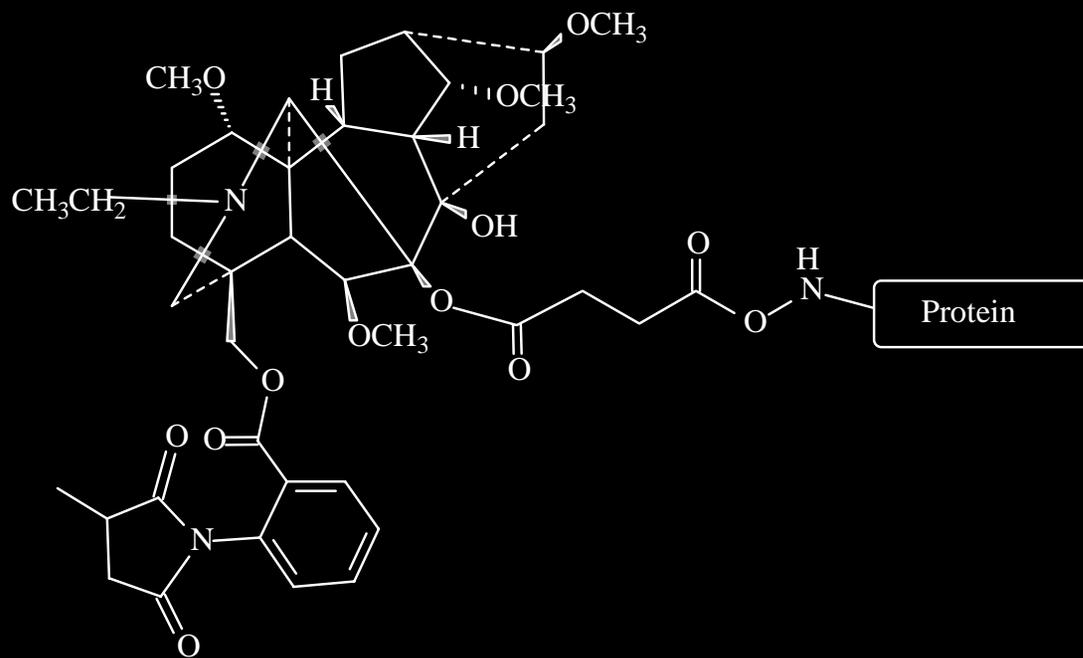
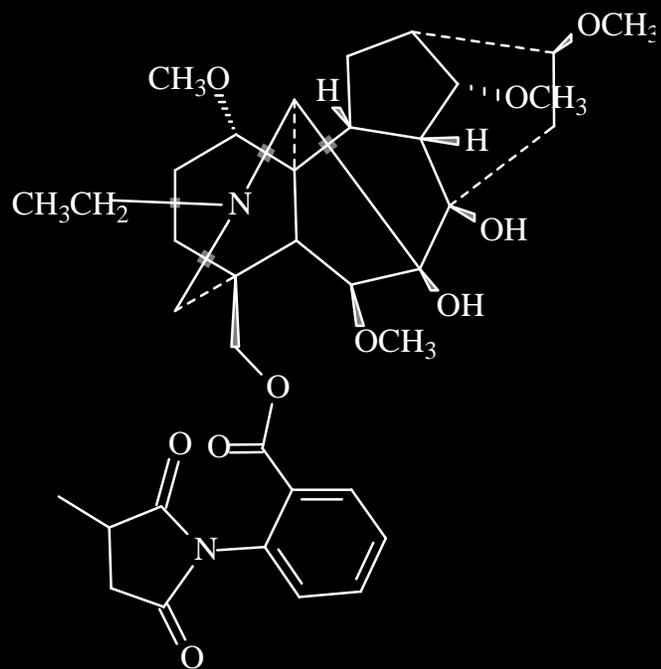
OH

OCH₃

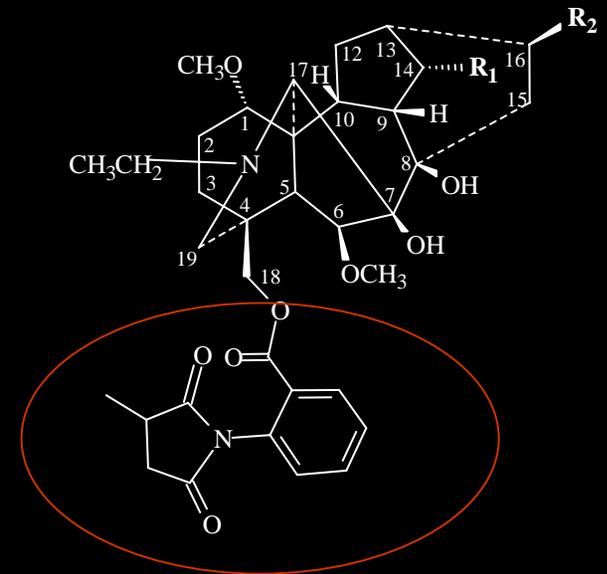
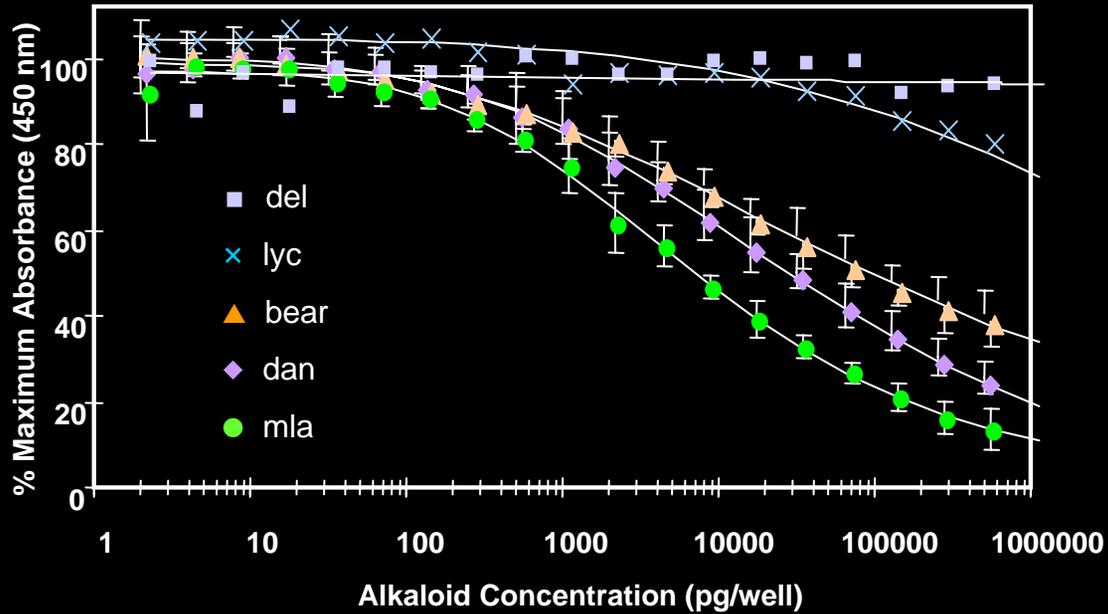
Bearline

OH

Oac



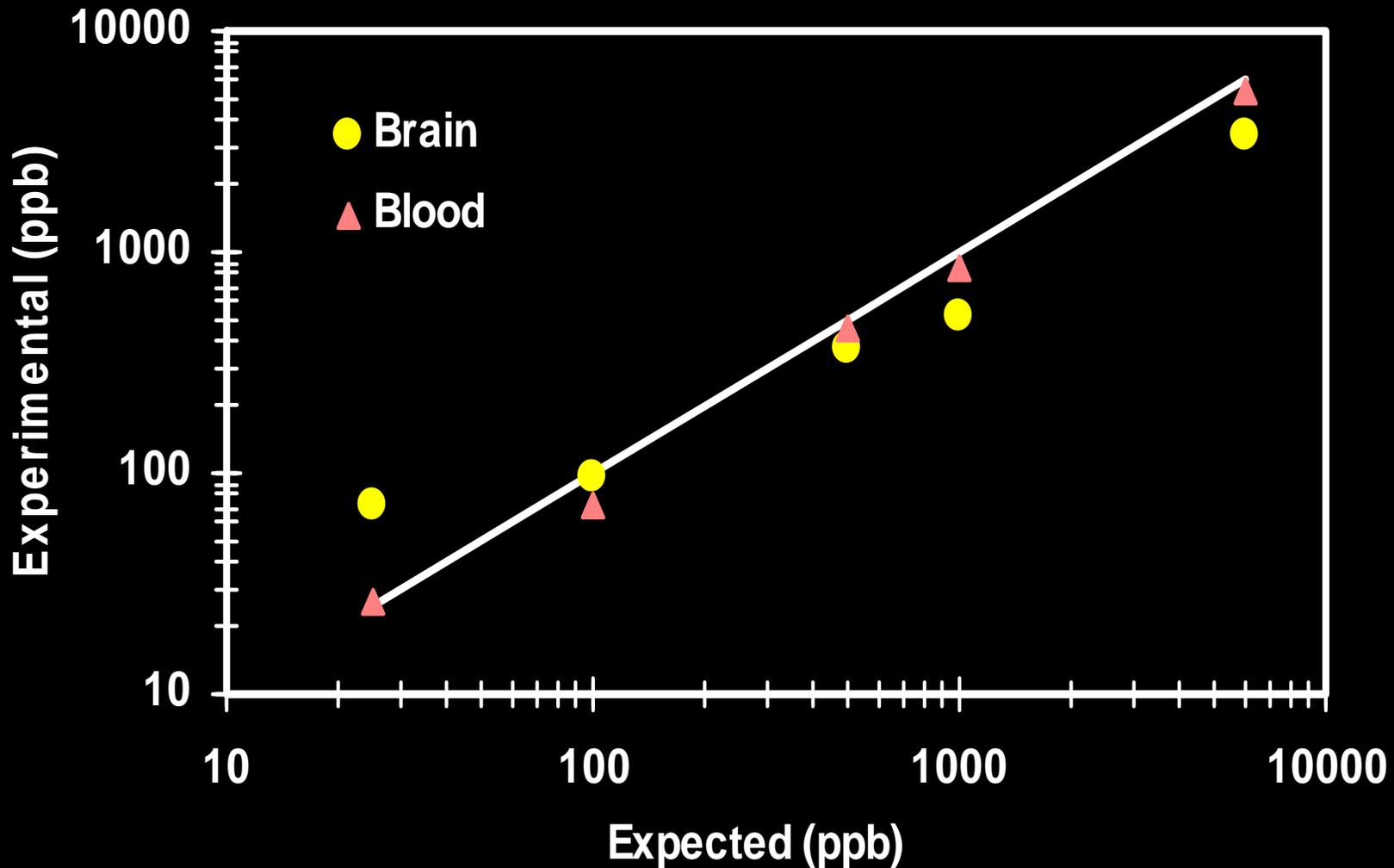
14-Deacetylindicauline Assay



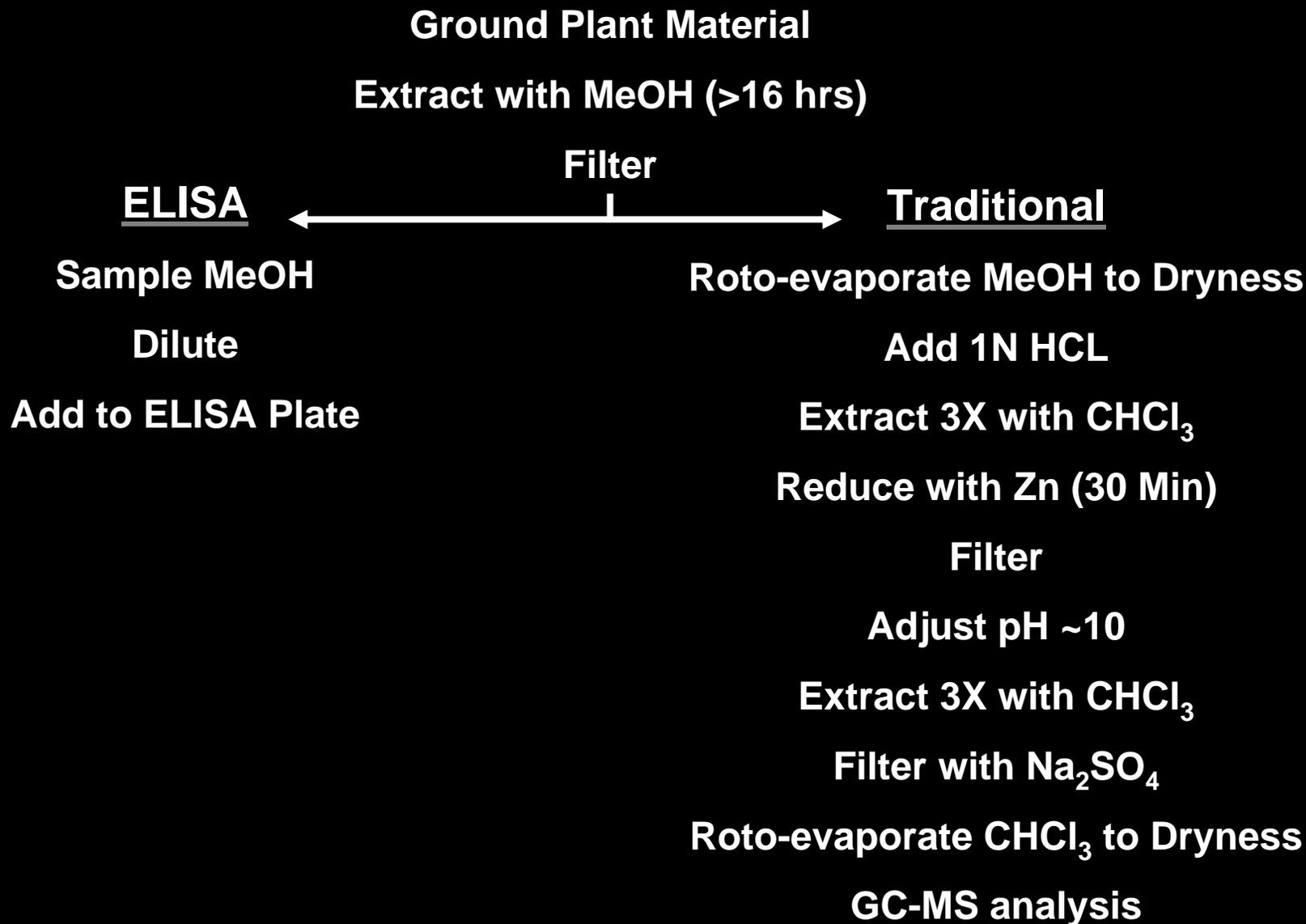
Results - 14-Deacetyl nudicauline Assay

- ▶ Methyllycaconitine, 14-deacetyl nudicauline and bearline are recognized by antibodies
- ▶ Antibodies have reduced affinities to lycoctonine and deltaline
- ▶ The primary recognition site is the *N*-(methylsuccinimido)anthranilic ester group
- class specific assay for these compounds
- ▶ Limit of detection for methyllycaconitine is 409.8 pg, 14-deacetyl nudicauline is 217.1 pg, and bearline is 581.6 pg

Determination of Methyllycaconitine in Blood and Brain Tissue



Extraction and Analysis of Plant Material for Pyrrolizidine Alkaloids







Rachel stares at her positive pregnancy test as her friend Monica looks on, (*Friends*, 2001).



This worked great. I wouldn't spend a lot on pregnancy tests. This showed a positive result at 3 weeks of pregnancy, and before the lab could confirm my positive result with a blood test. The blood test was done again a few days later, and was positive.

-- Anonymous on February 26, 2007



This worked great. I can't afford to spend a lot on Larkspur tests. However, this test was inexpensive and showed a positive result in 3 minutes. The test was done again at a lab again about a week later, and was also positive.
-- Joe Rancher on June 26, 2050

Vaccines Against Plant Toxins?

Experimental Design: