Chapter 84

N-Alkylated Derivatives of Swainsonine

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Introduction

Locoweeds (*Astragalus* and *Oxytropis* spp.) continue to be a significant rangeland poisonous plant problem (Ralphs and James 1999). The toxin in the plant has been identified as the polyhydroxy indolizidine alkaloid named swainsonine **1** (Colegate *et al.* 1979; Molyneux and James 1982). Swainsonine inhibits cellular α-mannosidase activity (Dorling *et al.* 1978) and alters glycoprotein biosynthesis (Elbein 1989), resulting in inhibition of mannosidase II and lysosomal storage disease. Clinical signs of intoxication in animals are numerous and include depression, anorexia, weight loss, intention tremors, seizures, nervousness, dull hair coat, infertility, and abortion (Stegelmeier *et al.* 1999).

The prevention of locoweed intoxication is currently best achieved through the application of a number of management recommendations that may include rotational pasture grazing, cyclic grazing, flash grazing, delayed grazing, careful observation and removal of animals eating locoweed, culling and removal of reproductive open cows in the fall, creation of locoweed-free pasture areas through selective herbicide use, and aversion conditioning (Allison and Graham 1999). The idea of a vaccination program for use against locoweed intoxication would seem attractive; however, protective immunization against plant toxins has been of only limited success (Culvenor 1978; Payne *et al.* 1992; Edgar 1994; Than *et al.* 1998; Lee *et al.* 2003) because of the small molecular size of most plant toxins. In order to enable the low molecular weight plant toxins to provoke an immune response they must be linked to a larger carrier molecule such as a protein.

The linkage of swainsonine **1** to a large carrier molecule (protein) for the purpose of the induction of immunological activity has proven difficult because of the lack of chemical 'handles' on such a small molecule. Swainsonine has been linked via the hydroxyl groups but no swainsonine-specific antibodies were produced. It was assumed that linkage through one of the hydroxyl groups likely alters the three dimensional structure that characterizes the swainsonine-specific epitope. We sought an alternate approach by searching for a possible linkage through the nitrogen with a diverse carbon skeleton such as a 4-carbon allylic system and 4–8-carbon alkyl systems. The resulting alkyl-halide swainsonine derivatives are linked to several amino acids as a model system for future work with proteins.

Methods

Reaction scheme I. Swainsonine acetonide

Swainsonine acetonide was prepared by the reaction of swainsonine **1** with 2,2-dimethoxy propane in acetone with the pH adjusted to 3–4 by dropwise addition of methyl sulfonic acid. The reaction was refluxed for 6 hours with a Dean-Stark trap to remove the methanol formed. The reaction mixture was worked up by the removal of the solvent and extraction by base/acid/base partitioning to yield the final product, swainsonine acetonide **2** (> 90% yield).

Reaction scheme II. *N*-bromo-2-butene swainsonine and derivatives

To a 100 mL round bottom flask, equipped with a magnetic stirrer and condenser, was added 250 mg of swainsonine acetonide, 50 mL of benzene and 500 mg of 1,4-dibromo-2-butene. The reaction was refluxed for 48 hours. Diethyl ether was added, the solvent decanted, and the solid residue washed with diethyl ether/hexane, dried under vacuum to yield 395 mg of *N*-bromo-2-butene swainsonine acetonide **3**. A portion (100 mg) of the product was stirred with 2 mL water and 2 mL of 38% hydrobromic acid in acetic acid for 24 hours at 40°C. The solvent was removed by evaporation under vacuum to give a slightly yellow solid *N*-bromo-2-butene swainsonine **4**. High resolution electrospray mass spectrometry (HRESMS) $M^+ = 306.0705$; calculated for $C_{12}H_{21}BrNO_3 = 306.07048$.

Reaction scheme IIa. *N*-hydroxy-2-butene swainsonine

A small portion of **4** (15 mg) was dissolved in 1.5 mL of tetrahydrofuran and 1.5 mL water and the mixture stirred for 24 hours at room temperature and then for 3 hours at 40°C. The solvents were removed under vacuum to give 12 mg of *N*-hydroxy-2-butene swainsonine **5**.

Reaction scheme IIb. *N*-amino-2-butene swainsonine

To a small portion of **4** (50 mg) was added 1 mL of ammonium hydroxide and 1 mL of water. The mixture was stirred for 12 hours at 40°C and the solvent removed under vacuum to give 38 mg of *N*-amino-2-butene swainsonine **6**.

Reaction scheme IIc. *N*-(serine)-2-butene swainsonine

To 25 mg of **4** was added 1 mL of water and 0.5 mL of DMSO and 50 mg of serine. The reaction mixture was stirred for 48 hours, and the solvent removed under vacuum. The residue was washed twice with 2 mL of diethyl ether and 0.5 mL of hexane to remove excess serine and DMSO. The remaining residue was found to be a mixture of corresponding alcohol **5** (5%) and the serine derivative **7** (95%). If the reaction is conducted in pure water the yield of **7** is reduced to 60% and to 40% for the alcohol **5**.

Reaction scheme III. *N*-iodobutane swainsonine and derivatives

Swainsonine acetonide (200 mg) was dissolved in 30 mL of acetonitrile to which 200 mg of 1,4-diiodobutane was added. The reaction mixture was refluxed for 4 days. The solvent was mostly removed by a flow of argon, leaving a yellow residue. The residue was washed with diethyl ether and hexane (1/1) and dried under vacuum to give 50 mg of *N*-iodobutane swainsonine acetonide **8**. The acetonide group was removed by hydrolysis with iodic acid (0.5 mL 50% iodic acid) and 2 mL water upon stirring for 48 hours. The solvents were removed under vacuum to give 27 mg of *N*-iodobutane swainsonine **9**. HRESMS gave $M^+ = 356.0710$; calculated for $C_{21}H_{23}INO_3 = 356.07227$.

Reaction scheme IIIa. *N*-hydroxybutane swainsonine

To 10 mg of **9** was added 0.5 mL of DMSO, 0.5 mL water and 2 mg K_2CO_3. The reaction was stirred at 40°C for 24 hours. The reaction mixture was continuously extracted with methylene chloride for 24 hours, and the solvent removed to give *N*-hydroxybutane swainsonine **10**.

Reaction scheme IIIb. *N*-(serine)-butane swainsonine

In 0.5 mL of DMSO and 2 mL water was added 4 mg of **9**, 5 mg of serine, and 1 mg of K_2CO_3. The reaction was stirred for 48 hours at 40°C to yield *N*-(serine)-butane swainsonine **11** (50%) plus other products.

Reaction scheme IIIc. *N*-(glutathione)-butane swainsonine

To 0.5 mL of DMSO and 2 mL water was added 20 mg of **9**, 35 mg of glutathione and 3 mg of $NaHCO_3$. The mixture was stirred for 48 hours at 40°C. The solvent was removed under vacuum and the residue washed with 2 mL of dry methanol. The methanol was removed and the residue washed three times with diethyl ether and hexane (1/1) to yield a mixture of starting material **9** (5%), hydroxyl derivative **10** (5%), and *N*-glutathione-butane swainsonine **12** (90%).

Results

The overall reaction scheme is summarized in Figure 1. Initially, all reactions were carried out using swainsonine acetonide to enhance solubility in organic solvents and for protection of the hydroxyl groups. A number of *N*-alkylated derivatives of swainsonine could be produced by the reaction of swainsonine acetonide with 1,4-dibromo-2-butene and 1,4-diiodobutane. Alkyl addition on the nitrogen did occur, as evidenced by complete acetylation of the unprotected swainsonine derivatives (**4** and **9**) to give the corresponding triacetate compounds (Figure 2). Two possible isomers are produced, which are the *cis* and *trans* (relative to H-8a) adducts on the nitrogen, in a ratio of approximately 3:1. Both isomers are observed in LC/MS analysis of the product (Figure 3).

Compounds **4** and **9** were reacted to yield multiple functional groups on the end of the alkyl chain, thus providing multiple chemistry options to be used later in conjugation with the carrier molecules. In general the bromo derivatives were more reactive to a variety of functional groups than the iodo derivatives. However, we also noticed that with time the bromo reactions seemed reversible and the starting material was regenerated. Complete removal of Br anions from the reaction mixture might stop the conversion back to the alkyl bromide. The iodo derivative appeared to be more stable, but also less reactive towards OH and NH functional groups. *N*-iodobutane swainsonine did seem reactive towards SH groups as evidenced by the reaction with glutathione.

After confirmation that the above reactions were only occurring on the nitrogen atom, we recently completed the *N*-alkylation reaction directly with swainsonine, thus eliminating the use of the acetonide derivative. Identical products (**4** and **9**) were produced. Variable chain length alkyl compounds can also be used. 1,6-Diiodohexane was easily incorporated into the reaction scheme to produce the iodohexane swainsonine derivative. Future work will focus on coupling reactions to the carrier protein, injection into an animal model and testing for swainsonine-specific antibodies.

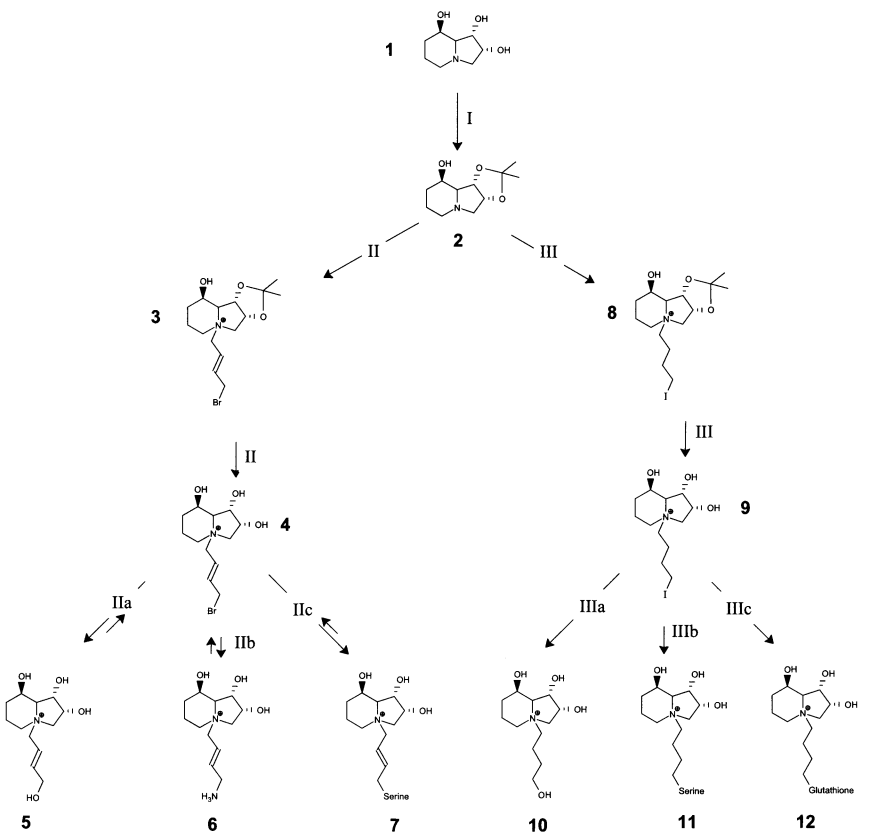

Figure 1. Overall reaction scheme for production of *N*-alkyl derivatives of swainsonine.

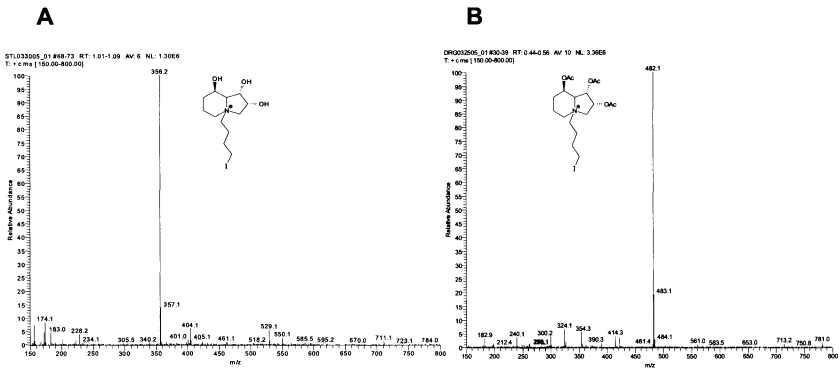

Figure 2. (A) ESMS spectrum of *N*-iodobutane swainsonine **9**; mw = 356 and (B) conversion to the triacetate, mw = 482 to prove *N*-alkylation.

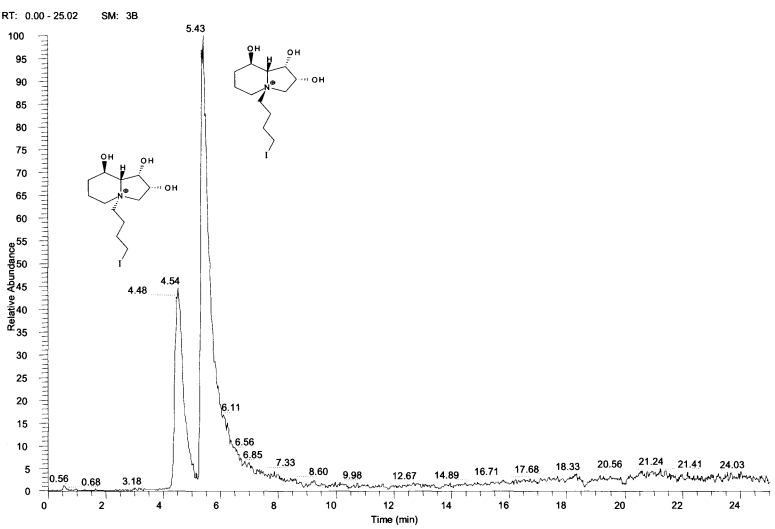

Figure 3. LC/MS separation of *cis* and *trans* *N*-iodobutane swainsonine.

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