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Analysis of Swainsonine and Swainsonine *N*-Oxide as Trimethylsilyl Derivatives by Liquid Chromatography−Mass Spectrometry and Their Relative Occurrence in Plants Toxic to Livestock

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ABSTRACT: There are limited data concerning the occurrence of swainsonine *N*-oxide in plants known to contain swainsonine and its relative impact on toxicity of the plant material. A liquid chromatography−mass spectrometry method based on a solvent partitioning extraction procedure followed by trimethylsilylation and analysis using reversed phase high-pressure liquid chromatography−mass spectrometry was developed for the analysis of swainsonine and its *N*-oxide. The concentrations of each were measured in several swainsonine-containing taxa as well as two endophytic isolates that produce swainsonine. In vegetative samples the relative percent of *N*-oxide to free base ranged from 0.9 to 18%. In seed samples the *N*-oxide to free base ratio ranged from 0 to 10%. The measured concentrations of swainsonine *N*-oxide relative to swainsonine only slightly increases the actual toxicity of the various plant samples in a combined assay of both compounds.

KEYWORDS: *swainsonine, swainsonine N-oxide, locoweeds, HPLC-MS, trimethylsilyl derivative*

INTRODUCTION

Swainsonine, **1** (Figure 1), a trihydroxy indolizidine alkaloid,[1] has been reported in species of *Ipomoea*[2] and *Turbina*[3] (Convolvulaceae) and *Astragalus*,[4] *Oxytropis*,[5] and *Swainsona*[1] (Fabaceae) and in *Sida carpinifolia* (Malvaceae).[6] Ingestion of these swainsonine-containing plants causes a toxicosis in livestock leading to a chronic wasting disease, depression, altered behavior, decreased libido, infertility, and death.[7,8] Other species within these genera do not contain swainsonine, and many are important forages that pose no swainsonine-related risk to livestock.

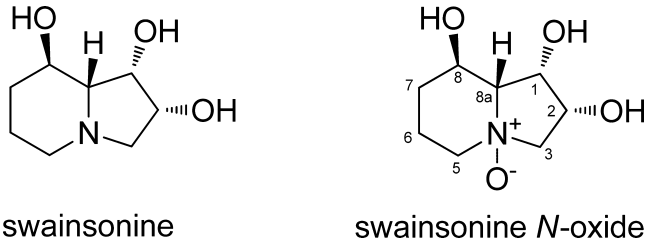

Figure 1. Chemical structures of swainsonine, **1**, and swainsonine *N*-oxide, **2**.

Recent studies provide evidence that swainsonine is not a plant-derived secondary metabolite but is produced by fungal endophytes associated with all swainsonine-containing taxa investigated to date.[8] *Astragalus*, *Oxytropis*, and *Swainsona* spp. are associated with fungal endophytes belonging to *Alternaria* spp. section *Undifilum* (Pleosporales),[9−14] whereas *Ipomoea carnea* has been shown to be associated with a fungal endophyte belonging to order Chaetothyriales.[15] Thus far, plants that lack the association with the respective endophyte do not contain swainsonine.[15−17]

There are several reported methods of analysis for swainsonine that include TLC,[5] HPLC,[18−21] and MS[22] methods for the direct analysis of the free base alkaloid as well as gas chromatography methods for the analysis of the trimethylsilyl ether or the triacetyl derivatives of swainsonine.[23−25] In addition to the occurrence of the free base swainsonine, swainsonine *N*-oxide, **2** (Figure 1), has been reported to occur in several locoweed plants based on isolation and a qualitative TLC method.[4,5,26] Qualitative TLC estimates suggested that some locoweeds had similar concentrations of swainsonine and its *N*-oxide, whereas the *N*-oxide was not detected by gas chromatography in several *Swainsona* species tested.[23] Additionally, it has been shown that swainsonine *N*-oxide was as effective as swainsonine in inhibiting α-mannosidase.[4] To better define the relative contribution of swainsonine *N*-oxide to the hydroxylated indolizidine alkaloid content in plants, a method for the simultaneous detection of swainsonine and swainsonine *N*-oxide was developed and used to determine the relative concentrations of each in a variety of swainsonine-containing taxa.

MATERIALS AND METHODS

Materials. Swainsonine was obtained by extraction and purification from *Astragalus lentiginosus* as previously described.[27] Solvents used for HPLC were purified water using a Water Pro PS, purified to 18.2 ΩM (Labconco, Kansas City, MO, USA), and HPLC grade acetonitrile (Sigma-Aldrich, St. Louis, MO, USA). All other solvents and reagents were of analytical reagent grade. Ammoniated methanol used in the solid phase extraction procedure was prepared by dilution of 50 mL of stock ammonia-saturated methanol (stored at 4 °C) into 450 mL of additional methanol. NMR spectroscopic data were acquired using a Bruker

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Table 1. Concentrations of Swainsonine and Swainsonine *N*-Oxide in Vegetative Material from a Collection of Several Swainsonine-Containing Taxa

species	variety	location	swainsonine *N*-oxide (%)	swainsonine (%)
Astragalus lentiginosus	*araneosus*	Milford, UT, USA	0.02	0.13
	diphysus	St. Johns, AZ, USA	0.02	0.26
	lentiginosus	Juntura, OR, USA	0.01	0.19
	wahweapensis	Notom, UT, USA	0.01	0.17
Astragalus mollissimus	*earlei*	Alpine, TX, USA	0.02	0.33
	mollissimus	Farley, NM, USA	0.007	0.18
		Kenton, OK, USA	0.008	0.18
	thompsonae	Sanders, AZ, USA	0.0002	0.002
Astragalus pubentissimus		Green River, WY, USA	0.02	0.35
Astragalus wootoni		Fort Davis, TX, USA	0.02	0.38
Oxytropis sericea		Des Moines, NM, USA	0.003	0.06
		Park Valley, UT, USA	0.003	0.05
		Virgindale, CO, USA	0.007	0.09
Swainsona canescens[a]			0.02	0.11
Ipomoea carnea		Patos, Paraíba, Brazil	0.0008	0.05
		Marajó, Pará, Brazil	0.0009	0.1
Ipomoea riedelii		Zebele, Bahia, Brazil	0.008	0.21
Ipomoea sericophylla		Zebele, Bahia, Brazil	0.003	0.2
Ipomoea verbascoidea		Sertânia, Pernambuco, Brazil	0.0003	0.005
Turbina cordata		Juazeiro, Bahia, Brazil	0.0006	0.05

[a]The plant material was from greenhouse-grown plants derived from seeds of *S. canescens* collected in Western Australia.

Table 2. Concentrations of Swainsonine and Swainsonine *N*-Oxide in Seeds from a Collection of Several Swainsonine-Containing Taxa

species	variety	location	swainsonine *N*-oxide (%)	swainsonine (%)
Astragalus lentiginosus	*araneosus*	Milford, UT, USA	0.001	0.12
	diphysus	St. Johns, AZ, USA	nd	0.04
	wahweapensis	Notom, UT, USA	0.001	0.46
Astragalus mollissimus	*earlei*	Alpine, TX, USA	0.0007	0.41
	mollissimus	Farley, NM, USA	0.0007	0.13
		Kenton, OK, USA	0.01	0.59
	thompsonae	Sanders, AZ, USA	nd	nd
Astragalus pubentissimus		Green River, WY, USA	0.002	0.04
Astragalus wootoni		Fort Davis, TX, USA	0.0001	0.11
Oxytropis sericea		Des Moines, NM, USA	nd	0.009
		Park Valley, UT, USA	0.0005	0.04
Swainsona canescens		*a*	0.002	0.02
Ipomoea carnea		Patos, Paraíba, Brazil	nd	0.07
Ipomoea riedelii		Zebele, Bahia, Brazil	0.001	0.42
Ipomoea verbascoidea		Sertânia, Pernambuco, Brazil	0.0003	0.05
Turbina cordata		Juazeiro, Bahia, Brazil	nd	0.05

[a]Seeds of *S. canescens* were purchased from B&T World Seeds.

Avance III HD spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C) (Bruker Biospin, Billerica, MA, USA) using solutions in deuterium oxide (Sigma-Aldrich) (δ_H 4.70) and with acetonitrile (δ_C 1.47) as the chemical shift reference.

Plant and Endophyte Materials. Field samples, vegetative and floral material if available, of the following swainsonine-containing taxa were collected: *Astragalus lentiginosus* var. *araneosus*, *diphysus*, *lentiginosus*, and *wahweapensis*; *Astragalus mollissimus* var. *earlei*, *mollissimus*, and *thompsonae*; *Astragalus pubentissimus*; *Astragalus wootoni*; *Oxytropis sericea*; *Swainsona canescens*; *Ipomoea carnea*; *Ipomoea riedelii*; *Ipomoea sericophylla*; *Ipomoea verbascoidea*; and *Turbina cordata* (Table 1). Seeds were also collected of select taxa when available (Table 2). Plant material was dried and ground and subsequently analyzed for swainsonine and its *N*-oxide. Mycelia from fungal cultures grown on potato dextrose agar of the swainsonine-producing isolates of *Alternaria* section *Undifilum oxytropis* cultured from *O. sericea* and the Chaetothryiales isolate cultured from *I. carnea* were analyzed for swainsonine and its *N*-oxide.[10,15,17] All plant and fungal materials were sampled from known locations and populations with previously identified voucher specimens known to contain swainsonine.

Preparation of Swainsonine *N*-Oxide. Swainsonine (10 mg; 0.058 mmol) was dissolved in absolute ethanol (1 mL) in a 7 mL screw-cap vial. To this solution was added hydrogen peroxide (30%) (0.2 mL) and the vial sealed with a Teflon-lined cap. After 16 h at room temperature, the reaction was checked for completeness by flow injection esi(+)MS (see high-resolution MS instrumentation below). The presence of unreacted swainsonine (relative abundance = 7%) was evident by the observation of the protonated molecule (MH^+) at m/z 174, so the reaction mixture was heated to 60 °C for 4 h, after which the absence of an ion at m/z 174 in the esi(+)MS spectrum indicated completeness of the reaction. The reaction mixture was diluted with 1% acetic acid in water (1 mL) and applied to a strong cation exchange (SCX) solid phase extraction (SPE) column, Strata SCX (500 mg) (Phenomenex, Torrance, CA, USA) conditioned as per the

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manufacturer's guidelines. The loaded column was rinsed with water (10 mL) and methanol (10 mL) before the swainsonine *N*-oxide was eluted with ammoniated methanol (10 mL). The ammoniated methanol was evaporated under a flow of nitrogen at 60 °C to give swainsonine *N*-oxide (10.8 mg; 0.057 mmol; 98% yield): high-resolution esi(+)MS, *m*/*z* 190.10671 ($C_8H_{16}NO_4$ requires 190.10738); ^{1}H NMR (D_2O, 500 MHz; reference to HDO at δ 4.70) δ_H 4.53 (ddd, J = 8.5, 6.8, 2.2 Hz, H-2), 4.41 (dd, J = 6.7, 4.3 Hz, H-1), 4.28 (td, J = 10.5, 4.7 Hz, H-8), 3.74 (dd, J = 12.7, 8.2 Hz, H-3α), 3.49 (dd, J = 12.8, 2.1 Hz, H-3β), 3.32 (dd, J = 12.1, 4.2 Hz, H-5α), 3.08 (td, J = 12.8, 3.6 Hz, H-5β), 2.94 (dd, J = 10.1, 4.2 Hz, H-8a), 2.1 (m, H-7α), 2.02 (m, H-6β), 1.77 (br d, J = 14.8 Hz, H-6α), 1.34 (qd, J = 13.1, 4.6 Hz, H-7β); ^{13}C NMR (D_2O, 125 MHz) (spectrum referenced to added acetonitrile at δ 1.47) δ_C 77.0 (CH, C-8a), 76.2 (CH_2, C-3), 70.7 (CH, C-1), 69.2 (CH, C-2), 63.2 (CH_2, C-5), 63.1 (CH, C-8), 31.0 (CH_2, C-7), 19.4 (CH_2, C-6), consistent with prior literature.[4]

Extraction and Derivatization. Powdered dry plant material (100 mg) was treated, as previously described,[28] with 2% acetic acid (5.0 mL) and chloroform (4 mL) in a Teflon-lined screw-capped 15 mL centrifuge tube for 16 h by mechanical rotation. The samples were centrifuged, and the upper aqueous layer was removed to a screw-cap vial. An aliquot (100 μL) of this aqueous acid extract was placed into an 8 mL screw-cap vial, and ammoniated methanol (1.5 mL) was added to ensure no swainsonine salts were present; then the solvent was removed by evaporation under a flow of nitrogen at 60 °C. To the vial was then added dry pyridine (200 μL) and *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) silylation reagent (50 μL) (Supelco, Bellefonte, PA, USA), and the vial was capped and heated for 30 min at 60 °C. After heating, the samples were diluted with dimethylformamide (DMF) (1.0 mL), and the samples were heated again for 30 min at 60 °C. For HPLC-esi(+)MS analysis an aliquot (25 μL) of the solution was further diluted with DMF (975 μL) in an LC autosampler vial.

Caibration Standards. A swainsonine-negative matrix solution was prepared from alfalfa (100 mg) extracted with 2% acetic acid and chloroform as described above. The aqueous fraction was then used to prepare standard stock solutions of swainsonine (50 ppm) and swainsonine *N*-oxide (5 ppm). An aliquot (100 μL) of each of the standard stock solutions was mixed with ammoniated methanol (1.5 mL) in an 8 mL vial, evaporated to dryness under a flow of nitrogen at 60 °C, and derivatized as described above. An aliquot (100 μL) of the final DMF solution was then further diluted with DMF (1.9 mL) and serially diluted (1:1) to provide a series of standards at 200, 100, 50, 25, 12.5, 6.25, and 3.12 ng/mL for swainsonine and at 20, 10, 5, 2.5, 1.25, 0.625, and 0.312 ng/mL for swainsonine *N*-oxide.

Spike and Recovery. For extraction recovery analysis, samples of powdered, dry plant material (100 mg) were spiked with swainsonine solution (1.0 mg/mL) (100 μL) and swainsonine *N*-oxide solution (0.100 mg/mL) (100 μL). The samples (n = 4) were then extracted as outlined above and analyzed by HPLC-esi(+)MS.

HPLC-esi(+)MS Analysis. The HPLC column used was a 50 × 2.1 mm, 2.6 μm, Kinetex EVO C18 column (Phenomenex) used in a gradient elution mode with a mixture of acetonitrile (A) and 20 mM ammonium acetate (B) at a flow rate of 0.400 mL/min. The linear gradient was as follows: 50% A (0−1 min); 50−98% A (1−10 min); 98−50% A (10−11 min); 50% A (11−16 min), with a sample injection volume of 5 μL.

For high-resolution MS, a Thermo Scientific (Thermo Scientific, San Jose, CA, USA) Exactive Plus Orbitrap mass spectrometer was coupled with a Dionex Ultimate 3000 HPLC and autosampler and heated electrospray ion source. The MS instrument was calibrated via the manufacturer's standard procedures. Operational parameters included the sheath gas flow (35), auxiliary gas flow (10), spray voltage (4 kV), capillary temp (320 °C), S-lens Rf (35), and auxiliary heater temperature (300 °C). The mass spectrometer scanned a mass range of *m*/*z* 200−800 at a resolution of 35,000. Peak areas were measured from reconstructed ion chromatograms for protonated swainsonine-$(TMS)_3$ (*m*/*z* 390.22813) and protonated swainsonine-$(TMS)_3$ *N*-oxide (*m*/*z* 406.22308) with a mass range of ±10 ppm.

For unit mass resolution and MS/MS data a Thermo Scientific Velos Pro LTQ mass spectrometer was coupled to a model 1260 Agilent HPLC and autosampler (Agilent Instruments, Santa Clara, CA, USA) and heated electrospray ion source. The MS instrument was calibrated via the manufacturer's standard procedures. Operational parameters included the sheath gas flow (40), auxiliary gas flow (5), spray voltage (3.5 kV), capillary temp (275 °C), and auxiliary heater temperature (300 °C). The mass spectrometer scanned a mass range of *m*/*z* 200−800.

RESULTS AND DISCUSSION

Preparation of Swainsonine *N*-Oxide. Swainsonine *N*-oxide was prepared by oxidation of swainsonine with hydrogen peroxide. The complete ^{1}H NMR data have not been previously reported, but with regard to the similarity to the free base, significant downfield shift of H-3, H-5, and H-8a protons was noted.[4,29]

Analytical Method Development for Swainsonine and Swainsonine *N*-Oxide. The only method that previously reported the detection of swainsonine *N*-oxide in plant material was a qualitative TLC method used to screen some of the reported locoweed plant materials at the time.[5] A method based on GC-FID, using a 2 m × 2 mm glass column, 3% OV-1 on Chromosorb W, was reportedly used for swainsonine *N*-oxide detection (but failed to detect any *N*-oxide in any *Swainsona* species);[23] however, because of the highly polar nature of swainsonine *N*-oxide, even derivatized, it is not expected to be a good candidate for modern capillary GC type analysis. Thus, despite observing the TMS derivative of swainsonine, the TMS derivative of swainsonine *N*-oxide was not observed using capillary GC conditions, J&W DB-5 MS, 30 m × 0.25 mm, and MS detector. As for HPLC type analyses, swainsonine *N*-oxide is not retained under reversed phase liquid chromatography conditions.[30] Attempts to replicate a reported hydrophilic interaction liquid chromatography (HILIC) method for swainsonine[30] were unsuccessful. Upon the investigation of several other HPLC columns, a 100 mm × 2 mm, 5 μm, Porous Graphitic Carbon (Thermo Scientific) produced the best retention and separation, but the peak shape was less than ideal (Figure 2) for swainsonine *N*-oxide.

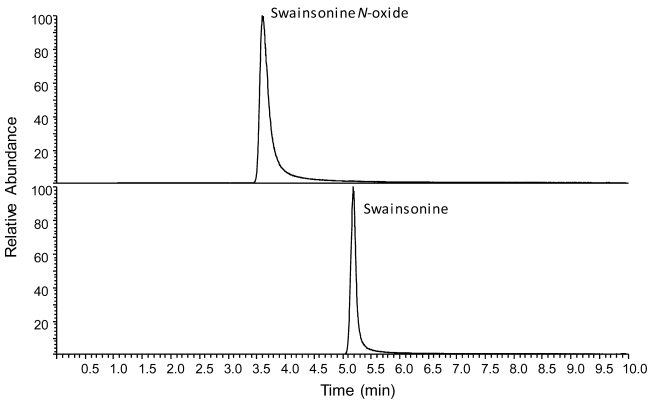

Figure 2. HPLC-esi(+)MS chromatograms for swainsonine and swainsonine *N*-oxide (nonderivatized compounds) separated on a Hypercarb column.

Analyte derivatization is often useful to improve chromatographic properties. The phenyl boronate derivative of swainsonine and its *N*-oxide, conjugating with the *cis*-diol groups,[27] were not stable in aqueous solutions within the chromatographic analysis time. The trimethylsilyl derivatives of swainsonine have been previously used in GC analysis, but the use of TMS derivatives in reversed phase HPLC is very limited

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due to the instability of the derivatives in the presence of active hydrogens and thus aqueous environments. However, the trimethylsilyl derivatives of swainsonine and its *N*-oxide displayed good chromatographic retention and separation as well as good esi(+)MS detector response using reversed phase HPLC-MS column and conditions. The relatively short Kinetex EVO C18 base column gave good separation of swainsonine and its *N*-oxide tri-TMS derivatives, with a reasonably rapid elution time of <6 min (Figure 3).

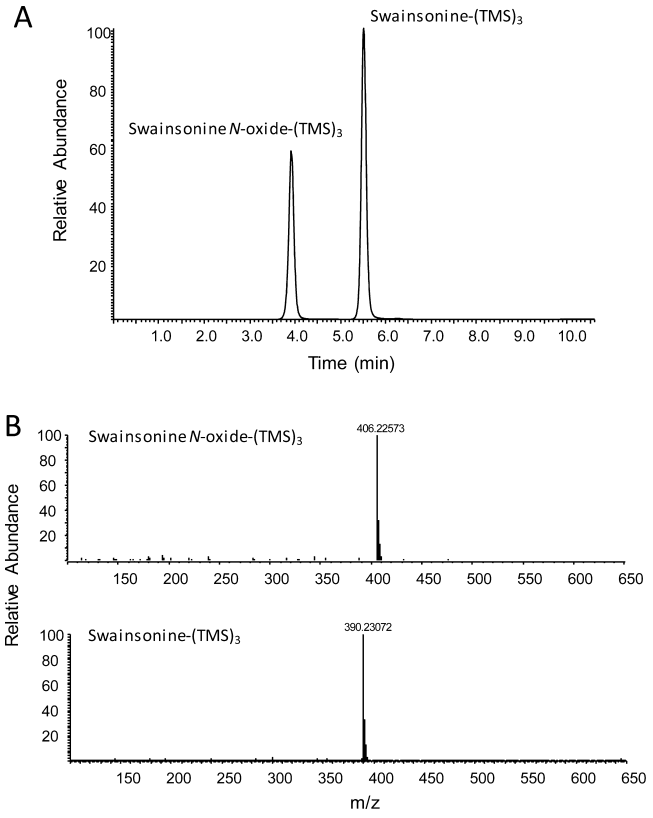

Figure 3. (A) HPLC-esi(+)HRMS reconstructed ion chromatogram for the trimethylsilyl (TMS) derivatives of swainsonine (MH^+ *m/z* 390) and swainsonine *N*-oxide (MH^+ *m/z* 406); (B) corresponding HRMS spectra.

Some initial problems with the stability of the trimethylsilyl derivatives were observed. The derivatized compounds were stable within a single chromatographic run but significant loss of signal was observed within 2 h if samples were prepared in solvents containing any water or protic solvents. The aprotic solvent dimethylformamide was used for sample dilution and the derivatized samples were stable for at least 16 h (Figure 4) which is sufficient for running a number of samples in an overnight analysis.

Consequently, the quantitative analysis of the trimethylsilylated derivatives of swainsonine and its *N*-oxide was achieved using reconstructed ion chromatograms, displaying the respective protonated molecules with a mass tolerance of ±10 ppm, from the high-resolution mass spectrum (Figure 3). For comparative purposes of different MS detection modes, the esi(+)MS/MS spectrum of swainsonine-$(TMS)_3$, under CID conditions, was dominated by loss of one *O*-silyl ether group (*m/z* 300; $MH^+ - 90$) (Figure 5A). The *N*-oxide likewise gave a major ion at *m/z* 316 with the loss of the *O*-silyl ether along with possible loss of $HOCH_3$ at *m/z* 374. Smaller fragment ions (*m/z*

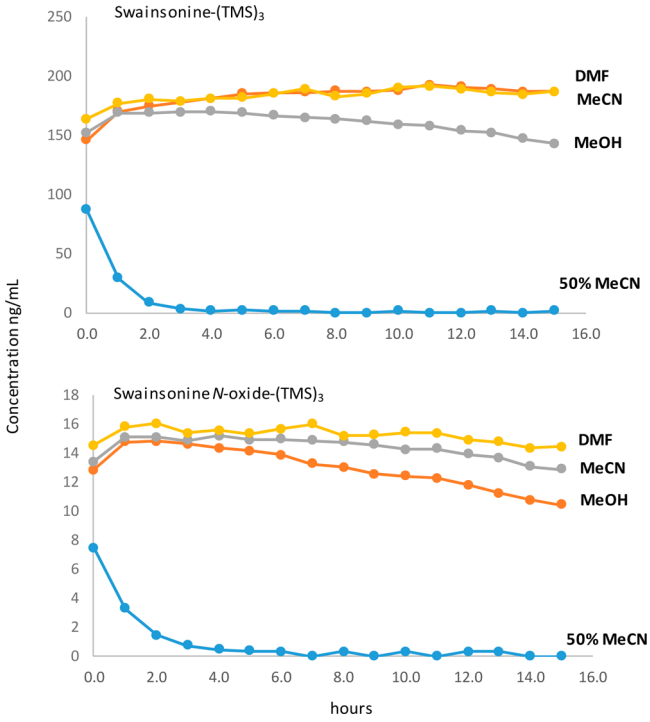

Figure 4. Measurement of swainsonine-$(TMS)_3$ and swainsonine-$(TMS)_3$ *N*-oxide stability in four different analytical solutions: acetonitrile (MeCN), dimethylformamide (DMF), methanol (MeOH), and 50% acetonitrile in water (50% MeCN). Repeat measurements were made over a 16 h period.

96, 114, 120, 143, 184, 210) were more pronounced under higher energy collision-induced dissociation (HCD) conditions (Figure 5B).

The limit of detection (LOD) was found to be similar between using the reconstructed ion chromatograms from the high-resolution MS and the MS/MS ion chromatograms (data not reported). Under the reported extraction protocol the LODs in dry plant material were estimated to be 0.13 μg of swainsonine/100 mg and 0.03 μg of swainsonine *N*-oxide/100 mg (Table 3). The resulting analytical method was found to be repeatable (RSD = 2.6−7.8%), and recovery was acceptable (recovery = 109−122%).

Swainsonine and Swainsonine *N*-Oxide in Various Plant Samples. Swainsonine and its *N*-oxide were detected in all vegetative plant material of the Fabaceae and Convolvulaceae taxa surveyed. Concentrations of swainsonine measured ranged from 0.002% (dw, dry weight basis) in *A. mollissimus* var. *thompsonae* to 0.38% in *A. wootoni*, consistent with previous reports for these respective taxa.[30] Concentrations of swainsonine were consistent with reports in regard to other taxa surveyed.[8,13,30] Concentrations of swainsonine *N*-oxide ranged from 0.0002 to 0.02% (dw) with a swainsonine *N*-oxide to swainsonine ratio of 0.9−18% among the taxa surveyed. The ratio of swainsonine *N*-oxide to swainsonine was greatest in *A. lentiginosus* var. *araneosus* and *S. canescens*, whereas they were generally smallest in the *Ipomoea* and *Turbina* spp. surveyed. In general, taxa with greater swainsonine concentrations had greater concentrations of swainsonine *N*-oxide. Swainsonine *N*-oxide was detected in *Astragalus* and *Oxytropis* spp., consistent with that previously reported;[5,26] however, the semiquantitive estimates of swainsonine and swainsonine *N*-oxide reported previously differed from those reported here. Swainsonine *N*-

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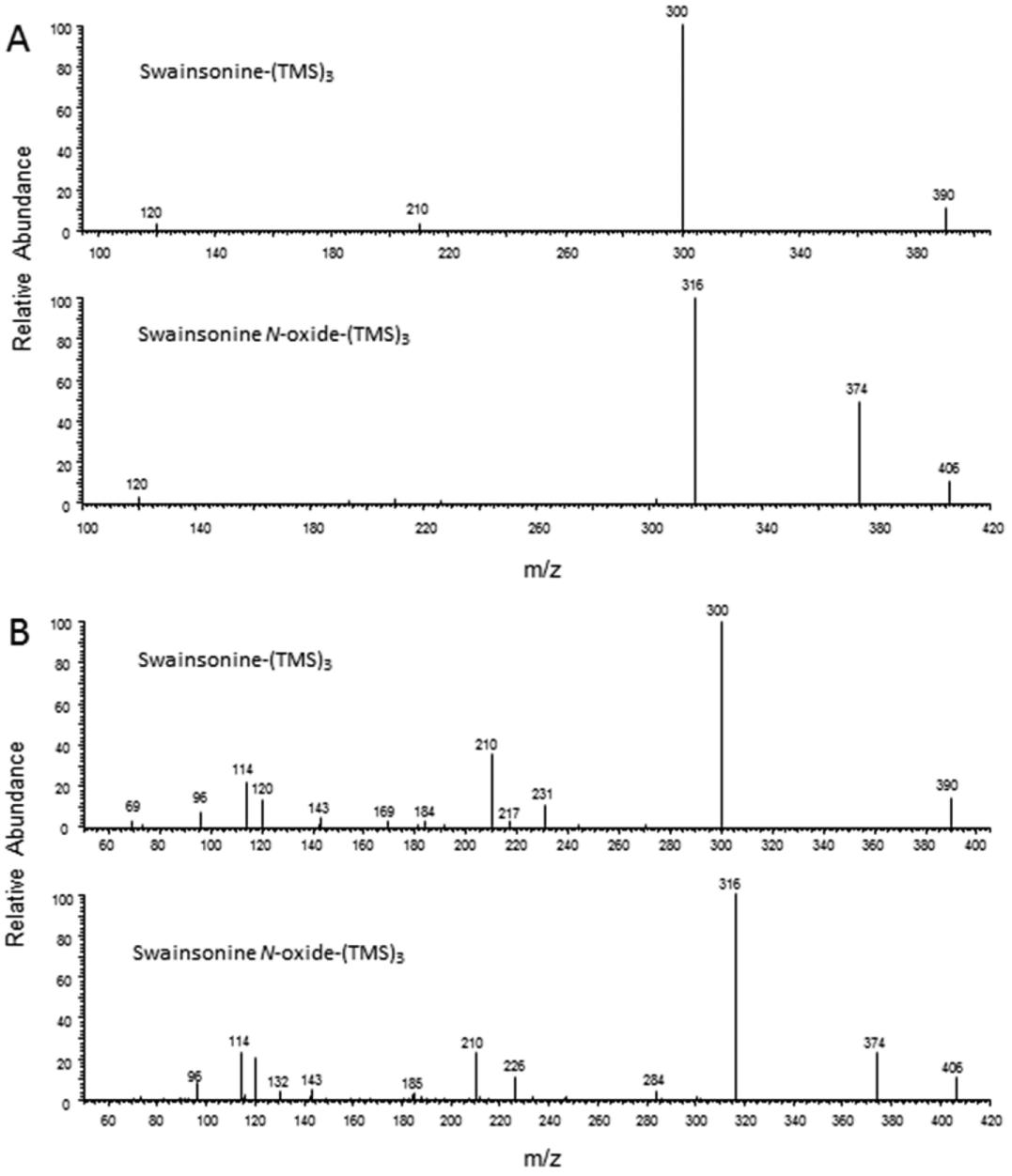

Figure 5. HPLC-esi(+)MS/MS spectra of swainsonine-$(TMS)_3$ and swainsonine-$(TMS)_3$ *N*-oxide under (A) CID- and (B) HCD-induced fragmentation.

Table 3. Measurements of Recovery and Limits of Detection (LOD) for Swainsonine and Swainsonine *N*-Oxide in Dry Plant Material

analyte	unfortified, $n = 4$ (μg/100 mg)	fortified, $n = 4$ (μg/100 mg)	spike amount (μg)	% recovery[a]	LOD,[b] ng/mL (μg/100 mg)
swainsonine	105.4 ± 5.0	214.2 ± 5.6	100	109	0.05 (0.13)
RSD (%)	4.8	2.6			
swainsonine *N*-oxide	5.88 ± 4.45	18.13 ± 0.48	10	122	0.01 (0.03)
RSD (%)	7.8	2.7			

[a]Recovery (%) = $[(C_F - C_U)/C_A] \times 100$, where C_F = concentration of analyte measured in fortified sample, C_U = concentration of analyte measure in unfortified sample, and C_A = concentration of analyte added. [b]LOD = ng/mL of analyte injected on-column and then converted to concentration (μg/100 mg) in original sample material given the dilution factors of the extraction procedure.

oxide was detected in *S. canescens*, in contrast to a previous study in which no swainsonine *N*-oxide was detected in *S. canescens*.[23] We suspect that differences between the data reported here and in previous studies is likely due to improved analytical instrumentation.

Swainsonine was detected in all seed samples (0.009−0.59%, dw) with the exception of *A. mollissimus* var. *thompsonae* sample, whereas swainsonine *N*-oxide ranged from "not detected" to 0.002%. The ratio of swainsonine *N*-oxide to swainsonine was 0.09−0.1% among the taxa with detectable amounts. In general,

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there was less swainsonine *N*-oxide detected in seed samples, resulting in a lower ratio of swainsonine *N*-oxide to the free base swainsonine compared to the samples of vegetative material from the same taxon.

Swainsonine and its *N*-oxide were detected in *Undifilum oxytropis* cultured from *O. sericea* and the Chaetothryiales isolate cultured from *I. carnea* in ratios similar to those found in plant material of the respective host taxa (data not shown). These data would suggest that swainsonine and its *N*-oxide are produced by the endophyte associated with the respective plant hosts and that the amounts found in planta are reflective of the concentrations produced by the respective endophytes. Endophytes are associated with seed material of each respective species and are the means of transmission to new plant material. We have no known explanation as to the apparent lower ratios of swainsonine *N*-oxide to swainsonine that were measured in seed material versus plant material other than seed samples in some cases were not necessarily sampled directly from their associated vegetative material (i.e., seed and leaf samples from the same plant) or further metabolism and/or possible selective transportation of swainsonine to the reproductive parts of the plant.

In summary, a method was developed to detect swainsonine and its *N*-oxide. The relative concentrations of each were determined in several swainsonine-containing taxa as well as two endophytic isolates that produce swainsonine. All swainsonine-containing taxa analyzed in the Fabaceae and Convolvulaceae contained swainsonine *N*-oxide. Among the samples analyzed, the concentrations of swainsonine *N*-oxide relative to swainsonine were sufficiently low that the addition of the *N*-oxide compound would only slightly increase the overall effective toxicity of the various plant samples. Therefore, the continued practice of plant sample analyses in which only swainsonine is the target analyte is sufficient to determine potential toxicity of plants.

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Notes

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