Toxicity and Chemical Phenology of Norditerpenoid Alkaloids in the Tall Larkspurs (*Delphinium* Species)

Gary D. Manners*

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710

Kip E. Panter, Michael H. Ralphs, James A. Pfister, John D. Olsen, and Lynn F. James

Poisonous Plant Research Laboratory, Agriculture Research Service, U.S. Department of Agriculture, 1150 East 1400 North, Logan, Utah 84321

Eleven norditerpenoid alkaloids found in three tall larkspur species (*Delphinium barbeyi*, *Delphinium occidentale*, *Delphinium glaucescens*) were evaluated as mammalian toxins in a mouse bioassay. Two methylsuccinimido anthranoyllycoctonine (MSAL) norditerpenoid alkaloids (methyllycaconitine and 14-deacetylnudicauline) were determined to have equivalent high toxicity. A third MSAL norditerpenoid, barbinine, was 5 times less toxic. Other norditerpenoid alkaloids tested were found to be significantly less toxic than the MSAL alkaloids. The chemical phenology of the alkaloids in the three larkspurs and the effect of collection site on alkaloid occurrence in *D. barbeyi* were determined.

INTRODUCTION

The larkspurs (*Delphinium* sp.) constitute one of the largest poisonous plant threats to rangeland livestock grazing mountain ranges of western North America (Cronin and Nielsen, 1978). Livestock deaths attributable to these plants are valued in the millions of dollars each year. Two general categories of larkspurs have been recognized—low larkspurs and tall larkspurs. The low larkspurs occur in drier, lower elevation rangelands, while the tall larkspurs are more prevalent in deep, moist soils of the high mountain rangelands. The tall larkspurs are considered to be a greater threat to livestock than the low larkspurs because of higher concentrations of toxic alkaloids (Beath, 1925). The tall larkspurs display their highest concentration of alkaloids during the early vegetative stages of growth; the alkaloid levels diminish through the growing season. The low larkspurs display a relatively constant level of alkaloids throughout their shorter growing season (Williams and Cronin, 1966).

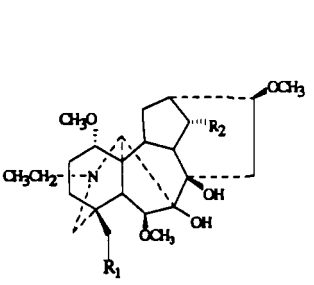
The presence of more than 40 norditerpenoid alkaloids has been reported in species of western tall and low larkspurs. One of these alkaloids, methyllycaconitine, has been established as a potent neuromuscular blocking agent in animals, including cattle (Benn and Jacyno, 1983; Nation et al., 1982), and has been linked to cattle deaths caused by the low larkspurs *Delphinium bicolor* (Kulanthaivel et al., 1986) and *Delphinium nuttallianum* (Majak and Engelsjord, 1988) and the tall larkspur *Delphinium brownii* (Aiyar et al., 1979). This compound has also been isolated from extracts of another low larkspur, *Delphinium andersonii* (Pelletier and Kulanthaivel, 1989). Methyllycaconitine displays an acute mammalian toxicity of 3.0–3.5 mg/kg (iv) in mice (Dozortseva, 1959; Benn and Jacyno, 1983) and has been estimated to have an LD_{50} of 5–6.3 mg/kg in cattle (Olsen et al., 1990) when administered intraruminally. Nudicauline, an alkaloid structurally similar to methyllycaconitine, is an additional suspected toxin in the low larkspur *D. nuttallianum* (Majak and Engelsjord, 1988); however, this alkaloid has not been toxicologically evaluated.

Additional evidence for the mode of action of methyllycaconitine is described in a recent study which reveals a higher competitive binding potency of methyllycaconitine to rat brain neuronal nicotinic acetylcholine receptors (nAChR) than to vertebrate muscle nAChR (Ward et al., 1990). The difference in sensitivity was attributed to unique structural features of methyllycaconitine (Ward et al., 1990; Aiyar et al., 1979). The demonstrated insecticidal properties of this toxic alkaloid have also been attributed to its potent competitive (nAChR) binding character (Jennings et al., 1986).

Distinct site and elevation effects on alkaloid level have been observed for the low larkspur *D. nuttallianum* on four sites (Majak and Engelsjord, 1988). Amounts of methyllycaconitine were found to be higher in reproductive vs vegetative tissues in this low larkspur. We reported (Manners et al., 1992) the phenological distribution of "total alkaloids" in the three tall larkspur species and the occurrence of three nontoxic norditerpenoid alkaloids in *D. barbeyi*. Highest total alkaloid yields were observed during the early growth stages and in the reproductive tissues of the plant. The levels of the nontoxic alkaloids were generally found to decrease with plant maturation.

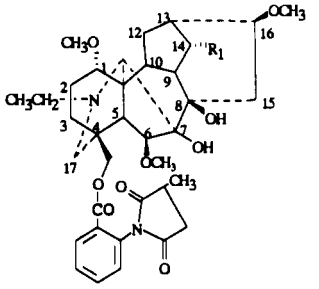
Previous chemical examinations of the three tall larkspurs [*D. barbeyi* (Pelletier et al., 1989), *D. glaucescens* (Pelletier et al., 1981), *D. occidentale* (Kulanthaivel and Pelletier, 1988)] detected the presence of methyllycaconitine in *D. barbeyi* and *D. glaucescens* but not in *D. occidentale*. Rat bioassays of extracts from early bud stage plant material from these three larkspurs have shown *D. barbeyi* to be 4 times more toxic than *D. glaucescens* and 10 times more toxic than *D. occidentale* (Olsen, 1977). *D. barbeyi* is considered the most toxic of all larkspurs to livestock (Cronin and Nielsen, 1978).

Less than 10% of the more than 200 norditerpenoids identified in all species of *Delphinium* examined chemically have been toxicologically evaluated (Benn and Jacyno, 1983), and toxicological data are available for only 6 of the 27 norditerpenoid alkaloids reported to occur in the tall larkspurs (Olsen and Manners, 1989). Using a mouse bioassay, we now report toxicity data for 11 norditerpenoid alkaloids. No toxicological data have previously been reported for six of these alkaloids, which include two structural analogs of methyllycaconitine. We



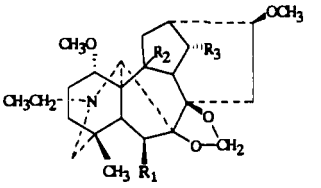
Lycoctonine Norditerpenoid Alkaloid

	R_1	R_2
Lycoctonine	OH	OMe
14-Dehydrobrowniine	OMe	=O
Browniine	OMe	OH
Anthranoyllycoctonine		OMe



Methylsuccinimidoanthranoyllycoctonine (MSAL) Norditerpenoid Alkaloid

	R_1
Methyllycaconitine	OMe
14-Deacetylnudicauline	OH
Barbinine	=O
Nudicauline	OAc



7,8-Methylenedioxylycoctonine (MDL) Norditerpenoid Alkaloid

	R_1	R_2	R_3
Deltaline	OAc	OH	OMe
Deltamine	OH	OH	Ome
6-Dehydrodeltamine	=O	OH	OMe
Dictyocarpine	OAc	OH	OH
14-OAcDictyocarpine	OAc	OH	OAc
Delpheline	H	H	H

Figure 1. Norditerpenoid alkaloids occurring in the tall larkspurs.

also report the chemical phenology of these alkaloids and two additional alkaloids obtained in amounts insufficient for toxicity testing in three tall larkspur species and the effect of site differences on the chemical phenology of these alkaloids in *D. barbeyi*.

EXPERIMENTAL PROCEDURES

Isolation of Alkaloids. Thirteen norditerpenoid alkaloids were obtained from an 80% ethyl alcohol extract of air-dried ground *D. barbeyi* plant material. Crude alkaloid fractions were obtained from the plant material according to a modified extraction procedure (Manners et al., 1992) originally described by Pelletier (1981). The concentrated alcohol extract (dissolved in chloroform) was extracted with 10% HCl, and the resulting aqueous acid (alkaloid containing) extract was basified (pH 8) with 20% aqueous NaOH. The resulting aqueous basic extract was extracted with ether and subsequently with chloroform to obtain two crude alkaloid enriched extracts.

Individual alkaloids were obtained from the ether and chloroform extracts by chromatographic techniques similar to those previously described (Pelletier et al., 1985, 1989) and were authenticated by comparison of spectral data to published data (Pelletier et al., 1984; Pelletier and Joshi, 1991) and by chromatographic comparisons with authentic specimens kindly supplied by Dr. Pelletier. The 7,8-methylenedioxylycoctonine (MDL) norditerpenoid alkaloids (deltaline, dictyocarpine, deltamine, 14-*O*-acetyldictyocarpine, 6-dehydrodeltamine, delpheline) and the lycoctonine norditerpenoid alkaloids (lycoctonine, browniine, 14-dehydrobrowniine) were obtained from the ether extract; the methylsuccinimido anthranoyllycoctonine (MSAL) norditerpenoid alkaloids (methyllycaconitine, 14-deacetylnudicauline, barbinine) and anthranoyllycoctonine were isolated from the chloroform extract. Two alkaloids (delpheline, 6-dehydrodeltamine) were obtained in yields too low to permit toxicological evaluation.

For toxicological evaluation, 11 of the 13 individual alkaloids were purified by chromatographic methods and recrystallized, if possible. Purity of the individual alkaloids was established by gas chromatographic (GC) or high-performance liquid chromatographic analytical methods (HPLC) and comparison of NMR spectra with spectra of standard compounds or published spectra. On the basis of these criteria, the 11 norditerpenoid alkaloids were considered pure. Chemical structures of the alkaloids are presented in Figure 1.

Chemical Phenology Analysis. Plant samples were collected in conjunction with a study of larkspur grazing by sheep (Ralphs et al., 1991) during vegetative, bud, and flowering stages for the three species at five different sites [(*D. barbeyi* (Ferron, Salina, and Cedar City, UT), *D. occidentale* (Oakley, ID), *D. glaucescens* (Ruby River Valley, MT)]. The collection sites ranged in elevation from 2200 to 3200 m. Specific locations and habitat descriptions of the plant collection sites are described in the sheep grazing study. A composite sample of larkspur leaf and head (bud, flower, pod) plant material was collected from 15–20 plants found within multiple transects established at each site for the sheep grazing study.

The chemical phenology of the 13 alkaloids present in plant tissues of the three tall larkspurs was determined by GC and HPLC analysis of crude alkaloid fractions obtained by Soxhlet extraction of 1-g air-dried plant samples. The lycoctonine and MDL norditerpenoid alkaloids present in the ether extracts were effectively quantified by GC (Manners and Ralphs, 1989), while the MSAL norditerpenoid alkaloids and anthranoyllycoctonine present in chlorform extracts were analyzed by a newly developed normal-phase HPLC analytical method (Manners and Pfister, 1993). The analysis results are reported as a percent of dry plant material weight Table II.

Alkaloid Toxicity Testing in Mice. Individual alkaloids from larkspur were suspended in physiological buffered saline, and the pH was lowered with 40% HCl to achieve solubility. The MSAL norditerpenoid alkaloids were solubilized over a pH range of 4.0–4.5, while the MDL norditerpenoid alkaloids dissolved over the pH range 5.4–6.6. The difference in the solubilization characteristics of the two groups of alkaloids reflects their difference in basicity. The solutions were stored in injection vials for toxicity testing.

Swiss Webster white male mice (30–40 g) were weighed after a 24-h fast and injected subcutaneously (sc) or intravenously (iv). Time of injection, clinical effects, and time of death were noted and recorded. The number of mice per group was variable depending on the amount of compound available. An LD_{50} was calculated using the probit method (Miller and Tainter, 1944) for alkaloids available in sufficient quantities to test adequate numbers of mice (methyllycaconitine, 14-deacetylnudicauline, deltaline). No LD_{50}s were calculated on the other eight alkaloids, but ranges or individual data were recorded (Table I).

RESULTS AND DISCUSSION

Toxicological Evaluation of Norditerpenoid Alkaloids. Toxicological data for 11 norditerpenoid alkaloids obtained from tall larkspur plant extracts are presented in Table I. The MDL norditerpenoid alkaloids (deltaline, deltamine, dictyocarpine, 14-*O*-acetyldictyocarpine) evaluated in the mouse bioassay demonstrated very low toxicity. For example, the estimated LD_{50} of dictyocarpine in mice approached 2000 mg/kg. The acute toxicity levels of the other MDL norditerpenoid alkaloids is in excess of 700 mg/kg. The lycoctonine norditerpenoid alkaloids (lycoctonine, anthranoyllycoctonine, browniine, 14-dehydrobrowniine) were found to be about twice as toxic as the 7,8-methylenedioxy norditerpenoid alkaloids; however,

Table I. Toxicological Evaluation of Norditerpenoid Alkaloids from Tall Larkspurs

compound	no. of mice	LD_{50},[b] mg/kg	clinical signs of toxicity[c]
methyllycaconitine	31 sc	31.8 (7.5 iv)	4
	16 iv		
14-deacetylnudicauline	30	32.0	4
barbinine[a]	4	175–200	4
anthranoyllycoctonine[a]	2	107.6–365.8	102.6 mg/kg = 1 at 1
			365.8 mg/kg = 1 at 4
lycoctonine[a]	27	>392	3
browniine[a]	2	>720	0
14-dehydrobrowniine[a]	2	>254	0
deltaline	24 sc	720 (132.5 iv)	4
	12 iv		
deltamine[a]	2	>777	2
dictyocarpine[a]	27	1584–1914	1584 mg/kg = 33% at 4
			1914 mg/kg = 50% at 4
14-*O*-acetyldictyocarpine[a]	12	>765	765 mg/kg = 33% at 4

[a] Not enough material to calculate LD_{50}; the LD_{50} is estimated or a range reported with clinical observations. [b] Injections were sc unless specified iv. [c] Clinical signs: 0–4; 0 = no effect; 1 = slight, signs include mild nervousness, elevated respiration; 2 = moderate, signs include 1 progressing to ataxia temporary sternal recumbancy; 3 = severe, signs include 1 and 2 progressing to sternal or lateral recumbancy for 5–10 min; 4 = death after progressing through 1, 2, and 3. Time of death varies depending on dosage and method of injection, i.e., sc vs iv.

this level of toxicity is still considered to be relatively low. The least toxic lycoctonine alkaloid, browniine, has a toxicity level comparable to that of the MDL norditerpenoid alkaloids.

The MSAL norditerpenoid alkaloids methyllycaconitine and 14-deacetylnudicauline exhibit acute toxic effects at least 10 times greater than any other norditerpenoid alkaloids administered to mice. The acute toxicity levels of methyllycaconitine and anthranoyllycoctonine were 31.8 and 365 mg/kg sc, respectively, in this study. This compares to an iv toxicity of 3.0–3.5 mg/kg for methyllycaconitine (Dozortseva, 1959; Benn and Jacyno, 1983) and 20 mg/kg (estimated) for anthranoyllycoctonine (Olsen and Manners, 1989). Subcutaneous administration of these alkaloids appears to result in a 7–10 fold lowering in sensitivity as contrasted with iv injection. Our measurement of iv toxicity for methyllycaconitine (7.5 mg/kg) was found to be more than twice those reported by Dozortseva (1959) and Benn and Jacyno (1983). The difference may be accounted for by differences in the chemical character of administered methyllycaconitine [(methyllycaconitine hydroiodide "mellictine" vs methyllycaconitine hydrochloride (this study)], animal size, number of animals, animal source, individual animal susceptibility, mouse strain, and injection protocol.

The toxic equivalency of methyllycaconitine and 14-deacetylnudicauline, their significantly higher toxicity compared to that of anthranoyllycoctonine, and the reported nicotinic impotency of lycoctonine (Ward et al., 1990) are consistent with the important participation of the methylsuccinimido group attached to the anthranilic acid ester moiety of the MSAL alkaloids in the toxic mechanism of these alkaloids in mammalian systems. Modeling studies on the competitive binding of methyllycaconitine, acetylcholine, and cytisine to rat brain and vertebrate muscle nAChR led Ward et al. (1990) to suggest that the ester carbonyl of the methylsuccinimido anthranilic acid ester group and the nitrogen of the lycoctonine moiety of methyllycaconitine were necessary for specific binding at the nicotinic receptor site. However, the lower toxicity of anthranoyllycoctonine (Table I) indicates that the ester carbonyl and the lycoctonine nitrogen alone do not impart toxicity to a norditerpenoid alkaloid but rather that the toxicity of these compounds results from a combination of specific structural features. The reduced toxicity of barbinine vs methyllycaconitine and 14-deacetylnudicauline and the lowered toxicity of anthranoyllycoctonine vs methyllycaconitine demonstrate the importance of the electronic or stereochemical factors associated with substituents at C-14, the presence of the methylsuccinimido group, and probably the ester carbonyl and lycoctonine nitrogen to the toxicity of the norditerpenoid alkaloids. The low toxicity observed for barbinine clearly establishes that not all MSAL norditerpenoid alkaloids are equally toxic in mice.

Chemical Phenology of Norditerpenoid Alkaloids. The chemical phenology of norditerpenoid alkaloids in the three tall larkspurs *D. barbeyi* (three sites), *D. glaucescens*, and *D. occidentale* shown in Table II describes significant inter- and intraspecies variations. The data illustrate the general decline in the amount of norditerpenoid alkaloids in the plant tissues of all three species throughout the growing season. In *D. occidentale*, for example, the amount of deltaline declined by 60% in the leaves between the vegetative and the flowering stages. Similar reductions occurred in many of the other MDL norditerpenoid alkaloids in all three species. Changes in the amounts of the lycoctonine norditerpenoid alkaloids during the growing season were less pronounced. Methyllycaconitine occurred in highest amount in the vegetative tissues of the larkspurs during the early stages of plant development. The amount of this toxic alkaloid subsequently decreased during the growing season with higher amounts of this alkaloid detected in the buds and flowers of the plants than in leaf material in their intermediate growth stages. This reduction in amount is in contrast to the fairly constant yield of methyllycaconitine reported in the low larkspurs (Majak and Engelsjord, 1988). Barbinine and 14-deacetylnudicauline occur in low yields in *D. barbeyi*, and their occurrence cannot be consistently related to the occurrence of methyllycaconitine in the samples of this study. The presence of these two MSAL norditerpenoid alkaloids in *D. occidentale* and *D. glaucescens* has not been established. The identification of methyllycaconitine in *D. occidentale* constitutes the first report of this compound in this tall larkspur.

Clear differences in the amounts of methyllycaconitine present in the three tall larkspur species were observed. At all stages of growth, *D. occidentale* produced significantly less methyllycaconitine than *D. barbeyi*. *D. glaucescens* and *D. occidentale* had similar amounts of methyllycaconitine in their leaves, but the bud and flower tissues of *D. glaucencens* had about 10 times more methyllycaconitine than the same tissue of *D. occidentale*. These data are in accord with reports that *D. occidentale* is 2.5 times less toxic than *D. glaucescens* and 10 times less toxic than *D. barbeyi* (Olsen, 1977).

The comparative chemical phenology of the norditerpenoid alkaloids among *D. barbeyi* samples from three different sites indicate a significant influence of environmental and climatic conditions on alkaloid level. While seasonal trends in alkaloid levels were comparable among the sites, the amounts of alkaloid present in the plant tissue at a particular growth stage varied considerably between the sites. At all three sites, methyllycaconitine was found to decline in both vegetative and reproductive

Table II. Distribution of Norditerpenoid Alkaloids in Three Species of Tall Larkspurs

				norditerpenoid alkaloids[a,b]											
				MSAL type[e,f]			MDL type[e,g]							lycoctonine type[e,g]		
species[c]	site[d]	part	stage	MLYC	14-DAN	Barb	Anth	Delph	DAM	Delt	6-DHD	14-OAD	Dicty	Lycoc	Brown	14-DHB
DB	Ferr	stem	veg	0.12			0.05	0.01	0.03	0.96	0.02	0.16	0.24	0.60	0.13	0.02
DB	Ferr	leaf	veg	0.21	0.03		0.12	0.01	0.04	1.17	0.04	0.39	0.34	0.26	0.21	0.04
DB	Ferr	leaf	bud	0.02			0.05	0.01	0.02	0.58	0.01	0.03	0.14	0.09	0.03	0.04
DB	Ferr	leaf	flwr	0.03			0.01		0.02	0.46		0.07	0.08	0.06	0.01	0.01
DB	Ferr	bud	bud	0.04			0.01		0.01	0.18		0.01	0.08	0.17	0.04	0.02
DB	Ferr	flwr	flwr	0.02			0.02			0.19		0.02	0.04	0.05	0.02	0.01
DB	Sal	leaf	bud	0.07			0.03			0.13		0.05	0.06	0.20	0.27	0.01
DB	Sal	leaf	flwr	0.04			0.02	0.03	0.01	0.15		0.02	0.01	0.15	0.12	0.03
DB	Sal	bud	bud	0.08			0.05			0.09		0.03	0.03	0.25	0.40	0.03
DB	Sal	flwr	flwr	0.04	0.01		0.01			0.06		0.02	0.01	0.26	0.13	0.04
DB	Ced	leaf	bud	0.08	0.03	0.01	0.03				0.01	0.01	0.11	0.18	0.28	0.01
DB	Ced	leaf	flwr	0.09			0.03					0.01	0.09	0.19	0.36	0.01
DB	Ced	bud	bud	0.19			0.03					0.01	0.10	0.10	0.33	0.01
DB	Ced	flwr	flwr	0.01			0.03						0.05	0.15	0.18	0.01
DG	Ruby	leaf	bud	0.01			0.03						0.13	0.10	0.18	0.02
DG	Ruby	leaf	flwr	0.01			0.01						0.03	0.07	0.05	0.01
DG	Ruby	bud	bud	0.11			0.04					0.01	0.26	0.28	0.44	0.02
DG	Ruby	flwr	flwr	0.10			0.01			0.03			0.15	0.21	0.17	0.01
DO	Oak.	leaf	veg	10.02			0.07	0.02	0.05	1.20	0.02	0.02	0.25	0.29	0.26	0.02
DO	Oak.	leaf	bud	10.02			0.06		0.01	0.51	0.01	0.02	0.14	0.14	0.13	0.02
DO	Oak	leaf	flwr				0.08			0.47			0.05	0.07	0.08	0.01
DO	Oak.	bud	bud	0.01			0.04		0.01	0.27	0.01	0.01	0.22	0.25	0.13	0.01
DO	Oak.	flwr	flwr	0.01			0.04	0.01		0.32	0.01		0.12	0.21	0.07	0.01

[a] Perent of dry weight. [b] MLYC, methyllycaconitine; 14-DAN, 14-deacetylnudicauline; Barb, barbinine; Anth, anthranoyllycoctonine; Delph, delpheline; DAM, deltamine; Delt, deltaline; 6-DHD, 6-dehydrodeltamine; 14-OAD, 14-*O*-acetyldictyocarpine; Dicty, dictyocarpine; Lycoc, lycoctonine; Brown, browniine; 14-DHB, 14-dehydrobrowniine. [c] DB, *D. barbeyi*; DO, *D. occidentale*; DG, *D. glaucescens*. [d] Ferr, Ferron, UT; Oak., Oakley, ID; Sal, Salina, UT; Ruby, Ruby River Valley, MT; Ced, Cedar City UT. [e] Blank entries indicate alkaloid levels ≤0.005%. [f] Analysis by HPLC; detection limit 300 ng. [g] Analysis by GC; detection limit 2 ng.

tissues as the plants matured. Large amounts of deltaline were found in *D. barbeyi* from Ferron and Salina, UT, sites, but none was detected in this larkspur at Cedar City, UT. Higher amounts of other MDL norditerpenoid alkaloids also were found in the Ferron, UT, *D. barbeyi* samples. The distribution of the MDL alkaloids appears to be similar to that of deltaline. The amounts of lycoctonine norditerpenoid alkaloids were not consistent, however; lycoctonine and browniine seem to be more abundant in the Salina and Cedar City, UT, samples. These trends are consistent with the observed regional variations in the toxicity of the tall larkspurs to livestock.

The large amounts of deltaline observed in the tall larkspurs suggest its potential contribution to the "total toxicity" of these plants. However, the comparative toxicity of this compound compared to that of methyllycaconitine [i.e., deltaline is 16.7 times less toxic than methyllycaconitine (iv)] and the indication (Table II) that the amount of deltaline lowers in concert with reductions in the level of methyllycaconitine support the minimal contribution of deltaline to the total toxicity of the tall larkspurs. The low toxicity of *D. occidentale* (high levels of deltaline, low levels of methyllycaconitine) further supports the minimal contribution of deltaline to the total toxicity of these plants.

Estimating Tall Larkspur Toxicity to Livestock. If the toxicity of the tall larkspurs can be attributed solely to methyllycacontine and 14-deacetylnudicauline, estimates of the toxic threat to cattle of the tall larkspurs can be made. The analysis of *D. barbeyi* plant tissues (Table II), for example, establishes a maximum availability of methyllycaconitine and 14-deacetylnudicauline of 0.24% in dry plant material collected at Ferron, UT, during the plant's vegetative stage (equivalent to approximately 0.05% in fresh plant material). The amount of methyllycaconitine necessary to cause poisoning of cattle is considered to be approximately 6 mg/kg (Olsen et al., 1990). Therefore, an average yearling cow (250 kg) which consumes about 10 kg of vegetation daily would be exposed to a toxic dose of MSAL norditerpenoid alkaloids (1.5 g) by ingesting only 3 kg of fresh *D. barbeyi* available at the Ferron, UT, site, roughly one-third of its daily food requirement. HPLC analysis of a recently collected sample of *D. barbeyi* from a Colorado rangeland area, where a poisoning episode resulted in the death of 30 head of cattle in August 1991, showed methyllycaconitine amounts in excess of 0.75% (dry wt basis) in the leaves of the plant 10 days before the poisoning. Leaf material from plants from this area during the vegetative growth stage (2 months earlier) was found to contain methyllycaconitine in excess of 1.4% (Manners and Pfister, unpublished results). On the basis of the above calculation, rangeland animals exposed to levels greater than 0.8% could be exposed to a toxic dose of the MSAL norditerpenoid alkaloids through the consumption of a few plants. In the past 5 years, nearly 150 cattle have been lost to larkspur poisoning in our Colorado study area.

This investigation establishes methyllycaconitine and 14-deacetylnudicauline as potent mammalian toxins in the tall larkspurs. The significantly lower toxicity of the structurally homologous MSAL norditerpenoid alkaloid, barbinine, indicates that not all norditerpenoid alkaloids containing the unique methylsuccinimido anthranylic acid ester group are toxic and demonstrates the importance of the C-14 functionality to the mechanism of the MSAL norditerpenoid alkaloid toxicity in mammalian systems. The isolation and chemical modification of other MSAL type norditerpenoid alkaloids and their subsequent toxic evaluation should further define the structure/activity relationships of these alkaloids and perhaps suggest alternative strategies for reducing livestock losses.

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