Poisonous Properties of Larkspur (*Delphinium* spp.)

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Resum

OLSEN, J. D., G. D. MANNERS & S. W. PELLETIER. Propietats tòxiques dels esperons de cavaller (*Delphinium* spp.). Collect. Bot. (Barcelona) 19: 141-151.

Alguns membres de la tribu *Delphineae* Warming són emprats com a farratge per a bestiar de pastura o com a font de medicaments per a l'home. Tanmateix, sota determinades circumstàncies, el seu consum esdevé intoxicació, aparentment per causa d'alcaloides norditerpènics. Al menys 150 alcaloides diterpènics han estat identificats en *Delphinium*. En aquest treball es discuteixen els factors que influeixen en el contingut alcaloídic, i, per tant, en la toxicitat de la planta. Es reporten els canvis relatius en el contingut de vuit alcaloides en quatre estadis de creixement de *Delphinium occidentale* (Watson) Watson. Els alcaloides dominants en nou espècies de *Delphinium* es presenten en una llista. La toxicitat de vuit espècies és comparada en estadi de floració, emprant assaigs en rata. *D. barbeyi* (Huth) Huth és unes quatre vegades més tòxic que *D. glaucescens* Rydb. o *D. geyeri* Greene, i unes deu vegades més tòxic que un cultivar de *Consolida, D. occidentale* o *Aconitum columbianum* var. *columbianum* Nutt. (en estadi vegetatiu). L'stress causat per infestació d'àfids o per una baixa disponibilitat d'aigua tenen un mínim efecte, si en tenen, sobre la toxicitat de *D. barbeyi* i *D. occidentale,* respectivament. A partir d'anàlisis químiques de *D. barbeyi* prèviament assajades en bestiar, la DL_{50} per a una única dosi intraruminal de metillicaconitina, ha estat estimada com a inferior a 6.3 mg/kg de pes.

Mots clau: *Delphineae, Delphinium, Aconitum, Consolida,* Emmetzinament, Alcaloides diterpènics, Toxicitat, Bioassaig, Bestiar, Pastura "rangeland".

Abstract

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Some members of the tribe *Delphineae* Warming are useful as forage for grazing cattle or as a source of medicaments for man. However, under certain circumstances their consumption results in poisoning, apparently because of norditerpenoid alkaloids. At least 150 diterpenoid alkaloids have been identified in *Delphinium*. In this report, factors

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influencing the alkaloid content and the toxicity of the plant are discussed. Relative change in content of eight alkaloids at four stages of growth of *Delphinium occidentale* (Watson) Watson is reported. The most dominant alkaloids in nine species of *Delphinium* are listed. Toxicity of eight species was compared at the flower stage of growth using a mouse assay with *D. barbeyi* (Huth) Huth being about four times more toxic than *D. glaucescens* Rydb. or *D. geyeri* Greene and about ten times more toxic than *Consolida* cv., *D. occidentale,* or *Aconitum columbianum* var. *columbianum* Nutt. (vegetative stage). Stress by aphid infestation or relatively low water availability had little if any effect on toxicity of *D. barbeyi* and *D. occidentale,* respectively. From chemical analysis of *D. barbeyi* tested previously in cattle, the LD_{50} for a single intraruminal dose of methyllycaconitine was estimated to be less than 6.3 mg/kg body wt.

Keywords: *Delphineae, Delphinium, Aconitum, Consolida,* Poisoning, Diterpenoid alkaloids, Toxicity, Bioassay, Cattle, Grazing, Rangeland.

INTRODUCTION

Some members of the tribe *Delphineae* have been, and continue to be, a source of medicaments used by man (e.g., XIAO, 1980; BISSET, 1981, BENN & JACYNO, 1983; CHEN, 1984). As is the case for drugs in general, excessive consumption or administration of the plant or plant products can result in poisoning, apparently in this case because of norditerpenoid alkaloids. These plants can be useful forage for animals (PFISTER & AL., 1988) but, under certain circumstances, poisoning occurs (OLSEN & MANNERS, 1989). Indeed, poisoning from consumption of larkspur is a major cause of acute cattle deaths on mountain and high plains rangelands of the western U.S.A. (NIELSEN & RALPHS, 1988). Poisoning of cattle by larkspur appears clinically to be primarily a failure of the skeletal muscle motor unit (OLSEN, 1978a; NATION & AL. 1982). As a result, the animal has initially signs of acute episodic weakness which may progress to a generalized paralysis, with severity being highly dose dependent (OLSEN & SISSON, 1990). If asphyxiation does not occur due to bloat, respiratory paralysis, or regurgitation and inhalation of rumen contents, the animal usually recovers the ability to stand within 24 hours (OLSEN & SISSON, 1990). For unknown reasons, reports of poisoning of livestock by these plants in other parts of the world are rare.

The major objectives of this paper are briefly to discuss the determinant factors influencing the poisonous properties of larkspur as reported previously and to present results of recent studies, with emphasis on those species which cause poisoning of livestock grazing rangeland in the U.S.A.

DITERPENOID ALKALOIDS IN LARKSPUR

Most of the diterpenoid alkaloids identified in *Delphinium* and *Aconitum* have been structurally characterized in the last 25 years. At least 150 diterpenoid alkaloids have been identified in *Delphinium* (for reference, see OLSEN & MANNERS, 1989, SOUTHON & BUCKINGHAM, 1989). The principal alkaloids in *Delphinium* form three distinct structural classes: (A) norditerpene alkaloids, (B) C_{20}-diterpenoid alkaloids, and (C) bis-diterpenoid alkaloids.

The norditerpenes can be divided into *aconitine-type* (1), *lycoctonine-type* (2), *pyrodelphinine-type* (3), or *heteratisine-type* (4) according to the pattern of oxidation of the C-7, C-8, C-14 and C-15 carbons of the alkaloid skeleton (Fig. 1). Most, if not all, of the poisonous properties of *Delphinium* spp. are due to norditerpenes. Only alkaloids of structural types (1) and (2) have been found to occur naturally in *Delphinium* with the predominant number being of the lycoctonine-type (OLSEN & MANNERS, 1989), whereas all four skeletal types have been found in *Aconitum.* Most of the C_{20}-diterpenoid alkaloids reported in *Delphinium* are structural variants

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of hetisine with the remainder being of the atisine or delnudine type. Eight bis-diterpenoid alkaloids have been characterized from the seeds of *Delphinium staphisagria*.

"Total alkaloid" content of *Aconitum* and *Delphinium* species has been estimated by gravimetric (PELLETIER & AL., 1981; MANNERS & RALPHS, 1989), titrimetric (WILLIAMS & CRONIN, 1963; SUN, W. & AL., 1984), fluorometric (LUO & AL., 1983), and near-infrared spectrophotometric (CLARCK & AL., 1987) analytical methods. Total alkaloid, as a percentage of dry weight, is usually reported to be highest in rapidly growing plant, such as young vegetative leaves and stem and reproductive parts of the raceme, then declining generally as the plant ages. Our recent measurements of total alkaloid in nine collections of *Delphinium* by both gravimetric and titrimetric methods showed reasonably good agreement overall (Table 1). However, certain samples showed considerable variation. In our experience, *D. occidentale* tended to have a relatively greater content of total alkaloid than the other *Delphinium* spp. Although the relationship between toxicity and total alkaloid appears to be complex (OLSEN, 1983), further study may reveal circumstances where toxicity can be estimated by measurement of "total alkaloid" content, provided the most toxic alkaloids change in a direct relationship with that of the total alkaloids.

Table 1. Comparison of total alkaloid concentration (percentage air-dry plant weight) determined by gravimetric and titration methods.

Sample[a]	Gravimetric[b]	Titration[c]	Difference (G - T)
1.	5.05[d]	4.05	1.00
2.	2.29	2.2	.09
3.	4.50	3.85	.65
4.	4.40	4.3	.10
5.	1.23	2.5	−1.27
6.	3.61	2.5	1.11
7.	4.50	3.8	.70
8.	1.43	0.9	.53
9.	2.06	2.11	−.05
Mean	3.23	2.91	0.32
s.d.	1.48	1.14	0.72

[a] Large collections of above-ground parts of whole plants air-dried in direct sunlight, ground (1 mm screen), mixed thoroughly, and stored in closed plastic bags until analyzed and used in other experiments.

[b] Ethanol (80 %) soxhlet extract, concentrated in vacuo, partitioned with chloroform and aqueous HCl (10 %), aqueous fraction basified (pH 8, 20 % NaOH) and extracted first with ether then with $CHCl_3$, dried with $MgSO_4$, and finally evaporated to dryness under a stream of N_2 (MANNERS & RALPHS, 1989).

[c] Benzene-chloroform (9:1) extraction, partitioned with aqueous acid, basified aqueous fraction partitioned with benzene, alkaline benzene fraction containing crystal violet indicator titrated to yellow endpoint using perchloric-glacial acetic acid, ml acid required represented amount of total alkaloid according to a standard curve (WILLIAMS & CRONIN, 1963).

[d] Percentage of air-dry weight of plant.

We recently studied the relative content of certain individual alkaloids in the leaf-petiole portion of *D. occidentale* at four stages of growth (Table 2). Following visual transect lines through the collection area, one individual stalk conforming to the desired growth stage was selected from each plant clump as encountered until a total of 50 stalks was collected for each sampling time, repeated at about 2 week intervals. The stalks were collected into closed plastic

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Table 2. Relative concentration of diterpenoid alkaloids in the leaf-petiole part of *Delphinium occidentale* during growth.[a]

Growth Stage	Total Alkaloid[b] (g %)	Delpheline (mg %)	Deltamine (mg %)	Deltaline (mg %)	14 Acetyl-Dictyocarpine (mg %)	Dictyo-carpine[c] (mg %)	Lycoctonine (mg %)	Browniine (mg %)	14 Dehydro-browniine (mg %)
Vegetative	3.06	3[d]	18	1044	9	43	6	24	6
Early Bud	1.91	0	8	682	23	44	0	19	36
Late Bud	1.54	0	25	989	6	14	23	12	57
Full Flower	1.07	3	9	463	5	12	8	11	2
Mean		2	15	794	11	28	9	16	25
s.d.		2	8	272	8	18	10	6	26

[a] Analysis of underivated alkaloids measured by capillary gas chromatography (MANNERS & RALPHS, 1989). Mixed individual alkaloids standard solution used to establish an external standard concentration/response factor. Resultant calibrated weighted response factor used to quantify the area under the G. C. curve for each alkaloid.

[b] Gravimetric (MANNERS & RALPHS, 1989).

[c] Retention time for dictyocarpine and dictyocarpinine is very similar in the gas chromatography method used, therefore they are presently indistinguishable and appear together as a single peak (MANNERS & RALPHS, 1989).

[d] Derived from a percentage of the total alkaloid. The weighted response factor and the area under the G. C. curve was used to quantify each alkaloid (MANNERS & RALPHS, 1989).

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Table 3. Abbreviated protocol for extraction of alkaloids from a collection of *Delphinium barbeyi*[a].

A. Dried, ground plant (1.4 kg); extracted with ethanol, 80 %, 2X; after evaporation, yielded tar-residue (231.4 g).
B. Tar-residue (231.4 g) extracted with ice-cold toluene; yielded toluene soluble and insoluble fractions.
C. Gradient pH fractionation of the toluene soluble and toluene insoluble fractions; total yield 32.79 g:
   1. Toluene soluble fractions:
      a. 8.28 g (pH 6)
      b. 0.62 g (pH 9)
      c. 0.066 g (pH 13)
   2. Toluene insoluble:
      d. 21.84 g (pH 6)
      e. 1.3 g (pH 9)
      f. 0.685 g (pH 13)
D. Vacuum liquid chromatography of fraction *a* (8.28 g); total yield 6.882 g (subfractions 2-25).
E. Vacuum liquid chromatography of a part of fraction *d* (14.2 f); total yield 11.909 g (subfractions 2-22).
F. Vacuum liquid chromatography of combined fractions *c* + *f* (0.751 g); total yield 0.333 g (subfractions 7-15).
G. Vacuum liquid chromatography and(or) centrifugally accelerated, radial, thin-layer chromatography of particular subfractions of *a, d,* and *c* + *f.*

[a] Adapted from PELLETIER & AL., (1989).

bags, weighed for moisture content, separated into plant parts [raceme (apex), stem, and combined leaf and petiole], oven-dried (49 to 55 °C), and ground (1 mm screen). The air-dry plant was stored in closed plastic bags until analyzed. Of the eight alkaloids identified thus far, deltaline, dictyocarpine (dictyocarpinine), and browniine had a general decline in content analogous to the decline in toxicity that occurs as the plant matures. However, these alkaloids are thought to be relatively nontoxic (compare AIYAR & AL., 1979; BENN & JACYNO, 1983).

We also studied the relative amount of alkaloids in a collection of *D. barbeyi* (PELLETIER & AL., 1989; KULANTHAIVEL & AL., 1990). The above-ground parts (328 lbs, air-dry) were collected predominantly in the bud-stage of growth, although stage of growth varied from plants having no conspicuous flower buds to those having well-formed buds and occasionally flowers. The entire dried collection was ground and mixed thoroughly in a commercial feed grinder. Chemical investigation (Table 3) resulted in the quantitative isolation of four new and 13 known alkaloids (Table 4). The same collection of plant was used to determine the LD_{50} for cattle (OLSEN, 1978a). Therefore, an inference can be made concerning response of cattle to the alkaloids contained in the plant (see below).

The relative content of individual alkaloids may eventually have value for determining taxonomic relationship, if the patterns for relative abundance of dominant alkaloids as identified thus far prove stable for a particular plant population (Table 5). In this regard also, we have observed a presently unidentified alkaloid that occurred only in *D. occidentale* x *barbeyi* collections, according to current plant classification. Further, KULANTHAIVEL & BENN (1985) identified hetisine from native *D. nudicaule* Torr. and Gray but did not isolate it from a horticultural cultivar. From data of Table 2, one can note that repeated determinations at several stages of growth (and standardized environment) may be necessary to establish taxonomically meaningful relationship. One can note also (Table 6), that the toxicity of a hybrid population of *D. occidentale* x *barbeyi* was intermediate to that of *D. barbeyi* and *D. occidentale* populations. Alteration of the genetics of a population of larkspur, perhaps by a biogenetic engineering mechanism, may eventually provide a means of altering the alkaloid make-up in the plant and thus modifying the toxicity to reduce the risk of poisoning.

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Table 4. Relative abundance of alkaloids identified in extract from a collection of *Delphinium barbeyi.*[a]

	Fractions[b]				Total	
	a	*d*	*d*-Adjusted[c]	*d*+*f*	Identified[d]	% of Plant[e]
Deltaline	3.38[f]	5.99[f]	9.21		12.9	.8993
Methyllycaconitine	1.45	.87	1.34		2.79	.1993
14 Acetyldictyocarpine	.50	.48	.74		1.24	.0886
14 Deacetylnudicauline		.25	.38		.38	.0271
14 Dehydrobrowniine	.10	.13	.20		.30	.0214
Browniine	.10	.11	.17		.27	.0193
Delcosine		.12	.18		.18	.0129
Barbisine[g]	.03	.10	.15		.18	.0129
6 Dehydrodeltamine				.14	.14	.0100
Dictyocarpine	.04	.06	.09		.13	.0093
Barbinine		.06	.09		.09	.0064
Delelatine		.05	.08		.08	.0057
Barbinidine		.02	.03	.01	.04	.0029
Delpheline		.02	.03		.03	.0021
Glaucenine		.02	.03		.03	.0021
Glaucerine		.01	.02		.02	.0014
Barbeline		.01	.02			
Total Identified		5.57	8.18			

[a] Large collection of entire above-ground parts mostly in bud stage of growth, air-dried in direct sunlight, ground, mixed thoroughly, stored in closed plastic bags at 5° C until analyzed (PELLETIER & AL., 1989; KULANTHAIVEL & AL., 1990) or used in experiments.
[b] Gradient pH fractions according to protocol of Table 3 (PELLETIER & AL., 1989).
[c] Since only a part (14.2 g) of the total (21.84 g) of fraction *d* was analyzed, an adjusted value was calculated to represent the amount present in the total of fraction *d*.
[d] Summation of the amount of a particular alkaloid identified in the respective total fractions (eg., *a* + *d*- adjusted).
[e] Total amount of alkaloid identified, calculated as a percentage of the air-dry weight of the starting plant material.
[f] Data in column calculated for total present in initial vacuum liquid chromatography subfraction assuming an 83 % yield.
[g] First reported N-C(19)-seco C_{20}-diterpenoid alkaloid from *Delphinium*, KULANTHAIVEL & AL., 1990.

FACTORS INFLUENCING THE TOXICITY OF LARKSPUR

The alkaloid content in larkspur, and thus the toxicity, varies according to the plant species, stage of plant growth, plant part, and plant environment.

Species. Toxicity of three larkspur species *(D. barbeyi > D. glaucescens > D. occidentale)* was compared previously for large collections of whole plant, ranging from vegetative to flowering stage of growth (OLSEN, 1977b). We now report recent studies, using the same methods but with mice, comparing the relative toxicity of eight species of rangeland and two horticultural larkspur varieties at the flower-stage of growth (Table 6). *Delphinium barbeyi* was about four times more toxic than *D. glaucescens* or *D. geyeri* and about ten times more toxic than *Consolida* cv., *D. occidentale,* or *Aconitum columbianum* var. *columbianum* (vegetative stage).

Stage of Growth. In general, toxicity of larkspur declines throughout the growing seasons as does the alkaloid content. However, decline in toxicity may not be directly proportional to the

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Table 5. Ranking of dominant alkaloids according to abundance as identified in whole plant collections of *Delphinium* species.

Species	Stage of Growth	Ranking 1.	Ranking 2.	Ranking 3.	Reference
D. barbeyi	bud	Deltaline Deltaline	Methyllycaconitine Lycoctonine	14 Acetyldictyocarpine 14 Acetyldictyocarpine	PELLETIER & AL., 1989[a] MANNERS & OLSEN, this paper[b]
D. glaucescens	bud	Lycoctonine Dictyocarpine[c]	Dictyocarpine Browniine/Lycoctonine	Browniine Deltaline	PELLETIER & AL., 1981[a] MANNERS & OLSEN, this paper[b]
D. occidentale	bud	Deltaline	Dictyocarpine[c]/ 14 Dehydrobrowniine	Browniine/Deltamine	MANNERS & OLSEN, this paper[b]
D. geyeri	flower	Browniine	14 Acetylbrowniine	Gyerine	GRINA & AL., 1986[a]
D. andersonii	flower	Methyllyacaconitine	Delavaine/nudicauline	Andersonine/takaosamine	PELLETIER & AL., 1988[a]
D. nuttallianum	flower	Methyllycanonitine	Nudicauline	Unknown (peak 3)	MAJAK & AL., 1987[d]
D. bicolor	flower	Methyllycaconitine	Delcosine/isotalitizidine	Condelphine/6 Acetylbicolorine	KULANTHAIVEL & AL., 1986[a]
D. carolinianum	flower?	Decaroline	Ajaconine	Browniine	PELLETIER & AL., 1981[a]
D. macrocentrum	vegetative	Delcosine	Methyllycaconitine	Desacetylnudicauline	BENN & AL., 1989[a]

[a] Quantified by chemical isolation.
[b] Quantified by gas chromatograpy peak separation (method of MANNERS & RALPHS, 1989).
[c] Retention time for dictyocarpine and dictyocarpinine was very similar in the gas chromatography method used, therefore they are presently indistinguishable and appear together as a single peak (MANNERS & RALPHS, 1989).
[d] Quantified by high pressure liquid chromatography peak separation.

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Table 6. Relative toxicity of leaf-petiole extract among *Delphinium, Consolida,* and *Aconitum* at the flowering stage of growth compared by mouse assay[a].

Species	LD_{50}[b]	Confidence Interval[c]
D. barbeyi	2.0	(1.8 - 2.3)
D. barbeyi (aphid infested)[d]	3.8	(3.2 - 4.5)
D. barbeyi (aphid noninfested)[d]	4.6	(3.9 - 5.5)
D. glaucescens (leaf-petiole)	7.8	(5.0 - 6.7)
(raceme)	4.0	(2.2 - 3.3)
(stem)	11.5	(10.0 - 13.2)
D. occidentale x barbeyi	13.6	(12.1 - 15.4)
D. geyeri	14.6	(12.2 - 17.4)
D. hybridum cv. ?[e]	22.9	(18.1 - 28.9)
D. tricorne[f]	27.6	(25.2 - 30.0)
Consolida sp. cv. ?[g]	35.4	(28.8 - 43.5)
D. occidentale (Moist site)[h]	35.7	(33.7 - 37.8)
D. occidentale (Dry site)[h]	38.8	(36.0 - 41.9)
A. columbianum[i]	39.2	(35.8 - 42.9)

[a] Air-dry ground plant extracted with ethanol (95 %), evaporated to dryness, extracted with buffered saline, filtered saline extract injected subcutaneously, 1 ml saline extract contained all of the alkaloid present in 1 g of plant except for that lost during extraction (OLSEN, 1977a).

[b] Microliters of saline extract per g body wt, calculated according to the method of WEIL (1952).

[c] Estimation of a confidence interval that will encompass the LD_{50} 95 times in 100 determinations.

[d] Plants for J.D.O. collected in 1986 from rangeland used for cattle grazing, by A. M. Peterson, D. V. M., Yampa Colorado, U.S.A. See text, Environmental Factors.

[e] Unclassified horticultural variety, 1.2 to 1.8 m height, most likely "Pacific giant", collected from private residential floral garden. (Anson B. Call, Logan, Utah, U.S.A.)

[f] Plant collected for J.D.O. in 1988 from pasture used for cattle grazing, by C.D. Halsey, D.V.M., Abingdon, Virginia, U.S.A.

[g] Unclassified horticultural variety, about 0.6 m height, collected from private residential floral garden. Anson B. Call.

[h] Part of a serial collection of plants during a growing season, see text. Environmental Factors.

[i] Vegetative stage, 0.5 to 0.6 m height, only sample compared presently.

change in the total alkaloid content (OLSEN, 1983), in agreement with the expectation that toxicity of particular individual alkaloids in the plant is the major determinant of the plant's toxicity.

Plant Part. Toxicity of plant parts generally parallels total alkaloid content, with rapidly growing parts (or parts having a high metabolic activity) and reproductive parts of the plant being relatively more toxic per g dry wt. (eg., *D. glaucescens,* Table 6).

Environment. Physiographic, ecological, macro- and micro-climatic aspects may have an influence on the alkaloid content of larkspur similar to that reported for other plants (WALLER & NOWACKI, 1978; GERSHENZON, 1984). However, the range of influence of these factors is yet to be determined. Using methods reported previously (OLSEN, 1983), we now report the toxicity of flowering larkspur plants *(D. occidentale)* growing in two contiguous areas: 1. Among a population of *Veratrum californicum* Dur. in a moist-seep area and, 2. In a higher area having an increased slope and drainage where the entire plant community, because of lack of moisture, was showing signs of stress such as wilting, arrest of bud maturation of a part of the raceme, and increased rate of senescence. Toxicity was similar for the green leaves from 50 stalks of plants collected at the same time from each collection site and at similar phenological development, viz., mid-flowering (Table 6). Aphid or other host-specific insect infestation of larkspur represents an aspect of the ecosystem which might affect plant alkaloid content and result in a change of plant toxicity or acceptability as forage for cattle (OLSEN, 1984). Such

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effects may indeed have potential as a biological mechanism to influence the risk of poisoning. An initial study of this relationship was done by collecting simultaneously about 50 stalks each of aphid-infested and contiguous noninfested *D. barbeyi* plants. The plant material was prepared in the usual manner (OLSEN, 1977a) for mouse assay of toxicity. Toxicity of extract from the leaf-petiole parts was not significantly different although the aphid-infested plants appeared to be slightly more toxic (Table 6). It is not known whether the noninfested plants contained a feeding deterrent (GRINA & AL., 1986) or if the alkaloid content of the infested plants changed in response to attack by the aphids. Further study is warranted.

Individual alkaloids. The relative toxicity of each alkaloid in *Delphinium* spp. is presently conjectural, as it relates to poisoning of grazing cattle. All larkspur analyzed thus far contained a mixture of alkaloids and specific alkaloids have varied effects in the intact animal. The integrated effect of the alkaloids and overall response of the body systems in a particular animal determines the resultant degree of poisoning though no doubt certain alkaloids are more influential in view of the variation of 2 orders of magnitude in reported LD_{50} values (Table 7).

Table 7. Relative acute toxicity of larkspur alkaloids in mice (LD_{50}, mg/kg i.v.)[a]

Methyllycaconitine:	3
Delsemine	6[b]
Anthranoyllycoctonine	20[b]
Condelphine	35[b]
Delcosine	109
Delsoline	175
Lycoctonine	350[b]
Deltaline	>300

[a] For reference to original studies see Olsen and Manners, 1989.
[b] Estimated values for i.v. route interpolated from related measurements for other parenteral routes and(or) other species.

An ultimate goal of our investigation of larkspur poisoning has been to estimate the toxicity of individual larkspur alkaloids for cattle (OLSEN, 1978b & 1984; OLSEN & MANNERS, 1989; PELLETIER & AL., 1989). We here estimate that the LD_{50} of methyllycaconitine for cattle is 5.0 to 6.3 mg/kg or less, when given intrauminally as a single dose of plant; provided all of the toxicity in *D. barbeyi* tested previously was due equally to methyllycaconitine and 14 deacetylnudicauline (compare AIYAR & AL., 1979; BENN & JACYNO, 1983) and that the chemical extraction (PELLETIER & AL., 1989) accurately reflected the plant composition (viz., methyllycaconitine + 14 deacetylnudicauline = 0.23 % of air dry weight of the plant, Table 4). This estimate was calculated using the relative dominance of alkaloids of *D. barbeyi* listed in Table 4, because that collection of plant was used to estimate the LD_{50} of *D. barbeyi* for cattle (2.5 g dry plant/kg body wt., 95 % confidence interval = 2.2 to 2.8) reported previously (OLSEN, 1978a). The estimate of lethal dose for methyllycaconitine can be used to predict risk of poisoning of cattle when the alkaloid content and biomass of the larkspur plant is known or can be estimated.

It seems prudent that future chemical investigations of members of the tribe *Delphineae* include quantification (or at least approximation) of the alkaloids isolated in relation to the amount of starting plant material. Also, one should select homogeneous plant material in terms of plant part, stage of growth, and growth environment. Such practices would greatly enhance knowledge of the relationships between alkaloid content, taxonomy, and toxicity.

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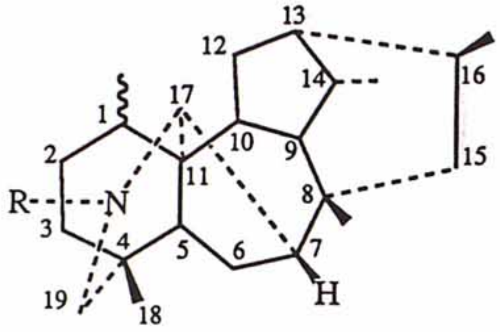

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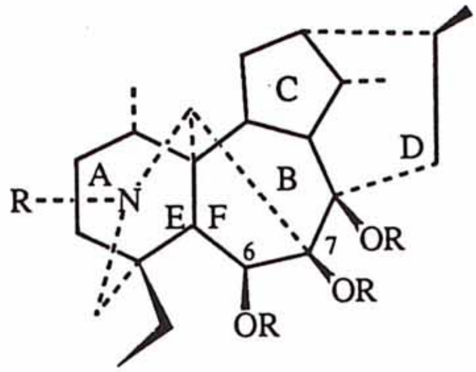

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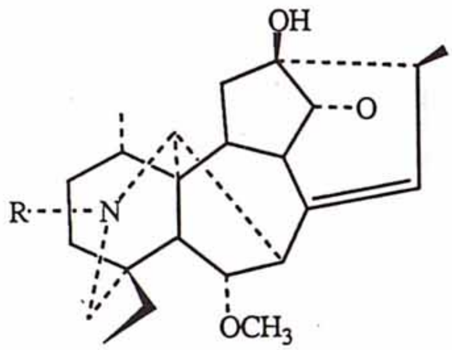

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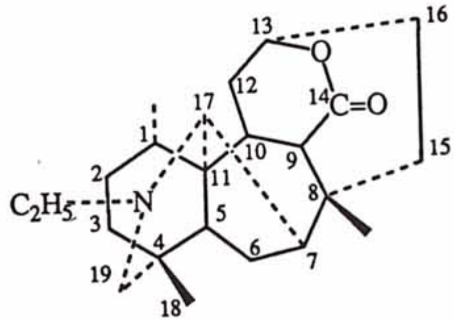

4

Fig. 1.– Structural classes of norditerpene alkaloids: (1) *aconitine-type,* (2) *lycoctonine-type,* (3) *pyrodelphinine-type,* or (4) *heteratisine-type,* according to the pattern of oxidation of the C-7, C-8, C-14 and C-15 carbons of the alkaloid skeleton.

Bibliography

AIYAR, V. N., M. H. BENN, T. HANNA, J. JACYNO, S. H. ROTH, & J. . WILKENS (1979). The principal toxin of *Delphinium brownii* Rydb., and its mode of action. Experientia 35:1367-1368.

BENN, M. H. & J. M. JACYNO (1983). The toxicology and pharmacology of diterpenoid alkaloids. In: S. W. PELLEITER, (Ed.), Alkaloids, Chemical and Biological Perspectives, Vol. 1, John Wiley & Sons, New York, pp. 153-210.

BISET, N. G. (1981). Arrow poisons in China. Part II. Aconitum botany, chemistry, and pharmacology. J. Ethnopharmacol. 4:247-36.

CHEN, D. (1984). Biological activities and medical prospect of diterpene alkaloids. Zhongcaoyao 15(4):180-184.

CLARK, D. H., M. H. RALPHS, & R. C. LAMB (1987). Total alkaloid determinations in larkspur and lupine with near infrared reflectance spectroscopy. Agronomy J. 79:481-485.

GERSHENZON, J. (1984). Changes in the levels of plant secondary metabolites under water and nutrient stress. In: B. M. TIMMERMANN & AL. (Eds.), Recent Advances in Phytochemistry, Phytochemical Adaptations to Stress. Plenum Press, New York, pp. 273-320.

GRINA, J. A., D. R. SCHROEDER, E. T. WYDALLI, & F. R. STERMITZ (1986). Alkaloids from *Delphinium geyeri.* Three new C_{20}-diterpenoid alkaloids. J. Org. Chem. 51:390-394.

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KULANTHAIVEL, P., M. BENN, & W. MAJAK (1986). The C_{19}-diterpenoid alkaloids of *Delphinium bicolor.* Phytochemistry 25:1511-1513.

KULANTHAIVEL, P., E. M. HOLT, J. D. OLSEN, & S. W. PELLETIER (1990). Barbisine, a C_{20}diterpenoid alkaloid from *Delphinium barbeyi.* Phytochemistry 29:293-295.

LUO, S., S. WU, & J. TANG (1983). A simple fluorochromatographic method for the identification of total alkaloids in *Delphinium.* Zhongcaoyao 14:399-400.

MANNERS, G. D. & M. H. RALPHS (1989). Capillary gas chromatography of *Delphinium* diterpenoid alkaloids. J. Chrom. 466:427-432.

NATION, P. N., M. H. BENN, S. H. ROTH, & J. L. WILKENS (1982). Clinical signs and studies of the site of action of purified larkspur alkaloid, methyllycaconitine, administered parenterally to calves. Canad. Vet. J. 23:264-266.

NIELSEN, D. B. & M. H. RALPHS (1988). Larkspur: economic considerations. In: L. F. JAMES & AL. (Ed.), The ecology and economic impact of poisonous plants on livestock production. Westview Press, Boulder, Colorado, U.S.A., pp. 119-129.

OLSEN, J. D. (1977a). A rat bioassay for estimating toxicity of plant material from larkspur (*Delphinium* spp). Amer. J. Vet. Res. 28:277-279.

OLSEN, J. D. (1977b). Toxicity of extract from three larkspur species measured by rat bioassay. J. Range Manage. 30:237-238.

OLSEN, J. D. (1978a). Tall larkspur poisoning in cattle and sheep. J. Amer. Vet. Med. Assoc. 173:762-765.

OLSEN, J. D. (1978b). Larkspur Toxicosis: A review of current research. In: R. F. KEELER & AL. (Ed.). Effects of Poisonous Plants on Livestock. Academic Press, New York, pp. 535-543.

OLSEN, J. D. (1983). Relationship of relative total alkaloid concentration and toxicity of Duncecap Larkspur during growth. J. Range Manage. 36:550-552.

OLSEN, J. D. (1984). Larkspur poisoning: potential for management. In: E. G. SEIMER & R. H. DELANEY (Ed.), Proceedings of the Second Intermountain Meadow symposium. Colorado St. Univ. Exp. Sta. Special Series 34:67-72.

OLSEN, J. D. & G. D. MANNERS (1989). Toxicology of diterpenoid alkaloids in rangeland larkspurs (*Delphinium* spp.). In: P. CHEEKE (Ed.), Toxicants of Plant Origin. Vol. I, Alkaloids, CRC Press, Inc., Boca Raton, FL, U.S.A., pp. 291-326.

OLSEN, J. D. & D. V. SISSON (1990). Rating scale for the clinical response of cattle poisoned by larkspur. Amer. J. Vet. Res. (In Press).

PELLETIER, S. W., O. D. DAILY, Jr., N. V. MODY, & J. D. OLSEN (1981). Isolation and structure elucidation of the alkaloids of *Delphinium glaucescens* Rybd. J. Org. Chem. 46:3284-3293.

PELLETIER, S. W., A. M. PANU, P. KULANTHAIVEL, & J. D. OLSEN (1988). New alkaloids from *Delphinium andersonnii* Gray. Heterocycles 27:2387-2393.

PELLETIER S. W., P. KULANTHAIVEL, & J. D. OLSEN (1989). Alkaloids of *Delphinium barbeyi.* Phytochemistry 28:1521-1525.

PFISTER, J. A., G. D. MANNERS, M. H. RALPHS, Z. X. HONG & M. A. LANE (1988). Effects of phenology, site, and rumen fill on tall larkspur consumption by cattle. J. Range Manage. 41:509-514.

SUN, W., X. MU, & Z. YANG (1984). Assay of total alkaloids and aconitine in the root of *Aconitum carmichaeli* and *Aconitum kusnezoffii,* Zhongyao Tongbao 9:125-127.

SOUTHON, I. W. & J. BUCKINGHAM (1889). Dictionary of alkaloids. Champman and Hall, London.

WALLER, G. R. & E. K. NOWACKI (1978). Alkaloid Biology and Metabolism in Plants. Plenum Press, New York and London, pp. 85-119.

WEIL, C. S. (1952). Tables for convenient calculation of median-effective dose (LD_{50} or ED_{50}) and instructions in their use. Biometrics 8:249-263.

WILLIAMS, M. C. & E. H. CRONIN (1963). Effect of Silvex and 2,4,5-T on alkaloid content of tall larkspur, Weeds, 11:317-319.

XIAO, P. (1980). A preliminary study of the correlation between phylogeny, chemical constituents and pharmaceutical aspects in the taxa of Chinese *Ranunculaceae.* Acta Phytotaxonomica Sinica 18:142-153.

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