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Note

Capillary gas chromatography of *Delphinium* diterpenoid alkaloids

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Tall larkspur (*Delphinium barbeyi*) and duncecap larkspur (*Delphinium occidentale*) are poisonous plants indigenous to the Western mountains of the U.S.A.[1]. These plants are poisonous to cattle and cause annual losses as high as 12% on mountain grazing ranges[2]. Alcohol extracts of *D. barbeyi* and *D. occidentale* were found to have a median lethal dose (LD_{50}) of 4.5 and 40.4 mg/g body weight respectively when administered subcutaneously to rats[3].

Diterpenoid alkaloids have been established as toxic components occurring in the larkspurs. Five diterpenoid alkaloids (anthranoyllycoctonine, delpheline, deltaline, dictyocarpine and lycoctonine) have been identified in *D. barbeyi*[4–6] while deltaline, deltamine, dictyocarpine, dictyocarpinine, delcosine, 14-dehydrodelcosine, browniine, 14-dehydrobrowniine, glaucerine, glaucenine, glaucedine, hetisine, hetisione, delsoline, delpheline, 6-acetyldelpheline, occidentaline and occidentalidine and have recently been described in *D. occidentale*[7]. The toxic character of various diterpenoid alkaloids, including anthranoyllycoctonine, lycoctonine, deltaline and delcosine, has been reviewed[8]. Toxicological data for the majority of diterpenoid alkaloids reported in *Delphinium* sp. is lacking and the correlation of specific larkspur diterpene alkaloids with the mortality of cattle has not been described.

While the specific toxicants occurring in tall and duncecap larkspur have not been identified, livestock grazing management based upon total alkaloid occurrence has been formulated[9]. It is recognized that more efficient management of livestock on larkspur infested grazing lands could be achieved if the identity and phenological variation of specific toxic alkaloids were available.

Capillary gas chromatography (GC) has been shown to be a sensitive and efficient method for the analyses and detection of quinolizidine alkaloids obtained from lupin[10] and for the screening of drug abuse alkaloids[11]. We now describe a capillary GC method for the analysis of underivatized diterpenoid alkaloids occurring in larkspur. This analytical method provides a rapid quantification of specific diterpenoid alkaloids occurring in larkspur and will allow the correlation of the phenological occurrence of these alkaloids with observed rangeland livestock toxicity.

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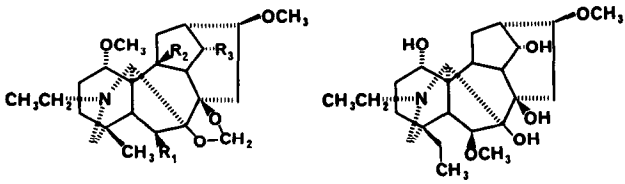

Scheme 1. Delpheline (1), R_1 = OH, R_2 = H, R_3 = OCH_3; deltamine (2), R_1 = OH, R_2 = OH, R_3 = OCH_3; deltaline (3), R_1 = $OOCCH_3$, R_2 = OH, R_3 = OCH_3; 14-acetyldictyocarpine (4), R_1 = $OOCCH_3$, R_2 = H, R_3 = $OOCCH_3$; dictyocarpine (5), R_1 = $OOCCH_3$, R_2 = H, R_3 = OH; dictyocarpinine (6), R_1 = OH, R_2 = OH, R_3 = OH. Structure on the right shows delcosine (7).

EXPERIMENTAL

Apparatus and operation conditions

A Hewlett-Packard 5890A gas chromatograph equipped with a hydrogen flame ionization detector and an HP on-column injector was used for all analyses.

A 30 m × 0.32 mm I.D. flexible fused-silica SE-30 capillary column (J&W Scientific) was used with the following operating conditions: detector temperature 300°C; initial temperature 85°C (0.1 min.), 40°C/min to 175°C, 5°C/min. to 300°C, final temp. 300°C (20 min); helium carrier gas flow-volume, 2.13 ml/min.

Alkaloid standards and reagents

Deltaline, dictyocarpine, deltamine, and delcosine standards were kindly supplied by Dr. S. William Pelletier. These four diterpenoid alkaloids were also isolated and purified from extracts of *D. occidentale* and *D. barbeyi* in our laboratory and served as standards for analytical analyses. Dictyocarpinine obtained from *D. occidentale* and 14-acetyldictyocarpine and delpheline obtained from *D. barbeyi* in our laboratory were also used as analytical standards. Chemical structures for the alkaloids are shown in Scheme 1. The hydrocarbon standard mixture was obtained from J&W Scientific. Solvents used were reagent grade and used without purification.

TABLE I

MEAN WEIGHT RESPONSE FACTORS AND RELATIVE RETENTION TIMES OF DITERPENOID ALKALOIDS

Alkaloid	*Mean wt. response factor (ng/count)*	*Observed retention time (min)*	*Rel. retention time*[a]
Delpheline (1)	0.012	24.22	$C_{28.93}$
Deltamine (2)	0.026	24.85	$C_{29.58}$
Deltaline (3)	0.011	25.34	$C_{29.91}$
14-Acetyldictyocarpine (4)	0.013	25.92	$C_{30.35}$
Dictyocarpine (5)	0.023	26.90	$C_{31.31}$
Dictyocarpinine (6)	0.021	26.90	$C_{31.31}$
Delcosine (7)	0.004	29.16	$C_{32.67}$

[a] Retention times relative to C_{28}–C_{34} hydrocarbons.

External standard calibration of capillary GC

A standard stock solution (acetone containing approximately 600 n/μl of each alkaloid) was prepared and analyzed to determine the retention times of the individual alkaloids (Table I). Aliquots of the stock solution of the standard alkaloid solutions were combined to produce a mixed alkaloid standard.

The mixed standard was used to calibrate the gas chromatograph. Samples of 1 μl of sequentially diluted aliquots of the mixed standard solution were applied to GC to establish an external standard concentration *vs.* response curve for each of the alkaloids. Weight response factors of the individual alkaloids were obtained (Table I). The response data was accumulated and stored using a Hewlett-Packard ChemStation (Model 5859A) computer interfaced to the GC system.

A standard mixture of alkanes (C_{28}–C_{34}) was added to the standard alkaloid mixture and the combined standards were applied to the gas chromatograph. Retention times for the standard alkaloids relative to the standard alkanes were established (Table I).

Quantitative analysis of alkaloids in larkspur extracts

A total alkaloid extract was obtained from ground, air-dried, whole plant material of *Delphinium occidentale* and *Delphinium barbeyi* through the modification of a procedure used by Pelletier *et al.*[12]. The ground plant material (10 g) was exhaustively extracted (80% ethanol) in a soxhlet apparatus and the resulting extract was concentrated (*in vacuo*) to a syrup. The syrup was dissolved in chloroform (200 ml) and extracted with 10% hydrochloric acid (100 ml). Two additional extractions with 10% hydrochloric acid (50 ml) were performed and the acidic extracts were combined. The chloroform extract was discarded. The acid extract was made basic (pH 8) with 20% sodium hydroxide, cooled and extracted with ether (three times with 100 ml). The ether extract was dried (magnesium sulfate) and concentrated (*in vacuo*). The remaining basic solution was further extracted with chloroform (three times with 100 ml). The chloroform extract was dried (magnesium sulfate) and concentrated (*in vacuo*). The ether and chloroform solutions were combined and concentrated to dryness under a stream of nitrogen. The combined extracts were considered to contain the total alkaloids of the plant extract.

The total alkaloid extract was dissolved and diluted to 50 ml in acetone. A suitable aliquot from the solution was diluted to afford a sample solution containing approximately 200 n/μl. A 1-μl sample of this solution was injected for analysis. The sample was compared to the calibrated external standard data base to establish the quantities of known alkaloids present (Table II).

RESULTS AND DISCUSSION

The capillary GC separation of the standard alkaloid mixture is shown in Fig. 1. The figure shows six peaks for the seven standard alkaloids applied to the chromatographic column. It was found that the diterpenoid alkaloids dictyocarpine and dictyocarpinine have identical retention times in the chromatographic system and are therefore unresolved. The five remaining standard alkaloids are clearly resolved. Calibration curves for all of the standard alkaloids were linear over the range of concentrations used. The minimum range of detectability for the standard alkaloids

TABLE II

DITERPENOID ALKALOID COMPOSITION OF *D. OCCIDENTALE* AND *D. BARBEYI*

Values are given as % of total alkaloid extract.

Alkaloid	*D. occidentale*	*D. barbeyi*
Delpheline (1)	0.1	2.6
Deltamine (2)	0.5	2.3
Deltaline (3)	33.0	37.5
14-Acetyldictyocarpine (4)	0.6	8.2
Dictyocarpine/dictyocarpinine (5,6)	19.5	4.2
Delcosine (7)	0.2	>0.1

was 2–12 ng. Correlation coefficients, r (response ratio *vs.* amount of alkaloid), were from 0.999 to 1.000 for all calibrations. Dictyocarpine and dictyocarpinine were independently calibrated and found to be closely comparable in detectable response. Therefore, a mixed standard containing delpheline, deltamine, deltaline, 14-acetyldictyocarpine, dictyocarpine (representing the unresolved mixture) and delcosine was used to calibrate the GC system for analysis of alkaloid mixtures from larkspur.

Total alkaloid extract yields for the two larkspur species varied according to the phenological growth stage of the samples. Yields (based upon air dry plant weight) ranged from 0.5 to 5% for *D. occidentale* and from 0.4 to 3% for *D. barbeyi*. Figs. 2 and 3 show the GC separation of total alkaloids extracts obtained from early growth leaf

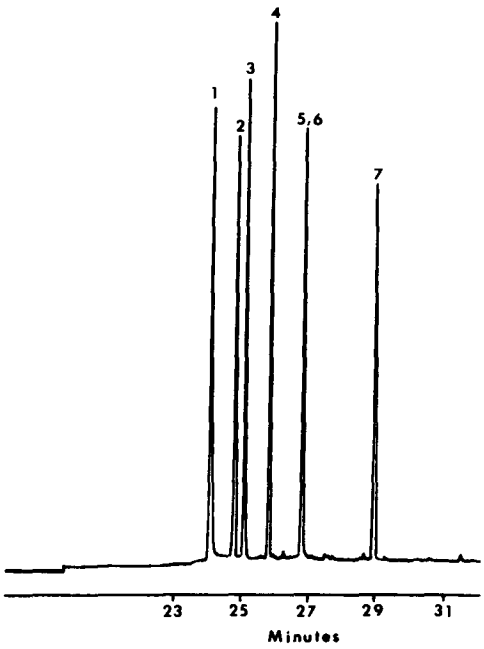

Fig. 1. Capillary GC trace of seven diterpenoid alkaloids. Peaks: 1 = delpheline; 2 = deltamine; 3 = deltaline; 4 = 14-acetyldictyocarpine; 5,6 = dictyocarpine and dictyocarpinine; 7 = delcosine.

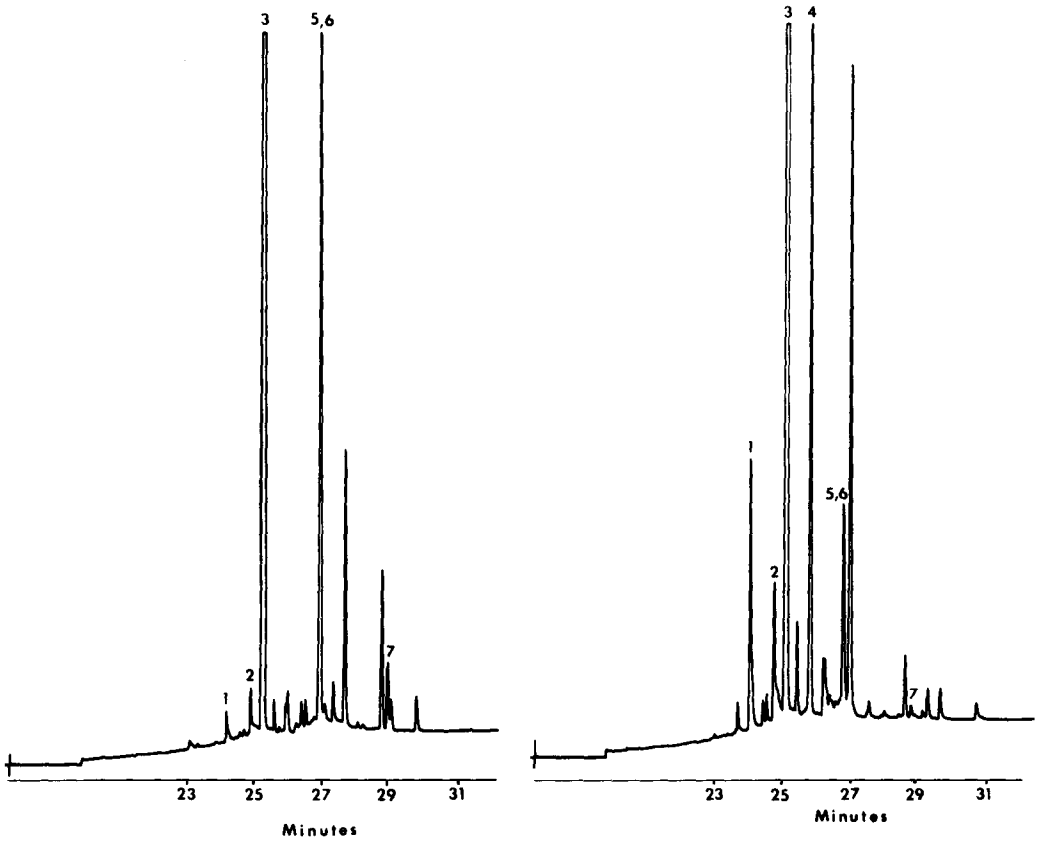

Fig. 2. Capillary GC trace of total alkaloid extract of *Delphinium occidentale*. Peaks: 1 = delpheline; 2 = deltamine; 3 = deltaline; 5,6 = dictyocarpine and dictyocarpinine; 7 = delcosine.

Fig. 3. Capillary GC trace of total alkaloid extract of *Delphinium barbeyi*. Peaks: 1 = delpheline; 2 = deltamine; 3 = deltaline; 4 = 14-acetyldictyocarpine; 5,6 = dictyocarpine and dictyocarpinine; 7 = delcosine.

material of *D. occidentale* and *D. barbeyi* respectively. Results of the quantification of the known diterpenoid alkaloids in these extracts is presented in Table II. The results clearly indicate that the seven standard alkaloids comprise over 50% of the total alkaloid extract obtained from *D. occidentale* and *D. barbeyi* although the occurrence of dictyocarpinine in *D. barbeyi* is negligible (established by thin-layer chromatography) and the occurrence of 14-acetyldictyocarpine and delpheline is very low in *D. occidentale*. Each of the larkspur species shows the occurrence of an unidentified constituent (*D. occidentale*, 27.83 min; *D. barbeyi*, 27.14 min) which appears in negligible yield in the other species. Isolation and identification of these compounds and the other unidentified constituents present in the alkaloid extract of these larkspur species is currently underway in our laboratory. The identification of 14-acetyldictyocarpine in *D. barbeyi* is the first reported natural occurrence of this diterpenoid alkaloid.

CONCLUSION

The results of this investigation show that diterpenoid alkaloids occuring in extracts of *Delphinium* can be detected (2 ng minimum) and quantified by capillary GC. The quantification of alkaloids from *D. occidentale* and *D. barbeyi* extracts illustrates the speed and sensitivity of this technique and its potential for the accurate assessment of the phenological variation of *Delphinium* sp. diterpenoid alkaloids in relation to their toxicity to rangeland cattle.

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