ALKALOIDAL ASSAYS OF DELPHINIUM ANDERSONII, GRAY.*

BY M. R. MILLER.†

A number of species of Delphinium, the larkspurs, have been investigated chemically on account of their physiological activity. Poisoning of range animals, particularly in the West, has led to the examination of suspected species with the result that a number are known definitely to contain poisonous alkaloids, while others have only been reported as poisonous.

The properties of mixtures of alkaloids obtained from *Delphinium bicolor*, *D. menziesii*, *D. scopulorum*, and *D. nelsonii* have been described by Heyl.[1] The quantities in which the alkaloids were present varied from 0.27 to 1.20 per cent. Keller[2] obtained alkaloids from the seeds of *D. consolida*, but not in the blossoms. *Delphinium nelsonii* was reported by Loy, Heyl, and Hepner[3] to contain crude alkaloids as follows: flower, 0.79 per cent.; pod, 0.60 per cent.; seed, 1.27 per cent.; leaf, 0.34 per cent.; and root, 0.48 per cent. In *D. glaucum* there was found: flower, 0.77 per cent.; leaf, 0.62 per cent.; and root, 1.79 per cent. In *D. geyeri:* leaf and stem, 1.15 per cent.; and root, 0.93 per cent. The physical properties of the poisons obtained by Beath[4] from *D. geyeri* and *D. subalpinium* (*D. barbeyi*) were found to present striking resemblances. *Delphinium geyeri* yielded amorphous alkaloidal products only, while *D. subalpinium* yielded a crystalline alkaloid only in the early stages of growth. It was reported by this author that the crystalline alkaloids combine with an acid similar to aconitic acid to form an amorphous alkaloidal product with a greater toxicity than the crystalline alkaloid. *Delphinium geyeri* was found to contain crude alkaloids to the extent of 1.5 per cent. in the stems and leaves of early growth. The mature stems and flowers contained 0.7 per cent. crude alkaloids. *Delphinium subalpinium* contained smaller quantities, *viz.*, 0.7 per cent. crude alkaloids in the early growth and 0.3 per cent. in the mature leaves, stems and flowers. The crude alkaloids found in the stems and leaves of *D. glaucescens* at the early growth stage is reported as 0.6 per cent. The mature leaves, stems and flowers were found to contain less than in the early growth, namely, 0.25 per cent.

Thus, while the literature contains data on a number of species of Delphinium, nothing has been found in regard to the amounts or kinds of alkaloids to be found in *D. andersonii*. *Delphinium andersonii*, one of the low larkspurs, so named from its habit of growth in distinction to the tall larkspurs as represented by *D. glaucescens*, is found in abundance in certain areas of Nevada and has been found to be responsible for cattle losses under certain conditions. The roots of this species have been reported harmless by Marsh, Clawson and Marsh[5] when fed to sheep but the above-ground parts have been found, at this Station, to be toxic to cattle.

As there was not sufficient material available for the preparation of the alkaloids, alkaloidal assays were made on the leaves and stems of the plant. The plants had been collected for use in feeding tests at the stage just before flowering from an area near Carson City, Nevada, and the samples received at the laboratory were in a badly wilted condition. The material, comprising principally the above-

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† Chemist, Nevada Agricultural Experiment Station.

ground portions, were allowed to become air-dried after which the material was finely powdered and moisture determinations made. From the moisture data the contents of alkaloids found were calculated to dry material basis.

For the determination of the alkaloids, two methods were employed: (*a*) the method used by Beath,[4] (*b*) the U. S. P. method for the assay of belladonna leaves. The methods are given briefly as follows:

(*a*) Twenty-five grams of powdered air-dried material are percolated free from alkaloids with 90% alcohol. The percolate, concentrated to approximately one-third its volume under reduced pressure, is made faintly acid with 1% sulphuric acid and concentrated further by means of a current of air. The concentrated aqueous solution, after washing with petroleum ether, is made alkaline with ammonium hydroxide and the alkaloids extracted with chloroform. The alkaloidal extract after removal of the solvent, is taken up with $N/50$ sulphuric acid, the excess titrated with $N/50$ potassium hydroxide (lacmoid indicator) and the alkaloidal content calculated as delphinin ($C_{31}H_{49}O_7N$).

(*b*) Twenty-five grams of powdered air-dried material are extracted in the presence of a little ammonium hydroxide with a chloroform-ether mixture, first by shaking in a flask and then by percolation. The percolate is run into dilute sulphuric acid and when percolation is complete the alkaloids are removed from the solvent by shaking. The acid solution is then made alkaline with ammonium hydroxide and the alkaloids extracted with chloroform. The chloroform is driven off, the residue taken up with standard sulfuric acid and the excess titrated with a volumetric solution of potassium hydroxide, using cochineal or iodeosin as indicator. The alkaloidal content is calculated as delphinin as above.

The results obtained by the two methods were:

No.	Method.	Alkaloid, %
1	(*a*)	1.90
2	(*a*)	1.75
3	(*a*)	1.76
4	(*b*)	1.64

In the application of method (*a*) the aqueous solution could not be washed by petroleum ether to a yellow color, as was found by Beath, but retained enough coloring matter to cause it to be of a dark color. Troublesome emulsions were encountered during the chloroform extractions. Cochineal proved to be more satisfactory as an indicator than lacmoid or iodeosin as advised in the procedures.

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