POISONOUS PROPERTIES OF BIKUKULLA CUCULLARIA (DUTCHMAN'S-BREECHES) AND B. CANADENSIS (SQUIRREL-CORN)[1]

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INTRODUCTION

Since the time of the early settlements in the mountains of Virginia frequent fatal cases of poisoning have occurred among cattle grazing in the mountain pastures in early spring. It has long been believed that certain early spring plants popularly known as "staggerweeds" have been the cause of these fatalities, since these plants are among the first to appear in the pastures and are often eaten by cattle when other forage is not abundant. Suspicion has chiefly centered upon the plants commonly called larkspur (*Delphinium tricorne* Michx.), dutchman's-breeches (*Bikukulla cucullaria* (L.) Millsp.), squirrel-corn (*B. canadensis* (Goldie) Millsp.), and wild bleeding heart (*B. eximia* (Ker) Millsp.). In the literature relating to poisonous plants the toxic character of *D. tricorne* has long been recognized, but the American species of Bikukulla appear to have received comparatively little attention from chemists and practically none from toxicologists.

The probable poisonous character of species of Bikukulla was first brought to the attention of the Department of Agriculture in June, 1920, by Prof. H. S. Stahl, of the Virginia Polytechnic Institute. He submitted specimens of "little staggerweed," later identified as *Bikukulla cucullaria*, with the statement that this plant was believed to be responsible for the recent death of a number of cattle in the mountain pastures of Bland County, Va. An extract of the plant prepared in the laboratory was found to be highly toxic, and in order to secure material for further study a representative of the department visited the locality where the cases of poisoning had occurred. At the request of Dr. A. W. Drinkard, jr., Director of the Virginia Agricultural Experiment Station, arrangements were made to conduct some cooperative experiments with a view to determine by feeding tests the effect upon cattle of the plant material collected for chemical examination.

As a result of these investigations *Bikukulla cucullaria* has been shown to contain a poisonous alkaloid heretofore unrecognized, and the toxicity of this plant for cattle has been demonstrated. Poisonous alkaloids have also been found in *B. canadensis*, but this species is much less toxic than *B. cucullaria* and apparently is not likely to cause any damage to cattle.

[1] Accepted for publication Sept. 13, 1921. The plants referred to in the title are also known in botanical literature under the following genus names: Dicentra, Bicuculla, Dielytra, Diclytra, Corydalis, Fumaria, and others.

SYSTEMATIC POSITION OF "LITTLE STAGGERWEEDS"

BIKUKULLA

Perennial and smooth herbs with basal ternately compound, dissected delicate leaves; watery juice; horizontal rootstocks and scapose, racemose, nodding inflorescence. Flowers flattened, either deciduous or withering persistent. Pedicels 2-bracted. Sepals 2, scalelike. Petals 4, the two outer spurred at base, loosely united; the two inner pair narrower, and their callous-crested tips united over the stigmas. Stamens 6, in two groups opposite the outer petals, hypogynous; their filaments often united; middle anthers of each set 2-celled, the lateral ones 1-celled. Pod 10 to 20 seeds, seeds crested.

A genus of about 13 species, ranging across North America to Eastern Asia. Six species are known on the Pacific slope of America and 3 on the Atlantic watershed.

The bleeding heart (*Bikukulla spectabilis* (L.) Coville) of the gardens is an Asiatic species.

KEY TO ATLANTIC SPECIES

Racemes simple; rootstocks tuber-bearing.
- Rootstocks long, with scattered cornlike, yellow tubers; flowers cordate; spurs rounded; inner petals conspicuously crested.....1. *B. canadensis.*
- Rootstocks much shortened; tubers gathered in a scaly, granulated bulb, white, becoming pink and dark red; flowers sagittate; spurs spreading, elongated; inner petals minutely crested.....2. *B. cucullaria.*

Racemes compound; rootstocks scaly, not tuber-bearing.....3. *B. eximia.*

1. *Bikukulla canadensis* (Goldie) Millsp. Squirrel-corn. Turkey-corn. Turkey-pea. Little blue stagger. Trembling stagger. Staggerweed. Colicweed. Wild hyacinth.

Foliage bright green; scapes erect 6 to 12 inches high, overtopping the leaves; flowers greenish white, tinged with pink. On vegetable mold in woods. April and May. Nova Scotia, Ontario, Minnesota, Missouri, and south along the mountains to North Carolina and Tennessee. In Virginia it occurs abundantly on northern slopes in the Alleghany Mountains and often in the Blue Ridge.

It rises to 4,500 feet altitude on White Top Mountain, although it seems most at home in the neighborhood of 3,000 feet. Gorge of the Potomac above Washington. Rare.

2. *Bikukulla cucullaria* (L.) Millsp. Dutchman's-breeches. Little blue stagger. Trembling stagger. Staggerweed. Colicweed. Soldier's-cap. Whitehearts. Indian boy-and-girls. Butterfly banners, etc.

Foliage often glaucescent; scapes 6 to 12 inches high, usually overtopped by the leaves; flowers white with yellowish tips. April and May.

On vegetable mold in the woods. Growing in Virginia with *Bikukulla canadensis* and *Delphinium tricorne* but usually preferring better drained soil than the former. Nova Scotia, Ontario, Minnesota, Nebraska, Kansas, and Missouri, and south in the mountains to Alabama. Common on the northern slopes of the Alleghany Mountains and often in the Blue Ridge, being more abundant around 3,000 feet altitude and rising on White Top Mountain to about 4,500 feet. Gorge of the Potomac above Washington.

3. *Bikukulla eximia* (Ker) Millsp. Wild bleeding heart. Staggerweed. Turkey-corn.

Leaves dark green; scapes 1 to 2 feet high; longer than the leaves; flowers deep pink. Mountain rocks and river gorges, southern Appalachian Mountains from Wills Mountain, Allegany County, Md., south to North Carolina and Tennessee.

CHEMICAL EXAMINATION

As might be expected in a group so nearly related to the poppy family, species of Bikukulla have been found to contain alkaloids. Wenzell (*7*)[2] in a study of *Bikukulla canadensis* (*Corydalis formosa*) reports the presence of an alkaloid which he assumed, on very insufficient evidence, to be corydaline. Fischer and Soell (*3*) made an examination of *B. cucullaria* (*Dicentra cucullaria*) and were able to identify protopine and also to isolate minute quantities of two other alkaloids in crystalline form, which they characterized only by their melting points and certain other physical properties. Battandier (*2*) discovered protopine in *B. formosa* (DC.) Howell (*D. formosa*). Heyl (*5*) made a more thorough study of the same plant and observed that protopine was the chief alkaloid, besides isolating two others which he differentiated by their physical properties.

Gadamer (*4*) found protopine in *B. spectabilis* (*D. spectabilis*), with a yield of 1 per cent, and surmised the presence of other alkaloids. Asahina (*1*) obtained a crystalline alkaloid from *B. pusilla* (Sieb. & Zucc.) Coville (*D. pusilla*), which he named dicentrine, and also isolated protopine from the plant. He made a very complete chemical study of dicentrine and concluded that it was identical with one of the unnamed alkaloids found by Heyl in *B. formosa* having a melting point of 169° C. Dicentrine was studied from a pharmacological standpoint by Iwakawa (*6*), who found that it produced narcosis when administered to small animals in moderate doses, whereas larger quantities caused convulsions, weakening of the heart, and respiratory paralysis.

A search of the literature has failed to reveal any publication on the constituents of *Bikukulla eximia*.

Table I brings out the more essential facts in the preceding summary.

TABLE I.—*Comparative chemical analyses of species of Bikukulla made by various investigators*

	BIKUKULLA CUCULLARIA, Fischer and Soell.		
Alkaloids	Protopine.		
Melting point	206° C.	231° C.	215° C.
Solubility	Soluble in alcohol. Soluble in ether.	Insoluble in alcohol. Soluble in $CHCl_3$.	Soluble in alcohol.
Form	Cryst. pr.	Needles, rosettes	Fine granular.
Color tests:			
Concentrated H_2SO_4.	.....	Red to Brown.	
Erdmann's	.....	Red to violet.	
Froede's	.....	Red to violet.	
Concentrated HNO.	.....	Red to yellow.	

[2] Reference is made by number (italic) to "Literature cited," p. 78.

TABLE I.—*Comparative chemical analyses of species of Bikukulla made by various investigators*—Continued.

		BIKUKULLA FORMOSA, G. Heyl.	
Alkaloids	Protopine		
Melting point	201° to 202° C.	168.5° C.	142.5° C.
Solubility		Soluble in H Br. salt. Insoluble in alcohol.	Soluble in H Br. salt. Soluble in alcohol.
Form	Needles	Yellow needles	White needles.
Color tests:			
Concentrated H_2SO_4.	Yellow	Colorless to red violet.	Colorless.
Erdmann's	Yellow to green	Blue	Weak green.
Froede's	Violet	Deep blue	Blue green.
Concentrated HNO.	Colorless	Colorless to brown	Brown.

	BIKUKULLA PUSILLA, Y. Asahina.	
Alkaloids	Protopine	Dicentrine.
Melting point	207° C.	168–9° C.
Solubility	Soluble in alcohol. Soluble in ether.	Soluble in hot alcohol. Soluble in $CHCl_3$.
Form	Prisms	Prisms.
Color tests:		
Concentrated H_2SO_4	Yellow to blue	Colorless to violet.
Erdmann's	Yellow to violet	Blue.
Froede's	Violet to blue	Deep blue.
Concentrated HNO	Colorless to brown	Colorless to brown.

It will be seen from Table I that protopine seems to be the alkaloid most common to the members of the genus, and if Asahina's (*1*) supposition is correct dicentrine is common to *Bikukulla formosa* and *B. pusilla* and possibly may be contained in others of the group. It seems not improbable that the protopine of Heyl (*5*) and Gadamer(*4*), melting at 201° C., may be a different body from that of Fischer(*3*) and Asahina(*1*), melting at 207° C. Some of the work summarized above is very incomplete, with conclusions often based on color reactions, which are notoriously deceptive. The physiological action of these alkaloids had not been studied, with the exception of protopine, which has long been known as one of the opium group, and dicentrine in Iwakawa's(*6*) report. Neither of these two alkaloids would seem to possess sufficiently toxic qualities to account for the symptoms of poisoning in animals fed with *B. cucullaria* (Pl. 1, A) and *B. canadensis* (Pl. 1, B).

The material on which the following chemical work was done was gathered at Round Bottom, Va., in April, 1921, just previous to the flowering stage. The tops and roots of *Bikukulla cucullaria*, and only the roots of *B. canadensis* were used in the experiments. The tops of the *B. cucullaria* were dried and in good condition, but the roots of both plants were contaminated with soil which was removed by sieving in a stream of running water. They were then dried, first in the air and then in an oven at 100° C., and ground fine in a mill.

The object of the study here reported was to determine what constituents of the plants were responsible for their toxic effect, and as it was known that both contained alkaloids it was obviously necessary to isolate and test the toxicity of these compounds. As a preliminary experiment a few grams of the ground roots of the two plants were allowed to macerate in Prolius' solution for 48 hours. The solution was then decanted and evaporated spontaneously until nearly dry, and the residue was extracted with dilute hydrochloric acid. Both these solutions gave a heavy precipitate when tests were made with Mayer's reagent, showing the presence of alkaloids. The acid solutions were then made strongly ammoniacal, which threw down some of the dissolved material as a white flocculent precipitate, and were shaken out exhaustively with ether. After the ether evaporated, the residue was again taken up with very dilute hydrochloric acid. One-half cc. of each of these two solutions, which represented the raw alkaloids of *Bikukulla cucullaria* and *B. canadensis*, was injected subcutaneously into two white mice. The animal receiving the dose of *B. canadensis* gave some slight evidence of narcosis but suffered no other ill effects. The mouse injected with the *B. cucullaria* extract also behaved in a sleepy manner for some time and died unobserved inside of two hours. Another mouse was then given a similar injection of the same *B. cucullaria* extract, and his reactions were carefully followed. The animal showed no striking symptoms in the course of an hour, then suddenly grew restless for a few minutes, was seized with acute convulsions, and died almost immediately. These results seemed to indicate that the roots of *B. cucullaria* were poisonous, whereas those of *B. canadensis* were harmless, but upon further investigation this conclusion was modified.

An approximate assay of the total alkaloids in the material which was available, namely, the tops and roots of *Bikukulla cucullaria* and the roots of *B. canadensis* was undertaken as follows: Of each of these three samples 5 gm., dried and finely ground, were macerated for 48 hours in 50 cc. of Prolius' solution, the solution was decanted off and the residue washed with a little alcohol and added to the solution, which was then extracted with 50 cc. of 5 per cent H_2SO_4 in three portions. The acid extract was made alkaline with ammonia and shaken out exhaustively with ether. The ether solution was distilled to small bulk, transferred to a tared beaker, and allowed to evaporate spontaneously, whereupon it was completely dried in a desiccator and weighed. Table II shows the results of the three determinations.

TABLE II.—*Assay of total alkaloids found in Bikukulla cucullaria and B. canadensis*

Plant.	Weight of plant.	Alkaloid.		Character of residue.
			Per cent.	
B. cucullaria:	*Gm.*	*Gm.*		
Tops	5	0.062	1.24	Amorphous.
Roots	5	.080	1.60	Amorphous, with some needles.
B. canadensis:				
Roots	5	.157	3.14	Amorphous, with rosettes.

With known weights of the raw alkaloids from definite quantities of the three samples, the way was now open for comparative tests of their toxicity. To this end all three total alkaloidal residues were dissolved in exactly 5 cc. of very dilute acetic acid, and 0.5 cc. of each was injected subcutaneously into three mice. The result was the same in each instance the animal died almost instantly in violent convulsions. The dosage was as follows:

B. cucullaria:
Tops.....0.5 gm. plant=0.0062 gm. alkaloid.
Roots.....0.5 gm. plant= .0080 gm. alkaloid.
B. canadensis:
Roots.....0.5 gm. plant= .0157 gm. alkaloid.

By testing these solutions at gradually increasing dilutions it was possible to determine within narrow limits the minimal fatal dose of the three with respect to mice of about 20-gm. weight, as shown in Table III.

TABLE III.—*Minimal fatal dose of Bikukulla cucullaria and B. canadensis for mice of 20-gm. weight*

Plant.	Weight of plant.	Alkaloid.	Total dose per kilogram of body weight.
B. cucullaria:	*Gm.*	*Gm.*	*Gm.*
Tops	0.040	0.0005	0.02
Roots	.031	.0005	.02
B. canadensis:			
Roots	.250	.0100	.40

From these figures it can be estimated that the combined alkaloids of *Bikukulla cucullaria* roots and those of the tops are of approproximately the same degree of toxicity.

This finding runs counter to observations made in the field tests on calves, as reported elsewhere, in which 60 pounds of the tops were fed without effect. A possible explanation of this result is that the bulk and succulence of the fresh tops so dilutes the poison that its action is minimized and it is absorbed so gradually that the animal suffers no ill effects. Nevertheless the fact remains that the tops contain the poison and must be held partially accountable for the poisoning of grazing stock which eat the whole plant. Applying the foregoing figures to a comparison of the relative toxicity of the roots of *Bikukulla cucullaria* and *B. canadensis*, it will be seen that the alkaloids of *B. cucullaria* are about 20 times as poisonous as those of *B. canadensis*, but as there is a considerably larger percentage of alkaloids in *B. canadensis* the ratio of toxicity between the two plants would be reduced to approproximately 6 to 1.

As a preliminary experiment to acquire some information about the character of the alkaloids in the two plants under investigation, 100 gm. of each were extracted in a Sohxlet apparatus with the selective solvents, ether, chloroform, alcohol, and acidified water. In the case of *Bikukulla cucullaria* the acidified water gave only the faintest of tests with Mayer's reagent, showing that the other three solvents had made a complete extraction. All three solutions contained appreciable quantities of alkaloids. From the chloroform and alcohol fractions only amorphous

products were obtained, but from the ether extract a crystalline alkaloid was isolated. After the ether was distilled off the residue was triturated with dilute hydrochloric acid until all the alkaloid was removed and the acid solution was made ammoniacal and shaken out with ether. The etherial solution was concentrated to small bulk, the same volume of alcohol was added, and on slow evaporation there were deposited warty masses of colorless crystals. These were removed and pressed between filters and recrystallized from a mixture of alcohol and chloroform. After one recrystallization they melted at 168° C., which was not changed by a further purification of the same kind. It was at once assumed that the dicentrine of Asahina (*1*) had been isolated, but after testing the compound on mice it was evident that it was another substance, and one of much higher degree of toxicity. By methods already described, it was found that 0.04 mgm. of the alkaloid was fatal to a mouse. The animal exhibited a period of intense excitement followed by convulsions and death, whereas Iwakawa in trying out dicentrine on mice found the fatal dose to lie between 5 and 10 mgm., and his animals experienced a prolonged period of narcosis before death. These facts would seem sufficient to differentiate the two compounds.

Another alkaloid is known with a melting point of 169° C., namely, Y-homochelidonine, found chiefly in *Sanguinaria canadensis* L. It has always been prepared in conjunction with its physical isomeride B-homochelidonine, melting point 159°, but no trace of the latter was found in our product. Moreover, the physiological action of Y-homochelidonine is like morphine, chiefly narcotic. It is therefore reasonable to assume that the alkaloid isolated from *Bikukulla cucullaria* is new, and we propose to name it provisionally cucullarine. Unfortunately, the small quantity at our disposal has made it impossible to make a complete study of it, but this we intend to do later when larger quantities of material are available. We have been able, however, to note the following properties: It crystallizes in characteristic warty masses of prisms; it is colorless when first prepared but changes to pink in the light; it is soluble in ether and chloroform, but not so soluble in alcohol; it is precipitated by the usual alkaloid reagents, Mayer's, iodin in potassium iodid, picric acid (crystalline), and platinum chlorid; with concentrated sulphuric acid a small crystal dissolves with difficulty, producing a violet coloration turning brown.

Another portion of *Bikukulla cucullaria* was exhaustively extracted with 95 per cent alcohol containing a little acetic acid, the alcohol was removed by vacuum distillation, and the residue was taken up in dilute acetic acid and allowed to stand for some time. The small quantity of resin which separated was filtered off, and the solution was made alkaline with ammonia and shaken out first with ether then with chloroform. Very little alkaloid was recovered from the latter extract, the great bulk being removed by the ether. The etherial solution was set aside for several days with the expectation that protopine would crystallize out. This did not occur, however. The ether was then distilled off, and the raw alkaloid fractioned in various ways with the production only of amorphous products. Our failure to isolate protopine, reported by Fischer and Soell in *B. cucullaria*, may be due to the comparatively small quantities of material on which the work was done or to the stage of maturity of the plant. Fischer and Soell do not say when their *B. cucullaria* was gathered and are rather obscure in their description of the technic they employed.

As with *Bikukulla cucullaria* it has not been possible as yet to give the alkaloids of *B. canadensis* a complete survey. It seems highly probable that the latter plant contains cucullarine, as the physiological effects produced on mice by the raw alkaloids of both plants are so similar. If present in *B. canadensis* it undoubtedly must be there in very minute quantity, as the toxicity of *B. cucullaria* is so much greater. This may account for the failure so far to isolate it. However, a crystalline alkaloid from the roots of *B. canadensis* has been prepared. The material was percolated with 95 per cent alcohol, plus acetic acid, and the solution was treated in the usual way. By extracting this preparation with ether which contained a small quantity of alcohol a bright yellow compound was obtained, crystallizing in bundles of silky needles. It has a melting point of 210° C. and gave reactions with the customary alkaloid reagents and a crystalline precipitate with picric acid. It proved to be nontoxic when injected into a mouse. A dose of 0.6 mgm. caused the animal no apparent inconvenience.

It is proposed to make a further study of the alkaloids of *Bikukulla cucullaria*, *B. canadensis* and *B. eximia* in more detail with larger quantities of material.

FEEDING EXPERIMENTS

Feeding experiments with both *Bikukulla cucullaria* and *B. canadensis* were made by the representative of the Virginia Agricultural Experiment Station on a farm near Merriam, Bland County. The material used was collected on this farm and fed in a fresh condition. The animals used were healthy yearling steers, weighing approximately 275 pounds each.

At first these plants only were fed, but the animals ate of them so sparingly that it was found necessary to mix them with grass before they were taken freely. Account was kept of the weight of the grass and of the plants fed to each animal and of the portion which remained uneaten, from which the approximate weight of the suspected plants eaten was calculated. A detailed account of the experiments follows.

STEER 2.—The whole plant of *Bikukulla cucullaria* was given this animal. Feeding was begun at 2 p. m., April 19, 1921, but on that day only ½ pound of the plant was eaten; April 20, 3½ pounds were consumed between 7.30 a. m. and 5 p. m., without noticeable effects. Feeding was resumed at 5.15 p. m.; and at 5.30, when the animal had eaten about another pound of the plants, symptoms of poisoning were first exhibited. He suddenly began to tremble, ran backward then forward several times with the head held very high. He was frothing at the mouth and several times ejected partly digested stomach contents a distance of several feet. The trembling which occurred all over the body became more violent, convulsions ensued, and at 5.40, 10 minutes after the first seizure, he fell and was unable to rise. He lay upon the left side, with the head thrown back as in opisthotonos, and with the legs extended and rigid. He moaned as though in great pain, the eyes were glassy, breathing was very difficult, and the bowels were lax. About 5.50 relaxation began; the animal struggled but was unable to rise until 6.10, when he got up; and although very weak and nervous he was able to walk about. From this time his condition improved rapidly, and, when no other symptoms of poisoning appeared, he was returned to the pasture at noon, April 21.

STEER 5.—The bulbs alone of *Bikukulla cucullaria* were fed to this animal. Feeding was begun at 2 p. m., April 19, but practically none of

the material was eaten until the following day when approximately 5 pounds were consumed between 7.30 a. m. and 5.30 p. m. Then trembling, convulsions and all other symptoms manifested by steer 2 were exhibited. As in the case of steer 2 this aninmal was eating heartily of the bulbs at the time of seizure.

There was no appreciable difference in the time both animals were down or in their condition during recovery. These two cases agree so closely in every respect that they are regarded as practically identical.

STEER 4.—Between April 19 and April 25 this animal received 60 pounds of *Bikukulla cucullaria* tops which included the flowers. There were no ripe seeds in the material. The quantity consumed daily ranged from 4 to 11½ pounds. No symptoms of poisoning were observed during the seven days the animal was under observation.

STEER 3.—This animal consumed approximately 55 pounds of the entire plant of *Bikukulla canadensis* between 5.30 p. m., April 19 and 12.30 p. m., April 27. The quantity consumed daily ranged from 3½ to 9½ pounds. Aside from a slight restlessness and uneasiness on the second day of feeding, when 7½pounds of the plant were eaten, no injurious effect was observed.

STEER 6.—This animal was brought in for feeding on the afternoon of April 24, and was given the entire plant of *Bikukulla canadensis*. Feeding was continued until noon, April 27, up to which time he had consumed 16 pounds of this material. Six and one-half pounds of the plant were eaten on April 24, and on April 25 the animal was restless and uneasy practically all day, but no other symptoms were manifested.

These experiments confirm the popular opinion that *Bikukulla cucullaria* is poisonous to cattle, but more definite feeding experiments must be performed with *Bikukulla canadensis* before it can be positively stated that this species constitutes a menace to live stock.

CONDITIONS UNDER WHICH POISONING MAY OCCUR

Most of the cases of poisoning occur early in the spring. The danger is usually past by the middle of May, although in some of the higher altitudes poisoning may occur as late as June. Poisoning is said to occur most frequently following heavy rains, when, the soil being soft, the bulbs of the plant are mostly likely to be pulled up and eaten. The experiments show that both species of *Bikukulla* used are unpalatable to cattle and therefore unlikely to be eaten in harmful quantities when suitable forage is available.

SUMMARY

(1) *Bikukulla culcullaria* and *B. canadensis*, in Virginia popularly called "little staggerweeds," have long been considered poisonous to cattle.

(2) Chemical examination has shown that both these plants contain toxic alkaloids and that the tops as well as the bulbs of *B. cucullaria* are poisonous.

(3) *B. cucullaria* contains at least one alkaloid of a highly poisonous nature. This alkaloid, heretofore apparently unknown, has been named cucullarine, and its properties are described.

(4) Cucullarine probably occurs in *B. canadensis* also, since its physiological effect on mice closely resembles that of *B. cucullaria*.

(5) Feeding experiments show that *B. cucullaria* is toxic for cattle.

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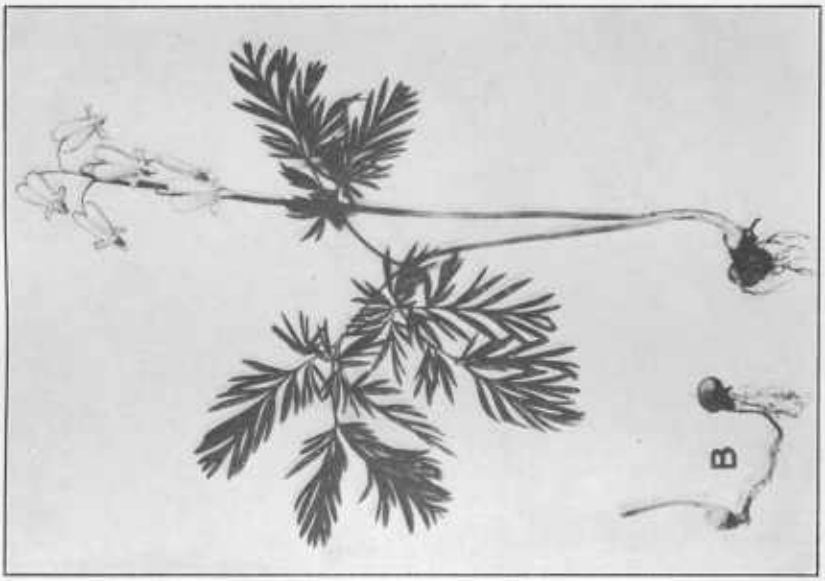
PLATE I

A.—*Bikukulla cucullaria*, Dutchman's-breeches.
B.—*Bikukulla canadensis*, squirrel-corn.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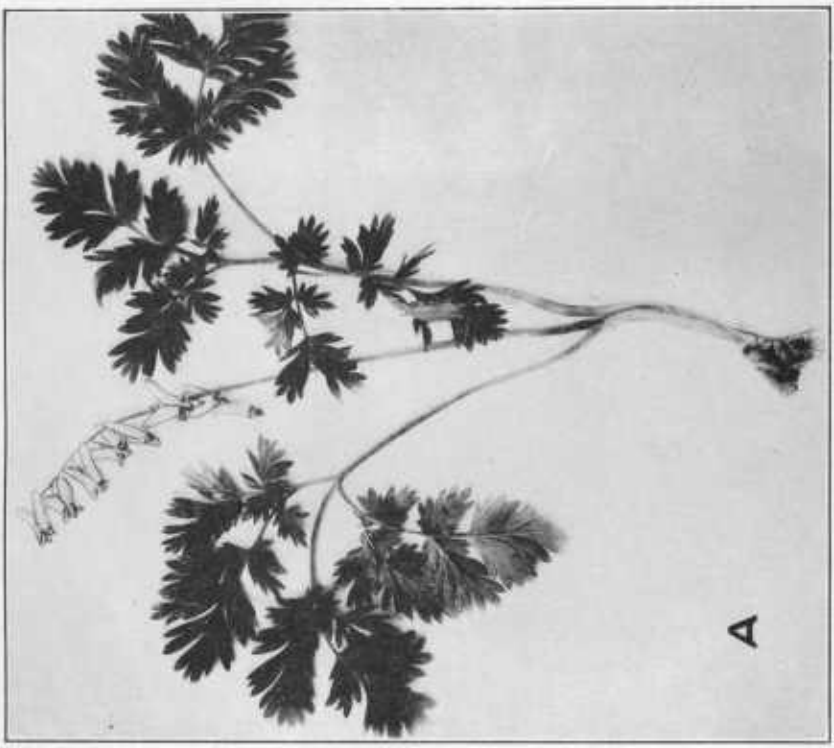
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