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A BOTANICAL AND CHEMICAL STUDY OF BIKUKULLA EXIMIA, WITH A KEY TO NORTH AMERICAN SPECIES OF BIKUKULLA¹

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INTRODUCTION

In 1921 a study was made of the alkaloids of *Bikukulla cucullaria* and *B. canadensis* in relation to their poisonous properties to grazing animals² and from the former there was isolated a new and extremely toxic alkaloid to which was given the name cucullarine. *B. canadensis* was also found to be poisonous but to a less degree. In view of these results it was thought worth while to make a chemical study of *B. eximia*, since no such study had previously been reported.

All the members of the *Bikukulla* group on which chemical work has been reported are known to contain alkaloids, which is not surprising since they are closely allied to the poppy family. Few of the alkaloids, however, have been examined with sufficient care to prevent confusion as to their identity, and, with the exception of protopine, dicentrine, cucullarine, and an unnamed alkaloid isolated from *B. canadensis*, practically nothing is known about their toxicity.

BIKUKULLA EXIMIA

BOTANICAL DESCRIPTION

Bikukulla eximia (Ker) Millsp. Fringed bleedingheart. Also known as wild bleedingheart, staggerweed, and turkey corn. (Fig. 1.) Perennial, smooth herb, with dark-green basal leaves ternately compound, finely cut into oblong or ovate segments; scaly rootstocks; flower pedicels rising from the root 1 to 2 feet high, taller than the leaves, in compound lengthened clusters; flowers flattened, usually withering and persistent; sepals 2, scalelike; petals 4, the outer produced into rounded sacs at the base, 2 inner petals crested; stamens 6, in two groups, their filaments often united; pods several seeded, splitting; seeds black, warty, crested. Habitat, mountain rocks and river gorges.

This species was found at Junius, Seneca County, N. Y., by H. P. Startwell more than a century ago, but it has never been found so far north since. Otherwise the plant ranges in the southern Appalachian Mountains from Wills Mountain, Allegany County, Md., south to Georgia.

EXPERIMENTAL WORK

The material on which the work was done consisted of leaves and twigs of *Bikukulla eximia* collected near Deerfield in Augusta County, Va., by the senior writer in June, 1923. This material, which was in

¹ Received for publication Mar. 19, 1929; issued October, 1929.

² BLACK, O. F., EGGLESTON, W. W., KELLY, J. W., and TURNER, H. C. POISONOUS PROPERTIES OF BIKUKULLA CUCULLARIA (DUTCHMAN'S-BREECHES) AND *B. CANADENSIS* (SQUIRREL-CORN). *Jour. Agr. Research* 23: 69-78, illus. 1923.

good condition when received, was ground to a moderately fine powder. A few grams of the powdered product was extracted with Prollius's solution, and the evaporated extract was taken up with

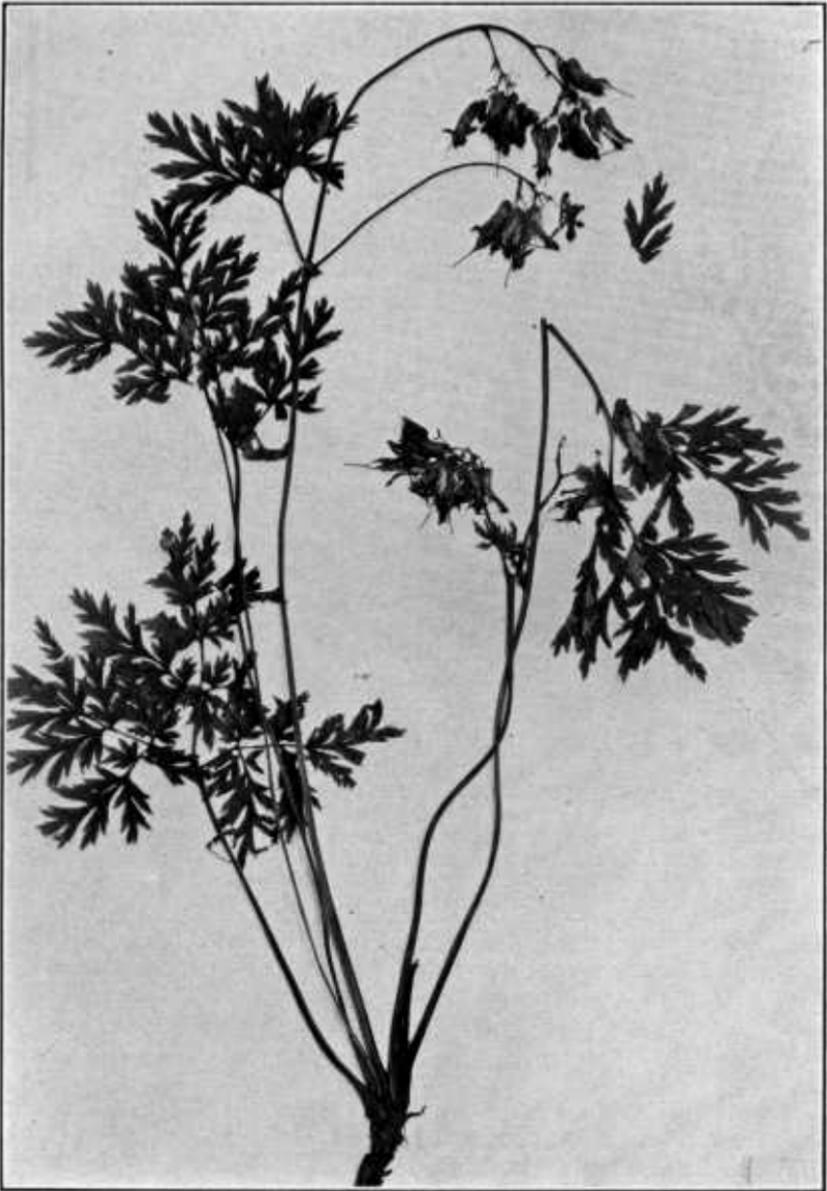


FIGURE 1.—*Bikukulla eximia* in flower. $\times 35$

dilute hydrochloric acid, filtered, and tested with Mayer's reagent. This gave a heavy flocculent precipitate, indicating the presence of alkaloids in quantity.

As the primary object of the work was to find out whether this plant is toxic to animals, the next step was to make a preparation that would contain the total alkaloids from a known weight of the plant and to test the toxicity of the preparation by administering definite doses of it to white mice. To this end 30 gm. of the finely ground plant was moistened with alcohol and acetic acid, packed in a percolator, and thoroughly extracted with 95 per cent alcohol. The resulting extract was evaporated by distillation under reduced pressure, and the residue was taken up with dilute acetic acid and then made alkaline with ammonia. The alkaloid was shaken out with chloroform, which entirely removed it. The chloroform extract was concentrated to a few cubic centimeters, and then extracted with dilute acetic acid. The last extract was made alkaline with ammonia and again shaken out with chloroform, the solvent was evaporated in a tared dish, and the residue was weighed. The residue consisted of an amorphous light-yellow substance weighing 0.7 gm., equivalent to 2.3 per cent of the dry plant. The crude alkaloid was next treated with very dilute hydrochloric acid, which, it was observed, did not effect complete solution. The insoluble portion was dried and weighed. One-half cubic centimeter of the solution, representing 8 mgm. of alkaloid, was then injected subcutaneously into a mouse weighing about 15 gm. No marked symptoms of any kind were observed in the animal, with the possible exception of a slight drowsiness. As 8 mgm. is a very large dose for an animal of 15 gm. weight, it was concluded that the alkaloids contained in *Bikukulla eximia* were of slight physiological activity.

The portion of the crude total alkaloid that did not dissolve in the hydrochloric acid weighed 0.19 gm. and represented approximately 15 per cent of the whole. This was redissolved in a little alcohol and ammonia, and on the addition of water the free alkaloid separated in fine white needle crystals. The crystals were washed on a filter with water, dried, and redissolved in alcohol, in which they were readily soluble, and purified by conversion into the chloride and recovery of the free base. As thus prepared the compound was found to have a melting point of 165° C. (uncorrected) and consisted of colorless needles, very soluble in alcohol and chloroform but insoluble in water. With gold and platinum chlorides it gave only amorphous precipitates, but with picric acid it formed a finely crystalline salt which could be recrystallized from hot alcohol and which had a melting point of 175° C. (uncorrected). The alkaloid, therefore, shows every evidence of its chemical individuality and is differentiated from other alkaloids hitherto found in this group of plants by its insoluble hydrochloric acid salt as well as by other physical properties. There is every reason, then, to believe it to be a new alkaloid, and the name *eximine* is proposed for it.

The physiological action of this new alkaloid was tested by dissolving a weighed quantity in very dilute acetic acid and injecting the solution subcutaneously into a white mouse. A dose of 3 mgm. produced only a slight restlessness, which soon wore off, and no further symptoms could be detected. This alkaloid, therefore, is not of a dangerously poisonous character. As the other alkaloidal contents of the plant were likewise found to be innocuous, it seems safe to conclude that *Bikukulla eximia* should not be classed as a poisonous plant.

Table 1 summarizes the chief chemical and physical characteristics of the alkaloids of the Bikukulla group that have been reported, including eximine, the new compound that has been obtained from *B. eximia*.

TABLE 1.—Comparison of the characteristics of previously reported alkaloids of *Bikukulla* species with those of *B. eximia*

Alkaloid	Species in which the alkaloid occurs	Melting point (° C.)	Solubility	Form
Protopine.....	<i>B. cucullaria</i>	206-208.	Ether chloroform.....	Needles and prisms.
	<i>B. pusilla</i>			
	<i>B. formosa</i>			
	<i>B. spectabilis</i>			
Dicentrine.....	<i>B. pusilla</i>	168-169.	Hot alcohol chloroform.	Prisms.
Cucullarine.....	<i>B. cucullaria</i>	168.....	Ether chloroform.....	Do.
Eximine.....	<i>B. eximia</i>	165.....	Alcohol chloroform.....	Neeeles.
Unnamed.....	<i>B. canadensis</i>	210.....	Alcohol.....	Silky, yellow needles.
Do.....	<i>B. cucullaria</i>	235.....	Alcohol (insoluble).....	Needles.
Do.....	do.....	215.....	Alcohol (soluble).....	Granular.
Do.....	<i>B. formosa</i>	168.5.....	Alcohol (insoluble).....	Yellow needles.
Do.....	do.....	142.5.....	Alcohol (soluble).....	White needles.

Alkaloid	Color tests				Physiological action
	Concentrated H ₂ SO ₄	Erdman's	Froede's	Concentrated HNO ₃	
Protopine.....	Yellow.....	Yellow to violet.	Violet.....	Colorless.....	Narcotic.
Dicentrine.....	Violet.....	Blue.....	Deep blue.....	do.....	Toxic.
Cucullarine.....	Yellow.....	Colorless.....	Green to blue.....	Orange.....	Very toxic.
Eximine.....	Crimson.....	Pale yellow.....	do.....	Brown.....	Nontoxic.
Unnamed.....	Light yellow.....		Green.....	Orange.....	Do.
Do.....	Red to brown.....	Red to violet.....	Red to violet.....	Red to yellow.....	
Do.....	Violet.....	Blue.....	Deep blue.....	Colorless.....	
Do.....	Colorless.....	Green.....	Blue green.....	Brown.....	

FIELD NOTES ON THE GENUS BIKUKULLA

Bikukulla is largely a North American group of plants, with three species in the East (*B. cucullaria*, *B. canadensis*, and *B. eximia*) and several species on the Pacific coast.

An extended field survey was made in the mountain counties of southwestern Virginia in April, 1921, and in May and June, 1923. *Bikukulla eximia* was not observed in 1921, but in 1923 it was collected at Deerfield and also on the Peaks of Otter. *B. cucullaria* and *B. canadensis* were found in abundance in many places in Giles, Bland, Tazewell, Russell, and Washington Counties. They are more abundant in these localities than anywhere else in their range, but they were even more abundant before the forests were generally removed.

A better field knowledge of the western species is being acquired with a view to obtaining material for chemical study. *Bikukulla formosa*, the first species discovered on the Pacific slope, was observed by the senior writer in a number of places in the Sierra Nevada in California, but only a few plants at a time. This species is much more abundant in the moist woods along the streams flowing into Puget Sound and is common in the vicinity of Glacier, Wash. *B. chrysantha*, one of the yellow-flowered species of California, ranges

from central to southern California and is abundant in Bull Run Canyon, Sequoia Forest, Kern County. Three other species (*B. ochroleuca*, *B. uniflora*, and *B. pauciflora*) are of less importance and as yet have not been observed in any quantity. The western form of *B. cucullaria*, considered by many botanists as a distinct species, and perhaps rightly so, should be studied chemically in comparison with the eastern species.

KEY TO NORTH AMERICAN SPECIES OF BIKUKULLA

- Stems leafy, tall; flowers yellow; corolla deciduous; seeds crestless.
 Flowers sulphur yellow; outer petals spreading or recurving to the middle; widely scattered. California and southern Oregon..... 1. *B. chrysantha*.
 Flowers straw yellow or cream colored; outer petals erect or only tips spreading; Santa Ynez Mountains to the Santa Monica Mountains.
 2. *B. ochroleuca*.
- Stems naked; leaves basal; flowers white or pink, nodding; seeds crested.
 Rootstocks tuber bearing; racemes simple.
 Rootstocks long, with scattered cornlike yellow tubers; flowers cordate; spurs rounded..... 3. *B. canadensis*.
 Rootstocks much shortened; tubers at base of stem.
 Tubers gathered in a scaly, granulated bulb, white to dark red; flowers several; sagittate; spurs long, spreading..... 4. *B. cucullaria*.
 Tubers in a fascicle, elongate, tapering toward the base; flowers 1 or 2, outer petals long, tips recurving beyond the body; small; alpine.
 5. *B. uniflora*.
- Rootstocks not tuber bearing.
 Racemes simple; flowers 1 to 3; petals distinct, outer petals narrow, recurved; small; alpine..... 6. *B. pauciflora*.
 Racemes compound.
 Appalachian species; flowers oblong; crest of inner petals projecting.
 7. *B. eximia*.
 Pacific coast species; corolla ovate cordate; petals united. 8. *B. formosa*.

