[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF WYOMING.]

ANALYSIS OF ZYGADENUS INTERMEDIUS.

(FIRST PAPER.)

BY FRED. W. HEYL AND L. CHAS. RAIFORD.

Received November 28, 1910.

The poisonous character of the several species of plants designated as death camas has long been known to the stockmen of certain western states. These plants belong to the genus *Zygadenus* and are found in Montana, Wyoming, Colorado, and other states of the Northwest, where they have from time to time[1] caused heavy stock losses, particularly among sheep.

In Wyoming the most common species is the *intermedius*, and the greatest stock losses noted have usually occurred in the early spring when this plant is in bloom, and before ordinary forage plants are plentiful. In addition to this, there prevails the idea that poisoning is more frequent immediately after rain-storms and it has been suggested that this might be accounted for on the general knowledge that in some plants the active constituent is found chiefly in the roots,[2] and that when the ground is moist and soft the bulbous portion could be more easily uprooted by cattle. Such a view, however, is scarcely supported by our experiments, for analyses show that the bulb and the leaf differ but little in the amount of alkaloid contained. It will be shown that the flower contains a higher percentage of alkaloid than any other part of the plant. In the light of these facts the more probable explanation of the greater frequency of poisoning immediately after rain-storms would seem to be that at such a period either the flowers are more numerous, or the animal then eats a greater quantity of the leaves than usual. A possible explanation of the tendency to eat such a plant at all when anything else is available, at which some surprise has been expressed, may be found in the fact that the plant as a whole has a relatively high food value, while the bulb, which is doubtless eaten to some extent, contains a high percentage of sugar.

A knowledge of the constituents of the several species of the genus *Zygadenus*, or of the details of the behavior of animals subjected experimentally to their influence, is confined to a report of a study of the stock-poisoning plants of Montana, by Chesnut and Wilcox[3] who conducted feeding and other experimental tests with extracts of *Zygadenus venenosus;* and to a preliminary chemical study of the constituents of the same species

[1] Chestnut and Wilcox, Div. Bot., U. S. Dept. Agr., *Bull.* 26, 34 (1901).

[2] Blankinship, Montana Exp. Sta., *Bull.* 45, 91 (1903), states that the bulb of death camas is the most poisonous part of the plant, but his statement is not supported by experimental evidence.

[3] *Loc cit.*

by Slade.[1] The latter obtained, by means of the color tests described by Merck,[2] and Wright and Luff,[3] evidence which led him to conclude that the poisonous character of the plant is due to the presence of the alkaloids sabadine, sabadinine, and veratralbine.

The present paper reports the results of the proximate analyses of the different parts of the species available in Wyoming. In the preliminary work, various methods have been used in securing the alkaloid with a view of subsequently isolating a larger quantity by the method found most suitable. We shall report later upon the properties of this and other constituents obtained.

Experimental Part.

Preparation of Material.—Portions of *Zygadenus intermedius* which together weighed 5 kilograms in the green state were collected between May 26 and June 2, 1910. The first lot consisted of plants that had not reached the flowering stage, while the last contained many plants with flowers, though the latter were not mature. In preparing for analysis the leaves and flowering tops were separated from the bulbs, and the latter in all cases deprived of the outer layer and the roots. The average weight of the plant was 7.4 grams, and moisture determinations at 95–100° showed losses of 75.50 per cent. and 68.93 per cent. on the leaves and flowering tops, and on the bulbs and roots, respectively. The various parts were allowed to dry in the air at a somewhat elevated temperature, under which conditions the leaves dried readily, while the bulbs long remained sticky and had to be sliced. Six weeks were required to dry them to such a state that they could be ground easily. The grinding and sieving necessitated the shielding of one's face in order to avoid the extremely irritating dust, the character of which was probably due to one or more of the sternutatory veratrum alkaloids, possibly veratralbine.

The investigation was begun by extracting separate portions of 2 grams each in a Soxhlet apparatus with ligroin, ether, and alcohol, respectively. The quantities removed are given in percentages in Table I.

TABLE I.

Extract.	Leaf.	Flower.	Bulb.	Root.
Ligroin extract (dried at 93°)	2.04	2.80	0.62	0.89
Ether extract, total	5.47	5.21	3.18	3.05
Ether extract, volatil (110°)	1.33	1.07	1.30	0.80
Alcohol extract (dried at 110°)	35.12	46.27	39.60	lost

The proximate analyses were conducted in accordance with the usual[4] methods, and gave the results stated in Table II.

[1] *Am. J. Pharm.*, **77**, 262 (1905).
[2] *Arch. Pharm.*, **229**, 164 (1891).
[3] *J. Chem. Soc.*, **35**, 415 (1879).
[4] U. S. Dept Agr., Bur. of Chem., *Bull.* **107** (Revised).

TABLE II.

	Leaf.	Flower.	Bulb.	Root.
Moisture	6.91	6.13	7.50	7.56
Starch by diastase	absent	absent	23.53	absent
Pentosans	10.81	7.95	4.41	12.04
Crude fiber	16.17	10.58	5.08	21.53
Protein	13.25	19.73	6.19	6.78
Ash	8.12	8.91	4.29	18.41

The material removed by extraction with alcohol, the amounts of which were given in Table I, was examined for resin (insoluble in water), for sucrose and hexose sugars. The results are given in Table III, to which have been added the figures representing the dextrin determinations.

TABLE III.

	Leaf.	Flower.	Bulb.	Root.
Resin	5.03	undet.	2.58	5.00
Sucrose	5.60	undet.	18.44	1.02
Reducing sugar[1]	5.89	undet.	6.69	2.45
Dextrin	3.26	trace	1.40	0.34

Determination of Alkaloid in the Leaf.—The presence of an alkaloid in the leaf having been detected by a preliminary assay, it was decided to make determinations in more than one way. Accordingly, portions of leaf were next assayed by three different methods, as follows:

I. The first method[2] tried is that official in the United States Pharmacopeia for the assay of belladonna leaves, which we modified to the extent of using ether instead of chloroform in the final extraction. Duplicate determinations in this way gave crude alkaloidal residues weighing 0.0883 and 0.0910 gram, which required for neutralization 1.13 and 1.07 cc., respectively, of 0.1 *N* sulfuric acid.

The residues so obtained, which were contaminated with conspicuous amounts of resin that was insoluble in dilute acid, were united, the mixture acidified and filtered, and the clear filtrate precipitated with Mayer's reagent. The insoluble mercury compound that resulted was collected on a filter, washed with water, suspended in dilute sulfuric acid, and decomposed by hydrogen sulfide. After standing over night the mixture was filtered, the mercuric sulfide washed with water, the filtrate made alkaline with ammonia and extracted with ether. Evaporation of the ether left a crystallin alkaloidal residue that weighed 0.0815 gram, and which corresponded to 0.41 per cent. of the drug employed. The combined, partially exhausted drug residues obtained in the first part of the operation were next exhausted by percolation with 5 per cent. sulfuric acid. The percolate was made alkaline with ammonia, and worked up for alkaloid in the usual way. A further quantity of alkaloidal residue

[1] Calculated as invert sugar.

[2] Given in detail in U. S. Disp., 19th Ed., p. 228.

weighing 0.0230 gram and requiring 0.11 cc. 0.1 *N* acid for neutralization, was obtained.

II. A second portion of leaf weighing ten grams was assayed by the method specified above and modified to the extent of using Prolius'[1] solution instead of the ether-chloroform mixture as a menstruum. The crude alkaloidal residue obtained by this method weighed 0.0807 gram, and required 1.04 cc. 0.1 *N* acid, and 0.78 cc. Mayer's reagent. Decomposition of the mercury salt in the manner already described gave 0.0280 gram of crystallin residue, representing 0.28 per cent. of the original material. Further percolation of the partially exhausted drug with 5 per cent. sulfuric acid yielded an alkaloidal residue that weighed 0.0107 gram and sufficient to neutralize 0.10 cc. 0.1 *N* acid.

III. A third portion of leaf weighing 40 grams was exhausted as far as possible by repeated extractions with boiling 95 per cent. alcohol. The extract was then concentrated under reduced pressure to a volume of 200 cc., the dissolved solids determined in an aliquot of 5 cc., and the remainder poured into a mixture of 40 cc. *N* sulfuric acid, and 460 cc. water. A brown, resinous mass amounting to 4.9 per cent. of the weight of the drug was precipitated. This was collected on a filter and washed with dilute acid.

The acid filtrate was now made alkaline with ammonia, and worked up for alkaloid in the usual way. The crude residue obtained weighed 0.2382 gram, and required for neutralization 3.70 cc. 0.1 *N* acid, and for precipitation 7.49 cc. Mayer's reagent. The residue was known to be contaminated with resin, and in order to correct for this the mercury compound was decomposed as described above, and a crystallin alkaloid obtained. The latter weighed 0.1033 gram, and represented 0.26 per cent. of the drug.

The residue of leaf left after extraction with alcohol was percolated with 5 per cent. sulfuric acid until the percolate no longer reacted with Mayer's reagent. A further yield of alkaloid amounting to 0.0139 gram and neutralizing 0.18 cc. 0.1 *N* acid was secured.

Determination of Alkaloid in the Bulb.—When three separate portions of ten grams each of the bulb were subjected to the process designated as I under the leaf, there were obtained crude alkaloidal residues that weighed 0.0560, 0.0530, and 0.0450 gram, and which neutralized 0.32, 0.34, and 0.36 cc., respectively, of 0.1 *N* acid. As with the leaf, the partially exhausted drug residues from twenty grams were percolated to exhaustion with 5 per cent. sulfuric acid, and the percolate yielded 0.0168 gram crude alkaloidal residue that neutralized 0.13 cc. 0.1 *N* acid, and precipitated 0.37 cc. Mayer's reagent.

II. Two ten-gram portions of bulb were assayed in accordance with

[1] Alcohol 8 cc., ether 88 cc., ammonia (10 per cent.) 4 cc.

method II outlined under the leaf and gave impure alkaloidal residues weighing 0.0876 and 0.0770 gram, equivalent to 0.97 and 0.96 cc. 0.1 *N* acid, and 1.90 and 1.51 cc., respectively, of Mayer's reagent. Decomposition of the mercury salt of the first of these in the usual way gave 0.0390 gram of crystallin residue, equivalent to 0.39 per cent. of the drug.

III. Fifty grams of bulb were exhausted as nearly as possible by repeated extraction with boiling 95 per cent. alcohol. When the first portion of the extract, measuring 500 cc., was concentrated, it deposited crystals of sucrose. The combined extracts were then concentrated under reduced pressure to a small bulk and finally allowed to dry out over sulfuric acid in a desiccator. The residue was next boiled with 150 cc. 95 per cent. alcohol and the undissolved portion (which had a sticky consistency) removed and boiled with a second portion of alcohol. The final residue which was granular in character weighed 3.771 grams and represented 7.54 per cent. of the bulb. When heated upon platinum foil the substance produced caramel; tested with Fehling's solution, it showed slight reduction. Examined with the polariscope it showed the constants for cane sugar mixed with a trace of reducing sugar. The specific rotation found was $[\alpha]_D = +62.70$ at 20°, while that for the pure sucrose is +66.48.

The alcoholic extract obtained above, which had a strong reducing action on Fehling's solution, was now concentrated to the consistency of a fluid extract, and then diluted with a mixture of 10 cc. *N* sulfuric acid and 240 cc. water. A brown, flocculent resin precipitated. This was collected on a filter, washed with water and dried. It weighed 1.2875 grams and was equivalent to 2.58 per cent. of the bulb.

The acid filtrate obtained above was now shaken repeatedly with ether, which removed a brown tar, weighing 0.2922 gram, equivalent to 0.58 per cent. The liquid was then made alkaline with ammonia, and the alkaloid removed by extraction with ether as already described. From the ether the alkaloid was recovered by shaking out with sulfuric acid in the usual way. The liquid obtained was made alkaline with ammonia a second time, extracted with ether and the latter evaporated off. The residue weighed 0.0905 gram, was completely soluble in 0.1 *N* acid, of which 1.10 cc. were required for neutralization, and 2.90 cc. of Mayer's reagent for precipitation.

It was found in the determination just specified that ether failed to remove all the alkaloid from the second ammoniacal liquid. A further extraction with chloroform yielded 0.0307 gram of alkaloid that required 0.23 cc. 0.1 *N* acid, in which it was completely soluble, and 1.30 cc. Mayer's reagent for precipitation. The combined alkaloidal residues constitute 0.24 per cent. of the bulb.

The flower and root were next assayed by the method designated above

as I. Duplicate determinations on ten-gram portions of the flower gave crude alkaloidal residues weighing 0.1358, and 0.1842 gram, which required 1.82 and 2.32 cc. 0.1 *N* acid for neutralization, and 3.79 and 3.60 cc., respectively, of Mayer's reagent for precipitation.

Assays of duplicate portions of the root in the same way gave crude alkaloidal residues weighing 0.0504, and 0.0422 gram. These required for neutralization 0.52, and 0.56 cc. 0.1 *N* acid, and 1.15, and 1.15 cc., respectively, of Mayer's reagent for precipitation.

The work will be continued in this laboratory.

LARAMIE, WYOMING.

[CONTRIBUTION FROM THE DEPARTMENT OF PLANT AND ANIMAL CHEMISTRY, MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION.]

THE SOLUBLE CARBOHYDRATES IN ASPARAGUS ROOTS.

BY FRED W. MORSE.

Received November 17, 1910.

This paper is a simple statement of progress in a study of the composition of the asparagus plant, and is part of an investigation of the fertilizer requirements of asparagus now being conducted at this agricultural experiment station.

The nutrition of asparagus shoots in early spring necessarily depends on the material stored in the roots, since the mode of growth of the young shoots up to the time of cutting for the table renders assimilation from the atmosphere nearly impossible. Hence, roots were selected as the first portion of the plant to be studied.

A search of the literature of asparagus failed to show anything about the composition of the roots, beyond a few scattering ash analyses and a brief article by Vines[1] on the reserve proteins.

Very recently, however, Wichers and Tollens[2] have reported an extensive study of asparagus roots, and called attention to similar work by Tanret,[3] brief abstracts of whose articles had been overlooked.

Since the work has been wholly independent of that just mentioned, it is believed that this report of progress will be of value at this time.

All the material for the work here reported was prepared ni other divisions of the department, and consisted of finely pulverized samples of individual root systems. All of the plant below the surface had been dug up, freed from earth and dried at about 50°. The roots were secured in November of the second year after setting when translocation from the tops was believed to be complete. For subsequent study of the effects

[1] *Proc. Roy. Soc. London*, **52**, 130–2. Abstr. *J. Chem. Soc.*, **64**, 431.

[2] *J. Landw.*, **58**, 101–16.

[3] *Bull. soc. chim.*, [4] **5**, 889, 893. *Compt. rend.*, **149**, 48–50. Abstr. *J. Chem. Soc.*, **1909**, 634. *C. A.*, **3**, 2677.