which carboxyl group in polybasic acids is ionizing. The experimental study of these place factors is now in progress and will be extended to other classes of compounds.

URBANA, ILL.

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WYOMING EXPERIMENT STATION.]

CHEMICAL EXAMINATION OF WOODY ASTER.

BY L. CHAS. RAIFORD.

Received May 15, 1911.

During the past several years thousands[1] of sheep have died in Wyoming through what is supposed to have been the eating of poisonous plants on the ranges. For the last two or three seasons the losses have occurred, in many instances, in localities where the areas grazed were somewhat circumscribed, and in several of these places a certain plant that later came to be suspected as poisonous was found in enormous numbers. It is notable that the heaviest losses for the seasons of both 1908 and 1909 occurred in approximately the same localities, and that in nearly all of these places the suspected plant was abundant.

The facts stated above were secured through a preliminary investigation of the conditions that prevailed, during the seasons mentioned, by both botanist and veterinarian of this station; and, it may be added, the data collected tended to indicate a plant known as the woody aster as the possible source of the trouble. The idea that the losses were due to poisonous plants seemed to be favored indirectly by the inconclusive results of two veterinarians from the Bureau of Animal Industry, who studied the question during the seasons of 1907 and 1908, and who made careful search for animal parasites.[2] Still further support of the view at present entertained was secured from data[3] obtained by the veterinarian of this station during the season of 1910, when extracts prepared in this laboratory from the aster in question were fed to sheep kept under such conditions that the effect of the poison, if any, could be noted. Tests upon other experimental animals are now being conducted with these extracts, and the results so far obtained are in harmony with what has been recorded above.

The suspected plant belongs to the genus *Xylorrhiza*,[4] species of which have been collected in Wyoming, Colorado, and Utah.[5] These plants

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[1] Tenth Annual Report of State Board of Sheep Commissioners, 7 (1908).

[2] The losses were at first attributed to the so-called "grub-in-the-head."

[3] *Wyoming Exp. Sta.*, *Bull.* **88**.

[4] Pammel, "Manual of Poisonous Plants," p. 138 (1910), designates this plant as the *Aster Parryi Gray*.

[5] According to the statements of herders, stock usually avoid this plant on the range, although analysis shows that it has a relatively high food value.

appear to be confined to the arid regions, and to soils in which only the so-called alkali-enduring vegetation will thrive. The most abundant species in Wyoming is the *Parryi*, which does not, however, occur over the entire state, but is restricted to certain districts characterized by what is usually known as the adobe or gumbo type of soil, intermixed somewhat with gravel and containing larger or smaller quantities of the salts commonly designated as "alkali." A consideration of further interest in this connection is that this particular plant is not only confined to the type of soil named, but that Wyoming is the center of its distribution. More remarkable[1] yet, perhaps, is the fact that this aster is almost invariably more or less infected with a fungus[2] (*Puccinia Xylorrhiza*); and that in the season of 1910, during which time the sheep losses in Wyoming from suspected poisoning were fewer than in either of the previous years mentioned, this aster was quite free from the fungus. Among the other species of the genus *Xylorrhiza*, of which three or four have been described, only the *villosa*,[3] so far as the writer is aware, has been known[4] to be infected with this fungus; and no other has been suspected of having poisonous properties.[5]

A consideration of the above-mentioned facts seemed to warrant a chemical examination of the suspected plant; and with this in view a considerable quantity of material consisting of both roots and tops[6] was collected during the early part of June, 1910. In grazing, the animal probably gets but a trifle of the root when the top is taken, but it was thought worth while to use both in the preliminary examination.

In the present paper the results of the proximate analyses of both parts of the plant are reported, to which have been added the constituents of the ash in each case. For tests[7] with experimental animals extracts were prepared with different solvents. The results secured seem to indicate that the material extracted by alcohol (from which almost all the alcohol was removed before administration to the animal) had the most pronounced poisonous effect. In a later paper the results of a detailed examination of this extract will be given.

Experimental Part.

Preparation of Material.—As soon as the plant was collected[8] the roots

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[1] *Press Bull.* 10, *Wyo. Agr. Exp. Sta.*

[2] This fungus has not been shown to be poisonous, but has been suspected in this connection.

[3] According to some authorities this is a variety of *Parryi.*

[4] Examination made by Dr. A. Nelson, of this Station.

[5] Dr. P. A. Rydberg, New York Botanical Gardens, private communication.

[6] This includes everything above the surface of the ground.

[7] Conducted by the veterinarian of this Station.

[8] This was done while the plant was in bloom.

were separated from the leaves and tops, and each was spread in a thin layer in the open air and dried thus until all danger of heating up in packages was passed, after which the different parts were placed in separate sacks and sent to the laboratory. When thoroughly air dry all old stems, representing the growth of a previous year, were removed from the leaves and tops, and the remainder was then ground and sieved. The root was cleaned and prepared in the usual way.

Extraction with Different Solvents.—The examination was begun by extracting separate portions of about 2.5 grams each in a Soxhlet apparatus with ligroin (boiling point 25–50°), ether, and alcohol, respectively. The quantities removed are indicated in percentages in Table I.

TABLE I.

	Leaf.		Root.	
	93°[1].	110°.	93°.	110°.
Ligroin (25–50°)	1.66	1.63	0.96	0.90
Ether	2.94	2.86	3.48	3.29
Ether extract, volatil at 110°	...	0.08	...	0.19
Alcohol	28.98	27.90	13.73	13.29

When the solvents ligroin, ether, chloroform, ethyl acetate, and alcohol were used successively on the same samples of drug, the quantities removed were as follows:

TABLE II.

	Leaf.		Root.	
	93°.	110°.	93°.	110°.
Ligroin	1.60	1.59	0.75	0.72
Ether	1.63	1.57	0.88	0.83
Chloroform	1.15	1.14	0.35	0.33
Ethyl acetate	1.88	1.74	0.86	0.82
Alcohol	17.69	16.81	7.61	7.20
Total	23.95	22.85	10.45	9.90

It will be noted that the material removed by alcohol alone (Table I) weighed more than that extracted by all the solvents mentioned in Table II. This difference, which is indicated in Table III, is interpreted to mean that this material became oxidized or otherwise changed during extraction in such a way as to render it partially insoluble; and that the change was facilitated by the increased length of time required for successive extraction with several solvents as against that necessary for treatment with alcohol alone. With the last named liquid the extract began, before extraction was complete, to deposit a solid that could not afterward be dissolved by alcohol. This behavior was noted with both leaf and root, and will be considered later.

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[1] Boiling point of water at the altitude of this Station.

TABLE III.

	Leaf.		Root.	
	93°.	110°.	03°.	110°.
Alcohol	28.98	27.90	13.73	13.29
Solvents of Table II	23.95	22.85	10.45	9.90
Difference	5.03	5.05	3.28	3.39

Proximate Analysis.—The proximate analyses of the plant parts were conducted in accordance with official methods. The results are given in Table IV.

TABLE IV.

	Leaf.		Root.	
	(1)	(2)	(1)	(2)
Moisture[1]	8.27	8.27	6.08	6.02
Ash	10.89	10.98	17.37	17.98
Crude fiber	13.34	13.18	29.40	30.10
Pentosans	12.20	12.25	14.43	14.74
Protein (N × 6.25)	23.00	23.00	8.43	8.62
Dextrin	1.15	1.13	2.32	1.96
Starch by malt	None	None	None	None

Preparation and Analysis of Ash.—Portions of the ground and sieved leaf and root, respectively, were ignited below red heat in a muffle furnace, under which conditions the root gave an ash that was almost white.[2] In case of the leaf the charred material was extracted with hot water and filtered, the residue then burned to a white ash, the filtrate mixed with this ash, and the whole evaporated to dryness on a water bath. The last portions of water were expelled by heating just below redness over a free flame. The resulting solid was at once powdered and preserved in glass stoppered bottles until analyzed.

In general the official methods of the A. O. A. C. were followed in the analyses of these ashes. Certain modifications, however, were necessary. In accordance with the official method it is expected that the filtrate[3] obtained when sand, soluble silica and carbon are removed will contain all iron present and other ash components soluble in hydrochloric acid. As a matter of fact, with the ashes here involved, a very considerable quantity of iron (and probably other substances) did not go into solution under the conditions prescribed, and was invariably found in that portion of the material in which soluble silica was determined. In order to account for this, the filtrate obtained during the soluble silica determination was mixed with the first one (Solution A), and the resulting

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[1] Determined by heating to constant weight in a stream of hydrogen at the boiling point of water.

[2] Though this ash did not fuse when heated at the higher temperature required by the official method for residues from which soluble matter had been extracted at this stage, a control showed that considerable material was volatilized.

[3] Designated by the official method as Solution A.

liquid used for the estimation of iron, aluminum, phosphorus pentoxide, etc. But it was thought worth while, before the last named determinations were made, to evaporate the combined filtrates to dryness and remove the remaining silica. This[1] was done in the usual way, and the results obtained, in addition to those secured in accordance with the official method, are given in Table V. Duplicate determinations are reported on the ashes of both leaf and root.

TABLE V.—SOLUBLE SILICA.

		Official method.	Second evaporation.	Total.
Leaf	1	3.43	1.24	4.67
	2	4.09	0.53	4.62
Root	1	12.30	1.42	13.72
	2	12.67	1.03	13.70
Root[2]	1	13.69	0.51	14.20
	2	13.23	1.07	14.30

Average = 0.96 per cent.

It will be seen from these results that the duplicate determinations did not agree in any series when the work was done in strict accordance with the official method, but that close agreement was secured in all

TABLE VI.—CONSTITUENTS OF ASH.

	Leaf.			
	(1)	(2)	(3)	(4)
Moisture	0.67	...		
Chlorine	10.91	10.89		
Carbon dioxide	14.16	14.23		
Sand	2.39	2.56		
Carbon	0.31	0.29		
Soluble silica	4.67	4.62		
Sulfur trioxide[3]	4.89	4.91	10.51[4]	11.08
Phosphorus pentoxide	5.31	5.10		
Ferric oxide	1.42	1.62		
Aluminum oxide	1.70	1.30		
Manganese	trace	trace		
Calcium oxide	12.10	12.42		
Magnesium oxide	4.90	4.95		
Sodium oxide	20.92	20.71		
Potassium oxide	17.41	17.19		

[1] Not required by the official method.

[2] The second series of results from root ash were obtained from material that had not been specially prepared for these analyses, and no other determinations were made on it. Silica estimations were made for the sole purpose of confirming conclusions already reached.

[3] This was determined after iron and aluminum had been removed. See *Z. anal. Chem.*, **19**, 419; *J. prakt. Chem.*, **39**, 321; and THIS JOURNAL, **24**, 658 (1902).

[4] These figures represent total sulfur determined by the peroxide method, and calculated as sulfate in terms of the ash.

cases when the method was modified to include the silica removed by a second evaporation. The average additional amount obtained thus was 0.96 per cent. The difference in the total amount of iron that resulted through mixing the filtrate from the determination of soluble silica with Solution A was not estimated, but the color of this filtrate indicated that the additional amount of iron was probably as great as in the case of silica.

The composition of the leaf ash is given in Table VI.

Table VII.—Constituents of Ash.

	Root.			
	(1)	(2)	(3)	(4)
Moisture	1.25	1.23		
Chlorine	0.79	0.91		
Carbon dioxide	6.42	6.59		
Sand	39.63	39.17		
Carbon	0.84	0.91		
Soluble silica	13.72	13.70		
Sulfur trioxide	1.37	1.45	2.93	2.81[1]
Phosphorus pentoxide	1.52	1.43		
Ferric oxide	...	2.56		
Aluminum oxide	...	5.53		
Manganese	trace	trace		
Calcium oxide	19.32	19.22		
Magnesium oxide	2.80	2.60		
Sodium oxide	2.62	2.35		
Potassium oxide	4.28	4.31		

The composition of the root ash is given in Table VII.

A consideration of the figures representing the various analyses of the woody aster shows that the results of duplicate determinations do not agree very well, and that this disagreement is more marked in the case of the root. These differences are apparently due to the difficulty in securing a uniform sample, a condition which probably depends, so far as the root is concerned, upon the relatively high percentage of sand present in the prepared material. The large amount of substance removed in the analysis of the root at the point where, in accordance with the official method, sand should be obtained led at first to the suspicion that a portion of this substance might be barium sulfate. This suspicion was based largely on the fact that only a small percentage of sulfate was found in the combined filtrate (Solution A) left after determining sand, soluble silica, etc.; and upon the probable presence of considerable quantities of sulfates in the soil[2] where the plant grew. In order to determine

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[1] Represents total sulfur determined by peroxide method.

[2] The soil from which this particular sample of aster was collected has not been examined by the writer, but the presence of relatively large amounts of "alkali" (consisting largely of the chloride and sulfate of sodium) in all the soils in that vicinity leaves little doubt of the presence of sulfates in the "aster patches."

the presence or absence of barium sulfate, a portion of the original root ash was fused with a mixture of sodium and potassium carbonates, and total silica determined in the usual way. After being ignited and weighed, the resulting mass was heated with hydrofluoric and sulfuric acids until constant in weight.[1] The amount of sand and soluble silica determined by the modification of the official method already described was 52.87 per cent., while determination of total silica by fusion gave 49.87 per cent. Now, the presence of iron in the filtrate (containing soluble silica) from sand, previously noted, made it seem almost certain that the latter, also, contained iron. This view was confirmed by showing that the filtrate from total silica by fusion contained a larger amount of iron than did Solution A. The percentage of ferric oxide in the ash, as determined in Solution A, was 2.56 per cent.; that in the filtrate from total silica was 2.88 per cent. Undoubtedly a redetermination of other bases would have shown sufficient increases to account for the difference between the sum of sand and soluble silica in one analysis, and total silica in the other.

An assay of the leaf having shown that alkaloids were absent, attention was next directed to the alcoholic extract. It is worthy of note that in the preliminary consideration of this extract some of the matter (of which a large part seemed to be resin) removed was changed by oxidation or otherwise so rapidly that before the drug was exhausted the extract began to deposit a solid that could not afterward be dissolved in alcohol. In future work the amount separating at this stage will be determined as nearly quantitatively as possible, and other properties will be examined. This behavior, taken in connection with the statement already given regarding the poisonous character of the extracts prepared with various solvents, makes necessary a detailed study of the alcoholic extract. The work is now in progress.

LARAMIE, WYOMING.
May, 1911.

[CONTRIBUTION FROM THE HAVEMEYER LABORATORIES, COLUMBIA UNIVERSITY, NO. 193.]

STUDIES ON AMYLASES: III. EXPERIMENTS UPON THE PREPARATION AND PROPERTIES OF PANCREATIC AMYLASE.[2]

BY H. C SHERMAN AND M. D. SCHLESINGER.

Investigations in this laboratory during the three years, 1907–1910, resulted in the development of a method for the determination of amylolytic power[3] and a somewhat extended quantitative study, by means of this method, of the action of pancreatic amylase.[4] On the basis of

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[1] The slight non-volatile residue left contained iron, but spectroscopic examination failed to show even a trace of barium.

[2] Read before the New York Section, American Chemical Society, May, 1911.

[3] Sherman, Kendall and Clark, THIS JOURNAL, 32, 1073.

[4] Kendall and Sherman, *Ibid.*, 32, 1087.