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.

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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.

of different fertilizers, the individual samples were separately analyzed; but for this report detailed results are unnecessary.

The average proximate composition of the dry matter of 16 roots was as follows:

Protein (nitrogen × 6.25)	11.03 per cent.
Fat	1.00 per cent.
Fiber	15.39 per cent.
Nitrogen-free extract	66.34 per cent.
Ash[1]	6.24 per cent.

The proximate composition showed clearly that the soluble non-nitrogenous matter included most of the reserve material of the roots.

The methods of the Association of Official Agricultural Chemists[2] for sugars, starch, pentosans and galactans were employed for estimating the different carbohydrates in the reserve material.

An examination of 25 roots showed 12 to contain no reducing sugars while most of the others had only traces present; therefore, reducing sugars were not estimated but reckoned with total sugars. The latter were especially abundant and ranged from 26.4 per cent. to 50.8, only 2 samples containing less than 35 per cent. calculated to dry matter. Pentosans were determined in 16 samples and ranged from 7.32 per cent. to 10.68 per cent. in the dry matter. Galactans were determined in 4 individual samples and in a composit sample, but were insignificant in amount, averaging only 1.04 per cent.

In the estimation of starch by the diastase method, it was found that there was no more glucose obtained than was accountable from the diastatic extract. Subsequent examination revealed it in only microscopic traces. Six different samples, after having undergone the diastase treatment as for starch, were filtered and washed, and the residues then subjected to 2 hours' boiling under reflux condensers, with 100 cc. of hydrochloric acid of approximately 6 per cent. After cooling the solutions they were nearly neutralized with sodium hydroxide, and made up to 250 cc. The reducing sugars were then determined by Fehling's solution and the weights of copper calculated to glucose. The 6 samples averaged 8.6 per cent. of glucose by this hydrolysis; but since the same samples averaged 8.67 per cent. of pentosans reckoned from furfural-phloroglucide, it is improbable that there are any hydrolyzable carbohydrates unaccounted for by the usual analytical methods.

From these different analyses it was found that the dry matter of 16 roots contained:

Sugars calculated as invert sugar	41.43 per cent.
Pentosans	8.78 per cent.
Galactans	1.04 per cent.

[1] Ash determinations were made in the fertilizer division of the department.

[2] U. S. Dept. Agr., Bur. of Chem., *Bull.* 107, 38–56.

The carbohydrate forming over 40 per cent. of the dry matter was at first assumed to be sucrose. The analytical procedure had shown it to be soluble in cold water and inactive to Fehling's solution until hydrolyzed, which was easily accomplished by dilute acids. Repeated attempts to recover sucrose by means of strontium hydroxide[1] resulted in securing only very small quantities of a straw-colored sirup which could not be crystallized like sucrose, but did not reduce Fehling's solution.

Methyl alcohol was found to extract considerable quantities of the sugar from the roots, which suggested raffinose; but no mucic acid could be obtained by oxidation with nitric acid, although a parallel test with lactose under the same conditions yielded it in abundance.

Osazones were prepared from both methyl alcohol and water extracts, before and also after inversion. The characteristic yellow, crystallin precipitate was easily obtained in every case. Five such precipitates had their melting points determined and they ranged between 203 and 210° and were accompanied by an evolution of gas. Glucosazone was evidently the only one formed.

About 100 grams of roots were extracted by cold water and the extract concentrated on the water-bath to a thick, black, tenacious sirup, which was strongly reducing to Fehling's solution. Heat and probably acid salts had brought about a nearly complete hydrolysis during the evaporation. This extract failed to yield mucic acid, but oxalic acid was readily formed.

Portions of the sirup were subjected to distillation with hydrochloric acid of 1.06 sp. gr. and yielded a small quantity of furfural. The furfural-phloroglucide, after being dried and weighed, was found to lose about two-thirds of its weight by solution in hot 93 per cent. alcohol, indicating that it was largely methyl-furfural.

The action of polarized light was observed upon freshly prepared water extracts of two different roots, and upon three sirups which had been fractionated by strontium hydroxide. The solutions were clarified by lead subacetate and the readings were made in a Schmidt and Haensch triple-shade saccharimeter through a 200 mm. tube. The solutions were then inverted and again polarized together with two solutions of the dense water extract above mentioned.

Subsequent to the readings, the actual strength of sugar in each solution was determined with Fehling's solution. The solutions were necessarily dilute, because the roots on moistening swelled to a large volume and small charges had to be used. The three sirups were small in amount as before mentioned, and the black sirup from the water extract was difficult to clarify to a point where light would pass through it.

[1] E. Schulze, *Z. physiol. Chem.*, 20, 513–5.

POLARIZATION BEFORE HYDROLYSIS.

	Sugar in 100 cc. Grams.	Saccharimeter reading.	Sp. rotatory power.
Root 34	1.738	+ 0.5	+ 5°
Root 40	2.259	— 1.4	— 10°
Sirup A	2.623	+ 2.88	+ 18.9°
Sirup B	2.775	— 1.6	— 10°
Sirup C	0.858	zero	zero

POLARIZATION AFTER HYDROLYSIS.

	Invert sugar in 100 cc. Grams.	Saccharimeter reading.	Sp. rotatory power.
Root 34	0.893	— 2.33	— 45°
Root 40	1.189	— 4.10	— 59°
Sirup A	1.381	— 3.45	— 49°
Sirup B	1.461	— 5.25	— 62°
Sirup C	0.452	— 1.3	— 49°
Extract 1	0.936	— 3.0	— 55°
Extract 2	2.350	— 7.8	— 57°

The action on polarized light both before and after inversion excludes the possibility of the carbohydrate being pure sucrose, while the failure to secure it with strontium hydroxide renders its absence probable.

Fructose was clearly demonstrated by the osazone and the negative optical activity, also by fine reactions with resorcinol and hydrochloric acid. Glucose is indicated by the osazone and the fact that the specific rotatory power of the inverted solutions is not high enough for pure fructose. Fructose clearly predominates over the glucose, and the non-reducing property before hydrolysis indicates some condensation product formed between them. The behavior of individual root extracts does not ponit to any fixed proportion of the two sugars.

These results are, on the whole, in close agreement with those of Wichers and Tollens. There was, however, a marked difference in the behavior of the water extract of the roots, which contained the sugar-like carbohydrate. Wichers and Tollens used boiling water and state that only a portion of this carbohydrate was soluble in water when extractions were made on the water bath. Their solutions also reduced Fehling's solution before hydrolysis.

My extractions were all made with water at 20° and, until hydrolyzed, either had no reducing action or precipitated no more than traces of copper.

This difference in solubility and reducing action is doubtless due to the stage of development of the roots, since Wichers and Tollens worked upon roots gathered in April and July instead of in November.

Tanret isolated two distinct crystallin carbohydrates from the root sap, one of which had a rotation of —35.1° and the other +30.3°.

Sirups A and B fractionated with strontium hydroxide showed opposit rotations before inversion, but lack of material has given no opportunity to confirm further his observations.

Grateful acknowledgment is made of suggestions received from Dr. Joseph B. Lindsey during the progress of this investigation.

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[FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY OF THE UNIVERSITY OF ILLINOIS.]

FASTING STUDIES: I. NITROGEN PARTITION AND PHYSIOLOGICAL RESISTANCE AS INFLUENCED BY REPEATED FASTING.[1]

BY PAUL E. HOWE AND P. B. HAWK.

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Historical.

The literature upon fasting is large and may be divided into two classes, that presenting data upon (*a*) simple fasts of (1) short duration, and dealing with changes in body weight, nitrogen partition, the blood, temperature, secretions, and the effect of drugs, or only as a preparation for the study of normal or pathological metabolism; (2) more or less profound fasts, which include in addition to those changes mentioned above, losses in the weight and differences in composition of the organs as compared to the normal organs; and (*b*) repeated fasts in which (1) the object was to study the effect of repeated fasting; or (2) fasts in which the main object was not the study of repeated fasting, but in which the subject was used repeatedly in various investigations, no regard being given to the original conditions, such as body weight, nitrogen equilibrium, etc.

Experiments on Repeated Fasting.—Investigations upon fasting have been limited mainly to the lower animals, while those upon repeated fasting are confined entirely to them unless the fasts of Succi[1] are to be considered as repeated fasts, which is hardly proper, since each fast was conducted under different circumstances as regards original condition, body weight, time of year, climate, etc.

Repeated fasts have been conducted upon dogs by Albitsky.[3] His investigations were made upon four dogs, two fasting absolutely, *i. e.*, without water, to a loss in body weight of 40.4 per cent. and 37.6 per cent., in twenty-nine days, and fifteen days respectively, and the second time with water *ad libitum*, with a loss of 53.2 per cent. and 45.8 per cent. in fifty-one and thirty days, respectively. The other two dogs fasted first

[1] The expense of this research was borne by the Department of Animal Husbandry of the College of Agriculture. It was presented in abstract at the Boston meeting of the Society of Biological Chemists. It was also presented by Mr. Howe to the Graduate School of the University of Illinois, in partial fulfi'ment of the requirement for the degree of Doctor of Philosophy.