ON THE CARBOHYDRATES OF THE EDIBLE TUBERS OF JAPAN

BY

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The present paper aims at giving a summary of the results of my investigation on the carbohydrates of the tubers which are at present produced and consumed in Japan as food material.

The following species are commonly known in Japan as edible tubers:—

Amorphophallus Konjac K. KOCH. (コンニャク); *Apios Fortunei* MAXIM. (ホド); *Colocasia antiquorum* SCHOTT. (サトイモ); *Corydalis ambigua* CHAM. et SCH. (エゾノエンゴサク); *Dioscorea Batatas* DECNE. *form. typica* MAK. (ナガイモ); *Dioscorea Batatas* DECNE. *form. Tsukune* MAK. (ツクネイモ); *Dioscorea japonica* THUNB. (ヤマノイモ); *Eleocharis plantaginea* R. BR. (クログワヰ); *Helianthus tuberosus* L. (キクイモ); *Ipomaea Batatas* LAM. (サツマイモ); *Sagittaria sagittifolia* L. *form. sinensis* MAK. (クワヰ); *Solanum tuberosum* L. (ジャガタライモ); and *Stachys Sieboldi* MIQ. (チョロギ).

In regard to the chemical nature of the carbohydrates, which compose the greater part of the edible tubers, the following special investigations have been made. C. TSUJI [1] and Y. KINOSHITA [2] studied mannan in tubers of *Amorphophallus Konjac*. K. YOSHIMURA [3] reported that the mucilage of the tubers of *Colocasia antiquorum* consisted only of a polyanhydride of d-glucose. J. ISHII [4] studied the chemical nature of the mucilage of the tubers of *Dioscorea Batatas* and concluded that the mucilage belongs to the class of mucins.

1) Bull. Imp. Coll. Agric., Tokyo, **2** (1894), 2, pp. 103–105.
2) Ibid., **2** (1895), 4, pp. 205–206.
3) Ibid., **2** (1895), 4, pp. 207–208.
4) Ibid., **2** (1894), 2, pp. 97–100.

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But his results were considered not fully conclusive, as he made no detailed study concerning the presence of carbohydrate group in his preparation. K. OSHIMA in conjunction with T. TADOKORO[1] proved the presence of the glucosamin group in the same mucilage. The presence of inulin and its allied substances in tubers of *Helianthus tuberosus* have been made known by the examination of C. TANRET.[2] F. H. STORER[3] reported the presence of mannan in tubers of the same plant. W. E. STONE[4] found sucrose in tubers of *Ipomaea Batatas*, while K. MIYAKE[5] proved the presence of glucose and fructose besides sucrose in the same tubers. The latter[6] found glucose, fructose, sucrose and raffinose in tubers of *Sagittaria sagittifolia* f. *sinensis*. The tubers of *Solanum tuberosum* are known to contain a large amount of starch, and a very small amount of glucose and sucrose. A. v. PLANTA and E. SCHULZE[7] obtained stachyose from the tubers of *Stachys Sieboldi* which was proved by C. TANRET[8] to be identical with manneotetrose. No further investigation of the carbohydrates of other edible tubers has been undertaken. Consequently, the author has studied and examined the chemical nature of carbohydrates in tubers of *Apios Fortunei*, *Colocasia antiquorum*, *Corydalis ambigua*, *Dioscorea Batatas*, *Eleocharis plantaginea* and *Helianthus tuberosus*.

The present investigation was undertaken at the suggestion of Prof. K. OSHIMA, to whom the author wishes to express his hearty thanks for the kind advice he has given in the work.

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1) Jour. Coll. Agric., Tohoku Imp. Univ., Sapporo, **4** (1911), pp. 243–249; Jour. Tokyo Chem. Soc, Tokyo, **33** (1912), pp. 131–138.

2) Compt. rend., **116** (1893), pp. 514–517; abs. in Ber. D. Chem. Ges., Berlin, **26** (1893), Ref., p. 233; Compt. rend., **117** (1893), pp. 50–53; abs. in Ber. D. Chem. Ges, Berlin, **26** (1893), Ref., p. 691; Bull. Soc. Chim., (3) T, **9** (1893), pp. 227–234; abs. in Ber. D. Chem. Ges., Berlin, **26** (1893), Ref., p. 772.

3) Bull. Bussey Inst., **3** (1902), pp. 13–45; abs. in Chem Zentralbl., Berlin, **73** (1902), 2, p. 1155.

4) Ber. D. Chem. Ges., Berlin, **23** (1890), pp. 1406–1408.

5) Jour. Biol. Chem., New York, **21** (1915), pp. 503–506.

6) Jour. Biol. Chem., New York, **15** (1913), pp. 221–230; Trans. Sapporo Nat. Hist. Soc, Sapporo, **5** (1913), pp. 23–35.

7) Ber. D. Chem. Ges., Berlin, **23** (1890), pp. 1692–1699; ibid, **94** (1891), pp. 2705–2709; Landw. Vers.-Stat., **40** (1892), p. 277; ibid., **41** (1893), p. 123; ibid, **55** (1902), p. 419.

8) Compt. rend, **136** (1903), pp. 1569–1571; Bull. Soc. Chim., (3) T, **29** (1903), p. 888.

I. APIOS FORTUNEI MAXIM.

Apios Fortunei MAXIM. (ホド) largely grows wild and is to be seen in Honshu and the southern part of Hokkaido in Japan. The people of the above mentioned regions eat the tuberous rootstocks of this plant as food in May and June. The tubers used for the present investigation were collected in the province of Oshima, Hokkaido, (北海道渡島國松前郡福山町), in the middle part of May, 1916.

A quantitative analysis was made of the edible part of the tubers with the following results:—

	Air-dry substance.	Water-free substance.
	%	%
Water	68.60	—
Protein	4.19	13.25
Fat	0.19	0.60
Crude fiber	1.20	3.83
Nitrogen-free extract	24.52	78.19
Ash	1.30	4.13
Total nitrogen	0.67	2.12
Protein nitrogen	0.25	0.80
Non-protein nitrogen	0.42	1.32
Reducing sugar	1.15	3.65
Non-reducing sugar	2.85	9.07
Dextrin	0.99	3.15
Starch	18.30	58.30
Galactan	1.02	3.24
Pentosan	1.46	4.65

The presence of starch, galactan and pentosan was proved by the potassium iodide iodin solution, mucic acid reaction, and spectral reaction with phloroglucin and hydrochloric acid respectively. The amount of galactan

was estimated according to the American "Provisional method[1]" as modified by K. MIYAKE.[2] The determination of pentosan was made by the method of TOLLENS and KRÖBER[3].

From the above table, we may observe that the main constituents of the tubers are carbohydrates, which form about 82% of water-free substance, and that about 63% of the total nitrogen is in the form of non-protein. Of the carbohydrates, starch is prominent, its amount attaining about 58% of water-free substance. Sugars are also present in no slight quantity, reaching the amount of about 13% of water-free substance, and the weight of non-reducing sugar is about 2.5 times that of reducing sugar.

Sugars of the Tubers.

1. Preparation of the Syrup.

500 grams of the finely pulverized material of the edible part of the tubers were mixed with 1000 c.c. of 95 % alcohol. The alcoholic solution was found to show a weak acid reaction. Hence it was neutralized with ammonia, heated in a boiling water bath for 5 hours, using a reflux-condenser, and then filtered with suction. With the residue the same treatment was repeated twice to make the extraction complete. Another 500 grams of the sample were extracted in the same manner. The extracts were combined and evaporated, with the addition of a little calcium carbonate, to about 500 c.c. After allowing to stand for about 20 hours, the brown colored transparent solution was decanted and concentrated to a small volume. The concentrated solution was again purified with 95 % alcohol and evaporated down to a syrupy consistency.

2. Qualitative Reactions of the Syrup.

The above prepared syrup showed the following qualitative reactions:—

1) U. S. Department of Agric., Bureau of Chem.—Bull No. 107 (Revised), Official and Provisional Methods of Analysis. (1912), pp. 55-56.

2) Jour Coll. Agric., Tohoku Imp. Univ., Sapporo, **4** (1912), pp. 337-345.

3) Zs. physiol. Chem., Strassburg, **36** (1902), pp. 239-243; ibid., **36** (1902), Anhang, pp. 1-9

a) It was very sweet and of a yellow color.

b) It gave MOLISCH's reaction with α-naphthol and sulphuric acid distinctly.

c) It reduced FEHLING's solution strongly; after inversion with hydrochloric acid, the reducing power was much enhanced.

d) It rotated the plane of polarization toward the right, but after inversion, toward the left.

e) It gave no characteristic absorption-spectrum of pentose with phloroglucin and hydrochloric acid.

f) It gave BRAUN's reaction by heating with picric acid and a small quantity of caustic soda solution.

g) It gave PINOFF's reaction for ketose with ammonium molybdate and acetic acid; after inversion, the reaction was more distinct.

h) It gave SELIWANOFF's reaction for ketose with resorcin and hydrochloric acid very distinctly.

i) It produced no mucic acid upon oxidation with nitric acid of 1.15 sp. gr.

j) It produced no characteristic mannosephenylhydrazone with phenylhydrazin, either before or after inversion.

k) Five drops of the syrup were placed on an object glass, and the drops were seeded with crystals of glucose, galactose, mannose, sucrose, and maltose, respectively. After a few days, the drops which had been seeded with glucose and sucrose showed the formation of new crystals respectively, while the others remained unchanged.

From the above qualitative reactions, we may conclude that the syrup here examined contains both reducing and non-reducing sugars, and that the presence of glucose, fructose and sucrose is most probable. On the other hand, the absence of pentose, galactose and mannose is also probable.

3. Isolation of Sucrose.

After a month, the syrup was found thickly laden with large crystals. A

small amount of 95 % alcohol was added to the syrup, well mixed, filtered with suction, and washed with 95 % alcohol and ether. Upon recrystallization from alcohol, the crystals became perfectly white and left no ash on ignition. The weight of sugar obtained was about 2 grams.

The aqueous solution of the sugar showed no reducing power, but after inversion, it reduced FEHLING's solution. It was very sweet. It gave the ketose reaction according to SELIWANOFF.

0.4 gram of the dried sugar was dissolved in water, a few drops of alumina cream added, and then made up to 10 c.c. and polarized in a 100 mm.-tube in a SCHMIDT and HAENSCH half-shadow polariscope. A dextro-rotation of 7.6 on the scale was observed.

The specific rotatory power of the sugar is

$$[\alpha]_D = +\frac{7.6 \times 0.346 \times 10}{0.4 \times 1} = +65.74°$$

0.2 gram of the dried sugar was dissolved in about 5 c.c. of water, acidified with a few drops of dilute hydrochloric acid, and heated in a water bath for 30 minutes. After inversion, it was neutralized with sodium carbonate, a few drops of alumina cream added, and then made up to 10 c.c. and polarized in a 100 mm.-tube. Strong bi-rotation was observed. After 24 hours, the rotation was 1.2 on the scale toward the left.

The specific rotatory power is

$$[\alpha]_D = -\frac{1.2 \times 0.346 \times 10}{0.2 \times 1} = -20.76°$$

Consequently, the sugar under examination is sucrose.

The mother-liquor, filtered off from the crystals of sucrose, was again concentrated to a syrup and left for a long time. New crystals of sugar were separated, and their properties were determined and found to be identical with those of sucrose.

4. Phenylosazone Tests.

The mother-liquor, filtered off from the second crystals of sucrose, was again evaporated to a syrup. It did not show any sign of forming new crys-

tals even after long standing. An attempt was made to separate and detect the sugars as phenylosazones, according to the method of FISCHER[1] as follows:—

a) 2 grams of the syrup, 2 grams of phenylhydrazin hydrochloride, 3 grams of sodium acetate and 20 c.c. of water were mixed in a large test tube and heated in a boiling water bath. Yellow crystals of osazone began to form in 15 minutes. The heating was continued for an hour and a half. When cooled, the crystals were filtered off and washed with a little water. By repeating the recrystallization from 60 % alcohol, about 0.55 gram of pure osazone was obtained, which was dried over concentrated sulphuric acid in a vacuum.

The determination of nitrogen in the osazone gave the following result:—

0.2692 g. substance gave 0.0413644 g. N.

		N.
$C_{18}H_{22}N_4O_4$	Calculated	15.64 %
	Found	15.37 %

The melting point of the osazone was determined and found to be 203—205°C.

The crystalline form, the quantity of nitrogen and the melting point indicate that the osazone in question is glucosephenylosazone.

The mother-liquor, separated from the crystals of glucosephenylosazone, was again evaporated in a boiling water bath to a small volume. A small amount of crystals of osazone, which were identical with those of glucosephenylosazone first obtained, was produced.

b) 2 grams of the syrup were dissolved in 20 c.c. of water and inverted with hydrochloric acid in a boiling water bath for about 30 minutes. After inversion, the solution was neutralized with sodium carbonate, and 2 grams of phenylhydrazin hydrochloride and 3 grams of sodium acetate were added to it and the mixture was heated in a boiling water bath for an hour and a half.

1) Ber. D. Chem. Ges., Berlin, **17** (1884), 1, pp. 579–584.

When cooled, the crystals were filtered off and washed with a little water. By repeating the recrystallization from 60 % alcohol, about 1.2 grams of pure osazone were obtained, which were dried over concentrated sulphuric acid in a vacuum.

The determination of nitrogen in the osazone gave the following result:—

0.253 g. substance gave 0.039296 g. N.

		N.
$C_{18}H_{22}N_4O_4$	Calculated	15.64 %
	Found	15.53 %

The melting point of the osazone was determined and found to be 203—204°C.

Therefore I am sure that the osazone thus isolated is glucosephenylosazone.

The mother-liquor, separated from the crystals of glucosephenylosazone, was again evaporated in a boiling water bath to a small volume. New crystals of osazone were formed. They coincide, in the melting point and in the quantity of nitrogen, with the crystals of glucosephenylosazone first obtained.

Glucosephenylosazone may be formed either from glucose, fructose, mannose or sucrose. The presence of mannose is excluded by the qualitative test already mentioned. The presence of sucrose was ascertained in the previous experiment. Maltosephenylosazone, if present, has a melting point similar to that of glucosephenylosazone but can easily be distinguished from the latter by its characteristic crystalline form. From the results of my experiment maltose can hardly be expected to exist. Consequently, it may be concluded that the reducing sugar consists of glucose, or fructose, or both, while the non-reducing sugar is sucrose.

5. Fructose Tests.

The sample was extracted with water, and the extract gave SELIWANOFF's and PINOFF's reactions for ketose. Also it reduced FEHLING's solution very strongly, and rotated the plane of polarization toward the right.

The presence of fructose in the free as well as the combined form was to be supposed. But the attempt to isolate the fructose as the methylphenylosazone after NEUBERG's method[1] was not successful and hence it may be inferred that the amount of free fructose, if present, is very little.

Products of Hydrolysis of Hemicellulose.

1. Preparation of the Sample.

1600 grams of air-dry finely pulverized substance of the edible part of the tubers were extracted with boiling 95 % alcohol. The residue was put into 9 liters of water and well stirred. After several hours, it was filtered. The residue was heated with water in a boiling water bath until the starch became gelatinized. When cooled to 60°C., malt extract was added to it, and the mixture was maintained at 60–63°C. to saccharify the starch, and then filtered. The treatment with malt extract was repeated until it gave no starch reaction with iodin, and then filtered and well washed with water. The residue was then extracted with 10 liters of 0.25 % caustic soda solution. After 2 days, it was filtered and well washed with water until the filtrate became neutral to litmus. The residue was dried and used as the sample for the hydrolysis.

2. Method of Hydrolysis.

140 grams of the sample above prepared were hydrolyzed with 4 liters of 3 % sulphuric acid in a boiling water bath for about 13 hours. When cooled, the solution was filtered and well washed with water. The filtrate was neutralized with calcium carbonate and left overnight. The following morning, it was filtered with suction and well washed with water. The filtrate was evaporated to about 100 c. c. The warm solution thus obtained was put into 500 c. c. of 95 % alcohol, well shaken and allowed to stand for about 20 hours. The clear solution was then decanted and concentrated again to about 100 c. c. The purification with alcohol was repeated twice in

1) Ber. D. Chem. Ges., Berlin, **37** (1904), pp. 4616–4618.

the same manner as described above. The final syrup is indicated as the syrup (I). The residue, remaining after hydrolysis with the acid, was about 60 grams in weight as air dry substance. The residue was again hydrolyzed with 1000 c. c. of 5 % sulphuric acid in the same manner as already described. After purifying with alcohol several times, the final syrup is indicated as the syrup (II).

3. Qualitative Reactions of the Syrup.

Both syrups, (I) and (II), showed the following qualitative reactions respectively:—

a) They were very sweet and of a yellow color.

b) They gave MOLISCH's reaction.

c) They reduced FEHLING's solution very strongly.

d) They rotated the plane of polarization toward the right strongly.

e) They gave the spectral reaction for pentose with phloroglucin and hydrochloric acid.

f) They gave neither SELIWANOFF's nor PINOFF's reaction for ketose.

g) They produced mucic acid in abundance upon oxidation with nitric acid of 1.15 sp. gr.

h) They did not produce mannosephenylhydrazone with phenylhydrazin.

i) BERTRAND's reaction[1] was applied to detect xylose. The crystals obtained were white and boat shaped. The aqueous solution gave the reactions of cadmium and bromin. But the amount of crystals was too small to allow an analysis.

j) Five drops of each syrup were placed on an object glass, and the drops were seeded with crystals of mannose, galactose, arabinose, xylose, and rhamnose, respectively. After a few days, the drop which had been seeded with arabinose showed the formation of new crystals, while the others

1) Bull Soc. Chim., Paris, (3) T, **5** (1891), pp. 554–557, Abs. in Ber. D. Chem. Ges., Berlin, **24** (1891), Ref., p. 530.

remained unchanged.

From the above reactions, it is safe to conclude that the syrups examined contain both galactose and pentose, but neither mannose nor fructose.

4. Isolation of Diphenylhydrazones of Arabinose and Glucose.

An attempt was made to isolate arabinose as diphenylhydrazone[1] in the following manner:—

2.5 grams of the syrup (I) were dissolved in a small amount of water, to which 1 gram of diphenylhydrazin and a sufficient quantity of absolute alcohol were added to form a perfectly clear solution. The mixture was boiled for 30 minutes in a water bath, using a reflux-condenser. When the solution became cool, white, needle crystals of diphenylhydrazone were abundantly produced. They were separated by filtration with suction and washed with 95 % alcohol. After the recrystallization from a small quantity of 95 % alcohol, the crystals were separated by filtration and dried.

When a small amount of the crystals was heated with dilute hydrochloric acid, it produced furfurol, which was detected by anilin acetate. The melting point of the crystals was determined and found to be 203–204°C.

According to TOLLENS and his associates,[2] the melting point of l-arabinosediphenylhydrazone is 204–205°C.

Consequently, the diphenylhydrazone in question is arabinosediphenylhydrazone.

The mother-liquor, separated from the crystals of arabinosediphenylhydrazone, was slowly evaporated to a small volume, when white needle crystals were again formed. They were filtered, washed with a little water, and then dissolved in 5 c.c. of hot water. After cooling, the recrystallized diphenylhydrazone was separated by filtration and dried.

1) Ber. D. Chem Ges., Berlin, **33** (1900), pp. 2243–2254.
2) Ber. D. Chem. Ges., Berlin, **37** (1904), pp. 311–315; ibid., **38** (1905), pp. 500–501; ibid., **39** (1906), pp. 3576–3581; ibid., **39** (1906), pp. 3581–3582.

A small amount of the crystals was heated with dilute hydrochloric acid, but the solution gave neither furfurol reaction with anilin acetate, nor spectral reaction for pentose with phloroglucin and hydrochloric acid. The crystals produced no mucic acid upon oxidation with nitric acid.

The melting point of the diphenylhydrazone was determined and found to be 158–160°C.

According to STAHEL,[1] the melting point of d-glucosediphenylhydrazone is 161°C.

Therefore, it will be clear that the diphenylhydrazone in hand is that of glucose.

5. Isolation of l-Arabinose.

After a week, the syrup (I) was found thickly laden with fine crystals. To the syrup, 95% alcohol was added, well mixed, filtered with suction, and washed with 95% alcohol and ether. The sugar thus obtained was recrystallized from alcohol. After drying, it became perfectly white, left no ash on ignition and was about 2.5 grams in weight.

The aqueous solution of the sugar was colorless and very sweet. It reduced FEHLING's solution very strongly, and also gave a spectral reaction for pentose very distinctly.

0.1 gram of the dried sugar was dissolved in water, a few drops of alumina cream added, and then made up to 10 c.c. and polarized in a 100 mm.-tube. Strong birotation was observed. After 24 hours, the rotation was 3.0 on the scale toward the right.

The specific rotatory power of the sugar is

$$[\alpha]_D = + \frac{3.0 \times 0.346 \times 10}{0.1 \times 1} = + 103.8°$$

0.1 gram of the dried sugar was dissolved in a small amount of water, to which a solution of 0.2 gram of diphenylhydrazin, dissolved in absolute

1) LIEBIG's Ann. Chem., (1890), **258**, pp. 242–245; abs. in Ber. D. Chem. Ges., Berlin, **23** (1890), Ref., p. 282.

alcohol, was added. The mixture was heated in a boiling water bath. When cooled, white, needle crystals, melting at 203–204°C., were produced. By heating with phenylhydrazin and acetic acid, the sugar produced easily osazone, which is soluble in hot water. After recrystallization from hot water, the melting point was determined and found to be 159–160°C.

The specific rotatory power and the melting points of diphenylhydrazone as well as of phenylosazone indicate that the sugar under examination is l-arabinose.

The filtrate from the crystals of l-arabinose was again concentrated to a syrup. After a month, new crystals of sugar were separated, their properties were determined and ascertained to be identical with those of l-arabinose.

6. Phenylosazone Tests.

1 gram of the syrup, remaining after the separation of second crystals of l-arabinose, was mixed with 2 grams of phenylhydrazin hydrochloride, 3 grams of sodium acetate and 20 c.c. of water, and the mixture was heated in a boiling water bath for an hour and a half. When cooled, crystals of osazone were formed in abundance. They were filtered and washed with water, and then separated into two parts by hot water. The osazone soluble in hot water melted at 159–160°C., which coincides with that of l-arabinosephenylosazone. The osazone insoluble in hot water was again separated into two parts by hot dilute alcohol. The alcohol-soluble osazone, melted at 188–189°C., while the insoluble one melted at 204°C. The former seems to be galactosephenylosazone, and the latter, glucosephenylosazone, the amount of which was the least of the three.

Judging from the melting points of osazones, it is to be supposed that the syrup here examined contains arabinose, galactose and a small amount of glucose.

Summary.

The results of the above investigation are summarized as follows:—

1) The principal constituents of the edible part of the tubers of *Apios*

Fortunei are carbohydrates, the chief of which are starch and sugars.

2) Glucose and sucrose seem to compose the principal sugar in the tubers; the latter exceeds the former in amount. The presence of fructose in free form is probable, but its amount is very small even if present.

3) Hemicellulose of the edible part of the tubers is made up of both galactan and araban. As to the other constituents of the hemicellulose, I have not sufficient data to decide.

II. COLOCASIA ANTIQUORUM SCHOTT.

Colocasia antiquorum SCHOTT. is cultivated to a large extent in the central and the southern parts of Japan and its tuberous rootstocks are widely consumed as a valuable food material. There are several names commonly given to the tubers of this plant according to their forms. The name of the sample used for my investigation is "Satoimo" (サトイモ) (an ordinary variety).

A quantitative analysis was made of the edible part of the tubers, and the results are as follows:—

	Air-dry substance. %	Water-free substance. %
Water	79.47	—
Protein	2.44	11.75
Fat	0.16	0.80
Crude fiber	0.76	3.70
Nitrogen-free extract	16.24	79.24
Ash	0.93	4.51
Total nitrogen	0.39	1.88
Protein nitrogen	0.29	1.42
Non-protein nitrogen	0.10	0.46
Reducing sugar	0.12	0.60
Non-reducing sugar	0.16	0.79
Dextrin	0.14	0.68

Starch	14.67	71.44
Galactan	0.60	2.94
Pentosan	0.66	3.23

From the above table, we may observe that the main constituents of the tubers are carbohydrates, which form about 83 % of water-free substance, and that about 24 % of the total nitrogen is in the form of non-protein. Of the carbohydrates, starch is a prominent member, which forms about 71 % of water-free substance.

Sugars of the Tubers.

1. Preparation of the Syrup.

200 grams of the finely pulverized material of the edible part of the tubers were mixed with 600 c. c. of 95 % alcohol and heated in a boiling water bath for 5 hours, using a reflux-condenser, and filtered with suction. With the residue the same treatment was repeated twice to make the extraction complete. 1600 grams of the sample were extracted by the above process and the combined extracts of the repeated treatments were concentrated, with the addition of a little calcium carbonate, to about 500 c. c. After allowing to stand for about 20 hours, a brownish gummy substance was deposited. The brown colored clear solution was decanted and concentrated to a small volume. The concentrated solution was again purified with 95 % alcohol and evaporated to a syrup.

2. Qualitative Reactions of the Syrup.

The syrup gave the following qualitative reactions:—

a) It was very sweet and of a yellow color.

b) It gave MOLISCH's reaction.

c) It reduced FEHLING's solution strongly; after inversion, the reducing power was much enhanced.

d) It rotated the plane of polarization toward the right; even after inversion, toward the right, weakly.

e) It gave no spectral reaction for pentose.

f) It gave BRAUN's reaction.

g) It gave both PINOFF's and SELIWANOFF's reactions for ketose.

h) It gave no mucic acid reaction, but the saccharic acid test was positive.

i) It produced no mannosephenylhydrazone with phenylhydrazin, either before or after inversion.

j) Five drops of the syrup were placed on an object glass and the drops were seeded with crystals of glucose, galactose, mannose, sucrose, and maltose, respectively. After a few days, the drops which had been seeded with glucose and sucrose showed the formation of new crystals, while the others remained unchanged.

From the above qualitative reactions, we may conclude that the syrup here examined contains both reducing and non-reducing sugars and that the presence of glucose, fructose and sucrose is most probable. On the other hand, the absence of pentose, galactose and mannose is also probale.

3. Isolation of Sucrose.

When the syrup was allowed to evaporate at a low temperature, it was found thickly laden with fine white crystals. A small amount of 95 % alcohol was added to the syrup, well mixed, filtered with suction, and washed with 95 % alcohol and ether. The mother-liquor, filtered off from the crystals, was again evaporated to a syrup and left to stand till the following day. New crystals were found in it. They were examined under a microscope and found to have the same crystalline form as that of the sugar first obtained. Upon recrystallization from alcohol, the crystals became perfectly white and left no ash on ignition. The weight of the sugar was about 4.5 grams.

The aqueous solution of the sugar showed no reducing power, but after inversion, it reduced FEHLING's solution. It was very sweet. It gave the ketose reaction with resorcin and hydrochloric acid.

0.9282 gram of the dried sugar was dissolved in water, a few drops of

alumina cream added, and then made up to 25 c. c. and polarized in a 100 mm.-tube. A dextro-rotation of 7.1 on the scale was observed.

The specific rotatory power of the sugar is

$$[\alpha]_D = +\frac{7.1 \times 0.346 \times 25}{0.9282 \times 1} = +66.2°$$

Consequently, the sugar under examination is sucrose.

4. Phenylosazone Tests.

a) 1 gram of the syrup was mixed with 2 grams of phenylhydrazin hydrochloride, 3 grams of sodium acetate and 20 c. c. of water and heated in a boiling water bath for an hour and a half. Yellow, needle crystals of osazone were formed. After recrystallization from 60 % alcohol, the melting point was determined and found to be 204–206°C. The mother-liquor, separated from the crystals of the osazone, was again concentrated. New crystals of osazone were produced. After drying, their melting point was determined and found to be 204°C.

Consequently, the osazone under examination is glucosephenylosazone.

b) 1 gram of the syrup was dissolved in 20 c. c. of water and inverted with hydrochloric acid in a boiling water bath for about 30 minutes. After neutralization with sodium carbonate, the solution was heated with phenylhydrazin hydrochloride and sodium acetate as in the above experiment. Yellow crystals of osazone were formed and found to be quite identical with those of glucosephenylosazone obtained before. After recrystallization from alcohol, the melting point was determined and found to be 204–205°C. The mother-liquor, filtered off from the first crystals of osazone, was again concentrated. New crystals of osazone, the melting point of which is 204°C., were formed.

Consequently, all the osazone in question is glucosephenylosazone.

No other osazone than that of glucose was observed.

The absence of mannose and the presence of sucrose were proved in the previous experiments. Maltose can hardly be expected to exist, since the

characteristic crystalline form of maltosephenylosazone could not be observed. From the results, it may be inferred that the reducing sugar consists of glucose or fructose or both, while the non-reducing sugar is sucrose. The presence of fructose in the free form is most probable as I have described in the qualitative reactions, but its amount, if present, must be very small, as the attempt to isolate the fructose as methylphenylosazone after NEUBERG's method was not successful.

Products of Hydrolysis of Hemicellulose.

1. Preparation of the Sample.

2000 grams of air-dry finely pulverized substance of the edible part of the tubers were extracted with boiling 95 % alcohol. The residue was heated with water until the starch was gelatinized. And then, the gelatinized starch was saccharified with malt extract several times. The residue was again extracted with 0.25 % caustic soda solution, filtered and well washed with water until the filtrate became neutral to litmus. The residue was dried and used as the sample for the hydrolysis.

2. Method of Hydrolysis.

The sample above prepared was hydrolyzed with 6 liters of 3 % sulphuric acid in a boiling water bath for about 8 hours. When cooled, the solution was filtered and neutralized with calcium carbonate. The filtrate was evaporated to a small volume and purified with 95 % alcohol in the usual manner. The final syrup, indicated as the syrup (I), was about 60 grams in weight.

The residue, remaining after hydrolysis with sulphuric acid, was again hydrolyzed with 2 liters of 4 % sulphuric acid according to the process above described. After purifying with alcohol several times, the final syrup was about 17 grams, indicated as the syrup (II).

3. Qualitative Reactions of the Syrup.

Both syrups, (I) and (II), gave the following qualitative reactions respectively: –

a) They were sweet and of a brown color.

b) They gave MOLISCH's reaction.

c) They reduced FEHLING's solution very strongly.

d) They rotated the plane of polarization toward the right strongly.

e) They gave the spectral reaction for pentose.

f) They gave neither SELIWANOFF's nor PINOFF's reaction for ketose.

g) They produced mucic acid in abundance upon oxidation with nitric acid of 1.15 sp. gr.

h) They did not produce mannosephenylhydrazone with phenylhydrazin.

i) BERTRAND's reaction was applied to detect xylose. White, boat-shaped crystals, which gave the reactions of cadmium and bromin, were formed, but the amount of the crystals was too small to allow an analysis.

j) Five drops of each syrup were placed on an object glass, and the drops were seeded with crystals of mannose, galactose, arabinose, xylose and rhamnose, respectively. Even after 3 days, they did not show any sign of forming new crystals.

From the above reactions, it may safely be concluded that these syrups contain both galactose and pentose, but neither mannose nor fructose.

4. Isolation of Arabinosebenzylphenylhydrazone.

An attempt was made to isolate arabinose by the use of benzylphenylhydrazin, according to the method of RUFF and OLLENDORFF.[1]

7 grams of the syrup (I) were dissolved in 20 grams of 70 % alcohol, to which a solution of 5 grams of benzylphenylhydrazin in 9 grams of absolute alcohol was added, and the mixture well shaken. The fluid slowly became turbid and after an hour and a half, white, fine crystals were formed. After 10 hours, the crystals were separated by filtration with suction, washed with a small amount of 75 % alcohol and finally recrystallized from 95 % alcohol.

1) Ber. D. Chem. Ges., Berlin, **32** (1899), pp. 3234–3237.

The product obtained in this manner was perfectly white and was observed to be fine needle crystals under a microscope. The weight of the crystals was about 0.4 gram, when dried over concentrated sulphuric acid in a vacuum.

The melting point of the hydrazone was determined and found to be 169–170°C., which coincides with that of arabinosebenzylphenylhydrazone.

0.134 gram of the hydrazone was dissolved in 50 c. c. of methyl alcohol and polarized in a 200 mm.-tube. A laevo-rotation of 0.2 on the scale was observed.

The specific rotatory power of the hydrazone is

$$[\alpha]_D = -\frac{0.2 \times 0.346 \times 50}{0.134 \times 2} = -12.9°$$

The specific rotatory power found indicates that the benzylphenylhydrazone in hand is that of arabinose.

The mother-liquor, separated off from the crystals of arabinosebenzylphenylhydrazone, was slowly evaporated to a small volume, but no other crystals of benzylphenylhydrazone were formed.

5. Phenylosazone Tests.

1 gram of the syrup (I) was treated with phenylhydrazin hydrochloride and sodium acetate, to isolate sugars as phenylosazones. Yellow crystals of osazone were formed. An attempt was made to separate the osazones by their solubility and to determine the melting points. The osazone soluble in hot water melted at 159–160°C. The osazone insoluble in hot water was separated by using hot alcohol. The osazones, soluble in hot 30% alcohol, in hot 60% alcohol, and in hot 85% alcohol, melted at 159–160°C, 189–190°C., and 190°C., respectivery.

From the above experiments, two kinds of osazone were isolated. The one melting at 159–160°C. coincides with the osazone of arabinose, while the other melting at 189–190°C. with that of galactose.

Mucilaginous Substance.

1. Separation of Mucilage.

The edible part of the tuber was chopped into three or four pieces, and those pieces were put in a jar containing a sufficient quantity of water. After 24 hours, the extract was carefully decanted and then filtered through linen-cloth repeatedly. The starch was tested with potassium iodide iodin solution under a microscope, but the filtrate was free from starch. To the starch-free filtrate, strong alcohol was added, when a colorless mucilaginous substance was easily separated. The mucilage thus prepared was dried and used for the following investigation.

2. Qualitative Reactions of the Mucilage.

The mucilage gave the following qualitative reactions :—

a) It gave no starch reaction.

b) When the preparation was boiled with water, it became pasty. On warming with MILLON'S reagent, it gave no color reaction for protein.

c) MOLISCH'S reaction was positive.

d) On boiling with 5 % sulphuric acid, it was easily hydrolyzed, and the solution showed a reducing power very strongly.

e) Upon oxidation with nitric acid of 1.15 sp. gr., it produced mucic acid in abundance.

f) It gave spectral reaction for pentose.

From the above reactions, we may conclude that the mucilage mainly consists of galactan and pentosan.

According to my analysis, the amount of galactan is about four times that of pentosan.

3. Method of Hydrolysis.

15 grams of the preparation were heated with 100 c. c. of 5 % sulphuric acid in a boiling water bath for about 8 hours, using a reflux-condenser.

When cooled, the solution was filtered and the filtrate was neutralized with calcium carbonate. The neutral solution obtained was evaporated to a small volume, twice purified with alcohol and then evaporated to a syrup.

4. Qualitative Reactions of the Syrup.

The syrup above prepared by the hydrolysis of the mucilage gave the following qualitative reactions:—

a) It was very sweet and reduced FEHLING's solution very strongly.

b) It rotated the plane of polarization toward the right.

c) It gave both mucic acid reaction for galactose and spectral reaction for pentose.

5. Isolation of d-Galactose.

The syrup above prepared was found thickly laden with white crystals after 3 days. A small amount of 95 % alcohol was added to the syrup, well mixed, filtered with suction, and washed with 95 % alcohol and ether. The sugar was again recrystallized from strong alcohol and dried over concentrated sulphuric acid. The sugar thus isolated became perfectly white and was about 0.85 gram in weight.

The aqueous solution of the sugar was very sweet and reduced FEHLING's solution very strongly. SELIWANOFF's reaction was negative. Upon oxidation with nitric acid of 1.15 sp. gr., mucic acid was produced.

0.281 gram of the dried sugar was dissolved in water and then made up to 10 c.c. and polarized in a 100 mm.-tube. Bi-rotation was observed. After 24 hours, the rotation was 6.5 on the scale toward the right.

The specific rotatory power of the sugar is

$$[\alpha]_D = +\frac{6.5 \times 0.346 \times 10}{0.281 \times 1} = +80.0^\circ$$

From the specific rotatory power and other properties, the sugar in hand is considered as d-galactose.

6. Phenylhydrazone and Phenylosazone Tests.

The mother-liquor, filtered off from the crystals of d-galactose, was again concentrated to a syrup, to which phenylhydrazin was added, and the mixture was well stirred and set aside in a cold room. White, fine, needle crystals of hydrazone were easily produced. After drying, the melting point was determined and found to be 158°C., which coincides with that of the galactose-phenylhydrazone. The mother-liquor, separated from the crystals of the hydrazone, was acidified with acetic acid and heated in a boiling water bath for an hour and a half. When cooled, the crystals of osazone produced were filtered and washed with water. The osazone obtained was separated into two parts by its solubility in water. The osazone soluble in hot water melted at 159°C., which coincides with that of arabinosephenylosazone. The insoluble osazone melted at 191–193°C., which seems to agree with that of galactose-phenylosazone.

From the above experiments, we may conclude that the osazones consist of galactose-and arabinosephenylosazone, in which the former exceeds the latter in quantity. The absence of mannose and glucose is also probable.

Summary.

The results of the above investigation are summarized as follows:—

1) The principal constituents of the edible part of the tubers of *Colocasia antiquorum* are carbohydrates, the chief of which is starch.

2) Sugars in the tubers consist of glucose, fructose, and sucrose.

3) Hemicellulose of the edible part of the tubers is made up of both galactan and araban.

4) The mucilaginous substance of the tubers consists of galactan and pentosan, in which the former exceeds the latter in amount. The pentosan seems to consist chiefly of araban, although no further study other than that of osazone could be made on account of the small amount of the material.

III. CORYDALIS AMBIGUA CHAM. ET SCH.

Corydalis ambigua CHAM. et SCH. (エゾノエンゴサク) is largely distributed in a wild state in Hokkaido. Its tubers are utilized as food material in the time of famine. The tubers used for the present investigation were taken in Sapporo in the middle part of October, 1915.

A quantitative analysis was made of the whole tubers with the following results:—

	Air-day substance. %	Water-free substance. %
Water	57.86	—
Protein	2.19	5.13
Fat	0.22	0.52
Crude fiber	1.20	2.85
Nitrogen-free extract	37.93	90.07
Ash	0.60	1.43
Total nitrogen	0.35	0.82
Protein nitrogen	0.24	0.58
Non-protein nitrogen	0.11	0.24
Reducing sugar	1.32	3.13
Non-reducing sugar	5.69	13.50
Dextrin	2.30	5.45
Starch	26.29	62.38
Galactan	Trace	Trace
Pentosan	0.98	2.33

From the above table, we may observe that the main constituents of the tubers are carbohydrates, which form about 93 % of water-free substance, and that about 30 % of the total nitrogen is in the form of non-protein. Of the carbohydrates, starch forms about 62 % of water-free substance. Total sugar comprises about 17 % of water-free substance, and the weight of non-reducing sugar is about 4 times that of reducing sugar.

Sugars of the Tubers.

1. Preparation of the Syrup.

1500 grams of the finely pulverized material of the tubers were extracted with ether. 500 grams of the fat-free sample were mixed with 1500 c.c. of 95 % alcohol and heated in a boiling water bath for 10 hours, using a reflux-condenser, and then filtered with suction. The residue was treated three more times by the same process to complete the extraction. Another 1000 grams of the sample were extracted in the same manner. The extracts were combined and evaporated to a small volume. (Syrup (I)).

A portion of the syrup (I) was dissolved in water, to which a small quantity of basic lead acetate solution was added, and the mixture was well stirred. The precipitate was removed. The filtrate, to which a large amount of basic lead acetate and ammonia were added, formed a large amount of a flocculent, white substance. The insoluble lead compound was collected, well washed with water, suspended in water and decomposed by hydrogen sulphide. After the decomposition was complete, it was filtered, well washed with water, and then the filtrate was evaporated to a small volume, purified with 95 % alcohol and concentrated to a syrupy condition. (Syrup (II)).

2. Qualitative Reactions of the Syrup.

Both syrups, (I) and (II), gave the following qualitative reactions respectively:—

a) They gave MOLISCH'S reaction.

b) They reduced FEHLING'S solution strongly; after inversion, the reducing power was much enhanced.

c) They rotated the plane of polarization toward the right, but after inversion, toward the left.

d) They gave no spectral reaction for pentose.

e) They gave BRAUN'S reaction.

f) They gave no PINOFF'S reaction for ketose, but after inversion, the reaction was positive.

g) They gave SELIWANOFF's reaction for ketose.

h) They gave no mucic acid reaction.

i) They produced no mannosephenylhydrazone with phenylhydrazin, either before or after inversion.

From the above qualitative reactions, we may conclude that these syrups contain both reducing and non-reducing sugars, and that pentose, galactose, mannose and fructose are absent.

3. Isolation of Sucrose.

When the syrup (II) was allowed to stand for a month, it was found thickly laden with large crystals. The crystals were separated from the mother-liquor, and recrystallized from alcohol. After drying, the crystals became perfectly white and left no ash on ignition.

The aqueous solution of the sugar showed no reducing power, but after inversion, it reduced FEHLING's reaction. It was very sweet. It gave SELIWANOFF's reaction for ketose.

0.1904 gram of the dried sugar was dissolved in water, a few drops of alumina cream added, and then made up to 10 c. c. and polarized in a 100 mm.-tube. A dextro-rotation of 3.6 on the scale was observed.

The specific rotatory power of the sugar is

$$[\alpha]_D = +\frac{3.6 \times 0.346 \times 10}{0.1904 \times 1} = +65.4^\circ$$

Consequently, the sugar under examination is sucrose.

4. Phenylosazone Tests.

The mother-liquor, filtered off from the crystals of sucrose in the previous experiment, was again concentrated to a syrupy condition, and an attempt was made to isolate sugars as phenylosazones by the method of FISCHER.

a) 1 gram of the syrup was treated with phenylhydrazin hydrochloride and sodium acetate. Yellow crystals of osazone were formed. After recrystallization from alcohol, about 0.1 gram of pure osazone was obtained. The

determination of nitrogen in the osazone above obtained gave the following result:—

0.08 g. substance gave 0.01219 g. N.

		N.
$C_{18} H_{22} N_4 O_4$	Calculated	15.64 %
	Found	15.24 %

The melting point of the osazone was determined and found to be 204°C.

The crystalline form, the quantity of nitrogen and the melting point indicate that the osazone in hand is glucosephenylosazone.

b) 1 gram of the syrup was dissolved in 20 c. c. of water and inverted with hydrochloric acid. After neutralization with sodium carbonate, the solution was heated with phenylhydrazin hydrochloride and sodium acetate as in the above experiment. Yellow crystals of osazone were formed. After recrystallization from alcohol, about 0.6 gram of pure osazone was obtained. The determination of nitrogen in the osazone gave the followihg result:—

0.1782 g. substance gave 0.02726 g. N.

		N.
$C_{18} H_{22} N_4 O_4$	Calculated	15.64 %
	Found	15.30 %

The melting point of the osazone was determined and found to be 204–206°C.

Consequently, the osazone under examination is glucosephenylosazone.

The presence of sucrose and the absence of fructose and mannose were ascertained in the previous experiments. The presence of maltose is also excluded as the characteristic crystalline form of maltosephenylosazone could not be observed.

Consequently, it is certain that the reducing sugar consists of glucose while the non-reducing sugar is sucrose.

Summary.

The results of the above investigation are summarized as follows:—

1) The principal constituents of the tubers of *Corydalis ambigua* are carbohydrates, the chief of which are starch and sugars.

2) Glucose and sucrose seem to compose the principal part of the sugars in the tubers.

3) Pentosan is present, but the presence of galactan is uncertain.

IV. DIOSCOREA BATATAS DECNE.

Dioscorea Batatas DECNE. (ツクネイモ) used for the present investigation is produced in Hokkaido.

A quantitative analysis was made of the edible part of the tubers, and the following results were obtained:—

	Air-dry substance. %	Water-free substance. %
Water	70.50	—
Protein	3.00	10.19
Fat	0.11	0.36
Crude fiber	0.65	2.20
Nitrogen-free extract	24.76	83.93
Ash	0.98	3.32
Total nitrogen	0.48	1.63
Protein nitrogen	0.32	1.07
Non-protein nitrogen	0.16	0.56
Reducing sugar	0.70	2.38
Non-reducing sugar	1.51	5.11
Dextrin	0.49	1.67
Starch	18.23	61.81
Galactan	None	None
Pentosan	0.66	2.25

From the above table, we may observe that the main constituents of the tubers are carbohydrates, which form about 86% of water-free substance,

and that about 35 % of total nitrogen is in the form of non-protein. Of the carbohydrates, starch is a prominent constituent, comprising about 62 % of water-free substance. Total sugar forms about 8 % of water-free substance, and the weight of non-reducing sugar is about 2 times that of reducing sugar.

Sugars of the Tubers.

1. Preparation of the Syrup.

400 grams of the finely pulverized material of the tubers were mixed with 1200 c. c. of 95 % alcohol and heated in a boiling water bath for 4 hours, using a reflux-condenser, and filtered with suction. The residue was treated twice by the same process to complete the extraction. The combined extracts were concentrated to a small volume and then dissolved in water. The insoluble precipitate was removed and the clear solution was evaporated to about 30 grams of the syrup.

2. Qualitative Reactions of the Syrup.

The syrup above prepared gave the following qualitative reactions :—

a) It was very sweet and of a yellow color.

b) It gave MOLISCH'S reaction.

c) It reduced FEHLING'S solution strongly ; after inversion, the reducing power was much enhanced.

d) It rotated the plane of polarization toward the right, but after inversion, toward the left.

e) It gave no spectral reaction for pentose.

f) It gave BRAUN'S reaction.

g) It gave no PINOFF'S reaction for ketose, but after inversion, the reaction was positive.

h) It gave the ketose reaction with resorcin and hydrochloric acid.

i) It gave no mucic acid reaction.

j) It produced no mannosephenylhydrazone with phenylhydrazin, either before or after inversion.

From the above qualitative reactions, we may conclude that the syrup examined contains both reducing and non-reducing sugars, and that the presence of pentose, galactose, mannose and fructose is excluded.

3. Phenylosazone Tests.

a) 1 gram of the syrup was treated with phenylhydrazin hydrochloride and sodium acetate according to FISCHER's method. Yellow, needle crystals of osazone were formed. After recrystallization from alcohol, the melting point was determined and found to be 204–205°C.

Consequently, the osazone here examined is glucosephenylosazone.

The mother-liquor, separated from the crystals of glucosephenylosazone, was again evaporated to a small volume. No other crystals of osazone than those of glucosephenylosazone were formed.

b) 1 gram of the syrup was dissolved in 20 c. c. of water and inverted with hydrochloric acid. After neutralization with sodium carbonate, the solution was heated with phenylhydrazin hydrochloride and sodium acetate as in the previous experiment. Yellow crystals of osazone were formed and found to be all uniform and quite identical with those of osazone obtained in the previous experiment. After recrystallization from alcohol, the melting point was determined and found to be 204°C. The mother-liquor, separated from the crystals of the above osazone, was again concentrated to a small volume. Crystals of osazone, melting at 204°C., were again isolated.

Consequently, the osazones under examination are glucosephenylosazone.

No other crystalline forms of osazone than those of glucosephenylosazone were found in the further evaporation of the filtrate.

Mannose and fructose are absent as proved in the previous experiments, and no maltosephenylosazone could be found. Therefore it is certain that the reducing sugar consists of glucose while the non-reducing sugar is sucrose.

Summary.

The results of the above investigation are summarized as follows:—

1) The principal constituents of the edible part of the tubers of *Dioscorea Batatas* are carbohydrates, the chief of which are starch and sugars.

2) Sugars in the tubers consist of glucose and sucrose.

3) Pentosan is present, but no galactan.

V. ELEOCHARIS PLANTAGINEA R. BR.

Eleocharis plantaginea R. Br. (クログワヰ) mostly grows wild in the central part of Japan and is cultivated also in some localities. The tubers used for the present investigatinn were collected in the province of Yechigo, Niigataken, (新潟縣越後國北蒲原郡中條町), in February, 1915, where they had been cultivated.

A quantitative analysis was made of the edible part of the tubers, and the results are as follows:—

	Air-dry substance. %	Water-free substance. %
Water	68.52	—
Protein	2.25	7.19
Fat	0.19	0.61
Crude fiber	1.00	3.17
Nitrogen-free extract	26.46	84.01
Ash	1.58	5.02
Total nitrogen	0.36	1.15
Protein nitrogen	0.27	0.86
Non-protein nitrogen	0.09	0.29
Reducing sugar	0.24	0.75
Non-reducing sugar	1.06	3.38
Dextrin	0.60	1.90
Starch	18.75	59.57
Galactan	None	None
Pentosan	0.79	2.52

From the above table, we may observe that the main constituents of the

tubers are carbohydrates, which form about 87 % of water-free substance, and that about 25 % of the total nitrogen is in the form of non-protein. Of the carbohydrates, starch is a prominent member, which comprises about 60 % of water-free substance. Total sugar forms 4.13 % of water-free substance, and the weight of non-reducing sugar is about 4.5 times that of reducing sugar.

Sugars of the Tubers.

1. Preparation of the Syrup.

500 grams of the finely pulverized material of the tubers were mixed with 1200 c. c. of 95 % alcohol, were heated in a boiling water bath for 5 hours, using a reflux-condenser, and filtered with suction. The residue was treated four more times by the same process to make the extraction complete. Another 1000 grams of the sample were extracted in the same manner. The extracts were combined and concentrated to a small volume. (Syrup (I)).

A portion of the syrup (I) was dissolved in water, to which a small quantity of basic lead acetate solution was added and the mixture well stirred. The precipitate was removed. The excess of lead compound in the solution was removed by precipitating with hydrogen sulphide. The clear solution, after neutralization with calcium carbonate, was evaporated to a small volume, twice purified with 95 % alcohol and concentrated to a syrupy consistency. (Syrup (II)).

2. Qualitative Reactions of the Syrup.

Both syrups, (I) and (II), gave the following qualitative reactions respectively :—

a) They were sweet and of a reddish-brown color.

b) They gave MOLISCH's reaction.

c) They reduced FEHLING's solution strongly; after inversion, the reducing power was much enhanced.

d) They rotated the plane of polarization toward the right; after inversion, toward the left weakly.

e) They gave no spectral reaction for pentose, and no mucic acid reaction for galactose.

f) They gave BRAUN'S reaction.

g) They gave both PINOFF'S and SELIWANOFF'S reactions for ketose.

h) They produced no mannosephenylhydrazone with phenylhydrazin, either before or after inversion.

i) Five drops of each syrup were placed on an object glass and the drops were seeded with crystals of glucose, galactose, mannose, sucrose and maltose, respectively. After a few days, the drops which had been seeded with glucose and sucrose showed the formation of new crystals, while the others remained unchanged.

From the above qualitative reactions, it may be said that these syrups contain both reducing and non-reducing sugars, and that the presence of glucose and sucrose, and the absence of pentose, galactose, and mannose are highly probable. The presence of free fructose is also probable according to PINOFF'S reaction.

3. Phenylosazone Tests.

a) 1 gram of the Syrup (II) was treated with phenylhydrazin hydrochloride and sodium acetate according to FISCHER'S method. Yellow, needle crystals of osazone were formed. After recrystallization from 60% alcohol, the melting point was determined and found to be 205°C. The crystalline form and the melting point indicate that the osazone in hand is glucosephenylosazone. No other crystals of osazone than those of glucosephenylosazone were formed in the further evaporation of the filtrate.

b) 1 gram of the Syrup (II) was dissolved in 20 c. c. of water and inverted with hydrochloric acid. After neutralization with sodium carbonate, the solution was heated with phenylhydrazin hydrochloride and sodium acetate as in the previous experiment. Yellow, needle crystals of osazone were formed and found to be quite identical with the osazone obtained in the previous experiment. After recrystallization from 60% alcohol, the melting

point was determined and found to be 204–205°C.

Consequently, the osazone under examination is glucosephenylosazone.

Even after a further evaporation of the filtrate, no other crystals of osazone than those of glucosephenylosazone were found.

Mannose is absent in the syrup as I already mentioned, while maltosephenylosazone could not be found among the osazones formed. Therefore, it is certain that the reducing sugar consists of glucose or fructose or both, wihle the non-reducing sugar is sucrose.

4. Isolation of Sucrose.

After a month, the syrup (II) was found thickly laden with fine crystals.

To the syrup, 95% alcohol was added, well mixed, filtered with suction, and then washed with 95% alcohol and ether. The crystals of sugar thus separated were recrystallized from alcohol. The product obtained in this manner was perfectly white and weighed about 3 grams.

The aqueous solution of the sugar showed no reducing power, but after inversion, it reduced FEHLING'S solution. It was very sweet. It gave SELIWANOFF'S reaction for ketose.

0.4 gram of the dried sugar was dissolved in water, a few drops of alumina cream added, and then made up to 10 c. c. and polarized in a 100 mm.-tube. A dextro-rotation of 7.7 on the scale was observed.

The specific rotatory power of the sugar is

$$[\alpha]_D = +\frac{7.7 \times 0.346 \times 10}{0.4 \times 1} = +66.6^\circ$$

Consequently, the sugar in hand is sucrose.

The mother-liquor, filtered off from the crystals of sucrose, was again evaporated to a small volume and left to stand for about 2 months. It did not show a sign of forming new crystals of other sugar than those of sucrose.

Products of Hydrolysis of Hemicellulose.

1. Preparation of the Sample.

1850 grams of air-dry finely pulverized substance of the tubers were ex-

tracted with boiling 95 % alcohol. The residue was heated with water until the starch became gelatinized. Next the gelatinized starch was saccharified with malt extract several times. The residue was extracted with 0.25 % caustic soda solution, filtered and well washed with water until the filtrate became neutral to litmus. The residue was dried and used as the sample for the hydrolysis.

2. Method of Hydrolysis.

The sample above prepared was hydrolyzed with 6 liters of 3 % sulphuric acid in a boiling water bath for about 15 hours. When cooled, it was filtered and well washed with water. The solution was neutralized with calcium carbonate and the filtrate was evaporated to a small volume. To the solution here obtained, a small quantity of basic lead acetate solution was added and the mixture was well stirred. The precipitate was removed by filtration with suction. The excess of lead compound in the solution was removed by precipitating with hydrogen sulphide. The clear solution, after neutralization with calcium carbonate, was evaporated to a small volume, twice purified with 95 % alcohol; and then concentrated to a syrupy condition.

The residue, remaining after hydrolysis with 3 % sulphuric acid, was again hydrolyzed with 2 liters of 5 % sulphuric acid in the same manner as mentioned before. After purifying with basic lead acetate and alcohol, the solution was concentrated to a syrupy condition. The syrups obtained were combined and used for the following experiment.

3. Isolation of d-Glucose.

When the syrup was allowed to stand for about 2 weeks, it was found thickly laden with large, white crystals. They were separated from the mother-liquor, and recrystallized from alcohol. The crystals thus obtained were dried at 60–80°C. and then in the desiccator in a vacuum. After drying, the crystals became perfectly white and left no ash on ignition.

The aqueous solution of the sugar was very sweet, reduced FEHLING's solution very strongly, and produced osazone in the usual manner. The form of the crystals and their melting point were identical with those of glucosephenylosazone.

0.9 gram of the dried sugar was dissolved in water, a little alumina cream added, and then made up to 25 c. c. and polarized in a 200 mm.-tube. Bi-rotation was observed. After 24 hours, the rotation was 11.0 on the scale toward the right,

The specific rotatory power of the sugar is

$$[\alpha]_D = +\frac{11.0 \times 0.346 \times 25}{0.9 \times 2} = +52.3°$$

The melting point of the sugar was determined and found to be 146–148°C.

Consequently, the sugar in hand is d-glucose.

The mother-liquor, filtered off from the crystals of d-glucose, was again concentrated to a syrupy condition, from which the crystals of sugar were isolated thrice. Their specifie rotatory powers were determined and found to be +51.9°, +52.94°, and +52.37° respectively. The qualitative reactions of the crystals were equal to those of the crystals first obtained.

All the sugar thus isolated is therefore d-glucose.

The mother-liquor, separated from the fourth crystals of d-glucose, was evaporated to a syrup.

The syrup gave the following qualitative reactions:—

a) It was sweet and of a yellowish-brown color.

b) It gave MOLISCH's reaction.

c) It reduced FEHLING's solution very strongly.

d) Its aqueous solution rotated the plane of polarization toward the right.

e) It gave the spectral reaction for pentose.

f) It gave neither SELIWANOFF's nor PINOFF's reaction for ketose.

g) It gave no mucic acid reaction.

h) It did not produce mannosephenylhydrazone with phenylhydrazin.

i) BERTRAND'S reaction was applied to detect xylose. White, boat-shaped crystals, which gave the reactions of cadmium and bromin, were formed, but the amount was too small to allow an analysis.

From the above reactions, it may safely be said that the syrup contains pentose but not any mannose, fructose, or galactose.

4. Isolation of Arabinosediphenylhydrazone.

0.5 gram of the syrup was dissolved in a small amount of water, to which 0.5 gram of diphenylhydrazin and a sufficient quantity of absolute alcohol were added to form a perfectly clear solution. The mixture was heated in a water bath and then cooled. White, needle crystals of diphenylhydrazone were produced. They were separated by filtration and again recrystallized from 95% alcohol. The melting point of the diphenylhydrazone was determined and found to be 203–204°C., which exactly coincides with that of arabinosediphenylhydrazone.

Consequently, it is certain that the isolated diphenylhydrazone is that of arabinose.

The mother-liquor, separated from the crystals of arabinosediphenylhydrazone, was evaporated down to a small volume. But no other forms of crystals than those of arabinosediphenylhydrazone were formed.

Summary.

The results of the above investigation are summarized as follows:—

a) The principal constituents of the tubers of *Eleocharis plantaginea* are carbohydrates, the chief of which are starch, sugar and pentosan.

b) Glucose and sucrose seem to compose the principal part of the sugars in the tubers. The presence of free fructose is also probable.

c) Araban forms the principal constituent of the hemicellulose.

VI. HELIANTHUS TUBEROSUS L.

Helianthus tuberosus L. (キクイモ), used for the present investigation, was collected in Sapporo, Hokkaido, in the middle part of November, 1914.

A quantitative analysis was made of the edible part of the tubers, and the following results were obtained :—

	Air-dry substance. %	Water-free substance. %
Water	82.27	—
Protein	2.31	13.19
Fat	0.14	0.81
Crude fiber	0.88	4.98
Nitrogen-free extract	13.28	74.72
Ash	1.12	6.30
Total nitrogen	0.37	2.11
Protein nitrogen	0.20	1.14
Non-protein nitrogen	0.17	0.97
Reducing sugar	0.41	2.30
Non-reducing sugar	1.74	9.82
Inulin	6.51	36.74
Galactan	0.58	3.28
Pentosan	0.79	4.46

From the above table, we may observe that the main constituents of the tubers are carbohydrates, which form about 80 % of water-free substance, and that about 46 % of the total nitrogen is in the form of non-protein. Of the carbohydrates, inulin is a prominent constituent, which comprises about 37 % of water-free substance. Total sugar forms about 12 % of water-free substance, and the weight of non-reducing sugar is about 4.5 times that of reducing sugar.

Sugars of the Tubers.

1. Preparation of the Syrup.

1200 grams of the finely pulverized material of the edible part of the tubers were extracted with 95 % alcohol, and the extracts were concentrated, purified and finally made into a syrup in the usual manner. The syrup is indicated as the syrup (I).

A portion of the syrup (I) was dissolved in water, to which a small quantity of basic lead acetate solution was added, and the mixture was well stirred, filtered, and the excess of lead compound in the filtrate was removed by hydrogen sulphide. The clear solution, after neutralization with calcium carbonate, was evaporated to a small volume, purified with 95 % alcohol and concentrated to a syrup which is indicated as the syrup (II).

2. Qualitative Reactions of the Syrup.

Both syrups, (I) and (II), gave the following qualitative reactions respectively:—

a) They were very sweet and of a yellowish-brown color.

b) They gave MOLISCH's reaction.

c) They reduced FEHLING's solution strongly; after inversion, the reducing power was much enhanced.

d) They rotated the plane of polarization toward the right; after inversion, toward the left weakly.

e) They gave no spectral reaction for pentose nor any mucic acid reaction for galactose.

f) They gave BRAUN's reaction.

g) They gave no PINOFF's reaction for ketose directly, but after inversion the reaction was positive.

h) They gave SELIWANOFF's reaction for ketose.

i) They produced no mannosephenylhydrazone with phenylhydrazin, either before or after inversion.

From the above qualitative reactions, it may be said that these syrups contain both reducing and non-reducing sugars, and that pentose, fructose, galactose and mannose are absent.

3. Phenylosazone Tests.

a) 1 gram of the syrup (II) was treated with phenylhydrazin hydrochloride and sodium acetate according to FISCHER's method. Yellow crystals of osazone were formed. After recrystallization from 60% alcohol, the melting point of the osazone was determined and found to be 204°C. The crystalline form and the melting point indicate that the osazone in hand is no other than glucosephenylosazone.

The mother-liquor, separated from the crystals of glucosephenylosazone, was again concentrated to a small volume. New crystals of osazone were formed, but their form was quite identical with that of glucosephenylosazone first obtained. The melting point was found to be 204°C.

Consequently, the osazone under examination is glucosephenylosazone.

b) 1 gram of the syrup (II) was dissolved in 20 c.c. of water and inverted with hydrochloric acid. After neutralization with sodium carbonate, the solution was heated with phenylhydrazin hydrochloride and sodium acetate. Yellow crystals of osazone were formed and found to be all uniform with the melting point of 204–206°C. The filtrate, separated from the crystals of the above osazone, was again concentrated to a small volume. New crystals of osazone were formed, which were quite identical in form and melting point with the osazone first obtained.

Consequently, the osazone above obtained is glucosephenylosazone.

Mannose and fructose are absent as shown in the previous experiment, and also no maltosephenylosazone could be found. From the above data, it is certain that the reducing sugar consists of glucose, while the non-reducing sugar is sucrose.

4. Isolation of Sucrose.

According to SCHULZE's method,[1] a portion of the syrup (II) was dissolved in hot 90 % alcohol and then heated to boiling with the addition of a hot saturated solution of strontium hydrate, using over 3 parts of strontium hydrate for 1 part of the syrup used. After boiling for 30 minutes, the precipitate produced was filtered hot and then, after suspending in water, decomposed with a stream of carbon dioxide. The solution, filtered from strontium carbonate, was evaporated to the state of syrup, and then was purified with 95 % alcohol several times. When it was seeded with the crystals of sucrose and left for about a week, new crystals were formed. After recrystallization from alcohol, the crystals became perfectly white and left no ash on ignition.

The aqueous solution of the sugar showed no reducing power, but after inversion, it reduced FEHLING's solution very strongly. It was very sweet. It gave SELIWANOFF's reaction for ketose. It rotated the plane of polarization toward the right, but after inversion, toward the left. From the above date, it is concluded that the sugar in hand is sucrose.

Products of Hydrolysis of Hemicellulose.

1. Preparation of the Sample.

1200 grams of air-dry finely pulverized substance of the edible part of the tubers were extracted with boiling 95 % alcohol. The residue was extracted with water and then with 0.25 % caustic soda solution, filtered and well washed with water until the filtrate became neutral to litmus. The residue was once more extracted with hot water, filtered, dried and used as the sample for the hydrolysis.

2. Method of Hydrolysis.

The sample above prepared was hydrolyzed with 6 liters of 3 % sulphu-

1) Landw. Vers.-Stat., **34** (1887), pp. 403-407; abs. in Ber. D. Chem. Ges., Berlin, **21** (1888), Ref, p. 299.

ric acid in a boiling water bath for about 15 hours. When cooled, it was filtered and the residue well washed with water. The solution was neutralized with calcium carbonate and filtered. To the filtrate, a small quantity of basic lead acetate solution was added, and the mixture was well stirred. The precipitate was removed by filtration with suction. The excess of lead compound in the solution was removed by hydrogen sulphide, The clear solution, after neutralization with calcium carbonate, was evaporated down to a small volume, purified with 95 % alcohol three times and then concentrated to the state of syrup which is indicated as the syrup (I).

The residue, remaining after hydrolysis with 3 % sulphuric acid, was again hydrolyzed with 2 liters of 5 % sulphuric acid. After purifying with basic lead acetate and alcohol, the solution was concentrated to the state of syrup which is indicated as the syrup (II).

3. Qualitative Reactions of the Syrup.

Both syrups, (I) and (II), gage the following qualitative reactions respectively :—

a) They were sweet aud a brown color.

b) They gave MOLISCH's reaction.

c) They reduced FEHLING's solution very strongly.

d) They rotated the plane of polarization toward the right strongly.

e) They gave spectral reaction for pentose.

f) They produced mucic acid in abundance upon oxidation with nitric acid of 1.15 sp. gr.

g) They gave neither SELIWANOFF's nor PINOFF's reaction for ketose.

h) They did not produce mannosephenylhydrazone with phenylhydrazin.

i) BERTRAND's reaction was used for the detection of xylose. White, boat-shaped crystals were formed, which gave the reactions of cadmium aud bromin, but the amount was too small to allow an analysis.

j) Six drops of each syrup were placed on an object glass and the

drops were seeded with the crystals of mannose, galactose, glucose, arabinose, xylose, and rhamnose, respectively. Even after a few days, they did not show any sign of forming new crystals.

From the above reactions, it may be concluded that these syrups contain both galactose and pentose, but no mannose nor fructose.

4. Isolation of Arabinosebenzylphenylhydrazone.

1 gram of the syrup (I) was treated with benzylphenylhydrazin according to the method of RUFF and OLLENDORFF. The fluid slowly became turbid, and in the course of 2 hours, fine, white, crystals were formed. After 15 hours, the crystals were separated by filtration with suction, and then recrystallized from 95% alcohol. The product obtained in this manner was perfectly white and left no ash on ignition. The melting point of the hydrazone thus obtained was determined and found to be 169–170°C., which coincides with that of arabinosebenzylphenylhydrazone.

0.0368 gram of the hydrazone was dissolved in 15 c.c. of methyl alcohol and polarized in a 200 mm.-tube. A laevo-rotation of 0.2 on the scale was observed.

The specific rotatory power of the hydrazone is

$$[\alpha]_D = -\frac{0.2 \times 0.346 \times 15}{0.0368 \times 2} = -14.1°$$

Consequently, the benzylphenylhydrazone in hand is that of arabinose.

The mother-liquor, separated from the crystals of arabinosebenzylphenylhydrazone, was slowly evaporated to a small volume, but no other form of crystals than those of arabinosebenzylphenylhydrazone was formed.

5. Phenylosazone Tests.

1 gram of the syrup, (I)+(II), was treated with phenylhydrazin hydrochloride and sodium acetate according to FISCHER'S method. Yellow crystals of osazone were formed. An attempt was made to separate the osazone into two parts by hot water. The osazone soluble in hot water melted at

157–159°C., the insoluble one at 193°C. The former coincides with the osazone of arabinose, and the latter with that of galactose.

Summary.

The results of the above investigation are summarized as follows:—

1) The principal constituents of the edible part of the tubers of *Helianthus tuberosus* are carbohydrates, the chief of which are inulin and sugars.

2) Sugars consist of glucose and sucrose.

3) Hemicellulose of the edible part of the tubers is made up of both galactan and araban.