On the Constituents of Rush-pith (Tōshin).

BY

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A large number of investigations have been undertaken in late years, on the chemistry of cellulose-tissues of plants; little, however, is known as yet of the exact nature of the substances which enter into the composition of plant pith. So far as the author is aware, maize (*Zea Mays* L.) and elder (*Sambucus nigra* L.) are the only plants whose pith has been subjected to thorough chemical research.[1] Further investigations concerning the chemistry of the piths of other plants are therefore necessary and desirable from the scientific, if not from the practical standpoint.

Rush-pith has long been used in Japan as wick for oil lamps,[2] the commercial article being known under the name of *Tōshin* which means literally lamp-wick. It has also been used as wick for Japanese candles.[3] *Tōshin* is nothing but the pith of a rush—*Juncus effusus* L., var. *decipiens* F. Buch. The pith is easily obtained by a simple but ingenious process of splitting the stem of the rush lengthwise. The pith consists of stellate parenchyma cells of soft and delicate nature, with large intercellular spaces between.

The *Tōshin* used for the present investigation was white and spongy, of about 2 mm. in diameter and about 400 mm. in length and free from admixtures. The material was placed at my disposal through the courtesy of MR. S. KAWAMURA of Akita, to whom I here express my sincere thanks.

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1) C. A. BROWNE and TOLLENS—Berlin, Ber. D. chem. Ges. 35 (1902) pp. 1457–1467.

2) Rape seed oil is commonly used. The light is necessarily dim, but soft and restful to the eye. Though in common use in Japan under the old régime, its use is confined, at present, chiefly to temples and Buddhist services.

3) In this connection, it is interesting to note that according to TYLOR (*Anthropology*, 1881, p. 273) the rush light, made of the pith of the rush dipped in melted fat, was in common use in Pliny's time.

Qualitative Tests.

Qualitative reactions were examined at the outset of the investigation, to get a general idea about the chief constituents of the rush-pith.

Microchemical tests were applied to determine the presence of starch, reducing sugar, free cellulose and lignin. None of the reactions characteristic of those compounds was shown. The absence of lignin was rather unexpected and repeated trials were made with phloroglucin-hydrochloric-acid,[1] anilin sulphate, and permanganate-reaction,[2] with a negative result in every case. According to BROWNE's test (*l.c.*), the pith of maize, as well as that of elder, shows lignin reactions very distinctly.

A small amount of the substance was heated in a test tube, with dilute hydrochloric acid (1 : 3) for about 10 minutes and then filtered. The filtrate gave the characteristic absorption-spectrum of pentose, upon warming with phloroglucin and hydrochloric acid. On distilling the substance with hydrochloric acid of 1.06 sp. gr., a distillate was obtained which gave characteristic furfurol reaction with anilin acetate. The distillate was also tested for the presence of methyl furfurol by the spectrum reaction of the author and TOLLENS.[3] To about 10 c.c. of the distillate, a little phloroglucin and equal volume of concentrated hydrochloric acid were added and filtered after standing for 5 minutes. The clear filtrate obtained showed characteristic absorption-spectrum of methyl furfurol. Both pentosan and methyl pentosan are therefore present in the rush-pith.

Zinc-chloride-iodin solution was used to test for free cellulose. When applied directly to sections of the pith, the result was negative, as already stated. Pentosan-free fiber prepared after KÖNIG'S method[4] gave at once a dark blue color with the solution, showing that the cellulose here was in the free form. The cellulose in rush-pith evidently holds pentosan and methyl pentosan in combination.

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1) Hadromal-reaction of CZAPEK—HOPPE-SEYLERS Zs. physiol. Chem., Strassburg. 27 (1899) p. 141.

2) MÄULE—Verhalten verholz. Memb. gegen Kalium-permanganat, Habilitationsschrift, Stuttgart, 1901.

3) OSHIMA u. TOLLENS—Berlin, Ber. D. chem. Ges. 34 (1901) pp. 1425–1426.

4) KÖNIG—Zs. Unters. Nahrungsmittel, Berlin, 1 (1898) pp. 3–16. By this method methyl pentosan is removed as well. Details on this point will be published later.

Quantitative Analysis.

Analysis was made by the Weende method, commonly adopted for food stuffs. The results follow :

	Air-dry substance. %	Water-free substance. %
Water	7.15	—
Ash	4.39	4.73
Protein	1.73	1.86
Fat	6.55	7.05
Crude fiber	33.16	35.72
Nitrogen-free extract	47.02	50.64
Pentosan-free fiber	25.35	27.30
Pentosan...	35.02	37.72
Methyl pentosan	2.82	3.04

The crude fiber obtained by the Weende method contained still a considerable amount of pentosan, as could be easily shown with phloroglucin and hydrochloric acid. KÖNIG'S method (*l.c.*) was consequently tried to determine pentosan-free fiber by treating the substance with glycerin of 1.23 sp. gr., and containing 2 grams of sulphuric acid in 100 c.c. The fiber obtained by this method gave no pentosan reaction with phloroglucin and hydrochloric acid, nor did it show that of methyl furfurol on testing its distillate by the spectrum reaction. Treated with zinc-chloride-iodin solution, the fiber assumed at once a dark blue color, indicating the presence of free cellulose.

Determination of pentosan and methyl pentosan was made according to the method of ELLETT and TOLLENS.[1] The substance was distilled with hydrochloric acid of 1.06 sp. gr., adding 30 c.c. of the acid into the distilling flask, whenever 30 c.c. were distilled over. The distillation was continued until the distillate gave no more characteristic reactions of furfurol and

1) Berlin, Ber. D. chem. Ges. 38 (1905) pp. 492–499.

methyl furfurol. The former was tested with anilin acetate, the latter by the spectrum reaction of the author and TOLLENS (*l.c.*). Calculated amount of phloroglucin was added to the distillate, to precipitate all of the furfurol and methyl furfurol present as phloroglucides. On the following day the mixture of the phloroglucides was filtered, and weighed after drying. The methyl furfurol phloroglucide was then extracted with 95 % alcohol, at about 60°, and the residue dried and weighed. This gives the weight of furfurol phloroglucide, while the difference shows the amount of methyl furfurol phloroglucide. For the calculation of pentosan from furfurol phloroglucide, KRÖBER'S formula[1] was used; for that of methyl pentosan from methyl furfurol phloroglucide, the formula of ELLETT and TOLLENS (*l.c.*) was applied.

It is interesting to compare the composition of the pith of the rush with that of maize-pith and elder-pith.

In 100 parts of dry substance.

	Rush-pith.	Maize-pith.[2]	Elder-pith.[2]
Ash	4.73	4.48	1.93
Protein	1.86	3.31	2.50
Fat	7.05	1.40	1.19
Crude fiber	35.72	42.41	69.05
Cellulose	27.30	39.93	41.96
Pentosan	37.72	27.04	18.81
Methyl pentosan	3.04	none	present

Considerable differences are to be noticed in the amounts of pentosan and cellulose, in different kinds of pith. Pentosan is richest in rush-pith and least in elder-pith, while the amount of cellulose is in reversed order. Noticeable is also the large amount of fat (ether extract) in rush-pith. In general, the composition of rush-pith approaches more closely to that of maize-pith than that of elder-pith. This is what we should expect, since both rush and maize belong to Monocotyledonae, while elder is a Dicotyledonous plant.

1) HOPPE-SEYLERS Zs. physiol. Chem., Strassburg. 36 (1903), Anhang.
2) BROWNE and TOLLENS (*l.c.*)

Products of Hydrolysis.

(1) Method of Hydrolysis.

90 grams of the pith and 1800 c.c. of 5 % sulphuric acid were put into a porcelain jar. The jar and the contents were heated in a boiling water bath for 20 hours. During the heating the jar was kept covered, the contents being stirred from time to time. At the end of the stated period, the tissues of the pith seemed to have been disintegrated and the smell of furfurol was appreciable. When cooled it was filtered through muslin. The yellowish filtrate was neutralized with pure calcium carbonate and allowed to stand overnight. On the following morning, the calcium sulphate was filtered off through a "Nutsch" filter with suction and the filtrate was concentrated, with the addition of a little calcium carbonate to about 100 c.c., in partial vacuum. The warm solution thus obtained was put into a dry flask with 500 c.c. of 85% alcohol and allowed to stand for about 10 hours, when a blackish gummy substance adhered to the sides and bottom of the flask. The fluid was decanted and concentrated again in partial vacuum to about 100 c.c. To the remaining syrup, about 500 c.c. of 95% alcohol were added. This produced a second precipitate of yellowish gummy substance. After standing for a few hours, the clear solution was decanted and concentrated to a small volume. The syrup was once more purified by shaking with about 200 c.c. of absolute alcohol. The clear solution was decanted and evaporated down to about 70 c.c. The syrup thus prepared was preserved for further investigation, being indicated as syrup A. Another portion of the syrup was prepared from 150 grams of the pith, with 3000 c.c. of 5% sulphuric acid, in exactly the same manner as described above. This is designated as syrup B in the statement below.

(2) Detection of Xylose.

Syrup A produced no crystals, even at the end of two weeks after preparation. It gave the following reactions;

1) It reduced Fehling's solution very strongly.
2) It rotated the plane of polarization toward the right.
3) It gave the characteristic absorption-spectrum of pentose with phloroglucin and hydrochloric acid.

4) It produced neither mucic acid nor saccharic acid, upon oxidation with nitric acid of 1.15 sp. gr.

5) It gave no ketose reaction with resorcin and hydrochloric acid.

6) It produced no characteristic mannose-phenylhydrazone with phenylhydrazin.

7) 5 drops of the syrup were placed on an object glass and were seeded respectively with a crystal of xylose, arabinose, dextrose, galactose and mannose. After 36 hours, the drop seeded with xylose showed the formation of new crystals, while all the rest remained unchanged.

From the above reactions it is safe to conclude that the syrup did not contain any dextrose, levulose, galactose or mannose. On the other hand, the presence of xylose was highly probable. To ascertain its presence, BERTRAND'S reaction[1] was made use of.

5 grams of the syrup were dissolved in 15 c.c. of water, in a small flask, and 7 grams of cadmium carbonate and 3 grams of bromin were added and well mixed. After standing for 20 hours, the mixture was warmed and when all the bromin was driven out it was heated to boiling, filtered hot and the residue washed with hot water. The filtrate was concentrated, and when nearly dry, 25 c.c. of 95% alcohol were added. After about half an hour, the formation of fine crystals was observed. When examined under microscope, the crystals proved to be the characteristic boat shaped needles of cadmium bromoxylonate. After a few hours, the crystals were separated from the mixture by spreading upon an unglazed porcelain plate. These were then recrystallized from alcohol, using animal charcoal. Perfectly white crystals were thus obtained, which on filtering were washed with alcohol and ether and finally dried over sulphuric acid in vacuum.

Analysis of the cadmium salt was made with the following result :

0.2480 gm. substance gave	0.0948 gm. Cd S.	
0.3158 „ „ „	0.1575 „ Ag Br.	
For $(C_5H_9O_6CdBr+H_2O)$[1] ...	Cd %	Br %
Calculated	29.86	21.32
Found	29.73	21.22

1) Paris, Bul. Soc. Chim. (3) 5 p. 554.

1) MAQUENNE—Les Sucres et principaux dérivés, Paris, 1900, p. 815. BERTRAND (*l.c.*) gives the formula $(C_5H_9O_6)_2Cd+CdBr_2+2H_2O$.

The result shows clearly that the substance on hand was cadmium bromoxylonate. The presence of xylose in the syrup is thus fully confirmed.

3) Isolation of Xylose.

Syrup B was left untouched nearly two months, when, to the agreeable surprise of the author, it was found thickly laden with fine crystals. A little amount of 85% alcohol was added to the syrup, well mixed, filtered with suction and washed with absolute alcohol and ether. The sugar thus obtained was 4.2 grams in weight and slightly yellowish in color, but, upon recrystallization from alcohol with use of animal charcoal, it became perfectly white and left no ash on ignition.

1 gram of the carefully dried sugar was dissolved in water and made up into 25 c. c. and polarized in 100 mm. tube, in the SOLEIL-VENTZKE Polariscope. Strong bi-rotation was observed. After 24 hours the rotation was 2.1 on the scale toward the right. The specific rotatory power is

$$[a]\ D = \frac{2.1 \times 0.346 \times 25}{1 \times 1} = +\ 18.2^\circ \text{ (at } 21^\circ)$$

The mother-liquor filtered off from the crystals formed again a considerable quantity of new crystals in a few days. At the end of a week, when the formation of crystals seemed to be at a standstill, the crystals were separated by filtration with suction and washed with absolute alcohol and ether. The yield of sugar was 1.4 grams. Upon recrystallization from alcohol and careful drying, its specific rotatory power was determined and found to be

$$[a]\ D = \frac{3.0 \times 0.346 \times 25}{0.7215 \times 2} = +\ 18.1^\circ \text{ (at } 19^\circ)$$

The calculated specific rotatory power of 4% xylose solution at 20°, according to TOLLENS[1] is

$$[a]\ D = 18.095 + 0.06986\ p = 18.4^\circ$$

The sugar under examination is consequently xylose.

1) Handbuch d. Kohlenhydrate II, Breslau, 1895, p. 70.

4) Isolation of Arabinose.

The mother-liquor filtered off from the second crystals of xylose was allowed to evaporate slowly by itself. It did not show any sign of forming new crystals of its own accord, after long standing. Trial was made to induce the formation of crystals by seeding with xylose or arabinose, but the effort was in vain in both cases. Attempt was then made to separate and detect arabinose by use of benzylphenylhydrazin, according to the method of RUFF and OLLENDORF.[1] Benzylphenylhydrazin easily forms with arabinose a hydrazone, which is hardly soluble in 75% alcohol, while xylose-hydrazone is easily soluble in the same medium. Thus it affords an excellent means of separating the two sugars from each other.

7 grams of the syrup 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 soon became turbid and in course of 3 hours abundant crystalline precipitates were formed. The crystals were separated by filtration with suction, washed with a small amount of 75% alcohol and finally recrystallized from 95% alcohol. The product obtained in this manner was perfectly white and weighed 1.45 grams when dried over sulphuric acid in vacuum. The melting point was found to be 169°–170° which coincides with that of arabinose-benzylphenylhydrazone.

0.2054 gram of the substance was dissolved in 50 c.c. of methyl alcohol and polarized in 200 mm. tube. A levo-rotation of 0.3 on the scale was observed. The specific rotatory power is

$$[a]\ D = \frac{0.3 \times 0.346 \times 50}{0.2054 \times 2} = -\ 12.6^\circ \text{ (at } 15^\circ)$$

The specific rotatory power of arabinose-benzylphenylhydrazone, according to VAN EKENSTEIN and DE BRUYN[2] is −14.6°, while BROWNE and TOLLENS[3] found it to be −12.1°.

For the separation of arabinose from its hydrazone, benzaldehyde was used. The operation was carried out in the following manner. A mixture

1) Berlin, Ber. D. chem. Ges. 32 (1899) p. 3234.
2) Berlin, Ber. D. chem. Ges. 29 Ref. p. 911.
3) *Ibid*, 35 (1902) p. 1461.

of 1.2 grams of arabinose-benzylphenylhydrazone, 10 grams of 95% alcohol, and 1 gram of benzaldehyde was heated on water bath, in a small flask provided with a reflux condenser. At the end of $4\frac{1}{2}$ hours an oily liquid was formed, which on cooling solidified into a crystalline mass. On recrystallization from alcohol, fine needles were formed whose melting point was found to be 110°. The melting point coincides with that of benzal-benzylphenylhydrazone and shows that decomposition of arabinose-benzylphenylhydrazone had taken place. The filtrate from benzal-hydrazone was shaken with ether in a separating funnel three times and the watery solution was then evaporated. On cooling, crystals began to show themselves in the syrup. They were separated by filtration, washed with absolute alcohol and ether and finally dried over sulphuric acid in vacuum. The sugar thus obtained was slightly colored, but further purification was not attempted because of its small amount.

0.2566 gram of the sugar was dissolved in water, a few drops of alumina cream added and then made up into 25 c.c. and polarized in 200 mm. tube. Bi-rotation was observed. At the end of 24 hours a dextro-rotation of 6.1 on the scale was noted, The specific rotatory power is

$$[a]\ D = \frac{6.1 \times 0.346 \times 25}{0.2566 \times 2} = + 102.8° \text{ (at } 20°)$$

The observed specific rotatory power coincides closely with that of arabinose. Isolation of arabinose from the syrup is hereby fully demonstrated.

Summary.

The chief results of the present investigation will be here recapitulated.

We have succeeded, first of all, in isolating xylose (in comparatively large amount), as well as arabinose, from the hydrolysis product, by tedious and painstaking processes. The pentosan of rush-pith is therefore made up of both xylan and araban, the former, however, predominating in amount over the latter.

The fact that both xylan and araban were proved to exist together in the pith, is of special interest. So far, the co-existence of two kinds of

pentosans (xylan and araban) has been positively proved only in four cases, namely in brewers' grain (barley),[1] and in maize-pith, elder-pith and cherry-gum.[2] Rush-pith may now be added to the list.

The presence of methyl pentosan in rush-pith is also worthy of notice. A wide distribution of methyl pentosan in nature, accompanying pentosan, has been shown in late years, by the investigations of WIDTSOE, OSHIMA, ELLETT and TOLLENS, SOLLIED, VOTOCEK, and Y. SUZUKI[3] in co-operation with the author.

The cellulose in rush-pith is not in free form, but is, in all probability, in combination with pentosans (xylan and araban) and methyl pentosan. The cellulose, together with pentosans and methyl pentosan, forms the main bulk of the pith—namely, about 68% of its dry matter.

1) STONE and TOLLENS--Zs. Ver. D. Zuckerindustrie, 38 p. 1135.
2) BROWNE and TOLLENS (*l.c.*)
3) TRANS, SAPPORO NAT. HIST. SOC., 1 (1906). pp. 119-123.