AMERICAN
JOURNAL OF BOTANY

VOL. II OCTOBER, 1915 NO. 8

THE UTILIZATION OF CERTAIN PENTOSES AND COMPOUNDS OF PENTOSES BY GLOMERELLA CINGULATA*

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Compounds of the pentose sugars are generally present in the cell walls of plants. These compounds apparently may be composed of pentoses alone or in combination with sugars of other groups. The compounds of pentoses alone are frequently called pentosans. When they are considered to be derived from a single pentose, they may be referred to more specifically, as, for example, araban and xylan, which are considered to be the polysaccharides of arabinose and xylose respectively. The widespread occurrence of these compounds in plants has led to numerous investigations as to their utilization by organisms. The question as to whether or not animals and plants secrete enzymes capable of splitting the complex pentosans to their constituent sugars has also received some attention. A discussion of the results of some of these investigations will follow. For a more comprehensive bibliography the work of Swartz[1] may be consulted.

It has been shown by Lindsey and Holland,[2] Slowtzoff,[3] Goetze and Pfeiffer,[4] Swartz,[5] and others that pentosans as such disappear in

* Published by permission of the Secretary of Agriculture.

[1] Swartz, Mary Davies. Nutrition Investigations on the Carbohydrates of Lichens, Algae, and related substances. Trans. Conn. Acad. Arts and Sciences **16**: 247–382. 1911.

[2] Lindsey, J. B., and Holland, E. B. Concerning the Digestibility of the Pentosans. Ann. Rep. of Mass. Agr. Exp. Sta. 1894: 175–188.

[3] Slowtzoff, B. Ueber das Verhalten des Xylans im Thierkörper. Zeitschr. Physiol. Chemie **34**: 181–193. 1901–2.

[4] Goetze, K., and Pfeiffer, Th. Beiträge zur Frage über die Bildung resp. das Verhalten der Pentaglykosen im Pflanzen- und Tierkörper. Landw. Versuchs-Stationen **47**: 59–93. 1896.

[5] Swartz, Mary Davies. *Loc. cit.*

[The **Journal** for July (**2**: 311–374) was issued 18 Aug 1915]

passing through the alimentary canal of higher animals, but it seems doubtful from the work of Seillière[6] and of Swartz that the digesting enzymes are secreted by the higher animals themselves. Seillière attempted to show that the digestion of xylan by various animals was not carried out by the intestinal or pancreatic juices of these animals but by the bacteria present in the intestines. His work seems to be corroborated by the investigations of Swartz. Seillière[7] showed further, however, that the extracts of the digestive organs of snails hydrolyzed xylan, and later obtained similar results with other molluscs[8] and also with the larvae of certain Coleoptera.[9]

The question of the utilization of pentoses and pentosans by plants, especially by fungi, has received some attention. Behrens[10] has shown that *Botrytis vulgaris* and some Penicilliums can utilize both arabinose and xylose as a source of carbon. Went[11] obtained good results when growing *Monilia sitophila* (Mont.) Sacc. upon xylose. Cross, Bevan and Smith,[12] Schöne and Tollens,[13] and Cross and Tollens[14] consider that the disappearance of pentoses in fermenting mixtures of sugars is due to the utilization of pentoses in the building up of the yeast plant. Krüger[15] has recently shown that a species of Gloeosporium parasitic upon apple, which he calls *Gloeosporium fructigenum germanicum*, can utilize arabinose. Other writers have

[6] Seillière, G. Sur la digestion de la xylane chez quelques mammifères herbivores. Compt. Rend. Soc. Biol. **64**: 941–943. 1908; Sur la digestion de la xylane chez les mammifères. Compt. Rend. Soc. Biol. **66**: 691–693. 1909.

[7] Seillière, G. Sur la présence d'une diastase hydrolysant la xylane dans le suc gastro-intestinal de L'Escargot. Compt. Rend. Soc. Biol. **58**: 409–410. 1905.

[8] Seillière, G. Sur la présence de la xylanase chez différents Mollusques gastéropodes. Compt. Rend. Soc. Biol. **59**: 20–22. 1905.

[9] Seillière, G. Sur une diastase hydrolysant la xylane dans le tube digestif de certaines larves de Coléoptères. Ibid. **58**: 940–941. 1905.

[10] Behrens, J. Beiträge zur Kenntnis der Obstfäulnis. Centralbl. Bakter. und Parasit. Abt. II **4**: 547–553. 1898.

[11] Went, F. A. F. C. *Monilia sitophila* (Mont.) Sacc., ein technischer Pilz Javas. Centralbl. Bakter. und Parasit. Abt. II **7**: 591–598. 1901.

[12] Cross, C. F., Bevan, E. J., and Smith, Claude. The Carbohydrates of Barley Straw. Journ. Chem. Soc. **73**: 459–463. 1898.

[13] Schöne, A., und Tollens, B. Ueber die Gärung der Pentosen. Journ. Landw. **49**: 29–40. 1901.

[14] Cross, W. E., und Tollens, B. Versuche über das Verhalten der Pentosen in gärenden Mischungen. Journ. Landw. **59**: 419–428. 1911.

[15] Krüger, Frederich. Beiträge zur Kenntniss einiger Gloeosporien I und II. Arbeit. Kaiserl. Biol. Anstalt Land und Forstwirt. **9**: 233–323. 1913.

shown that the pentoses can be used by fungi, but it is generally agreed that they can not be fermented.

In regard to the utilization of pentosans by fungi it seems probable from the work of Czapek[16] and Schörstein[17] that certain components of the cell walls of woody tissues are rendered soluble and utilized by some fungi. The last mentioned writer reaches the conclusion that xylan is digested by *Merulius lachrymans*. He bases his conclusion upon the observed difference in optical rotation of extracts of wood before and after it had been acted upon by the fungus. Swartz[18] found apparently that the pentosans of *Rhodymenia palmata*, a marine alga, were hydrolyzed to a reducing substance by "Taka diastase." Duggar and Davis[19] recently announced that they were unable to demonstrate the presence of a pentosanase in Fucus.

It seems then that pentosans and pentoses are of some value as food for higher animals but that these organisms probably secrete no enzymes capable of hydrolyzing pentosans. Some of the invertebrates can utilize pentoses readily and seem to be able to hydrolyze some pentosans. Pentoses have been found to be a good source of carbon for certain fungi and there is evidence that some pentosans may be broken down through the action of enzymes secreted by fungi. The products of such enzyme action have apparently not been identified.

It was to obtain more information upon the effect of parasitic fungi on pentoses and their compounds that the experiments described in this paper were planned and carried out. The study includes a series of experiments on the effect of *Glomerella cingulata* (Stonem.) S. & v. S. upon pentose compounds in the apple fruit, a series on the utilization of pentoses and certain of their compounds as sources of carbon for this fungus, and experiments upon the effect of the extract of the fungus mycelium under aseptic conditions on xylan.

The fungus was isolated from apples kindly furnished by Dr. C. L. Shear and was maintained in stock culture in tubes of cornmeal agar throughout the study. In the experiments on the effect of the fungus upon the pentose-containing compounds in apples practically

[16] Czapek, F. Zur Biologie der holzbewohenden Pilze. Bericht. Deutsch. Bot. Ges. 17: 166–170. 1899.

[17] Schörstein, J. Zur Biochemie der Holzpilze. Centralbl. Bakter. und Parasit. Abt. II 9: 446–447. 1902.

[18] Swartz, Mary Davies. *Loc. cit.*

[19] Duggar, B. M., and Davis, A. R. Enzyme Action in *Fucus vesiculosus* L. Ann. Mo. Bot. Gard. 1: 419–426. 1914.

the same methods of sampling and analysis were used as in a former study of peaches.[20] The different compounds were determined in the two halves of the same fruit, one portion of which had been inoculated with the fungus while the other was retained sterile as a control. In the determination of the furfurol-yielding material it was found that the percentage of this substance in the rotten half was considerably less than in the sound portion. The fungus apparently used the furfurol-yielding constituents of the apple fruit. Several apples were then prepared and inoculated. After two weeks the sound and rotten halves were sliced up and extracted with alcohol. The furfurol-yielding material was then determined in the extract and solid portions separately. The results are given in Table I. All data were calculated as percentage of the original wet weight of the portion of the apple used.

TABLE I

Percentage of Alcohol-soluble, Alcohol-insoluble, and Total Furfurol-yielding Material in Sound and Rotten Halves of Apple, Each Substance Determined in Sound and Rotten Halves of Same Fruit

Alcohol-soluble		Alcohol-insoluble		Total	
Sound half	Rotten half	Sound half	Rotten half	Sound half	Rotten half
0.12	0.18	0.62	0.49	0.74	0.67
0.12	0.16	0.71	0.50	0.83	0.66
0.14	0.27	1.11	0.93	1.25	1.20

From the data in Table I it is apparent that the total percentage of furfurol-yielding material and the percentage of alcohol-insoluble furfurol-yielding material were higher in the sound portion than in the corresponding rotten half of the apple, but that the percentage of alcohol-soluble furfurol-yielding material was higher in the rotten half.

Pentose sugars are readily soluble in 80 percent alcohol while most of the furfurol-yielding material in the sound apple is not. The increase in alcohol-soluble furfurol-yielding material during the early stages of the rot therefore indicates that some compounds containing pentoses were broken down. That this was due to the action of the fungus and not to the autolysis of the dead apple tissue was evident from the fact that when portions of apple in which the cells had been killed with chloroform were allowed to stand under aseptic

[20] Hawkins, Lon A. Some Effects of the Brown Rot Fungus upon the Composition of the Peach. Amer. Journ. Bot. 2: 71–81. 1915.

conditions for three weeks they contained practically the same percentage of both alcohol-soluble and alcohol-insoluble pentosans as control portions of the same apples in which the cells had not been killed.

When the fungus was allowed to act upon the apple four weeks or more the percentages of furfurol-yielding material in the alcohol extract and in the solid residue were both higher in the sound half. It is evident then that the fungus is able to break down the pentose-containing compounds, whether they be pentosans or compounds of the pentoses with other sugars, and to use the pentoses. There is no evidence at hand, however, to show which pentoses can be used or what compounds of the pentoses are most readily broken down by the fungus. This was made the subject of further experimentation. An attempt was made to determine the value of xylose, arabinose, xylan and arabin as compared with glucose as sources of carbon for this fungus.

The fungus was grown on a solution of nutrient salts to which the sugars and compounds of the pentoses were added. The medium was prepared according to a formula used by Hasselbring[22] in his work on Penicillium. It was composed of NH_4NO_3 1 g., KH_2PO_4 0.5 g., $MgSO_4$ 0.25 g., and 100 cc. H_2O. To this was added the carbohydrate usually in sufficient quantity to make a 1 percent concentration. Three hundred cc. Erlenmeyer flasks were used for the cultures. In each of these 100 cc. of the culture solution was placed. These solutions were inoculated with the fungus by transferring conidiospores from the stock cultures with a sterile needle. After a suitable period the felt of mycelium was removed from each flask separately, washed with a little water, placed in a tared, glass-stoppered weighing bottle and covered with alcohol. The alcohol was driven off at low temperature, about 60° C., and the weighing bottles with mycelium dried to constant weight in a vacuum drying oven at 78° C.

The sugars, the arabin, and a small part of the xylan used in these experiments were purchased from a chemical supply house. The rest of the xylan was prepared from rye straw, most of it according to a method similar to that used by Schöne and Tollens[23] for the prepara-

[22] Hasselbring, H. The Carbon Assimilation of Pencillium. Bot. Gaz. 45: 176–193. 1908.

[23] Schöne, A., und Tollens, B. Untersuchungen über die Pentosane der Jute, der Luffa, und der Biertreber. Journ. Landw. 49: 21–28. 1901.

tion of xylan from jute. The ground straw was extracted with 2 percent solution of ammonia and then digested 48 hours with a 6 percent solution of KOH, 6 liters to each original half kilogram of straw. The extract was pressed out, filtered through cloth and the xylan precipitated by adding an equal volume of 95 percent alcohol to the solution. It was then filtered and the precipitate neutralized with a solution of HCl in alcohol. The xylan was washed in a Buchner funnel with 60 percent alcohol until the washings gave no test for chloride with silver nitrate. It was then dried with absolute alcohol and ether, ground up in a mortar, passed through a fine sieve and was ready for use. In order to prove that the preparation was a compound of xylose sugar, a quantity of the xylan was hydrolyzed with sulphuric acid according to the method of Wheeler and Tollens.[24]

The mixture of xylan and 5 percent sulphuric acid was heated on the steam bath for six hours. The acid was then neutralized with magnesium-free calcium carbonate, filtered, and the filtrate evaporated nearly to dryness under reduced pressure. The residue was taken up with hot alcohol and treated with animal charcoal to decolorize. The solution was concentrated in a vacuum desiccator over sulphuric acid and in a few days the sugar crystallized out. It was recrystallized from alcohol several times. The crystals were white and had the characteristic form of xylose. They melted at 143°–144° C., uncorrected. The sugar had a specific rotation, $[a]_D$, of + 18.92 as compared with + 19.22[25] for xylose. It reduced Fehling's solution, formed furfurol which was precipitated as the phloroglucid when boiled with a hydrochloric acid solution of phloroglucin. The osazone was prepared in the usual way and crystallized as bright yellow needle-like crystals, insoluble in cold water, soluble in hot, and melting at 160° C,, uncorrected, as compared with 160° C. for xylosazone according to Tollens.[26] The sugar was apparently xylose.

In the preparation of xylan, Salkowski's method[27] was also used.

[24] Wheeler, H. J., und Tollens, B. Ueber die Xylose oder den Holzzucker, eine zweite Penta-glycose. Zeitschr. Vereins Rübenzucker-Industrie Deutsch. Reichs **39**: 848–868. 1889.

[25] Parcus, E., und Tollens, B. Ueber die Mehr- oder Weniger-Drehung (Multi-Rotation oder sog. Birotation und Halbrotation) der Zuckerarten. Liebig Ann. **257**: 160–178. 1890.

[26] Tollens, B., et al. Untersuchungen über Kohlenhydrate. Zeitschr. Vereins Rübenzucker-Industrie Deutsch. Reichs **41**: 885–911. 1891.

[27] Salkowski, E. Über die Darstellung des Xylan. Zeitschr. Physiol. Chem. **34**: 162–180. 1901–02.

According to this method the ground straw was boiled in 5 percent KOH for 1 hour, the extract pressed out with a fruit press and filtered through cloth. Fehling's solution was added in the proportion of one half liter to every 300 g. of straw used. The precipitate was separated by filtration, washed and treated with an alcoholic solution of hydrochloric acid. It was washed several times with 60 percent alcohol, dried and dissolved in dilute KOH and again precipitated with Fehling's solution. It was then washed and acidified as before and finally freed from chloride and dried.

Salkowski considered that xylan prepared according to this method was practically free from araban and other hemicelluloses, cellulose and starch, and that it was nearly pure.

This preparation yielded 74.0 percent xylan, according to Krober's tables,[28] when analyzed according to Tollens' phloroglucid method for the determination of pentosans, which is one percent more than was found in the xylan prepared according to Schöne and Tollens' method and analyzed in the same way. The commercial preparation of xylan used in these experiments gave 86.1 percent of the theoretical amount. The xylan prepared according to Salkowski's method dissolved in 1 percent KOH had a specific rotation, $[a]_D$, of $-83°$. It contained 0.5 percent ash. Swartz[29] mentions some which she prepared which yielded 72.0 percent of the theoretical amount according to Krober's tables. She found the specific rotation of one sample to be $-83°$. Tollens gives the specific rotation of xylan from wheat straw as $-84.1°$. Swartz's determinations of ash in the xylan were somewhat higher than those obtained in the present study while Salkowski reports that some of his preparations contained as low as 0.7 percent ash.

The xylans prepared by both Tollens's and Salkowski's methods were tested for galactan and methyl pentosans with negative results. No starch was present. It is apparent from the above described experiments that the compounds prepared were composed largely of xylose.

The first series of experiments was made with the two pentose sugars as compared with glucose as a source of carbon. The sugars were added to the solutions of nutrient salts in quantity to make

[28] Wiley, H. W., et al. Official and Provisional Methods of Analysis. Association of Official Agricultural Chemists. U. S. Dept. Agr., Bur. Chem. Bull. 107. 1907.

[29] Swartz, Mary Davies. *Loc. cit.*

one percent concentration. In preparing the culture media the solutions of salts were made up at a somewhat higher concentration than finally desired and sterilized in the autoclave, then diluted to the proper concentration by the addition of the sugar solutions which had been sterilized separately in a steamer. The culture solutions were inoculated and the fungus allowed to grow twelve days in an incubator at about 28° C. At the end of this time the mycelium was removed and dried to constant weight. The culture solutions were tested for reducing sugars, and the solutions which originally contained xylose or arabinose were tested for pentoses. Traces only were found. The sugar had been almost entirely removed from the solution by the fungus. The weight of the mycelium from the twelve flasks is shown in Table II.

TABLE II

Comparative Yields of Mycelium, Expressed in mg. Dry Weight, from Cultures of Glomerella cingulata on Nutrient Salt Solutions with Glucose, Arabinose or Xylose as Source of Carbon

Glucose	Arabinose	Xylose
365.1	382.0	401.4
347.5	372.3	383.8
317.3	337.2	380.1
365.3	349.5	386.1

The pentoses seem to be slightly better sources of carbon than glucose. Xylose is apparently the best, though the difference is not great. It is very evident that the pentoses are readily utilized by this fungus.

A series of culture solutions was next made using arabin and two xylans, one prepared from rye straw and the other the commercial preparation. The yield in dry weight of fungus mycelium with the amount of furfurol-yielding material used in each case is given in Table III. The amount of arabin or xylan used was determined by

TABLE III

Comparative Yields of Mycelium and Amount of Pentosan Used with Cultures of Glomerella cingulata Grown upon Nutrient Salt Solutions with Xylan or Arabin as the Source of Carbon

Commercial Xylan		Xylan Prepared from Rye Straw		Arabin	
Yield, mg.	Percentage of Xylan Used	Yield, mg.	Percentage of Xylan Used	Yield, mg.	Percentage of Arabin Used
202.2	86.0	230.9	88.0	74.3	15.0
196.0	85.0	....	...	64.6	14.0
179.9	90.0	....	...	98.2	13.0
205.4	87.0	....	...	79.8	15.0

difference between the amount remaining in the culture solutions and the amount recovered from uninoculated control flasks, prepared in exactly the same way as the flasks for cultures. The flasks were kept in an incubator at 28° C. throughout the course of the experiment, which lasted fifteen days.

It is obvious from Table III that these compounds of pentose sugars can be utilized by the fungus as sources of carbon. It is apparent that the yield of mycelium was less after fifteen days with either arabin or one of the xylans as the source of carbon than in the case of any one of the three sugars in twelve days. A much larger amount of the compound remained in the solution than in the case of the sugars which were removed almost quantitatively. It is evident then that xylan and arabin are not as readily available for the fungus as the pentose sugars themselves.

A comparison of xylan and arabin shows that the yield of dried mycelium was considerably more than twice as great from the cultures containing xylan, while a much higher percentage of the material was removed from the solution. The conclusion is obvious then from the table that xylan, although not as good a source of carbon as xylose, arabinose, or glucose is readily available and can be utilized by the fungus. It also seems probable that in the utilization of the xylan it is broken down into simpler compounds and that one or several steps in its digestion and assimilation is the hydrolysis of xylan to xylose, the sugar. If this is the case there is probably an enzyme secreted by the fungus which brings about this hydrolysis.

A series of experiments was planned and carried out to see if the extract of the fungus was able to hydrolyze xylan to xylose under aseptic conditions. The fungus was grown on the mixture of nutrient salts already described with gum arabic or glucose as a source of carbon. The cultures were allowed to grow about three weeks before the mat of mycelium was removed. It was separated from the culture solution, washed with a little water and ground up in a mortar. The resulting pulp was then placed in a flask with water and a little chloroform and allowed to stand with frequent shaking for 24 hours. It was then filtered and the extract used in the digestion experiments. The xylan was prepared by weighing small quantities, 0.2 or 0.3 g., into 100 cc. flasks to which 25 cc. of water was added. The mixture was then boiled, cooled, and 25 cc. of the extract from the fungus mycelium was added to each flask. The mixtures were neutral to

litmus. Several of the flasks containing the xylan and extract of the fungus were again boiled as controls. Chloroform was finally added to all the preparations as an antiseptic. The flasks were then placed in an incubator at a temperature of approximately 30° C. and allowed to remain with frequent shaking for the required time. To see whether the preparations were contaminated with microorganisms frequent inoculations were made from the mixtures to various culture media. No organisms were found.

The first series of experiments was carried out with six preparations for each experiment, all alike excepting that three had been boiled. After these had remained in the constant temperature chamber from five to eight days they were removed, and the contents of the flasks washed into beakers with 95 percent alcohol. Sufficient alcohol was added to bring the mixture up to about 80 percent alcohol, thus precipitating the unchanged xylan. The mixture was filtered and the alcohol evaporated from the filtrate on the steam bath. One pair of preparations consisting of one unboiled and one boiled control was used for the determination of the furfurol-yielding substance. A similar pair was used for the determination of the reducing substance. These last were cleared with neutral lead acetate, filtered, the excess lead precipitated as oxalate, and the mixture filtered again. The sugar was determined in the filtrate, using Allihn's modification of Fehling's solution.[30] The dry cuprous oxide was weighed directly. The third pair of preparations was usually used for the preparation of the phenylhydrazine derivative which will be taken up later. It has been shown earlier in this paper that xylose is soluble in 80 percent alcohol, while xylan is not; also xylose is a reducing sugar, forms furfurol when boiled with HCl and reacts with phenylhydrazine to form a characteristic osazone. It is apparent then that if the amount of alcohol-soluble furfurol-yielding material and reducing substance is greater in the unboiled preparation it will be evidence that a pentose sugar results from the action of the extract of fungus mycelium upon the xylan. This evidence will be strengthened if the phenylhydrazine derivative is similar to xylosazone. The results of this series of experiments are shown in Table IV.

The phenylhydrazine derivative was prepared in the usual manner in the third unboiled preparation in all the experiments given in Table IV. In all cases it proved to be the same, bright yellow needle-

[30] Wiley, H. W., et al. *Loc. cit.*

TABLE IV

Comparative Effect of Boiled and Unboiled Extract of Fungus Mycelium upon Xylan from Rye Straw as Shown by Alcohol-soluble Furfurol-yielding Material and Substance Reducing Fehling's Solution

Duration of Experiment	Amount of Xylan in Each Preparation	Cuprous Oxide Derived from Material Reducing Fehling's Solution.		Amount of Pentoses	
		Unboiled	Boiled	Unboiled	Boiled
	g.	mg.	mg.	mg.	mg.
6 days	0.3	123.8	31.5	67.5	9.2
8 "	0.3	93.6	18.1	52.1	8.3
8 "	0.2	70.5	12.1	46.7	8.1
10 "	0.3	133.0	31.4	67.8	9.5
10 "	0.2	108.2	17.6	47.4	9.5

shaped crystals soluble in hot water, insoluble in cold, and melting at 161°–162° C. uncorrected, as compared with 161° C. for xylosazones prepared from the xylose sugar obtained from rye straw and from the commercial xylose as used in the experiments already described. Some of the control preparations were treated in a like manner, but the quantity of osazone formed was so small that it was impossible to identify it with certainty.

It is obvious from Table IV that there is much more alcohol-soluble substance which reduces Fehling's solution and furfurol-yielding substances in the unboiled preparations than in the boiled controls. That this increase is not due to the autolysis of the filtered extract of the fungus mycelium itself was proved by the fact that when the extract was accorded exactly the same treatment as the unboiled preparations no appreciable amount of reducing substance was found and no measurable amount of furfurol-yielding material. The above mentioned considerations and the fact that the phenylhydrazine derivative is similar to the osazone of xylose seems to show that xylose results from this action of the unboiled extract of fungus mycelium upon the xylan.

Whether this hydrolysis takes place immediately or the amount of alcohol-soluble reducing substance and furfurol-yielding material present at the completion of the experiment is the result of a gradual breaking down of the xylan is now shown by the results already given. Further experimentation was necessary to obtain evidence upon this point. In these experiments twelve or more similar preparations were made, as already described, several of which were boiled as

before for controls. The flasks were placed in an incubator and shaken frequently. At intervals some of the preparations, both unboiled and boiled, were removed from the incubator, the xylan precipitated and alcohol-soluble furfurol-yielding substances determined.

In the experiments, the results of which are given in Table V, both

TABLE V

Production of Alcohol-soluble, Furfurol-yielding Material, and Substance Reducing Fehling's Solution, by Action of Extract of Fungus Mycelium upon Xylan from Rye Straw. 0.3 *g. Xylan in Each Preparation. Yield in mg. Pentoses and mg. Cuprous Oxide*

Duration of Action	Unboiled Preparations		Boiled Preparations	
	Cuprous Oxide	Pentoses	Cuprous Oxide	Pentoses
	mg.	mg.	mg.	mg.
Beginning.....	.....	.....	23.7	9.5
4 days.....	71.4	57.2	.....	.....
8 "	99.8	63.5	19.4	9.0
12 "	100.4	66.5	.....	.....
16 "	100.8	66.3	20.6	9.4

the reducing substance and the furfurol-yielding material are given. In the other experiments (Table VI) only the latter was determined.

It is evident from Tables V and VI that the hydrolysis of the

TABLE VI

The Production of Alcohol-soluble, Furfurol-yielding Substances by Action of Extract of Fungus Mycelium upon Xylan. Yield as mg. Pentoses

No. of Experiment	Amount of Xylan in Each Preparation in g.	Duration of Experiment	Unboiled		Boiled Control
			1	2	
			mg.	mg.	mg.
1	0.3	At beginning	.....	.....	9.7
		20 hours	34.1	35.0	.....
		44 "	40.0	38.2	10.5
		68 "	41.2	43.3	.....
		106 "	47.3	44.0	8.8
2	0.3	At beginning	.....	.....	9.9
		20 hours	19.0	19.0	.....
		42 "	25.1	25.4	.....
		66 "	31.0	31.1	.....
		80 "	38.7	34.3	8.1
3	0.2	At beginning	.....	.....	8.8
		12 hours	13.5	13.8	.....
		36 "	15.9	16.1	.....
		70 "	17.2	17.1	.....
		94 "	17.8	17.7	8.8

xylan did not take place immediately but that the amount of alcohol-soluble furfurol-yielding substance (xylose) increased the longer the extract was allowed to act. The rate of hydrolysis was much more rapid at first and gradually decreased almost, if not quite, to zero. In contrast to the unboiled preparation there seemed to be no increase in the alcohol-soluble furfurol-yielding material in the boiled controls; that is, heating to 100° C. apparently rendered the extract incapable of affecting the xylan.

While it seemed probable that xylose was liberated by the action of the extract of the fungus upon xylan it was deemed advisable to attempt to crystallize the sugar from the alcohol-soluble portion of the preparation. In order to obtain a sufficient quantity, a number of flasks of the xylan and extract of the fungus mycelium were prepared in the usual manner excepting that larger quantities were used.

These preparations were kept in the incubator for about a week, the unchanged xylan precipitated as before, the alcohol extract concentrated under reduced pressure, decolorized with animal charcoal and filtered. The filtrate was allowed to evaporate slowly over sulphuric acid in a desiccator and crystals were formed. These crystals were separated from the mother liquor and recrystallized. The substance did not melt sharply but apparently fused between 141° and 144° C. This was probably due to impurities present. The crystals reduced Fehling's solution and formed the characteristic phloroglucid when boiled with a hydrochloric acid solution of phloroglucin. The phenylhydrazine derivative was similar to the osazone of xylose as prepared in this study in color, crystal form, solubility and melting point. The compound when dissolved in water and treated with cadmium carbonate and bromine formed the characteristic crystals of the double salt of cadmium xylonate and cadmium bromide described by Widstoe and Tollens[31] and observed in the present study with xylose. These properties all agree closely with the properties of xylose.

It is evident then that xylan is hydrolyzed under aseptic conditions by the extract of the fungus and that xylose is formed. No attempt was made to determine whether intermediate products of hydrolysis were present as might well be the case.

In these experiments on the effect of the fungus *Glomerella cingulata*

[31] Widstoe, J. A., und Tollens, B. Ueber Arabinose, Xylose und Fucose aus Traganth. Bericht. Deutsch. Chem. Ges. **33**: 132–143. 1900.

upon pentoses and compounds of pentose sugars it has been shown that the amount of furfurol-yielding material in the apple is decreased when the apple is rotted by this fungus. This decrease is brought about by the action of the fungus on the compounds in the apple which contain pentoses. These compounds are broken down and the furfurol-yielding material at least is used by the fungus. In this process the alcohol-soluble furfurol-yielding material is increased, which would seem to indicate that the pentose sugars are split off from the compounds in which they exist in the fruit.

The fungus is able to utilize either glucose, xylose, arabinose, arabin or xylan as a sole source of carbon. The three sugars are most efficiently utilized, xylose perhaps the best. The fungus grows better on xylan than on arabin.

The filtered extract of the fungus mycelium is able to act on xylan under aseptic conditions with the formation of alcohol-soluble substance which reduces Fehling's solution, forms furfurol when boiled with HCl and possesses other properties of xylose. This ability of the extract is lost on boiling. The breaking down of the xylan takes place gradually and the alcohol-soluble furfurol-yielding material is found to increase the longer the extract acts upon the xylan, the rate being much more rapid, however, during the early part of the action.

A crystalline compound was obtained from the alcohol-soluble portion of the preparation of xylan which had been acted on by the extract of the fungus mycelium. This compound was apparently xylose. It is evident then that there is a xylanase present in the extract of fungus mycelium which hydrolyzes xylan to xylose.

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