14. Studies on the Alcoholization of Cellulose Materials. (VIII)

Methods of Paper Chromatography of Sugars in Saccharified Solutions of Mulberry-tree

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The authors have already reported on the chemical components of the saccharified solution of mulberry-tree hydrolysed by dilute acid solution at high temperature and pressure. In the present paper, sugars in the solution are investigated by methods of paper chromatography.

The saccharified solutions of mulberry-tree are prepared as follows : (1) saccharification is carried out 4 times successively, (II) saccharified by Scholler's method—— (o) saccharified solution, (a) saccharified solution is neutralized by $Ca(OH)_2$ and (b) fermented solution of (a) by yeast. (III) (a) neutral solution and (b) fermenet solution of sulfite pulp liquor are chosen as comparison.

As is shown in the table, the presence of glucose, mannose, arabinose and xylose was verified in all saccharified solutions on paper chromatograms, but any kind of pentose was never detected in sulfite pulp liquor.

The authors are convinced that all these sugars in the saccharified solutions are available for microbiological industries. The amount of unkown sugars was little and these non fermentable sugars are supposed to be secondary product of saccharification.

Rf values of sugars in various saccharlfied solutions.

Saccharified Solution \ Developer		Phenol 8 H_2O 2	Pyridine 2 *n*-BuOH 3 H_2O 1.5	Aceton 13 AcOH 3 H_2O 5	*iso*-PrOH 1 *n*-BuOH 1 H_2O 1	Corresponding Sugars
I	1st run	50	17[1], 42, 46, 51, 56[2]	58[4], 64	17[1], 38	Glucose, mannose, arabinose, xylose
	2nd run	35[5], 43[5]	17[1], 42, 46, 51, 56[2]	61, 66	38, 44	Glucose, mannose, arabinose, xylose
	3rd run	35[5]	47	62	36	Glucose
	4th run	35[5]	45[3]	62	36	glucose
II	(o) No treatment		42, 47, 57[2]	62	38	Glucose, arabinose
	(a) After neutralization		42, 47, 52, 57[2]	62	33, 38	Glucose, mannose, arabinose
	(b) After alc. fermentation		42, 51, 57[2]		34	Arabinose, xylose
III	(a) After neutralization		42, 51, 56[2]			Glucose, mannose
	(b) After alc. fermentation		42, 56[2]			Glucose

Note : For spray reagent Horrock's benzidine reagent is used.

1) These spots are suggested to correspond to gentiobiose which is not tested.

2), 3), 4), and 5) These spots are never recorded but these are supposed to be polymers of sugar.

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15. Studies on the Urinary Phosphatase

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Man's urine (4 l.) was adjusted to pH 4 with acetic acid. The urinary enzyme was absorbed with 100 g. of *bolus alba* (or kaolin) and eluted at pH 8. After dialysing at pH 4, it was precipitated with $(NH_4)_2SO_4$ of 60 % saturation. The precipitated enzyme was dialysed and used in the experiments (15 ml.). It was regarded as the prostatic enzyme (Kutscher *et al.*, *Z. physiol. Chem.* **236**, 237 (1953) ; Abul-Fadl., *Biochem. J.* **45**, 51 (1949)). A mixture of 2 % β-glycerophosphate, acetate buffer solution and enzyme was incubated at pH 5.5 and 37°C and then the free P was measured by Allen's method. In order to distinguish the urinary phosphatase from the acidic kidney phosphatase the test mixtures were digested at 37°C for 60 mins. with EtOH or AcOAc. The results showed that the former was more resistant against HCHO and more sensible to EtOH andAcOAc, while the latter was inactivated by HCHO and not influenced by AcOAc and EtOH. To confirm the effects of pH upon the enzymes, the acetate buffer of several pH values was added to the enzyme solution and then the enzyme-buffer solutions were kept at 37°C for 90 mins.; after adjusting pH to 5.5 they were mixed with substrate and digested in order to examine the enzyme activities. It was confirmed that the enzyme activities were not decreased at all in pH range from 3 to 7, but at the higher pH value, their activities were lost. So the enzyme appeared to be very stable at the acid condition. In a centrifuge tube, precipitation of the urinary enzyme by acetone was performed at several pH ranges. 0.2 ml. of enzyme, 0.2 ml. of acetate-buffer and 5.0 ml. of cold AcOAc were mixed, and the mixture was kept at 0°C for 30 mins. and on centrifuging the supernatant was removed; to the precipitant, 1 ml. of acetate-buffer of pH 5.5 and 0.2 ml. of 2% β-glycerophosphate solution were added and digested under toluene to measure the activity of the enzyme. At pH 7 its activity was observed to remain sufficiently well, but it was decreased suddenly by the acid treatment and greatly damaged below pH 4 (Fig. 1). To ascertain the activity of the supernatant, it was aerated to evaporate AcOAc, the residual matter was dissolved in a buffer of pH 5.5 and its enzymatic activity was measured. No activity was observed in the acid side supernatants,