

The Formation of Trisaccharides during Degradation of Potato Starch by Pancreatic Amylase

JAKOB BLOM and CARL OLOF ROSTED*

The Laboratory of the Tuborg Breweries, Copenhagen, Denmark

In 1941, Smits van Waesberghe¹ showed that during degradation of potato starch by bacterial amylase apart from glucose and maltose small quantities of a third fermentable sugar were formed, presumably a trisaccharide. The separation was carried out by fermentation with *Zygosaccharomyces marxianus*, *Saccharomyces uvarum* and *Saccharomyces cerevisiae*. The first only ferments glucose, the second glucose and maltose and the third all three sugars. Our preceding work was interrupted because we were unable to separate the fermentable sugars. It was resumed in 1944—45 when we got cultures of *S. uvarum* and *Z. marxianus*.

It may be concluded from the table in the preceding paper that the unknown carbohydrates must be present in maximum amount when the difference between % TMG and % TMR of fermentable carbohydrates is greatest. This is the case at a degradation of about 65 % TMR. Experiments confirmed our assumption; only the experiments in the following table will be mentioned; others gave similar results. *S. uvarum* does not attack the unknown carbohydrates which are fermented by *S. cerevisiae*. The degradation products of starch hydrolyzed to 64 % TMR contain no glucose, about 33 % disaccharides, about 31 % trisaccharides and about 36 % dextrins. The RM of the unknown sugars indicates that we have to deal with trisaccharides. They can easily be separated from lower molecular sugars; a separation from higher molecular components involves great difficulties. By precipitations and extractions with methyl- and ethylalcohol of different concentrations we

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Table 1. Degradation products of potato starch by pancreatic amylase. Starch paste 3 %, pH = 6.9, 20°, [Cl⁻] = 0.005. Degree of hydrolysis 63.8 % TMR.

	% TMG	% TMR	RM	[α] _D ²⁰
<i>Zygosaccharomyces marxianus</i>				
Glucose	0.0	0.2	—	—
<i>Saccharomyces uvarum</i>				
Glucose + disaccharides	32.8	34.4	105	138°
<i>Saccharomyces cerevisiae</i>				
Glucose + disaccharides + trisaccharides	63.3	56.9	90	150
Trisaccharides	30.5	22.5	74	162°
Dextrins	36.7	6.9	19	—

Abbreviations, see preceding paper.

Reductometrical determination. Potassium copper carbonate method ⁷.

obtained a dry product in a purity of 84 % and a yield of 45 g from 450 g potato starch. But crystallisation failed. By means of measurements before and after fermentation with *S. cerevisiae* it was possible to calculate the following constants of the trisaccharides [α]_D²⁰ = 163°, mol. wt. (freezing point) 500 (theory: 522), equiv. wt. (iodine) 265 (theory 261), RM = 71. The determinations were controlled by parallel experiments with glucose and maltose, specially prepared. The constants show that *the unknown carbohydrates must be aldotrisaccharides*, and since starch only contains glucoserests they must be glucotrisaccharides. The trisaccharides are hydrolyzed by enzymes from pancreas and yeast, not or only very slowly by β-amylase from barley. The first step of hydrolysis of glucotrisaccharides must yield glucose and disaccharides. The formation of glucose starts at a starch-degradation of about 65 % TMR (see preceding paper). By comparison of the described starch-degradation interrupted at 63.8 % TMR and a starch-

Table 2. Formation of glucose from potato starch by pancreatic amylase. Starch paste 3 % pH = 6.9, [Cl⁻] = 0.005.

	20°	33°
Degree of hydrolysis	63.8 % TMR	91.8 % TMR
Glucose	0 » TMG	11.3 » TMG
Disaccharides	32.8 » »	51.5 » »
Trisaccharides	30.5 » »	12.5 » »
Dextrins	36.7 » »	24.7 » »

Abbreviations, see preceding paper.

Reductometrical determination. Potassium copper carbonate method ⁷.

degradation stopped at 91.8 % TMR (table 2) it is found that in this step about 11 % glucose and about 19 % disaccharides were formed. The trisaccharides decreased simultaneously about 18 % and the dextrans about 12 %. These facts are best explained by assuming the formation of trisaccharides from dextrans and a partial hydrolysis of trisaccharides into glucose and disaccharides. The hydrolysis of the trisaccharides is most certainly a property of the amylase itself, experiments to eliminate this property by partial heat-inactivation of the pancreatic enzymes being without result.

The isolation of trisaccharides with similar $[\alpha]_D = 150-170^\circ$ from starch or glycogen has been denied almost as often as it has been reported. We only want to cite a few examples. Barbour² stated that he had isolated a trisaccharide from glycogen, $[\alpha]_D = 154^\circ$. The result could, however not be confirmed³. Ling and Nanji⁴ reported that they had isolated a trisaccharide from starch with the constants $RM = 66$; $[\alpha]_D = 165^\circ$. Later investigations by Ling⁵ showed however, that the trisaccharide formerly prepared was a mixture. Smits van Waesberghe¹ isolated small amounts of a similar trisaccharide as ours $[\alpha]_D = 154^\circ$. Using bacteriaamylase, which easily splits the trisaccharides, the yield was very poor.

The trisaccharide, isolated by Myrbäck and Ahlborg⁶ from starch, $[\alpha]_D = 124^\circ$, must be of a different constitution.

SUMMARY

The work of the preceding paper was continued in 1944—45. The degradation products of starch, hydrolyzed to about 64 %, contain 1/3 disaccharides, 1/3 trisaccharides and 1/3 dextrans. The glucotrisaccharides, $[\alpha]_D^{20} = 163^\circ$, $RM = 71$, mol. wt. 500 (theory 522), equiv. wt. 265 (theory 261), are hydrolyzed by pancreatic amylase under formation of disaccharides and glucose.

REFERENCES

1. Smits van Waesberghe, *Onderzoekningen over Microben-Amylasen*. Delft (1941).
2. Barbour, A. D. *J. Biol. Chem.* **85** (1929) 29.
3. Walker, A. M. and Young, F. G. *Biochem. J.* **32** (1938) 94.
4. Ling, A. R., and Nanji, D. R. *J. Chem. Soc.* (1923) 2666; (1925) 629.
5. Ling, A. R. *Journ. Inst. Brew.* **44** (1938) 419.
6. Myrbäck, K., and Ahlborg, K. *Biochem. Z.* **307** (1940) 69.
7. Blom, J., and Rosted, C. O. *Acta Chem. Scand.* **1** (1947) 32.

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