STUDIES ON THE UNFERMENTABLE SUGARS (VIII) : IDENTIFICATION OF SAKEBIOSE, NIGEROSE AND Y-SUGAR

STUDIES ON THE UNFERMENTABLE SUGARS (VIII)

* IDENTIFICATION OF SAKÉBIOSE, NIGEROSE AND *Y*-SUGAR

By

Kazuo MATSUDA, Goro HIROSHIMA, Kazuo SHIBASAKI and Kiyoshi ASO

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received October 30, 1954*)

The authors(1) previously reported on the physical constants of crystalline octaacetates of isomaltose and sakébiose which were the unfermentable sugars in koji extract. Since the properties of sakébiose were similar to those of nigerose which had been isolated by Barker and his co-workers (2) from the hydrolyzate of mycodextran (nigeran), a hot water soluble intracellular polysaccharide of *Asp. niger* (strain 152), and also to those of *y*-sugar which had been isolated as crystalline acetate from acid reversion products of D-glucose by Thompson and his co-workers (3), it was suggested in our report that they may be probably the same sugar *i.e.* 3-*O*-α-D-glucopyranosyl-D-glucopyranose. But, there are some differences among the constants of these sugars. The present study was made to dissolve the question. Namely, the melting point of sakébiose octaacetate was compared with those of nigerose acetate prepared in pursuance of the procedure described by Barker *et al* and *y*-acetate which was kindly provided by Dr. Thompson. The results, as given in Table 1, did not show the depression

Table 1. Melting points and mixed melting points of the acetates of sakébiose, nigerose and *y*-sugar

Name	m.p °C (uncorrected)
Sakébiose octaacetate (I)	150
Nigerose octaacetate (II)	150
Y-acetate (III)	150
Mixed m.p. of I and II	150
" of I and III	150
" of II and III	150

* Japanese report: The Journal of Fermentation Technology, Osaka, Japan, Vol 32, No. 12, 498, 1954.

of the melting point on admixture. So it was decided that they were the same disaccharide.

According to Barker *et al*, the melting point of nigerose octaaceate is 111~113°C, while that of the acetate obtained by us is 150°C (uncorrected), but that of *y*-acetate was reported to be 155~157°C by Thompson *et al*. It may be suggested that the nigerose acetate prepared by Barker *et al* was probably contaminated with maltose acetate. We have written to Dr. Thompson with regards to whether *y*-sugar might be a disaccharide having 1,3-α linkage from aforementioned results and have received his reply that this sugar is identical with 3-*O*-α-D-glucopyranosyl-D-glucopyranose.

Experimental

[1] *Preparation of Nigeran*

Nigeran was prepared according to Yuill's report(4). The employed strain was selected among *Asp. niger* sp., *Asp. niger* NRRL 337, *Asp. Awamori* and *Asp. Usamii* which are preserved in our laboratory, because Yuill's strain 152 was not able to be obtained.

The medium was a solution containing per liter; sucrose 150 g, NH_4NO_3, 2.5 g, KH_2PO_4 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, 1 ml of 1N HCl, and traces of Fe and Zn. It was put in a flask (300 ml Erlenmeyer) and sterillized as usual. The above stated four strains were inoculated into each of the five flasks and then incubated for ten days at 28~30°C. The grown moulds were taken out from the flasks, washed thoroughly with water, and steamed with 500 ml of water for 30 minutes at atmospheric pressure and filtered while hot. The residues were steamed again with 300 ml of water and filtered, and the filtrate was put together with the previous filtrate.

After standing over night in a cold place, the white precipitates were filtered, washed with alcohol and then with ether, and dried.

The yields of nigeran by the four strains are shown in Table 2.

They were very inferior to strian 152, but since *Asp. niger* NRRL 337 was the best among them, much nigeran was prepared with this strain.

Table 2. The Yields of Nigeran

Strain	*Asp. niger* NRRL 337	*Asp. niger sp.*	*Asp. Usamii*	*Asp. Awamori*
Classen (dry matter)	7.3	6.4	6.0	6.6
Nigeran (g) (g)	2.10	0.15	0.45	0.50

[2] *Paper Chromatography*

About 0.5 g of nigeran was hydrolyzed with 10 ml of 1N H_2SO_4 on a water bath for 30 minutes. The hydrolyzate was neutralized with barium carbonate

and developed on the filter paper by multiple (four run) paper chromatography. The part of paper containing nigerose which had an Rf value similar to that of laminaribiose (2) was cut off and nigerose was eluted with water from it. The resulted nigerose solution was used for the following examinations. Namely, to test whether nigerose was hydrolyzed with β-glucosidase, emulsin was added to the above sample in the same manner as had been reported previously (5), and as the result it was found that nigerose could not be hydrolyzed as well as sakébiose by paper chromatographic analysis. Then the multiple (three run) paper chromatography of sakébiose, nigerose and laminaribiose, which was kindly provided by Dr. E. J. Bourne, was carried out. Their chromatograms are shown in Fig. 1, which indicates that the Rf value of nigerose was identical with that of sakébiose, but the Rf value of laminaribiose was higher than that of nigerose and equal to that of galactose. Nigerose was not fermented by baker's dry yeast.

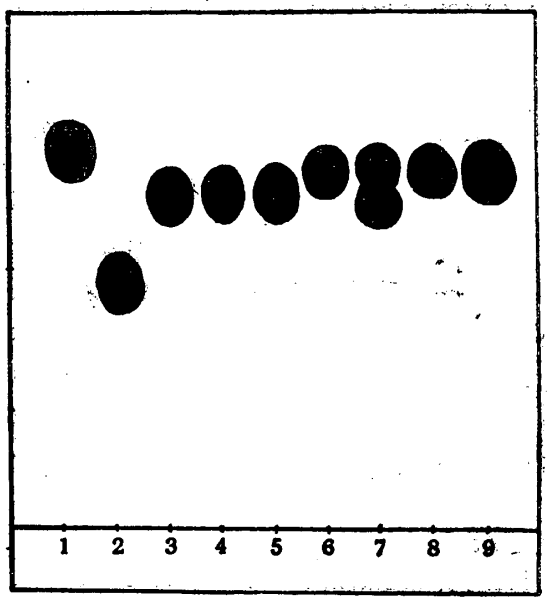

Fig. 1.
Paper chromatogram of sugars
1. glucose.
2. isomaltose.
3. sakébiose.
4. nigerose.
5. sakébiose mixed with nigerose.
6. laminaribiose.
7. laminaribiose mixed with nigerose
8. galactose.
9. laminaribiose mixed with galactose

[3] *Identification of Octaacetates*

About 40 g of crude nigeran was hydrolyzed on a water bath with 800 ml of 1 N H_2SO_4 for 30 minutes. The hydrolyzate was passed through an ion exchanger and concentrated until its sugar content had risen to about 10 percent. The resulted mixture of saccharides was fractionated on a charcoal column as described in the previous report (1). They were eluted with water, 5 percent ethanol and finally with 10 percent ethanol successively. The sugar components of each fraction were analyzed by paper chromatography. The first water fraction contained only glucose, and the next contained isomaltose and maltose. The 5 percent ethanol fraction gave maltose and nigerose, and the 10 percent ethanol fraction gave higher polysaccharides.

The 5 percent ethanol fraction was concentrated to a sirup, and then dissolved in methanol. After the insoluble substances were removed, the liquor was concentrated again and dried in a vacuum desiccator. About 1 g of white powder was obtained. The powder was acetylated with 5 ml of acetic anhydride and 0.5 g of anhydrous sodium acetate for 3 hours at 105~110°C, and about 2 g of acetate was obtained.

Then, 1 g of acetate was developed on Magnesol-Celite (5 : 1) column (35×200 mm) with 2000 ml of benzene-*t*-butanol (100 : 1) as reported previously (1). Two zones were indicated with 1 percent alkaline $KMnO_4$ and each zone was cut off, and eluted with acetone.

From the lower zone (near the bottom of the column) 0.4 g of sirupy acetate was obtained which was crystallized from ethanol. The melting point of recrystallized acetate was 158°~159°C and was not depressed on admixture with known β-maltose octaacetate. While, from the upper zone (40–80 mm from the top of the column) 0.2 g of sirupy acetate was obtained and this was also crystallized from ethanol. Twice recrystallized acetate melted at 150°C and it did not present the depression of melting point on admixture with sakébiose octaacetate and also with *y*-acetate supplied from Dr. Thompson.

Summary

Nigerose was isolated from the partial hydrolyzate of nigeran which was extracted from the mycelium of *Asp. niger* NRRL 337 according to the method reported by Yuill, and it was compared with sakébiose and *y*-acetate obtained by Thompson *et al.* Since nigerose had an Rf value equal to that of sakébiose, and the acetate of the above two and *y*-acetate had the same melting point of 150°C (uncorrected) which did not show the depression on admixture, it was confirmed that the above sugars were identical, and are 3-*O*-α-D-glucopyranosyl-D-glucopyranose.

Acknowledgement: The authors express their gratitude to Drs. A. Thomposn, S. A. Barker and E. J. Bourne for providing the samples and the necesarry literatures. The work was aided in part by a grant from the Scientific Research Expenditure of the Ministry of Education.

References

1) Matsuda, K. & Aso, K. (1954). This Journal Vol. V. No. **2**, 123.
2) Barker, S. A., Bourne, E. J. & Stacey, M. (1953). J. Chem. Soc., 3084.
3) Thompson, A., Anno, K., Wolfrom, M. L., & Inatome, M. (1954): J. Am. Chem. Soc., **76**, 1307.
4) Yuill, J. L. (1953). Chem. and Ind., **31**, 755.
5) Matsuda, K. & Aso, K. (1953): J. Ferm. Tech. (Japan) **31**, 211.