# ACETOLYSIS OF POLYSACCHARIDES I. ISOLATION OF NIGEROSE FROM THE ACETOLYSATE OF A DEXTRAN PRODUCED BY LEUCONOSTOC MESENTEROIDES NRRL B-421

# ACETOLYSIS OF POLYSACCHARIDES
# I. ISOLATION OF NIGEROSE FROM THE ACETOLYSATE OF A DEXTRAN PRODUCED BY *LEUCONOSTOC MESENTEROIDES* NRRL B-421*

By

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Nigerose was isolated from the acetolysate of a dextran produced by *Leuconostoc mesenteroides* NRRL B-421 by carbon: Celite column chromatography. From that only a small amount of isomaltose could be isolated as compared with nigerose, it was presumed that 1, 6-linkage was much less stable than 1, 3-linkage to acetolysis. This was further confirmed by another experiment using isomaltose and nigerose.

Occurrence of 1, 3-$\alpha$ linkage in some dextrans was first suggested by Abdel-Akher *et al.* (1) and also by Lohmar (2) almost at the same time from the results of periodate oxydation analysis. Barker *et al.* (3) observed that about 26 per cent of 2, 4-dimethyl glucose was present in the acid hydrolysate of a methylated dextran produced by *Betacoccus arabinosaseous* (Birmingham strain). They presumed that the 2, 4-dimethyl glucose might be derived from 1, 3-branch point in the above dextran.

Fractionation of the partial acid hydrolysate of this dextran was also attempted by the same authors (3) and the presence of a small amount of nigerose in the hydrolysate was recognized by paper chromatography. However, isolation and identification of nigerose from the hydrolysate have not been successful.

Jeanes *et al.* (4) have later characterized dextran from 96 strains of bacteria by periodate oxydation analysis and classified them into several groups on the basis of the proportions of 1, 3-like linkages. According to their results, 29 of them appeared to contain 1, 3-linkages more than 10 per cent and a few of them showed extremely high content of 1, 3-linkages (more than 30 per cent). In spite of the above observations, however, no gluco di- or oligosaccharides

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containing 1, 3-linkage have so far been isolated from the hydrolysates of the dextran.

We presumed that the failure to isolate 1, 3-linked fragments might be due to the difference of the stability of 1, 6- and 1, 3-linkage to acid hydrolysis. It has been already reported by Wolfrom *et al.* (5) that 1, 6-$\alpha$ linked isomaltose was four times as resistant as 1, 4-$\alpha$ linked maltose against acid hydrolysis. Kobayashi *et al.* (6) have compared the behaviors of dextran and amylose against acid hydrolysis and observed that the latter was less stable to hydrolysis. As to 1, 3-linkage, there is no report concerning the behavior of this linkage against hydrolysis. However, it may be presumed that 1, 3-linkage is also less stable to hydrolysis than 1, 6-linkage, because 1, 3-linkage is also a linkage attached to a secondary hydroxyl group. Actually, we have now observed that 1, 3-linkage is less stable than 1, 6-linkage just like 1, 4-linkage. Therefore, it may be very difficult to isolate 1, 3-linked fragments from dextrans which are mainly composed of 1, 6-linkages.

We have now attempted to apply acetolysis to breaking down the dextrans. By this method, we have now been able to isolate nigerose from the acetolysate of a dextran produced by *Leuconostoc mesenteroides* NRRL B-421. Furthermore, it became obvious from our results that 1, 6-linkage is much less stable to acetolysis than 1, 3-linkage. This fact was ultimately confirmed by comparing the behaviors of isomaltose and nigerose against acetolysis. Namely, when both sugars were submitted to acetolysis at 25°C, the former was completely broken down into glucose in six hours whereas the latter remained unchanged.

## Experimental

### (1) *Preparation of the dextran*

Dextran was prepared by cultivating a strain of *Leuconostoc mesenteroides* NRRL B-421 in the culture medium as shown in Table 1.

**Table 1.** Composition of the culture medium g/*l*.

| | |
|---|---|
| Sucrose | 125 |
| $(NH_4)_2SO_4$ | 0.6 |
| $KH_2PO_4$ | 5 |
| NaCl | 1 |
| $MgSO_4 \cdot 7H_2O$ | 0.2 |
| Yeast Extract | 1 |
| Peptone | 1 |
| pH | 7.4 |

After seven days of cultivation, the broth was mixed with twice volume of 95 per cent ethanol. The precipitated dextran was washed with 60 per cent ethanol and purified by reprecipitation. The yield of the purified dextran was

about 40~50 g from 2 *l* of the broth.

(2) *Periodate oxydation analysis of the dextran*

The above dextran was oxydized by sodium metaperiodate according to the method of Kobayashi *et al* (7). The dextran consumed 1.58 mol of periodate and produced 0.77 mol of formic acid per anhydroglucose unit when oxydized for 72 hours at 25°C. From these results, the proportions of 1, 6-, 1, 4- and 1, 3-like linkages were calculated as 77, 4 and 19 per cent respectively.

(3) *Paper Chromatography of the Acetolysate and the Hydrolysate of the Dextran*

a) *Acetolysis*: Applying the procedure reported by Wolfrom *et al.* (8) for the acetolysis of amylopectin, 1 g of the finely powdered dextran was added to a cooled mixture of 4.8 *ml* of acetic anhydride, 3.2 *ml* of glacial acetic acid and 0.6 *ml* of conc. sulfuric acid. After standing at 30°C for seven days, the mixture was heated slowly to 80°C and maintained at this temperature for 30 minutes. The dark red solution was poured into 500 *ml* of ice and water, neutralized with sodium carbonate and allowed to stand overnight. The whole mixture was then extracted with chloroform and the extract was washed with water and dried over anhydrous sodium sulfate. Removal of the solvent left 1.6 g of sirupy acetolysate. This sirupy acetolysate was then dissolved in 19 *ml* of dry methanol and cooled to 0°C. A solution of 1 *ml* of 1 *N* sodium methoxide was added and the whole was kept at 0°C overnight. Sufficient cold water was then added to this solution to dissolve the precipitate and the ionic materials was removed by adding a small amount of ion exchanger Amberlite IR 120. The deionized solution was evaporated under reduced pressure and then submitted to paper chromatography.

b) *Hydrolysis*: One gram of the same dextran was suspended in 35 *ml* of 1 *N* sulfuric acid and was heated in a boiling water bath for two hours.

A slight excess of barium carbonate was added to this hydrolysate and the precipitate was filtered off. The filtrate was then treated with a small amount of ion exchanger Amberlite IR 120 and evaporated under reduced pressure and submitted to paper chromatography.

c) *Paper chromatography*: The above prepared actolysate and hydrolysate were spotted on a sheet of Toyo filter paper No. 2 and developed three times with a mixture of pyridine: butanol: water (4:6:3).

Aniline hydrogen phthalate was used as a spraying reagent.

As shown in Fig. 1, considerable amounts of glucose and nigerose were observed on the chromatogram of the acetolysate and isomaltose was hardly observed. On the contrary, only a series of 1, 6-linked sugars including glucose, isomaltose, isomaltotriose etc. was observed on the paper chromatogram of the hydrolysate.

(4) *Examination of the reversion*

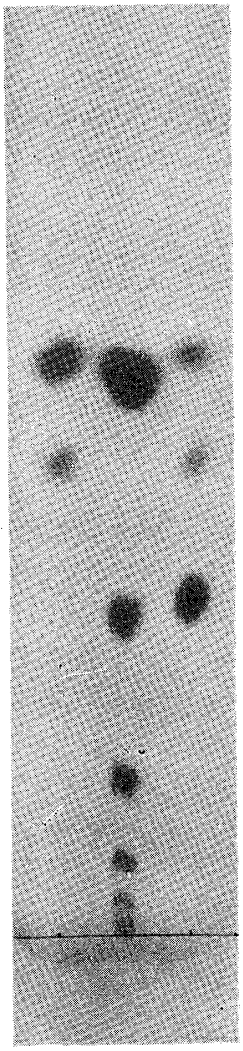


Fig. 1. Paper chromatogram of the hydrolysate and the acetolysate of the dextran.
1. Acetolysate.
2. Hydrolysate.
3. Standard sugars : Glucose, nigerose and isomaltose.

It has been already known that a considerable amount of reversion products were produced during the hydrolysis under some conditions. (9)

As to the acetolysis, no report has been observed concerning reversion.

To ascertain whether a reversible reaction had occurred during the acetoysis, 1 g of anhydrous glucose was treated exactly as described above. However, no reversion product was detected by paper chromatography.

(5) *Fractionation of the acetolysate by carbon : Celite column chromatography*

Twenty grams of the finely powdered dextran was added to a cooled mixture of 96 *ml* of acetic anhydride, 64 *ml* of glacial acetic acid and 12 *ml* of conc. sulfuric acid in small portions. After being kept at 30°C for seven days, the mixture was treated in the same manner as described above. Twenty six grams

of sirupy acetolysate was obtained. This acetolysate was then deacetylated as usual, 12 g of the deacetylated products were obtained.

Thirty grams of the deacetylated products was dissolved in 300 *ml* of water and poured on a chromatographic column of a mixture of 300 g of carbon (Shirasagi brand) and the same amount of Celite (No. 545).

Fractionation was carried out by the usual method of gradient elution using water and five per cent ethanol. The results are shown in Table 2.

**Table 2.** Fractionation of the acetolysate of the dextran by carbon : Celite column chromatography.

| Fraction | Solvent used for elution | Paper chromatography | Yield (g) |
|---|---|---|---|
| 1 | Water | — | — |
| 2～ 7 | 〃 | Glucose | 17 |
| 8～ 9 | 〃 | — | — |
| 10 | 5% Ethanol | — | — |
| 11 | 〃 | Isomaltose | 0.4 |
| 12 | 〃 | Isomaltose + Nigerose | 0.5 |
| 13～18 | 〃 | Nigerose | 7.8 |

* Each fraction was caught in 2*l*.

Fraction 11 which contained only isomaltose was evaporated to dryness (0.4g) and acetylated by the usual method. 0.6 g of crude acetate was obtained. This crude acetate was crystallized from ethanol in fine prisms. Recrystallized product showed m.p. 144°C which was undepressed on admixture with the known specimen. of β-isomaltose octaacetate.

7.8 g of the amorphous powder obtained from the fractions 13—18 was acetylated in the same manner to give 14.5 g of the crude acetate which was crystallized from ethanol. Recrystallized product showed m.p. 149°C, undepressed on admixture with the known specimen of nigerose octaacetate.

(6) *Comparison of the behaviors of isomaltose and nigerose against hydrolysis and acetolysis*

a) *Comparison of the behaviors against hydrolysis with dilute sulfuric acid*

80 mg of the each acetate of isomaltose and nigerose was dissolved in 1 *ml* of 0.05 *N* sodium methoxide solution and kept at 0°C overnight.

After the precipitate was dissolved by adding a small amount of water, the solutions were neutralized by a few drops of glacial acetic acid and evaporated under reduced pressure and filled up to 100 *ml*.

Each 5 *ml* of the above solutions was mixed with the same amount of 0.4 *N* sulfuric acid and heated on a boiling water bath for the appointed hours. The reducing powers at each stage were determined by Somogyi's method (10). The reducing power of the complete hydrolysate was determined in the same way

after being hydrolyzed with 2.5 per cent hydrochloric acid for two hours. From these results, the apparent degrees of hydrolysis were calculated. True degrees of hydrolysis were obtained by applying the following equation.

$$\alpha = \frac{100(a - a_0)}{100 - a_0}$$

$\alpha$ = True degree of hydrolysis
$a$ = Apparent degree of hydrolysis
$a_0$ = Apparent degree of hydrolysis at 0 hour

The degrees of hydrolysis at each stage of hydrolysis are plotted as shown in Fig. 2. It became obvious that nigerose is less stable to hydrolysis than isomaltose. Namely, 96 per cent of the former was hydrolyzed in six hours

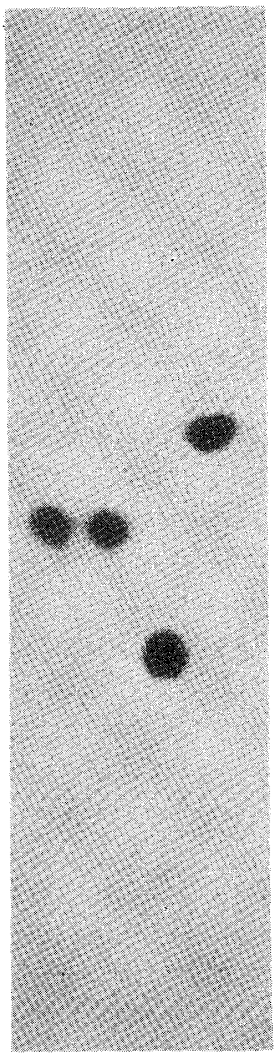


Fig. 3. Chromatogram of isomaltose and nigerose before and after acetolysis.
1. Nigerose before acetolysis
2. 〃 after acetolysis
3. Isomaltose before acetolysis
4. 〃 after acetolysis

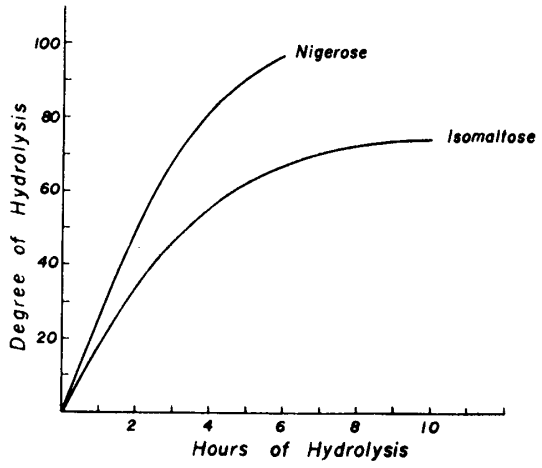


Fig. 2. Hydrolysis of isomaltose and nigerose.

whereas only 66 per cent of the latter was hydrolyzed in the same hours.

b) *Comparison of the behaviors against acetolysis*

Twenty mgs of the each acetate of isomaltose and nigerose was dissolved in 0.1 *ml* of the above described acetolysis mixture and kept at 25°C for six hours. The reactants were treated in the same manner as before and examined by paper chromatography. As shown in Fig. 3, isomaltose was completely broken down into glucose by this treatment whereas nigerose was not changed under the same condition.

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## References

1) Abdel-Akher, M., J.K. Hamilton, R. Montogomery and F. Smith (1952). J. Am. Chem. Soc., **74**, 4970.
2) Lohmar, R. (1952). J. Am. Chem. Soc., **74**, 4974.
3) Barker, S.A., E.J. Bourne, G.T. Bruce, W.B. Neely and M. Stacey (1954). J. Chem. Soc., 2395.
4) Jeanes, A., W.C. Haynes, C.A. Wilham, J.C. Rankin, E.H. Melvin, M.J. Austin, J.E. Cluskey, B.E. Fisher, H.M. Tsuchiya and C. E. Rist (1954). J. Am. Chem. Soc., **76**, 5041.
5) Wolfrom, M.L., E.N. Lassettre, and A.N. O'Neill (1951). J. Am. Chem. Soc., **73**, 595.
6) Kobayashi, T. and Y. Tsukano (1951). J. Agr. Chem. Soc. Japan, **25**, 424.
7) Kobayashi, T. and Y. Tsukano (1953). Ibid., **27**, 33.
8) Wolfrom, M.L., J.T. Tyree, T.T. Galkowski and A.N. O'Neill (1951). J. Am. Chem. Soc., **73**, 4927.
9) Thompson, A., K. Anno, M.L. Wolfrom and M. Inatome (1954). J. Am. Chem. Soc., **76**, 1309.
10) Somogyi, M. (1945). J. Biol. Chem., **160**, 61.