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## Note

# Partial hydrolysis of α-D-glucans with acid in the presence of 1,1,3,3-tetramethylurea

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For elucidation of the structure of polysaccharides, partial hydrolysis with acid is usefully employed, in combination with acetolysis, to obtain molecular information from the structures of the oligosaccharide fragments formed.

It is desirable to obtain these molecular fragments in high yields, keeping a constant level during the course of the reaction. In previous work on acetolysis[1] we found that addition of 1,1,3,3-tetramethylurea ($Me_4u$) to the acid medium gave constant production of acetylated oligomers from D-glucans.

## EXPERIMENTAL

*Materials.* — Amylose A (mol. wt. 2,900), amylose B (mol. wt. 16,000), and dextran A (mol. wt. 177,000) were used.

*Standard samples and reagents.* — D-Glucose, malto-oligosaccharides, and MeCN (Nakarai Chemicals, Ltd.); $Me_4u$ (Tokyo Kasei Kogyo Co., Ltd.); and Amberlite IRA-47 and -45 (Japan Organo Co., Ltd.) were used.

*Procedure.* — Each polysaccharide (800 mg) was weighed into a 200-mL round-bottomed flask, fitted with a condenser and a rotor; then, 100 mL of 165mM $H_2SO_4$ or 165mM $H_2SO_4$ containing 5, 7.5, or 10% respectively of $Me_4u$ was added, and the mixture was stirred vigorously at 100°. From the reaction mixture, aliquots (5 mL each) of the hydrolyzate were removed at 20-min intervals for testing. The hydrolyzate was placed in a 100-mL round-bottomed flask, made neutral with ion-exchange resin, and filtered, and the filtrate was evaporated to dryness *in vacuo* at 40°.

*L.c. analysis.* — (*1*) *Apparatus.* Degasser, ERC-3110; pump, Beckman 110 A; damper, Bellows; injection, Rheodyne 7125; column, NH 4.6 mm i.d. × 250 mm; Senshu Pak-$NH_2$-2251; detector, RI ERC-7520; integrator, SIC Chromatocorder 11; eluant, 3:2 MeCN–$H_2O$; and column temp., 40°.

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(2) *Calibration curve.* Six kinds (15 mg each) of oligosaccharide, from D-glucose to maltohexaose, were weighed out and dissolved together in $H_2O$ (5 mL). The solution (20 $\mu$L) was injected into the l.c. apparatus. Then, 30 mg of the oligosaccharides were treated in the same way. The calibration curve was drawn up automatically by the integrator supplied with the l.c. apparatus. It was calibrated with the average of 5 measurements each. This calibration curve was also used in quantitative analysis of the series of isomalto-oligosaccharides in the hydrolyzates.

(3) *Application of the hydrolyzed samples to l.c.* The degraded samples were respectively dissolved in $H_2O$ (1 mL), and this solution (20 $\mu$L each) was injected for l.c.

## RESULTS AND DISCUSSION

Usually, partial acid hydrolysis of polysaccharides is performed by heating with a dilute mineral acid (0.05–0.5M $H_2SO_4$ or 0.1–1M HCl) at 80–100°. In the

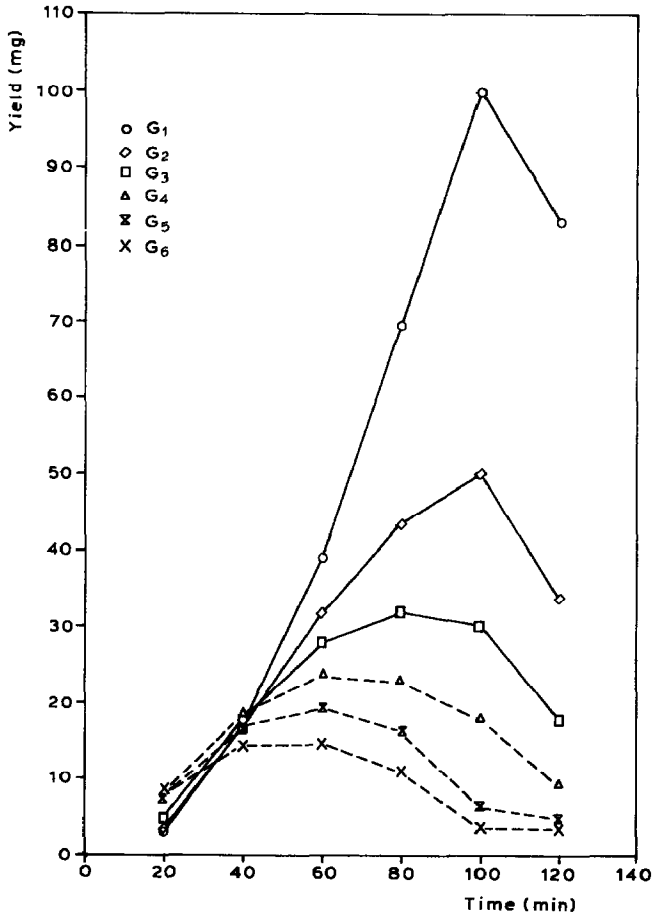


Fig. 1. Time-course yield for the ordinary, partial hydrolysis of amylose A with acid. Key: $G_1$, D-glucose; $G_2$, maltose; $G_3$, maltotriose; $G_4$, maltotetraose; $G_5$, maltopentaose; and $G_6$, maltohexaose.

partial acid hydrolysis of a polysaccharide, the concentration of the acid is a more important factor than the temperature and the reaction time.

Using acid in higher concentration may yield oligosaccharide fragments without leaving any of the polysaccharide in the reaction mixture, but the oligosaccharides formed are rapidly decomposed into the monomers. The decomposition of oligomers must be controlled by the reaction temperature. However, it is not easy to select the most suitable reaction temperature to keep the oligomers in high yields. The addition of 5–10% of $Me_4u$ to the acid resulted in a more stable formation of oligosaccharides than is obtained by the ordinary method. This is not the simple effect of lowering acidity by the addition of $Me_4u$, because dilution of acid concentration to the same extent in the ordinary method gave no significant difference in the pattern of formation of hydrolyzed fragments. $Me_4u$ would protect the oligosaccharide molecules from drastic decomposition and irregular degradation during the course of acid hydrolysis at high temperature.

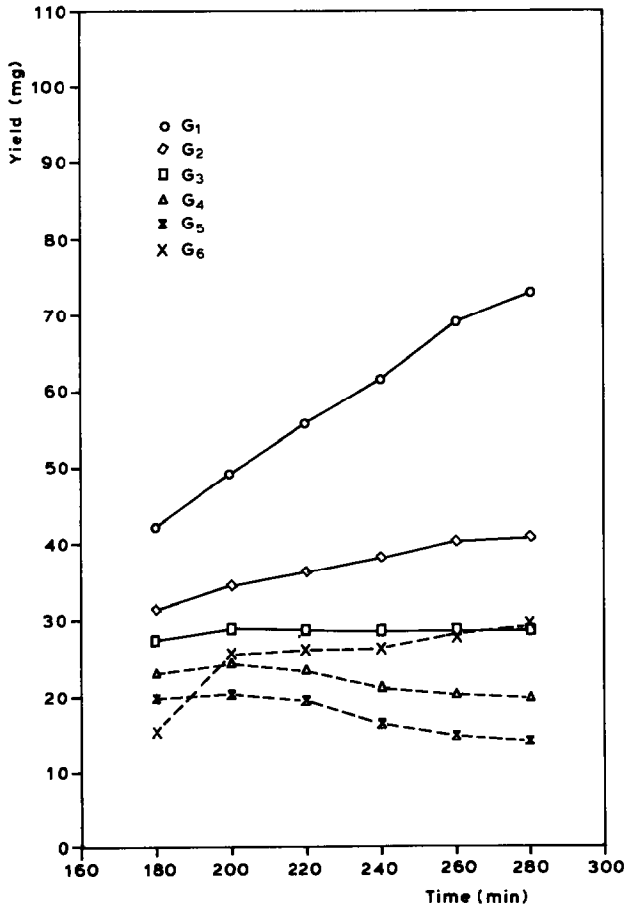


Fig. 2. Time-course yield for the modified, partial acid hydrolysis of amylose A in the presence of 10% of $Me_4$urea. $G_1$–$G_6$, as indicated in Fig. 1.

Amylose A (mol. wt. 2,900), having α-D-(1→4) linkages, was hydrolyzed with 165mM $H_2SO_4$ at 100° by the ordinary method, and by the addition of 5 and 10%, respectively, of $Me_4u$.

The yields of mono- and oligo-saccharides ($G_2$–$G_6$) were respectively determined by l.c. every 20 min. In ordinary acid hydrolysis, the oligomers ($G_4$–$G_6$) reach their maximum yields at 60 min, whereas the monomer and the small molecular oligomers ($G_2$ and $G_3$) reach their maximum yields at 100 min (see Fig. 1). On the other hand, in the presence of $Me_4u$, although there was a slight extension of reaction time, the yield of each oligomer was higher than in the case of the ordinary method, keeping the level almost constant, irrespective of the reaction time (180–280 min), and suppressing the increased formation of monomer (see Fig. 2).

The result was almost the same in the case of the higher molecular amylose B (mol. wt. 16,000).

Dextran (mol. wt. 177,000), having α-D-(1→6) linkages, was subjected to acid hydrolysis under the modified conditions in comparison with the ordinary proce-

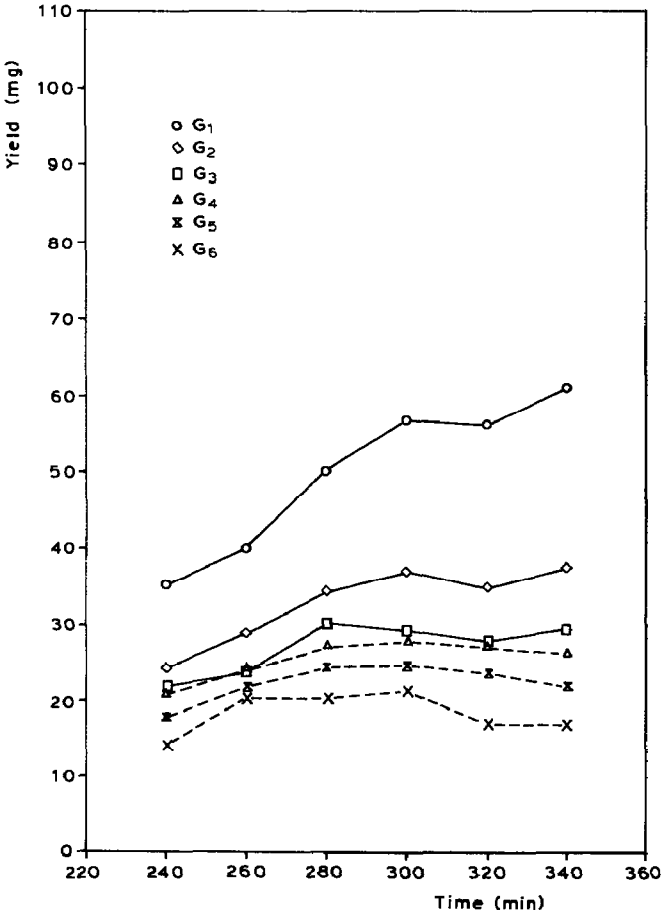


Fig. 3. Time-course yield for the ordinary, partial hydrolysis of dextran with acid. Key: $G_1$, D-glucose; $G_2$, isomaltose; $G_3$, isomaltotriose; $G_4$, isomaltotetraose; $G_5$, isomaltopentaose; and $G_6$, isomaltohexaose.

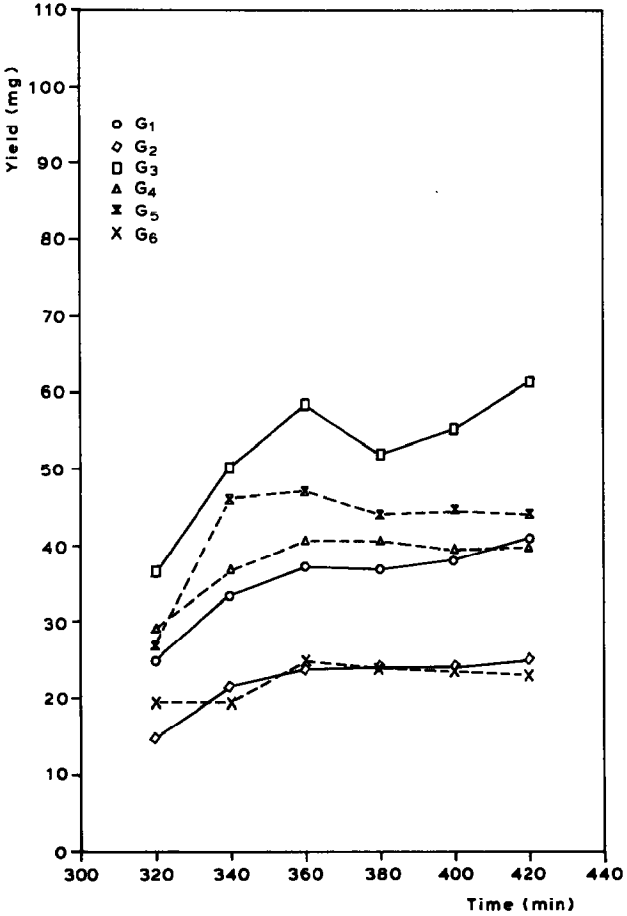


Fig. 4. Time-course yield for the modified partial acid hydrolysis of dextran in the presence of 5% of $Me_4$urea. $G_1$–$G_6$, as indicated in Fig. 3.

dure. In the presence of 5% of $Me_4u$, the yields of isomalto-oligosaccharides ($G_3$, $G_4$, and $G_5$) in the hydrolyzate of dextran were almost double those given by the ordinary method (see Figs. 3 and 4). Reaction took a longer time than for the α-D-(1→4)-glucans, as the α-D-(1→6) is more stable towards acid hydrolysis than the α-D-(1→4) linkage[2,3].

In summary, the modified acid hydrolysis in the presence of $Me_4u$ showed the following advantages. (*1*) Higher yields of oligosaccharide fragments are obtained than by the ordinary method. (*2*) The oligosaccharides of higher $\overline{d.p.}$ are obtained in an almost constant level, irrespective of the course of reaction time. (*3*) Secondary decomposition and irregular degradation are suppressed, affording higher yields of the oligosaccharide fragments, and preventing caramelization of the reaction mixture.

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