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Note

Acetolysis of α-D-glucans in the presence of 1,1,3,3-tetramethylurea

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Acetolysis[1] and partial hydrolysis[2] with acid are often applied to the chemical degradation of polysaccharides in order to elucidate their chemical structures by obtaining oligosaccharide fragments of various molecular sizes.

On acetolysis, α-D-(1→6)-glycosidic linkages are cleaved relatively faster than α-(1→4), α-(1→3), and α-(1→2) linkages[3,4]. whereas, on acid hydrolysis, the α-D-(1→4)-glycosidic linkages are the most readily cleaved[5,6].

Acetolysis is usually performed at low temperature in order to avoid irregular reaction and caramelization, therefore, it requires a long period of time to complete the reaction. Recently, in connection with our previous studies on the effect of 1,1,3,3-tetramethylurea (Me_4U) on the alkylation of polysaccharides[7,8], we found that, by addition of 5–10% of Me_4U, acetolysis can be conducted at a higher temperature (80–100°) within a remarkably short reaction-time without inducing irregular degradation and caramelization.

TABLE I

REAGENTS USED FOR ACETOLYSIS[a]

Acetolysis reagent	*Acetic acid*	*Acetic anhydride*	*H_2SO_4*	*Me_4U*
A	9.0	9.5	1.5	0
B	9.75	9.5	0.75	0
C	8	9.5	1.5	1.0 (5%)
D	7	9.5	1.5	2.0 (10%)

[a]Figures indicate the volumetric ratio.

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EXPERIMENTAL

Reagents. — All of the reagents and solvents were of Analytical Reagent grade.

Materials and standard samples. — Glucose, maltose, malto-oligosaccharides, isomalto-oligosaccharides (G_3–G_6), amylose A (mol. wt. 2,900), amylose B (mol. wt. 16,000), amylose (mol. wt. 150,000), and dextran (mol. wt. 5,000,000–40,000,000) were products of Nakarei Chem. Reagent Co.

Procedure. — An α-D-glucan (1 g) was dissolved in 20 mL of the chosen acetolysis reagent (A, B, C, or D, respectively). Each reaction mixture was stirred in a stoppered 200-mL round-bottomed flask either for 96 h at room temperature, or for 20 min at 100°, respectively. In the latter case, 2.5 mL of each test solution was removed from the reaction mixture at 1-min intervals from 10 to 13 min after start of the reaction up to 20 min. The chloroform-soluble acetolyzed mixture was mixed with methanol (15 mL) and stirred for 15 min at room temperature. Then, the mixture was subjected to *O*-deacetylation with sodium methoxide solution [0.5 g of Na metal dissolved in dry methanol (100 mL)] under stirring for 1 h at room temperature. The hydrolyzate was dissolved in the minimum volume of distilled water, the base was neutralized with Amberlite IR-120 (H^+) ion-exchange resin. The suspension was filtered and the filtrate was evaporated to a syrup which was dried for 12 h at 40° at 13.3 mPa. The products were checked by thin-layer chromatography, and the yields were determined by liquid chromatography.

Thin-layer chromatography. — Thin-layer chromatography was conducted on a Whatman LKP-KF linear-K high-performance plate (10 × 10 cm), using 5:5:3 BuOH–pyridine–water as the developing solvent, and 40% H_2SO_4 as the detecting reagent. The development was performed three times.

Liquid chromatography. — Liquid chromatography was performed under conditions 1 and 2, using an ERC-3110 degasser (Erma Optical Co.); a 110 A (Beckman) pump; a Bellows-type dumper; and a 7125 (Rheodyne) injector.

	Condition 1	*Condition 2*
Column	ODS (4.6 mm × 25 cm) Senshu-Pak (Senshu Sci. Co.)	Amino-bound silica gel (6.0 mm x 25 cm) Senshu-Pak
Detector	ERC-7520(RI) (Erma Optical Co.)	
Integrator	SIC-7000A	SIC Chromatocoder-11
Flow rate	1.5 mL/min	2.0 mL min
Eluant	3:2 CH_3CN–H_2O	
Column temp.	r.t.	40°

Calibration curve. — Fifteen mg each of D-glucose and malto- and isomalto-oligosaccharides (d.p. 2–6) was dissolved in water (5 mL). Then, 30 mg of the

oligosaccharides were treated in the same way. The calibration curve was drawn automatically by the integrator provided with the l.c. equipment. The experiments were repeated 5 times each to obtain an average value.

RESULTS AND DISCUSSION

The acetolysis of dextran A at room temperature, with or without Me_4U, required 96 h for completion; at 70°, 42–50 min without Me_4U and 152 min with 5% of Me_4U were needed. At the reaction temperature of 100°, acetolysis was complete within 20 min for both cases, but, without Me_4U, only D-glucose and isomaltose were produced with high caramelization, and in the presence of 10% of Me_4U, several oligomers were afforded without caramelization.

The acetolysis of amylose (mol. wt. 150,000) in the presence of 10% of Me_4U at 100° was complete within 20 min to afford D-gluco-oligomers of higher molecular size, keeping a well balanced production ratio (see Fig. 1), whereas, without Me_4U,

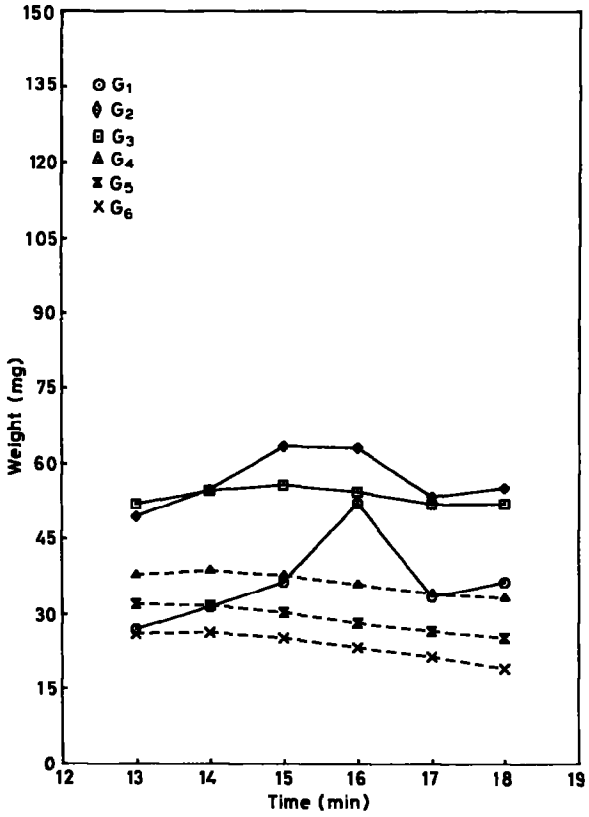

Fig. 1. L.c. determination of the time course of yields of acetolysis products from amylose of mol. wt. 150,000 by the modified method in the presence of 5% of Me_4U at 100°. Key: G_1, D-glucose; G_2, maltose; G_3, maltotriose; G_4, maltotetraose; G_5, maltopentaose; and G_6, maltohexaose.

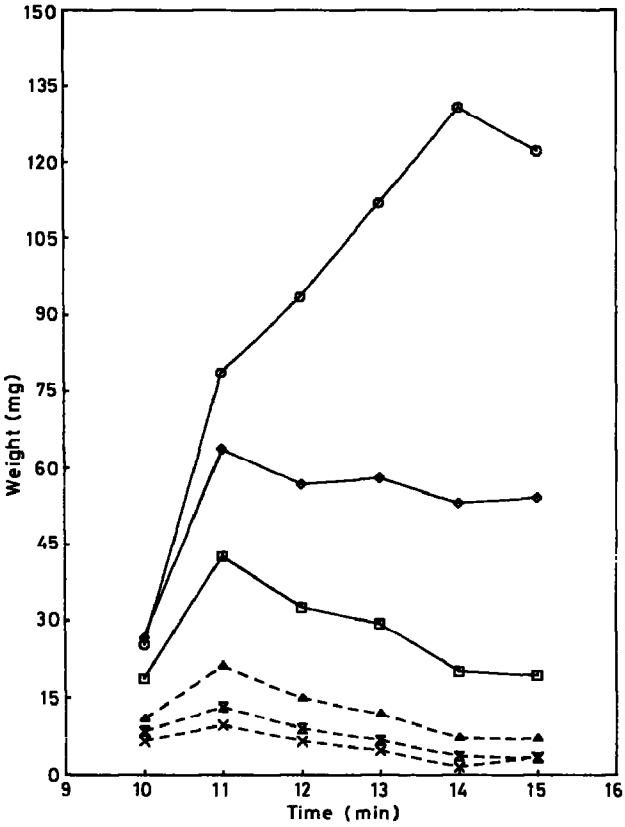

Fig. 2. L.c. determination of the time course of yields of acetolysis products from amylose of mol. wt. 150,000 by the ordinary method at 100°. Key: as in Fig. 1.

the reaction mixture was highly caramelized and contained more D-glucose, maltose, and maltotriose, and less of the oligomers of higher molecular sizes (see Fig. 2).

The presence of Me_4U in the acetolysis reagent suppressed the secondary and irregular reactions even at the higher reaction temperature.

Because of the ready availability of standard samples of the oligomers, we performed the present acetolysis experiments using mainly α-D-glucans, but the procedure could be extended to other polysaccharides under the same conditions.

In conclusion, the present acetolysis, modified by the addition of Me_4U, brings the following advantages. (*1*) The acetolysis is complete within a short reaction time to give satisfactory production of the oligosaccharide fragments; (*2*) the oligosaccharide fragments of larger molecular size are formed in a well balanced ratio; and (*3*) Me_4U suppresses irregular degradation by preventing caramelization and the secondary formation of monosaccharide from oligomers even at the higher reaction temperature.

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