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Note

Methanolysis of polysaccharides: a new method

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In depolymerization of polysaccharides by cleavage with an acid, methanolysis may be preferable to hydrolysis, as loss of neutral sugars (*e.g.*, by reactions of reducing groups with amino groups) is avoided because of anomeric protection through glycosidation.

Methanolysis using hydrogen chloride and methanol has been found useful in the identification and determination of uronic acid groups in complex polysaccharides[1–3] and in glycosaminoglycans[4,5]. Under acid hydrolysis, mild conditions will not readily break the glycosidic bonds between uronic acids, whereas conditions which are severe enough to break these bonds will additionally decarboxylate the acid. A similar methanolysis procedure has been used to determine amino sugars in glycopeptides, with subsequent *N*-reacetylation for g.l.c. analysis[6], and in the analysis of polysaccharide–peptide complexes[7] by t.l.c. Methanolysis has been used in lipopolysaccharide analysis[8]. Fatty acids and sugars of lipopolysaccharides have been simultaneously analyzed by use of methanolysis and subsequent (trifluoroacetyl)ation for g.l.c. analysis on a fused-silica capillary column[9].

The usual procedure for methanolysis of polysaccharides[10] uses methanolic hydrogen chloride, and requires the generation of hydrogen chloride gas. Silver carbonate is generally employed to remove the excess of hydrogen chloride when the reaction is complete. Because silver can complex with sugars, some precipitant such as hydrogen sulfide has usually been added to remove the silver ions. More recently, hydrogen chloride has been removed by distillation with *tert*-butanol[11]; ion-exchange resins have also been used[2], especially where quantitation is not desired[3,4]. Yields from these procedures are seldom quantitative.

A procedure for the methanolysis of starch using a 10% solution of sulfuric acid in methanol has been described[12]. In this procedure, methanol containing sulfuric acid and starch is heated under pressure at 100°; this procedure is therefore somewhat unsuitable for routine laboratory work, and is difficult to perform quantitatively. In the method reported here, a polysaccharide is dissolved in methanolic

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sulfuric acid. Less-soluble polysaccharides (such as cellulose and bagasse) are presolubilized in 72% aqueous sulfuric acid, to break the polymer into soluble oligosaccharides. Procedures are monitored by h.p.l.c. in order to check that hydrolysis to monosaccharides does not occur.

Methanolysis of cellulose has, to the best of our knowledge, not hitherto been reported. The literature describes methanolysis of cellulose triacetate[13] and cellulose nitrates[14], and the analysis of permethylated cellulose has been used in studies of structure[15,16] and of reaction mechanisms[17].

The traditional problem in methanolysis, that of removing water from the reaction mixture to prevent hydrolysis, has been solved by the use of a drying agent. The procedure described here is relatively simple, with only slight destruction of sugars, and uses a simple method for removal of the acid catalyst. High yields of methanolyzed products were obtained; the procedure is suitable for methanolysis of simple as well as complex polysaccharides.

EXPERIMENTAL

Methanolysis. — Sulfuric acid (5 mL, 72%; Baker Analytical-Reagent Grade) was added to the polysaccharide (cotton cellulose, sugarcane bagasse, citrus pectin, gum arabic, or indigenous sugarcane polysaccharide, 0.5 g). After stirring at room temperature until complete dissolution had occurred (usually 2–3 h), methanolic sulfuric acid (5 mL, 72% w/w) was added and the stirring was continued for a further 5–6 h at room temperature. Methanol (300 mL) and Drierite (5 g, recently dried) were added and the mixture was boiled under reflux for 16 h.

In polysaccharides containing hexuronic acids, it is necessary to use twice the amount of 72% aqueous sulfuric acid and 72% methanolic sulfuric acid in the solubilization stage; otherwise the procedure is identical to that for cellulose.

In methanolysis of polysaccharides that are more easily dissolved in methanolic sulfuric acid, the presolubilization step may be omitted. Thus, corn starch (Staley Co., 0.5 g) or T-2000 dextran (0.5 g) was treated with sulfuric acid in methanol (10 mL, 72% w/w) for 6 h, after which complete dissolution had occurred. Methanolysis was then continued as already described.

The methanolysis mixture was cooled, and filtered through Celite, which was then washed with methanol. The filtrate and washings were combined, concentrated in a rotary evaporator to ~150 mL, water (100 mL) was added, and the solution was passed through IR-45 (OH^-) ion-exchange resin (Rohm and Haas Co., 100 g). The resin was washed with water (100 mL), and the eluate and washings were combined, concentrated to a small volume, and the concentrate freeze-dried. The yields from the methanolysis were virtually quantitative.

Base hydrolysis of methanolyzed polysaccharides containing uronic acids. — A solution of methanolyzed uronic acid-containing polysaccharide (0.2 g) in M aqueous barium hydroxide (25 mL) was boiled under reflux for 4 h, and cooled; carbon dioxide was bubbled through and the barium carbonate was removed by

filtration through Celite which was then washed with water. The filtrate and washings were combined, concentrated to a small volume, and then freeze-dried, yielding ~0.2 g of material.

Chromatographic analysis. — For g.l.c. analysis, the methanolyzates were (trimethylsilyl)ated[18], and the mixture of trimethylsilylated methyl glycosides was analyzed by using a Hewlett–Packard model 5880 instrument fitted with a 30-m fused-silica capillary column coated with DB-5, operated for 4 min at 210° and then programmed at 4°/min until the end of the run. For h.p.l.c. analysis of mono-, di-, and tri-saccharides, a Waters Associates Sugar Analyzer system was used, incorporating refractive-index detection, a WISP auto-injector (Waters Associates), and an HPX-87C ion-exchange type of column in the calcium form (BioRad Corp.). The solvent was water (MilliQ-treated) containing calcium acetate (20 p.p.m.). For h.p.l.c. analysis of oligosaccharides of up to d.p. 12, refractive-index detection, a Model 6000A pump, a WISP autoinjector, a DextroPak column in a radial-compression module (Waters Associates), and Milli-Q-treated water as solvent were used.

Fig. 1. H.p. liquid chromatogram of (A) cellulose and (B) pectin solubilized in concentrated sulfuric acid. A. Cotton cellulose was dissolved in 72% sulfuric acid. The column employed was HPX87H, the solvent was 3.5mM sulfuric acid, and detection was by refractive index. Glucose (at 8.76) and glucose sulfate (at 9.26) were not observed. B. Pectin was dissolved in 72% sulfuric acid. The column employed was HPX87H, the solvent was 3.5mM sulfuric acid, and detection was by refractive index. Galacturonic acid (at 8.20) was not observed.

RESULTS AND DISCUSSION

The presolubilization procedure was monitored by h.p.l.c. Formation of oligomers was observed, but no monomeric species were observed during the solubilization process. H.p.l. chromatograms of cellulose and pectin dissolved in sulfuric acid are shown in Fig. 1.

During the methanolysis some methyl hydrogensulfate is formed. Because the barium salt of methyl hydrogensulfate is soluble in water, Ba^{2+} cannot be used to remove the acid catalyst as it is in hydrolysis procedures[1,2]. Instead, an ion-exchange procedure was chosen.

In order to remove the acid catalyst with an anion-exchange resin, it was found necessary, after methanolysis was complete, to evaporate half of the methanol and replace it with water. The quantity of resin used to remove the acid was so chosen as to be sufficient to give an effluent pH near 7.0.

After removal of the acid catalyst the solutions were concentrated; the freeze-

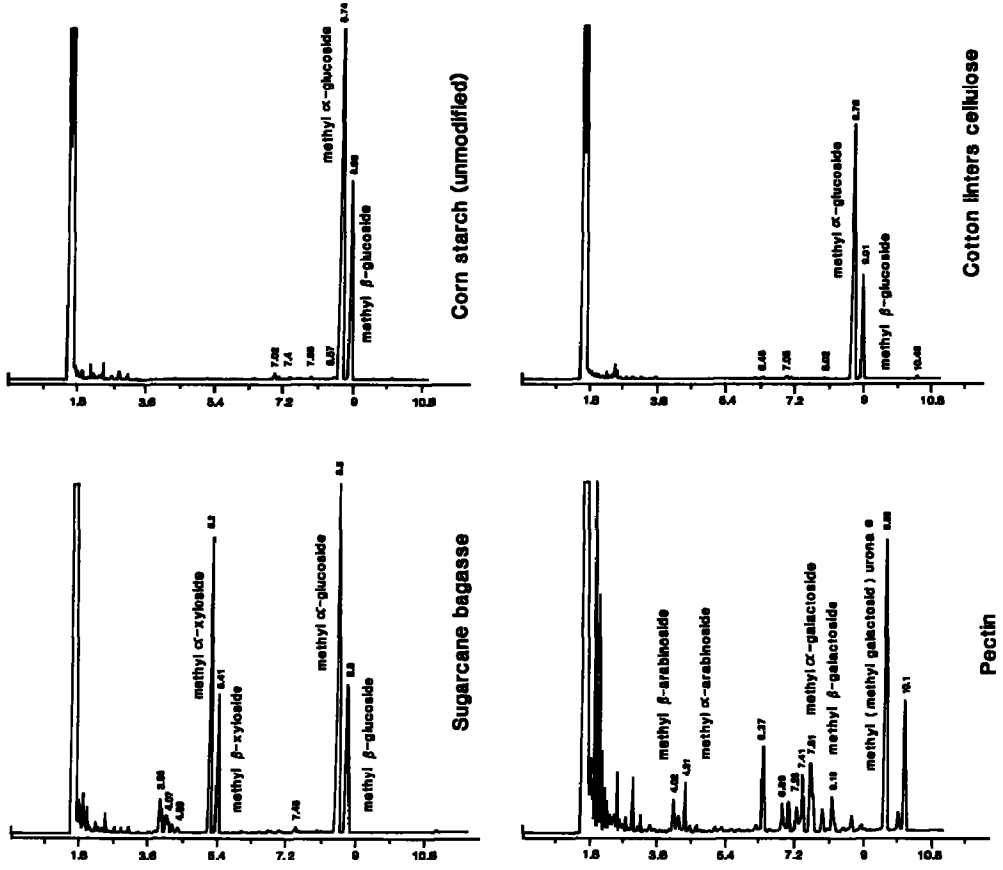

Fig. 2. G.l. chromatogram of methanolyzed starch, cellulose, bagasse, and pectin.

dried products were analyzed by g.l.c. or by h.p.l.c. The methanolysis products were identified by retention time and peak enhancement with known compounds. G.l. chromatograms of methanolyzed cellulose, bagasse, pectin, and starch are shown in Fig. 2.

Cellulose. — The crystalline structure of cellulose renders it insoluble in most solvents. The solubilization step described here destroys the crystalline structure and fragments the chain. Soluble oligosaccharides are then methanolyzed (see Fig. 1). Although not crystalline, soluble-with-difficulty, uronic acid-containing polysaccharides also require presolubilization (see Fig. 1). During methanolysis, carboxyl groups of the uronic acids are converted into methyl esters or lactones. For this reason, it is necessary to hydrolyze the methanolysis products with base in order to improve the accuracy of quantitation by limiting the number of peaks in g.l.c. Barium hydroxide is the base of choice because the excess of barium ions is readily removed with CO_2. Methyl esters and lactones are hydrolyzed to afford the barium salts of the carboxyl groups, which are readily silylated for g.l.c. analysis.

Starch. — Starch contains no uronic acids and no crystalline structure, and is soluble in 72% (w/w) methanolic sulfuric acid. Only the expected methyl α- and β-D-glucosides were observed by g.l.c.

Dextran. — Dextran, like starch, contains no uronic acids and is therefore soluble in 72% (w/w) methanolic sulfuric acid. The T-2000 dextran used contains >95% of α-D-(1→6) linkages with no more than 5% of α-D-(1→3) or α-D-(1→4) branching. G.l.c. analysis of the methanolyzed dextran showed only the methyl α- and β-D-glucosides, as expected. The yield of methyl glucosides was 95.5% of the theoretical, assuming 5% of moisture in the starting material.

Tables I and II show the percentage composition of sugars in methanolyzed polysaccharides. In Table I, both starch and dextran T-2000 show a higher proportion of methyl α-D-glucoside than that reported after methanolysis by hydrogen chloride[3], or known to be the equilibrium concentration[19].

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TABLE I

PERCENTAGE COMPOSITION OF SUGARS IN METHANOLYZED STARCH AND DEXTRAN

Substrate	*Compounds liberated*	*α-Pyranoside (%)*	*β-Pyranoside (%)*	*Total (%)*
Starch	glucose	72.6	27.4	97.3
	unidentified			2.7
Dextran	glucose	71.9	28.1	95.7
	unidentified			4.3

TABLE II

PERCENTAGE COMPOSITION OF SUGARS IN METHANOLYZED POLYSACCHARIDES

Substrate	*Compounds liberated*	*α-Glycoside (%)*	*β-Glycoside (%)*	*Total (%)*
Cotton cellulose	glucose	72.2	27.8	96.3
	unidentified	—	—	3.7
Bagasse, internodal	glucose	72.8	27.2	56.1
	xylose	67.9	32.1	34.2
	arabinose[a]	39.4	60.6	5.1
	unidentified	—	—	4.6
Bagasse, nodal	glucose	71.5	28.5	52.8
	xylose	66.6	33.4	35.5
	arabinose[a]	34.3	65.7	6.6
	unidentified	—	—	5.1
Bagasse, whole	glucose	72.8	27.2	55.8
	xylose	67.9	32.1	34.5
	arabinose[a]	41.5	54.5	5.8
	unidentified	—	—	3.9
Pectin	galacturonic acid	—	—	30.1
	arabinose	73.1	26.9	4.1
	galactose	70.8	29.2	14.3
Other peaks not identified				
Gum arabic	galactose	71.2	28.8	38.6
	arabinose	26.9	73.1	31.0
	rhamnose	81.5	18.5	14.7
	4-methyl ether	—	—	8.4
	glucuronic acid	—	—	1.6
	glucose	70.9	29.1	1.5
	unidentified	—	—	4.2
I.S.P. (a cell-wall polysaccharide of sugarcane)	glucose	72.8	27.2	67.9
	arabinose	25.8	74.2	9.7
	galactose	84.5	15.5	4.4
	rhamnose	68.8	31.2	3.4
	xylose	68.3	31.7	2.0
	glucuronic acid	—	—	0.4
	unidentified	—	—	12.2

[a]Arabinose peaks are very small; integration error may account for low α/β ratio.

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