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Note

General and facile method for distinguishing 4-linked aldopyranosyl residues from 5-linked aldofuranosyl residues*

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Methylation analysis of complex carbohydrates does not distinguish between 4-linked aldopyranosyl and 5-linked aldofuranosyl residues, because the substitution pattern of the *O*-methyl groups is the same in both of the derivatives produced[1]. For example, methylation of either a 5-linked hexofuranosyl or a 4-linked hexopyranosyl residue yields the same 2,3,6-tri-*O*-methylhexosyl derivative. These two types of glycosyl residue cannot be distinguished by methylation analysis, even if the glycosyl residues are further substituted; for example, methylation analysis of a 3,4-linked hexopyranosyl residue or a 3,5-linked hexofuranosyl residue yields the same 2,6-di-*O*-methylhexosyl derivative.

Previous methods for obtaining information about the ring size of glycosyl residues have involved the susceptibility of furanosyl residues to mild acid hydrolysis[2], specific degradations[2–4], and isolation of characterizable oligosaccharides[5]. These methods are not general and, when they can be applied, often require involved procedures.

This paper describes an easy-to-use, general method for distinguishing between aldo-furanosyl and -pyranosyl residues. The method can be performed on small amounts (~1 mg) of any complex carbohydrate, and uses only the techniques and apparatus commonly employed to perform routine methylation analyses.

The method is based on converting the glycosyl residues of the complex carbohydrate being analyzed into residues whose ring form can be readily ascertained. The first steps in this procedure are to methylate the complex carbohydrate and then, by partial hydrolysis, to convert the permethylated complex carbohydrate into a random mixture of methylated oligosaccharides. In the next steps, the aldehyde at the reducing end of each partially methylated oligosaccharide fragment is reduced to a primary alcohol, and all of the unsubstituted hydroxyl groups produced by the partial hydrolysis and reduction are ethylated. All of the glycosyl residues are then

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converted into partially alkylated alditol acetates by successive hydrolysis, reduction, and acetylation.

The ring form of any 4-linked aldopyranosyl or 5-linked aldofuranosyl residue in the complex carbohydrate can be ascertained if either the glycosidic linkage from C-1 or the glycosidic linkage to O-4 (or O-5) is cleaved by the partial hydrolysis. This is true because the partially methylated, partially ethylated alditol acetates derived in this way from 4-linked aldopyranosyl and 5-linked aldofuranosyl residues will have different substitution patterns of *O*-methyl and *O*-ethyl groups. The method is illustrated with xanthan, the well characterized polysaccharide secreted by *Xanthomonas campestris*[6-9], and also with beet arabinan.

RESULTS AND DISCUSSION

Xanthan contains three glycosyl residues whose ring form, either furanosyl or pyranosyl, cannot be determined by methylation analysis. These residues are 4-linked D-glucopyranosyl, 3,4-linked D-glucopyranosyl, and 4-linked D-glucopyranosyluronic (Fig. 1). Methylation analysis does not establish that these residues are not 5-linked glucofuranosyl, 3,5-linked glucofuranosyl, and 5-linked glucofuranosyluronic residues, respectively. Our method is described here with the 4-linked glucopyranosyl residue of xanthan as an example, although the method is equally applicable for all glycosyl residues.

Xanthan (1 mg) was methylated and the carboxyl groups were reduced[6]. Partial cleavage conditions for the xanthan, resulting in ~25–50% cleavage of the glycosidic linkages of the glycosyl residues, were established as described[6]. These conditions involved heating for 30 min at 90° in 0.5 mL of 90% formic acid. This operation gave ~25% cleavage of each of the glycosidic linkages of the methylated and carboxyl-reduced xanthan[6].

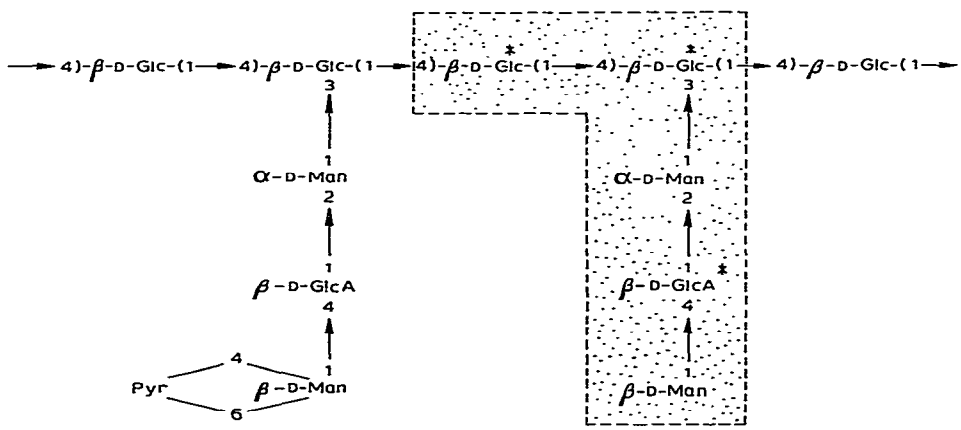

Fig. 1. The structure of xanthan [6-9]. An asterisk (*) indicates those glycosyl residues whose ring size cannot be determined by methylation analysis. Approximately two thirds of the terminal mannosyl groups are not substituted by pyruvic acid.

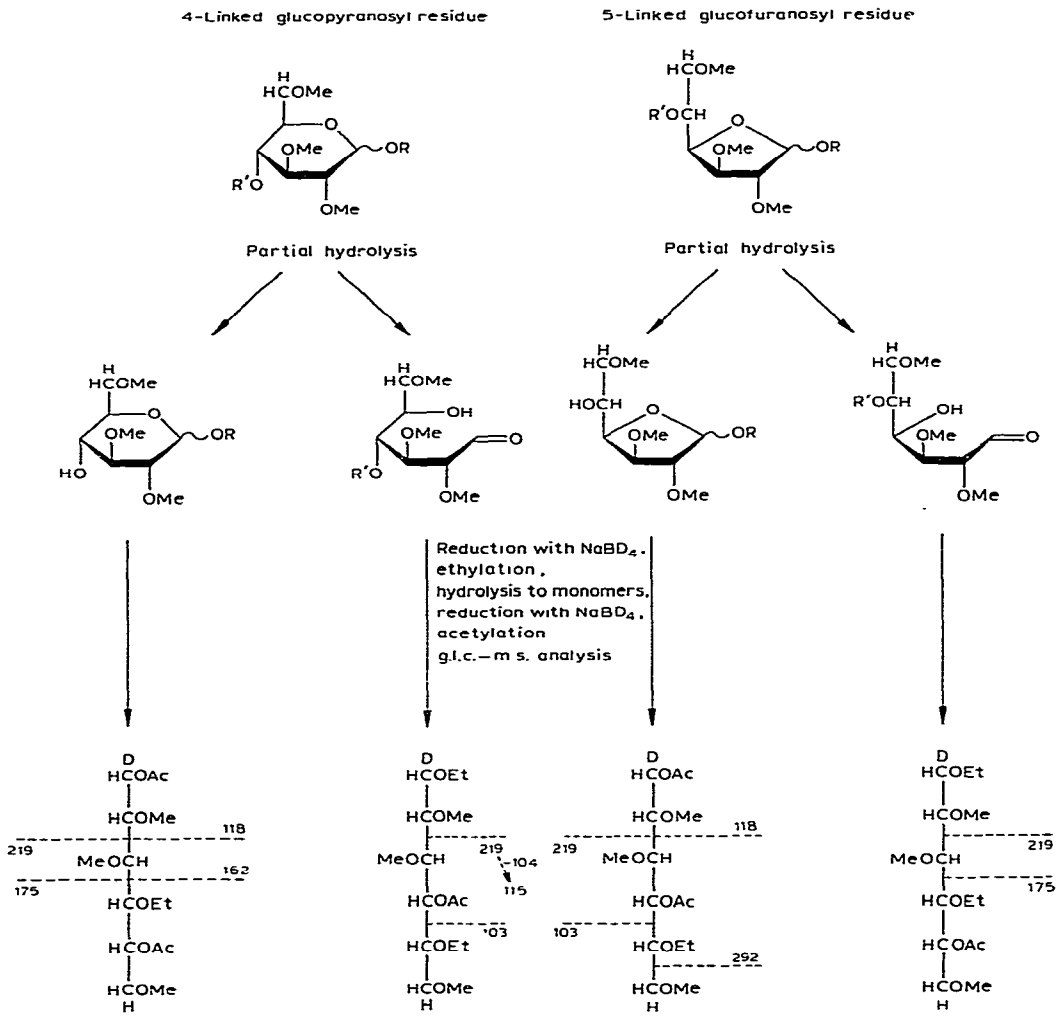

Fig. 2. Theoretical cleavage-products from 4-linked glucopyranosyl and 5-linked glucofuranosyl residues after partial cleavage and derivatization of these products to allow g.l.c.–m.s. analysis. R and R^1 = glycosyl residues.

The partial formolysis of xanthan frequently results in cleavage of one, but not two, of the glycosidic linkages to or from any given glycosyl residue. The two possible cleavages of the glycosidic linkages connecting the 4-linked glucopyranosyl residues are illustrated in Fig. 2. The two analogous cleavages of the glycosidic linkages to or from a 5-linked glucofuranosyl residue are also illustrated in Fig. 2.

The methylated, carboxyl-reduced, and partially cleaved xanthan is successively reduced with sodium borodeuteride, ethylated, and hydrolyzed[6]. The resulting mixture of partially methylated, partially ethylated aldoses and alditols is reduced with sodium borodeuteride and then acetylated[6] (Fig. 2). The resulting, partially

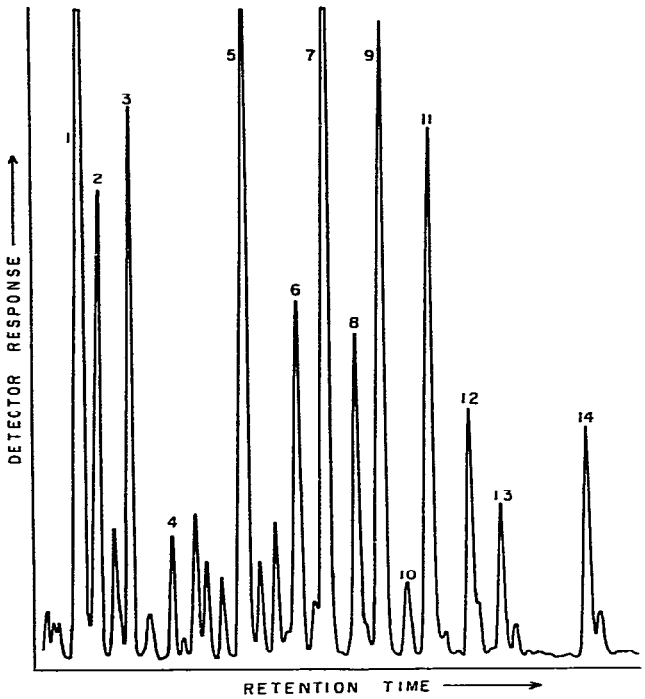

Fig. 3. Gas chromatogram of the partially alkylated alditol acetates obtained from analysis of xanthan, to determine the ring size of the glycosyl residues. The derivatives in the numbered peaks are listed in Table I. The chromatogram was obtained on a 25-m, S.E. 30 glass-capillary column programmed from 170–240° increasing at 2° per min.

TABLE I

IDENTIFICATION OF THE PARTIALLY ALKYLATED ALDITOL ACETATES IN THE G.L.C. PEAKS IN FIG. 3

Peak	*Position of* O-*ethyl groups*	*Position of* O-*methyl groups*	*Position of* O-*acetyl groups*	*Alditol*
1	1,5	3,4,6	2	Mannitol
2	1,5	2,3,6	4	Glucitol
3	1,3,5	2,6	4	Glucitol
4	1,5,6	2,3	4	Glucitol[a]
5	2	3,4,6	1,5	Mannitol
	4	2,3,6	1,5	Glucitol
6	3,4	2,6	1,5	Glucitol
7	4,6	2,3	1,5	Glucitol[a]
8		3,4,6	1,2,5	Mannitol
9		2,3,6	1,4,5	Glucitol
10	4	2,6	1,3,5	Glucitol
11	3	2,6	1,4,5	Glucitol
12	6	2,3	1,4,5	Glucitol[a]
13		2,6	1,3,4,5	Glucitol
14		2,3	1,4,5,6	Mannitol

[a]Originates from carboxyl-reduced glycosyluronic residues, as demonstrated by the presence of two deuterium atoms at C-6.

methylated, partially ethylated alditol acetates are analyzed by g.l.c. and g.l.c.–m.s. The g.l.c. profile of the partially methylated, partially ethylated alditol acetates obtained from xanthan is shown in Fig. 3; the identity of the peaks in Fig. 3 is summarized in Table I.

The ring form of the 4-linked glucopyranosyl residue of xanthan was established by structurally characterizing the derivative produced by cleavage of the glycosidic linkage from C-1 (peak 2 in Fig. 3), and also by characterizing the derivative produced by cleavage of the glycosidic linkage to O-4 (peak 5 in Fig. 3). In the derivative resulting from cleavage of the glycosidic linkage from C-1 of the 4-linked glucopyranosyl residue, ethoxyl groups were detected at C-1 and C-5 (and not at C-4) indicating that C-5 was involved in forming the ring. In the derivative resulting from cleavage of the glycosidic linkage attached to O-4 of the 4-linked glucopyranosyl residue, an ethoxyl group at C-4 and the absence of an ethoxyl group at C-1 demon-

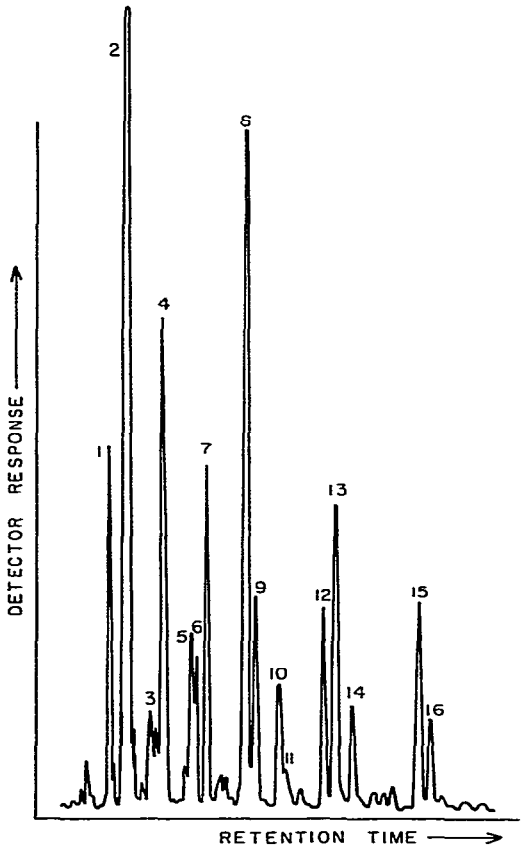

Fig. 4. Gas chromatogram of the partially alkylated alditol acetates obtained from analysis of arabinan, to determine the ring size of the glycosyl residues. The derivatives in the numbered peaks are listed in Table II. The chromatogram was obtained on a 25-m, S.E. 30 glass-capillary column programmed from 170–240° per min.

strated that C-4 was involved in the linkage to another sugar and not in forming the ring. Identification of either of these derivatives established the ring form of the 4-linked glucopyranosyl residue.

The ring forms of the 3,4-linked glucopyranosyl and 4-linked glucopyranosyluronic residues of xanthan were established similarly (see peaks 3, 4, 6, 7, and 10 in Fig. 3 and Table I). No derivatives from furanosyl residues were detected (Table I).

Beet arabinan contains four differently linked arabinosyl residues whose ring forms cannot be determined by methylation analysis[10]. We have now established that these residues are 5-linked, 2,5-linked, 3,5-linked, and 2,3,5-linked arabinofuranosyl residues rather than the corresponding pyranosyl residues. The ring form of these residues was determined in a manner exactly analogous to that used for xanthan. Beet arabinan was successively methylated, partially hydrolyzed (2M trifluoroacetic acid, 2 h at 70°), reduced with sodium borodeuteride, and ethylated. The g.l.c. profile of the resulting partially methylated, partially ethylated alditol acetates is shown in Fig. 4; the identity of the peaks in Fig. 4 is summarized in Table II. The partially alkylated alditol acetates identified in peaks 1 and 4 (Fig. 4 and Table II) established the presence in beet arabinan of 5-linked arabinofuranosyl residues. Similarly, the partially alkylated alditol acetates in peak 5, peaks 6 and 9, and peak 10 established, respectively, the presence of 2,5-linked arabinofuranosyl, 3,5-linked arabinofuranosyl, and 2,3,5-linked arabinofuranosyl residues. No derivatives from pyranosyl residues were detected, although the presence of small proportions of pyranosyl residues in this complex polysaccharide could not be ruled out.

TABLE II

IDENTIFICATION OF THE PARTIALLY ALKYLATED ALDITOL ACETATES IN THE G.L.C. PEAKS IN FIG. 4

Peak	*Position of O-ethyl groups*	*Position of O-methyl groups*	*Position of O-acetyl groups*	*Alditol*
1	1,4	2,3	5	Arabinitol
2		2,3,5	1,4	Arabinitol
3	3	2,5	1,4	Arabinitol
4	5	2,3	1,4	Arabinitol
5	1,4	3	2,5	Arabinitol
6	1,4	2	3,5	Arabinitol
7		2,5	1,3,4	Arabinitol
8		2,3	1,4,5	Arabinitol
9	5	2	1,3,4	Arabinitol
10	1,4		2,3,5	Arabinitol
11	3	2	1,4,5	Arabinitol
12		2,3,4,6	1,5	Galactitol
13		2	1,3,4,5	Arabinitol
14		3	1,2,4,5	Arabinitol
15	{		1,2,3,4,5	Arabinitol
	{	2,3,6	1,4,5	Galactitol
16		2,3,6	1,4,5	Glucitol

EXPERIMENTAL

Methylation of polysaccharides. — Beet arabinan (1 mg) was purified and methylated as described[10]. Xanthan (1 mg) was methylated and its carboxyl groups were reduced as described[6].

Determination of conditions for partial cleavage of the glycosidic linkages of xanthan and arabinan. — The conditions for partial formolysis of xanthan and partial hydrolysis conditions of arabinan in order to cleave between 25–50% of the glycosidic linkages were determined as described[6].

Reduction, ethylation, and preparation of alkylated alditol acetates. — The partially methylated oligosaccharides, produced by partial cleavage of xanthan and arabinan, were reduced and ethylated, and their alkylated alditol acetates prepared as described[6]. The partially methylated, partially ethylated alditol acetates were separated and identified[6] by g.l.c. and g.l.c.–m.s. by using a 25-m open-tubular, glass-capillary column containing SE-30.

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REFERENCES

1 H. Björndal, C. G. Hellerqvist, B. Lindberg, and S. Svensson, *Angew. Chem., Int. Ed. Engl.*, 9 (1970) 610–619.
2 B. Lindberg, B. Lindqvist, J. Lönngren, and W. Nimmich, *Carbohydr. Res.*, 58 (1977) 443–451.
3 J. P. Kamerling, B. Lindberg, J. Lönngren, and W. Nimmich, *Acta Chem. Scand., Ser. B*, 29 (1975) 593–598.
4 M. Curvall, B. Lindberg, J. Lönngren, and W. Nimmich, *Carbohydr. Res.*, 42 (1975) 73–82.
5 C. Erbing, L. Kenne, B. Lindberg, J. Lönngren, and I. W. Sutherland, *Carbohydr. Res.*, 50 (1976) 115–120.
6 B. S. Valent, A. G. Darvill, M. McNeil, B. K. Robertsen, and P. Albersheim, *Carbohydr. Res.*, 79 (1980) 165–192.
7 C. J. Lawson and K. C. Symes, *Carbohydr. Res.*, 58 (1977) 433–438.
8 L. Melton, L. Mindt, D. Rees, and G. Sanderson, *Carbohydr. Res.*, 46 (1976) 245–257.
9 P. Jansson, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 45 (1975) 275–282.
10 L. Weinstein and P. Albersheim, *Plant Physiol.*, 63 (1979) 425–432.