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Simplified and improved methylation analysis of saccharides, using a modified procedure and thin-layer chromatography

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Abstract

Methylation analysis used in determining the positions of glycosidic linkages in oligo- and poly-saccharides has been greatly simplified by eliminating two of the usual reactions, reduction and acetylation of the methylated monosaccharide residues. The saccharides are methylated by the Hakomori procedure, followed by acid hydrolysis and analysis by thin-layer chromatography (TLC). The procedure is illustrated by analysis of four known disaccharides, cyclomaltohexaose, and eleven polysaccharides. 2,3,4,6-Tetra-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-galactose, and 2,3,4,6-tetra-*O*-methyl-D-mannose, three isomeric tri-*O*-methyl-D-glucoses, tri-*O*-methyl-D-mannoses, and tri-*O*-methyl-D-galactoses, three isomeric di-*O*-methyl-D-glucoses, and mono-*O*-methyl-D-glucose are resolved by the TLC system. The *O*-methylated sugars can be quantitated directly on the TLC plate by using imaging densitometry. The new procedure permits any laboratory to carry out the analyses with a minimum of expense and without the need of dedicated, elaborate laboratory instrumentation. The procedure is sufficiently micro that it can be carried out on 1 mg of sample. © 1996 Elsevier Science Ltd.

Keywords: Methylation analysis; Hakomori reagent; Oligosaccharides; Polysaccharides; Thin-layer chromatography; Densitometry

1. Introduction

Methylation analysis is the principal method used to determine the position of linkages between monosaccharide residues and the presence of branching in oligo- and

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poly-saccharides [1]. The method was greatly improved by the development of the Hakomori procedure [2]. The saccharide is treated with methylsulfinyl carbanion to form polyalkoxide ions from the free hydroxyl groups, which are then methylated with methyl iodide. The Hakomori reagent solubilizes most oligo- and poly-saccharides and gives complete methylation in only one step. The methylated saccharides are acid hydrolyzed and the methylated monosaccharides are converted into highly volatile compounds for gas–liquid chromatography (GC) or gas–liquid chromatography–mass spectrometry (GCMS) by reduction and acetylation [3]. These additional reactions of reduction and acetylation, coupled with analysis by GC or GCMS are tedious, time-consuming, and fraught with problems due to incomplete reduction and acetylation, and selective losses of volatile compounds during evaporation and transfer. The interpretation of the mass spectra is often problematic. Further, the analyses require a dedicated GC system and highly expensive MS instrumentation. These problems have inhibited the occasional user in performing methylation analyses.

We have, therefore, developed a greatly simplified procedure by eliminating the extra reactions of reduction and acetylation. Methylation is carried out by the Hakomori procedure, followed by trifluoroacetic acid hydrolysis, and the direct analysis of the methylated products by thin-layer chromatography (TLC) to give qualitative and quantitative analyses of the types of linkages found in oligo- and poly-saccharides. The procedure is simple and inexpensive and can be performed without the need of a dedicated GC instrument that is only occasionally used and without highly sophisticated GCMS instrumentation.

2. Experimental

Materials.—Analytical grade dimethyl sulfoxide (Me_2SO) was dried by adding coarse CaH_2. Methyl iodide and trifluoroacetic acid was obtained from Aldrich Chemical Co., Milwaukee, WI. Whatman K6 plates were obtained from Fisher Scientific Co., Chicago, IL. Various polysaccharides (B-512F, B-742CA, and B-742CB dextrans, alternan, and cyclomatohexaose) were enzymatically synthesized in the laboratory. Other polysaccharides (yeast mannan, birch xylan, and arabinogalactan) were obtained from Sigma Chemical Co., St. Louis, MO. Arabinogalactan and birch xylan were treated with 1 M CF_3CO_2H for 15 h at 40 °C. Pullulan was obtained from Hayashibara Biochemical Laboratories, Inc. Okayama, Japan. All other chemicals were of highest grade obtainable commercially.

Methods.—Hakomori reagent. Sodium hydride (55% coated with mineral oil, 1.5 g) was washed three times in a sealed container with 30-mL portions of *n*-pentane; the *n*-pentane was removed by evacuation, interspersed by flushing with nitrogen; 15 mL of dry Me_2SO was added to the NaH and the solution was heated to 50 °C and sonicated for 2–4 h, until the solution became green and evolution of hydrogen had ceased; 2–5 mL was transferred to cryovials and 0.5 mL of mineral oil was added. The reagent was stored at −20 °C.

Methylation procedure. Vacuum-dried saccharides (10 mg) were dissolved in dry Me_2SO (0.8 mL) by stirring until a clear solution was obtained. The solution was placed into a tube that was then closed with a septum. Hakomori's reagent (0.2 mL) was added.

A gel was immediately formed and it slowly dissolved over ~ 18 h. The dissolution may be accelerated by sonication (0.25–2 h). Methyl iodide (0.2 mL) was then added, whereupon the solution turned from a gray-green to a turbid yellow. The reaction was allowed to proceed 2 h to give a darker yellow to brown color; 4 mL of water was then added to the yellow/brown Me_2SO solution. This mixture was extracted three times with 2 mL of $CHCl_3$. The $CHCl_3$ extracts were combined and extracted 2 times with 10 mL of water containing a crystal of $Na_2S_2O_3$. The $CHCl_3$ layer was rotary-evaporated *in vacuo* at 30 °C or lower; 1 mL of 4 M CF_3CO_2H was added and the solution was transferred to a screw-cap tube that was autoclaved for 2 h at 121 °C. The sample was taken to a syrup by rotary evaporation *in vacuo* at 30 °C or lower and the syrup was dissolved in 1 mL of MeOH.

TLC analysis of the methylated products.—Aliquots (1–3 μL) were placed on Whatman K6 TLC plates using a AIS TLC Multi-spotter (Analtech, Newark, DE); the TLC plate was irrigated at room temperature with two ascents of MeCN–$CHCl_3$–MeOH, 3:9:1 (v/v/v) at room temperature. The plate was thoroughly dried between each ascent. The compounds were developed on the plate by rapidly dipping the plate into a solution containing 3 g of *N*-(1-naphthyl)ethylenediamine and 50 mL of concentrated H_2SO_4 in 1 L of MeOH. The plate was dried and then placed in an oven for 10 min at 120 °C; blue-black spots appeared on a white background [4].

Methylation analysis of micro-amounts of sample. Samples of 1 mg to 10 μg were methylated by using the standard procedure, but instead of applying 2 μL of methylated sample to the TLC plate, 20 μL was applied using the Multi-spotter.

Preparation of methylated standards.—2,3,4,6-tetra-*O*-methyl-D-glucose was obtained from Sigma Chemical Co., 2,3,6-tri-*O*-methyl-D-glucose by methylating cyclomaltohexaose, and 2,3,4-tri-*O*-methyl-D-glucose by methylating B-512F dextran (Sigma Chemical Co.). Solutions (1 mL) each of the methylated glucoses (~ 10 mg) were streaked across the center 14 cm of a 20 × 20 cm preparative TLC plate (1500 μm thick preparative silica gel plate obtained from Analtech, Newark, DE). The plates were irrigated 2 times with 47:3 (v/v) $CHCl_3$–MeOH; 3 cm were cut from each end of the plate and dipped into the *N*-(1-naphthyl)ethylenediamine reagent for visualization. The developed plates were placed next to the undeveloped plate and lines were drawn with a pencil on either side of the undeveloped compounds. The silica gel between the lines was removed and placed into a tube to which 5 mL of 50% (v/v) aqueous MeOH was added. The tubes were centrifuged and the supernatants removed and filtered through a 5-μm membrane. The solutions of pure compound were pooled and evaporated to dryness in tared tubes. The solid material was dissolved in 50% aqueous MeOH.

Quantitative determination of O*-methylated sugars.*—The quantitative determination of the *O*-methylated sugars directly on the TLC plate was achieved by scanning the plate with a Bio-Rad Imaging Densitometer, model GS-670 (Bio-Rad Laboratories, Hercules, CA).

3. Results

The TLC analysis of the *O*-methylated monosaccharides obtained from methylation and acid hydrolysis of four disaccharides and cyclomaltohexaose are shown in Fig. 1.

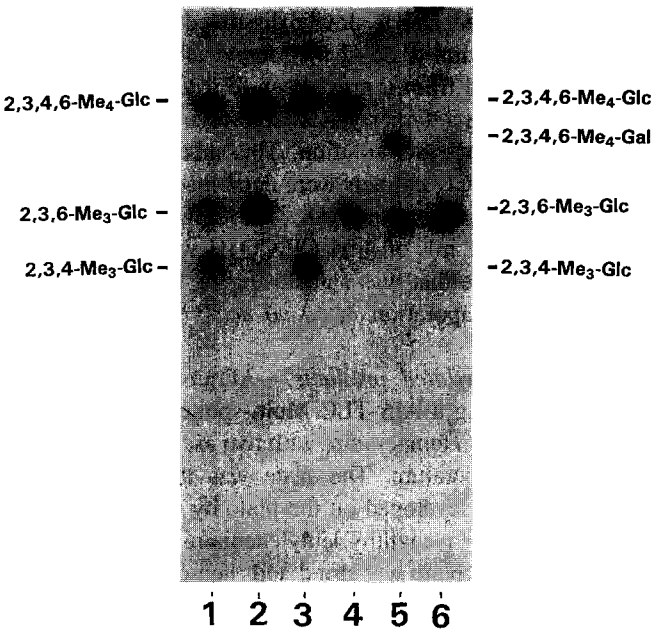

Fig. 1. TLC separation of *O*-methylated-D-monosaccharides from oligosaccharides. Lane 1, standards from top to bottom of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose; lane 2, *O*-methylated products from maltose; lane 3, *O*-methylated products from isomaltose; lane 4, *O*-methylated products from cellobiose; lane 5, *O*-methylated products from lactose; and lane 6, *O*-methylated product from cyclomaltohexaose.

Lane 1 contains the standards, 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,4-tri-*O*-methyl-D-glucose that migrate in order with R_f values of 0.83, 0.63, and 0.50, respectively. R_f is defined conventionally as the distance migrated from the origin divided by the distance from the origin to the top of the TLC plate. Lane 2 shows the methylation analysis of maltose, which gives 2,3,4,6-tetra-*O*-methyl-D-glucose from the nonreducing end and 2,3,6-tri-*O*-methyl-D-glucose from the reducing end. Lane 3 shows the methylation analysis of isomaltose, which gives 2,3,4,6-tetra-*O*-methyl-D-glucose from the nonreducing end and 2,3,4-tri-*O*-methyl-D-glucose from the reducing end. Lane 4 shows the methylation analysis of cellobiose, which as expected, gives the same *O*-methyl-D-glucoses as does maltose. Lane 5 shows the methylation analysis of lactose that gives 2,3,4,6-tetra-*O*-methyl-D-galactose from the nonreducing-end and 2,3,6-tri-*O*-methyl-D-glucose from the reducing-end. Lane 6 shows the methylation analysis of cyclomaltohexaose that gives a single methylated product, 2,3,6-tri-*O*-methyl-D-glucose. This is the only expected *O*-methylated product from the nonreducing, cyclic maltodextrins with only α-(1 $\rightarrow$ 4) linked glucose residues.

The methylation analysis of eight glucans, containing different kinds of glycosidic linkages, are shown in Fig. 2A. Lane 1 contains the same *O*-methylated-D-glucose standards used in Fig. 1. Lane 2 shows the methylated products obtained from amylopectin. The major methylated glucose is 2,3,6-tri-*O*-methyl-D-glucose which re-

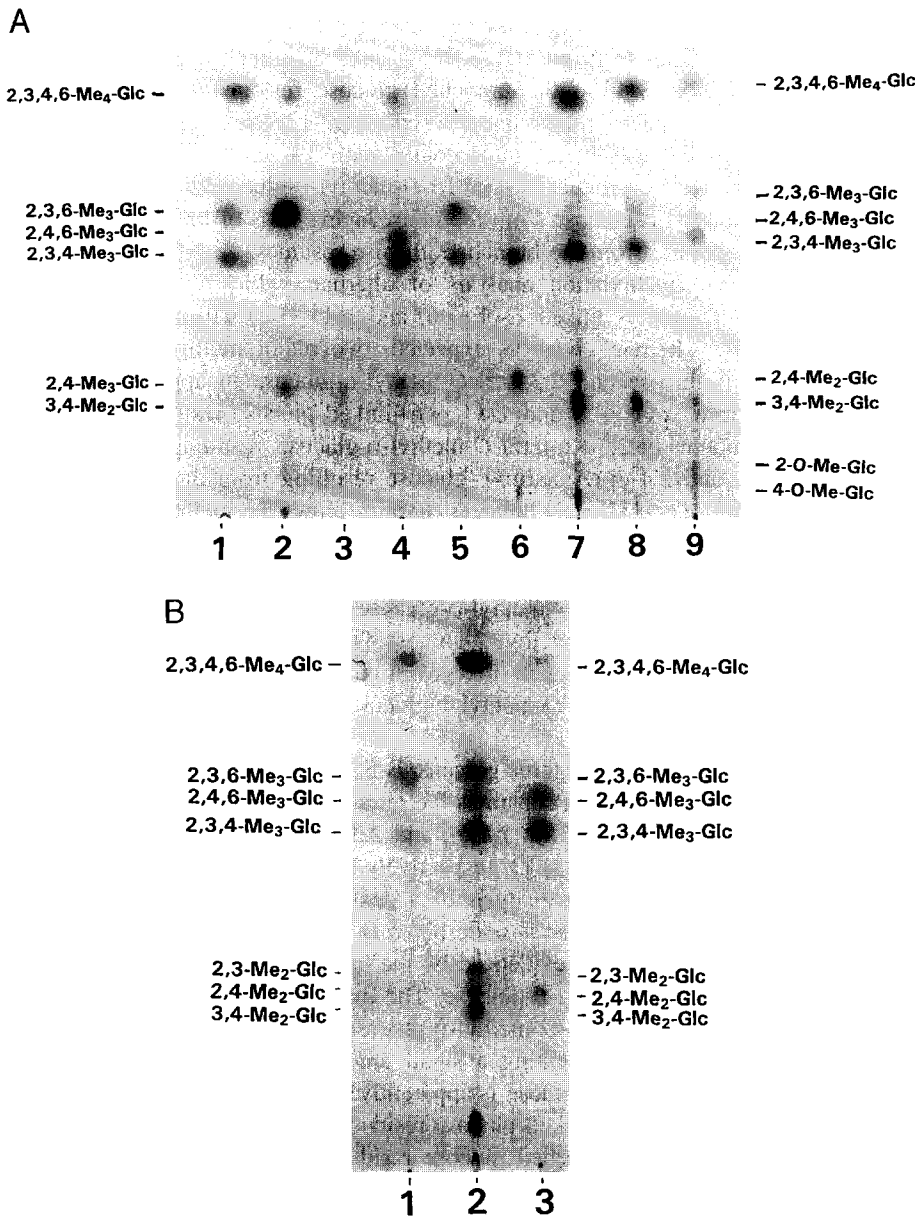

Fig. 2. **A**, TLC separation of *O*-methylated-D-glucoses obtained from the methylation analysis of various glucans. Lane 1, standards from top to bottom of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose; lane 2, *O*-methylated products from amylopectin; lane 3, *O*-methyl-D-glucoses from B-512F(M) dextran; lane 4, *O*-methyl-D-glucoses from alternan; lane 5, *O*-methyl-D-glucoses from pullulan; lane 6, *O*-methyl-D-glucoses from B-742CA dextran; lane 7, *O*-methyl-D-glucoses from B-743CB dextran; lane 8 *O*-methyl-D-glucoses from B-1299CA dextran. **B**, TLC analysis of a complex, highly branched glucan, showing the resolution of three isomeric tri-*O*-methyl-D-glucoses and three di-*O*-methyl-D-glucoses; lanes 1 and 3, standards; lane 2, *O*-methyl-D-glucoses from the methylation analysis of the complex glucan.

sults from the major glucose residue linked α-(1 → 4); smaller amounts of 2,3,4,6-tetra-*O*-methyl-D-glucose comes from the nonreducing ends of branched chains, and 2,3-di-*O*-methyl-D-glucose comes from the branched glucose residues. Lane 3 shows the methylation analysis of *Leuconostoc mesenteroides* B-512F dextran that is known to have 95% α-(1 → 6) linkages in the main chains with 5% α-(1 → 3) branch linkages [5,6]. The major methylated product is 2,3,4-tri-*O*-methyl-D-glucose with minor amounts of 2,3,4,6-tetra-*O*-methyl-D-glucose from the nonreducing ends of branched chains, and 2,4-di-*O*-methyl-D-glucose from the branched glucose residues.

Lane 4 shows the methylation analysis of alternan, which is a glucan having D-glucose residues alternately linked α-(1 → 6) and α-(1 → 3) with ~ 11% α-(1 → 3) branch linkages [7]. Alternan gives, as expected, two major methylated compounds, 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose in approximately equal amounts coming from α-(1 → 3) and α-(1 → 6)-linked glucose residues, respectively. There are lesser amounts of 2,3,4,6-tetra-*O*-methyl-D-glucose, resulting from the ends of the branch chains, and 2,4-di-*O*-methyl-D-glucose resulting from the glucose residues that contain the branched α-(1 → 3) linkages. Lane 5 shows the methylation analysis of pullulan, which is a poly(maltotriose) glucan with maltotriose units joined end-to-end by an α-(1 → 6) glycosidic linkage [8,9]. Pullulan, also as expected, gives two major methylated D-glucoses, 2,3,6-tri-*O*-methyl-D-glucose from the two α-(1 → 4) linked glucose residues and 2,3,4-tri-*O*-methyl-D-glucose from the α-(1 → 6) linked glucose residues. There are no apparent branch linkages in pullulan, and consequently there is a very small amount of 2,3,4,6-tetra-*O*-methyl-D-glucose from the nonreducing ends of the polysaccharide chains.

The methylation analysis of the three glucans, B-512F dextran, alternan, and pullulan, demonstrates that the TLC system separates the tetra-*O*-methyl-D-glucose and the three major tri-*O*-methyl-D-glucoses, 2,3,6-, 2,4,6-, and 2,3,4-tri-*O*-methyl-D-glucoses, which have R_f values of 0.52, 0.49, and 0.43, respectively.

Lane 6 shows the methylation analysis of a dextran synthesized by a constitutive dextransucrase elaborated by *Leuc. mesenteroides* B-742CA [11]. This glucan shows a very high degree of branching as evidenced by the large amount of 2,4-di-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose. The major linkage in the main chains is α-(1 → 6), which gives 2,3,4-tri-*O*-methyl-D-glucose with a very small amount of 2,3,6-tri-*O*-methyl-D-glucose, indicating a small amount of α-(1 → 3)-linked glucose residues in the main chains. This glucan is apparently the highly branched, regular comb dextran that has the α-(1 → 6) linked glucose residues in the main chain substituted at the 3-position with a single branched glucose residue [10]. There also appears to be some mono-*O*-methyl-D-glucose, indicating multiple branching on a single glucose residue, tentatively identified as substitution on positions 2 and 3 to give 4-mono-*O*-methyl-D-glucose. Lane 7 shows the methylation analysis of a dextran synthesized by a constitutive dextransucrase elaborated by *Leuc. mesenteroides* B-742CB [10]. This glucan also shows a high degree of branching, as evidenced by the large amounts of 3,4-di-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose, indicating that the major branch linkage in this dextran is (1 → 2), although there are some α-(1 → 4) branch linkages as evidenced by the formation of 2,3-di-*O*-methyl-D-glucose, and a smaller

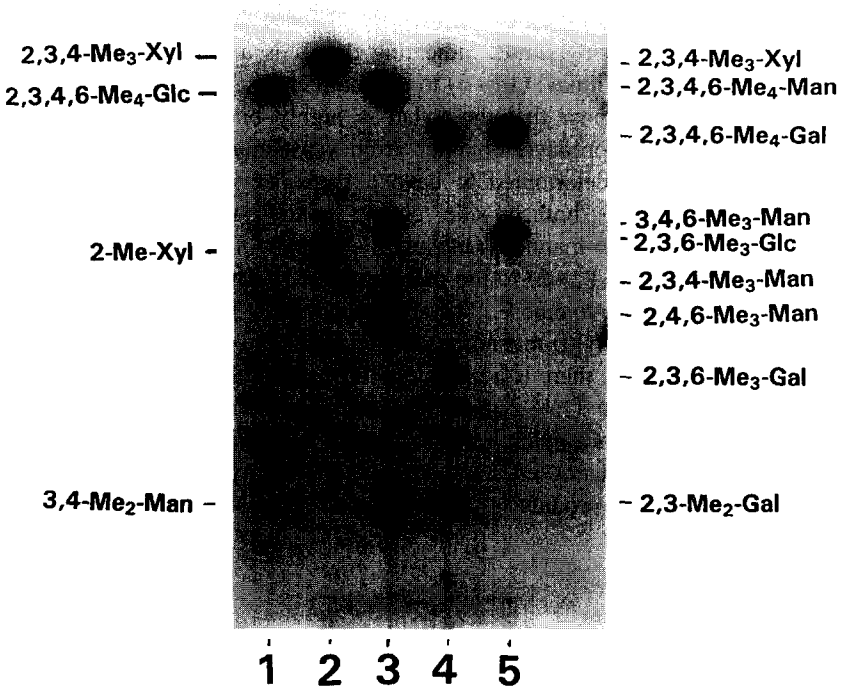

Fig. 3. TLC separation of *O*-methylated monosaccharides from various polysaccharides. Lane 1, 2,3,4,6-tetra-*O*-methyl-D-glucose standard; lane 2, *O*-methylated products from xylan; lane 3, *O*-methylated products from mannan; lane 4, *O*-methylated products from galactan; lane 5, *O*-methylated standards from lactose.

amount of α-(1 → 3) linkages in the main chains as evidenced by the formation of a small amount of 2,4,6-tri-*O*-methyl-D-glucose.

Lane 8 shows the methylation analysis of *Leuc. mesenteroides* B-1299CA, a mutant that is constitutive for the elaboration of dextransucrase [12]. The synthesized glucan has (1 → 3), (1 → 4), and (1 → 6) glycosidic linkages in the main chains, as shown by the formation of 2,4,6-tri-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,4-tri-*O*-methyl-D-glucose, respectively. The latter is the predominant tri-*O*-methyl-D-glucose residue, indicating that the (1 → 6) linkage is dominant. This glucan is also highly branched through a (1 → 2) linkage, as shown by the formation of 3,4-di-*O*-methyl-D-glucose from the branched glucose residue and 2,3,4,6-tetra-*O*-methyl-D-glucose from the nonreducing-ends of the branched chains. This dextran is known to contain a high percentage of α-(1 → 2) branch linkages [7,11]. The methylation analysis of a complex glucan is shown in lane 2 of Fig. 2B in which three isomeric tri-*O*-methyl-D-glucoses and three di-*O*-methyl-D-glucoses are shown. This glucan has (1 → 4), (1 → 3), and (1 → 6) glycosidic linkages in the main chains and three types of branch linkages, (1 → 6), (1 → 3), and (1 → 2).

Fig. 3 shows the methylation analysis of three polysaccharides, birch xylan, yeast mannan, and galactan. Lane 1 contains the 2,3,4,6-tetra-*O*-methyl-D-glucose standard. Lane 2 shows the methylated D-xyloses from birch xylan; the fastest migrating product

is 2,3,4-tri-*O*-methyl-D-xylopyranose with an R_f of 0.86; the other methylated product is 2-mono-*O*-methyl-D-xylopyranose, indicating that it is a (1 → 4) linked xylan with single xylopyranosyl units linked (1 → 3) to the main chain. The relative amounts of the two methylated xyloses show that the xylan is highly branched. Lane 3 shows the *O*-methylated D-mannose products obtained from yeast mannan. The structure of this polysaccharide has been determined to have a backbone chain of α-(1 → 6) linked D-mannose residues with branch side chains of α-D-mannopyranosyl-(1 → 2)-D-mannopyranose and α-D-mannopyranosyl-(1 → 3)-α-D-mannopyranosyl-(1 → 2)-D-mannose chains linked α-(1 → 2) to the main chain [13]. The fastest migrating product is 2,3,4,6-tetra-*O*-methyl-D-mannose. There are three tri-*O*-methyl-D-mannoses: 3,4,6-, 2,3,4- and 2,4,6-tri-*O*-methyl-D-mannoses, arising from (1 → 2), (1 → 6), and (1 → 3)-linked D-mannose residues; there is a relatively large amount of 3,4-di-*O*-methyl-D-mannose that comes from the (1 → 2)-branched mannose residue. Because of the high degree of branching there is a much lower amount of 2,3,4-tri-*O*-methyl-D-mannose than the other tri-*O*-methyl-D-mannoses. Lane 4 shows the methylated products from galactan. The fastest migrating product is 2,3,4,6-tetra-*O*-methyl-D-galactose with an R_f of

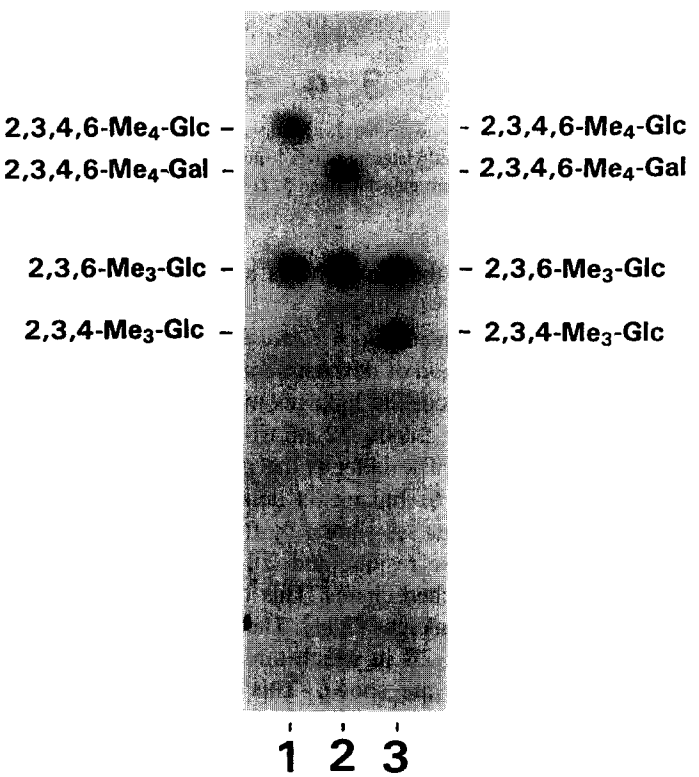

Fig. 4. TLC separation of the methylation analysis of 1 mg of maltose, lactose, and pullulan. Lane 1, *O*-methylated sugars from maltose; lane 2, *O*-methylated sugars from lactose; and lane 3, *O*-methylated sugars from pullulan.

Table 1
Quantitative determination of *O*-methylated sugars by TLC plate imaging densitometry

Maltose		Lactose		Pullulan	
2,3,4,6-Me_4-Glc	27.85	2,3,4,6-Me_4-Gal	18.23	2,3,6-Me_3-Glc	9.47 [a]
2,3,6-Me_3-Glc	21.35	2,3,6-Me_3-Glc	18.37	2,3,6-Me_3-Glc	4.74 [a]
exp. ratio [b]	1.30	exp. ratio [b]	0.99	exp. ratio [b]	1.99
theor. ratio [c]	1.00	theor. ratio [c]	1.00	theor. ratio [c]	2.00

[a] Density value obtained using a Bio-Rad Imaging Densitometer, model GS-670.
[b] Ratio of the experimental values of two *O*-methyled compounds.
[b] Theoretical ratio based on the structures of the saccharides.

0.77; there are two tri-*O*-methyl-D-galactoses, 2,3,6-tri-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-galactose, resulting from residues having (1 → 4) and (1 → 6) glycosidic linkages that are well resolved. There also is a significant amount of (1 → 6) branching, as shown by the presence of 2,3-di-*O*-methyl-D-galactose. Lane 5 contains the standards, 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-glucose obtained from methylated lactose.

Fig. 4 shows the TLC analysis of the methylation and hydrolysis of 1 mg of maltose, lactose, and pullulan. The quantitative determination by densitometry of the *O*-methylated sugars obtained from these saccharides is given in Table 1. The relative ratios of each of the methylated sugars were determined and compared with the expected theoretical ratios. The tri-*O*-methyl- and the di-*O*-methyl-D-glucoses were very close to the theoretical ratios. Tetra-*O*-methyl-D-glucose, however, was consistently high by a factor of 1.3. The densitometric measurement of this *O*-methylated sugar could be corrected by multiplying the densitometric value for tetra-*O*-methyl-D-glucose by 0.77.

4. Discussion

Methylation analysis of the linkages found in oligo- and poly-saccharides has been greatly simplified by eliminating the reduction and acetylation reactions that have traditionally followed the methylation and acid hydrolysis reactions. The analysis has been further simplified by separating the methylated monosaccharides by TLC. The method is a micro procedure, as the amounts of methylated monosaccharides that can be detected on the TLC plate are in the nanogram range. We show here the methylation analysis of 1 mg samples, but samples as small as 0.01 mg can be analyzed. The amount of sample added to the TLC plate should have a total of about 3,000 ng.

The most highly methylated monosaccharides, the tetra-*O*-methyl-D-glucose, -D-galactose, and -D-mannose and the tri-*O*-methyl-D-xylopyranose migrate in the upper third of the TLC plate. The intermediate methylated monosaccharides, the tri-*O*-methyl -D-glucoses, -D-galactoses, and -D-mannoses and the mono-*O*-methyl-D-xylopyranose migrate in the middle third of the TLC plate. In addition, the various isomeric tri-*O*-methyl monosaccharides can be resolved so that the various types of glycosidic linkages can be determined, such as the (1 → 4) linkage by 2,3,6-tri-*O*-methyl monosaccharide, the (1 → 3) linkage by 2,4,6-tri-*O*-methyl monosaccharide, and the (1 → 6)

linkage by 2,3,4-tri-*O*-methyl monosaccharide. The di- and mono-*O*-methyl monosaccharides, that arise from branched residues, migrate in the bottom third of the TLC plate and the various isomeric forms can also be resolved.

Commercial 2,3,4,6-tetra-*O*-methyl-D-glucose standard is contaminated with small but detectable amounts of 2,3,4- and 2,4,6-tri-*O*-methyl-D-glucose. Pure 2,3,4,6-tetra-*O*-methyl-D-glucose may be obtained by methylating and hydrolyzing α,α-trehalose. Other standards can be readily prepared by methylating and hydrolyzing cyclomaltooligosaccharides, which give exclusively 2,3,6-tri-*O*-methyl-D-glucose; by methylating and hydrolyzing B-512F dextran, which gives mainly 2,3,6-tri-*O*-methyl-D-glucose; by methylating and hydrolyzing alternan that predominantly gives 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose; and by methylating and hydrolyzing lactose, which gives 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-glucose.

Many carbohydrate structure determinations may be simply achieved by a qualitative examination of the type of *O*-methylated products formed. For example, the two trisaccharides, 6^2-α-D-glucopyranosylmaltose and 4^2-α-D-glucopyranosylisomaltose give the same *O*-methyl-D-glucoses. However, after reduction and methylation analysis, the trisaccharides can be distinguished in that the former gives 2,3,4-tri-*O*-methyl-D-glucose and the latter gives 2,3,6-tri-*O*-methyl-D-glucose. Another example is the differentiation of a branched cyclomaltooligosaccharide from a nonbranched cyclomaltooligosaccharide, containing the same number of monosaccharide residues. The former would give 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-glucose in addition to 2,3,6-tri-*O*-methyl-D-glucose, while the latter gives only 2,3,6-tri-*O*-methyl-D-glucose. The determination of carbohydrate structures may, thus, often be made by qualitative examination of the various types of *O*-methylated monosaccharides observed on the TLC plate. When necessary, the quantitative determination of the distribution of the *O*-methylated monosaccharides in the mixture can be obtained by using TLC imaging densitometry. The TLC analysis can be readily and inexpensively performed by any laboratory, without the necessity of performing several tedious reactions and without the need of dedicated, expensive, and sophisticated instrumentation.

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