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IDENTIFICATION OF FURANOSE AND PYRANOSE RING FORMS OF CARBOHYDRATES BY METHYLATION, GAS–LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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SUMMARY

New data on the furanose and pyranose ring transformations of glucose, arabinose and galactose in dimethyl sulfoxide solvent have been obtained by the combined technique of methylation, gas–liquid chromatography and mass spectrometry. The ratios of the furanose to pyranose ring forms in the monosaccharides were 1:1.7 for arabinose, 1:1.2 for galactose and 1:99 for glucose. The method has been used to detect furanose and pyranose ring forms in the reducing units of two disaccharides of galactose and arabinose. Thus, the furanose and pyranose ratios of the reducing units of α- and β-D-galactopyranosyl-(1,3)-D- or -L-arabinose are 2:1 for the α-linked and 1:4 for the β-linked disaccharide. The acyclic form of arabinose could also be detected by the analytical technique. The combined methylation, gas–liquid chromatography and mass spectrometry procedure should be useful for determining the ring forms of the reducing units of other oligosaccharides and for identifying acyclic forms of reducing sugars.

INTRODUCTION

Originally, furanose and pyranose ring forms of monosaccharides were identified by methylation[1] and specific chemical degradation of the methyl derivatives[2] while transformations of ring forms were investigated by optical rotation measurements[3]. More recent methods for identifying furanose and pyranose ring forms of monosaccharides are ^{1}H NMR spectroscopy[4,5], ^{13}C NMR spectroscopy[6,7], gas–liquid chromatography (GLC) of trimethylsilyl derivatives[8,9] and polarimetry coupled with GLC[10]. These methods have been discussed admirably in a recent review[11].

In the present communication we are reporting new results obtained by a combined method of analysis based on methylation, GLC and mass spectrometry (MS) for the identification and the quantitation of the ring forms of glucose, galactose, arabinose and the reducing units of α- and β-D-galactopyranosyl-(1,3)-D- or L-arabinose. Up to the present, the identification of ring forms of the reducing units of oligosaccharides has been difficult or impossible to achieve. The analysis for ring

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forms has been performed in a basic solvent which is known to favor furanose ring formation[12] and, accordingly, kinetic measurements were not attempted.

In brief, the combined method consists of solution of the carbohydrate in dimethyl sulfoxide, generation of alkoxide ions with dimethylsulfinyl sodium and methylation with methyl iodide. The methylated product was purified by chromatography if necessary, hydrolyzed in acid, and then reduced, acetylated and subjected to GLC and MS. The partially methylated alditol acetates were identified by the retention times on GLC and by the type of *m/e* fragments produced on MS.

EXPERIMENTAL

Monosaccharides and derivatives

D-Arabinose was purified by recrystallization from methanol–isopropanol (7:3). D-Galactose was purified by recrystallization from ethanol after incubation of a solution of the galactose with glucose oxidase for 30 min and removal of the impurities in the crystallization solvent. L-Arabinose was purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.) and was not purified further. L-Galactose was provided by Dr. H. S. Isbell (Catholic University, Washington, DC, U.S.A.) and L-glucose was purchased from Pfanstiehl Lab. (Waukegan, IL, U.S.A.). Ethyl β-D-galactofuranoside was provided by Dr. A. S. Perlin (Chemistry Department, McGill University, Montreal, Canada). Methyl β-D-arabinoside, methyl β-D-galactoside and gum arabic were purchased from Sigma (St. Louis, MO, U.S.A.).

The dithioacetal derivatives of D-arabinose and D-galactose which were needed as reference compounds were prepared by a method used in earlier studies from this laboratory[13]. The derivatives were prepared by dissolving 0.02 g of the carbohydrate in 0.4 ml of concentrated hydrochloric acid and adding 0.25 ml of ethyl mercaptan. The reaction mixture was maintained at room temperature for 15 min in which time crystalline derivatives were formed. The derivatives were separated from the reaction mixture by centrifugation and recrystallized from hot water to constant melting points: m.p. of the dithioacetal of arabinose, 118°C, and m.p. of the dithioacetal of galactose, 139°C.

Oligosaccharides

The disaccharides used in this study were prepared by an enzymatic transfer reaction with the appropriate substrate and cosubstrate or by partial acid hydrolysis of a polysaccharide of known structure[14,15]. The β-D-galactopyranosyl-(1,3)-D-arabinose was prepared by the enzymatic method utilizing yeast galactosyl transferase and *o*-nitrophenyl β-D-galactoside as the substrate and arabinose as the cosubstrate. The procedure for the enzymatic synthesis of the oligosaccharide, the isolation of the compound by column paper chromatography and evidence for the structure have been presented earlier[14]

The α-D-galactopyranosyl-(1,3)-L-arabinose was isolated from a partial acid hydrolyzate of gum arabic[15]. The hydrolysis was performed in 0.02 *M* hydrochloric acid for 15 min at 100°C. Under these conditions the hydrolysis of furanosyl linkages between the galactosyl–arabinofuranosyl moieties and the main chain of the gum arabic was essentially complete while the hydrolysis of other types of linkages in the gum was minimal. The hydrolyzate of gum arabic prepared as described above con-

tained the disaccharide of galactose and arabinose, some arabinose and high-molecular-weight products. The hydrolyzate was neutralized with solid sodium carbonate and concentrated to a small volume. Samples of 0.2 ml of the concentrate were placed on paper for preparative paper chromatography and the disaccharide was separated by three ascents of *n*-butanol–pyridine–water (6:4:3)[16]. The position of the oligosaccharide on the paper was located with marker strips and the compound was extracted with water. The oligosaccharide isolated by this procedure was homogenous on paper chromatography and on acid hydrolysis was converted to arabinose and galactose. The compound was also converted to galactose and arabinose by α-galactosidase but not by β-galactosidase. The above results and the data of methylation analysis described in a later section establish the structure for the new disaccharide to be α-D-galactopyranosyl-(1,3)-L-arabinose. The sample of disaccharide was dried by lyophilization and used for subsequent experiments.

Methylation

Methylation of the monosaccharides and disaccharides was effected by the Hakomori method[17] and analysis was performed by the Lindberg procedure[18]. The modifications utilized in our laboratory have been described in an earlier publication[19]. In this method, samples of 1 to 5 mg of the monosaccharides or disaccharides were placed under nitrogen and then thoroughly dried under vacuum. The dried sample was dissolved in 1 ml of dimethyl sulfoxide and subjected to sonication for 15 to 20 min to aid in the solution of the sample. The solution was mixed with 0.2 ml of dimethylsulfinyl carbanion and maintained at room temperature for 6 h in which time formation of alkoxyl ions from the hydroxyl groups was complete. Next, 0.3 ml of dry methyl iodide was added and the reaction was allowed to proceed for 6 h at room temperature. The methylated product was recovered by extraction with chloroform, purified by chromatography on Sephadex LH-20 if needed, hydrolyzed in formic acid and sulfuric acid, reduced with sodium borohydride and acetylated with acetic anhydride in pyridine. The final product was dissolved in chloroform and subjected to GLC in a Varian Aerograph series 1400 chromatograph equipped with a stainless-steel 6 ft. × 1/8 in. column containing 3% OV-225 on 80–100 mesh Supelcoport. GLC was performed at 190°C and the effluents were monitored with a flame ionization detector.

Mass spectrometry

The components from the gas chromatograph were subjected to fragmentation and analysis in the mass spectrometer. A DuPont 21-490 spectrometer attached to the gas chromatograph was used. Fragmentation was effected at source temperature of 250°C and an ionization potential of 70 eV. The mass fragments and mass marker tracing were recorded on a Bell-Howell oscillograph. The spectra of reference carbohydrate derivatives were also obtained and used for identifying the carbohydrate derivatives. The preparation of reference derivatives is described in the preceding sections.

RESULTS AND DISCUSSION

The methylated derivatives of arabinose, galactose and glucose were prepared

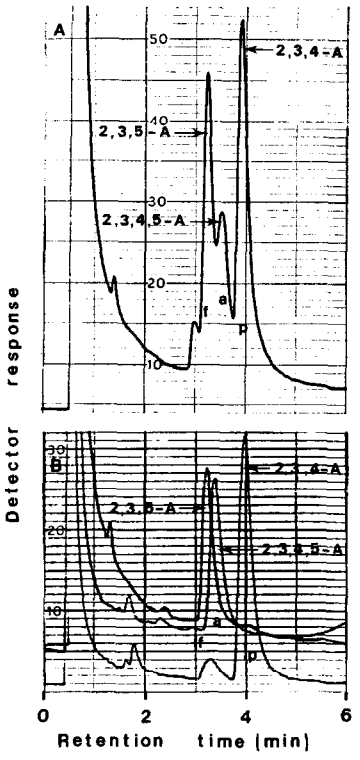

Fig. 1. GLC patterns of the methylated alditol acetates of arabinose (A) and of reference standards (B). 2,3,5-A = 1,4-Di-O-acetyl-2,3,5-tri-O-methyl arabinitol; 2,3,4,5-A = 1-O-acetyl-2,3,4,5-tetra-O-methyl arabinitol; 2,3,4-A = 1,5-di-O-acetyl-2,3,4-tri-O-methyl arabinitol; f and p = derivatives from furanose and pyranose ring forms, respectively; a = acyclic form.

TABLE I

RELATIVE RETENTION TIMES (RRT) ON OV-225 AT 190°C RELATIVE TO 1,5-DI-O-ACETYL-2,3,4,6-TETRA-O-METHYL GLUCITOL AND CHARACTERISTIC ION FRAGMENTS IN THE MASS SPECTRA

Position of OCH_3	*RRT*	*m/e*							
		89	*101*	*117*	*129*	*141*	*145*	*161*	*205*
Arabinose									
2,4	1.07	–	30	100	–	–	40	20	10
2,5	0.89	–	40	100	30	–	–	–	–
2,3,4	0.61	–	70	100	20	–	–	30	–
2,3,5	0.59	–	60	100	40	–	–	30	–
2,3,4,5	0.55	–	60	100	–	40	–	–	–
Galactose									
2,3,4,6	1.19	–	100	80	60	–	40	60	30
2,3,5,6	1.07	80	100	90	10	–	–	–	20
Glucose									
2,3,4,6	1.00	–	100	60	60	–	40	70	30

and analyzed for furanose and pyranose ring forms by GLC and MS. The GLC patterns for the derivatives of arabinose and reference standards are reproduced in Fig. 1. Fig. 1A shows the derivatives from arabinose to be 2,3,5-tri-O-methyl arabinose, 2,3,4,5-tetra-O-methyl arabinose and 2,3,4-tri-O-methyl arabinose identified as the methylated arabinitol acetates. Reference methylated derivatives of arabinose were prepared from gum arabic, dithioacetal arabinose and methyl-β-D-arabinopyranoside. The GLC patterns for the reference derivatives are shown in Fig. 1B. For ease of comparison, the patterns for the reference derivatives have been combined. The GLC relative retention times and the MS *m/e* fragments of the derivatives are given in Table I. The numbers represent relative abundance with the most abundant fragment assigned a value of 100. The values in the table are in agreement with literature values[20,21].

The methylation analysis has been conducted in a dimethyl sulfoxide solvent, and the results have been used to calculate the relative amounts of the various forms of arabinose in this solvent. Thus the furanose, the pyranose and the acyclic forms comprise 30%, 52% and 18%, respectively, of the arabinose in solution. The method can be used therefore to detect acyclic forms of monosaccharides which are difficult

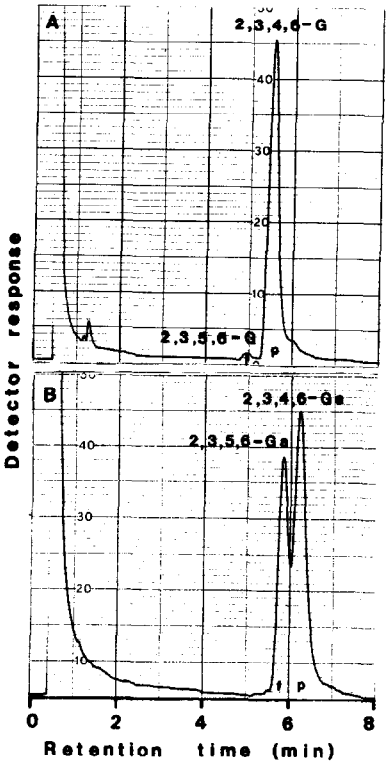

Fig. 2. GLC patterns of the methylated alditol acetates of glucose (A) and galactose (B). 2,3,5,6-G peak = probably 1,4-Di-O-acetyl-2,3,5,6-tetra-O-methyl glucitol; 2,3,4,6-G = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl glucitol; 2,3,5,6-Ga = 1,4-di-O-acetyl-2,3,5,6-tetra-O-methyl galactitol; 2,3,4,6-Ga = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl galactitol; f and p = derivatives from furanose and pyranose ring forms, respectively.

to detect by NMR spectroscopy or other physical methods[11]. The acyclic forms of monosaccharides have been considered to be intermediates in the α and β anomeric interconversions of monosaccharides[3] and the experimental verification of the presence of the acyclic form of a monosaccharide in solution has been achieved.

In a comparable series of experiments with glucose and galactose, the methylated derivatives of glucose are shown by the GLC pattern in Fig. 2A and of galactose the pattern is shown in Fig. 2B. It is noted in Fig. 2A that one major derivative, 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl glucitol, and one minor derivative representing less than 1% of the compound were obtained from glucose. The latter derivative may be 1,4-di-O-acetyl-2,3,5,6-tetra-O-methyl glucitol and would arise from the furanose form of glucose. The identity of this derivative has not yet been established. In Fig. 2B it can be seen that the galactose derivatives were 2,3,5,6-tetra-O-methyl galactose and 2,3,4,6-tetra-O-methyl galactose identified as the methylated galactitol acetates. Methyl β-D-galactopyranoside and ethyl β-D-galactofuranoside were used for preparing standard methylated derivatives of galactose, and these yielded analytical values identical to those for the derivatives shown in Fig. 2B. By integration of the areas under the peaks in the pattern of Fig. 2B it was calculated that approx-

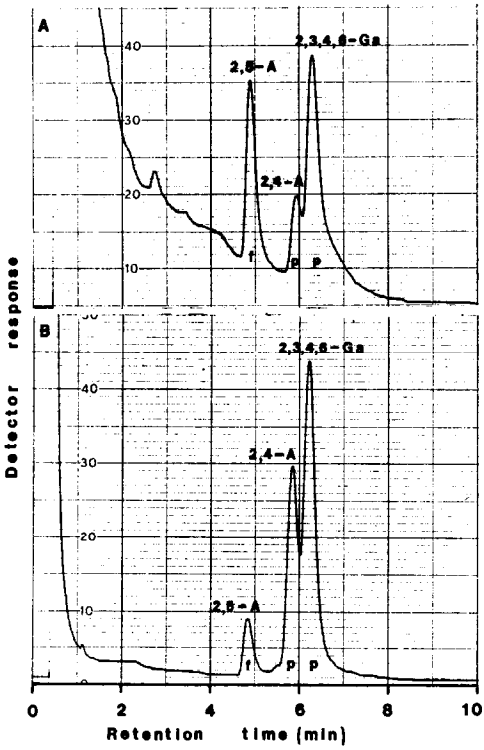

Fig. 3. GLC patterns of partially methylated alditol acetates of α-D-galactopyranosyl-(1,3)-L-arabinose (A), and β-D-galactopyranosyl-(1,3)-D-arabinose (B). 2,5-A = 1,3,4-Tri-O-acetyl-2,5-di-O-methyl arabinitol; 2,4-A = 1,3,5-tri-O-acetyl-2,4-di-O-methyl arabinitol; 2,3,4,6-Ga = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl galactitol; f and p = derivatives from furanose and pyranose ring forms, respectively.

imately one-half of galactose occurs in the furanose form and one-half in the pyranose form.

It should be pointed out that the corresponding L forms of glucose, galactose and arabinose all yielded values for the furanose and pyranose ring forms which were comparable to the values for the D series. Evidently the same steric factors are influencing the anomeric transformations and ring formations of the D and L series of compounds.

The disaccharides of galactose and arabinose were subjected to methylation analysis to determine if this method of analysis is applicable to the identification of the furanose and pyranose ring forms in the reducing units of oligosaccharides. The GLC patterns for the analysis of the methylated products from these disaccharides are shown in Fig. 3. It will be noted in the figure that both disaccharides yield two dimethyl derivatives of arabinose, the 2,3-dimethyl and the 2,4-dimethyl and one derivative of galactose, the 2,3,4,6-tetramethyl. Thus the arabinose units of the α- and β-linked disaccharides exist in the furanose and pyranose ring forms. It will be noted that the amounts of the two arabinose derivatives obtained from the two disaccharides differed markedly. The disaccharide which contains the α-glycosidic linkage yielded a ratio of 2:1 for the furanose and pyranose isomers while the disaccharide with a β-glycosidic linkage yielded derivatives in a ratio of 1:4.

The percentages of the furanose and pyranose isomers in the carbohydrates analyzed by methylation, GLC and MS have been calculated from the analytical data and these values are recorded in Table II. For comparison purposes literature data

TABLE II

PERCENT COMPOSITION AND RATIOS OF FURANOSE AND PYRANOSE RING FORMS IN MONOSACCHARIDES

Ara = Arabinose; Gal = galactose; Glc = glucose; DMSO = dimethyl sulfoxide; f = furanose; p = pyranose.

Compound	*Solvent*	*Method*	*f (%)*	*p (%)*	*Ratio f/p*	*Ref.*
Ara*	DMSO	Methylation	30*	52*	0.58	This study
	DMSO	NMR	33	67	0.49	22
	^2H_2O	NMR	4	96	0.04	5
Gal	DMSO	Methylation	45	55	0.85	This study
	DMSO	NMR	15	85	0.18	22
	Pyridine	Silylation	37	63	0.58	24
	Pyridine	Silylation	24	76	0.32	8
	^2H_2O	NMR	10	90	0.11	5
	H_2O	Silylation	5	95	0.05	8
	H_2O	Silylation	4	96	0.04	9
	H_2O	Rotation	4	96	0.04	10
	H_2O	NMR	1	99	0.01	5
Glc	DMSO	Methylation	1	99	0.01	This study
Gal(α-1,3)-Ara	DMSO	Methylation	65	35	1.90	This study
Gal(β-1,3)-Ara	DMSO	Methylation	20	80	0.25	This study

* Approximately 18% of Ara is present in the acyclic form in DMSO and none in ^2H_2O.

have been included in the table. The values in the table show that the carbohydrates which undergo ring transformations readily are present in amounts of 15% to 45% in the furanose ring form in basic solvents and only 1% to 10% in non-basic solvents such as H_2O or ^2H_2O. It should be noted in Table II that the reducing units of two disaccharides of galactose and arabinose exist in a high proportion in the furanose ring form and that the configuration of the glycosidic linkage in the disaccharide has a pronounced effect on the furanose and pyranose ratio. The latter observation emphasizes the need for special analytical techniques for the structural characterization of oligosaccharides in which the reducing units occur in the furanose and pyranose ring forms.

The differences in the furanose–pyranose ratios that have been observed with the monosaccharides analyzed to date can be explained on the basis of the combined contributions from conformation, configuration and solvent stabilization. The orientation of hydroxyl groups of monosaccharides in the pyranose ring in water solution is such that hydrogen bonding can occur readily between the hydroxyl groups of this ring and the structured water molecules attracted to the ring[22,23]. As a result, a lesser degree of disorientation of the solvent molecules occurs and at the same time a greater stability is imparted to the pyranose ring. Accordingly, in non-basic solvents monosaccharides tend to assume the pyranose ring structure and the furanose ring isomer is present in low concentrations. However in basic solvents, the interactions of the solvent with the two ring forms are equivalent and under these conditions the concentration of the furanose ring form of the monosaccharide does increase.

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