Monosaccharide permethylation products for gas chromatography – mass spectrometry: how reaction conditions can influence isomeric ratios

Daniel Derbie Asres and Hélène Perreault

Abstract: Methylation analysis has been widely used for determination of carbohydrate structures by mass spectrometry. Permethylation of monosaccharides yields mixtures of anomeric pyranosides and furanosides. This paper discusses the influence of some of the permethylation reaction parameters on the proportions of isomeric products obtained. The ratios of three five- and six-membered ring products obtained from two permethylated monosaccharides, D-galactose and L-fucose, have been determined as a function of reaction parameters. The method of Ciucanu and Kerek (1) (methyl iodide in dimethyl sulfoxide (DMSO) in the presence of sodium hydroxide (NaOH)) was used as a starting point. The "conventional" method consists of mixing all of the reagents with the substrate and allowing the reaction to proceed with stirring. Both D-galactose and L-fucose under these conditions produced two main permethylated isomers, a furanoside and a pyranoside, along with two other minor isomeric components. We have investigated the effect on the proportion of products obtained of mixing DMSO, substrate, and NaOH for various times prior to the addition of methyl iodide. Results for D-galactose showed that shorter times enhanced the formation of permethylated furanoside isomers, while reducing the proportion of pyranosides. In other sets of experiments, the time and temperature of reaction, following the addition of methyl iodide, were studied. The indication is that 15 min are sufficient to produce complete methylation, with longer reaction times yielding the same results. Again for D-galactose, low reaction temperatures (ca. 10°C) favored formation of furanoside products. Higher temperatures yielded higher pyranoside/furanoside ratios. Higher quantities of NaOH also favored formation of the main galactopyranoside product. As for L-fucose, the ratio of the main furanoside vs. pyranoside products obtained by permethylation varied in a way similar to permethylated galactoside. Thus, higher temperatures and longer reaction times favored the main fucopyranoside product. Gentler conditions (i.e., shorter reaction times and lower temperatures) significantly favored the formation of the main fucofuranoside product. These results are interesting as they show the possibility of controlling the relative abundance of permethylated isomers of fucose and galactose. They also constitute a warning to chemists who use methylation procedures in their analyses, to the effect that permethylation products may vary considerably if the reaction conditions are not carefully controlled.

Key words: glucose, galactose, fucose, TLC, GC–MS, permethylation, monosaccharides.

Résumé : On a fait appel à la méthode fréquemment utilisée d'analyse par méthylation pour déterminer les structures de carbohydrates par spectrométrie de masse. La perméthylation des monosaccharides fournit des mélanges de pyranosides et de furanosides anomères. Dans ce travail, on discute de l'influence de quelques paramètres de la réaction de perméthylation sur les proportions des produits isomères obtenus. On a déterminé les rapports des trois produits à cinq et à six chaînons obtenus à partir de deux monosaccharides perméthylés, D-galactose et L-fucose, en fonction des paramètres de réaction. Comme point de départ, on a utilisé la méthode de Ciucanu et Kerek (iodure de méthyle dans le diméthylsulfoxyde (DMSO) en présence d'hydroxyde de sodium (NaOH)). La méthode «conventionnelle» consiste à mélanger tous les réactifs avec le substrat et à laisser la réaction se produire sous agitation. Dans ces conditions, le D-galactose et le L-fucose conduisent tous les deux à deux isomères perméthylés principaux, un furanoside et un pyranoside, avec deux autres composants isomériques mineurs. On a examiné l'influence que peut avoir le mélange du DMSO, du substrat et du NaOH, pour des temps variables avant l'addition de l'iodure de méthyle, sur la proportion des produits obtenus. Les résultats avec le D-galactose ont montré que les temps les plus courts augmentent la formation des isomères furanosides perméthylés avec une réduction concomitante de la proportion des pyranosides. Dans d'autres ensembles d'expériences, on a étudié l'influence du temps et de la température de la réaction après l'addition de l'iodure de méthyle. Les résultats indiquent que 15 min sont suffisantes pour conduire à une méthylation complète; des temps de réaction plus longs conduisent aux même résultats. Encore une fois, avec le D-galactose, les températures de réaction plus basses (environ 10°C) favorisent la formation de produits furanosides. Les températures plus élevées conduisent

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D.D. Asres and H. Perreault.[1] Department of Chemistry, University of Manitoba, 144 Dysart Road, Winnipeg, MB R3T 2N2, Canada.

[1] Author to whom correspondence may be addressed. Telephone: (204) 474-7418. Fax: (204) 474-7608. E-mail: perreau@cc.umanitoba.ca

à des rapports pyranoside/furanoside plus élevés. Des quantités plus élevées de NaOH favorisent aussi la formation de galactopyranoside comme produit principal. Dans le cas du L-fucose, le rapport du furanoside principal et des produits pyranosides obtenus par perméthylation varient d'une façon semblable à celle observée avec les galactosides perméthylés. Ainsi, aux températures les plus élevées et aux temps de réaction plus longs, les produits fucopyranosides principaux sont favorisés. Des conditions plus douces (c'est-à-dire des temps de réaction plus courts et des températures plus basses) favorisent fortement la formation du fucofuranoside comme produit principal. Ces résultats sont intéressants parce qu'ils démontrent la possibilité de contrôler les quantités relatives d'isomères perméthylés du fucose et du galactose. Ils constituent aussi un avertissement pour les chimistes qui utilisent les méthodes de méthylation dans leurs analyses; en effet, la nature des produits de perméthylation peut varier d'une façon significative si les conditions réactionnelles ne sont pas bien contrôlées.

Mots clés : glucose, galactose, fucose, CCM, CG–SM, perméthylation, monosaccharides.

[Traduit par la rédaction]

Introduction

The analysis of carbohydrates by mass spectrometric techniques often requires derivatization of the samples in order to enhance sensitivity and improve the quantity and quality of information obtained. The Ciucanu and Kerek (1) permethylation method figures amongst the most popular derivatization methods and has been used in many laboratories for the analysis of carbohydrates and glycolipids. This method yields fully methylated sugars in remarkably short reaction times, on the order of 15 min, based on optimal proportions of powdered sodium hydroxide (NaOH), dimethyl sulfoxide (DMSO), methyl iodide (CH_3I), and substrate.

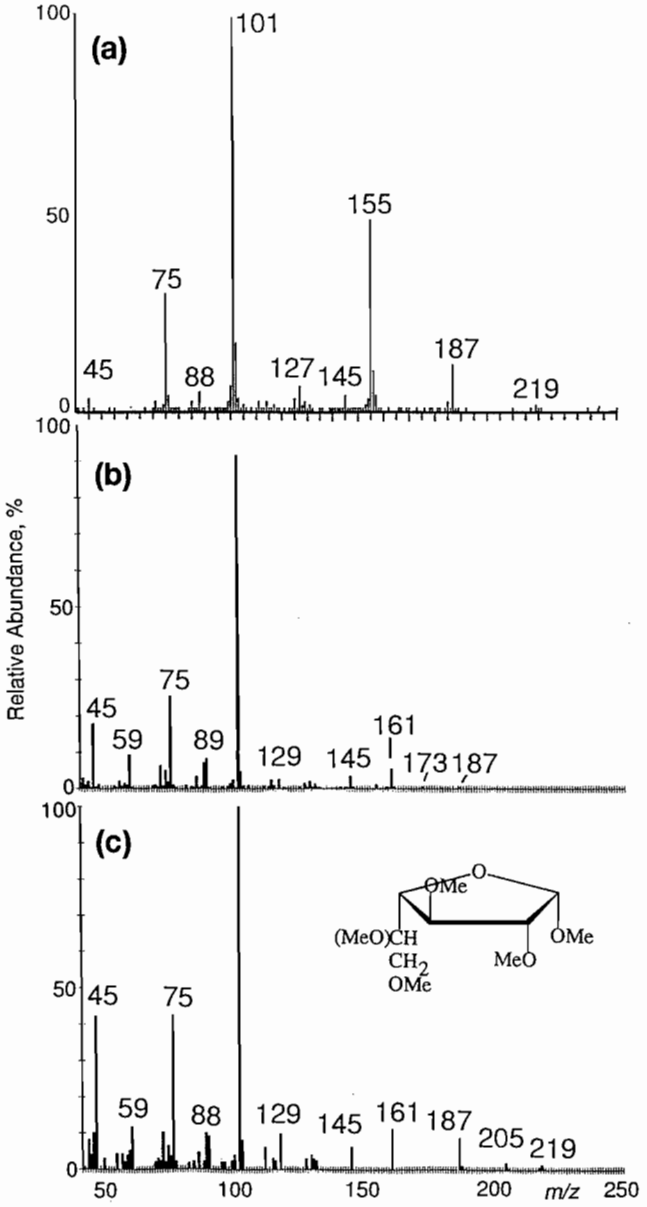
In a recent publication (2), we discussed the gas chromatographic – mass spectrometric (GC–MS) data for permethylated monosaccharides and observed the presence of two or more isomeric products. Similar results had been reported earlier (3, 4). We observed that permethylated D-glucose, D-galactose, and L-fucose each yielded two major GC–MS peaks. In the case of D-glucose, the two peaks corresponded to the α and β anomers of the permethylated glucopyranoside species, as reported by Ciucanu and Kerek (1). In the cases of D-galactose and L-fucose, the two peaks had been tentatively assigned to pyranoside and furanoside, or open-ring structures (2), although the products were not fully characterized. Pyranoside-type products yielded electron impact (EI) mass spectra that corresponded quite well to the spectra found in the NIST library (5), while the other type of products (furanoside or open ring) could not be readily identified in the collection of reference spectra, including those of furanosides.

We have recently compared a collection of mass spectra of permethylated monosaccharides obtained using our Finnigan MAT 800 ion trap GC–MS system to those from our HP 5988A GC–MS system. We observed from this comparison that the Finnigan MAT system in general produced less fragmentation than the HP instrument, although similar ionization conditions (EI, 70 eV) were used. The nature of the fragment ions was also different from one instrument to the other. These differences agree well with the fact that some EI-formed ions can undergo chemical ionization or cooling while in the trap, thus producing different extents and patterns of fragmentation relative to EI-formed ions travelling through a magnetic sector or quadrupole mass analyzer. The HP spectra matched the NIST library spectra (5) much better and more reliably than the Finnigan MAT spectra, which were discussed in our previous publication (2). In general, we found that the EI spectra obtained with the ion trap system were not always reliable for identification of unknowns, or for diverse classes of compounds. From the HP data obtained with a quadrupole analyzer, we have reassigned the permethylation products of D-galactose and L-fucose as pyranosides and furanosides, eliminating the possibility of open-ring structures. As an example, Fig. 1 shows comparative spectra for a furanoside product from permethylation of D-galactose, where (*a*) is the Finnigan MAT-acquired spectrum, (*b*) is the HP-acquired spectrum, and (*c*) shows the library spectrum that best matched the HP spectrum. Library data (5) allowed for preliminary assignments of permethylation products. Accordingly, the compound of interest in Fig. 1 was identified as methyl 2,3,5,6-tetra-*O*-methyl-α-D-galactofuranoside (compound **3**). The other permethylation products of D-galactose were identified as methyl 2,3,5,6-tetra-*O*-methyl-β-D-galactofuranoside (compound **1**) and methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside (compound **2**). The retention times of these compounds using the Finnigan MAT system were on average 20:42, 20:50, and 21:00 min for **1**, **2**, and **3**, respectively. These identity assignments were confirmed with the use of the corresponding permethylated galactoside standards. Perfect matches between retention times and mass spectra were obtained between standards and galactose permethylation products.

The present paper discusses the influence of some of the permethylation reaction parameters on the proportions of compounds **1**, **2**, and **3** found. The overall quantity of compound **1**, a minor product, did not vary significantly with any of the parameters. The other expected product, methyl 2,3,4,6-tetra-*O*-methyl-α-D-galactopyranoside, was found only in relative amounts lower than 1% and eluting simultaneously with compound **2**.

In the case of L-fucose (6-deoxy-L-galactose), no reference mass spectra were available for preliminary identification of permethylated fucofuranoside anomers. In our earlier publication (2), we showed that permethylation of fucose produced two major products, and according to the features of their mass spectra, they should correspond to fucopyranose (compounds **4** and **5**, retention times 19:15 and 19:20) and fucofuranose structures (compound **6**, 19:30 min). In this paper, the proportions of these three compounds (**4**, **5**, and **6**) were determined as a function of reaction parameters. Further GC–MS experiments were performed with permethylated fucoside standards in order to confirm the identity of the products. Compounds **4**, **5**, and **6** were identified as methyl 2,3,4-tri-

Fig. 1. Mass spectra of product **3**, tentatively identified as permethylated α-D-galactofuranoside; (*a*) obtained using the Finnigan MAT ion trap system; (*b*) obtained with the Hewlett Packard system; and (*c*) from the spectrum library (5).



O-methyl β-L-6-deoxygalactopyranoside, methyl 2,3,4-tri-*O*-methyl α-L-6-deoxygalactopyranoside, and methyl 2,3,5-tri-*O*-methyl α-L-6-deoxygalactofuranoside, respectively. A fourth compound, methyl 2,3,5-tri-*O*-methyl β-L-6-deoxygalactofuranoside, was not taken into account as its relative abundance never exceeded 3%. This compound eluted earlier than the other three.

Permethylation of the monosaccharides D-galactose and L-fucose was carried out using the Ciucanu and Kerek (1) method, and the effects of varying the following parameters were investigated: time of mixing (of DMSO, NaOH, and monosaccharide) prior to addition of CH_3I, time of reaction in the presence of CH_3I, temperature of reaction, quantity of NaOH, and quantity of DMSO and CH_3I.

When saccharides are dissolved in solution there is a slow equilibration between anomeric forms as well as an equilibrium between pyrano- (six-membered ring) and furano- (five-membered ring) forms. Our results suggest that the nature of permethylation products depends on the proportions of the four possible species (or their anions) in solution at the time the CH_3I is added. These proportions are dependent on the length of time the solution is stirred before CH_3I is added, on the alkalinity of the solution, and on the temperature of the solution.

Experimental

Chemicals

Monosaccharide standards D-galactose (99.9%) and L-fucose (99%) were purchased from Sigma Chemicals (St. Louis, Mo.) and used without further purification. Dimethyl sulfoxide (DMSO) was obtained from Pierce Chemicals (Rockford, Ill.) and methyl iodide from Fluka (Buchs, Switzerland). All solvents (acetonitrile, methanol, dichloromethane, and chloroform) were glass-distilled and obtained from Mallinckrodt (Paris, Ky.). Distilled, deionized nanopure water was utilized.

Standards

Methyl D-galactopyranoside (α and β) and methyl L-fucopyranoside (α and β) were obtained from Sigma. Methyl D-galactofuranoside (α and β) and methyl L-fucofuranoside (β only) were purchased from Color Your Enzyme (Bath, Ont.). All of these standards were permethylated using the Ciucano and Kerek (1) method as outlined below. No anomerization and epimerization were observed according to GC–MS analyses, as a single sharp peak was obtained for each permethylated standard.

Permethylation

This derivatization was conducted using the method of Larsson et al. (6), which itself was adapted from Ciucanu and Kerek's method (1). Briefly, galactose or fucose (ca. 5 mg) was dissolved in (450–1000) μL of DMSO, followed by addition of (32–82) mg of powdered NaOH. The mixture was sonicated at room temperature (0–60 min). Methyl iodide (100–200 uL) was then added and the mixture was sonicated for (15–180) min at 10–50°C. The underlined numbers represent parameters used in our typical permethylation procedure, at room temperature. The extent of variation of each parameter is indicated by the two hyphenated numbers. Permethylated monosaccharides were recovered by chloroform or dichloromethane extraction.

Gas chromatography – mass spectrometry

The main GC–MS system used in this study consisted of a Varian 3400 gas chromatograph (Varian Chromatographic Systems, Walnut Creek, Calif.) coupled to a Finnigan Mat 800 ion trap detector (Finnigan Corp., San Jose, Calif.) equipped with an ITD 4.10 applications data system. The second system used was a HP 5890 gas chromatograph (Hewlett Packard Canada, Calgary, Alta.) coupled to a HP 5988A single quadrupole mass analyzer. For both GC–MS instruments, repetitive 1 s scans were effected on a range of 50–650 Da. Each gas chromatograph was equipped with a 30 m × 0.25 mm i.d. DB-5 column (J & W Scientific Inc., Folsom, Calif.), with a

Table 1. Experimental conditions for permethylation of D-(+) galactose, based on the method of Ciucanu and Kerek (1); relative proportions[a] of the products.

Method no.	Galactose (mg)	DMSO (μL)	NaOH (mg)	1st sonic. (min)	CH_3I (μL)	React. time (min)	Temp. (°C)	Prod. **1**[b] (rel. conc., %)	Prod. **2**[c] (rel. conc., %)	Prod. **3**[d] (rel. conc., %)
1	4.7	450	35.7	60	100	180	20–50	12	67	22
2	4.9	450	35.6	45	100	85	20–50	7	61	32
3	4.7	450	35.5	20	100	85	20–50	7	45	48
4	5	500	31.8	0	100	85	20–50	9	20	71
5	5	500	31.8	0	100	15	20–25	10	23	68
6	5.3	500	82.4	0	100	15	20–25	5	32	63
7	8.2	1000	81.9	0	100	10	10–14	5	14	81

[a]Relative proportions: ±2%.
[b]Methyl 2,3,5,6-tetra-*O*-methyl β-D-galactofuranoside.
[c]Methyl 2,3,4,6-tetra-*O*-methyl β-D-galactopyranoside.
[d]Methyl 2,3,5,6-tetra-*O*-methyl α-D-galactofuranoside.

0.25 mm film thickness. The oven temperature program was as follows: the initial temperature of 80°C was held for 10 min, followed by a linear temperature ramp to 260°C at a rate of 10°C/min, and was then held constant at 260°C for 10 min.

Results and discussion

The GC–MS trace that we had previously obtained for permethylated D-glucose (2) is very similar to the chromatogram published earlier by Ciucanu and Kerek (1), for the same compound. The trace (not shown) features two major peaks, which, according to these authors (1), respectively correspond to β- and α-glucopyranoside, in a ratio of ca. 2 to 1. The mass spectra obtained at the apex of each peak with our HP GC–MS system matched the NIST library spectra and suggested permethylated β- and α-glucopyranoside products for the respective peaks.

Ciucanu and Kerek (1) had briefly discussed the effect of pH on the equilibrium between the different forms of D-glucose in solution, but no specific details were given by these authors regarding the initial equilibrium in a basic solution of D-glucose in DMSO. More recently, Angyal (7) reported the proportions of glucopyranosides and glucofuranosides observed when D-glucose is dissolved in DMSO, without a base. The relative amounts reported were 45% α-glucopyranose and 55% β-glucopyranose, with no mention of the two other possible isomers. Proportions close to the above were also found when D-glucose was dissolved in pyridine. In this solvent, the amount of β-glucopyranose was decreased by 2% and both glucofuranosides made up for that 2%. These proportions, although measured in a nonbasic environment, reflect the numbers reported by Ciucanu and Kerek (1) and our laboratory (2) for the permethylated derivatives.

Permethylation products of D(+)-galactose

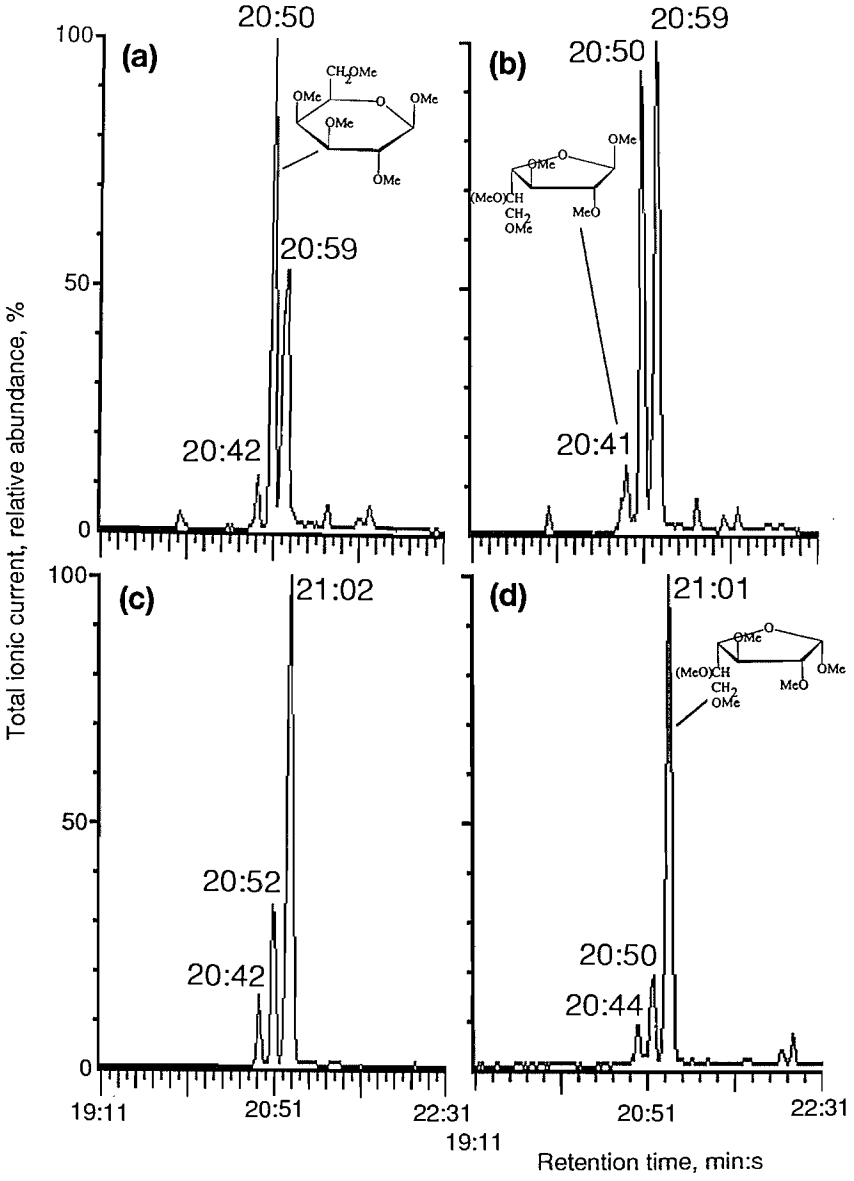
Angyal (7) also reported the following proportions for the forms of D-galactose in pyridine: 31% α-galactopyranose, 46% β-galactopyranose, 5% α-galactofuranose, and 18% β-galactofuranose, in comparison to 30, 64, 2.5, and 3.5%, respectively, in water (8). Unfortunately, no such data were given for galactose in DMSO. According to Ferrier and Collins (9), aprotic solvents (e.g., *N,N*-dimethylformamide (DMF) and DMSO) do not solvate sugars as well as water does, and as a consequence they enhance the anomeric effect and yield a higher proportion of five-membered ring compounds. The figures given above for galactose in pyridine agree with this statement.

As for the effect of a basic environment on the proportions of hexose species, it has been suggested (8) that in aqueous solution it will influence the rate of mutarotation, and thus the rate of approach of the anomers in solution to an equilibrium mixture. It is difficult to evaluate the pH values of basic solutions in DMSO, but typically DMSO solutions of NaOH are much more basic than the corresponding aqueous solutions. In assuming that the permethylation method of Ciucanu and Kerek (1) is performed at an effective pH > 14, substrates such as D-glucose (pK_a = 12.28) and D-galactose (pK_a = 12.30) should be in their monoanionic forms (10), if not dianionic. The proportions of anomeric anionic species, as opposed to electrically neutral species, has not been discussed in detail in the literature. The ability of hydroxide ions vs. silver, barium, and strontium oxides to act as acid receptors has been discussed as a possible factor influencing the proportions of ionic–nonionic substrate molecules in methylation reactions (9). According to Walker et al. (11), D-galactose, permethylated using methyl iodide and silver oxide, yields the following products: 80% α-D galactofuranoside, 10% β-D-galactofuranoside, and 10% (α and β)-galactopyranosides.

Gee (4) performed thin-layer chromatography on permethylated galactosides and reported separation into three spots, corresponding to β-D-galactofuranoside, α-D-galactofuranoside overlapping with β-D-galactopyranoside, and α-D-galactopyranoside. The compounds were prepared using Kuhn's procedure (12) (DMF as the solvent), and no mention of the relative proportions was made.

In our hands (2), using our former routine permethylation method (method 2 in Table 1), D-(+) galactose yielded two major products and a third minor component. A composition of (7 ± 2)% of galactofuranoside (**1**), (61 ± 2)% of galactopyranoside (**2**), and (32 ± 2)% of galactofuranoside (**3**) was found. Figure 2(*a*) shows the GC–MS total ion current (TIC) obtained from permethylation method 2 (Table 1) as a bench-

Fig. 2. Total GC–MS ion chromatograms obtained for the permethylation products of D-galactose. Refers to Table 1, (*a*) method 2, (*b*) method 3, (*c*) method 5, and (*d*) method 7. The three time-labelled peaks on each chromatogram correspond to products **1**, **2**, and **3**.



mark for starting the present investigation. A similar trace was obtained from method 1, which involved longer sonication times than method 2, both before and after addition of methyl iodide.

The influence of sonication time of the (DMSO–galactose–NaOH) mixture was originally investigated as part as an attempt to shorten the overall reaction time. This step was thus aimed at making the (DMSO–galactose–NaOH) solution as homogeneous and base saturated as possible. Interestingly, a shorter sonication time (see method 3, Table 1) was found to have an important impact on the nature of the products (Fig. 2(*b*)).

While initially performing the experiments, not much thought was given to the kinetics of mutarotation and anomerization at the pH and temperature used. Previous reports, however, demonstrated the importance of such factors. Collins and Ferrier (9) reported that when galactose is heated under reflux in methanol containing 2% HCl, 12 h or more are necessary to reach an equilibrium between the different glycoside forms. It seems reasonable to assume a similar situation exists for anionic galactose in basic solution, in which case the first sonication time involved in methods 1–3 would be too short for an equilibrium to be reached. If the addition of CH_3I "interrupts" the progression toward equilibrium, a systematic variation in the proportions of permethylated galactosides should be observed, as appears to be the case, from method 3 to method 1. The proportions of products **1** and **2** increase, while product **3** becomes less abundant.

Smirnyagin and Bishop (13) studied the kinetics of glycosylation of D-galactose. Their results confirmed an earlier observation for D-xylose (14): when D-galactose is subjected to methanolysis in methanolic HCl, galactofuranosides are preferentially formed first, and their relative concentrations decay as they isomerize into galactopyranoses or anomerize.

Table 2. Experimental conditions for the permethylation of L(−) fucose, based on the method of Ciucanu and Kerek (1) and relative proportions[a] of the products obtained.[b]

Method no.	1st sonic. (min)	React. time (min)	Temp. (°C)	Prod. **4**[c] (rel. conc., %)	Prod. **5**[d] (rel. conc., %)	Prod. **6**[e] (rel. conc., %)
8	60	60	20–50	100	0	0
9	30	15	20–30	100	0	0
10	20	15	20–27	79	4	17
11	10	15	20–25	44	10	46
12	1	15	20–23	29	12	59
13	0	15	20–23	28	14	58
14	0	25	20–33	40	13	47
15	0	60	20–50	54	14	32
16	0	60	10–20	36	12	52

[a]Relative proportions: ±2%.
[b]Fucose: 4.5–4.8 mg; DMSO: 450 μL; NaOH: 50 mg; CH_3I: 100 μL.
[c]Methyl 2,3,4-tri-*O*-methyl β-L-6-deoxygalactopyranoside.
[d]Methyl 2,3,4-tri-*O*-methyl α-L-6-deoxygalactopyranoside.
[e]Methyl 2,3,5-tri-*O*-methyl α-L-6-deoxygalactofuranoside.

These authors also showed the effect of temperature on the relative concentrations of galactosides over more than 200 h.

It is clear that in a methanolic HCl solution, glycosides would be far from equilibrium after 45 min or 1 h of reflux, which corresponds more with our times used for the first sonication. Our experimental conditions are far different from those used by these authors (14). However, this rationalization helps in understanding that our starting material in alkaline DMSO is changing with time and temperature. Thus, method 3 yields different proportions of products relative to methods 2 and 1, which involve longer prereaction sonication times.

The sonication time was further reduced to zero with yet another increase in the proportion of α-galactofuranoside **3** to 71% of the total (see method 4, Table 1).

In the fifth experiment (see method 5, Table 1) the reaction time with CH_3I was reduced from 85 to 15 min. The results were very similar to those of method 4, indicating that the reaction was complete after 15 min. Figure 2(*c*) illustrates the results. Collins and Ferrier (8) indicated the possibility that permethylated hexosides may mutarotate in pyridine, but no mention was made of DMSO. The similarity in the proportion of products obtained with method 4 (85 min reaction time) and method 5 (15 min) suggests that no mutarotation took place once permethylation was complete.

When the amount of NaOH was increased (see method 6, Table 1), there was a small increase in the amount of β-galactopyranoside **2** (rt 20:50 min), at the expense of **1** and **3**. The effects of bases on hexoses in aqueous solutions are well documented (8). Epimerization, although it is the most common effect, did not seem to occur in our experiments. The permethylated hexosides obtained with methods 1–7 had GC retention times and mass spectra that characterized galactosides.

In aqueous solutions where the concentration of the base exceeds 1%, sugars may be converted into isomeric deoxyaldonic acids (saccharinic and isosaccharinic acids) (8). Also, prolonged treatment of hexoses with bases may yield 3-carbon fragments such as 2-hydroxypropionaldehyde, pyruvic acid, methyl glyoxal, and lactic acid (8). No such information could be found for solutions of sugars in DMSO. However, these facts may explain the presence of side products after long first sonication – reaction times. We made no further attempt to identify the side products, present at low levels relative to the galactosides (e.g., Fig. 2(*a*) and (*b*)).

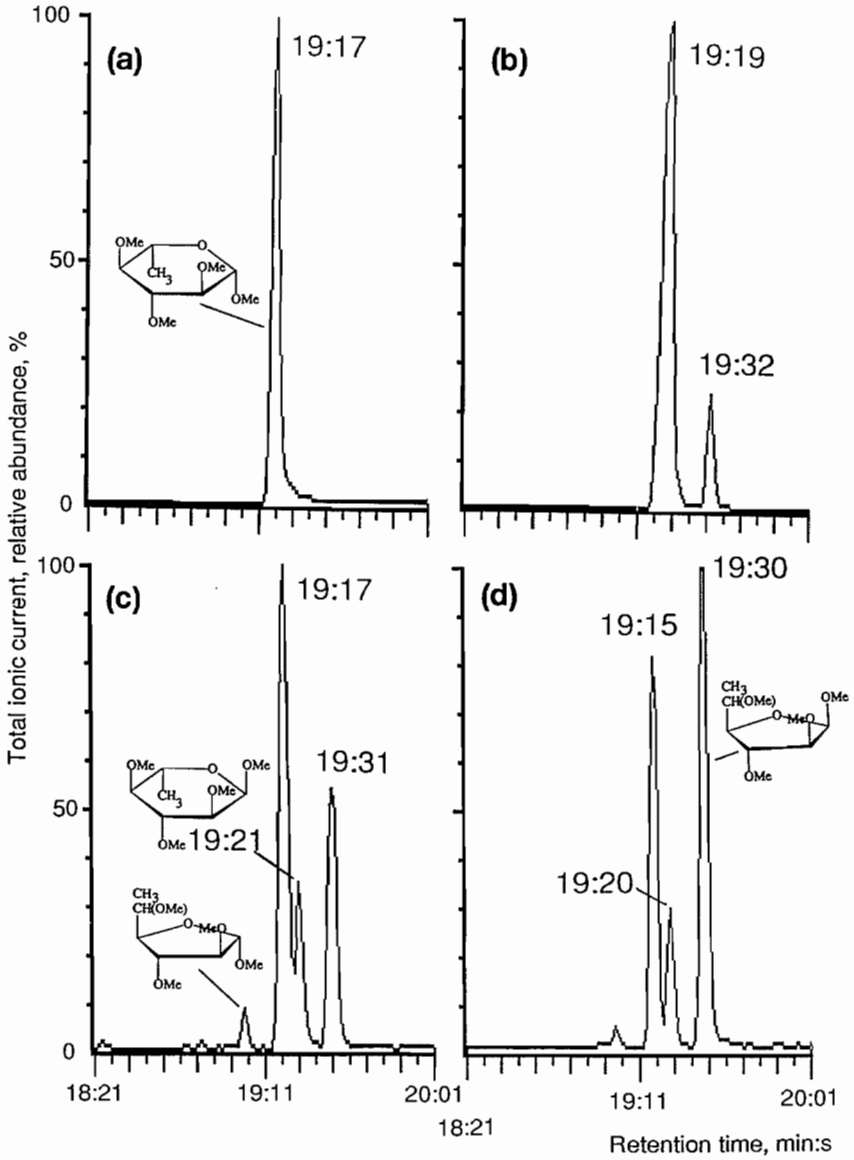
The effect of altering the temperature of the methylation reaction was investigated next. In previous experiments (see methods 1–6, Table 1), the reactions were started at room temperature (ca. 22–25°C); however, the temperature of the sonication bath gradually increased, reaching 50°C after 1 h of reaction. Holding the temperature of this reaction constant at 50, 30, 20, 15, and 10°C showed that low temperatures (15 and 10°C) favored formation of the main galactofuranoside **3** and almost eliminated the six-membered ring species **2** (Fig. 2(*d*)). Temperatures above 15°C (20–50°C, methods 4 and 5) all yielded approximately the same proportions of products. Although the reaction appeared to be complete after 15 min at the lower temperatures, we felt that it was safer to allow 1 h for permethylation, as indicated in method 7, Table 1. We believe that a lower starting temperature will alter the initial proportions of galactosides, as observed by Smirnyagin and Bishop (13) for methyl galactosides at 25 and 44°C. Conducting permethylation at a low temperature, such as 10°C, is also likely to slow down by-product formation processes.

The effect of altering the amounts of DMSO and methyl iodide relative to the other reagents was also investigated. No significant changes in the nature or proportions of the products were observed.

Method 7 (Table 1) provided an 81% relative yield of galactopyranoside **3** (Fig. 2(*d*)), indicating that this method might be preparatively useful.

Vain attempts were made to prepare the galactopyranoside **2** *exclusively*. Large concentrations of NaOH and long sonication times before addition of methyl iodide were investigated, but the results were very similar to those of method 1, i.e., the relative yield of product **2** peaked at approximately 67%. The only effect associated with excess NaOH and long permethylation–sonication times was the appearance of small amounts of unidentified side products, possibly saccharic-type acids and smaller fragments as discussed above.

Fig. 3. Total GC–MS ion chromatograms obtained for the permethylation products of L-fucose. Refers to Table 2, (*a*) method 8, (*b*) method 10, (*c*) method 15, and (*d*) method 16. The time-labelled peaks on the chromatograms correspond to products **4**, **5**, and **6**.



Permethylation products of L(−)-fucose

Results from the GC–MS TIC trace previously obtained for the three major products of permethylated L(−)-fucose (2) are reported in Table 2, method 11. A minor peak appears first and corresponds to a permethylated fucofuranoside. This product has not been taken into account in the following discussion since it constitutes less than 3% of the total product mixture. With method 11, fucopyranosides **4** and **5** made up 54% of the three main products, with the remainder (46%) being the fucofuranoside anomer **6**. The permethylated fucopyranosides **4** and **5** were first tentatively identified by library comparison to the spectra obtained on the HP system (5), as methyl 2,3,4-tri-*O*-methyl-6-deoxy-β- and α-galactopyranosides. Although no library spectra were available for permethylated fucofuranosides, they were identified by the similarity of spectral features between the fucose and galactose-derived products: permethylated fuco- and galactofuranosides each yield a main peak at *m/z* 101. More systematic identification was carried out with the use of the corresponding standards.

Varying the reaction parameters for the permethylation of L(−)-fucose was accomplished based on the results obtained for D(+)-galactose. Hence, the relative amounts of DMSO, CH_3I, NaOH, and fucose used remained constant in all experiments. These quantities are indicated in Table 2. Parameters subjected to variation were: time of first sonication, reaction time, and temperature. Table 2 lists nine different methods, along with the corresponding proportions of products (**4**, **5**, and **6**) obtained. In methods 8–12, fucose, NaOH, and DMSO were sonicated prior to addition of CH_3I. The general trend shows increasing proportions of **5** and **6** relative to **4** with shorter prereaction sonication times. In methods 13–15, the first sonication step was omitted and the reaction time was incremented. Longer reaction times (25 min, method 14 and 60 min, method 15) had the effect of increasing the propor-

tions of **4** vs. **5** and **6**, i.e., the effect was similar to that of longer first sonication times. Lowering the reaction temperature (method 16) favored product **6**, and brought down the relative concentration of **4** from that observed using method 15. Interestingly, the relative proportion of product **5** was not significantly altered with methods 11–16.

The results obtained from methods 8 and 9 (e.g., Fig. 3(*a*)) suggest that longer mixing times of the (fucose–DMSO–NaOH) solution yield 100% of β-fucopyranoside, neutral or anionic, prior to the addition of CH_3I. Methods 8 and 9 are therefore advisable if preparation of β-fucopyranoside as a single isomer is desired.

Shorter mixing of the ternary solution, as in methods 10–13, showed a change in the proportions of fucosides, with a tendency towards α-fucopyranoside **5** and fucofuranoside **6** relative to the previously predominant β-fucopyranoside. Figure 3(*b*) illustrates this trend. Here compound **5** is a minor constituent and has not been resolved from **4**. The slight shoulder at the right base of the peak assigned to **4** led us to assign a proportion of 4% to compound **5**.

So far, these results are in agreement with the general trend observed for the galactose permethylation reactions. However, much less information is available about the chemistry of fucose in solution than about galactose and therefore interpretation of the results is more difficult.

In methods 8–13, we estimated that a 15 min reaction time was the minimum required to ensure complete permethylation of the sugar. We also assumed that no further isomerization took place once the products were formed. In methods 14 and 15, where the reaction time was deliberately pushed above 15 min, isomerization seemed to occur, either during permethylation itself, or once the reaction was completed. It could also be possible that one (or more) of the isomers is selectively being degraded. This would change the ratio of isomers and give the impression that equilibration between isomers was occurring. This process overall favored product **4**, to the detriment of **5** and **6** (Fig. 3(*c*)).

Lowering the reaction temperature as in method 16 seemed to slow down the process and the proportions were pushed in favor of product **6**. Figure 3(*d*) shows the corresponding chromatogram.

Conclusion

It has been shown that permethylation conditions, based on the method of Ciucanu and Kerek (1), have a profound influence on the composition of the products obtained from permethylation of D-galactose and L-fucose. For both of the monosaccharides, permethylation produced a mixture of four isomers: two pyranoside and two furanoside α and β anomers. In each case, three compounds out of the four were predominant and well separated on the GC–MS chromatograms. The fourth component in each case was very minor and its proportions did not appear to vary from one set of conditions to another. Changes in the relative proportions of the three main compounds were reported as different reaction parameters were varied. The most influential parameter appeared to be the time the DMSO solution of the sugar and NaOH was mixed by sonication before reaction with methyl iodide. The initial temperature of reaction also had an important effect. It is assumed that these two parameters influenced the initial proportion of the four possible isomers, or isomeric anions, of the monosaccharides in solution prior to permethylation.

In both cases (galactose and fucose), formation of the β-pyranoside product was noticeably favored when longer pre-reaction sonication times and higher temperatures were used. Opposite trends in the conditions favored formation of the α-furanoside product.

Above all, it has been shown that care has to be taken when performing permethylation of sugars as a routine operation. The reaction conditions must be very carefully controlled in order to obtain reproducible meaningful results.

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