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Identification of monosaccharides and related compounds by gas chromatography–Fourier transform infrared spectroscopy of their trimethylsilyl ethers

R.G. Veness*, C.S. Evans

School of Biological and Health Sciences, University of Westminster, 115 New Cavendish St., London WIM 8JS, UK

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Abstract

Trimethylsilyl (TMS) ethers are widely used in gas chromatography (GC) and gas chromatography–mass spectroscopy (GC–MS) for the identification of carbohydrates; this work shows that gas chromatography–Fourier transform infrared spectroscopy (GC–FT-IR) can also be used, with some advantages, for this purpose. A selection of 42 monosaccharides and related compounds were examined and in each case unique spectra were obtained for the differing compounds and their isomeric forms, allowing unambiguous identification. No relationship between the observed spectra and anomer form was seen. There was a statistically significant difference in the intensity of the peak at 1108 ± 25 cm^{-1} distinguishing the furanose and pyranose conformers.

Keywords: Carbohydrates; Monosaccharides

1. Introduction

The analysis of carbohydrates continues to be of considerable importance in the biological sciences. The diversity of structure and function of carbohydrates in organisms contributes to the difficulty of analysis of these materials.

There exist a number of techniques for the analysis of carbohydrates, but no one technique has universal applicability. For the identification of constituent monosaccharides, GC–MS of either trimethylsilyl (TMS) ethers or alditol acetates are widely used. Stereoisomers of alditol acetates give mass spectra with minor differences in relative intensities rather than in fragmentation patterns [1]. For TMS ethers it is necessary to rely on relative intensities to differentiate stereoisomers whilst anomers are not differentiated [2].

Mass spectrometry is extremely good at differentiating on the basis of molecular size but less so at differentiating on the basis of stereochemical changes. Infrared (IR) spectroscopy is effective at differentiating stereochemical changes but is less able to differentiate on the basis of molecular size as in homologous series [3].

In recent years a number of workers have demonstrated the ability of IR spectroscopy to

* Corresponding author.

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handle real applications in carbohydrate analysis. Near-infrared spectroscopy has been used to detect adulteration of orange juice [4] and to determine the sugar content of intact peaches [5]. Fourier transform infrared spectroscopy (FT-IR) was used to detect fructose and glucose in fermentation liquor [6], to determine the concentrations of sucrose, glucose and fructose in the soft drinks industry [7] and for quantitative analysis of powdered sugar mixtures [8]. In addition, GC analysis has been used to determine the levels of alditols in vinegar after first converting them to their TMS ethers [9].

For GC–FT-IR the use of TMS ethers has advantages over alditol acetates as the Si-X IR absorptions are approximately five times as strong as the equivalent C-X absorption [3]. This results in enhanced sensitivity. A further advantage of GC–FT-IR is that it has the ability to record a series of time-sliced spectra throughout the width of the GC peak. Hence, if adjacent peaks are not fully resolved, it is possible to record the spectrum from the "heart" of the peak and thus obtain a spectrum of the pure component. Even if the separation is such that a peak appears as a shoulder on an adjacent peak, spectra of the pure components can be obtained by difference [10].

The signal-to-noise ratio (S/N) determines the sensitivity of the method. With FT-IR the S/N is governed by the quantity of material present, the intensity of the absorptions and the number of scans taken (n). Increasing n increases S/N by a factor of $\sqrt{n}$. Hence, to obtain a ten-fold increase in S/N, and thus sensitivity, requires 100 scans. Increasing the number of scans also increases the slice width (time to acquire), unless the resolution is decreased. Techniques such as coaddition of spectra, classical least squares [11] and peak holding can also be employed to increase S/N.

GC–FT-IR of TMS and siloxyl compounds has been carried out by other workers [12,13], but no systematic study of carbohydrates appears to have been done. For these reasons it was decided to investigate the use of GC–FT-IR of the TMS ethers as an analytical procedure for identifying monosaccharides.

2. Experimental

All chemicals were obtained from Sigma (Poole, Dorset, UK). The compounds examined are listed in Table 1. TMS ethers were prepared by one of two methods. For the salts of sugar acids and acetamido sugars, derivatives were prepared using N,O-bis(trimethylsilyl) acetamide (BSA) and trimethylchlorsilane (TMCS) [14]. All other compounds were derivatised using hexamethyldisilazane (HMDS) and TMCS [15]. Between 5 and 10 mg of each compound was used together with 1–2 mg of myo-inositol as an internal standard. After derivatising, the dried residue was dissolved in 50–200 μl of n-hexane. Each compound was prepared either as part of a mixture or as a single compound at least twice (mean 2.5 times) and each preparation was analysed at least twice (mean 2.3 times). Thus a minimum of four replicates was obtained for each compound.

Between 1 and 3 μl was injected in splitless mode into a 8600 gas chromatograph coupled to a 1720X FT-IR spectrometer by means of a 1700 GC–IR interface, all equipment from Perkin-Elmer (Beaconsfield, Bucks., UK). The FT-IR

Table 1
Compounds examined by GC–FT-IR

Oxalic acid	Psicose
Glyceraldehyde	Rhamnose
2,3-Dihydroxypropane	Tagatose
Glycerol	Fructose
Glyceric acid	Sorbose
Erythrose	Fucose
Threose	2-Deoxy-D-glucose
Arabinose	2-Deoxy-D-ribose
Xylose	3-O-methyl-D-glucose
Lyxose	Methyl-α-D-mannopyranoside
Ribose	N-acetylglucosamine
Ribulose	N-acetylgalactosamine
Xylulose	Glucuronic acid
Allose	Galacturonic acid
Altrose	Gluconic acid
Glucose	Galactonic acid
Mannose	Glucaric acid
Talose	Glucuronic lactone
Galactose	Gluconic 1,5-lactone
Idose	Glucaric 1,4-lactone
Gulose	Myo-inositol

was fitted with a mercury cadmium telluride (MCT) detector cooled with liquid nitrogen. The GC was fitted with a fused-silica capillary column, 25 m × 0.53 mm I.D., with 5 μm of BP1 phase [SGE (UK), Milton Keynes, UK]. Carrier gas was helium at 86 kPa, the injector and flame ionisation detector were operated at 300°C and the GC was programmed from 165°C, hold 1 min, ramp 3°C min^{-1} to 250°C, hold 20 min. The light and transfer pipes were operated at 250°C. Make up gas for the FT-IR was helium at 34 kPa. The FT-IR was continuously purged with nitrogen at a flow-rate of 20 ml min^{-1}. The spectrometer was set to make four scans at a resolution of 2 cm^{-1} with a slice width of 0.24 min and an interval of 0.25 min. All spectra were recorded on-the-fly from chromatography of either a mixture or a single compound, and in many cases from both. None of the techniques to enhance *S/N* were used.

3. Results and discussion

The GC separation of the compounds was comparable to that reported by previous workers [15,16]. A typical gas chromatogram of TMS ethers of a sugar mix is shown in Fig. 1. Peaks 1–4 are from the four cyclic forms of arabinose, and indicate that under the GC conditions used total separation was not achieved. By virtue of the fact that the interval between slices was 0.25 min, which was less than the peak widths, it was possible to obtain spectra from the heart of the peaks. In this particular example slices 5, 7, 9 and 11 corresponded to the middle of the four peaks. This resulted in 0.25-min intervals between the peaks when the unresolved mixture would have eluted. As the furanose spectra were statistically significantly different from the pyranose spectra, we were able to check that the spectra thus obtained were for essentially pure components.

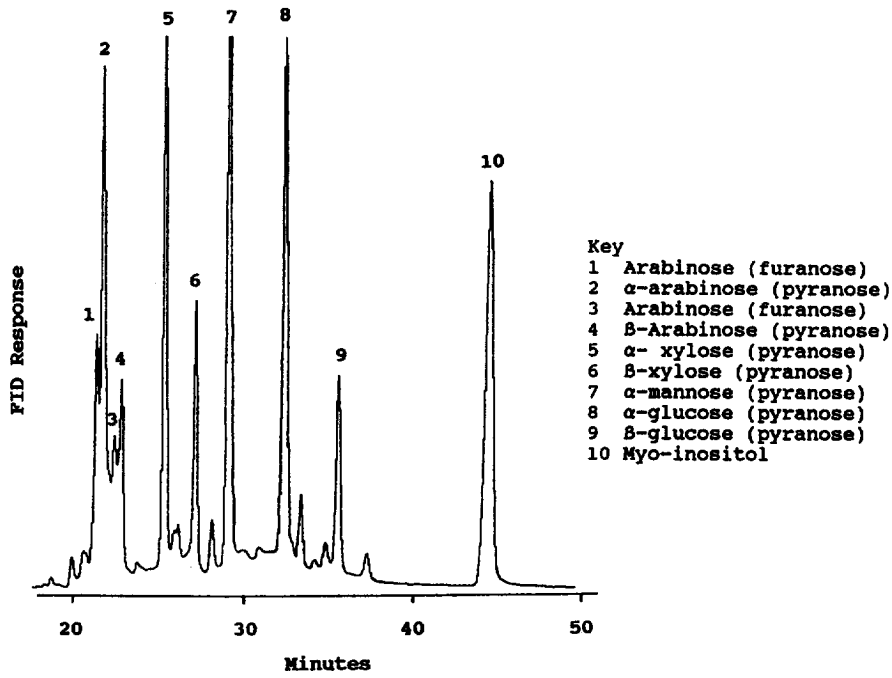

Fig. 1. Gas chromatogram of TMS ethers of a typical sugar mixture.

Most other peaks were fully resolved and resolution could be improved by reducing the ramp rate from 3 to 2°C min^{-1} (data not shown). The GC trace also illustrates that not all of the compounds used as standards were 100% pure, as a number of small peaks are evident which could not be assigned to anomers of the sugars present.

Under the conditions we employed it was found that optimal spectra were obtained from samples with approximately 20 μg of each component. Recognisable spectra, but with poor *S/N* ratios, were obtainable with sample sizes down to around 500 ng. By increasing the *S/N*, it is estimated that we could probably obtain spectra from as little as 100 ng, but we do not believe that with our equipment we could achieve the 10 ng sensitivity reported for some siloxane esters [17]. Thus the level of sensitivity of GC–FT-IR is several orders of magnitude below that achievable by using flame ionisation or MS as detectors.

From the 42 compounds examined, some 100 unique spectra were obtained representing the various stereoisomers. Representative spectra are given in Figs. 2 and 3.

The FT-IR spectrum obtained from D-threose (Fig. 2a) was typical of all the cyclic sugar forms, showing five prominent "groups" of peaks. Table 2 gives the position of the major peak(s) in each group for the compounds depicted in Figs. 2 and 3.

Group 1 was normally a double peak, as shown, with the major absorbance at 2962 ± 2 cm^{-1} and a minor peak at 2906 ± 4 cm^{-1}. These peaks could be assigned to the asymmetrical and symmetrical stretching modes of C–H in the methyl groups.

Group 2 was normally a doublet (see also Figs. 2f and 3a, of α- and β-D-glucopyranose) with peaks at 1266 ± 1 and 1258 ± 2 cm^{-1} and could be assigned to $Si(CH_3)_3$ symmetrical deformations [3]. This group was not found to be diagnostic.

Group 3 was one of the most diagnostic groups, often showing multiple peaks, with the most intense peak of the group at 1108 ± 25 cm^{-1}. This group of peaks can be attributed to the complex Si–O–C stretching modes. In the sugars analysed they appear at a higher wavenumber than the 1020–1090 cm^{-1} normally quoted [3]. This is almost certainly due to the higher freedom of the molecules in the gas phase compared with that in the liquid or solid phases.

Group 4 normally contained the most intense peak of the spectrum at 849 ± 2 cm^{-1}. It sometimes appeared as a single peak, as here, or as a complex of peaks as in α-xylose (Fig. 2d). This group could be ascribed to $Si–CH_3$ stretching vibrations.

Group 5 was always a single weak peak at 759 ± 5 cm^{-1} caused by $Si–CH_3$ and had no diagnostic value.

A weak peak was sometimes seen at 1370–1390 cm^{-1} (Fig. 2d), which was due to C–H symmetrical deformation of the methyl groups. This also had no diagnostic value. The hemiacetal–C–O–C–stretching mode was not specifically observed in the spectra of cyclic sugars. This was due to its appearing at the same position as the group-3 absorptions, and being much weaker they would be masked by them.

The spectrum of D-erythrose (Fig. 2b) showed how group-3 absorptions varied significantly, compared to Fig. 2a. There was an additional peak at 1742 cm^{-1} caused by C = O stretching, which signified that this was an acyclic form. This was confirmed by an additional peak at 2821 cm^{-1}, caused by the C–H stretch of tertiary C–H. Thus the additional peaks were consistent with the presence of a–CHO group.

Fig. 2c shows the spectrum of 2-deoxy ribose. This again was an acyclic form, as shown by the C = O stretch at 1735 cm^{-1}. Group-1 absorptions consisted of five peaks which could be explained as C–H stretches, two from CH_3, two from CH_2 (C_2 of the ring being deoxy) and the remaining peak from tertiary C–H (–CHO).

The spectrum of α-xylose in Fig. 2d showed how complex groups 3 and 4 could be and why these two were major diagnostic groups. It was also one of the few spectra where the intensity of the major peak in group 3 at 1084 cm^{-1} was greater than that of the group-4 peak at 848 cm^{-1}.

The ribulose spectrum in Fig. 2e could be

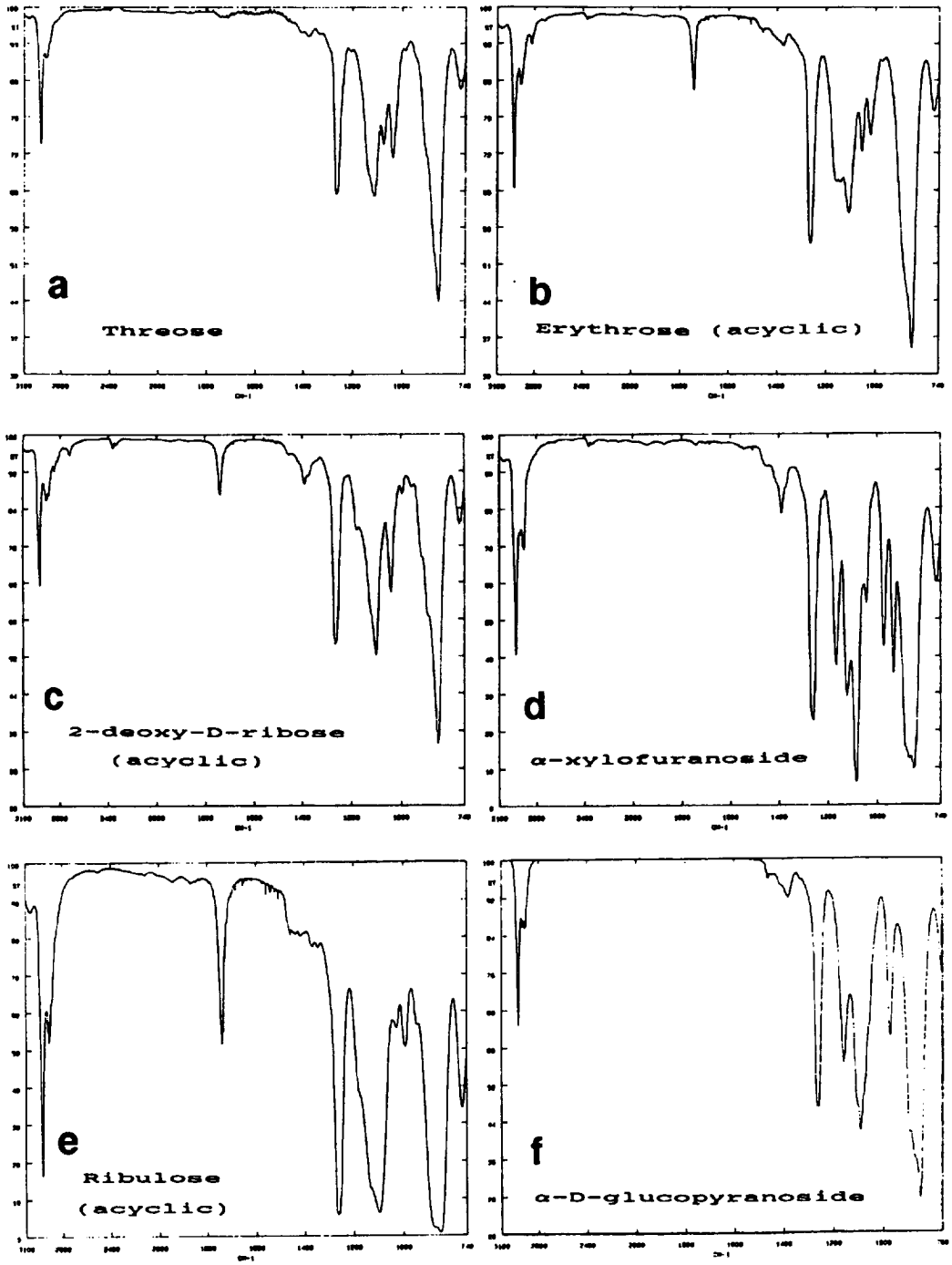

Fig. 2. Vapour-phase FT-IR spectra of TMS ethers of (a) threose, (b) erythrose (acyclic), (c) 2-deoxy-D-ribose (acyclic), (d) α-xylofuranoside, (e) ribulose (acyclic) and (f) α-D-glucopyranoside. Spectra obtained from chromatographing 10–30 μg of the sugars on a 25 m × 0.53 mm column with 5 μm of BP1 phase. The spectra were recorded at 2 cm^{-1} resolution from four scans using an MCT detector at −196°C.

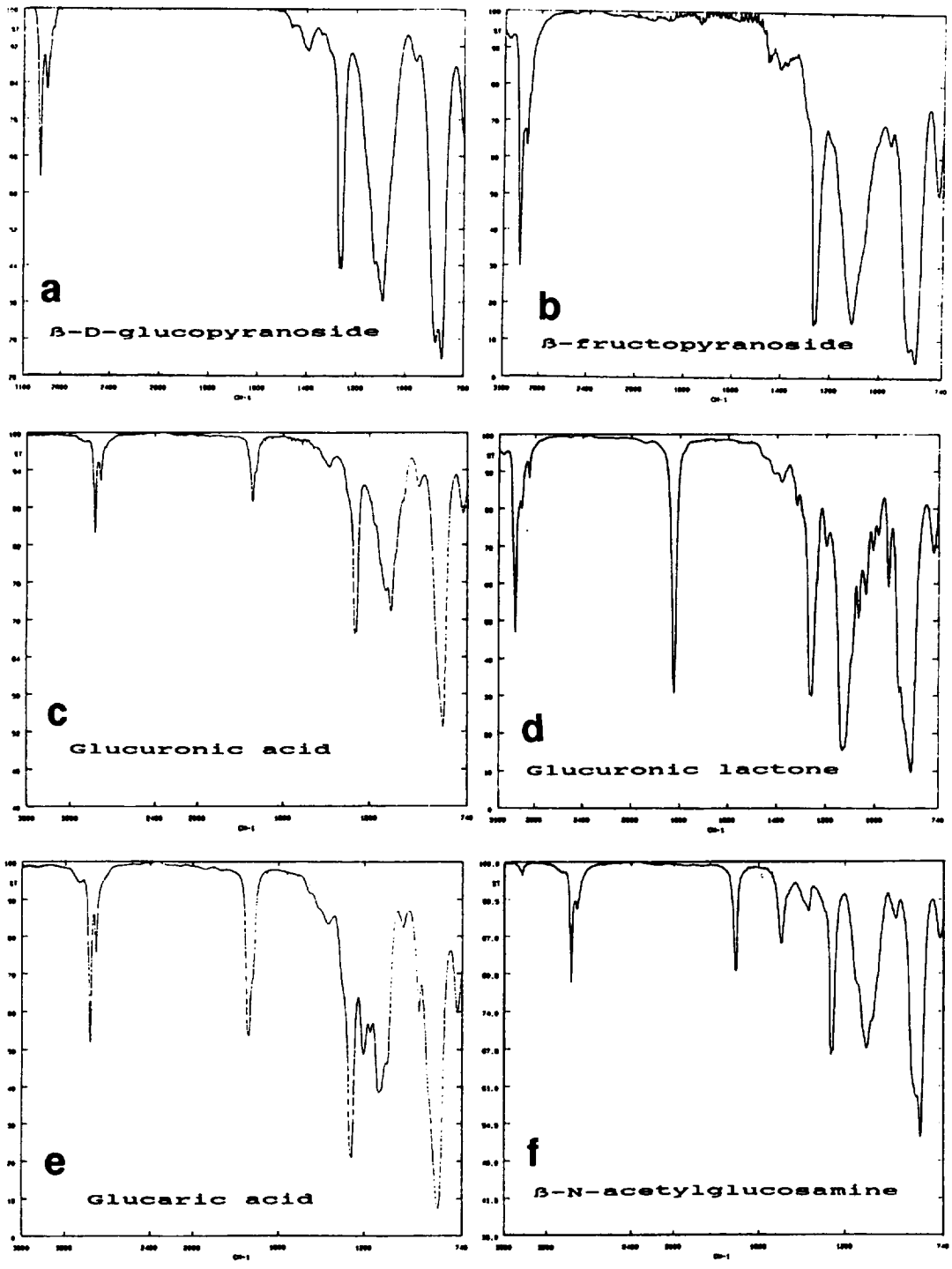

Fig. 3. Vapour phase FT-IR spectra of TMS ethers of (a) β-D-glucopyranoside, (b) β-fructopyranoside, (c) glucuronic acid, (d) glucuronic lactone, (e) glucaric acid and (f) β-N-acetylglucosamine. Conditions as in Fig. 2.

Table 2
Position of the major peaks in each group for the compounds in Figs. 2 and 3

Compound	Group 1	Group 2	Group 3	Group 4	Group 5	Others
Threose	2963	1265	1111	850[a]	760	
Erythrose	2962	1260	1108	849	758	C=O 1742
2-Deoxy ribose	2963	1265	1099	848	754	C=O 1735
α-Xylose	2963	1258	1084	848	756	
Ribulose	2963	1265	1097	850	759	C=O 1740
α-Glucose	2962	1265 1257[b]	1089	848	758	
β-Glucose	2961	1266 1257[b]	1090	848	760	
β-Fructose	2962	1266	1111	848	759	
Glucuronic acid	2963	1266	1096	851	760	C=O 1742
Glucuronic lactone	2963	1260	1133	852	761	C=O 1824
Glucaric acid	2963	1259	1132	854	764	C=O 1737
N-Acetyl glucosamine	2962	1266	1101	848	758	Amide I 1707 Amide II 1498

All peak positions given in cm^{-1}.
[a] The major absorption in group 4 was normally the most intense peak of the spectrum.
[b] The group-2 peak was normally a doublet, in these cases the two peaks were of equal intensity. In all other cases only the most intense peak is recorded here.

readily identified as the open-chain (acyclic) form of this keto sugar. The C=O stretch appeared at 1740 cm^{-1}, which was somewhat higher than normally associated with this group, again probably due to the spectrum being acquired from the vapour phase.

Figs. 2f and 3a provide the spectra of the α and β anomers of glucopyranoside, clearly showing the doublet nature of group 2 and the significant differences that were seen between anomers.

Figs. 3c, 3d and 3e show the spectra of glucuronic acid, glucuronic lactone and glucaric acid, respectively. In Figs. 3c and 3e the wavenumber scale was extended to 3600 cm^{-1} to show the absence of a peak at ≈3300 cm^{-1} caused by O–H stretching. This demonstrated that the silylation was complete. This check was also made on all other compounds analysed, with the same result (data not shown). For Figs. 3c and 3e the peak at ≈1740 cm^{-1} corresponds to the C=O stretch from the COOH group. For glucaric acid, with two carboxyl groups, this peak was more intense than for glucuronic acid, with one carboxyl group. For the lactone the C=O stretch moved to the higher wavenumber of 1824 cm^{-1}.

Fig. 3f is the spectrum of β-N-acetyl-glucosamine. In addition to the normal groups of peaks three additional peaks were evident. A very weak peak at 3433 cm^{-1} caused by N–H stretching and the peaks at 1707 and 1498 cm^{-1}, corresponding to the amide I and amide II absorptions of the acetamido group. These two bands were also displaced from their normal positions: amide I (mainly C=O stretch) normally 1630–1680 cm^{-1}; amide II (mainly N–H deformation) normally 1515–1570 cm^{-1}. This shift could also be accounted for by the additional freedom conferred in the vapour phase. The presence of amide II and the weak N–H stretch indicated that the N–H of the acetamido group was not silylated, confirming a previous report [18]. This was probably due to steric hinderance as BSA has the ability to silylate acetamides [14].

For comparison of peak intensities the % transmission T was converted to absorption using the relationship $A = \log 100/T$. The major peak

in group 4 was assigned a relative intensity of 1.00 and the intensities of the other groups were calculated relative to this.

Detailed examination of all of the spectra revealed no correlation between peak positions or intensities for the α and β anomers. Comparison of the major peak (1108 cm^{-1}) in group 3 did show a statistically significant difference (Mann–Whitney U test $P < 0.0001$), with the furanose peaks being lower in intensity than the pyranose forms. One possible reason that the furanose forms gave lower intensities for the C–O–Si vibration was that the greater degree of strain in the five-membered furanose ring inhibited the vibration.

These results indicate that GC–FT-IR of the trimethylsilyl ethers of monosaccharides is a useful analytical technique for their identification. The resultant spectra are unique, easy to interpret, and stereoisomeric forms are readily differentiated.

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