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High energy collision-induced dissociation (CID) product ion spectra of isomeric polyhydroxy sugars

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Abstract

High energy (4 keV) collision-induced dissociation (CID) product ion spectra have been obtained for a series of isomeric sugar molecules of close structural similarity. The reproducibility of the approach has been established and the spectra shown to have significant differences. These differences have been rationalised in terms of conventional mass spectrometric fragmentation rules. The data have also been subjected to analysis using chemometric methods, which require no specialist mass spectrometric input. The resulting classification of the data shows good agreement with the conventional interpretation approach.

Keywords: CID; Product ion; High energy; Sugar; Isomer; Chemometrics; PCA

1. Introduction

Differentiation of isomers of close structural similarity using mass spectrometry has often been identified as a difficult problem. Energy imparted by electron impact (EI) ionisation can often obscure subtle differences in structure. The use of soft ionisation methods can help to maintain the structural integrity of the molecule ion but often little useful fragmentation is observed. In order to obtain more structural information, collision-induced dissociation (CID) product ion spectra can be generated from the molecule ion. High energy CID is often favoured for these experiments since it has been observed that subtle structural differences can lead to differences in the product ion spectra obtained. This has been shown for the characterisation of side chains in the CID spectra from peptides [1] and in the identification of complex glycosylation patterns in post translational modified proteins [2]. In this work, high energy (4 keV) CID product ion spectra have been obtained for a number of polyhydroxy sugar compounds. These molecules are commonly used as feedstocks in many industrial processes and often exist as isomeric mixtures with a wide concentration variation [3,4]. Analytical methods based on gas chromatography–mass spectrometry (GC–MS) have been utilised for some isomers but these methods make use of the chromatography retention time to differentiate isomers since the electron impact mass spectra are very similar [5]. Liquid chromatography–mass spectrometry has also been used but, in this case, only molecule ions are observed [6]. The various isomers can easily be differentiated by nuclear magnetic resonance (NMR) spectroscopy when pure but the ability of mass spectrometry (and tandem mass spectrometry), especially when coupled with chromatography, to characterise complex mixtures of these compounds with a wide dynamic range has significant potential practical advantages.

2. Experimental

The product ion spectra were obtained in a ZAB-T four sector mass spectrometer of BEBE geometry (Micromass UK Ltd., Manchester, UK) [7,8], operated at an accelerating potential of 8 kV. A schematic of the instrument is shown in Fig. 1. The second electrostatic analyser (E_2) has an inhomogeneous design, which, by varying the voltages applied, can be employed to change the focal point, focal-plane

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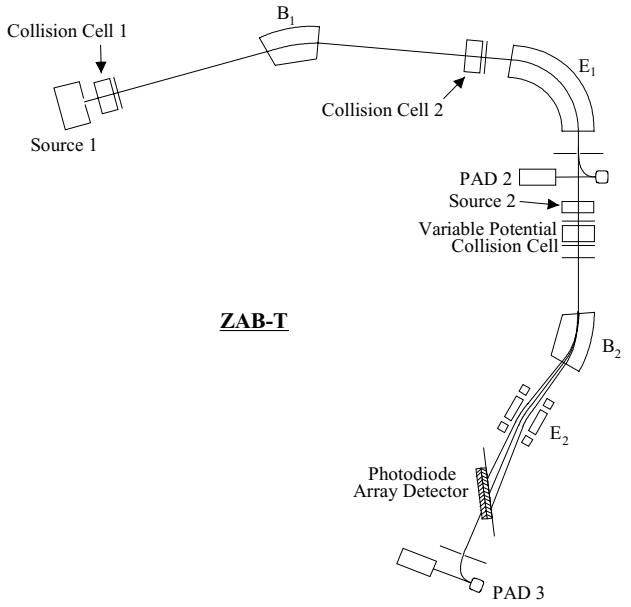


Fig. 1. Schematic of ZAB-T four sector instrument.

angle and the focal-plane flatness of MS2. Ionisation was performed using liquid secondary ion-mass spectrometry (LSI-MS) with 35 keV caesium ions and a gun current of 1 μA. The liquid matrix employed was glycerol.

Precursor ions for product ion scanning experiments were selected at greater than unit mass resolution (10% valley definition) by means of MS1 (B_1E_1). The ion beam was attenuated by 80% with argon at 4 keV collision energy in an electrically floated collision cell. The fragment ions formed were re-accelerated by an energy of 4 keV and passed to the detector by a linked scan of MS2. The 75 mm photodiode array detector in the fifth field-free region of the instrument was set at an angle of 30° to the incoming ion beam to give a mass ratio of 1.225:1. Acquisition of 8–10 scans under control of the OPUS data system was used to produce the product ion spectra.

The sugar molecules (see structures shown in Fig. 2) were purchased commercially (Sigma) or synthesised using standard methods [9]. All structures of pure materials were confirmed using NMR spectroscopy.

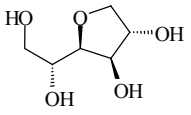
3. Results and discussion

3.1. $C_6H_{10}O_4$ isomers

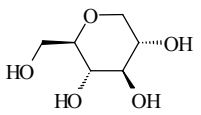
The spectra obtained from the collision-induced decomposition of the protonated precursor (MH^+) ions (mass-to-charge ratio (*m*/*z*) 147) of these two compounds, 1,4:3,6-dianhydro-D-mannitol and 1,4:3,6-dianhydro-D-glucitol [**1(A–C)**, **2(A–C)**], of molecular formula $C_6H_{10}O_4$, together with their structures, are shown in Figs. 3 and 4. In order to evaluate the reproducibility of the method, three replicate spectra were obtained (at different times) from each precursor ion. The excellent reproducibility obtained therefore enables significant differences between spectra to be used for classification. The spectra are very similar in most respects. The major differences between these spectra are that the spectrum given by **1** has a peak at *m*/*z* 103, which is barely visible in the spectrum of **2**, and the *m*/*z* 71 peak is somewhat more prominent in the spectrum of **1**. These differences, although small, are quite reproducible and may

1,4:3,6-dianhydro-D-mannitol,**1**

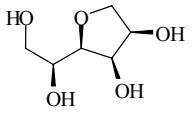
1,4:3,6-dianhydro-D-glucitol,**2**



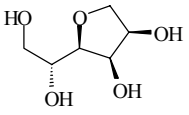
1,4-anhydro-D-glucitol,**3**



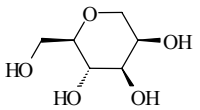
1,5-anhydro-D-glucitol,**4**



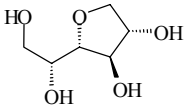
3,6-anhydro-D-glucitol,**5**



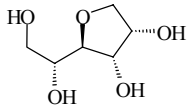
1,4-anhydro-D-mannitol,**6**



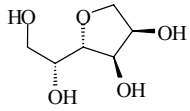
1,5-anhydro-D-mannitol,**7**



1,4-anhydro-D-galactitol,**8**



1,4-anhydro-D-allitol,**9**



3,6-anhydro-D-altritol,**10**

Fig. 2. Structure and names of compounds studied.

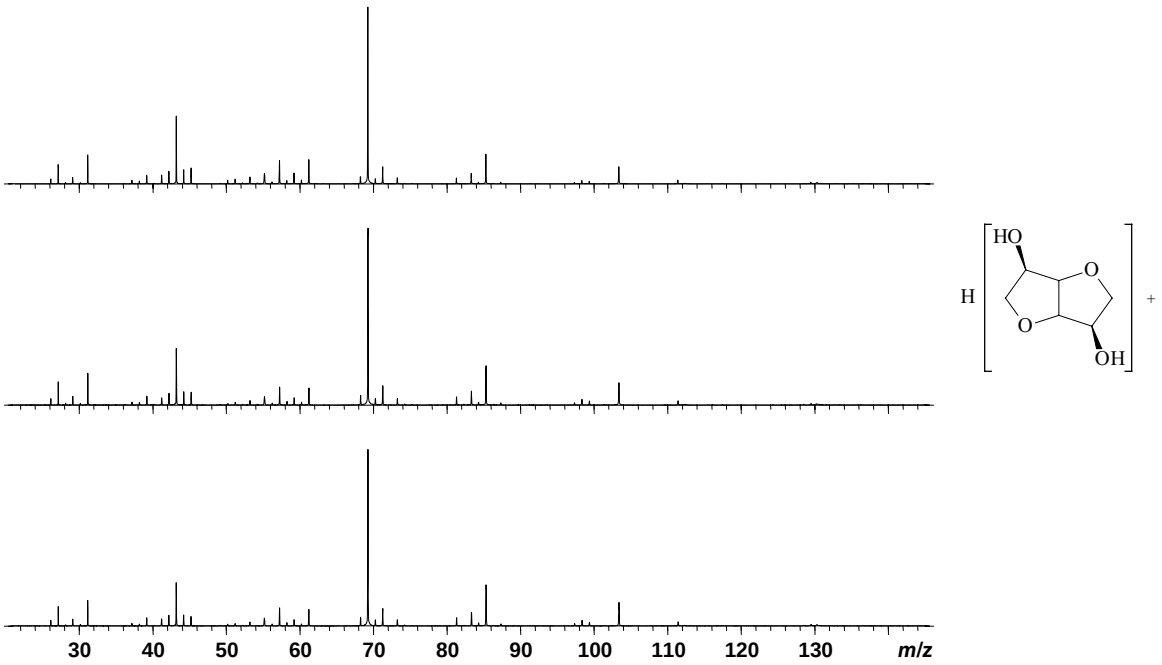


Fig. 3. MS/MS spectra from 1,4:3,6-dianhydro-D-mannitol.

be used to distinguish between the two compounds. The *m*/*z* 103 ion, formed by the loss of 44 Da, probably arises from ring cleavage with the loss of CH_2=CHOH, which rearranges to acetaldehyde, giving the $C_4H_6O_3^+$ ion, which may lose a methanol molecule to give the $C_3H_3O_2^+$ ion of *m*/*z* 71. The difference in relative intensities of these ions in the two CID spectra probably arises from slight differences in the internal energies with which the two precursor ions are formed.

The major fragmentation product in each case is the *m*/*z* 69 ion which is tentatively assumed to have the composition C_4H_5O and to have the structure of protonated furan. This requires the loss of 78 Da, [$C_2H_6O_3$], which may correspond to the rapid, successive loss of two units of formaldehyde

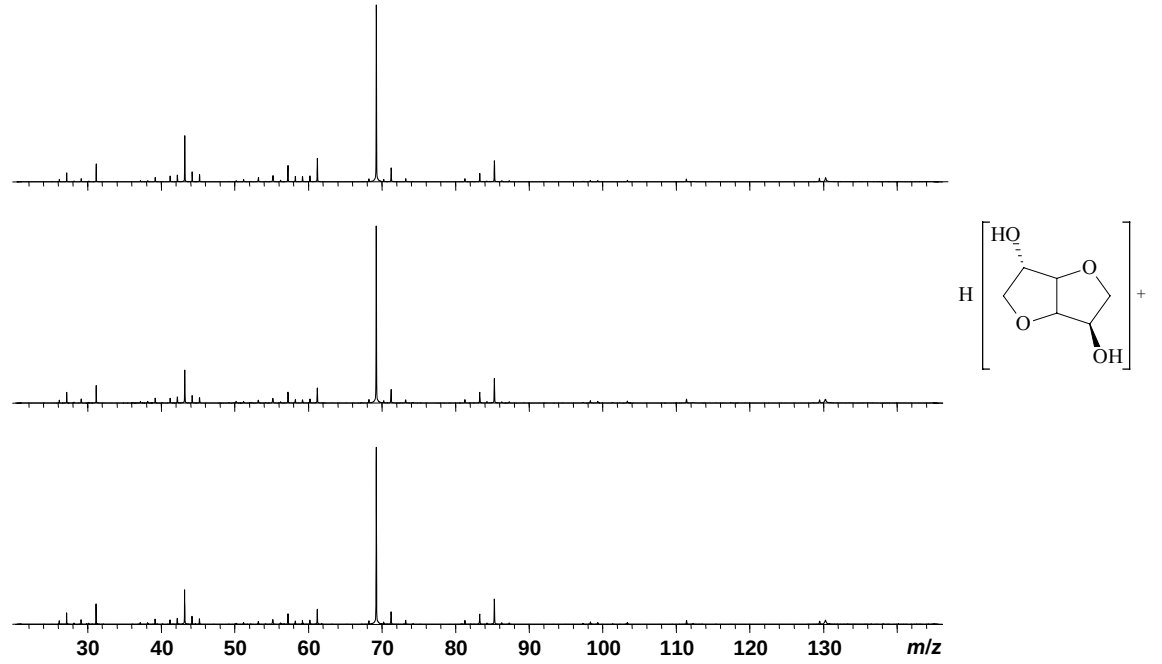


Fig. 4. MS/MS spectra from 1,4:3,6-dianhydro-D-glucitol.

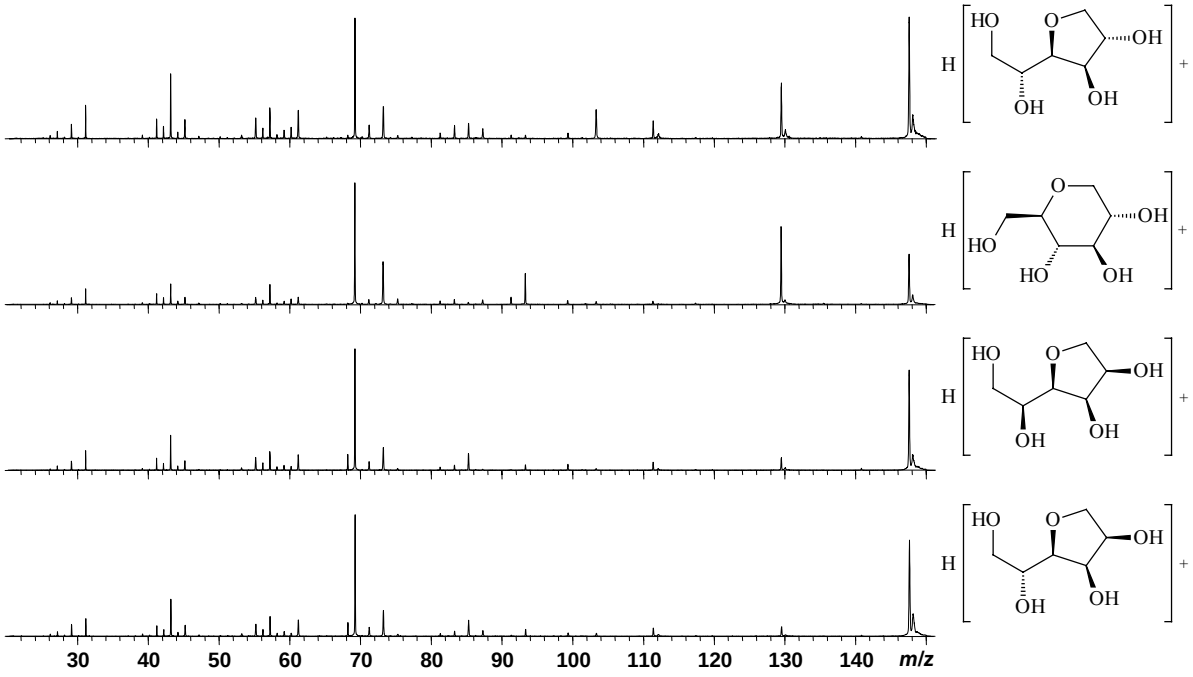


Fig. 5. MS/MS spectra from monoanhydrohexitols (1).

and water. The only other high mass ion of any consequence is the *m*/*z* 85 ion which may arise by the loss of ethylene glycol after protonation of an oxygen atom from the ring.

3.2. $C_6H_{12}O_5$ isomers

In order to evaluate the potential of the approach for the differentiation of closely related structures, a sample set of eight isomeric sugars was chosen. The CID spectra of the protonated precursor ion of the eight compounds, together with their structures, are shown in Figs. 5 and 6.

The eight samples (**3**–**10**) can be divided into two categories:

(1) Two that contain a 6-membered ring, **4** and **7** (1,5-anhydro-D-glucitol and 1,5-anhydro-D-mannitol).

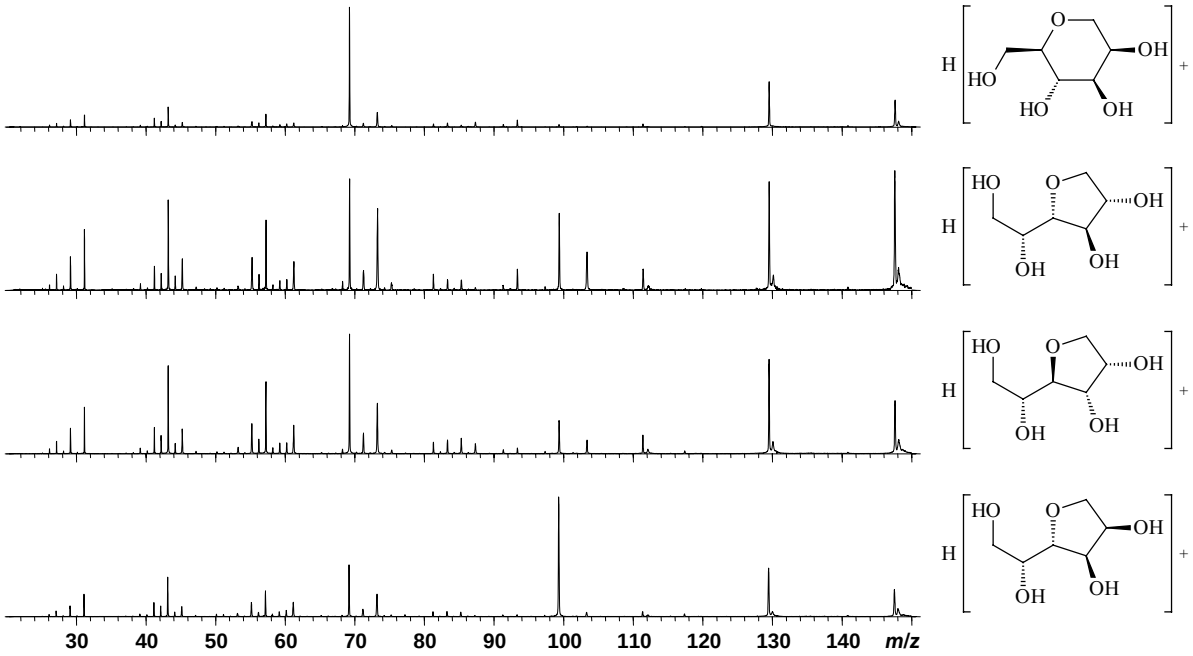


Fig. 6. MS/MS spectra from monoanhydrohexitols (2).

(2) Six that contain a 5-membered ring with a –CH(OH)–CH_2OH group attached to a carbon atom adjacent to the oxygen atom in the ring.

The two 6-membered ring compounds **4** and **7** have spectra dominated by the *m*/*z* 69 ion but differ from the two compounds discussed above, **1** and **2**, by the presence of relatively intense peaks due to the *m*/*z* 129 ion. The very low abundance or absence of *m*/*z* 61 and 85 ions in the spectra from **4** and **7** distinguishes them from the 6 samples that contain one 5-membered ring (**3**, **5**, **6**, **8**, **9** and **10**). Distinguishing between **4** and **7** is difficult but the product ion spectrum from **4** has somewhat more prominent fragment ions of *m*/*z* 73, 93 and 129 than that from **7**.

The samples **5** and **6** can be distinguished from **3**, **8**, **9** and **10** by reference to the abundance of the *m*/*z* 99 ion which is very low for the first group and among the most abundant fragment ions of the latter group. Within the subgroup, **3** can be recognised by the presence of a significant *m*/*z* 103 peak. Considering **8**, **9**, and **10**, the spectrum of **10** can be distinguished from the other two by the fact that the base peak is *m*/*z* 99 and the *m*/*z* 69 peak is only of moderate intensity, the lowest observed in the spectra under consideration. Samples **8** and **9** can be distinguished from each other by the relative intensities of the peaks due to the two pairs of ions *m*/*z* 129 and 99, and *m*/*z* 111 and 103 which are approximately 1.1 and 0.4 for **8** and 3.0 and 1.3 for **9**, respectively.

The above analysis therefore allows one to distinguish between the eight isomeric samples of RMM 164 Da on the basis of their CID spectra. Major differences in structure leading to the two classes indicated above are to some extent reflected in the differences in the spectra, but it is not straightforward to rationalise these differences in mechanistic terms.

Protonation is expected to occur on one of the four-hydroxyl groups or on the ring oxygen atom. The relatively high abundance of *m*/*z* 147 ions suggests that loss of water following protonation of a hydroxyl group is an important primary fragmentation process. The loss of a second water molecule to form the *m*/*z* 129 ion is most prominent for **8**–**10** and least important for **6**. Loss of a third water molecule to give the *m*/*z* 111 ion is of significance only for compounds **3**, **8** and **9**. The same three compounds also yield *m*/*z* 103 ions of moderate abundance and could arise from the loss of ethylene glycol from the ring in a process resembling that suggested for the formation of the *m*/*z* 85 ion in the spectra of **1** and **2**.

The *m*/*z* 99 ion is important in the spectra of **8**–**10** (base peak in **10**) and corresponds to the loss of 66 Da by the MH^+ ion. The most plausible explanation for the formation of this ion is protonation of a hydroxyl group leading to the loss of two molecules of water followed by ring cleavage to lose a formaldehyde molecule formed from the oxygen atom together with the adjacent methylene group.

The *m*/*z* 85 ion is of fairly low abundance in the spectra of **3**, **6**, **8** and **9** ($\leq$20%) but is absent from the spectra given by **4** and **7** that contain the six-membered ring, suggesting that its formation is associated with the presence of the –CH(OH)–CH_2OH side chain, although it is essentially absent from the spectrum of **10**.

The *m*/*z* 73 ion is present in the spectra of all eight isomers and corresponds to the $[MH^+ - 92]^+$ ion. It seems probable that this is the $C_3H_5O_2^+$ ion, which in the case of the 5-membered ring isomers could be formed by the side chain and the carbon atom of the ring to which it is attached, with the transfer of a hydrogen atom to the neutral species lost.

The most important ion in most of the spectra is the *m*/*z* 69 ion which is almost certainly the $C_4H_4OH^+$ ion, i.e., protonated furan, formed by loss of 96 Da. In the case of the 5-membered ring isomers, this could arise from the loss of two water molecules together with the side-chain and a hydrogen atom transfer from the ring but a more extensive rearrangement is required to rationalise its formation from the 6-membered ring species.

4. Chemometric analysis

The above discussion of the origin of the major ions formed in the CID spectra of the various isomers requires a significant prior knowledge of mass spectrometry fragmentation mechanisms. Chemometric approaches to the analysis of the data have been employed in order to establish whether classification of the data was possible without expert knowledge.

The product ion mass spectra have been subjected to principal component analysis (PCA) [10]. This technique identifies the mass values, which account for most of the spectral variance in the data set, and produces a (hopefully small) number of principal components (PC), which can be linearly combined to reproduce as much of the original data variance as possible. Each PC is a vector with an intensity (positive or negative) corresponding to each mass number in the spectrum. The assumption underlying PCA is that any spectrum in the original data set can be estimated by a linear combination of a relatively small number of PCs. Any given spectrum or sample can then be represented in a "scores" plot, which displays the sample's coordinate in principal component space.

The data from all structures were pre-processed by normalising the most intense product ion peak to 100. No other pre-processing was performed prior to analysis. First, to illustrate the use of PCA for classification purposes, spectra were acquired in triplicate from **1** and **2** and subjected to PCA. Just one PC accounted for over 70% of the variance, and Fig. 7 shows how the spectra form two distinct clusters when plotted in terms of their score for PC1. This shows that the differences between the replicate spectra were small compared with the differences between the spectra from **1**

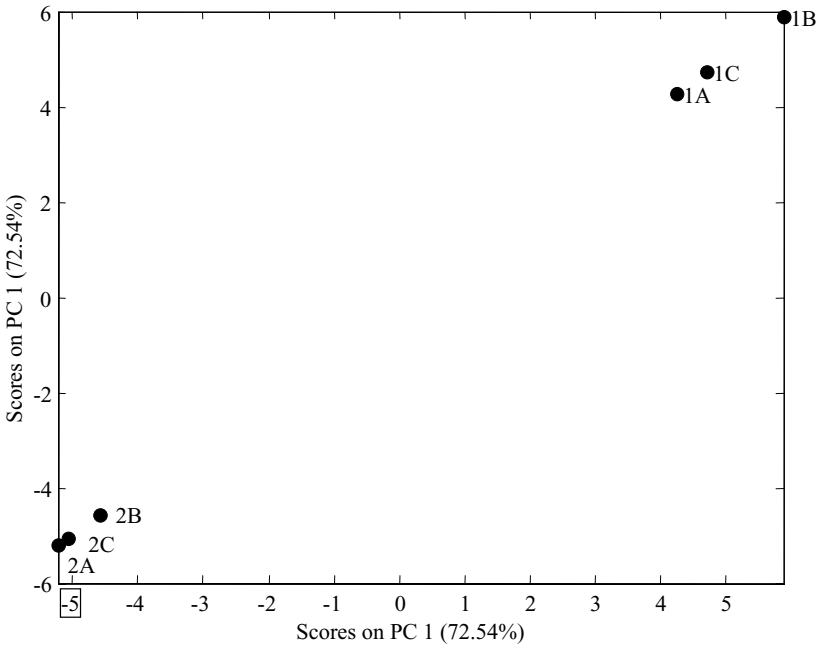


Fig. 7. Principal component plot for $C_6H_{10}O_4$ isomers.

and **2**. On this basis, we applied PCA to the spectra from **3**–**10** and found that three PC's accounted for ∼95% of the spectral variance. In this case we initially applied cluster analysis, which classifies samples based on their proximity in PC space. In this work the classification was made using the so-called K-nearest neighbour approach [10]. Fig. 8 shows the results in the form of a dendrogram, which is a convenient graphical tool for displaying similarity. Objects which form clusters on the left hand side of the plot are closer together, and hence more spectrally similar, than those to the right. It is, therefore, apparent that the spectra are clustered as [**5**, **6A**, **6B**], [**4**, **7**], and [**8**, **9**]. The spectrum from **3** is more similar to [**5**, **6A**, **6B**] than any other group, while the spectrum from **10** is dissimilar to all the other data.

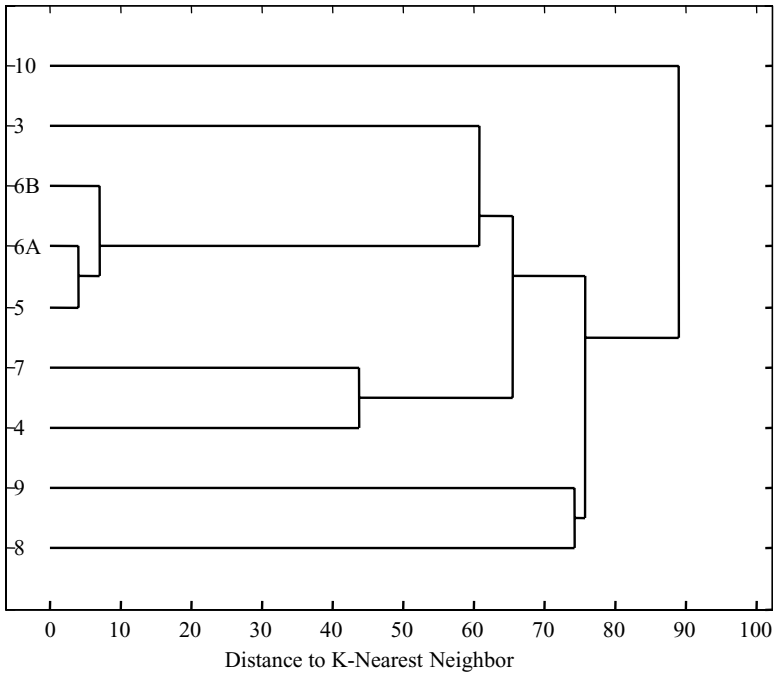


Fig. 8. Dendogram using no scaling and distance on three PCs.

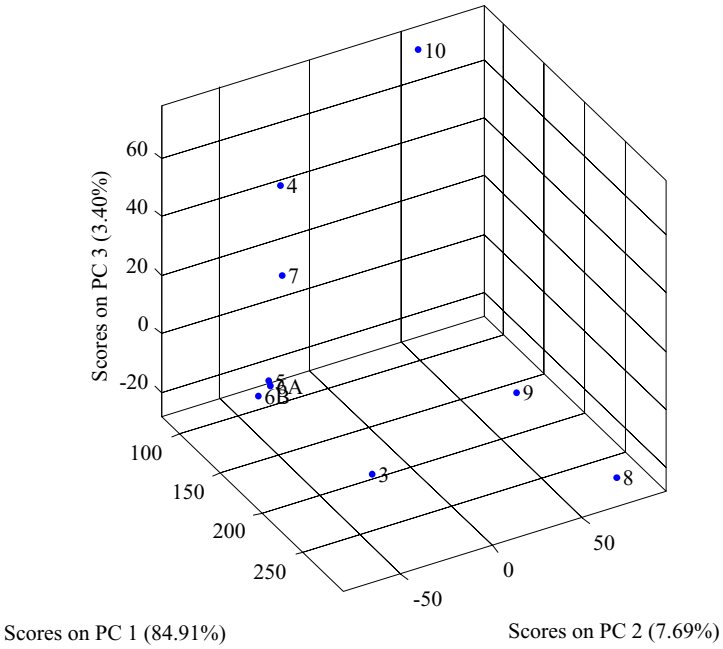


Fig. 9. Principal component plot for $C_6H_{12}O_5$ isomers.

Fig. 9 shows the scores plot in 3D from one particular perspective, and again indicates that the primary clusters are [**5**, **6A**, **6B**], [**4**, **7**], and [**8**, **9**], with **10** as an obvious outlier. This provides useful guidance as to where the spectral similarities lie within the dataset.

Although scores and cluster plots show how the spectra group, they do not identify which aspects of the spectra actually differentiate the groupings. Some useful indicators can be obtained from a PC biplot, which shows not only the scores for each sample but also shows the correlation between mass number and PC. In other words, it shows which mass numbers most strongly influence each PC. Using this approach (but not shown here) it can be confirmed that the pair **4** and **7** have relatively intense peaks at *m*/*z* 69 and 129. Differentiation of the other clusters is more difficult but can be made easier by rotating the biplot in space and viewing it from different perspectives. By this method, it is apparent a particularly intense peak at *m*/*z* 99 differentiates **10** and *m*/*z* 43 is important in the clustering of **8** and **9**.

The samples can therefore be seen, from the chemometric data, to form three, distinct groups:

4 and **7**: 6-membered ring compounds;
5, **6**: 5-membered rings;
8, **9**: 5-membered rings.

10 is quite distinct due to the dominant ion at *m*/*z* 99 and **3** is intermediate in character, but, from the chemometric data, closer to **5**, **6A** and **6B** than any other cluster.

5. Conclusion

High-energy CID product ion spectra can be used to differentiate a series of structurally similar isomeric sugars. A high degree of reproducibility of the spectra is essential if small differences in product ion intensity are to be used in differentiating the closely related structures. The chemometric analysis gives results that are in good general agreement with that obtained from interpreting the spectra. This approach however assumes no detailed spectroscopic knowledge of the data. It is therefore a useful approach for both expert and novice alike in initial classification of spectral data. Use of the biplots can be made by the experienced spectrometrists to provide interpretation aids to the peaks used in differentiation.

Interpretation of the spectra from first principles can assist in the differentiation but would be difficult to carry out on unknown compounds without labelling experiments. A combination of interpretation and classification methods provides a powerful approach to the characterisation of complex, structurally similar systems.

Appendix A. Opening of four sector ZAB-T instrument in May 1990 by Prof. John Beynon

The four sector instrument described in the above paper had BEBE geometry and utilised an array detector in order to improve sensitivity. The instrument was installed at the ICI laboratory in Wilton (England) and was officially opened by Prof. John Beynon in May 1990. The photographs show the mass spectrometry research group at Wilton, John (with the ICI Research Manager Prof. David Clark) and various British mass spectrometrists assembled for the day. This was particularly appropriate since John had spent such a long part of his career with ICI and had been very closely involved in the design of the ZAB series of instruments.

References

[1] K. Biemann, Biomed. Environ. Mass Spectrom. 16 (1988) 99.
[2] D.J. Harvey, R.H. Bateman, M.R. Green, J. Mass Spectrom. 32 (1997) 167.
[3] P. Collins, R. Ferrier, Monosaccharides, Wiley, New York, 1998.
[4] Z. Gyorgydeak, I.F. Pelyvas, Monosaccharide Sugars, Academic Press, New York, 1998.
[5] M. Tetsuo, et al., J. Chromatogr. B 731 (1) (1999) 111.
[6] T. Niwa, K. Tohyama, Y. Kato, J. Chromatogr. Biomed. Appl. 613 (1) (1993) 9.
[7] A.T. Jackson et al., Eur. Mass Spectrom. 3 (113) 1997
[8] J.H. Scrivens et al., RCMS 6 (272) 1992.
[9] H.B. Sinclair, Carbohydr. Res. 1984. 127(1) 146.
[10] M.A. Sharaf, D.L. Illman, B.R. Kowalski, Chemometrics, Wiley, New York, 1986.