Mass Spectrometry of Carbohydrates

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For mass spectrometry of carbohydrates, several types of derivatives are used, e.g. peracetyl esters, permethyl ethers, pertrimethylsilyl ethers (Lönngren & Svensson, 1974). Mainly electron-impact ionization is applied, but chemical ionization seems to be very promising (Horton *et al.*, 1974). The occurrence of a vast amount of isomers is a severe complication in carbohydrate chemistry. Only a part of the structural problems in this field can be solved by mass spectrometry. Classes of monosaccharides which differ in molecular weight can easily be recognized. Within a class of compounds, furanoid and pyranoid ring forms can be distinguished from each other. This holds also for the methyl glycosides, e.g. as encountered in methanolysis.

The characterization of the members of a class of monosaccharides, is complicated owing to the similarity of the spectra. For pertrimethylsilyl derivatives of aldohexoses (Vink *et al.*, 1972) and 2-acetamido-2-deoxy-aldohexoses (Vink *et al.*, 1974) we could demonstrate that the various isomers are distinguishable on the basis of small, but distinct and reproducible, differences in intensities of a number of peaks. The differences become more pronounced when intensity ratios are compared.

In disaccharides, the position of the glycosidic linkage has a distinct influence on the spectra. For pertrimethylsilyl derivatives of several classes of disaccharides, we could derive empirical rules for the determination of the position of the glycosidic bond (Kamerling & Vliegenthart, 1974). These rules are based partly on the presence or absence of specific peaks and partly on ratios of peak intensities. Undoubtedly also the configuration of the glycosidic bond has an influence on the spectrum, although to a lesser extent than its position. The sequence of the monosaccharide residues in a disaccharide can only be determined when both units differ in molecular weight. The spectra of higher oligosaccharides bear a lot of structural information, but the unambiguous interpretation is complicated. The investigation of a great number of isomers would be necessary to obtain sufficient reference data.

For acetylated and methylated saccharides, sometimes in combination with an aromatic aglyconic stabilizing group, other authors published similar studies (for a review see Lönngren & Svensson, 1974). Another approach to the mass-spectrometric

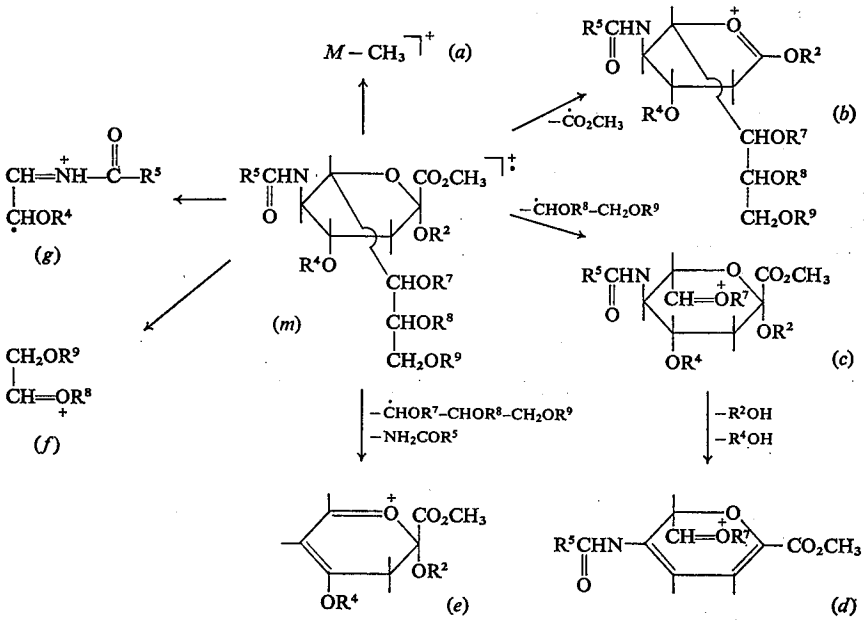

Fig. 1. *Fragmentation pattern of sialic acid*

The mass values of the fragments *a–g* are characteristic for the occurrence and position of substituents. R^2 = Me or Me_3Si; R^4, R^7, R^8, R^9 = Me_3Si and/or Ac; R^5 = Me or CH_2OMe_3Si.

Table 1. *List of identified trimethylsilyl sialic acid methyl esters*

1. *N*-Acetylneuraminic acid
2.* 2-*O*-Methyl-*N*-acetylneuraminic acid
3. 4-*O*-Acetyl-*N*-acetylneuraminic acid
4. 9-*O*-Acetyl-*N*-acetylneuraminic acid
5. 7,9-Di-*O*-acetyl-*N*-acetylneuraminic acid
6. 4,9-Di-*O*-acetyl-*N*-acetylneuraminic acid
7.* 9-*O*-Acetyl-2-*O*-methyl-*N*-acetylneuraminic acid
8.* 4,9-Di-*O*-acetyl-2-*O*-methyl-*N*-acetylneuraminic acid
9.* 4-*O*-Methyl-*N*-acetylneuraminic acid
10. 9-*O*-Lactyl-*N*-acetylneuraminic acid
11. *N*-Glycolylneuraminic acid
12. 4-*O*-Acetyl-*N*-glycolylneuraminic acid
13. 9-*O*-Acetyl-*N*-glycolylneuraminic acid
14. 2-Deoxy-2,3-dehydro-*N*-acetylneuraminic acid

* Synthetic compounds. The other compounds are isolated from natural sources.

analysis of carbohydrates comprises the investigation of reduced oligosaccharides, by using the same types of derivatives as mentioned above. In higher oligosaccharides, such as those occurring in glycosphingolipids, the sequence of the constituting classes of monosaccharides can be established (Karlsson *et al.*, 1974). In general, however, the

positions of the glycosidic bonds cannot be deduced. The 'alditol acetate method' is in a large number of cases suited to solve this type of problem (Björndal *et al.*, 1970). The method consists of permethylation of the compound (oligosaccharide, polysaccharide, glycoconjugate, etc.) followed by hydrolysis, reduction and finally by acetylation of the liberated hydroxyl groups. The obtained mixture of partially methylated alditol acetates can be analysed by g.l.c.–mass spectrometry.

Recently we developed a mass-spectrometric method for the identification of sialic acids. To this purpose use has been made of the trimethylsilyl derivatives of the sialic acid methyl esters. Based on the highly specific fragmentation pattern of these compounds, seven peaks could be selected which furnish the information necessary to determine the type and position of substituents (Fig. 1 and Table 1) (Kamerling *et al.*, 1975).

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