Gas chromatography-mass spectrometry of sugars and related hydroxy acids as trimethylsilyl derivatives[1]

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KEYWORDS: Monosaccharides, Hydroxy acids, Gas chromatography, Mass spectroscopy, Pulping.

SUMMARY: The application of gas chromatography and mass spectrometry has contributed to the rapid progress of carbohydrate chemistry during the past ten years. The methods are indispensable tools in current research on carbohydrate reactions during pulping processes.

Acyclic ether, ester, and oxime trimethylsilyl derivatives are useful for gas chromatographic analysis of alditols, aldoses, ketoses, aldonic acids, aldaric acids and uronic acids. These derivatives have been studied in detail by mass spectrometry and shown to be advantageous for structural analysis. The mass spectrometric fragmentation of glycosidic trimethylsilyl derivatives has also been investigated. An important application is the identification of products from methylation analysis.

☐ Användning av gaskromatografi och masspektrometri har bidragit till en snabb utveckling inom kolhydratkemin under de senaste tio åren. Dessa metoder är oumbärliga hjälpmedel i pågående forskning om kolhydraternas reaktioner vid cellulosaprocesser.

Acykliska eter-, ester- och oxim-trimetylsilylderivat är mycket användbara för gaskromatografisk analys av alditoler, aldoser, ketoser, aldonsyror, aldarsyror och uronsyror. Dessa derivat har studerats ingående med masspektrometri och visats vara fördelaktiga för strukturanalys. Den masspektrometriska fragmenteringen för glykosidiska trimetylsilylderivat har också undersökts. En viktig tillämpning är identifieringen av produkter från metyleringsanalys.

☐ Die Anwendung von Gaschromatographie und Massenspektrometrie hat zu einer schnellen Entwicklung der Chemie der Kohlenhydrate in den letzten zehn Jahren beigetragen. Diese Methoden sind unentbehrliche Hilfsmittel in der Forschung der Kohlenhydratreaktionen in Celluloseprozessen geworden.

Azyklische Äther-, Ester- und Oxim-Trimethylsilylderivate sind zur gaschromatographischen Analyse von Alditolen, Aldosen, Ketosen, Aldonsäuren, Aldarsäuren und Uronsäuren sehr geeignet. Diese Derivate wurden massenspektrometrisch eingehend studiert und haben sich für die Strukturanalyse als sehr günstig erwiesen. Die massenspektrometrische Fragmentierung glykosidischer Trimethylsilylderivate ist auch untersucht worden. Eine wichtige Anwendung ist die Identifizierung der Produkte der Methylierungsanalyse.

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The progress in wood chemistry including the chemistry of the pulping processes during the last two decades depends to a large extent on the advent of new and improved analytical methods. Among these, various chromatographic techniques and mass spectrometry are particularly important.

During the sixties, paper chromatography was gradually replaced by gas chromatography (GC) as the standard method for the separation of sugars. The increased versatility, speed and sensitivity of the GC technique outweighed the disadvantage that volatile and thermally stable derivatives must be prepared. A decisive step in favour of GC was the introduction of trimethylsilyl (TMS) derivatives as described by Sweeley et al. (1) in 1963. The present-day importance of GC of carbohydrates is clear from a recent review (2) which refers to more than 700 papers within the last ten years though not all aspects of the subject are covered. The majority of these papers deal with TMS derivatives.

Until mass spectrometry (MS) was introduced, convenient micro-scale methods for structural analysis of sugars were lacking. The first comprehensive papers which demonstrated the potential of MS in this field appeared in 1963 and the early work was reviewed in 1966 (3, 4). The common instrument separates (according to mass) and records positively charged ions formed from the gaseous sample ($\sim 10^{-3}$ Pa) by impact of high-energy (usually 70 eV) electrons. Since both GC and MS are gas phase methods requiring some volatility of the compounds studied, they are suitably combined. An instrument capable of recording spectra directly from the components in the effluent from a gas chromatographic column was constructed in 1964 (5). The GC-MS combination permits simultaneous separation and structure determination of components in microgram quantities in complicated mixtures and is, in many respects, an ideal analytical method. By now, GC-MS is a standard technique in carbohydrate analysis. The MS fragmentation of sugar derivatives and the structural information obtained by MS have been reviewed (6).

The basic problem in all work is to convert each species into a derivative which gives the desired information on subsequent GC and MS analysis. The main task is then to collect appropriate data (GC and MS) and to relate these data to structural features. A major part of this work is to rationalize MS fragmentations which is necessary in order to make full use of the capability of MS. Since TMS derivatives are at present the most widely used derivatives for a great variety of natural products, the value of such studies is not restricted to carbohydrate analysis.

This paper describes GC and MS investigations made primarily to meet the analytical requirements of research on pulping processes. Since the first demonstration of the utility of GC-MS for aldonolactones (7), it has been applied to most of the relevant types of monosaccharides and hydroxy acids and is now an indispensable analytical tool in this field. However, the analytical problems are similar in all carbohydrate research and lately the analytical methods involving TMS derivatives and GC-MS have brought about notable progress particularly in biochemistry and medicine (6).

GC-MS of acyclic derivatives

Gas chromatography

The use of the same type of derivative for all common sugar-related compounds is highly desirable in GC work. Ideally, each compound should be converted into a derivative separable as one GC peak from other components and suitable for MS characterization. The TMS derivatives have permitted methods to be devised (I) which very nearly fulfill these requirements.

Aldoses and ketoses can be conveniently analysed as acyclic oxime TMS derivatives (I). A separation of some

[1] Inaugural dissertation based on references I—X.

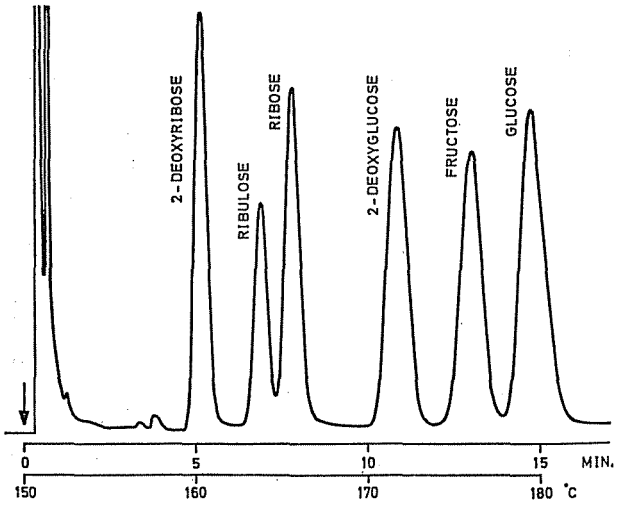

Fig. 1. Programmed gas chromatographic analysis on OV-1 of a mixture of related aldoses, ketoses, and 2-deoxyaldoses as acyclic oxime trimethylsilyl derivatives.

important sugars is illustrated in *fig. 1.* Although *syn* and *anti* isomers are formed for each sugar, analytical conditions making them coincide can usually be found. The oxime derivatives are of particular value for ketoses since other suitable methods are lacking.

On analysis of hydroxy acids, the possibility of preparing TMS esters and ethers simultaneously offers many advantages (I). Multiple peaks from lactone-forming acids are avoided by preparing the derivatives from salts. In *fig. 2* an analysis of a series of aldonic acids is shown. A previously developed technique which makes use of aldonolactone TMS ethers for GC (8) and MS (7, 9) is useful in combination with this more universal approach.

Uronic acids can be analysed as acyclic TMS derivatives of their oximes (I).

Mass spectrometry

In MS analysis the acyclic derivatives are advantageous because their spectra are usually the easiest to interpret. The MS fragmentation and the use of MS for structural

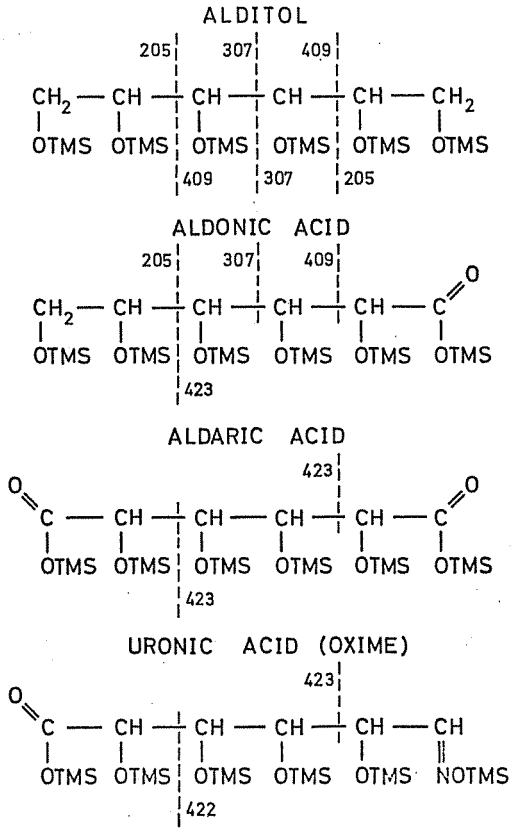

Fig. 3. Characteristic mass spectrometric chain cleavage fragmentations of acyclic trimethylsilyl derivatives of C_6 compounds.

analysis were dealt with in papers on alditols (II), aldonic and deoxyaldonic acids (III), and aldaric and deoxyaldaric acids (IV). The MS characteristics of the oxime TMS derivatives of aldoses and ketoses (I) are analogous to those of the *O*-methyloxime TMS derivatives recently reported by Laine and Sweeley (10).

The large number of fragmentation-promoting functional groups in the derivatives causes facile fragmentation of the initially formed molecular ion of mass M

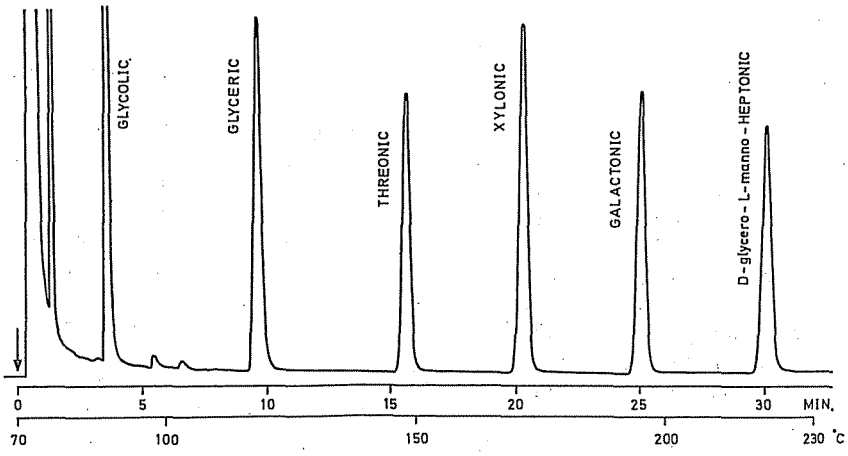

Fig. 2. Programmed gas chromatographic analysis on OV-1 of a series of aldonic acids as acyclic trimethylsilyl derivatives.

and a low abundance of the high-mass ions. However, the molecular weight of the TMS derivatives can usually be deduced from M-15 peaks due to ions formed by the loss of a silicon-linked methyl group.

The origin and the masses of some of the structurally most significant fragment ions are shown in *fig. 3* for representative derivatives. These ions are formed by cleavage of a bond in the carbon chain and provide direct structural evidence. Characteristic mass shifts make possible the identification and localization of carbon-linked as well as oxygen-linked substituents. Favoured chain cleavage and charge retention adjacent to ether oxygen atoms is observed and can be used to determine the position of deoxy groups.

The ether-ester derivatives in particular exhibit unusual rearrangement fragmentations (IV). Structurally most diagnostic is a McLafferty-type rearrangement of a TMS group in α,β-dihydroxy carbonyl compounds *(fig. 4)*. Since this rearrangement was judged to be of general interest in organic mass spectrometry it was made the subject of a detailed investigation (V).

In a GC-MS study of the TMS derivatives obtained on direct silylation of tetrulose and pentuloses (VI), the rearrangement was indicative of *keto* forms. Furanose and enediol derivatives, characterized by MS, were also obtained, emphasizing the advantages of oxime TMS derivatives for ketoses. The rearrangement also aided in the identification of previously unknown products from methanolysis of galacturonic acid (VII) and oxygen-alkali treatment of methyl β-glucopyranoside (11).

GC-MS of cyclic derivatives

Two or more isomeric species are usually obtained when derivatives are prepared from sugars in the hemiacetal form. Although this complicates analysis, such derivatives are extensively used both in GC and MS work.

Mass spectrometry

The first comprehensive MS studies of TMS derivatives of pentoses (VIII) and hexoses (IX) demonstrated close analogies with the spectra of methylated sugars (3). The fragmentation pattern was deduced from the spectra (X) of TMS derivatives of a large number of partially methylated aldoses. The results were confirmed by De Jongh et al., in studies based on specifically deuterated sugars (12).

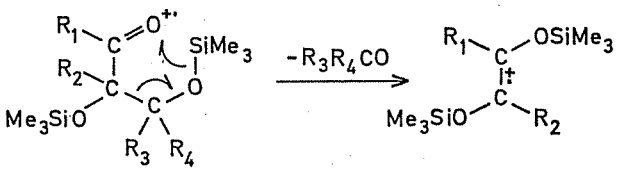

Fig. 4. McLafferty-type rearrangement of a trimethylsilyl group in α,β-dihydroxy carbonyl compounds.

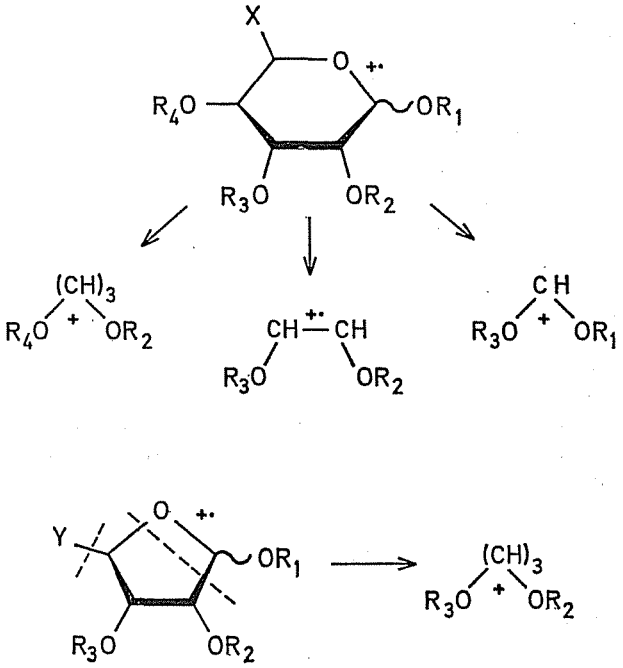

Fig. 5. Prominent ions formed from pyranoid and furanoid trimethylsilyl and methyl derivatives.

Three characteristic, abundant ions are obtained from the pyranose derivatives whereas one predominant ion is formed from the furanose derivatives *(fig. 5)*. Different *X* and *Y* groups do not influence these basic fragmentations appreciably. They are the same with uronic acids (VII) and many other classes of carbohydrates. The spectra of diastereomers are similar.

Methylation analysis

A standard method for the structure elucidation of oligo- and polysaccharides is methylation followed by hydrolysis or methanolysis and analysis of the partially methylated sugars formed. The basic analytical problem is the determination of the number and position of the methyl groups in these sugars.

The discovery of the fragmentation analogies of methyl and TMS derivatives made possible the deduction of this information from mass spectra of the TMS derivatives. Simple and universally applicable identification schemes were worked out for the pyranose forms of aldopentoses (VIII) and aldohexoses (IX). These are based on the mass shifts of 58 mass units resulting from the exchange of a methyl group for a TMS group in fragment ions. The most essential information is obtained directly from the masses of the ions depicted in fig. 5. Ethylated sugars can be analysed similarly (IX). The method described is applicable to glycosides (e.g. from methanolysis) and involves no loss of structural information by chemical conversions. This contrasts with MS methods introduced later which make

use of alditol acetates (13) and *O*-methyloxime TMS derivatives (10).

Application of GC-MS in wood chemistry and pulping research

All pulping and bleaching processes are accompanied by some degradation of the cellulose and a better knowledge of the reactions involved improves the possibility of controlling the yield and quality of wood pulps. Equally, and sometimes even more important are the reactions of the hemicellulose. Here, the insufficiently known structures of several important types of hemicelluloses complicate the study of their reactions.

Much progress has been made during the last few years regarding the cellulose and hemicellulose reactions and the structure elucidation of hemicelluloses. Of great importance in this research is the determination of individual carboxyl and carbonyl groups present along the polysaccharide chains and as end-groups. Relevant information can also be obtained from studies of hydroxy acids and other compounds released into the solution. In itself an increased knowledge of the composition of the spent liquors is important in regard to water pollution and recycling in the pulp mill.

In this laboratory, the GC-MS methods described have been combined successfully with chromatography on ion exchangers in applied analytical work. The technique has been used for several years in detailed studies of the carbohydrate reactions during pulping processes. Information on end-groups and other modified units in the polysaccharides is obtained by analysis of the corresponding compounds formed on hydrolysis. Among the studies relying to a large extent on GC-MS are investigations of pine sulfate (14), spruce sulfite (15), and several other pulps. End-groups formed during alkali-cooking of cellulose were analysed unmodified and after reduction to alditol end-groups (16). Oxygen-alkali treatment of cellulose (17, 18) and birch xylan (19) was studied under conditions simulating oxygen bleaching. Spent liquors from oxygen-alkali cooking of birch (20) and oxygen-alkali treatment of cellulose (18, 21) and xylan (19) were analysed, and monocarboxylic and dicarboxylic (18, 20) acids, including several new species, were identified by GC-MS. Model studies of the products and reactions of sugars of low molecular weight are also informative as demonstrated for oxygen-alkali treatment of cellobiitol and glucose (22). Similar GC-MS methods have been adopted by other research groups for related studies (23, 24).

The usefulness of the procedure for methylation analysis was demonstrated in identifying aldobiouronic acid anhydrides (25) and oligomeric acids from hemicelluloses (26, 27). The technique has been extended to the identification of ethylated (28) and hydroxyethylated (29) glucoses in studies of cellulose derivatives. The knowledge of the general fragmentation behaviour of glycosidic TMS derivatives was also used in the identification of isomerization products of uronic acids (30, 31) and for structure determination of glucopyranosylglycolic acids (32).

Acknowledgements

I wish to express my special thanks to Professor Olof Samuelson for his constant encouragement and constructive criticism. I am also greatly indebted to Miss Mary Lundin and Mr Åke Andersson for experimental assistance and to other colleagues and friends for valuable assistance and stimulating cooperation.

Thanks are due Professor Erik von Sydow for his kind permission to use the mass spectrometer at the Swedish Institute for Food Preservation Research.

The financial support of the Swedish Board for Technical Development is gratefully acknowledged.

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(Manuscript received March 22, 1974)