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MASS SPECTROMETRY OF THE TRIMETHYLSILYL ETHERS OF 2-KETOHEXOSES

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Abstract—The mass spectra of the trimethylsilyl ethers of D-fructose, L-sorbose, methyl-D-fructopyranose and methyl-L-sorbofuranose are presented. The interpretation of the fragmentation is supported by the use of deuterium labelled analogues. The preparation of 1,1-d_2 and 6,6-d_2-L-sorbose is reported. The mass spectra of the trimethylsilyl ethers offers a method to distinguish 2-ketohexoses from aldohexoses, and to identify the existing ring structure. The characteristic peaks are 2-ketohexoses *m/e* 437; aldohexoses *m/e* 435, pyranose ring *m/e* 204, furanose ring *m/e* 217.

MASS spectrometry has found many useful applications in carbohydrate chemistry. The earlier results were summarized in a review by Kochetkov and Chizhov.[1] Recently, the use of trimethylsilyl (TMS) ethers has been introduced and the fragmentation patterns of the TMS ethers of aldoses[2, 3] di- and oligosaccharides[2, 4] and various other sugar derivatives[5] have been published. After the completion of our work a fine detailed analysis of the mass spectra of the TMS ethers of aldohexoses appeared by DeJongh and co-workers.[3] Furthermore, the mass spectra of the ketohexose TMS ethers were published.[6] No detailed analysis was offered, but, the pyranose, furanose and open chain isomers were separated and identified.

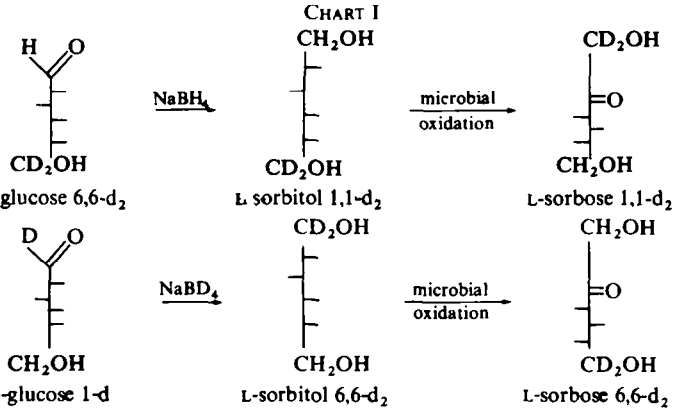
In connection with another problem, we were interested in the mass spectrometry of ketohexoses. In this paper we report our analysis of the fragmentation of the TMS ethers of 2-ketohexoses and their methyl glycosides.

The mass spectra of these compounds were distinctly different from those of the isomeric aldose[2, 3] derivatives. There were, however, several common fragments with analogous genesis. As in the case of other sugars,[1] the fragmentation of stereoisomers differed only in the relative intensity of some peaks. Thus, the spectra of the TMS ethers of D-fructose and L-sorbose as well as the corresponding methyl pyranosides, were almost identical. The analogous mode of fragmentation of the ketoses and the methyl pyranosides showed clearly that both penta-TMS-fructose and sorbose possess a pyranose structure.* Furanose derivatives such as 2-methylfructofuranose gave rise to an entirely different type of spectrum.

As it will be shown later, the spectra of the deuterium labelled derivatives, heptadeuteriofructose, 1,1-d_2- and 6,6-d_2-sorbose, shed light on the genesis of some important peaks. The two dideuteriosorboses were prepared from appropriately labelled glucoses by the reduction-oxidation sequence shown on Chart 1.

* Minor products of the silylation were the corresponding furanose and open chain derivatives.[6]

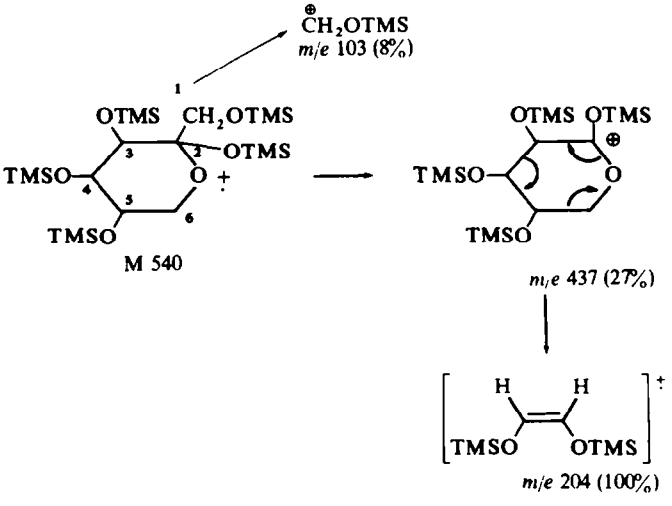
CHART I



Fructose and sorbose (Fig 1)

The molecular ion of penta-TMS-sorbose (or fructose) was barely observable. Loss of a silicon bonded Me group, however, gave rise to a small M-15 peak, at *m/e* 525.† Fission of the C—1–C—2 bond produced an intense peak at *m/e* 437. Since this cleavage is negligible in the case of aldohexoses,[2, 3] the intense *m/e* 437 peak is characteristic of 2-ketohexoses. The fragment retained both deuteriums attached to C-6 (6,6-d_2-sorbose) and none of the deuteriums at C-1 (1,1-d_2-sorbose). The *m/e* 103 peak arose only partially by this fission because the spectrum of 1,1-d_2-sorbose showed both *m/e* 103 and *m/e* 105 in about equal intensity. The common dominant peak of pyranose derivatives[2, 3] (aldo-, keto-, and glycosides) *m/e* 204, can form from *m/e* 437 or from the molecular ion by cyclic fragmentation as shown on Path A. This fragment must contain carbons 3, 4 or 5 since it retained none of the deuteriums introduced at C-1 or C-6.

Path A



† Because of their low intensity (ca. 0·05%) the M-15 peaks are not shown in the figures.

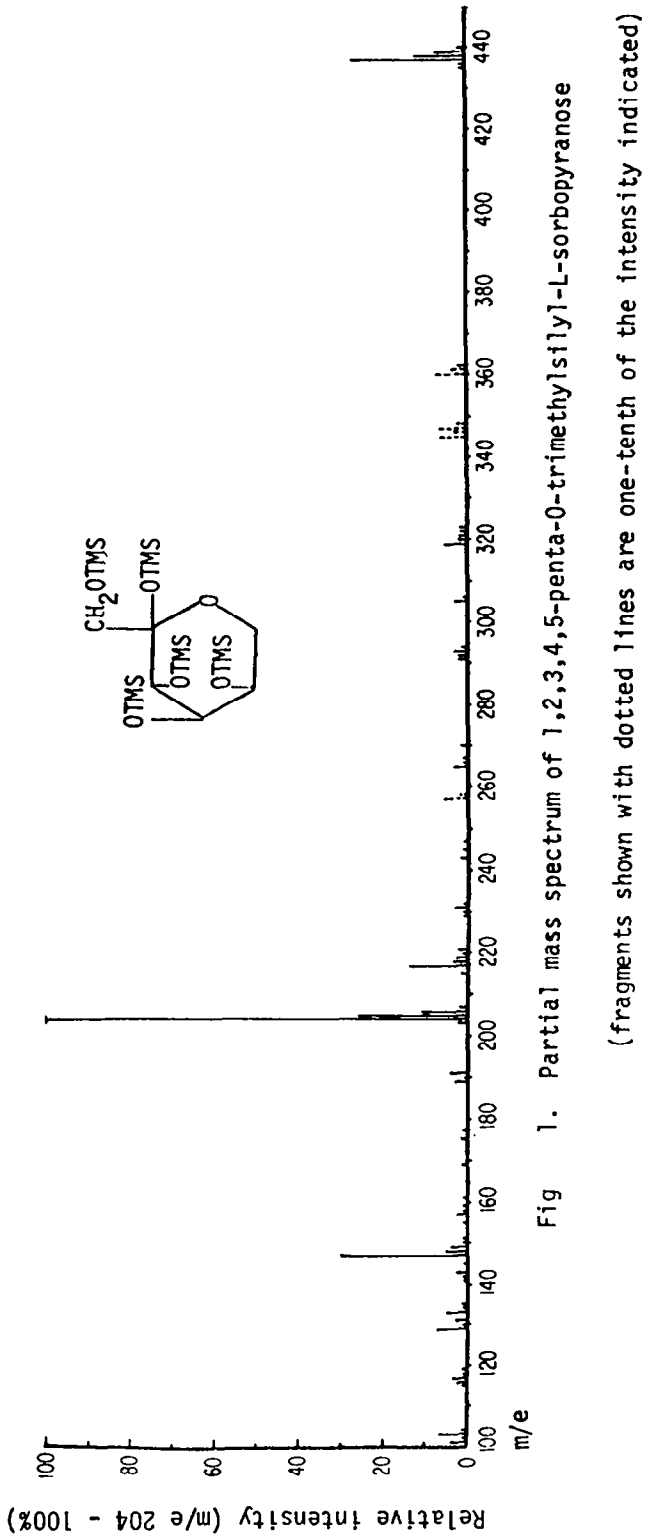
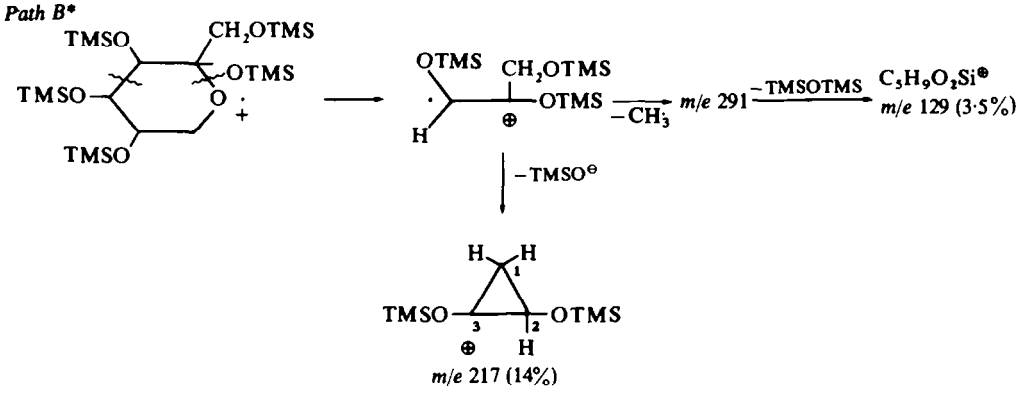


Fig 1. Partial mass spectrum of 1,2,3,4,5-penta-O-trimethylsilyl-L-sorbopyranose

(fragments shown with dotted lines are one-tenth of the intensity indicated)

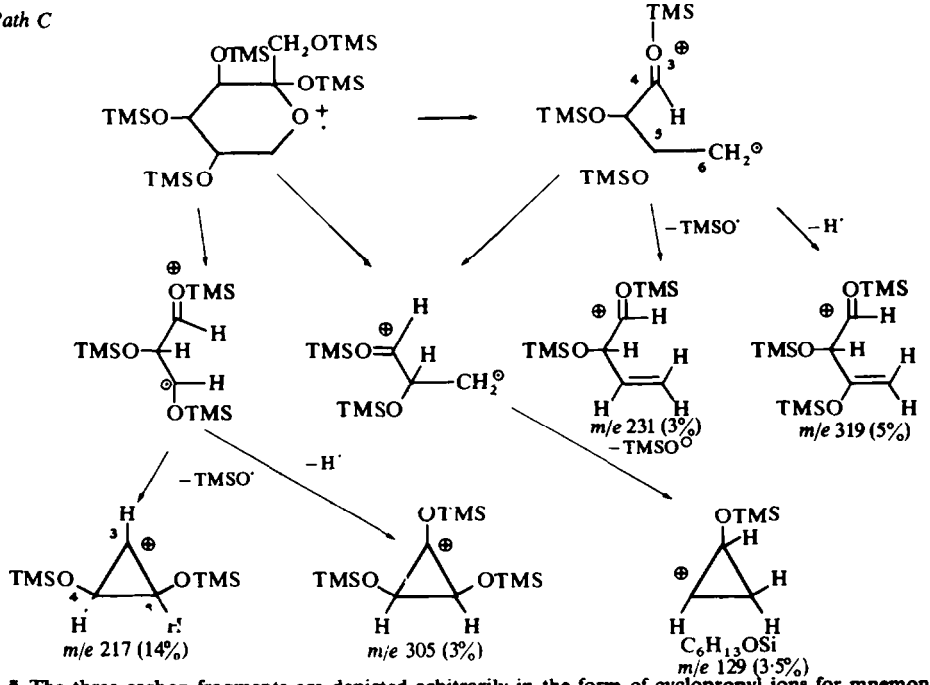
A route leading to fragments *m/e* 217 and 129 is depicted on Path B. The pathway to *m/e* 129, originally proposed by DeJongh[3] in the aldose series, is supported by the high resolution spectrum where *m/e* 129 appeared as an equal intensity doublet corresponding to $C_5H_9O_2Si$ and $C_6H_{13}OSi$. This, and the presence of *m/e* 129 and 131 in about equal intensity in the spectrum of both 1,1-d_2- and 6,6-d_2-sorbose indicates that there is another route leading to these ions which involves C-6.

*Path B**



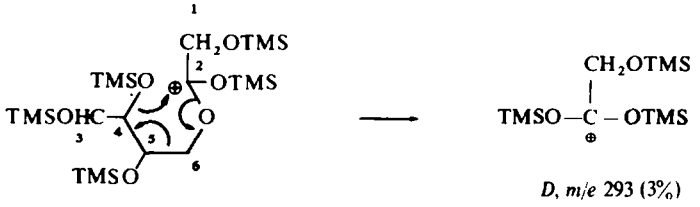
The spectra of the labelled analogues indicated that *m/e* 217 also originated from two different sources: C-1, C-2, C-3 (Path B) and C-3, C-4, C-5 (Path C). The C-6 containing fragments the genesis of which is depicted on Path C showed the expected two mass unit shift in the spectrum of 6,6-d_2-sorbose.

Path C



* The three carbon fragments are depicted arbitrarily in the form of cyclopropyl ions for mnemonic reasons.

The origin of *m/e* 305 was somewhat confusing. The spectra of 6,6-d_2-sorbose exhibited peaks at *m/e* 305, 306 and 307 (2:1:2) while fructose-d_7 showed only one peak at *m/e* 307. This indicated that only part of *m/e* 305 formed according to Path C. The other part which contains C-6 must be the result of a rearrangement involving TMSO migration. Another rearrangement ion, *m/e* 293 (D) was formed by TMSO migration as shown.

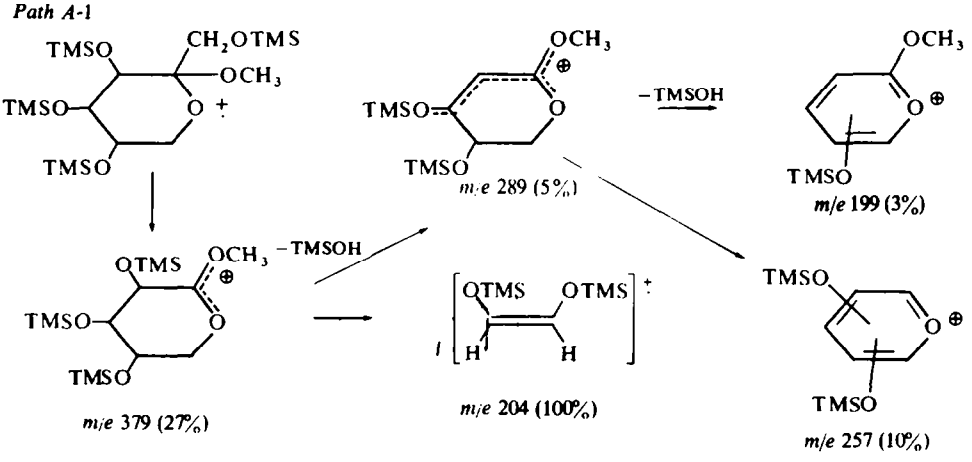


Analogous rearrangements were reported with other carbohydrate derivatives.[1-3] Furthermore, rearrangement ion D retained the deuteriums at C-1 and shifted to *m/e* 235 in the spectrum of the methylglycosides. (Path B-1.)

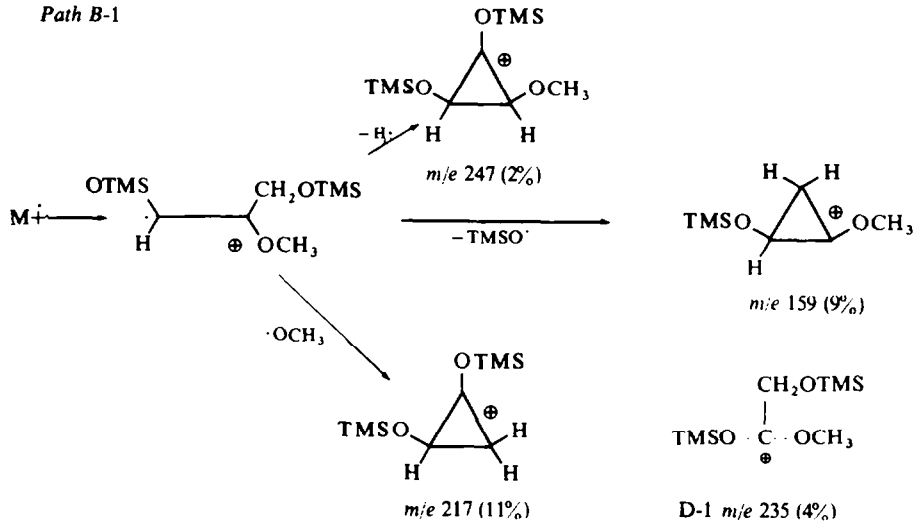
It was reassuring to compare the fragmentation pattern of sorbose or fructose with that of heptadeuteriofructose. All fragments showed the shift required by the assigned structure.

Methylglycosides, pyranoses (Fig 2)

As mentioned earlier TMS ethers of the isomeric methyl pyranosides (α- and β-D-fructo and L-sorbo) gave rise to practically identical mass spectra and the fragmentation followed paths analogous to the 2-ketohexose TMS ethers. The spectral distinctions were readily explained by: (a) the shift of 58 mass unts for ions containing OMe instead of OTMS, and (b) additional peaks representing loss of both MeOH and TMS-OH, where previously, only TMS-OH was eliminated. Loss of the former seems to be favored in the formation of the two ions shown on Path A-1.



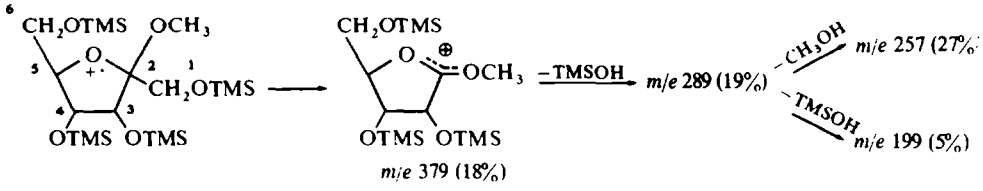
Path B-1 also led to two high intensity fragments.



Fragmentation analogous to Path C explained the genesis of *m/e* 305 (3%), 129 (9%) and also showed an alternative for *m/e* 217 (11%). Rearrangement ion D-1 showed the expected 58 mass unit shift and appeared at *m/e* 235.

Furanoses (Fig 3)

The TMS ethers of the two isomeric 2-methylfructofuranoses showed a characteristically different fragmentation from the pyranose derivatives. The most striking difference was that *m/e* 204, the base peak of the pyranoses, was absent from the spectrum of methylfructofuranose TMS ethers; instead, *m/e* 217 dominated the spectra. Loss of C-1 gave rise to fragment *m/e* 379 which, being a furan, cannot undergo a cyclic fragmentation to produce *m/e* 204 (as the isomeric pyrans favor) but instead, by subsequent loss of TMS-OH and methanol gave abundant ions: *m/e* 289, 257 and 199. Again, the loss of methanol over TMS-OH was favored.



The formation of *m/e* 159, 217 and 247 followed the same pathway as shown on Path B-1.

The *m/e* 147 fragment, one of the dominant peaks of the spectra, has been thoroughly covered in previous papers; [3, 7] it needs no further comment. Likewise, *m/e* 89, which forms analogously in the case of the methyl glycosides,[7] and *m/e* 73, common to the spectra of all TMS ethers need not be discussed. The latter two are not shown in the partial spectra presented.

$(CH_3)_2\overset{\oplus}{S}iOSi(CH_3)_3$ *m/e* 147 (30–40%)

$(CH_3)_2\overset{\oplus}{S}iOCH_3$ *m/e* 89 (8%)

$(CH_3)_3\overset{\oplus}{S}i$ *m/e* 73 (70–100%)

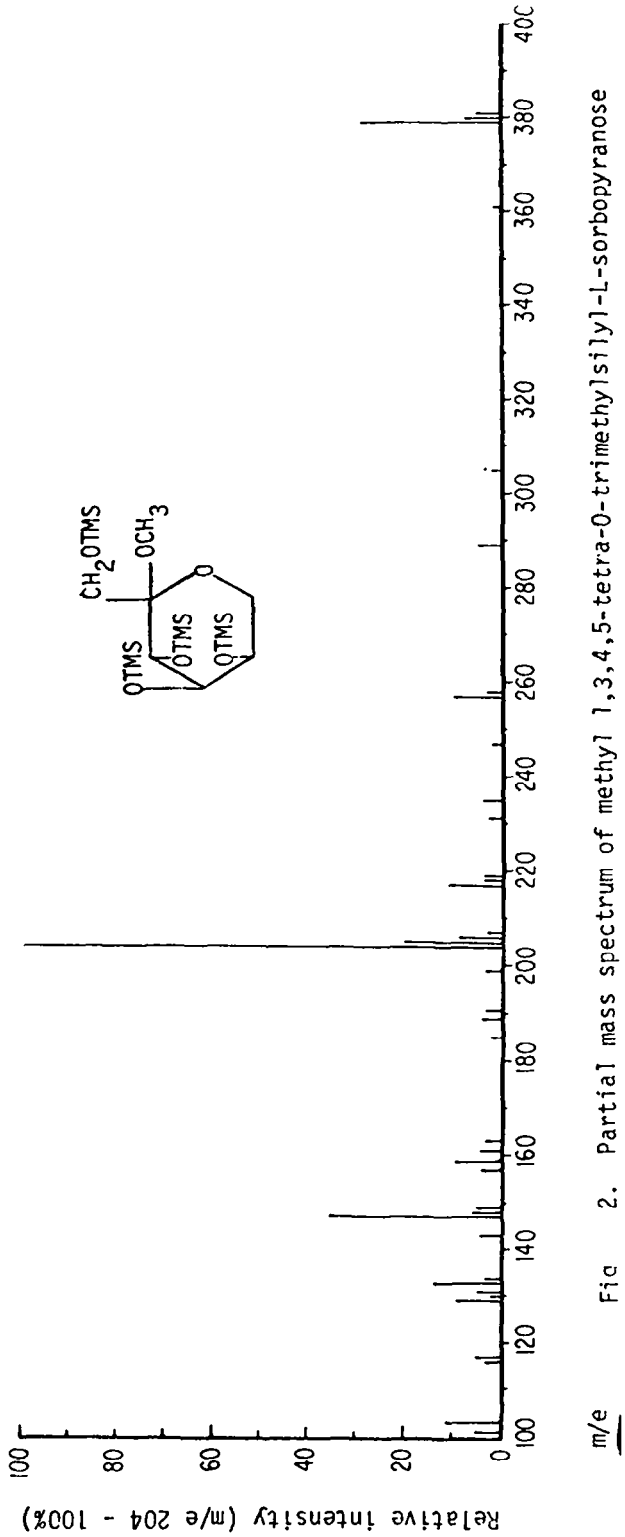


Fig. 2. Partial mass spectrum of methyl 1,3,4,5-tetra-O-trimethylsilyl-L-sorbopyranose

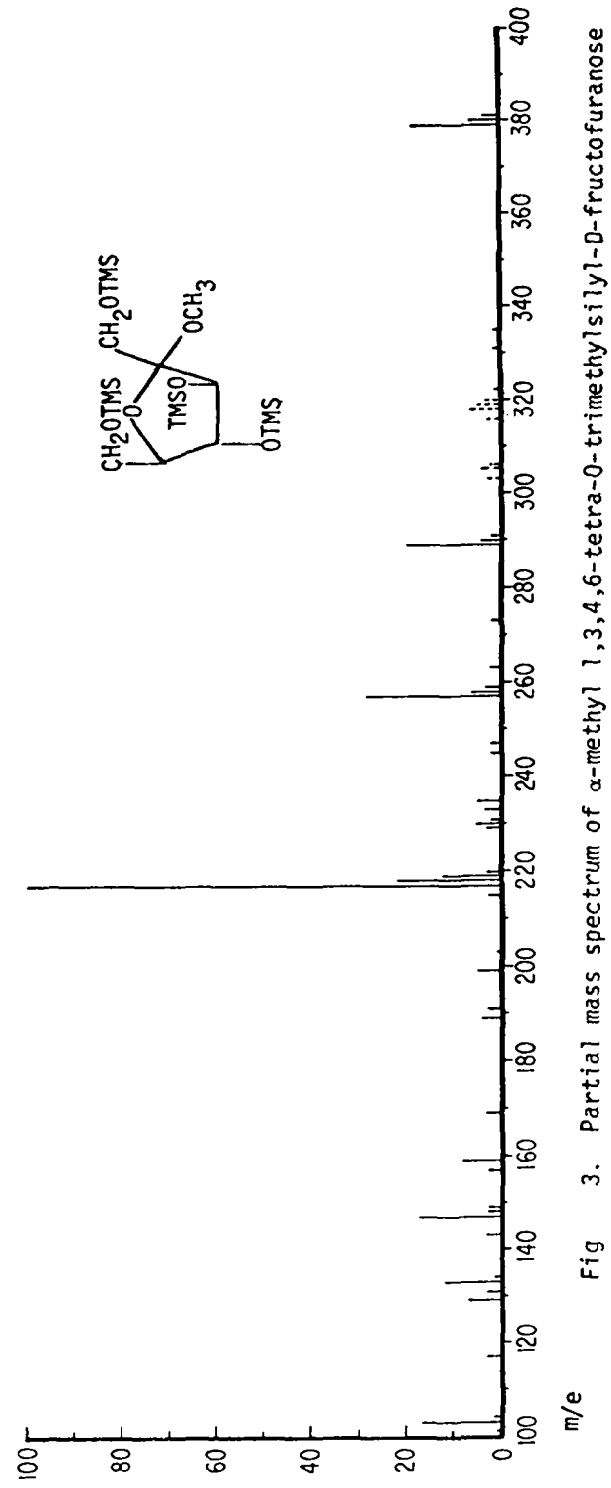


Fig 3. Partial mass spectrum of α-methyl 1,3,4,6-tetra-O-trimethylsilyl-D-fructofuranose

(fragments shown with dotted lines are one-tenth of the intensity indicated)

Mass spectrometry offers a convenient method to distinguish aldohexoses from ketohexoses and to identify the existing ring structure. In the following we summarize the main features which distinguish the mass spectra of these compounds.

In all cases investigated, pyranosides gave a large *m/e* 204 peak while the spectrum of furanosides was dominated by the *m/e* 217 fragment.[6] Cleavage of the C-1–C-2 bond in the 2-ketohexose series gave characteristic fragments which appeared at *m/e* 437 for silylated ketoses and at *m/e* 379 for the methyl glycosides. This cleavage was minimal in the aldose series;[2, 3] instead, consecutive loss of $CH_3^{\cdot}$ and TMSOH gave rise to *m/e* 435 for aldohexoses and *m/e* 377 for the methyl glycosides. 2-Ketopyranosides, then, are recognized by the combination of *m/e* 204 and 437 fragments, while 2-ketofuranosides[6] are identified by the *m/e* 217 and 437 peaks. In case of simple substituted derivatives the fragments shift according to the substitution. Thus, ethylgalactofuranoside[3] gives rise to the characteristic *m/e* 217 and 391 fragments.

EXPERIMENTAL

All mass spectra were recorded on an L.K.B. mass spectrometer at 70 EV and 8 sec scan-time. The silylation mixtures were introduced through the VPC inlet and the spectra of the pure TMS ethers (major peak) were measured at approximately the same intensity.

Preparation of trimethylsilyl ethers.[8] To a soln of the appropriate sugar (40 mg) in dry pyridine (5 ml) was added 0·5 ml hexamethyldisilazane and 0·5 ml trimethylchlorosilane. The mixture was allowed to stand overnight at room temp. A small portion of the pyridine soln was diluted with ether and injected into the VPC inlet.

L-*Sorbose*-1,1-d_2. Sodium borohydride (10 mg) was added to the soln of D-glucose-6,6-d_2[9] (50 mg) in 1 ml water. The soln was allowed to stand at room temp for 1 hr then a drop of AcOH was added and evaporated to dryness. A control experiment indicated that all the starting material at this point had been converted to sorbitol. The residue was dissolved in 5 ml MeOH and evaporated to dryness. This procedure was repeated 5 times to insure complete removal of boric acid. The crude L-sorbitol-1,1-d_2 was subjected to microbial oxidation utilizing *acetobacter suboxydans.*[10] The culture utilized was thoroughly washed with distilled water to remove traces of sugars or sugar alcohols. The fermentation broth was filtered and the filtrate was freeze-dried, yielding a solid which was used directly for silylation.

L-*Sorbose*-6,6-d_2. This material was prepared by the procedure described above utilizing D-glucose-1-d[9] as starting material. The reduction was carried out with sodium borodeuteride in D_2O.

Methylglycosides. The preparation of α- and β-methylfructofuranoside and α-methylfructopyranoside was carried out by the method of Augestad *et al.*[11] Methylsorboside was prepared according to Arragon.[12]

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