CHROM. 5688

A gas chromatographic–mass spectrometric study on TMS derivatives of sorbose, 2-keto-L-gulonic acid and ascorbic acid

Within the scope of our work on the oxidation of monosaccharides[1], we investigated the platinum-catalysed oxidation by oxygen of sorbose to 2-keto-L-gulonic acid (KGA) in an aqueous alkaline medium. In order to follow the course of the reaction we are developing a quantitative analytical method for sorbose and KGA, based on previous work on the gas chromatography of silylated monosaccharides and their corresponding acidic oxidation products[2]. However, especially for KGA, we obtained poor correlation between the concentration in a sample and the corresponding response in a flame ionisation detector (FID) of a gas chromatograph. Moreover, their is an overall discrepancy between the actual responses in the FID and predicted calculated ones[2]. These two phenomena are schematically illustrated in Fig. 1.

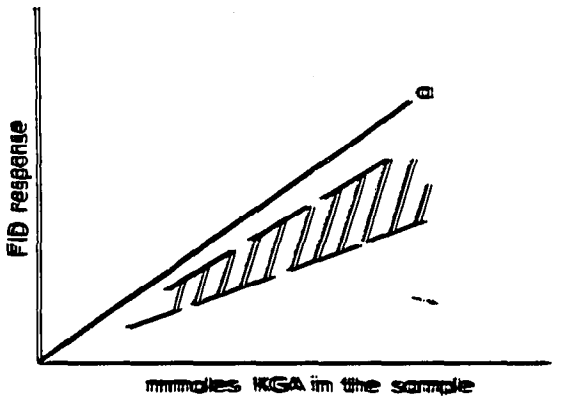


Fig. 1. FID response *vs.* concentration of KGA. Line a represents the response calculated for fully silylated KGA according to ref. 2. The shaded area represents the responses measured.

The KGA signal appeared to get influenced by both the column temperature and the method of injection, which can be either direct on the column packing or in a free space in the injection port.

Experimental

Gas chromatography. In order to investigate the phenomena mentioned above a standard sample of silylated KGA was prepared. After dissolving 50 mg Na-KGA in 200 ml dimethyl sulphoxide (DMSO), 150 μl trimethylsilyl chloride and 300 μl hexamethyldisilazane were added. The resulting two-phase system was shaken for 15 min and allowed to stand for 24 h at room temperature in order to complete the silylation reaction and to separate the two phases. Subsequently, 0.5 μl of the upper layer, which consisted mainly of silylated KGA and the excess of silylating agent, was injected into the gas chromatograph direct on the column packing.

A typical chromatogram is shown in Fig. 2. The relative abundance of the two smaller peaks P1 and P2 as a function of the column temperature is given in Fig. 3. The total signal (P1 + P2 + P3), as compared with the signal of an added internal standard *m*-C_{12}, is hardly affected by the column temperature. (Retention time data are given in Table I.)

Gas chromatography–mass spectrometry. In order to elucidate the structure of P1, P2 and P3, GC-MS has been applied to the sample. The experimental conditions were: apparatus, LKB (Shimadzu) 9000; GLC conditions, as given in Fig. 2; column temperature, 135°; molecular separator, Ryhage type, 200°; temperature of ion source, 200°.

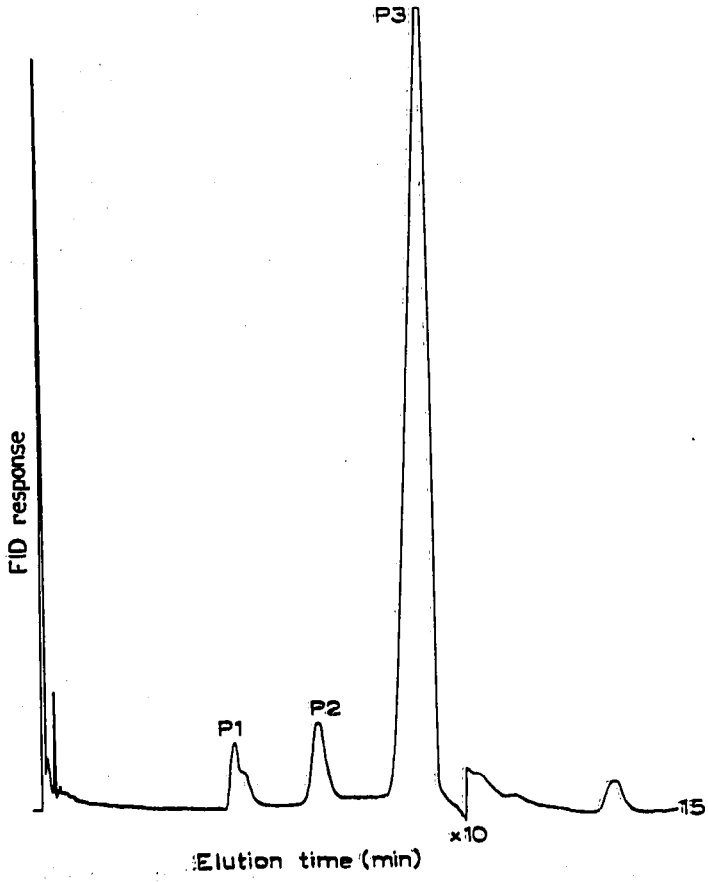


Fig. 2. Gas chromatogram of silylated 2-keto-L-gulonic acid. Apparatus: Shimadzu; column: 3 m × 3 mm I.D. stainless steel; stationary phase: 80–100 mesh HP Chromosorb G; liquid phase: 1% OV-17; carrier gas: He, 35 ml/min; FID: air, 1 l/min and hydrogen 45 ml/min; column temperature: 130–190°.

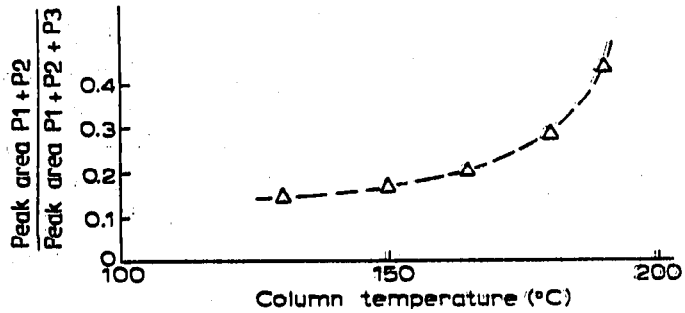


Fig. 3. Relative abundance of P1 + P2 as a function of the column temperature.

The resulting total ion monitor chromatogram was essentially the same as the chromatogram given in Fig. 2. The mass spectra of P1, P2 and P3 are shown in Figs. 4–6.

TABLE I

RETENTION TIME DATA RELATIVE TO α-GLUCOPYRANOSE (= 32 min)

Column: 3 m × 3 mm I.D. OV-17 on a.w. Chromosorb; temperature, 130°.

	Relative retention time
Sorbose	
α- or β-sorbofuranose	0.58
β-sorbopyranose (main peak)	0.85
2-Keto-L-gulonic acid KGA	
α- or β-furanose form of KGA (P1)	0.90
α-pyranose form of KGA (P2)	1.22
β-pyranose form of KGA (P3)	1.64
Ascorbic acid[1] (The same peak results from silylated ascorbic acid-Na salt.)	1.83

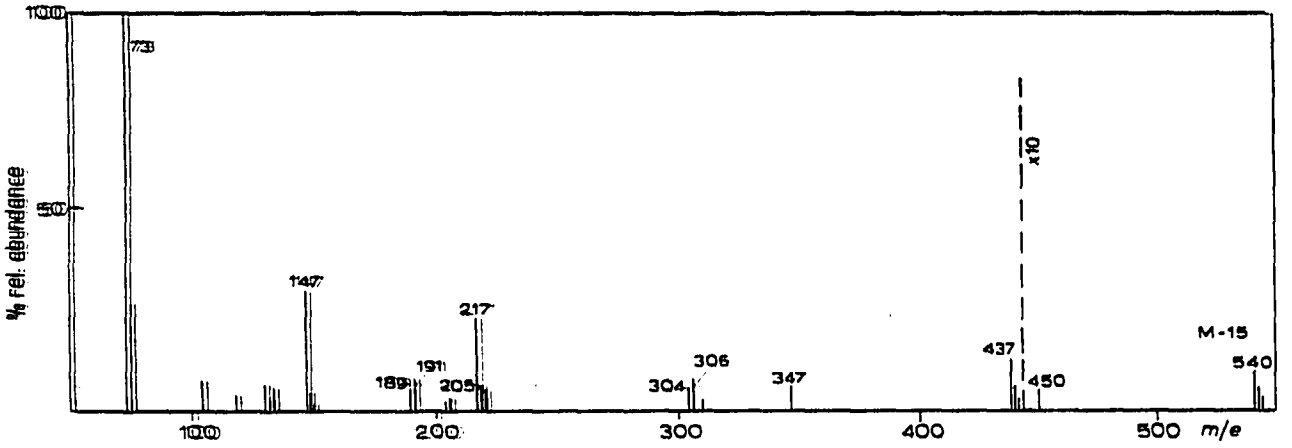


Fig. 4. Mass spectrum of P1.

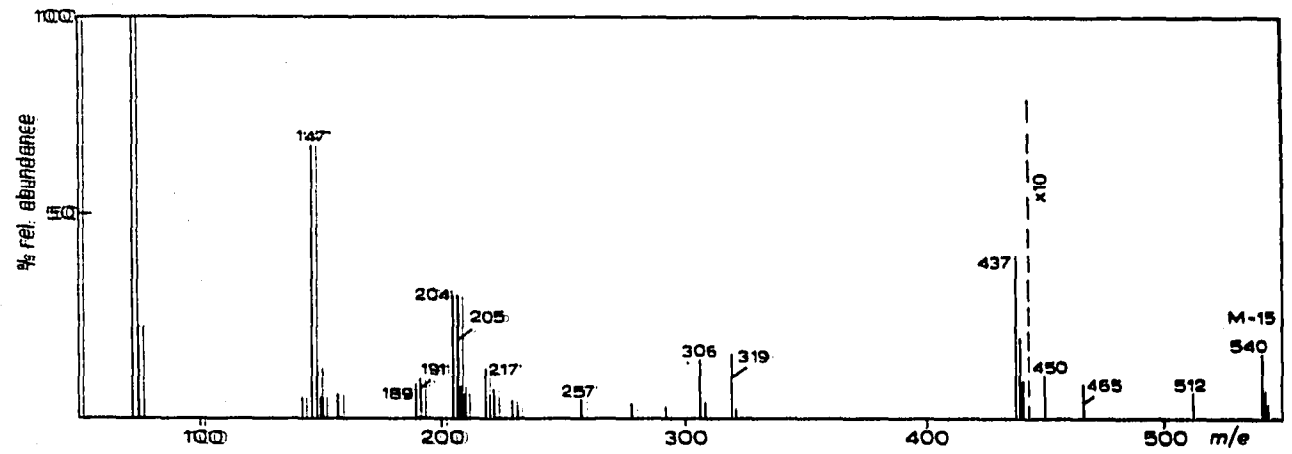


Fig. 5. Mass spectrum of P2.

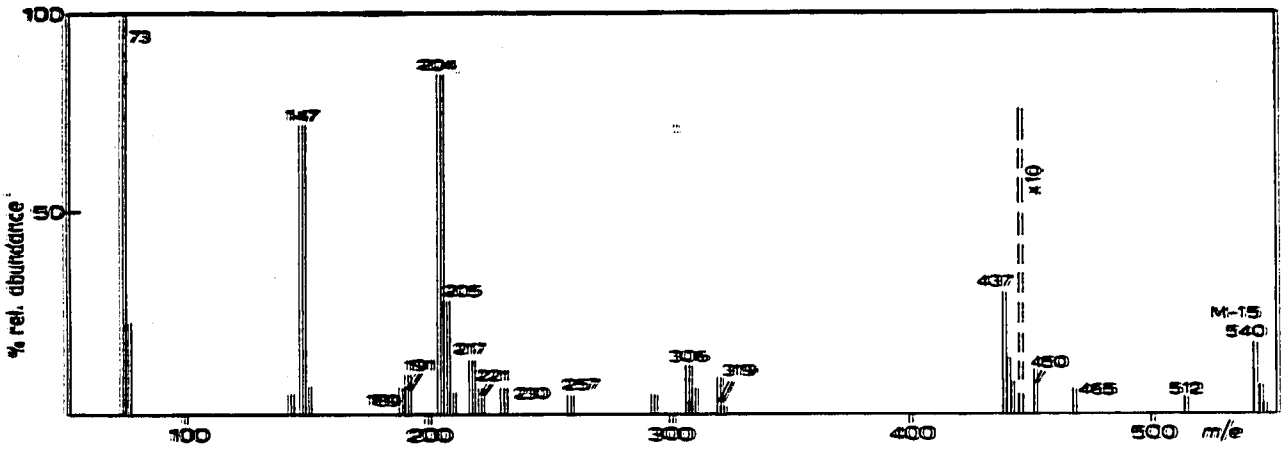


Fig. 6. Mass spectrum of P3.

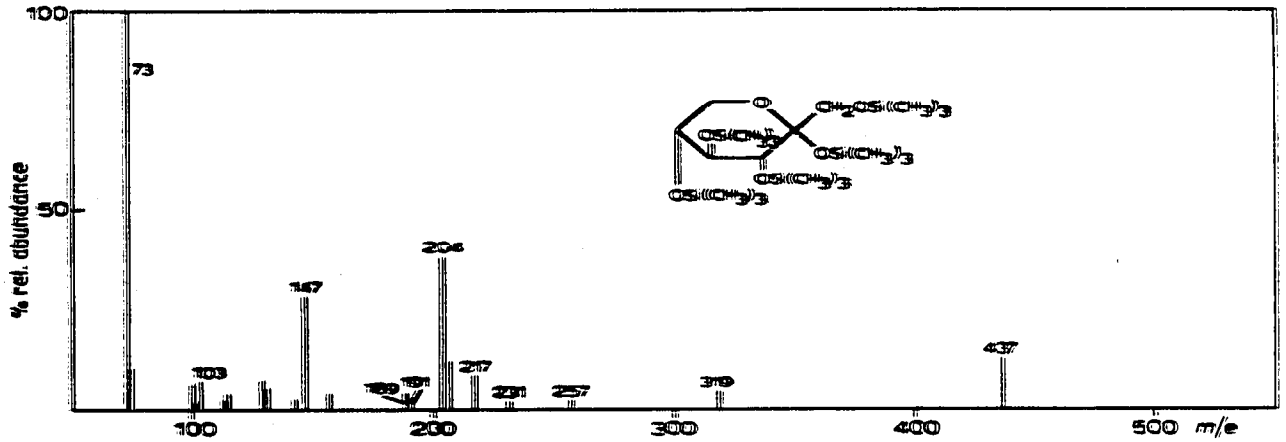


Fig. 7. Mass spectrum of sorbopyranose (agrees qualitatively with previously reported data[8]).

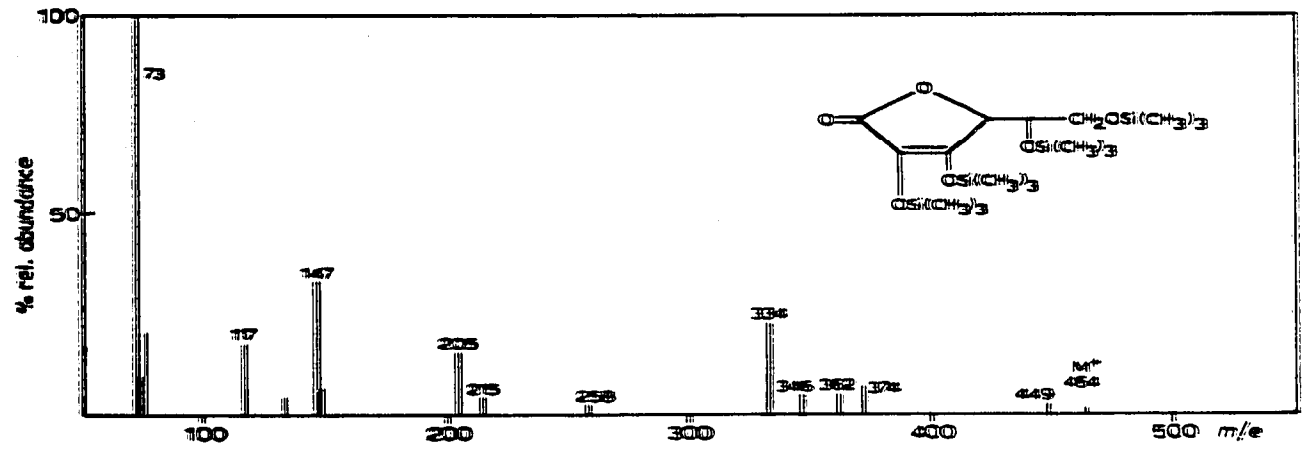
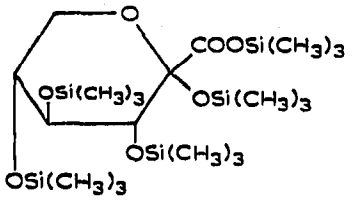


Fig. 8. Mass spectrum of ascorbic acid.

Discussion

The spectrum of P3 (Fig. 6) can be understood when the corresponding compound is assumed to be the silylated 2-keto-L-gulonic acid in a pyranose ring structure (I) (mol. wt. 554.7; nominal 554).



I

In accordance with the general pattern of mass spectra of silylated carbohydrates[3], the fragment with the highest mass number, *m/e* 539, represents the M-15 ion. The relatively high abundance of the *m/e* 437 fragment follows from the easy rupture of the C-1–C-2 bond by which the carboxyl group is expelled ($M^+ \rightarrow 437^+ + 117$). An analogous fragmentation has been reported very recently on silylated straight-chain aldonic acids[4]. The pyranose structure of the ring follows conclusively from the ratio of the relative intensities of the ions *m/e* 204 and *m/e* 217 (ref. 3). The ions *m/e* 73 and *m/e* 147 are typical of silylated compounds and do not have any structural significance. A more extensive identification of the main fragments is given in Table II.

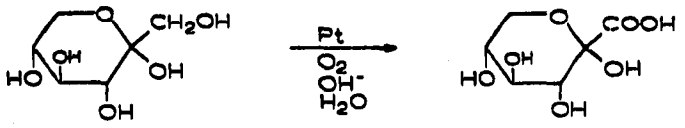
TABLE II

IDENTIFICATION OF MAIN FRAGMENTS[4,6]

T = $Si(CH_3)_3$.

m/e	*Identity*
539	M-15
512	M-15-CO
467	not established
449	M-15-$HOSi(CH_3)_3$
437	M-COOT
319	H_2COT–HCOT–COT=CH$^+$
306	$\cdot$HCOT$_{3}$–HCOT$_{4}$–HCOT$_{5}$–COT$^+_{6}$
292, 257, 230, 221	not established
217	HCOT=HC–HCOT$^+$
204	$\cdot$HCOT–HCOT$^+$
191	TO–HCOT+
189	$(CH_3)_2$Si$^+$OCH=CHOT
147	$(CH_3)_2$Si$^+$–OT
75	HOSi–$(CH_3)_2$
73	Si$^+(CH_3)_3$

The remarkable congruency between the spectra of silylated sorbopyranose (Fig. 7) and the main silylation product of KGA indicates that the oxidation reaction of sorbose to KGA with Pt in aqueous alkaline solution proceeds in the pyranose form.



Moreover, as the sorbose used for this process is known to be the β-sorbopyranose, the resulting KGA will most probably be in the β-anomeric form.

As the result of the considerations mentioned above, P3 can be identified as 1,2,3,4,5-penta-O-TMS-β-L-xylo-hexulopyranosonic acid.

The mass spectrum of P2 (Fig. 5) is qualitatively and quantitatively almost identical with that of P3. As mass spectra of α and β anomers are known to be the same, peak P2 can most probably be identified as the α anomer of P3. This supposition is supported by the order of elution of P2 and P3, which is in accordance with the general sequence of elution of α and β anomers.

The identification of P1 has not yet been established and is hampered by the fact that probably P1 covers at least two compounds (indicated by the shape and width of the peak). In some respect the mass spectrum of P1 represents a furanose compound, because the intensity of the *m/e* 217 ion is significantly greater than that of the *m/e* 204 fragment. A clear mass spectrum of P4 could not be obtained, due to a very low concentration of the compound in the sample. However, in analogous experiments[5,8] the open-chain form of sorbose and fructose exhibited the highest retention time, which suggests that P4 might represent the open-chain form of the silylated KGA. This suggestion is in accordance with the generally observed very low concentrations of aldehydo structures in neutral aqueous solutions[7].

From the relative abundance of (P1 + P2) as a function of the GLC column temperature, it follows that some conversion of P3 occurred in the column. This process seems to be limited only to the very first part of the column, because the ultimately resulting chromatogram shows clear-cut peaks of the reaction products P1 and P2 instead of a broad undefined band. From the identity of P2 and P3, as established by mass spectroscopy, the (main) process at the beginning of the GLC column can be specified as a partial conversion of an initially injected β-pyranose form of KGA into the α-pyranose form in the presence of the solvent DMSO. Consequently, as soon as the very volatile solvent has been separated from the silylated sugars in the very first part of the elution, the process stops. This hypothesis is supported by (a) an observed very slow increase of the ratio (P1 + P2)/P3 in the sample as a function of time (weeks) at room temperature, (b) an increase of the ratio when some additional DMSO is injected during the elution of the sugar components, and (c) the absence of P1 and P2 when pyridine is used as solvent.

As the plotted FID signal in Fig. 1 concerns only P3, the scattering of points within the shaded area is partly explained by a varying degree of conversion of P3 into P2 (and P1). The degree of conversion depends on the time prior to the separation of the DMSO and the silylated compounds; hence on column temperature and method of injection.

However, even when the peak areas of P1 and P2 are added to P3, there remains a discrepancy between the FID responses and these calculated for the free acid (line a in Fig. 1). The discrepancy may be caused by gradual decomposition of the silylated KGA in the column, most probably due to the rather unstable TMS ester bond in the carboxyl group at C_1. For ascorbic acid, the enol-lactone form of KGA in which the carboxyl group is absent, excellent agreement has been obtained between the calculated and observed FID responses. (The mass spectrum of silylated ascorbic acid is given in Fig. 8.)

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