ACTA CHEMICA SCANDINAVICA 26 (1972) 2205–2215

Partition Chromatography and Mass Spectrometry of Tetrulose and Pentuloses

JAROSLAV HAVLICEK, GÖRAN PETERSSON and OLOF SAMUELSON

Department of Engineering Chemistry, Chalmers Tekniska Högskola, Göteborg, Sweden

Partition chromatography on ion exchange resins in aqueous ethanol is an excellent tool for the separation of tetrulose and pentuloses for both analytical and preparative purposes. The analysis of complex mixtures is facilitated by the fact that the ketoses are recorded by both the orcinol and periodate-formaldehyde methods whereas under proper conditions aldoses are only recorded in the orcinol channel.

In gas chromatography the trimethylsilylated 3-pentuloses gave rise to only one prominent peak which as revealed by mass spectrometry corresponded to the keto form. Tetrulose and the 2-pentuloses gave two or more peaks. The 1,2-enediol derivative obtained from tetrulose was identical with that recorded for threose.

A study of the degradation products obtained from xylan by partition chromatography on ion exchange resins in aqueous ethanol revealed that under certain conditions pentoses and pentuloses formed by the isomerization of xylose were present. As shown previously this chromatographic method gives excellent separations of the pentoses.[1] No results with pentuloses have been reported previously and the information available about their behaviour in other methods is scanty. The present paper describes their separation for preparative and analytical purposes by partition chromatography on ion exchange resins coupled with automatic analysis of the eluates as well as gas chromatography–mass spectrometry of trimethylsilyl (TMS) derivatives of pentuloses. Some related substances were included for comparison.

EXPERIMENTAL

The eluate fractions obtained from partition chromatography on a preparative scale containing the ketoses were evaporated at 35° leaving the ketoses as amorphous masses. The sugars were found to give single peaks when rechromatographed on analysis columns with lithium and sulphate resins. All prepared ketoses as well as the commercial ketoses gave a strong response in the periodate-formaldehyde channel.[2] The pH of the periodate solution was 2. The conditions were chosen so that little or no response was recorded with aldoses.

After reduction with sodium borohydride [3] *threo*-3-pentulose gave arabinitol, whereas each of the other pentuloses gave the two expected alditols. The alditols were identified by partition chromatography on both the lithium and sulphate resins.[4] The paper chromatographic behaviour of *threo*-2-pentulose was in agreement with that reported by Touster.[5]

The TMS derivatives were prepared by the addition of excess bis(trimethylsilyl)trifluoroacetamide (BSTFA) to 1 – 5 mg of the sugars in dry pyridine. Trimethylchlorosilane (TMCS) was added for the ketoses. The reaction was completed by shaking the reaction mixtures for a few hours at room temperature. The pyridine was removed and the derivatives were dissolved in diethyl ether.

The mass spectrometric studies were performed on an LKB 9000 gas chromatograph – mass spectrometer. The introductory column was of the same type as that used for the determination of GLC data. The column temperature ranged from 115° to 140°. The mass spectrometric data were: temperature of molecule separator 210°, temperature of ion source 270°, accelerating voltage 3.5 kV, trap current 60 μA, exit slit 0.05 mm, collector slit 0.10 mm.

SEPARATIONS ON ION EXCHANGE RESINS

A commercial sample of D-*erythro*-2-pentulose gave rise to several significant peaks when chromatographed in aqueous ethanol on an anion exchanger in its sulphate form. There were no signs of interfering decomposition during the chromatographic run which means that the sample contained a complicated mixture of sugars. The two diastereomeric pentuloses were, therefore, prepared by isomerization of D-xylose and D-arabinose in boiling pyridine.[5] In a study of this reaction Fedoronko and Linek [6] found that xylose gave lyxose, arabinose, ribose, *threo*-2-pentulose, *erythro*-2-pentulose, and minor amounts of diastereomeric 3-pentuloses. The products were isolated by a combination of partition chromatography on a cellulose column and permeation chromatography in aqueous solution on a cation exchanger in its barium form.

When chromatographed in aqueous ethanol on an anion exchanger in the sulphate form (Fig. 1A) all these reaction products were recorded in a single run. The five main reaction products could be determined accurately. A rough estimate could be obtained for the minor products (3-pentuloses) as well. A trace compound which appeared ahead of the other peaks was not identified. The same compounds were present in the reaction mixture obtained in a parallel experiment with arabinose (Fig. 1B). As expected the main isomerization products were ribose and *erythro*-2-pentulose.

In these analyses advantage is taken of the fact that all sugars give a strong response in the orcinol channel whereas at low pH of the periodate solution the aldoses give only a very weak response in the periodate-formaldehyde channel.[2] As can be seen from the chromatograms the 2-pentuloses give a strong response in this channel whereas the response is even stronger for the 3-pentuloses than that recorded in the orcinol channel. This can be explained by the fact that the 3-pentuloses cannot give any cyclic hemiketals, which means that theoretically, two moles of formaldehyde are formed for each mole of 3-pentulose. Hence, the two-channel analyzer facilitates the identification of the separated species and makes it possible to determine *erythro*-2-pentulose and ribose quantitatively although these species overlap seriously.

As can be seen from Fig. 1, chromatography on a sulphate resin offers a convenient method for the isolation of *threo*-2-pentulose on a preparative scale

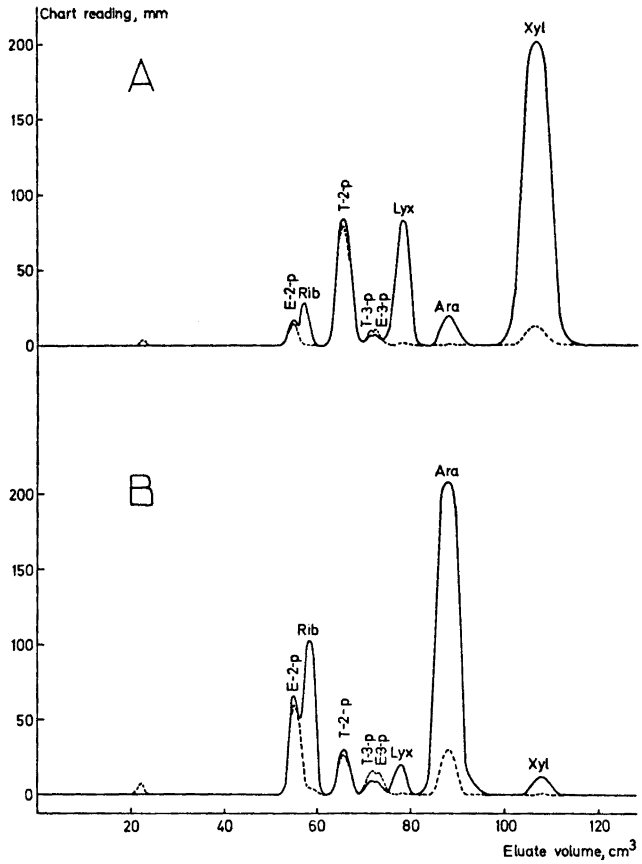

Fig. 1. Chromatographic analysis of the reaction mixture obtained by pyridine catalysed isomerization of 0.5 mg of xylose (A) and 0.5 mg of arabinose (B) in 85 % ethanol (w/w) at 75° on an anion exchanger in its SO_4^{2-} form. Resin bed, 4.3 × 740 mm; Technicon T5C, 14 – 17 μ. Flow rate, 2.8 cm min^{-1} (calculated for an unpacked column). Orcinol method, full line; periodate-formaldehyde method, broken line. Ara = arabinose, Lyx = lyxose, Rib = ribose, Xyl = xylose, E-2-p = *erythro*-2-pentulose, E-3-p = *erythro*-3-pentulose, T-2-p = *threo*-2-pentulose, T-3-p = *threo*-3-pentulose.

from the reaction mixture obtained after isomerization of xylose. The isolation of *erythro*-2-pentulose (after isomerization of arabinose) by this technique is less favourable (Fig. 1B) but by applying a longer column (1000 mm) and repeating the separation, this technique could be used for the isolation of this isomer in a pure form. The same technique was used for the isolation of *glycero*-tetrulose which was obtained together with threose by isomerization of erythrose in pyridine.

The isolation of the 3-pentuloses from the isomerization mixture might be performed by a group separation on a sulphate resin followed by rechromatography at a high ethanol concentration on a cation exchange resin in its lithium form. As can be seen from Fig. 1, the yields of these products were

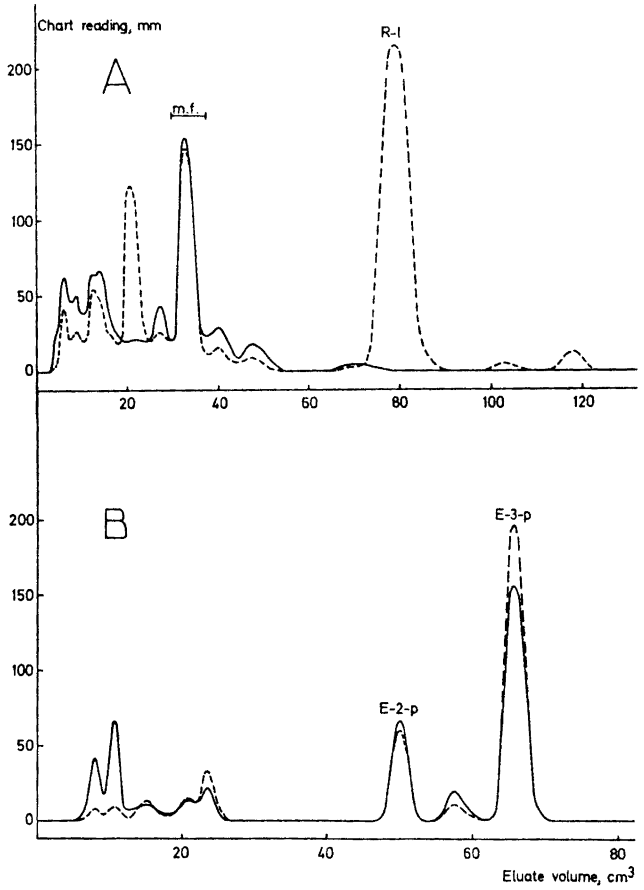

Fig. 2. Chromatographic separation of the reaction mixture obtained after oxidation of ribitol according to Stoodley [7] "method B". Orcinol method, full line; periodate-formaldehyde method, broken line. A. Separation on a cation exchanger in its Li^+ form, Dowex 50W-X8, 4.5 × 815 mm, 14 – 17 μ at 75° in 92.4 % ethanol. Flow rate, 3.0 cm min^{-1}. R-1 = ribitol, m.f. = main fraction. B. Rechromatography on an anion exchanger in its SO_4^{2-} form, T5C, 4.3 × 740 mm, 14 – 17 μ at 75° in 85 % ethanol of the main fraction (denoted m.f. in Fig. 2A) from the lithium column. Flow rate, 2.8 cm min^{-1}. E-2-p = *erythro*-2-pentulose; E-3-p = *erythro*-3-pentulose.

very small, however, and for this reason mercuric acetate oxidation of ribitol and arabinitol [7] was used for their preparation. The unreacted starting material as well as a number of by-products were removed by chromatography on a lithium resin (Fig. 2A). The fraction containing the main reaction product was evaporated and then rechromatographed on a sulphate resin (Fig. 2B) to obtain a pure fraction. The results given in Fig. 2 refer to the oxidation of ribitol. It is seen that besides the desired reaction product, *erythro*-3-pentulose, appreciable amounts of *erythro*-2-pentulose and other products were obtained.

Table 1. Distribution coefficients of some monosaccharides at 75°.

Saccharides	SO_4^{2-}-resin, T5C (14 – 17 μ) 85 % EtOH	Li^+-resin, Dowex 50W-X8 (14 – 17 μ) 85 % EtOH	92.4 % EtOH
Erythrose	2.20	1.16	1.80
Threose	2.71	0.92	1.38
glycero-Tetrulose	3.29	1.24	1.96
Arabinose	7.86	2.24	3.24
Lyxose	6.94	1.62	2.45
Ribose	5.15	2.45	3.55
Xylose	9.62	1.62	2.47
erythro-2-Pentulose	4.75	1.69	2.78
threo-2-Pentulose	5.80	1.44	2.31
erythro-3-Pentulose	6.47	1.38	2.19
threo-3-Pentulose	6.32	1.61	2.55
2-Deoxy-D-*threo*-pentose [a]	1.83	0.67	1.06
Galactose	17.31	3.51	5.67
Glucose	20.48	2.79	4.48
Mannose	12.28	2.78	4.45
Fructose	10.24	2.73	4.91
Psicose [b]	7.24	2.40	4.30
Sorbose	11.55	2.33	4.03
Tagatose	10.55	2.17	3.85
Glucoheptose	30.12	3.90	7.90
Mannoheptulose	19.41	3.32	6.29
Sedoheptulosan	6.52	3.10	5.57

[a] Prepared by Ruff degradation of 3-deoxy- D-*xylo*hexonic acid.
[b] Kindly supplied by Dr. Emma J. McDonald, Southern Regional Res. Lab., New Orleans, La.

threo-3-Pentulose was prepared from arabinitol and isolated in the same manner. As expected both *threo*-2-pentulose and *erythro*-2-pentulose were formed as by-products.

The volume distribution coefficients (D_v) of the isolated ketoses as well as those of 2-deoxy-D-*threo*-pentose and some commercial sugars were calculated [8] from the peak elution volumes determined with single species and mixtures on both a sulphate resin and a lithium resin (Table 1). The results show that the ketoses are eluted in the order of increasing number of hydroxyl groups on both resins: Dihydroxyacetone [4] < tetrulose < pentuloses < hexuloses < mannoheptulose. With few exceptions this rule holds true for aldoses and alditols as well.[9] Moreover, it is seen that 2-deoxy-D-*threo*-pentose is held less firmly than the tetroses on both resins. Its position reflects the negative contributions to the D_v values from hydrophobic groups.

It is interesting to note that a reversed order of elution of the diastereomeric pentuloses is obtained on the lithium column compared to the sulphate column. These results confirm the earlier observation that the distribution coefficients of the sugars are not only determined by the differences in their solubilities at the ethanol concentrations in the external solution and in the resin but also by the fact that the ions present inside the resin exert a direct influence upon the equilibrium distribution. Like fructose and tagatose, *erythro*-

3-pentulose and *threo*-3-pentulose exhibit very small differences in their distribution coefficients on the sulphate resin but are well separated on the lithium column at a high ethanol concentration. For most of the other compounds listed in Table 1 the separation is most favourable on the sulphate resin. By applying both types of resins all species can be conveniently separated for both analytical and preparative purposes.

GAS CHROMATOGRAPHY – MASS SPECTROMETRY

Tetroses. Erythrose yielded one and threose three major gas chromatographic peaks (Table 2).

Table 2. Gas chromatography of trimethylsilyl derivatives identified by mass spectrometry. Adjusted retention times on QF-1 at 120° relative to the glucitol derivative (32 min). Instrument: Perkin-Elmer Model 900 with FID detector. Column: 300 × 0.2 cm i.d., stainless steel, packed with 3 % DC QF-1 on 100 – 120 mesh Gas Chrom Q. Carrier gas: Purified nitrogen, 30 ml/min. Temperatures: Injector 200°, column oven 120°, manifold 200°. Marker: Tetra-OTMS-erythritol (retention relative to hexa-OTMS-glucitol: 0.118).

		Rel. retention
Erythrose	furanose	0.100
Threose	furanose	0.085
	furanose [a]	0.095
	1,2-enediol [a]	0.134
Tetrulose	1,2-enediol	0.134
	keto [a]	0.280
erythro-2-Pentulose	furanose [a]	0.229
	furanose	0.304
	1,2-enediol	0.363
	keto [a]	0.645
threo-2-Pentulose	furanose	0.178
	keto	0.666
erythro-3-Pentulose	*keto* [a]	0.642
threo-3-Pentulose	*keto*	0.654

[a] Mass spectra given in Figs. 3 and 4.

The similarity of their spectra showed that two of the components from threose and the one from erythrose were diastereomers. The expected furanoside structure was confirmed by the spectra recorded for the fully trimethylsilylated sugars (*cf.* V in Fig. 3). The peaks in the upper mass region are compatible with the proper mass (M; *m/e* 336) of the molecular ion. The peak at M – 15 – 90 is explained by the loss of a silicon-linked methyl group accompanied by elimination of trimethylsilanol. The trimethylsiliconium (*m/e* 73) and pentamethyldisiloxonium (*m/e* 147) ions give rise to the two most intense peaks. These ions are indicative of TMS derivatives rather than of individual structures. They are formed in high abundance at the electron energy 70 eV for all the compounds discussed, but exhibit a much lower relative abundance at 20 eV. The formation of the abundant structure-specific

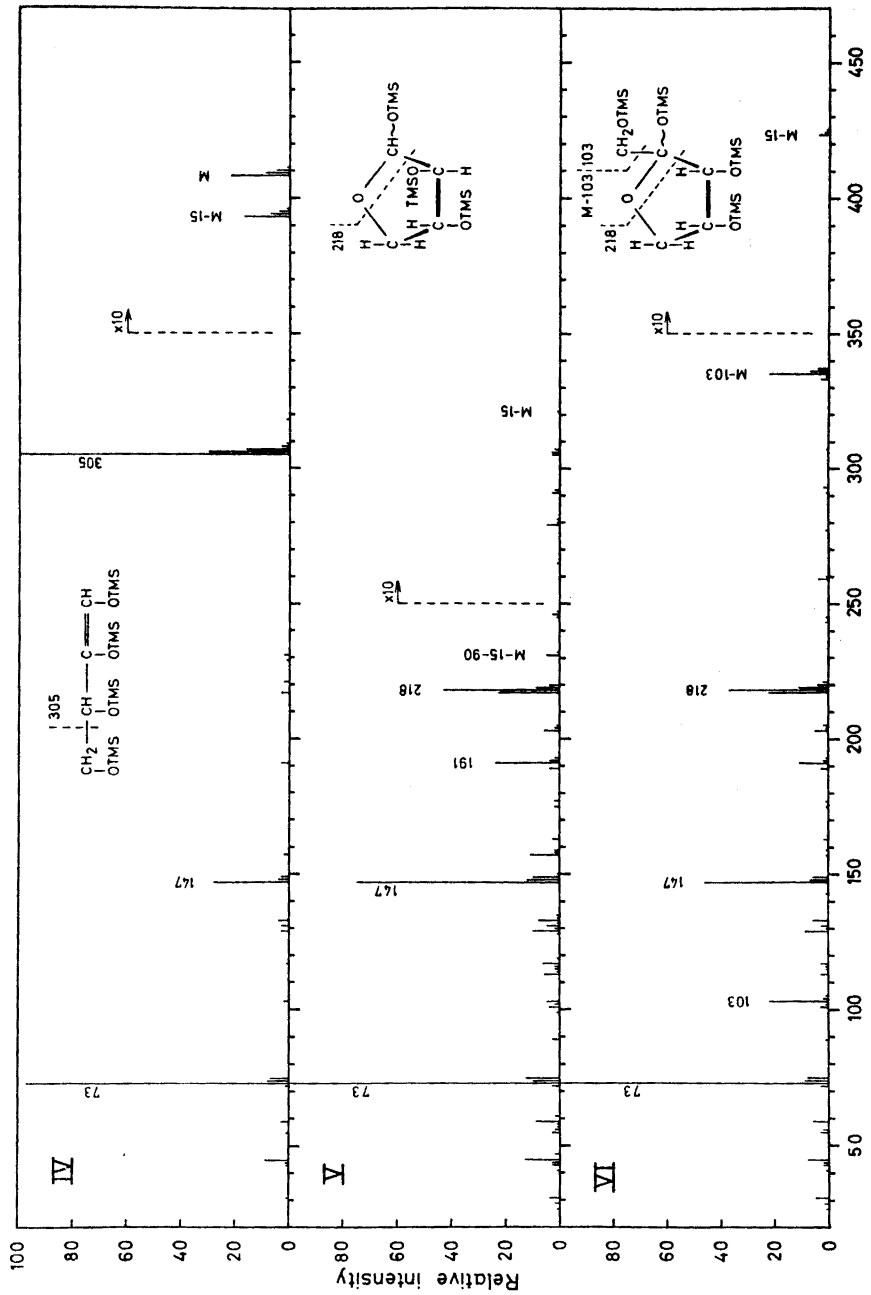

Fig. 3. Mass spectra at 70 eV of TMS derivatives of a 1,2-enediol of a tetrose (IV), a furanose anomer of threose (V), and a furanose anomer of *erythro*-2-pentulose (VI).

m/e 218 ion can be ascribed to the loss of trimethylsilyl formate from the molecular ion as indicated in Fig. 3. This fragmentation indicates that cleavage of the C-1 to C-2 bond is the most important initial fragmentation step, not only for the ether derivatives of aldopyranoses [10,11] but also for those of aldofuranoses. The analogues of the *m/e* 218 ion in the spectra of methyl [10] and TMS [12] derivatives of aldopentofuranoses studies previously are much less abundant. This can be ascribed to further decomposition.[10] The *m/e* 218 ion is also likely to be a precursor to several ions of lower mass and its decomposition by rearrangement to give *m/e* 191 ions is indicated by metastable peaks ($m^* = 167 - 168$; calc. 167.3).

The spectrum (IV in Fig. 3) of the threose component with the longest retention time suggests that the peak at *m/e* 408 arises from the molecular ion. This assignment is supported by the peak at *m/e* 393 (M − 15) corresponding to the characteristic loss a methyl group for TMS derivatives. The M value suggests an enediol derivative. Confirmatory evidence for a 1,2-enediol structure is obtained from the very abundant *m/e* 305 ion to be expected from allylic cleavage. The favourable resonance structure of the ion explains its predominance. The *m/e* 73 and *m/e* 147 ions give rise to the two other prominent peaks.

Tetrulose. Gas chromatography demonstrated the formation of two different TMS derivatives from tetrulose. One of these was identical with the 1,2-enediol derivative from threose as demonstrated by very similar mass spectra and retention data (Table 2). The spectrum (I in Fig. 4) of the other component indicates *m/e* 336 for the molecular ion. The expected acyclic structure is confirmed by other features of the spectrum, *e.g.* the *m/e* 205 peak indicating a vicinal diol end group. The formation of an acyclic *keto* derivative on silylation of mutarotated fructose has been demonstrated previously by gas chromatography – mass spectrometry.[13]

Immediately after silylation the *keto* form predominated. On storing the diethyl ether solution with an excess of the reagent bis(trimethylsilyl)trifluoroacetamide (BSTFA) in sealed glass capillaries the ratio between the two components changed and after some time the *keto* form had vanished completely. This observation can be interpreted as a successive silylation of a tautomeric enol form of the initially formed TMS *keto* derivative. The non-fluorine-containing reagent BSA is known to favour the formation of enol derivatives from ketones.[14] It is likely that the use of other silylating agents would lead to a different distribution of the various types of TMS derivatives for the sugars discussed.

2-Pentuloses. The formation of four different TMS derivatives from *erythro*-2-pentulose was demonstrated by gas chromatography.

The two components with the shortest retention times exhibited similar mass spectra suggesting that they were the derivatives of the furanose anomers. The main features of the spectra (*cf.* VI in Fig. 3) confirm the furanoside structure. Thus the peak at *m/e* 423 (M − 15) indicates the proper molecular weight. The intense peak at *m/e* 218 is ascribed to the same characteristic fragmentation as with the tetrofuranoside derivatives. The M − 103 ion is formed by the loss of the CH_2OTMS group linked to C-2. This fragmentation has been demonstrated earlier for TMS derivatives of furanose and pyranose forms of

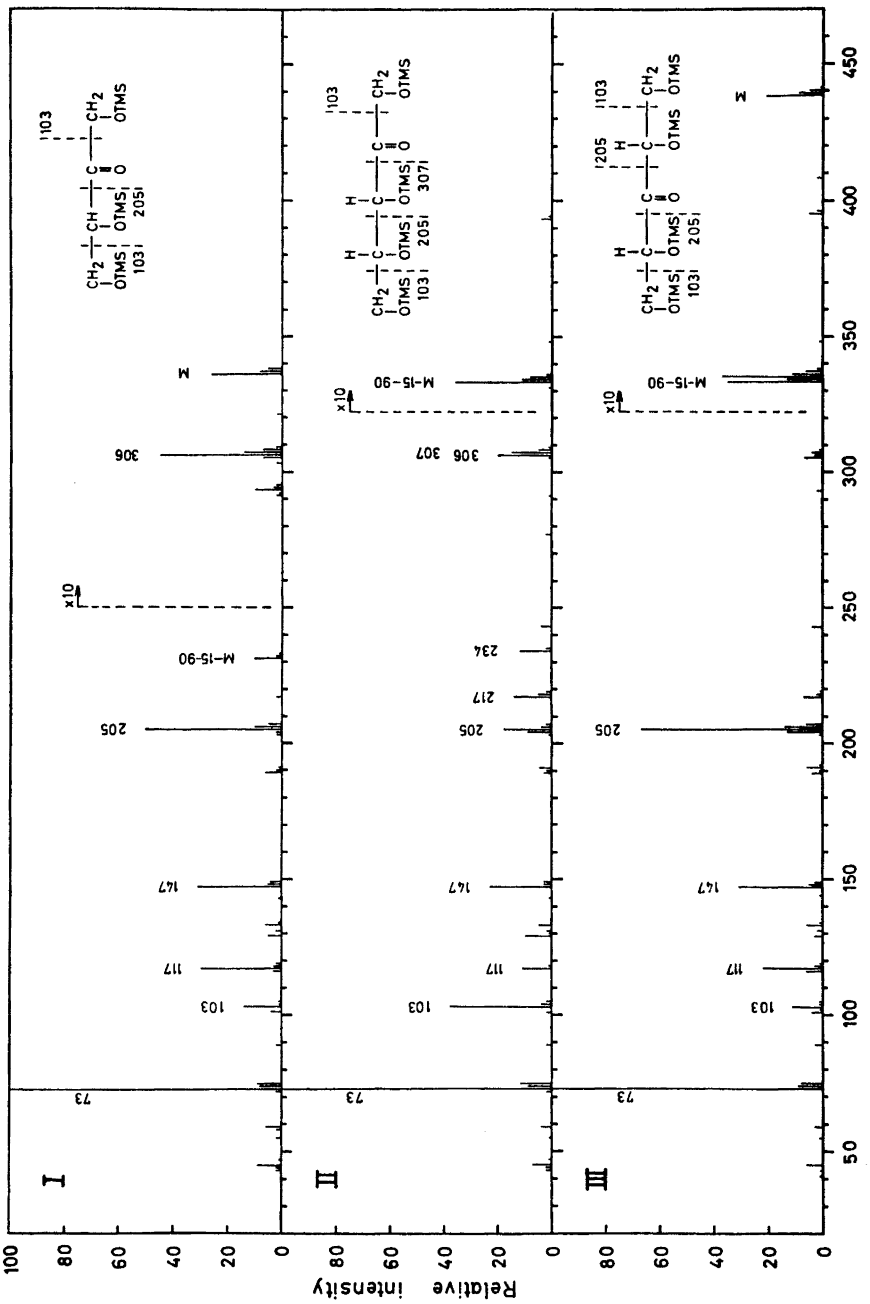

Fig. 4. Mass spectra at 70 eV of TMS derivatives of the *keto* forms of tetrulose (I), *erythro*-2-pentulose (II), and *erythro*-3-pentulose (III).

hexuloses.[13,15] The structure-specific ions of masses 218 and M – 103 are the two most abundant in the 20 eV spectrum. From an analytical point of view it is important that the spectra differ markedly from those of the TMS derivatives of the furanose and pyranose forms of aldopentoses.

The component of longest retention time was identified as the *keto* derivative from the abundant chain cleavage ions of masses 103, 205, and 307 in its spectrum (II in Fig. 4). The observed peak from the molecular ion (*m/e* 438) was less than 0.1 % of the base peak. The peak at *m/e* 234 is ascribed to an ion from the McLafferty rearrangement. The abundant *m/e* 306 ion is formed by the analogous migration of a TMS group to the carbonyl oxygen with concurrent β-cleavage. The *m/e* 306 ion for tetrulose is formed in the same way ($m^* = 278 - 279.5$; calc. 278.7 for *m/e* 336→*m/e* 306). This type of rearrangement has been described previously for TMS derivatives of aldonic acids.[16]

For the fourth component, a mass number of 510 was indicated for the molecular ion by distinct peaks at *m/e* 510 and *m/e* 495 (M – 15) in its spectrum. A predominant peak at *m/e* 305 and the absence of other prominent peaks gave further evidence that the compound was a 1,2-enediol derivative of the sugar. In the 20 eV spectrum only the base peak at *m/e* 305 and its isotope peaks exceeded 2 % relative intensity which emphasizes the favourable features of the characteristic allylic cleavage. In analogy with the results for tetrulose it was found that the *keto* derivative was successively transformed to the enediol derivative on storing.

The main component from *threo*-2-pentulose was identified as a furanose derivative and the component with a longer retention time as the *keto* derivative. Both spectra were similar to those of the diastereomeric derivatives from *erythro*-2-pentulose. For all the compounds studied the retention times on QF-1 of the derivatives identified were shortest for the cyclic forms and longest for the *keto* forms (Table 2).

3-Pentuloses. Only one major component was obtained from each of the two diastereomeric 3-pentuloses after silylation. The spectra (*cf.* III in Fig. 4) were very similar. The anticipated *keto* structure was confirmed by peaks from the molecular ion and by the formation of an abundant *m/e* 205 ion characteristic of vicinal diol end groups. Metastable peaks for the 3-pentuloses as well as for the *keto* derivatives of tetrulose and the 2-pentuloses indicate that *m/e* 205 ions decompose to *m/e* 147 ions ($m^* = 105 - 106$; calc. 105.4) and *m/e* 117 ions ($m^* = 66.6 - 68$; calc. 66.8). The precursor ion in these prominent fragmentations is likely to be a rearranged oxonium ion (Fig. 5). The spectra in Fig. 4 demonstrate that the *keto* derivatives of 3-pentuloses are readily distinguished from those of 2-pentuloses by mass spectrometry.

The results given above show that gas chromatography – mass spectrometry of the TMS derivatives is an excellent tool for the identification of the studied sugars. Most compounds give rise to more than one chromatographic band, each containing the TMS derivative of a single form which can be easily identified by mass spectrometry. With *erythro*-2-pentulose the two furanose anomers as well as a 1,2-enediol and the straight chain *keto* form were recorded. The relative amounts of the different forms depend upon the working conditions applied in preparing the TMS derivatives. A successive transformation of the 2-*keto* derivatives to the enediol derivatives upon storing the

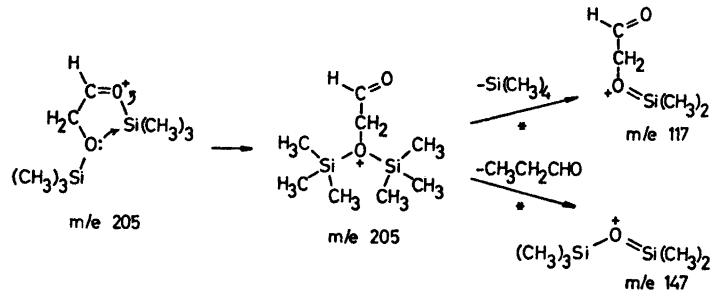

Fig. 5. Mass spectrometric fragmentation of the *m/e* 205 ion from the *keto* derivatives of ketoses.

reaction mixture shows that enediol derivatives of high stability are formed. It is interesting to note that a 1,2-enediol derivative was obtained from threose and tetrulose as well, whereas with erythrose, which is known to be more stable, the corresponding peak was virtually absent.

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

REFERENCES

1. Martinsson, E. and Samuelson, O. *J. Chromatog.* **50** (1970) 429.
2. Samuelson, O. and Strömberg, H. *Carbohyd. Res.* **3** (1966) 89.
3. Lewis, B. A., Smith, F. and Stephen, A. M. *Methods Carbohyd. Chem.* **2** (1963) 68.
4. Samuelson, O. and Strömberg, H. *Acta Chem. Scand.* **22** (1968) 1252.
5. Touster, O. *Methods Carbohyd. Chem.* **1** (1962) 98.
6. Fedoronko, M. and Linek, K. *Collection Czech. Chem. Commun.* **32** (1967) 2177.
7. Stoodley, R. J. *Can. J. Chem.* **39** (1961) 2593.
8. Samuelson, O. *Ion Exchange Separations in Analytical Chemistry*, Almqvist and Wiksell, Stockholm, and Wiley, New York 1963.
9. Samuelson, O. In Marinsky, J., Ed., *Ion Exchange II*, Dekker, New York 1969.
10. Kochetkov, N. K. and Chizhov, O. S. *Tetrahedron* **21** (1965) 2029.
11. Heyns, K., Grützmacher, H. F., Scharmann, H. and Müller, D. *Fortschr. Chem. Forsch.* **5** (1966) 448.
12. Petersson, G. and Samuelson, O. *Svensk Papperstid.* **71** (1968) 77.
13. Curtius, H.-C., Müller, M. and Völlmin, J. A. *J. Chromatog.* **37** (1968) 216.
14. Klebe, J. F., Finkbeiner, H. and White, D. M. *J. Am. Chem. Soc.* **88** (1966) 3390.
15. Karady, S. and Pines, S. H. *Tetrahedron* **26** (1970) 4527.
16. Petersson, G. *Tetrahedron* **26** (1970) 3413.

Received October 26, 1971.