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# GAS–LIQUID AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSES OF THE ACID-CATALYZED DEHYDRATION REACTION OF XYLITOL

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## SUMMARY

Reactions of xylitol with concentrated hydrochloric acid or diluted sulfuric acid were studied under various conditions. Complete separation of reaction products was achieved by means of gas–liquid chromatography on a capillary column coated with SE-30. The chemical structures of the compounds obtained were assigned using mass spectrometry. The major components of reaction mixtures were separated by high-performance liquid chromatography.

The analytical methods so developed were applied to estimate the usefulness of preparative methods.

## INTRODUCTION

Anhydroalditols and their derivatives are widely used in chemical industry. Some of them are pharmacologically active[1,2]. Anhydroalditols are present in the cerebrospinal fluid[3] and in some plants[4]. It is therefore advisable to develop efficient methods for the separation of isomeric anhydroalditol for the analysis of reaction products from which these compounds are obtained.

Developing new synthetic methods on a preparative scale necessitates analytical studies of reaction products and efficient methods for the separation of products and the identification of their chemical structures.

A fundamental method for obtaining anhydroalditols is the acid-catalyzed alditol dehydration reaction, which gives rise to a series of isomeric products. It is known from the chemical literature that pentitols treated with concentrated hydrochloric acid yield solely 1,4-anhydroalditols, and the reaction of xylitol is considerably slower than that of D,L-ribitol. In investigating the reaction products of D-mannitol with concentrated hydrochloric acid we used gas chromatography–mass spectrometry (GC–MS) with good results[5]. The results of a similar chromatographic analysis of the action of hydrochloric and sulfuric acids on xylitol are presented here.

## EXPERIMENTAL

A 200-mg amount of xylitol (Fluka, Buchs, Zwitzerland) and 1 ml of concentrated hydrochloric acid were heated under reflux at 100° for 3, 12 or 24 h. Identical mixtures were heated simultaneously in sealed glass ampoules at 100° for 3, 6, 12 or 72 h.

The samples so obtained were evaporated under reduced pressure. To the residue was gradually added 1 ml of anhydrous ethanol, and the residue was again evaporated in a nitrogen stream. The samples were kept for 24 h in a desiccator over potassium hydroxide, then dried, purified from hydrochloride residue, and acetylated as follows. 2 mg of the oily product was dissolved in 200 $\mu$l of freshly distilled acetic anhydride containing *ca.* 1 mg of anhydrous sodium acetate, and heated at 100° for 1 h. The resulting solutions were subjected to chromatographic analysis.

The dehydration reaction with sulfuric acid was performed for 200 mg of xylitol dissolved in 1 ml of 5% sulfuric acid and heated at 160° in a sealed ampoule for 10, 30 or 60 min. The reaction products were purified by the action of excess barium carbonate. The solution was separated from the residue, concentrated in a nitrogen stream, and converted into the acetyl derivative as described above.

### *Gas chromatography*

Chromatographic separations were performed using a capillary column coated with SE-30 with Silanox[6]. Column efficiency determined for *n*-tetracosan at 250° was 180,000 theoretical plates (column 60 m × 0.025 cm I.D.). Other analysis conditions had been described in a previous report[7].

### *Mass spectrometry*

Mass spectra were measured using an LKB 2091 mass spectrometer coupled with a PDP-11 minicomputer. The mass spectrometer was linked with a gas chromatograph equipped with a capillary column coated with SE-30.

The ion source had a temperature of 260°, the molecular separator a temperature of 250°. Ionization energy was 70 eV. The scan was from *m/z* 10 to 680 in 2 sec.

### *High-performance liquid chromatography (HPLC)*

The products of the xylitol dehydration reaction were transformed into acetyl derivatives and separated by means of a Siemens S 100 high-performance liquid chromatograph equipped with a 25 × 0.8 cm column packed with 5 $\mu$m Partisil and a refractometric detector. Elution was performed using ethyl acetate–hexane (1:5). 200 $\mu$l of solution containing 20 mg of reaction mixture were injected each time into the column. The flow-rate through the column was 3 ml/min, and the pressure 60 kg/cm$^2$.

### *Nuclear magnetic resonance (NMR) spectra*

The fractions collected from the liquid chromatograph were concentrated under reduced pressure at 40°, then dissolved in deuterochloroform. The solutions obtained, each containing 20 mg of the compound, were used to measure NMR

spectra with an internal tetramethylsilane standard, using a Jeol Model JNM-4H NMR spectrometer.

## RESULTS AND DISCUSSION

First we identified the chemical structures of the products of the hydrochloric acid reaction with xylitol. The mixture formed after heating for 3 h at 100° in a sealed ampoule was chosen as the most typical sample. Chromatographic analysis showed that this contained all compounds (Fig. 1) formed under different conditions of the dehydration reaction.

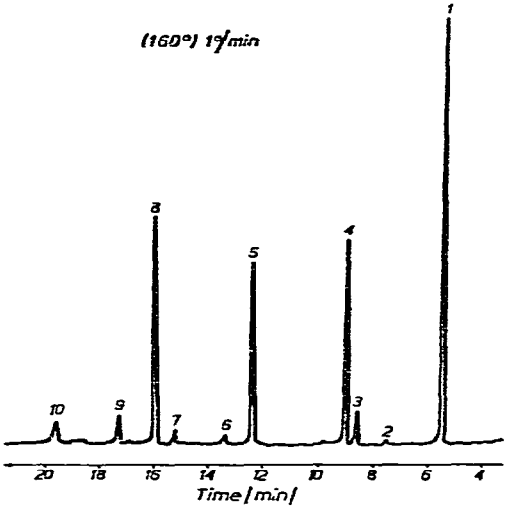
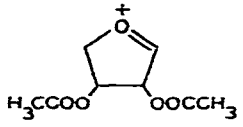



Fig. 1. Gas chromatogram of product mixture formed after heating xylitol with hydrochloric acid in sealed ampoule at 100° for 3 h. For peaks, see Table I.

The substance with the shortest retention time (peak 1) was characterized by a mass spectrum containing a small number of ions (Fig. 2). The ion with an odd number of *m/z* 201 has the largest mass; however, it is an ion derived from fragmentation. The next in the spectrum was the *m/z* 187 ion which, in the standard sample of 1,4-anhydropentitol obtained on a preparative scale, corresponds to the ion with the structure




The mass difference of the two ions corresponds to the $CH_2$ group. During formation of the *m/z* 201 ion there must have been a splitting of the $C_5$–X bond. Similar splitting was observed in the fragmentation of the acetyl-1,4-anhydro-6-chloro-6-deoxy-D-mannitol[5]. By using the coinjection method, we found that the reaction mixture contained, 1,4-anhydroxylitol appearing as peak 4 in the chromatogram.

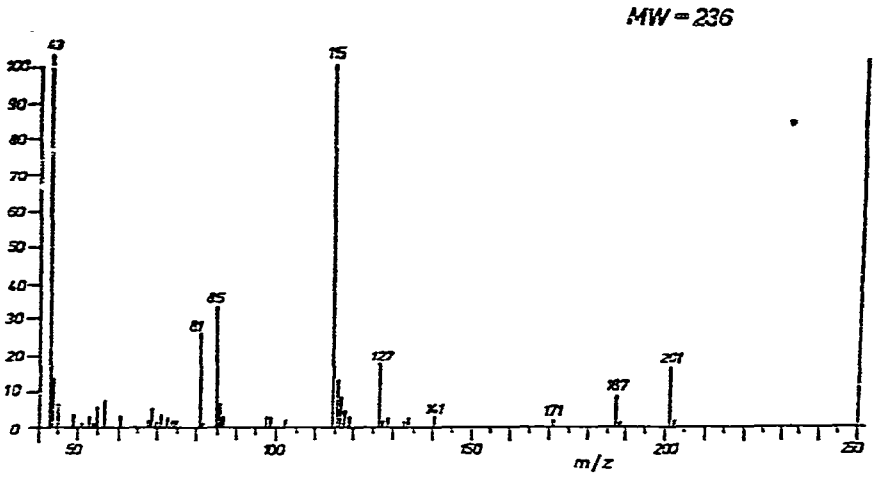



Fig. 2. Mass spectrum of GC peak 1 in Fig. 1.

The retention time might have been shorter because of the substitution of the hydroxyl group with the chlorine atom. All this led us to ascribe the structure of the acetyl 1,4-anhydro-5-chloro-5-deoxyxylitol derivative to substance 1.

The chemical identities of peaks 2, 4 and 10 has been established as 1,5-anhydro (peak 2), 1,4-anhydro (peak 4), and xylitol (peak 10). 1,5-Anhydro- and 1,4-anhydro-xylitols were obtained by preparative methods as described in the chemical literature[8,9].

Peaks 3 and 4 (1,4-anhydro) whose mutual 1:12 intensity is constant, independent of the conditions of dehydration by hydrochloric acid are characterized by identical mass spectra (Figs. 3 and 4). On this basis the two compounds have been ascribed the structure of 1,4-anhydropentitol. When subjected to chromatographic analysis as its acetyl derivative, the initial xylitol did not reveal the presence of any other pentitol[10] or of any other contaminants, despite considerable "overload" of the column.

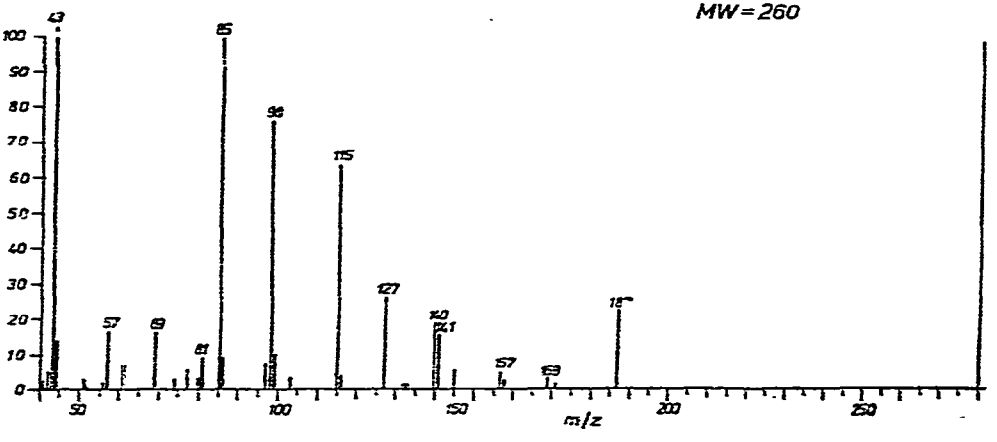



Fig. 3. Mass spectrum of GC peak 3 in Fig. 1.

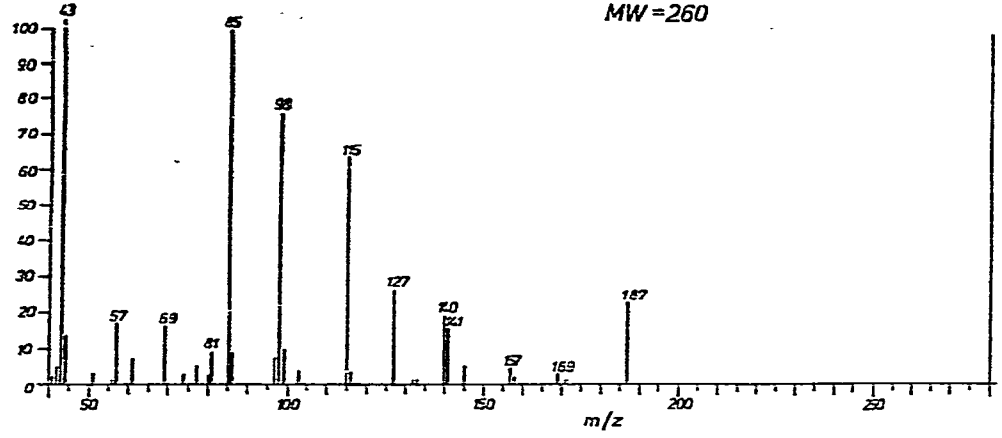



Fig. 4. Mass spectrum of GC peak 4 in Fig. 1.

The appearance of two 1,4-anhydroxylitol isomers is of interest. Results from model experiments show that 1,4-anhydro-D-xylitol and 2,5-anhydro-L-xylitol are identical. On the other hand, 1,4-anhydro-L-xylitol is identical with 2,5-anhydro-D-xylitol[11]. As the two pairs are optical antipodes, they cannot undergo separation on the optically inactive phase. It seems therefore rational to assume that epimerization had occurred during the dehydration reaction. Our next report will deal with these processes.

Dichloro derivatives should elute between anhydropentitols and monochloro derivatives; and indeed, two peaks (numbers 5 and 6) are observed in that range, with peak 5 being of greater intensity. The mass spectra (Figs. 5 and 6) of the two components are similar, apart from ion *m/z* 115 $\left(\begin{matrix}\mathrm{CH{=}CH{-}CH} \\ | \qquad\quad \| \\ \mathrm{OH} \qquad +\mathrm{OOCCH_3}\end{matrix}\right)$ characterizing alditol derivatives, which is not present in the spectrum of the compound

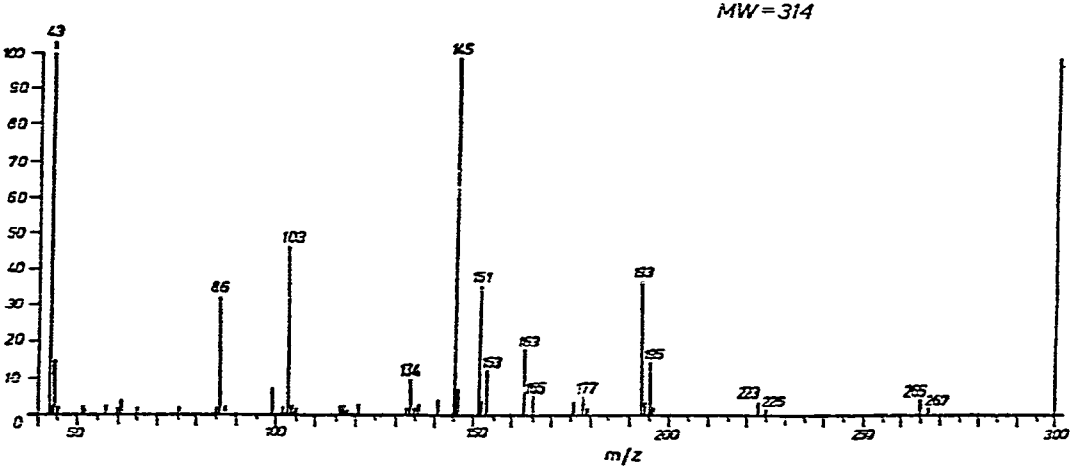



Fig. 5. Mass spectrum of GC peak 5 in Fig. 1.

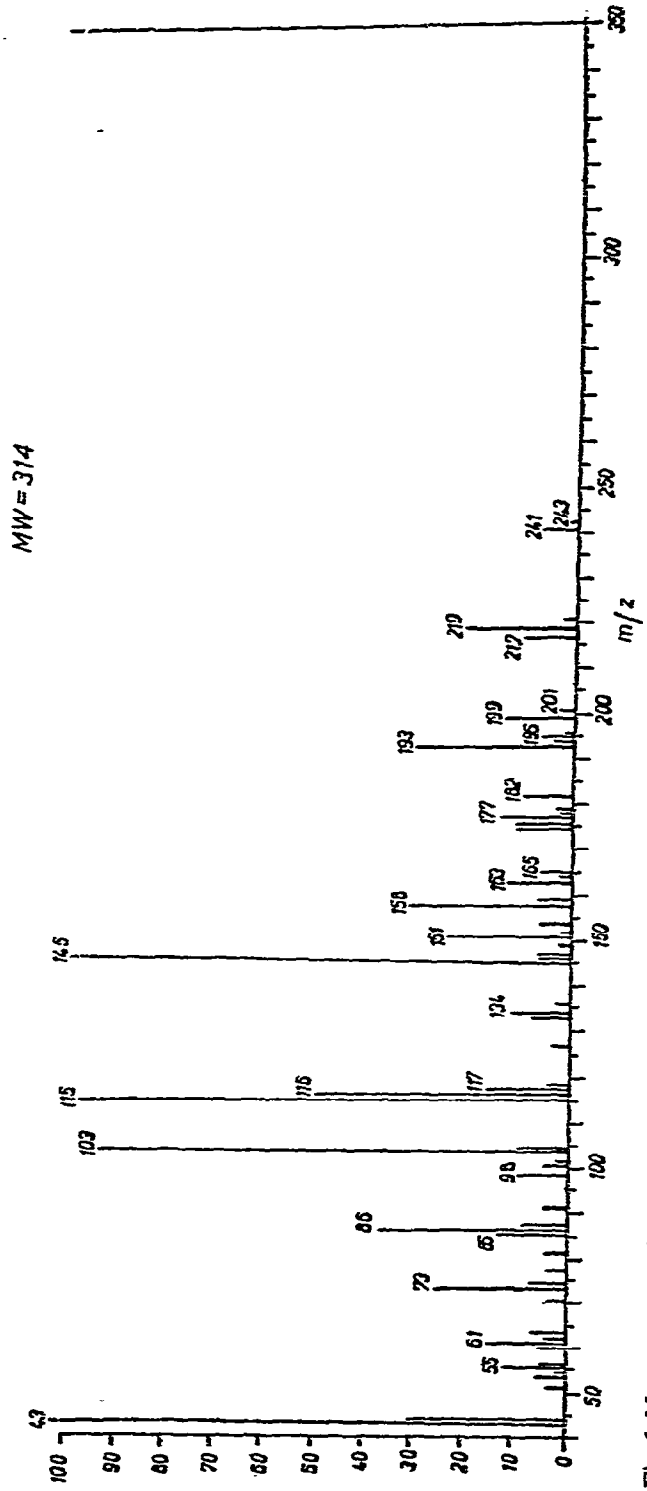



Fig. 6. Mass spectrum of GC peak 6 in Fig. 1.

eluting as peak 5. That ion arises from the part of a molecule with three carbon atoms in the chain and three acetoxy groups. The ion common to the two compounds is *m/z* 193/195 (*M*—121) which loses ketene to give ion *m/z* 151/153, whose intensity is greater in peak 5. Ion *m/z* 163/165 also occurs in the spectrum of the monochloro derivative, but here it arises via the following sequence

$$M \xrightarrow{-49} m/z\ 265/267 \xrightarrow{-102} m/z\ 163/165$$

The differences found in the spectra however do not allow the determination of the isomers of the derivatives examined.

The chromatographic peaks 7, 8 and 9 have identical mass spectra (Figs. 7, 8 and 9), where the most intense ions are *m/z* 217 (*M*—121), *m/z* 193/195 (*M*—145), *m/z* 163/165 (*M*—73—102) and *m/z* 115 ($C_5H_7O_3$). The characteristic ions *m/z* 163/156 and 193/195 led us to assign the monochloropentitol derivative structure to the three compounds. At the same time, the presence of ion *m/z* 217 rules out the chloro derivative in position 3.

It remains only to differentiate the 1-chloro and 2-chloro derivatives. What is surprising, however, is the occurrence of three components, while, theoretically, there can be only two. This can be accounted for in a similar manner as for 1,4-anhydropentitol (peaks 3 and 4) by epimerization at the second carbon atom during formation of the halogen derivative. Unfortunately, neither mass spectra nor chromatographic properties (lack of standards) allowed as to differentiate between the isomers.

The structure of the main product arising from an interaction of hydrochloric acid and xylitol under drastic conditions (peak 1, Fig. 1) was confirmed by HPLC (Fig. 10), which revealed two components, as had GC analysis. The structure of the main fraction was confirmed from its mass spectrum (peak 1, Fig. 10). Thus, the doublet observed in the NMR spectrum at $\tau = 6.28$ (Fig. 11) was assigned to protons of the chloromethyl group which are outside the chain. This was done after comparing the spectrum of the compound investigated with the spectrum of the per-O-acetyl-1,4-anhydroxylitol derivative. In the compound examined, the acetyl group causes a considerably greater deshielding effect, manifesting itself by a shift of the proton signals $H_5'$ and $H_5''$ from $\tau = 6.28$ to $\tau = 5.5$. We assigned the resonance for proton $H_4$ to the quartet at $\tau = 6.0$, because irradiation of protons $H_5'$ and $H_5''$ simplified the signal of proton $H_4$ and produced a doublet. We assigned the multiplet at $\tau = 5.35$–$5.65$ to protons $H_3$ and $H_2$, in agreement with the integral curve (two protons). This assignment of these signals was supported by simplification of that multiplet irradiation of protons $H_4$ and $H_1$. The resolving power of the apparatus did not allow detailed analysis of that multiplet. The signals at $\tau = 4.65$ came from protons $H_1'$ and $H_1''$. Irradiation of the multiplet at $\tau = 5.35$–$5.65$ simplifies the signal of protons $H_1'$ and $H_1$, producing a doublet, and also irradiation at $\tau = 4.65$ simplifies the complex multiplet. The 5-chloro-5-deoxy-1,4-anhydroxylitol structure for peak 1 in Fig. 1 is further supported by the fact that the NMR spectrum of that compound (Fig. 11) shows only signals of the two methyl groups derived from acetyl substituents. One cannot assume that the signals of these groups are superposed, because the NMR spectrum of the per-O-acetyl-1,4-anhydro-

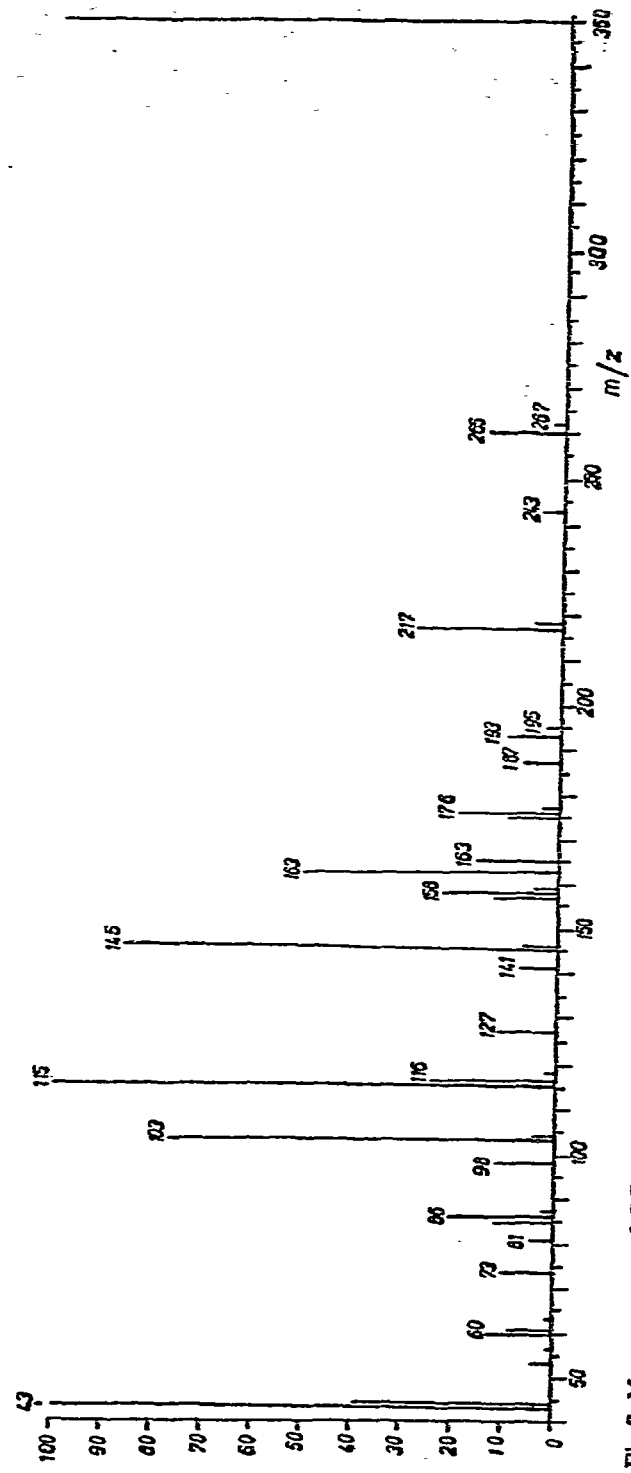



Fig. 7. Mass spectrum of GC peak 7 in Fig. 1.

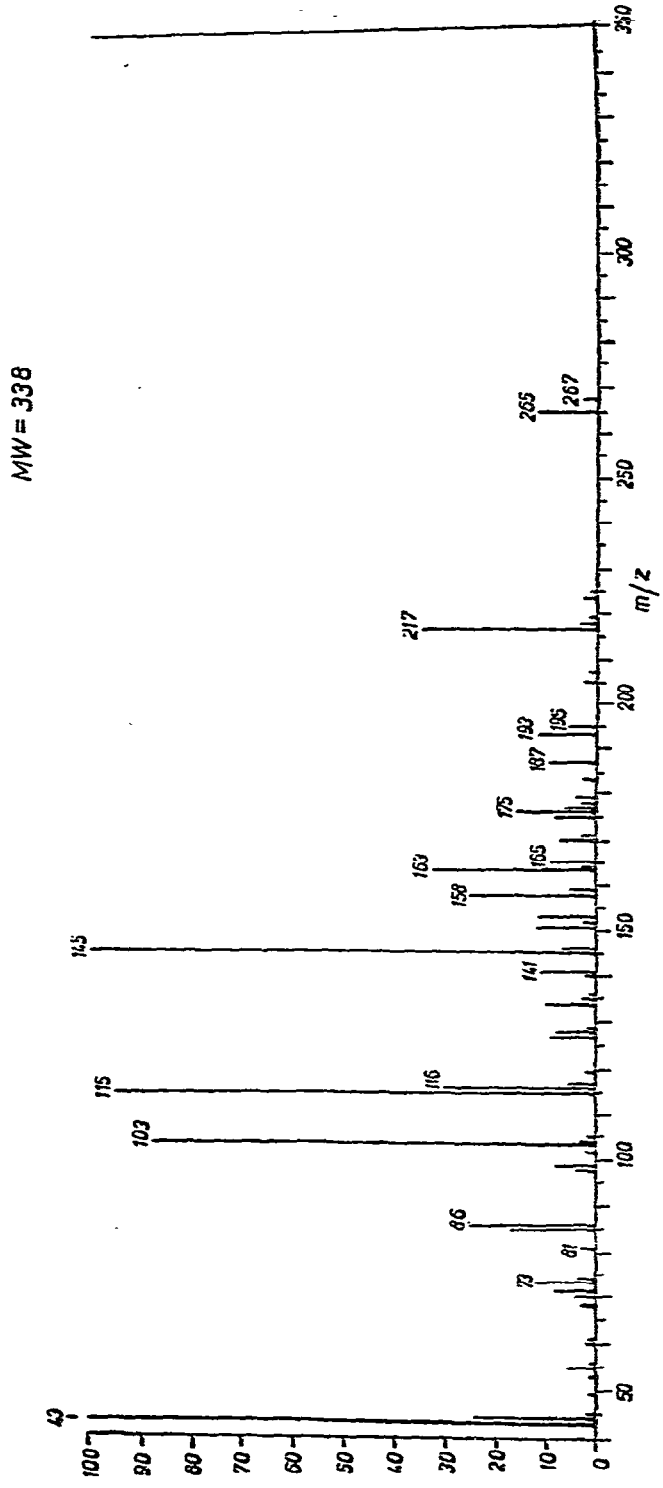



Fig. 8. Mass spectrum of GC peak 8 in Fig. 1.

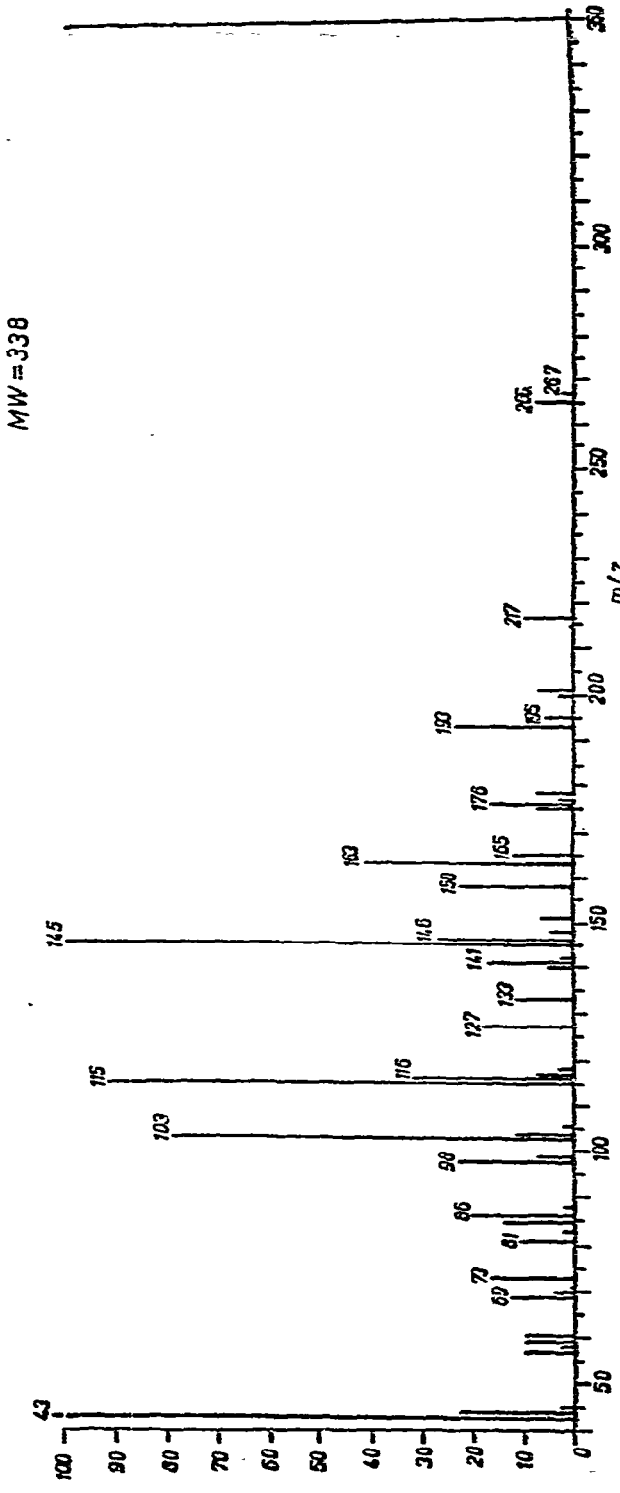



Fig. 9. Mass spectrum of GC peak 9 in Fig. 1.

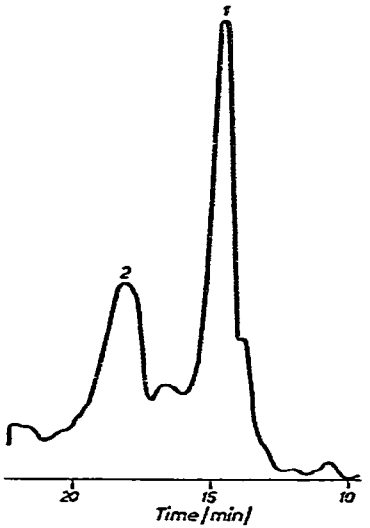



Fig. 10. HPLC separation of product mixture formed after heating xylitol with hydrochloric acid in sealed ampoule at 100° for 72 h.

xylitol derivative shows separate signals for the three methyl groups derived from acetyl substituents.

In discussing the structure of the compounds we use the term pentitol without defining the configuration. Configurational assignment will be dealt with in a later study.

Heating under reflux with concentrated hydrochloric acid at 100° for 3 h

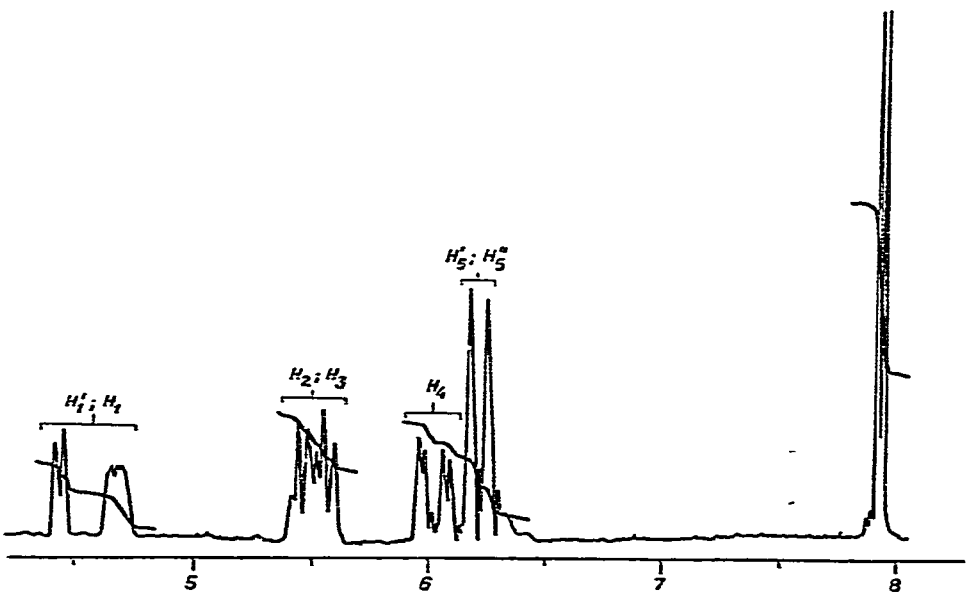



Fig. 11. NMR spectrum of GC peak 1 in Fig. 1.

TABLE I

PRODUCT YIELDS FROM XYLITOL UNDER VARIOUS REACTION CONDITIONS

| *GC peak in Fig. 1* | *Acetyl derivative of* | *Yield *of reactions %* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Conc. HCl solution refluxed at 100° for* | | *Conc. HCl solution heated in glass sealed ampoule at 100° for* | | *5% $H_2SO_4$ solution heated in glass sealed ampoule at 160° for* | | |
| | | *3 h* | *24 h* | *3 h* | *72 h* | *10 min* | *30 min* | *60 min* |
| 1 | 5-Chloro-5-deoxy-1,4-anhydropentitol | 0.3 | 17.9 | 33.5 | 69.0 | — | — | — |
| 2 | 1,5-Anhydroxylitol | 0.1 | 0.2 | 0.1 | 0.1 | — | — | — |
| 3 | 1,4-Anhydropentitol | 6.5 | 6.3 | 1.7 | 0.2 | 0.4 | 1.7 | 4.5 |
| 4 | 1,4-Anhydropentitol | 79.5 | 75.3 | 17.9 | 0.4 | 6.5 | 30.7 | 79.5 |
| 5 | Dichlorodideoxypentitol | — | — | 19.2 | 29.6 | — | — | — |
| 6 | Dichlorodideoxypentitol | — | — | 0.2 | 0.2 | — | — | — |
| 7 | Monochloromonodeoxypentitol | — | — | 1.5 | — | — | — | — |
| 8 | Monochloromonodeoxypentitol | 0.5 | 0.3 | 22.2 | 0.5 | — | — | — |
| 9 | Monochloromonodeoxypentitol | 0.1 | — | 2.2 | — | — | — | — |
| 10 | Xylitol | 13.0 | — | 1.5 | — | 93.1 | 67.6 | 16.0 |

* Calculated from GC peak areas.

leads to *ca.* 87% reaction, giving rise mainly to two 1,4-anhydropentitol compounds, whereas heating under identical conditions for 24 h makes xylitol disappear, giving rise to a large number of 5-chloro-5-deoxy-1,4-anhydropentitols. Within 3 h the hydroxyl groups are substituted by halogen both in the initial xylitol and in the cyclization product. Heating for 72 h under similar conditions gives rise to only two products (98% in all), namely 5-chloro-5-deoxy-1,4-anhydropentitol and dichloro-dideoxypentitol (Table I).

As 5-chloro-5-deoxy-1,4-anhydropentitol is a substrate for obtaining dianhydropentitol[12], it must be pure. Attempts were made (according to the literature) to purify the product of the concentrated hydrochloric acid action on xylitol at 100° during 72 h by distillation under reduced pressure, both in the form of an acetyl derivative and with free hydroxyl groups. In neither case did GC analysis show enrichment of the distillate with the required product. Purification was possible by using the HPLC method.

There are reports in the literature[9] of some dehydration methods in which 5% sulfuric acid is used at 160°. Chromatographic analysis of such a mixture (the reaction product was isolated by using barium carbonate to remove sulfuric acid) showed after 10, 30 and 60 min a markedly simpler composition. Apart from the initial xylitol, the mixture contained only various amounts of two 1,4-anhydropentitols. These results suggest that the best way to obtain 1,4-anhydropentitols alone is to heat xylitol under reflux at 100° for 6 h in concentrated hydrochloric acid, or at 160° for 2 h in a sealed ampoule with 5% sulfuric acid.

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