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IDENTIFICATION OF ACETYL DERIVATIVE ISOMERS OF 1,5-ANHYDRO-D-XYLITOL BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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SUMMARY

High-resolution gas chromatography and mass spectrometry have been employed to separate selectively acetylated 1,5-anhydro-D-xylitol derivatives and to study their chemical structures.

Complete separation of reaction mixtures was achieved on a capillary column coated with an SE-30 liquid phase. The combination of gas chromatography-mass spectrometry with oxidation reactions allowed the assignment of the chemical structures of all the derivatives, obtained.

INTRODUCTION

The relative reactivities of hydroxyl groups present a fundamental problem in the synthesis and stereochemical study of carbohydrates¹. Analytical methods have been considerably improved in recent years to allow more accurate measurements of product distribution. Relative reactivity has been found to be influenced by electronic and steric factors.

The selective behaviour of anhydroalditols in catalytic oxidation, acylation and acetylation has been studied². The present paper deals with the study of the selective acetylation reaction of 1,5-anhydro-D-xylitol by means of high-resolution gas chromatography and mass spectrometry (GC-MS).

We used capillary column GC-MS because we had obtained good results in the separation of alditols³ and anhydroalditols⁴ on a capillary column.

This paper is the first report of our studies of the thermodynamic characteristics of the relative reactivities of hydroxyl groups in carbohydrate derivatives.

EXPERIMENTAL

Reaction 1

To 2.68 mg of 1,5-anhydro-D-xylitol (0.02 mM) dissolved in 200 μ l of dry pyridine was added 1.9 μ l (0.02 mM) of acetic anhydride. The solution was heated for 1 h at 100°, evaporated to dryness in a nitrogen stream, and treated with 200 μ l

of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). After this mixture had been heated for 15 min at 100°, it was used for GC and GC-MS analyses.

Reaction 2

The above procedure was repeated using 2.7 mg (0.02 mM) of anhydroxylitol and 3.8 μ l (0.04 mM) of acetic anhydride.

Reaction 3

The above procedure was repeated using 2.7 mg (0.02 mM) of anhydroxylitol and 5.6 μ l (0.06 mM) of acetic anhydride.

The three reactions were repeated using labelled acetic anhydride (d_6) to generate derivatives selectively labelled in the acetyl groups.

Reaction 4

To 5 mg (0.056 mM) of anhydroxylitol dissolved in 400 μ l of pyridine was added 5.3 μ l (0.056 mM) of acetic anhydride. The solution was heated and divided into two equal samples. The first was derivatized and analysed. The second was oxidized with 5.8 mg (0.027 mM) of sodium periodate in water (400 μ l) at room temperature for 24 h. Then the solution was evaporated to dryness and the residue treated with 200 μ l of BSTFA.

GAS CHROMATOGRAPHY

Column preparation

Borosilicate (Pyrex) glass capillaries (80 m \times 0.7 mm O.D. \times 0.3 mm I.D.) were drawn from 1.5-m tubes (6.2 mm O.D. \times 3.0 mm I.D.) using a Hewlett-Packard glass drawing and coiling apparatus. Prior to drawing, the tubes were rinsed successively with acetone, methylene chloride, 1% aqueous potassium hydroxide and methanol, and dried under vacuum⁵. The diameter of the capillary coil was 10 cm⁶.

The glass capillaries were silanized with dimethyldichlorosilane (DCMS) and coated with SE-30 containing Silanox 101 (Cabot Corp., Boston, Mass., U.S.A.) by the German and Horning⁷ method and prepared by the dynamic method. In the first step, a plug consisting of Silanox dispersed in a dilute solution of the SE-30 phase in carbon tetrachloride was forced through a column immersed in an ultrasonic bath. After the second coating, the last 20 m of the capillary column was broken off.

The columns were conditioned in a flow of carrier gas (argon) by temperature programming at 1°/min from 40° to 280°, keeping the temperature at 280° for 2 h, then lowering it to 200°, programming it again at 1°/min to 300°, and keeping it at 300° for 1 h. The theoretical plate efficiencies of the resulting columns (for *n*-tetracosane at 250°) exceed 130 000.

Instrumental conditions

The compounds studied were separated with a Varian Aerograph series 1400 gas chromatograph modified to include a glass inlet system with splitter and 60 m glass capillary columns. The inlet system (see Fig. 1) was designed as a very simple, inexpensive, home-made unit to fit all GC injection ports. It contains 2.2 mm I.D. glass tubing in the form of a precolumn packed with glass wool and 3% SE-30 on

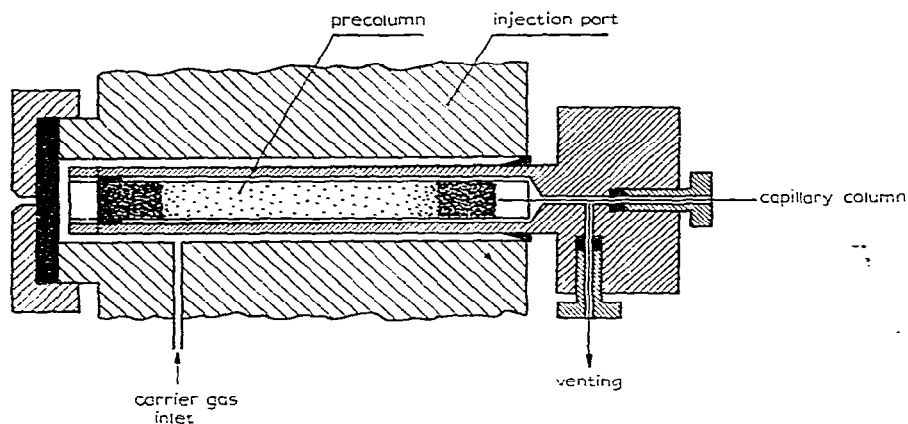


Fig. 1. A modified inlet system.

Gas-Chrom Q. Two capillary columns (60 m and 12 m), were connected to the inlet system. The 12-m column was used as restrictor for venting the carrier gas flow at a splitting ratio of 1:5. The end of the 60-m column was connected to a make-up system (Fig. 2) which in turn fed a flame ionization detector. This system, with an additional argon flow of 20 ml/min, assures maximum column efficiency.

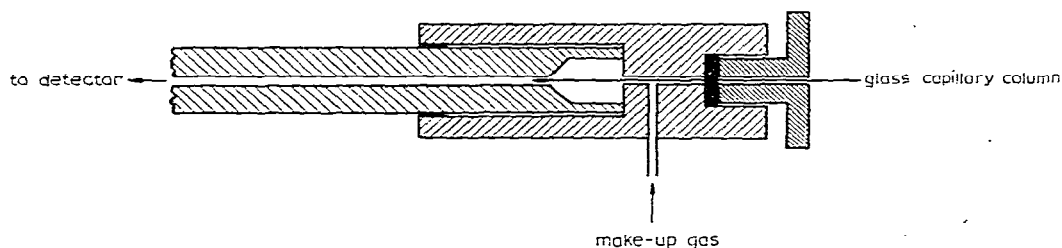


Fig. 2. Make-up system.

A Philips PM 8220 recorder was used. Further GC conditions adopted were: GC oven, 150° (isothermal); precolumn inlet splitter, 250°; detector block, 260°; column inlet pressure, 1.5 kg/cm², resulting in a carrier gas (argon) linear velocity of 16–17 cm/sec (200°); hydrogen flow-rate, 30 ml/min; air flow-rate, 250 ml/min.

Quantitative analysis

Calculations were performed by hand, and area measurements by multiplying the height of the peak by its width at half height. The weight response factors were assumed to be equal for all isomers studied.

MASS SPECTROMETRY

The mass spectra were recorded on a LKB 2091 mass spectrometer linked to a mini-computer PDP-11. The mass spectrometer was equipped with a 25-m capillary

column coated with an SE-30 liquid phase. The spectrometer was operated at a source temperature of 260°, the molecular separator at 250°; the ionizing voltage was 70 V; a scan from m/e 10 to 680 took 2 sec.

RESULTS AND DISCUSSION

1,5-Anhydro-D-xylitol has a plane of symmetry that decreases the number of products because positions 2 and 4 are equivalent. All the hydroxyl groups are equatorial.

The reaction of 1,5-anhydro-D-xylitol with one, two or three equivalents of acetic anhydride (the third reaction corresponds to a molar ratio of one anhydride molecule to one hydroxyl group) was studied. The mixture shows five GC peaks (Fig. 3) in a unimolar reaction (reaction 1). The GC peak labelled F was assigned to per-O-silyl derivative on the grounds of the mass spectrum (Fig. 4) and of the retention time.

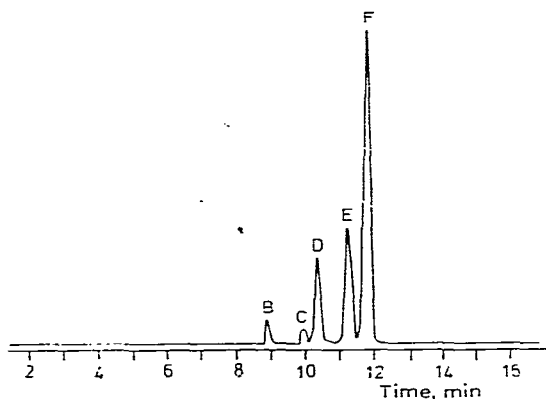


Fig. 3. GLC of the mixture 1.

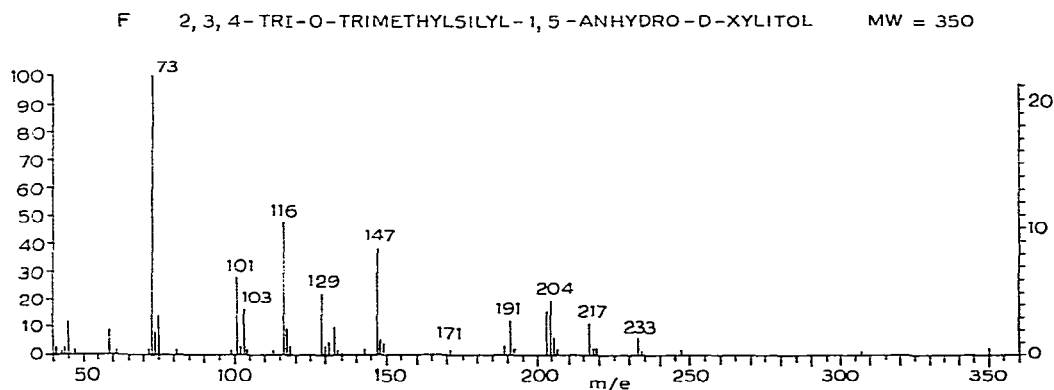


Fig. 4. Mass spectrum of GC peak F.

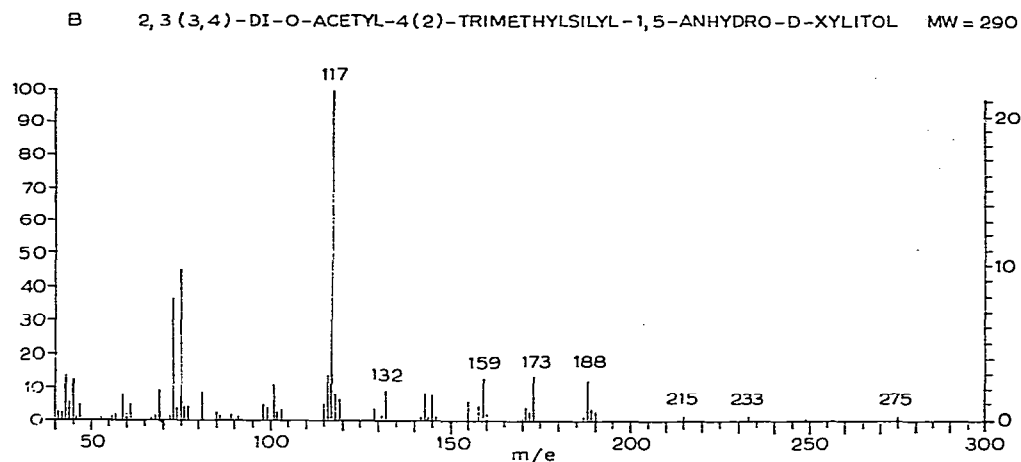


Fig. 5. Mass spectrum of GC peak B.

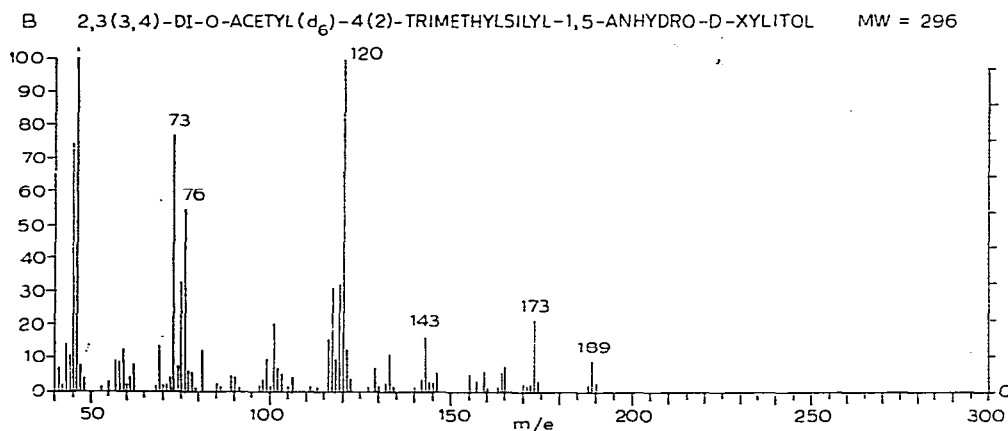


Fig. 6. Mass spectrum of GC peak B (labelled isomer).

The peak labelled B is 2,3(3,4)-di-O-acetyl-4(2)-O-trimethylsilyl-1,5-anhydro-D-xylitol. This structure has been confirmed by mass spectrometry (Figs. 5 and 6). The m/e 188/189 ion corresponds to loss of acetic acid (m/e 230/233) followed by ketene elimination. This sequence is only possible for an acetyl derivative with two acetoxy groups on adjacent carbon atoms.

The next GC peak (C) can be assumed to be 2,4-di-O-acetyl-3-O-trimethylsilyl-1,5-anhydro-D-xylitol. The mass spectra (Figs. 7 and 8) contain the ions m/e 275/281 ($M-15$) and m/e 170/170 ($M-2 \times 60$). Owing to the presence of the plane of symmetry there are only two possible structures for the di-O-acetyl derivatives of 1,5-anhydro-D-xylitol, namely 2,3- and 2,4-. Comparison of the spectra (Figs. 5 and 6 and 6 and 7) eliminated the first structure (2,3-di-O-acetyl) because the GC peak B had previously been shown to be of this derivative.

Thus, the monoacetylsilyl derivatives (peaks D and E) appear in the gas chromatogram before the per-O-silyl derivative (peak F). The mass spectra of both

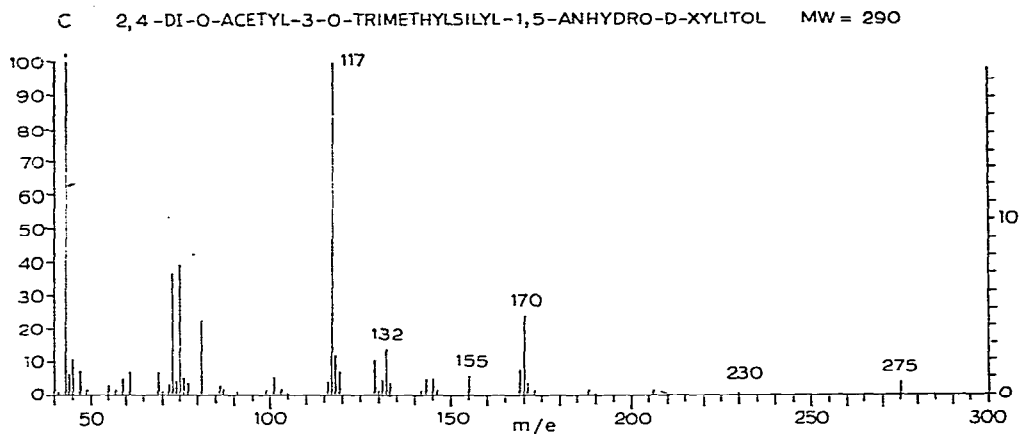


Fig. 7. Mass spectrum of GC peak C.

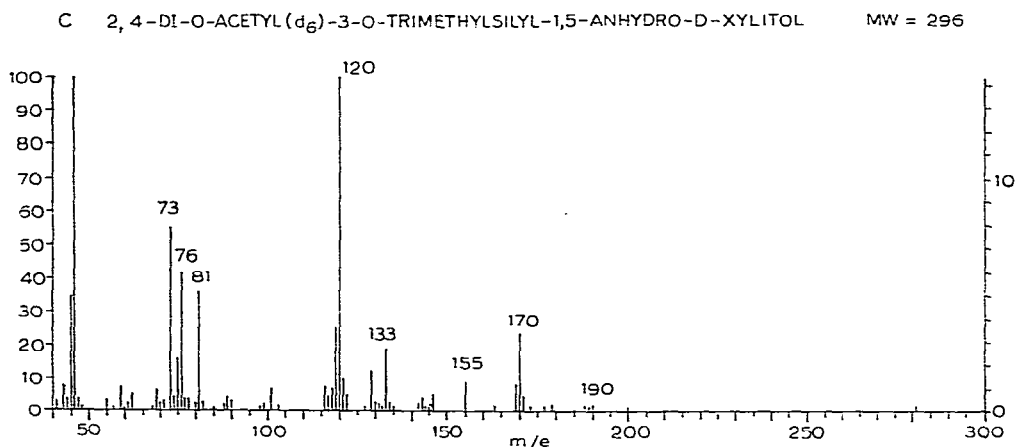


Fig. 8. Mass spectrum of GC peak C (labelled isomer).

compounds (Figs. 9–12) reveal significant differences. The second (GC peak E) is assumed to be the 2(4)-O-acetyl-per-O-silyl derivative because its mass spectra (unlabelled and labelled derivatives) exhibit abundant m/e 147 ion. The m/e 147 ion in the mass spectra of trimethylsilylated sugar ethers has been shown⁸ to arise from the $(M - 15)$ ion by means of a cyclic structure. Havlicek *et al.*⁹ have shown by determining the number and type of cyclic processes which provide the $\text{TMSiO} = \text{SiMe}_2$ ion that it is possible to predict which of an isomeric pair will show a stronger m/e 147 ion. 1,2-Diequatorial derivatives (1,2-*trans*) have been assigned the value 0.75, and 1,3-diaxial (1,3-*cis*) derivatives the value 1.50. The cyclic structure of the 1,3-diaxial group involves a change of ring conformation. As the mass spectra did not differentiate between the two possible monoacetyl isomers, we decided to discover the position of the acetyl group by a chemical method. 1,2-Glycols are known to be oxidized much more easily than 1,3-glycols¹⁰. A gas chromatogram run for a sample following

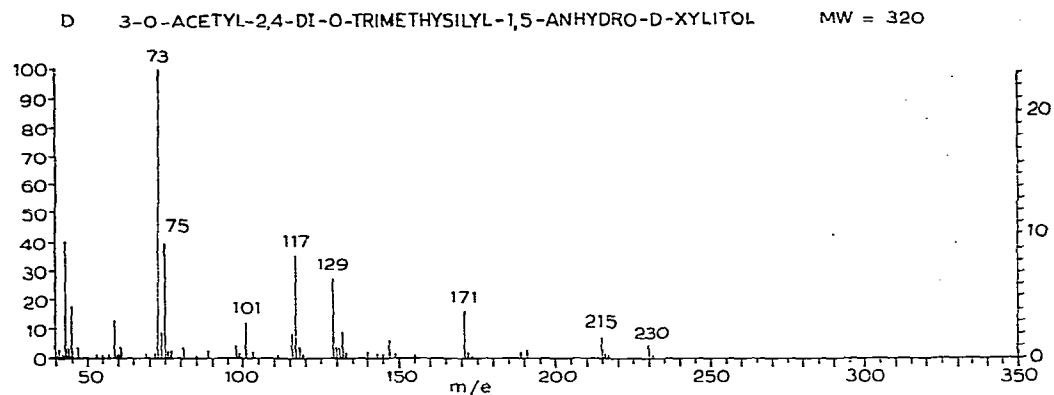


Fig. 9. Mass spectrum of GC peak D.

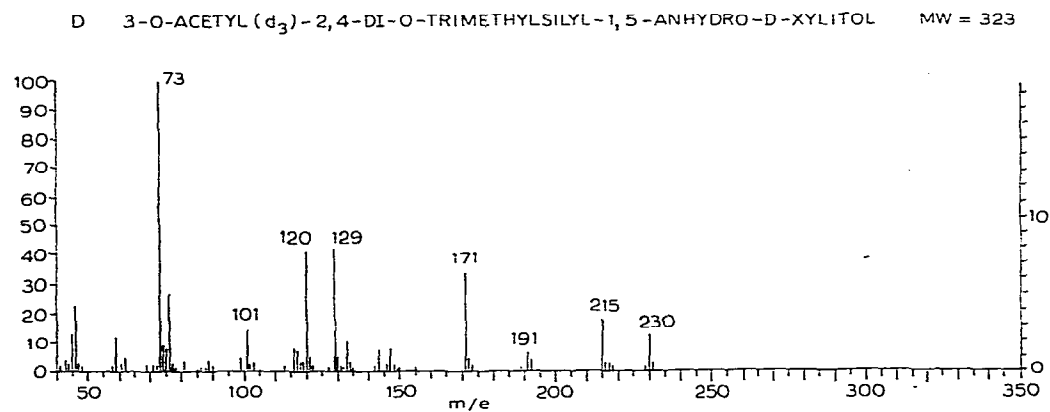


Fig. 10. Mass spectrum of GC peak D (labelled isomer).

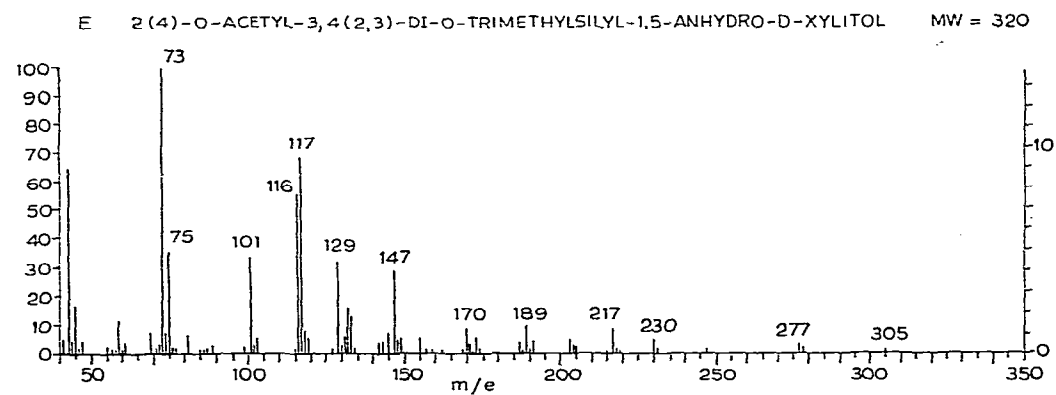


Fig. 11. Mass spectrum of GC peak E.

E 2(4)-O-ACETYL(d_3)-3,4(2,3)-DI-O-TRIMETHYLSILYL-1,5-ANHYDRO-D-XYLITOL MW = 323

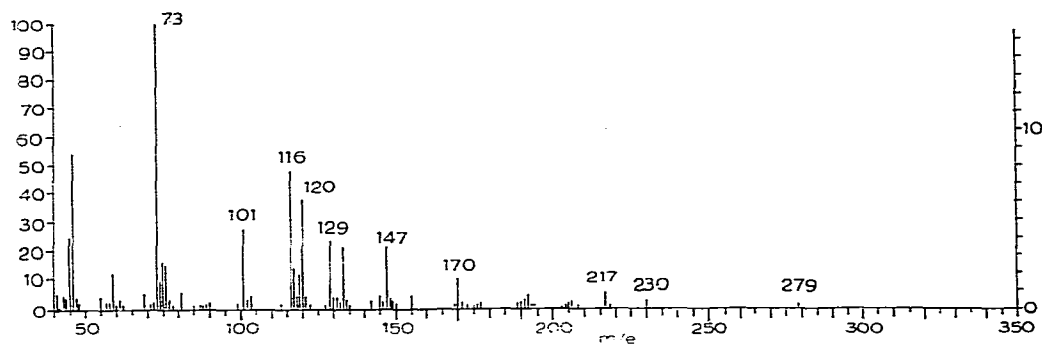


Fig. 12. Mass spectrum of GC peak E (labelled isomer).

sodium periodate oxidation is shown in Fig. 13. In this chromatogram, the GC peaks E and F disappeared completely. This showed that the 2(4)-monoacetyl structure may be assigned to the GC peak E, as peak F has previously been assigned as silyl derivative of 1,5-anhydro-D-xylitol.

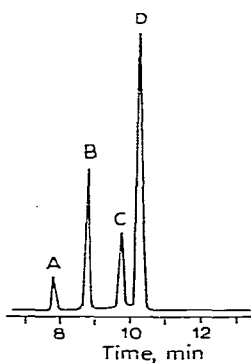


Fig. 13. GLC of the mixture 4.

TABLE I

THE COMPOSITION OF REACTION MIXTURES 1, 2 AND 3

GC peak	Silyl derivative of	Reaction mixture					
		1		2		3	
		Yield* (%)	Molar yields	Yield* (%)	Molar yields	Yield* (%)	Molar yields
A	Per-O-acetyl-1,5-anhydro-D-xylitol**	—	—	1.0	0.5	22.5	8.5
B	2,3(3,4)-Di-O-acetyl-1,5-anhydro-D-xylitol	3.0	1.0	7.0	2.5	24.5	8.5
C	2,4-Di-O-acetyl-1,5-anhydro-D-xylitol	2.0	0.7	7.0	2.5	22.5	8.0
D	3-O-Acetyl-1,5-anhydro-D-xylitol	14.0	4.5	17.5	5.5	17.0	5.0
E	2(4)-O-Acetyl-1,5-anhydro-D-xylitol	20.5	6.5	29.0	9.0	10.0	3.0
F	1,5-Anhydro-D-xylitol	60.5	17.5	38.5	11.0	3.5	1.0

* Weight yields from GC peaks area.

** Simple acetyl derivative of 1,5-anhydro-D-xylitol.

The product distributions in samples 2 and 3 are shown in Figs. 14 and 15. If more acetic anhydride is used then more of the per-O-acetyl and di-O-acetyl derivatives is obtained. This confirms the previous structural assignments.

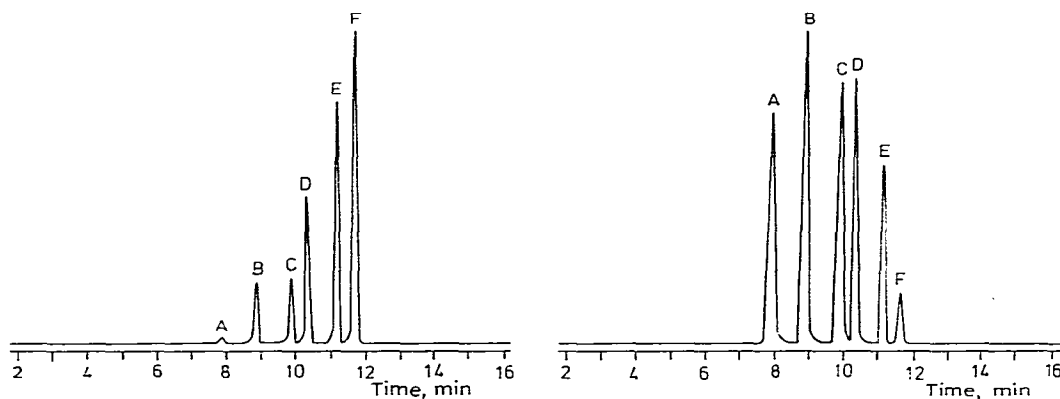


Fig. 14. GLC of the mixture 2.

Fig. 15. GLC of the mixture 3.

Study of the reaction of 1,5-anhydro-D-xylitol with one molar equivalent of acetic anhydride (reaction 1) in pyridine for 50 min at 100° showed that a 48% reaction occurred with a degree of substitution at O-3 and O-2(4) of 1.38 and 1.0, respectively (Table I). A similar ratio of reactivities has been obtained for a 1:2 molar ratio (1.22:1). The degrees of substitution were computed as molar yield ratios, keeping in mind that positions 2 and 4 are equivalent.

We shall publish further conclusions in a paper reporting kinetic studies.

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