

ANALYSIS OF TRIMETHYLSILYL DERIVATIVES OF SUGARS DERIVED FROM THE HYDROLYSATES OF WOOD PULP, SHOULDER MATERIAL AND UNFRACTIONATED CELLULOSE TRIACETATE BY GAS LIQUID CHROMATOGRAPHY – PART II

F. Mahmud* and E. Catterall

Department of Applied Chemistry Coventry (Lanchester) Polytechnic Coventry, England

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Wood pulp-based cellulose triacetate samples have shown a shoulder on the high molecular weight side of the gel permeation chromatographic (GPC) distribution curve. Material isolated from this region by fractional precipitation was found to be highly enriched in mannose and xylose, attributed to the presence of a hemicellulose derivative. Cellulose triacetate from cotton linters did not show this behaviour.

The hemicellulose derivative was hydrolysed using mineral acid and the hydrolysate was subjected to trimethylsilylation reaction in order to obtain volatile trimethylsilyl (TMS) derivatives. These TMS derivatives were analysed by gas liquid chromatography against known calibration standards.

INTRODUCTION

Most earlier studies of the preparation of volatile derivatives of polyhydroxy compounds were restricted to the use of either *o*-methyl ether or acetyl-derivatives as has been reviewed by Bishop^[1] and others.^[2,3] Gas liquid chromatography of trimethylsilyl derivatives of various sugars was reported by Bayer^[4] and Sweeley *et al.*,^[5] who extended the method used first by Makita and Wells^[6] for the preparation of trimethylsilyl (TMS) derivatives of bile acids.

Brewer *et al.*^[7] have reported the gas chromatography of TMS-derivatives of sugars in materials rich in hemicellulose, but have not presented any experimental details of the silylation process.

Trimethylsilyl derivatives of glucose, mannose and xylose and of the unknown samples in the present work were made to the standard conditions recommended by Sweeley *et al.*^[5]

EXPERIMENTAL

Materials. The chemicals and solvents used in this work were all analytical grade materials.

Procedure for the Preparation of Trimethylsilyl Derivatives. 10 mg of the sugar was dissolved in 1 ml of anhydrous pyridine (kept over KOH pellets) followed by the addi-

tion of 0.20 ml of hexamethyldisilazane and 0.10 ml of trimethylchlorosilane. The reaction was carried out in 5 ml plastic stoppered graduated flasks and the contents were vigorously shaken for 2-3 min at room temperature prior to chromatography.

The solution became cloudy on the addition of trimethylchlorosilane probably due to the precipitation of ammonium chloride and no attempt was made to remove it, as it did not interfere with the gas liquid chromatographic analysis of the TMS derivatives. Dissolution of the unknown samples in pyridine was effected by slightly warming the mixtures to 30-35° for 5 min prior to the addition of hexamethyldisilazane and trimethylchlorosilane.

The above procedure for the preparation of TMS-derivatives of pure sugars and unknown samples gave products which showed a single peak on the gas chromatogram, but when the amount of trimethylchlorosilane was doubled, small amounts of the respective anomeric forms were observed in most cases.

Analysis of trimethylsilyl derivatives by gas liquid chromatography. The trimethylsilyl (TMS) derivatives were analyzed using a Perkin Elmer F11 chromatograph, with a hydrogen flame ionization detector. Three coiled 5 ft stainless steel columns (OD ¼ inch) packed with 2.5% SE-30 on 100-200 mesh universal B, 8% carbowax 1540 on 80-100 mesh chromosorb-W, and 2.5% OV-17, on 80-100 mesh chromosorb G were employed. It was found that SE-30 operating at 180° gave better resolution of the anomeric forms of the various sugars studied. The resolution of the

*Present Address: Research Institute, University of Petroleum and Minerals, Dhahran, Saudi Arabia.

Table 1. Gas liquid chromatographic analysis of TMS-derivatives of sugars from hydrolysates of wood pulp, shoulder material, unfractionated cellulose triacetate and triacetate fraction free of shoulder material.

Sample as TMS derivative	Per Cent composition of each anomeric species						Per cent of shoulder mate- rial in GPC Chromatogram of each sample	Unidentified peaks Retention in mm	per cent of each
	Glucose		Mannose		Xylose				
	α -Glucose	β -Glucose	β -Mannose	β -Mannose	β -Xylose	α -Xylose			
Wood Pulp	51.60	17.10	1.01	0.30	0.61	0.20	—	147	29.00
Unfractionated Cellulose Triacetate	80.00	16.40	0.74	—	1.40	—	3.08	147	0.74
Shoulder Material	20.00	10.00	1.60	—	6.00	3.00	12.4	58.62	16.42
Cellulose Tri- acetate free of Shoulder Material	91.20	6.70	—	—	—	—	—	149	2.10

other two columns mentioned above was not very good by comparison.

RESULTS

Gas liquid chromatography of trimethylsilyl-derivatives of sugars from wood pulp, shoulder material and unfractionated cellulose triacetate. The results obtained from gas-liquid chromatography of various trimethylsilyl derivatives are given in Table 1.

The percentage composition of the component sugars was determined as the product of peak heights and widths at half heights.

Fig. 1 illustrates the chromatogram of the TMS-derivatives of "shoulder material*" on a 2.5% SE-30 column. The data for the various sugars, identified as TMS-derivatives, show that the presence of hemicellulose in cellulose triacetate sample and "shoulder material" is associated with the starting material or wood pulp.

The anomerization of the sugars shown in Table 1 is attributed to their relatively poor solubility in pyridine at room temperature, which necessitated heating the sugars with pyridine prior to silylation.

*Gel permeation chromatography (GPC) of cellulose triacetate made from wood pulp shows a small peak or "shoulder" on the high D_p side of the GPC distribution. Material isolated from this shoulder area by both fractional precipitation and preparative GPC is highly enriched in mannose and xylose.

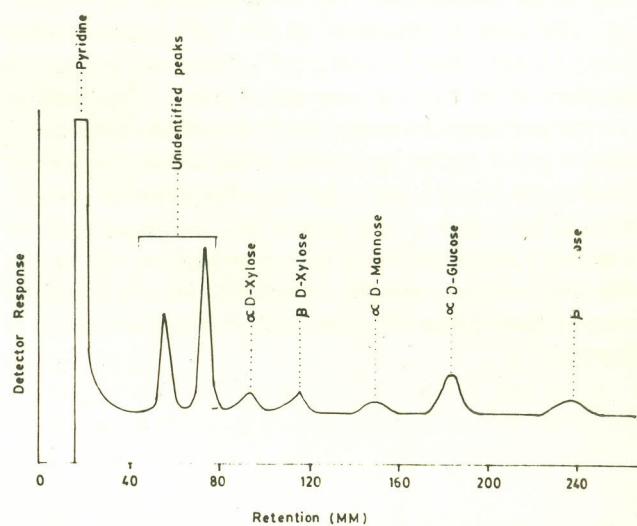


Fig. 1. Chromatogram of Hydrolysed and (Trimethylsilylate) "Shoulder Material" (Column 2.5% SE-30) temperature 180°C, Nitrogen Pressure; 14 psi.

DISCUSSION

Gas Liquid Chromatography of Trimethylsilyl Derivatives. The data from the gas liquid chromatography of TMS-derivatives of various sugars present in the hydrolysates of wood pulp, unfractionated cellulose triacetate, shoulder material and cellulose triacetate free of shoulder material are presented in Table 1.

The multiplicity of peaks indicated in Fig. 1 is believed to be due to the presence of small amounts of β -anomers

rather than incomplete silylation. An excess of trimethylchlorosilane was employed to reduce the possibility of incomplete silylation. The hydrolysates had low solubility in the reaction medium (pyridine) at room temperature and thus necessitated the application of heat.

According to Sweeley, *et al.*, [5] anomerization of sugars does occur in pyridine solution. Anomerization of xylose and sugars from aqueous equilibrium solutions has also been reported by these workers. [5]

The absence of β -anomers of both mannose and xylose in unfractionated cellulose triacetate and of β -mannose in the "shoulder material" is probably due to exceedingly small amount of the β -anomers in the samples in question. Any attempt to increase the sample size and/or the sensitivity of the instrument to establish the presence of the β -anomers resulted in an undesirable increase of other peaks, particularly α -glucose.

Further comparison of results in Table 1 shows the presence of an unidentified peak with retention of 147-149 mm. This peak is typical of all the hydrolysates studied except for the "shoulder material", where two peaks with retention of 58 and 62 mm are observed. The peak at 147-149 mm might have resulted from cellobiose which is a product of the partial hydrolysis of the sample in question. The absence of this peak in the "shoulder material" reflects the total hydrolysis of this sample. The two peaks with low retention stated above must have formed from compounds with low molecular weights. The sample free of "shoulder material" gave peaks for α - and β -glucose and cellobiose as expected.

CONCLUSION

Both paper [8] and gas liquid chromatography of the hydrolysates of different samples (as sugars or TMS derivatives) has shown that the hemicellulose present in the unfractionated cellulose triacetate and "shoulder material" resulted from the starting material (wood pulp) used for making commercial cellulose derivatives.

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