that the chart movement was irregular. Recorders 6 and 7 gave substantially less irregularity than the other five.

Recorders 1 to 5 were similar models from one manufacturer having a chart width of 10 in. Recorder 6 was of different manufacture but similar specification while recorder 7 was a 20 cm width flat-bed model. The most obvious difference between the recorders was that 1–5 included a chain and sprockets in the chart-drive mechanism whereas 6 had worms and pinions and 7 had an entirely spur-gear train, but it has not so far been possible to prove that the chain is the source of the irregularity. The fitting of an improved type of chain supplied by the manufacturer made no significant difference to the chart travel.

The irregularities demonstrated could cause substantial errors in analysis and it is evident that for precise work careful testing and selection of recorders is important.

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Analysis of sugar mixtures by gas–liquid chromatography

The separation of sugar derivatives by gas–liquid(GLC) chromatography was actively studied recently[1]. Remarkable results were obtained for the separation of sugars as the trimethylsilyl and permethylated derivatives[2]. The only disadvantage of the method is the complicated pattern of the chromatogram as each sugar yields up to four isomers during methylation or trimethylsilylation. Thus GLC analysis of sugars as trimethylsilyl and methyl derivatives is efficient for simple mixtures only and for the identification of preseparated monosaccharides. GLC of derivatives giving only a single peak on the chromatogram for parent monosaccharide would be preferable for sugar determination. TMS derivatives of aldono-1,4-lactones and diethyldithioacetal acetates[3,4] satisfy this demand but are rather difficult to prepare. Acetates of polyols[5,6] have been suggested for GLC and are available for quantitative sugar analysis. However it should be noted that the elution time for the compounds is considerable due to their higher boiling points.

This paper describes the use of permethylated polyols and permethylated methyl glycosides for qualitative and quantitative analysis of sugars by GLC.

Hitherto the absence of a simple and effective procedure for producing permethylated polyols prevented the use of these derivatives for GLC analysis. The new and effective procedure of carbohydrate methylation in the presence of sodium hydride and dimethyl sulphoxide[7] has been recently applied to a number of carbohydrate types[8].

Our data also show excellent results in the case of polyol methylation. Monosaccharides were also methylated by essentially the same procedure.

Experimental

Apparatus. The analysis was carried out using a "Tswet I-64" (Dzerjinsk, U.S.S.R.) gas chromatograph. Experimental conditions are given in Table.I

TABLE I

APPARATUS AND EXPERIMENTAL CONDITIONS

Chromatograph	"Tswet I-64" (Dzerjinsk, U.S.S.R.)
Detector	Flame-ionization
Dosator temperature	250°
Column	Steel, 1 m, I.D. 4 mm
Carrier gas	Nitrogen
Gas flow rate	75 ml/min
Solid support	Celite 545 (80–100 mesh) Chromosorb W (30–60 mesh)
Chart rate	1 cm/min
Sample size	3–5 μl 10–20 % in chloroform

Derivatives. Permethylated monosaccharides and polyols were obtained by the HAKOMORI procedure.[7] The monosaccharide or polyol (0.1 g) in dimethylsulphoxide (5 ml) was added to a solution of methylsulphinyl carbanion prepared by stirring sodium hydride (0.1 g) in dimethyl sulphoxide (5 ml) for 0.5 h at 65°. The reaction mixture was stirred for 10 min at 20° and treated with methyl iodide (0.5 ml), stirring continuously for 20 min, then chloroform (20 ml) was added. The resulting mixture was washed with water (4 × 10 ml), dried over sodium sulphate and evaporated *in vacuo*. Permethylation was checked by I.R. spectra and thin-layer chromatography on silica gel with 10 % ethanol in chloroform as the developer. The detection reagent was 2 % $K_2Cr_2O_7$ in conc. sulphuric acid (110°/15 min).

Reduction procedure. 1 ml of 10 % KBH_4 in 50 % aqueous methanol was added to the sugar mixture (10 mg). The mixture was stirred for 15 min at 20°, treated with Amberlite IR-120 and evaporated several times in the presence of methanol. The resulting polyols were treated by continuously stirring with methyl sulphinyl carbanion (1 ml) prepared as above and then with methyl iodide (0.2 ml) for 20 min; chloroform (10 ml) was added to the mixture and dimethyl sulphoxide was washed out with water. In a similar way, monosaccharide mixtures (10 mg) were methylated to permethylated methyl glycosides. Solutions obtained were used for GLC analysis.

Results and discussion

A number of liquid phases and carriers were used for GLC separation of carbohydrates as their permethylated derivatives. The best results are given in Table II and Fig. 1. As may be seen some monosaccharides can be separated as their corresponding methyl ethers by gas–liquid chromatography. However, unsatisfactory resolution of xylitol from arabitol and mannitol from sorbitol were observed. Therefore, the GLC analysis of monosaccharides as their permethylated derivatives was used showing a distinctive pattern for the above sugars (Table III). As a result, 4–5

TABLE II

RELATIVE RETENTION TIMES OF METHYLATED POLYOLS

Column: A = 20% of Apiezon L on Celite 545, 180°.
B = 20% of Apiezon L on Celite 545, 158°.
C = 15% of PEGA[a] on Chromosorb W, 152°.
D = 10% of Apiezon L and 10% of methylated cellulose mixture on Celite 545, 138°.

	A	*B*	*C*	*D*
Meso-erythritol	0.20	0.23	—	0.10
Arabinitol	0.47	0.37	0.48	0.39
Xylitol	0.49	0.39	0.49	0.37
Rhamnitol	0.62	0.48	0.68	0.48
Mannitol	0.98	1.03	0.97	1.08
Sorbitol	1.00 (11.4 min)	1.00 (15.3 min)	1.00 (12.5 min)	1.00 (54.5 min)
Galactitol	1.18	1.17	0.95	1.27

[a] Polyethyleneglycol adipate.

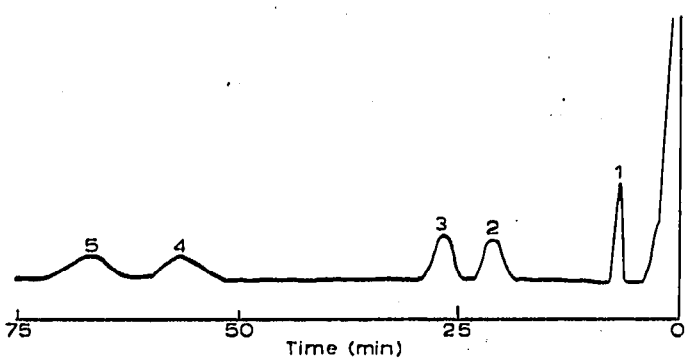

Fig. 1. Chromatogram of methylated polyols separated at 138° on a column packed with a mixture of 10% Apiezon L and 10% methylated cellulose on 80–100 mesh Celite 545. 1 = Meso-erythritol; 2 = arabinitol; 3 = rhamnitol; 4 = mannitol; 5 = galactitol.

TABLE III

RETENTION TIMES OF METHYLATED METHYL GLYCOSIDES IN MIN × 10

Compound	*PEGA[a] (20%) on Chromosorb W 152°*	*Apiezon M (20%) on Celite 545 184°*	*Apiezon L (20%) on Celite 545 152°*
Galactose	114[b], 142	84[b], 116	67, 130[b], 200
Glucose	56[b], 88	66[b], 82	89[b], 119
Xylose	21[b], 30, 54	29[b], 34, 42[b], 50	33[b], 41, 57[b], 78
Arabinose	40[b], 60[b]	30, 33[b], 40[b], 52	47[b], 55, 66
Mannose	75[b], 124[b]	66[b], 75[b]	109[b], 128[b]
Rhamnose	—	37[b]	—

[a] Polyethyleneglycol adipate.
[b] Most intense peak.

TABLE IV

GLC ANALYSIS OF TWO MONOSACCHARIDE MIXTURES
Column D, see Table II.

Component[a]	% *recovery*	*Component*[b]	% *recovery*
Erythrose	102.4	Erythrose	103.2
Arabinose	101.7	Xylose	98.5
Mannose	99.1	Glucose	97.8
Galactose	98.3	Galactose	98.9

[a] 7.5 mg of each component.
[b] 2.5 mg of each component.

component monosaccharide mixtures could be separated and identified. In the case of simple methylated polyol mixtures a quantitative analysis can be carried out successfully. Several synthetic monosaccharide mixtures were quantitatively analysed by reduction to polyols followed by conversion to their permethylated derivatives. The resulting mixtures of permethylated derivatives were separated and quantitated with rhamnitol as internal standard. Calculations were carried out by measuring the ratio between the substance and the standard peak areas (Table IV).

Data obtained indicate that both reduction and methylation of monosaccharides are quantitative and that the method is suitable for analysis of monosaccharide mixtures.

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