up to 5.0 ng for similar samples applied to the F & M instrument. The data indicated that a 3 % silicone GE XE-60 column would be preferable, because of the decreased retention time characteristics and because of minimal "bleed" problems after about 150 h of column conditioning.

Preliminary studies have been made with this gas chromatographic procedure, in which fresh papayas were fortified with Captan and Phaltan at the 1.0 p.p.m. level. Cleanup of the benzene extract of the papaya consisted of a 5-minute contact of the extract with Nuchar C-190 carbon (3 g carbon per 100 g whole fresh fruit). Papaya plant extractives did not affect the pattern of the Captan and Phaltan curves chromatographed on the GE XE-60 column, and recoveries of the fungicides from the extracts ranged from 85–95 %.

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Gas–liquid chromatography of methylated D-galactose derivatives

Gas–liquid chromatography (GLC) of methylated methyl glycosides, and GLC of methyl-D-galactosides[1–3] in particular, is widely used for structural polysaccharide investigation by methylation procedure[4,5]. This paper describes the further investigations of the experimental conditions of GLC-separations of methylated methyl-D-galactosides.

Experimental

Apparatus. The analysis was carried out using a Pye Argon Chromatograph. Experimental conditions are given in Table I.

TABLE I

EXPERIMENTAL CONDITIONS

Detector	β-ionization (80 μC radium D)
Columns	Glass tubes: 1.2 m, I.D. 5 mm
Carrier gas	Argon
Solid support	Acid washed Chromosorb W (60–80 mesh)
Sample size	0.025 μl *ca.* 5 % in chloroform
Liquid phases	A = 5 % neopentylglycol succinate
	B = 5 % polyethyleneglycol adipate
	C = 10 % Apiezon M

Derivatives. The samples of methylated methyl-D-galactosides with the exception of the 3,4,6-tri-O-methyl-derivative were synthesized as described previously[6,7]. In all cases, methylation was carried out using a modified Kuhn's procedure[8]. All solutions were evaporated *in vacuo* at 40°. The course of reactions was checked by thin-layer chromatography (TLC) on Silica Gel KSK (200 mesh and more) using 10 % ethanol in chloroform or methylethyl ketone, saturated with 1 % ammonia, as developer. The detection reagents were conc. sulphuric acid or a saturated solution of ammonium sulphate (120°/15 min in both cases). Methyl ethers of D-galactose were also chromatographed on Whatman 3 MM or Leningrad factory "Goznak" papers using methyl ethyl ketone as developer. The detection reagent was aniline hydrogen phthalate. The purification of methylated D-galactose derivatives was achieved using column chromatography on Silica Gel KSK (100–200 mesh) by gradient elution with suitable solvents, as usual. Fractions collected were examined by TLC as above. 3,4,6-Tri-O-methyl-D-galactose had been prepared earlier by methylation of 1,2-isopropylidene-D-galactose[9]; recently it has been obtained in methylation studies of synthetic galactan[10].

In the present work the above sample was synthesized as follows: 3,4,6-tri-O-acetyl-1,2-O-(ethylorthoacetyl)-α-D-galactopyranoside (1 g) obtained as described previously[11] was treated with ammonia saturated in absolute methanol (50 ml) overnight at room temperature. The mixture was evaporated; the slightly yellowish syrup revealing only one spot on chromatograms was permethylated as above. The reaction product was hydrolyzed with 0.1 *N* HCl for 2 h at 100°, deionized and evaporated. 3,4,6-Tri-O-methyl-D-galactose was obtained as a colourless syrup, $(\alpha)_D^{20}$ —5° (in CH_3OH, yield 0.2 g); showed a positive test with WALLENFELS AND BONNER's[13] reagents; 2-O-tosyl-3,4,6-tri-O-methyl-β-methyl-D-galactose[14], prisms, m.p. 160°.

Results and discussion

A number of liquid phases, operation temperatures and carriers were used for the GLC-separation of methylated methyl-D-galactosides. The best results obtained using the above experimental conditions (Table I) are given in Table II and Fig. 1. Column A allows satisfactory separation of the most isomeric and anomeric derivatives to be achieved. Columns B and C are suitable for separation of a number of isomers. However, on these latter columns, the dimethyl derivatives have been

TABLE II

RETENTION TIMES[a] OF METHYLATED METHYL-D-GALACTOSIDES

	Methyl galactoside	*Anomer*	*Ring form*	*A**	*B***	*C****
1	2,3,5,6-tetra-O-methyl-	α	F	0.82	0.89	0.81
2		β	F	0.82	0.89	0.81
3	2,3,4,6-tetra-O-methyl-	β	P	1.00	1.00	1.00
4		α	P	1.00 (6.2 min)	1.00 (11.9 min)	1.00 (7.01 min)
5	3,4,6-tri-O-methyl-	β	P	1.54	—	—
6		α	P	1.62	—	—
7	2,3,6-tri-O-methyl-	α	F	1.70	—	—
8		β	F	1.80	—	—
9		β	P	2.18	1.34	2.59
10		α	P	2.30	1.34	2.59
11	2,3,5-tri-O-methyl-	α	F	2.17	—	—
12		β	F	2.23	—	—
13	2,4,6-tri-O-methyl-	β	P	2.19	1.32	2.59
14		α	P	2.60	1.66	2.72
15	2,3,4-tri-O-methyl-	β	P	3.16	1.62	3.21
16		α	P	3.64	1.73	3.35
17	2,6-di-O-methyl-	β	P	4.89	—	—
18		α	P	5.63	—	—
19	2,3-di-O-methyl-	β	P	6.51	1.89	—
20		α	P	7.72	1.93	—
21	3,4-di-O-methyl-	β	P	10.9	—	—
22		α	P	11.1	—	—

[a] Relative to 2,3,4,6-tetra-O-methyl-β-methyl-D-galactoside. F=furanoside; P=pyranoside.
* On column A at 167° with a flow rate of 62 ml/min.
** On column B at 151° with a flow rate of 70 ml/min.
*** On column C at 159° with a flow rate of 70 ml/min.

assigned to the wide overlapping peaks with very long retention times. Since each methyl ether of galactose may give rise to two to four anomeric glycosides, one pure anomer was obtained, wherever possible, to facilitate assignment of the peaks. As usual the furanoside ring form has been assigned to the faster peak, and the pyranoside ring form to the slower peak. As for the methyl galactopyranosides, when the two anomers were separable, the β-anomer was eluted before the α-anomer, while the reverse pattern was observed for the methyl galactofuranosides. The above GLC-separation was used successfully in methylation studies of some pectic substances and will be described elsewhere.

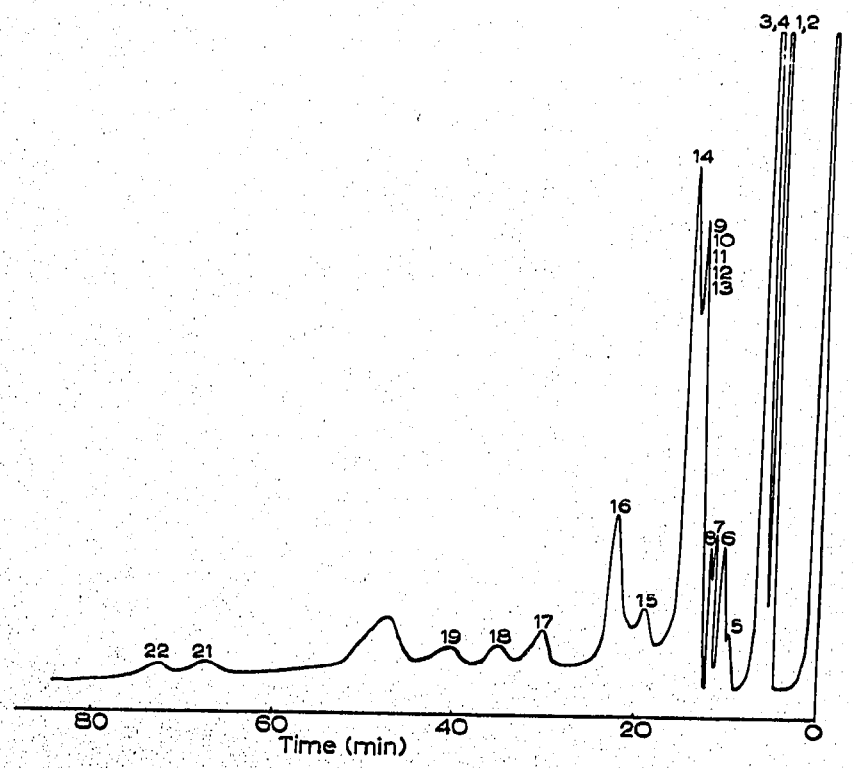

Fig. 1. Separation of the mixture methylated methyl-D-galactosides by gas–liquid chromatography on column A. For the numeration of peaks see Table II.

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