GAS CHROMATOGRAPHY OF THE METHYL EHTERS OF METHYL (METHYL-α-D-GALACTO- AND MANNOPYRANOSID)URONATES

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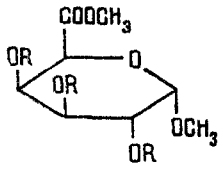
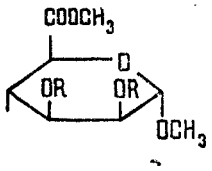
Conditions are proposed for the gas-chromatographic separation of the methyl ethers of methyl (methyl α-D-galacto- and mannopyranosid)uronates in the form of their complete acetates on the stationary phases OV-17, GE-XE-60, and QF-1.

In spite of the vigorous development of the ^{13}C NMR spectroscopy of carbohydrates, methylation remains one of the most important methods of establishing the structures of complex polysaccharides and other carbohydrate-containing substances.

In the analysis of glycosides and of oligo- and polysaccharides containing D-galacturonic or D-mannuronic acid residues by the methylation method, after methanolysis it is mainly the α anomers of the corresponding methyl ethers of methyl (methyl-D-galactopyranosid)uronates (I) or (methyl D-mannopyranosid)uronates (II) that are obtained, these being thermodynamically more stable because of the anomeric effect:



I. R = H, CH_3



II. R = H, CH_3

The identification of compounds of this class by the usual method is very difficult and is practically impossible when only a small amount of carbohydrate-containing material is available.

With the aim of using gas chromatography and chromato-mass spectrometry to identify these compounds, we have studied the conditions for the gas-chromatographic separation of the methyl ethers of methyl (methyl α-D-galacto- and mannopyranosid)uronates in the form of their complete acetates.

In the literature on the separation of methyl ethers of methyl (methyl α-D-glucopyranosid)-uronate [1] it is shown that the use of the acetates as the volatile derivatives gives the best results, while the trifluoroacetyl and trimethylsilyl derivatives are separated considerably less well, and methyl ethers with several free hydroxy groups may be irreversibly adsorbed on the column. Furthermore, the acetates are readily obtained and are stable on storage.

In the selection of the conditions for gas chromatography, we have shown that the methyl (methyl O-acetyl-O-methyl-α-D-mannopyranosid)uronates are separated completely on the polymethylphenylsiloxane OV-17 and on the methyl-β-cyanoethylsiloxane rubber GE-XE-60, while on the polymethyltrifluoropropylsiloxane QF-1 only the 2,3- and 2,4-di-O-methyl ethers are practically irresolvable, the other methyl esters being separated well.

The methyl (methyl O-acetyl-O-methyl-α-D-galactopyranosid)uronates are also readily separated when chromatographed on OV-17, XE-60, and QF-1. However, on the phase XE-60, the 2- and 4-O- and also the 3-O- and 2,4-di-O-methyl ethers cannot be resolved. The first pair

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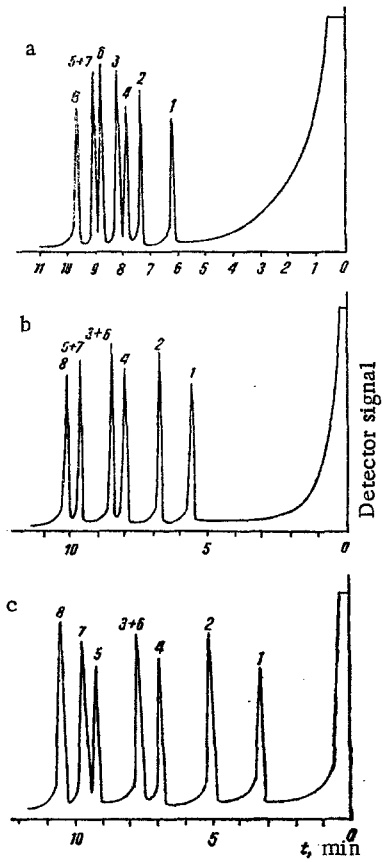

Fig. 1

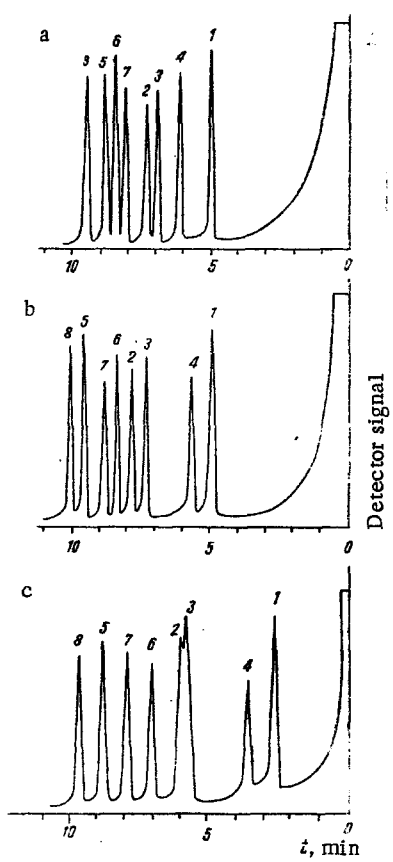

Fig. 2

Fig. 1. Separation of acetates of methyl ethers of methyl (methyl α-D-galactopyranosid)uronates: a) on the phase OV-17; b) on the phase GE-XE-60; c) on the phase QF-1. (The numbers above the peaks correspond to the numbers of the substances in Table 1).

Fig. 2. Separation of acetates of methyl ethers of methyl (methyl α-D-mannopyranosid)uronates: a) on the phase OV-17; b) on the phase GE-XE-60; c) on the phase QF-1. (The numbers above the peaks correspond to the numbers of the substances in Table 2).

is separated on the phase QF-1, and the second on the phase OV-17. In the general case, the phases XE-60 and QF-1 are preferable because of the greater difference in the relative retention times of the compounds under consideration.

Figure 1 shows typical examples of the separation of methyl (methyl O-acetyl-O-methyl-α-D-galactopyranosid)uronates on the phases OV-17, XE-60, and QF-1. Figure 2 illustrates the separation of methyl (methyl O-acetyl-O-methyl-α-D-mannopyranosid)uronates on the phases OV-17, XE-60, and QF-1.

EXPERIMENTAL

The synthesis of the methyl esters of methyl (methyl α-D-galactopyranosid)uronate has been described by us [2-4], and the synthesis of the methyl ethers of methyl (methyl α-D-mannopyranosid)uronate in [5, 6].

The acetates were obtained by treating the methyl ethers with an excess of acetic anhydride in pyridine at 70°C for 3 h followed by evaporation in vacuum.

Gas chromatography was carried out on a Chrom 41 chromatograph (Czechoslovakia). We used glass columns 1.2 m × 3 mm filled with: a) 3% of OV-17 on Inerton-Super (0.16-0.20 mm); b) 3% of GE-XE-60 on Gas Chrom Q (100-120 mesh); and c) 3% of QF-1 on Chromaton N (80-100 mesh). Working conditions: temperature of the evaporator 260°C, of the detector 290°C, and of the column initially 140°C and after 2 min programmed up to 250°C at the rate of 10°C/min

for the phases OV-17 and XE-60 and 8°C/min for the phase QF-1. The carrier gas was nitrogen, 40 ml/min. The substances were dissolved in ethyl acetate.

The relative retention times for the acetates of the methyl ethers of the methyl (methyl α-D-galacto- and mannopyranosid)uronates are given below. The retention times of methyl (methyl 2,3,4-tri-O-methyl-α-D-galacto- and mannopyranosid)uronates were taken as 1.00.

We give the relative retention times of the acetates of the methyl ethers of methyl (methyl α-D-galactopyranosid)uronate (I):

Compound	Phase		
	OV-17	*XE-60*	*QF-1*
1) 2,3,4-Tri-O-methyl- (I)	1,00[a]	1,00[b]	1,00[c]
2) 4-O-Acetyl-2,3-di-O-methyl- (I)	1,18	1,20	1,41
3) 3-O-Acetyl-2,4-di-O-methyl- (I)	1,32	1,48	2,06
4) 2-O-Acetyl-3,4-di-O-methyl- (I)	1,26	1,40	1,84
5) 3,4-Di-O-acetyl-2-O-methyl- (I)	1,46	1,68	2,46
6) 2,4-Di-O-acetyl-3-O-methyl- (I)	1,41	1,48	2,06
7) 2,3-Di-O-acetyl-4-O-methyl- (I)	1,46	1,68	2,58
8) 2,3,4-Tri-O-acetyl- (I)	1,55	1,77	2,76

The absolute retention times of compound (I) were: a) 6.25 min; b) 5.70 min; and c) 3.80 min.

Below we give the relative retention times of the acetates of the methyl ethers of methyl (methyl α-D-mannopyranosid)uronate (II):

Compound	Phase		
	OV-17	*XE-60*	*OF-1*
1) 2,3,4-Tri-O-methyl- (II)	1,00[a]	1,00[b]	1,00[c]
2) 4-O-Acetyl-2,3-di-O-methyl- (II)	1,38	1,63	2,30
3) 3-O-Acetyl-2,4-di-O-methyl- (II)	1,32	1,52	2,20
4) 2-O-Acetyl-3,4-di-O-methyl- (II)	1,18	1,16	1,41
5) 3,4-Di-O-acetyl-2-O-methyl- (II)	1,61	1,98	3,30
6) 2,4-Di-O-acetyl-3-O-methyl- (II)	1,55	1,74	2,70
7) 2,3-Di-O-acetyl-4-O-methyl- (II)	1,51	1,81	3,00
8) 2,3,4-Tri-O-acetyl- (II)	1,71	2,08	3,60

The absolute retention times of compound (II) were: a) 5.0 min; b) 4.8 min; c) 2.7 min.

SUMMARY

Conditions for the gas-chromatographic separation of the methyl ethers of methyl (methyl α-D-galacto- and mannopyranosid)uronates in the form of their complete acetates on the phases OV-17, XE-60, and QF-1 are proposed.

LITERATURE CITED

1. D. Anderle and P. Kovač, J. Chromatogr., 91, 463 (1974).
2. V. I. Grishkovets and V. Ya. Chirva, First Bratislava Symposium on Saccharides, Smolenice, Abstracts, Bratislava (1981), p. 143.
3. V. I. Grishkovets, A. E. Zemlyakov, and V. Ya. Chirva, Khim. Prir. Soedin., 279 (1982).
4. V. I. Grishkovets, A. E. Zemlyakov, and V. Ya. Chirva, Khim. Prir. Soedin., 283 (1982).
5. V. I. Grishkovets, A. E. Zemlyakov, and V. Ya. Chirva, Khim. Prir. Soedin., No. 4, 432 (1983).
6. V. I. Grishkovets, A. E. Zemlyakov, and V. Ya. Chirva, Khim. Prir. Soedin., No. 5, 555 (1983).