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SEPARATION OF SOME METHYL O-METHYL-D-XYLOFURANOSIDES BY GAS CHROMATOGRAPHY

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SUMMARY

The separation of the methyl furanosides of O-methyl-D-xyloses, which may be formed upon methanolysis of a methylated polysaccharide containing D-xylopyranose as a structural unit, is described. The order of elution of the compounds under investigation follows, with no exceptions, the rule generally accepted for methyl O-methyl-glycopyranosides.

INTRODUCTION

Methylation analysis, despite its known shortcomings, has been of utmost importance in the elucidation of the structure of polysaccharides. Gas chromatography (GC) solves many of the problems encountered when complex mixtures of carbohydrate derivatives formed upon methanolysis and/or hydrolysis of methylated polysaccharides are analyzed[1–4] and is virtually the major analytical technique in methylation analysis.

One of the groups of compounds most frequently used for GC analysis in the investigation of the structure of polysaccharides is the methyl glycosides which are obtained upon methanolysis. These substances are fairly stable and, except those of the methyl mono-O-methylhexosides, are sufficiently volatile to be injected directly. However, when a methanolyzate is analyzed, identification of the four stereoisomers (two methyl pyranosides and two methyl furanosides) which may be formed from certain of the sugar moieties on methanolysis of a fully methylated polysaccharide can create a problem.

Methanolysis of some of the O-methyl ethers of D-xylose has been studied by BISHOP AND COOPER[5] who identified and determined, by GC, the amounts of the individual glycosides formed after exhaustive methylation as methyl per-O-methyl-D-xylosides. This procedure, for obvious reasons, is not suitable for the analysis of the methanolyzate of a methylated polysaccharide, where it is necessary to have a method of separation and reasonable resolution for all the possible components in the mixture. Data useful for the identification of methyl O-methyl-D-xylopyranosides have been summarized by BISHOP[3] and ASPINALL[6]. As all the possible methyl O-methyl-D-xylofuranosides which may be produced upon methanolysis of a methylated polysaccharide containing D-xylopyranose as a structural unit are now known[7,8],

we now wish to present data helpful in the identification of these compounds in methylation analysis.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Research Chromatograph, Model 5750 G, with a dual column system and dual flame ionization detector was employed. Relative retention times were taken from the record of a Hewlett-Packard Integrator 3370 A.

Operating conditions

Gas chromatography was carried out on the following columns: (A) 6 ft. × 1/8 in. (O.D.), packed with 10% Apiezon L on Gas-Chrom Z 80–100 mesh (Applied Science Laboratories), flow rate 32 ml/min, temperature programmed 100–290° (8°/min); (B) 6 ft. × 1/4 in. (O.D.), packed with 10% Carbowax 20 M–terephthalic acid (Hewlett-Packard) on Chromaton N AW-DMCS 70–80 mesh (Lachema, Brno), flow rate 32 ml/min, temperature programmed 100–240° (8°/min); (C) 6 ft. × 1/4 in. (O.D.), packed with 10% diethylene glycol succinate (Lachema, Brno) on Chromaton N AW-DMCS, flow rate 35 ml/min, temperature programmed 100–216° (8°/min); (D) 6 ft. × 1/8 in. (O.D.), packed with 3% ECNSS-M (Applied Science Laboratories) on Chromaton N AW-DMCS, flow rate 25 ml/min, temperature programmed 100–210° (4°/min).

Derivatives

Methyl α- and β-D-xylofuranoside was made according to AUGESTAD AND BERNER[9]. Methyl 2,3,5-tri-O-methyl-D-xylofuranosides were made according to BISHOP AND COOPER[10]. Methyl 2-O-, 3-O-, and 2,3-di-O-methyl-D-xylofuranosides were made as described by KOVÁČ AND PETRÍKOVÁ[7,8]. The compounds were injected in methanol (2% solution).

RESULTS AND DISCUSSION

The problems regarding the separation of the complex mixture of stereoisomers present in a methanolyzate of a methylated polysaccharide arise particularly when the polysaccharide subjected to methanolysis contains pentoses. BISHOP *et al.*[5,10,11] found that methyl furanosides, resulting from methanolysis, occur to a larger extent in the case of pentoses than in the case of hexoses. It is known that D-xylose occurs, as a structural unit of polysaccharides, exclusively in its pyranose form. Thus, methanolysis of such a polysaccharide results in the possible formation of methyl furanosides of 2-O-, 3-O-, and 2,3-di-O-methyl-D-xylose. Therefore, our attention was focussed mainly on the separation of these substances.

Identification of the products of methanolysis is aided by the application of certain generalizations concerning the pattern in which the components of the mixture are eluted from the column. Based on empirical data, BISHOP[3] concluded that in the case of fully methylated glycosides, furanosides precede pyranosides in the series of arabinosides, galactosides and fructosides, whereas in the series of xylosides and glucosides, the order of elution is reversed. Another important generalization was

TABLE I

RELATIVE RETENTION TIMES OF METHYL O-METHYL-D-XYLOFURANOSIDES

No.	*Compound*	*Column*			
		A	*B*	*C*	*D*
1	Me 2,3,4-tri-O-Me-β-D-xylopyranoside	1.00[a]	1.00[b]	1.00[c]	1.00[d]
2	Me 2,3,5-tri-O-Me-β-D-xylofuranoside	1.18	1.18	1.20	1.34
3	Me 2,3,5-tri-O-Me-α-D-xylofuranoside	1.32	1.29	1.29	1.54
4	Me 2,3-di-O-Me-β-D-xylofuranoside	1.55	1.55	1.69	2.34
5	Me 2,3-di-O-Me-α-D-xylofuranoside	1.66	1.66	1.79	2.62
6	Me 2-O-Me-β-D-xylofuranoside	1.85	1.96	2.15	3.36
7	Me 2-O-Me-α-D-xylofuranoside	1.87	2.35	2.87	5.08
8	3-O-Me-β-D-xylofuranoside	2.05	2.44	2.87	5.28
9	Me 3-O-Me-α-D-xylofuranoside	1.80	1.85	2.01	3.11
10	Me β-D-xylofuranoside				6.68
11	Me α-D-xylofuranoside				5.92

[a] 6.61 min.
[b] 6.64 min.
[c] 7.45 min.
[d] 3.49 min.

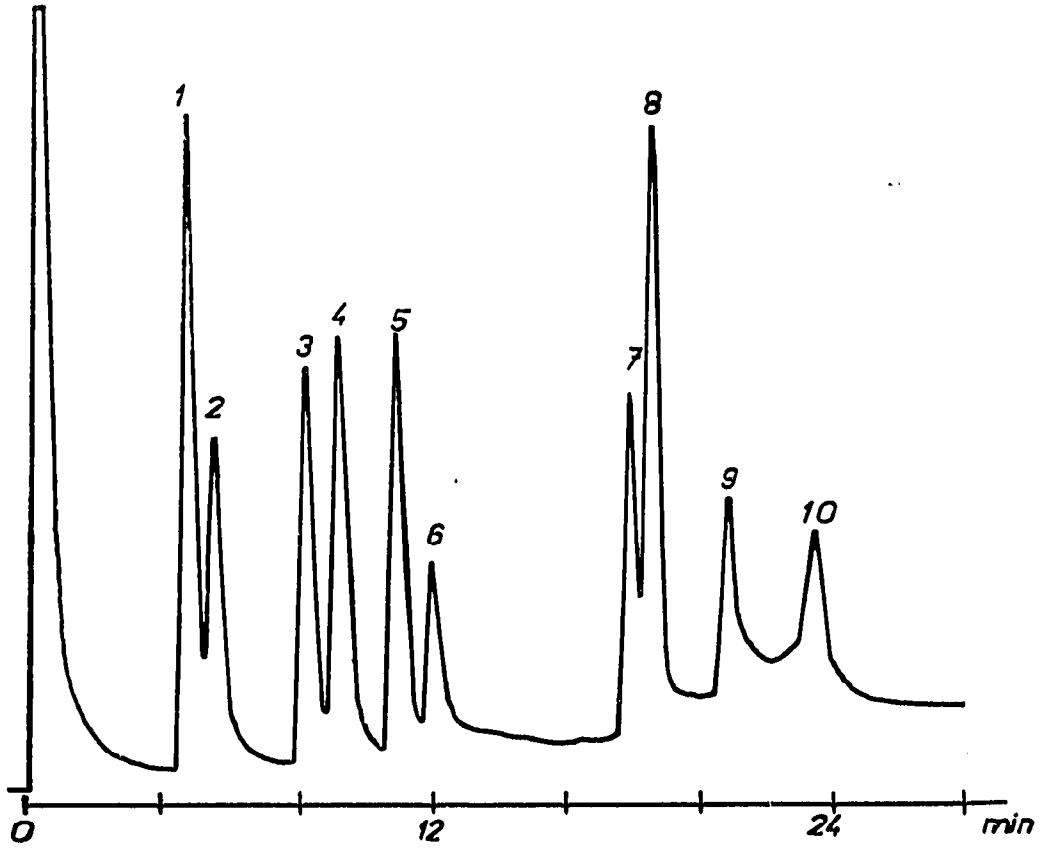

Fig. 1. Separation of methyl O-methyl-D-xylofuranosides. Column D. 1 = Methyl 2,3,5-tri-O-methyl-β-D-xylofuranoside; 2 = methyl 2,3,5-tri-O-methyl-α-D-xylofuranoside; 3 = methyl 2,3-di-O-methyl-β-D-xylofuranoside; 4 = methyl 2,3-di-O-methyl-α-D-xylofuranoside; 5 = methyl 3-O-methyl-α-D-xylofuranoside; 6 = methyl 2-O-methyl-β-D-xylofuranoside; 7 = methyl 2-O-methyl-α-D-xylofuranoside; 8 = methyl 3-O-methyl-β-D-xylofuranoside; 9 = methyl α-D-xylofuranoside; 10 = methyl β-D-xylofuranoside.

made for the series of partially methylated methylglycopyranosides. According to BISHOP[3], in the case of all the methyl O-methylglycopyranosides examined and on all liquid phases, that anomer in which the glycosidic methoxyl group is in a *cis* position relative to the methoxyl group at C-2 has the higher retention volume. When the C-2 methoxyl group is unsubstituted, the order in which the two anomers are eluted is reversed. At the time BISHOP wrote his review, and as far as the authors know at present, there are insufficient data to show whether this generalization is valid for methyl O-methylglycofuranosides as well.

In the work presented we tried to ascertain whether the same rule, as far as the order of elution of anomers is concerned, can be applied for the methyl O-methyl-D-xylofuranosides investigated. In Table I the retention times, on four columns of different polarity, relative to the retention time of methyl 2,3,4-tri-O-methyl-β-D-xylofuranoside are summarized. It follows from the presented data that BISHOP's generalization for the order in which methyl O-methylglycopyranosides are eluted can be extended to the methyl O-methyl-D-xylofuranosides under investigation.

Of the four liquid phases used for the separation of the methyl O-methyl-D-xylofuranosides investigated, ECNSS-M was found to be the most suitable (see Fig. 1), as both separation and resolution on the column D was the best. Here, even methyl D-xylofuranosides were resolved satisfactorily for quantitative work.

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