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Note

Gas–liquid chromatographic separation of methyl ethers of L-rhamnose as their methyl glycosides, trifluoroacetylated L-rhamnitols and acetylated L-rhamnononitriles

FRANTIŠEK JANEČEK, RUDOLF TOMAN, ŠTEFAN KARÁCSONYI and DUŠAN ANDERLE

Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava (Czechoslovakia)

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L-Rhamnose is a constituent sugar of many polymers, especially in the plant kingdom[1,2]. The mode of linkage and the distribution of L-rhamnose residues in these macromolecules has a considerable influence on their size and shape[3]. Among the methods used in the investigation of these residues, methylation analysis is still of fundamental importance. After methylation and subsequent hydrolysis, the resulting mixture of methylated sugars is converted into volatile derivatives and analyzed by gas–liquid chromatography (GLC) or combined GLC–mass spectrometry (MS). Several types of derivatives have been used, *e.g.*, methyl glycosides[4], acetates[5], alditol acetates[6] and aldononitrile acetates[7,8]. Nevertheless, a prerequisite for the unambiguous qualitative and quantitative evaluation of the complex mixtures, the knowledge of retention data and response factors of the individual derivatized sugar methyl ethers, is presumed.

The separations of all possible O-methyl derivatives of L-rhamnose as their corresponding partially methylated alditol acetates have already been described[9]. We now extend these investigations to the corresponding methyl glycosides, trifluoroacetylated alditols (TFAA) and acetylated aldononitriles (AAN) which appeared more suitable for GLC and GLC–MS analyses, through their better separation, ease of preparation, duration of analyses, etc.

EXPERIMENTAL

Derivatives

Methyl ethers of methyl α-L-rhamnopyranoside were prepared as described by Toman *et al.*[10]. Hydrolysis with 90% formic acid for 3 h at 100° and subsequent purification by thin-layer chromatography on silica gel G using chloroform–methanol (9:1) and light petroleum (b.p. 35–50°)–acetone (5:2) afforded the corresponding methyl ethers of L-rhamnose. A small amount (1–3 mg) of each derivative was reduced with sodium borohydride (3–10 mg) and worked-up in the usual way. The dry residue was then trifluoroacetylated with 0.3 ml of trifluoroacetic anhydride in the presence of 3 μl of dry pyridine. The reaction mixture was stirred vigorously for *ca.* 1 min and left at room temperature for 1 h.

L-Rhamnononitrile acetates were prepared as follows. A sample of methylated L-rhamnose (1–3 mg) was dissolved in a solution (150 μl) of hydroxylamine hydrochloride in pyridine (100 mg in 1 ml) and heated at 110° for 15 min. Acetic anhydride was added (200 μl) and the reaction mixture was kept at 110° for 30 min. The mixture was then cooled and, after addition of water (1 ml), evaporated to dryness. Chloroform (100 μl) was added and the resulting solution was directly injected into the column.

Apparatus

GLC of methyl ethers of methyl α-L-rhamnopyranoside and of the corresponding TFAA was performed with a Hewlett-Packard Model 5711 A chromatograph equipped with a flame ionization detector (FID). The separations of O-acetyl-O-methyl-L-rhamnononitriles were carried out on a Hewlett-Packard Model 5754 G instrument with an FID.

Columns and operating conditions

The following columns were used:

(A) 3% ECNSS-M on Chromaton N AW DMCS (80–100 mesh) at a programmed temperature range of 110° (8 min) to 150° at 2°/min, flow-rate 25 ml/min;

(B) 10% DEGS on Chromosorb W AW DMCS (80–100 mesh) at 180°, flow-rate 34 ml/min;

(C) 5% BDS on Gas-Chrom Z (80–100 mesh) at 135°, flow-rate 41 ml/min;

(D) 4% XE-60 on Gas-Chrom Z (80–100 mesh) at 130°, flow-rate 24 ml/min;

(E) 3% OV-225 on Chromosorb W AW DMCS (80–100 mesh) at 110°, flow-rate 39 ml/min;

(F) 20% SF-96 on Chromosorb W AW DMCS (60–80 mesh) at 120°, flow-rate 29 ml/min;

(G) 3% QF-1 on Gas-Chrom Q (100–120 mesh) at 165°, flow-rate 8 ml/min;

(H) 1% XE-60 on Gas-Chrom Q (100–120 mesh) at 165°, flow-rate 6.1 ml/min;

(J) 5% NPGS on Gas-Chrom Z (80–100 mesh) at 165°, flow-rate 9.8 ml/min.

The columns (stainless steel) had the following sizes: 200 × 0.316 cm O.D. (A–F); 300 × 0.316 O.D. (G, H); and 180 × 0.316 O.D. (J). Nitrogen was used as carrier gas throughout.

RESULTS AND DISCUSSION

As mentioned above, the GLC separation of all possible O-acetylated O-methyl-L-rhamnitols is relatively poor, and does not allow a proper quantitative evaluation. This fact, together with our need to perform the methylation analysis on oligomers containing high proportions of L-rhamnose residues, prompted us to search for other derivatives more suitable for GLC analyses.

Glycosidation (methanolysis) of methyl ethers of L-rhamnose gives rise to α-anomers only and in addition, when the reaction is performed under more vigorous conditions (heating with 7% methanolic hydrogen chloride for 8 h), the content of furanosides is negligible. Hence, the separation is much simplified, since each derivative gives only one peak on GLC, and might be useful in methylation analysis of certain types of oligo- and polysaccharides.

TABLE I

RELATIVE RETENTION TIMES $t'_{R,2\text{-O-MeRh}}$ OF METHYL O-METHYL-α-L-RHAMNOPYRANOSIDES AND O-TFA-O-METHYL-L-RHAMNITOLS

Positions of O-methyl groups	*Columns*					
	Methyl α-L-rhamnosides				*TFA L-rhamnitols*	
	A	*B*	*C*	*D*	*E*	*F*
2, 3, 4	0.11	0.11	0.10	0.21	1.06	2.17
2, 3	0.47	0.41	0.41	0.56	1.00	1.43
2, 4	0.36	0.32	0.31	0.51	1.56	1.72
3, 4	0.26	0.27	0.25	0.39	1.17	1.53
2	1.00	1.00	1.00	1.00	1.00	1.00
3	1.12	1.05	1.12	1.09	1.17	0.65
4	1.22	1.18	1.30	1.40	1.50	0.91
$t'_{,2\text{-O-MeRh}}$ (min)	13.46	17.48	31.26	16.46	5.67	11.42

The best separation of all possible methyl ethers of methyl α-L-rhamnopyranoside has been achieved on column A (Table I). In this case, the difference in relative retention times (Δt) is not lower than 0.1 which enables, using columns of suitable efficiency, a convenient quantitative estimation. If $\Delta t < 0.1$, the peaks are not well resolved. This situation occurred when columns B, C and D were used for the separations. In this case, a combination of two different columns is necessary to obtain the desired resolution.

O-Trifluoroacetyl (TFA)-O-methyl-L-rhamnitols chromatographed on column F (Table I) also provide an unambiguous qualitative and quantitative evaluation. Somewhat poorer results, but still comparable with the corresponding alditol acetates[9], have been obtained using column E.

Finally, attempts have been made to separate the methyl ethers of L-rhamnose as AAN. As is evident from Table II, separations also enabling quantitative estimation have not been achieved, since several compounds form pairs separable only on the

TABLE II

RELATIVE RETENTION TIMES $t'_{R,2\text{-O-MeRh}}$ AND RETENTION INDICES *I* OF O-ACETYL-O-METHYL-L-RHAMNONONITRILES

Positions of O-methyl groups	*Columns*					
	$t'_{R,2\text{-O-MeRh}}$			*I*		
	G	*H*	*J*	*G*	*H*	*J*
2, 3, 4	0.33	0.39	0.46	2025	2055	2133
2, 3	0.60	0.64	0.80	2117	2122	2208
2, 4	0.64	0.70	0.80	2129	2133	2208
3, 4	0.92	0.93	1.00	2184	2173	2238
2	1.00	1.00	1.00	2198	2183	2238
3	1.30	1.28	1.42	2239	2216	2286
4	1.27	1.21	1.50	2235	2209	2293
	1.56	1.39	1.70	2267	2228	2310
$t'_{R,2\text{-O-MeRh}}$ (min)	28.35	43.07	27.48			

highly efficient columns. The retention indices were also determined for these derivatives. Such indices have not yet been routinely used in GLC identification of sugar derivatives.

Obviously, for both qualitative and quantitative investigations of mixtures of methyl ethers of L-rhamnose, the methyl glycosides and TFA derivatives are more advantageous than alditol acetates. Better separations are obtained, GLC requires less time and in the case of methyl glycosides the preparation of samples is very simple compared with the alditol acetates. AAN of methylated L-rhamnoses are separated almost as well as the corresponding alditol acetates. Moreover, the reaction can be performed in one step and, after evaporation and dissolution in a suitable solvent, the reaction mixture is directly injected into the column.

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