

CHROM. 12,866

## Note

### Preparative liquid column chromatography of methyl ethers of methyl $\alpha$ -L-rhamnopyranoside

E. V. EVTUSHENKO\*, N. M. VAKHRUSHEVA and Yu. S. OVODOV

*Pacific Institute of Bio-organic Chemistry, Far East Science Centre, U.S.S.R. Academy of Science, Vladivostok (U.S.S.R.)*

(Received April 3rd, 1980)

In connection with the difficulty of synthesizing methyl ethers of monosaccharides in recent years there has been a tendency to obtain methyl ethers by separation of the products of the partial methylation of methyl glycosides<sup>1-5</sup>. Usually micro-scale preparative gas-liquid chromatography (GLC) has been used for this purpose<sup>1,2</sup>. The load on the column was typically 10 mg and the yields of methyl ethers were about 1-3 mg. Semi-micropreparative GLC of the methyl ethers of  $\beta$ -D-xylopyranoside<sup>4</sup> and methyl  $\alpha$ -L-rhamnopyranoside have also been described<sup>5</sup>. In this instance the load on the column was 300-600 mg.

Toman *et al.*<sup>6</sup> suggested a convenient method for obtaining methyl ethers from methyl  $\alpha$ -L-rhamnopyranoside based on liquid chromatography on silica gel of mixtures of 2- and 3-mono- and 2,4- and 3,4-di-O-methyl ethers of methyl  $\alpha$ -L-rhamnopyranoside, obtained by partial diazomethane methylation of methyl  $\alpha$ -L-rhamnopyranoside and methyl 4-O-methyl  $\alpha$ -L-rhamnopyranoside, respectively. A drawback of this method is that a three-step synthesis is required for the initial 4-O-methyl ether.

In this paper we describe a convenient method for obtaining six methyl ethers of methyl  $\alpha$ -L-rhamnopyranoside using liquid chromatography on silica gel of the product of partial methylation of methyl  $\alpha$ -L-rhamnopyranoside by the Haworth method<sup>4</sup>.

## EXPERIMENTAL

Synthesis of the methyl ethers was performed as described earlier<sup>4</sup>, except that the volume of methylated reagents was reduced 3-fold.

Specific rotations were measured on chloroform solutions using a Perkin-Elmer Model 141 instrument. Melting temperatures were measured on a Boethius device. Thin-layer chromatography (TLC) was performed on silica gel (5  $\mu$ m; Chemapol, Prague, Czechoslovakia) with light petroleum (b.p. 70-100°C)-acetone (60:40). Identification was carried out in 30% sulphuric acid in methanol.

GLC was performed on a Tsvet-106 chromatograph provided with a flame-ionization detector and double U-shaped columns (200  $\times$  0.3 cm). The liquid stationary phase was 3% NPGS on Chromatone N AW HMDS (0.125-0.160 mm).

The flow-rate of the carrier gas (argon) was 60 ml/min. The temperature of the column was maintained thermostatically at 145°C.

Column chromatography was carried out on silica gel L (100–160  $\mu\text{m}$ ; Chema-pol), previously ground and sieved, containing 10% of water. The column (45  $\times$  3 cm I.D.) was filled with a suspension of silica gel in light petroleum (b.p. 70–100°C) and acetone (9:1). The column loading was 14 g of mixed methyl ethers of methyl  $\alpha$ -L-rhamnopyranoside. The column was eluted stepwise with 0.5 l of a gradient of acetone in light petroleum (b.p. 70–100°C) (10, 20, 25, 30, 40, 50 and 100%) at a rate of 7 ml/min. Fractions of 50 ml were taken and evaporated to dryness in a vacuum.

## RESULTS AND DISCUSSION

Table I gives  $R_F$  values and relative retention times (RRT) for methyl ethers of methyl  $\alpha$ -L-rhamnopyranoside. These results show that the TLC separation of methyl ethers is satisfactory, except for 2- and 3-monomethyl ethers.

TABLE I

$R_F$  VALUES AND RELATIVE RETENTION TIMES (RRT) OF METHYL ETHERS OF METHYL  $\alpha$ -L-RHAMNOPYRANOSIDE

Parameter	Methyl ether							$\alpha$ -L-Rha
	2,3,4-	3,4-	2,4-	2,3-	4-	2-	3-	
$R_F$	0.65	0.53	0.50	0.45	0.36	0.29	0.29	0.13
RRT*	0.30	0.47	0.85	1.00	1.29	1.47	1.25	1.76

\* Relative to 2,3-di-O-methyl ether.

Table II shows the compositions of the fractions obtained by liquid column chromatographic separation, and their yields. Despite the small discrepancies in the chromatographic mobilities between 3,4- and 2,4-di-O-methyl ethers, these substances were well separated with good yields.

As the 2- and 3-monomethyl ethers could not be separated under the conditions used, the following method was used to obtain the 2-O-methyl ether. Fractions 61–65 (3.45 g) were acetylated with acetic anhydride in pyridine and the 2-O-methyl

TABLE II

YIELDS AND PROPERTIES OF METHYL ETHERS OF METHYL  $\alpha$ -L-RHAMNOPYRANOSIDE OBTAINED BY LIQUID CHROMATOGRAPHY

Methyl ether	Content in initial mixture (%)	No. of fractions	Yield (g)	$[\alpha]_D^{20}$
2,3,4-	27.7	10–13	3.90	–55.6°
3,4-	4.8	25–27	0.65	–89.3°
2,4- + 3,4-		28–29	0.49	
2,4-	17.4	30–38	1.86	–61.0°
2,3-	18.7	40–50	2.56	–23.6°
4-	7.1	56–60	0.65	–87.3°
2- + 3- + 4-		61–65	3.45	
$\alpha$ -L-Rha	0.7	70–75	0.10	

ether acetate was crystallized from a mixture of ethyl acetate and light petroleum (b.p. 70–100°C). The yield was 2.0 g (9.2% of the initial mixture), the melting point 70°C and  $[\alpha]_D^{20} -74^\circ$ .

Identification of methyl ethers was performed by comparison with known samples using GLC of the acetates. The GLC results demonstrated the chromatographic purity of the methyl ethers obtained. The elemental and functional group composition corresponded to the theoretical values.

#### REFERENCES

- 1 B. Fournet and J. Montreuil, *J. Chromatogr.*, 75 (1973) 29.
- 2 B. Fournet, J. M. Dhalluin, Y. Leroy, J. Montreuil and H. Mayer, *J. Chromatogr.*, 153 (1978) 91.
- 3 P. A. Mied and Y. C. Lee, *Anal. Biochem.*, 49 (1972) 534.
- 4 E. V. Evtushenko and Yu. S. Ovodov, *J. Chromatogr.*, 97 (1974) 99.
- 5 E. V. Evtushenko, N. M. Vakhrusheva and Yu. S. Ovodov, *Khim. Prir. Soedin.*, (1979) 142.
- 6 R. Toman, S. Karacsonyi and R. Palovcik, *Carbohydr. Res.*, 56 (1977) 191.