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Note

Micropreparative gas–liquid chromatography of methylated sugars

I. Preparative separation of partial methylation products from methyl β-D-xylopyranoside

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Recently, we have shown[1] that, depending on the procedure and conditions used for the partial methylation of methyl β-D-xylopyranoside, different methylated derivatives are produced. Analytical gas–liquid chromatography (GLC) was successfully used for the quantitative and qualitative analysis of methylated methyl glycosides as the corresponding acetates. These mixtures may serve as starting materials for obtaining authentic methylated sugar samples which are essential for a structural study of the carbohydrate chain by methylation.

While we were completing this work, Fournet and Montreuil[2] reported that GLC can be used for the preparative separation of small amounts of products obtained by partial methylation of methyl α-D-mannopyranoside.

In this work, methyl β-D-xylopyranoside was used to show the possibility of and to select suitable conditions for the separation on a preparative scale of considerable amounts of methylated methylglycoside acetates (MMGA) obtained by partial methylation.

Previous results from the study of the partial methylation of methyl β-D-xylopyranoside by different methods[1] were used to obtain the various methyl ethers.

EXPERIMENTAL

Partial methylation of methyl β-D-xylopyranoside was achieved by the procedures of Kuhn and Trischman[3], Haworth[4] and Purdie and Irvine[5].

Kuhn and Trischman methylation

The starting xyloside (4 g) was dissolved in dimethylformamide (120 ml) with subsequent addition of barium oxide (24 g), barium hydroxide octahydrate (1.9 g) and methyl iodide (24 ml). The solution was stirred for 1.5 h in the dark. The reaction mixture was treated as described previously[1] to yield 5.1 g of MMGA.

Haworth methylation

The starting xyloside (2 g) was dissolved in water (10 ml) and stirred on a

TABLE I

CHARACTERIZATION OF METHYLATED METHYL β-D-XYLOPYRANOSIDE ACETATES (MMGA) AS PRODUCTS OF PARTIAL METHYLATION FOLLOWED BY GLC SEPARATION*

Methyl ether	$T_{a.r.rel}$**	*M.p. (°C)***	$[\alpha]_D^{20}$ *in CHCl₃* *	*Yield in accordance with procedure of*								
				Kuhn and Trischmann[3]			*Haworth*[4]			*Purdie and Irvine*[5]		
				Preparative data		*Analytical data*	*Preparative data*		*Analytical data*	*Preparative data*		*Analytical data*
				mg	*%*	*%*	*mg*	*%*	*%*	*mg*	*%*	*%*
2, 3, 4	0.19	48.5–49.5 (49–50°, ref. 6)	−68.3° [3.0] (−73°, ref. 6)	27	4.5	4.0	110	18.3	22.2	2	0.3	1.4
2, 3	0.64	51.5–52.5	−79.4° [3.6]	56	9.3	21.9	31	5.2	10.1	8	1.3	2.3
2, 4	1.00 (5.8 min)	Syrup	−60.9° [3.6]	105	17.5	32.0	151	25.2	42.8	20	3.3	8.8
2	1.72	94–95	−35.3° [3.6]	53	8.8	14.0	46	7.7	12.7	68	11.3	21.0
4	2.28	119–121°	−80.2° [1.5]	19	3.2	7.3	30	5.0	10.9	43	7.2	12.3
3	2.83	86–88	−77.5° [2.6]	40	6.7	14.3	3	0.5	1.3	36	6.0	13.9
Parent	4.72	118–119.5 (115°, ref. 7)	−60.1° [3.1] (−60.8°, ref. 7)	27	4.5	6.5				186	31.0	40.3
			Total yield	327	54.5	100.0	371	61.9	100.0	361	60.4	100.0

* 600 mg of MMGA were separated by GLC.

** Retention times relative to peracetate of methyl 2,4-di-O-methyl-β-D-xylopyranoside (analytical column).

*** Reference data are given in parentheses. Concentrations are given in brackets.

magnetic mixer in an argon flow with subsequent dropwise addition of dimethyl sulphate (20 ml) and 30% aqueous sodium hydroxide solution (34 ml) for 2 h. Following degradation of dimethyl sulphate and neutralization of the solution, its volume was made up to 100 ml with water and extracted with chloroform (2 × 200 ml). The mixture of methyl ethers obtained was acetylated as usual to yield 1.5 g of MMGA.

Purdie and Irvine methylation

Methyl β-D-xyloside (5 g) was dissolved in methanol (100 ml) with subsequent addition of methyl iodide (20 ml) and inactivated silver oxide (30 g). The solution was stirred on a magnetic mixer for 2 h in the dark. The reaction mixture was treated as described previously[1] to yield 8.2 g of MMGA.

Gas–liquid chromatography

A Tsvet-106 (U.S.S.R.) chromatograph with dual columns (100 × 0.3 cm) and flame ionization detectors was used for analysis. The columns were packed with butanediol succinate (10%) on 80–100 mesh Chromosorb W. The thermostat and batcher temperatures were 160° and 300°, respectively. The carrier gas was argon at a flow-rate of 30 ml/min.

A Tsvet-3-66 (U.S.S.R.) chromatograph with U-shaped stainless-steel columns, A (100 × 0.8 cm) and B (200 × 1.4 cm), equipped with a preparative attachment and flame ionization detector was used for GLC on a micro-preparative scale. The columns were packed with butanediol succinate (10%) on 60–80 mesh Chromosorb A. The temperature in the evaporator and collector was 240°. The thermostat temperature was 150° when using column A and 170° when using column B. The argon flow-rates with columns A and B were 200 and 300 ml/min, respectively. Straight glass tubes (6 × 0.5 cm) having narrow openings and connected with receivers served as traps. When operating column A, up to 100 mg of the mixture of MMGA were introduced into the chromatograph as a concentrated solution in chloroform. When operating column B, up to 600 mg of the MMGA mixture were introduced into the chromatograph as a concentrated solution in chloroform (*ca.* 0.8 ml). The specific rotation of MMGA (in chloroform) was measured on a Perkin-Elmer 141 instrument.

RESULTS AND DISCUSSION

When the 100-cm column packed with 10% butanediol succinate on 60–80 mesh Chromosorb W was used for analysis, complete separation of the methylated derivatives of methyl β-D-xylopyranoside as the corresponding acetates was achieved. It should be noted that the 3,4-dimethyl ether does not result from partial methylation.

The conditions for the micro-preparative GLC were virtually the same as those in the analytical version. The load on column A was *ca.* 100 mg of the mixture of MMGA, and the yield of individual methyl ethers acetates was about 5–10 mg, depending on their contents in the mixture. High loads resulted in a considerable decrease in column efficiency and did not permit effective separation of the methyl ether mixture. In order to compensate for the loss in efficiency, the length of the column was increased to 200 cm and the I.D. to 1.4 cm, and it was then possible to increase the load on the column to 600 mg of mixture without a noticeable decrease in efficiency.

At present, it is known that the principal factor responsible for decreased efficiency is the component displacement rate profile over the column section, this rate profile increasing as the column diameter increases. Hence the efficiency of the analytical column used is equal to 400 theoretical plates while for the 100- and 200-cm preparative columns, it is 90 and 150 theoretical plates, respectively. Thus, when the column diameter was increased from 0.8 to 1.4 cm, *i.e.*, 1.7-fold, and the column length 2-fold, the efficiency of a 2-m column increased 1.7-fold compared with a 1-m column. At the same time, the cross-sectional area increased 3-fold thus allowing the overall load to be increased at least 3-fold and the same specific load to be retained. The possibility of using even longer columns is handicapped by the increased time required in order to give the same component yield and the pressure differential in the column, which naturally results in a lower efficiency. Table I shows the yields and characteristics of methylated methyl β-D-xylopyranoside acetates obtained with partial methylation by the Kuhn and Trischman[3], Haworth[4] and Purdie and Irvine[5] procedures with subsequent separation by GLC on a micro-preparative scale. In each instance, the load on the column was 600 mg.

The MMGA isolated were individual compounds, and under analytical GLC conditions resulted in a single peak, the analytical and theoretical data virtually coinciding. It is noteworthy that all of the individual components obtained, except for the 2,4-dimethyl ether, instantly crystallized in a trap. We used small glass tubes as traps, bearing in mind that MMGA are high-boiling liquids, which do not require special cooling of receivers. The total recovery was about 70%, the chromatographic recovery factor for mono- and dimethyl ethers being about 60%.

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