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Note

Separation of trimethylsilyl ethers of methyl(methyl 4-deoxy-O-methyl-β-L-*threo*-hex-4-enopyranosid)uronate by gas chromatography

DUŠAN ANDERLE, PAVOL KOVÁČ and JÁN HIRSCH
Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava (Czechoslovakia)
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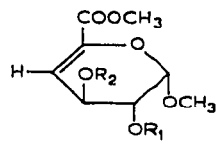
Olefinic sugar derivatives of the type



No.	R_1	R_2
3	CH_3	CH_3
4	CH_3	H
5	H	CH_3
6	H	H

may be found among the products of the methylation of D-glucuronic or D-galacturonic acid conjugates[1–3]. This class of substance is formed by β-elimination reactions of esterified uronic acid derivatives under alkaline[4–6] and neutral conditions[7] and may also be formed by the action of certain enzymes[8–10]. Elimination reactions in uronic acids have been extensively studied[11–14] and it can be concluded that this type of reaction is a general means of degradation of heteropolysaccharides and poly(hexopyranosid)uronates in nature.

As a result of β-elimination in hexuronic acids, the asymmetric centre at C4 is no longer present and, therefore, the same olefins are formed from D-glucuronic and D-galacturonic acid derivatives. The separation of O-methyl derivatives of methyl(methyl α-D-glucopyranosid)uronate has been described earlier[15]. In this paper, we describe the separation of methyl(methyl 2,3,4-tri-O-methyl-α-D-glucopyranosid)-uronate (1) and methyl (methyl 2,3,4-tri-O-methyl-α-D-galactopyranosid)uronate (2) in admixture with differently substituted olefinic saccharides (3–6). In view of the possibility of determining the number and the location of the methyl groups in the derivatives of methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid by mass spectrometry[16], this work is a contribution to the analysis and unequivocal identification of compounds 1–6 by combined gas chromatography–mass spectrometry (GC–MS).

EXPERIMENTAL

Apparatus

The instrument used for capillary column analysis was a Hewlett-Packard (Avondale, Pa., U.S.A.) Model 5750 G chromatograph equipped with a flame ionization detector and an inlet splitter (1:100). A Hewlett-Packard Model 5711 A

chromatograph was used with packed columns. Oxygen- and moisture-free nitrogen[17-19] was used as the carrier gas. The temperature of the injection port and that of the detector was 200°.

Columns and operating conditions

A stainless-steel capillary (45 m × 0.2 mm I.D.) coated with OV-17 (phenyl methyl silicone) was used. Coating was carried out by the plug coating method and the work was performed at $p_i = 0.7$ kp/cm^2. The packed columns were stainless-steel tubes (180 cm × 3.15 mm O.D.) packed with: (A) 10% UC-W 98 (methyl silicone) on HP Chromosorb W, 80–100 mesh (Hewlett-Packard) at an operating temperature of 172° and a nitrogen flow-rate of 12 ml/min; (B) 3% OV-17 on Gas-Chrom Q, 80–100 mesh (Applied Science Labs., State College, Pa., U.S.A.), at an operating temperature of 165° and a nitrogen flow-rate of 21 ml/min; and (C) 5% neopentyl glycol succinate (NPGS) on Gas-Chrom Z, 80–100 mesh (Applied Science Labs.), at an operating temperature of 172° and a nitrogen flow-rate of 19 ml/min.

Derivatives

The preparation of the olefins 3–6 and of methyl(methyl 2,3,4-tri-O-methyl-α-D-glucupyranosid)uronate (1) has been described elsewhere[14,20,21]. Methyl(methyl 2,3,4-tri-O-methyl-α-D-galactopyranosid)uronate was prepared according to Luckett and Smith[22].

Silylation of partially methylated 4,5-unsaturated 4-deoxyhexopyranuronates was carried out by the usual procedure[23].

RESULTS AND DISCUSSION

Methyl ethers of methyl(methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosid)uronate were separated in admixture with fully methylated derivatives of methyl α-D-glucuronic and methyl α-D-galacturonic acids. Other workers[11,12] have used gas chromatography for monitoring the course of reactions leading to this class of compounds, but the separation of a series of these substances had not yet been described.

The first approach for the analysis of compounds 1–6 was the use of packed columns containing packings of increasing polarity. Preliminary experiments showed that compounds 4–6 are unstable under the conditions used and are therefore not amenable for direct analysis by gas chromatography. The compounds bearing free hydroxyl groups were eluted as broad, severely tailing peaks, suggesting decomposition in the column. As silylation is a fast, quantitative and simple method for preparing volatile carbohydrate derivatives, trimethylsilyl (TMS) ethers were chosen. Depending upon the polarity of the liquid phase, substitution with this group alters markedly the relative retention times (Fig. 1) of the substances under investigation and therefore, when it is impossible to use a capillary column, a combination of several packed columns of different polarities renders the separation and identification of the substances possible.

It has been found that in order to achieve the desired minimum resolution ($R = 1$) of all components to be separated, the separating power of packed columns, for the pairs that are difficult to separate, was unsatisfactory.

The results obtained are presented in Table I in terms of relative retention

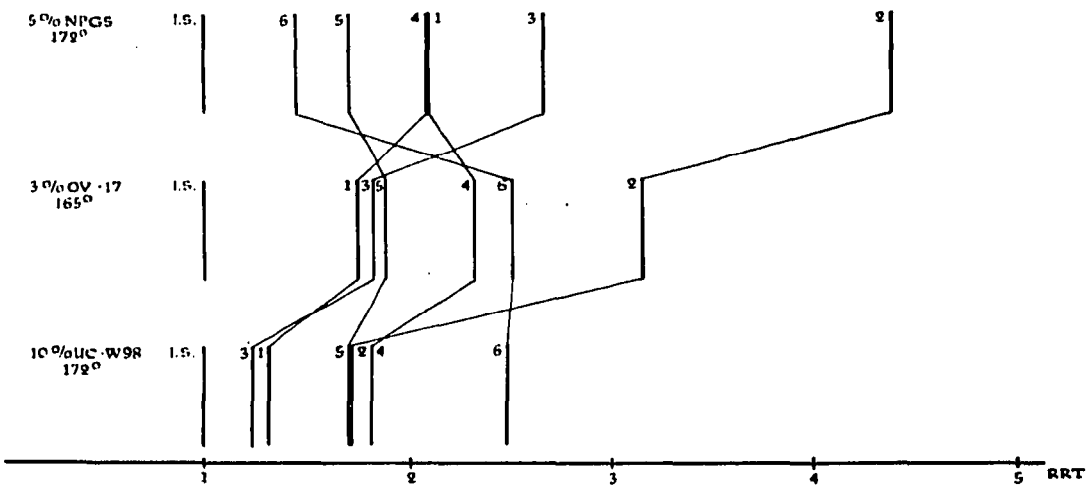


Fig. 1. Elution pattern of TMS derivatives of compounds 1–6 depending on the polarity of stationary phase. RRT = Relative retention time.

TABLE I
RETENTION DATA FOR COMPOUNDS 1–6 ON PACKED COLUMNS

*Compound**	*Packing*	*Temperature (°C)*	*Relative retention time*	*α*	*S*	*N*
I.S.	UC-W 98	172	1.00 (6.21 min)			
3			1.23			
1			1.34	1.086	5741	6500
5			1.71	1.266		
2			1.73	1.021	85,079	93,500
4			1.84	1.051	15,289	16,700
6			2.49	1.348		
I.S.	OV-17	165	1.00 (3.32 min)			
1			1.77			
3			1.82	1.038	26,900	30,000
5			1.87	1.035	31,500	35,000
4			2.32	1.233		
6			2.51	1.079	6700	7300
2			3.15	1.255		
I.S.	NPGS	172	1.00 (4.71 min)			
6			1.44			
5			1.72	1.187		
4			2.10	1.223		
1			2.14	1.008	57,200	625,000
3			2.65	1.262		
2			4.35	1.637		

* I.S. = methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside.

times. For pairs of substances that are very difficult to separate, Table I shows also the relative volatilities, α, separation factors, S (according to Purnell[24]), and the number of plates required, N, to achieve $R = 1.5$, of which only a capillary column is capable.

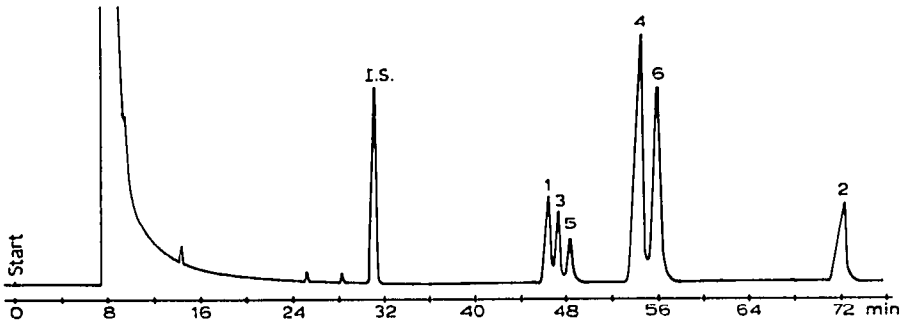


Fig. 2. Separation of compounds 1–6 on a capillary column coated with OV-17.

When GC–MS is to be applied, it is most advantageous to use a single column for the separation of all substances to be identified. In the case of the mixture under investigation, this situation is achieved with a capillary column coated with OV-17, on which all components were satisfactorily separated (Fig. 2, Table II). This phase was chosen because N calculated for a packed column had the lowest value.

Table II shows the results of the separation of compounds 1–6 and R values for the pairs of substances that are difficult to separate. These values are satisfactory for both quantitative analysis of the substances under investigation and GC–MS identification.

TABLE II

RETENTION DATA FOR COMPOUNDS 1–6 ON A CAPILLARY COLUMN COATED WITH OV-17

*Compound**	*Relative retention time*	α	S	N	R
I.S.	1.00 (23.69 min)				
1	1.65				
		1.024	65,500	92,500	1.19
3	1.69				
		1.033	35,200	49,300	1.48
5	1.75				
		1.148			
4	2.01				
		1.031	39,800	53,000	1.42
6	2.07				
		1.330			
2	2.75				

* I.S. = methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside.

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