Journal of Chromatography, 259 (1983) 159 163
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,549

Note

Gas–liquid chromatographic resolution of sugar enantiomers as diastereoisomeric methylbenzylaminoalditols

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(Received November 18th, 1982)

In previous papers[1,2] we presented a convenient way to determine the configuration of neutral monosaccharides based on liquid–solid chromatography of diastereoisomeric 1-(N-acetyl-α-methylbenzylamino)-1-deoxyalditol acetates, which was successfully applied to the sugar analysis of the plant gum separated from the sap of the lac tree (*Rhus vernicifera*)[3].

Recently two kinds of gas–liquid chromatographic (GLC) methods to separate sugar enantiomers have been published. One is capillary GLC with chiral liquid phases, for sugar derivatives such as trifluoroacetates[4–6] and heptafluorobutanoates[7]. In these cases the chromatograms were complicated due to the presence of anomers of sugars, as in the case of diastereoisomeric glycosides on glass capillary columns with achiral stationary phases[8,9]. The second method has been used to separate enantiomers of sugars as the trimethylsilyl (TMS) ethers or acetates of diastereoisomeric dithioacetals on an achiral fused-silica capillary column[10].

In this paper, GLC separation of sugar enantiomers as diastereoisomeric α-methylbenzylaminoalditols (MBA-alditols) is examined using fused-silica capillary columns[11,12]. Twenty of the 24 monosaccharides tested here, including ketoses and N-acetyl amino-sugars, are resolved as the TMS ethers of MBA-alditols on a Carbowax 20M column.

EXPERIMENTAL

The samples of MBA-alditol acetates were the same as those used in refs. 1 and 2. TMS ethers were prepared as follows. A mixture of a solution of a sugar (1 mg) in 50 μl of water and a solution of α-methylbenzylamine (MBA) (7 mg) and $NaBH_3CN$ (0.4 mg) in 50 μl of ethanol was kept at 40°C for 3 h. Several drops of acetic acid were added, and the mixture was evaporated and further coevaporated with 0.5 ml of methanol. The oily residue was dried over P_2O_5 in a vacuum desiccator and mixed with dry acetonitrile (100 μl) and N,O-bis-(trimethylsilyl)acetamide (BSA) (25 μl). After standing for 15 min at room temperature in a stoppered tube,

0021-9673/83/0000–0000/$03.00

the supernatant (0.2–1 μl) was injected into a GLC column. Identification of peaks was carried out by comparing chromatograms of the reaction product of a pure enantiomer with L-MBA and that with DL-MBA.

GLC was carried out with a Hitachi 063 instrument or a Hewlett-Packard 5790A chromatograph with a flame ionization detector in a split mode (splitting ratio *ca.* 100/1). Hewlett-Packard fused-silica WCOT columns coated with Carbowax 20M (25 m × 0.20 mm)* and silicone SE-54 (ULTRA No. 2, 50 m × 0.20 mm, thickness of liquid phase, d_f 0.11 μm) were used for separation of TMS-ethers and acetates of MBA-alditols, respectively.

RESULTS AND DISCUSSION

The separation of MBA-alditol acetates of several aldoses, which was not achieved in the previous study[2], was attempted on an SE-54 column. Although enantiomers of rhamnose, mannose and galactose were resolved, derivatives of D-mannose and D- and L-glucose could not be distinguished (Fig. 1).

Satisfactory results were obtained by GLC of TMS-ethers of MBA-alditols on a Carbowax 20M column. Except for erythrose, 2-deoxyribose, digitoxose and 4-O-methylglucose, most monosaccharides examined were found to be resolvable. 2-Deoxygalactose and 2-deoxyglucose, which cannot be resolved by liquid–solid chro-

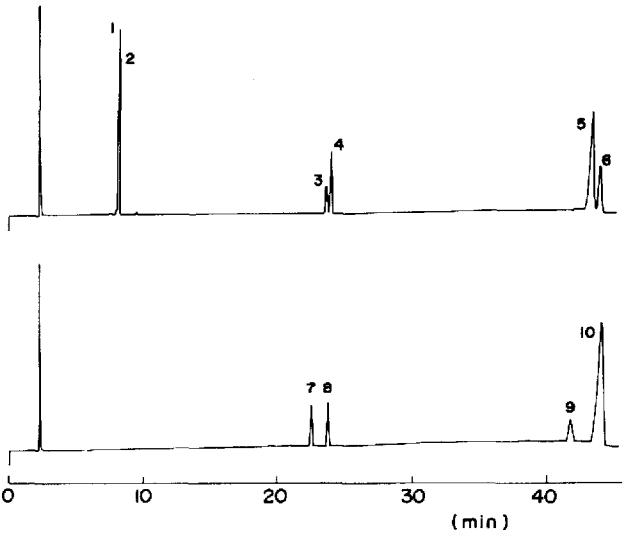

Fig. 1. Chromatograms of 1-(N-acetyl-L-α-methylbenzylamino)-1-deoxyalditol acetates on a fused-silica WCOT column with silicone SE-54 (Hewlett-Packard, ULTRA No. 2, 50 m × 0.2 mm, d_f = 0.11 μm) using hydrogen as the carrier gas ($\bar{u}$ 37 cm/sec) at 250°C. Derivatives: 1 = D-glyceraldelhyde; 2 = L-glyceraldehyde; 3 = L-arabinose; 4 = D-arabinose; 5 = D-galactose; 6 = L-galactose; 7 = L-rhamnose; 8 = D-rhamnose; 9 = L-mannose; 10 = D-mannose, D- and L-glucose.

* The characteristics of this column may be changed from those of a new one as a result of the introduction of the excess of silylating reagent; however, good reproducibility was obtained for analysis of TMS-ethers of MBA-alditols.

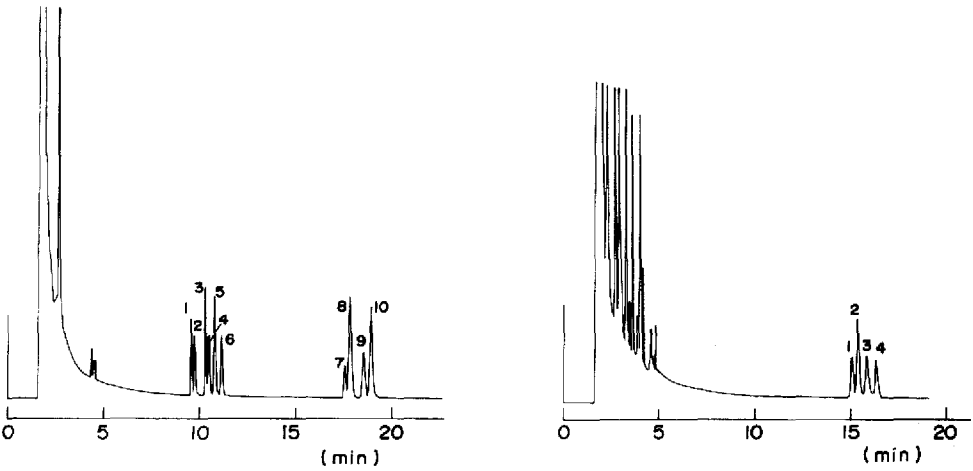

Fig. 2. Chromatogram of TMS-ethers of 1-(L-α-methylbenzylamino)-1-deoxyalditols of neutral aldoses on a fused-silica WCOT column with Carbowax 20M (Hewlett-Packard, 25 m × 0.2 mm) using helium as the carrier gas at 158°C. Derivatives: 1 = D-arabinose; 2 = L-arabinose; 3 = D-rhamnose; 4 = L-rhamnose; 5 = D-fucose; 6 = L-fucose; 7 = L-mannose; 8 = D-mannose and D-galactose; 9 = L-glucose; 10 = D-glucose and L-galactose.

Fig. 3. Chromatogram of TMS-ethers of 2-(L-α-methylbenzylamino)-2-deoxyalditols derived from the reaction product of L-sorbose with DL-MBA. Peaks 1 and 4 correspond to products from L-sorbose and L-MBA, and peaks 2 and 3 from L-sorbose and D-MBA. Conditions as in Fig. 2 except that the column temperature was 155°C.

matography (LSC) of MBA-alditol acetates, are also well resolved. Fig. 2 is an example of the separation the TMS-ethers of MBA-alditols of common neutral aldoses.

The method was extended to the analyses of ketose and N-acetyl amino-sugars. An enantiomer of ketose gives two C-2 epimers after reductive amination with L-MBA, *e.g.*, N-α-methylbenzyl-D-glucosaminitol and N-α-methylbenzyl-D-mannosaminitol are derived from D-fructose. Four peaks in a chromatogram of the reaction product of D- (or L-) ketose with DL-MBA are well separated for fructose, tagatose or sorbose (Fig. 3). Representative N-acetyl amino-sugars, N-acetylglucosamine,

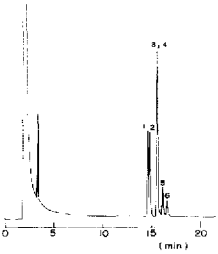

Fig. 4. Chromatogram of TMS-ethers of 1-(L-α-methylbenzylamino)-1-deoxyalditols of N-acetyl amino-sugars. Conditions as Fig. 2 except that the coluomn temperature was 190°C. Derivatives: 1 = N-acetyl-L-mannosamine; 2 = N-acetyl-D-glucosamine; 3 = N-acetyl-L-glucosamine; 4 = N-acetyl-D-mannosamine; 5 = N-acetyl-D-galactosamine; 6 = N-acetyl-L-galactosamine.

TABLE I

RETENTION TIME (min) AND SEPARATION FACTORS r, OF DIASTEREOISOMERS OF SUGARS WITH L-(−)-α-METHYLBENZYLAMINE

An adduct of a D-sugar and L-MBA is abbreviated as D-L*; the letter with the asterisk designates the configuration of the MBA added.

Sugar	*TMS-ethers**			*Acetates***		
	*D–L**	*L–L**	*r*	*D–L**	*L–L**	*r*
Glyceraldehyde	4.23	4.26	1.007	8.04	8.11	1.009
Erythrose	5.97		1.0	13.72	13.60	1.009
Arabinose	8.60	8.75	1.017	23.70	23.33	1.016
Xylose	8.76	8.83		24.80		1.0
Lyxose	8.88	8.94	1.007			
Ribose	8.98	9.24	1.029	23.10	22.82	1.012
Rhamnose	9.28	9.43	1.016	23.43	22.26	1.053
Fucose	9.64	9.93	1.030	23.23	22.85	1.017
6-Deoxyglucose	10.86	10.74	1.011***			
2-Deoxyribose	11.30		1.0***			
Digitoxose	10.91		1.0***			
Fructose	12.83	12.23				
	13.56	12.90				
Sorbose***	15.40	15.11				
	15.84	16.34				
Tagatose***	15.09	14.80				
	15.40	15.69				
4-O-Methylglucose	15.02		1.0			
Mannose	15.53	15.22	1.020	43.26	41.04	1.054
Glucose	16.36	16.04	1.020	43.26		1.0
Galactose	15.63	16.36	1.047	42.64	43.17	1.012
Allose	19.88	19.19	1.036			
2-Deoxyglucose	19.54	19.44	1.005***			
2-Deoxygalactose	16.72	16.96	1.014			
	19.77	20.09	1.016***			
N-Acetylglucosamine	14.96	15.74	1.052§			
N-Acetylgalactosamine	16.07	16.67	1.037§			
N-Acetylmannosamine	14.55	15.50	1.065§			

* Carbowax 20M, 0.2 mm × 25 m; at 158°C; helium carrier gas.
** Silicone SE-54, 0.2 mm × 50 m; at 250°C; hydrogen carrier gas.
*** At 155°C.
§ At 190°C.

N-acetylgalactosamine and N-acetylmannosamine, are also resolved as TMS-ethers of MBA-alditols (Fig. 4).

Characteristics of the separation of sugar enantiomers as MBA-alditols are summarized in Table I.

Due to the high efficiency of the column, diastereoisomers with a separation factor of as little as 1.007 could easily be distinguished.

In the present GLC separation, the order of elution of enantiomers of a certain monosaccharide cannot readily be related to the chemical structure, as has been done for LSC of MBA-alditol acetates[2]. This reflects the difference in separation mechanisms of GLC and LSC.

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