Journal of Chromatography, 216 (1981) 219–228
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,952

GAS–LIQUID CHROMATOGRAPHIC SEPARATION AND DETERMINATION OF THE COMPONENTS OF MALTITOL SYRUPS

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(First received February 18th, 1981; revised manuscript received April 29th, 1981)

SUMMARY

Relative retention times and detector responses of trimethylsilyl and trifluoroacetyl derivatives of the components of maltitol (maltotriitol) syrups on Dexsil GC 300 liquid phase are reported. Glucose, sorbitol, maltose, maltitol, maltotriose and maltotriitol gave a single main peak, whereas as trifluoroacetylated derivatives two or three separated peaks could be observed, which probably showed products of incomplete trifluoroacetylation. A method for rapid separation and quantitative estimations obtained by gas–liquid chromatography for the constituents of maltitol (maltotriitol) syrups are given.

INTRODUCTION

No method is available in the literature for the simultaneous determination of glucose, maltosaccharides and their reduced derivatives, though the problem is important owing to the more favourable sweetening properties of maltites, the reduced derivatives of products obtained by enzymatic hydrolysis of mainly maltose-containing mixtures. Maltitols are better artificial sweeteners than sorbitols.

For the chromatographic analysis of glucose and maltosaccharides numerous methods are known; they are paper chromatography[1–4], thin-layer chromatography[5–8] and liquid chromatography[9–14], including gel permeation chromatography[12,13], as well as gas chromatography[15–38].

Schmidt and Enevoldsen[13] separated the malto- and isomalto-oligosaccharides and the appropriate alditols by gel filtration. However, the separation of the reducing saccharide and the reduced product has not been achieved.

This paper deals with a gas chromatographic method for the separation and determination of the component of maltitol syrups.

The first step of the gas chromatographic analysis of saccharides is the preparation of suitable derivatives. Sweeley *et al.*[15] suggested a quick and simple method for the preparation of the trimethylsilyl (TMS) ethers of saccharides. As a result of their work, gas chromatography became an efficient tool in the analysis of carbohydrates. Subsequent studies[16–26] involve mainly modifications of silylating agents, and the use

0021-9673/81/0000–0000/$02.50

of various column packings for the preparation of the derivatives and the optimal preparation of different saccharides with different degrees of polymerization. It is noteworthy that the silyl ethers of saccharides, together with those of *cis*- and *trans*-oximes, were eluted in one peak by Zürcher *et al.*[27–29]. This advantage was also utilized by the analysis of the acylated derivatives. Monosaccharides can also be acylated directly with acetic anhydride[33], but those with a higher degree of polymerization must be treated with trifluoroacetic anhydride[30–32]. Acetic anhydride hydrolyses the glycoside bond, so higher members can be acylated only after transformation into oximes[34]. Reduction with sodium borohydride into polyalcohols[35–38] results in saccharides that give only a single peak. This laborious and time-consuming procedure is not suitable in our case.

MATERIALS AND METHODS

Materials and reagents

D-Glucose, D-maltose, maltotriose, maltitol and maltotriitol were purchased from Serva (Heidelberg, G.F.R.). Maltitol$_1$ and maltitol$_2$ are artificial sweeteners, commercial products of Japan. Maltitol$_3$ and maltitol$_4$ are Hungarian research intermediates, which are corn syrups, hydrolysed enzymatically at Phylaxia (Budapest, Hungary) and hydrated at Péti Nitrogénmüvek (Várpalota, Hungary).

Pyridine, trifluoroacetic acid (TFA) and methylene chloride were products of Reanal (Budapest, Hungary). Hexamethyldisilasane (HMDS) was from Applied Science Labs (State College, PA, U.S.A.) and trifluoroacetic anhydride (TFAA) from E. Merck (Darmstadt, G.F.R.), both of guaranteed grade.

Apparatus

The gas chromatograph used was a Chromatron Model G.C.H.F. 18.3 instrument equipped with a flame ionisation detector. Chromatographic peak area determinations were made with a Chinoin Model Digint-34μ computing integrator. Stainless-steel columns (2 m × 3 mmI.D.) were used. The packing materials were 3% silicone SE-30, 10% OV-17 and 15% Dexsil GC 300, all of them on 100–120 mesh Chromosorb WAWDMCS purchased from Applied Science Labs.

For the analysis of glucose, maltosaccharides and their reduced products only the packing containing 15% Dexsil as stationary phase was suitable.

Separation of TFA derivatives. The temperature of the injection and detector ports was 270°C. With a temperature-programmed analysis from 100 to 280°C at a heating rate of 12°C/min, it required 15 min to elute the TFA derivatives. The flow-rate of nitrogen was 40 cm^3/min.

Separation of the TMS derivatives. The temperatures of the injection and detector ports were 380°C and 410°C, respectively. With a temperature-programmed analysis from 120°C to 360°C at a heating rate of 16°C/min (with an isothermal period at 320°C for 5 min, it required 25 min to elute the TMS derivatives. The flow-rate of nitrogen was 45 cm^3/min.

Preparation of TFA derivatives. The solution containing 0.01–0.02 g of saccharides and/or alditols was evaporated to dryness in a rotary evaporator at 50–60°C. The dehydrated residue was trifuoroacylated with sodium trifluoroacetate* (*ca.* 10

* Sodium trifluoroacetate was prepared according to the procedure of Ueno *et al.*[30].

mg) and 10 cm^3 of TFAA by refluxing for 30 min at 60–70°C with occasional shaking. Excess TFAA was evaporated in a rotary evaporator at 15–20°C to dryness. Thereafter the TFA derivatives thus obtained were dissolved in 1.0 cm^3 of dry methylene chloride. Sodium trifluoroacetate was left as an insoluble residue. A 10-μl volume of the supernatant solution was injected into the gas chromatograph.

Preparation of TMS derivatives. The dehydrated sample (prepared as above) was dissolved in 500 μl of anhydrous pyridine and trimethylsilylated with a mixture of 900 μl of HMDS and 100 μl of TFA in a glass-stoppered test-tube with a volume of 2–3 cm^3. The mixture was heated at 70–72°C for 60 min, thereafter 5–10 μl of the clear solution was injected into the gas chromatograph.

RESULTS AND DISCUSSION

The joint aims of the model studies concerning the gas chromatographic analysis of glucose, maltosaccharides and their reduced derivatives were (a) the preparation of the derivatives and (b) the optimisation of the dimensions and packing of the column. The following facts were established.

(1) For the solution of our task only the packing with 15% Dexsil is suitable of

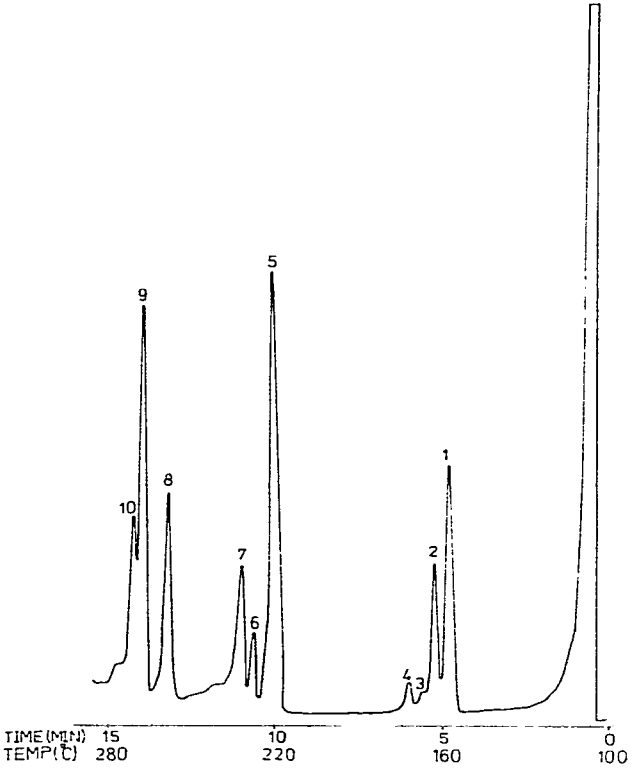

Fig. 1. Gas chromatogram of TFA derivatives. 1 = sorbitol; 2 = α-glucose + sorbitol; 3 = sorbitol; 4 = β-glucose; 5 = maltitol; 6 = α-maltose + maltitol; 7 = β-maltose; 8 = maltotriitol; 9 = α-maltotriose; 10 = β-maltotriose.

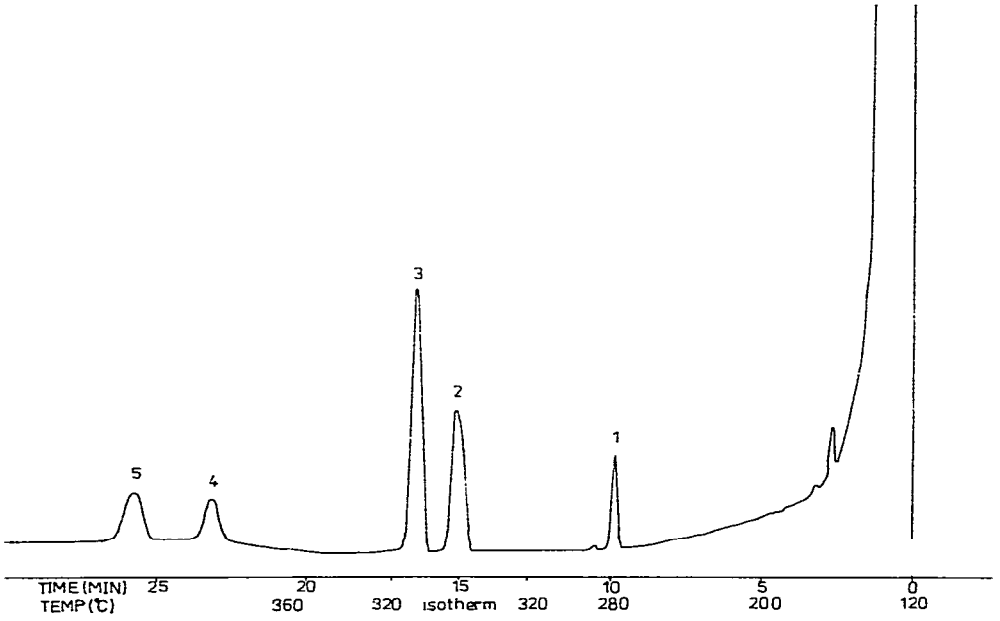

Fig. 2. Gas chromatogram of TMS derivatives. 1 = sorbitol; 2 = maltose; 3 = maltitol; 4 = maltotriose; 5 = maltotriitol.

the three packings investigated for both the trifluoroacylated and the silyl ether derivatives.

(2) On a 2-m long column favourable for the separation of maltose and maltitol, saccharides and their reduced derivatives higher than maltotriose and maltotriitol cannot be measured. On a column packed with 3% SE-30 on 100–120 mesh Chromosorb W AW DMCS (65 cm × 3 mm I.D.) the saccharide content of hydrolysed mixtures could be determined up to maltopentose.

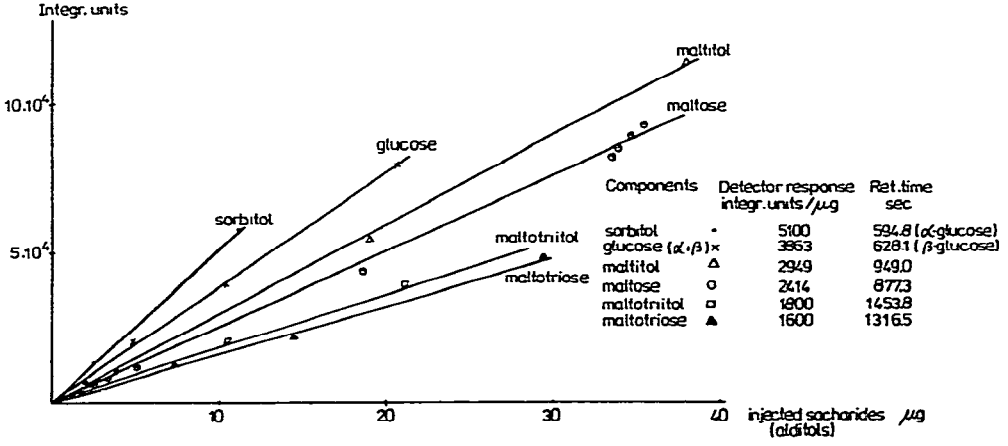

Fig. 3. Detector response of TMS derivatives.

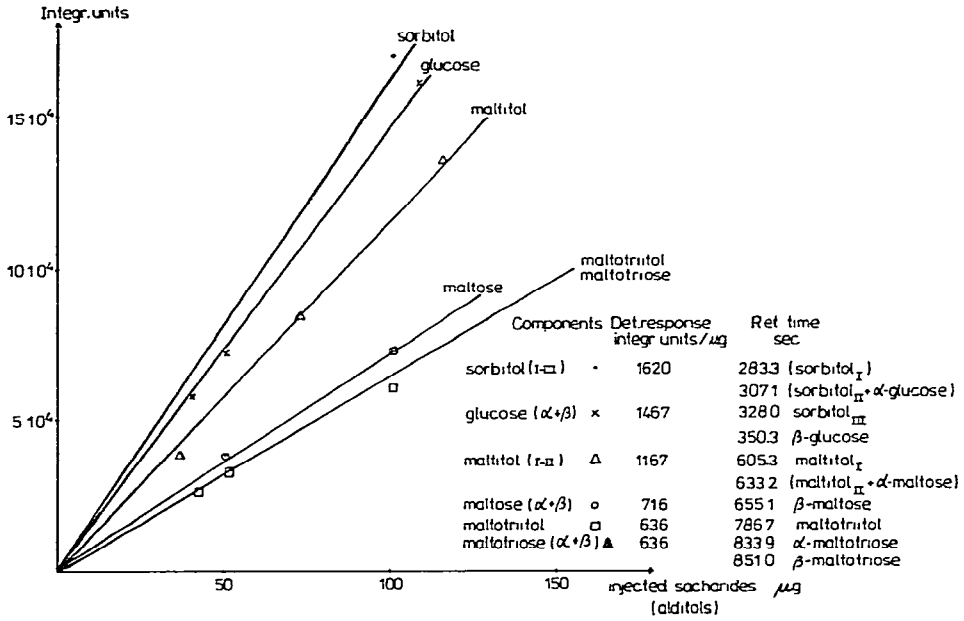

Fig. 4. Detector response of TFA derivatives.

(3) The analysis of silyl ethers is more advantageous from every point of view than that of the trifluoroacetyl derivatives, for several reasons. (a) Much care has to be taken when working with TFAA; (b) Trifluoroacylation is more laborous than the preparation of the silyl ethers; (c) The individual saccharides and their reduced products yield several peaks after trifluoroacylation (Fig. 1), whereas silyl ethers are eluted in a single peak (Fig. 2). as expected from literature data[36]; (d) The most important advantage is that the detector response of silyl ethers is two or three times greater (Fig. 3) than that of trifluoroacylated derivatives (Fig. 4). The low maltose content of maltitol cannot be detected when using the trifluoroacylated derivatives. The efficiency of the analysis usely TMS derivatives was such that less than 0.5 μg of maltose could be measured against a 100-fold amount of maltitol, and less than 0.5 μg of maltitol against a 100-fold amount of maltose. The compositions of model solutions prepared from Serva maltose and Serva maltitol are shown in Fig. 5. (recorded at 320°C isothermally); reproducibility is illustrated in Table I.

(4) Our experiences concerning the detector responses of TFA and TMS derivatives were that (a) the detector response of the reducing saccharide is smaller than that of the corresponding alditol, and (b) the detector response decreases with increasing polymerisation.

(5) The compositions of the maltitols studied, and the components of impurities in maltotriitol, are summerised in Table II. This table shows that in terms of residual reducing power (which is mainly due to maltose), the Serva maltitol and the commercial Japanese sweeteners (maltitol$_1$, maltitol$_2$) are of the same quality. The sorbitol and maltotriitol contents of the latter two are higher than those of the Serva maltitol. The firm Serva does not mention the saccharide impurities in its products.

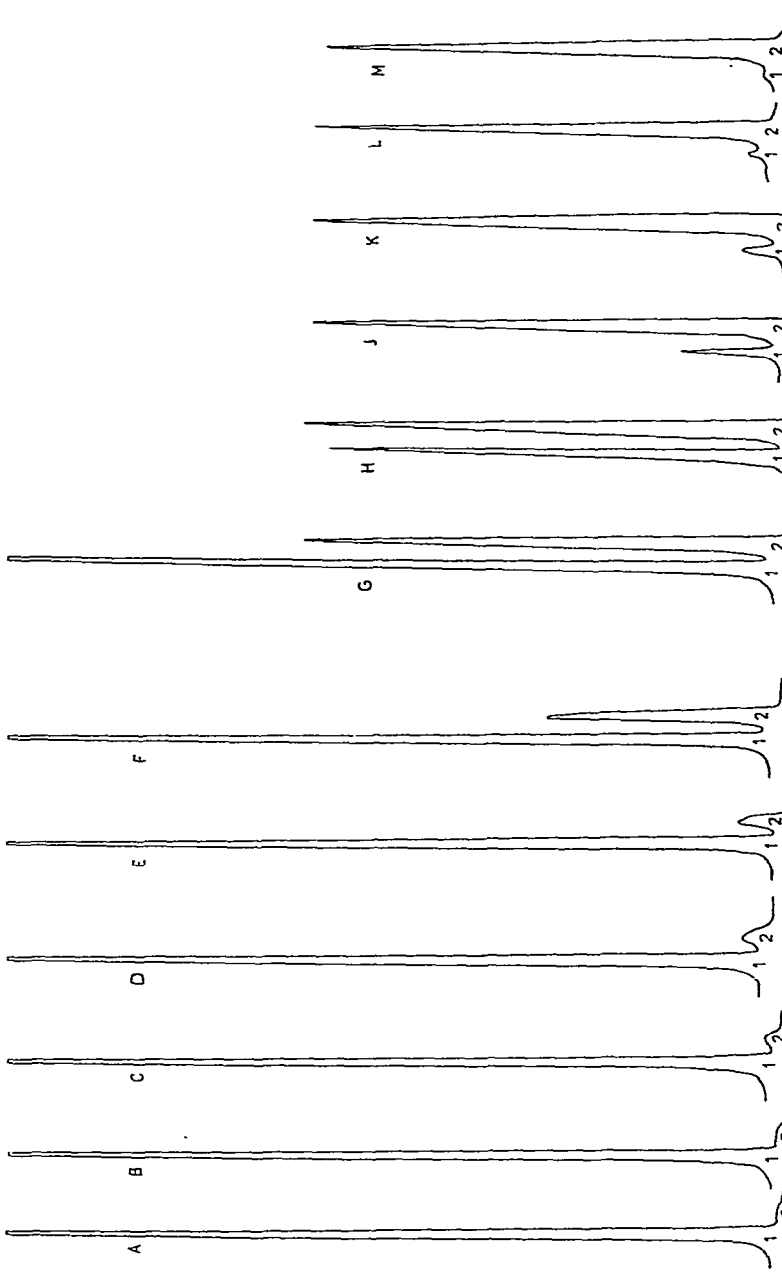

Fig. 5. Gas chromatograms of different amounts of TMS derivatives of maltose and maltitol in the presence of each other. (Detailed data in Table I.)

TABLE I

REPRODUCIBILITY OF JOINT DETERMINATION OF MALTOSE AND MALTITOL AS TMS DERIVATIVES ON THE BASIS OF FIGS. 3 AND 6

$\bar{x}$ = average; S.D. = standard deviation; C.V. = coefficient of variation

Sample	*Components*													
	Maltitol (Serva)						*Maltose*							
	Amount injected (μg)	*Integrator units obtained*					*Amount injected (μg)*			*Integrator units obtained*				
		Total	*Equiv. to 1 μg*				*From maltitol**	*Added*	*Total*	*Total*	*Equiv. to 1 μg*			
			Indiv.	$\bar{x}$	*S.D.*	*C.V.*					*Indiv.*	$\bar{x}$	*S.D.*	*C.V.*
A	38.0	113,813	2995				1.71	0.0	1.71	**				
B	38.0	114,654	3017				1.71	0.34	2.05	4428	2160			
C	38.0	115,816	3048				1.71	0.67	2.38	5741	2412			
D	38.0	112,856	2970				1.71	1.69	3.40	8500	2500			
E	38.0	107,247	2822				1.71	3.37	5.08	11,603	2284			
F	38.0	106,893	2813	2949	114.9	3.9	1.71	16.9	18.6	40,311	2167	2414	152.7	6.3
G	38.0	115,336	3035				1.71	33.7	35.4	93,458	2640			
H	19.0	54,159	2850				0.85	33.7	34.6	88,171	2548			
I	3.80	10,687	2812				0.17	33.7	33.9	84,910	2505			
K	1.90	5940	3126				0.09	33.7	33.8	83,796	2479			
L	0.76	**					0.04	33.7	33.7	81,002	2404			
M	0.38	**					0.02	33.7	33.7	82,797	2457			

* See Table II.

** Peak areas resulting from low concentrations of saccarides were not measured reproducibly with the electronic integrator. The area of these peaks was determined by triangulation.

TABLE II

IMPURITIES IN MALTITOL AND MALTOTRIITOL SAMPLES

1: Determined as TFA derivatives; 2: determined as TMS derivatives.

Sample		*Impurity compounds, expressed in % of the total dry material content*								
		Sorbitol	*Glucose*	*Maltose*	*Matitol*	*Maltotriose*	*Maltotriitol*	*Reducing power**		*Dry material content (% w/v)*
								a	*b*	
Maltitol$_1$	1	7.1	–				9.7	1.3		74.8
	2	5.9	–	2.8			11.8		1.5	
Maltitol$_2$	1	5.1					14.6	1.8		74.6
	2	5.7	–	3.0			14.4		1.6	
Maltitol$_3$	1	12.7	5.8	18.6			4.2	11.9		74.9
	2	12.6	4.4	16.5		2.0	4.1		13.1	
Maltitol$_4$	1	22.1					2.0	2.3		43.2
	2	19.4	1.0	1.9			2.1		2.0	
Maltitol	1	2.6					7.2	1.2		71.3
(Serva)	2	2.3		2.6			7.5		1.4	
Maltotriitol	1	2.5			18.6			**	**	**
(Serva)	2	3.2			19.5					

* Expressed in glucose: (a) determined according to the procedure of Kolthoff[38]; (b) total of reducing saccharides, determined by GLC as TMS derivatives.

** Not measured.

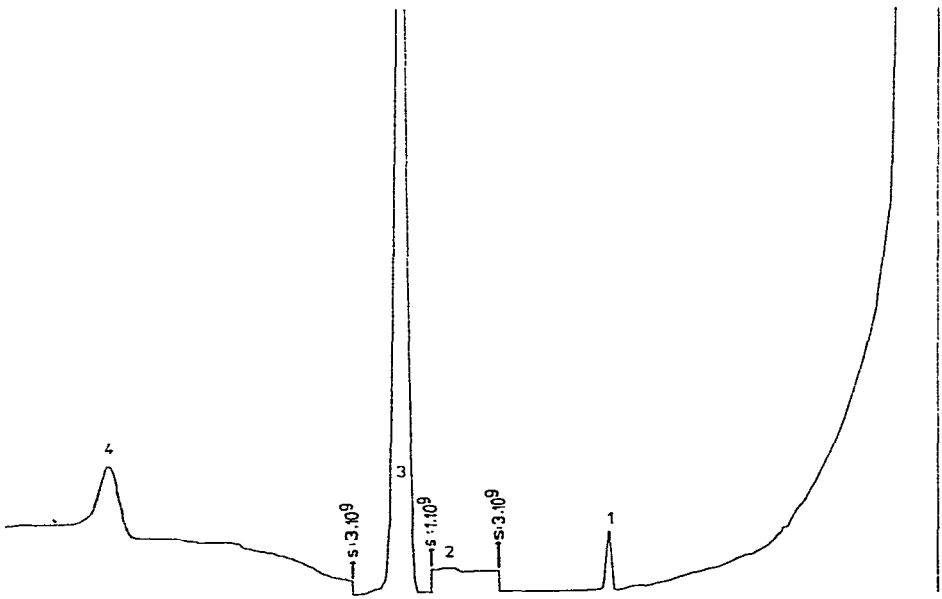

Fig. 6. Gas chromatogram of TMS derivatives of Serva maltitol. 1 = sorbitol; 2 = maltose; 3 = maltitol; 4 = maltotriitol.

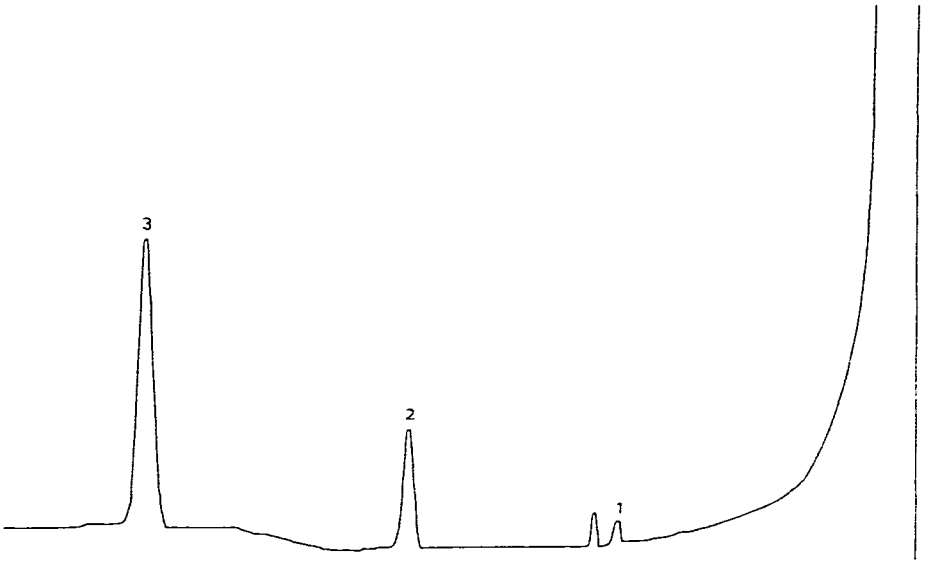

Fig. 7. Gas chromatogram of TMS derivatives of Serva maltotriitol. 1 = sorbitol; 2 = maltitol; 3 = maltotriitol.

Among its products only sorbitol and glucose proved to be gas chromatographically pure. We applied this product for the preparation of the calibrating solutions. The following impurities were found: in maltose, 0.7% glucose, 12.5% maltotriose and 3.9% maltotetrose; in maltotriose, 0.9% glucose, 1.6% maltose and 2.1% maltotetrose. The compositions of the reduced products are shown in Table II, and gas chromatograms of the TMS derivatives of maltitol and maltotriitol are shown in Figs. 6 and 7, respectively.

ACKNOWLEDGEMENTS

We acknowledge the generous gift of maltitol and maltotriitol by I. Petróczy and A. Kővágó (Phylaxia, Hungary). This work was supported by Phylaxia and Péti Nitrogénmüvek, Hungary.

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