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Gas–liquid chromatographic assay of the components of electrochemically reduced glucose solutions

Impurities in sorbitol samples

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ABSTRACT

Relative retention times and detector responses of the trimethylsilyl ether and oxime derivatives of the components of electrochemically reduced glucose syrups and commercial sorbitol samples are reported. D-Glucose, 2-deoxy-D-glucose, D-glucitol and 2-deoxy-D-glucitol each gave a single main peak on Dexsil 300 stationary phase a second peak of D-glucose was about one quarter the height of the main peak. Amounts of 0.13–13 μg of all four components in the presence of each other, even in ratios of 1:100, have been measured with relative standard deviations of 2.5–5.3%. The amount of 2-deoxy-D-glucitol in commercial sorbitols varied from >0.1 to 0.8% (w/w).

INTRODUCTION

No method could be found in the literature for the simultaneous determination of D-glucose and its derivatives obtained either by catalytic hydrogenation or by electrolysis. The production of sorbitol by an electrochemical process (used on an industrial scale [1] before the second world war) has been re-examined recently [2–4] owing to its several advantages in comparison with catalytic hydrogenation procedures.

Owing to the very similar characteristics of the byproducts obtained in the reduction processes, such as deoxy sugars and/or deoxy alditols, the analytical methods used [5,6] proved to be unsatisfactory in both qualitative and quantitative analysis. Our previously described gas–liquid chromatographic (GLC) method [7–9] has therefore been applied, after optimization of the conditions, for the simultaneous determination of D-glucose, 2-deoxy-D-glucose, D-glucitol and 2-deoxy-D-glucitol.

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EXPERIMENTAL

Materials and reagents

D-Glucose and 2-deoxy-D-glucose were purchased from Serva (Heidelberg, F.R.G.). Sorbitol samples 1–6 were products of various companies as follows: Péti Nitrogén Művek, Pét, Hungary (sorbitol 1), Atlas Powder, Miami, FL, U.S.A. (sorbitol 2), Anil Starch Products, Ahmedabad, India (sorbitol 3), SPA, Milan, Italy (sorbitol 4), an unknown Japanese company (sorbitol 5) and Carlo Erba, Milan, Italy (sorbitol 6).

2-Deoxy-D-glucitol was prepared from 2-deoxy-D-glucose by reduction with sodium tetrahydroborate as described by Lehrfeld [10].

Test samples 2–7 were electrochemically reduced glucose solutions (1 *M*) containing sodium sulphate (0.5 *M*) or, in test sample 5, lithium nitrate (0.5 *M*).

The electrochemial reductions were carried out in an Electro MP Cell (500 cm^3) equipped with an ion-exchange membrane (Nafion XR 423) and separate cathode and anode compartments (Electro Cell, Täby, Sweden). Electrolyses were performed with current densities of 8 A per 200 cm^3 for 6 h (test sample 2) and of 4 A per 200 cm^3 for 6.7 h (3–5), for 4 h (6) and for 4 × 6.7 h (7). Test sample 1, an unelectrolysed solution of D-glucose, served as the basis of comparison.

Apparatus

The gas chromatograph used was a Chromatron Model G.C.H.F. 18.3 instrument equipped with a flame ionization detector. Chromatographic peak-area determinations were made with a Chinoin Model Digint 34 μ computing integrator. Stainless-steel columns (3 m × 4 mm I.D.) were used. The packing materials were 15% Dexsil GC 300 on 80–100-mesh Chromosorb W AW DMCS, purchased from Applied Science Labs. (State College, PA, U.S.A.).

In the separation of the trimethylsilyl (TMS) alditol and TMS oxime derivatives, the temperatures of the injection and detector port were 380 and 400°C, respectively. With temperature programming from 180 to 300°C at 8°C/min, the TMS derivatives were eluted in 20 min. The flow-rate of the carrier gas (nitrogen) was 60 cm^3/min.

Preparation of the TMS sugar–oxime derivatives

The solutions, containing 0.02–2.00 mg of saccharides and/or alditols, were evaporated to dryness in a rotary evaporator at 50–60°C. The dried sample was dissolved in 500 μl of anhydrous pyridine containing hydroxylamine hydrochloride (25 mg/cm^3) and the mixture was heated for 30 min at 70–72°C. The oxime derivatives were then trimethylsilylated with a mixture of hexamethyldisilazane (900 μl) and trifluoroacetic acid (100 μl) in Reacti-vials with a volume of 2–5 cm^3. The mixture was heated at 70–72°C for 60 min; thereafter, 5–10 μl of the clear 1.5-cm^3 solution were injected into the gas chromatograph.

RESULTS AND DISCUSSION

Our model investigations, performed with authentic saccharides in order to find the optimum derivatization and GLC conditions, gave the following results:

Columns 0.5, 2 and 3 m long (each of 4 mm I.D.) coated with 15% Dexsil GC 300

or 3% SP 2250 were tested in parallel. [Columns 0.5 m long served only for testing saccharides of higher degree of polymerization (DP); in the present analysis the proportion of saccharides and/or alditols with DP >1 was <0.1 wt.-% in all the samples tested.]

Optimum separation was achieved on the 15% Dexsil columns (3 m × 4 mm I.D.) with the sugars as their TMS oxime derivatives (Table I). The differences in retention times of the components are summarized and the chromatogram is simplified, as a single peak is obtained for the 2-deoxy-D-glucose derivative. In addition, the responses of the TMS derivatives are *ca.* 25–30% smaller than those of the corresponding TMS oximes.

TABLE I

RETENTION TIMES OF TMS D-GLUCITOL AND 2-DEOXY-D-GLUCITOL AND TMS OXIMES OF D-GLUCOSE AND 2-DEOXY-D-GLUCOSE ON 15% DEXSIL GC 300

The temperature programme for the 2-m column was from 120 to 300°C at 12°C/min and that for the 3-m column as given in the text.

Sample	Retention time (min)			
	TMS derivatives		TMS oxime derivatives	
	2-m column	3-m column	2-m column	3-m column
2-Deoxy-D-glucitol	649	588		
2-Deoxy-D-glucose	621	535	694	649
	670	604		
D-Glucitol	720	675		
D-Glucose	708	657	750	716
	765	729	768	746

The column packing of 3% SP 2250 (useful in the GLC analysis of sugars as alditol acetates) gave unsatisfactory separations of the alditol derivatives in this present work on columns of any length.

Under our optimum conditions, all components of the electrochemical reduction of D-glucose were measured with good reproducibility even when present in a ratio of approximately 100:1 to one another (Table II, Fig. 1).

Regarding the components of the electrochemically reduced glucose solutions, the amounts of 2-deoxy-D-sorbitol were surprisingly high in all test samples (Table III, tests 1–7). As the biological and/or physiological effect of 2-deoxy-D-glucitol is unknown, it would be of interest to clarify it, especially because the commercial sorbitols (Table III, sorbitols 1 6) also contain 0.1–0.9% of 2-dcoxy-D-glucitol. Concerning the composition of test 1, it was a commercial dextrose sample which contains as contaminants all three components at levels of 0.02%. The (D-glucitol + 2-deoxy-D-glucitol)/D-glucose ratio, even with the most effective procedure (test sample 7), was about 3.2, *i.e.*, (7.97 + 2.10)/3.17. Hence results obtained with test samples 1–7 could be regarded as a first step in a systematic study performed in order

TABLE II

REPRODUCIBILITY OF JOINT DETERMINATION OF D-GLUCOSE, 2-DEOXY-D-GLUCOSE, D-GLUCITOL AND 2-DEOXY-D-GLUCITOL AS TMS ETHER AND TMS OXIME DERIVATIVES ON THE BASIS OF FIG. 1

	Components									
	D-Glucose					2-Deoxy-D-glucose				
	Amount injected (μg)	Integrator units obtained, equiv. to 1 μg[a]				Amount injected (μg)	Integrator units obtained, equiv. to 1 μg[a]			
		Individual	x^5	S.D.	R.S.D. (%)		Individual	x^5	S.D.	R.S.D. (%)
A	12.82	10015	10917	579.5	5.3	0.0		9990	479.8	4.8
B	12.69	9994				0.136	10450			
C	12.33	10913				0.324	10248			
D	11.81	10629				0.594	10436			
E	10.36	10898				1.30	9635			
F	8.65	10195				3.35	9288			
G	6.48	11046				6.75	9305			
H	4.30	10997				4.48	9535			
J	2.59	11446				2.21	10251			
K	1.14	11824				1.19	10010			
L	0.620	11355				0.65	10783			
M	0.260	10044				0.27	10793			
N	0.130	11568				0.130	10143			

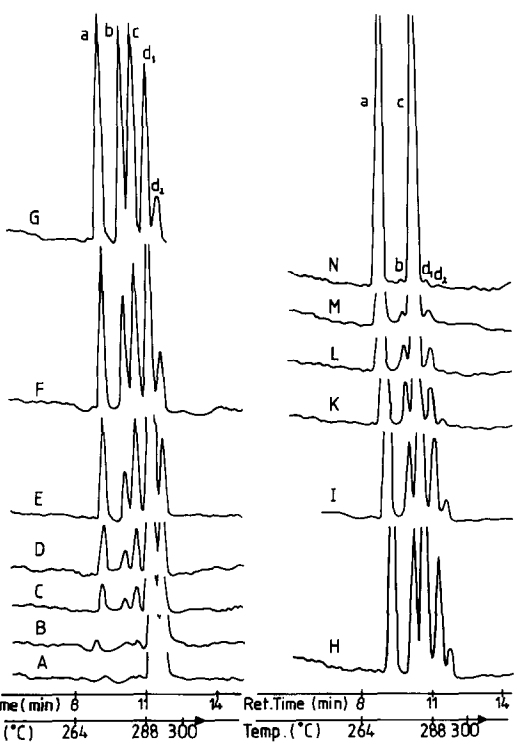

Fig. 1. Gas–liquid chromatograms (A–N) of different amounts of TMS ether (peaks a and c) and TMS oxime derivatives (peaks b, d_1 and d_2) of 2-deoxy-D-glucitol (peaks a), 2-deoxy-D-glucose (peaks b), D-glucitol (peaks c) and D-glucose (peaks d_1 and d_2) Detailed data in Table II.

D-Glucitol					2-Deoxy-D-glucitol				
Amount injected (μg)	Integrator units obtained, equiv. to 1 μg[a]				Amount injected (μg)	Integrator units obtained, equiv. to 1 μg[a]			
	Individual	x^5	S.D.	R.S.D. (%)		Individual	x^5	S.D.	R.S.D. (%)
0.130	9697	10591	323.7	3.1	0.136	4730	9828	240.7	2.5
0.259	10800				0.272	9977			
0.620	10805				0.653	10297			
1.14	11196				1.19	10090			
2.59	11124				2.72	10095			
4.30	10304				4.52	9842			
6.48	10344				6.80	9464			
8.65	10245				9.09	9677			
10.36	10646				10.88	9940			
11.81	10295				12.40	9564			
12.33	10525				12.95	9792			
12.69	10452				13.33	9678			
12.82	10360				13.46	9621			

[a] x^5 = Average; S.D. = standard deviation; R.S.D. = relative standard deviation.

to extend the effectiveness of the electrochemical process, possibly to quantitative reduction, to eliminate the formation of 2-deoxy-D-glucose and 2-deoxy-D-glucitol, to combine cathodic reduction with an anodic oxidation (if economically useful), in addition to the reduction of D-glucose, and to apply the procedure to other hexoses and disaccharides.

Concerning the purity of sorbitol samples, none proved to be free of 2-deoxy-D-glucitol and the variation in the quality and amount of impurities determined in products from different sources was large; sorbitol 6 proved to be of the highest purity.

TABLE III

COMPOSITION OF ELECTROCHEMICALLY REDUCED D-GLUCOSE SOLUTIONS AND COMMERCIAL SORBITOL SAMPLES

Sample	Compound (% of total dry material content)								
	D-Glucose	2-Deoxy-D-glucose	D-Glucitol	2-Deoxy-D-glucitol	Reducing power[a]		Dry material content (%, w/w, in total)		
					A	B	Weighed	Found	Difference (%)
Test 1	18.03	0.02	0.02	0.02	17.92	18.05	17.92	18.28	+2.0
Test 2	9.77	0.024	6.22	1.34	9.65	9.79	17.60	17.35	−1.4
Test 3	6.88	0.025	7.07	3.00	6.91	6.91	17.12	16.99	−0.8
Test 4	7.95	0.070	6.87	2.37	7.81	8.02	17.42	17.32	−0.6
Test 5	16.17	0.020	0.75	0.022		16.19	16.85	16.94	+0.1
Test 6	12.21	0.027	3.60	0.94	12.28	12.24	18.11	16.75	−7.5
Test 7	3.15	0.020	7.97	2.10	3.13	3.17	20.21	13.24	−3.5
Sorbitol 1	0.02	0.020	70	0.89					
Sorbitol 2	0.02	0.020	98	0.58					
Sorbitol 3	0.02	0.020	70	0.50					
Sorbitol 4	6.11	0.020	70	0.10	6.50	6.11			
Sorbitol 5	0.00	0.020	70	0.25					
Sorbitol 6	0.02	0.020	98	0.10					

[a] Expressed in glucose: (A) determined according to the procedure of Kolthoff [11]; (B) total of D-glucose and 2-deoxy-D-glucose, determined by GLC as TMS oxime derivatives.

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REFERENCES

1 Atlas Powder Comp., Wilmington, DE, *U.S. Pat.*, 1 653 004 (1927); 1 612 361 (1927); *Fr. Pat.*, 760 507 (1934); *Ger. Pat.*, 630 454 (1936); *U.S. Pat.*, 2 300 218 (1942); 2 280 887 (1942); 2 289 189 (1942); 2 289 190 (1942); 2 303 210 (1942); 2 458 895 (1942); 2 507 973 (1942).
2 A. Bin Kassim, C. L. Rice and A. T. Kuhn, *J. Appl. Electrochem.*, 11 (1981) 261.
3 A. Bin Kassim, C. L. Rice and A. T. Kuhn, *J. Chem. Soc., Faraday Trans.*, 77 (1981) 683.
4 A. Bin Kassim, *Ph.D. Thesis*, University of Salford, 1980.
5 M. L. Wolfrom, M. Konigsberg, F. B. Moody and R. M. Goepp, *J. Am. Chem. Soc.*, 68 (1946) 122.
6 M. Birkett and A. T. Kuhn, *Electrochim. Acta*, 21 (1976) 91.
7 I. Molnár-Perl and M. Pintér-Szakács, *J. Chromatogr.*, 216 (1981) 219.
8 I. Molnár-Perl, M. Pintér-Szakács, Á. Kövágó and J. Petróczy, *J. Chromatogr.*, 295 (1984) 433.
9 I. Molnár-Perl, M. Pintér-Szakács, Á, Kovágó and J. Petróczy, *Carbohydr. Res.*, 138 (1985) 83.
10 J. Lehrfeld, *Anal. Chem.*, 57 (1985) 346.
11 I. M. Kolthoff, *Z. Unters. Lebensm.*, 45 (1923) 131.