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ISOTACHOPHORETIC ANALYSIS OF SACCHARINIC ACIDS

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SUMMARY

The optimum conditions were found for isotachophoretic separation of organic acid mixtures formed by alkaline degradation of saccharides. In the reaction mixture after alkaline treatment of glucose, the following acids were identified and determined: formic, glycolic, glyceric, lactic, 2-hydroxybutyric, 2-hydroxyvaleric, 3-deoxy-D-ribohexonic (α-D-glucometasaccharinic), 3-deoxy-2-C-hydroxymethyl-D-*erythro*-pentonic (α-D-glucoisosaccharinic) and 3-deoxy-D-arabinohexonic (β-D-glucometasaccharinic). The isotachophoretic identification was confirmed by gas–liquid chromatography in conjunction with mass spectrometry using the per(O-trimethylsilyl) derivates of the acids.

INTRODUCTION

Hydroxy acids of the formula $C_nH_{2n}O_n$, *i.e.*, the saccharinic acids, are formed by alkaline degradation and redox disproportionation of poly-, oligo- and monosaccharides. Polyhydroxy acids derived from carbohydrates have been analysed by paper chromatography, thin-layer chromatography (TLC)[1,2], gas chromatography (GC)[3,4], high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS)[5]. Studies of other liquors obtained by alkaline treatments of polysaccharides revealed that none of these techniques can resolve the complex mixtures of organic acids produced and therefore additional information was obtained by applying a combination of ion-exchange chromatography[6] and GC–MS[7].

Isotachophoresis has been shown to be a suitable method for identification and determination of organic acids. It possesses some advantages over chromatographic methods, especially the minimum consumption of sample and the direct analysis without a preliminary sample modification. The possibility of the direct determination of the acids makes this method preferable to GC. Isotachophoresis also gives important information about the anionic nature of the separated acids.

EXPERIMENTAL

To minimize side reactions, degradations were carried out under mild condi-

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TABLE I

SEPARATING CONDITIONS

	I	*II*	*III*
Leading electrolyte			
Acid (HCl) concentration (*M*)	10^{-2}	$8 \cdot 10^{-3}$	10^{-2}
Counter ion	β-Alanine	Creatinine	L-Histidine
pH	3	5	6
Metal ion (Ca^{2+}) concentration (*M*)		$1 \cdot 10^{-3}$	
Additive methylhydroxypropylcellulose (MHPC)	0.1%	0.1%	0.1%
Terminating electrolyte			
Anion concentration (*M*)	Acetate $5 \cdot 10^{-3}$	Capronate $5 \cdot 10^{-3}$	
Counter ion		Tris	

tions using oxygen-free 0.1 *M* aqueous sodium hydroxide at 25°C over 50 days. The initial molar ratio of alkali to D-glucose was 5:1[8].

Degradation products were analysed by a two-capillary column isotachophoretic analyser at a driving current of 250 μA in the pre-separation column (0.8 mm I.D.) and 40 μA in the analytical column (0.3 mm I.D.) using a conductometric detector. The samples were injected into the isotachophoretic column by a Hamilton syringe (1–5 μl) or by means of a three-way valve (30 μl). Details of the electrolytes employed are given in Table I.

Degradation products were analysed as their per(O-trimethylsilyl) (TMS) derivatives by GC–MS using an HP-5840 A instrument with flame ionization detection. A glass column packed with Dexsil 300 (3%) was used. Temperature program: 343–493°K. Mass spectra of separated acids were recorded during the whole GC analysis using an HP-5985 A instrument. TMS derivatives were prepared using the reagents of Sweeley *et al.*[9], *i.e.*, hexamethyldisilazane (HMDS) with chlorotrimethylsilane (TMCS) in pyridine (2:1:10) at 333°K.

RESULTS AND DISCUSSION

Complex mixturess of hydroxycarboxylic acids were formed by alkaline degradation of saccharides. When analysed by isotachophoresis, different separation conditions must be tested to eliminate the possible formation of mixed zones of some acids. The following conditions were chosen:

1. Leading electrolyte III, terminating electrolyte II; separation takes place on the basis of molecular size (Fig. 1).

2. Leading electrolyte II, terminating electrolyte II; Ca^{2+} in the leading electrolyte acts as a complexation counter ion. Cations and Ca^{2+} form reversible complexes with anions of the hydroxycarboxylic acids, the mobilities of which are lower than those of the free anions (Fig. 2)

3. Leading electrolyte I, terminating electrolyte I; most acids are only partly ionized and are separated according to their pK_a values.

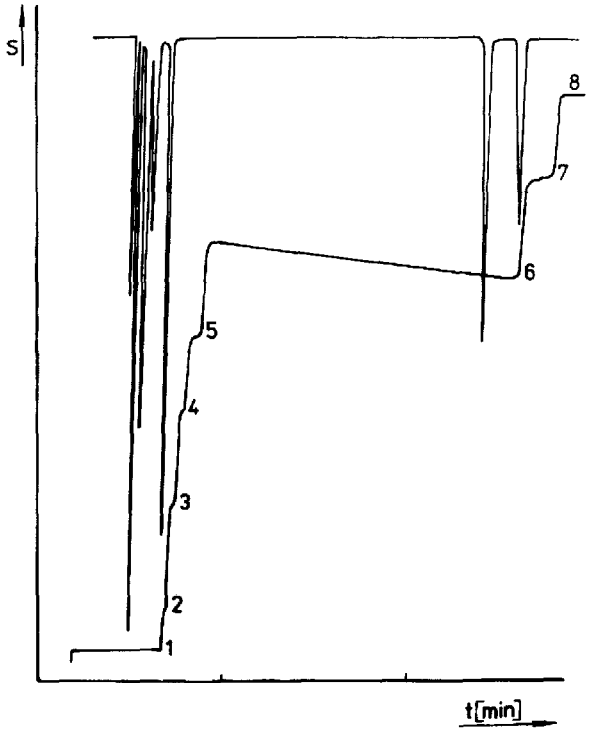
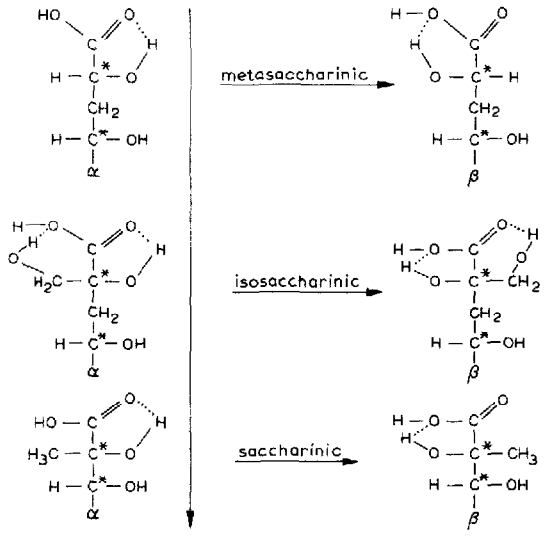


Fig. 1. Isotachopherogram of glucose after alkaline degradation. Dilution 1:4. Injected amount: 30 μl, 60% of lactic acid having been removed. Separation system 1. Zones: 1 = Cl^-; 2 = impurities; 3 = formic acid; 4 = unidentified; 5 = glycolic; 6 = lactic; 7 = 2-hydroxybutyric; 8 = caproic acid.

Quantitative analyses using all three systems were carried out by comparison of the relative height step of the sample zone, r_{sh}, with that of a relevant standard. Identification was effected by incremental additions of all identified acids to the analysed sample. The acids were then determined by the use of a calibration curve (Table II). Relative deviations were within the interval ± 1 to 5%.

The most effective separations of analysed samples were obtained with the electrolytic system[3]. The following acids were identified and determined: formic, glycolic, glyceric, lactic, 2-hydroxybutyric, 2-hydroxyvaleric, α-D-glucometasaccharinic (α-GMSA), α-D-glucoisosaccharinic (α-GISA) and β-GMSA (Table III).

Correct assignment of zones on an isotachopherogram (Fig. 3) to individual saccharinic acids can confirm from theoretical point of view justification of saccharinic acids strength, where are probably decisive associations of hydrogen-bonds. In the following scheme the strength of the acids decreases in the direction of the vertical:



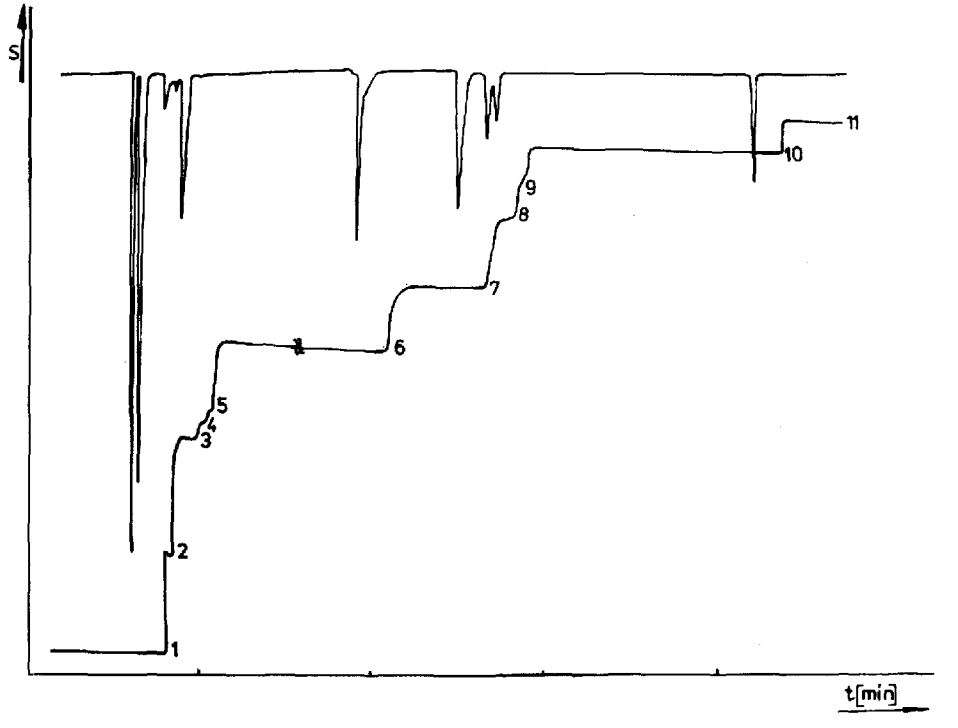


Fig. 2. Isotachopherogram of glucose after alkaline degradation. Injected amount: 30 μl, 80% of lactic acid having been removed. Separation system 2. Zones as in Fig. 1 except: 8 = 2-hydroxyvaleric acid; 9 = unidentified; 10 = GMSA; 11 = caproic acid.

TABLE II

PARAMETERS OF CALIBRATION LINE $y = a + bx$

a is the intercept (mm), b the slope (mm/mole) and r the correlation coefficient.

Acid	*Parameter*	*Separation system*		
		3	*2*	*1*
Formic	*a*	3.7	−2.4	0.6
	b	$4.12 \cdot 10^9$	$3.69 \cdot 10^9$	$3.32 \cdot 10^9$
	r	0.9991	0.9996	0.9999
Glycolic	*a*	1.2	0.7	0.8
	b	$4.11 \cdot 10^9$	$2.96 \cdot 10^9$	$2.45 \cdot 10^9$
	r	0.9999	0.9997	0.9969
Lactic*	*a*	−0.9	1.2	−0.6
	b	$6.12 \cdot 10^8$	$6.20 \cdot 10^8$	$4.23 \cdot 10^8$
	r	0.9992	0.9996	0.9994
2-Hydroxybutyric	*a*	0.5	−1.2	1.61
	b	$4.38 \cdot 10^9$	$4.15 \cdot 10^9$	$3.19 \cdot 10^9$
	r	0.9995	0.9987	0.9995
2-Hydroxyvaleric	*a*	0.7	−0.3	−0.03
	b	$4.29 \cdot 10^9$	$3.94 \cdot 10^9$	$3.38 \cdot 10^9$
	r	0.9996	0.9981	0.9990
GISA	*a*	−1.3		
	b	$0.955 \cdot 10^9$		
	r	0.9991		

* From pre-separation column.

TABLE III

DETERMINATION OF ACIDS AFTER REDOX DISPROPORTIONATION OF GLUCOSE

s = Estimate of deviation.

Acid	*Concentration (M)*	*s (M)*
Formic	$1.72 \cdot 10^{-3}$	$5 \cdot 10^{-5}$
Glycolic	$1.70 \cdot 10^{-3}$	$8 \cdot 10^{-5}$
Lactic	$2.01 \cdot 10^{-1}$	$4 \cdot 10^{-3}$
2-Hydroxybutyric	$9.14 \cdot 10^{-3}$	$5 \cdot 10^{-5}$
2-Hydroxyvaleric	$1.35 \cdot 10^{-4}$	$5 \cdot 10^{-5}$
α-GMSA	$7.18 \cdot 10^{-1}$	$3 \cdot 10^{-2}$
α-GISA	$1.78 \cdot 10^{-2}$	$8 \cdot 10^{-4}$
β-GMSA	$4.18 \cdot 10^{-1}$	$2 \cdot 10^{-3}$

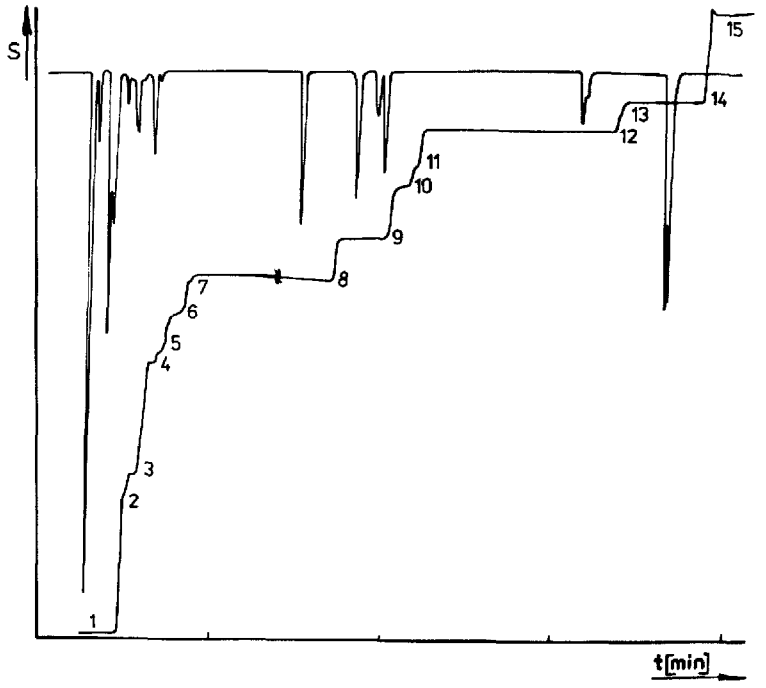


Fig. 3. Isotachopherogram of glucose after alkaline degradation. Conditions as in Fig. 2 except separation system was 3. Zones: 1 = Cl^-; 2 = impurities; 3 = formic acid; 4, 7, 11 = unidentified; 5 = glycolic; 6 = glyceric acid; 8 = lactic; 9 = 2-hydroxybutyric; 10 = 2-hydroxyvaleric; 12 = α-GMSA; 13 = α-GISA; 14 = β-GMSA; 15 = acetic acid.

Often saccharinic acids appear in the form of more stable lactones and an equilibrium between the lactone and the acid occurs. Thus the ratio lactone/acid was investigated at various pH values under the laboratory conditions (Table IV). Glucometasaccharinic acids were determined by means of a calibration curve, and the parameters of glucoisosaccharinic acid and lactonization were considered.

GC–MS was chosen as a comparative method for identification of organic acids in mixtures formed by redox disproportionation of glucose. The silanized mix-

TABLE IV

EQUILIBRIUM BETWEEN GISA AND ITS LACTONE AT VARIOUS pH VALUES

pH	*GISA*			*Lactone*	
	Calc. amount (mole)	*Found (mole)*	*%*	*Found (mole)*	*%*
3.28	$2 \cdot 10^{-10}$	$0.6 \cdot 10^{-10}$	30	$1.4 \cdot 10^{-10}$	70
4.15	$2 \cdot 10^{-10}$	$0.64 \cdot 10^{-10}$	32	$1.36 \cdot 10^{-10}$	68
5.08	$2 \cdot 10^{-10}$	$0.67 \cdot 10^{-10}$	34	$1.33 \cdot 10^{-10}$	66
5.68	$2 \cdot 10^{-10}$	$0.7 \cdot 10^{-10}$	35	$1.3 \cdot 10^{-10}$	65
7.85	$2 \cdot 10^{-10}$	$0.74 \cdot 10^{-10}$	37	$1.26 \cdot 10^{-10}$	63

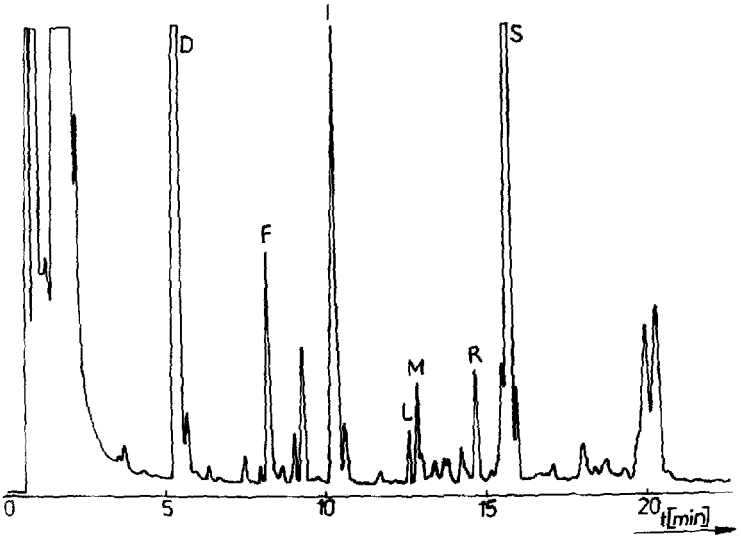


Fig. 4. Chromatogram of glucose after alkaline degradation followed by decationization, evaporation and derivatization with TMCS + HMDS in pyridine. A 1-μl volume was injected directly into the GC colum. Peaks: D = lactic acid; F = 2-hydroxy-1,4-butyrolactone; I = 2-hydroxybutyric; L, M = hydroxybutyric; R = isosaccharinic; S = metasaccharinic acid.

ture was separated chromatographically and on the base analysis of standards at the same conditions individual peaks were identified (Fig. 4). The identification was checked by mass spectrometry. Structural information on the analysed compounds was obtained from fragmentation spectra acquired by electron ionization and by comparison with reference spectra stored in a computer memory. TMS derivatives of GISA and GMSA have very similar retention times under the GC analysis conditions used (peaks R and S). The presence of both α- and β-GMSA and -GISA was established by the analysis of the fragmentation spectra of peaks R and S. These acids would be differentiated on the basis of fragmentation studies.

Silanized GISA γ-lactone, which has a CH_2OH group on the C-2 carbon, looses formaldehyde with rearrangement of the $SiMe_3$ group, resulting in a characteristic fragment [M − 30] with molecular weight 348. Such a splitting does not occur in the case of acids which do not have a hydroxymethyl group at C-2. In this way the presence of both α- and β-GISA in the analysed mixture was verified by the mass fragmentation. GISA were present in trace amounts.

These GC–MS results confirmed the isotachophoretic analysis of the same sample. Differentiation of the two forms (α, β) of GMSA and GISA under the isotachophoretic separation conditions[3] was more obvious. Identification by GC–MS of other reaction products in the mixture was not carried out.

Alkaline redox disproportionation of xylose, lactose, cellobiose and cellulose were also studied. The results obtained were applied in the analysis of a sample of sulphate black liquor. The amounts of saccharinic acids including lactones in this sample are relatively compensated. β-GMSA was not present.

REFERENCES

1 J. W. Green, *Tappi*, 39 (1956) 472.
2 A. N. Ivanov, L. G. Popova, V. N. Pijalkin and Ju. I. Cernousov, *Izv. Vyssh. Uchebn. Zaved., Lesnoj Zurn.*, 3 (1973) 118.
3 S. M. Aronovic, E. P. Crowell, D. M. Whalen and B. B. Burnett, *Tappi*, 54 (1971) 1693.
4 R. Malinen and E. Sjöström, *Paperi ja Puu*, 57 (1975) 728.
5 H. Kolmodin and O. Samuelson, *Sv. Papperstid.*, 73 (1970) 93.
6 L. Löwendahl and O. Samuelson, *Sv. Papperstid.*, 77 (1974) 593.
7 G. Petersson, *Carbohyd. Res.*, 33 (1974) 47.
8 M. MacLeod and L. R. Schroeder, *J. Wood Chem. Technol.*, 2 (1982) 187.
9 C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
10 G. Petersson, *Org. Mass. Spectrom.*, 6 (1972) 577.