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PERIODATE OXIDATION ANALYSIS OF CARBOHYDRATES

XIV*. SIMULTANEOUS DETERMINATION OF THE ANIONS RELATED TO OXIDATION OF CARBOHYDRATES BY ISOTACHOPHORESIS

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SUMMARY

An isotachophoretic method was devised, which allows rapid, simultaneous determination of 5–100 nmole samples of iodate, periodate, and formate ions with coefficients of variation less than 3.5%, and the usefulness of this method for the study of periodate oxidation of carbohydrates was demonstrated. The component aldehydes in the dialdehyde fragments formed on periodate oxidation of oligoglycosides were also determined simultaneously by isotachophoresis of the carboxylates derived thereof by subsequent bromine oxidation and hydrolysis.

INTRODUCTION

In the previous papers of this series, we described a potentiometric[1] and a photometric[2] procedure for the determination of periodate consumed by carbohydrates. We also reported a simple proton magnetic resonance (PMR) method[3] for the determination of formate liberated. Since both the amount of periodate consumed and formate liberated provide important complementary information on carbohydrate structure, it is highly desirable to determine both ions by one procedure.

Under these circumstances, application of isotachophoresis to this end[4] seems promising, although reproducible results under the conditions described could not be obtained. Better conditions for more reproducible isotachophoretic analysis of iodate, periodate, and formate ions have been found, and applied to this study of periodate oxidation of various carbohydrates. Further study of the products of oxidation has led us to devise a method of estimating carboxylates formed by subsequent bromine oxidation and hydrolysis.

EXPERIMENTAL

Materials

The samples of the iodate, glycolate, and glyoxylate ions were their sodium

* Part XIII, see ref. 15.

salts. Those of the formate and lactate ions were their free acids. The sample of the glycerate ion was its calcium salt. Sodium metaperiodate was used as the source of the periodate ion. All samples were obtained from Wako (Osaka, Japan). D-Erythronate was generated by the saponification of D-erythonolactone synthesized according to the literature[5]. All the chemicals used for the electrolyte solutions were of the highest grade commercially available.

Instrument

Isotachophoresis was performed on a Shimadzu IP-1B isotachophoretic analyzer equipped with a Shimadzu PGD-1 potential gradient detector. Potential gradients were recorded directly and also in the differential mode to ensure accurate determination of ions. A PTFE tube (20 cm × 0.5 mm I.D.) was used as the isotachophoresis cell throughout the work.

Periodate oxidation of carbohydrates

Equal volumes of 0.1 *M* sodium metaperiodate and a 0.01 *M* aqueous solution of a carbohydrate sample were mixed, and the mixture was maintained at 25° in the dark. The iodate, periodate, and formate ions in the samples were analyzed at intervals by injecting 2 μl-samples of the reaction mixture into the isotachophoresis cell.

Derivatization of the component aldehydes in dialdehyde fragments to carboxylates

A sample (*ca.* 1 μmole) of an oligoglycoside was dissolved in water (100 μl, methyl β-cellobioside) or methanol (100 μl, rutine), and 0.1 *M* sodium metaperiodate (100 μl), was added to this solution. The mixture was incubated for 3 h at 50°. The reaction mixture was deionized by passing it through a column of Amberlite CG-120 (H^+, 1 ml) and Amberlite CG-400 (CH_3COO^-, 1 ml), and the column was washed with water (20 ml). The combined eluate and the washing fluid were concentrated to a small volume, transferred to a small reaction tube, and evaporated to dryness. The syrupy residue was redissolved in water (100 μl), bromine (1 μl) and calcium carbonate (5 mg) were added to this solution, and the mixture was allowed to stand for 1 h. Then 4 *M* hydrochloric acid (1 ml) was added, and nitrogen was flushed into the reaction tube, which was then sealed and heated for 3 h on a boiling water bath. The hydrolysate was passed through a column of Amberlite CG-120 (H^+, 1 ml), and the column was washed with water (20 ml). The combined eluate and the washing fluid were evaporated to dryness, and redissolved in water (50 μl). A 2-μl sample of the resultant solution was injected into the isotachophoresis cell for analysis of carboxylates.

RESULTS AND DISCUSSION

Simultaneous determination of iodate, periodate, and formate

Among various couples of electrolytes tested, the combination of histidine hydrochloride (leading electrolyte) and *n*-caproic acid (terminating electrolyte) was the most appropriate for the separation of iodate, periodate, and formate ions. The pH values that gave the best separation were 3.0 and 3.4 for the leading and terminating electrolytes, respectively, and the best concentration was 0.01 *M* for both

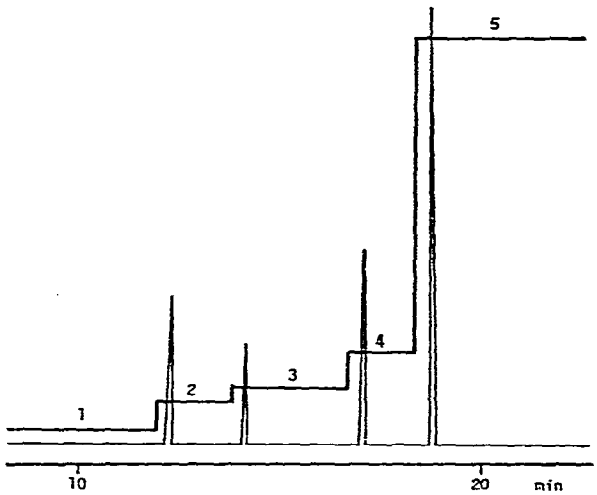

Fig. 1. Isotachopherogram of iodate, periodate, and formate. 1 = Chloride; 2 = periodate (potential unit value, 0.074); 3 = iodate (potential unit value, 0.116); 4 = formate (potential unit value, 0.208); 5 = *n*-caproate. Leading electrolyte: 0.01 *M* hydrochloric acid–histidine (pH 3.0) containing 0.5% polyvinylalcohol. Terminating electrolyte: 0.01 *M* *n*-caproic acid (pH 3.4). Applied current: 100 μA.

electrolytes. The leading electrolyte should contain 0.5% polyvinylalcohol to prevent diffusion of electrophoretic zones, but need not contain organic solvent such as dioxane[4]. Under these conditions these three ions were completely separated from each other (Fig. 1), and the calibration curves for all of these ions were linear in the range of 5–100 nmoles (Fig. 2). The coefficients of variation for iodate, periodate, and formate were 3.5, 2.1, and 1.7, respectively, when six determinations were completed for each ion at the 50-nmole level.

Fig. 3 shows an application of this isotachophoretic analysis to the determination of these ions in the reaction mixture of periodate oxidation of D-glucose in

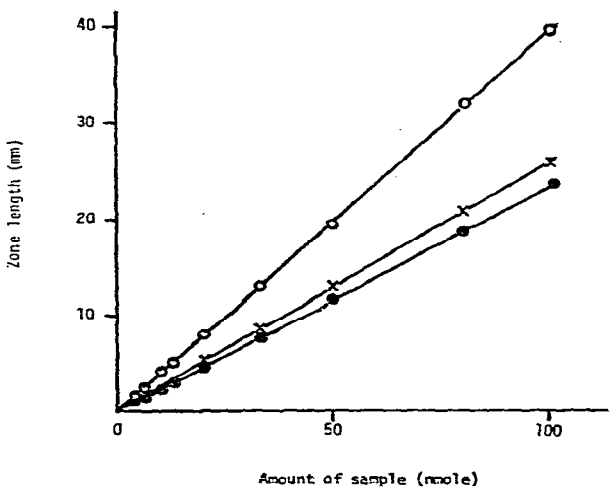

Fig. 2. Calibration curves of iodate, periodate, and formate. ○, iodate; ×, periodate; ●, formate.

water. It is indicated that the amounts of iodate and formate increased rapidly during the initial 30 min, giving values of 3.2 and 2.3 mole/mole of D-glucose, respectively. Thereafter, they continued to increase gradually to reach 4.9 and 4.3, respectively after 24 h. These results point out clearly that D-glucose was oxidized at first in the pyranose form to yield a dialdehyde fragment composed of formic acid and D-glyceraldehyde, then the dialdehyde was hydrolyzed and the resultant D-glyceraldehyde was further oxidized. The final values were consistent with those expected for the open-chain structure. It is noteworthy that periodate consumption could be estimated directly from the amounts of iodate formed, unlike in all other reported methods[1,2,6–9], in which periodate consumption is indirectly calculated from the amounts of periodate remaining after oxidation. It should also be noted that formate could be determined far more rapidly and simply than by any other methods[3,10]. The amounts of the remaining periodate in the present experiment exactly corresponded to those of iodate formed. The foregoing results demonstrate that this isotachophoretic method provides manifold information on carbohydrate structure by one determination.

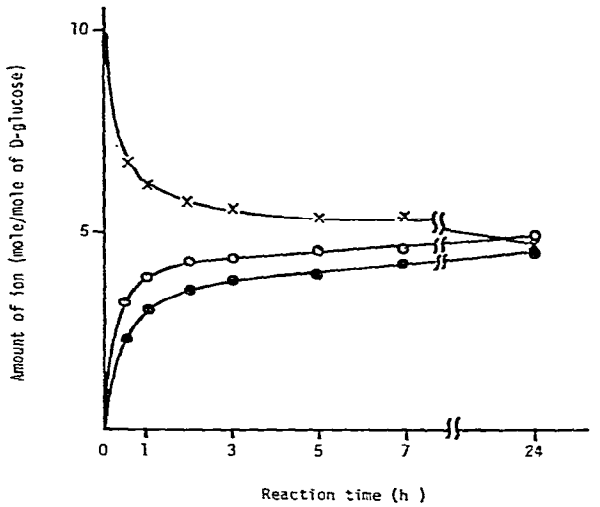

Fig. 3. Course of periodate oxidation of D-glucose. ○, iodate; ×, periodate; ●, formate.

Table I summarizes the data obtained for the oxidation of other selected carbohydrates. The alditols (D-xylitol and D-sorbitol) were oxidized rapidly to give approximately quantitative yields to iodate and formate. 2-Acetamido-2-deoxy-D-glucose gave approximately 3 and 2 moles of iodate and formate, respectively, after oxidation for 5 h, indicative of rapid cleavage of the C-3–C-4 bond, followed by hydrolysis of the resultant dialdehyde fragment to give D-glyceraldehyde and 2-acetamido-2-deoxymalonaldehyde. Further oxidation of the former component will give the observed values of iodate and formate. The latter component was presumably oxidized slower to yield additional 2 moles of iodate and formate after 24 h. The mode of oxidation of methyl α-D-glucopyranoside was simple, because the reducing end is blocked by the methyl group in this case. Only the C-2–C-3 and C-3–C-4 bonds were oxidized, and the amounts of the products were approximately theoretical

TABLE I

DETERMINATION OF PERIODATE CONSUMPTION AND FORMATE LIBERATION FOR SELECTED CARBOHYDRATES

Carbohydrate	*Periodate consumption* (mole/mole of carbohydrate)*				*Formate liberation (mole/mole of carbohydrate)*			
	Theo-retical	*Found*			*Theo-retical*	*Found*		
		3 h	*5 h*	*24 h*		*3 h*	*5 h*	*24 h*
D-Xylitol	4	4.15	4.25	4.25	3	3.05	3.15	3.33
D-Sorbitol	5	4.78	4.79	4.80	4	3.90	3.88	3.90
2-Acetamido-2-deoxy-D-glucose	5	2.53	2.95	4.90	4	1.90	2.00	4.00
Methyl α-D-glucopyranoside	2	1.95	1.95	2.05	1	0.88	0.98	1.18
Cellobiose	4	3.67	4.10	4.75	2	1.90	1.90	2.49

* Estimated from the amounts of iodate formed.

after 5 h. The oxidation of cellobiose for prolonged reaction time seems to involve overoxidation due to hydrolysis of the dialdehyde fragment. The hydrolysis of the formyl ester at the reducing D-glucose residue will reveal the erythrose moiety substituted at C-2, which is oxidizable at the C-3–C-4 bond to yield additional iodate. All the observations for the alditols[1–3], D-glucose[1–3], methyl α-D-glucopyranoside[1–3,11,12], and cellobiose[11] were consistent with those reported previously.

Simultaneous determination of carboxylates

The conditions used for the analysis of iodate, periodate, and formate also allowed separation of glyoxylate, glycerate, and lactate, which are typical carboxylates derivable from dialdehyde fragments formed on periodate oxidation of car-

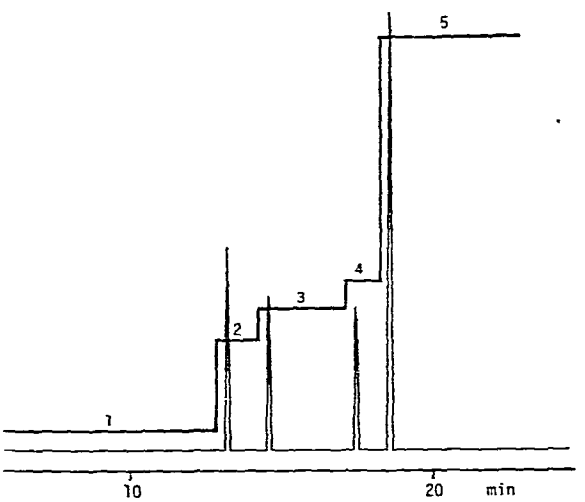

Fig. 4. Isotachopherogram of glyoxylate, glycerate, and lactate. 1 = Chloride; 2 = glyoxylate (potential unit value, 0.236); 3 = glycerate (potential unit value, 0.315); 4 = lactate (potential unit value, 0.379); 5 = *n*-caproate. Leading electrolyte: 0.01 *M* hydrochloric acid–histidine (pH 3.0) containing 0.5% polyvinylalcohol. Terminating electrolyte: 0.01 *M* *n*-caproic acid (pH 3.4). Applied current: 100 μA.

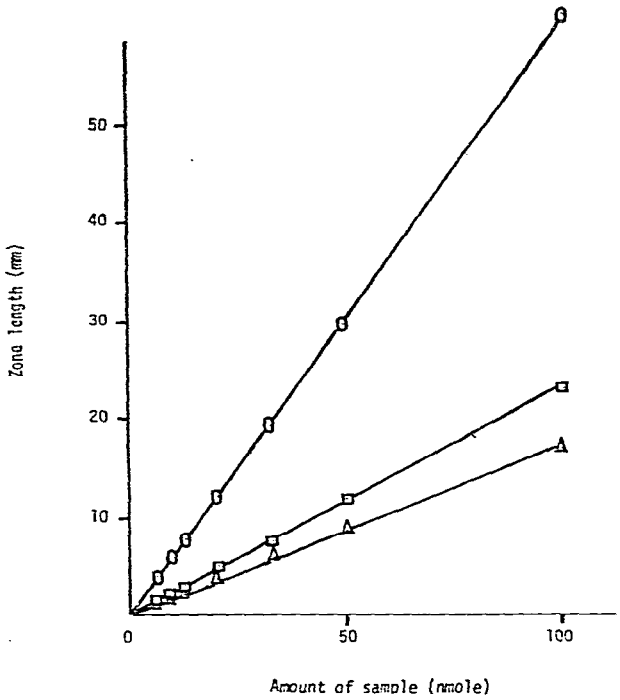

Fig. 5. Calibration curves of glyoxylate, glycerate, and lactate. □, glyoxylate; ○, glycerate; △, lactate.

bohydrates by further oxidation and hydrolysis (Fig. 4). The calibration curves of all of these carbohydrates were again linear in the same sample range (Fig. 5). The coefficients of variation for glyoxylate, glycerate, and lactate were 0.4, 1.5, and 2.4, respectively, at the 50-nmole level ($n = 6$ for all these ions).

On the basis of these results we investigated the procedure to derivatize the component aldehydes in dialdehyde fragments to carboxylates using various reagents. Since the removal of the excess reagents is essential, oxidation and hydrolysis were performed best with bromine and hydrochloric acid, respectively. Table II gives a few examples of its application. The molar proportions of glyoxylate, glycerate, and lactate, formed from the product of periodate oxidation of rutine approximately

TABLE II

MOLAR PROPORTIONS OF THE CARBOXYLATES DERIVED FROM OLIGOGLYCOSIDES BY SEQUENTIAL REACTIONS OF PERIODATE OXIDATION, BROMINE OXIDATION AND HYDROLYSIS

Oligoglycoside	*Carboxylate*	*Molar proportion*
Rutine	Glyoxylate	1.8
	Glycerate	1
	Lactate	1.1
Methyl β-cellobioside	Glyoxylate	2.1
	Glycerate	1
	Erythronate	detected

agreed with the theoretical values. The product from methyl β-cellobioside gave approximately equimolar amounts of glyoxylate and glycerate. It also gave erythronate, though its amount was unknown because the pure authentic specimen was unavailable. The above results conformed well with those obtained by the gas chromatographic analysis of the dithioacetal derivatives formed from the component aldehydes[11,13,14].

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