Simple Gas Chromatographic Identification of Monosaccharides Using a Curie-Point Type Pyrolyzer

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Simple and rapid identification of monosaccharides by gas chromatography was achieved by the application of Curie-point pyrolysis. Almost all monosaccharides were distinguishable under pyrolysis conditions of 358 °C for 3 s. Each sugar group, *e.g.* aldohexoses, aldopentoses, deoxysugars, uronic acids, sugar alcohols, and aminosugars, showed a characteristic pyrogram.

Keywords carbohydrate; Curie-point pyrolysis; monosaccharide; identification; gas chromatography; GC-MS; pyrolysis gas chromatography; pyrogram

Introduction

Gas chromatography (GC) is widely used in laboratories, but it has not been possible to analyze saccharides by GC without tedious chemical derivatization because of their low volatility. We therefore attempted to develop a facile GC identifcation of saccharides. We thought that pyrolysis of saccharides would give volatile molecules which would reflect the structure of the saccharides, and identification of saccharides might be possible on the basis of their GC pattern.

Some studies on analytical pyrolysis of saccharides have been reported, but most of them dealt with polysaccharides. In some cases, analytical pyrolysis of many kinds of monosaccharides was done under the same conditions for comparison, but these analytical pyrolysis data[1-4] are not adequate for our purpose.

In our study, Curie-point pyrolysis was adopted as the pyrolysis method because the pyrolysis conditions are precisely maintained in this method. We investigated the possibility of identification of monosaccharides by GC with Curie-point pyrolysis.

Materials and Methods

A JHP-2 Curie point pyrolyzer (Japan Analytical Industry) was used. GC was performed by using a Shimadzu GC-6AM with a 20% PEG1000 column (3 mm × 3 m). The column temperature was raised from 50 °C to 140 °C at the rate of five degree a minute. GC-Mass (MS) analysis was done with a Shimadzu-LKB 9000B at 70 eV ionization energy.

D-Glucose, D-mannose, D-fructose, D-xylose, D-glucuronic acid, *N*-acetyl-D-glucosamine, L-rhamnose (hydrate) and methyl-α-D-glucoside were purchased from Wako Pure Chemical Industries. D-Galactose, D-galacturonic acid (hydrate), 2-deoxy-D-glucose, 2-deoxy-D-ribose, D-arabinose, *N*-acetyl-D-galactosamine, *N*-acetyl-D-mannosamine (hydrate), D-sorbitol, D-mannitol, xylitol and methyl-β-D-glucoside were purchased from Nakarai Chemicals. D-Ribose used was produced by Kojin.

Two milligrams of each sugar sample was tightly folded in ferromagnetic alloy foil (pyrofoil), and placed in a quartz tube. The tube was preheated at 150 °C for 3 min, then the pyrofoil was irradiated at high frequency. It absorbed the energy, and the temperature was raised to the Curie-point. The ferromagneticity was lost at the temperature determined by the alloy composition. The temperature was maintained precisely during the irradiation period (3 s as the standard condition). The volatile pyrolysis products were carried immediately to the GC column by an N_2 gas flow (GC, 40 ml/min) or an He gas flow (GC-MS, 30 ml/min) through a heated pipe directly connected to the GC column.

Results and Discussion

First, the temperature dependence of the pyrolysis pattern was investigated to determine the optimal pyrolysis temperatures. Glucose, galactose, mannose and fructose were pyrolyzed for 3 s at various temperatures. The total amount of glucose pyrolysis products was very small at 235 °C. It increased with increasing pyrolysis temperature, but at 445 °C or above, some characteristic pyrolysis products (*e.g.* methanol from galactose) of each saccharide decreased, and the pyrograms of different saccharides became rather similar to each other (Fig. 1). It was thus thought that 358 °C was the optimal pyrolysis temperature for the identification of the monosaccharides.

Next, the identification of the pyrolysis products of 20 monosaccharides, *i.e.* glucose, galactose, mannose, xylose, ribose, arabinose, 2-deoxyglucose, 2-deoxyribose, rhamnose, glucuronic acid, galacturonic acid, fructose, sorbitol, mannitol, xylitol, *N*-acetylglucosamine, *N*-acetylgalactosamine, *N*-acetylmannosamine, α-methyl glucoside and β-methyl glucoside, at 358 °C was done by GC-MS. Almost all pyrolysis products were identified by comparing their MS spectra with those of authentic samples. For example, the pyrolysis products of glucose (Fig. 2) were identified as acetaldehyde, furan, acetone, acrolein, 2-methylfuran, di-

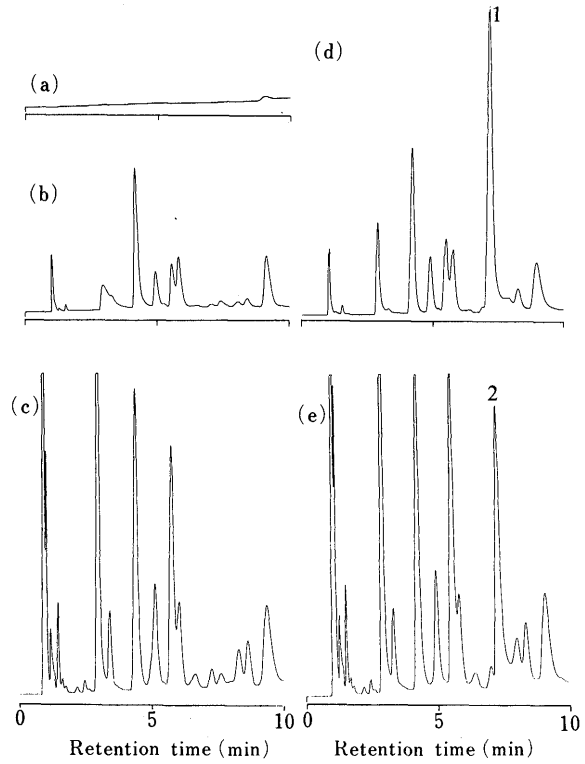

Fig. 1. Pyrolysis at Lower or Higher Temperature (Retention Time < 10 min)

a) Glucose (235°C, 3 s). b) Glucose (358°C, 3 s). c) Glucose (590°C, 3 s). d) Galactose (358°C, 3 s). e) Galactose (590°C, 3 s). 1) MeOH. 2) MeOH.

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acetyl, 2,3-pentadione, 3-methylfuran, acetol, glycol aldehyde and furfural. Relative standard deviations of the pyrolysis products were 10.5%, 6.2%, 7.5%, 7.2%, 3.9%, 7.2%, 9.3%, 5.7%, 4.2%, 10.1% and 5.0%, respectively, in five pyrolysis experiments with glucose (358 °C, 3 s).

Table I shows the number and amount of pyrolysis products (358 °C, 3 s) from the 20 monosaccharides. The pyrolysis products patterns of 8 sugar groups (aldohexoses, aldopentoses, deoxysugars, uronic acids, ketoses, sugar alcohols, aminosugars, glycosides) significantly differed. Thus, it was very easy to distinguish monosaccharides from different sugar groups. Some saccharides that belonged to the same sugar group could be distinguished by using particular pyrolysis products as follows.

The pyrograms of aldohexoses were similar to that of glucose, but methanol from galactose and ethanol from mannose were good indicators to distinguish them from glucose.

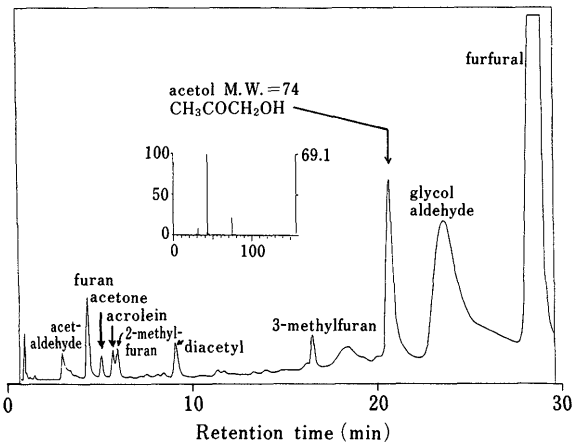

Fig. 2. Pyrogram of Glucose (358°C, 3 s)

Pyrograms of aldopentoses lacked the peak of 3-methylfuran, and had a smaller peak of furan or diacetyl than those of aldohexoses. In this group, ribose was distinguishable from xylose and arabinose as the peak of acetone, 2-methylfuran or methanol from ribose was larger than that from xylose or arabinose.

Deoxysugars gave large amounts of acetaldehyde. The amount of furfural was much less than those from other sugar groups except sugar alcohols. 2-Deoxysugars gave large amounts of furan, and small amounts of acetol and glycol aldehyde. On the other hand, 6-deoxysugar gave large amounts of acetol.

Uronic acids gave large amounts of furan and acetone, but small amounts of acrolein, acetol, and glycol aldehyde. Galacturonic acid did not give methanol, so was difficult to distinguish from glucuronic acid.

Sugar alcohols gave some unique pyrolysis products such as methyl ethyl ketone, 3-hydroxy-2-butanone and 1-hydroxy-2-butanone. They gave large amounts of acetaldehyde and 2,3-pentanedione but little furfural. Hexose alcohols, such as sorbitol or mannitol gave very similar pyrograms.

N-Acetyl aminosugars gave acetonitrile and 2-methyloxazole as indicator products. The amount of acetol was less than that in the case of aldohexoses. *N*-Acetylgalactosamine was distinguished from the characteristic peaks of acrolein, methanol and ethanol.

A large proportion of the pyrolysis products of glycosides was alcohol derived from the aglycone. Very small amounts of pyrolysis products were derived from the sugar residue. Thus, it was thought that the aldehyde or hemiacetal group played an important role during the pyrolysis reaction.

TABLE I. Pyrolysis Patterns of Monosaccharides (Relative Percentage)

		Acetaldehyde	Furan	Acetone	Acrolein	2-Methylfuran	2-Butanone	Methanol	Ethanol	Diacetyl	Acetonitrile	2-Methyloxazole	2,3-pentadione	3-Methylfuran	3-Hydroxy-2-butanone	Acetol	Glycol aldehyde	1-Hydroxy-2-butanone	Furfural
Aldohexose	Glucose	0.6	1.4	0.4	0.5	0.5				0.9			0.2	1.2		8.8	24.4		56.4
	Galactose	0.6	1.4	0.5	0.7	0.6		5.6		0.9			0.2	1.4		4.9	31.9		47.2
	Mannose	0.9	2.2	0.5	0.6	0.8			0.6	1.1			0.2	0.8		7.2	18.4		63.4
Aldopentose	Xylose	0.5	0.7	0.2	0.2	0.2		0.2		0.4			0.2			4.0	28.6		61.7
	Ribose	0.7	1.0	0.4	0.2	0.4		1.0		0.7						5.4	12.4		71.7
	Arabinose	0.6	0.7	0.2	0.2	0.2				0.5			0.2			3.9	24.3		66.6
Deoxysugar	2-Deoxyglucose	48.5	39.8		0.6			0.2	0.2	0.6						1.5	5.1		1.9
	2-Deoxyribose	74.0	15.3		0.5					0.5			0.2			1.7	2.0		0.7
	Rhamnose	19.4	0.2			0.2			0.5	0.7						59.9	15.2		2.2
Uronic acid	Glucuronic acid	0.9	4.2	6.3	0.3	1.1				0.7			0.3	0.6		1.9	6.4		62.7
	Galacturonic acid	0.9	3.2	8.6	0.2	0.6				0.6			0.5	0.5		1.0	3.1		69.8
Ketose	Fructose	0.6	0.7	0.4	0.5	0.6		0.3		0.5				0.8		6.4	17.4		65.0
Sugar alcohol	Sorbitol	8.1	3.3	2.0	0.4	0.4	1.0	1.9		3.0			1.4		1.4	32.9	15.7	9.7	3.0
	Mannitol	8.3	3.5	2.3	0.5	0.5	1.1	1.9		3.3			1.5		1.5	32.9	17.0	7.6	2.1
	Xylitol	7.1	3.5	1.8	0.4	0.4	0.9	2.1		2.3			2.6		1.3	25.9	16.4	11.6	8.1
Amino sugar	*N*-Ac Glucosamine	0.5	0.4	0.2						0.4	0.8	0.3				1.0	46.4		44.5
	N-Ac Galactosamine	1.2	0.7	0.7	0.3			2.9	8.6	0.7	2.3	0.7				2.1	24.1		50.2
	N-Ac Mannosamine	0.5	0.4	0.2					0.9	0.4	0.6	0.3				1.3	41.8		46.0
Glycoside	α-Methylglucoside			0.2				96.1								1.2			0.2
	β-Methylglucoside		0.2	0.7				88.5		0.3				0.4		1.4	0.4		0.5

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In conclusion, identification of monosaccharides by GC was possible by using Curie-point pyrolysis. The method is called pyrolysis gas chromatography (PGC). The PGC method does not require the tedious chemical pretreatment essential for ordinary identification of saccharides with GC, and is faster than paper chromatography. Thin layer chromatography (TLC) is a facile method to identify saccharides, but the reproducibility of *Rf* value of each saccharide is not so good. The PGC method is superior to the TLC method in this regard. High performance liquid chromatography (HPLC) is a good method for identification of saccharides, but it needs a special, expensive column to separate saccharides. In contrast, the PGC method uses a general, inexpensive column. Application of the PGC method to disaccharides may clarify the relationship between the pyrogram and constituent monosaccharides or linkage mode.

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