Reactions of Oligosaccharides. V. Pyrolysis-Gas Chromatography

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ABSTRACT

Pyrolysis-gas chromatography (pyrolysis-GC) distinguished between model carbohydrate compounds, a series of malto-oligosaccharides previously isolated in our laboratories from corn syrup. These sugars, namely maltose (G_2), maltotriose (G_3), maltotetraose (G_4), maltopentaose (G_5), and maltohexaose (G_6), were subjected to pyrolysis-GC under carefully controlled conditions; major volatile compounds were identified by mass spectrometry. The oligosaccharides gave similar decomposition products. However, when the pyrograms were normalized, the relative amounts of individual volatile components varied from one sugar to the next. This proved to be true also for other disaccharides and trisaccharides examined by this technique. Differences in the normalized patterns permitted differentiation between carbohydrates of varying molecular structure. Major volatile compounds identified were ethylene, ethane, propylene, acetaldehyde, furan, propionaldehyde, acetone, 2-methylfuran, and methyl ethyl ketone.

Pyrolysis products of sugars have been examined for many years, but only recently have advances in instrumental techniques permitted precise determination of identities and amounts of volatile degradation products. The new tools permit identification of sugar derivatives by examination of their pyrolytic decomposition products.

A further advance is reported here: identification of purified but underivatized individual oligosaccharides by a combination of pyrolysis and gas chromatography (GC), with assistance from mass spectrometry (MS).

Much of the early work on carbohydrate pyrolysis was focused on sucrose, which produces a highly volatile and odorous portion on heating to caramelization temperatures (Pucherna 1930, 1931; Trillat 1907).

Bryce and Greenwood (1963a,1963b,1963c) applied newly introduced GC techniques to the study of the pyrolysis of several sugars and starches. On the basis of yields of pyrolytic residues, they speculated that the α-1:6 bond may be more heat-stable than the α-1:4 bond.

Heyns and Klier (1968) showed that all carbohydrates tested (erythrose, xylose, arabinose, sorbose, lactose, cellobiose, raffinose, amylose, and amylopectin) give the same pyrolytic degradation products.

Myers and Smith (1972) examined the pyrolysis products from glucose, mannose, fructose, xylose, starch, and cellulose (among other materials of biological origin). They found that, in general, the same volatile products were produced from each (as evidenced by peak retention times) but in somewhat different amounts (as evidenced by peak heights). They felt that the carbohydrates examined were distinguishable by the simple means employed but that their results were not very reproducible.

Shafizadeh et al (1978, 1979) and Shafizadeh and Lai (1973a,1973b) extensively investigated the pyrolysis of cellulose, starch, sugars, and model compounds under varying thermal conditions. They used thermal gravimetric analysis coupled with gas liquid chromatography and thin-layer chromatography to follow the pathways of the formation of decomposition products.

These investigators were not especially directing their efforts toward the goal of identifying unknown starting compounds by examining the relationships of the pyrolyzate constituents to the starting materials.

Against this background, we decided to apply the pyrolysis-GC method, with assistance from MS, to a series of malto-oligosaccharides in an effort to determine whether this method would permit detection of distinctive degradative patterns for the various sugars.

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MATERIALS AND METHODS

Carbohydrates

The malto-oligosaccharides G_2, G_3, G_4, G_5, and G_6 were previously isolated in these laboratories and shown to be homogeneous, in accordance with Donnelly et al (1973). D-Glucose, cellobiose, gentiobiose, trehalose, and panose were purchased from Pfanstiehl Co. Isomaltotriose was a gift from the Hayashibara Co., Japan. Both thin-layer and gas chromatography showed these commercial samples to be not less than 95% pure.

GC Operating Conditions

A Perkin-Elmer pyrolysis accessory unit was coupled to a Hewlett-Packard 7620 A gas chromatograph. An 18-ft × 1/8-in stainless steel column containing 80–100 mesh Porapak Q was used to separate the pyrolysis volatile components. The GC conditions were: sensitivity, 16×10^2; detector, 290°C; injector, 290°C; column, 50°C/6 min; program, 20°C/min to 150°C and hold 20 min; program, 20°C/min to 170°C and hold 30 min. The gas flow rates were H_2, 30 ml/min; air, 300 ml/min; He, 22 ml/min. Integrator (Hewlett-Packard model/3370B) settings were: noise suppression, 2; recorder presentation, 1; slope sensitivity, 0.1/0.3; base line reset delay, 0.2; area threshold, 1,000; shoulder front, on; rear mV, 1,000; chart speed, 0.25 in./min.

Pyrolysis Conditions

A sugar sample (3.6 mg ± 0.1 mg), in a porcelain boat, was placed in the side arm of the pyrolysis unit. The oven section of this unit was heated to 600°C over a period of 25 min, after which carrier gas (He) was swept through the pyrolysis unit for 5 min. The sample was placed in the oven, and after exactly 1 min the carrier gas was switched to bypass the pyrolysis unit. At the same time, the temperature programmer, integrator, and chart recorder were switched on.

Pyrolysis-GC-MS

The pyrolysis unit was attached to a Varian MAT 111 GC-mass spectrometer. The pyrolysis-GC operating conditions were the same as described except for the following: sample weight, 11 mg; detector temperature, 250°C; inlet temperature to the mass spectrometer, 265°C. The MS operating conditions were: eV, 75; range, 0.1–1.0; gain, 2; M/sec, 50; ΔM (display mode), 100; vacuum 5×10^{-5} torr; scan mode, cyclic lin; Oscilloport chart speed, 0.1 × 100 cm/sec.

RESULTS AND DISCUSSION

The pyrograms of maltose (G_2) and maltohexose (G_6), which cover a 60-min time span on the chart, have peaks in the same locations as those in the pyrograms of dextrose (G_1), G_3, G_4, and G_5. The only major difference, in terms of visible peaks, is the appearance of an extra peak with retention time (R_t) of approximately 53 min in G_6. This peak appears just before the

TABLE I
Normalized Data for Sugar Pyrograms—Peak Areas as Percentages of Largest Peak

Sugar	Volatile Components								
	Ethylene	Ethane	Propylene	Acetaldehyde	Furan	Propionaldehyde	Acetone	2-Methylfuran	Methyl Ethyl Ketone
Glucose	100	95	36	44	16	33	19	6	13
Maltose	95	100	42	45	12	38	13	4	16
Maltotriose	100	96	41	44	10	30	16	2	14
Maltotetraose	100	85	29	24	6	19	13	3	12
Maltopentaose	100	93	35	32	8	25	18	4	14
Maltohexaose	100	92	42	38	12	36	21	8	20
Cellobiose (β-1:4)	91	100	46	64	19	50	20	12	19
Gentiobiose (β-1:6)	100	80	36	52	15	38	20	9	21
Trehalose (α-1:1)	100	94	46	73	17	50	16	9	19
Panose (α-1:6, α-1:4)	100	100	50	76	17	50	17	11	20
Isomaltotriose (α-1:6,α-1:6)	100	86	36	49	10	31	20	14	24

methyl ethyl ketone peak. The major volatile components resulting from the pyrolysis of these sugars were identified by MS analysis. The minor components were not identified. The homogeneity of the major peaks was determined by comparing the mass spectra of the upslope, top, and downslope of each peak. An exception was the propionaldehyde peak. This peak has a shoulder on its downslope that was not identified because of insufficient data. The R_ts of acetaldehyde, furan, propionaldehyde, acetone, 2-methylfuran, and methyl ethyl ketone were identical with those of authentic samples. Identification of ethylene, ethane, and propylene was based solely on their mass spectra.

The appearance of such compounds as acetaldehyde, acetone, furan, and 2-methylfuran agrees with published data on the pyrolysis of polysaccharides (Horton 1965). Our study shows that, through strict adherence to carefully controlled operating conditions, pyrograms can be produced that are in effect, "fingerprints" of individual carbohydrates. Although the pyrograms show the same sequence of peaks for G_1–G_6 (except for the extra peak in G_6), the "fingerprint" appears when the pyrograms of the individual sugars are normalized. The peak areas in each pyrogram are replotted as a percentage of the "base peak", that is, the peak with the largest area. Table I summarizes these data. These figures clearly indicate that each sugar has its unique pattern. The numbers given in Table II are the averages of five replicate analyses for each sugar. The standard deviation for most peaks is less than 6%, with the majority less than 3%. The exceptions are shown in Table II. Even these exceptions do not affect the overall uniqueness of the pyrogram as a "fingerprint" for the individual sugars.

The same technique can be used to distinguish structural differences between other disaccharides and trisaccharides. Table I shows the data obtained from the normalized pyrograms of gentiobiose, cellobiose, trehalose, and maltose. As can be seen, the major differences between all four sugars are in the relative amounts of ethylene and ethane produced when they are pyrolyzed. With gentiobiose and trehalose, the "base peak" is ethylene, whereas with cellobiose and maltose (G_2), the "base peak" is ethane. Differences also exist in the amounts of acetaldehyde and propionaldehyde produced from these sugars. The characteristic pyrograms for these disaccharides must be related to the differences in the carbohydrate linkages.

These linkage differences must also account for the unique pyrograms seen for the trisaccharides isomaltotriose and panose. Comparison of the pyrograms for the three trisaccharides shows that the relative peak areas of ethylene and ethane are the same in panose, but different in G_3 and isomaltotriose. The propylene, acetaldehyde, and propionaldehyde components also differ in relative peak areas among the three trisaccharides. Pyrolysis at 600°C and subsequent sampling of the products present at that temperature do not allow for characterization of the many intermediate compounds formed as thermal excitation increases.

Shafizadeh et al (1978) point out the complexity of carbohydrate pyrolysis in the statement

> intramolecular transglycosylation of polysaccharides is responsible for their depolymerization to monomeric compounds which can themselves undergo further transglycosylation, dehydration, disproportionation and polymerization reactions.

TABLE II
Standard Deviations (%) in Measurements of Volatile Components in Pyrograms of Oligosaccharides

Oligosaccharide	Volatile Components				
	Ethane	Propylene	Acetal-dehyde	Propion-aldehyde	Methyl Ethyl Ketone
G_1	3	2	5	4	1
G_2	...	4	6	6	2
G_3	9	7	13	14	5
G_4	2	4	2	3	1
G_5	4	3	3	3	2
G_6	6	12	11	14	8

When the pyrolysis conditions are carefully controlled in replicate analyses, pyrograms that represent "fingerprints" for the carbohydrates under examination can be produced. In this paper, such a procedure has been outlined for a number of carbohydrates that differ in chain length (the malto-oligosaccharides G_2–G_6) and linkages (the disaccharides and trisaccharides). This technique provides an analytical tool to characterize simple carbohydrates but could undoubtedly be applied also to other more complex carbohydrates such as starch and its derivatives.

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