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Note

Separation of malto-oligosaccharide derivatives by capillary supercritical fluid chromatography and supercritical fluid chromatography–mass spectrometry

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Pressure-programmed, capillary supercritical fluid chromatography (SFC) can be used to separate silylated malto-oligosaccharides of degree of polymerization (d.p.) 1 to beyond 20 in a single analysis. Peaks are observed as pairs, presumably through resolution of the anomers at each molecular weight. However, the retention between each pair varies, especially below d.p. 6. SFC–mass spectrometry was used to verify that both members of any pair below d.p. 7 have the same nominal mass. Analysis of maltoheptaose revealed the same two peaks thought to be the d.p. ~7 anomers in Maltrin M-100.

INTRODUCTION

Oligo- and poly-saccharides can be separated by a variety of chromatographic techniques, including various implementations of high-performance liquid chromatography (h.p.l.c.). Kainuma *et al.*[1], separated malto-oligosaccharides of d.p. up to 15 using a bonded-phase carbohydrate column. Cheetam and Teng used reversed-phase columns with the addition of surfactants to the mobile phase to separate malto-oligosaccharides; analysis times were shortened compared to unmodified mobile phases[2]. White *et al.*[3], separated similar oligosaccharides having d.p. up to 20 using a silica stationary phase dynamically modified with amines. Koizumi *et al.*[4], used a bonded-amine stationary phase to separated polysaccharide hydrolyzates to d.p. ~30.

In all of these examples, a refractive-index detector (RID) was used. Because the RID effectively prohibits the use of gradient elution, a compromise is necessary between resolution of the early-eluting peaks and the d.p. range that can be covered within an arbitrary maximum-analysis time. Gradient elution eliminates

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this inconvenience and is possible with a pulsed amperometric detector[5–7]. The possibility of using mobile-phase gradients also makes high-performance ion-exchange practical[7] for a d.p. range from 1 to ~45.

Despite this success, the field of carbohydrates is so wide that additional separation and detection methods are still sought. Capillary gas chromatography (g.l.c.) has the potential to achieve very high separation efficiency. Traitler *et al.*[8] successfully used g.l.c. to elute and separate silyl derivatives of low-molecular-weight oligosaccharides with outstanding peak resolution and much sample information, but only through d.p. ~6. Additional range is possible with the use of higher-temperature columns[9,10] and more-volatile derivatives. For example, Karlsson and Hansson[11] used high-temperature g.l.c. to separate permethylated isomalto-oligosaccharides up to d.p. 10. However, extending the upper temperature of the program beyond 400° will only add a few more members of the series to the chromatogram, and then only if the late-eluting peaks survive the high-temperature separation.

Capillary supercritical fluid chromatography (SFC) is a relatively new technique[12,13]. Commonly, CO_2 is used as the mobile phase above its critical temperature (31°). Thus, the application of high pressure does not cause condensation and formation of a liquid mobile phase. When highly compressed, the mobile phase nevertheless begins to act like a liquid in its ability to dissolve and solvate materials. The effective solvent strength depends on the density of the mobile phase at a particular temperature. By adjustment of the pressure, temperature, or both, the strength of the mobile phase can be set or programmed. Columns are similar to bonded-phase g.l.c. columns, but usually have a smaller inside diameter (50–100 μm) and a relatively thicker stationary-phase film in relation to the inside diameter. Films of 0.05–0.2 μm are typical.

SFC is capable of eluting much larger and less-volatile molecules than g.l.c. while using much lower column temperatures. In addition, the CO_2 mobile phase is compatible with many g.l.c. detectors, particularly the flame-ionization detector (FID). Thus, SFC is capable of eluting many organic solutes with a low-temperature mobile phase of programmable strength, and of providing universal detection for organic compounds. In addition, capillary SFC may be interfaced with conventional, differentially pumped mass spectrometers with little or no modification of the mass spectrometer. This combination of features makes SFC a valuable analytical tool capable of solving many problems beyond the current limits of g.l.c. and h.p.l.c.[14].

Most carbohydrates do not have sufficient solubility in the CO_2 mobile phase for successful separation and detection by SFC. However, trimethylsilyl derivatives of malto-oligosaccharides are readily formed by reaction with *N*-trimethylsilylimidazole and *N,O*-bis(trimethylsilyl)trifluoroacetamide, and are separated by pressure-programmed, capillary SFC using CO_2 as the mobile phase with a methylsilicone-coated column[15]. A typical chromatogram is shown in Fig. 1. The mass range is sufficient to elute malto-oligosaccharides with d.p. in the low to mid 20's,

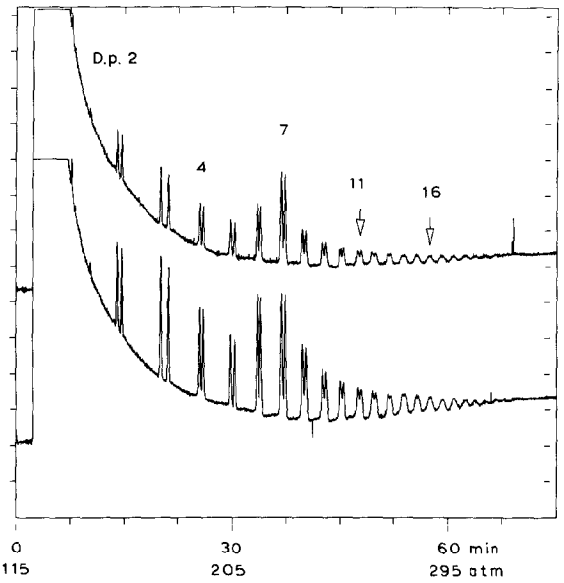

Fig. 1. SFC-FID chromatograms of trimethylsilylated Maltrin M-100, with (upper) and without (lower) mixing of trimethylsilylated maltoheptaose. Linear pressure programming was used, starting at 115 atm. and increasing at 3 atm./min. The column-oven temperature was 100°.

depending on the conditions chosen[16]. Pressure programming still allows good resolution at the low-molecular-weight end of the range. Resolution typically worsens with retention in SFC, because the mobile-phase velocity increases with pressure while solute diffusion-coefficients decrease with increasing molecular weight and increasing mobile-phase pressure. All of these phenomena lower the column efficiency through the course of the program.

We earlier assumed that the peak pairs evident in Fig. 1 are attributable to the two anomers possible for malto-oligosaccharides at each d.p. value[15]. However, the spacing of the peak pairs in Fig. 1 is not regular: the peaks of d.p. 3 and 5 are spaced wider than those of d.p. 2, 4, and 6. In this work we report on-line mass-spectrometric detection data for these peaks and the separation of the anomers in maltoheptaose.

EXPERIMENTAL

Materials and methods. — SFC-grade CO_2 (Scott Specialty Gases, Plumsteadville, PA, U.S.A.) was used without additional purification as the mobile phase.

When an FID was desired, separations were performed on a model 501 supercritical fluid chromatograph (Lee Scientific, Salt lake City, UT, U.S.A.) equipped with a flow-splitting injector and an FID. A 2-m long DB-1 column with a 50-μm inside diameter and a 0.20-μm stationary phase film thickness (J & W Scientific, Rancho Cordova, CA, U.S.A.) was used in this instrument.

The supercritical fluid chromatograph used with mass spectrometric detection was assembled following the work of Peadon *et al.*[17] and is similar to instruments described in previous papers[18,19]. However, in the present work, 0.06-μL samples were injected directly (without splitting) into a 50-cm inlet tube of untreated 50-μm (i.d.) fused silica. This was connected to a 10-m DB-1 column with a 50-μm inside diameter and a 0.20-μm stationary-phase film thickness (J & W Scientific) using a ZU.5 union (Valco, Houston, TX, U.S.A.).

The mass spectrometer interface used is based on a robot-pulled, tapered, fused-silica capillary restrictor[19] and is described in detail elsewhere[20,21]. It provides separate and adjustable heating of the restrictor stem and tip. The interface was inserted through the direct probe inlet of a Vacuum Generators model 30-253 quadrupole mass spectrometer of 3000-Da mass range. Mass resolution was decreased slightly by increasing the "high mass" peak-width tuning parameter from 1.02/Da to 1.20/Da in order to increase the ion transmission efficiency and the signal-to-noise ratio. Isotope multiplets merged above 1000 Da, and centroid mass-to-charge ratios were obtained. Ammonia chemical ionization was used.

The samples were Maltrin M-100 (Grain Processing Corp., Muscatine, IA, U.S.A.), and maltoheptaose (Sigma Chemical Co., St. Louis, MO, U.S.A.). For SFC-m.s. analysis, a 10-mg sample of Maltrin M-100 was silylated with 0.2 mL of a 5:1 mixture of *N*-trimethylsilylimidazole and *N,O*-bis(trimethylsilyl)trifluoroacetamide, 0.1 mL of pyridine, and 0.1 mL of dimethyl sulfoxide for 1 h at 80°. After cooling to room temperature, 0.6 mL of dry CH_2Cl_2 was added. For SFC analysis, a similar procedure involving 10 mg of Maltrin M-100 or 2 mg of maltoheptaose was used, with dilution to a final volume of 10 mL with dry CH_2Cl_2.

RESULTS AND DISCUSSION

Mass-spectrometric detection of the second through seventh peak pairs showed that both members of a given pair have the same nominal molecular weight, within the limits of the instrument's resolving power. This supports the proposition that the pairs represent anomers and that the unusual spacing of retention times is not due to species of differing mass. However, it does not rule out the possibility that one of the peaks in each pair represents a branched saccharide. Fig. 1 shows SFC-FID chromatograms of silylated Maltrin M-100 with and without the addition of silylated maltoheptaose. The addition of maltoheptaose (upper chromatogram) diluted all but the DP-7 peaks. There was no measurable difference in the widths of the DP-7 peaks before and after spiking. Analysis of the silylated maltoheptaose alone produced only the two expected peaks. An enlarged portion of that chromatogram is shown in Fig. 2. No indication of sample degradation by the derivatization process or of incomplete derivatization is evident in this chromatogram.

While branching or linkages other than α-(1→4) could lead to multiple peaks of the same mass in amylopectin hydrolyzates, we seen no significant additional peaks under the separation conditions used. The most likely reason for this absence

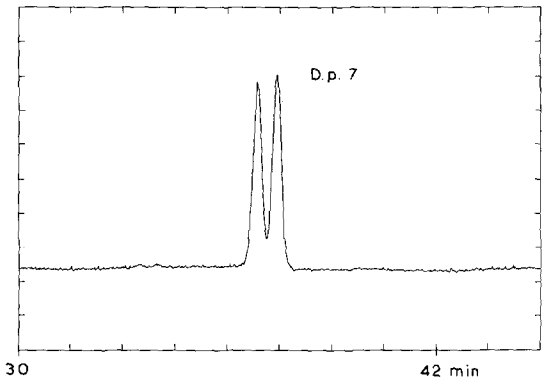

Fig. 2. Enlargement of the SFC-FID chromatogram of trimethylsilylated maltoheptaose. Chromatographic conditions were identical to those used in Fig. 1.

of additional peaks is that the levels of branched material are too low in Maltrin M-100 to be visible.

The starch from which Maltrin M-100 is made by acid hydrolysis is ~75% amylopectin and ~25% amylose. If the hydrolysis is completely random, then the relative number of α-(1→6) linkages should remain at ~4%, and, the saccharides of lower d.p. will tend to be exclusively by α-(1→4)-linked. There would be at most 4 α-(1→6) linkages in 20 molecules of d.p. 6 if none of the α-(1→6) linkages were hydrolyzed in manufacturing the product. At least 16 of these 20 molecules would be exclusively α-(1→4)-linked. Furthermore, if the location of any remaining branch points is random among molecules of a given d.p. and if the position of branching causes shifts in the retention time from the value for linear oligomers, the branched materials would be distributed among many possible isomer peaks, whereas the predominant linear materials would elute as one anomeric-peak pair at each d.p. value. Thus, evidence of branched material may not be visible under the chromatographic conditions used.

The mass range of the derivatives shown in Fig. 1 is ~7000 daltons. The mass spectrometer used had only a 3000-Da range. While this was not sufficient to see the entire range of malto-oligosaccharides in Maltrin M-100, it was sufficient to study the early-eluting peaks. Fig. 3 shows the reconstructed total-ion-current chromatogram (upper trace) and several mass spectra (lower traces). Although mass resolution was decreased slightly by purposely detuning the mass analyzer, this adjustment enhanced the signal-to-noise ratio by approximately a factor of ten at m/z 2000. The ion current is predominantly concentrated in ammonium adduct ions. The strongest spectra also display fragment ions at m/z 773 and 1152, which incorporate two and three glucose residues[21]. Mass spectra of peak pairs, that is, scans 785 and 812, 900 and 912, and 1141 and 1160, show identical nominal masses, despite the unusual peak spacing evident in the reconstructed chromatogram and in Fig. 1.

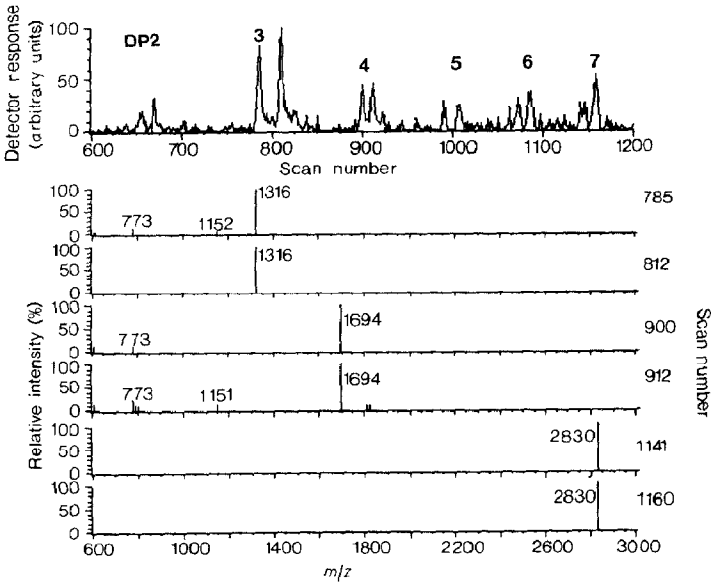

Fig. 3. Reconstructed total-ion chromatogram and mass spectra of selected peaks for silylated Maltrin M-100.

It is important to point out that a much greater mass-spectrometric analysis range (in terms of d.p.) is available when lower-mass derivatives are made, or when derivatization is avoided. For example, Reinhold, *et al.*[22], permethylated malto-oligosaccharides prior to SFC-MS analysis and were able to analyze peaks up to d.p. 10 within a 4000-Da mass range. However, it is just as important to point out that selectivity and chromatographic quality are also likely to change with the derivative used[22].

We conclude that SFC separates silylated linear malto-oligosaccharides by degree of polymerization with selectivity between anomers, especially at the lower end of the d.p. range. We cannot explain the unusual peak spacing noted between the anomer peaks from one d.p. to the next. Mass-spectrometric detection should not be needed, in general, for solving analysis problems concerning silylated malto-oligosaccharides separated by SFC. The chromatographic mass-range clearly exceeds the range of most mass spectrometers. Chromatographic resolution is much better when conditions are optimized for the separation rather than the detection step.

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