INFRARED AND RAMAN SPECTRA OF MALTOOLIGOSACCHARIDES[1]

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ABSTRACT

Infrared and Raman spectra of maltooligosaccharides (G_1–G_{12}) have been obtained. Maltooligosaccharides G_3 and higher could not be readily distinguished by their infrared spectra. The Raman spectra of crystalline glucose and maltose showed distinct vibrational bands, but maltotriose and all higher oligosaccharides exhibited such high fluorescence that the vibrational bands were obscured.

Infrared spectrometry has been a useful tool for identifying organic compounds because vibrations of definite groups of atoms characteristically show specific absorption at certain wavelengths of the spectrum. The normal O-H stretching band occurs at 3634 cm^{-1} when not hydrogen-bonded, but shifts to lower frequencies when the hydroxyl group becomes involved in hydrogen bonding; the greater the strength of the hydrogen bond, the lower will be the absorption frequency (1). The O-H stretching of the hydrogen-bonded OH group gives rise to a characteristic frequency at 3300 cm^{-1} in the spectrum of glucose.

Carbon-carbon and carbon-oxygen bonds (2) produce several closely spaced absorption bands between 1100 and 1000 cm^{-1} in the spectra of carbohydrates. Katon *et al.* (3) investigated infrared absorption in the 2000–200 cm^{-1} range for α-trehalose, for glucose mixed with fructose (1:1), and for lactose at two different temperatures, and found infrared spectra useful for identifying biological materials. Cael *et al.* (4,5) studied infrared and Raman spectra of α-D and β-D glucose and assigned absorption bands at 840 and 898 cm^{-1} to CH and CH_2 complex vibrational modes in each of the anomeric forms, respectively. They also observed absorption bands in the spectra of crystalline amylose and cellulose at 1432, 1334, and 1263 cm^{-1} and attributed these to vibrational motions of the CH_2, C-O-H, and C-C-H groups, respectively. Vasko *et al.* (6) studied the infrared and Raman spectra of D-glucose, maltose, cellobiose, and dextran; these investigators assigned various absorption bands to vibrational modes of C-O-H, CH_2, and C-H groups. But Hoover *et al.* (7), in studying infrared spectra of maltooligosaccharides, found the absorption spectra so similar that they were not useful in identifying the sugars of increasing chain-length.

During our study of physical and chemical properties of maltooligosaccharides, we prepared a homologous series of highly purified maltooligosaccharides (8). Infrared and Raman spectra were obtained for these materials.

MATERIALS AND METHODS

A partially hydrolyzed amylose corn starch, Morrex T. E. (CPC International

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Co., New York), was used as a source of oligosaccharides. The saccharides in Morrex were isolated on a carbon-Celite column and further purified by the use of macropaper chromatography (9).

Infrared Spectra

To obtain the infrared spectra, we used a Perkin-Elmer infrared spectrometer, Model 421. The oligosaccharide samples, G_1 to G_{12}, were blended and ground to a fine, uniform particle size in a stainless-steel capsule. We prepared samples for use in the infrared studies by two methods:

1) In studies of absorption in the region of 4000–2000 cm^{-1}, we deposited approximately 10 mg of each ground sample on a 25 mm diameter calcium fluoride (CaF_2) crystal window. To minimize scattering, we applied several drops of hexachloro-1,3-butadiene to a second window; the windows were sandwiched together and pressed to spread the sample uniformly between the windows. Hexachloro-1,3-butadiene has no strong absorption bands in the 4000–2000 cm^{-1} region.

2) In studies of absorption in the 2000–500 cm^{-1} region, we mixed each of the ground samples with petroleum jelly, using an agate mortar and pestle. The resulting "mull" was placed on the face of a 25 mm diameter potassium bromide (KBr) crystal window. To provide a uniform film, a second window was pressed on the first window supporting the sample. Petroleum jelly has only three strong normal bands in the 2000–500 cm^{-1} region. These regions are shaded in Figs. 1–3. Absorption in these shaded regions is principally that of the mulling agent; in all other regions the absorption is that of the sample. All samples were examined in triplicate.

Raman Spectra

Raman spectra of samples G_1 to G_{12} were obtained by using a Raman spectrometer Spex Model 1401, which has double dispersion to reduce stray light; the spectrometer we employed had an associated photodetector and counting circuits. The beam source consisted of a Coherent Radiation Model 52 argon ion laser. A Mosely Autograph Model 2D-2 X-Y recorded the spectra at a scan rate of 50 cm^{-1}/min^{-1}; the amplifier time constant was 2 sec, and the spectrometer slit width provided a spectral resolution of ± 4 cm^{-1}.

All sugar samples used in our Raman study were prepared in a similar manner: a small, stiff wire was used to force small amounts of each sample into 1 mm i.d. Kimax capillary tubes. After loosely tamping but not packing the samples, we sealed each sample tube with wax.

We used the laser's 5145A line for Raman excitation; at this wavelength, the laser had a maximum output of 750 mW. In most cases, the beam was attenuated by neutral density filters before it reached the sample.

RESULTS AND DISCUSSION

Infrared Spectra

The infrared spectra, G_1 to G_{12}, are shown in Figs. 1, 2, and 3. Basically, all the oligosaccharides absorbed infrared radiation in approximately the same general frequency ranges. A broad, deep absorption band between 3600 and 3100 cm^{-1}

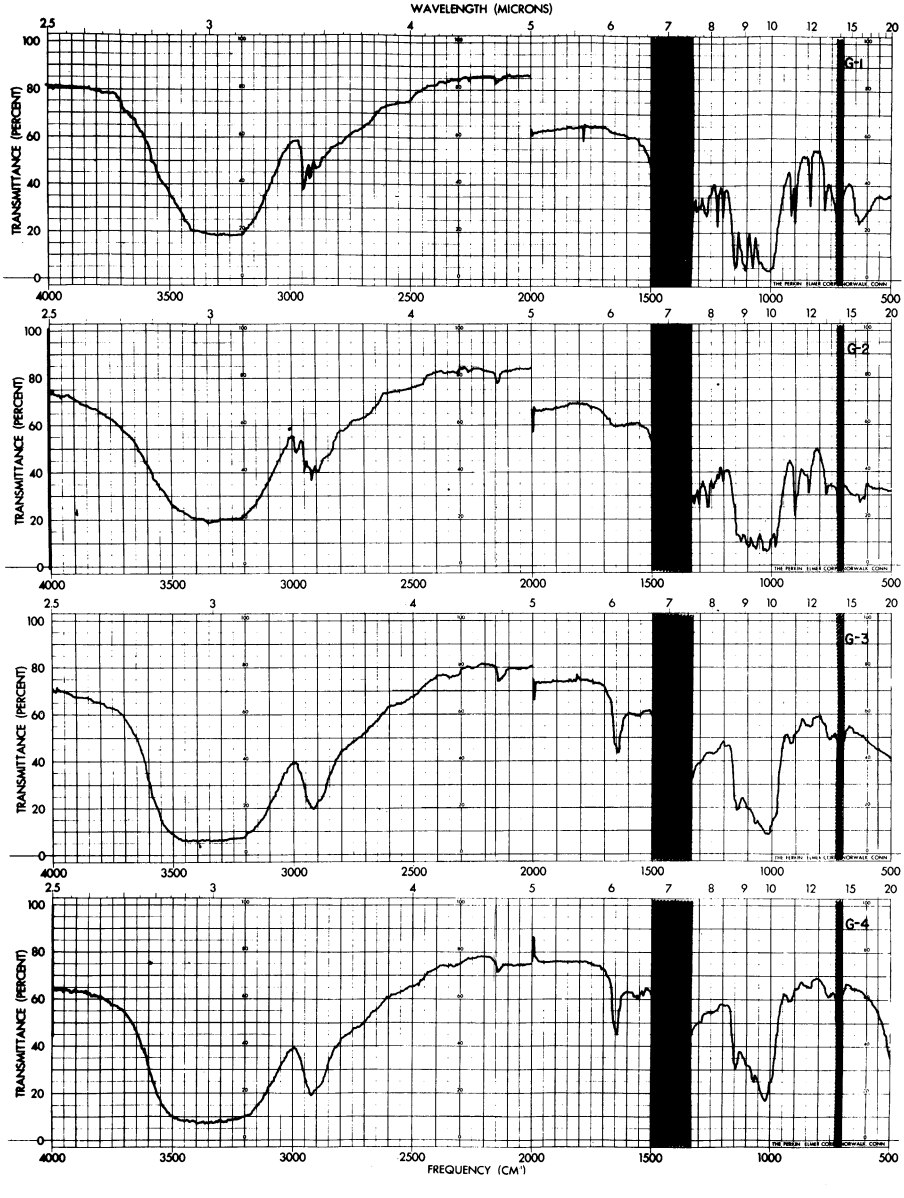

Fig. 1. Infrared spectra of glucose, maltose, maltotriose, and maltotetrose.

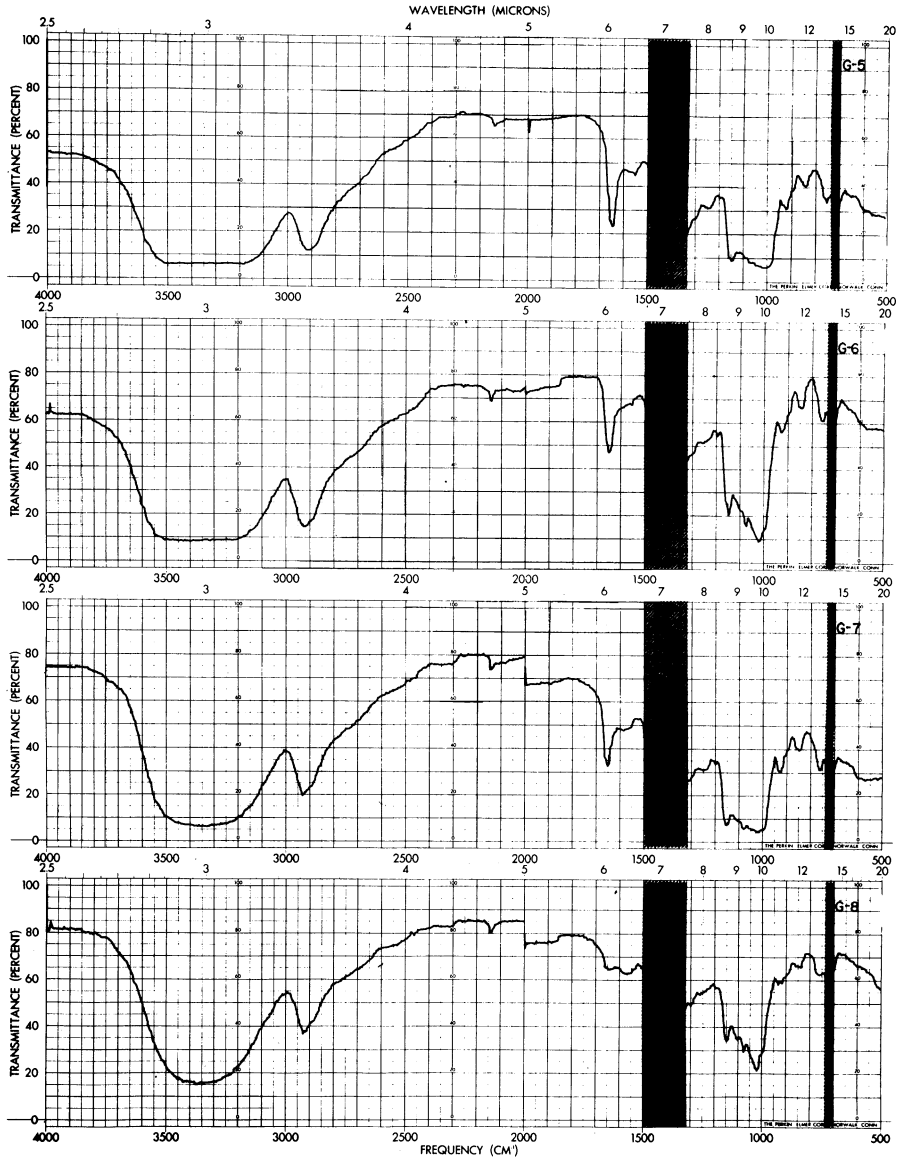

Fig. 2. Infrared spectra of maltopentaose, maltohexaose, maltoheptaose, and maltooctaose.

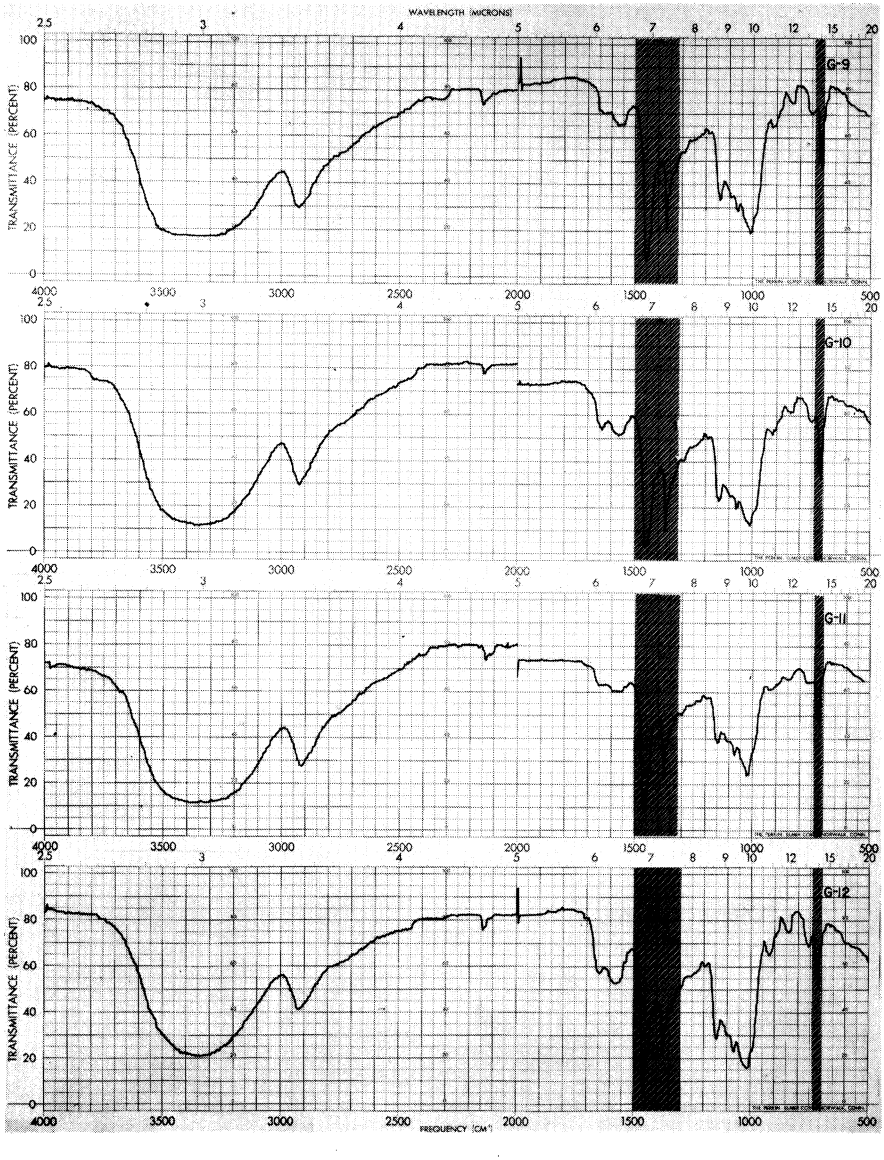

Fig. 3. Infrared spectra of maltononaose, maltodecaose, maltoundecaose, and maltododecaose.

can be associated with O-H stretching modes. The C-H stretching vibrations caused a small band at 2900 cm^{-1}. The absorption band at 1640 cm^{-1} is characteristic of the bending vibration in water (10). The absorption band at 1640 cm^{-1} in the spectra of the oligosaccharides (G_1 to G_{12}) varied in intensity. This absorption is most likely a result of absorption of water vapor from the atmosphere during sample preparation. The variation in intensity reflects a variation in amount of absorbed water (11). The oligosaccharides tended to be hygroscopic.

The bands between 1320 and 1220 cm^{-1} involve O-H in-plane and C-H, CH_2 bending modes. Glucose and maltose exhibited distinct absorption bands in this region but these distinctive absorption bands were absent in the higher oligosaccharides, G_3 to G_{12}. Absorption bands in this region became less distinct as chain-length increased. The bands between 1150 cm^{-1} and 980 cm^{-1} are broad, intense, and close together. The peak at 1147 cm^{-1} has been assigned to C-C in-plane bending and the peaks at 1105 and 1150 cm^{-1} have been identified as bands associated with C-O-C bending (12).

The region of absorption at 960–730 cm^{-1} indicates that those oligosaccharides were α-D-glucopyranosides (13,14). Although absorption peaks shifted only slightly in the 960–730 cm^{-1} region, band intensities of the infrared spectra of G_1 and G_2 differed appreciably from those of G_3–G_{12}, and this intensity variation could be used to distinguish the first two sugars from the other higher polymers.

Spectra for the oligosaccharides G_4–G_{12} resembled those of the G_3 spectrum. The infrared spectra could not be used to identify the higher polymers, possibly because the maltooligosaccharides contained the same functional groups and the same type linkages, which merely increased the number of nearly identical normal frequencies as the sugar polymer size increased.

Raman Spectra

Because the infrared spectra did not appear promising for distinguishing between the higher maltooligosaccharides, the normal Raman spectra corresponding to the Stokes shift were examined to see if they would be more distinctive. The Raman spectra of glucose, maltose, and maltooligosaccharides were studied in the spectral region 100 to 3600 cm^{-1}. Glucose and maltose showed distinct spectra, but maltotriose and higher oligosaccharides showed such high fluorescence throughout this spectral range that no satisfactory Raman spectra could be obtained. The Raman spectra of crystalline glucose and maltose are shown in Fig. 4.

We also attempted to obtain Raman spectra of maltooligosaccharides on the Antistokes (higher frequency) side of the laser exciting line in order to avoid the effects of fluorescence. Glucose and maltose showed a distinct structure and were distinguishable. The Antistokes spectra of G_3 through G_{12} appeared slightly different from one another; however, closer examination of the spectra of the compounds revealed that they had bands at the same frequencies but that the intensities of the bands varied slightly from one polymer to another. Thus, we concluded that neither Stokes nor Antistokes spectra provided a *simple* way to differentiate between the higher maltooligosaccharides.

In summary, pure preparations of glucose and maltose can be distinguished readily from higher maltooligosaccharides by either infrared or Raman spectra.

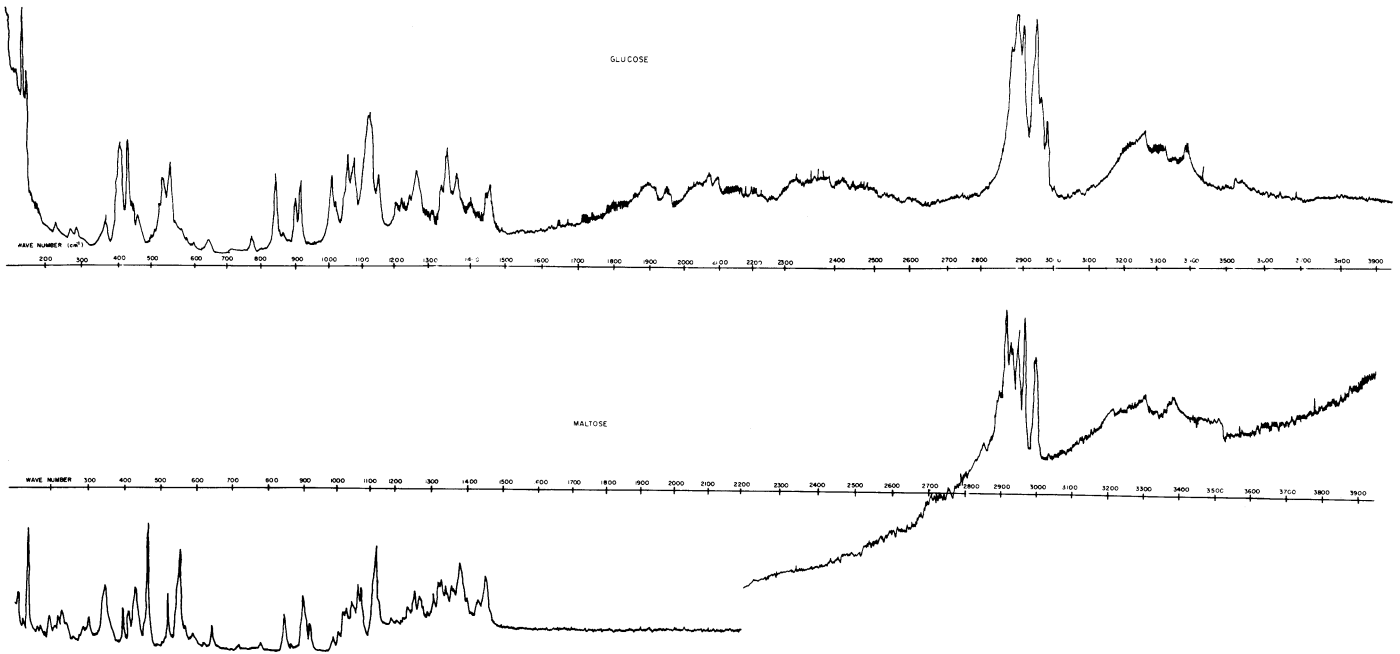

Fig. 4. Raman spectra of glucose and maltose.

The differences in the spectra of the higher maltooligosaccharides (G_3–G_{12}) are too subtle to be distinguished qualitatively by visual examination.

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