Physical and Chemical Properties of Oligosaccharides[1]

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ABSTRACT

Maltooligosaccharides (G_1 to G_{12}) from partially hydrolyzed amylose starch were partially separated on a Celite-carbon column and further separated and purified by macro-paper chromatography. The fractions were shown to be pure, straight-chain molecules of a homologous glucose series. Certain physical and chemical properties of these oligosaccharides were determined. Reducing power agreed with theoretical values and with values of some found in the literature. Specific gravity of solutions increased with chain length and concentration. Refractive indices did not increase with chain length but did with increasing concentrations. Solubility decreased with chain length. Oligosaccharides (G_9 and G_{10}) did not completely dissolve at 8 to 10% concentrations. Relative viscosity and hygroscopicity increased with molecular weight of the oligosaccharides.

Many procedures have been developed to separate maltooligosaccharides from starch hydrolysates and to measure certain physical and chemical properties of the fractions. Unfortunately, probably because of great difficulty in obtaining pure fractions, the values reported for physical properties vary widely. To establish physical and chemical properties of these substances would have obvious value to scientists and industrial users.

Many investigators have used carbon columns and ethanol solutions to separate the maltooligosaccharides (1–11).They obtained pure fractions of the lower-molecular-weight sugars with ease, but experienced increasing difficulty as they attempted to separate the higher-polymer fractions. Carbon mixed with Celite (diatomaceous earth) with ethanol gradients has been used (3,6,10,13,14). Celite aids in maintaining a uniform flow-rate. Paper chromatography, including cellulose columns, with a wide range in solvents, has been used to separate maltooligosaccharides (15–22). These separation methods, while precise, usually are applied to small quantities of sugars and for identification. Other procedures for isolating oligosaccharides have included forming of the borate complex (23), carbon-aluminum oxide columns (24), polyacrylamide gels (25), cross-linked starch (6), and gas chromatography (26). Derivatives of the sugars have limited value when physical and chemical properties of pure sugar polymers are to be measured.

Numerous investigators have measured the physical and chemical properties of the maltooligosaccharides but their data do not agree well. R_f values for maltooligosaccharides using paper chromatography with various solvents have been reported by Jeanes et al. (17) and by French and Wild (18). Hoover et al. (12) reported values for density, refractive index, viscosity, optical rotation, reducing power, and infrared spectra of the maltooligosaccharides from corn syrup. Some of their values for the higher-molecular-weight fractions were estimated by linear regression. Unusually high reducing values were reported. Generally, most measured values increased with chain length, but solubility decreased. Commerford and Scallet (27) found the dextrose equivalent to be higher than theoretical values for dextrose polymers, G_1 to G_6. They did not

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report on higher-molecular-weight polymers. Birch et al. (28) suggested that copper oxidation was associated with oxidation beyond the terminal reducing group of the polymer. Donnelly et al. (29) have shown that degree of polymerization has little relation to hygroscopicity.

In view of the broad interest and wide variation in existing data on physical and chemical properties relating to the maltooligosaccharides, we attempted to obtain highly purified samples that could be used to measure the physical and chemical properties of the homologous glucose series.

MATERIALS AND METHODS

A partially hydrolyzed amylose starch (Morrex T. E.)[3] was used as a crude source of maltooligosaccharides. Morrex is a dry powder consisting of glucose polymers from G_1 to G_{14} but mainly G_6 and G_7.

The polymers of Morrex were crudely separated on a large Celite-carbon column (6 in. × 12 ft.) filled with a mixture of Darco G-60 carbon, granulated carbon (20 mesh), and Celite 535 (4:4:2). The carbon-Celite was blended and water was added to form a thick slurry which was packed in the Pyrex-column. After being packed, the column was washed with one gallon of 40% hydrochloric acid, then with several gallons of distilled water until the eluant reached a pH of 3.5. The Celite-carbon column was then loaded with 450 g. of Morrex dissolved in 1 liter of water. The column was further washed with 20 gal. of distilled water before beginning elution with 5% aqueous ethanol. An elution pressure was created by sealing the top of the column with an appropriate cap and elevating the eluant reservoir 6 ft. above the column cap. The flow rate was approximately 0.3 gal. per hr. at first, but slowed to 0.08 gal. per hr. at the end of the elution with 50% aqueous ethanol.

Each gallon of eluant was analyzed for total carbohydrate by the method of Dubois et al. (30,31). Two milliliters of eluant of each gallon of the oligosaccharides was concentrated under reduced pressure and was spotted on No. 4 Whatman Chromatography paper, which was then irrigated with *n*-propanol-ethyl acetate-water (6:1:3) for 18 hr. After the paper was dried, the chromatogram was dipped successively in silver nitrate, sodium hydroxide in methanol and sodium thiosulfate solutions to detect and fix the sugar on the paper (32).

After the eluant had been collected and analyzed for sugar content and type of oligosaccharides, the fractions of similar types were combined and concentrated in a vacuum evaporator (a dry milk evaporator operated at 26 in. of Hg vacuum and 110° F.). The concentrated solution represented a mixture of neighboring polymers, cations, and anions originating from the Celite-carbon column.

The concentrated solutions were deionized by passing through Amberlite IR-100 (H^+ form) and Dowex IX-8 (CO_3-form) columns. The deionized, concentrated solution was further concentrated in a rotary evaporator and finally dried to a white powder by lyophylization.

The polymers were finally separated on washed 3MM Whatman Chromatography paper using the procedure of Commerford et al. (21). The paper was irrigated with *n*-propanol, ethyl acetate, and water (14:3:7); it took 3 to

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5 days to separate G_3 to G_7 and 8 to 14 days to separate G_7 to G_{12} oligosaccharides. After separating and identifying the polymers, the remaining paper was cut into strips and the individual polymers eluted with 5 to 10 ml. of deionized water. The water solution of the individual polymers was freeze-dried and collected in small vials, which were desiccated over phosphorus pentoxide. After collecting many samples of each oligosaccharide and combining them, they were dissolved in water, filtered and freeze-dried. The samples were further dried under vacuum and stored in a desiccator with phosphorus pentoxide until physical and chemical properties could be determined.

Reducing power was determined by the Somogyi method (33) with reagents prepared according to Hodge and Hofreiter (34). Calculations were made according to Commerford et al. (21).

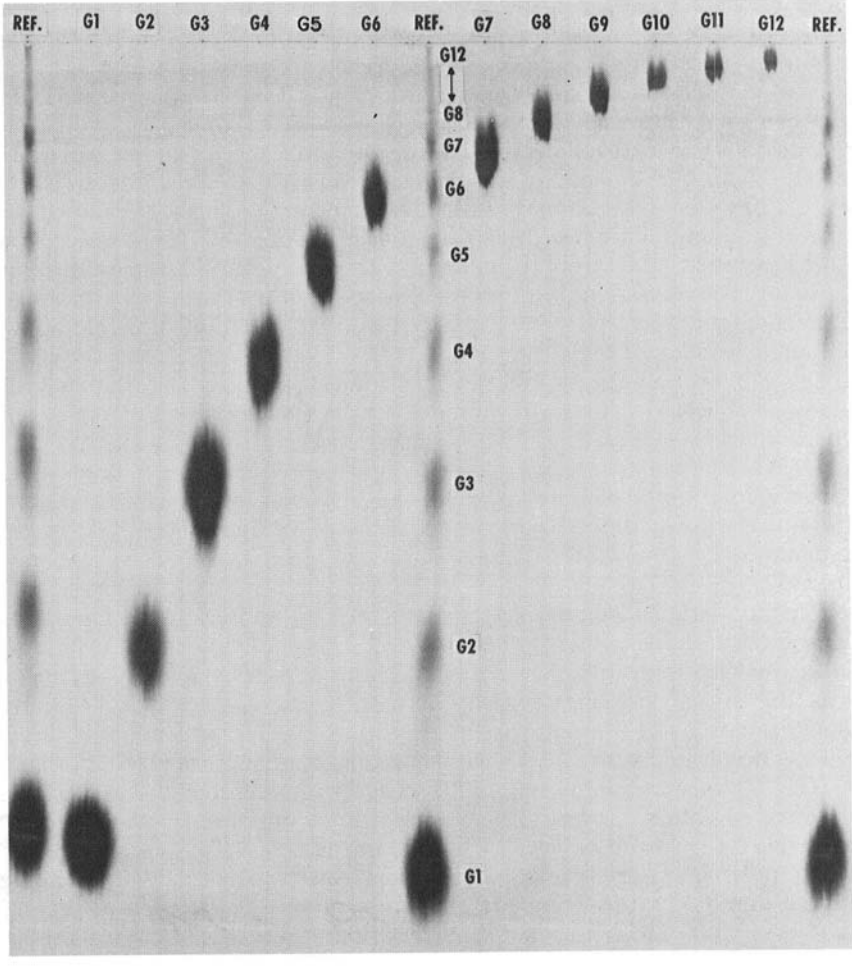

Fig. 1. Paper chromatograph of maltooligosaccharides separated from Morrex.

Specific gravity was measured by dissolving samples in water and diluting to 1, 2, 4, 8, and 10% concentrations. Specific gravities were measured in a 2-ml. pycnometer at 20°C. The specific gravities were corrected for buoyancy according to Hann (35).

The homogeneity of the fractions was established by paper chromatography (Fig. 1). The curvilinear nature of the R_f suggested a homogeneous glucose series of relatively high purity. In addition, linearity of the individual oligosaccharides was established by β-amylolysis (29). The even-numbered oligosaccharides, after 24 hr. of β-amylolysis, produced only maltose when tested by TLC, whereas the odd-numbered oligosaccharides produced mainly maltose with traces of glucose and maltotriose.

Hygroscopicity of the maltooligosaccharides was determined by placing a weighed quantity of each oligosaccharide in an aluminum dish that was placed in a desiccator containing sulfuric acid of known concentration to regulate the humidity at 60% r.h. (36). In addition, humidities were recorded with an Abbeon humidiscope and thermometer. Changes in weight of the dishes and polymers were determined periodically for 4.5 hr. Hygroscopicities were expressed as the percent increase in weight of the polymers by absorption of water.

RESULTS AND DISCUSSION

While glucose and maltose were present in Morrex in small quantities, they

TABLE I. DEXTROSE EQUIVALENTS (D.E.) AND STOICHIOMETRY OF MALTOOLIGOSACCHARIDES

Degree of Polymerization	Hoover et al.[1] (12)	Commerford and Scallet[2] (27)	Theoretical D. E. Value	Present Investigation D. E. Values	Stoichiometry of Polymers: Stoichiometry of Glucose[3]
G_1	100.08	100.00	100.00	100.39	1.00
G_2	71.95	58.10	52.63	54.34	0.98
G_3	61.63	39.50	35.71	37.83	0.99
G_4	52.27	29.80	27.03	29.58	1.03
G_5	53.97	24.20	21.74	23.19	1.01
G_6	41.14	20.80	18.18	20.36	1.06
G_7	38.04	...	15.63	15.69	0.93
G_8	33.84	...	13.70	13.65	0.93
G_9	31.83	...	12.20	12.57	0.95
G_{10}	28.67	...	10.99	10.91	0.93
G_{11}	...	...	10.00	9.63	0.89
G_{12}	...	...	9.17	8.42	0.84

[1]Ferricyonide procedure (12).
[2]Lane and Eynon procedure (27).
[3]Stoichiometry of the copper-sugar reaction (27).

TABLE II. TRUE SPECIFIC GRAVITY[1] OF SOLUTIONS OF MALTOOLIGOSACCHARIDES AT 20°C.

Degree of Polymerization	True Specific Gravity				
	1%	2%	4%	8%	10%
G_1	1.0018	1.0050	1.0131	1.0282	1.0348
G_2	1.0011	1.0047	1.0126	1.0266	1.0337
G_3	1.0013	1.0049	1.0129	1.0287	1.0364
G_4	1.0014	1.0051	1.0130	1.0289	1.0369
G_5	1.0015	1.0053	1.0130	1.0290	1.0369
G_6	1.0016	1.0057	1.0136	1.0286	1.0386
G_7	1.0019	1.0058	1.0138	1.0297	1.0386
G_8	1.0020	1.0062	1.0140	1.0296	...
G_9	1.0021	1.0057	1.0140	...	...
G_{10}	1.0020	1.0056	1.0139	...	...

[1]Specific gravity in vacuo.

were not collected when eluted by 10% aqueous ethanol. Both were obtained from commercial sources and purified with batch carbon treatment and crystallization. Maltotriose and maltotetraose were eluted together with 20% aqueous ethanol. Later fractions eluted with 20% ethanol contained maltotetraose, maltopentaose and maltohexaose. As the higher ethanol concentrations were used, higher members of the homologous glucose series were eluted but always as a mixture of the neighboring polymers.

The proximities of the R_f values of the longer-chain polymers (Fig. 1) suggest why complete separation by Celite-carbon columns was impossible. The proximities also explain why good separation could be achieved with 3MM paper chromatographs irrigated 7 to 10 days to separate the G_7 to G_{12} polymers.

The dextrose equivalents of the 12 maltooligosaccharides calculated from the reducing power are compared with theoretical and literature values (12,27) in Table I. In general, the values agree well with those reported by Commerford and Scallet (27) for polymers up to G_6 and with the theoretical values. High values reported by Hoover et al. (12) suggest a low degree of purity in separation or perhaps, oxidation by ferricyanide beyond the terminal reducing group. The stoichiometry was in agreement with values reported by Commerford and Scallet (27). Samples G_1 through G_6 gave approximately equal relative response (approximately 1.0) while G_7 through G_{12} tended to be 10 to 15% less than an equivalent amount of glucose. Samples G_7 through G_{12} may have needed longer time to react with copper than G_1 through G_6.

The relationships between true specific gravity (corrected for buoyance) for 1 to 10% sugar solutions and degree of polymerization are shown in Table II. There is a general linear relationship between specific gravity and degree of polymerization. With larger polymers (G_{10} through G_{12}) the specific gravity

TABLE III. REFRACTIVE INDICES OF MALTOOLIGOSACCHARIDE SOLUTIONS AT 20°C.

Degree of Polymerization	Refractive Index				
	Oligosaccharide concentration of solution				
	1%	2%	4%	8%	10%
G_1	1.3350	1.3367	1.3392	1.3451	1.3480
G_2	1.3349	1.3362	1.3390	1.3448	1.3480
G_3	1.3348	1.3360	1.3390	1.3448	1.3480
G_4	1.3348	1.3361	1.3390	1.3449	1.3482
G_5	1.3348	1.3361	1.3390	1.3450	1.3483
G_6	1.3348	1.3360	1.3390	1.3450	1.3483
G_7	1.3348	1.3360	1.3390	1.3450	1.3482
G_8	1.3348	1.3360	1.3390	1.3450	...
G_9	1.3348	1.3360	1.3390	...	...
G_{10}	1.3348	1.3360	1.3390	...	...

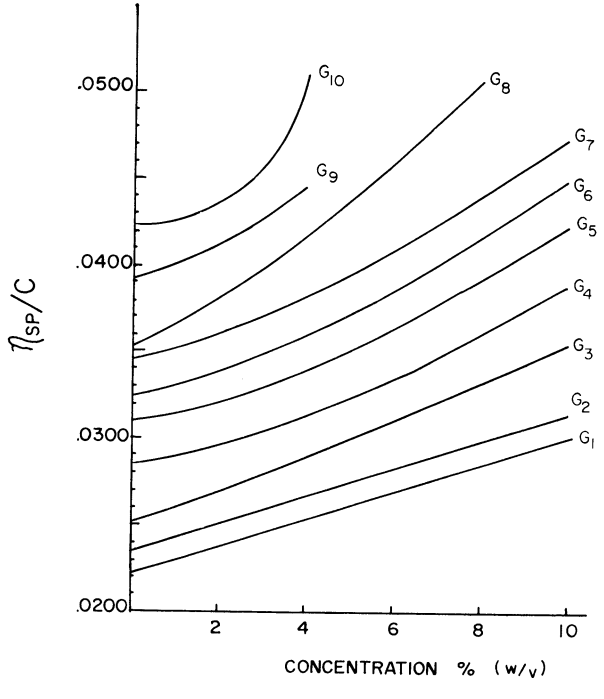

Fig. 2. The relationship of the ratio of specific viscosity to concentration of maltooligosaccharides.

TABLE IV. HYGROSCOPICITIES OF MALTOOLIGOSACCHARIDES (G_1 to G_{10}) AT 60 ± 5% RELATIVE HUMIDITY AT 26°C.

Degree of Polymerization	Moisture, %		
	15 min.	90 min.	270 min.
G_1	1.72	0.52	0.43
G_2	1.45	0.33	0.34
G_3	3.82	7.93	11.27
G_4	4.21	9.22	10.82
G_5	6.21	9.94	10.10
G_6	7.76	9.14	10.96
G_7	4.01	9.67	11.44
G_8	7.48	11.53	13.23
G_9	9.62	12.73	14.32
G_{10}	8.90	12.30	13.92

could not be measured because of the limited solubility of the polymers. As would be expected, the densities (Table II) of the solutions increased with concentration of solution.

Refractive indices of maltooligosaccharides of 1 to 10% solution of G_1 to G_{10} are summarized in Table III. These data indicate that refractive index did not increase as the size of the polymer increased but as expected increased with the concentration of the solution. Refractive indices of 8 and 10% concentrations of G_9 and G_{10} could not be measured because of limited solubility.

Viscosities of solutions of large polymers are defined as the ratio of sheer stress per square centimeter to the velocity gradient produced as the solution flows. Viscosities of various sugar solutions are listed in the International Critical Tables (37) and viscosities of maltooligosaccharides have been reported by Hoover et al. (12). Specific viscosity (ηsp.) frequently is used to express the relationship of polymer size to viscosity since specific viscosity depends on the volume occupied by the polymers (38). Specific viscosity can be used to express intrinsic viscosity of the polymers as the concentration approaches zero.

The relationship of intrinsic viscosity to concentration of oligosaccharides is shown in Fig. 2. Maltooligosaccharides G_8 to G_{10} and above had limited solubility. Therefore, few values were obtained for these maltooligosaccharides. The intrinsic viscosity increased linearly for the lower-molecular-weight oligosaccharides but for G_7 to G_{10} the intrinsic viscosity increased curvilinearly with increasing concentrations. The intrinsic viscosity values are generally higher than those reported by Hoover et al. (12) but lower than values reported for cellodextrins of equivalent chain length (39).

Hygroscopicity is a characteristic of sugar polymers because of the many residual valence forces that attract water through hydrogen bonding. Table IV lists percentages of water absorbed by the maltooligosaccharides when exposed

to relative humidity of 60 ± 5% at 26° C. These data indicate that hygroscopicity increased as the polymer became larger. Moisture increase was particularly large for polymers G_3 to G_{10}.

SUMMARY

Maltooligosaccharides G_1 to G_{12} were separated by Celite-carbon column and further separated and purified by macro-paper chromatography. They were proved to be free of any branched fractions.

Reducing power of the maltooligosaccharides agreed closely with theoretical values. Specific gravity tended to increase with increasing chain length and concentration but refractive indices were identical for all oligosaccharides and increased with concentrations. Intrinsic viscosity increased as polymerization and concentration increased. Hygroscopicity likewise increased with length of the glucose polymer.

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