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Note

Preparation of oligosaccharide aldonolactones and *N*-(2-aminoethyl)aldonamides*

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Aldonolactones (**1**) and *N*-(2-aminoethyl)aldonamides (**2**) of monosaccharides and oligosaccharides have been successfully grafted onto carriers containing either amino or carboxylic acid groups *via* amide linkages[2]. The method is applicable to multifunctional carriers such as polysaccharides, synthetic polymers, and polypeptides. We now report procedures for the larger-scale preparation of **1** and **2**, especially the purification and non-degradative derivatisation of malto-oligosaccharides of d.p. $\geqslant 5$, which are active as primers for phosphorylase.

The best starting material for primer-active malto-oligosaccharides is produced by degradation of amylose with alpha-amylase of *Bacillus subtilis*[3]. Under suitable conditions, the hydrolysis mixture contains mainly maltohexaose, maltoheptaose, and maltotriose. The higher oligosaccharides were required, because maltotetraose is no longer a primer when linked by amide bonds[4].

On ultrafiltration (Amicon UM, exclusion limit MG 500) of the mixture, considerable amounts of material with d.p. >5 were found in the filtrate, indicating a rather heterogeneous pore-size. Better results were obtained by precipitation of the mixture with ethanol from its solution in methyl sulphoxide, but the fractions containing the higher oligosaccharides were not completely free from maltotetraose and maltotriose. An excellent fractionation on a 5–10-g scale could be effected by gel filtration on Bio-Gel P-2 and P-4, using a column of 5-cm diameter and elution with water. With Bio-Gel P-2, the individual malto-oligomers G_3–G_{10} could be obtained in quantities up to 1 g and, with Bio-Gel P-4 (Fig. 1), the range could be extended up to G_{23}. The heterogeneity of peak G_6 was mainly due to a concentration effect, since the mixture contains mainly G_6 and G_7, and disappeared when lower concentrations were used.

The preparation of aldonolactones from the oligosaccharides was effected by hypoiodite oxidation and electrolytic oxidation[5]. The latter method was more advantageous, since the oxidising agent (bromine from calcium bromide) is continuously regenerated and therefore the concentration of inorganic salts is relatively

*Chemical Synthesis of Branched Polysaccharides: Part IX. For Part VIII, see ref. 1.

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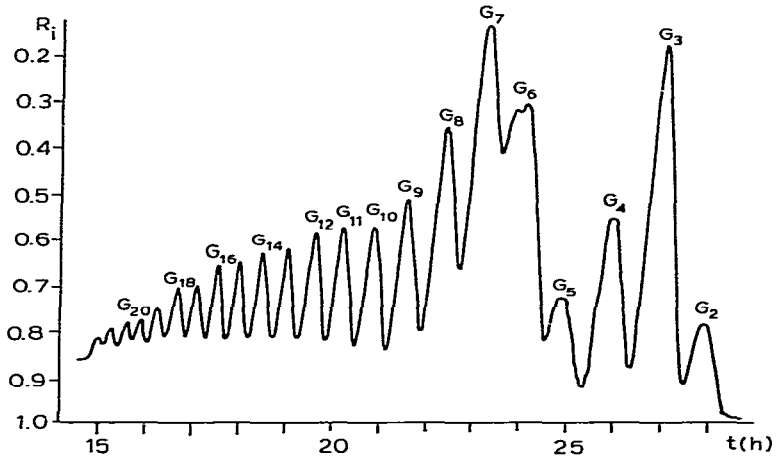

Fig. 1. Elution profile of an alpha-amylolytic digest on Bio-Gel P-4.

TABLE I

PRODUCTS FROM ELECTROLYTIC OXIDATION OF OLIGOSACCHARIDES

Starting compound		*Lactone*		
Oligosaccharide	*Weight (g)*	*Yield (%)*	*Carboxylic acid groups (%)*[a]	*Reducing groups (%)*[a]
Maltose	9	94.4	97.7	3.96
Maltose	17	97.5	95.5	6.10
Maltose	45	88.0	94.0	3.30
Cellobiose	8	99.4	94.3	4.66
Maltopentaose[b]	6	90.2	96.3	6.35
Malto-oligomer (d.p. 5.6)[c]	14	85.7	95.0	8.84
Malto-oligomer (d.p. 7.5)[d]	8.75	72.0	96.1	6.78
Malto-oligomer (d.p. 8.1)[d]	12.4	65.3	96.3	5.60

[a]Calculated on the basis of reducing end-groups before oxidation. [b]From degradation of amylose with alpha-amylase of *Bacillus licheniformis* (by courtesy of Dr. L. J. Hansen, DuPont de Nemours). [c]From fractional precipitation (see text). [d]Combined fractions G_5–G_{13} from gel filtration on Bio-Gel P-4.

low, thereby enabling their complete removal. The presence of calcium carbonate in the electrolytic oxidation procedure maintains a neutral pH throughout the reaction, thereby preventing hydrolysis of glycosidic bonds by hydrogen bromide. Also, the current applied can be easily controlled, thus making possible reproducible results. The purification procedure yielded the lactone without isolation of the calcium salt[5]. Typical data are listed in Table I. The yields were almost quantitative for disaccharides, but somewhat lower for the higher oligosaccharides.

From the kinetics of phosphorolytic synthesis studied with the lactones of maltotriose, maltotetraose, and maltopentaose, there is strong evidence that degradation has not occurred[4]. The residual reducing-activity, which has also been reported by others[6,7] for electrolytically oxidised sugars, does not necessarily reflect contamination by unoxidised material, but may be due to 5-hexulosonic acid[6]. However, reducing contaminants do not interfere in the subsequent step.

Terminal amino groups may be introduced into sugar derivatives by the catalysed reaction of aldonic acids with ethylenediamine[8]. With lactones, this addition takes place directly and at room temperature. The procedure used for maltopentaonolactone is typical and was a modification of that described by Geyer[9]. The reaction with ethylenediamine was effected in methyl sulphoxide at room temperature, with purification of the product by anion-exchange chromatography, to give *N*-(2-aminoethyl)maltopentaonamide.

EXPERIMENTAL

General methods. — Oxidation was performed with an apparatus consisting of a rectifier, a variable resistance (0–12 V), an ammeter (0–1 A), and two graphite electrodes (18-mm diameter). Reducing end-groups were determined by the Nelson–Somogyi method[10]. Carboxylic acid groups were determined by dissolving aldonic acids in an excess of 0.1M sodium hydroxide and back-titrating with 0.1M hydrochloric acid.

Gel-filtration was performed at 50°, using a column (5 × 150 cm) of Bio-Gel P-2 or P-4 (—400 mesh) and elution with deionised and degassed water. An aqueous 20% solution containing 3–10 g of a malto-oligosaccharide mixture, obtained by degradation of amylose with alpha-amylase from *Bacillus subtilis*[3], was applied to the column. Chromatography on Bio-Gel P-2 and P-4 was performed under pressure, to give flow rates of 100 and 80 ml/h, respectively.

Electrolytic oxidation of malto-oligosaccharides. — The following example is typical. To a solution of maltopentaose (6.0 g, 7.25 mmol) in water (150 ml) were added calcium bromide (1.3 g) and calcium carbonate (2.5 g), and oxidation was performed at a current of 0.5 A for 47 min, with stirring at room temperature. The mixture was then filtered, stirred with Amberlite IR-120 (H^+) resin for 30 min, and filtered. The filtrate was stirred with silver carbonate (2.5 g) for 30 min, filtered, and then applied to a column (2 × 15 cm) of Amberlite IR-120 (H^+) resin. Elution with water followed by freeze-drying gave the maltopentaonolactone (5.4 g, 90%), ν_{max}^{KBr} 1750 cm^{-1} (C=O, lactone).

N-(*2-Aminoethyl*)-*maltopentaonamide.* — To a solution of maltopentaonolactone (2.3 g, 2.77 mmol) in methyl sulphoxide (3.2 ml) at room temperature was added ethylenediamine (360 mg, 6 mmol). The solution was stirred overnight at room temperature and then diluted with 2-propanol (300 ml). The precipitate was collected, washed several times with 2-propanol and then with ether, and dried *in vacuo* at room temperature. A solution of the product [2 g; ν_{max}^{KBr} 1560, 1670 (Amide I and II), and

1610 cm^{-1} (NH, amine)] in water (10 ml) was applied to a column (2 × 7 cm) of Amberlite IR-120 (H^+) resin and eluted with water (200 ml). Fractions were monitored by using the anthrone test. Most of the by-products were eluted by the first 50 ml of water. The next 50 ml contained only traces of saccharides. Elution with 0.5M ammonia then gave the title compound, most of which was eluted in the first 250 ml. Elution was concluded after 500 ml. Ammonia was evaporated from the eluate (500 ml) at 30° under reduced pressure and the residual solution was freeze-dried, to give the title compound (0.96 g, 48%).

Anal. Calc.: C, 43.34; H, 6.54; N, 3.15. Found: C, 43.59; H, 6.59; N, 3.16.

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