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STRUCTURE ANALYSES OF OLIGOSACCHARIDES BY TAGGING OF THE REDUCING END SUGARS WITH A FLUORESCENT COMPOUND

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Summary: A potential aldehyde group of an oligosaccharide is combined with 2-aminopyridine by means of the reductive amination with sodium cyanoborohydride giving a fluorescent 2-aminopyridine derivative. The sugar derivative can be used for the purpose of the determination of 1, a degree of polymerization; 2, sequence of the sugar units; 3, the linkage points of the sugar units, and the preparation of finger prints.

The fluorescent 2-aminopyridine derivative of an oligosaccharide having a positive charge was found to be useful for the elucidation of the structure of oligosaccharides owing to its high sensitivity in the detection under UV-lamp and the stability of the 2-aminopyridine group on the hydrolysis reaction. The method includes the combination of 2-aminopyridine (1) with the reducing end of an oligosaccharide, partial degradation of the 2-aminopyridine derivative of an oligosaccharide followed by paper electrophoresis. The authors applied the method to some known oligosaccharides in order to test its usefulness.

MATERIALS AND METHODS

Materials: Maltooligomers were isolated from the partial hydrolysates of β-cyclodextrin. Isomaltooligomers were kindly donated by Dr.M.Torii(Osaka Univ.)
The Combination of Sugar Specimens with 2-Aminopyridine: About one mg of a sugar was dissolved in 20 µl of water. To this was added 80 µl of a reagent made by mixing 83 mg of 2-

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aminopyridine, 32 mg of sodium cyanoborohydride (2), 37 µl of acetic acid, and 0.33 ml of methanol. The solution was heated in a sealed tube at 75°C for 3-7 hr and then the reaction mixture was mixed with 1.3 ml of Dowex 50X2(H^+). The resin was washed with water and eluted with 0.6 M aqueous ammonia and the eluate was evaporated to dryness. Yield 80-90%.

Paper Electrophoresis: Two dimensional paper electrophoresis was performed at 40 V/cm, using buffer solutions of pH 5 (pyridine: acetic acid: water=45:30:900) and of pH 11(0.6 M sodium tetraborate(3), pH was adjusted by adding a sodium hydroxide solution). When the borate was used, the paper(Whatman No.1) was dried and allowed to stand in an atmosphere of acetic acid for several hr. The paper was dried and scanned with a UV-lamp (Toshiba F1-3L).

Analyses of Methylated Alditol Acetates: Methylated samples were hydrolyzed with 90% formic acid at 100°C for 2 hr and then with 0.25 N sulfuric acid at 100°C for 12 hr (4). Sulfuric acid was removed by passing through a Dowex 1X4(acetate) column. Component sugars were reduced with [$^2H_4$]borohydride, and the products were peracetylated with pyridine-acetic anhydride. Methylated alditol acetates thus obtained were analyzed(5) by a JEOL JMS D300 mass spectrometer with a JEOL JMA 2000 mass data analysis system connected to a gas chromatograph (OV-17, 1m). Mass spectra were taken at 30 eV ionization potential(1,4 kV acceleration voltage), 300 µA ionization current, 40-400 atomic mass unit range, 5 sec scan time. The column temperature was programmed from 180°C or 210°C at the rate of 5°C increment per min. A Shimadzu gas chromatograph GC 4APF was used at a column temperature of 180°C (SE-52,1m).

RESULTS

Determination of a Degree of Polymerization: Oligosaccharides (maltooligomers and isomaltooligomers) were combined with 2-aminopyridine, and the products were separated by paper electrophoresis at pH 5. The relative migration rates to the 2-aminopyridine derivative of glucose were: the derivatives of disaccharides, 0.72; those of trisaccharides, 0.57; those of tetrasaccharides, 0.52; those of pentasaccharides, 0.46. By two dimensional paper electrophoresis, a finger print of the 2-aminopyridine derivatives of the saccharides was obtained. An example is shown in Fig.1. About one nmole of a 2-aminopyridine derivative could be detected on a paper electrophoretogram.

Sequence Analyses: The 2-aminopyridine derivative of lactose, which moved as a derivative of a disaccharide as shown in Fig.1, was partially hydrolyzed (0.2 N HCl, 100°C, 30 min). The

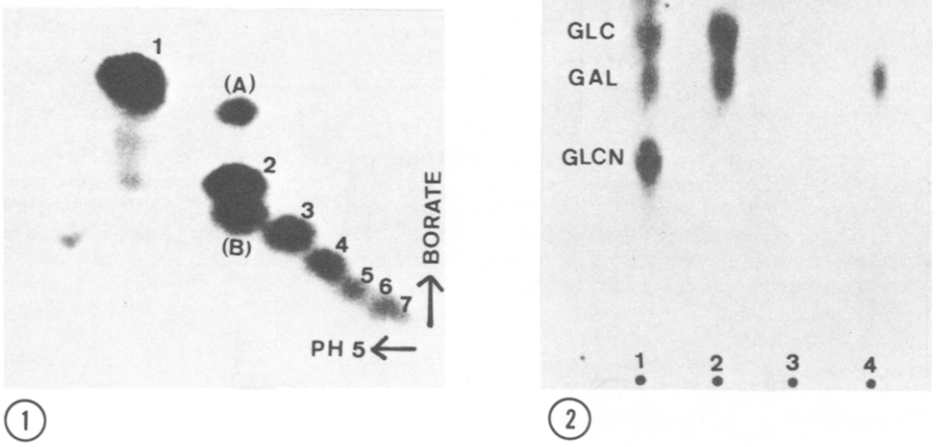

Fig.1. A two dimensional paper electrophoretogram of 2-aminopyridine derivatives of lactose(A), isomaltose(B), and maltooligomers. 1~7 correspond to mono-~ heptamers. The samples were applied to a paper (15 X 15 cm), and electrophoresis was performed first with the buffer solution of pH 5 and then with the borate solution pH 11.
Fig.2. A thin layer chromatogram in the structure analyses of lactose. 1, a standard mixture; 2, hydrolysates of lactose; 3, hydrolysates of the monosaccharide fraction obtained by paper electrophoresis; 4, hydrolysates of the disaccharide fraction. A thin layer plate DC-Fertigplatten Cellulose (E.Merck) was used with a solvent, ethyl acetate:pyridine:water(40:20:10, v/v) and a few drops of aqueous ammonia, developed three times. X, the 2-aminopyridine derivative of glucose.

hydrolysates were separated by paper electrophoresis at pH 5. Two spots corresponding to the derivatives of mono- and disaccharides were detected. Each spot was eluted from the paper, hydrolyzed (2N HCl, 100°C, 4 hr), and the component sugars of each spot were identified on a thin layer chromatogram by the method of Trevelyan (6) as shown in Fig.2. From the results, the sequence of this disaccharide is to be galactosyl-glucose.

Determination of the Linkage Points: The 2-aminopyridine derivative of isomaltotriose (1 mg) was permethylated by the

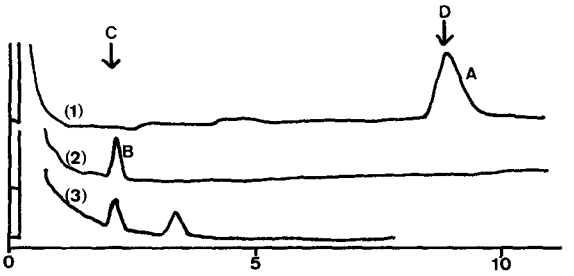

Fig.3. (1) a gas chromatogram of the monosaccharide fraction; (2) of the disaccharide fraction; (3) of the trisaccharide fraction. The arrow C indicates the position of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol; D, the permethylated 2-amino-pyridine derivative of glucose. Ordinate, detector response; abscissa, retention time(min).

method of Hakomori(7) and the product was purified by gel-filtration on a LH-20 column(8). The purified methylated sample was partially hydrolyzed (4% 12 N HCl in methanol, 100°C, 35 min) and the hydroxyl groups which newly emerged were methylated with [$^2H_3$]methyliodide. The neutral methylated saccharides carrying no 2-pyridylamino group were removed by passing through a column of Dowex 50X2(H^+). The methylated 2-aminopyridine derivatives, which had been adsorbed to the resin, were eluted from the column with 0.6 M aqueous ammonia and were separated on a thin layer chromatogram (DC-Alufolien Kieselgel 60, E.Merck; benzene:acetone =2:1, v/v). Three spots were detected and each spot was extracted with methanol. Di- and trisaccharide fractions were completely hydrolyzed and the methylated 2-aminopyridine derivative of glucose was removed by passing through a Dowex 50X2(H^+) column after borohydride reduction. The component sugars were analyzed as methylated alditol acetates. The monosaccharide fraction on the thin layer chromatogram was applied directly to gas chromatography-mass spectrometry. The results are shown in Fig.3 and 4. The peak A was identified with 1-deoxy-2,3,4,5-tetra-O-methyl-6-O-[$^2H_3$]methyl-1-[N-(2-

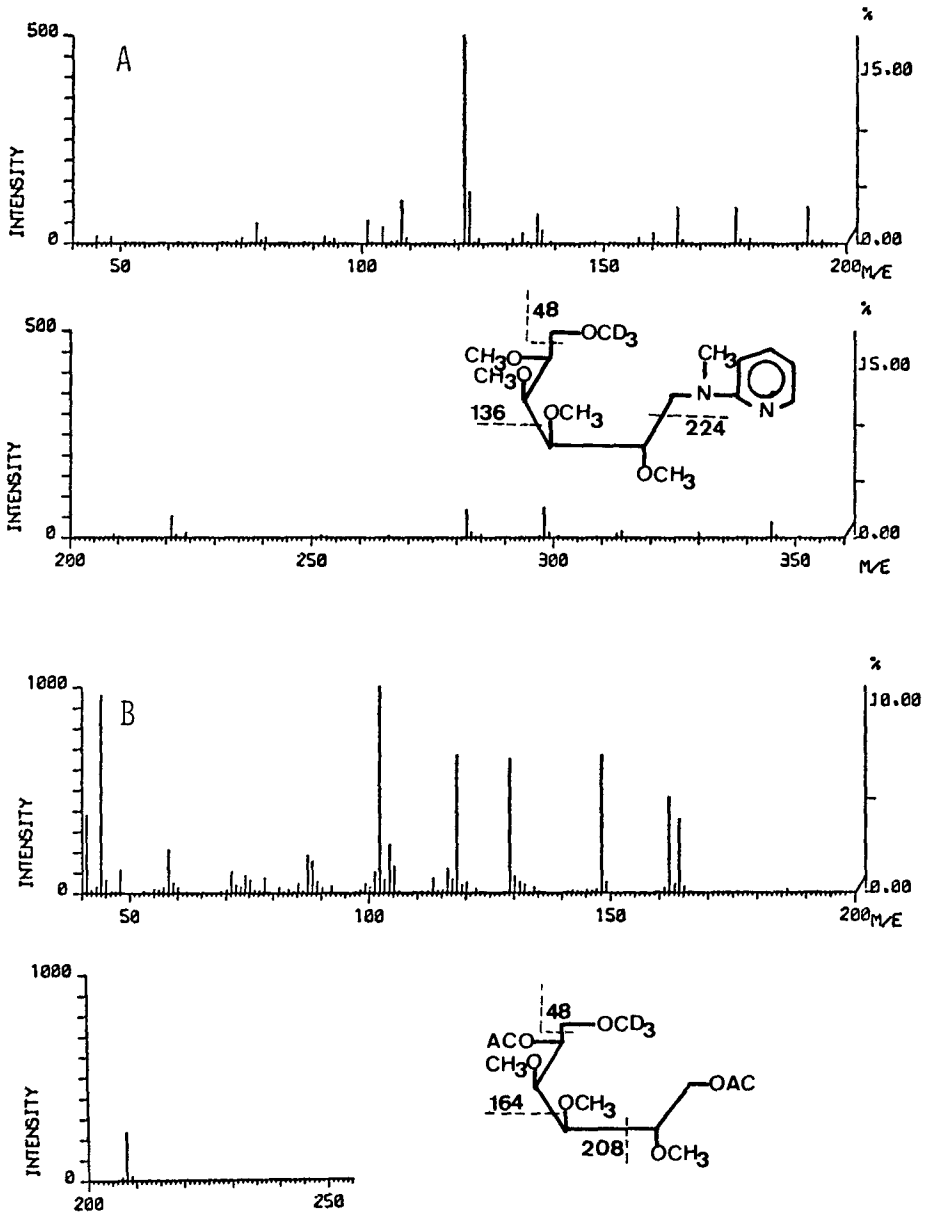

Fig.4. The mass spectra of the peaks in Fig.3. A, the mass spectrum of the peak A; B, of the peak B.

pyridyl)-methylamino]-D-glucitol by detection of the characteristic mass fragments; m/e=(48), 136, 224 (Fig.4A). The peak B was identified with 1,5-di-O-acetyl-2,3,4-tri-O-methyl-6-

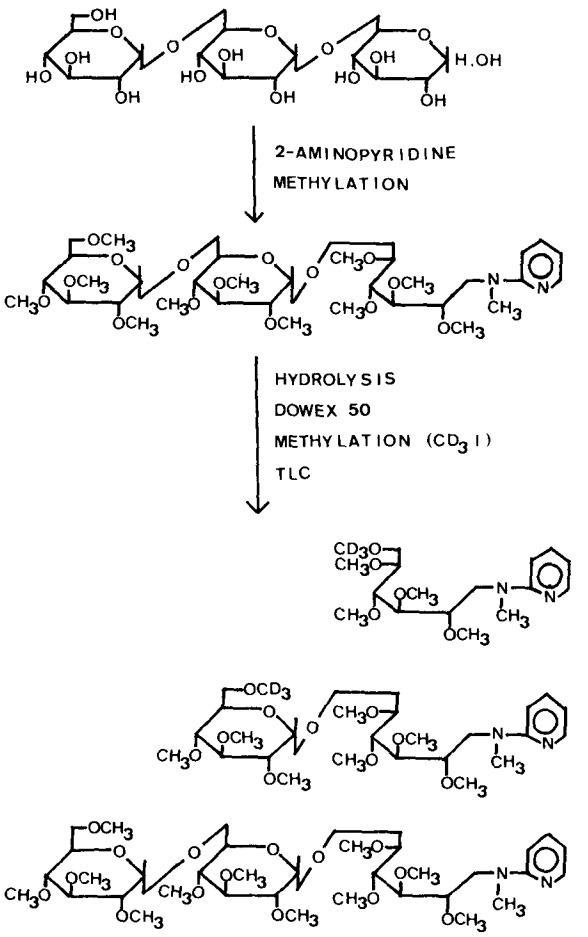

Fig.5. Reaction scheme of the determination of the linkage points of isomaltotriose.

O-[$^2H_3$]methyl-D-[1-^{2}H]glucitol by detection of the characteristic mass fragments; m/e=(48), 164, 208 (Fig. 4B). From these results, the structure of the trisaccharide was found to be glucosyl 1-6 glucosyl 1-6 glucose. The reaction scheme is shown in Fig.5.

DISCUSSION

The molecular weight determination of oligosaccharides by way of paper electrophoresis was reported by Baker et al (9) and by Frahn et al (10), showing that the migration rates of oligosaccharides are relative to their molecular weights and not

to their structures. The authors obtained the similar results using fluorescent 2-aminopyridine derivatives of oligosaccharides. 2-Aminopyridine derivatives of saccharides on a paper electrophoretogram gave a single spot with blue color under the UV-lamp. Owing to fluorescence, the present method was simpler and more sensitive than the previous methods (9,10) and no quantitative manipulation was required in contrast to the reported method (11). A finger print of 2-aminopyridine derivatives of saccharides was also obtained by two dimensional paper electrophoresis, in one direction in a buffer solution of pH 5 and in the other in a borate solution pH 11, which has been used for the separation of saccharides (12). Besides application to the determination of a degree of polymerization and the preparation of the finger prints of saccharides, the present method was used to determine the sequence and the linkage points of the unit monosaccharides as shown in this paper.

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