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A New Method for Simultaneous Determination of a Reducing End Group and Component Hexoses in Oligosaccharides by Gas Chromatography-Mass Spectroscopy

Simultaneous determination of a reducing-end hexose and the molar ratios of component hexoses of an oligosaccharide (containing mannose, glucose and/or galactose) can be achieved by mass fragmentography with gas chromatography–mass spectroscopy equipped with multiple ion detector.

In recent years, mass fragmentography[1] is being used in the biochemical field because of its high sensitivity and short time required for analysis but there is no convenient method for analysis of oligosaccharides by gas chromatography–mass spectrography (GC–MS) equipped with a multiple ion detector.

The present communication deals with analysis of oligosaccharides by mass fragmentography, which makes it possible to determine the reducing-end group and molar ratios of component hexoses in one process, using a minimum of 1 μg of an oligosaccharide, within an error of ±3%, and in a much shorter time than is required by the existing method of paper or gas chromatography.[2]

In this procedure, 1 μg to 1 mg sample of an oligosaccharide was contacted with $NaBD_4$ in D_2O (sample concentration, *ca.* 0.5%) for 2 hr in a sealed tube and excess $NaBD_4$ was de-

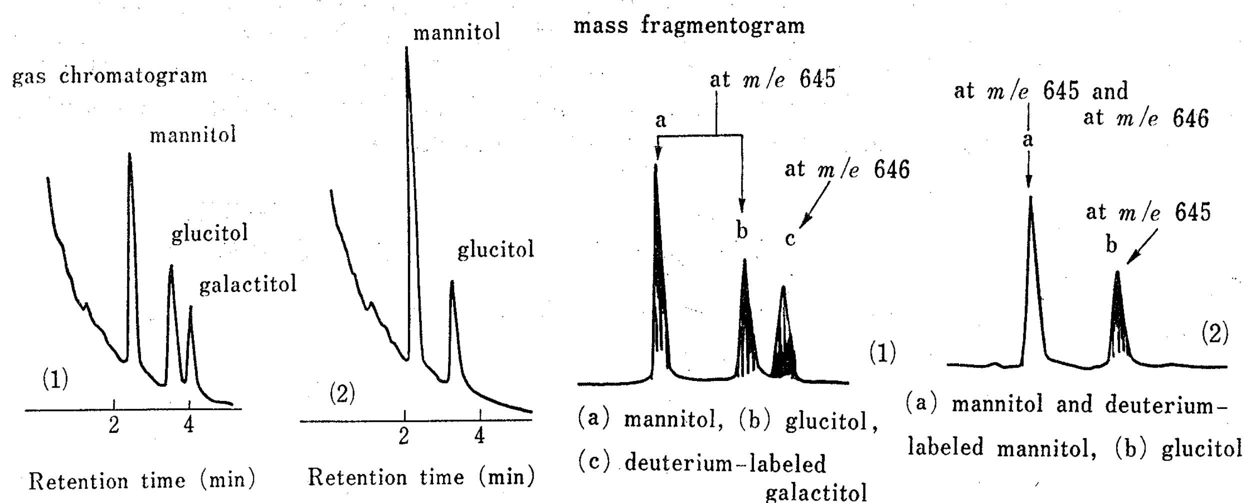

Fig. 1. Gas Chromatogram and Mass Fragmentogram of Alditol Trifluoroacetates of Hexoses constituting The Oligosaccharide (1); Man-Glc-Gal and (2); Man-Glc-Man

gas chromatography
column; 2 m coiled column packed with 2% Silicone XE-60 on Gas-Chrom P
column temp.; 150°
carrier gas; He, 25 ml/min
mass fragmentography
MID; *m/e* 645 and 646
electron energy; 70 eV
accelerating voltage; 3500 V
trap current; 60 μA
carrier gas separator; 220°

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Table I. Simultaneous Determination of Reducing-end Group and Molar Ratio of Component Hexoses in Oligosaccharides

Sample	Relative molar ratios: Calcd. Man	Calcd. Glc	Calcd. Gal	Found Man	Found Glc	Found Gal
Gal-Glc (Lactose)	0	1	1	0	1.01	0.99
Glc-Glc (Maltose)		1			0.98	
	0	1	0	0	1.03	0
*Man-Glc-Gal	1	1	1	0.97	1.01	0.99
	1			0.98		
*Man-Glc-Man	1	1	0	1.03	0.99	0
	1			0.97		
*Gal-Man-Glc-Man	1	1	1	1.02	1.01	1.00

Underlined are reducing-end group.
Oligosaccharides of these samples were obtained by hydrolysis of the polysaccharides from *E. Coli* of mucous strain in our laboratory [A. Kamei, S. Akashi, N. Takeuchi, and K. Nakazawa, *Seikagaku*, **46**, 418 (1974); A. Kamei, S. Akashi, N. Takeuchi, and K. Kagabe, *Seikagaku*, **47**, 743 (1975)].

stroyed by the addition of a cation-exchanger, Dowex 1×8 (50—100 mesh). The deuteration rate was constantly 89.6%. The cation-exchanger was removed by filtration and thoroughly washed with water. The filtrate and washings were combined and evaporated to dryness. Deuterium-labeled oligosaccharide was hydrolyzed[3] at 100° with 1N H_2SO_4. The excess acid was neutralized with $BaCO_3$ and the precipitated $BaSO_4$ was removed by centrifugation and filtration. The hydrolyzate was reduced with $NaBH_4$[4] by a similar procedure as above. After the combined filtrate was evaporated to dryness, the residue was treated with MeOH to remove H_3BO_3. The residue was dissolved in a small volume of water and its aliquot, containing about 0.01—1 μg of each component hexose, was transferred to a Teflon-capped reaction vial, dried, and trifluoroacetylated[5] at 80° for 15 min by vigorously stirring with 0.1 ml of trifluoroacetic anhydride in 0.1 ml of AcOEt. When cooled, a known volume of the solution was directly injected into the GC inlet of the mass spectrometer (Shimadzu LKB 9000). The reducing-end hexose is identified by determining the ratios of peak height at *m/e* 645 and at *m/e* 646 on a mass fragmentogram. The molar ratios of the component hexoses are determined by comparing their peak heights with the calibration curves prepared from authentic hexoses.

Experimental conditions for GC and MF are presented in Fig. 1 by giving analysis of Man-Glc-Gal and Man-Glc-Man (underlined component is the reducing-end) as examples. The fragment ions of *m/e* 645 and *m/e* 646 were produced by the loss of trifluoroacetoxyl moiety from the respective molecular ions. These fragment ions were highly intense and were not affected by other peaks because of the absence of fragment ions in adjacent area. From the exact correspondence of the sample quantity and peak height, these two fragment ions were used for the determination. Result obtained by the application of this technique to oligosaccharides of known composition are presented in Table I.

The present procedure can also be used for the determination of the number of component sugars in oligosaccharides composed of the same or mixed sugars, and it is thought to be a use-

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ful method for structural study of biogenetic polysaccharides which are available only in a minute quantity. It may be possible to use this procedure for the determination of oligosaccharides containing a mixture of pentoses, hexosamines, and hexoses.

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Synthesis of [27-Tyr]-Cholecystokinin-Pancreozymin (CCK-PZ)[1]

In a conventional manner, the tritriacontapeptide amide corresponding to the entire amino acid sequence of the desulfated form of porcine cholecystokinin-pancreozymin (CCKPZ) was synthesized by applying protecting groups removable by hydrogen fluoride. Synthetic peptide, though it is not too biological active, was smoothly and efficiently converted to the labeled compound with ^{125}I (specific activity 200—250 μci/μg).

In 1971, Mutt and Jorpes[2] elucidated the complete amino acid sequence of cholecystokinin-pancreozymin (CCK-PZ). The C-terminal dodecapeptide amide[3] and the N-terminal octapeptide[4] were synthesized for the structural confirmation. Radioimmunoassay of CCK-PZ has been encountered the difficulty because of the unavailability of pure CCK-PZ for labeling with radioactive iodine.

We wish to report the synthesis of tritriacontapeptide amide corresponding to the entire amino acid sequence of desulfated CCK-PZ, termed as [27-Tyr]-CCK-PZ (I), which seems to be the most ideal tracer for radioimmunoassay of this important upper intestinal hormone. As strategies for the synthesis of such a peptide containing Met, Lys and Trp residues, amino acid derivatives bearing protecting groups removable by hydrogen fluoride[5] were employed, *i.e.*, Lys(Z), Arg(Tos), and Asp(OBzl). The α-amino function of intermediates were protected by the Z(OMe) group[6] which is known to be cleaved by treatment with trifluoroacetic acid (TFA) without affecting side chain protecting groups employed. Anisole containing 2% ethanedithiol[7] rather than mercaptoethanol was employed to minimize destruction of the Trp residue

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1) Peptides and their derivatives mentioned in this communication are of the L-configuration. Abbreviations: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Tos=tosyl, OBzl=benzyl ester, ONP=p-nitrophenyl ester, ODNP=2,4-dinitrophenyl ester, OPCP=pentachlorophenyl ester, PCP–O–TCA=pentachlorophenyl trichloroacetate, DCC=dicyclohexylcarbodiimide, TFA=trifluoroacetic acid, DMF=dimethylformamide, DMSO=dimethylsulfoxide.
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