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Gas Chromatographic Analysis of Disaccharides. I.⁽¹⁾ Analysis of Disaccharides as Trimethylsilyl and Trifluoroacethyl Derivatives of Corresponding Alditols

Hiroshi Nakamura and Zenzo Tamura

Faculty of Pharmaceutical Sciences, University of Tokyo[2]

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Disaccharides in a mixture are reduced to the corresponding disaccharide alditols with sodium borohydride and analysis is performed as trimethylsilyl or trifluoroacethyl derivatives by gas chromatography.

Trifluoroacetylation of carbohydrates with trifluoroacetic anhydride is accomplished in N,N-dimethylformamide in a few minutes at room temperature.

Quantitative analysis in microscale is achieved both by the extraction of trimethylsilyl derivatives with *n*-hexane from the reaction mixture and by the use of an electron capture detector sensitive to TFA derivatives. Determination limits of 0.5 μg of disaccharide as TMS derivative and 0.1 μg as TFA derivative are successfully achieved.

In recent years, interest in gas chromatography (GC) of carbohydrates has been concentrated on the study of monosaccharides and related compounds,[3] despite the fact that man's carbohydrate intake is almost completely in the form of di-, oligo-, and polysaccharides. Whereas there has been intense interest in the clinical syndromes associated with disaccharide intolerance[4] and intestinal disaccharidase deficiency,[5] our present information for diagnosis and medical treatment are based mainly on *in vitro* assays of intestinal biopsy homogenates and on changes in blood sugar concentrations after ingestion of disaccharides. Little is known about the concentration of disaccharides in blood during the oral sugar-loading tests because of lack of proper analytical method for minute amount of disaccharides, though the analysis of disaccharides along with glucose in blood would give a direct and more detailed information for the function of intestinal mucosa.

There has been analytical methods for disaccharides, such as paper chromatography,[6] thin-layer chromatography,[7] paper electrophoresis,[8] column chromatography[9] and enzymic

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[2] Location: *Hongo, Bunkyo-ku, Tokyo.*

[3] *a*) C.T. Bishop, *Advan. Carbohyd. Chem.*, **19**, 95 (1964); *b*) *Idem*, "Methods of Biochemical Analysis," Vol. X, ed. by D. Glick, Interscience Publishers, Inc., New York, N.Y., 1962, pp. 1—42; *c*) H.W. Kircher, "Methods in Carbohydrate Chemistry," Vol. I, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York, 1962, pp. 13—20; *d*) W.W. Wells, R. Bentley and C.C. Sweeley, "Biomedical Applications of Gas Chromatography," ed. by H. Szymanski, Plenum Press, New York, 1964; *e*) C.C. Sweeley, R. Bentley, M. Makita and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).

[4] A. Holzel, T. Mereu and M.L. Thomson, *Lancet*, **1**, 1126 (1962); A. Dahlqvist, *Gastroenterology*, **43**, 694 (1962); F. Kern, Jr., J.E. Struthers, Jr. and W.L. Attwood, *ibid.*, **45**, 477 (1963); A. Dahlqvist, J.B. Hammond, R.K. Crane, J.V. Dunphy and A. Littman, *ibid.*, **45**, 488 (1963).

[5] A. Holzel, V. Schwarz and K.W. Satcliffe, *Lancet*, **1**, 1126 (1959); H.A. Weijers, J.H. Van de Kamer, D.A.A. Mossel and W.K. Dicke, *ibid.*, **2**, 296 (1960); H.A. Weijers, J.H. Van de Kamer, W.K. Dicke and J. Ijsseling, *Acta Paediat.*, **50**, 55 (1961); A. Pradel, S. Auricchio and G. Mürset, *Shweiz. med. Wochschr*, **91**, 465 (1961); U.P. Haemmerli, H.J. Kistler, R. Ammann, S. Auricchio and A. Prader, *Helv. Med. Acta*, **30**, 693 (1963).

[6] S.M. Partridge, *Biochem. J.*, **42**, 238 (1948); F. Frič and O. Kubániová, *J. Chromatog.*, **11**, 127 (1963); L.D. Ough, *Anal. Chem.*, **34**, 660 (1962).

[7] S. Chiba and T. Shimomura, *Agr. Biol. Chem.* (Tokyo), **29**, 486 (1965).

[8] H. Weigel, *Adv. Carbohyd. Chem.*, **18**, 61 (1963); B. Lindberg and B. Swan, *Acta Chem. Scand.*, **14**, 1043 (1960); E.J. Bourne, D.H. Huston and H. Weigel, *J. Chem. Soc.*, **1961**, 35.

[9] K. Asō and K. Shibasaki, *Nippon Nogeikagaku Kaishi*, **29**, 856 (1956); J.X. Khym and L.P. Zill, *J. Am. Chem. Soc.*, **74**, 2090 (1952).

method,[10] however, GC is ideally suited for the identification and the quantification of the carbohydrates in a mixture. Gas chromatographic separation of disaccharides have been performed as O-acetyl,[11] O-methyl,[12] O-trimethylsilyl (TMS)[3e] and O-trifluoroacethyl (TFA)[13] derivatives of disaccharides, but the reported methods require relatively much amount of sample for analysis and, in addition, the separation of disaccharides from each other is poor, since most disaccharides give multiple peaks in gas chromatogram resulting from anomeric and ring isomerization.

In recent years, to prevent multiplicity of peaks, analysis of monosaccharides as corresponding their reduction products has been successfuly performed,[14] and in the present paper, we have investigated the reduction of disaccharides and analysis of the derived disaccharide alditols as TMS or TFA derivatives by GC.

Experimental

Materials and Reagents——Lactulose (4-O-β-D-galactopyranosyl-D-fructose) and isomaltose (6-O-α-D-glucopyranosyl-D-glucose) were purchased from K&K Laboratories, Inc., Plainview, N.Y. All other carbohydrates used in this study were of guaranteed grade purity and were purchased from the following commercial sources, respectively. Lactose (4-O-β-D-galactopyranosyl-D-glucose) and sucrose (α-D-glucopyranosyl-β-D-fructofuranoside) were from Kanto Chemical Co., Inc., Tokyo. Maltose (4-O-α-D-glucopyranosyl-D-glucose), trehalose (α-D-glucopyranosyl-α-D-glucopyranoside), turanose (3-O-α-D-glucopyranosyl-D-fructose), cellobiose (4-O-β-D-glucopyranosyl-D-glucose), gentiobiose (6-O-β-D-glucopyranosyl-D-glucose), melibiose (6-O-α-D-galactopyranosyl-D-glucose) and phenyl-β-D-glucoside were from Tokyo Kasei Kogyo Co., Ltd., Tokyo.

Pyridine stored on KOH pellets and N,N-dimethylformamide (DMF) were used in the trimethylsilylation reaction. Ethyl acetate (AcOEt) and DMF, which were used in the trifluoroacethylation reaction, were stored on Wakogel Q-23 (silica gel for chromatography, 100—200 mesh, Wakō Pure Chemical Industries Ltd., Tokyo) or Molecular Sieve (3A, 1/16, Nishio Kogyo Co., Ltd., Tokyo) or anhydrous sodium sulfate (GR; Kanto Chemical Co., Inc.); these drying agents had been heated for 3 hr at 220° before use. All of above mentioned solvents were of guaranteed grade purity from Kanto Chemical Co., Inc.

Trifluoroacetic acid and *n*-hexane (GR; Tokyo Kasei Kogyo Co., Ltd.) were used directly.

Sodium borohydride ($NaBH_4$, GR; E Merck AG) was used directly. Hexamethyldisilazane (HMDS, EP), trimethylchlorosilane (TMCS, EP), trifluoroacetic anhydride (TFAA, GR) and pyrene (GR) were products of Tokyo Kasei Kogyo Co., Ltd. and were used directly.

Apparatus——Shimadzu Gas Chromatograph GC-1B, GC-1C, GC-4APF (all equipped with a hydrogen flame detector (HFID)) and GC-4APE equipped with an electron caputre detector (ECD) were used. The carrier gas was nitrogen. The glass tubes (1.8 m×4 mm i.d. or 1.5 m×4 mm i.d.) were packed with 2% OV-1, 2% OV-17, 2% SE-30, 2% GE-XF-1105, 2% QF-1 or 1% NGS coated on Gas Chrome P (80—100 mesh).

Reduction of Disaccharides (Sample Preparation)——To 0.5 ml of aqueous solution containing less than 500 μg of a mixture of disaccharides, 0.5 ml of 1% $NaBH_4$ in distilled water was added. The solution was stand for 10 min at room temperature and the excess $NaBH_4$ was destroyed by adding 0.4 ml of Amberlite CG-120 (H^+). The resin was removed by a sintered glass filter and was washed with 0.5 ml of MeOH. The filtrate and the washing were combined and evaporated to dryness below 40° *in vacuo*.[15] To the residue 2 ml of MeOH was added and evaporated to dryness to remove the borate as trimethyl borate. The final treatment was repeated three times.

Preparation of TMS Derivatives——The sample was dissolved in 0.1 ml of anhydrous pyridine and was trimethylsilylated with 0.1 ml of HMDS and 0.05 ml of TMCS for 1—2 min at 75° on a water bath.

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[10] R.G. Coffey and F.J. Reithel, *Anal. Biochem.*, 32, 229 (1969).

[11] W.J.A. VandenHeuvel and E.C. Horning, *Biochem. Biophys. Res. Commun.*, 4, 399 (1961).

[12] M. Gee and H.G. Walker, Jr., *Anal. Chem.*, 34, 650 (1962).

[13] *a*) M. Vilkas, Hiu-I-Jan, Mme G. Boussac and Mme M.C. Bonnard, *Tetrahedron Letters*, **1966**, **1441**; *b*) T. Ueno, N. Kurihara and M. Nakajima, *Agr. Biol. Chem.* (Tokyo), **31**, 1189 (1967).

[14] *a*) S.W. Gunner, J.K.N. Jones and M.B. Perry, *Chem. Ind.* (London), **1961**, **255**; *b*) J.S. Sawardeker, J.H. Sloneker and A. Jeanes, *Anal. Chem.*, **37**, 1602 (1965); *c*) Yu.S. Ovodov and E.V. Evtushenko, *J. Chromatog.*, **31**, 527 (1967); *d*) W.F. Lehnhardt and R.J. Winzler, *ibid.*, **34**, 471 (1968); *e*) M. Matsui, M. Okada, T. Imanari and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **16**, 1383 (1968); *f*) T. Imanari, Y. Arakawa and Z. Tamura, *ibid.*, **17**, 1967 (1969).

[15] All the evaporation procedures were performed with rotary evaporators below 40° *in vacuo* throughout this study except the cases denoted.

Then the reaction mixture was stand for 15 min at room temperature and 1—4 μl of it was injected directly to the gas chromatograph. In case of minor quantities (0.5—10 μg) of disaccharides, the reaction mixture was evaporated to dryness below 30°, and the TMS derivatives were extracted with 0.5—1 ml of *n*-hexane. The clear hexane extract was evaporated with nitrogen stream or by a evaporator at room temperature in a microtube (50 mm × 6 mm i.d.). After addition of 10—20 μl of hexane to the residue, 1—5 μl of the resulted solution was injected to the gas chromatograph.

Preparation of TFA Derivatives——The sample was treated with 50 μl of DMF and 30 μl of TFAA for 5 min at room temperature and 1—4 μl of the reaction mixture was injected to the gas chromatograph. In the analysis of disaccharides less than 1 μg, ECD was exclusively used and in this case the reaction mixture was added with appropriate amount of hexane and 1—3 μl of the resulted solution was injected.

Analysis of the Monosaccharide Components of Disaccharide Alditols——One mg of each reduction products of lactulose or turanose was dissolved in 2 ml of 25% trifluoroacetic acid and was hydrolyzed for 4 hr at 106° in a sealed glass tube. Evaporation of the hydrolysate was repeated with addition of water several times to remove the trifluoroacetic acid. Hexoses and alditols released were trifluoroacetylated and identified by GC on 2% GE-XF-1105 column at 140°.

Result

Reduction of Disaccharides

Since any detailed paper which described the process of determining the reductive conditions of carbohydrates with $NaBH_4$ in gas chromatographic scale has not been at hand, the reduction of reducing disaccharides and the stability of the derived disaccharide alditols and non-reducing disaccharide during the reduction were examined. As shown in Fig. 1, the rates of reduction of maltose and gentiobiose were dependent upon the concentration of $NaBH_4$ and the reducing disaccharides were completely reduced within 10 min with 0.5% $NaBH_4$.

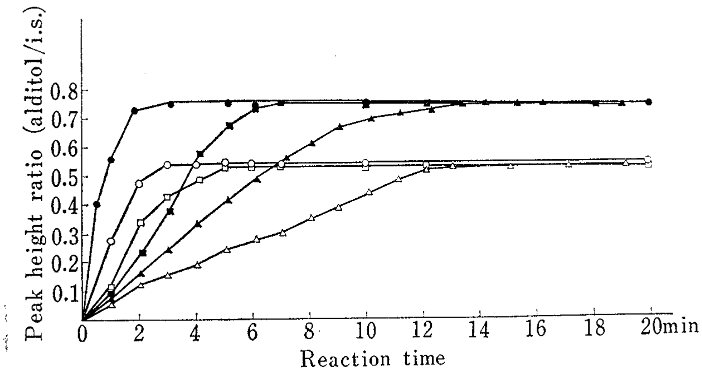

Fig. 1. Reduction of Maltose and Gentiobiose with $NaBH_4$

To 0.5 ml of aqueous solution containing sucrose (500 μg) and maltose (500 μg), 0.5 ml of 2.0, 1.0 or 0.5% $NaBH_4$ in distilled water was added respectively. Reduction was carried out at room temperature (20°) and the aliquots of the reaction mixture were treated by the successive procedures described in Experimental. The reaction was followed by the increasing peaks of TMS derivatives of maltitol using the sucrose as the internal standard (i.s.). Reduction of gentiobiose (500 μg) was carried out and followed in the same manner as that of maltose using trehalose (1250 μg) as the internal standard. Gas chromatography was performed on 2% OV-17(1.8 m × 4 mm i.d.) column at 210° for the analysis of maltitol and 225° for that of gentiobiitol. The flow rate of nitrogen was 90 ml/min.

—○—: maltose in 1.0% $NaBH_4$,
—●—: gentiobiose in 1.0% $NaBH_4$
—□—: maltose in 0.5% $NaBH_4$,
—■—: gentiobiose in 0.5% $NaBH_4$
—△—: maltose in 0.25% $NaBH_4$,
—▲—: gentiobiose in 0.25% $NaBH_4$

Sucrose and the produced disaccharide alditols were stable in 1% $NaBH_4$ within 24 hr period at room temperature.

The Trimethylsilylation Reaction

Sucrose, lactitol and maltitol were completely trimethylsilylated within 10 min with the method by Sweeley, *et al.*[3e] (Fig. 2).

The Trifluoroacethylation Reaction

The trifluoroacethylation of the carbohydrates with TFFA in DMF in place of AcOEt[14f] was accomplished within a few minutes at room temperature as shown in Fig. 3.

Separation of TMS Derivatives

The resolution of TMS derivatives of non-reducing disaccharides and disaccharide alditols was examined on several columns and relative retention times in the best conditions of each columns are given in Table I.

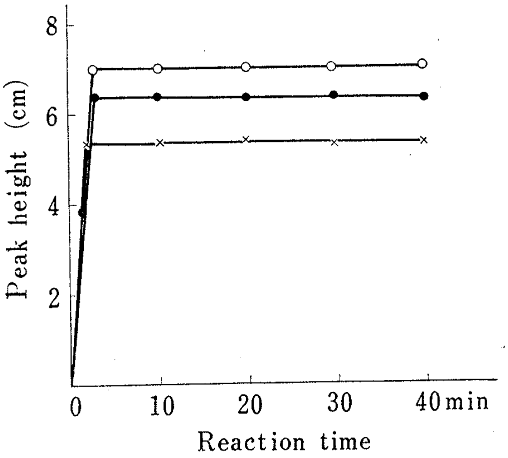

Fig. 2. Trimethylsilylation of Sucrose, Lactitol and Maltitol

Sucrose (8.0 mg), lactitol (10.7 mg) and maltitol (11.7 mg) were treated with 1.0 ml of anhydrous pyridine, 0.2 ml of HMDS and 0.1 ml of TMCS at room temperature (20°). conditions: column, 2% OV-17 (1.8 m×4 mm i.d.); temperature[a] T_c 210°, T_{inj} 250°, T_d 230°; N_2 95 ml/min

—○—: lactitol
—●—: sucrose
—×—: maltitol

a) The following abbreviations were used in this paper.
T_c: column temperature, T_{inj}: injection port temperature, T_d: detector temperature

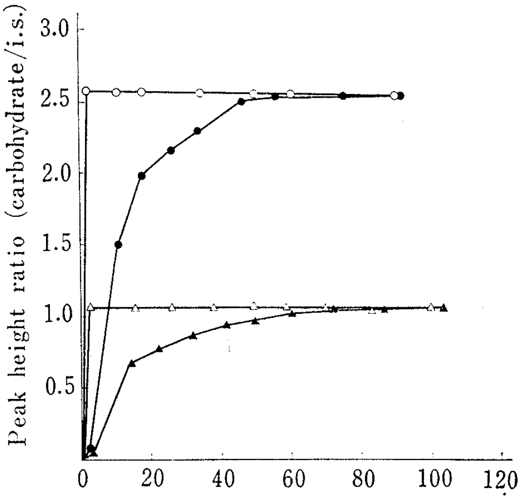

Fig. 3. Trifluroacetylation of Sucrose and Lactitol

Sucrose (500 μg) or lactitol (500 μg) was dissolved in 50 μl of DMF or AcOEt containing pyrene (50 μg, internal standard) and was treated with 30 μl of TFAA at room temperature (20°). conditions: column, 2% QF-1 (1.5 m×4 mm i.d.); T_c 190°, T_{inj} 200°, T_d 220°; N_2 60 ml/min

—○—: sucrose in DMF
—●—: sucrose in AcOEt
—△—: lactitol in DMF
—▲—: lactitol in AcOEt

TABLE I. Relative Retention Times for TMS Derivatives

Carbohydrate	2% OV-1 (1.5 m) 220°(*a*)	2% OV-17 (1.8 m) 210°(*b*)	2% SE-30 (1.8 m) 226°(*c*)	2% QF-1 (1.8 m) 190°(*d*)	2% GE-XF-1105 (1.8 m) 210°(*e*)	1% NGS (1.8 m) 170°(*f*)
Phenyl-β-D-glucoside	0.28	0.50	0.30	0.44	0.29	0.56
Sucrose	1.00 (11.80 min)	1.00 (11.24 min)	1.00 (15.0 min)	1.00 (4.95 min)	1.00 (26.8 min)	1.00 (12.4 min)
Lactitol	1.57	1.22	1.55	1.68	1.62	1.65
4-O-β-D-Galactopyranosyl-D-Mannitol	1.57	1.22	1.55	1.68	1.62	1.65
Cellobiitol	1.70	1.39	1.65	1.67	1.79	1.79
Trehalose	1.36	1.43	1.35	1.37	1.49	—
3-O-α-D-Glucopyranosyl-D-Mannitol	1.89	1.55	1.85	1.90	1.97	1.86
3-O-α-D-Glucopyranosyl-D-Glucitol	1.89	1.55	1.85	1.90	1.97	1.86
Maltitol	1.98	1.63	1.93	2.10	2.04	1.97
Gentiobiitol	2.47	2.26	2.32	2.52	2.78	3.13
Isomaltitol	2.61	2.34	2.48	2.42	2.77	—
Melibiitol	2.88	2.58	2.71	3.04	3.25	3.50

The flow rates of nitrogen are 55, 90, 65, 42, 120 and 150 ml/min in *a*), *b*), *c*), *d*), *e*), *f*) respectively.

Good separation was obtained on non-polar 2% OV-1, 2% OV-17 and 2% SE-30 columns, and also on polar 2% QF-1 and 1% NGS columns. The representative gas chromatograms obtained on 2% OV-1 and 2% QF-1 columns are shown in Figures 4 and 5, respectively.

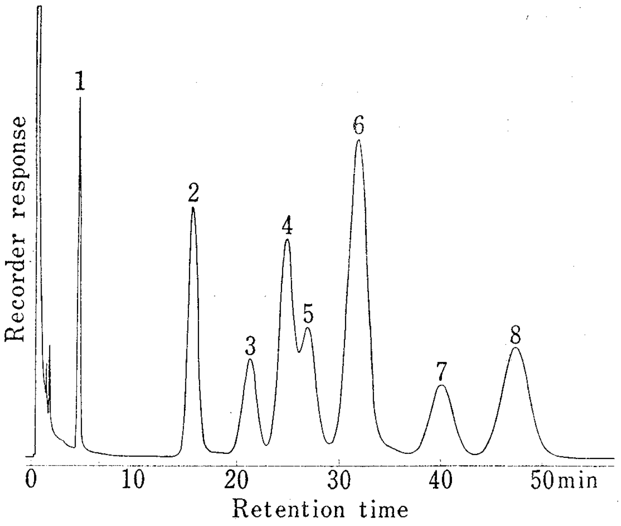

Fig. 4. Gas Chromatogram of TMS Derivatives

peak: 1. phenyl-β-D-glucoside, 2. sucrose, 3. trehalose, 4. lactitol, 5. cellobiitol, 6. maltitol, 7. isomaltitol, 8. melibiitol
conditions: column, 2% OV-1(1.5 m×4 mm i.d.); T_c 210°, T_{inj} 230°, T_d 240°; N_2 55 ml/min; sens. 10^3, range 6.4 V

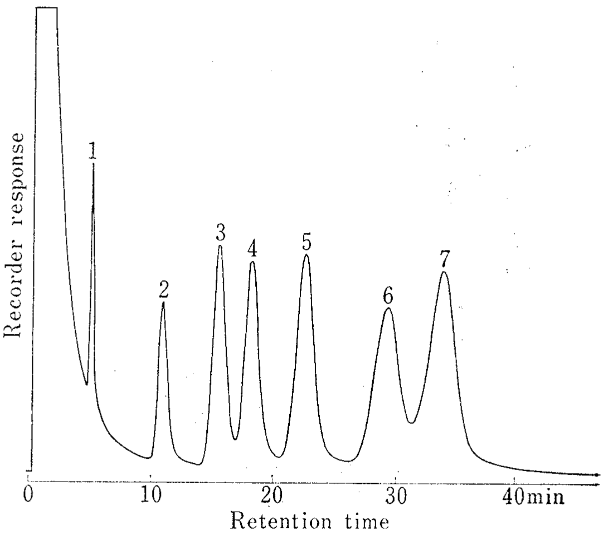

Fig. 5. Gas Chromatogram of TMS Derivatives

peak: 1. phenyl-β-D-glucoside, 2. sucrose, 3. trehalose 4. lactitol, 5. maltitol, 6. gentiobiitol, 7. melibiitol
conditions: column, 2% QF-1 (1.8 m×4 mm i.d.); T_c 190°, T_{inj} 230°, T_d 240°; N_2 42 ml/min: sens. 10^3, range 6.4 V

However, separations of maltitol from 3-O-α-D-glucopyranosyl-D-glucitol and 3-O-α-D-glucopyranosyl-D-mannitol (reduction products of turanose), isomaltitol from gentiobiitol were unsuccessful in all columns used.

Separation of TFA Derivatives

The best separation of TFA derivatives was achieved on 2% GE-XF-1105 column (Table II).

Table II. Relative Retention Times for TFA Derivatives

Carbohydrate	2% OV-1 (1.5 m) 140°, 60 ml/min	2% QF-1 (1.5 m) 180°, 55 ml/min	2% GE-XF-1105 (1.8 m) 205°, 90 ml/min
Sucrose	1.00 (6.78 min)	1.00 (9.78 min)	1.00 (6.14 min)
Trehalose	0.98	1.31	1.47
Melibiitol	0.93	0.96	1.50
3-O-α-D-Glucopyranosyl-D-mannitol	0.87	0.93	1.67
3-O-α-D-Glucopyranosyl-D-lucitol	0.87	0.93	1.67
Isomaltitol	—	—	1.67
Maltitol	0.99	1.00	2.12
Gentiobiitol	1.41	1.61	2.15
Cellobiitol	0.96	1.35	2.98
4-O-β-D-Galactopyranosyl-D-mannitol	1.22	1.46	3.39
Lactitol	1.22	1.63	3.96

Representative gas chromatograms are shown in Figures 6 and 7.

Thus seven TFA derivatives were completely separated from each other within 30 min, and the separations of maltitol from the reduction products of turanose, isomaltitol from gentiobiitol, which were not achieved with TMS derivatives on any liquid phases used, were completely obtained. Similarly, the two reduction products of lactulose, 4-O-β-D-galactopyranosyl-D-glucitol (lactitol) and 4-O-β-D-galactopyranosyl-D-mannitol, were separated as

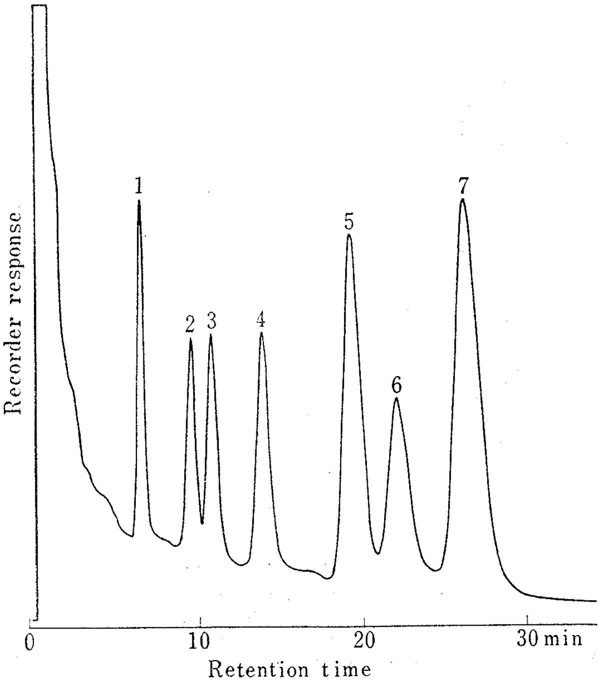

Fig. 6. Gas Chromatogram of TFA Derivatives with HFID

peak: 1. sucrose, 2. trehalose, 3. isomaltitol, 4. maltitol, 5. cellobiitol, 6. 4-O-β-D-galactopyranosyl-D-mannitol, 7. lactitol
conditions: column, 2% GE-XF-1105 (1.8 m×4 mm i.d.); T_c 205°, T_{inj} 220°, T_d 230°; N_2 90 ml/min; sens. 10^3, range 6.4 V

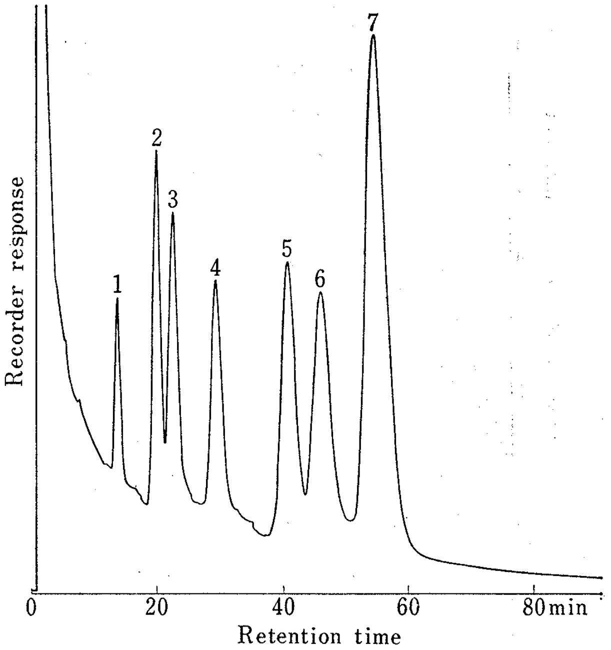

Fig. 7. Gas Chromatogram of TFA Derivatives with ECD

peak: 1. sucrose, 2. trehalose, 3. 3-O-α-D-glucopyranosyl-D-glucitol and 3-O-α-D-glucopyranosyl-D-mannitol, 4. maltitol, 5. cellobiitol, 6. 4-O-β-D-galactopyranosyl-D-mannitol, 7. lactitol
conditions: column, 2% GE-XF-1105 (1.5 m×4 mm i.d.); T_c 180°, T_{inj} 200°, T_d 200°; N_2 65 ml/min; sens. 10^3, range 1.6 V

TFA derivatives on 2% QF-1 and 2% GE-XF-1105 columns (Table II). Productions of the two isomers were confirmed by hydrolysis of the reduction product, which gave 2.5 mole of galactose, 1.5 mole of glucitol and 1.0 mole of mannitol. With turanose, it was similarly proved that 3-O-α-D-glucopyranosyl-D-glucitol and 3-O-α-D-glucopyranosyl-D-mannitol were produced in a ratio of about 2 to 1, though the separation of the isomers was not achieved by any gas chromatographic systems employed.

Quantitative Analysis

The internal standard was added to the disaccharide samples prior to the reduction and the analysis was performed with the peak height ratio method. The calibration curves with TMS derivatives for sucrose, lactose, maltose and melibiose using trehalose as an internal standard are shown in Fig. 8.

The determination limit of the TMS derivatives by a usual method in which the reaction mixture was directly injected to the gas chromatograph was about 5—10 μg, in contrast we could quantify 0.5 μg of disaccharide with the hexane extraction technique described in Experimental. The limit obtained is the smallest one ever achieved in TMS carbohydrates.

The calibration curves with TFA derivatives for some disaccharides are shown in Fig. 9 (HFID) and Fig. 10 (ECD) with good linearlity.

The determination limit of the TFA derivatives was 5—10 μg with HFID and 0.1 μg with ECD. The employment of the hexane extraction technique to the TFA derivatives gave no reproducible result.

An authentic mixture of disaccharides was analyzed as TMS derivatives on a 2% OV-17 column and the variation and the average recoveries of 10 samples are illustrated in Table III.

These data indicate that the method including the hexane extraction technique is accurate enough for quantitative analysis of disaccharides.

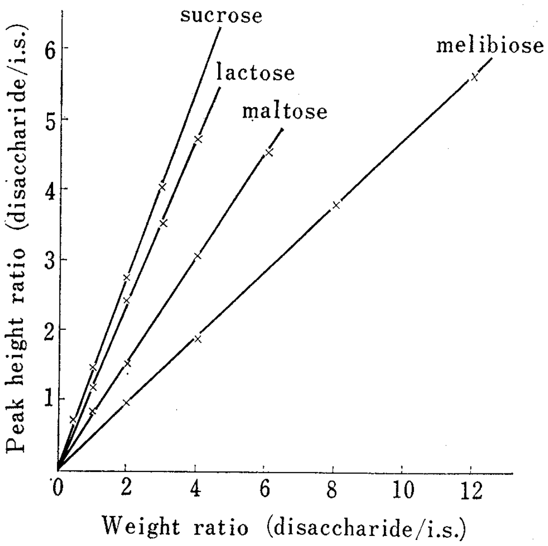

Fig. 8. Calibration Curves for Disaccharides using Trehalose as an Internal Standard (i.s.)

Analysis of TMS derivatives of the disaccaride and the disaccharide alditols was performed on 2% OV-17 (1.8 m×4 mm i.d.) column at 210°. The flow rate of nitrogen was 90 ml/min.

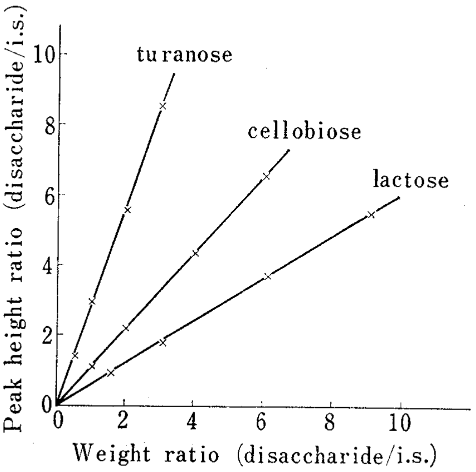

Fig. 9. Calibration Curves for Disaccharides using Maltose as an Internal Standard(i.s.) with HFID

Analysis of TFA derivatives of the disaccharide alditols was performed on 2% GE-XF-1105 (1.5 m×4 mm i.d.) column at 195°. The flow rate of nitrogen was 43 ml/min.

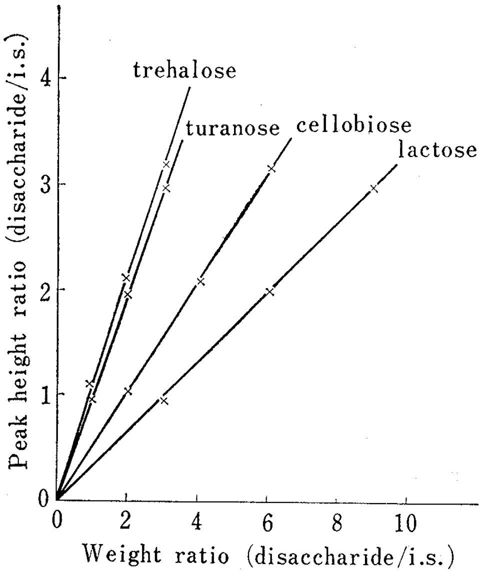

Fig. 10. Calibration Curves for Disaccharides using Maltose as an Internal Standard (i.s) with ECD

Analysis of TFA derivatives of disaccharide alditols was performed on 2% GE-XF-1105 (1.5 m×4 mm i.d.) column at 180° The flow rate of nitrogen was 65 ml/min.

Discussion

During the couse of our study, Kärkkäinen[16] reported the structural analysis of disaccharides as TMS derivatives of corresponding alditols by GC-mass spectrometry, however, he did not examine the conditions of separation and determination of minute amount of disaccharides. We achieved a good separation of TMS derivatives of disaccharide alditols on 2% OV-1, 2% OV-17 and 2% QF-1 columns, and the best separation was achieved as TFA derivatives on 2% GE-XF-1105 column, similarly to the separation of TFA monosaccharide alditols.[14e,f]

Disaccharides, especially one involves (1→3) or (1→4) linkage, are tend to be labile to alkalie treatment, but the successful reduction was performed in the solution of 0.5% $NaBH_4$ in a short time at room temperature. On the other hand, fructose residue is sensitive to acid, and the possibility of the methanolysis was pointed in the preparation of lactitol by reduction of lactose.[17] However, no degradation was occurred by removal of water and boric acid below 40°.

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16) J. Kärkkäinen, *Carbohyd. Res.*, **11**, 247 (1969).

17) J.R. Clamp, L. Hough, J.L. Hickson and R.L. Whistler, "Advances in Carbohydrate Chemistry," Vol. 16, ed. by M.L. Wolfrom, Academic Press, New York, N.Y., 1961, pp. 159—206.

Table III. Precision of Analysis

		Sucrose	Lactose	Maltose	Melibiose
Added (μg)		2.0	3.0	3.0	5.0
Found (μg)	1	2.02	2.99	3.08	4.86
	2	1.98	3.02	2.95	4.91
	3	1.96	2.89	2.91	4.87
	4	1.95	3.02	2.99	4.92
	5	1.96	2.94	2.93	4.95
	6	1.97	2.97	2.95	4.96
	7	1.89	2.95	3.05	4.99
	8	1.90	2.98	3.10	4.98
	9	1.99	2.95	2.98	4.89
	10	1.93	2.97	3.08	5.07
Mean		1.96	2.97	3.00	4.94
Standard deviation		0.030	0.037	0.065	0.060
Recovery (%)		98.0	98.9	100.0	98.8

For the purpose of microanalysis of TMS derivatives of methylglycosides, Yamakawa, *et al.*[18] added to the reaction mixture with chloroform and water, and the organic phase was condensed after removal of the upper aqueous layer and the resulting condensate was used for analysis, however this method was not employed to quantitative analysis. Use of the hexane extraction technique described in Experimental was found to offer several advantages. The reagents were first removed by evaporation of the reaction mixture of trimethylsilylation, and only TMS derivatives of carbohydrates and ammonium chloride were remained, therefore, the solution which contained exclusively the former was obtained by the successive hexane extraction. This resulted in marked decrease of determination limit to 0.5 μg of a disaccharide, and in reduction of tailing of pyridine and of damage on columns and detectors. The TMS derivatives were stable in hexane for a few months.

The preparation of TFA derivatives of some carbohydrates and related compounds was performed with TFAA and sodium trifluoroacetate in acetonitrile,[13a,19] or formamide[13b]. However, the reported methods were not always suited for microanalysis by GC with ECD. Recently, Imanari, *et al.*[14f] reported the trifluoroacetylation of monosaccharide alditols with TFAA in AcOEt without catalyst at room temperature, but the method was not suitable for disaccharides, because it took about 1 hr for complete trifluoroacethylation at room temperature. We overcame this difficulty by using DMF in place of AcOEt; the trifluoroacethylation reaction of disaccharides and disaccharide alditols with TFAA in DMF was accomplished in a few minutes at room temperature.

Only one report was described as to the use of ECD in gas chromatographic analysis of carbohydrates by Tamura, *et al.*,[20] who analyzed 1 ng of monosaccharide as TFA derivatives. We determined 0.1 μg and detected 0.1 ng of disaccharide with ECD.

In combination with the method by Matsui, *et al.*,[14e] the present method provides a simultaneous gas chromatographic analysis of ten monosaccharides and seven disaccharides as TFA derivatives of the corresponding alditols on 2% GE-XF-1105 column.

The validity of the present method will be further verified in the successive paper[21] by applying to analysis of disaccharides in human materials, such as serum, urine, milk, feces *etc.*

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18) T. Yamakawa, N. Ueta and I. Ishizuka, *Japan. J. Exp. Med.*, **34**, 231 (1964).

19) E.J. Bourne, C.E.M. Tatlow and J.C. Tatlow, *J. Chem. Soc.*, **1950**, 1367; E.J. Bourne, M. Stacey, C.E.M. Tatlow and J.C. Tatlow, *ibid.*, **1951**, 826.

20) Z. Tamura and T. Imanari, *Chem. Pharm. Bull.* (Tokyo), **15**, 246 (1967).

21) H. Nakamura and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), in preparation.